Supporting information

Development of a Simple High-Throughput Assay for Directed Evolution of Enantioselective Sulfoxide Reductases

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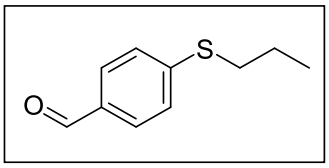
1. General

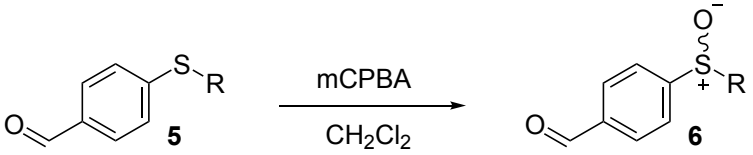
All starting reagents were commercially available and of analytical purity, which were used without further treatment unless otherwise stated. Racemic sulfoxides were prepared according to the literature procedures[1,2] unless otherwise stated. Solvents were dried according to standard methods. ^{1}H NMR spectra were obtained at 400 MHz. ^{13}C NMR spectra were obtained at 101 MHz and were ^{1}H decoupled. ^{19}F NMR spectra were obtained at 376 MHz. Chemical shifts (δ) are reported in ppm relative to the residual solvent peak or internal solvent peak ($(CD_3)_2SO$: δ_H = 2.50, δ_C = 39.5; $CDCl_3$: δ_H = 7.26 , δ_C = 77.2, CD_3OD: δ_H = 3.31, δ_C = 49.0) Chemical shifts for ^{19}F NMR are reported in terms of chemical shift in reference to an internal standard (fluorobenzene set to δ -112.96 ppm). Accurate mass measurements (HRMS) were obtained by ESI on an Agilent 6530 Q-TOF MS spectrometer. UV–Vis absorption spectra were recorded on a BioDrop Touch spectrophotometer. Analytical HPLC was performed under the following conditions: Agilent Eclipse plus C18 column (3.5 μL, 4.6×100 mm); UV/Vis detection at λ_{obs} = 254 nm or 220 nm; flow rate 0.4 mL/min; gradient elution method (0.1% aqueous formic acid – CH_3CN from 95:5 to 0:100 in 13 min. Analytical TLC was performed using a precoated silica gel 60 Å F_{254} plates (0.2 mm thickness) and visualized by irradiation with UV light at 254 nm or by dipping in stain solution ($KMnO_4$, CAM) followed by heating. Preparative column chromatography was carried out using silica gel 60 Å (particle size 0.063–0.200mm). Enantiomeric excesses were determined by chiral HPLC analysis using Daicel Chiralpak (IA, IC) and Chiralcel OD-H columns and a mixture of *n*-heptane/propan-2-ol as the eluent. The detailed conditions are given at the characterization part of the products. The absolute configurations of the products were determined by the comparison of chiral HPLC retention times with the literature. Selectivity factor *s* was calculated according to formula $s = \ln[(1-c)(1-ee)]/\ln[(1-c)(1+ee)]$.[3] Purifications by HPLC were performed under the following conditions: Agilent ZORBAX SBC18 column (5 μL, 9.4×150 mm); UV/Vis detection at λ_{obs} = 254 nm; flow rate 4 mL/min; gradient elution method (0.1% aqueous trifluoroacetic acid – CH_3CN from 95:5 to 0:100 in 20 min. Oligonucleotides were purchased from Sigma-Aldrich, restriction endonucleases and other enzymes for DNA cloning and mutagenesis were purchased from New England Biolabs or Agilent Technologies.

2. Preparation of 4-(Propylthio)benzaldehyde (**5a**)



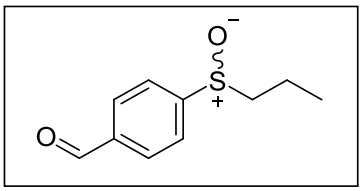
4-(propylthio)benzaldehyde **5a** was prepared according to a reported protocol.[4] A mixture of 1,2-ethanedithiol (188 mg, 2.0 mmol), 4-bromobenzaldehyde (185 mg, 1.0 mmol), $CuSO_4 \cdot 5H_2O$ (13 mg, 0.05 mmol), Cs_2CO_3 (1.625 g, 5.0 mmol) in DMSO (2 mL) was stirred at 90 ºC for 20 h under argon atmosphere. Then, the reaction mixture was cooled to the room temperature and 1-iodopropane (510 mg, 3 mmol) in DMF (1 mL) was added. The reaction mixture was stirred for another 5 h at room temperature, then diluted with water and extracted with EtOAc. Combined organic phase was washed with water, brine, dried over $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (cyclohexane/EtOAc=8:1) to afford **5a** in 83 % yield (150 mg) as a yellow-brown liquid: R_f 0.7 (cyclohexane/EtOAc=4:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.76 – 7.71 (m, 2H), 7.36 – 7.31 (m, 2H), 2.98 (t, J = 7.3, 2H), 1.74 (m, 2H), 1.06 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.3, 147.2, 133.2, 130.1, 126.5, 33.9, 22.2, 13.6. HRMS (ESI): *m/z* $[M+H]^+$calcd for $C_{10}H_{13}OS$ 181.0682, found: 181.0663.

3. General Procedure for the Oxidation of 4-(Alkylthio)benzaldehydes to Sulfoxides



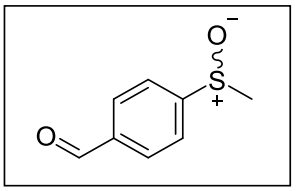
To a solution of 4-(alkylthio)benzaldehydes (2.0 mmol) in CH_2Cl_2 (20 mL) 3-chloroperbenzoic acid (415 mg, 2.4 mmol) was added portionwise at 0 °C over the period of 10 min. The reaction mixture was stirred at room temperature for 1 h. Then, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and extracted with aqueous Na_2SO_3 (saturated solution, 2 × 30 mL), aqueous $NaHCO_3$ (1M solution, 2 × 30 mL), water, brine, dried over $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel.

4-(Propylsulfinyl)benzaldehyde (6a)



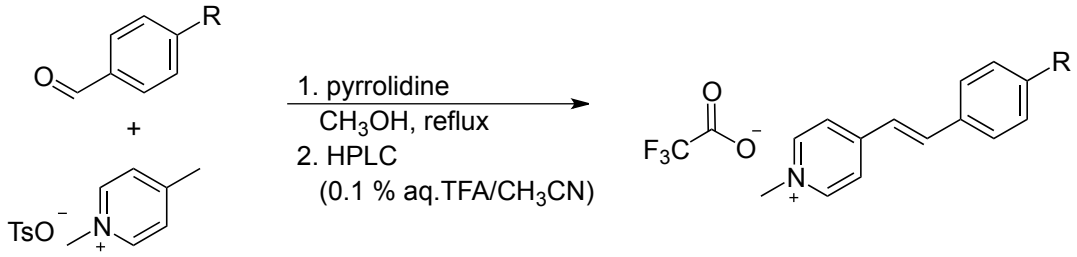
$C_{10}H_{12}O_2S$ (196.26 g/mol), 180 mg, pale yellow solid, R_f 0.3 (cyclohexane/EtOAc=2:3), 46 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.05 – 7.97 (m, 2H), 7.81 – 7.73 (m, 2H), 2.84 – 2.75 (m, 2H), 1.90 – 1.77 (m, 1H), 1.71 – 1.56 (m, 1H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.3, 151.1, 138.2, 130.3, 124.8, 59.1, 15.9, 13.3. HRMS (ESI): *m/z* $[M+H]^+$calcd for $C_{10}H_{13}O_2S$ 197.0631, found: 197.0635.

4-(Methylsulfinyl)benzaldehyde (6b)



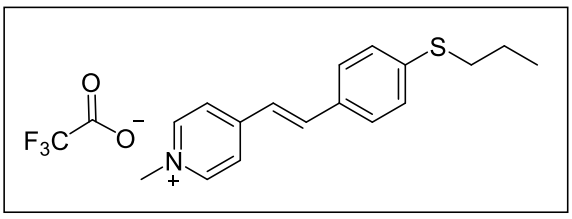
$C_8H_8O_2S$ (168.21 g/mol), 161 mg, white solid, R_f 0.3 (cyclohexane/EtOAc=2:3), 48 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.05 – 7.97 (m, 2H), 7.83 – 7.75 (m, 2H), 2.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.2, 152.6, 138.2, 130.5, 124.3, 43.9. The spectra are in agreement with reported data.[5]

4. General Procedure for the Knoevenagel Condensation



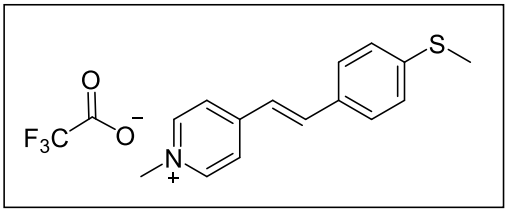
This procedure used a modification of the reported protocol.[6] Pyrrolidine (19 mg, 269 µmol) was added to the mixture of 1,4-dimethylpyridinium p-toluenesulfonate (50 mg, 179 µmol) and an aldehyde (538 µmol) in MeOH (4 mL) under argon atmosphere. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the mixture was concentrated by rotary evaporation under reduced pressure, the solid residue was washed with EtOAc (3 × 30 mL) and further purified by HPLC and lyophilized.

(*E*)-1-Propyl-4-(4-(methylthio)styryl)pyridin-1-ium 2,2,2-trifluoroacetate (3a)



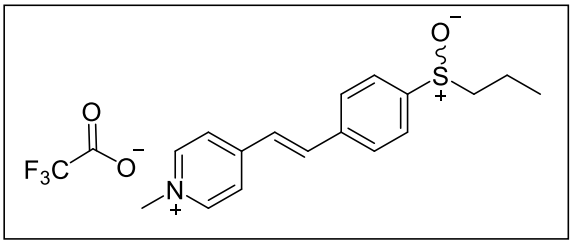
$C_{19}H_{20}F_3NO_2S$ (383.43 g/mol), 59 mg, pale yellow solid, R_f 0.7 (MeOH/NH_4Cl aq. (2M)/$MeNO_2$ 7:2:1), 87 % yield. HPLC t_R 12.7 min. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.84 (d, J = 6.8 Hz, 2H), 8.18 (d, J = 6.8 Hz, 2H), 7.97 (d, J = 16.3 Hz, 1H), 7.71 – 7.63 (m, 2H), 7.46 (d, J = 16.3 Hz, 1H), 7.43 – 7.35 (m, 2H), 4.24 (s, 3H), 3.03 (t, J = 7.2 Hz, 2H), 1.70 – 1.59 (m, 2H), 1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 152.5, 145.0, 140.4, 140.1, 131.95, 128.6, 127.1, 123.3, 122.3, 46.8, 33.0, 21. 8, 13.2. ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ -74.34. HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{17}H_{20}NS$ 270.1311, found: 270.1303. UV-Vis λ_{max} (phosphate buffer, pH = 7.4): 380 nm (ε/$M^{-1}cm^{-1}$: 19600).

(*E*)-1-Methyl-4-(4-(methylthio)styryl)pyridin-1-ium 2,2,2-trifluoroacetate (3b)



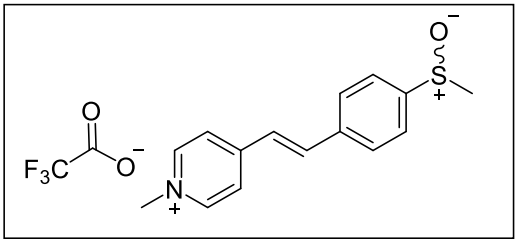
$C_{17}H_{16}F_3NO_2S$ (355.37 g/mol), 37 mg, pale yellow solid, R_f 0.7 (MeOH/NH_4Cl aq. (2M)/$MeNO_2$ 7:2:1), 58 % yield. HPLC t_R 10.7 min. ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 – 8.61 (m, 2H), 8.19 – 8.07 (m, 2H), 7.89 (d, *J* = 16.3 Hz, 1H), 7.74 – 7.60 (m, 2H), 7.37 (d, *J* = 16.3 Hz, 1H), 7.34 – 7.28 (m, 2H), 4.30 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 155.4, 146.0, 144.6, 142.6, 132.9, 129. 8, 127.0, 124.8, 122.6, 47.6, 14.9. ^{19}F NMR (376 MHz, (CD_3OD) δ -76.92. HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{15}H_{16}NS$ 242.0998, found: 242.0993. UV-Vis λ_{max} (phosphate buffer, pH = 7.4): 380 nm (ε/$M^{-1}cm^{-1}$: 21880).

(*E*)-1-Propyl-4-(4-(methylsulfinyl)styryl)pyridin-1-ium 2,2,2-trifluoroacetate (4a)- Propyl GreenOx



$C_{19}H_{20}F_3NO_3S$ (399.43 g/mol), 60 mg, pale yellow solid, R_f 0.6 (MeOH/NH_4Cl aq. (2M)/$MeNO_2$ 7:2:1), 84 % yield. HPLC t_R 8.1 min. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.96 – 8.86 (m, 2H), 8.29 – 8.20 (m, 2H), 8.06 (d, *J* = 16.4 Hz, 1H), 7.96 – 7.86 (m, 2H), 7.80 – 7.70 (m, 2H), 7.64 (d, *J* = 16.4 Hz, 1H), 4.27 (s, 3H), 2.98 (ddd, *J* = 13.3, 9.2, 6.4 Hz, 1H), 2.80 (ddd, *J* = 13.2, 9.2, 6.4 Hz, 1H), 1.76 – 1.58 (m, 1H), 1.58 – 1.40 (m, 1H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 152.0, 146.2, 145.3, 139.2, 137.3, 128.5, 125.0, 124.7, 123.8, 57.1, 47.0, 15.1, 12.9. ^{19}F NMR (376 MHz, $(CD_3)_2SO$)) δ -74.50. HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{17}H_{20}NOS$ 286.1260, found: 286.1284. UV-Vis λ_{max} (phosphate buffer, pH = 7.4): 340 nm (ε/$M^{-1}cm^{-1}$: 29900).

(*E*)-1-Methyl-4-(4-(methylsulfinyl)styryl)pyridin-1-ium 2,2,2-trifluoroacetate (4b)- Methyl GreenOx



$C_{17}H_{16}F_3NO_3S$ (371.37 g/mol), 41 mg, pale yellow solid, R_f 0.6 (MeOH/NH_4Cl aq. (2M)/$MeNO_2$ 7:2:1), 61 % yield. HPLC t_R 6.9 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 – 8.86 (m, 2H), 8.30 – 8.21 (m, 2H), 8.06 (d, *J* = 16.4 Hz, 1H), 7.97 – 7.90 (m, 2H), 7.85 – 7.77 (m, 2H), 7.64 (d, *J* = 16.4 Hz, 1H), 4.27 (s, 3H), 2.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.0, 148.2, 145.3, 139.2, 137.3, 128.6, 125.0, 124.4, 123.8, 47.0, 43.0. ^{19}F NMR (376 MHz, $(CD_3)_2SO$)) δ -74.36. HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{15}H_{16}NOS$ 258.0947, found: 258.0930. UV-Vis λ_{max} (phosphate buffer, pH = 7.4): 340 nm (ε/$M^{-1}cm^{-1}$: 32200).

5. Absorption and Fluorescence Spectra of Propyl GreenOx (**4a**), Methyl GreenOx (**4b**), **3a** and **3b**

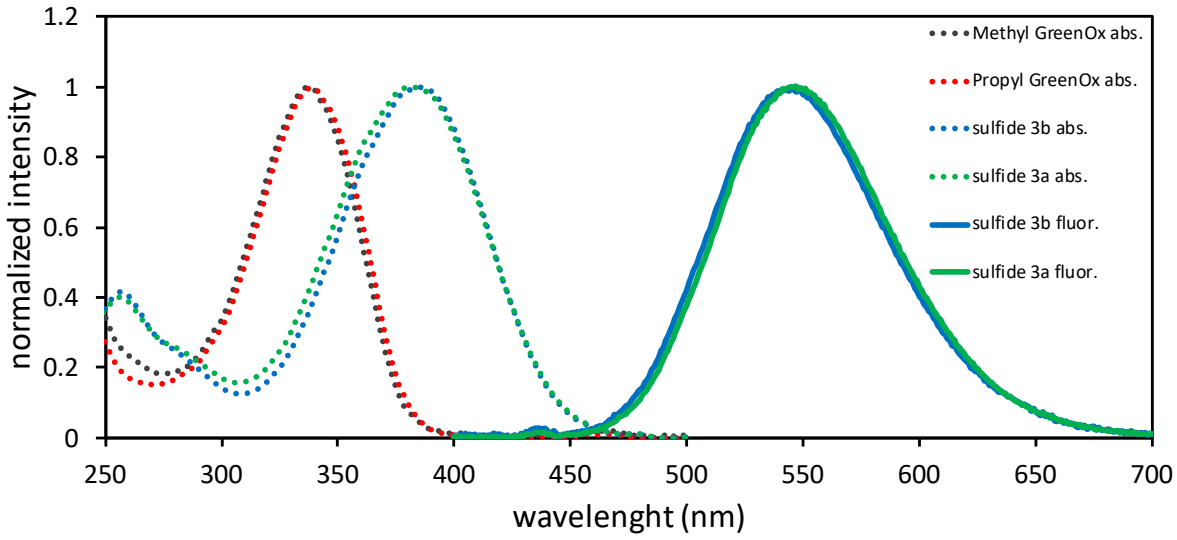


Figure S1. Normalized absorption and emission spectra (λ_{exc} = 380 nm) of Propyl GreenOx (**4a**), Methyl GreenOx (**4b**), **3a** and **3b** recorded in phosphate buffer (pH 7.4) at room temperature.

6. Quantum Yields Determination

Quantum yields were calculated by measuring the integrated emission area of the fluorescence spectra when excited at 380 nm in PBS and comparing it to the area measured for fluorescein in 0.1 M NaOH when excited at 470 nm (Φ = 0.92).[7] Quantum yields were calculated using the equation: $\Phi_X = \Phi_S \left(\frac{\mathrm{Grad}_X}{\mathrm{Grad}_S}\right)\left(\frac{\eta_X^2}{\eta_S^2}\right)$, where *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance and η the refractive index of the solvent. The subscript *S* and *X* represent the standard dye (fluorescein) and the tested dye, respectively. For the determination of the relative fluorescence quantum yields in solution only dilute solutions with an absorbance below 0.1 at the excitation wavelength (470 nm or 380 nm) were used.

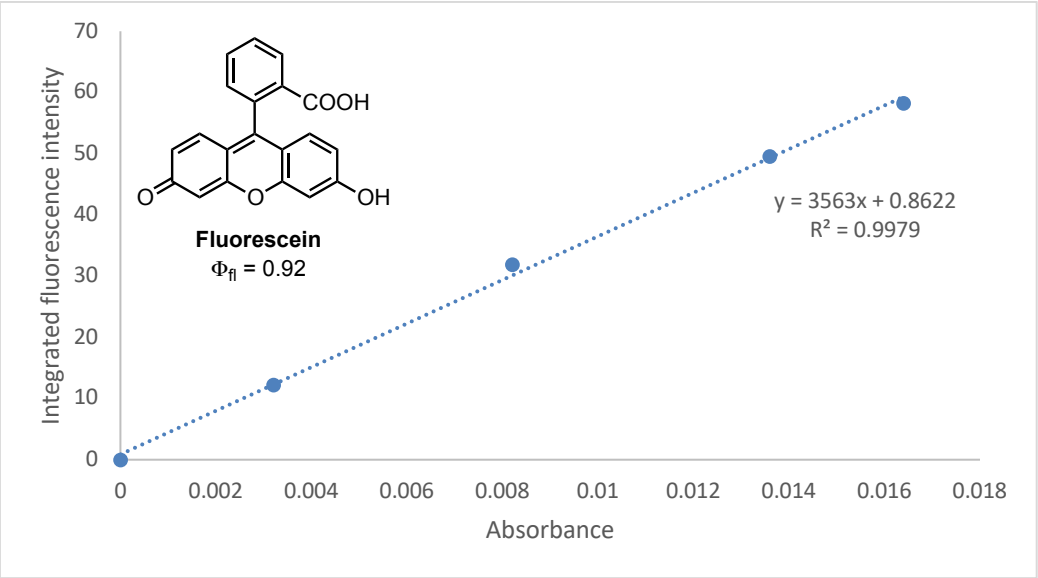


Figure S2. Plot of integrated fluorescence intensity *vs* absorbance of fluorescein.

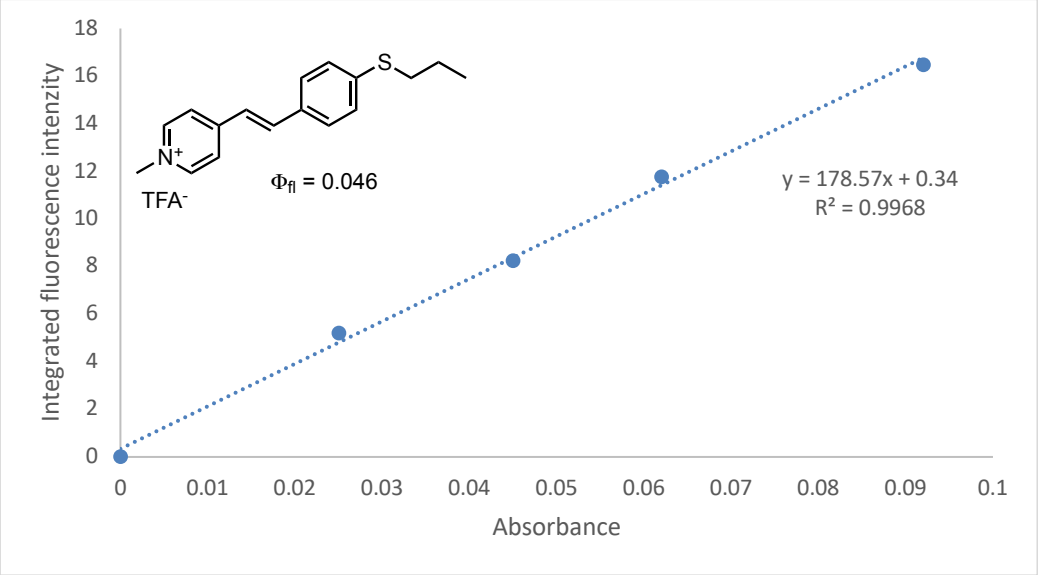


Figure S3. Plot of integrated fluorescence intensity *vs* absorbance of sulfide **3a**.

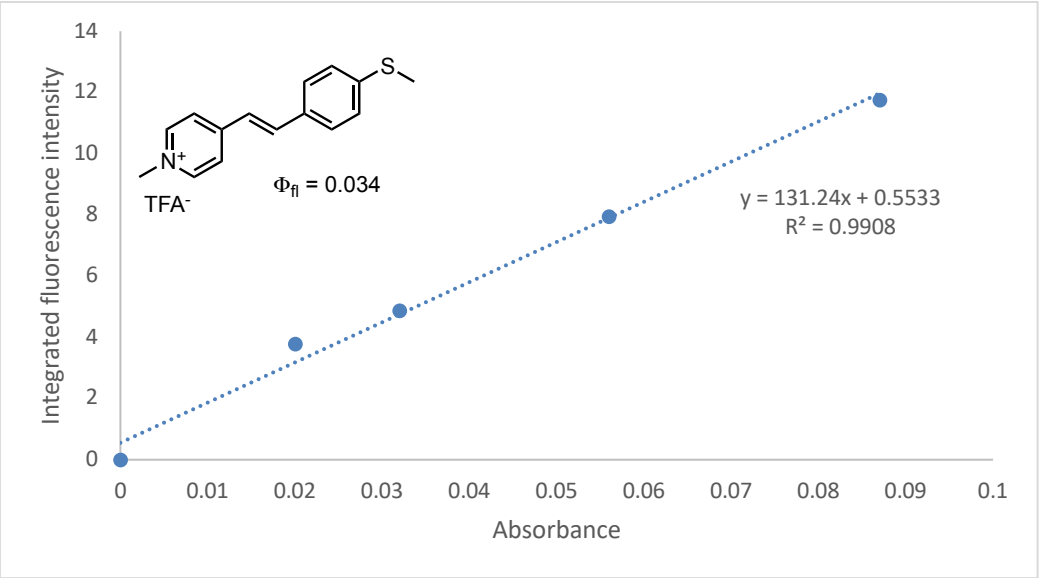


Figure S4. Plot of integrated fluorescence intensity *vs* absorbance of sulfide **3b**.

7. Preparation of MsrA

NdeI/XhoI ends were added to a 639 bp fragment containing the complete methionine sulfoxide reductase A gene (*msrA*) with PCR (Red Taq2x Master Mix, VWR) using two primers (5'-ATACATATGAGTTTATTTGATAAAAAGCATCTGGT and 5'-AATACTCGAGCTATGCTTCCGGCGGC) and pGEX/MsrA[8] construct as a template. The amplified fragment was cloned into pET-16b vector (GenScript), previously cut with NdeI/XhoI restriction endonucleases. Ligated vector was used to transform *E. coli* 5-alpha competent (New England Biolabs) via electroporation. 100 µL of the electroporation mixture was plated on LB agar plate with ampicillin (100 mgL^{-1}). 10 single colonies were selected and the correct sequence of the construct was confirmed by DNA sequencing analysis. This construct of *msrA* in pET-16b vector was used to transform *E. coli* BL21 (DE3) cells (Agilent Technologies). A 25 mL preculture of transformed *E. coli* BL21 (DE3) cells was used to inoculate 400 mL of LB medium with ampicillin (100 mgL^{-1}). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} reached 0.8. Then, the production of the enzyme was induced with 0.5 mM IPTG, and the culture was incubated at 37 °C, 200 rpm for an additional 3 h until. Cells were then harvested by centrifugation (3 500 × g for 30 min at 4 °C). The cell pellet was suspended in PBS buffer (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3) and lysed by sonication. Cell debris was removed by centrifugation (4 000 × *g* for 30 min at 4 °C), the soluble fraction was filtered with 0.22 µm filter and loaded onto Nickel agarose column (Ni-NTA Agarose, Invitrogen) and the resulting suspension was gently mixed for 30 min at room temperature. The column was washed with 3 × 10 bed volumes of loading buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8.0) and finally the protein was eluted with 3 × 1 bed volume of elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole, pH 8.0). Elution buffer was exchanged to Tris buffer (50 mM Tris-HCl buffer, pH 8.0) with Amicon Ultra-15 centrifugal

filter (10 000 NMWL). This procedure typically yielded ~20 mg of protein per liter of culture. Protein concentration was determined from the absorbance at 280 nm using calculated extinction coefficient or by SDS PAGE quantification using bovine serum albumin (BSA) as a standard (Figure S5).

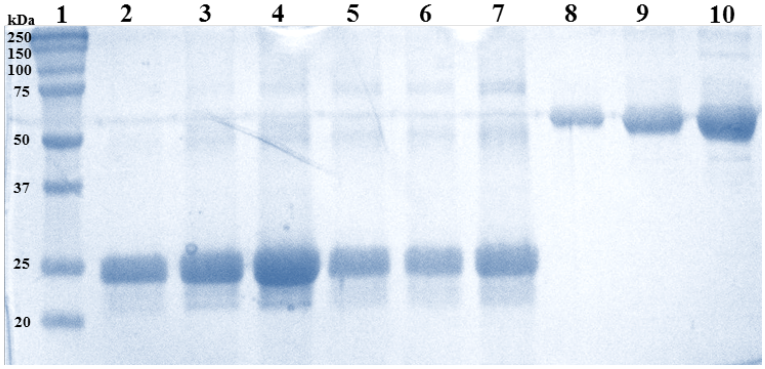


Figure S5. SDS PAGE quantification of purified wt MsrA and F52L MsrA: (1) protein mass marker; (2) 2 µL of F52L MsrA; (3) 4 µL of F52L MsrA; (4) 8 µL of F52L MsrA; (5) and (6) 5 µL of wt MsrA; (7) 8 µL of wt MsrA; (8) 2 µg of BSA; (9) 4 µg of BSA; (10) 8 µg of BSA.

8. Preparation of a Library and Screening

MsrA in pET-16b vector was used as the template for error-prone PCR using standard conditions of GeneMorph II EZ Clone Domain Mutagenesis Kit (Agilent Technologies) and a set of primers (5'-GCGGCCATATCGAAGGTC and 5'-CGGGCTTTGTTAGCAGCC). Error-prone PCR was carried out using GeneMorph II enzymes and the following cycling protocol: 1 × (95 °C, 2 min.), 30 × (95 °C, 1 min.; 60 °C, 1 min.; 72 °C, 1 min.), 1 × (72° C, 10 min). PCR product (megaprimers) was purified on agarose gel and used for the cloning reaction using EZ Clone kit and *msrA* in pET-16b vector as a template. The resulting gene library in pET-16b vector was transformed into *E. coli* 5-alpha competent cells competent cells via electroporation, plated on LB agar plates with ampicillin (100 mgL^{-1}) and incubated overnight at 37 °C. Then, the plates with colonies were homogenously sprayed with the solution of Propyl GreenOx (10 mM in 10 × TBE) and incubated at room temperature for 10-30 min. Then, the plates were visually inspected under UV light (366 nm handlamp, 8W) and the glowing colonies were collected for further analysis.

Protein sequences of variants (mutations indicated in red)

wt-MsrA

MGHHHHHHHHHHSSGHIEGRHMSLFDKKHLVSPADALPGRNTPMPVATLHAVNGH
SMTNVPDGMEIAIFAMGCFWGVERLFWQLPGVYSTAAGYTGGYTPNPTYREVCSGD
TGHAEAVRIVYDPSVISYEQLLQVFWENHDPAQGMRQGNDHGTQYRSAIYPLTPEQD

AAARASLERFQAAMLAADDDRHITTEIANATPFYYAEDDHQQYLHKNPYGYCGIGGI
GVCLPPKA

clone1-MsrA

MGHHHHHHHHHHSSGHIEGRHMSLFDKKHLVSPADALPGCNTPMPVATLHAVNGH
SMTNVPDGMEIAIFAMGCLWGVERLFWQLPGVYSTAAGYTGGYTPNPTYREVCSGD
TGHAEAVRIVYDPSVISYEHLLQVFWENHDPAQGMRQGNDHGTQYRSAIYPLTPEQD
AAARASLERFQAAMLAADDDRHITTEIANATPFYYAEDDHQQYLHKNPYGYCGIGGI
GVCLPPKA

clone2-MsrA

MGHHHHHHHHHHSSGHIEGRHMSLFDKKHLVSPADALPGRNTPMPVATLHAVNGH
SMTNVPDGMEIAIFAMGCLWGVERLFWQLPGVYSTAAGYTGGYTPNPTYREVCSGD
TGHAEAVRIVYDPSVINYEQLLQVFWENHDPAQGMRQGNDHGTQYRSAIYPLTPEQ
DAAARASLERFVAAMLAADDDRHITTEIANATPFYYAEDDHQQYLHKNPYGYCGIG
GIGVCLPPKA

9. Preparation of F52L single mutant of MsrA

The single mutation F52L was introduced to the wild type MsrA by Q5 Site-Directed Mutagenesis Kit (New England Biolabs) with *msrA* in pET-16b vector as a template and a set of primers (5'-GATGGGTTGTCTCTGGGGTGT and 5'-GCAAAAATGGCAATCTCCATTCC). The procedure was carried out according to the manufacturer's protocol. The presence of the F52L mutation was confirmed by DNA sequencing analysis.

10. MsrA Activity Assay

Purified wild type and mutant MsrA were used in the assay. To a solution of Propyl GreenOx (100 µM Propyl GreenOx and 2 mM DTT in 1 × TBE, 90 µl) in a cuvette 10 µl of the enzyme (15 mg/mL) was added and absorbance at 400 nm was measured over the period of 30 min (Figeure S6).

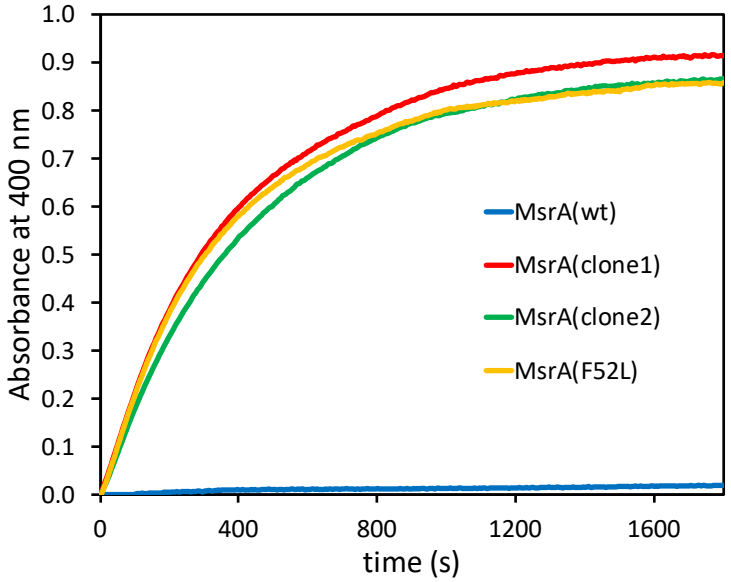
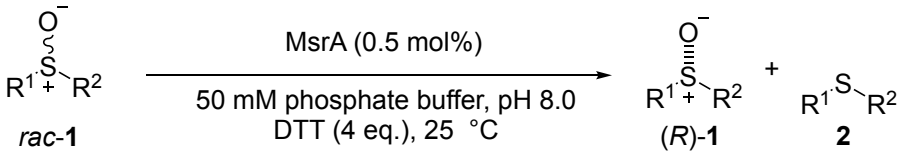


Figure S6. Kinetics of reduction of Propyl GreenOx (100 µM) catalyzed by wt MsrA and its three mutants measured by the change of absorbance at 400 nm.

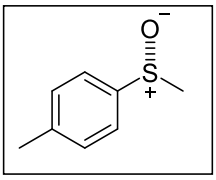
11. General Procedure for the Kinetic Resolution of Racemic Sulfoxides with Wild Type and F52L MsrA



A 1.5mL Eppendorf tube was charged with individual solutions of phosphate buffer (214 µL, 50mM Na_2HPO_4/NaH_2PO_4, 50mM NaCl, pH = 8) and DTT (250 µL of 52 mM, 4 equivs., 26 mM final concentration) in phosphate buffer and a racemic sulfoxide (10 µL of 324 mM, 3.2 µmol, 6.5 mM final concentration) in CH_3CN. The reaction was initiated by the addition of MsrA (25.6 µL of 635 µM, 0.5 mol%, 32.5 µM final concentration) in Tris buffer (50 mM Tris-HCl buffer, pH 8.0). The reaction mixture was flushed with argon and incubated at 25 °C. The conversion of the reaction was determined by analytical HPLC. After 24 h, the reaction mixture was extracted with EtOAc (4 mL), washed with water (0.5 mL), brine (0.5 mL), dried over

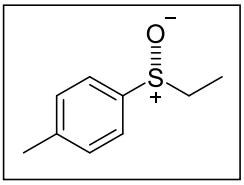
$MgSO_4$ and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess of the crude product was determined by chiral HPLC.

(*R*)-1-Methyl-4-(methylsulfinyl)benzene (1a)[1]



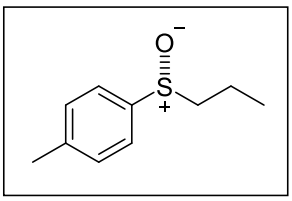
$C_8H_{10}OS$ (154.23 g/mol), wt MsrA: conversion 50 %, *ee* > 99 %, *s* > 100; F52L MsrA: conversion 50 %, *ee* > 99 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (98:2); flow rate 1mL/min; 25 °C; 254 nm; t_r= 31.3 and 34.1 min).

(*R*)-1-(Ethylsulfinyl)-4-methylbenzene (1b)[1]



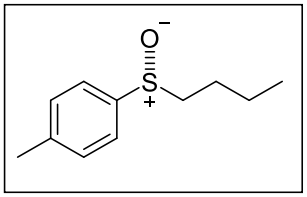
$C_9H_{12}OS$ (168.25 g/mol), wt MsrA: conversion 50 %, *ee* > 99 %, *s* > 100; F52L MsrA: conversion 50 %, *ee* > 99 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 16.4 and 17.9 min).

(*R*)-1-(Propylsulfinyl)-4-methylbenzene (1c)[1]



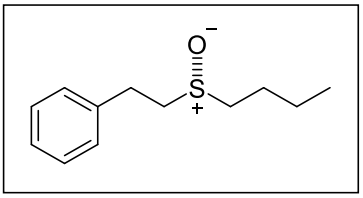
$C_{10}H_{14}OS$ (182.28 g/mol), wt MsrA: conversion <3 %, *ee* = 3 %, *s* not determined; F52L MsrA: conversion 50 %, *ee* = 99 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 14.7 and 16.0 min).

(*R*)-1-(butylsulfinyl)-4-methylbenzene (1d)[9]



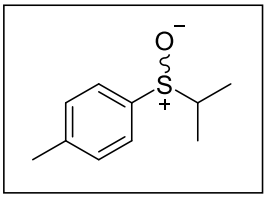
$C_{11}H_{16}OS$ (196.31 g/mol), wt MsrA: conversion <3 %, *ee* = 0 %, *s* = not determined; F52L MsrA: conversion 50 %, *ee* > 99 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 14.2 and 15.5 min).

(*R*)-(2-(butylsulfinyl)ethyl)benzene (1e)[10]



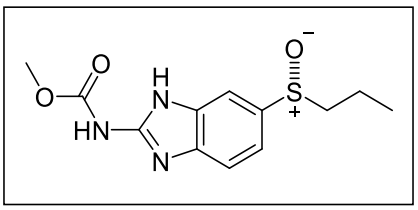
$C_{12}H_{18}OS$ (210.34 g/mol), wt MsrA: conversion <3 %, *ee* = 0 %, *s* not determined; F52L MsrA: conversion 50 %, *ee* > 99 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 17.1 and 23.0 min).

1-(Isopropylsulfinyl)-4-methylbenzene (1f)[2]



$C_{10}H_{14}OS$ (182.28 g/mol), wt MsrA: conversion <3 %, *ee* = 0 %, *s* not determined; F52L MsrA: conversion <3 %, *ee* = 0 %, *s* not determined; The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 13.8 and 15.1 min).

Albendazole-(*R*)-sulfoxide (1g)[11]



$C_{12}H_{15}N_3O_3S$ (281.33 g/mol), stock solution was prepared in *N*-methyl-2-pyrolidone instead of CH_3CN and 1.2 mol% of MsrA at 37 °C was used, wt MsrA : conversion = 6 %, *ee* = 6 %, *s* not determined; F52L MsrA: conversion = 49 %, *ee* = 94 %, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (70:30); flow rate 1 mL/min; 25 °C; 294 nm; t_r= 24.5 and 31.4 min).

12. References

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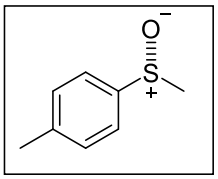
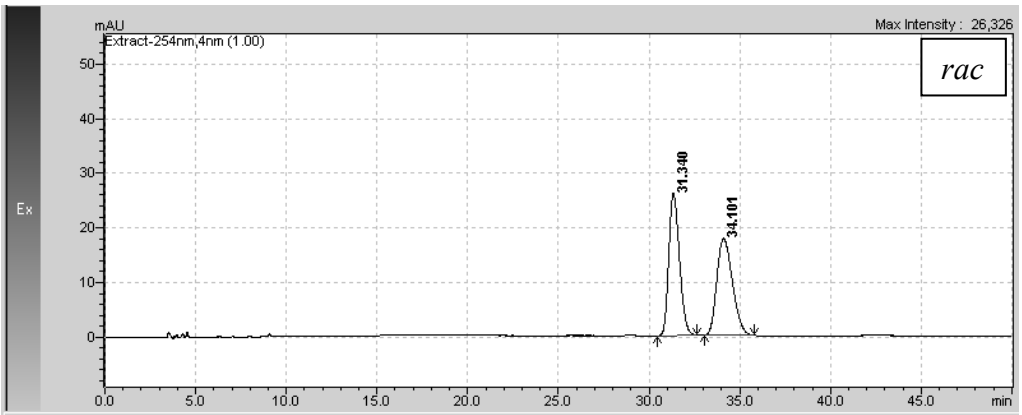
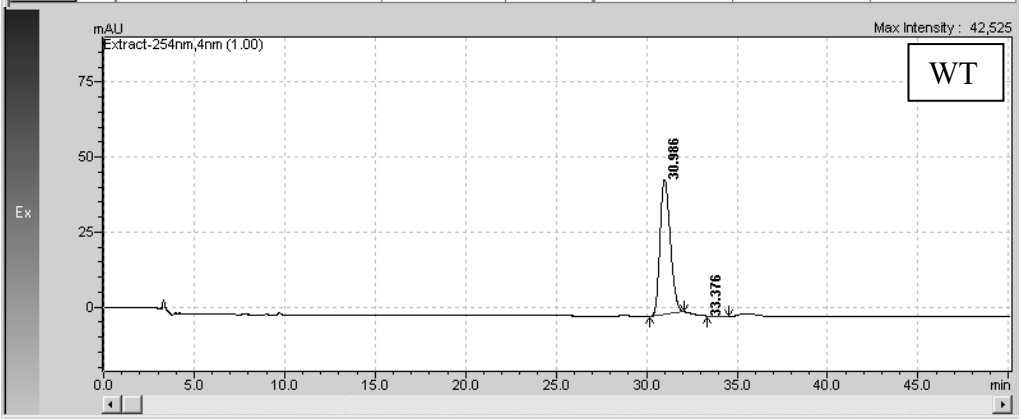
13. HPLC Traces: Kinetic Resolution with MsrA (WT and F52L)





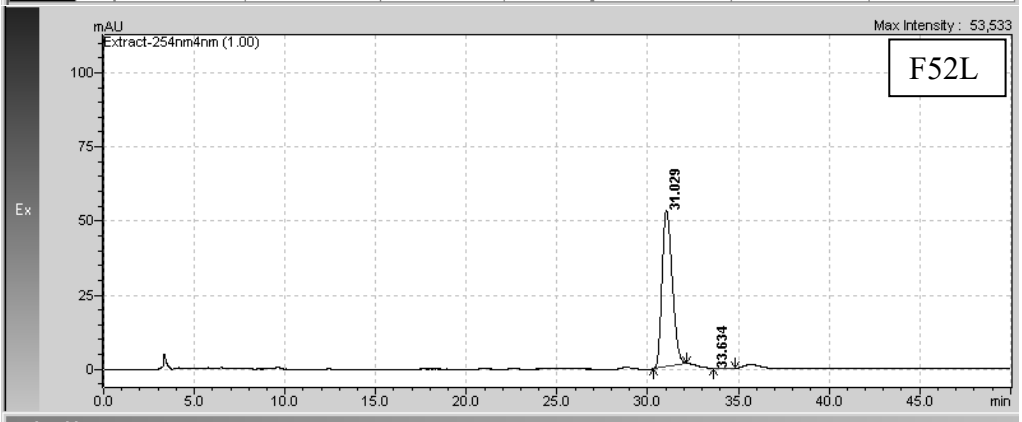
Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		31.340	50.07008	1063959	26056	30.485	32.651	50.0701
2		34.101	49.92992	1060981	17749	33.035	35.829	49.9299



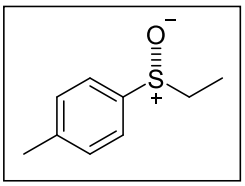
Peak Table

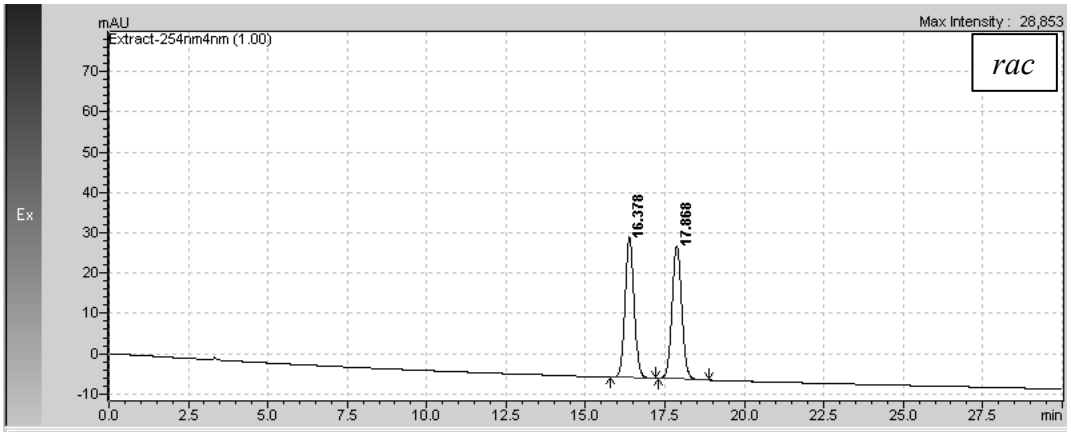
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		30.986	99.77246	1783397	44938	30.155	32.053	99.7725
2		33.376	0.22754	4067	178	33.365	34.539	0.2275



Peak Table

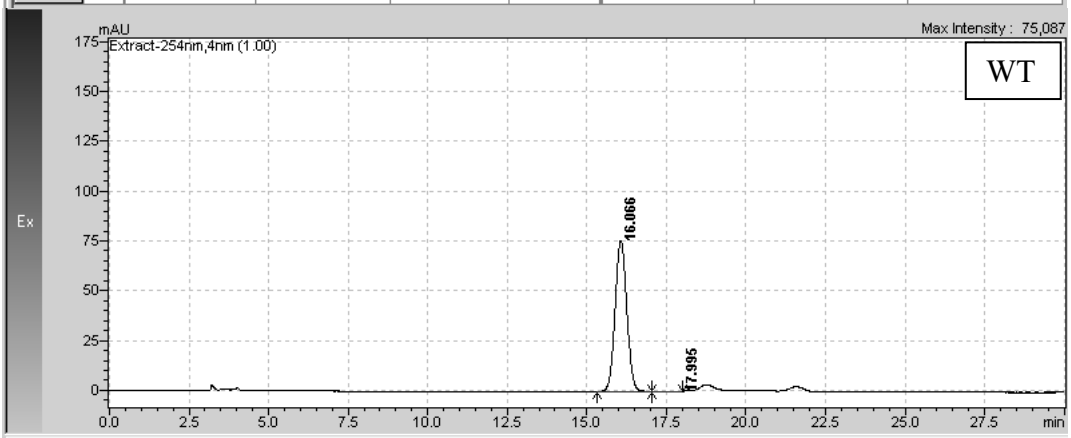
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		31.029	99.83648	2062672	52578	30.315	32.149	99.8365
2		33.634	0.16352	3378	137	33.632	34.805	0.1635





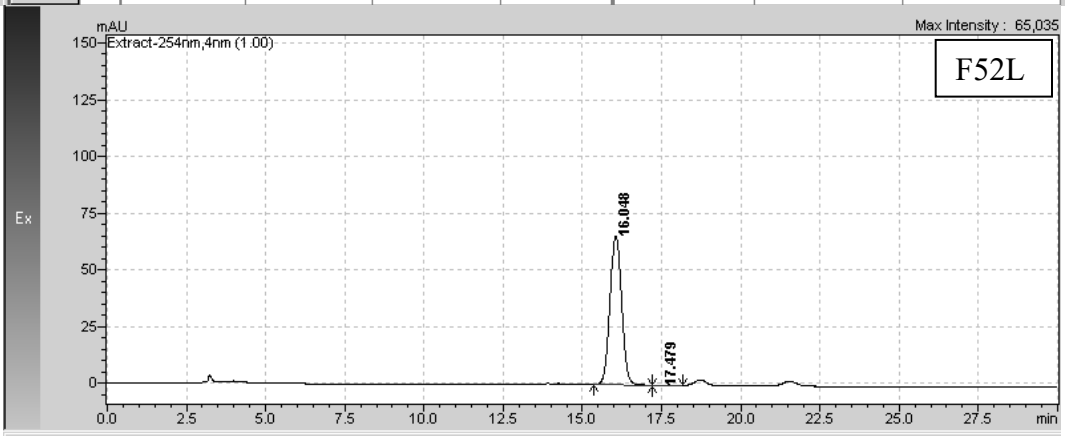
Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		16.378	50.02220	697034	34843	15.797	17.195	50.0222
2		17.868	49.97780	696415	32947	17.280	18.880	49.9778



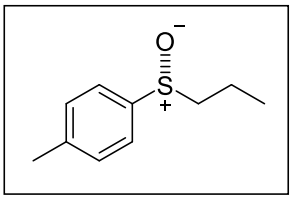
Peak Table

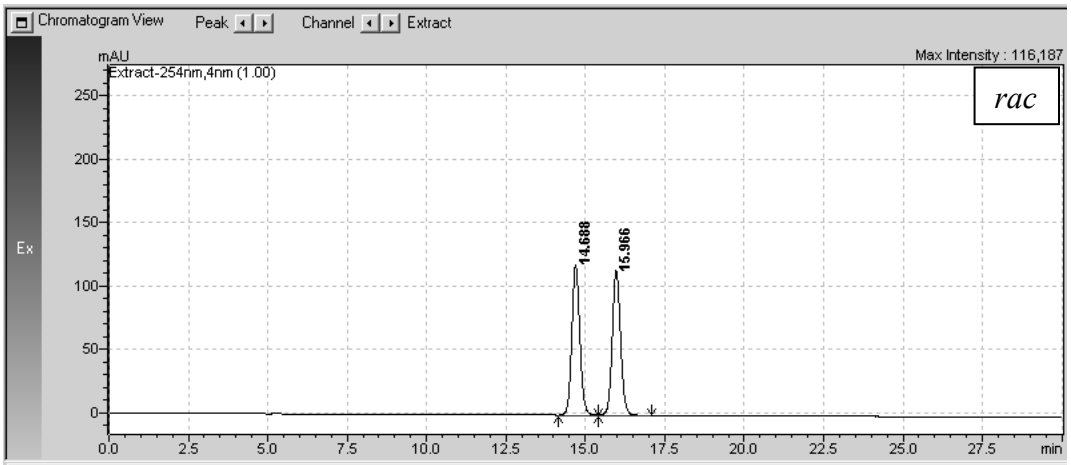
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		16.066	99.91349	1944906	75741	15.339	17.045	99.9135
2		17.995	0.08651	1684	44	17.045	18.005	0.0865



Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		16.048	99.93190	1676830	65501	15.371	17.195	99.9319
2		17.479	0.06810	1143	76	17.205	18.165	0.0681





Peak Table

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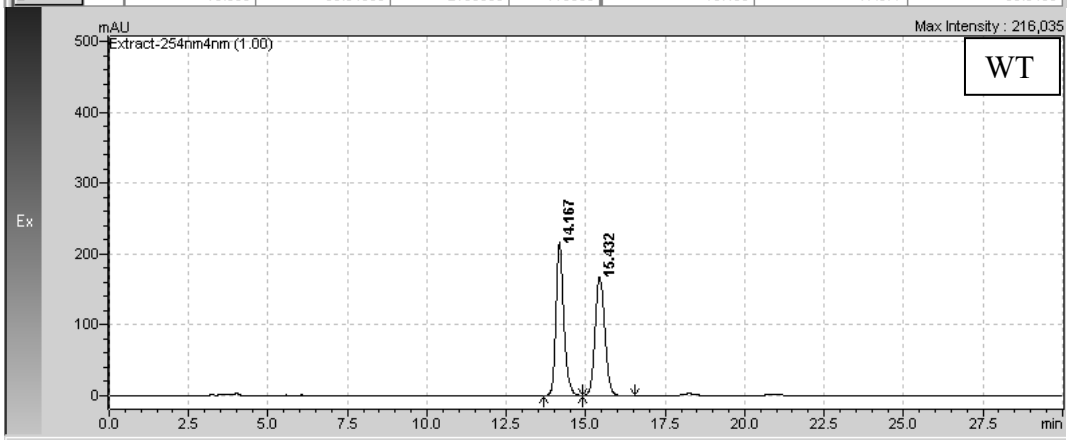
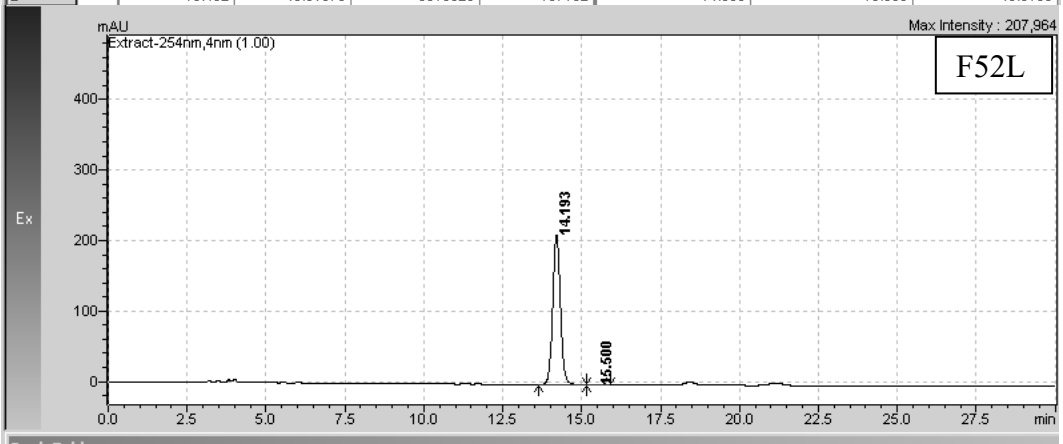


Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		14.167	51.48922	3729352	215984	13.675	14.880	51.4892
2		15.432	48.51078	3513625	167132	14.880	16.555	48.5108



Peak Table

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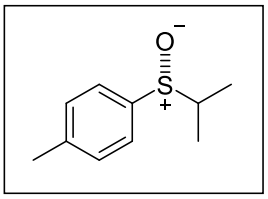
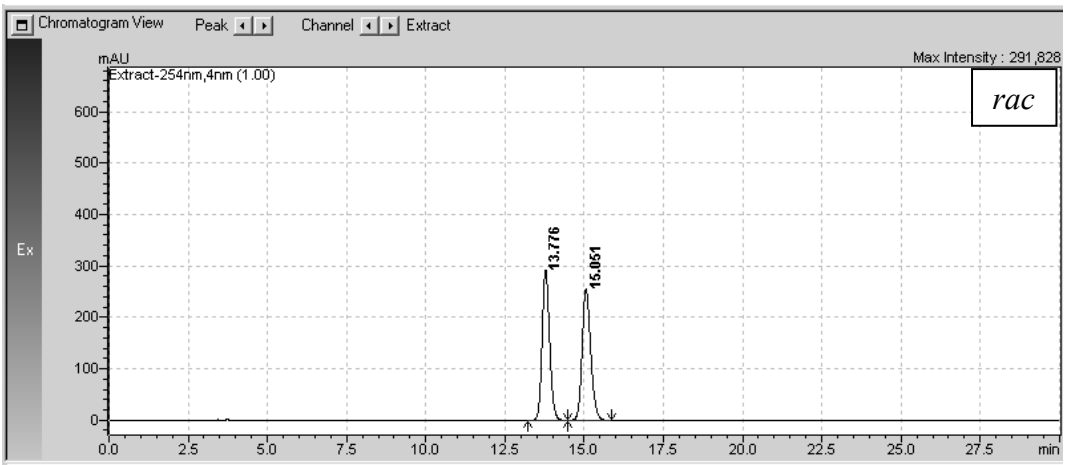
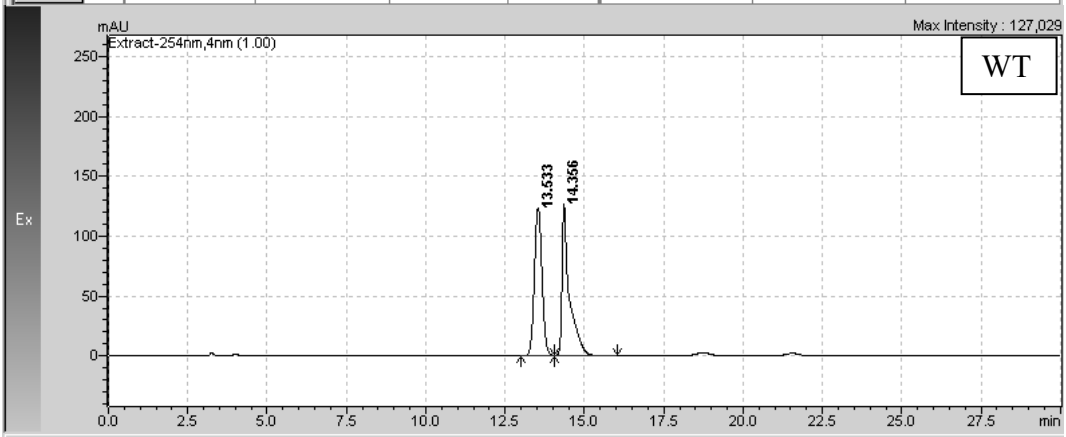
2		15.500	0.43740	15870	785	15.157	15.925	0.4374





Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		13.776	50.02701	4901629	292600	13.216	14.485	50.0270
2		15.051	49.97299	4896336	254998	14.485	15.851	49.9730



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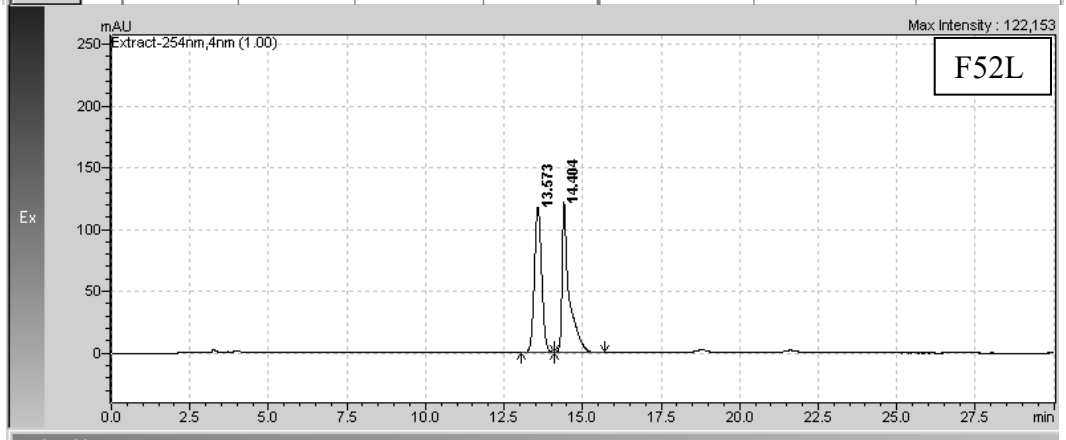
2		14.356	50.24945	2046126	127021	14.059	16.053	50.2495



Peak Table

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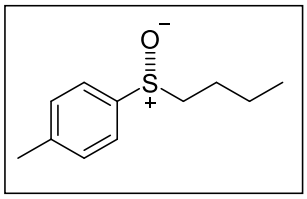
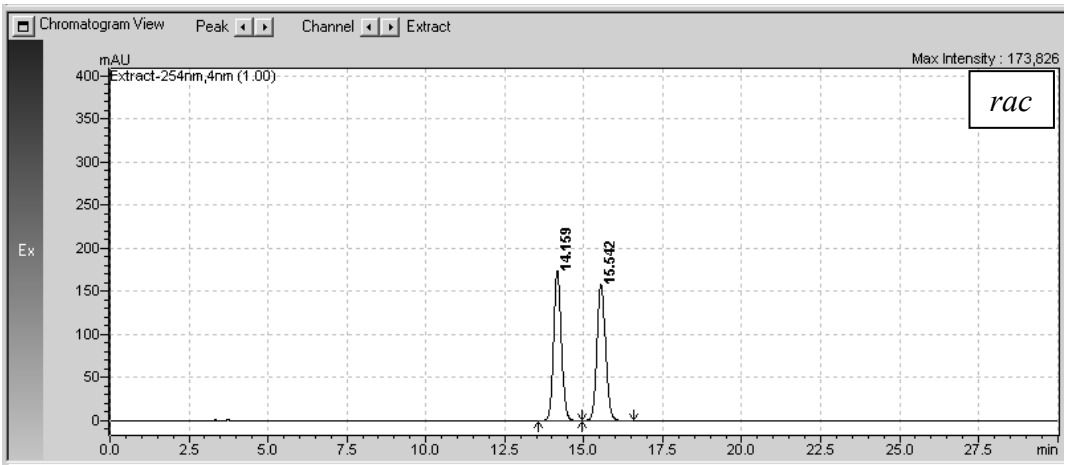
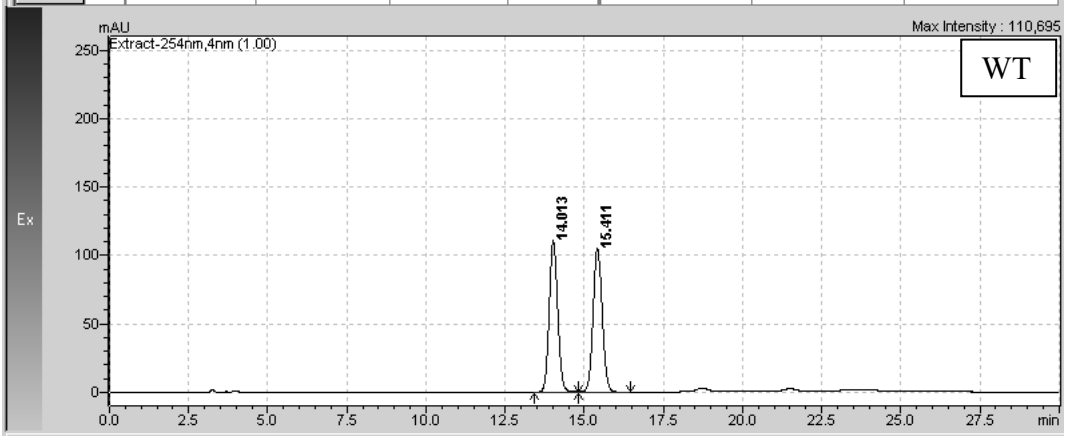




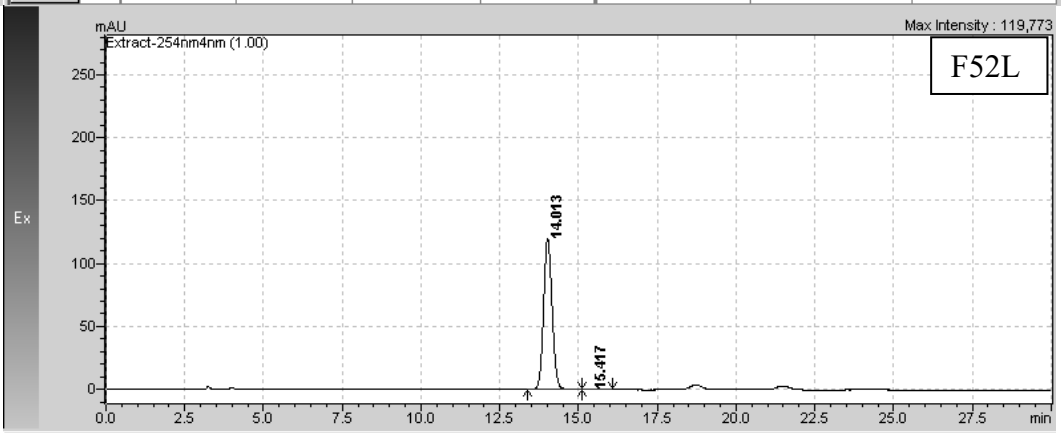
Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		14.159	49.99147	3015231	174373	13.568	14.965	49.9915
2		15.542	50.00853	3016260	158343	14.965	16.576	50.0085



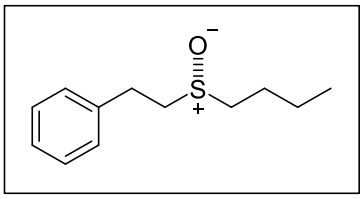
Peak Table

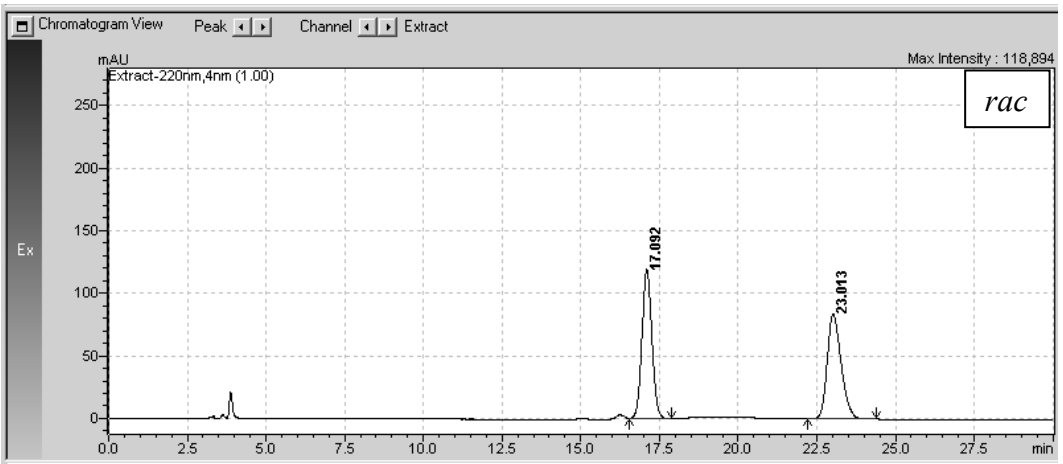
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		14.013	50.05404	2105439	110773	13.440	14.816	50.0540
2		15.411	49.94596	2100893	105435	14.816	16.448	49.9460



Peak Table

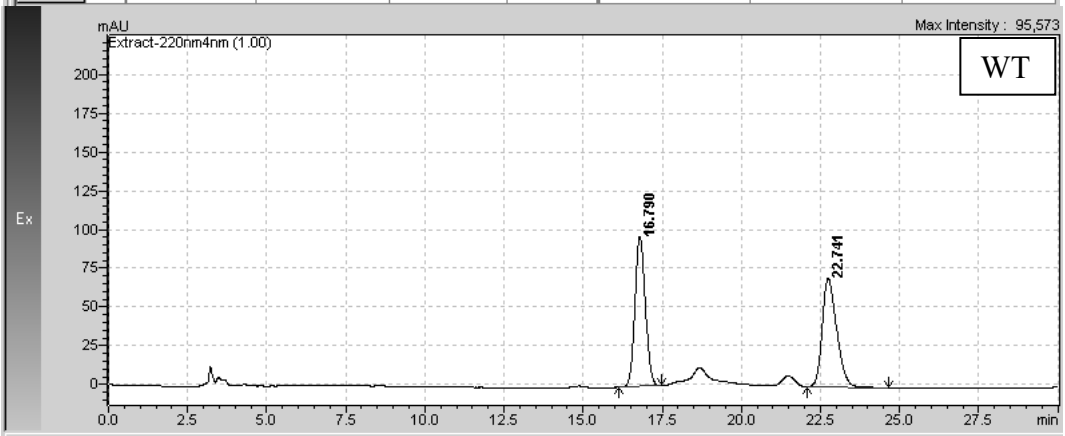
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		14.013	99.86996	2285685	119966	13.397	15.104	99.8700
2		15.417	0.13004	2976	186	15.115	16.075	0.1300





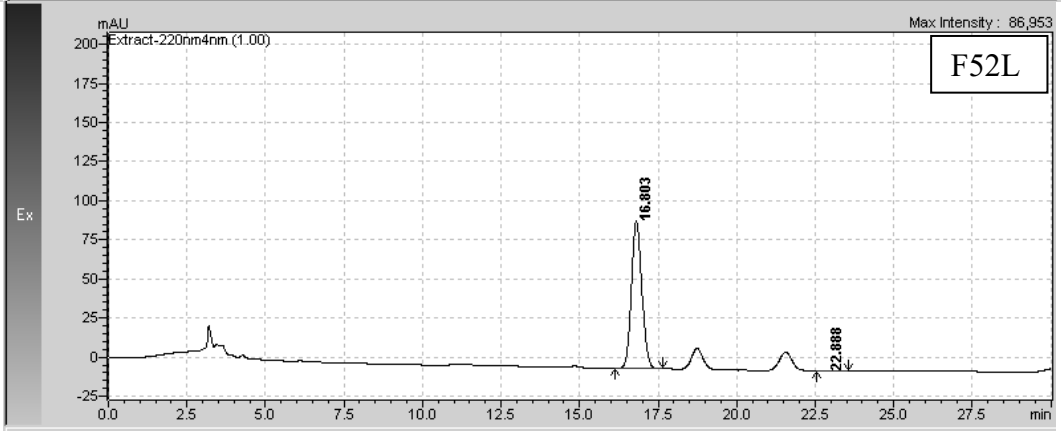
Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		17.092	50.27525	2617798	119151	16.544	17.867	50.2752
2		23.013	49.72475	2589134	83983	22.219	24.373	49.7248



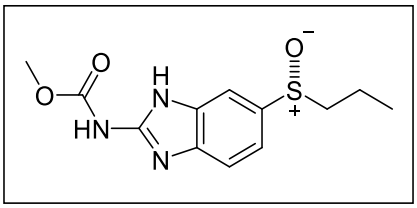
Peak Table

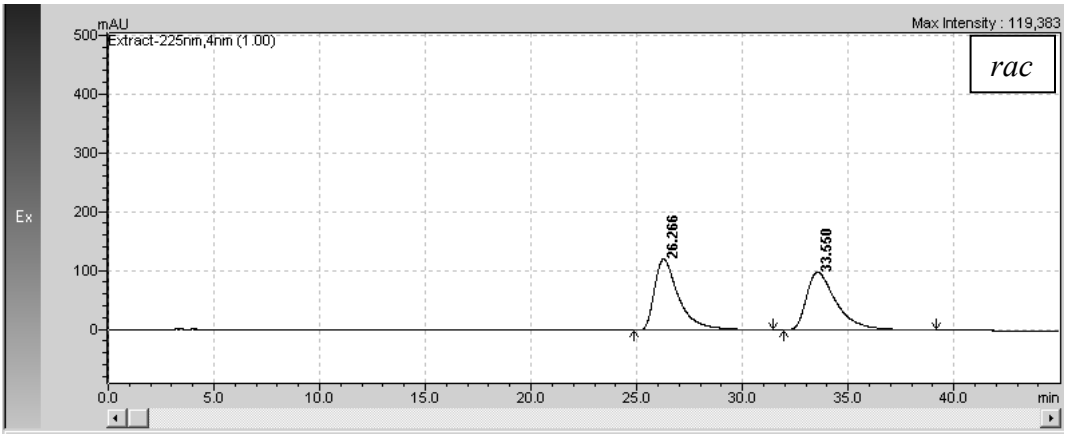
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		16.790	49.93502	2221104	96904	16.139	17.472	49.9350
2		22.741	50.06498	2226884	69832	22.069	24.672	50.0650



Peak Table

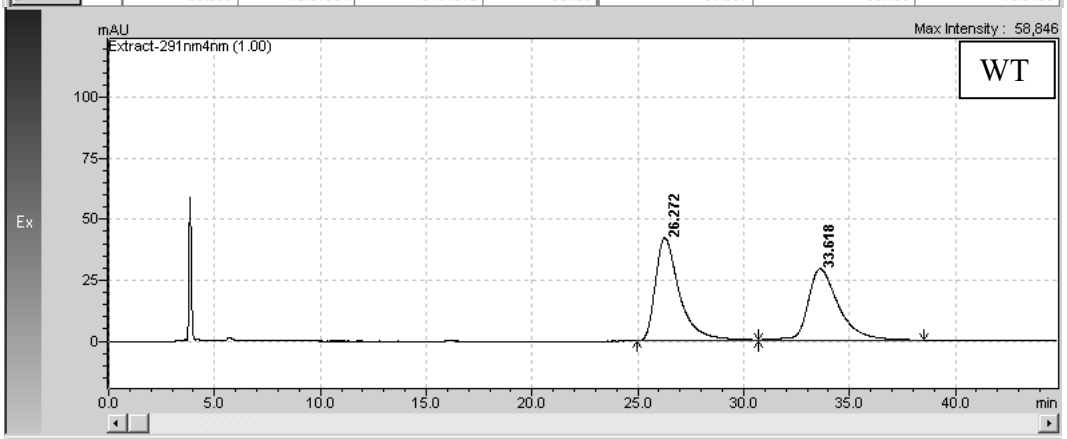
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		16.803	99.68400	2186712	94322	16.128	17.664	99.6840
2		22.888	0.31600	6932	280	22.528	23.552	0.3160





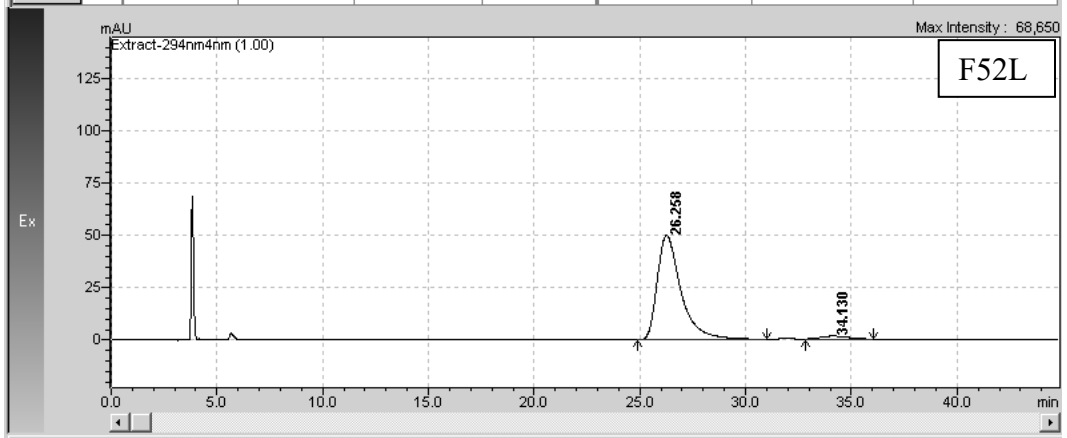
Peak Table

Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		26.266	50.18346	9782597	121181	24.875	31.424	50.1835
2		33.550	49.81654	9711072	98108	31.957	39.136	49.8165



Peak Table

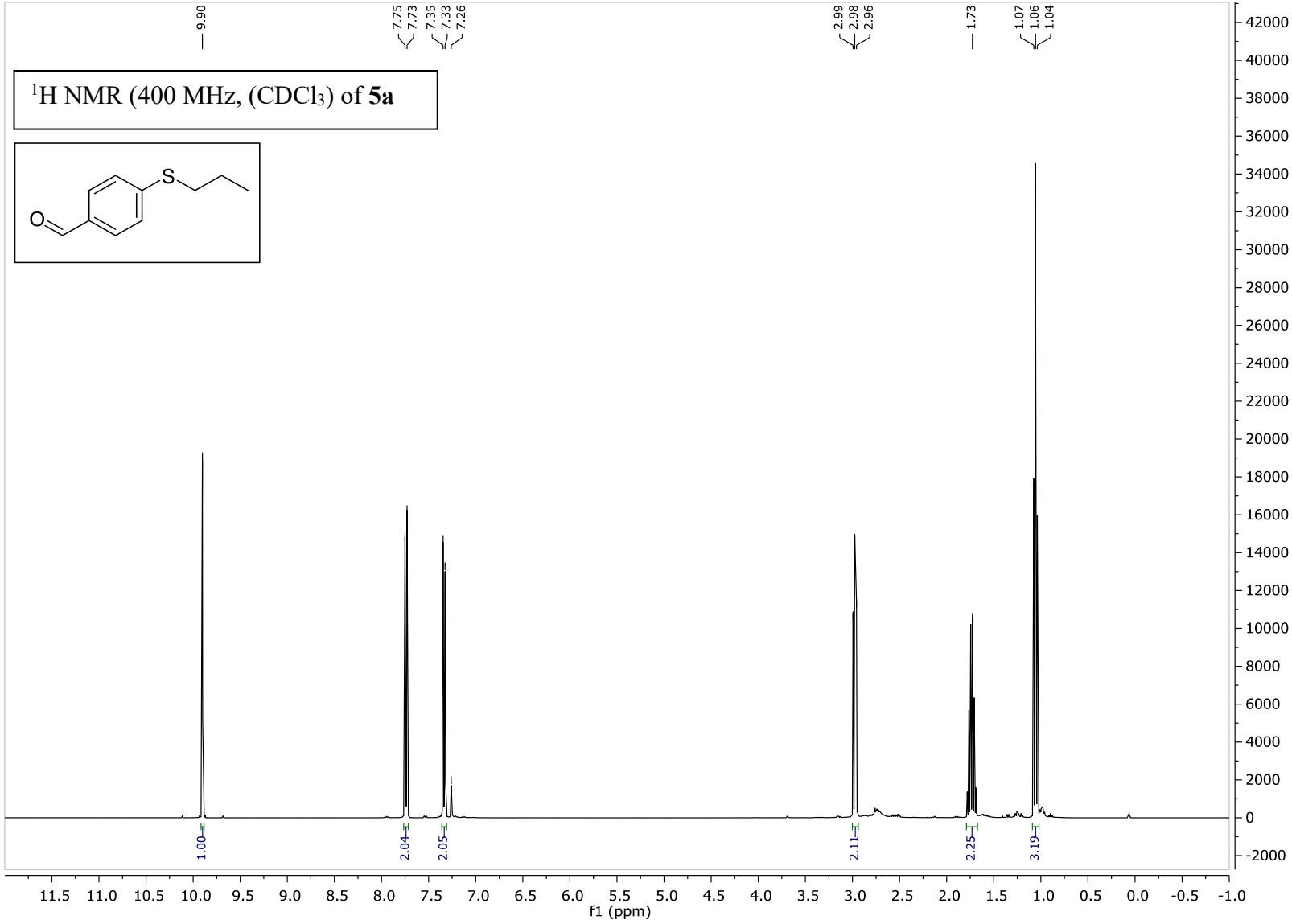
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		26.272	52.98070	3391019	42245	24.949	30.677	52.9807
2		33.618	47.01930	3009460	29475	30.677	38.485	47.0193

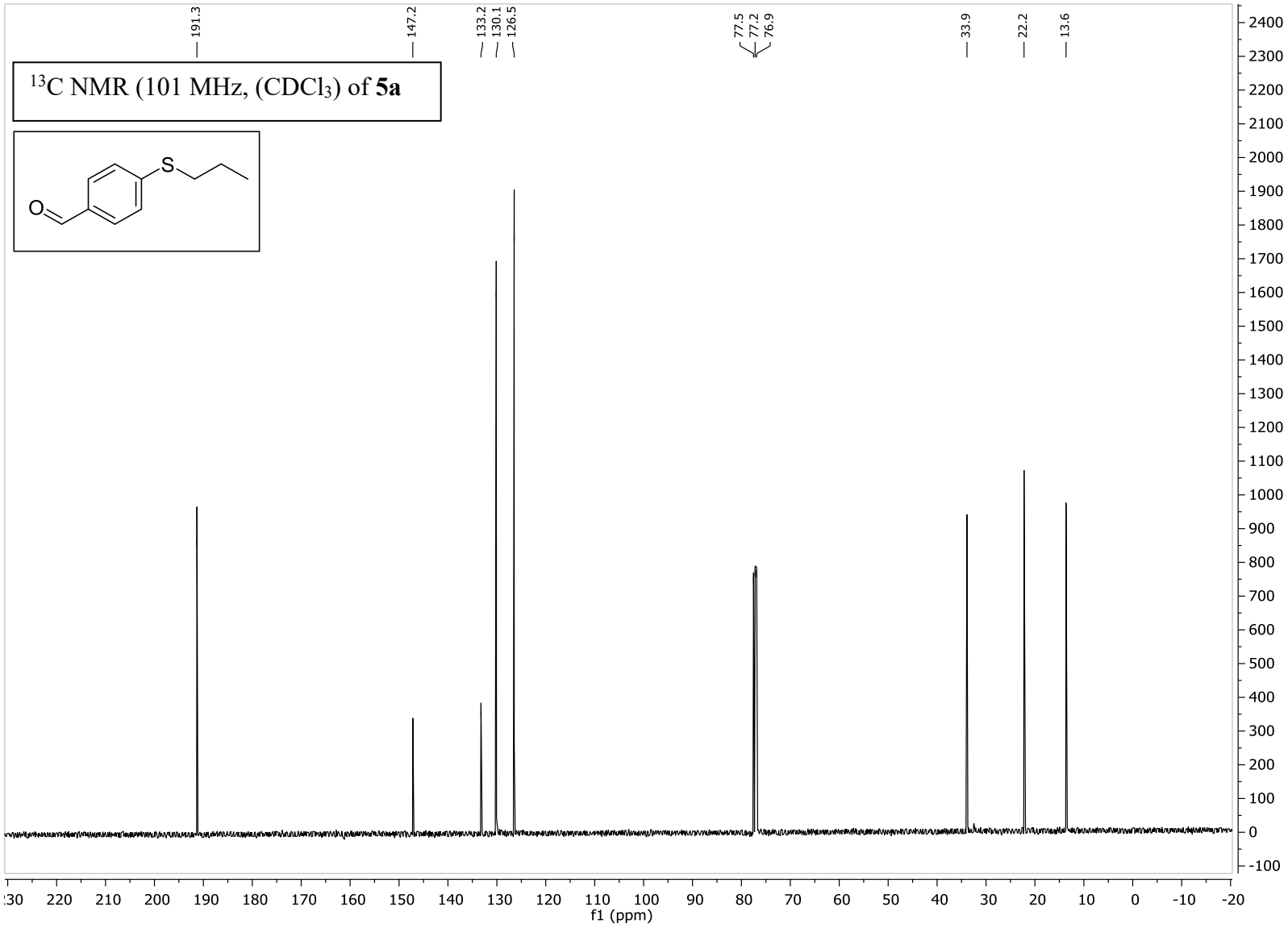


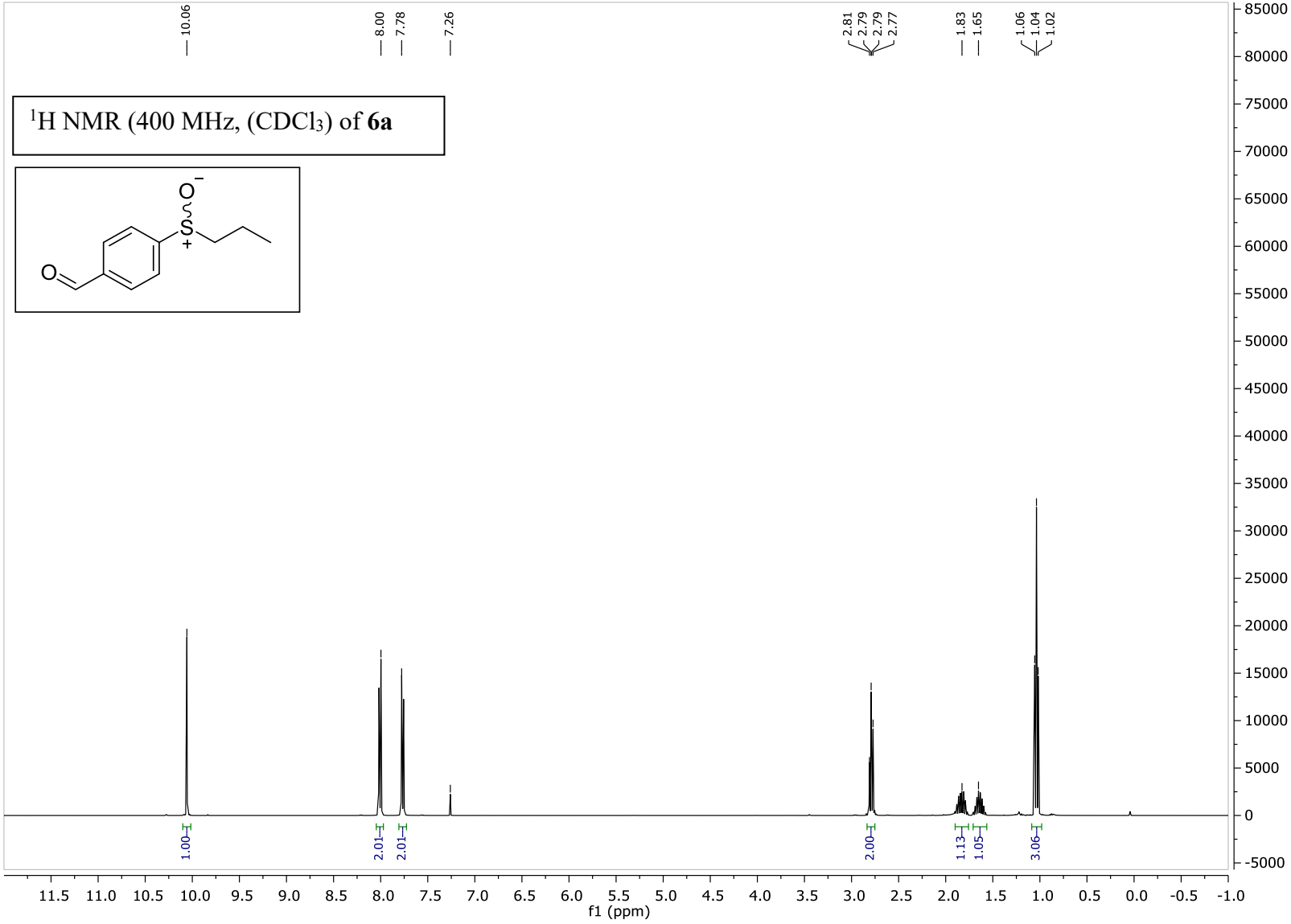
Peak Table

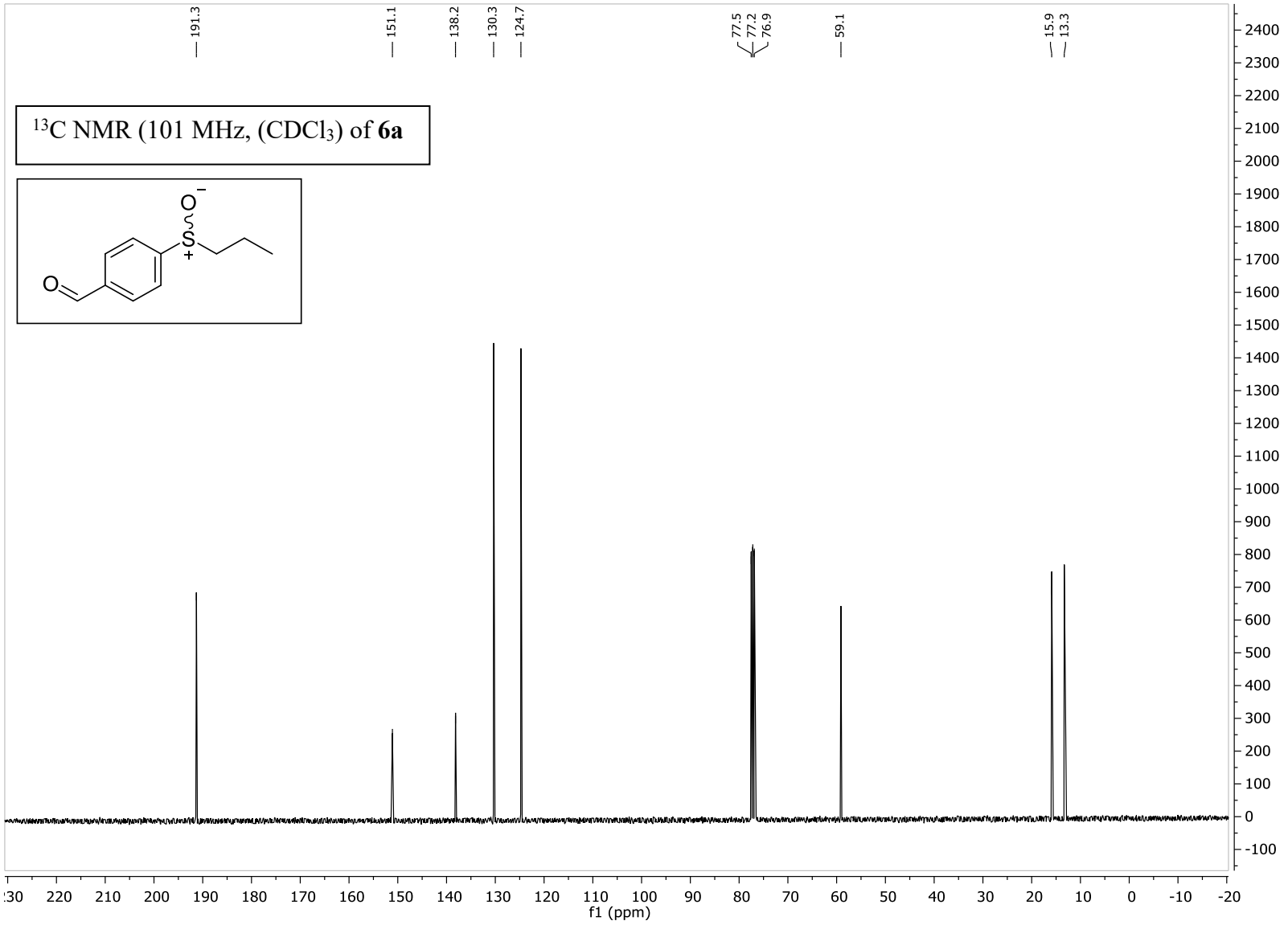
Peak#	ID#	Ret. Time	Conc.	Area	Height	Peak Start	Peak End	Area%
1		26.258	96.74304	3904835	49982	24.917	30.997	96.7430
2		34.130	3.25696	131460	1447	32.832	36.053	3.2570

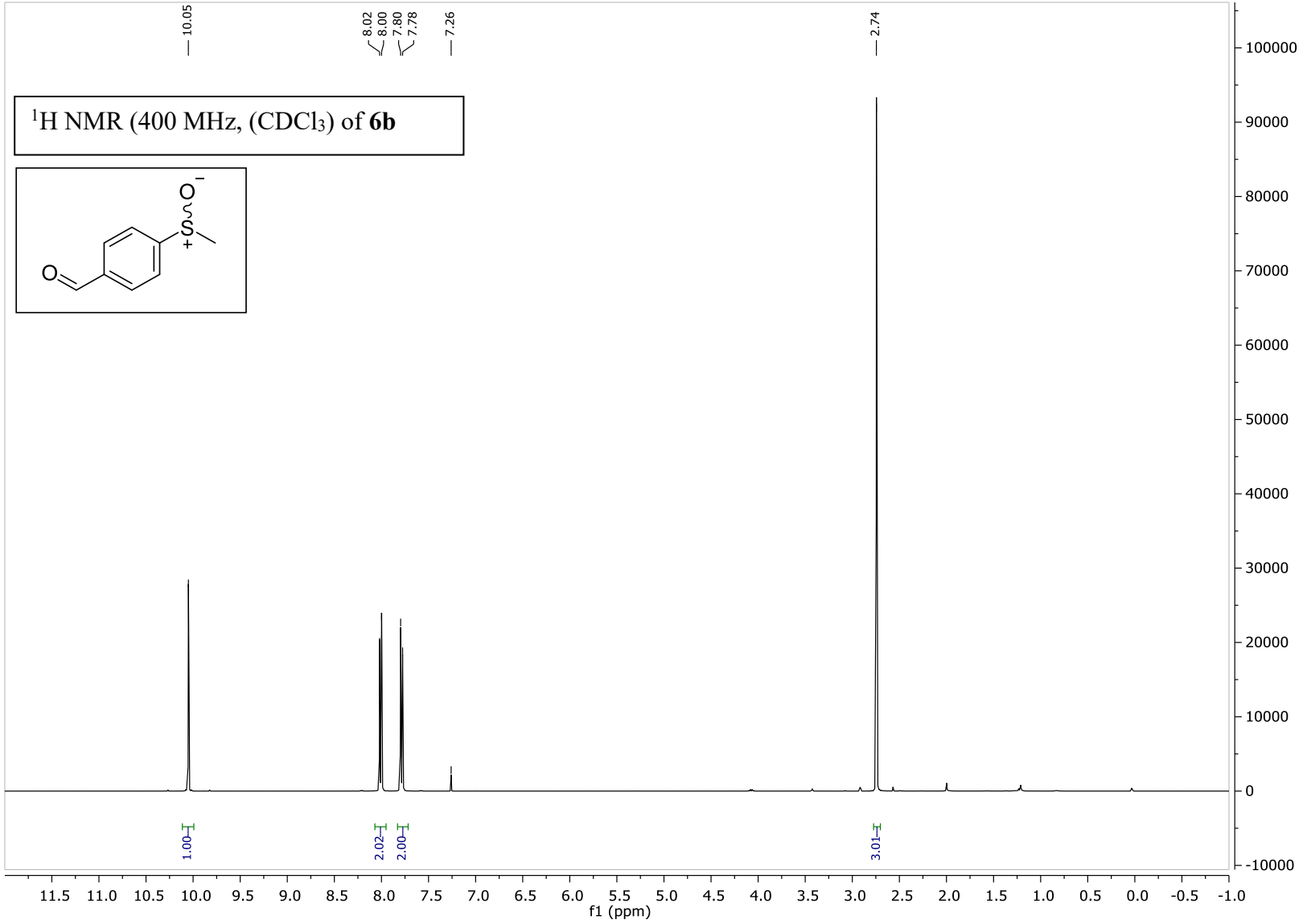
14. NMR Spectra

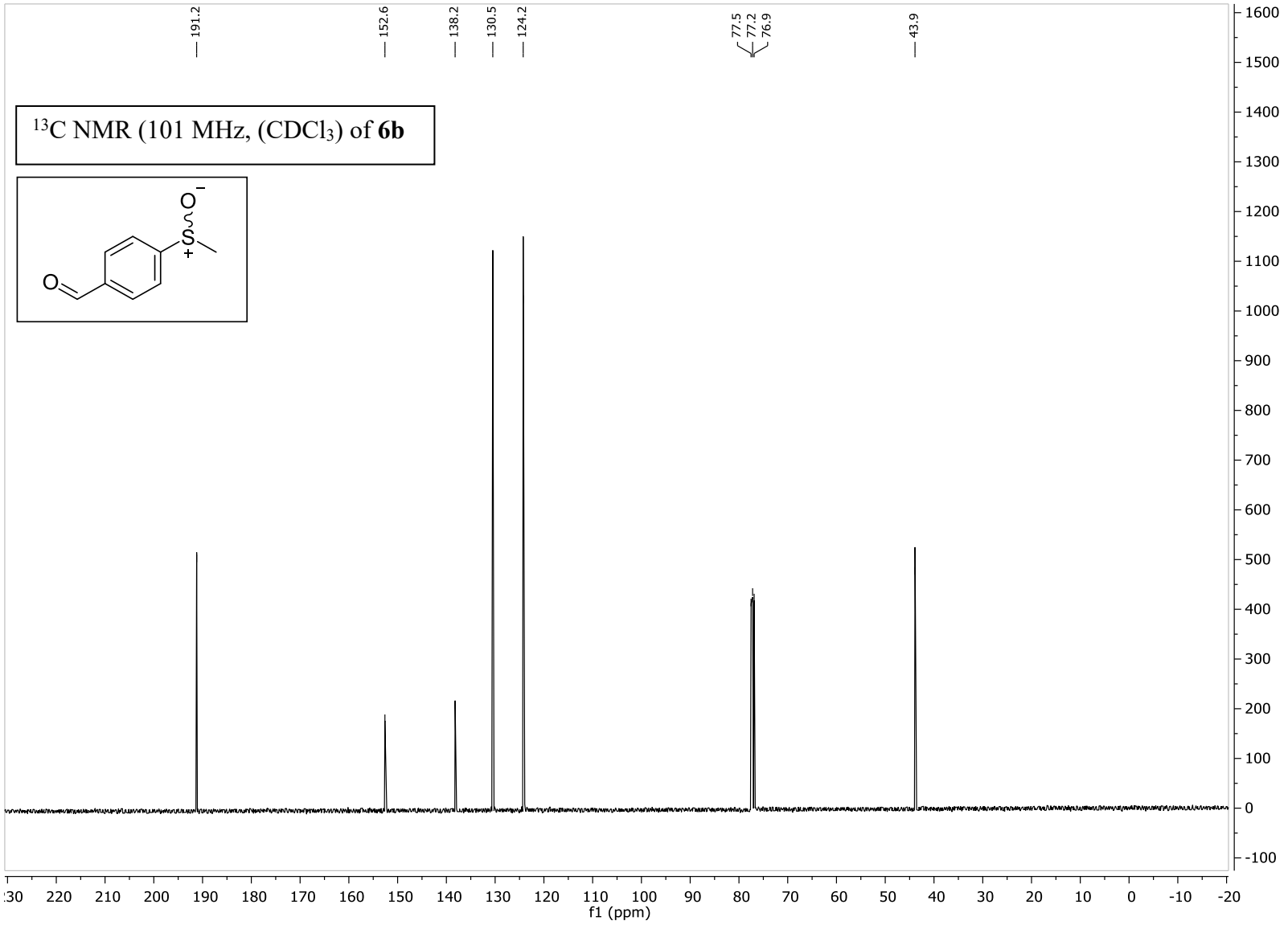

9.90
7.75
7.73
7.35
7.33
7.26
2.99
2.98
2.96
1.73
1.07
1.06
1.04
1H NMR (400 MHz, (CDCl3) of 5a
S
O
1.00
2.04
2.05
2.11
2.25
3.19
f1 (ppm)

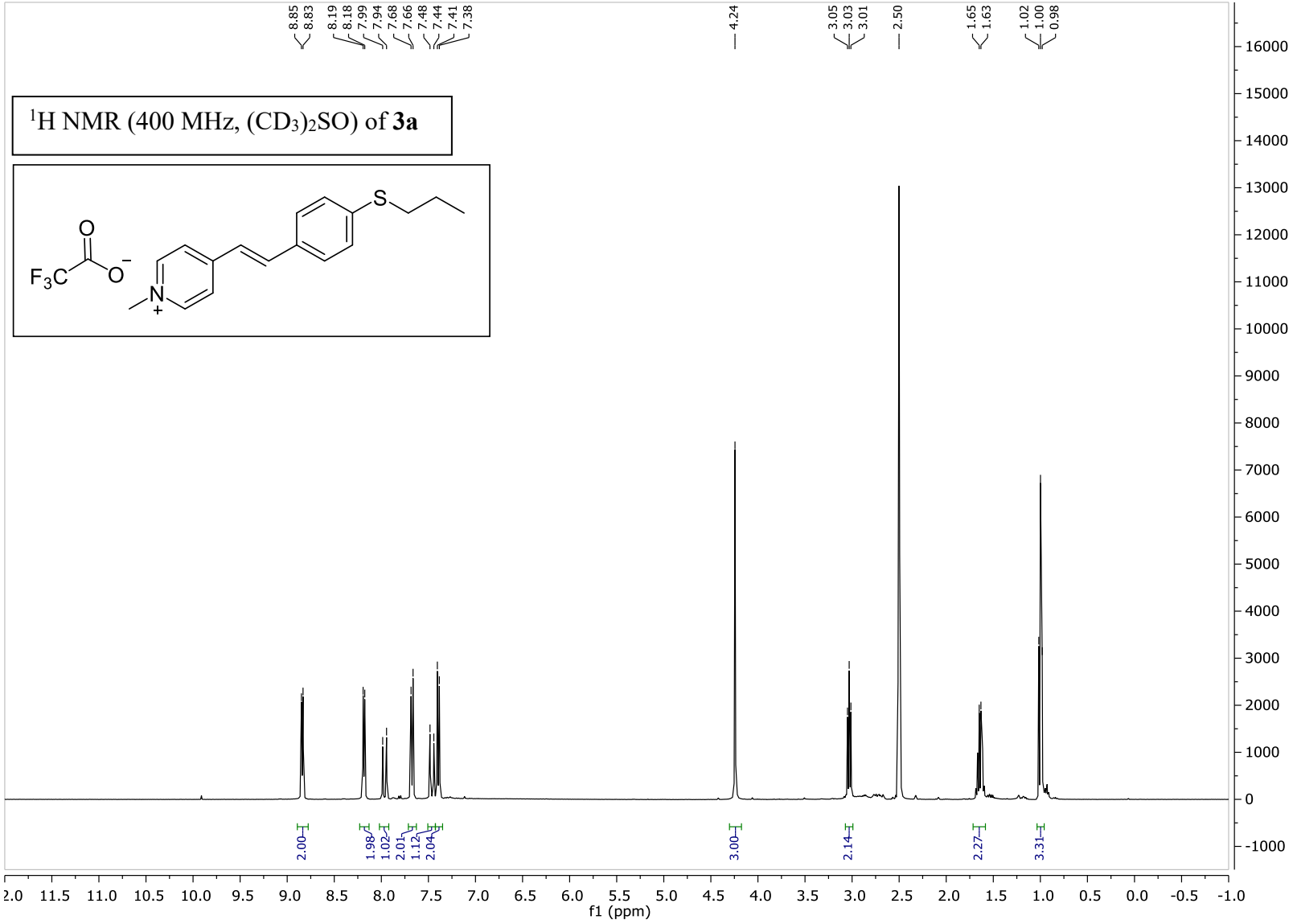

191.3
147.2
133.2
130.1
126.5
77.5
77.2
76.9
33.9
22.2
13.6
^{13}C NMR (101 MHz, ($CDCl_3$) of **5a**
O
S
f1 (ppm)

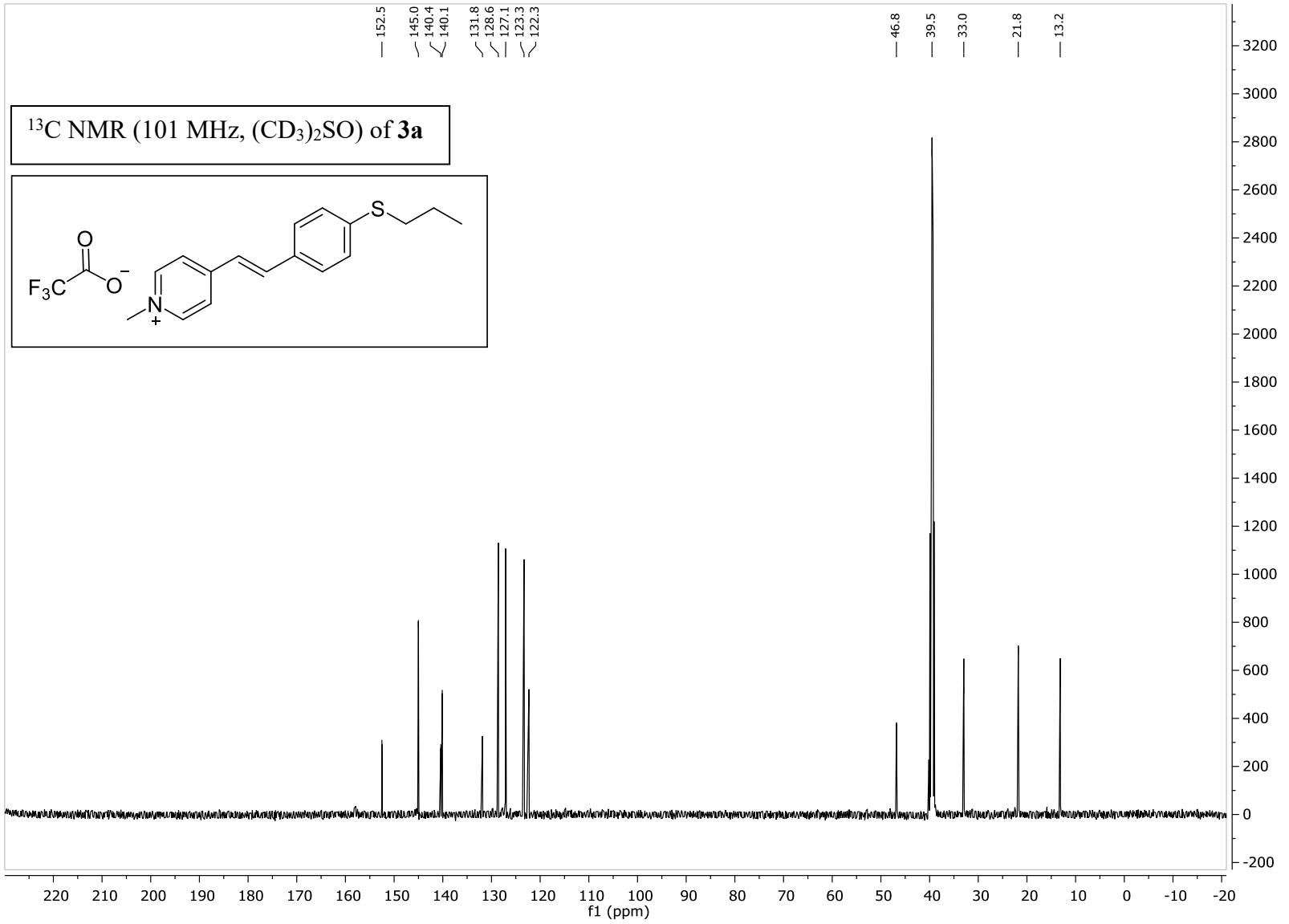

10.06
8.00
7.78
7.26
2.81
2.79
2.79
2.77
1.83
1.65
1.06
1.04
1.02
1H NMR (400 MHz, (CDCl3) of 6a
O
S
O
1.00
2.01
2.01
2.00
1.13
1.05
3.06
f1 (ppm)

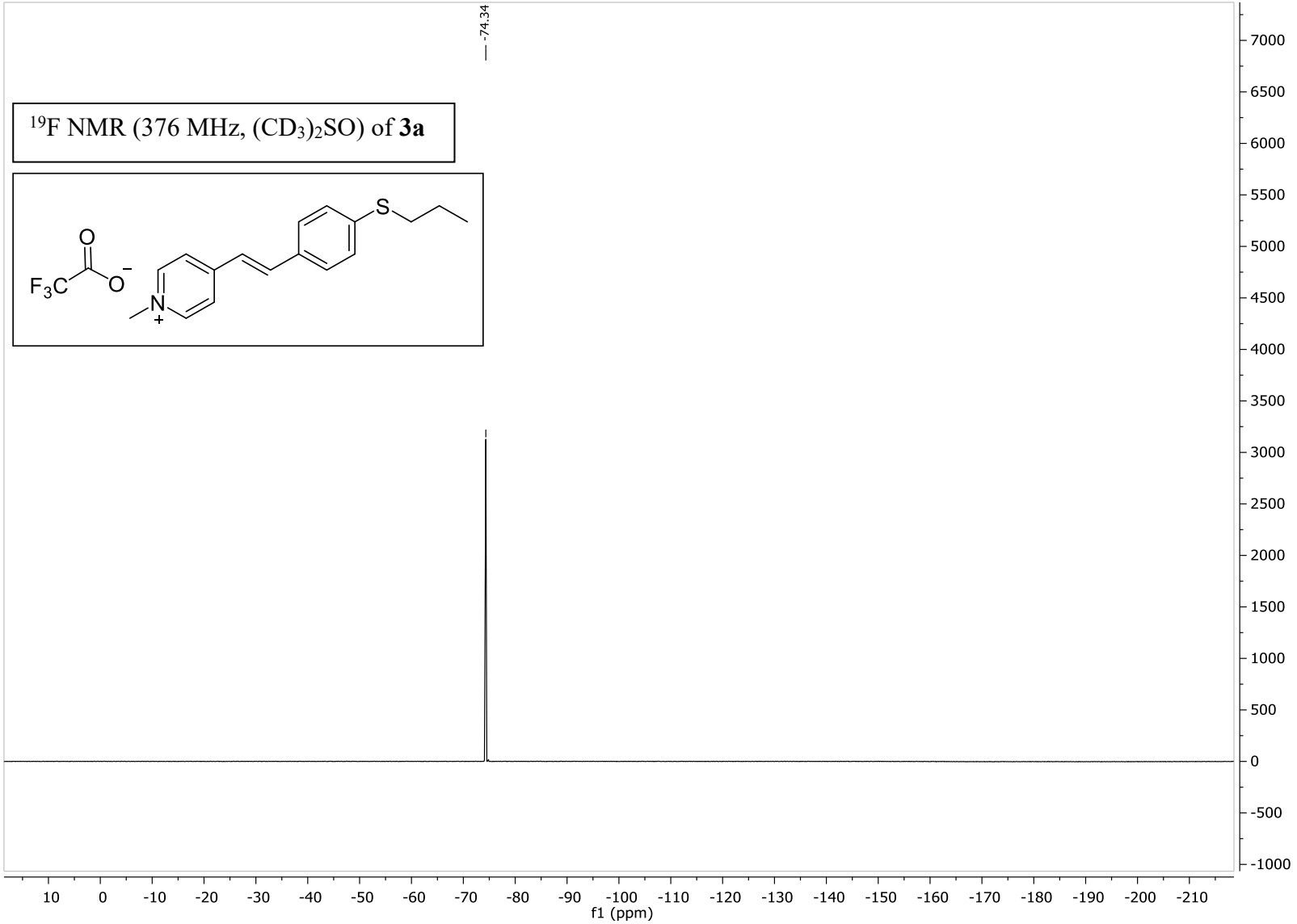

191.3
151.1
138.2
130.3
124.7
77.5
77.2
76.9
59.1
15.9
13.3
^{13}C NMR (101 MHz, ($CDCl_3$) of **6a**
O
O
S
f1 (ppm)

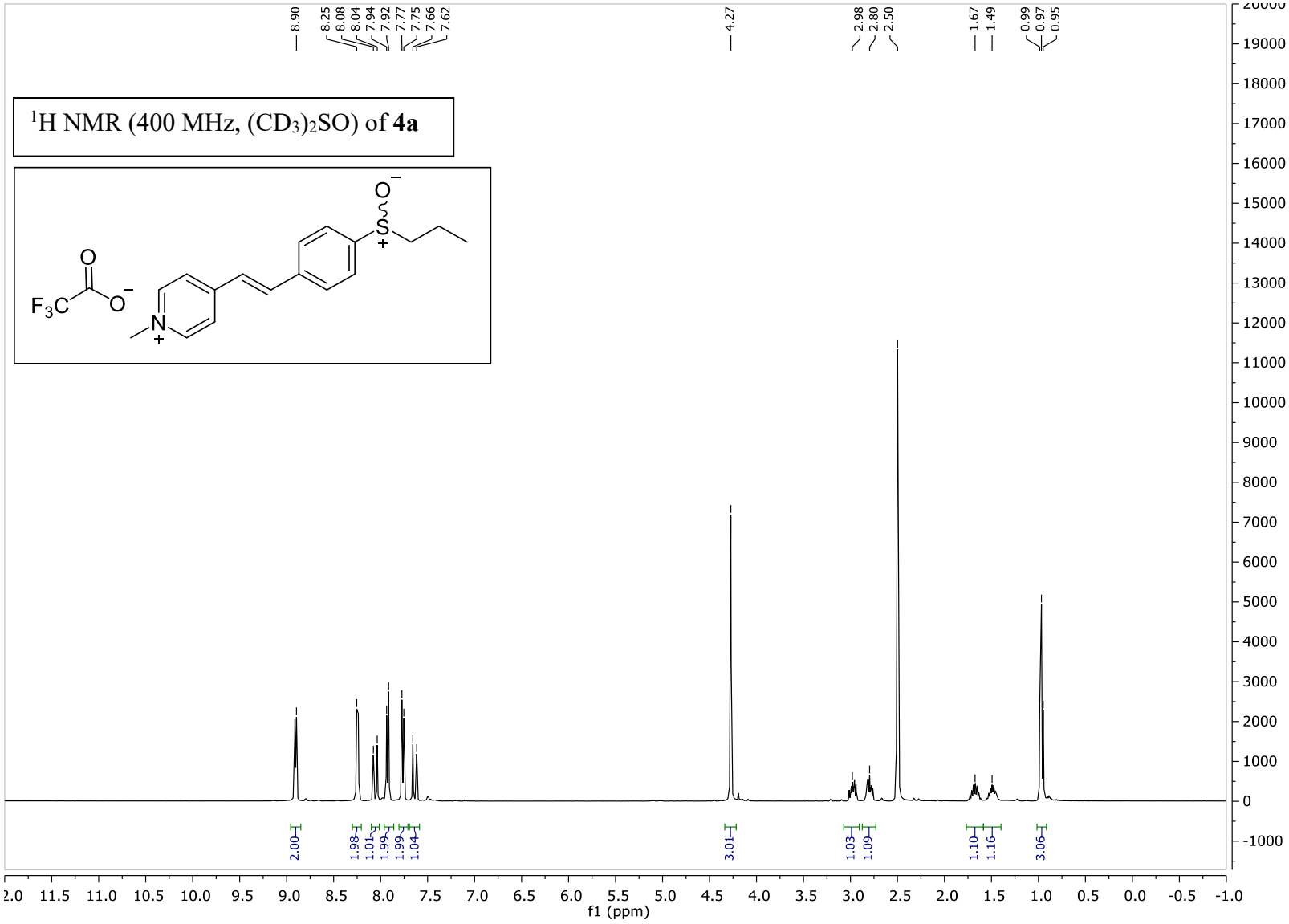

10.05
8.02
8.00
7.80
7.78
7.26
2.74
1H NMR (400 MHz, (CDCl3) of 6b
O
S
O
1.00
2.02
2.00
3.01
f1 (ppm)


191.2
152.6
138.2
130.5
124.2
77.5
77.2
76.9
43.9
^{13}C NMR (101 MHz, ($CDCl_3$) of **6b**
O
S
O
f1 (ppm)

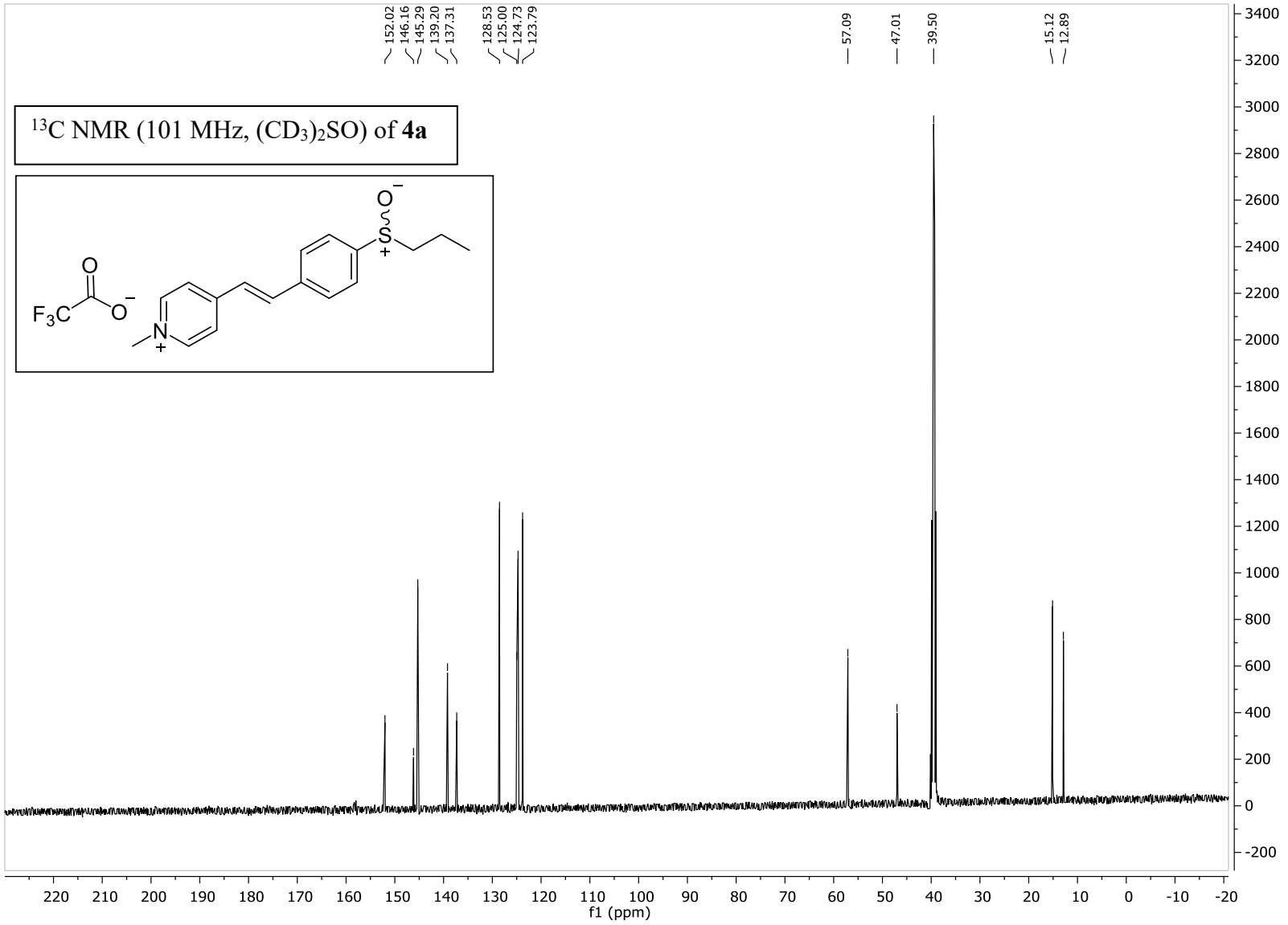

8.85
8.83
8.19
8.18
7.99
7.94
7.68
7.66
7.48
7.44
7.41
7.38
4.24
3.05
3.03
3.01
2.50
1.65
1.63
1.02
1.00
0.98
^{1}H NMR (400 MHz, $(CD_3)_2SO$) of **3a**
O
F_3C
O
N
+
S
2.00
1.98
1.02
2.01
1.12
2.04
3.00
2.14
2.27
3.31
f1 (ppm)

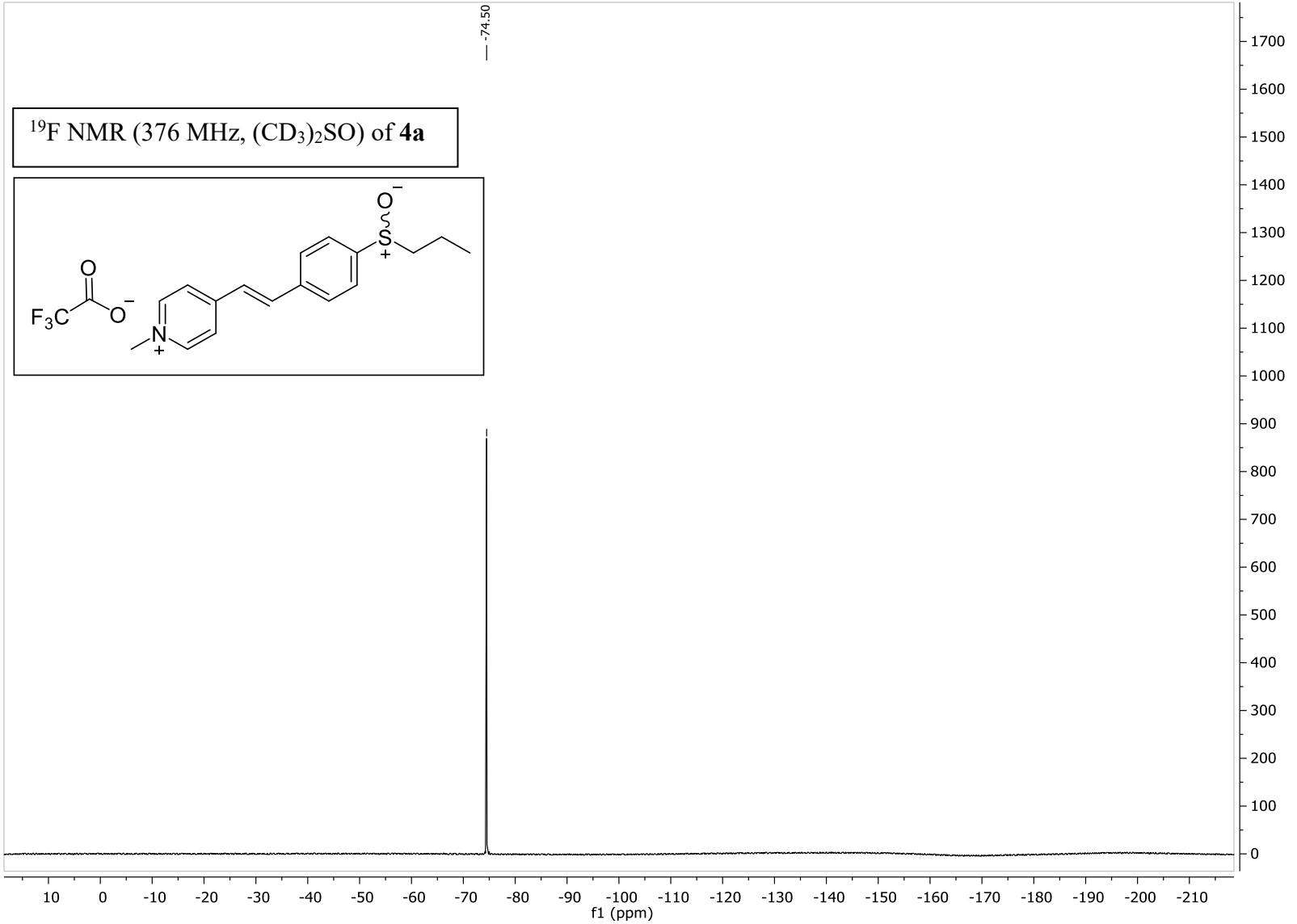

152.5
145.0
140.4
140.1
131.8
128.6
127.1
123.3
122.3
46.8
39.5
33.0
21.8
13.2
^{13}C NMR (101 MHz, $(CD_3)_2SO$) of **3a**
O
F_3C
O
N
+
S
f1 (ppm)

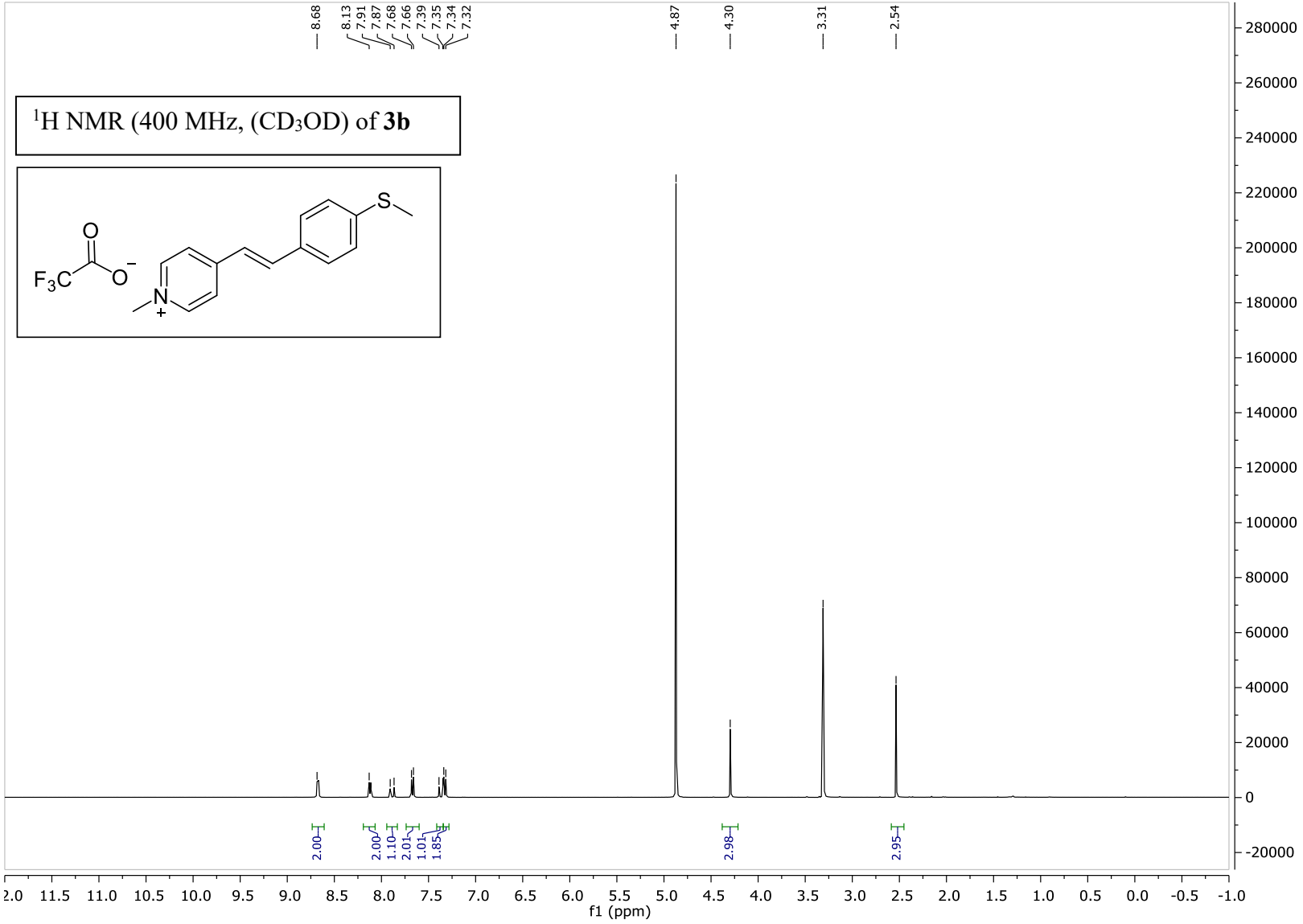

-74.34
^{19}F NMR (376 MHz, $(CD_3)_2SO$) of **3a**
O
F_3C
O
S
N
+
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
-1000
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

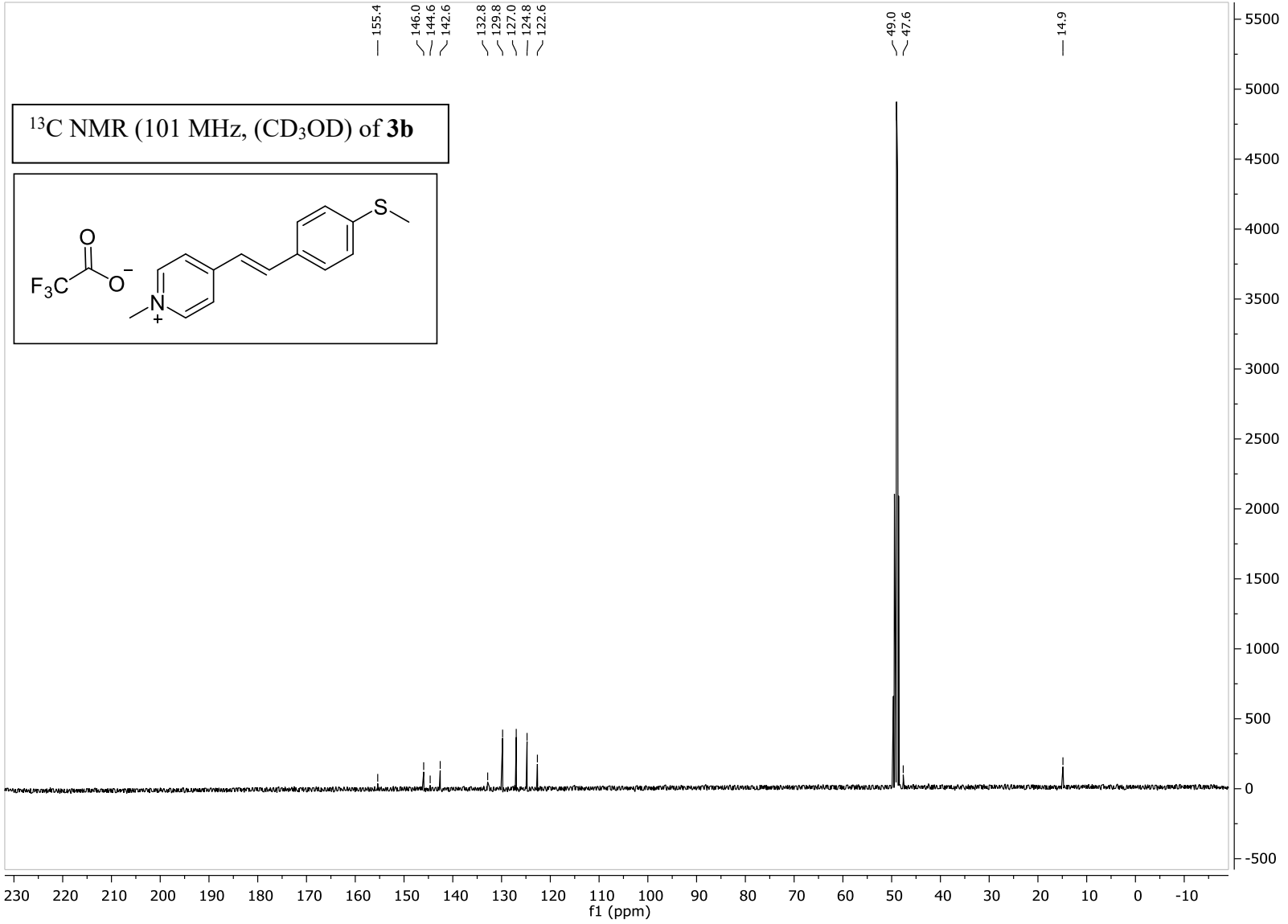


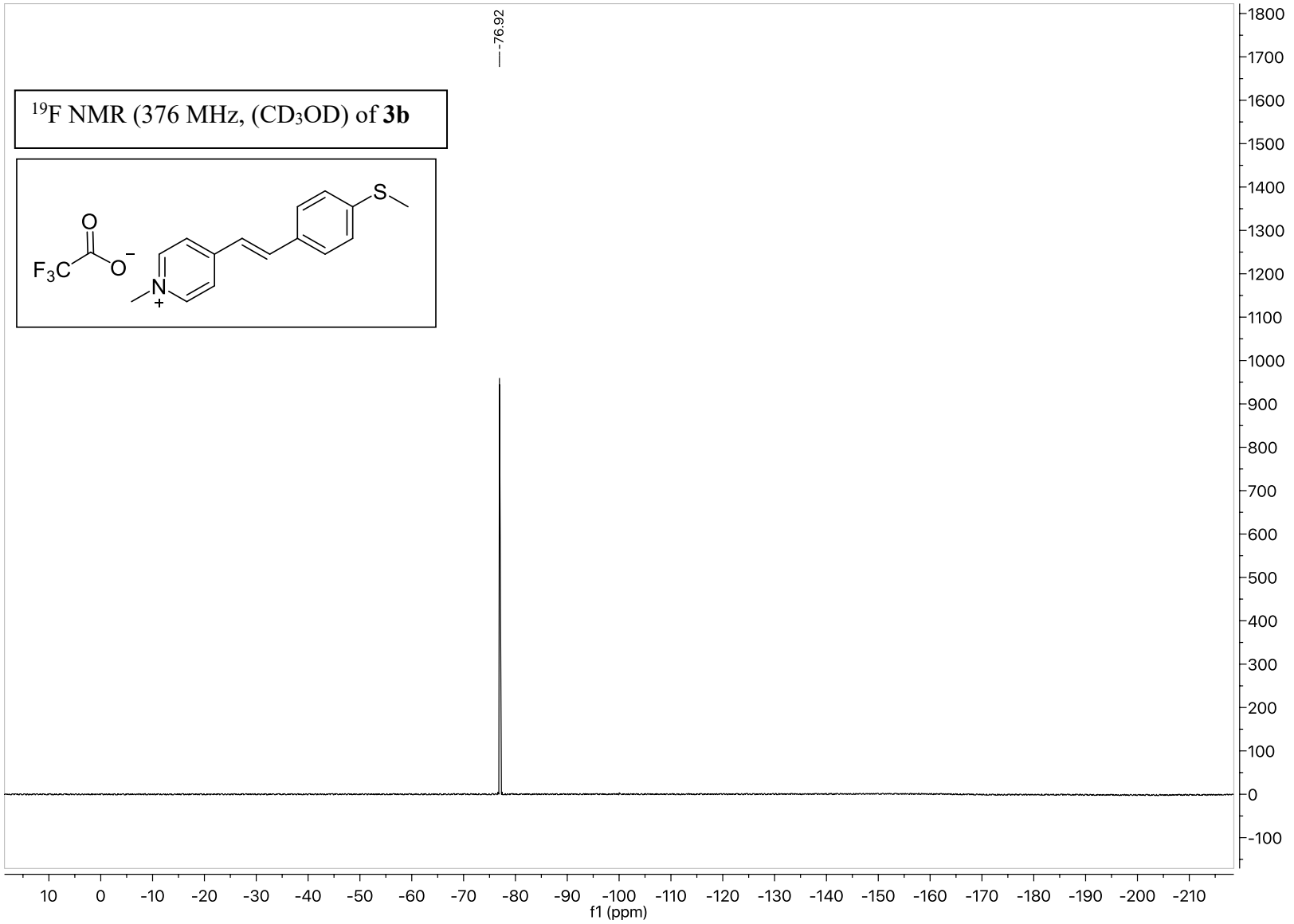
^{1}H NMR (400 MHz, $(CD_3)_2SO$) of **4a**

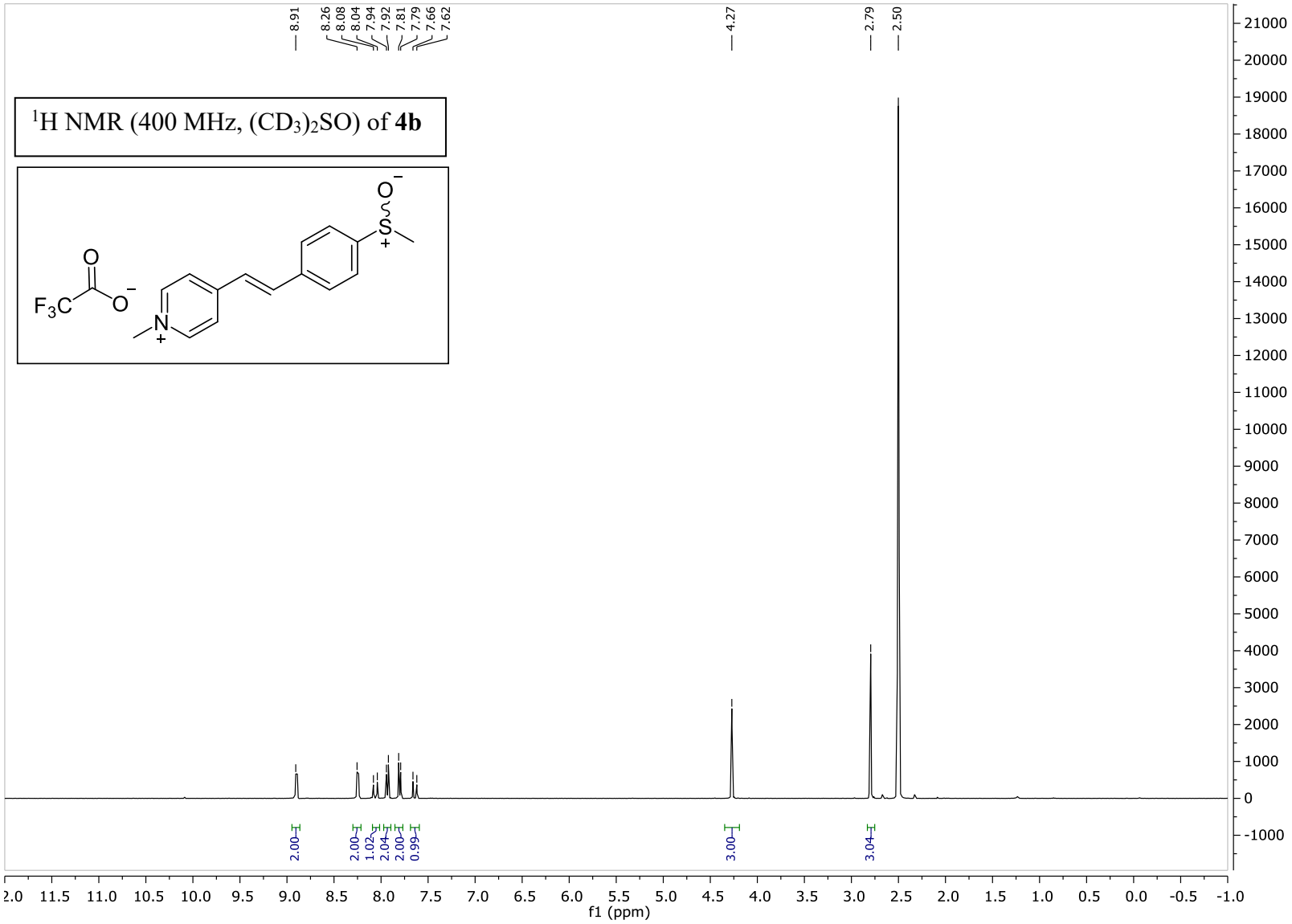

152.02
146.16
145.29
139.20
137.31
128.53
125.00
124.73
123.79
57.09
47.01
39.50
15.12
12.89
^{13}C NMR (101 MHz, $(CD_3)_2SO$) of **4a**
f1 (ppm)

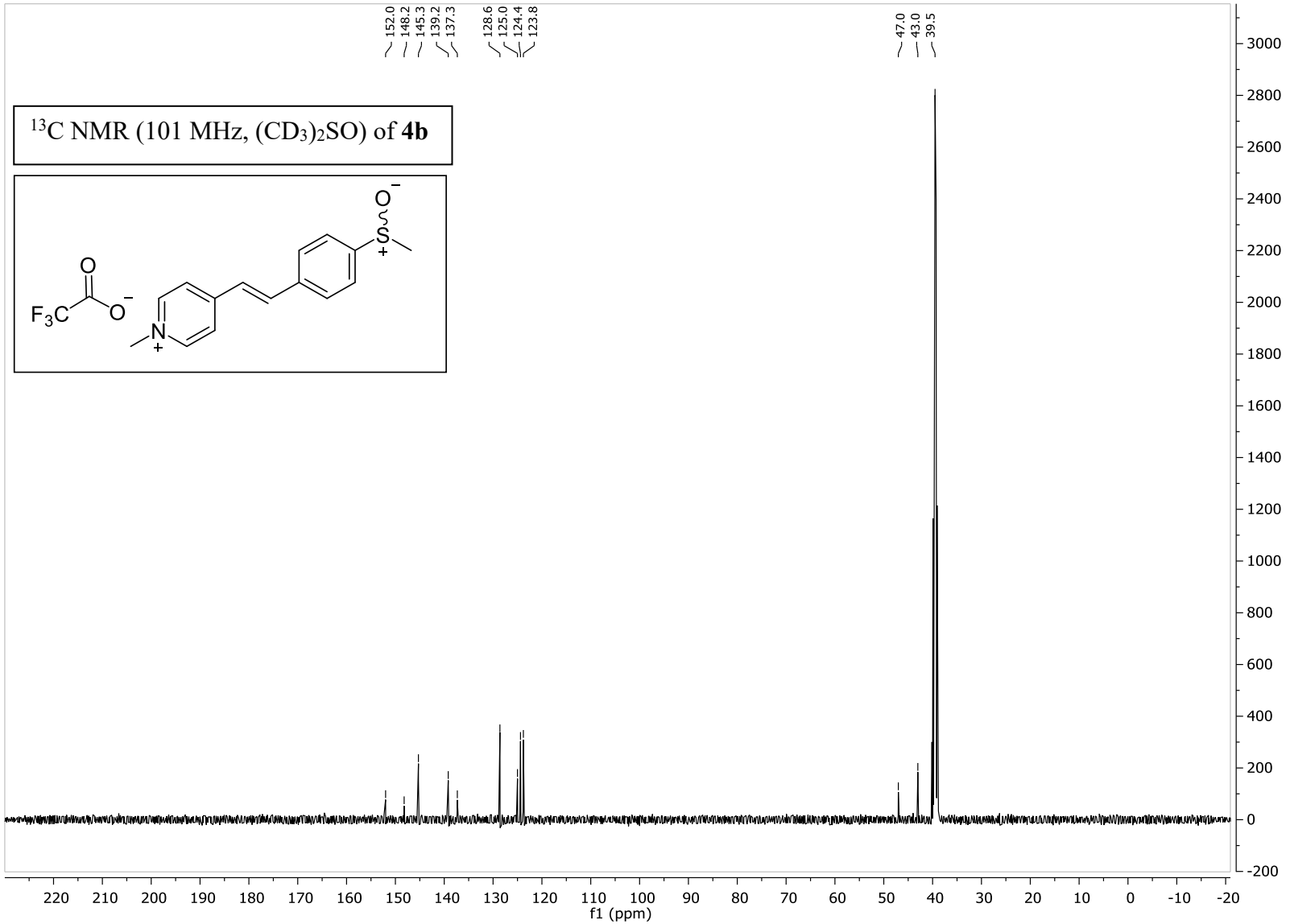

-74.50
^{19}F NMR (376 MHz, $(CD_3)_2SO$) of **4a**
O
O⁻
S⁺
F3C
O⁻
N⁺
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

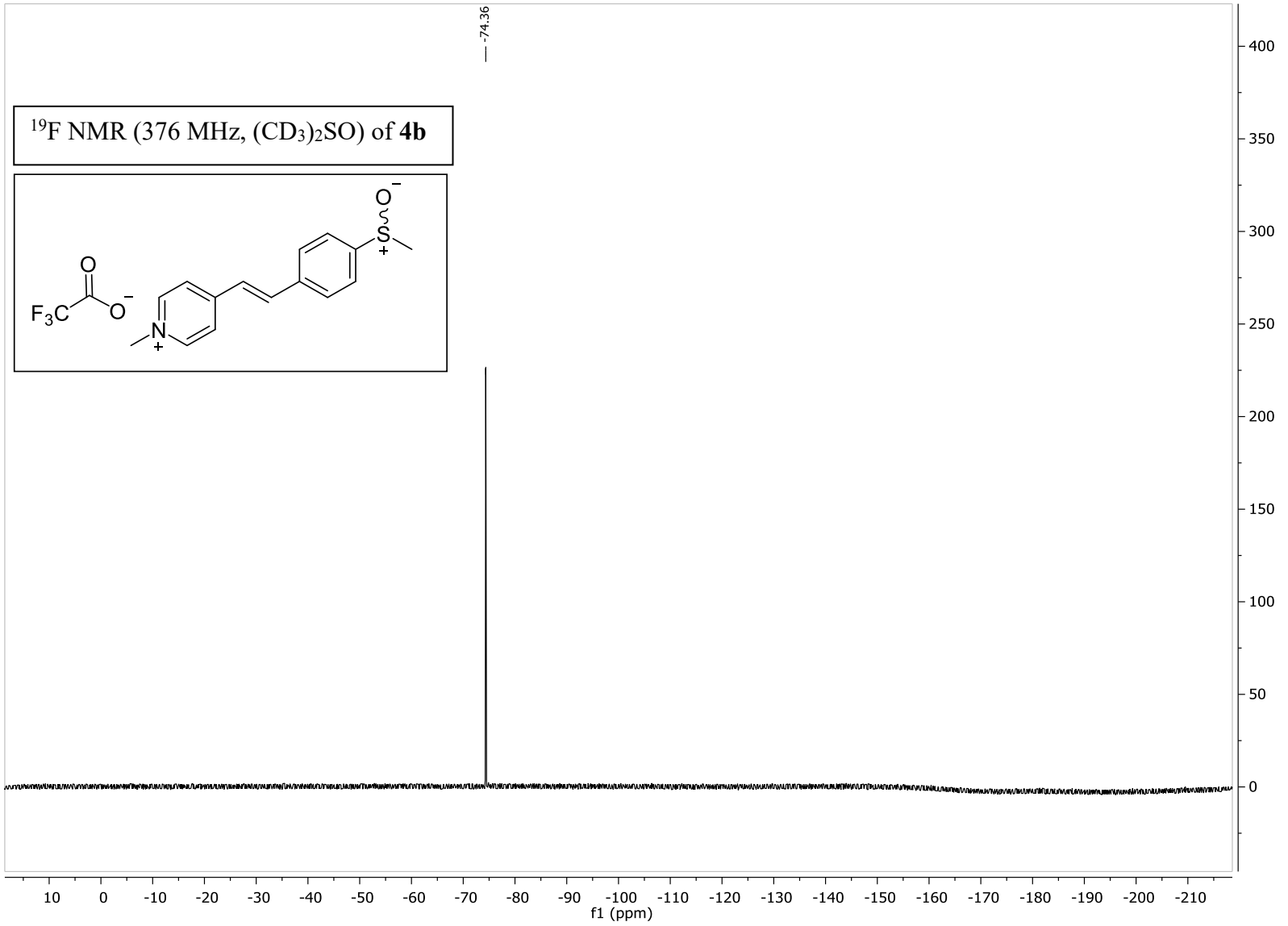

[1]H NMR (400 MHz, (CD3OD) of 3b
8.68
8.13
7.91
7.87
7.68
7.66
7.39
7.35
7.34
7.32
4.87
4.30
3.31
2.54
2.00
2.00
1.10
2.01
1.01
1.85
2.98
2.95
f1 (ppm)


155.4
146.0
144.6
142.6
132.8
129.8
127.0
124.8
122.6
49.0
47.6
14.9
^{13}C NMR (101 MHz, (CD$_3$OD) of **3b**
F3C
O
O
S
N
+
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


-76.92
^{19}F NMR (376 MHz, (CD_3OD) of **3b**
O
F_3C
O
N
+
S
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)


8.91
8.26
8.08
8.04
7.94
7.92
7.81
7.79
7.66
7.62
4.27
2.79
2.50
^{1}H NMR (400 MHz, $(CD_3)_2SO$) of **4b**
F_3C
O
O
N
S
2.00
2.00
1.02
2.04
2.00
0.99
3.00
3.04
f1 (ppm)


152.0
148.2
145.3
139.2
137.3
128.6
125.0
124.4
123.8
47.0
43.0
39.5
^{13}C NMR (101 MHz, $(CD_3)_2SO$) of **4b**
F_3C
O
O
N
S
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


-74.36
^{19}F NMR (376 MHz, $(CD_3)_2SO$) of **4b**
400
350
300
250
200
150
100
50
0
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)