Supporting Information

Selective inhibitors of class II microbial fructose bis-phosphate aldolases as a potential new family of antibiotics

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General Information

All reactions were performed in open flasks, unless otherwise stated.

Solvents used were dry solvents. Dichloromethane and acetonitrile were distilled over CaH_2; tetrahydrofuran, toluene, diethylether were distilled over sodium chips using benzophenone as indicator of dryness; dimethyl formamide was distilled at reduced pressure over anhydrous BaO. All other solvents were used as purchased. Commercial reagents were used as purchased without any further purification. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was carried out using SDS silica gel (35 – 70 μm). Thin layer chromatography was carried out using Merck Kieselgel 60 F_{254} (230-400 mesh) fluorescent treated silica which were visualized under UV light (240 nm) or by staining with aqueous basic potassium permanganate solutions. NMR spectra were recorded in deuterated solvents on Bruker spectrometers, with residual protic solvent as the internal standard. ^{13}C and ^{31}P NMR spectra were recorded under broad-band irradiation of the protons. Interpretation of the spectra was made possible by use of 2D (proton-proton and proton-carbon) and DEPT experiments.

All atom numbering used in this section is arbitrary and does not follow any particular convention.

Biochemical evaluation of inhibitors 1 and 2

Enzymes:

Glycerophosphate dehydrogenase from rabbit muscle (GPDH, 274 $U.mL^{-1}$), , triose-phosphate isomerase from rabbit muscle (TIM, 32,000 $U.mL^{-1}$), rabbit muscle Fba ($8U.mg^{-1}$) were commercial preparations, available from Sigma or Fluka. Microbial Fbas were recombinant enzymes expressed in *E. coli*. *H. pylori* Fba was kindly provided by Pr Jurgen Sygusch,

Université de Montréal (Canada). *Candida albicans* Fba was kindly provided by Dr Jean-Michel Bruneau, NOVEXEL, Romainville (France). *Mycobacterium tuberculosis* and *Yersinia pestis* Fbas were kindly provided by Dr Mary Jackson, Colorado State University; Fort-Collins (USA).

Solutions:

Glycylglycine buffer (0.1 M, pH 7.4, with 0.2 M potassium acetate for class II aldolases).

NADH 1.41 mM in buffer.

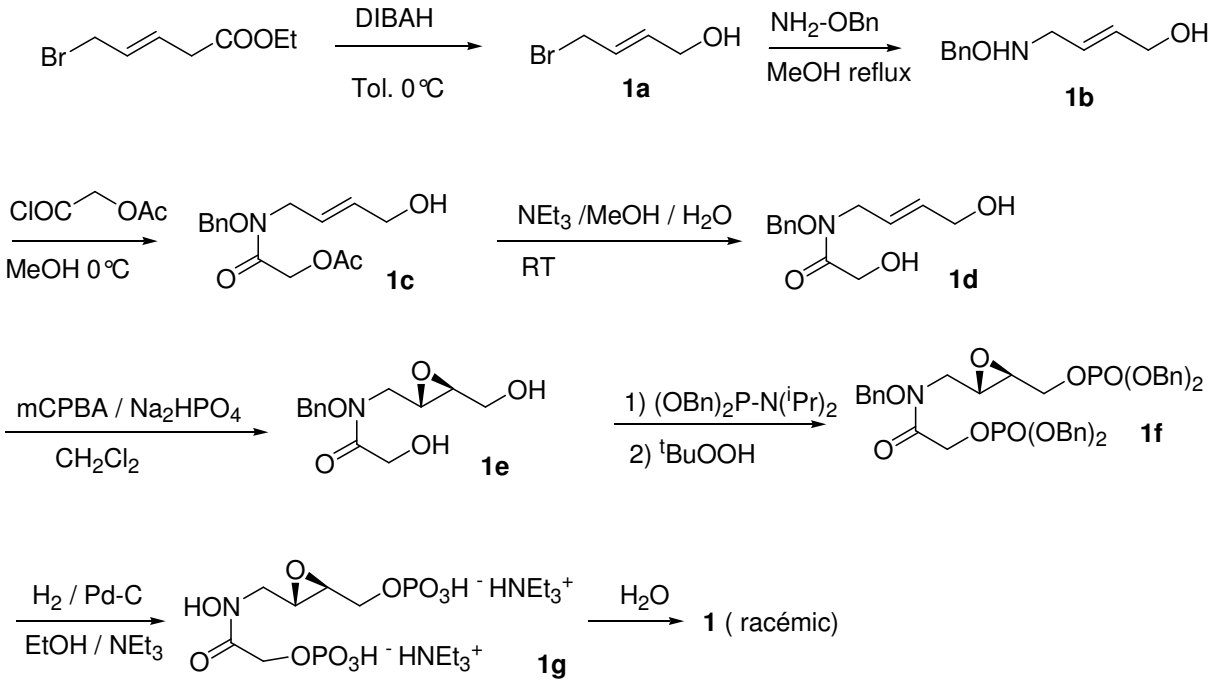
Fructose bisphosphate 2 mM in buffer.

Enzymatic tests:

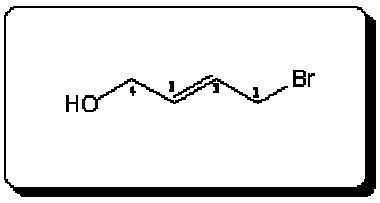
Fructose bisphosphate and inhibitor at the convenient concentration, NADH (0.12 mM), GPDH (11 U) TIM (4 U) and aldolase (4 mU)

were placed in a cuvette to give a final volume of 1.2 mL. The decrease in absorbance of NADH at 340 nm was monitored on a spectrophotometer over 5 min.

Synthesis of compound 1 (Compounds 1, 1d, 1e, 1f were synthesized as racemic mixtures. Only one enantiomer is represented in the scheme).



1a



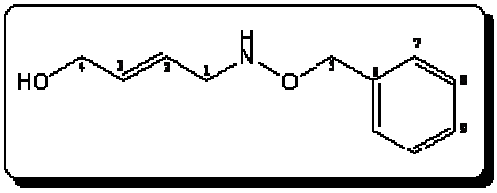
Ethyl bromocrotonate (6.75g, 35 mmol) was dissolved in in anhydrous toluene (120 mL). To this solution stirred under argon at 0°C was added drop-wise a commercial 1M solution of DIBAH in cyclohexane (97 mL, 97 mmol, 2.8 eq.) within 2 h. The mixture was kept at 0° C for an additional hour. Acetic acid in water (50% solution, 10 mL) was then added to the medium at RT. The mixture was filtered on celite and the solid washed with acetone. 1a was obtained as a black oil after evaporation of the solvents (4.9g, 93%; Rf 0.37 Pentane:AcOEt 6:4). The product was used without further purification. NMR data in accordance with

litterature[1]

^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 2.4 (s, 1H, OH), 3.98 (d, 2H, H-1, *J* 5.6 Hz), 4.18 (d, 2H, H-4, *J* 3.5 Hz), 5.96-5.92 (m, 2H, H-2, H-3)

^{13}C NMR ($CDCl_3$, 63 MHz) δ_C 32.22 (C-1), 62.19 (C-4), 127.12 (C-2), 134.23 (C-3)

1b

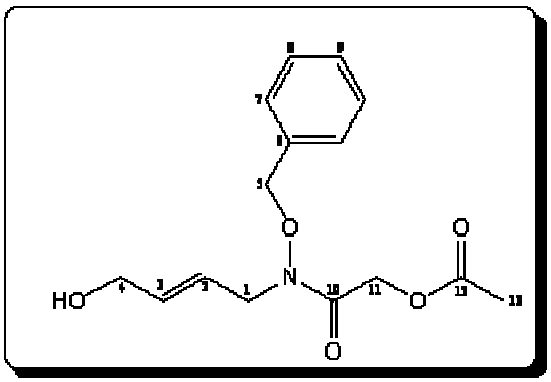


N-benzylhydroxylamine (7.6 g, 60.3 mmol, 2.5 eq.) was dissolved in refluxing methanol (180 mL). 1a (3.64 g, 24.12 mmol, dissolved in methanol (60 mL) was added drop-wise, and the mixture was stirred under reflux overnight. After evaporation of the solvent, the formed benzylhydroxylammium bromhydrate was precipitated by addition of ethyl acetate (200 mL) and recovered by filtration. The filtrate was evaporated and the residue was redissolved in diethylether. This organic solution was washed with 1M aqueous sodium hydrogenocarbonate (3 x 20 mL), dried over sodium sulfate and evaporated. The title compound was purified by flash-chromatograohy (pentane:ethyl acetate 6:4; *Rf* 0.23), and obtained as a pale yellow oil (1.57 g, 34%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 3.53 (d, 2H, H-1, *J* 5.24 Hz), 4.08 (d, 2H, H-4, *J* 4.40 Hz), 4.72 (s, 2H, H-5), 5.81-5.75 (m, 2H, H-2, H-3), 7.37-7.32 (m, 5H, Ph)

^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 53.6(C-1), 62.84 (C-4), 76.16 (C-5), 127.09 (C-2), 127.92 (C-9), 128.42 (C-7, C-7'), 128.48 (C-8, C-8'), 133.04 (C-3), 137.74 (C-6)

1c

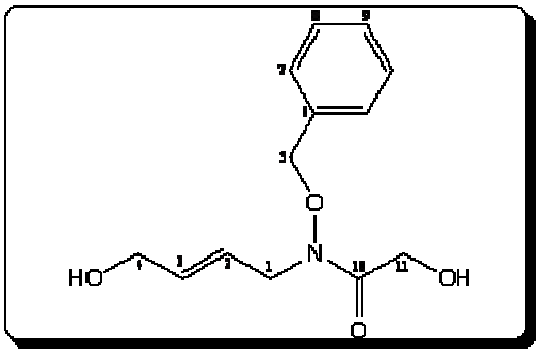


1b (1.96 g, 10.2 mmol) and triethylamine (1.23 g, 12.2 mmol, 1.2 eq.) were dissolved in anhydrous methanol at 0°C (22 mL). Acetoxyacetyl chloride (1.66 g, 12.2 mmol, 1.2 eq.) dissolved in anhydrous dichloromethane (5 mL) was added drop-wise to this solution. After 30 min, dichloromethane (20 Ml) and water (20 mL) were added to the mixture. The organic phase was separated, washed three times with water, dried and evaporated to yield the title compound as a pale yellow oil (2.99 g, *Rf* 0.5, AcOEt). The compound was used without further purification, although it was contaminated by a small amount of methyl acetoxyacetate, resulting from the methanolysis of acetoxyacetyl chloride.

^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 2.12 (s, 3H, H-13), 4.08 (d, 2H, H-1, *J* 4.65 Hz), 4.2 (d, 2H, H-4, *J* 5.67 Hz), 4.7 (s, 2H, H-5), 4.85 (s, 2H, H-11), 5.8-5.72 (m, 2H, H-2, H-3), 7.39-7.32 (m, 5H, Ph). (Methyl acetoxyacetate: ^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 2.13 (s, 3H, CH_3), 3.73(s, 3H, CH_3), 4.6 (s, 2H, CH_2))

^{13}C NMR ($CDCl_3$, 63 MHz) δ_C 20.6 (C-13), 48.36(C-1), 61.42 (C-4), 62.52 (C-11), 77.1 (C-5), 124.02 (C-2), 128.84(C-7,C-7'), 129 (C-9), 129.35 (C-8, C-8'), 134 (C-6), 134.13 (C-3) 168.91(C-10), 170.71(C-12). (Methyl acetoxyacetate: ^{13}C NMR ($CDCl_3$, 63 MHz) δ_C 20.6 (CH_3), 53.5 (CH_3), 60.64 (CH_2)).

1d

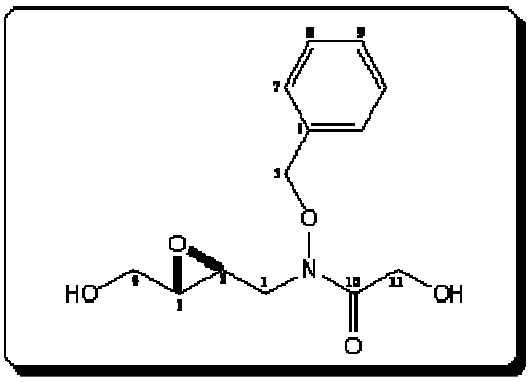


1c (2.99 g, 10.2 mol) was dissolved in a 8:2:1 mixture of Methanol / Triethylamine / water (33 mL) and the mixture was stirred overnight at RT, and evaporated to yield the title compound as a pale yellow oil (2.425 g, 97%; *Rf* 0.35 AcOEt). The product was used without further purification.

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 4.12 (d, 2H, H-1, *J* 4.94 Hz), 4.23 (s, 2H, H-11), 4.26 (d, 2H, H-4, *J* 4.87 Hz), 4.82 (s, 2H, H-5), 5.9-5.7 (m, 2H, H-2, H-3), 7.4-7.3 (m, 5H, Ph)

^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 48.55 (C-1), 60.3 (C-4), 62.2 (C-11), 76.77 (C-5), 123.6 (C-2), 128.8 (C-7, C-7'), 129.3 (C-9), 129.4 (C-8, C-8'), 133.9 (C-6), 134.26 (C-3), 174.1(C-10)

1e



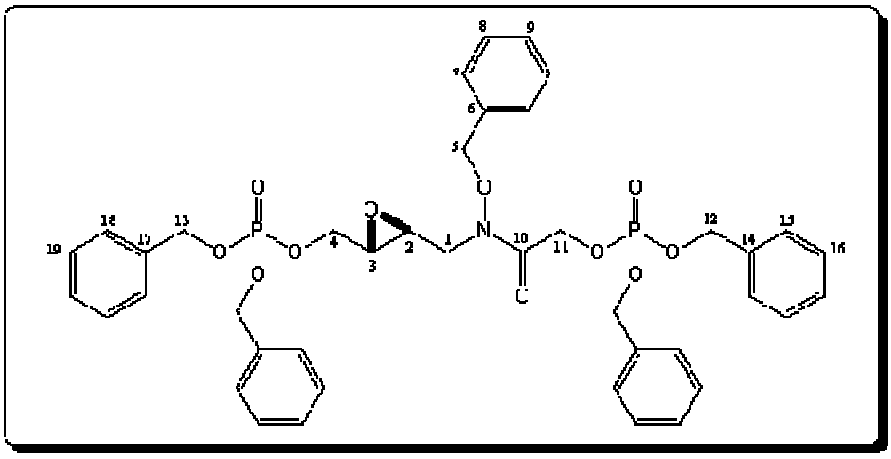
1d (2.425g, 9.67 mmol) was dissolved in anhydrous dichloromethane at 0°C and under argon (50 mL). Solid sodium di-hydrogenophosphate (10.56 g, 59 mmol, 6 eq.) was added, followed

by mCPBA (70% pure, 5.45 g, 22 mmol, 2.2 eq.). The mixture was vigorously stirred overnight. Dichloromethane (40 mL) and saturated aqueous sodium carbonate (40 mL) were added. The organic phase was separated, washed further with sat. sodium carbonate (3 x 25 mL), dried over anhydrous sodium sulfate and evaporated. The product was purified by flash chromatography (Ethyl acetate) to yield the title compound as a pale yellow oil (1.27 g, 48%; *Rf* 0.26 pentane:AcOEt 6:4).

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H), 3.07-3.05 (m, 1H, H-2), 3.25-3.21 (m, 3H, H-3, C-4-OH, C-11-OH), 3.67-3.58 (m, 2H, H-1), 3.9- 3.85 (dd, 1H, H-4, *J* 14.4 Hz , *J* 2.9 Hz,), 4.1(brd, 1H, H-4, *J* 14.4 Hz), 4.24 (s, 2H, H-11), 4.87 (s, 2H, H-5), 7.38 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$, 90.5 MHz) δ_C 49.56 (C-1), 52.51 (C-2), 57.39 (C-3), 60.3 (C-11), 61.07 (C-4), 77.13 (C-5), 128.9 (C-7, C-7'), 129.3 (C-9), 129.5 (C-8, C-8'), 133.8 (C-6), 174.77 (C-10)

1f



Dibenzyl-diispropylphosphoramidite (6.56 g, 19 mmol, 2eq.) and **1e** (1.27 g, 4.75 mmol) were mixed together in a flask and left under vacuum (0.1 torr) during 1.5 h. Triazole (0.98 g, 14 mmol, 1.5 eq.), imidazole (0.65 g, 9.5 mmol, 1 eq.) and anhydrous acetonitrile were then

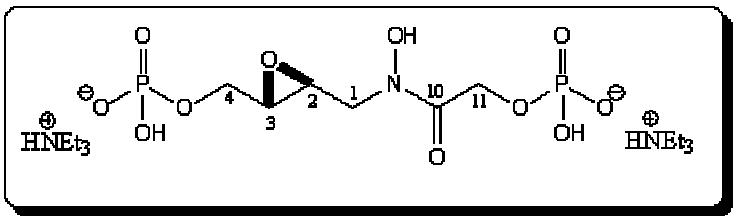
added and the mixture stirred at RT overnight under argon. 70% aqueous ^{t}butylhydroperoxide (2.6 mL, 19 mmol, 2 eq.) was added, and the mixture was stirred for 3 h. 1M sodium thiosulfate (25 mL) was added, and the mixture was extracted with dichloromethane (3 x 25 mL). The combined organic phases were washed with saturated aqueous sodium hydrogenocarbonate, dried over sodium sulfate and evaporated. The product was purified by flash-chromatography (pentane : AcOEt 6:4, *Rf* 0.23) to yield the title compound as a yellow oil (2.725 g, 73%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 3.09-3.08 (m, 2H, H-2, H-3), 3.5 (dd, 1H, H-1, *J* 15.3 Hz, *J* 6.3 Hz), 3.94-3.92 (m, 1H, H-4) 4.21- 4.1 (m, 2H, H-1', H-4'), 4.68 (dd, 2H, H-11, *J* 2.1 Hz, *J* 11.4 Hz), 4.82 (s, 2H, H-5), 5.08 (d, 4H, H-12, H-12'or H-13, H-13', *J* 8.4 Hz), 5.14 (d, 4H, H-13, H-13'or H-12, H-12', *J* 7.8 Hz) 7.36 (m, 25H, Ph)

^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 49.39 (C-1), 52.87 (C-2), 54.72, 54.61 (C-3), 64.22, 64.16 (C-11), 66.76, 66.69 (C-4), 69.54 (C-13, C-13', C-12, C-12'), 77.3 (C-5), 128.04 (C-7,C-7'), 128.57 (C-15, C-18), 128.66 (C-16, C-19), 128.89 (C-9), 129.5 (C-8, C-8'), 133.7 (C-6), 135.61, 135.7, 135.77, 135.87 (C-14,C-14'), 169.45(C-10)

^{31}P NMR ($CDCl_3$, 101.2 MHz) δ_P -0,97 (s, 2P)

1g



1f (0.219 g, 0.28 mmol) and triethylamine (0.056 g, 0.56 mmol, 2 eq.) were dissolved in methanol (7 mL). 10% palladium on charcoal (22 mg) was added, and the mixture was

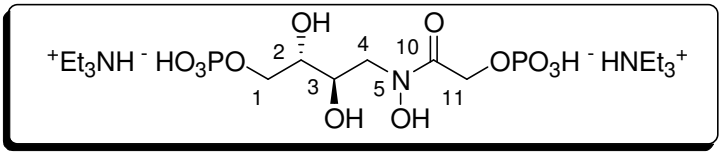
vigorously stirred under dihydrogen (1 bar) at RT until consumption of hydrogen had ceased (around 31 mL). The solid was filtered on celite and the filtrate was evaporated to dryness to yield 1g (0.133 g, 88%).

^{1}H NMR (D2O, 250 MHz) δ_H 1.13 (t, 18H, CH_3 $HNEt_3^+$), 3.05(q, 12H, CH_2 $HNEt_3^+$), 3.23-3.18 (m, 2H, H-2, H-3), 3.71-3.6 (m, 2H, H-1,H-4) 3.91-3.86 (m, 1H, H-1') 4.06-4.02 (m, 1H, H-4'), 4.6 (d, 2H, H-11, *J* 6.9 Hz)

^{13}C NMR (D2O, 63 MHz) δ_C 8.21 (CH_3 $HNEt_3^+$), 46.5 (CH_2 $HNEt_3^+$), 49.46 (C-1), 53.22(C-2) 55.89, 56.02, (C-3), 62.06 (C-11), 64.14, 64.2(C-4), 171.11, 171.17 (C-10)

^{31}P NMR (D2O, 101 MHz) δ_P 0.67 (s, 2P)

1



1g was dissolved in pure water and left at RT for 72 h. 1 was recovered quantitatively by evaporation or freeze drying of the water solution.

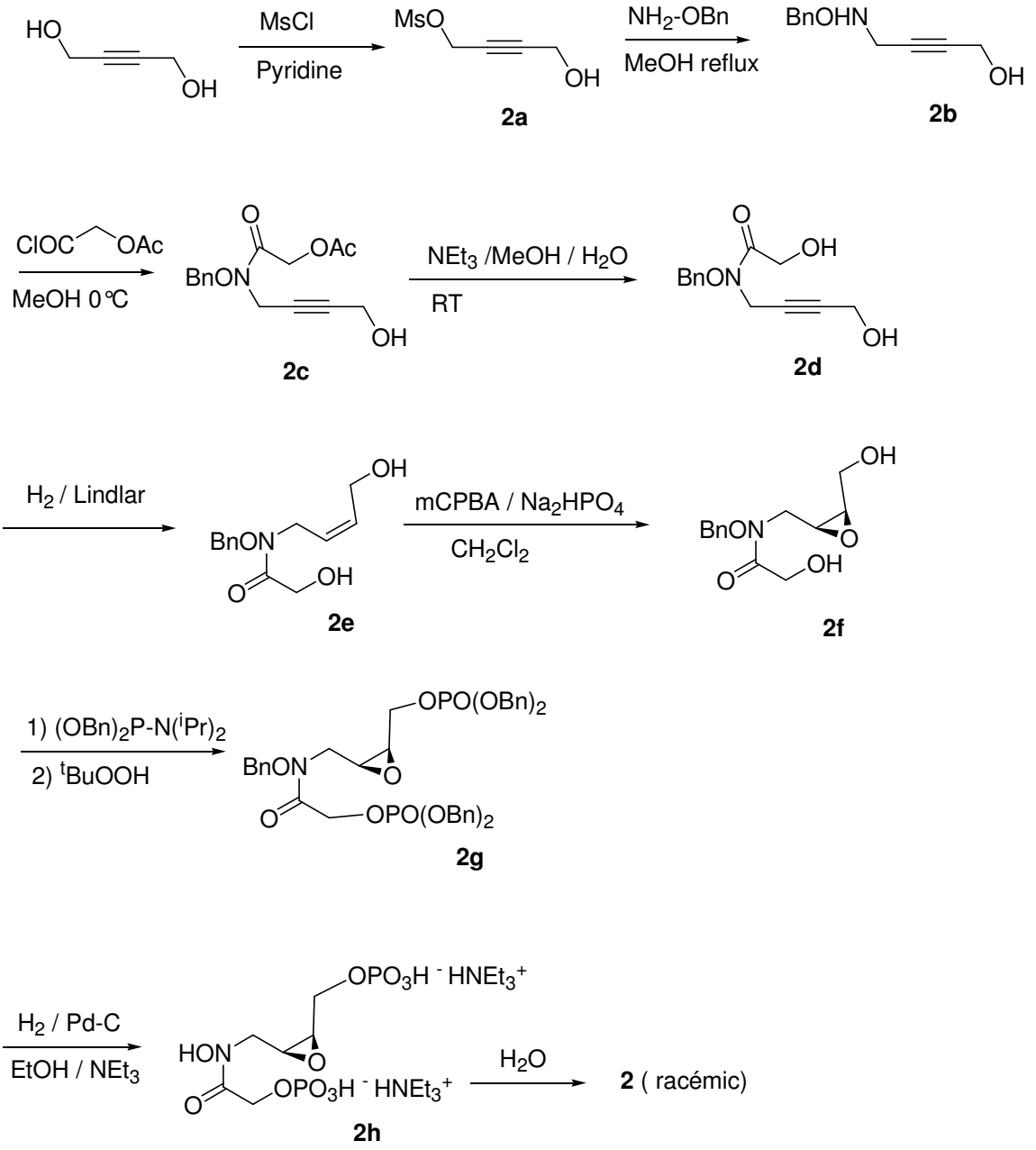
^{1}H NMR (D2O, 250 MHz): δ_H 1.11 (t, 18H, CH_3 $HNEt_3^+$), 3.03 (q, 12H, CH_2 $HNEt_3^+$), 3.6 (m, 2H, H-2, H-3) 3.85-3.76 (m, 4H, H-1, H-4), 4.03-4.02 (m, 1H, H-1'), 4.6 (d, 2H, H-11, *J* 7.25 Hz).

^{13}C NMR (D2O, 63 MHz): δ 8.15 (CH_3 $HNEt_3^+$), 46.49 (CH_2 $HNEt_3^+$), 51.06 (C-1), 62.11 (C-11), 65.75, 65.82 (C-4), 67.54 (C-2), 70.6, 71.7 (C-3) 171.23 (C-10).

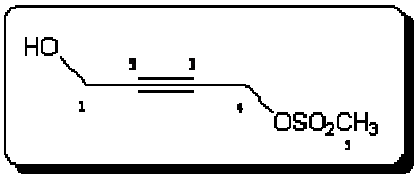
^{31}P NMR (D2O, 101 MHz): 0.67, 0.19 (2s, 2P).

HR-MS (ESI) calcd for $(C_6H_{14}NO_{12}P_2)^-$: 353.9991; found: 353.9986

Synthesis of compound 2 (Compounds 2, 2f, 2g, 2h were synthesized as racemic mixtures. Only one enantiomer is represented in the scheme).



2a

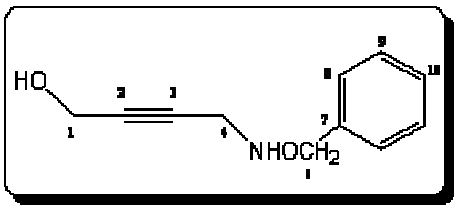


2-butyne-1.4-diol (6.45 g, 75 mmol) and mesylchloride (1.26 g, 11 mmol, 0.15 eq.) were dissolved in anhydrous dioxanne (80 mL) at 0°C and under argon. Anhydrous triethylamine (4.55 g, 45 mmol, 0.6 eq.) was added drop-wise withi 30 min. The mixture was further stirred at 0°C for 30 min and evaporated. The residue was redissolved in water (50 mL) and the solution was extracted with dichloromethane (3 x 50 mL). The organic phase was dried over sodium sulfate and evaporated. The product was purified by flash chromatography (toluene : acetone 8:2, *Rf* 0.18) to yield the title compound as a yellow oil (0.37 g). NMR data in accordance with litterature[2]

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H 3.11 (s, 3H, H-5), 4.28 (t, 2H, H-1, *J* 1.68 Hz), 4.87 (t, 2H, H-4, *J* 1.69 Hz), 4.9 (s, 1H, OH).

^{13}C NMR ($CDCl_3$, 91 MHz) δ_C 38.8 (C-5), 50.5 (C-1), 57.98 (C-4), 77.5(C-3), 88.4 (C-2).

2b



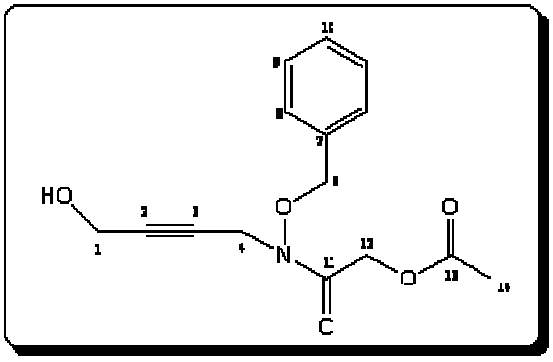
A solution of **2a** (1.23 g, 7.5 mmol) in methanol (60 mL) was added drop-wise to a refluxing solution of O-benzyl hydroxylamine(1.85 g, 15 mmol, 2 eq.) in methanol (100 mL). The mixture was heated overnight and the solvent was evaporated. The formed benzyl hydroxylammonium mesylate was precipitated by addition of ethyl acetate (200mL) and

recovered by filtration. The filtrate was evaporated, the residue redissolved in diethyl ether (50 mL) and this solution was washed with 1M sodium bicarbonate (3 x 20 mL), then dried over sodium acetate and evaporated. The product was purified by flash chromatography (toluene : acetone : NEt_3 9 : 1:0.01, *Rf* 0.15) to yield the title compound as a pale yellow oil (1.15 g, 80%).

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H 3.2 (s, 1H, OH), 3.7 (s, 2H, H-4), 4.24 (s, 2H, H-1), 4.8 (s, 2H, H-6), 7.4-7.38 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$, 91 MHz) δ_C 41.55 (C-4), 50.8 (C-1), 76.3 (C-6), 81.6 (C-3), 82.52 (C-2), 128.03 (C-10), 128.46 (C-9, C-9'), 128.55 (C-8, C-8'), 137.54 (C-7).

2c



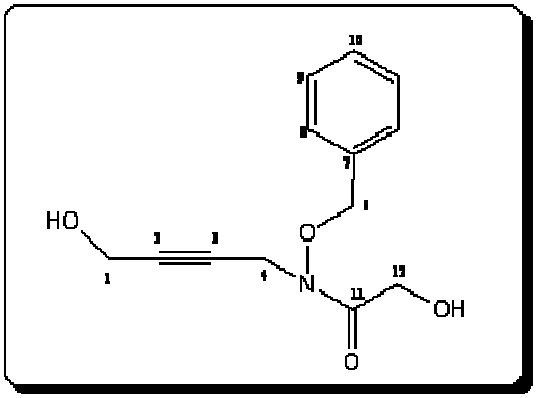
2b (1.16 g, 6.1 mmol) and triethylamine (0.74 g, 7.3 mmol, 1.2 eq) were dissolved in anhydrous methanol at 0°C (13 mL). Acetoxyacetyl chloride (1 g, 7.3 mmol, 1.2 eq.) dissolved in anhydrous dichloromethane (5 mL) was added drop-wise to this solution. After 30 min, dichloromethane (20 mL) and water (20 mL) were added to the mixture. The organic phase was separated, washed three times with water, dried and evaporated to yield the title compound as a pale yellow oil (1.73 g, 99%; *Rf* 0.73, AcOEt). The compound was used without further purification, although it was contaminated by a small amount of methyl

acetoxyacetate, resulting from the methanolysis of acetoxyacetyl chloride.

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H 2.14 (s, 3H, H-14), 2.8 (s, 1H, OH), 4.3 (s, 2H, H-4), 4.4 (s, 2H, H-1), 4.71 (s, 2H, H-6), 5.01 (s, 2H, H-12), 7.14 (m, 5H, Ph). .(Methyl acetoxyacetate: ^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 2.16 (s, 3H, CH_3), 3.76(s, 3H, CH_3), 4.6 (s, 2H, CH_2)).

^{13}C NMR ($CDCl_3$, 91 MHz) δ_C 20.5 (C-14), 37.42 (C-4), 50.8 (C-1), 61.43 (C-12), 77.88 (C-6), 78.87 (C-3), 82.84 (C-2), 128.85 (C-8,C-8'), 129.3 (C-10), 129.49 (C-9, C-9'), 134 (C-7), 169.76 (C-11), 170.62(C-13). .(Methyl acetoxyacetate: ^{13}C NMR ($CDCl_3$, 63 MHz) δ_C 20.41 (CH_3), 52.22 (CH_3), 60.64 (CH_2)).

2d



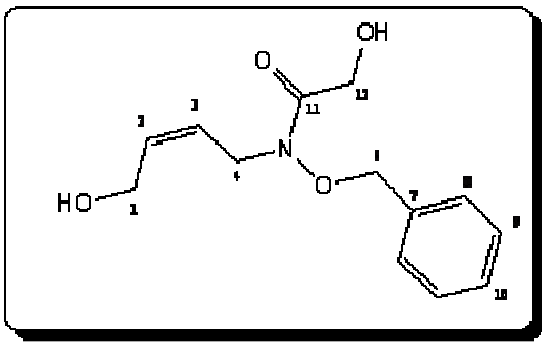
2c (1.73 g, 5.9 mol) was dissolved in a 8:2:1 mixture of Methanol / Triethylamine / water (33 mL). The mixture was stirred overnight at RT, and evaporated to yield the title compound as a pale yellow oil (1.43 g, 97%; *Rf* 0.6 AcOEt). The product was used without further purification.

^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 3.7 (s, 1H, OH), 4.2 (s, 4H, H-1, H-12), 4.4 (s, 2H, H-4), 4.92 (s, 2H, H-6), 7.4 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$, 63 MHz) δ_C 37.58 (C-4), 50.5 (C-1), 60.54 (C-12), 77.75 (C-6), 78.53 (C-

3), 83.03 (C-2), 128.86(C-8, C-8'), 129.37 (C-10), 129.54 (C-9, C-9'), 133.8 (C-7), 175 (C-11).

2e

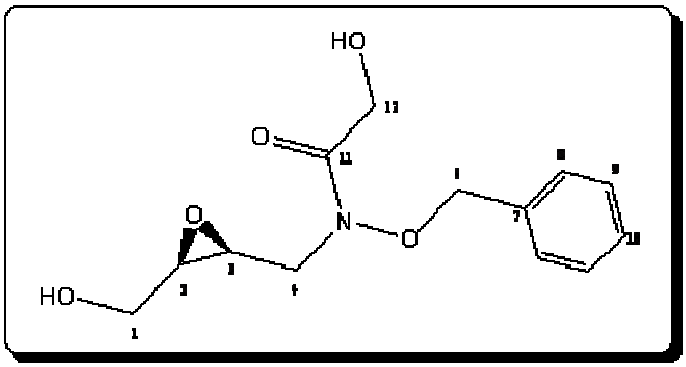


2d (1.18 g, 4.74 mmol) was dissolved in ehtanol (75 mL). Palladium on calcium carbonate and lead (5% Pd, 60 mg) was added and the mixture was vigorously stirred under a dihydrogen atmosphere (1 bar) during 30 min, at which time 106 mL of hydrogen had been consumed. The solid was filtered on celite, the filtrate evaporated and the product purified by flash chromatography (Pentane : AcOEt : NEt_3 4:6:0.01 *Rf* 0.25). The product was recovered as a pale yellow oil (0.5 g, 42%).

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H 2.9 (s, 1H, OH), 3.2 (s, 1H, OH), 4.23 (brs, 4H, H-1, H-12), 4.36 (d, 2H, H-4, *J* 7.27 Hz), 4.86 (s, 2H, H-6), 5.65-5.62 (m, 1H, H-3), 5.9-5.87 (m, 1H, H-2), 7.43-7.38 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$, 91 MHz) δ_C 44.39 (C-4), 58.09 (C-1), 60.43 (C-12), 77.32 (C-6), 124.37 (C-3), 128.9(C-8, C-8'), 129.38 (C-9, C-9'), 133.55 (C-2), 133.82 (C-7), 174.5 (C-11).

2f

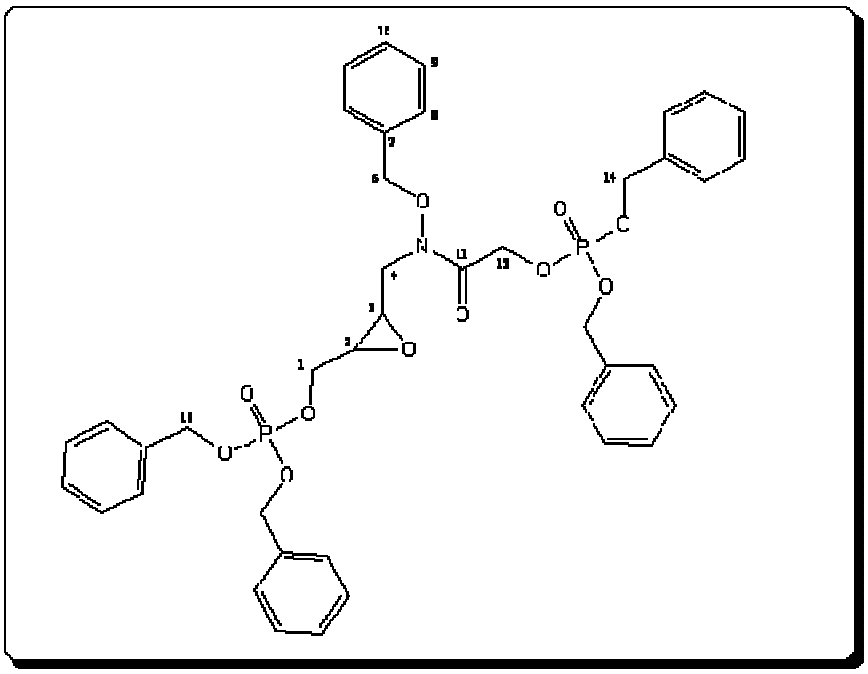


2e (2.425g, 9.67 mmol) was dissolved in anhydrous dichloromethane at 0°C and under argon (50 mL). Solid sodium di-hydrogenophosphate (10.56 g, 59 mmol, 6 eq.) was added, followed by mCPBA (70% pure, 5.45 g, 22 mmol, 2.2 eq.). The mixture was vigorously stirred overnight. Dichloromethane (40 mL) and saturated aqueous sodium carbonate (40 mL) were added. The organic phase was separated, washed further with sat. sodium carbonate (3 x 25 mL), dried over anhydrous sodium sulfate and evaporated. The product was purified by flash chromatography (Ethyl acetate) to yield the title compound as a pale yellow oil (1.27 g, 48%; *Rf* 0.26 pentane:AcOEt 6:4).

^{1}H NMR ($CDCl_3$, 360 MHz) δ_H 3.22-3.19 (m, 2H, H-2, H-3) 3.82 (brs, 2H, 2OH), 4.06-3.74 (m, 4H, H-1, H-4), 4.3 (s, 2H, H-12), 4.87 (s, 2H, H-6), 7.38 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$, 91 MHz) δ_C 45.8 (C-4), 53.25 (C-3), 56.4 (C-2), 59.74 (C-12), 60.3 (C-4), 61.07 (C-1), 77.3 (C-6), 128.9 (C-9, C-9'), 129.5 (C-10), 129.6 (C-8, C-8'), 133.6 (C-7), 175 (C-11).

2g



Dibenzyl-diispropylphosphoramidite (2.36 g, 6.83 mmol, 2eq.) and **2f** (0.456 g, 1.71 mmol) were mixed together in a flask and left under vacuum (0.1 torr) during 1.5 h. Triazole (0.354 g, 5.12 mmol, 1.5 eq.), imidazole (0.233 g, 3.42 mmol, 1 eq.) and anhydrous dichloromethane (15 mL) were then added and the mixture stirred at RT overnight under argon. 70% aqueous ^{t}butylhydroperoxide (0.93 mL, 6.83 mmol, 2 eq.) was added, and the mixture was stirred for 3 h. 1M sodium thiosulfate (25 mL) was added, and the mixture was extracted with dichloromethane (3 x 25 mL). The combined organic phases were washed with saturated aqueous sodium hydrogenocarbonate, dried over sodium sulfate and evaporated. The product was purified by flash-chromatography (pentane : AcOEt 6:4, *Rf* 0.25) to yield the title compound as a yellow oil (0.6 g, 45%).

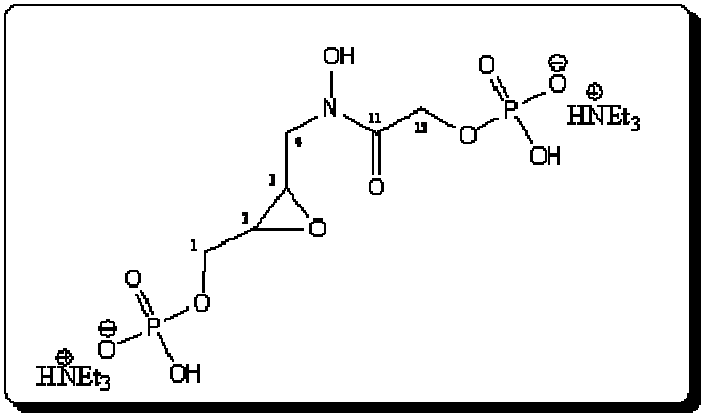
^{1}H NMR ($CDCl_3$, 250 MHz) 3.24-3.22 (m, 2H, H-2, H-3), 3.5 (dd, 1H, H-4, *J* 15.25 Hz, *J* 7 Hz), 4.08-4.003 (m, 2H, H-1, H-4'), 4.23- 4.18 (m, 1H, H-1'), 4.72-4.65 (m, 2H, H-12) 4.8 (s, 2H, H-6), 5.09 (d, 4H, H-14, H-14'or H-16, H-16', *J* 8.56 Hz), 5.15 (d, 4H, H-14, H-14'or H-

16, H-16', *J* 7.86 Hz) 7.38-7.35 (m, 25H, Ph).

^{13}C NMR ($CDCl_3$, 63 MHz): δ 46.23 (C-4), 53.3 (C-3), 54.23-54.1 (C-2), 64.25, 64.18 (C-12), 65.62, 65.54 (C-1), 69.69, 69.6 (C-14, C-14', C-16, C-16') 77.32 (C-6), 128.07, 128.14, 128.6, 128.7, 128.93, 129.45, 129.62, 133.5, 135.77, 135.88 (C Ph), 169.48, 169.39 (C-11)

^{31}P NMR ($CDCl_3$, 101 MHz) δ_P -0.83 (s, 2P)

2h



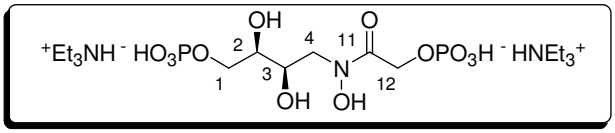
2g (0.219 g, 0.28 mmol) and triethylamine (0.056 g, 0.56 mmol, 2 eq.) were dissolved in methanol (7 mL). 10% palladium on charcoal (22 mg) was added, and the mixture was vigorously stirred under dihydrogen (1 bar) at RT until consumption of hydrogen had ceased (around 31 mL). The solid was filtered on celite and the filtrate was evaporated to dryness to yield 2h (0.102 g, 68%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 1.1 (t, CH_3 $HNEt_3^+$), 3.035 (q, CH_2 $HNEt_3^+$)', 3.27-3.26 (m, 2H, H-2, H-3), 3.84-3.68 (m, 3H, H-1, H-4), 4.08-4.02 (m, 1H, H-1') 4.56 (d, 2H, H-12, *J* 7.33 Hz).

^{13}C NMR (D2O, 75 MHz): δ 8.15 (t, CH_3 $HNEt_3^+$), 46.5 (CH_2 $HNEt_3^+$), 46.97 (C-4), 53.73 (C-3) 55.11, 55.22 (C-2), 62.09 (C-12), 63.16-, 63.21 (C-1), 171.16, 171.23 (C-11).

^{31}P NMR (D2O, 121 MHz): 0.19 (s, 2P)

2



2h was dissolved in pure water and left at RT for 72 h. 2 was recovered quantitatively by evaporation or freeze drying of the water solution.

^{1}H NMR (D2O, 360 MHz): δ_H 1.23 (t, 18H, CH_3 $HNEt_3^+$), 3.15 (q, 12H, CH_2 $HNEt_3^+$), 3.6 (dd, 1H, H-4, *J* 14.4 Hz *J* 4.32 Hz,) 3.8-3.76 (m, 1H, H-3), 3.93-3.87 (m, 3H, H-1, H-2, H-4'), 4.03-4.02 (m, 1H, H-1'), 4.68 (d, 2H, H-12, *J* 7.47 Hz).

^{13}C NMR (D2O, 75 MHz): δ 8.29 (CH_3 $HNEt_3^+$), 46.68 (CH_2 $HNEt_3^+$), 51.02 (C-4), 62.21, 62.25 (C-1), 65.84, 65.89 (C-12), 67.15 (C-3), 70.53, 70.61(C-2), 171.16 , 171.13 (C-11).

^{31}P NMR (D2O, 121 MHz): 0.5, 0.26 (2s, 2P).

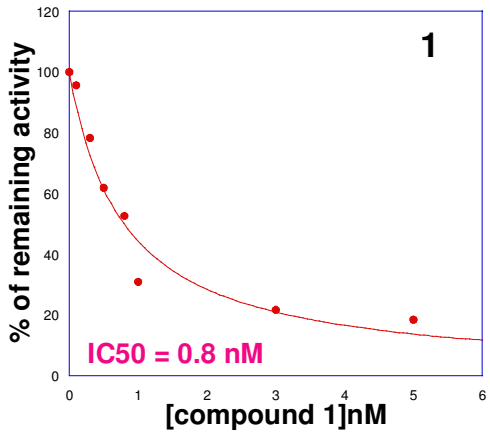
HR-MS (ESI) calcd for $(C_6H_{14}NO_{12}P_2)^-$: 353.9991; found: 354.0005

References

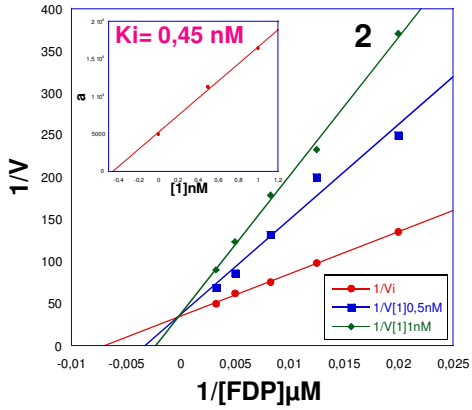
1. Kinoshita, M.; Masahiko, M.; Tanigushi, M.; Shimizu, J. *Bull. Soc. Chim. Jpn.* 1987, *60*, 4005.
2. Shirrokova, E.A.; Trussova, N.B.; Shipitsin, A.V., Semizarov, D.G.; Krayevsky, A.A. *J. Med. Chem.* 1994, *37*, 3739.

Enzymatic test on *Candida albicans* Fba; compound 1

Determination of IC_{50}

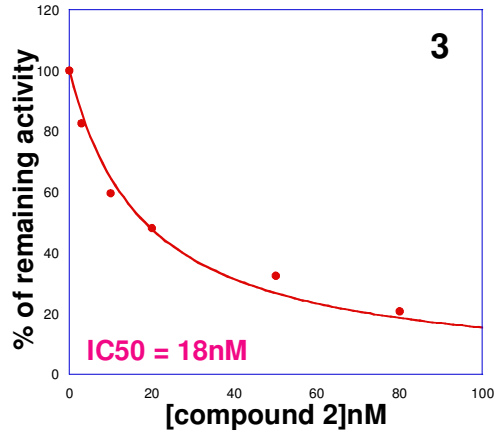


Determination of Ki

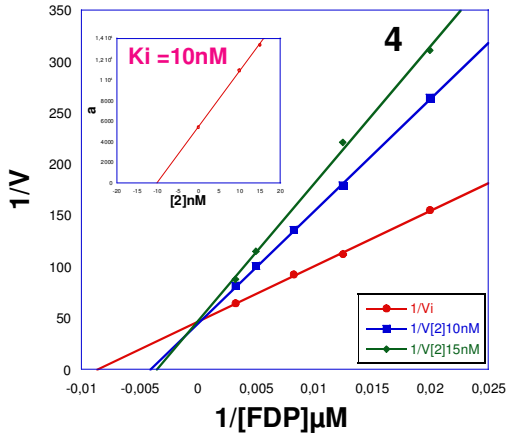


Enzymatic test on *Candida albicans* Fba; compound 2

Determination of IC_{50}

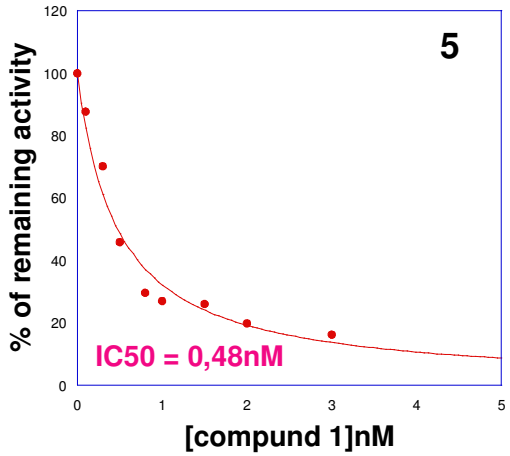


Determination of Ki

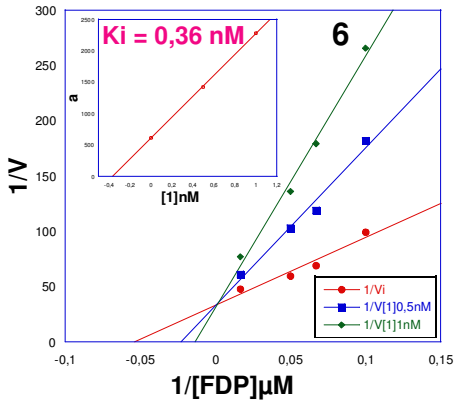


Enzymatic test on *Mycobacterium tuberculosis* Fba; compound 1

Determination of IC_{50}

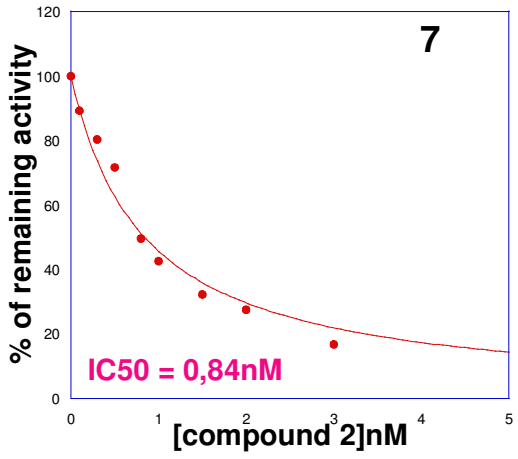


Determination of Ki

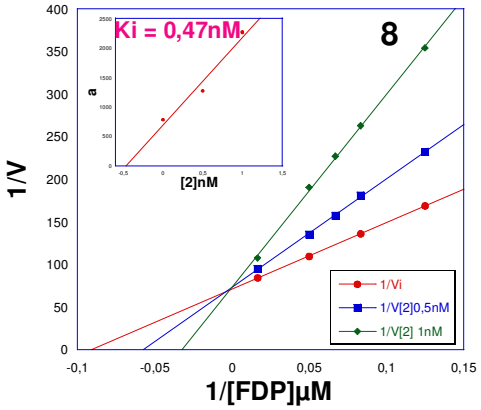


Enzymatic test on *Mycobacterium tuberculosis* Fba; compound 2

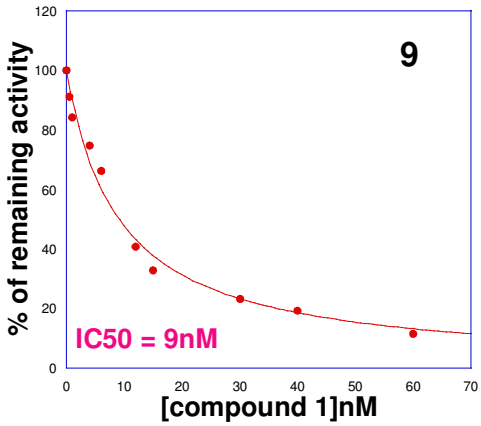
Determination of IC_{50}

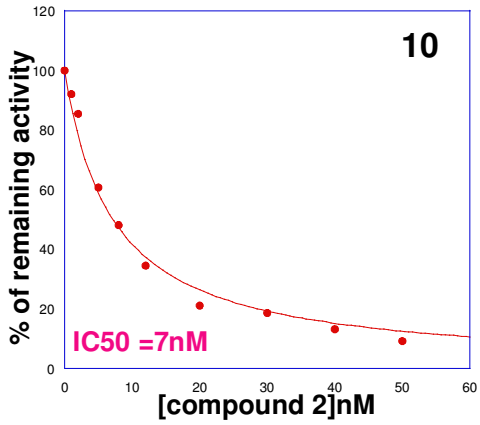


Determination of Ki

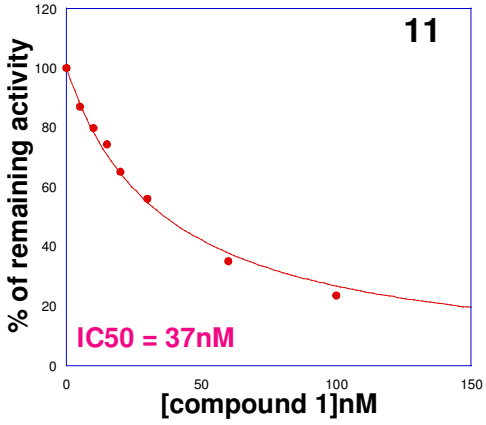


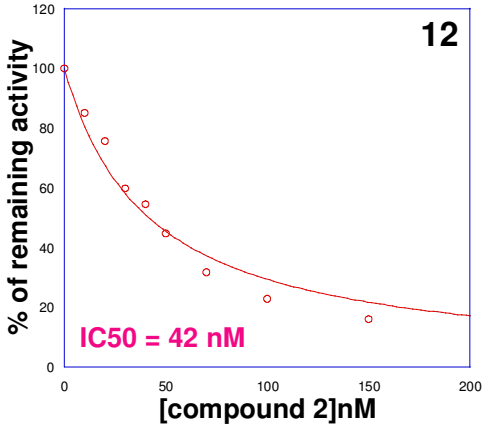
Enzymatic tests on *Yesinia pestis* Fba; compounds 1 and 2. Determination of IC_{50}





Enzymatic tests on *Helicobacter pylori* Fba; compounds 1 and 2. Determination of IC_{50}





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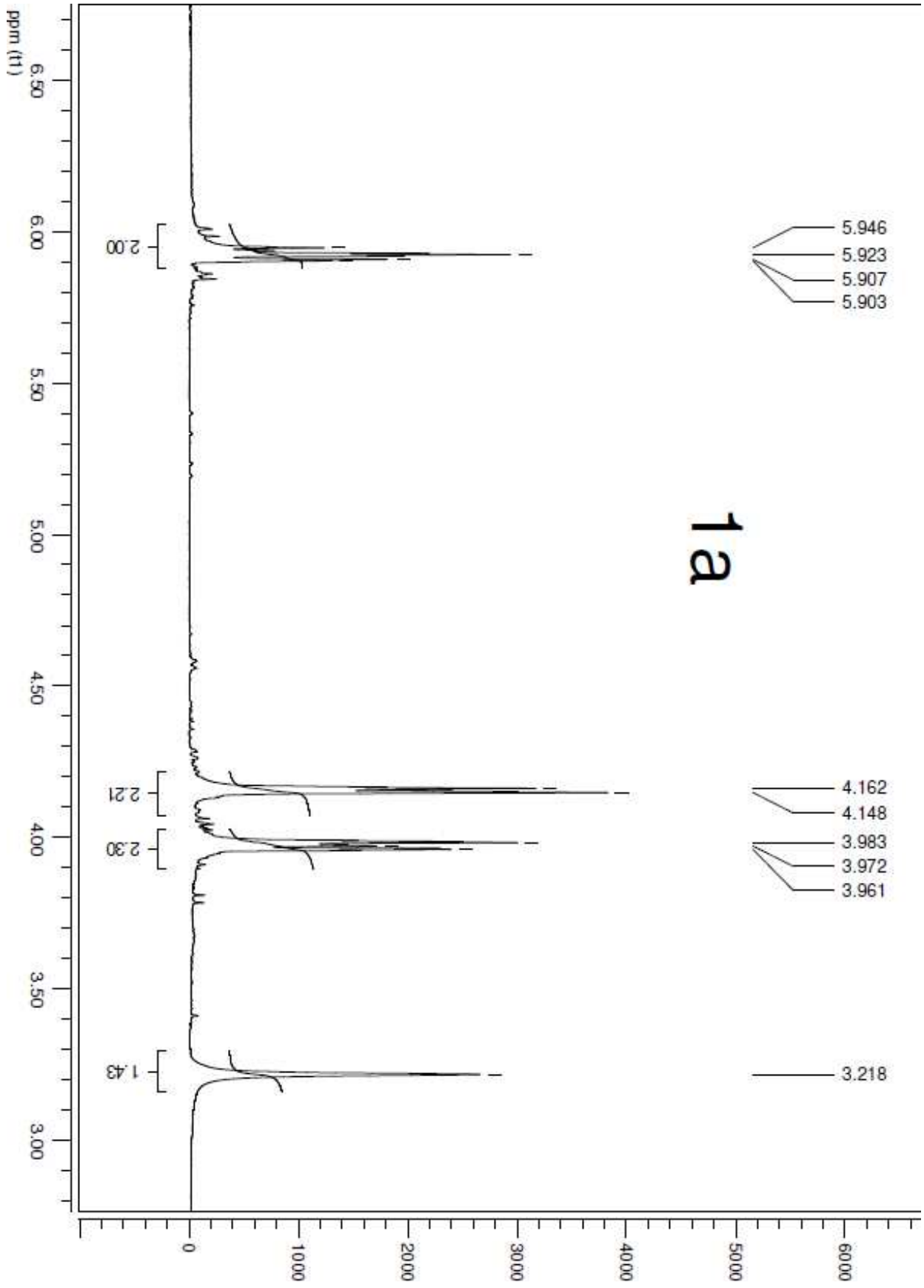
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2.21
2.30
1.43
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4.00
3.50
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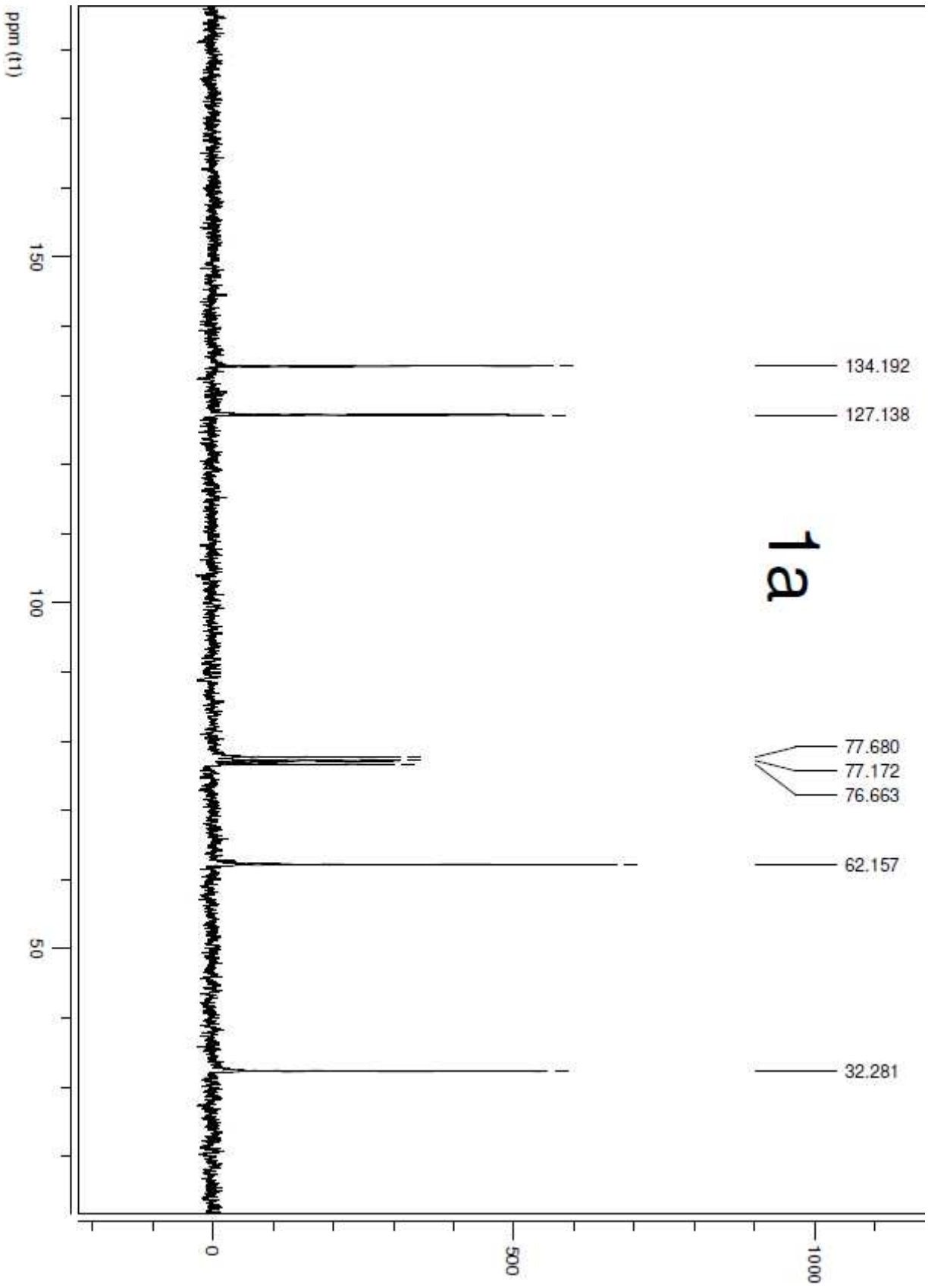
5000
6000

^{13}C NMR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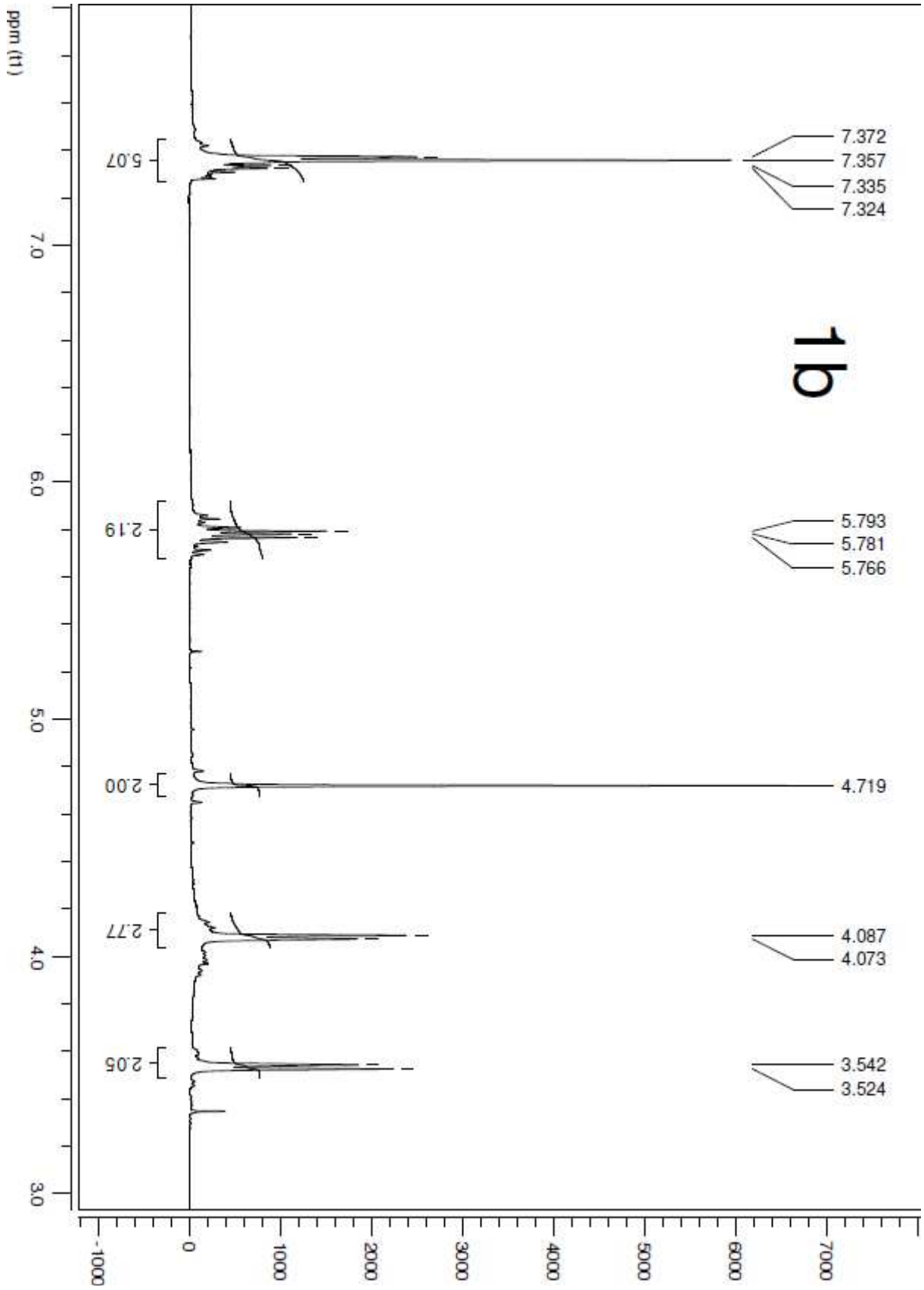
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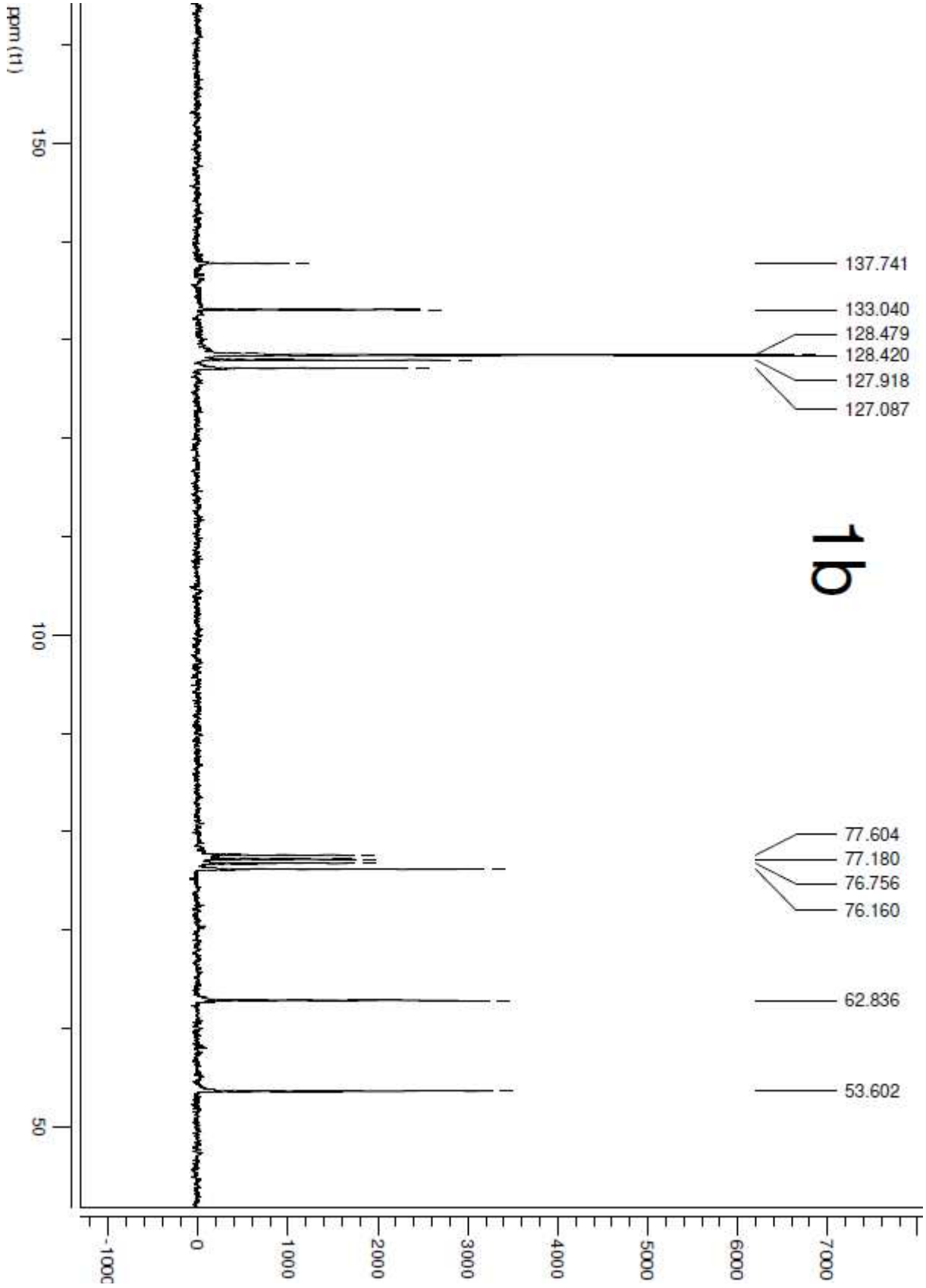
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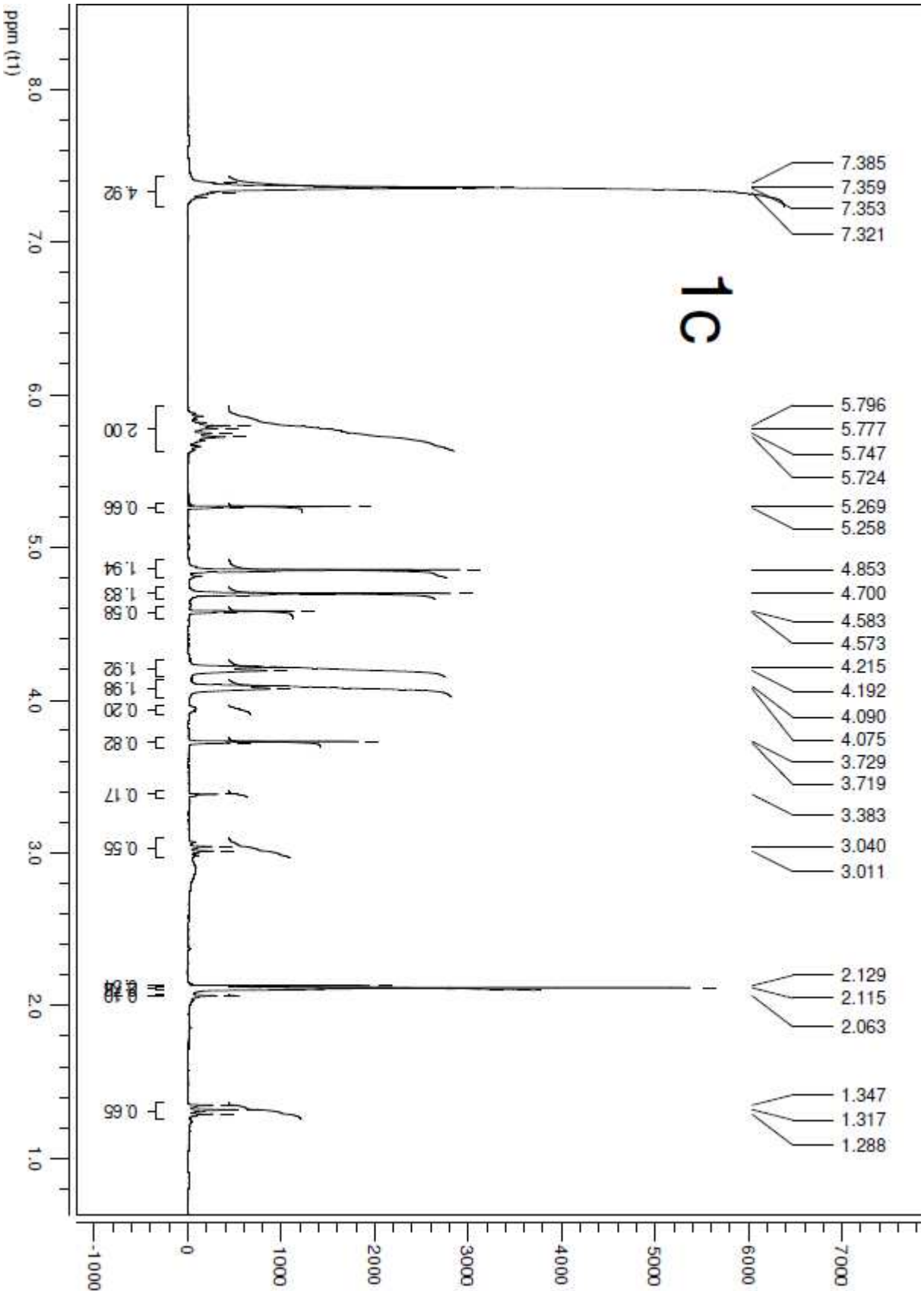
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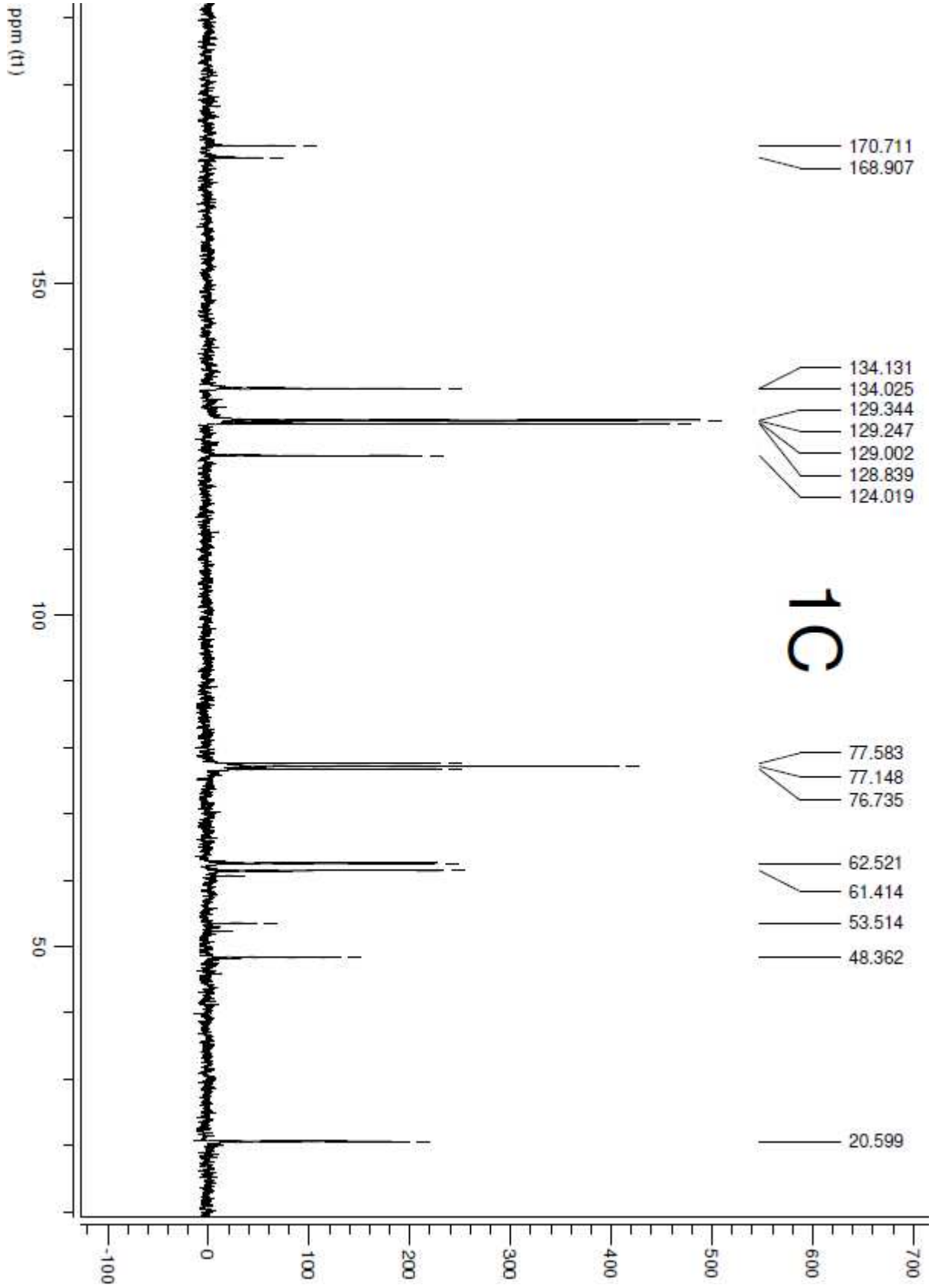
ppm (t1)

^{13}C NMR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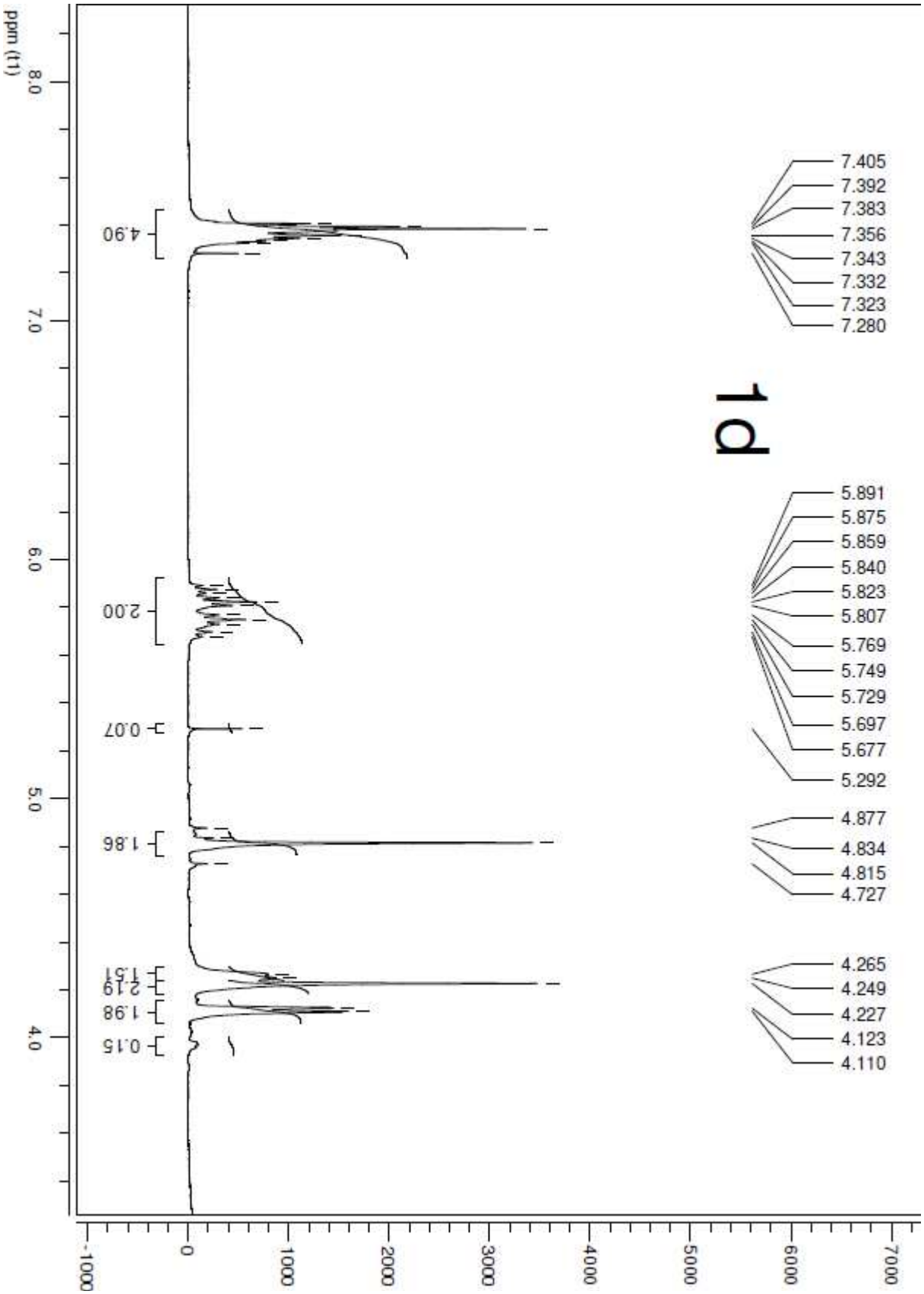
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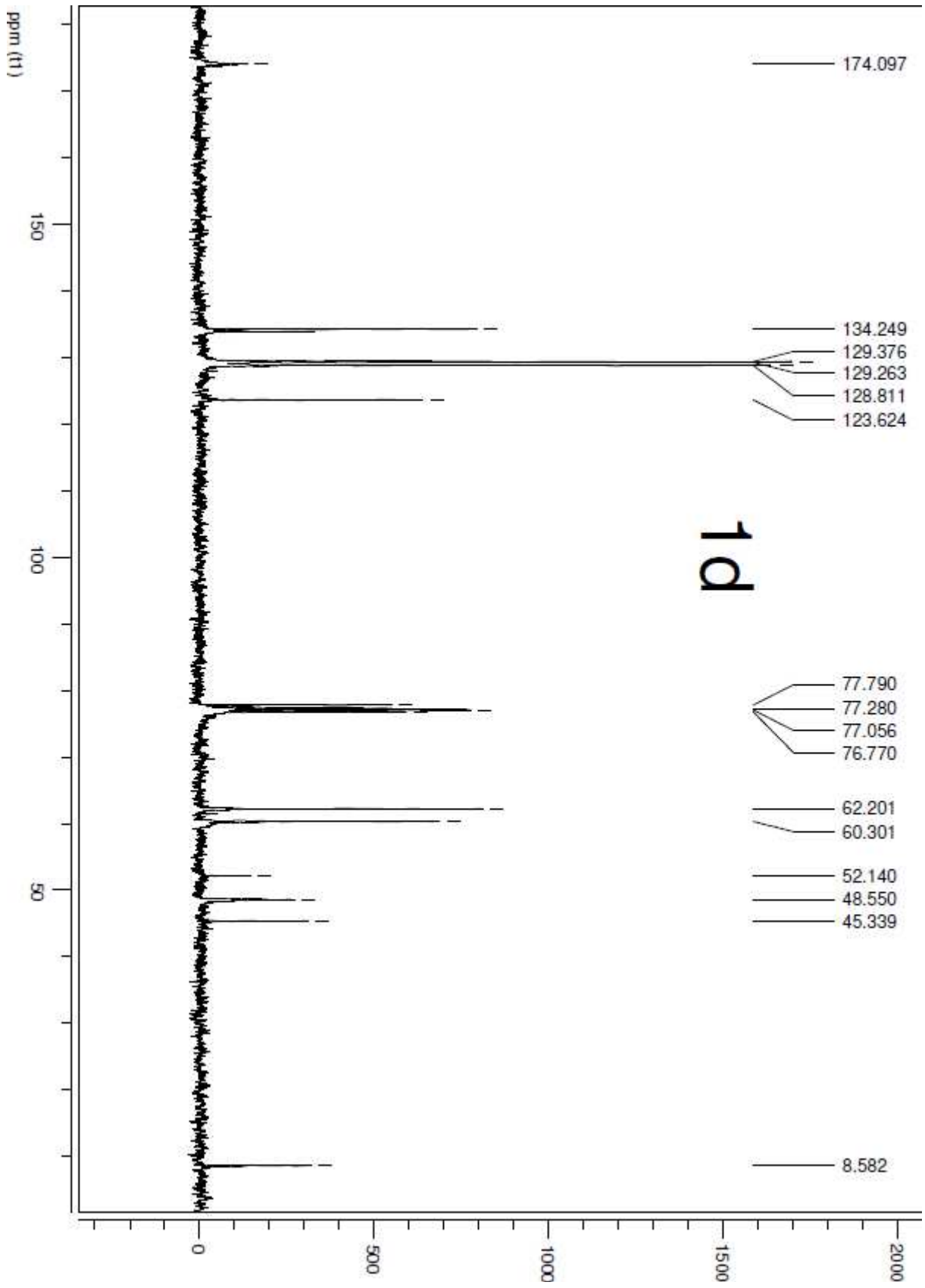
^{13}C NMR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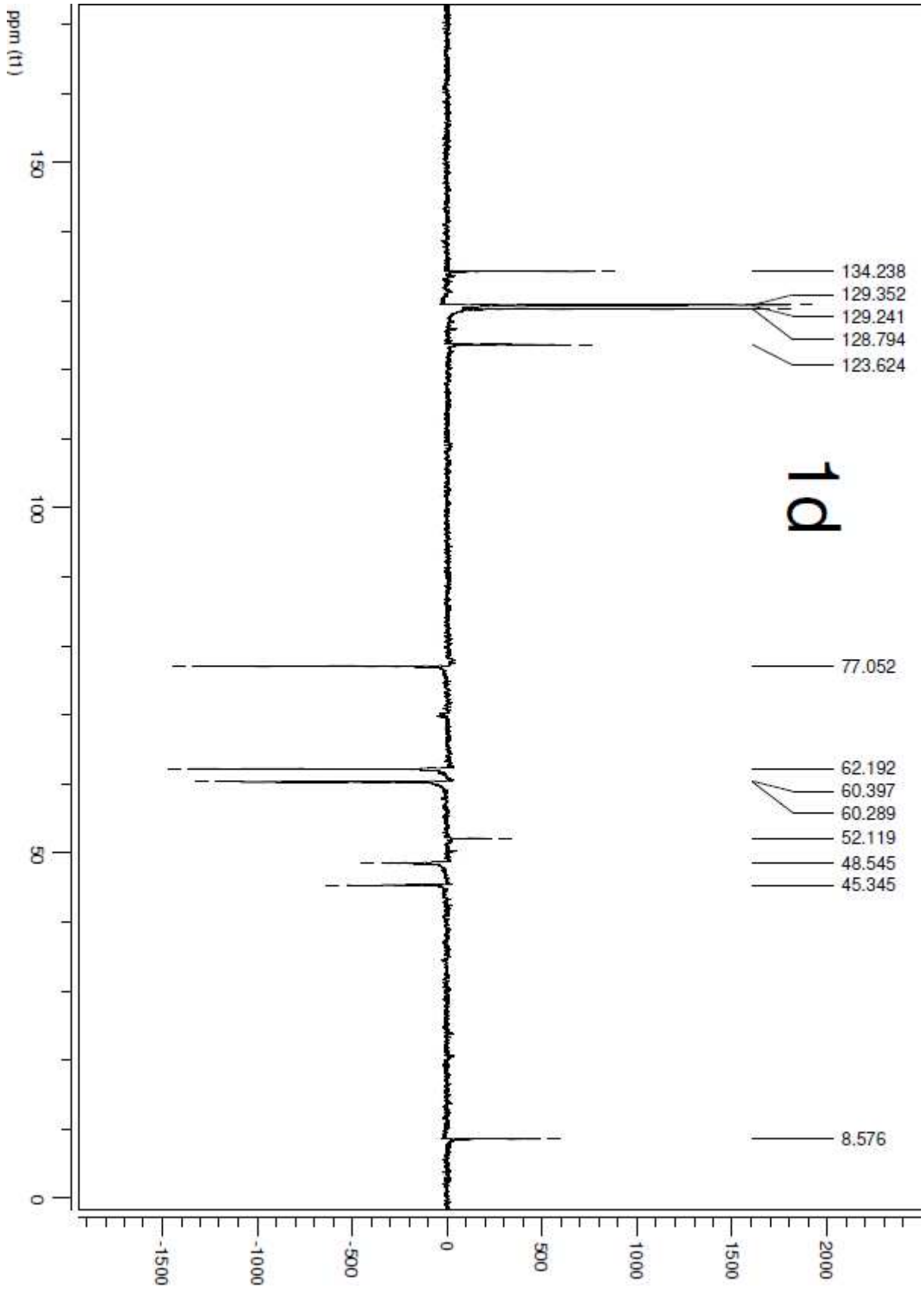
DEPT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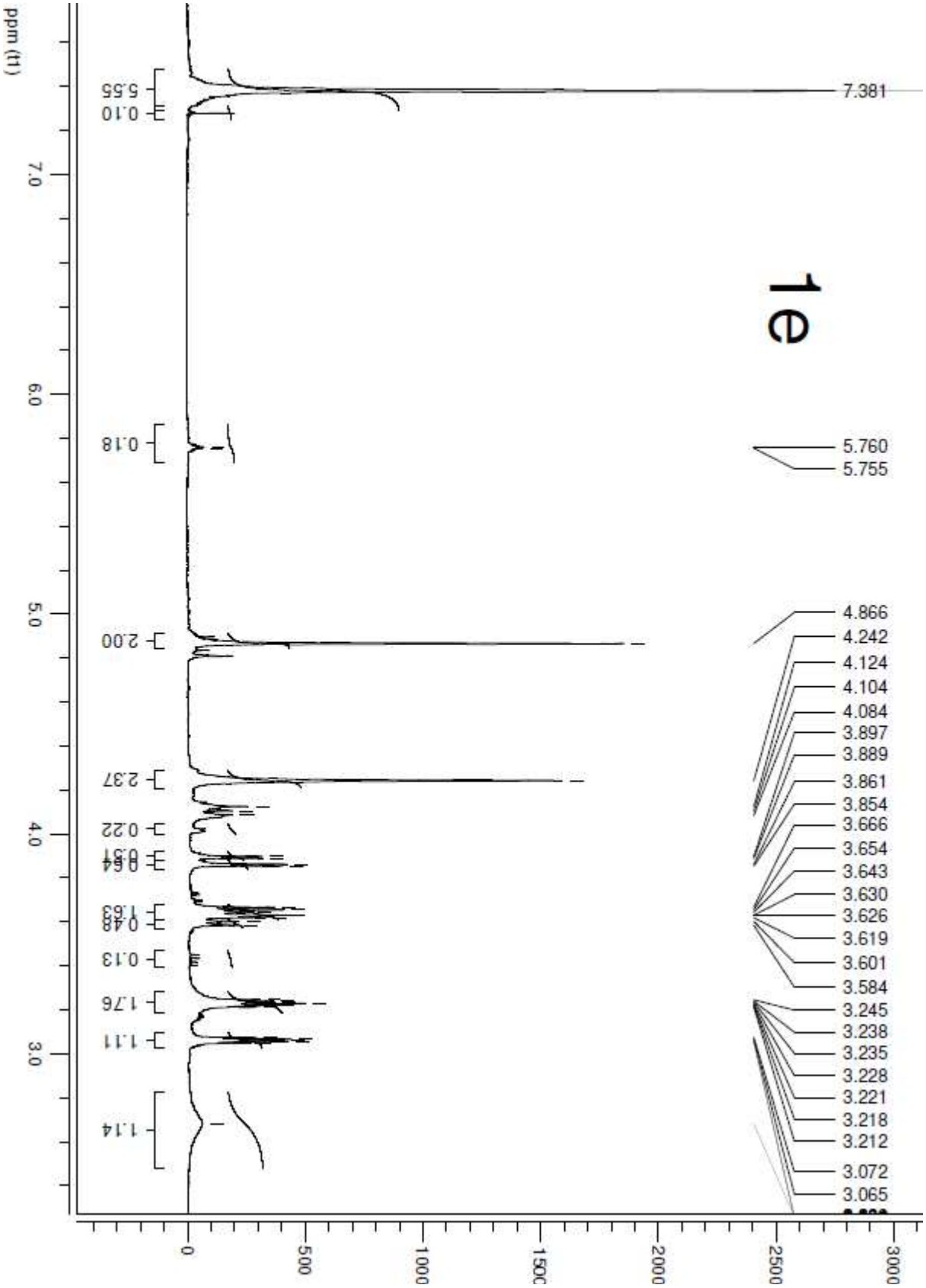
^{1}H NMR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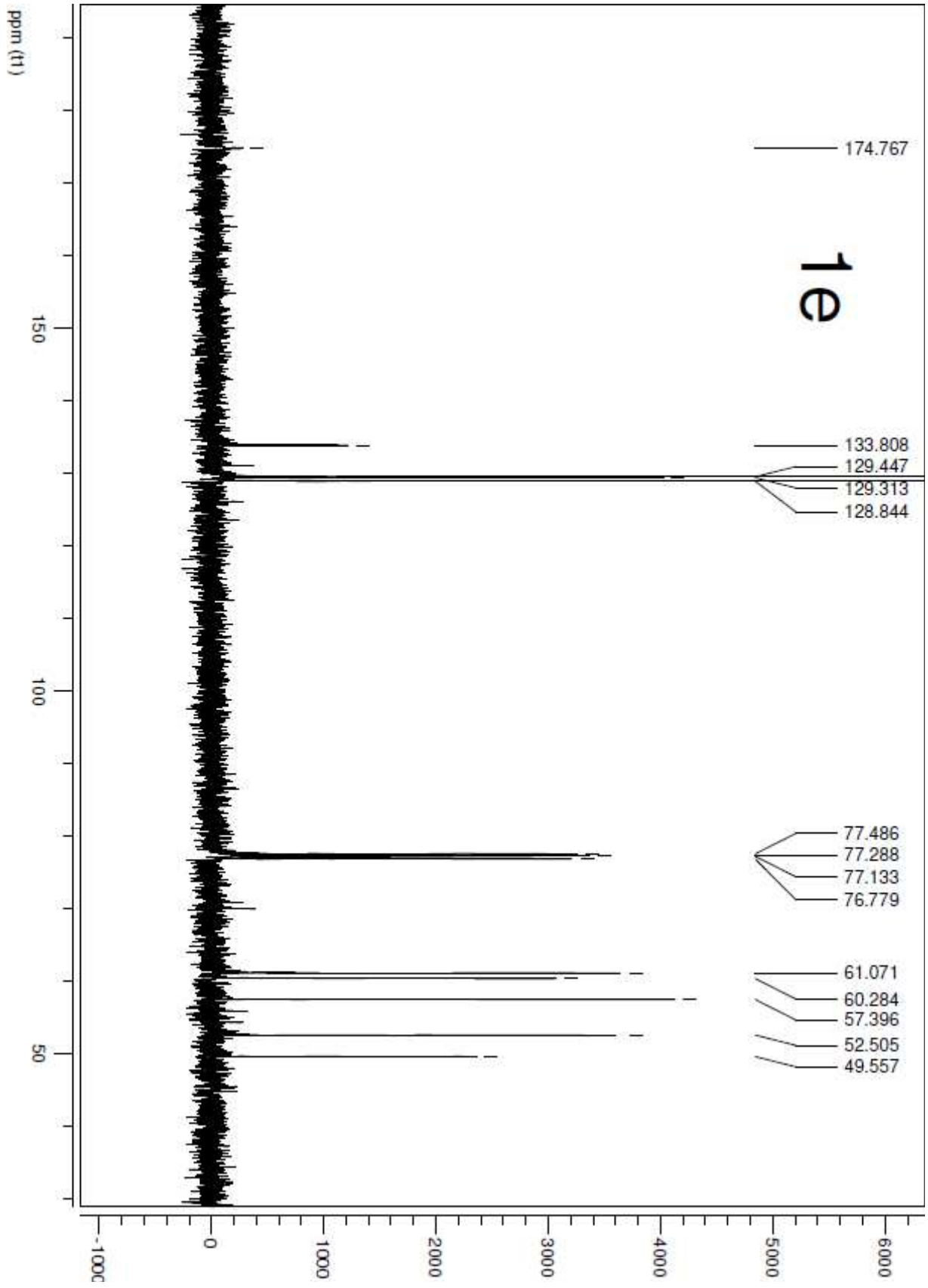
^{13}C NMR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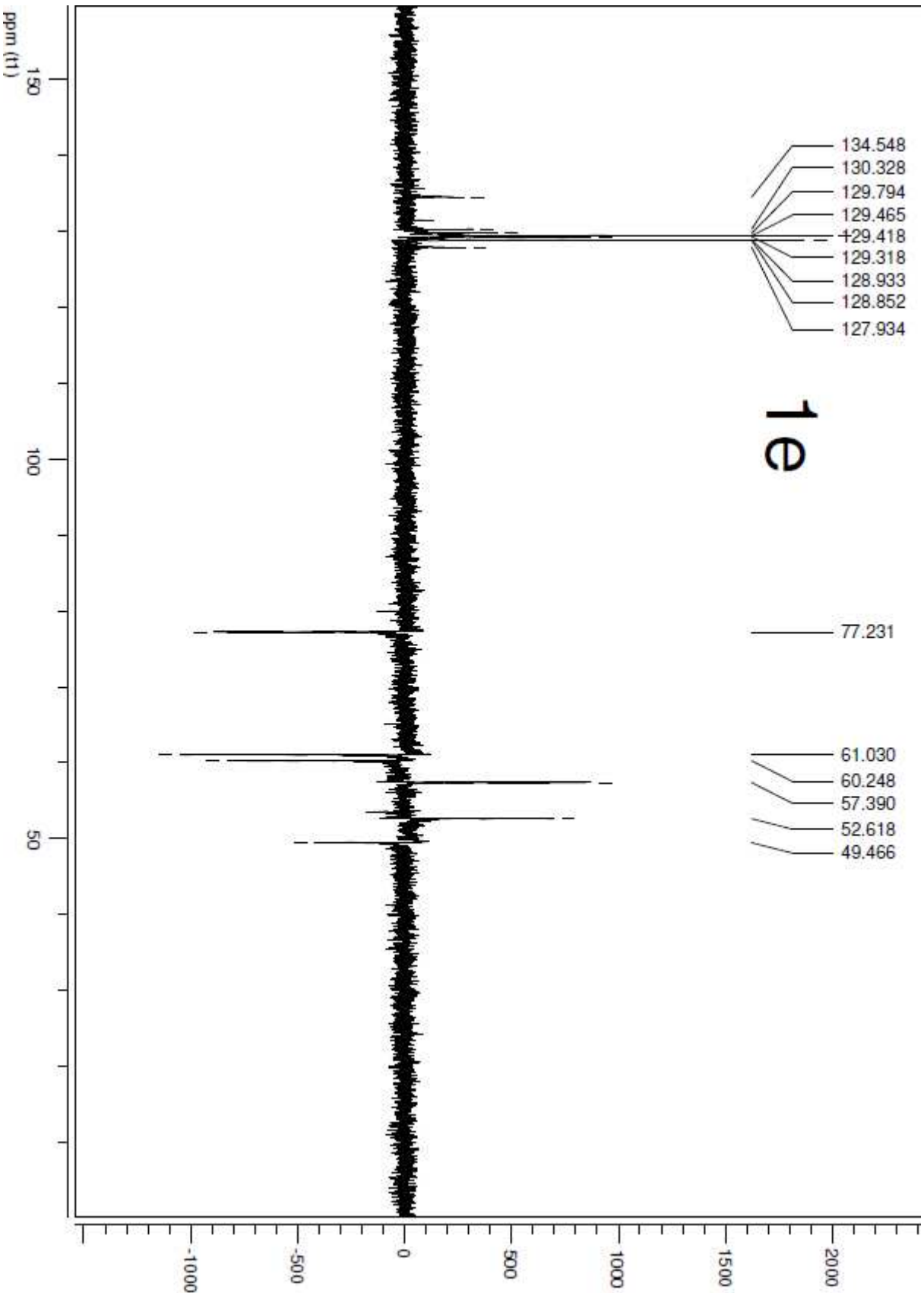
DEPT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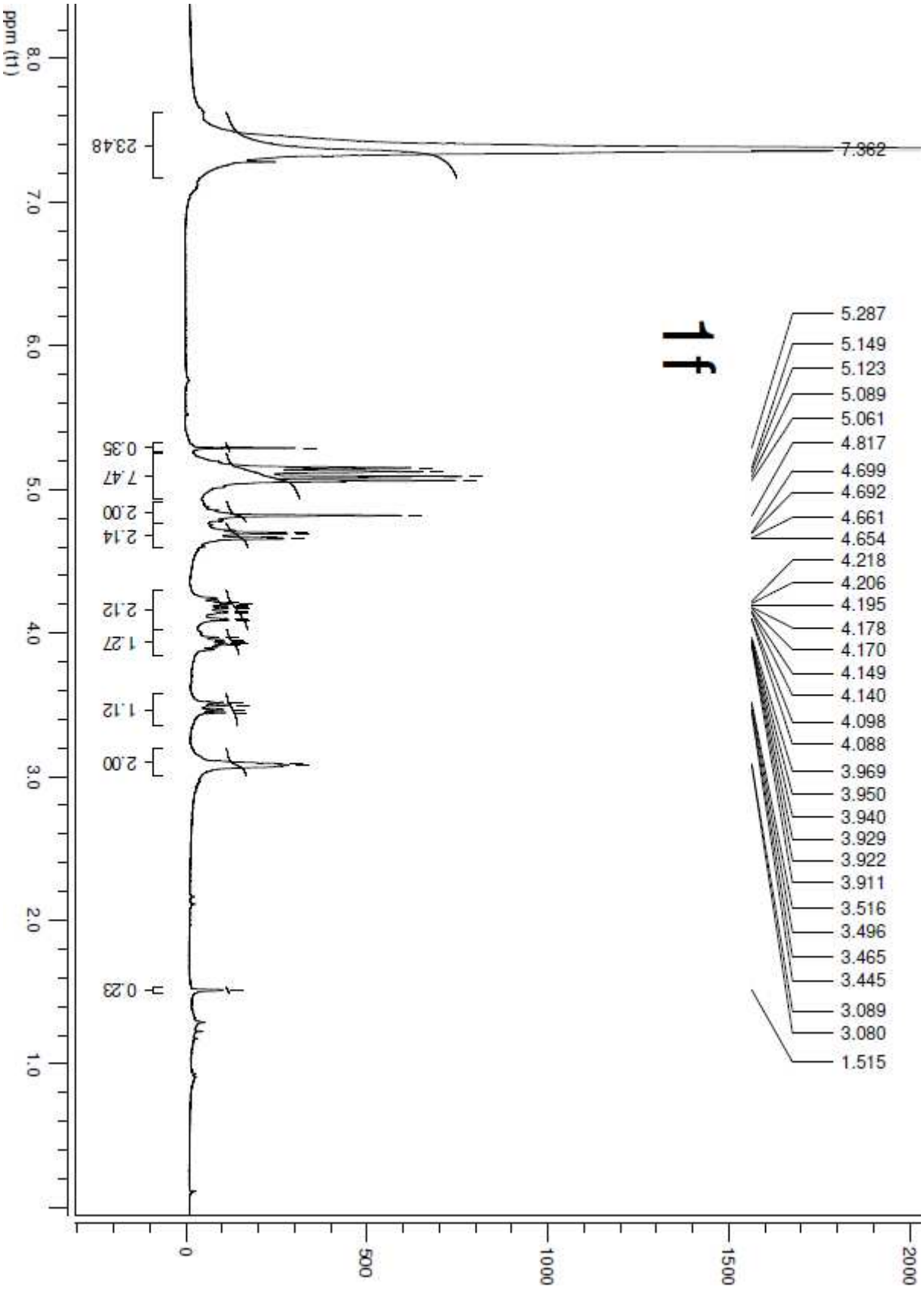
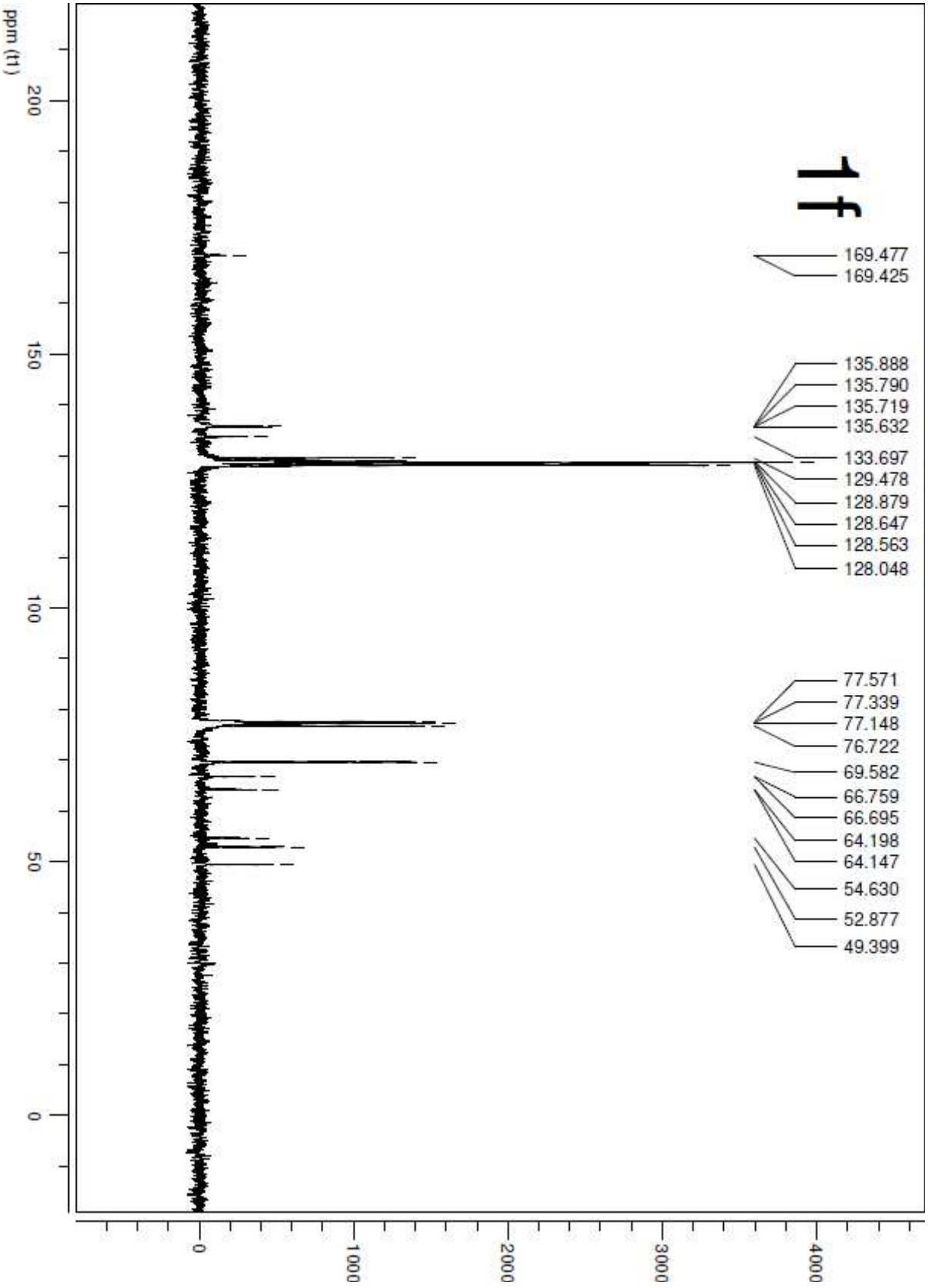
1000
1500
2000

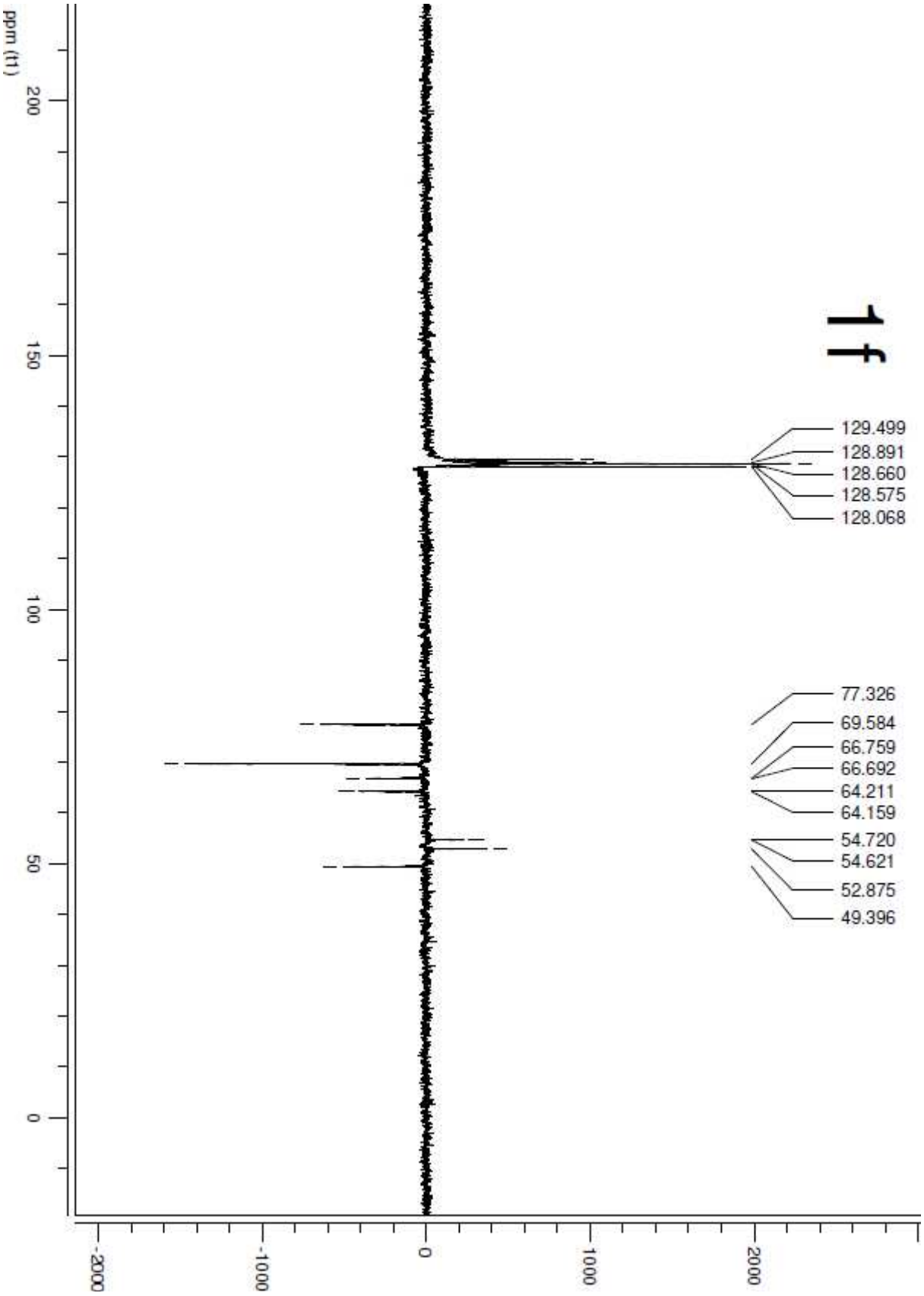
^{1}H NMR


ppm (t1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
500
1000
1500
2000
23.48
0.35
7.47
2.00
2.14
2.12
1.27
1.12
2.00
0.23
7.362
5.287
5.149
5.123
5.089
5.061
4.817
4.699
4.692
4.661
4.654
4.218
4.206
4.195
4.178
4.170
4.149
4.140
4.098
4.088
3.969
3.950
3.940
3.929
3.922
3.911
3.516
3.496
3.465
3.445
3.089
3.080
1.515

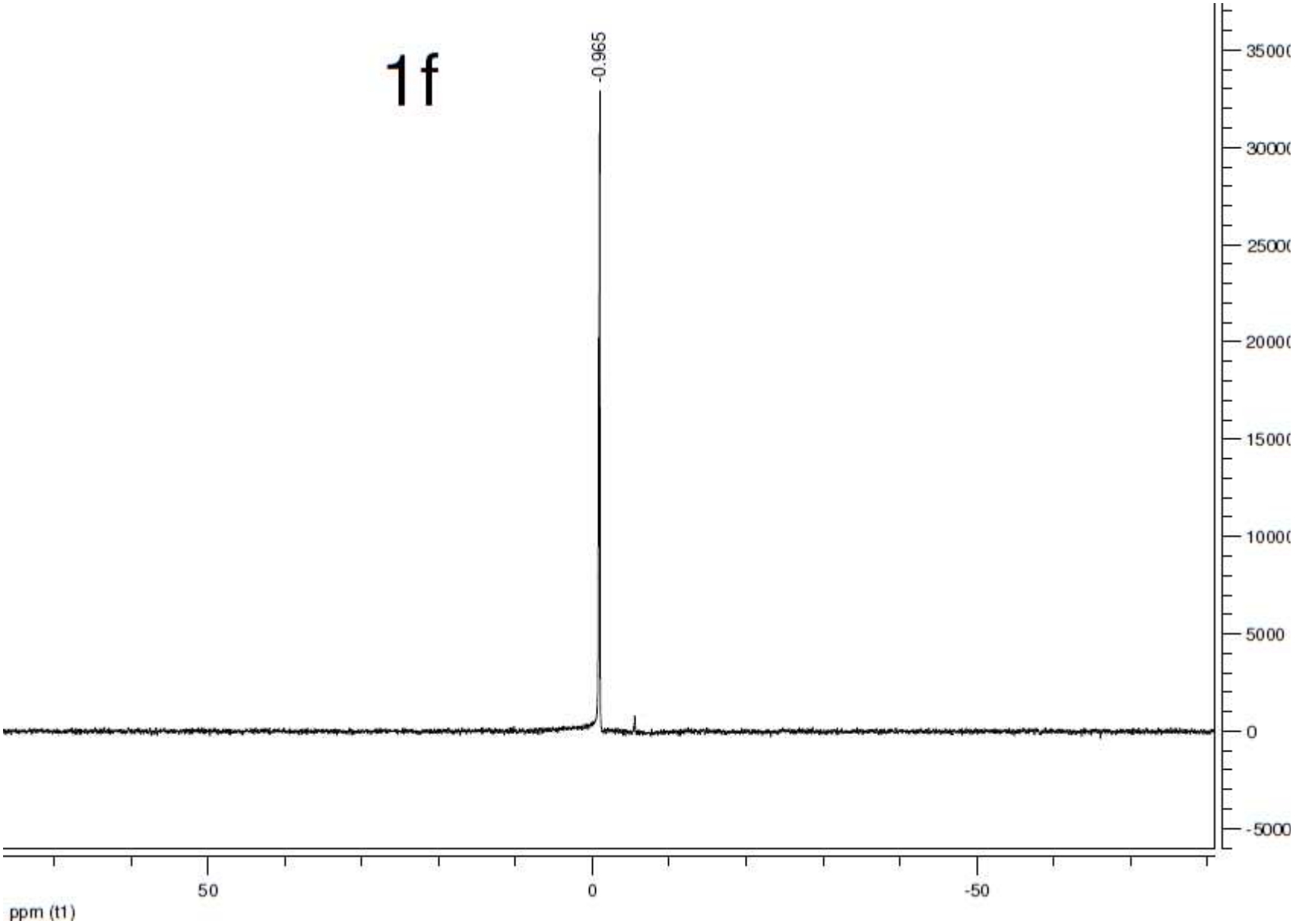
^{13}C NMR


1h
169.477
169.425
135.888
135.790
135.719
135.632
133.697
129.478
128.879
128.647
128.563
128.048
77.571
77.339
77.148
76.722
69.582
66.759
66.695
64.198
64.147
54.630
52.877
49.399
ppm (t1)
200
150
100
50
0
0
1000
2000
3000
4000

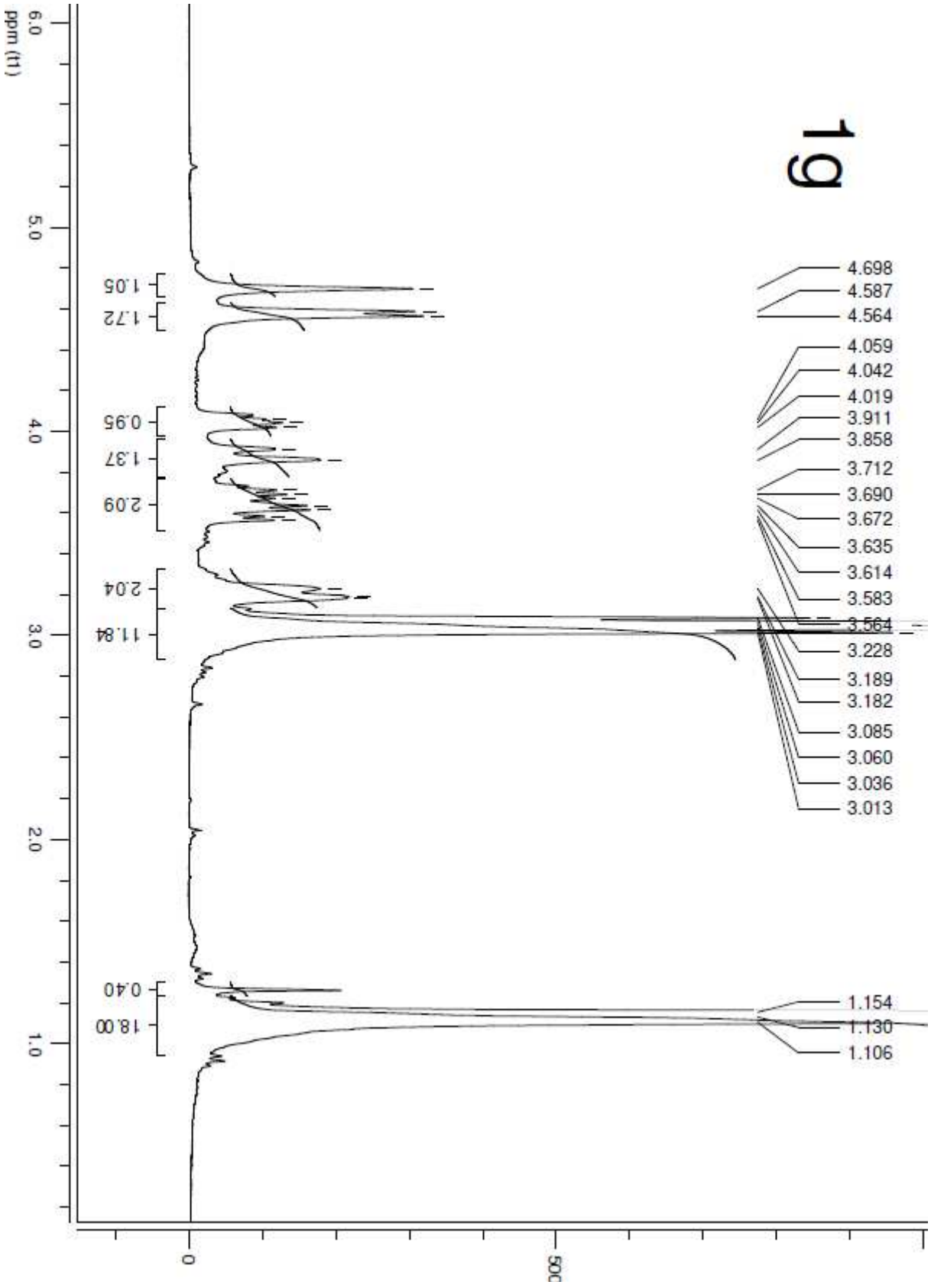
DEPT


ppm (t1)
200
150
100
50
0
-2000
-1000
0
1000
2000
129.499
128.891
128.660
128.575
128.068
77.326
69.584
66.759
66.692
64.211
64.159
54.720
54.621
52.875
49.396

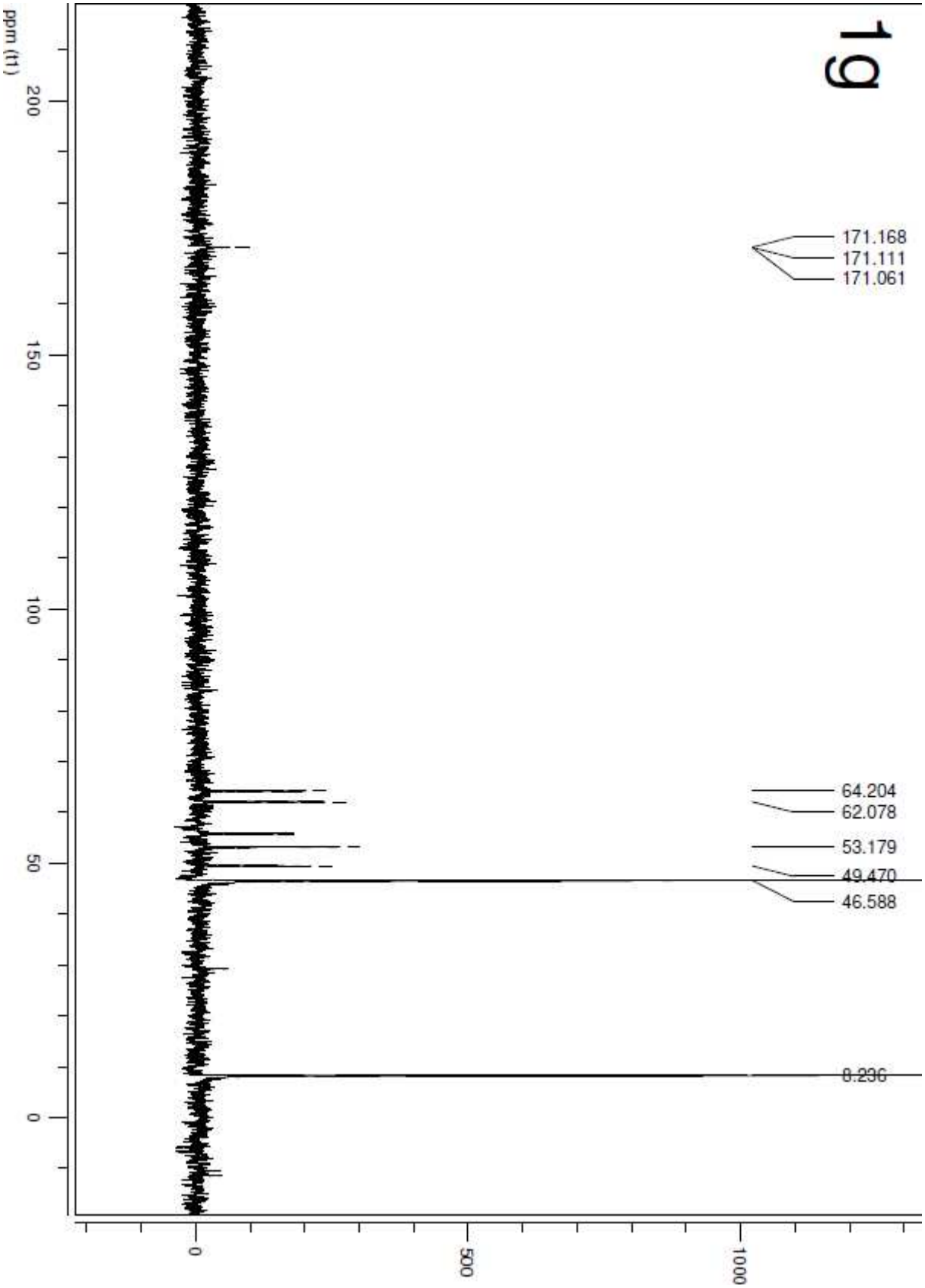
^{31}P-NMR


1f
-0.965
35000
30000
25000
20000
15000
10000
5000
0
-5000
50
0
-50
ppm (t1)

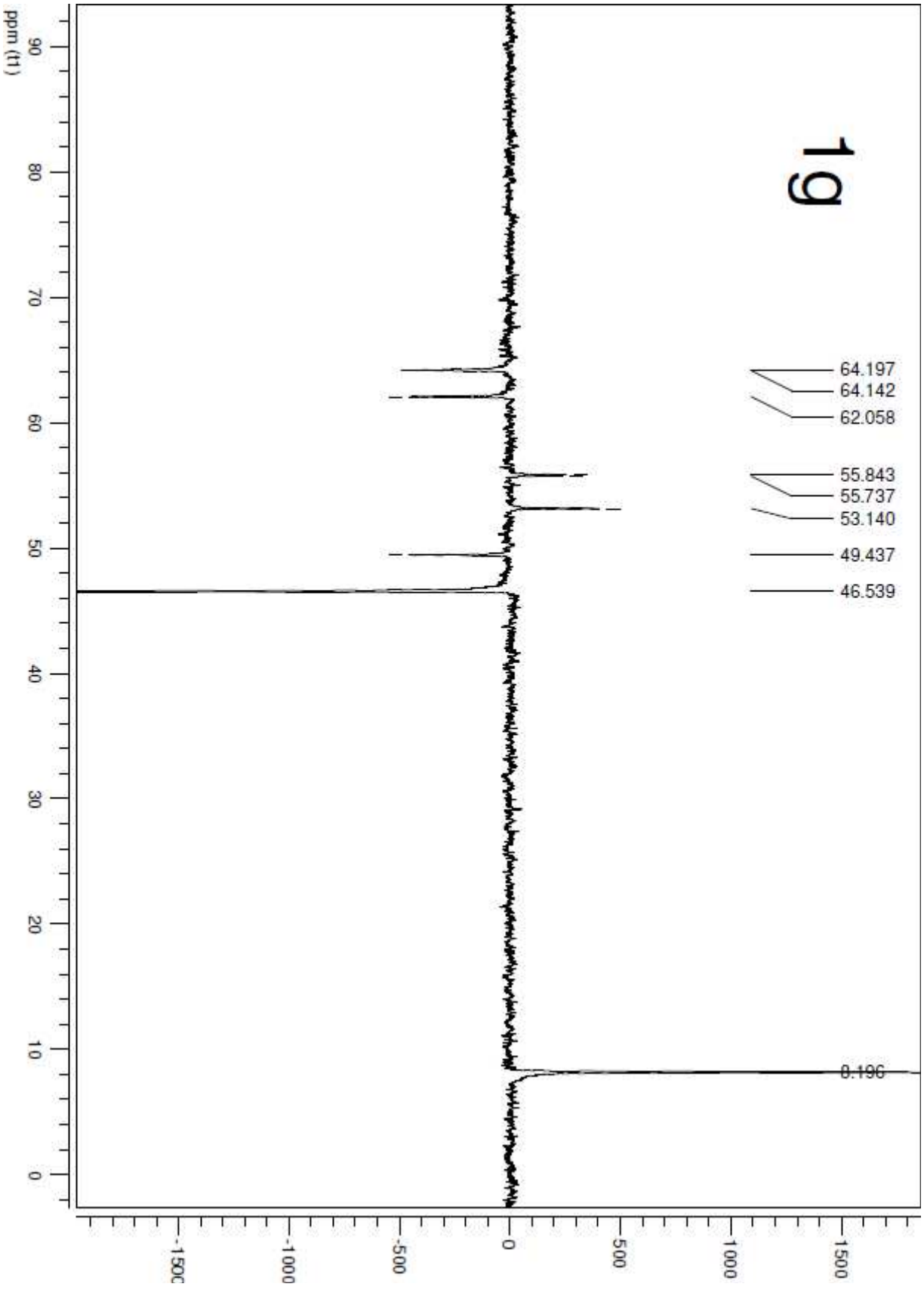
^{1}H NMR


1g
4.698
4.587
4.564
4.059
4.042
4.019
3.911
3.858
3.712
3.690
3.672
3.635
3.614
3.583
3.564
3.228
3.189
3.182
3.085
3.060
3.036
3.013
1.154
1.130
1.106
1.05
1.72
0.95
1.37
2.09
2.04
11.84
0.40
18.00
ppm (t1)
6.0
5.0
4.0
3.0
2.0
1.0
0
500

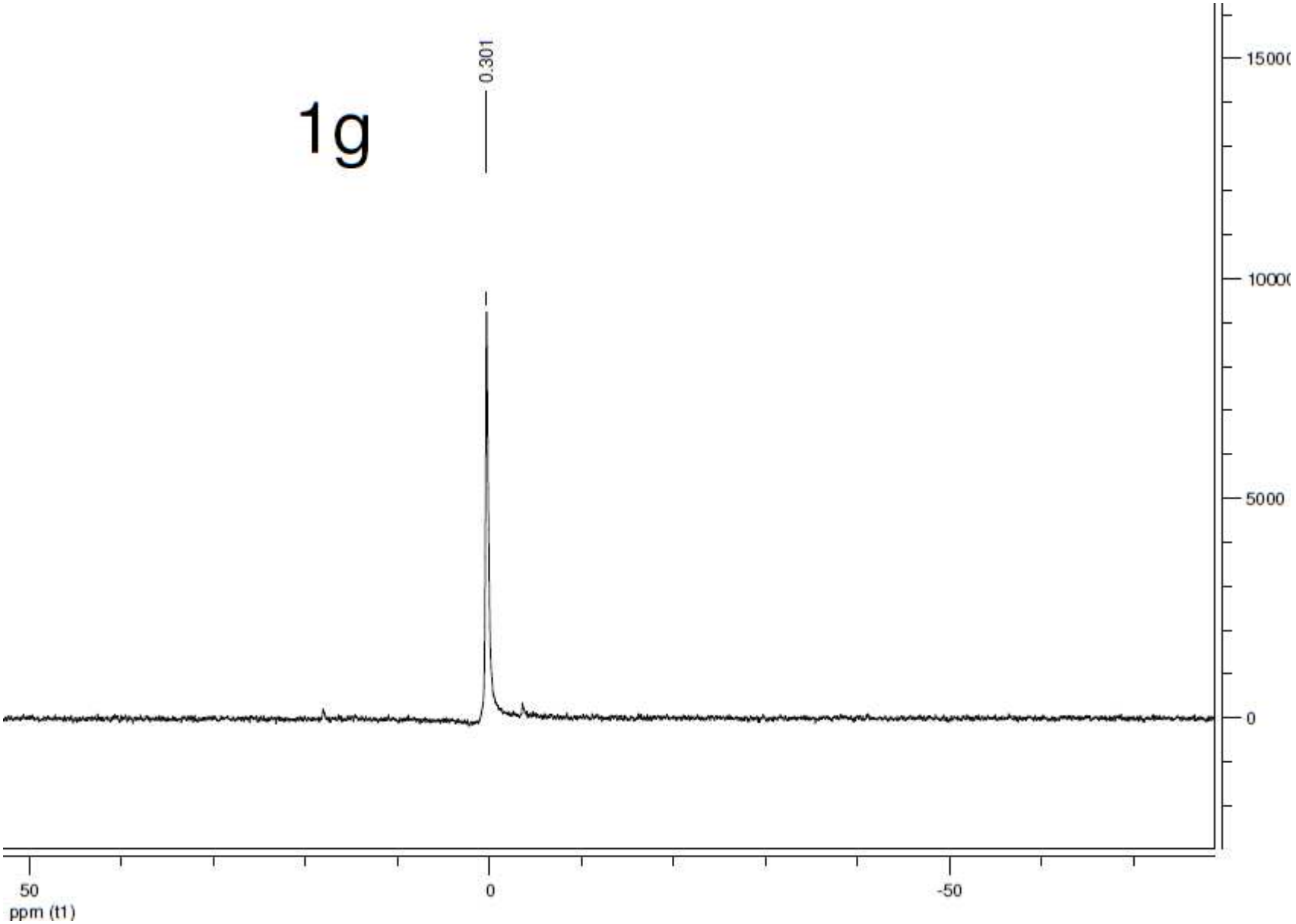
^{13}C NMR


1g
171.168
171.111
171.061
64.204
62.078
53.179
49.470
46.588
0.236
ppm (t1)
200
150
100
50
0
0
500
1000

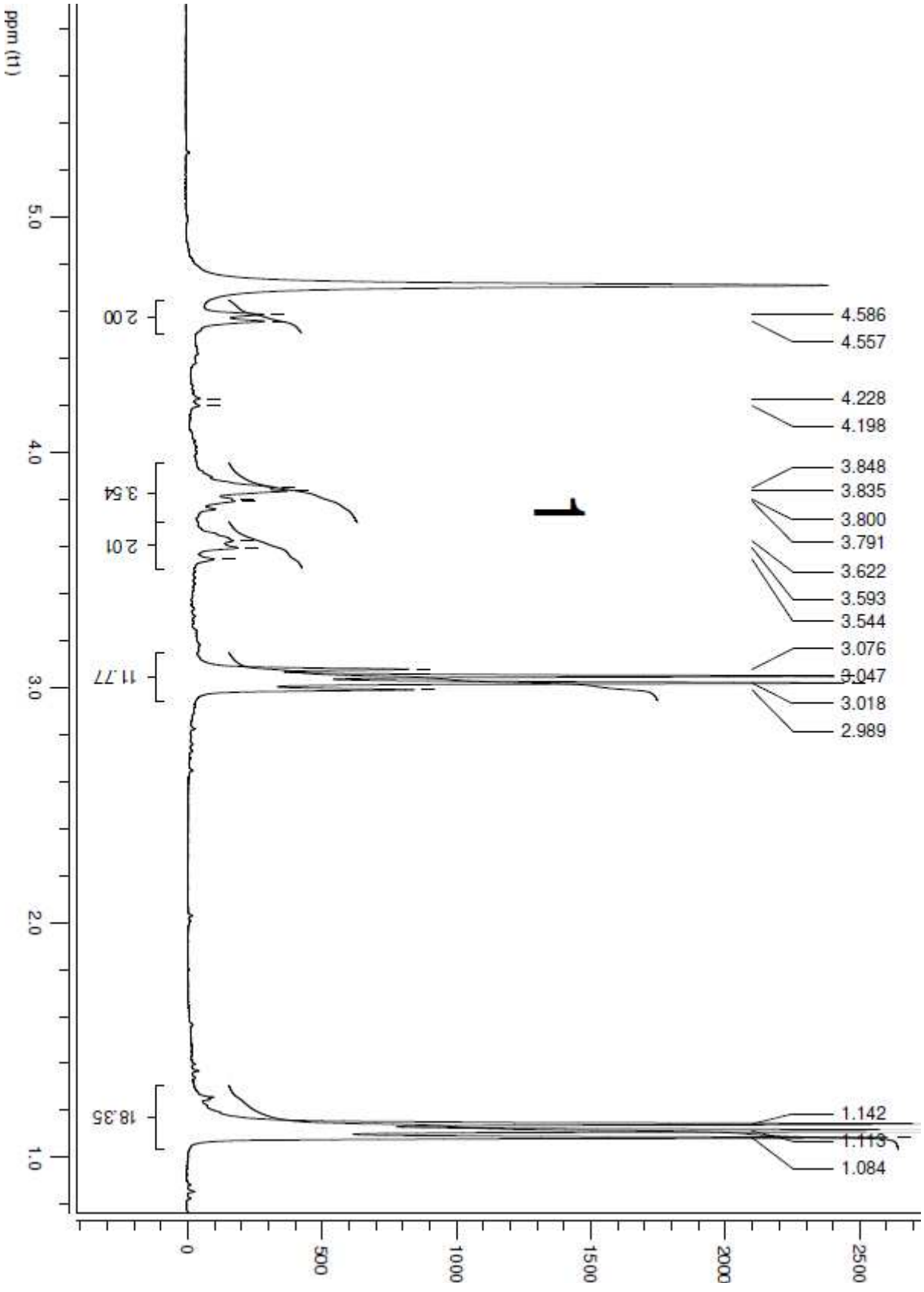
DEPT


19
64.197
64.142
62.058
55.843
55.737
53.140
49.437
46.539
8.196
ppm (t1)

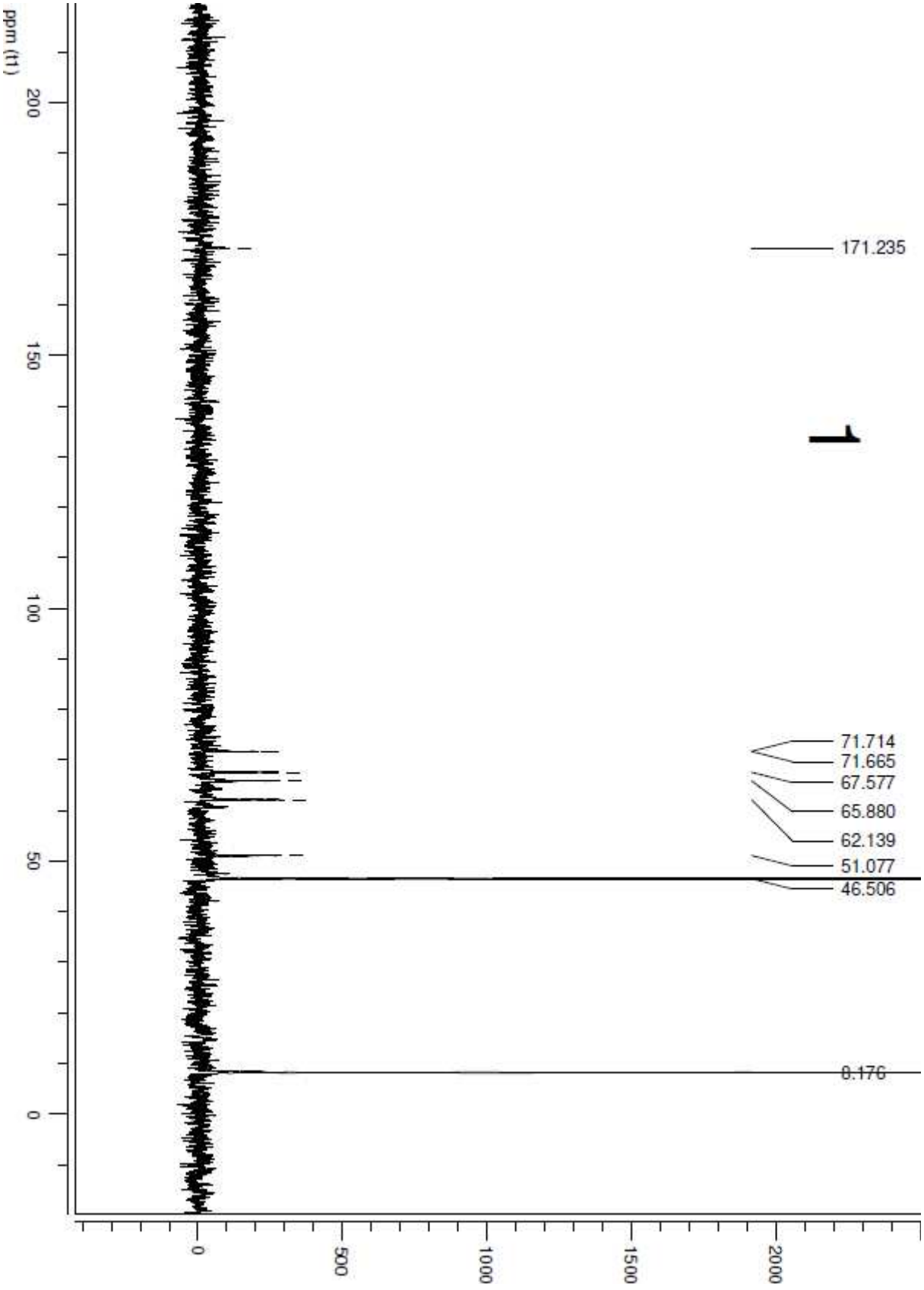
^{31}P-NMR


1g
0.301
15000
10000
5000
0
50
ppm (t1)
0
-50

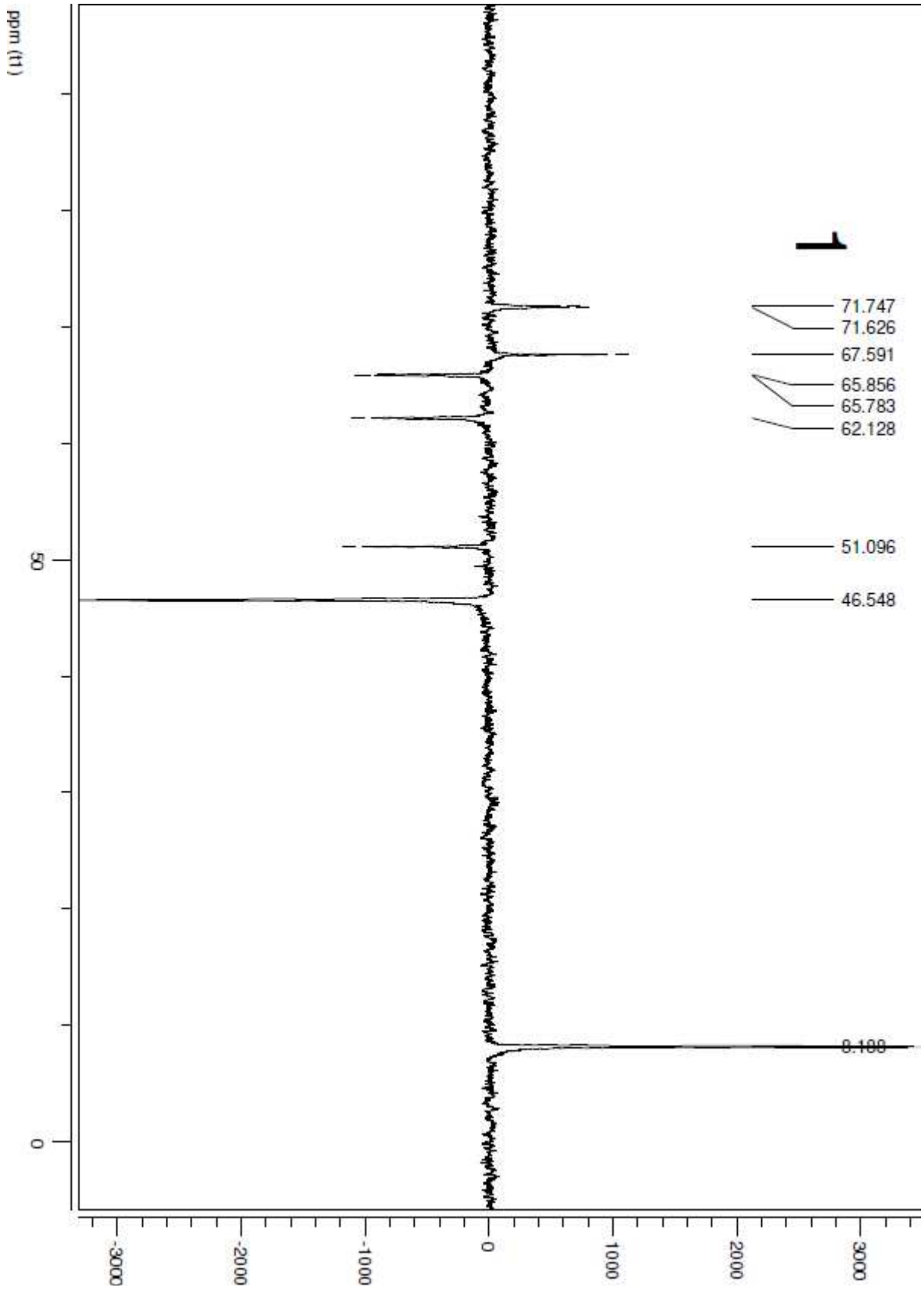
^{1}H NMR


ppm (t1)
5.0
4.0
3.0
2.0
1.0
2.00
3.54
2.01
11.77
18.35
4.586
4.557
4.228
4.198
3.848
3.835
3.800
3.791
3.622
3.593
3.544
3.076
3.047
3.018
2.989
1.142
1.113
1.084
0
500
1000
1500
2000
2500

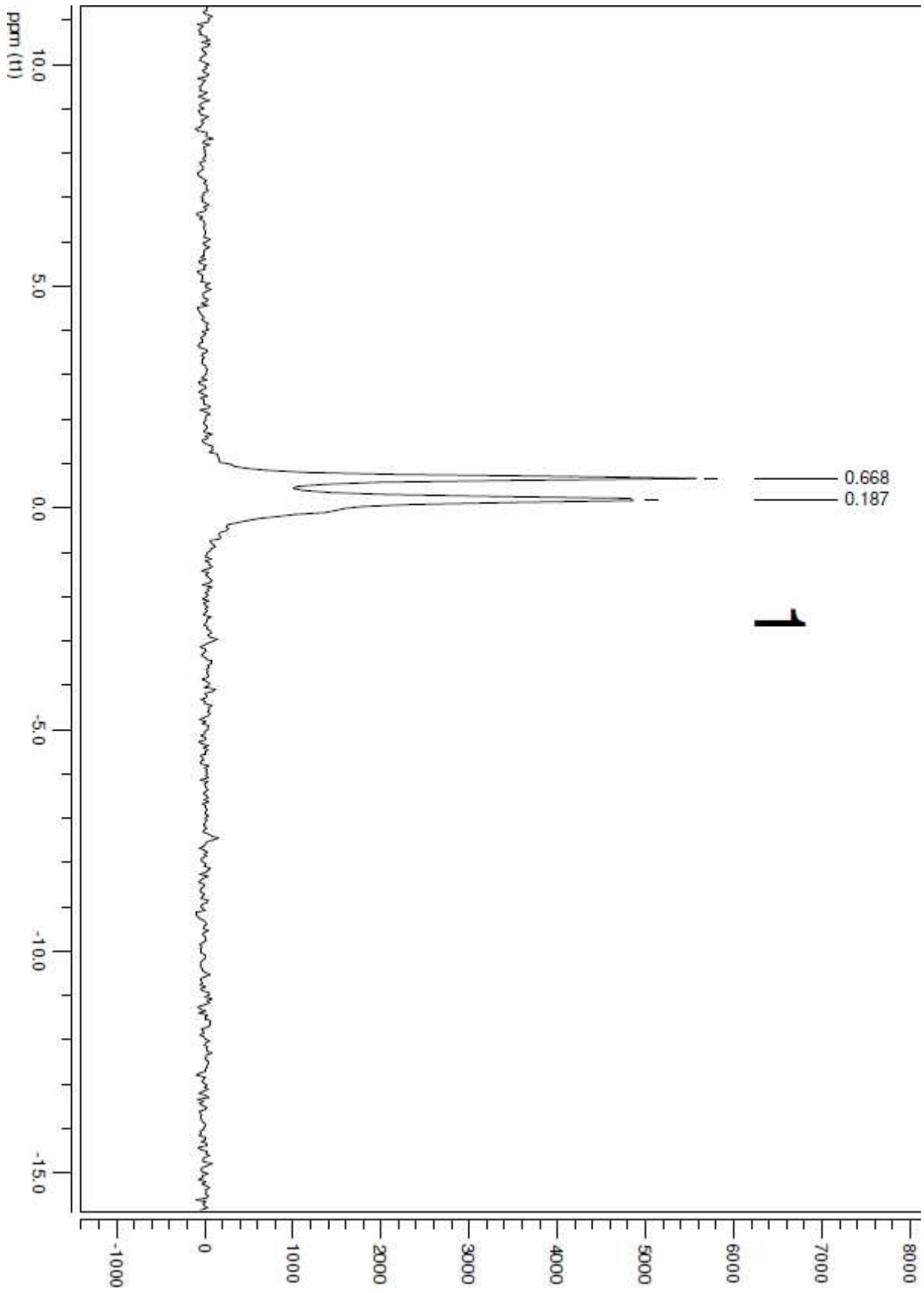
^{13}C NMR


ppm (t1)
200
150
100
50
0
171.235
71.714
71.665
67.577
65.880
62.139
51.077
46.506
0.176
0
500
1000
1500
2000

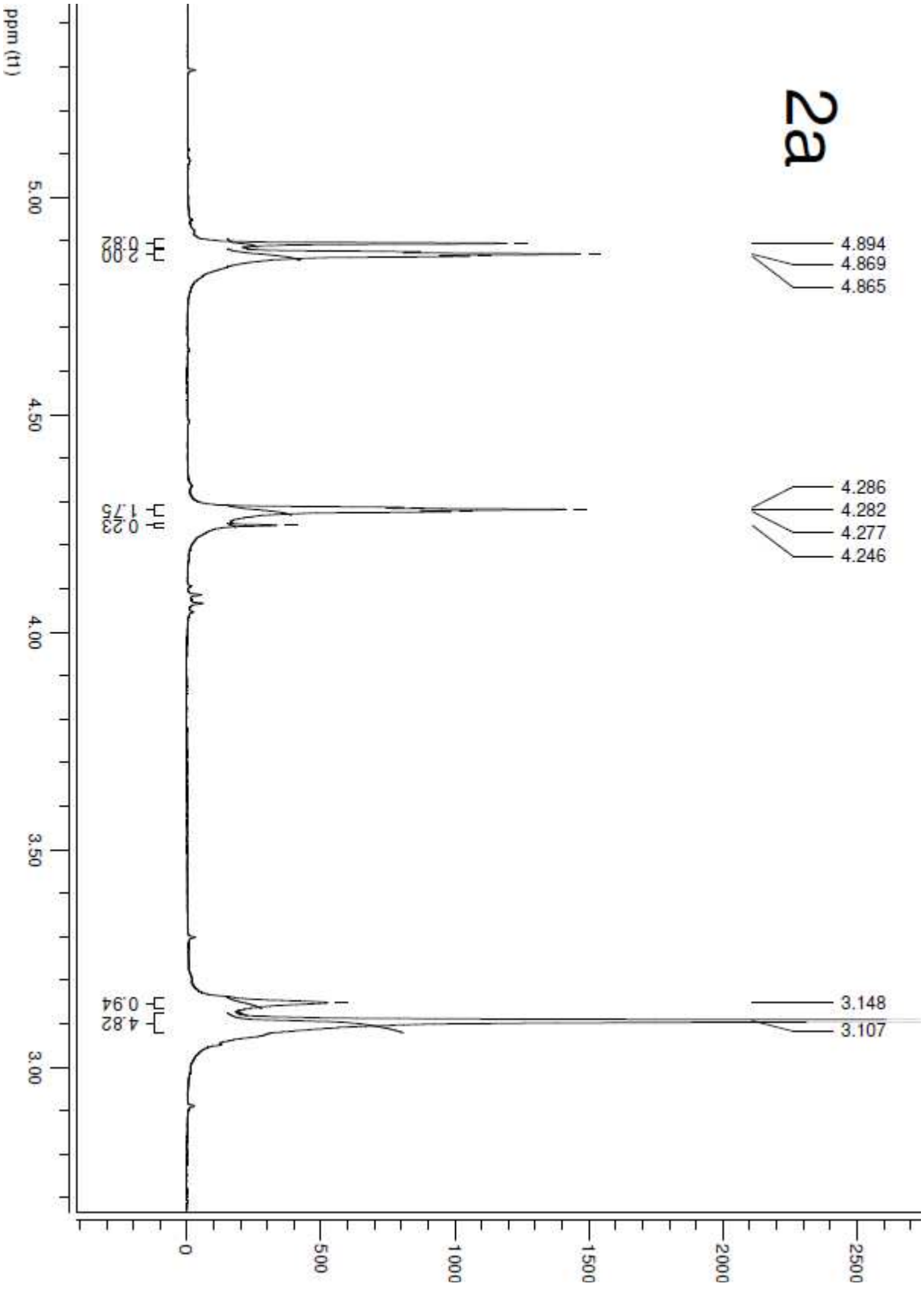
DEPT


ppm (t1)
50
0
71.747
71.626
67.591
65.856
65.783
62.128
51.096
46.548
-3000
-2000
-1000
0
1000
2000
3000

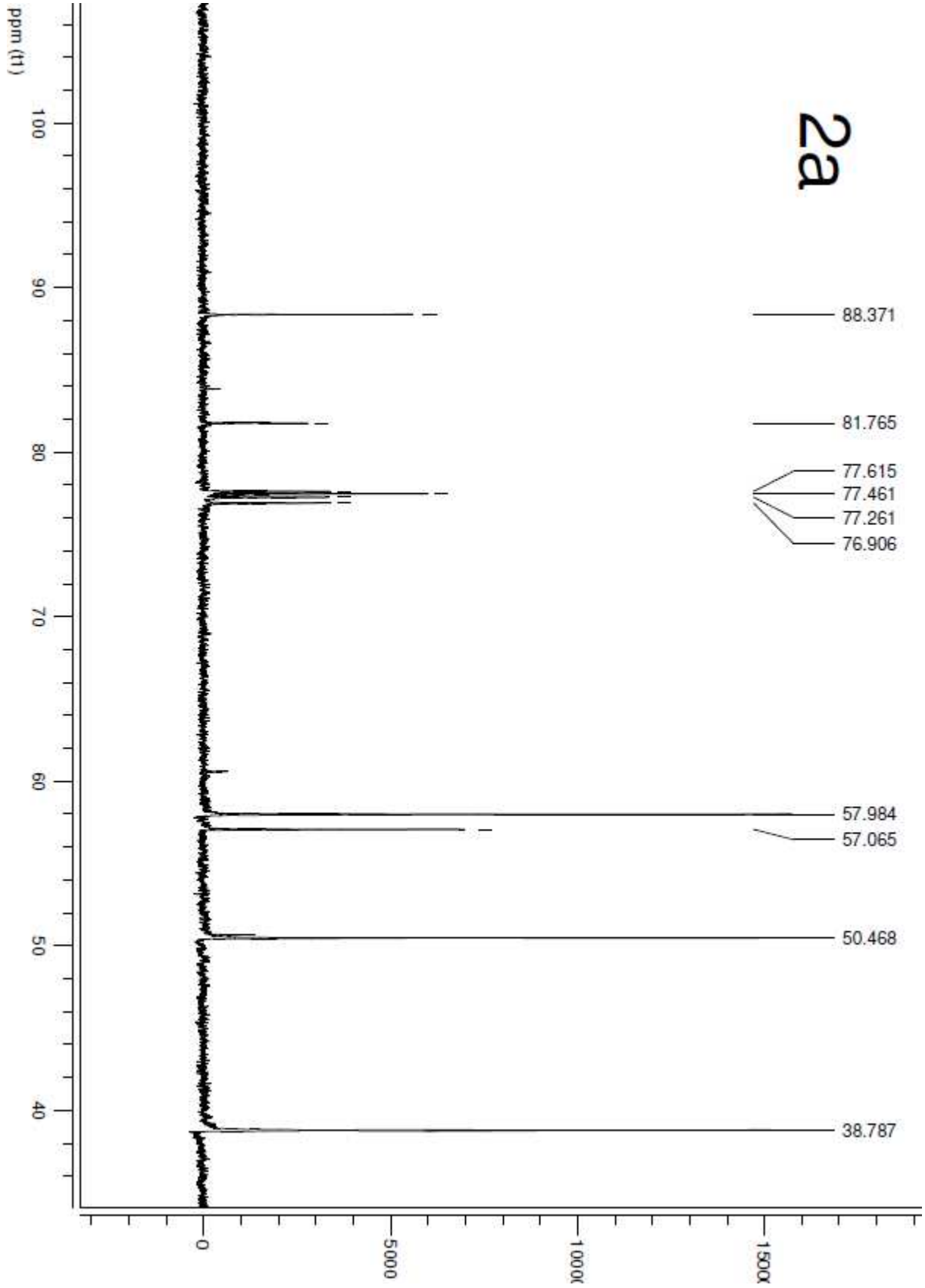
^{31}P-NMR


ppm (t1)
10.0
5.0
0.0
-5.0
-10.0
-15.0
0.668
0.187
1
-1000
0
1000
2000
3000
4000
5000
6000
7000
8000

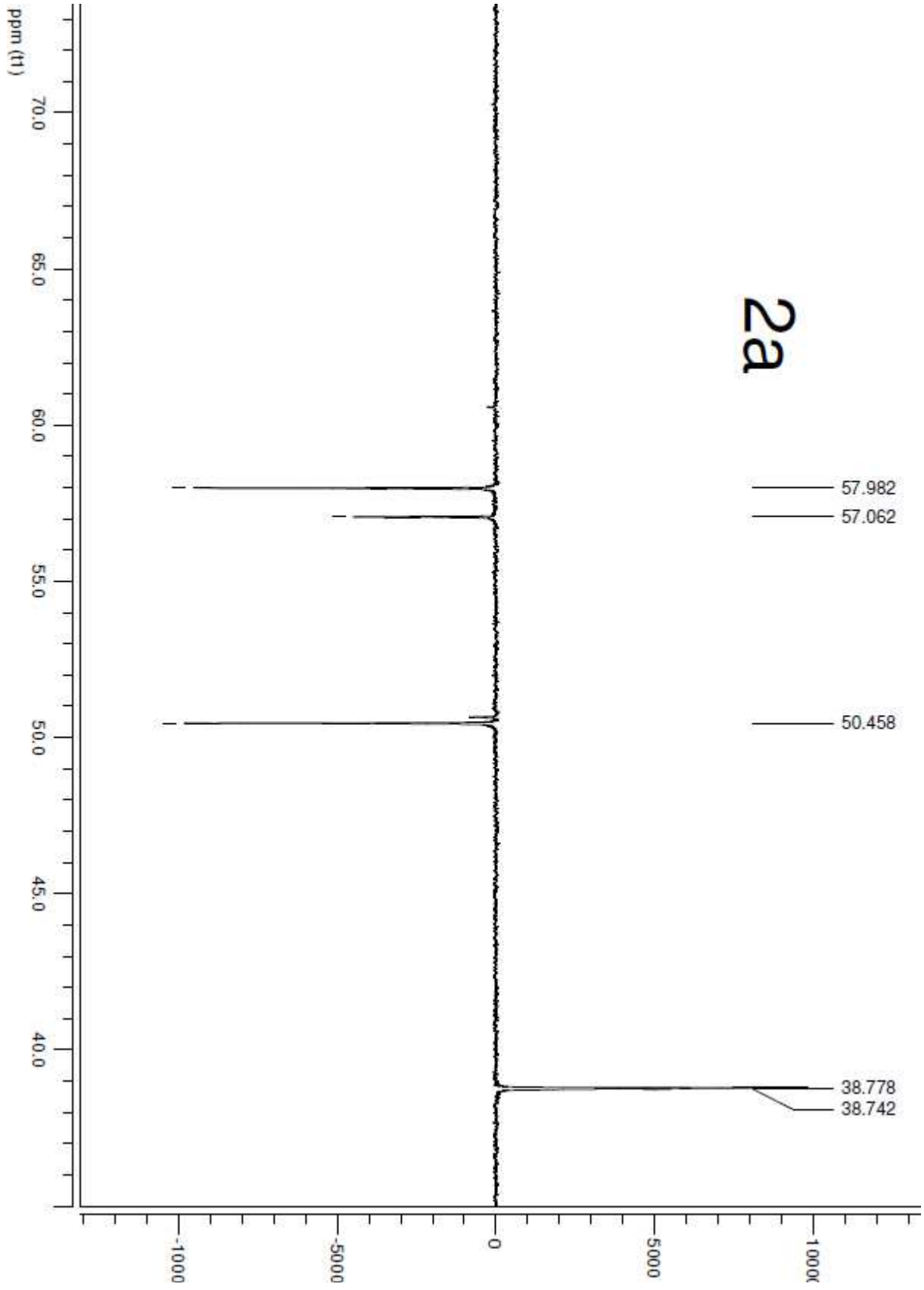
^{1}H NMR


2a
4.894
4.869
4.865
4.286
4.282
4.277
4.246
3.148
3.107
2.00
6.82
0.23
1.75
4.82
0.94
ppm (t1)
5.00
4.50
4.00
3.50
3.00
0
500
1000
1500
2000
2500

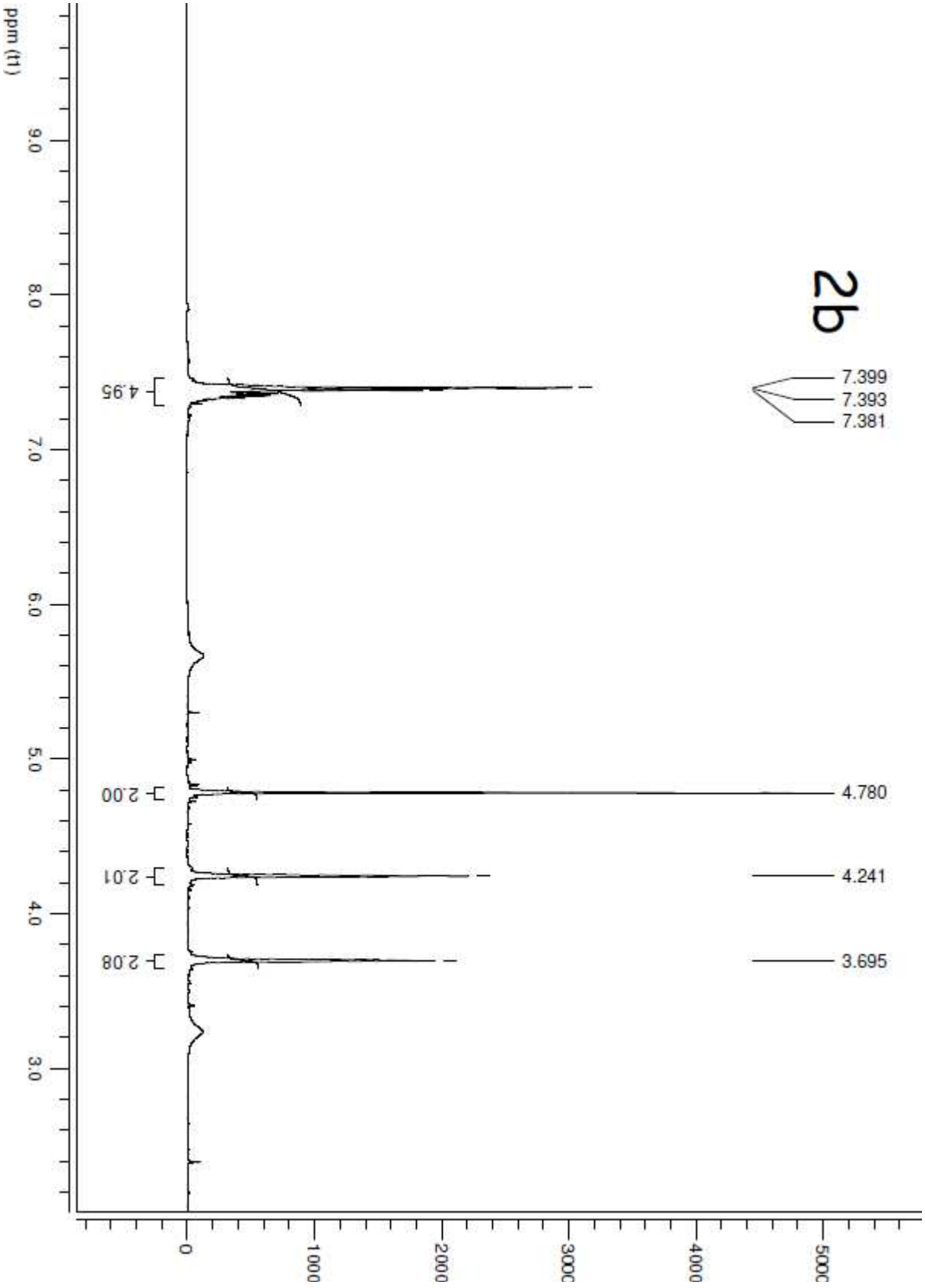
^{13}C NMR


2a
88.371
81.765
77.615
77.461
77.261
76.906
57.984
57.065
50.468
38.787
ppm (t1)
100
90
80
70
60
50
40
0
5000
1000
1500

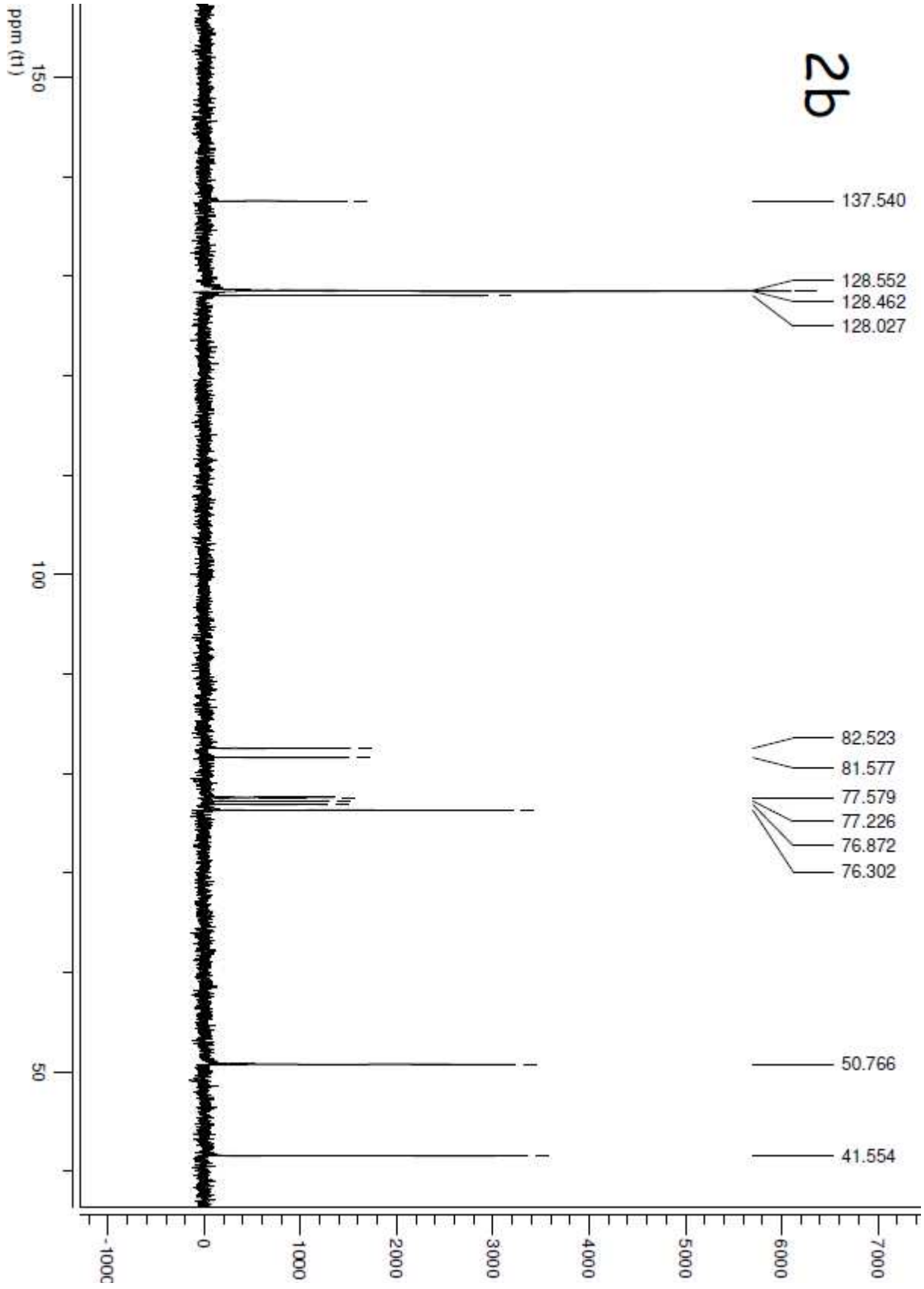
DEPT


ppm (t1)
70.0
65.0
60.0
55.0
50.0
45.0
40.0
2a
57.982
57.062
50.458
38.778
38.742
-1000
-5000
0
5000
1000

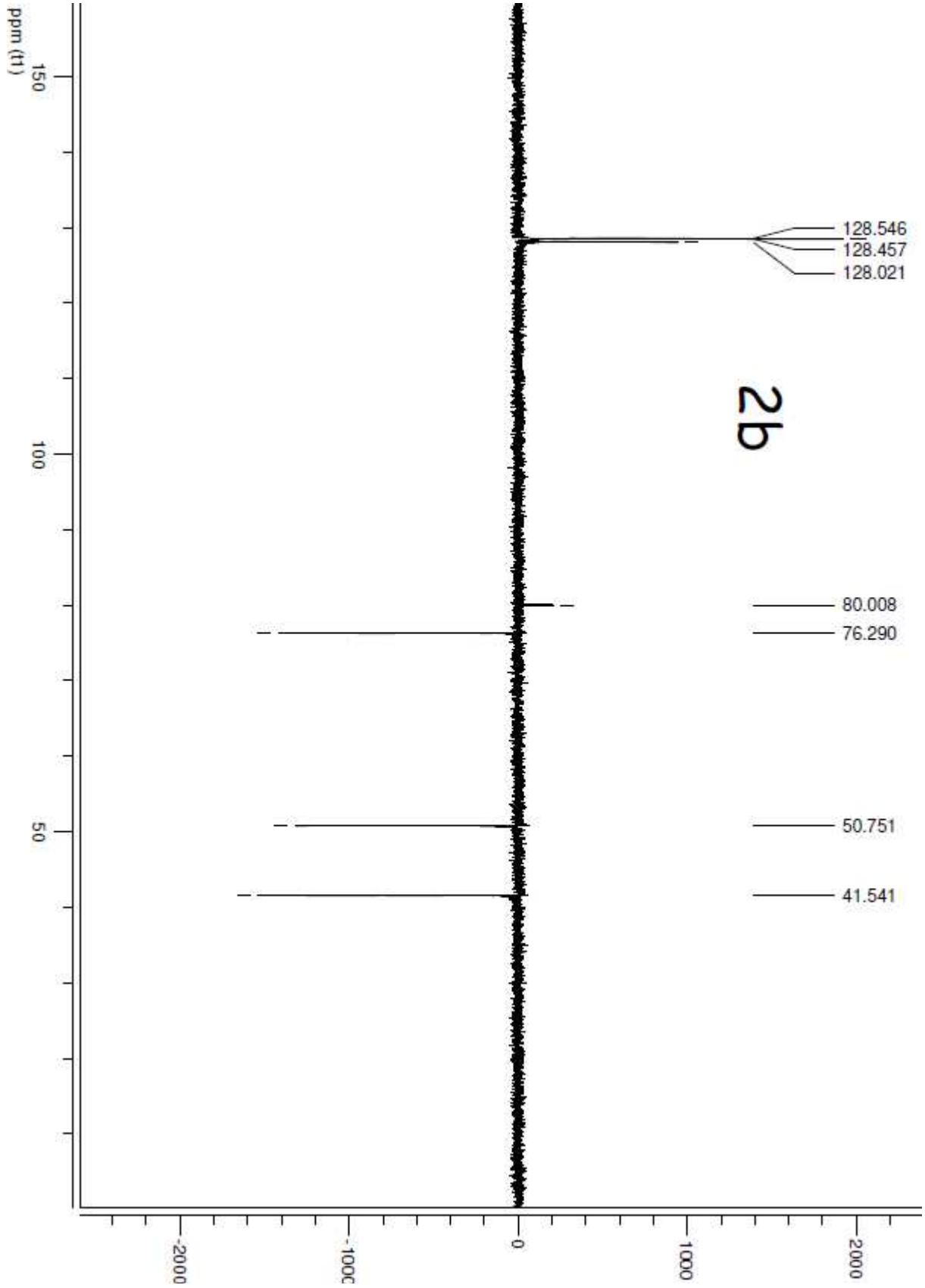
^{1}H NMR


2b
7.399
7.393
7.381
4.780
4.241
3.695
4.95
2.00
2.01
2.08
ppm (t1)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
0
1000
2000
3000
4000
5000

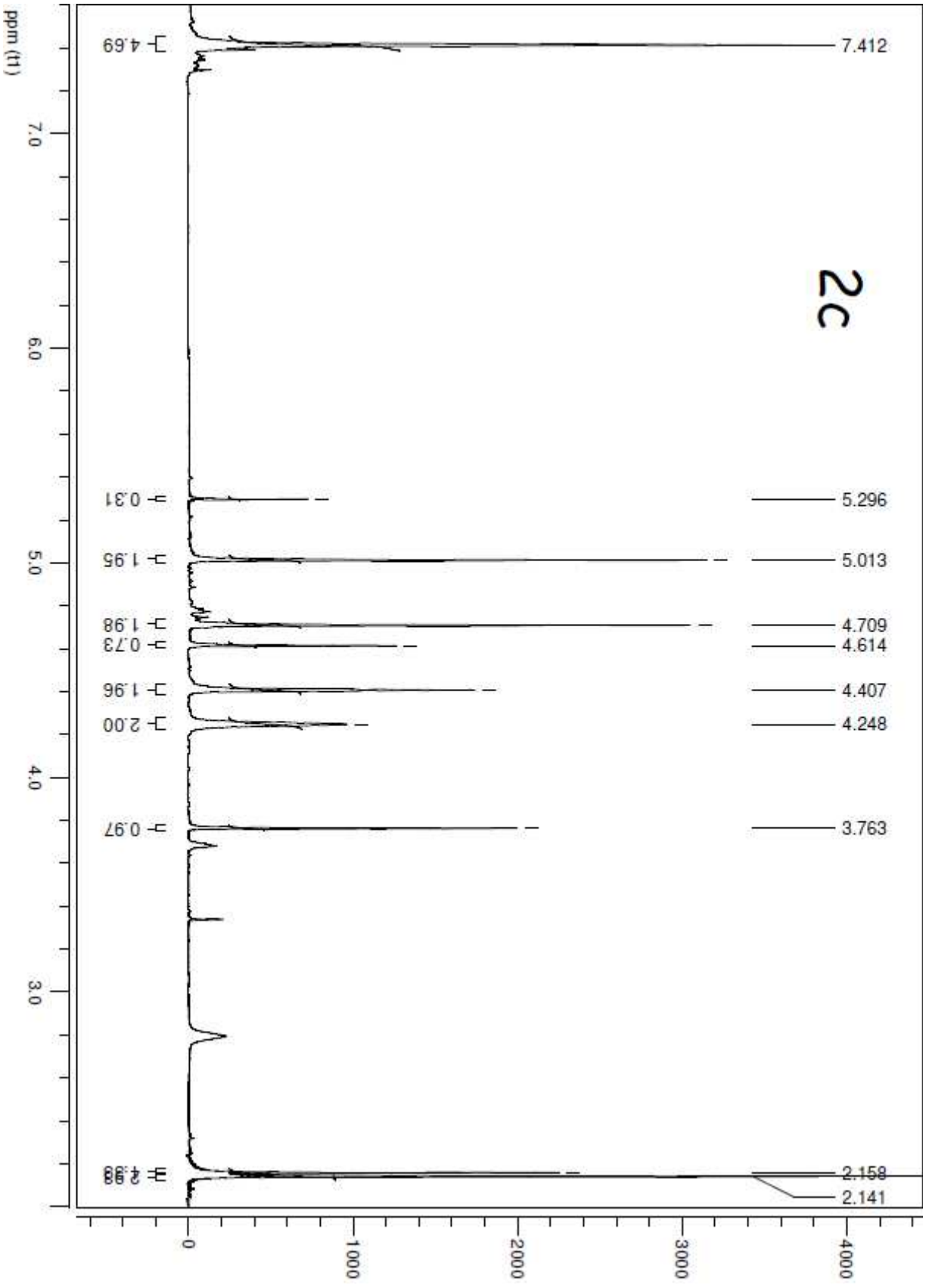
^{13}C NMR


2b
ppm (t1)
150
100
50
137.540
128.552
128.462
128.027
82.523
81.577
77.579
77.226
76.872
76.302
50.766
41.554
-1000
0
1000
2000
3000
4000
5000
6000
7000

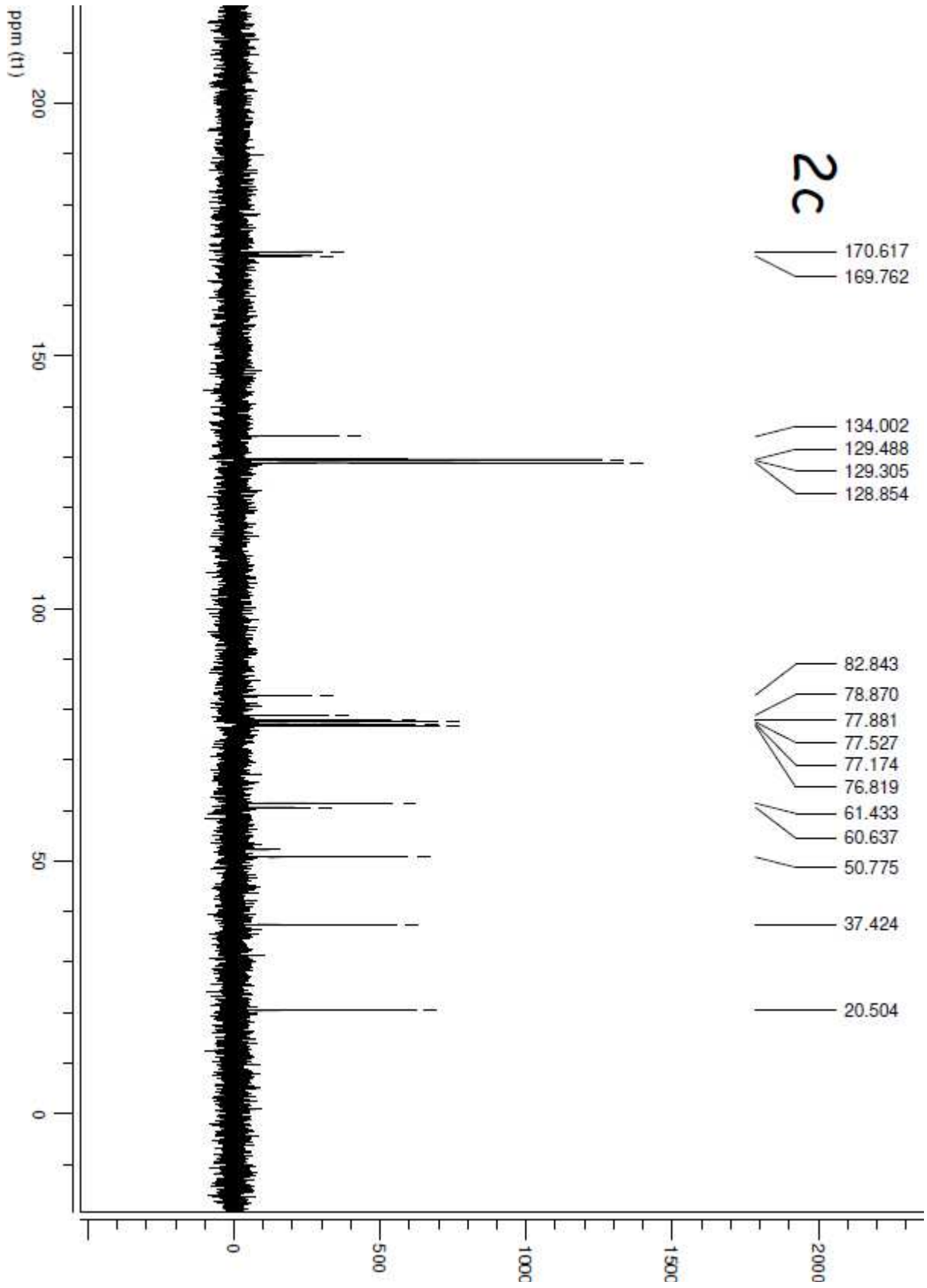
DEPT


ppm (t1)
150
100
50
-2000
-1000
0
1000
2000
128.546
128.457
128.021
2b
80.008
76.290
50.751
41.541

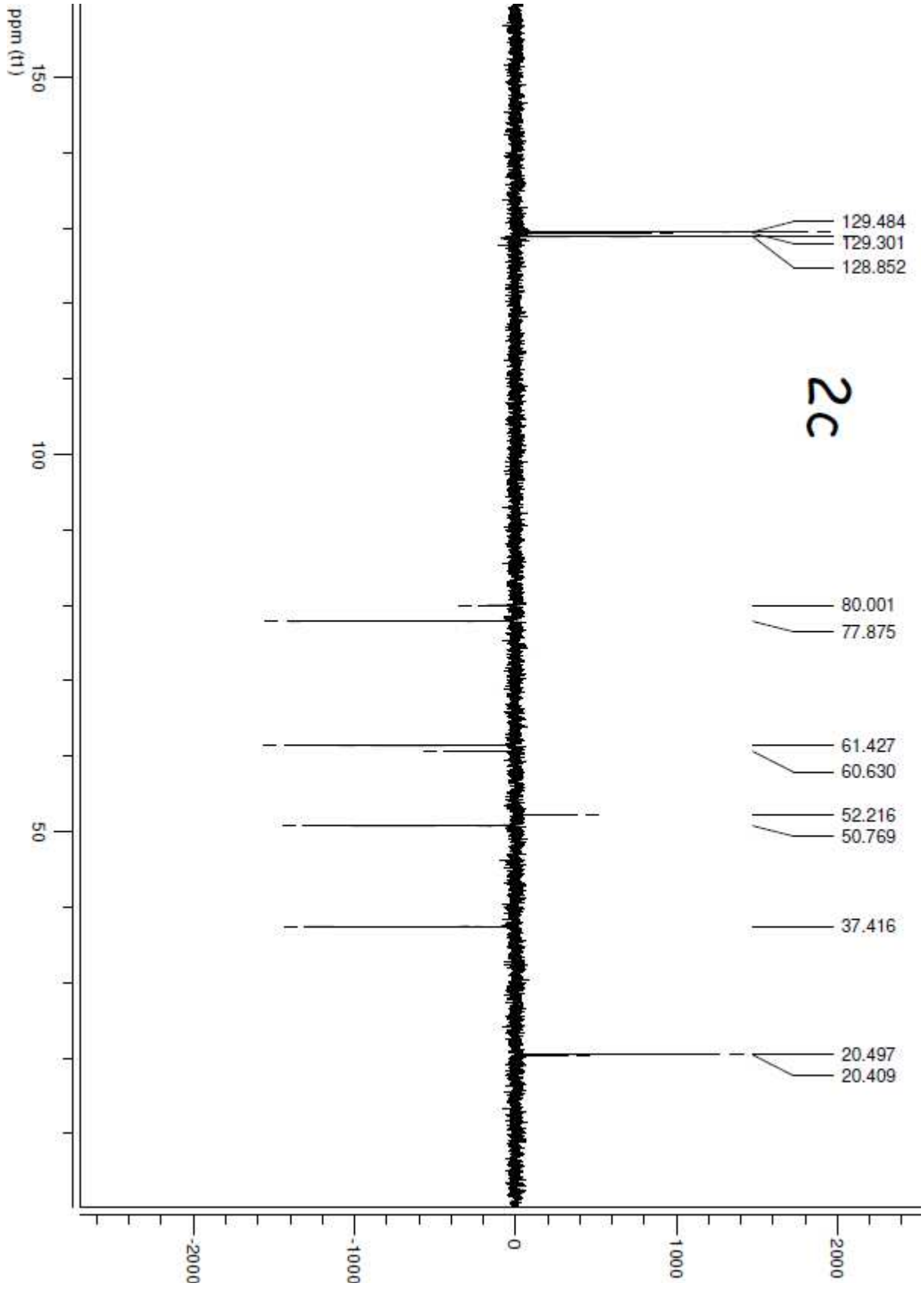
^{1}H NMR


2c
7.412
5.296
5.013
4.709
4.614
4.407
4.248
3.763
2.158
2.141
4.69
0.31
1.95
1.98
0.73
1.96
2.00
0.97
2.98
ppm (t1)
7.0
6.0
5.0
4.0
3.0
0
1000
2000
3000
4000

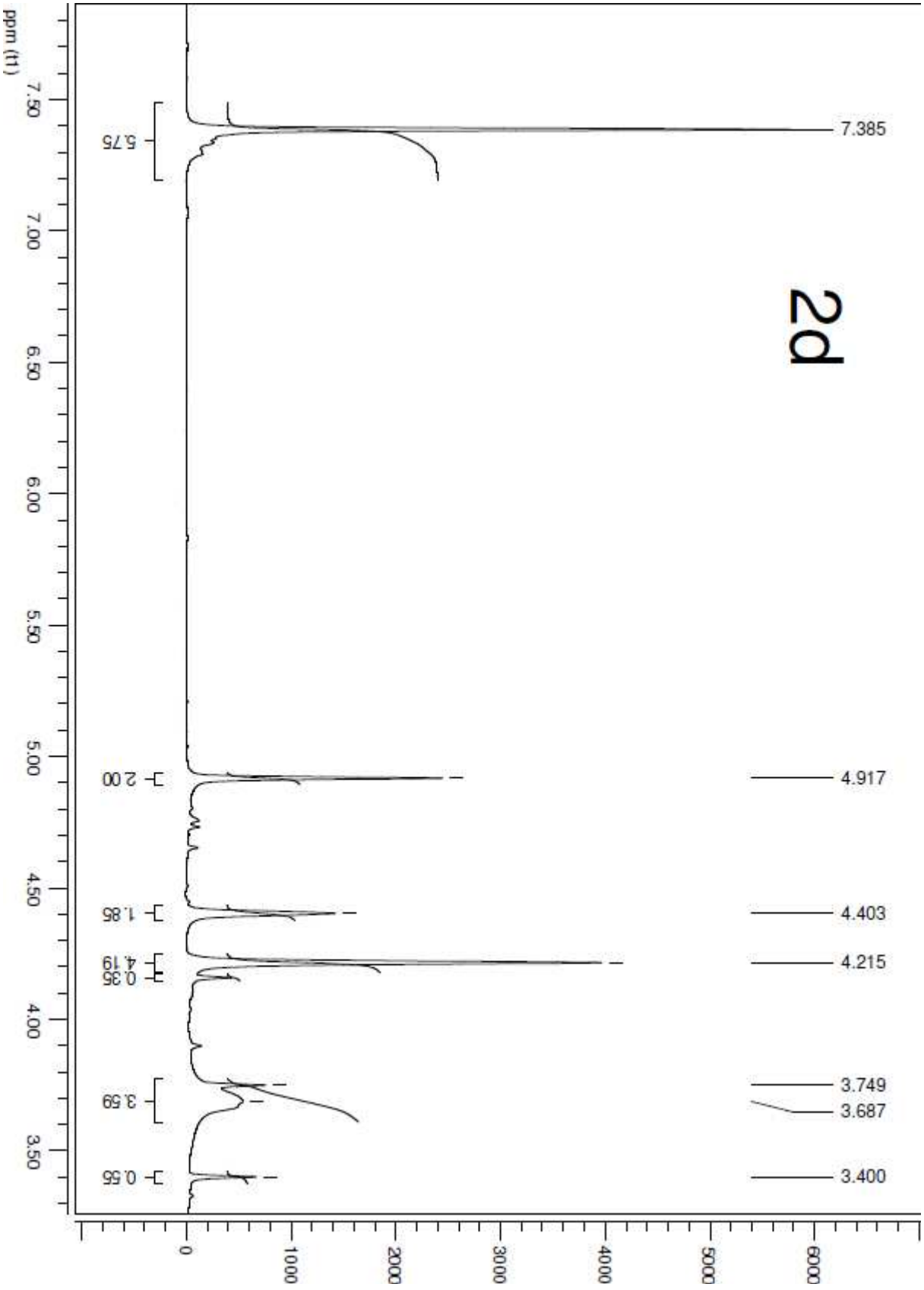
^{13}C NMR


2c
170.617
169.762
134.002
129.488
129.305
128.854
82.843
78.870
77.881
77.527
77.174
76.819
61.433
60.637
50.775
37.424
20.504
ppm (t1)
200
150
100
50
0
0
500
1000
1500
2000

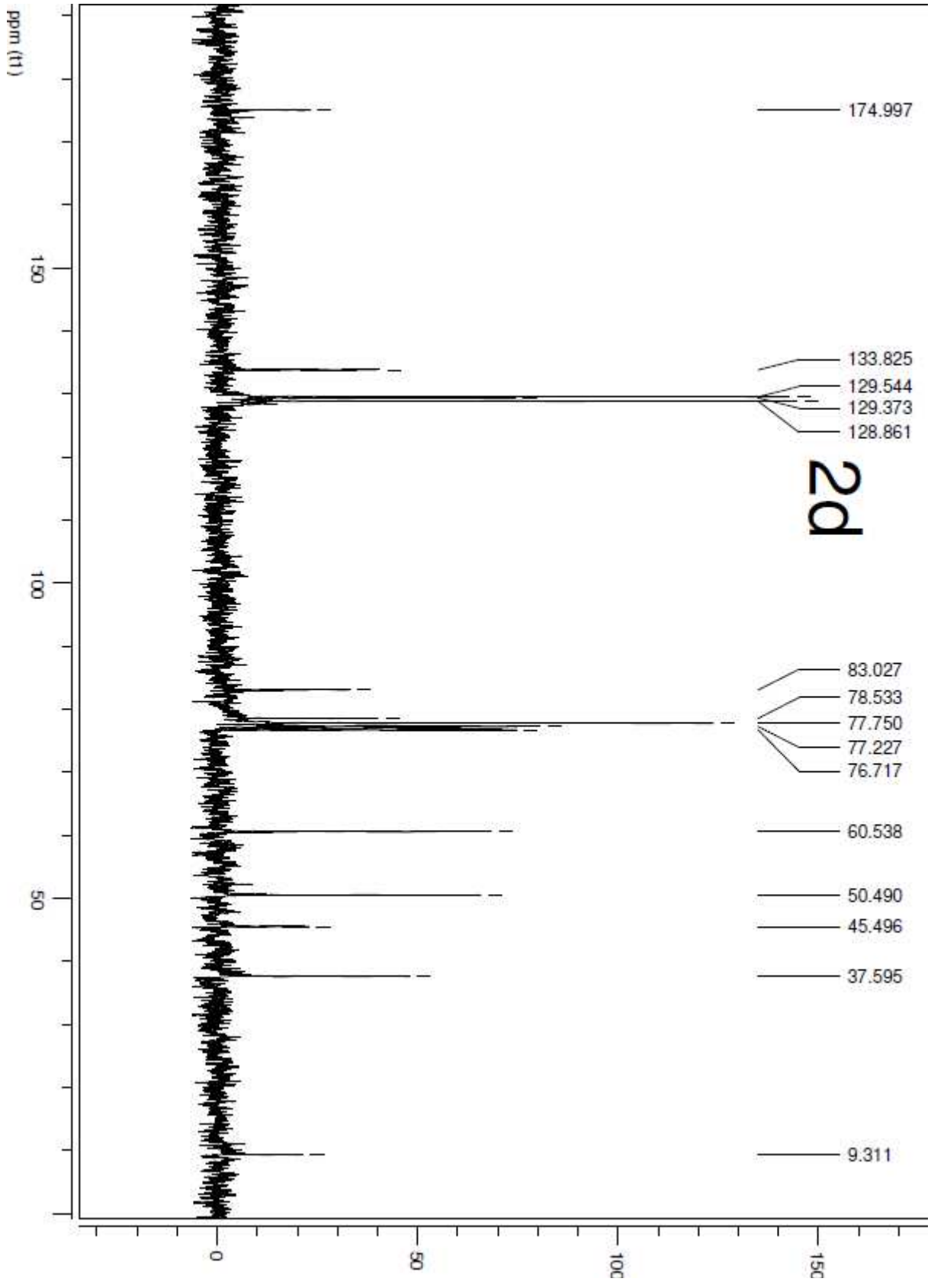
DEPT


ppm (t1)
150
100
50
-2000
-1000
0
1000
2000
129.484
129.301
128.852
2c
80.001
77.875
61.427
60.630
52.216
50.769
37.416
20.497
20.409

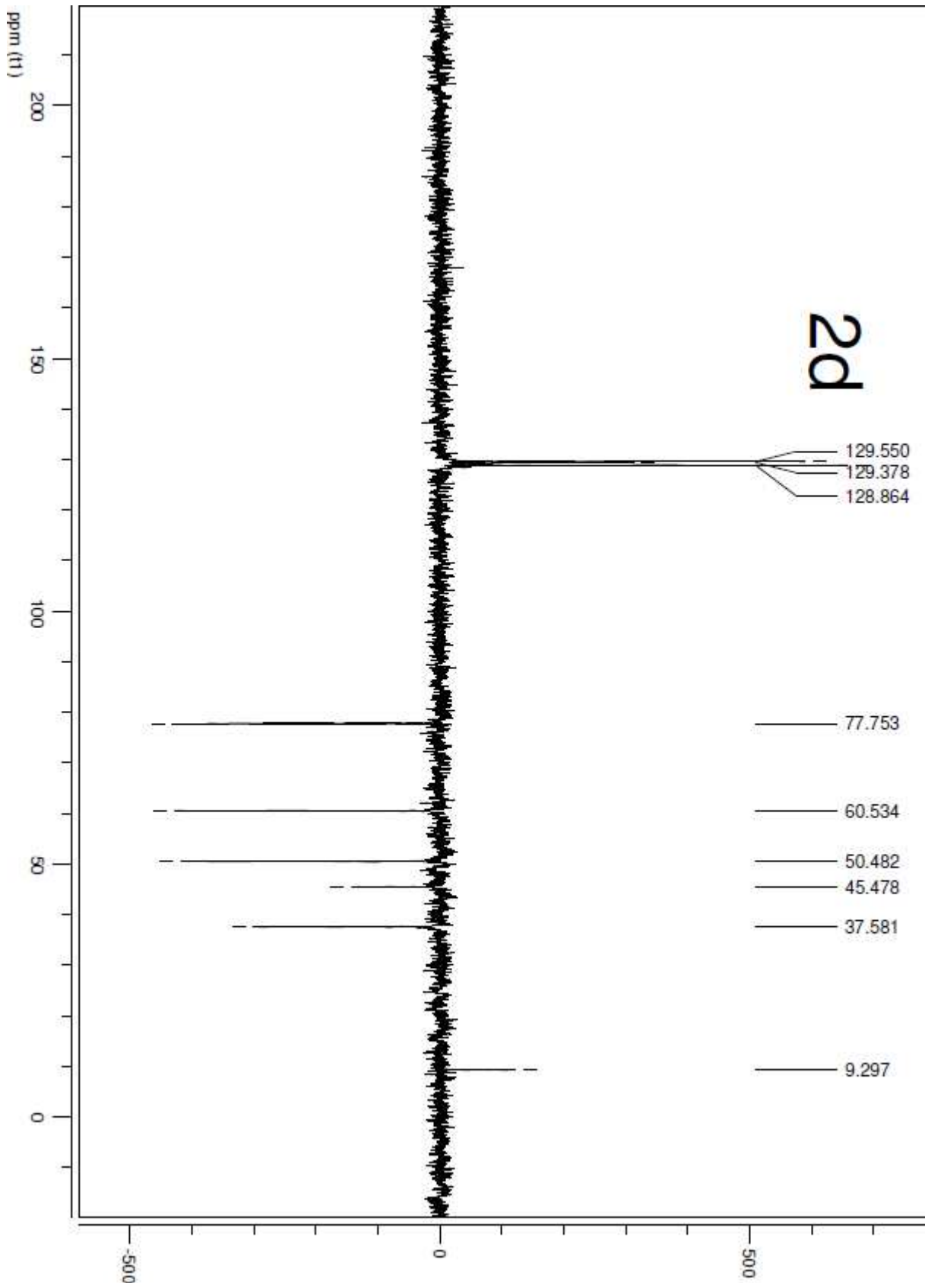
^{1}H NMR


2d
7.385
4.917
4.403
4.215
3.749
3.687
3.400
5.75
2.00
1.86
4.19
0.35
3.59
0.55
ppm (t1)
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
0
1000
2000
3000
4000
5000
6000

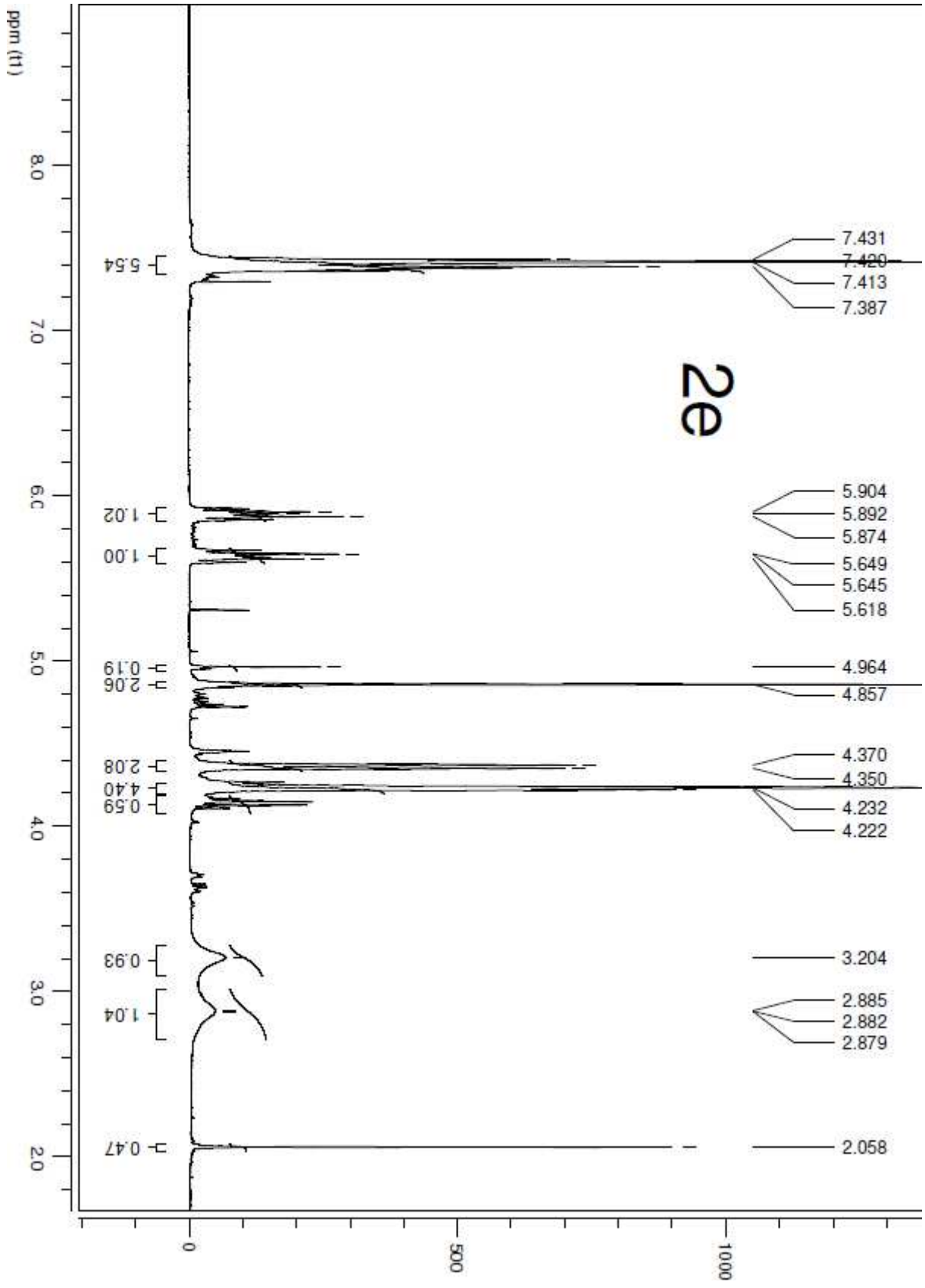
^{13}C NMR


ppm (t1)
150
100
50
0
50
100
150
174.997
133.825
129.544
129.373
128.861
2d
83.027
78.533
77.750
77.227
76.717
60.538
50.490
45.496
37.595
9.311

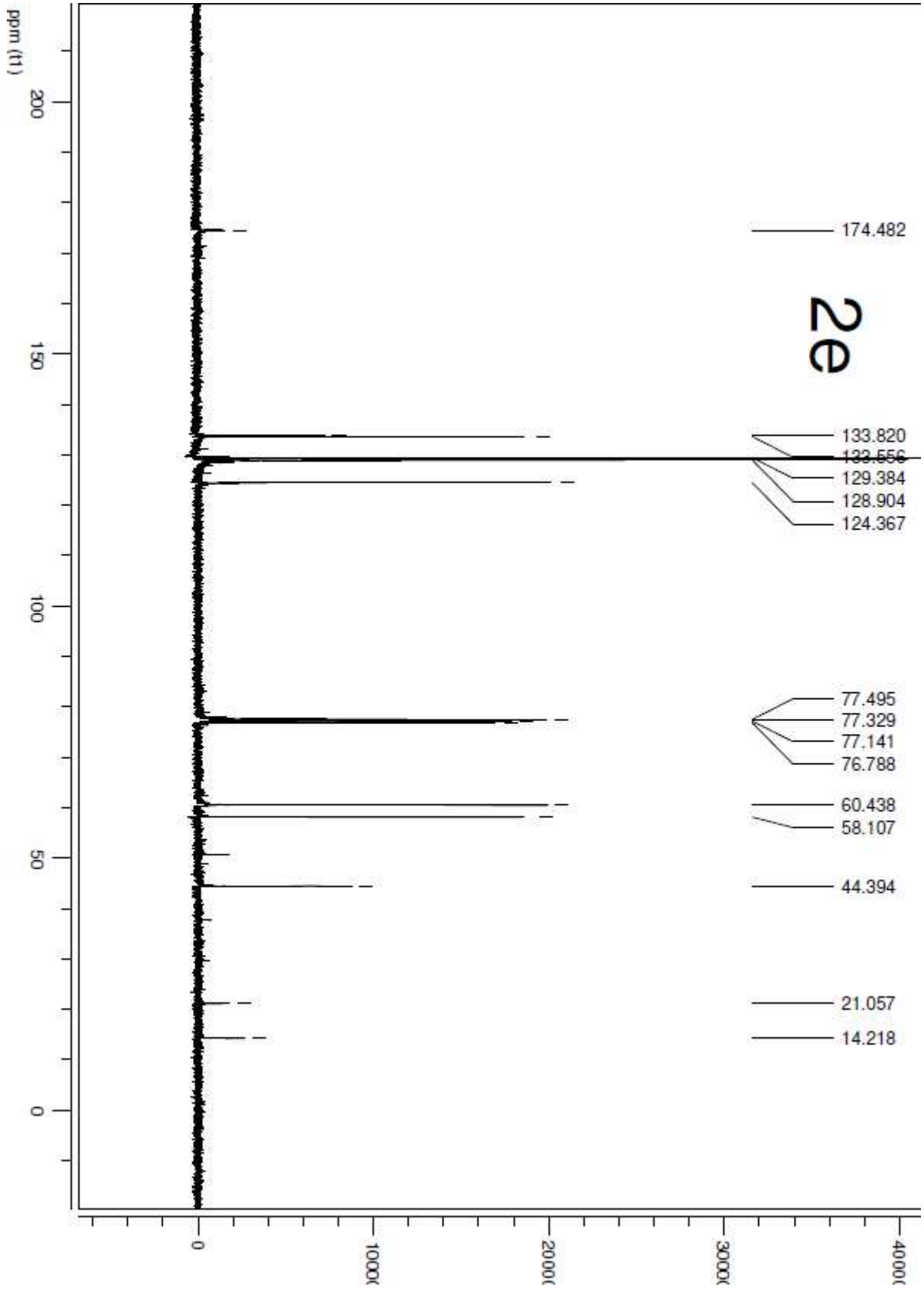
DEPT


2d
ppm (t1)
200
150
100
50
0
-500
0
500
129.550
129.378
128.864
77.753
60.534
50.482
45.478
37.581
9.297

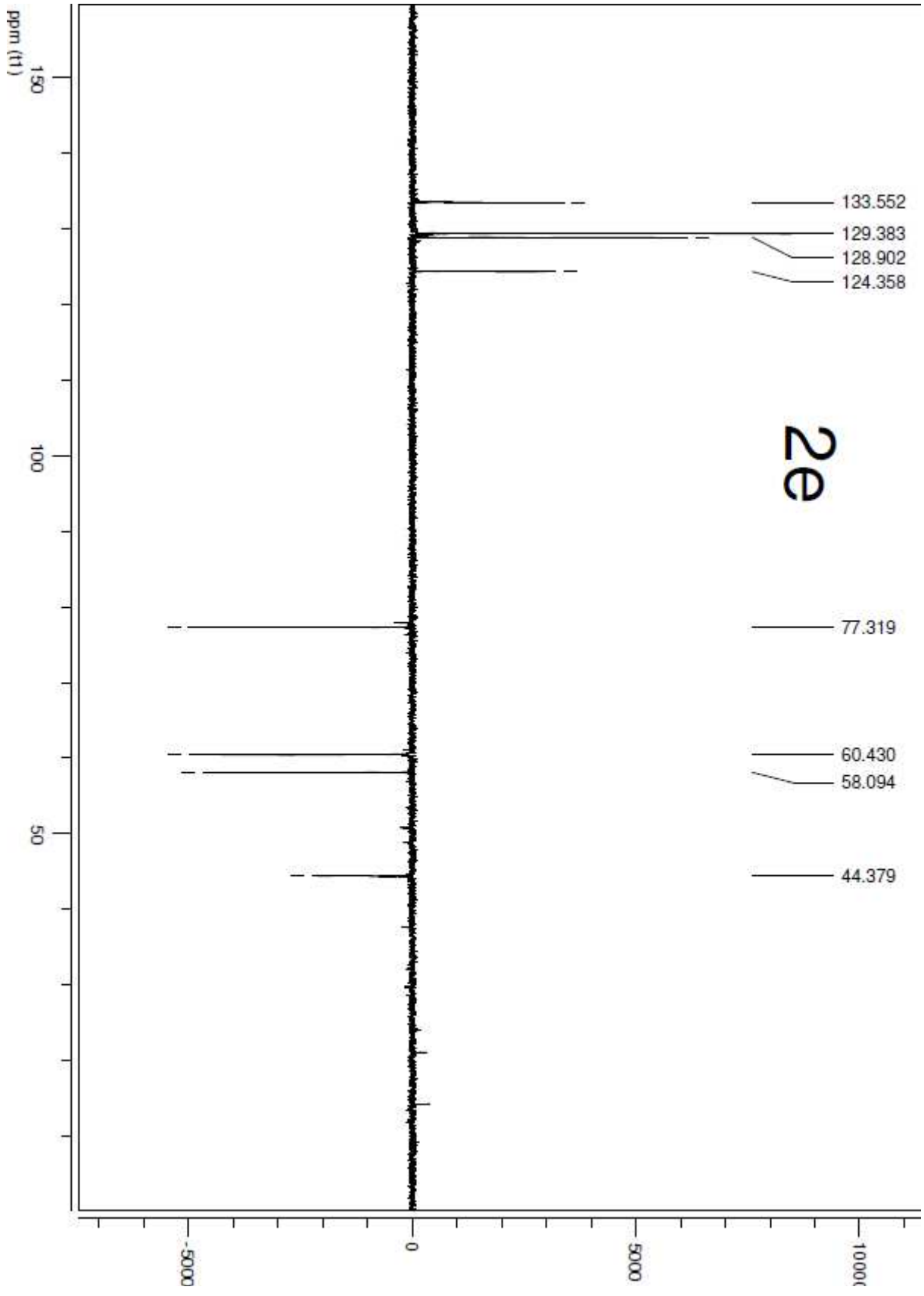
^{1}H NMR


ppm (t1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
500
1000
5.54
1.02
1.00
0.19
2.06
2.08
4.40
0.59
0.93
1.04
0.47
7.431
7.420
7.413
7.387
5.904
5.892
5.874
5.649
5.645
5.618
4.964
4.857
4.370
4.350
4.232
4.222
3.204
2.885
2.882
2.879
2.058
2e

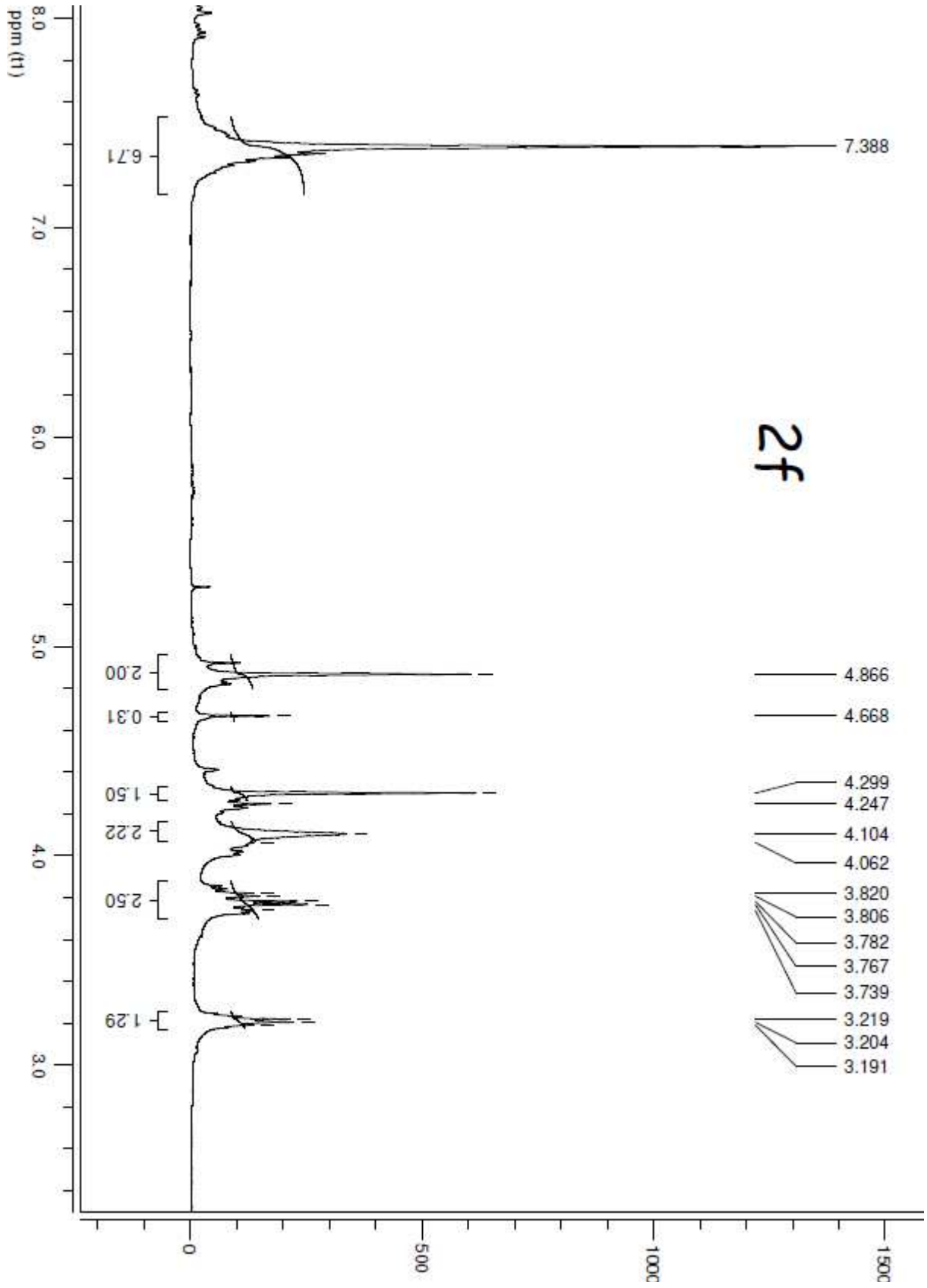
^{13}C NMR


ppm (t1)
200
150
100
50
0
174.482
2e
133.820
133.556
129.384
128.904
124.367
77.495
77.329
77.141
76.788
60.438
58.107
44.394
21.057
14.218
0
10000
20000
30000
40000

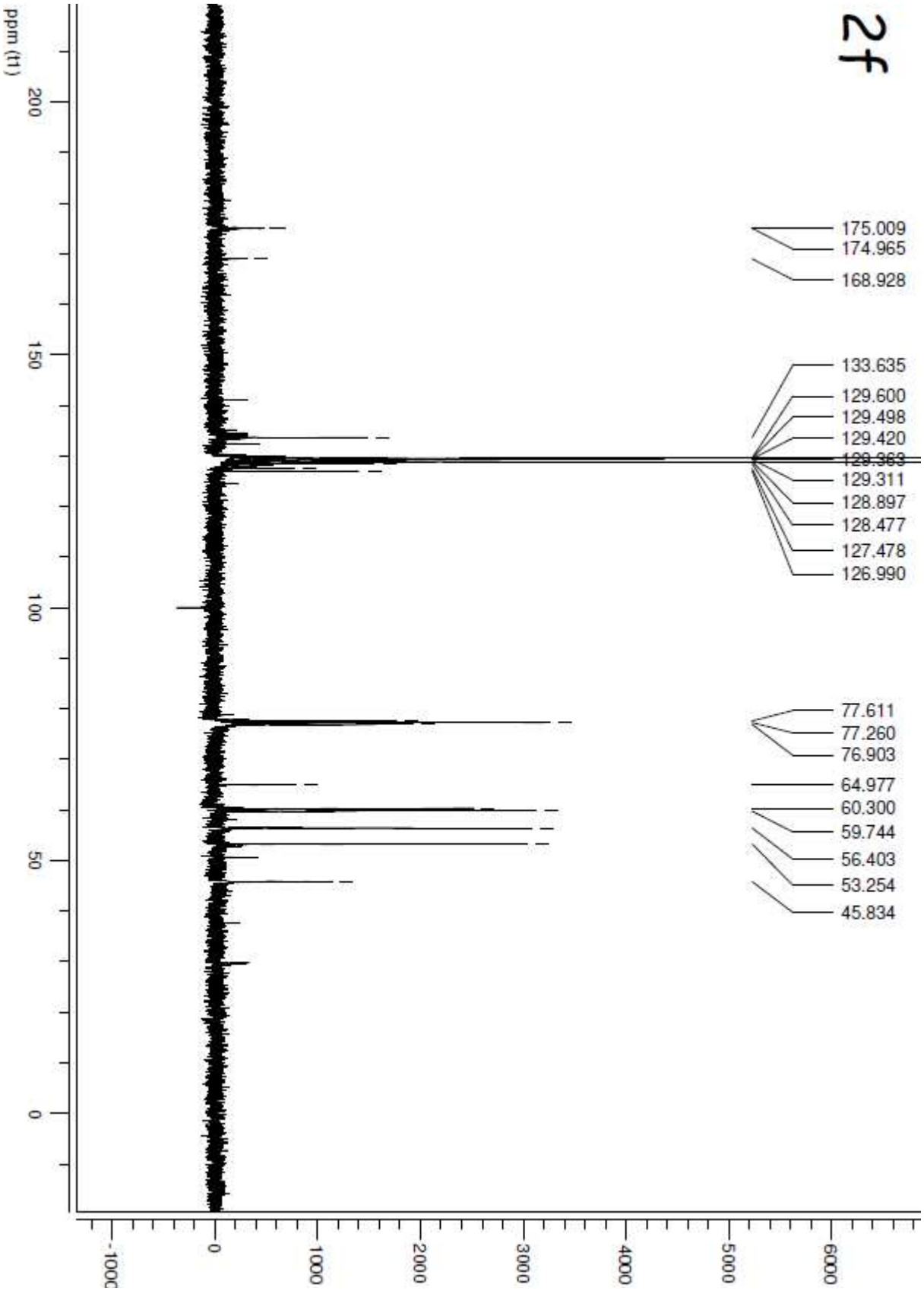
DEPT


ppm (t1)
150
100
50
-5000
0
5000
10000
133.552
129.383
128.902
124.358
77.319
60.430
58.094
44.379
2e

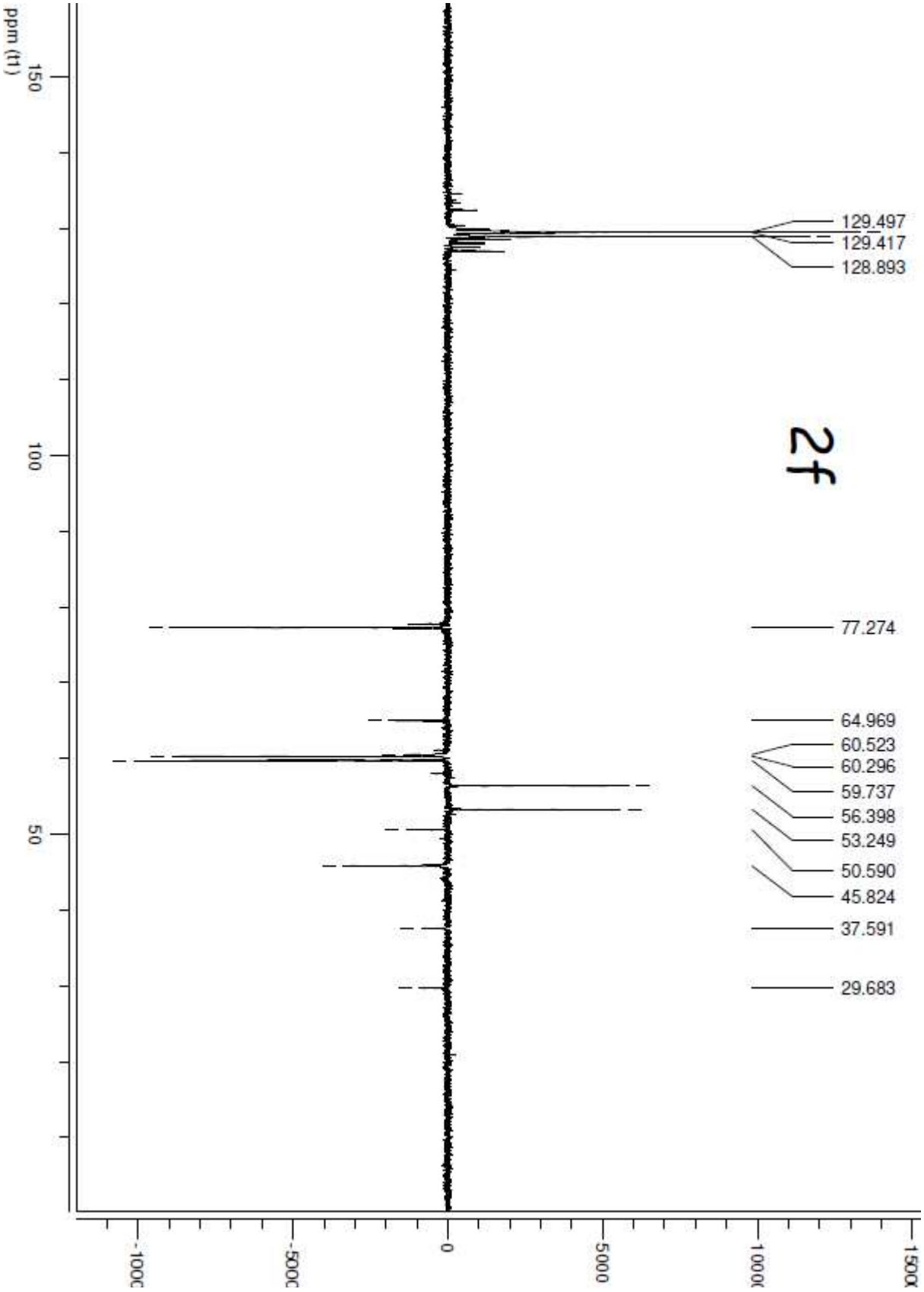
^{1}H NMR


2f
7.388
6.71
4.866
4.668
4.299
4.247
4.104
4.062
3.820
3.806
3.782
3.767
3.739
3.219
3.204
3.191
2.00
0.31
1.50
2.22
2.50
1.29
ppm (t1)
8.0
7.0
6.0
5.0
4.0
3.0
0
500
1000
1500

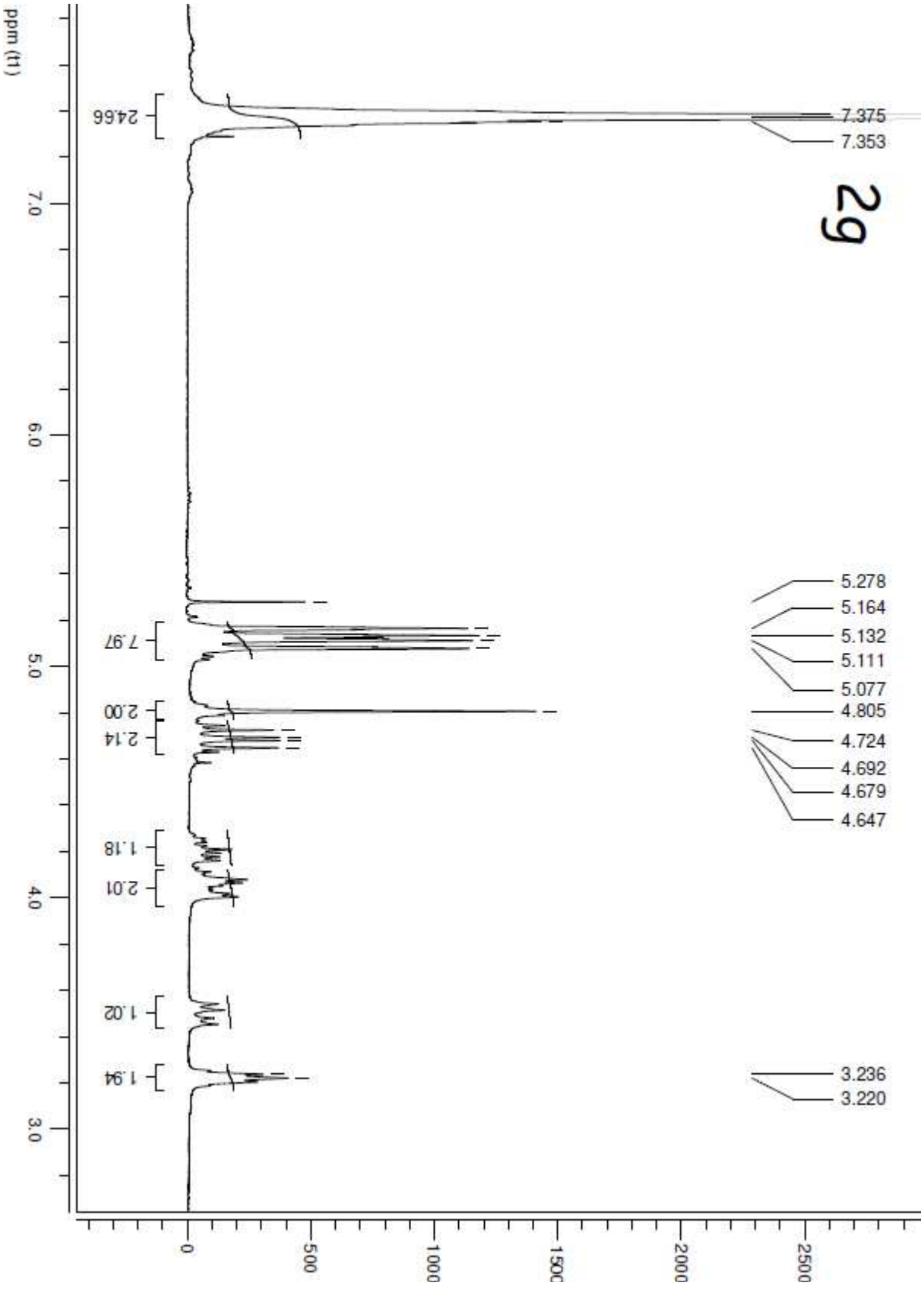
^{13}C NMR


2f
ppm (t1)
200
150
100
50
0
175.009
174.965
168.928
133.635
129.600
129.498
129.420
129.363
129.311
128.897
128.477
127.478
126.990
77.611
77.260
76.903
64.977
60.300
59.744
56.403
53.254
45.834
-1000
0
1000
2000
3000
4000
5000
6000

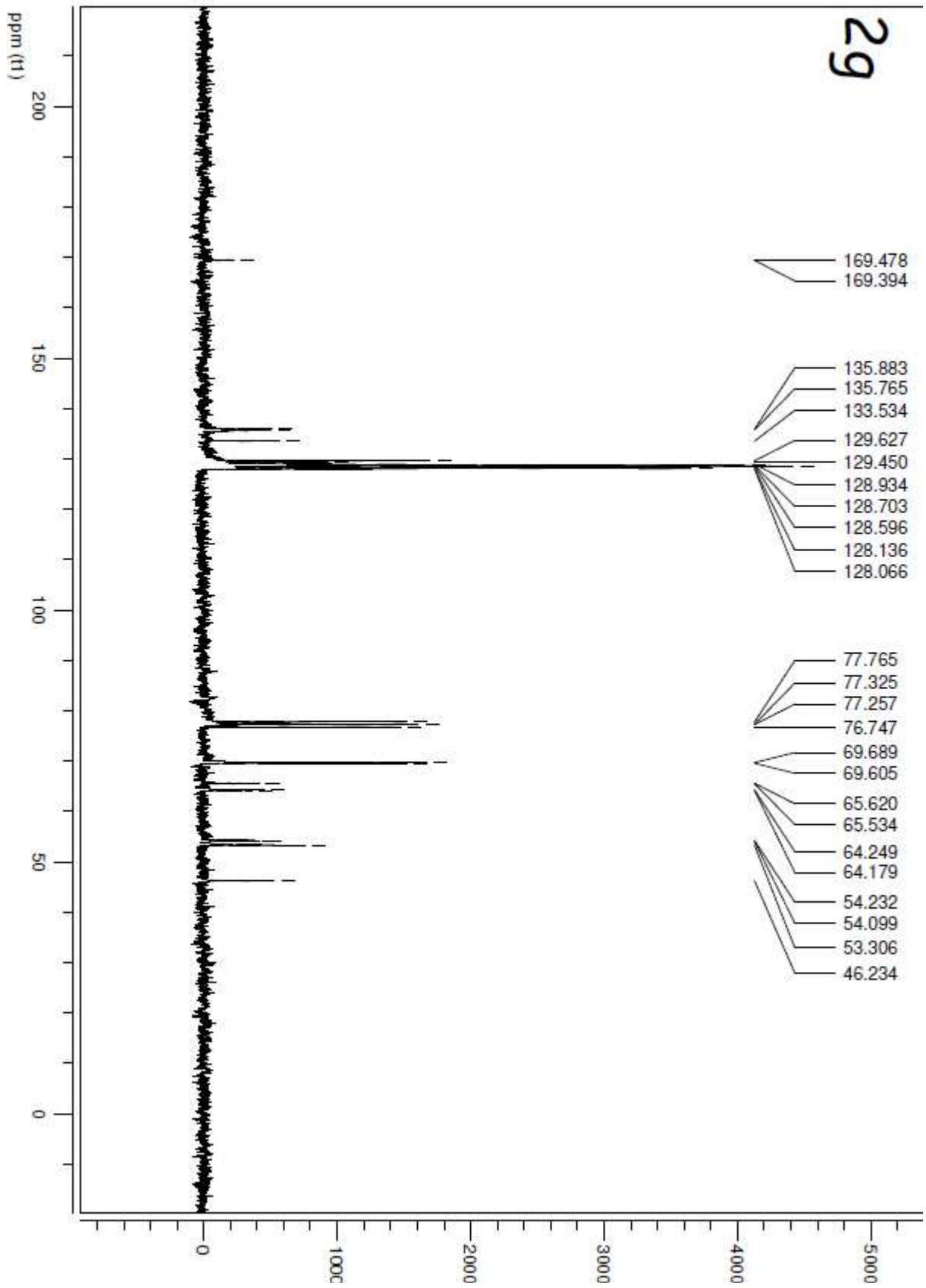
DEPT


ppm (t1)
150
100
50
-1000
-500
0
5000
10000
15000
129.497
129.417
128.893
2f
77.274
64.969
60.523
60.296
59.737
56.398
53.249
50.590
45.824
37.591
29.683

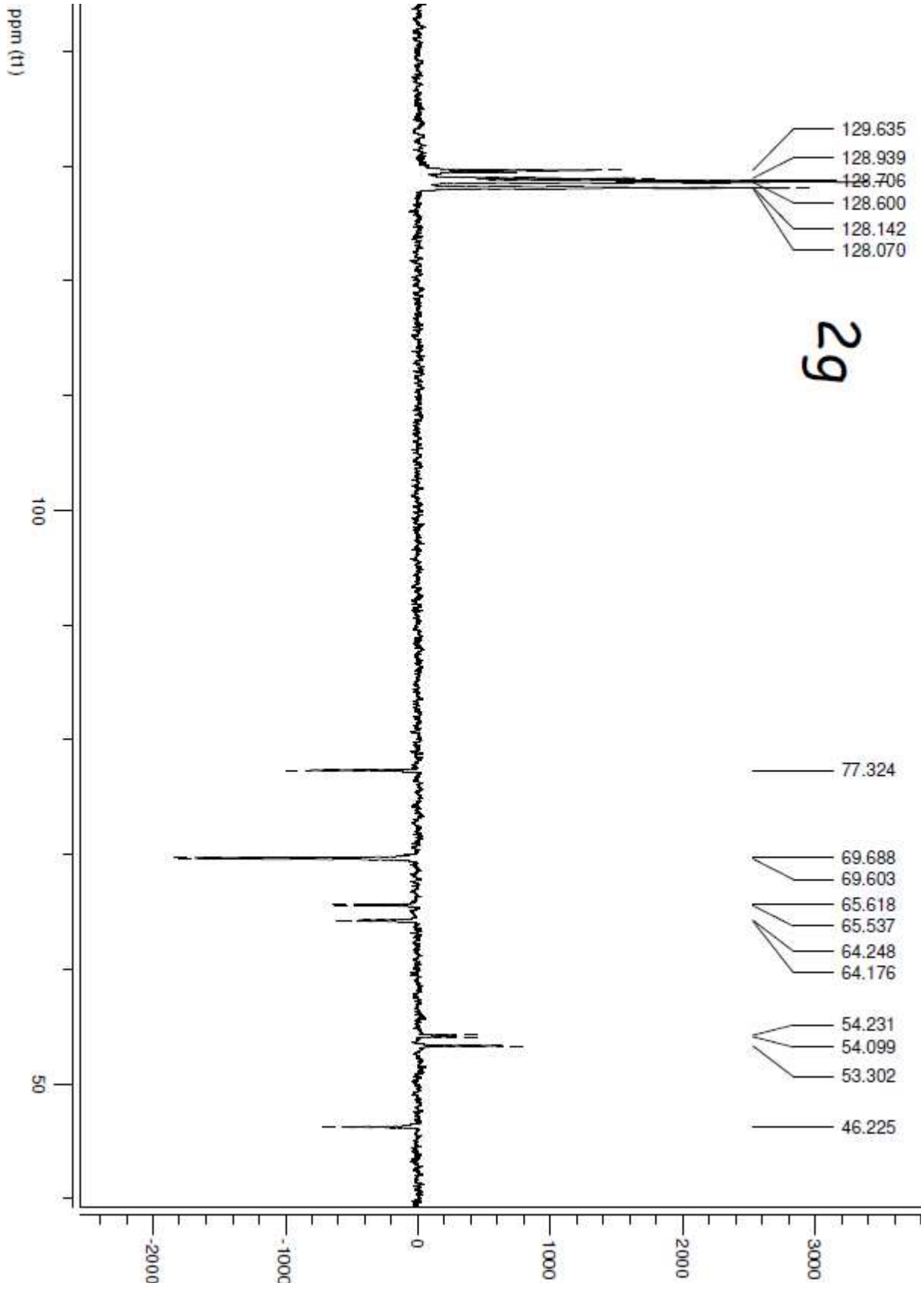
^{1}H NMR


ppm (t1)
7.0
6.0
5.0
4.0
3.0
24.66
7.97
2.00
2.14
1.18
2.01
1.02
1.94
7.375
7.353
29
5.278
5.164
5.132
5.111
5.077
4.805
4.724
4.692
4.679
4.647
3.236
3.220
0
500
1000
1500
2000
2500

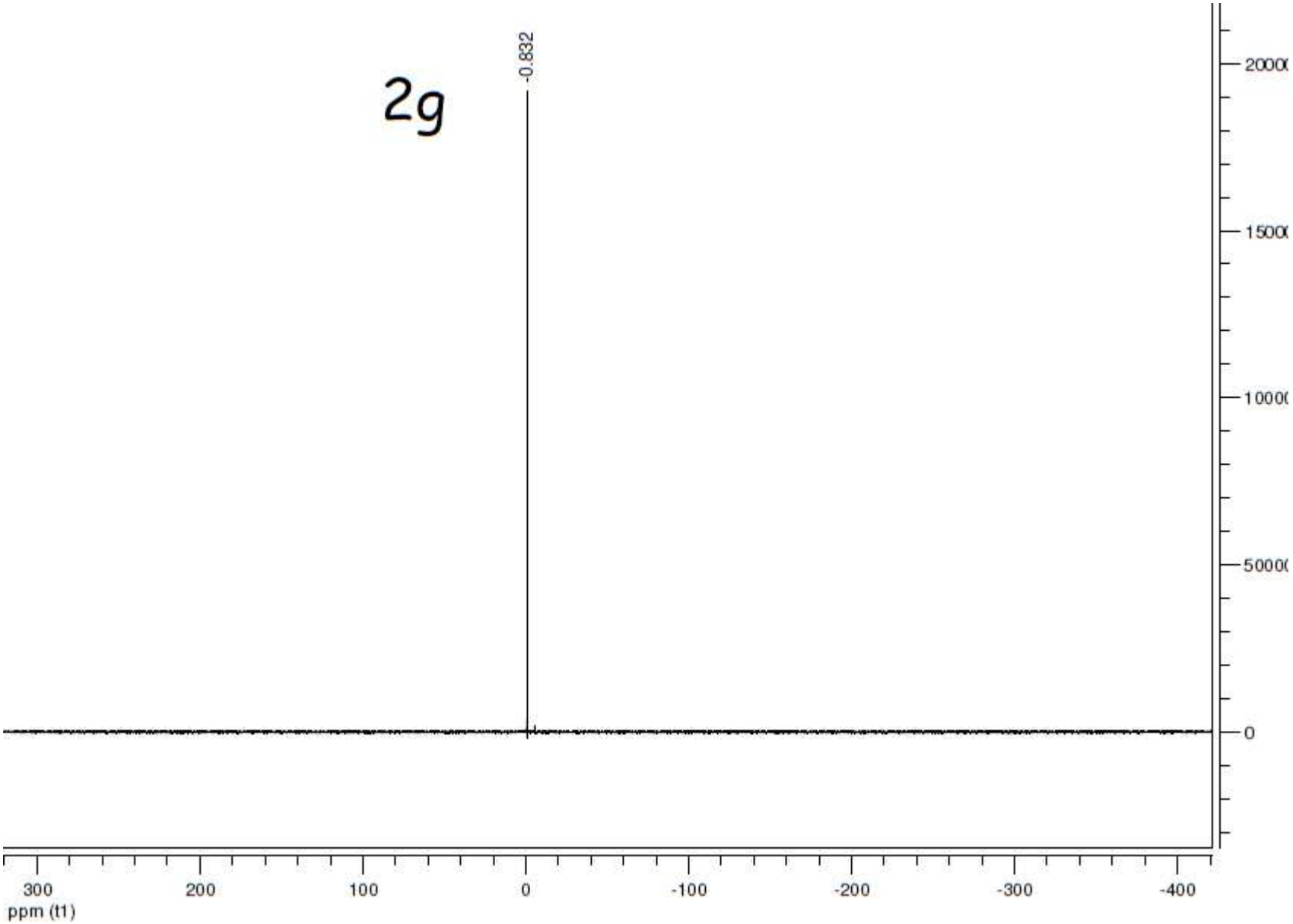
^{13}C NMR


2g
ppm (t1)
200
150
100
50
0
169.478
169.394
135.883
135.765
133.534
129.627
129.450
128.934
128.703
128.596
128.136
128.066
77.765
77.325
77.257
76.747
69.689
69.605
65.620
65.534
64.249
64.179
54.232
54.099
53.306
46.234
0
1000
2000
3000
4000
5000

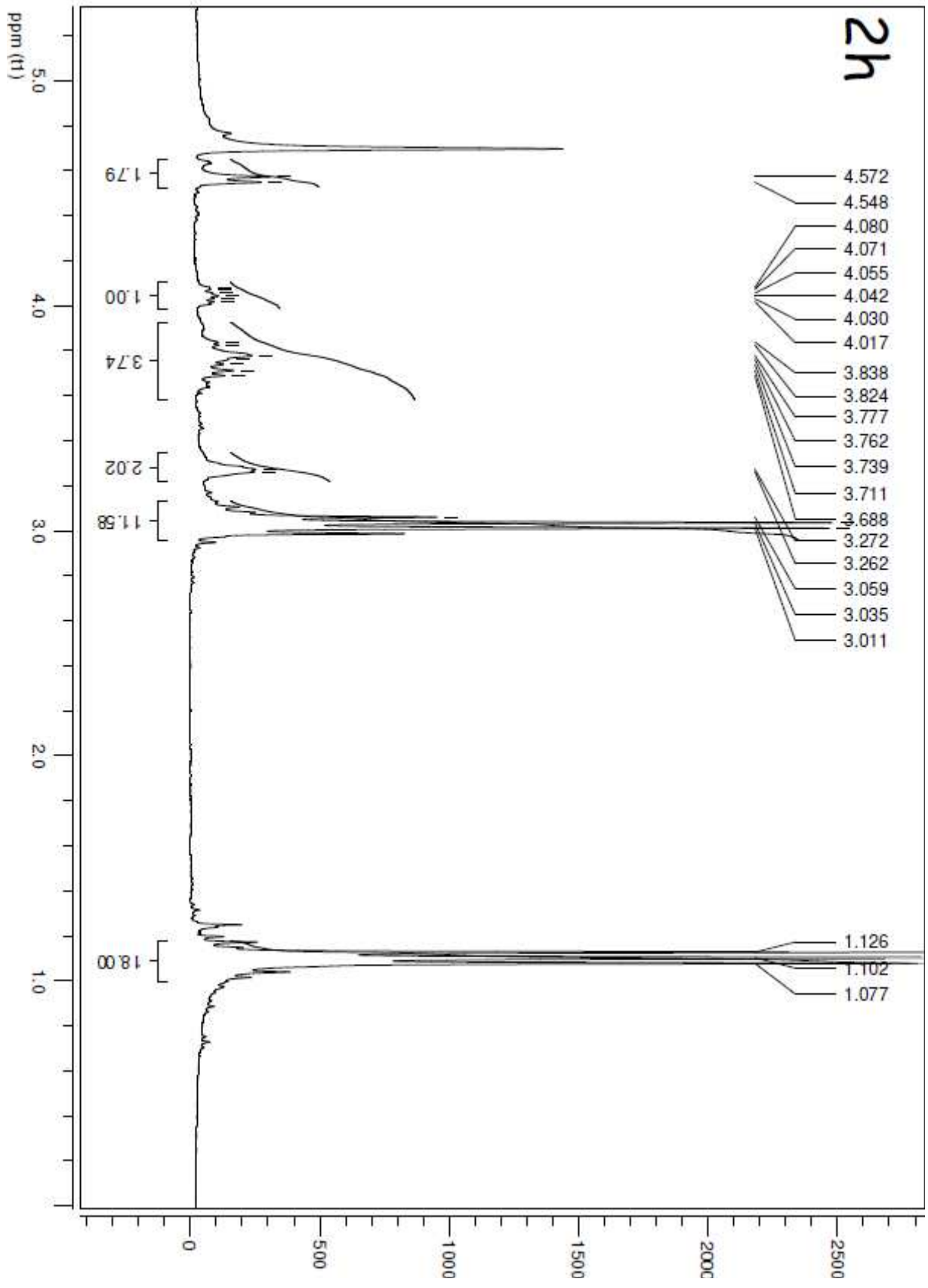
DEPT


ppm (t1)
100
50
129.635
128.939
128.706
128.600
128.142
128.070
29
77.324
69.688
69.603
65.618
65.537
64.248
64.176
54.231
54.099
53.302
46.225
-2000
-1000
0
1000
2000
3000

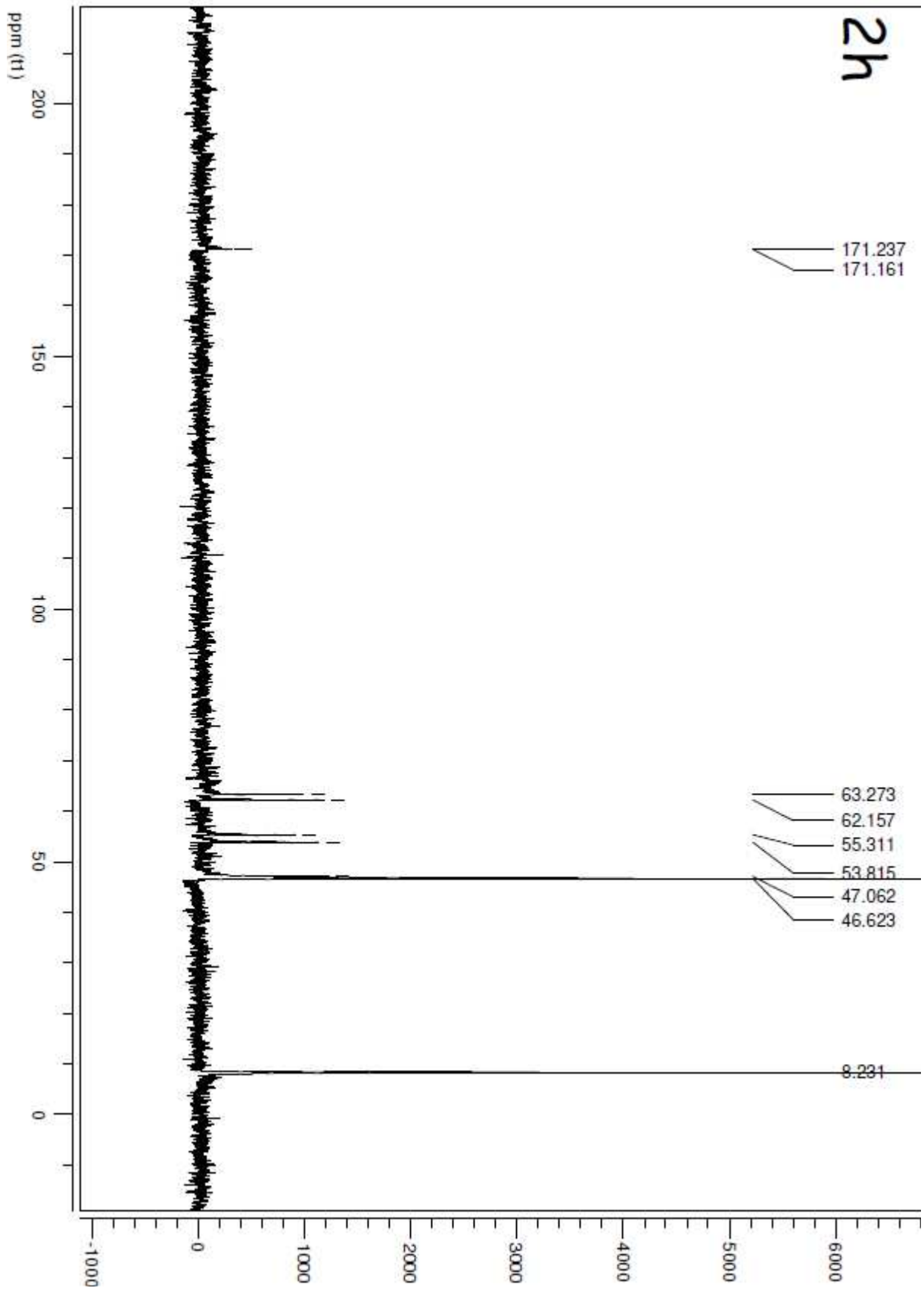
^{31}P-NMR


2g
-0.832
2000
1500
1000
5000
0
300
200
100
0
-100
-200
-300
-400
ppm (t1)

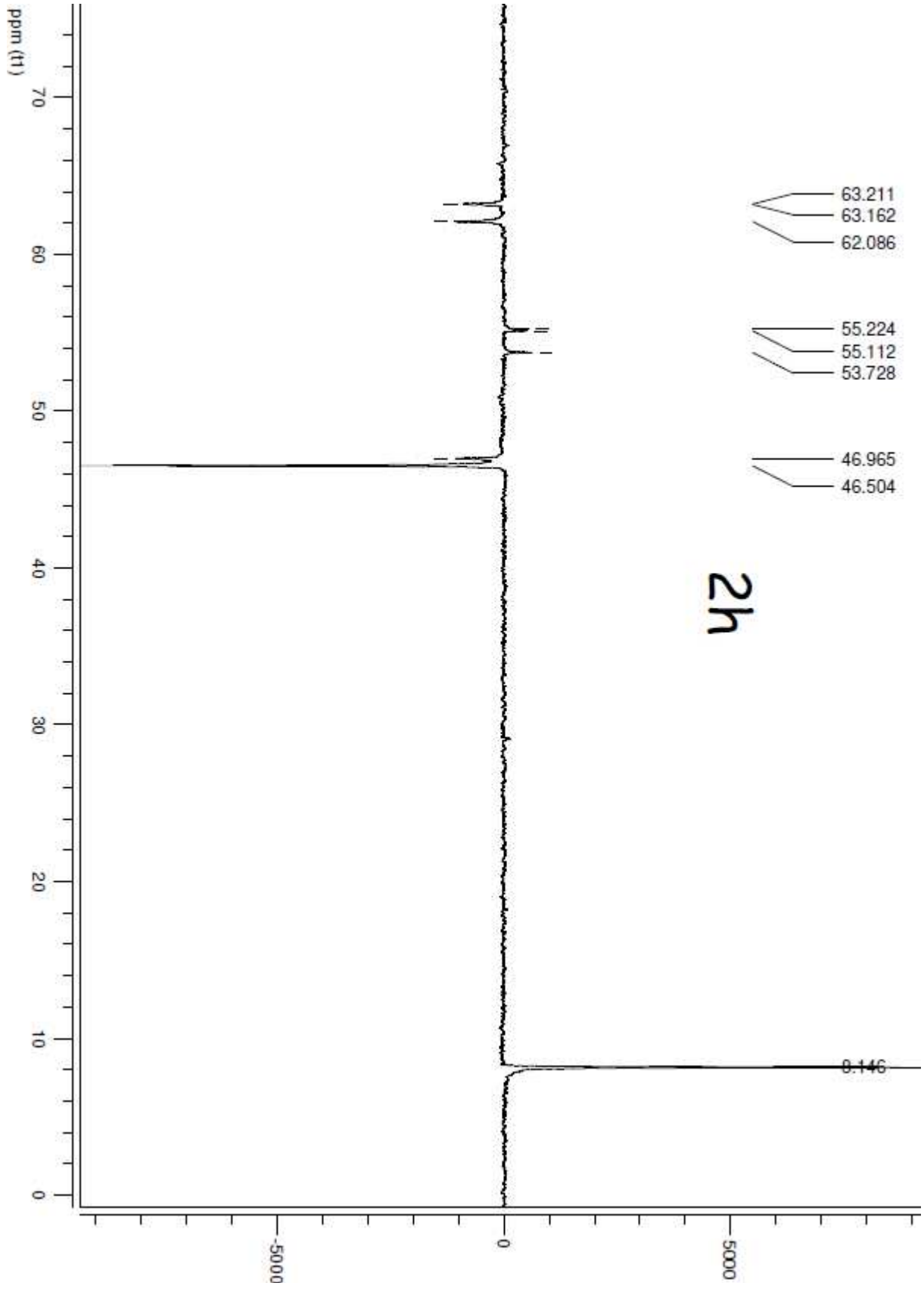
^{1}H NMR


2h
ppm (t1)
5.0
4.0
3.0
2.0
1.0
1.79
1.00
3.74
2.02
11.58
18.00
4.572
4.548
4.080
4.071
4.055
4.042
4.030
4.017
3.838
3.824
3.777
3.762
3.739
3.711
3.688
3.272
3.262
3.059
3.035
3.011
1.126
1.102
1.077
0
500
1000
1500
2000
2500

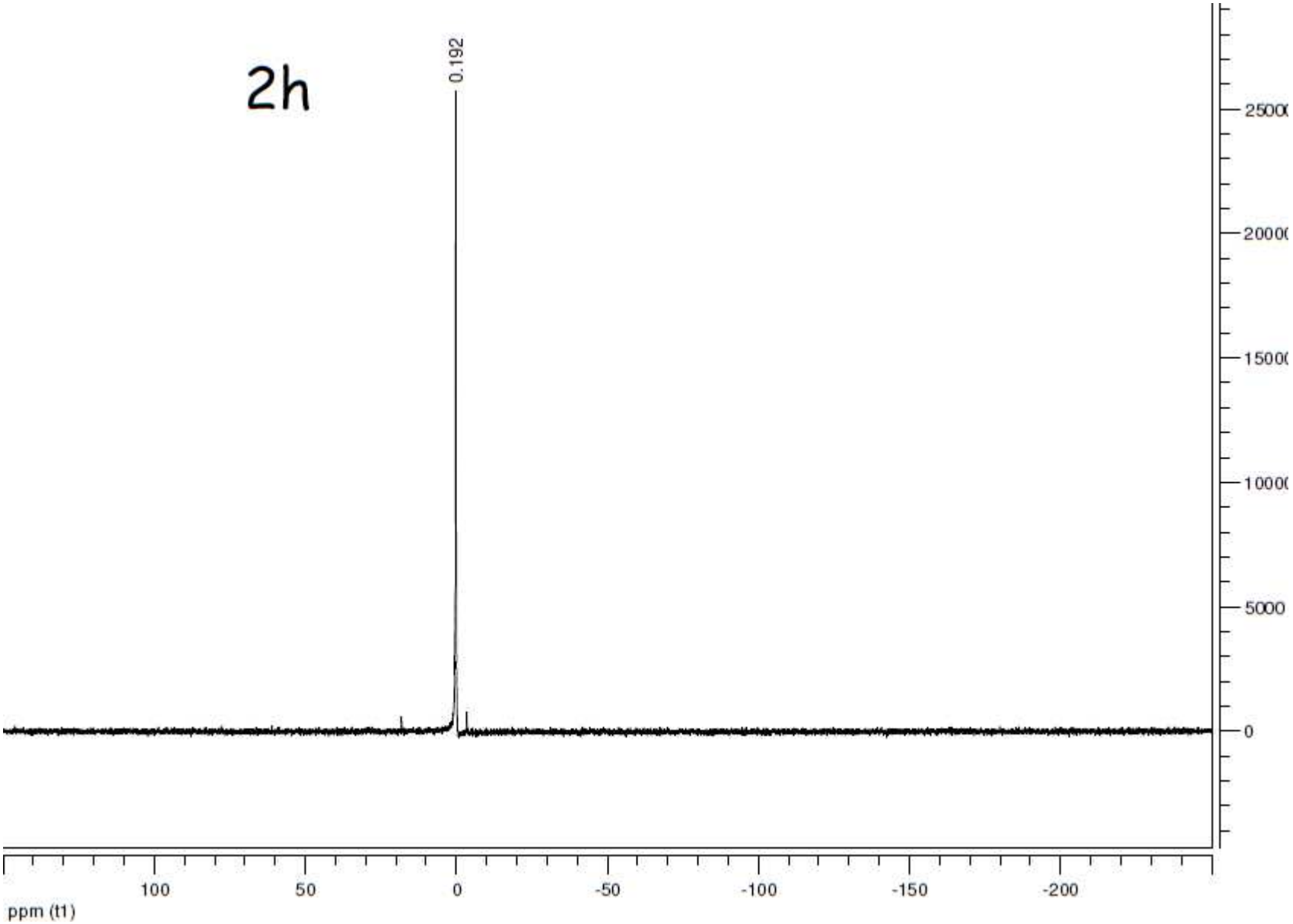
^{13}C NMR


2h
ppm (t1)
200
150
100
50
0
171.237
171.161
63.273
62.157
55.311
53.815
47.062
46.623
8.231
-1000
0
1000
2000
3000
4000
5000
6000

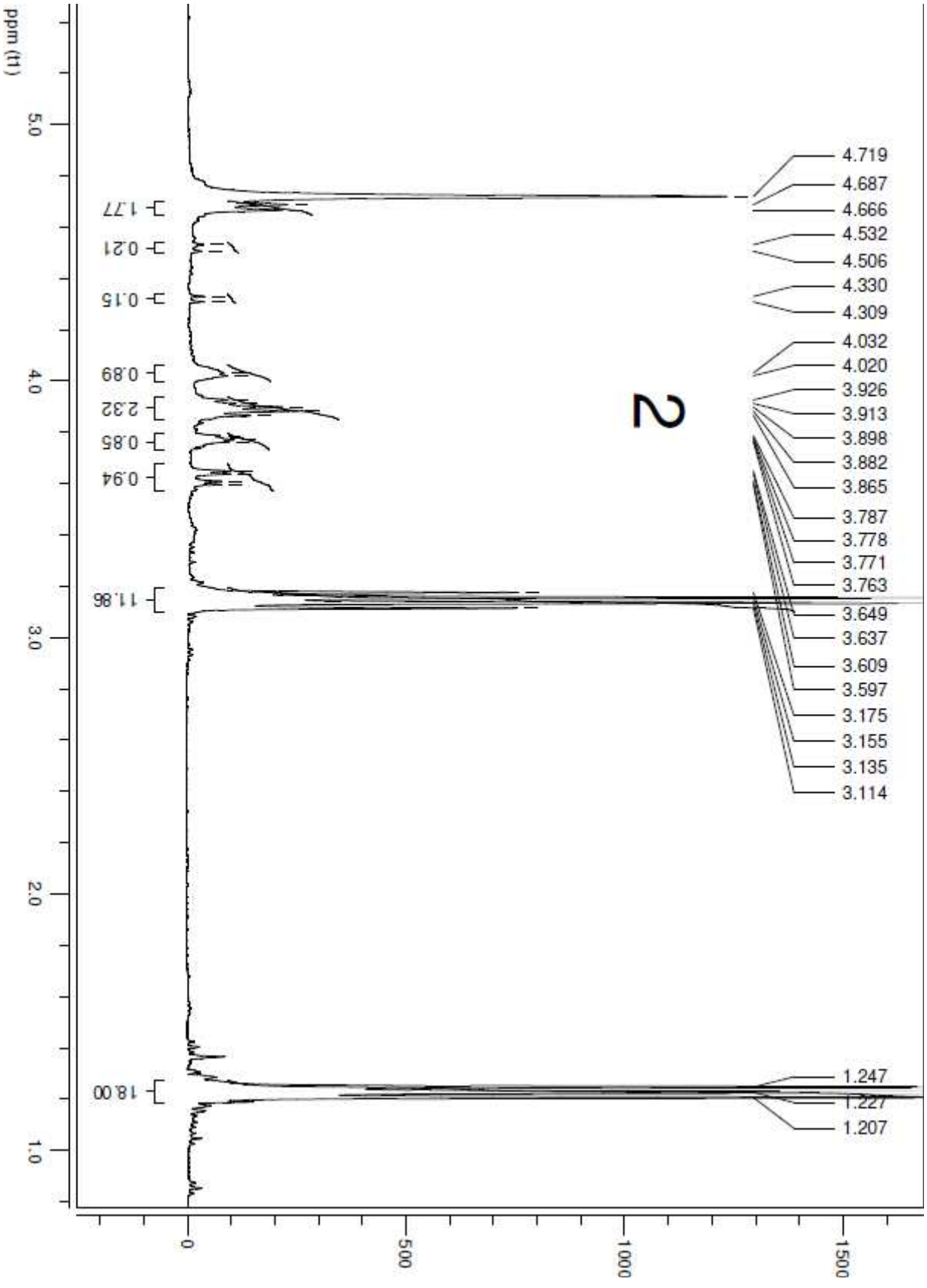
DEPT


ppm (t1)
70
60
50
40
30
20
10
0
-5000
0
5000
63.211
63.162
62.086
55.224
55.112
53.728
46.965
46.504
2h
8.146

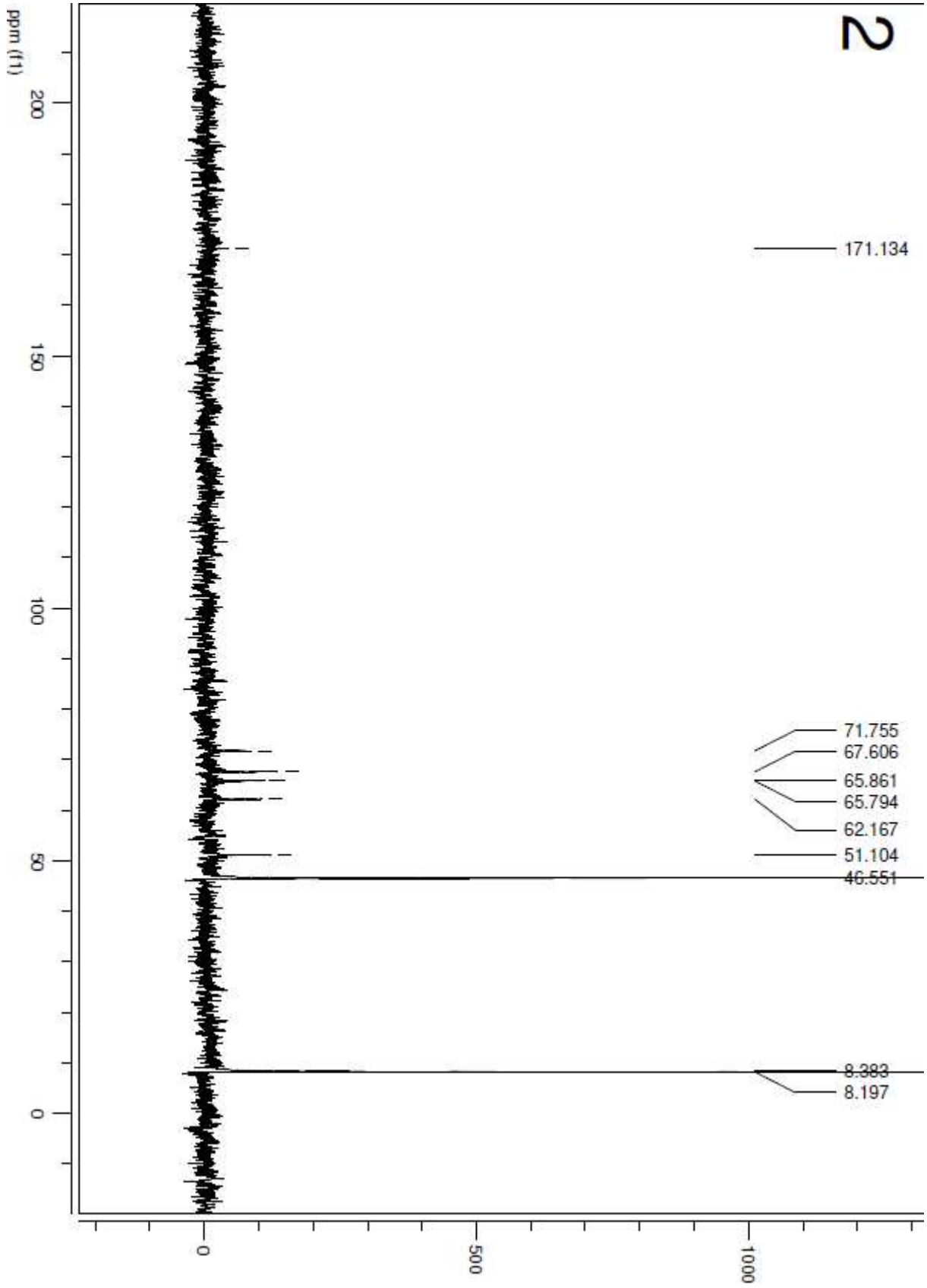
^{31}P-NMR


2h
0.192
2500
2000
1500
1000
5000
0
100
50
0
-50
-100
-150
-200
ppm (t1)

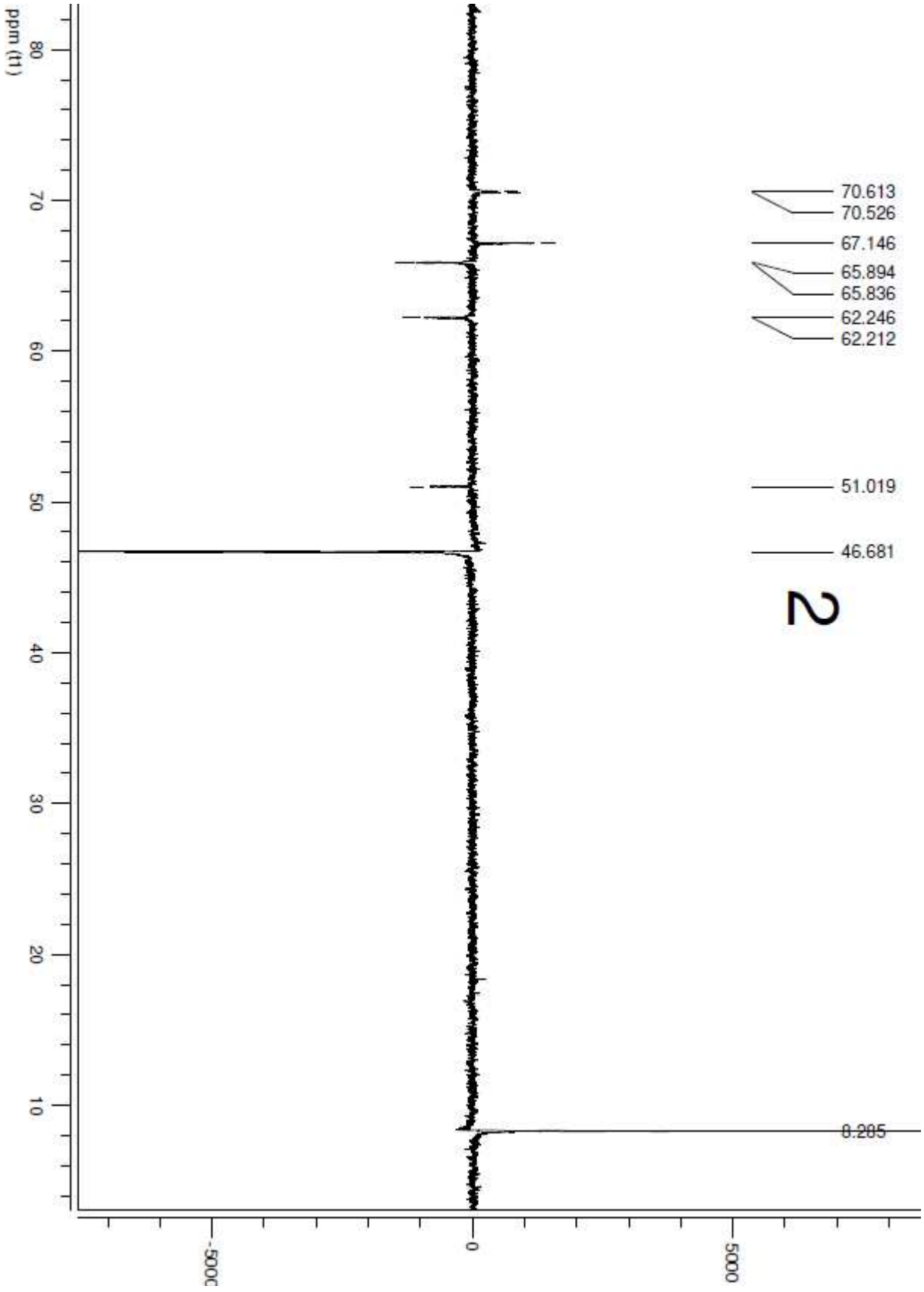
^{1}H NMR


ppm (t1)
5.0
4.0
3.0
2.0
1.0
0
500
1000
1500
1.77
0.21
0.15
0.89
2.32
0.85
0.94
11.86
18.00
2
4.719
4.687
4.666
4.532
4.506
4.330
4.309
4.032
4.020
3.926
3.913
3.898
3.882
3.865
3.787
3.778
3.771
3.763
3.649
3.637
3.609
3.597
3.175
3.155
3.135
3.114
1.247
1.227
1.207

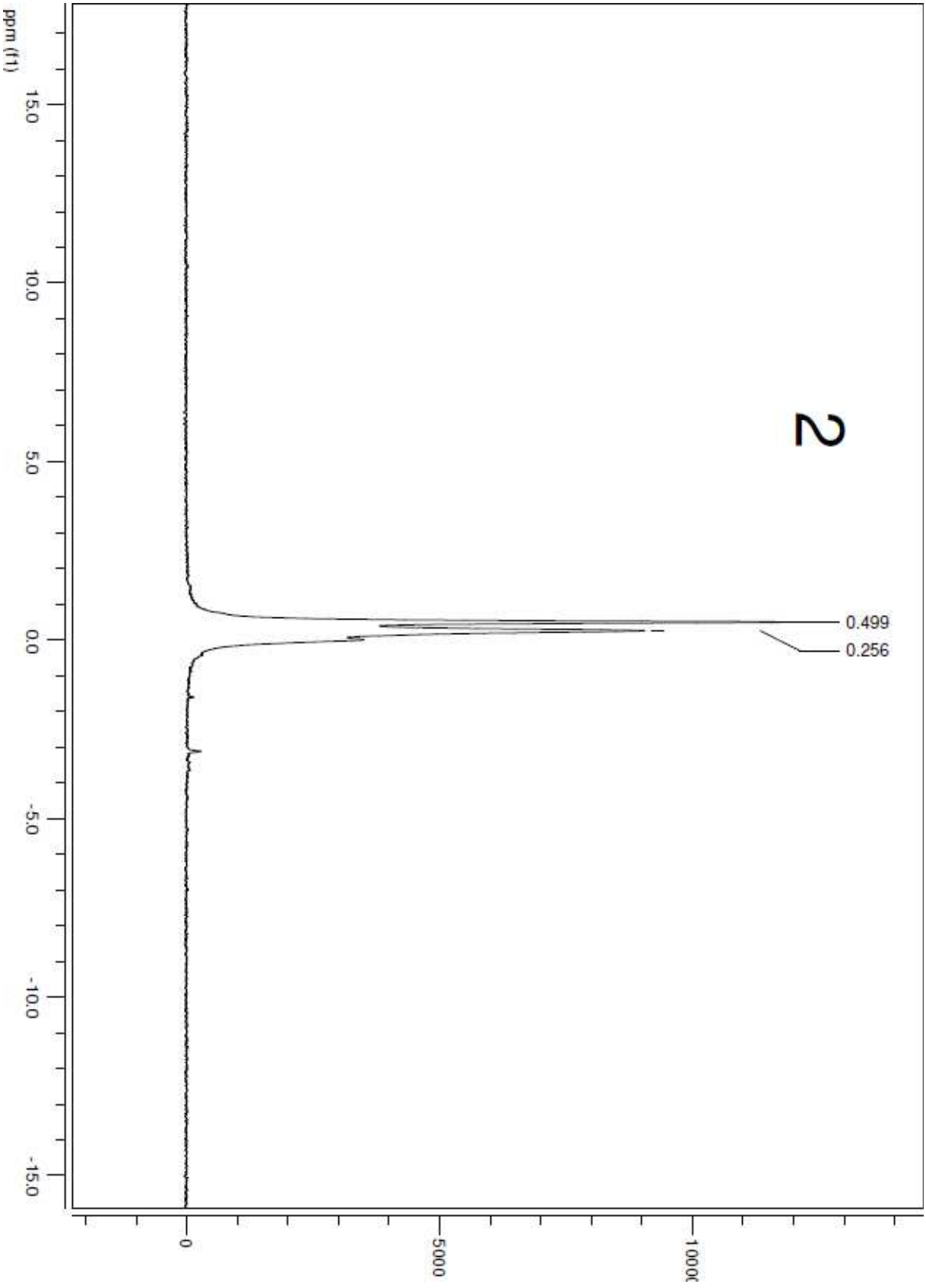
^{13}C NMR


2
ppm (f1)
200
150
100
50
0
171.134
71.755
67.606
65.861
65.794
62.167
51.104
46.551
8.383
8.197
0
500
1000

DEPT


ppm (t1)
80
70
60
50
40
30
20
10
-5000
0
5000
70.613
70.526
67.146
65.894
65.836
62.246
62.212
51.019
46.681
2
0.285

^{31}P-NMR


ppm (f1)
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
0
5000
10000
2
0.499
0.256