Supporting Information

for

Synthesis of Coenzyme A Thioesters Using Methyl Acyl Phosphates in an Aqueous Medium[†]

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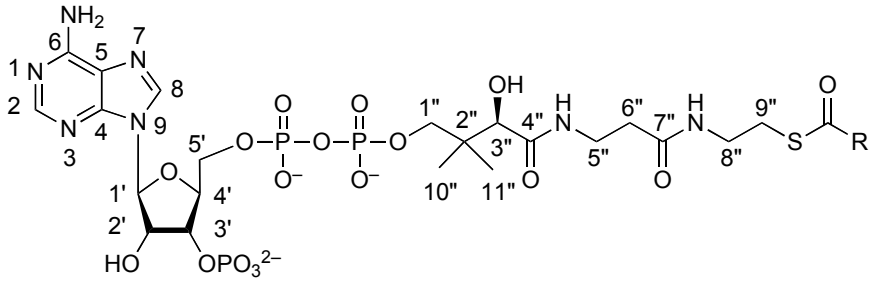
MATERIALS AND METHODS

General. Trimethyl phosphate, sodium iodide, acetyl chloride, butyric acid, isovaleric acid, caprylic acid, 3-mercaptopropanoic acid, 4-mercaptobenzoic acid, and ibuprofen were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Fenoprofen was purchased from Fluka Analytical (Buchs, Switzerland). Palmitic acid was purchased from Fisher Scientific (Ottawa, ON, Canada). The trilithium salt of CoA was purchased from BioShop Canada Inc. (Burlington, ON, Canada). Acetonitrile (MeCN, HPLC grade) was purchased from Fischer Scientific (Ottawa, ON, Canada). Tetrahydrofuran (THF) was dried and distilled over sodium/benzophenone and acetone was dried and distilled over dry potassium carbonate prior to use. Concentration under reduced pressure refers to the removal of solvent in a rotary evaporator, unless stated otherwise. All NMR spectra were obtained using either a Bruker AVANCE 300 or 500 spectrometer. Chemical shifts (δ) for proton (^{1}H) and carbon (^{13}C) are reported in ppm relative to the internal standard trimethylsilane (TMS) for spectra obtained in $CDCl_3$, or relative to the HOD signal for spectra obtained in D_2O. Chemical shifts (δ) for phosphorous (^{31}P, ^{1}H-decoupled) spectra are reported in ppm relative to the deuterium signal for spectra obtained in $CDCl_3$ or D_2O, calibrated to an external standard of 85% aqueous solution of H_3PO_4. Low resolution (LR) and high resolution (HR) electrospray ionization (ESI) mass spectra (MS) were collected using a Bruker microTOF Focus orthogonal ESI-TOF mass spectrometer operating in either negative or positive ion mode as indicated in the experimental procedures below. Melting points (uncorrected) were determined using an Electrothermal Melt-Temp model 1201D capillary melting point apparatus (Barnstead International, Dubuque, IA, USA). Numbering for acyl-CoA compound are shown below as reported previously by Weeks *et al*.[1]



Synthesis of sodium methyl acyl phosphates

Step 1: General procedure for preparation of various aliphatic and aromatic acid chlorides

The aliphatic or aromatic acid (2.5 g, **5b** – **5i**) was dissolved in thionyl chloride (15 mL) and refluxed for 3 h (**5b**, **5c**, **5f**, **5h**, & **5i**), 5 h (**5g** & **5e**), or 10 h (**5d**). Thionyl chloride was distilled off under reduced pressure and, with the exception of the butyric acid and isovaleric acid chlorides, the crude light yellow/brown oil was further concentrated using a high vacuum (oil pump). The remaining thionyl chloride was removed from the butyric acid and isovaleric acid chlorides using fractional distillation. Fractions boiling at 102 °C or 115 °C were collected to obtain pure butyryl chloride or isovaleryl chloride, respectively. For the synthesis of fenoprofenoyl chloride, $CHCl_3$ was used as a solvent.[2]

Butyryl chloride[3] **(6b)**: Colorless oil; yield (1.90 g, 63%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (t, C$\underline{H}_3$CH$_2$, ^3J(HH) = 7.5 Hz, 3H), 1.70-1.82 (m, CH$_3$C$\underline{H}_2$, 2H), 2.91 (t, C$\underline{H}_2$CO, ^3J(HH) = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.13 (C$\underline{H}_3$CH$_2$), 18.82 (CH$_3$C$\underline{H}_2$), 48.99 (C$\underline{H}_2$CO), 173.82 (CO) ppm.

Isovaleryl chloride[3] **(6c)**: Colorless oil; yield (1.82 g, 62%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (d, (C$\underline{H}_3$)$_2$CH, ^3J(HH) = 7.0 Hz, 6H), 2.18-2.27 (m, (CH$_3$)$_2$C$\underline{H}$, 1H), 2.76 (d, C$\underline{H}_2$CO, ^3J(HH) = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.92 (C$\underline{H}_3$)$_2$CH), 26.11 ((CH$_3$)$_2$C$\underline{H}$), 55.66 (CHC$\underline{H}_2$), 172.91 (CO) ppm.

Octanoyl chloride[4] **(6d)**: Brown oil; yield (2.69 g, 95.6%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, C$\underline{H}_3$CH$_2$, ^3J(HH) = 7.0 Hz, 3H), 1.25-1.36 (m, CH$_3$(C$\underline{H}_2$)$_4$, 8H), 1.68-1.74 (m, C$\underline{H}_2$CH$_2$CO, 2H), 2.88 (t, CH$_2$C$\underline{H}_2$CO, ^3J(HH) = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.15

($\underline{C}H_3CH_2$), 22.67 (CH_2), 25.22 (CH_2), 28.54 (CH_2), 28.87 (CH_2), 31.65 (CH_2), 47.27 ($\underline{C}H_2CO$), 174.0 (CO) ppm.

Palmitoyl chloride[5] **(6e)**: Brown oil; yield (2.58 g, 96.5%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, $C\underline{H}_3CH_2$, ^3J(HH) = 7.5 Hz, 3H), 1.26 (m, $CH_3(C\underline{H}_2)_{12}$, 24H), 1.68-1.74 (m, $C\underline{H}_2CH_2CO$, 2H), 2.87 (t, $CH_2C\underline{H}_2CO$, ^3J(HH) = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.25 ($\underline{C}H_3CH_2$), 22.84 (CH_2), 25.23 (CH_2), 28.59 (CH_2), 29.22 (CH_2), 29.48 (CH_2), 29.51 (CH_2), 29.67 (CH_2), 29.76 (CH_2), 29.81 $(CH_2)_4$, 32.08 ($\underline{C}H_2CH_2CO$), 47.27 ($\underline{C}H_2CO$), 173.97 (CO) ppm.

Ibuprofenoyl chloride[6] **(6f)**: Yellow oil; yield (2.62 g, 93.4%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (d, $(C\underline{H}_3)_2CH$, ^3J(HH) = 6.5 Hz, 6H), 1.63 (d, $C\underline{H}_3CH$, ^3J(HH) = 7.0 Hz, 3H), 1.86-1.97 (m, $(CH_3)_2C\underline{H}$, 1H), 2.52 (d, $CHC\underline{H}_2$, ^3J(HH) = 7.0 Hz, 2H), 4.14 (q, $CH_3C\underline{H}$, ^3J(HH) = 7.0 Hz, 1H), 7.2 (d, Ar$\underline{H}$, ^3J(HH) = 8.0, 2H), 7.24 (d, Ar$\underline{H}$, ^3J(HH) = 8.0, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.87 ($\underline{C}H_3CH$), 22.57 (($\underline{C}H_3)_2CH$), 30.36 (($CH_3)_2\underline{C}H$), 45.25 ($CH\underline{C}H_2$), 57.30 ($CH_3\underline{C}H$), 127.85 ($C_{aromatic}$), 129.98 ($C_{aromatic}$), 134.83 ($C_{aromatic}$), 142.01 ($C_{aromatic}$), 175.93 (CO) ppm.

Fenoprofenoyl chloride[2] **(6g)**: Brown oil; yield (2.45 g, 91%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (d, $C\underline{H}_3CH$, ^3J(HH) = 7.0 Hz, 3H), 4.10 (q, $CH_3C\underline{H}$, ^3J(HH) = 7.0 Hz, 1H), 6.97-7.05 (m, ArH, 5H), 7.14-7.17 (m, ArH, 1H), 7.33-7.39 (m, ArH, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.66 ($\underline{C}H_3CH$), 57.28 ($CH_3\underline{C}H$), 118.36 ($C_{aromatic}$), 118.54 ($C_{aromatic}$), 119.16 ($C_{aromatic}$), 122.67 ($C_{aromatic}$), 123.73 ($C_{aromatic}$), 129.97 ($C_{aromatic}$), 130.44 ($C_{aromatic}$), 139.42 ($C_{aromatic}$), 156.85 ($C_{aromatic}$), 157.96 ($C_{aromatic}$), 175.31 (CO) ppm.

3-Phenylpropanoyl chloride[7] **(6h)**: Colorless oil; yield (2.6 g, 92.8%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.07 (t, $ArC\underline{H}_2CH_2$, ^3J(HH) = 7.5 Hz, 2H), 3.26 (t, $C\underline{H}_2CO$, ^3J(HH) = 7.5 Hz, 2H), 7.25-7.32 (m, ArH, 3H), 7.36-7.39 (m, ArH, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.09 ($Ar\underline{C}H_2CH_2$), 48.58 ($\underline{C}H_2CO$), 126.92 ($C_{aromatic}$), 128.39 ($C_{aromatic}$), 128.85 ($C_{aromatic}$), 138.71 ($C_{aromatic}$), 173.11 (CO) ppm.

***trans*-Cinnamoyl chloride**[8] **(6i)**: Dark brown oil; yield (2.65 g, 94.6%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.65 (d, ArCH=C$\underline{H}$, ^3J(HH) = 26 Hz, 1H), 7.41-7.56 (m, ArH, 3H), 7.56-7.59 (m, ArH, 2H), 7.85 (d, ArC$\underline{H}$=CH, ^3J(HH) = 26 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 122.52

(ArCH=CH), 129.21 ($C_{aromatic}$), 129.37 ($C_{aromatic}$), 132.15 ($C_{aromatic}$), 133.19 ($C_{aromatic}$), 150.80 (ArCH=CH), 166.24 (CO) ppm.

Step 2: General procedure for preparation of aliphatic and aromatic substituted dimethyl acyl phosphates

A suspension of sodium dimethyl phosphate (1.48 g, 0.01 mol, from trimethyl phosphate and sodium iodide) and the acid chloride (**6a** – **6i**, 0.01 mol) in dry THF (20 mL) was stirred for 24 h at room temperature under argon.[9] The solution was filtered and the THF was removed under reduced pressure. The resulting crude liquid was analyzed using NMR and used for the subsequent demethylation step without purification. With the exception of dimethyl fenoprofenoyl phosphate, the dimethyl acyl phosphates were reasonably pure and contaminated by a minor amount of dimethyl phosphate appearing as a doublet at ~4.3 ppm (^3J(PH) = 11.5 Hz) and a singlet at ~3.2 ppm in the ^{1}H and ^{31}P NMR spectra, respectively, and free acid arising from hydrolysis. In some cases, an unidentified phosphorus-containing by-product was also observed at ~–10.6 ppm in the ^{31}P NMR spectra. These crude were used directly in the subsequent demethylation reaction.

Dimethyl acetyl phosphate[9] (7a): Colorless liquid; yield (1.45 g, 86%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.20 (d, C$\underline{H}_3$CO, ^4J(PH) = 1.5 Hz, 3H), 3.87 (d, (C$\underline{H}_3$O)$_2$P, ^3J(PH) = 11.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.89 (d, $\underline{C}H_3CO$, ^3J (CCOP) = 7.5 Hz), 55.48 (d, ($\underline{C}H_3O)_2P$, ^2J (COP) = 5.0 Hz), 165.20 (d, CO, ^2J (COP) = 8.7 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –6.0 ppm.

Crude dimethyl butyryl phosphate (7b): Yellow liquid; yield (1.33 g, 68%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.994 (t, C$\underline{H}_3$CH$_2$, ^3J(HH) = 7.5 Hz, 3H), 1.66-1.73 (m, CH$_3$C$\underline{H}_2$, 2H), 2.45 (t, C$\underline{H}_2$CO, ^3J(HH) = 7.0 Hz, 2H), 3.89 (d, (C$\underline{H}_3$O)$_2$P, ^3J(PH) = 12 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.47 ($\underline{C}H_3CH_2$), 17.96 ($CH_3\underline{C}H_2$), 36.84 (d, $\underline{C}H_2CO$, ^3J (CCOP) = 6.25 Hz), 55.42 (d, ($\underline{C}H_3O)_2P$, ^2J (COP) = 6.25 Hz), 167.90 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.75 ppm.

Crude dimethyl isovaleryl phosphate (7c): Yellow liquid; yield (1.55 g, 74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.0 (d, (C$\underline{H}_3$)$_2$CH, ^3J(HH) = 7.0 Hz, 6H), 2.09-2.17 (m, (CH$_3$)$_2$C$\underline{H}$, 1H), 2.33 (d,

$C\underline{H}_2CO$, ^3J(HH) = 7.0 Hz, 2H), 3.88 (d, $(C\underline{H}_3O)_2P$, ^3J(PH) = 11.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.31 $(\underline{C}H_3)_2CH$), 25.51 $(CH_3)_2\underline{C}H$), 43.89 (d, $(\underline{C}H_2CO)$, ^3J (CCOP) = 7.5 Hz), 55.41 (d, $(\underline{C}H_3O)_2P$, ^2J (COP) = 5.0 Hz), 167.29 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.75 ppm.

Crude dimethyl octanoyl phosphate (7d): Brown liquid; yield (2.37 g, 94%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, $C\underline{H}_3CH_2$, ^3J(HH) = 7.0 Hz, 3H), 1.27-1.35 (m, $CH_3(C\underline{H}_2)_4$, 8H), 1.62-1.68 (m, $C\underline{H}_2CH_2CO$, 2H), 2.45 (t, $CH_2C\underline{H}_2CO$, ^3J(HH) = 7.5 Hz, 2H), 3.88 (d, $(C\underline{H}_3O)_2P$, ^3J(PH) = 11.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.21 $(\underline{C}H_3CH_2)$, 22.75 (CH_2), 24.45 (CH_2), 28.94 (CH_2), 29.0 (CH_2), 31.75 $(\underline{C}H_2CH_2CO)$, 35.09 (d, $(\underline{C}H_2CO)$, ^3J (CCOP) = 7.5 Hz), 55.49 (d, $(\underline{C}H_3O)_2P$, ^2J (COP) = 6.25 Hz), 168.13 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.77 ppm.

Crude dimethyl palmitoyl phosphate (7e): Light brown semi-solid; yield (3.50 g, 96%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, $C\underline{H}_3CH_2$, ^3J(HH) = 6.5 Hz, 3H), 1.26 (m, $CH_3(C\underline{H}_2)_{12}$, 24H), 1.61-1.70 (m, $C\underline{H}_2CH_2CO$, 2H), 2.46 (t, $CH_2C\underline{H}_2CO$, ^3J(HH) = 7.2 Hz, 2H), 3.90 (d, $(C\underline{H}_3O)_2P$, ^3J(PH) = 11.7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.03 $(\underline{C}H_3CH_2)$, 22.62 (CH_2), 24.19 (CH_2), 28.73 (CH_2), 29.09 (CH_2), 29.29 (CH_2), 29.30 (CH_2), 29.49 (CH_2), 29.56 (CH_2), 29.59 $(CH_2)_4$, 31.86 $(\underline{C}H_2CH_2CO)$, 34.83 (d, $\underline{C}H_2CO$, ^3J (CCOP) = 7.50 Hz), 55.21 (d, $(\underline{C}H_3O)_2P$, ^2J (COP) = 5.0 Hz), 167.87 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.75 ppm.

Crude dimethyl ibuprofenoyl phosphate (7f): Yellow oil; yield (2.72 g, 87%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (d, $(C\underline{H}_3)_2CH$, ^3J(HH) = 6.5 Hz, 6H), 1.53 (d, $C\underline{H}_3CH$, ^3J(HH) = 7.0 Hz, 3H), 1.79-1.88 (m, $(CH_3)_2C\underline{H}$, 1H), 2.45 (d, $CHC\underline{H}_2$, ^3J(HH) = 7.5 Hz, 2H), 3.74 (m, $CH_3C\underline{H}$, 1H), 3.74 (dd, $(C\underline{H}_3O)_2P$, ^3J(PH) = 11.7 Hz, ^7J(HH) = 1 Hz, 6H), 7.11 (d, Ar$\underline{H}$, ^3J(HH) = 8.0, 2H), 7.19 (d, Ar$\underline{H}$, ^3J(HH) = 8.0, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.22 $(\underline{C}H_3CH)$, 22.51 $(\underline{C}H_3)_2CH$), 30.36 ($(CH_3)_2\underline{C}H$), 45.21($CH\underline{C}H_2$), 46.22 (d, $CH_3\underline{C}H$, ^3J (CCOP) = 6.25 Hz), 55.36 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 55.47 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 127.49 ($C_{aromatic}$), 129.83 ($C_{aromatic}$), 136.09 ($C_{aromatic}$), 141.47 ($C_{aromatic}$), 169.05 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –6.06 ppm.

Crude dimethyl fenoprofenoyl phosphate (7g): Brown oil; yield (2.98 g, 85%); although the presence of the product in the crude compound was evident in the ^{1}H and ^{13}C NMR spectra, there were also substantial contaminants present, which were removed in the subsequent step.

Crude dimethyl 3-phenylpropanoyl phosphate (7h): Light yellow oil; yield (1.69 g, 65.6%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.82 (t, ArC$\underline{H}_2$CH$_2$, ^{3}J(HH) = 7.5 Hz, 2H), 3.0 (t, C$\underline{H}_2$CO, ^{3}J(HH) = 7.5 Hz, 2H), 3.88 (d, (C$\underline{H}_3$O)$_2$P, ^{3}J(PH) = 11.5 Hz, 6H), 7.22-7.25 (m, Ar$\underline{H}$, 3H), 7.31-7.33 (m, Ar$\underline{H}$, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.36 (Ar$\underline{C}$H$_2$), 36.58 (d, ($\underline{C}$H$_2$CO), ^{3}J (CCOP) = 7.5 Hz), 55.42 (d, ($\underline{C}$H$_3$O)$_2$P, ^{2}J (COP) = 5.0 Hz), 126.69 ($C_{aromatic}$), 128.36 ($C_{aromatic}$), 128.75 ($C_{aromatic}$), 139.45 ($C_{aromatic}$), 167.17 (d, CO, ^{2}J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.97 ppm.

Crude dimethyl *trans-c*innamoyl phosphate[10] (7i): Brown liquid: yield (1.28 g, 50%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.95 (d, (C$\underline{H}_3$O)$_2$P, ^{3}J(PH) = 12.0 Hz, 6H), 6.41 (dd, ArCH=C$\underline{H}$, ^{3}J(HH) = 16.0 Hz, ^{4}J (PH) = 1.5 Hz, 1H), 7.41-7.56 (m, ArH, 5H), 7.80 (d, ArC$\underline{H}$=CH, ^{3}J(HH) = 16.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.52 (d, ($\underline{C}$H$_3$O)$_2$P, ^{2}J (COP) = 5.0 Hz), 115.90 (d, ArCH=$\underline{C}$H, ^{2}J (COP) = 10.0 Hz), 128.71 ($C_{aromatic}$), 129.36 ($C_{aromatic}$), 133.63 ($C_{aromatic}$), 149.55 (ArC$\underline{H}$=CH), 160.85 (d, CO, ^{2}J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –6.24 ppm.

Step 3: General procedure for preparation of aliphatic and aromatic substituted sodium methyl acyl phosphates

A solution of sodium iodide (0.892 g, 5.95 mmol) in dry acetone (10 mL) was added to a solution of dimethyl acyl phosphate (**7a** – **7i**, 5.95 mmol) in dry acetone (10 mL). The dark brown solution was allowed to stand overnight at room temperature. The precipitate of sodium acetyl methyl phosphate **(8a)** or sodium methyl palmitoyl phosphate **(8e)** were collected by filtration on a sintered-glass funnel and washed with dry acetone to obtain pure **8a** and **8e**. For the reactions with **7b**, **7c**, and **7f** – **7i**, the acetone was evaporated to give a dark brown liquid, which was decolorized using activated carbon in chloroform. After removal of the carbon by filtration through celite, the solvent was evaporated *in vacuo*, and the resulting light brown liquid was then purified using solid-phase extraction (SPE, Strata C18 columns, 10 g/60 mL,

Phenomenex, Torrance, CA). After washing the SPE columns with MeCN and water (60 mL each), the aqueous solution containing the crude methyl acyl phosphate (**8b**, **8c** and **8f** – **8i**) was applied to the column and eluted consecutively with aqueous solutions containing 0%, 2%, 5%, 10%, 15%, 20%, 25%, 50%, and 100% MeCN (60 mL each). The specific elution conditions for each compound (**8b**, **8c** and **8f** – **8i**) are given below. Solvent was removed from the aqueous fractions by lyophilization, giving white solids. The products (**8a** – **8i)** were stored at 4 °C.

Sodium methyl acetyl phosphate[9] **(8a)**: White solid; yield (0.793 g, 76%); ^{1}H NMR (500 MHz, D_2O) δ 2.16 (s, C$\underline{H}_3$CO, 3H), 3.64 (d, C$\underline{H}_3$OP, ^{3}J(PH) = 11.0 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 21.42 (d, $\underline{C}H_3CO$, ^{3}J (CCOP) = 6.25 Hz), 53.62 (d, $\underline{C}H_3OP$, ^{2}J (COP) = 6.25 Hz), 170.01 (d, CO, ^{2}J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –6.06 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 152.9952, found $[M\text{-}Na]^-$ *m/z* 152.9961.

Sodium methyl butyryl phosphate (8b): Elution conditions: three fractions of water (20 mL each) were collected. Fraction 2 (20 mL) of 100% H_2O contained pure product; white solid; yield (0.411 g, 34%); ^{1}H NMR (300 MHz, D_2O) δ 0.910 (t, C$\underline{H}_3CH_2$, ^{3}J(HH) = 7.2 Hz, 3H), 1.54-1.66 (m, $CH_3C\underline{H}_2$, 2H), 2.42 (t, C$\underline{H}_2$CO, ^{3}J(HH) = 7.2 Hz, 2H), 3.62 (d, C$\underline{H}_3$OP, ^{3}J(PH) = 11.4 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 12.72 ($\underline{C}H_3CH_2$), 17.75 ($CH_3\underline{C}H_2$), 36.76 (d, $\underline{C}H_2CO$, ^{3}J (CCOP) = 6.25 Hz), 53.67 (d, $\underline{C}H_3OP$, ^{2}J (COP) = 6.25 Hz), 172.72 (d, CO, ^{2}J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –5.89 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 181.0266, found $[M\text{-}Na]^-$ *m/z* 181.0275. (The additional peak present at 180.9782 in the HR-ESIMS arises from sodium formate trimer (calcd. $[M_3\text{-}Na]^-$, m/z = 180.973) used to standardize the instrument.)

Sodium methyl isovaleryl phosphate (8c): Elution conditions: three fractions of water (20 mL each) were collected. Fraction 2 (20 mL) of 100% H_2O contained pure product; white solid; yield (0.519 g, 40%); ^{1}H NMR (500 MHz, D_2O) δ 1.03 (d, $(C\underline{H}_3)_2CH$, ^{3}J(HH) = 7.0 Hz, 6H), 2.11-2.16 (m, $(CH_3)_2C\underline{H}$, 1H), 2.40 (d, C$\underline{H}_2$CO, ^{3}J(HH) = 7.0 Hz, 2H), 3.71 (d, C$\underline{H}_3$OP, ^{3}J(PH) = 11.5 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 21.50 $(\underline{C}H_3)_2CH$), 25.28 $(CH_3)_2\underline{C}H$), 43.91 (d, $\underline{C}H_2CO$), ^{3}J (CCOP) = 5.0 Hz), 53.68 (d, $\underline{C}H_3OP$, ^{2}J (COP) = 5.0 Hz), 172.19 (d, CO, ^{2}J (COP) =

10 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –5.93 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 195.0422, found $[M\text{-}Na]^-$ *m/z* 195.0424.

Sodium methyl octanoyl phosphate (8d): Elution conditions: 5% MeCN/H_2O; white solid; yield (0.897 g, 58%); ^{1}H NMR (500 MHz, D_2O) δ 0.92 (t, $C\underline{H}_3CH_2$, ^3J(HH) = 7.0 Hz, 3H), 1.34-1.38 (m, $CH_3(C\underline{H}_2)_4$, 8H), 1.68 (m, $C\underline{H}_2CH_2CO$, 2H), 2.52 (t, $CH_2C\underline{H}_2CO$, ^3J(HH) = 7.0 Hz, 2H), 3.71 (d, $C\underline{H}_3OP$, ^3J(PH) = 11.5 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 13.40 ($\underline{C}H_3CH_2$), 21.98 (CH_2), 24.11 (CH_2), 28.04 (CH_2), 28.10 (CH_2), 30.95 (CH_2), 34.90 (d, $\underline{C}H_2CO$, ^3J (CCOP) = 6.25 Hz), 53.68 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 172.95 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –5.93 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 237.0891, found $[M\text{-}Na]^-$ *m/z* 237.0897.

Sodium methyl palmitoyl phosphate (8e): Light yellow solid: yield (1.20 g, 54%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.917 (t, $C\underline{H}_3CH_2$, ^3J(HH) = 7.0 Hz, 3H), 1.29 (m, $CH_3(C\underline{H}_2)_{12}$, 24H), 1.62 (t, $C\underline{H}_2CH_2CO$, ^3J(HH) = 6.5 Hz, 2H), 2.39 (t, $CH_2C\underline{H}_2CO$, ^3J(HH) = 7.5 Hz, 2H), 3.64 (d, $C\underline{H}_3OP$, ^3J(PH) = 11.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.32 ($\underline{C}H_3CH_2$), 22.92 (CH_2), 24.82 (CH_2), 29.48 (CH_2), 29.61 (CH_2), 29.74 (CH_2), 29.93 (CH_2), 30.01 $(CH_2)_6$, 32.17 (CH_2), 35.80 (d, $\underline{C}H_2CO$, ^3J (CCOP) = 5.0 Hz), 53.70 (d, $\underline{C}H_3OP$, ^2J (COP) = 5.0 Hz), 173.10 (d, CO, ^2J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.79 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 349.2143, found $[M\text{-}Na]^-$ *m/z* 349.2145.

Sodium methyl ibuprofenoyl phosphate (8f): Elution conditions: 20% MeCN/H_2O; white solid; yield (0.440 g, 23%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (d, $(C\underline{H}_3)_2CH$, ^3J(HH) = 6.5 Hz, 6H), 1.42 (d, $C\underline{H}_3CH$, ^3J(HH) = 7.0 Hz, 3H), 1.80-1.85 (m, $(CH_3)_2C\underline{H}$, 1H), 2.42 (d, $CHC\underline{H}_2$, ^3J(HH) = 7.0 Hz, 2H), 3.25 (d, $C\underline{H}_3OP$, ^3J(PH) = 11.0 Hz, 3H), 3.71 (q, $CH_3C\underline{H}$, ^3J(HH) = 7.0 Hz, 1H), 7.05 (d, ^3J(HH) = 8.0, ArH, 2H), 7.20 (d, ^3J(HH) = 8.0, ArH, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.28 ($\underline{C}H_3CH$), 22.50 ($\underline{C}H_3)_2CH$), 30.33 ($(CH_3)_2\underline{C}H$), 45.21($CH\underline{C}H_2$), 46.41 (d, $CH_3\underline{C}H$, ^3J (CCOP) = 5.0 Hz), 53.38 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 127.67 ($C_{aromatic}$), 129.43 ($C_{aromatic}$), 137.66 ($C_{aromatic}$), 140.62 ($C_{aromatic}$), 173.55 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5

MHz, $CDCl_3$) δ –5.77 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 299.1048, found $[M\text{-}Na]^-$ *m/z* 299.1044.

Sodium methyl fenoprofenoyl phosphate (8g): Elution conditions: 5–10% MeCN/H_2O; white solid; yield (0.554 g, 26%); ^{1}H NMR (500 MHz, D_2O) δ 1.53 (d, C$\underline{H}_3$CH, ^3J(HH) = 7.0 Hz, 3H), 3.52 (d, C$\underline{H}_3$OP, ^3J(PH) = 11.5 Hz, 3H), 3.94 (q, $CH_3C\underline{H}$, ^3J(HH) = 7.0 Hz, 1H), 7.05-7.14 (m, ArH, 4H), 7.22-7.29 (m, ArH, 2H), 7.45-7.50 (m, ArH, 3H); ^{13}C NMR (125 MHz, D_2O) δ 17.0 (CH_3), 46.06 (d, $CH_3\underline{C}H$, ^3J (CCOP) = 5.0 Hz), 53.63 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 117.89 ($C_{aromatic}$), 118.05 ($C_{aromatic}$), 119.02 ($C_{aromatic}$), 123.02 ($C_{aromatic}$), 124.03 ($C_{aromatic}$), 130.2 ($C_{aromatic}$), 130.51 ($C_{aromatic}$), 141.94 ($C_{aromatic}$), 156.58 ($C_{aromatic}$), 157.20 ($C_{aromatic}$), 172.52 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –5.99 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 335.0684, found $[M\text{-}Na]^-$ *m/z* 335.0688.

Sodium methyl 3-phenylpropanoyl phosphate (8h): Elution conditions: 0–5% MeCN/H_2O; white solid; yield (0.570 g, 36%); ^{1}H NMR (500 MHz, D_2O) δ 2.87 (t, ArC$\underline{H}_2CH_2$, ^3J(HH) = 7.5 Hz, 2H), 3.03 (t, C$\underline{H}_2$CO, ^3J(HH) = 7.5 Hz, 2H), 3.61 (d, C$\underline{H}_3$OP, ^3J(PH) = 11.5 Hz, 3H), 7.33-7.45 (m, ArH, 5H); ^{13}C NMR (125 MHz, D_2O) δ 29.97 (Ar$\underline{C}H_2CH_2$), 36.29 (d, ($\underline{C}H_2$CO), ^3J (CCOP) = 5.0 Hz), 53.65 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 126.53 ($C_{aromatic}$), 128.44 ($C_{aromatic}$), 128.79 ($C_{aromatic}$), 140.52 ($C_{aromatic}$), 171.66 (d, CO, ^2J (COP) = 10 Hz); ^{31}P NMR (202.5 MHz, D_2O) δ –6.05 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 243.0422, found $[M\text{-}Na]^-$ *m/z* 243.0420.

Sodium methyl *trans*-cinnamoyl phosphate (8i): Elution conditions**:** 0–5% MeCN/H_2O; white solid; yield (0.50 g, 32%); ^{1}H NMR (300 MHz, D_2O) δ 3.77 (d, C$\underline{H}_3$OP, ^3J(PH) = 11.4 Hz, 3H), 6.50 (dd, ArCH=C$\underline{H}$, ^3J(HH) = 15.9 Hz, ^4J (PH) = 2.1 Hz, 1H), 7.55 (m, ArH, 5H), 7.90 (d, ArC$\underline{H}$=CH, ^3J(HH) = 16.0 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 53.77 (d, $\underline{C}H_3OP$, ^2J (COP) = 6.25 Hz), 117.06 (d, ArCH=$\underline{C}$H, ^2J (COP) = 7.5 Hz), 128.64 ($C_{aromatic}$), 129.15 ($C_{aromatic}$), 131.25 ($C_{aromatic}$), 133.82 ($C_{aromatic}$), 148.34 (Ar$\underline{C}$H=CH), 164.68 (d, CO, ^2J (COP) = 8.75 Hz); ^{31}P NMR (202.5 MHz, $CDCl_3$) δ –5.52 ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 241.0265, found $[M\text{-}Na]^-$ *m/z* 241.0261.

General procedure for *S*-acylation of 3-mercaptopropanoic acid (1) and 4-mercaptobenzoic acid (3) in aqueous conditions with sodium methyl acetyl phosphate

To a solution of 3-mercaptopropanoic acid (0.106 mg, 1 mmol) or 4-mercaptobenzoic (0.154 g, 1 mmol) in borate buffer (pH 9.0, 20 mL) was added sodium methyl acetyl phosphate (0.176 g, 1 mmol) and the pH of solution was adjusted to 9.0 by addition of 6 M NaOH. The reaction mixture was stirred for 3 h, then acidified to pH 2.0 with 2 M HCl and the product was extracted using ethyl acetate (3 × 20 mL). After removal of the solvent *in vacuo*, the crude product was dissolved in 10 mL chloroform, filtered to remove insoluble material, and the filtrate was evaporated *in vacuo* to give pure product.

3-(Acetylthio)propanoic acid[11] **(2)**: Light yellow oil; yield (0.107 g, 72%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.33 (s, $COCH_3$, 3H), 2.67 (t, $C\underline{H}_2CH_2S$, ^{3}J(HH) = 7.0 Hz, 3H), 3.10 (t, CH_2S, ^{3}J(HH) = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.96 ($\underline{C}H_2CH_2S$), 30.50 (CH_2S), 34.16 ($COCH_3$), 176.43 (COOH), 195.70 (SCO) ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 147.0116 found $[M\text{-}Na]^-$ *m/z* 147.0117.

4-(Acetylthio)benzoic acid[12] **(4)**: White solid; yield (0.091 g, 65%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.46 (s, $COCH_3$, 3H), 7.53 (d, ArH, ^{3}J(HH) = 8.0, 2H), 8.13 (d, ArH, ^{3}J(HH) = 8.0, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.61 ($COCH_3$), 130.13 ($C_{aromatic}$), 130.84 ($C_{aromatic}$), 134.17 ($C_{aromatic}$), 134.77 ($C_{aromatic}$), 170.74 (COOH), 192.63 (SCO) ppm; HR-ESIMS calcd. $[M\text{-}Na]^-$ *m/z* 195.0116, found $[M\text{-}Na]^-$ *m/z* 195.0121.

General procedures for *S*-acylation of coenzyme A (CoA) by sodium methyl acyl phosphates under aqueous conditions

Sodium methyl acyl phosphates (**8a**, 0.18 mmol and **8b** – **8i**, 0.06 mmol) were added to a solution of CoA (0.05 g, 0.06 mmol) in borate buffer (pH 9.0, 15 mL) and the pH of solution was adjusted to 9.0 using 6 M NaOH. The mixture was stirred at room temperature for 6 h for **8a** and 24 h for **8b** – **8i**. The reaction was then quenched by the addition of 1 M HCl until the pH of the solution was 2.0. Free acid (formed due to slow hydrolysis of the methyl acyl phsphate) was removed from the reaction mixture using liquid-phase extraction with $CHCl_3$ (5 × 15 mL), and

the solvent was removed by lyophilization to give a white powder. CoA thioesters were purified using solid-phase extraction (SPE, Strata C18 columns, 5 g/20 mL, Phenomenex, Torrance, CA). After washing the SPE columns with MeCN and water (20 mL each), the aqueous phase (5 mL) was applied to the column and eluted consecutively with aqueous solutions containing 0%, 2%, 5%, 10%, 15%, 20%, 25%, 50%, and 100% MeCN (20 mL each). All the compounds were purified twice using the same protocol. Specific elution conditions for each compound (**10a** – **10i**) are given below. Solvent was removed from the aqueous fractions by lyophilization, giving white solids. The products (**10a** – **10i)** were stored at –20 °C.

Acetyl-CoA[1, 13, 14] **(10a)**: Elution conditions: 4 × 5 mL fractions of water were collected. The third fraction contained ~90% pure acetyl CoA: white solid (17.2 mg, 33%); ^{1}H NMR (300 MHz, D_2O) δ 0.82 (s, $H_{11''}$, 3H), 0.94 (s, $H_{10''}$, 3H), 2.39 (s, $COCH_3$, 3H), 2.47 (t, $H_{6''}$, J = 6.6 Hz, 2H), 3.01 (t, $H_{9''}$, J = 6.6 Hz, 2H), 3.37 (t, $H_{8''}$, J = 6.3 Hz, 2H), 3.49 (t, $H_{5''}$, J = 6.3 Hz, 2H), 3.60-3.63 (m, $H_{1''}$, 1H), 3.87-3.91 (m, $H_{1''}$, 1H), 4.01 (s, $H_{3''}$, 1H), 4.29 (m, $H_{5'}$, 2H), 4.64 (s, $H_{4'}$, 1H), 4.85-4.94 (m, $H_{2'}$ and $H_{3'}$, 2H,), 6.23 (d, $H_{1'}$, J = 6.3 Hz, 1H), 8.35 (s, H_2, 1H), 8.62 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 403.5550, found $[M\text{-}2H]^{2-}$ m/z 403.5560.

Butyryl-CoA (10b): Elution conditions: 0 – 2% MeCN/H_2O; white solid (10 mg, 19%); ^{1}H NMR (300 MHz, D_2O) δ 0.93 (s, $H_{11''}$, 3H), 0.95 (t, $C\underline{H}_3CH_2$, J = 7.2 Hz, 3H), 0.99 (s, $H_{10''}$, 3H), 1.66 (m, $CH_3C\underline{H}_2$, 2H), 2.49 (t, $H_{6''}$, J = 6.6 Hz, 2H), 2.63 (t, $C\underline{H}_2CO$, J = 7.2 Hz, 2H), 3.05 (t, $H_{9''}$, J = 6.3 Hz, 2H), 3.39 (t, $H_{8''}$, J = 6.3 Hz, 2H), 3.51 (t, $H_{5''}$, J = 6.3 Hz, 2H), 3.66-3.71 (m, $H_{1''}$, 1H), 3.88-3.95 (m, $H_{1''}$, 1H), 4.08 (s, $H_{3''}$, 1H), 4.33 (m, $H_{5'}$, 2H), 4.66 (s, $H_{4'}$, 1H), 4.94-4.96 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.27 (d, $H_{1'}$, J = 5.4 Hz, 1H), 8.50 (s, H_2, 1H), 8.71 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 417.5707, found $[M\text{-}2H]^{2-}$ m/z 417.5709.

Octanoyl-CoA (10d): Elution conditions: 2 – 5% MeCN/H_2O; white solid (14.8 mg, 26%); ^{1}H NMR (300 MHz, D_2O) δ 0.78 (s, $H_{11''}$, 3H), 0.87 (t, $C\underline{H}_3CH_2$, J = 7.2 Hz, 3H), 0.91 (s, $H_{10''}$, 3H), 1.26 (m, $CH_3(C\underline{H}_2)_4$, 8H), 1.61 (m, $(CH_2)_4C\underline{H}_2$, 2H), 2.46 (t, $H_{6''}$, J = 6.6 Hz, 2H), 2.62 (t, $C\underline{H}_2CO$, J = 7.2 Hz, 2H), 3.03 (t, $H_{9''}$, J = 6.3 Hz, 2H), 3.37 (t, $H_{8''}$, J = 6.3 Hz, 2H), 3.49 (t, $H_{5''}$, J = 6.3 Hz, 2H), 3.56-3.61 (m, $H_{1''}$, 1H), 3.84-3.89 (m, $H_{1''}$, 1H), 4.06 (s, $H_{3''}$, 1H), 4.27 (m, $H_{5'}$, 2H), 4.60

(s, $H_{4'}$, 1H), 4.83-4.86 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.20 (d, $H_{1'}$, J = 6.9 Hz, 1H), 8.31 (s, H_2, 1H), 8.61 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 445.6020, found $[M\text{-}2H]^{2-}$ m/z 445.5997.

Ibuprofenoyl-CoA[15] **(10f)**: Elution conditions: 5 – 10% MeCN/H_2O; white solid (15 mg, 24%); ^{1}H NMR (300 MHz, D_2O) δ 0.75 (s, $H_{11''}$, 3H), 0.86 (d, $(C\underline{H}_3)_2CH$, ^3J(HH) = 6.6 Hz, 6H), 0.90 (s, $H_{10''}$, 3H), 1.50 (d, $C\underline{H}_3CH$, ^3J(HH) = 7.2 Hz, 3H), 1.74-1.85 (m, $(CH_3)_2C\underline{H}$, 1H), 2.27 (t, $H_{6''}$, J = 6.0 Hz, 2H), 2.45 (d, $CHC\underline{H}_2$, ^3J(HH) = 7.2 Hz, 2H), 2.91-2.99 (m, $H_{9''}$, 1H), 3.03-3.12 (m, $H_{9''}$, 1H), 3.30-3.37 (m, $H_{5''}$ and $H_{8''}$, 4H), 3.55-3.60 (m, $H_{1''}$, 1H), 3.84-3.89 (m, $H_{1''}$, 1H), 4.04 (q, $CH_3C\underline{H}$, ^3J(HH) = 6.9 Hz, 1H), 4.06 (s, $H_{3''}$, 1H), 4.27 (m, $H_{5'}$, 2H), 4.61 (s, $H_{4'}$, 1H), 4.81-4.86 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.19 (d, $H_{1'}$, J = 6.9 Hz, 1H), 7.19 (d, ArH, ^3J(HH) = 8.1, 2H), 7.26 (d, ArH, ^3J(HH) = 8.1, 2H), 8.27 (s, H_2, 1H), 8.60 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 476.6098, found $[M\text{-}2H]^{2-}$ m/z 476.6094.

Fenoprofenoyl-CoA[15] **(10g)**: Elution conditions: 5% MeCN/H_2O; white solid (25.4 mg, 40%); ^{1}H NMR (300 MHz, D_2O) δ 0.73 (s, $H_{11''}$, 3H), 0.89 (s, $H_{10''}$, 3H), 1.47 (d, $C\underline{H}_3CH$, ^3J(HH) = 6.9 Hz, 3H), 2.29 (t, $H_{6''}$, J = 6.9 Hz, 2H), 2.90-3.10 (m, $H_{9''}$, 2H), 3.28-3.38 (m, $H_{5''}$ & $H_{8''}$, 4H), 3.54-3.59 (m, $H_{1''}$, 1H), 3.83-3.88 (m, $H_{1''}$, 1H), 3.98 (q, $CH_3C\underline{H}$, ^3J(HH) = 6.9 Hz, 1H), 4.03 (s, $H_{3''}$, 1H), 4.27 (m, $H_{5'}$, 2H), 4.59 (s, $H_{4'}$, 1H), 4.82-4.85 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.17 (d, $H_{1'}$, J = 6.6 Hz, 1H), 6.96-7.04 (m, ArH, 4H), 7.11-7.23 (m, ArH, 2H), 7.35-7.45 (m, ArH, 3H), 8.23 (s, H_2, 1H), 8.57 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 494.5916, found $[M\text{-}2H]^{2-}$ m/z 494.5912.

3-Phenylpropanoyl-CoA[16] **(10h)**: Elution conditions: 2% MeCN/H_2O; white solid (15 mg, 26%); ^{1}H NMR (300 MHz, D_2O) δ 0.78 (s, $H_{11''}$, 3H), 0.92 (s, $H_{10''}$, 3H), 2.39 (t, $H_{6''}$, J = 6.6 Hz, 2H), 2.96-3.0 (m, $C\underline{H}_2C\underline{H}_2$ and $H_{9''}$, 6H), 3.29 (t, $H_{8''}$, J = 6.0 Hz, 2H), 3.45 (t, $H_{5''}$, J = 6.6 Hz, 2H), 3.57-3.60 (m, $H_{1''}$, 1H), 3.85-3.90 (m, $H_{1''}$, 1H), 4.06 (s, $H_{3''}$, 1H), 4.27 (m, $H_{5'}$, 2H), 4.60 (s, $H_{4'}$, 1H), 4.81-4.86 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.18 (d, $H_{1'}$, J = 6.6 Hz, 1H), 7.23-7.37 (m, ArH, 5H), 8.26 (s, H_2, 1H), 8.56 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M\text{-}2H]^{2-}$ m/z 448.5785, found $[M\text{-}2H]^{2-}$ m/z 448.5777.

***trans*-Cinnamoyl-CoA**[17] **(10i)**: Elution conditions: 10 – 20% MeCN/H_2O; white solid (17.4 mg, 30%); ^{1}H NMR (300 MHz, D_2O) δ 0.88 (s, $H_{11''}$, 3H), 0.99 (s, $H_{10''}$, 3H), 2.50 (t, $H_{6''}$, J = 6.3 Hz,

2H), 3.17 (t, $H_{9''}$, J = 6.6 Hz, 2H), 3.45-3.54 (m, $H_{5''}$ and $H_{8''}$, 4H), 3.65-3.70 (m, $H_{1''}$, 1H), 3.91-3.95 (m, $H_{1''}$, 1H), 4.08 (s, $H_{3''}$, 1H), 4.33 (m, $H_{5'}$, 2H), 4.64 (s, $H_{4'}$, 1H), 4.89-4.94 (m, $H_{2'}$ and $H_{3'}$, 2H), 6.15 (d, $H_{1'}$, J = 6.0 Hz, 1H), 6.79 (d, ArCH=C<u>H</u>, ^{3}J(HH) = 15.9 Hz, 1H), 7.42-7.55 (m, ArH and ArC<u>H</u>=CH, 6H), 8.34 (s, H_2, 1H), 8.64 (s, H_8, 1H) ppm; HR-ESIMS calcd. $[M-2H]^{2-}$ m/z 447.5707, found $[M-2H]^{2-}$ m/z 447.5713.

Figure 1S: ^{1}H NMR spectrum of butyryl chloride (**6b**)

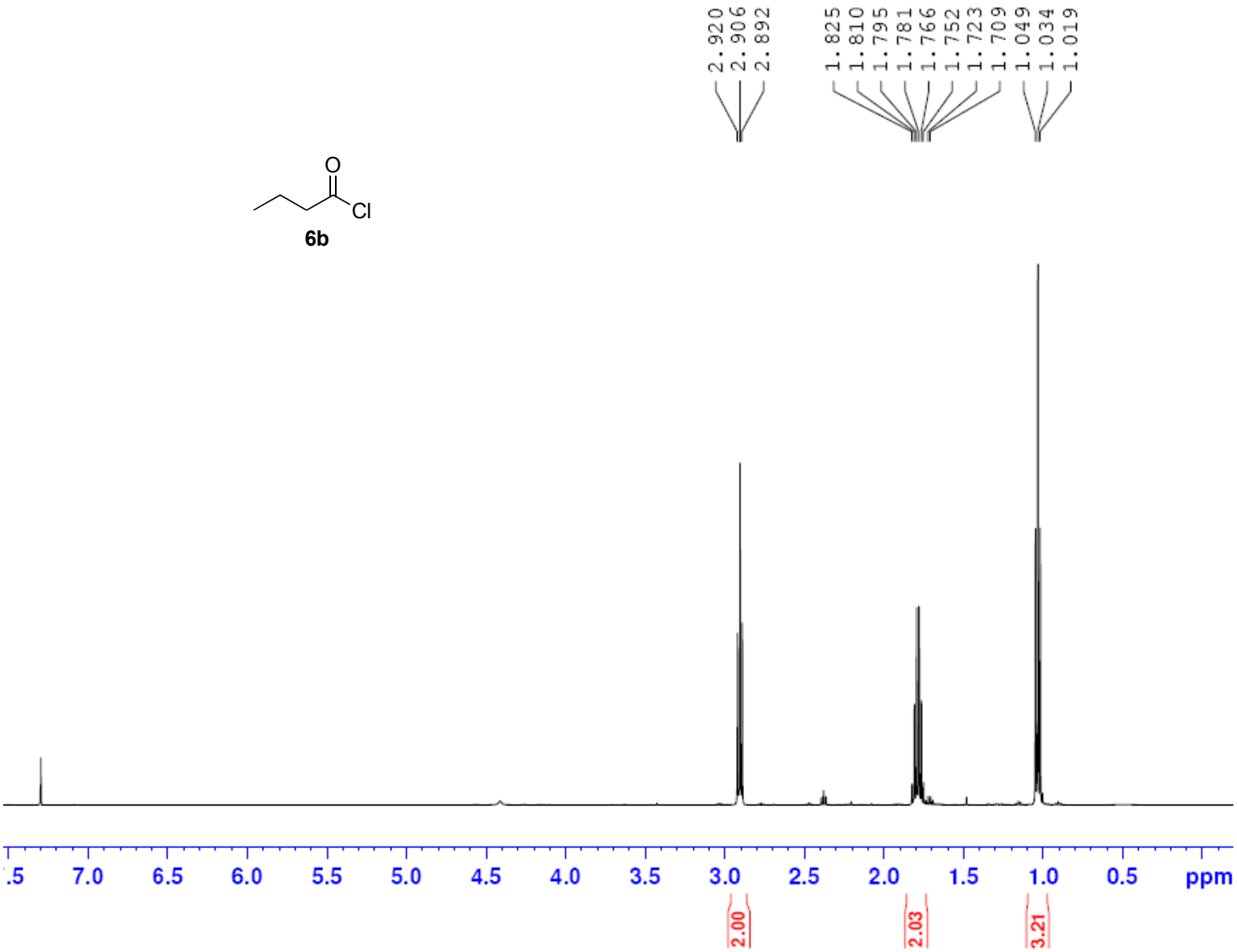


Figure 2S: ^{13}C NMR spectrum of butyryl chloride (**6b**)

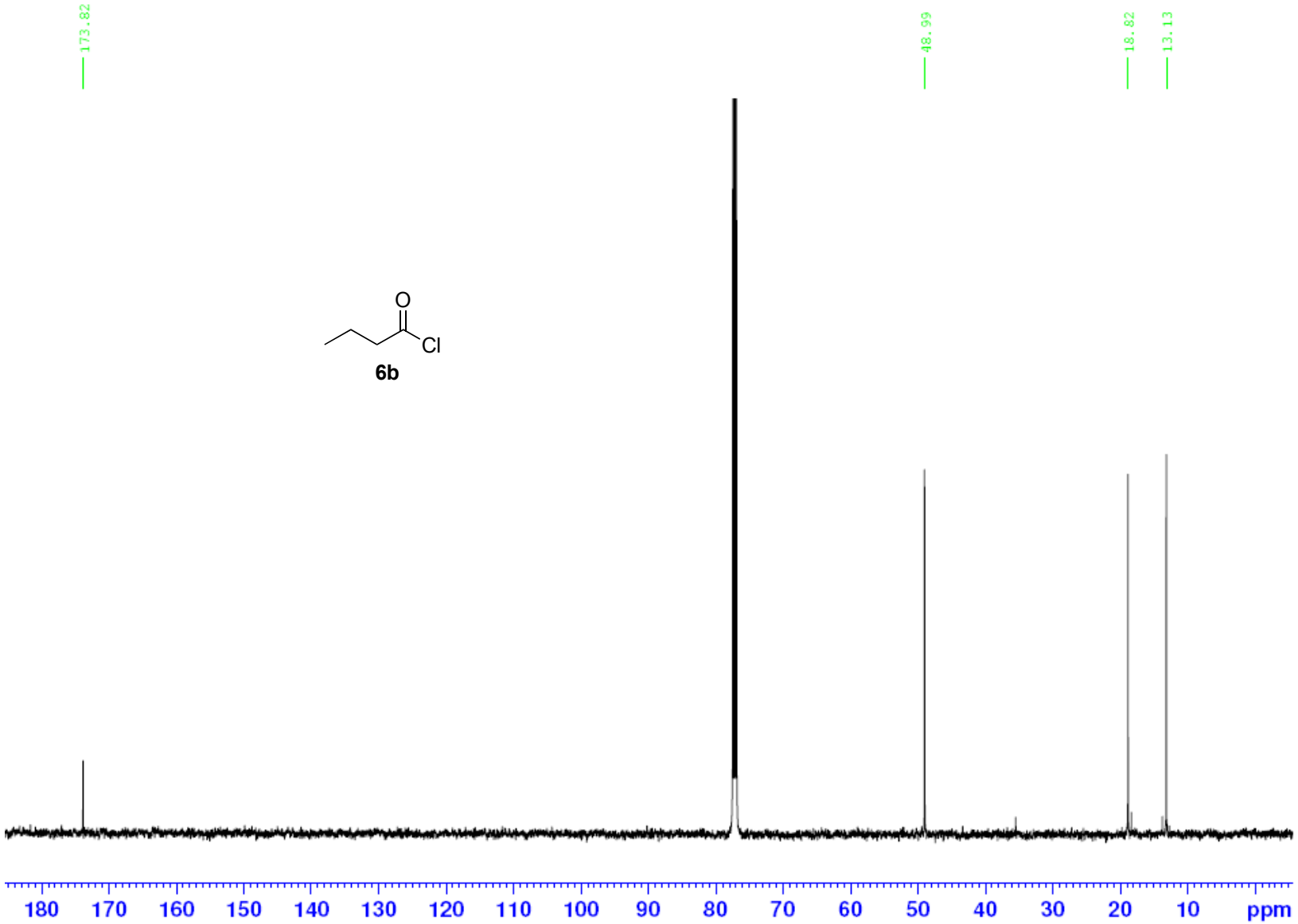


Figure 3S: ^{1}H NMR of spectrum isovaleryl chloride (**6c**)

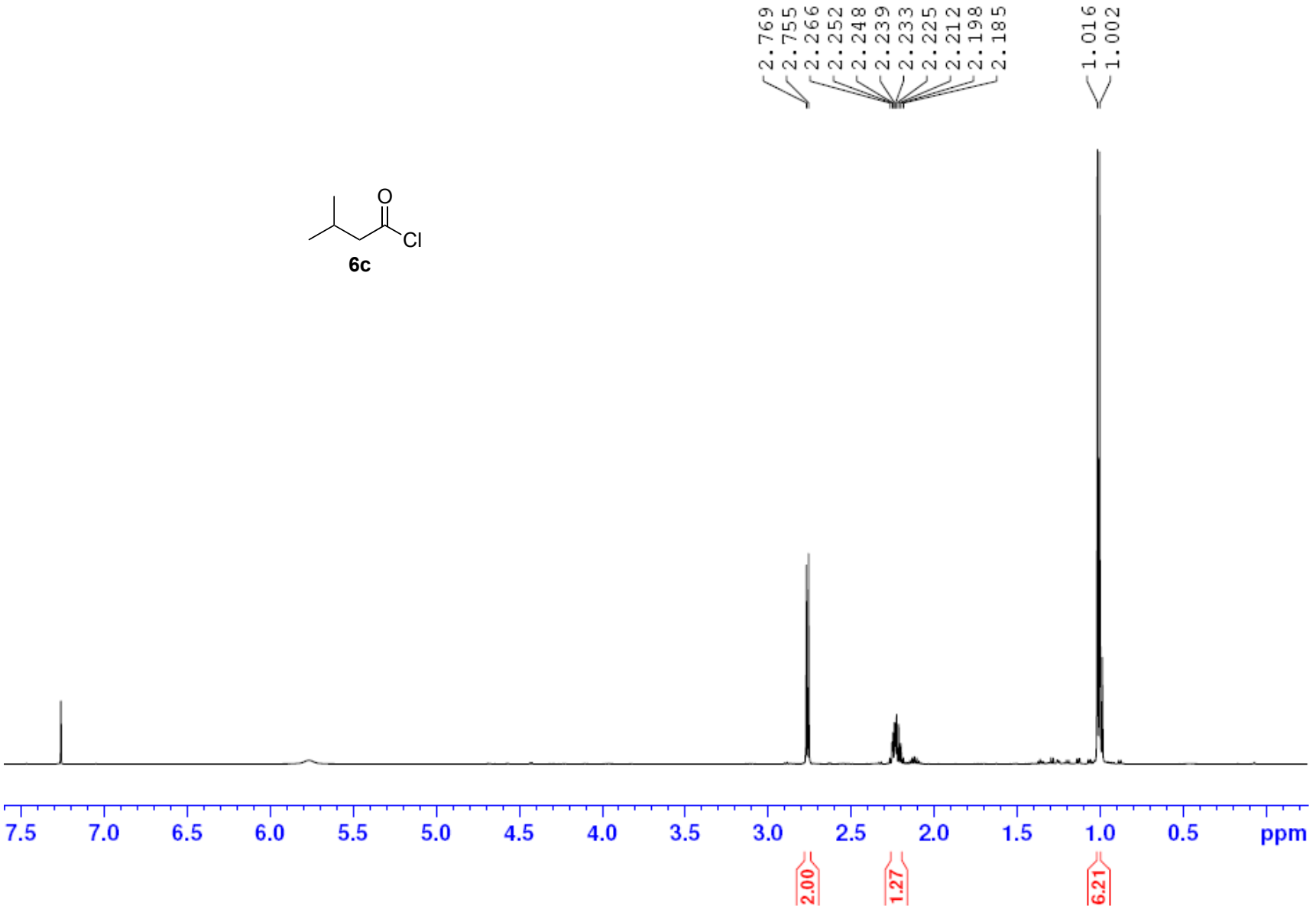


Figure 4S: ^{13}C NMR spectrum of isovaleryl chloride (**6c**)

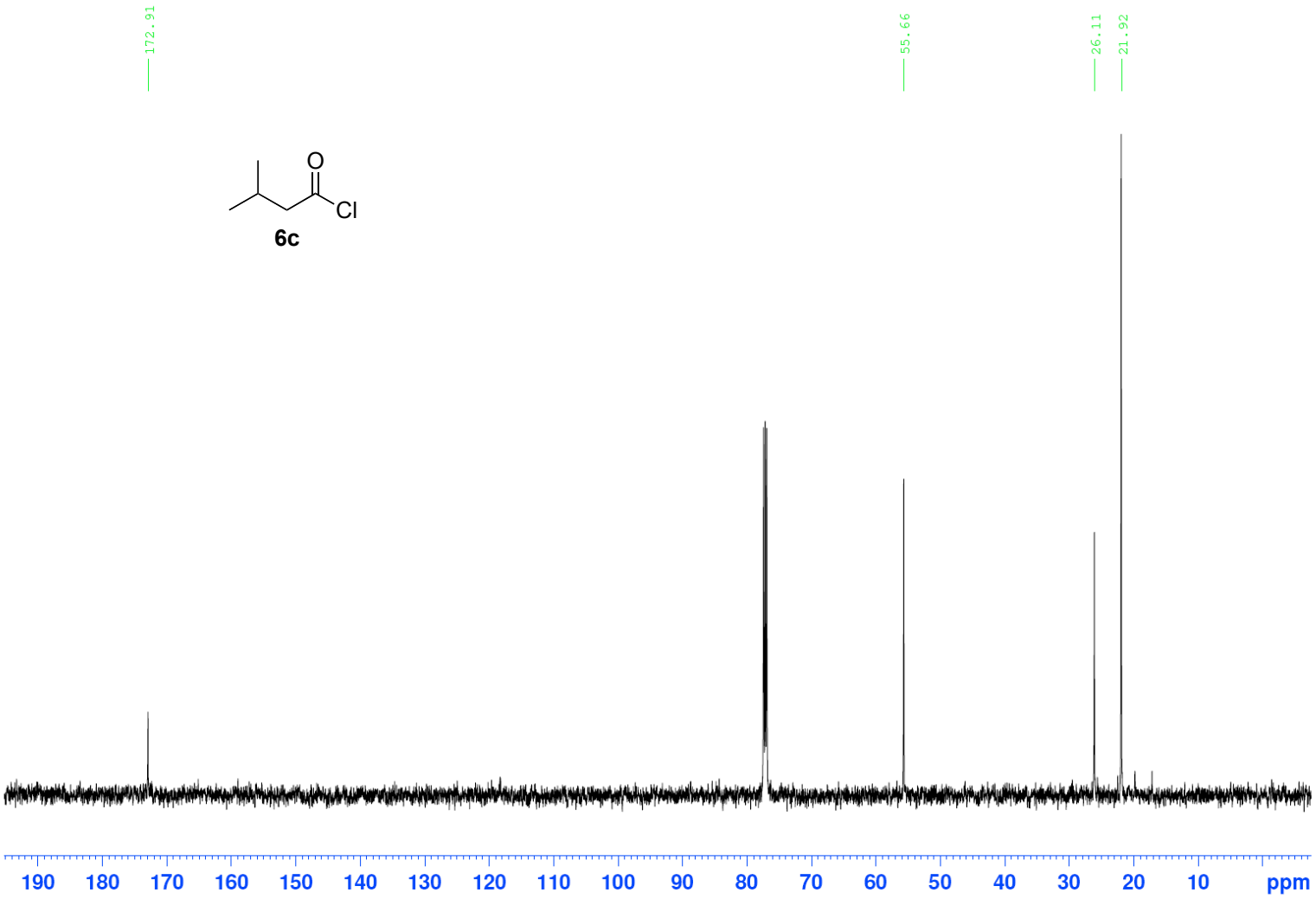


Figure 5S: ^{1}H NMR spectrum of octanoyl chloride (**6d**)

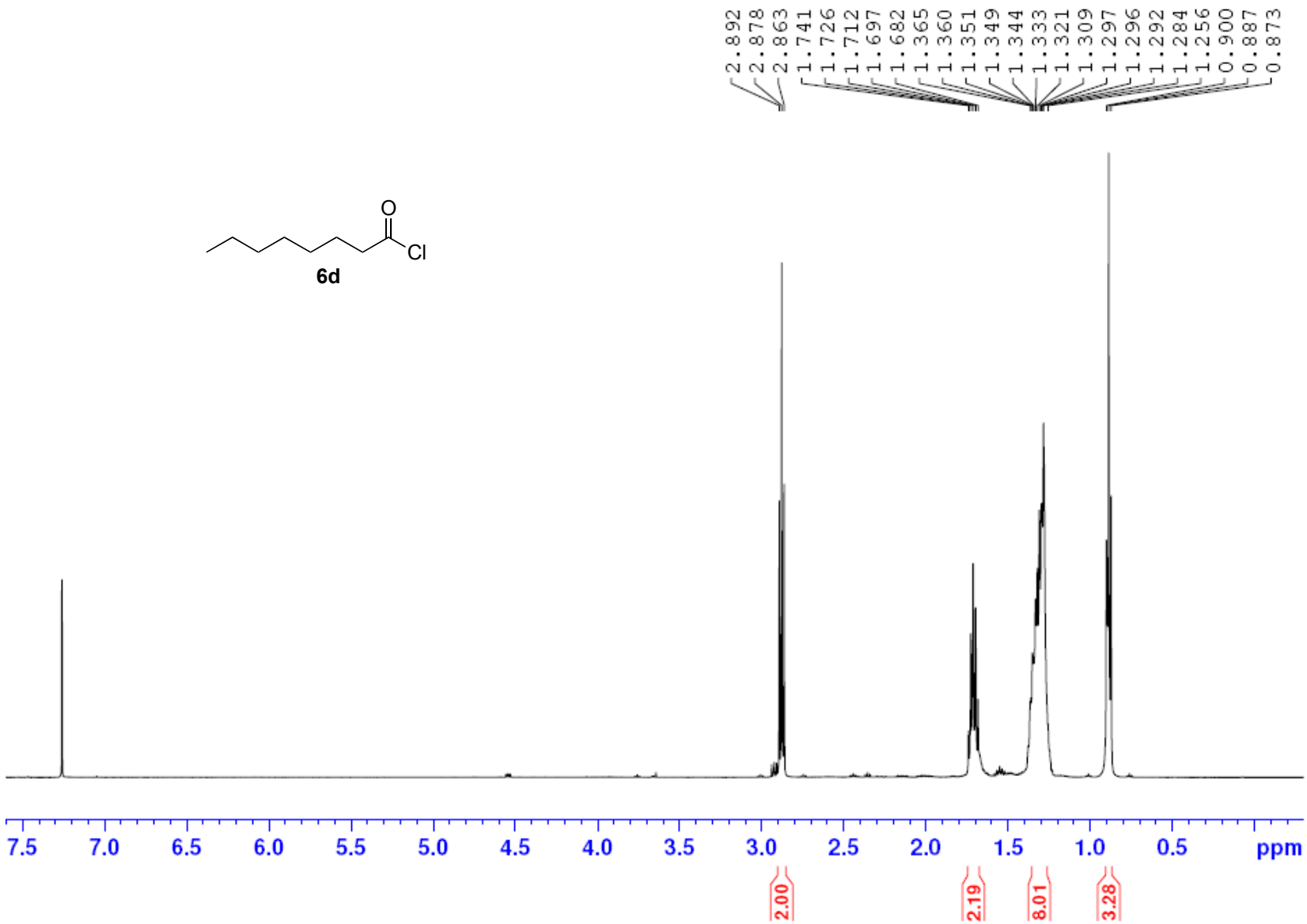


Figure 6S: ^{13}C NMR spectrum of octanoyl chloride **(6d)**

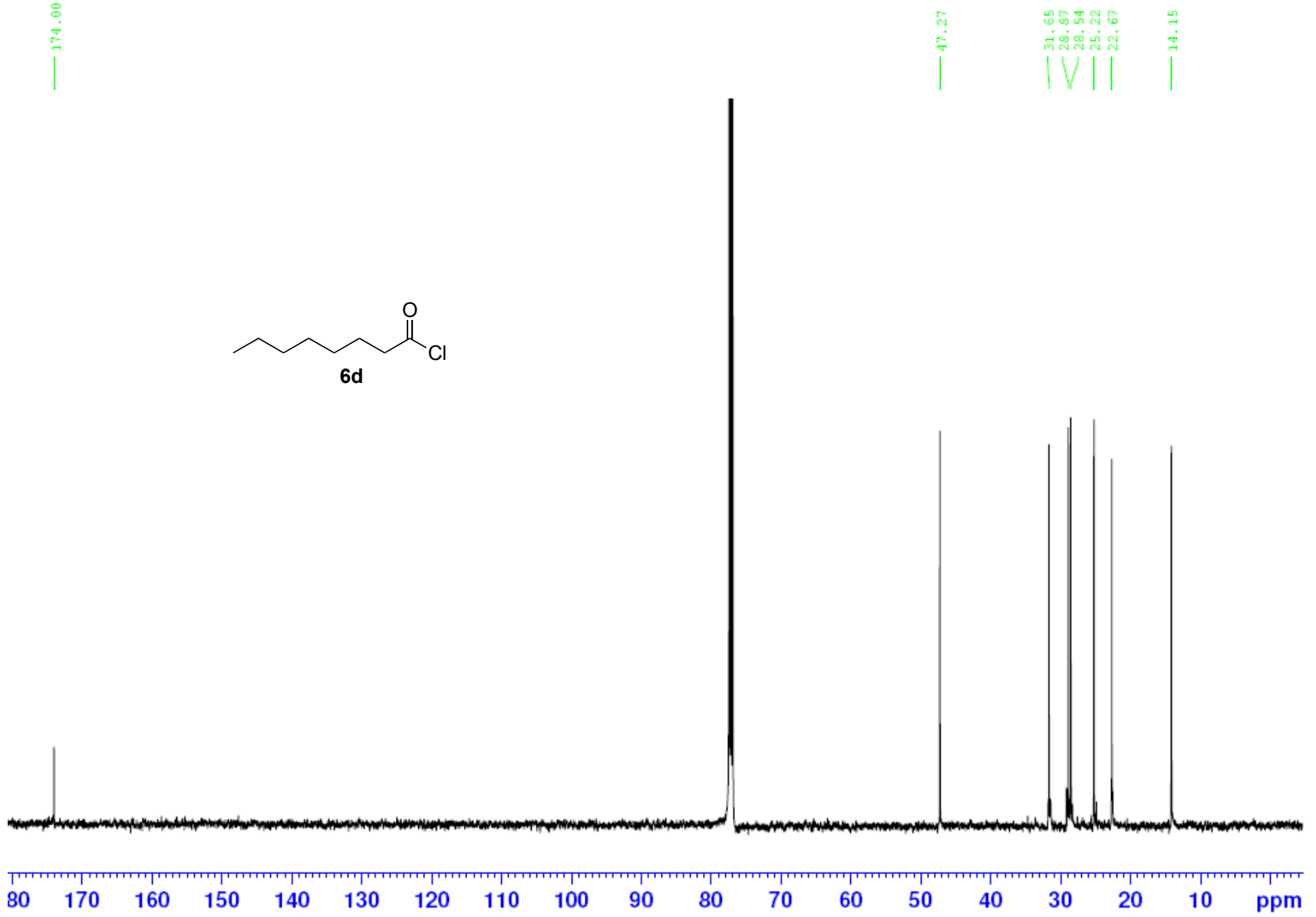


Figure 7S: ^{1}H NMR spectrum of palmitoyl chloride **(6e)**

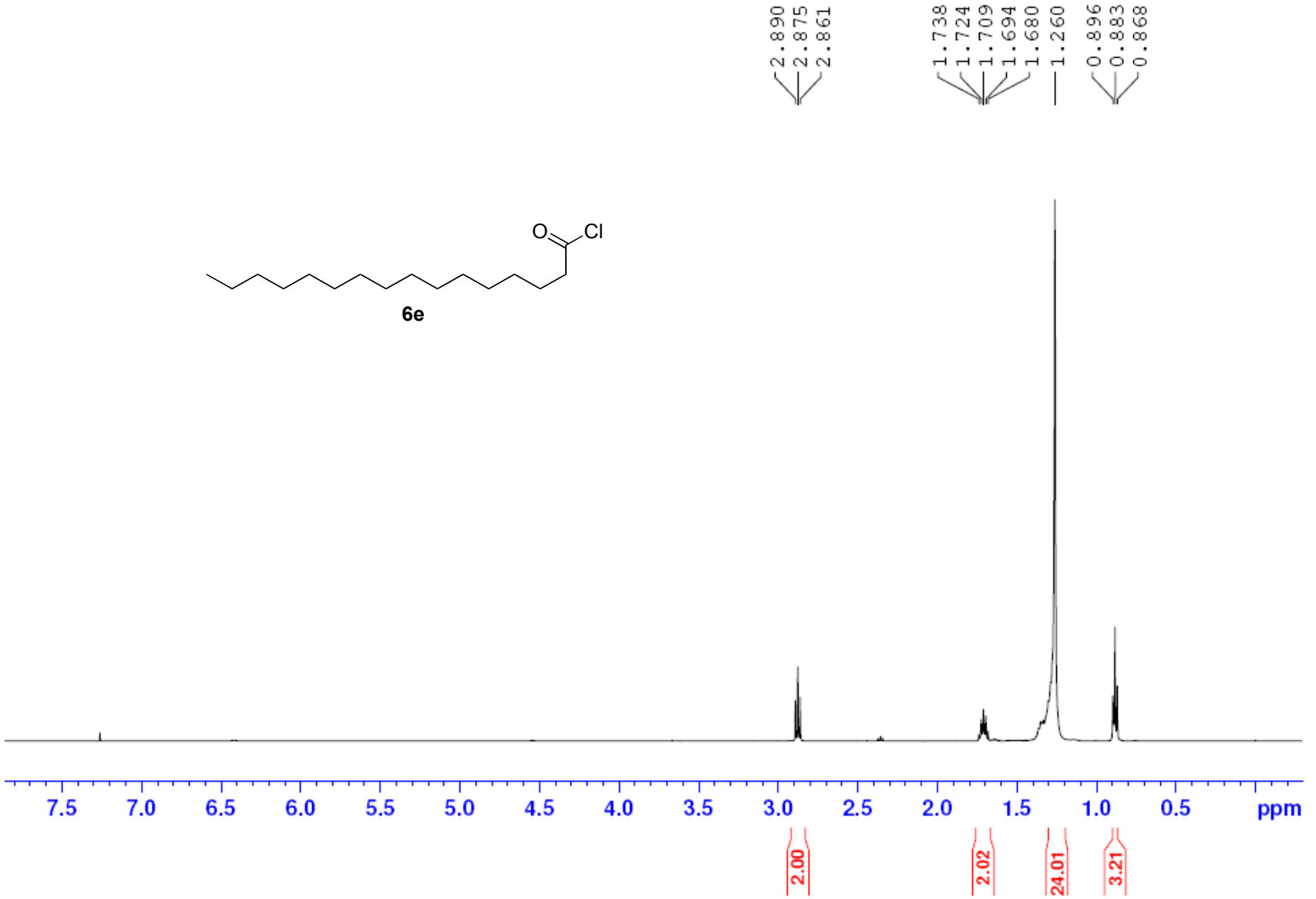


Figure 8S: ^{13}C NMR spectrum of palmitoyl chloride **(6e)**

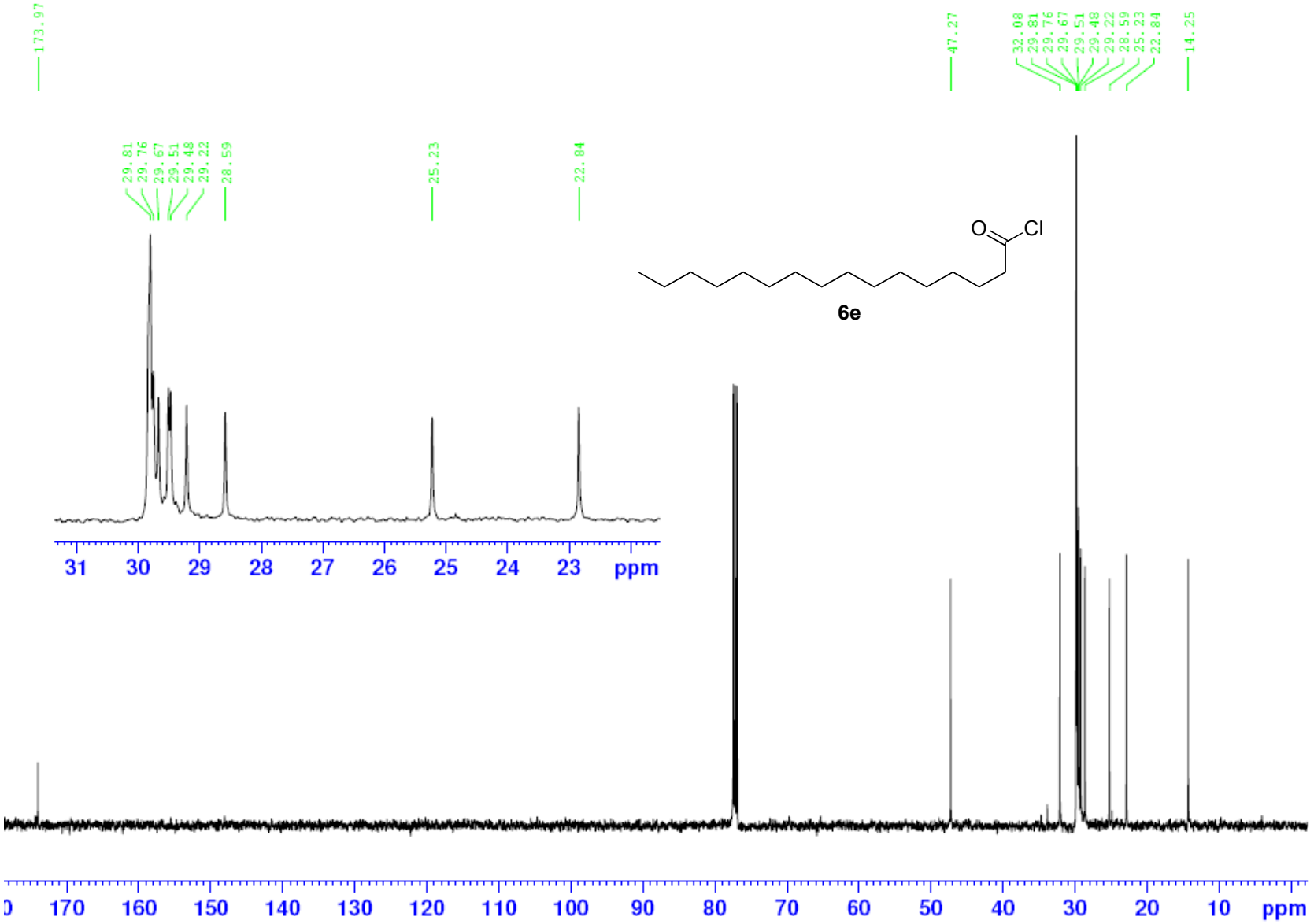


Figure 9S: ^{1}H NMR spectrum of ibuprofenoyl chloride **(6f)**

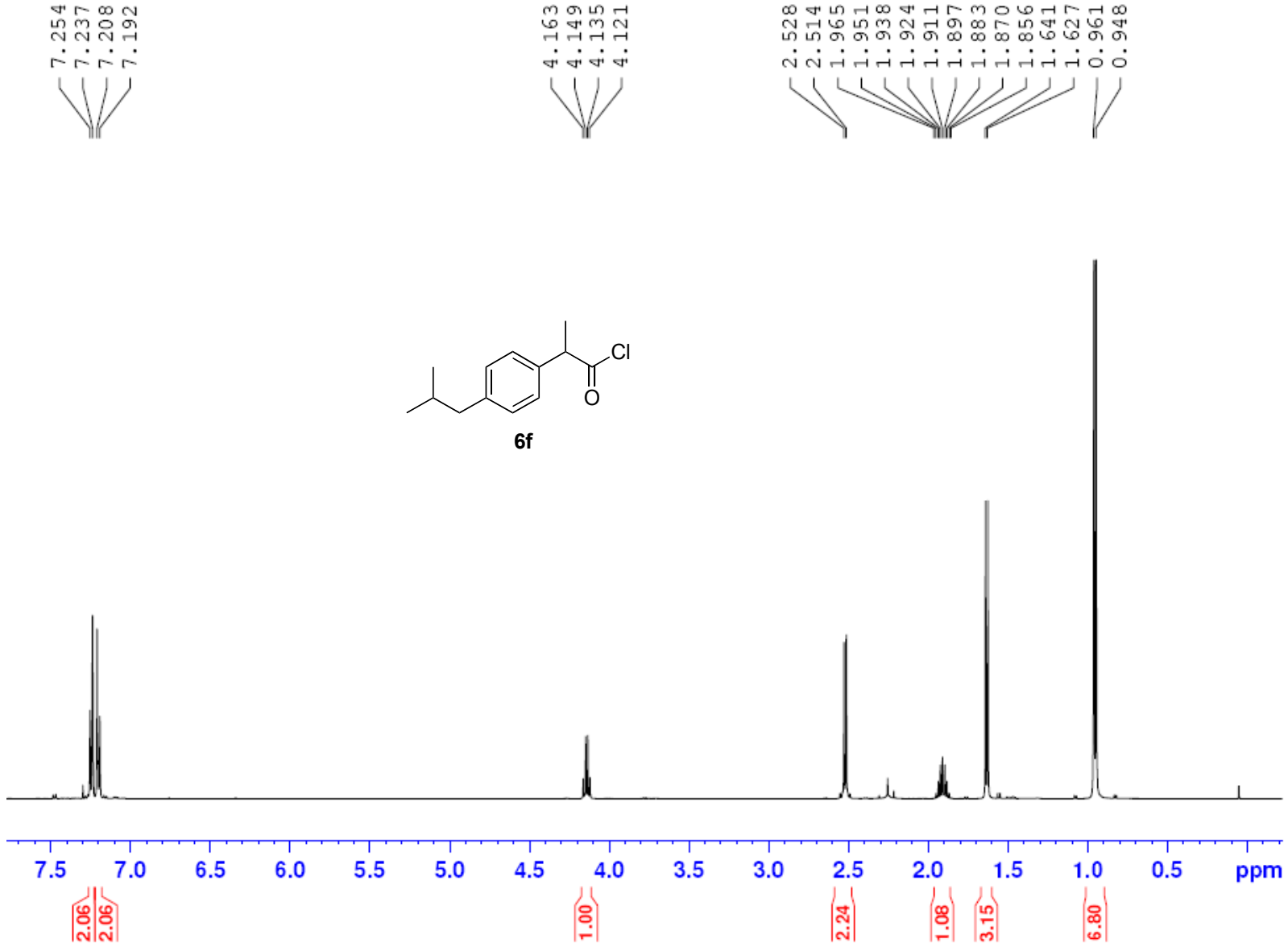


Figure 10S: ^{13}C NMR spectrum of ibuprofenoyl chloride **(6f)**

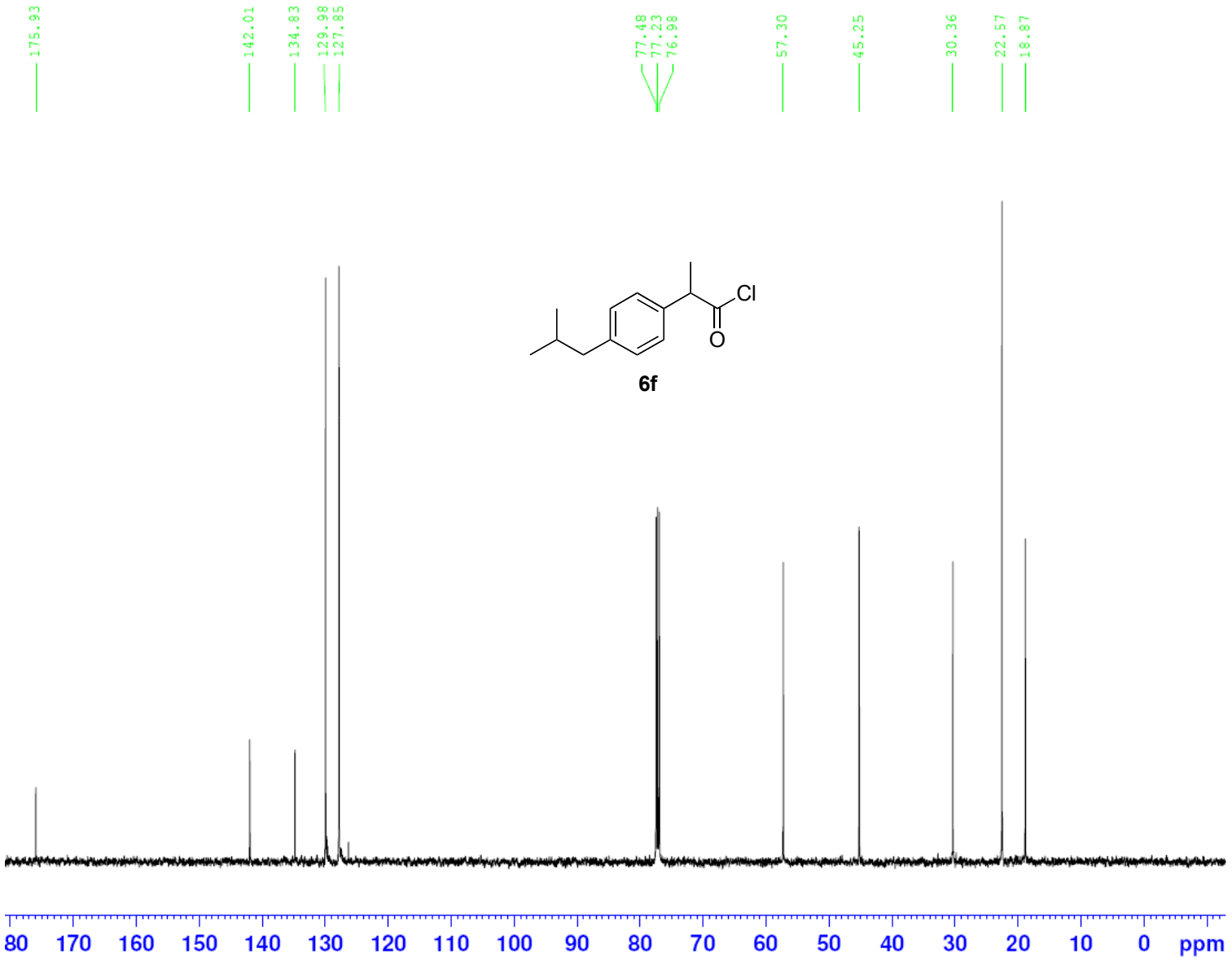


Figure 11S: ^{1}H NMR spectrum fenoprofenoyl chloride **(6g)**

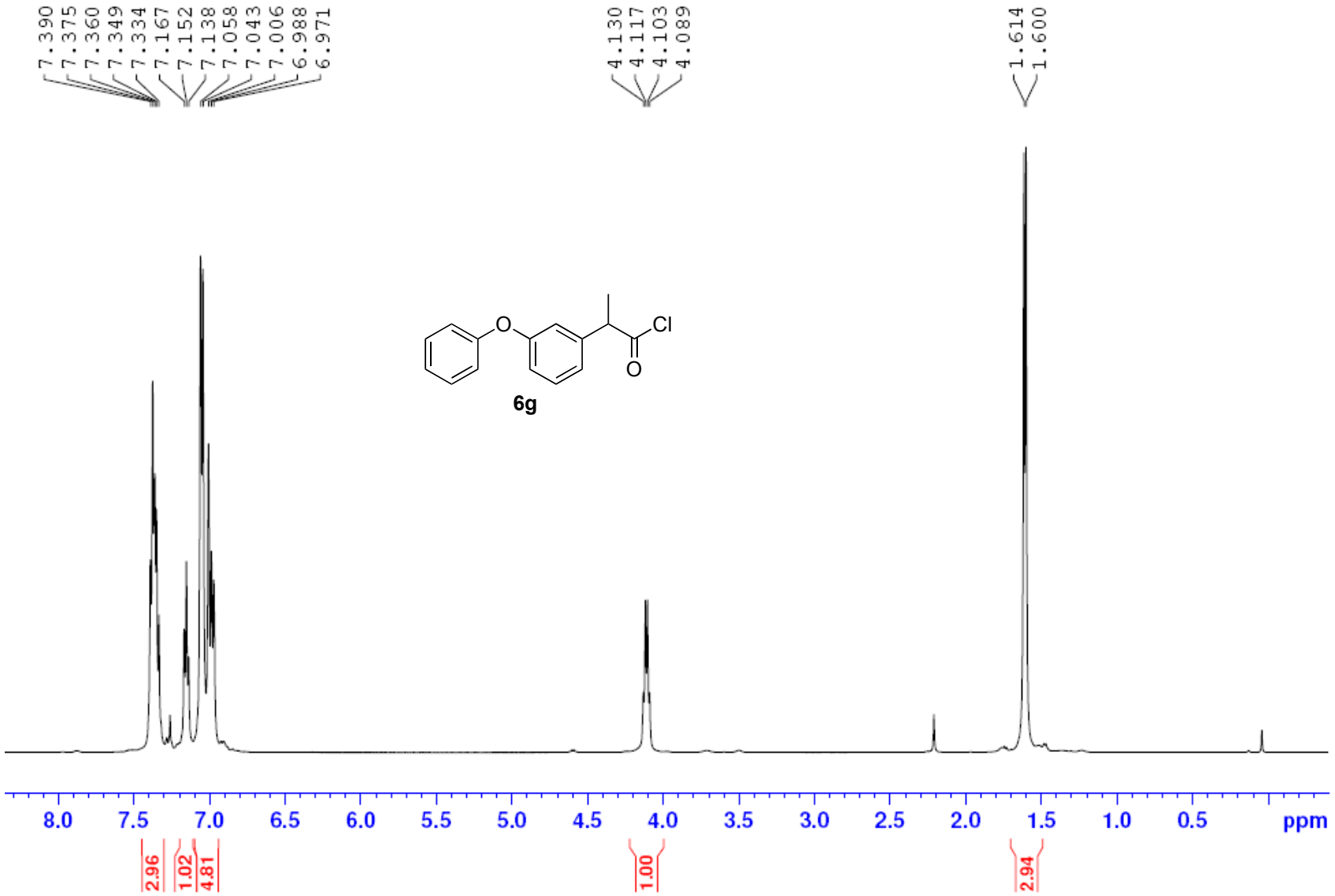


Figure 12S: ^{13}C NMR spectrum fenoprofenoyl chloride **(6g)**

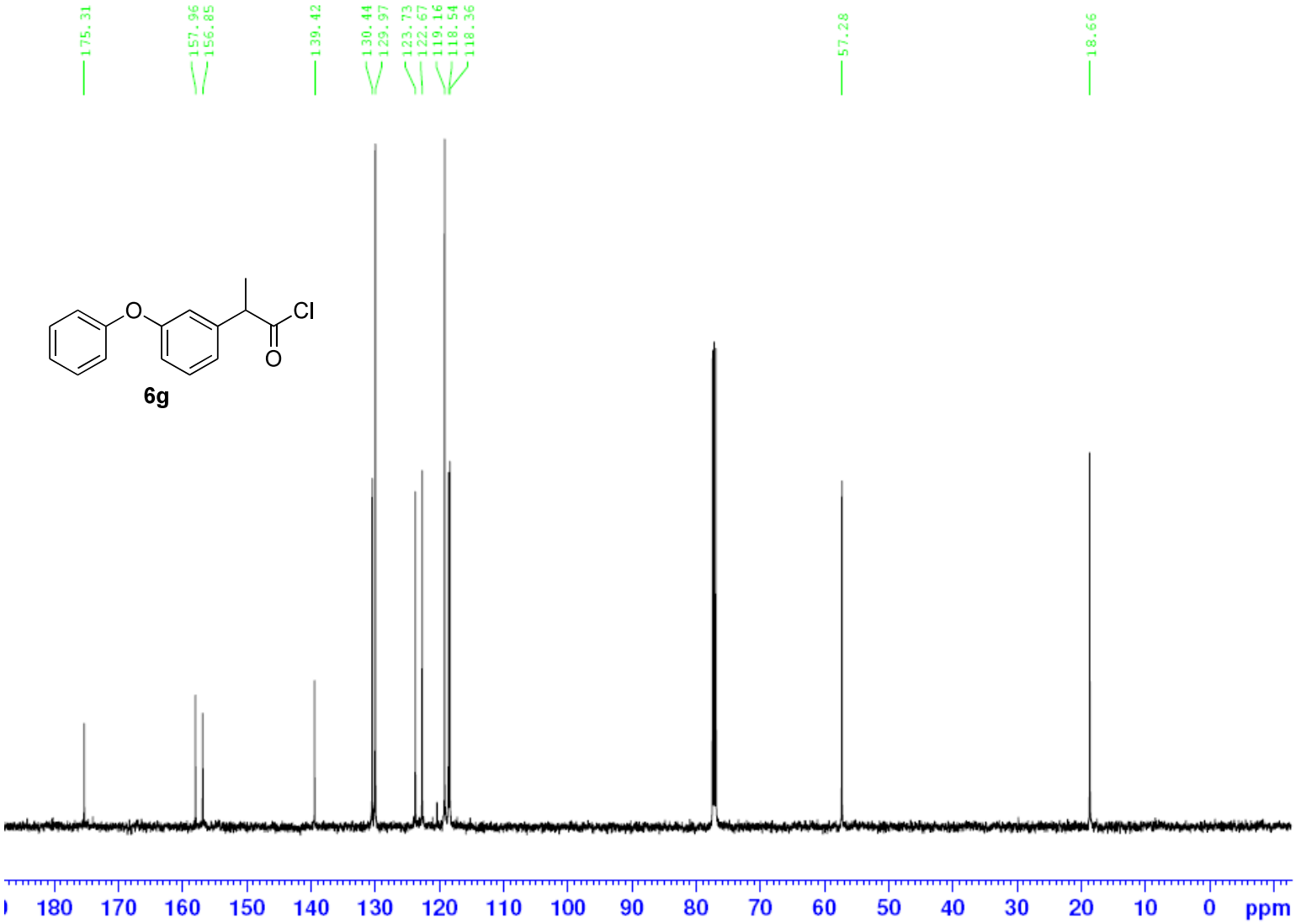


Figure 13S: ^{1}H NMR spectrum of 3-phenylpropanoyl chloride **(6h)**

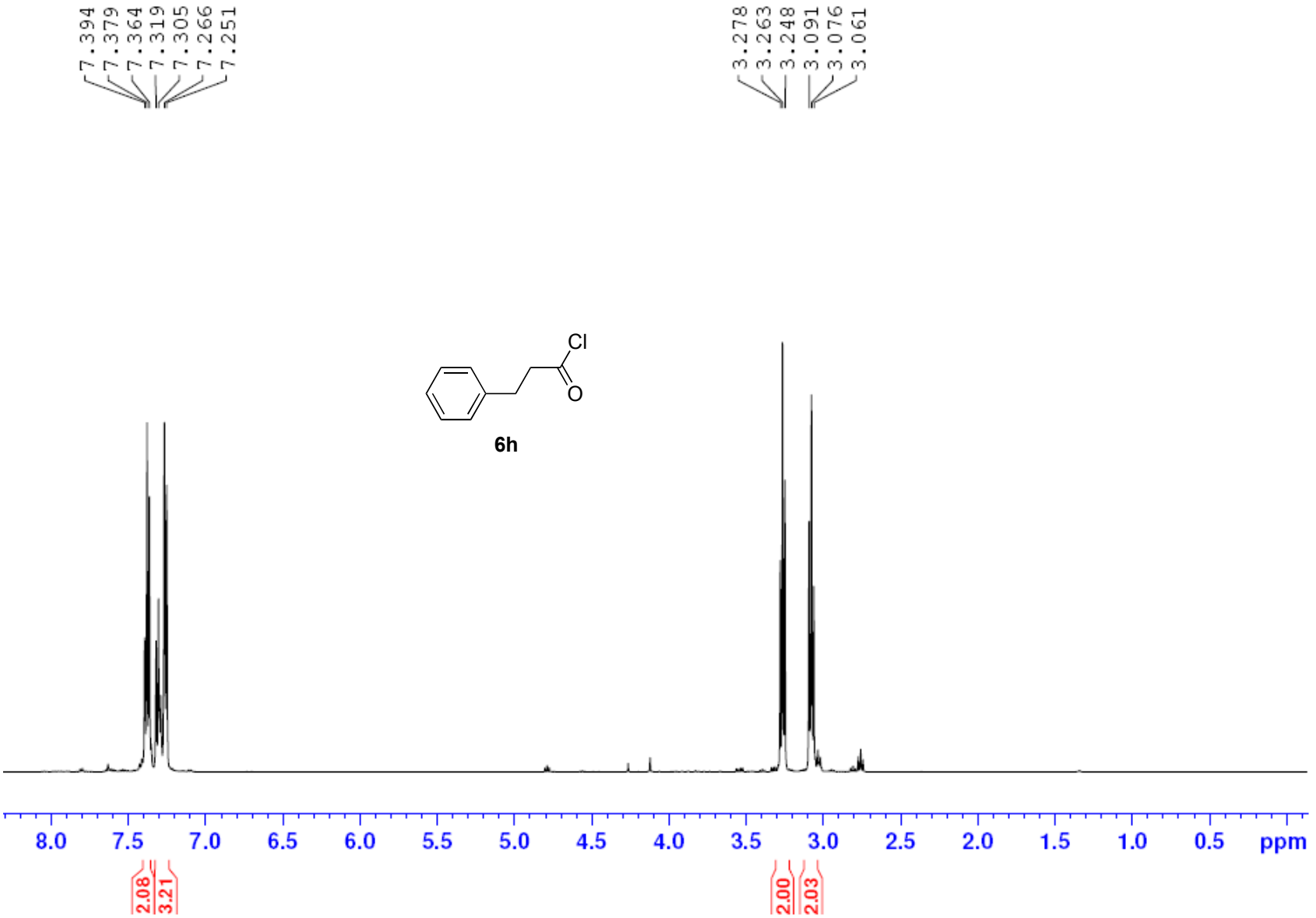


Figure 14S: ^{13}C NMR spectrum of 3-phenylpropanoyl chloride **(6h)**

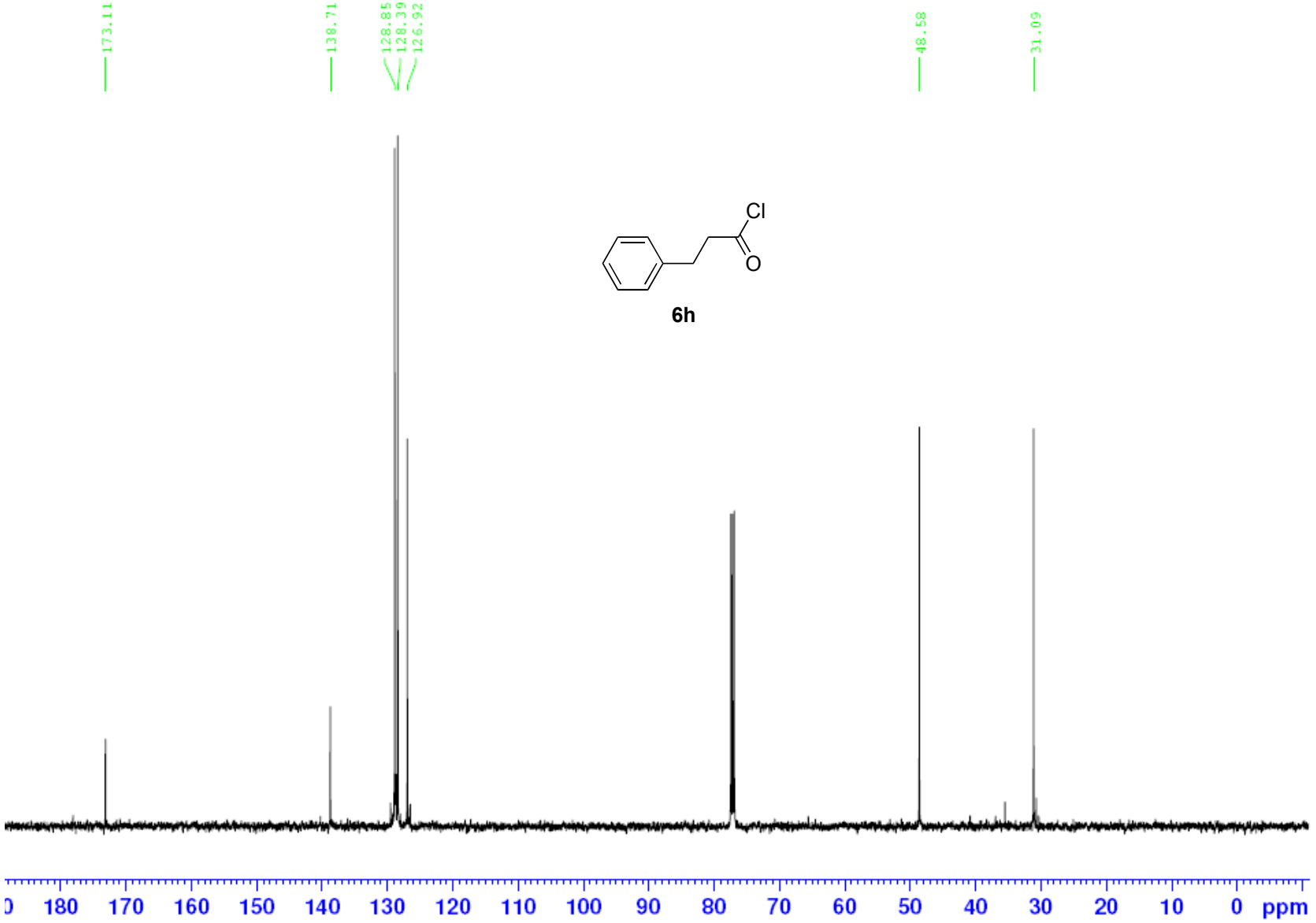


Figure 15S: ^{1}H NMR spectrum of *trans*-cinnamoyl chloride **(6i)**

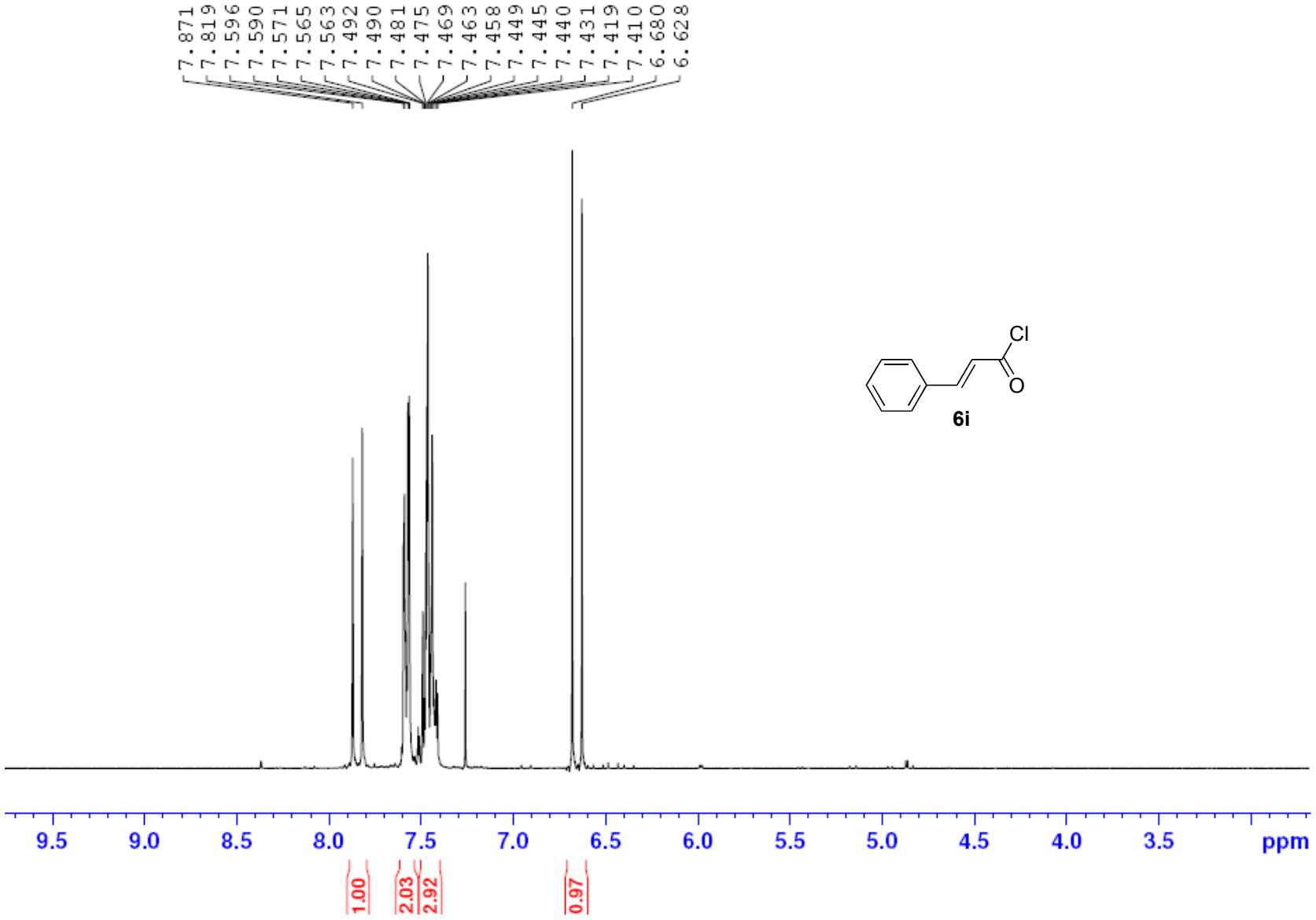


Figure 16S: ^{13}C NMR spectrum of *trans*-cinnamoyl chloride **(6i)**

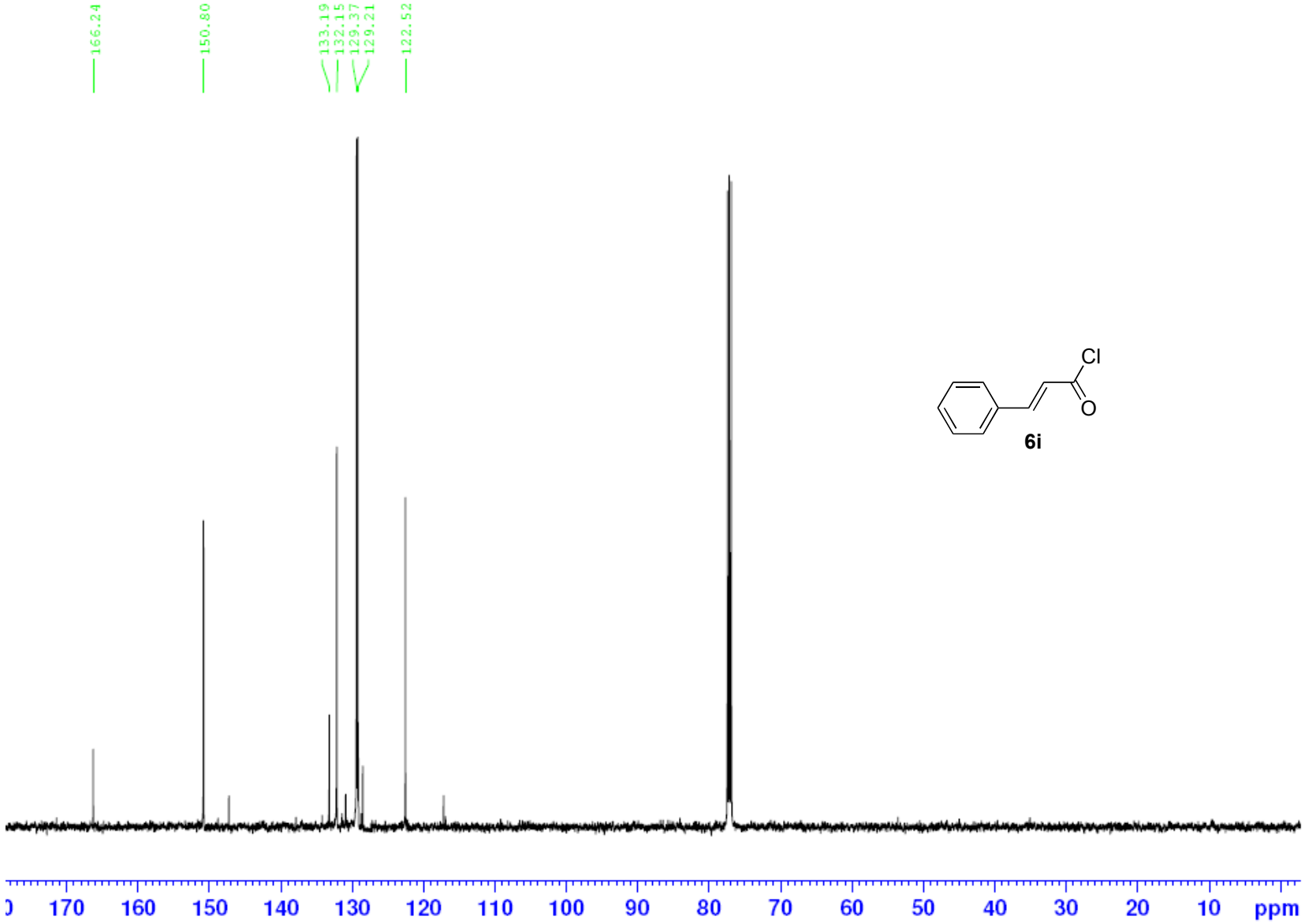


Figure 17S: ^{1}H NMR spectrum of crude dimethyl acetyl phosphate **(7a)**

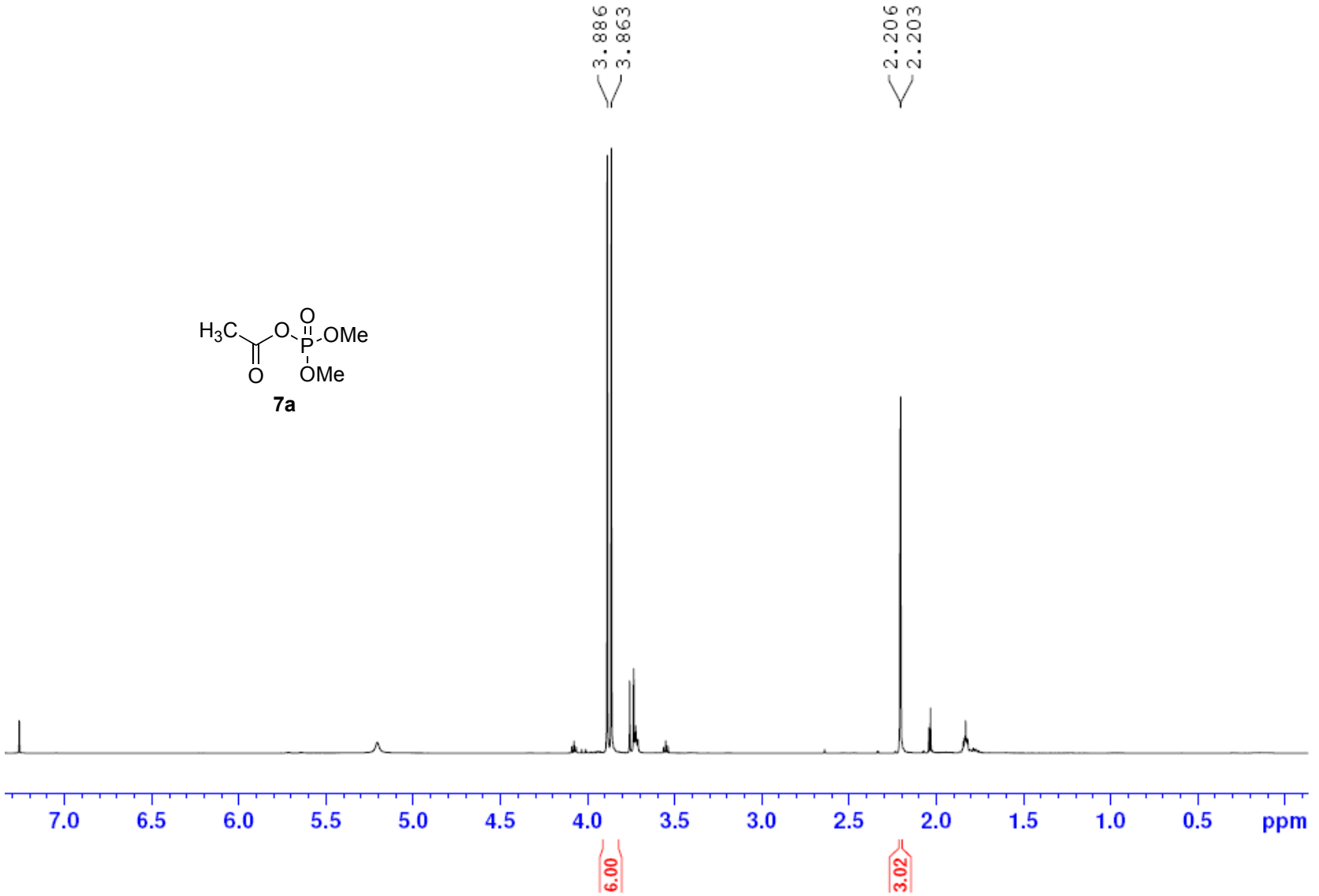


Figure 18S: ^{13}C NMR spectrum of crude dimethyl acetyl phosphate **(7a)**

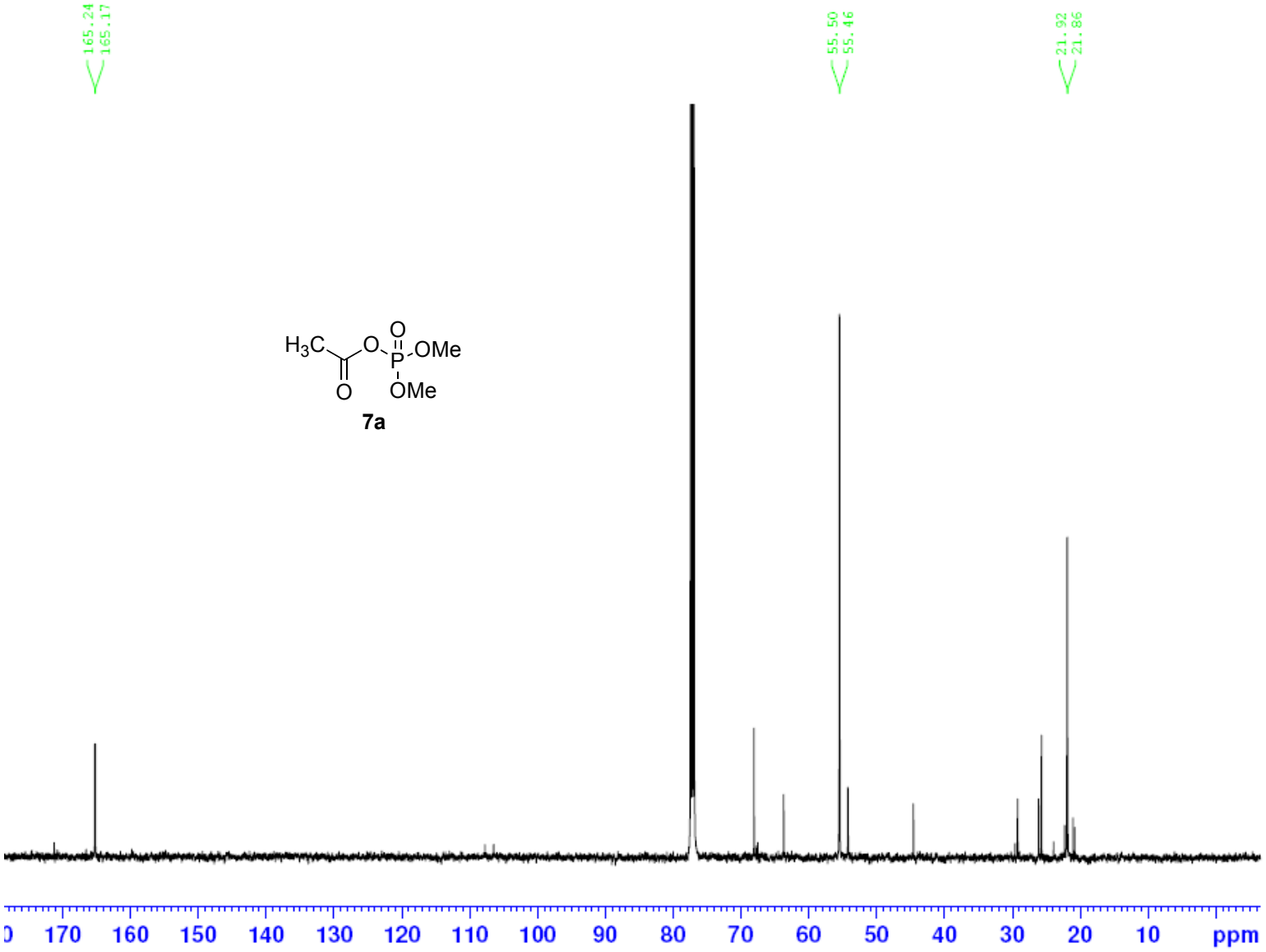


Figure 19S: ^{31}P NMR spectrum of dimethyl acetyl phosphate **(7a)**

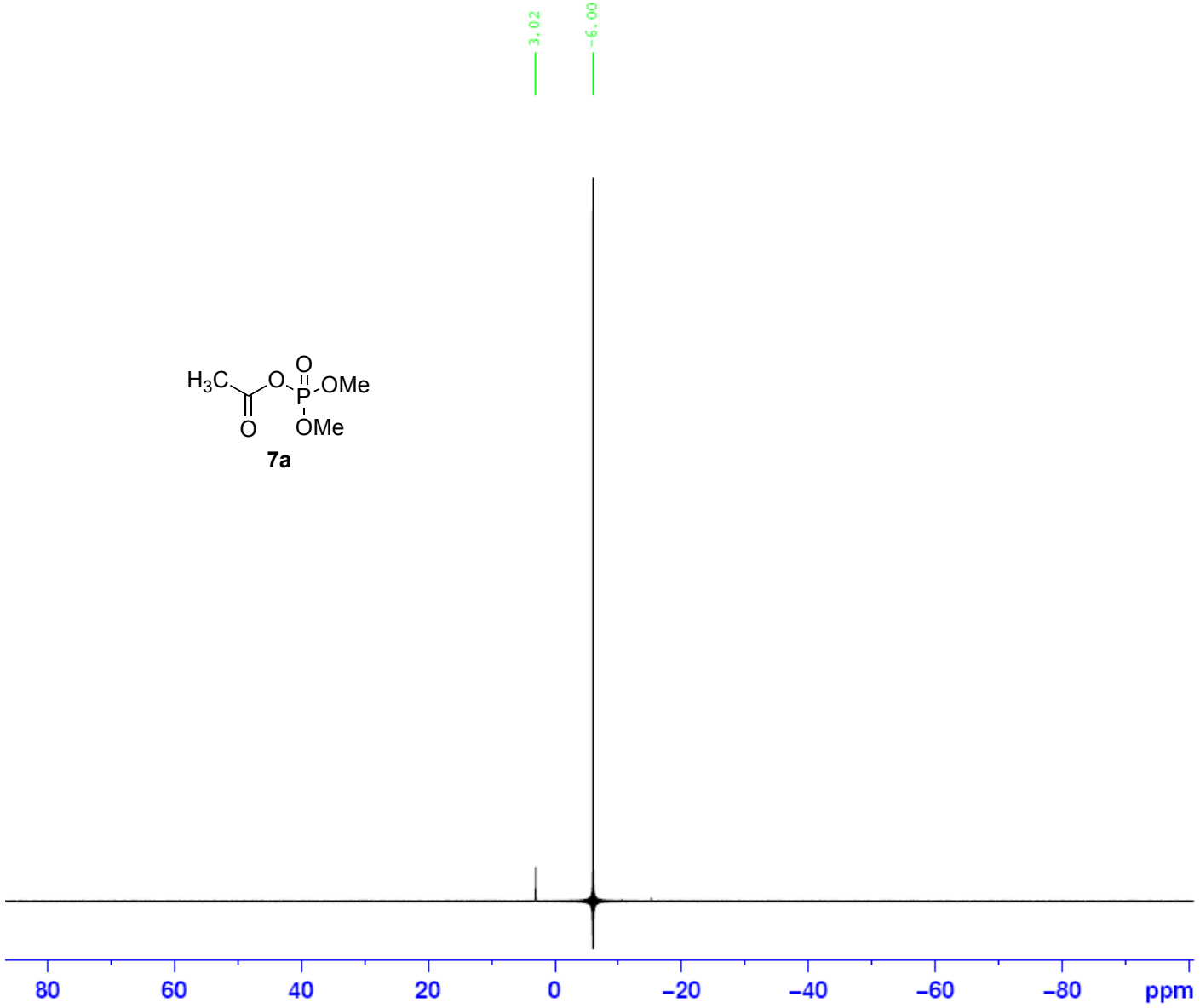


Figure 20S: ^{1}H NMR spectrum of crude dimethyl butyryl phosphate **(7b)**

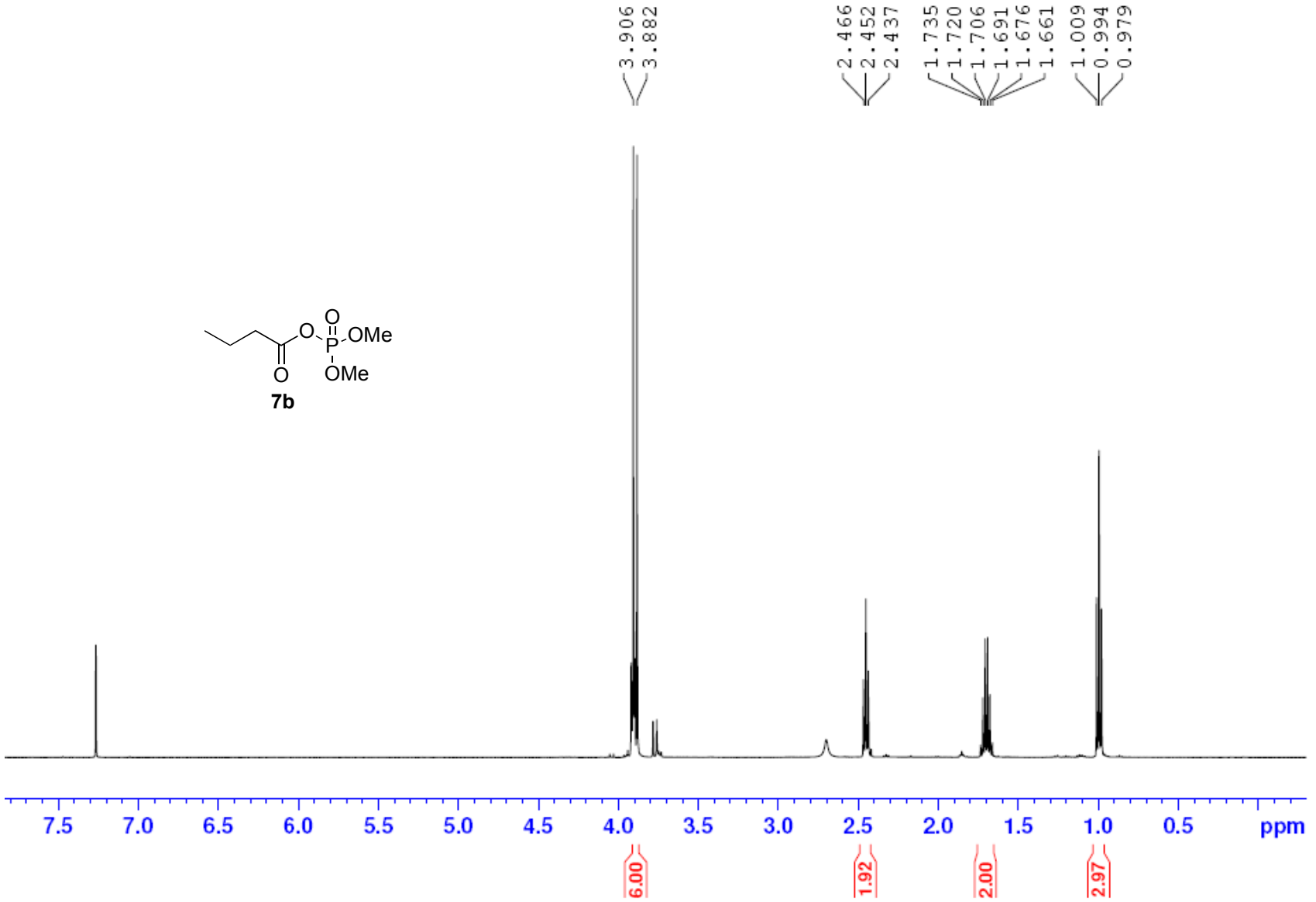


Figure 21S: ^{13}C NMR spectrum of crude dimethyl butyryl phosphate **(7b)**

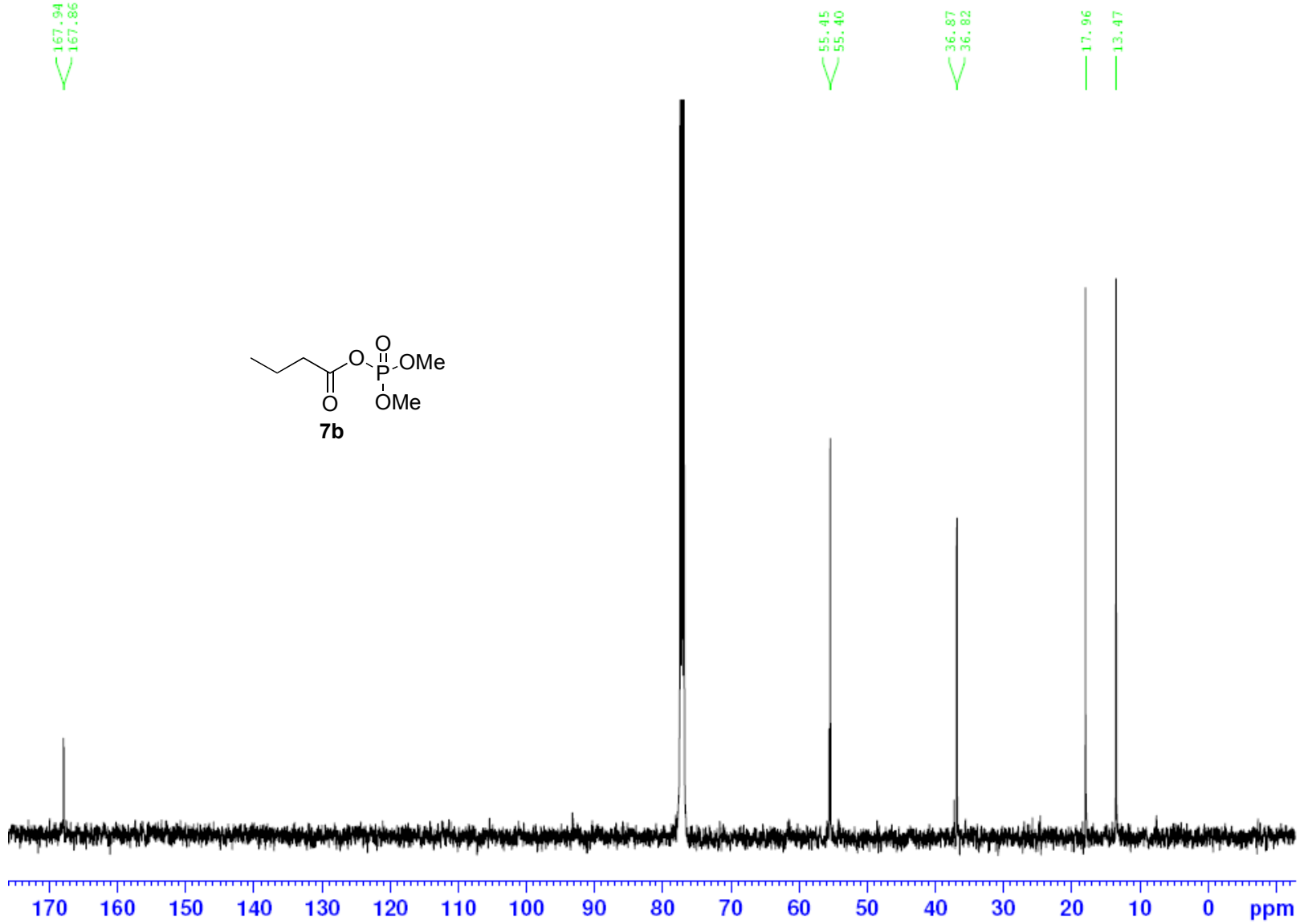


Figure 22S: ^{31}P NMR spectrum of crude dimethyl butyryl phosphate **(7b)**

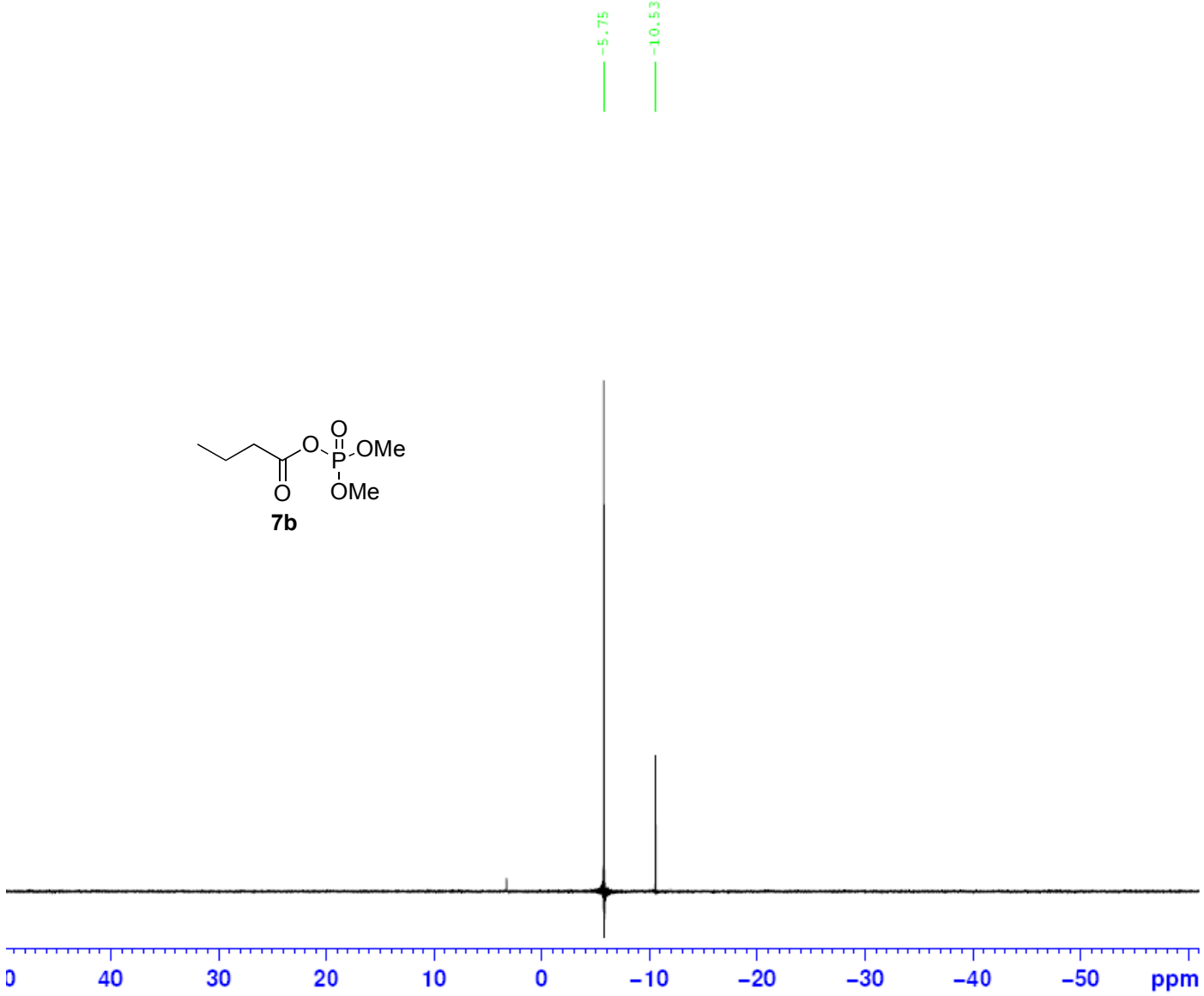


Figure 23S: ^{1}H NMR spectrum of crude dimethyl isovaleryl phosphate **(7c)**

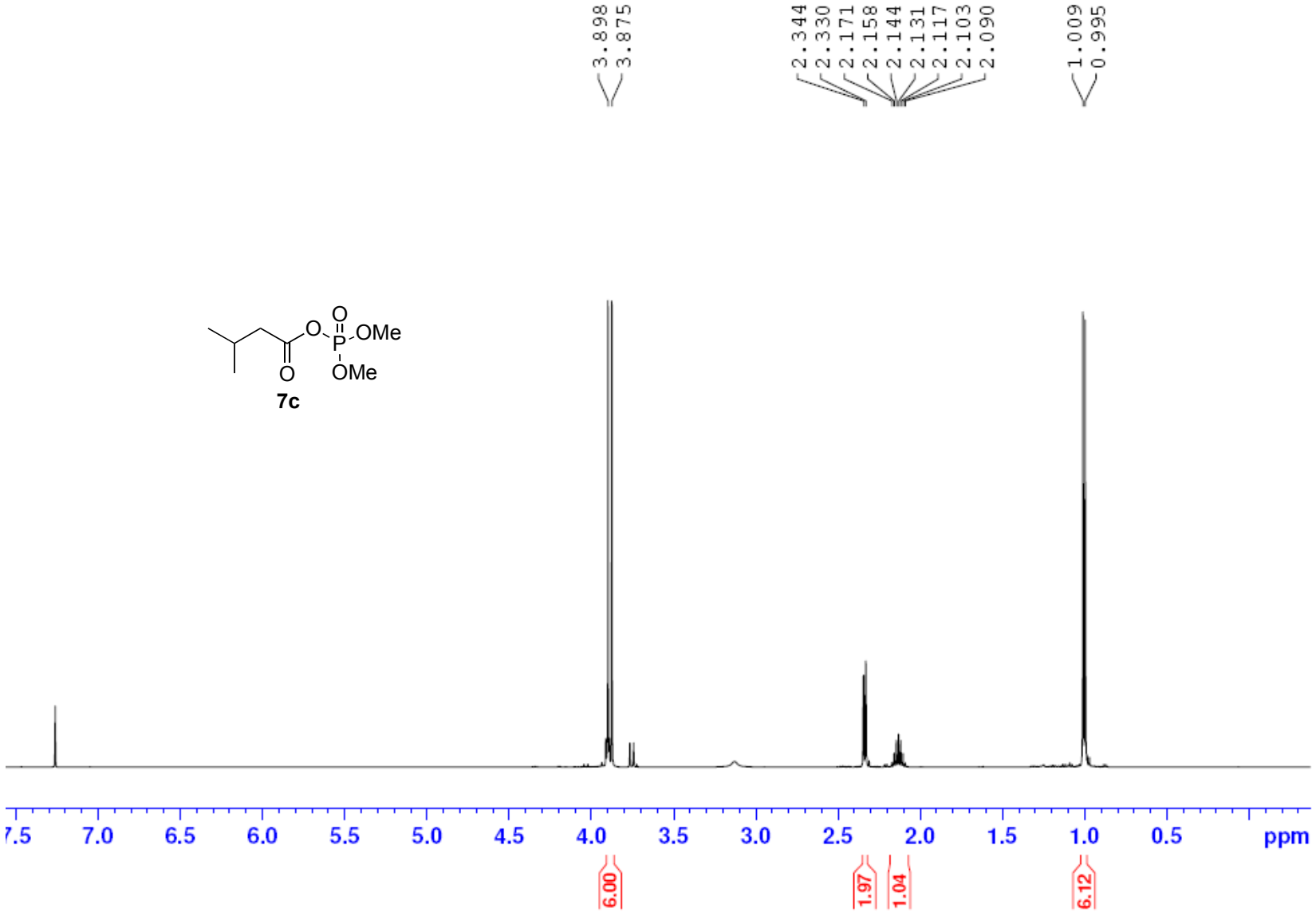


Figure 24S: ^{13}C NMR spectrum of crude dimethyl isovaleryl phosphate **(7c)**

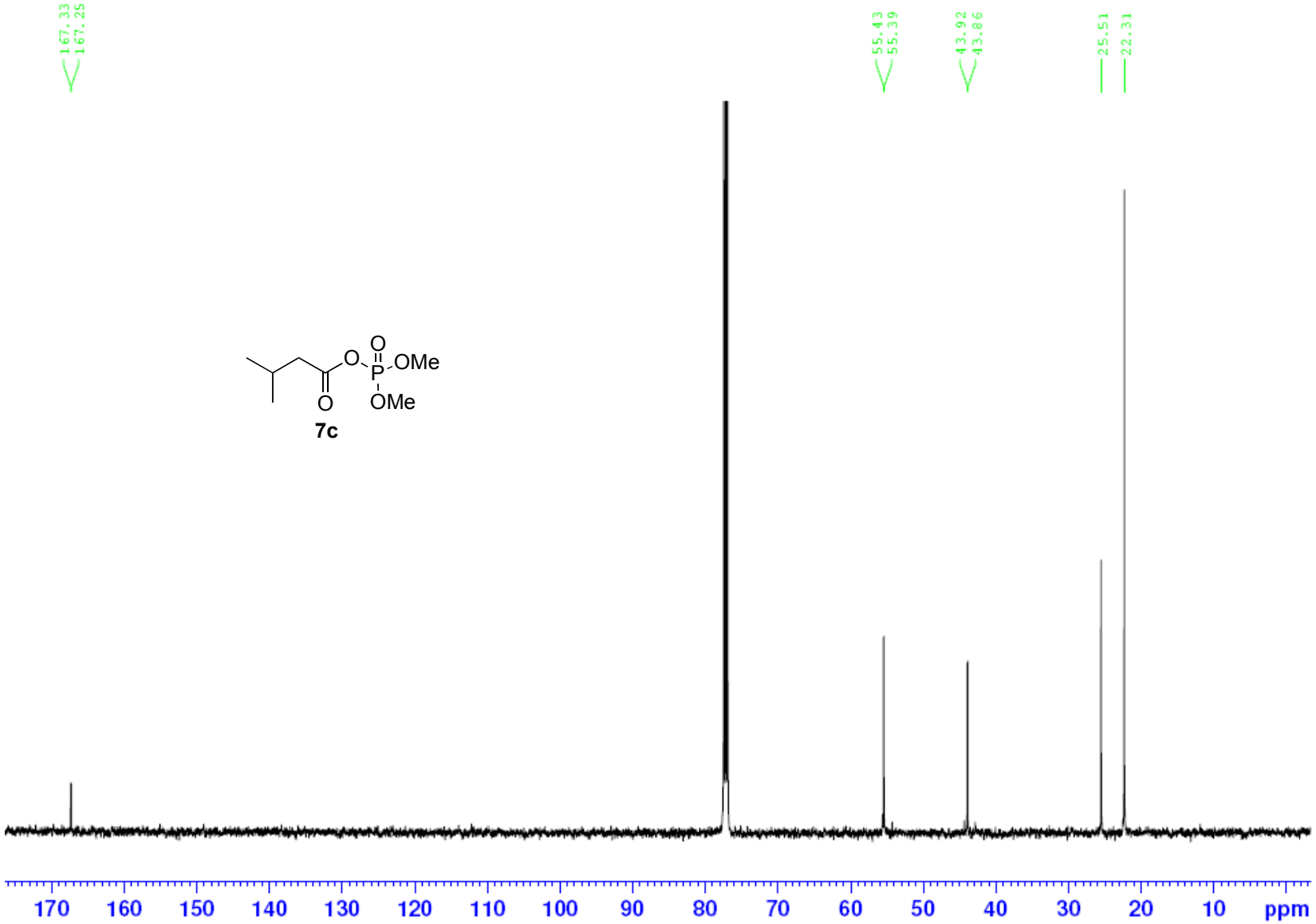


Figure 25S: ^{31}P NMR spectrum of crude dimethyl isovaleryl phosphate **(7c)**

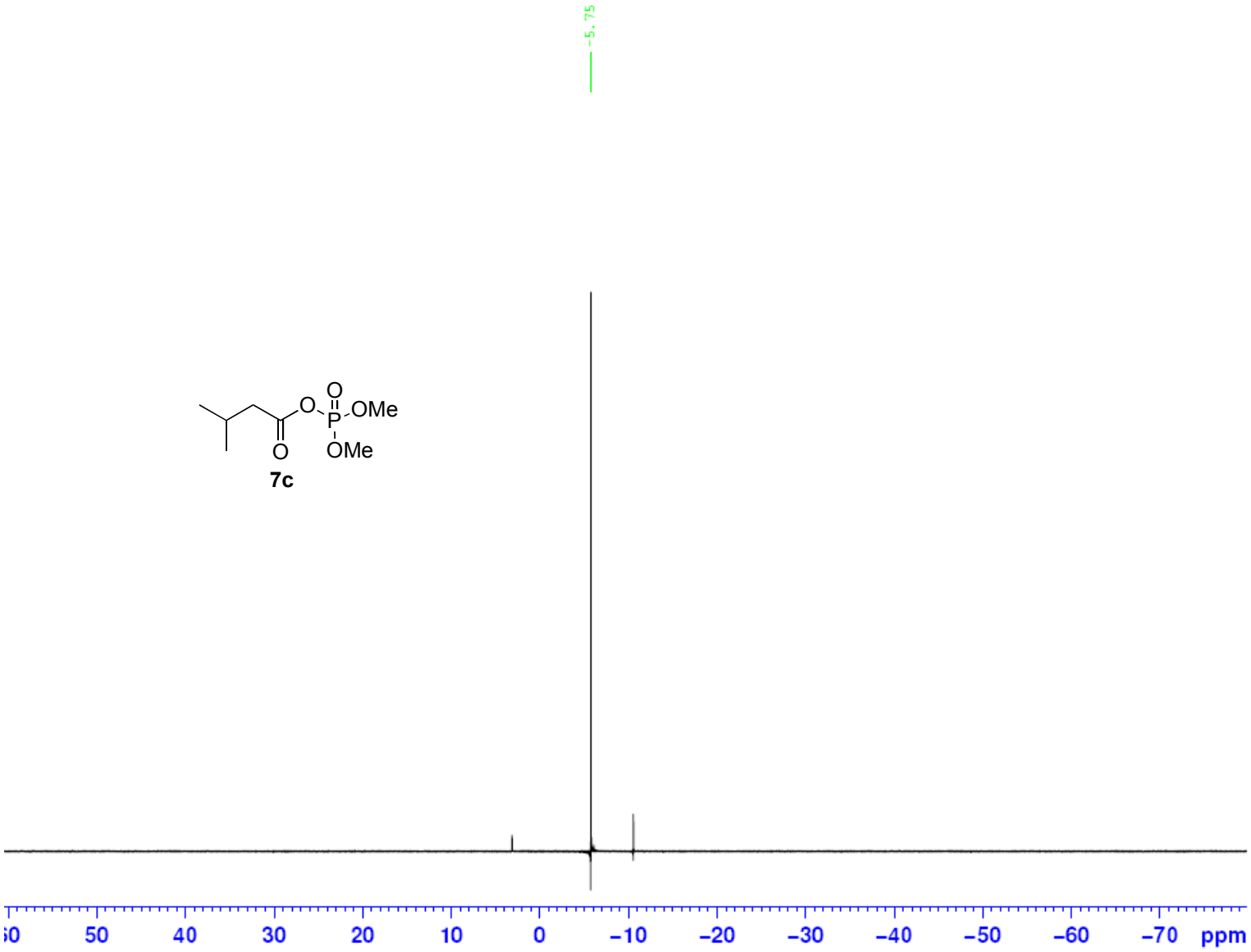


Figure 26S: ^{1}H NMR spectrum of crude dimethyl octanoyl phosphate **(7d)**

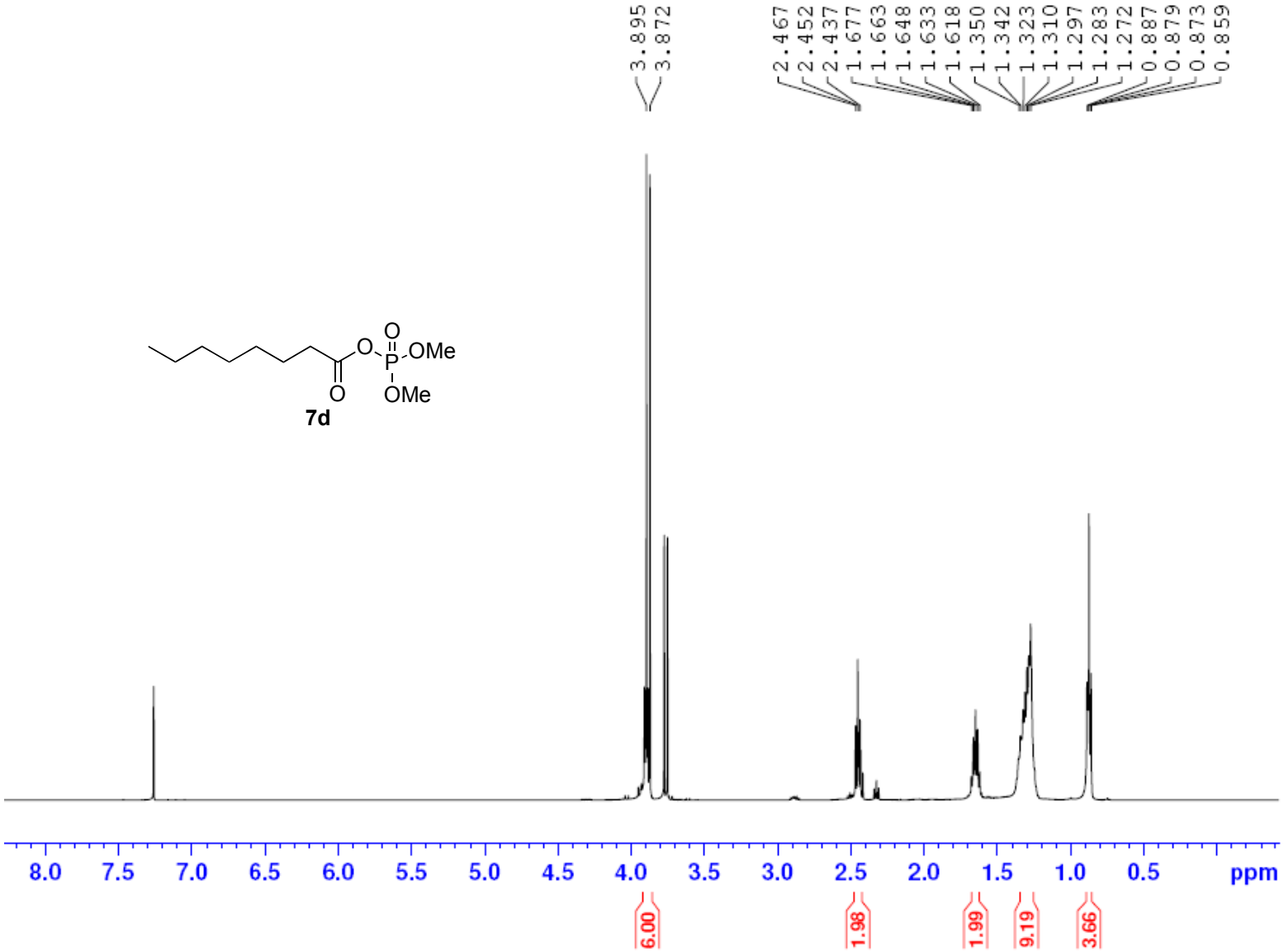


Figure 27S: ^{13}C NMR spectrum of crude dimethyl octanoyl phosphate **(7d)**

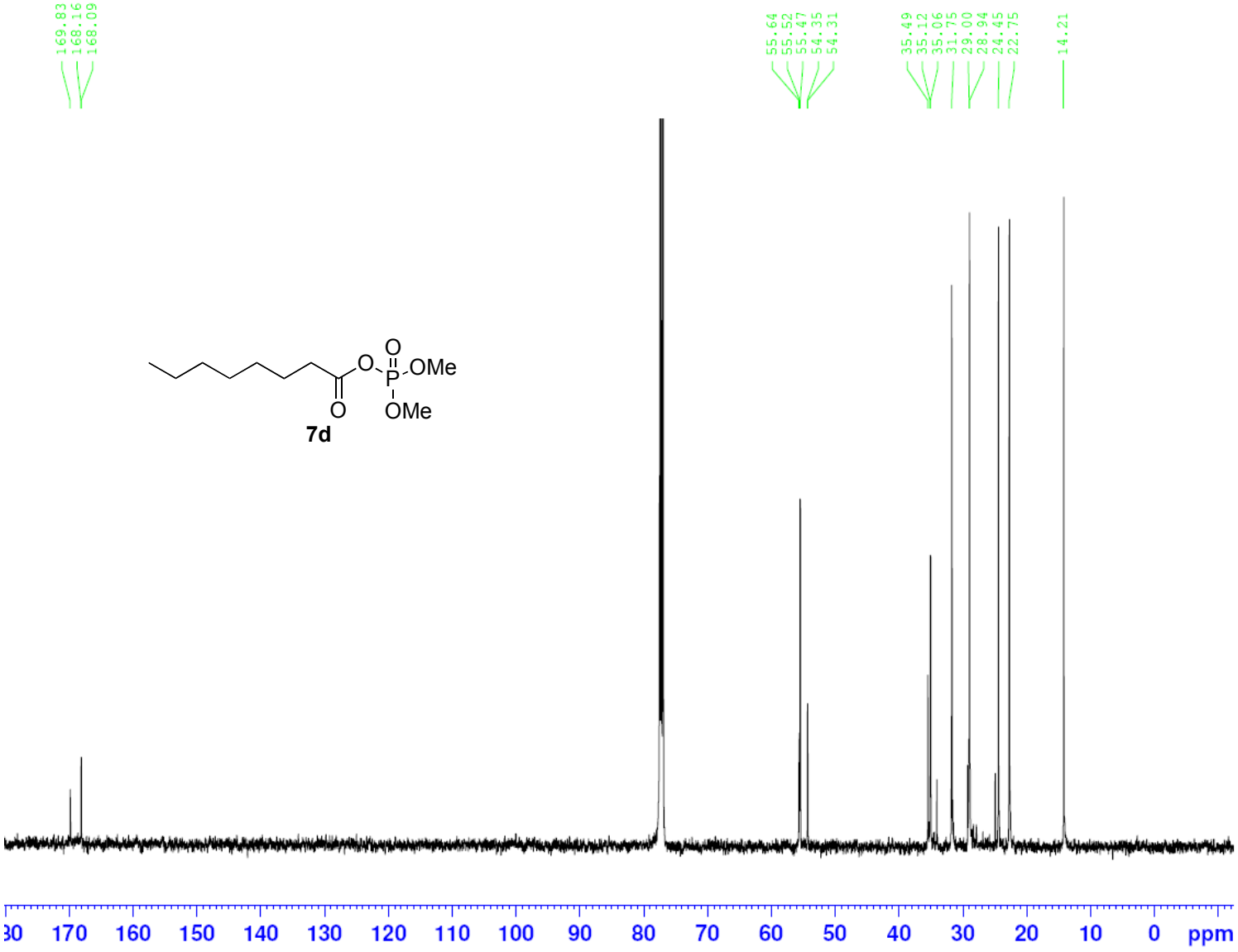


Figure 28S: ^{31}P NMR spectrum of crude dimethyl octanoyl phosphate **(7d)**

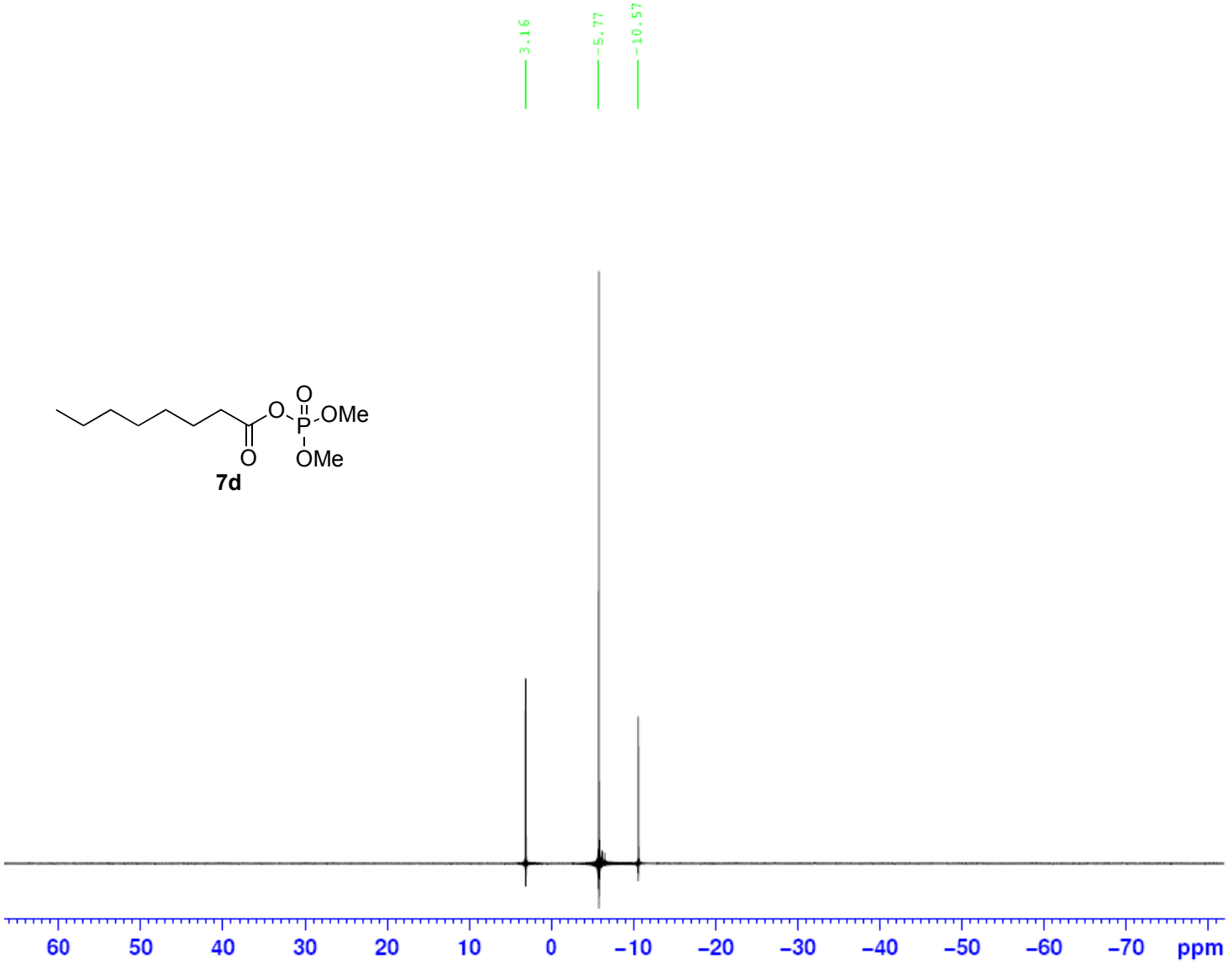


Figure 29S: ^{1}H NMR spectrum of crude dimethyl palmitoyl phosphate **(7e)**

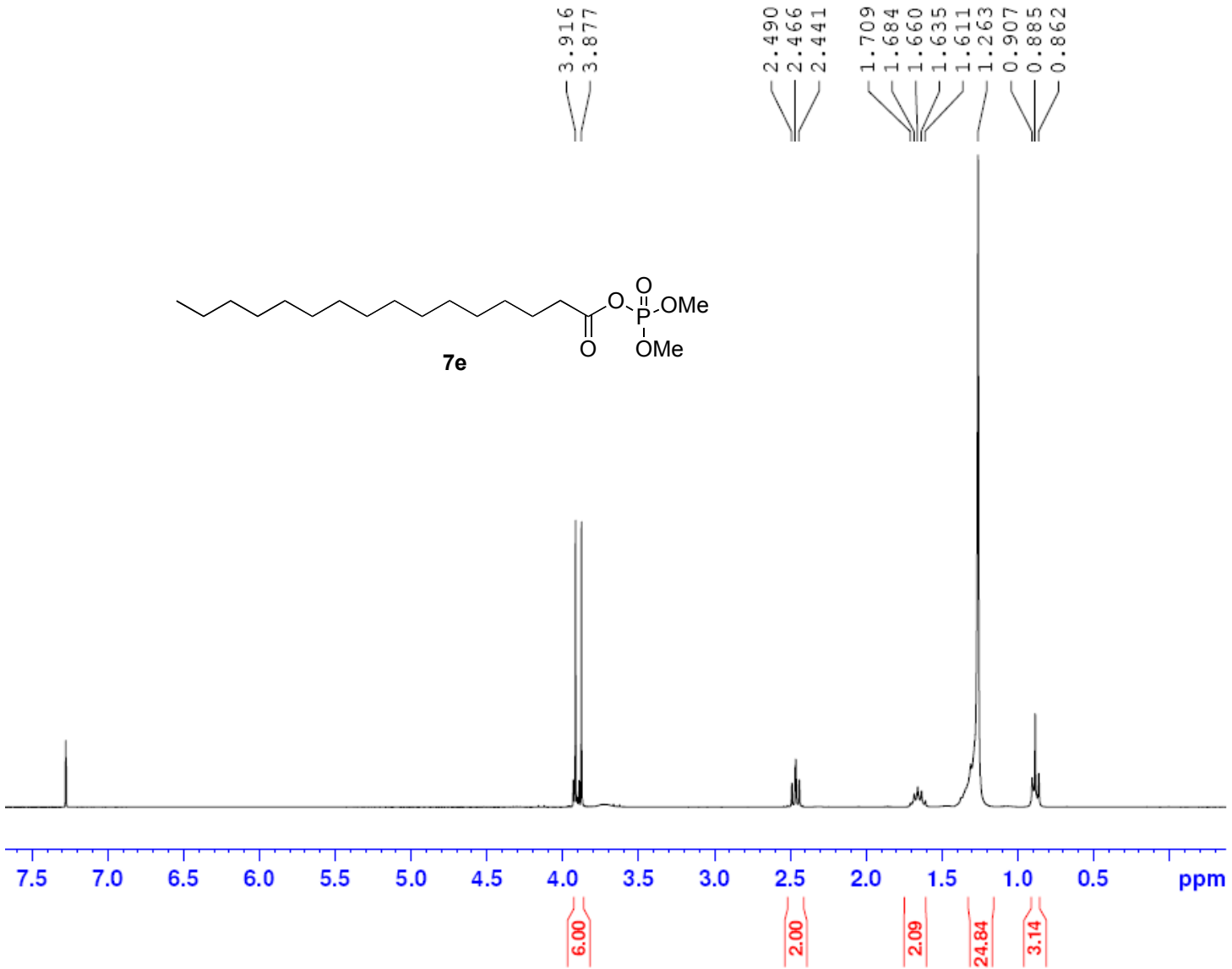


Figure 30S: ^{13}C NMR spectrum of crude dimethyl palmitoyl phosphate **(7e)**

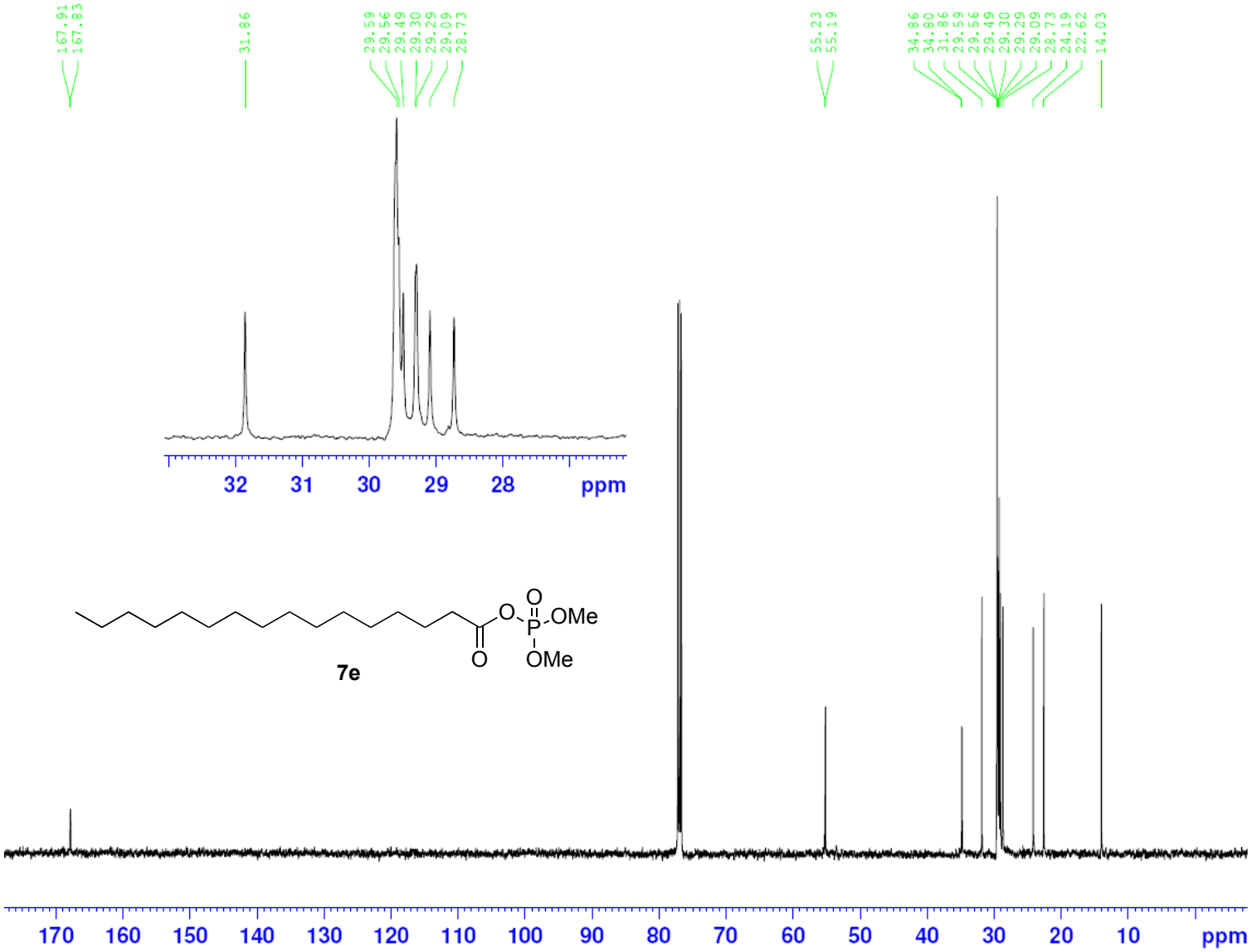


Figure 31S: ^{31}P NMR spectrum of crude dimethyl palmitoyl phosphate **(7e)**

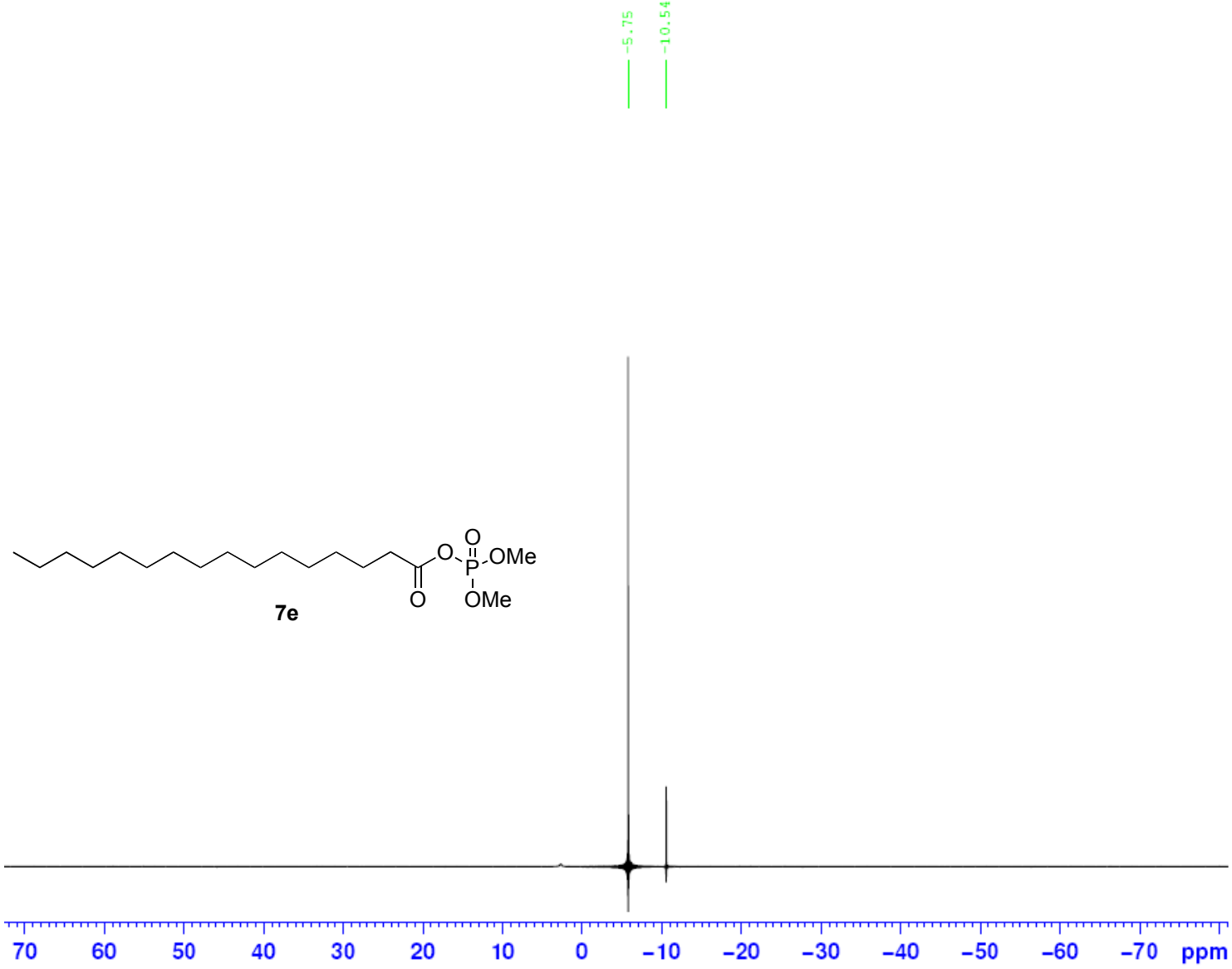


Figure 32S: ^{1}H NMR spectrum of crude dimethyl ibuprofenoyl phosphate **(7f)**

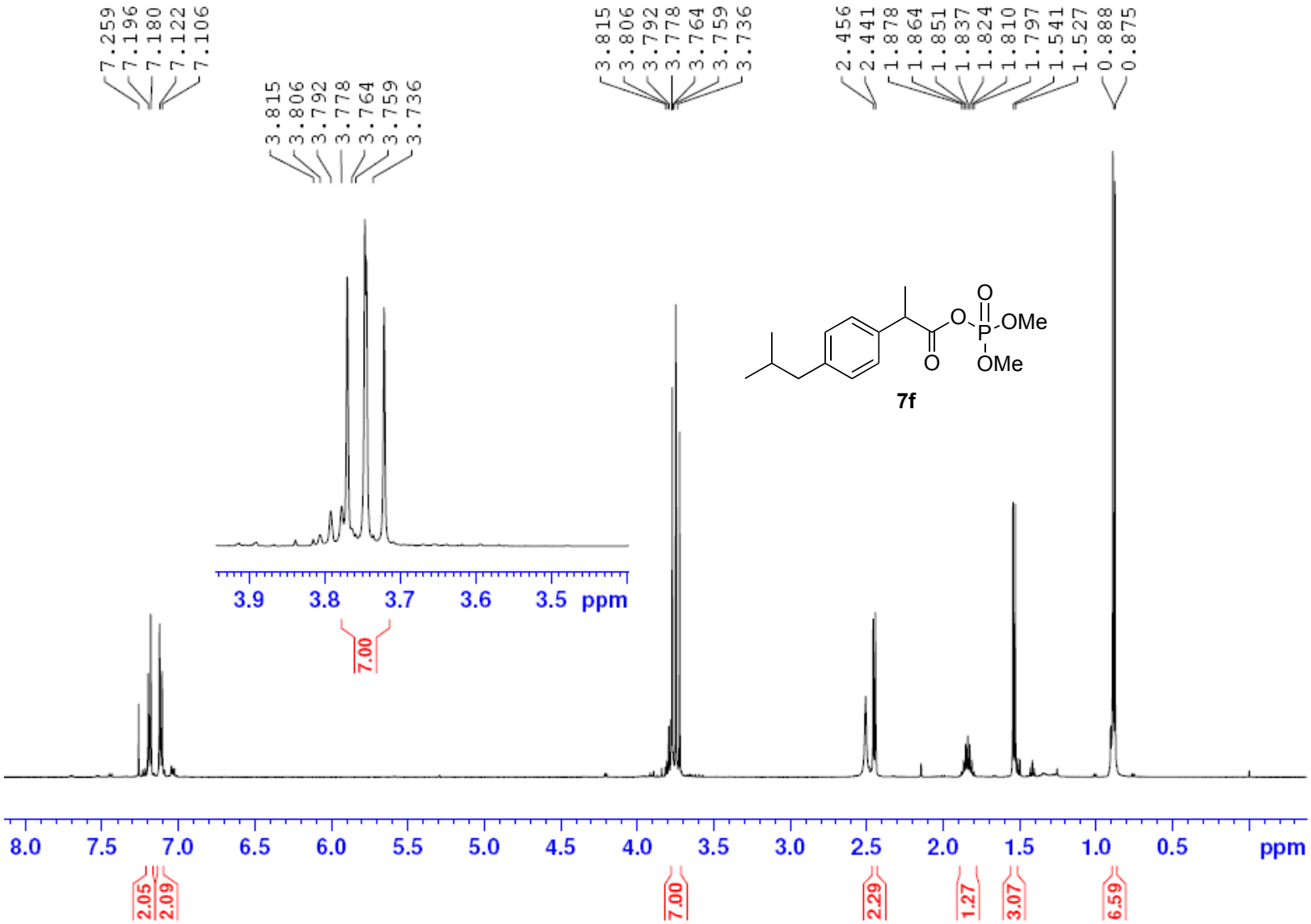


Figure 33S: ^{13}C NMR spectrum of crude dimethyl ibuprofenoyl phosphate **(7f)**

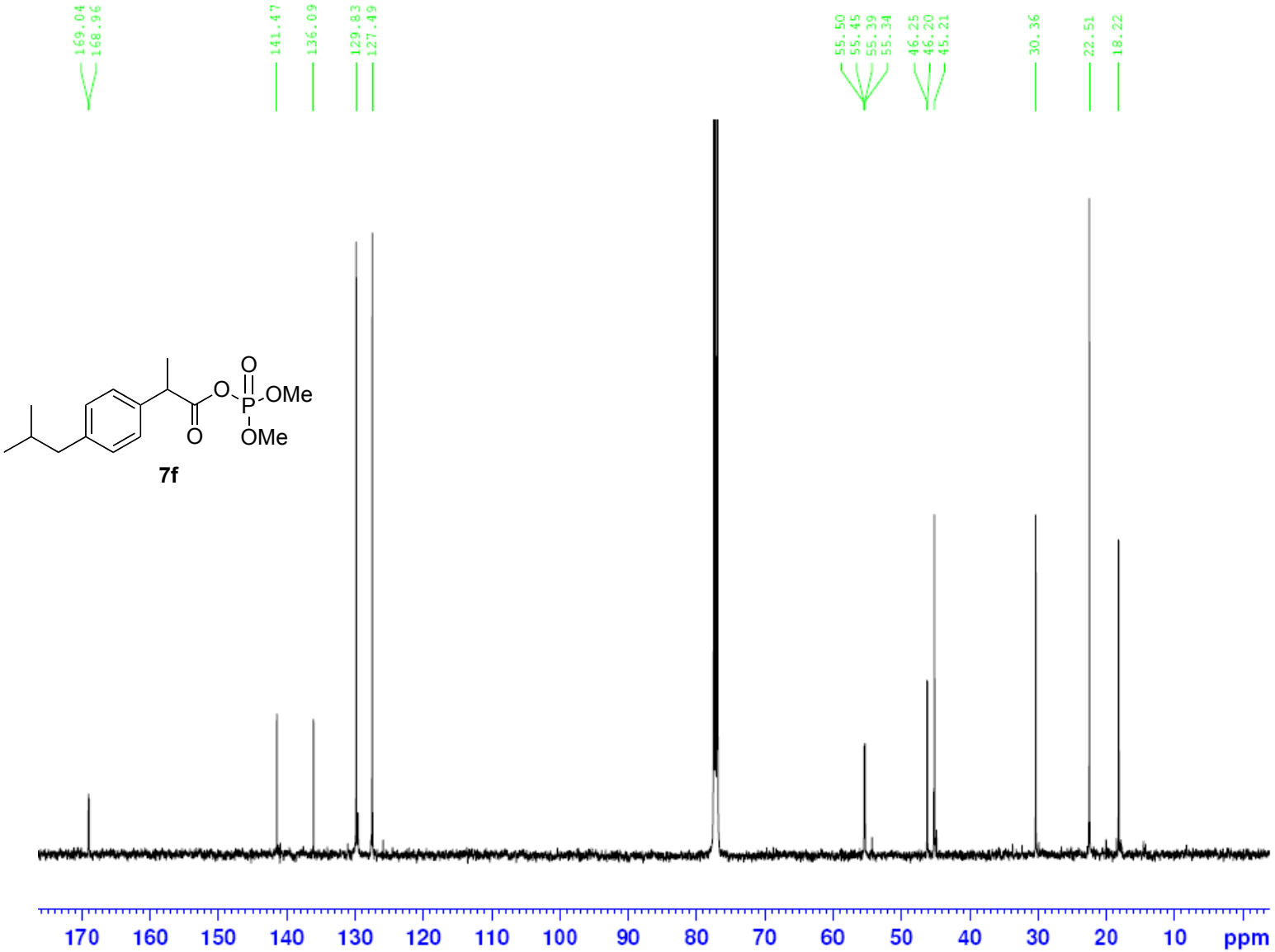


Figure 34S: ^{31}P NMR spectrum of crude dimethyl ibuprofenoyl phosphate **(7f)**

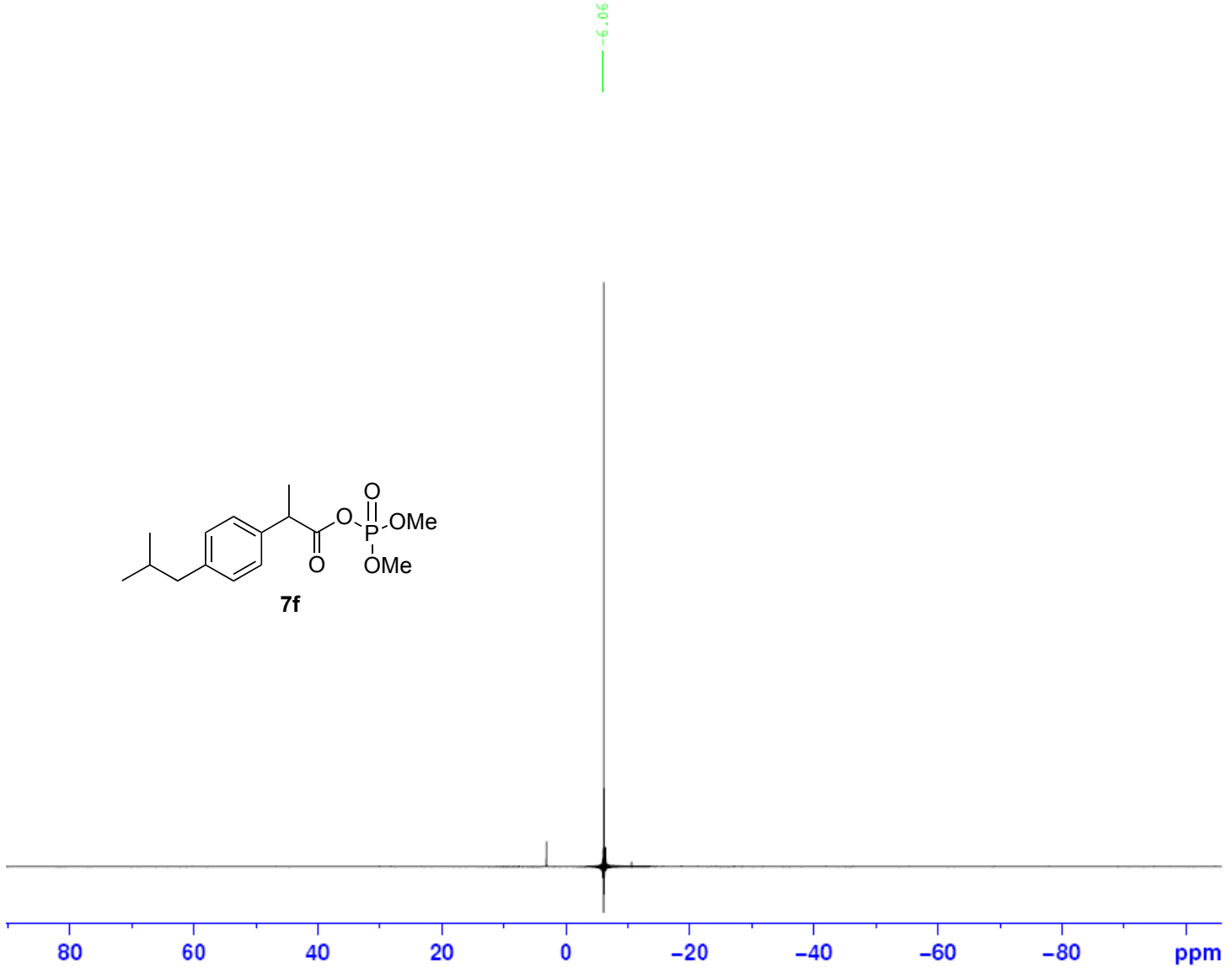


Figure 35S: ^{1}H NMR spectrum of crude dimethyl fenoprofenoyl phosphate **(7g)**

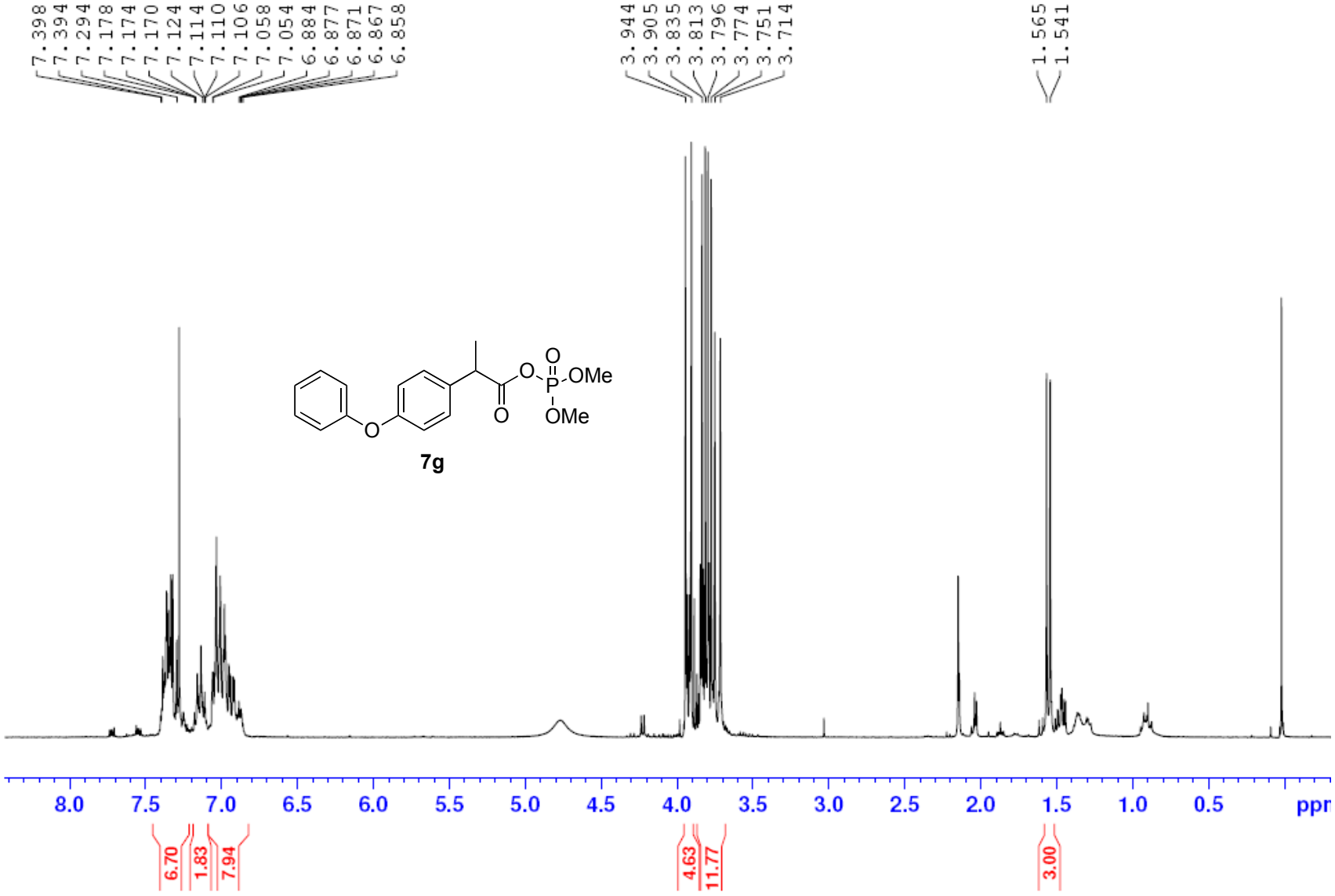


Figure 36S: ^{31}P NMR spectrum of crude dimethyl fenoprofenoyl phosphate **(7g)**

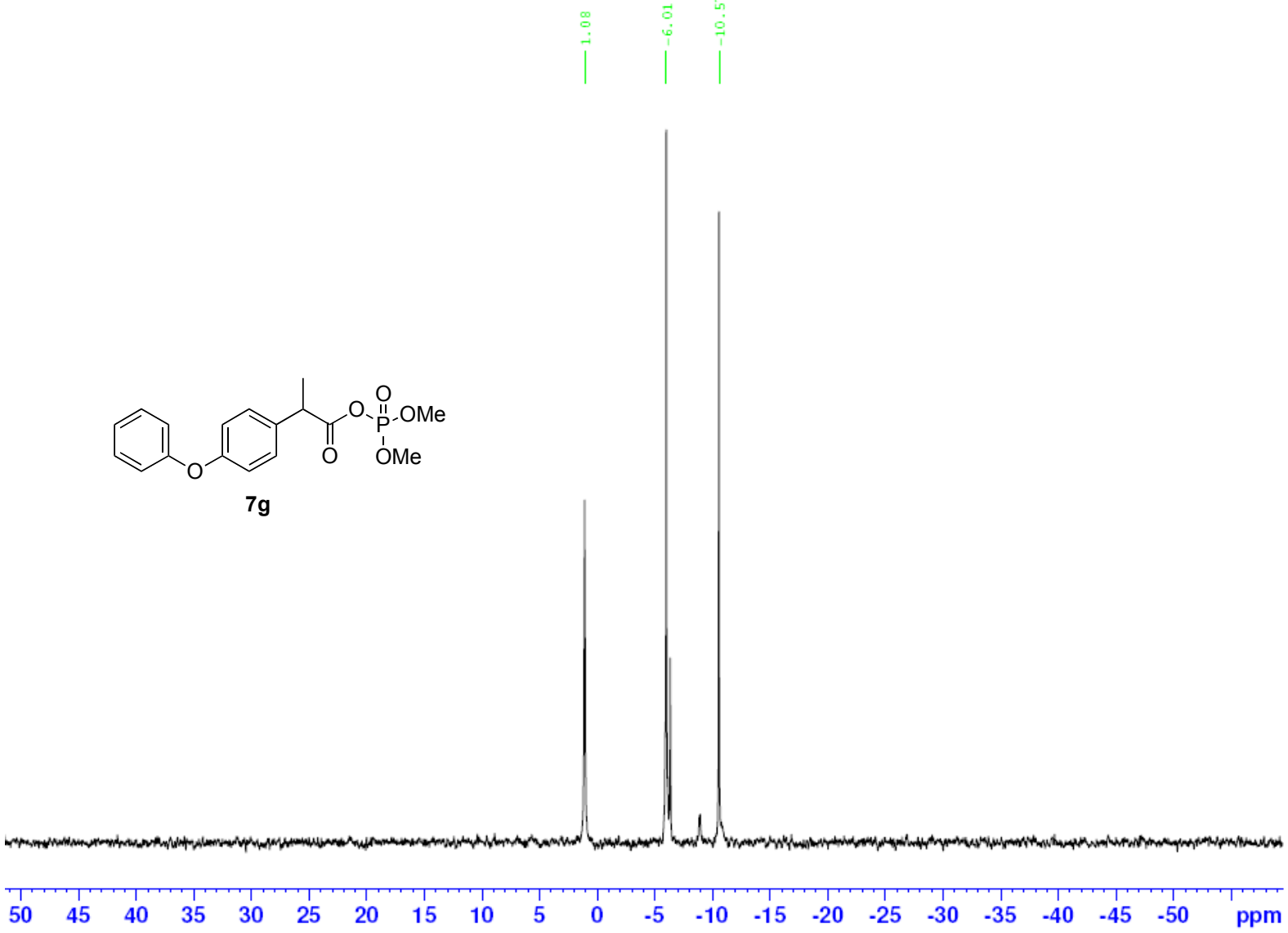


Figure 37S: ^{1}H NMR spectrum of crude dimethyl 3-phenylpropanoyl phosphate **(7h)**

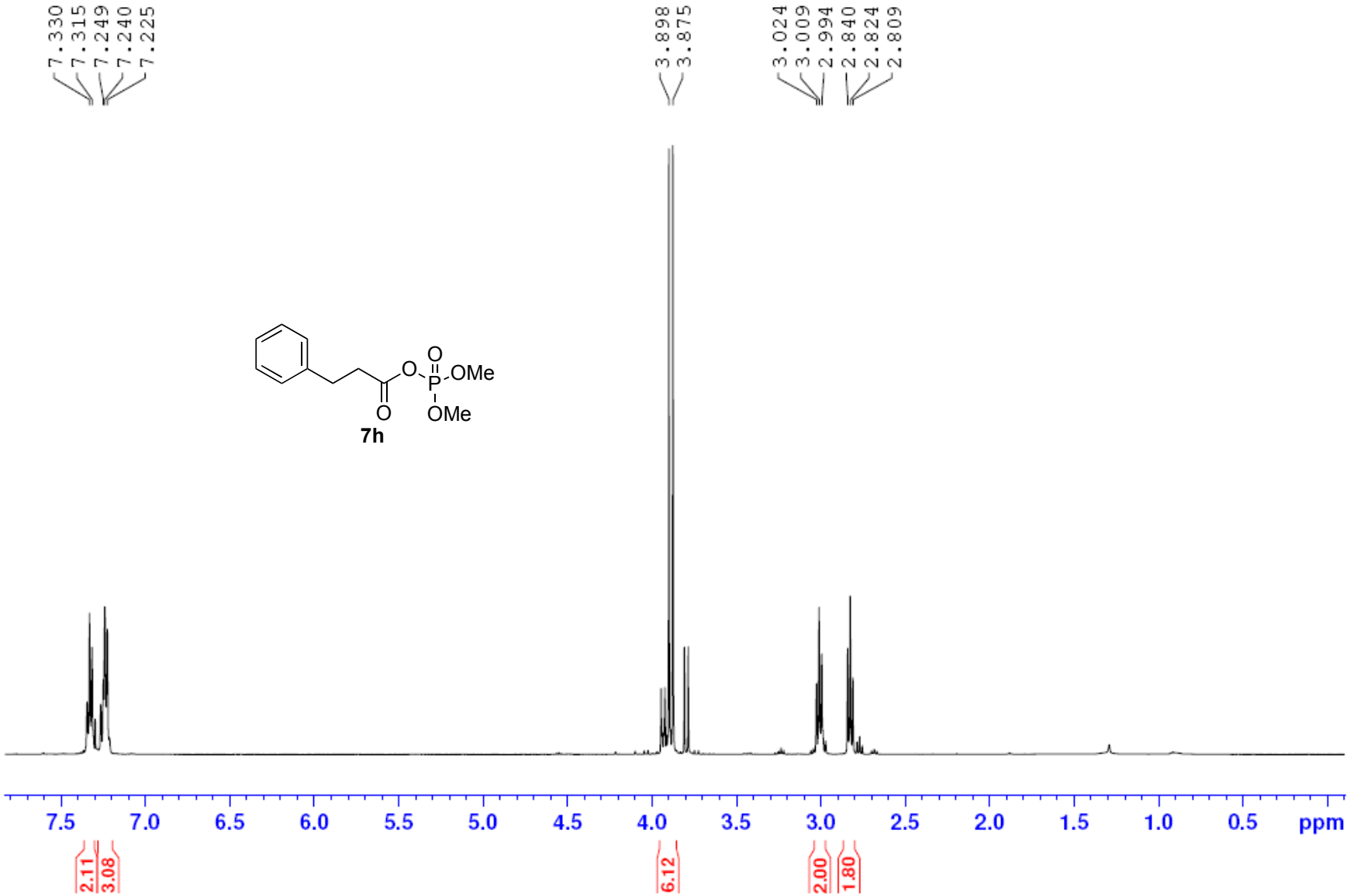


Figure 38S: ^{13}C NMR spectrum of crude dimethyl 3-phenylpropanoyl phosphate **(7h)**

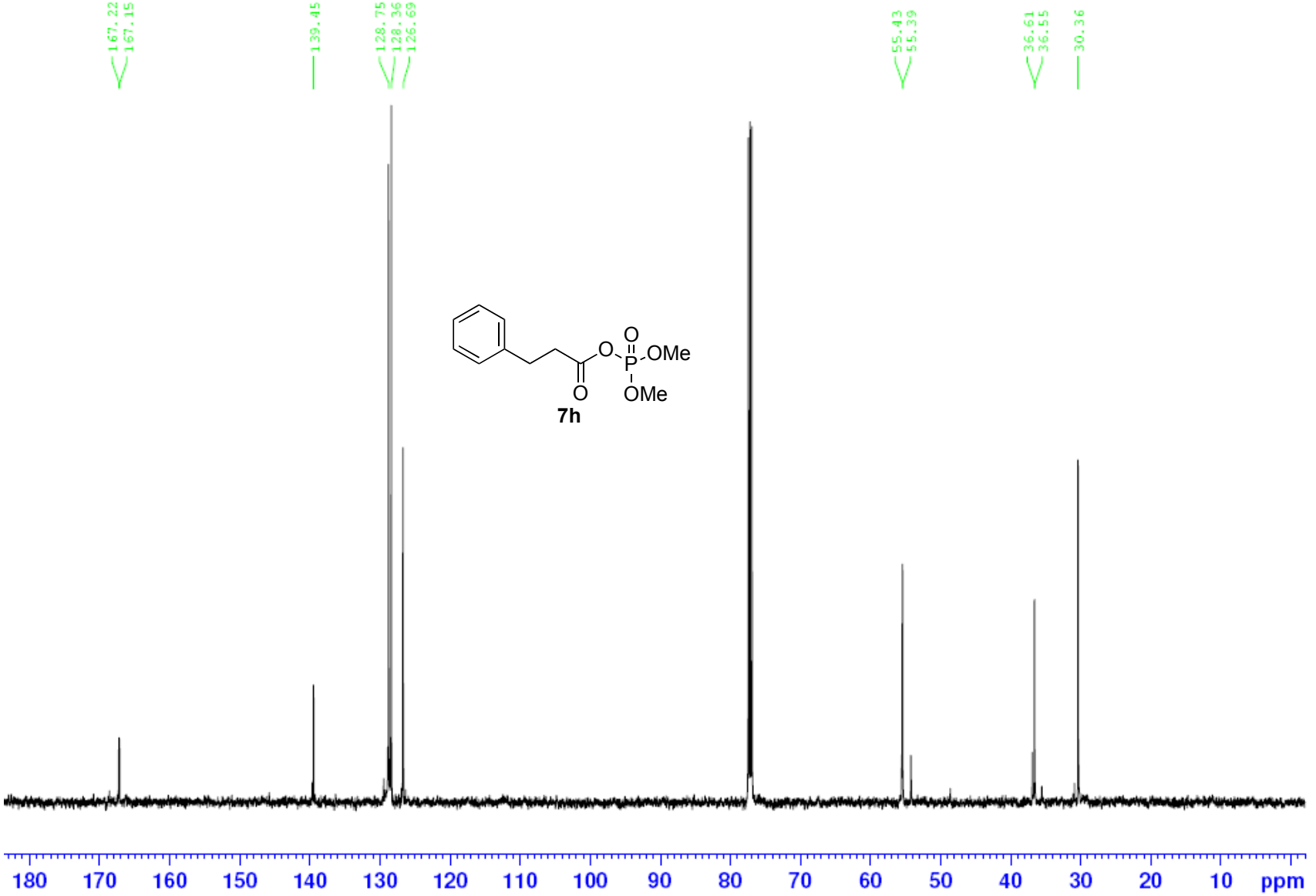


Figure 39S: ^{31}P NMR spectrum of crude dimethyl 3-phenylpropanoyl phosphate **(7h)**

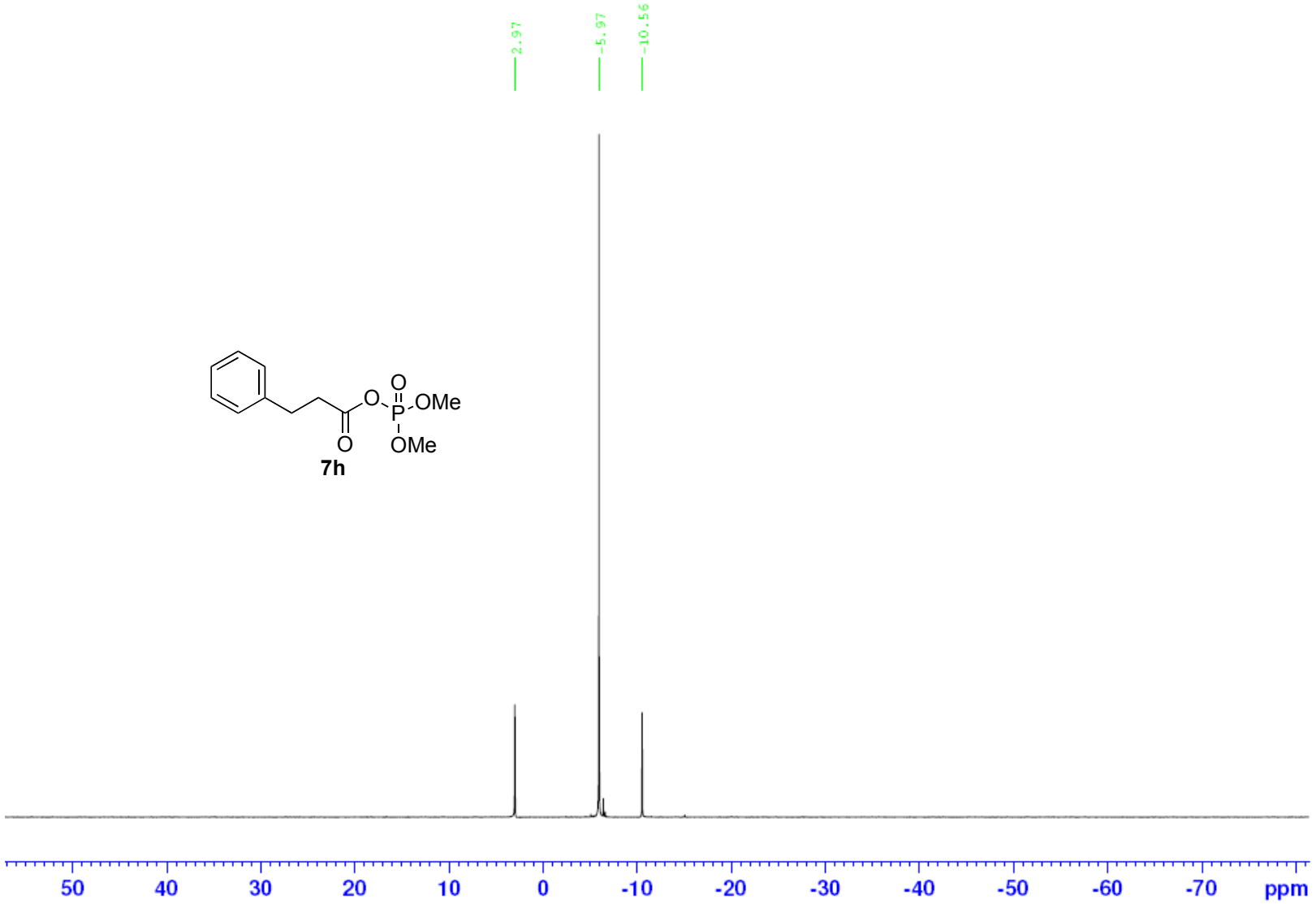


Figure 40S: ^{1}H NMR spectrum of crude dimethyl *trans*-cinnamoyl phosphate **(7i)**

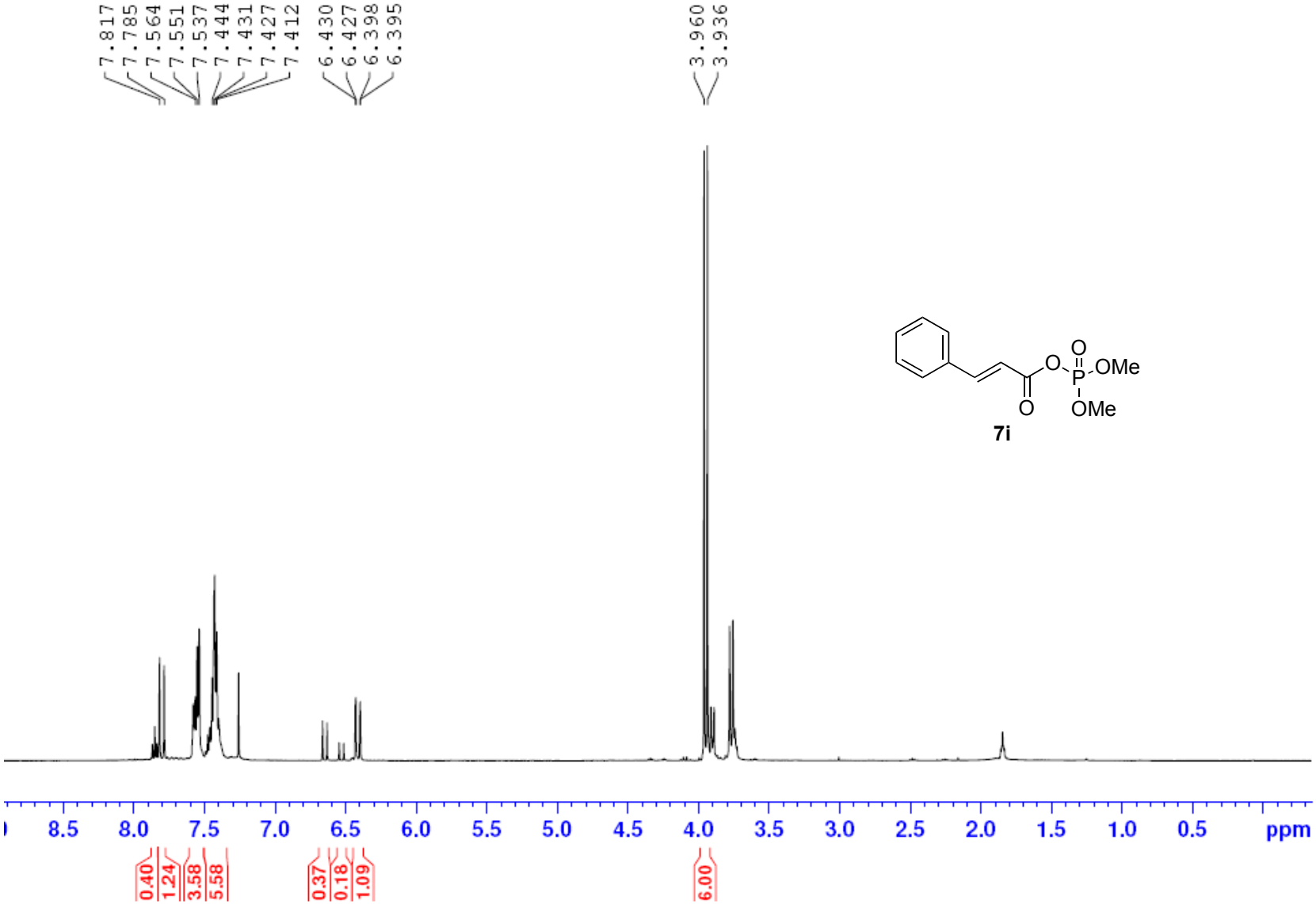


Figure 41S: ^{13}C NMR spectrum of crude dimethyl *trans*-cinnamoyl phosphate **(7i)**

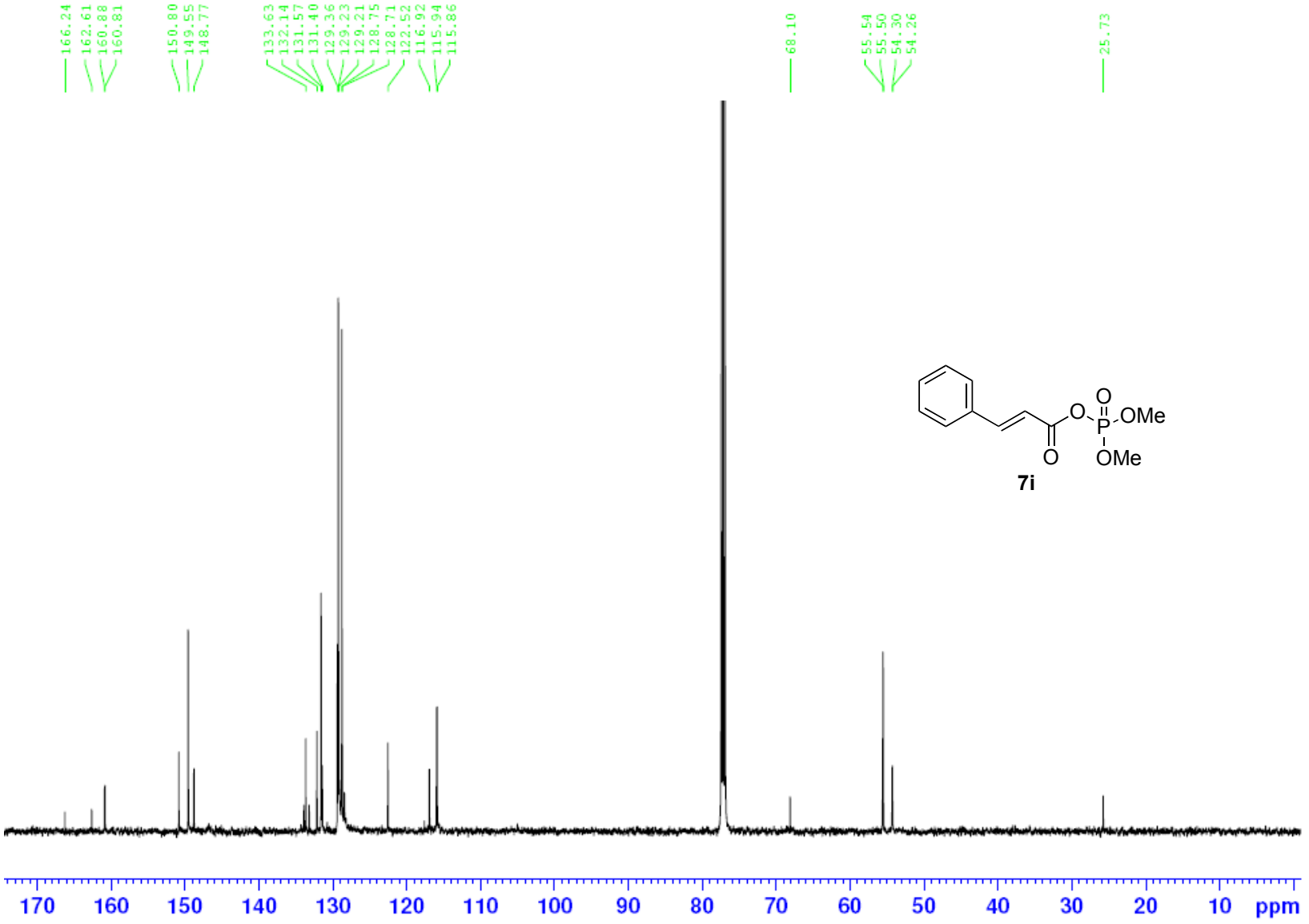


Figure 42S: ^{31}P NMR spectrum of crude dimethyl *trans*-cinnamoyl phosphate **(7i)**

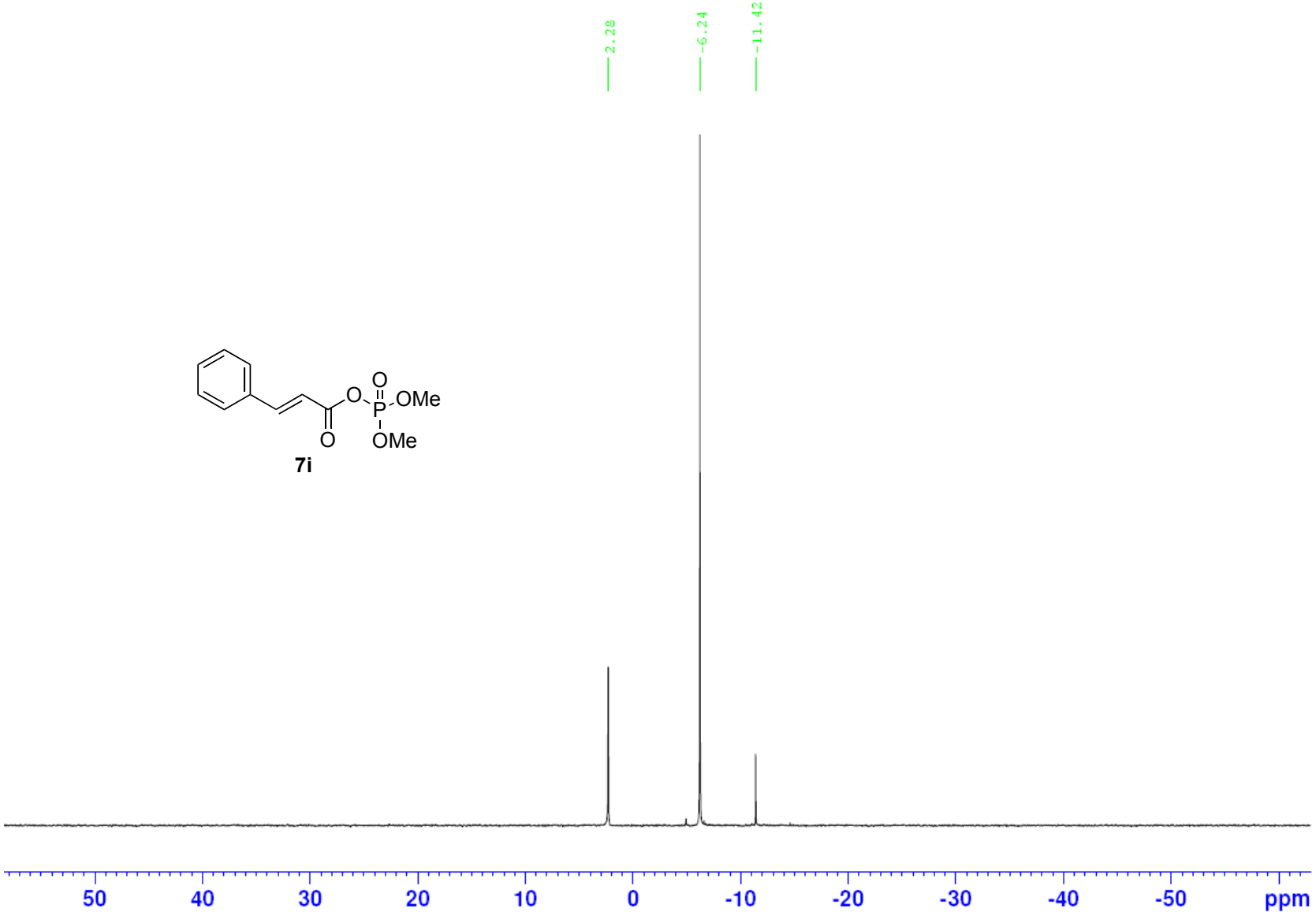


Figure 43S: ^{1}H NMR spectrum of sodium methyl acetyl phosphate **(8a)**

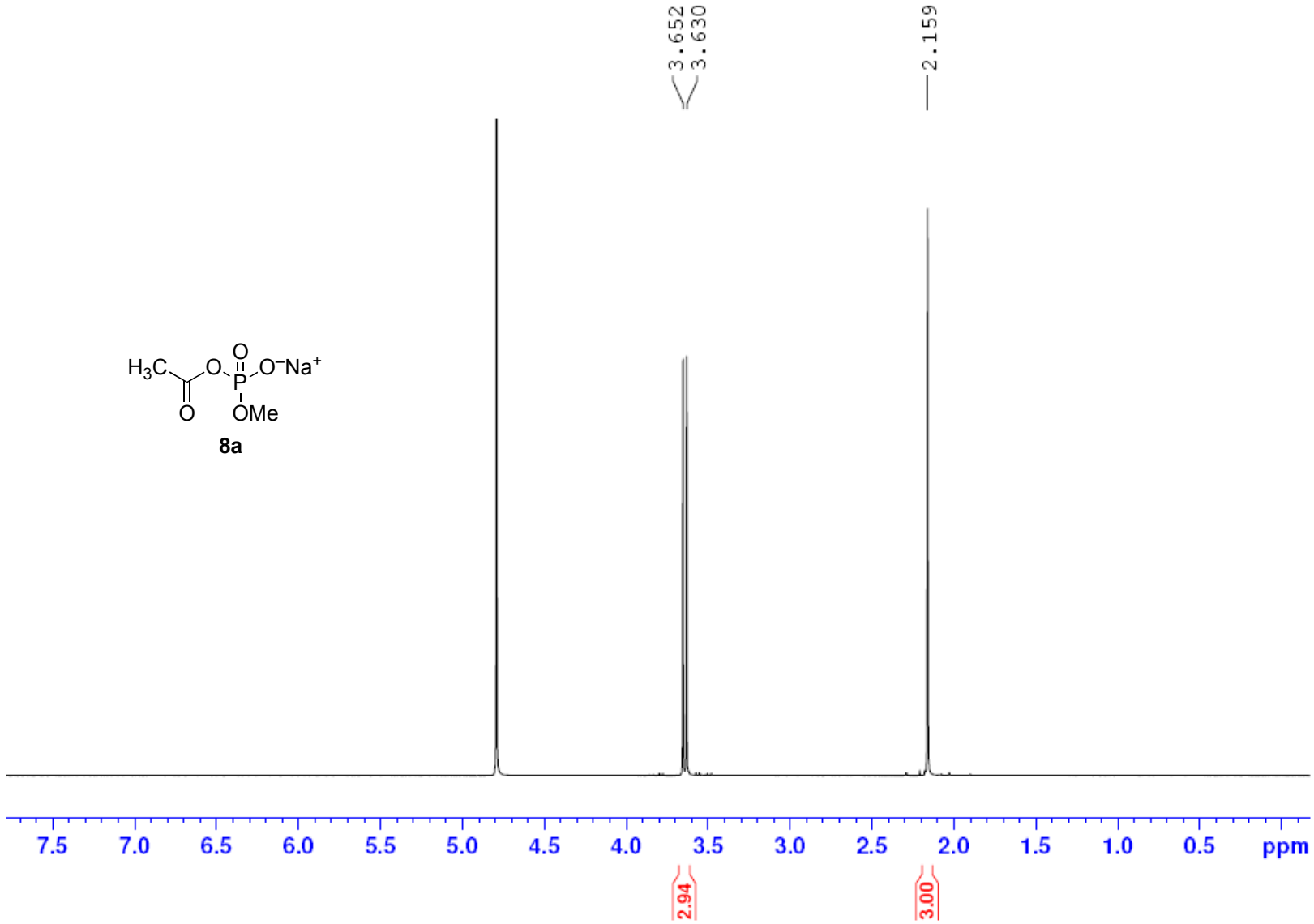


Figure 44S: ^{13}C NMR spectrum of sodium methyl acetyl phosphate **(8a)**

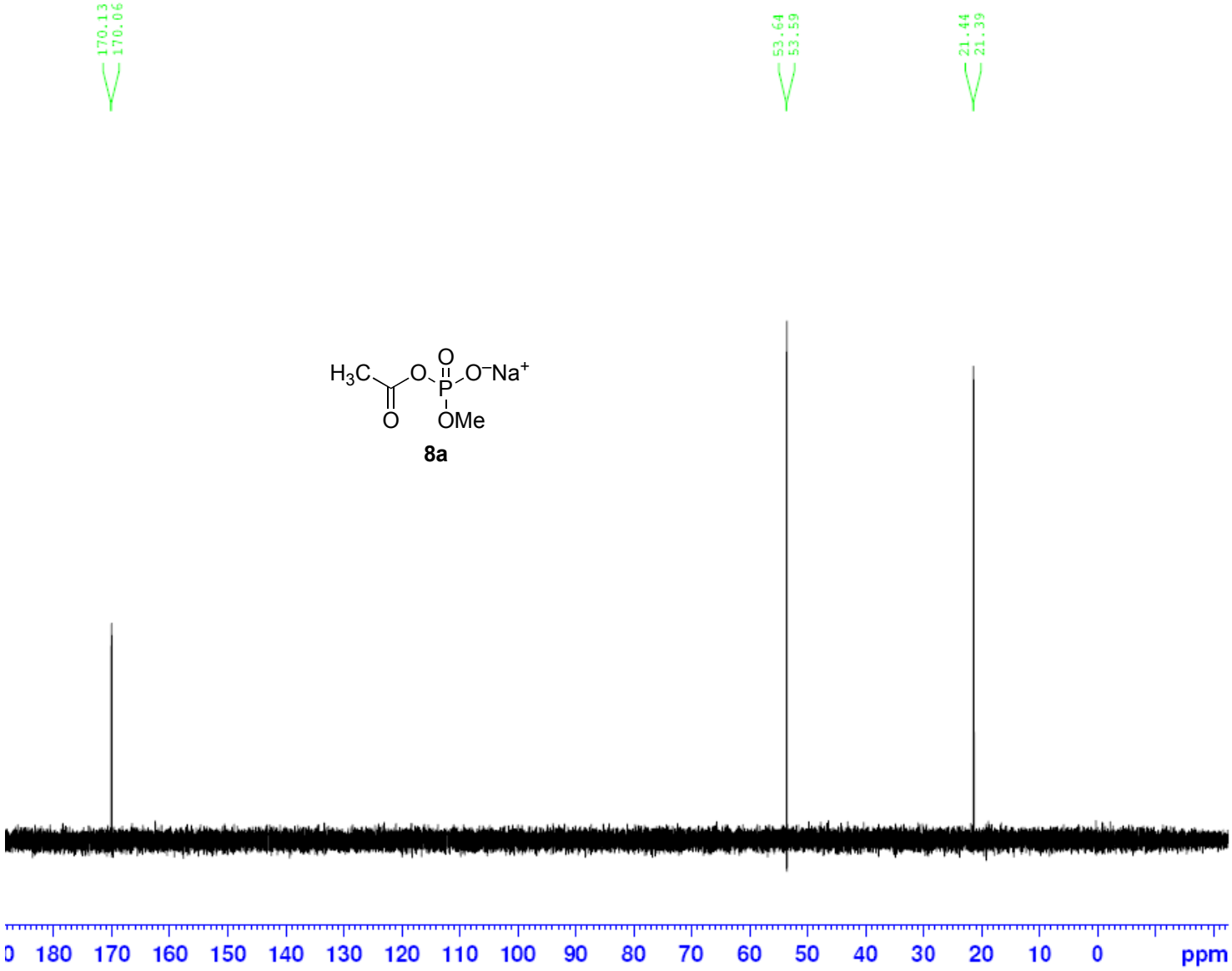


Figure 45S: ^{31}P NMR spectrum of sodium methyl acetyl phosphate **(8a)**

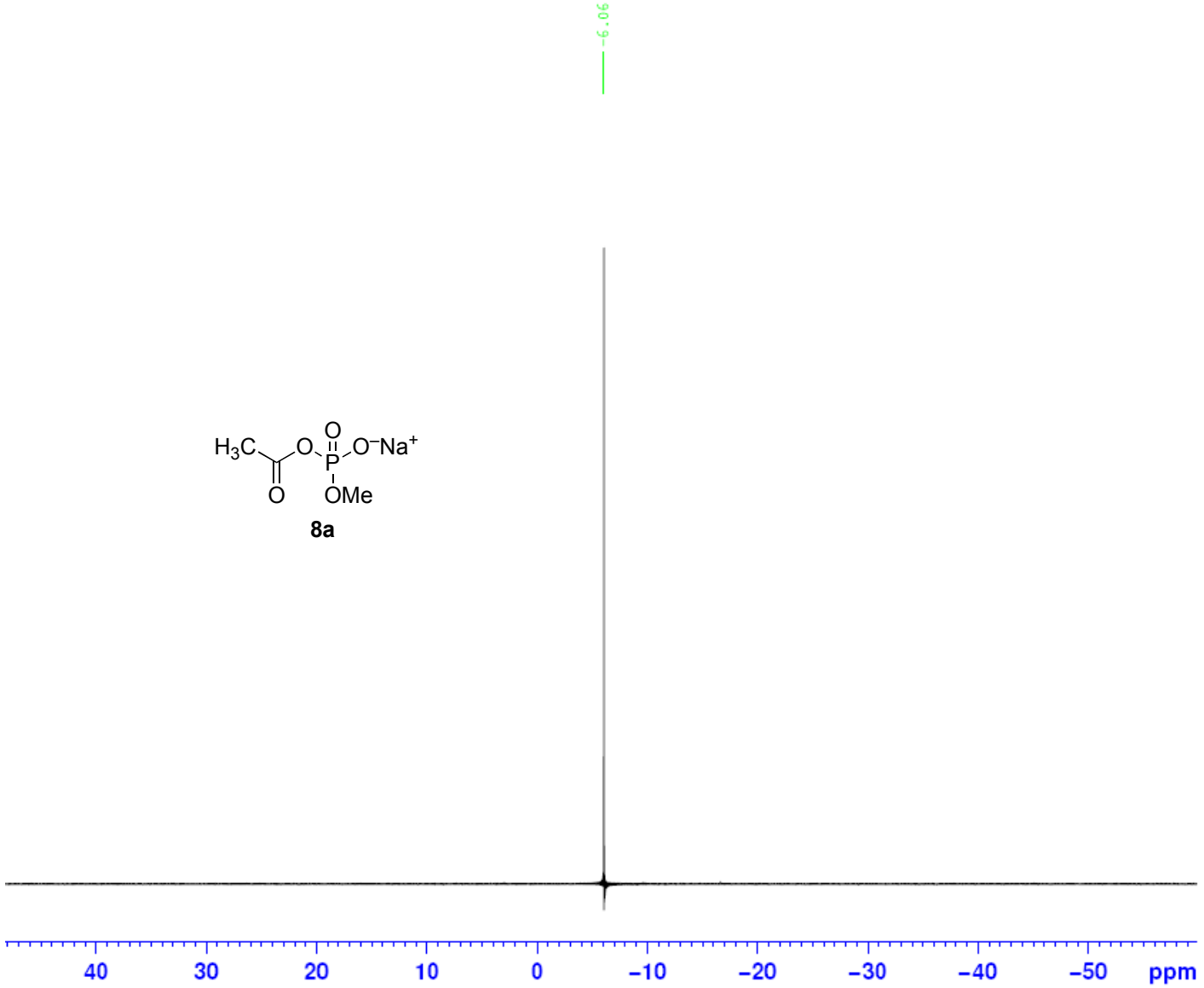


Figure 46S: ^{1}H NMR spectrum of sodium methyl butyryl phosphate **(8b)**

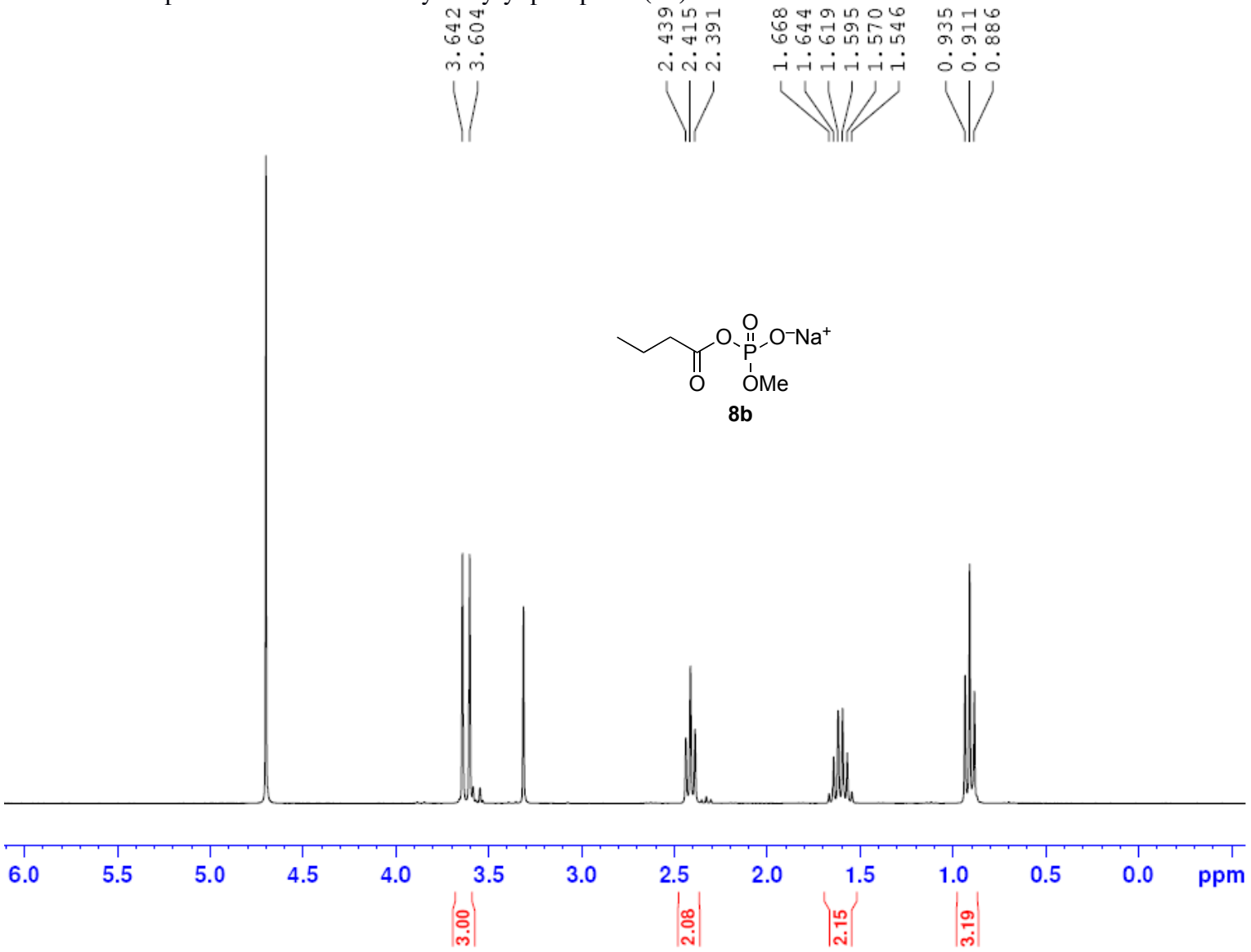


Figure 47S: ^{13}C NMR spectrum of sodium methyl butyryl phosphate **(8b)**

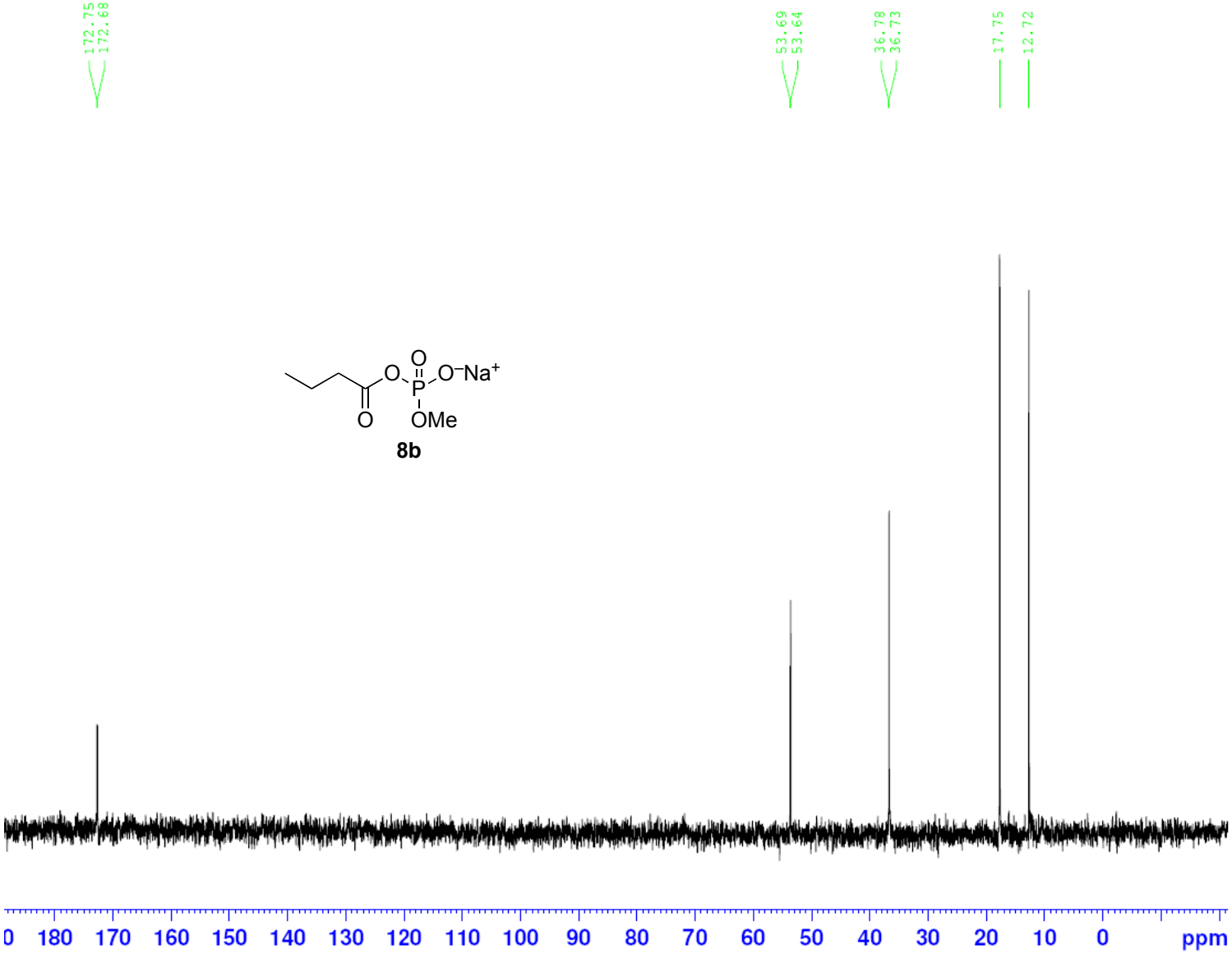


Figure 48S: ^{31}P NMR spectrum of sodium methyl butyryl phosphate **(8b)**

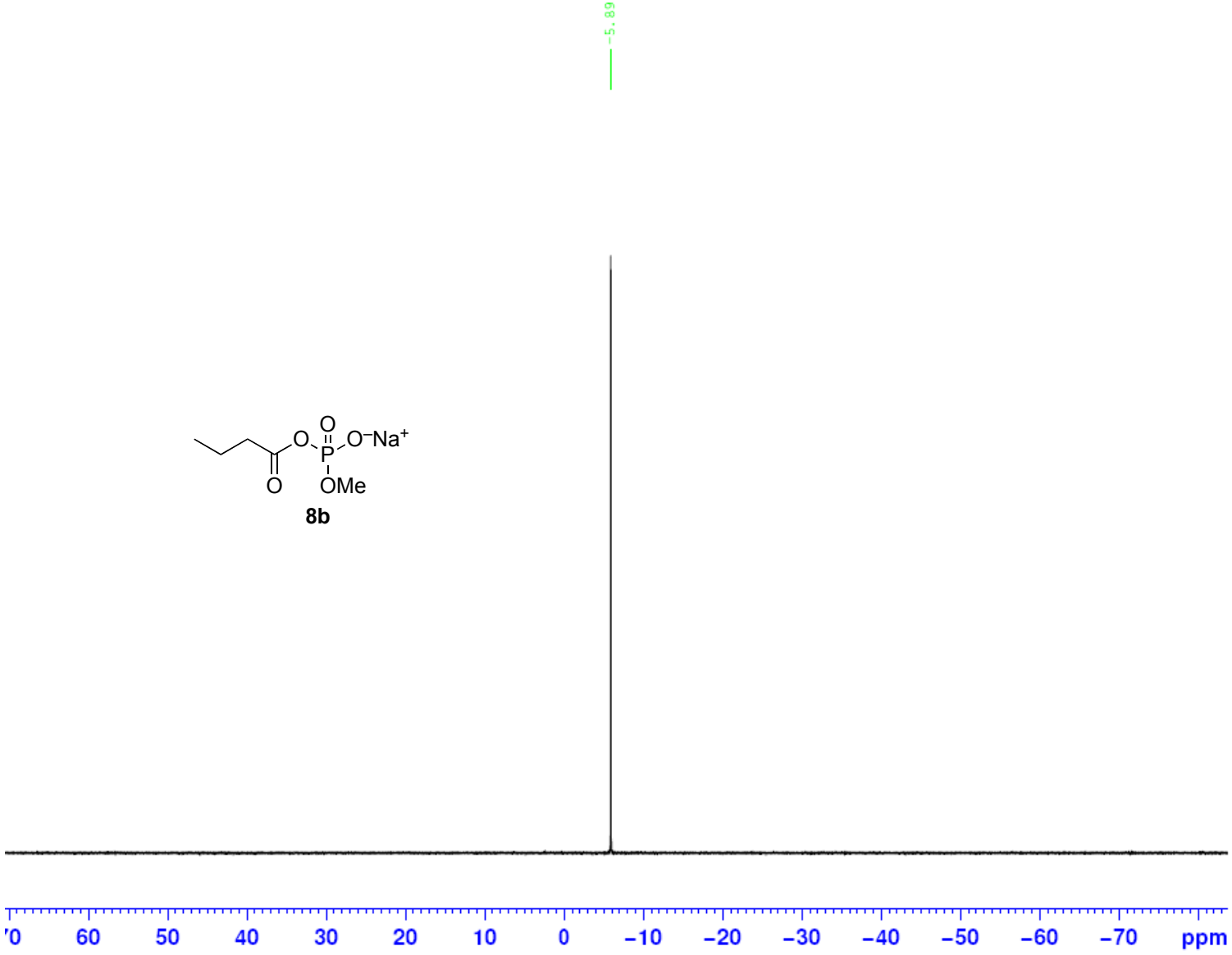


Figure 49S: ^{1}H NMR spectrum of sodium methyl isovaleryl phosphate **(8c)**

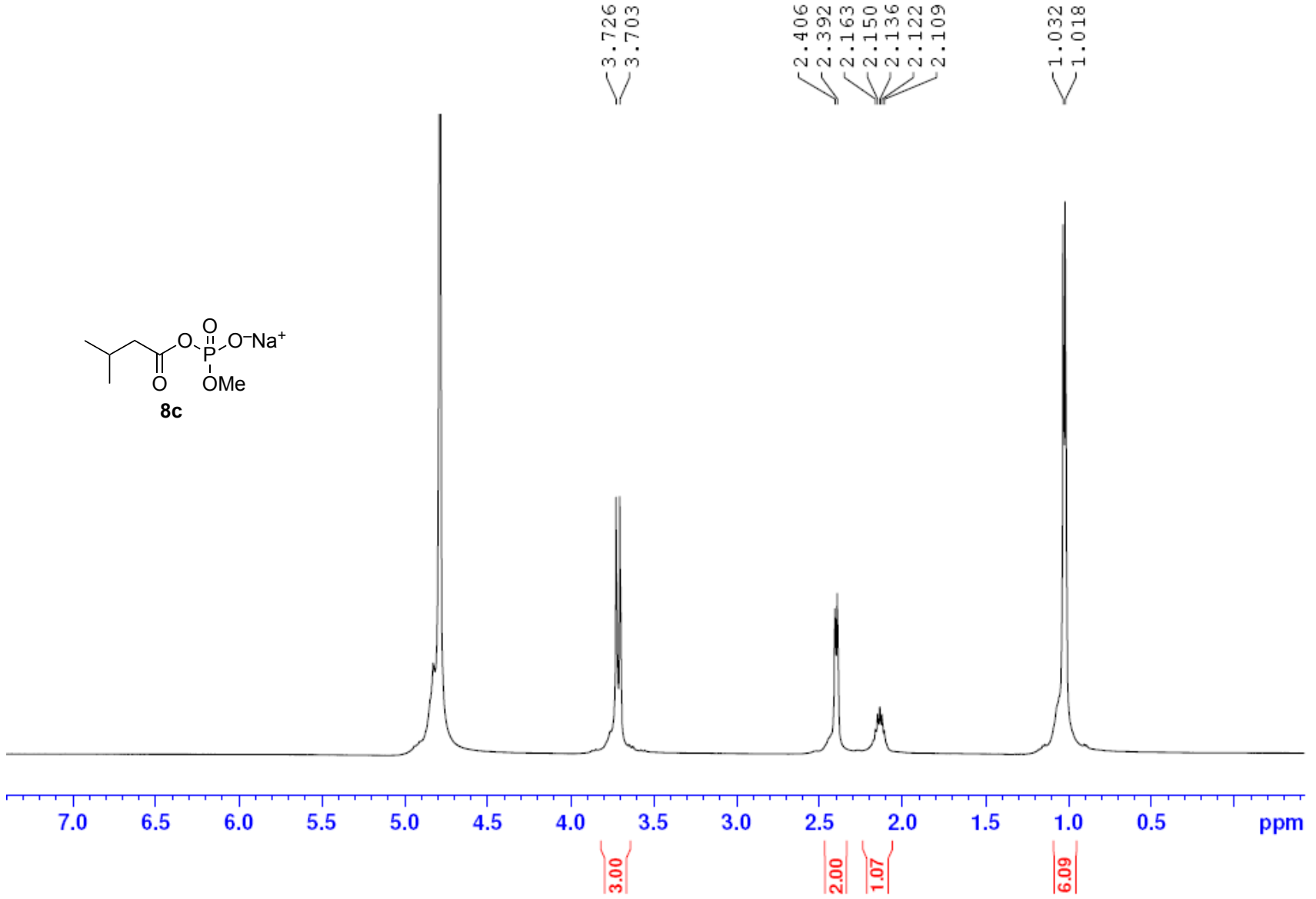


Figure 50S: ^{13}C NMR spectrum of sodium methyl isovaleryl phosphate **(8c)**

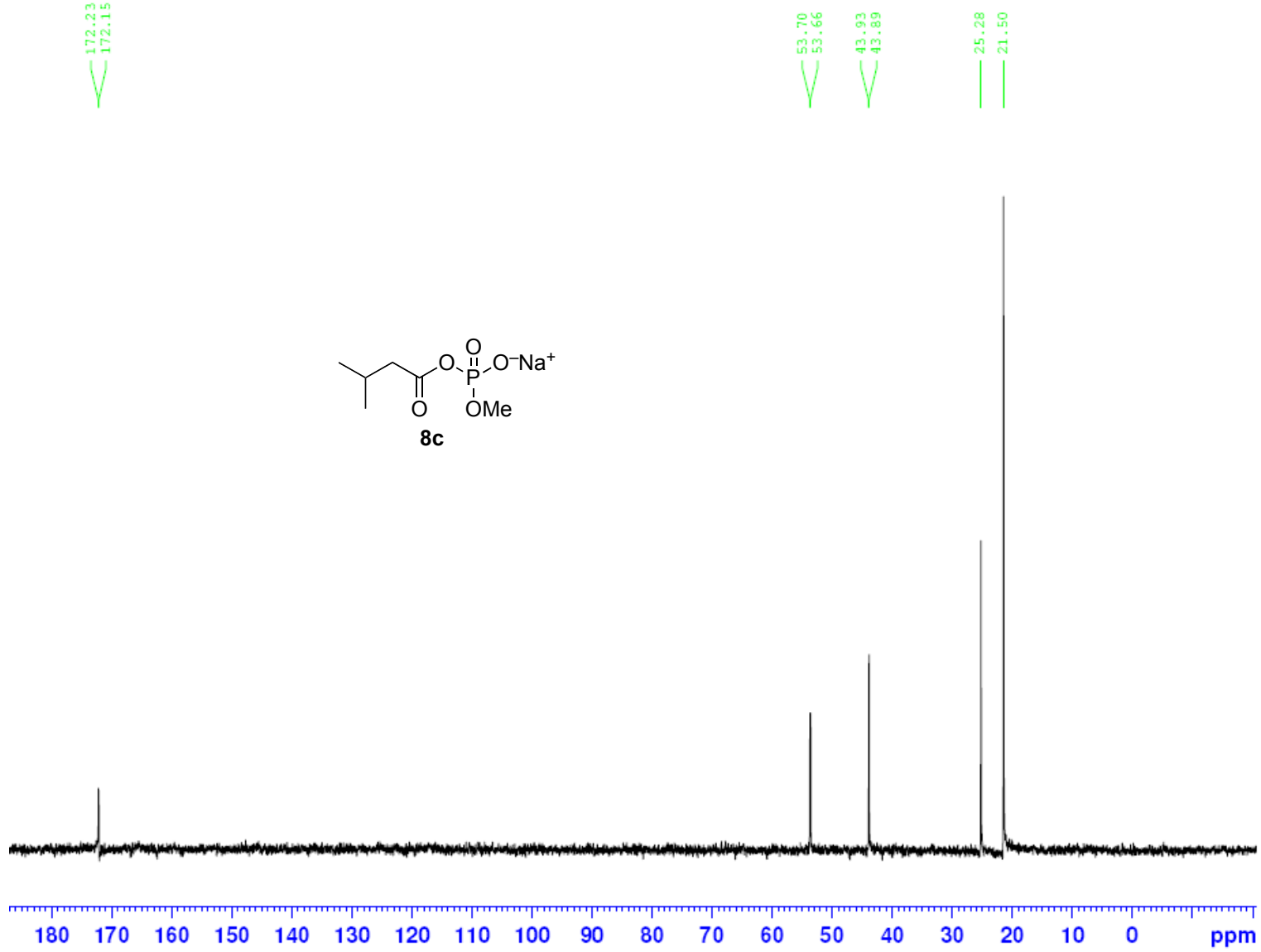


Figure 51S: ^{31}P NMR spectrum of sodium methyl isovaleryl phosphate **(8c)**

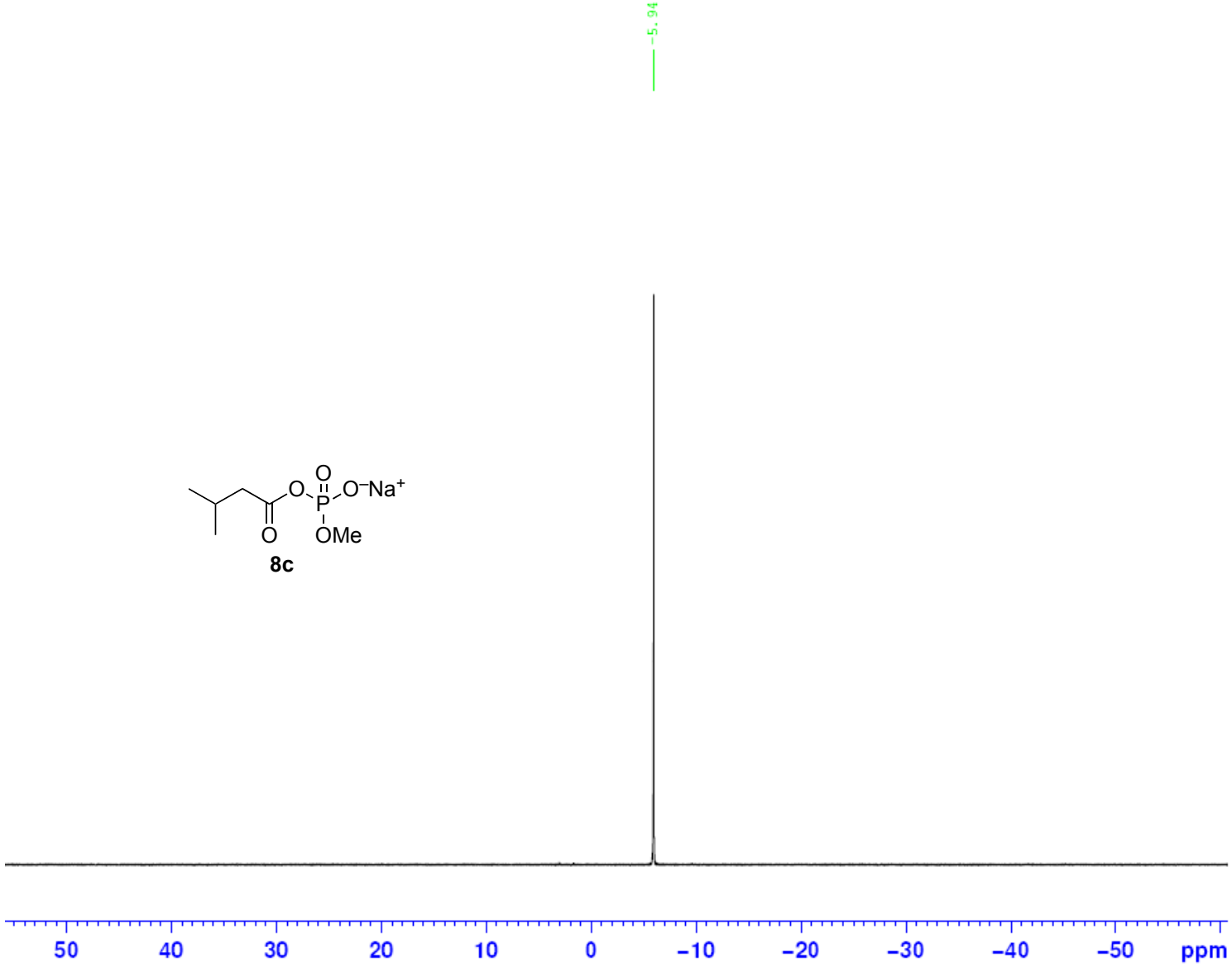


Figure 52S: ^{1}H NMR spectrum of sodium methyl octanoyl phosphate **(8d)**

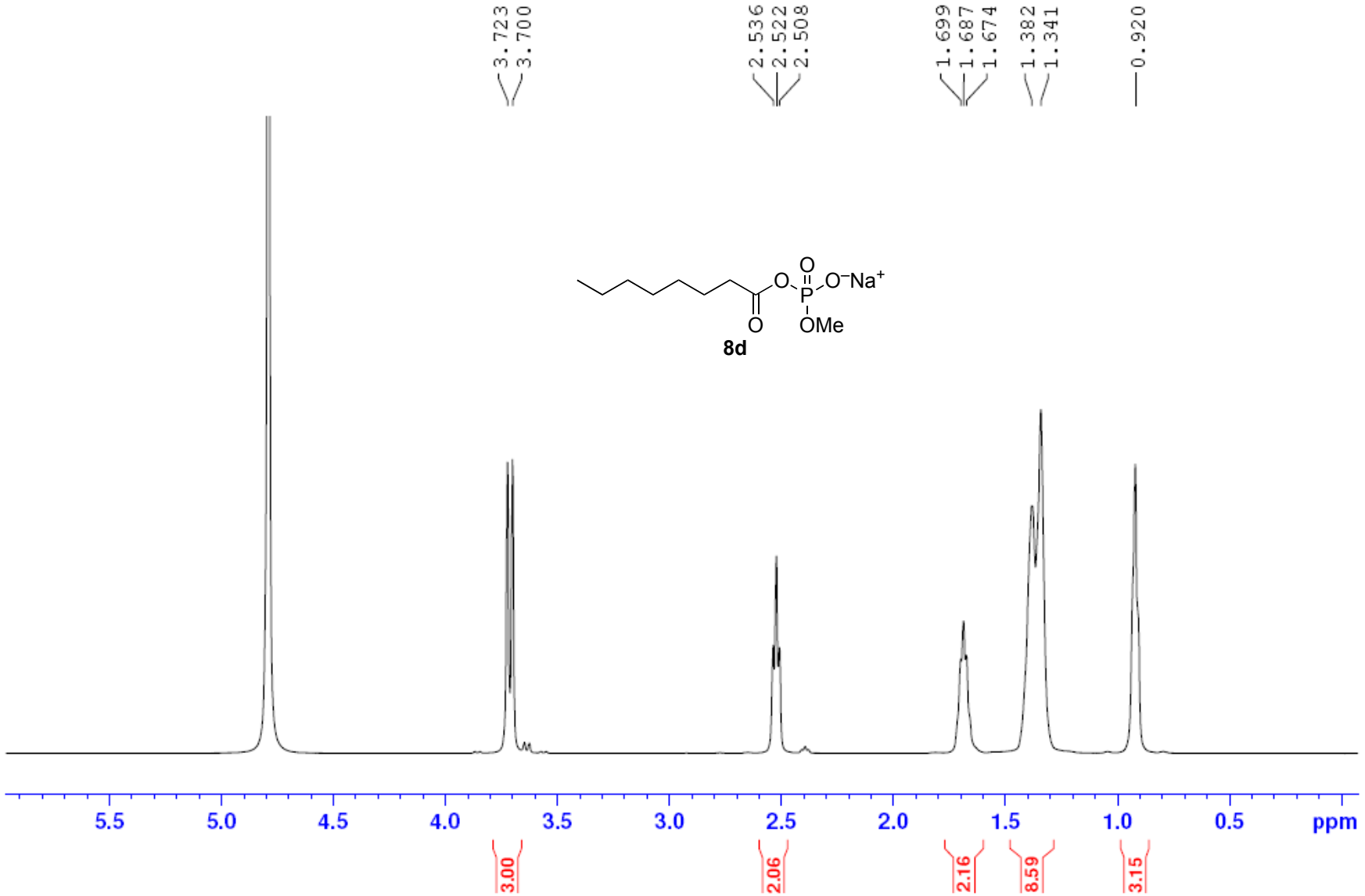


Figure 53S: ^{13}C NMR spectrum of sodium methyl octanoyl phosphate **(8d)**

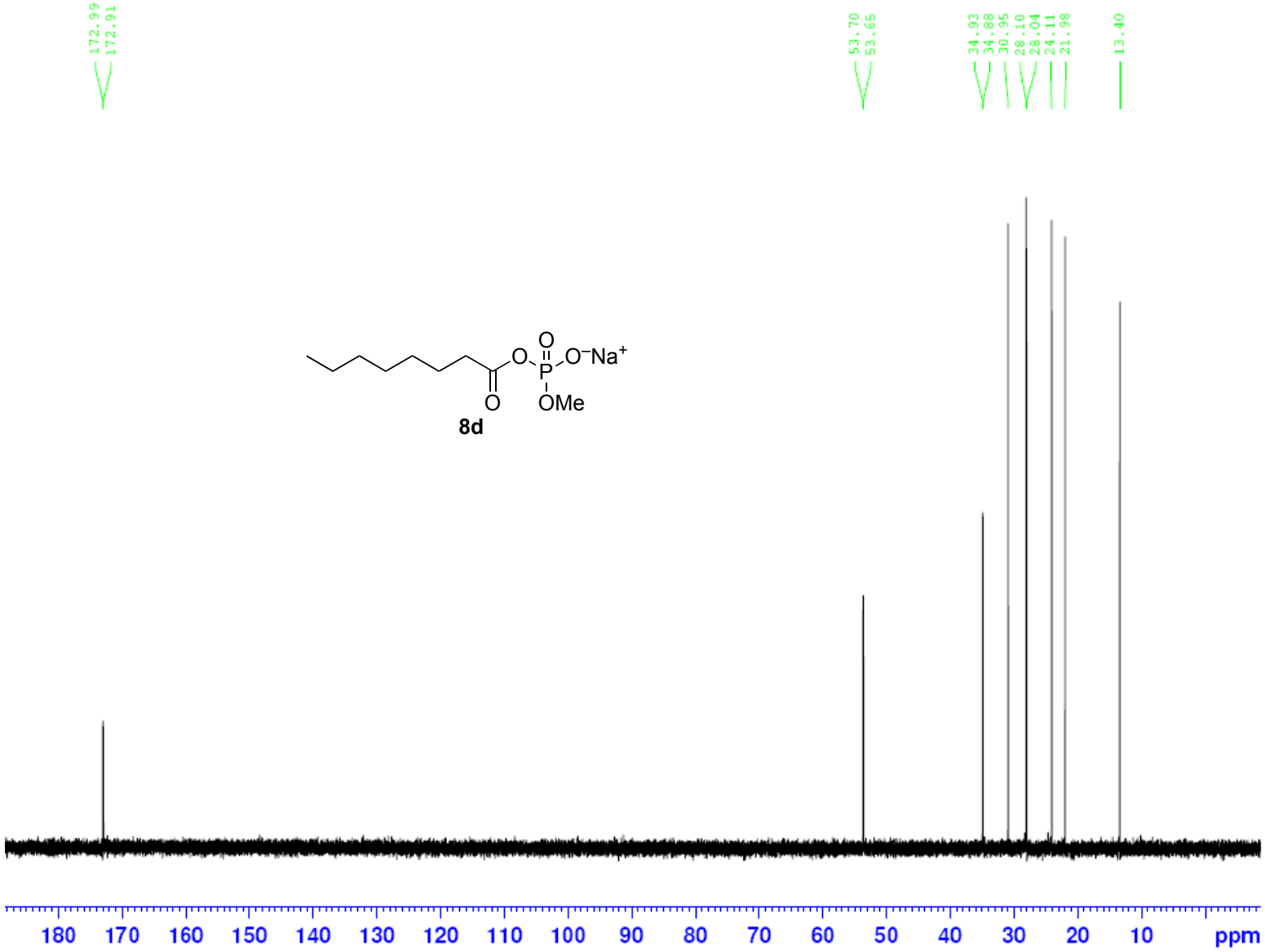


Figure 54S: ^{31}P NMR spectrum of sodium methyl octanoyl phosphate **(8d)**

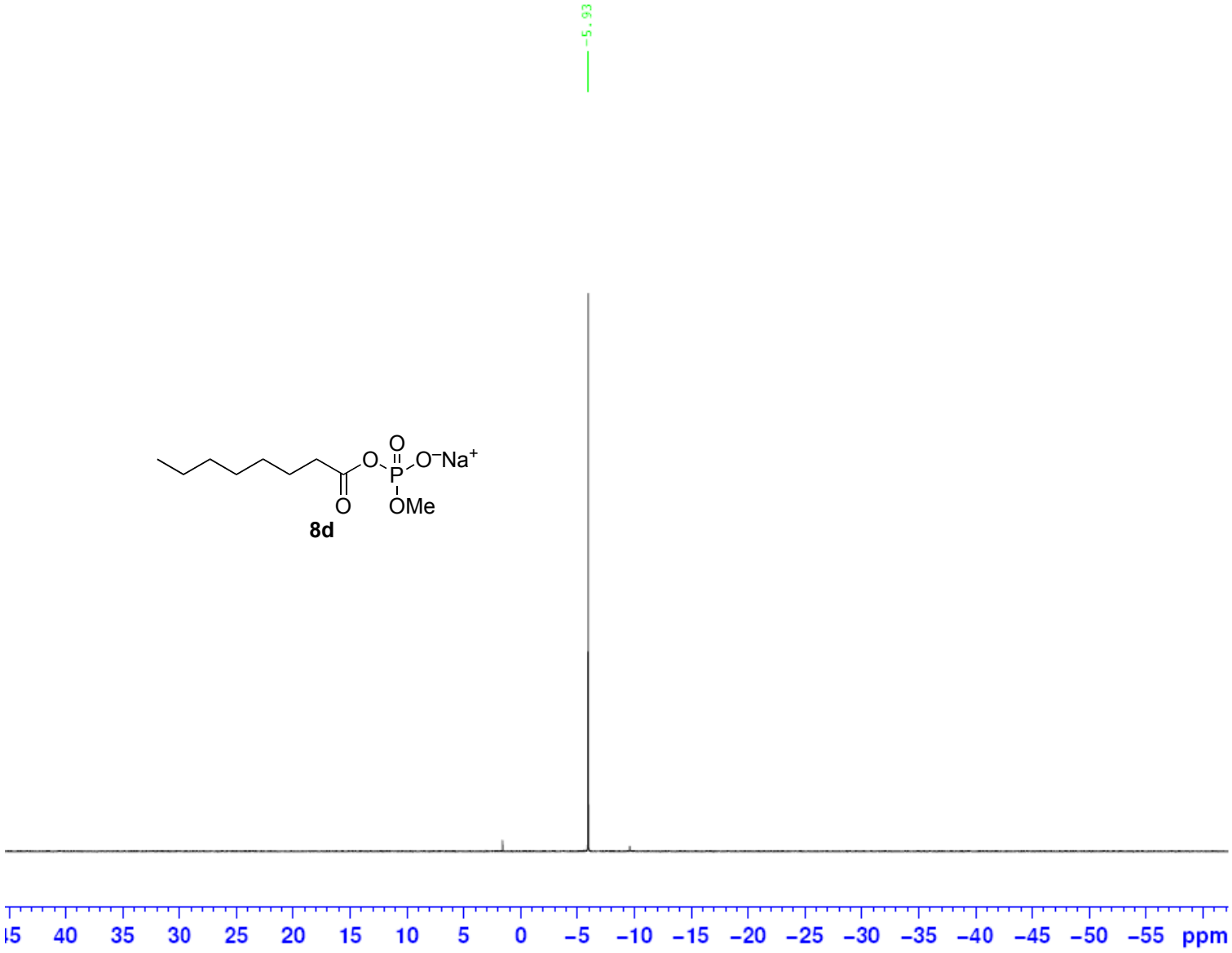


Figure 55S: ^{1}H NMR spectrum of sodium methyl palmitoyl phosphate **(8e)**

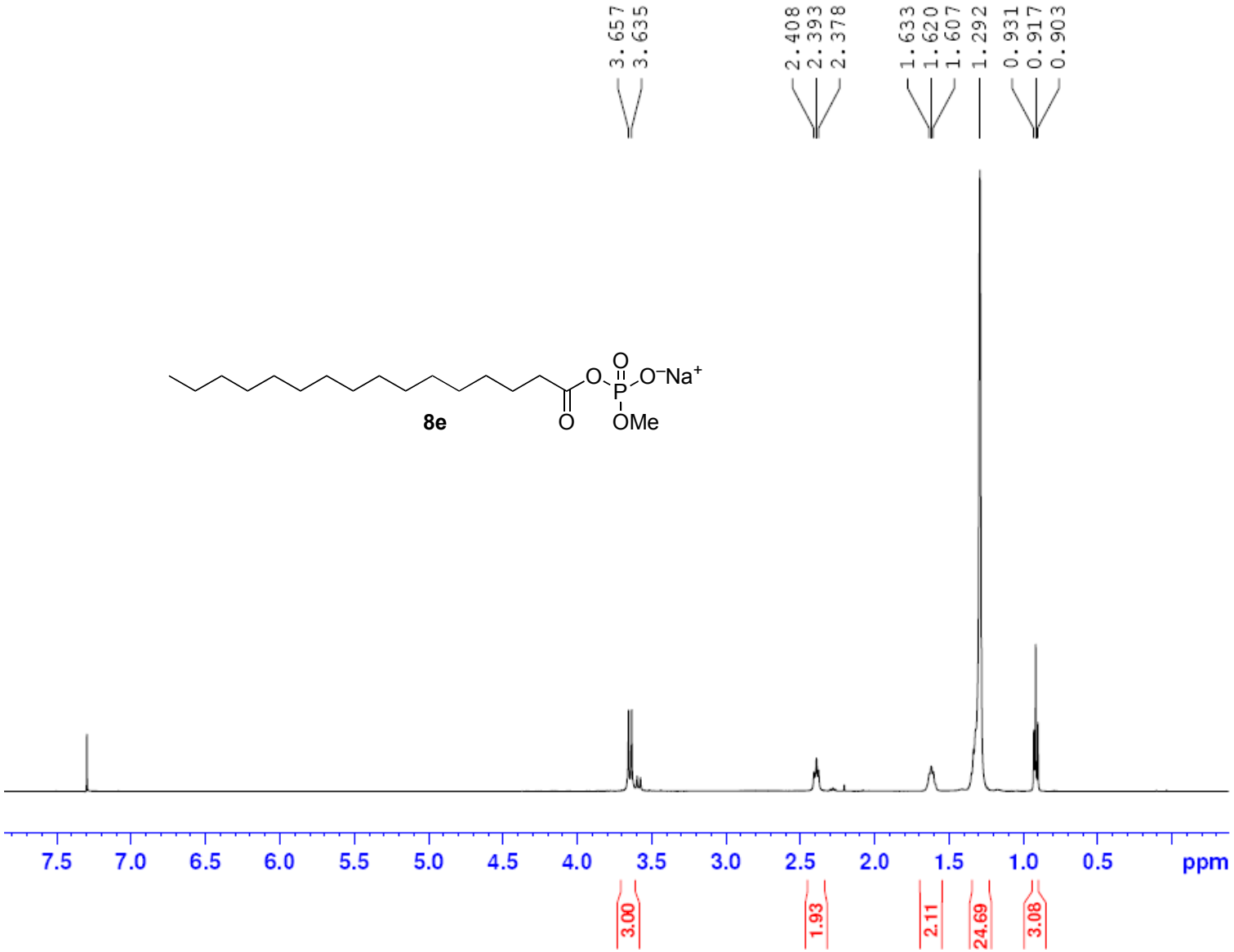


Figure 56S: ^{13}C NMR spectrum of sodium methyl palmitoyl phosphate **(8e)**

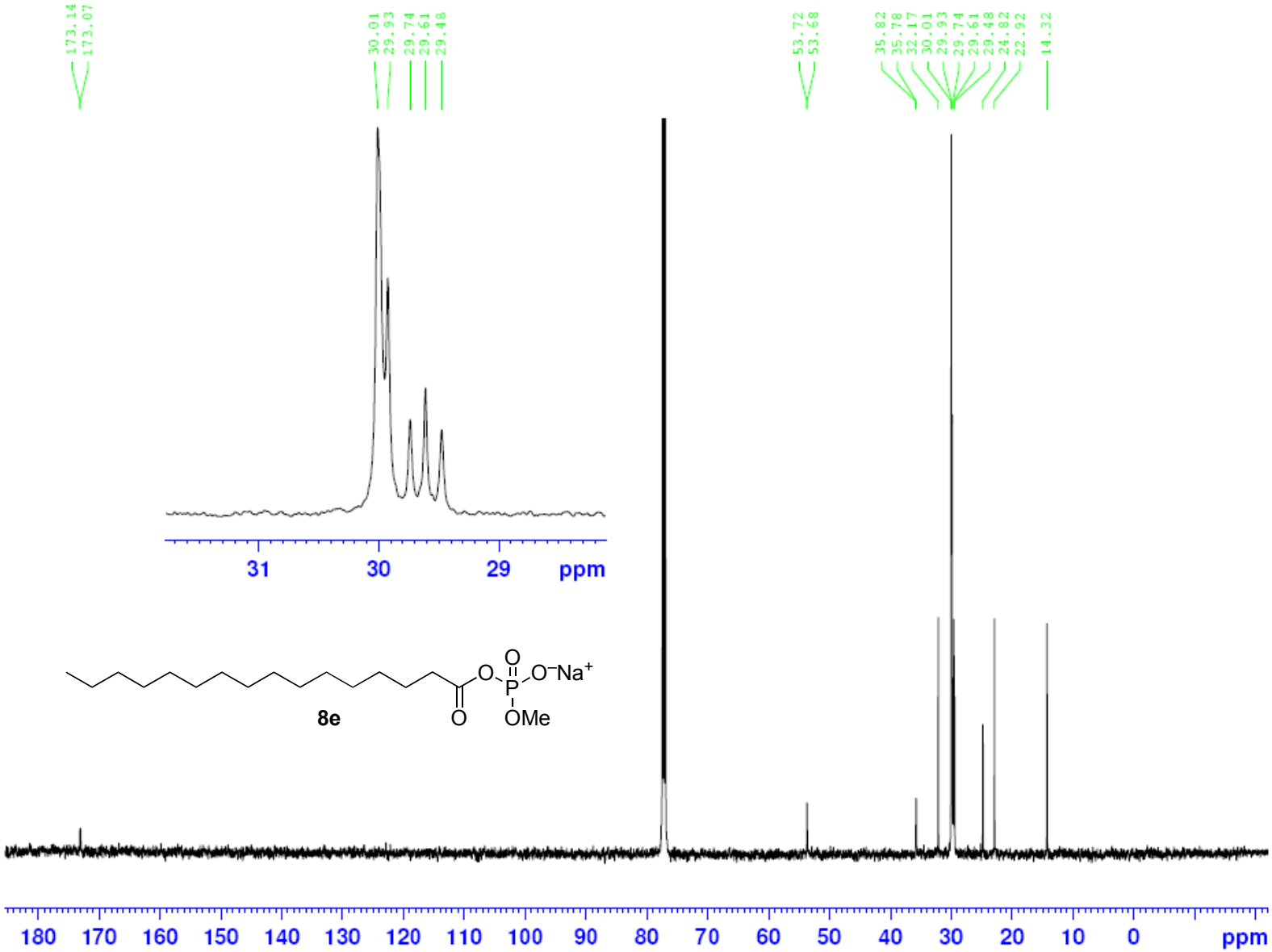


Figure 57S: ^{31}P NMR spectrum of sodium methyl palmitoyl phosphate **(8e)**

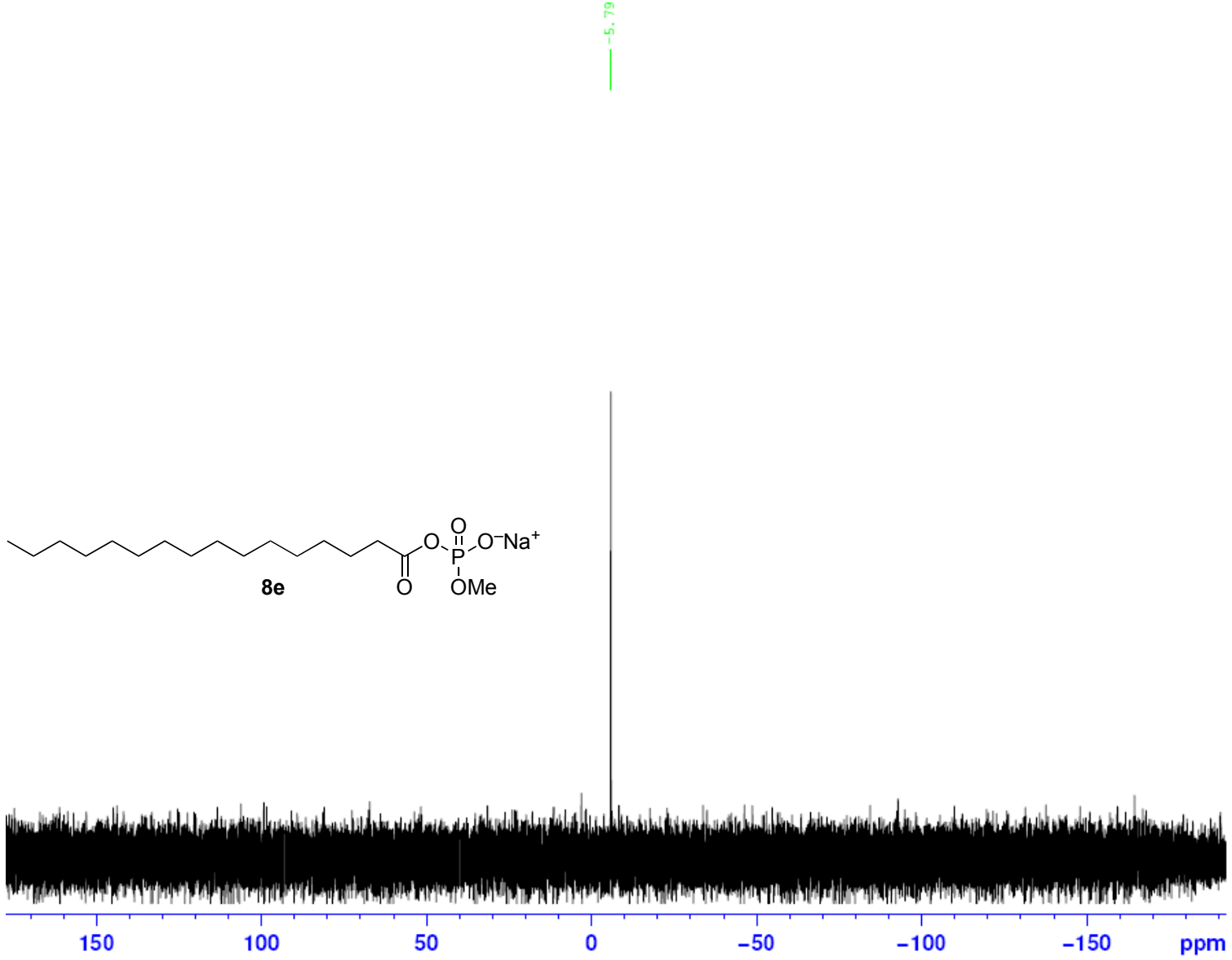


Figure 58S: ^{1}H NMR spectrum of sodium methyl ibuprofenoyl phosphate **(8f)**

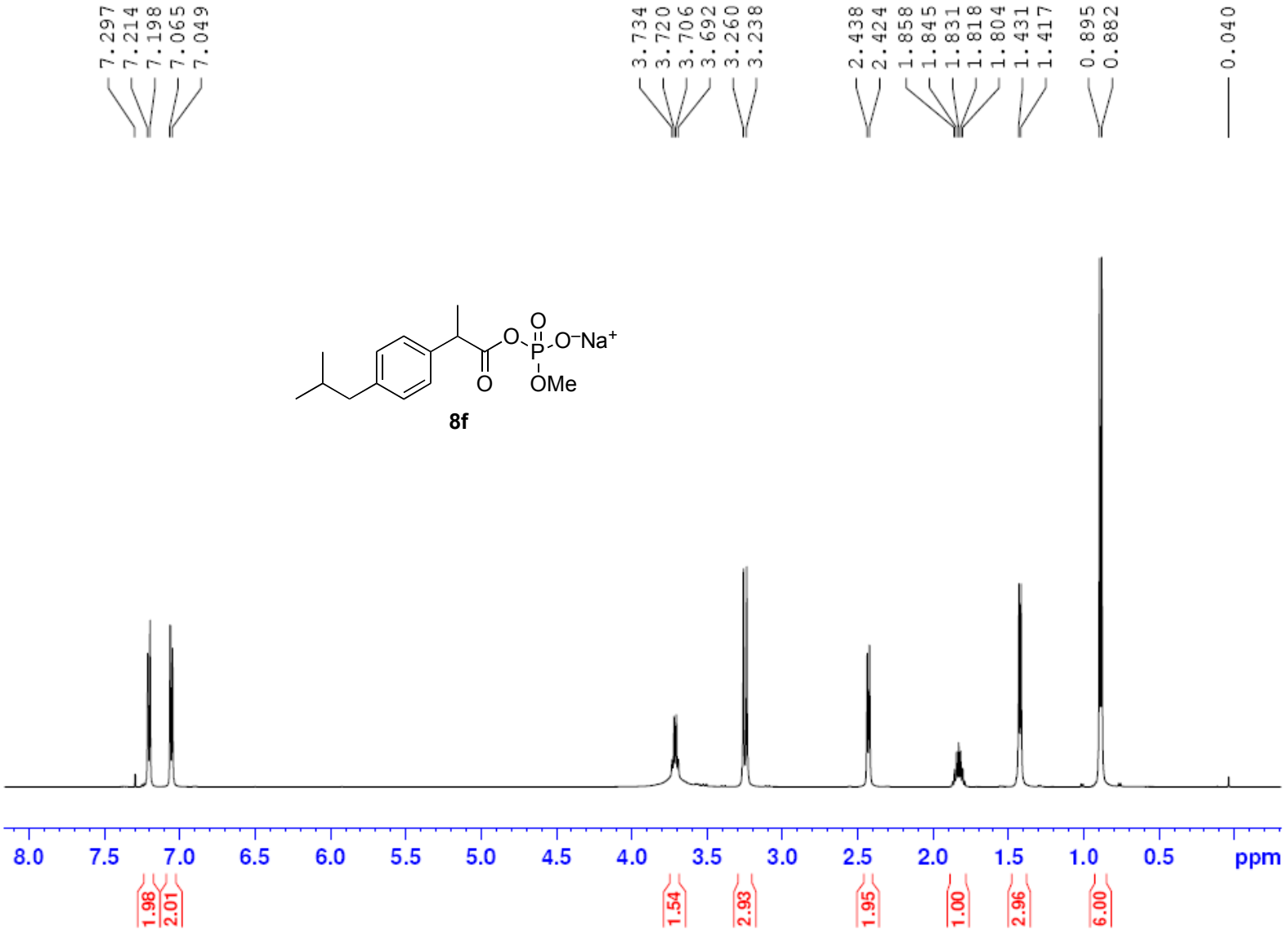


Figure 59S: ^{13}C NMR spectrum of sodium methyl ibuprofenoyl phosphate **(8f)**

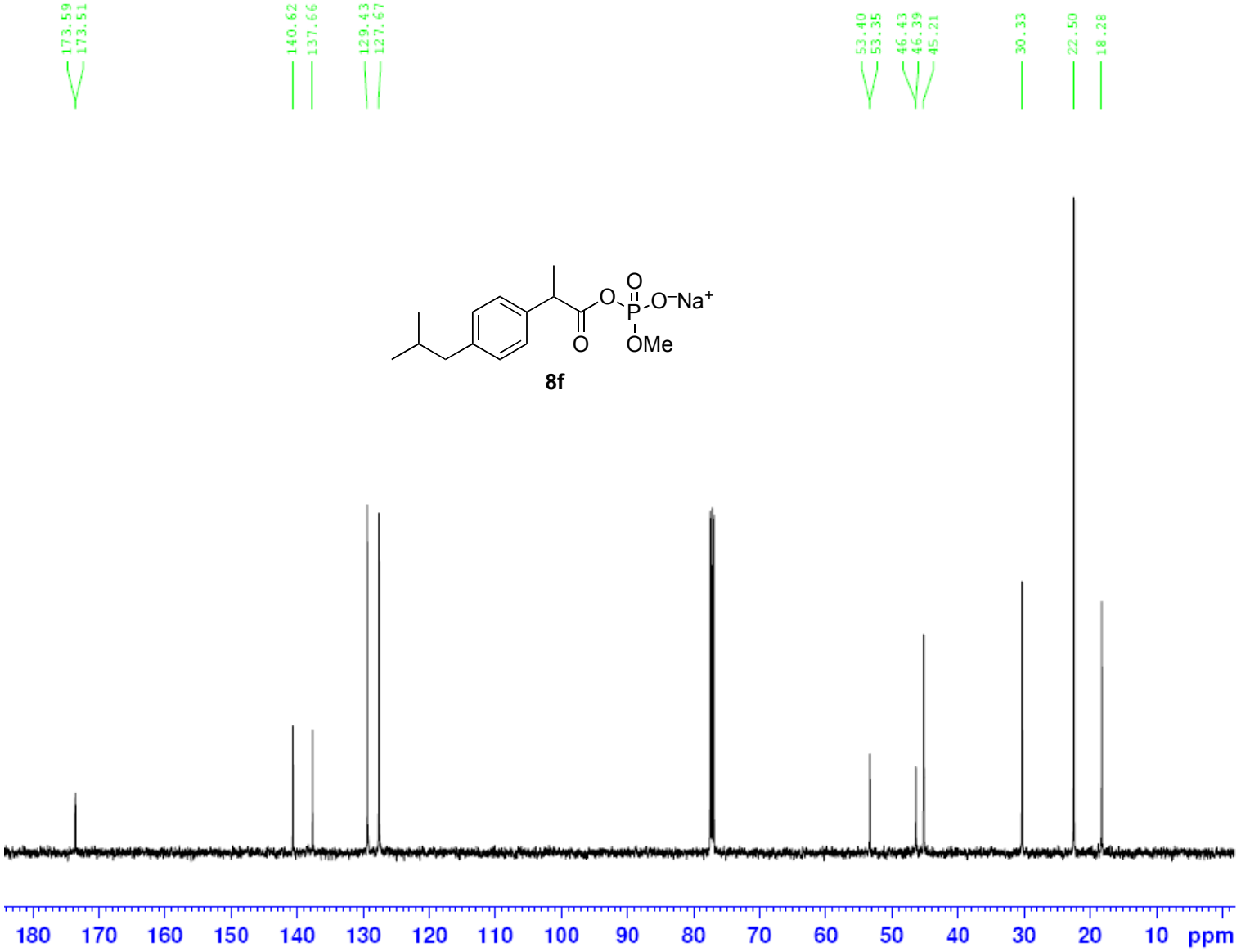


Figure 60S: ^{31}P NMR spectrum of sodium methyl ibuprofenoyl phosphate **(8f)**

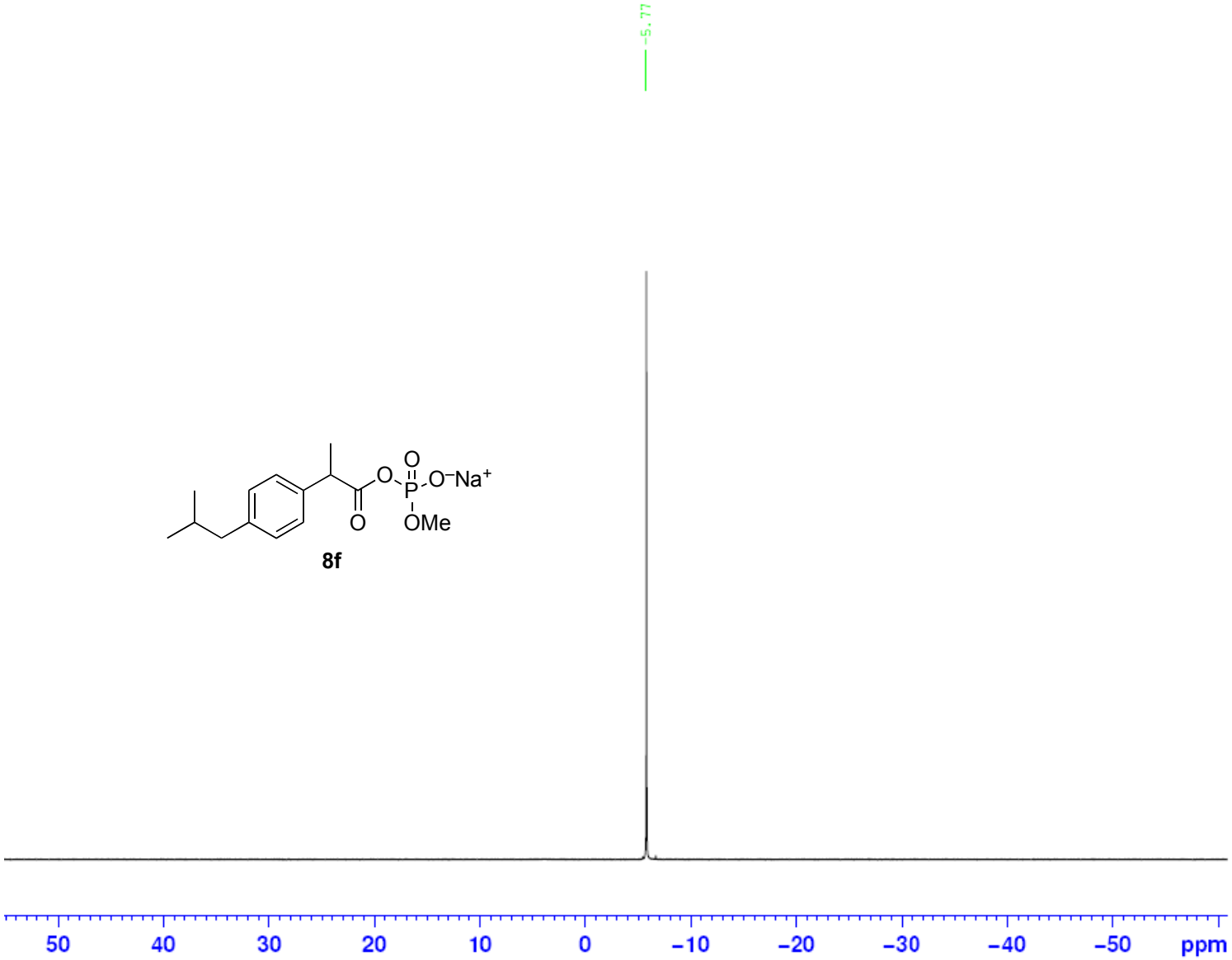


Figure 61S: ^{1}H NMR spectrum of sodium methyl fenoprofenoyl phosphate **(8g)**

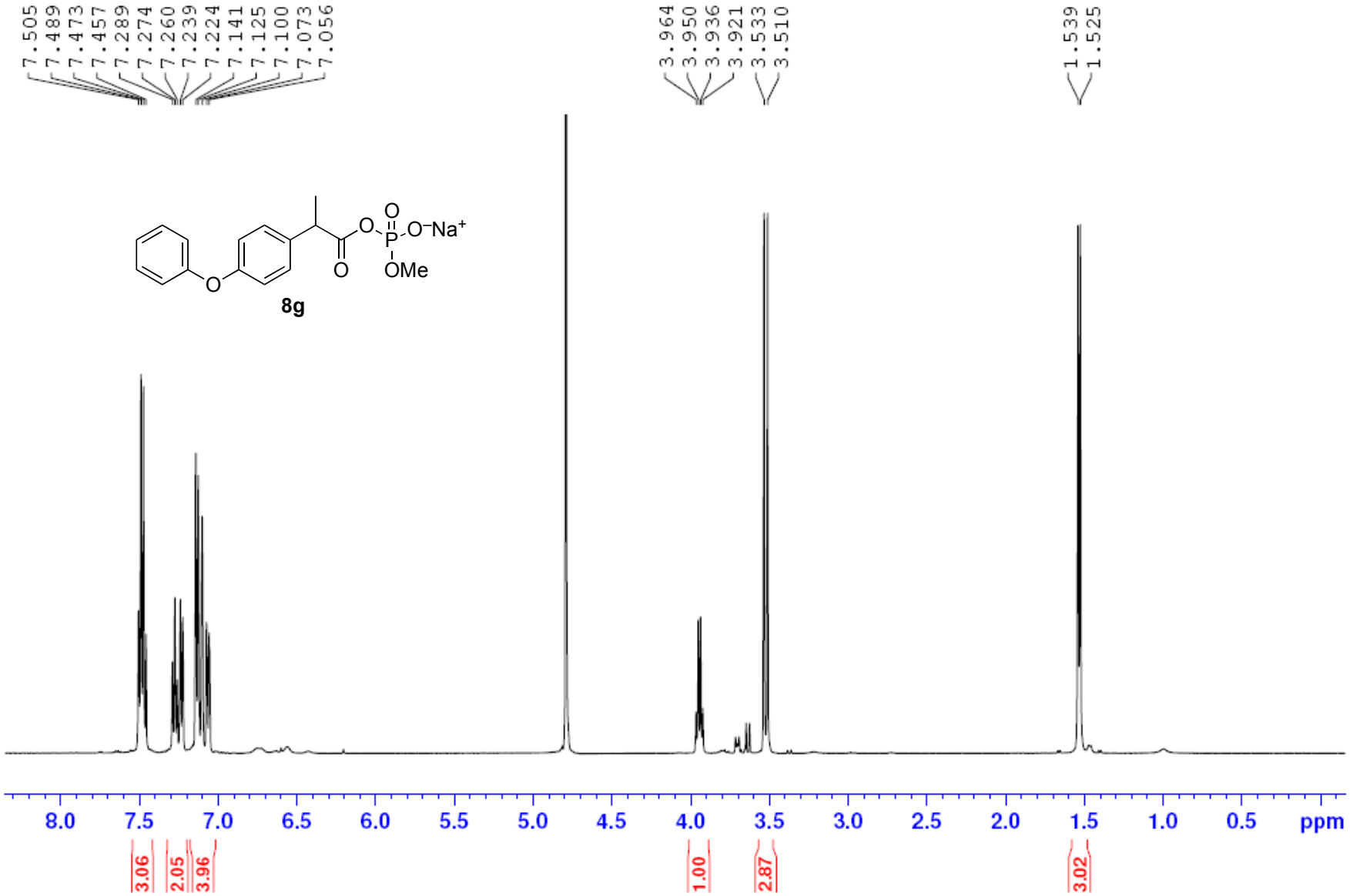


Figure 62S: ^{13}C NMR spectrum of sodium methyl fenoprofenoyl phosphate **(8g)**

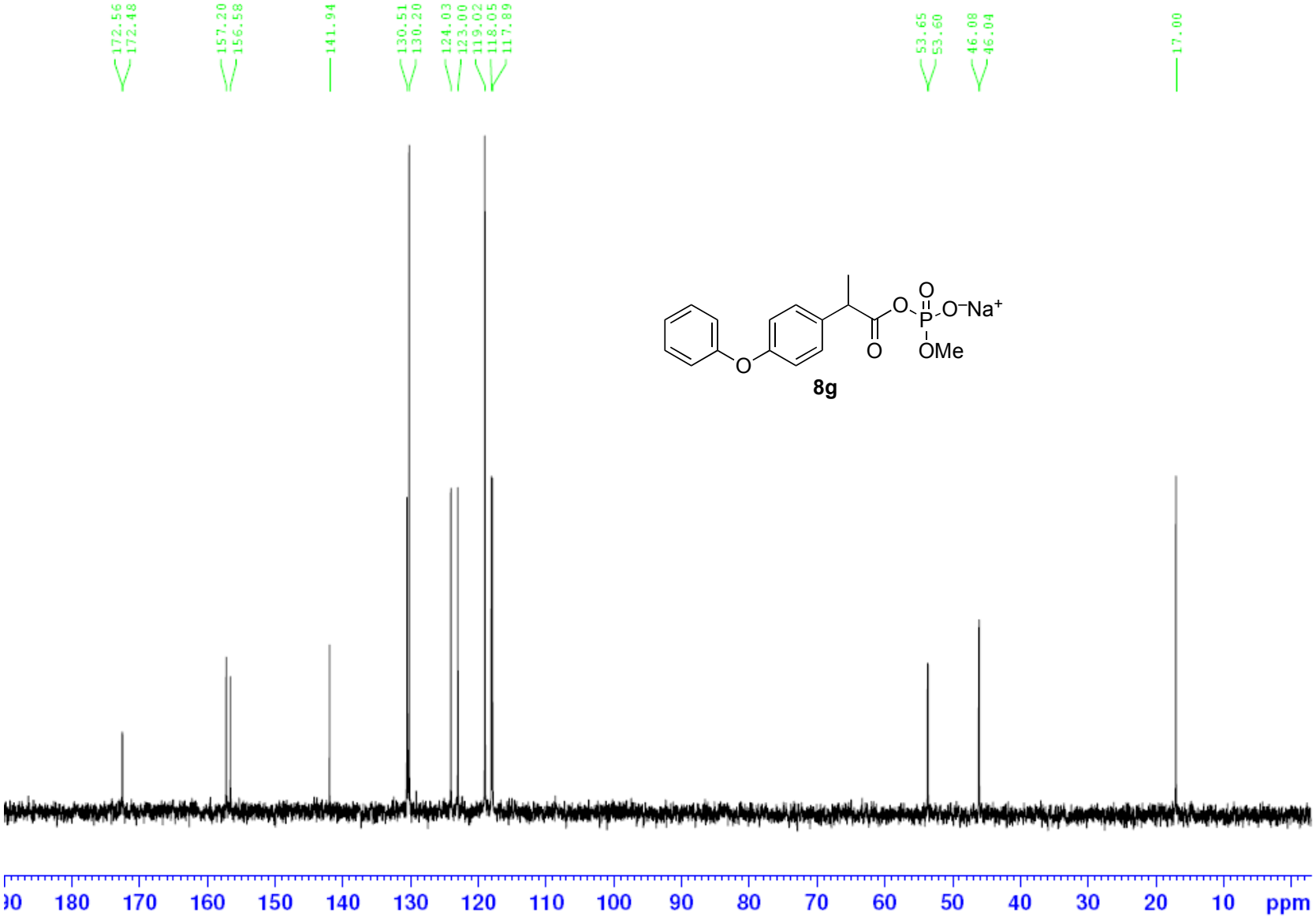


Figure 63S: ^{31}P NMR spectrum of sodium methyl fenoprofenoyl phosphate **(8g)**

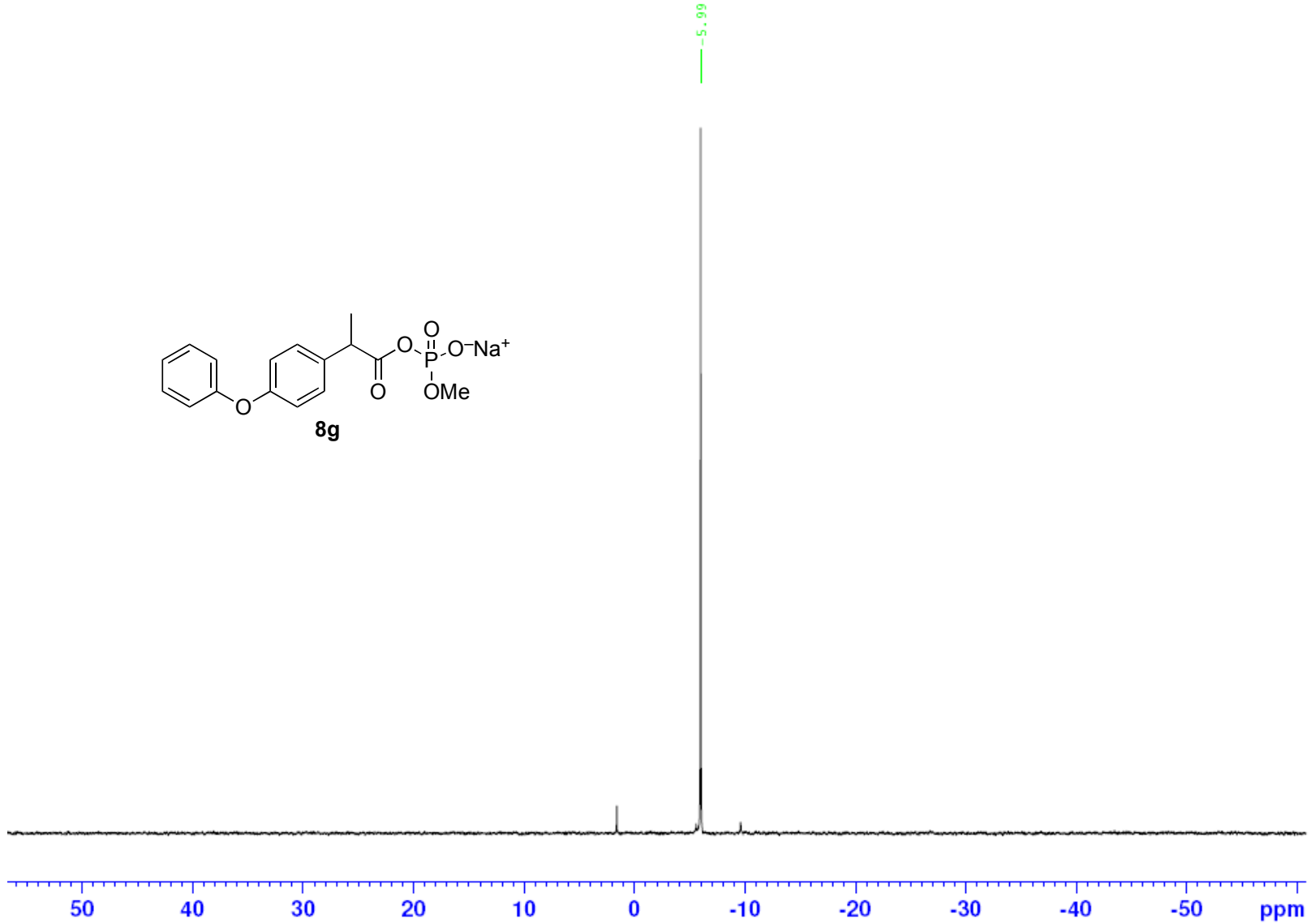


Figure 64S: ^{1}H NMR spectrum of sodium methyl 3-phenylpropanoyl phosphate **(8h)**

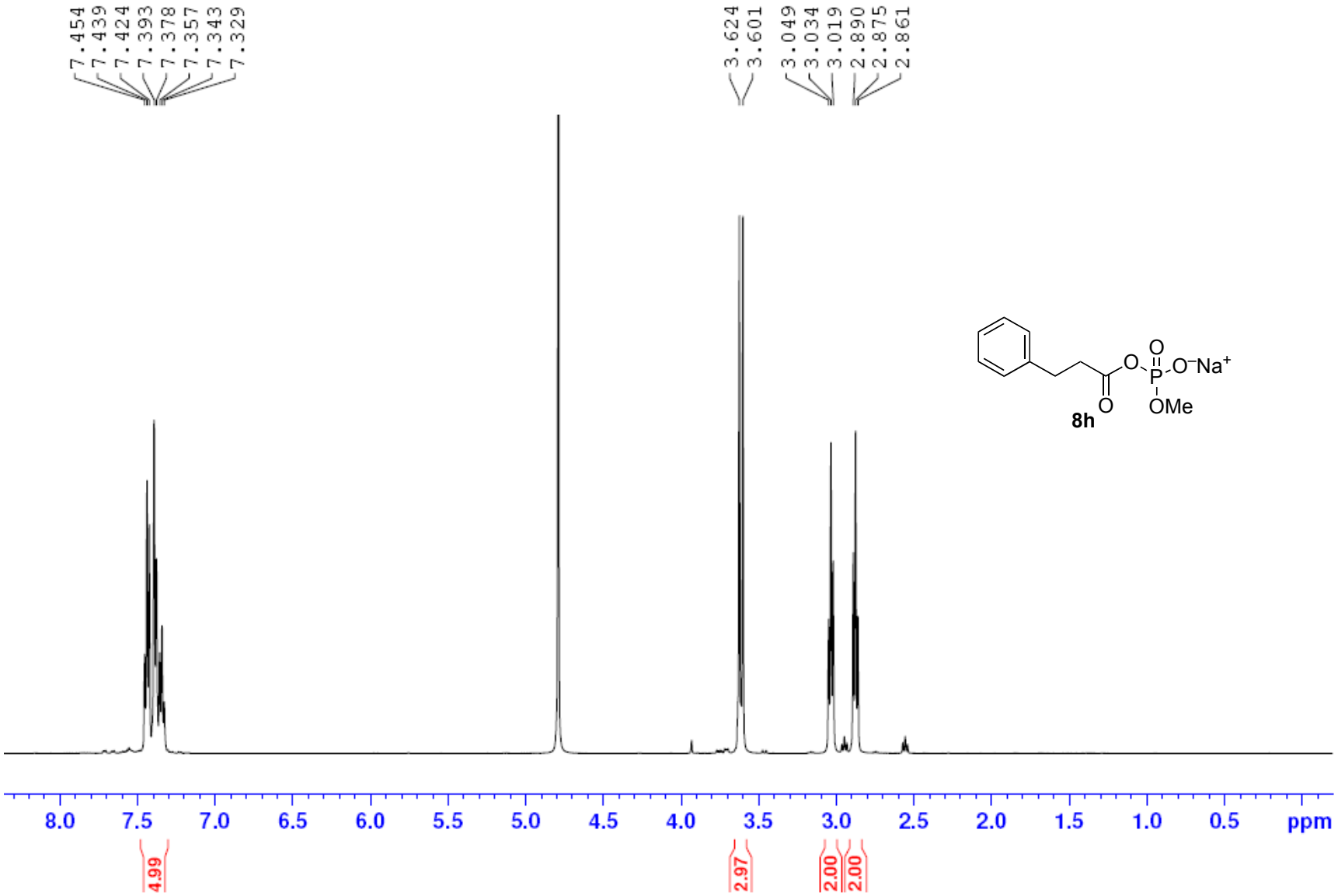


Figure 65S: ^{13}C NMR spectrum of sodium methyl 3-phenylpropanoyl phosphate **(8h)**

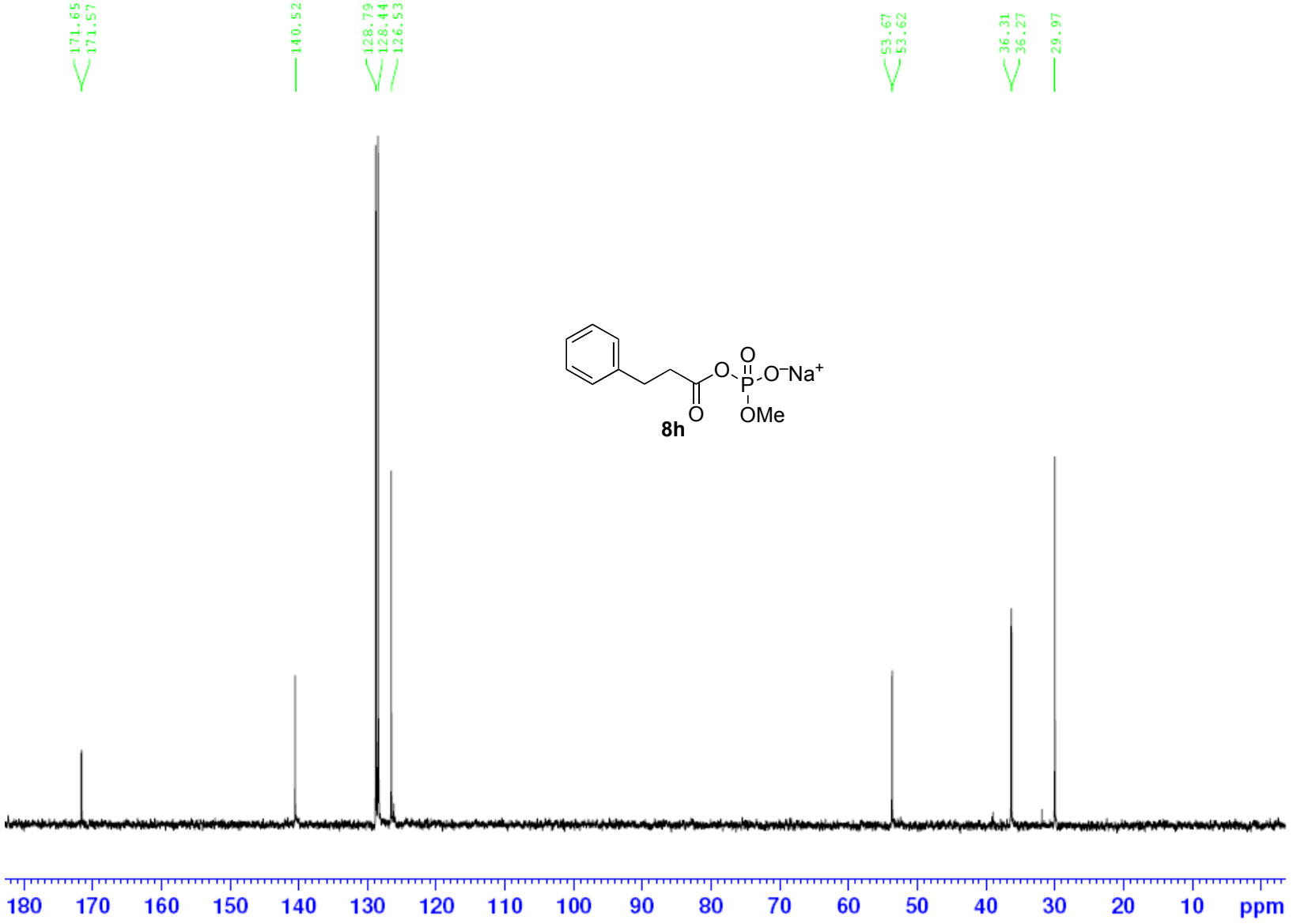


Figure 66S: ^{31}P NMR spectrum of sodium methyl 3-phenylpropanoyl phosphate **(8h)**

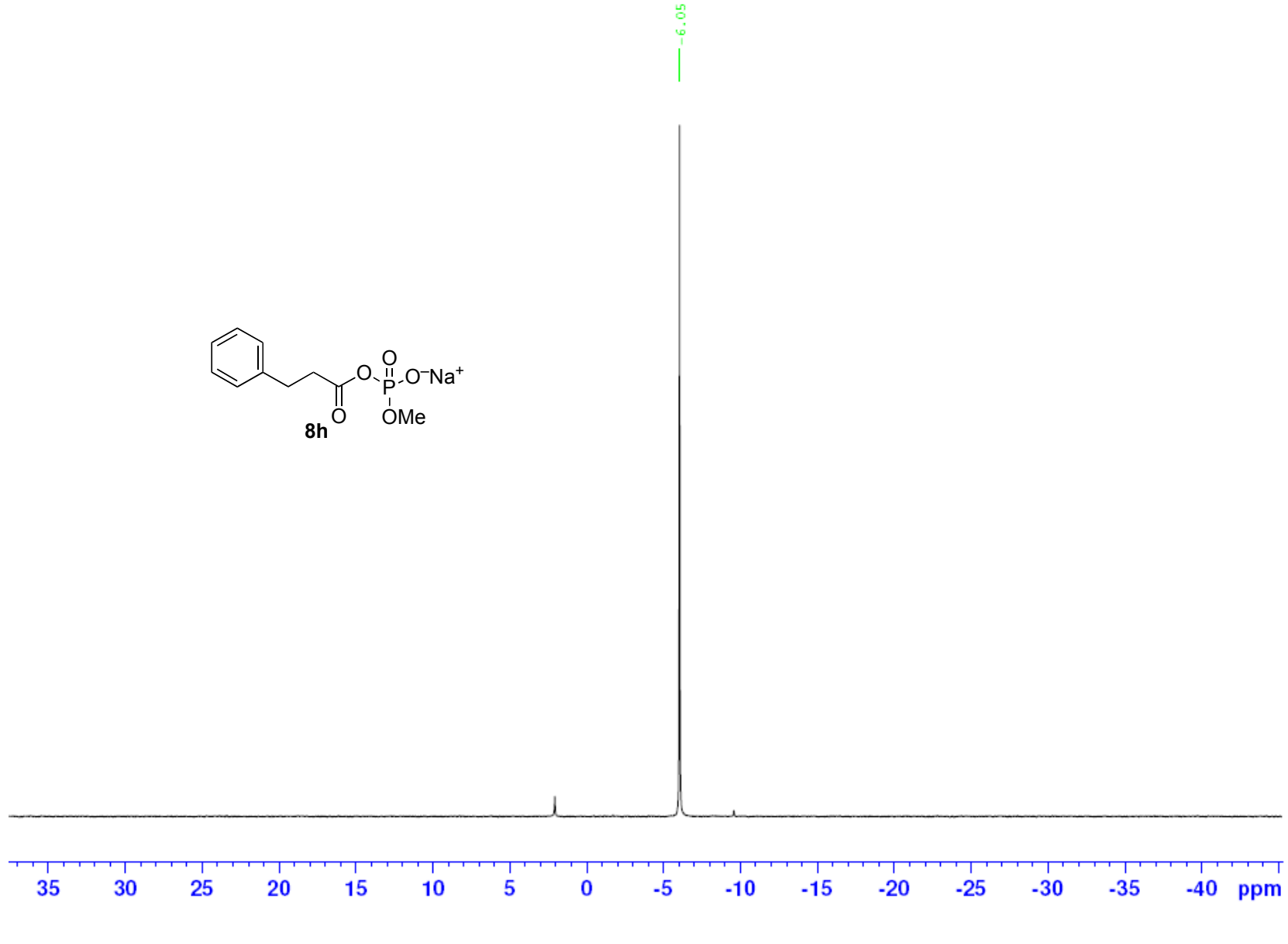


Figure 67S: ^{1}H NMR spectrum of sodium methyl *trans*-cinnamoyl phosphate **(8i)**

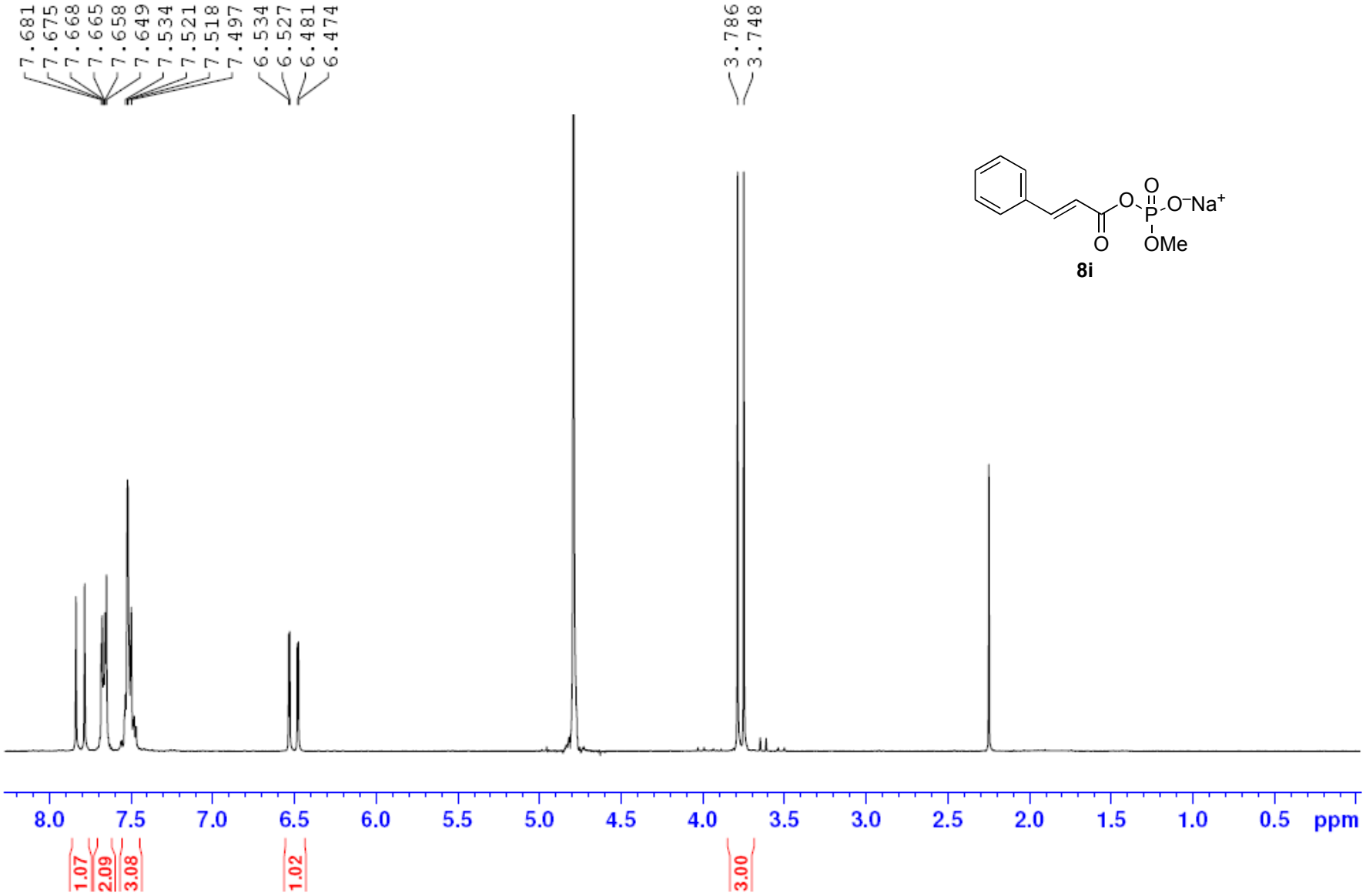


Figure 68S: ^{13}C NMR spectrum of sodium methyl *trans*-cinnamoyl phosphate **(8i)**

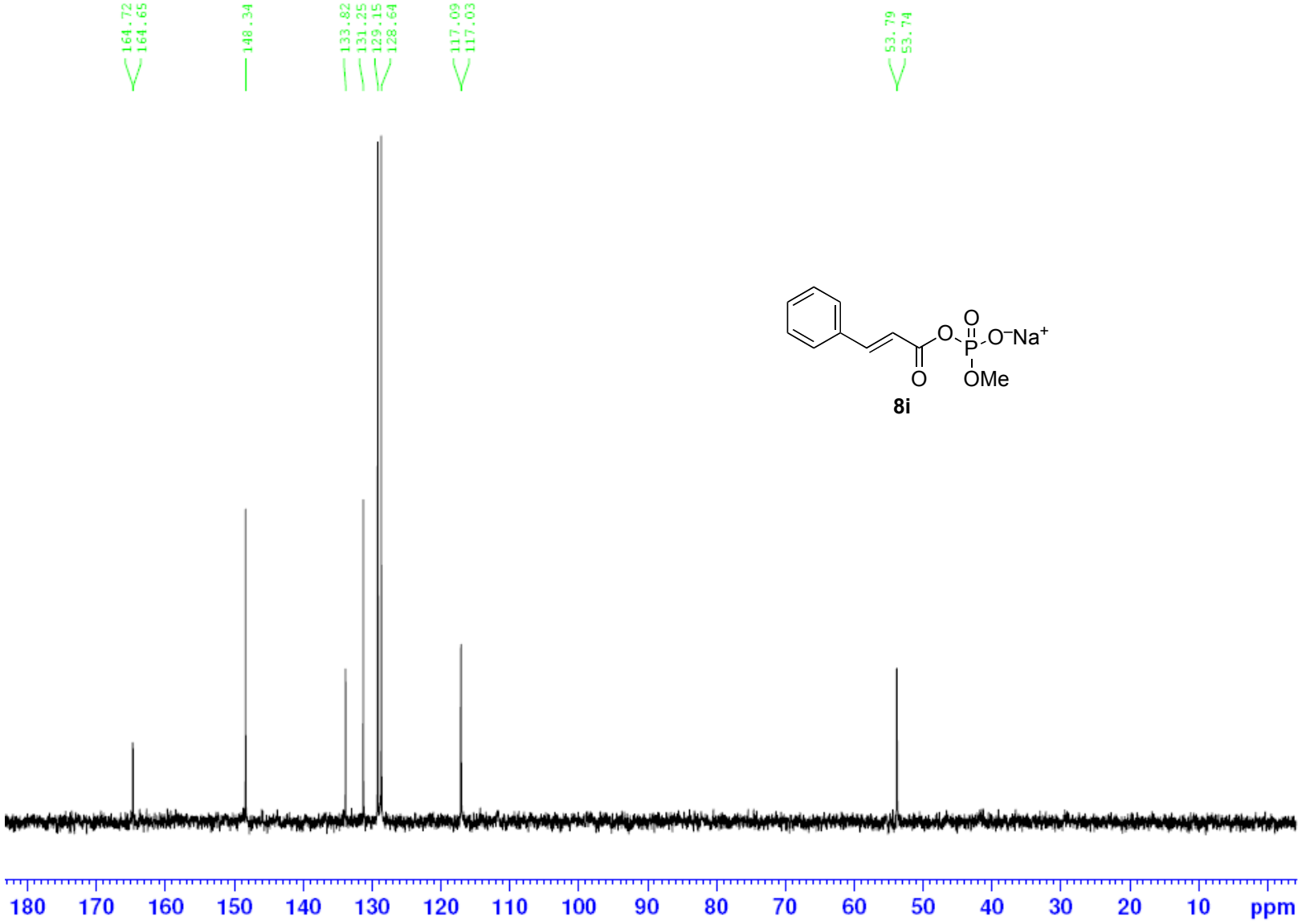


Figure 69S: ^{31}P NMR spectrum of sodium methyl *trans*-cinnamoyl phosphate **(8i)**

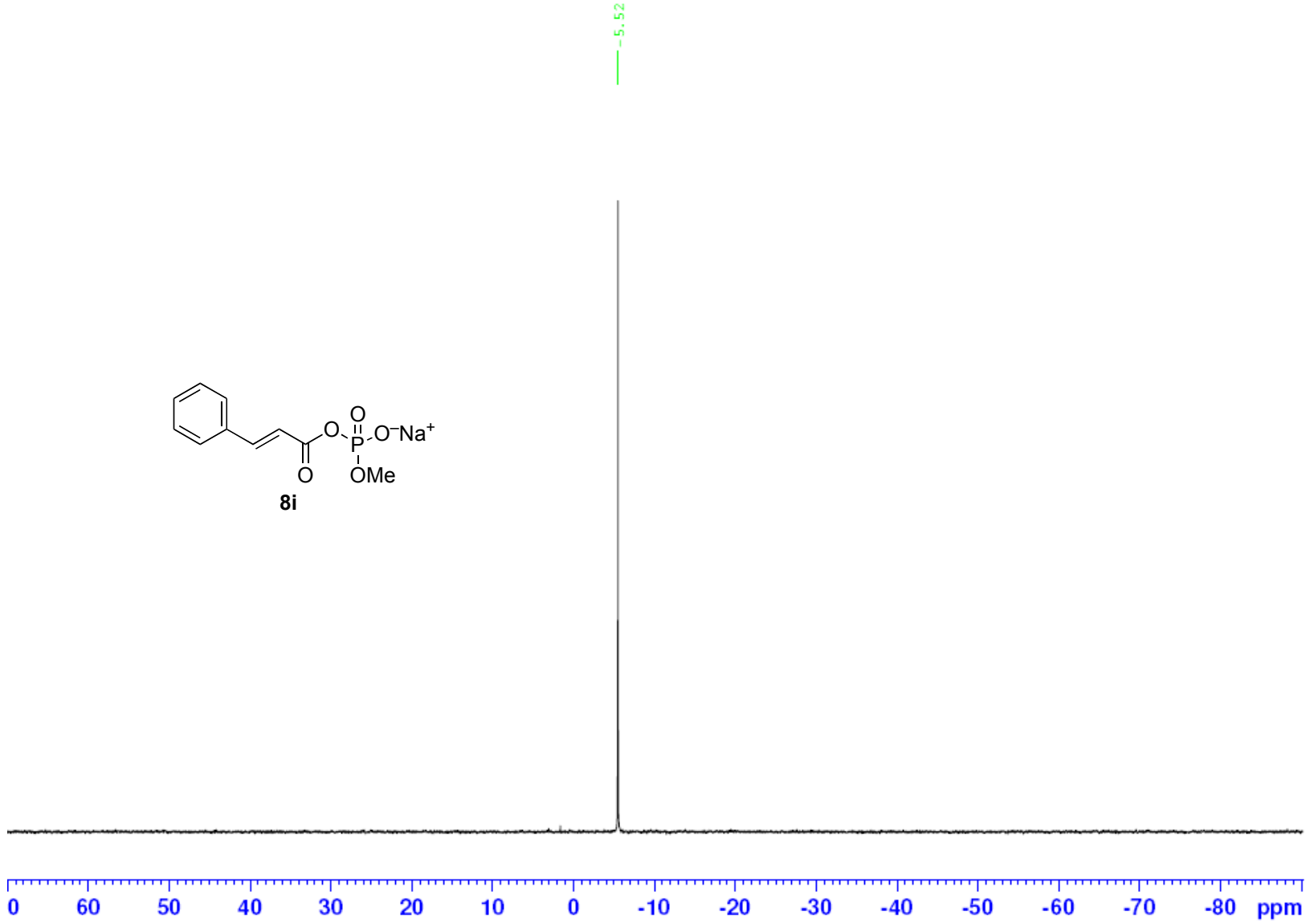


Figure 70S: ^{1}H NMR spectrum of 3-(acetylthio)propanoic acid **(2)**

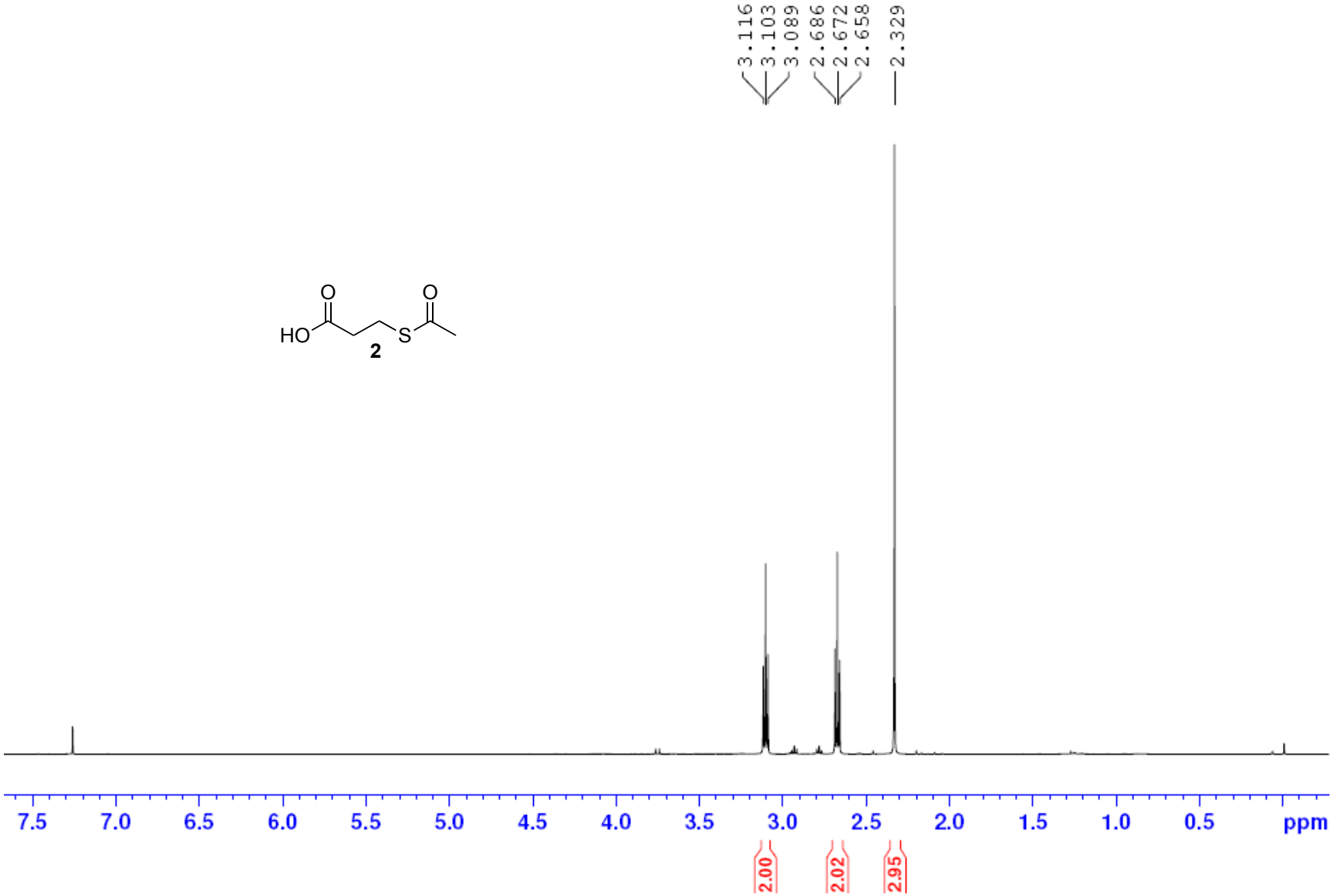


Figure 71S: ^{13}C NMR spectrum of 3-(acetylthio)propanoic acid **(2)**

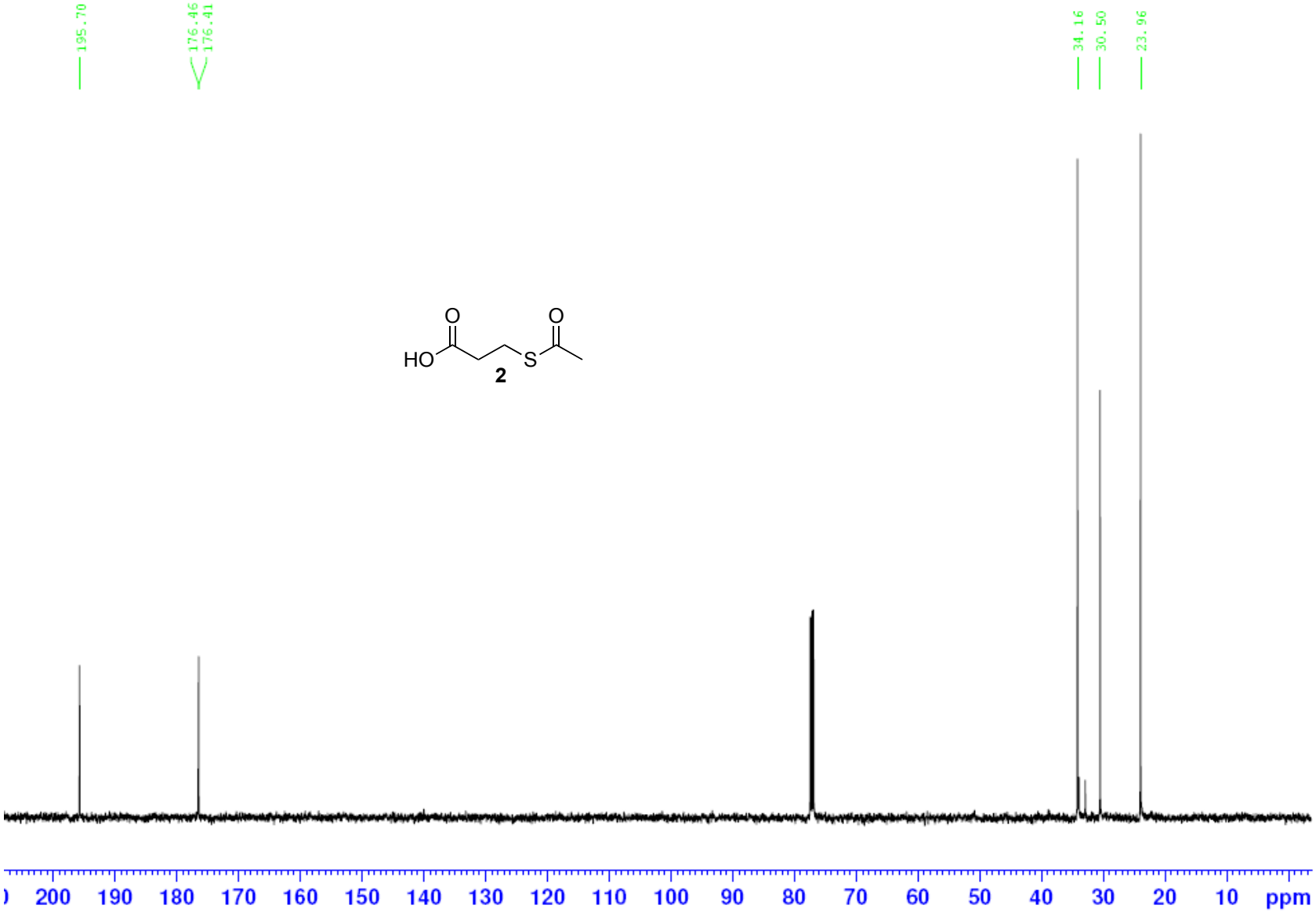


Figure 72S: ^{1}H NMR spectrum of 4-(acetylthio)benzoic acid **(4)**

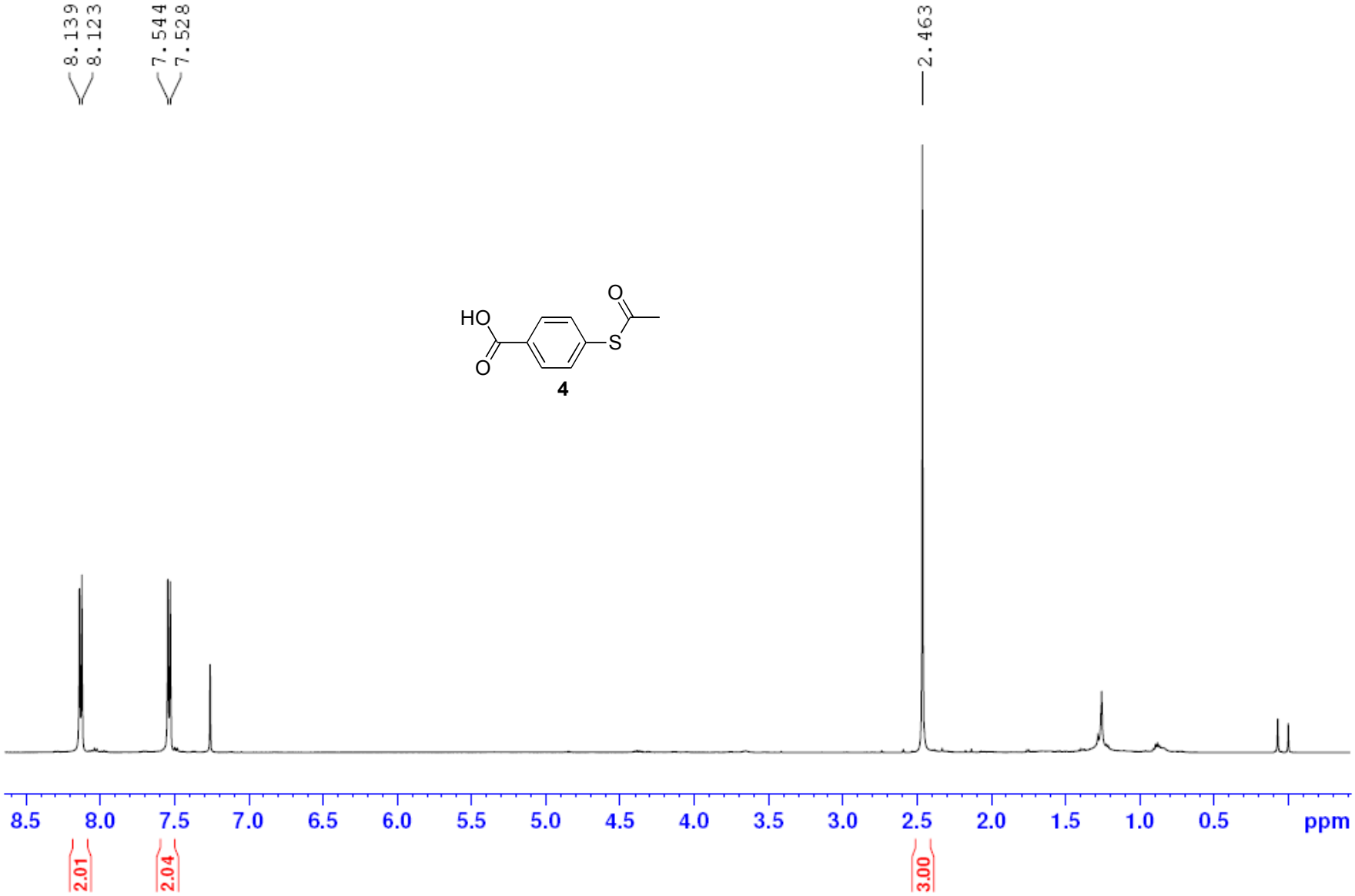


Figure 73S: ^{13}C NMR spectrum of 4-(acetylthio)benzoic acid **(4)**

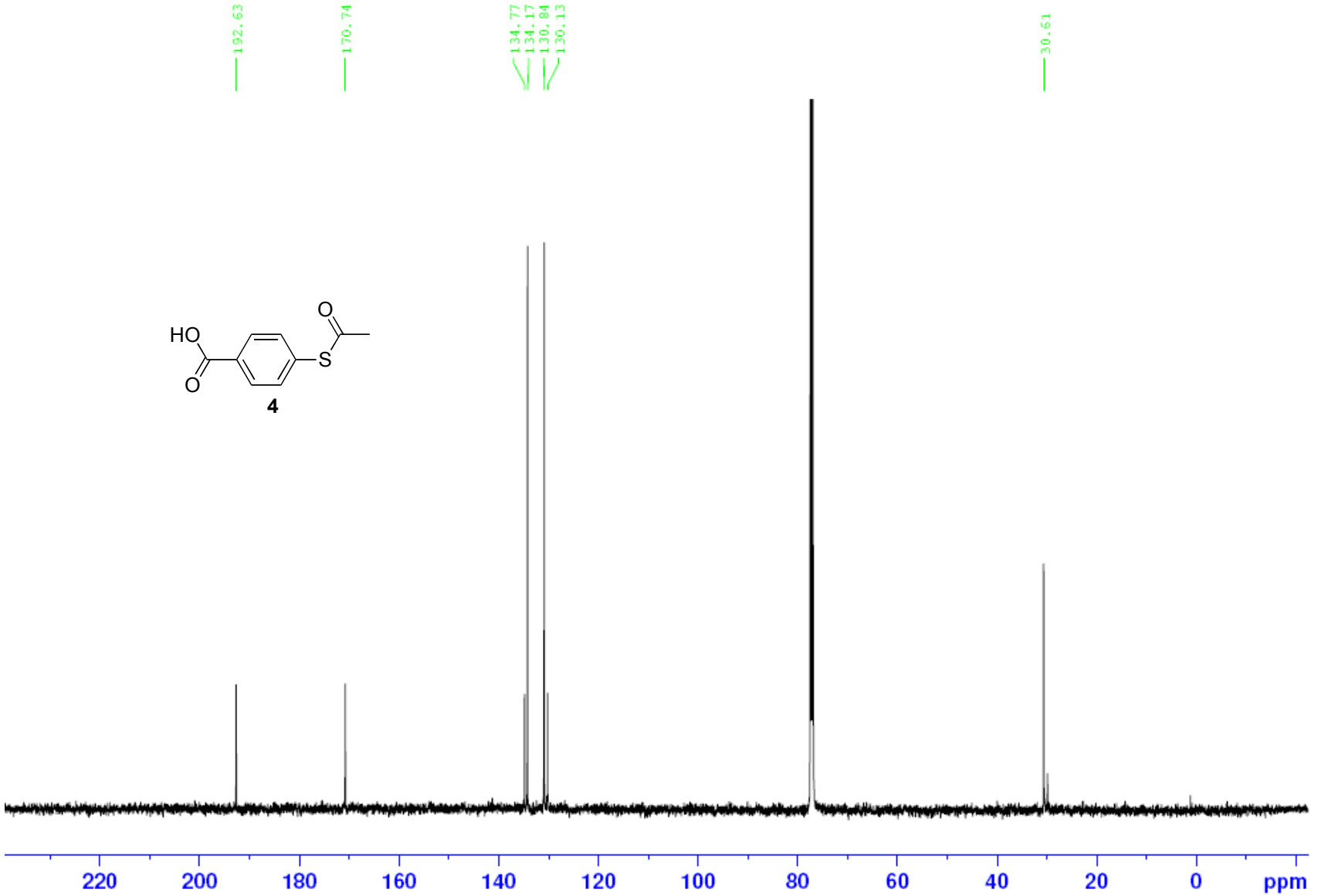


Figure 74S: ^{1}H NMR spectrum of acetyl-CoA **(10a)**

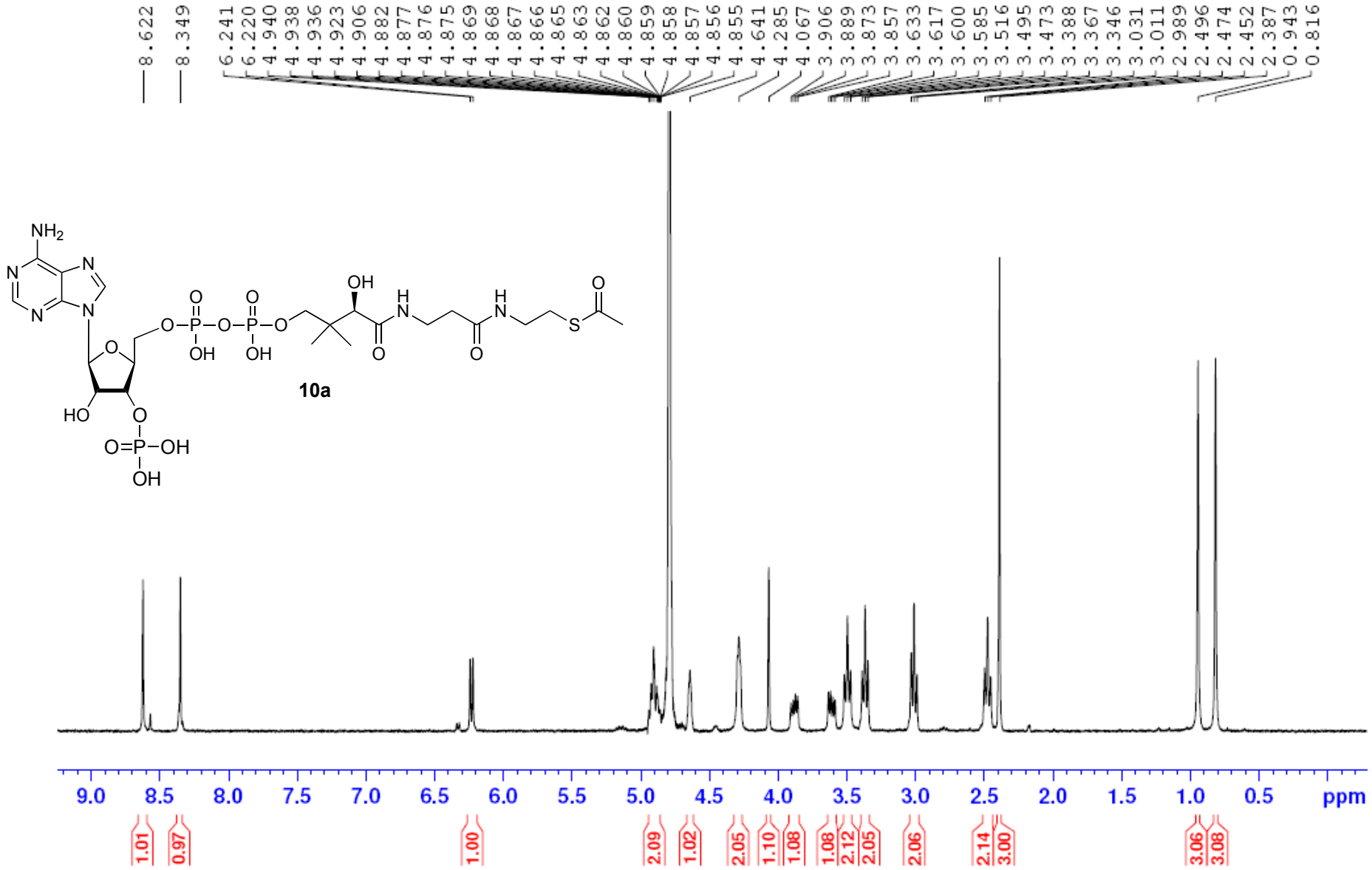


Figure 75S: ^{1}H NMR spectrum of butyryl-CoA **(10b)**

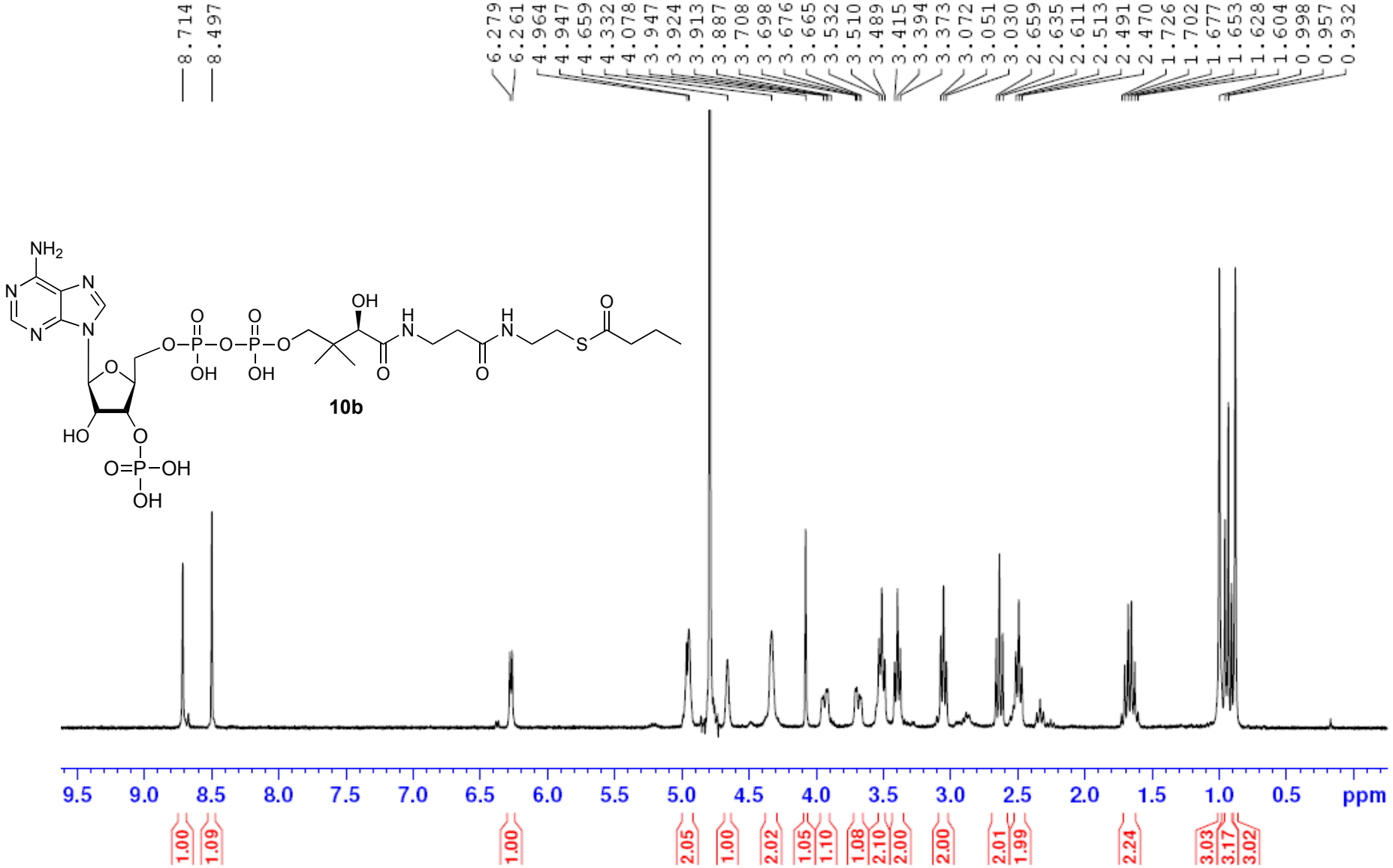


Figure 76S: ^{1}H NMR spectrum of octanoyl-CoA (**10d**)

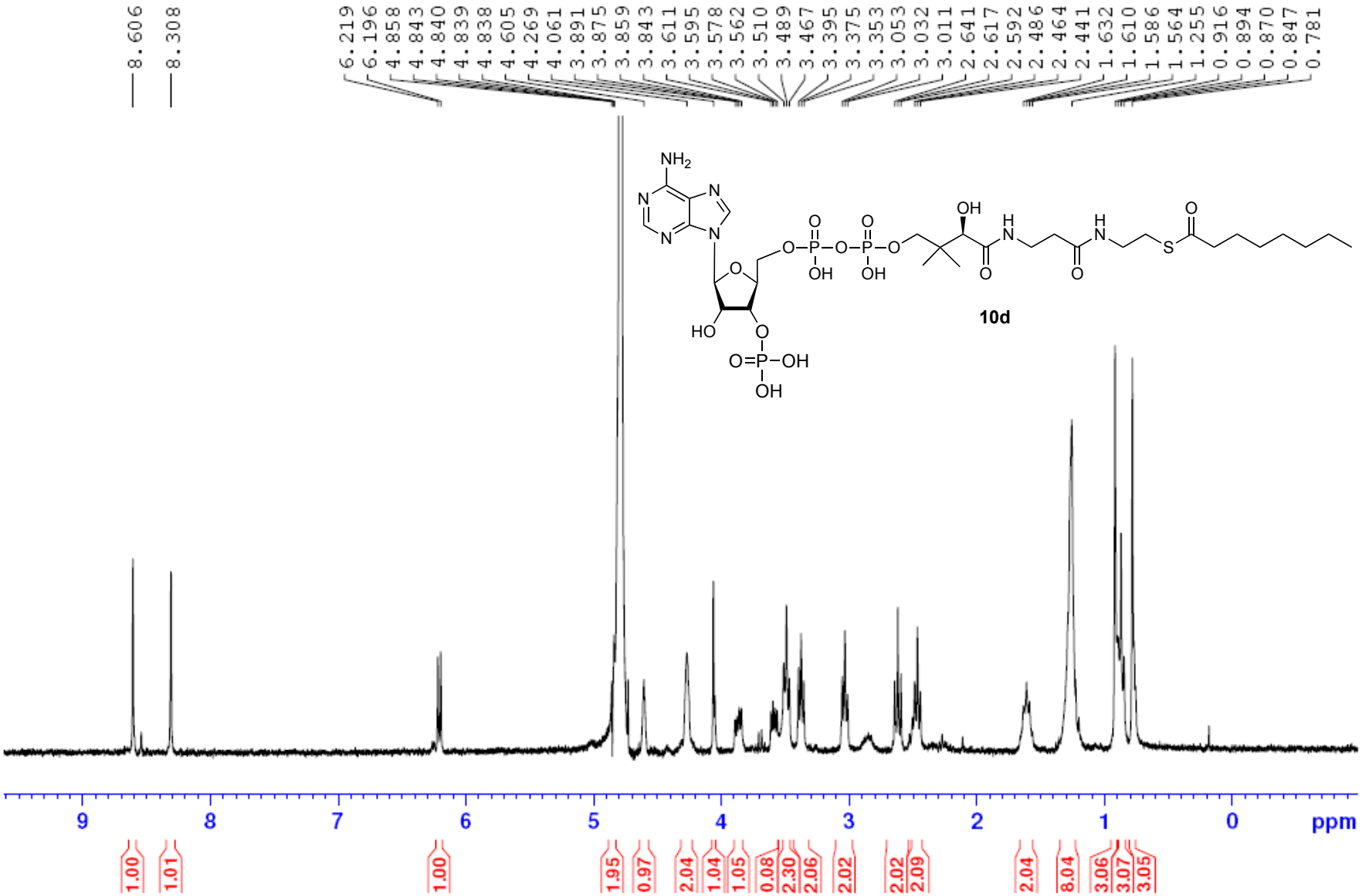


Figure 77S: ^{1}H NMR spectrum of ibuprofenoyl-CoA **(10f)**

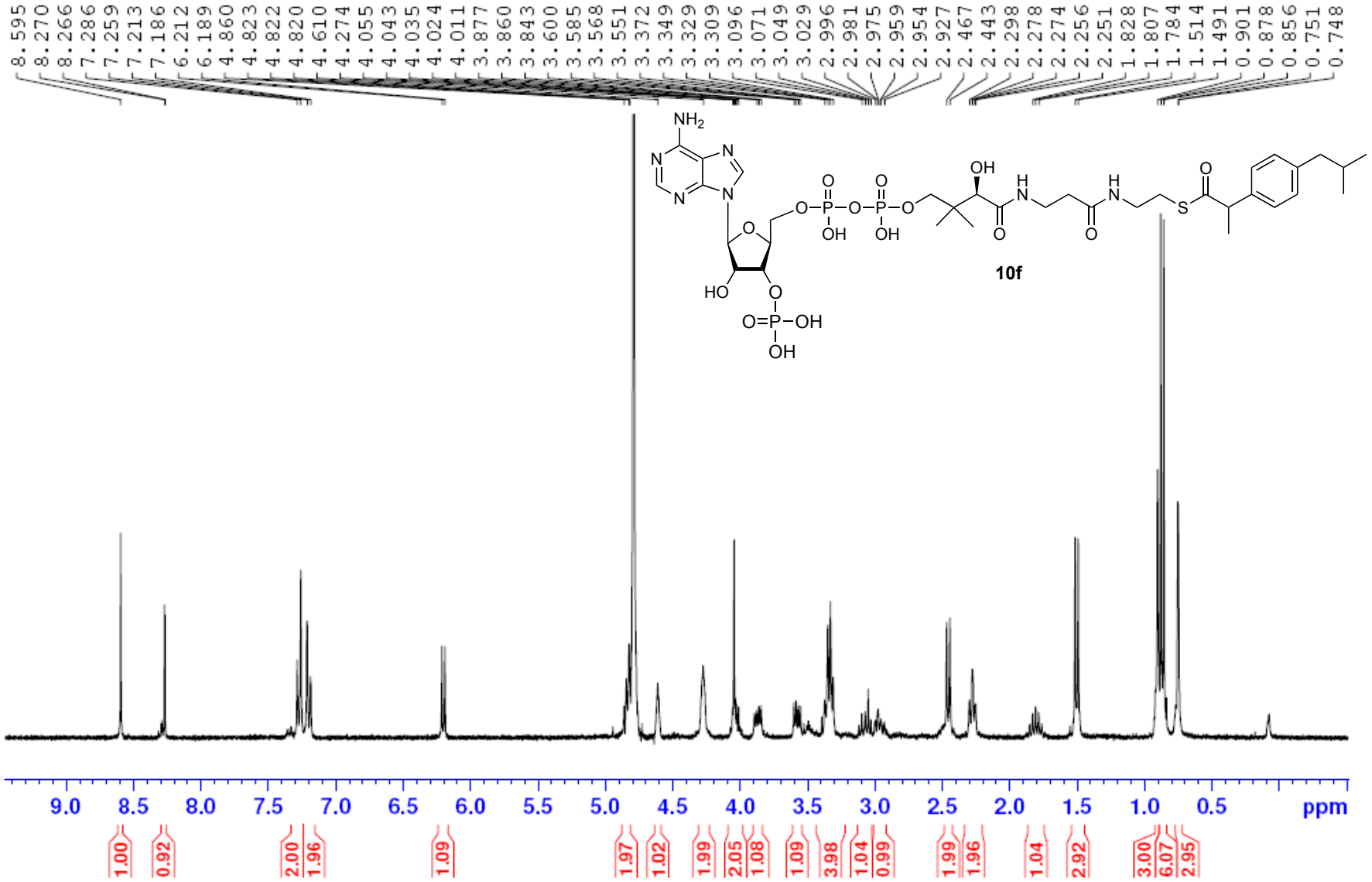


Figure 78S: ^{1}H NMR spectrum of fenoprofenoyl-CoA (**10g**)

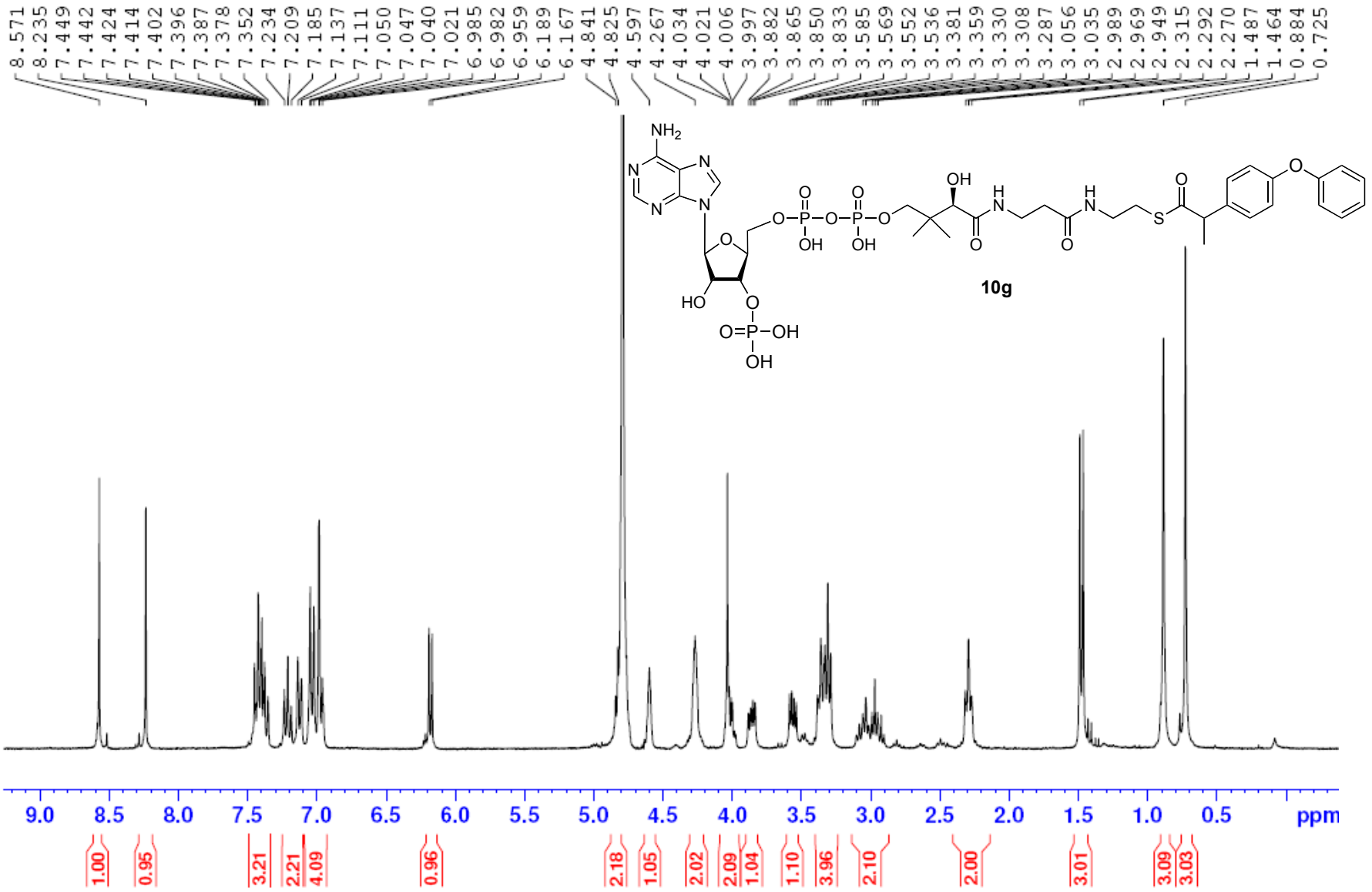


Figure 79S: ^{1}H NMR spectrum of 3-phenylpropanoyl-CoA **(10h)**

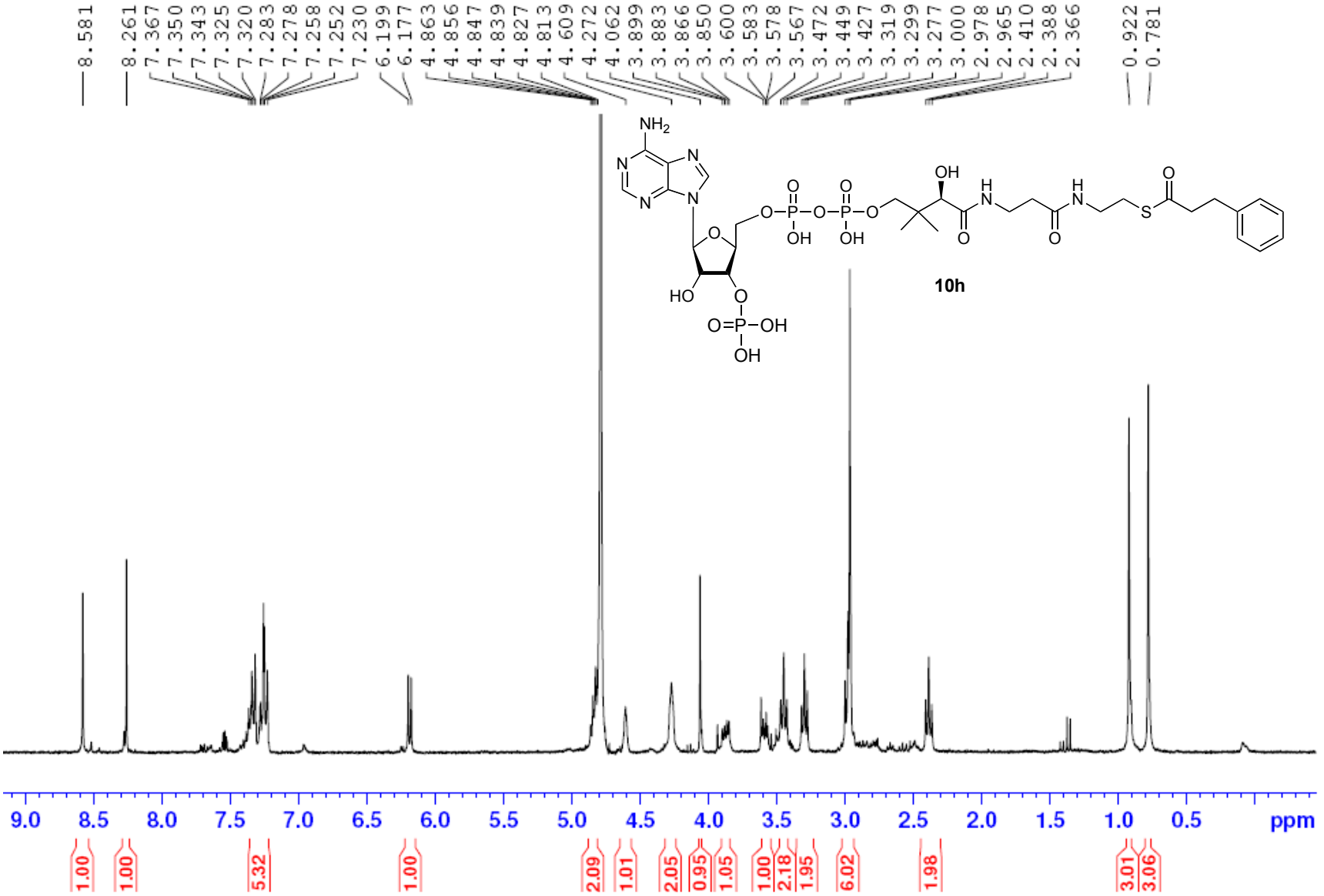


Figure 80S: ^{1}H NMR spectrum of *trans*-cinnamoyl-CoA **(10i)**

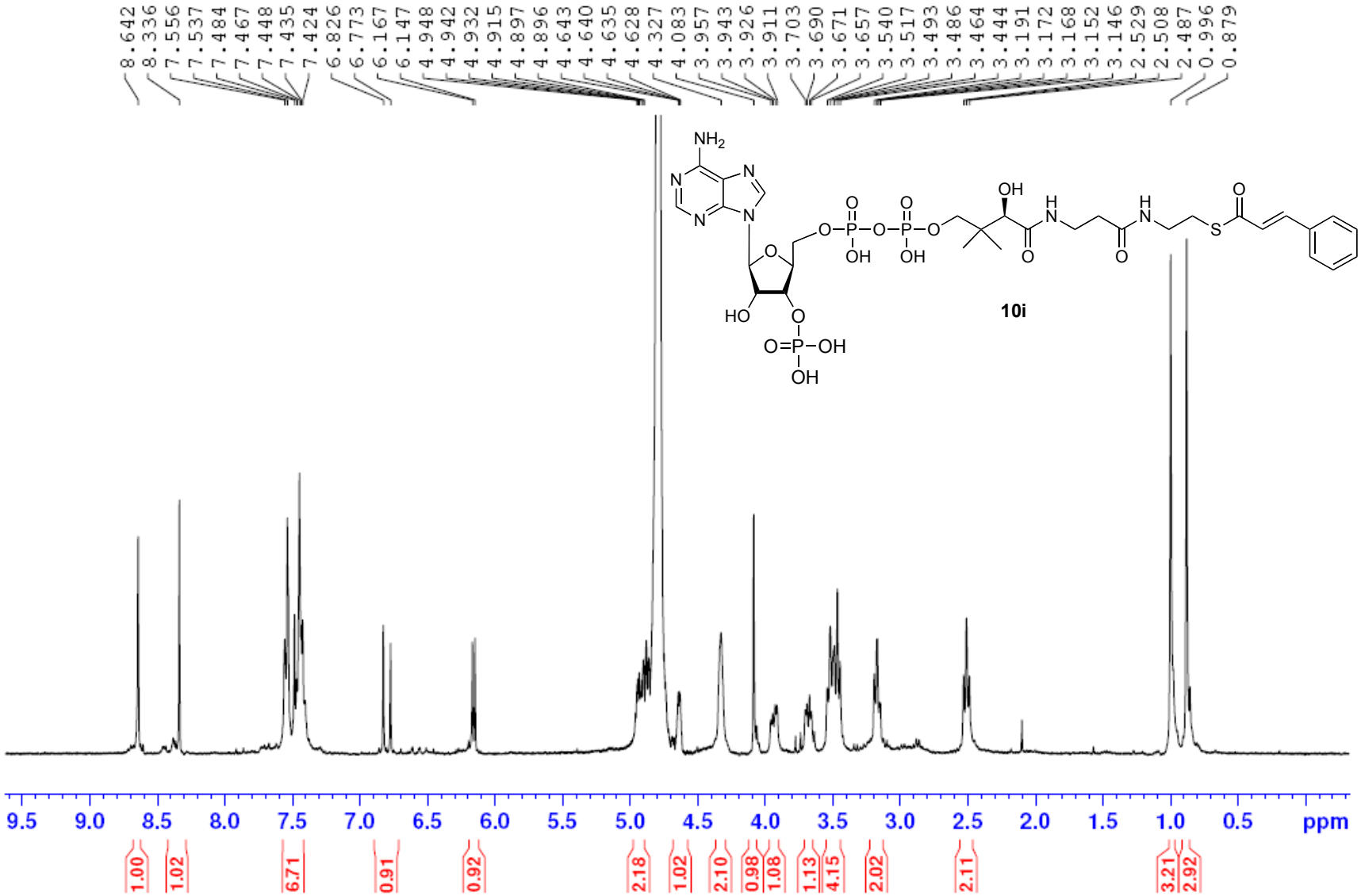


Figure 81S: High resolution mass spectrum of sodium methyl acetyl phosphate (**8a**)

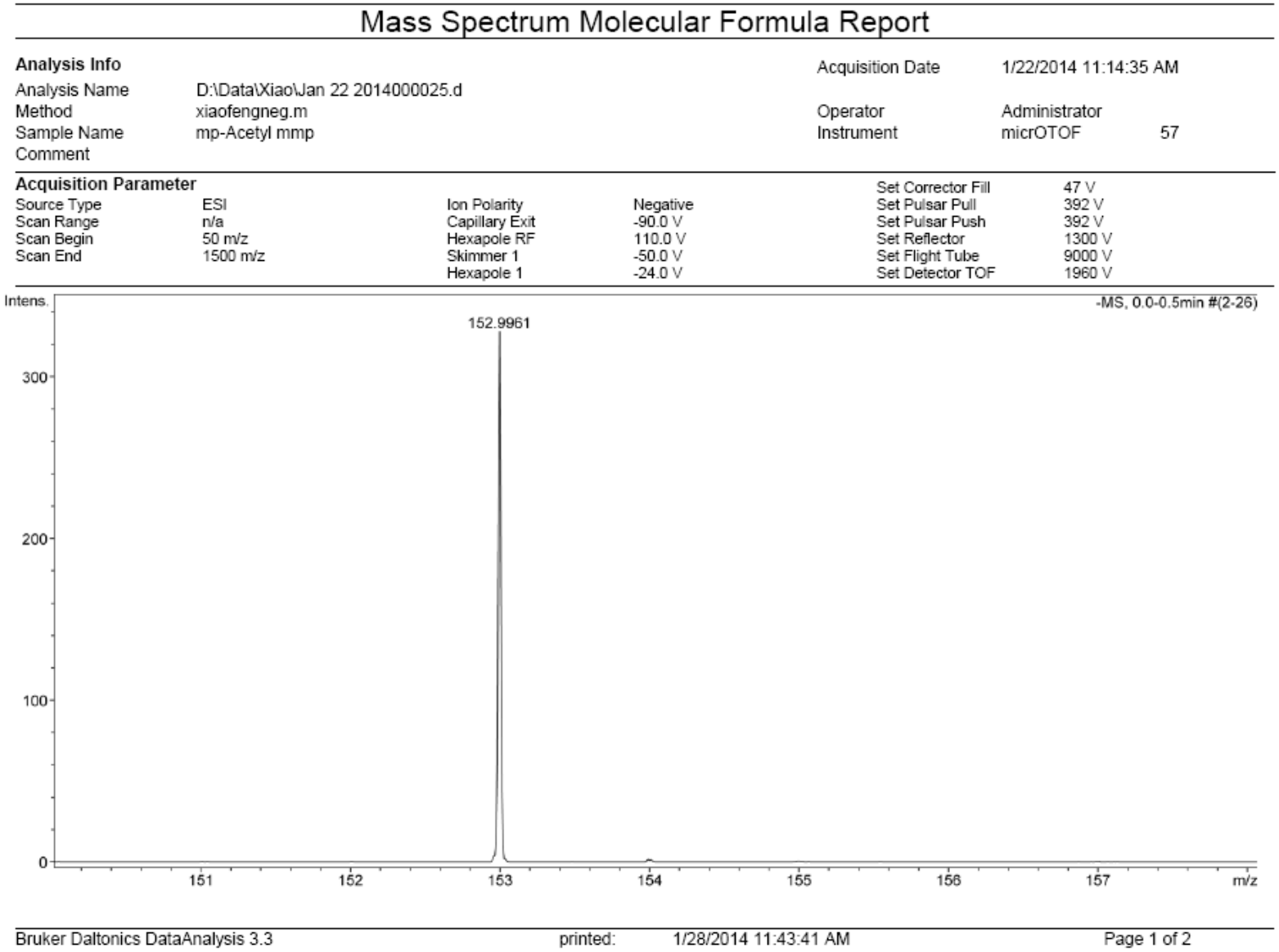


Figure 82S: High resolution mass spectrum of sodium methyl butyryl phosphate (**8b**)

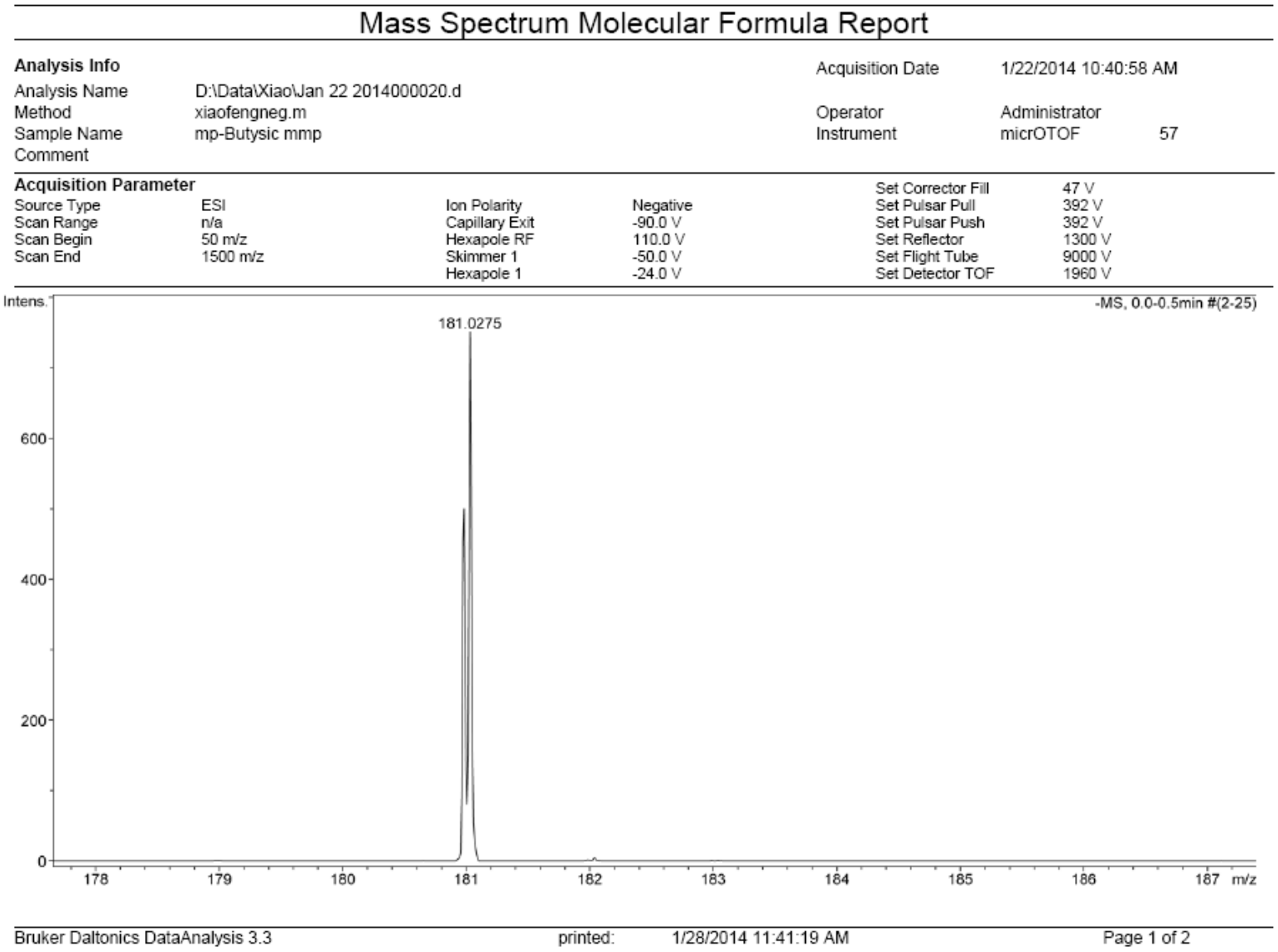


Figure 83S: High resolution mass spectrum of sodium methyl isovaleryl phosphate (**8c**)

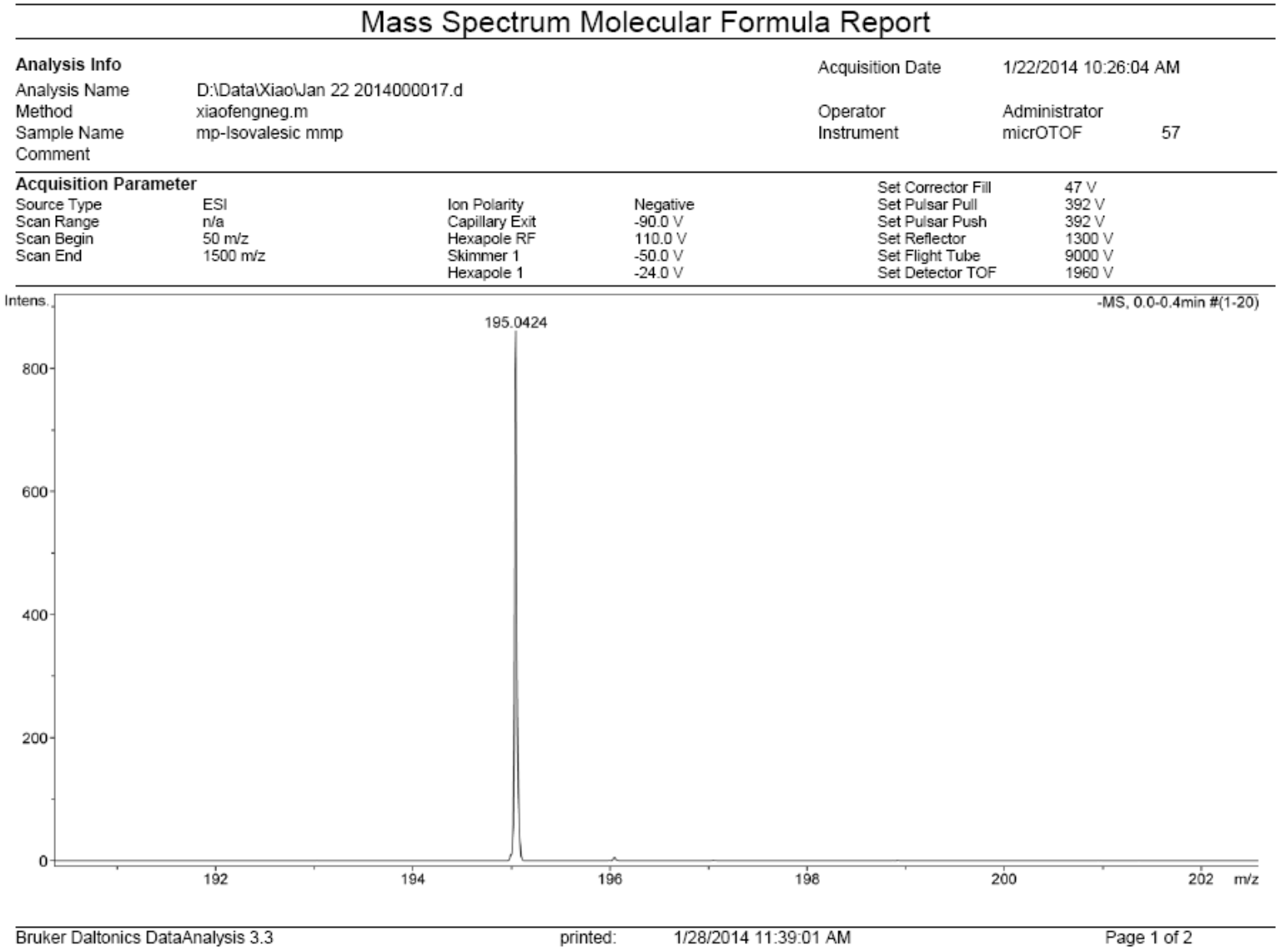


Figure 84S: High resolution mass spectrum of sodium methyl octanonyl phosphate (**8d**)

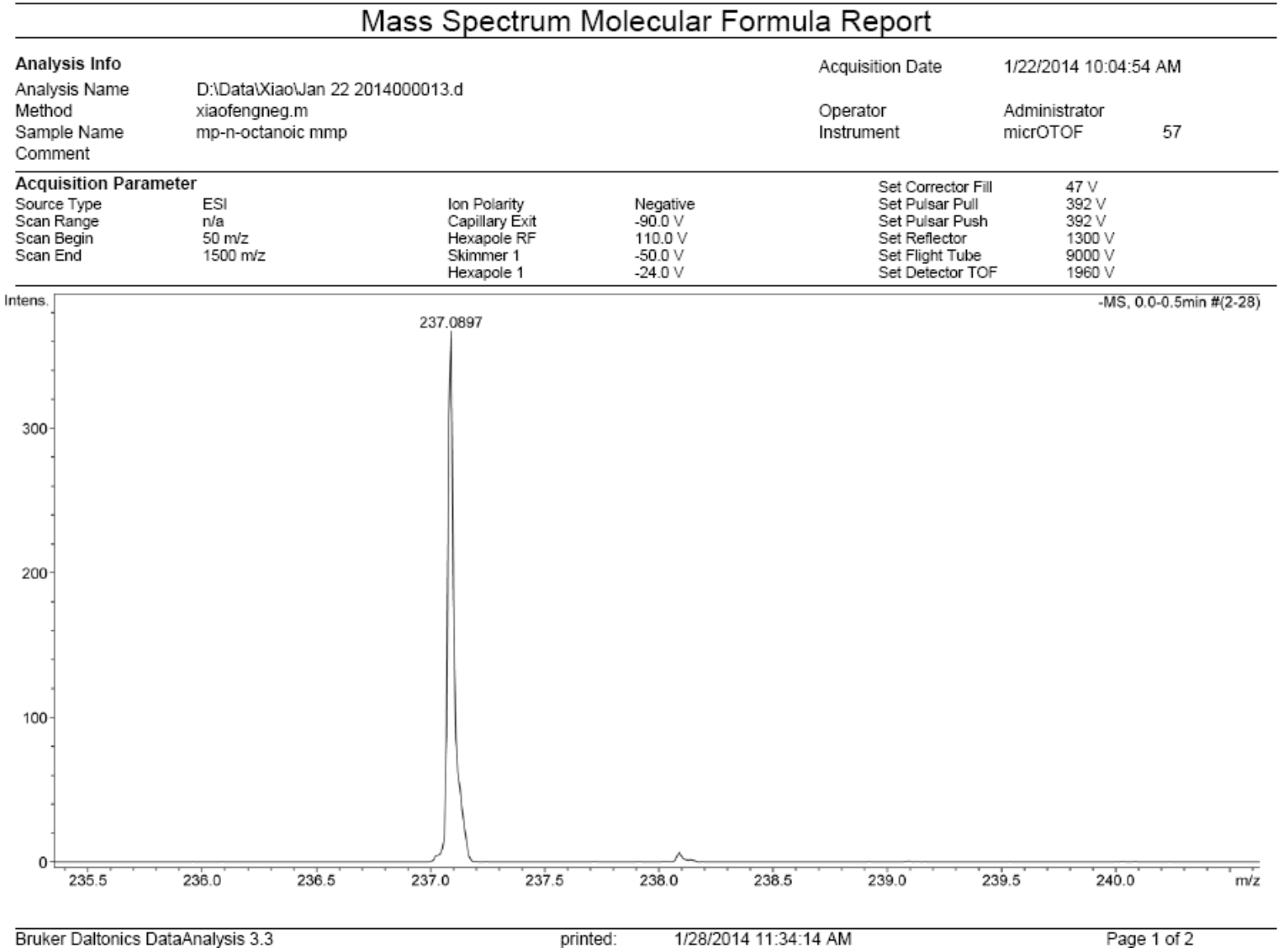


Figure 85S: High resolution mass spectrum of sodium methyl palmitoyl phosphate (**8e**)

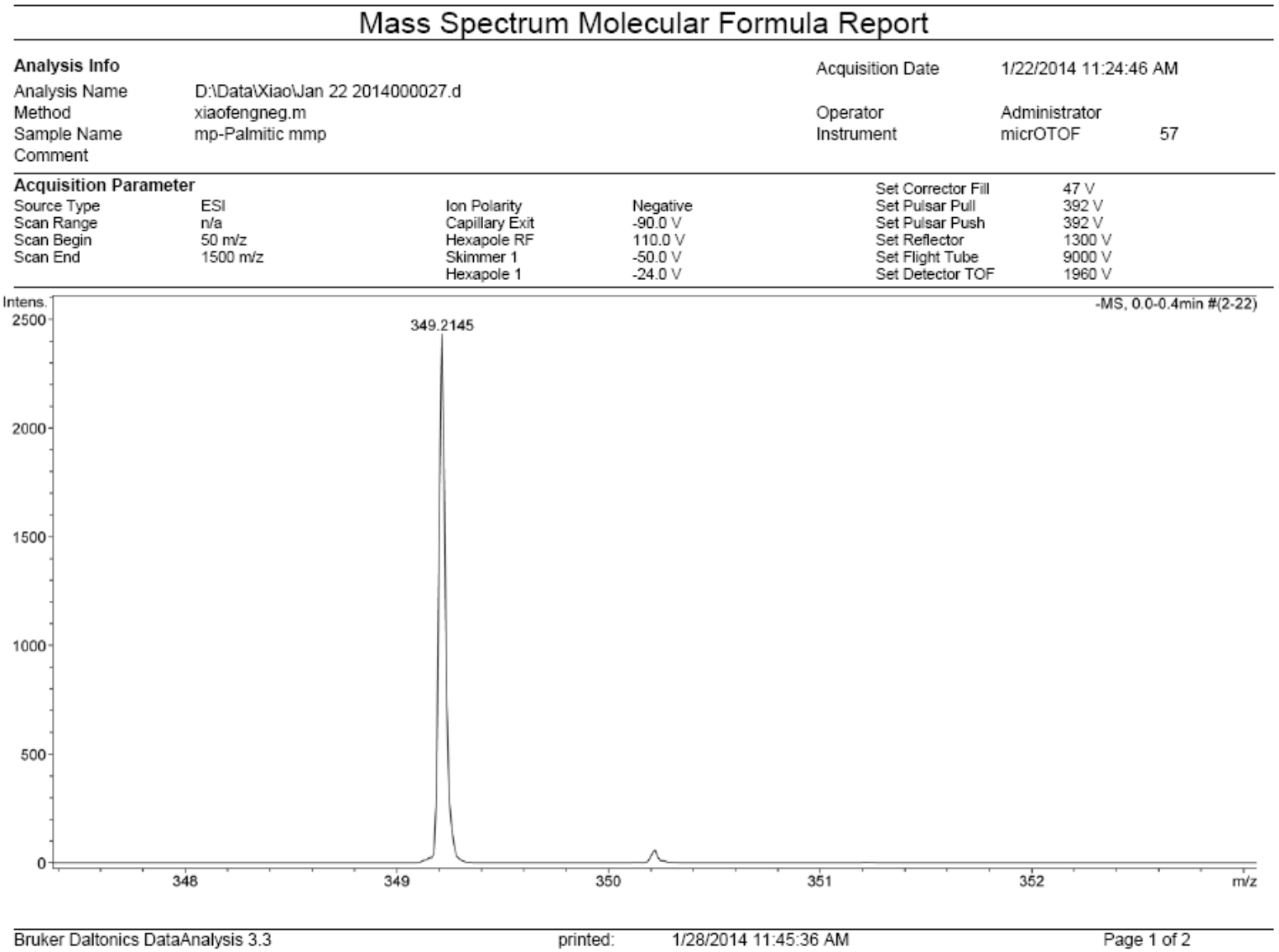


Figure 86S: High resolution mass spectrum of sodium methyl ibuprofenoyl phosphate (**8f**)

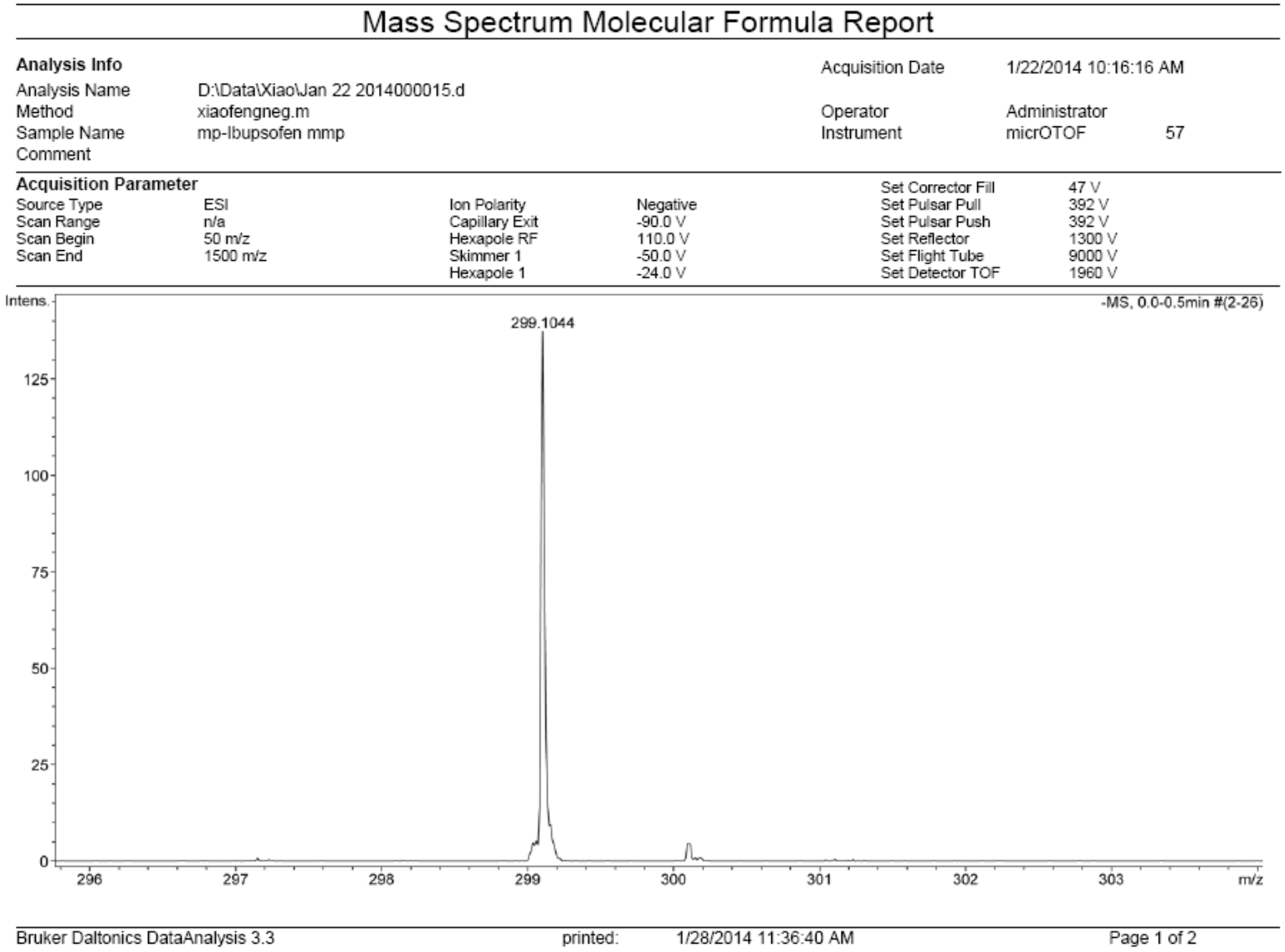


Figure 87S: High resolution mass spectrum of sodium methyl fenoprofenoyl phosphate (**8g**)

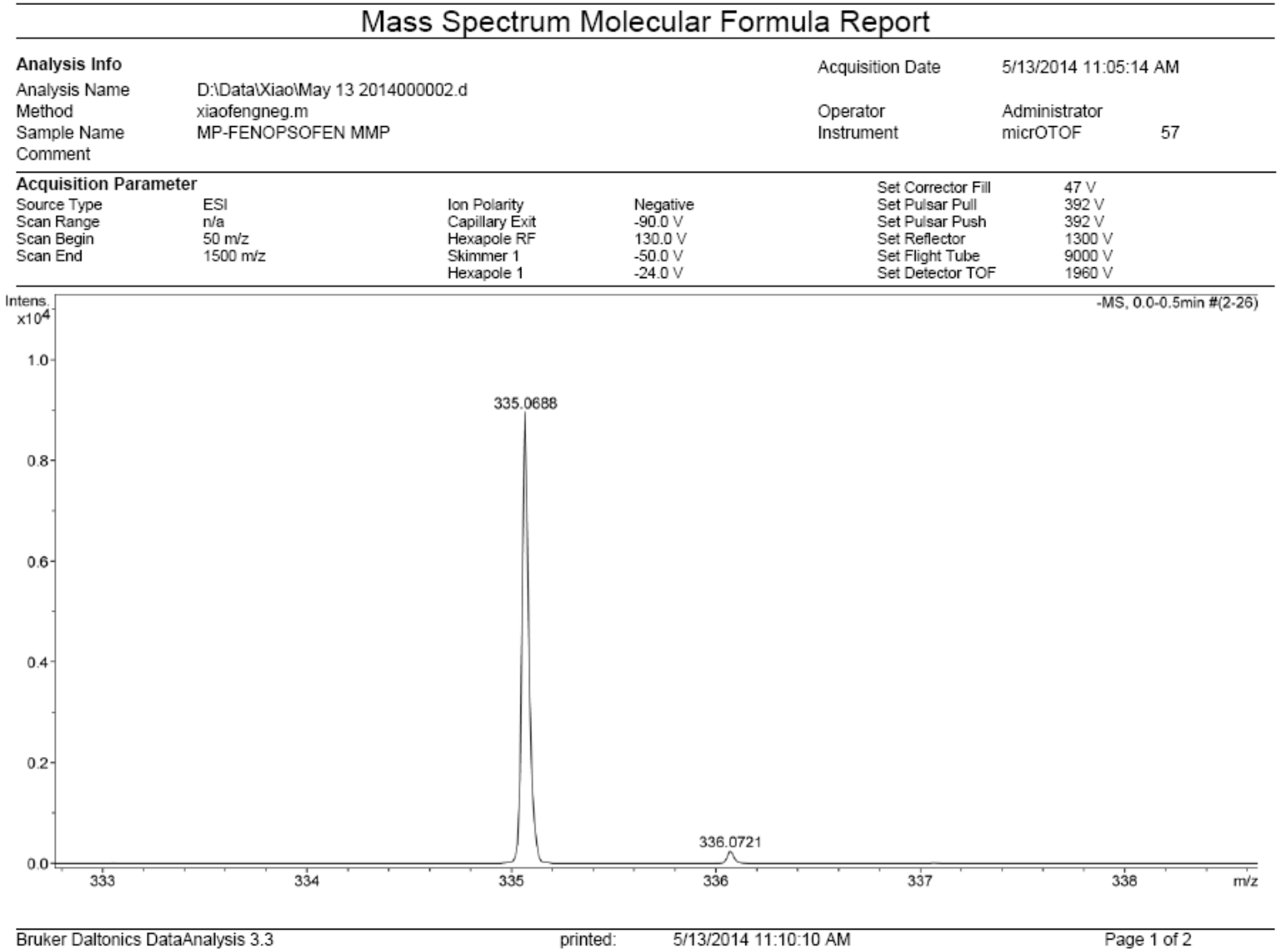


Figure 88S: High resolution mass spectrum of sodium methyl 3-phenylpropanoyl phosphate (**8h**)

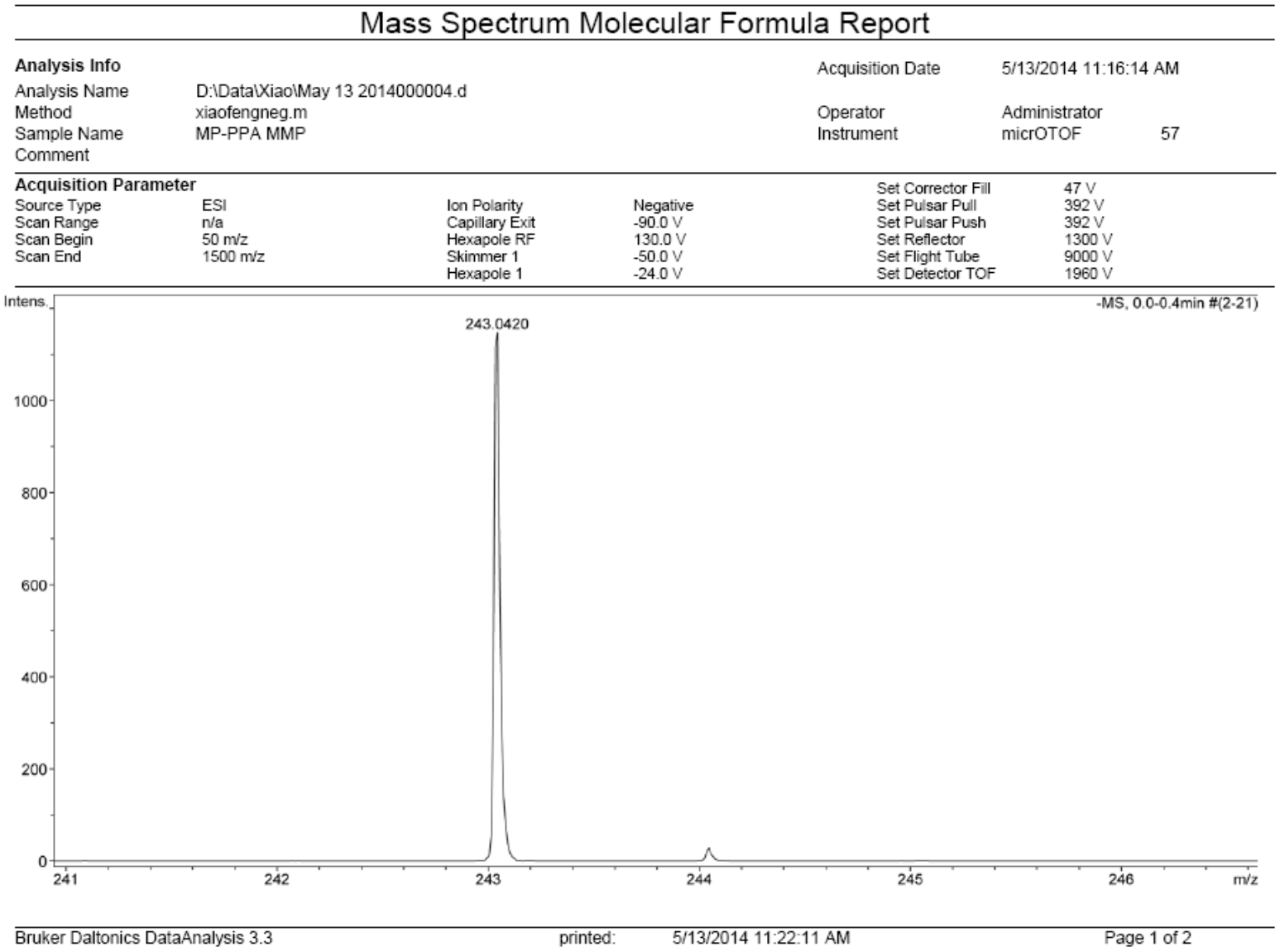


Figure 89S: High resolution mass spectrum of sodium methyl *trans*-cinnamoyl phosphate (**8i**)

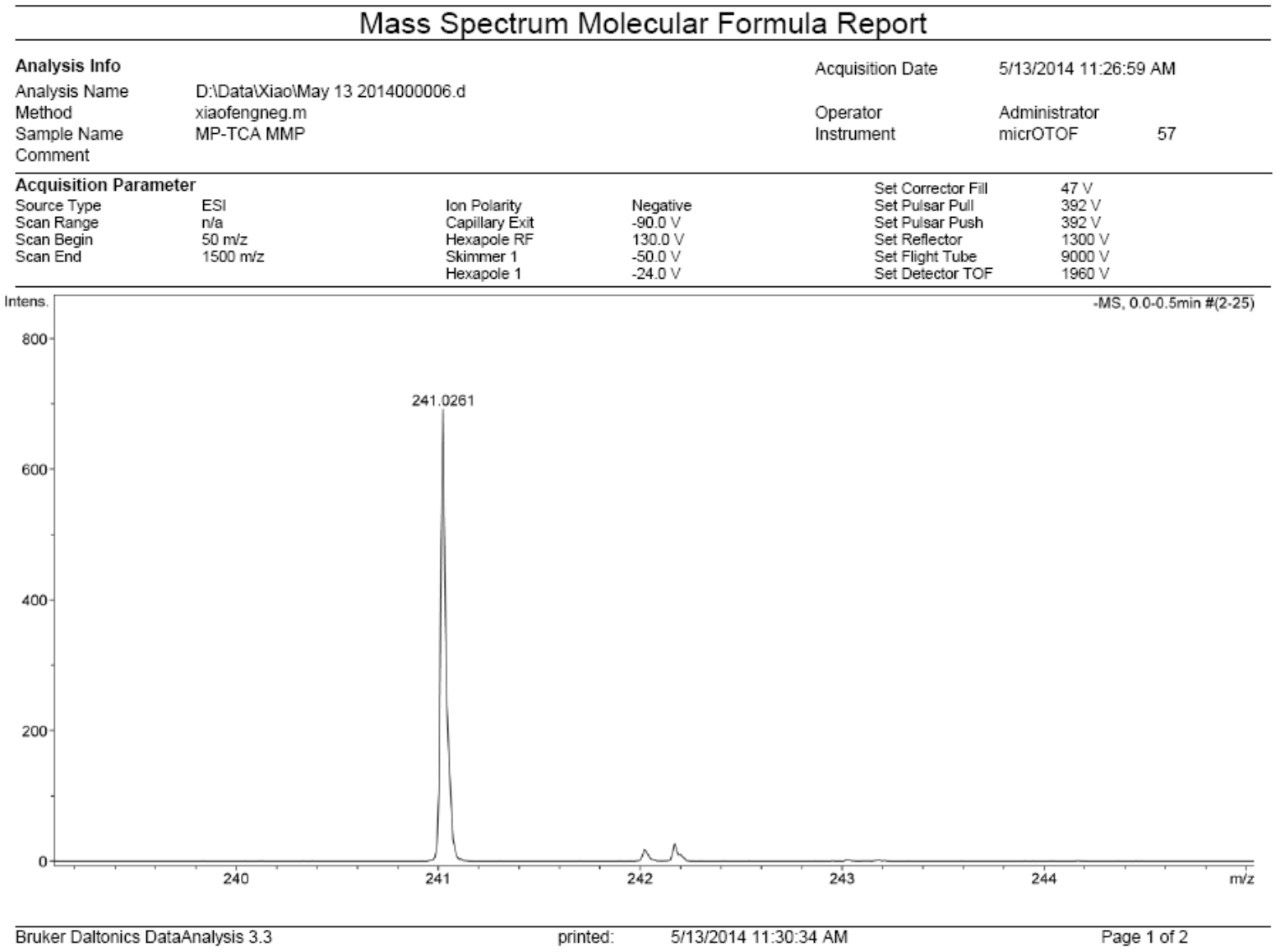


Figure 90S: High resolution mass spectrum of 3-(acetylthio)propanoic acid (**2**)

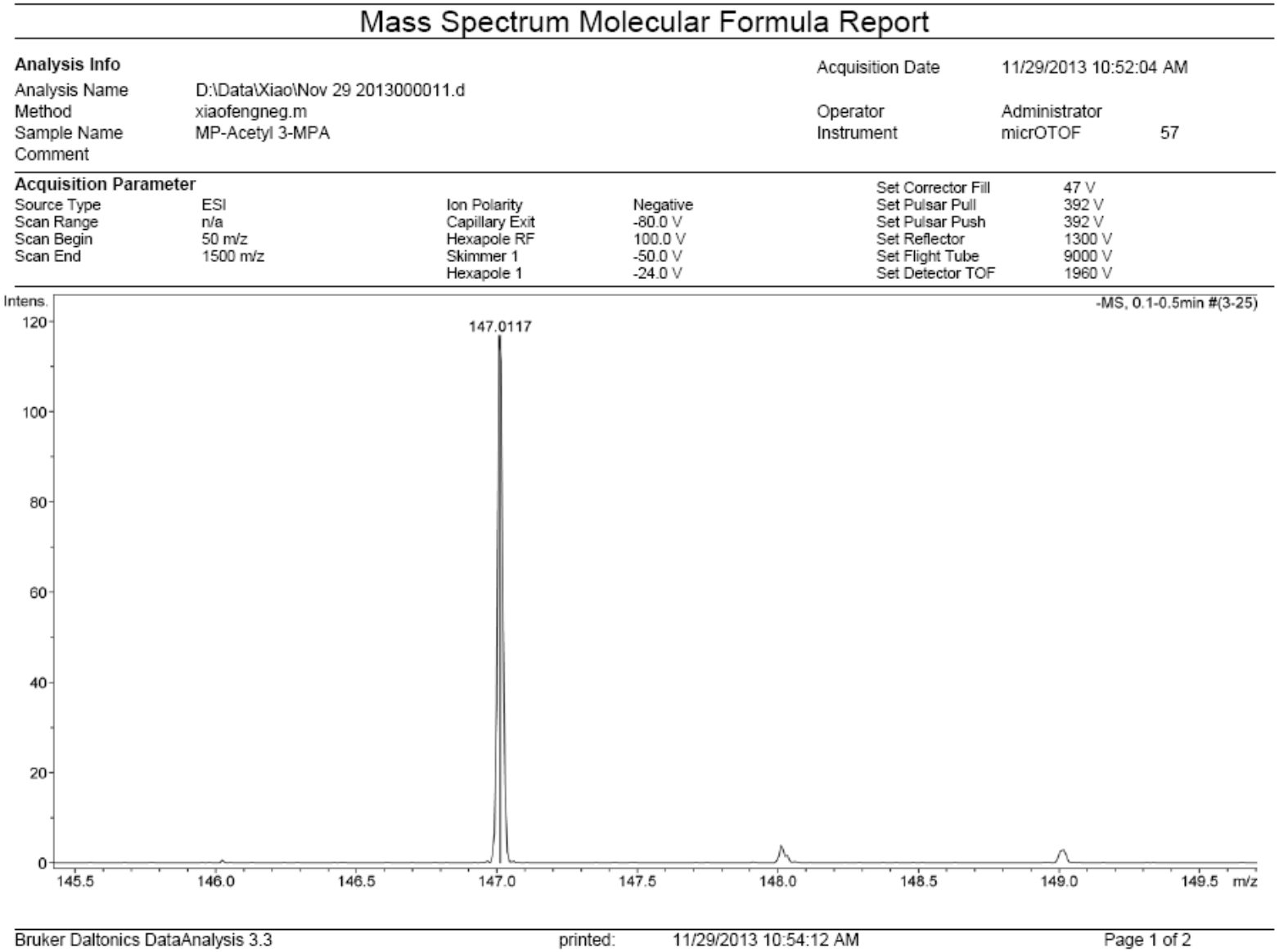


Figure 91S: High resolution mass spectrum of 4-(acetylthio)benzoic acid (**4**)

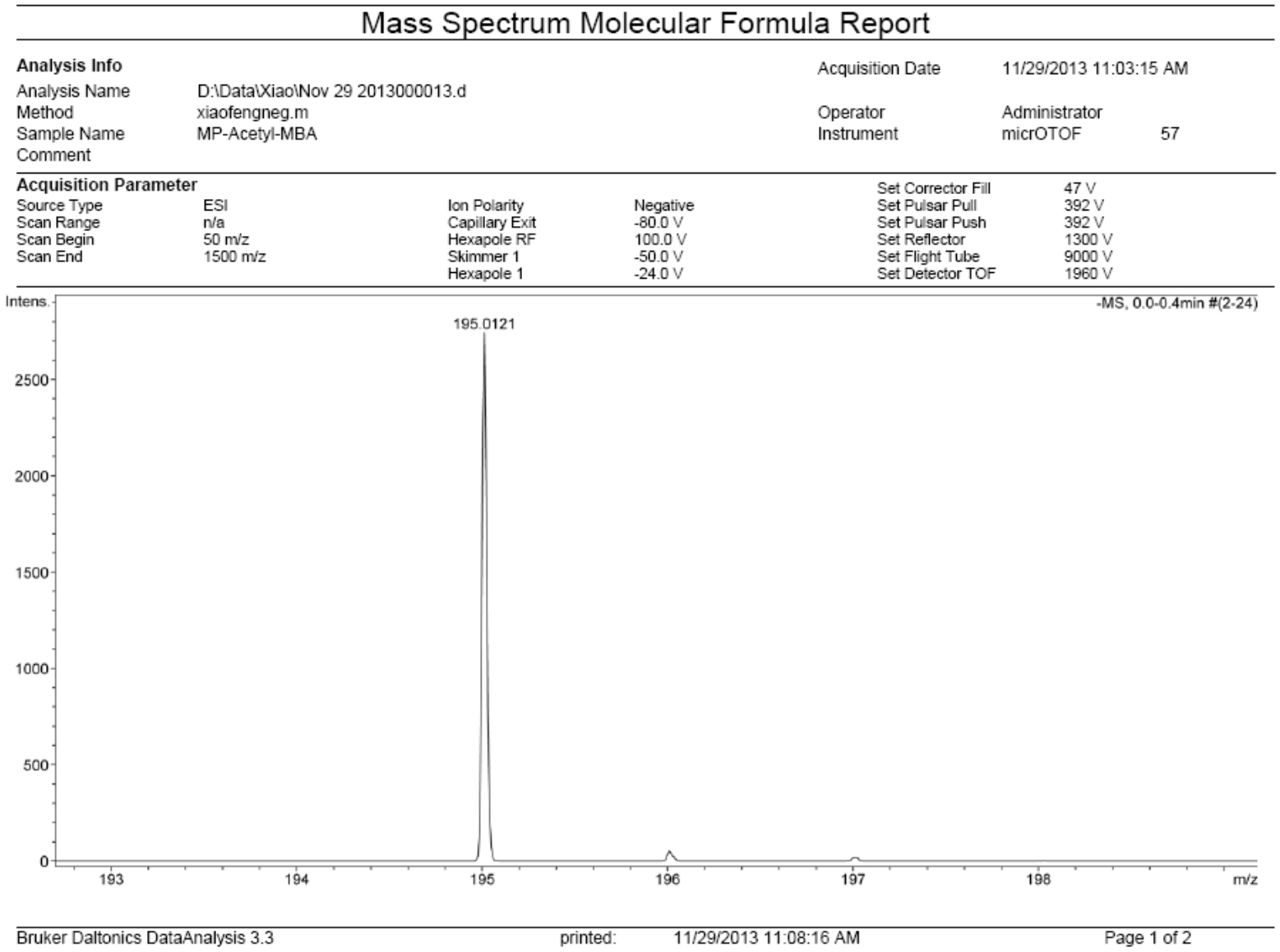


Figure 92S: High resolution mass spectrum of acetyl-CoA (**10a**)

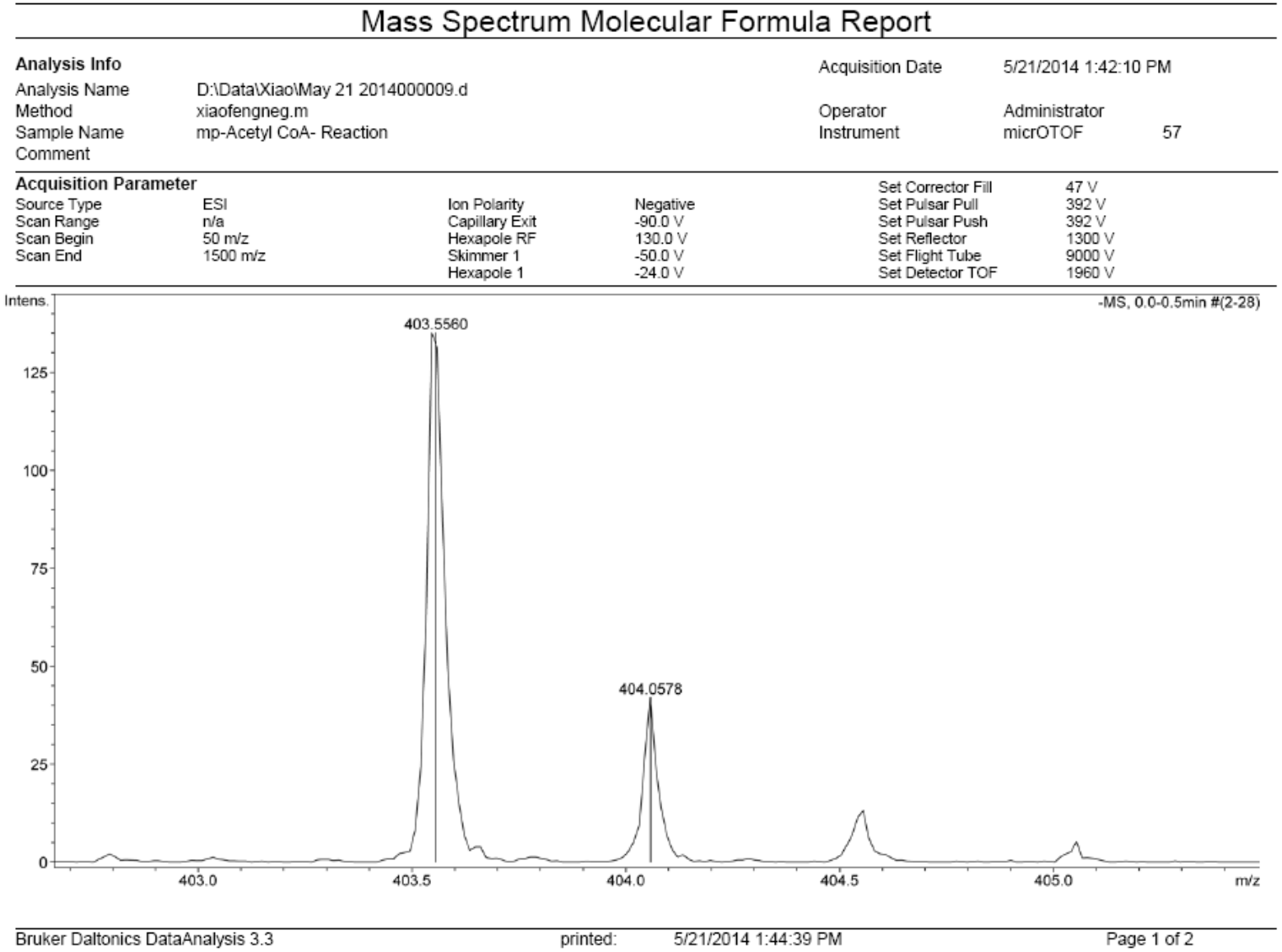


Figure 93S: High resolution mass spectrum of butyryl-CoA (**10b**)

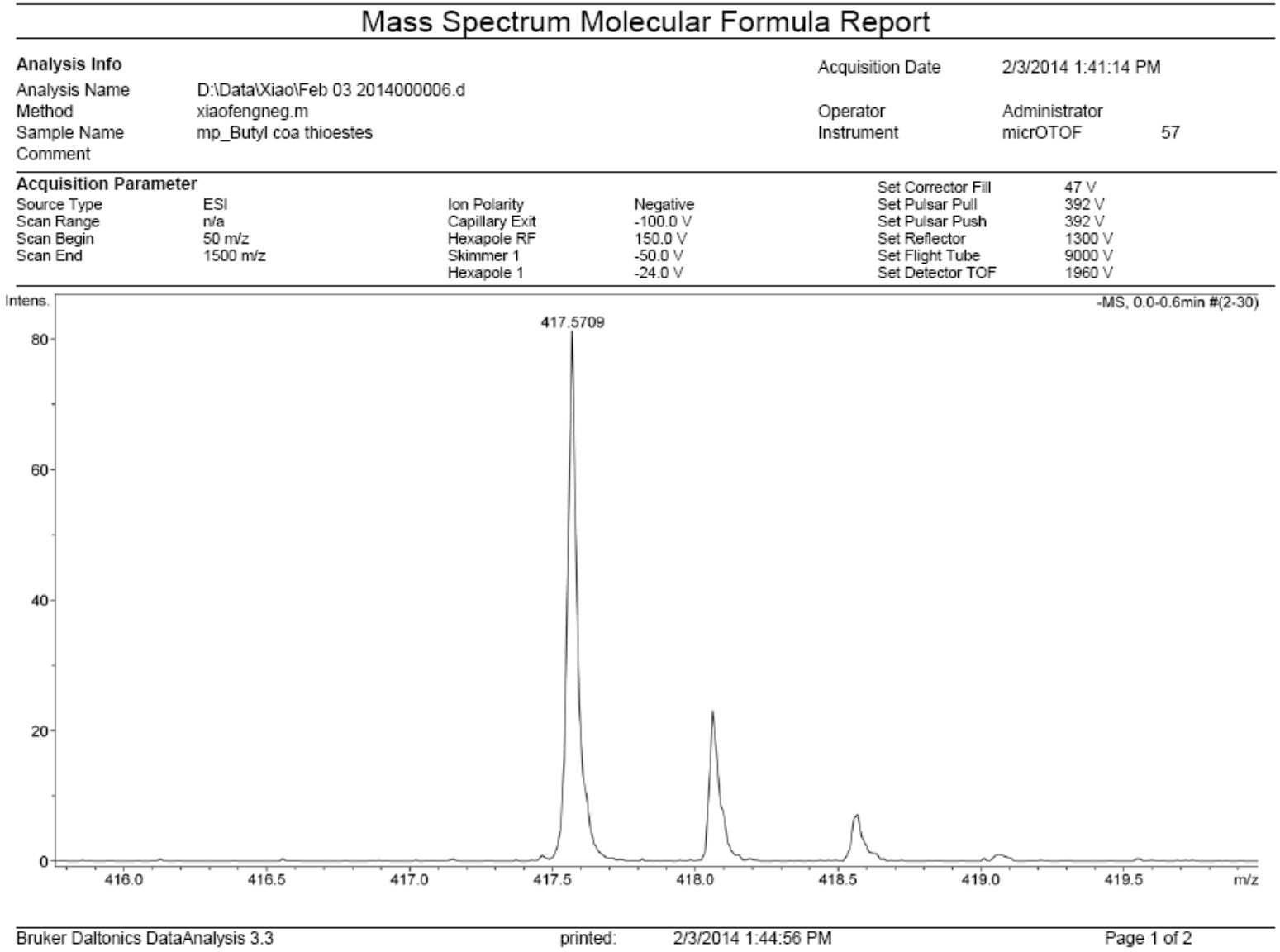


Figure 94S: High resolution mass spectrum of octanoyl-CoA (**10d**)

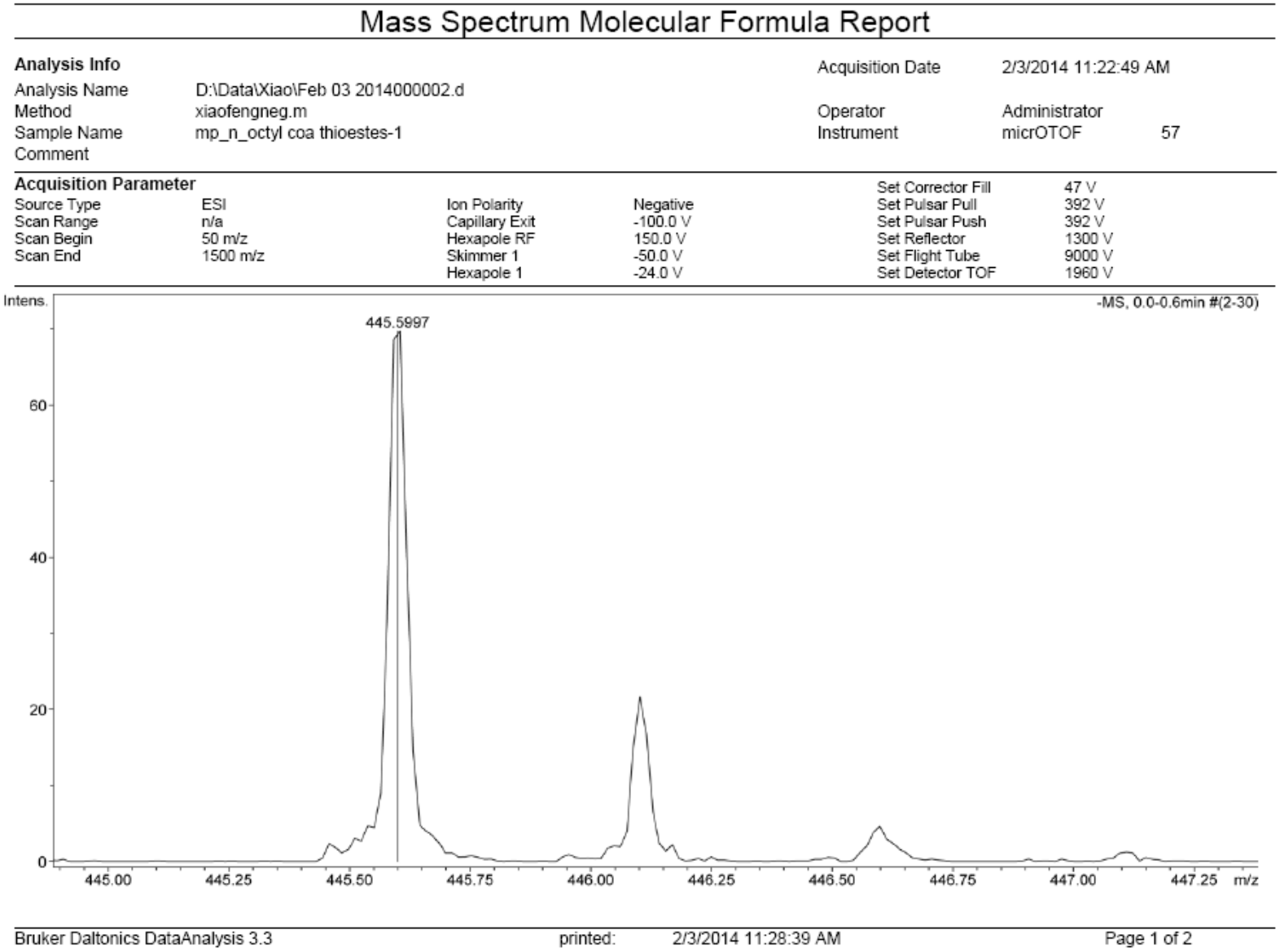


Figure 95S: High resolution mass spectrum of ibuprofenoyl-CoA (**10f**)

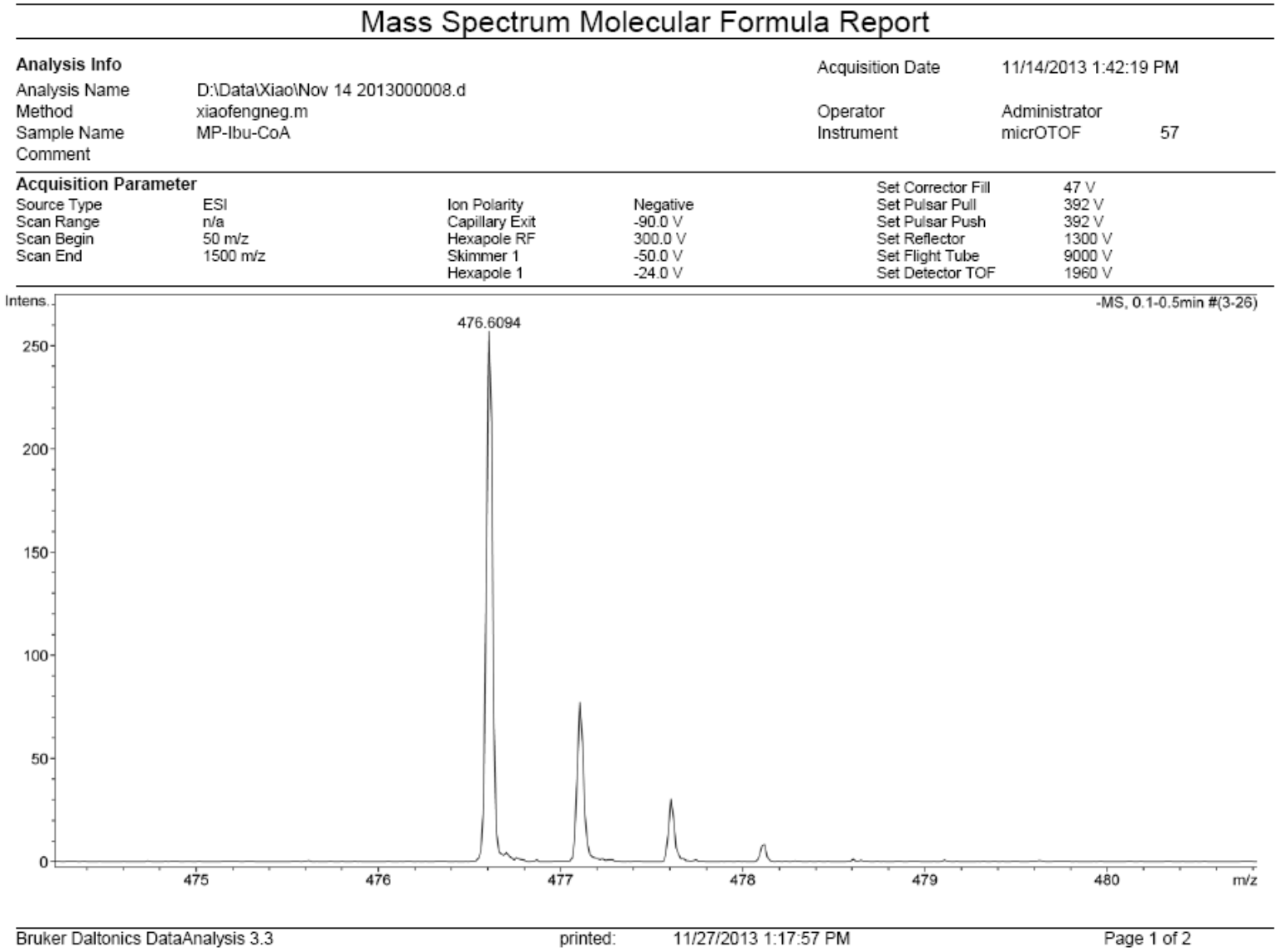


Figure 96S: High resolution mass spectrum of fenoprofenoyl-CoA **(10g)**

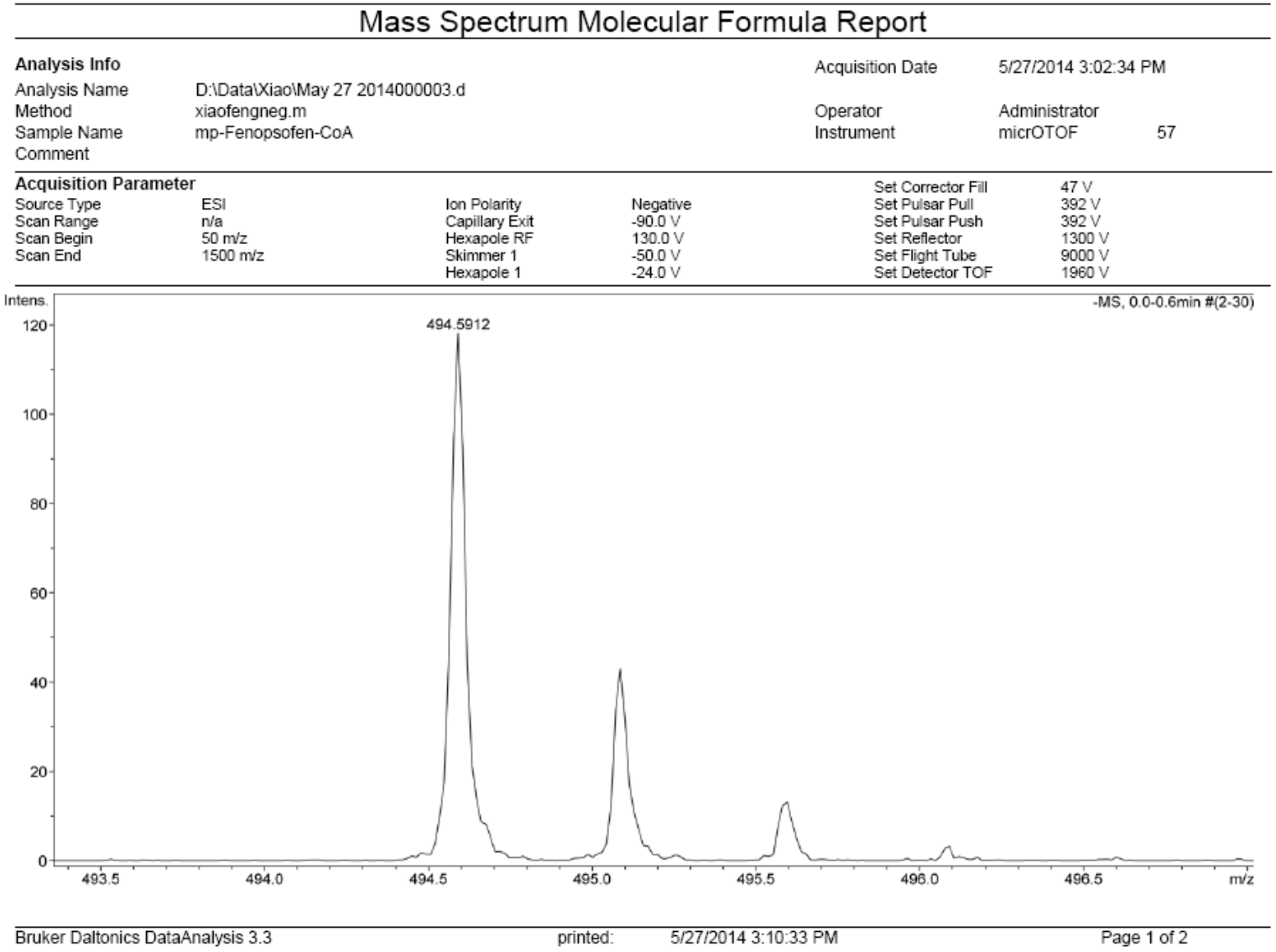


Figure 97S: High resolution mass spectrum of 3-phenylpropanoyl-CoA (**10h**)

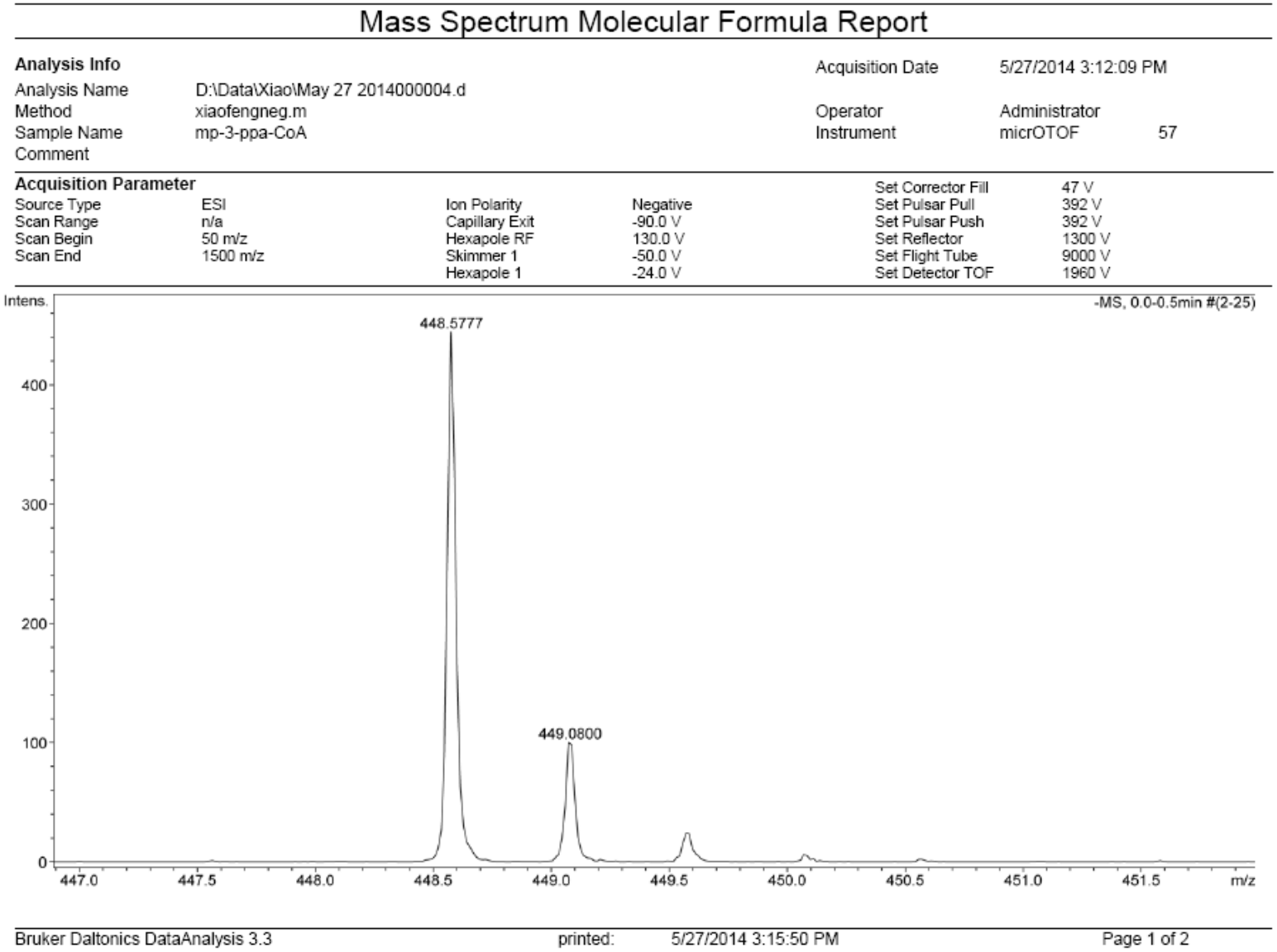
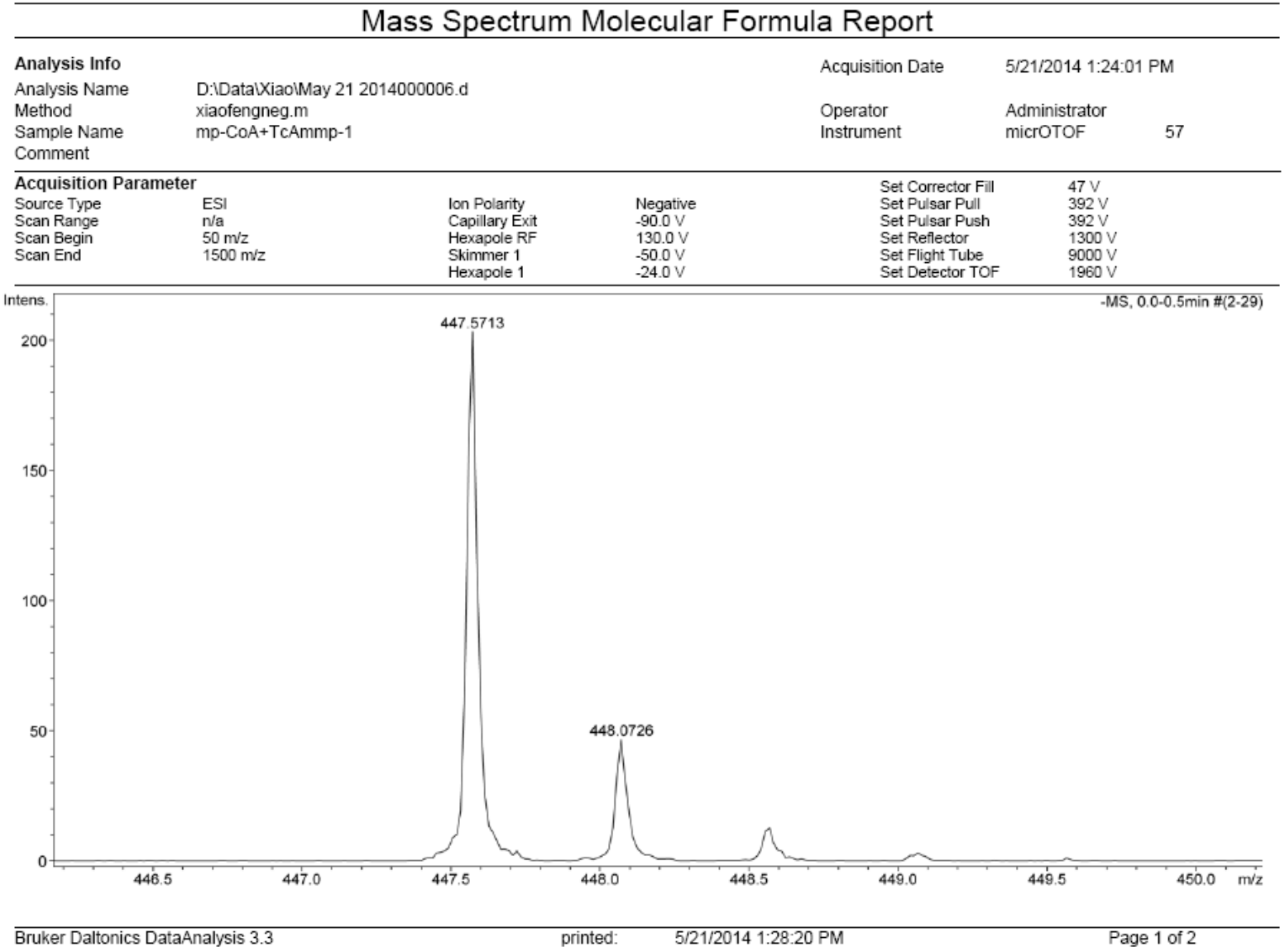


Figure 98S: High resolution mass spectrum of *trans*-cinnamoyl-CoA (**10i**)



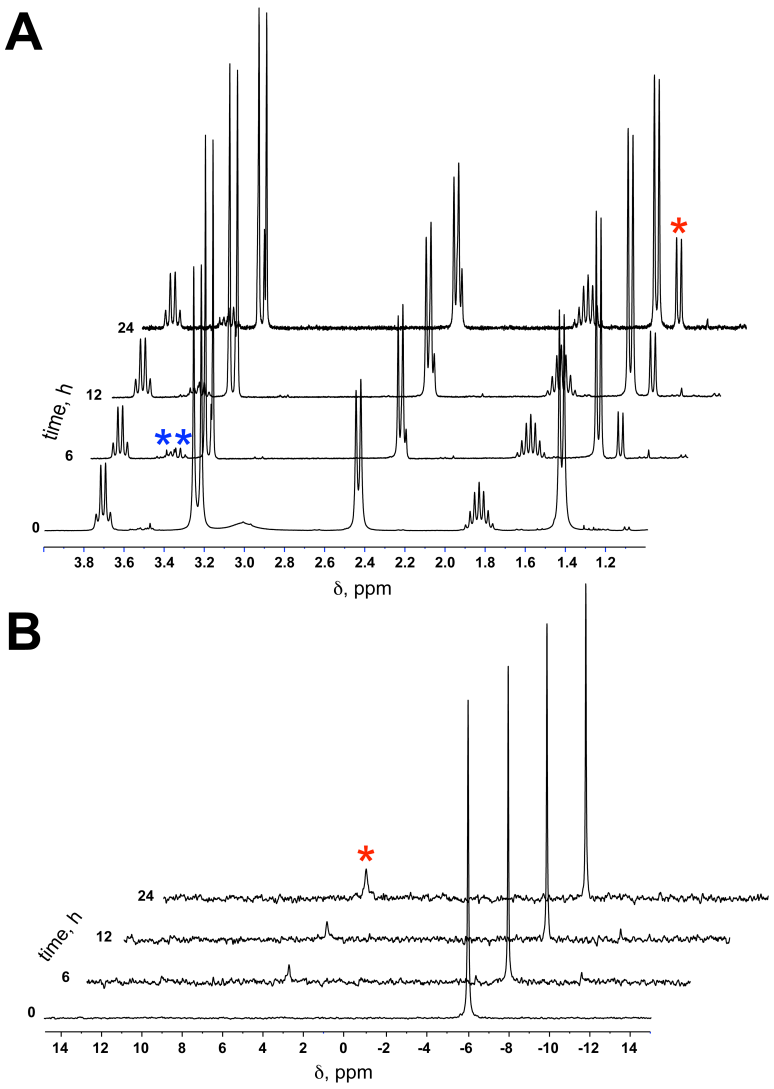


Figure 99S: Time course for the hydrolysis of methyl ibuprofenoyl phosphate (**8f**) in borate buffer. A solution of methyl ibuprofenoyl phosphate (20 mg) in 5 mL borate buffer (pD = 9.0, 100 mM, prepared in D_2O) was stirred for 24 h. Samples were analyzed at 0, 6, 12, and 24 h using ^{1}H (**A**) and ^{31}P (**B**) NMR spectroscopy. In panel A, the appearance of a doublet at δ ~1.3 ppm (*) and the quartet at δ ~3.4 ppm (**) correspond to the α-CH_3 and α-H of ibuprofen. Integration of the doublet at 1.3 ppm and 1.4 ppm were used to calculate the degree of hydrolysis. At 0, 6, 12, and 24 h, the values for the % hydrolysis were 0%, 15%, 19%, and 25%, respectively. In panel B, the signal at 4.94 ppm (*) corresponds to methyl phosphate, and the large signal at –5.95 arises from **8f**.

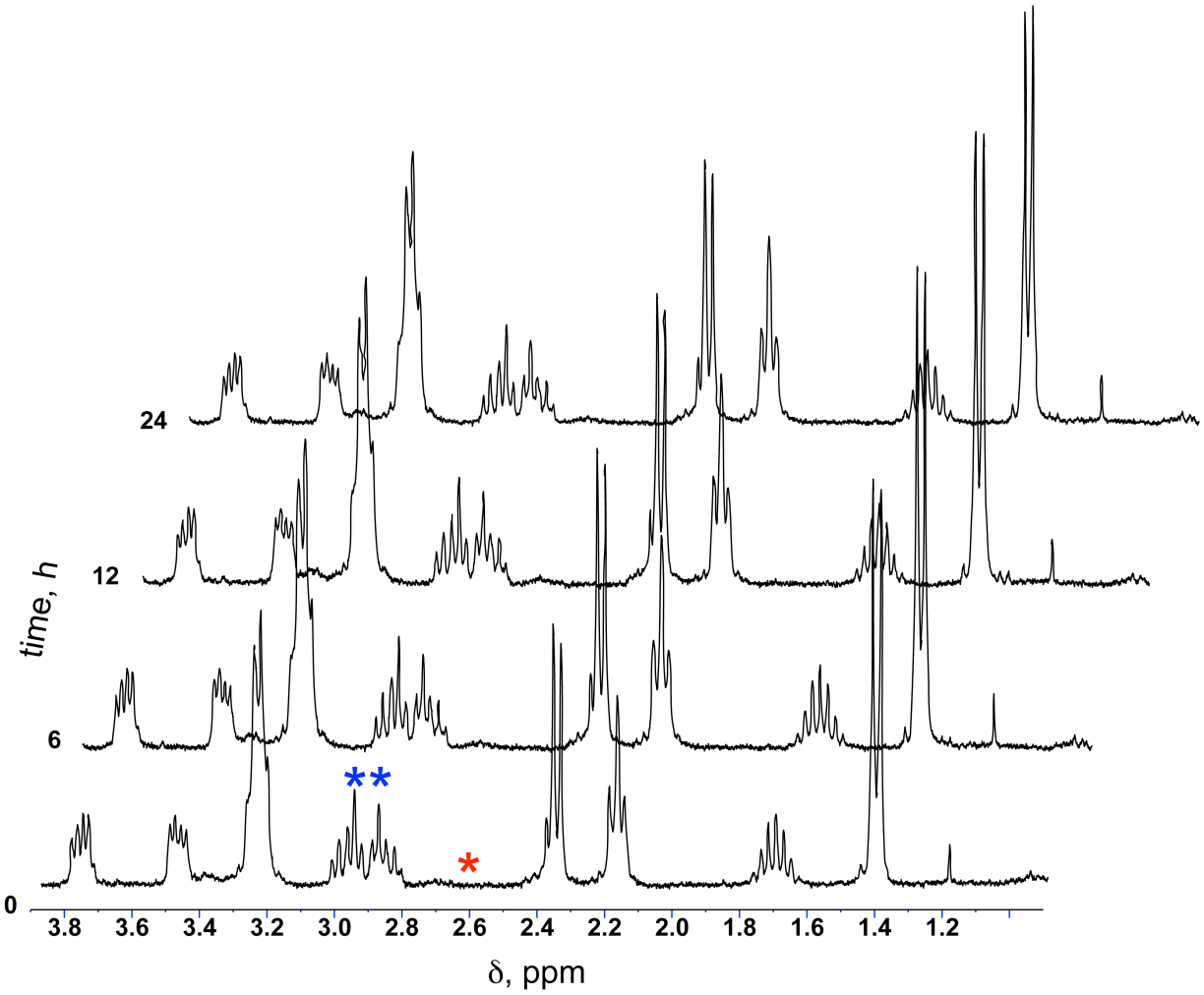


Figure 100S: Time course showing the stability of ibuprofenoyl-CoA (**10f)** in borate buffer. A solution of ibuprofenoyl-CoA (20 mg) in 5 mL borate buffer (pD = 9.0, 100 mM, prepared in D_2O) was stirred for 24 h. Samples were analyzed at 0, 6, 12, and 24 h using ^1H NMR spectroscopy. The multiplet centered at 2.9 ppm (**) corresponds to the 9′′ methylene protons of ibuprofenoyl-CoA, and the absence of a multiplet at ~2.6 ppm (*), corresponding to the 9′′ methylene protons of CoA, indicates that no detectable amount of hydrolysis of the thioester occurred over 24 h.

Table 1S: Percentage conversions for the preparation of CoA thioesters (**10**)

Compound	**CoA-thioester**	**% conversion**[a]
10a	acetyl-CoA	37
10b	butyryl-CoA	30
10c	isovaleryl-CoA	< 10
10d	octanoyl-CoA	30
10e	palmitoyl-CoA	0
10f	ibuprofenoyl-CoA	32
10g	fenoprofenoyl-CoA	55
10h	3-phenylpropanoyl-CoA	40
10i	*trans*-cinnamoyl-CoA	50

[a] Conversions, prior to purification, calculated from integration of the multiplets in the ^{1}H NMR spectra centered at 2.9 – 3.0 ppm and 2.6 ppm arising from the 9″ methylene protons of the acyl-CoA and CoA, respectively (using a 1:1 ratio for methyl acyl phosphate to CoA in the synthesis).

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