Supporting Information

ROS Self-Scavenging Polythiophene Materials for Cell Imaging

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Experimental Section

Materials and Measurements: All chemicals were purchased from Sigma-Aldrich Chemical Company, Alfa-Aesar, J&K Scientific Ltd or Beijing Chemical Works and used as received. All organic solvents were purchased from Beijing Chemical Works and used as received. Compound **1, 4, 5** were synthesized according to the procedure in literatures.[1,2] Rat aortic endothelial cells were obtained from Pricells (Wuhan, China). Bovine serum albumin (BSA) was puerchesed from Sigma-Aldrich. Fetal bovine serum (FBS), culture medium, cell additives and Penicillin-Streptomycin for primary cells were also purchased from Pricells (Wuhan, China). Phosphate buffer saline (PBS) was purchased from Hyclone (Beijing, China). Water was purified with a Millipore filtration system. HotMaster Taq DNA Polymerase, TRNzol Reagent and FastQuant RT Kit (with gDNAase) were purchased from Tiangen Biotech (Beijing, China). LysoTracker labeling solution (1 mM) and DiD were purchased from Life Technologies (Beijing, China). Cell counting kit 8 (cck-8) was obtained from Tiangen (Beijing, China). Primers were synthesized by Invitrogen (Beijing, China). The ^{1}H NMR and ^{13}C NMR were performed by Bruker Avance 400 MHz spectrometer. MALDI-TOF mass spectra were obtained using Bruker AotoFlex Ⅲ system. UV-Vis absorption spectra were measured on JASCO V-550 spectrophotometer. Fluorescence spectra were measured on Hitachi F-4500 fluorometer equipped with a xenon lamp excitation source. The molecular weights of the polymers

were obtained from Waters 1515 GPC with polystyrene as the standard and DMF as the eluent. The cell viability analysis for estimating cytotoxicity was collected using a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 490 nm. Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan). For phototoxicity test, the white light source was equipped with a metal halogen lamp (MVL-210, Mejiro Genossen, Japan). The absorbance for cck-8 analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 490nm. Polymerase chain reaction (PCR) was operated on My Cycler (Bio-Rad, USA). RNA quantitative test was measured on Nanodrop 2000 (Thermo Scientific, USA). A radiometer (Photoelectric Instrument Factory of Beijing Normal University) was used to estimate the intensity of the incident beam. Automated cell counter (Countess, Invitrogen) was employed for cell counting. Zeta potentials and size were carried out on a Nano ZS (ZEN3600) system.

Synthesis of Compound 2. To a solution of compound **1** (1.89 g, 12 mmol) in 6 mL toluene was added piperidine (0.83 mL). After stirring for 10 min, 2-(trifluoromethyl)benzaldehyde (1.26 mL, 12 mmol) was added and stirred at 50 ^{o}C for 1 h, finally, AcOH (0.33 mL) was added to the mixture. After 12 hours, the reaction was cooled down to room temperature. The mixture was washed by 1 mL saturated $NaHCO_3$ aqueous solution twice and 2 mL deionized water once. Then 3 mL CH_2Cl_2 was used to extract the product. The organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified by silica gel chromatography with petroleum ether/ acetic ether/ dimethoxyethane (10:2:1) as the eluent to get a white solid (1.81 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.942 (d, 1H), 7.74 (m, 3H), 4.25 (t, 2H), 3.85 (t, 2H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.2, 166.2, 139.3, 137.7, 132.1, 129.3, 126.2,

106.0, 99.1, 72.3, 70.8, 66.8, 59.2, 26.5. HR-MS (MALDI, $[M+Na]^+$): Calcd. For $C_{14}H_{13}F_3O_4$: 325.06636; Found: 325.06553.

Synthesis of Compound 3. The reaction was operated in Soxhlet extractor. After the molecular sieves were added to the extractor to absorb water molecule, to a solution of compound **2** (0.785 g, 2.6 mmol) in 30 mL of isopropanol was added 3-amino-2-butenoic acid ethyl ester (0.194 g, 1.5 mmol) in a round flask and stirred at 100 ^oC for 10 h. The solvent was removed and got the residue. To the solution of residue (100 mg, 0.242 mmol) in 15 mL of CH_2Cl_2 was added propynoic acid (66 mg, 0.726 mmol) and 4-dimethylaminopyridine (5 mg, 0.0363 mmol). The solution was stirred at -18 ^oC for 15 min. Dicyclohexylcarbodiimide (74.1 mg, 0.363 mmol) was dissolved in 15 mL of CH_2Cl_2 and dropped into the solution over 1 h, meanwhile, the temperature was kept at -18℃. The resulting yellow solution was stirred at room temperature for another 8 h. After the white precipitate was separated by centrifugation, the solvent was removed under reduced pressure. The resulting residue was purified by silica gel chromatography with petroleum ether/ ethyl acetate (3:1) as the eluent to afford a colorless liquid (59 mg, 53%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, 1H), 7.50 (d, 1H), 7.40 (t, 1H), 5.64 (s, 1H), 5.57 (s, 1H), 4.38 (m, 2H), 4.16 (m, 2H), 4.00 (m, 1H), 2.88 (s, 1H), 2.31 (s, 1H), 1.70 (t, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.51, 167.04, 152.40, 146.63, 144.77, 143.11, 131.90, 131.12, 126.47, 105.31, 103.93, 105.31, 103.93, 63.79, 60.71, 59.81, 35.54, 19.57, 19.21, 13.94. ESI-MS: Calcd. For $C_{23}H_{22}F_3NO_6$: 465013992, Found: 464.13081 $(M-H)^+$.

Synthesis of Compound PT. Compound **4** (29.9 mg, 0.1 mmol), compound **5** (71.6 mg, 0.234 mmol) and $FeCl_3$ (216.4 mg, 1.336 mmol) were added to 10 mL of anhydrous oxygen-free $CHCl_3$, and the solution was stirred at room temperature for 36 h. After the reaction was quenched by

adding 1 mL of methanol, the solvent was removed. The black precipitate was dissolved in 11 mL of DMSO/$CHCl_3$/CH_3OH (1:5:5), and 1 mL of hydrazine hydrate was added to separated the $FeCl_3$. The solvent was removed under vaccum, and 10 mL of deionzed water was added to the residue, then dialyzed in water through a membrane with a molecular weight cutoff of 3500 g/mL for three days to give an orange solid (26 mg, 26 %). ^{1}H NMR (400 MHz, D_2O+CD_3OD) δ 7.27-7.09 (b, 1H), 3.869 (s, 2H), 3.87-3.61 (d, 15H), 3.11 (s, 10H).

Synthesis of PTDHP. To a solution of **PT** (10 mg) in 2 mL of oxygen-free DMSO was added compound **3** (4 mg, 8.6 mmol), $CuSO_4$•$5H_2O$ (10 mg, 40 mmol), sodium ascorbate (20 mg, 0.1 mol). The mixture was stirred at room temperature for 2 days and dialyzed in deionzed water through a membrane with a molecular weight cutoff of 3500 g/mL for three days to give an orange solid. The solid was further purified by precipitate in ethyl acetate to get rid of the unreacted compound **3** and gave an orange solid (11 mg). ^{1}H NMR (400 MHz, D_2O+CD_3OD) δ 7.42-7.16 (b, 1H), 3.92 (s, 1H), 3.67 (b, 10H), 3.19 (b, 10H), 2.42 (b, 1H), 1.332 (b, 0.5H), 1.05-1.03 (b, 0.7H).

The measurement of the molecular weight. 1 mg of polymer was dissolved in 1 ml of DMF with 0.05M LiBr followed by filtered with the 0.45 um filter. The sample was added after baseline calibration, and the DMF with 0.05M LiBr was used as the mobile phase. Meanwhile, polystyrene was employed as the standard. Herein, the temperatures of the UV detector and chromatographic column were 50 °C and 85 °C, respectively.

Cell culture. (1) For ordinary culture: rat aortic endothelial cells were cultured in primary cell culture medium with 10% fetal bovine serum (FBS), 1% cell additives and 1% Penicillin-

Streptomycin at 37 °C in a humified atmosphere containing 5% carbon dioxide. **(2)** For experimental culture: rat aortic endothelial cells were in culture medium with 1% bovine serum albumin (BSA) and 1% Penicillin-Streptomycin (conditioned medium) at 37°C in a humified atmosphere containing 5% carbon dioxide.

PTDHP and PT photo-stability experiments. PTDHP and PT were dissolved in H_2O respectively with the final concentration of 3×10^{-5} M. The solution was exposed to white light with the dose of 6 mW · cm^{-2} for 14 min, and the fluorescence of the two compounds were respectively recorded every minute.

In vitro cell viability assay. To estimate the toxicity of PTDHP and PT to rat aortic cells, the cell viability assay was operated in this work. Rat aortic cells were seeded in 96-well plate with 8×10^3 cells/well, and incubated with the gradient concentration of PTDHP/PT ($2\sim64 \times 10^{-6}$ M) in culture medium at 37 °C for 24 h. 10 μL of Cell Counting Kit 8 (cck-8) was added to each well, another 4 h were needed. After shaking the plate for 2 min, the absorption was recorded by a microplate reader at 490 nm. The cell viability rate (VR) was estimated as following equation:

$$VR = \frac{A}{A_0} \times 100\%$$

where A is the absorbance of experimental groups reacted with PTDHP, which deducted the absorbance of cck-8 and PTDHP. A_0 is the absorbance of the control groups without incubation with PTDHP, which deducted the absorbance of cck-8.

To judge the photo-toxicity of PT and PTDHP toward cells, we also measured the cell viability assay in this work. Cells were seeded in 96-well plate with 8×10^3 cells/well, and treated with the

gradient concentration of PT-DHP/PT (2~64 × 10^{-6} M) in culture medium for 6 h. After removing the culture medium mixed with polymer, 100 μL of new medium was added to each well. Followed by exposed to white light with the dose of 1 mW · cm^{-2} for 15 min, cells were incubated for another 24 h. The following procedures were the same as above.

DLS measurement. PTDHP was dissolved in deionized water with a final concentration of 1 × 10^{-6} M, and the size was recorded on Nano ZS system respectively. As negative control, PT was managed under the same conditions.

Confocal laser scanning microscopy (CLSM) characterization. For PT, DHP and PTDHP: after rat aortic cells were incubated with PTDHP (2 × 10^{-5} M), DHP (5 × 10^{-6} M) or PT (2 × 10^{-5} M) in conditioned medium for 9 h at 37 °C, cells were washed with PBS for three times. LysoTracker (5 × 10^{-7} M) in culture medium without any additive was applied to label lysosome for 1 h, and washed with PBS for three times. DiD (5 × 10^{-6} M) in culture medium without any additive was applied to label cell membrane, and washed with PBS for three times as well. The specimens were examined by confocal laser microscopy using a 405 nm laser for DHP, 488 nm laser for PTDHP or PT, 559 nm for LysoTracker and 635 nm for DiD. The fluorescence of PTDHP and PT were highlighted in green, DiD was highlighted in red, and LysoTracker was highlighted in magenta.

References

[1] Satoh, Y.; Ichihashi, M.; Okumura, K. *Chem. Pharm. Bull.* (Tokyo) 1992, *40*, 912-919.

[2] Wang, F.; Liu, Z.; Wang, B.; Feng, L.; Liu, L.; Lv, F.; Wang, Y.; Wang, S. *Angew. Chem., Int. Ed.* 2014, 53, 424-428.

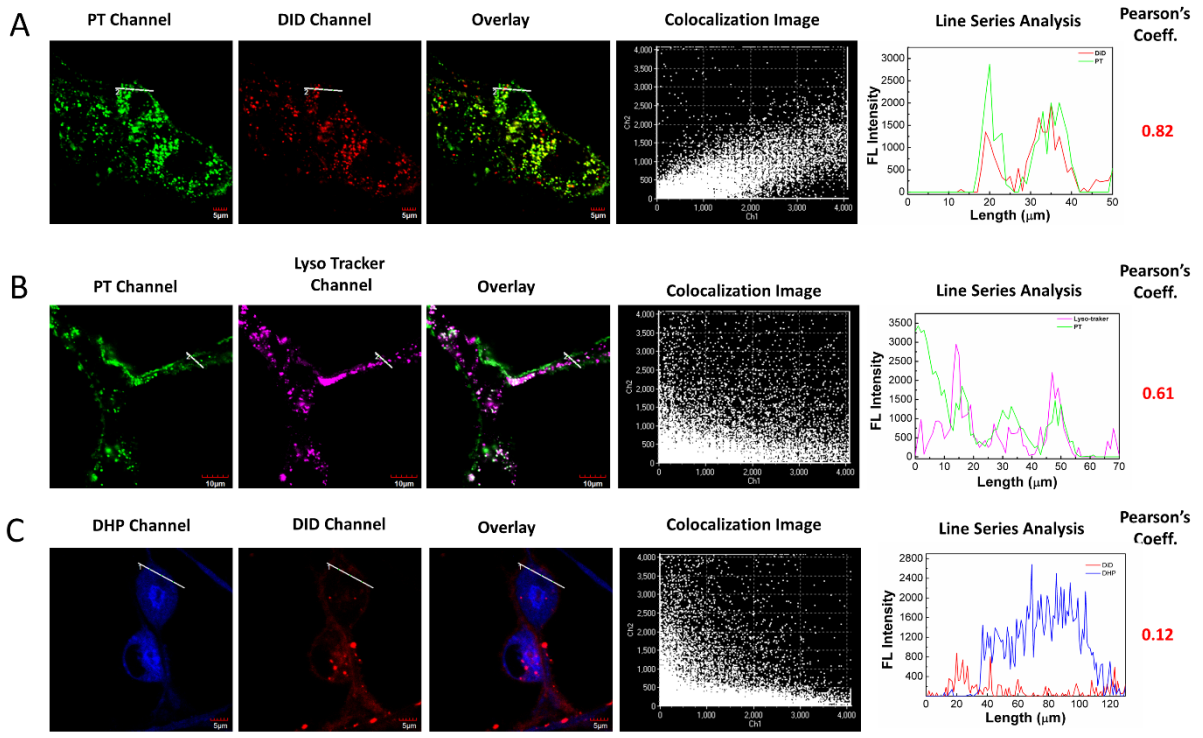
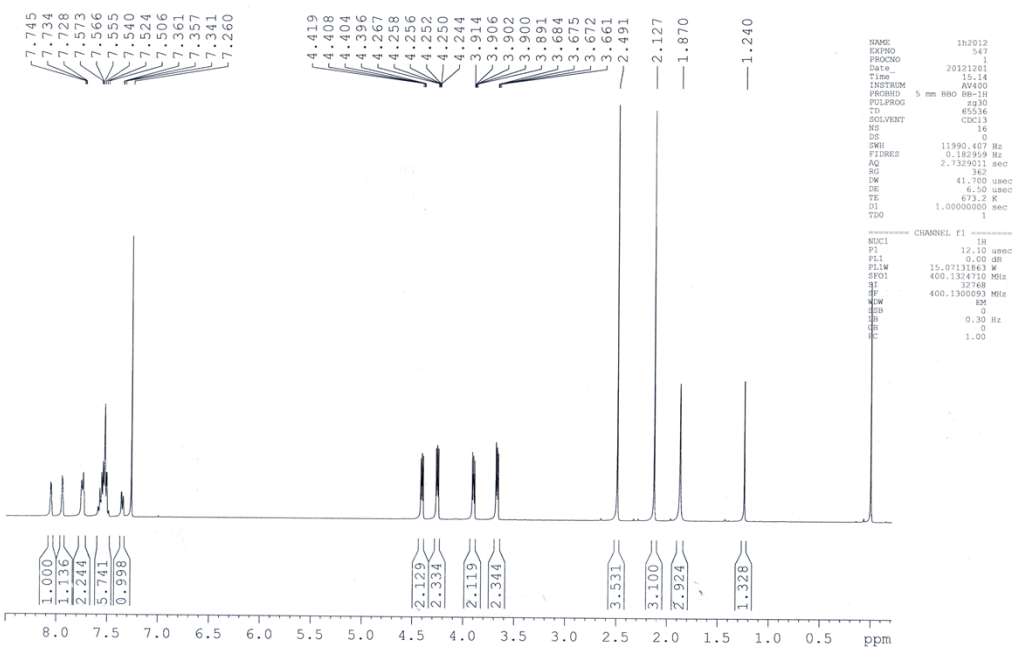
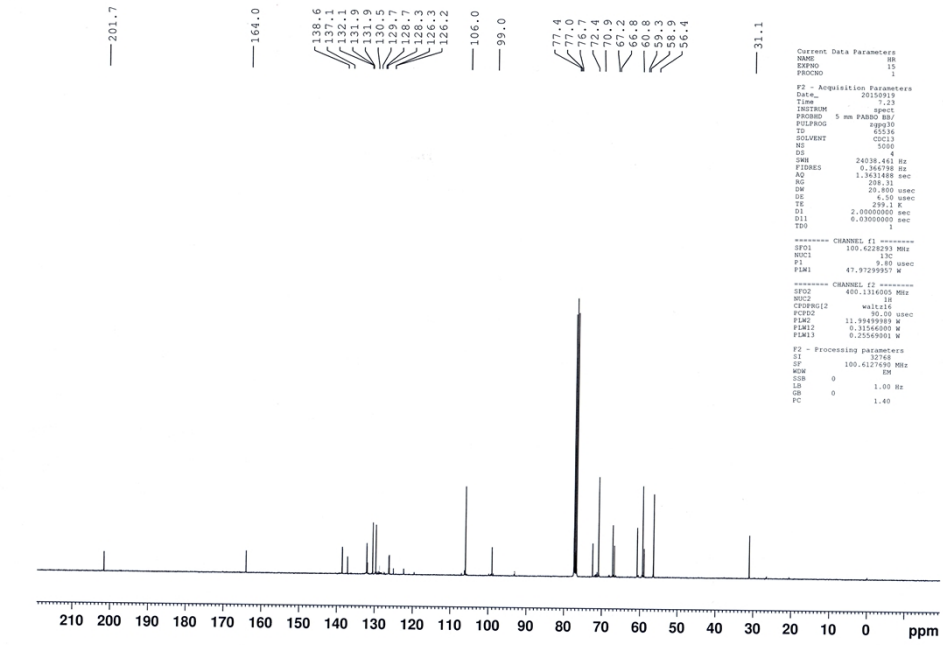


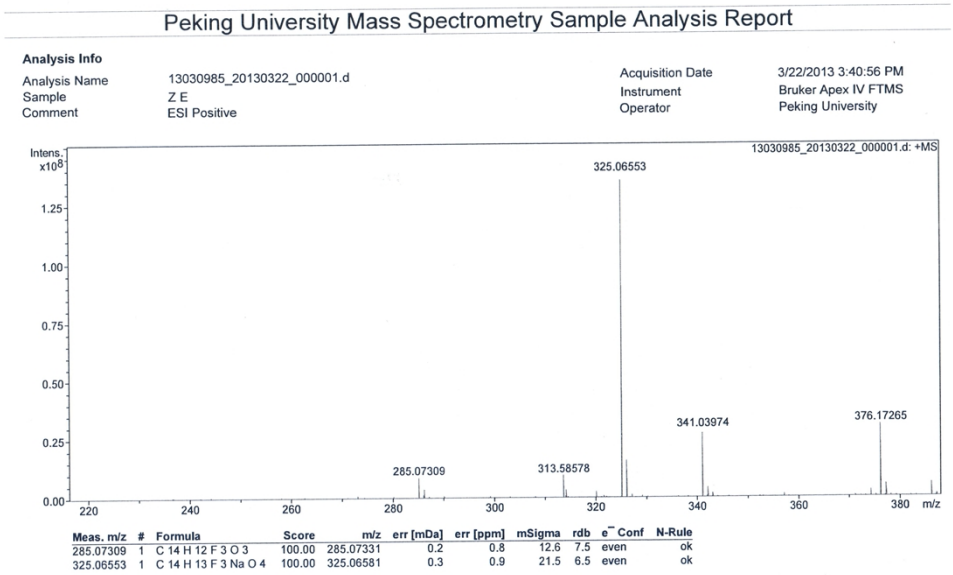
Figure S1. Cellular location of PT and DHP in rat aortic endothelial cells. CLSM images of rat aortic endothelial cells, line series analysis within ROI and colocalization of PT with DiD probe after incubation with PT for 9 h, followed by incubated with DiD for 0.5 h (A) or LysoTracker for 1 h (B) at 37 ^{o}C. (C) CLSM images of rat aortic endothelial cells, line series analysis within ROI and colocalization of DHP with DiD probe after incubation with DHP for 9 h, followed by incubated with DiD for 0.5 h . [PT] = 2.0 × 10^{-5} M in RUs. [DiD] = 5 × 10^{-6} M, [LysoTracker] = 5 × 10^{-7} M, [DHP] = 5.0 × 10^{-6} M. PT was highligted in green, LysoTracker was highlighted in magenta, DHP was highlighted in blue and DiD was highlighted in red. Colocalization, line series analysis and pearson's correlation coefficient were evaluated by OlympusFluoview.



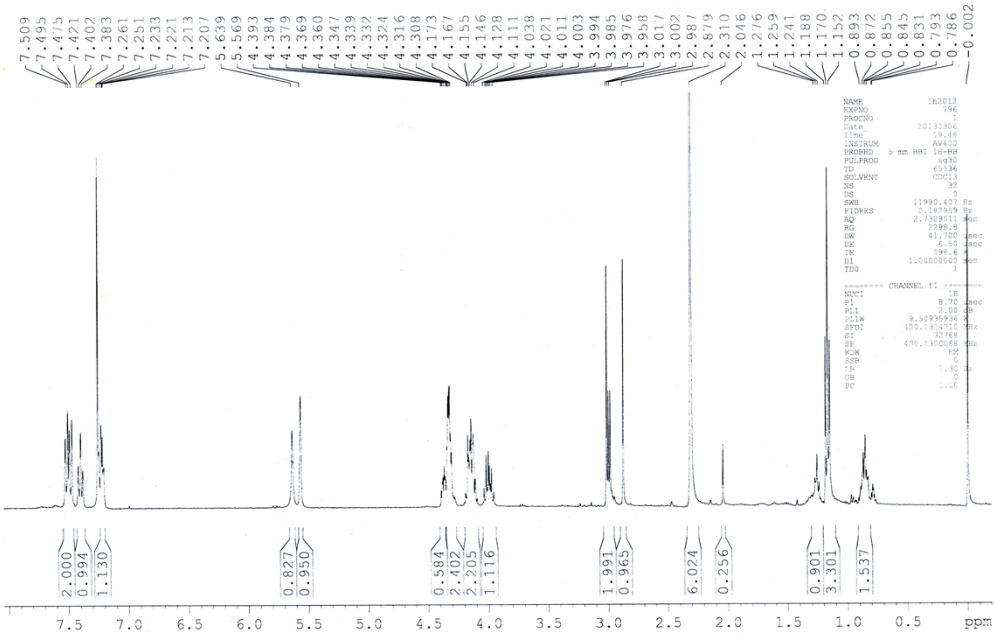
The ^{1}H NMR spectrum of compound 2



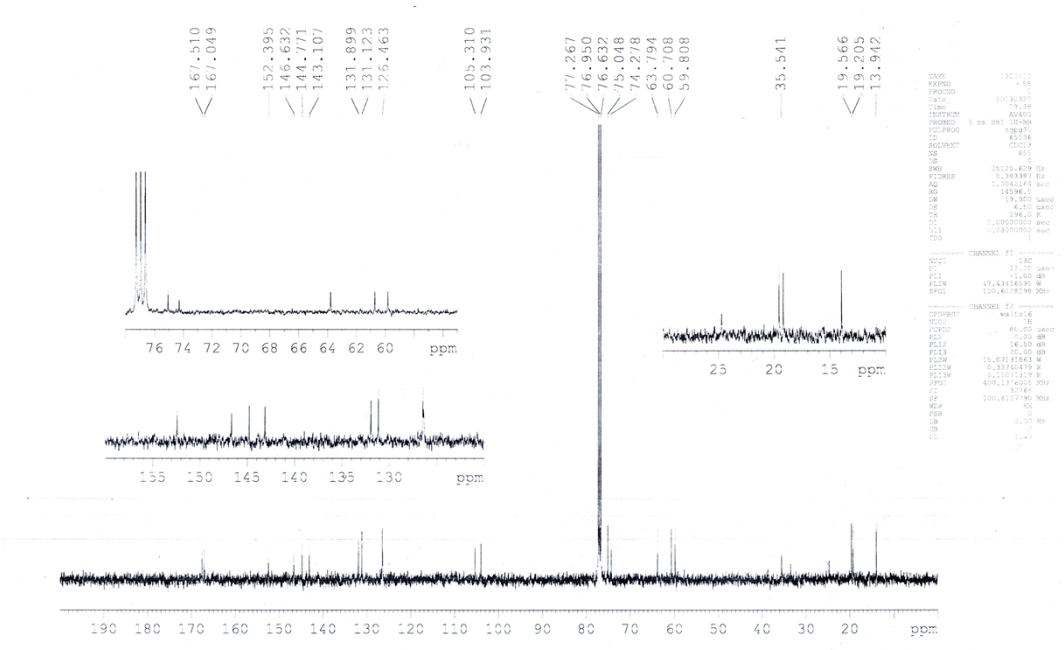
The ^{13}C NMR spectrum of compound 2



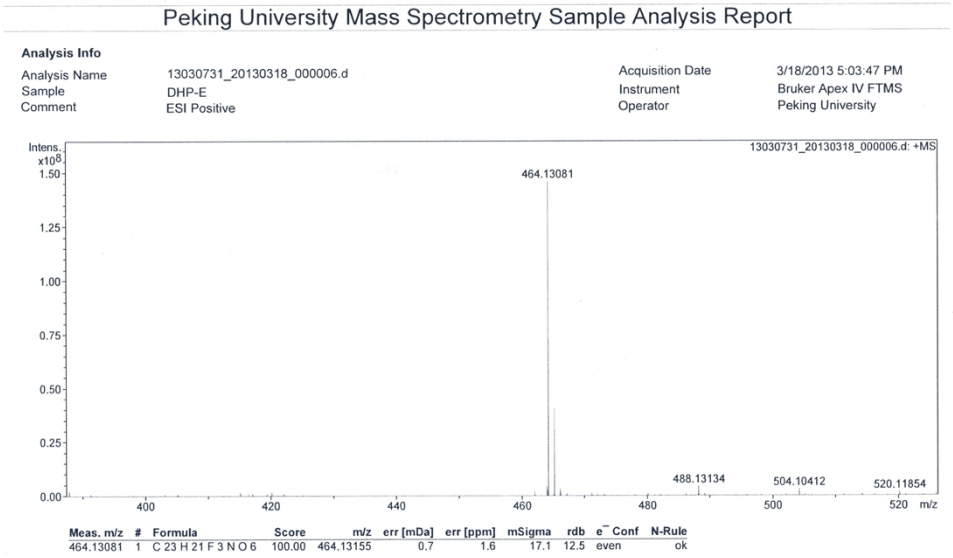
The high resolution mass spectrum of compound 2



The ^{1}H NMR spectrum of compound 3



The ^{13}C NMR spectrum of compound 3



The high resolution mass spectrum of compound 3

Figure S2. The original H NMR, C NMR and MS spectra of compound 2 and 3.