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Anticarcinogenic and metal chelation properties of novel hydroxybenzylidene-1-indanone derivatives in the U-251 glioblastoma cell line

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SUPPORTING INFORMATION

Contents

Experimental section

General materials and methods

All reagents and solvents were obtained from commercial suppliers (Sigma Aldrich México, Fluka México) and used without further purification. $Fe_2(CO)_9$ was synthesized from $Fe(CO)_5$ according to the literature.[1] All compounds were characterized by IR spectra, recorded on a Perkin-Elmer 283B or 1420 spectrophotometer, by the KBr technique, and all data are expressed in wavenumbers (cm^{-1}). Melting points were obtained on a Melt-Temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded with a Bruker AV 400 or JEOL Eclipse +300 spectrometer. Chemical shifts for the ^1H NMR spectra were recorded in parts per million from tetramethylsilane with the solvent resonance as the internal standard (chloroform, δ = 7.25 ppm). Chemical shifts for the ^{13}C NMR spectra were recorded in parts per million from tetramethylsilane using the central peak of $CDCl_3$ (δ = 77.1 ppm) as the internal standard. Mass spectra were recorded with a JEOL JMSAX 505 HA spectrometer at 70 eV using the electronic impact (EI), (DART) and fast atom bombardment (FAB^+) technique.

Crystallographic structure determination

A suitable X-ray-quality crystal for **6** and **8** were grown by slow evaporation of an *n*-hexane-CH_2Cl_2 mixture at room temperature. The crystal was mounted on a glass fiber at room temperature, and then placed on a Bruker Smart Apex CCD diffractometer, equipped with Mo Kα radiation; decay was negligible. Structure solutions and refinements were performed using SHELXTL V6.10.[2,3]

Cytotoxicity Assay.

The compounds were screened *in vitro* against six human cancer cell lines: U251 (human glioblastoma), HCT-15 (human colorectal adenocarcinoma), and SKLU-1 (human lung adenocarcinoma), PC-3 (human prostatic adenocarcinoma), K562 (human chronic myelogenous leukemia), MCF-7 (human mammary adenocarcinoma), cell lines were supplied by National Cancer Insitute (USA). The human tumor cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in microculture assay to measure cell growth, as described in the protocols established by the NCI. The cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10000 units/mL penicillin G sodium, 10000 µg/mL streptomycin sulfate, and 25 µg/mL amphotericin B (Gibco) and 1% nonessential aminoacids (Gibco). They were maintained at 37° in humidified atmosphere with 5% CO_2. The viability of the cells used in the experiments exceeds 95% as determined with trypan blue. The cells were removed from the tissue culture flask and diluted with fresh media. Of this cell suspension, 100 µL containing 5000 or 10000 cells per well, were pipetted into 96-well microtiter plates (Costar) and the material was incubated at 37 °C

for 24 h in a 5% CO_2 atmosphere. Subsequently, 100 µL of a solution of the test compounds obtained by diluting the stocks were added to each well. The cultures were exposed for 48 h to the drug at concentrations ranging from 1.0 to 50 µM. After the incubation period, cells were fixed to the plastic substratum by the addition of 50 µL of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4 °C for 1 h. Washed with tap H_2O, and air-dried. The trichloroacetic-acid-fixed cells were stained by the addition of 0.4% SRB. Free SRB solution was removed by washing with 1% aqueous acetic acid. The plates were airdried, and the bound dye was solubilized by the addition of 10 µM unbuffered tris based (100 µL). The plates were placed on a shacked for 5 min, and the absorption was determinate at 515 nm using an ELISA plates reader (Bio-Tex Instruments).

Polymophonuclear (PMN) assay

For the test of PMN the protocol reported by Ivan. J. Fuss et al.[4] was followed.

Statistical analysis

All biological experiments were performed at least twice with triplicates in each experiment. Representative results were depicted in this report. Data were presented as means + standard deviations.

Lipid peroxidation inhibition

Animals. Adult male Wistar rat (200-250 g) was provided by the Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM). Procedures and care of animals were conducted in conformity with Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). They were maintained at 23±2 °C on a 12/12 h light-dark cycle with free access to food and water**.**

Rat Brain Homogenate Preparation. Animal sacrifice was carried out avoiding unnecessary pain. Ten rats were sacrificed with CO_2 to carry out all experiments. The cerebral tissue (whole brain), was rapidly dissected and homogenized in phosphate buffered saline (PBS) solution (0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 2.16 g of $NaHPO_4 \cdot 7\ H_2O$/l, pH adjusted to 7.4) as reported elsewhere [5,6] to produce a 1/10 (w/v) homogenate. Then, the homogenate was centrifuged for 10 min at 800 rcf (relative centrifugal field) to yield a pellet that was discarded. The supernatant protein content was measured using the Folin and Ciocalteu's phenol reagent [7] and adjusted with PBS at 2.666 mg of protein/ml.

Induction of lipid peroxidation and Thiobarbituric Acid Reactive Substances (TBARS) Quantification.

As an index of lipid peroxidation, TBARS levels were measured using rat brain homogenates according to the method described by Ng et al.[8], with some modifications. Supernatant (375 µl) was added with 50 µl of 20 µM EDTA and 50 µl of each sample concentration solved in DMSO (50 µl of DMSO for control group) and incubated at 37 °C for 30 min. Lipid peroxidation was started adding 50 µL of freshly prepared 100 µM $FeSO_4$ solution (final concentration 10 µM), or AAPH and incubated at 37 °C for 1 hour. The TBARS content was determined as described by Ohkawa et al.[9] with some modifications. 500 µl of TBA reagent (1% 2-thiobarbituric acid in 0.05 N NaOH and 30% trichloroacetic acid, in 1:1 proportion) was added at each tube and the final suspension was cooled on ice for 10 min, centrifugated at 13400 rcf for 5 min and heated at 80°C in a water bath for 30 min. After cooling at room temperature, the absorbance of 200 µL of supernatant was measured at λ=540 nm in a Bio-Tek Microplate Reader Synergy HT. Concentration of TBARS was calculated by interpolation in a standard curve of tetra-methoxypropane (TMP) as a precursor of MDA. Results were expressed as nmoles of TBARS per mg of protein. The inhibition ratio (I_R[%]) was calculated using following formula $I_R=(C-E)*100/C$, where *C* is the absorbance of control and *E* is the absorbance of the test sample. Butylated hydroxytoluene (BHT) and α-tocopherol were used as positive standards.

All data were represented as mean± standard error (SEM). Data were analyzed by one-way ANOVA followed by Dunnett´s test for comparison against control. Values of $p \leq 0.05$ (*) and $p \leq 0.01$ (**) were considered statistically significant. The inhibitory concentration 50 (IC_{50}), was estimated by means of a linear regression.

Synthesis and characterizations

General procedure for the synthesis of 2-benzyliden-1-indanones derivatives.

The reaction between *o*-phthalaldehyde and acetophenone in a basic medium was performed to produce the 2-benzyliden-1-indanone type compound under simpler and faster conditions. *O*-phthalaldehyde was added slowly to a cool sodium hydroxide (1.5 eq.) ethanolic solution with the appropriate amount of acetophenone. The reaction mixture was stirred at room temperature for approximately 3 h, and then poured into a mixture of ice and commercial hydrochloric acid (pH was adjusted to about 7). The resulting solid was filtered and in some cases purified by column chromatography using hexane/ethyl acetate. All compounds are stable to aire and temperature, they are soluble in ether, CH_2Cl_3, $CHCl_3$, DMSO, ethyl acetate and partially soluble in ethanol.

General procedure for the synthesis of iron (III) complex of (Z)-2-(1-arylhydroxiliden)-1-indanone

a) A solution of (Z)-2-(1-arylhydroxiliden)-1-indanone in anhydrous ethyl ether (20 mL) was treated with $Fe_2(CO)_9$ or $Fe(CO)_5$ at room temperature for 2-3 h, under an inert atmosphere. After the reaction was complete, the crude product was filtered off through a Celite column, washed with ethyl ether and dissolved in dichloromethane. The solvent was evaporated to dryness. The iron complex (III) was obtained pure.

b) A solution of (Z)-2-(1-arylhydroxiliden)-1-indanone in anhydrous ethyl ether (10mL) was treated with $FeCl_3$ or $FeCl_2$ at room temperature for 3h, under an inert atmosphere. After the reaction was complete, the crude product was filtered off through a Celite column, washed with ethyl ether and crystallized in dichloromethane/hexane. All compounds are stable to aire and temperature, they are soluble in CH_2Cl_3, $CHCl_3$, DMSO and ethyl acetate.

General procedure for the synthesis of cupper (II) complex of (Z)-2-(1-arylhydroxiliden)-1-indanone

A mixture 2 eq. of (Z)-2-(1-arylhydroxiliden)-1-indanone and 1 eq. of cupper (0) in DMSO (20 mL) at room temperature was agitated during 7 days, under an inert atmosphere. After the reaction was complete, the crude product was filtered, washed with ethyl ether and dissolved in dichloromethane. The solvent was evaporated to dryness. The cupper complex (II) was obtained pure. Compound is stable to aire and temperature, it is soluble in CH_2Cl_3, $CHCl_3$, DMSO and ethyl acetate.

Compound 1 ($C_{16}H_{12}O_2$, *M*=236 g/mol) was prepared starting from *o*-phthalaldehyde (0.5 g, 3.73 mmol), acetophenone (0.48g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid, **mp**. 90°C, (0.73g, 83%). **IR**: ν 1604, 1564 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 15.067(s, 1H, OH), 7.49, 7.5(d, 1H, 1), 7.54 (ddd, 1H, 2), 7.40 (dd,1H,3), 7.85(d, 1H, 4), 7.91(m, 1H,10), 7.48 (m,3H,11,12,13), 7.91(m, 1H,14), 3.86 (sa, 2H, 15). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.54 (C1), 133.27 (C2), 127.40 (C3), 123.36 (C4), 137.85 (C5), 195.70 (C6), 109.41 (C7), 170.79 (C8), 134.79 (C9), 128.07 (C10), 128.55 (C11), 131.21 (C12), 128.55 (C13), 128.07 (C14), 32.19 (C15), 148.51(C16). **MS (EI):** m/z (%) = 236 (1.9%). **HRMS** (FAB^+): calculated for $C_{16}H_{12}O_2$: 237.0821. Found: 237.0823.

Compound 2 ($C_{16}H_{11}ClO_2$, *M*=270.5 g/mol) was prepared starting from *o*-phthalaldehyde (0.5 g, 3.73 mmol), 4'-chloroacetophenone (0.69g, 4.48mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale brown solid, **mp**. 162-164°C, (0.86g, 85%). **IR**: ν 1605, 1559 cm^{-1}. **^{1}H NMR** (300

MHz, $CDCl_3$): δ= 15.03 (s, 1H, OH), 7.54 (d, 1H9, 1), 7.60 (d, 1H, 2), 7.44 (dd, 1H, 3), 7.86 (dd, 1H, 4), 7.89 (d, 2H, 10, 14), 7.48 (d, 2H, 11, 13), 3.91 (s, 2H, 15). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.45 (C1), 133.35 (C2), 127.35 (C3), 123.19 (C4), 137.44 (C5), 195.63 (C6), 109.29 (C7), 169.14 (C8), 132.99 (C9), 129.22 (C10), 128.70 (C11), 137.22 (C12), 128.70 (C13), 129.22 (C14), 31.95 (C15), 148.19 (C16). **MS (EI):** m/z (%) = 270 (15%). **HRMS** (FAB^+): calculated for $C_{16}H_{11}ClO_2$: 271.0526. Found: 271.0522.

Compound 3 ($C_{17}H_{14}O_2$, *M*=250 g/mol) was prepared starting from *o*-phthalaldehyde (0.5 g, 3.73 mmol), 4'-methylacetophenone (0.60g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid, **mp**. 96-98°C, (0.79g, 85%). **IR:** ν 1604, 1540 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 15.17 (s, 1H, OH), 7.50 (d, 1H, 1), 7.55 (dd, 1H, 2), 7.41 (dd, 1H, 3), 7.85(d, 1H, 4), 7.84 (d, 1H, 10), 7.30 (d, 2H, 11,13), 7.84 (d, 1H, 14), 3.89 (s, 2H, 15), 2.41 (s, 3H, 17). **^{13}C NMR** (75MHz, $CDCl_3$): δ =125.50 (C1), 133.11 (C2), 127.35 (C3), 123.27 (C4), 137.95 (C5), 195.45 (C6), 109.01 (C7), 171.09 (C8), 131.97 (C9), 128.08 (C10), 129.29 (C11), 141.89 (C12), 129.29 (C13), 128.08 (C14), 32.32 (C15), 148.39 (C16), 21.52 (C17). **MS (EI)**: m/z (%) = 250 (20%). **HRMS** (FAB^+): calculated for $C_{17}H_{14}O_{2:}$ 251.1072. Found: 251.1079.

Compound 4 ($C_{17}H_{14}O_3$, *M*=266 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.73 mmol), 4'-methoxyacetophenone (0.672g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid, **mp**.112-114 °C, (0.79 g, 80%). **IR**: ν 1601, 1566 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 15.33(s, 1H, OH), 7.47 (d, 1H, 1), 7.51 (dd, 1H, 2), 7.37 (dd, 1H,3), 7.82 (d, 1H, 4), 7.89 (d, 2H, 10, 14), 6.95 (d, 2H, 11, 13), 3.82 (s, 2H, 15), 3.83 (s, 3H, 17). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.37 (C1), 132.85 (C2), 127.23 (C3), 123.03 (C4), 137.87 (C5), 194.84 (C6), 108.32 (C7), 170.04 (C8), 127.00 (C9), 129.99 (C10), 113.88 (C11), 162.06 (C12), 113.88 (C13), 129.99 (C14), 32.45 (C15), 148.09 (C16), 55.30 (C17). **MS (EI)**: m/z (%) = 266 (15%). **HRMS** (FAB^+): calculated for $C_{17}H_{14}O_{3:}$ 267.1021. Found: 267.1023.

Compound 5 ($C_{16}H_{11}O_2Br$, *M*=314 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.73 mmol), 4'-bromoacetophenone (0.89g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid **mp**.160-162 °C, (0.46 g, 40%). **IR**: ν 1622, 1555 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 15.03(s, 1H, OH), 7.57 (m, 1H, 1, 2, 4, 11, 13), 7.44 (dd, 1H, 3), 7.81 (d, 1H, 10), 7.89 (d, 1H, 14), 3.90 (s, 3H, 15). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.67 (C1), 133.61 (C2), 127.62 (C3), 123.57 (C4), 137.74 (C5), 194.04 (C6), 109.57 (C7), 169.34 (C8), 130.00 (C9), 131.94 (C10), 129.63 (C11), 135.12 (C12),

129.63 (C13), 131.94 (C14), 32.20 (C15), 148.44 (C16). **MS (EI)**: m/z (%) = 314 (20%). **HRMS** (FAB^+): calculated for $C_{16}H_{12}O_2Br$ [$M^{+\cdot}$]: 315.0021 Found: 315.0017.

Compound 6 ($C_{14}H_{10}O_2S$, *M*=242 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.73 mmol), 2-thioacetophenone (0.57g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid **mp**.130-132 °C, (0.7 g, 77%). **IR**: ν 1709, 1604 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 14.83(s, 1H, OH), 7.56 (m, 2H, 1, 2), 7.44 (dd, 1H, 3), 7.67 (d, 1H, 4), 7.81 (d, 1H, 10), 7.22 (m, 1H, 11), 7.85 (d, 1H, 12), 3.89 (s, 2H, 13). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.68 (C1), 133.20 (C2), 127.54 (C3), 123.21 (C4), 138.31 (C5), 194.12 (C6), 108.07 (C7), 166.09 (C8), 135.12 (C9), 128.29 (C10), 130.79 (C11), 131.62 (C12), 32.18 (C13), 147.83 (C14). **MS (EI)**: m/z (%) = 242 (50%). **HRMS** (FAB^+): Found: $C_{15}H_{10}O_2S$ [$M^{+\cdot}$] 243.0480.

Compound 7 ($C_{15}H_{11}O_2N$, *M*=237 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.7mmol), 2-acetylpyridine (0.54g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid **mp**.136-138 °C, (0.7 g, 70%). **IR**: ν 1615, 1559 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 14.75(s, 1H, OH), 7.50 (m, 3H, 10, 11, 12), 7.78 (d, 1H, 1), 8.1 (d, 1H, 2), 7.66 (dd, 1H, 3), 8.44 (d, 1H, 4), 8.65 (d, 1H, 13), 3.87 (s, 2H, 14). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.02 (C1), 130.11 (C2), 126.82 (C3), 127.02 (C4), 133.64 (C5), 183.11 (C6), 114.31 (C7), 168.11 (C8), 153.12 (C9), 122.36 (C10), 139.96 (C11), 124.56 (C12), 149.16 (C13), 35.59 (C14), 144.63 (C15). **MS (EI):** m/z (%) = 237 (100%). **HRMS** (FAB^+): calculated for $C_{15}H_{12}O_2N$ [$M^{+\cdot}$]: 238.0868 Found: 238.0874.

Compound 8 ($C_{20}H_{16}O_2Fe$, *M*=344 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.7mmol), acetylferrocene (1.02g, 4.48 mmol) and *t*BuOK (0.42g, 3.73mmol) and was obtained as a red solid **mp**.140-141 °C, (0.60 g, 45%). **IR**: ν 1708, 1603 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 14.98(s, 1H, OH), 7.87 (d, 1H, 4), 7.58 (m, 2H, 1, 2), 7.47 (dd, 1H, 3), 4.97 (s, 2H, 10,13), 4.58 (s, 2H, 11,12), 4.21 (s, 5H, 14), 3.80 (s, 2H, 15). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.59 (C1), 132.5 (C2), 127.43 (C3), 122.97 (C4), 138.62 (C5), 192.33 (C6), 107.94 (C7), 177.57 (C8), 132.5 (C9), 71.91 (C10, 13), 69.27 (C11, 12), 70.27 (C14), 31.90 (C15), 147.43 (C16). **MS (DART):** m/z (%) = 344 (20%). **HRMS** (ESI^+): calculated for $C_{20}H_{17}O_2Fe$ [$M^{+\cdot}$]: 345.05780 Found: 345.05734.

Compound 9 ($C_{20}H_{14}O_2$, *M*=286 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.73 mmol), 2-acetylnaphthone (0.76g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid **mp**.102-104 °C, (0.80 g, 75%). **IR**: ν 3056, 1674, 1617 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 15.23(s, 1H, OH), 7.94 (m, 7H), 7.55 (m, 4H), 4.00 (s, 2H, H15). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 195.8, 170.8, 148.6, 137.9, 133.4, 120.2, 126.8, 125.7, 124.3, 109.8, 32.43. **MS (DART):** m/z (%) = 287 (360%). **HRMS** (ESI^+): calculated for $C_{20}H_{15}O_2$ [$M^{+\cdot}$]: 287.10720 Found: 287.10694.

Compound 10 ($C_{15}H_{11}O_2N$, *M*=237 g/mol) was prepared starting from *o*-phthalaldehyde (0.5g, 3.7mmol), 4-acetylpyridine (0.54g, 4.48 mmol) and NaOH (0.22 g, 5.5 mmol) and was obtained as a pale yellow solid **mp**.136-138 °C, (0.7 g, 80%). **IR**: ν 1615, 1559 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ= 14.75(s, 1H, OH), 7.50 (m, 3H, 10, 11, 12), 7.78 (d, 1H, 1), 8.1 (d, 1H, 2), 7.66 (dd, 1H, 3), 8.44 (d, 1H, 4), 8.65 (d, 1H, 13), 3.87 (s, 2H, 14). **^{13}C NMR** (75MHz, $CDCl_3$): δ = 125.02 (C1), 130.11 (C2), 126.82 (C3), 127.02 (C4), 133.64 (C5), 183.11 (C6), 114.31 (C7), 168.11 (C8), 153.12 (C9), 122.36 (C10), 139.96 (C11), 124.56 (C12), 149.16 (C13), 35.59 (C14), 144.63 (C15). **MS (EI):** m/z (%) = 237 (100%). **HRMS** (FAB^+): calculated for $C_{15}H_{12}O_2N$ [$M^{+\cdot}$]: 238.0868 Found: 238.0874.

Compound 1a ($C_{48}H_{33}FeO_6$, *M*=761 g/mol) was prepared starting from **1** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.21 g, 0.56 mmol) and was obtained as a purple solid, **mp**. 244-246°C, (0.425 g, 80 %). **MS (FAB^+):** m/z (%) = 761 (2%). **HRMS** (FAB^+): calculated for $C_{32}H_{22}O_4Fe$ [$M^{+\cdot}$]: 526.0867. Found: 526.0877.
Compound 2a ($C_{48}H_{30}Cl_3FeO_6$, *M*=863 g/mol) was prepared starting from **2** (0.5g, 2.1 mmol), and $Fe_2(CO)_9$ (0.18 g, 0.56 mmol) and was obtained as a purple solid, **mp**. 232-234°C, (0.48 g, 90%). **MS (FAB^+)**: m/z (%) = 864 (2%). **HRMS** (FAB^+): calculated for $C_{32}H_{20}O_4ClFe$ [$M^{+\cdot}$]: 594.0088. Found: 594.0092.

Compound 3a ($C_{51}H_{39}FeO_6$, *M*=803 g/mol) was prepared starting from **3** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.19 g, 0.56 mmol) and was obtained as a purple solid, **mp**. 238-240 °C, (0.33 g, 61%). **MS (FAB^+)**: m/z (%) = 803 (5%). **HRMS** (FAB^+): calculated for $C_{34}H_{26}O_4Fe$ [$M^{+\cdot}$]: 554.1180. Found: 554.1187.

Compound 4a ($C_{51}H_{39}FeO_9$, *M*=851 g/mol) was prepared starting from **4** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.18 g, 0.56 mmol) and was obtained as a purple solid, **mp**. 234-236°C, (0.37 g, 70%). **MS (FAB^+)**: m/z (%) = 851 (2%). **HRMS** (FAB^+): calculated for $C_{34}H_{26}O_6Fe$ [$M^{+\cdot}$]: 586.1079. Found: 554.1082.

Compound 7a ($C_{45}H_{30}FeN_3O_6$, *M*=764 g/mol) was prepared starting from **8** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.21 g, 0.56 mmol) and was obtained as a dark green solid, **dp**. >280°C, (0.52 g, 97%). **IR**: ν 3059, 1590, 1552 cm^{-1}**MS (FAB$^+$)**: m/z (%) = 765 (5%), 528 (40%).

Compound 8a ($C_{60}H_{45}Fe_4O_6$, *M*=1085 g/mol) was prepared starting from **9** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.14g, 0.56 mmol) and was obtained as a deep red solid, **dp**. 220-222°C, (0.49 g, 95%). **IR**: ν 3082, 1589, 1551 cm^{-1}. **MS (FAB$^+$)**: m/z (%) = 1085 (5%), 742 (10%). HRMS (FAB+): calculated for $C_{40}H_{30}O_4Fe_3$ [M+.]: 742.0192. Found: 742.0189.

Compound 9a ($C_{60}H_{39}FeO_6$, *M*=911 g/mol) was prepared starting from **10** (0.5 g, 2.1 mmol), and $Fe_2(CO)_9$ (0.17 g, 0.56 mmol) and was obtained as a deep red solid, **mp**. 196-198°C, (0.45 g, 85%). **IR**: ν 3051, 1583, 1554 cm^{-1}. **MS (FAB$^+$)**: m/z (%) = 911 (5%), 626 (10%). HRMS (FAB+): calculated for $C_{40}H_{26}FeO_4$ [$M^{+\cdot}$] : 626.1180. Found: 626.1189.

Compound 1b ($C_{32}H_{22}O_4Cu$, *M*=533.5 g/mol) was prepared starting from 1 (0.5g, 2.1mmol) and Cu (0.05g, 0.79mmol) in DMSO and was obtained as a green solid, mp. >200 °C **IR**: ν 3058, 1593, 1561. **MS (FAB$^+$)**: m/z (%) = 534 (10%). **HRMS** (FAB$^+$): calculated for $C_{32}H_{23}O_4Cu$ [$M^{+\cdot}$]: 534.0892. Found: 534.0883

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natural products from plants. Life Sci., 2000, 66, 709–723.
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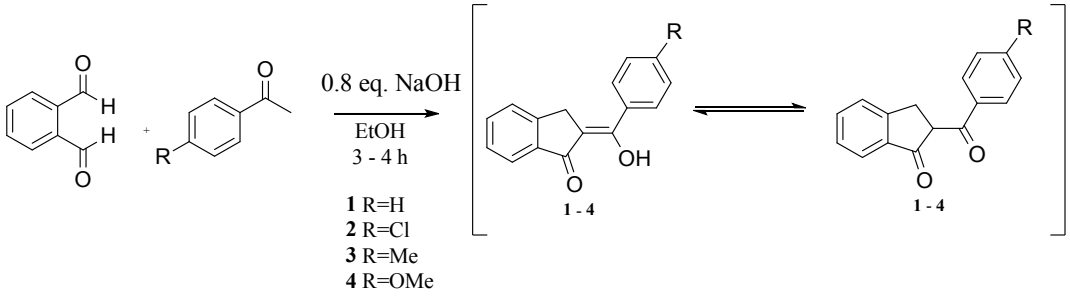
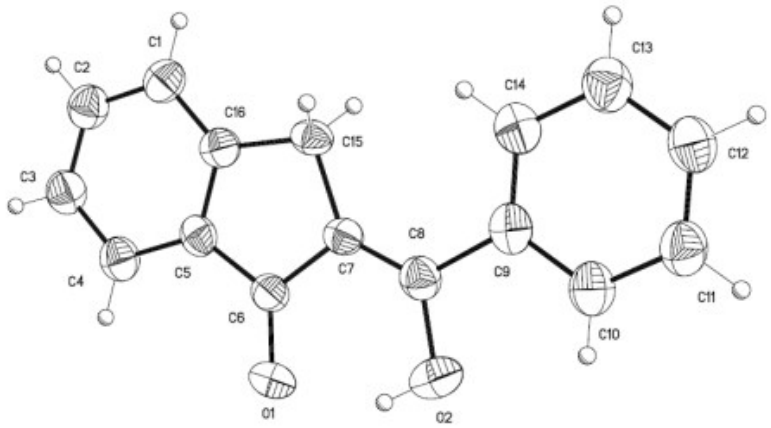
10) M. Turek, D. Szczesna, M. Koprowski and P. Balczewski, Beilstein J. Org. Chem., 2017, 13, 451–494.

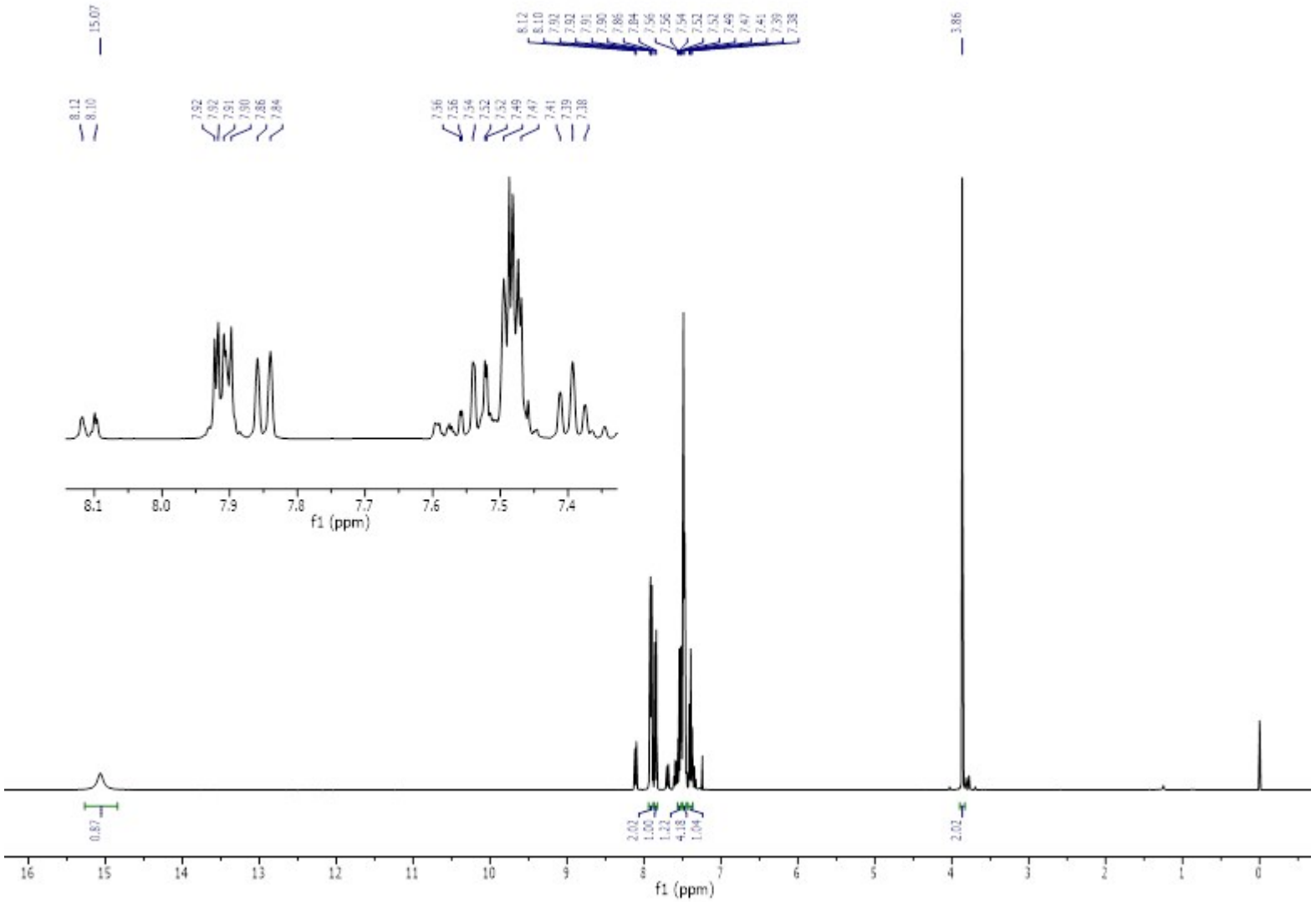
RMN Spectrum



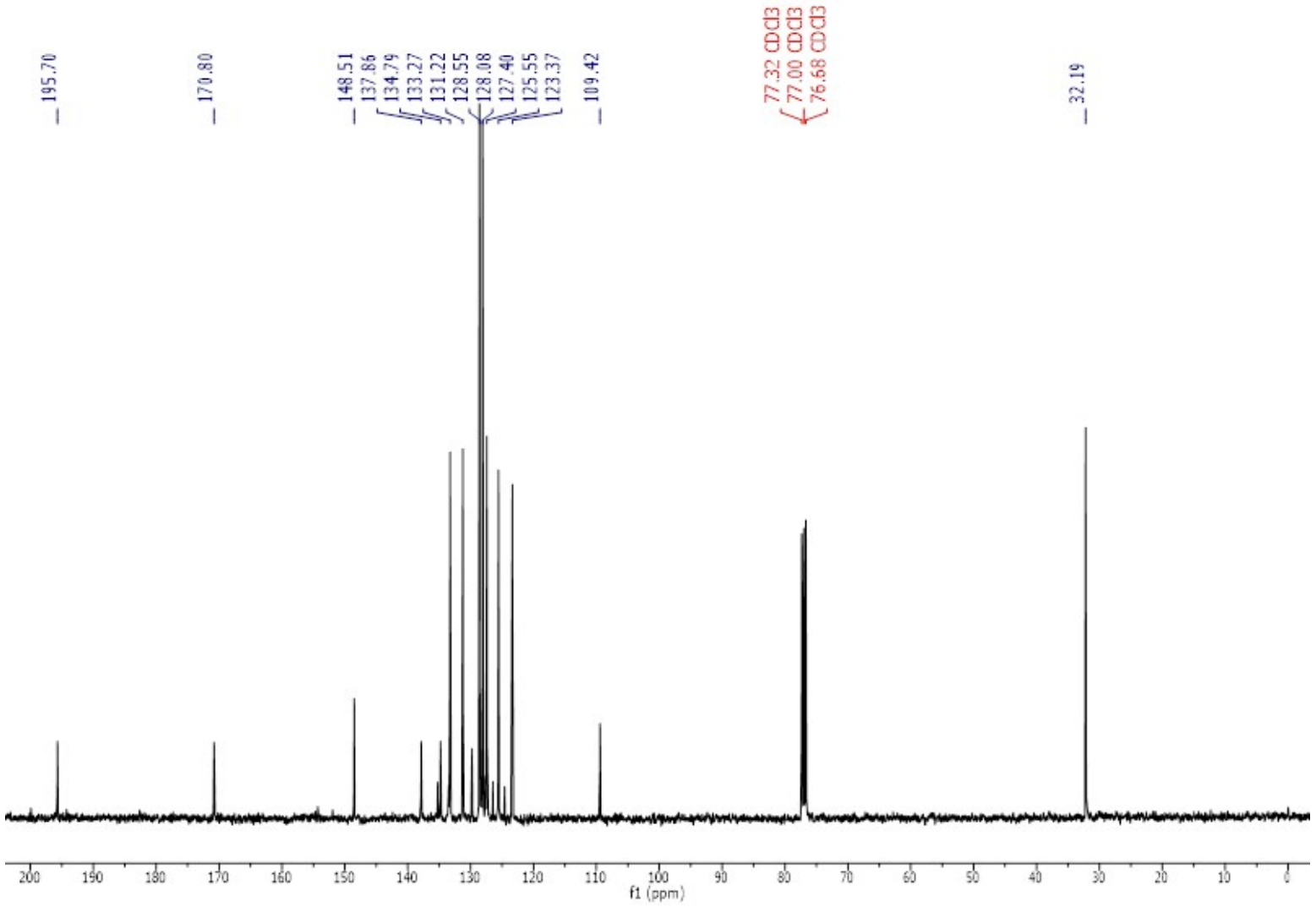
Exist an equilibrium keto-enolic, enol form is greatly favored (90-95%)


C1
C2
C16
C15
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
O1
O2

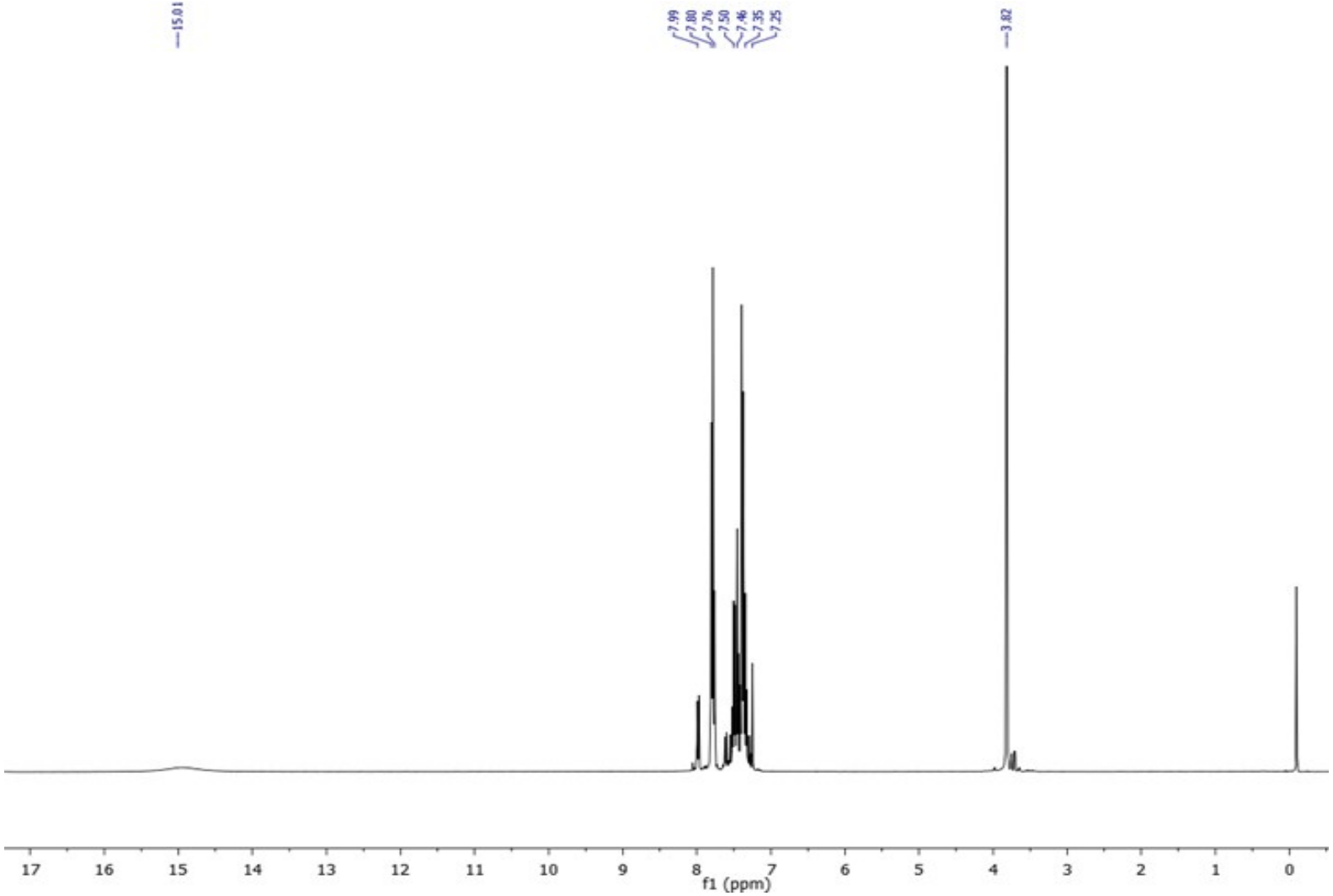
^{1}H RMN /Compound **1** ($CDCl_3$) 400MHz



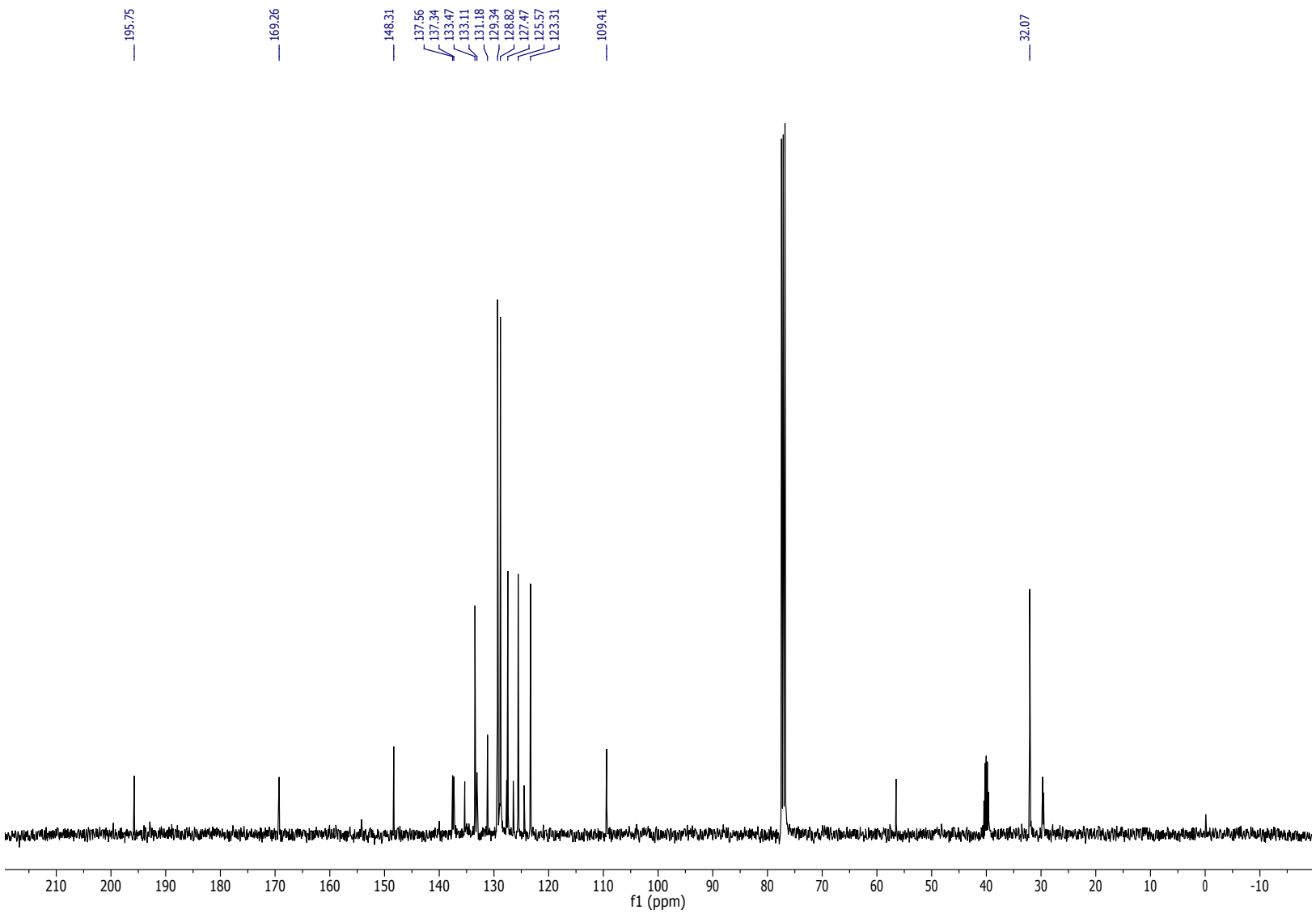
^{13}C RMN /Compound **1** ($CDCl_3$) 100MHz



^{1}H RMN /Compound **2** ($CDCl_3$) 400MHz



^{13}C RMN /Compound **2** ($CDCl_3$) 100MHz

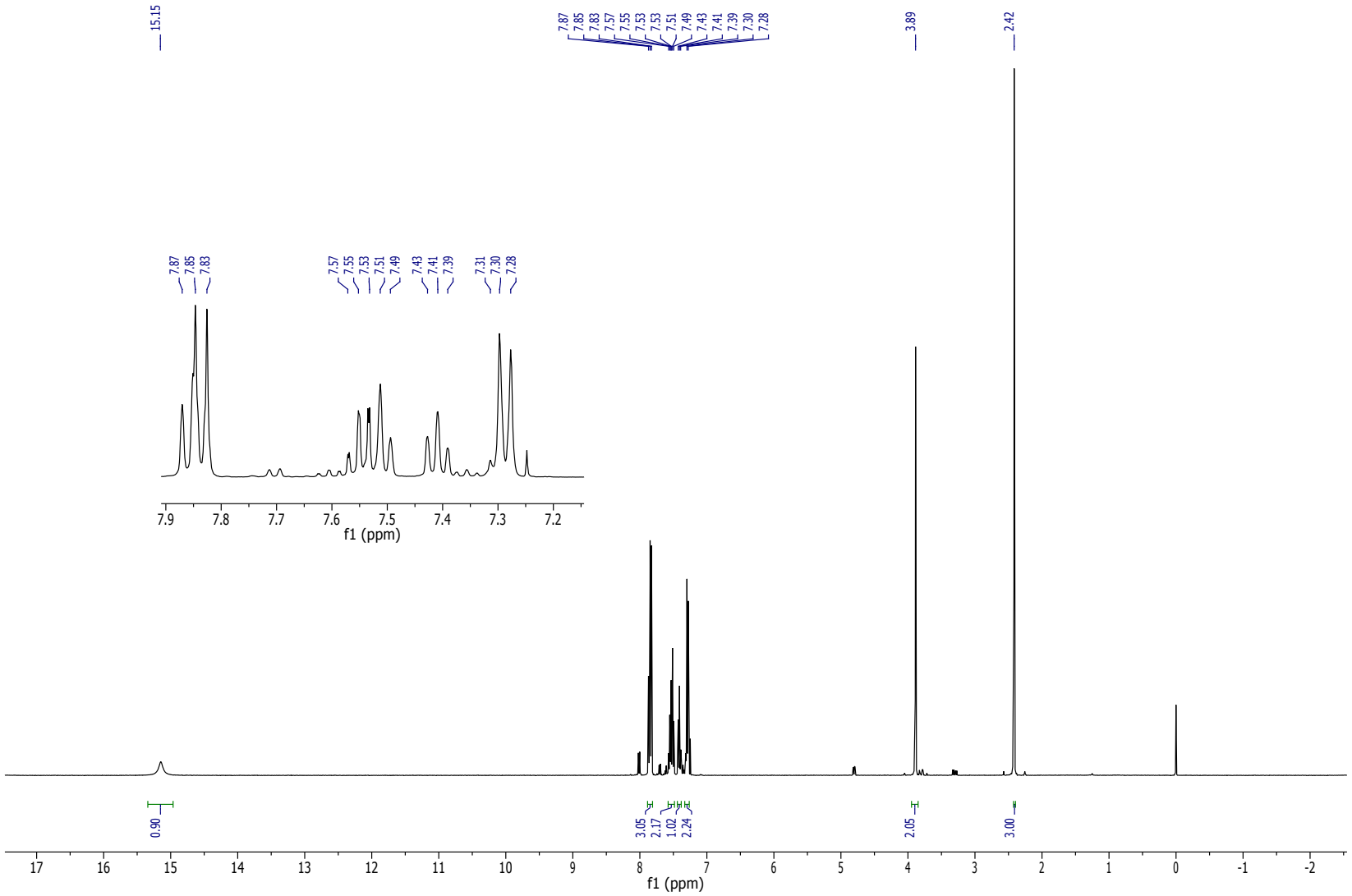

195.75
169.26
148.31
137.56
137.34
133.47
133.11
131.18
129.34
128.82
127.47
125.57
123.31
109.41
32.07
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Elemental Composition Compound **2**

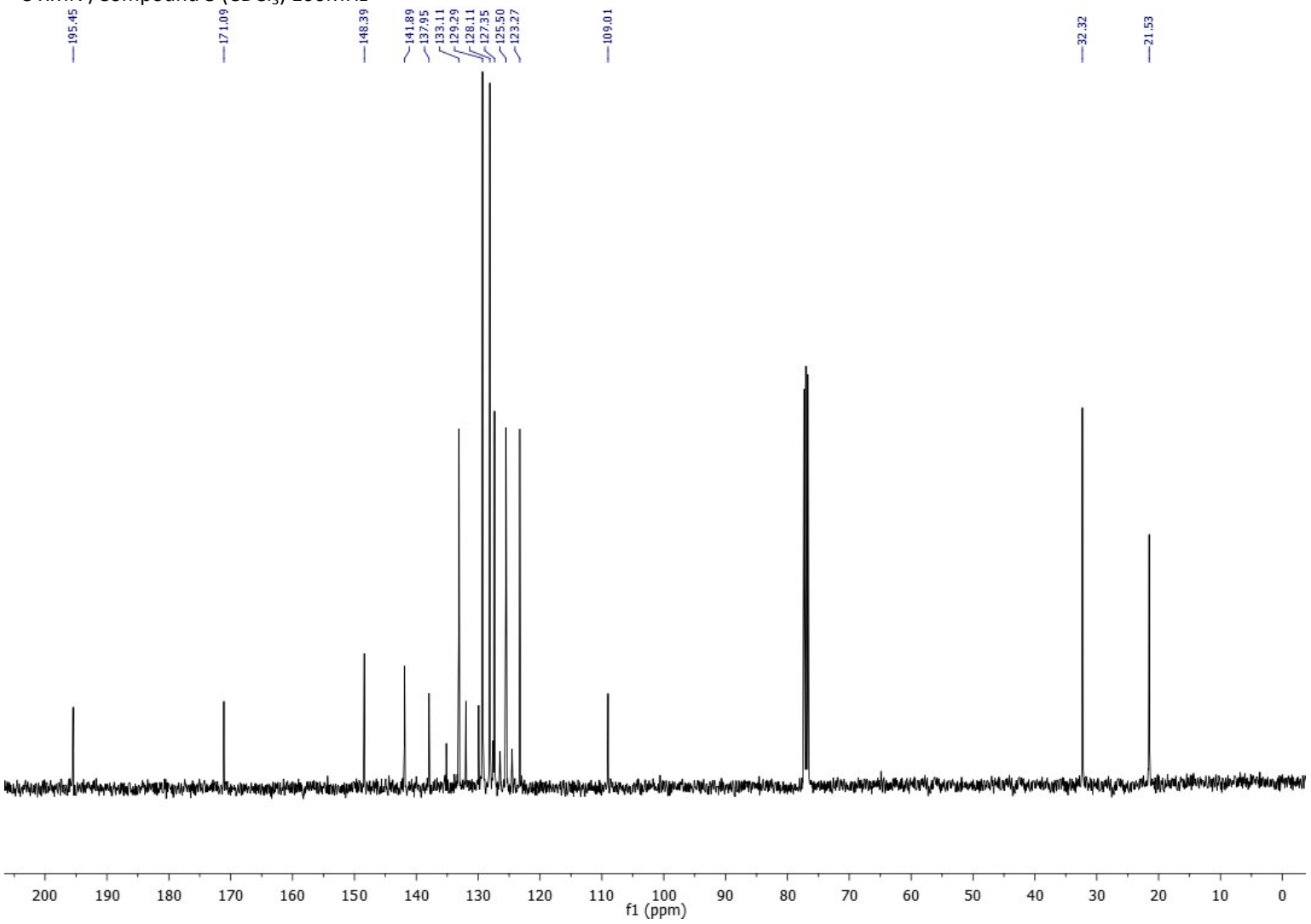
```
Sample: 2013        LCl-ME
Note : -luis-velasco
Inlet : Direct                          Ion Mode : FAB+
RT : 5.53 min                           Scan#: 14
Elements : C 40/0, H 58/0, O 6/1, Cl 3/0
Mass Tolerance       : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 0.0 - 25.0

Observed m/z   Int%
  271.0522     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     Cl
  271.0526       -1.2      10.5    16    12     2      1
```

^{1}H RMN /Compound **3** ($CDCl_3$) 400MHz



^{13}C RMN /Compound **3** ($CDCl_3$) 100MHz

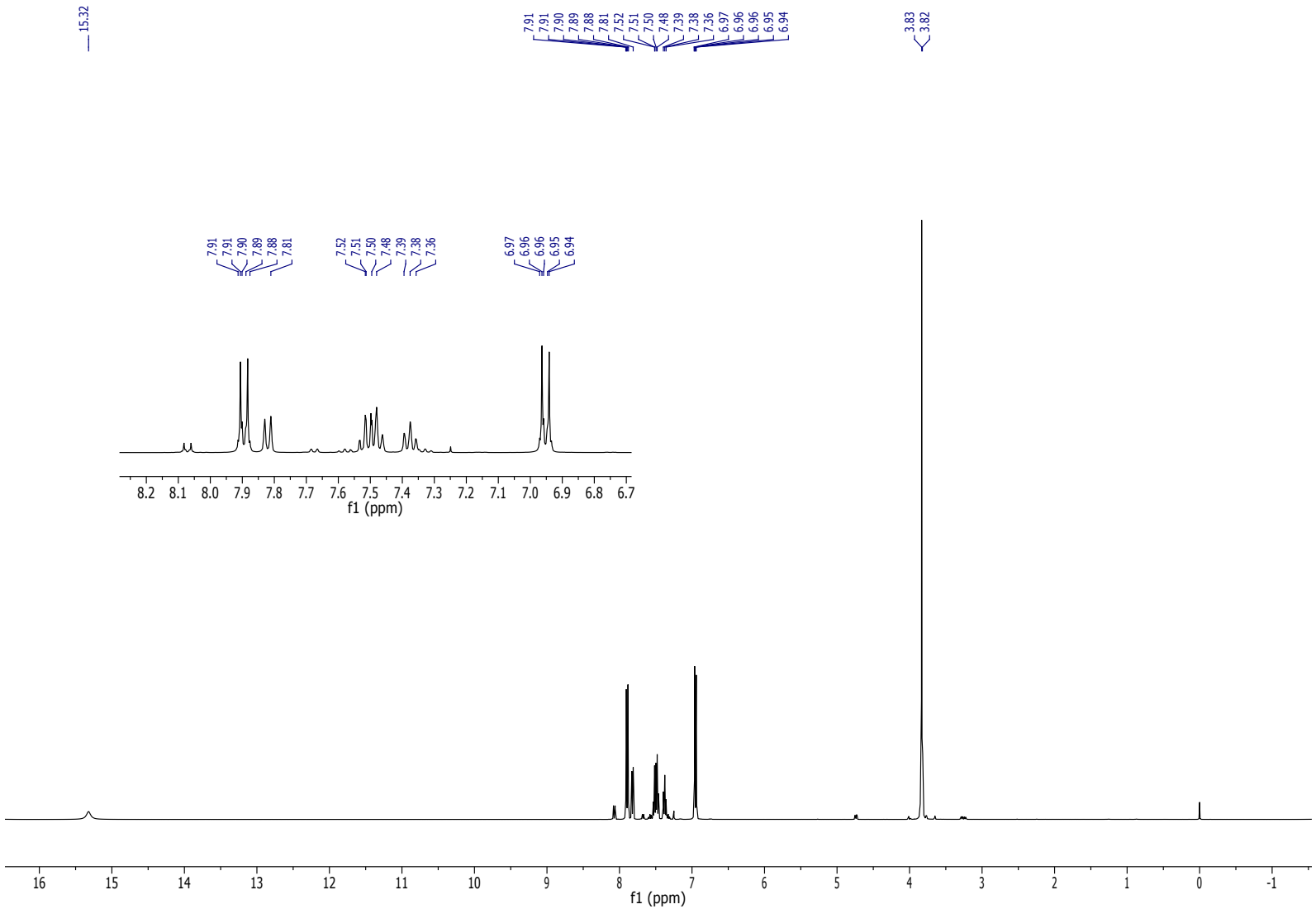


Elemental Composition Compound **3**

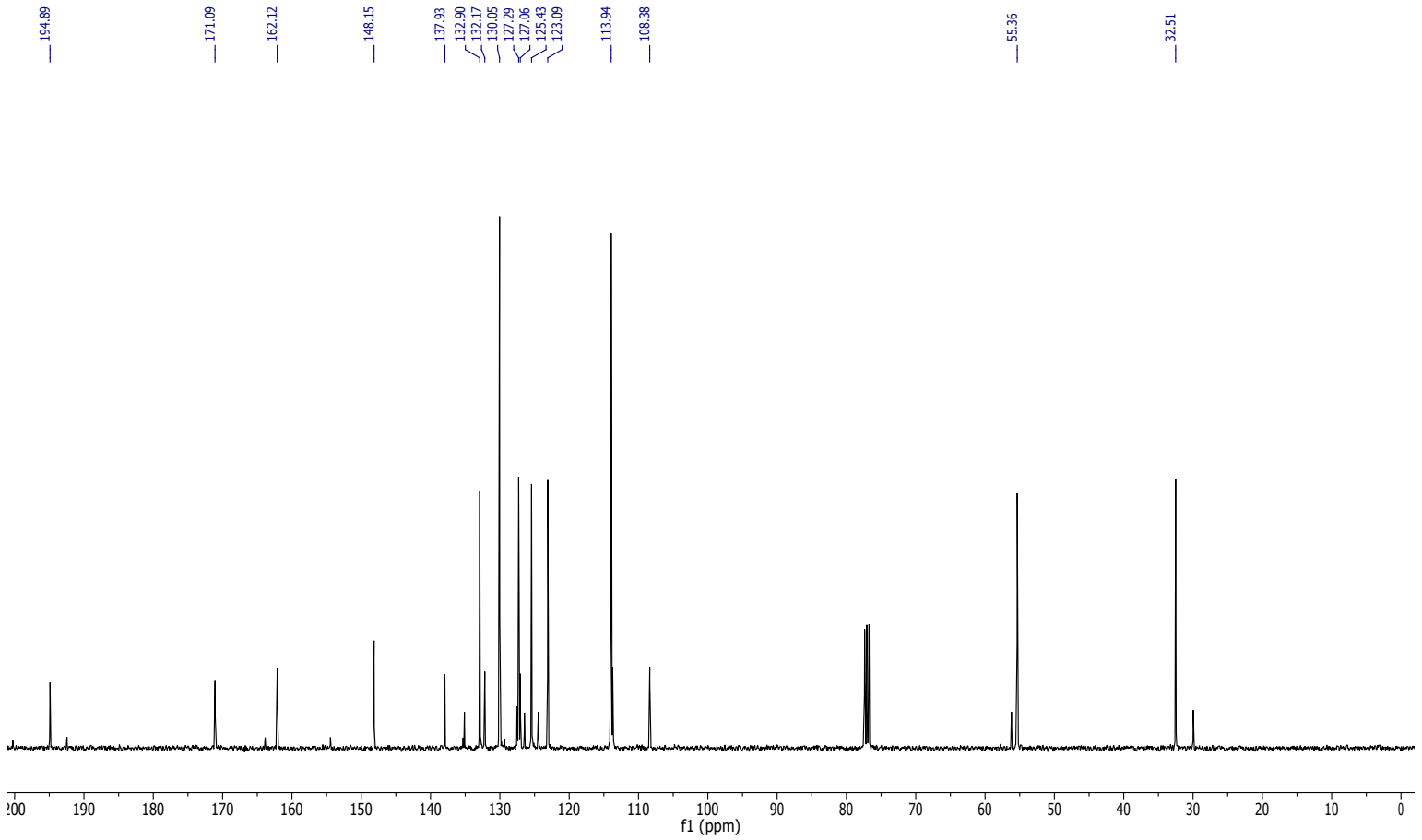
```
Sample: 1407     C3Me
Note : -luis-velasco
Inlet : Direct                          Ion Mode : FAB+
RT : 5.78 min                           Scan#: (14,16)
Elements : C 40/0, H 49/0, O 9/0
Mass Tolerance       : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 4.0 - 12.0

Observed m/z   Int%
  251.1079     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O
  251.1072       +2.7      10.5    17    15     2
```

^{1}H RMN /Compound **4** ($CDCl_3$) 400MHz



^{13}C RMN /Compound **4** ($CDCl_3$) 100MHz

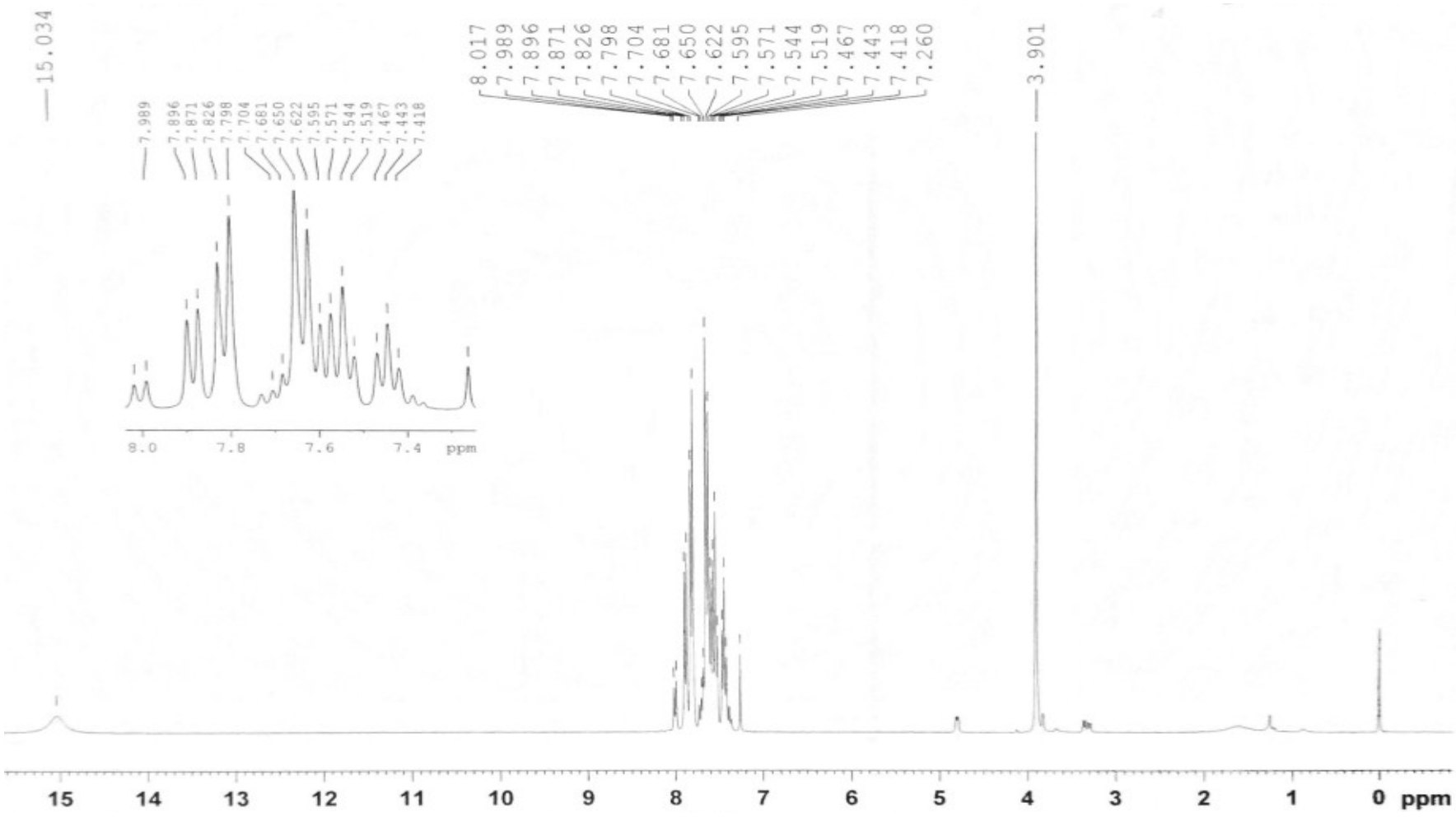


Elemental Composition Compound **4**

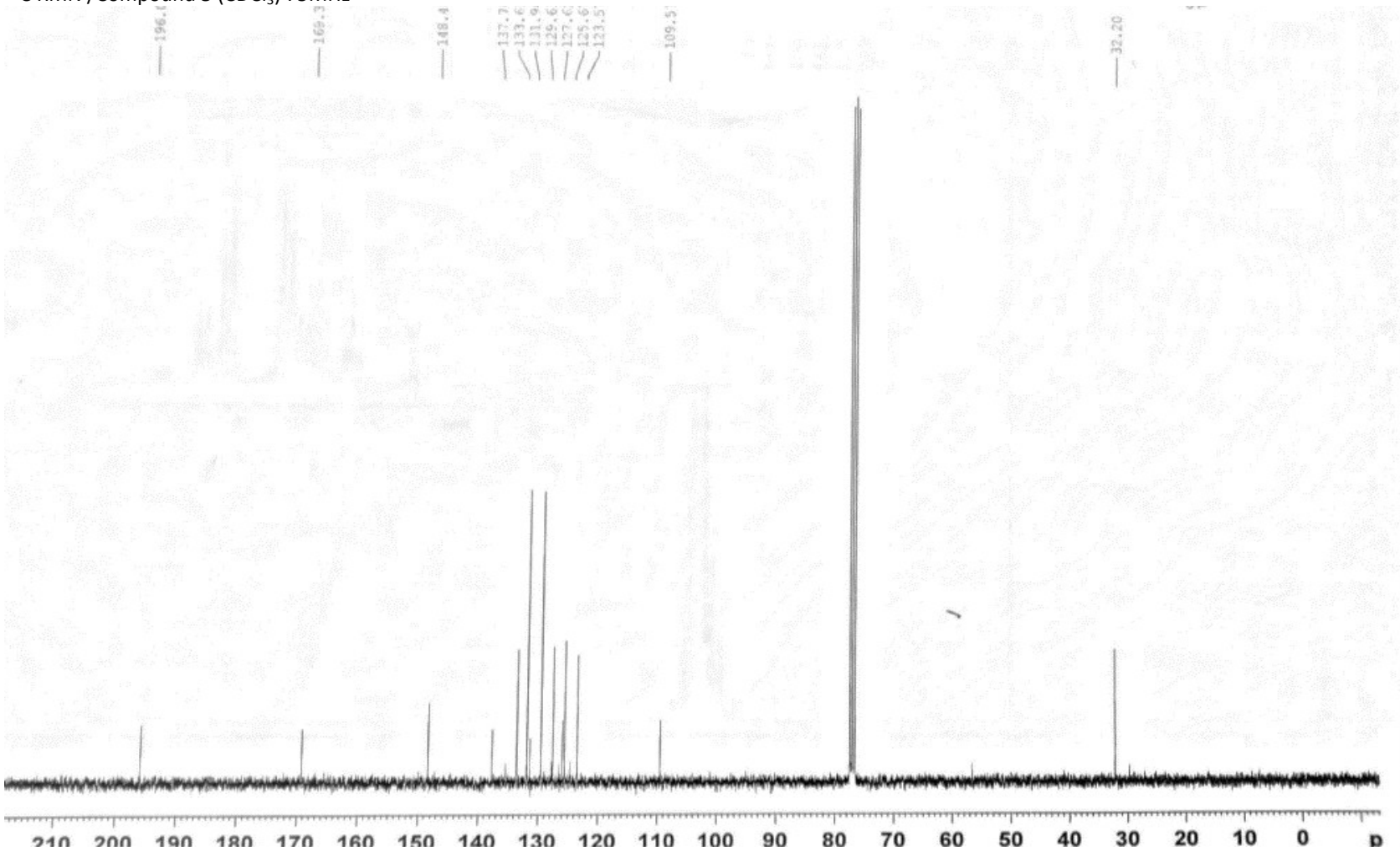
Sample: 1408 C4OMe
Note : -luis-velasco
Inlet : Direct Ion Mode : FAB+
RT : 2.89 min Scan#: 8
Elements : C 40/0, H 49/0, O 9/0
Mass Tolerance : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 4.0 - 12.0

Observed m/z	Int%				
267.1022	100.0				
Estimated m/z	Error[ppm]	U.S.	C	H	O
267.1021	+0.3	10.5	17	15	3

^{1}H RMN /Compound **5** ($CDCl_3$) 300MHz



^{13}C RMN /Compound **5** ($CDCl_3$) 75MHz

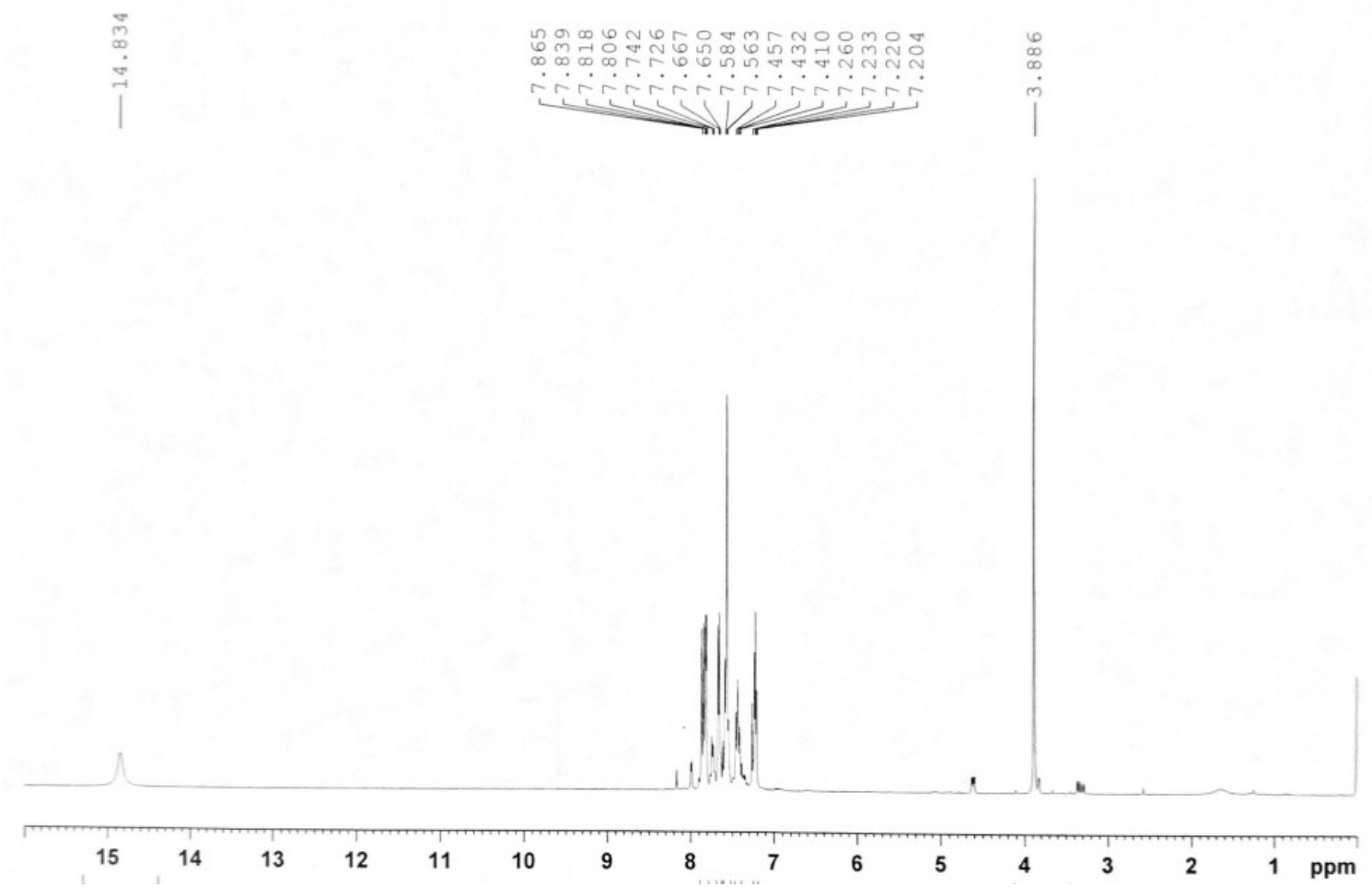


Elemental Composition Compound **5**

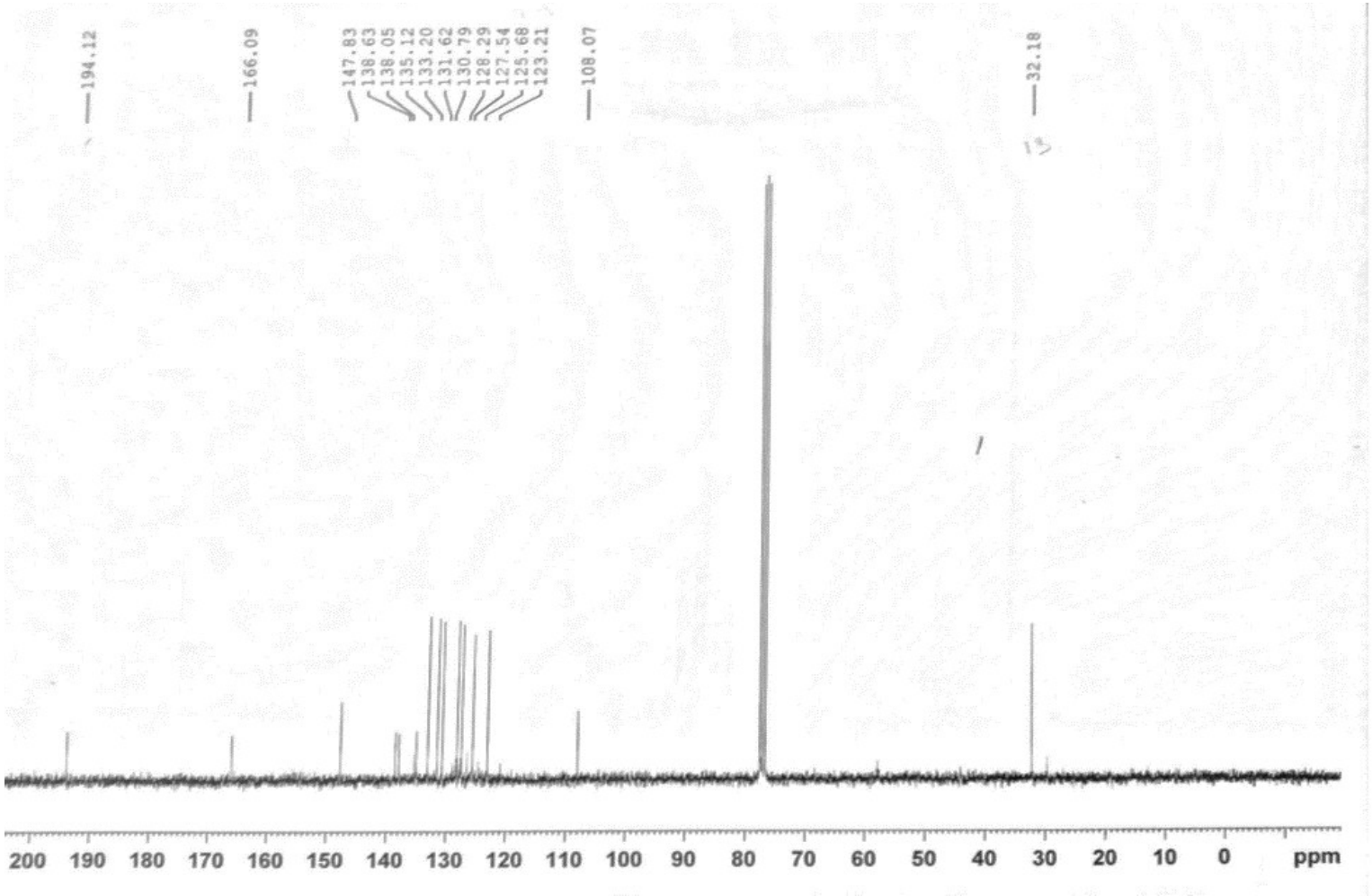
Sample: 1409 C5Br
Note : -luis-velasco
Inlet : Direct Ion Mode : FAB+
RT : 6.88 min Scan#: (19,21)
Elements : C 40/0, H 49/0, O 9/0, Br 3/0
Mass Tolerance : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 4.0 - 12.0

Observed m/z	Int%					
315.0017	25.3					
Estimated m/z	Error[ppm]	U.S.	C	H	O	Br
315.0021	-1.1	10.5	16	12	2	1

^{1}H RMN /Compound **7** ($CDCl_3$) 300MHz



^{13}C RMN /Compound **7** ($CDCl_3$) 75MHz

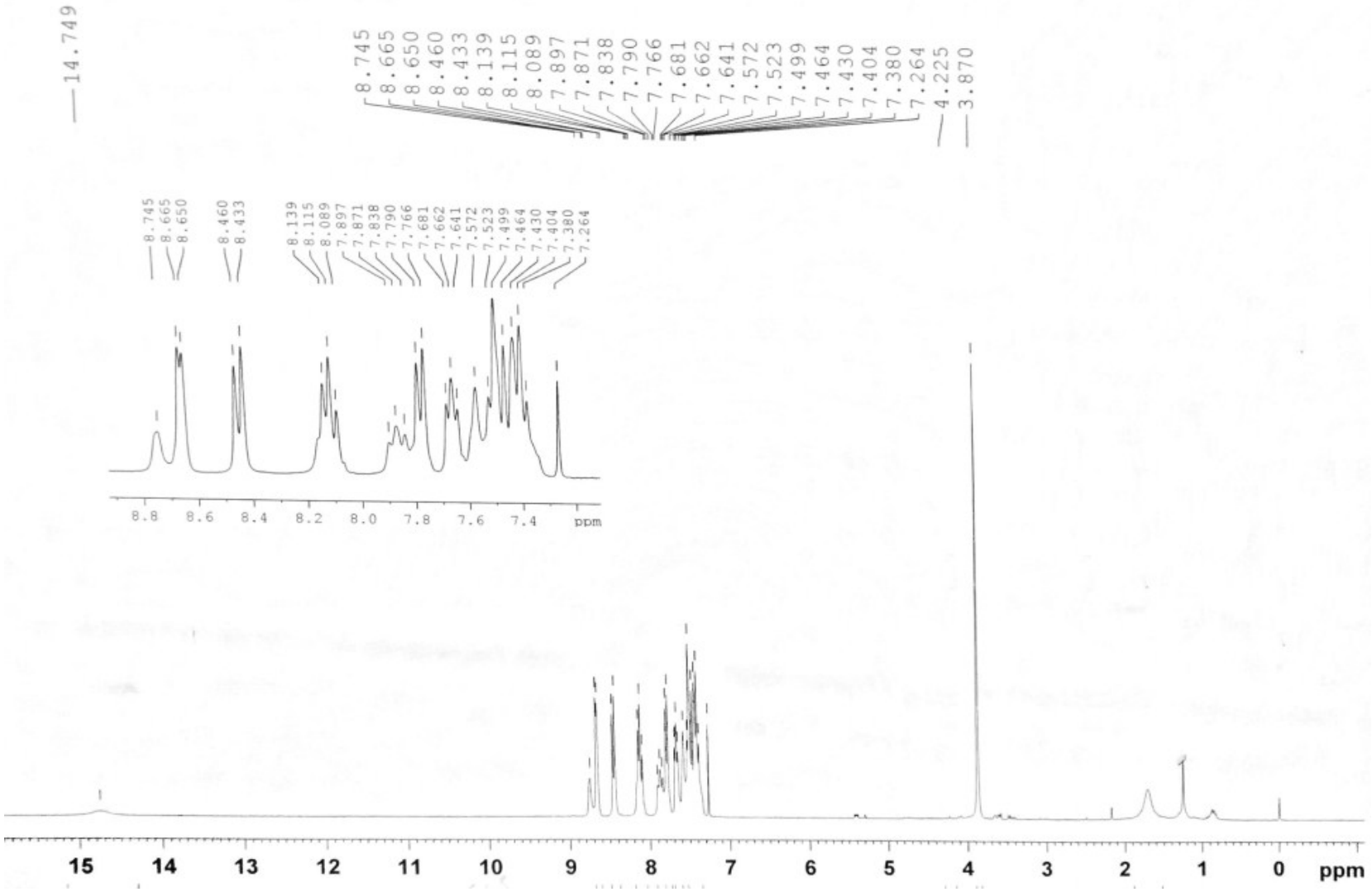


Elemental Composition of Compound **7**

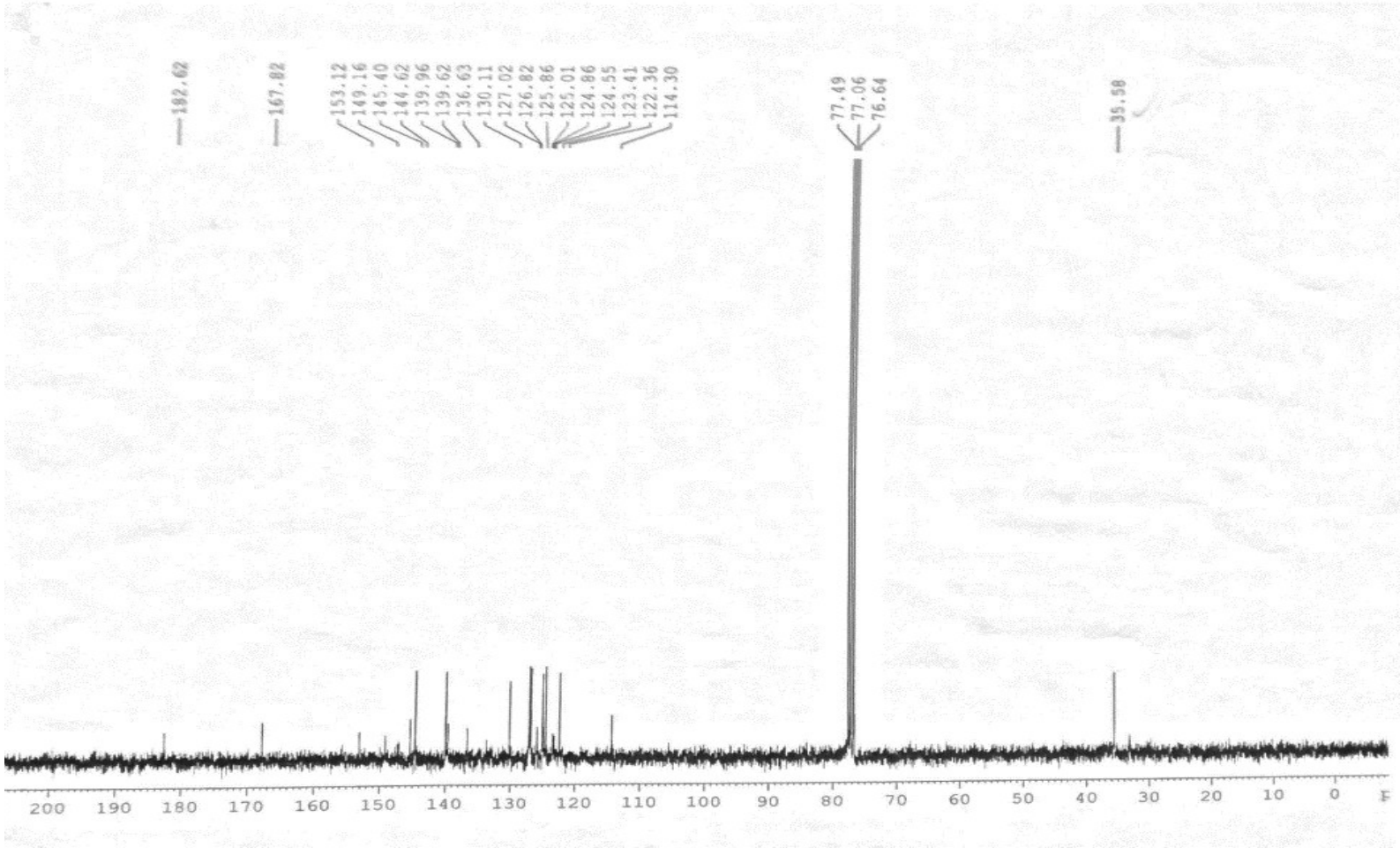
Instrument : MStation
Sample : 229 L7
Note : -
Inlet : Direct Ion Mode : FAB+
RT : 0.90 min Scan# : (5,8)
Elements : C 24/0, H 49/0, O 3/0, S 2/0
Mass Tolerance : 1000ppm, 2mmu if m/z > 2
Unsaturation (U.S.) : -0.5 - 20.0

	Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
1	243.0480	100.00	+0.1 / +0.0	10.5	C14 H11 O2 S

^{1}H RMN /Compound **8** ($CDCl_3$) 300MHz



^{13}C RMN /Compound **8** ($CDCl_3$) 75MHz

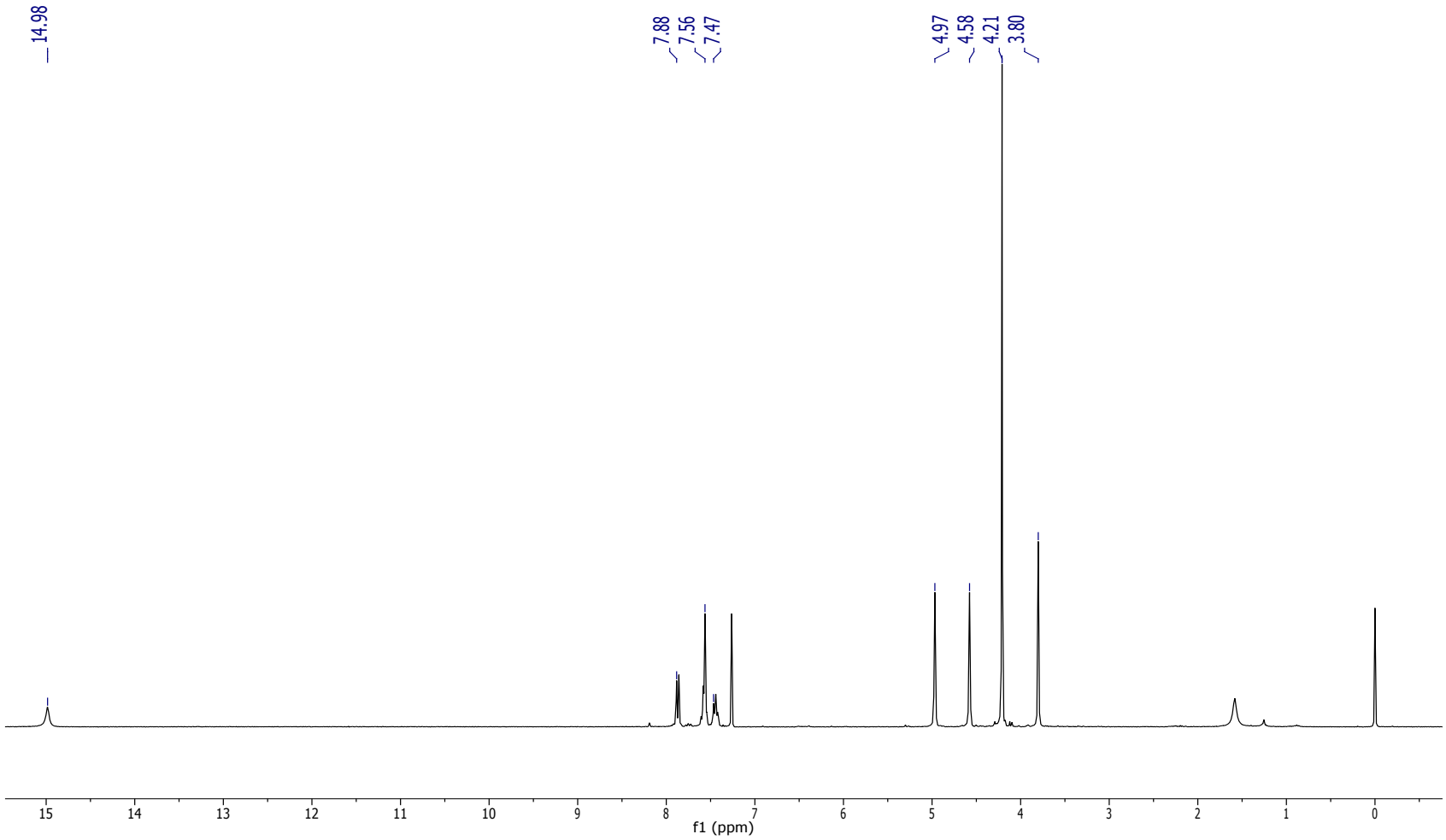


Elemental Composition Compound **8**

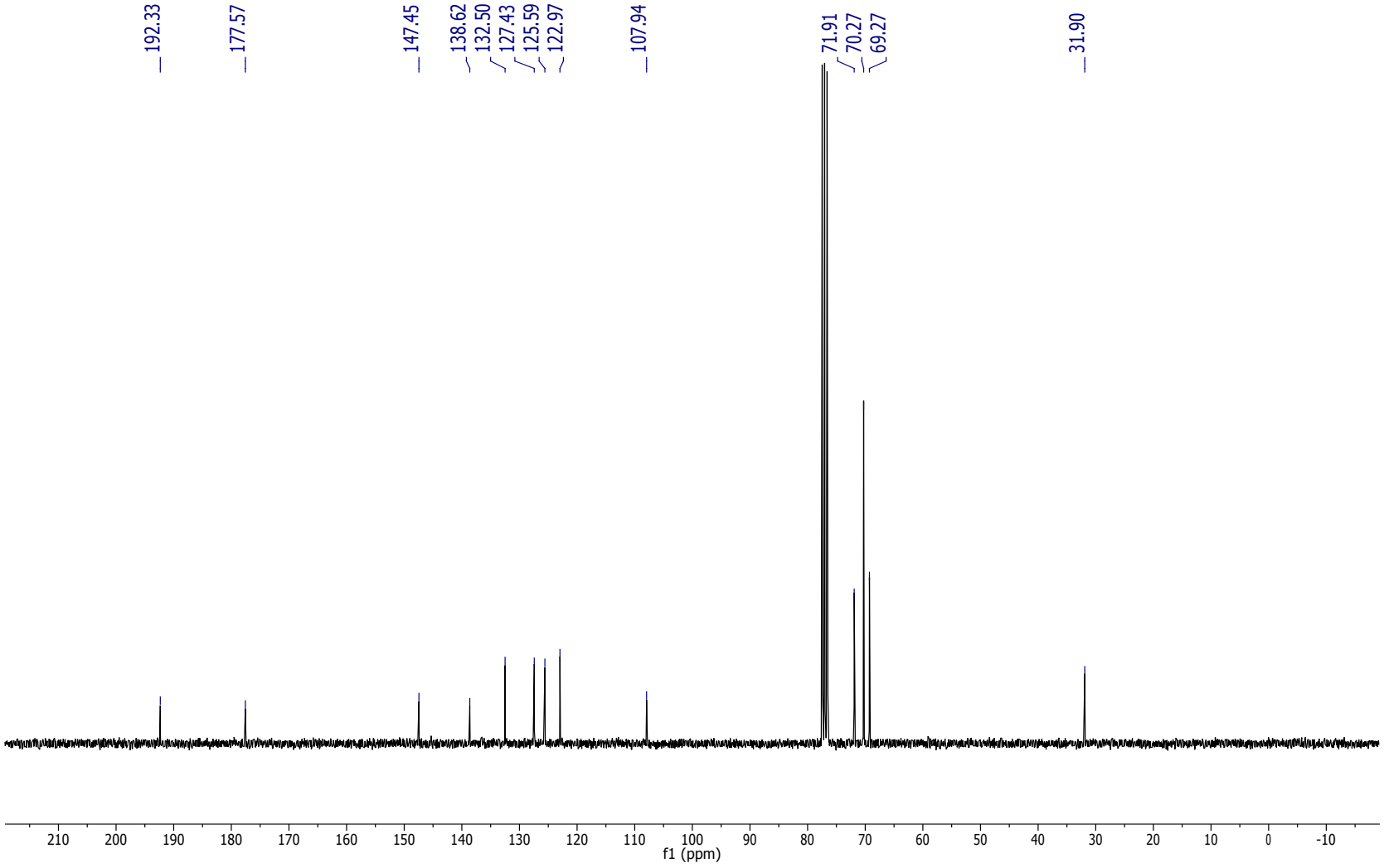
Sample: 2855 L Py
Note : -luis-velasco
Inlet : Direct
Ion Mode : FAB+
RT : 3.98 min
Scan#: (10,11)
Elements : C 44/0, H 49/0, O 5/0, N 2/1
Mass Tolerance : 20ppm, 1mmu if m/z > 50
Unsaturation (U.S.) : 0.0 - 23.0

Observed m/z	Int%					
238.0874	100.0					
Estimated m/z	Error[ppm]	U.S.	C	H	O	N
238.0868	+2.7	10.5	15	12	2	1

^{1}H RMN /Compound **9** ($CDCl_3$) 300MHz



^{13}C RMN /Compound **9** ($CDCl_3$) 75MHz



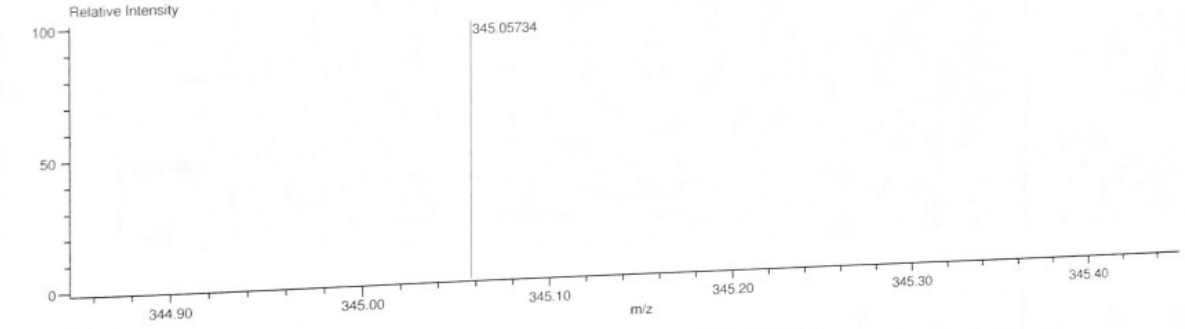
Elemental Composition of Compound **9**

Sample Name:Dr Alvarez Cecilio
Description:
Ionization Mode:ESI+
History:Determine m/z[Peak Detect[Centroid,30,Area];Correct Base[5.0%]];Correct Base[5.0%];Average(MS[1] 0.6...

Mass Calibration data:Cal_Peg_600
Created:10/27/2014 1:57:46 PM
Created by:AccuTOF

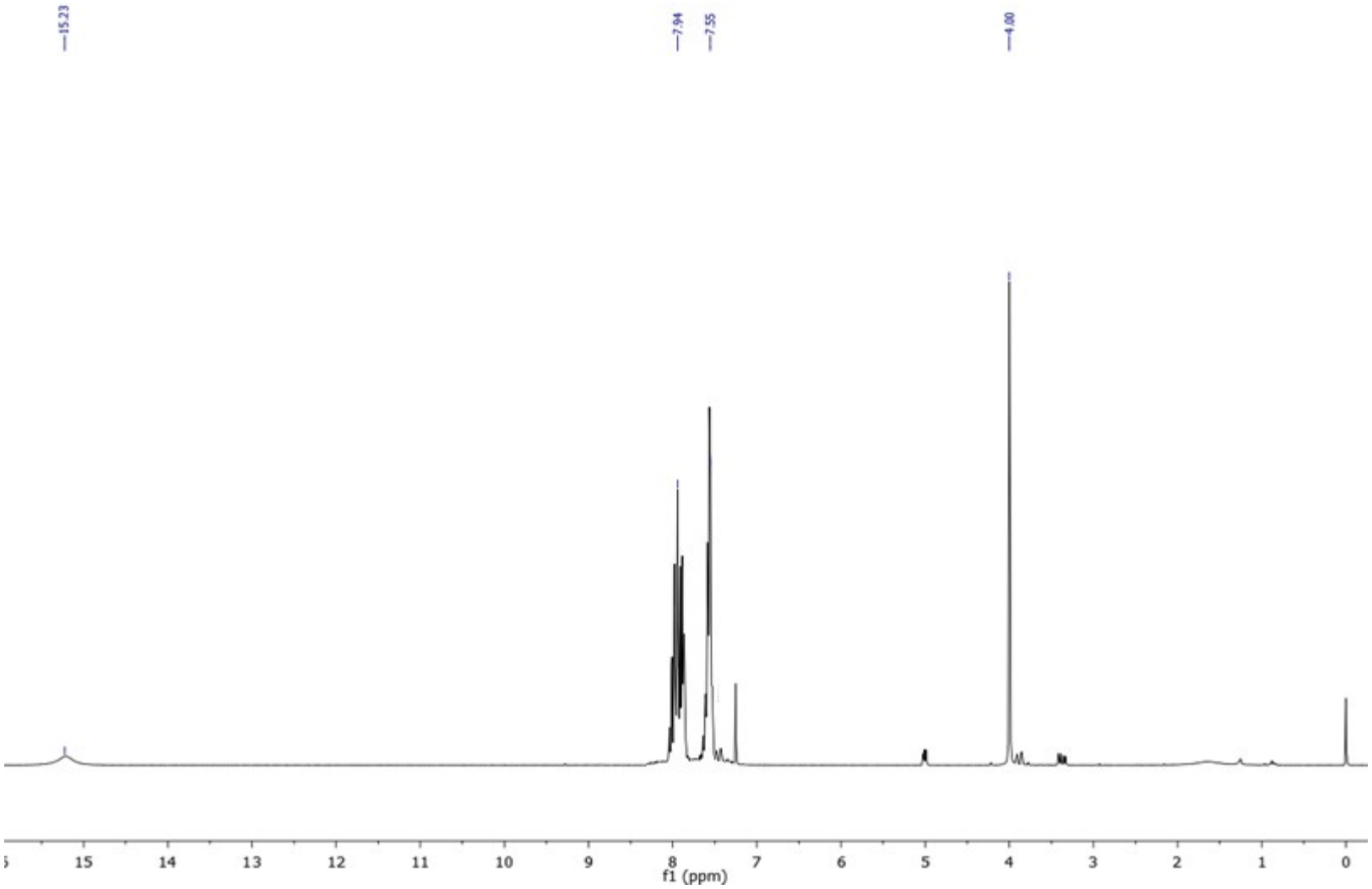
Unsaturation Number:0.0 .. 14.0 (Fraction:Both)

Charge number:1
Tolerance:3.00(mmu)
Element:^{12}C:0 .. 50, ^{1}H:0 .. 120, ^{56}Fe:0 .. 1, ^{16}O:0 .. 3

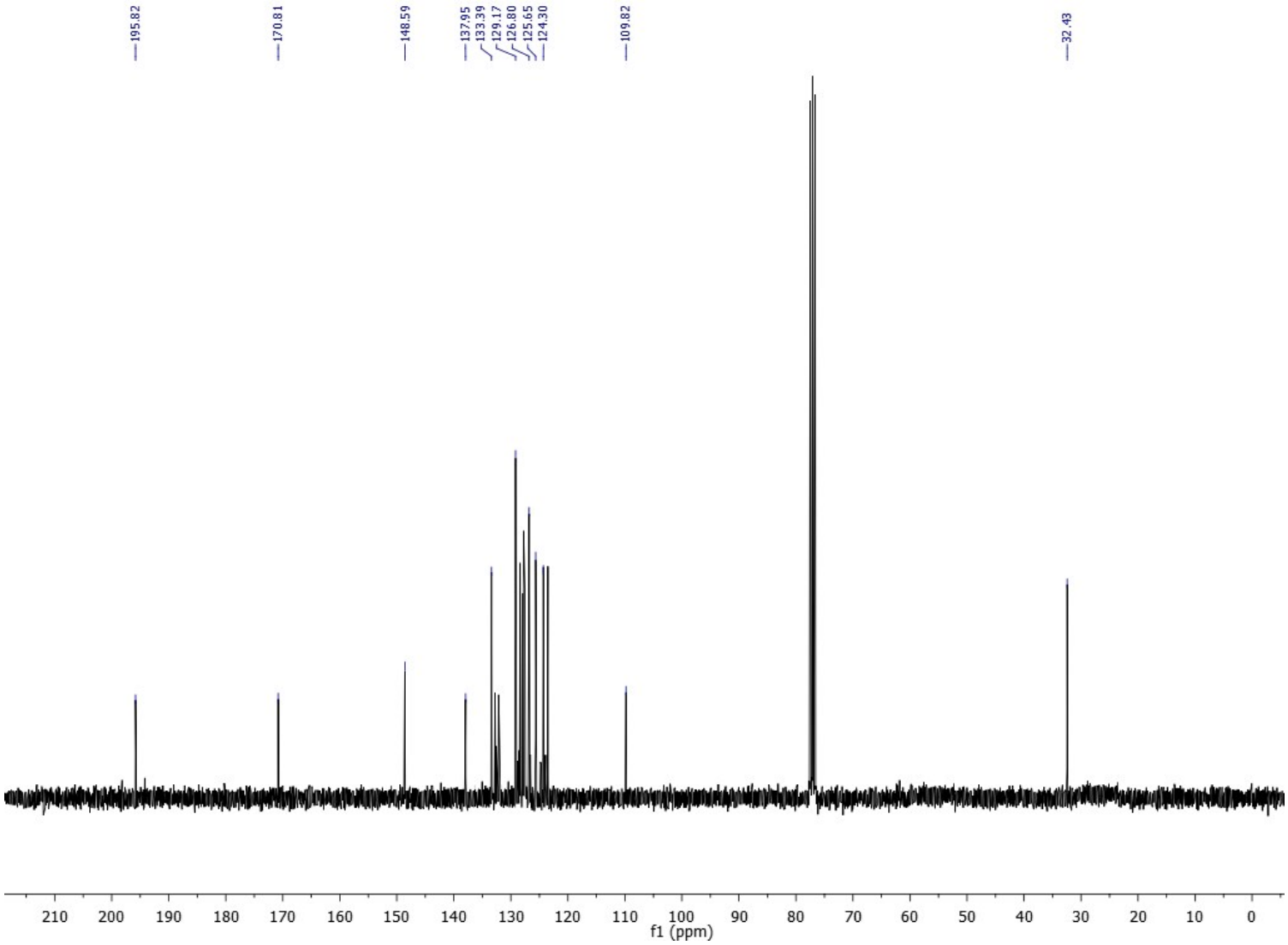


Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Possible Formula	Unsaturation Number
345.05734	1002154.12	345.05780	-0.45	$^{12}C_{20}{}^{1}H_{17}{}^{56}Fe_{1}{}^{16}O_{2}$	13.0

^{1}H RMN /Compound **10** ($CDCl_3$) 300MHz



^{13}C RMN /Compound **9** ($CDCl_3$) 75MHz



Elemental Composition of Compound **10**

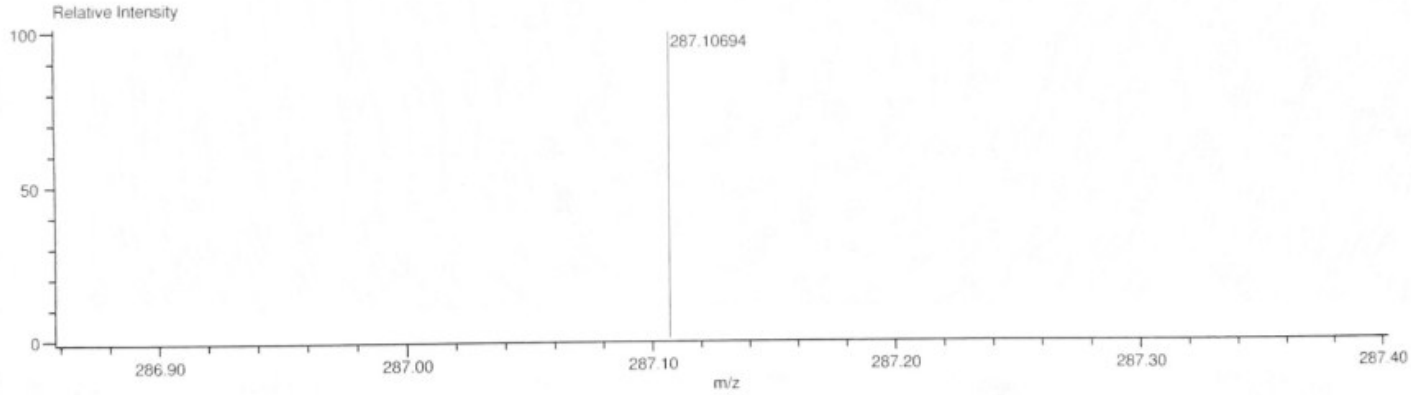
Description:
Ionization Mode:ESI+
History:Determine m/z[Peak Detect[Centroid,30,Area];Correct Base[5.0%]];Correct Base[5.0%];Average(MS[1] 0.4...

Mass Calibration data:Cal_Peg_600
Created:10/27/2014 1:54:42 PM
Created by:AccuTOF

Charge number:1
Element:^{12}C:0 .. 50, ^{1}H:0 .. 120, ^{16}O:0 .. 3

Tolerance:3.00(mmu)

Unsaturation Number:0.0 .. 14.0 (Fraction:Both)



Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Possible Formula	Unsaturation Number
287.10694	838467.25	287.10720	-0.26	$^{12}C_{20}{}^{1}H_{15}{}^{16}O_2$	13.5

Elemental Composition of fragment $C_{32}H_{22}O_4Fe$ [$M^{+\cdot}$] Compound **1a**

```
Sample: 2017   LC8NFe
Note : Luis-Velasco
Inlet : Direct                          Ion Mode : FAB+
RT : 5.30 min                           Scan#: (17,31)
Elements : C 40/0, H 49/0, O 10/1, Fe 5/1
Mass Tolerance      : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : -0.5 - 35.0

Observed m/z   Int%
  526.0877     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     Fe
  526.0867       +1.8      22.5    32    22     4      1
```

Elemental Composition of fragment $C_{32}H_{20}O_4ClFe$ [$M^{+\cdot}$] Compound **2a**

```
Sample: 2009        FeLC8Cl-ME
Note : -luis-velasco
Inlet : Direct                          Ion Mode : FAB+
RT : 0.75 min                           Scan#: (3,6)
Elements : C 40/0, H 58/0, O 6/1, Cl 3/0, Fe 3/0
Mass Tolerance      : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 13.0 - 25.0

Observed m/z   Int%
  594.0092     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     Cl    Fe
  594.0088       +0.6      22.5    32    20     4     2     1
```

Elemental Composition of fragment $C_{34}H_{26}O_4Fe$ [$M^{+\cdot}$] Compound **3a**

```
Sample: 2010          FeLC8Me-ME
Note : -luis-velasco
Inlet : Direct                          Ion Mode : FAB+
RT : 0.43 min                           Scan#: 3
Elements : C 40/0, H 58/0, O 6/1, Fe 3/0
Mass Tolerance       : 1000ppm, 1mmu if m/z > 1
Unsaturation (U.S.) : 13.0 - 25.0

Observed m/z   Int%
  554.1187     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     Fe
  554.1180       +1.1      22.5    34    26     4      1
```

Elemental Composition of fragment $C_{34}H_{26}O_6Fe$ [$M^{+\cdot}$] Compound **4a**

Elemental Composition of fragment $C_{40}H_{30}O_4Fe_3$ [$M^{+\cdot}$] Compound **9a**

Instrument : MStation
Sample : 230 L Fc Fe
Note : –
Inlet : Direct Ion Mode : FAB+
RT : 0.00 min Scan# : (1,7)
Elements : C 50/0, H 49/0, O 5/0, Fe 4/0
Mass Tolerance : 1000ppm, 3mmu if m/z > 3
Unsaturation (U.S.) : 25.0 – 30.0

Observed m/z	Int%
742.0189	80.16

	Estimated m/z	Err[ppm / mmu]	U.S.	C	H	O	Fe
1	742.0192	-0.4 / -0.3	27.5	40	30	4	3

Elemental Composition of fragment $C_{40}H_{26}O_4Fe$ [$M^{+\cdot}$] Compound **10a**

Instrument : MStation
Sample : 227 LNaphFe
Note : –
Inlet : Direct Ion Mode : FAB+
RT : 0.35 min Scan# : (4,5)
Elements : C 50/0, H 49/0, O 5/0, Fe 2/0
Mass Tolerance : 1000ppm, 2mmu if m/z > 2
Unsaturation (U.S.) : 22.0 – 35.0

Observed m/z	Int%
626.1189	22.51

	Estimated m/z	Err[ppm / mmu]	U.S.	C	H	O	Fe
1	626.1180	+1.4 / +0.9	28.5	40	26	4	1

Elemental Composition of Compound **1b**

Note : -luis-velasco
Inlet : Direct
RT : 6.53 min
Ion Mode : FAB+
Scan#: (24,35)
Elements : C 44/0, H 49/0, O 7/2, Cu 2/0
Mass Tolerance : 20ppm, 5mmu if m/z > 250
Unsaturation (U.S.) : 12.0 - 23.0

Observed m/z	Int%					
534.0883	8.2					
Estimated m/z	Error[ppm]	U.S.	C	H	O	Cu
534.0892	-1.7	21.5	32	23	4	1