Supporting Information

Fluorescence monitoring of STING using a coumarin-based chemigenetic probe

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Experimental Section

General notes

Unless specified otherwise, all reactions were conducted in flame-dried glassware under an argon atmosphere. Commercial reagents and solvents were utilized as received unless otherwise indicated. Flash column chromatography was performed with silica gel cartridges (40-65 μm particle size). The progress of reactions was monitored via thin-layer chromatography (TLC) using an ultraviolet (UV) lamp for detection. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were acquired on a Bruker NMR spectrometer (400/101 MHz) in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard, expressed as δ values. High-resolution mass spectrometry (HRMS) analyses were performed using a Waters LCT spectrometer. Ultraviolet-visible (UV-Vis) spectra were recorded on a PUMADA P7 instrument, and fluorescence spectra were obtained using a Shimadzu RF6000 instrument. The syntheses of **Y5** (RF15) and **Y6** (RF16) were conducted according to the previous reference[1, 2]. All synthesized compounds were determined to be ≥ 95% pure by ^{1}H NMR.

Synthesis of chemical probes Y1-Y6.

8-(1,3-dioxan-2-yl)-4-methyl-2-oxo-2H-chromen-7-yl acetate (**Y1**). Compound **2** (80 mg, 0.3 mmol), acetyl chloride (40 mg, 0.5 mmol) and potassium carbonate (108 mg, 0.6 mmol) in 5 mL ACN were added into a 25 mL Schlenk tube equipped with a magnetic stirring bar. Under reduced pressure, the tube was filled with argon for three times and the reaction was stirred at room temperature for 12 h. The reaction mixture was concentrated and purified by flash chromatography on silica gel using DCM/CH_3OH (v/v = 50:1) to afford **Y1** (75 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.7 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 6.31 (s, 1H), 6.28 (s, 1H), 4.32 – 4.17 (m, 2H), 4.04 (td, J = 12.3, 2.2 Hz, 2H), 2.42 (s, 3H), 2.38 (s, 3H), 2.23 (qt, J = 12.7, 5.0 Hz, 1H), 1.51 (d, J = 13.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.35, 159.80, 152.19, 152.04, 151.54, 125.66, 120.70, 119.01, 117.99, 114.45, 95.59, 67.76, 26.00, 20.98, 18.92.

7-(benzyloxy)-8-(1,3-dioxan-2-yl)-4-methyl-2H-chromen-2-one (**Y2**). Compound **2** (80 mg, 0.3 mmol), bromomethyl-benzene (85 mg, 0.5 mmol) and potassium carbonate (108 mg, 0.6 mmol) in 5 mL ACN were added into a 25 mL Schlenk tube equipped with a magnetic stirring bar. Under reduced pressure, the tube was filled with argon for three times and the reaction was stirred at room temperature for 12 h. The reaction mixture was concentrated and purified by flash chromatography on silica gel using DCM/CH_3OH (v/v = 50:1) to afford **Y2** (80 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.49 (m, 3H), 7.46 – 7.31 (m, 3H), 6.94 (d, J = 8.9 Hz, 1H), 6.40 (s, 1H), 6.15 (s, 1H), 4.28 (dd, J = 11.3, 4.8 Hz, 2H), 4.04 (td, J = 12.4, 2.4 Hz, 2H), 2.37 (s,

3H), 2.32 (td, J = 7.9, 3.9 Hz, 1H), 1.44 (d, J = 11.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.66, 160.02, 152.59, 152.31, 136.50, 128.45, 127.91, 127.09, 126.27, 114.62, 114.13, 112.36, 109.69, 96.13, 71.02, 67.78, 25.81, 18.80.

7-(benzyloxy)-8-(5-(hydroxymethyl)-5-methyl-1,3-dioxan-2-yl)-4-methyl-2H-chromen-2-one (**Y3**). Compound 3 (80 mg, 0.26 mmol), bromomethyl-benzene (85 mg, 0.5 mmol) and potassium carbonate (108 mg, 0.6 mmol) in 5 mL ACN were added into a 25 mL Schlenk tube equipped with a magnetic stirring bar. Under reduced pressure, the tube was filled with argon for three times and the reaction was stirred at room temperature for 12 h. The reaction mixture was concentrated and purified by flash chromatography on silica gel using DCM/CH_3OH (v/v = 50:1) to afford **Y3** (60 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 8.9 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.46 – 7.40 (m, 2H), 7.40 – 7.34 (m, 1H), 6.95 – 6.90 (m, 1H), 6.29 (s, 1H), 6.16 (q, J = 1.2 Hz, 1H), 5.24 (s, 2H), 4.11 – 4.02 (m, 2H), 3.91 (s, 2H), 3.72 – 3.64 (m, 2H), 2.36 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.07, 159.09, 153.69, 152.84, 136.97, 129.00, 128.38, 127.86, 127.55, 114.10, 113.97, 112.01, 110.09, 96.08, 73.45, 70.63, 64.61, 63.78, 35.34, 18.78, 17.26.

8-(1,3-dioxan-2-yl)-4-methyl-7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2H-chromen-2-one (**Y4**). Compound 2 (80 mg, 0.3 mmol), 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (150 mg, 0.5 mmol) and potassium carbonate (108 mg, 0.6 mmol) in 5 mL ACN were added into a 25 mL Schlenk tube equipped with a magnetic stirring bar. Under reduced pressure, the tube was filled with argon for three times and the reaction was stirred at room temperature for 12 h. The reaction mixture was concentrated and purified by flash chromatography on silica gel using DCM/CH_3OH (v/v = 50:1) to afford **Y4** (90 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 7.7 Hz, 2H), 7.55 (dd, J = 23.5, 8.3 Hz, 3H), 6.92 (d, J = 8.9 Hz, 1H), 6.40 (s, 1H), 6.15 (d, J = 1.5 Hz, 1H), 5.24 (s, 2H), 4.28 (dd, J = 11.4, 4.8 Hz, 2H), 4.04 (td, J = 12.3, 2.4 Hz, 2H), 2.37 (s, 3H), 1.44 (d, J = 13.4 Hz, 1H), 1.37 (s, 12H), 1.27 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.73, 159.99, 152.53, 152.40, 139.60, 134.90, 126.31, 126.19, 114.53, 114.12, 112.33, 109.59, 96.16, 83.88, 70.86, 67.80, 25.83, 24.89, 18.80. HRMS (TOF-ESI$^+$): m/z calcd for $C_{27}H_{31}O_7BNa$ [M + Na^+]: 501.2061; found: 501.2055.

8-(5-(hydroxymethyl)-5-methyl-1,3-dioxan-2-yl)-4-methyl-7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2H-chromen-2-one (**Y5**). A mixture of compound 3 (100 mg, 0.32 mmol), 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2 dioxaborolane (192 mg, 0.65 mmol), and $CsCO_3$ (325 mg, 1.0 mmol) in Acetonitrile (10 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated and the residue was purified by silica gel flash chromatography using DCM/ Methanol (v/v =

100:1) to afford **Y5** as a white powder (82 mg, 48%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.80 (m, 2H), 7.57 – 7.44 (m, 3H), 6.89 (d, *J* = 9.0 Hz, 1H), 6.29 (s, 1H), 6.16 (q, *J* = 1.2 Hz, 1H), 5.26 (s, 2H), 4.14 – 4.04 (m, 2H), 3.99 (s, 2H), 3.74 – 3.64 (m, 2H), 2.37 (s, 3H), 1.36 (s, 12H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.66, 159.44, 152.84, 152.29, 139.06, 135.10, 126.79, 126.33, 114.31, 114.02, 112.47, 109.31, 95.96, 83.95, 74.17, 71.04, 66.65, 34.94, 24.89, 18.82, 17.24. HRMS (TOF-ESI$^+$): m/z calcd for $C_{29}H_{35}O_8BNa$ [M + Na^+]: 545.2323; found: 545.2309.

18-chloro-3,6,9,12-tetraoxaoctadecyl ((5-methyl-2-(4-methyl-2-oxo-7-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2H-chromen-8-yl)-1,3-dioxan-5-yl)methyl) malonate (**Y6**). A mixture of Y5(50 mg, 0.10 mmol), 22-chloro-3-oxo-4,7,10,13,16-pentaoxadocosanoic acid (45 mg, 0.11 mmol), and 4-Dimethylaminopyridine (10 mg, 0.09mmol) in THF (5 mL) was stirred at icy water. Dicyclohexylcarbodiimide (30 mg, 0.15 mmol) was added and stir at room temperature for 12 h. The reaction mixture was concentrated and the residue was purified by silica gel flash chromatography using DCM/ Methanol (v/v = 100:1) to afford **Y6** as a white powder (70 mg, 40%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.82 (m, 2H), 7.55 – 7.46 (m, 3H), 6.90 (d, *J* = 8.9 Hz, 1H), 6.29 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 5.26 (s, 2H), 4.36 – 4.27 (m, 2H), 4.14 – 4.05 (m, 2H), 3.98 (s, 2H), 3.77 (s, 2H), 3.75 – 3.62 (m, 12H), 3.62 – 3.39 (m, 8H), 2.41 – 2.35 (s, 3H), 1.80 (dq, *J* = 8.0, 6.7 Hz, 2H), 1.62 (dq, *J* = 8.0, 6.7 Hz, 3H), 1.51 – 1.38 (m, 4H), 1.37 (s, 12H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.91, 160.65, 159.44, 152.83, 152.29, 139.05, 135.10, 126.81, 126.33, 114.31, 114.00, 112.48, 109.30, 95.94, 83.95, 74.19, 71.26, 70.66, 70.64, 70.61, 70.59, 68.86, 52.54, 45.07, 41.23, 34.93, 32.56, 29.46, 26.71, 25.44, 24.89, 18.83, 17.25. HRMS (TOF-ESI$^+$): m/z calcd for $C_{49}H_{64}BClO_{15}Na$ [M + Na^+]: 925.3924; found: 925.3937.

Fluorescence response of compound 1, Y1- Y6.

Stock solutions of each compound were prepared in DMSO at a concentration of 50 mM. For assessing the absorption and fluorescence properties, working solutions (5 μM) of each compound were prepared by diluting the stock solutions into a DMSO/PBS mixture (v/v = 1:99). Subsequently, 200 μL aliquots of these working solutions were used for recording UV-vis and fluorescence spectra of compound **1**, **Y1**, **Y2**, **Y3**, **Y4**, **Y5**, and **Y6** at various conditions.

Following that, 1000 μM H_2O_2 was added to a DMSO/PBS solution (v/v = 1:99) of **Y4** and **Y5** at 5 μM respectively, and the fluorescence changes were recorded at various time course (0, 1, 2, 3, 5, and 10 min) and the time-dependent fluorescence spectra were recorded at each time point.

Construction of STING 139-340_Halo_pET28a, STING 139-379_Halo_pET28a, GFP_Halo_pET28a

plasmids

The sequence of STING 139-340 gene was amplified by polymerase chain reaction (PCR) using primers and inserted in the Halo_pET28a(+) vector using restriction enzyme. The construction of STING 139-340_Halo_pET28a plasmid was verified by DNA gel and Sanger sequencing results. STING 139-379_Halo_pET28a, and GFP_Halo_pET28a were constructed using the same method.

Transformation, culture, and purification of POIs

STING 139-340_Halo_pET28a, STING 139-379_Halo_pET28a, GFP_Halo_pET28a plasmids were transformed into *E. coli* BL21(DE3) strain cells according to the standard protocol by manufactures (TIANGEN). Hexahistidine (His6)-tagged POI was induced for expression in *E. coli* BL21(DE3) strain cells growing in 500 mL fresh LB medium with 0.1 mM IPTG at 30 °C for 16 h. After culture, cells were spun down and resuspended in 30 mL lysis buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 10 mM imidazole) containing one protease inhibitor tablet for 30 min, then sonicated for complete lysis. After removal of the cell debris via centrifugation, the supernatant was incubated with Ni-NTA agarose (1 mL) at 4 °C overnight. The following day, samples containing Ni-NTA agarose were flowed through a gravity column to remove the untagged protein, washed with 12-column volumes of washing buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 50 mM imidazole), then eluted using a high imidazole elution buffer (20-column volumes, 50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 250 mM imidazole). Fractions were analyzed via SDS−PAGE. Finally, the fractions containing pure POI were pooled, concentrated, and aliquoted into small volume for long-term storage at -80 °C.

Conjugation of various of proteins with Y6 to obtain Y6_POI

In a 100 μL reaction, different concentrations (0.25μg/μL, 0.5 μg/μL ,1 μg/μL) of proteins (STING 139-349_Halo, STING 139-379_Halo, GFP_Halo) were mixed with **Y6** for 1 h at room temperature, respectively. The untreated or **Y5**-treated proteins were used as control in the same condition. After the reaction, the mixture was added with 400 μL of NaH_2PO_4 buffer (50 mM NaH_2PO_4, 300 mM NaCl), transferred to a 10 kDa cutoff ultrafiltration column (Vivaspin 500) for the removal of untagged molecules. The ultrafiltration column was centrifuged at 1,3000 g until the remaining volume was 100 μL. The solution from the bottom tube was retrieved and named as “F”. The ultrafiltration column was refilled with 400 μL of NaH_2PO_4 buffer for additional wash and centrifuged at 1,3000 g until the remaining volume was 100 μL. The solution from the bottom tube after centrifugation was retrieved again and named as “W”. The final protein complex in the ultrafiltration column was transferred to a clean tube and named as “E”. SDS-PAGE was used to monitor the

above “F”, “W”, or “E” fragments from the samples at various conditions. Specifically, aliquot was taken from each sample, treated with 1 mM H_2O_2 for 30 min, boiled, and loaded on the SDS-PAGE. The fluorescence gel was taken by irradiating the gel at 302 nm.

Fluorescence response of Y6_POI_Halo

Y6_POI_Halo was purified from the reaction mixture containing POI_Halo protein (0.25, 0.5, or 1 μg/μL) and **Y6** (100 μM), respectively. After that, **Y6_POI_Halo** in NaH_2PO_4 solution (50 mM NaH_2PO_4, 300 mM NaCl) was incubated with H_2O_2 (0-1000 μM) for 10 min, and the fluorescence changes were recorded by a Shimadzu RF6000 instrument. To compare the fluorescence of **Y6_POI_Halo** purified from different reaction conditions in parallel, we divided the fluorescence intensity (I) of **Y6_POI_Halo** in response of various concentrations of H_2O_2 by the initial fluorescence intensity (I_0) of **Y6_POI_Halo** and applied the fluorescence ratio (I/I_0) as a function against POI concentration in aqueous solution.

Antibodies and reagents.

Antibodies against STING (Proteintech, rabbit polyclonal) and α-Tubulin (Beyotime, mouse monoclonal) were obtained commercially.

Cell culture.

HeLa and 293T cells were cultured at 37 °C in a 5% CO_2 incubator using Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Lonsera), 100 U mL^{-1} penicillin G sodium, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and 100 μg mL^{-1} streptomycin sulfate. HeLa and 293T cells were passaged every 24-36 h depending on the confluence of cells on the culture dishes. All of the cellular experiments were carried out using HeLa and 293T cells from the second to fourth passage to maintain experimental reproducibility.

Cytotoxicity assay.

The cell cytotoxicity of **Y1**, **Y2**, **Y3**, **Y4**, **Y5**, and **Y6** to HeLa or 293T cells were evaluated by 2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) (CCK-8) assay (Solarbio). Cells were plated in 96-well plates at a density of 1×10^4 cells per well for 12 h before treatment. The following day, cells were treated with **Y1**, **Y2**, **Y3**, **Y4**, **Y5**, and **Y6** at 20 μM for 24 h and applied to CCK-8 assay according to the factory’s instruction. The absorbance at 450 nm was measured by a microplate reader (SpectraMax 190, Molecular Devices). Each experiment was conducted in triplicate and measured from three independent experiments.

Protein isolation and immunoblotting.

Cells expressing STING_Halo protein were lysed in radio-immunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors. Following centrifugation, the protein concentration of the supernatants was quantified using the bicinchoninic acid (BCA) assay. Samples were boiled in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and subjected to SDS-PAGE analysis. Separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes, which were then blocked with 5% (wt/vol) nonfat milk in Tris-buffered saline (TBS) prior to immunoblotting with appropriate primary and secondary antibodies. For STING detection, membranes were first incubated with a rabbit polyclonal STING primary antibody at 4 °C overnight, washed three times with Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST), and subsequently incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody at room temperature for 1 hour. After three additional washes with TBST, immunoblots were visualized using BeyoECL Plus chemiluminescence reagent. α-Tubulin served as a loading control and was detected using the same protocol, with a mouse monoclonal α-Tubulin primary antibody and HRP-conjugated goat anti-mouse IgG secondary antibody.

Confocal microscopy.

HeLa or 293T cells were counted and plated onto glass-bottom petri dishes at $0.5x10^5$ cells/well for 12 h before treatment. Live cells were treated with **Y1**, **Y2**, **Y3**, **Y4**, or **Y5** at 20 μM for different time courses, washed with PBS for 3 times, fixed in 4% paraformaldehyde solution for 15 min, and washed with PBS again. Cell images were obtained using a Nikon Ti2-E+A1 confocal microscope. The fluorescence was excited at 488 nm, observed at the emission wavelength from 500 to 550 nm, and expressed as green.

Next, HeLa or 293T cells were counted and plated onto glass-bottom petri dishes at $0.5x10^5$ cells/well for 12 h before treatment. STING_Halo plasmid was transfected for 36 hours. Live cells overexpressing STING_Halo were treated with 20 μM **Y5** or **Y6** for 2 h, then replaced with fresh media and added an extra wash and medium replacement during the additional 2-h incubation time. After treatment, cells were washed with PBS for 3 times, fixed in 4% paraformaldehyde solution for 15 min, and washed with PBS again. Cell images were obtained using a Nikon Ti2-E+A1 confocal microscope. The fluorescence was excited at 488 nm, observed at the emission wavelength from 500 to 550 nm, and expressed as green.

Statistical Analysis

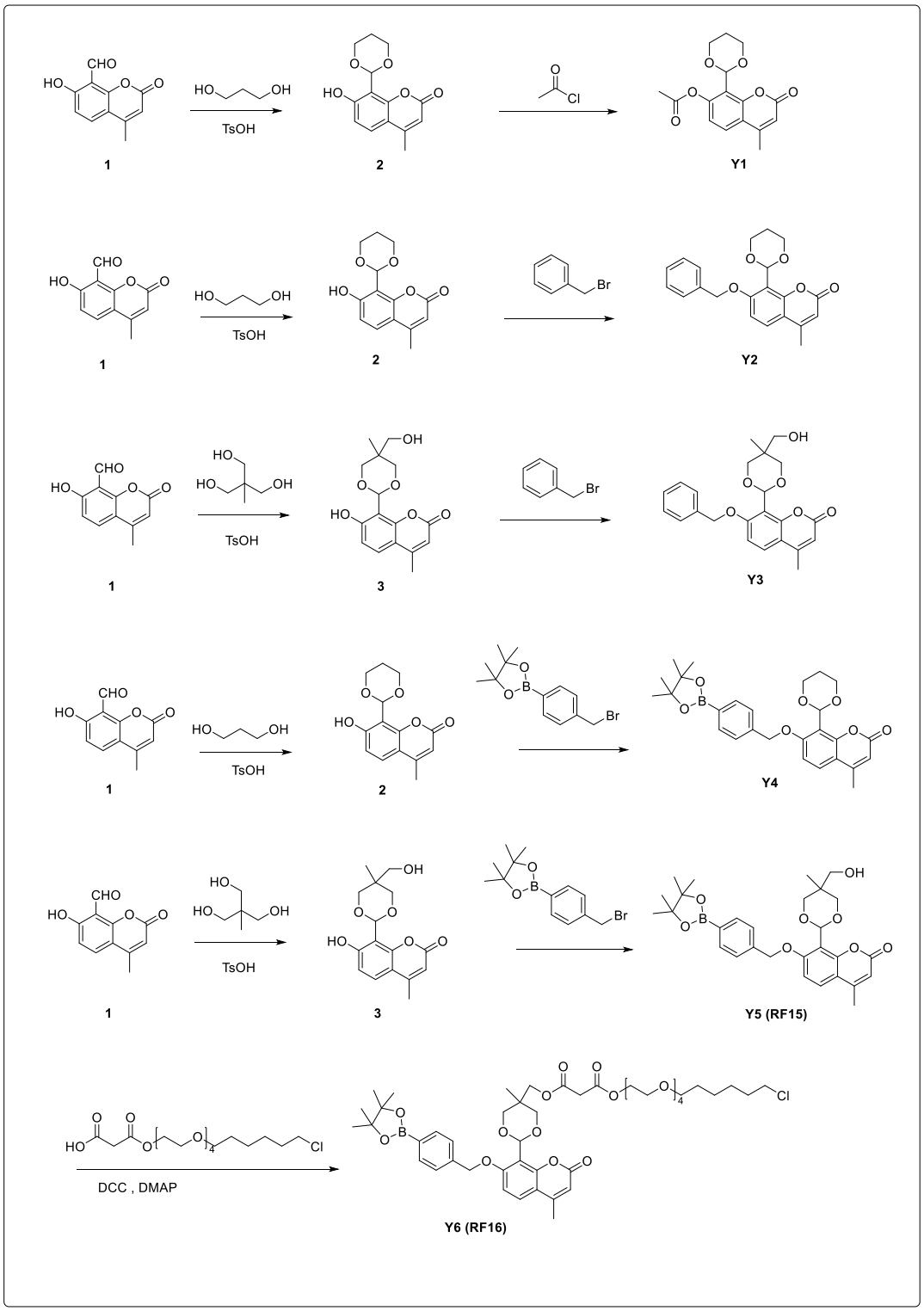
The t-test analysis was used to evaluate data. A *p* value of less than 0.05 was considered significant.

1. Shao, A.; Kang, C. W.; Tang, C. H.; Cain, C. F.; Xu, Q.; Phoumyvong, C. M.; Del Valle, J. R.; Hu,

C. C., Structural Tailoring of a Novel Fluorescent IRE-1 RNase Inhibitor to Precisely Control Its Activity. *Journal of medicinal chemistry* **2019,** *62* (11), 5404-5413.

2. Jiang, Y.; Li, R.; Ren, F.; Yang, S.; Shao, A., Coumarin-Conjugated Macromolecular Probe for Sequential Stimuli-Mediated Activation. *Bioconjugate chemistry* **2024,** *35* (1), 72-79.

Supplementary Figures and Legends



Scheme S1 The synthetic routes of **Y1, Y2, Y3, Y4**, **Y5**, and **Y6**.

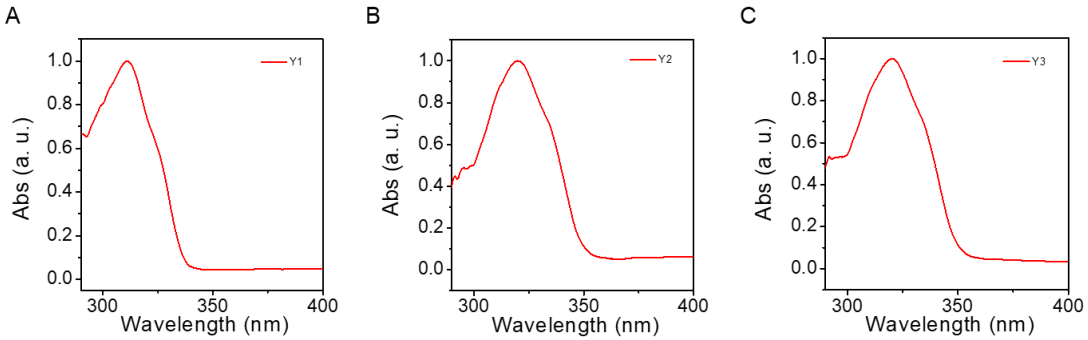


Fig. S1. The absorption spectra of (A) **Y1**, (B) **Y2**, and (C) **Y3** in DMSO/PBS solution (v/v = 1:99).

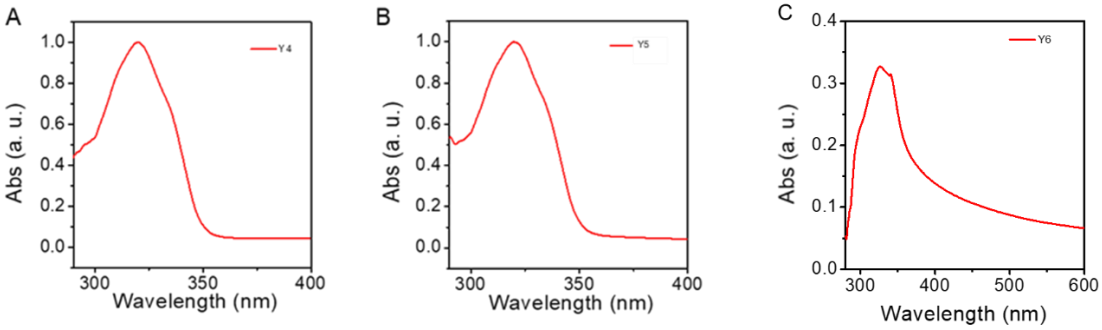


Fig. S2. The absorption spectra of (A) **Y4** and (B) **Y5**, and (C) **Y6** in DMSO/PBS solution (v/v = 1:99).

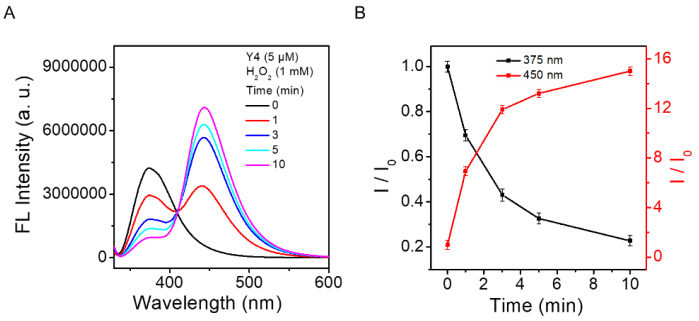


Fig. S3. (A) Fluorescence response of **Y4** (5 μM) against 1 mM H_2O_2 in DMSO/PBS solution (v/v = 1:99) for 0 - 10 min, E_x = 320 nm. (B) The fluorescence ratio (I/I_0) was plotted as a function against each time point in DMSO/PBS (v/v = 1:99) solution. I_0 was the initial fluorescence intensity of **Y4** at 5 μM.

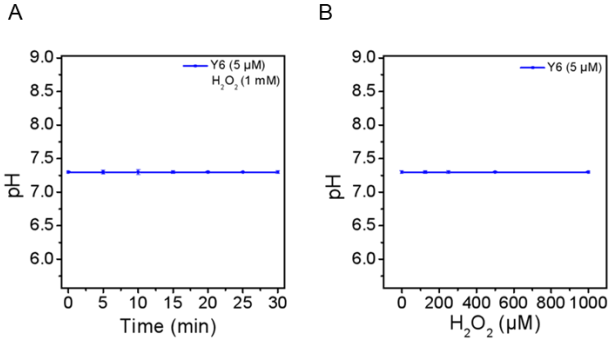


Fig. S4. The pH effect on the response of Y6 for H_2O_2 when incubating Y6 (5 μM) with H_2O_2 (A: 1 mM for 0-30 min) or different amounts of H_2O_2 (B: 0-1000 μM for 30 min).

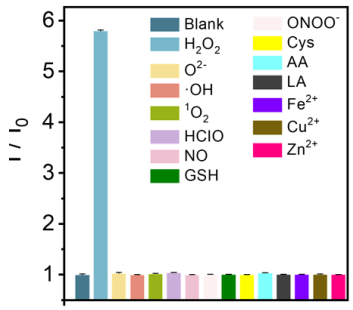


Fig. S5. The fluorescence response of **Y6** in the presence of various ROS types (H_2O_2, O^{2-}, ·OH, HClO, ONOO-, NO, each at 1 mM), GSH at 1 mM, Cys at 1 mM, AA (Ascorbic acid at 1 mM), LA (Lipoic acid at 1 mM), or metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+}, each at 1 mM).

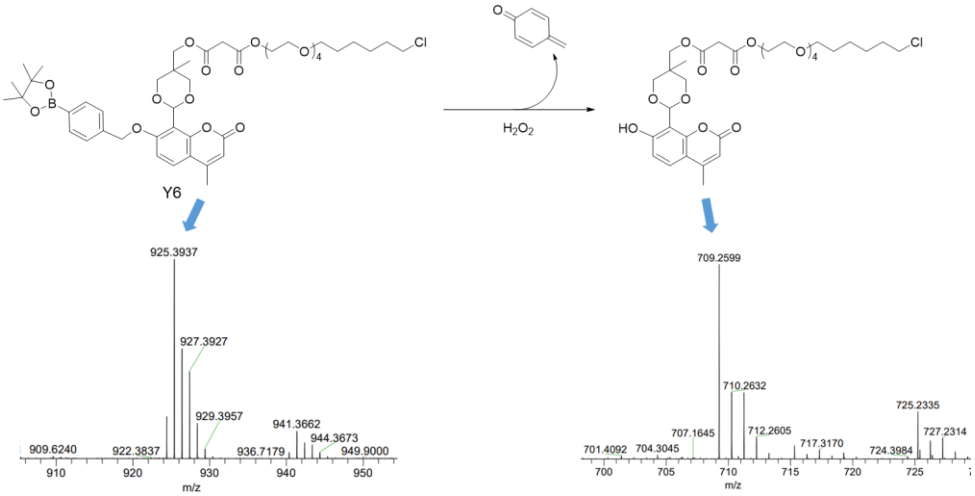


Fig. S6. The response mechanism of Y6 for H_2O_2.

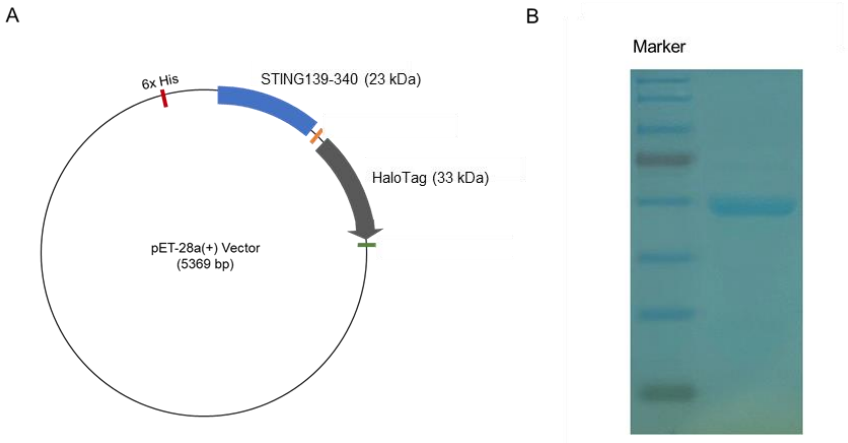


Fig. S7. (**A**) A diagram showing the construction of STING 139-340_Halo_pET28a plasmid. (**B**) The SDS-PAGE of STING 139-340_Halo protein.

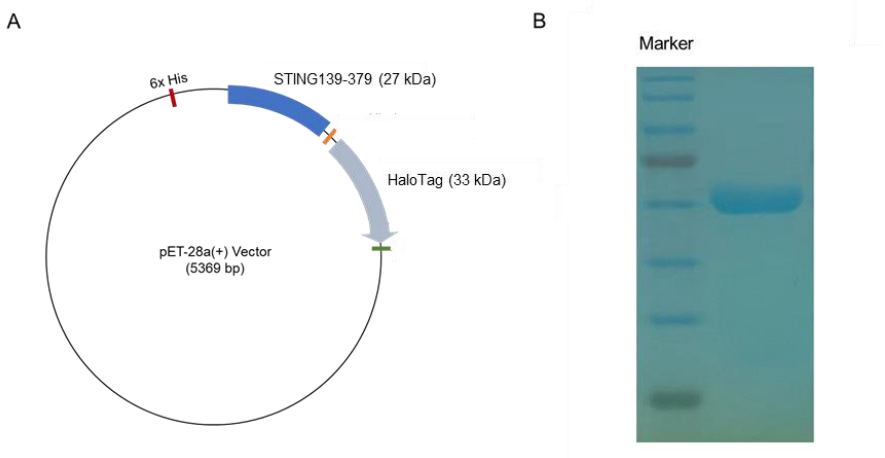


Fig. S8. (**A**) A diagram showing the construction of STING 139-379_Halo_pET28a plasmid. (**B**) The SDS-PAGE of STING 139-379_Halo protein.

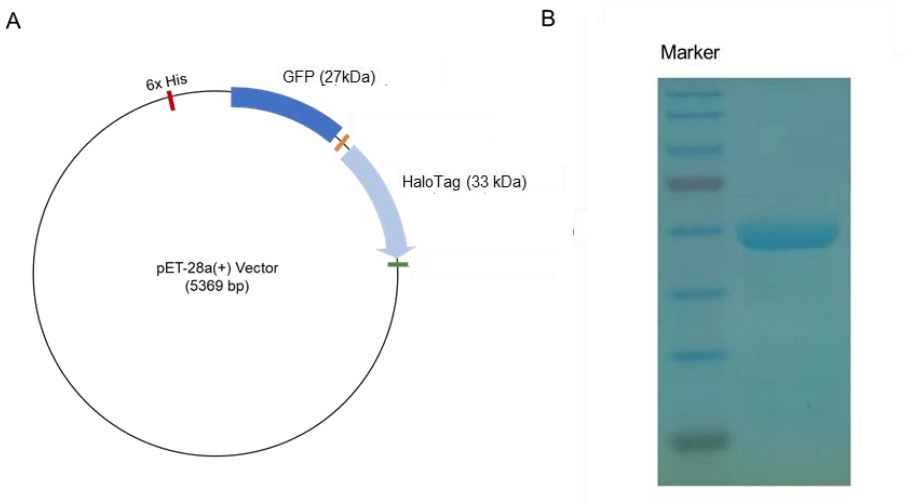


Fig. S9. (**A**) A diagram showing the construction of GFP_Halo_pET28a plasmid. (**B**) The SDS-PAGE of GFP_Halo protein.

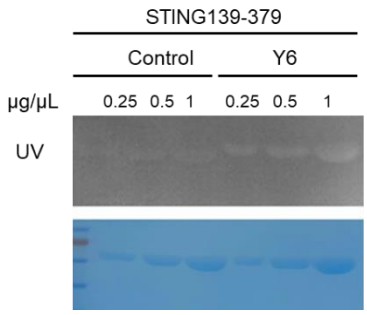


Fig. S10. The SDS-PAGE results from the reaction between different amounts of STING139-379_Halo protein with **Y6** (100 μM) at room temperature for 1 h. Aliquots were taken, treated with 1 mM H_2O_2 for 30 min, boiled, and loaded on the SDS-PAGE. The fluorescence gel was taken by irradiating the gel at 302 nm. Note: The same amount of protein without Y6 in the labeling reaction was used as control.

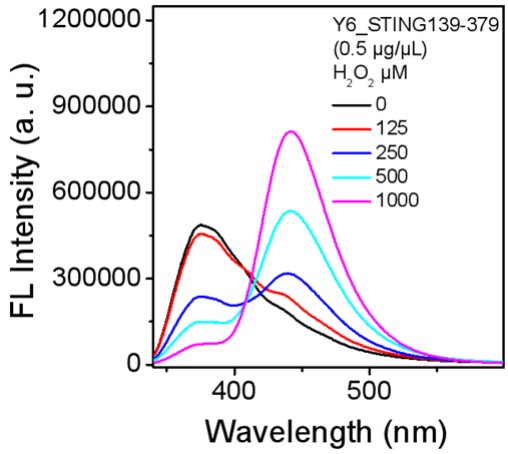


Fig. S11. Fluorescence response of **Y6**_STING139-379 upon incubation of **Y6**_STING139-379 (**Y6** at 100 μM + STING139-379_Halo protein at 0.5 μg/μl, E_x= 320 nm) with increasing concentrations of H_2O_2 at room temperature for 30 min.

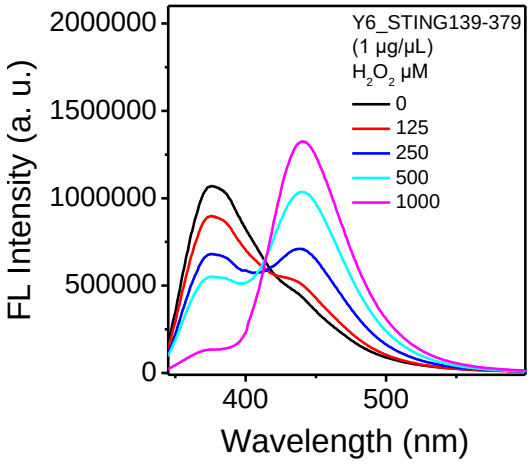


Fig. S12. Fluorescence response of **Y6**_STING139-379 upon incubation of **Y6**_STING139-379 (**Y6** at 100 μM + STING139-379_Halo protein at 1 μg/μl, E_x= 320 nm) with increasing concentrations of H_2O_2 at room temperature for 30 min.

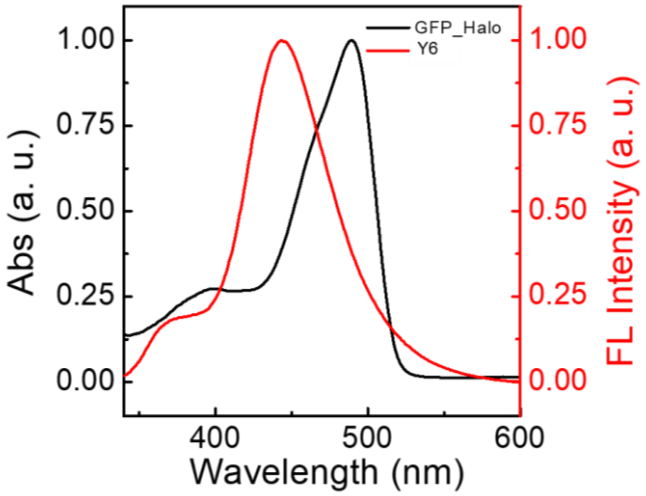


Fig. S13. The normalized results of the absorbance of GFP_Halo and the fluorescence intensity of activated **Y6**, E_x = 320 nm.

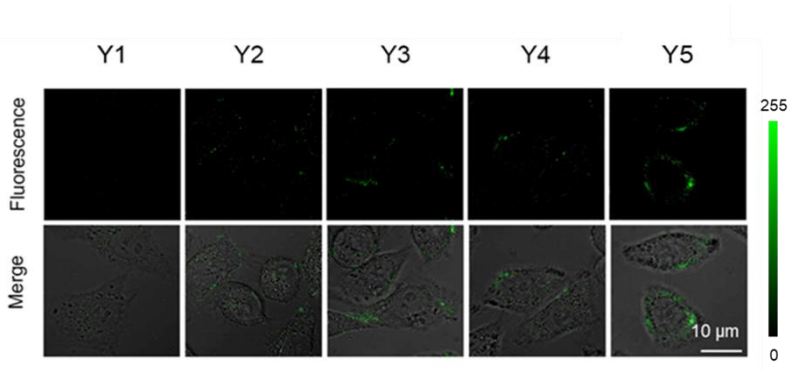


Fig. S14. HeLa cells were treated with **Y1-Y5** at 20 μM for 2 h, cells were fixed and analyzed by confocal microscopy. The fluorescence was recorded in the range of 500–550 nm. Scale bar = 10 μm.

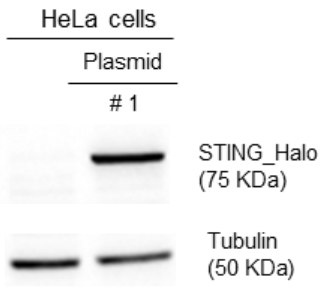


Fig. S15. HeLa cells were transfected with STING_Halo plasmid for 36 h, lysed, and analyzed for indicated proteins by immunoblots.

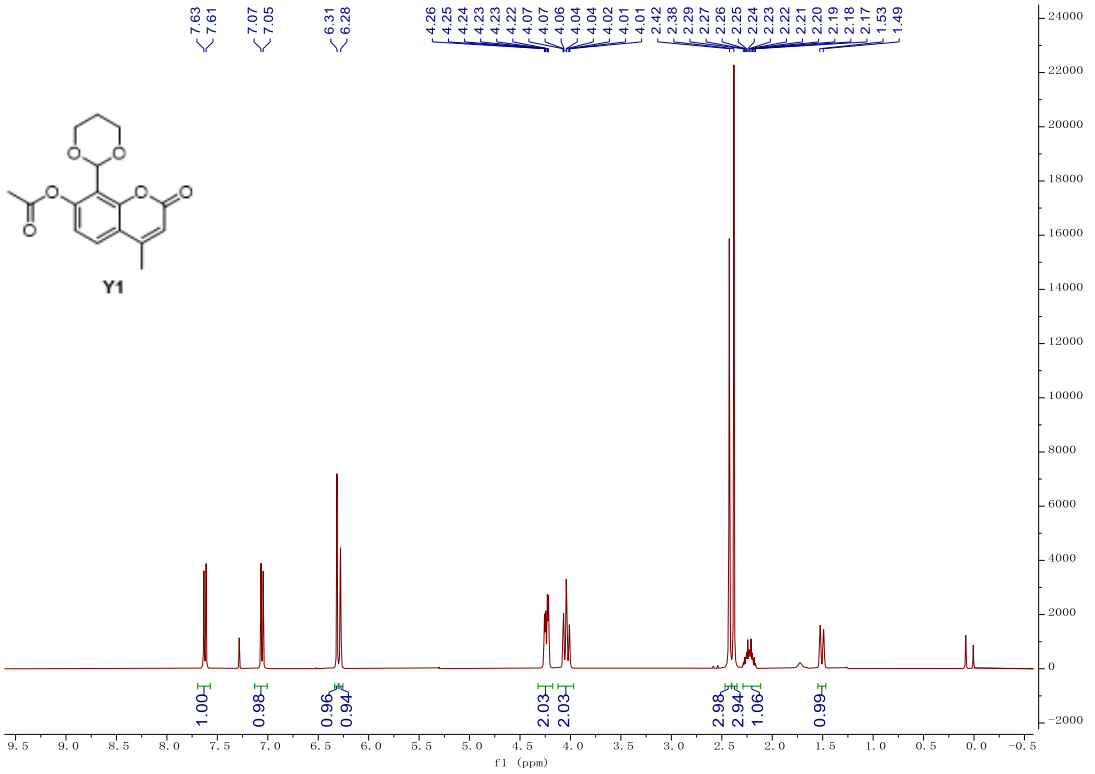


Fig. S16. The ^{1}H NMR spectra of **Y1** in $CDCl_3$.

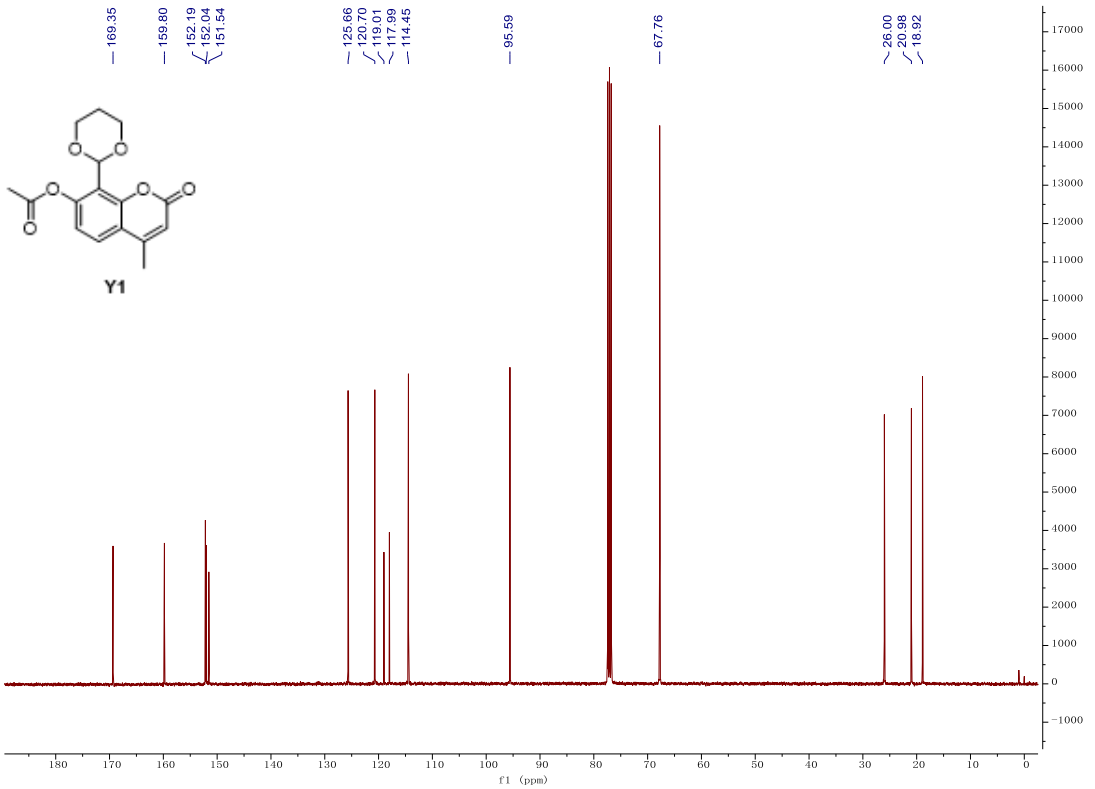


Fig. S17. The ^{13}C NMR spectra of **Y1** in $CDCl_3$.

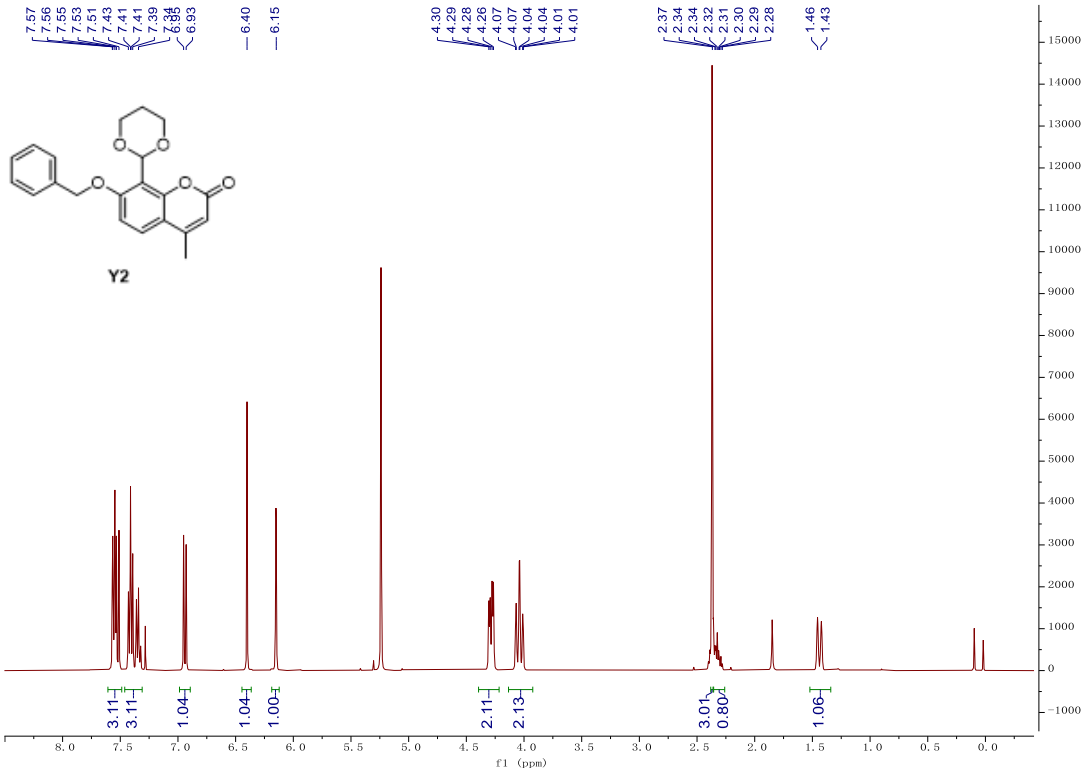


Fig. S18. The ^{1}H NMR spectra of **Y2** in $CDCl_3$.

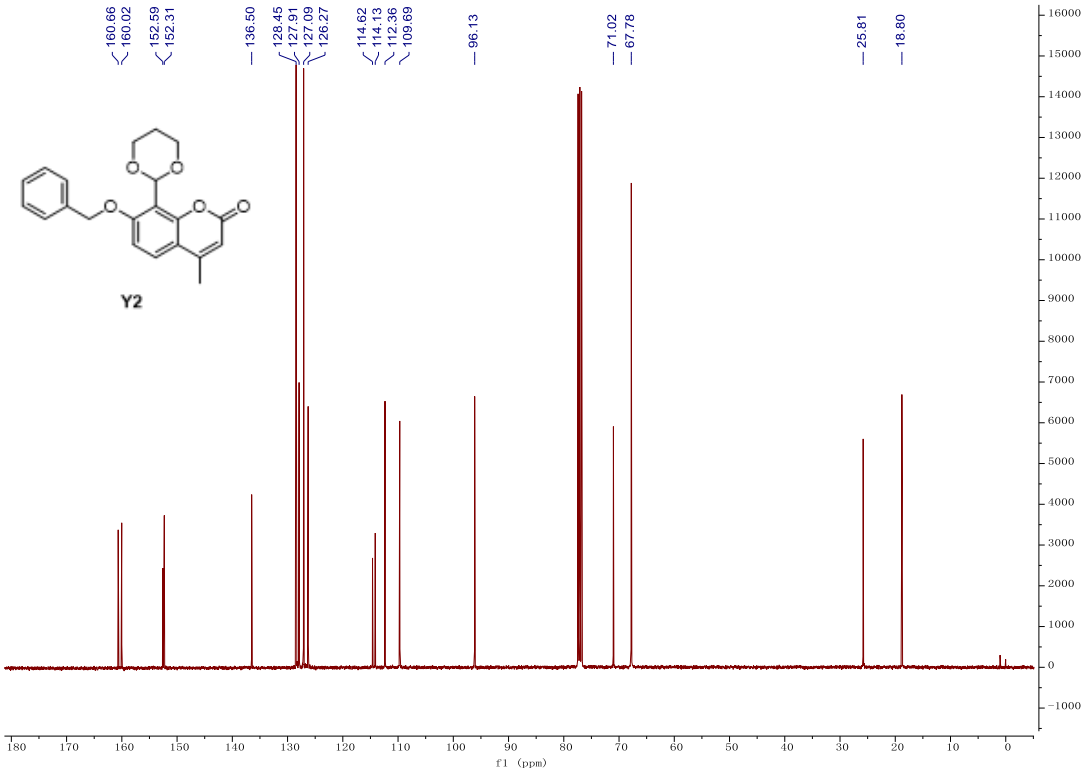


Fig. S19. The ^{13}C NMR spectra of **Y2** in $CDCl_3$.

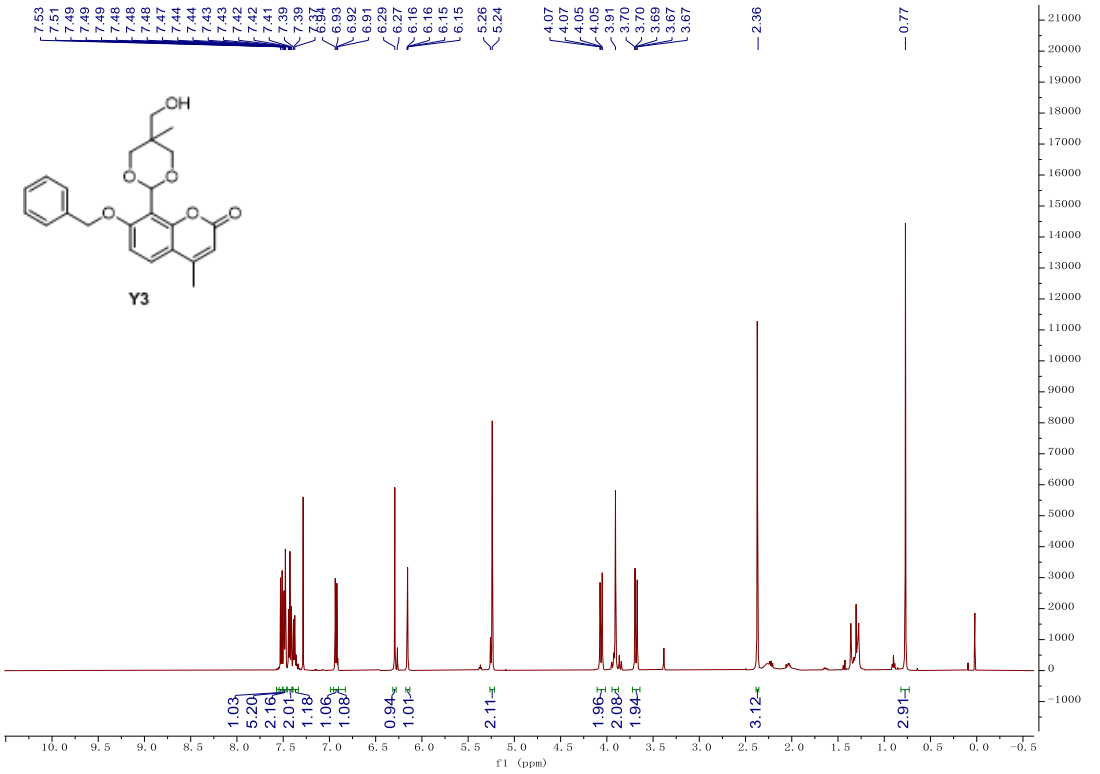


Fig. S20. The ^{1}H NMR spectra of **Y3** in $CDCl_3$.

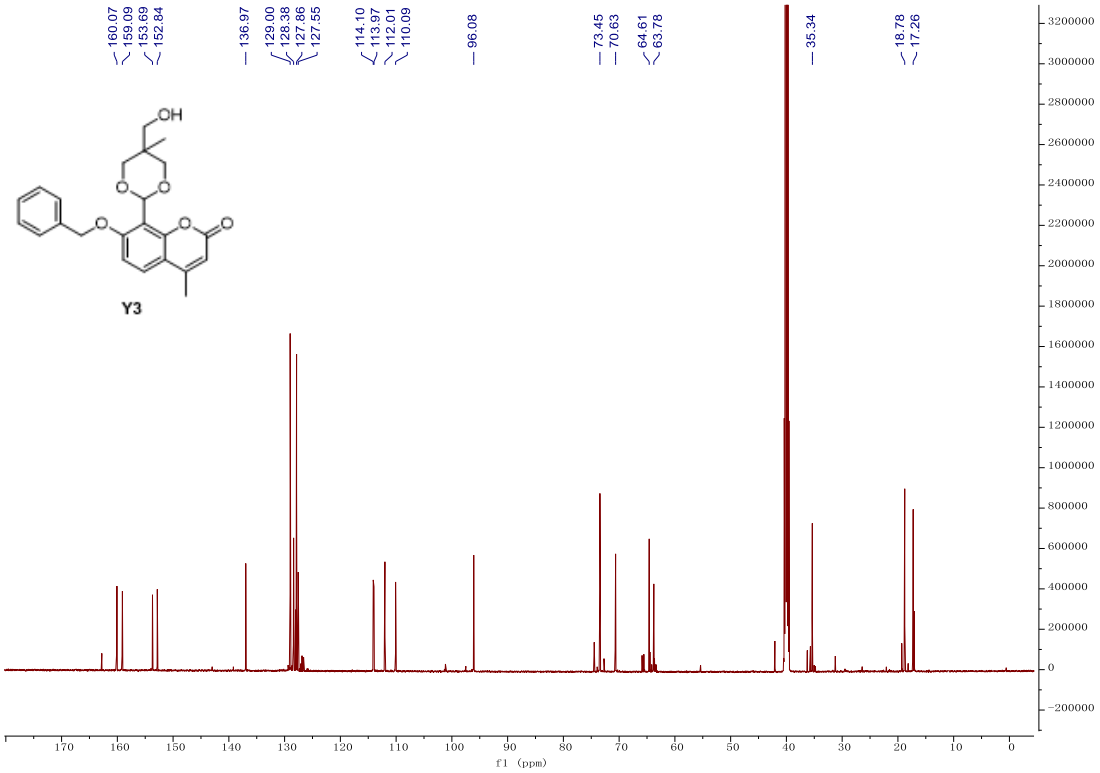


Fig. S21. The ^{13}C NMR spectra of **Y3** in DMSO-d_6.

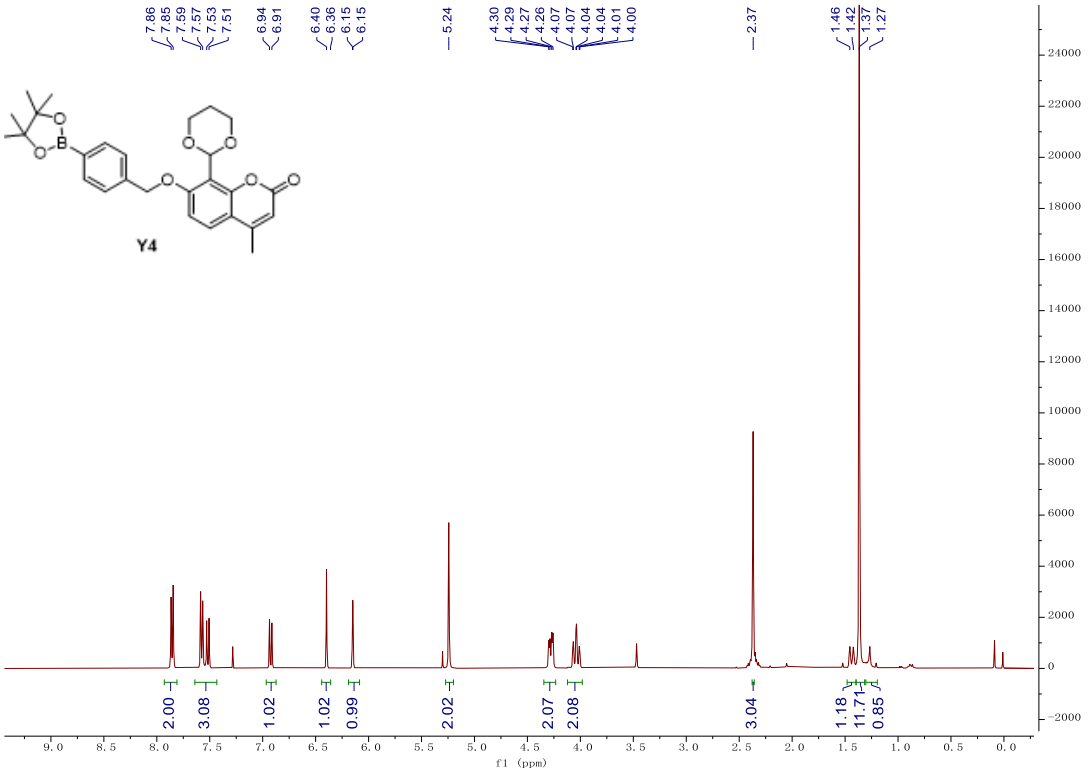


Fig. S22. The ^{1}H NMR spectra of **Y4** in $CDCl_3$.

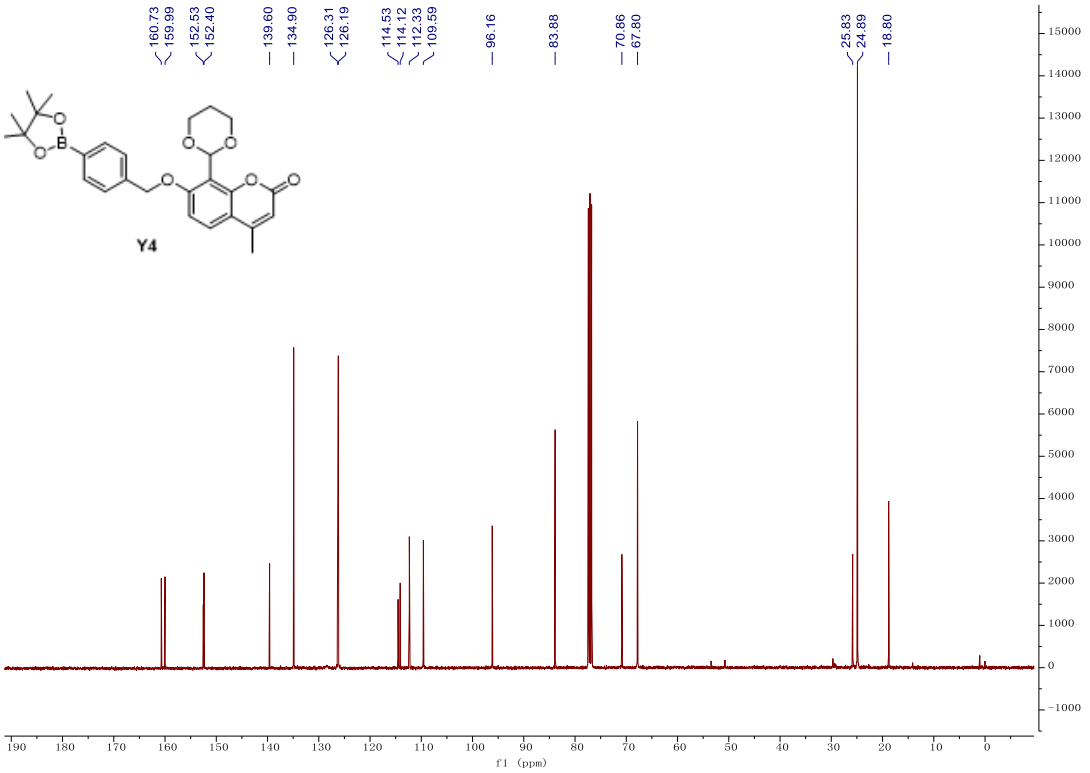


Fig. S23. The ^{13}C NMR spectra of **Y4** in $CDCl_3$.

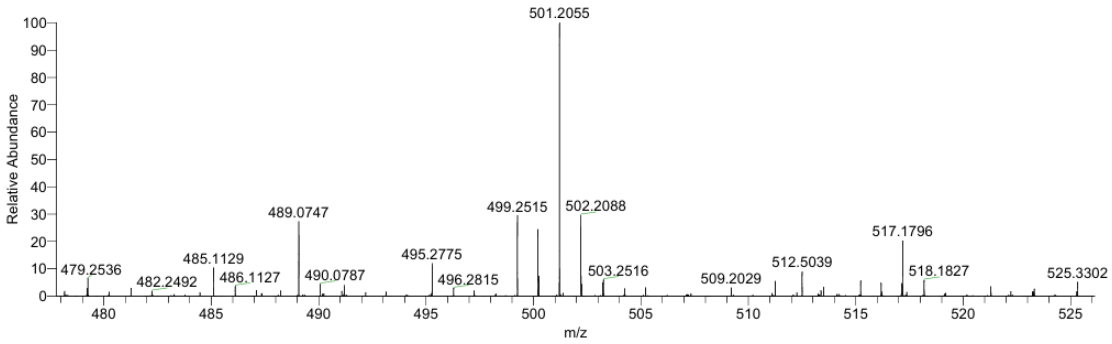


Fig. S24. HRMS spectrum of compound **Y4**.

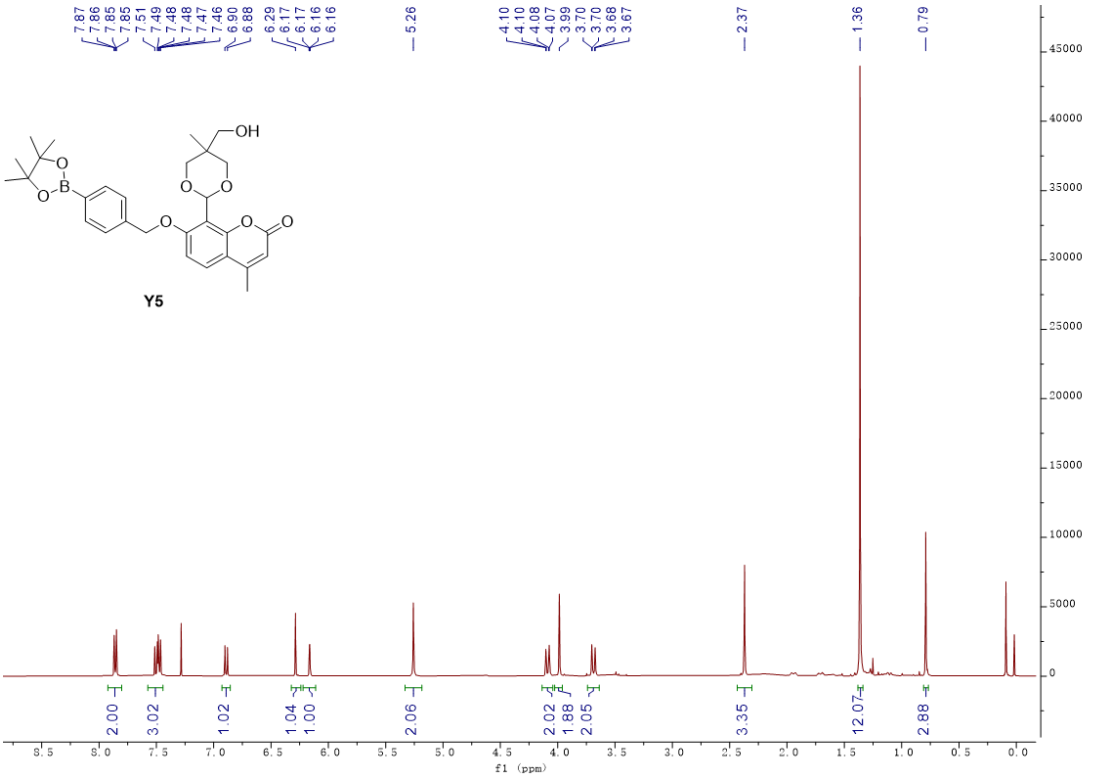


Fig. S25. The ^{1}H NMR spectra of **Y5** in $CDCl_3$.

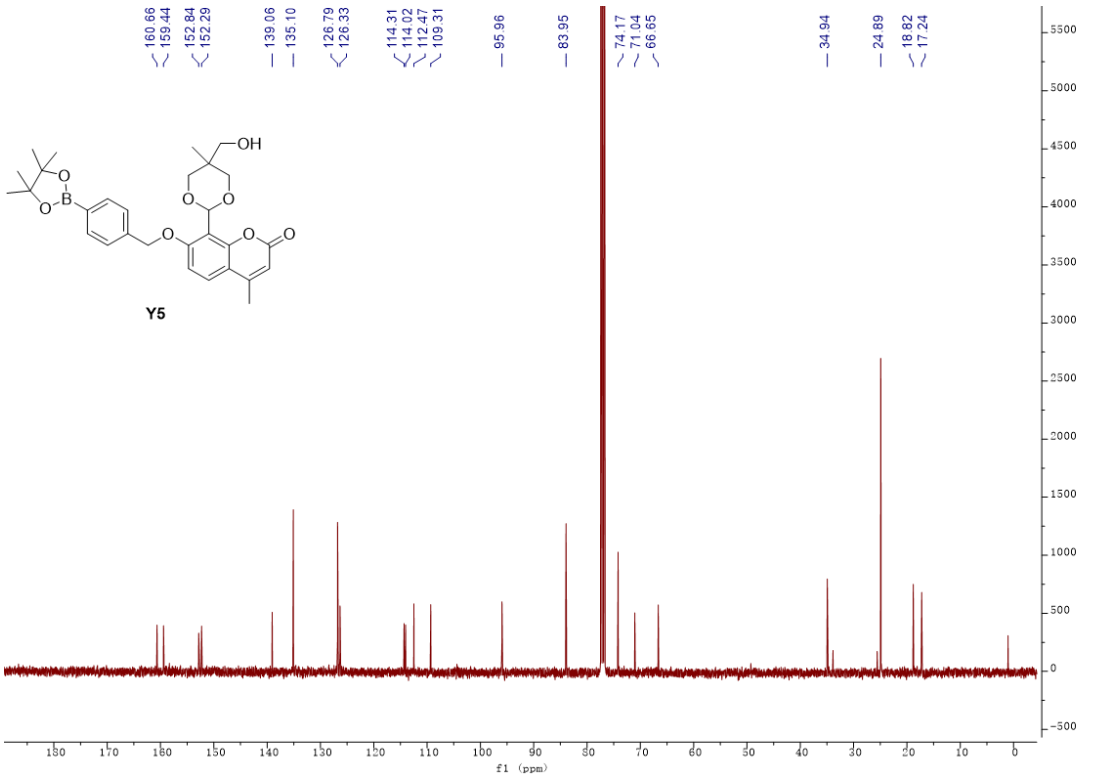


Fig. S26. The ^{13}C NMR spectra of **Y5** in $CDCl_3$.

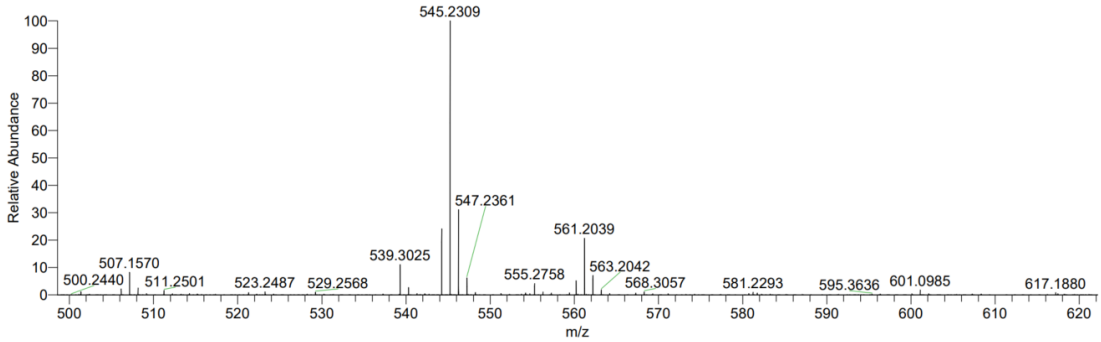


Fig. S27. HRMS spectrum of compound **Y5**.

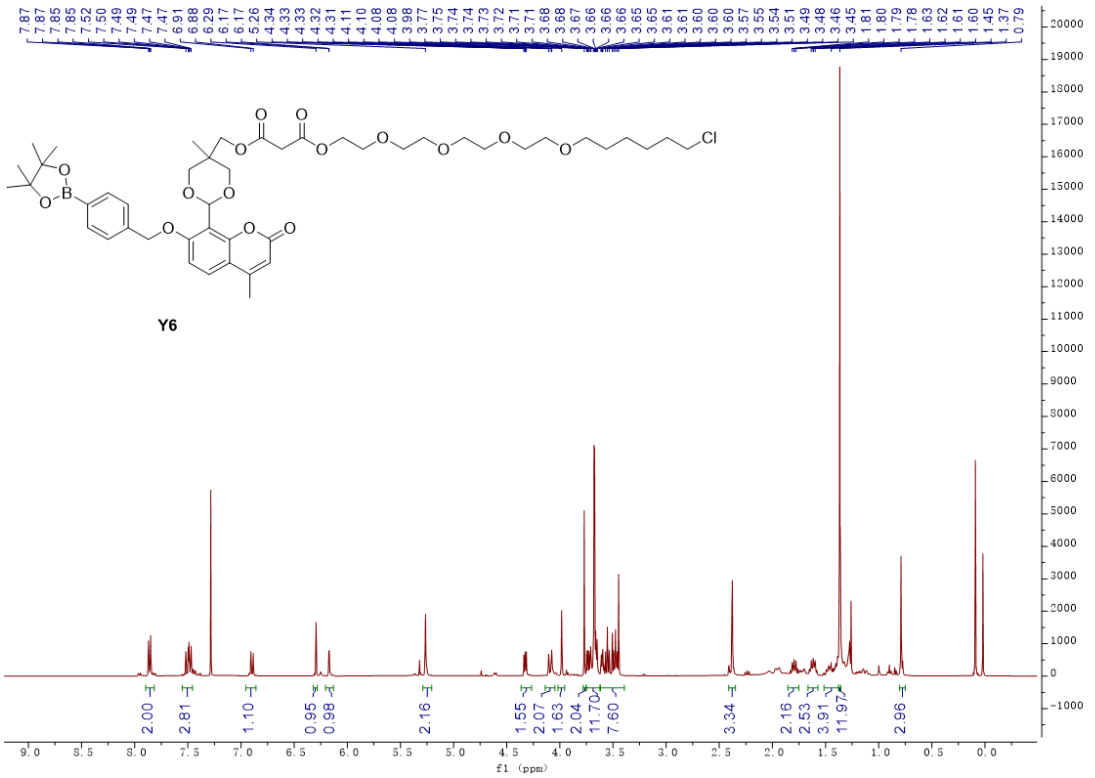


Fig. S28. The ^{1}H NMR spectra of **Y6** in $CDCl_3$.

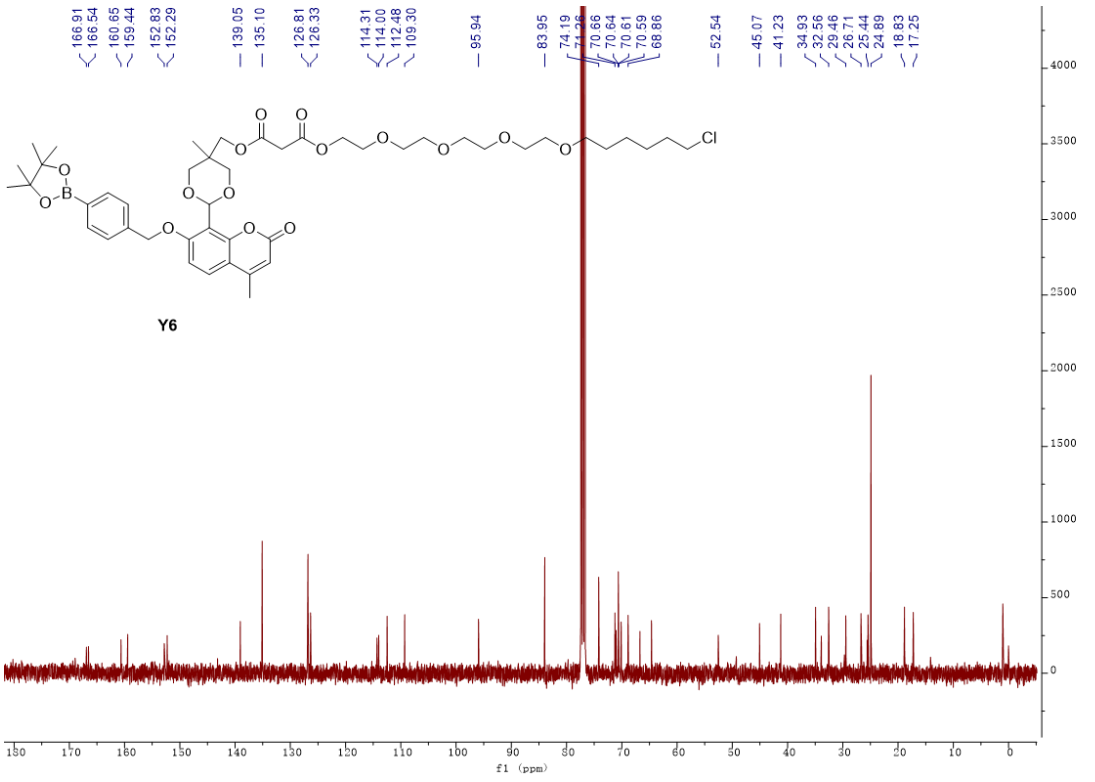


Fig. S29. The ^{13}C NMR spectra of **Y6** in $CDCl_3$.

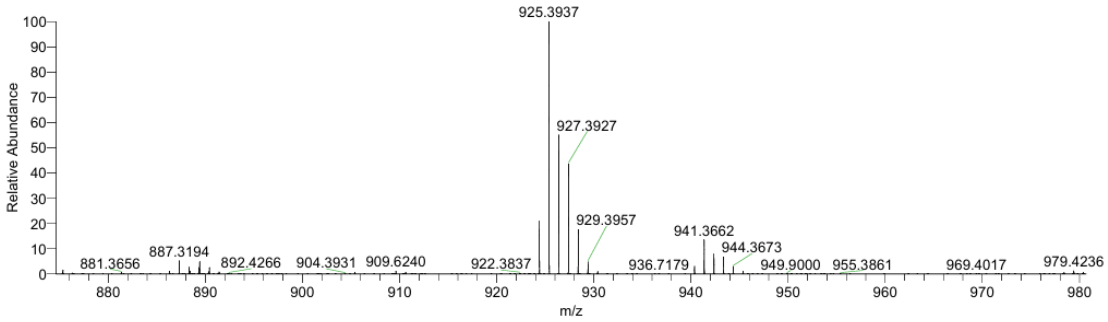


Fig. S30. HRMS spectrum of compound **Y6**.