

Electrochemical sulfonylation of thiols with sulfonyl hydrazides: a metal- and oxidant-free, protocol for the synthesis of thiosulfonates

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1. General Information

Unless otherwise noted, all reagents and solvents were obtained commercially and used without further purification. Column chromatography on silica gel (300-400 mesh) was carried out using technical grade 60-90 °C petroleum ether and analytical grade EtOAc (without further purification). ^1H and ^{13}C spectra were recorded on a 400 MHz or 500 MHz spectrometer. Chemical shifts were reported in ppm. ^1H NMR spectra were referenced to CDCl_3 (7.26 ppm), and ^{13}C -NMR spectra were referenced to CDCl_3 (77.0 ppm). Peak multiplicities were designated by the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet and J, coupling constant in Hz. HRMS spectra were recorded with Micromass QTOF2 Quadrupole/Time-of-Flight Tandem mass spectrometer using electron spray ionization. All devices of the electrolysis were purchased according to the Xu's group (Ref. 9f-9i in the manuscript).

2. Studies on anti-tumor mechanism

2.1 Induction of cell cycle arrest

The cell cycle consists of two stages, namely, interphase and split phase (M phase). The M phase is further divided into three stages: genetic material DNA pre-synthesis stage (G1 stage), DNA synthesis period (S phase), and protein synthesis period (G2 stage), which is the late stage of DNA synthesis. Cell multiplication plays a vital role in tumor cell regeneration; as such, the compound that induces cell cycle arrest can inhibit cell proliferation. Our previous study indicated that compound **3ag** could effectively inhibit the proliferation of T-24 cells, respectively. Therefore, flow cytometry analysis was conducted in the present work to investigate cell cycle distribution in T-24 cells treated with compound **3ag**.

As shown in Fig. S1, upon exposure to compound **3ag**, the percentage of T-24 cells in the G2/M phase increased from 7.52% to 27.40%; This finding indicated that compound **3ag** significantly induced cell cycle arrest at the G2/M phase in T-24 cells.

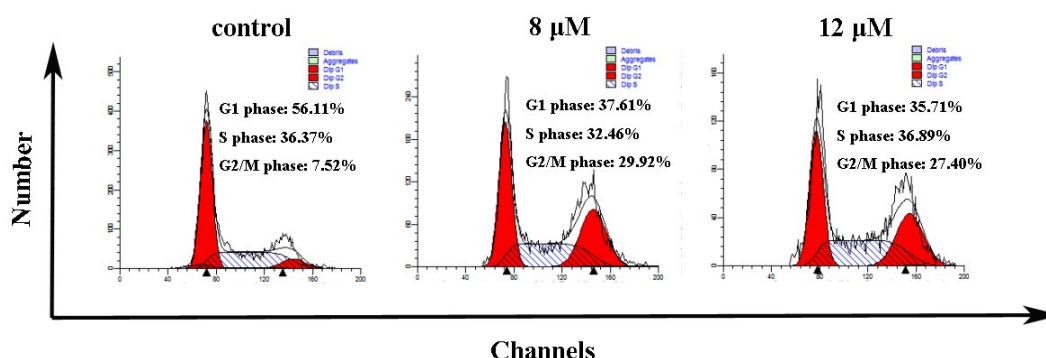


Figure S1. Cell cycle distribution in T-24 cells treated with compound **3ag** for 24 h.

2.2 Hoechst 33342 nucleic acid staining

Hoechst 33342 staining is a fluorescent dye that binds sturdily to nucleus and detect the nuclear damage or chromatin condensation. The hoechst 33342 stains the apoptotic cells as bright colored owing to the condensed nucleus which is a distinctive apoptotic characteristic. Hence, it was of our interest to detect nuclear damage or chromatin condensation persuaded by the compound **3ag** in T-24 cells. Hoechst 33342 staining technique was performed according to earlier reported method. The results from Fig. S2 illustrated that the nuclear structure of untreated cells was intact whereas compound **3ag** treated cells exhibited condensed, or fragmented nuclei.

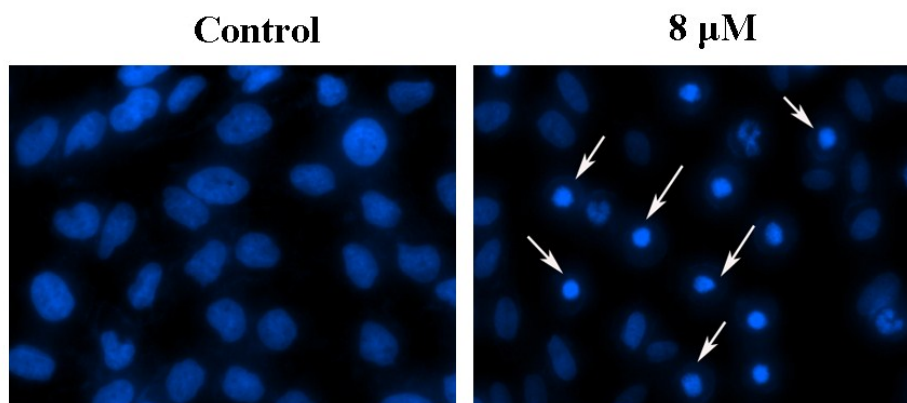


Figure S2. Assessment of nuclear morphological changes by hoechst 33342 staining in T-24 cells after 24 h. Compound **3ag** treated T-24 cells have displayed nuclear apoptotic characteristics such as nuclear fragmentation and shrunken nuclei.

2.3 Detection of released calcium ions

Ca^{2+} as a death signaling molecule is involved in important life activities, such as cell shrinkage, movement, secretion, and division. When cells are stimulated by specific signals, calcium channels (mitochondria and endoplasmic reticulum) will be opened, resulting in a rapid increase in intracellular calcium concentration. Intracellular Ca^{2+} levels were determined by fluorescence microscope with Fluo-3 AM staining kit, which could pass through the cell membrane and be cut into Fluo-3 by esterase. Fluo-3 could bind to the calcium ions to produce strong green fluorescence. As shown in Fig. S3, after treatment with compound **3ag** (0 and 8 μM), the green fluorescence intensity increased significantly in T-24 cells. Hence, compound **3ag** can increase the intracellular levels of Ca^{2+} .

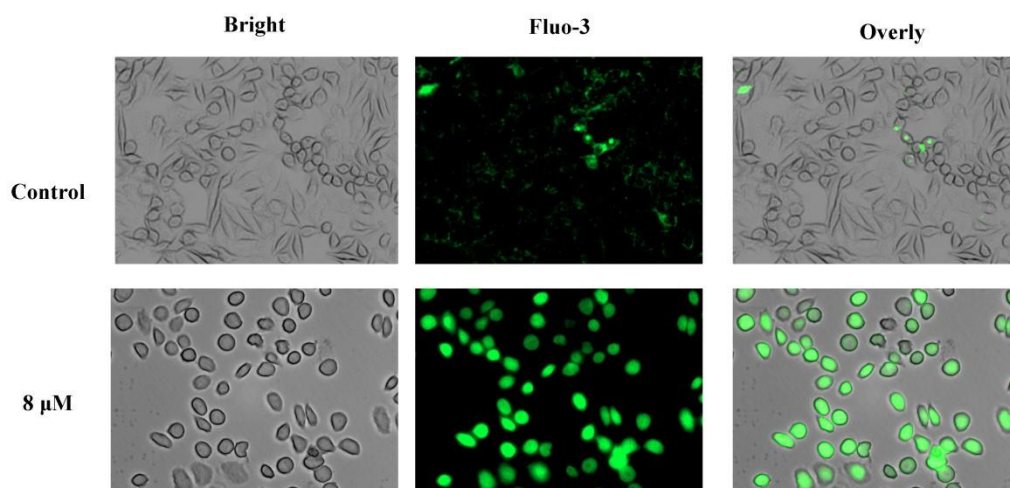


Figure S3. Changes in Ca^{2+} concentration in T-24 cells treated with compound **3ag** determined with a Fluo-3AM staining kit under a fluorescence microscope. Scale bar: 100 μm .

2.4 Intracellular ROS

The DCFH-DA (2',7'-dichlorofluorescein diacetate) probe was used to detect ROS content in cells. DCFH-DA cannot produce fluorescence but can freely pass through the cell membrane. Upon entry to the cell, DCFH-DA can be hydrolyzed by esterase into DCFH, which will not permeate the cell membrane but accumulate in the cell. ROS in the cells can oxidize the nonfluorescent DCFH to produce a green fluorescent DCF. The intensity of green fluorescence is proportional to the ROS level. Therefore, the green fluorescence intensity can reflect the concentration of ROS in cells.

As shown in Fig. S4, after treatment with compound **3ag** (0 and 8 μM) for 24 h, the green fluorescence in T-24 cells were enhanced compared with that in the untreated controls. Hence, compound **3ag** can increase the level of ROS in T-24 cells, respectively.

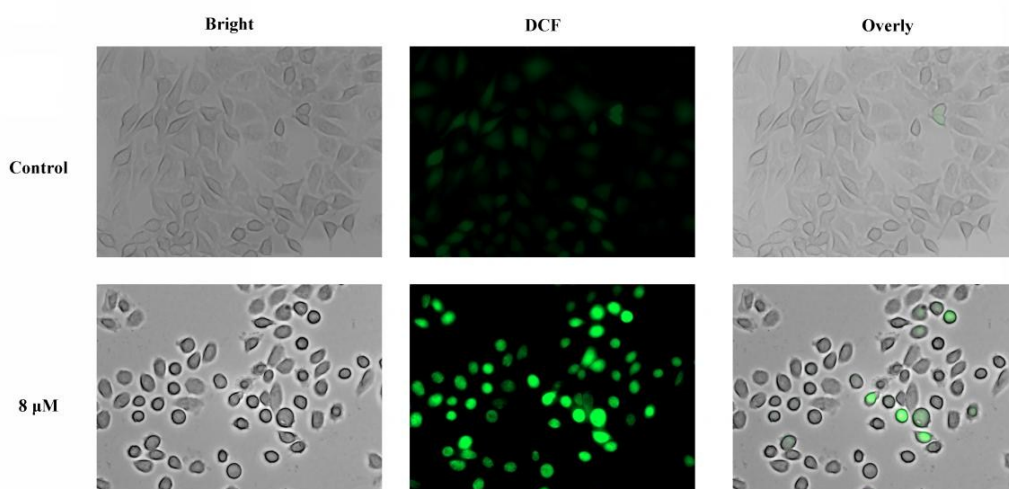


Figure S4. Changes in ROS concentration in T cells treated with compound **3ag** determined with a DCFH-DA staining kit under a fluorescence microscope. Scale bar: 100 μm .

2.5 Inhibition of polymerization of microfilaments

T-24 cells were treated with compound **3ag** (0 and 8 μM) for 24 h and stained with hoechst 33342 and anti- β -tubulin antibody (EPR16774, ab179513). As shown in Fig. S3, cells in the control group exhibited normal filamentous microtubules arrays.

However, after treatment with compound **3ag**, the cells showed reduced microtubule networks, indicating the decreased expression of the microtubule protein. Hence, compound **3ag** can inhibit the polymerization of microtubules and disrupt their organization (Fig. S5). The result indicated that compound **3ag** induced the collapse of the microtubule network and interfered with the mitosis of T-24 cells.

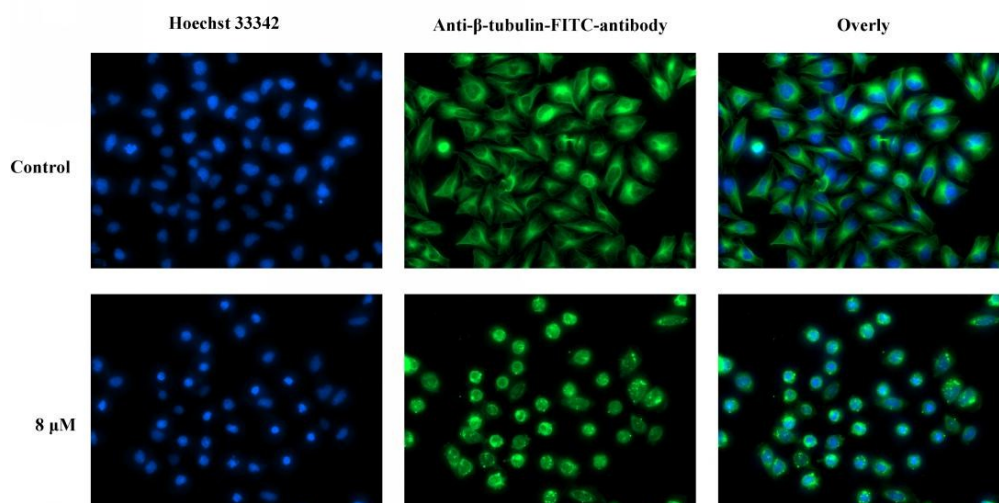


Figure S5. Fluorescence microscopic (Cytation 5 Cell Imaging Multi-Mode Reader, BioTek Instruments, Inc., USA) images of T-24 cells stained with hoechst 33342 and anti-β-tubulin antibody after treatment with compound **3ag** 24 h.

2.6 Wound healing assay

Metastasis is a multistep process involving cancer cell motility and invasion which accounts for more than 90% of cancer related deaths. Microtubule or tubulin network plays an important role in cell motility and migration. As the compound **3ag** inhibits tubulin polymerization, we have investigated their migration inhibition capacity through wound healing assay. In this assay, standardized scratches (wounds) were made in confluent monolayers of T-24 cells and incubated with increasing concentrations of the compound **3ag** (0,4 and 8 μM). The number of cells migrated in to the wound area were captured using phase contrast microscope after 0 and 24 h incubation. As shown in Fig. S6, the compound **3ag** treatment resulted in significant inhibition of migration capacity of T-24 cells and the effect was more prominent after 24 h. For instance, compound **3ag** treatment at 4 and 8 μM concentration led to strong inhibition of migration in to the scratch area in relative comparison to control after 24 h. It can be inferred from the results that the compound **3ag** suppresses the migration potential of the T-24 cells in dose dependent manner.

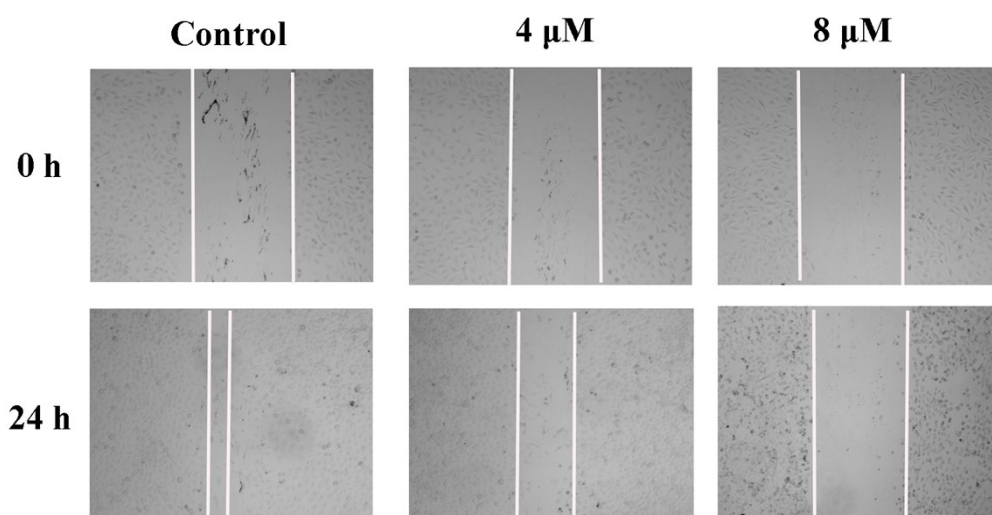
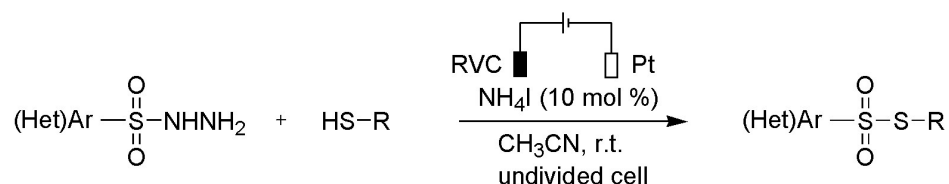


Figure S6. Effect of compound **3ag** on in vitro migration potential of T-24 prostate cancer cells. Scratches were created with sterile 200 μ L pipette and images were captured using fluorescence microscopic (Cytation 5 Cell Imaging Multi-Mode Reader, BioTek Instruments, Inc., USA) at 0 h and 24 h after treatment with 0, 4 and 8 μ M of compound **3ag**.

3. Experimental Section

3.1 Synthesis of unsymmetrical thiosulfonates



The sulfonyl hydrazides (0.3 mmol, 1.0 equiv), thiols (0.3 mmol, 1.0 equiv) and NH_4I (0.03 mmol, 0.01 equiv) were placed in a 10 mL three-necked round-bottomed flask. The flask was equipped with a condenser, a RVC (100 PPI, 1 cm x 1 cm x 1.2 cm) anode and a platinum plate (1 cm x 1 cm) cathode. MeCN (6.0 mL) were added. The electrolysis was carried out at room temperature using a constant current of 10 mA until complete consumption of the substrate (monitored by TLC, about 3 h). The reaction mixture was concentrated and the residue was chromatographed through silica gel eluting with ethyl acetate/petroleum ether to give the products.

3.2 Anticancer activity assay

The 180 μ L cell suspensions (4500-5000 cells/mL) was seeded in 96-well plates and

incubated for 24 h. All compounds and 5-FU were dissolved in the Phosphate Buffered Saline (PBS) with 1% DMSO to give various concentrations (2.5, 5, 10, 20, and 40 μ M, respectively) to 96-well plates and control wells contained supplemented media with 1% DMSO. Continue incubating for 48 h at 37 °C in 5% CO₂ atmosphere and then the MTT solution (10 μ L, 5 mg/mL) was added into each well and the cultures were incubated further for 4~6 h. After removal of the supernatant, DMSO (100 μ L) was added to dissolve the formazan crystals. The absorbance was read by enzyme labeling instrument with 570/630 nm double wavelength measurement. The cytotoxicity was estimated based on the percentage cell survival in a dose dependent manner relative to the negative control. The final IC₅₀ (a drug concentration killing 50% cells) values were calculated by the Bliss method. All the tests were repeated in at least three independent experiments.

3.3 Cell cycle analysis

T-24 cells were treated with different concentrations of compound **3ag** (0, 8, and 12 μ M). After incubating for 24 h. The cells were collected and used PBS washed twice, fixed with 9 ml 70% ice-ethanol at -20 °C overnight. The ethanol was removed and replaced with the 0.5 mL RnaseA (10 mg/ml, Sigma) for 30 min at 37 °C, then added 20 μ L PI (1 mg/mL, Sigma) for 15 min in dark. Flow cytometry (FACScan, Becton Dickinson, San Jose, CA) was used to analyze the cell cycle distribution.

3.4 Hoechst 33342 nucleic acid staining

Nuclear morphological changes were observed through hoechst 33342 staining. After treatment with compound **3ag** for 24 h in T-24 cells, cells were washed with PBS and permeabilized with 0.1% Tween 20 for 10 min followed by staining with hoechst 33342 (2.5 μ g/mL). Control and treated cells were observed with fluorescence microscope (Cytation 5 Cell Imaging Multi-Mode Reader, BioTek Instruments, Inc., USA.).

3.5 Measurement of intracellular reactive oxygen species

Intracellular reactive oxygen species (ROS) was detected by using fluorescence microscope by with 2',7'-dichlorofluorescein diacetate (DCHF-DA) staining kit. T-24 cells were seeded at 2×10⁶/well in 10% FBS/DMEM into 6-well plates and treated with different concentrations of compound **3ag** (0 and 8 μ M) for 24 h, then cells were washed twice with serum free DMEM and

incubated with DCHF-DA for 30 minutes at 37 °C in dark. The fluorescence microscope was used to measure the enhanced green fluorescent intensity in cells, the intensity of the microscope is 488 nm, and the emission wavelength is 525 nm.

3.6 Measurement of intracellular Ca^{2+} levels

The intracellular Ca^{2+} levels were determined by fluorescent with Fluo-3 AM staining kit, which could pass through the cell membrane and be cut into Fluo-3 by the esterase. The Fluo-3 could bind with calcium ions to produce strong green fluorescence. T-24 cells were seeded at 2×10^6 /well in 10% FBS/DMEM into 6-well plates and incubation for 24 hours. After the cells were treated with compound **3ag** (0 and 8 μM) for 24 h, the Fluo-3 AM (5.0 μM) was added and incubated for 30 min at 37 °C. The fluorescence microscope was used to measure the enhanced green fluorescent intensity in cells, the intensity of the microscope is 506 nm, and the emission wavelength is 526 nm.

3.7 Tubulin polymerization assay

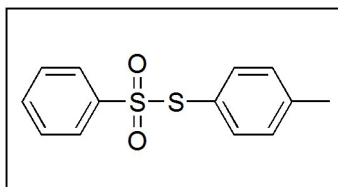
The effects of compound **3ag** on tubulin were detected using an anti-beta tubulin antibody (EPR16774, ab179513). The T-24 cells were seeded in 6-well plates (2×10^6 cells/well) and cultured for 24 h. After treated with compound **3ag** (0 and 8 μM) for 24 h, the cells were fixed with 4% paraformaldehyde for 10min, and were permeabilized with 0.1% Triton X-100 for 10min. Then incubation with tubulin proteins (1/1000) at 4 °C overnight. Followed by anti-rabbit Alexa Fluor 488 secondary antibody at 1/5000. Confocal image showing cytoplasmic staining on T-24 cells. The nuclears were stained by hoechst 33342 (2.5 $\mu\text{g/mL}$) for 30 min. Fluorescence microscope was used to observed the change of microtubule networks in cells.

3.8 Wound healing assay

T-24 cells (5×10^5 cells/well) were grown in petridishes for 24 h. Scratches were made in confluent monolayers using 200 μL pipette tip. Then, wounds were washed twice with PBS to remove non-adherent cell debris. The media containing different concentrations (0, 4 and 8 μM) of the compound **3ag** were added to the petridishes. Cells which migrated across the wound area were photographed under the fluorescence microscope (Cytation 5 Cell Imaging Multi-Mode Reader, BioTek Instruments, Inc., USA) after 0 and 24 h treatment.

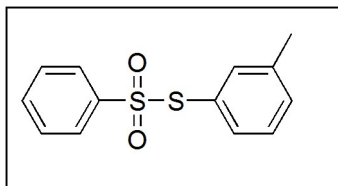
4. ^1H , ^{13}C NMR, MP and MS Data of all products

Benzenethiosulfonic acid S-p-tolyl ester (**3aa**)



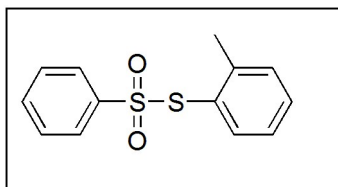
Yellow oil (82%, 65.0 mg). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.63 – 7.52 (m, 3H), 7.46 – 7.39 (m, 2H), 7.27 – 7.18 (m, 2H), 7.16 – 7.10 (m, 2H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 143.1, 142.1, 136.5, 133.5, 130.2, 128.8, 127.5, 124.3, 21.4. HRMS (*m/z*) (ESI): calcd for $\text{C}_{13}\text{H}_{12}\text{NaO}_2\text{S}_2$ [$\text{M}+\text{Na}$] $^+$ 287.0176, found 287.0169.

Benzenethiosulfonic acid S-m-tolyl ester (**3ab**)



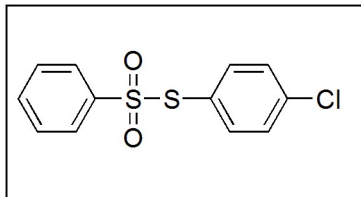
Yellow oil (78%, 61.8 mg). ^1H NMR (500 MHz, Chloroform-*d*) δ 7.60 – 7.55 (m, 3H), 7.45 – 7.41 (m, 2H), 7.28 – 7.25 (m, 1H), 7.22 – 7.19 (m, 1H), 7.16 – 7.11 (m, 2H), 2.29 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 142.9, 139.4, 137.1, 133.5, 133.5, 132.2, 129.1, 128.7, 127.6, 127.4, 21.1. HRMS (*m/z*) (ESI): calcd for $\text{C}_{13}\text{H}_{12}\text{NaO}_2\text{S}_2$ [$\text{M}+\text{Na}$] $^+$ 287.0176, found 287.0167.

Benzenethiosulfonic acid S-o-tolyl ester (**3ac**)



Yellow oil (75%, 59.4 mg). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.62 – 7.52 (m, 3H), 7.46 – 7.30 (m, 4H), 7.24 – 7.19 (m, 1H), 7.18 – 7.11 (m, 1H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 144.1, 143.3, 138.3, 133.6, 131.9, 130.9, 128.9, 127.4, 127.0, 126.9, 20.5. HRMS (*m/z*) (ESI): calcd for $\text{C}_{13}\text{H}_{12}\text{NaO}_2\text{S}_2$ [$\text{M}+\text{Na}$] $^+$ 287.0176, found 287.0168.

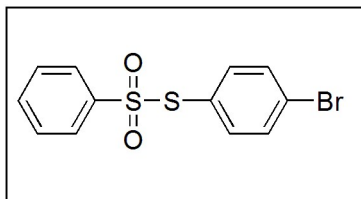
Benzenethiosulfonic acid S-(4-chloro-phenyl) ester (**3ad**)



Yellow oil (72%, 61.34 mg). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.64 – 7.56 (m, 3H), 7.48 – 7.43 (m, 2H), 7.34 – 7.26 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3) δ 142.8, 138.3, 137.7, 133.8, 129.7, 128.9, 127.6, 126.3. HRMS (*m/z*) (ESI): calcd for $\text{C}_{12}\text{H}_9\text{ClNaO}_2\text{S}_2$ [$\text{M}+\text{Na}$] $^+$ 306.9630,

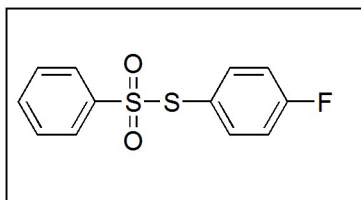
found 306.9624.

Benzenethiosulfonic acid S-(4-bromo-phenyl) ester (**3ae**)



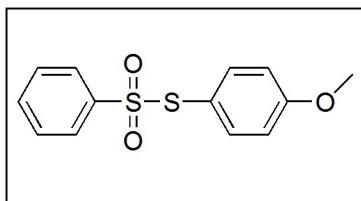
Yellow oil (65%, 63.9 mg). **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.63 – 7.57 (m, 3H), 7.49 – 7.43 (m, 4H), 7.23 – 7.18 (m, 2H). **¹³C NMR** (125 MHz, CDCl₃) δ 142.9, 137.9, 133.8, 132.7, 129.0, 127.6, 126.9, 126.7. **HRMS** (m/z) (ESI): calcd for C₁₂H₉BrKO₂S₂ [M+K]⁺ 366.8864 and 368.8844, found 366.8861 and 368.8839.

Benzenethiosulfonic acid S-(4-fluoro-phenyl) ester (**3af**)



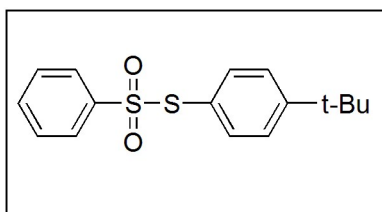
White oil (70%, 56.3 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.62 – 7.55 (m, 3H), 7.47 – 7.41 (m, 2H), 7.37 – 7.29 (m, 2H), 7.07 – 6.98 (m, 2H). **¹³C NMR** (100 MHz, CDCl₃) δ 164.8 (d, J = 253.9 Hz), 142.8, 138.9 (d, J = 9.0 Hz), 133.8, 128.9, 127.6, 123.5 (d, J = 3.3 Hz), 116.8 (d, J = 22.2 Hz). **HRMS** (m/z) (ESI): calcd for C₁₂H₉FNaO₂S₂ [M+Na]⁺ 290.9926, found 290.9920.

Benzenethiosulfonic acid S-(4-methoxy-phenyl) ester (**3ag**)



White solid (85%, 71.4 mg), mp 56 – 57 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.61 – 7.55 (m, 3H), 7.4 – 7.39 (m, 2H), 7.27 – 7.22 (m, 2H), 6.86 – 6.81 (m, 2H), 3.82 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 162.3, 142.9, 138.3, 133.5, 128.8, 127.5, 118.5, 114.9, 55.4. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₂NaO₃S₂ [M+Na]⁺ 303.0126, found 303.0118.

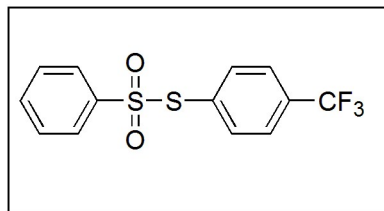
Benzenethiosulfonic acid S-(4-tert-butyl-phenyl) ester (**3ah**)



White solid (83%, 76.2 mg), mp 60 – 61 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.60 – 7.54 (m, 3H), 7.43 – 7.38 (m, 2H), 7.36 – 7.32 (m, 2H), 7.29 – 7.25 (m, 2H), 1.31 (s, 9H). **¹³C NMR** (100

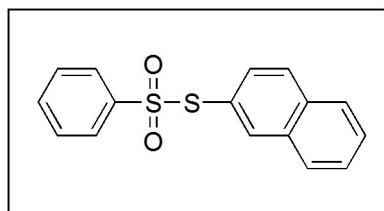
MHz, CDCl₃) δ 155.2, 143.0, 136.3, 133.5, 128.7, 127.5, 126.5, 124.3, 34.9, 31.1. **HRMS** (m/z) (ESI): calcd for C₁₆H₁₉O₂S₂ [M+H]⁺ 307.0826, found 307.0819.

Benzenethiosulfonic acid S-(4-trifluoromethyl-phenyl) ester (**3ai**)



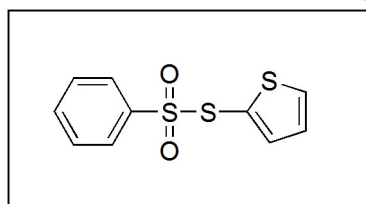
White solid (78%, 74.4 mg). mp 68 – 69 °C. **¹H NMR** (500 MHz, Chloroform-*d*) δ 7.64 – 7.55 (m, 5H), 7.54 – 7.43 (m, 4H). **¹³C NMR** (125 MHz, CDCl₃) δ 142.8, 136.7, 134.0, 133.2 (q, J = 32.8 Hz), 132.2, 129.0, 127.5, 126.2 (q, J = 3.6 Hz), 123.5 (q, J = 273.3 Hz). **HRMS** (m/z) (ESI): calcd for C₁₃H₉F₃NaO₂S₂ [M+Na]⁺ 340.9894, found 340.9883.

Benzenethiosulfonic acid S-naphthalen-2-yl ester (**3aj**)



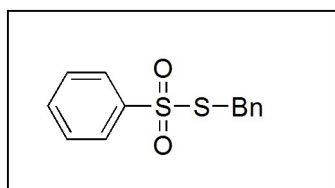
Yellow oil (60%, 54.1 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.88 – 7.83 (m, 2H), 7.80 – 7.77 (m, 1H), 7.76 – 7.72 (m, 1H), 7.61 – 7.52 (m, 5H), 7.41 – 7.35 (m, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.9, 137.6, 134.0, 133.6, 133.2, 131.7, 129.1, 128.8, 128.4, 128.2, 127.7, 127.6, 126.9, 124.9. **HRMS** (m/z) (ESI): calcd for C₁₆H₁₃O₂S₂ [M+H]⁺ 301.0357 found 301.0344.

Benzenethiosulfonic acid S-thiophen-2-yl ester (**3ak**)



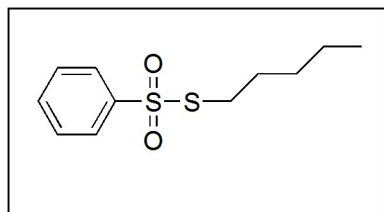
Yellow oil (76%, 58.36 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.68 – 7.58 (m, 4H), 7.51 – 7.42 (m, 2H), 7.17 – 7.11 (m, 1H), 7.09 – 7.07 (m, 1H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.2, 139.4, 135.2, 133.9, 128.9, 128.4, 127.8, 125.0. **HRMS** (m/z) (ESI): calcd for C₁₀H₈NaO₂S₃ [M+Na]⁺ 278.9584, found 278.9579.

Benzenethiosulfonic acid S-benzyl ester (**3al**)



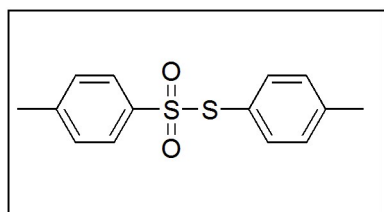
White solid (72%, 57.0 mg). mp 63 – 64 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.87 – 7.81 (m, 2H), 7.63 – 7.55 (m, 1H), 7.51 – 7.44 (m, 2H), 7.24 – 7.14 (m, 5H), 4.27 (s, 2H). **¹³C NMR** (100 MHz, CDCl₃) δ 144.9, 133.6, 133.5, 129.1, 129.1, 128.8, 128.0, 126.9, 40.4. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₂NaO₂S₂ [M+Na]⁺ 287.0176, found 287.0165.

Benzenethiosulfonic acid S-pentyl ester (**3am**)



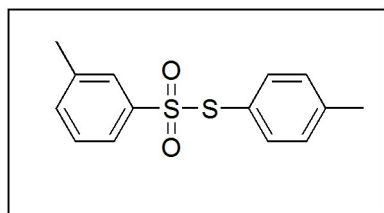
Yellow oil (78%, 57.11 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.99 – 7.90 (m, 2H), 7.66 – 7.59 (m, 1H), 7.58 – 7.51 (m, 2H), 3.03 – 2.91 (m, 2H), 1.63 – 1.54 (m, 2H), 1.28 – 1.21 (m, 4H), 0.86 – 0.78 (m, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 145.0, 133.6, 129.3, 127.0, 36.1, 30.6, 28.3, 22.0, 13.8. **HRMS** (m/z) (ESI): calcd for C₁₁H₁₆NaO₂S₂ [M+Na]⁺ 267.0489, found 267.0483.

Toluene-4-thiosulfonic acid S-p-tolyl ester (**3ba**)



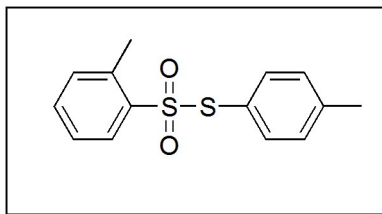
Yellow oil (85%, 70.9 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.48 – 7.43 (m, 2H), 7.25 – 7.19 (m, 4H), 7.16 – 7.11 (m, 2H), 2.42 (s, 3H), 2.37 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 144.6, 142.0, 140.4, 136.5, 130.2, 129.3, 127.6, 124.5, 21.6, 21.4. **HRMS** (m/z) (ESI): calcd for C₁₄H₁₄KO₂S₂ [M+K]⁺ 317.0072, found 317.0061.

Toluene-3-thiosulfonic acid S-p-tolyl ester (**3ca**)



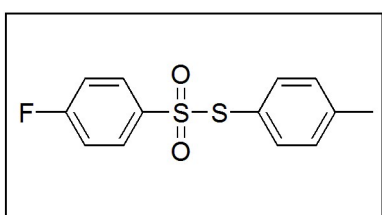
White solid (80%, 66.7 mg). mp 46 – 47 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.40 – 7.33 (m, 3H), 7.32 – 7.27 (m, 1H), 7.26 – 7.20 (m, 2H), 7.17 – 7.12 (m, 2H), 2.38 (s, 3H), 2.34 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.9, 142.1, 139.1, 136.6, 134.3, 130.2, 128.6, 127.9, 124.7, 124.6, 21.5, 21.2. **HRMS** (m/z) (ESI): calcd for C₁₄H₁₄NaO₂S₂ [M+Na]⁺ 301.0333, found 301.0324.

Toluene-2-thiosulfonic acid S-p-tolyl ester (**3da**)



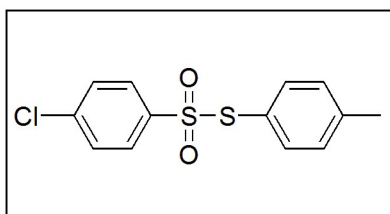
Yellow oil (70%, 58.4 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.47 – 7.39 (m, 2H), 7.35 – 7.30 (m, 1H), 7.18 – 7.06 (m, 5H), 2.70 (s, 3H), 2.34 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.0, 140.4, 137.8, 136.4, 133.7, 132.8, 130.2, 130.1, 125.7, 124.3, 21.4, 20.5. **HRMS** (m/z) (ESI): calcd for C₁₄H₁₄NaO₂S₂ [M+Na]⁺ 301.0333, found 301.0334.

4-Fluoro-benzenethiosulfonic acid S-p-tolyl ester (**3ea**)



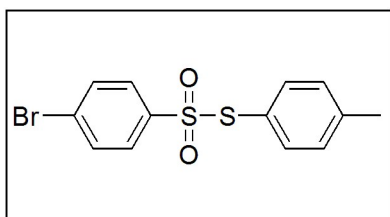
White solid (70%, 59.2 mg). mp 40 – 42 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.61 – 7.56 (m, 2H), 7.25 – 7.21 (m, 2H), 7.18 – 7.13 (m, 2H), 7.12 – 7.06 (m, 2H), 2.38 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 165.5 (d, J = 256.6 Hz), 142.4, 139.1 (d, J = 3.1 Hz), 136.5, 130.5 (d, J = 9.6 Hz), 130.4, 124.2, 116.1 (d, J = 22.8 Hz), 21.5. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₁FN₂O₂S₂ [M+Na]⁺ 305.0082, found 305.0071.

4-Chloro-benzenethiosulfonic acid S-p-tolyl ester (**3fa**)



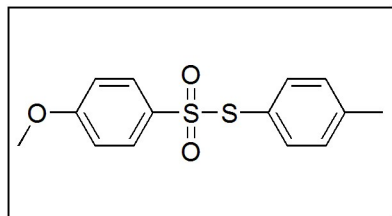
White solid (73%, 65.3 mg). mp 45 – 46 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.52 – 7.49 (m, 2H), 7.42 – 7.38 (m, 2H), 7.27 – 7.23 (m, 3H), 7.18 – 7.14 (m, 2H), 2.39 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.4, 141.5, 140.2, 136.5, 130.4, 129.1, 129.0, 124.1, 21.5. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₁ClNaO₂S₂ [M+Na]⁺ 320.9787, found 320.9771.

4-Bromo-benzenethiosulfonic acid S-p-tolyl ester (**3ga**)



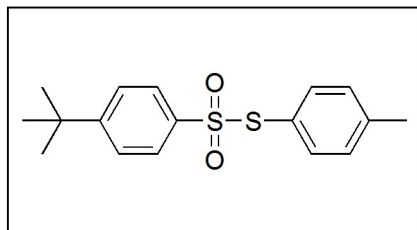
Yellow oil (78%, 80.0 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.59 – 7.54 (m, 2H), 7.45 – 7.40 (m, 2H), 7.28 – 7.23 (m, 2H), 7.19 – 7.14 (m, 2H), 2.39 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.4, 142.1, 136.4, 132.0, 130.4, 129.0, 128.7, 124.0, 21.5. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₁BrNaO₂S₂ [M+Na]⁺ 364.9281 and 366.9261, found 364.9272 and 366.9261.

4-Methoxy-benzenethiosulfonic acid S-p-tolyl ester (**3ha**)



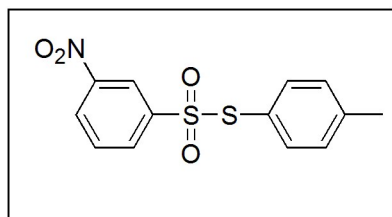
White solid (68%, 60.0 mg), mp 67-68 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.53 – 7.46 (m, 2H), 7.26 – 7.21 (m, 2H), 7.17 – 7.11 (m, 2H), 6.89 – 6.83 (m, 2H), 3.86 (s, 3H), 2.37 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 163.5, 142.0, 136.5, 135.1, 130.1, 129.9, 124.7, 113.8, 55.7, 21.4. **HRMS** (m/z) (ESI): calcd for C₁₄H₁₄NaO₃S₂ [M+Na]⁺ 317.0282, found 317.0272.

4-tert-Butyl-benzenethiosulfonic acid S-p-tolyl ester (**3ia**)



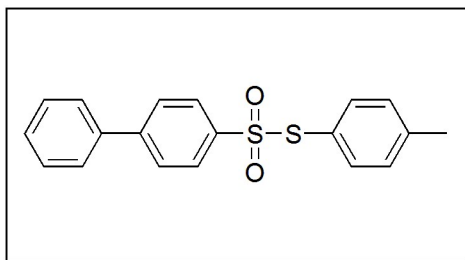
Yellow oil (78%, 74.8 mg), 97 – 98 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.52 – 7.48 (m, 2H), 7.44 – 7.40 (m, 2H), 7.25 – 7.22 (m, 2H), 7.15 – 7.10 (m, 2H), 2.38 (s, 3H), 1.33 (s, 9H). **¹³C NMR** (100 MHz, CDCl₃) δ 157.6, 142.0, 140.3, 136.5, 130.1, 127.4, 125.7, 124.6, 35.2, 31.0, 21.5. **HRMS** (m/z) (ESI): calcd for C₁₇H₂₁O₂S₂ [M+H]⁺ 321.0983, found 321.0972.

3-Nitro-benzenethiosulfonic acid S-p-tolyl ester (**3ja**)



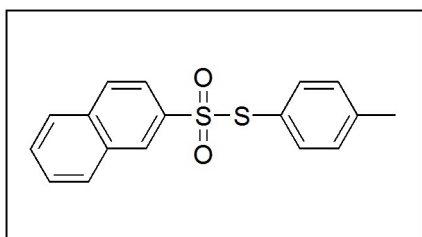
White solid (70%, 64.9 mg), mp 87-88 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 8.45 – 8.40 (m, 1H), 8.30-8.25 (m, 1H), 7.94 – 7.87 (m, 1H), 7.72 – 7.63 (m, 1H), 7.26 – 7.22 (m, 2H), 7.20 – 7.16 (m, 2H), 2.39 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 147.9, 144.6, 143.1, 136.4, 132.7, 130.7, 130.3, 127.9, 123.5, 122.9, 21.5. **HRMS** (m/z) (ESI): calcd for C₁₃H₁₁NNaO₄S₂ [M+Na]⁺ 332.0027, found 332.0031.

Biphenyl-4-thiosulfonic acid S-p-tolyl ester (**3ka**)



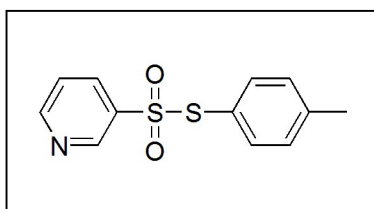
White solid (75%, 76.5 mg). mp 107 – 108 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.65 – 7.98 (m, 6H), 7.51 – 7.42 (m, 3H), 7.32 – 7.27 (m, 2H), 7.19 – 7.14 (m, 2H), 2.39 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 146.3, 142.2, 141.7, 138.9, 136.5, 130.2, 129.1, 128.7, 128.0, 127.3, 127.2, 124.4, 21.4. **HRMS** (m/z) (ESI): calcd for C₁₉H₁₇O₂S₂ [M+H]⁺ 341.0670, found 341.0661.

Naphthalene-2-thiosulfonic acid S-p-tolyl ester (**3la**)



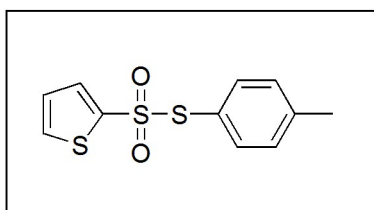
Yellow oil (80%, 75.4 mg). mp 105 – 106 °C. **¹H NMR** (400 MHz, Chloroform-*d*) δ 8.00 – 7.97 (m, 1H), 7.92 – 7.88 (m, 2H), 7.82-7.78 (m, 1H), 7.69 – 7.64 (m, 2H), 7.61 – 7.56 (m, 1H), 7.22 – 7.17 (m, 2H), 7.11 – 7.04 (m, 2H), 2.35 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 142.1, 139.7, 136.5, 135.0, 131.6, 130.2, 129.4, 129.3, 129.2, 129.2, 127.9, 127.6, 124.5, 122.4, 21.4. **HRMS** (m/z) (ESI): calcd for C₁₇H₁₅O₂S₂ [M+H]⁺ 315.0513, found 315.0505.

Pyridine-3-thiosulfonic acid S-p-tolyl ester (**3ma**)



Yellow oil (72%, 57.2 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 8.82 – 8.76 (m, 1H), 8.70 – 8.65 (m, 1H), 7.92 – 7.85 (m, 1H), 7.44 – 7.37 (m, 1H), 7.29 – 7.22 (m, 2H), 7.20 – 7.15 (m, 2H), 2.39 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 176.5, 153.8, 148.2, 142.8, 139.5, 136.4, 134.9, 130.6, 123.6, 21.46. **HRMS** (m/z) (ESI): calcd for C₁₂H₁₂NO₂S₂ [M+H]⁺ 266.0309, found 266.0302.

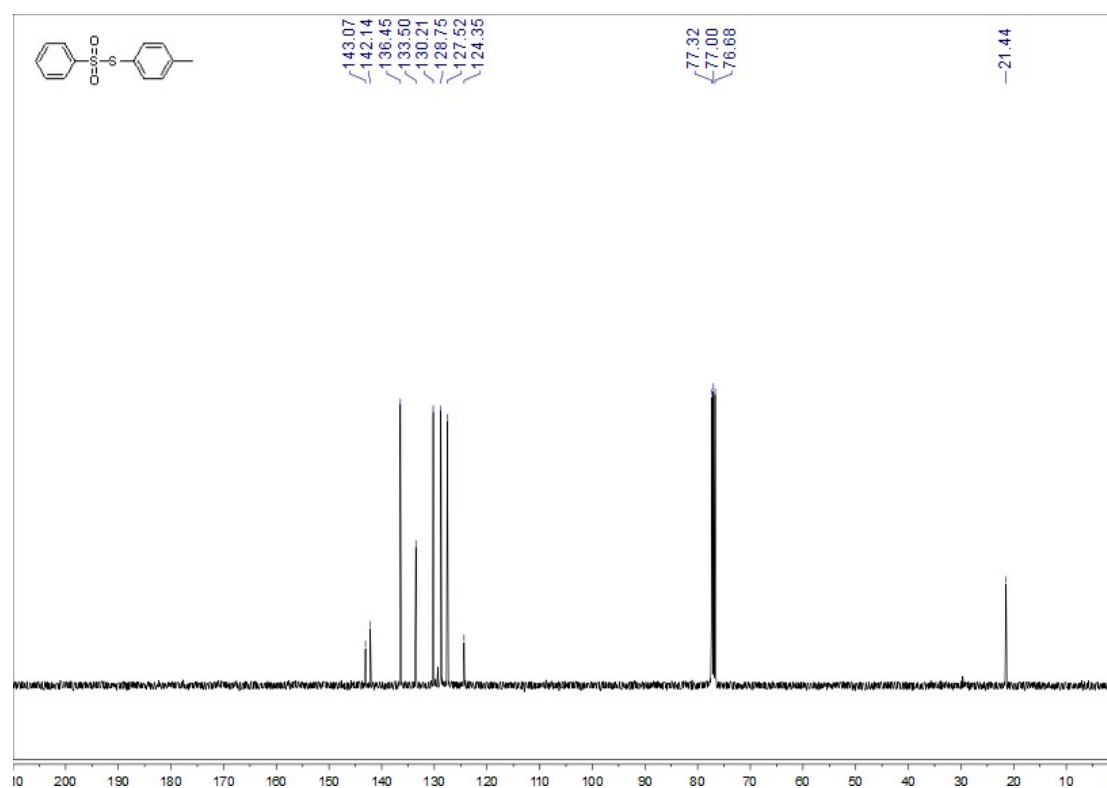
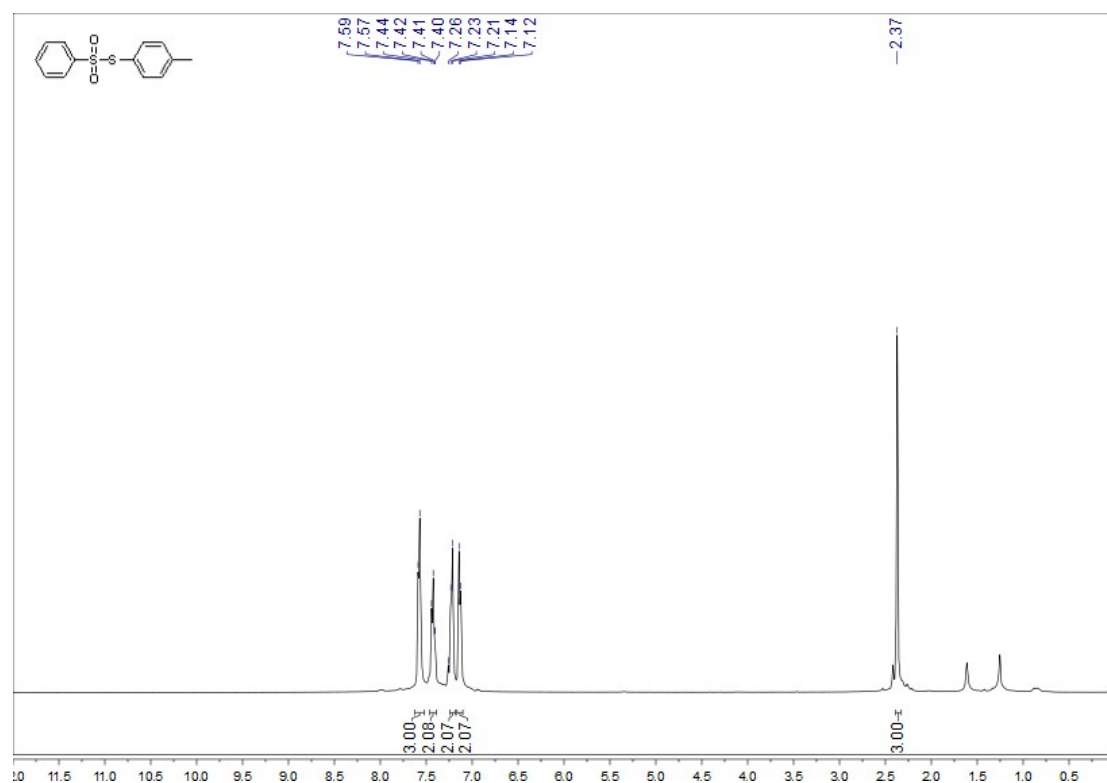
Thiophene-2-thiosulfonic acid S-p-tolyl ester (**3na**)



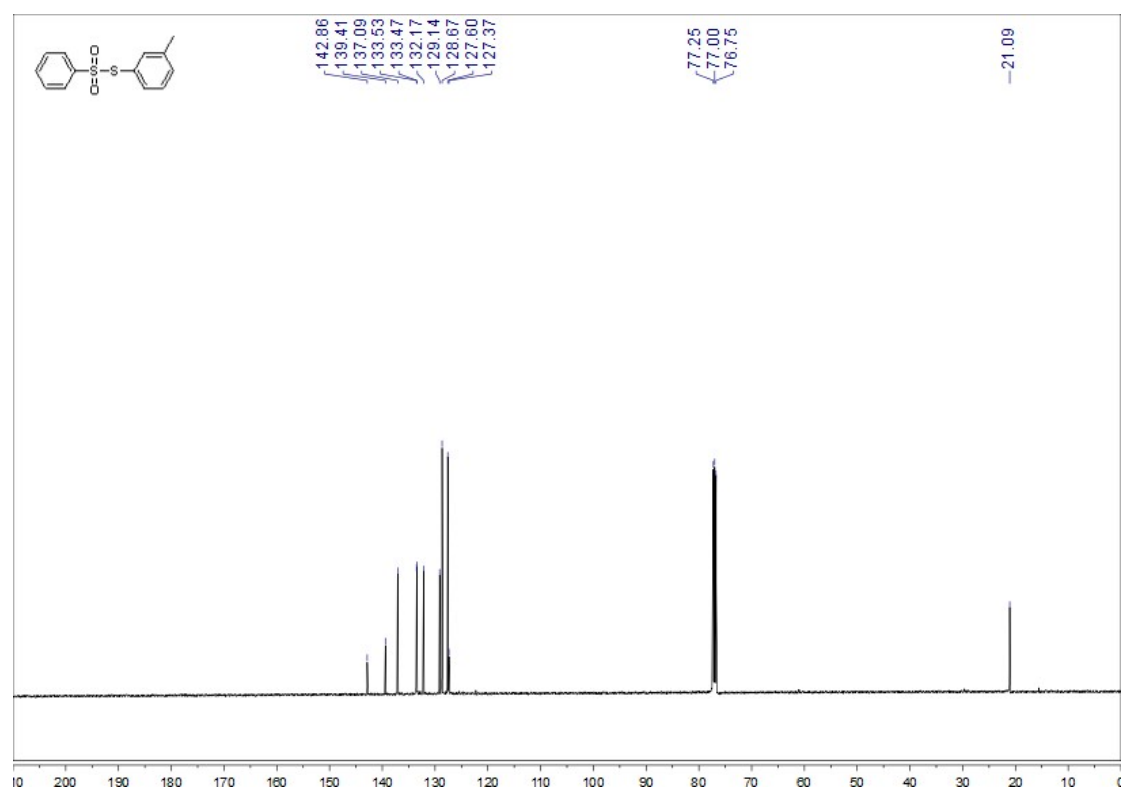
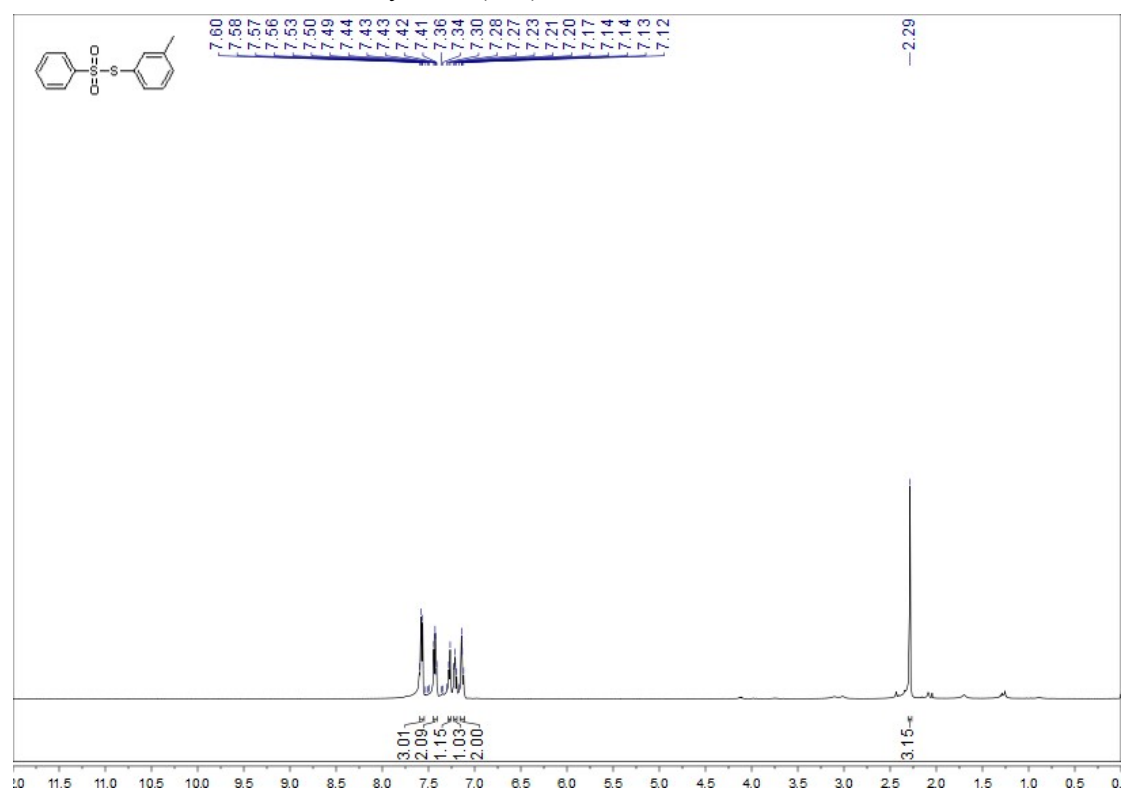
Yellow oil (78%, 63.18 mg). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.65 – 7.60 (m, 1H), 7.33 – 7.27 (m, 3H), 7.20 – 7.15 (m, 2H), 7.02 – 6.98 (m, 1H), 2.38 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 143.7, 142.4, 136.4, 133.9, 133.7, 130.3, 127.0, 124.5, 21.5. **HRMS** (m/z) (ESI): calcd for C₁₁H₁₀NaO₂S₃ [M+Na]⁺ 292.9741, found 292.9729.

Copies of ^{13}C and ^1H NMR spectra for all products

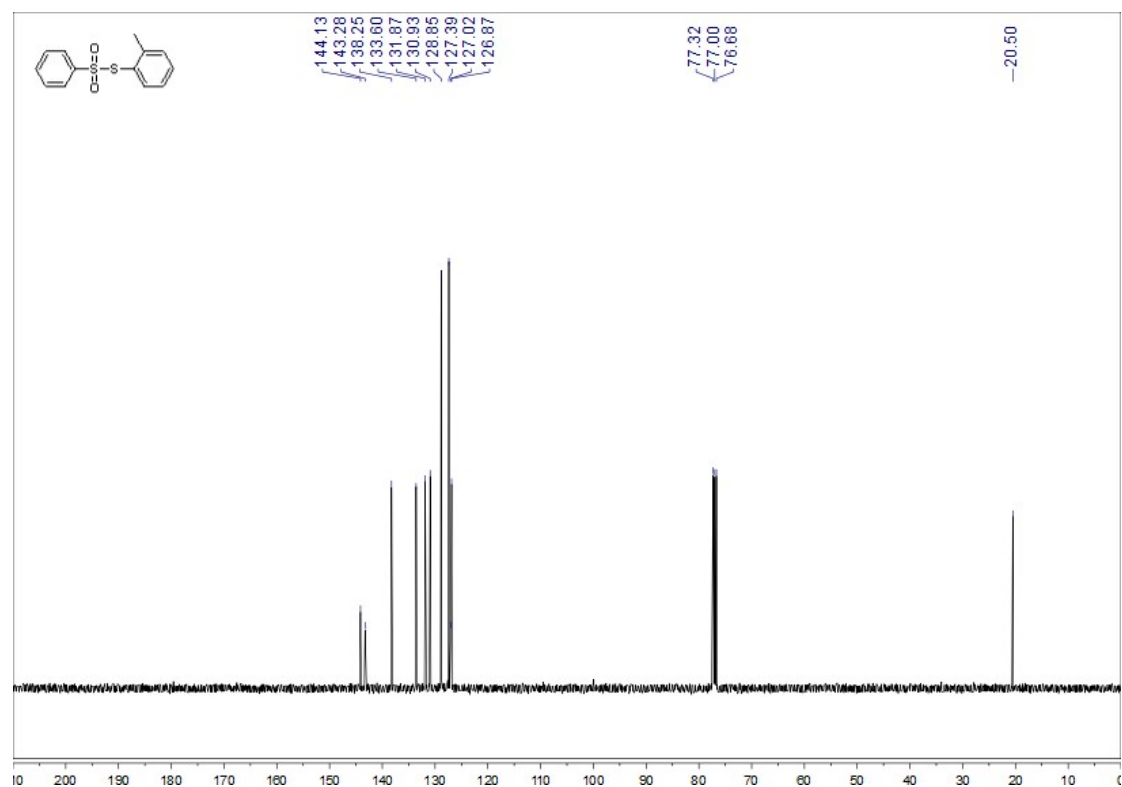
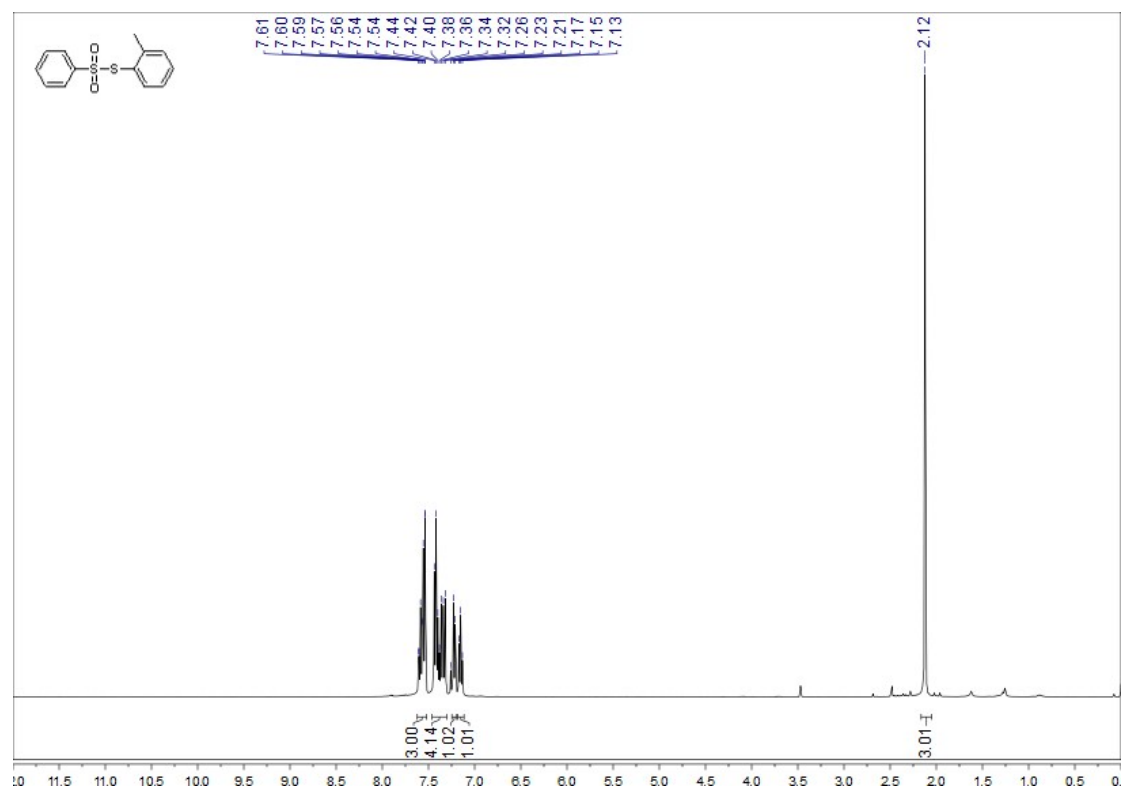
Benzenethiosulfonic acid S-p-tolyl ester (**3aa**)



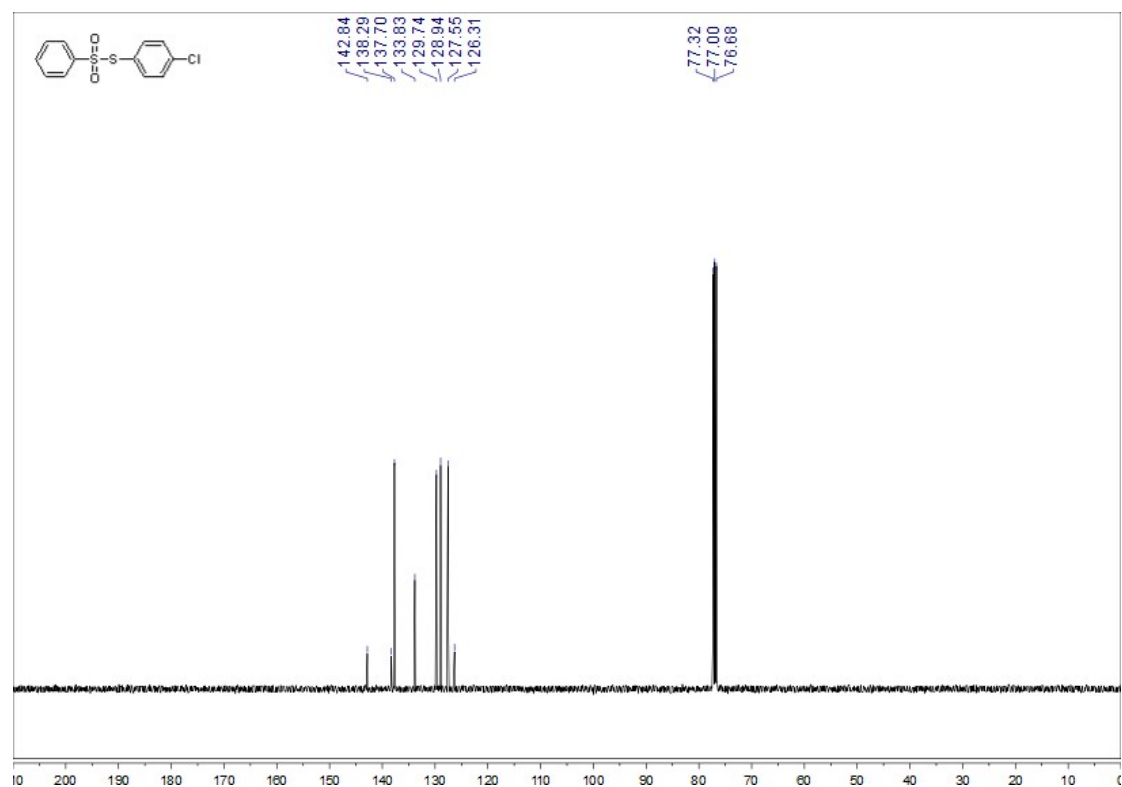
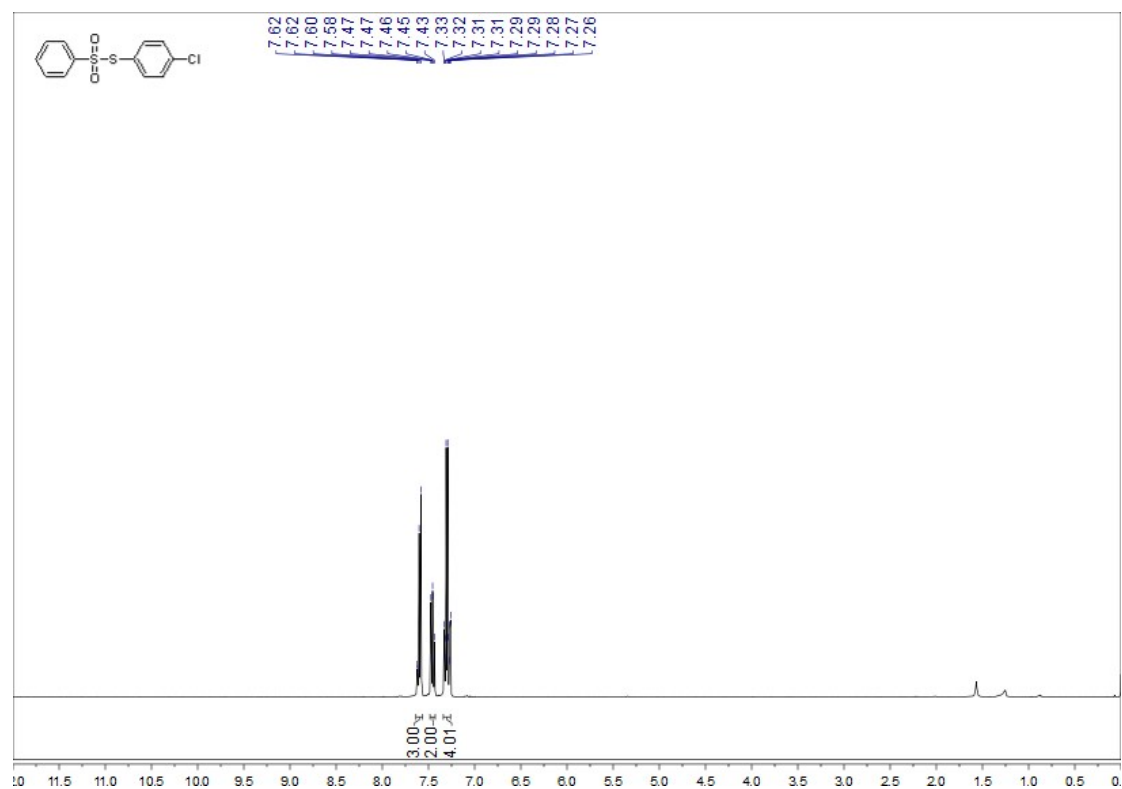
Benzenethiosulfonic acid S-m-tolyl ester (**3ab**)



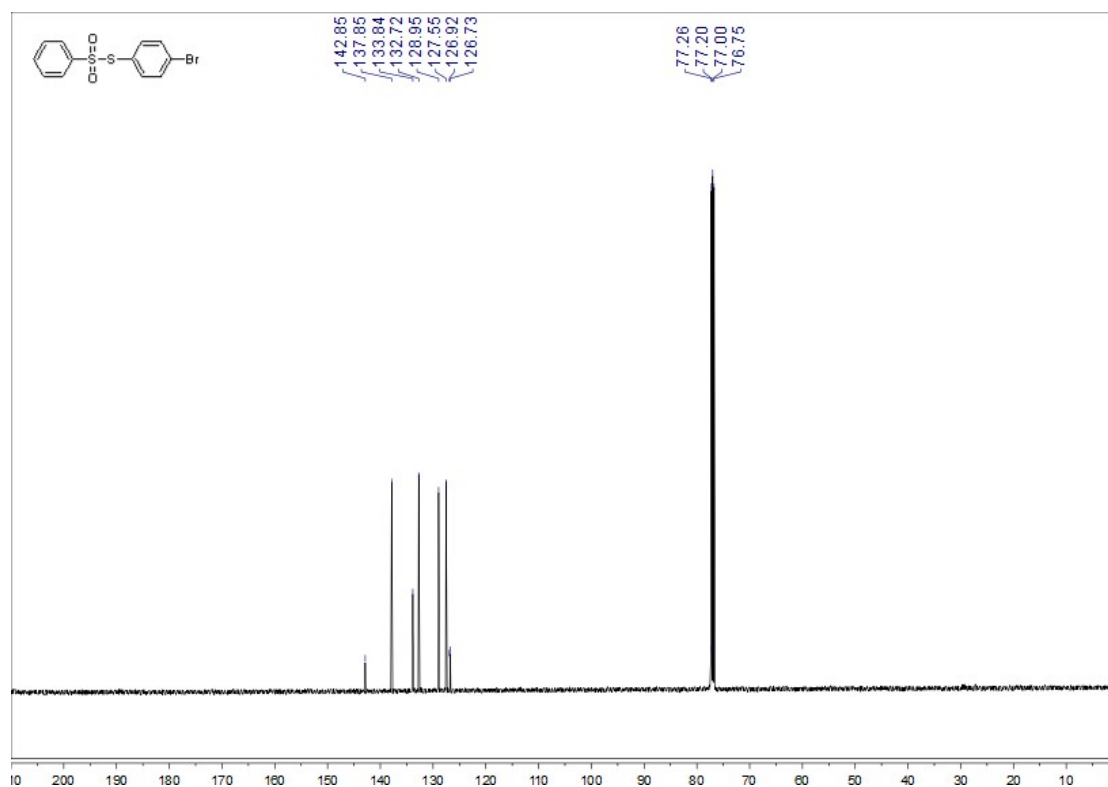
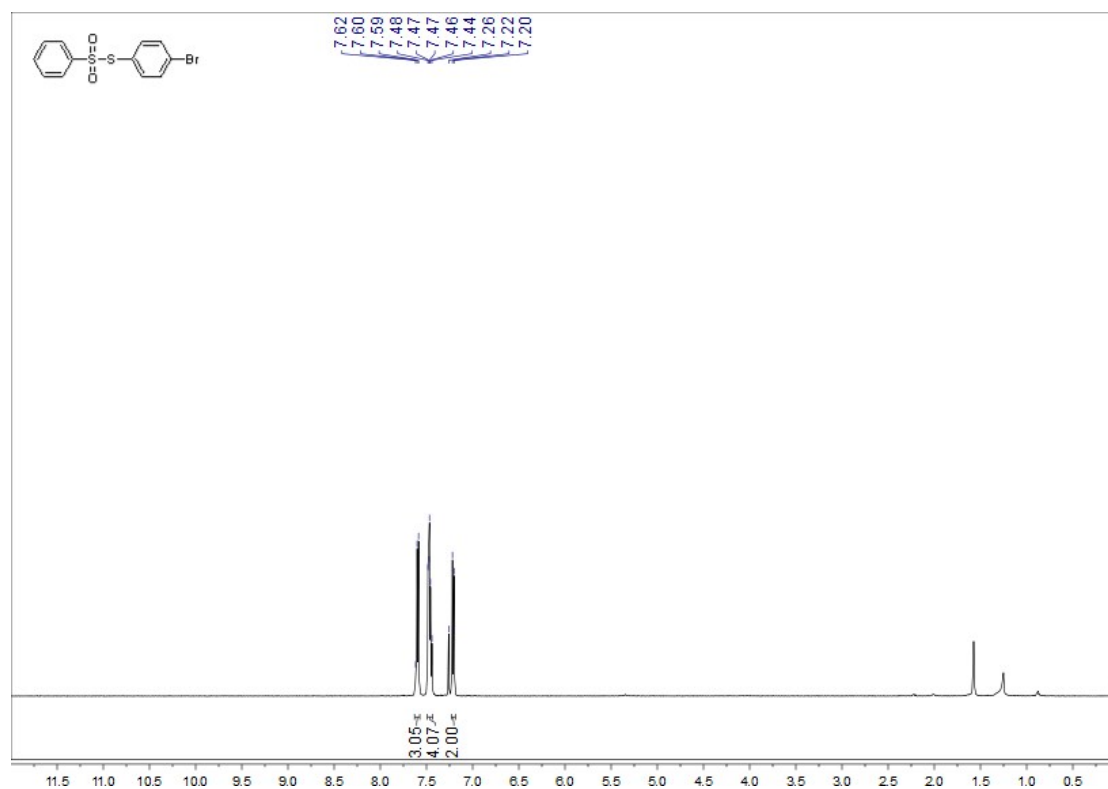
Benzenethiosulfonic acid S-o-tolyl ester (**3ac**)



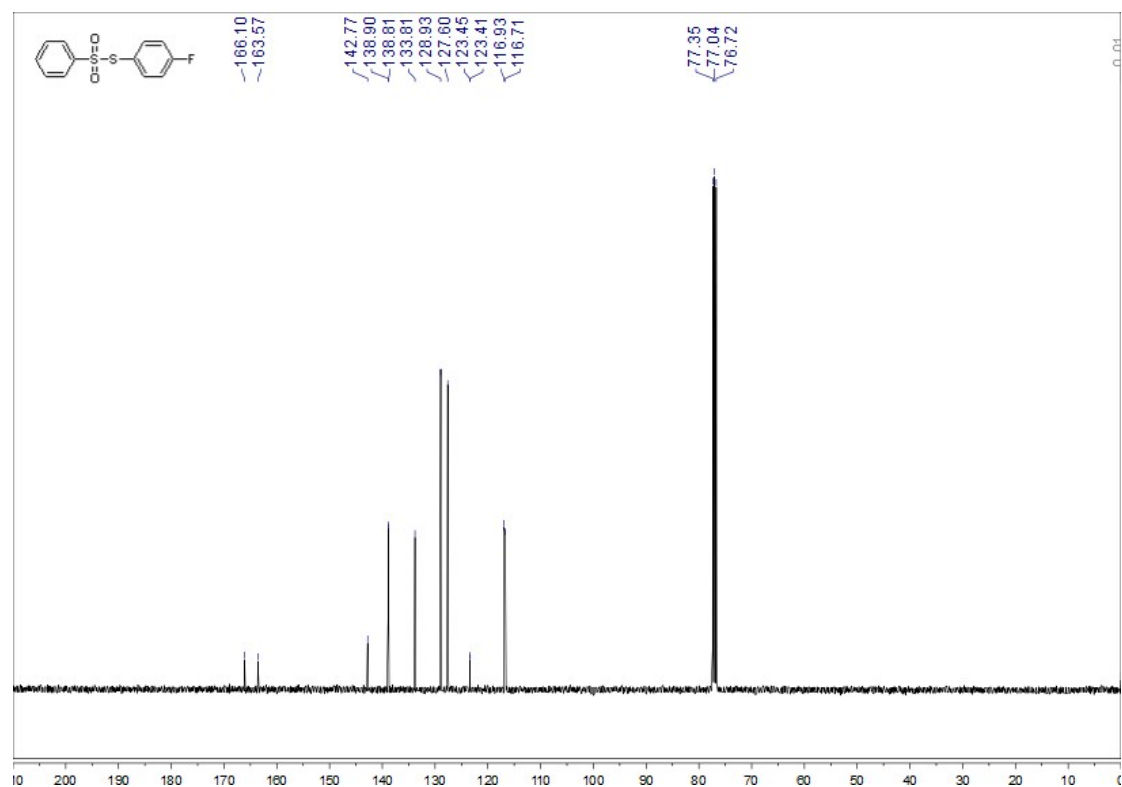
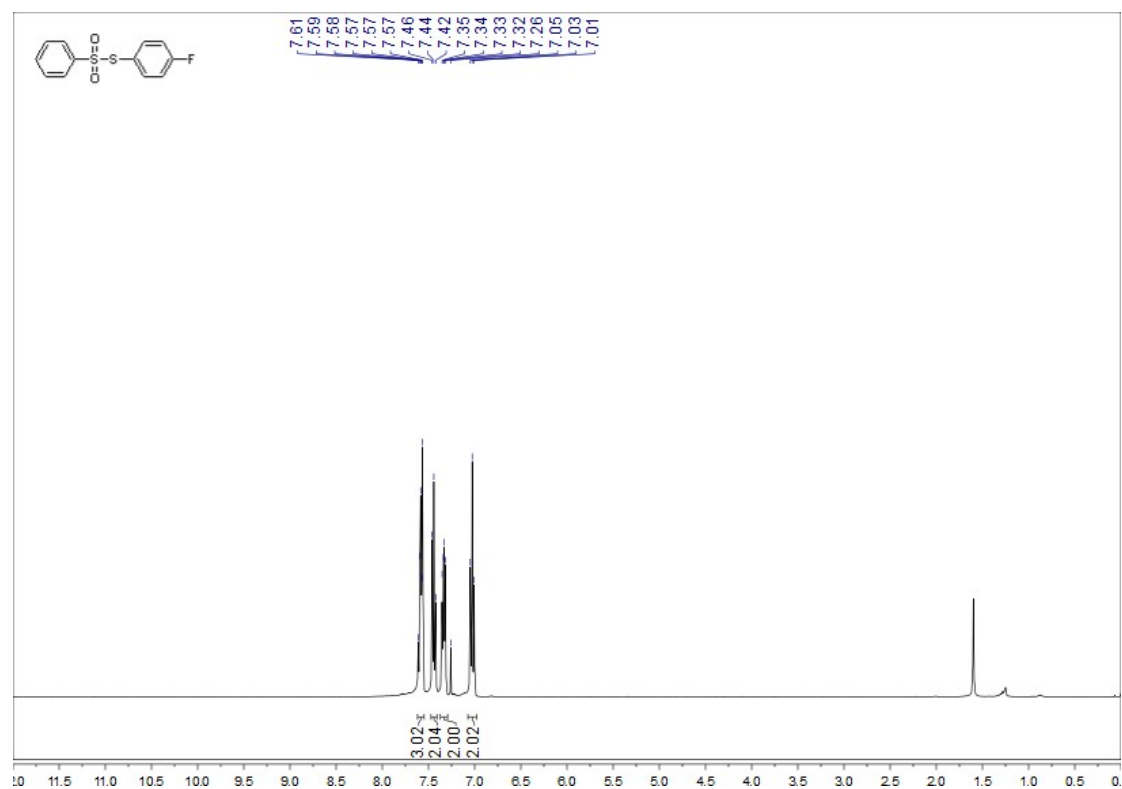
Benzenethiosulfonic acid S-(4-chloro-phenyl) ester (**3ad**)



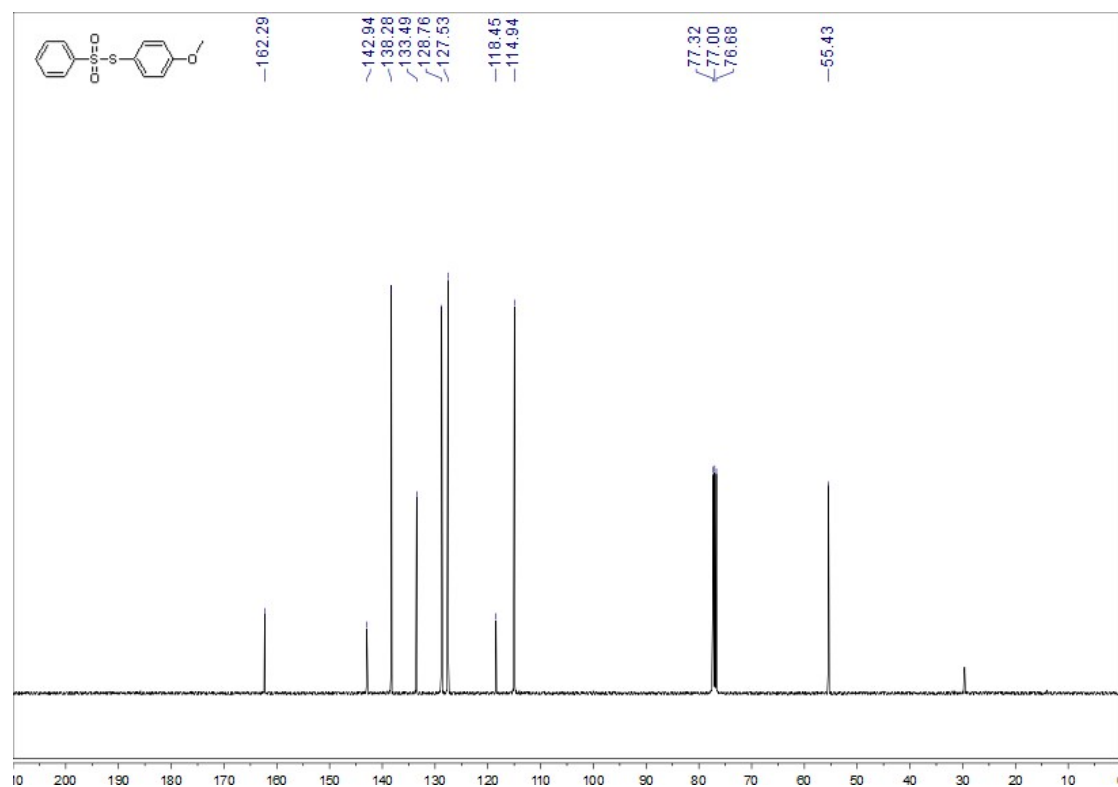
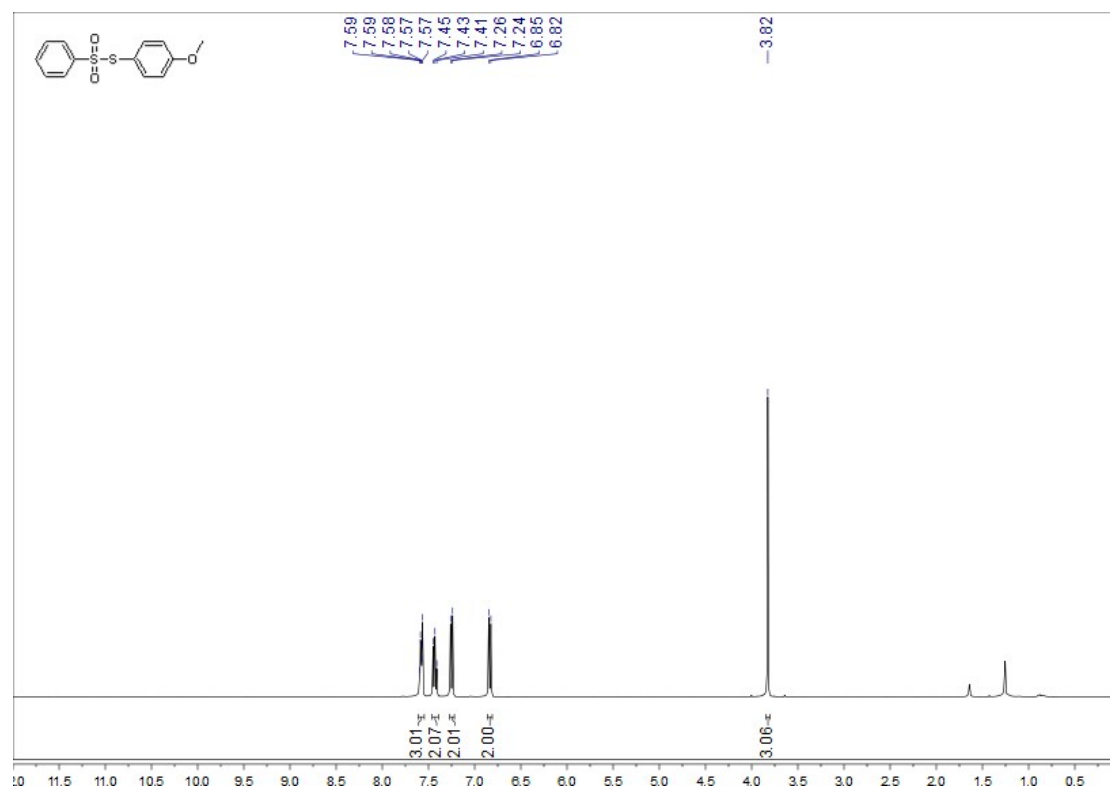
Benzenethiosulfonic acid S-(4-bromo-phenyl) ester (**3ae**)



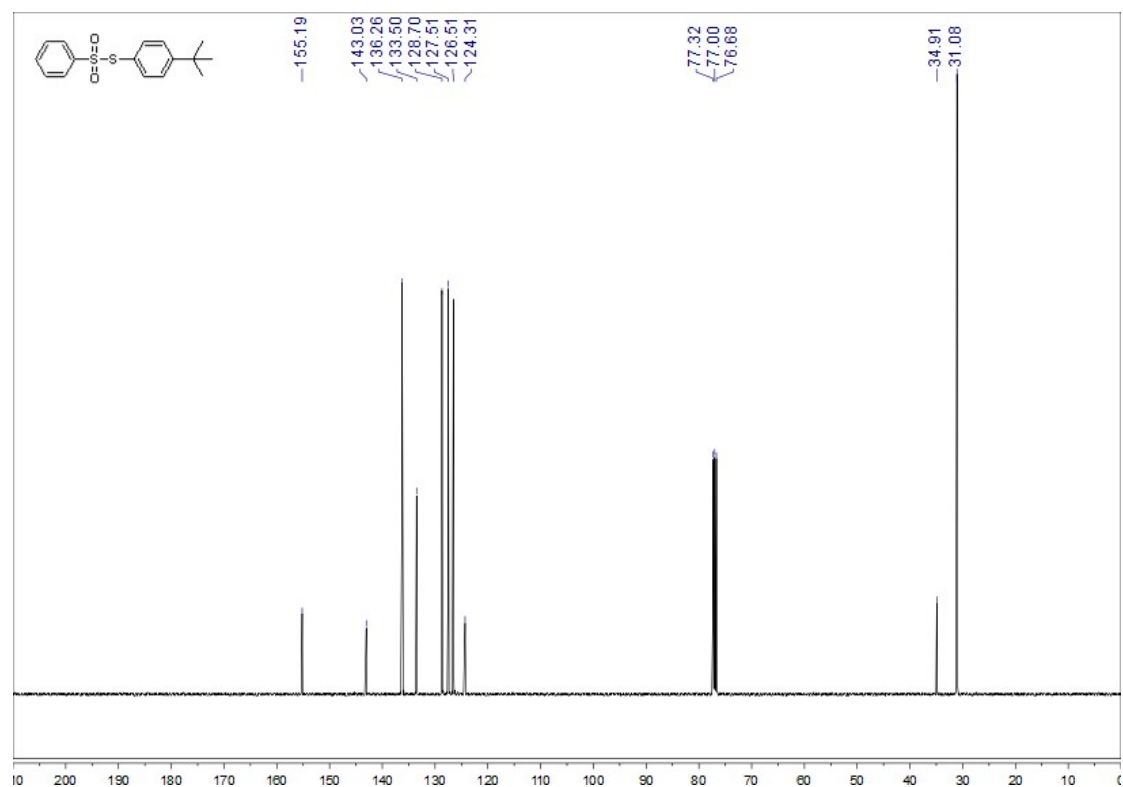
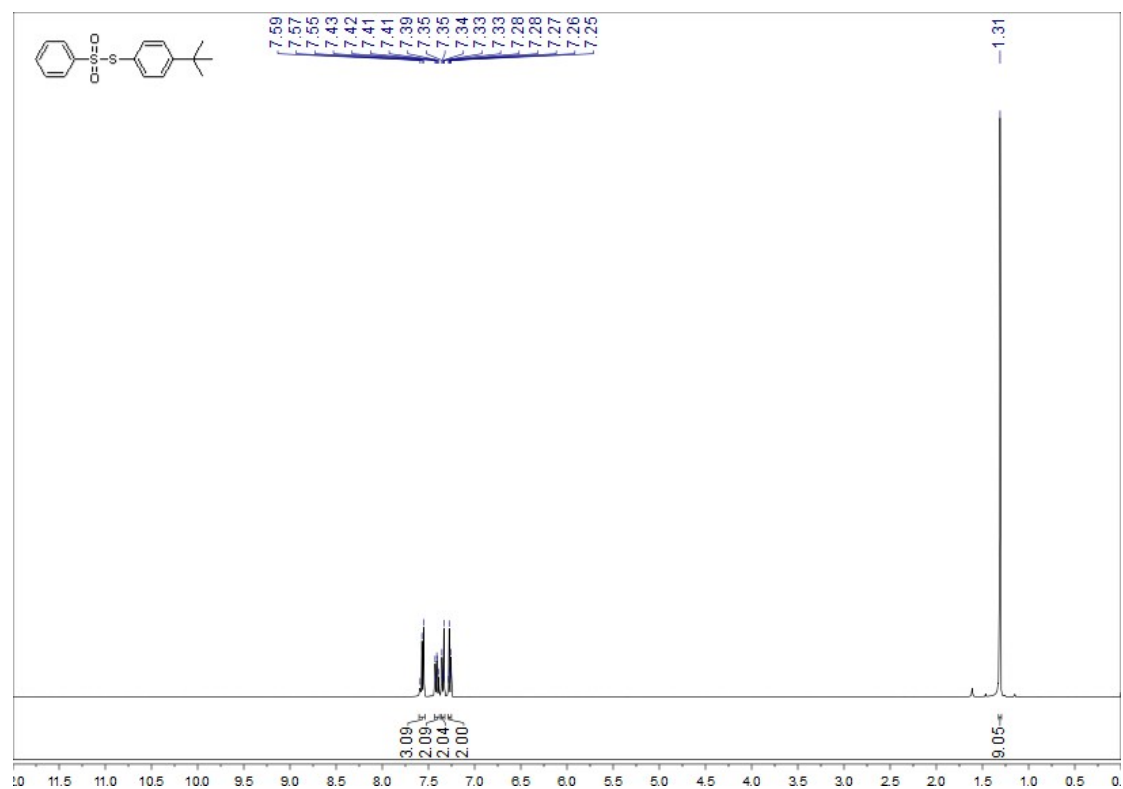
Benzenethiosulfonic acid S-(4-fluoro-phenyl) ester (**3af**)



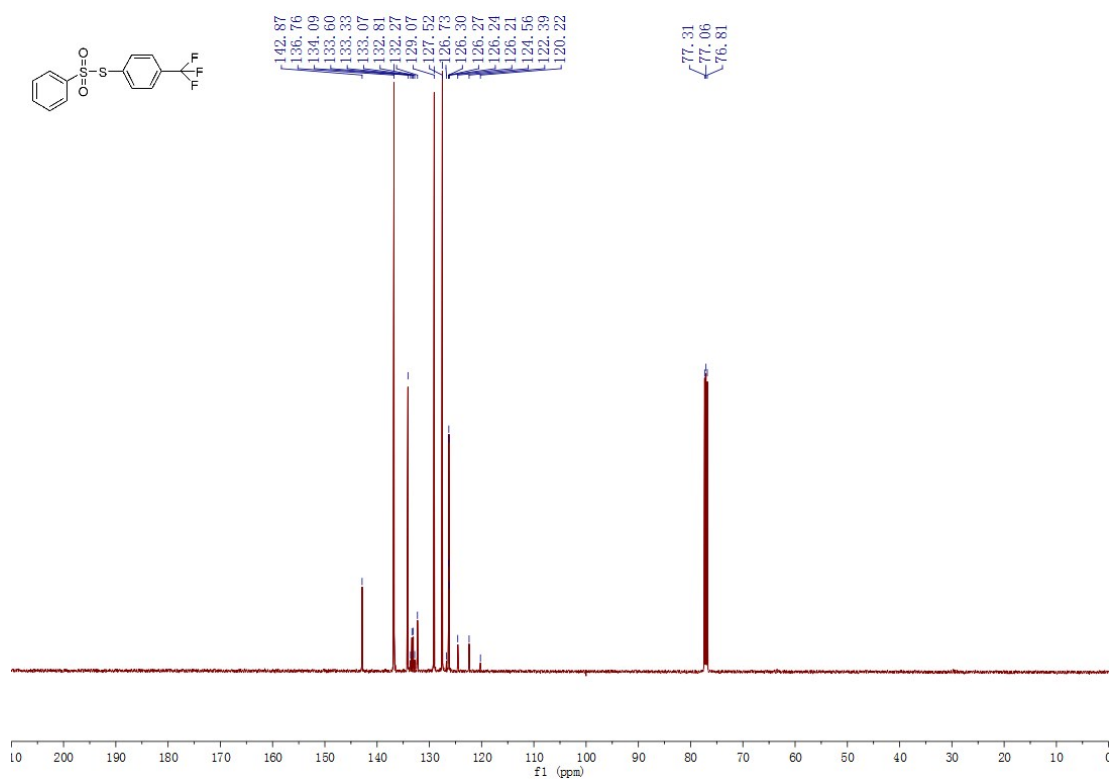
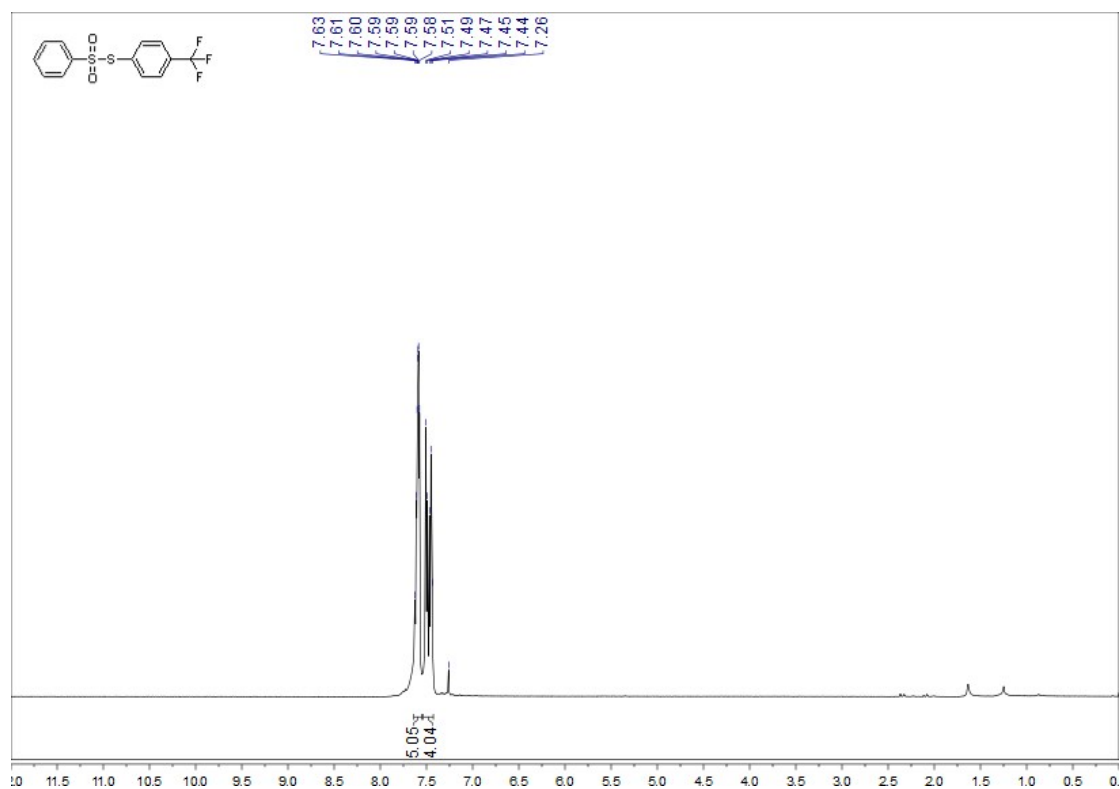
Benzenethiosulfonic acid S-(4-methoxy-phenyl) ester (**3ag**)



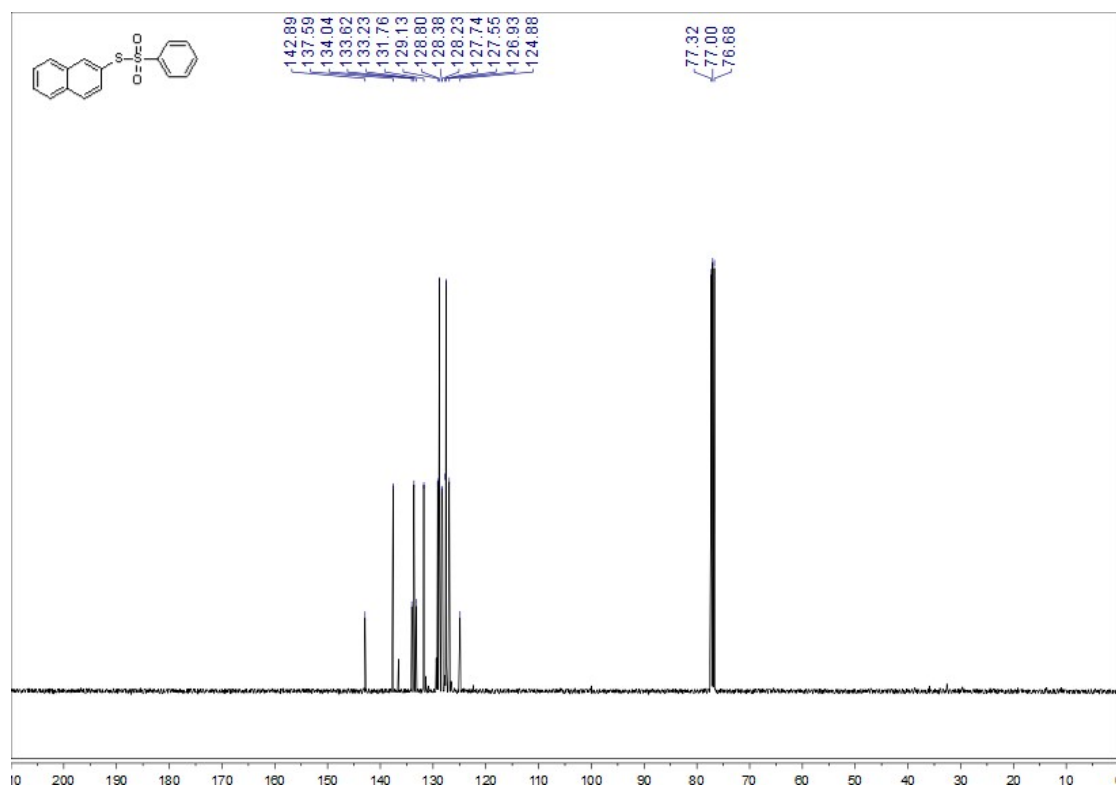
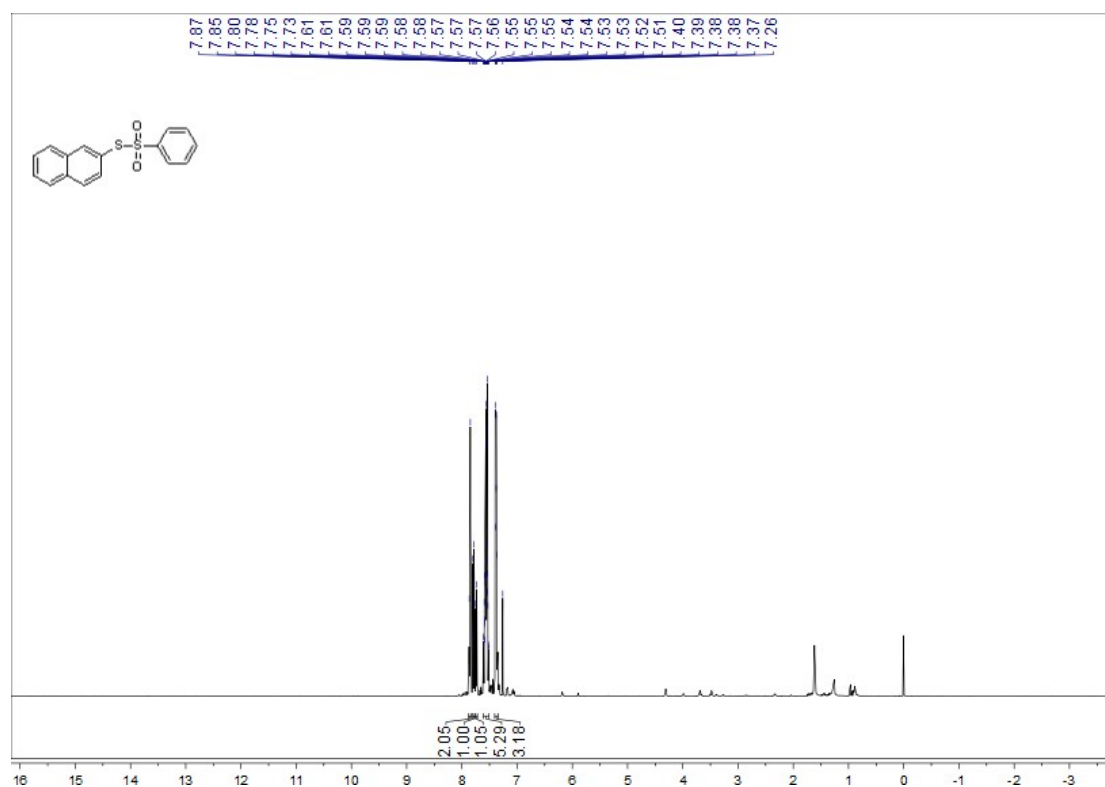
Benzenethiosulfonic acid S-(4-tert-butyl-phenyl) ester (**3ah**)



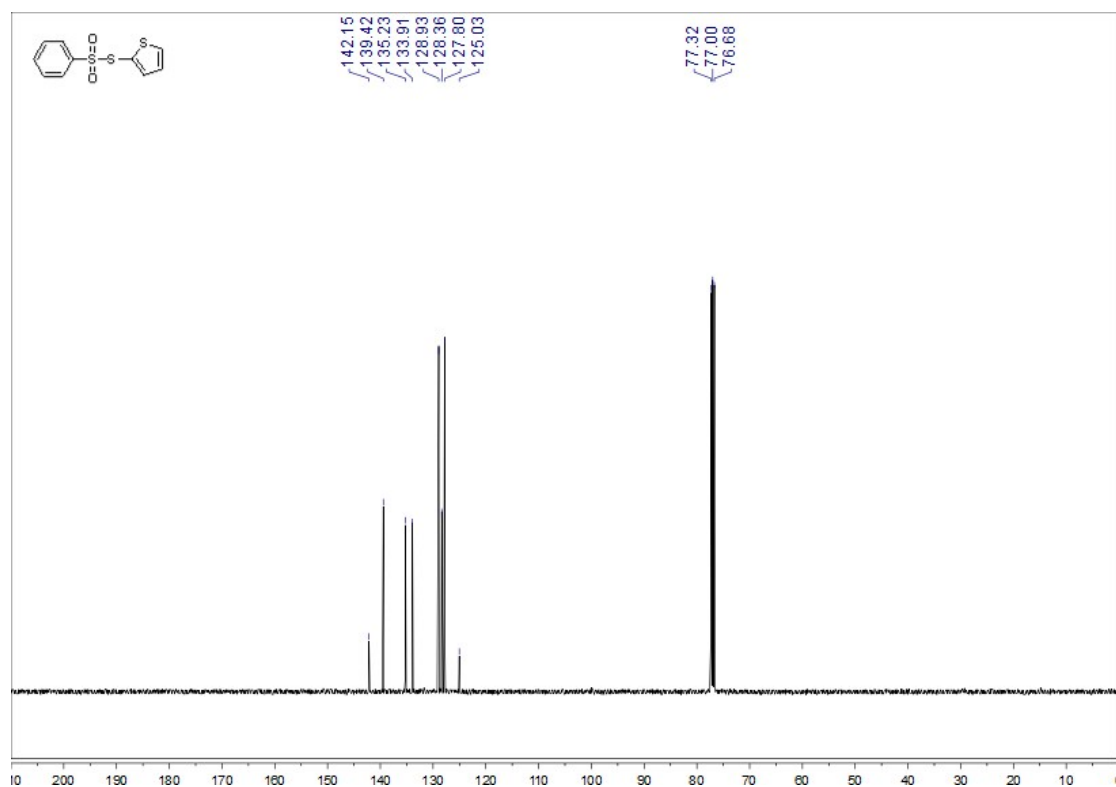
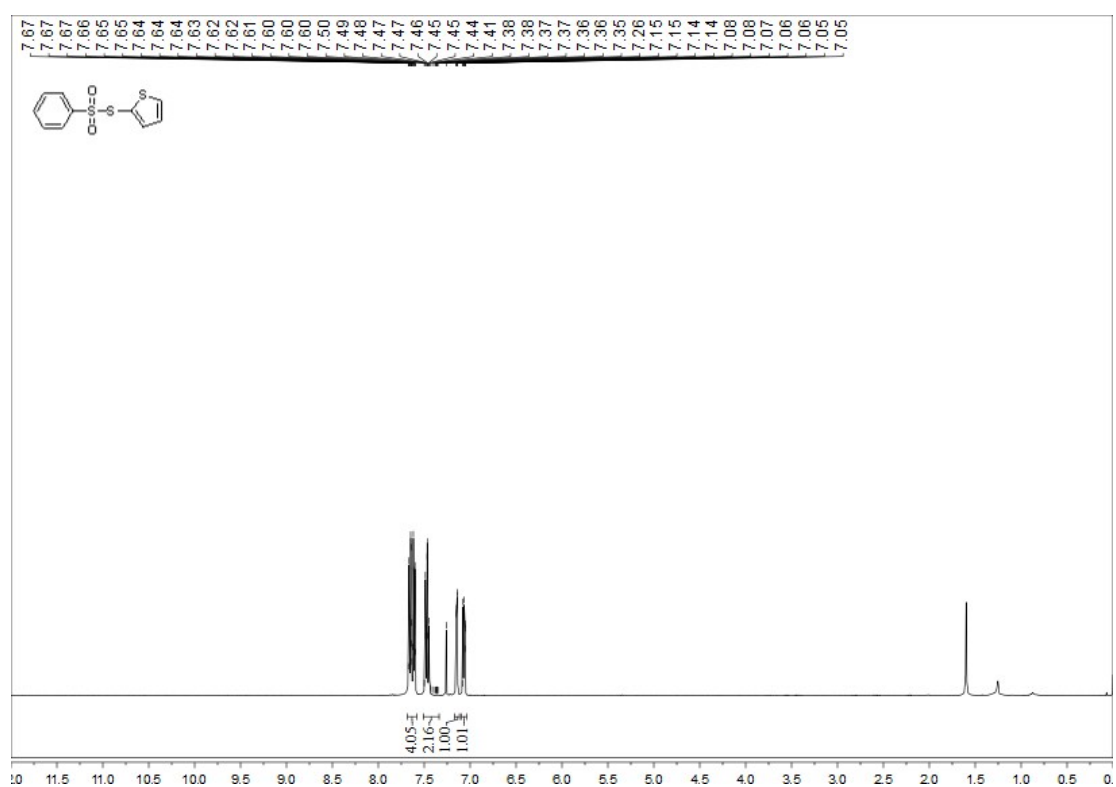
Benzenethiosulfonic acid S-(4-trifluoromethyl-phenyl) ester (**3ai**)



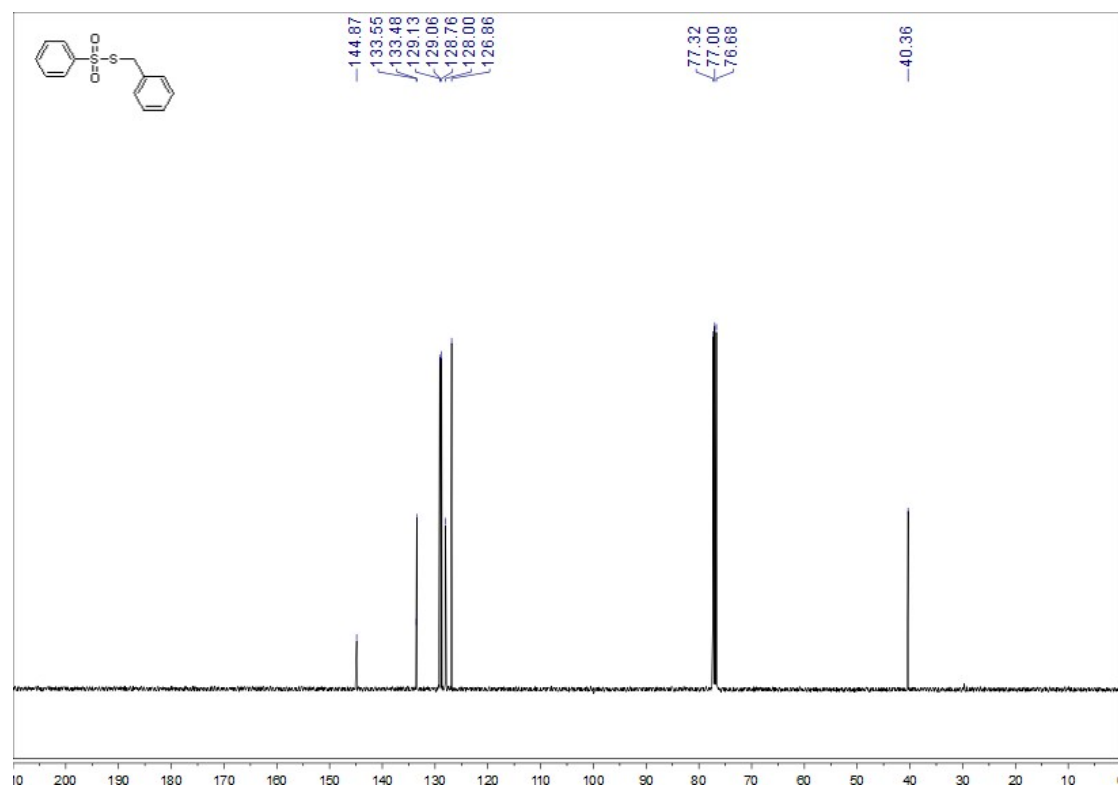
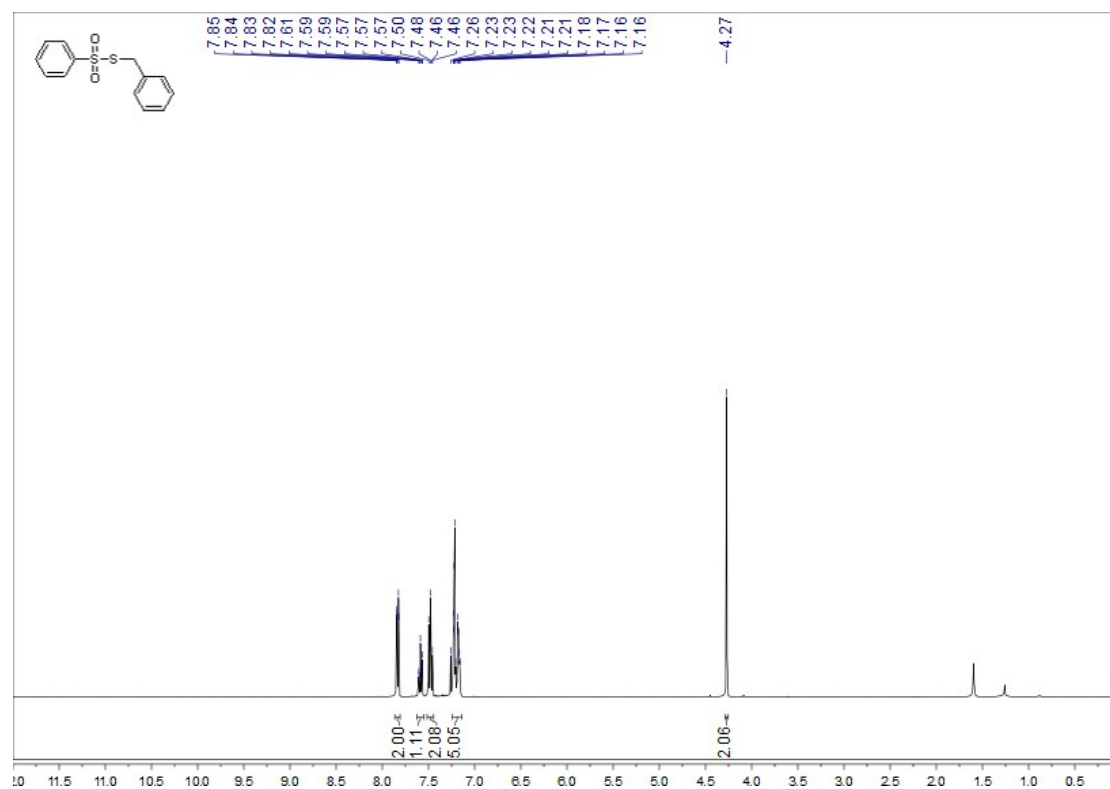
Benzenethiosulfonic acid S-naphthalen-2-yl ester (**3aj**)



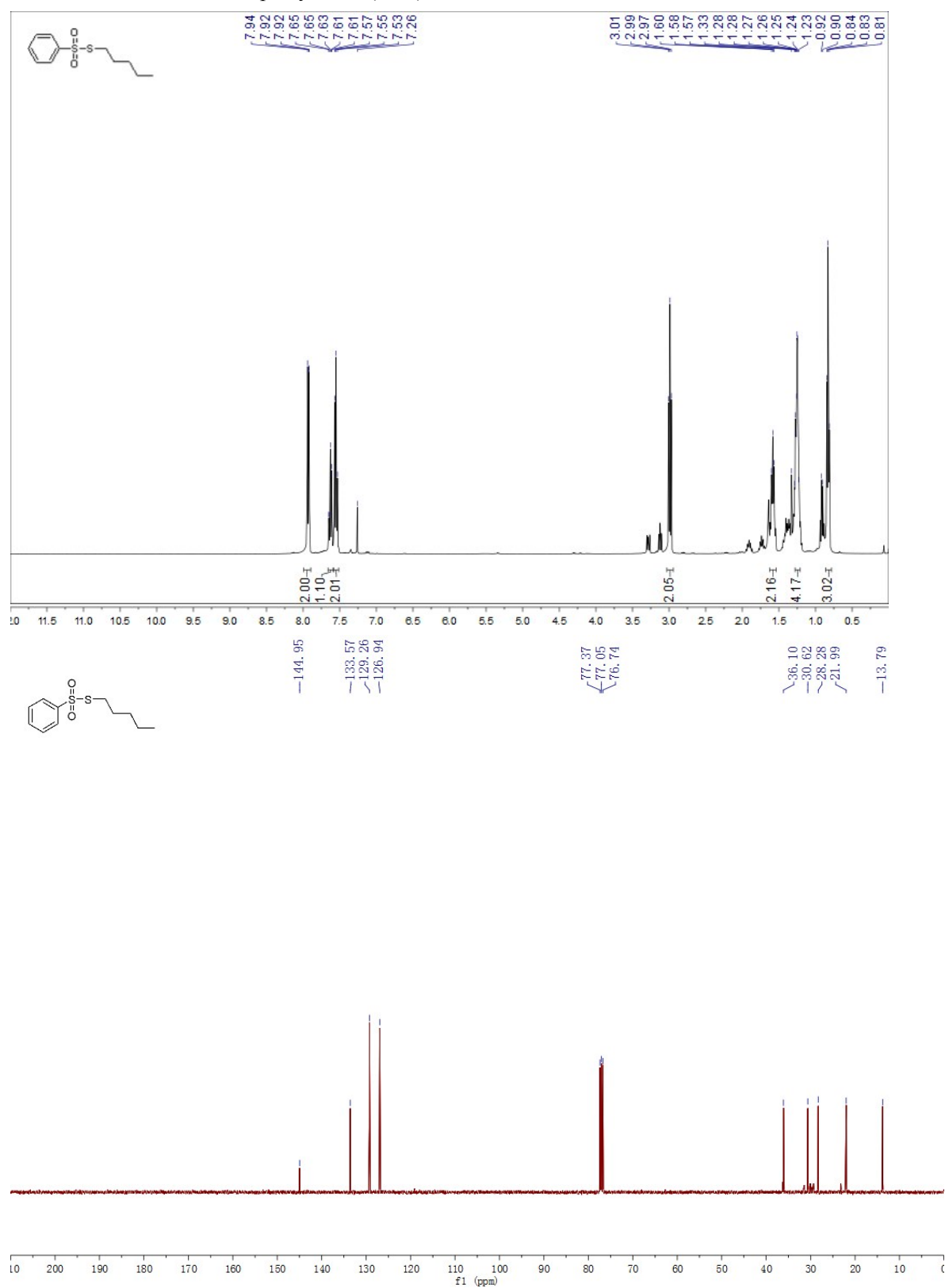
Benzenethiosulfonic acid S-thiophen-2-yl ester (**3ak**)



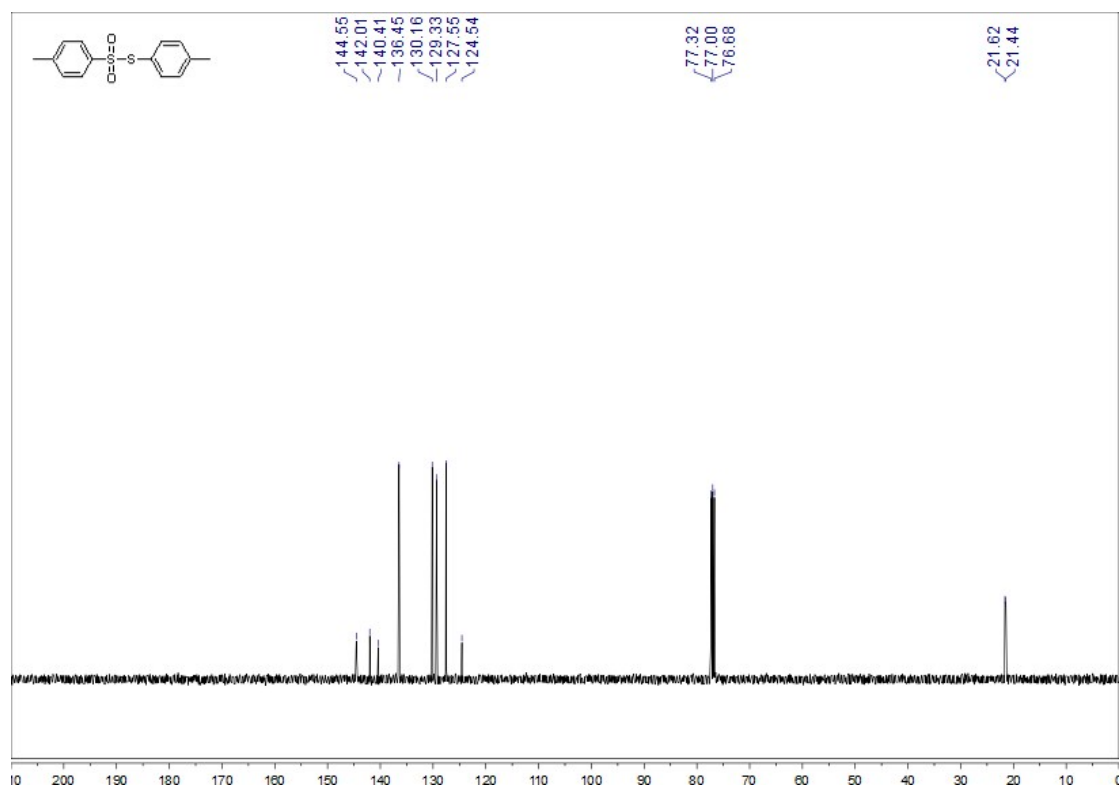
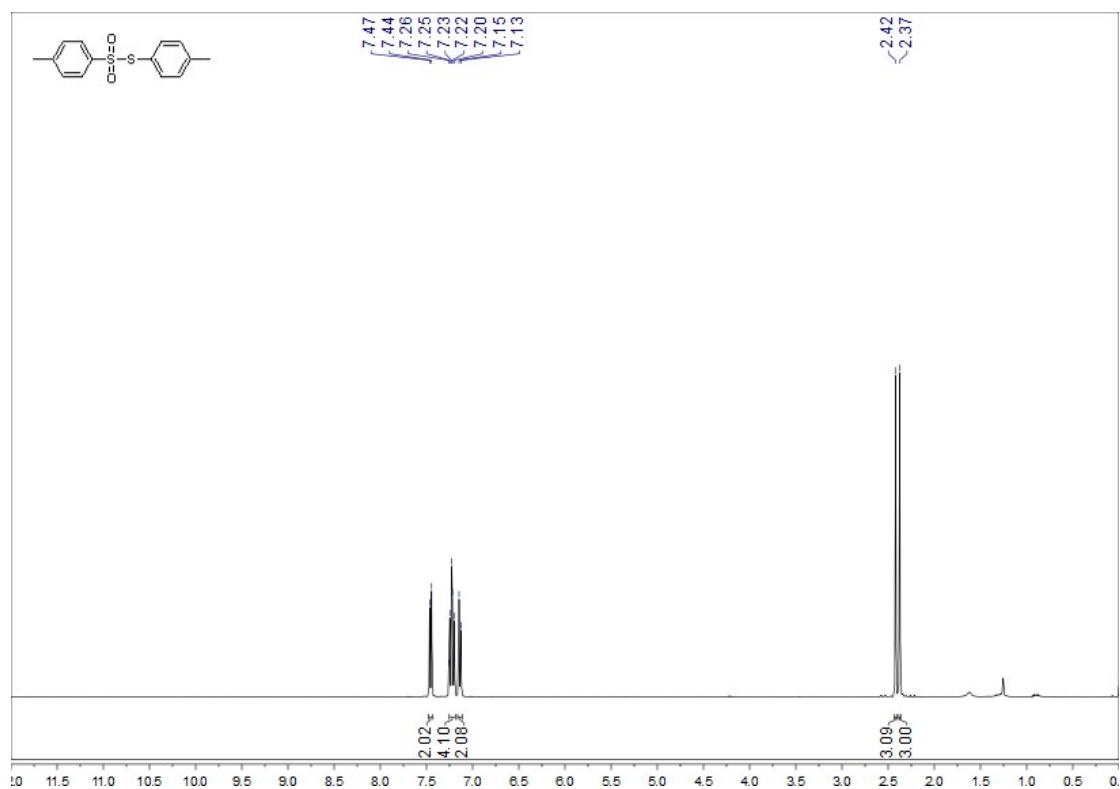
Benzenethiosulfonic acid S-benzyl ester (**3al**)



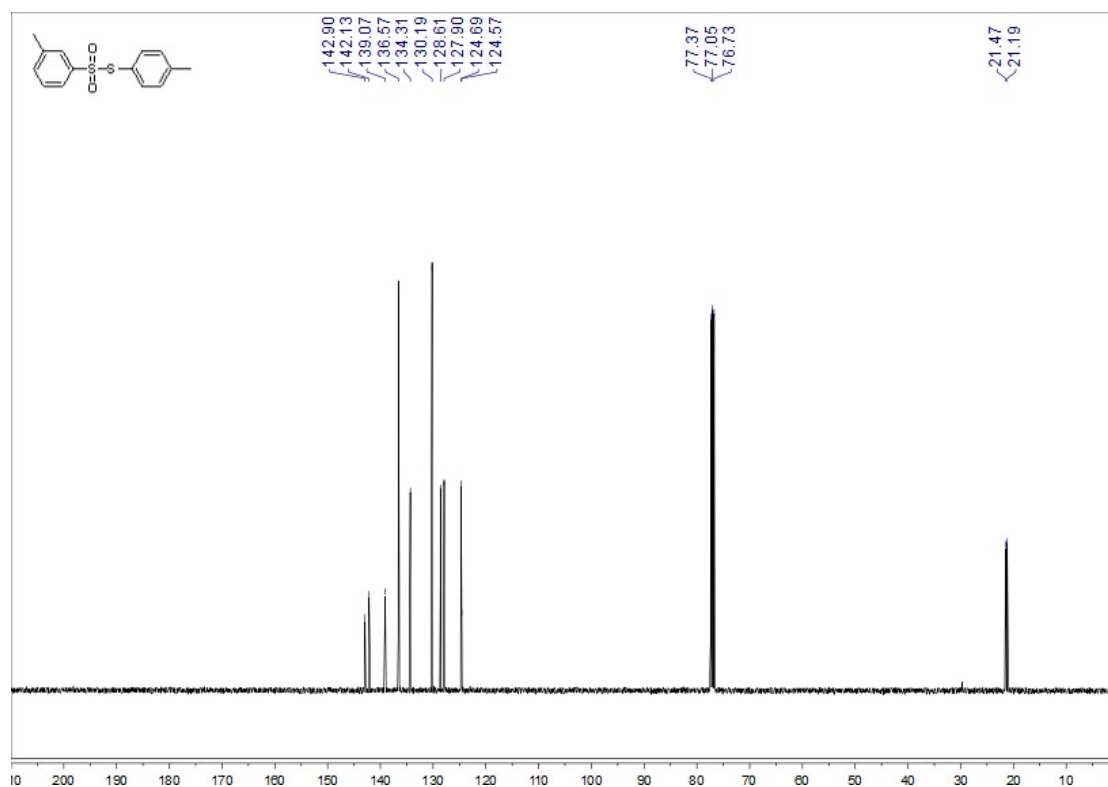
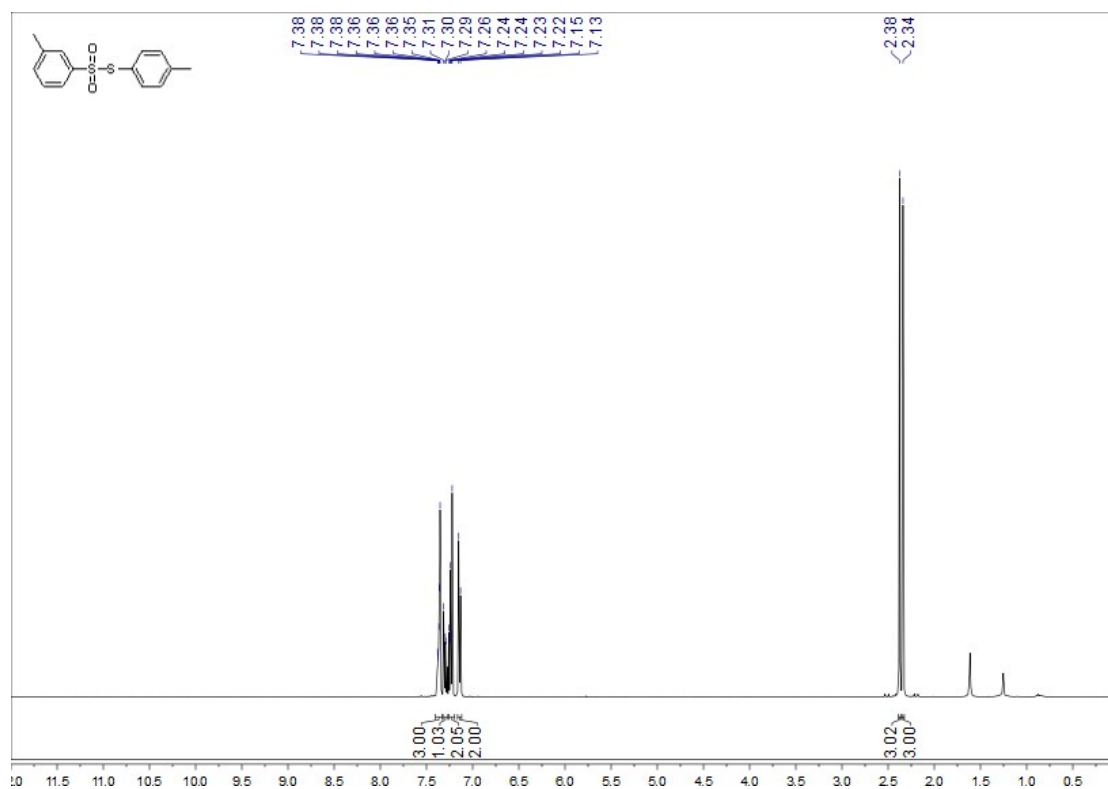
Benzenethiosulfonic acid S-pentyl ester (**3am**)



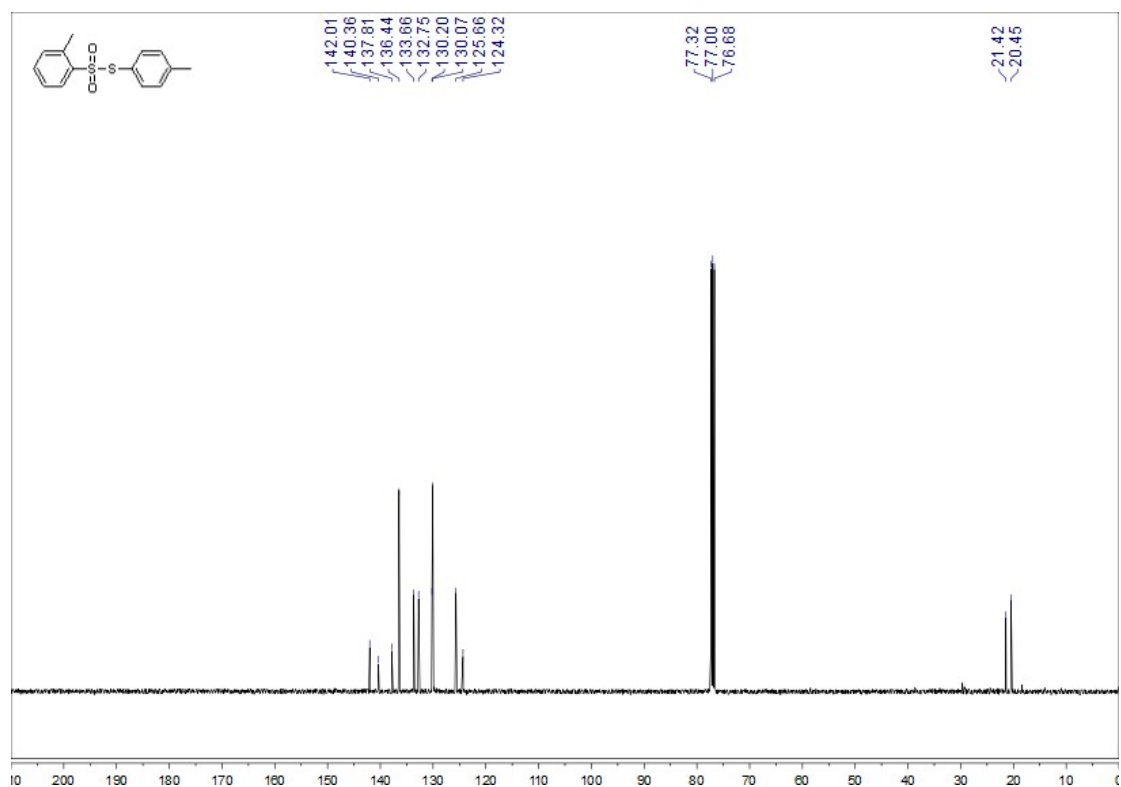
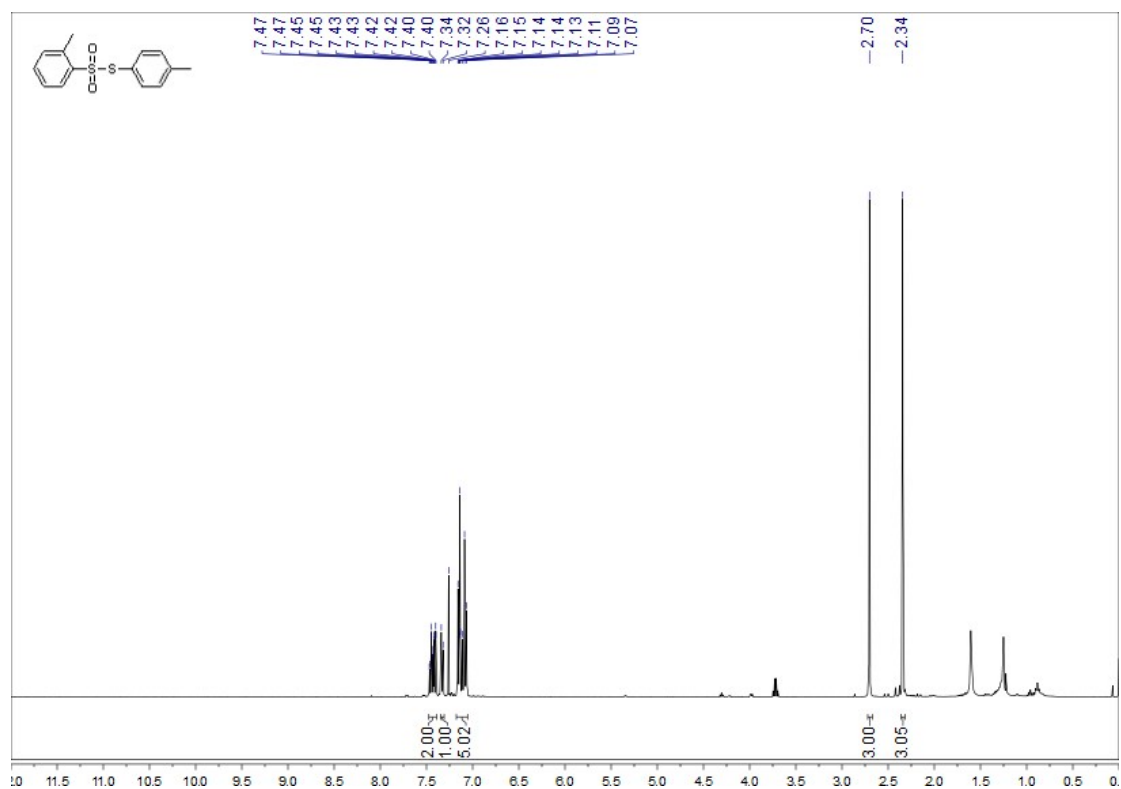
Toluene-4-thiosulfonic acid S-p-tolyl ester (**3ba**)



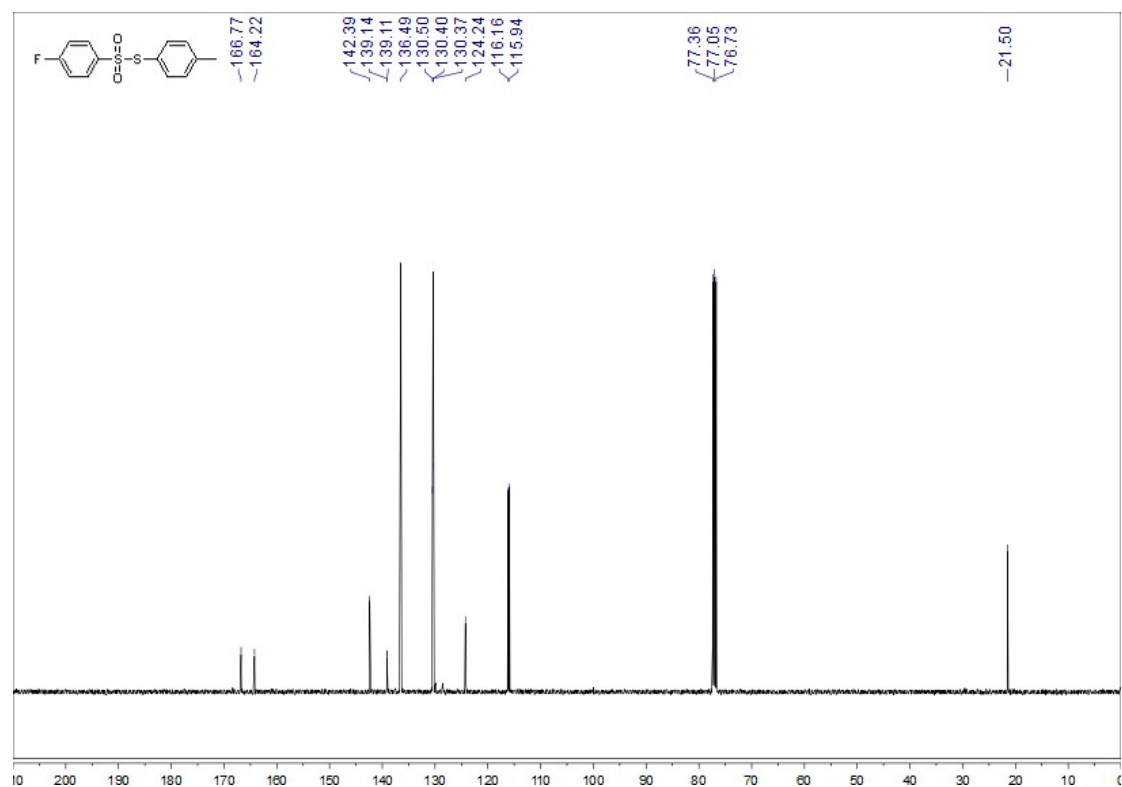
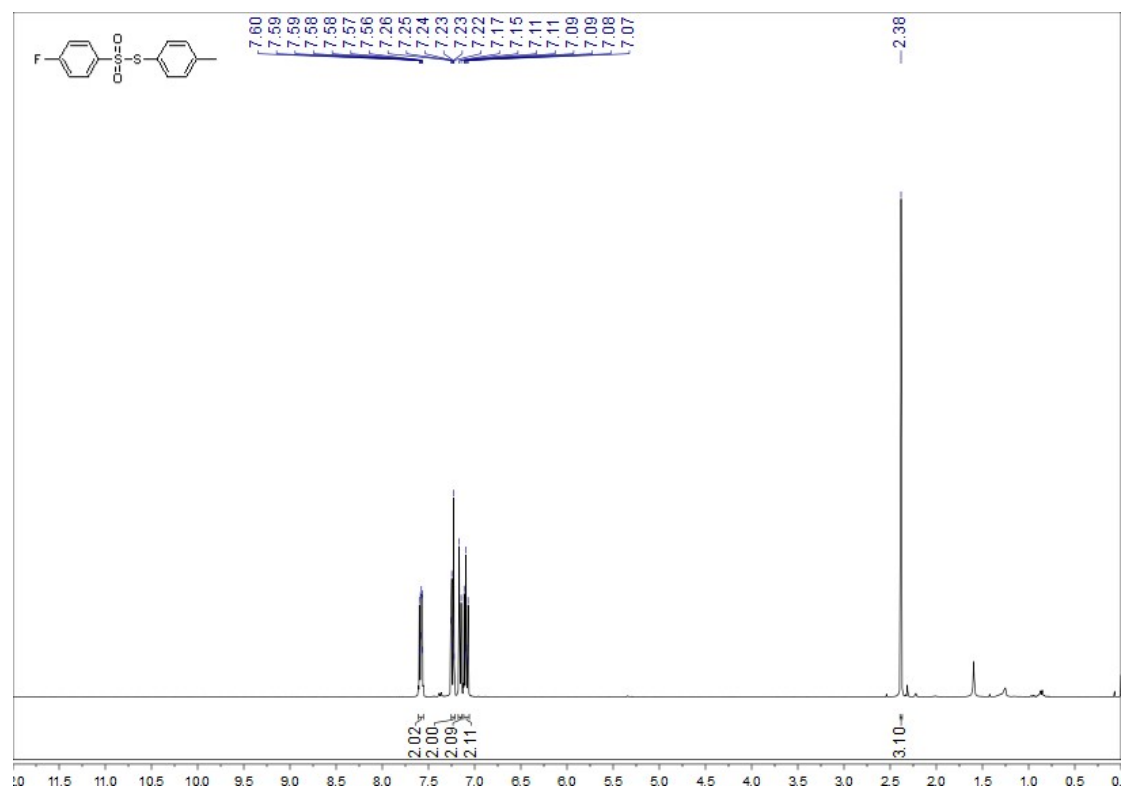
Toluene-3-thiosulfonic acid S-p-tolyl ester (**3ca**)



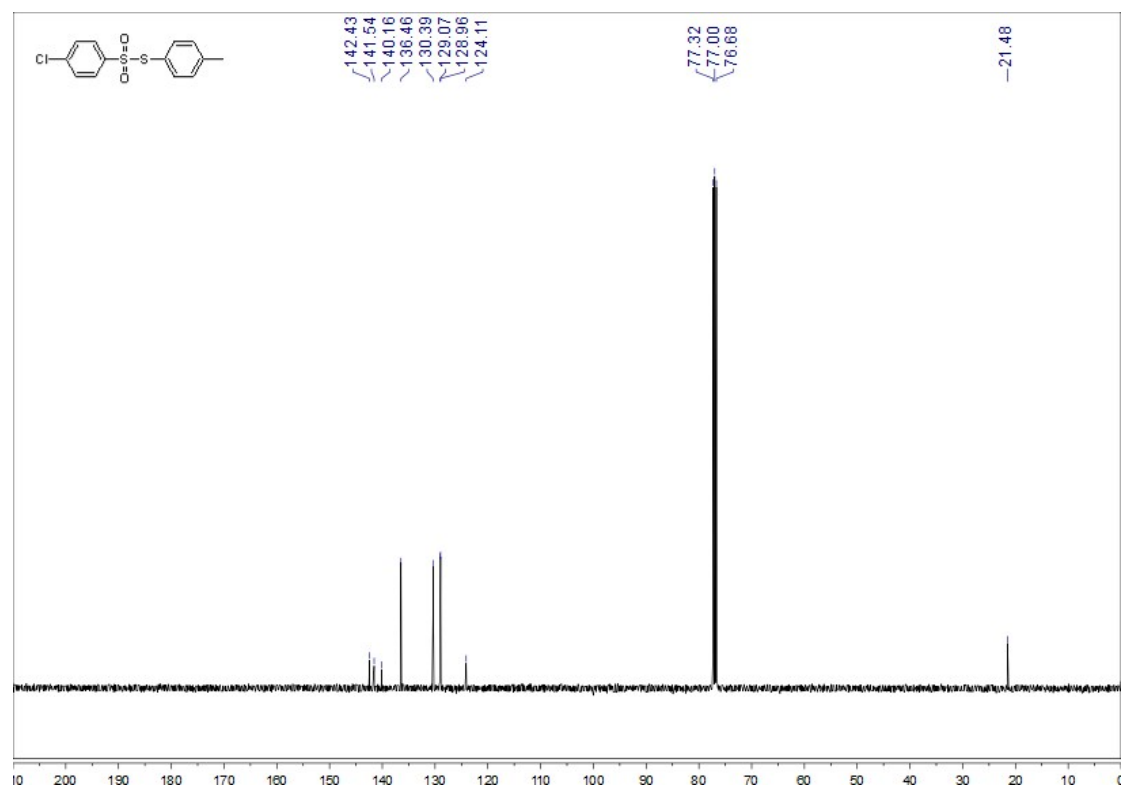
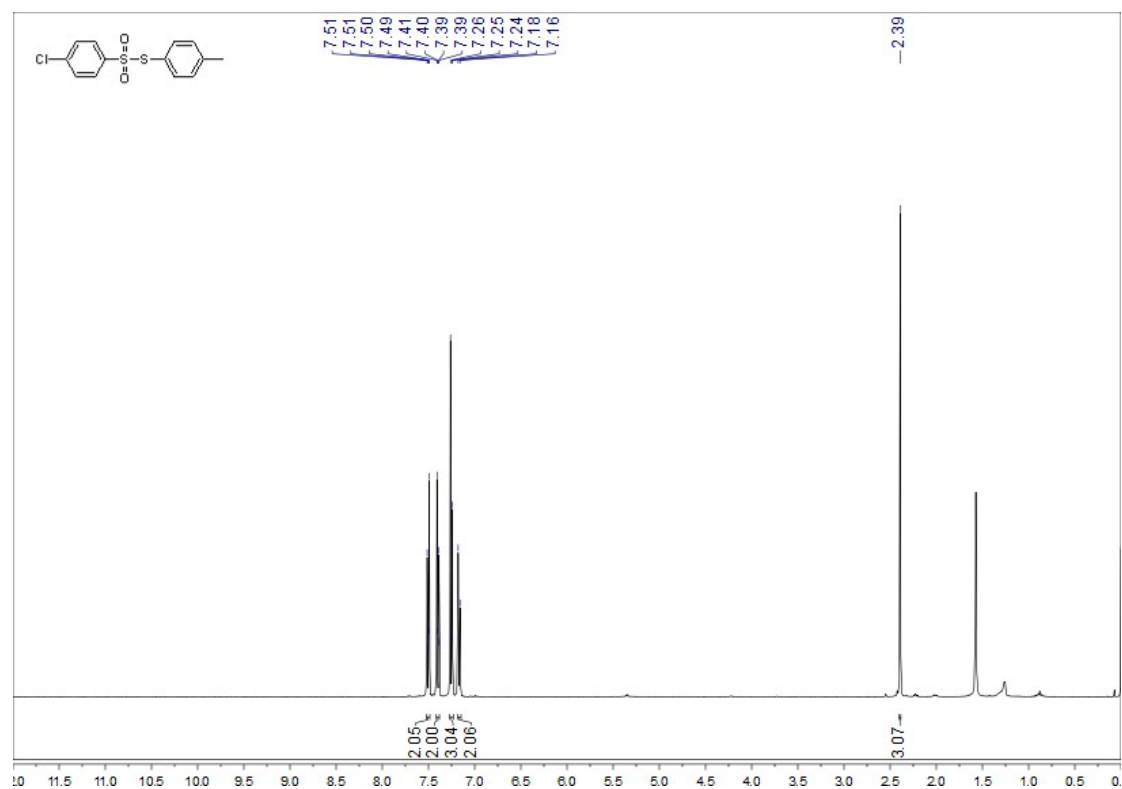
Toluene-2-thiosulfonic acid S-p-tolyl ester (**3da**)



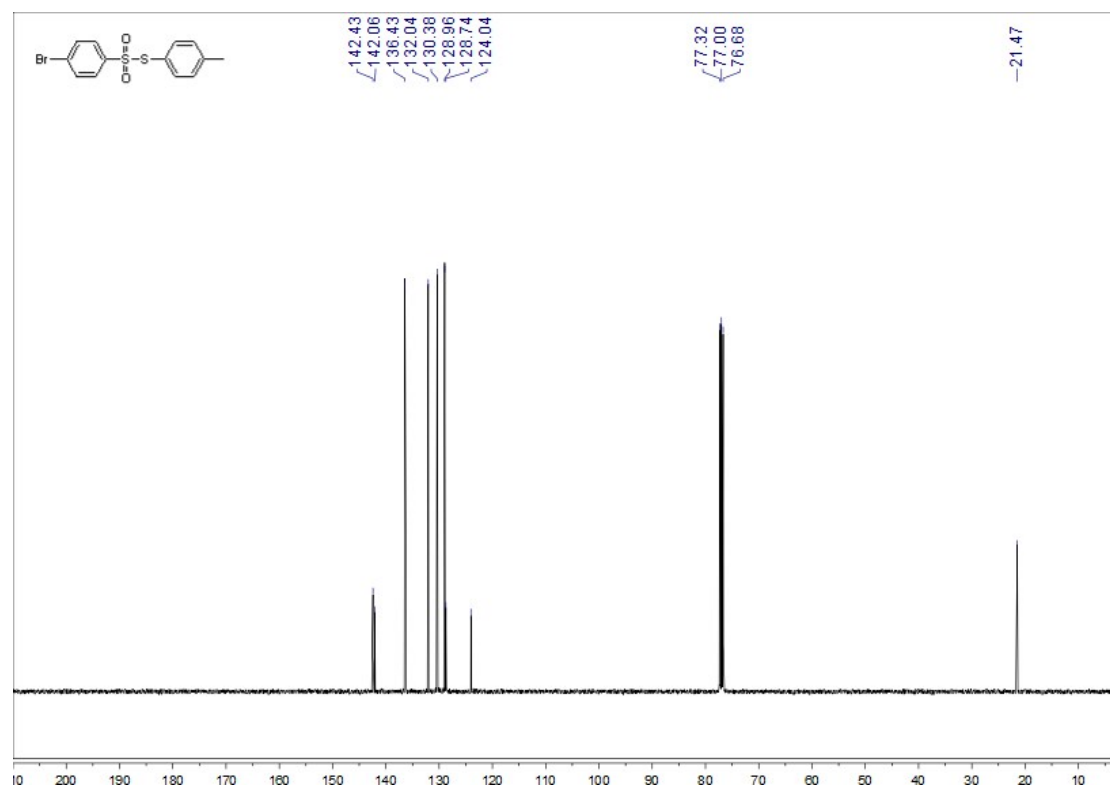
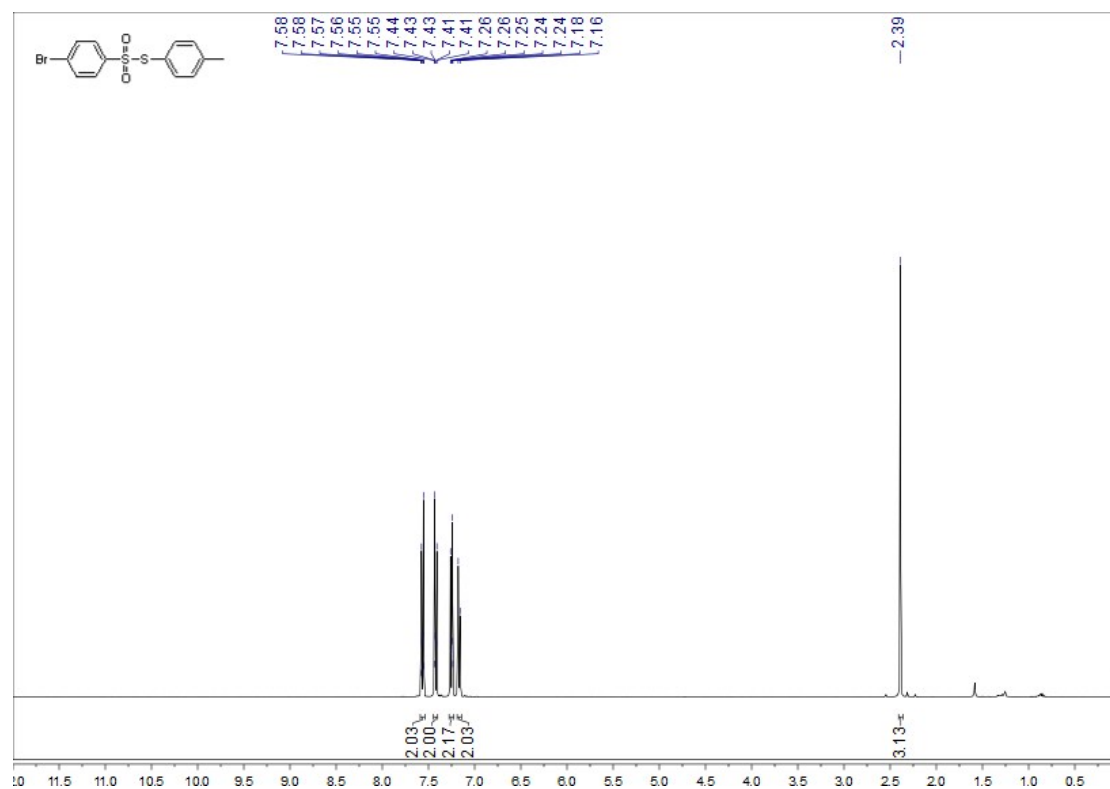
4-Fluoro-benzenethiosulfonic acid S-p-tolyl ester (**3ea**)



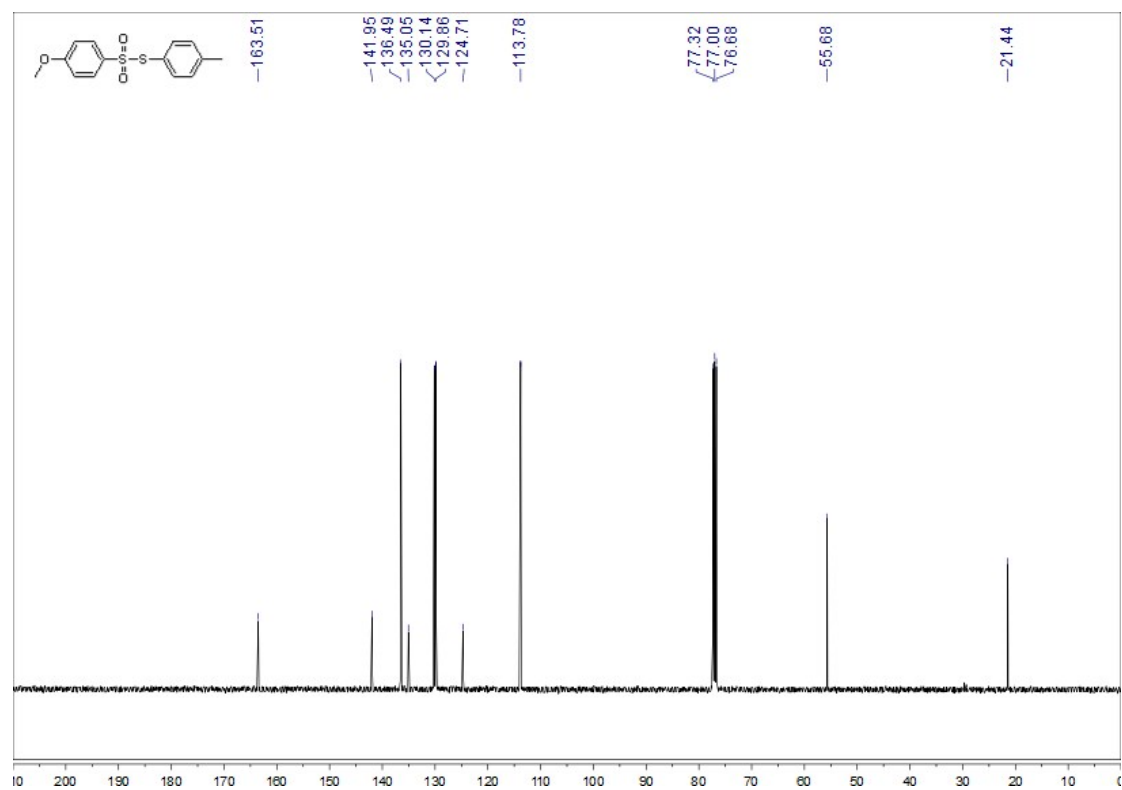
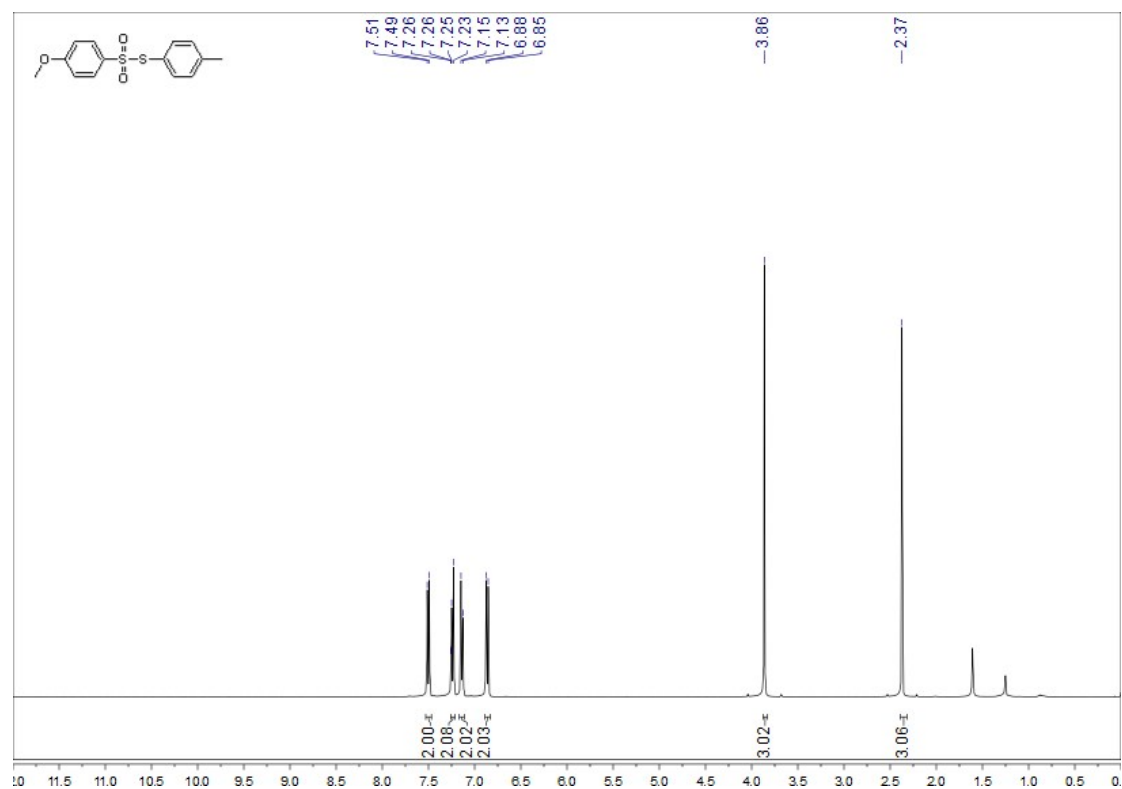
4-Chloro-benzenethiosulfonic acid S-p-tolyl ester (**3fa**)



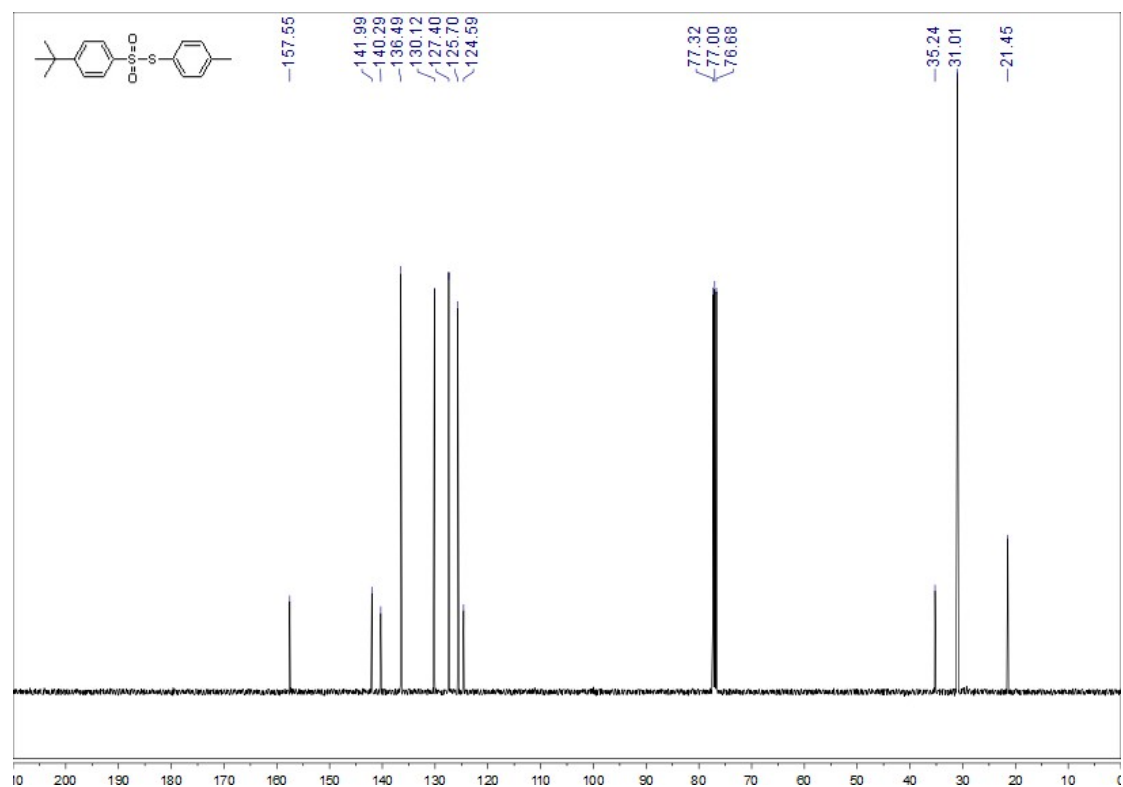
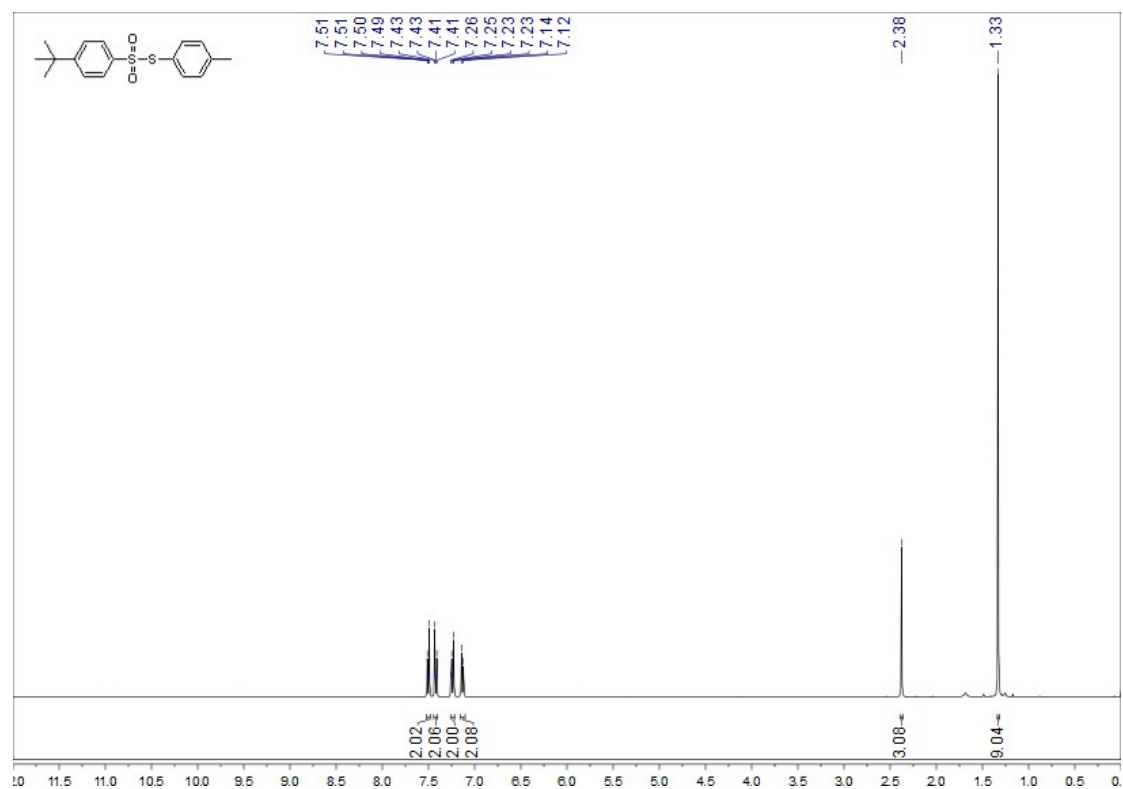
4-Bromo-benzenethiosulfonic acid S-p-tolyl ester (**3ga**)



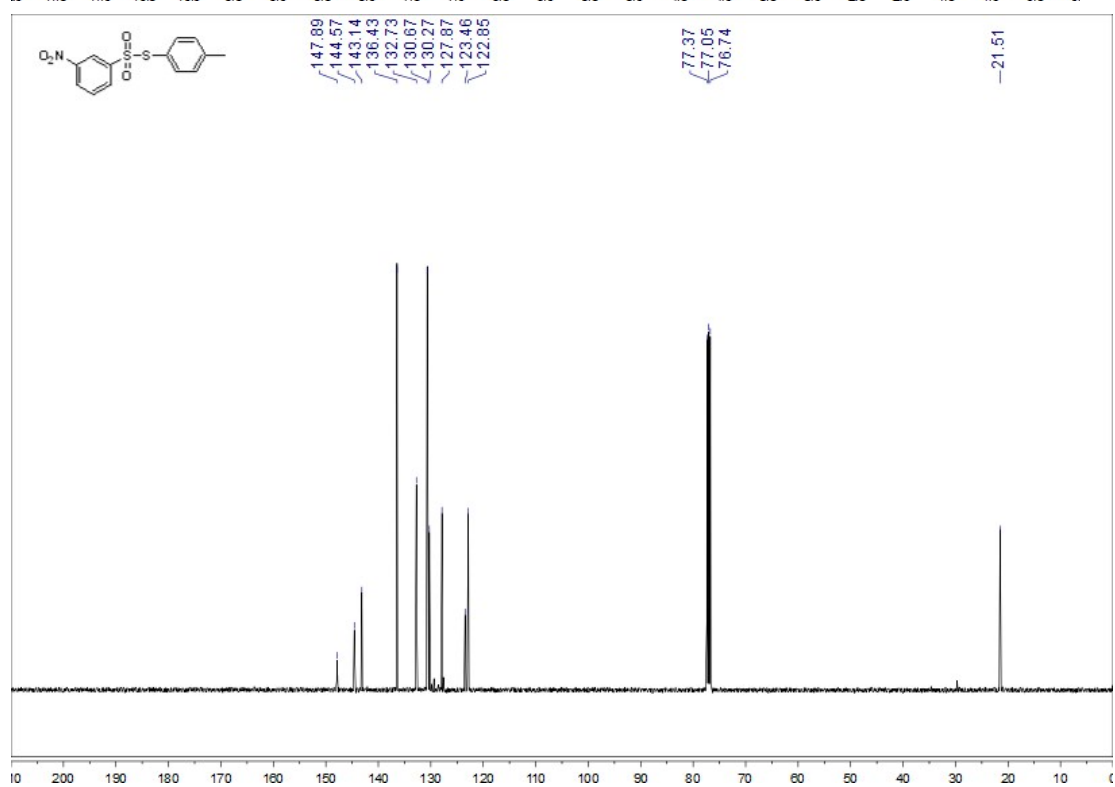
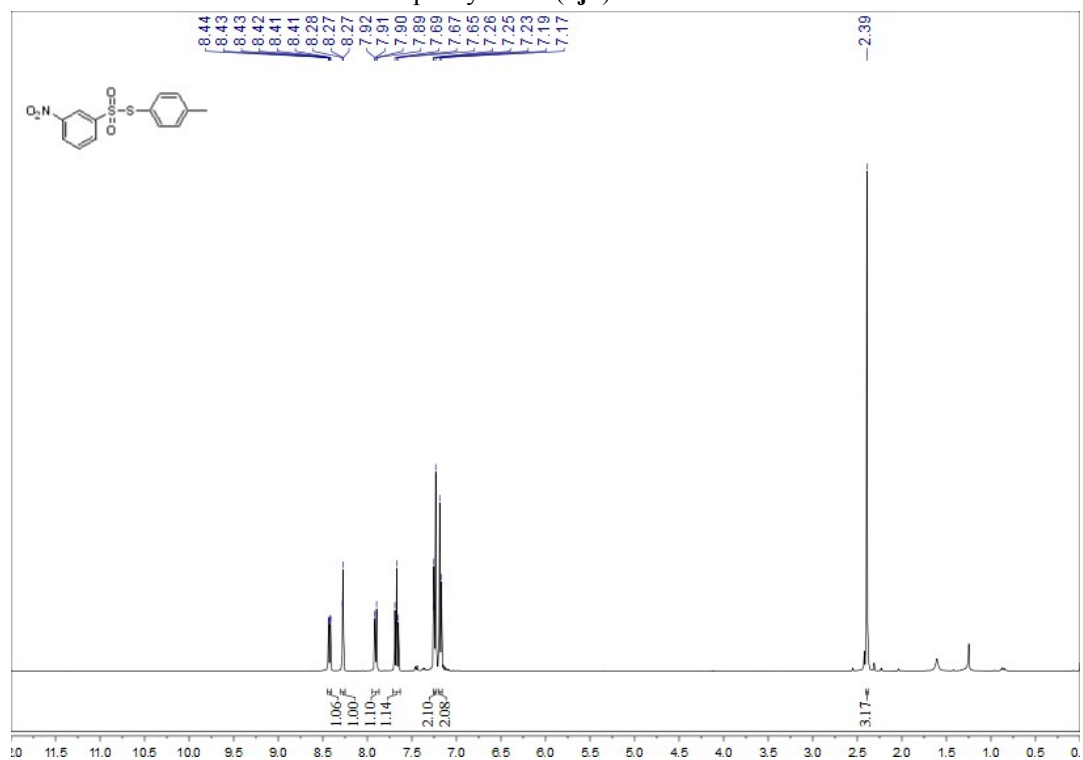
4-Methoxy-benzenethiosulfonic acid S-p-tolyl ester (**3ha**)



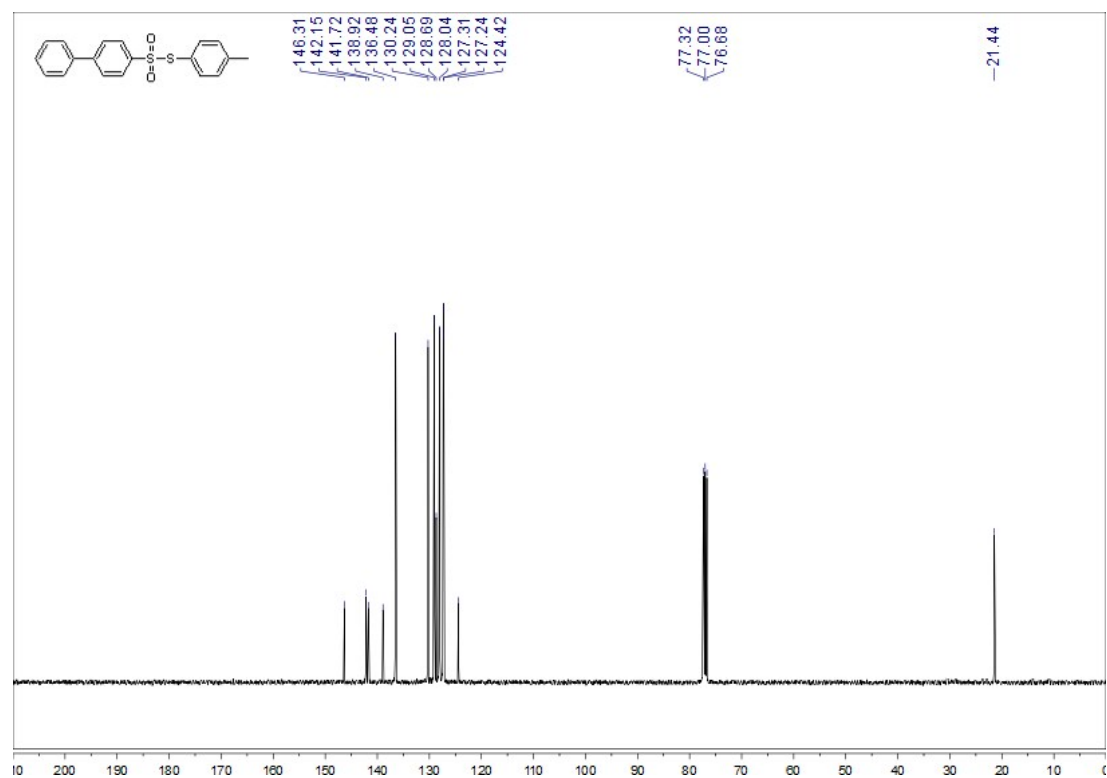
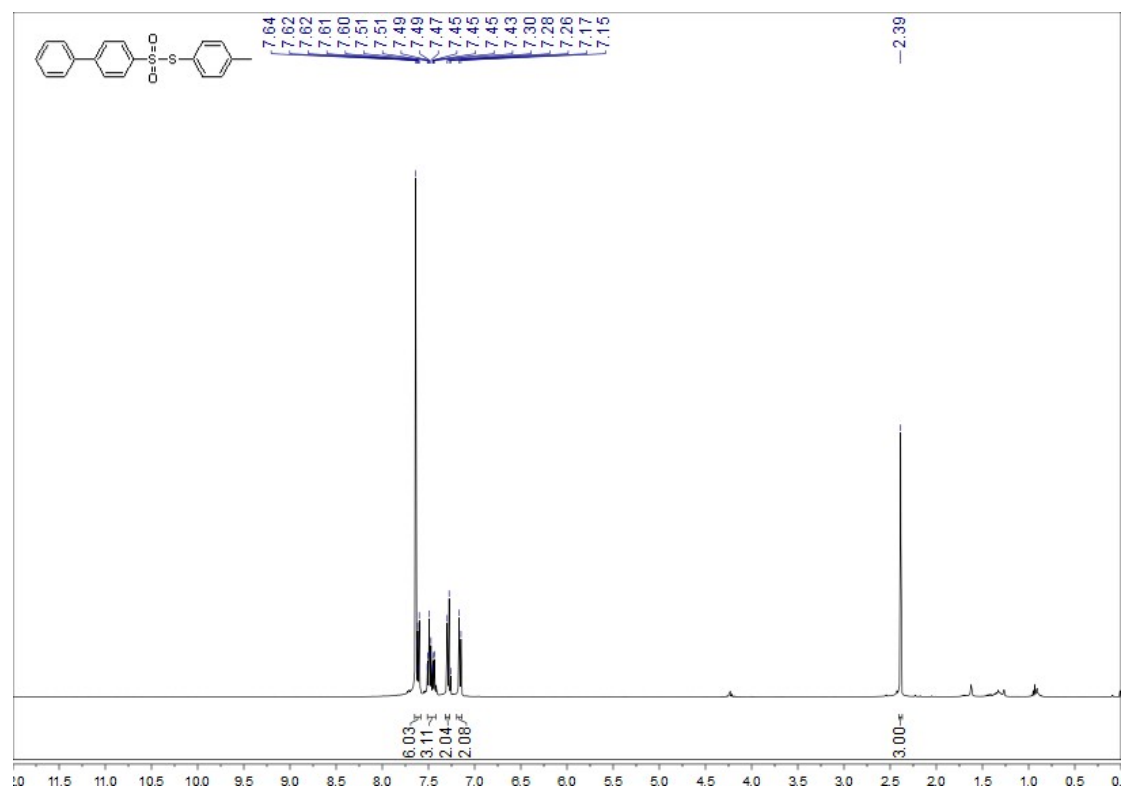
4-tert-Butyl-benzenethiosulfonic acid S-p-tolyl ester (**3ia**)



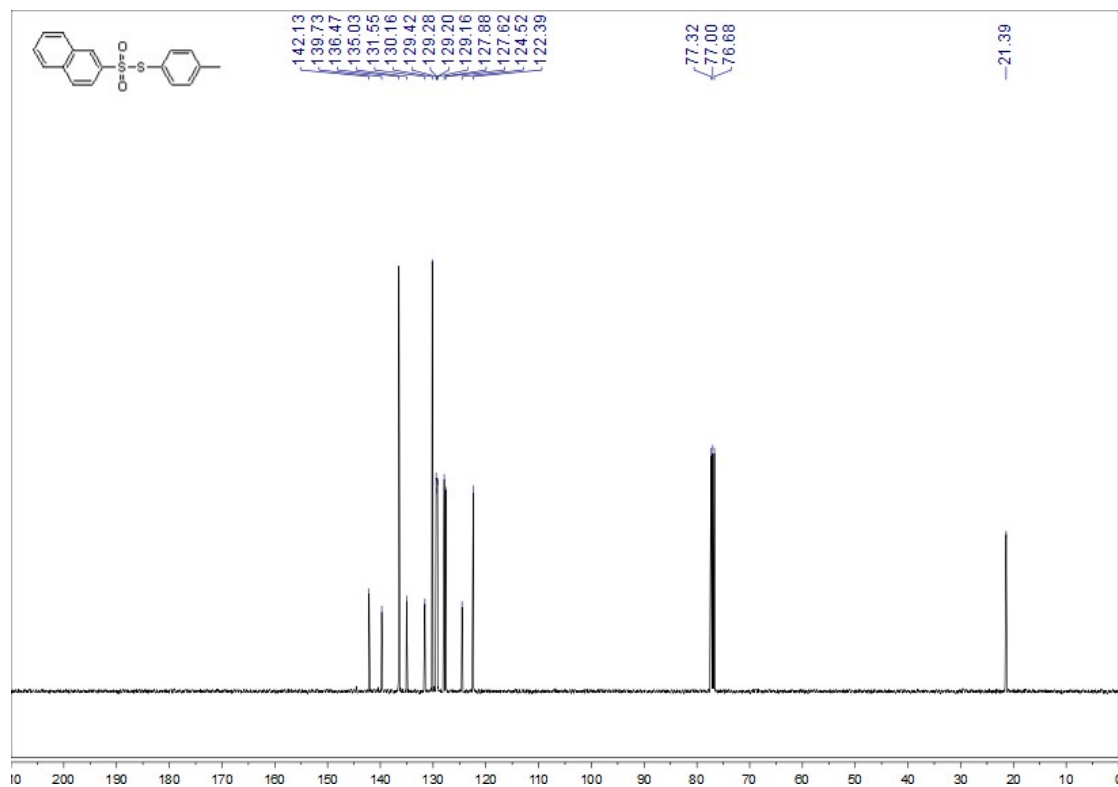
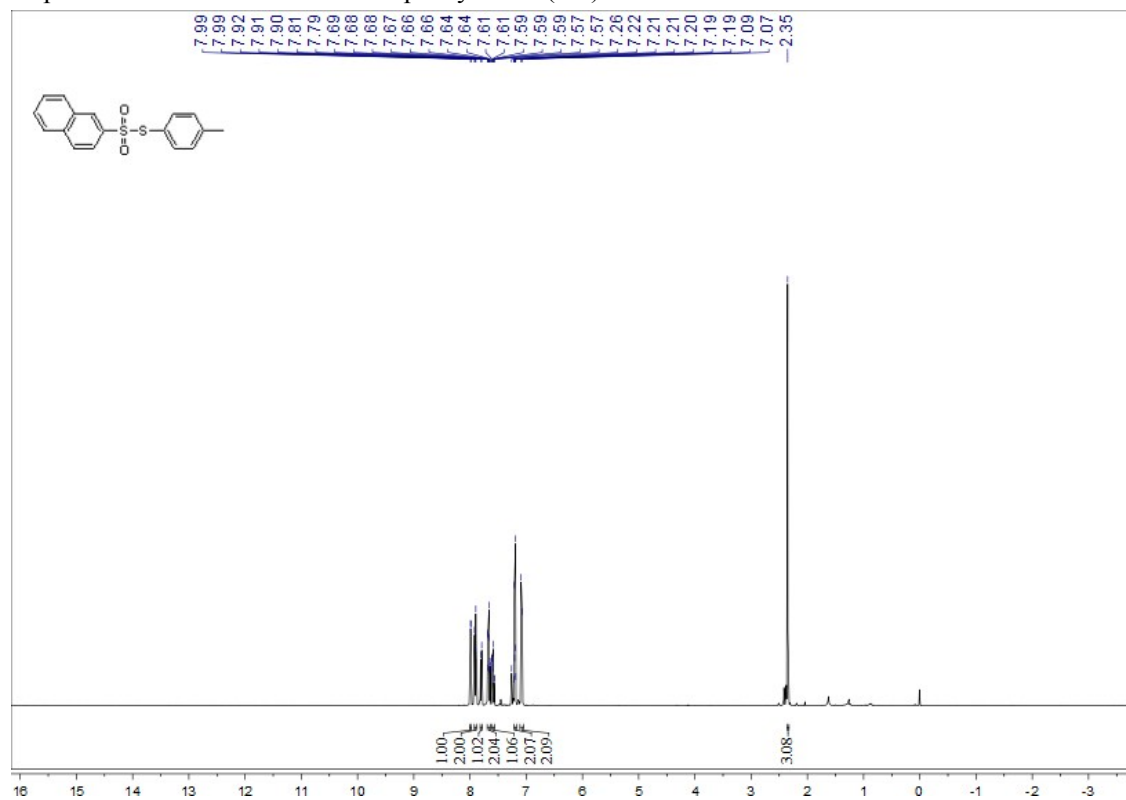
3-Nitro-benzenethiosulfonic acid S-p-tolyl ester (**3ja**)



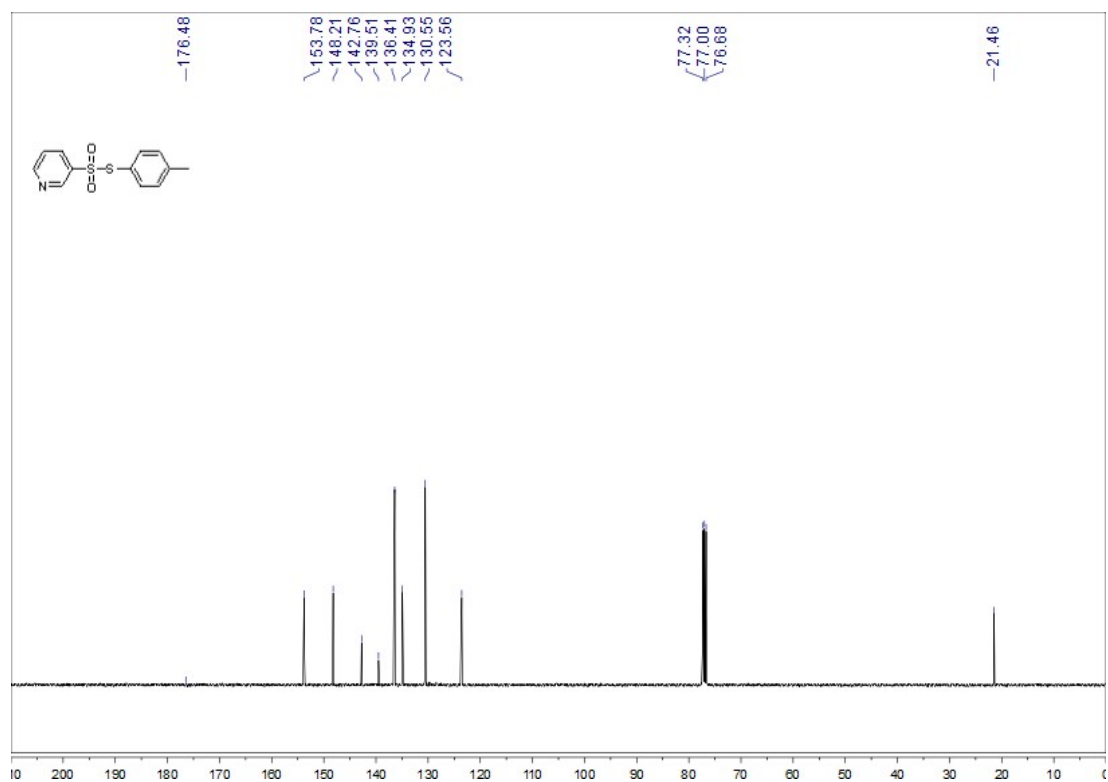
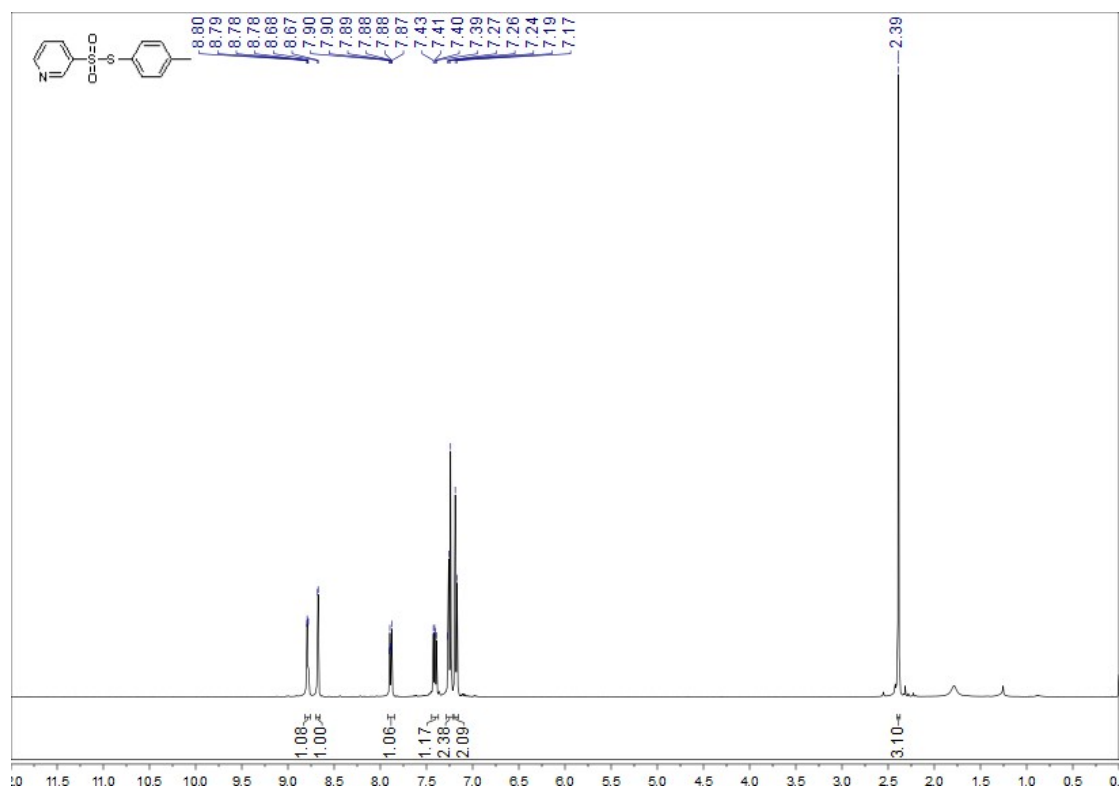
Biphenyl-4-thiosulfonic acid S-p-tolyl ester (**3ka**)



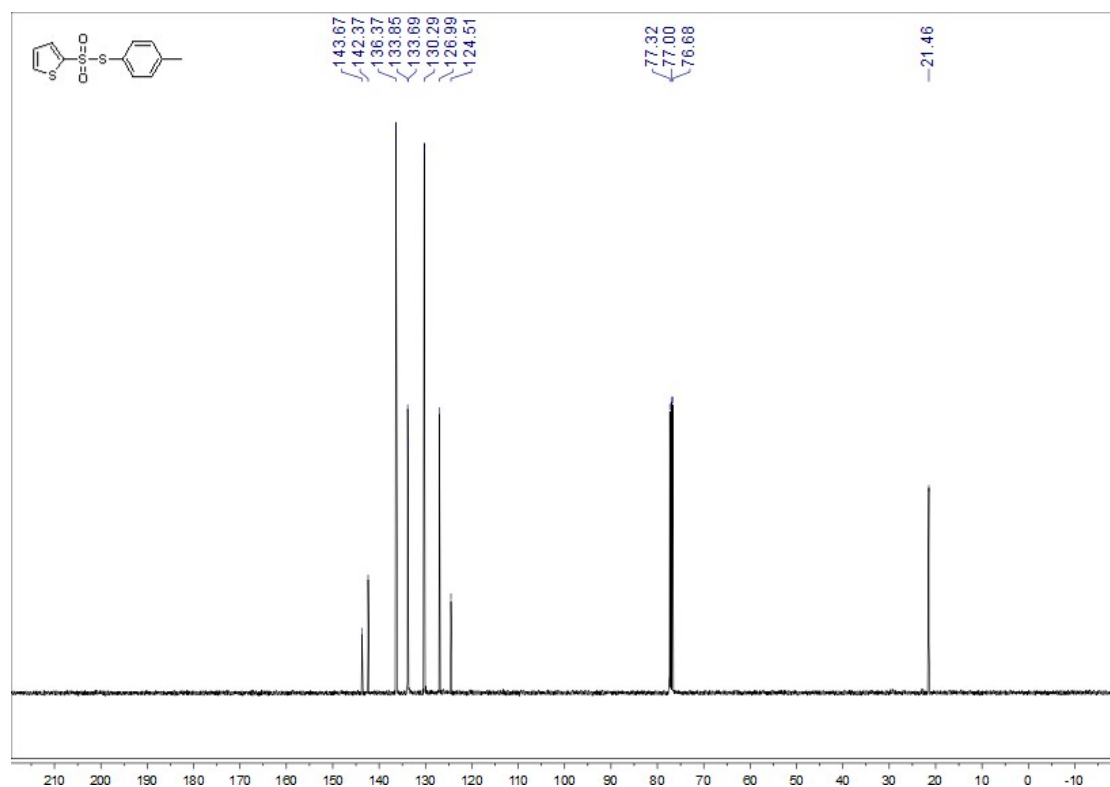
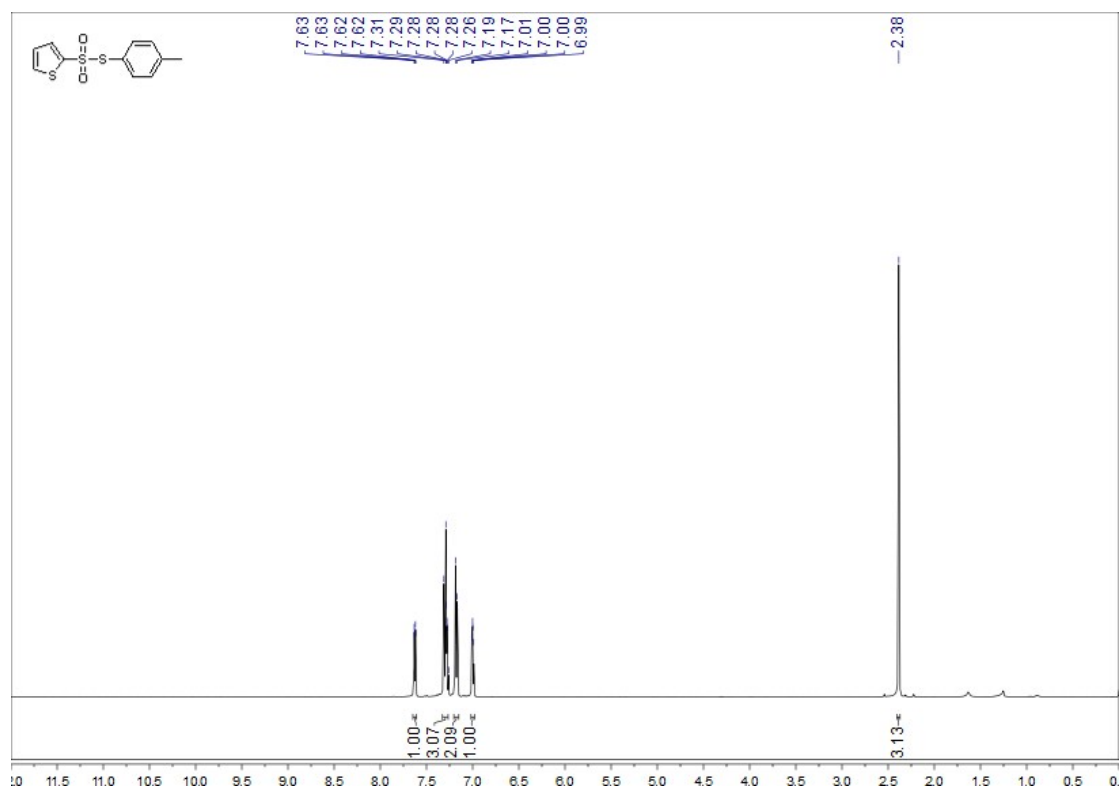
Naphthalene-2-thiosulfonic acid S-p-tolyl ester (**3la**)



Pyridine-3-thiosulfonic acid S-p-tolyl ester (**3ma**)



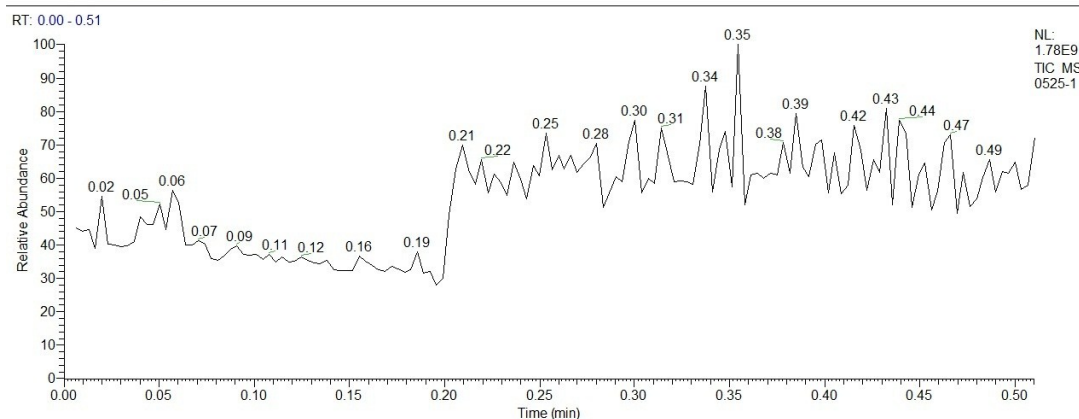
Thiophene-2-thiosulfonic acid S-p-tolyl ester (**3na**)



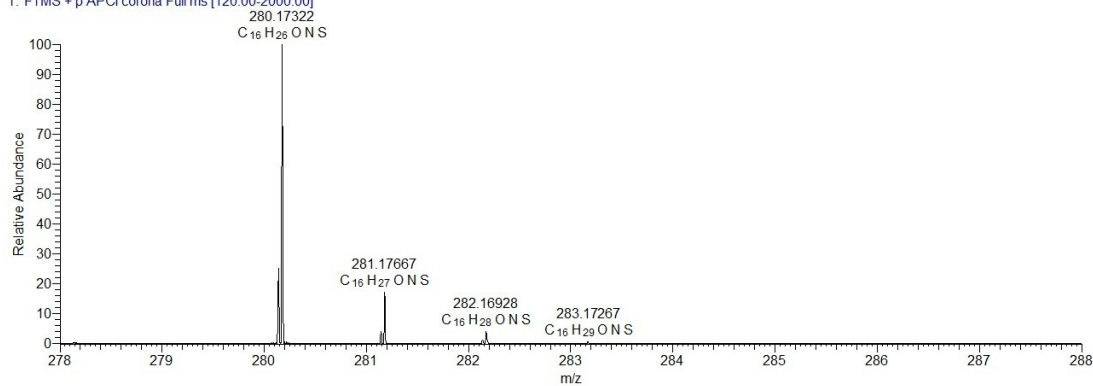
The HRMS spectra of **4a**, **4b**, **6a**, and **6b**

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05/25/18 11:42:00

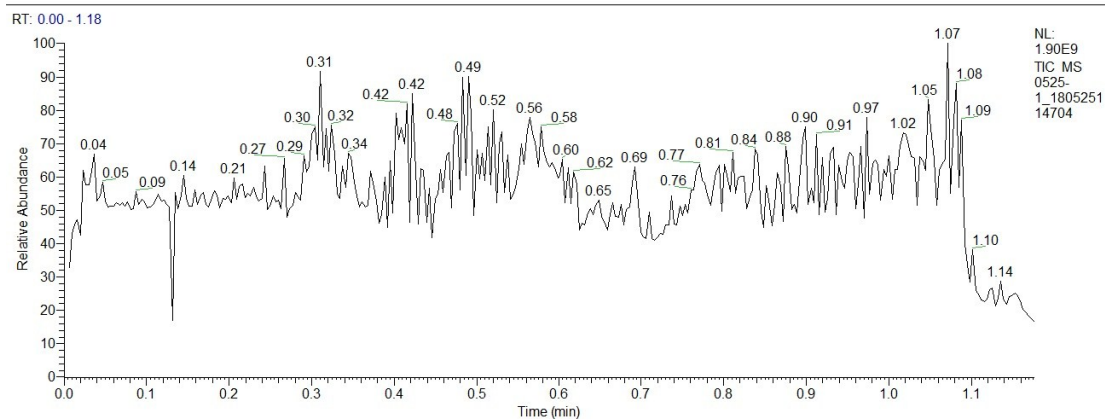


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T: FTMS + p APCI corona Full ms [120.00-2000.00]

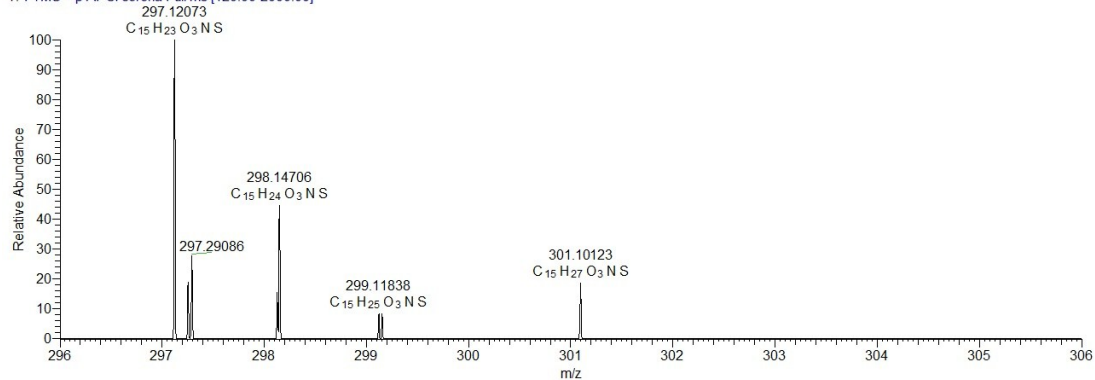


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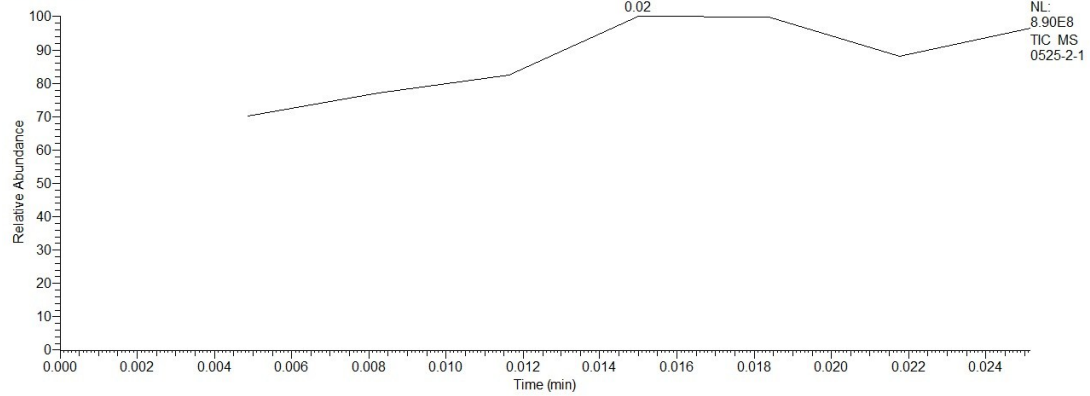
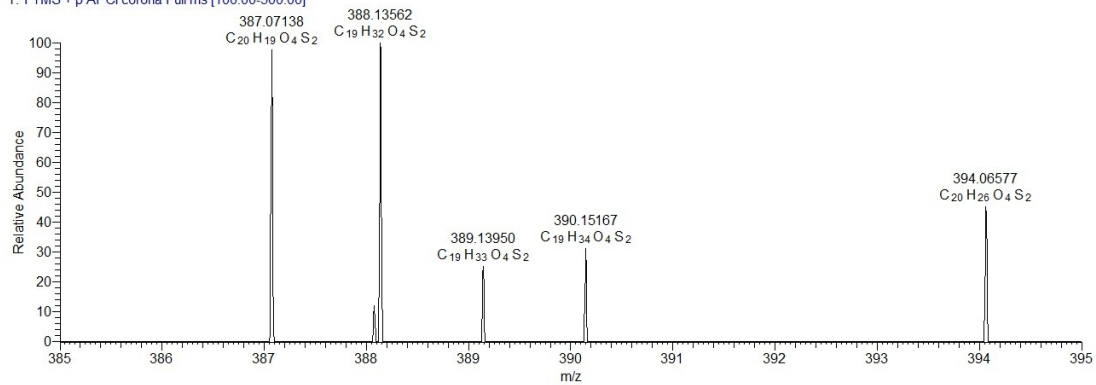
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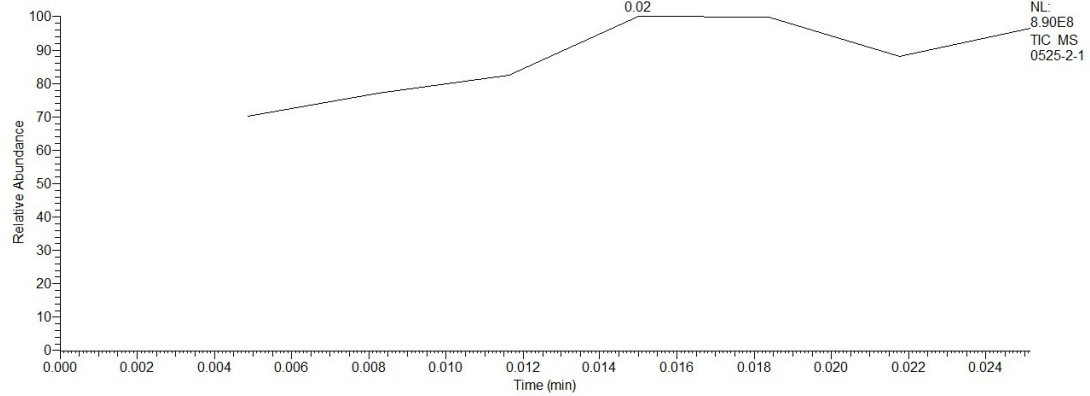
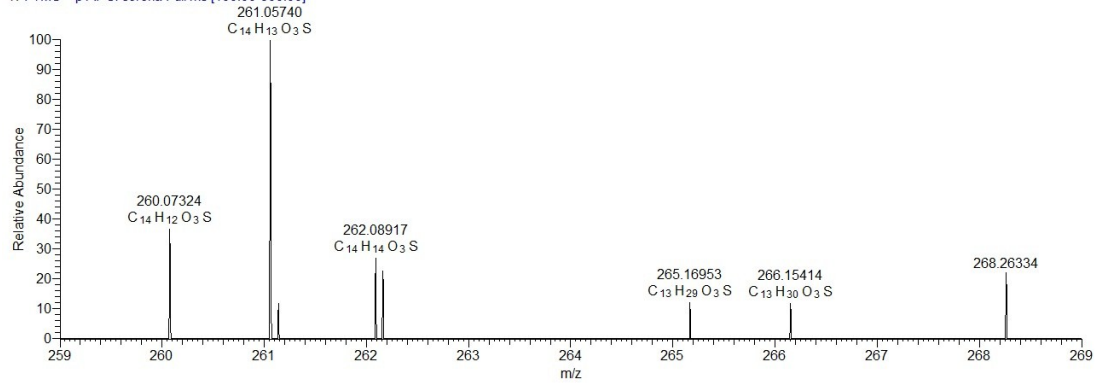
0525-1_180525114704 #2 RT: 0.01 AV: 1 NL: 7.34E5
T: FTMS + p APCI corona Full ms [120.00-2000.00]



RT: 0.00 - 0.03

0525-2-1 #1 RT: 0.00 AV: 1 NL: 6.67E5
T: FTMS + p APCI corona Full ms [100.00-500.00]

RT: 0.00 - 0.03

0525-2-1 #1 RT: 0.00 AV: 1 NL: 6.26E5
T: FTMS + p APCI corona Full ms [100.00-500.00]

The F-NMR spectra of **3af**, **3ai** and **3ea**

