Supporting Information

Synthesis and functionalisation of cyclic sulfonimidamides, a novel chiral heterocyclic carboxylic acid bioisostere

Nils Pemberton,* Henrik Graden, Emma Evertsson, Emma Bratt , Matti Lepistö, Petra Johannesson and Per Svensson.

Medicinal Chemistry, AstraZeneca R&D Mölndal, Pepparedsleden 1, S-431 83 Mölndal, Sweden.
Nils.Pemberton@astrazeneca.com

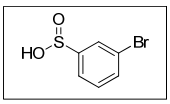
Table of contents:

General Methods. All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Commercially available reagents were used without further purification. Thin-layer chromatography was performed using Merck silica gel 60 F254 plates and visualized under a UV lamp at 254 nm. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 500 and 400 machines (at 500 MHz and 400 MHz respectively). Data for ^{1}H-NMR spectra are reported as follows: chemical shift (δ, ppm), multiplicity, coupling constants (Hz) and integration. Data for ^{13}C-NMR spectra are reported in terms of chemical shift (δ, ppm). Flash column chromatography was performed using prepacked Biotage SNAP columns on a Biotage SP4 system using HPLC grade solvents.

Melting points were measured in a Stuart scientific smp3 melting point apparatus and are uncorrected. High resolution mass spectrometry was performed by Compound Analysis & Plate Purification group, AstraZeneca R&D Mölndal. The method of ionization is given in parentheses.

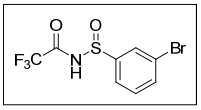
Experimental procedures

3-bromobenzenesulfinic acid (4).



$NaHCO_3$ (50.0 g, 595 mmol) was added to a stirred solution of sodium sulfite (37.5 g, 297 mmol) in water (600 mL) at rt. The reaction was heated to 75 °C and 3-bromobenzenesulfonyl chloride (43.9 mL, 297 mmol) **3** in THF (60 mL) was added over 30 minutes under vigorous stirring. The reaction mixture was stirred at 75 °C for 30 minutes and then cooled to rt. The resulting solution was washed with MTBE. The aqueous phase was acidified by adding by adding 3.8 M HCl, and the aqueous layer was extracted with MTBE. The combined organic layers were dried and concentrated to yield **4** as a white solid (60.0 g, 91 %) ; mp: 87-88 °C; ^{1}H NMR (400 MHz, CD_3CN) δ 8.24 (s, 1H), 7.85 (t, *J* = 1.7 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (101 MHz, CD_3CN) δ 151.2, 135.8, 132.1, 128.3, 124.6, 123.5; HRMS (ESI^+) $[M+H]^+$, calcd for $C_6H_4BrO_2S$: 218.9116 Found: 218.9086.

***N*-(3-bromophenylsulfinyl)-2,2,2-trifluoroacetamide (6).**



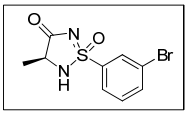
Thionyl chloride (60.7 mL, 832 mmol) was added to 3-bromobenzenesulfinic acid **4** (46.0 g, 208 mmol) in CH_2Cl_2 (600 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and then

allowed to attain rt and stirred for an additional 30 minutes. The reaction mixture was concentrated and the residue was co-evaporated twice with toluene to give 3-bromobenzenesulfinic chloride **5** (49.8 g, 208 mmol) as a yellow oil which was used in the next step without further purification.

n-Butyllithium 1.6 M in hexane (200 mL, 320 mmol) and 2.5 M in hexane (41.6 mL, 104 mmol) were added to 2,2,2-trifluoroacetamide (23.5 g, 208 mmol) in THF (40 mL) at -78 °C. The internal temperature was never allowed to rise above -20 °C during addition. After the addition was complete the reaction was stirred for 10 minutes and then 3-bromobenzenesulfinic chloride (49.8 g, 208 mmol) **5** in THF (100 mL) was added drop-wise over 20 minutes at -78 °C. After the addition was complete the mixture was stirred for 1h at -78 °C.

The reaction was quenched by addition of water. Brine was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried and concentrated to yield 80 g of a crude material, which was dissolved in cold saturated aqueous $NaHCO_3$ and washed with Et_2O. The aqueous layer was acidified by adding saturated aqueous $KHSO_4$, and extracted with EtOAc. The combined organic layers were dried and concentrated to yield **6** (45 g, 142 mmol, 68 %) as a colourless oil; ^{1}H NMR (500 MHz, CD_3CN) δ 10.12 (br singlet), 7.96 (t, *J* = 1.8 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (126 MHz, CD_3CN) δ 158.6 (q, 40 Hz), 145.0, 136.6, 132.3, 128.8, 125.0, 123.8, 116.0 (q, 288 Hz); ^{19}F NMR (470 MHz, CD_3CN) δ -76.1; HRMS (ESI^-) $[M-H]^-$, calcd for $C_8H_4BrF_3NO_2S$: 313.9104 Found: 313.9106.

Synthesis of (8ab).



N-Chlorosuccinimide (0.76 g, 5.69 mmol) was added to **6** (1.50 g, 4.75 mmol) in MeCN (30 mL) at -10 °C. The reaction was stirred for 25 minutes at -10 °C. To the solution was slowly added via a dropping funnel a solution of L-Alanine methyl ester hydrochloride (1.25 g, 8.97 mmol) and DIPEA (1.58 mL, 9.02 mmol) in acetonitrile (5 mL) (that had been stirred together for 40 minutes at rt and filtered before addition). The resulting mixture was allowed to attain rt and stirred for 4h. The reaction mixture was concentrated, dissolved in CH_2Cl_2 washed with water. The organic layer was dried, filtered and concentrated to yield crude **7**, which was used in the next step without further purification.

Sodium (1.31 g, 57.0 mmol) was added to dry MeOH (20 mL). Crude **7** dissolved in MeOH (20 mL) was added to the sodium methoxide solution at rt. The reaction was refluxed for 1h and then concentrated to half the volume. Water was added and the aqueous layer was washed with Et_2O. The aqueous layer was acidified by adding saturated aqueous $KHSO_4$, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried, filtered and concentrated. The residue was purified by column chromatography to yield **8ab** (1.00 g, 73 %) as a mixture of diastereomers ~ 1:1. The diastereomers was separated by HPLC on a Kromasil Cellucoat™ column (Heptane:2-propanol 80:20).

Diastereomer **8a**, mp: 158-160 °C; $[\alpha]_D$ 44.5 (*c* 1.0, MeCN); ^{1}H NMR (400 MHz, DMSO) δ 8.04 (m, 1H), 7.98 (ddd, *J* = 1.0 Hz, 2.0 Hz, 8.0 Hz, 1H), 7.91 (ddd, *J* = 1.0 Hz, 2.0 Hz, 8.0 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 4.44 (q, *J* = 6.7 Hz, 1H), 1.37 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 179.3, 141.1, 137.0, 131.9, 129.4, 126.5, 122.5, 59.4, 19.5; HRMS (ESI^+) $[M+H]^+$, calcd for $C_9H_{10}BrN_2O_2S$: 288.9641 Found: 288.9646.

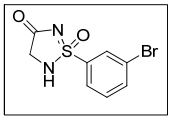
Diastereomer **8b**; mp: 169-171 °C; $[\alpha]_D$ -49.5 (*c* 1.0, MeCN); ^{1}H NMR (400 MHz, DMSO) δ 7.99 (ddd, *J* = 0.9, 1.9, 8.0 Hz, 1H), 7.96 (t, *J* = 1.9 Hz, 1H), 7.86 (ddd, *J* = 0.9, 1.9, 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 4.37 (q, *J* = 6.8 Hz, 1H), 1.32 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 179.7, 141.1, 137.0, 132.2, 129.3, 126.3, 122.4, 57.9, 18.4; HRMS (ESI^+) $[M+H]^+$, calcd for $C_9H_{10}BrN_2O_2S$: 288.9641 Found: 288.9649.

General procedure for the synthesis of cyclic sulfonimidamides (8c-f).

N-Chlorosuccinimide (1.2 equiv.) was added to **6** (1 equiv.) in MeCN (0.13 mmol/mL) at 0 °C. The reaction was stirred for 15 minutes at 0 °C. To the solution was slowly added via a dropping funnel a solution of ester protected amino acid hydrochloride (2 equiv.) and DIPEA (2 equiv) in MeCN (1.6 mmol/mL) (that had been stirred together at rt and filtered prior to addition). The resulting mixture was allowed to attain rt and stirred for 3-20h (until completion as monitored by LC-MS). The reaction mixture was concentrated, CH_2Cl_2 was added and washed with water. The organic layer was dried, filtered and concentrated to yield crude sulfonimidamide, which was used in the next step without further purification.

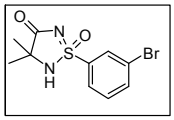
The crude sulfonimidamide was dissolved in MeOH (0.13 mmol/mL) and sodium tert-amylate (4 equiv.) was added at rt. The reaction was refluxed for 1-3 h (until completion as monitored by LC-MS) and then concentrated to half the volume. Water was added and the aqueous layer was washed with MTBE. The aqueous layer was acidified by adding saturated aqueous $KHSO_4$, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried and concentrated. The residue was purified by column chromatography to yield **8c-f** in 40-79% yield.

Compound (8c).



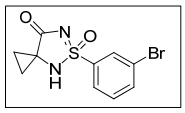
By following the general procedure described above. **6 (**2.20 g, 6.96 mmol) and glycine methyl ester hydrochloride (1.75 g, 13.92 mmol) gave **8c** as a white solid (760 mg, 40%); mp: 150-153 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.04 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 4.19 (d, *J* = 14.3 Hz, 1H), 4.10 (d, *J* = 14.3 Hz, 1H), 3.40 (br singlet, 1H); ^{13}C NMR (126 MHz, DMSO) δ 176.8, 141.2, 136.9, 131.9, 129.5, 126.5, 122.5, 51.7; HRMS (ESI) $[M+H]^+$, calcd for $C_8H_8BrN_2O_2S$: 274.9484 found: 274.9493.

Compound (8d).



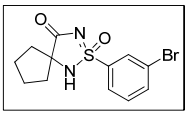
By following the general procedure described above. **6 (**495 mg, 1.57 mmol) and methyl 2-amino-2-methylpropanoate hydrochloride (481 mg, 3.13 mmol) gave **8d** as a white solid (225 mg, 47%); mp: 187-189 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.61 (br s, 1H), 7.98 - 8.04 (m, 1H), 7.96 (t, *J* = 1.8 Hz, 1H), 7.84 - 7.90 (m, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 1.44 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 181.8, 141.1, 137.0, 132.2, 129.2, 126.2, 122.4, 65.5, 27.6, 25.6; HRMS (ESI^+) $[M+H]^+$, calcd for $C_{10}H_{12}BrN_2O_2S$: 302.9797 found: 302.9800.

Compound (8e).



By following the general procedure described above. **6 (**500 mg, 1.58 mmol) and methyl 1-aminocyclopropanecarboxylate hydrochloride (480 mg, 3.16 mmol) gave **8e** as a white solid (375 mg, 79%); mp: 195-197 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.76 (br s, 1H), 7.99 - 8.05 (m, 1H), 7.96 (t, *J* = 1.9 Hz, 1H), 7.85 - 7.88 (m, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 1.14 - 1.27 (m, 4H); ^{13}C NMR (126 MHz, DMSO) δ 178.8, 140.9, 137.1, 132.3, 129.0, 126.1, 122.6, 47.3, 12.1,11.9; HRMS (ESI) $[M+H]^+$, calcd for $C_{10}H_{10}BrN_2O_2S$: 300.9641 found: 300.9647.

Compound (8f).

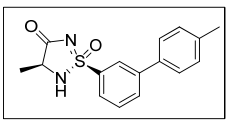


By following the general procedure described above. **6 (**370 mg, 1.17 mmol) and methyl 1-aminocyclopentanecarboxylate hydrochloride (335 mg, 1.86 mmol) gave **8f** as a white solid (160 mg, 42%); mp: 168-171 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.73 (br s, 1H), 7.99-8.01 (m, 1H), 7.93 (t, *J* = 1.9 Hz, 1H), 7.81-7.84 (m, 1H) 7.65 (t, *J* = 8.1 Hz, 1H), 2.03 - 2.16 (m, 2H), 1.62 - 1.89 (m, 6H); ^{13}C NMR (126 MHz, DMSO) δ 182.0, 141.1, 136.9, 132.2, 129.2, 126.2, 122.5, 74.8, 38.0, 24.85, 24.80; HRMS (ESI) $[M+H]^{+}$, calcd for $C_{12}H_{14}BrN_2O_2S$: 328.9954 found: 328.9955.

General procedure for the Suzuki coupling to prepare (9a-c).

A glass vial was charged with Aryl Halide **8b** (1 equiv.), arylboronic acid (1.5 equiv.), potassium carbonate (2 equiv.), water (0.4 mmol/mL), and MeCN (0.4 mmol/mL) and the reaction mixture was degassed. 1,1'-bis(di-tert-butylphosphino)ferrocene palladium dichloride (0.1 equiv.) was added and the reaction mixture was degassed once again and heated to 80 °C for 2h. Aqueous $KHSO_4$ (1M) and DCM were added and the organic phase was separated and concentrated. The residue was purified by column chromatography to yield **9a-c** in 87-88%.

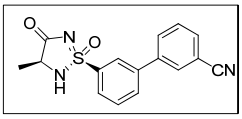
Compound (9a).



By following the general procedure described above, **8b (**50 mg, 0.17 mmol) and *p*-tolylboronic acid (35 mg, 0.26 mmol) gave **9a** as a white solid (45.0 mg, 87%); mp: 212-213 °C; $[\alpha]_D$ -49.6 (*c* 1.0, MeCN); ^{1}H NMR (500 MHz, DMSO) δ 9.42 (s, 1H), 8.02 – 8.06 (m, 1H), 7.99 (t, 1H), 7.81 – 7.84 (m, 1H), 7.74 – 7.80 (m, 1H), 7.61 (d, *J*=7.8 Hz, 2H), 7.33 (d, *J*=7.8 Hz, 2H), 4.38 (q, *J*=6.8 Hz, 1H), 2.36 (s, 3H), 1.36 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (126

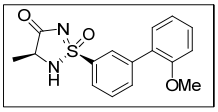
MHz, DMSO) δ 179.8, 141.6, 139.7, 138.1, 135.2, 132.0, 130.7, 129.9, 126.7, 125.7, 124.5, 57.8, 20.7, 18.5; HRMS (ESI) $[M+H]^+$, calcd for $C_{16}H_{17}N_2O_2S$: 301.1011 found: 301.1013.

Compound (9b).



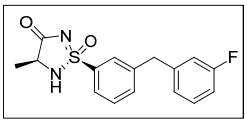
By following the general procedure described above, **8b (**50 mg, 0.17 mmol) and 3-cyanophenylboronic acid (38 mg, 0.26 mmol) gave **9b** as a white solid (47.0 mg, 87%); mp: 136-138 °C; $[\alpha]_D$ -44.6 (*c* 1.0, MeCN); ^{1}H NMR (500 MHz, DMSO) δ 9.43 (s, 1H), 8.26 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 8.12 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.91 (t, *J* = 8.5 Hz, 2H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 4.38 (q, *J* = 6.8 Hz, 1H), 1.36 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 179.8, 140.0, 139.6, 139.2, 132.7, 132.1, 131.9, 130.9, 130.8, 130.4, 126.9, 125.3, 118.6, 112.3, 57.8, 18.4; HRMS (ESI) $[M+H]^+$, calcd for $C_{16}H_{14}N_3O_2S$: 312.0807 found: 312.0810.

Compound (9c).



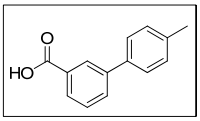
By following the general procedure described above, **8b (**50 mg, 0.17 mmol) and 2-methoxyphenylboronic acid (39 mg, 0.26 mmol) gave **9c** as a white solid (48.0 mg, 88%); mp: 137-139 °C; $[\alpha]_D$ -34.0 (*c* 1.0, MeCN); ^{1}H NMR (500 MHz, DMSO) δ 9.42 (s, 1H), 7.92 (t, *J* = 1.7 Hz, 1H), 7.81 – 7.88 (m, 2H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.41 – 7.45 (m, 1H), 7.36 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 7.06 – 7.11 (m, 1H), 4.37 (q, *J* = 6.8 Hz, 1H), 3.78 (s, 3H), 1.35 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 179.8, 155.9, 139.4, 138.7, 134.6, 130.3, 130.1, 129.9, 127.5, 127.3, 125.7, 121.1, 112.0, 57.9, 55.6, 18.5; HRMS (ESI) $[M+H]^+$, calcd for $C_{16}H_{17}N_2O_3S$: 317.0960 found: 317.0970.

Compound (9d).



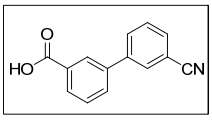
Aryl Halide **8b** (50 mg, 0.17 mmol) and tetrakis(triphenylphosphine)palladium (7.99 mg, 0.007 mmol) were dissolved in THF (0.5 mL) and degassed. (3-fluorobenzyl)zinc(II) bromide (0.519 mL, 0.26 mmol) was added and the rection mixture was heated to 60 °C under N_2 for 3h. (3-fluorobenzyl)zinc(II) bromide (0.519 mL, 0.26 mmol) was added once again and stirred at 60 °C for another 2h. The residue was purified by column chromatography to yield **9d** (45.0 mg, 82 %) as a white solid; ; $[\alpha]_D$ -43.8 (*c* 1.0, MeCN); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 – 7.75 (m, 2H), 7.41 – 7.51 (m, 2H), 7.22 – 7.30 (m, 1H), 6.89 – 6.97 (m, 2H), 6.81 – 6.87 (m, 1H), 4.35 (q, *J* = 6.8 Hz, 1H), 4.03 (s, 2H), 1.42 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.5, 163.0 (d, *J* = 247 Hz), 142.5, 141.7 (d, *J* = 7.2 Hz), 138.4, 134.8, 130.3 (d, *J* = 8.4 Hz), 129.7, 127.9, 125.8, 124.6 (d, *J* = 2.8 Hz), 115.8 (d, *J* = 21.4 Hz), 113.7 (d, *J* = 21.4 Hz), 58.2, 41.1 (d, *J* = 1.6 Hz), 18.9; HRMS (ESI^+) $[M+H]^+$, calcd for $C_{16}H_{16}FN_2O_2S$: 319.0917 found: 319.0914.

4'-methylbiphenyl-3-carboxylic acid (10a).



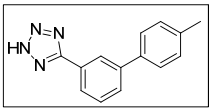
By following the general Suzuki procedure described above, 3-bromobenzoic acid (100 mg, 0.50 mmol) and 4-tolylboronic acid (101 mg, 0.75 mmol) gave **10a** as a white solid (85 mg, 81 %); ^{1}H NMR (500 MHz, DMSO) δ 13.05 (s, 1H), 8.15 (s, 1H), 7.89 (dd, *J* = 10.3, 8.9 Hz, 2H), 7.51 - 7.62 (m, 3H), 7.29 (d, *J* = 7.9 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.3, 140.4, 137.3, 136.4, 131.4, 130.8, 129.7, 129.3, 127.9, 127.0, 126.6, 20.7; HRMS (ESI^-) $[M-H]^-$, calcd for $C_{14}H_{11}O_2$: 211.0759 found: 211.076.

3'-cyanobiphenyl-3-carboxylic acid (10b).



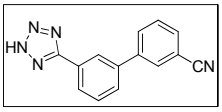
By following the general Suzuki procedure described above, 3-bromobenzoic acid (116 mg, 0.58 mmol), 3-cyanophenylboronic acid (127 mg, 0.87 mmol), gave **10b** as a white solid (69 mg, 53 %); ^{1}H NMR (500 MHz, DMSO) δ 13.16 (br s, 1H), 8.18 (s, 1H), 8.11 (s, 1H), 7.91 - 8.03 (m, 3H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 167.6, 140.7, 138.8, 132.1, 132.0, 131.9, 131.8, 130.8,130.7, 130.0, 129.6, 127.9, 119.1, 112.5; HRMS (ESI^{-}) $[M-H]^{-}$, calcd for $C_{14}H_8NO_2$: 222.0555 found: 222.0546.

5-(4'-methylbiphenyl-3-yl)-2H-tetrazole (10c).



By following the general Suzuki procedure described above, 5-(3-bromophenyl)tetrazole (200 mg, 0.89 mmol) and 4-tolylboronic acid (181 mg, 1.33 mmol) gave **10c** as an amorphous solid (100 mg, 48 %); ^{1}H NMR (500 MHz, MeOD) δ 8.26 (s, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.81 (d, *J* = 7.9Hz, 1H), 7.62 (t, *J* = 7.7Hz, 1H), 7.59 (d, *J* = 8.0Hz, 2H), 7.30 (d, *J* = 8.0Hz, 2H), d 2.39 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 157.5, 143.7, 139.1, 138.2, 131.0, 130.8, 130.7, 127.9, 126.7, 126.5, 125.9, 21.1; HRMS (ESI^{+}) $[M+H]^{+}$, calcd for $C_{14}H_{13}N_4$: 237.1140 found: 237.1137.

3'-(2H-tetrazol-5-yl)biphenyl-3-carbonitrile (10d).



By following the general Suzuki procedure described above, 5-(3-bromophenyl)tetrazole (200 mg, 0.89 mmol) and 3-cyanophenylboronic acid (196 mg, 1.33 mmol) gave **10d** as an amorphous solid (60 mg, 27 %); ^{1}H NMR (500 MHz, acetone-d6) δ 8.48 (t, *J* = 1.7 Hz, 1H),

8.17 - 8.25 (m, 2H), 8.11 - 8.16 (m, 1H), 7.99 (m, 1H), 7.85 (m, 1H), 7.77 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR (126 MHz, acetone-d6) δ 142.0, 140.7, 132.4, 132.3, 131.4, 131.2, 130.6, 127.8, 126.7, 126.6, 119.2,114.1; HRMS (ESI^+) $[M+H]^+$, calcd for $C_{14}H_{10}N_5$: 248.0936 found: 248.0939.

Measurement of permeability using caco-2 monolayers.

The Caco-2 cell monolayers are washed once with HBSS (Hanks balanced saline solution) prior to start. TEER (Trans Epithelial Electrical Resistance) is measured both before and after performing all the transport experiments. Experiments are made in apical (A) to basolateral (B) direction and B to A direction in singlicates.

For AB direction: A solution of HBSS (800 µL, pH 7.4) is first dispensed to the basal side of the monolayer. The assay is then initiated when 200 µL of a 1 µM solution of test compound in HBSS (pH 7.4, with 1% DMSO as co-solvent) is added to the apical side. The mixture is placed in a shaking incubator at 480 rpm and 37 °C. Donor samples are withdrawn at 0 and 60 minutes post addition of test compound and receiver samples are withdrawn at 60 minutes (2 μL and 200 μL are withdrawn from the apical (donor) compartment and the basolateral (receiver) compartment, respectively.

For BA direction: A solution of HBSS (200 µL, pH 7.4) is first dispensed to the apical side of the monolayer. The assay is then initiated when 800 µL of a 1 µM solution of test compound in HBSS (pH 7.4, with 1% DMSO as co-solvent) is added to the basal side. The mixture is placed in a shaking incubator at 480 rpm and 37 °C. Donor samples are withdrawn at 0 and 30 minutes post addition of test compound and receiver samples are withdrawn at 30 minutes. (1 and 100 µL are withdrawn from the basal (donor) compartment and the apical (receiver) compartment, respectively.

The analytes are separated on a short reversed-phase HPLC column with rapid gradient elution and detected by LCMS. All samples are analysed directly after being withdrawn.

The apparent permeability coefficient (P_{app}) for the two transport directions (AB and BA) are calculated according to the following equation:

$P_{app} = (\Delta Q/\Delta t)/(A \times C_D)$ [cm/s]

Where ($\Delta Q/\Delta t$) [cm/s] is the cumulative amount test compound transported over time, A is the surface area of the monolayer membrane (cm^2) and C_D is the average drug concentration in the donor chamber over the period which ($\Delta Q/\Delta t$) was determined (0 - 60 minutes for the AB direction and 0 – 30 minutes for the BA direction).

Recovery during the experiment is calculated as:

Recovery [%] = $[(QD_{end} + QR_{end})/QD_0] \times 100$

Where ($QD_{end} + QR_{end}$) is the final amount of compound found in both donor and receiver sides and (QD_{0min}) is the amount in the donor side at start (0 minutes).

The efflux-ratio (ER) is unitless and the ratio is calculated as:

P_{app} BA / P_{app} AB.

pKa measurement using pressure-assisted capillary electrophoresis.

Ionisation constants were measured using a high throughput pKa screening assay by pressure-assisted capillary electrophoresis (CE) and mass spectrometry (MS). The fundamental principle relies on measuring the ionic effective mobility of the solute in a series of different pH background electrolytes with the same ionic strength. The pKa was obtained by non-linear fitting of the effective mobility as a function of pH (see ref 23. for a more detailed description of the method).

Compounds (2 µl, 10 mM) in 96-plates (including references atenolol, lidocaine, nicotine, papaverine, propanolol and quinidine) were pooled into a 1.5 ml glass vial using 50% ethanol-water as a generic solvent. 14 buffers (1 mL) with a pH ranging from 2.3 to 10.8 were

prepared and placed in the CEMS. MS data were converted into migration times and the final pKa values were calculated using effective mobility versus pHs using different pKa-fit models. This method provides good accuracy and good reproducibility (±0.2 units).

CEMS- CE instrument is HPCE3D (Agilent Technologies) coupled on-line with an 1100 Series LC/MSD Trap (SL) and a binary LC pump). The MSD trap mass analyzer was scanned from 70-660 m/z; Averages: 10; Tune: smart mode; drying gas: 5 l/min; nebulizer gas: 5 psi, drying temperature: 150 °C. DMSO was used as a neutral marker.

Sheath- Sheath flow was 0.3 ml/min with a split of 1:100. For positive detection, sheath liquid compositions were NH_4HCO_2 (5 mM in H_2O-MeOH (50:50 %)). For negative detection, sheath liquid compositions were NH_4OH (5 mM in H_2O-MeOH (50:50 %)) and benzyl alcohol was used instead of DMSO, as a neutral marker.

Capillary- Untreated fused silica capillaries (Skandinaviska GeneTec AB, Sweden) were used. The capillary was thermostated at 25 °C by utilizing an external water bath connected to the sample tray. New capillaries (50 μm I.D., 53-58 cm) were pretreated by flushing with NaOH (1.0 M, 180 min.), then with H_2O (10 min) and finally with NH_4OH (0.2 M, 20 min). Before starting the analytical sequence the capillary was preconditioned by flushing with NH_4OH (0.2 M, 5 min.) then with a pH 10.5 buffer (flush 5 min. and subsequently another 20 min. under an applied high voltage of 20 kV). After the sequence, the capillary was washed with NH_4OH (0.2 M, 5 min.), then EtOH (50 %, 5 min.) and finally with H_2O (5 min). Samples were introduced by a standard pressure of 50 mbar. Sequence run orders were set from high pH to low pH to minimize the effect of carbonate.

DATA ANALYSIS

Effective mobility was calculated according to the equation below:

$$m_{eff} = m_{obs} - m_{eof} = \frac{L_{tot} * L_{eff}}{V} * (1/t_{obs} - 1/t_{eof})$$

m_{eff} the effective mobility of the analyte ($cm^2/V\ s$)

m_{obs} the apparent or observed mobility of the analyte ($cm^2/V\ s$)

m_{eof} the mobility of the electroosmotic flow (neutral marker) ($cm^2/V\ s$)

L_{tot} the total length of capillary (cm)

L_{eff} the effective separation length from injection to detector (cm)

V the applied high voltage (V)

t_{obs} the observed migration time of the analyte (s)

t_{eof} the observed migration time of the neutral marker (DMSO) (s).

Calculation of pKa by non-linear fitting

The pKa calculations are based on equations listed in the table below. An in-house written program was employed to evaluate pKa values for a number of compounds simultaneously.

Model equations used for pK_a calculation

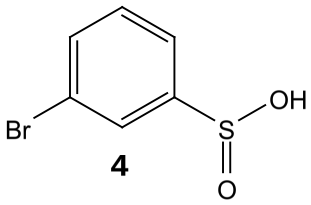
Ionization type	Model equation
Mono-Acid	$m_{eff} = \frac{M_a 10^{-pKa}}{10^{-pKa} + 10^{-pH}}$
Mono-Base	$m_{eff} = \frac{M_b 10^{-pH}}{10^{-pKa} + 10^{-pH}}$
Mono-Acidic-Mono-Basic Ampholyte	$m_{eff} = \frac{M_{b1}[10^{-pH}]^2 + M_{a1} 10^{-pKa1} 10^{-pKa2}}{[10^{-pH}]^2 + 10^{-pKa1} 10^{-pH} + 10^{-pKa1} 10^{-pKa2}}$
Di-Acid	$m_{eff} = \frac{M_{a1} 10^{-pKa1} 10^{-pH} + M_{a2} 10^{-pKa1} 10^{-pKa2}}{[10^{-pH}]^2 + 10^{-pKa1} 10^{-pH} + 10^{-pKa1} 10^{-pKa2}}$
Di-Base	$m_{eff} = \frac{M_{b2}[10^{-pH}]^2 + M_{b1} 10^{-pKa1} 10^{-pH}}{[10^{-pH}]^2 + 10^{-pKa1} 10^{-pH} + 10^{-pKa1} 10^{-pKa2}}$
Tri-Acid	$m_{eff} = \frac{M_{a1} 10^{-pKa1}[10^{-pH}]^2 + M_{a2} 10^{-pKa1} 10^{-pKa2} 10^{-pH} + M_{a3} 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}{[10^{-pH}]^3 + 10^{-pKa1}[10^{-pH}]^2 + 10^{-pKa1} 10^{-pKa2} 10^{-pH} + 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}$
Tri-Base	$m_{eff} = \frac{M_{b3}[10^{-pH}]^3 + M_{b2} 10^{-pKa1}[10^{-pH}]^2 + M_{b1} 10^{-pKa1} 10^{-pKa2} 10^{-pH}}{[10^{-pH}]^3 + 10^{-pKa1}[10^{-pH}]^2 + 10^{-pKa1} 10^{-pKa2} 10^{-pH} + 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}$
Di-Acidic-Mono-Basic Ampholyte	$m_{eff} = \frac{M_{b1}[10^{-pH}]^2 + M_{a1} 10^{-pKa1} 10^{-pKa2} 10^{-pH} + M_{a2} 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}{[10^{-pH}]^3 + 10^{-pKa1}[10^{-pH}]^2 + 10^{-pKa1} 10^{-pKa2} 10^{-pH} + 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}$
Mono-Acidic-Di-Basic Ampholyte	$m_{eff} = \frac{M_{b2}[10^{-pH}]^2 + M_{b1} 10^{-pKa1}[10^{-pH}]^2 + M_{a1} 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}{[10^{-pH}]^3 + 10^{-pKa1}[10^{-pH}]^2 + 10^{-pKa1} 10^{-pKa2} 10^{-pH} + 10^{-pKa1} 10^{-pKa2} 10^{-pKa3}}$

Reference: Wan, H; Holmén, A; Wang, YD; Lindberg, W; Englund, M; Någård, M; Thompson, R. High throughput screening of pKa values of pharmaceuticals by pressure-assisted capillary electrophoresis and mass spectrometry. *Rapid Communications in Mass Spectrometry.* **2003**,1, 2639-2648.

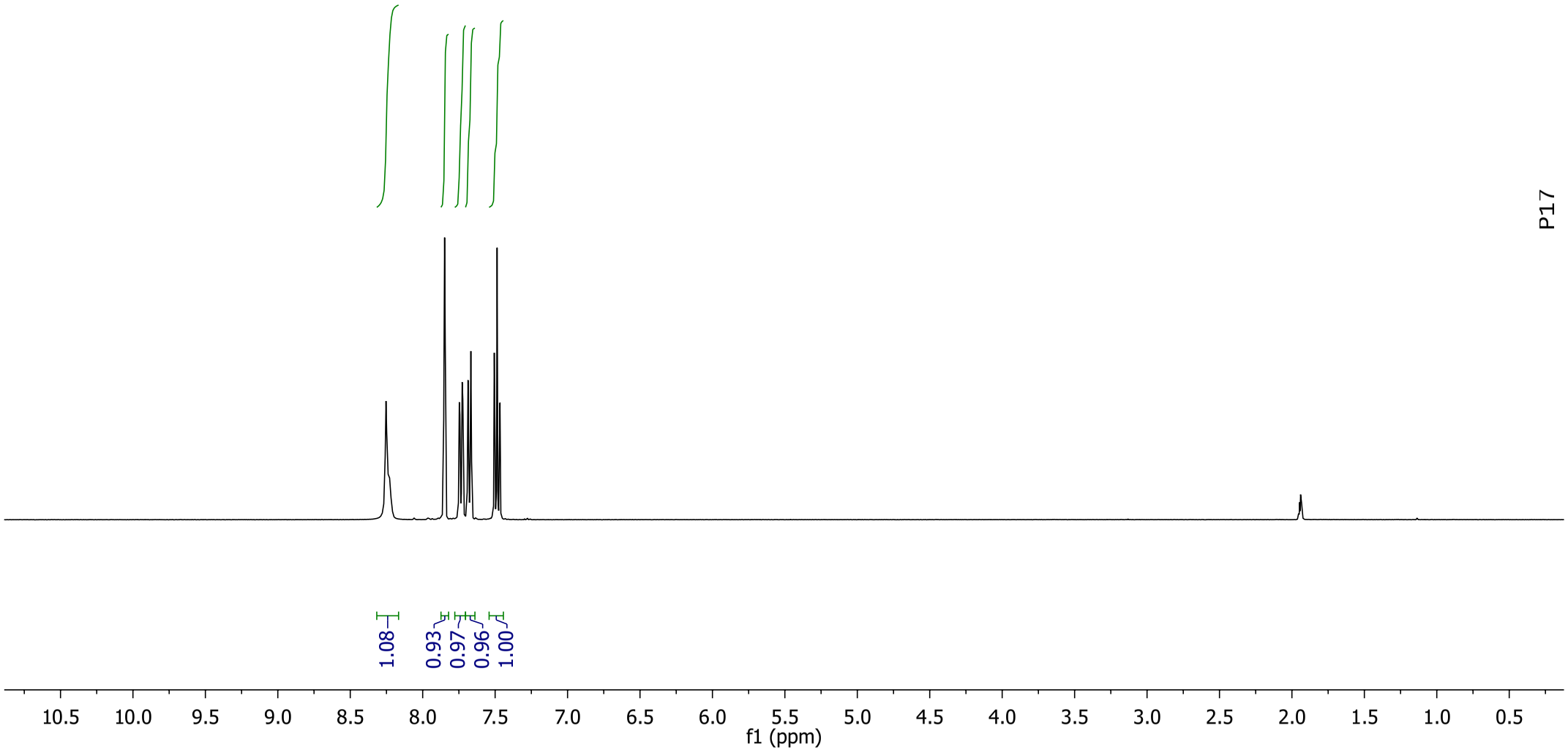
log D measurement

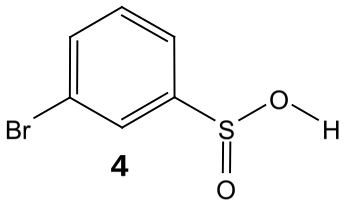
log D measurements were made using a shake-flask method where the extent of partitioning between pH 7.4 buffer and octanol was measured. Compounds were dissolved in a known volume buffer, and following the addition of a known amount of octanol, the solutions were

shaken for 30 min. Following centrifugation, analysis of the aqueous layer was performed by LC–UV to quantify the amount of compound in solution and then compared to analysis of the compound in solution before the addition of octanol to calculate the partitioning coefficient, D.

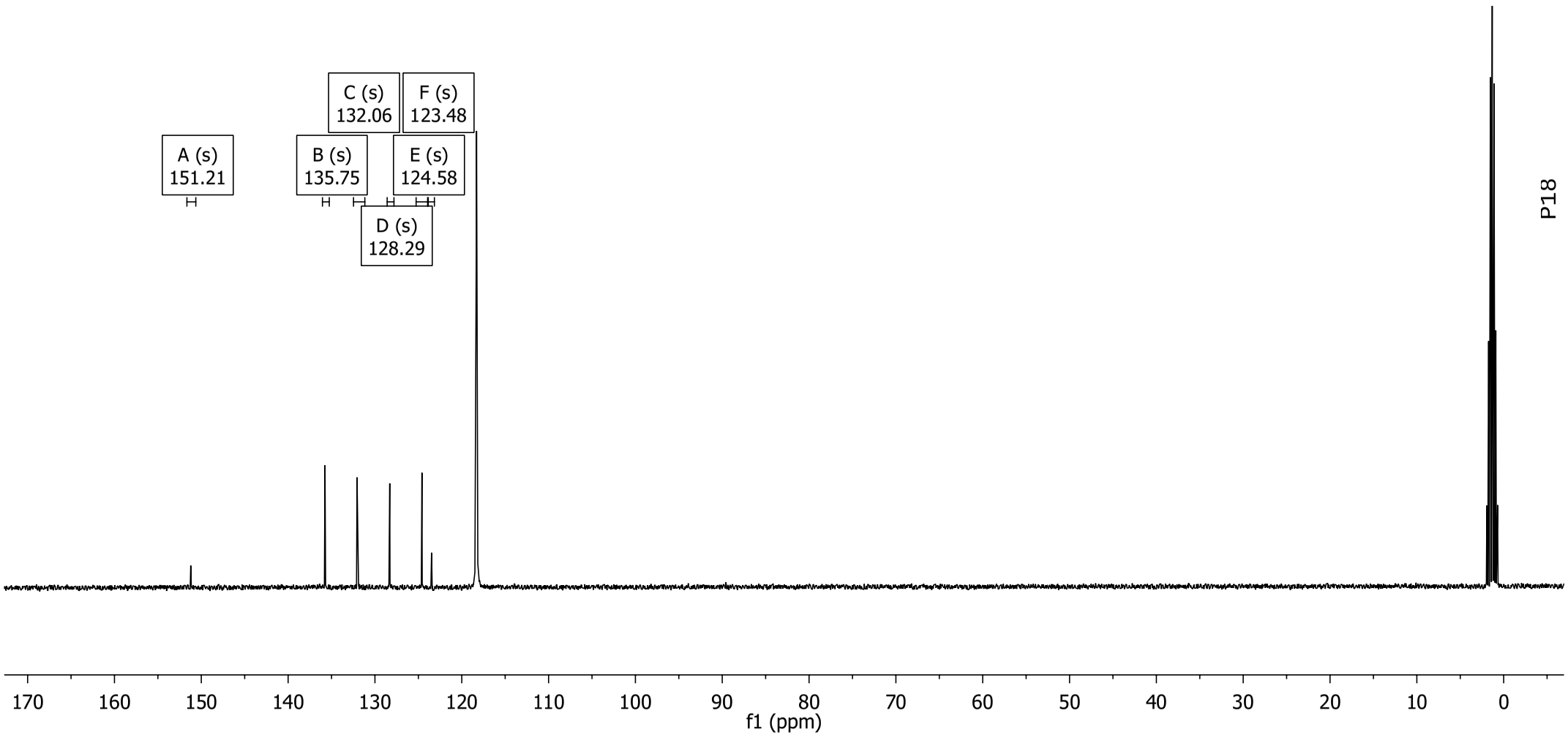

Br
S
OH
O

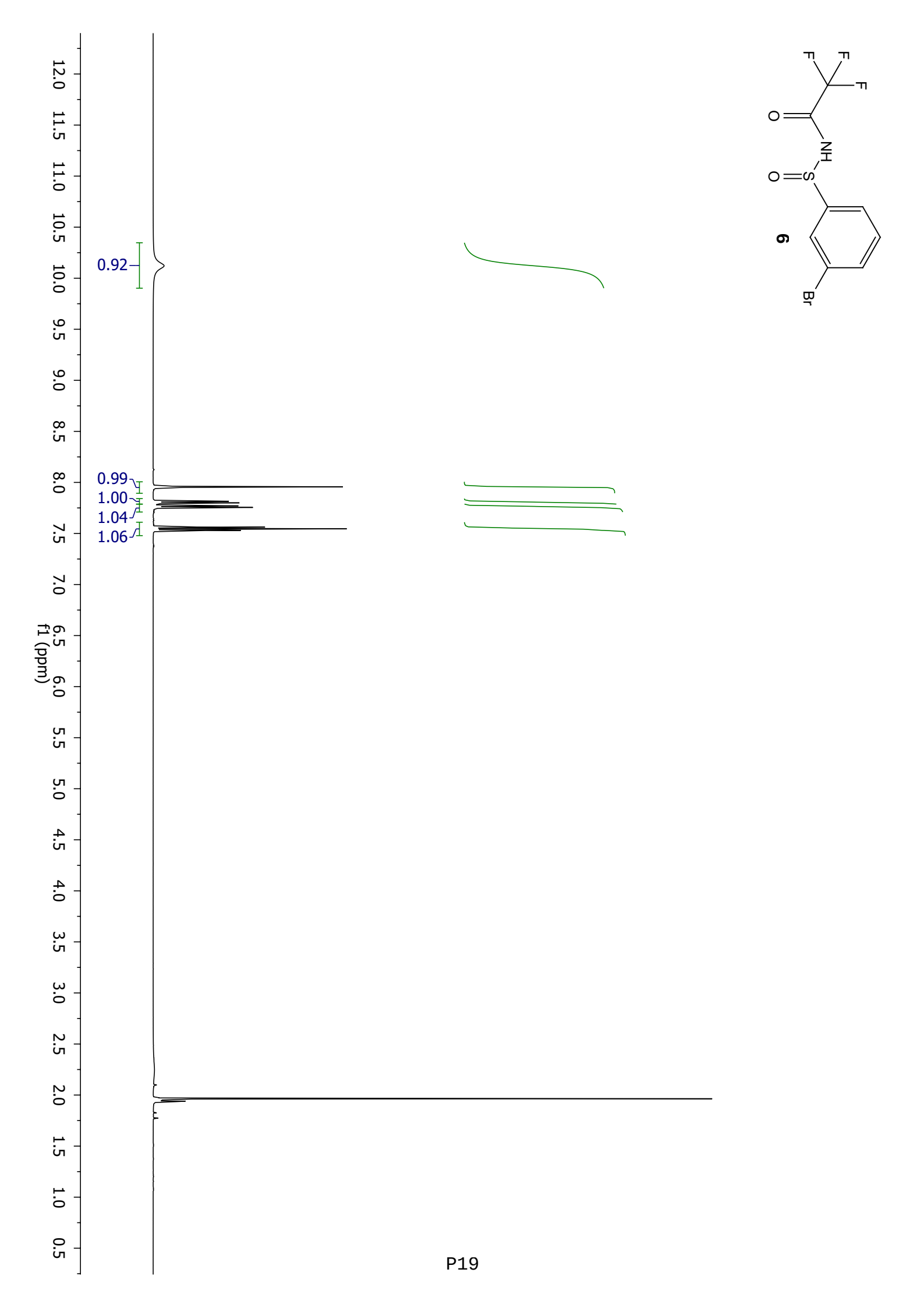
4

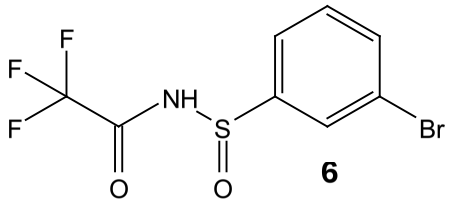

P1.7
1.08
0.93
0.97
0.96
1.00
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


Br
S
O
H
O

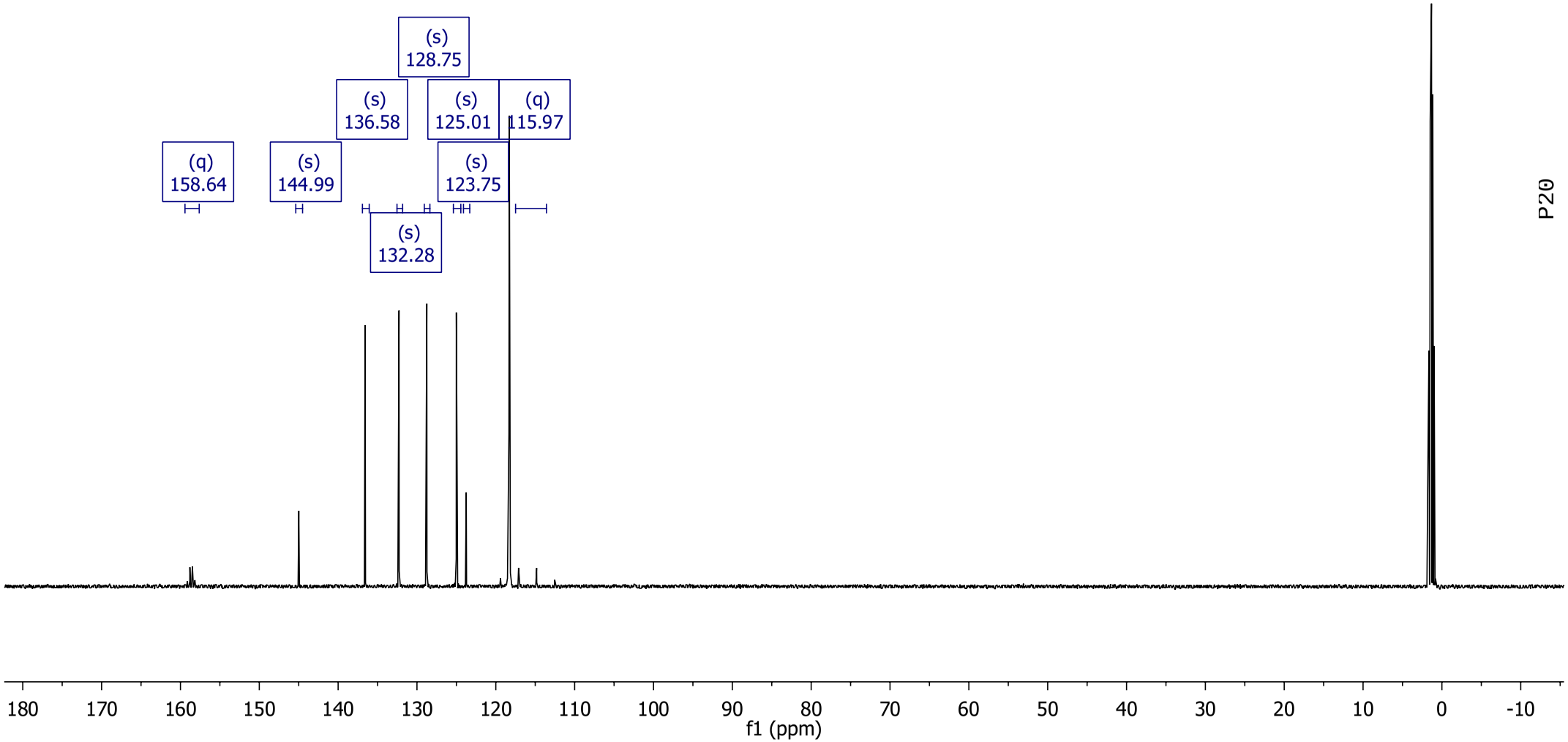
4

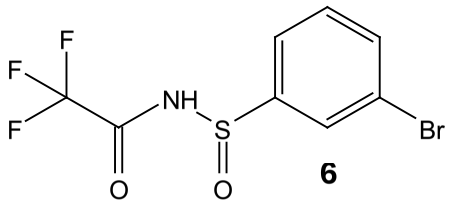

A (s)
151.21
B (s)
135.75
C (s)
132.06
D (s)
128.29
E (s)
124.58
F (s)
123.48
P18
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


0.92
0.99
1.00
1.04
1.06
f1 (ppm)
12.0
11.5
11.0
10.5
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
F
F
F
O
NH
S
O
Br
6

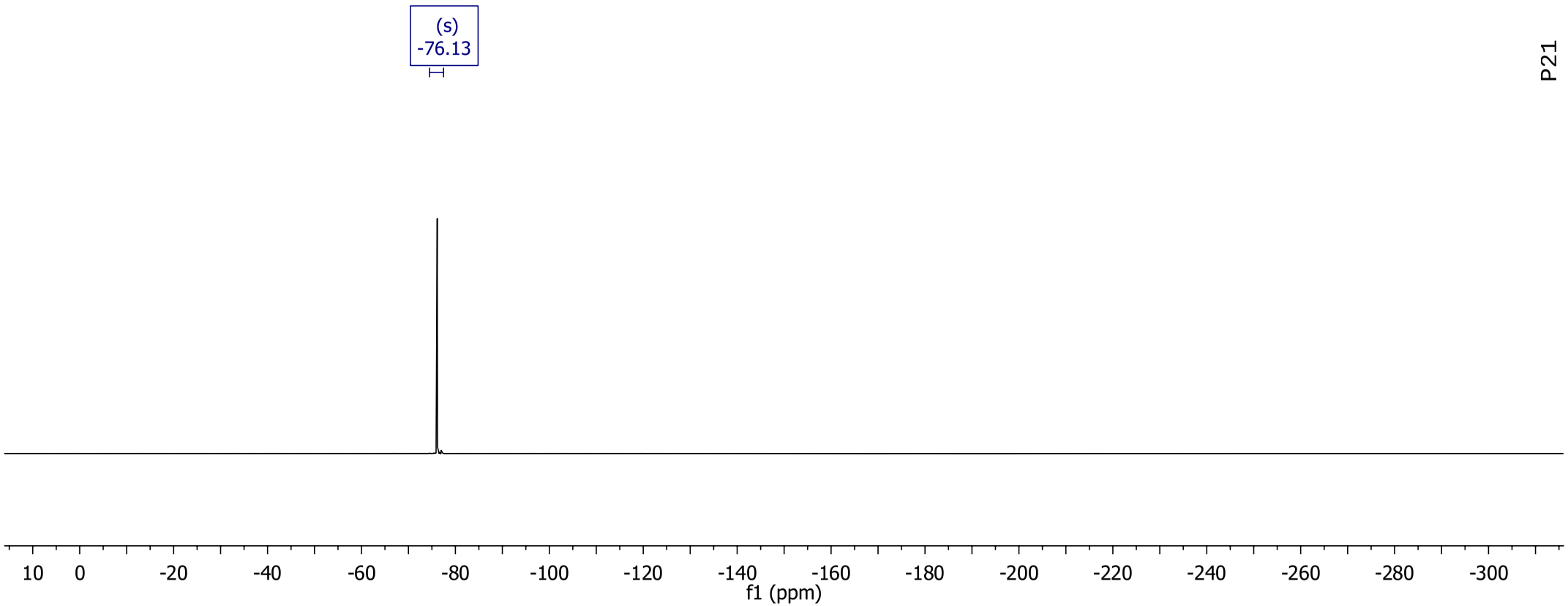

F
F
F
NH
S
O
O
Br

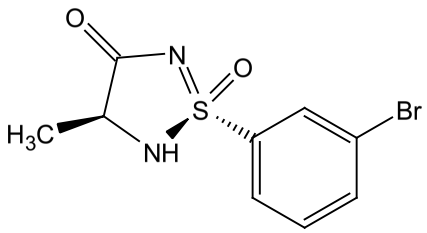
6


(s)
128.75
(s)
136.58
(s)
125.01
(q)
115.97
(q)
158.64
(s)
144.99
(s)
123.75
(s)
132.28
P20
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

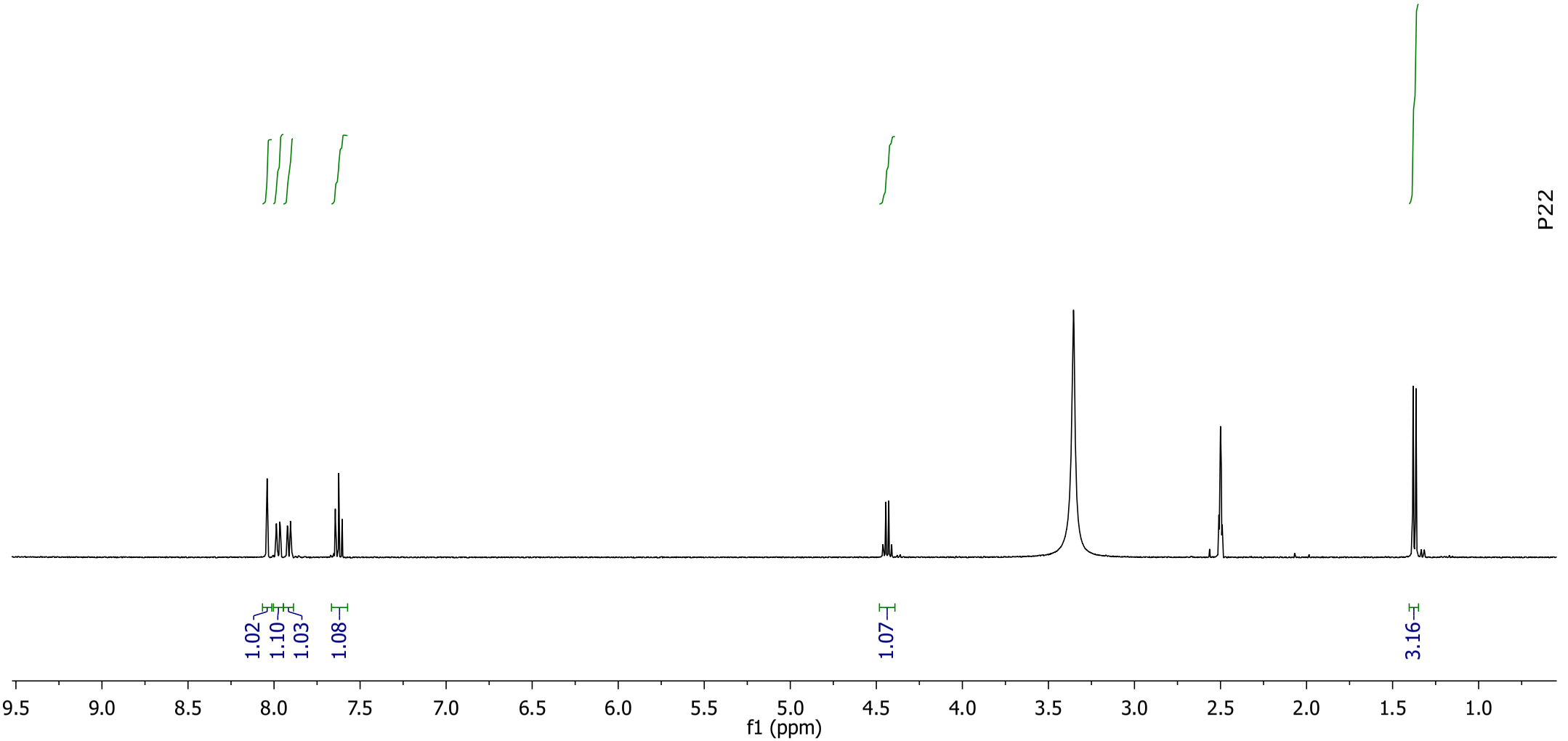

F
F
F
NH
S
O
O
Br

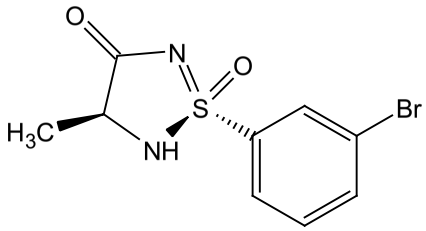
6


(s)
-76.13
P21
10
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
f1 (ppm)


O
N
O
S
Br
H_3C
NH

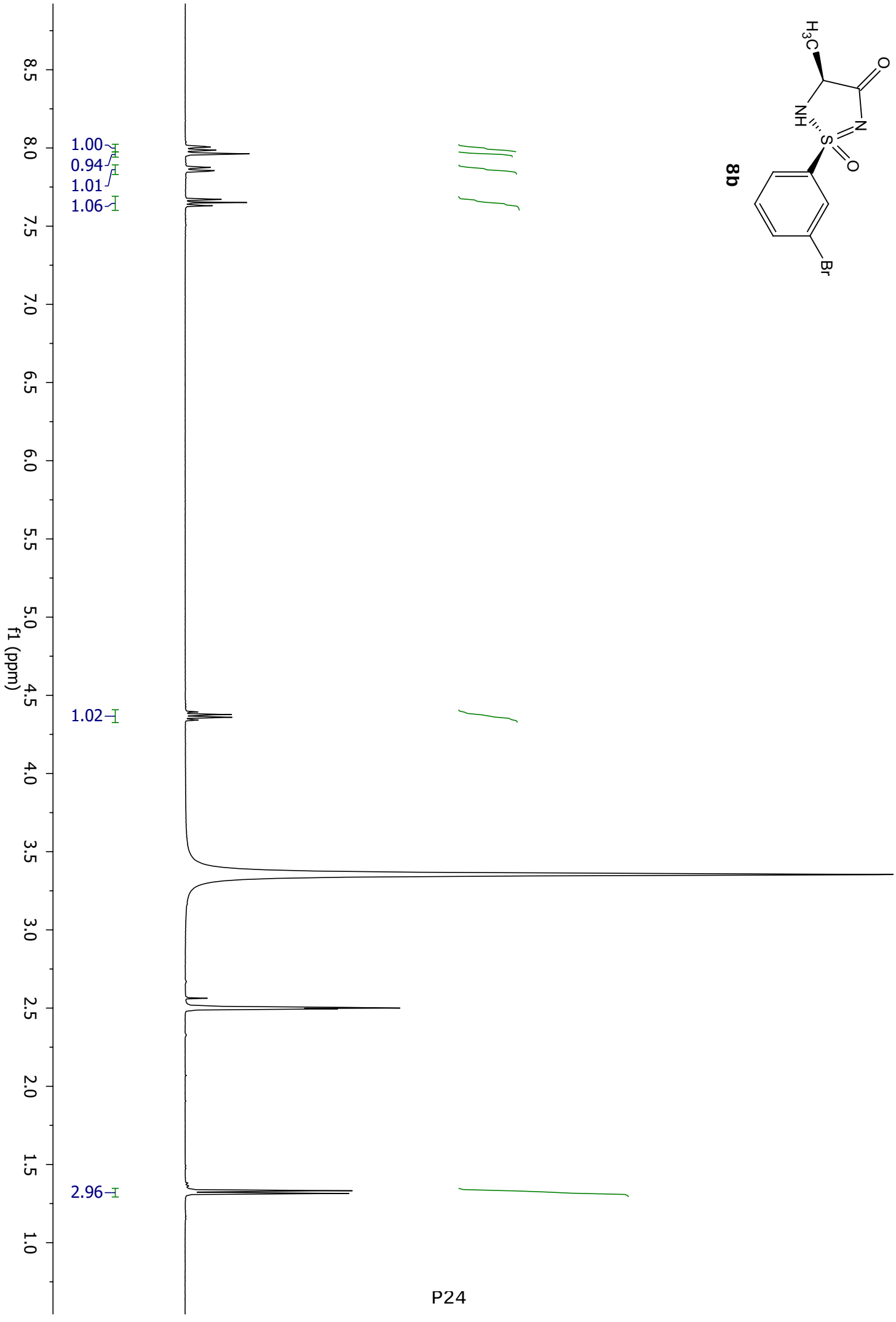
8a


P22
1.02
1.10
1.03
1.08
1.07
3.16
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
f1 (ppm)

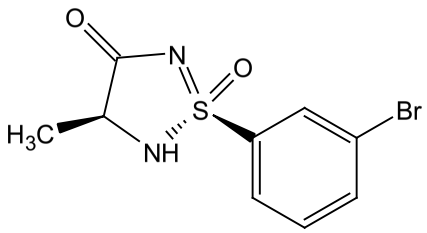

O
N
O
S
Br
H_3C
NH

8a

(s)
179.25
(s)
136.97
(s)
126.47
(s)
141.05
(s)
131.91
(s)
122.54
(s)
129.39
(s)
59.39
(s)
19.51
P23
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


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
f1 (ppm)
1.00
0.94
1.01
1.06
1.02
2.96
H3C
O
NH
N
S
O
Br

8b



8b

(s) 179.74

(s) 141.13

(s) 136.99

(s) 132.23

(s) 129.34

(s) 126.33

(s) 122.43

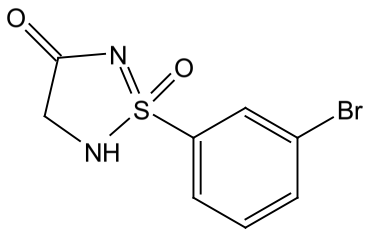
(s) 57.94

I (s) 18.38

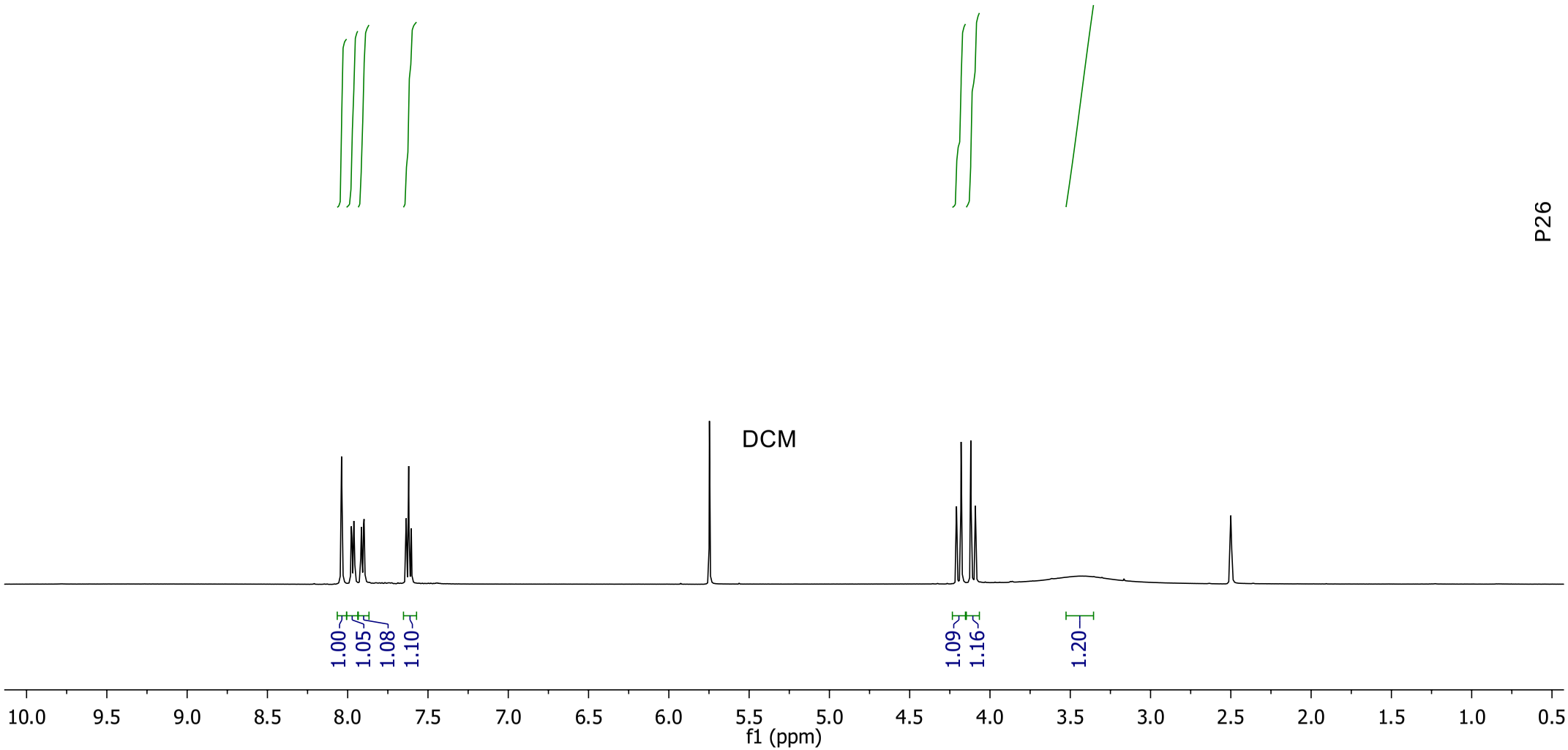
P25

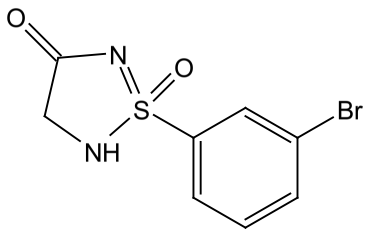
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

f1 (ppm)

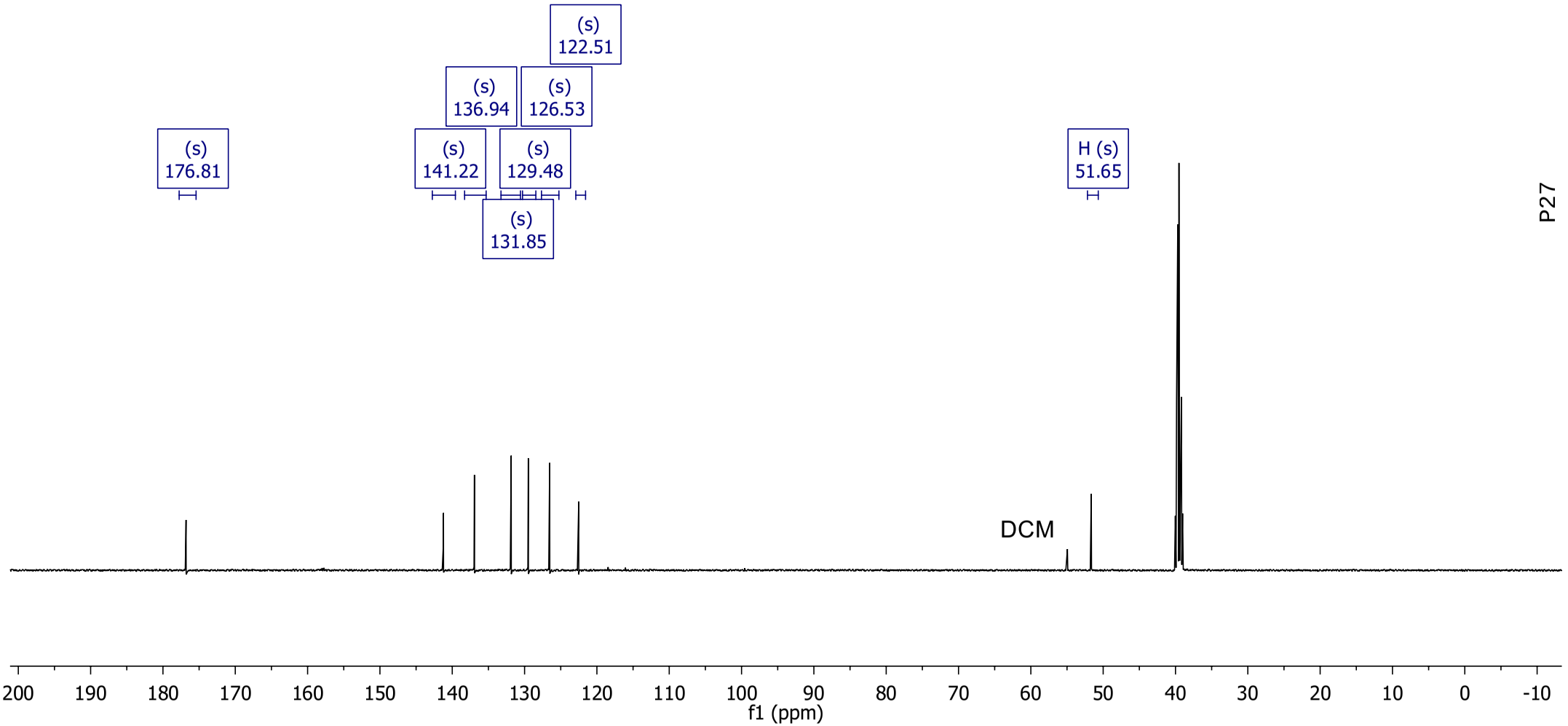

O
N
O
S
Br
NH

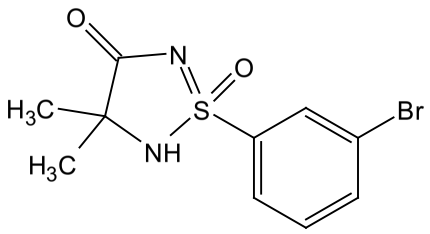
8c


P26
DCM
1.00
1.05
1.08
1.10
1.09
1.16
1.20
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
f1 (ppm)


O
N
O
S
Br
NH

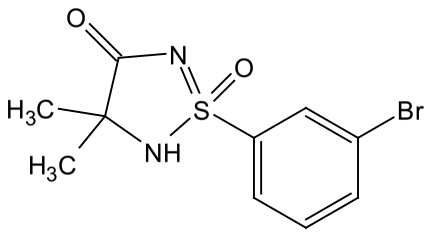
8c


(s)
122.51
(s)
136.94
(s)
126.53
(s)
176.81
(s)
141.22
(s)
129.48
(s)
131.85
H (s)
51.65
DCM
P27
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


O
N
O
S
H3C
Br
H3C
NH

8d

P28
0.73
1.01
1.00
1.02
1.05
3.11
3.16
11.5 11.0 10.5 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
f1 (ppm)



8d

(s) 181.79

(s) 141.08

(s) 136.95

(s) 132.24

(s) 126.19

(s) 129.21

(s) 122.44

(s) 65.53

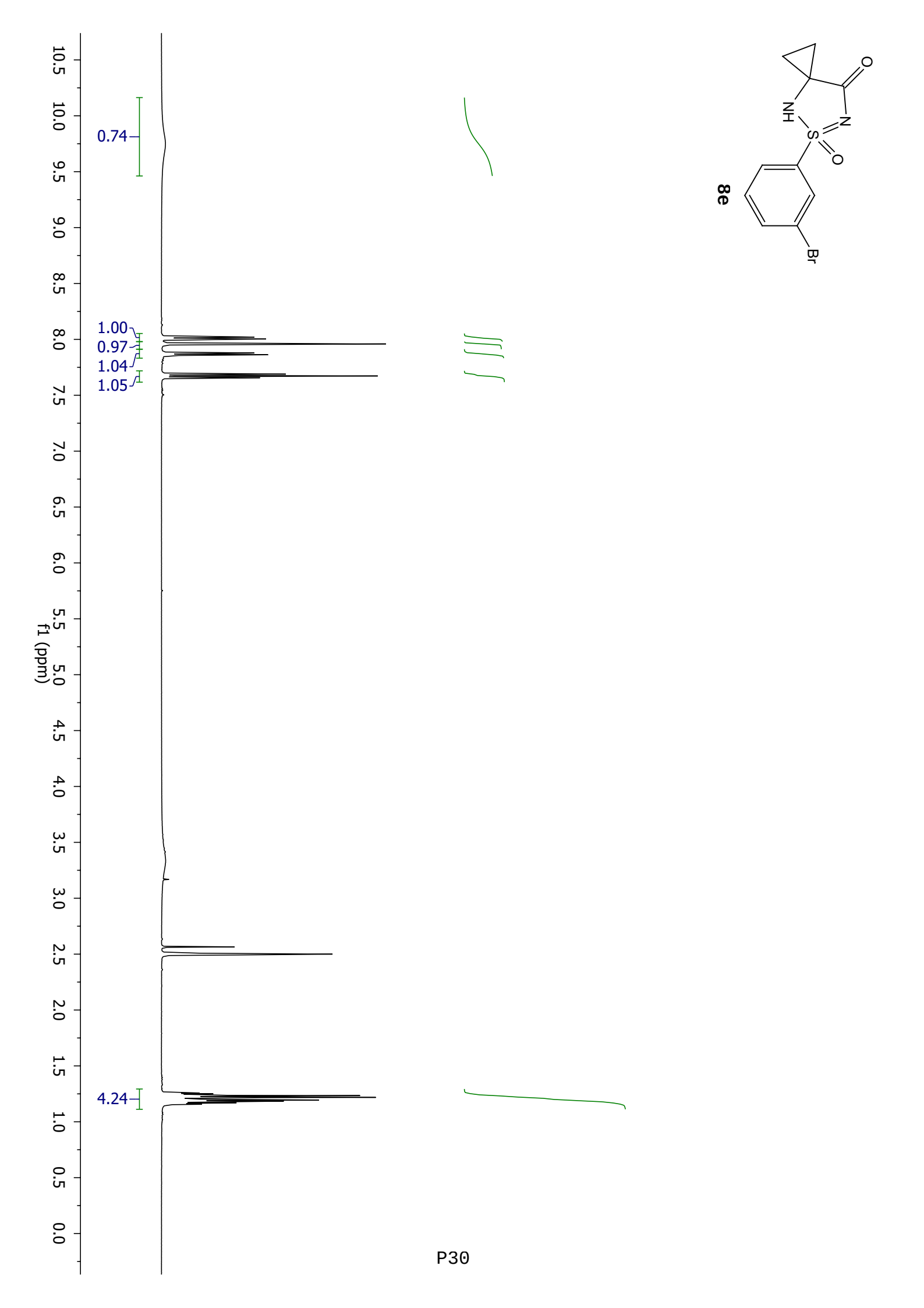
(s) 27.62

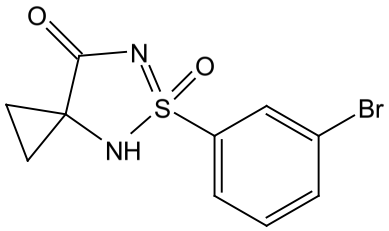
(s) 25.55

P29

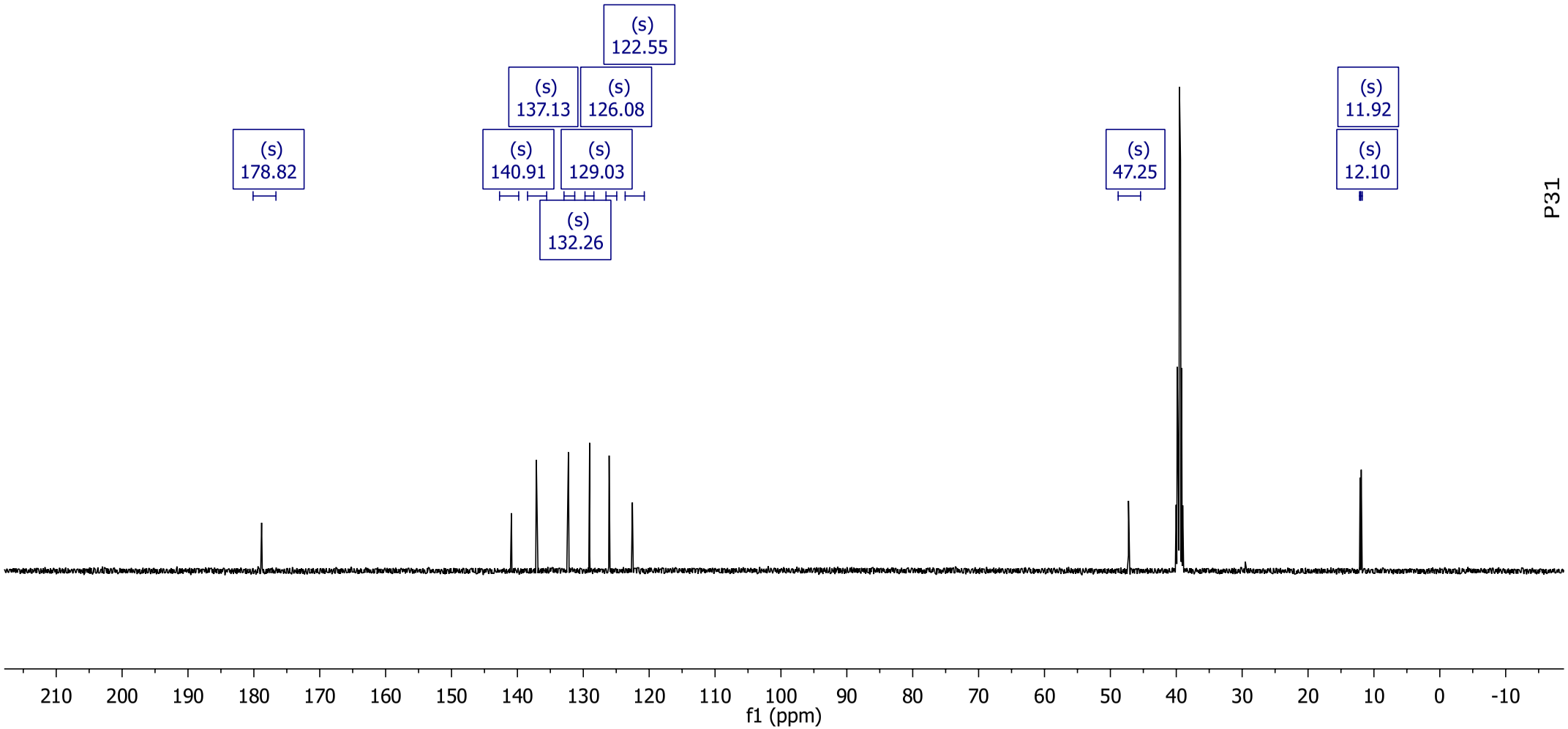
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

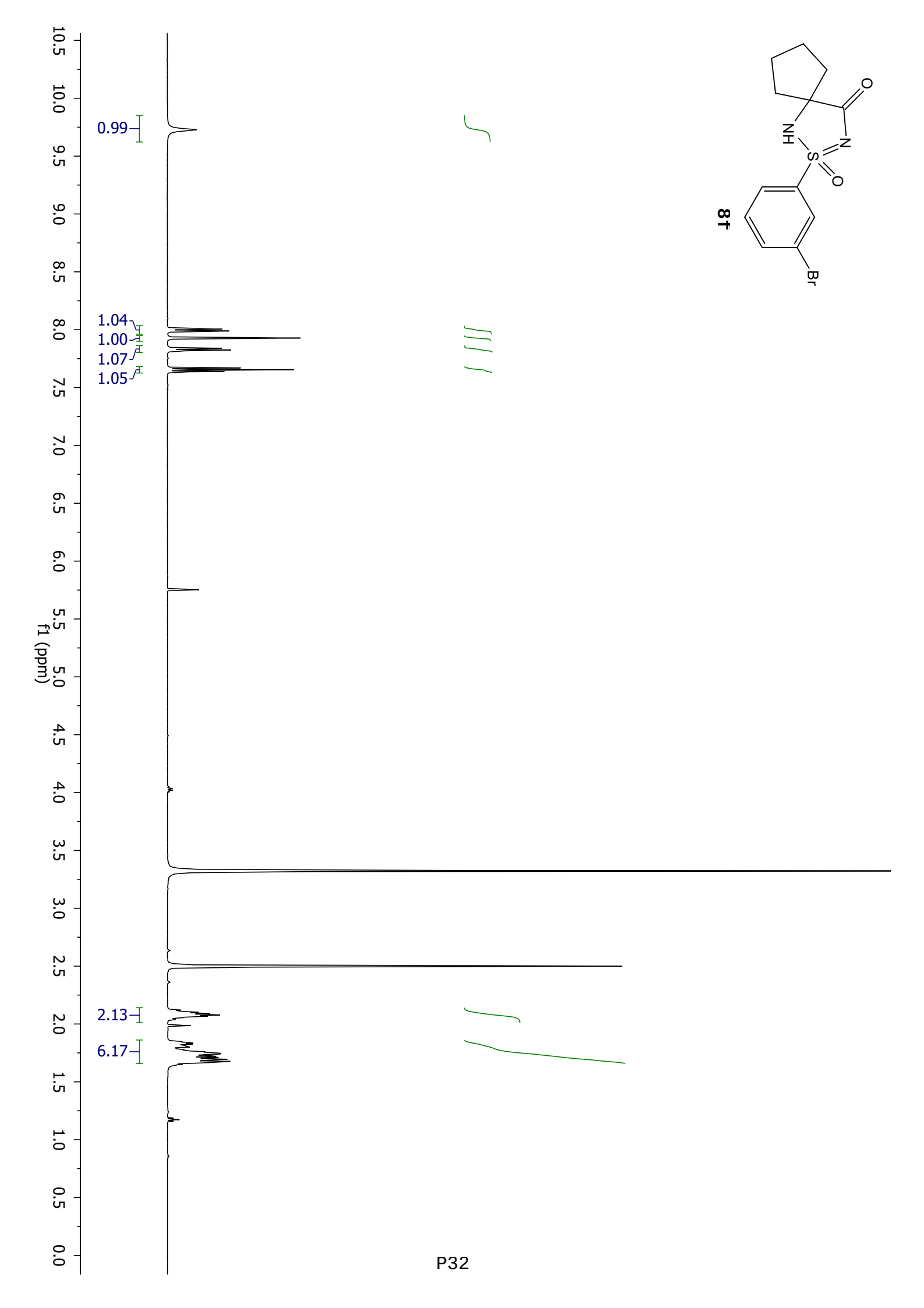
f1 (ppm)

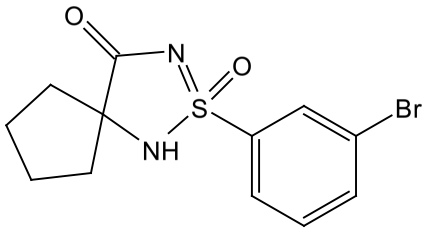

0.74
1.00
0.97
1.04
1.05
4.24
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
O
NH
N
S
O
Br
8e


O
N
O
S
Br
NH

8e


(s)
178.82
(s)
140.91
(s)
137.13
(s)
132.26
(s)
129.03
(s)
126.08
(s)
122.55
(s)
47.25
(s)
12.10
(s)
11.92
P31
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)


0.99
1.04
1.00
1.07
1.05
2.13
6.17
f1 (ppm)
NH
N
S
O
O
Br
8f

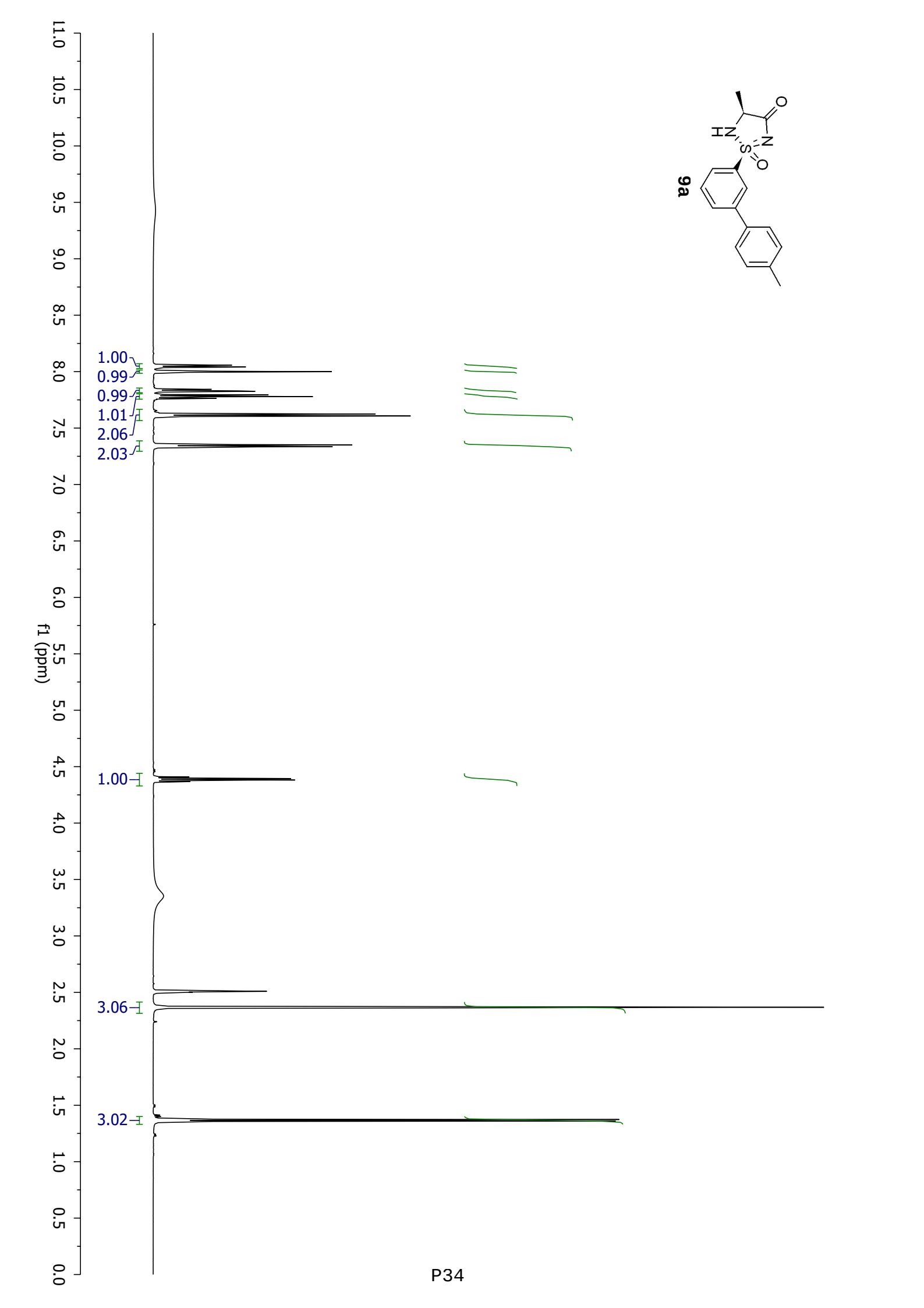


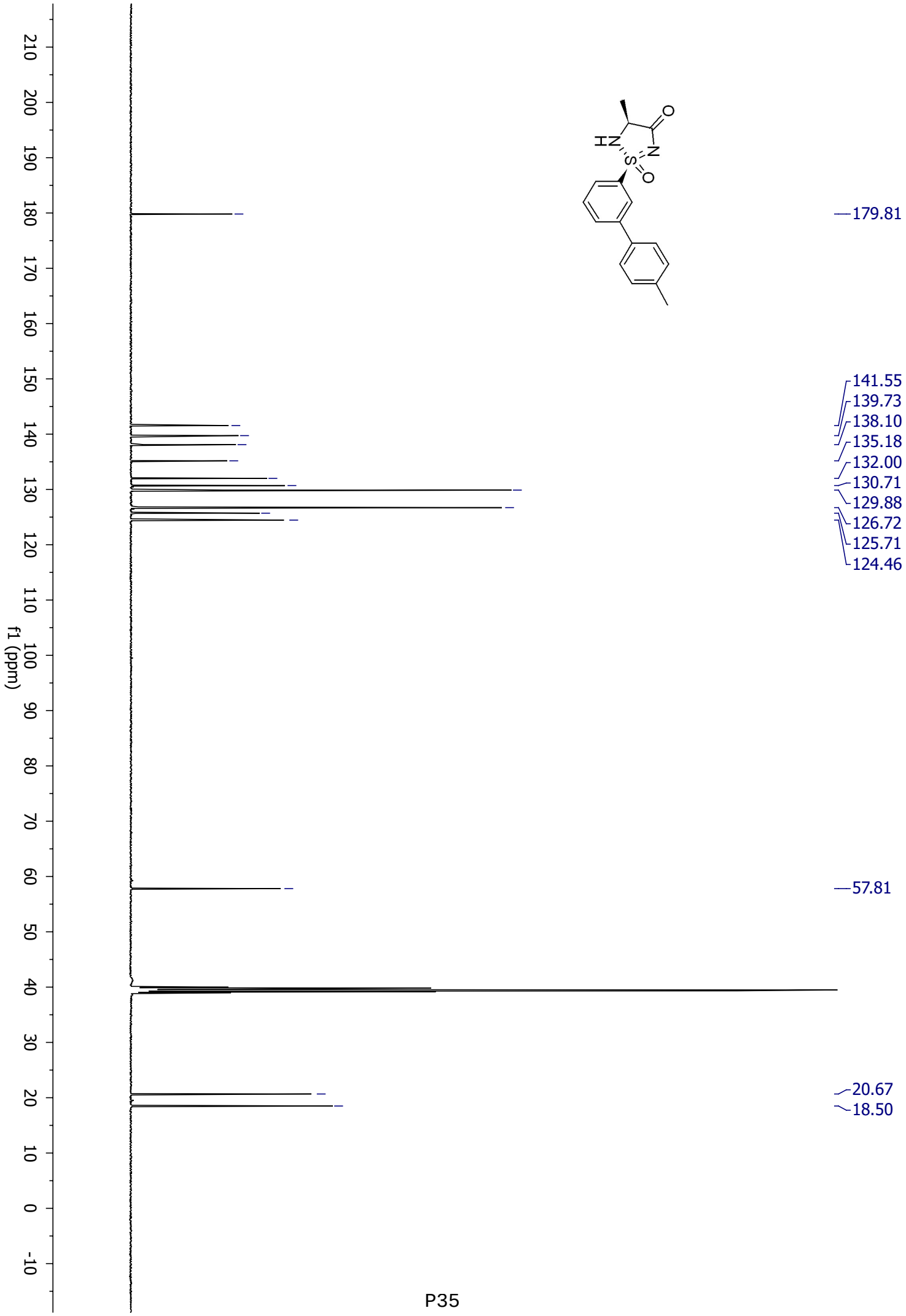
8f

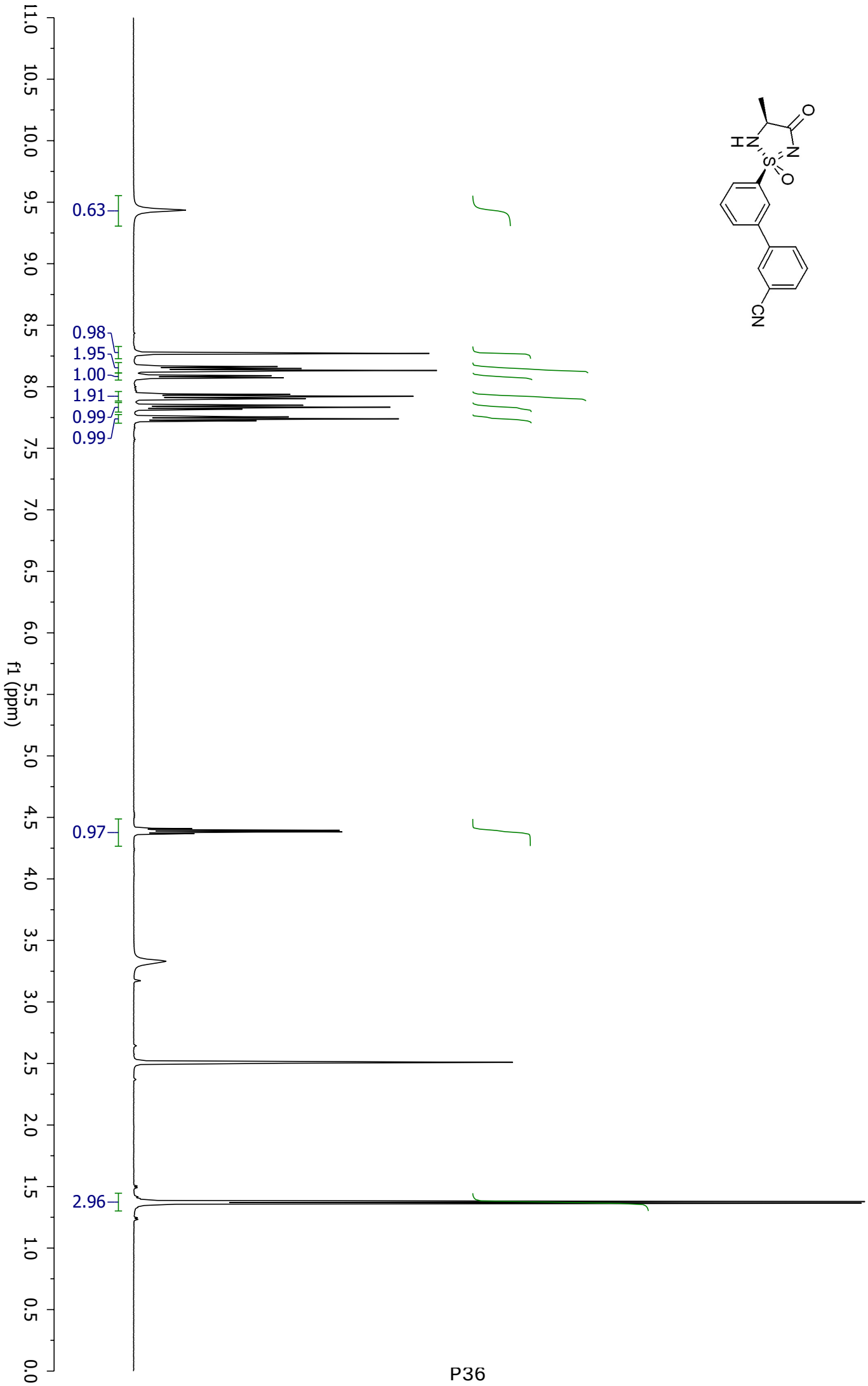
(s) 182.03
(s) 141.07
(s) 136.93
(s) 132.24
(s) 129.19
(s) 126.17
(s) 122.45
(s) 74.78
(s) 38.00
(s) 24.85
(s) 24.80

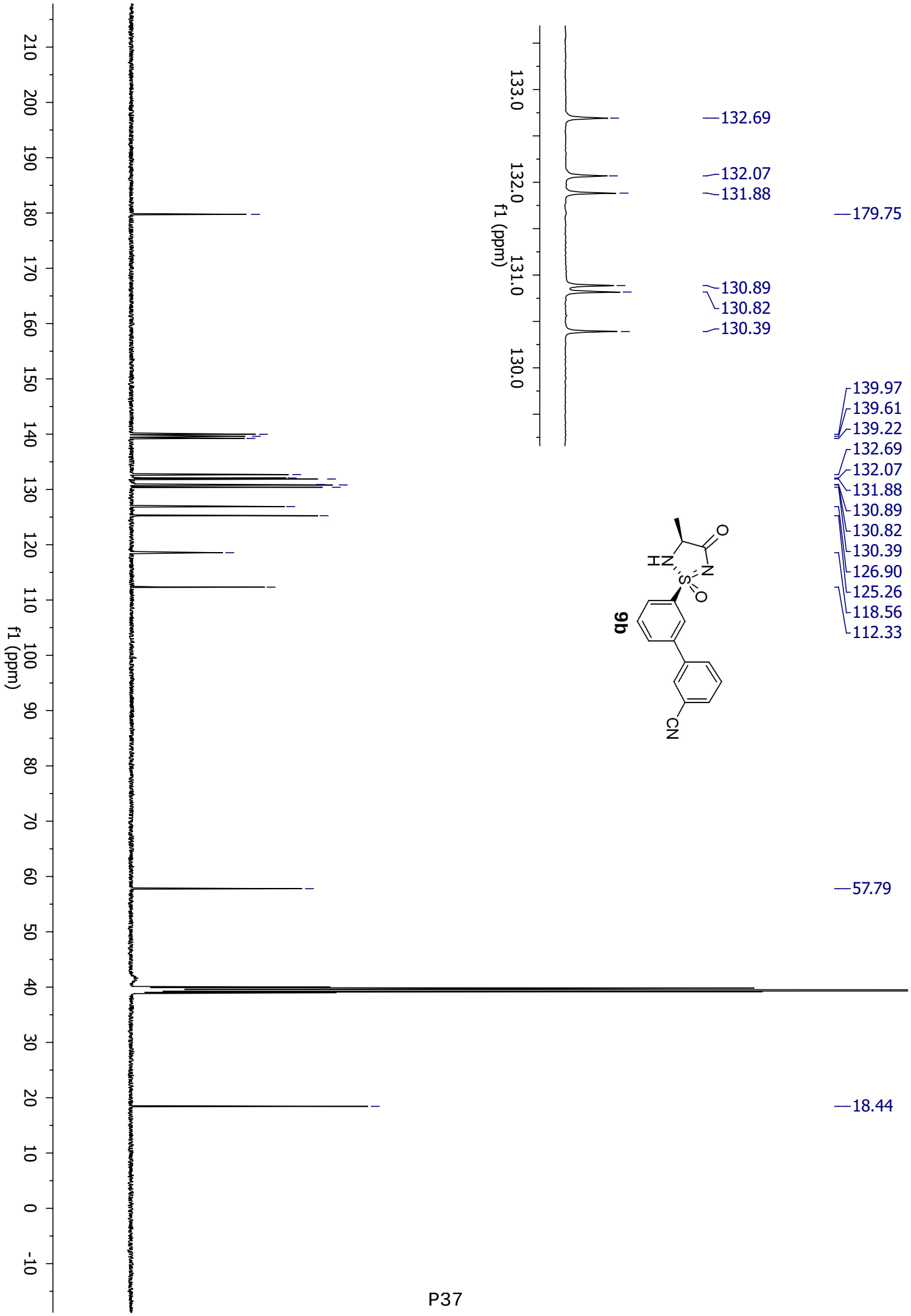
P33

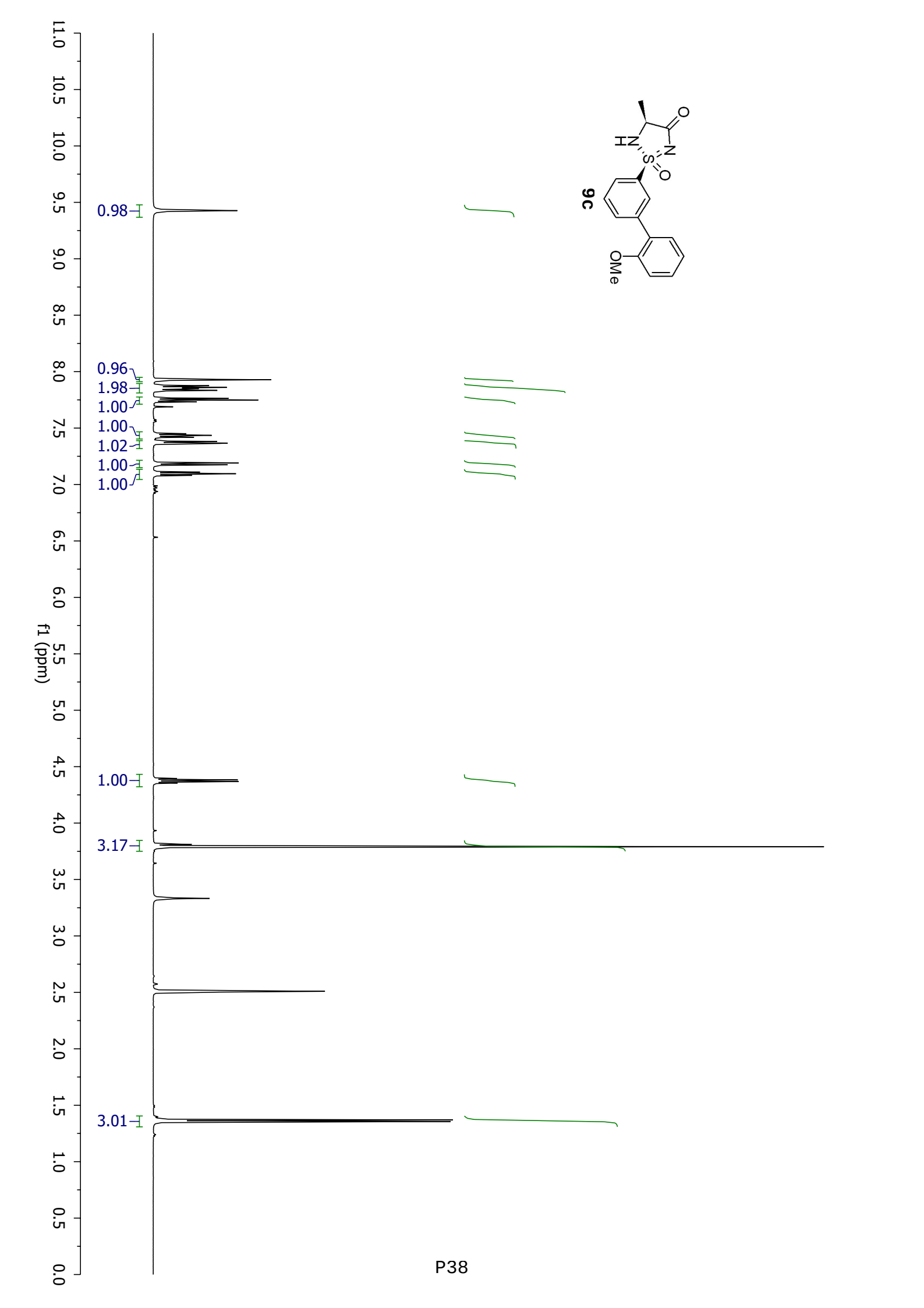
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

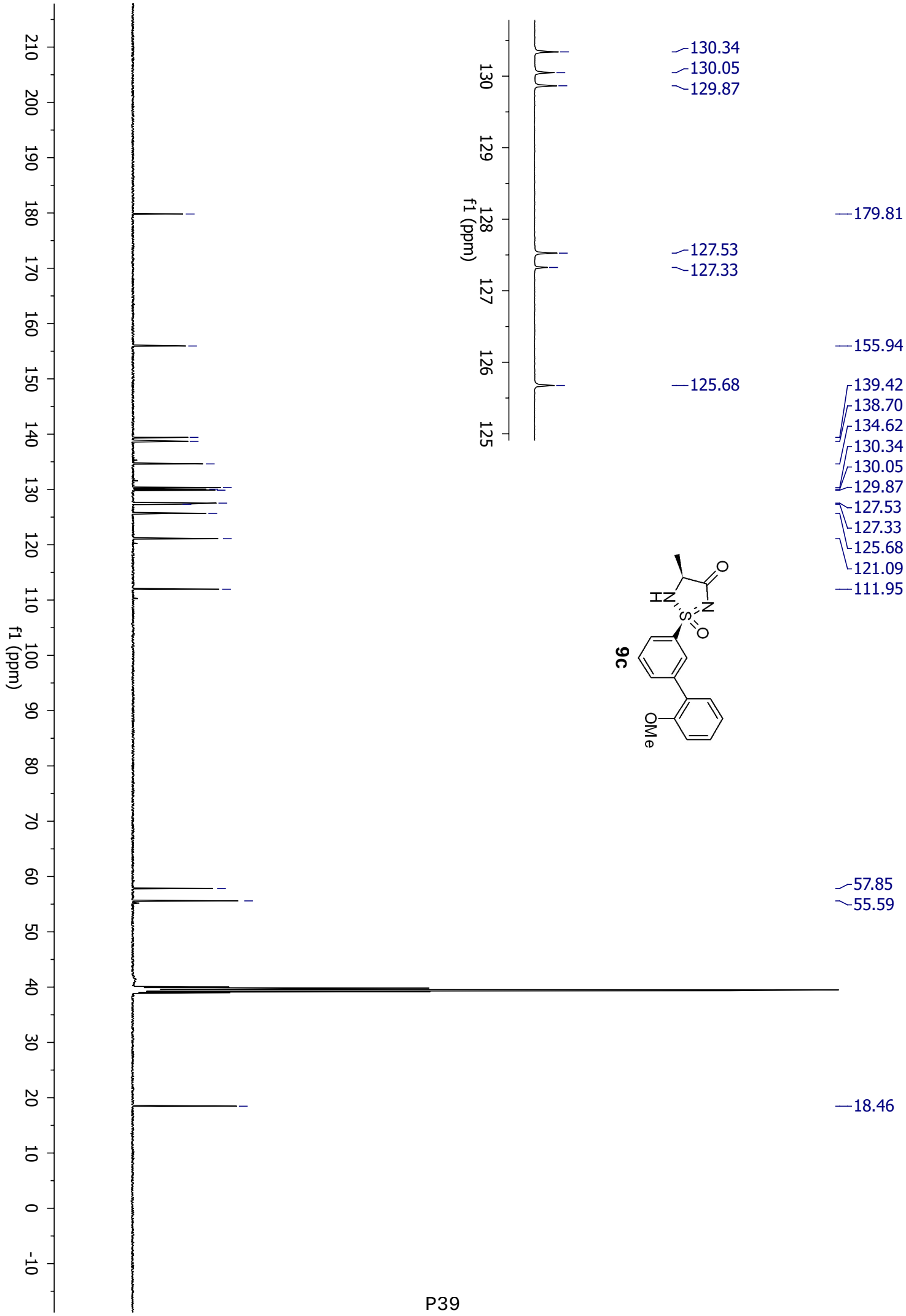

11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
1.00
0.99
0.99
1.01
2.06
2.03
1.00
3.06
3.02
O
N
H
S
O
N
9a

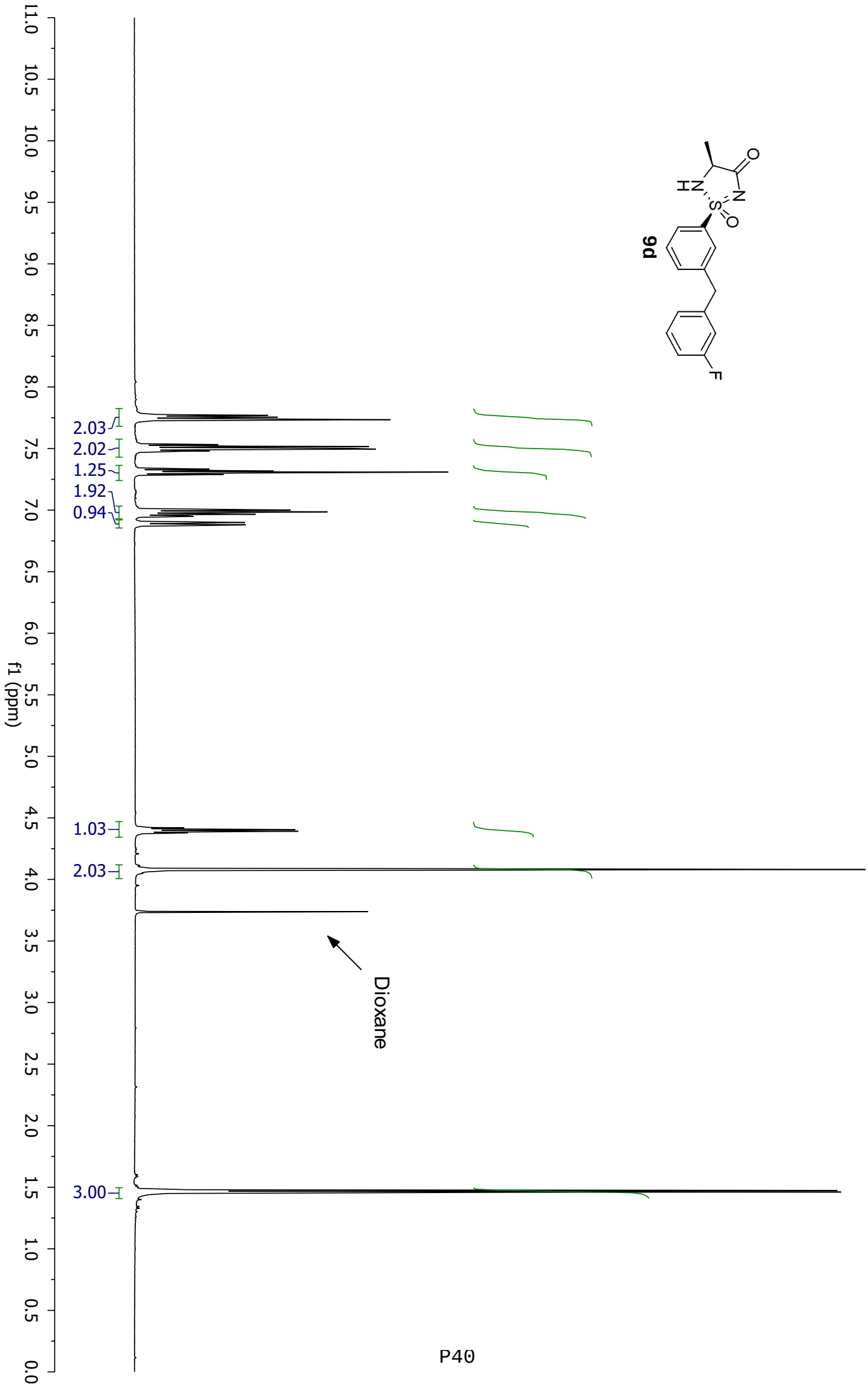

179.81
141.55
139.73
138.10
135.18
132.00
130.71
129.88
126.72
125.71
124.46
57.81
20.67
18.50
f1 (ppm)

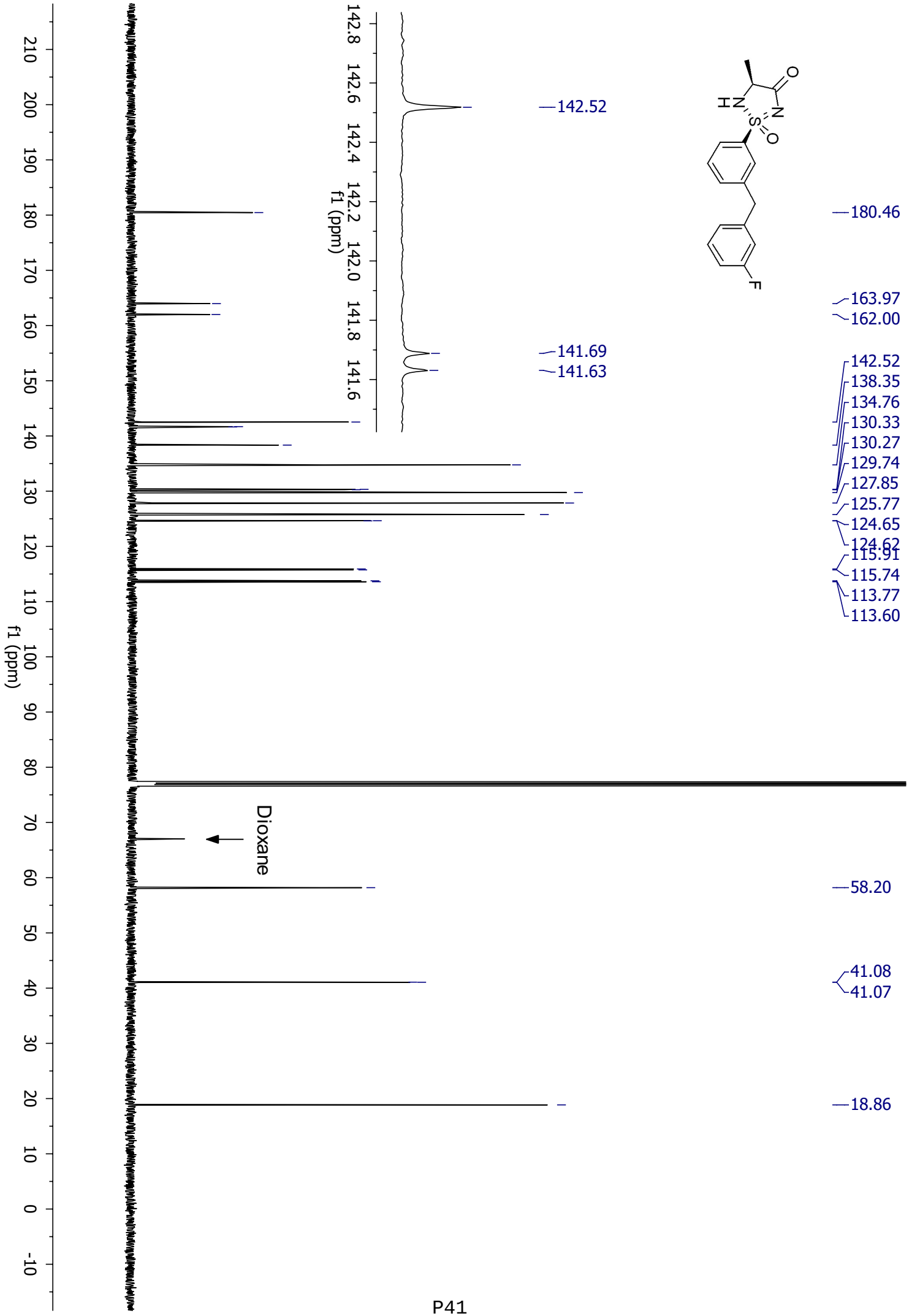

0.63
0.98
1.95
1.00
1.91
0.99
0.99
0.97
2.96
f1 (ppm)
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
O
N
H
S
O
N
CN

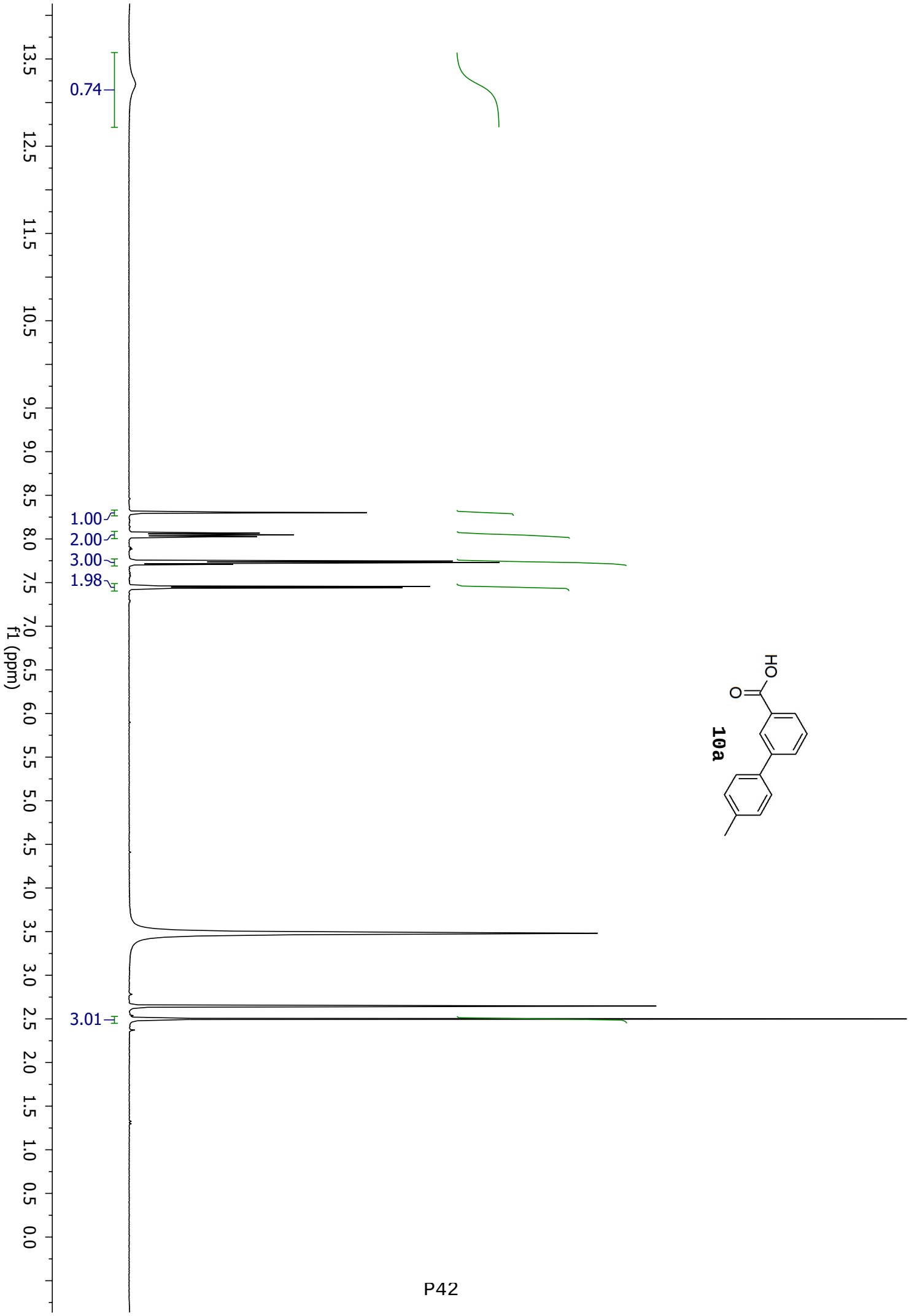

f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
133.0
132.0
131.0
130.0
132.69
132.07
131.88
130.89
130.82
130.39
179.75
139.97
139.61
139.22
132.69
132.07
131.88
130.89
130.82
130.39
126.90
125.26
118.56
112.33
57.79
18.44
9b
O
N
H
S
O
N
CN

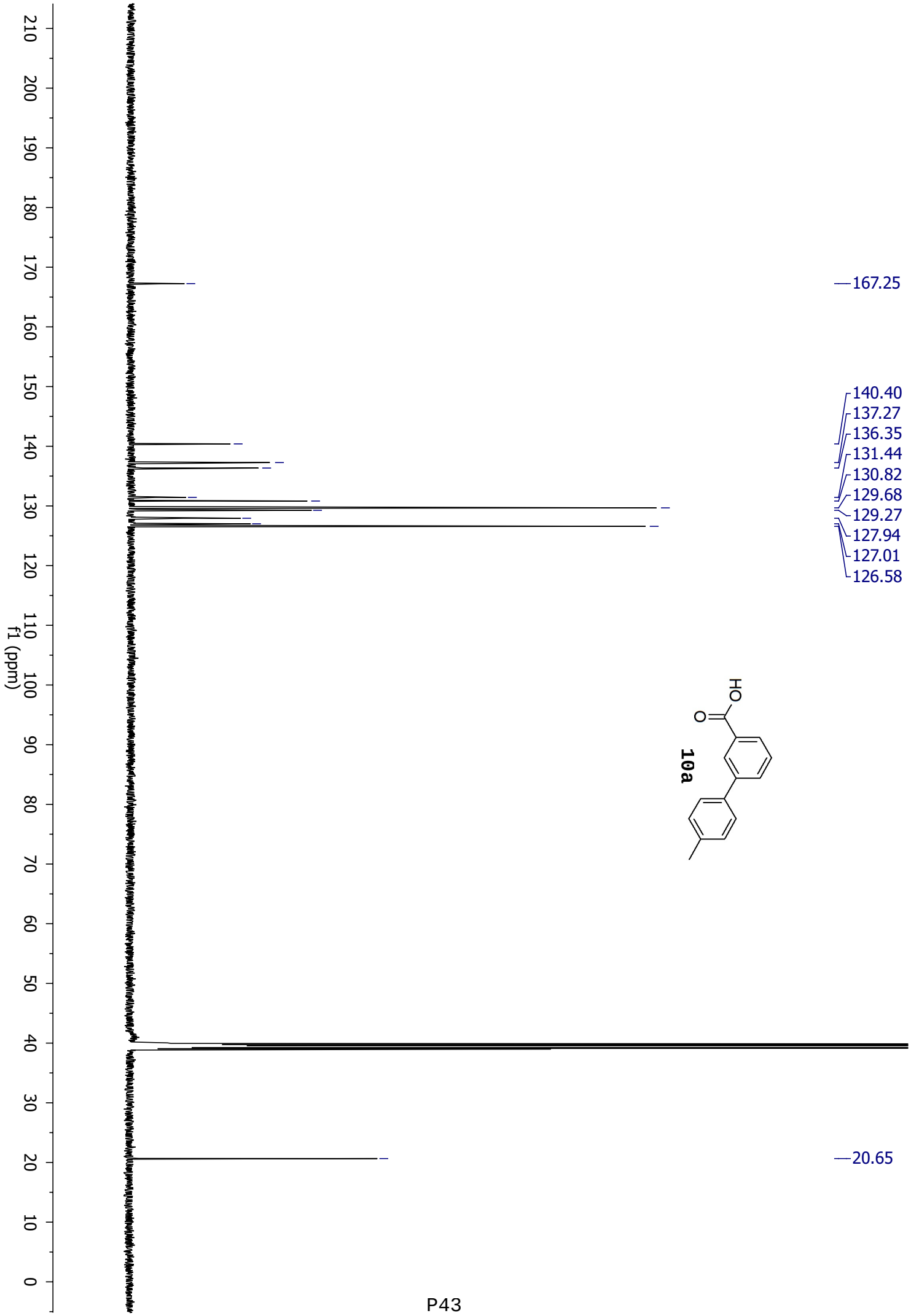

0.98
0.96
1.98
1.00
1.00
1.02
1.00
1.00
1.00
3.17
3.01
f1 (ppm)
9c
OMe

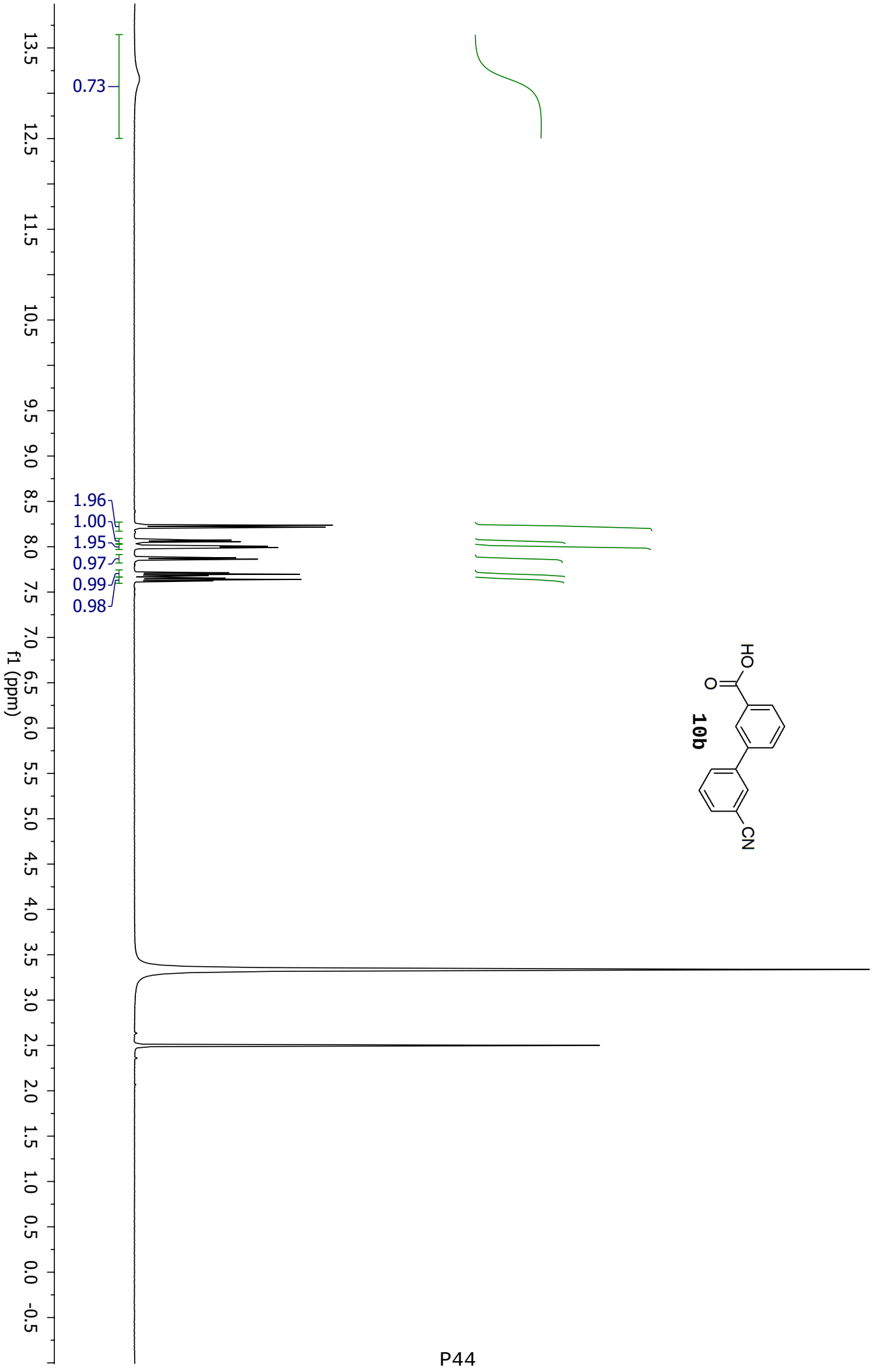

130.34
130.05
129.87
127.53
127.33
125.68
179.81
155.94
139.42
138.70
134.62
130.34
130.05
129.87
127.53
127.33
125.68
121.09
111.95
57.85
55.59
18.46
9c
OMe
f1 (ppm)

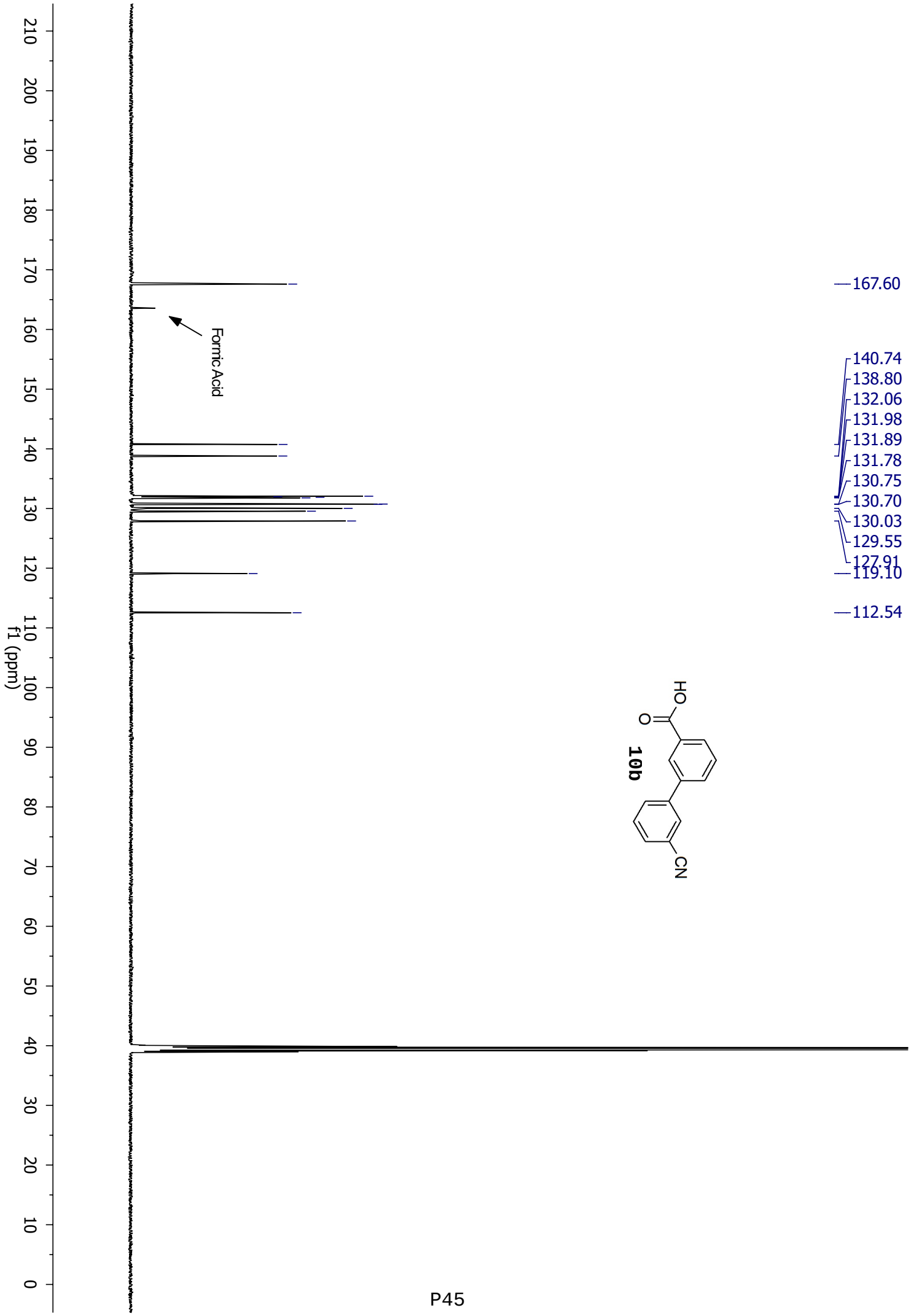

9d
2.03
2.02
1.25
1.92
0.94
1.03
2.03
3.00
Dioxane
f1 (ppm)

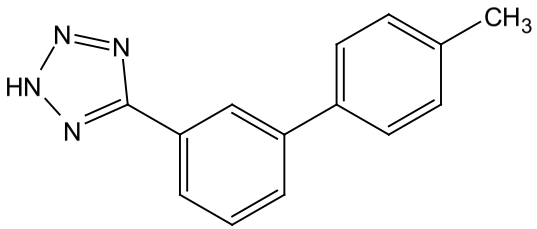

f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Dioxane
142.52
141.69
141.63
180.46
163.97
162.00
142.52
138.35
134.76
130.33
130.27
129.74
127.85
125.77
124.65
124.62
115.91
115.74
113.77
113.60
58.20
41.08
41.07
18.86
142.8
142.6
142.4
142.2
142.0
141.8
141.6
f1 (ppm)
O
N
H
S
O
N
O
F


0.74
1.00
2.00
3.00
1.98
3.01
f1 (ppm)
HO
O
10a

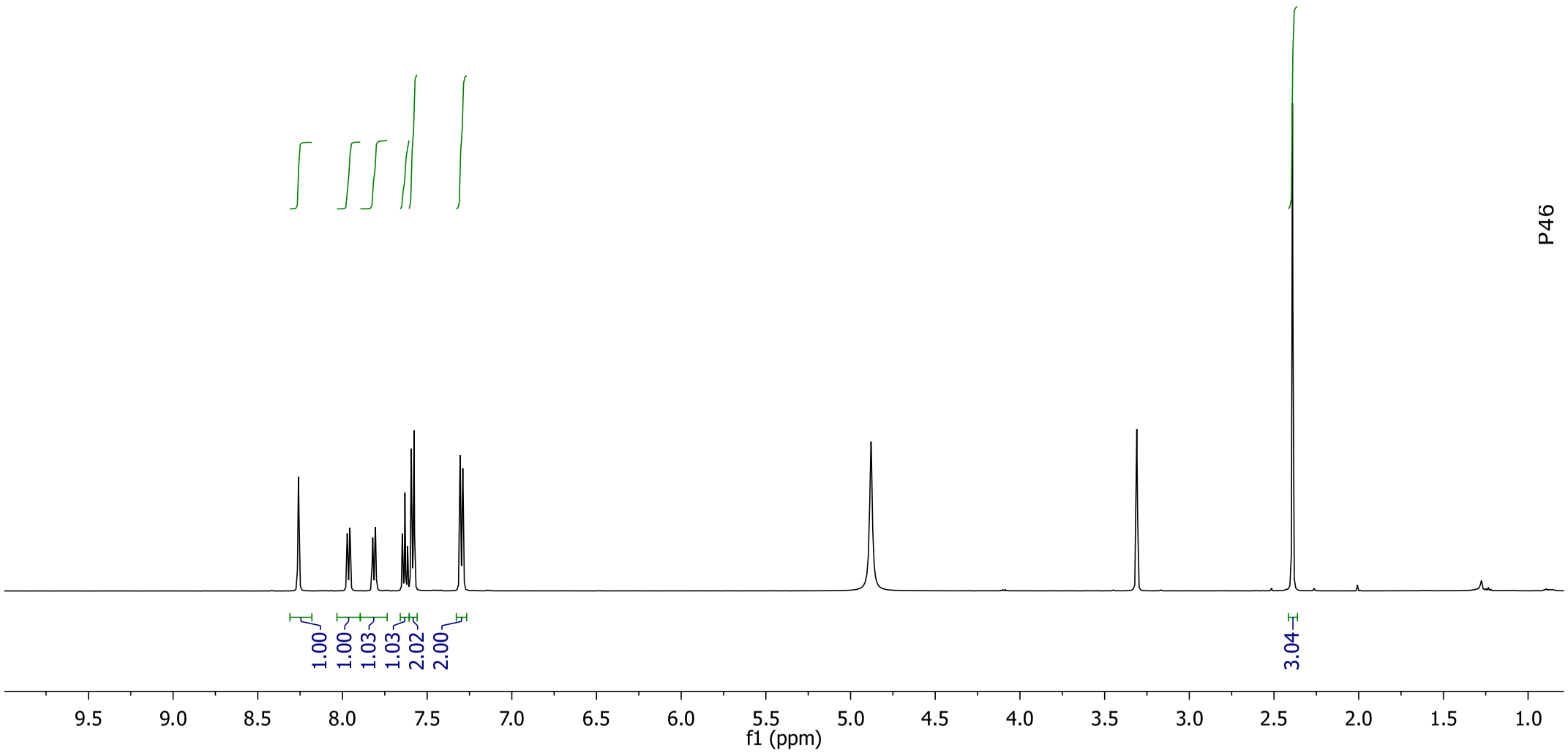

167.25
140.40
137.27
136.35
131.44
130.82
129.68
129.27
127.94
127.01
126.58
20.65
HO
O
10a
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

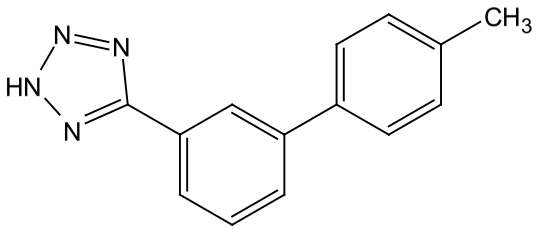

0.73
1.96
1.00
1.95
0.97
0.99
0.98
f1 (ppm)
HO
O
10b
CN


167.60
140.74
138.80
132.06
131.98
131.89
131.78
130.75
130.70
130.03
129.55
127.91
119.10
112.54
Formic Acid
HO
O
CN
10p
f1 (ppm)


N
N
HN
N
CH3

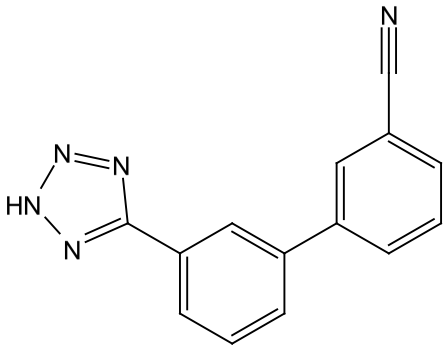
10c


P46
1.00
1.00
1.03
1.03
2.02
2.00
3.04
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
f1 (ppm)


N
N
HN
N
CH3

10c

(s)
126.45
(s)
130.74
(s)
139.14
(s)
130.83
(s)
157.52
(s)
143.74
(s)
131.03
(s)
138.19
(s)
127.90
(s)
125.65
(s)
125.86
L (s)
21.14
P47
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



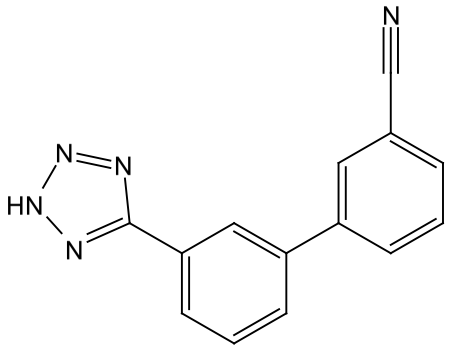
10d

P48

1.00 2.01 1.06 1.03 1.00 2.11

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

f1 (ppm)



10d

(s) 126.71
(s) 127.83
(s) 131.15
(s) 132.34
(s) 114.07
(s) 132.44
(s) 119.22
(s) 141.97
(s) 131.44
(s) 140.73
(s) 130.56
(s) 126.61

P49

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)