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# Electronic Supplementary Information (ESI)

# for

# A highly selective sulfinate ester probe for thiol bioimaging

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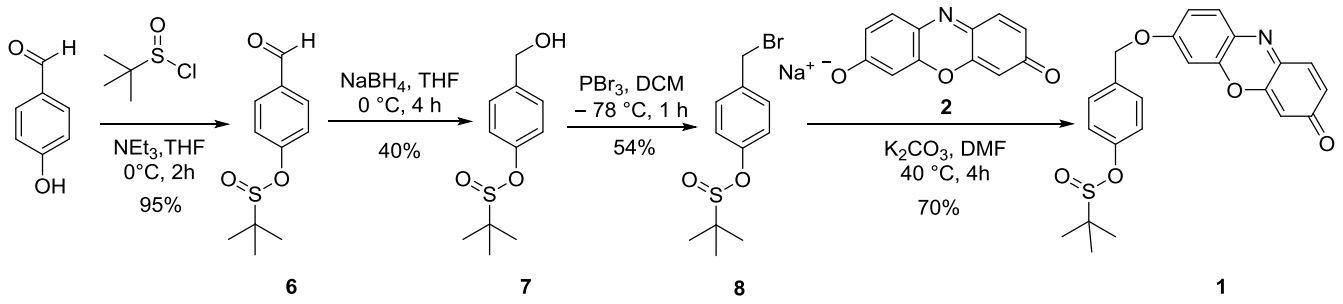
## General

All chemicals were purchased from commercial sources and used as received unless stated otherwise. All reactions were conducted in nitrogen atmosphere. Petroleum ether (PE) and ethyl acetate (EtOAc), for chromatography were distilled before use. THF was dried using a sodium wire and distilled before use. Dichloromethane (DCM), was pre-dried over calcium hydride and then distilled under reduced pressure. Column chromatography was performed using Merck silica gel (60-120/100–200 mesh) as the solid support. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a JEOL 400 MHz (or 100 MHz for $^{13}C$) spectrometer using either residual solvent signals as an internal reference or with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. High-resolution mass spectra (HRMS) were obtained using a HRMS-ESI-Q-Time of Flight LC/MS (Synapt G2, Waters). Infrared spectra (IR) were obtained using NICOLET 6700 FT-IR spectrophotometer using a KBr disc. Melting points were measured using a VEEGO melting point apparatus in open glass capillary and values reported are uncorrected. High performance liquid chromatography (HPLC) was performed on a Dionex ICS-3000 model with Phenomenex C-18 reverse phase column (250 × 4.6 mm, 5 μm) with 1:1 ACN/$H_2O$ isocratic gradient at wavelength, λ = 254 nm. Absorption spectra were recorded on a PerkinElmer, Lambda 45 UV-Vis spectrophotometer. Steady State fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on a Fluoromax 4 instrument (Horiba Jobin Yvon).




**Scheme S1.** Synthesis of **1**, a sulfinate ester.

**4-(3-Oxo-3H-phenoxazin-7-yl)oxy)methyl)phenyl 2-methylpropane-2-sulfinate (1).**[2] To a solution of resorufin sodium salt **2** (0.015 g, 0.06 mmol) in dry DMF (3 mL), a solution of **6** (0.018 g, 0.06 mmol) in dry DMF (2 mL) was added dropwise at room temperature, followed by $K_2CO_3$ (0.012 g, 0.08 mmol). The reaction mixture was heated to 40 °C oil bath temperature and stirred for 3h. Then to the reaction mixture, 75 mL of water was added and extracted in EtOAc (3 × 15 mL). The combined organic layer was dried on $Na_2SO_4$, filtered and the resulting filtrate was concentrated under reduced pressure. The crude was purified by silica gel column chromatography (EA/PE, 1:4) to afford **1** (0.019 g, 70 %) as a orange solid: mp 189-190 °C; FTIR ($\nu_{max}$, $cm^{-1}$): 2925, 2853, 1623, 1569, 1507, 1466, 1316, 1266, 1195; $^1$H NMR (400 MHz, $CDCl_3$): δ 7.71 (d, *J* = 8.9 Hz, 1H), 7.43-7.41 (m, 3H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.99 (dd, *J* = 2.6, 9.0 Hz, 1H), 6.86 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 1.9, 9.7 Hz, 1H), 6.32 (d, *J* = 2.0 Hz, 1H), 5.14 (s, 2H), 1.35 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 186.3, 162.5, 154.7, 149.8, 145.8, 145.6, 134.7, 134.3, 132.4, 131.7, 129.2, 128.6, 120.6, 114.2, 106.8, 101.1, 70.3, 58.8, 21.7; HRMS (ESI-TOF): $C_{23}H_{21}NO_5S$ $[M+ Na]^+$ : 446.1038. Found: $[M+ Na]^+$ : 446.1033.

**4-Tolyl-2-methylpropane-2-sulfinate (3).**[1] To a solution of 4-methylphenol (0.05 g, 0.46 mmol) in dry THF (3 mL) at 0 °C, *tert*-butylsulfinyl chloride (0.082 mL, 0.66 mmol) and triethylamine (0.092 mL, 0.66 mmol); as solutions in 2 mL dry THF, were added dropwise and reaction mixture was stirred at 0 °C for 2 h. After completion of reaction (TLC analysis) 5 mL of water was added and extracted in EtOAc (3×10 mL). The combined organic layer was dried on $Na_2SO_4$, filtered and the resulting filtrate was concentrated under reduced pressure. The crude was purified by silica gel column chromatography (EA/PE, 1:4) to afford **8** (0.045 g, 46%) as a colorless oil: FTIR ($\nu_{max}$, $cm^{-1}$): 1505, 1364, 1196; $^1$H NMR (400 MHz, $CDCl_3$): δ 7.13 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 2.32 (s, 3H), 1.33 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ

152.4, 135.0, 130.3, 120.2, 58.5, 21.7, 20.8; HRMS (ESI-TOF): $C_{11}H_{16}O_2S$ $[M+H]^+$ : 213.0949. Found: $[M+H]^+$ : 213.0951.

***S*-phenyl 2-methylpropane-2-sulfinothioate (5).**[3] To a the solution of *tert*-butylsulfinyl chloride (0.158 mL, 1.27 mmol) in dry $CCl_4$ (6 mL), dry pyridine (0.102 mL, 1.27 mmol) was added at –20 °C. After 10 min. solution of thiophenol (0.100 mL, 0.90 mmol) in $CCl_4$ (6 mL) was added dropwise at -20 °C. The reaction mixture was warmed to rt and stirred for 1.5 h. After completion of reaction, RM was filtered and the residue was washed with $CCl_4$ (3 × 2 mL each). The filtrate was washed with water ( 2 × 5 mL) and brine, dried on $Na_2SO_4$, filtered and the resulting filtrate was concentrated under reduced pressure. The resultant crude was purified by silica gel column chromatography (EA/PE, 1:9) to afford **5** ( 0.150 g, 77%) as a colorless oil: FTIR ($\nu_{max}$, $cm^{-1}$): 2959, 1580, 1474, 1439, 1391, 1365, 1303, 1173; $^1$H NMR (400 MHz, $CDCl_3$): δ 7.65-7.62 (m, 2H), 7.42-7.37 (m, 3H), 1.47 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 135.1, 129.9, 129.7. 129.5, 60.3, 24.2; HRMS (ESI-TOF): $C_{10}H_{14}OS_2$ $[M+ Na]^+$ :237.0384. Found: $[M+Na]^+$ :237.0384.

**4-Formylphenyl 2-methylpropane-2-sulfinate (6).**[1] To a solution of 4-hydroxybenzaldehyde (0.2 g, 1.63 mmol) in dry THF (5 mL) at 0 °C, *tert*-butylsulfinyl chloride (0.29 mL, 2.34 mmol) and triethylamine (0.326 mL, 2.34 mmol) as solutions in 2 mL dry THF were added dropwise. The reaction mixture stirred at 0 °C for 2 h. After completion of reaction (TLC analysis), 5 mL of water was added and extracted in EtOAc (3×10 mL). The combined organic layer was washed with brine, dried over $Na_2SO_4$, filtered and the filtrate was concentrated under reduced pressure to afford **6** (0.350 g, 95%) as a colorless oil. FTIR ($\nu_{max}$, $cm^{-1}$): 1693, 1596, 1365, 1301, 1210; $^1$H NMR (400 MHz, $CDCl_3$): δ 9.97 (s,1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 1.37 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 190.8, 159.6, 133.2, 131.9, 120.0, 59.3, 21.7; HRMS (ESI-TOF): $C_{11}H_{14}O_3S$ $[M+H]^+$ : 227.0742. Found $[M+H]^+$ : 227.0744.

**4-(Hydroxyl methyl ) phenyl 2-methylpropane-2-sulfinate (7).** To a solution of **6** (0.400 g, 1.76 mmol) in dry THF (10 mL) at 0 °C, sodium borohydride (0.073 g, 1.94 mmol) was added portion-wise under a $N_2$ atmosphere and the reaction mixture was stirred at RT for 4 h. After completion of reaction (TLC analysis), 5 mL of water was added and THF was evaporated under reduced pressure; extracted in EtOAc (3×15 mL). The combined organic layer was washed with brine, dried on $Na_2SO_4$ filtered and the filtrate was concentrated under reduced pressure. The crude was purified by silica gel column chromatography (EA/PE,

1:4) to afford **7** (0.160 g, 40 %) as a colorless oil: FTIR ($\nu_{max}$, $cm^{-1}$): 3451, 2928, 1605, 1504, 1475, 1459, 1365, 1195; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.32 (d, *J* = 8.5 Hz, 2H ), 7.12 (d, *J* = 8.5 Hz, 2H ), 4.65 (s, 2H), 1.32 (s, 9H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 154.0, 138.1, 128.5, 120.3, 64.6, 58.7, 21.7; HRMS (ESI-TOF): $C_{11}H_{16}O_3S$ $[M + Na]^+$ : 251.0718. Found $[M+Na]^+$ : 251.0718.

**4-(Bromomethyl)phenyl 2-methylpropane-2-sulfinate (8).** To a solution of **7** (0.070 g, 0.30 mmol) in dry DCM (3 mL), $PBr_3$ ( 9.50 µL, 0.101 mmol) solution in dry DCM (1mL) was added dropwise at -78 °C and reaction mixture was stirred at same temperature for 1h. After completion of reaction, 5 mL of saturated $NaHCO_3$ was added and extracted in another 2× 10 mL of DCM. The combined organic layer was washed with brine, dried over $Na_2SO_4$, filtered and filtrate concentrated under reduced pressure. The crude was purified by silica gel column chromatography (EA/PE, 1:4) to afford **8** (0.048 g, 54 %) as a white solid: mp 87-88 °C; FTIR ($\nu_{max}$, $cm^{-1}$): 2973, 1598, 1500, 1471, 1413, 1363, 1227; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.30 (d, *J* = 8.6 Hz, 2H ), 7.06 (d, *J* = 8.4 Hz, 2H ), 4.41 (s, 2H), 1.27 (s, 9H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 154.6, 134.8, 130.7, 120.5, 58.8, 32.8, 21.7; HRMS (ESI-TOF): $C_{11}H_{15}BrO_2S$, $[M + Na]^+$: 312.9874. Found $[M+Na]^+$ : 312.9874.

**Methyl (4-nitrobenzoyl)cysteinate (9).**[5] To a solution of L-cystiene methyl ester hydrochloride (0.640 g, 3.7 mmol ) in ACN ( 18 mL) and water (6 mL), triethylamine ( 0.104mL, 7.4 mmol) and (1H-benzo[d][1,2,3]triazol-1-yl)(4-nitrophenyl)methanone[4] (1g, 3.7 mmol ) were added at rt. After completion of reaction (2h), the solvent was evaporated under reduced pressure; 5 mL of water was added to the crude and extracted with EtOAc (3×10 mL). The combined organic layer was washed with 2N HCl (2 × 10 mL), dried over $Na_2SO_4$ filtered and the resulting filtrate was concentrated under reduced pressure. The crude was purified by silica gel column chromatography (EA/PE, 1:9) to afford **10** (0.48 g, 48 %) as a white solid: mp 141-143 °C; FTIR ($\nu_{max}$, $cm^{-1}$): 3312, 2930, 2375, 1742, 1642, 1599, 1530, 1438, 1349, 1320, 1221; $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.33 (d, *J* = 8.7 Hz, 2H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.12 (d, *J* = 8.1 Hz, 1H), 5.11-5.07 (m, 1H), 3.86 (s, 3H), 3.18 (dd, *J* = 0.8, 3.7 Hz, 1H ), 3.16 (dd, *J* = 1.0, 3.8 Hz, 1H ), 1.39 (t, *J* = 9.0 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 170.4, 165.0, 149.9, 139.1, 128.4, 124.6, 54.1, 53.2,.26.8; HRMS (ESI-TOF): $C_{11}H_{12}N_2O_5S$ $[M+ Na]^+$ : 307.0364. Found: $[M+Na]^+$ : 307.0358.

**Dimethyl 3,3'-disulfanediylbis(2-(4-nitrobenzamido)propanoate) (10).**[6] To a solution of methyl (4-nitrobenzoyl)cysteinate **9** ( 50 mg, 0.17mmol) in ethyl acetate (3 mL), NaI (1.31 mg, 0.008 mmol) and 30%

$H_2O_2$ (20 µL, 0.17 mmol) were added at RT, reaction mixture stirred at rt. After 1h, 20 mL saturated solution of sodium thiosulphate was added and extracted in EtOAc (3×10 mL). The combined organic layer was dried over $Na_2SO_4$, filtered and the resulting filtrate was concentrated under reduced pressure to afford **10** ( 0.046 g, 92 %) as a white solid: mp 172-174 °C; FTIR ($\nu_{max}$, $cm^{-1}$): 3278, 1741, 1646, 1601, 1528, 1519, 1460, 1430, 1345, 1242; $^1$H NMR (400 MHz, $CDCl_3$): δ 9.30 (d, *J* = 7.6 Hz, 2H), 8.29 (d, *J* = 8.8 Hz, 4H), 8.04 (d, *J* = 8.9 Hz, 4H), 4.81-4.78 (m, 2H), 3.67 (s, 6H), 3.32-3.27 (m, 2H), 3.14-3.08 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 171.2, 165.3, 149.7, 139.3, 129.3, 124.1, 52.9, 52.4, 38.8; HRMS (ESI-TOF): $C_{22}H_{22}N_4O_{10}S_2$ $[M+H]^+$ : 567.0856. Found: $[M+H]^+$ : 567.0848.

**Selectivity study:** First 1 mM stock solutions of probe and various analytes were prepared in DMSO and phosphate buffer pH 7.4 (10 mM) respectively. Then 10 µL of probe was added to 990 µL (100 µL analyte + 890 µL PB pH 7.4) of reaction mixture, 1% DMSO/PB to get final conc. 10 µM and incubated at 37 $^o$C for 30 min; fluorescence intensity measured at $\lambda_{ex}$ = 550 nm/$\lambda_{em}$ = 585 nm.

**Detection limit:** The detection limit was determined based on the fluorescence titration and slit was adjusted to 5.0 nm/5.0 nm. To determine the S/N ratio, the emission intensity of probe (10 µM) without Cys was measured 6 times and the standard deviation of blank measurements was determined. The probe was then treated with cysteine (2-10 µM) and a nearly linear relationship was observed ($R^2$ = 0.9931). The detection limit is then calculated with the equation: detection limit = $3\sigma_{bi}/m$, where $\sigma_{bi}$ is the standard deviation of blank measurements, *m* is the slope between intensity versus sample concentration. The detection limit was measured to be 2.77 × $10^{-8}$ M at *S/N* = 3 (signal-to-noise ratio of 3:1).

**Stability Study:** 10 µM solutions of probe **1** in 1% DMSO/PB (10mM) in different pH: 5.0, 6.0, 6.5, 7.0, 7.4, 8.0 and 9.0 were incubated at 37 °C for 1h and fluorescence intensity measured at λex = 550 nm/ λem = 585 nm, no significant rise was observed, suggesting that probe is stable in various pH ranging from 5-9.

**HPLC Study:**

**1. Reaction of p-tolyl 2-methylpropane-2-sulfinate (3) with thiophenol:**

100 µM of compound was treated with 20/100/1000 µM of thiophenol, with final composition 2 % DMSO in PB pH 7.4, then RM incubated at 37 °C and followed by HPLC.

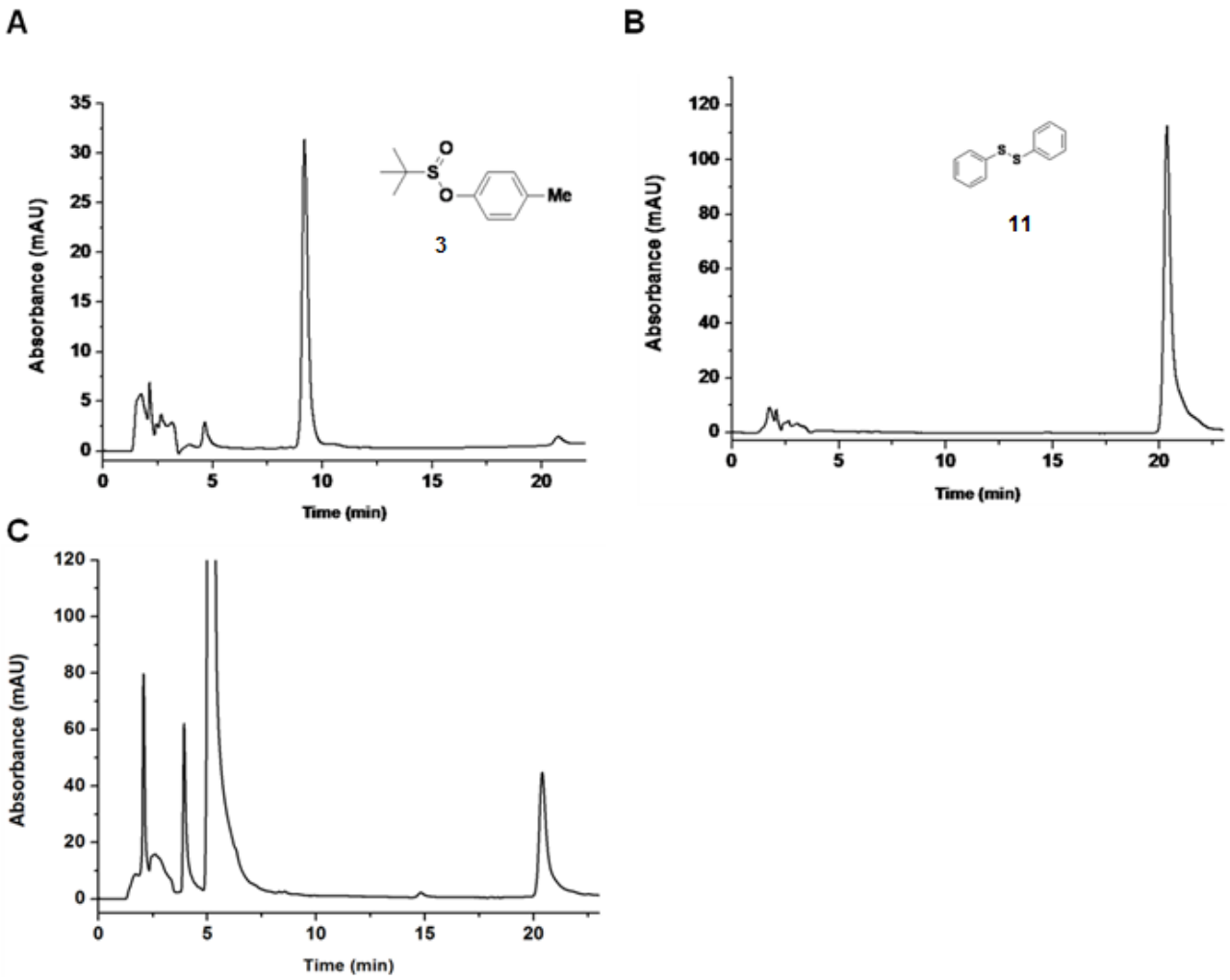



**Figure S1.** (a) Authentic p-tolyl 2-methylpropane-2-sulfinate (**3**, 100 µM); (b) Authentic sample of thiophenol disulfide (**11**); (c) **3** (100 µM) was treated with thiophenol (10 equiv) and incubated at 37 °C. The reaction mixture after 30 min of was analyzed by HPLC and shows complete disappearance of **3** with formation of **11**.

**2. Reaction of p-tolyl 2-methylpropane-2-sulfinate (3) with modified cysteine:**

100 µM of compound was treated with 1mM of modified cysteine with final composition 2 % DMSO/PB pH 7.4, then RM incubated at 37 °C and followed by HPLC.

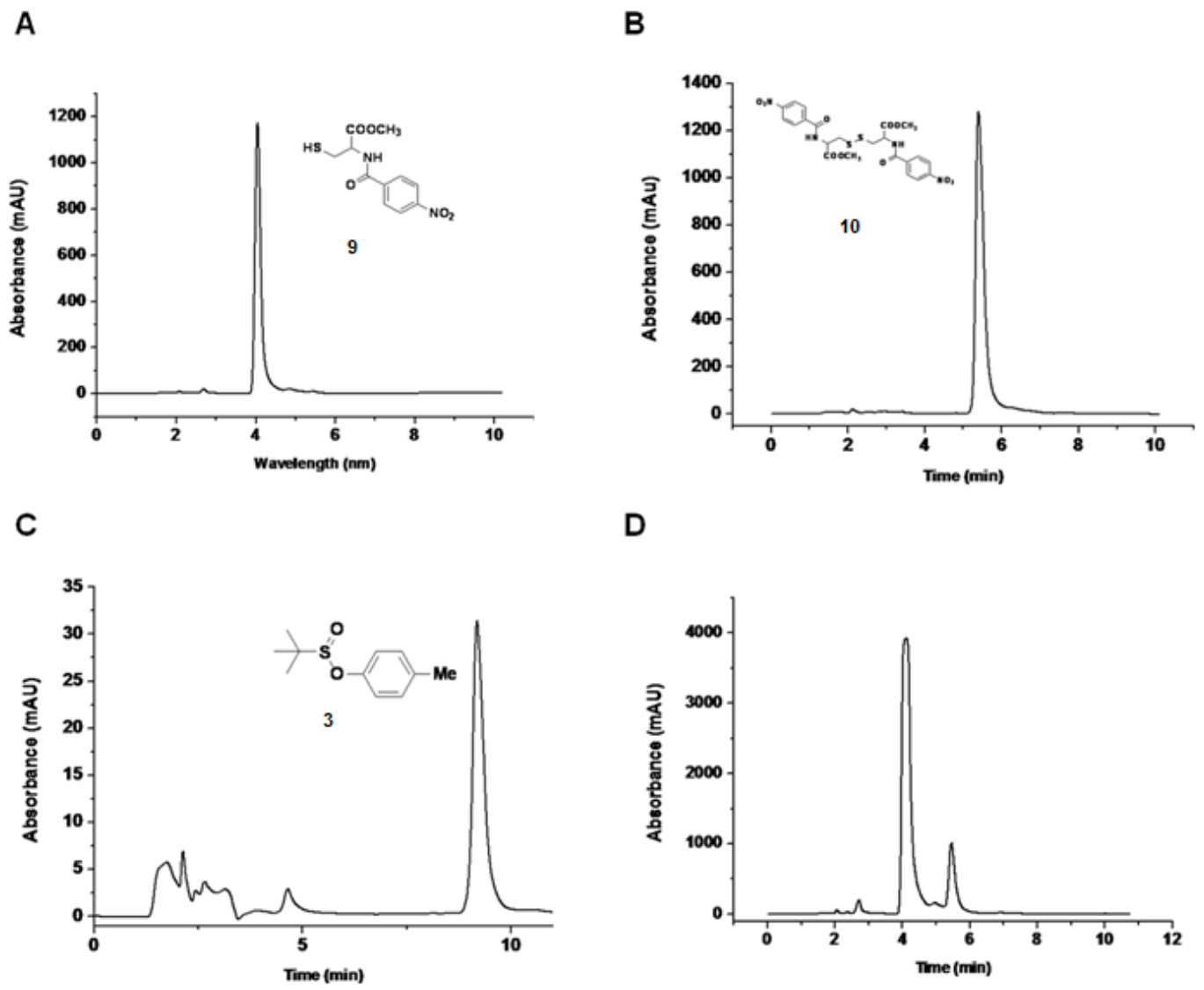



**Figure S2.** (a) Authentic methyl (4-nitrobenzoyl)cysteinate (**10**); (b) Authentic sample of Dimethyl 3,3'-disulfanediylbis(2-(4-nitrobenzamido)propanoate) (**10**); (c) Authentic p-tolyl 2-methylpropane-2-sulfinate (**3**, 100 µM); (d) HPLC analysis of this reaction mixture: 100 µM of p-tolyl 2-methylpropane-2-sulfinate **3** was treated with 1 mM of **9** in pH 7.4 phosphate buffer with final composition 2% DMSO/PB pH 7.4 and incubated at 37 °C for 30 min. Complete disappearance of **3** with concomitant formation of **10** is observed.

**Cell Viability Assay.** The in vitro cytotoxicity of **1** on DLD1 cells (colorectal cancer cell) was determinedby MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay. DLD1cells were seeded in 96-well flat-bottom culture plates at a density of 5000 cells per well in 100µL RPMIcomplete medium (Gibco). The cells were allowed to adhere and grow for 24 h at 37 ºC in a $CO_2$ incubator. The medium was replaced with 100 µL fresh reduced serum medium (OptiMEM) containing various concentrations of **1** (0 to 20 µM). The assay was performed in quadruplicate for each concentration. Cells were then incubated for 12 h, after which the culture medium was removed and 100 µL of 0.5 mg/ml MTT reagent in DPBS (Dulbecco's Phosphate-Buffered Saline) was added to each well and the plate was incubated for 4 h. To remove the unreduced MTT plate was centrifuged at 2000 rpm and supernatant was removed. Purple formazan crystals were dissolved in 100 µL DMSO per well, which was then measured spectrophotometrically using a microplate reader (Biorad, USA) at 570 nm. The cytotoxic effect of each treatment was expressed as percentage of cell viability relative to the untreated control cells. The following formula was used to calculate viability of cell growth.

Cell viability (%) = (means of Absorbance value of treated group/ means of Absorbance value of untreated control) × 100.

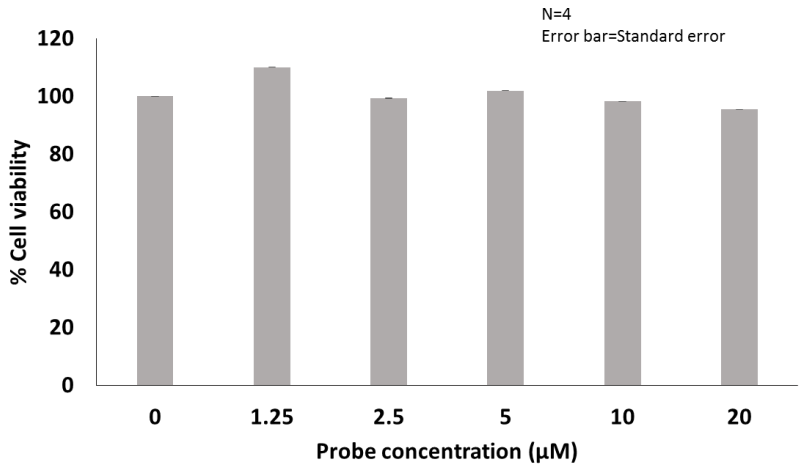



**Figure S3.** Cell Viability assay

**Confocal imaging**: 0.4 million DLD-1 cells were seeded in live cell imaging chamber in RPMI-1640 medium supplemented with 10% fetal bovine serum. Cells were grown at 37 $^{o}$C in a $CO_2$ incubator for 24 h. For probe treatment, RPMI was replaced with 1 ml of reduced serum medium OptiMEM (Gibco) and cells were incubated with probe at 20 µM concentration in 1:1000 (DMSO:OptiMEM) medium for 30 min at 37 $^{o}$C, washed with DPBS (*Dulbecco`s Phosphate Buffered Saline*) three times to remove the remaining probe. During probe treatment cells were also treated with nuclear staining dye Hoechst33258 for last 10 min. Imaging was done on Zeiss LSM 710 Confocal microscope with 63 × oil- immersion objective. For probe, excitation wavelength of laser was 561 nm, and emissions were obtained in full range (568-797 nm). For Hoechst 33258 stain, excitation wavelength was 360 nm and emissions were obtained in full range (426-797 nm).

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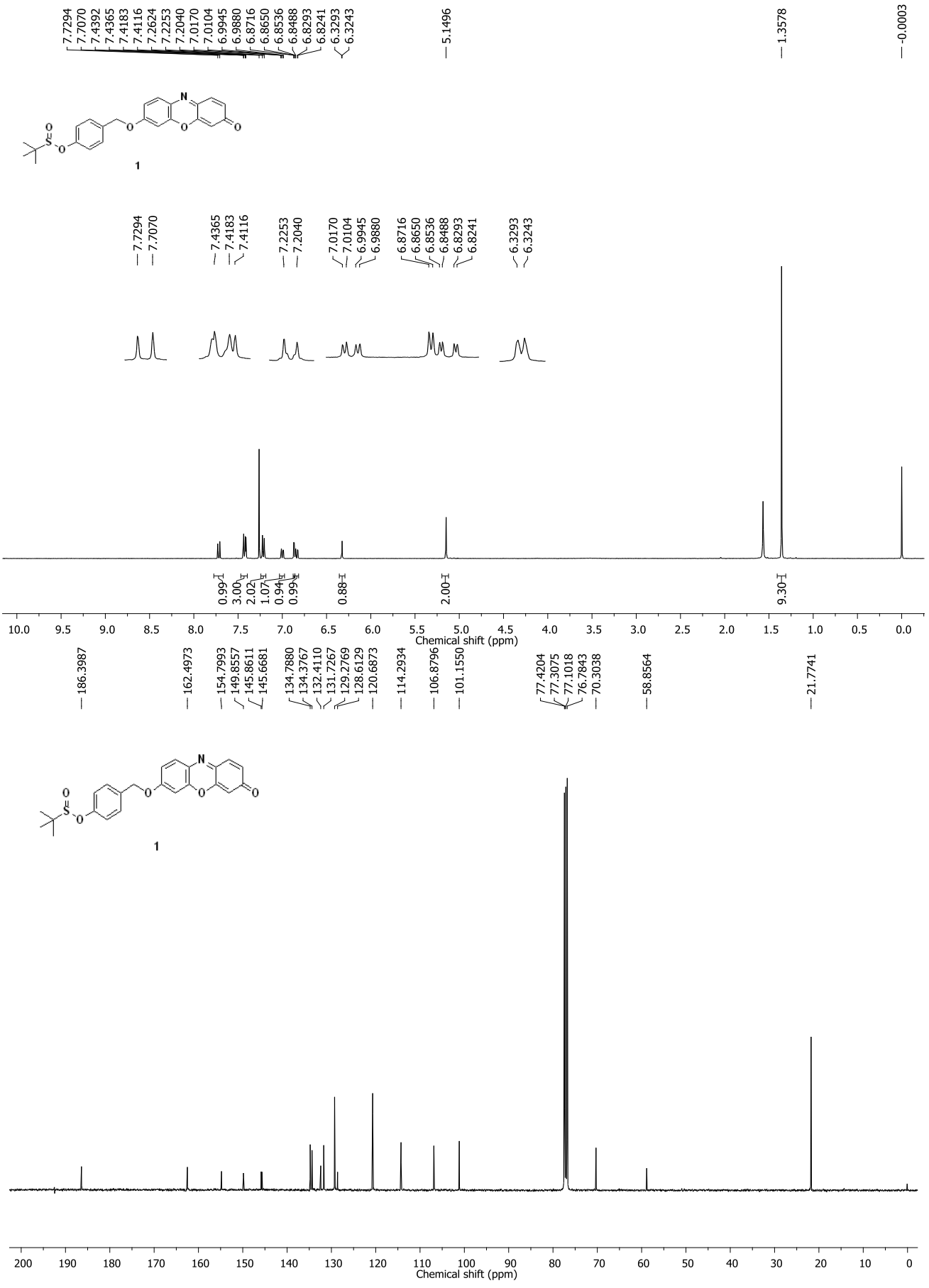
NMR Spectra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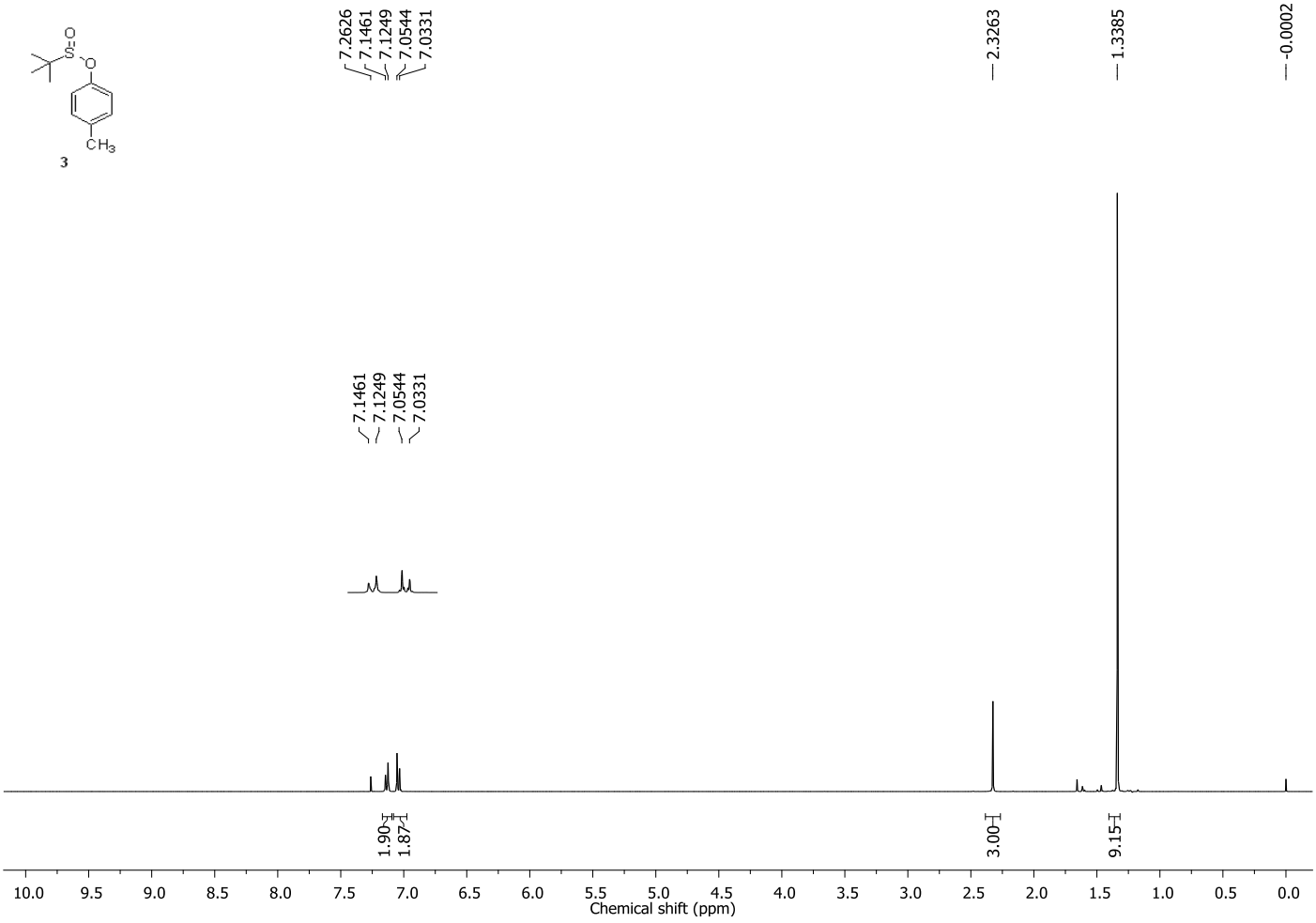
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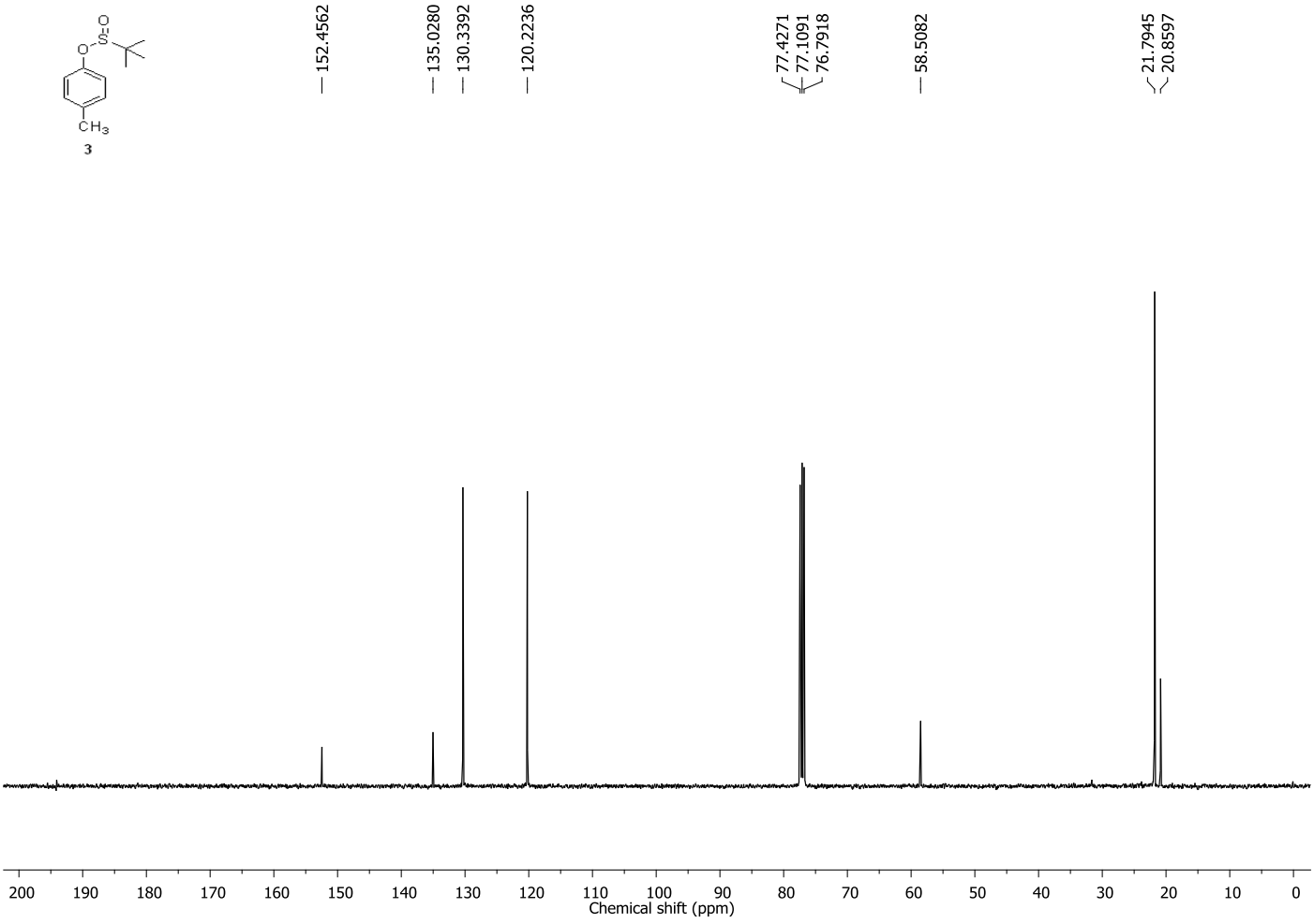
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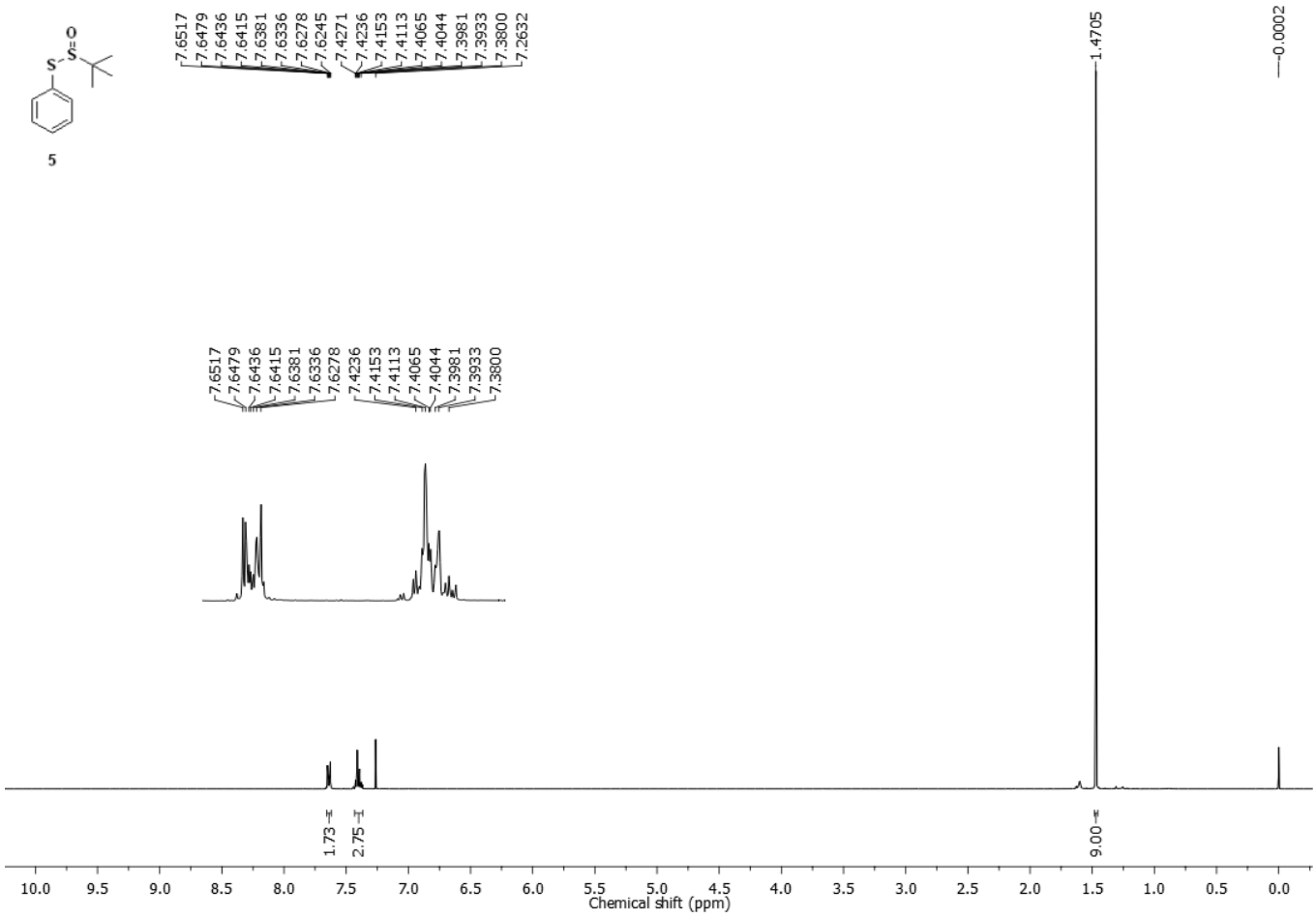
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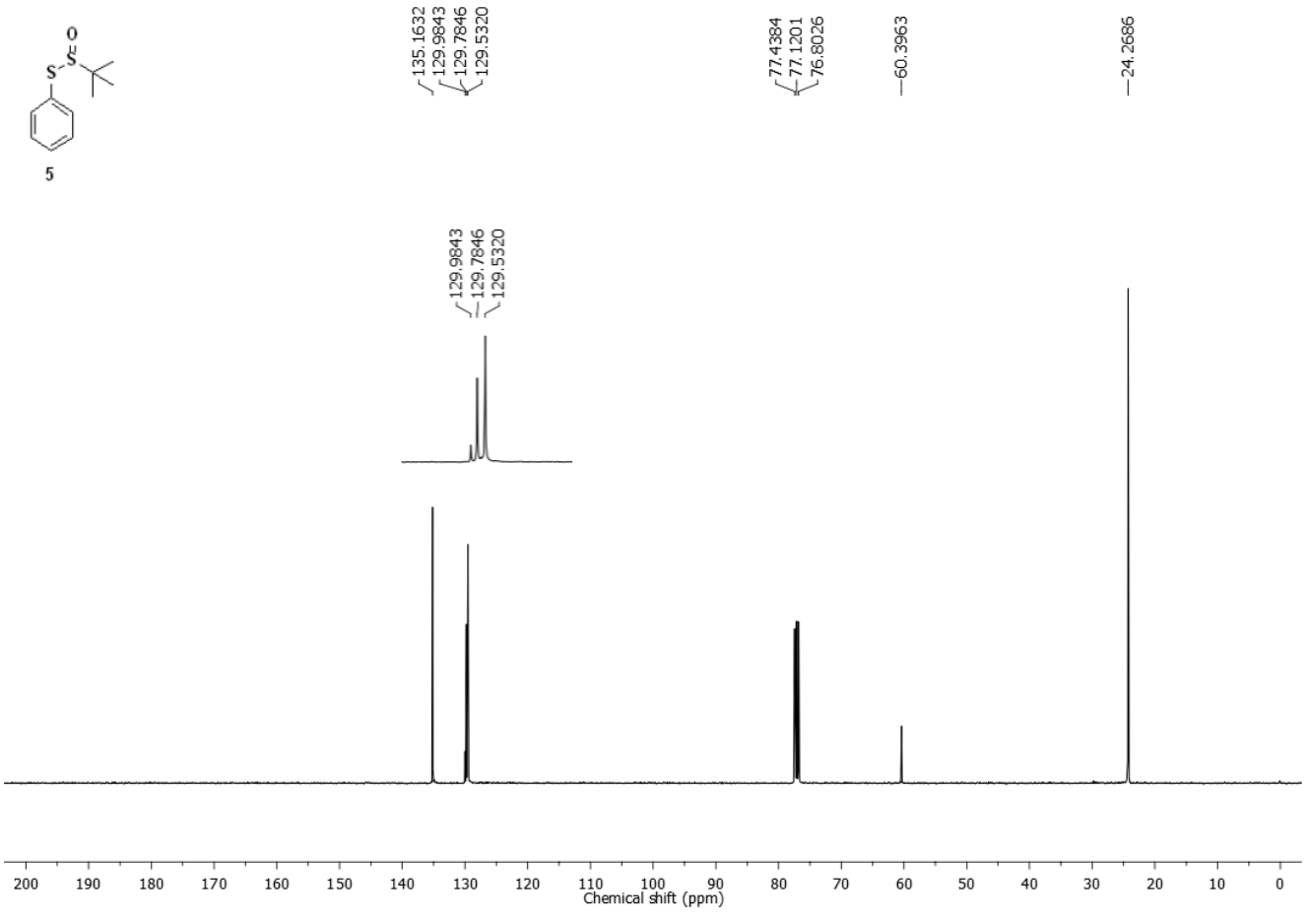
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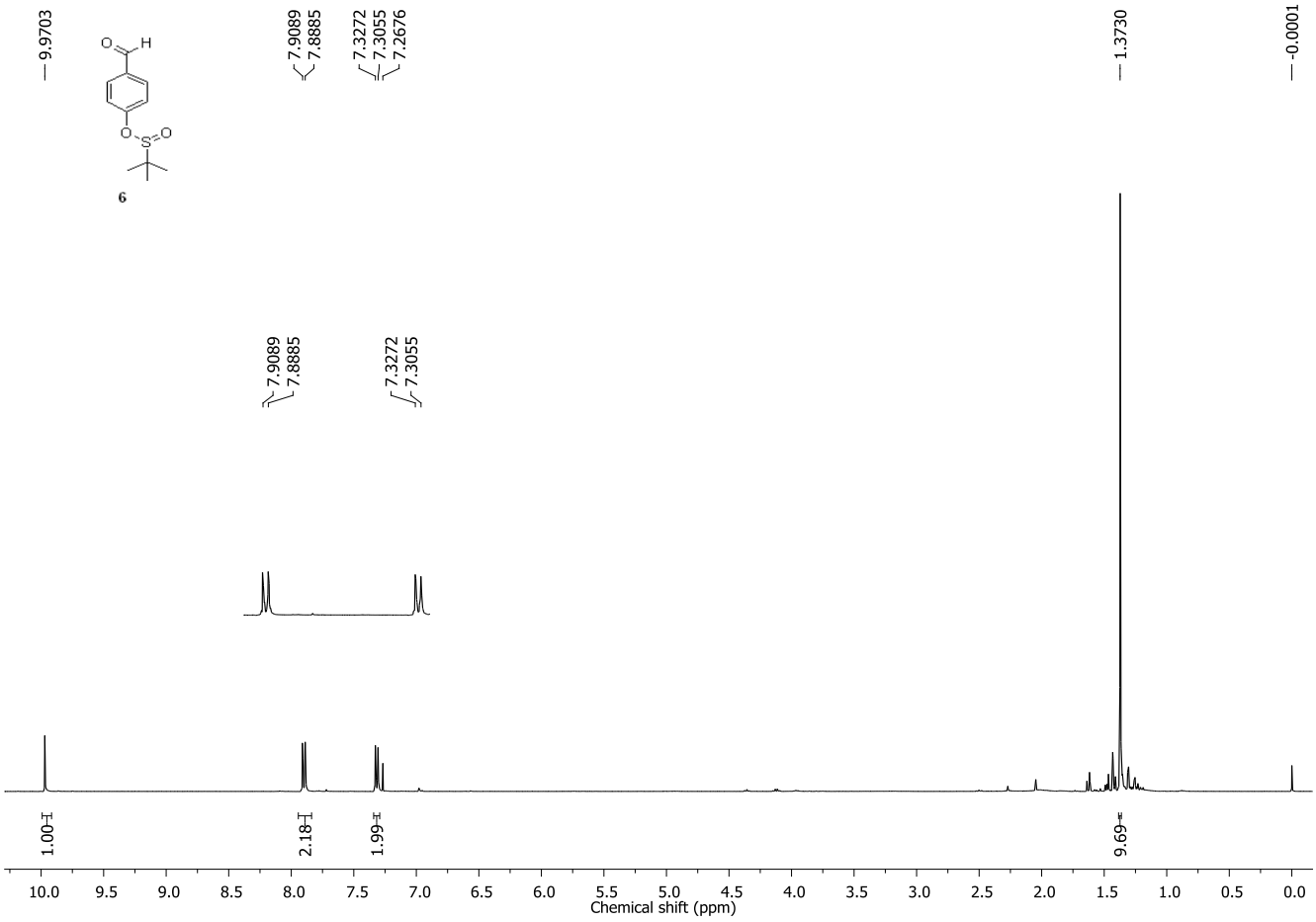
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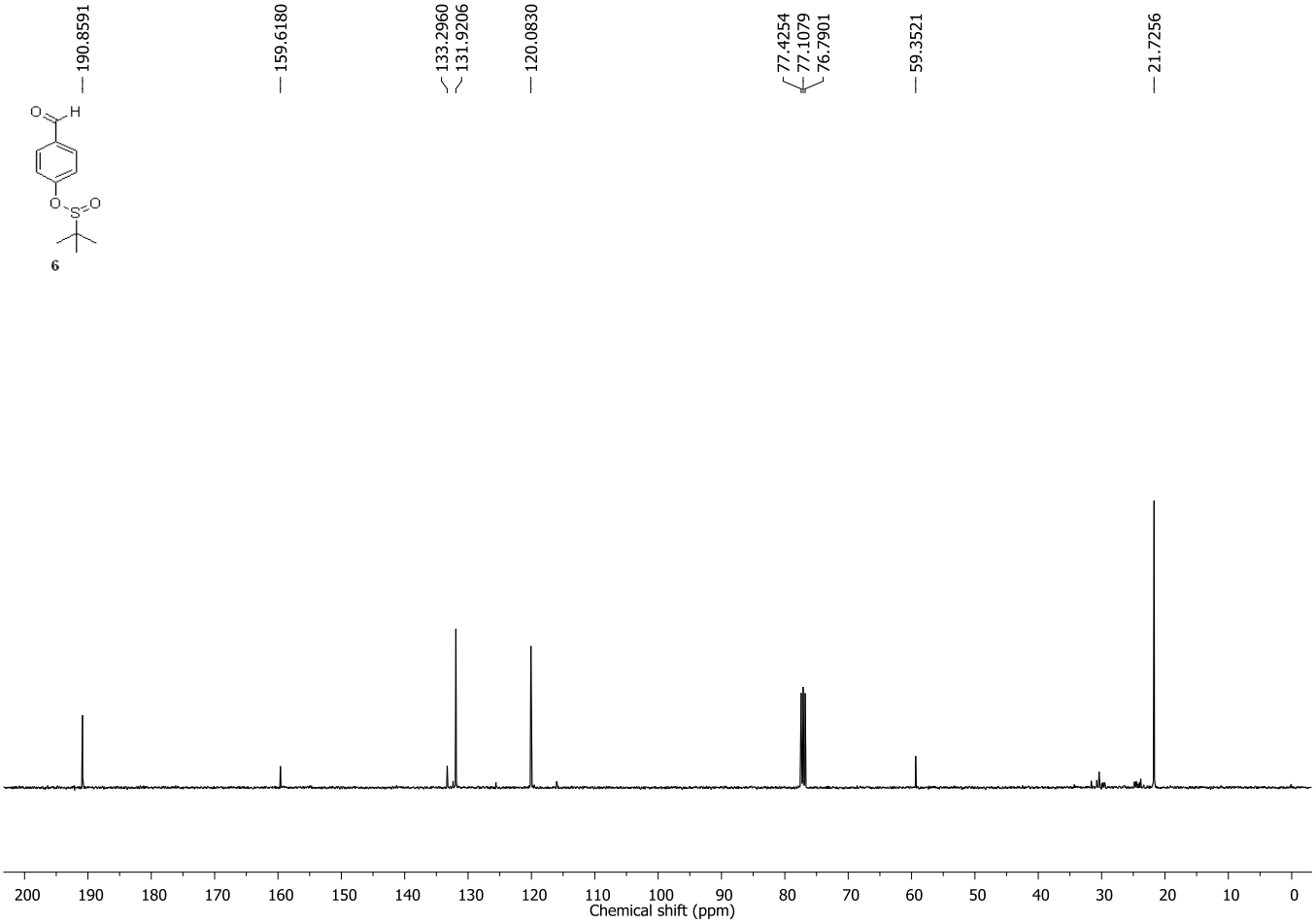
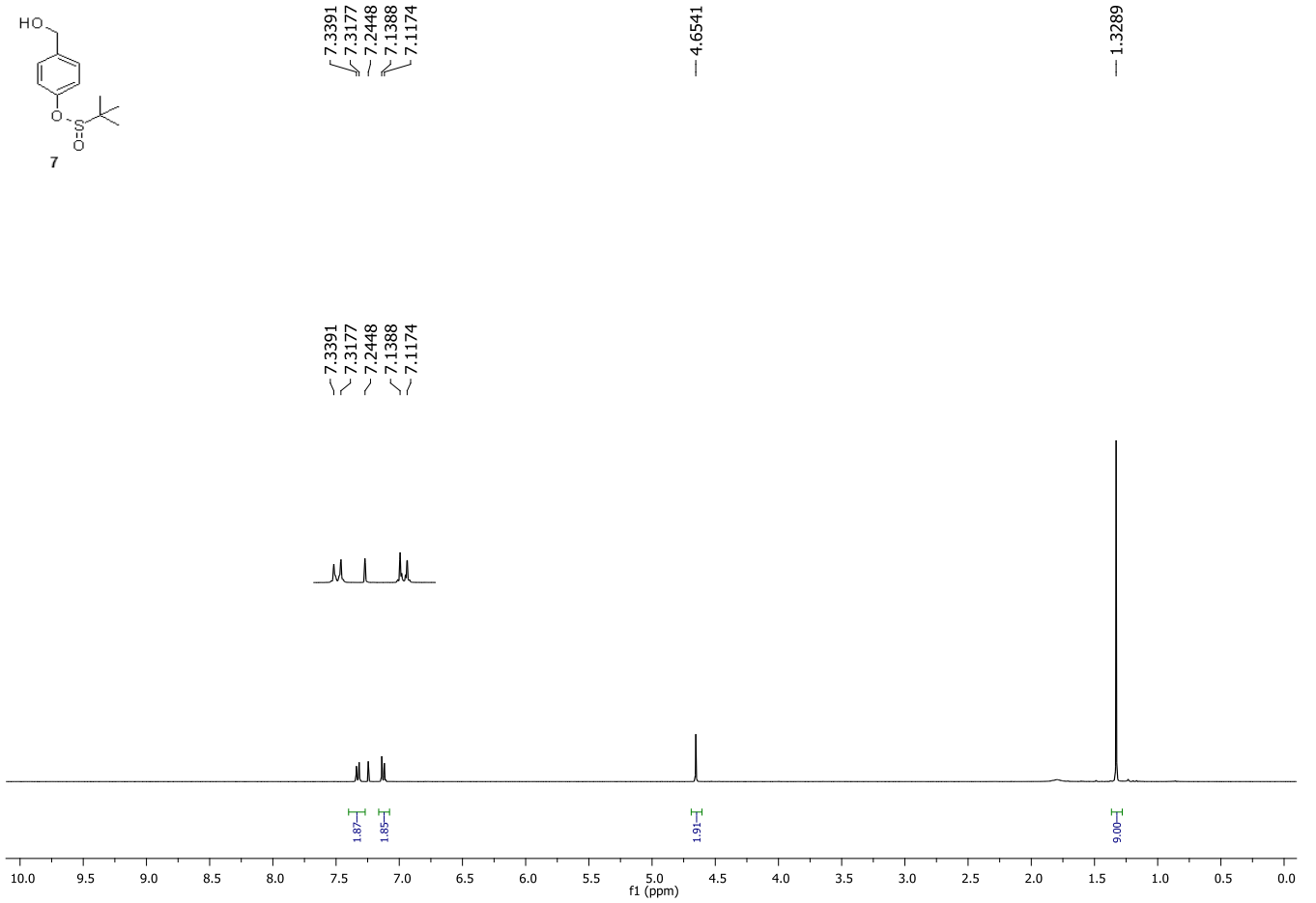
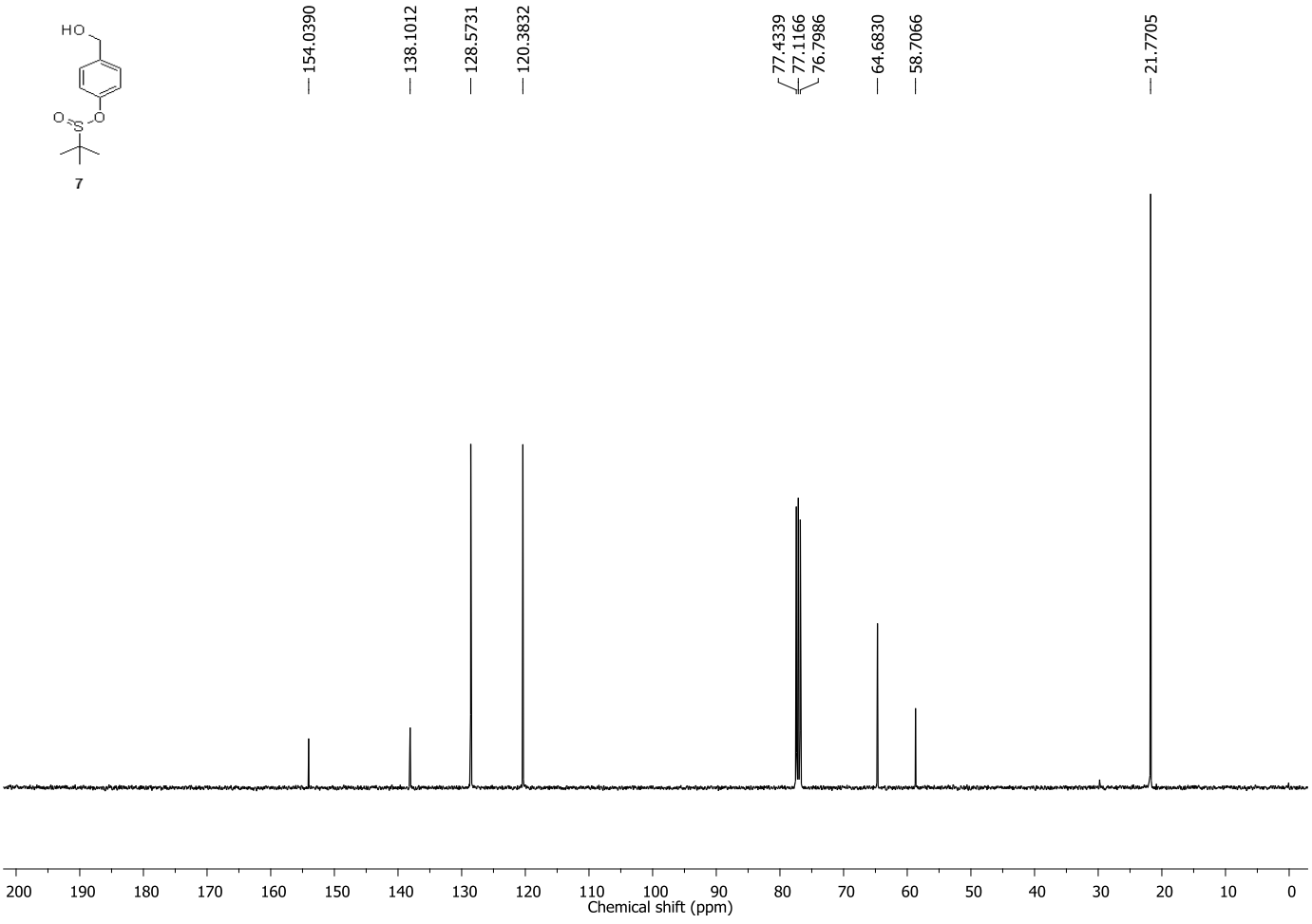
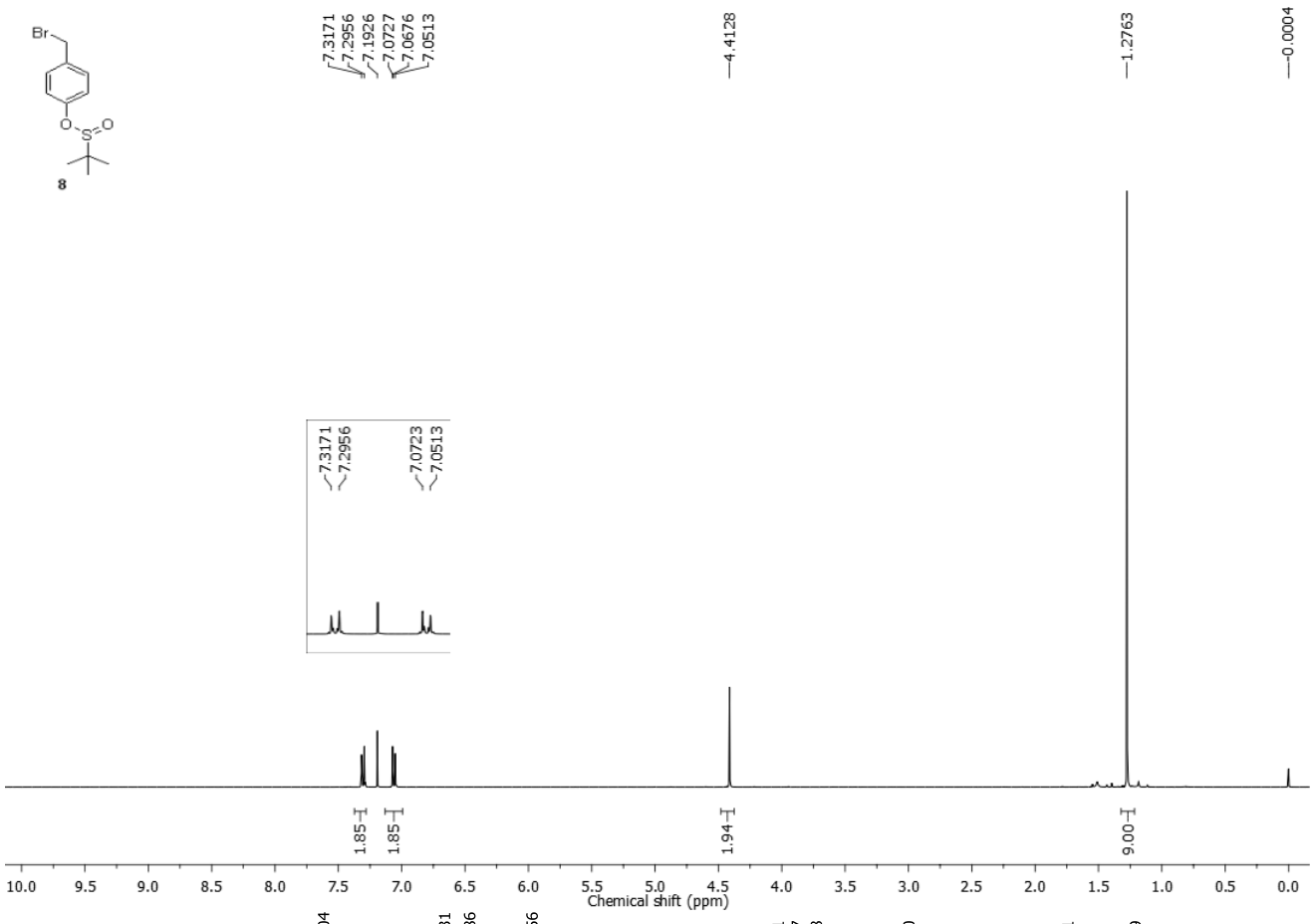
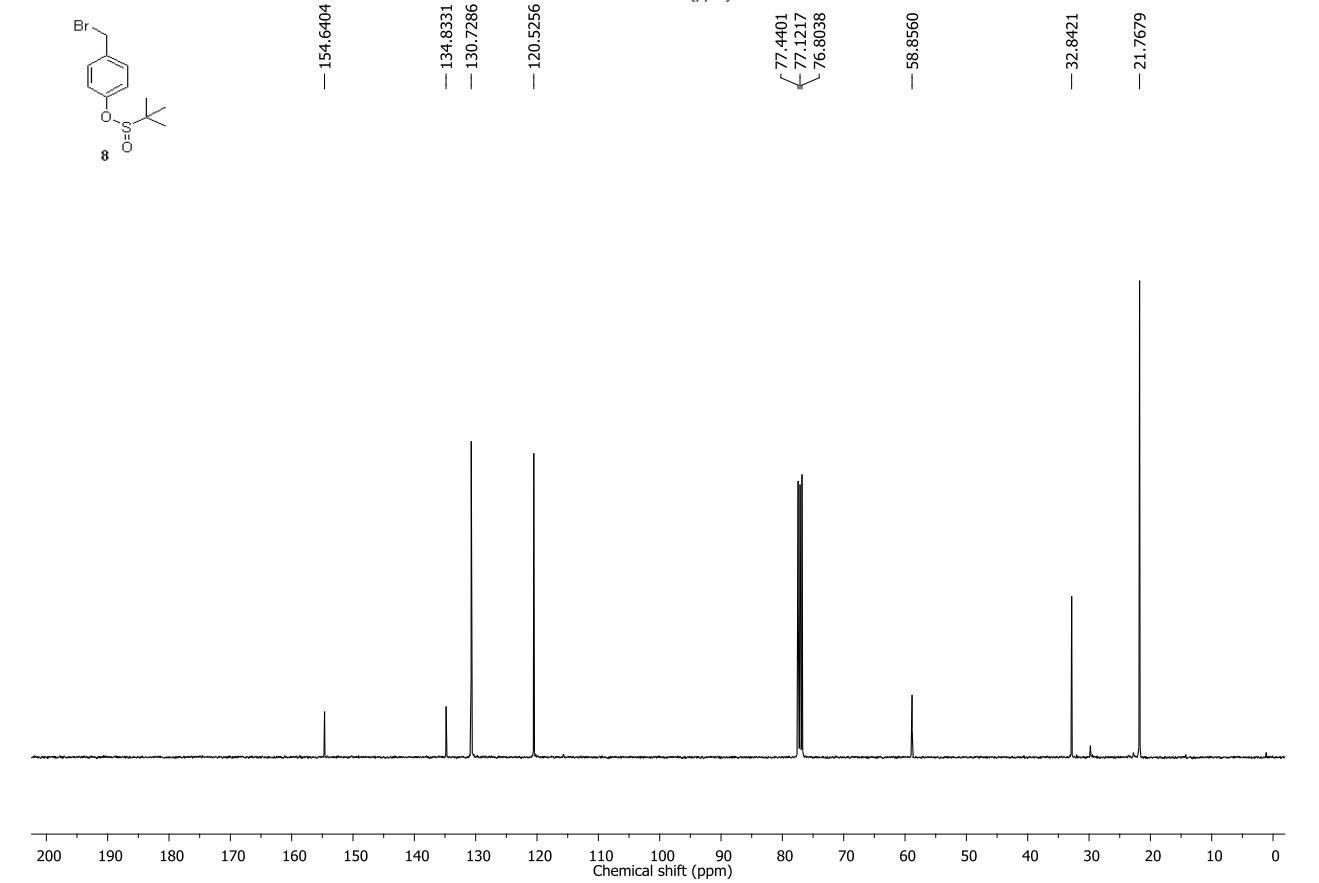
Chemical shift (ppm)

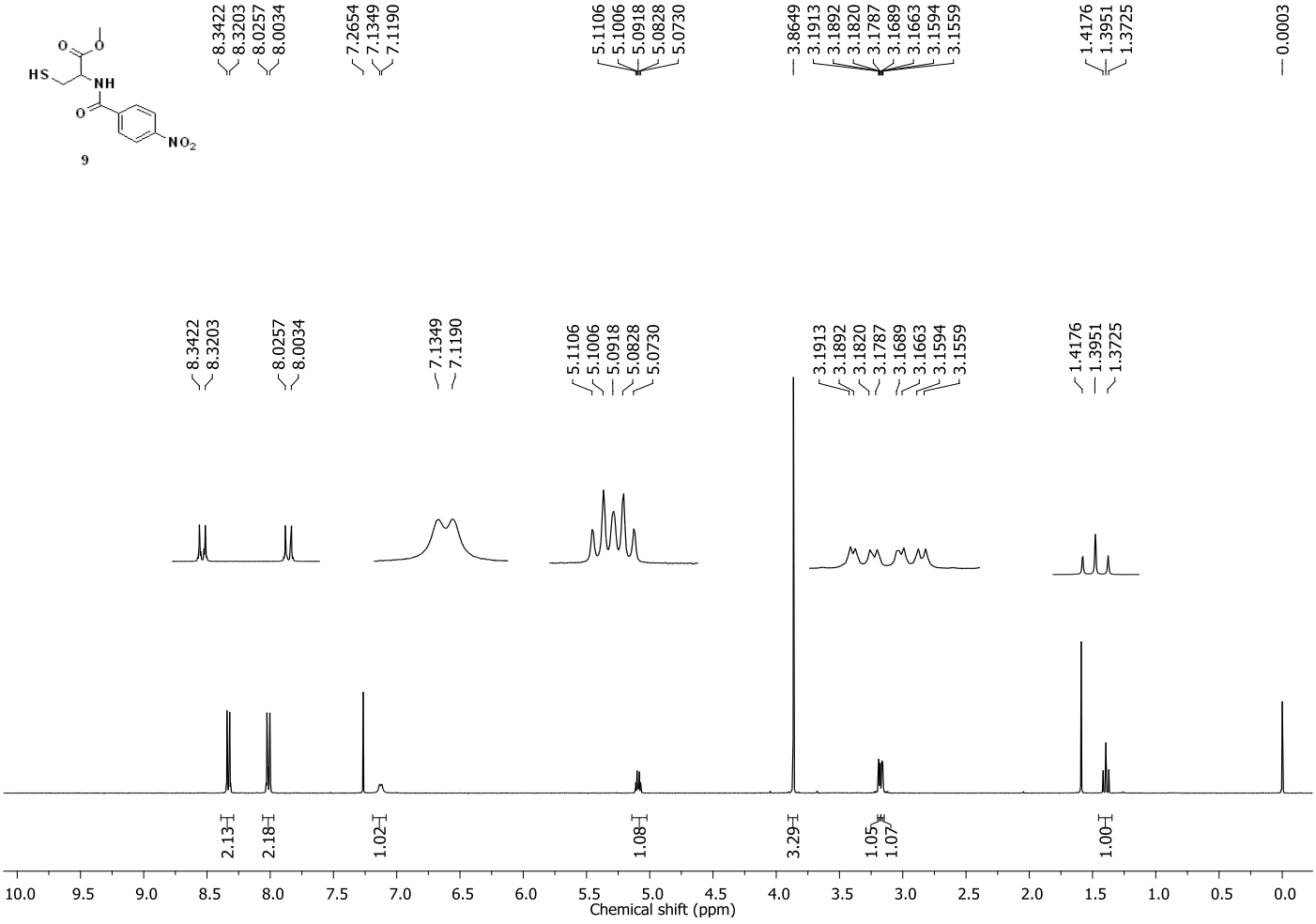

190.8591
159.6180
133.2960
131.9206
120.0830
77.4254
77.1079
76.7901
59.3521
21.7256
6
Chemical shift (ppm)

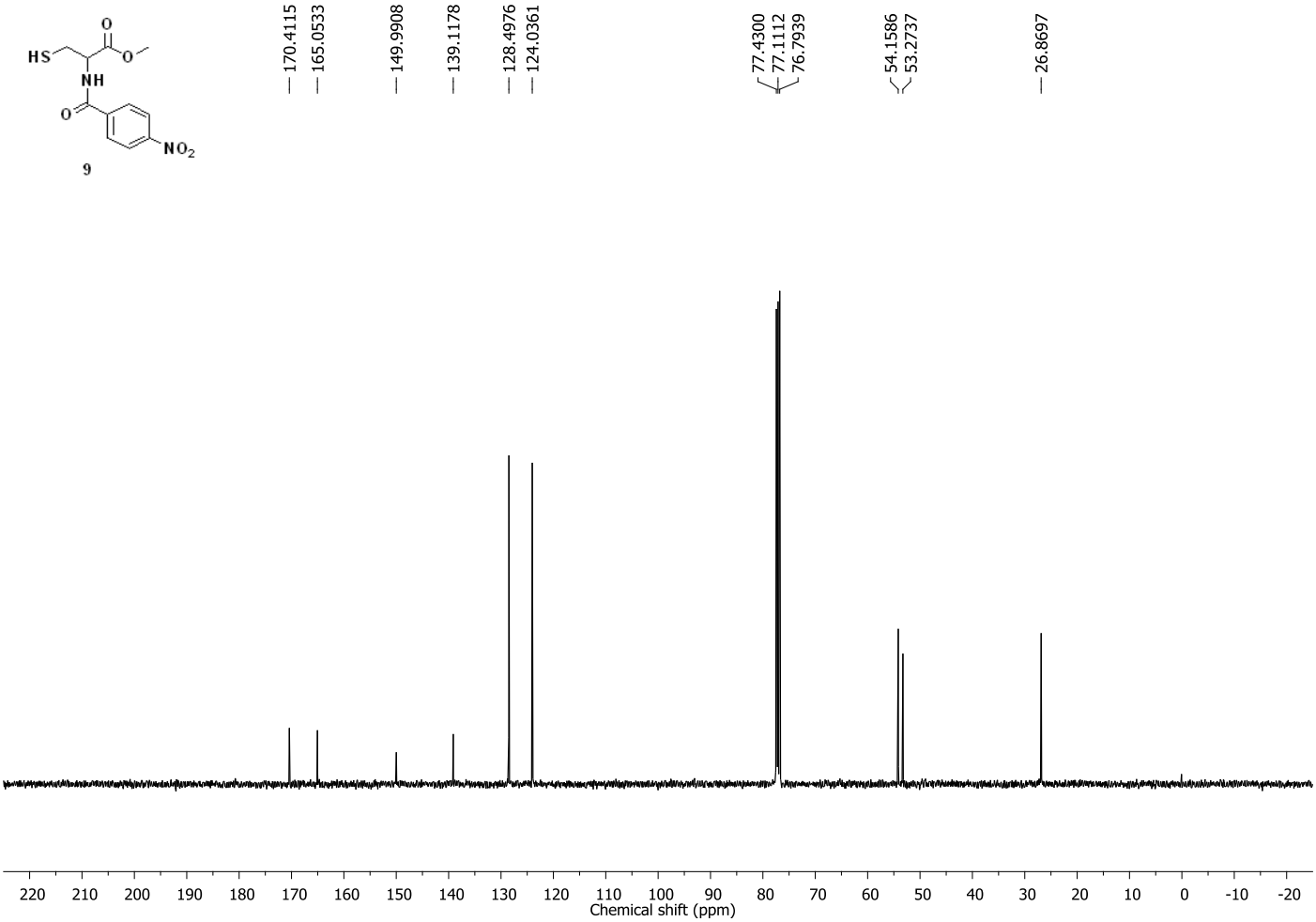

HO
O
S
O
7
7.3391
7.3177
7.2448
7.1388
7.1174
4.6541
1.3289
1.87
1.85
1.91
9.00
f1 (ppm)

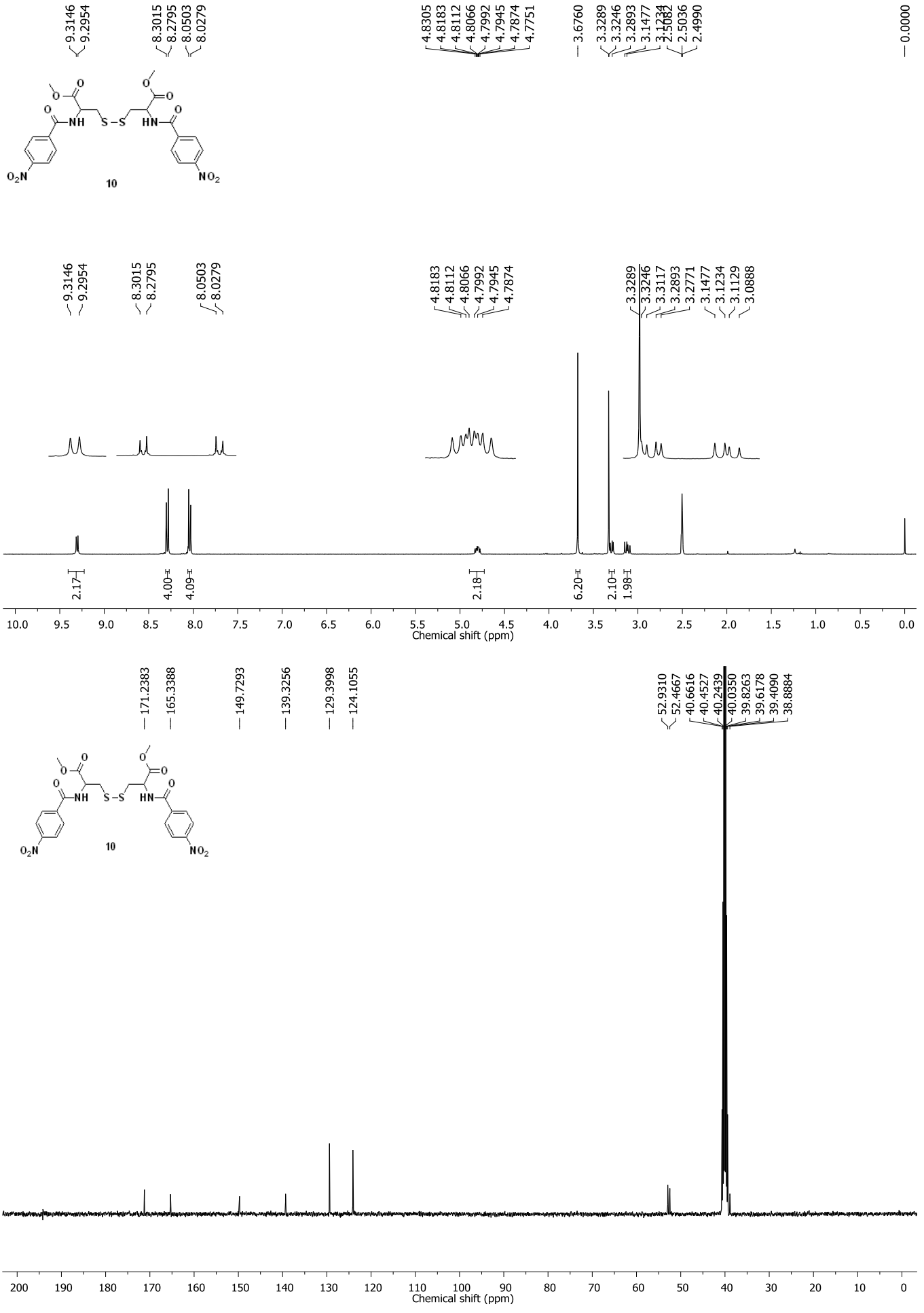

HO
O
S
O
7
154.0390
138.1012
128.5731
120.3832
77.4339
77.1166
76.7986
64.6830
58.7066
21.7705
Chemical shift (ppm)


Br
O
S
O
8
7.3171
7.2956
7.1926
7.0727
7.0676
7.0513
4.4128
1.2763
0.0004
7.3171
7.2956
7.0723
7.0513
1.85
1.85
1.94
9.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical shift (ppm)


Br
O
S
O
8
154.6404
134.8331
130.7286
120.5256
77.4401
77.1217
76.8038
58.8560
32.8421
21.7679
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical shift (ppm)


8.3422
8.3203
8.0257
8.0034
7.2654
7.1349
7.1190
5.1106
5.1006
5.0918
5.0828
5.0730
3.8649
3.1913
3.1892
3.1820
3.1787
3.1689
3.1663
3.1594
3.1559
1.4176
1.3951
1.3725
0.0003
HS
NH
O
NO2
9
2.13
2.18
1.02
1.08
3.29
1.05
1.07
1.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical shift (ppm)


170.4115
165.0533
149.9908
139.1178
128.4976
124.0361
77.4300
77.1112
76.7939
54.1586
53.2737
26.8697
HS
NH
O
NO2
9
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
Chemical shift (ppm)


9.3146
9.2954
8.3015
8.2795
8.0503
8.0279
4.8305
4.8183
4.8112
4.8066
4.7992
4.7945
4.7874
4.7751
3.6760
3.3289
3.3246
3.2893
3.1477
3.1234
3.1082
2.5036
2.4990
0.0000
10
2.17
4.00
4.09
2.18
6.20
2.10
1.98
Chemical shift (ppm)
171.2383
165.3388
149.7293
139.3256
129.3998
124.1055
52.9310
52.4667
40.6616
40.4527
40.2439
40.0350
39.8263
39.6178
39.4090
38.8884
10
Chemical shift (ppm)