Supporting Information

for

Carbocyclic substrate analogs reveal kanosamine biosynthesis begins with the α-anomer of glucose 6-phosphate

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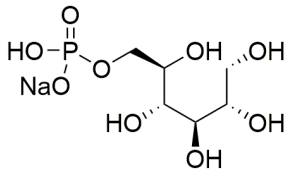
Complete experimental methods, supplementary figures, and NMR spectra

Compounds in this manuscript



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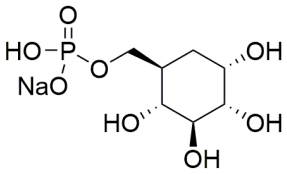

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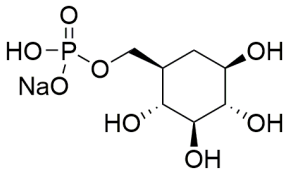

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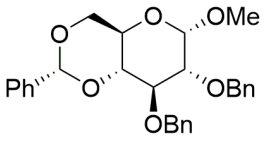

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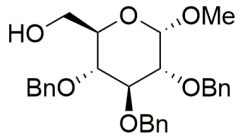

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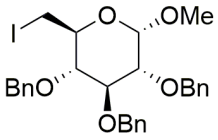

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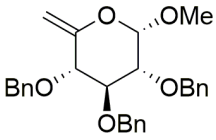

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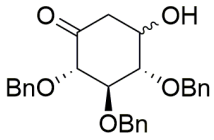

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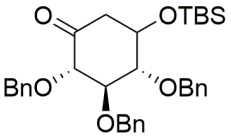

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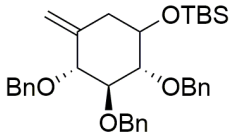

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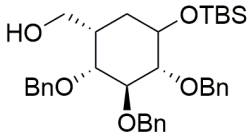

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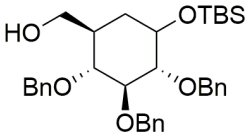

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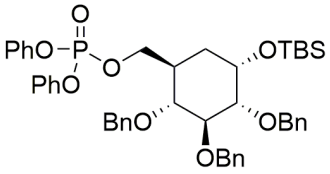

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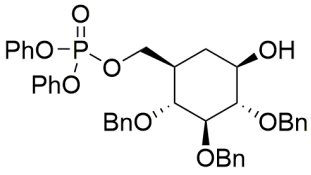

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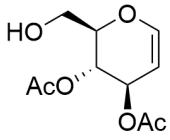

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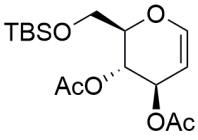

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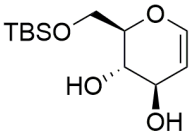

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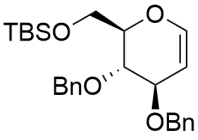

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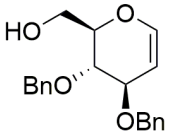

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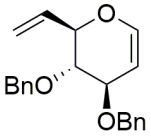

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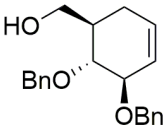

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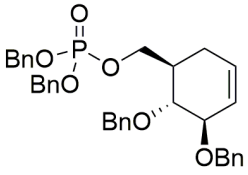

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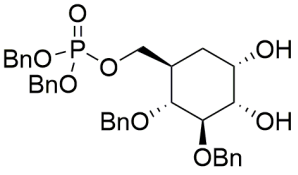

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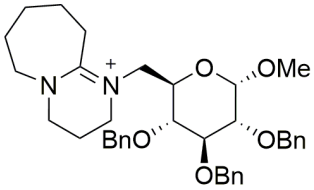
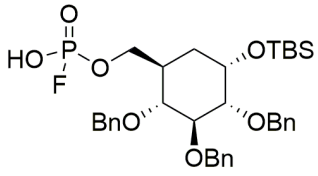


S27



S28

Experimental Methods

Lipase from *Candida rugosa* (CRL) type VII was purchased from Sigma-Aldrich (Oakville, ON) as a lyophilized powder (700 U/mg); tri-*O*-acetyl D-glucal and diphenyl ether were purchased from Fisher Scientific Company (Ottawa, ON); tetrabenzyl pyrophosphate and potassium osmate were purchased from AK Scientific, Inc. (Union City, CA). All other chemicals were obtained from Sigma-Aldrich (Oakville, ON) unless otherwise stated, and were of reagent grade or higher. Deionized distilled water was purified using a Barnstead NANOpure® DIamond™ (UV/UF) Ultrapure Water system at 18.2 MΩ. UV experiments were carried out on an Agilent 8453 UV-vis spectrophotometer, equipped with a multi-cell transport unit and circulating water bath; data was collected using Agilent ChemStation Plus version 8.0. NMR experiments, ^{1}H (500 MHz), ^{13}C{^{1}H}-DEPTQ (125 MHz), ^{31}P (202 MHz) and ^{19}F (470 MHz), were recorded on a 500 MHz Bruker Avance NMR spectrometer and are reported in ppm relative to their residual solvent signals. The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Sorbitol 6-phosphate (1)

To a solution of G6P (0.21 g, 0.70 mmol) in H_2O (4.5 ml) was added $NaBH_4$ (66 mg, 1.8 mmol) and the reaction stirred at room temperature for 1 hour. The reaction was quenched with the addition of 1 M AcOH (1-2 ml) until the pH reached 7.5. The resulting solution was applied to a column of anion exchange resin (Bio-Rad AG1-X8, acetate form, 15 mL bed volume), washed successively with 30 mL of H_2O and 15 mL of 0.1 M CH_3COONa (pH 6.0), and eluted with 30 mL each of 0.2, 0.4, 0.6, and 1.0 M CH_3COONa (pH 6.0). Fractions (~7 mL each) were collected, freeze-dried, and tested by ^{1}H NMR. Fractions containing pure S6P were pooled and dissolved in 50 mL of H_2O. Cation exchange resin (Bio-Rad AG50W-X2, H^+ form) was added with gentle stirring until the pH reached 2.0−2.5. The solution was filtered and freeze-dried to afford 0.20 g (98%) S6P as a white sticky solid. Contaminating borate salts were further removed by trituration in MeOH at 55 °C while under a mild vacuum, and the resulting solids filtered and dried to give 0.16 g (80%) pure S6P as a sticky white solid.

^{1}H ($CDCl_3$, δ): 3.93 (ddd, 1H, *J* = 2.7, 5.7, 11.1 Hz), 3.86 (ddd, 1H, *J* = 5.1, 6.3, 11.1 Hz), 3.77-3.67 (m, 3H), 3.65-3.55 (m, 2H), 3.53-3.44 (m, 1H). ^{13}C: 72.9, 10.1, 69.7 (d, $J_{C\text{-}P}$ = 8.0 Hz), 69.5, 66.1 (d, $J_{C\text{-}P}$ = 5.3 Hz), 62.3. ^{31}P: 1.03. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_6H_{14}O_9Na_2P$, 307.0165; found, 307.0162.

Methyl 2,3-di-*O*-benzyl-4,6-benzylidene-α-D-glucopyranoside (5)

To a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **4** (1.50 g, 5.31 mmol) in dry DMF (15.6 mL) at 0 °C under inert atmosphere was added dry sodium hydride (0.32 g, 13.27 mmol). The solution was stirred at 0 °C for 10 min and then warmed to room temperature over 20 min, at which point benzyl bromide (1.45 mL, 12.2 mmol) was added dropwise, and stirred at room temperature for another 3.5 hrs. The resulting reaction mixture was poured into 50 mL of a 1:1 mixture of saturated $NaHCO_3$ and EtOAc, and the aqueous layer extracted 2 x 25 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and evaporated to give 2.62 g crude product as a pale yellow solid. FCC (10% EtOAc/Hexanes) afforded 2.34 g (95%) of pure product as a bright white solid.

^{1}H ($CDCl_3$, δ): 7.54-7.53 (m, 2H), 7.43-7.26 (m, 13H), 5.57 (s, 1H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.87 (d, *J* = 12.3 Hz, 1H), 4.86 (d, *J* = 11.3 Hz, 1H), 4.72 (d, *J* = 12.3 Hz, 1H), 4.61 (d, *J* = 3.6 Hz), 1H, 4.28 (dd, *J* = 4.8, 10.2 Hz), 1H, 4.07 (t, *J* = 9.2 Hz, 1H), 3.85 (ddd, *J* = 4.8, 9.4, 10.2 Hz, 1H), 3.72 (t, *J* =

10.2 Hz, 1H), 3.62 (t, *J* = 9.4 Hz, 1H), 3.58 (dd, *J* = 3.6, 9.2 Hz, 1H), 3.42 (s, 3H). ^{13}C: 138.8, 138.3, 137.5, 129.0, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 126.1, 101.4, 99.3, 82.2, 79.3, 78.7, 75.5, 73.9, 69.2, 62.4, 55.5. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{28}H_{30}O_6Na$, 485.1934; found, 485.1926.

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (6)

Regioselective reduction of the 4,6-benzylidene followed the procedure of Daragics et al.[1] Briefly, **5** (4.99 g, 10.8 mmol) was dissolved in anhydrous DCM under inert atmosphere, and BH_3·THF (25.0 mL, 25.0 mmol) and trimethylsilyl triflate (0.27 mL, 1.5 mmol) were added at room temperature. The reaction was stirred at room temperature for 1.5 hours until complete by TLC (40% EtOAc/hexanes). The reaction was quenched with triethylamine (10 mL) and the dropwise addition of MeOH until H_2 bubbling ceased. The mixture was then concentrated by rotary evaporation, and co-evaporated three times with 50 mL portions of MeOH, giving 7.54 g of crude product as a yellow oil. FCC (25% EtOAc/Hexanes) afforded 4.83 g (96%) of pure product as a bright white solid.

^{1}H ($CDCl_3$, δ): 7.40-7.26 (m, 15H), 4.99 (d, 1H, *J* = 10.9 Hz), 4.89 (d, 1H, *J* = 11.0 Hz), 4.84 (d, 1H, *J* = 10.9 Hz), 4.81 (d, 1H, *J* = 12.1 Hz), 4.67 (d, 1H, *J* = 12.1 Hz), 4.64 (d, 1H, *J* = 11.0 Hz), 4.57 (d, 1H, *J* = 3.6 Hz), 4.01 (t, 1H, *J* = 9.5 Hz), 3.77 (dd, 1H, *J* = 2.7, 11.7 Hz), 3.69 (dd, 1H, *J* = 3.9, 11.6 Hz), 3.65 (ddd, 1H, *J* = 2.7, 3.9, 9.7 Hz), 3.53 (dd, 1H, *J* = 9.5, 9.7 Hz), 3.50 (d, 1H, *J* = 3.7, 9.5 Hz), 3.37 (s, 3H). ^{13}C: 138.8, 138.2, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 128.0, 127.8, 98.3, 82.1, 80.1, 77.5, 75.9, 75.2, 73.6, 70.8, 62.0, 55.3. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{28}H_{32}O_6Na$, 487.2091; found, 487.2108.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-iodo-α-D-glucopyranoside (7)

To a solution of **6** (7.13 g, 15.3 mmol) in dry toluene (95 mL) was added PPh_3 (10.01 g, 38.1 mmol) and imidazole (5.22 g, 76.6 mmol). Iodine chips (7.79 g, 30.6 mmol) were then added, and the reaction heated to 80 °C and stirred for 45 min until complete by TLC (25% EtOAc/hexanes). The reaction was cooled to room temperature, and the toluene layer decanted from the yellow residue, which was then washed three times with 50 mL EtOAc. The combined organic fraction was evaporated to give 17.02 g of crude as a pale yellow oil. FCC (10% EtOAc/hexanes) gave 8.68 g (98%) of pure iodo product as a clear, colorless oil that solidified upon standing at -20 °C.

^{1}H ($CDCl_3$, δ): 7.40-7.27 (m, 15H), 5.00 (d, 1H, *J* = 10.8 Hz), 4.95 (d, 1H, *J* = 10.9 Hz), 4.82 (d, 1H, *J* = 10.8 Hz), 4.81 (d, 1H, *J* = 12.3 Hz), 4.69 (d, 1H, *J* = 10.9 Hz), 4.67 (d, 1H, *J* = 12.3 Hz), 4.62 (d, 1H, *J* = 3.6 Hz), 4.03 (t, 1H, *J* = 9.2 Hz), 3.55 (dd, 1H, *J* = 3.6, 9.5 Hz), 3.50-3.44 (m, 2H), 3.43 (s, 3H), 3.35 (t, 1H, *J* = 9.2 Hz), 3.32-3.27 (m, 1H). ^{13}C: 138.6, 138.1, 138.1, 128.7, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.8, 98.2, 81.7, 81.6, 80.1, 75.9, 75.5, 73.6, 69.4, 55.6, 7.8. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{28}H_{31}O_5NaI$, 597.1108; found, 597.1117.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside (8)

Method 1: To a solution of **7** (9.46 g, 16.5 mmol) in dry DMF (175 mL) was added freshly distilled DBU (3.0 ml, 20.0 mmol), and the reaction heated to 80 °C and stirred for 5 hr. Another 1.2 ml of DBU was added, and the reaction stirred at 80 °C overnight. The reaction was cooled to room temperature, diluted with 100 mL EtOAc, and washed twice with saturated $NaHCO_3$. The aqueous layer was then extracted 3 x 100 ml EtOAc, and the combined organic fraction was washed 2 x 100 ml H_2O, dried over Na_2SO_4, filtered, and evaporated to give 13.5 g of crude as a yellow oil. FCC (40%

EtOAc/hexanes) gave 4.0 g (54%) of product as a pale yellow oil that showed a mixture of exo- and endo-alkene by ^{1}H NMR.

Method 2: To a solution of **7** (2.27 g, 3.96 mmol) in dry THF (100 ml) was added potassium *tert*-butoxide (1.33 g, 11.88 mmol) in one portion. A suspension formed and the reaction was stirred for 1 hour at room temperature until complete by TLC (5% EtOAc/Hexanes, repeat 3×). The suspension was filtered and the yellow cake washed with EtOAc (50 ml). The combined organic layer was washed with saturated NH_4Cl (50 ml) and brine (50 ml), dried over $MgSO_4$, filtered, and evaporated to give 2.18 g of crude product as a yellow oil. FCC (20% EtOAc/Hexanes) gave 1.76 g (99%) of pure 5-exo-methylene product as a clear, colorless oil.

^{1}H ($CDCl_3$, δ): 7.45-7.31 (m, 15H), 4.98-4.91 (m, 3H), 4.88 (d, 1H, *J* = 12.1 Hz), 4.85 (d, 1H, *J* = 11.4 Hz), 4.82 (d, 1H, *J* = 11.4 Hz), 4.78-4.75 (m, 1H), 4.73 (d, 1H, *J* = 12.1 Hz), 4.68 (d, 1H, *J* = 3.6 Hz), 4.03 (t, 1H, *J* = 9.2 Hz), 3.97 (dt, 1H, *J* = 1.9, 9.1 Hz), 3.66 (dd, 1H, *J* = 3.4, 9.3 Hz), 3.48 (s, 3H). ^{13}C: 153.7, 138.7, 138.1, 138.0, 128.51, 128.45, 128.4, 128.13, 128.07, 128.0, 127.9, 127.8, 127.7, 99.1, 96.9, 81.2, 79.6, 79.3, 75.8, 74.5, 73.6, 55.5. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{28}H_{30}O_5Na$, 469.199; found, 469.1988.

(2*S*,3*R*,4*S*,5*S*)- and (2*S*,3*R*,4*S*,5*R*)-2,3,4-tri-*O*-Benzyl-5-hydroxycyclohexanone (10)

To a solution of **8** (4.00 g, 9.00 mmol) in 1,4-dioxane/water (2:1, 60 ml) under inert atmosphere was added $PdCl_2$ (0.32 g, 1.8 mmol), and the mixture stirred at 60 °C for 3 hours. The reaction was cooled to room temperature, and diluted with EtOAc (50 ml). The organic layer was then washed with sat. $NaHCO_3$ (2 x 30 ml), H_2O (2 x 30 ml), and brine (30 ml), dried over Na_2SO_4, filtered and evaporated to give 4.60 g of crude product as a yellow waxy solid. FCC (20% EtOAc/hexanes) afforded 2.9 g (75%) of the ketone product as a 3:1 (α:β) mixture of isomers.

^{1}H ($CDCl_3$, δ): 7.42-7.27 (m, 30H), 5.03 (d, 1H, *J* = 11.3 Hz, β), 4.98-4.90 (m, 4H), 4.81 (d, 1H, *J* = 11.6 Hz, α), 4.79 (d, 1H, *J* = 11.6 Hz, α), 4.76 (d, 1H, *J* = 11.0 Hz, β), 4.72 (d, 1H, *J* = 11.6 Hz, α), 4.70 (d, 1H, *J* = 11.6 Hz, β), 4.56 (d, 1H, *J* = 11.6 Hz, α), 4.55 (d, 1H, *J* = 11.3 Hz, β), 4.24 (q, 1H, *J* = 3.3 Hz, α), 4.21-4.16 (m, 1H, β), 4.07-4.00 (m, 2H, α), 3.82-3.77 (m, 1H, α), 3.73-3.64 (m, 3H, β), 2.77 (dd, 1H, *J* = 4.5, 13.8 Hz, β), 2.68 (dd, 1H, *J* = 3.8, 14.6 Hz, α), 2.57-2.46 (m, 1H, β), 2.44 (dd, 1H, *J* = 3.2, 14.6 Hz, α). ^{13}C: 203.8 (α), 203.2 (β), 138.4 (α), 138.1 (β), 138.0 (β), 137.73 (α), 137.68 (α), 137.4 (β), 128.7, 128.6, 128.50, 128.48, 128.41, 128.39, 128.21, 128.16, 128.13, 128.12, 127.10, 127.99, 127.93, 127.89, 127.8, 127.7, 86.0 (β), 85.3 (α), 84.7 (β), 82.0 (β), 81.7 (α), 81.5 (α), 76.0 (α), 75.7 (β), 75.5 (β), 73.7 (β), 73.5 (α), 73.2 (α), 68.1 (β), 66.6 (α), 44.2 (β), 42.6 (α). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{27}H_{28}O_5Na$, 455.1828; found, 455.1818.

(2*S*,3*R*,4*S*,5*S*)- and (2*S*,3*R*,4*S*,5*R*)-2,3,4-tri-*O*-Benzyl-5-*tert*-butyldimethylsilyloxycyclohexanone (11a and 11b)

To a solution of **10** (2.7 g, 6.2 mmol) in dry DMF (54 ml) was added TBSCl (1.88 g, 12.5 mmol) and imidazole (0.85 g, 12.5 mmol). The mixture was heated to 50 °C and stirred under inert atmosphere for 20 hrs. The reaction was cooled to room temperature, diluted with EtOAc (100 mL), extracted with sat. aq. $NaHCO_3$ (3 x 25 mL), water (2 x 20 mL), dried over Na_2SO_4, filtered and evaporated to give 5.0 g of crude product as a yellow oil. FCC (10% EtOAc/Hexanes) gave 1.29 g (38%) of **11b** as a pale yellow oil and 1.67 g (49%) of **11a** as a white solid.

11a: ^{1}H ($CDCl_3$, δ): 7.45-7.25 (m, 15H), 4.96 (d, 1H, *J* = 11.6 Hz), 4.90 (d, 1H, *J* = 10.9 Hz), 4.85 (d, 1H, *J* = 10.9 Hz), 4.78-4.75 (m, 2H), 4.60 (d, 1H, *J* = 11.7 Hz), 4.33-4.29 (m, 1H), 4.09 (t, 1H, *J* = 9.0 Hz), 4.03 (d, 1H, *J* = 9.2 Hz), 3.70 (dd, 1H, *J* = 2.1, 8.7 Hz), 2.51 (dd, 1H, *J* = 4.0, 14.2 Hz), 2.44 (dd, 1H, *J* = 2.5, 14.2 Hz), 0.88 (s, 9H), 0.11 (d, 6H, *J* = 3.4 Hz). ^{13}C: 203.7, 138.3, 137.9, 128.6, 128.4, 128.34, 128.29, 128.28, 128.1, 127.71, 127.68, 127.6, 127.5, 127.4, 85.7, 82.2, 81.8, 75.7, 73.4, 73.0, 67.8, 45.1, 25.7, -4.7 (d, $J_{C\text{-}Si}$ = 66.9 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{33}H_{42}O_5NaSi$, 569.2693; found, 569.2694.

11b: ^{1}H ($CDCl_3$, δ): 7.40-7.20 (m, 15H), 4.89 (d, 1H, *J* = 11.9 Hz), 4.86 (d, 1H, *J* = 11.5 Hz), 4.78 (d, 1H, *J* = 11.0 Hz), 4.59 (d, 1H, *J* = 11.9 Hz), 4.54 (d, 1H, *J* = 12.3 Hz), 4.47 (d, 1H, *J* = 12.1 Hz), 4.40 (br. d, 1H, *J* = 3.2 Hz), 4.40-4.35 (m, 1H), 3.98 (dd, 1H, *J* = 3.6, 5.0 Hz), 3.82-3.78 (m, 1H), 2.82 (dd, 1H, *J* = 11.2, 13.0 Hz), 2.59 (ddd, 1H, *J* = 1.2, 5.0, 13.2 Hz), 0.91 (s, 9H), 0.08 (d, 6H, *J* = 9.8 Hz). ^{13}C NMR: 205.0, 138.5, 138.2, 137.9, 128.39, 128.35, 128.32, 127.8, 127.7, 127.6, 127.5, 81.3, 78.6, 78.3, 74.0, 73.5, 72.5, 69.8, 60.4, 45.3, 25.8, -4.7 (d, $J_{C\text{-}Si}$ = 19.1 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{33}H_{42}O_5NaSi$, 569.2693; found, 569.2683.

(2*S*,3*R*,4*S*,5*S*)- and (2*S*,3*R*,4*S*,5*R*)-2,3,4-tri-*O*-Benzyl-5-*tert*-butyldimethylsilyloxy-1-methylenecyclohexane (12a and 12b)

A solution of **11a** or **11b** (1 mmol) and pyridine (0.9 ml) in dry THF (5.1 ml) was cooled to -40 °C, and Tebbe's reagent (0.5 M solution in toluene, 2.5 mmol) was added dropwise over 10 minutes. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was cooled back to -40 °C, saturated $NaHCO_3$ (5 ml) was added, and the mixture stirred at room temperature for 30 min. The mixture was filtered through a short pad of celite, and solvents evaporated to give crude product as a bright yellow oil. Products were purified by FCC (5% EtOAc/Hexanes). From 2.55 g **11a**, obtained 2.18 g product (95% based on recovered starting material); from 1.22 g **11b**, obtained 0.98 g product (87% based on recovered starting material).

12a: ^{1}H ($CDCl_3$, δ): 7.49-7.30 (m, 15H), 5.36-5.33 (m, 1H), 4.98-4.95 (m, 1H), 4.93 (d, 1H, *J* = 10.7 Hz), 4.90 (d, 1H, *J* = 10.7 Hz), 4.82 (d, 1H, *J* = 11.5 Hz), 4.79 (d, 1H, *J* = 11.9 Hz), 4.78 (d, 1H, *J* = 11.9 Hz), 4.75 (d, 1H, *J* = 11.5 Hz), 4.22-4.18 (m, 1H), 3.92 (br. d, 1H, *J* = 8.7 Hz), 3.88 (t, 1H, *J* = 8.5 Hz), 3.48 (dd, 1H, *J* = 2.5, 8.5 Hz), 2.44 (dd, 1H, *J* = 4.1, 13.7 Hz), 2.15 (br. d, 1H, *J* = 13.5 Hz), 0.93 (s, 9H), 0.12 (d, 6H, *J* = 4.7 Hz). ^{13}C: 141.1, 139.1, 138.9, 138.7, 128.4, 128.3, 128.23, 128.16, 128.0, 127.63, 127.62, 127.4, 127.3, 110.8, 83.4, 83.3, 83.2, 75.7, 73.9, 72.7, 68.0, 38.5, 25.9, 18.3, -4.6 (d, $J_{C\text{-}Si}$ = 46.6 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{44}O_4NaSi$, 567.2901; found, 567.2897.

12b: ^{1}H ($CDCl_3$, δ): 7.48-7.34 (m, 15H), 5.34-5.31 (m, 1H), 5.07-5.04 (m, 1H), 4.99 (d, 1H, *J* = 10.7 Hz), 4.94 (d, 1H, *J* = 11.0 Hz), 4.87 (d, 1H, *J* = 10.7 Hz), 4.82 (d, 1H, *J* = 11.3 Hz), 4.76 (d, 1H, *J* = 11.3 Hz), 4.72 (d, 1H, *J* = 12.0 Hz), 4.00 (br. d, 1H, *J* = 9.5 Hz), 3.70-3.63 (m, 1H), 3.53 (t, 1H, *J* = 9.0 Hz), 3.45 (t, 1H, *J* = 9.0 Hz), 2.59 (dd, 1H, *J* = 5.2, 13.1 Hz), 2.17 (t, 1H, *J* = 11.9 Hz), 1.00 (s, 9H), 0.17 (d, 6H, *J* = 18.3 Hz). ^{13}C: 140.6, 139.1, 139.0, 138.5, 128.5, 128.33, 128.30, 128.24, 128.23, 128.0, 127.85, 127.81, 127.7, 127.6, 127.5, 127.3, 110.1, 86.8, 85.1, 83.3, 75.9, 75.8, 73.7, 73.6, 40.3, 26.0, 18.1, -4.4 (d, $J_{C\text{-}Si}$ = 12.3 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{44}O_4NaSi$, 567.2901; found, 567.2908.

***tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-5a-carba-L-idopyranoside (α, 13a and β, 13b)**

To an ice-cooled solution of **12a** or **12b** (1 mmol) in dry THF (5.5 ml) was added BH_3·THF (1 M solution in THF, 3 mmol). The reaction was warmed to room temperature, and stirred for 2 hrs. The reaction was cooled to 0 °C, aqueous NaOH (3 M, 2.8 ml) and H_2O_2 (30%, 2.8 ml) was added, and the mixture was allowed to stir for 30 min at room temperature. The reaction was diluted with DCM (50 ml), washed successively with sat. $NaHCO_3$ (3 x 20 ml), water (2 x 20 ml), and brine (12 ml), dried over $MgSO_4$, filtered and evaporated to give crude product as a white solid. Products were purified by FCC (10% EtOAc/Hexanes). From 2.13 g **12a**, obtained 2.09 g product (95%); from 0.98 g **12b**, obtained 0.72 g product (71%) as a 3:1 mixture of diastereomers (*ido*:*gluco*).

13a: ^{1}H ($CDCl_3$, δ): 7.40-7.26 (m, 15H), 4.84-4.68 (m, 5H), 4.60 (d, 1H, *J* = 11.5 Hz), 4.15-4.10 (m, 1H), 4.09-3.98 (m, 2H), 3.71-3.62 (m, 2H), 3.45-3.38 (m, 1H), 2.95 (br. s, 1H, OH), 2.30 (br. s, 1H), 2.01-1.90 (m, 1H), 1.54 (ddd, 1H, *J* = 3.5, 4.9, 13.9 Hz), 0.95 (s, 9H), 0.11 (d, 6H, *J* = 10.4 Hz). ^{13}C: 138.9, 138.7, 138.3, 128.5, 128.4, 128.21, 127.97, 127.92, 127.76, 127.7, 127.6, 127.4, 81.7, 81.5, 78.0, 74.6, 73.7, 73.1, 69.7, 65.6, 39.4, 30.8, 26.0, 18.2, -4.6 (d, $J_{C\text{-}Si}$ = 4.0 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{46}O_5NaSi$, 585.3006; found, 585.2991.

13b: ^{1}H ($CDCl_3$, δ): 7.4-7.26 (m, 15H), 4.93-4.82 (m, 4H), 4.76 (d, 1H, *J* = 11.3 Hz), 4.71 (d, 1H, *J* = 11.4 Hz), 4.03 (dd, 3H, *J* = 8.6, 11.1 Hz), 3.81-3.54 (m, 3H), 3.58-3.54 (m, 1H), 3.34 (t, 1H, *J* = 8.5 Hz), 2.47-2.40 (m, 1H), 1.89 (dt, 3H, *J* = 3.6, 13.6 Hz), 1.49 (ddd, 3H, *J* = 5.0, 11.7, 13.9 Hz), 0.92 (s, 9H), 0.09 (d, 6H, *J* = 6.5 Hz). ^{13}C: 138.9, 138.8, 137.9, 128.56, 128.36, 128.3, 128.2, 128.1, 128.0, 127.94, 127.88, 127.8, 127.7, 127.55, 127.50, 127.4, 86.8, 83.3, 82.2, 75.73, 75.70, 73.7, 70.7, 63.8, 37.5, 33.2, 25.9, 18.0, -4.5 (d, $J_{C\text{-}Si}$ = 17.3 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{46}O_5NaSi$, 585.3006; found, 585.3015.

***tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-5a-carba-D-glucopyranoside (α, 14a and β, 14b)**

To a solution of **13a** or **13b** (1 mmol) in dry DCM (12 ml) was added Dess-Martin periodinane (1.5 mmol), and the reaction stirred for 1.5 hrs at room temperature. The reaction was quenched with the addition of sat $NaHCO_3$ (6 ml) and diluted with DCM (60 ml). The aqueous layer was extracted with DCM (3 x 40 ml), and the combined organic fraction washed with brine, dried over Na_2SO_4, filtered and evaporated to give crude *ido*-aldehyde as a white solid. The crude aldehyde was then dissolved in MeOH (18 ml) and pyridine (9 ml), and stirred at 60 °C for 20 hrs. The reaction was concentrated under reduced pressure, and co-evaporated 3 times with MeOH and toluene to give the *gluco*-aldehyde as a yellow oil.

A solution of *gluco*-aldehyde (1 mmol) in dry MeOH (6 ml) was cooled to 0 °C, and $NaBH_4$ (1.5 mmol) was added portionwise with vigorous stirring. The reaction was stirred at 0 °C for 15 min, then at room temperature overnight. The reaction was concentrated under reduced pressure, and the resulting residue partitioned between 30 ml H_2O and 30 ml DCM. The aqueous layer was extracted 3 x 30 ml DCM, and the combined organic extracts washed with brine, dried over Na_2SO_4, filtered and evaporated to give crude product as a yellow oil. Products were purified by FCC (10% EtOAc/toluene) to give the carbaglucose as a white solid. From 2.05 g **13a**, obtained 1.05 g product (51%, 3 steps); from 0.72 g **13b**, obtained 0.47 g product (65%, 3 steps).

14a: ^{1}H ($CDCl_3$, δ): 7.30-7.16 (m, 15H), 4.93-4.86 (m, 2H), 4.73 (d, 1H, *J* = 10.8 Hz), 4.63 (d, 1H, *J* = 11.6 Hz), 4.59 (d, 1H, *J* = 11.1 Hz), 4.56 (d, 1H, *J* = 11.7 Hz), 4.12-4.08 (m, 1H), 3.90 (t, 1H, *J* =

9.2 Hz), 3.57 (br. d, 1H, *J* = 10.6 Hz), 3.45 (dd, 1H, *J* = 5.1, 10.7 Hz), 3.29 (dd, 1H, *J* = 9.1, 10.7 Hz), 3.19 (dd, 1H, *J* = 2.5, 9.6 Hz), 2.12-2.02 (m, 1H), 1.68 (br. s, 1H, OH), 1.55 (dt, 1H, *J* = 3.8, 13.9 Hz), 1.22-1.12 (m, 1H), 0.81 (s, 9H), -0.02 (d, 6H, *J* = 22.7 Hz). ^{13}C: 139.0, 138.7, 138.4, 128.6, 128.5, 128.4, 128.2, 128.05, 127.99, 127.6, 127.5, 127.4, 83.8, 83.5, 82.5, 75.5, 75.1, 72.5, 67.6, 65.0, 38.2, 32.1, 25.9, 18.2, -4.6 (d, $J_{C\text{-}Si}$ = 46.7 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{46}O_5NaSi$, 585.3006; found, 585.3003.

14b: ^{1}H ($CDCl_3$, δ): 7.32-7.15 (m, 15H), 4.92-4.84 (m, 3H), 4.79-4.74 (m, 2H), 4.60 (d, 1H, *J* = 11.0 Hz), 3.67-3.61 (m, 1H), 3.58-3.51 (m, 2H), 3.48 (t, 1H, *J* = 9.3 Hz), 3.41-3.34 (m, 1H), 3.29 (d, 1H, *J* = 9.2 Hz), 1.83 (br. s, 1H, OH), 1.71 (dt, 1H, *J* = 4.1, 13.1 Hz), 1.63-1.53 (m, 1H), 1.25 (q, 1H, *J* = 12.6 Hz), 0.84 (s, 9H), 0.01 (d, 6H, *J* = 11.5 Hz). ^{13}C: 139.0, 138.7, 138.1, 128.7, 128.40, 128.36, 128.3, 128.1, 127.8, 127.54, 127.51, 127.3, 86.9, 86.3, 82.3, 75.73, 75.68, 75.1, 73.3, 64.8, 40.2, 33.8, 25.9, 18.0, -4.4 (d, $J_{C\text{-}Si}$ = 4.1 Hz). ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{46}O_5NaSi$; found, 585.3018.

tert-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-5a-carba-α-D-glucopyranoside 6-(diphenylphosphate) (15a)

A solution of **14a** (0.86 g, 1.53 mmol) in DCM (50 ml) under N_2 atmosphere was cooled to 0 °C, and pyridine (17 ml) was added followed by diphenylphosphoryl chloride (1.11 ml, 5.37 mmol). The reaction was warmed to room temperature and stirred for 2 hrs. The reaction was cooled back to 0 °C and quenched with the addition of water (40 ml). The mixture was separated and the aqueous phase was extracted with DCM (4 x 50 ml). The combined organic fraction was washed with 2.5 M HCl (2 x 100 ml) with vigorous shaking over 10 minutes, then with sat. $NaHCO_3$, brine, and dried over Na_2SO_4. After filtration and evaporation, 1.02 g of crude product was obtained as a yellow semi-solid. FCC (40% EtOAc/Hexanes) afforded 1.19 g of product (99%) as a white solid.

^{1}H ($CDCl_3$, δ): 7.46-7.16 (m, 25H), 5.01 (d, 1H, *J* = 10.9 Hz), 4.96 (d, 1H, *J* = 10.5 Hz), 4.87 (d, 1H, *J* = 10.9 Hz), 4.77 (d, 1H, *J* = 11.7 Hz), 4.71 (d, 1H, *J* = 11.7 Hz), 4.60 (dt, 1H, *J* = 4.3, 9.5 Hz), 4.55 (d, 1H, *J* = 10.5 Hz), 4.38-4.31 (m, 1H), 4.21 (br. s, 1H), 4.00 (t, 1H, *J* = 9.5 Hz), 3.41 (t, 1H, *J* = 10.0 Hz), 3.28 (dd, 1H, *J* = 1.7, 9.9 Hz), 2.40-2.29 (m, 1H), 1.76 (dt, 1H, *J* = 3.8, 14.1 Hz), 1.42 (t, 1H, *J* = 13.2 Hz), 0.94 (s, 9H), 0.11 (d, 6H, *J* = 26.8 Hz). ^{13}C: 139.0, 138.7, 138.5, 129.9, 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 125.46, 120.20, 120.16, 120.12, 120.08, 83.4, 83.3, 80.1, 75.6, 75.5, 72.6, 69.5 (d, $J_{C\text{-}P}$ = 6.4 Hz), 67.6, 37.2 (d, $J_{C\text{-}P}$ = 8.2 Hz), 31.9, 25.9, 18.2, -4.6 (d, $J_{C\text{-}Si}$ = 57.8 Hz). ^{31}P NMR: -11.6. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{46}H_{55}O_8NaPSi$, 817.3296; found, 817.3293.

2,3,4-tri-*O*-Benzyl-5a-carba-β-D-glucose 6-(diphenylphosphate) (16)

Phosphorylation of **14b** followed the same procedure as above for **14a** resulting in 0.41 g (87%) of phosphorylated product, with loss of the TBS protecting group upon acidic workup.

^{1}H ($CDCl_3$, δ): 7.43-7.16 (m, 25H), 5.02 (d, 1H, *J* = 11.4 Hz), 4.96-4.91 (m, 2H), 4.89 (d, 1H, *J* = 10.6 Hz), 4.73 (d, 1H, *J* = 11.4 Hz), 4.55 (d, 1H, *J* = 10.6 Hz), 4.69 (dt, 1H, *J* = 4.9, 9.7 Hz), 4.34 (ddd, 1H, *J* = 2.5, 4.8, 9.6 Hz), 3.63-3.52 (m, 2H), 3.45 (t, 1H, *J* = 10.0 Hz), 3.29 (t, 1H, *J* = 9.2 Hz), 2.30 (br. s, 1H), 2.00 (dt, 4.0, 13.2 Hz), 1.90-1.78 (m, 1H), 1.44 (q, 1H, *J* = 12.5 Hz). ^{13}C: 138.4, 138.0, 129.9, 128.7, 128.5, 128.1, 128.0, 127.9, 127.75, 127.69, 125.5, 120.1 (d, $J_{C\text{-}P}$ = 4.9 Hz), 86.2, 86.1, 79.9, 75.6, 75.4, 70.9, 68.8 (d, $J_{C\text{-}P}$ = 6.2 Hz), 39.24, 39.17, 30.9 (d, $J_{C\text{-}P}$ = 8.8 Hz). ^{31}P NMR: -11.8. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{40}H_{41}O_8NaP$, 703.2431; found, 703.2447.

5a-Carba-α-D-glucose 6-phosphate (2)

To a solution of **15a** (0.20 g, 0.25 mmol) in MeOH (7 ml) was added 10% Pd/C (27 mg, 0.025 mmol) and HCl (5 M, 0.5 ml), and the mixture hydrogenated at 50 psi for 3 days with vigorous shaking. The reaction was filtered through celite, washed with methanol, and evaporated to give 0.19 g of crude debenzylated product, which was then dissolved in MeOH (5 ml) and H_2O (0.5 ml). PtO_2 (33 mg, 0.12 mmol) was added, and the mixture hydrogenated at 50 psi vigorous shaking. After 2 days, the reaction was filtered through celite, washed with H_2O, and evaporated to give 0.14 g crude C6P. The crude C6P was recrystallized from H_2O/EtOH to give 30 mg product (48% after two steps).

^{1}H (D_2O, δ): 4.07 (q, 1H, *J* = 2.9 Hz, H-1), 3.97 (ddd, 1H, *J* = 4.8, 5.3, 10.1 Hz, H-6), 3.89 (ddd, 1H, *J* = 2.9, 5.4, 10.0 Hz, H-6), 3.58 (t, 1H, *J* = 9.5 Hz, H-3), 3.44 (dd, 1H, *J* = 3.2, 9.9 Hz, H-2), 3.34 (dd, 1H, *J* = 9.5, 10.4 Hz, H-4), 1.98-1.90 (m, 1H, H-5), 1.87 (dt, 1H, *J* = 3.7, 14.8 Hz, H-5a), 1.56 (ddd, 1H, *J* = 2.2, 12.9, 14.8 Hz, H-5a). ^{13}C: 74.2, 73.8, 72.2, 68.8, 65.3 (d, J_{C-P} = 5.5 Hz), 37.1 (d, J_{C-P} = 7.7 Hz), 30.0. ^{31}P: 0.48. ESI-MS (*m/z*): $[M + 2Na]^+$ calcd for $C_7H_{14}O_8Na_2P$, 303.0216; found 303.0209.

5a-Carba-β-D-glucose 6-phosphate (3)

To a solution of **16** (0.36 g, 0.54 mmol) in 3:1 THF/2-propanol (25 ml) was added 10% Pd/C (54 mg, 0.054 mmol) and 20% $Pd(OH)_2$/C (54 mg, 0.10 mmol), and the mixture hydrogenated at atmospheric pressure for 18 h with vigorous stirring. The reaction was filtered through celite, washed with THF and 2-propanol, and evaporated to give 0.30 g of crude debenzylated product as a clear yellow oil, which was then dissolved in H_2O (3 ml) and glacial acetic acid (3 ml). PtO_2 (50 mg, 0.22 mmol) was added, and the mixture hydrogenated at atmospheric pressure for 24 hrs with vigorous stirring. After 24 hrs, another portion of PtO_2 (20 mg, 0.09 mmol) was added and the mixture stirred another 24 hrs under H_2 atmosphere. The reaction was filtered through celite, washed with H_2O, and evaporated to give 0.10 g crude C6P. The crude C6P was recrystallized from H_2O/iPrOH to give 13 mg product (10% after two steps).

^{1}H (D_2O, δ): 3.82-3.74 (m, 1H), 3.73-3.66 (m, 1H), 3.48-3.42 (m, 1H), 3.33 (t, *J* = 9.4 Hz, 1H), 3.20 (t, *J* = 9.3 Hz, 1H), 3.16 (t, *J* = 9.1 Hz, 1H), 1.88 (dt, *J* = 4.5, 12.3 Hz, 1H), 1.60-1.50 (m, 1H), 1.28 (dt, *J* = 11.9, 12.3 Hz, 1H). ^{13}C: 81.4, 76.7, 73.6, 70.0, 61.5, 29.2, 18.5. ^{31}P: -2.70. ESI-MS (*m/z*): $[M - H]^-$ calcd for $C_7H_{14}O_8P$, 257.0421; found, 257.0413.

3,4-Di-*O*-acetyl-D-glucal (18)

Following similar procedures in the literature,[2-3] tri-*O*-acetyl-D-glucal (**17**) (5.00 g, 18.36 mmol) was dissolved in acetone (50 ml) and potassium phosphate buffer (200 ml, 25 mM, pH 4). *C. rugosa* lipase (2.5 g, 731 U/mg) was added and the mixture stirred at 30 °C for 5 hrs. The reaction was filtered through celite, and the filtrate extracted four times with EtOAc. The organic layers were combined, dried over Na_2SO_4 and evaporated to give 4.62 g crude product as a colorless oil. FCC (20% EtOAc/Hexanes) afforded 2.64 g product (87% based on recovered starting material) as a colorless oil.

^{1}H ($CDCl_3$, δ): 6.44 (dd, 1H, *J* = 1.2, 6.2 Hz), 5.40-5.35 (m, 1H), 5.16 (dd, 1H, *J* = 6.5, 8.8 Hz), 4.76 (dd, 1H, *J* = 2.9, 6.2 Hz), 4.03-3.97 (m, 1H), 3.74 (dd, 1H, *J* = 2.9, 12.7 Hz), 3.69 (dd, 1H, *J* = 4.9, 12.7 Hz), 2.78 (br. s, 1H, OH), 2.07 (s, 3H), 2.01 (s, 3H). ^{13}C: 170.7, 170.4, 145.7, 98.8, 76.6, 68.1, 67.7, 60.4, 20.9, 20.7. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{10}H_{14}O_6Na$, 253.0682; found, 253.0689.

6-*O*-*tert*-Butyldimethylsilyl-3,4-di-*O*-acetyl-D-glucal (19)

To a solution of **18** (2.41 g, 10.45 mmol) in anhydrous DMF (95 ml) was added imidazole (2.13 g, 31.36 mmol) and TBSCl (4.73 g, 31.36 mmol). The reaction stirred under N_2 at room temperature overnight then concentrated under high vacuum. The resulting residue was dissolved in 50 ml EtOAc and washed three times with saturated $NaHCO_3$, twice with H_2O, and brine. After drying over Na_2SO_4 and evaporation, 3.77 g crude was obtained as a yellow oil. FCC (20% EtOAc/Hex) gave 3.24 g product (90%) as a colorless oil.

^{1}H ($CDCl_3$, δ): 6.41 (dd, 1H, *J* = 1.3, 6.3 Hz), 5.26 (m, 1H), 5.21 (dd, 1H, *J* = 5.7, 7.4 Hz), 4.72 (dd, 1H, *J* = 3.3, 6.2 Hz), 4.06-4.00 (m, 1H), 3.78-3.73 (m, 2H), 2.01 (s, 3H), 1.98 (s, 3H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C: 170.6, 169.5, 146.0, 98.3, 76.7, 67.59, 67.56, 61.2, 25.8, 21.1, 20.9, 18.3, -5.4. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{16}H_{28}O_6NaSi$, 367.1547; found, 367.1565.

6-*O*-*tert*-Butyldimethylsilyl-D-glucal (20)

Compound **19** (3.24 g, 9.40 mmol) was dissolved in anhydrous MeOH (30 ml) under N_2 and NaOMe (76 mg, 1.41 mmol) was added. The reaction was stirred at room temperature for 2 hours and then concentrated by rotary evaporation. The resulting slightly cloudy oil was dissolved in EtOAc, and filtered through a pad of silica gel and washed with 1:1 EtOAc/Hex to give 2.48 g crude product as a colorless oil which was used without further purification. The NMR data obtained was consistent with previous literature.[4-5]

^{1}H ($CDCl_3$, δ): 6.33 (dd, 1H, *J* = 1.7, 6.2 Hz), 4.74 (dd, 1H, *J* = 2.2, 6.2 Hz), 4.3-4.25 (m, 1H), 4.04-3.98 (m, 1H), 3.96-3.91 (m, 1H), 3.85-3.77 (m, 2H), 3.39 (br. s, 1H, OH), 2.79 (br. s, 1H, OH), 0.93 (s, 9H), 0.12 (s, 6H). ^{13}C: 144.2, 102.4, 76.7, 72.2, 69.3, 63.8, 25.9, 18.3, -5.4, -5.5.

6-*O*-*tert*-Butyldimethylsilyl-3,4-di-*O*-benzyl-D-glucal (21)

Compound **20** (2.44 g, 9.40 mmol) was dissolved in anhydrous DMF (30 ml) under N_2 and cooled to 0 °C. Dry NaH (0.63 g, 26.32 mmol) was added and the solution stirred at 0 °C for 10 min then room temperature for 1.5 hrs, at which time BnBr (3.35 ml, 28.20 mmol) was added. The reaction was stirred at room temperature for 2.5 hrs and concentrated under high vacuum. The resulting residue was partitioned between 50 ml H_2O and EtOAc and separated. The aqueous layer was extracted three times with EtOAc, and the combined organic phase washed with brine, dried over Na_2SO_4 and evaporated to give 4.65 g crude as a brown oil. FCC (10% EtOAc/Hex) gave 2.66 g product (64%). The NMR data obtained was consistent with previous literature.[5]

^{1}H ($CDCl_3$, δ): 7.47-7.32 (m, 10H), 6.45 (dd, 1H, *J* = 1.3, 6.1 Hz), 4.93 (d, 1H, *J* = 11.2 Hz), 4.90 (dd, 1H, *J* = 2.6, 6.2 Hz), 4.82 (d, 1H, *J* = 11.2 Hz), 4.71 (d, 1H, *J* = 11.7 Hz), 4.65 (d, 1H, *J* = 11.7 Hz), 4.30-4.25 (m, 1H), 4.06-4.01 (m, 1H), 4.00-3.94 (m, 3H), 0.98 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H). ^{13}C: 144.8, 138.53, 138.51, 128.47, 128.46, 128.0, 127.83, 127.77, 127.68, 99.8, 78.1, 75.9, 74.3, 74.0, 70.7, 61.8, 26.0, 18.4, -5.1, -5.3.

3,4-Di-*O*-benzyl-D-glucal (22)

Compound **21** (2.66 g, 6.04 mmol) was dissolved in dry THF (65 ml) under an inert atmosphere to which TBAF (1 M, 15.07 ml, 15.07 mmol) was added. The reaction was stirred for three days at room temperature. H_2O (100 ml) was added and the mixture extracted four times with EtOAc. The combined

organic layer was washed three times with water, brine and dried over Na_2SO_4, and after evaporation, 2.66 g of crude product was obtained as a yellow oil. FCC (10% EtOAc/Hexanes) gave 1.58 g product (80%) as a colorless oil.

^{1}H ($CDCl_3$, δ): 7.45-7.30 (m, 10H), 6.45 (br. d, 1H, *J* = 6.2 Hz), 4.94 (dd, 1H, *J* = 2.6, 6.1 Hz), 4.91 (d, 1H, *J* = 11.3 Hz), 4.77 (d, 1H, *J* = 11.3 Hz), 4.72 (d, 1H, *J* = 11.5 Hz), 4.62 (d, 1H, *J* = 11.5 Hz), 4.31-4.26 (m, 1H), 4.02-3.96 (m, 1H), 3.95-3.88 (m, 2H), 3.88-3.83 (m, 1H), 2.13 (br. s, 1H, OH). ^{13}C: 144.6, 138.2, 138.1, 128.55, 128.52, 128.1, 127.9, 127.82, 127.81, 100.2, 77.4, 7.6, 74.6, 73.8, 70.6, 61.8. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{20}H_{22}O_4Na$, 349.1410; found, 349.1417.

1,5-Anhydro-3,4-di-*O*-benzyl-2,6,7-trideoxy-D-*arabino*-hept-1,6-dienitol (23)

Following the procedures described by Gao et al.,[6] to a solution of **22** (1.36 g, 4.16 mmol) in dry ACN (80 ml) was added IBX (2.33 g, 8.33 mmol) and the mixture stirred at 60 °C for 2.5 hrs. The reaction was cooled to room temperature, filtered through celite and washed with EtOAc. The resulting filtrate was evaporated to give 1.41 g crude aldehyde as a yellow oil.

A suspension of CH_3PPh_3Br (4.48 g, 12.48 mmol) in dry THF (28 ml) was cooled to -78 °C. HMPA (2.2 ml, 12.64 mmol) and n-BuLi (2.5 M, 4.53 ml, 11.32 mmol) was added dropwise slowly and the mixture stirred at -78 °C for 10 min then room temperature for 2 hrs. The mixture was cooled back down to -78 °C and a solution of crude aldehyde in dry THF (25 ml) was added dropwise. The reaction was stirred at -78 °C for 30 min then room temperature for 1.5 hrs. The reaction was cooled to 0 °C and quenched with the addition of small ice chips and saturated NH_4Cl. The mixture was extracted four times with DCM, then washed with brine, dried over Na_2SO_4, and evaporated to give 7.58 g crude as a red oil. FCC (10% EtOAc/Hexanes) afforded 0.85 g of product (63%) as a colorless oil. NMR spectra were consistent with the literature.[6-7]

^{1}H ($CDCl_3$, δ): 7.45-7.31 (m, 10H), 6.49 (br. d, 1H, *J* = 6.1 Hz), 6.13 (ddd, 1H, *J* = 6.6, 10.7, 17.1 Hz), 5.50 (d, 1H, *J* = 17.1 Hz), 5.37 (d, 1H, *J* = 10.7 Hz), 4.95 (dd, 1H, *J* = 2.7, 6.1 Hz), 4.85 (d, 1H, *J* = 11.4 Hz), 4.76 (d, 1H, *J* = 11.4 Hz), 4.71 (d, 1H, *J* = 11.7 Hz), 4.65 (d, 1H, *J* = 11.7 Hz), 4.40 (t, 1H, *J* = 7.6 Hz), 4.31-4.25 (m, 1H), 3.68 (dd, 1H, *J* = 6.1, 8.6 Hz). ^{13}C: 144.6, 138.5, 138.2, 134.4, 128.46, 128.45, 128.0, 127.84, 127.78, 127.7, 118.4, 100.4, 78.3, 78.1, 75.5, 73.9, 70.7.

(3*R*,4*R*,5*R*)-3,4-Dibenzyloxy-5-(hydroxymethyl)cyclohexene (24)

Claisen rearrangement of **23** followed procedures described previously.[7-8] A mixture of 23 (0.15 g, 0.45 mmol) and Ph_2O (2.27 g) was heated to 210 °C and stirred for 2.5 hrs. The mixture was cooled to room temperature and $NaBH_4$ (0.71 g, 18.77 mmol) in 10 ml EtOH was added. The reaction was stirred for 20 min then 0.5 M HCl was added dropwise to quench. The mixture was diluted with DCM (50 ml) and 0.2 M HCl (20 ml), separated, and the aqueous layer extracted four times with DCM. The combined organic layer was washed with saturated $NaHCO_3$, brine, dried with Na_2SO_4 and evaporated to give 2.04 g of crude product as a yellow oil. FCC (30% EtOAc/Hexanes) gave 0.10 g of product (69%) as a colorless oil.

^{1}H ($CDCl_3$, δ): 7.55-7.31 (m, 10H), 5.86-5.79 (m, 1H), 5.79-5.74 (m, 1H), 5.04 (d, 1H, *J* = 11.4 Hz), 4.80 (d, 1H, *J* = 11.1 Hz), 4.78 (d, 1H, *J* = 11.1 Hz), 4.70 (d, 1H, *J* = 11.4 Hz), 4.32-4.26 (m, 1H), 3.74-3.67 (m, 2H), 3.65 (dd, 1H, *J* = 4.0, 11.0 Hz, H4), 2.73 (br. s, 1H, OH), 2.24-2.14 (m, 1H), 2.13-2.02 (m, 1H), 2.00-1.89 (m, 1H). ^{13}C: 138.5, 128.6, 128.5, 128.33, 128.27, 127.95, 127.92, 127.8, 126.0,

82.0, 81.2, 74.4, 71.3, 65.4, 40.7, 28.1. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{21}H_{24}O_3Na$, 347.1617; found, 347.1625.

(3*R*,4*R*,5*S*)-3,4-Dibenzyloxy-5-[[(dibenzyloxyphosphinyl)oxy]methyl]cyclohexene (25)

A solution of **24** (0.10 g, 0.31 mmol) in dry DMF (2.5 ml) was cooled to 0 °C under N_2, and NaH (21 mg, 0.86 mmol) was added. The reaction was stirred at 0 °C for 10 min then warmed to room temperature for 1 hr. Tetrabenzylpyrophosphate (0.33 g, 0.61 mmol) was added and the reaction stirred at room temperature overnight. The reaction was then diluted with EtOAc and H_2O (20 ml each), separated and extracted with EtOAc (3 x 20 ml). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated to give 0.33 g of crude phosphorylated product as an orange oil. FCC (20-40% EtOAc/Hexanes) gave 0.17 g (95%) of product as a clear, colorless oil.

^1H ($CDCl_3$, δ): 7.44-7.26 (m, 20H), 5.79-5.69 (m, 2H), 5.09-5.04 (m, 4H), 4.19 (d, 1H, *J* = 11.0 Hz), 4.72 (d, 1H, *J* = 11.6 Hz), 4.68 (d, 1H, *J* = 11.6 Hz), 4.65 (d, 1H, *J* = 11.0 Hz), 4.28 (ddd, 1H, *J* = 4.9, 5.1, 9.8 Hz), 4.23-4.15 (m, 2H), 3.61 (dd, 1H, *J* = 7.2, 10.1 Hz), 2.25-2.06 (m, 3H). ^{13}C: 138.6, 138.5, 135.9 (d, J_{C-P} = 6.6 Hz), 128.63, 128.60, 128.56, 128.5, 128.4, 127.98, 127.97, 127.94, 127.8, 127.7, 127.6, 126.1, 80.9, 78.4, 74.4, 71.4, 69.3 (d, J_{C-P} = 5.5 Hz), 68.0 (d, J_{C-P} = 6.0 Hz), 39.2 (d, J_{C-P} = 8.1 Hz), 27.8. ^{31}P NMR: 0.03. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{35}H_{37}O_6NaP$, 607.2219; found, 607.223.

3,4-Di-*O*-benzyl-5a-carba-α-D-glucose 6-(dibenzylphosphate) (26)

To a solution of **25** (0.74 g, 1.27 mmol) in 1:1 *t*-BuOH/H_2O (17.5 ml) was added $K_3Fe(CN)_6$ (1.25 g, 3.81 mmol), K_2CO_3 (0.52 g, 3.81 mmol) and $K_2OsO_2(OH)_4$ (9.3 mg, 25 μmol) and the mixture stirred at room temperature overnight, similar to procedures described by Minato et al. and Lee et al.[9-10] The reaction was quenched with a saturated solution of $NaHSO_3$ (10 ml) and stirred for an additional 2 hrs at room temperature. The reaction was concentrated *in vacuo*, and the residue taken up in 50 ml DCM and H_2O and separated. The aqueous layer was extracted with DCM (3 x 50 ml) and 3:1 $CHCl_3$/i-PrOH (3 x 50 ml), and the combined organic layer washed with brine, dried over Na_2SO_4, filtered and evaporated to give 0.79 g crude as an oily white solid. FCC (60-80% EtOAc/Hexanes) afforded 0.63 g product (90% based on recovered SM) as a colorless oil.

^1H ($CDCl_3$, δ): 7.42-7.27 (m, 20H), 5.12-5.02 (m, 4H), 5.00 (d, 1H, *J* = 11.8 Hz), 4.83 (d, 1H, *J* = 10.7 Hz), 4.70 (d, 1H, *J* = 11.8 Hz), 4.63 (d, 1H, *J* = 10.7 Hz), 4.36 (ddd, 1H, *J* = 3.9, 4.2, 9.9 Hz, H-7), 4.03 (q, 1H, *J* = 3.0 Hz, H-1), 3.99 (ddd, 1H, *J* = 2.5, 4.5, 9.9 Hz, H-7), 3.71 (t, 1H, *J* = 9.3 Hz, H-3), 3.43 (dd, 1H, *J* = 3.0, 9.3 Hz, H-2), 3.35 (dd, 1H, *J* = 9.3, 10.5 Hz, H-4), 2.25 (br. s, 2H, OH), 2.23-2.13 (m, 1H, H-5), 1.80 (dt, 1H, *J* = 3.7, 14.6 Hz, H-6), 1.42 (ddd, 1H, *J* = 2.5, 12.8, 14.8 Hz, H-6). ^{13}C: 138.6, 138.2, 135.83, 135.76, 128.8, 128.7, 128.65, 128.62, 128.5, 128.04, 127.97, 127.9, 127.85, 127.76, 83.3, 80.0, 75.3, 75.0, 69.42 (d, J_{C-P} = 5.0 Hz), 69.39 (d, J_{C-P} = 5.0 Hz), 67.5 (d, J_{C-P} = 6.3 Hz), 37.3 (d, J_{C-P} = 8.7 Hz), 29.6. ^{31}P NMR: 0.11. ESI-MS (*m/z*): $[M + Na]^+$ calcd for $C_{35}H_{39}O_8NaP$, 641.228; found, 641.2278.

5a-Carba-α-D-glucose 6-phosphate monosodium salt (2)

A solution of **26** (0.76 g, 1.23 mmol) and Pd/C (10% w/w, 0.26 g, 0.25 mmol) in MeOH (65 ml) and AcOH (4.5 ml) was purged with N_2 followed by H_2 three times at 45 psi with shaking. The mixture was hydrogenated with H_2 for 2 days at 50 psi with vigorous shaking. The resulting mixture was filtered through celite, washed with MeOH and H_2O, and evaporated to give 0.41 g crude C6P as the free acid.

The crude C6P was dissolved in 50 ml H_2O, and the pH adjusted carefully to pH 3.5 with 1M NaOH. The solution was filtered through a 0.20 μm syringe filter and freeze-dried to give 0.369 g (99%) C6P as the monosodium salt. Characterization of this product matched that as previously described (conversion of 15a to 2) with the exception of a shift in the phosphate peak in the ^{31}P spectrum due to the monosodium salt.

^1H (D_2O, δ): 4.07 (q, 1H, *J* = 2.9 Hz, H-1), 3.97 (ddd, 1H, *J* = 4.8, 5.3, 10.1 Hz, H-7), 3.89 (ddd, 1H, *J* = 2.9, 5.4, 10.0 Hz, H-7), 3.58 (t, 1H, *J* = 9.5 Hz, H-3), 3.44 (dd, 1H, *J* = 3.2, 9.9 Hz, H-2), 3.34 (dd, 1H, *J* = 9.5, 10.4 Hz, H-4), 1.98-1.90 (m, 1H, H-5), 1.87 (dt, 1H, *J* = 3.7, 14.8 Hz, H-6), 1.56 (ddd, 1H, *J* = 2.2, 12.9, 14.8 Hz, H-6). ^{13}C: 74.2, 73.8, 72.2, 68.8, 65.3 (d, J_{C-P} = 5.5 Hz), 37.1 (d, J_{C-P} = 7.7 Hz), 30.0. ^{31}P: 1.34. ESI-MS (*m/z*): $[M + 2Na]^+$ calcd for $C_7H_{14}O_8Na_2P$, 303.0216; found 303.0209.

Expression and Purification of NtdC and NtdA

Expression and purification of NtdC and NtdA followed the procedure described previously.[11] Purified protein was concentrated to 0.24 mg/mL (NtdC) and 4.76 mg/mL (NtdA) and stored at −80 °C in 100 μL aliquots. The concentration of protein was determined spectrophotometrically at 280 nm using theoretical extinction coefficients of 1.025 mL mg^{-1} cm^{-1} (42500 M^{-1} cm^{-1}) for NtdC and 0.725 mL g^{-1} cm^{-1} (37900 $M^{-1}cm^{-1}$) for NtdA.

Kinetic assays of NtdC and NtdA

Enzyme assays were carried out as previously described.[11] For kinetic assays of NtdC, reaction mixtures consisted of NAD (0.05-5.00 mM), C6P (0.05-20.0 mM), and Tris-HCl (100 mM, pH 9.00). Coupled NtdC-NtdA reactions also contained L-glutamate (10.0 mM) and NtdA (23.9 μg/ml, 450 nM). Product inhibition studies were carried out in the presence of 5.0-30.0 μM NADH. All reactions were initiated with the addition of NtdC (1.2 μg/ml, 29 nM).

Absorbance values at 340 nm were collected every 10 s over 5 min and used to determine initial velocities of the NtdC reaction. The resulting data was then fit to equations describing bisubstrate mechanisms (**Eqn. 1-5, Schemes S1-5**) using non-linear least squares regression in SigmaPlot v. 12.0 (Systat Software, Inc.).[12-13]

Sequential (steady-state ordered and random)

Eqn. 1 $$v = \frac{V_{max}[A][B]}{K_{ia}K_b + K_a[B] + K_b[A] + [A][B]}$$

Ping-Pong

Eqn. 2 $$v = \frac{V_{max}[A][B]}{K_a[B] + K_b[A] + [A][B]}$$

Rapid-equilibrium ordered

Eqn. 3 $$v = \frac{V_{max}[A][B]}{K_{ia}K_b + K_b[A] + [A][B]}$$

Substrate inhibition with dead-end BE complex

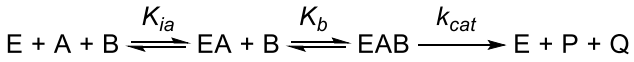
Eqn. 4 $$v = \frac{V_{max}[A][B]}{K_{ia}K_b + K_b[A] + K_a[B] + [A][B] + \frac{K_{ia}K_b[B]}{K_i}}$$

Substrate inhibition with dead-end BE and BEB complexes

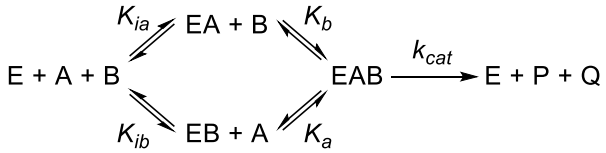
Eqn. 5 $$v = \frac{V_{max}[A][B]}{K_{ia}K_b + K_b[A] + K_a[B] + [A][B] + \frac{K_{ia}K_b[B]}{K_i} + \frac{K_a[B]^2}{\beta K_i}}$$

For **Eqn. 1-5**, v is the measured initial velocity of the reaction, V_{max} is the maximum velocity of the enzyme, [*A*] and [*B*] are the concentration of substrates *A* and *B*, respectively, K_a and K_b are the dissociation constants of *A* and *B* from the EAB complex, K_{ia} is the dissociation constant of *A* from the EA complex. For **Eqn. 4** and **5**, K_i and βK_i are the dissociation constants of *B* from the unproductive BE and BEB complexes, respectively.

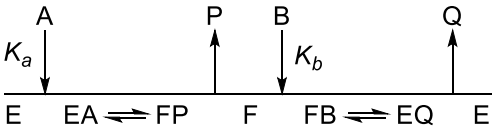
Scheme S1 - Ordered



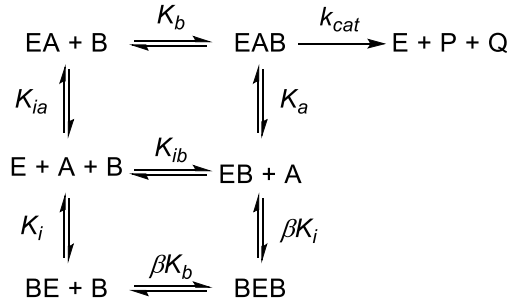
Scheme S2 - Random



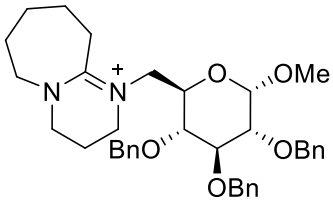
Scheme S3 - Ping-Pong



Scheme S4 - Dead-end BE and BEB Complexes

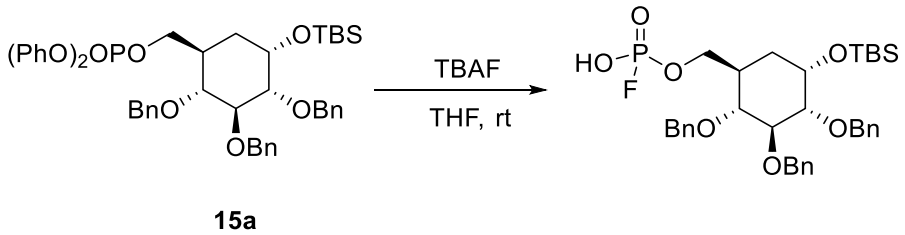


Proposed DBU nucleophilic adduct of 7 (S27)



ESI-MS (m/z): [M^+] calcd for $C_{37}H_{47}N_2O_5^+$, 599.34795; found, 599.3485

***tert*-Butyldimethylsilyl 2,3,4-tri-*O*-benzyl-α-D-carbaglucopyranoside 6-(fluorophosphate) (S28)**



15a

Attempts to remove the TBS protecting group from **15a** followed the same procedure as for compound **22** from **21**. Starting from 0.57 g of **15a** (0.72 mmol), 0.45 g (98%) of product was obtained, identified as the fluorophosphate shown above, and contaminated with tetrabutylammonium salts.

^{1}H (500 MHz, $CDCl_3$, δ): 7.40-7.20 (m, 15H), 4.92 (d, 1H, J = 11.4 Hz), 4.87 (d, 1H, J = 10.2 Hz), 4.85 (d, 1H, J = 11.4 Hz), 4.75 (d, 1H, J = 10.0 Hz), 4.69 (d, 1H, J = 11.8 Hz), 4.64 (d, 1H, J = 11.8 Hz), 4.44-4.37 (m, 1H), 4.20-4.15 (m, 1H), 4.03-3.97 (m, 1H), 3.94 (t, 1H, J = 9.4 Hz), 3.60 (t, 1H, J = 10.0 Hz), 3.27 (dd, 1H, J = 2.4, 9.6 Hz), 2.20-2.11 (m, 1H), 1.78-1.65 (m, 2H, overlapping with $(nBu)_4N^+$ in sample), 0.87 (s, 9H), 0.04 (d, 6H, J_{Si-H} = 16.9 Hz). ^{13}C (125 MHz, $CDCl_3$, δ): 139.1, 139.0, 128.6, 128.2, 128.13, 128.09, 127.73, 127.66, 127.3, 127.1, 127.0, 83.6, 83.5, 80.6, 75.4, 75.2, 72.5, 68.2, 50.7, 32.1, 29.7, 25.9, 13.7. ^{31}P (202 MHz, $CDCl_3$, δ): -5.03 (d, J_{F-P} = 920 Hz). ^{19}F (470 MHz, $CDCl_3$, δ): -78.2 (d, J_{F-P} = 920 Hz). ESI-MS (*m/z*): $[M - H]^-$ calcd for $C_{34}H_{45}O_7FSiP$, 643.26617; found, 643.2656.

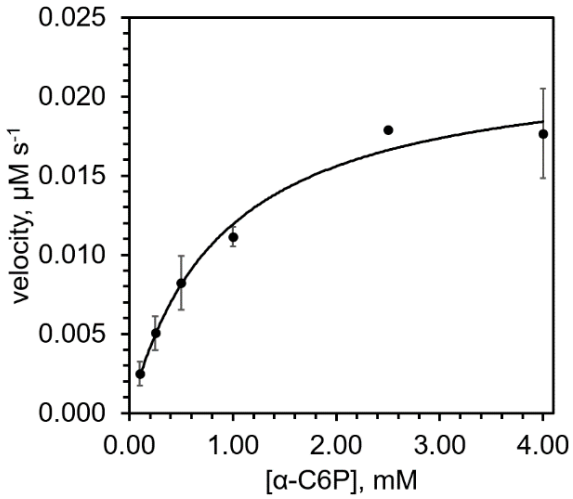
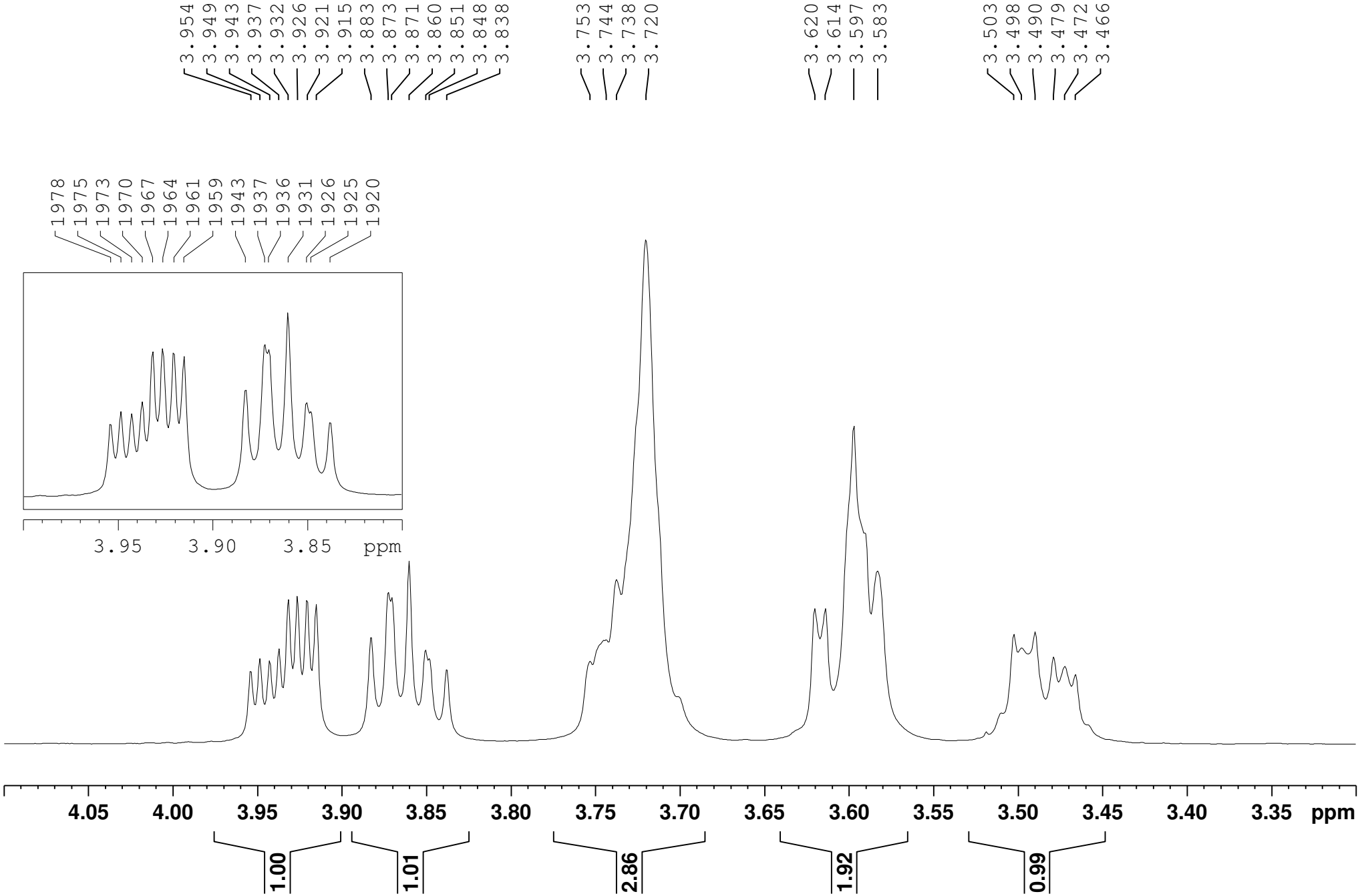
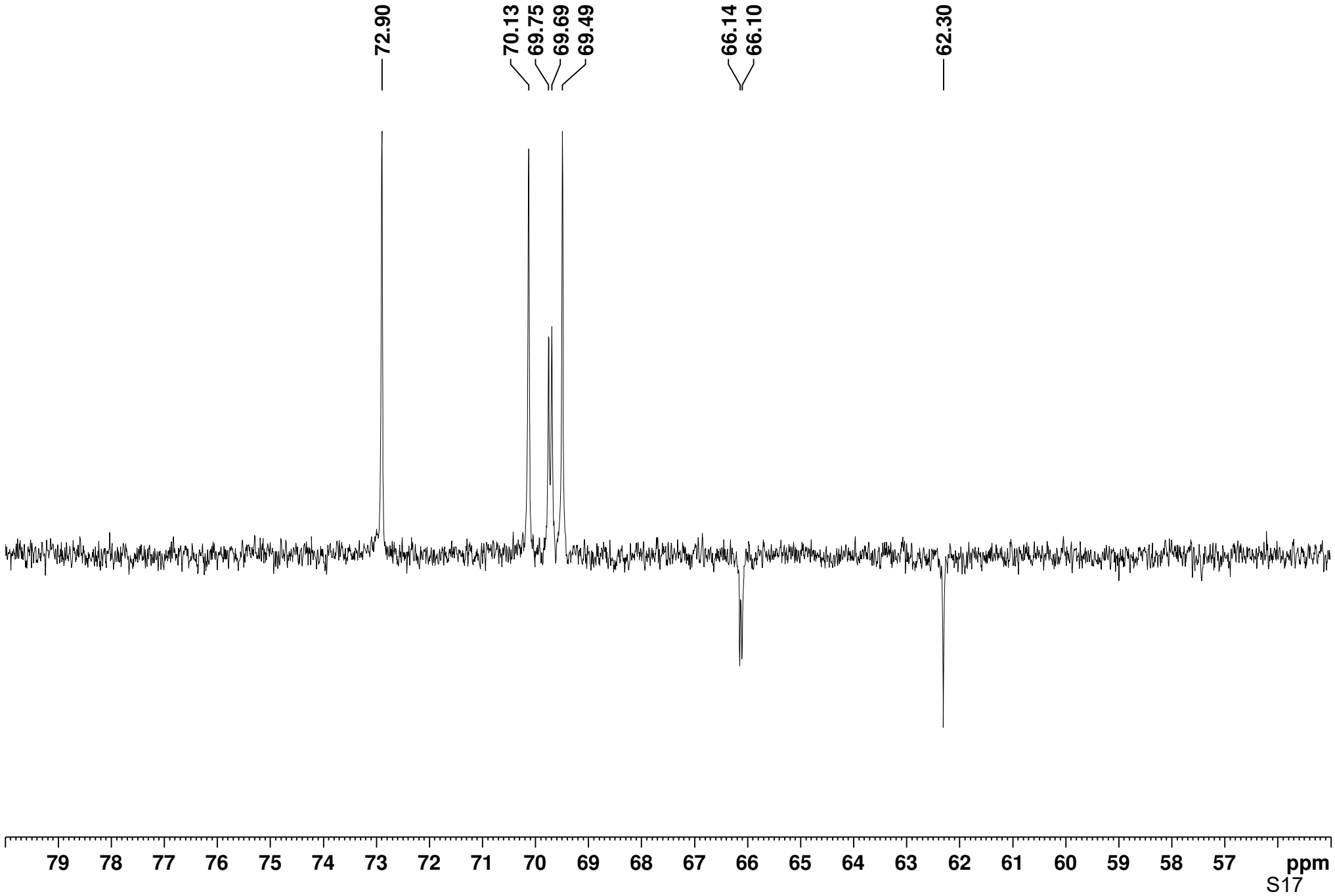


Figure S6. Activity of NtdC with α-C6P at 1 mM NAD, 100 mM Tris-HCl pH 9, and 29 nM NtdC.

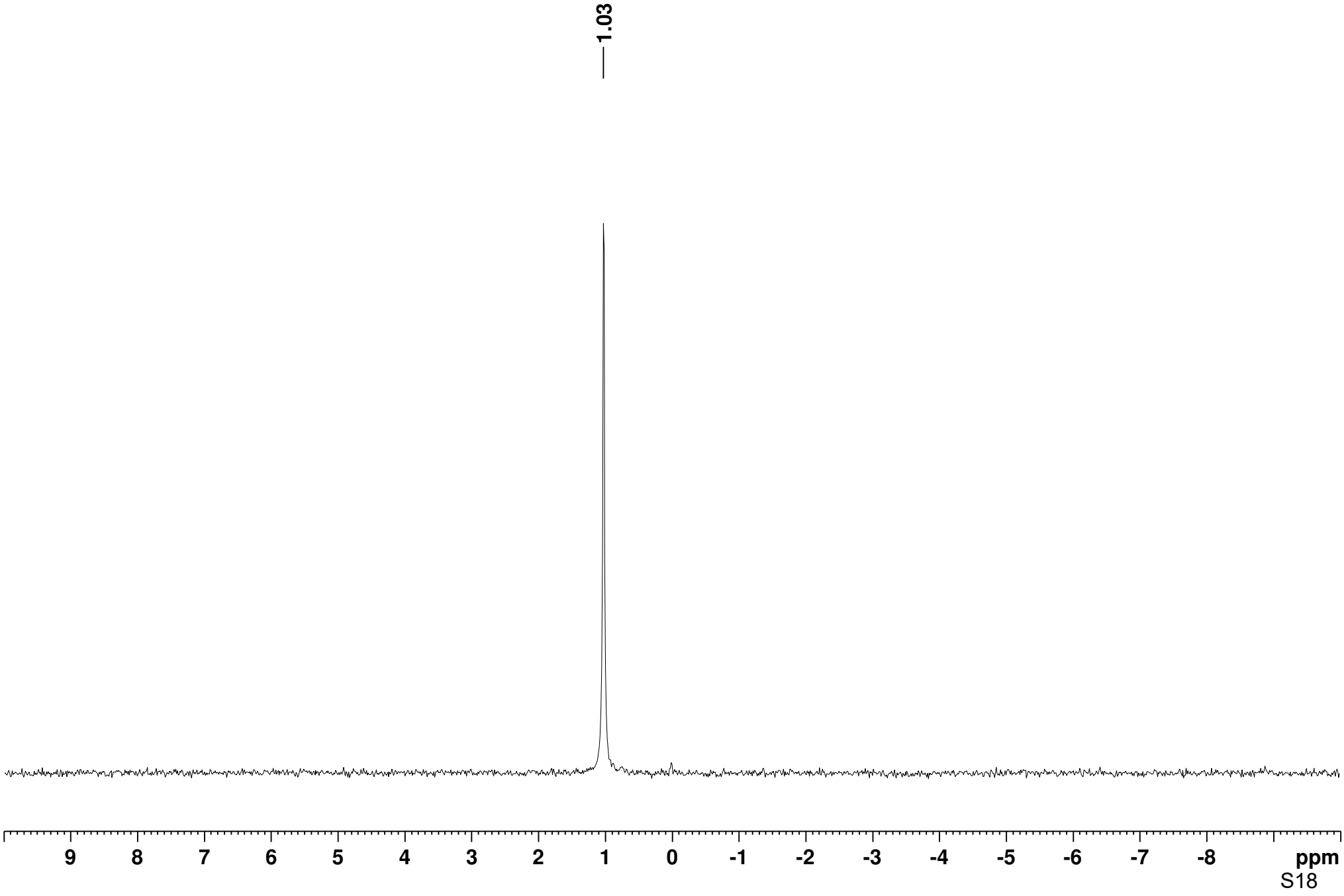
Compound 1 - 1H



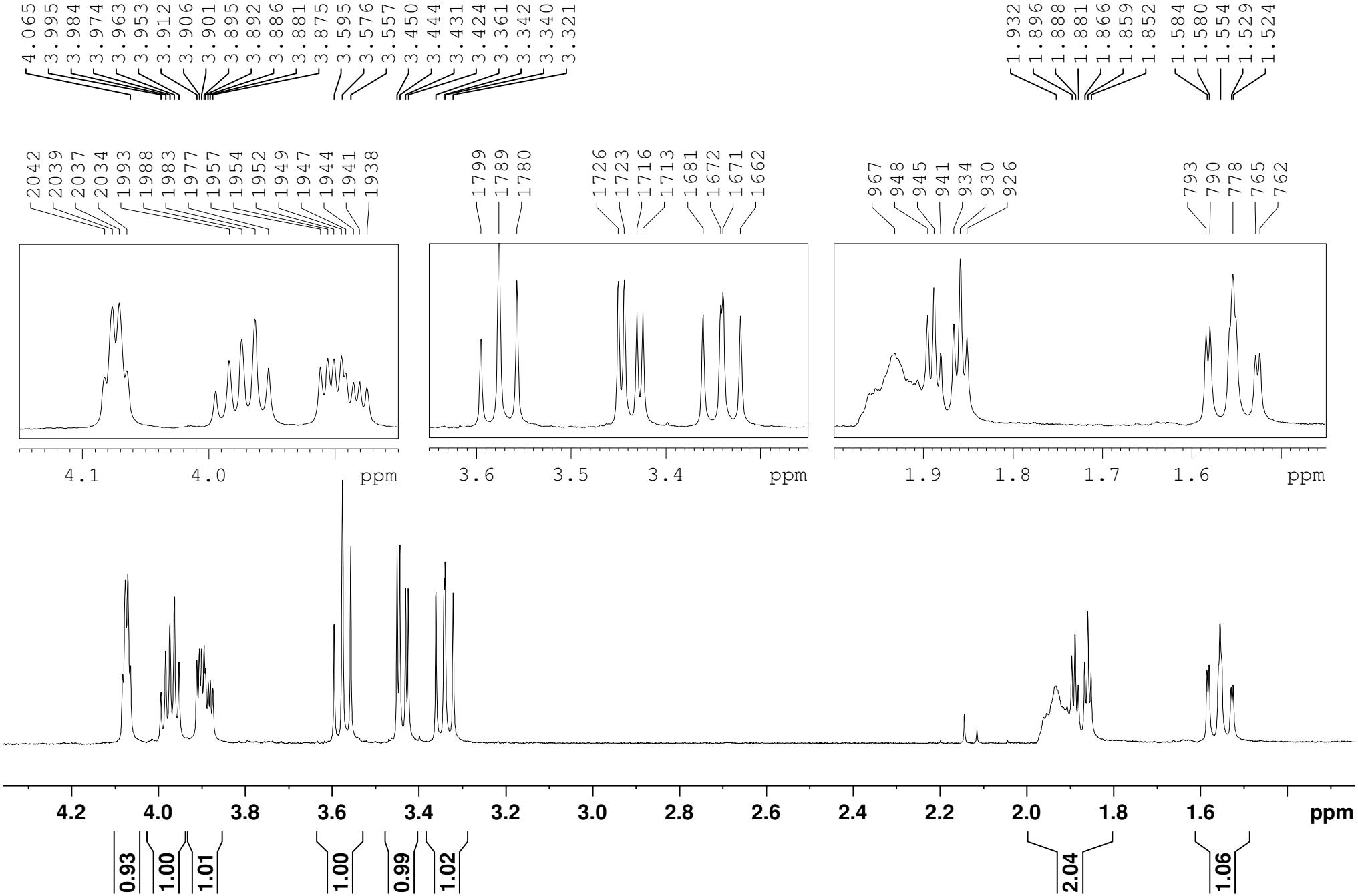
Compound 1 - 13C



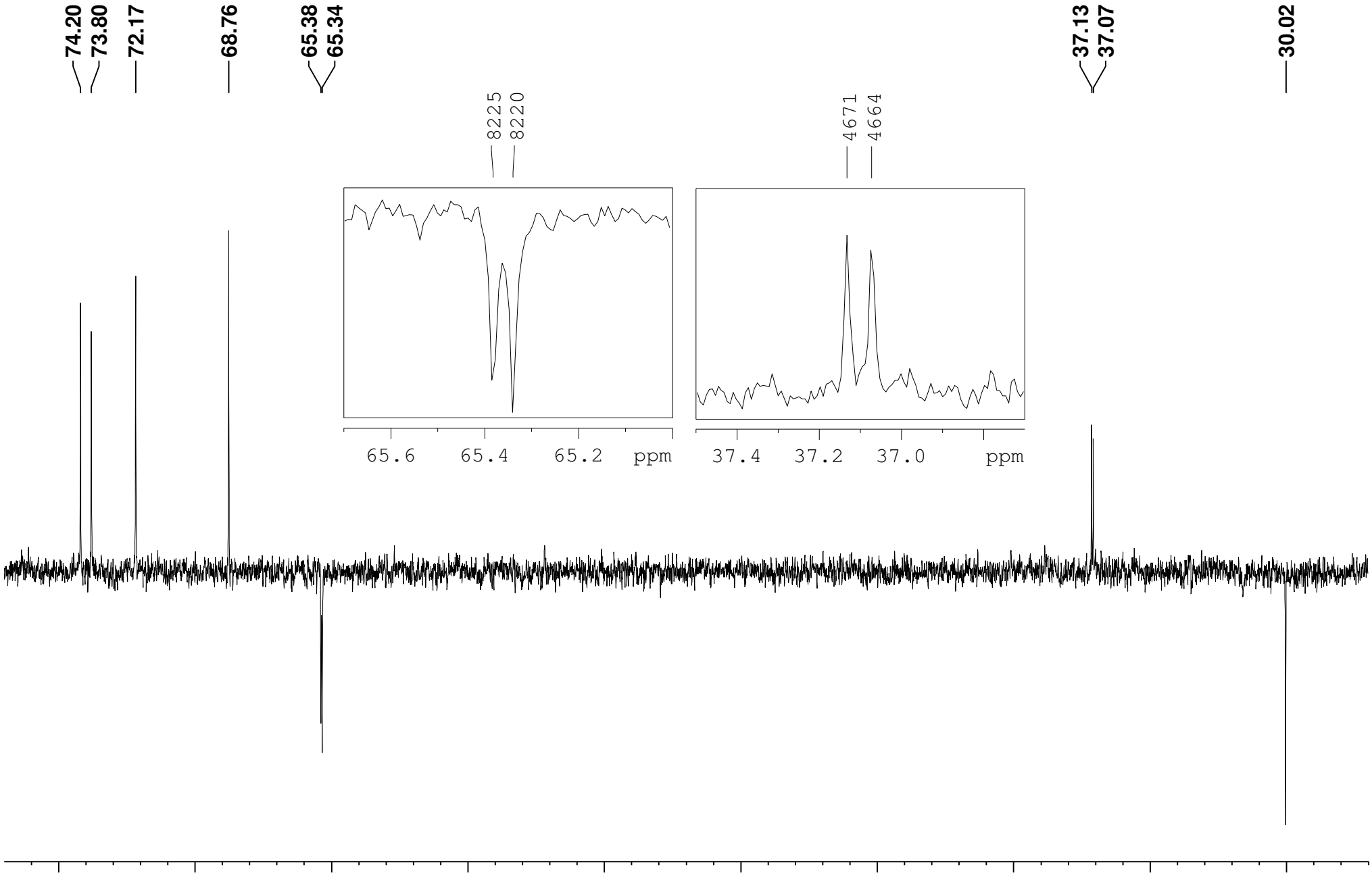
Compound 1 - 31P



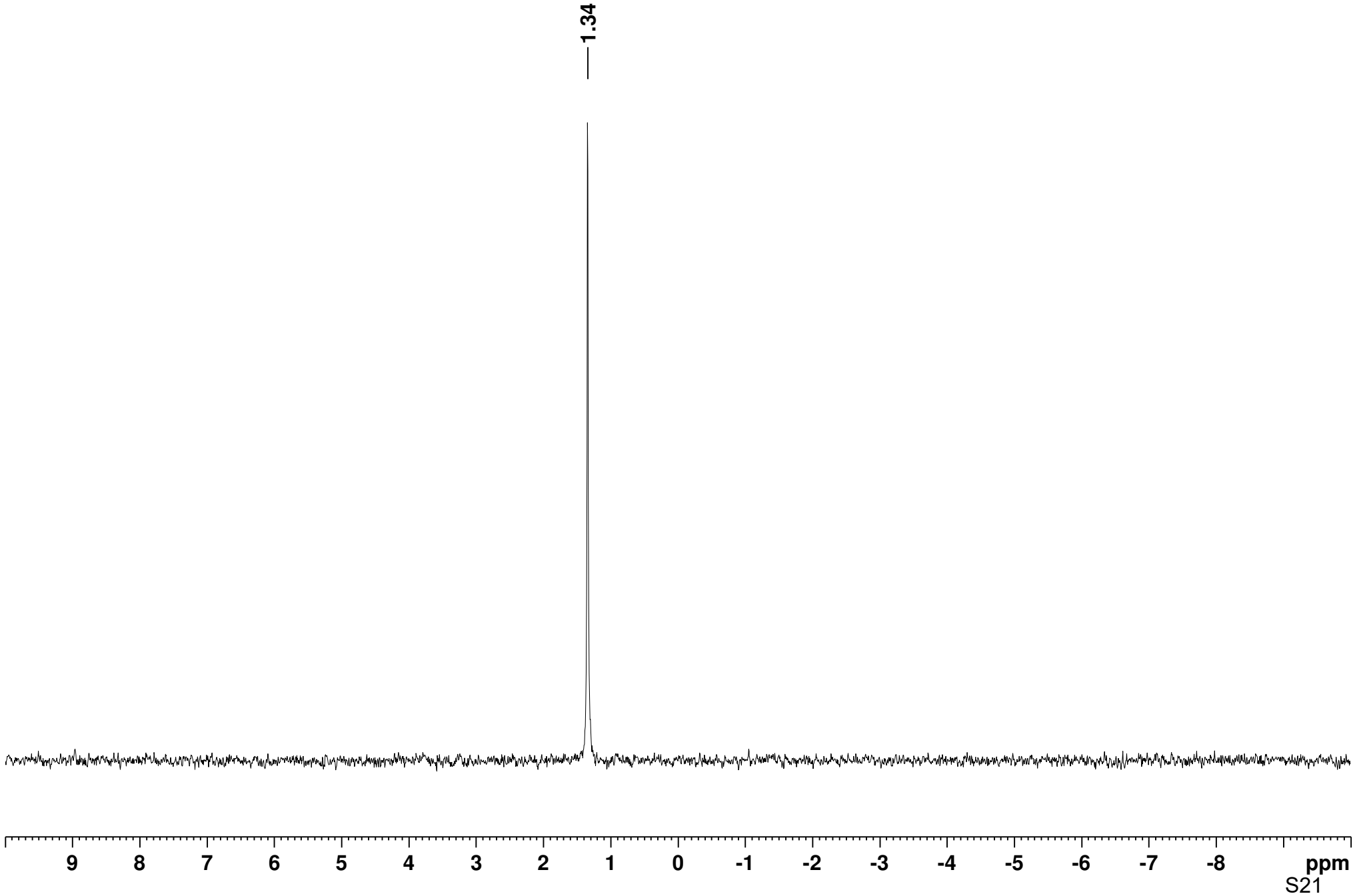
Compound 2 - 1H



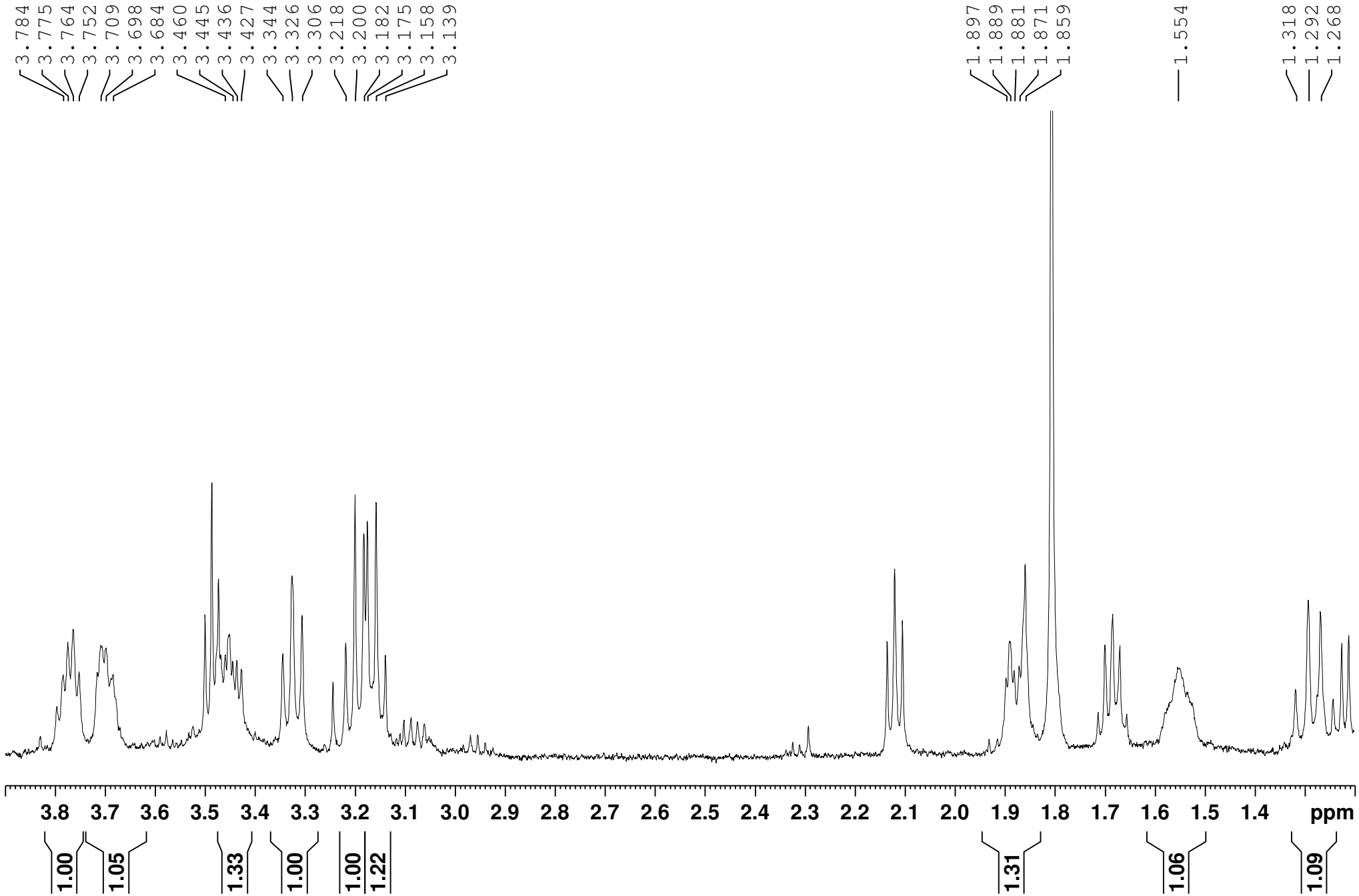
Compound 2 - 13C



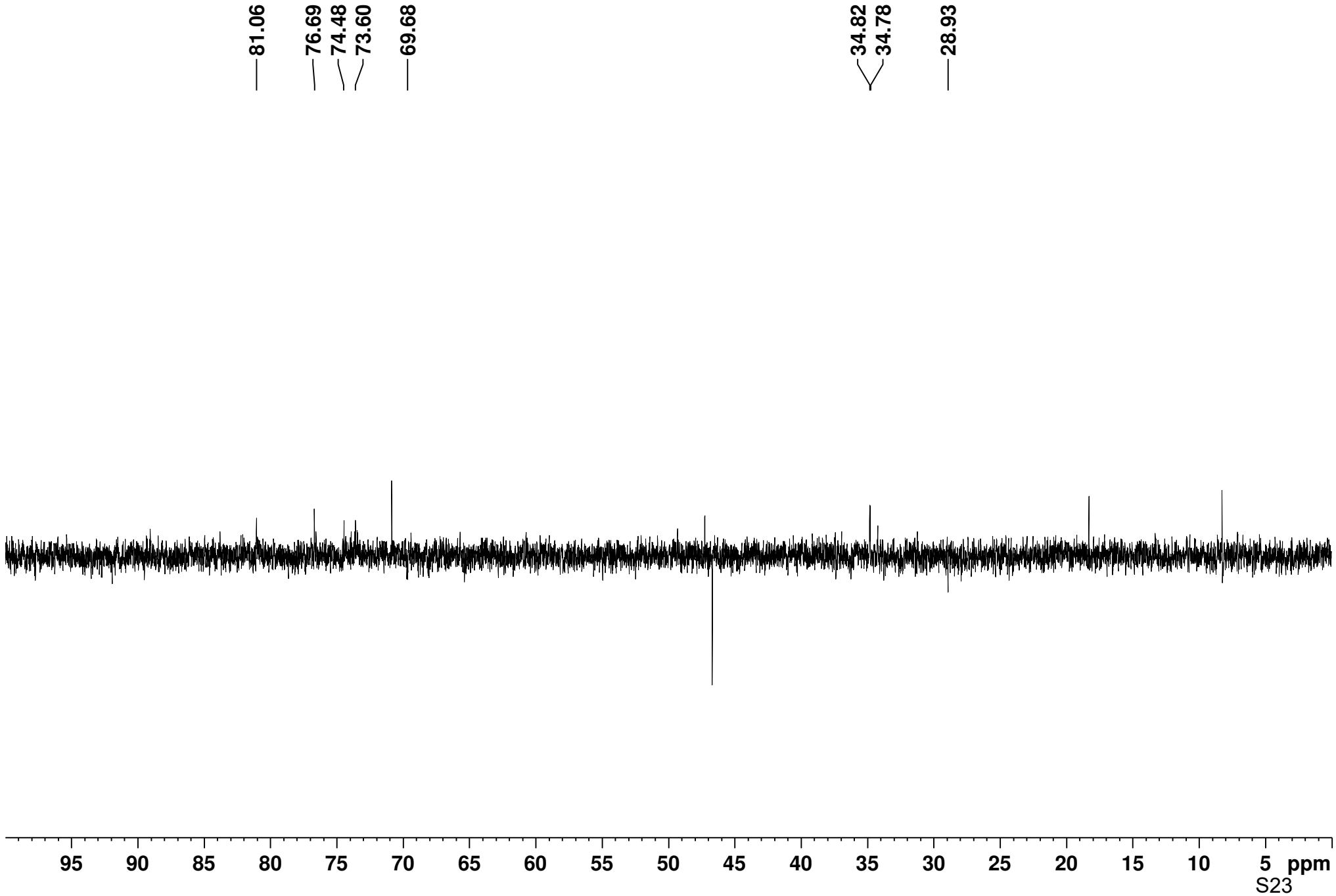
Compound 2 - 31P



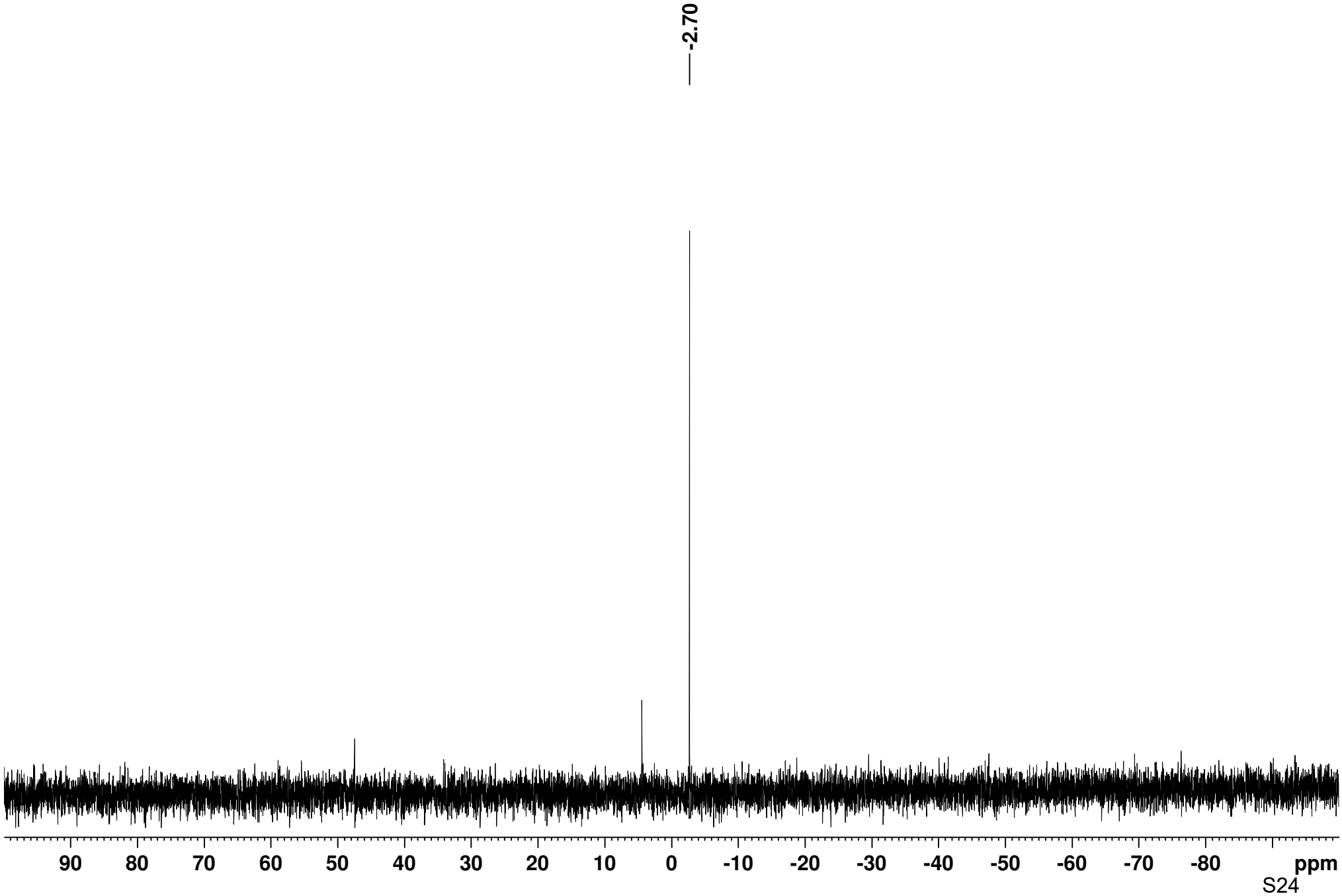
Compound 3 - 1H



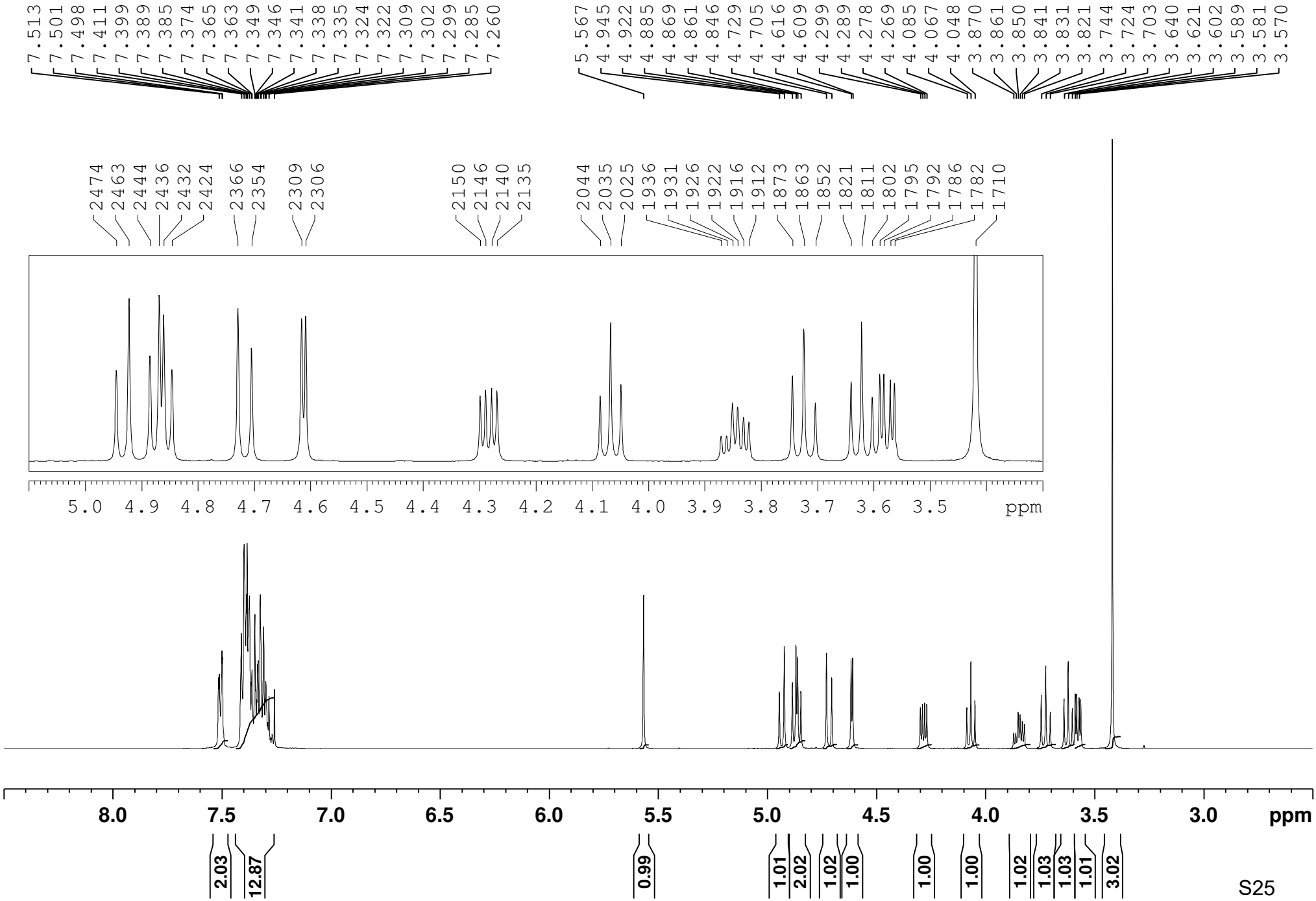
Compound 3 - 13C



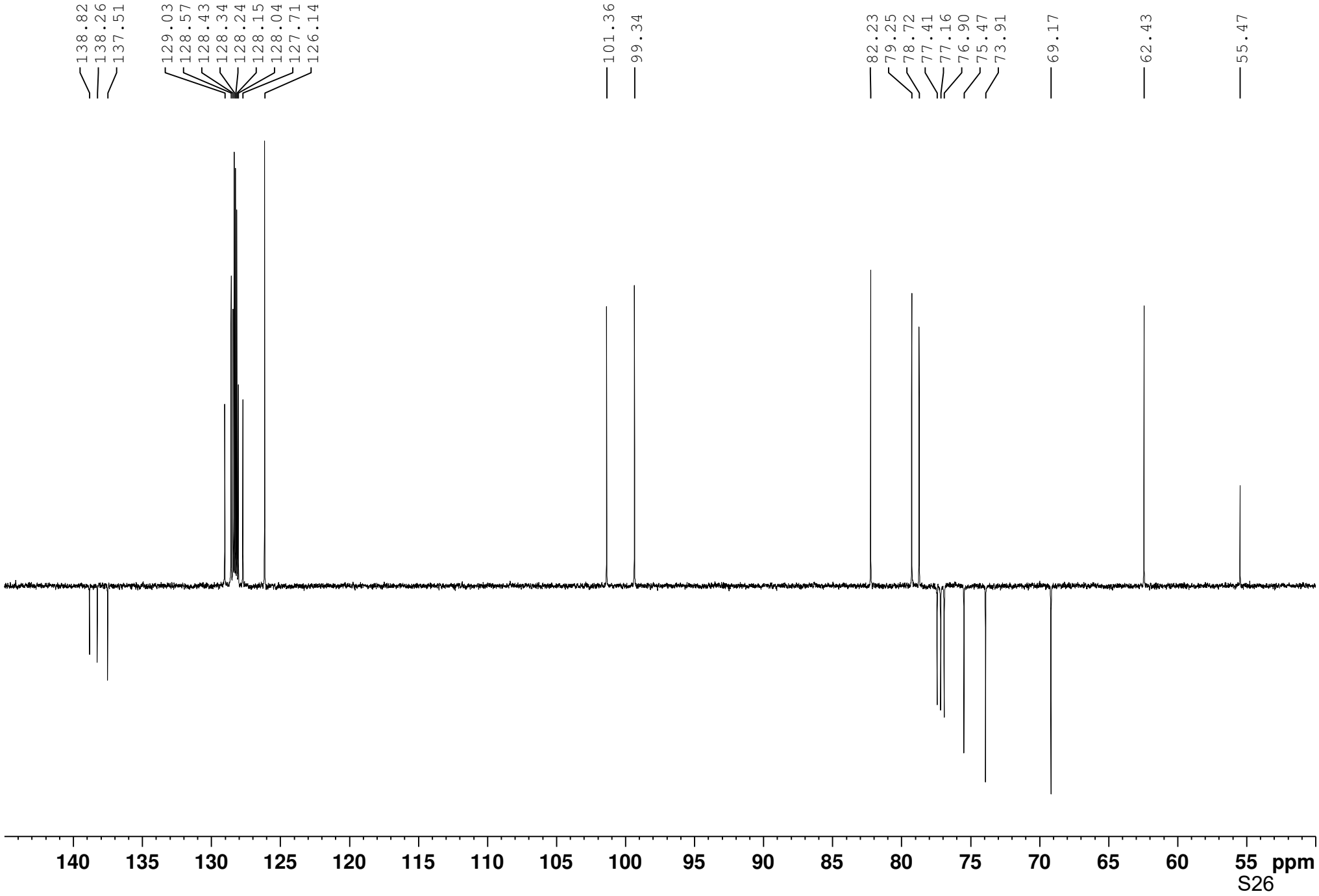
Compound 3 - 31P



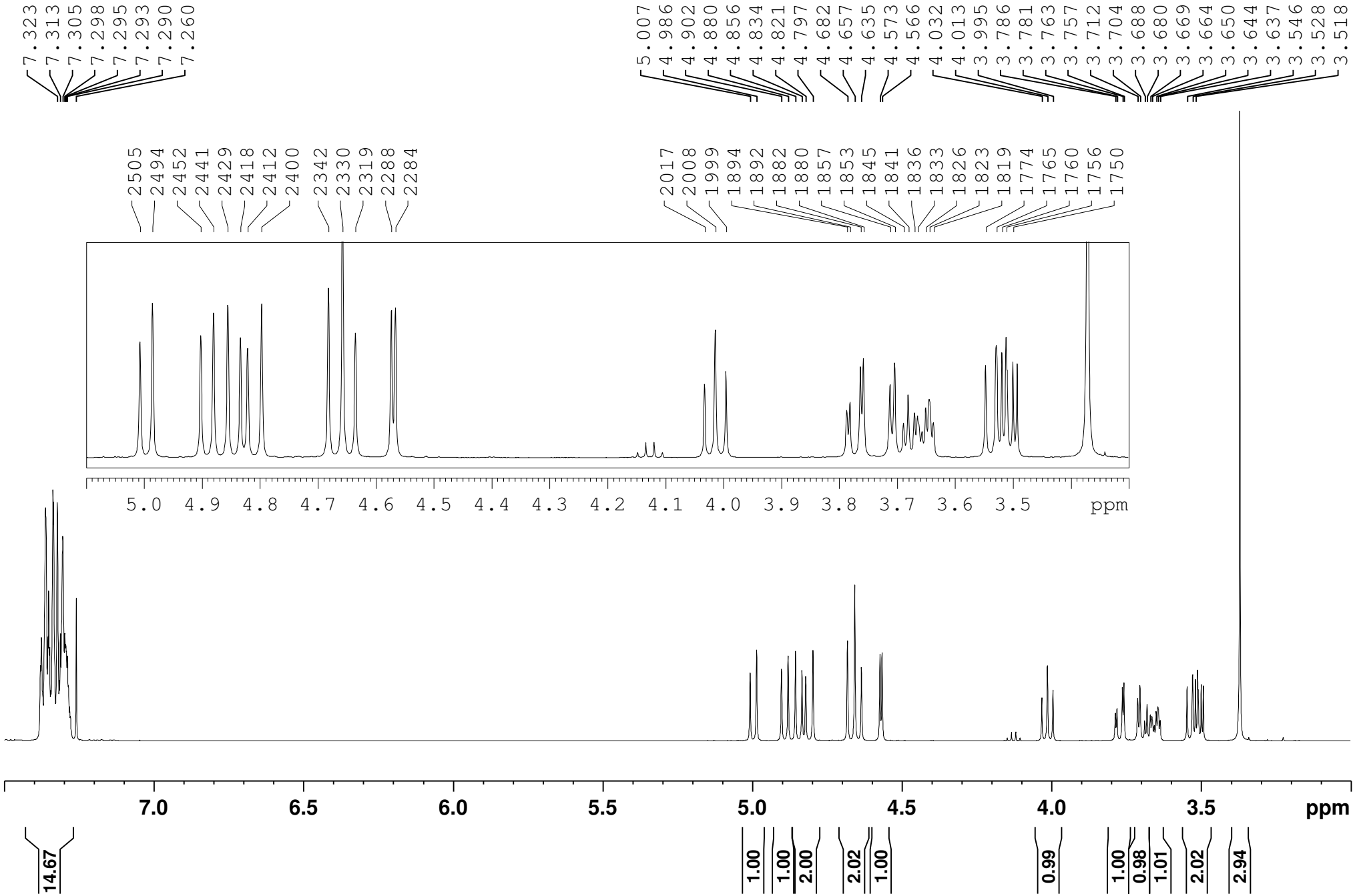
Compound 5 - 1H



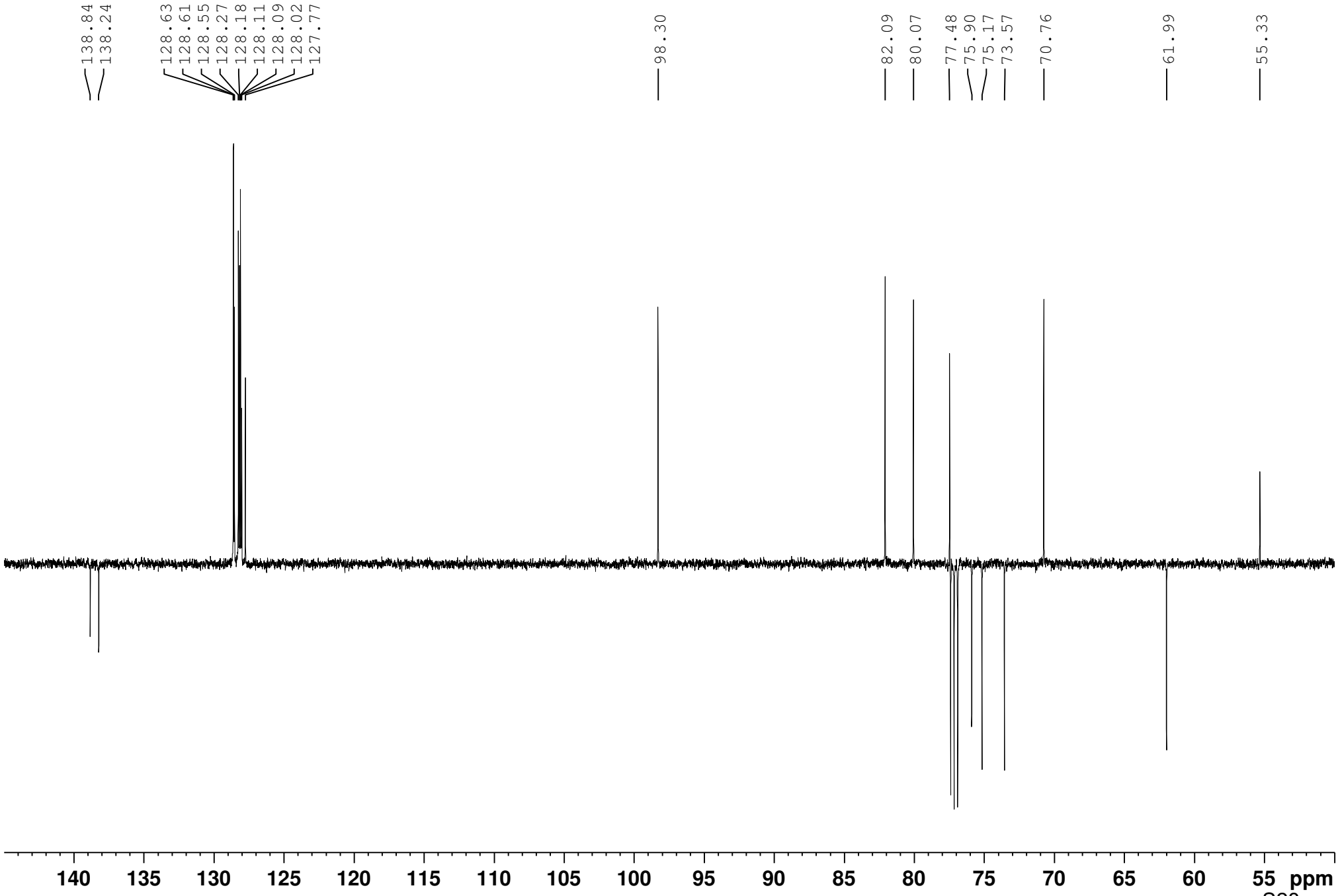
Compound 5 - 13C



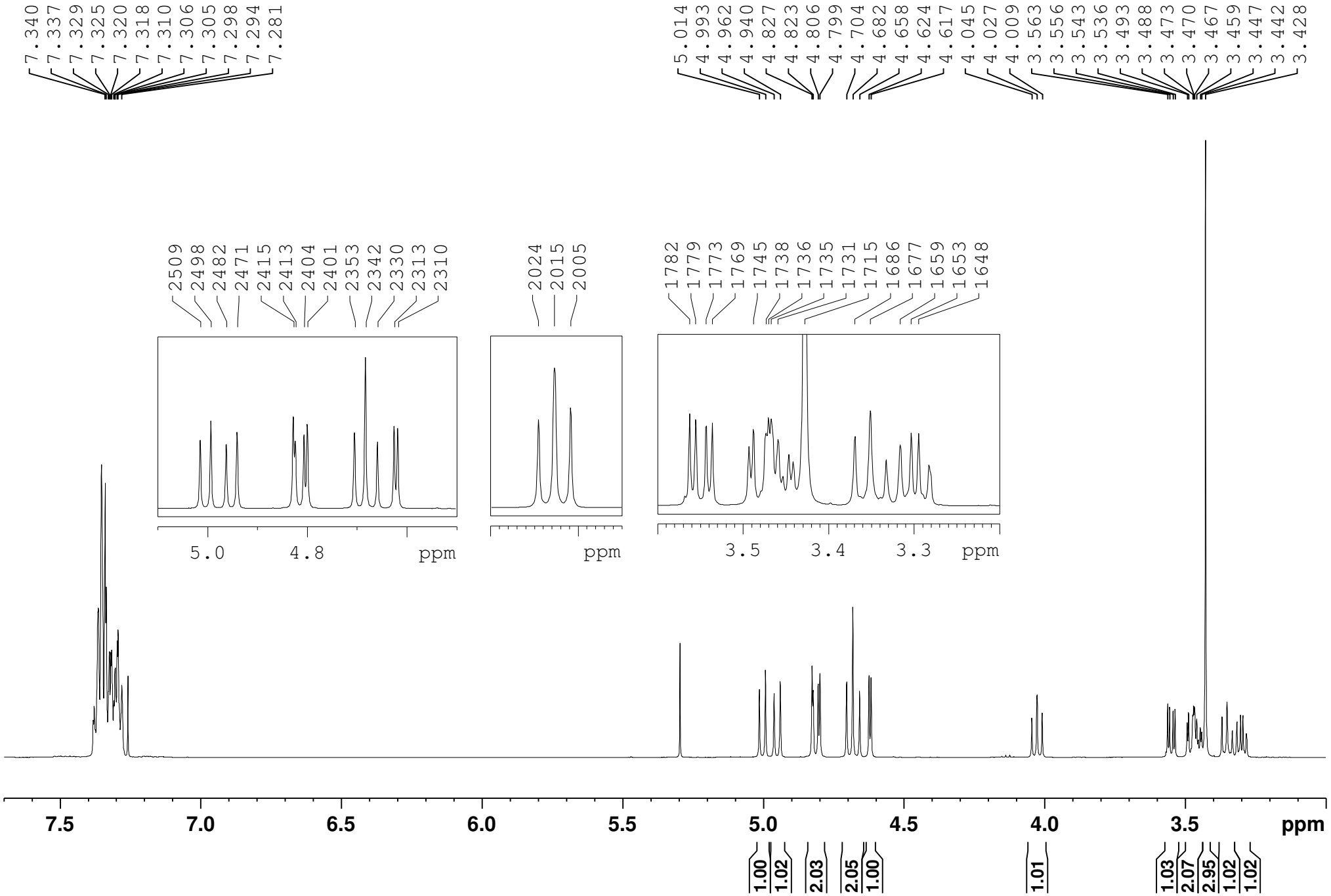
Compound 6 - 1H



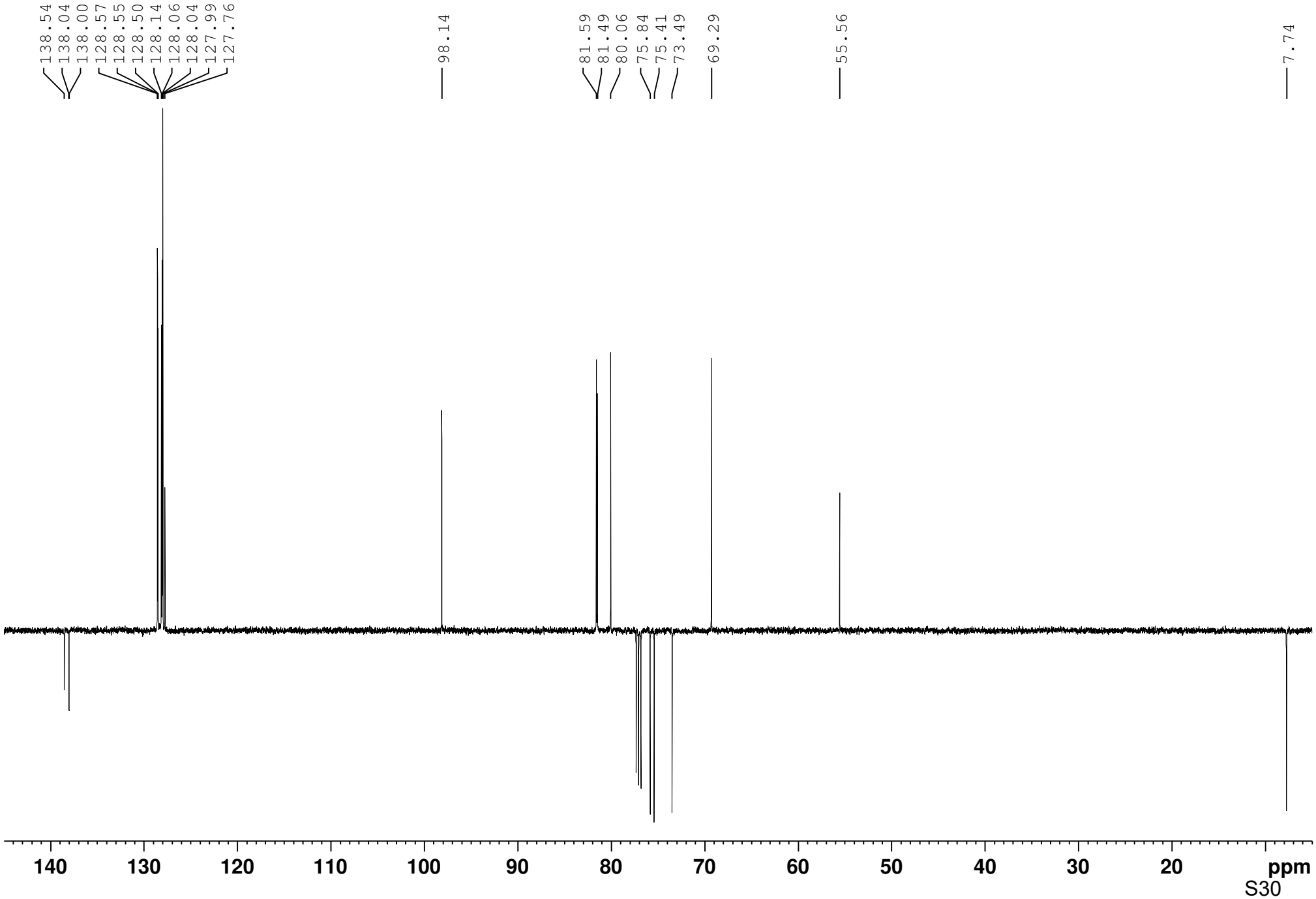
Compound 6 - 13C



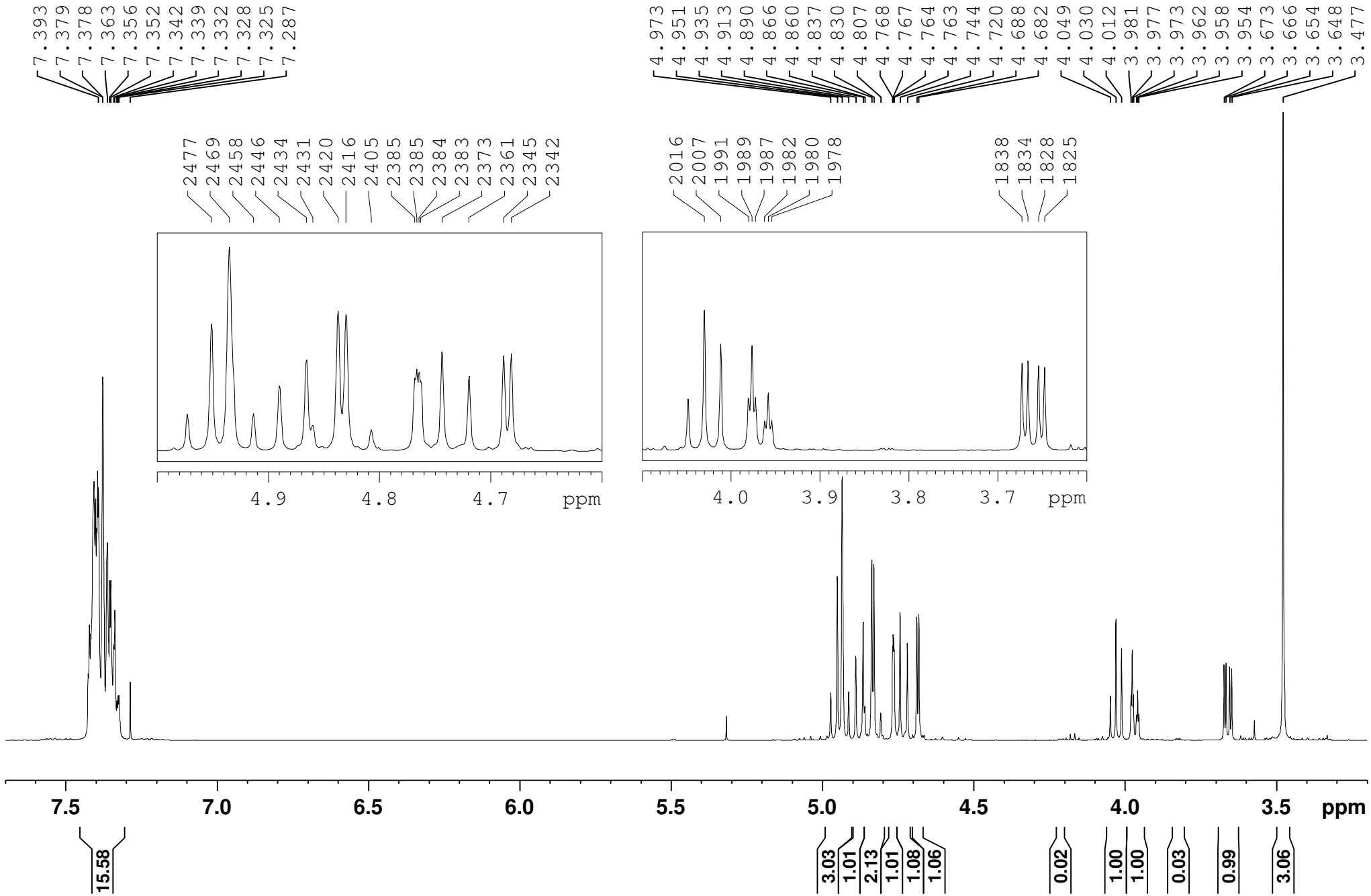
Compound 7 - 1H



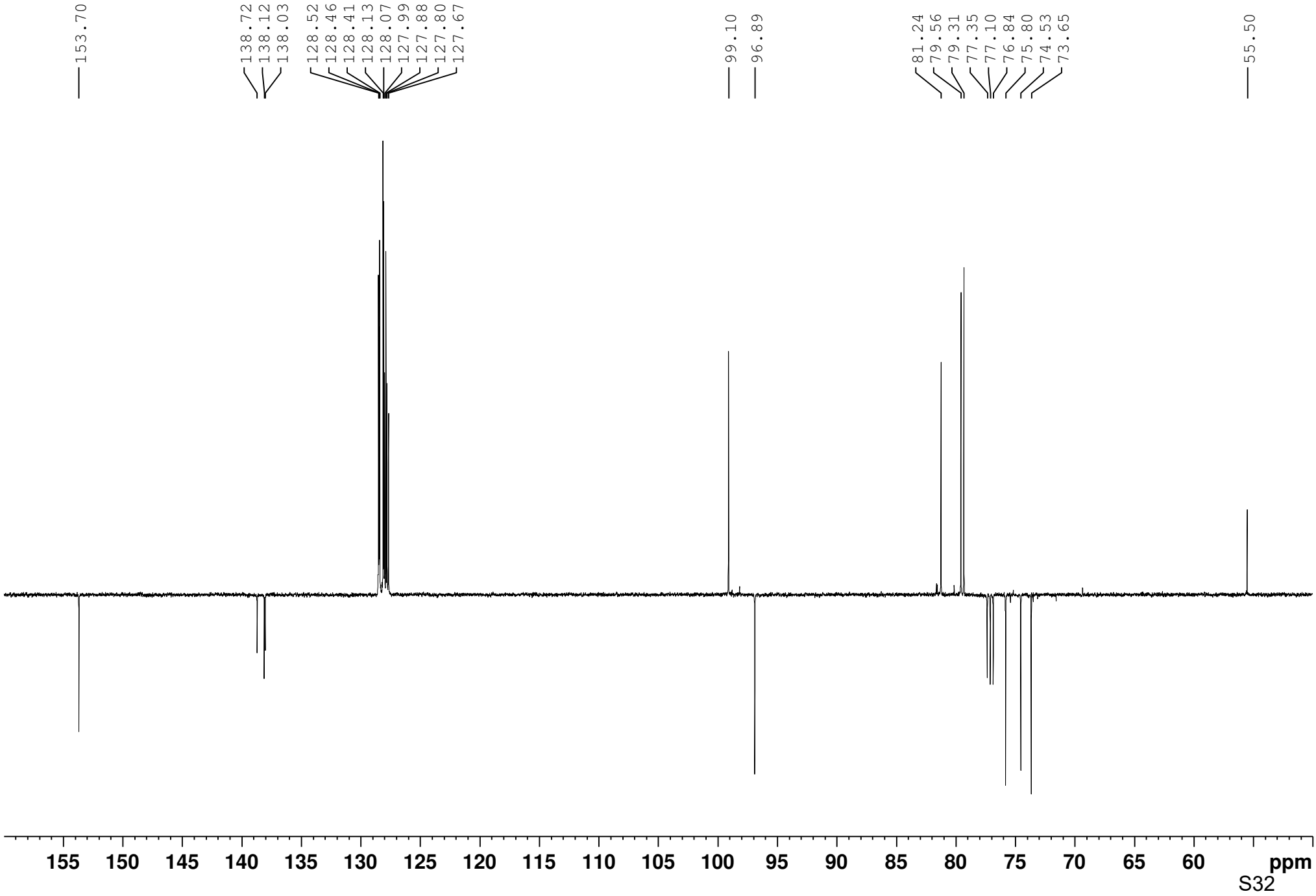
Compound 7 - 13C



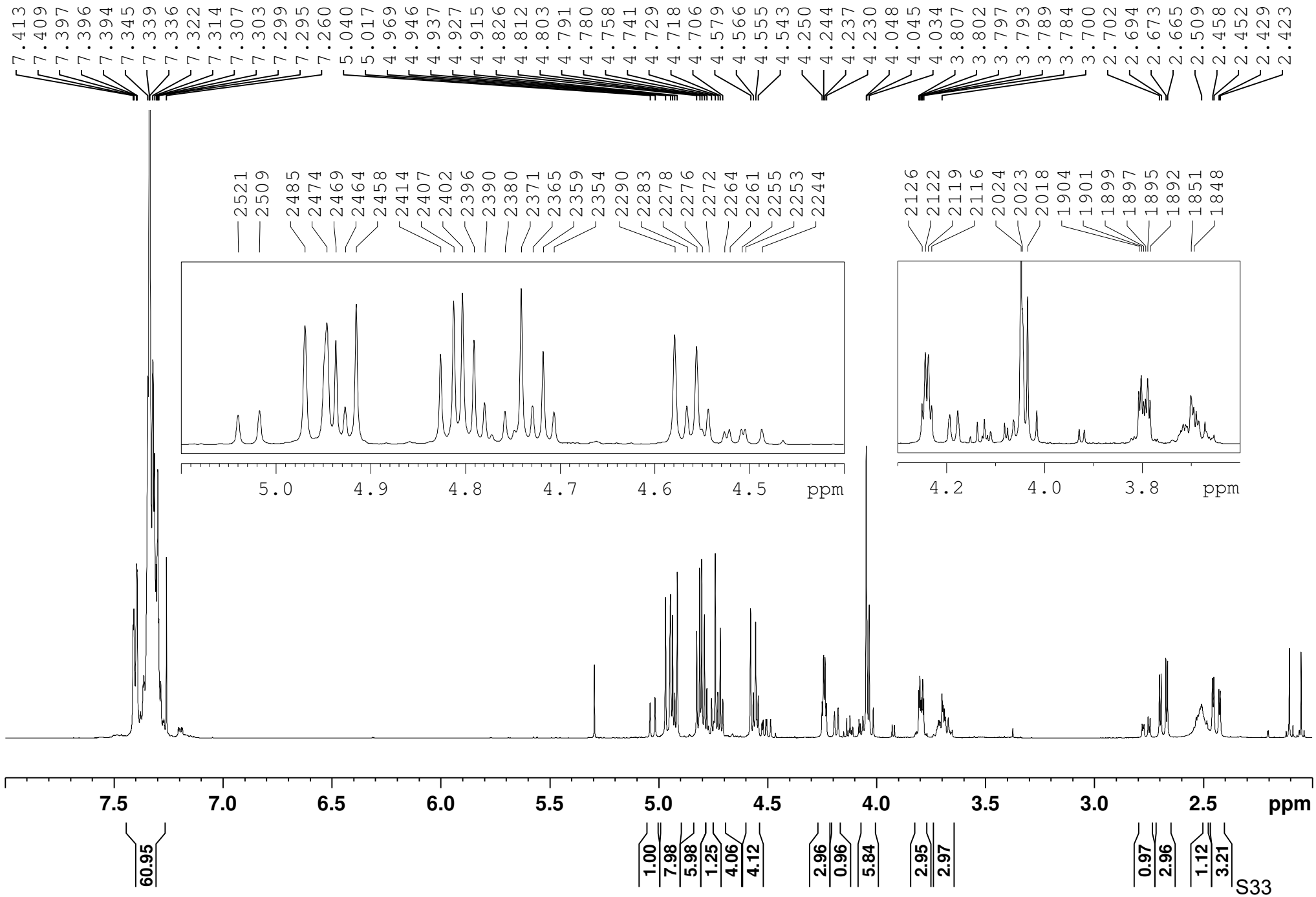
Compound 8 - 1H



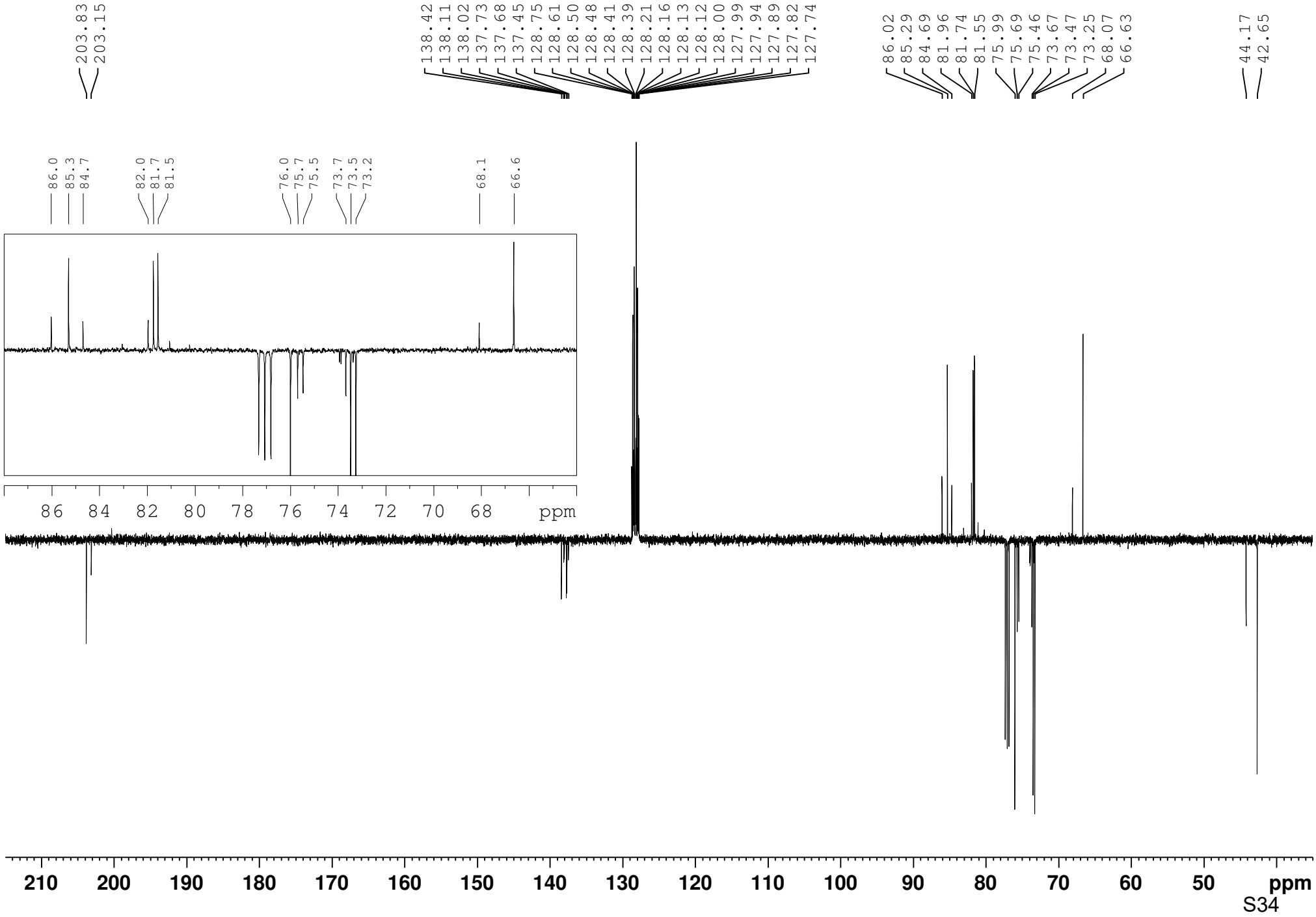
Compound 8 - 13C



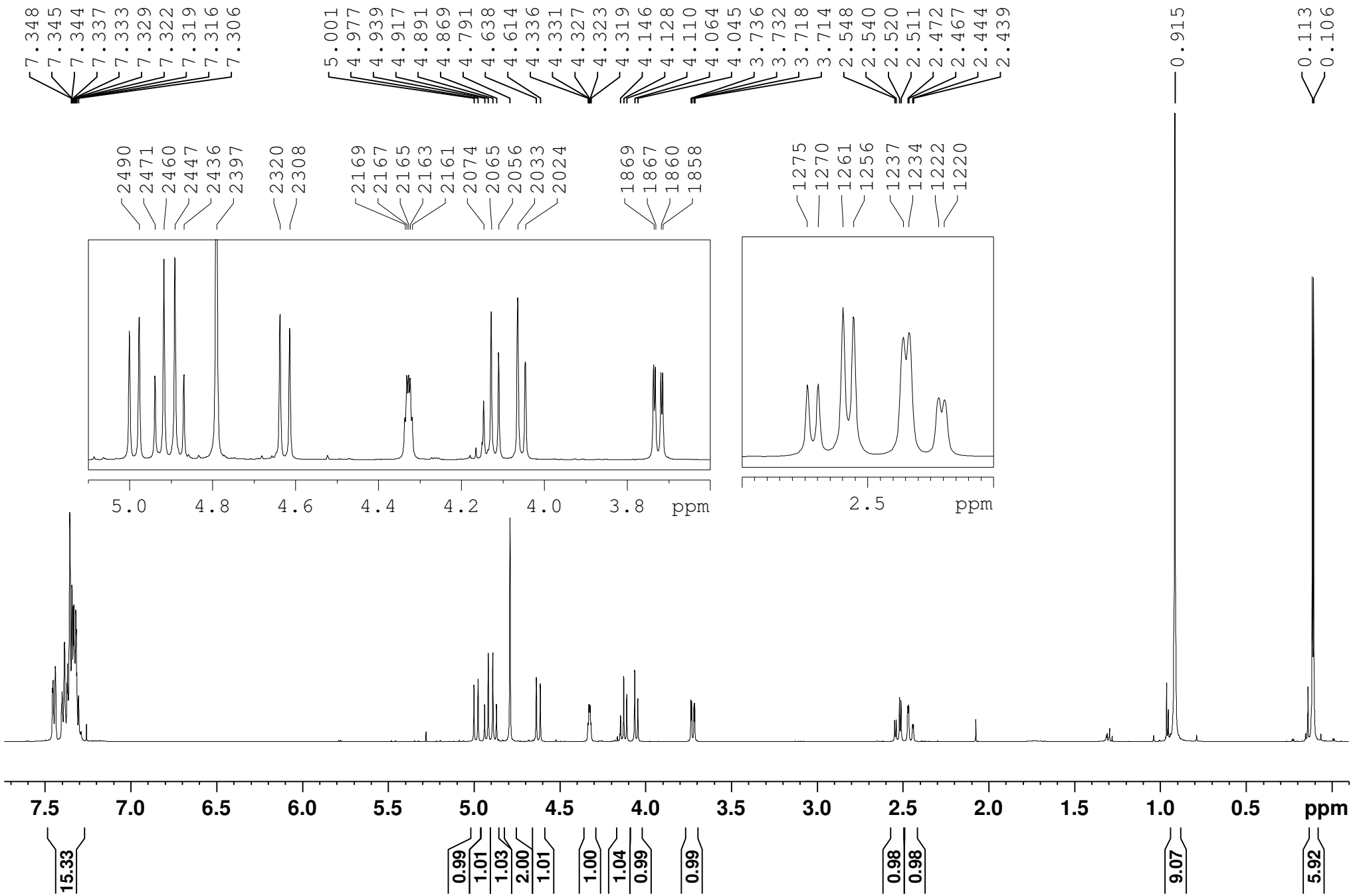
Compound 10 - 1H



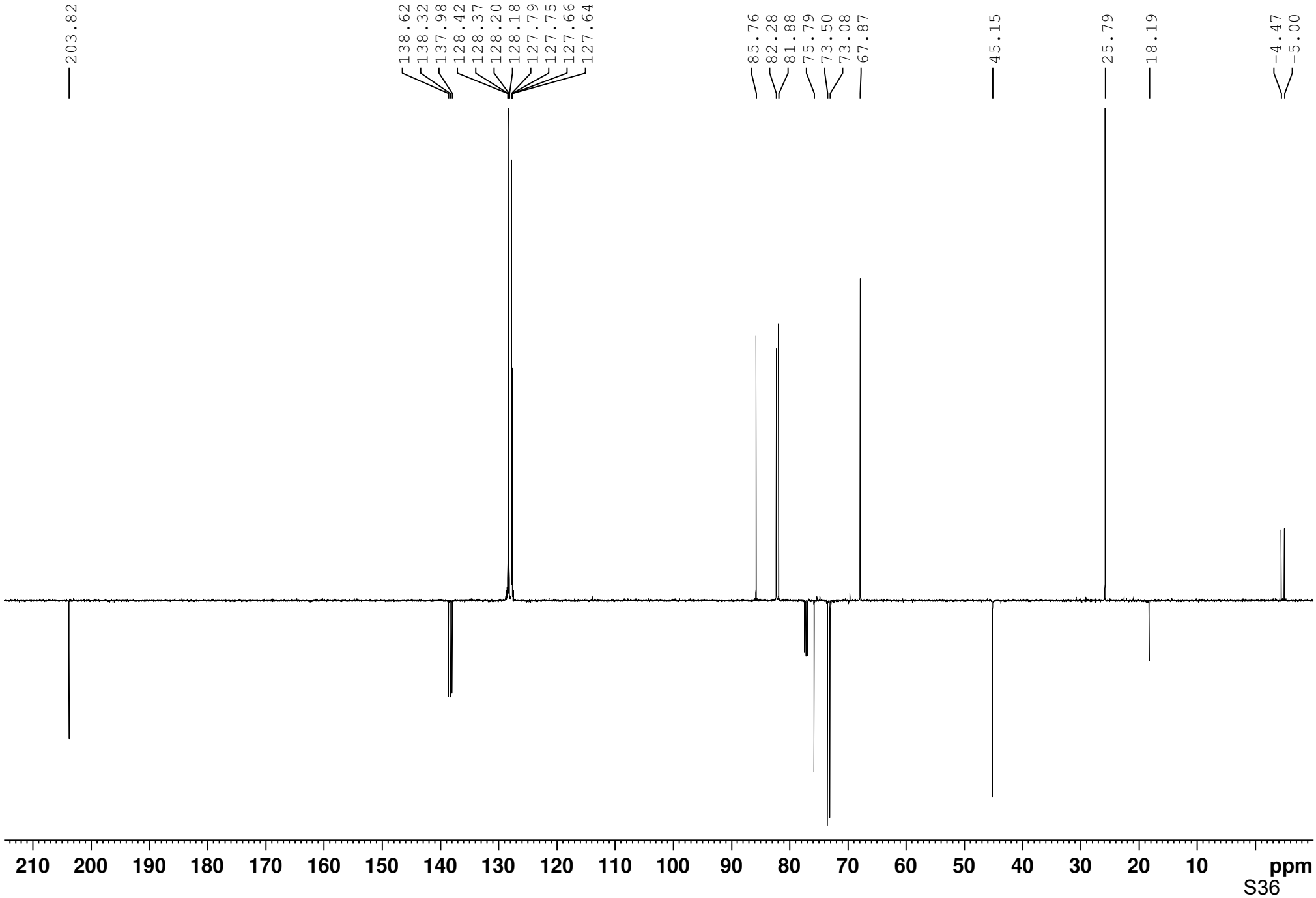
Compound 10 - 13C



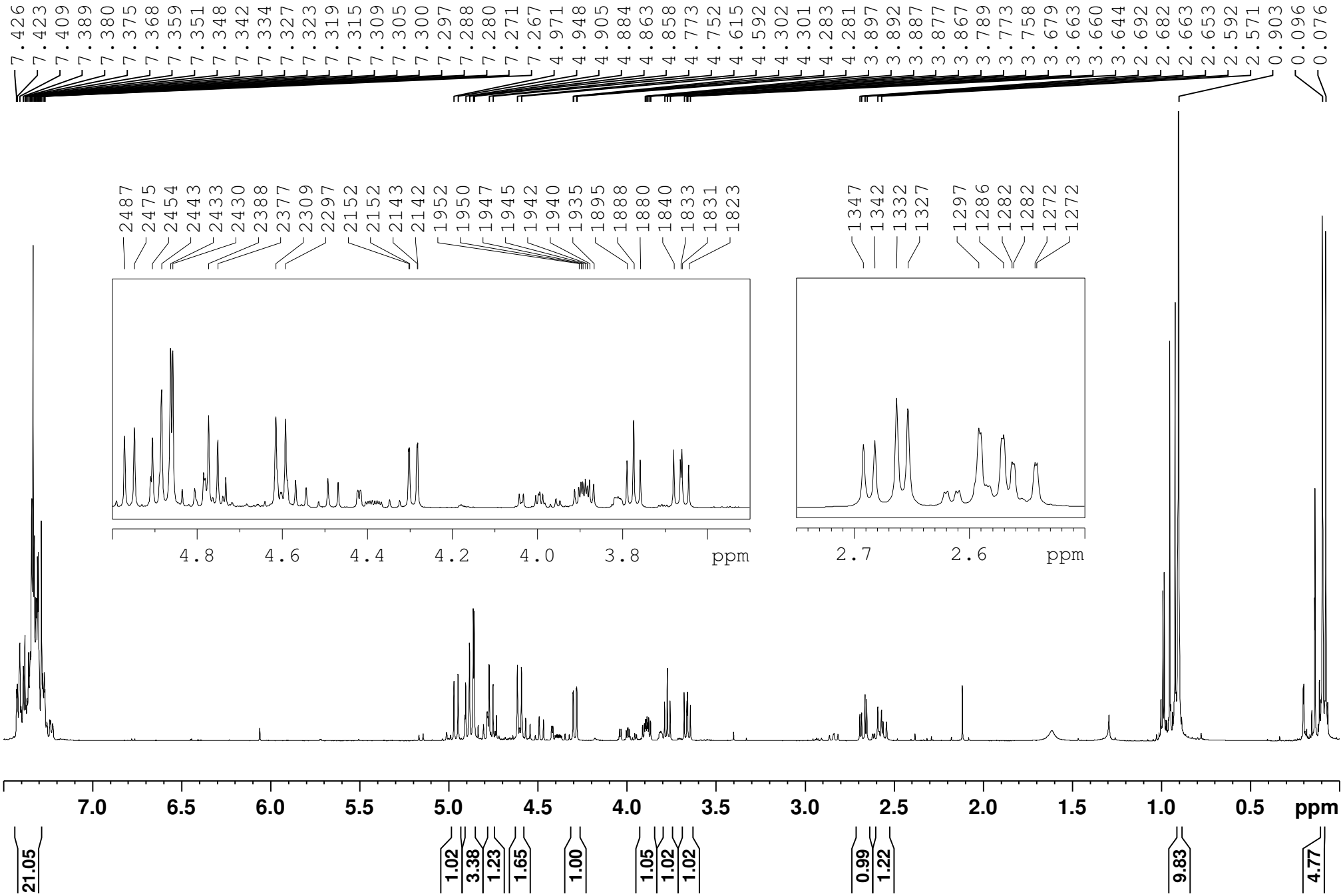
Compound 11a - 1H



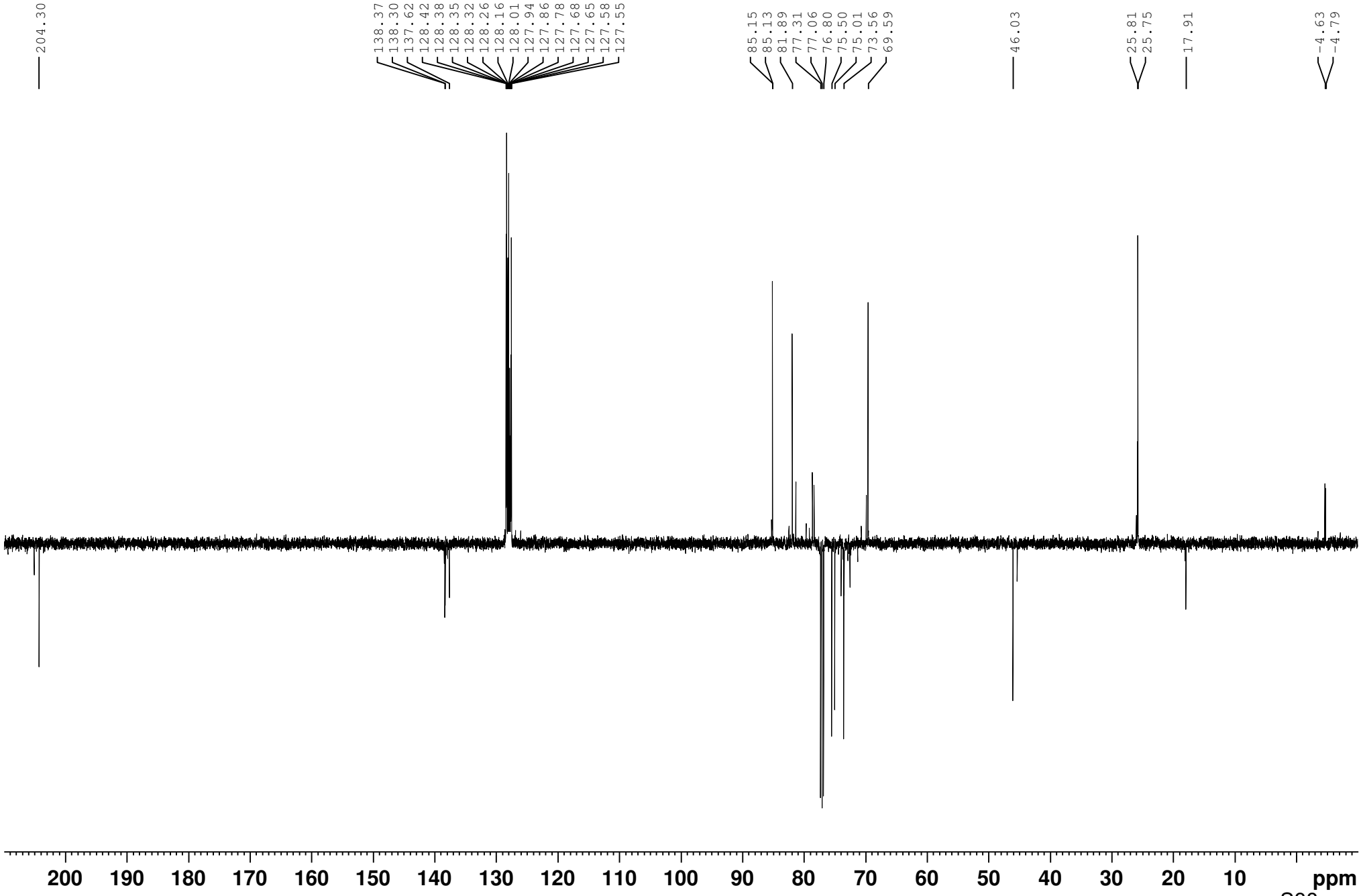
Compound 11a - 13C



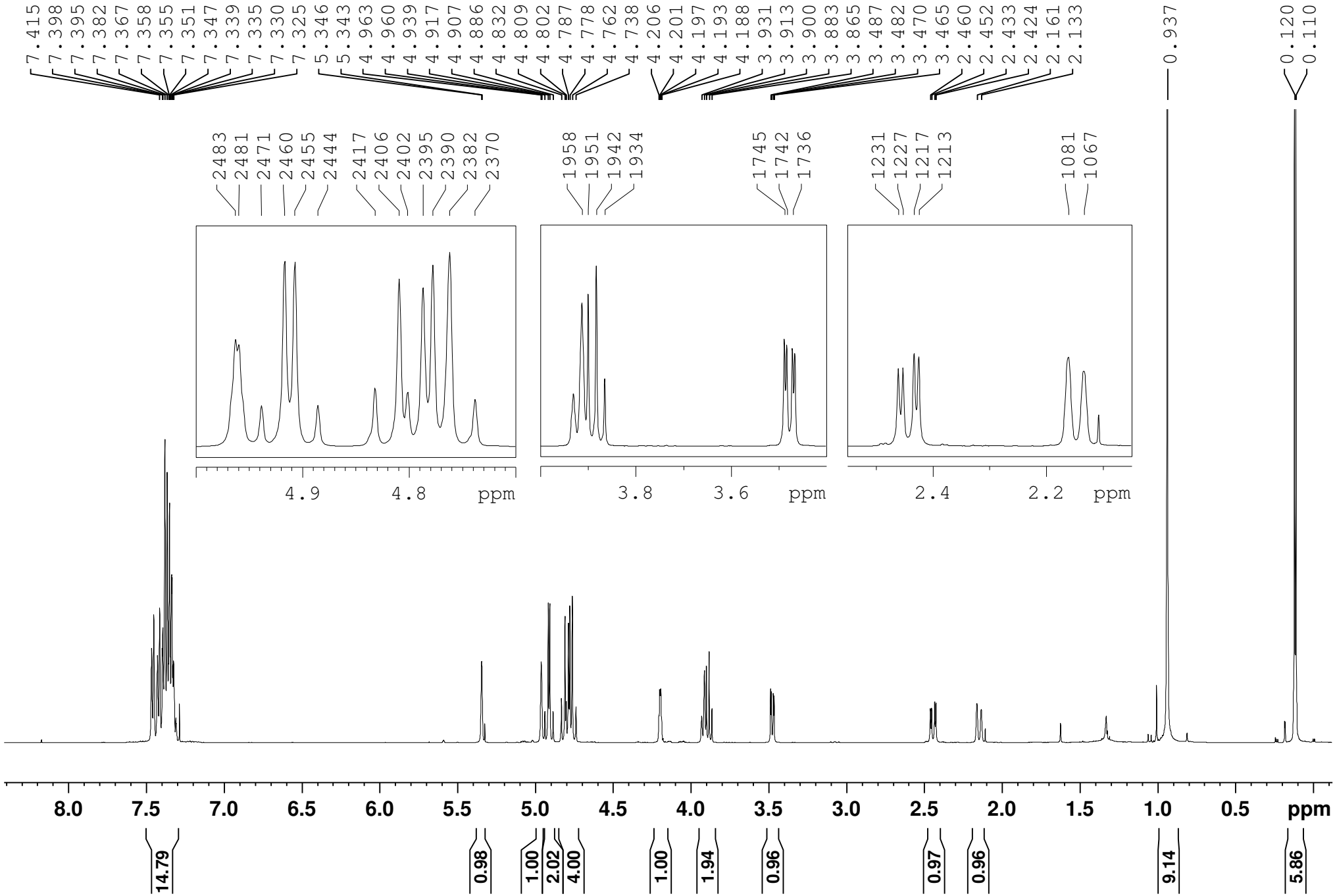
Compound 11b - 1H



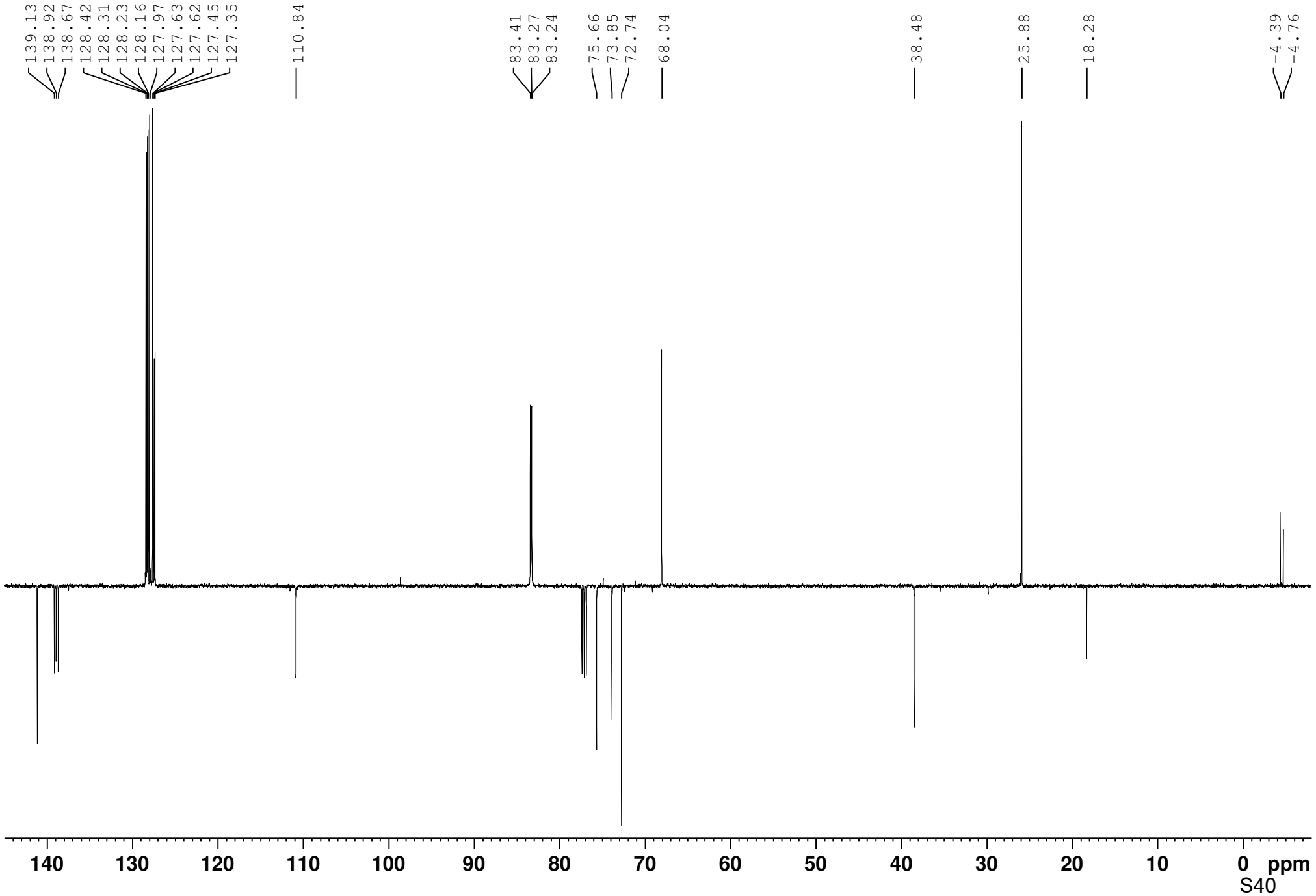
Compound 11b - 13C



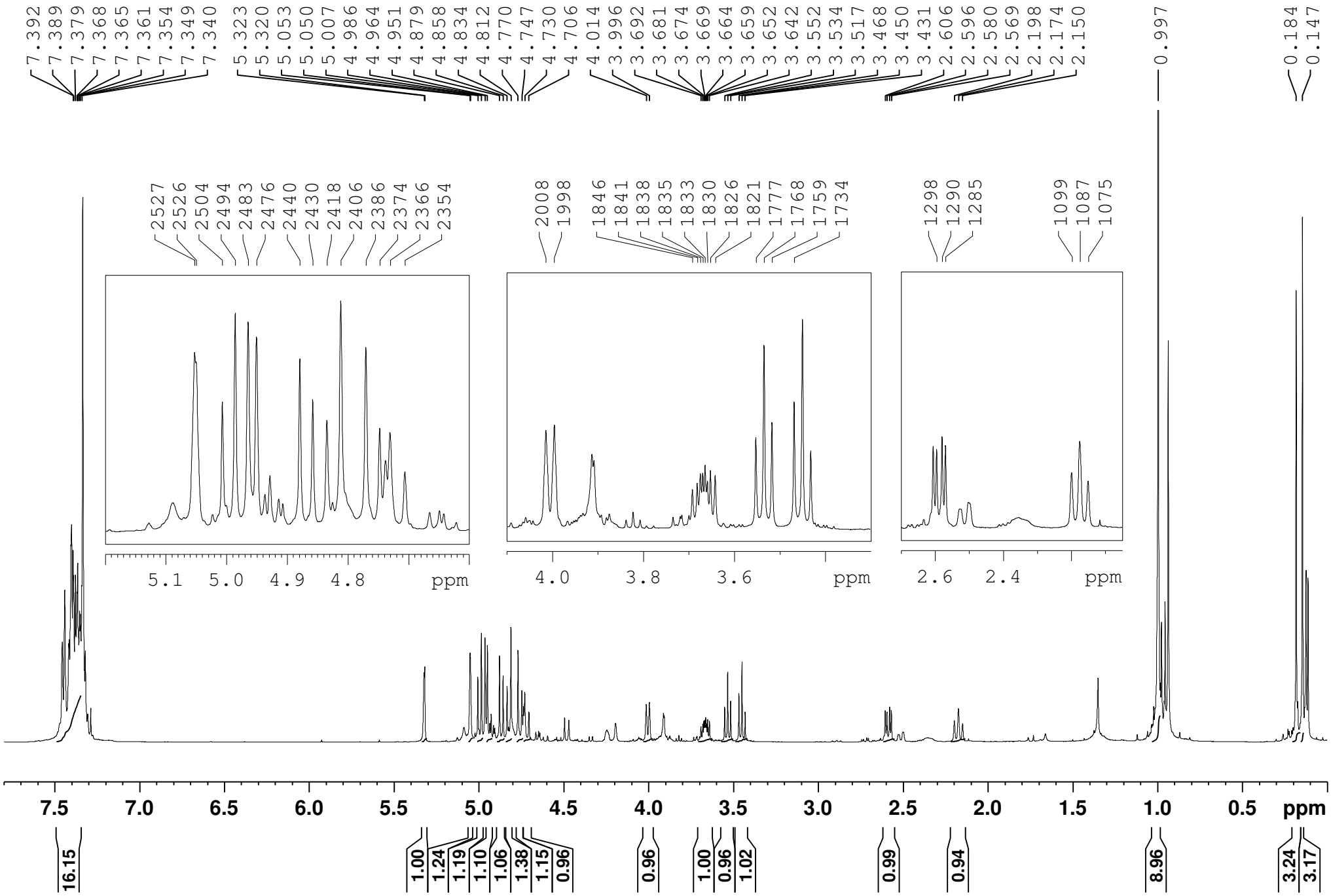
Compound 12a - 1H



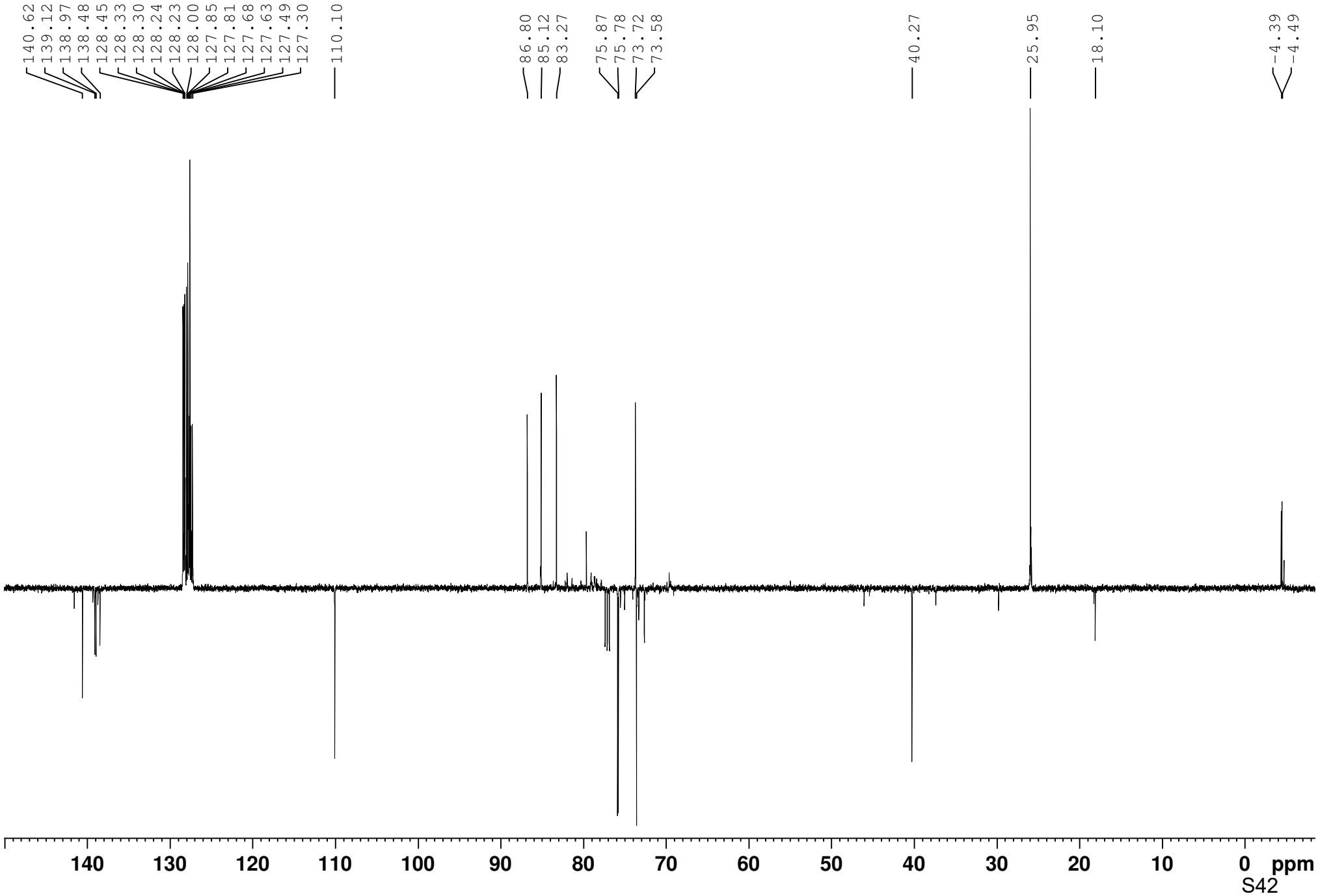
Compound 12a - 13C



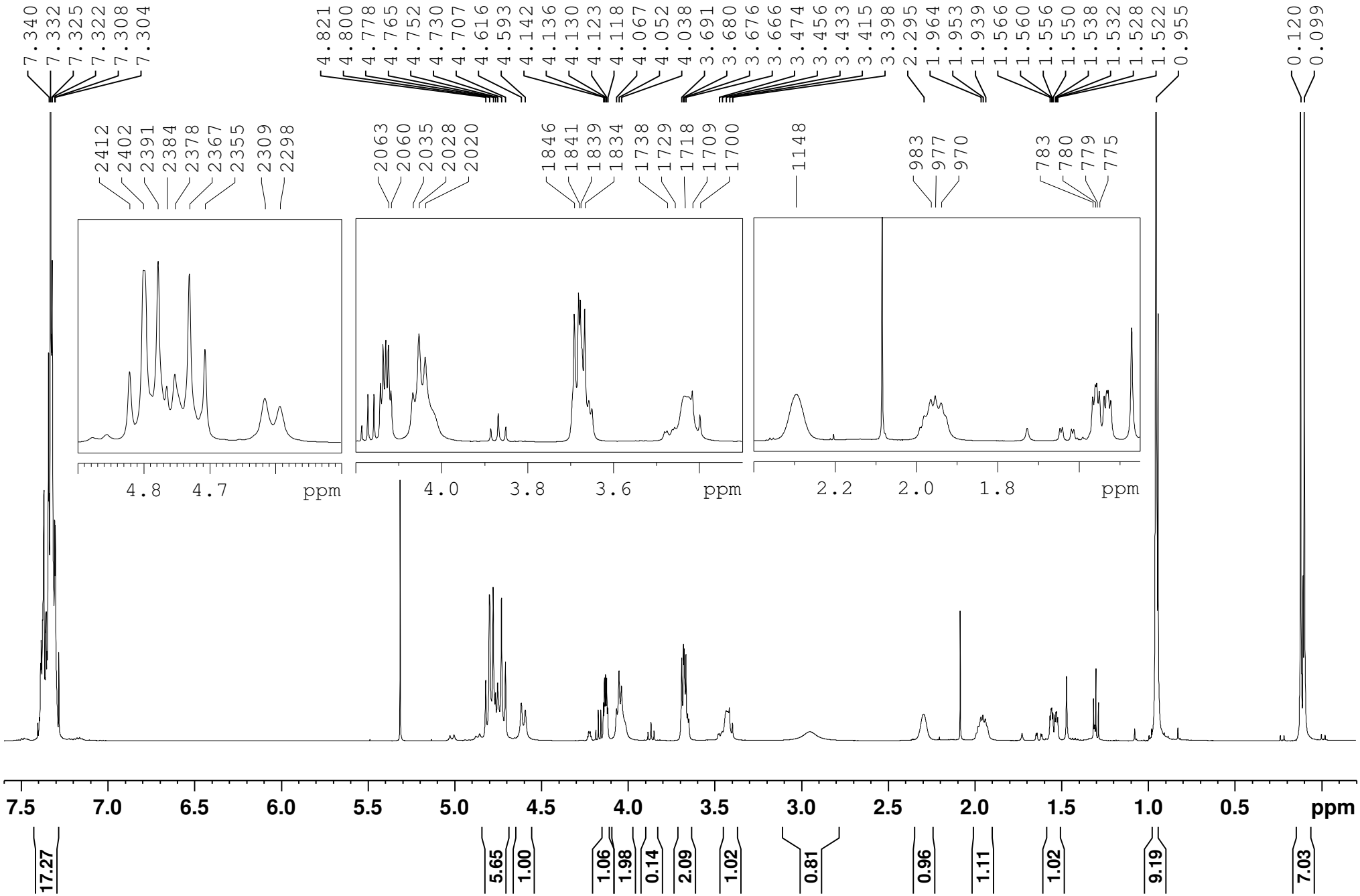
Compound 12b - 1H



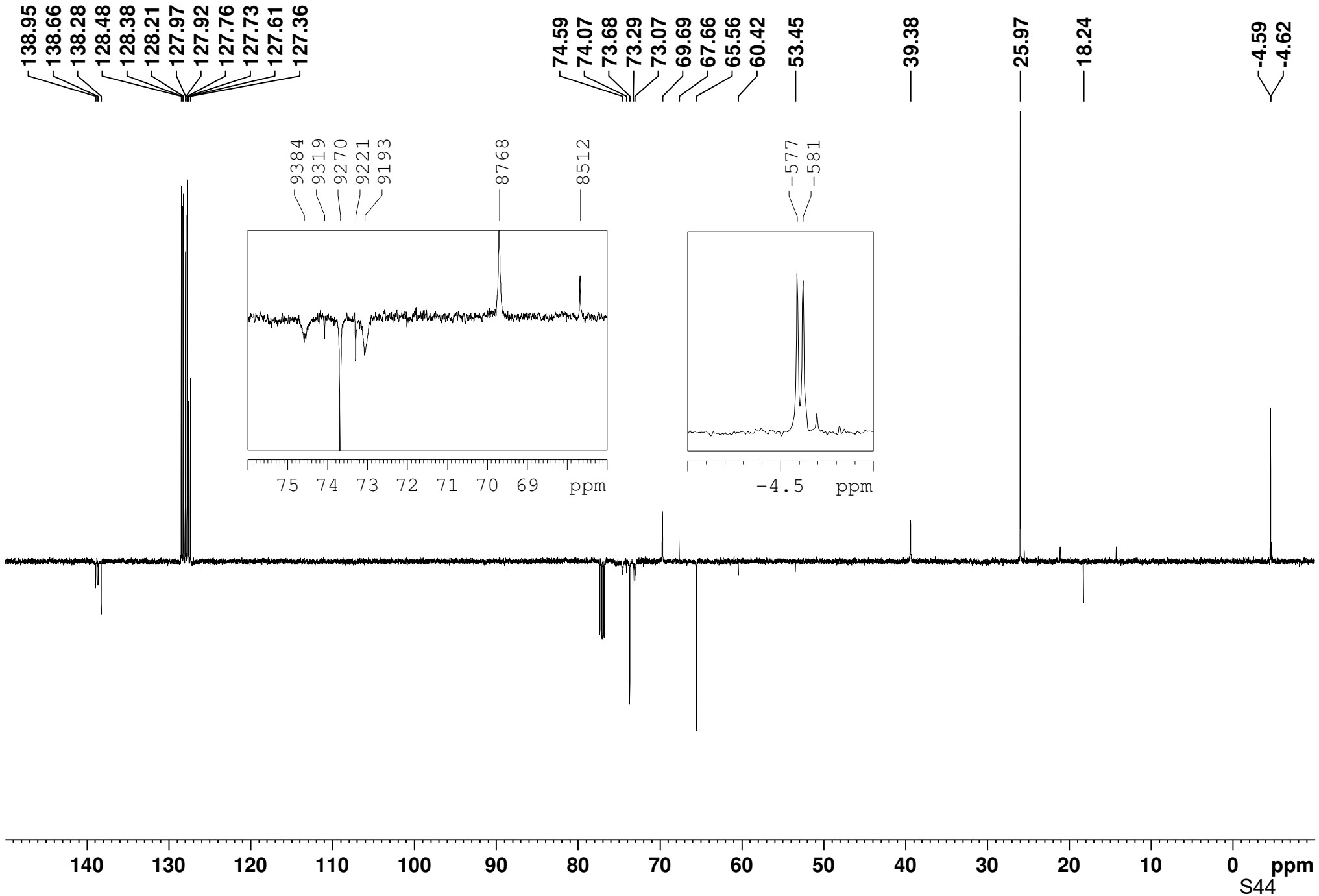
Compound 12b - 13C



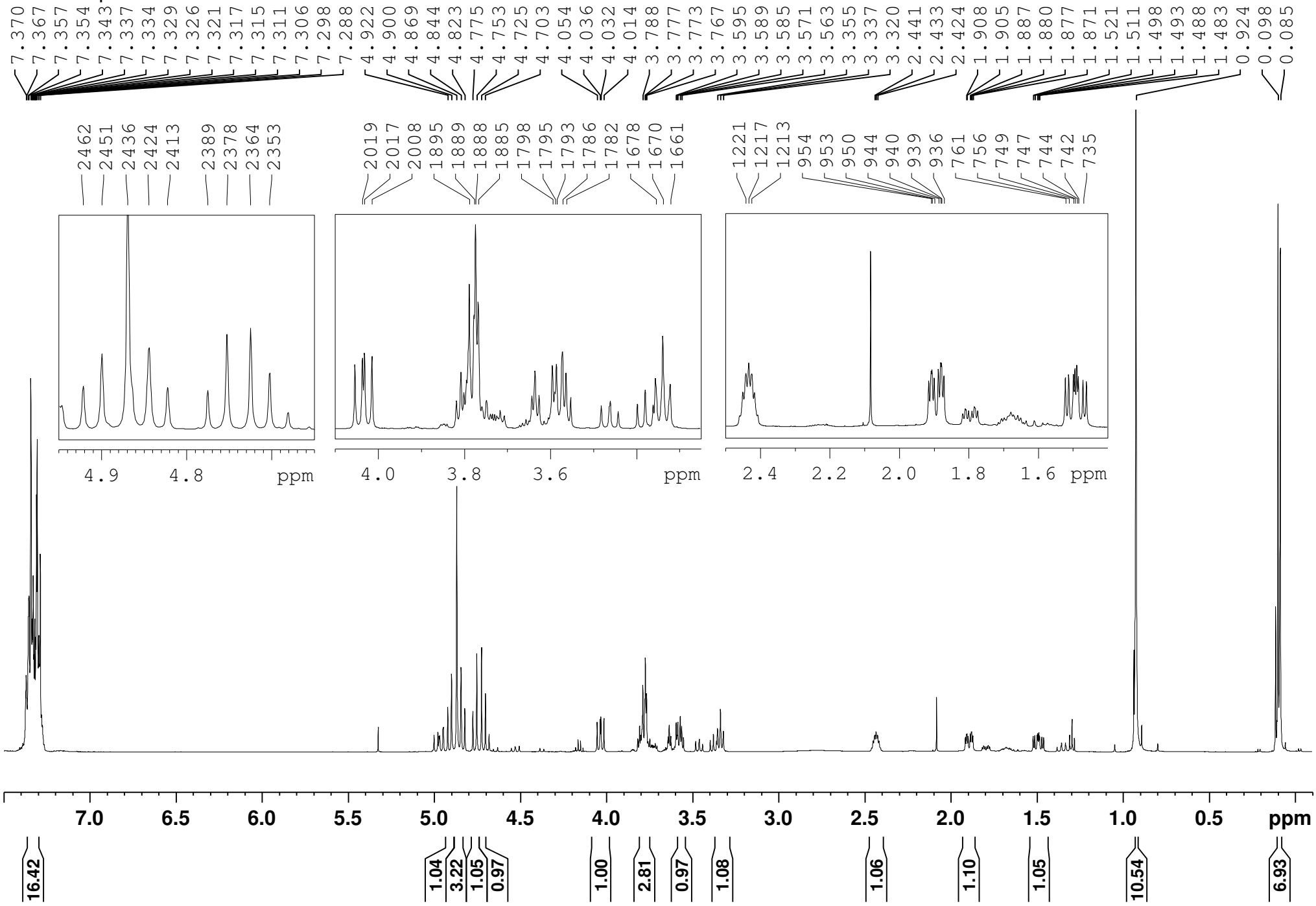
Compound 13a - 1H



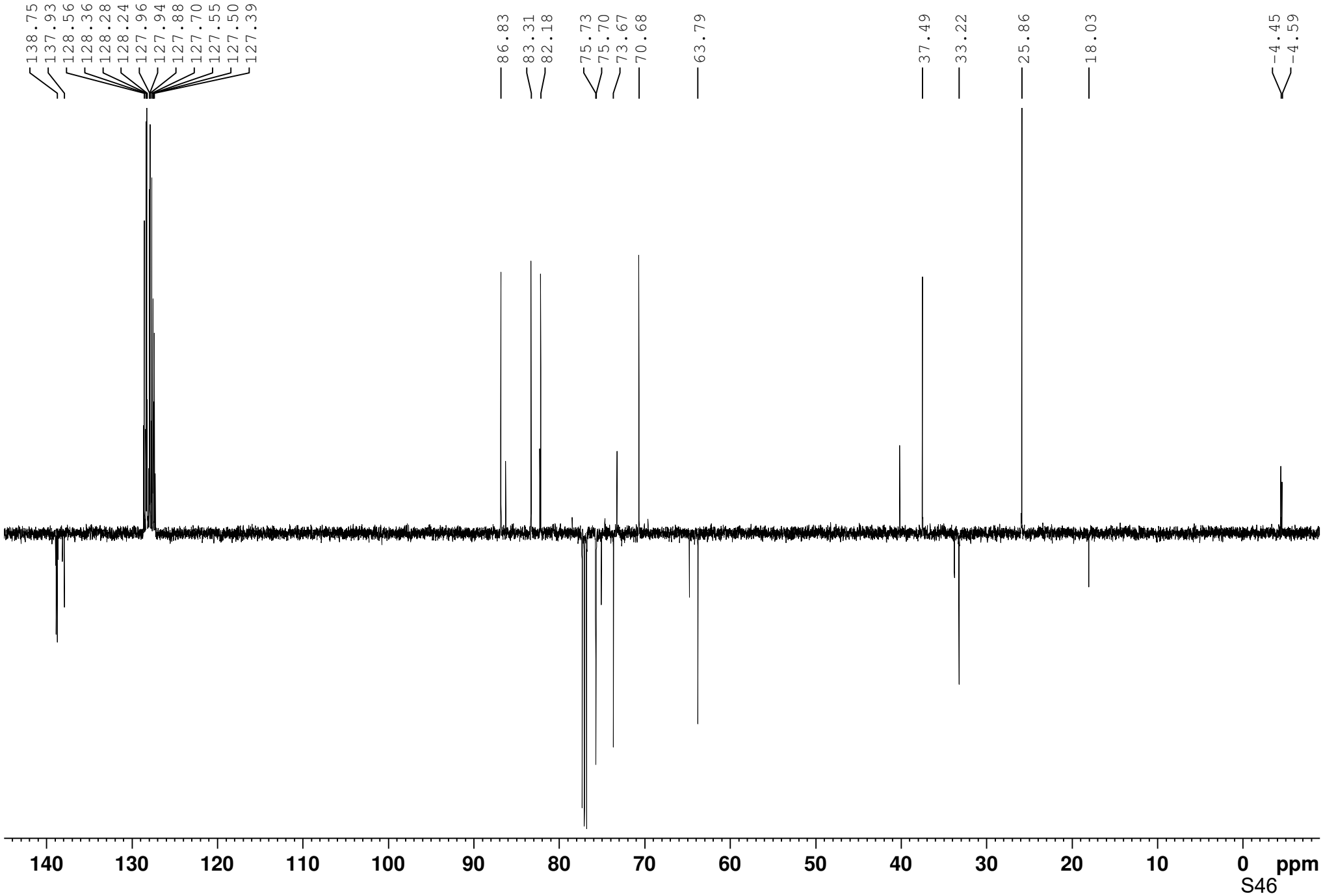
Compound 13a - 13C



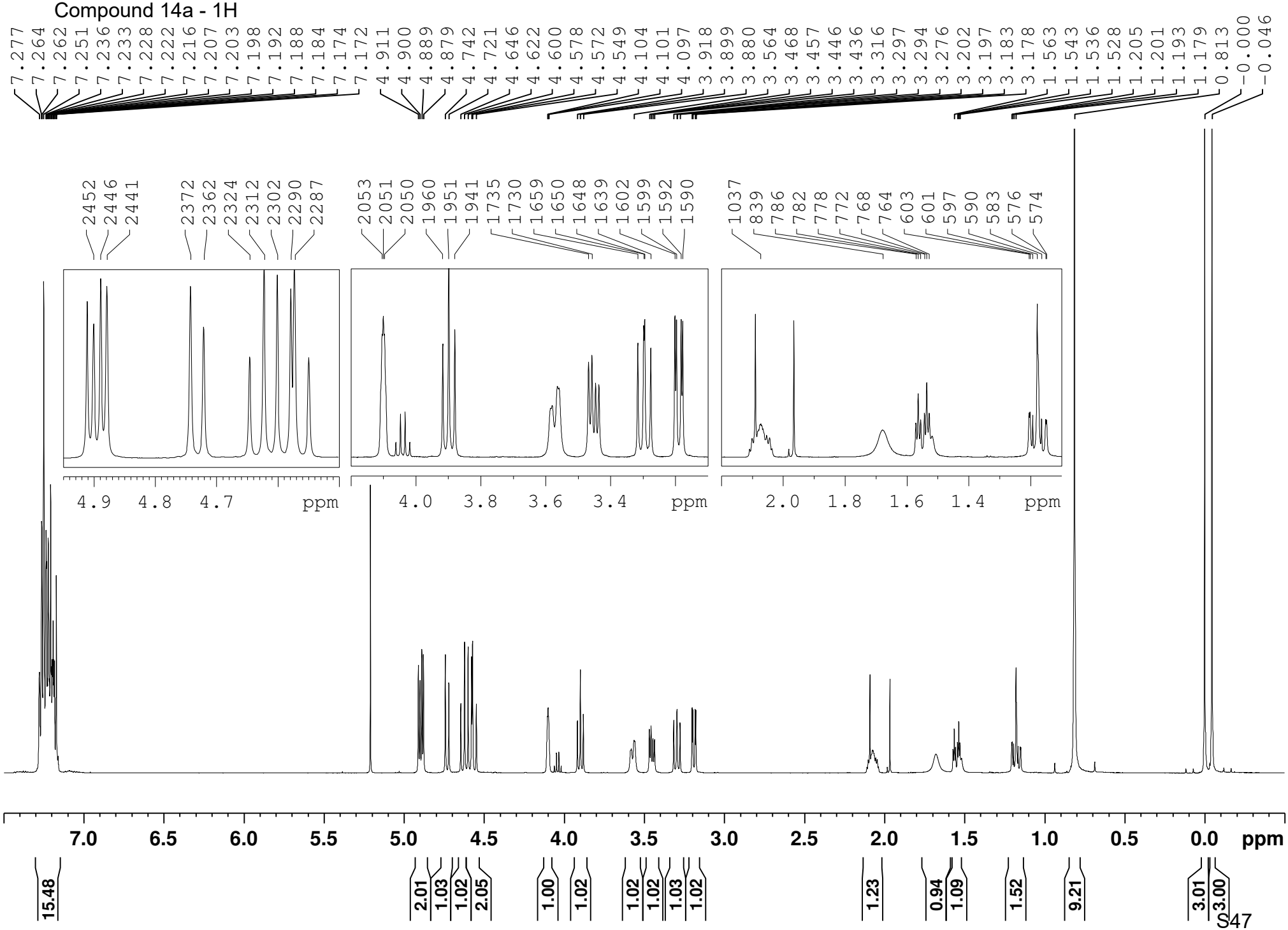
Compound 13b - 1H



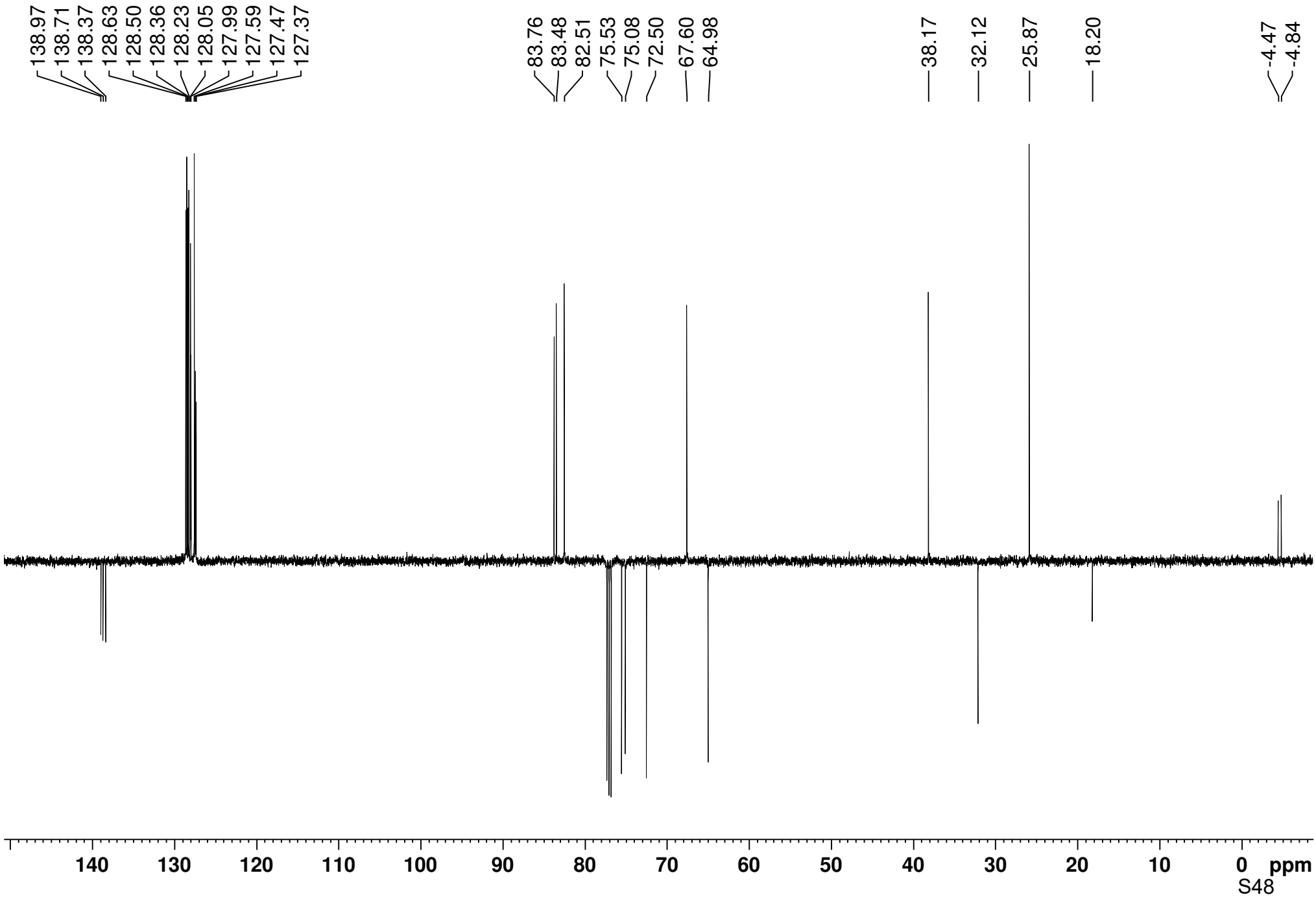
Compound 13b - 13C



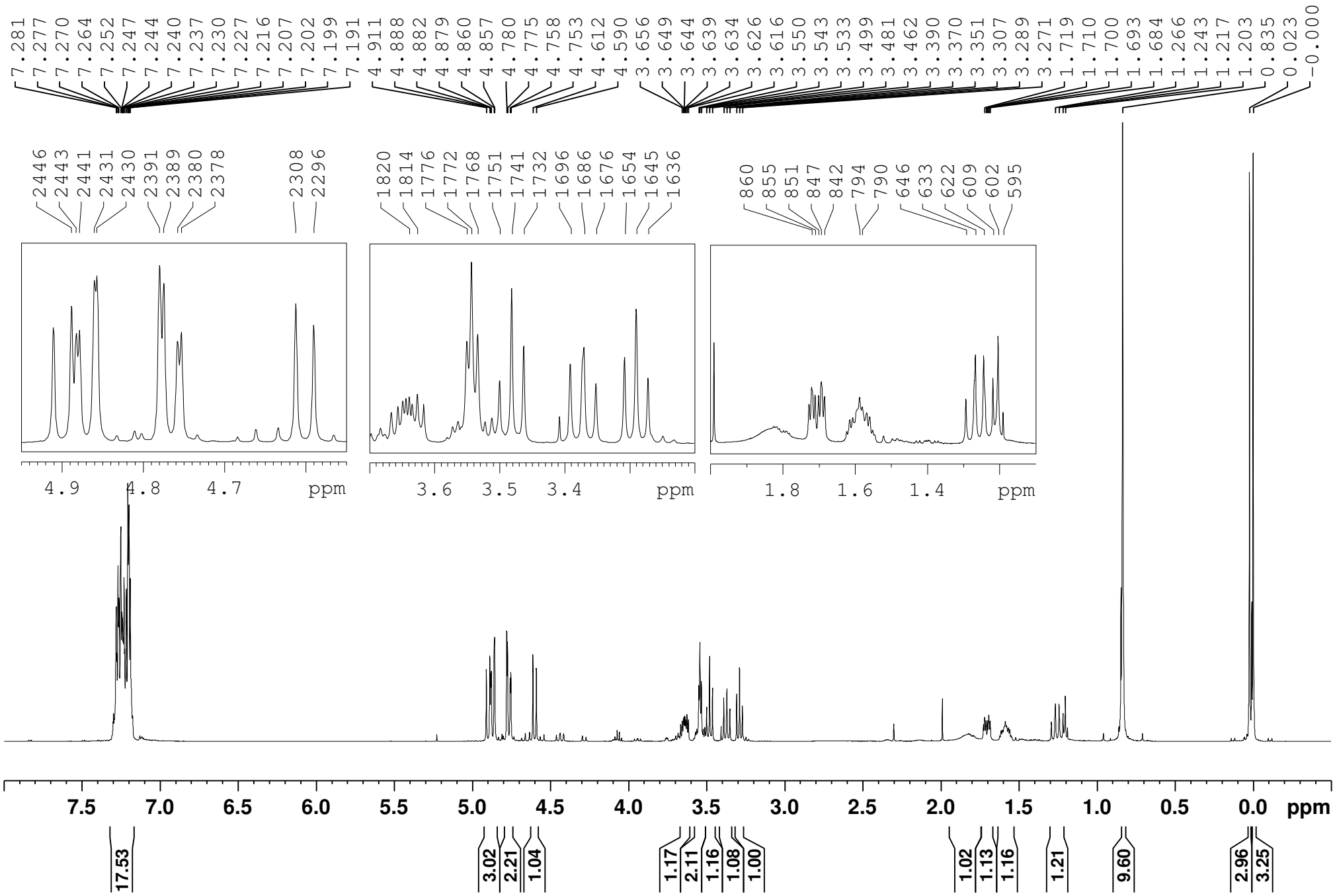
Compound 14a - 1H



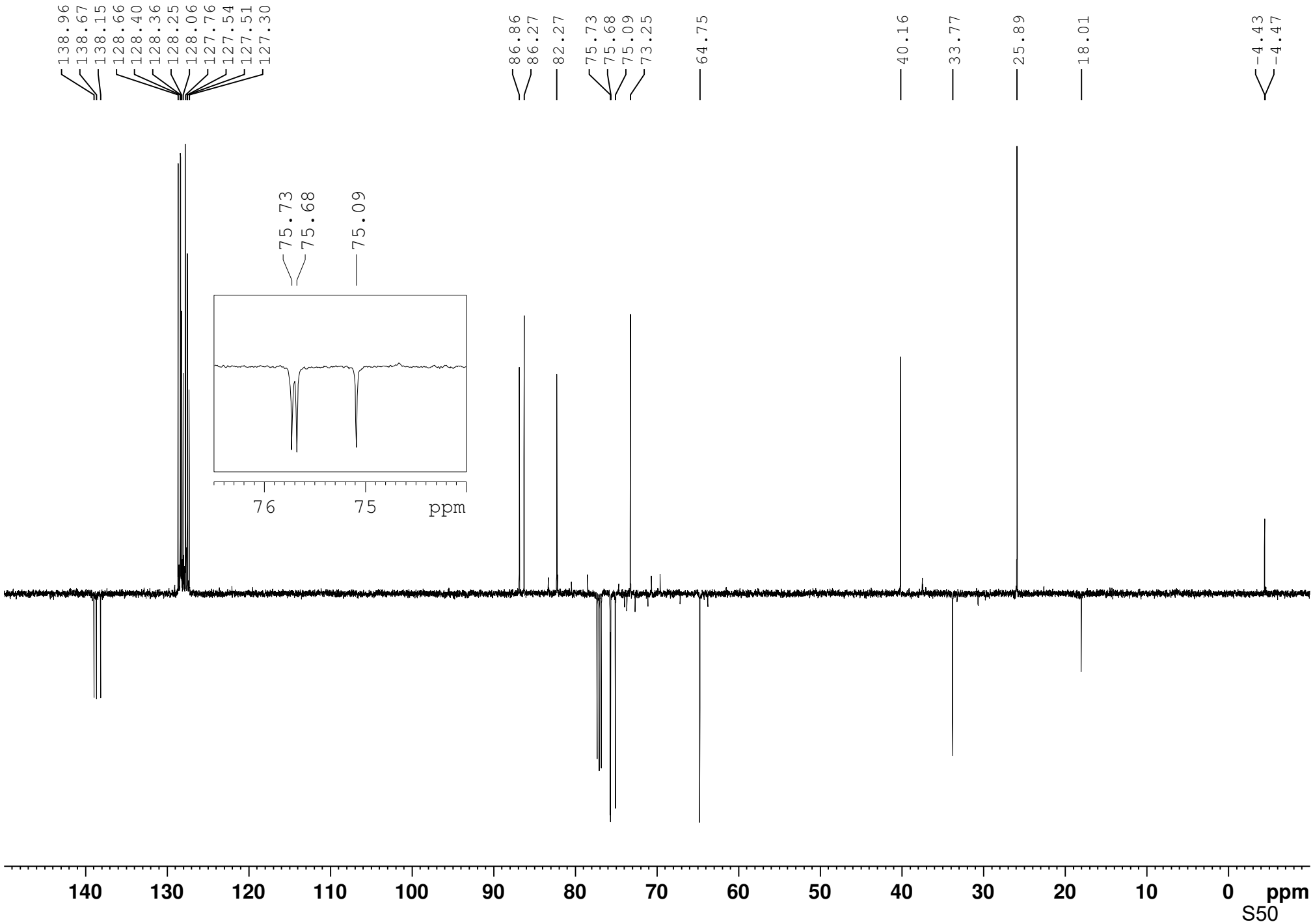
Compound 14a - 13C



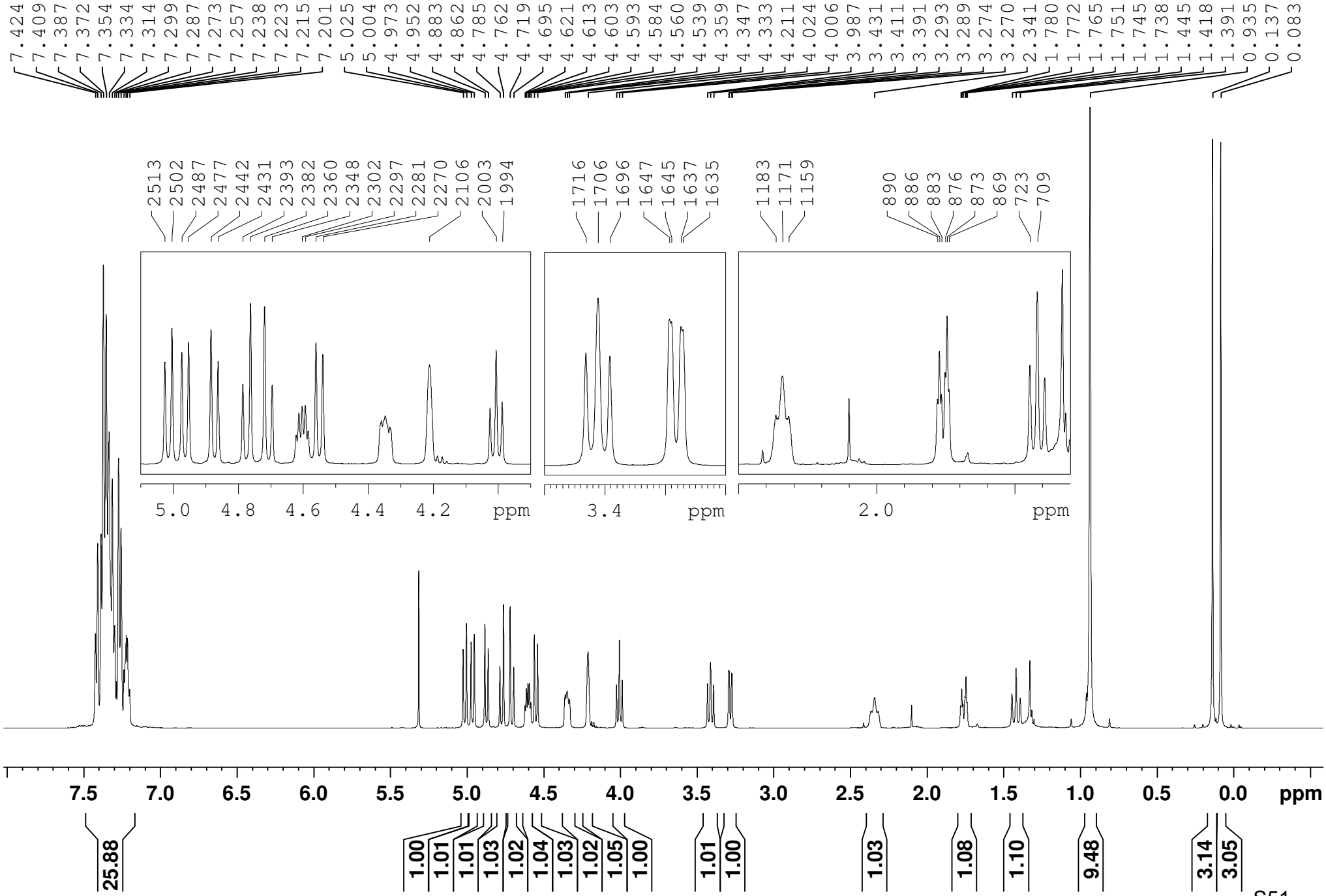
Compound 14b - 1H



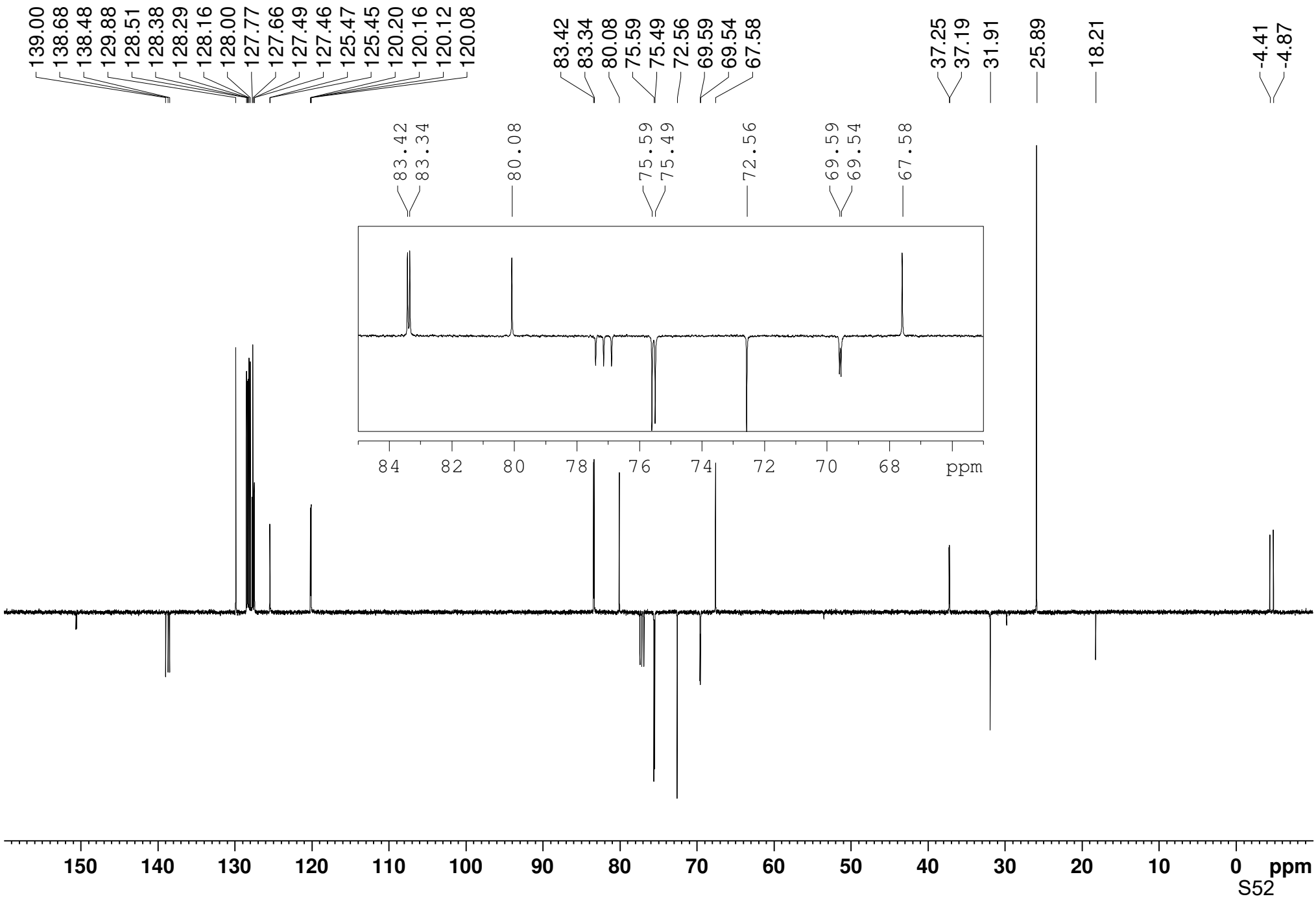
Compound 14b - 13C



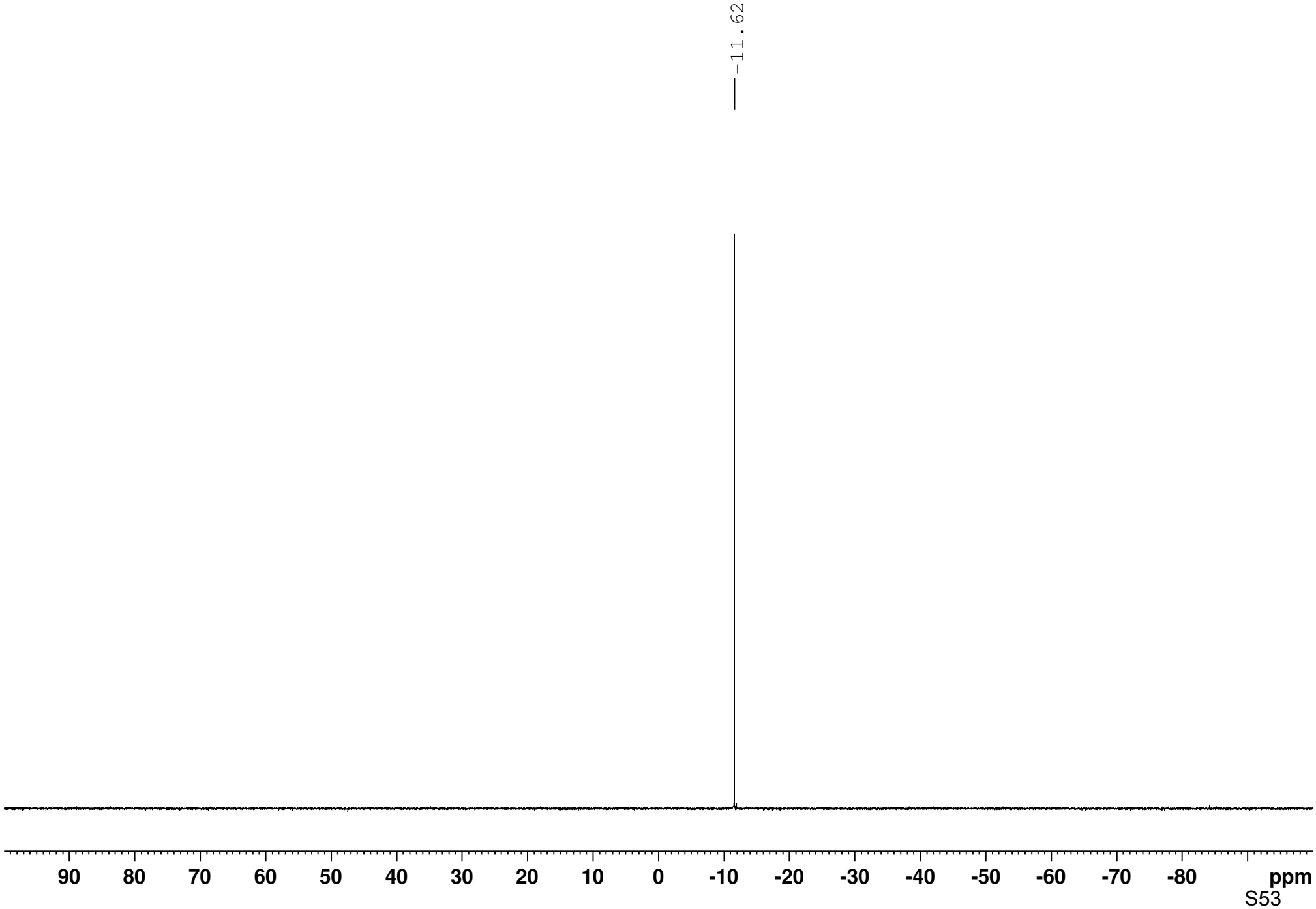
Compound 15a - 1H



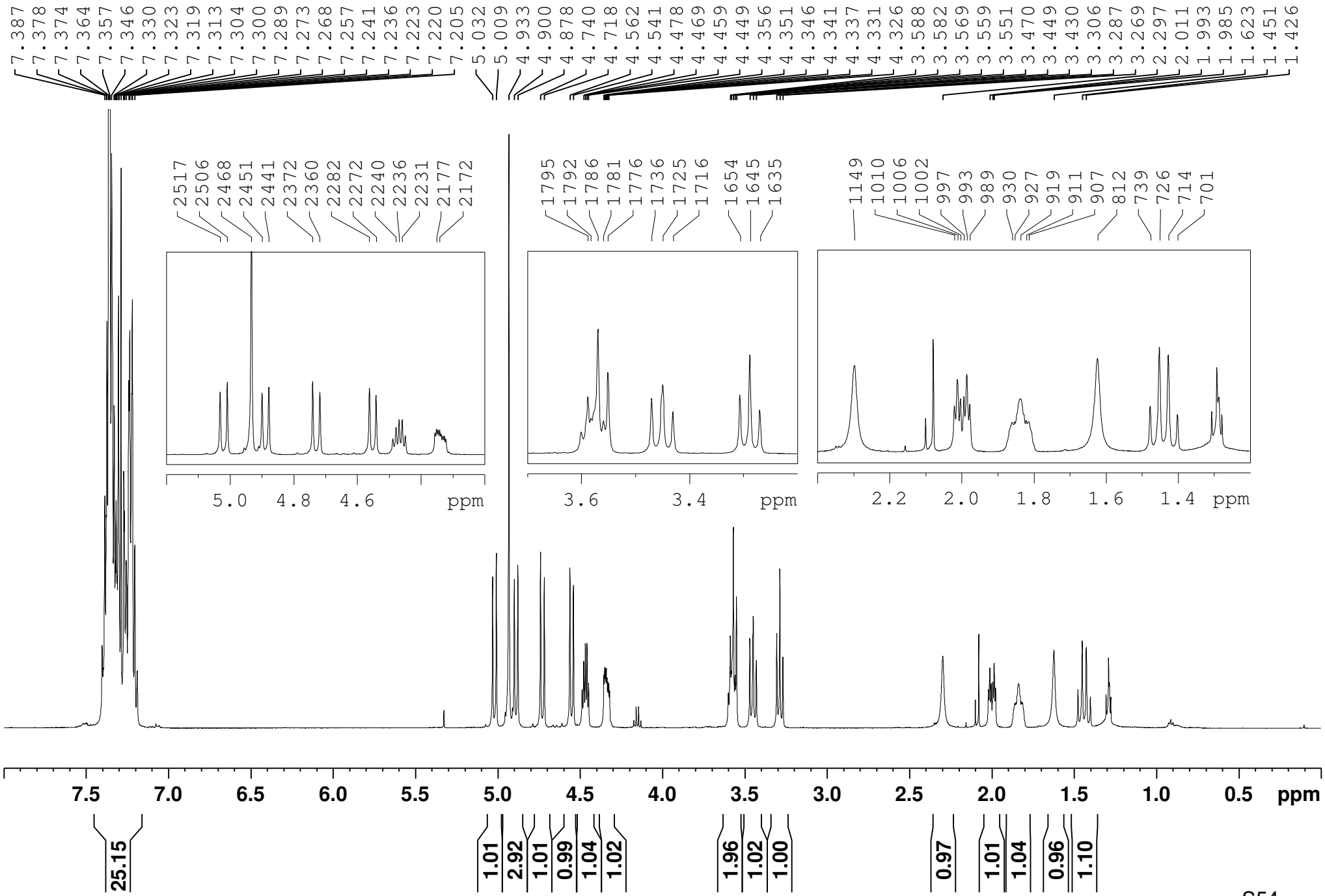
Compound 15a - 13C



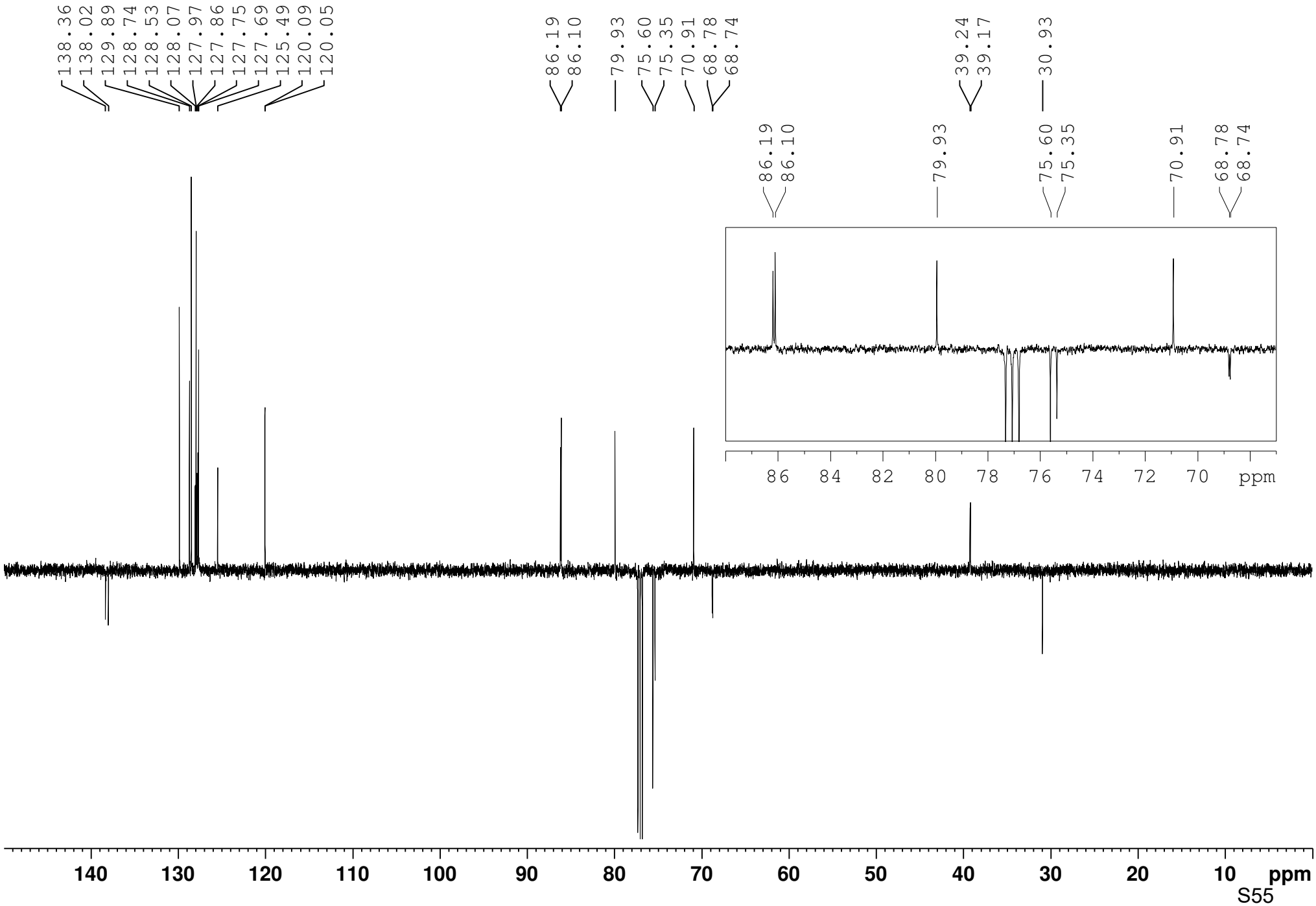
Compound 15a - 31P



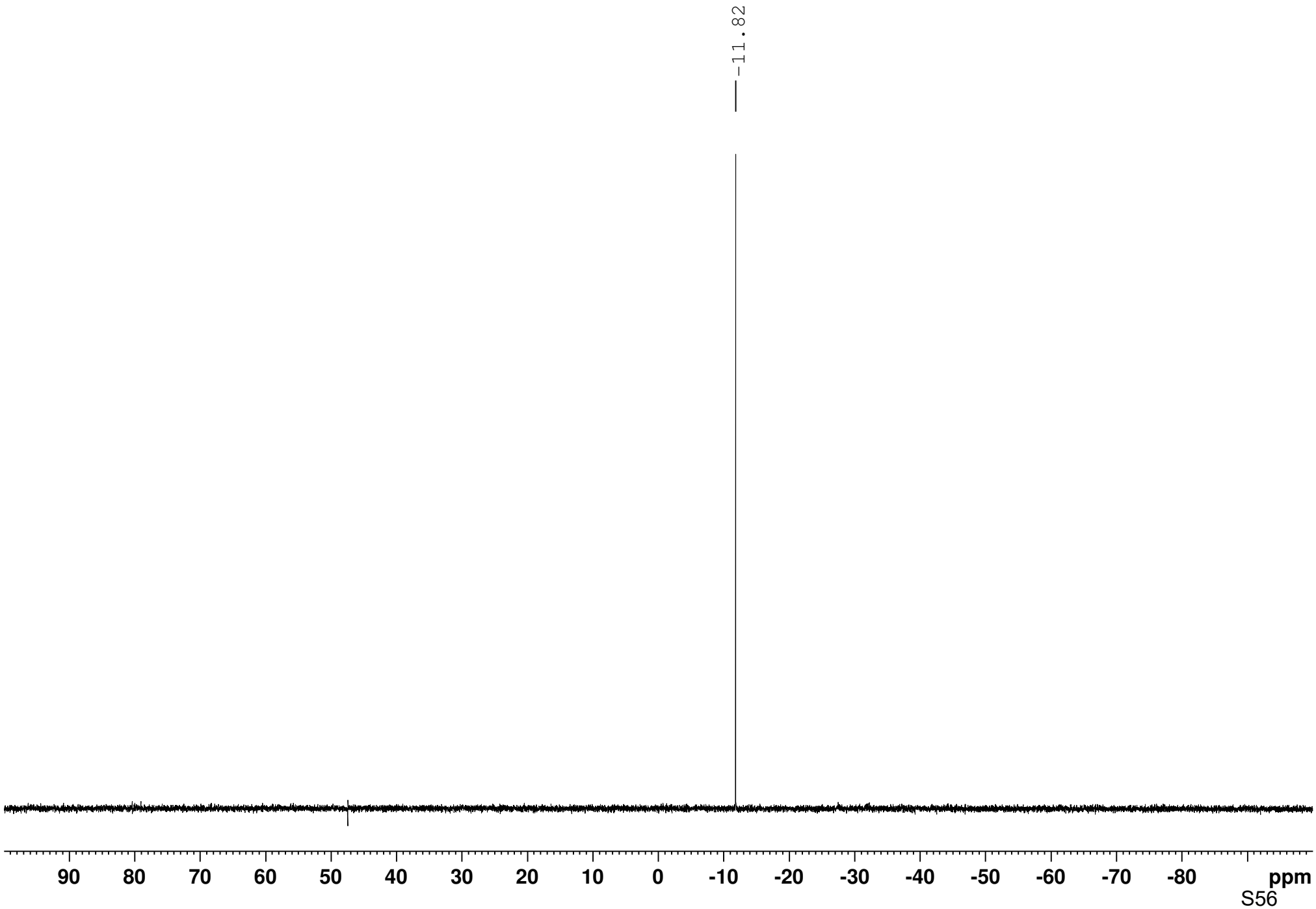
Compound 16 - 1H



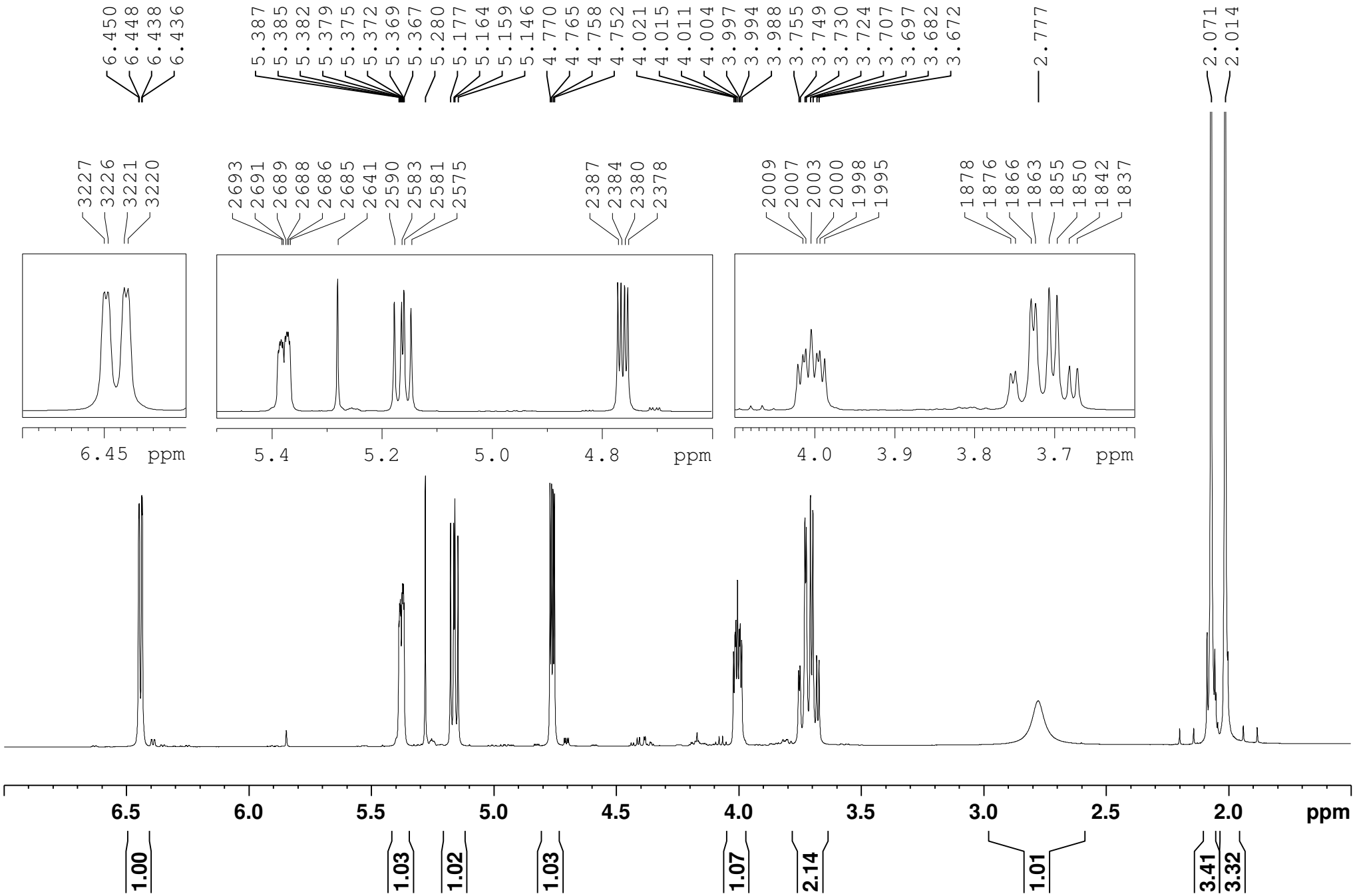
Compound 16 - 13C



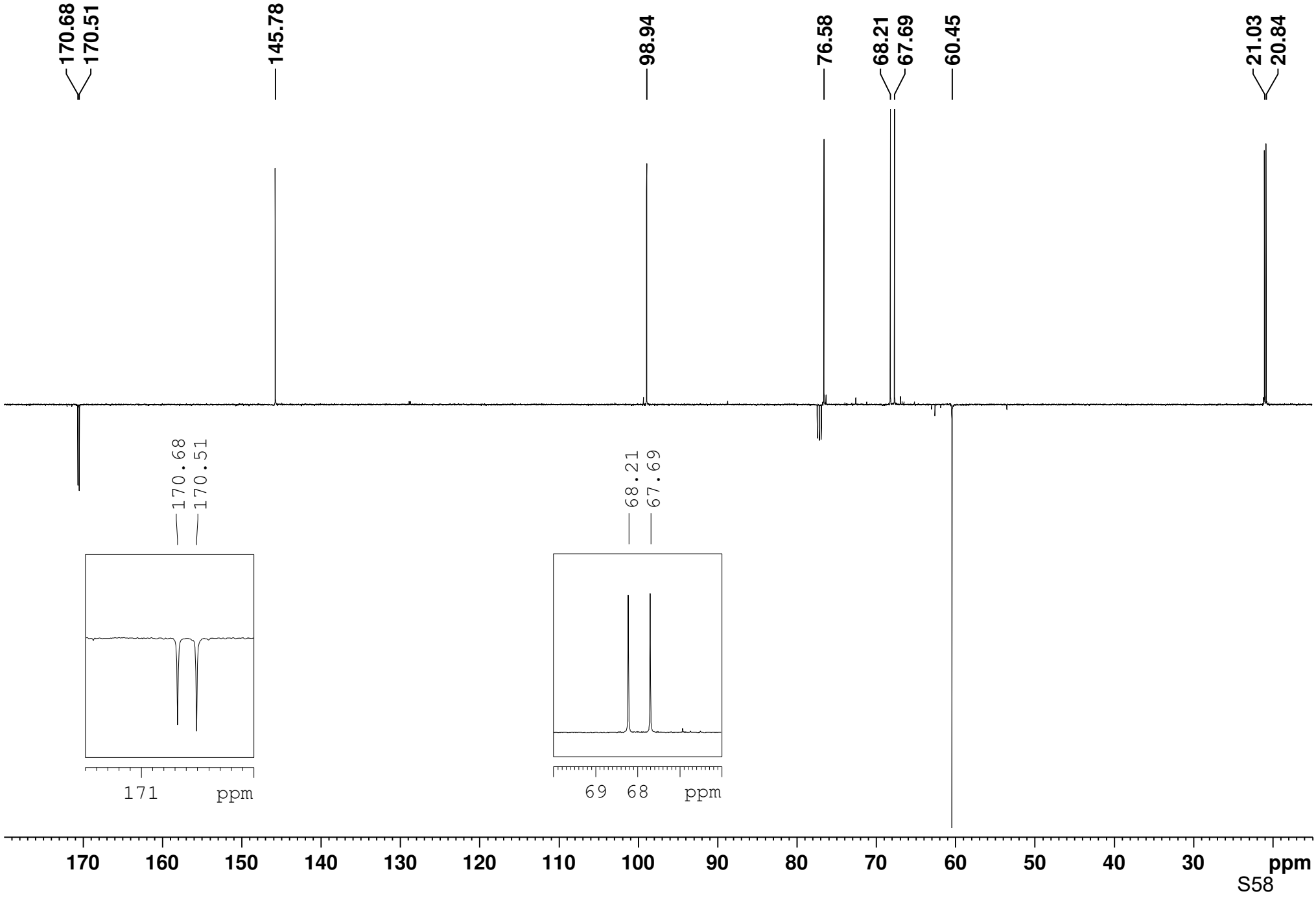
Compound 16 - 31P



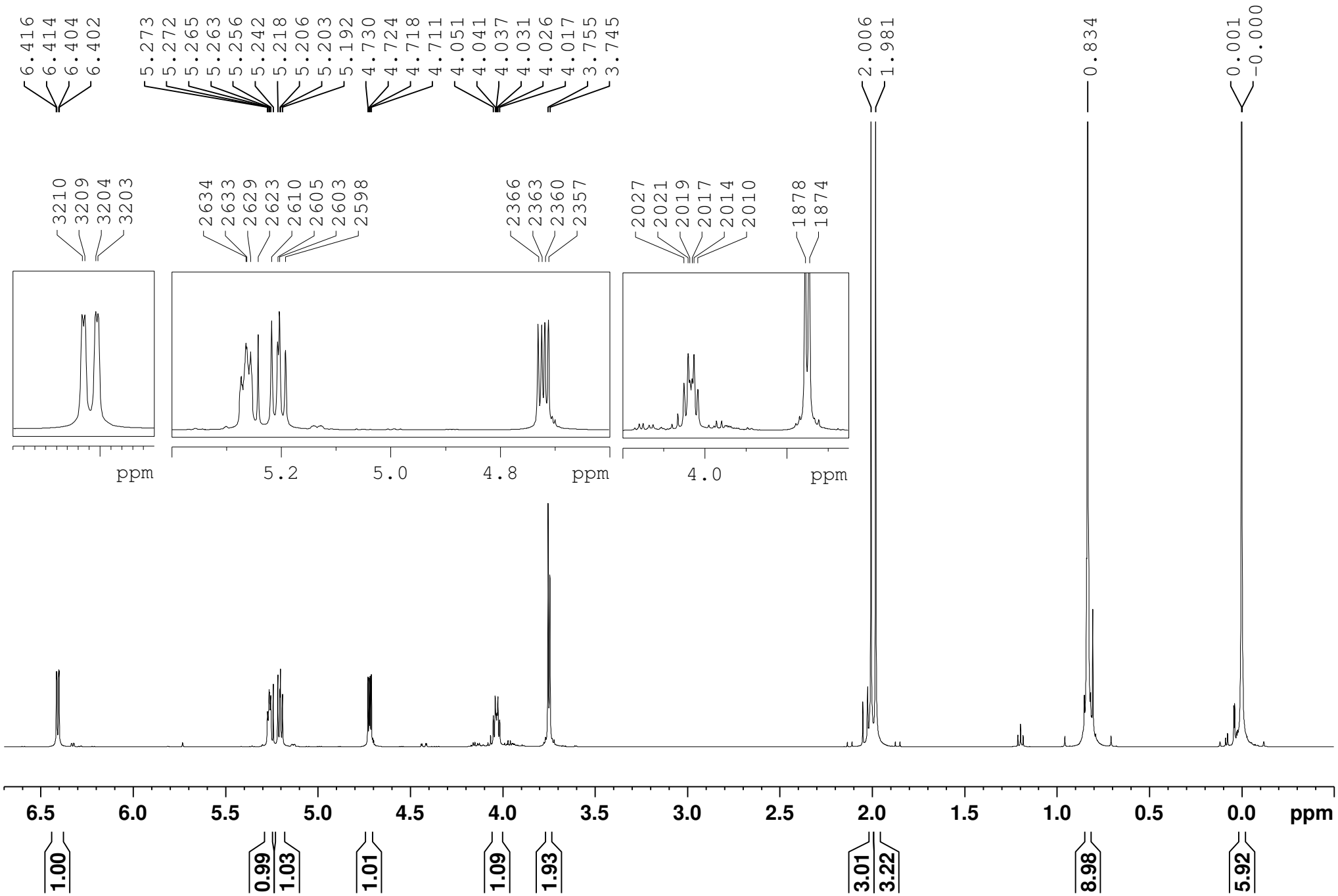
Compound 18 - 1H



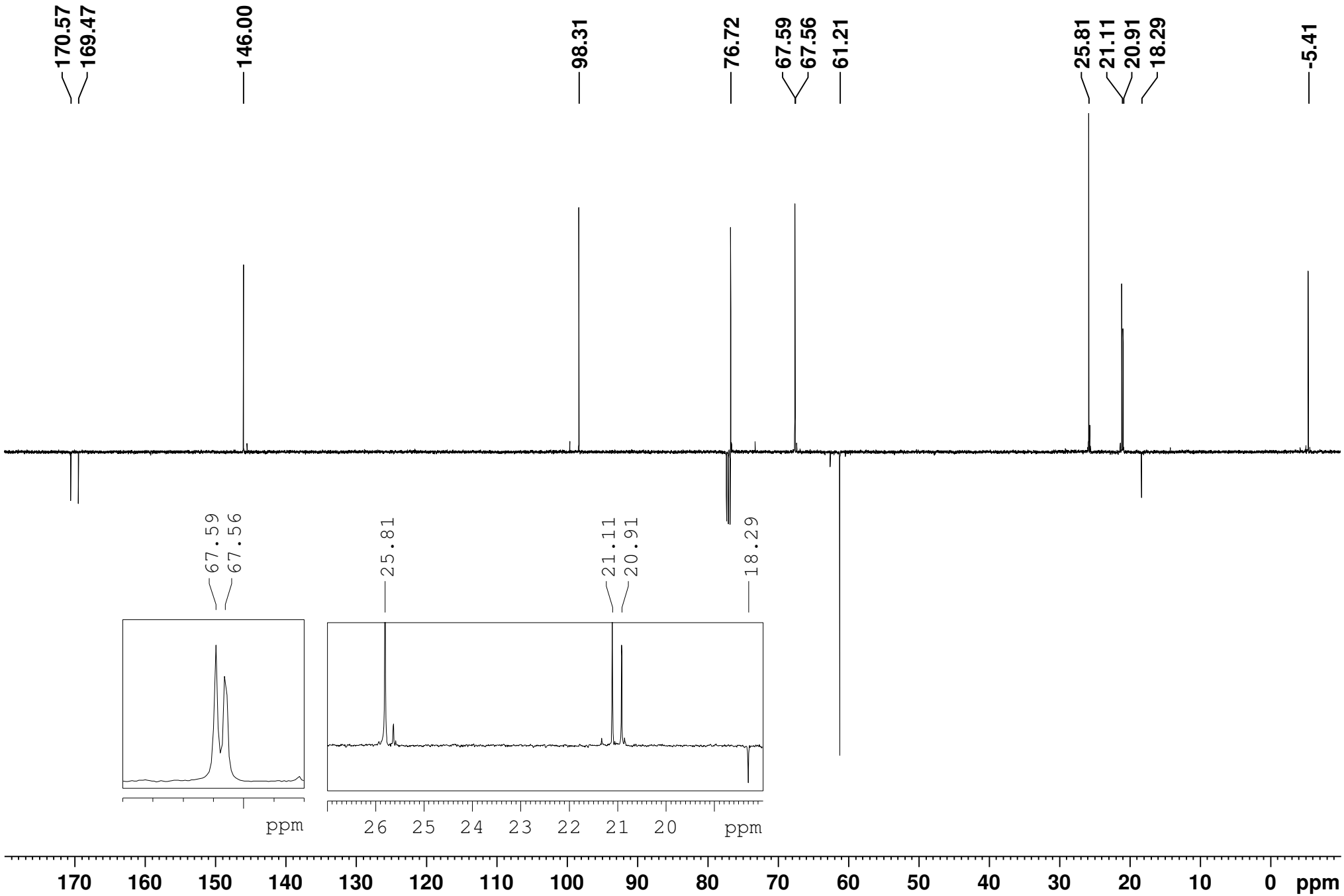
Compound 18 - 13C



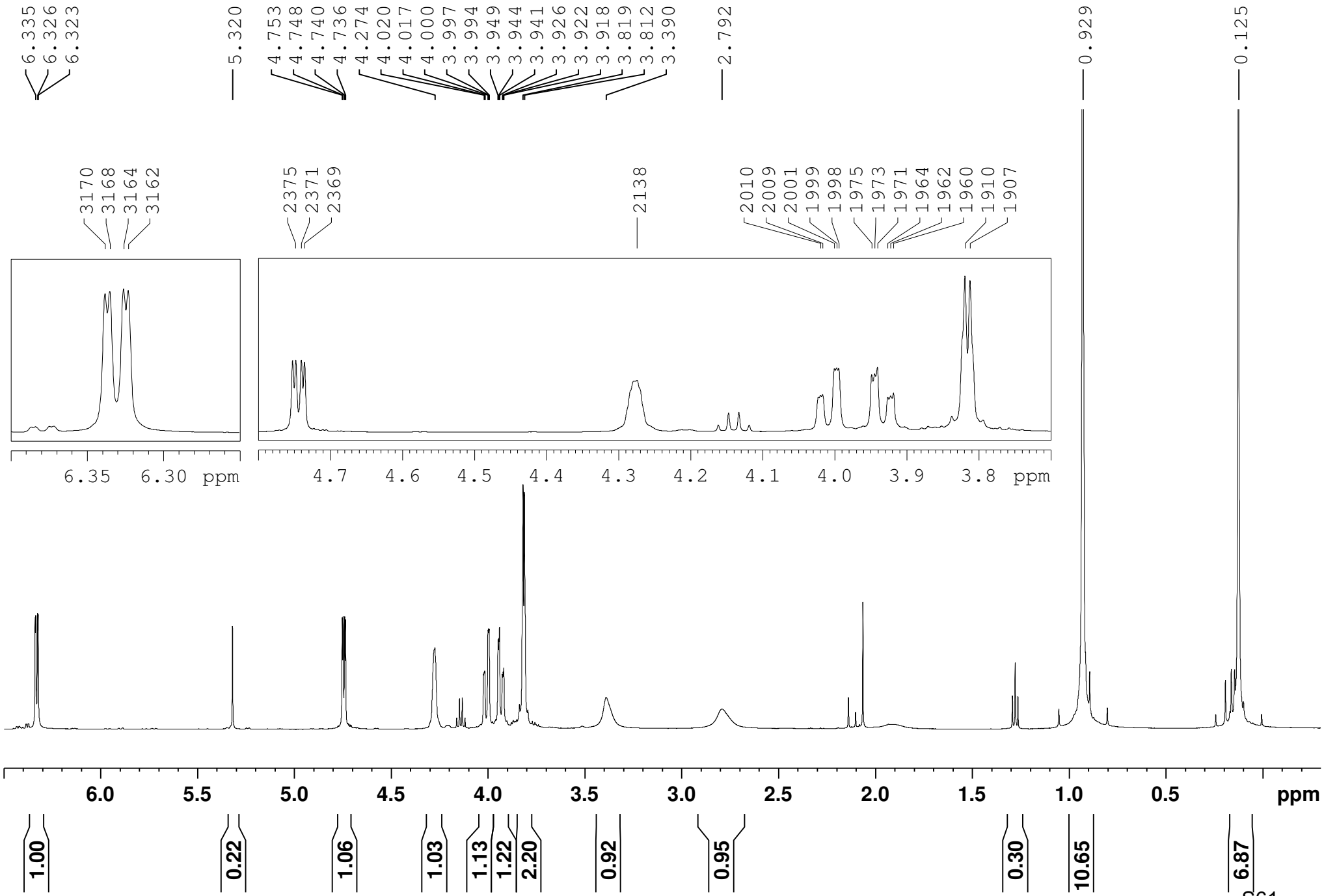
Compound 19 - 1H



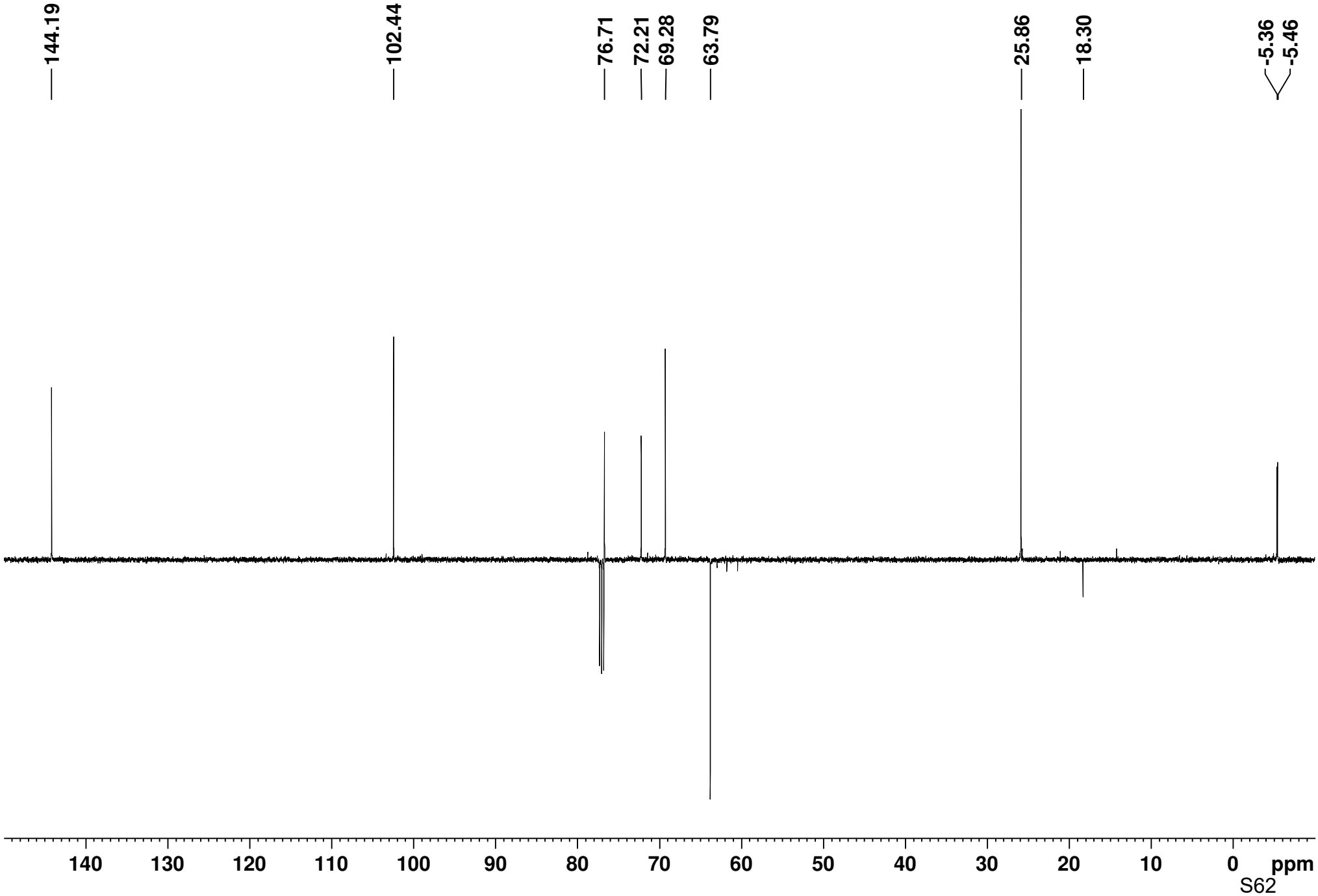
Compound 19 - 13C



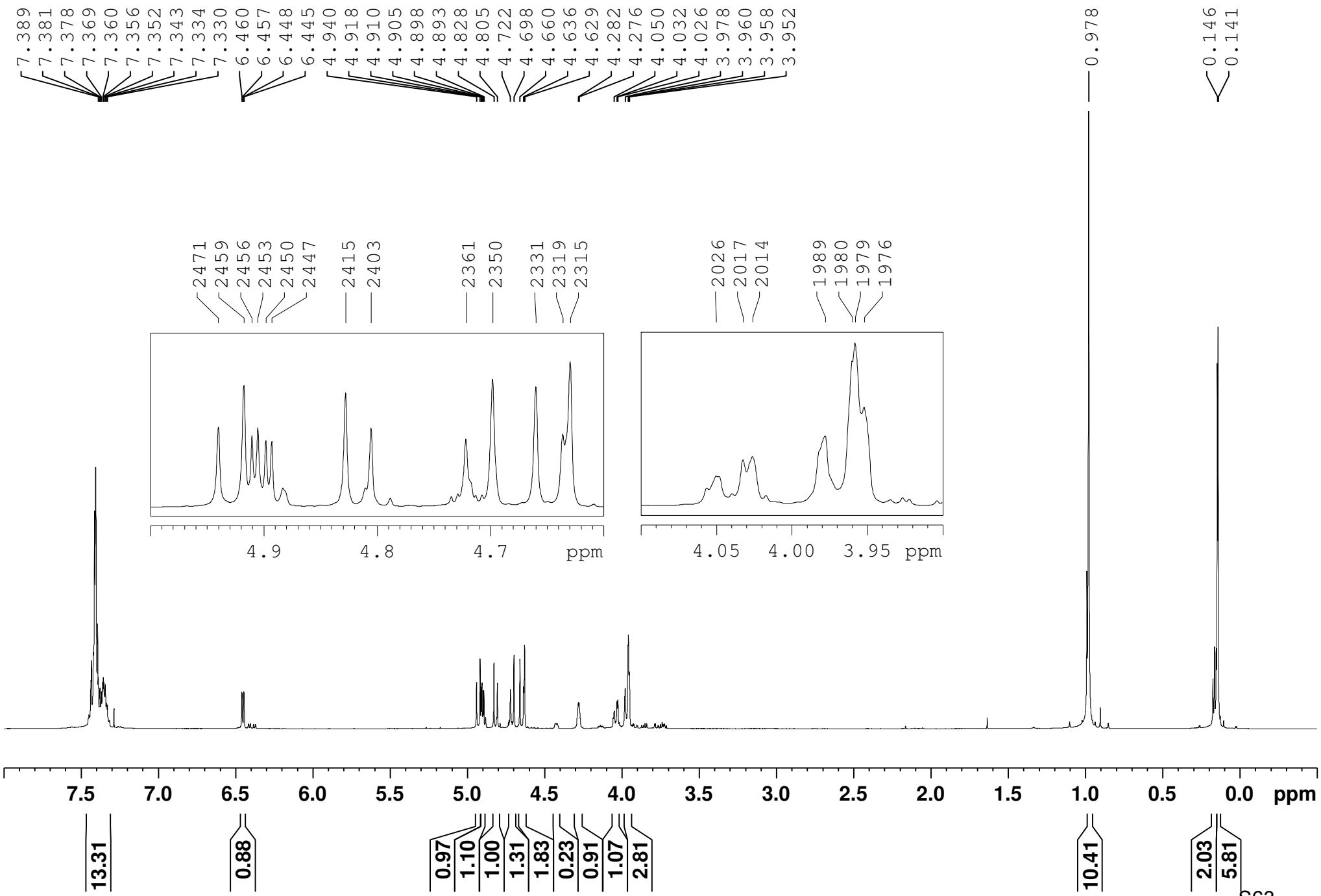
Compound 20 - 1H



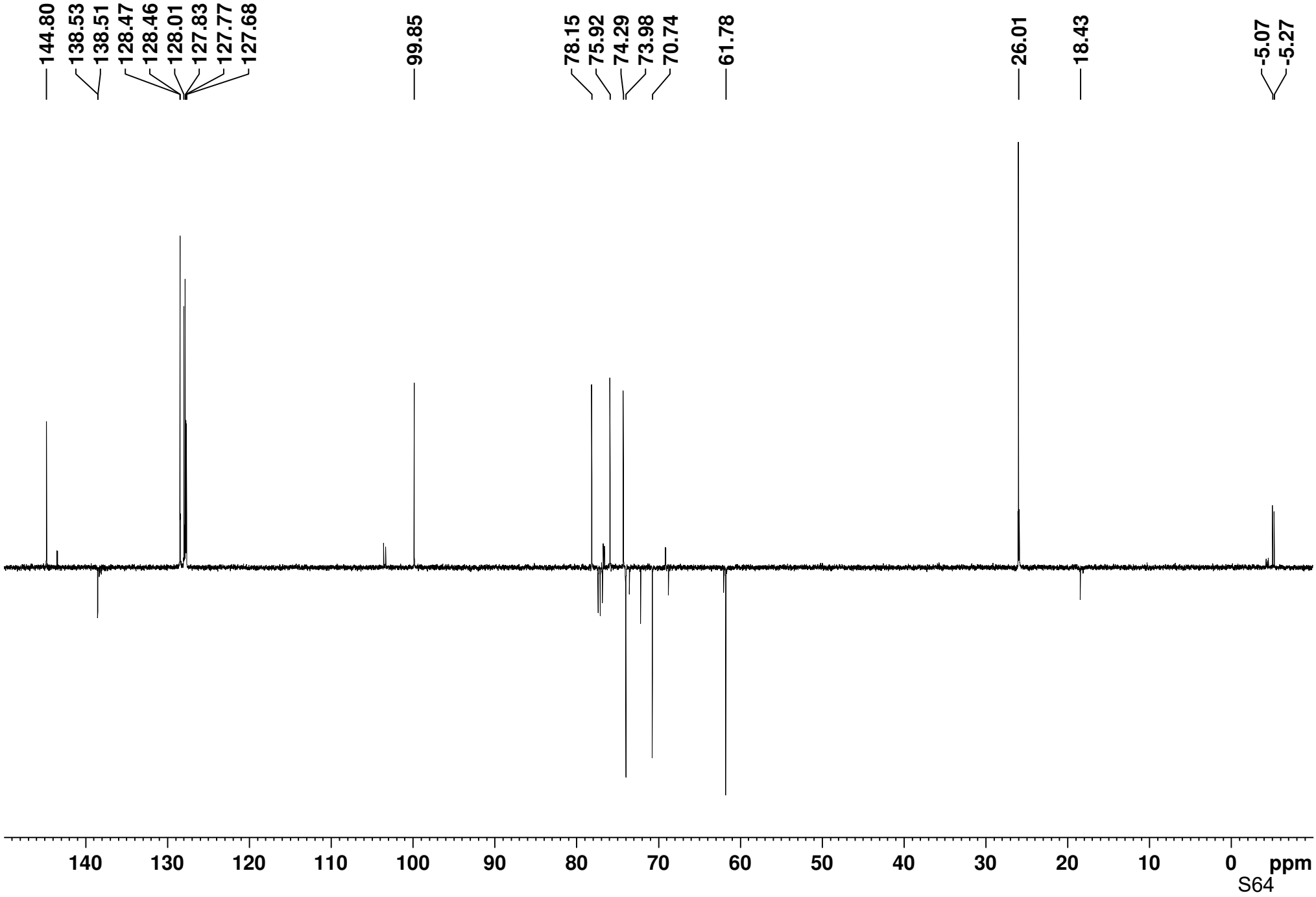
Compound 20 - 13C



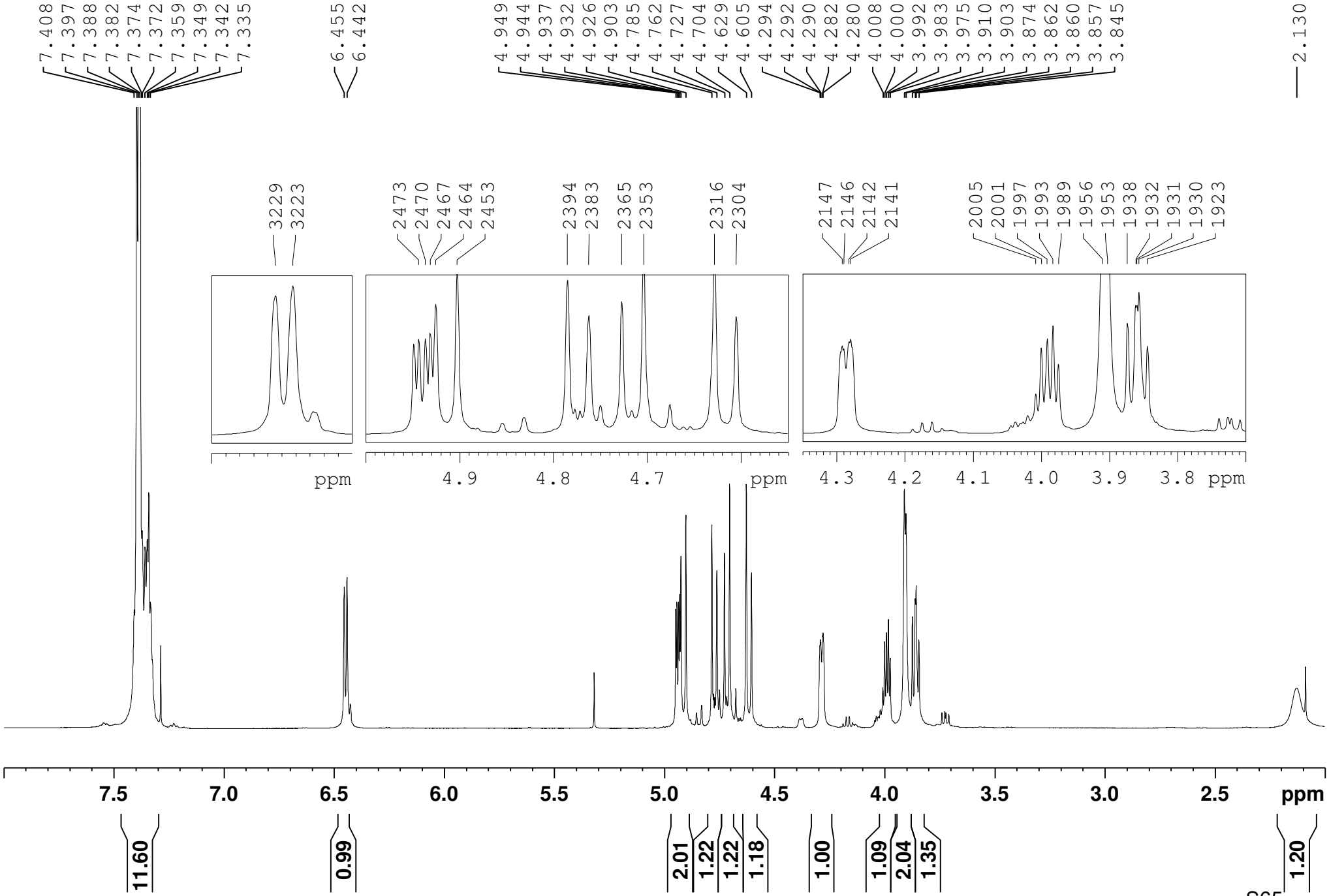
Compound 21 - 1H



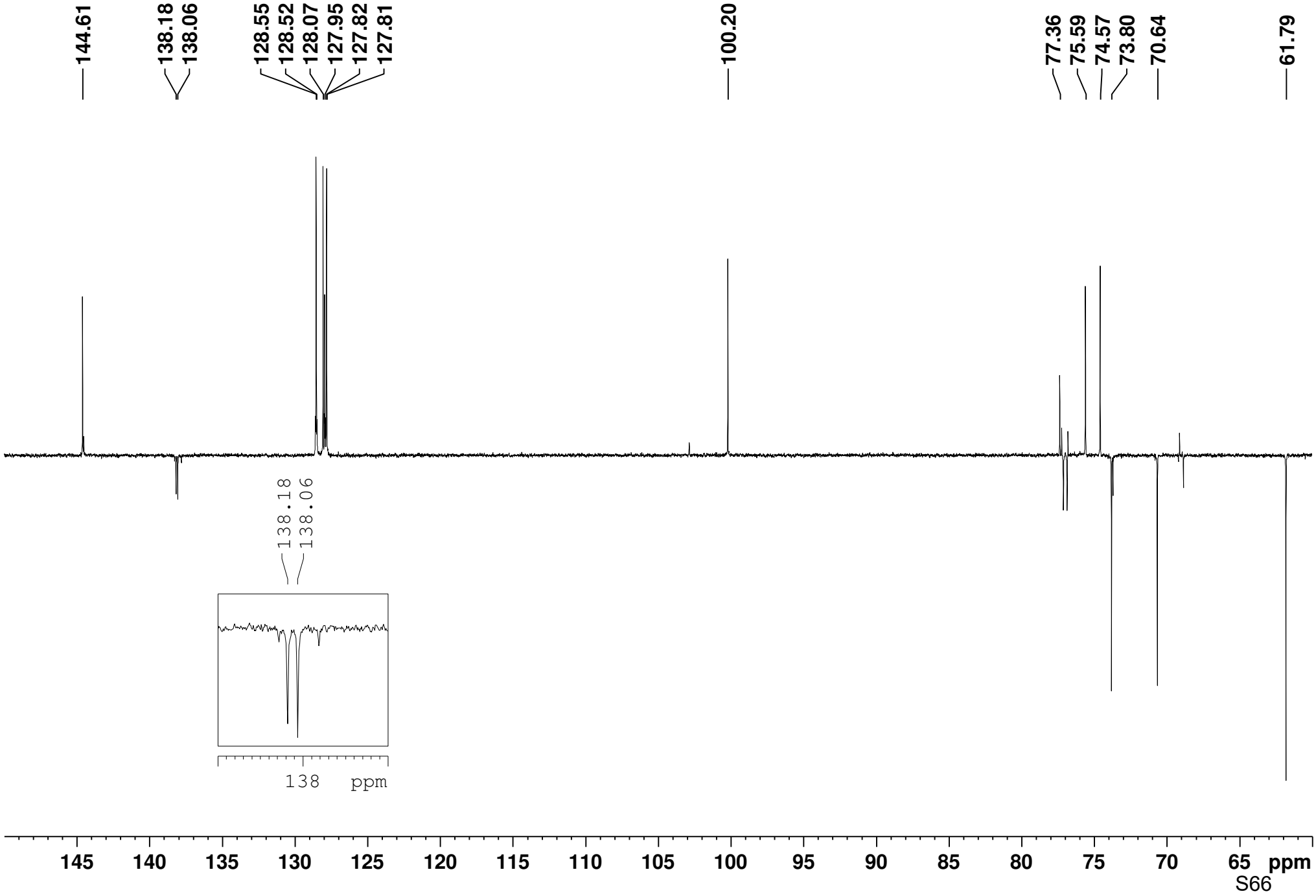
Compound 21 - 13C



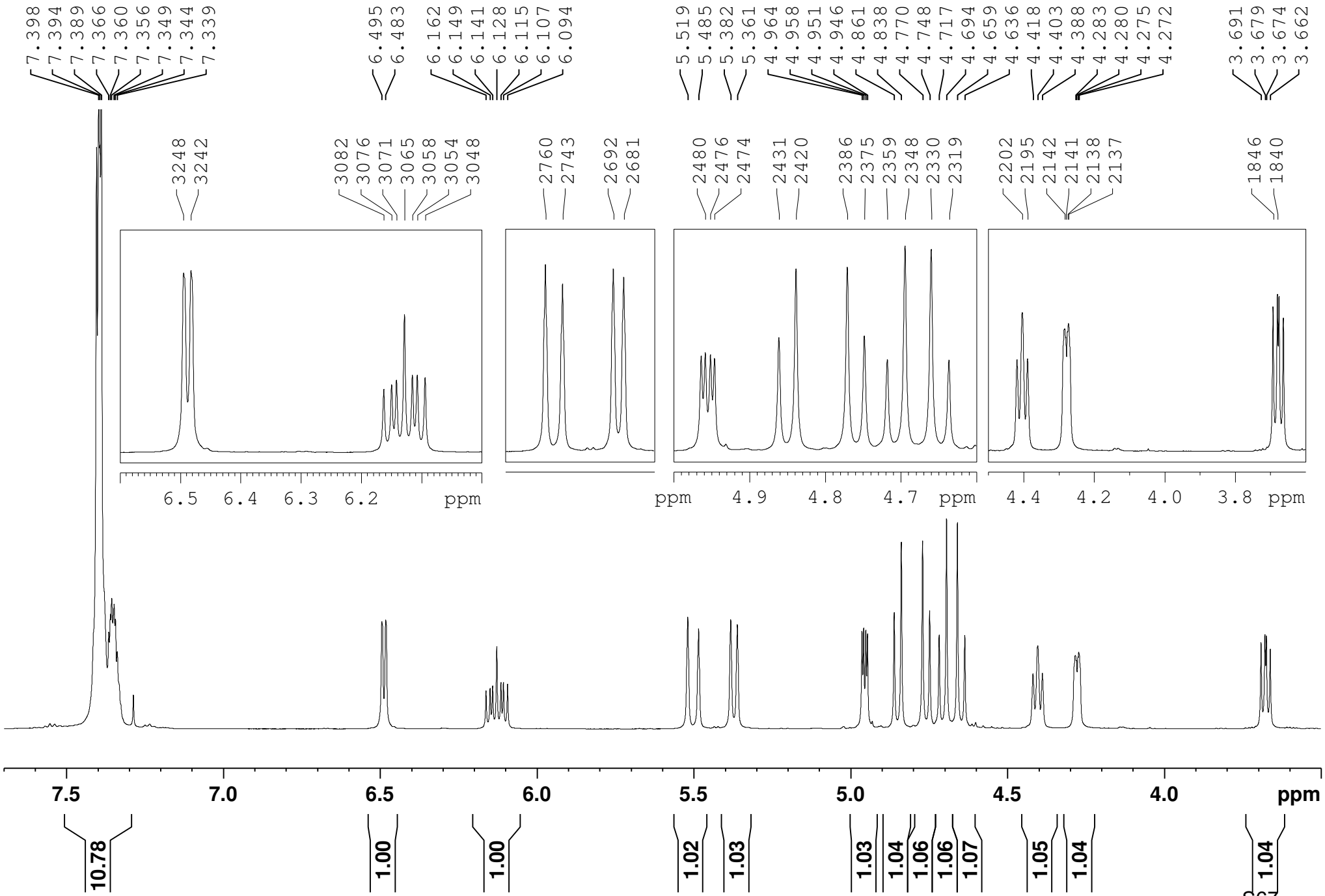
Compound 22 - 1H



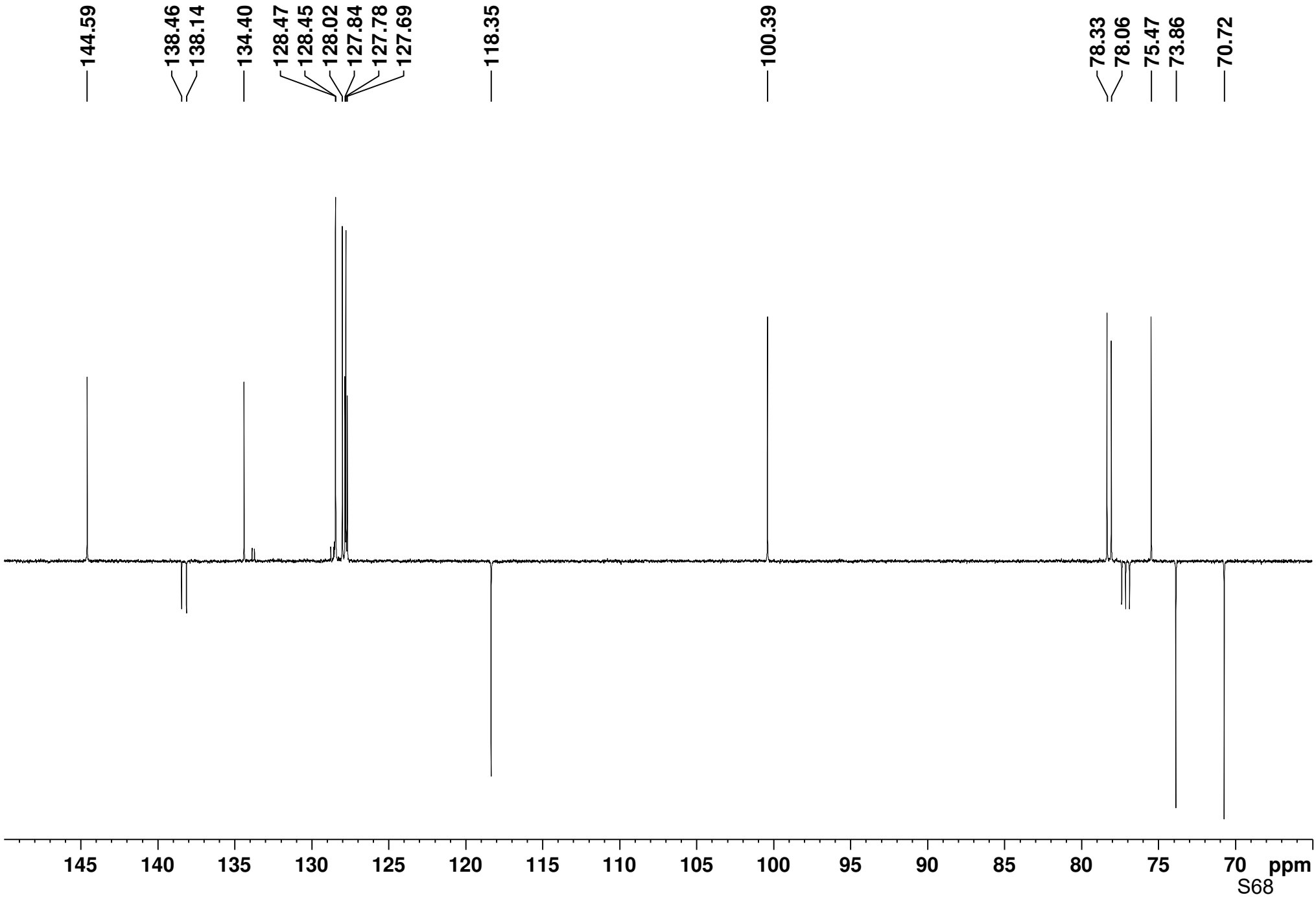
Compound 22 - 13C



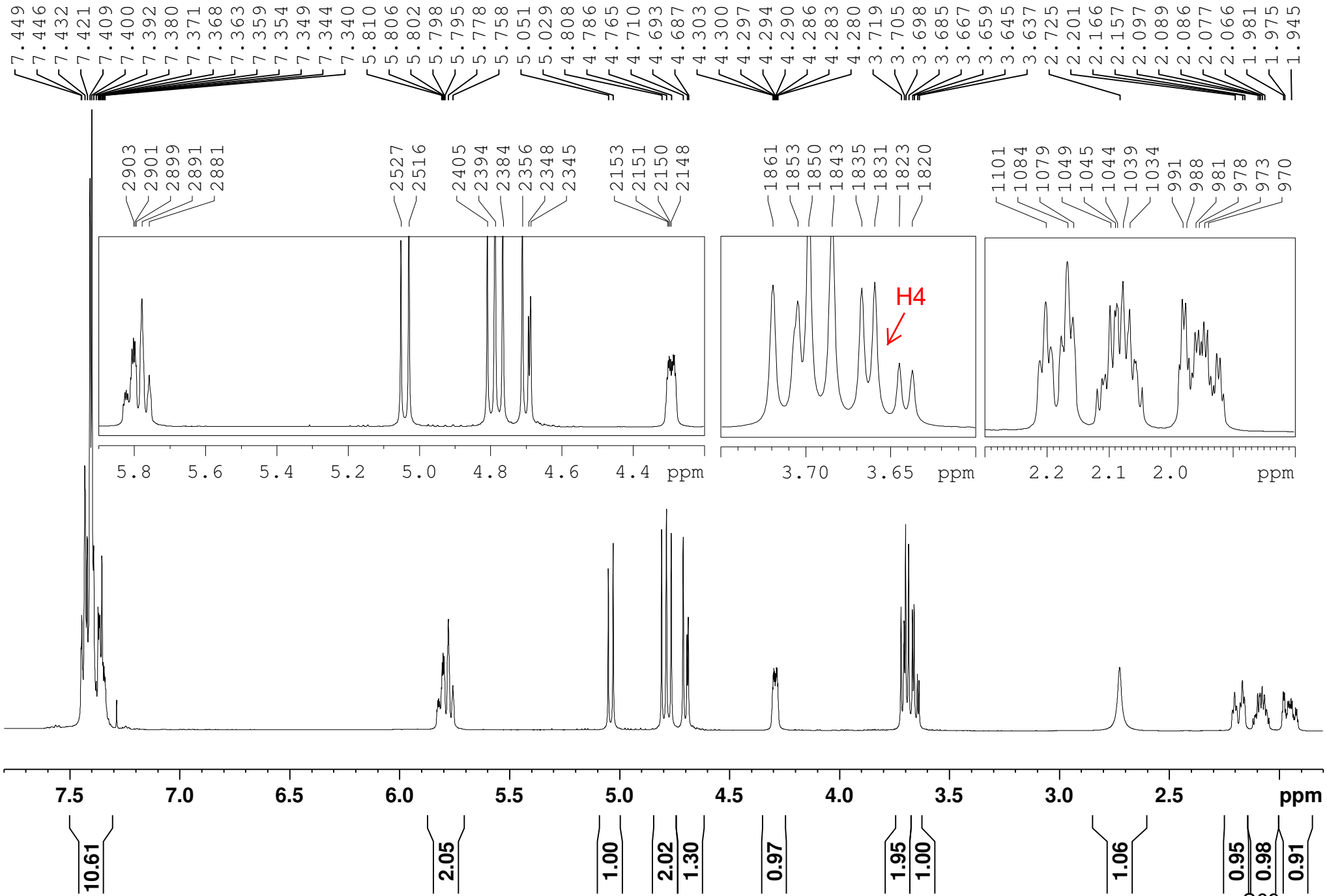
Compound 23 - 1H



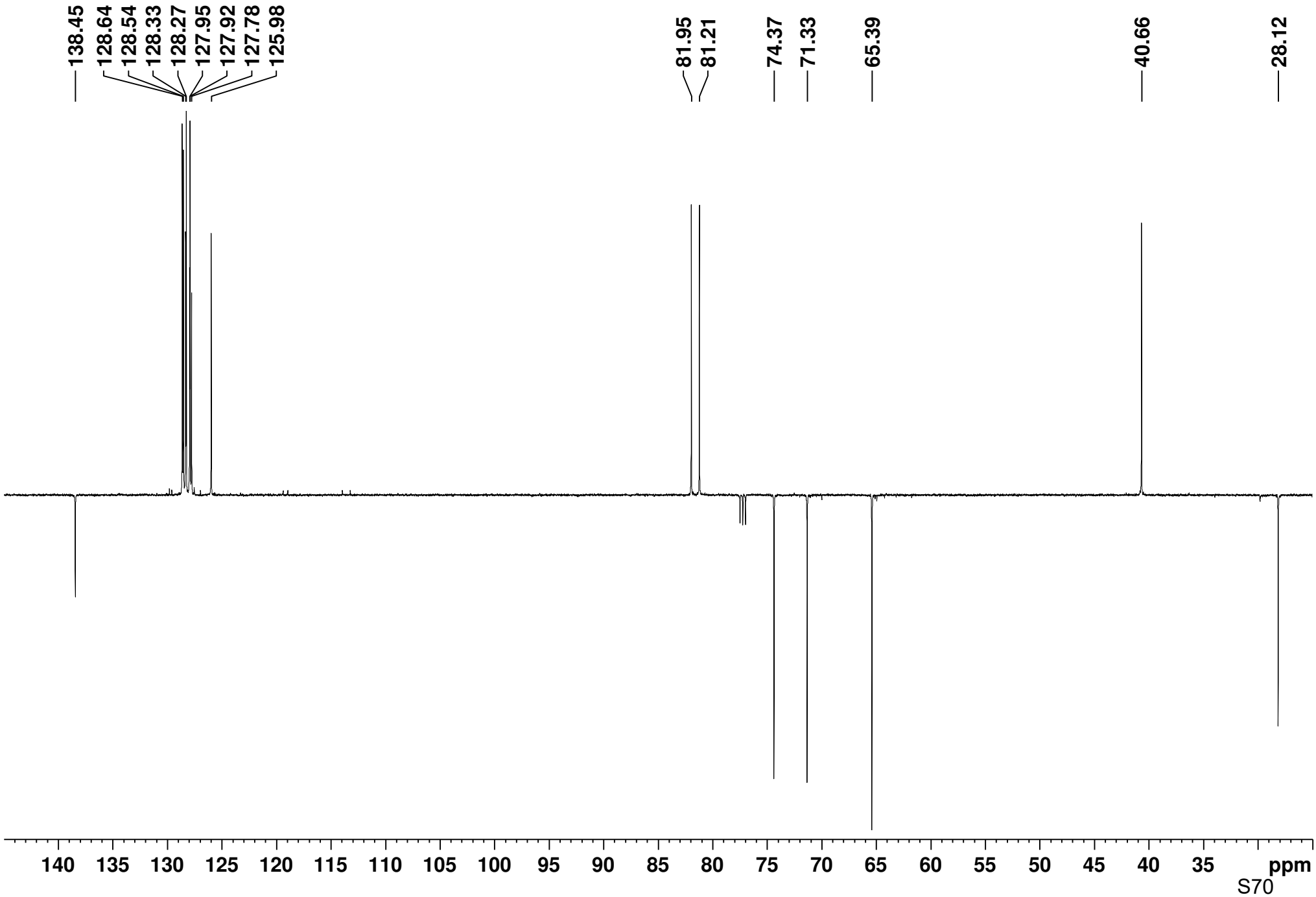
Compound 23 - 13C



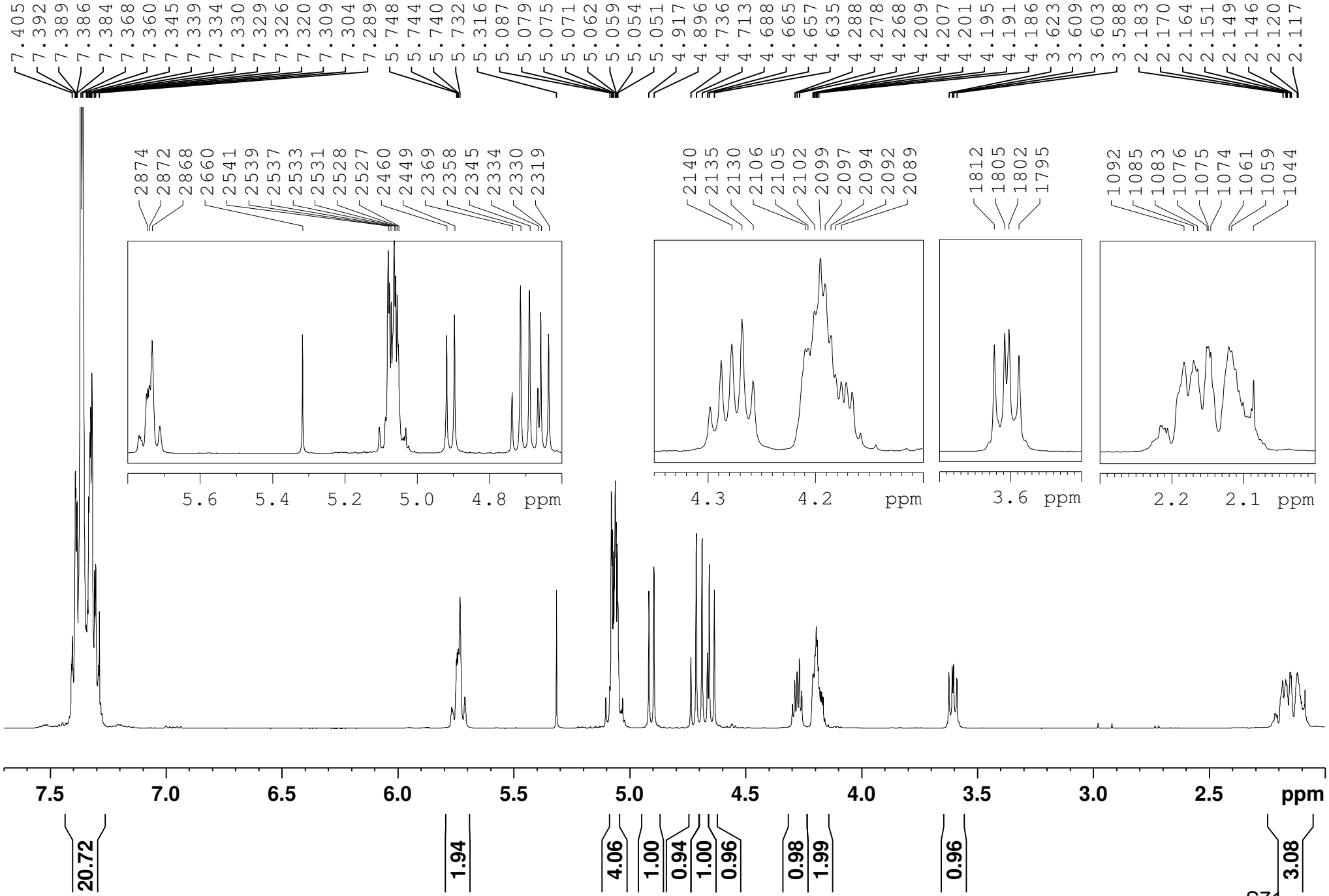
Compound 24 - 1H



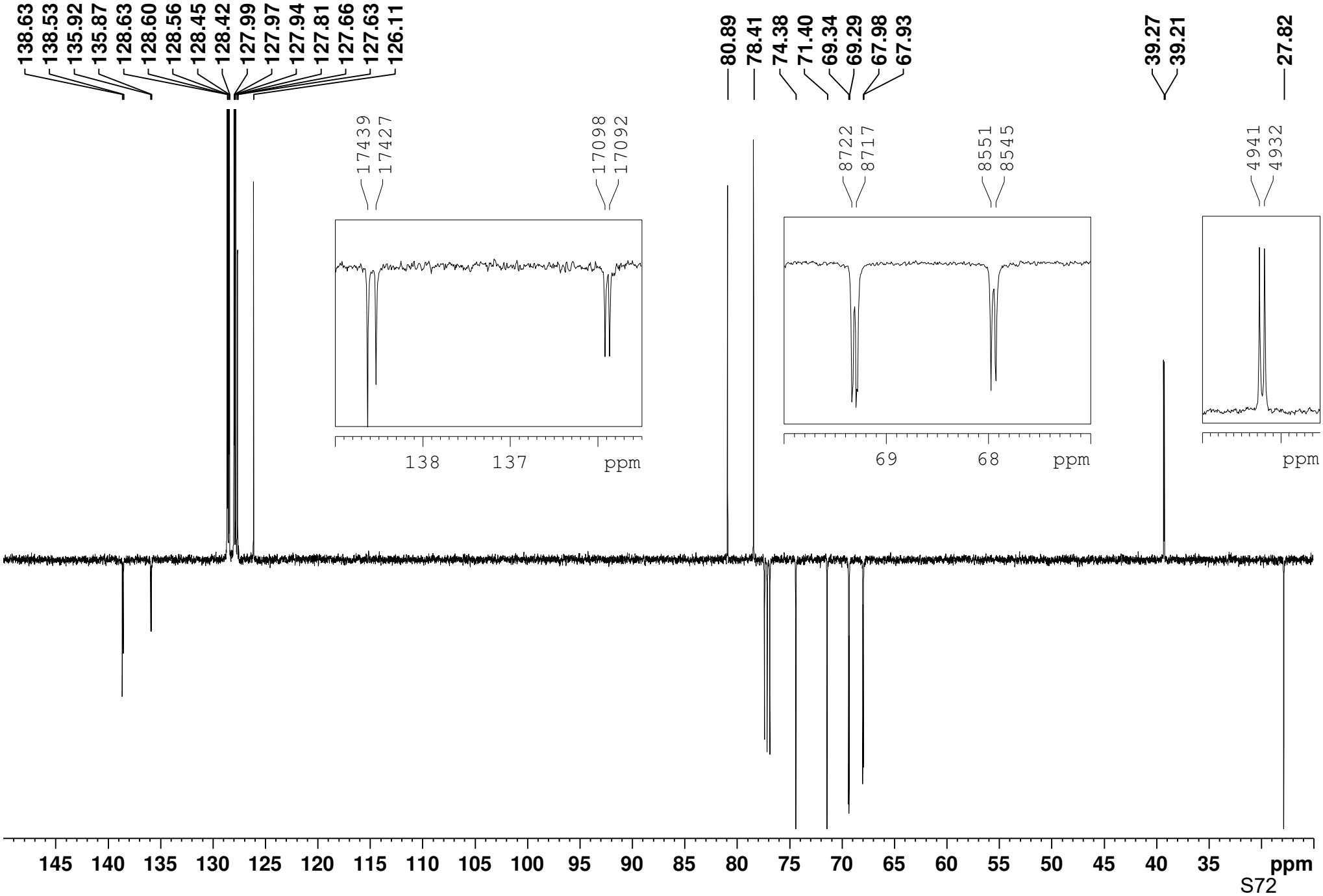
Compound 24 - 13C



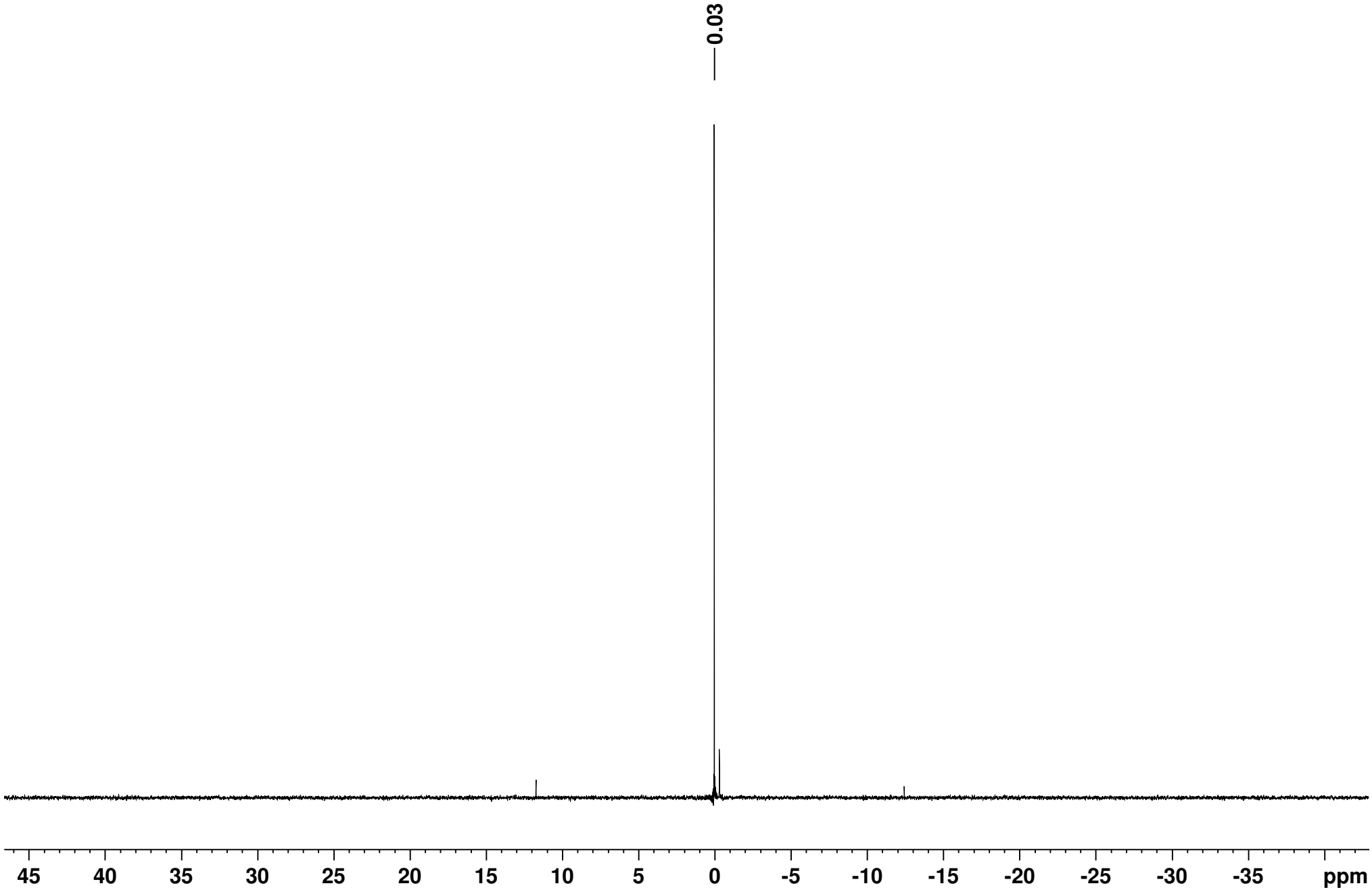
Compound 25 - 1H



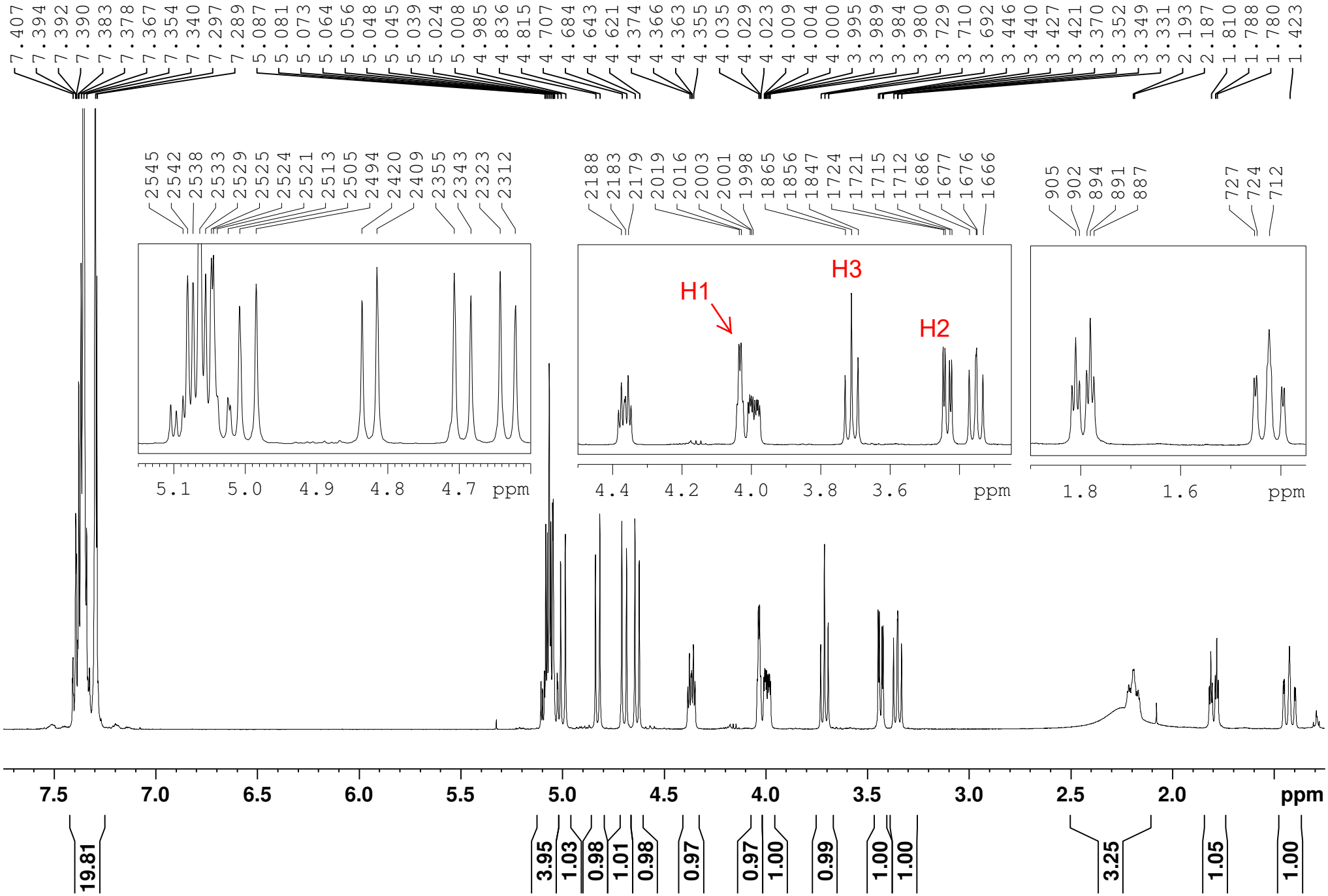
Compound 25 - 13C



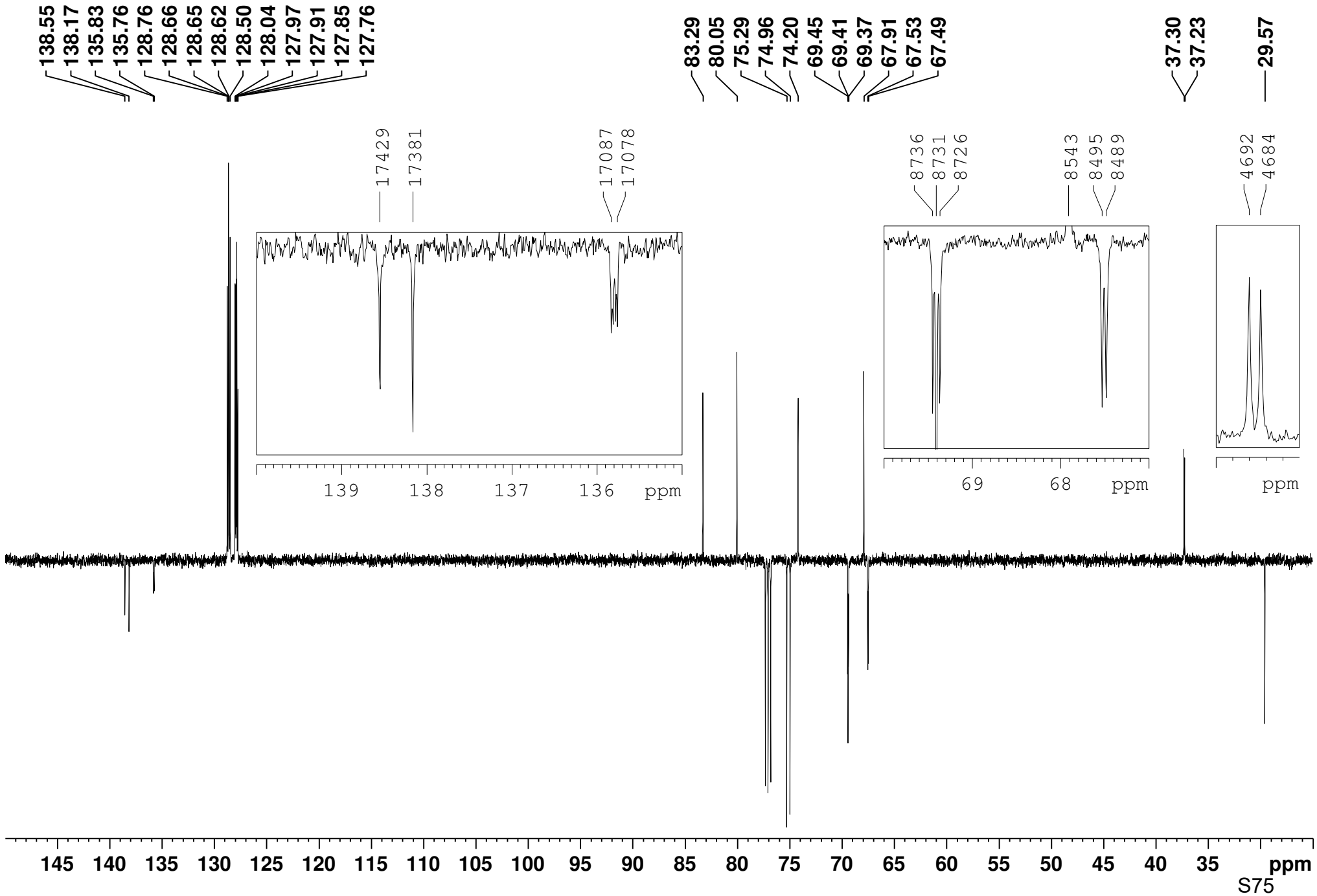
Compound 25 - 31P



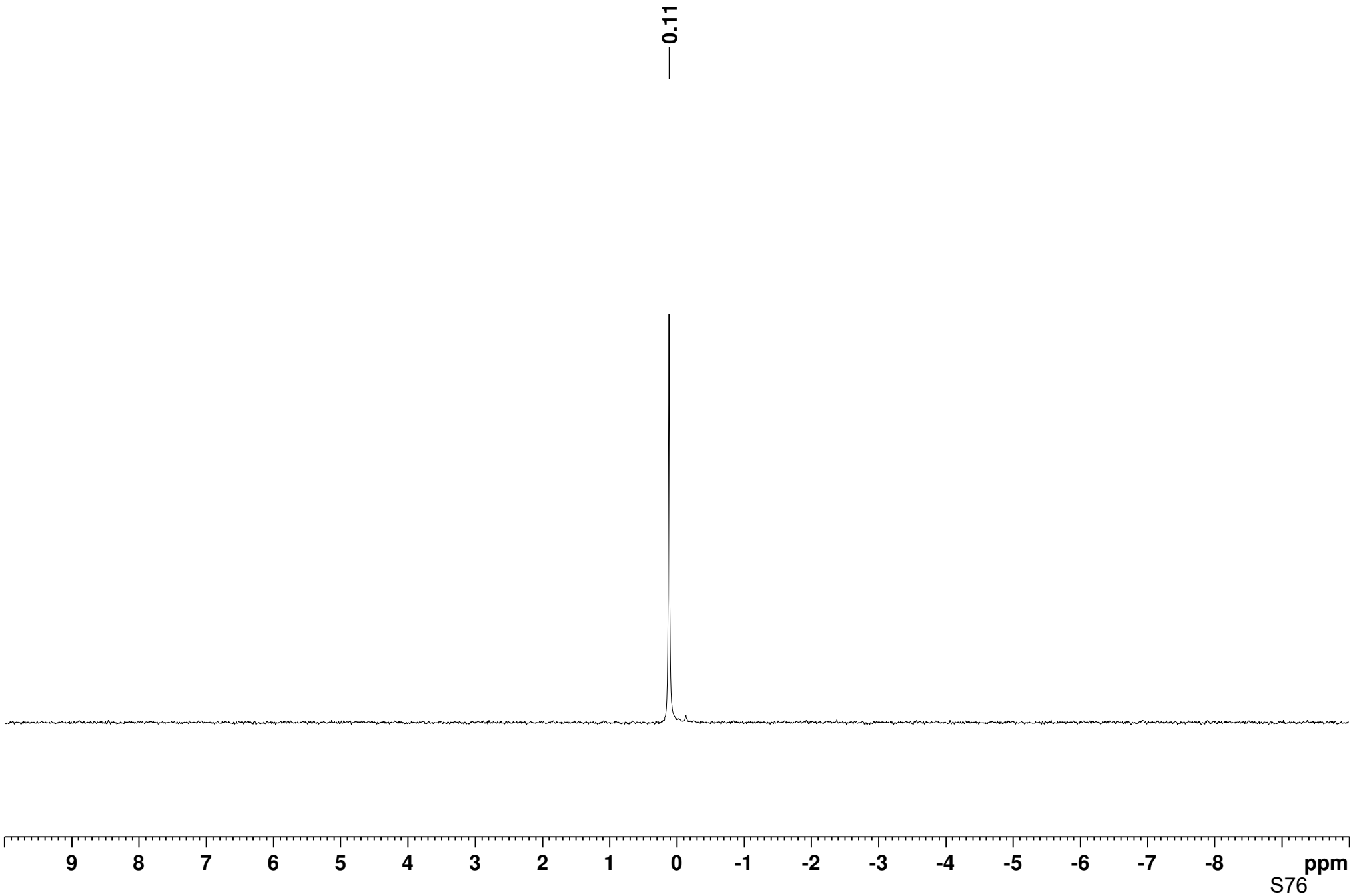
Compound 26 - 1H



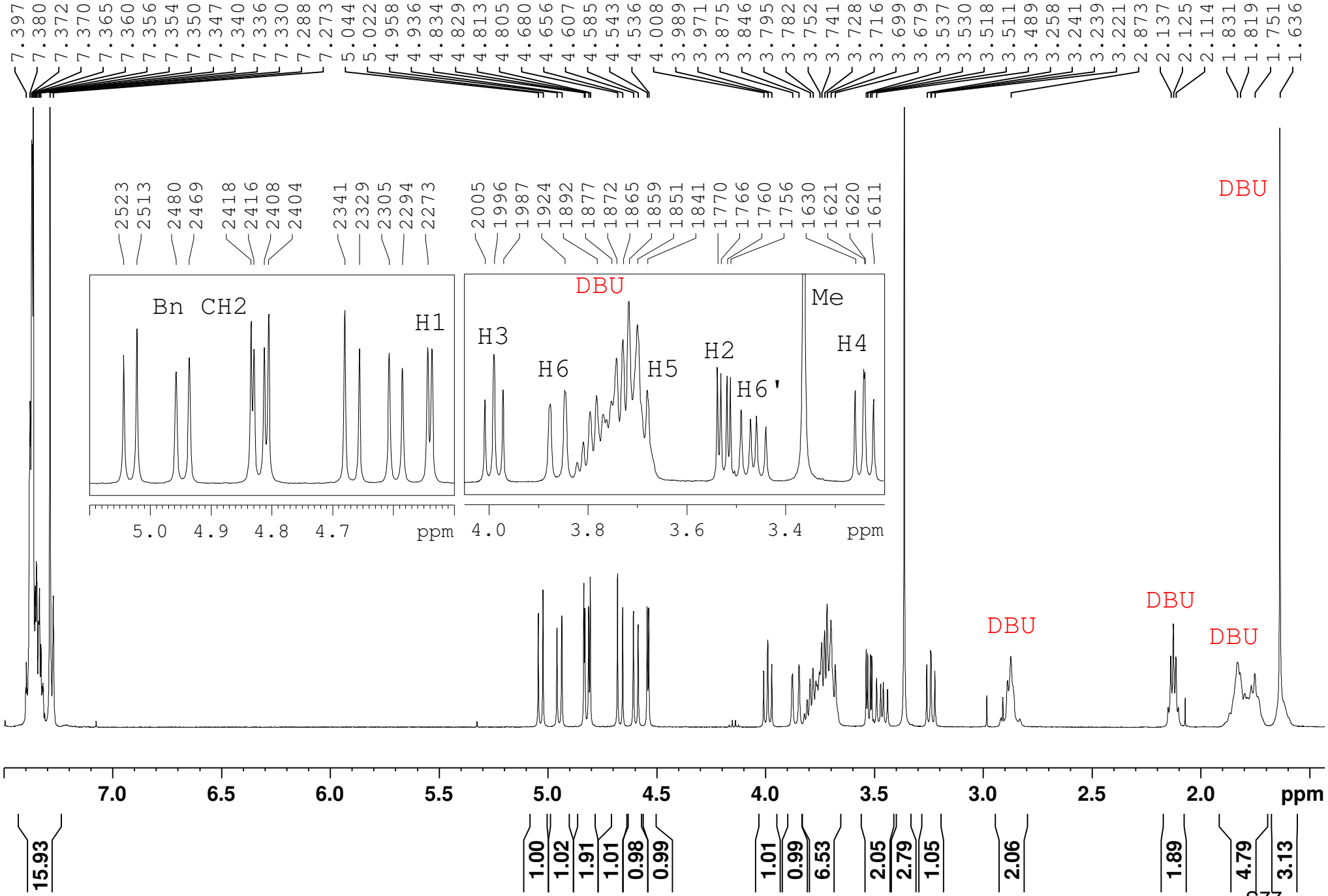
Compound 26 - 13C



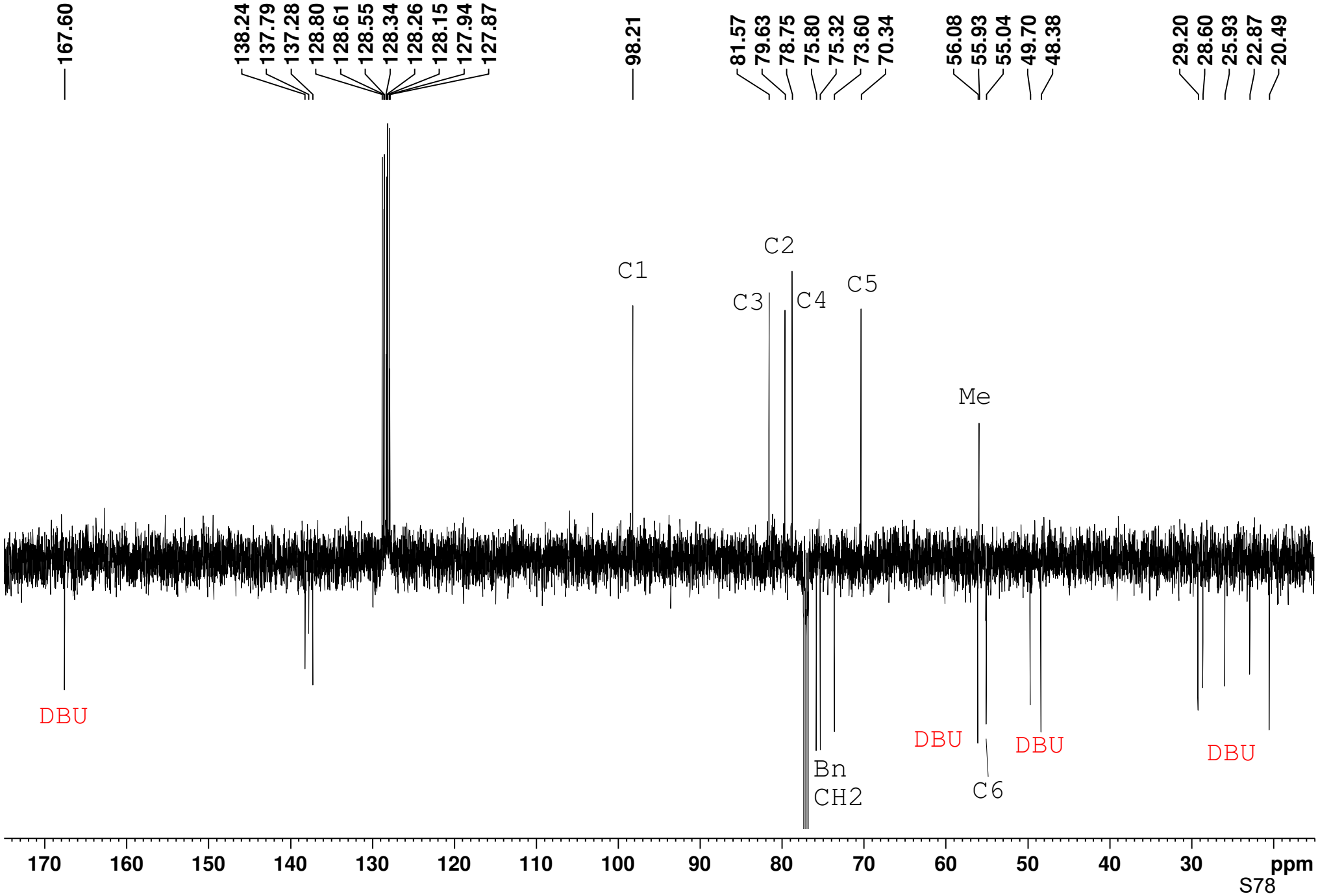
Compound 26 - 31P



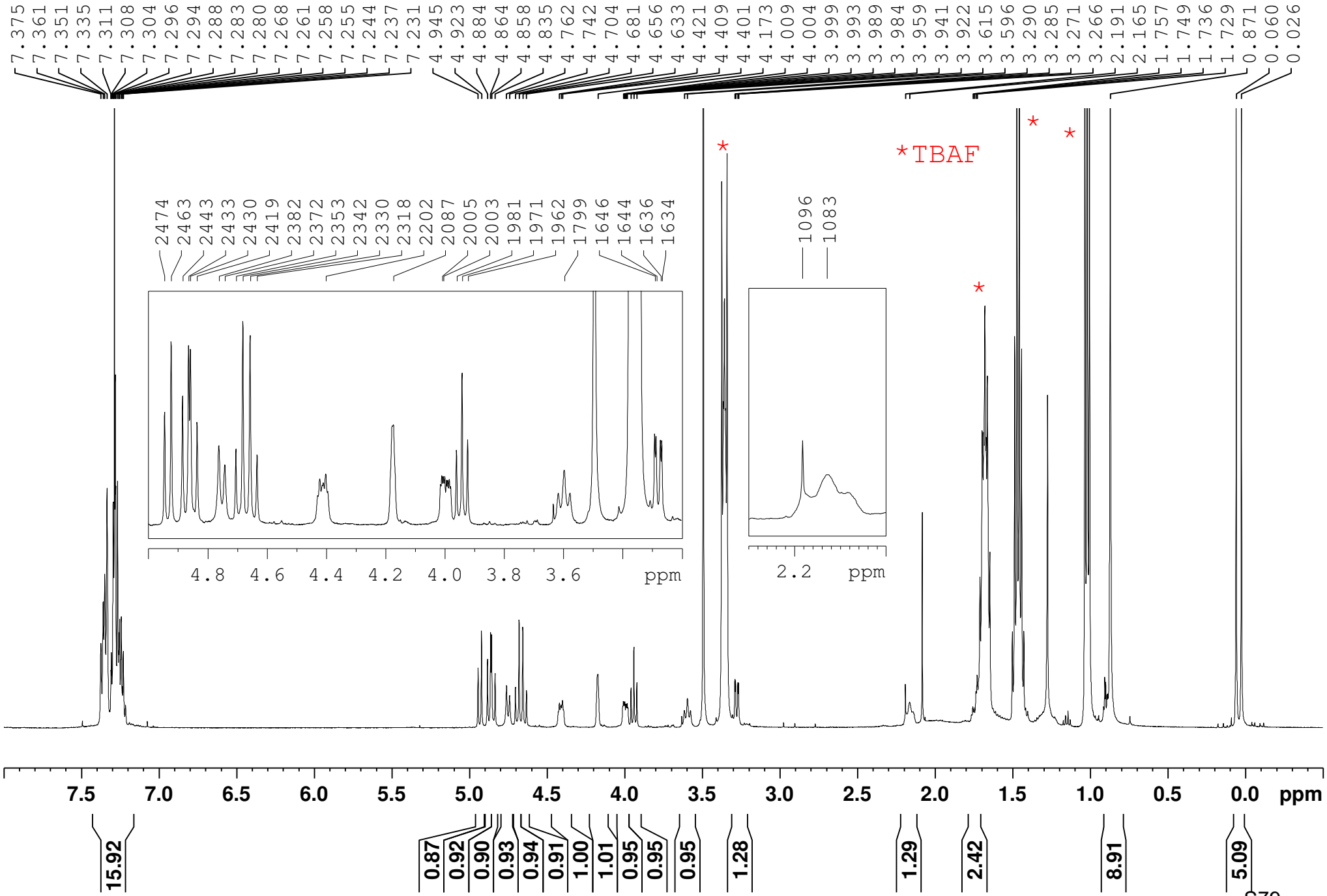
Compound S27 - 1H - DBU adduct



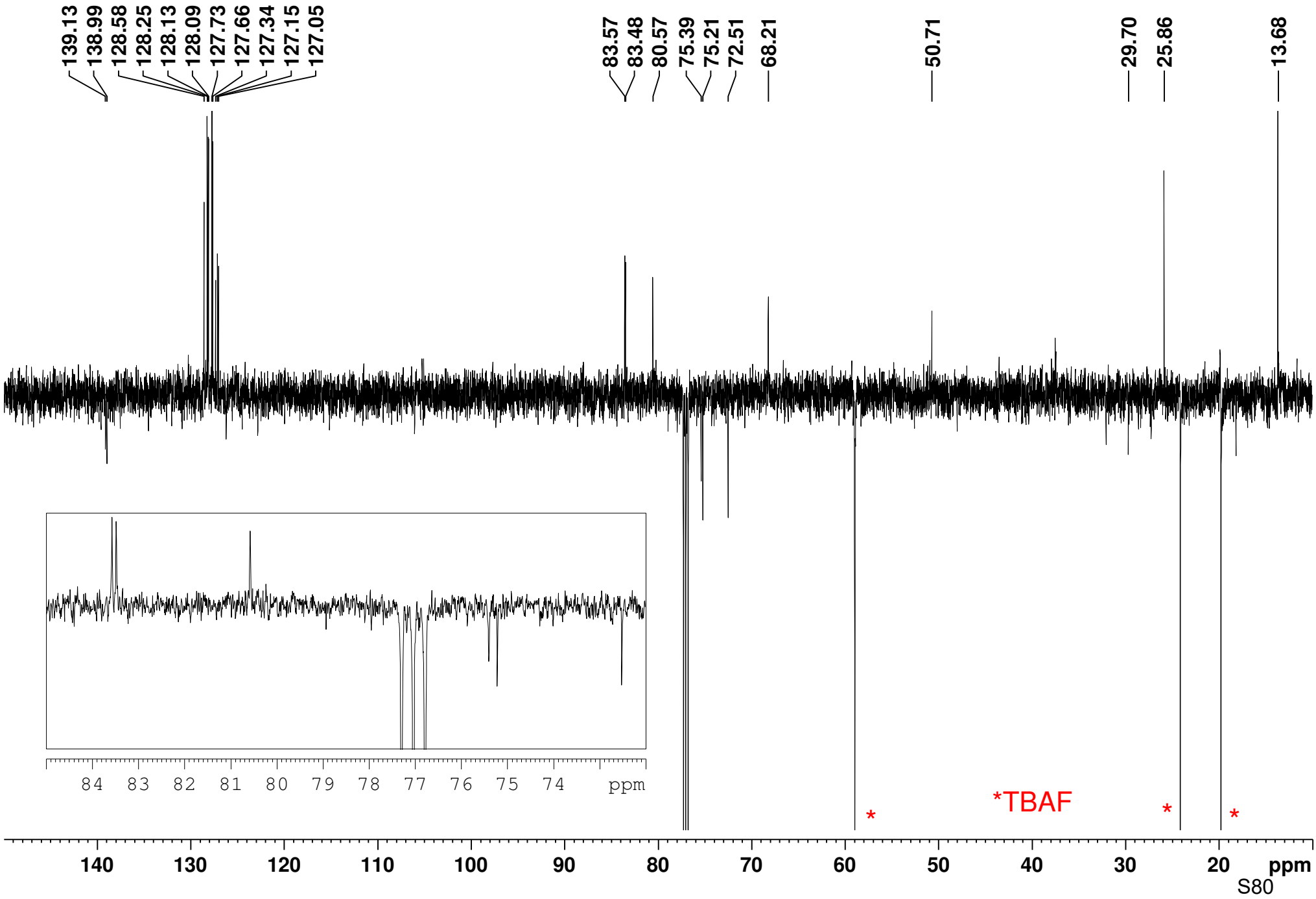
Compound S27 - 13C - DBU adduct



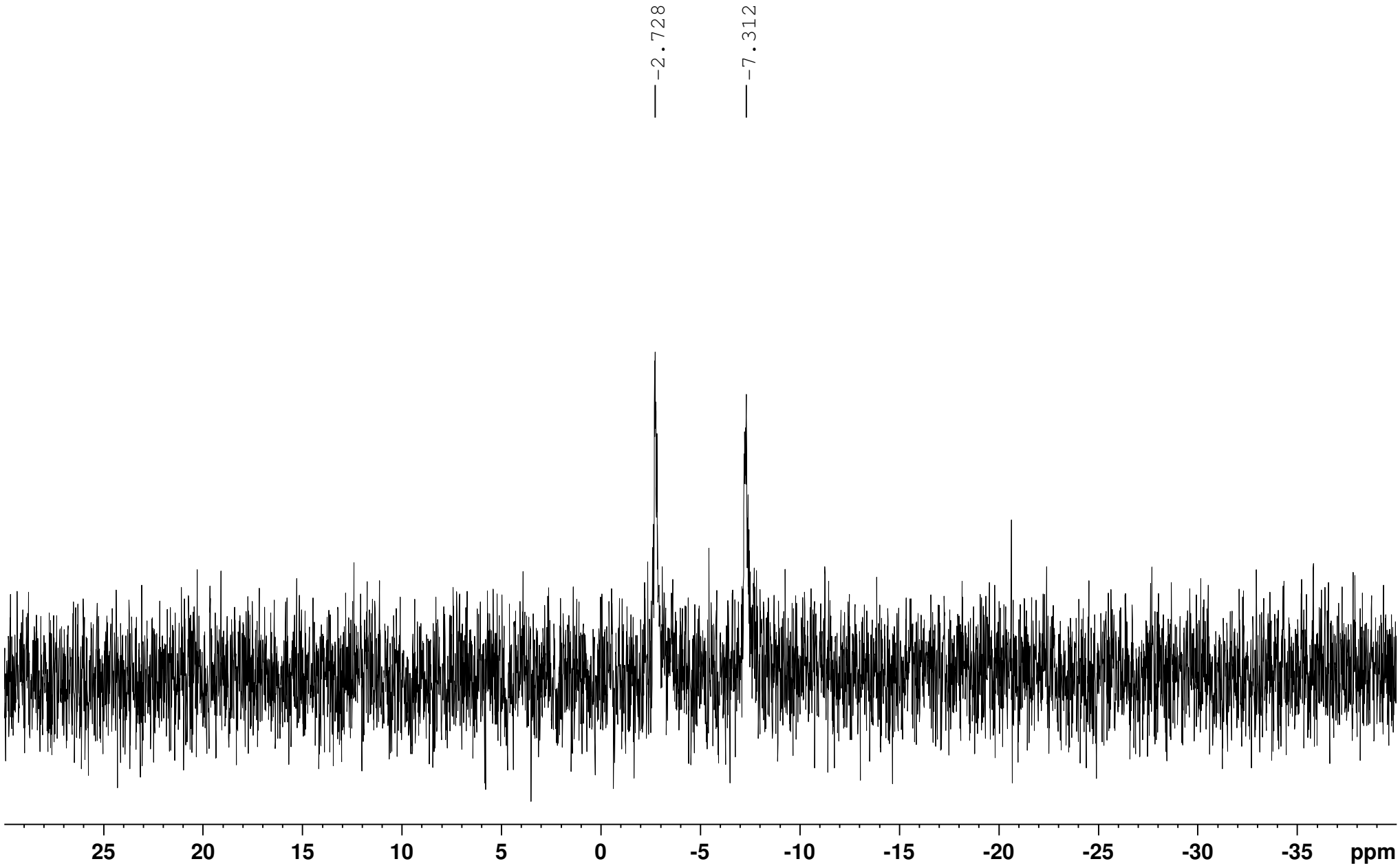
Compound S28 - 1H - Fluorophosphate



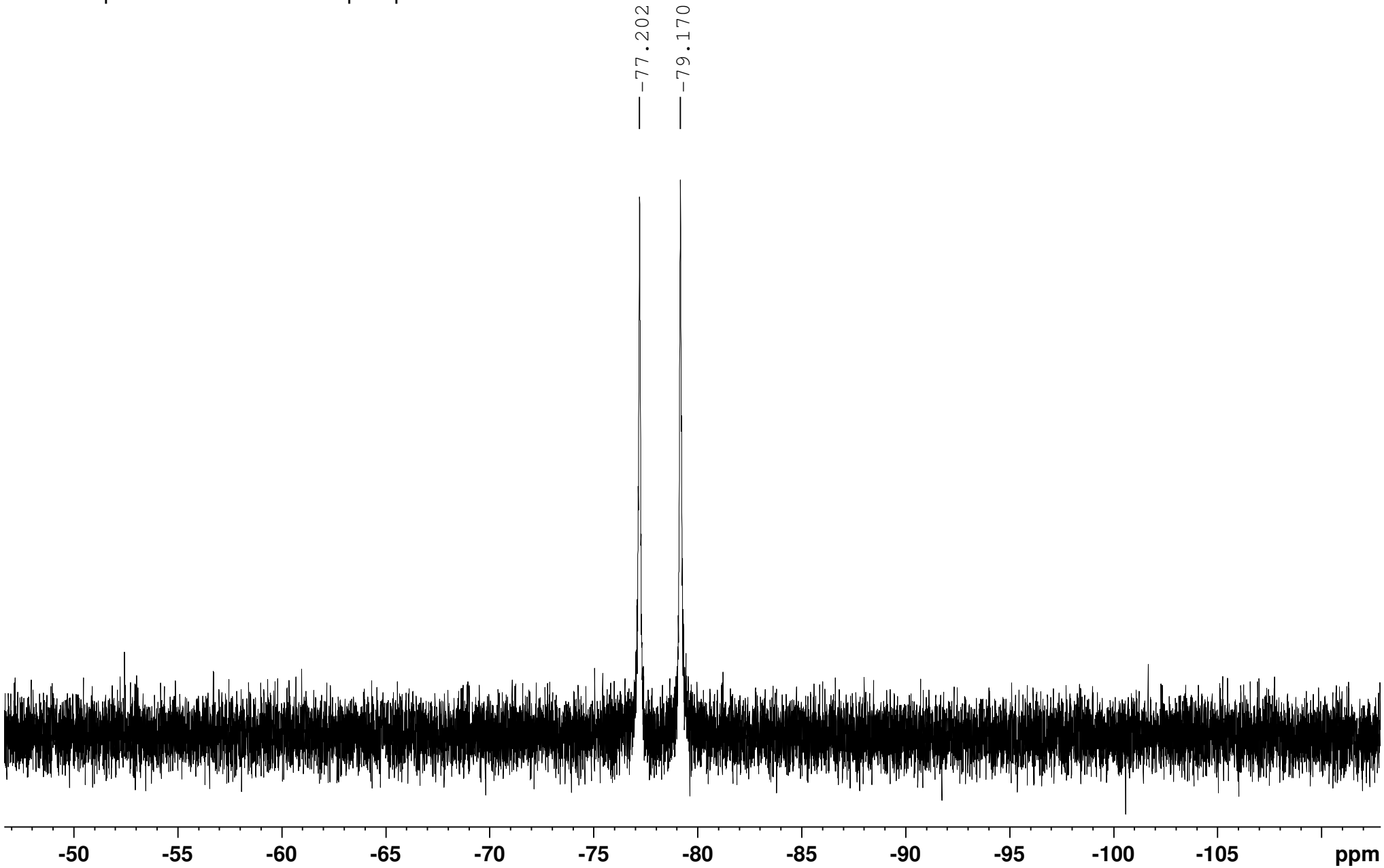
Compound S28 - 13C - Fluorophosphate



Compound S28 - 31P - Fluorophosphate



Compound S28 - 19F - Fluorophosphate



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