Supporting Information for

Controllable Thioester-Based Hydrogen Sulfide Slow-releasing Donors as Cardioprotective Agents

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Contents

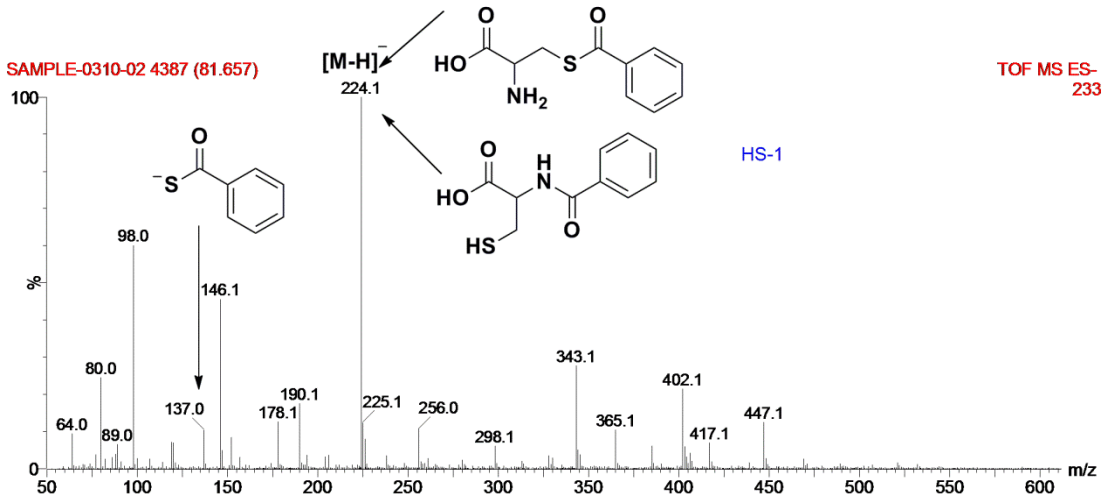


Figure S1. The production of intermediate **8** was confirmed by the formation of **9**. ESI-MS of products from the reaction between **5a** and 10 equiv Cys in PBS/THF (10:1) overnight at 37 °C.

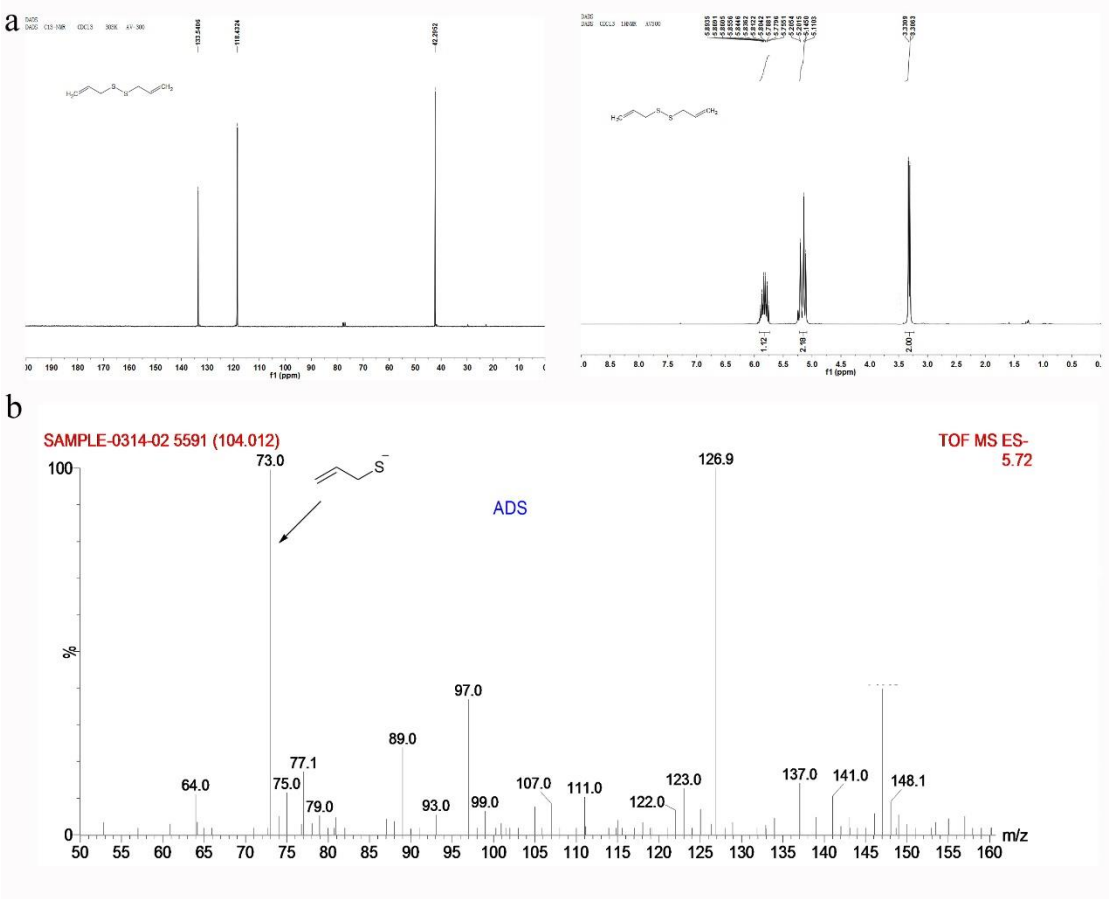


Figure S2. The formation of disulfide **10** from allyl mercaptan (**8**) was confirmed by mass spectra and NMR. (a) NMR spectra of the isolated oxidation product from the

solution of mercaptan and 10 equiv Cys in air overnight at 37 °C; (b) ESI-MS of product **10**.

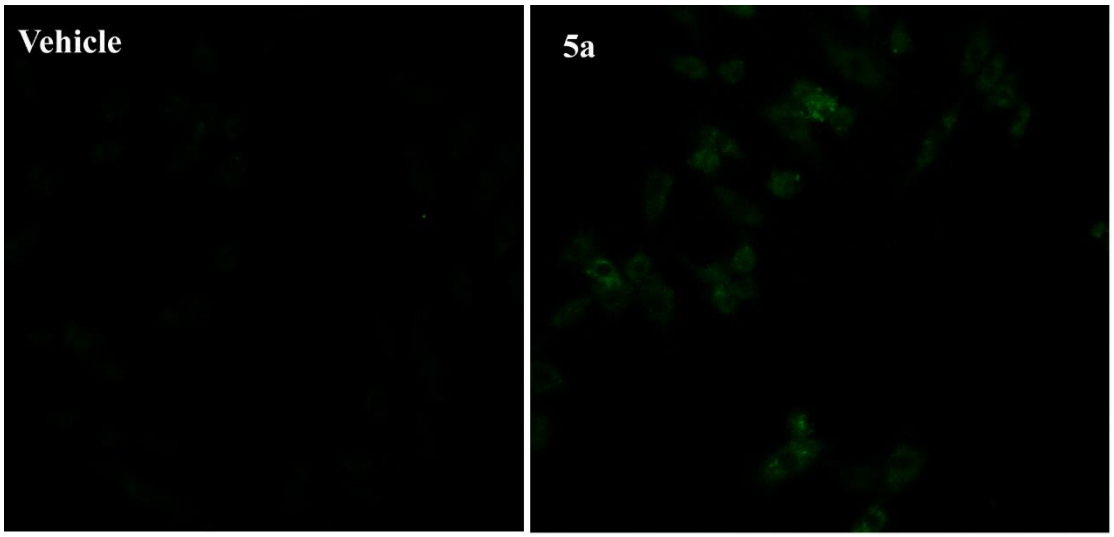


Figure S3. H_2S production from donor **5a** in H9c2 cells. Cells were stimulated with 400 μM H_2O_2 for 1 hour followed by treatment of 100 μM **5a** for another 5 hours. After removal of excess donors, 250 μM concentration of a H_2S fluorescent probe (WSP-1) was added. Images were taken after 30 min with a fluorescence microscope

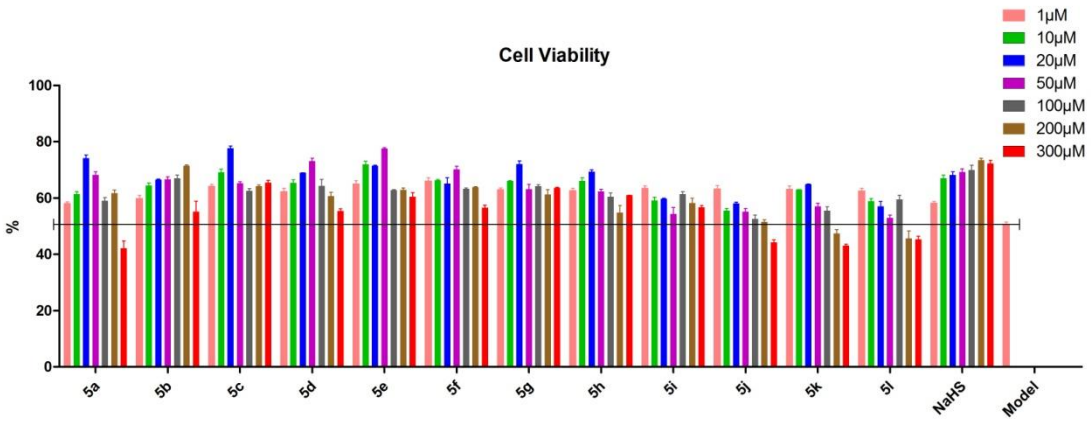


Figure S4. Bar graph of protective effect of compounds on H_2O_2-stimulated H9c2 cells.

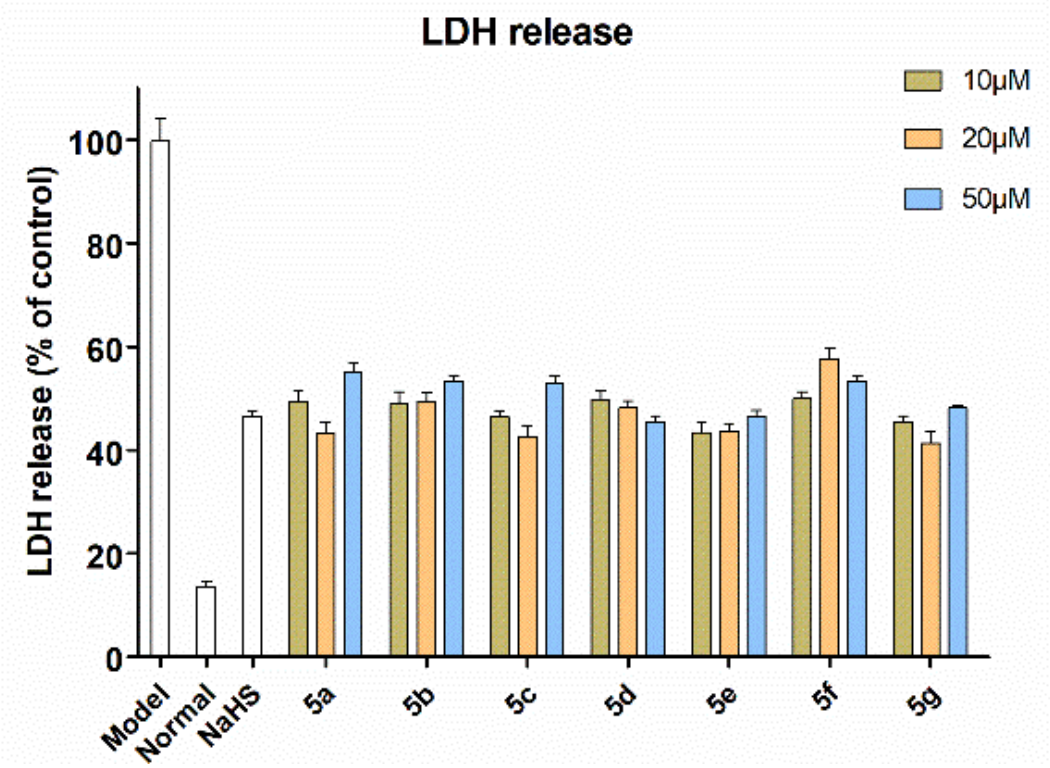


Figure S5. LDH assay of compounds **5a-g** on H9c2 cells. Cell viability was analyzed by LDH assay from culture media of indicated cells which were pretreated with different concentrations (10, 20, and 50 μM) of compounds for 5 h or NaHS (100 μM, 1 h). The data are expressed as percentages of H2O2-stimulated cells (model group).

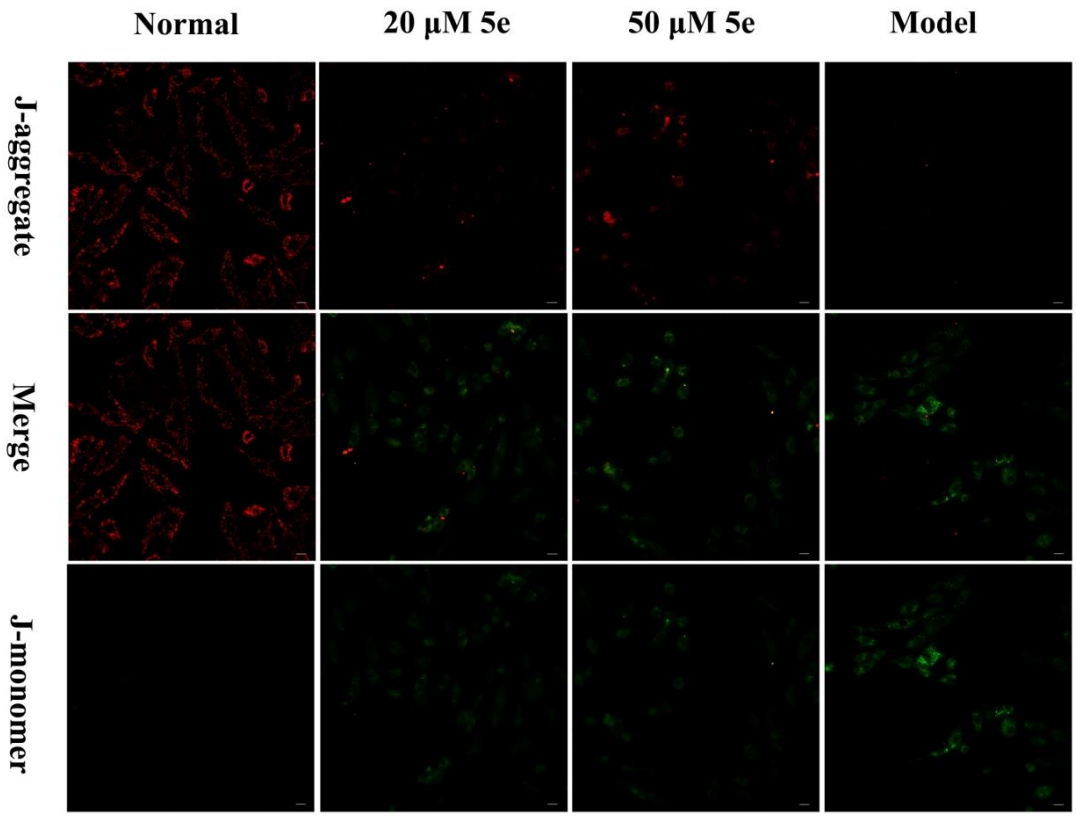


Figure S6. Protective effects of **5e** on H_2O_2-induced Δψm loss in H9c2 cells. Cells were pretreated with 20 or 50 μM **5e** in the presence of cysteine (400 μM) for 8 h prior to H_2O_2 treatment (400 μM, 1 h). Control group cells were pretreated with 400 μM cysteine for 8 h prior to H_2O_2 treatment. Cells were stained with JC-1 followed by fluorography to observe Δψm. Bars denote 10 μm.

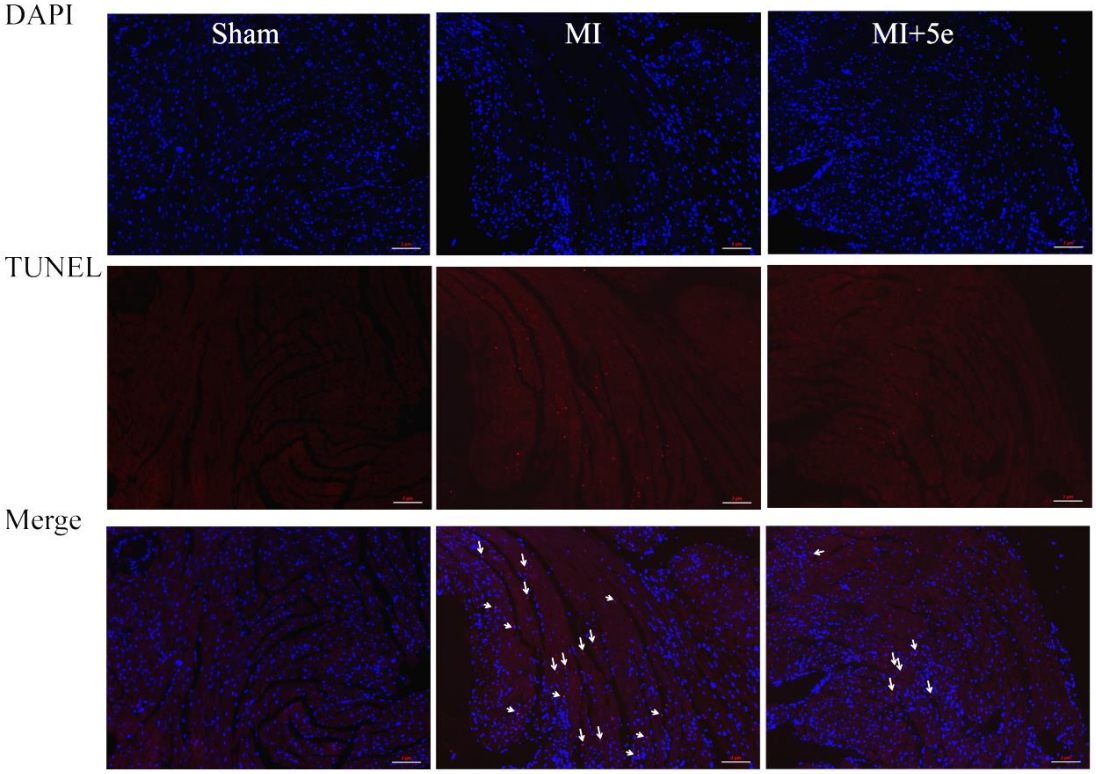


Figure S7. Cardiomyoblasts apoptosis in dangerous area were determined with TUNEL staining. Cellular nucleus (Blue dots) and TUNEL positive nucleus (Red dots) were captured *via* con-focal microscopy. White arrows showed TUNEL-positive staining. Scale bar: 2 μm.

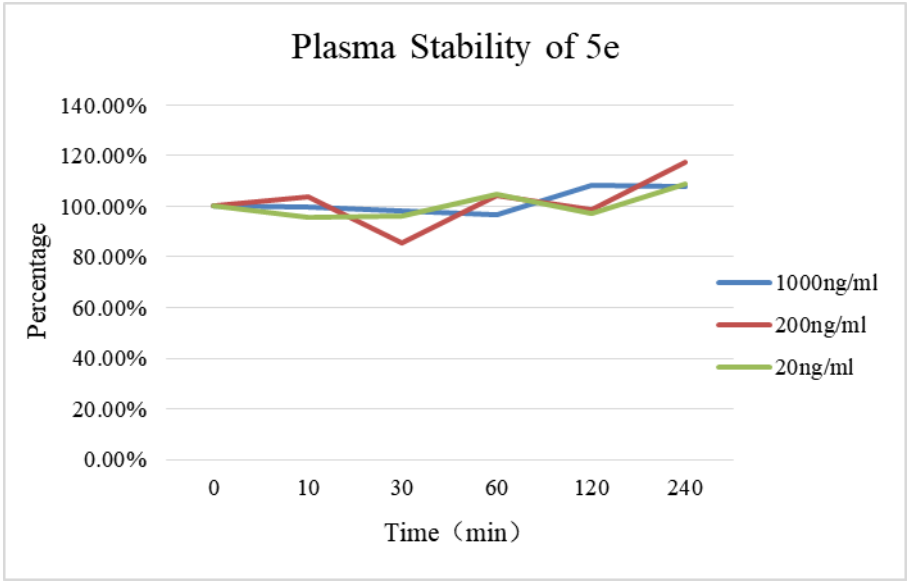


Figure S8. Plasma stability of **5e** was determined using LC-MS / MS.

Experimental section

Chemical syntheses

General information.

The reagents (chemicals) were purchased from commercial sources, and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15-0.2 mm thickness). All products were characterized by their NMR and MS spectra. ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) on 300 MHz instrument. Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t). Low-and high-resolution mass spectrums (LRMS and HRMS) were measured on Finnigan MAT 95 spectrometer (Finnigan, Germany). The purity (≥95%) of the compounds was verified by the HPLC (Aglilent Technologies 1260 Infinity) study performed on an Aglilent C18 column (4.6×150 mm, 5μm) using a mixture of solvent methanol/water (1:4) at the flow rate of 1.0 mL/min and peak detection at 240 nm under UV.

*General procedure for the preparation of **5a-l**.*

50 mL two neck round bottomed flask equipped with a magnetic stir bar and a dropping funnel was placed in an ice bath. To a stirred solution of 8 mM aryl acid (**1**) in 100 mL CH_2Cl_2 at 0 ^{o}C was added dropwise 16 mM thionyl chloride, then the mixture was stirred at room temperature for 1h. The resulting mixture was concentrated under reduced pressure to afford acid chloride **2** for further reaction without purification. Compound **2** and thioacetamide (5.3 mM) was dissolved in 100 mL toluene, the mixture was stirred at 30 ^{o}C for 3h. Compound **3** cannot be isolated due to its instability in air, it was hydrolyzed with 10% NaOH solution (30 mL) for 30 min, and then use 10% HCl to adjust the PH to 4. The mixture was extracted with CH_2Cl_2 and the extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford crude compound **4** for further reaction without purification. To a round-bottomed flask (50 mL) the crude compound **4** was dissolved in anhydrous acetonitrile (20mL) in ice bath, then TEA (2.2 mM) was added dropwise, the mixture was stirred for 10 min. To

a 50 mL two neck round bottomed flask equipped with a magnetic stir bar and a dropping funnel, add 1mM allylic bromide or benzyl bromide, and 10 mL anhydrous acetonitrile, the mixture was cooled to 0 ^{o}C. Then the mixture of compound **4** and TEA was added dropwise into the bromide or benzyl bromide solution. Then stirred at room temperature for 30 min. Evaporated the solvent from the reaction mixture and purified with flash chromatography on silica gel [eluent: petroleum ether] to obtain products as colorless liquid.

*S-allyl-benzothioate (**5a**)*

86% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 – 7.91 (m, 2H), 7.66 – 7.52 (m, 1H), 7.47 (t, J = 7.5 Hz, 2H), 5.93 (ddt, J = 16.9, 10.0, 6.9 Hz, 1H), 5.35 (dd, J = 16.9, 1.3 Hz, 1H), 5.18 (d, J = 10.0 Hz, 1H), 3.76 (d, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.25, 136.93, 133.43, 133.10, 128.64, 127.27, 118.13, 31.89. HRMS (ESI): calculated for $C_{10}H_{11}OS$ $[M+H]^+$: 179.0351, found: 179.0350.

*S-(3-methylbut-3-en-1-yl) benzothioate (**5b**)*

74% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 7.5 Hz, 2H),7.56 (t, J = 7.3 Hz, 1H), 7.44 (t, J = 7.7 Hz, 2H), 5.31 (t, J = 7.8 Hz, 1H), 3.73 (d, J = 7.9 Hz, 2H), 1.74 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.10, 137.12, 136.89, 133.25, 128.57, 127.21, 118.66, 27.36, 25.72, 17.88. HRMS (ESI): calculated for $C_{12}H_{15}OS$ $[M+H]^+$: 207.0838, found: 207.0838.

*(E)-ethyl-4-(benzoylthio)but-3-enoate (**5c**)*

88% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 – 7.77 (m, 2H), 7.48(t, J = 7.4 Hz, 1H), 7.35 (t, J = 7.6 Hz, 2H), 6.92 – 6.75 (m, 1H), 5.97 (d, J = 15.5 Hz,1H), 4.17 – 3.99 (m, 2H), 3.72 (d, J = 7.1 Hz, 2H), 1.17 (t, J = 7.1 Hz, 3H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 190.28, 165.97, 142.26, 133.74, 128.74, 127.33, 123.98, 60.51,29.76, 14.23. HRMS (ESI): calculated for $C_{13}H_{15}O_3S$ $[M+H]^+$: 251.0736, found:251.0733

*S-(cyclohex-1-en-1-ylmethyl) benzothioate (**5d**)*

79% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 7.5 Hz, 2H),

7.04 (t, *J* = 8.6 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 2H), 5.91 – 5.75 (m, 1H), 5.77 – 5.60 (m, 1H), 4.36 (d, *J* = 2.2 Hz, 1H), 2.12 – 1.92 (m, 3H), 1.90 – 1.75 (m, 1H), 1.76 – 1.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.79, 137.14, 133.27, 131.15, 128.58, 127.24, 126.21, 39.86, 29.60, 24.78, 20.00. HRMS (ESI): calculated for $C_{14}H_{17}OS$ $[M+H]^+$: 233.1000, found: 233.1004.

*S-allyl-4-fluorobenzothioate (**5e**)*

82% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 – 7.88 (m, 2H), 7.47 (t, *J* = 8.6 Hz, 1H), 5.86 – 5.78 (m, 1H), 5.25 (dd, *J* = 16.9 Hz, 1.3 Hz, 1H), 5.08 (dd, *J* = 10 Hz, 1.3 Hz, 1H), 3.65 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.76, 165.92 (d, *J* = 254.9 Hz), 132.93, 129.86, 129.74, 118.25, 115.77 (d, *J* = 22.1 Hz), 31.97. HRMS (ESI): calculated for $C_{10}H_{10}FOS$ $[M+H]^+$: 197.0431, found:197.0411.

*S-allyl-4-methoxybenzothioate (**5f**)*

83% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.8 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 5.86 – 5.72 (m, 1H), 5.23 (dd, *J* = 16.9 Hz, 0.7 Hz, 1H), 5.02 (d, *J* = 16.9 Hz, 1H), 3.72 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.77, 163.78, 133.38, 129.43, 117.89, 113.78, 55.51, 31.75. HRMS (ESI): calculated for $C_{11}H_{13}O_2S[M+H]^+$: 209.0631, found: 209.0632.

*S-allyl-4-(trifluoromethyl)benzothioate (**5g**)*

76% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 – 7.84 (m, 2H), 7.75 – 7.49 (m, 2H), 5.94 – 5.67 (m, 1H), 5.41 – 5.18 (m, 1H), 5.09 (d, *J* = 9.9 Hz, 1H), 3.78 – 3.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.44, 139.68, 132.52, 127.60, 125.76, 125.71, 118.60, 32.12. HRMS (ESI): calculated for $C_{11}H_{10}F_3OS$ $[M+H]^+$: 247.0399, found: 247.0405.

*S-4-methoxybenzyl benzothioate (**5h**)*

85% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 – 7.82 (m, 2H), 7.55 (dd, *J* = 7.4, 6.2 Hz, 1H), 7.42 (t, *J* = 6.9 Hz, 2H), 7.29 (d, *J* = 7.2 Hz, 2H), 6.92 – 6.75 (m, 2H), 4.27 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.54, 158.87,

136.87, 133.43, 130.16, 129.44, 128.64, 127.30, 114.07, 55.30, 32.89. HRMS (ESI): calculated for $C_{15}H_{14}NaO_2S$ $[M+Na]^+$: 281.0607, found: 281.0612.

*S-4-fluorobenzyl benzothioate (**5i**)*

72% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 – 7.91 (m, 2H), 7.58(t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 7.40 – 7.29 (m, 2H), 7.00 (t, J = 8.7 Hz, 2H), 4.28 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.16, 159.59, 133.07, 130.16, 130.05, 128.19, 126.82, 115.16, 114.87, 76.96, 76.54, 76.12, 32.05. HRMS (ESI): calculated for $C_{14}H_{12}FOS$ $[M+H]^+$: 247.0587, found: 247.0590.

*S-4-methylbenzyl benzothioate (**5j**)*

86% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 7.8 Hz, 2H), 7.57 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.3 Hz, 2H), 7.35 – 7.22 (m, 2H), 6.85 (d, J = 7.4 Hz, 2H), 4.28 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.92, 129.64, 128.13, 126.79, 113.56, 54.80, 32.38. HRMS (ESI): calculated for $C_{14}H_{15}OS$ $[M+H]^+$: 243.0844, found: 243.0841.

*S-4-(trifluoromethyl)benzyl benzothioate (**5k**)*

81% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 7.8 Hz, 2H), 7.65-7.57 (m, 3H), 7.54-7.42 (m, 4H), 4.36 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.28, 138.32, 136.00, 133.21, 131.93, 128.64, 128.24, 126.87, 125.69 – 124.84 (m), 123.69 (q, J = 3.8 Hz), 32.26. HRMS (ESI): calculated for $C_{15}H_{12}F_3OS$ $[M+H]^+$:297.0555, found: 297.0560.

*S-benzyl benzothioate (**5l**)*

80% yield, colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00-7.97 (m, 2H), 7.60-7.55 (m, 1H), 7.48-7.24 (m, 8H), 4.36 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.28, 137.63, 136.87, 133.56, 129.11, 128.78, 128.75, 127.45, 127.41, 33.44. HRMS (ESI): calculated for $C_{14}H_{13}OS$ $[M+H]^+$: 229.0682, found: 229.0685.

H_2S-releasing measurement.

H_2S generation was initiated by adding 100 μL of a donor's stock solution (30 mM in

THF) into a 30 mL PBS (pH 7.4, 50 mM)/THF (9:1) solution containing cysteine or GSH (1.0 mM). Then, 1.0 mL of reaction aliquots were periodically taken and transferred to UV cuvettes containing MB cocktail (100 μL of zinc acetate (1% w/v), 200 μL of *N*,*N*-dimethyl-1,4-phenylenediamine sulfate (20 mM in 7.2 M HCl), and 200 μL of ferric chloride (30 mM in 1.2 M HCl). The MB reaction was carried out for 15 min, and the absorbance (670 nm) of the resultant solution was determined using an UV-vis spectrometer (Thermo Evolution 300). The H_2S concentration of each sample was calculated against a calibration curve, which was obtained with a series of Na_2S solutions.

Cell culture.

H9c2 cells were purchased from ATCC. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) high glucose medium supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2.

Cell viability assay.

The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The optical density is therefore related with activity of dehydrogenase enzymes. Briefly, H9c2 cells were released from plate by trypsin, centrifuged and re-suspended in medium at 1×10^5 cell/mL. 100 μL of the dilutions were plated out into wells of a 96-well microtiter plate in triplicate. Three control wells of medium alone was used to provide the blanks for absorbance. Cells were incubated for 18 hours before treated with hydrogen donors.

H_2S donors were diluted with fresh cultural medium containing Cysteine to 1, 10, 20, and 50 μM. Cells were pre-treated with different concentrations of H_2S donors for another 4 hours. Then the medium were removed and washed with fresh medium, followed by stimulation with 400 μM hydrogen peroxide. Cells treated with NaHS were

used as positive control and cells treated with 1000 μM Cysteine alone were used as negative control. 100 μL of MTT Reagent was added to each well, including controls and plate was returned to cell culture incubator for 2 to 4 hours. When the purple precipitate is clearly visible under the microscope, the MTT regent was removed and 100 μL of DMSO was added to all wells to solve formazan. The absorbance in each well was measured at 570 nm in a microtiter plate reader.

LDH release detection.

LDH is a soluble cytoplasmic enzyme that is present in almost all cells and is released into extracellular space when the plasma membrane is damaged. LDH produces reduced NADH which can reduce tetrazolium salt into colored formazan which can be colorimetrically quantified. Briefly, cells were seeded in a 96-well flat bottom microtiter plate at a density of 5×10^4 cells/well in 100 μL of culture medium in triplicates and were incubated for 18 hours before treated with H_2S donors.

Cells were treated with different H_2S donors at different concentrations (10, 20, 50 μM) for 5 hours in the presence of 10 equiv. of Cys. Then the medium was removed and fresh medium was added and cells were stimulated with H_2O_2 for another 1 hour. Control cells were treated with same volume of ddH_2O and model cells were treated with H_2O_2 without pre-incubation of H_2S donors. After treatments, collect the medium, the left was washed with PBS for 3 times. Cells were lysed with cell lysis buffer and the protein concentration was determined with BCA colorimetric method. For each sample, add 50 μL of culture supernatant into 50 μL reconstituted 2X LDH assay buffer. Protected the assay plate from light and incubated the plate at room temperature (22-25°C) for 10-30 minutes. Absorbance between 490-520 nm was measured. The reading from the control and test wells is subtracted with the reading from the medium alone control wells. The relative LDH activity was calculated according to the following formula:

$$\text{Activity\%} = \frac{(\text{Test well OD} - \text{Medium alone well OD})}{\text{Test well protein content}} \Big/ \frac{(\text{Model well OD} - \text{Medium alone well OD})}{\text{Model well protein content}}$$

Detection of mitochondrial membrane potential (MMP) by JC-1.

MMP was determined with the dual-emission mitochondrial dye JC-1. H9c2 cells were pre-treated with 20 or 50 μM **5e** in the presence of Cysteine (400 μM) for 8 h prior to H_2O_2 treatment (400 μM). After washed with cold PBS (pH 7.4), cells were then loaded with 0.5 μg/mL JC-1 dye for 40 min at 37 °C. The dye was then removed and cells were washed with PBS buffer for three times. Samples were immediately observed under fluorescence microscope. The fluorescent signal of monomers is measured with an excitation wavelength of 490 nm and an emission wavelength of 535 nm (F_{535}). The fluorescent signal of aggregates is detected with an excitation wavelength of 525 nm and an emission wavelength of 600 nm (F_{600}).

Generation of myocardial infarction model (MI).

C57 mice weighing a minimum of 20g at an age of 8 to 12 weeks are purchased from SLAC Laboratory Animal Corporation (Songjiang, Shanghai). Mice are housed under conventional conditions, fed standard mouse pellets and water. All animals received human care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). The investigation was approved by Institutional Animal Care and Use Committee of China Pharmaceutical University.

Mice were randomly assigned into four groups: Sham operated, **5e** high dose (30 $mg \cdot kg^{-1} \cdot day^{-1}$) with MI, **5e** low dose (15 $mg \cdot kg^{-1} \cdot day^{-1}$) with MI and Model groups, with 8-10 rats in each group. We intraperitoneally injected mice with **5e** or vehicle (saline) into the abdomen from 7 d before acute myocardial infarction injury and until the mice were killed. Mice were anaesthetized with isoflurane. Adequacy of anaesthesia was monitored by pedal response. The mice were then cannulated the trachea with a polyethylene tube connected to a respirator with a tidal volume of 0.2 ml (110 breaths/min) and mechanically ventilated with oxygen-enriched room air mixed with isoflurane by a rodent respirator ventilated (Ugo, Comerio, Italy). A thoracotomy was performed at the fourth intercostal space, hearts were ‘‘popped out’’ from chest and the

left anterior descending artery (LAD) was permanently ligated with a 8-0 polypropylene suture under sterile conditions. Control animals underwent the same procedure except that the LAD was left untied. During the surgery, body temperature was maintained constant at 37 °C by a heating pad.

WSP-1 staining in H9c2 cell lines

Cells were stimulated with 400 μM H_2O_2 for 1 hour followed by treatment of 100 μM **5a** for another 5 hours. After removal of excess donors, 250 μM concentration of a H_2S fluorescent probe (WSP-1) was added. Images were taken after 30 min with a fluorescence microscope with a 465/515 nm and an excitation/emission filter set.

TCC staining

TTC (2,3,5-Triphenyltetrazolium chloride) staining measures tissue viability used to evaluate infarct size. On the eleventh day, animals were anaesthetized with isoflurane, exsanguinated by perfusion with normal saline supplemented with 40 mM KCl, and hearts were rapidly removed. Freshly isolated left ventricular tissues were snap-frozen in -80^{o}C, then the heart were cut into 4-5 transverse slices. The slices were incubated in 1% TTC solution, pH 7.4 at 37°C for 20 min. Then TTC staining buffer was removed and tissues are fixed in 10% PBS-buffered formalin overnight at 2-8°C and was photographed. Infarct area was determined by computerized planimetry using ImagePro Plus software (version 6.0, Media Cybernetics, Bethesda, MD, USA). The infarct area was expressed as a percentage of the left ventricular area.

Determination of myocardial injury and apoptosis.

We used H&E staining to determine myocardial injury and TUNEL staining to determine apoptosis, respectively. Freshly isolated hearts were fixed in 4% (w/v) formalin overnight. The hearts were processed for paraffin embedding and transverse cut into 5 μm thick sections along the center of the fibrotic scar.

For H&E staining, sections were dried, deparaffinized with xylene and hydrated by

gradient ethanol. After washed with tap water for 5 min, the sections were stained with hematoxylin for 10 minutes and washed with tap water. To remove the unspecific staining in cytoplasm, the sections were dipped in 0.1% HCl for 5 second and washed with tap water for 3-4 times to removed HCl. After that, sections were stained with Eosin for 3-5 min and washed with ddH_2O to remove Eosin solution. Then the sections were dried in oven at 55^oC for 1-2 hours followed by dehydrated in ethanol and xylene. Finally, the sections were mounted with resinene and covered with a cover glass. Optical microscope was used to observe the structure of cardiac fibrils. The normal myoplasm was stained into deep red and collagen was pink. Cellular nucleus was counter stained into deep blue or purple. Cardiomyoctes subjected with ischemic injury were stained into pale pink and infiltrated with massive fragmented nucleus.

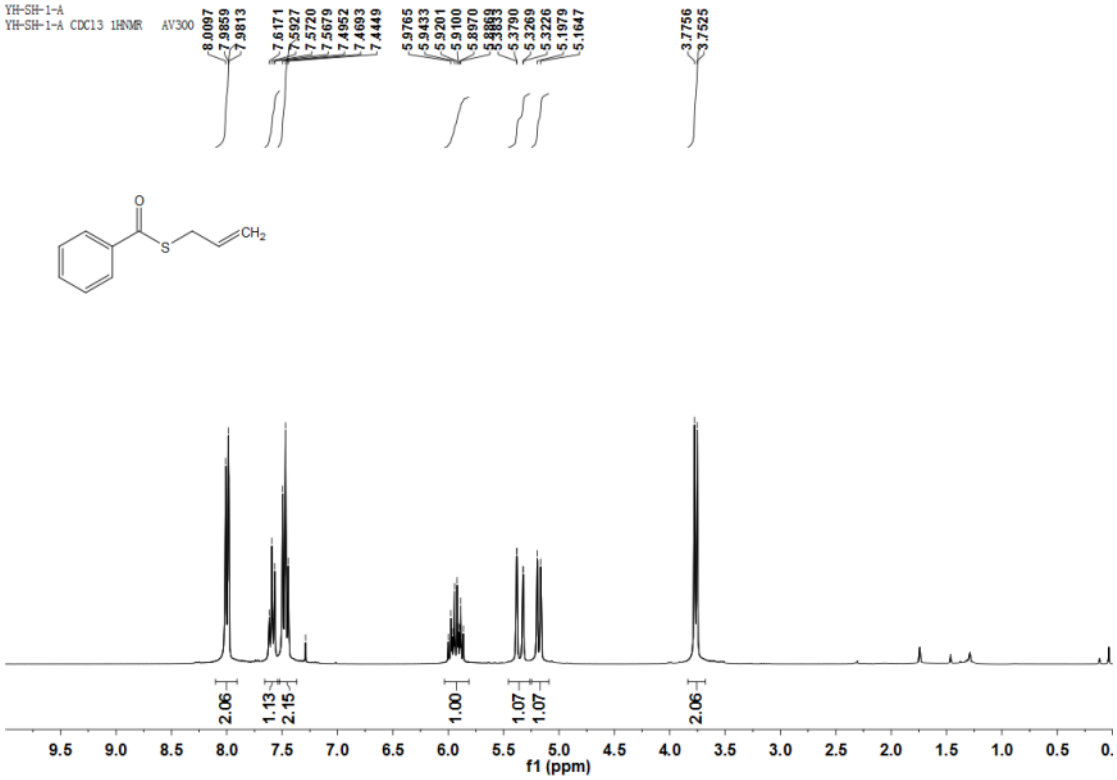
TUNEL staining relies on the ability of the enzyme terminal deoxynucleotidyl transferase to incorporate labeled dUTP into free 3'-hydroxyl termini generated by the fragmentation of genomic DNA, which occurs during cells programmed death. In this work, apoptosis was determined with a TUNEL staining kit (Roche Applied Science, Indianapolis, IN) according to the instruction. Besides deparaffinized and hydration, permeabilization of the sections with protease K was necessary for a better staining result. Following proteinase K treatment, slides were washed 3×5 min with ddH_2O. Endogenous peroxidases was then inactivated by covering sections with 2% hydrogen peroxide for 5 min at room temperature. Washed slides 3×5 min with ddH_2O and covered sections with TdT equilibration buffer for 10 min at room temperature, followed by incubating sections with TdT reaction buffer for 30 min at 37^oC. The reaction was stopped, sections were washed 3×5 min with PBS and nuclear were counter stained with DAPI for 5 min at room temperature. Sections were observed under fluorescence microscope with excitation wavelength at 585-600 nm. TUNEL positive nuclear were in red fluorescence and nuclear were in blue fluorescence.

Plasma stability

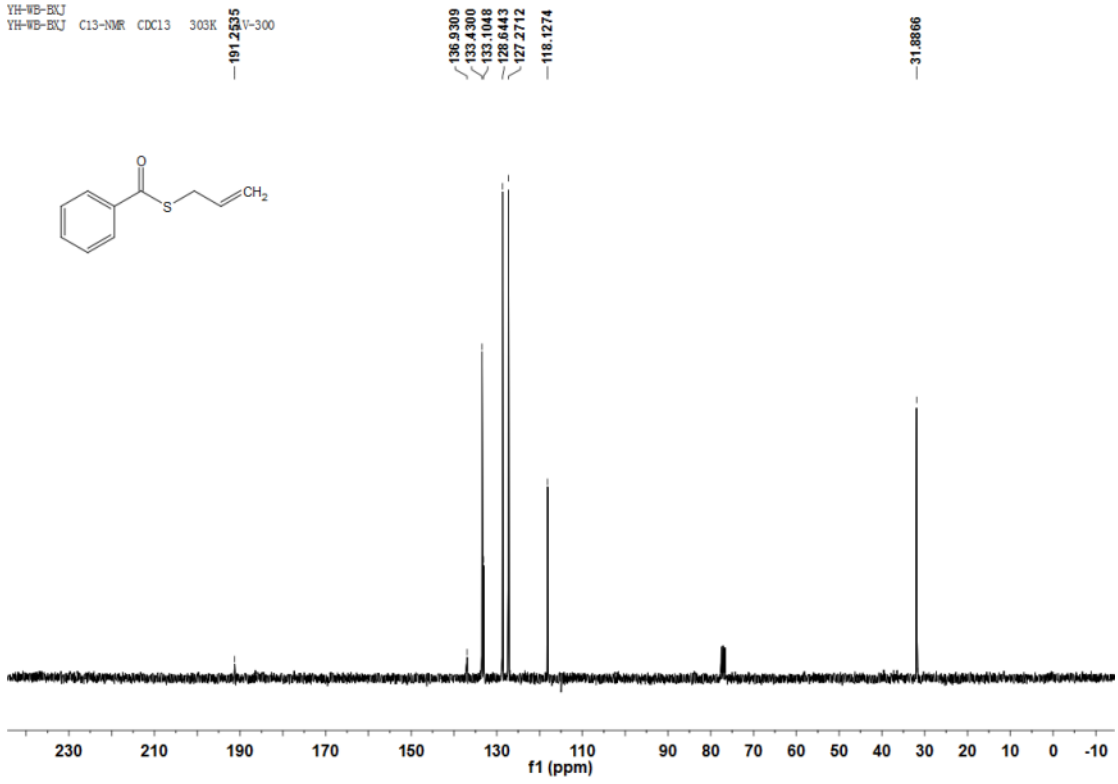
The stability of **5e** in rat plasma was determined using LC-MS/MS method.

Weighed accurately **5e** 1 mg, added 1mL DMSO to make a standard solution of 1mg/ml, diluted with methanol. Used blank plasma as matrix, prepare different concentrations (1000, 200, 20 ng/ml) of drug-containing plasma solution (drug solution: plasma 1:19), taked 50 μL of the plasma-drug solution to a microcentrifuge tube, incubated at 37 °C water bath. The incubations were terminated at 0, 10, 30, 60, 120, 240 min, and added 250 μL of a methanol solution containing an internal standard (diazepam, 50 ng / mL) to precipitate. The mixture was stirred vigorously and centrifuged (15,000 rpm, 10 min). Vortex for 10 minutes, centrifuged at 15,000 rpm for 10 minutes, and 5 μL of the supernatant was taken for LC-MS / MS determination.

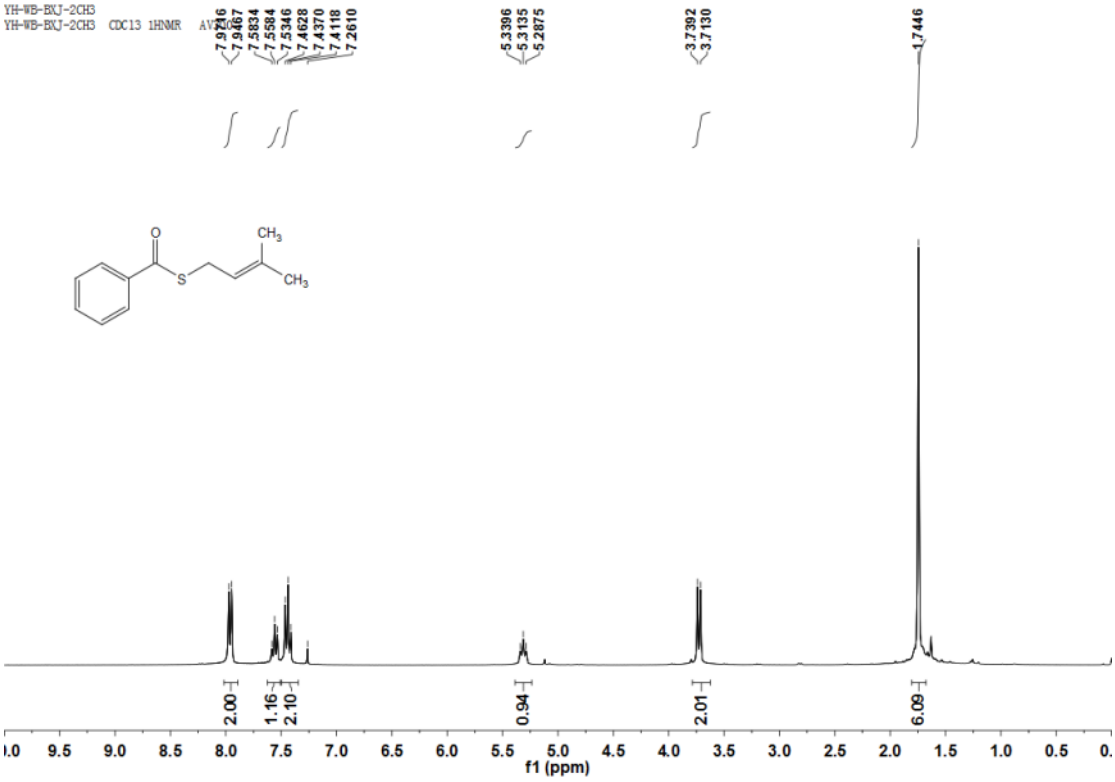
^{1}H NMR of 5a



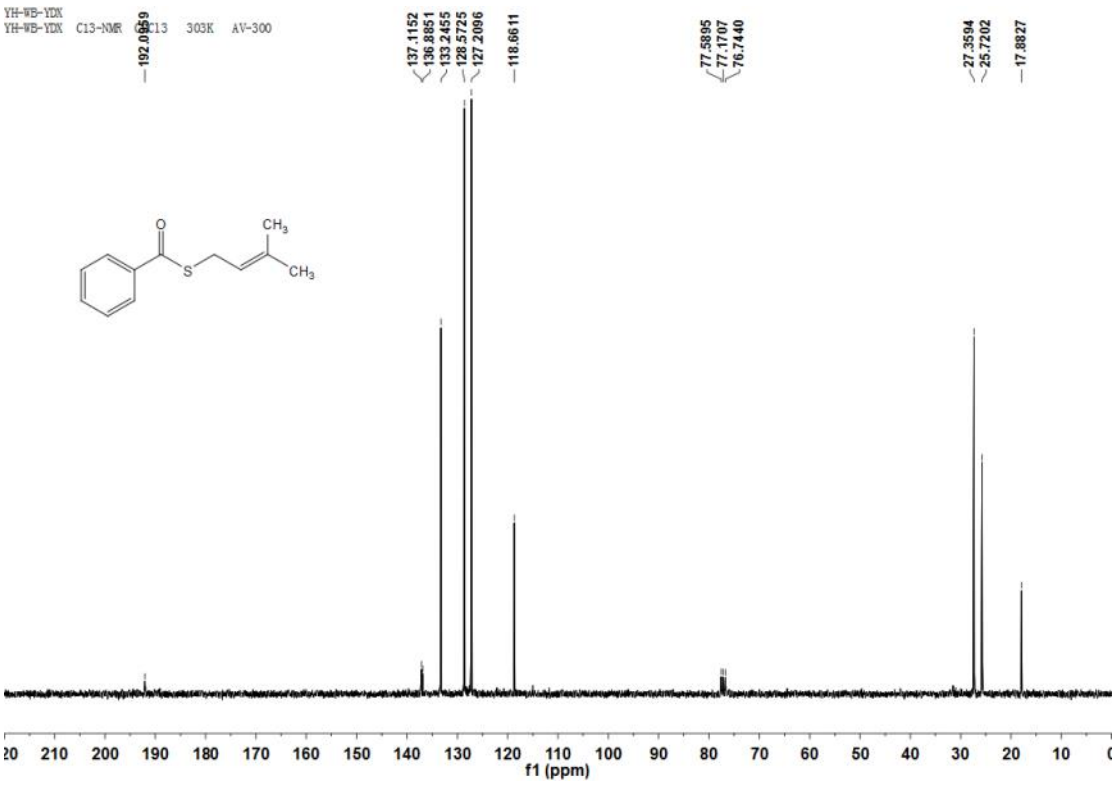
^{13}C NMR of 5a



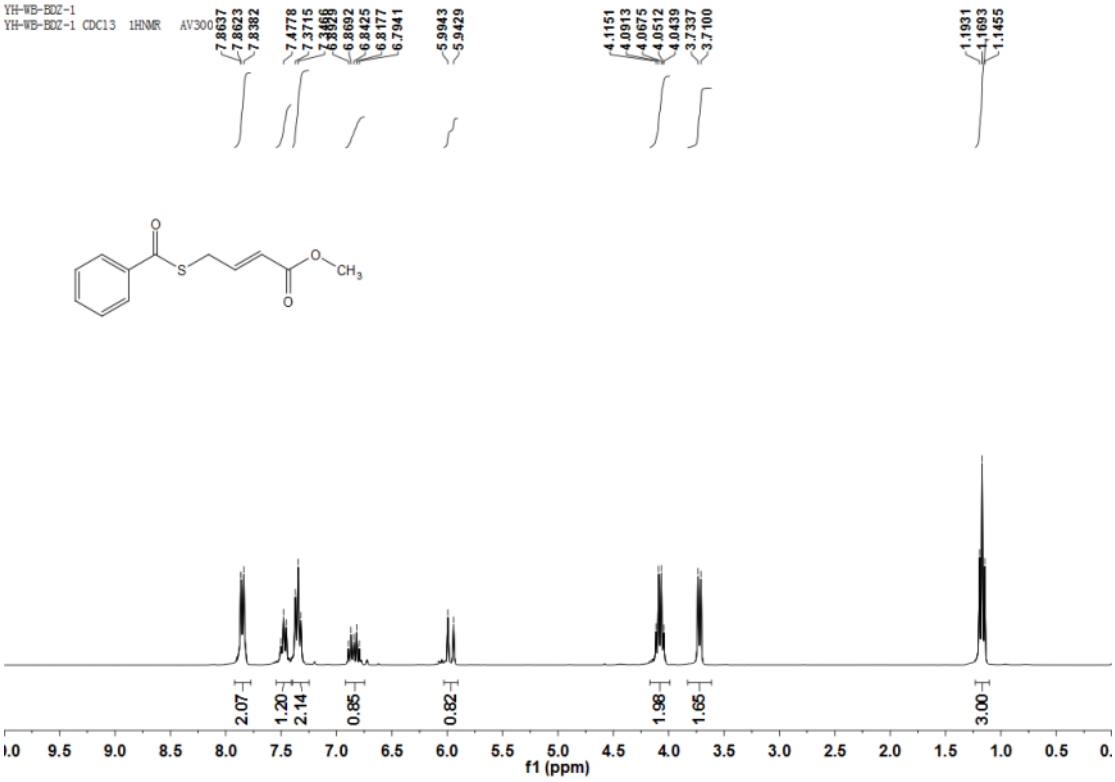
^{1}H NMR of 5b



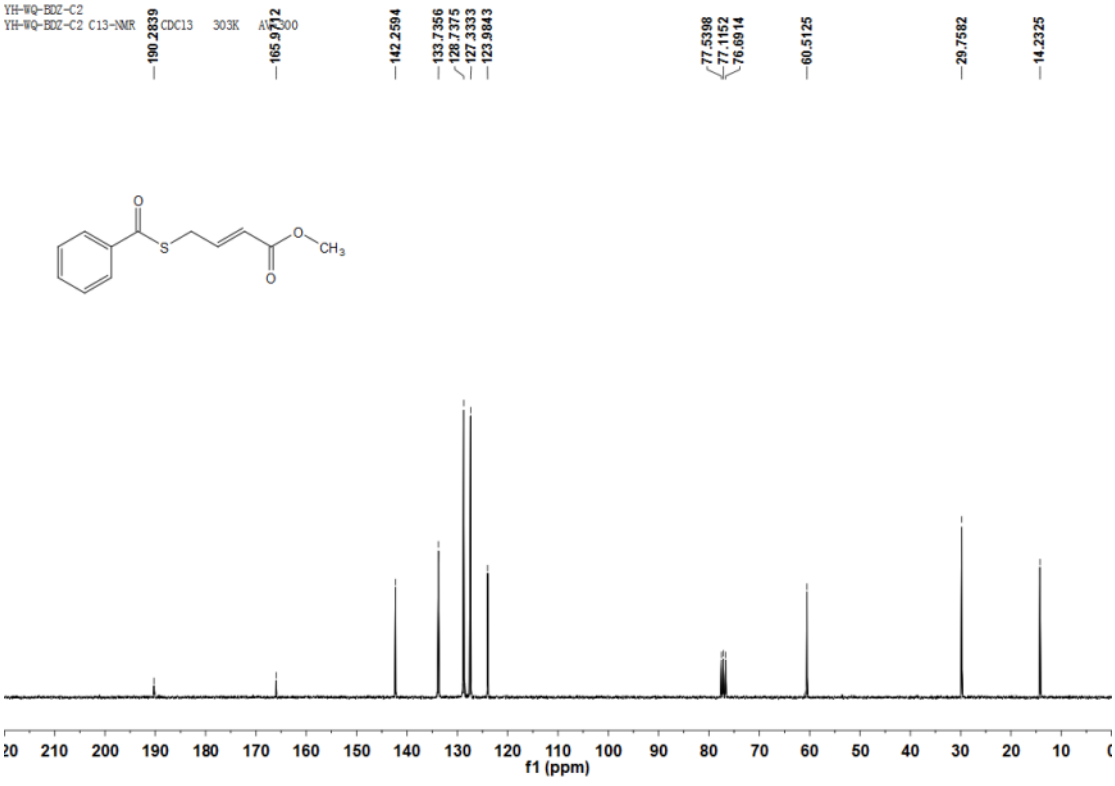
^{13}C NMR of 5b



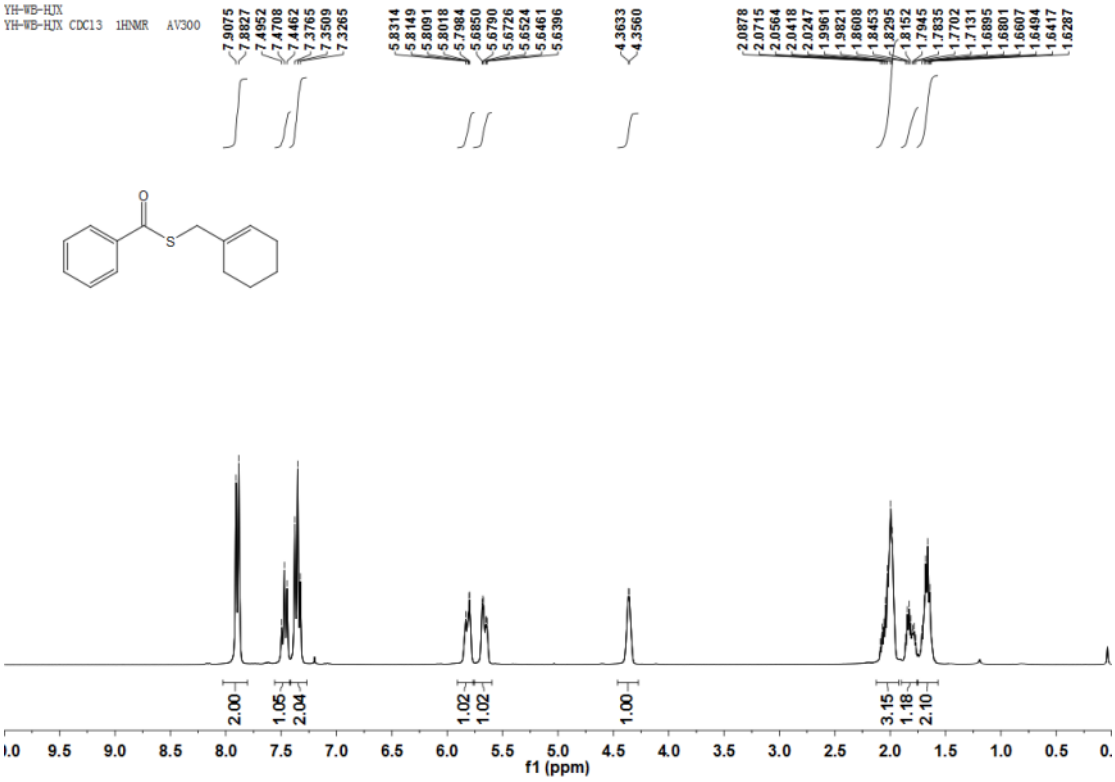
^{1}H NMR of 5c



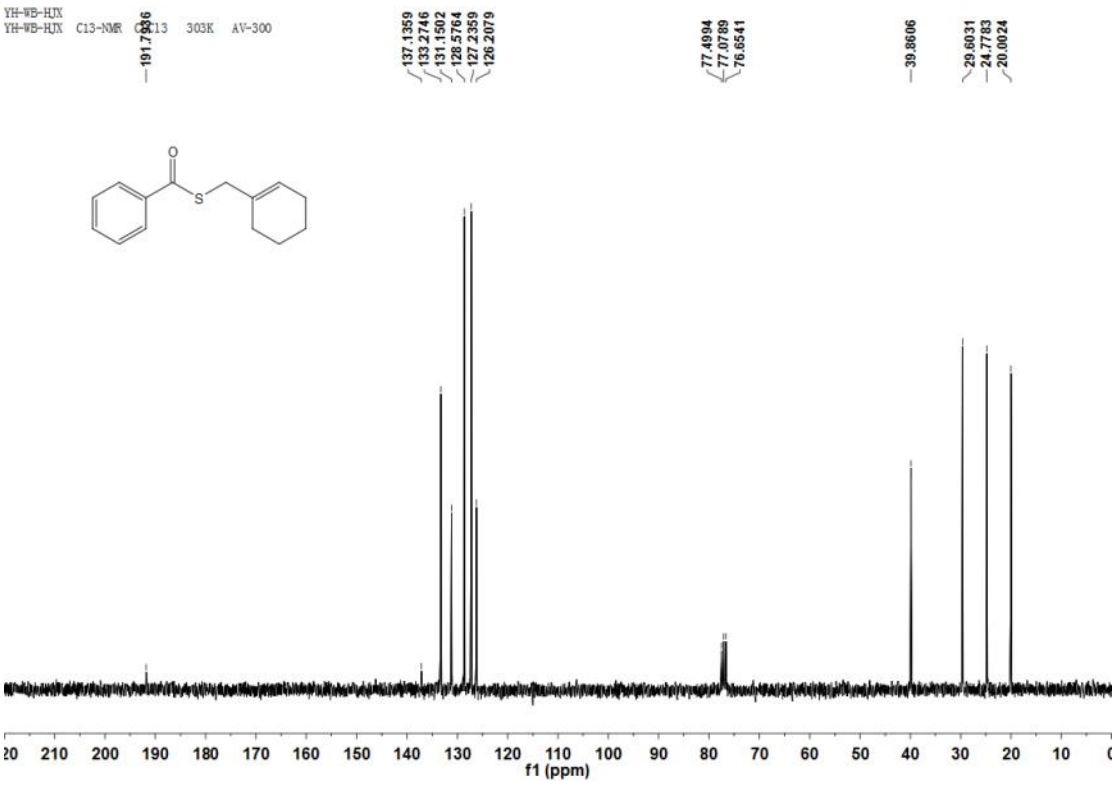
^{1}H NMR of 5c



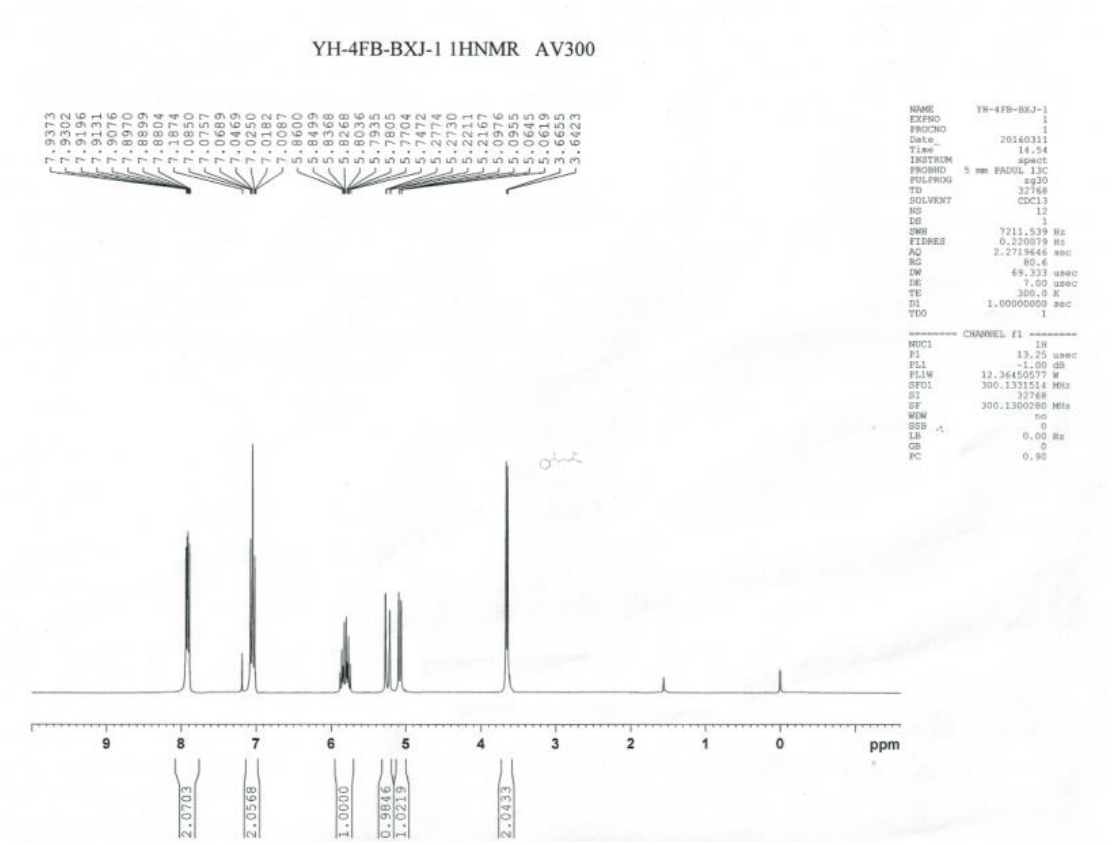
^{1}H NMR of 5d



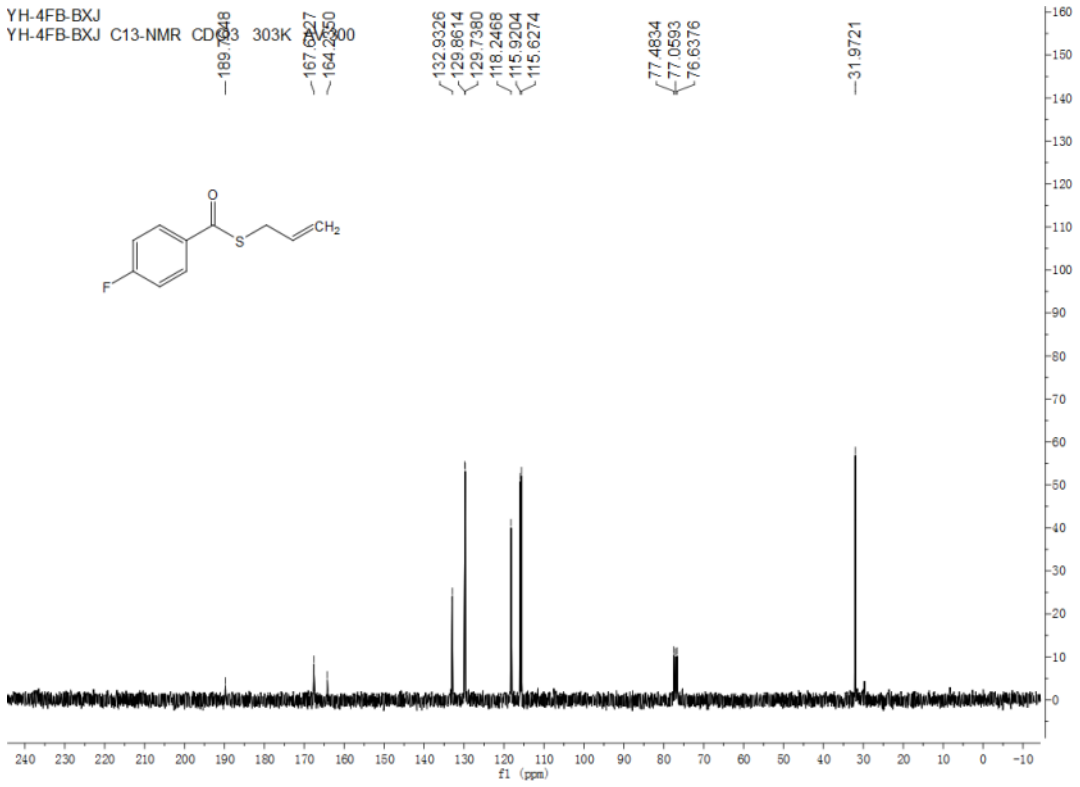
^{13}C NMR of 5d



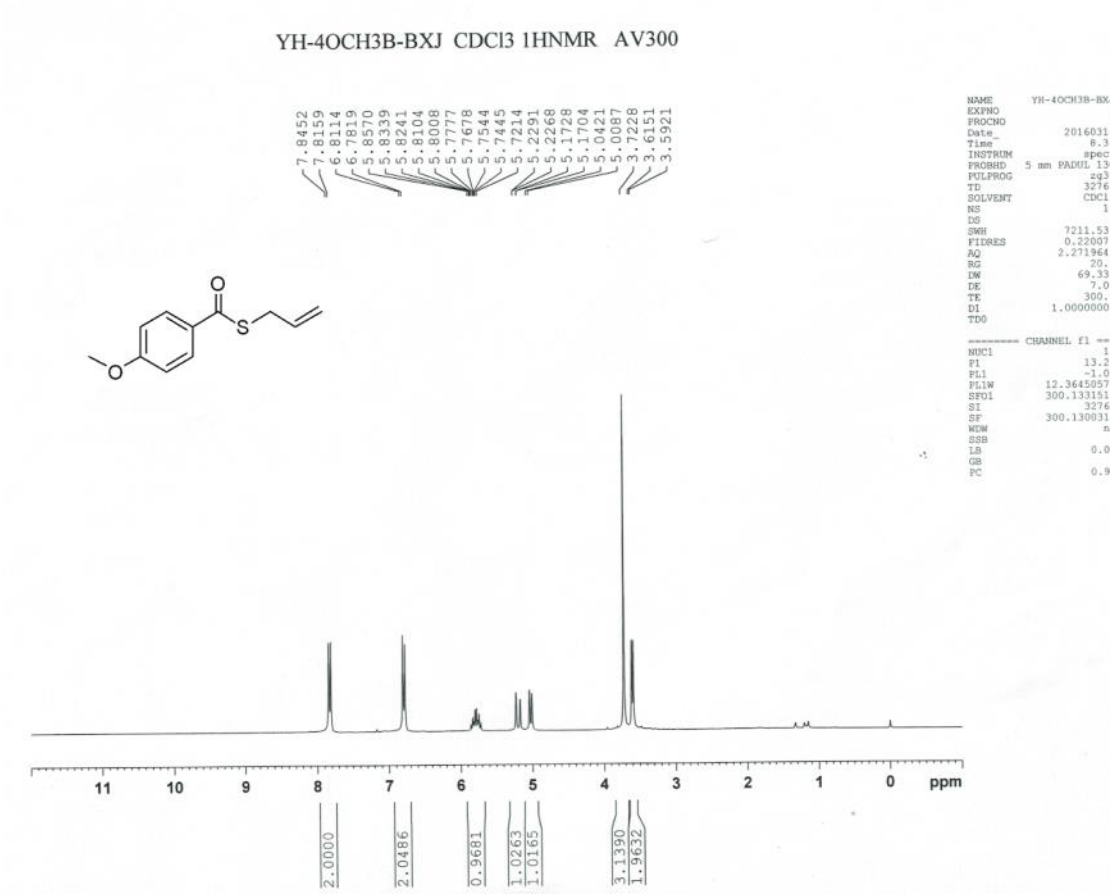
^{1}H NMR of 5e



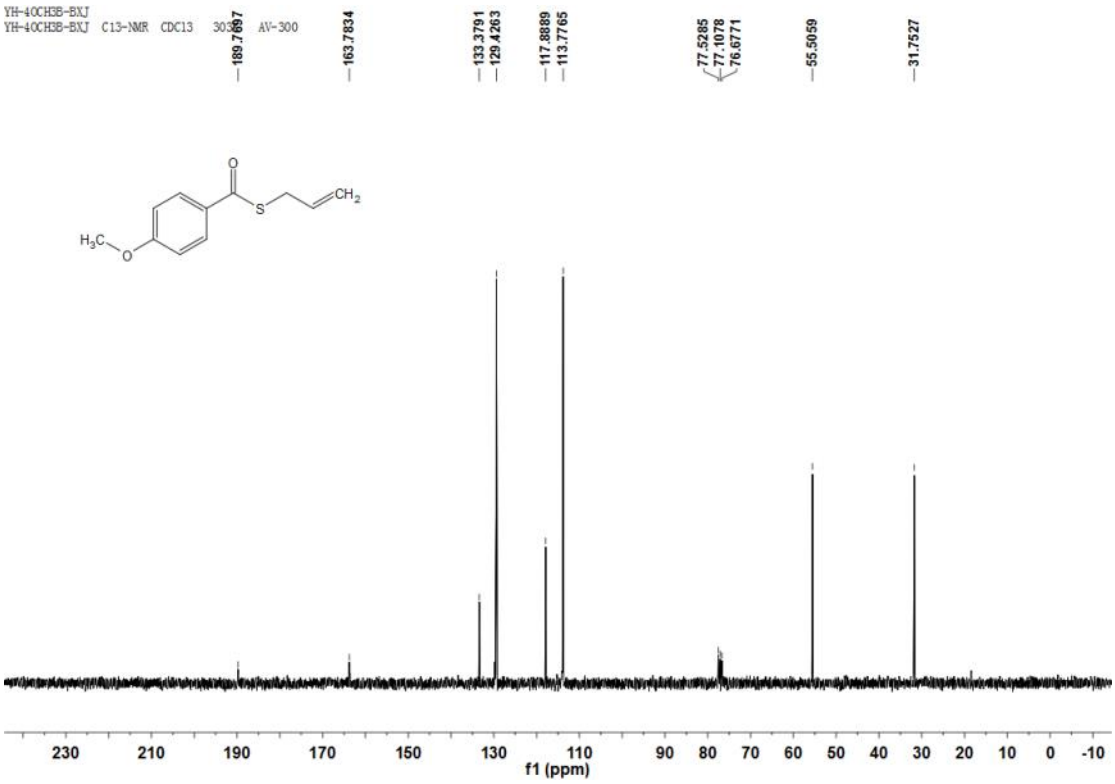
^{1}H NMR of 5e



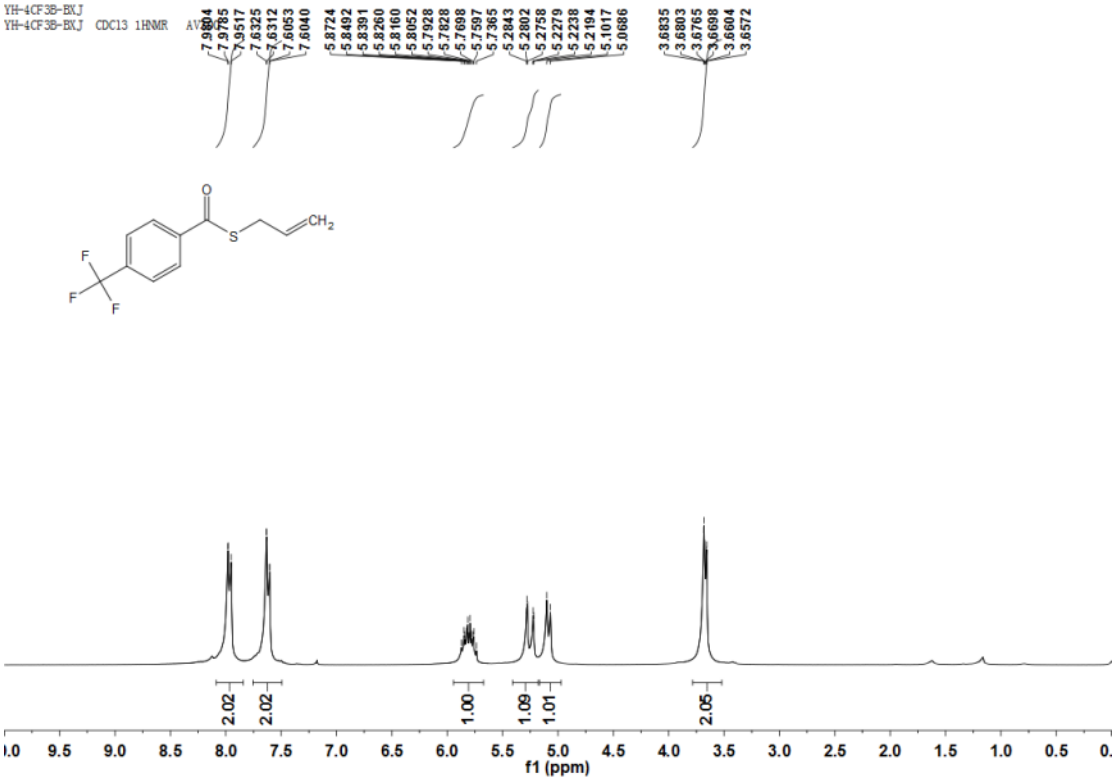
^{1}H NMR of 5f



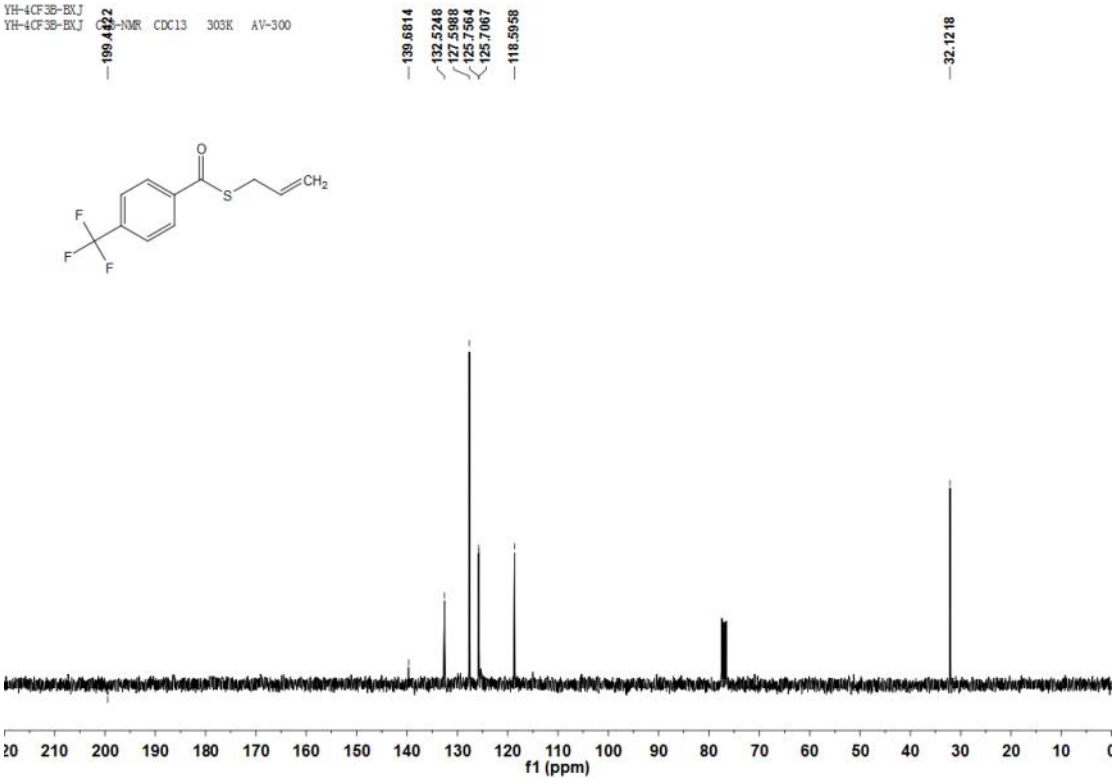
^{13}C NMR of 5f



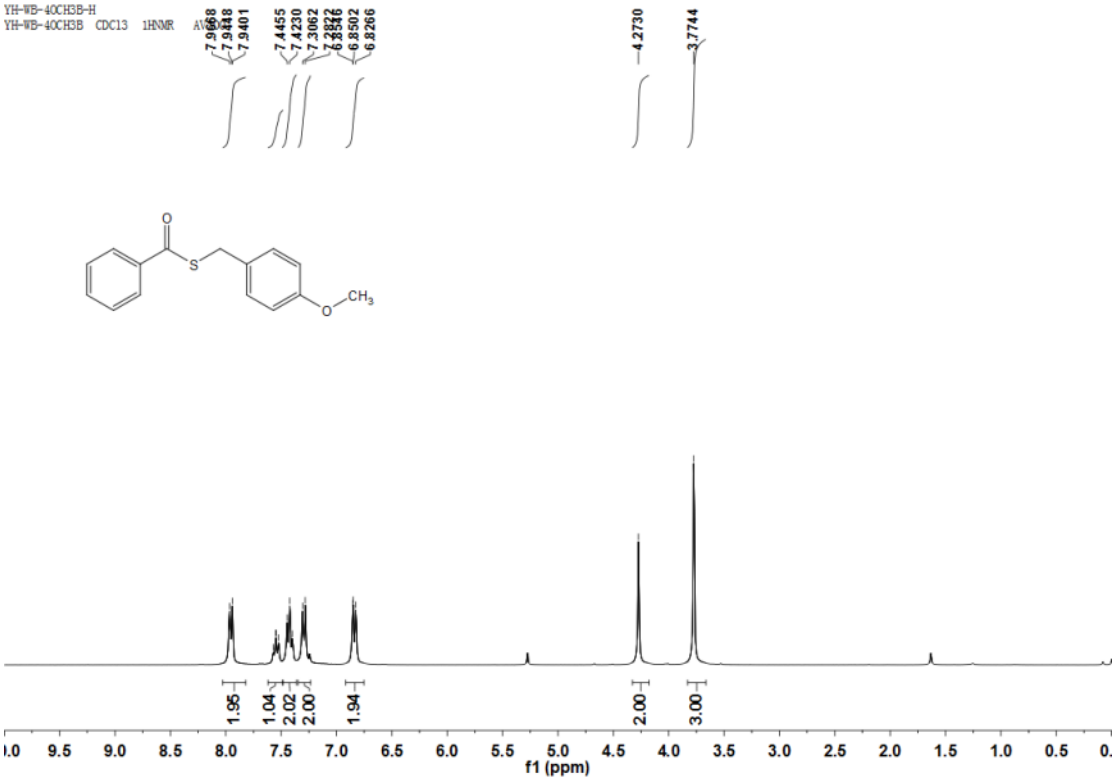
^{1}H NMR of 5g



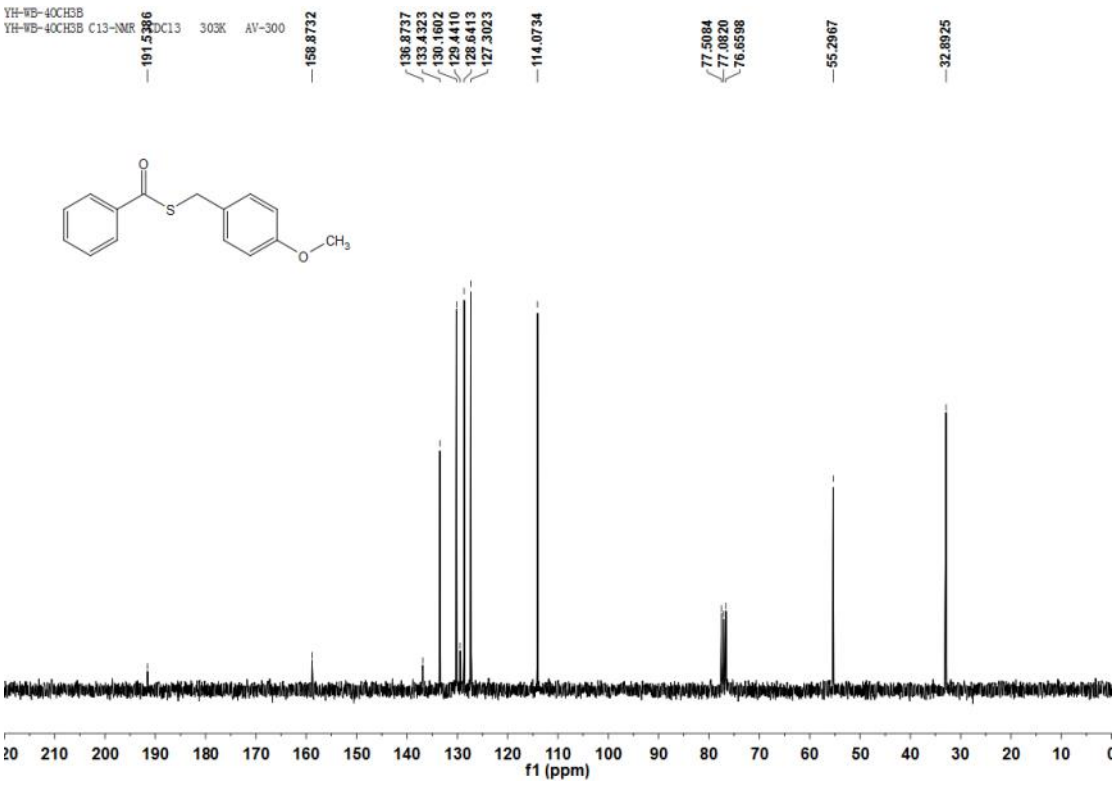
^{13}C NMR of 5g



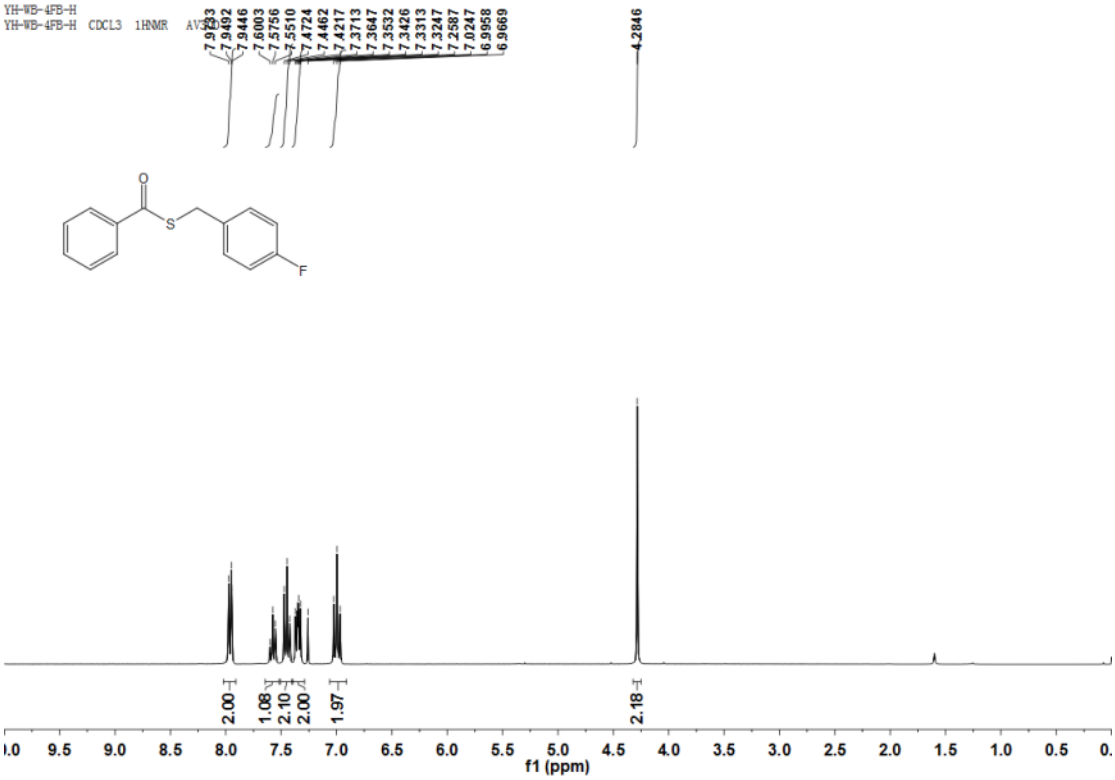
^{1}H NMR of 5h



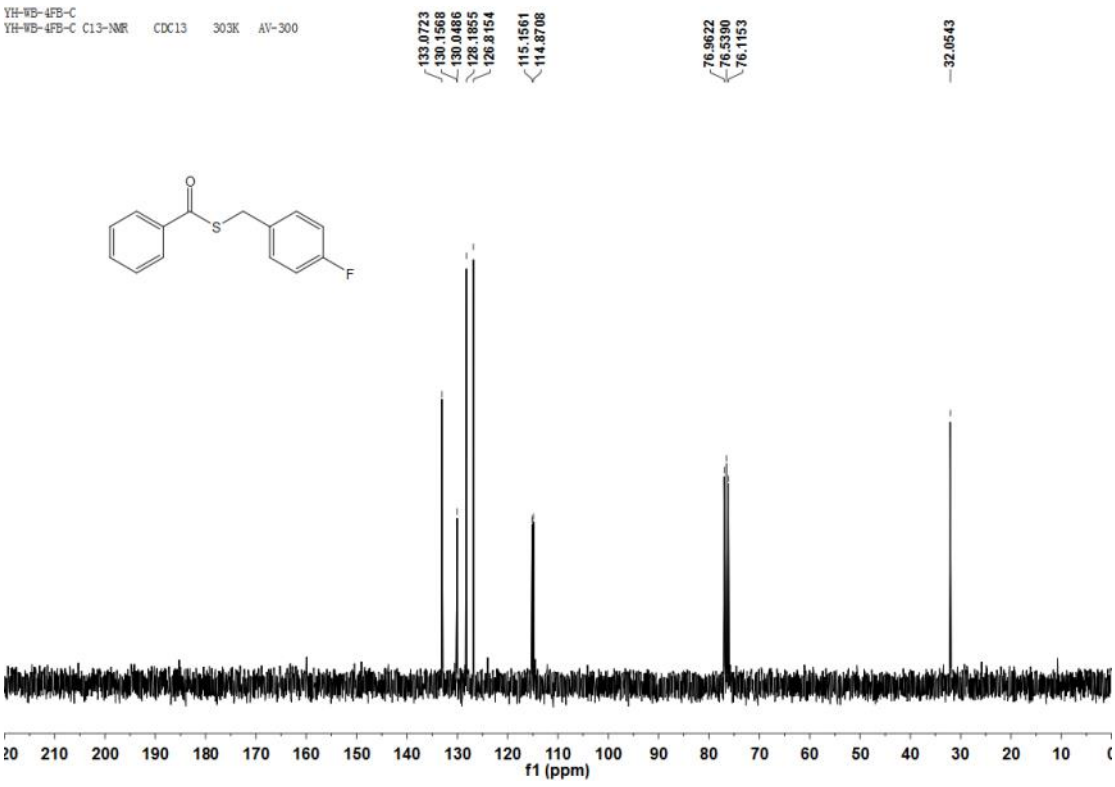
^{13}C NMR of 5h



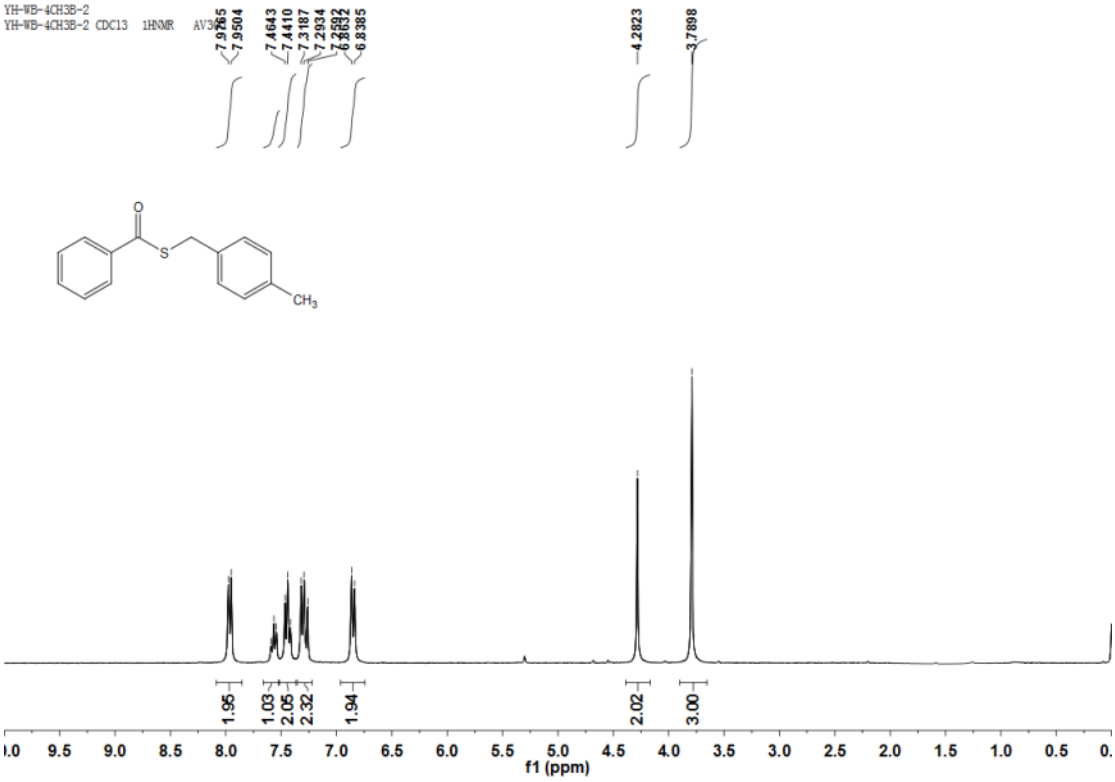
^{1}H NMR of 5i



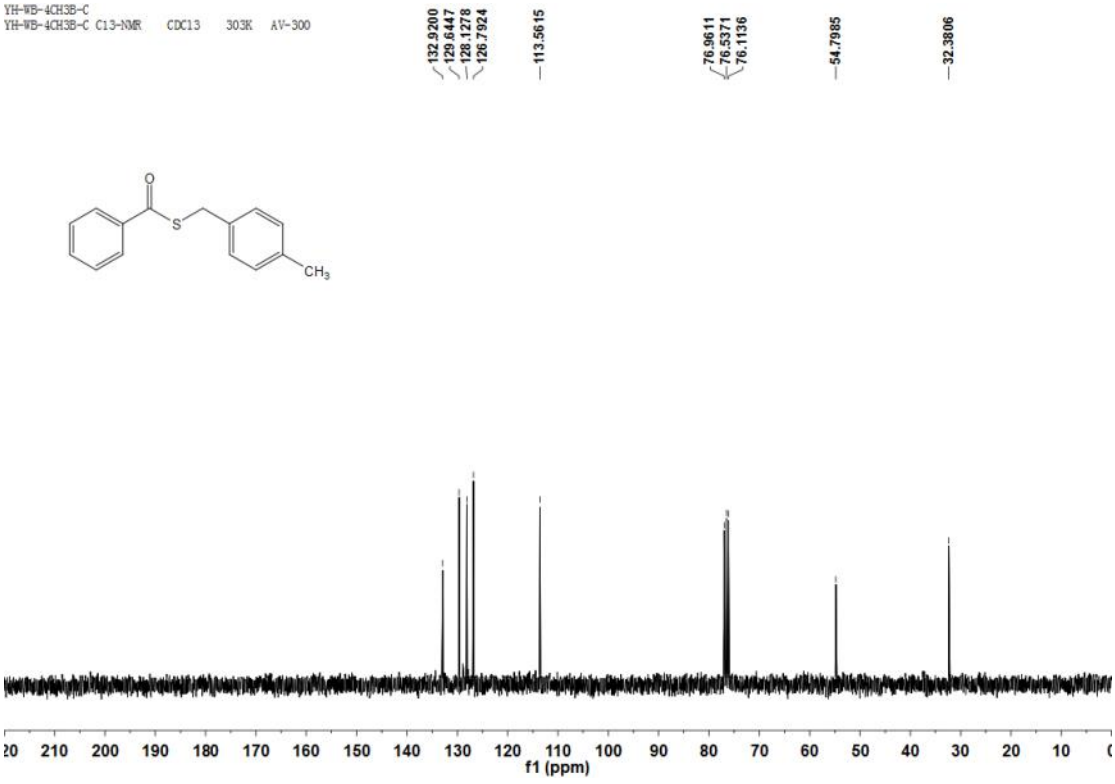
^{13}C NMR of 5i



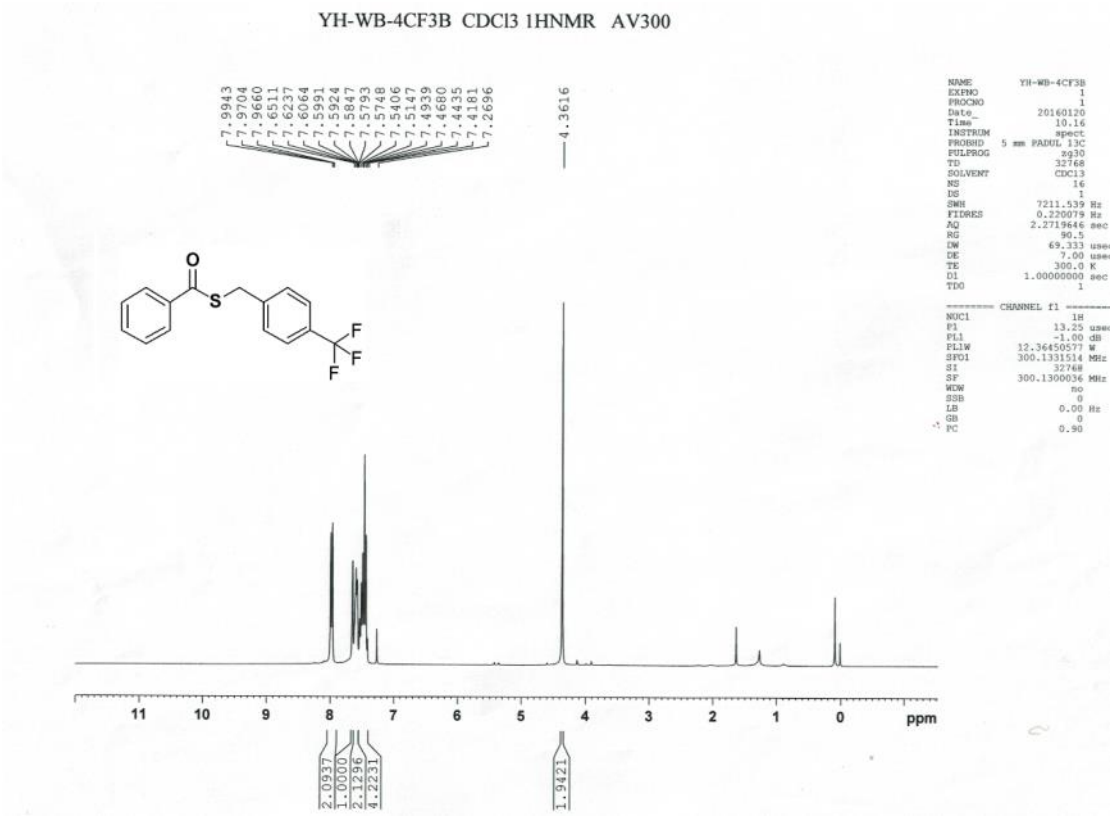
^{1}H NMR of 5j



^{13}C NMR of 5j



^{1}H NMR of 5k



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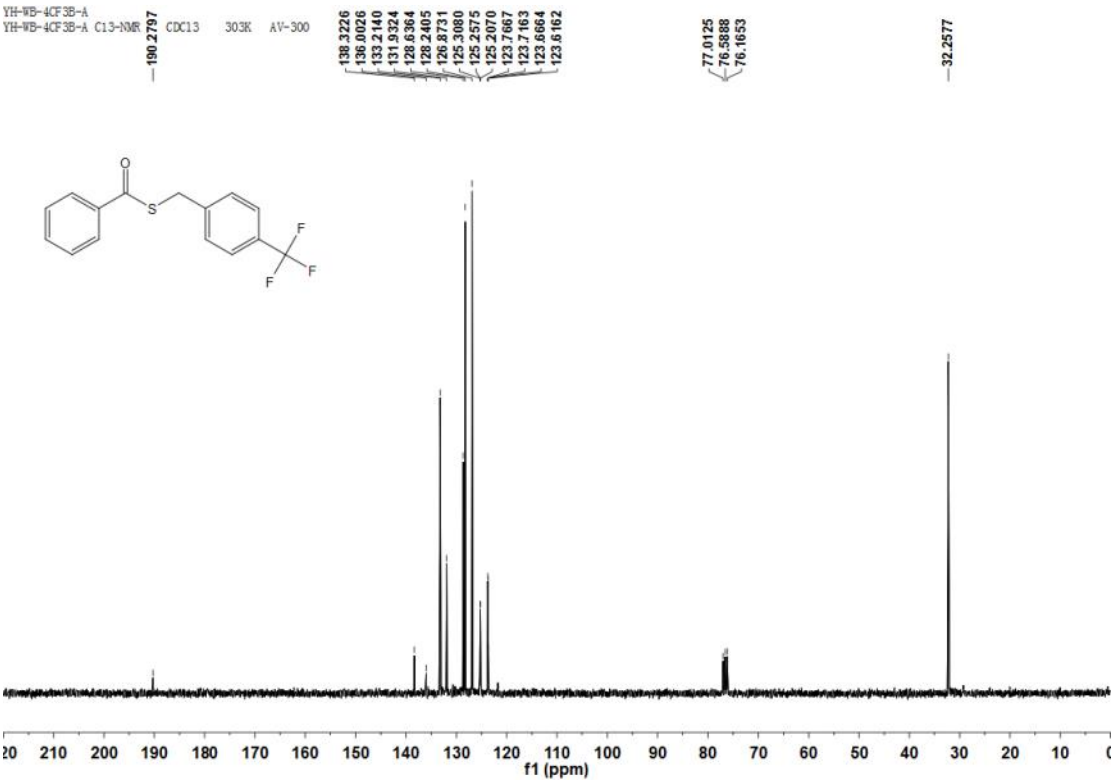
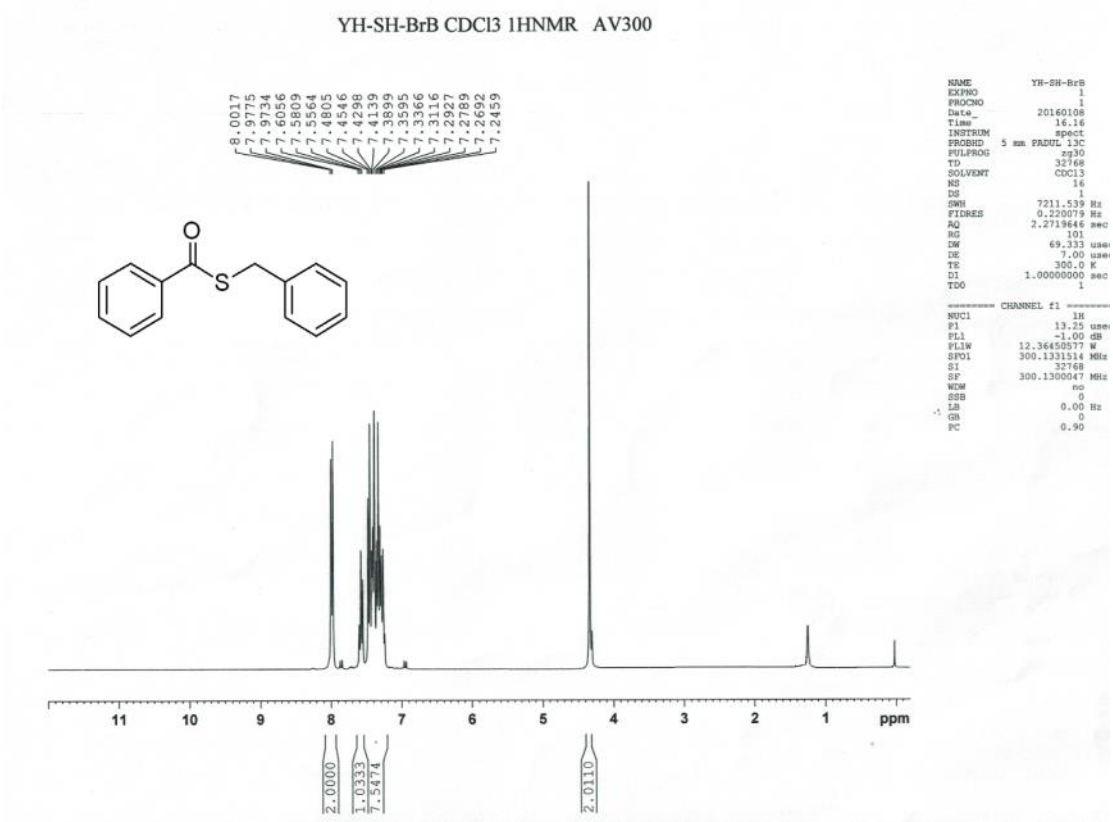


^{1}H NMR of 5l



^{1}H NMR of 5l