Mitochondria-targeted combine with methyl modification novel resveratrol derivatives enhances the anti-tumor activity

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Materials and methods

1. Reagents

Both the human cervix adenocarcinoma cell (HeLa) and human pulmonary adenocarcinoma cell (A549) were obtained from cell culture center of Chinese Academy of Medical Sciences, the Institute of Basic Medical Sciences (Beijing, China). Dulbecco's Modified Eagle Medium (DMEM, High Glucose) was purchased from Thermo Fisher Scientific. 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), 4',6-diamidino-2-phenylindole (DAPI), rhodamine 123 and 2',7'-dichlorofluorescein diacetate (DCFH-DA) were from Sigma-Aldrich. ATP detection kit, Fluo-3 AM, caspase 3 activity assay kit and the Mito-Tracker Red CMXRos were purchased from Beyotime. Apoptosis detection kit was purchased from BD Biosciences. Antibodies against cyclin D, cyclin E, p21, Bax, Bcl-2 and GAPDH were purchased from ABclonal.

2. Synthetic route employed for the preparation of the compounds A0-A6.

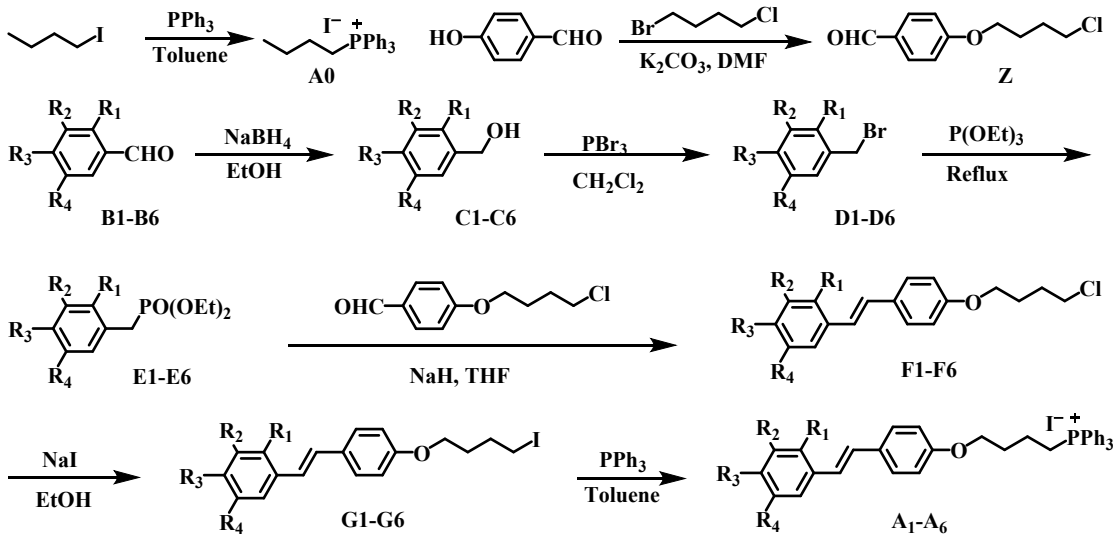
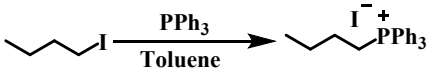
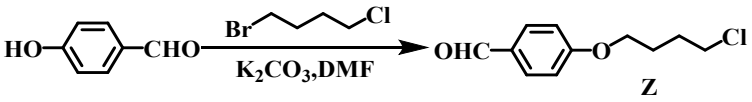


Figure S1 Synthesis of the compounds A0-A6.

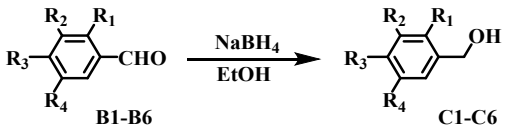
Synthesis details



Butyltriphenylphosphonium iodide 1-Iodobutane (0.3687 g, 2 mmol) and triphenylphosphine (2.6229 g, 10 mmol) were added to a 100 mL round-bottomed flask and dissolved in toluene (40 mL). Under nitrogen, the mixture was stirred for 6 h at 100℃. The toluene was removed by vacuum distillation, and the residue was washed five times with a mixed solution (diethyl ether: acetone = 100: 3), and the solid was dried under vacuum to obtain product **A0** as a white solid with yield of 73%.

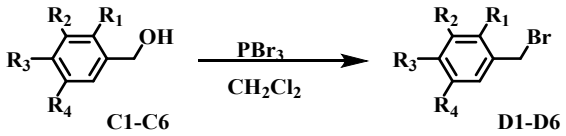


4-(4-Chlorobutoxy)benzaldehyde Z Under argon, the mixture of *p*-hydroxybenzaldehyde (2.4424 g, 20 mmol), potassium carbonate (3.0406 g, 22 mmol) was dissolved in DMF (60 mL) and stirred for 30 min. Then, 1-bromo-4-chlorobutane (5.1438 g, 30 mmol) was added to the mixture and stirred for 20 h at room temperature. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with saturated brine and was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (petroleum ether: ethyl acetate = 10: 1) to provide the colorless liquid compound **Z** with yield of 75%.

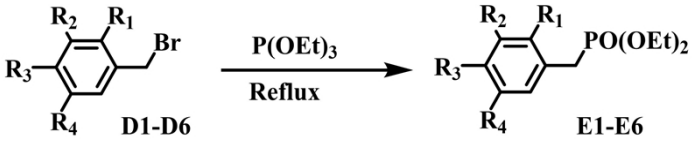


Compounds **C1-C6** To a solution benzaldehyde (B1-B6) (10 mmol) in ethanol (50 mL) was added sodium borohydride (10 mmol). After the reaction was stirred for 3 h at 80 ℃, and the reaction was quenched with water, and ethanol was removed in vacuum. The mixture was

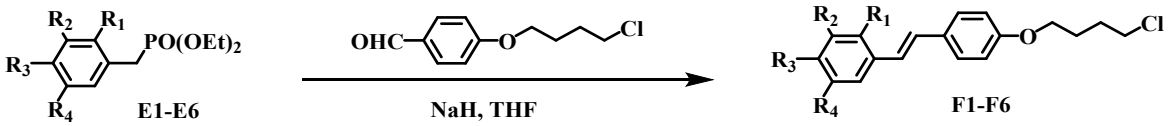
extracted with dichloromethane. The organic layers were washed with a solution of 2 M NaOH and water, dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel; petroleum/ethyl acetate 20/1) to afford the products **C1-C6.**



Compounds **D1-D6** To a solution of compounds **C1-C6** (10 mmol) in dichloromethane (50mL) was added dropwise phosphorus tribromide (1 mL) at 0℃, and the mixture was stirred for 1 h at room temperature. The reaction solution was poured into ice water (50 mL) to quench the reaction, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over Na_2SO_4, and evaporated under reduced pressure. The desired product **D1-D6** was used directly to next reaction without further purification.

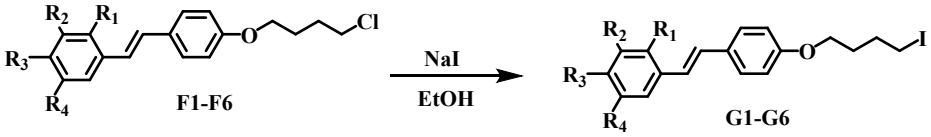


Compounds **E1-E6** Under argon, to the compound **D1-D6** (10 mmol) was added triethyl phosphite (1.9939 g, 12 mmol), and the solution was stirred and refluxed for 3h. The excess and unreacted triethylphosphite was removed by distillation under reduced pressure. The product **E1-E6** was obtained and used directly to next reaction without further purification.

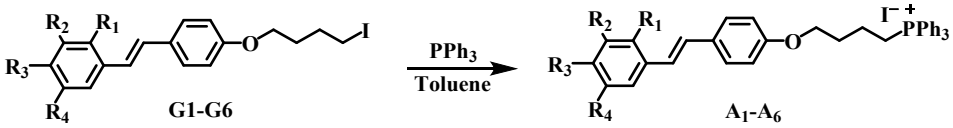


Compounds **F1-F6** Under argon, sodium hydride (0.8 g, 20 mmol) was dissolved in

anhydrous tetrahydrofuran (50 mL), and the solution was stired for 10min at room temperature. The solution of E1-E6 (10 mmol) in anhydrous tetrahydrofuran was added to the mixture and stirred for another 30 min, Then, the solution of **Z** (1.6965 g, 8 mmol) in anhydrous tetrahydrofuran, and the mixture was refluxed for 7 h. The reaction was quenched with water, and extracted with ethyl acetate. The organic layer was washed with saturated brine and was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and column chromatography (petroleum ether: ethyl acetate=100:1) to obtain the product **F1-F6**.



Compounds **G1-G6** To the solution of sodium iodide (0.7494 g, 5 mmol) in dry acetone (32 mL), was added F1-F6 (5 mmol), and refluxed for 20 h. The acetone was removed and the mixture was added water (50 mL) and extracted by ethyl acetate. The organic layer was removed, and the residue was recrystallized with petroleum ether and ethyl acetate to obtain the product **G1-G6**.



Compounds **A1-A6** Under argon, compounds **G1-G6** (2 mmol) and triphenylphosphine (2.6229 g, 10 mmol) were dissolved in toluene (40 mL). The mixture was heated to 100 ℃ and stirred for 6 h. The toluene was removed, and the residue was washed five times with a mixed solution (diethyl ether: acetone = 100: 3), and dried under vacuum to obtain the desired compound **A1-A6**.

3. Cell culture

All the cell lines were cultured in DMEM, with 10% (v/v) fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 units/mL), and kept at 37 °C in a 5 % CO_2 atmosphere. DMSO, as the effective solvent, was used to dissolve the compounds before experiments. The concentration of DMSO in cell suspension was less than 0.1 % (v/v).

4. Cell viability assay

The MTT assay was carried out to evaluate the cell viability (HeLa and A549 involved). The cells suspension was (5×10^4 cells/mL) added to 96-well flat microtiter plates. After culture for 24 h, the cells were treated with compounds **A0-A6** and resveratrol at gradient concentrations for 24 h. Then 100 μL DMEM medium with 10% MTT was added to each well, and incubated for another 4 h at 37 ℃ in the dark. Subsequently, remove the culture medium, and add 100 μL DMSO in each cell. The absorbance was determined at 570 nm using a microplate reader (Thermo Fisher 1510).

5. Cell Cycle assay

HeLa cells (2×10^5 cells/mL density) were cultured in a six-well plate for 24 h. Then the cells were treated with indicated concentrations of **A4** for another 24 h. Subsequently, the cells were fixed in 70% ethanol at 4 °C overnight. The cells were washed twice with ice-cold PBS, incubated with PI staining buffer for 30 min in the dark, and analyzed the cycle distribution by flow cytometry (BD FACSCalibur) lastly.

6. Cell apoptosis analysis

The apoptosis effect was detected by the AnnexinV-fluorescein isothiocyanate /propidium iodide (Annexin V-FITC/PI) apoptosis detection kit. The HeLa cells (2×10^5 cells/mL) were

cultured in six-well plates for 24 h. Then the culture medium was replaced by indicated concentrations of **A4** for another 24 h. Harvest the cells, wash with ice-cold PBS, and stained with annexin V-FITC/PI for 15 min in the dark. Lastly, the cells were detected by flow cytometry (BD FACS Calibur).

7. Morphological observation of nuclear

Fluorescence microscopy with DAPI staining was utilized to observe the morphological changes of nucleus. Firstly, HeLa cells (10×10^4 cells/mL) were cultured in a six-well plate with slide in each cell for 24 h. After treatment with indicated concentration of **A4** for another 24 h, the cells were fixed with 4 % paraformaldehyde overnight. DAPI staining for 10 min and the cells were identified by fluorescence microscopy (Olympus BX41).

8. Intracellular Fluorescence Imaging

1) Confocal fluorescence images of HeLa cells treated with A4 and Mito-Tracker Red CMXRos

HeLa cells (2.5×10^5 cells/mL) were cultured in a 20 mm confocal dish for 24 h. After **A4** (10 μM) treatment for 0.5 h, the Mito-Tracker Red CMXRos (70 nm) was added into the dish and incubated for 15 min. Wash 3 times using PBS, the fluorescence images were observed by confocal laser scanning microscopy.

2) Fluorescence images for probing mitochondrial transmembrane potential

HeLa cells were cultured in a six-well plate for 24 h. After A4 treatment with indicated concentrations for 18 h. Wash the cells with iced-PBS and resuspend it with Rhodamine 123 (3μM) for 0.5 h at 37 °C in the dark. Then the treated cells were washed by ice-cold PBS and detected the fluorescence intensity by fluorescence microscopy (Olympus BX41) immediately.

3) Fluorescence images for probing intracellular ROS levels

HeLa cells were cultured in a six-well plate for 24 h. After A4 treatment with indicated concentrations for 6 h. Wash the cells with iced-PBS and resuspend it with DCFH-DA for 0.5 h at 37 °C in the dark. Then the treated cells were washed by ice-cold PBS and detected the fluorescence intensity by fluorescence microscopy (Olympus BX41) immediately.

4) Fluorescence images for probing cytosolic Ca^{2+}

HeLa cells were cultured in a six-well plate for 24 h. After A4 treatment with indicated concentrations 6 h. Wash the cells with iced-PBS and resuspend it with Fluo-3 AM (5 μM) for 0.5 h at 37 °C in the dark. Then the treated cells were washed by ice-cold PBS and detected the fluorescence intensity by fluorescence microscopy (Olympus BX41) immediately.

9. Measurement of mitochondrial transmembrane potential

HeLa cells were cultured in a six-well plate for 24 h. Then the culture medium was replaced by indicated concentrations of **A4** for another 18 h/21 h/24 h. Collected and washed the cells, and resuspended them in PBS (3μM Rhodamine 123 containing) for 0.5 h at 37 °C in the dark. Then the treated cells were washed by ice-cold PBS and detected the fluorescence intensity by flow cytometry (BD FACSCalibur) immediately.

10. **ATP test**

The HeLa cells (2×10^5 cells/mL) were cultured in six-well plates for 24 h. After the cells were incubated with **A4** gradient concentration for 24 h, 200 μL lysis buffer from ATP detection kit was added into each cell to lyse them. The lysate were centrifuged at 12000 × g for 5 min at 4℃ and the supernatant was transferred to a 1.5 mL eppendorf tube for ATP test according to

the ATP detection kit.

11. Measurement of ROS

HeLa cells were cultured in a 6-well plate for 24 h, and were treated with indicated concentration of **A4** for another 3 h or 6 h. Collected and washed the cells, followed by staining with specific fluorescent probe, DCFH-DA for 0.5 h at 37 °C in the dark. Then the cells were washed by ice-cold PBS, and the fluorescence intensity was detected immediately by flow cytometry (BD FACSCalibur).

12. Determination of cytosolic Ca^{2+}

The fluorescent dye Fluo-3 AM can cross the cell membrane and be cleaved into Fluo-3, combining with the Ca^{2+} specifically, producing obvious fluorescence at 488 nm excitation wavelength. 2×10^5/mL cells were cultured in 6 well plates for 24 h. Then treated with indicated concentrations **A4** for another 6 h, collected and washed the cells, and then resuspended in Fluo-3 AM (5 μM) for 0.5 h at 37 °C in the dark. Then measured the fluorescence intensity by flow cytometry (BD FACSCalibur).

13. Caspase-3 activities assay

The lysates of treated HeLa cells with **A4** for 24 h were extracted and by caspase-3 activity assay kit. Then the caspase-3 activity was detected at 405 nm by a microplate reader (Thermo Fisher 1510), with Ac-DEVD-pNA as the substrate incubation for 2 h at 37 °C following the manufacturer's instructions.

14. Western blot analysis

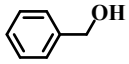
The lysates of treated HeLa cells with **A4** were extracted by RIPA Lysis Buffer containing PMSF (P0020, Solarbio, China) following the instructions. And the protein concentration was

measured by BCA protein assay kit. Then the SDS-PAGE loading buffer (1:1 v/v) was added into the proteins sample, and boiled for 5 min. The extracted proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane by a wet transfer method. After blocking with 5% nonfat milk in TBST for 1.5 h at room temperature, the PVDF membrane was incubated with primary antibodies (1:2000 against Bax, Bcl-2, Cyclin D, Cyclin E and 1:10000 against GAPDH) overnight at 4 °C, followed by the incubation with secondary antibody at room temperature for another 1.5 h. The immunoblots were visualized with an Ultra ECL kit (LK-U1421, MultiSciences Biotech Co., Ltd, China).

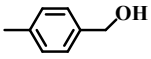
15. Statistical Analysis

All experiments were repeated at least three times. The results were expressed as mean ± SD and the differences were analyzed by ANOVA using SPSS 22.0.

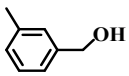
16. ^{1}H NMR, ^{13}C NMR and ESI-MS



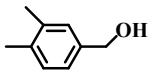
Phenylmethanol (C1) (yield: 95%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.37 (d, *J* = 4.4 Hz, 4 H), 7.33-7.28 (m, 1 H), 4.68 (s, 2 H), 1.98 (s, 1 H); **^{13}C-NMR** (100 MHz, acetone-d_6): δ = 141.0, 128.7, 127.8, 127.1, 65.4.



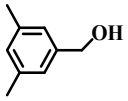
4-Methylbenzyl alcohol (C2) (yield: 94%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.21 (d, *J* = 8.0 Hz, 2 H), 7.14 (d, *J* = 8.0 Hz, 2 H), 4.60 (s, 2 H), 2.33 (s, 3 H), 2.12 (s, 1 H); **^{13}C-NMR** (100 MHz, acetone-d_6): δ = 138.0, 137.4, 129.3, 127.2, 65.18, 21.2.



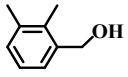
3-Methylbenzyl alcohol (C3) (yield: 92%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.26-7.23 (m, 1 H), 7.18 (s, 1 H), 7.15 (d, *J* = 4.2 Hz , 1 H), 7.1 (d, *J* = 7.6 Hz, 1 H), 4.64 (s, 2 H), 2.35 (s, 3 H) , 1.83 (s, 1 H); **^{13}C-NMR** (100 MHz, acetone-d_6): δ = 141.0, 138.4, 128.6, 128.5, 127.9, 124.2, 65.5, 21.5.



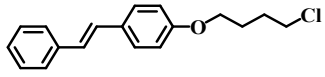
(3,4-dimethylphenyl)methanol (C4) (yield: 95%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.13 (d, *J* =8.4 Hz, 2 H), 7.10 (d, *J* =7.6 Hz, 1 H), 4.60 (d, *J* = 5.6 Hz, 2 H), 2.28 (s, 3 H), 2.27 (s, 3 H),1.72 (t, *J* =5.6 Hz, 1 H); **^{13}C-NMR** (100 MHz, $CDCl_3$-d_1): δ = 138.5, 136.9, 136.2, 129.9, 128.7, 124.7, 65.4, 19.9, 19.6.



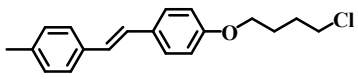
(3,5-dimethylphenyl)methanol (C5) (yield: 95%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 6.99 (s, 2 H),6.94 (s, 1 H), 4.62 (s, 2 H), 2.33 (s, 6 H), 1.80 (s, 1 H); **^{13}C-NMR** (100 MHz, $CDCl_3$-d_1): δ = 140.9, 138.3, 129.4, 124.9, 65.5.



(2,3-dimethylphenyl)methanol (C6) (yield: 92%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.2 (dd, *J* = 2, 2.4 Hz, 1 H), 7.17-7.09 (m, 1 H), 6.89-6.87 (m, 1 H), 4.69 (d, *J* = 5.6 Hz, 2 H), 2.32 (s, 3 H) , 2.27 (s, 3 H), 1.751 (t, *J* = 5.4 Hz, 1 H); **^{13}C-NMR** (100 MHz, $CDCl_3$-d_1): δ = 138.7, 137.3, 135.0, 129.7, 125.9, 125.7, 64.3, 20.4, 14.7.

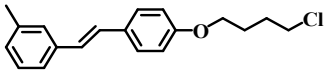


(*E*)-1-(4-Chlorobutoxy)-4-styrylbenzene (F1) (yield: 53%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.54 (dd, *J* = 12.0, 8.0 Hz, 4 H), 7.35 (dd, *J* = 8.0, 4.0 Hz, 2 H), 7.23 (t, *J* = 8.0 Hz, 1 H), 7.17 (s, 1 H), 7.09 (d, *J* = 20 Hz, 1 H), 6.94 (d, *J* = 8.0 Hz, 2 H), 4.03 (d, *J* = 4.0 Hz, 2 H), 7.71 (d, *J* = 4.0 Hz, 2 H), 1.86 (s, 4 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.3, 137.3, 129.6, 128.6, 128.0, 127.8, 127.1, 126.1, 126.0, 114.7, 66.7, 45.1, 39.9, 39.7, 39.5, 39.3, 39.1, 28.9, 26.1.

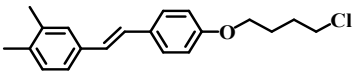


(E)-1-(4-chlorobutoxy)-4-(4-methylstyryl)benzene (F2) (yield: 26%) **^{1}H-NMR** (400 MHz, $CDCl_3$-d_1): δ = 7.44 (d, *J* = 8.8 Hz, 2 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 7.16 (d, *J* = 8.0 Hz, 2 H), 7.04-6.93 (m, 2 H), 6.88 (d, *J* = 7,2 Hz, 2 H), 4.02 (t, *J* = 5.6 Hz, 2 H), 3.64 (t, *J* = 6.2 Hz, 2 H), 2.36 (s, 3 H), 2.02-1.94 (m, 4 H); **^{13}C-NMR** (100 MHz, $CDCl_3$-

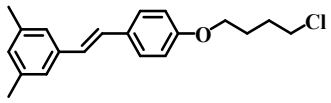
d_1): δ=158.5, 137.2, 134.9, 130.5, 129.5, 127.7, 127.3, 126.7, 126.3, 114.7, 67.1, 44,9, 29.4, 26.8, 21.4.



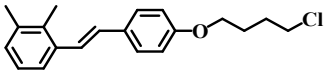
(E)-1-(4-(4-chlorobutoxy)styryl)-3-methylbenzene (F3) (yield: 19%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.52 (d, *J* = 8.8 Hz, 2 H), 7.36 (t, *J* = 7.6 Hz, 2 H), 7.25-7.16 (m, 2 H), 7.05 (t, *J* = 8.2 Hz, 2 H), 6.94 (d, *J* = 8.4 Hz, 2 H), 4.07 (t, *J* = 5.8 Hz, 2 H), 3.70 (t, *J* = 6.2 Hz, 2 H), 2.34 (s, 3 H), 2.06-1.92 (m, 4 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=159.6, 138.7, 138.5, 129.2, 130.3, 129.2, 128.7, 128.4, 127.6, 127.2, 124.2, 115.4, 67.7, 45.4, 21.3.



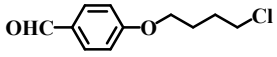
(E)-4-(4-(4-chlorobutoxy)styryl)-1,2-dimethylbenzene (F4) (yield: 55%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.45 (d, *J* = 8.8 Hz, 2 H), 7.28 (s, 1 H), 7.24 (d, *J* = 8.0 Hz, 1 H), 7.12 (d, *J* = 8.0 Hz, 1 H), 7.04-6.91 (m, 2 H), 6.88 (d, *J* = 8.8 Hz, 2 H), 4.02 (t, *J* = 5.8 Hz, 2 H), 3.63 (t, *J* = 6.2 Hz, 2 H), 2.29 (s, 3 H), 2.27 (s, 3 H), 2.02-1.94(m, 4 H); **^{13}C-NMR** (100 MHz, $CDCl_3$-d_1): δ=158.5, 136.9, 136.0, 135.4, 130.6, 130.1, 127.7, 127.6, 127.1, 126.8, 123.9, 114.2, 67.1, 44.9, 29.4, 26.78, 20.0, 19.7.



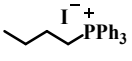
(E)-1-(4-(4-chlorobutoxy)styryl)-3,5-dimethylbenzene (F5) (yield: 20%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.50 (d, *J* = 8.8 Hz, 2 H), 7.14 (d, *J* = 16.4 Hz, 3 H), 6.99 (d, *J* = 16.8 Hz, 1 H), 6.93 (d, *J* = 8.8 Hz, 2 H), 6.87 (s, 1 H), 4.0 (t, *J* = 6 Hz, 2 H), 3.70 (t, *J* = 6.4 Hz, 2 H), 2.27 (s, 6 H), 1.92-1.79 (m, 4 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.2, 137.5, 137.2, 129.8, 128.8, 127.7, 126.2, 124.0, 114.7, 66.8, 45.2, 28.9, 26.2, 20.9.



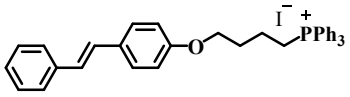
(E)-1-(4-(4-chlorobutoxy)styryl)-2,3-dimethylbenzene (F6) (yield: 38%) **^{1}H-NMR** (400 MHz, DMSO-d$_6$): δ = 7.54 (d, *J* = 8.8 Hz, 2 H), 7.44-7.42 (m, 1 H), 7.33 (d, *J* = 16.0 Hz, 1 H), 7.08-7.05 (m, 2 H), 6.98-6.92 (m, 3 H), 4.02 (t, *J* = 6.0 Hz, 2 H), 3.71 (t, *J* = 7 Hz, 2 H), 2.27 (s, 3 H), 2.26(s, 3H), 1.92-1.80(m, 4H); **^{13}C-NMR** (100 MHz, DMSO-d$_6$): δ=158.2, 136.4, 133.7, 130.0, 129.6, 128.7, 127.8, 125.5, 124.6, 123.2, 114.6, 66.8, 45.2, 28.9, 26.2, 20.3,15.1.



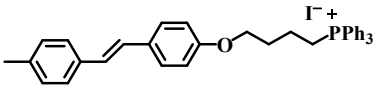
4-(4-Chlorobutoxy)benzaldehyde (Z) **^{1}H-NMR** (yield: 75%) (400 MHz, DMSO-d$_6$): δ = 9.90 (s, 1 H), 7.87 (d, *J* = 8.0 Hz, 2 H), 7.12 (d, *J* = 8.0 Hz, 2 H), 4.19 (dd, *J* = 8.0, 4.0 Hz, 2 H), 3.71 (dd, *J* =8.0, 4.0 Hz, 2 H), 2.06-1.98 (m, 5 H); **^{13}C-NMR** (100 MHz, DMSO-d$_6$): δ=191.0, 164.7, 132.4, 131.0, 115.6, 68.2, 45.4, 27.1.



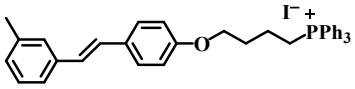
Butyltriphenylphosphonium iodide (A0) (yield:73%) **^{1}H-NMR** (400 MHz, DMSO-d$_6$): δ = 7.92-7.88 (m, 3 H), 7.84-7.75 (m, 12 H), 3.62-3.55 (m, 2 H), 1.54-1.46 (m, 4 H), 0.88 (t, *J* =6.8 Hz, 3 H); **^{13}C-NMR** (100 MHz, DMSO-d$_6$): δ=134.9, 134.9, 133.6, 133.5, 130.3, 130.2, 119.0, 118.1, 23.9, 23.8, 23.2, 23.0, 20.3, 19.8, 13.3.



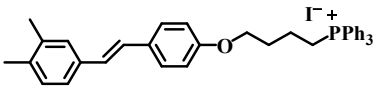
(*E*)-Triphenyl(4-(4-styrylphenoxy)butyl)phosphonium iodide (A1) (yield: 89%) **^{1}H-NMR** (400 MHz, DMSO-d$_6$): δ = 7.93-7.89 (m, 3 H), 7.85-7.74 (m, 12 H), 7.57-7.52 (m, 4 H), 7.35 (dd, *J* = 7.2, 8.0 Hz, 2 H), 7.23-7.19 (m, 2 H), 7.12 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 2 H), 4.07 (dd, *J* = 6.4, 6.0 Hz, 2 H), 3.71-3.65 (m, 2 H), 1.95-1.90 (m, 2 H), 1.73 (t, *J* = 7.6 Hz, 2 H); **^{13}C-NMR** (100 MHz, DMSO-d$_6$): δ=158.1, 137.3, 134.9, 133.6, 133.5, 130.3, 130.2, 129.7, 128.6, 128.0, 127.7, 127.2, 126.1, 118.9, 118.0, 114.7, 66.0, 29.2, 29.0, 20.0, 19.5, 18.4; ESI-MS: calcd for $C_{36}H_{34}OP^{+}$ $[M-I]^{+}$ *m/z* 513.23, found 513.2348.



(E)-(4-(4-(4-methylstyryl)phenoxy)butyl)triphenylphosphonium iodide (A2) (yield: 48%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.91-7.89 (m, 3 H), 7.84-7.75 (m, 12 H), 7.52 (d, *J* = 8.8 Hz, 2 H), 7.45 (d, *J* = 8.0 Hz, 2 H), 7.12 (t, *J* = 13.4 Hz, 2H), 7.04 (d, *J* = 16.4 Hz, 2 H), 6.87 (d, *J* = 8.8 Hz, 2 H), 4.06 (t, *J* = 6.2 Hz, 2 H), 3.67 (t, *J* = 15 Hz, 3 H), 2.30 (s, 3 H), 1.94-1.90 (m, 2 H), 1.73 (t, *J* = 7.4 Hz, 2 H); **^{13}C-NMR** (100 MHz, acetone-d_6): δ=158.0, 136.5, 134.9, 134.5，133.6, 133.5, 130.3, 130.2, 129.9, 129.3, 127.6, 127.0, 126.1, 118.9, 118.1, 114.7, 114.2, 66.0, 30.7, 29.3, 20.1, 19.9, 19.2, 18.5; ESI-MS: calcd for $C_{37}H_{36}OP^{+}$ $[M-I]^{+}$ *m/z* 527.25, found 527.2507.

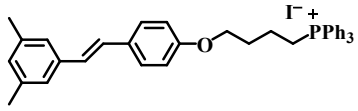


(E)-(4-(4-(3-methylstyryl)phenoxy)butyl)triphenylphosphonium iodide (A3) (yield: 56%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.91-7.89 (m, 3 H), 7.84-7.75 (m, 12 H), 7.52 (d, *J* = 8.4 Hz, 8 H), 7.34 (t, *J* = 10.2 Hz, 2H), 7.26-7.15 (m, 2 H), 7.05 (t, *J* = 11.8 Hz, 2 H), 6.89 (d, *J* = 8.8 Hz, 2 H), 4.06 (t, *J* = 6.0 Hz, 2 H), 2.32 (s, 3 H), 1.93 (t, *J* = 6.6 Hz, 2 H), 1.73 (t, *J* = 7.6 Hz, 2 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.1, 137.7, 137.2, 134.9, 133.6, 133.5, 130.3, 130.2, 129.8, 128.6, 127.9, 127.8, 127.7, 126.6, 126.6, 126.2, 123.5, 118.9, 118.1, 114.7, 67.0, 29.2, 29.0, 21.0, 19.4, 18.5; ESI-MS: calcd for $C_{37}H_{36}OP^{+}$ $[M-I]^{+}$ *m/z* 527.25, found 527.2505.

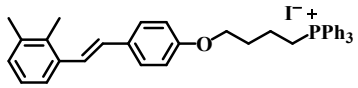


(E)-(4-(4-(3,4-dimethylstyryl)phenoxy)butyl)triphenylphosphonium iodide (A4) (yield: 54%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.93-7.88 (m, 3 H), 7.85-7.74 (m, 12 H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 7.6 Hz, 1 H), 7.13 (d, *J* = 9.2 Hz, 1 H), 7.01 (s, 1 H), 7.0 (d, *J* =

16.4 Hz,1 H), 6.89 (d, *J* = 8.4 Hz, 2 H), 4.06 (t, *J* = 6.2 Hz, 2 H), 3.72 (d, *J* = 7.6 Hz, 2 H), 2.23 (s, 3 H), 2.20 (s, 2 H), 1.95-1.90 (m, 2 H), 1.75-1.70 (m, 2 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.0, 136.3, 135.3, 134.9, 133.6, 133.5, 130.3, 130.2, 129.9, 129.7, 127.5, 127.2, 126.7, 126.2, 123.7, 118.7, 118.0, 114.7, 66.0, 30.7, 29.2, 29.0, 20.0, 19.4, 19.1, 18.5; ESI-MS: calcd for $C_{38}H_{38}OP^+$ $[M-I]^+$ *m/z* 541.27, found 541.2656.



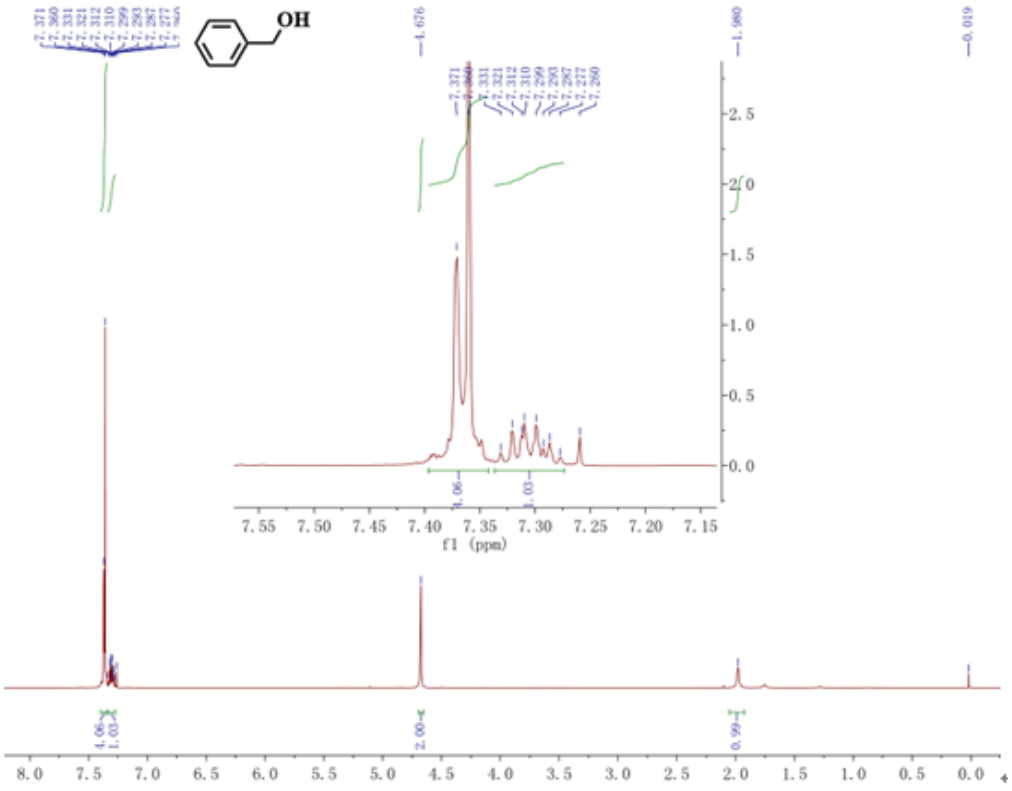
(E)-(4-(4-(3,5-dimethylstyryl)phenoxy)butyl)triphenylphosphonium iodide (A5) (yield: 58%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.91-7.89 (m, 3 H), 7.84-7.76 (m, 12 H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 14.8 Hz, 3 H), 7.0 (d, *J* = 16.4 Hz, 1 H), 6.88 (t, *J* = 6 Hz, 3 H), 4.06 (t, *J* = 6.0, 2 H), 3.71-3.63 (m, 2 H), 2.27 (s, 6 H), 1.93 (t, *J* = 6.6 Hz, 2 H) , 1.73 (t, *J* = 7.4 Hz, 2 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.0, 137.5, 137.2, 134.9, 133.6, 133.5, 130.3, 130.2, 129.9, 128.9, 128.8, 128.2, 127.7, 127.6, 126.3, 124.0, 118.9, 118.0,114.8, 66.0, 29.2, 29.0, 20.9, 20.0, 19.5, 18.5; ESI-MS: calcd for $C_{38}H_{38}OP^+$ $[M-I]^+$ *m/z* 541.27, found 541.2657.

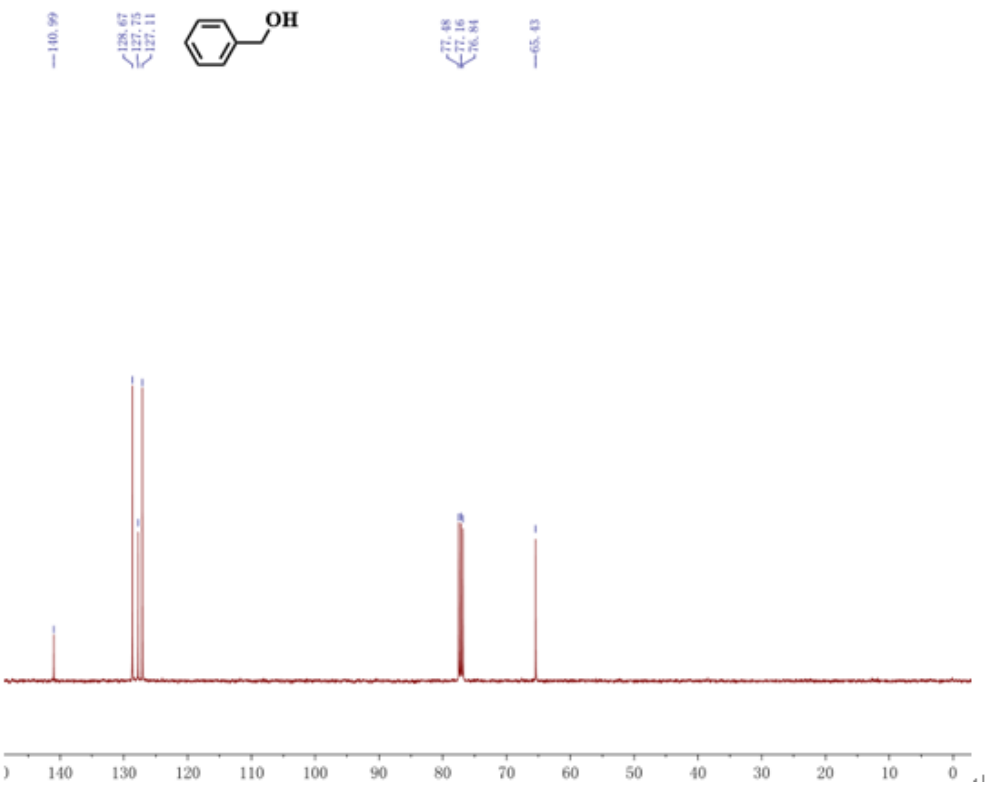


(E)-(4-(4-(2,3-dimethylstyryl)phenoxy)butyl)triphenylphosphonium iodide (A6) (yield: 63%) **^{1}H-NMR** (400 MHz, DMSO-d_6): δ = 7.93-7.89 (m, 3 H), 7.85-7.76 (m, 12 H), 7.85-7.74 (m, 12 H), 7.55 (d, *J* = 8.8 Hz, 2 H), 7.42 (d, *J* = 6.4 Hz, 1 H), 7.33 (d, *J* = 16.4 Hz, 1H), 7.06 (t, *J* = 3.2 Hz, 2 H), 6.96 (d, *J* = 16 Hz, 1 H), 6.95 (d, *J* = 8.8 Hz, 2 H), 4.06 (t, *J* = 6.0 Hz, 2 H), 3.74-3.67 (m, 2 H), 2.27 (s, 3 H), 2.25 (s, 3 H), 1.96-1.91 (m, 2 H), 1.73 (t, *J* = 7.4 Hz, 2 H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ=158.0, 136.4, 136.3, 134.9, 133.7, 133.6, 133.5,

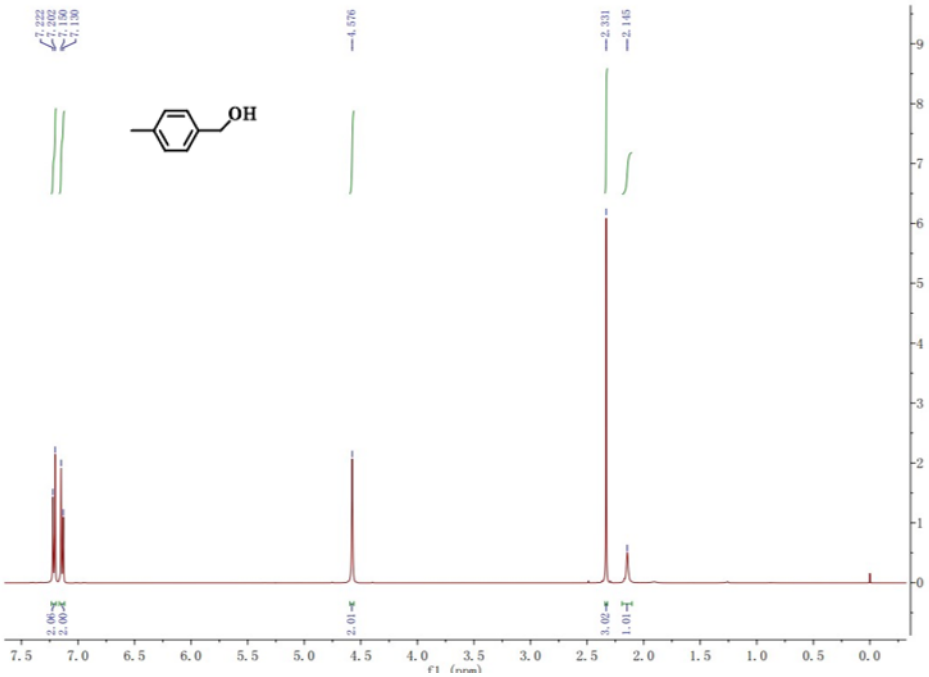
130.3, 130.2, 130.1, 129.5, 128.7, 127.8, 125.4, 124.6, 123.2, 118.9, 118.0, 114.7, 66.0, 20.4, 20.0, 19.5, 18.5, 15.1, 20.0, 19.5, 18.5, 18.4; ESI-MS: calcd for C38H38OP+ [M-I]+ m/z 541.27, found 541.2657.

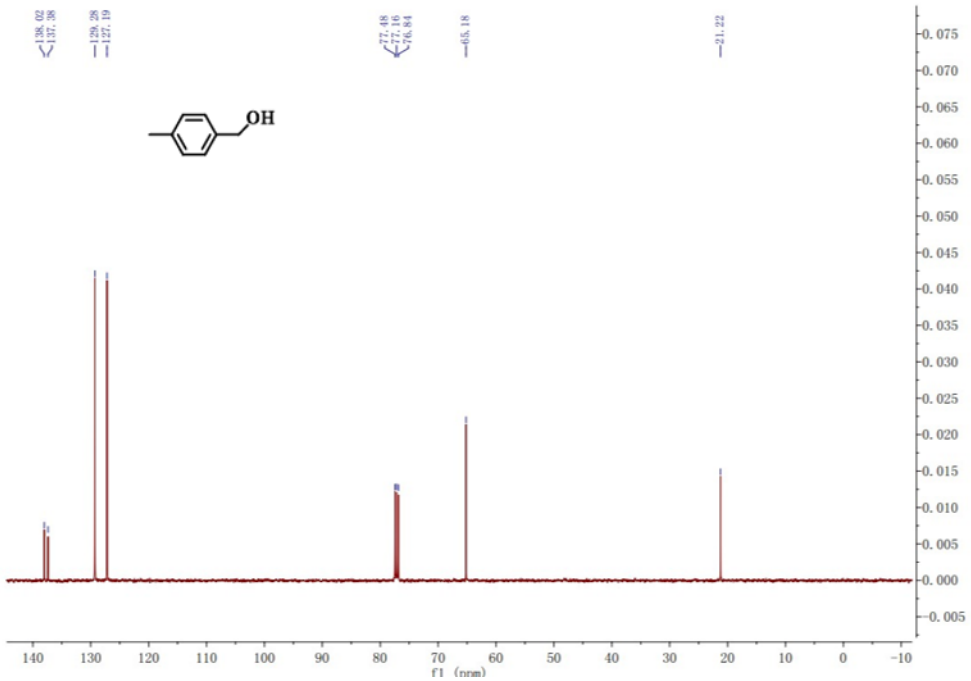
^{1}H NMR and ^{13}C NMR of compound C1



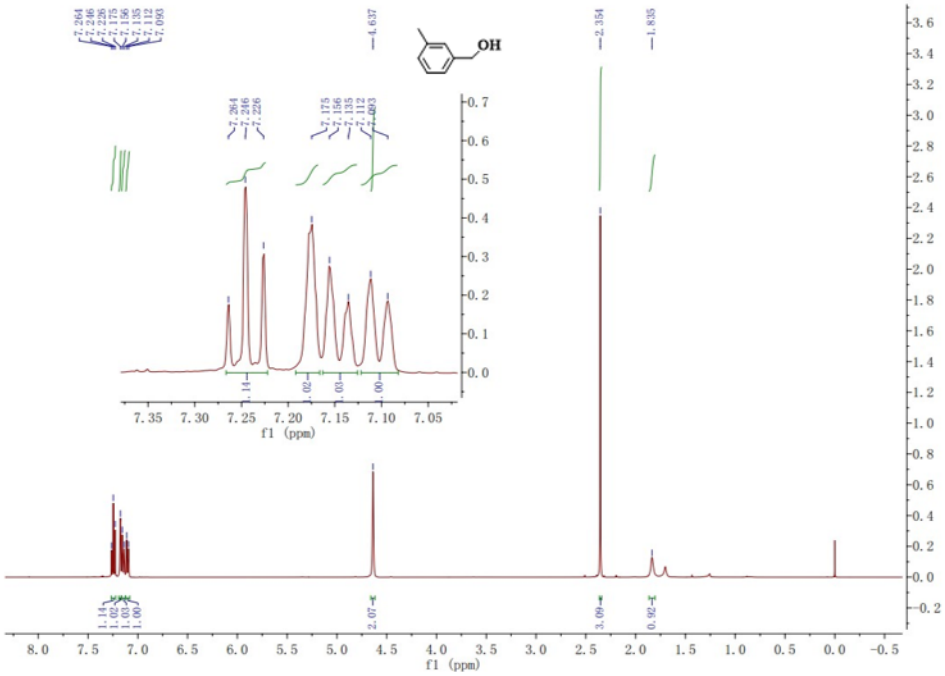


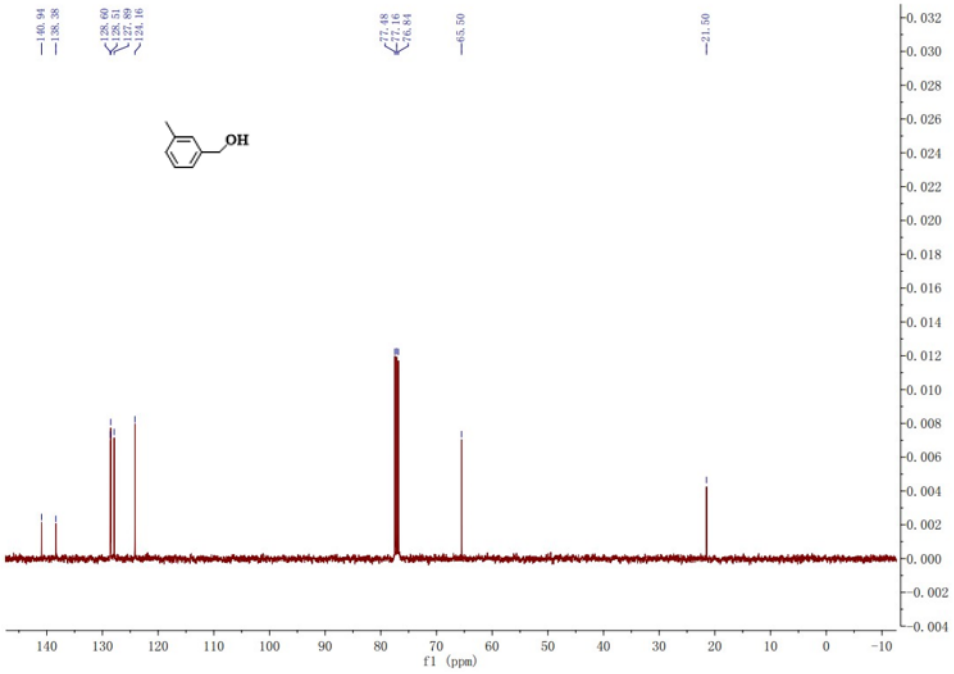
^{1}H NMR and ^{13}C NMR of compound C2



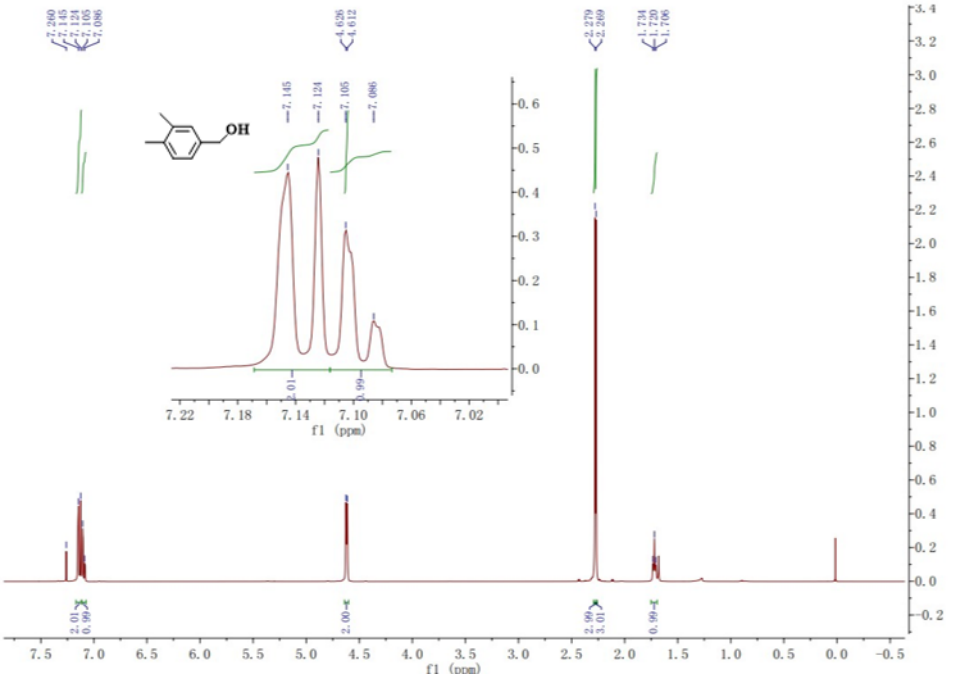


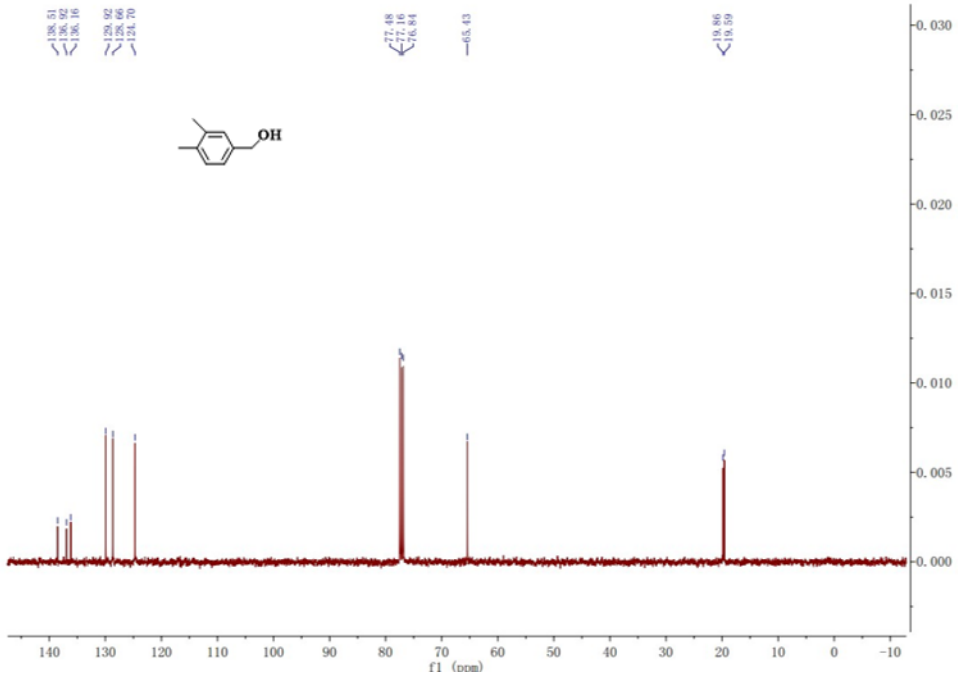
^{1}H NMR and ^{13}C NMR of compound C3



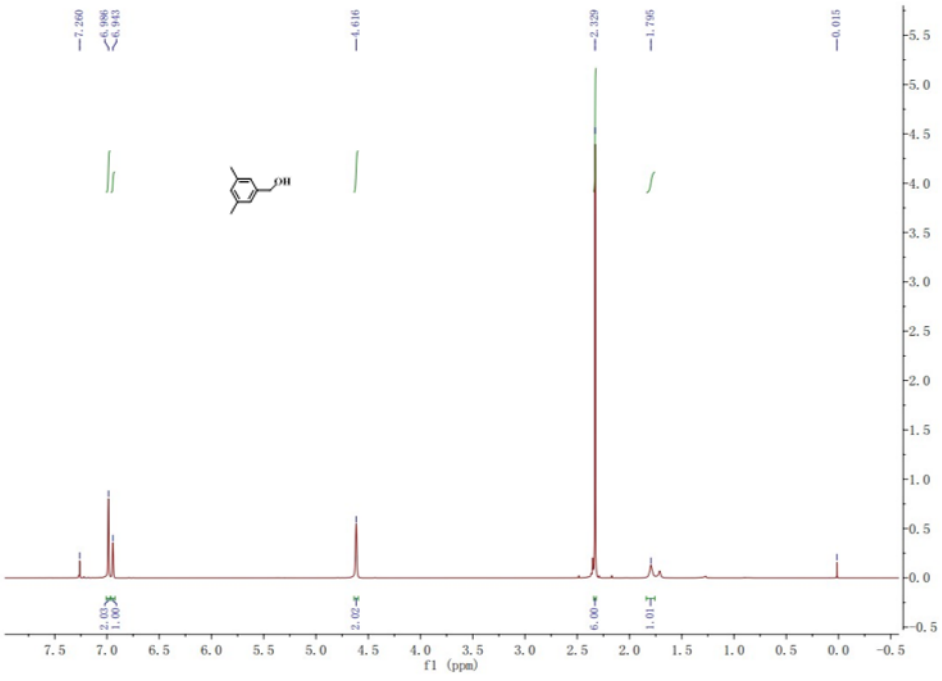


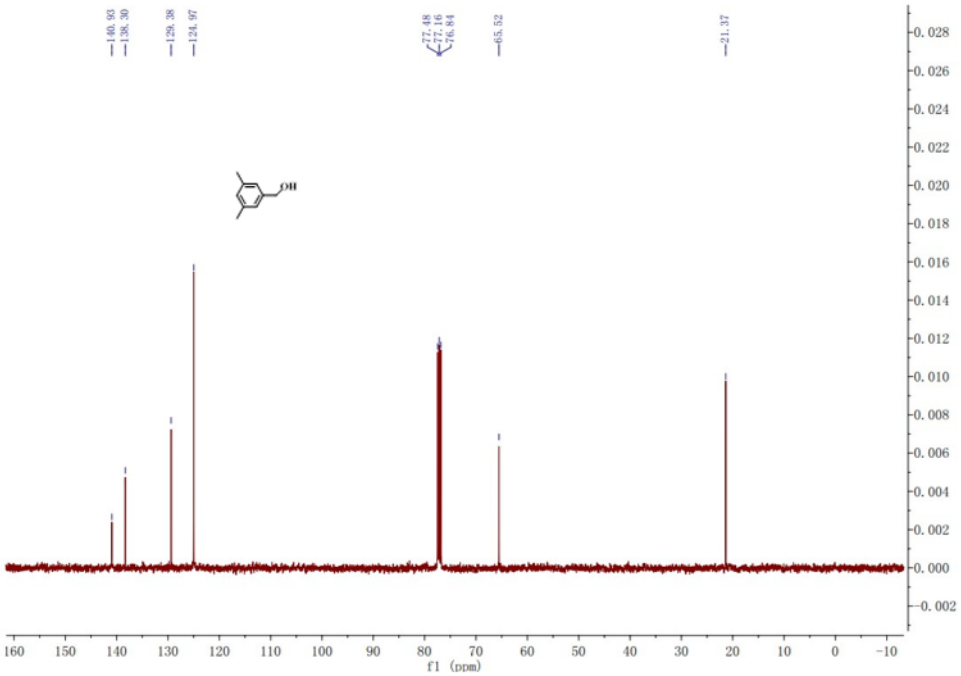
^{1}H NMR and ^{13}C NMR of compound C4



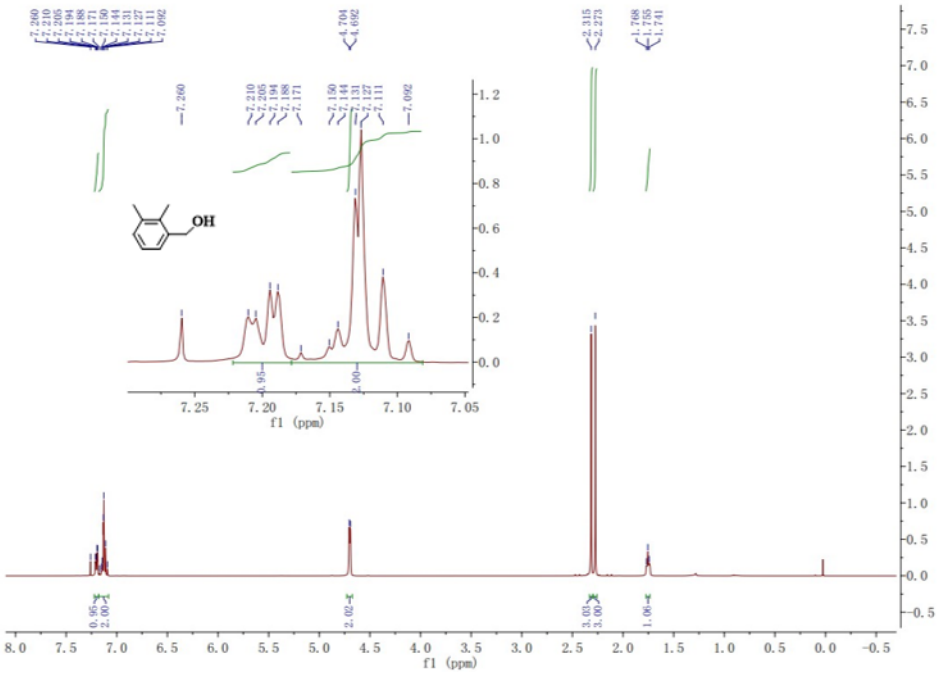


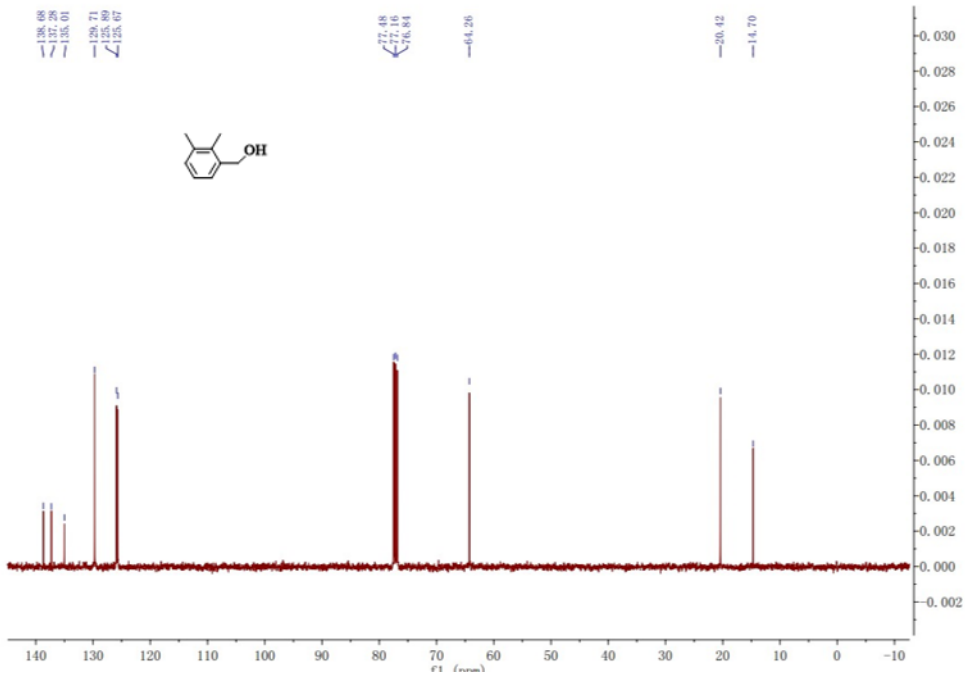
^{1}H NMR and ^{13}C NMR of compound C5



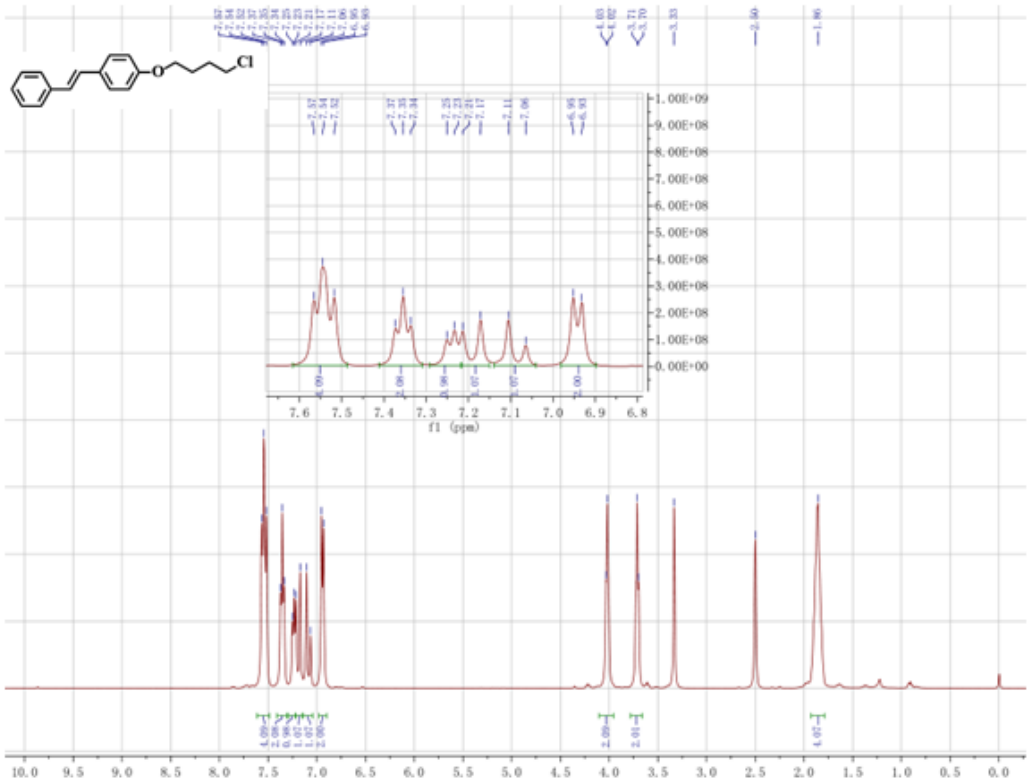


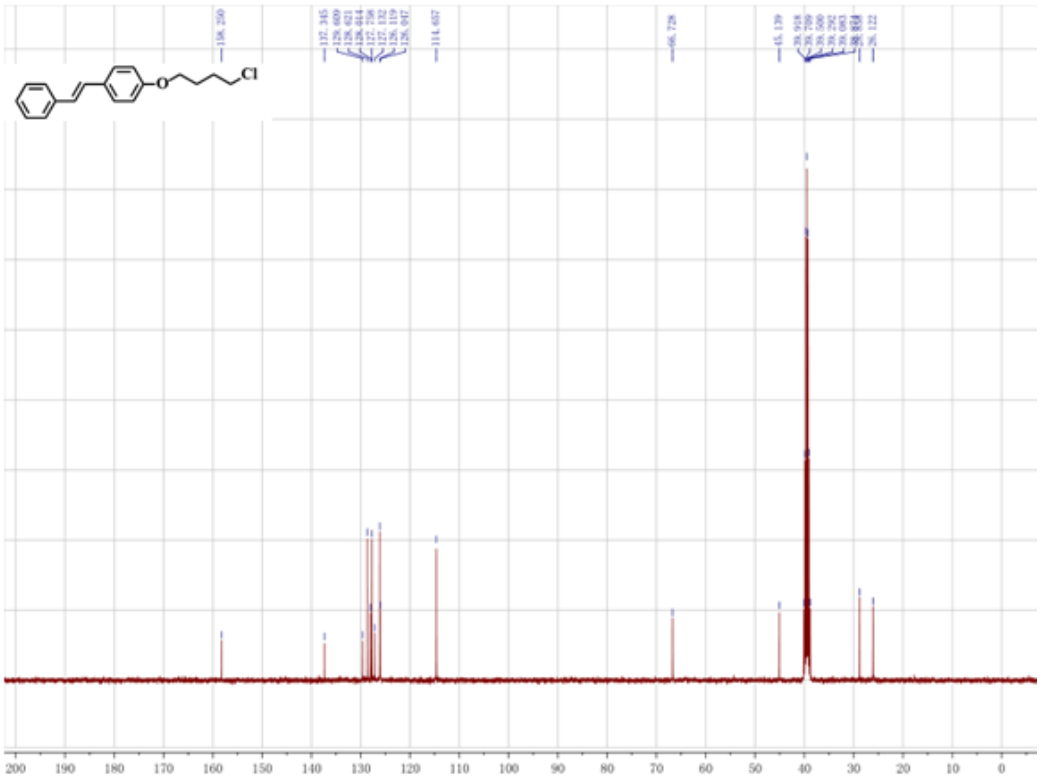
^{1}H NMR and ^{13}C NMR of compound C6



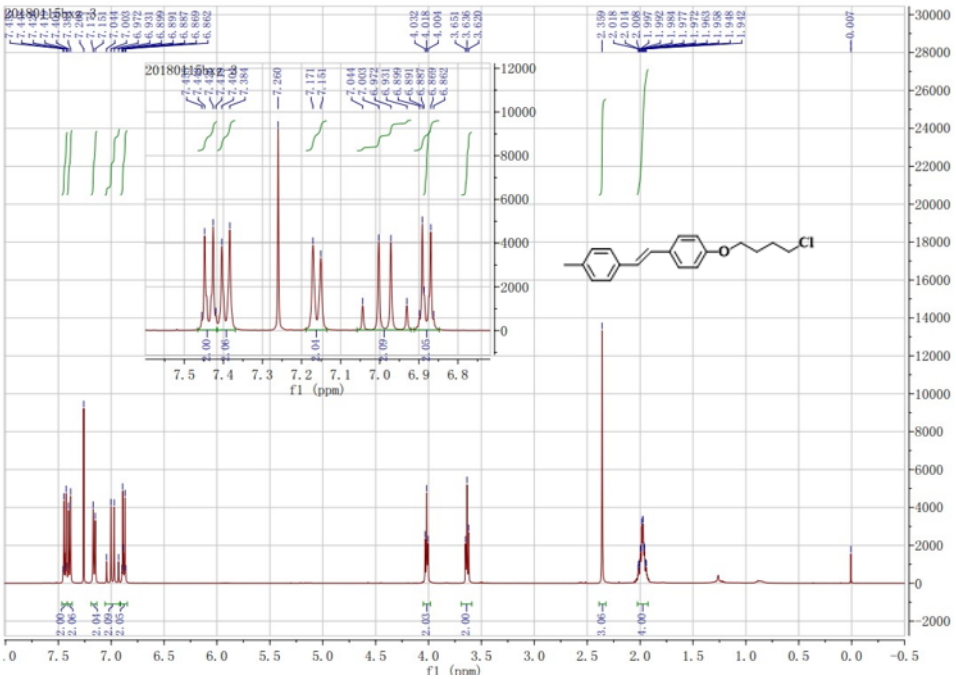


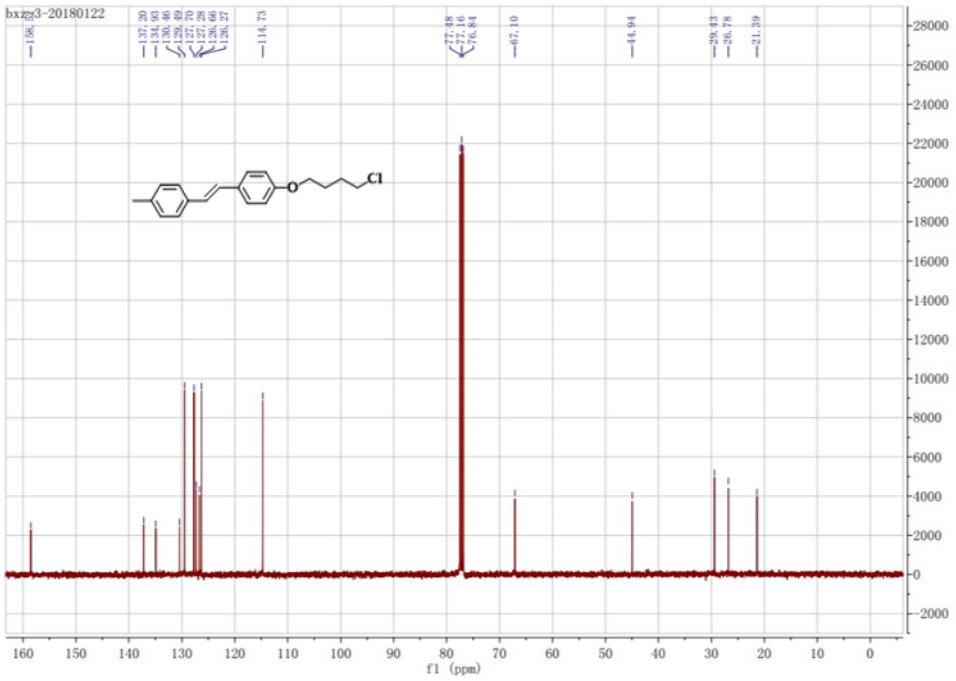
^{1}H NMR and ^{13}C NMR of compound F1



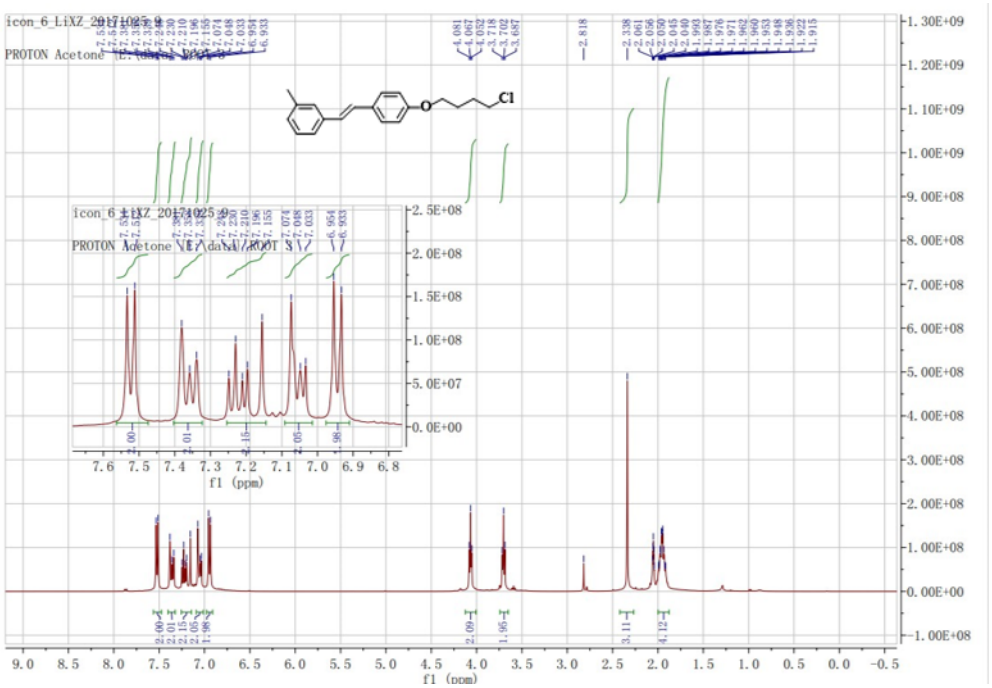


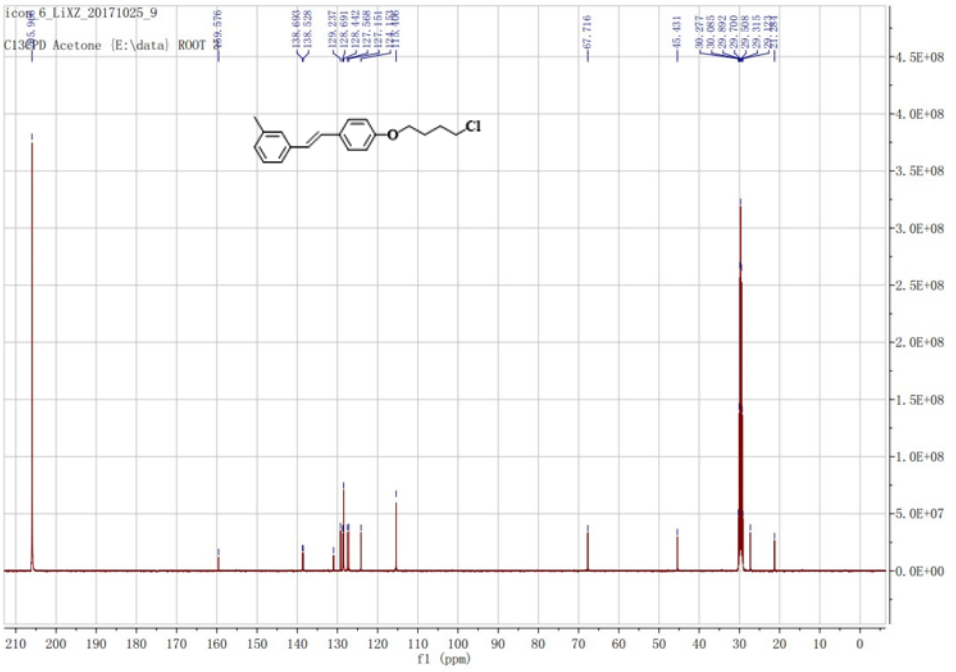
^{1}H NMR and ^{13}C NMR of compound F2



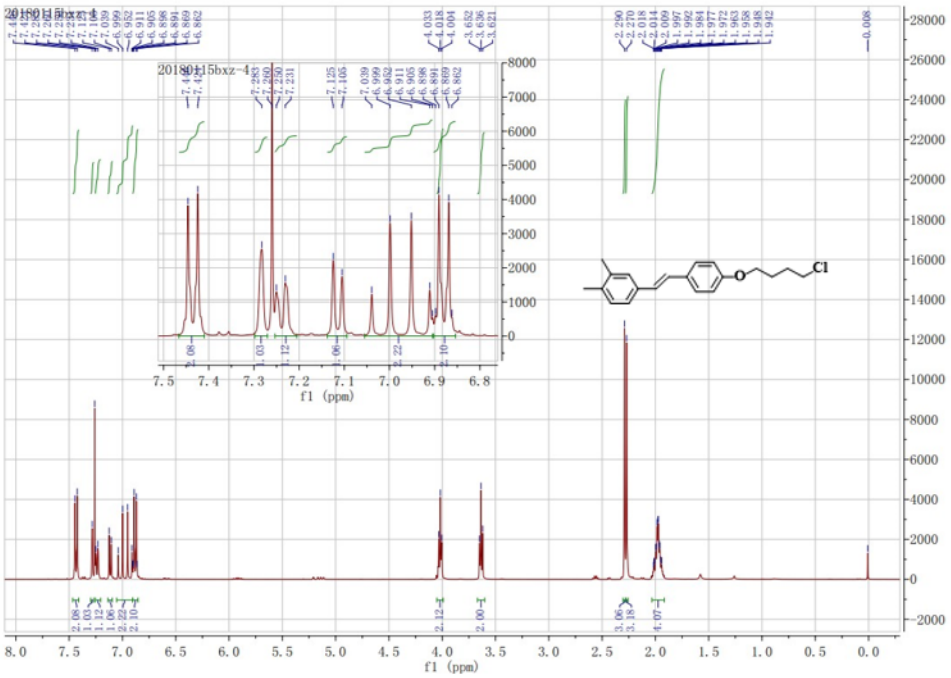


^{1}H NMR and ^{13}C NMR of compound F3





^{1}H NMR and ^{13}C NMR of compound F4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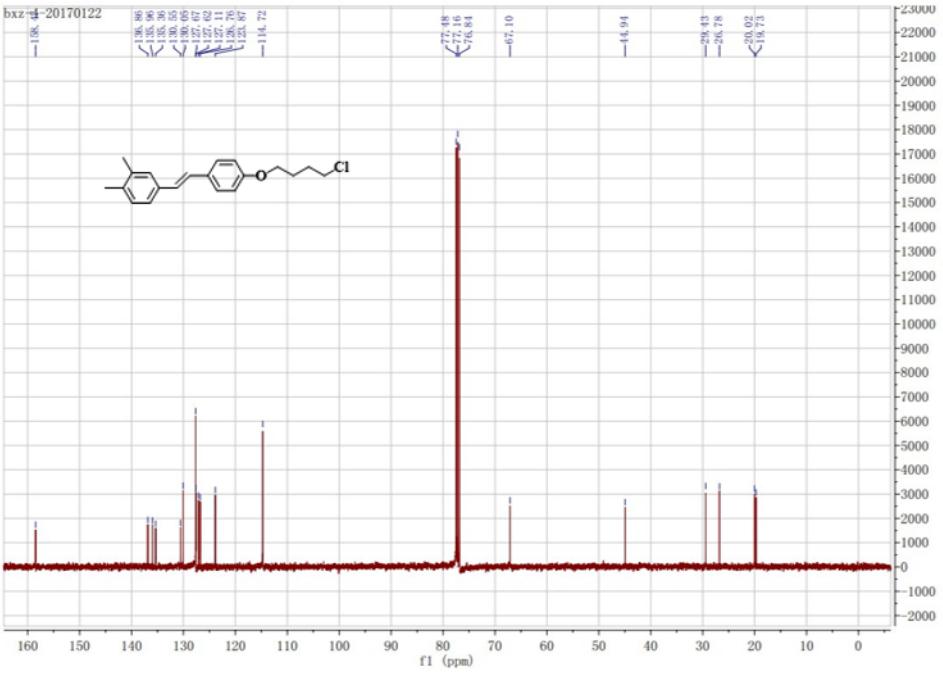
Cl
O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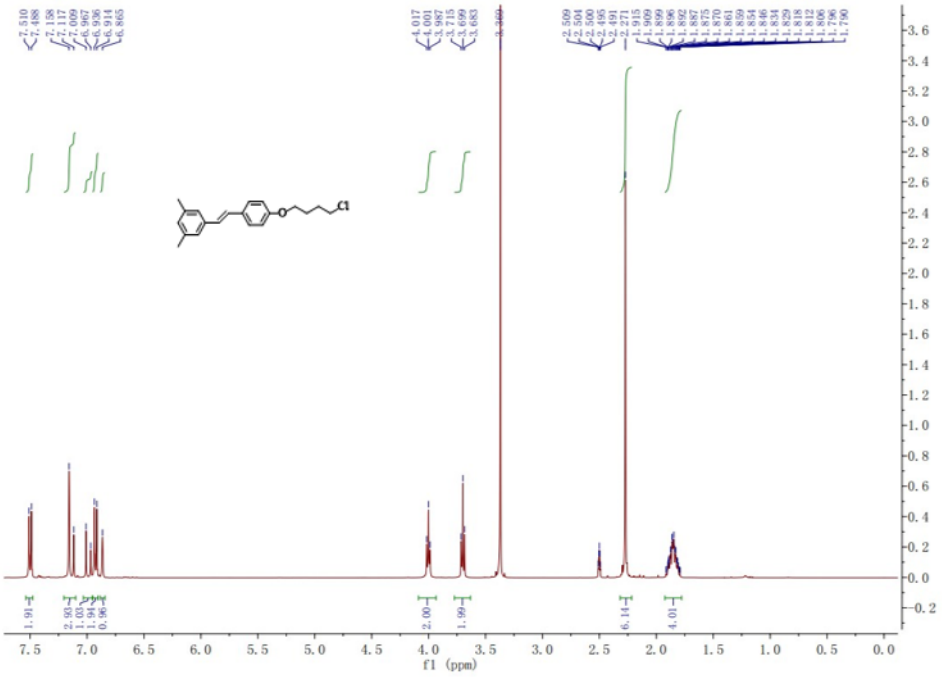
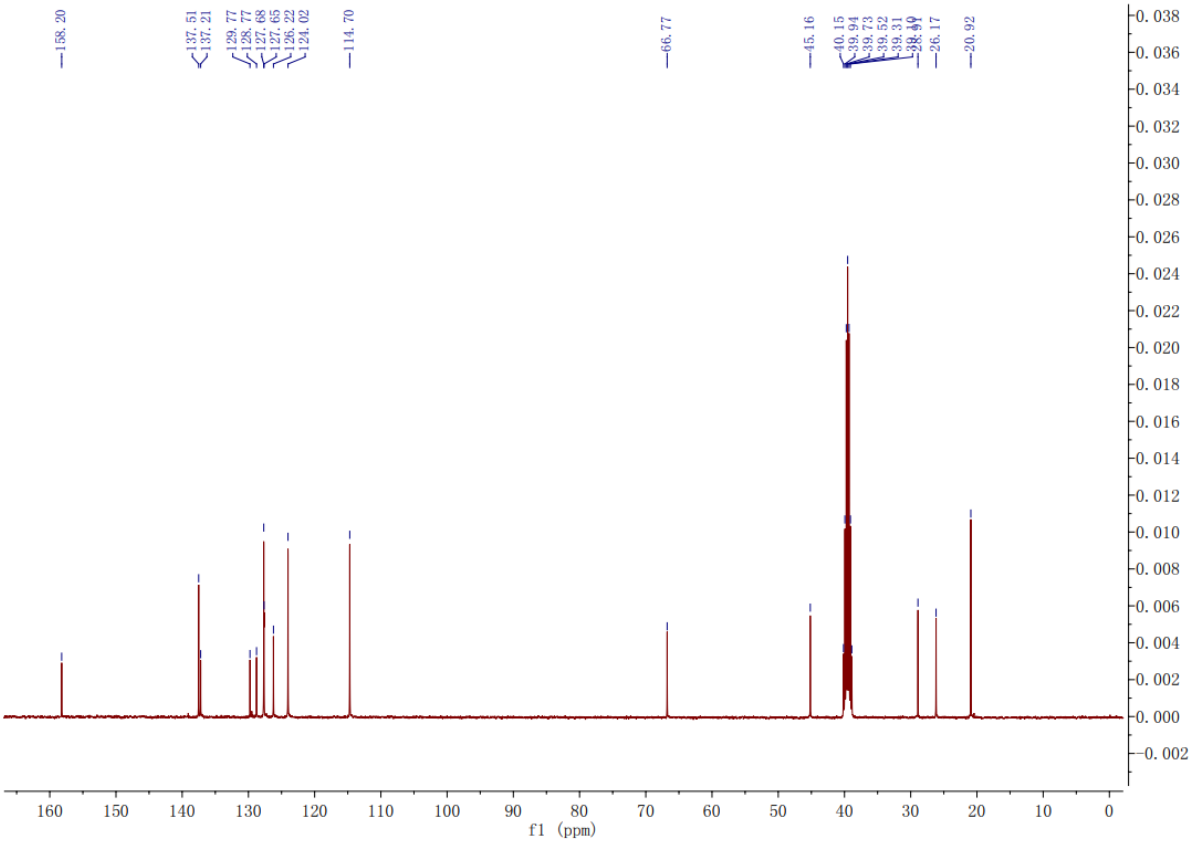
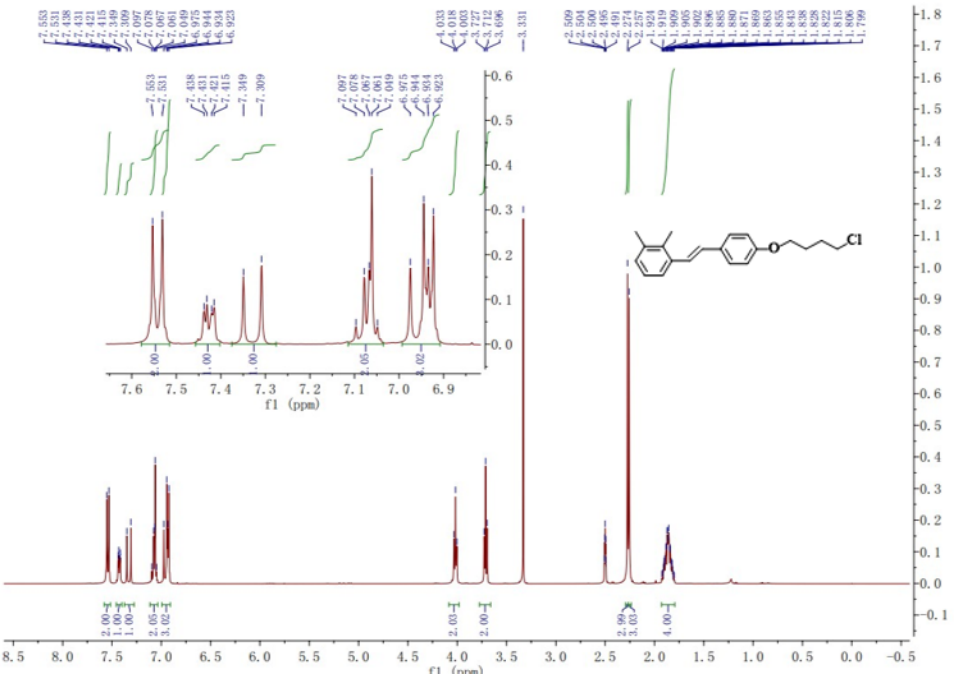
f1 (ppm)
Cl
O

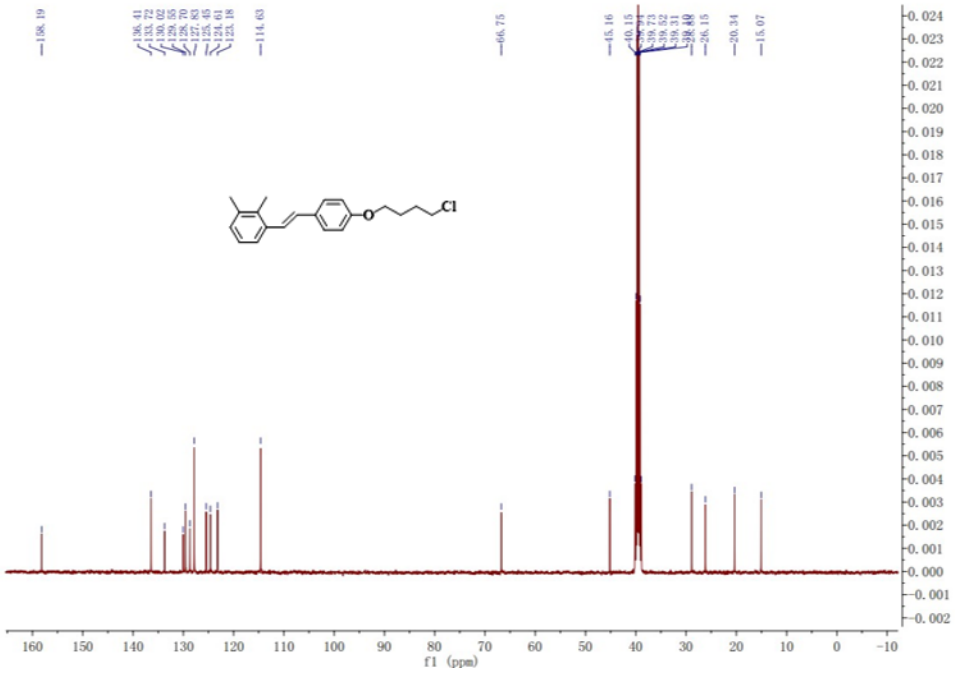
^{1}H NMR and ^{13}C NMR of compound F5



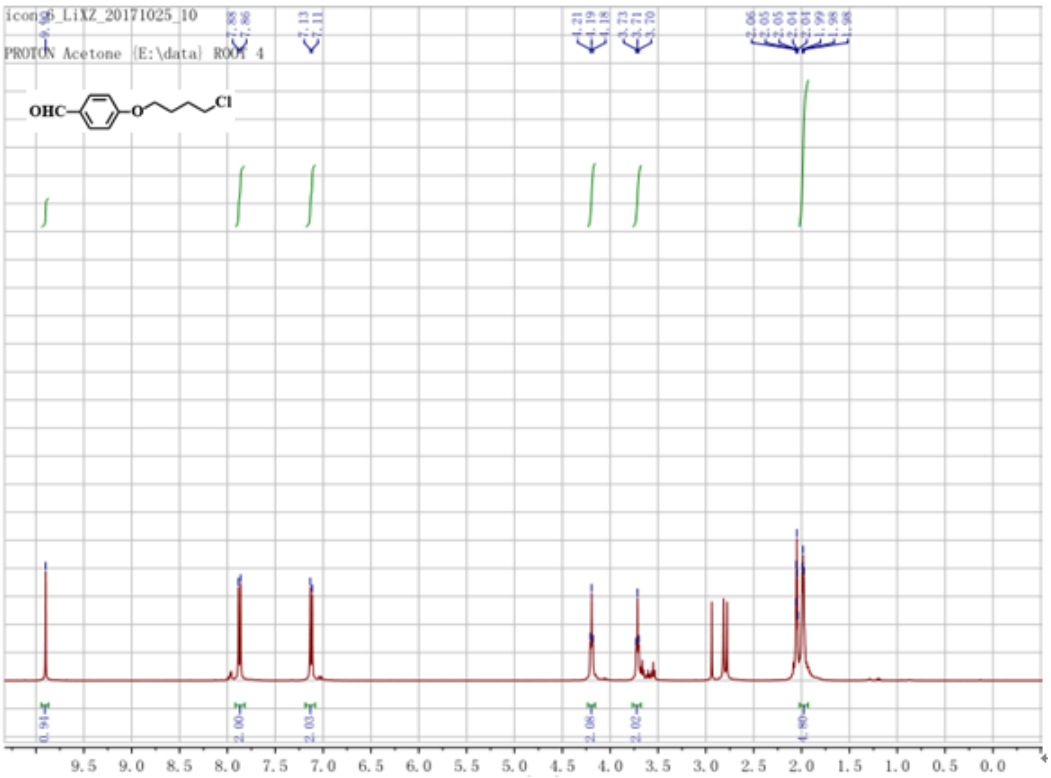


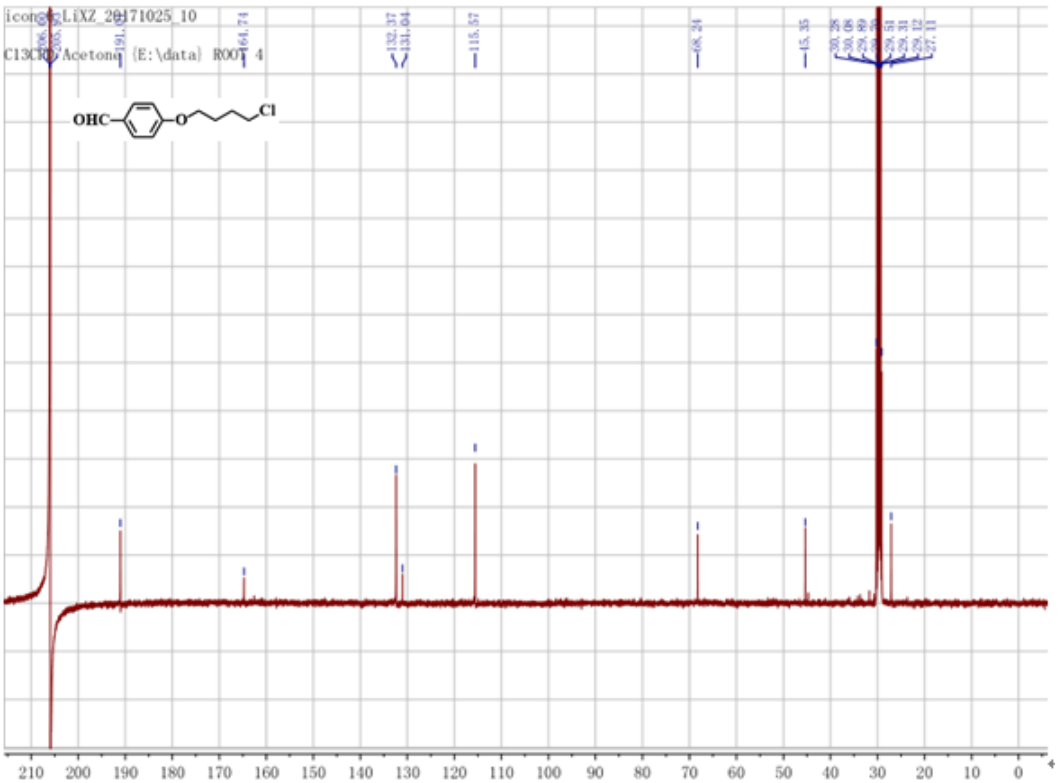
^{1}H NMR and ^{13}C NMR of compound F6



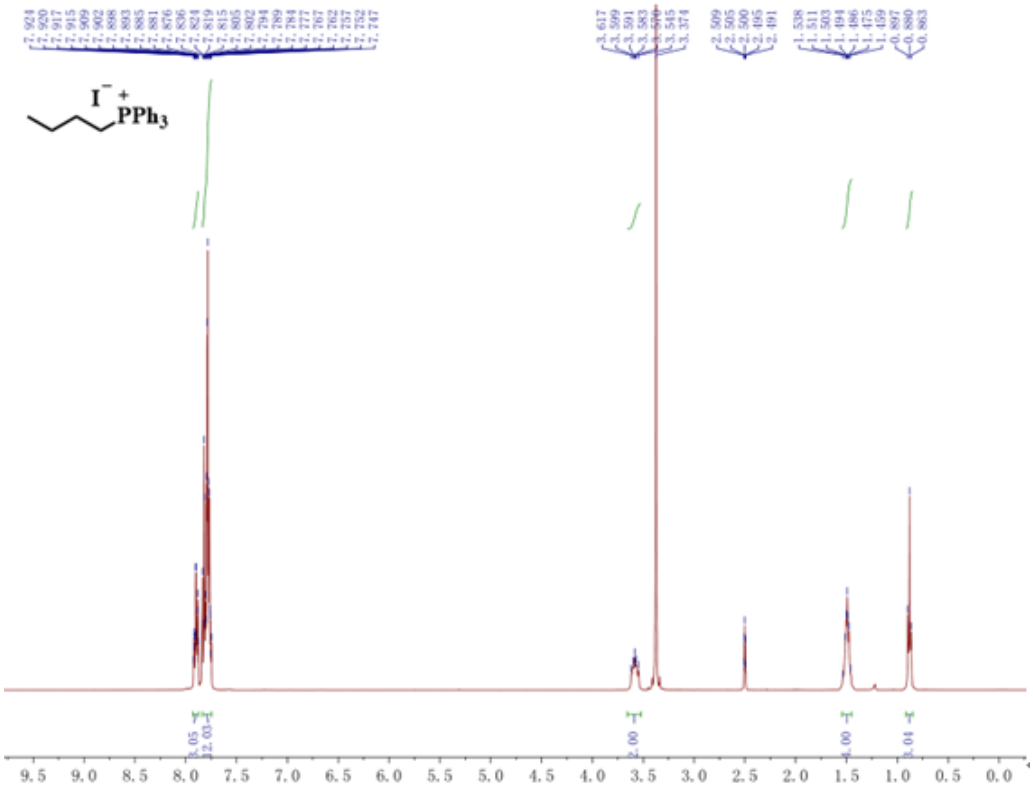


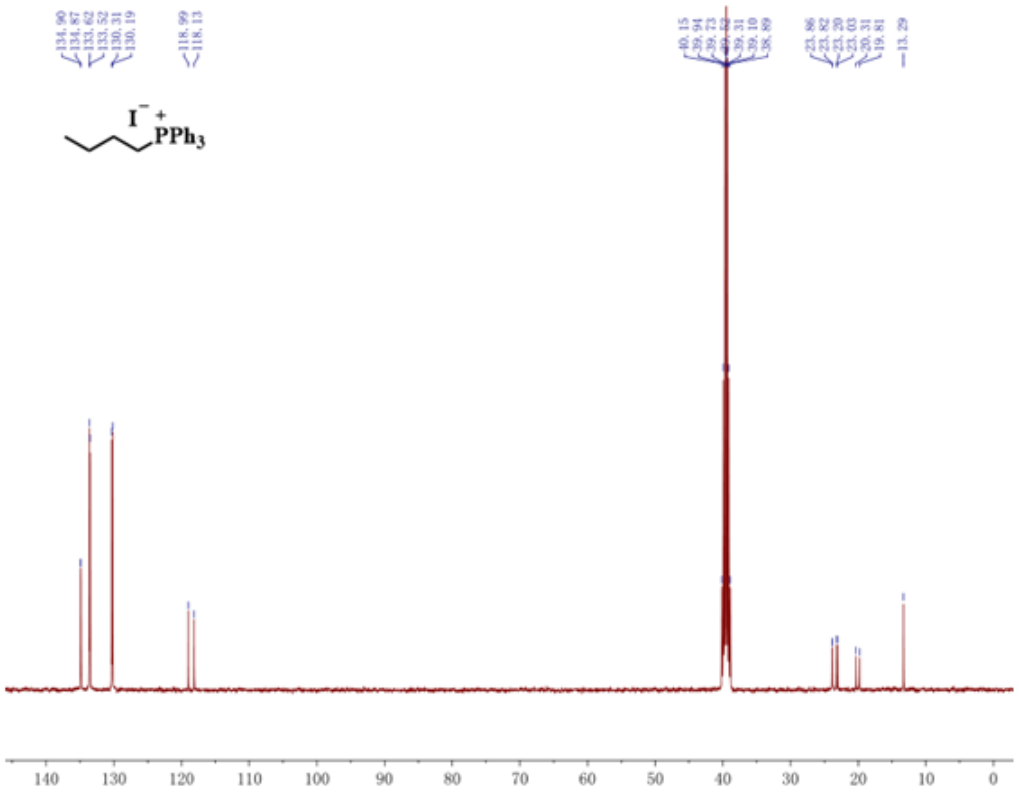
^{1}H NMR and ^{13}C NMR of compound Z



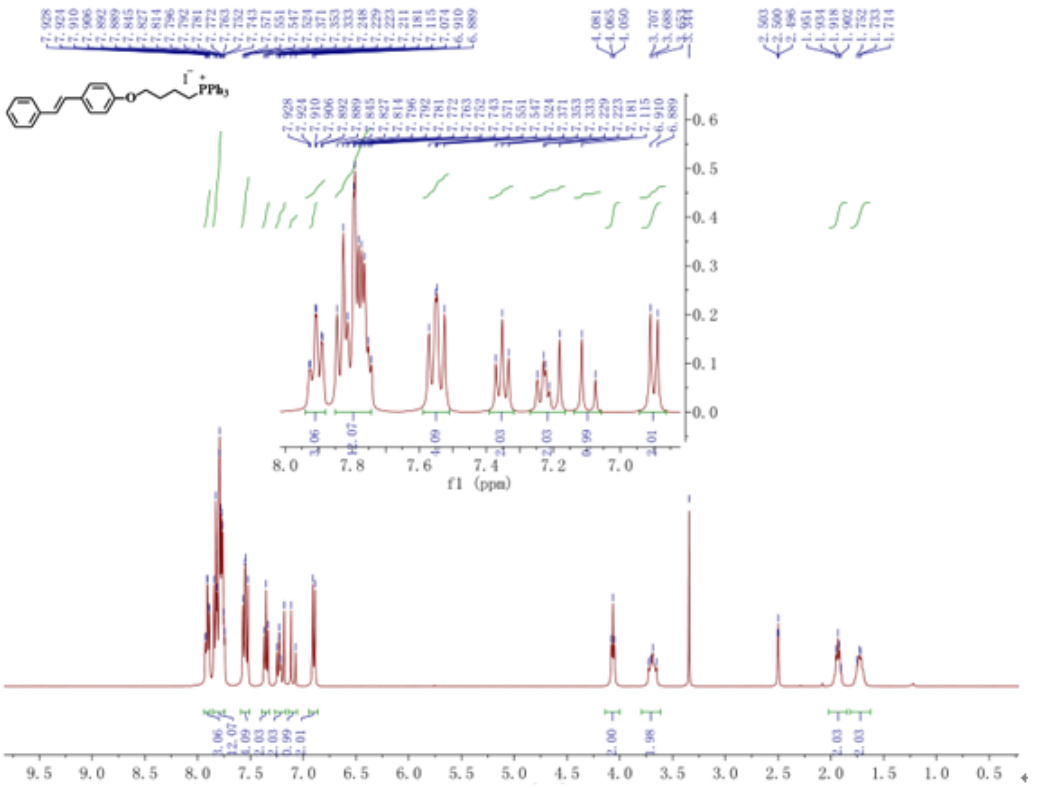


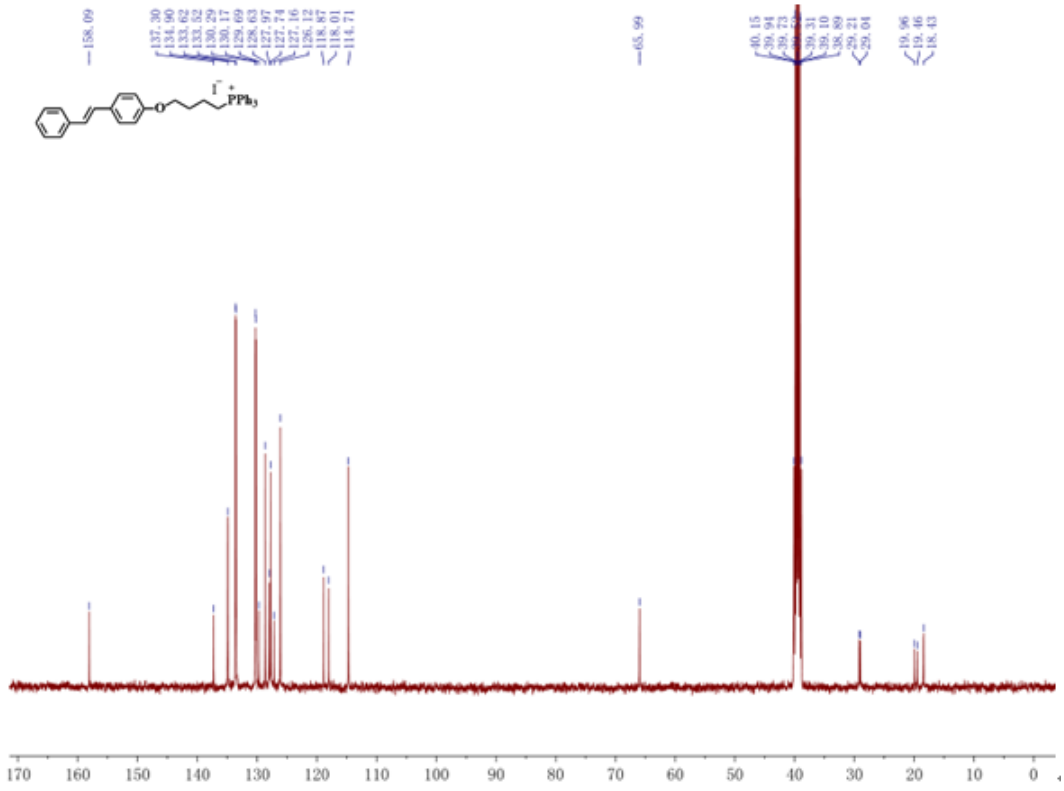
^{1}H NMR and ^{13}C NMR of compound A0



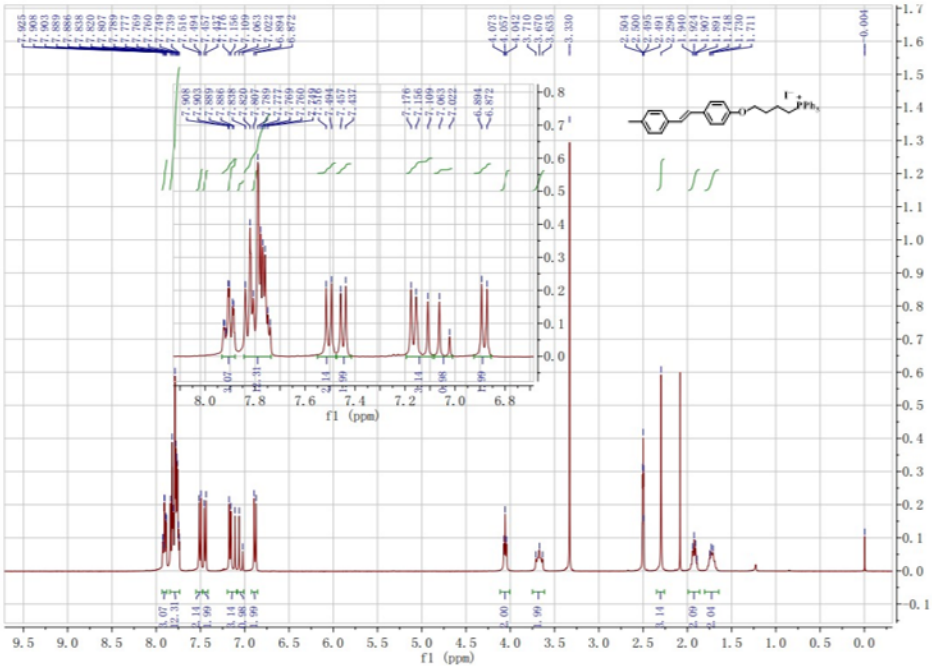


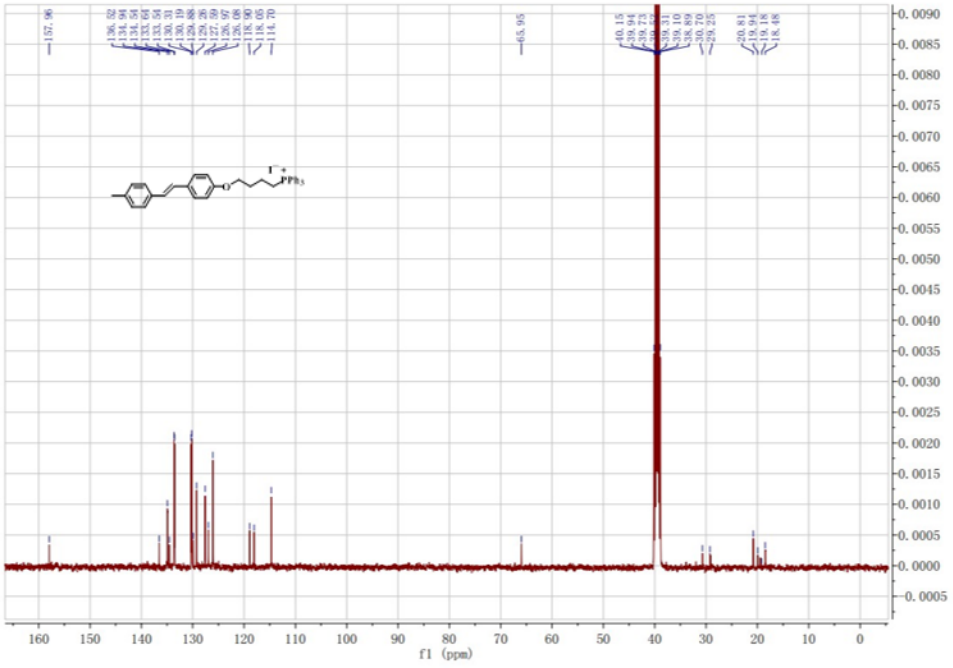
^{1}H NMR and ^{13}C NMR of compound A1



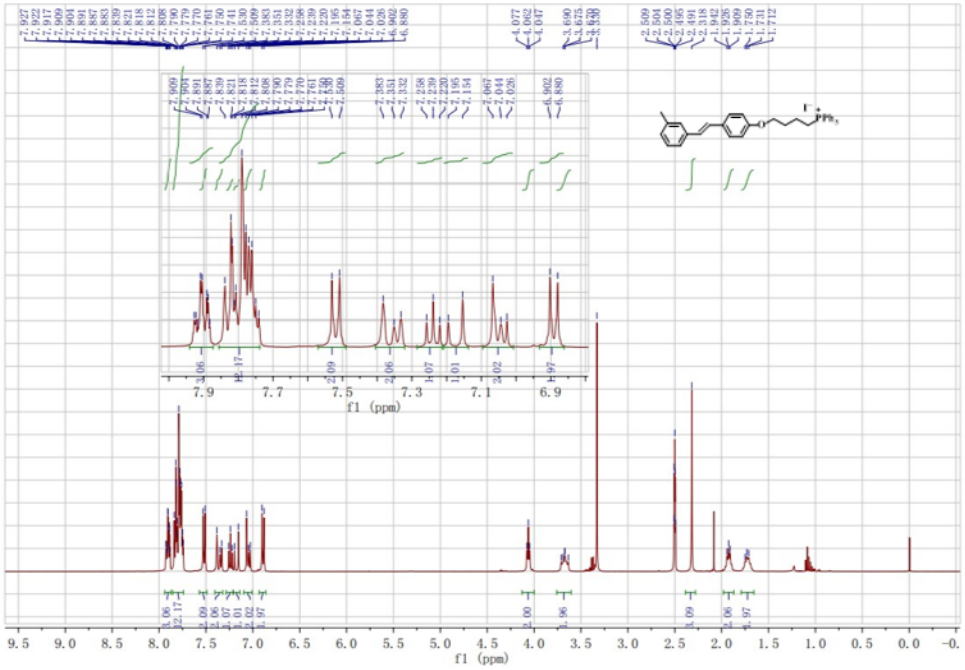


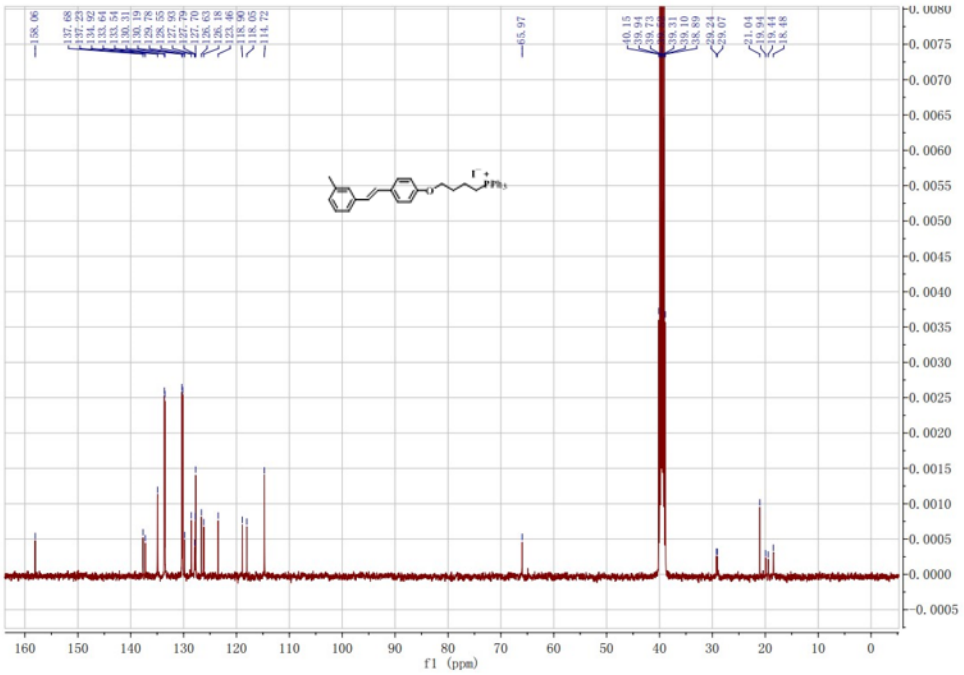
^{1}H NMR and ^{13}C NMR of compound A2



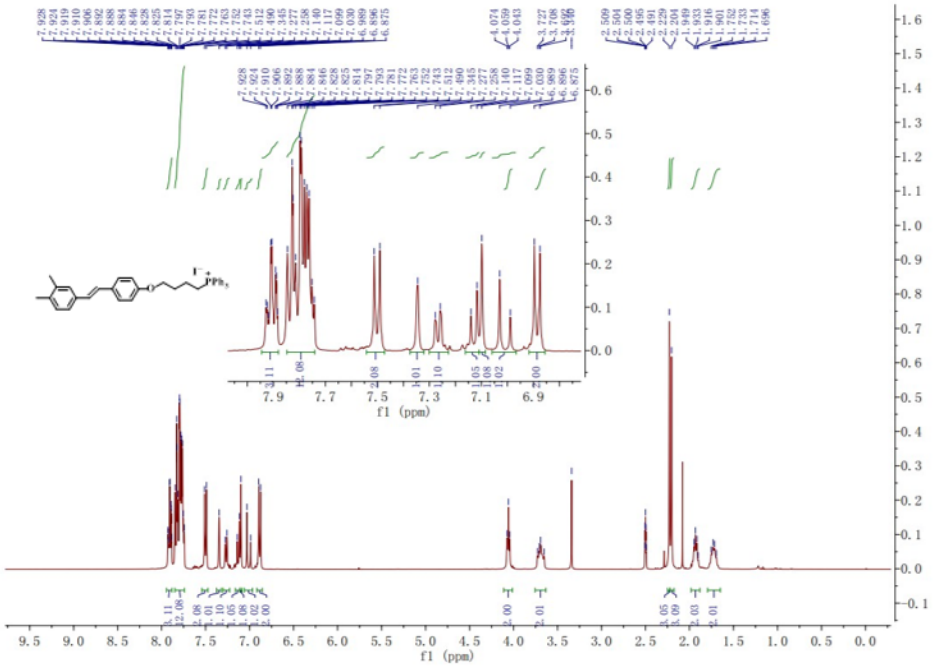


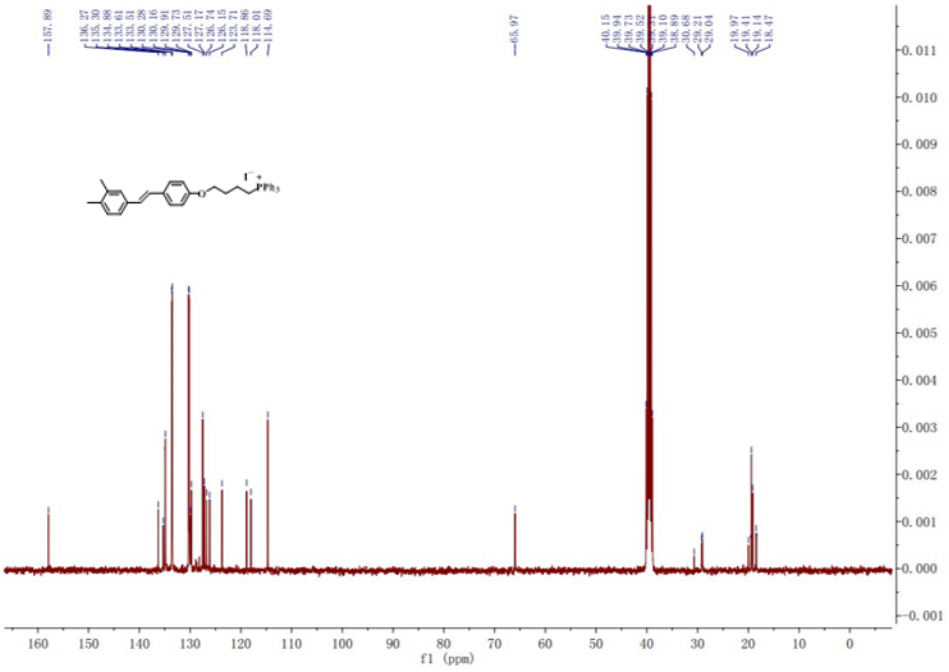
^{1}H NMR and ^{13}C NMR of compound A3



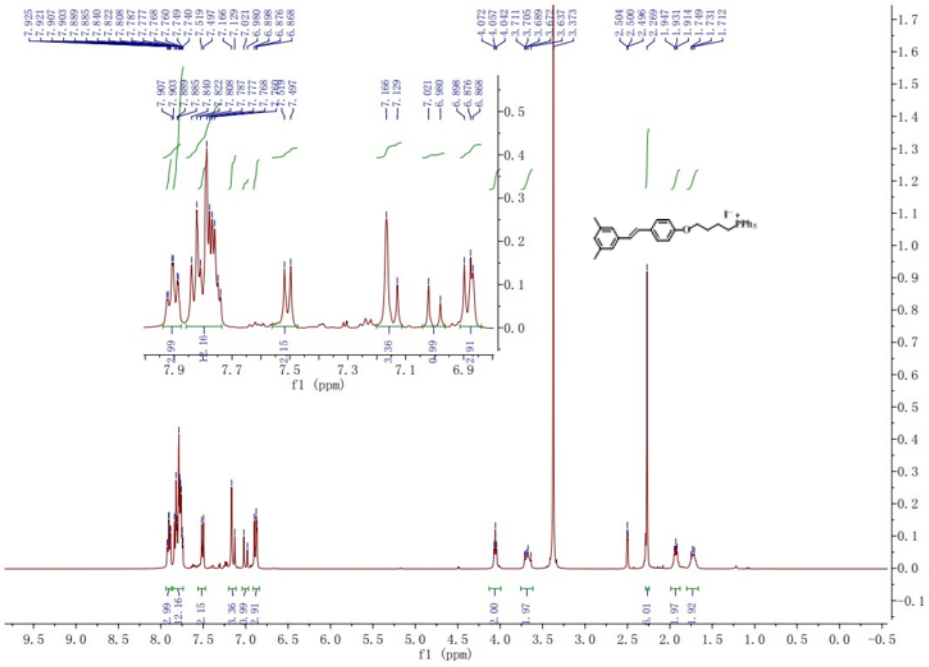


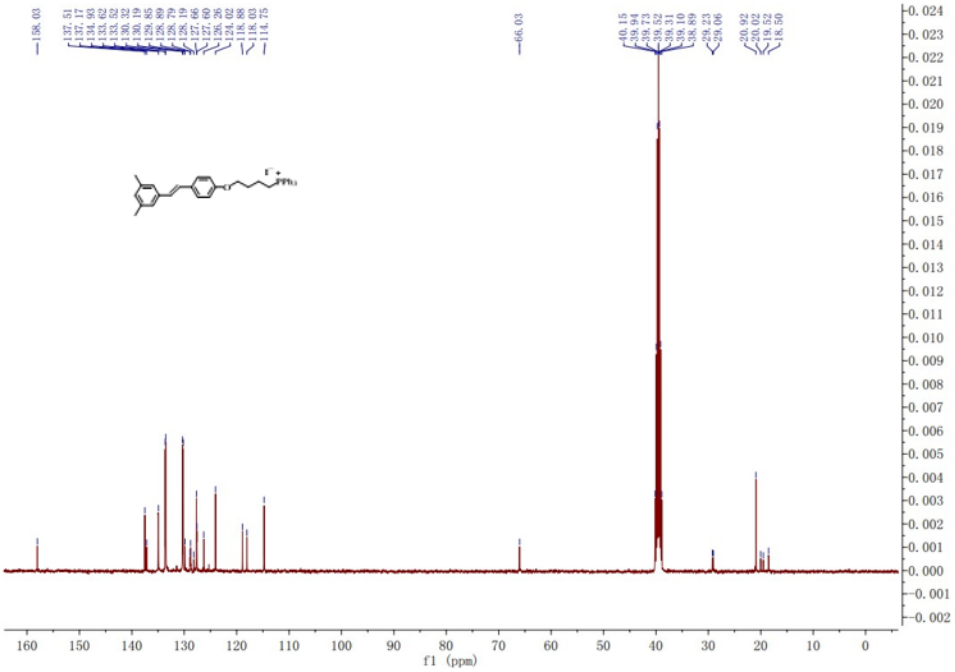
^{1}H NMR and ^{13}C NMR of compound A4



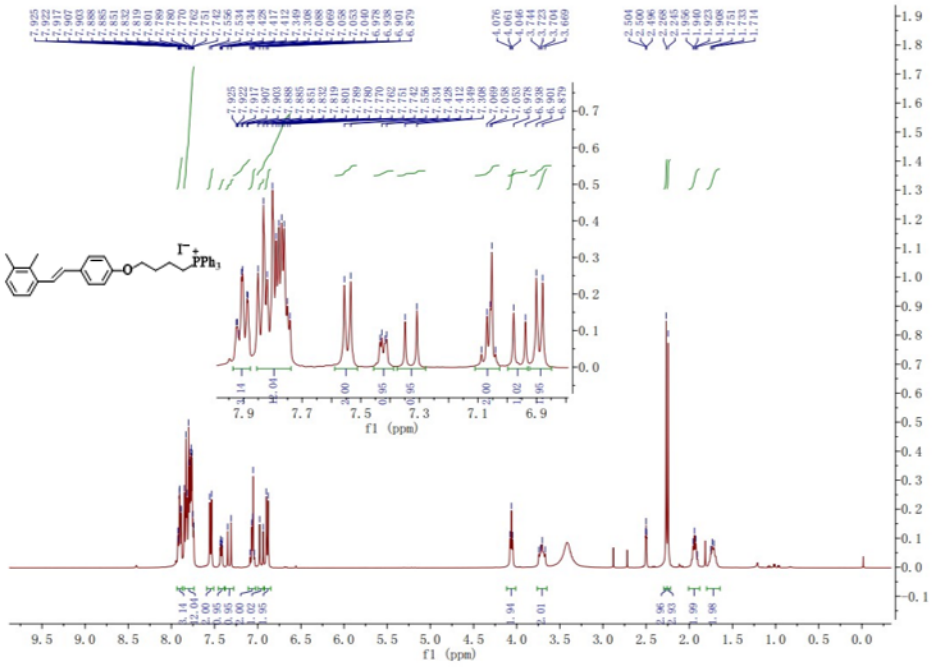


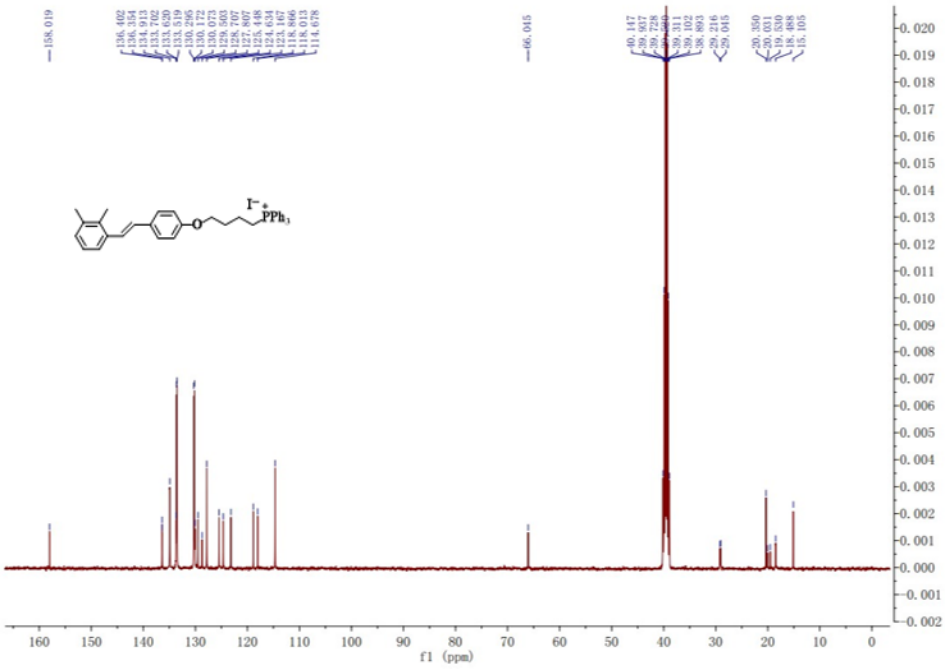
^{1}H NMR and ^{13}C NMR of compound A5



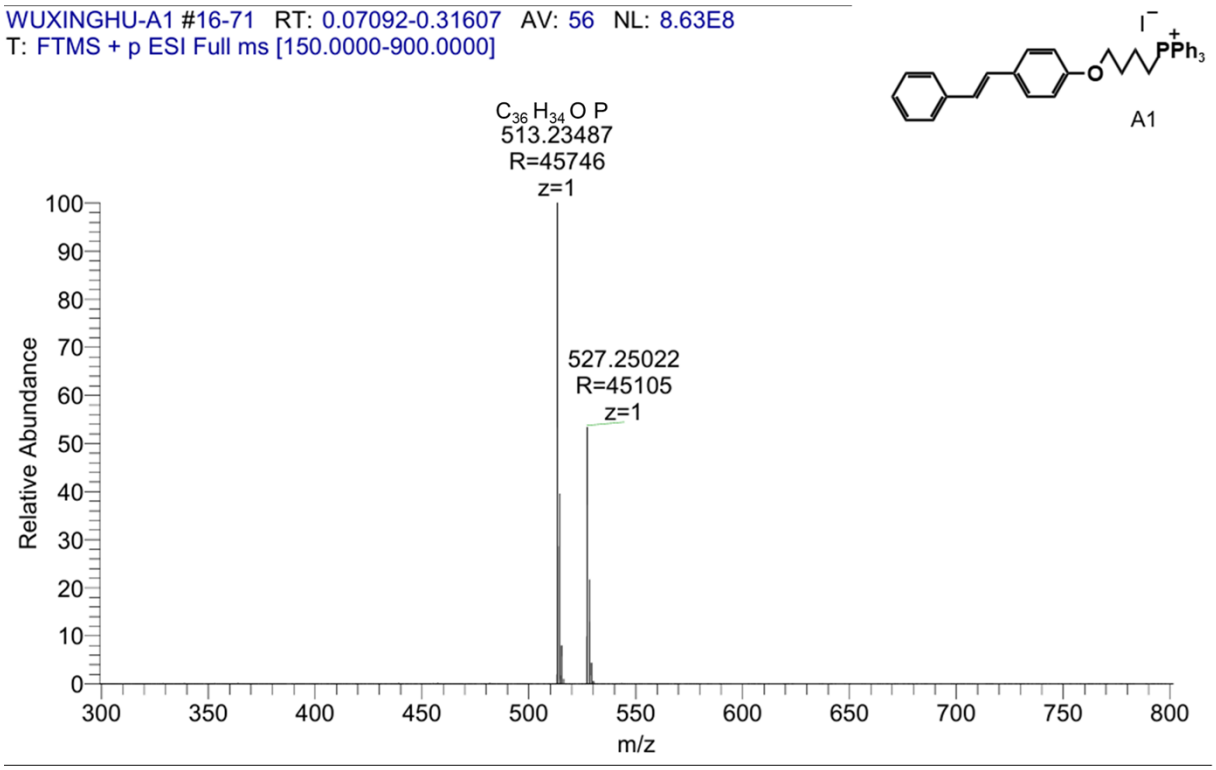


^{1}H NMR and ^{13}C NMR of compound A6

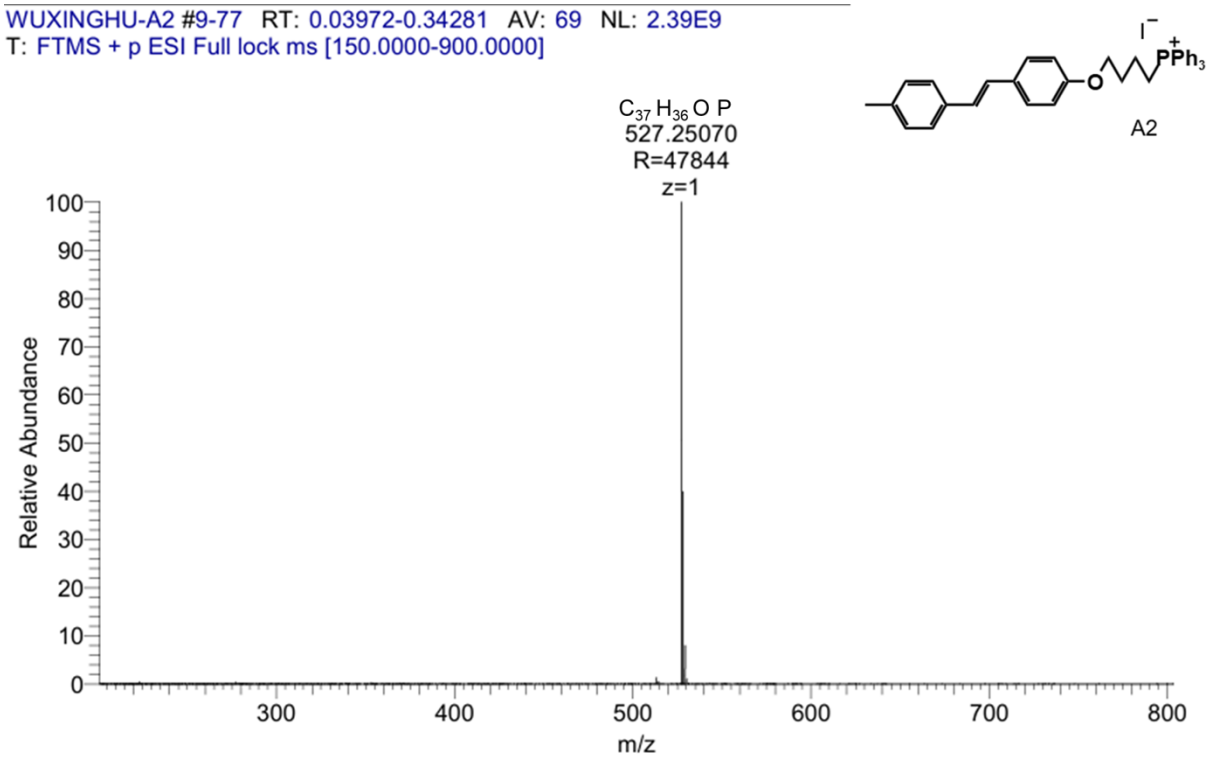




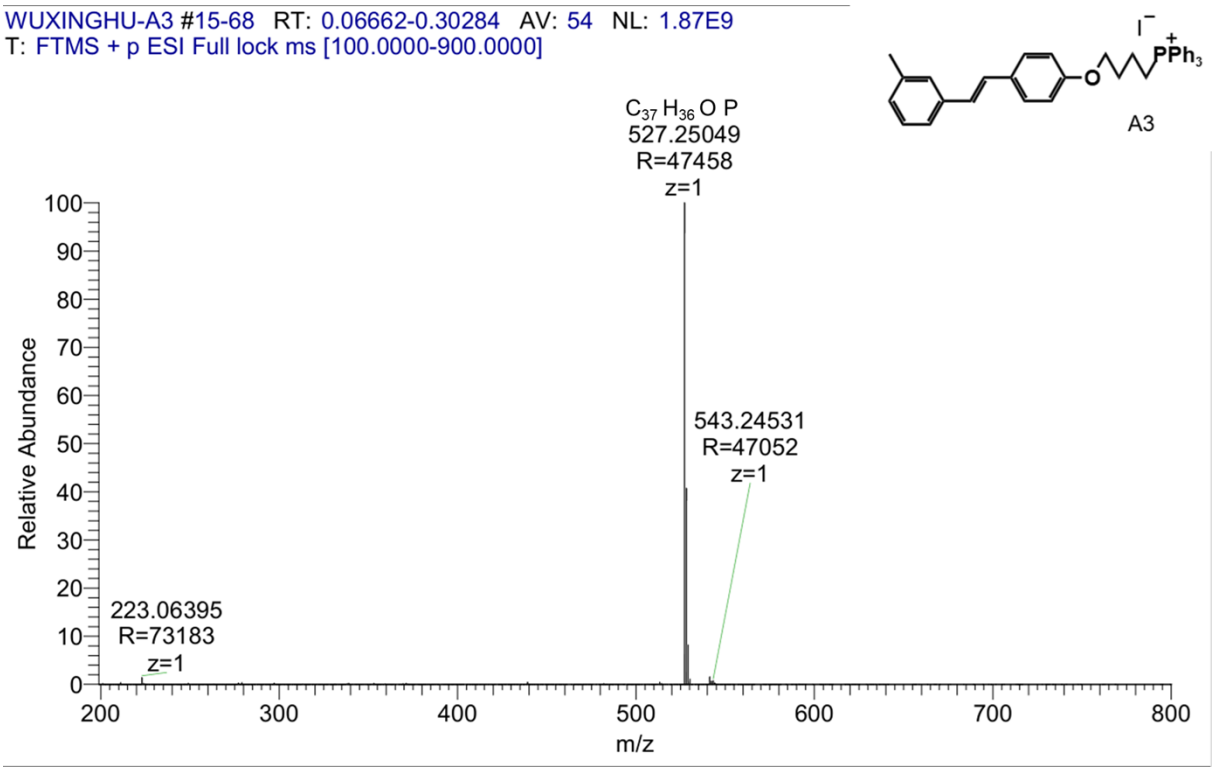
ESI-MS of compound A1



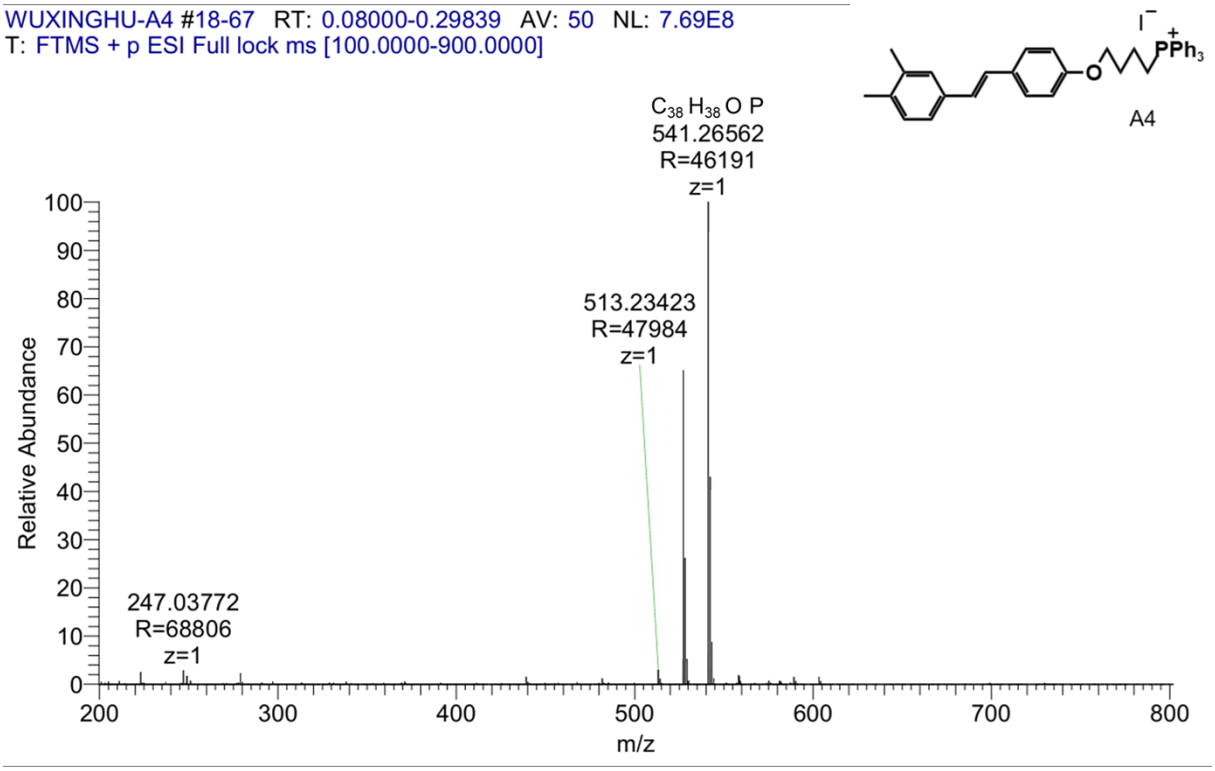
ESI-MS of compound A2



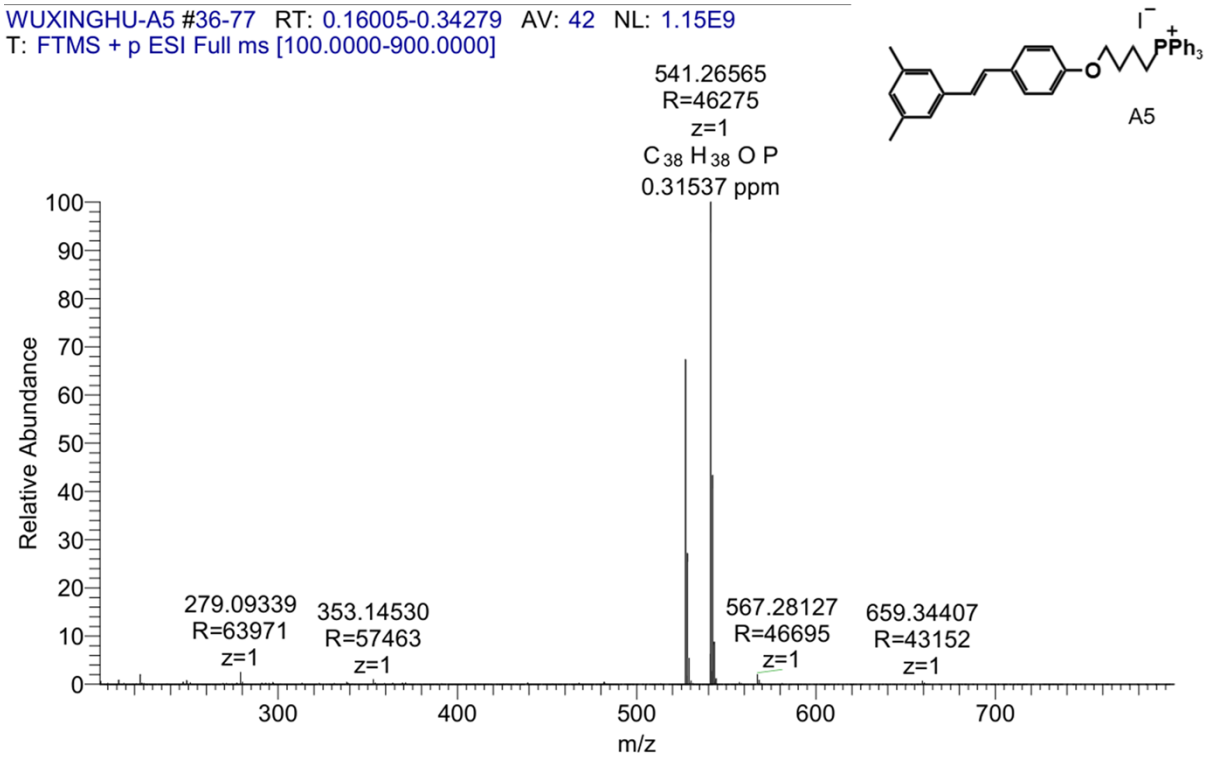
ESI-MS of compound A3



ESI-MS of compound A4



ESI-MS of compound A5



ESI-MS of compound A6