Electronic Supplementary Material (ESI) for RSC Advances.


# Synthetic investigation on chirally pure Mannich derivatives of pseudophenylpropanolamine and their anticancer properties against HepG-2 cells with inhibition of JAK2/STAT3

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## Supporting information

## 1.0 Equipments and analytical instruments

Melting points were measured using a Veego melting point apparatus model VMP-PM. Specific optical rotations (c=1.0%., methanol) were measured on Rudolph Autopol® V polarimeter at 25 °C using the sodium Dline (589 nm) lamp. TLC was carried out using pre-coated Merck TLC Silica gel 60 $F_{254}$ and spots were detected using UV light. GC analysis was performed on SHIMADZU GC-17A with column DB-1, length 30 meters, 0.53 mm inner dia, 1.5 µ film thickness with FID as detector and GC solution as chromatographic data processor.

The purities of the synthesized Mannich derivatives have been obtained with LC-2010AHT, LC-10ATVP with SPD-M10 (DAD detector) with Inertsil ODS-3V-250 x 4.6 mmm 5.0 µm HPLC column and sodium dihydrogen phosphate buffer with acetonitrile as mobile phase and CLASS-VP as chromatography data processor. The chiral purity of the prepared □-amino alcohols have been obtained with Chiralpak-ADH-250 x 4.6 mm, 5.0 µm HPLC chiral column using 1% ethanol in *n*-hexane with 0.1% of diethylamine at a flow rate of 1.0 mL/min using UV variable wavelength detector.

IR spectra were recorded (KBr pellet) on Shimadzu Prestige 21 FTIR instrument in the range of 400 to 4000 $cm^{-1}$. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded using a Bruker-Avance FT-NMR spectrometer (300 MHz and 100 MHz respectively) using DMSO-$d_6$ and $CDCl_3$ as solvents and TMS as an internal standard. Chemical shifts ($\delta$) were mentioned in parts per million (ppm). The following abbreviations are used: s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet. Mass spectra were recorded on Agilent 6110 LC/MS mass spectrophotometer using ESI mode. The elemental analysis was done (sample thoroughly dried under vacuum) using a Thermo Fischer Flash 1112 Series elemental analyzer. Single crystal XRD data were obtained using Bruker X8 KAPPA APEX.

## 2.0 Single crystal XRD data of compound 3c′

**Table 1. Crystal data and structure refinement parameters of compound 3c′**

| PARAMETERS | VALUES |
|---|---|
| Empirical formula | $C_{18}H_{21}Cl_2NO_2.H_2O$ |
| Formula weight | 372.27 |
| Temperature | 293(2) K |
| Wavelength | 0.71073 Å |
| Crystal system, space group | Monoclinic, C 2 |
| Unit cell dimensions | a = 18.7740(3) Å alpha = 90 °. |
| | b = 7.1164(2) Å beta = 118.663(2) °. |
| | c = 16.2465(4) Å gamma = 90 °. |
| Volume | 1904.59(8) $A^3$ |
| Z, Calculated density | 4, 1.298 $Mg/m^3$ |
| Absorption coefficient | 0.356 $mm^{-1}$ |
| F(000) | 784 |
| Crystal size | 0.210 x 0.150 x 0.120 $mm^3$ |
| Theta range for data collection | 1.428 to 28.594 °. |
| Limiting indices | -14<=h<=24, -9<=k<=7, -21<=l<=20 |
| Reflections collected / unique | 9141 / 4250 [R(int) = 0.0234] |
| Completeness to theta = 25.242 | 100.00% |
| Absorption correction | Semi-empirical from equivalents |
| Max. and min. transmission | 0.959 and 0.929 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 4250 / 1 / 236 |
| Goodness-of-fit on $F^2$ | 1.045 |
| Final R indices [I>2sigma(I)] | R1 = 0.0331, wR2 = 0.0917 |
| R indices (all data) | R1 = 0.0356, wR2 = 0.0944 |
| Absolute structure parameter | 0.956(19) |
| Extinction coefficient | 0.0143(13) |
| Largest diff. peak and hole | 0.220 and -0.276 $e.A^{-3}$ |

## 3.0 IR, 1H-NMR, 13C-NMR and Mass spectrums

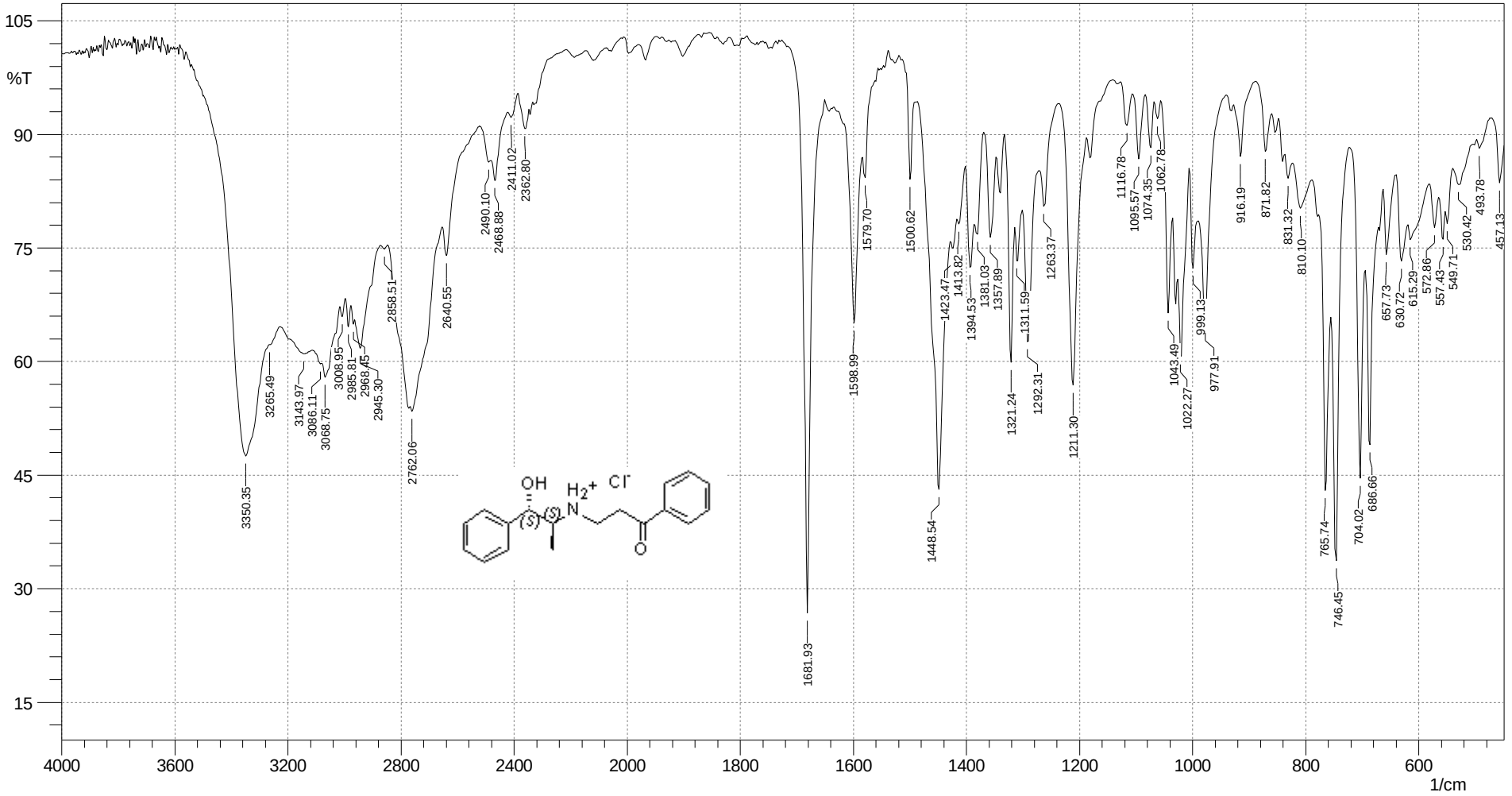



**Figure 1:** IR Spectrum of Compound 3a

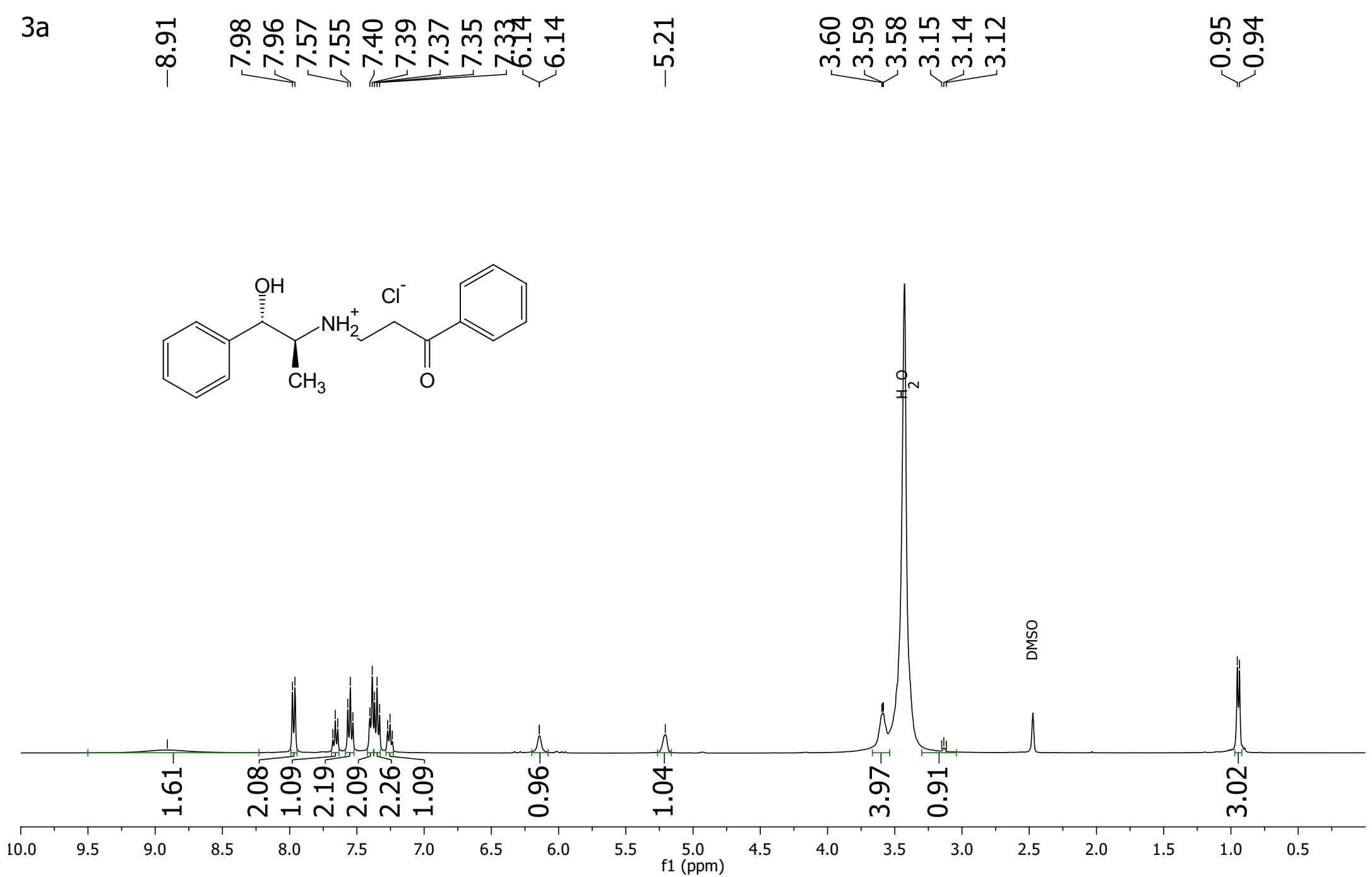



**Figure 2**: $^{1}$H NMR spectrum of Compound 3a

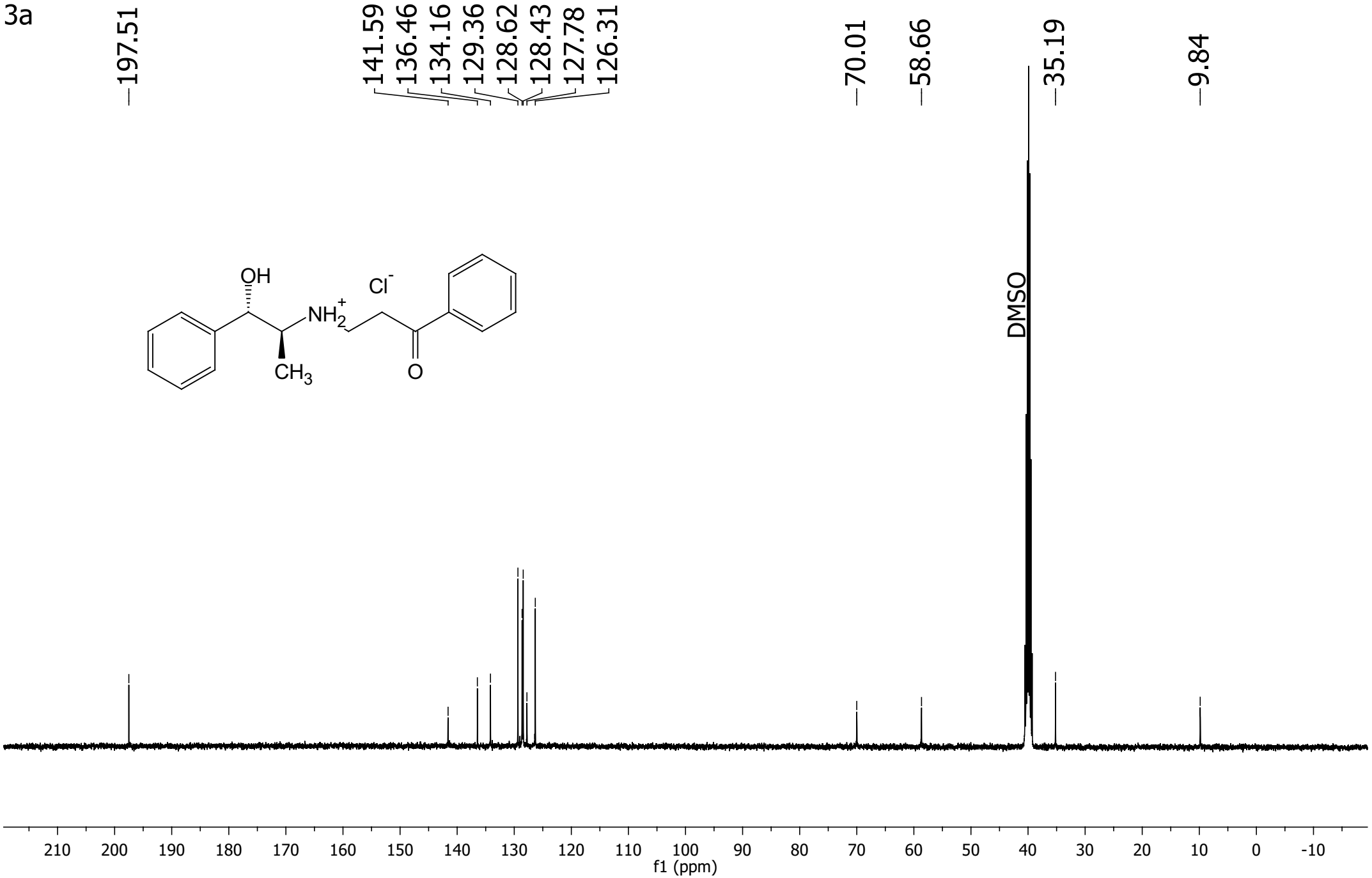



**Figure 3**: $^{13}C$ NMR spectrum of Compound 3a

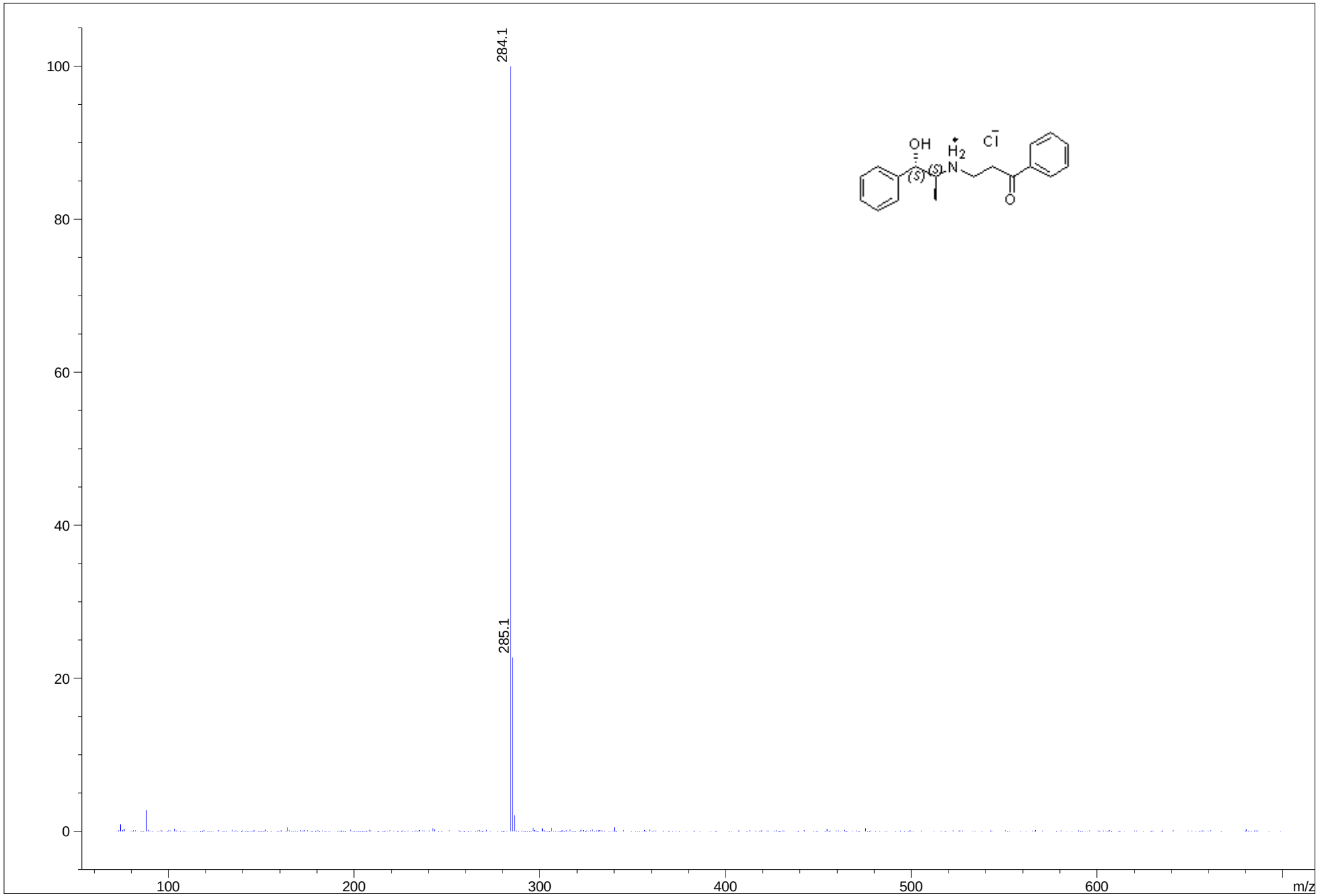



**Figure 4:** Mass Spectrum of Compound 3a

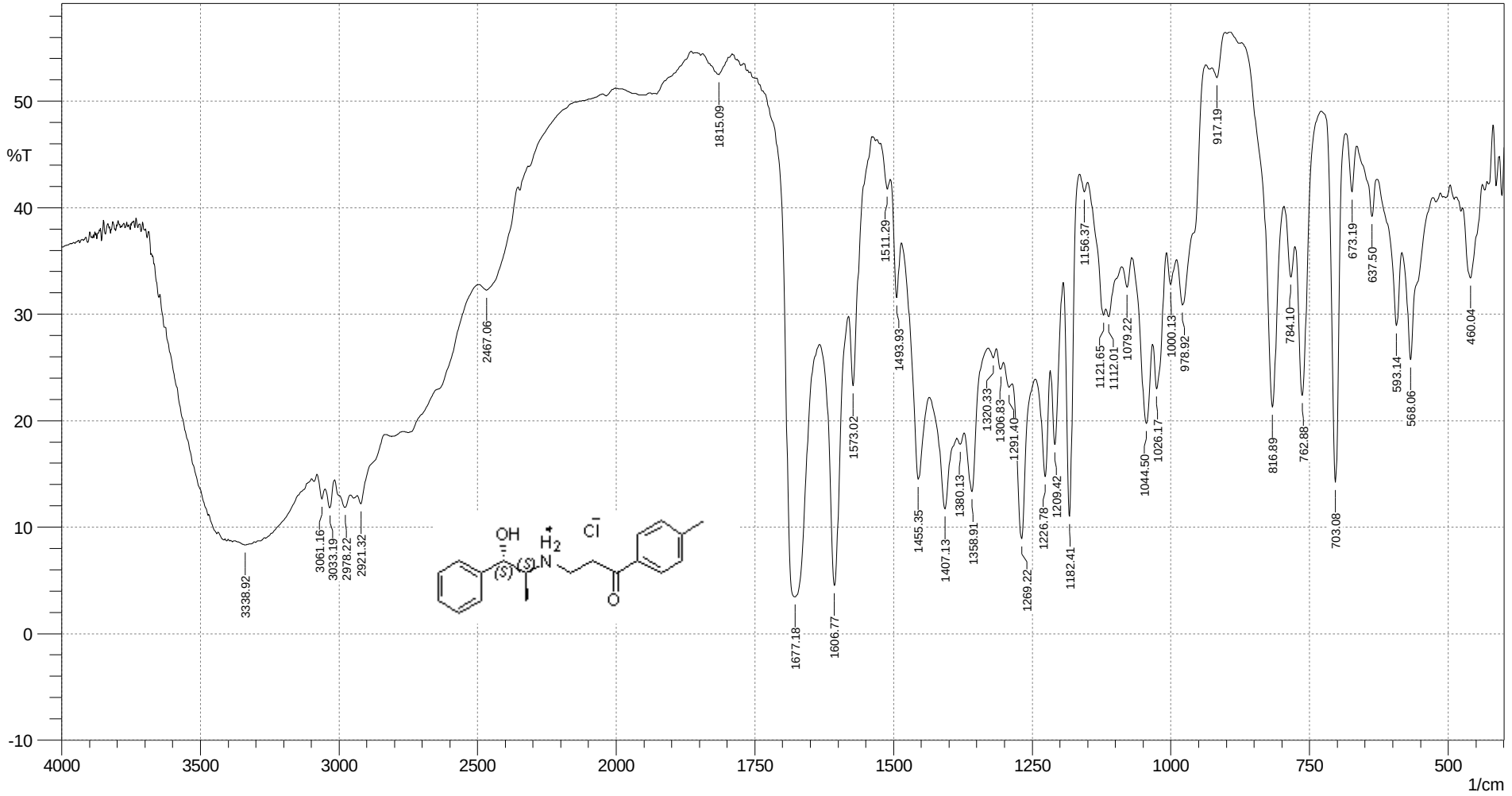



**Figure 5:** IR Spectrum of Compound 3b

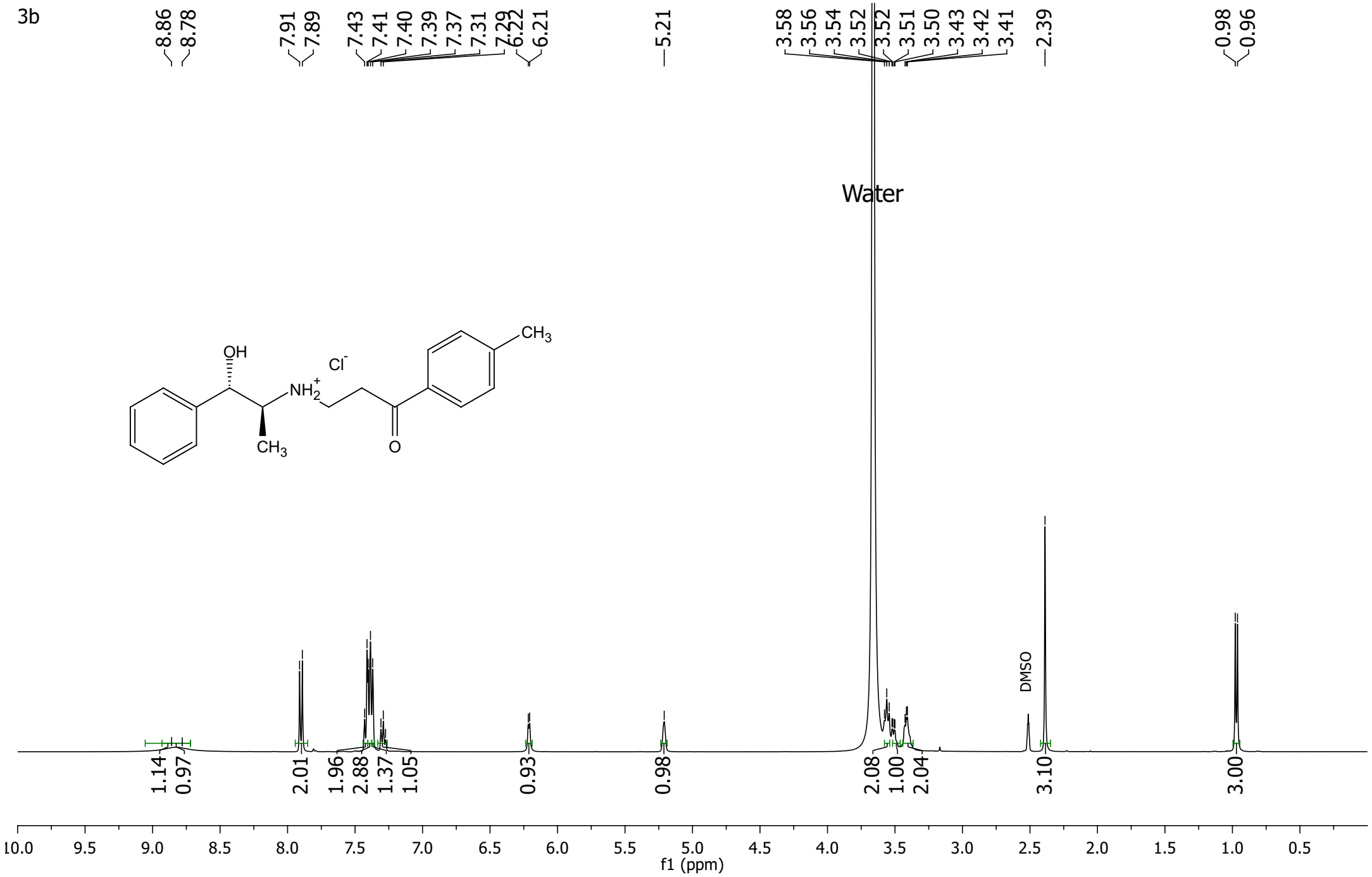



**Figure 6:** $^{1}$H-NMR Spectrum of Compound 3b

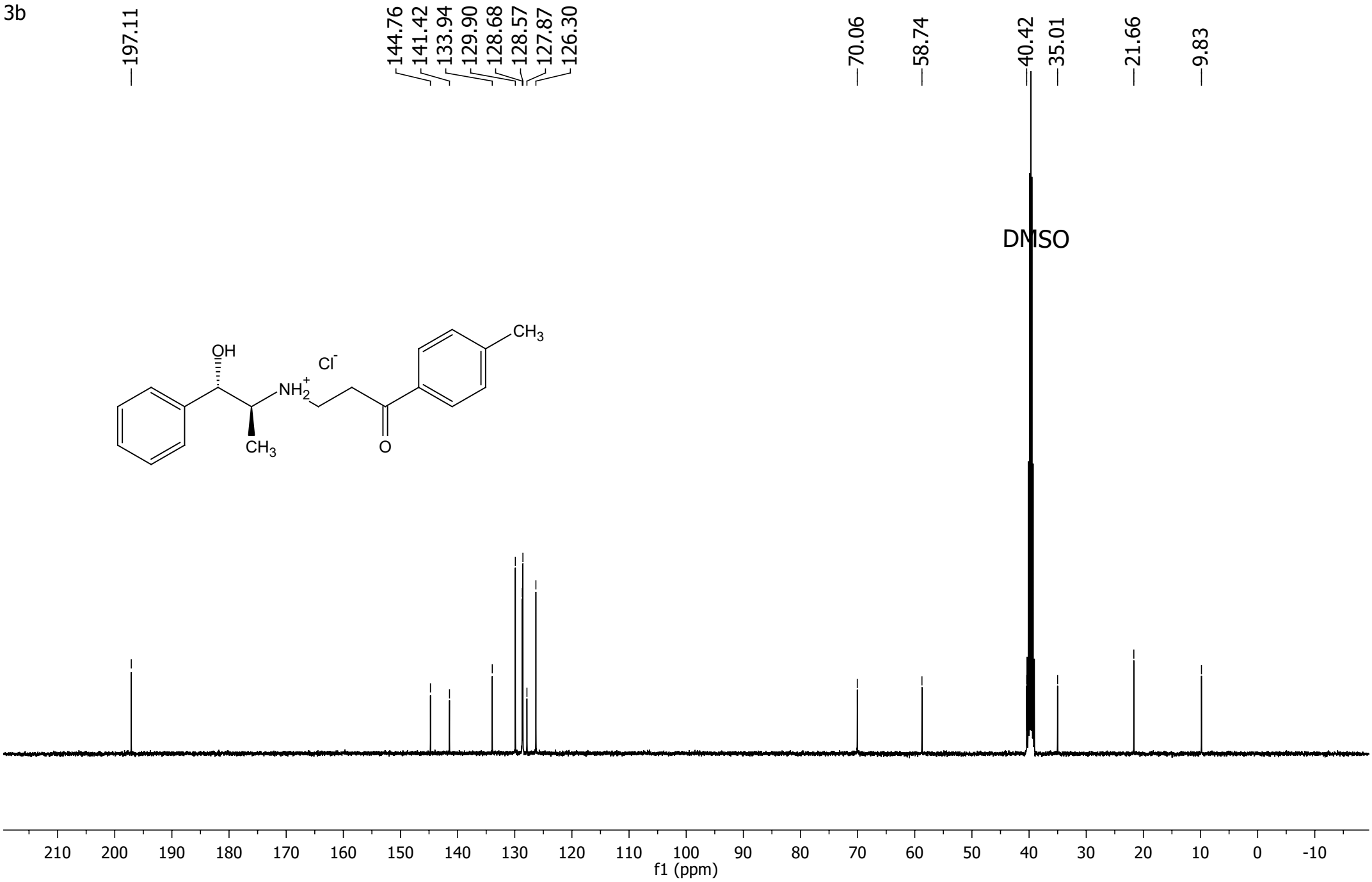



**Figure 7:** $^{13}$C-NMR Spectrum of Compound 3b

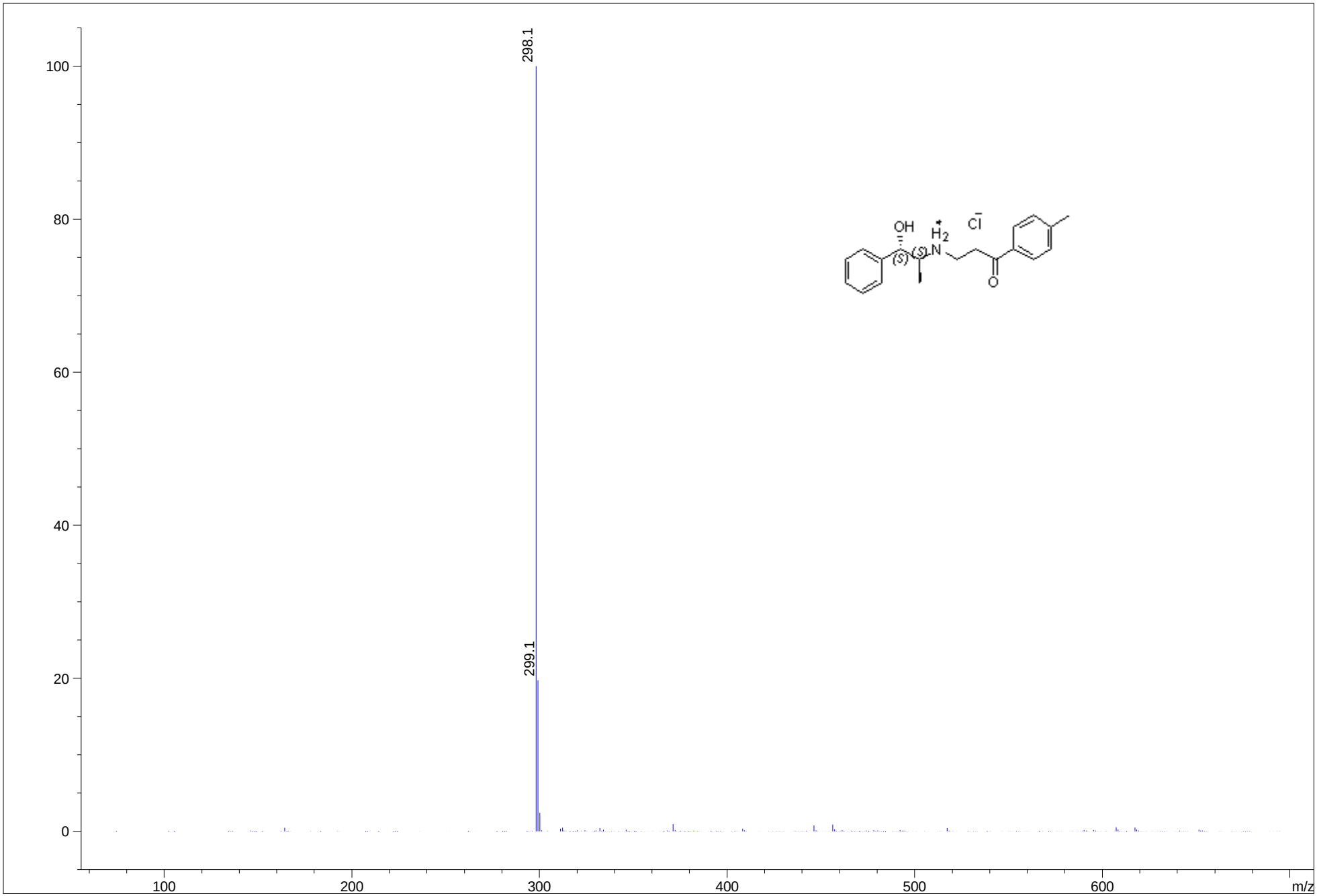



**Figure 8:** Mass Spectrum of Compound 3b

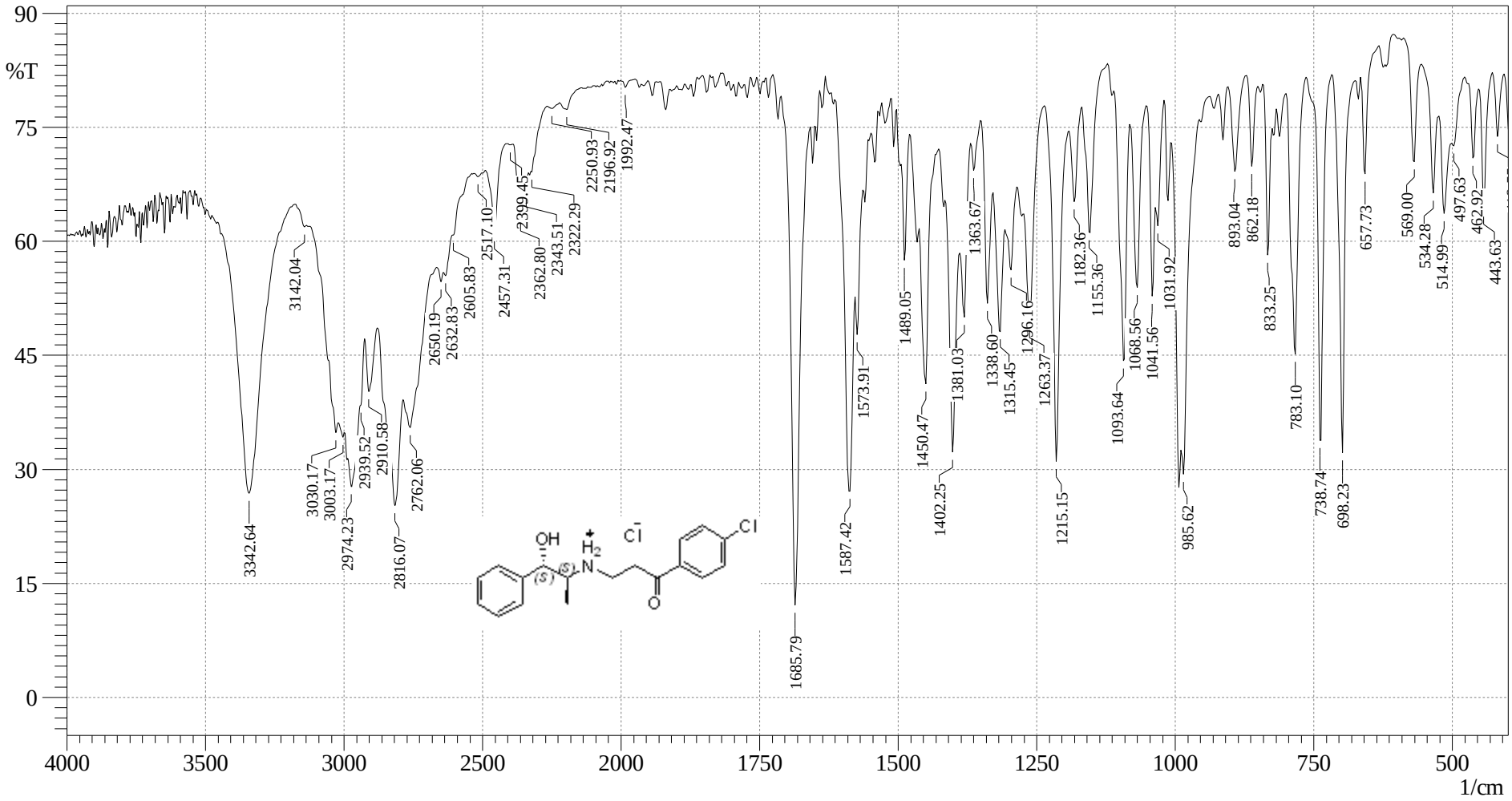



**Figure 9:** IR Spectrum of Compound 3c

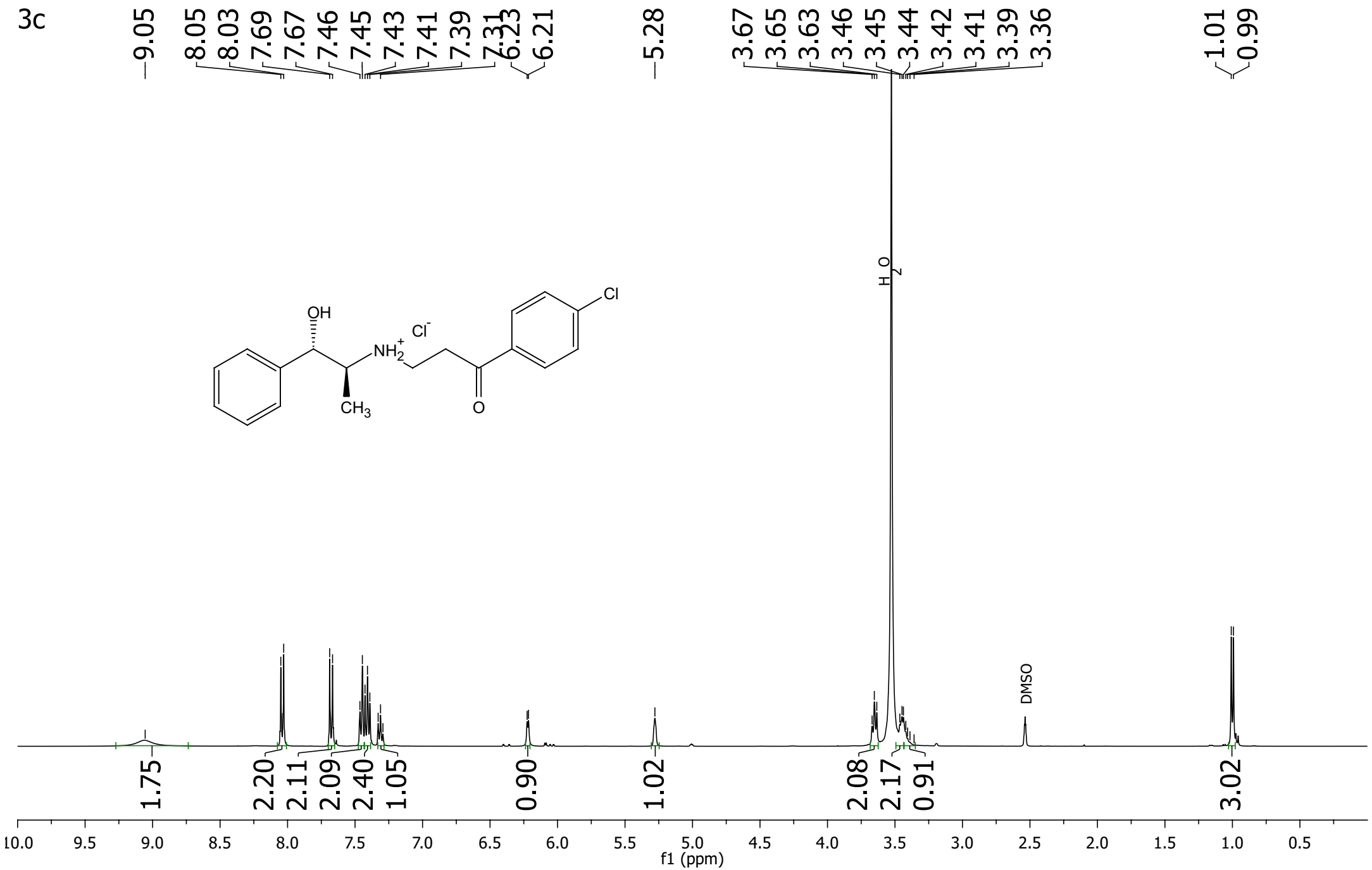



**Figure 10:** $^{1}$H-NMR Spectrum of Compound 3c

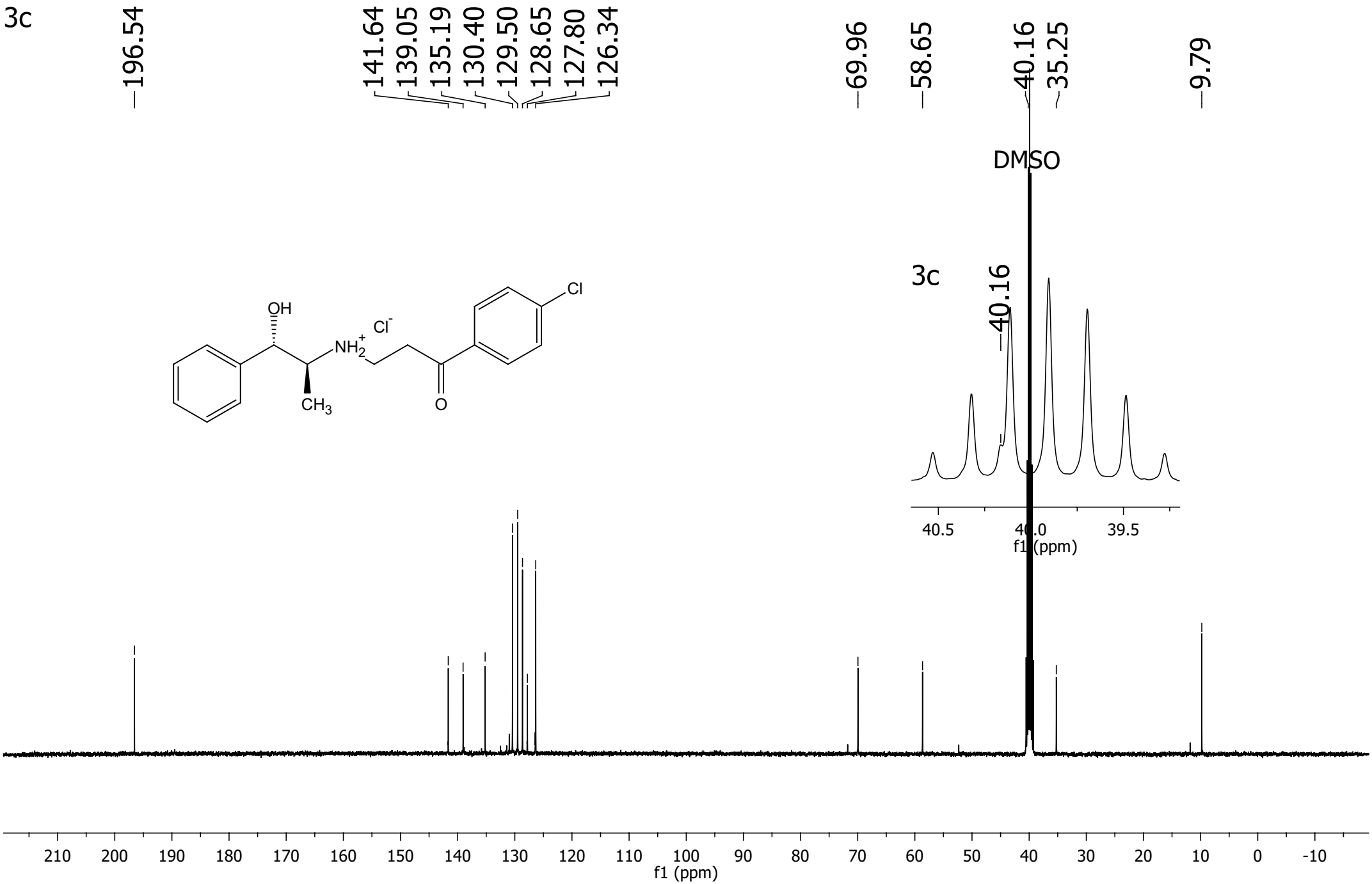



**Figure 11:** $^{13}$C-NMR Spectrum of Compound 3c

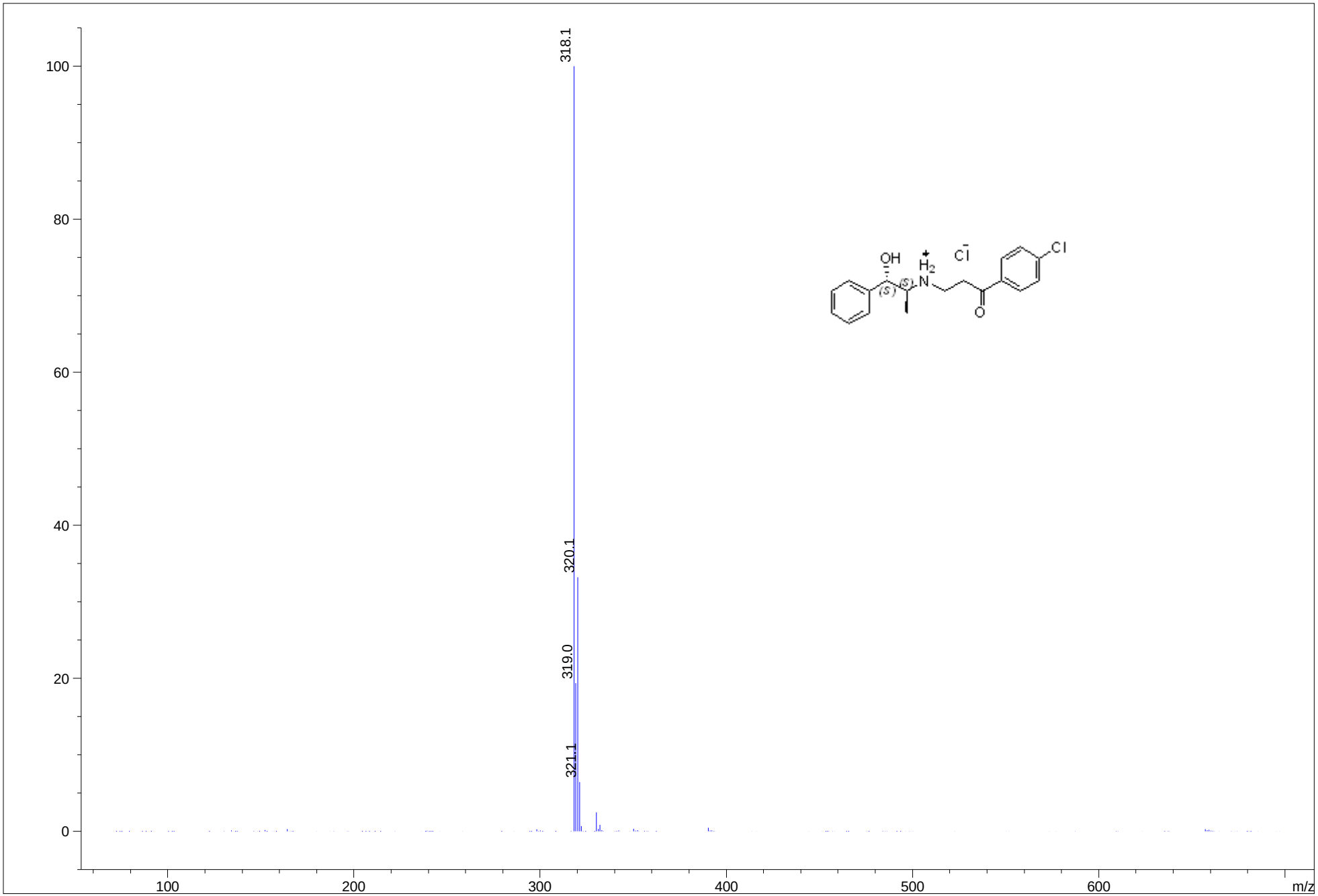



**Figure 12:** Mass Spectrum of Compound 3c

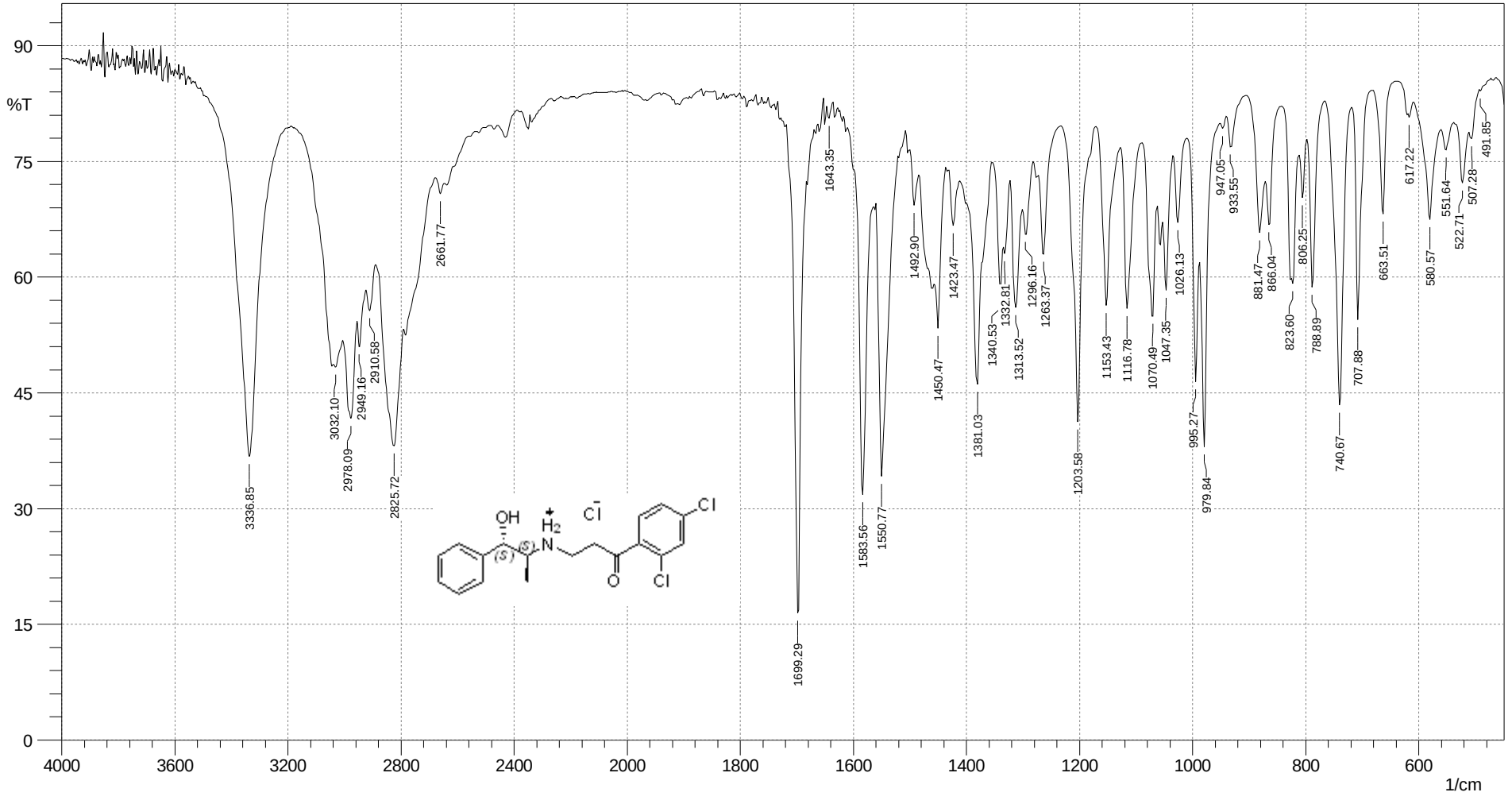



**Figure 13:** IR Spectrum of Compound 3d

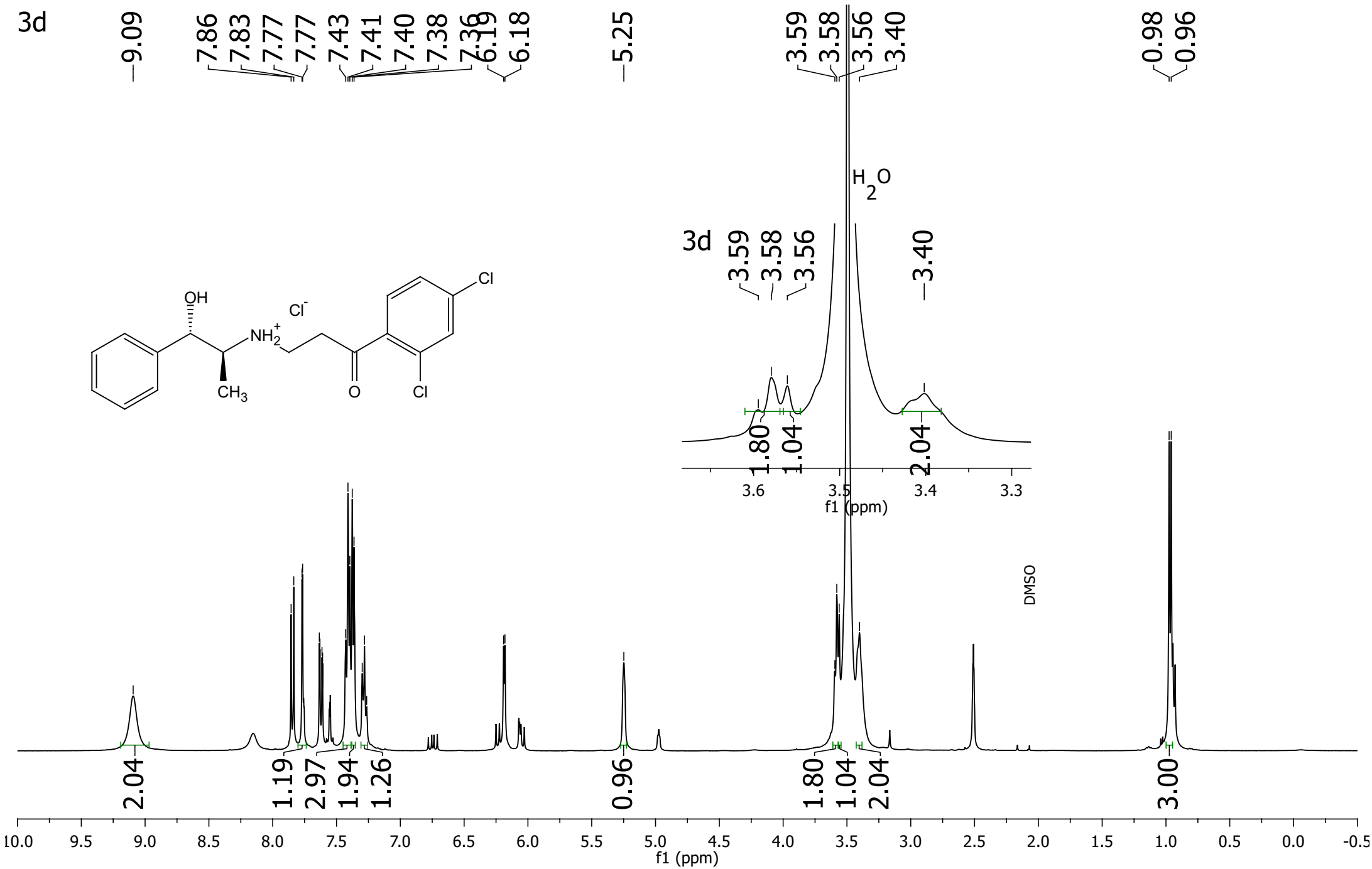



**Figure 14:** [1]H-NMR Spectrum of Compound 3d

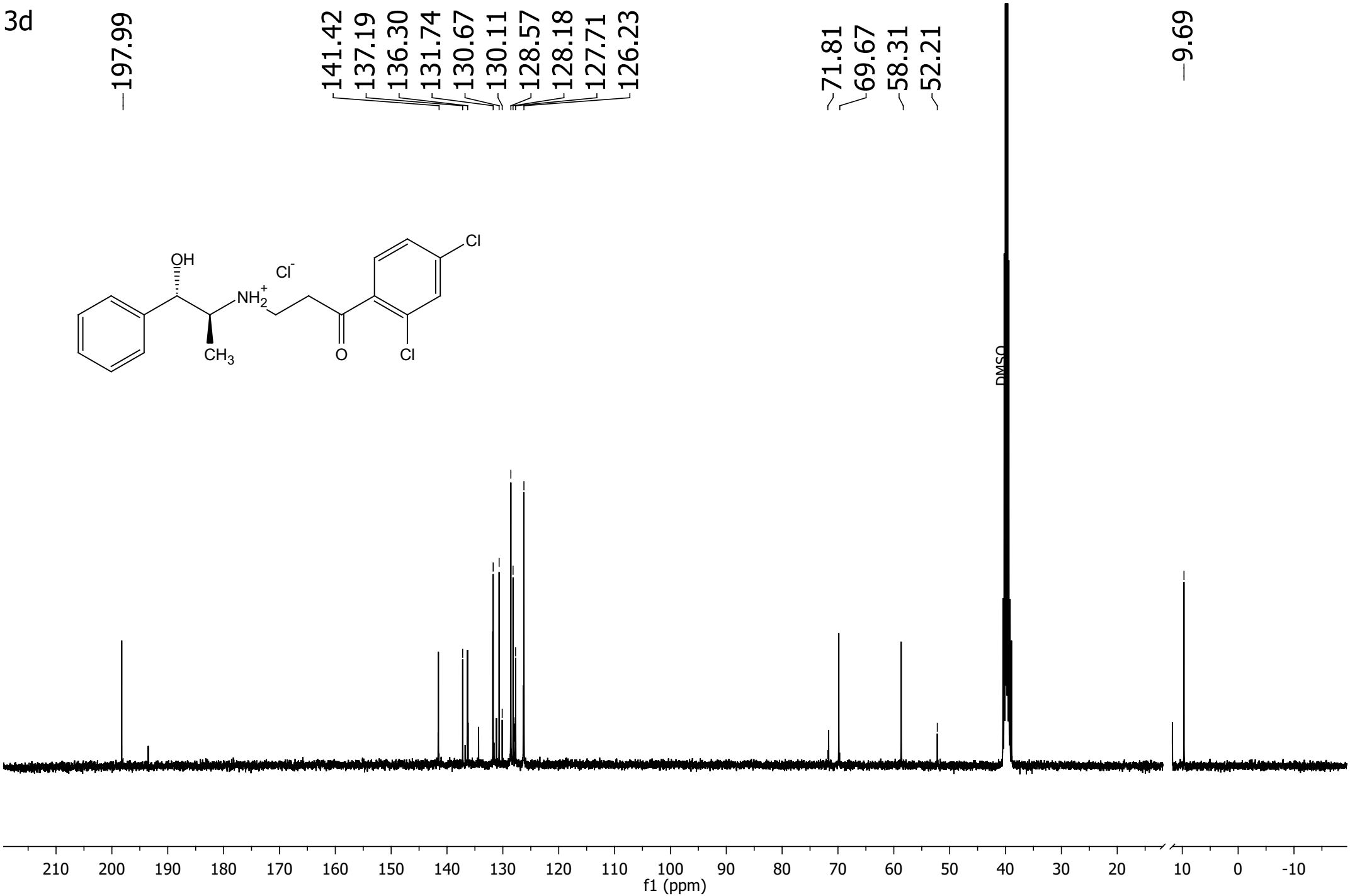



**Figure 15:** $^{13}$C-NMR Spectrum of Compound 3d

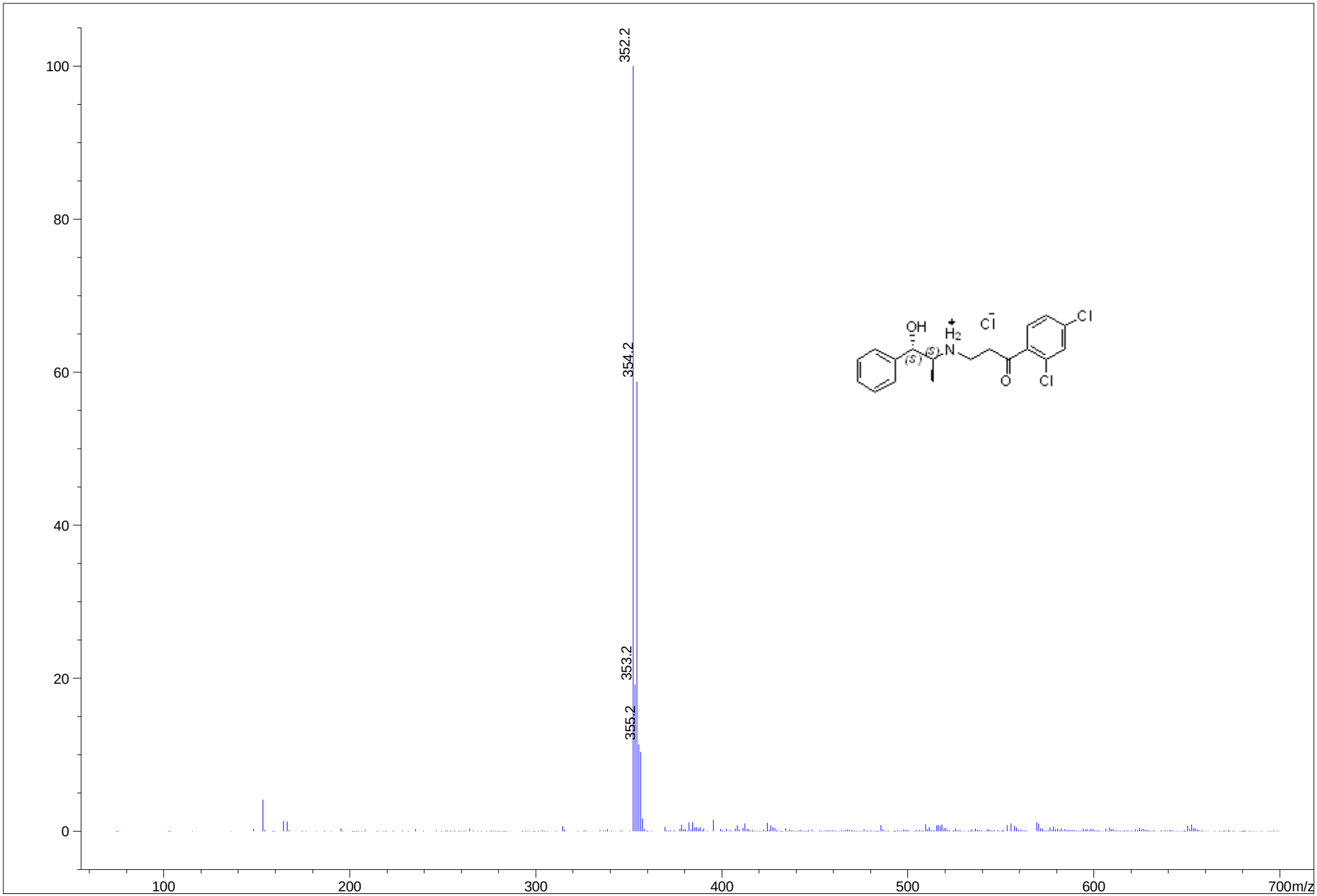



**Figure 16:** Mass Spectrum of Compound 3d

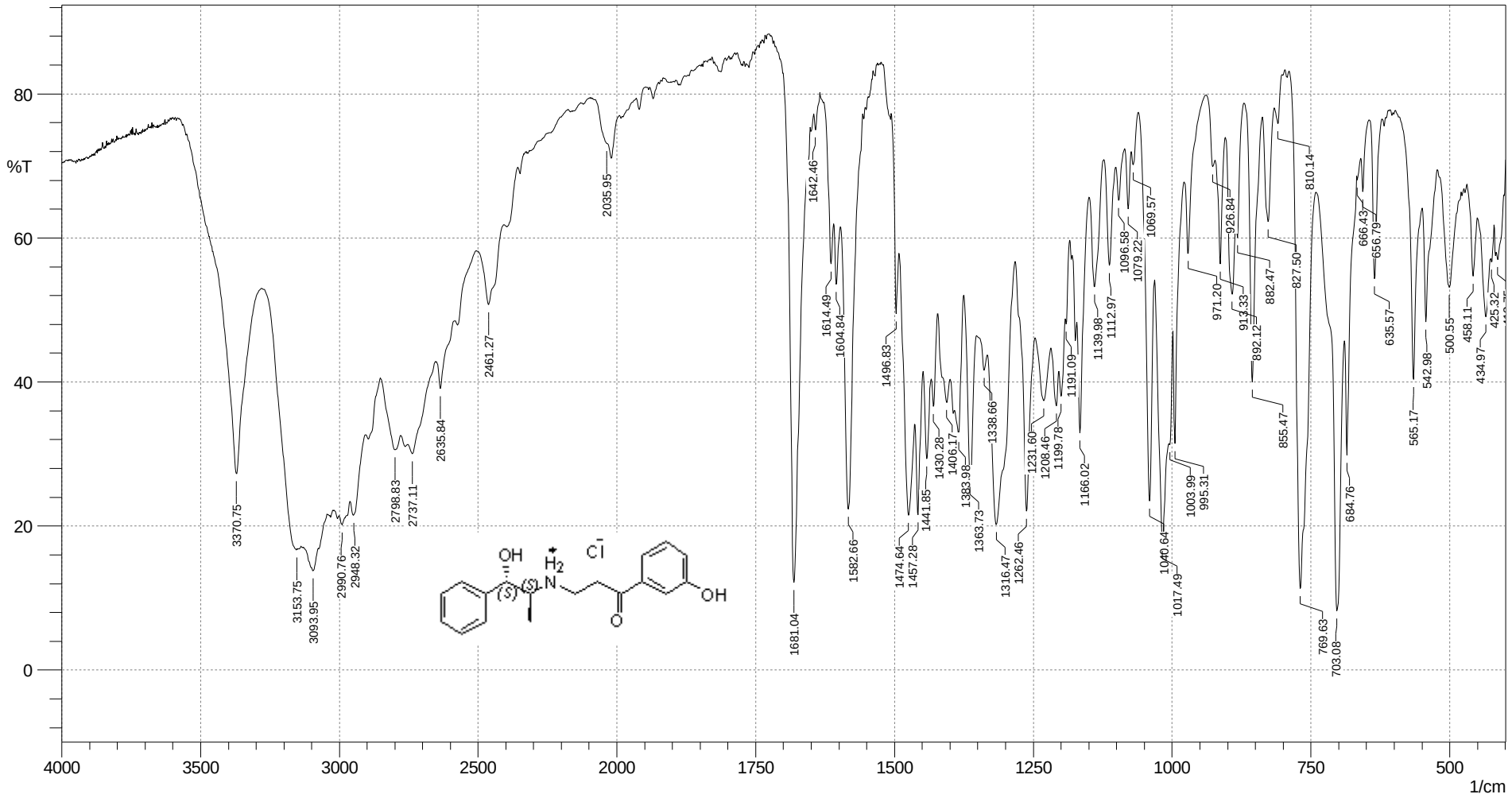



**Figure 17:** Mass Spectrum of Compound 3e

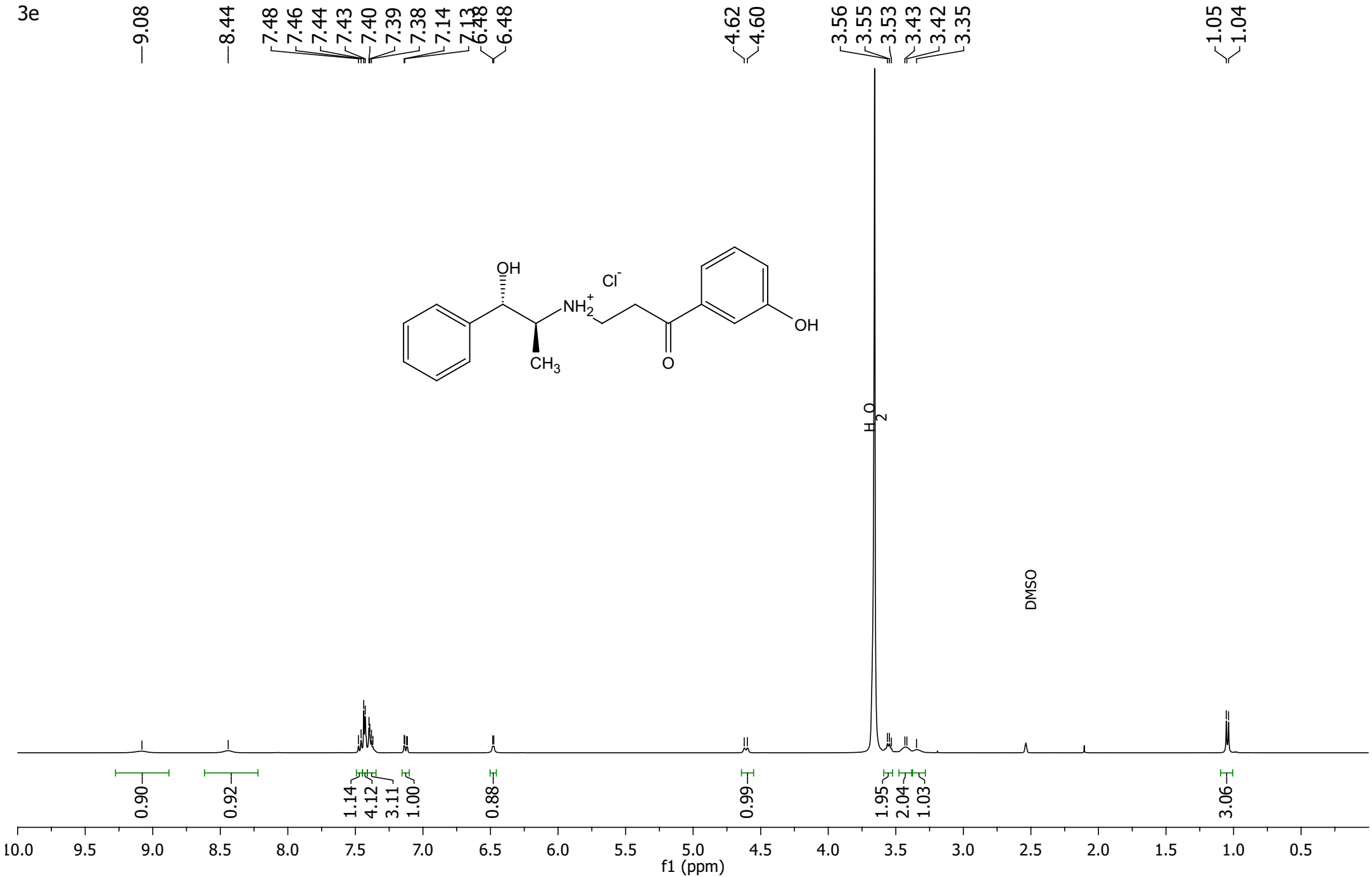



**Figure 18**: $^{1}$H-NMR Spectrum of Compound 3e

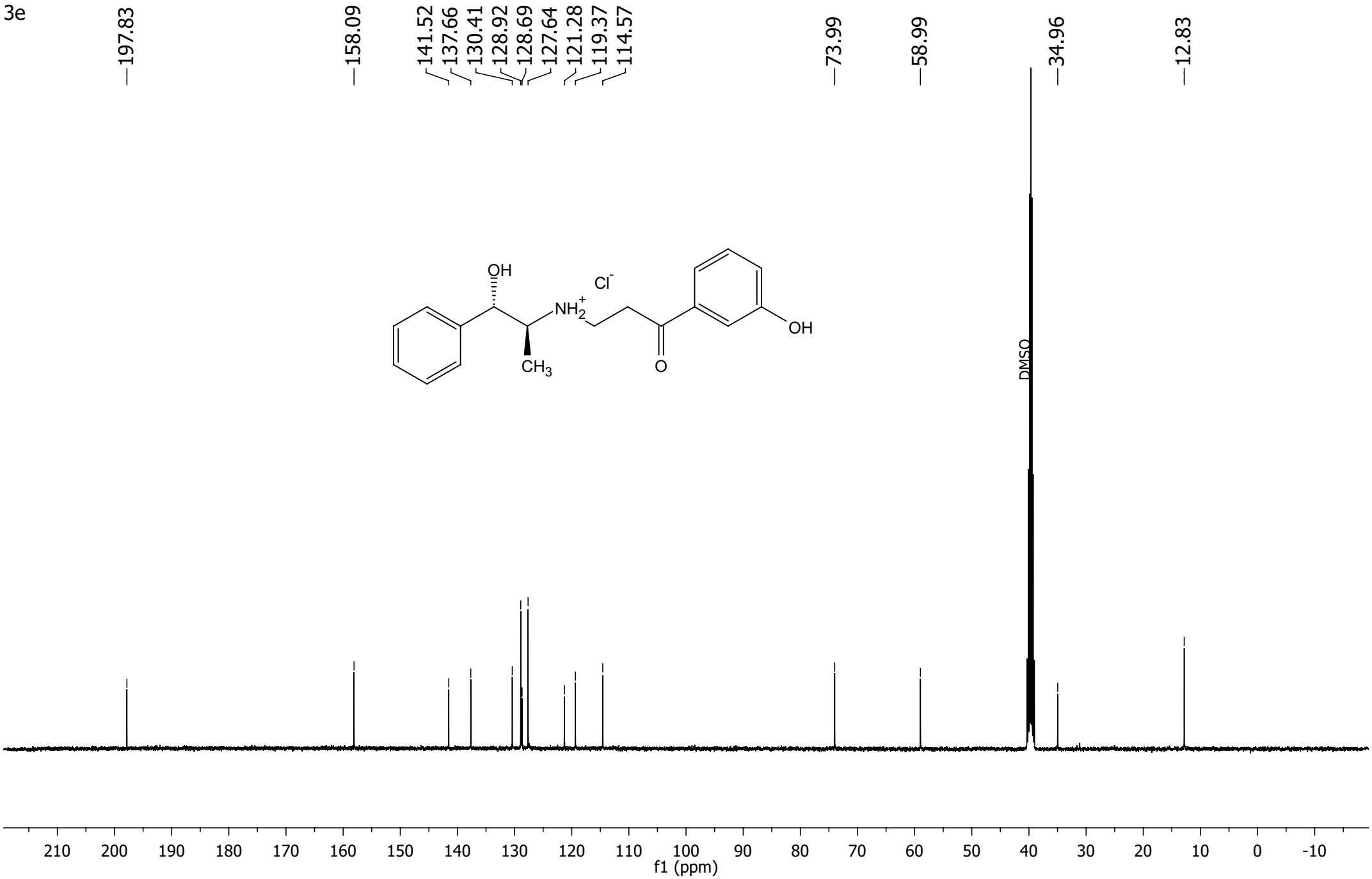



**Figure 19**: $^{13}$C-NMR Spectrum of Compound 3e

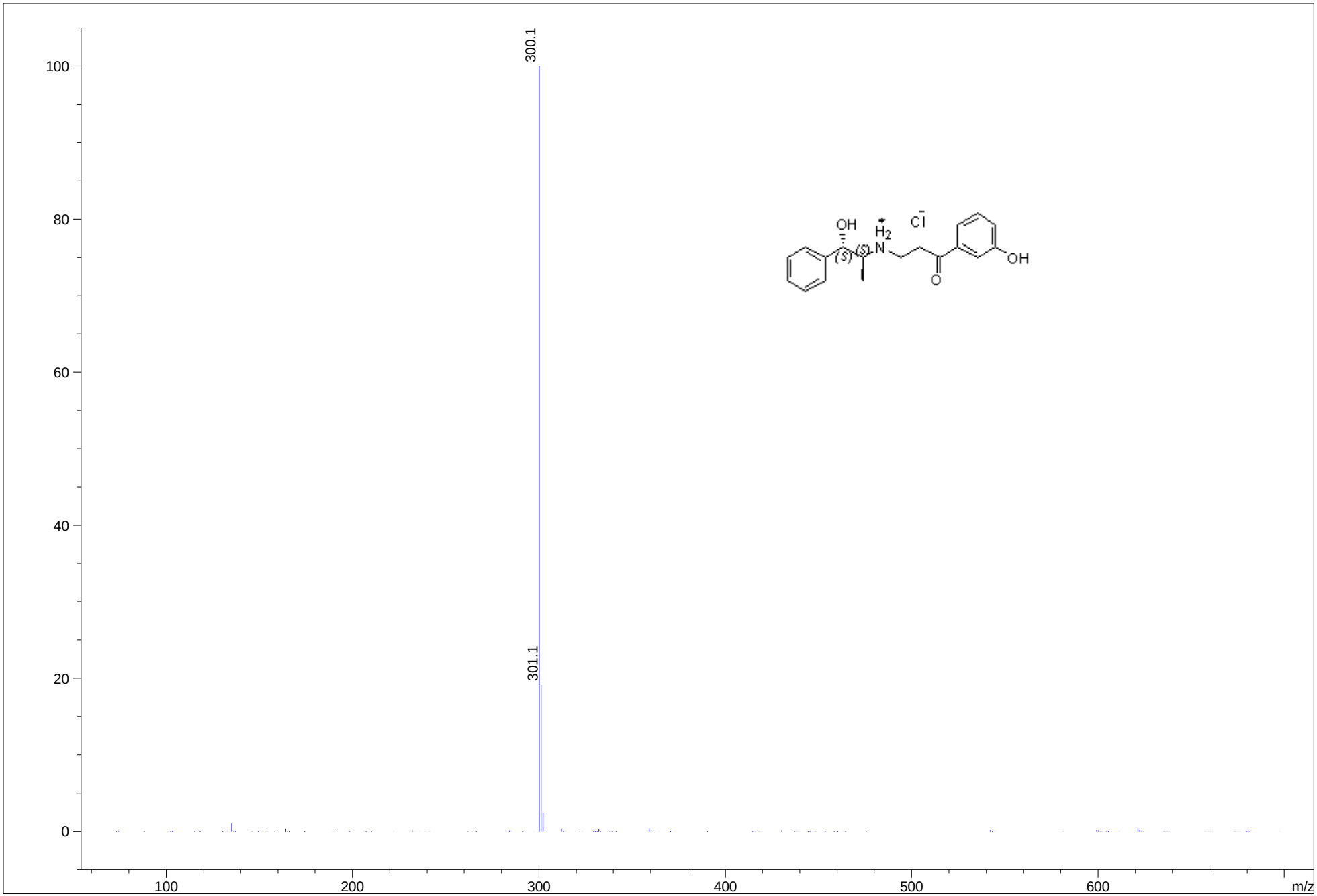



**Figure 20:** IR Spectrum of Compound 3e

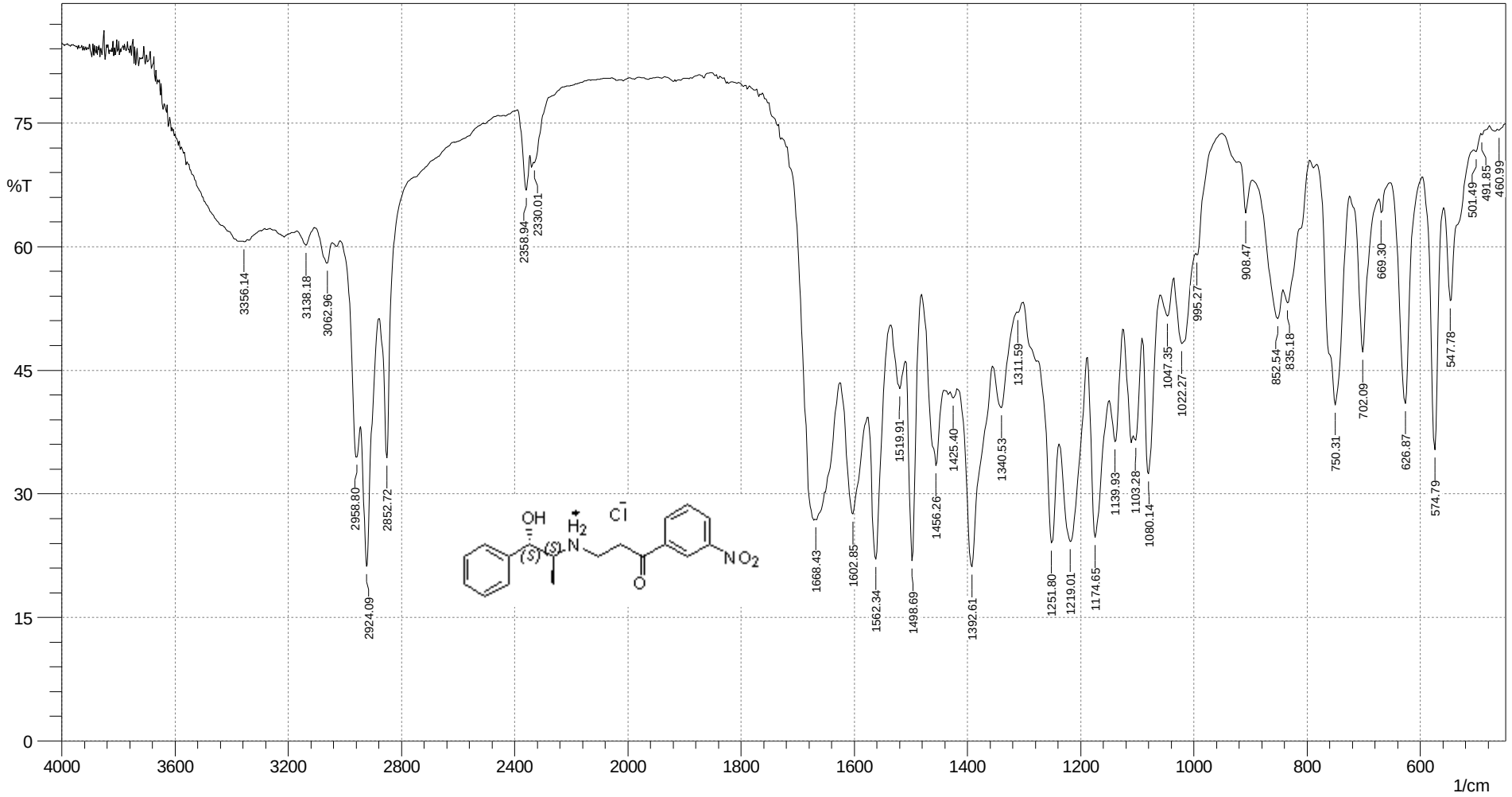



**Figure 21:** IR Spectrum of Compound 3f

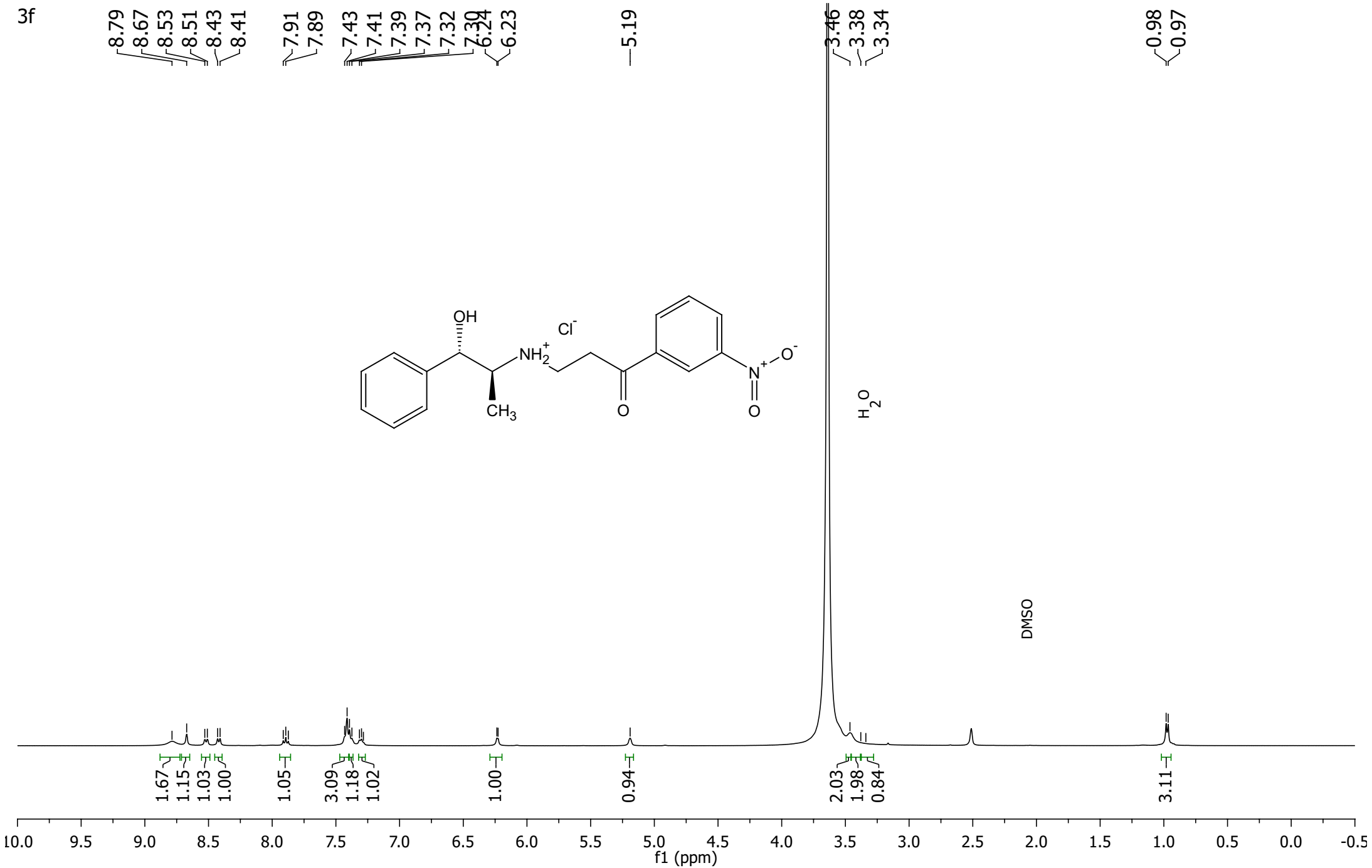



**Figure 22**: $^{1}$H-NMR Spectrum of Compound 3f

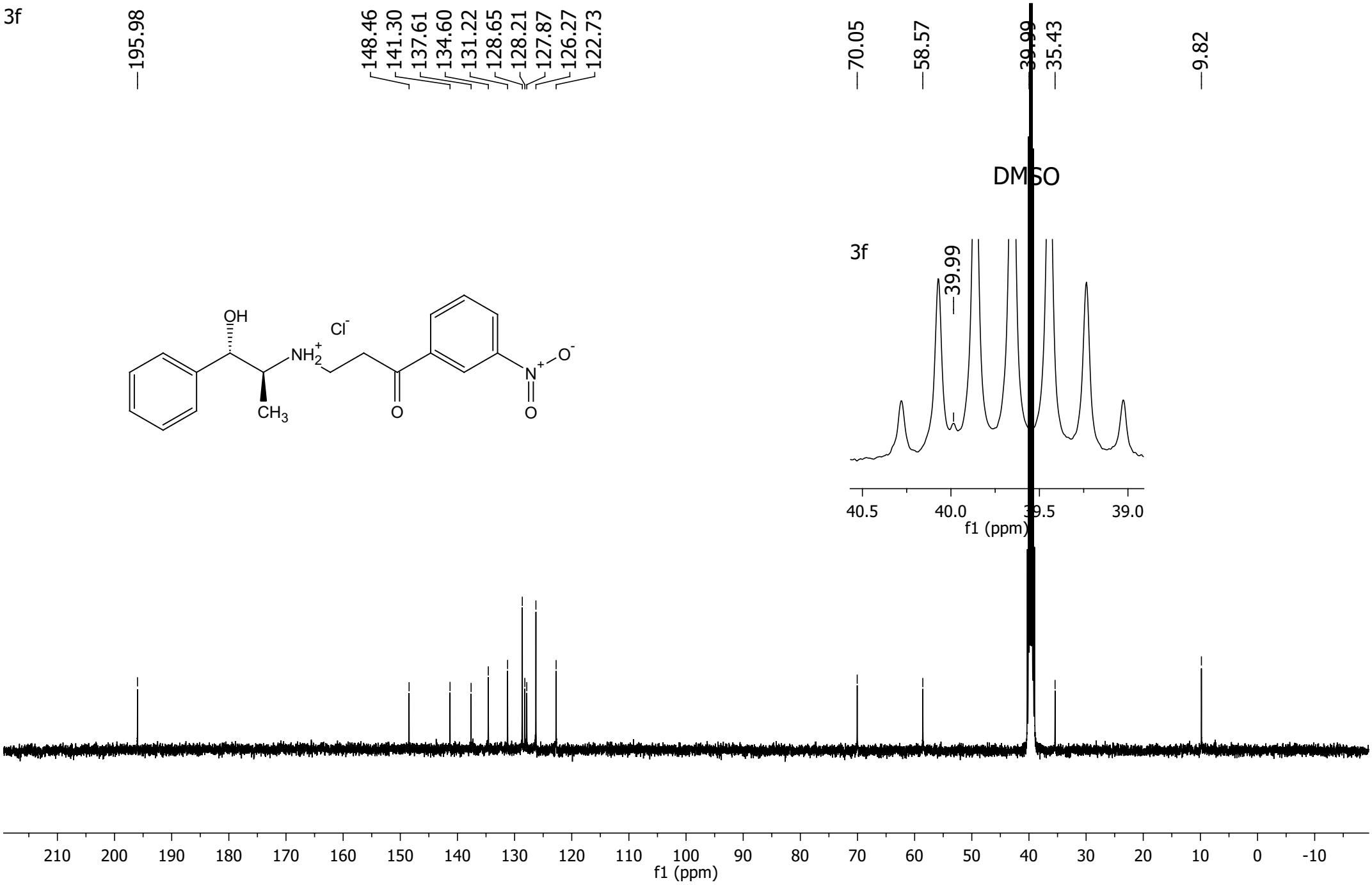



**Figure 23**: $^{13}$C-NMR Spectrum of Compound 3f


**Figure 24:** Mass Spectrum of Compound 3f

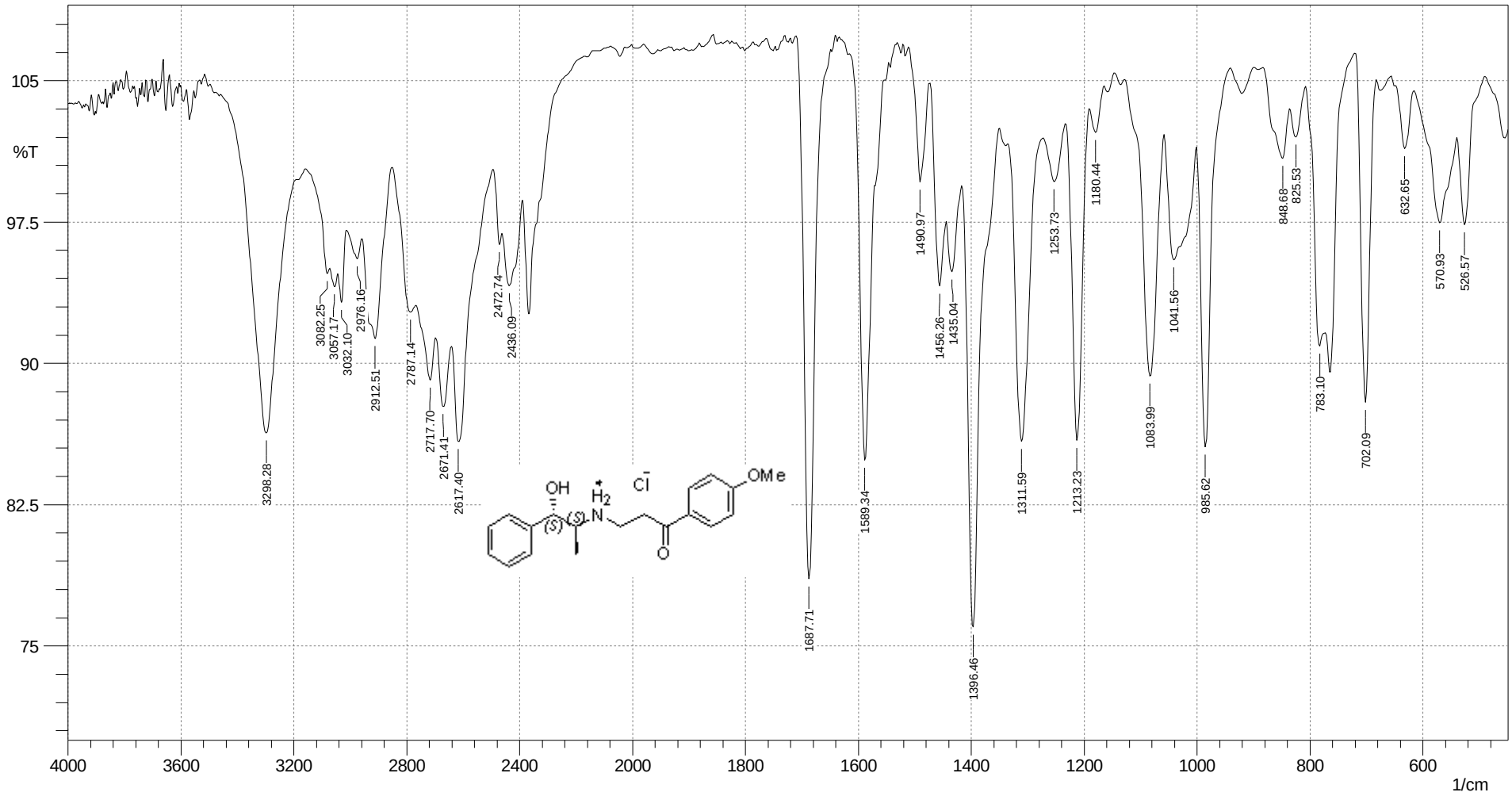



**Figure 25:** IR Spectrum of Compound 3g

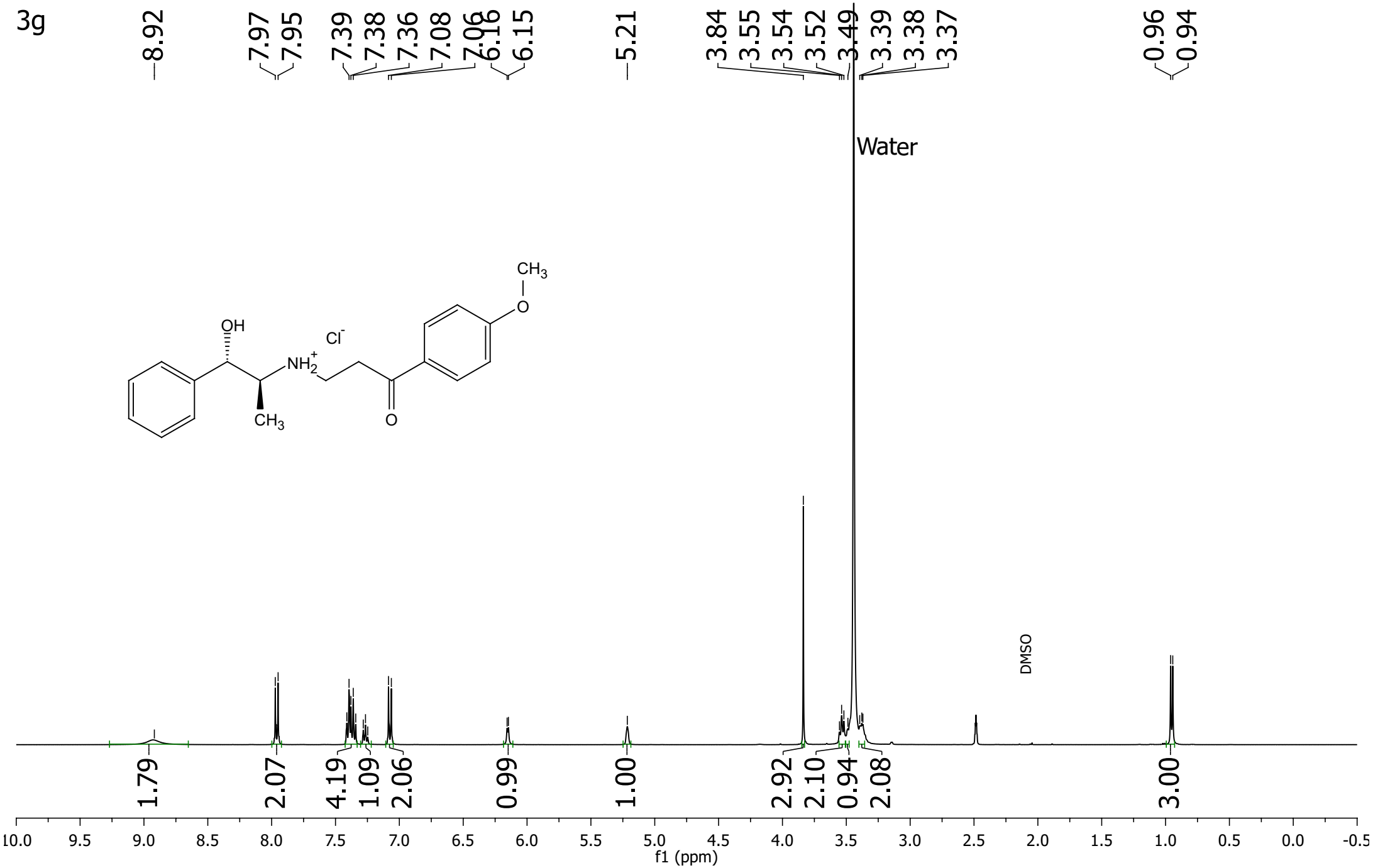



**Figure 26**: $^{1}$H-NMR Spectrum of Compound 3g

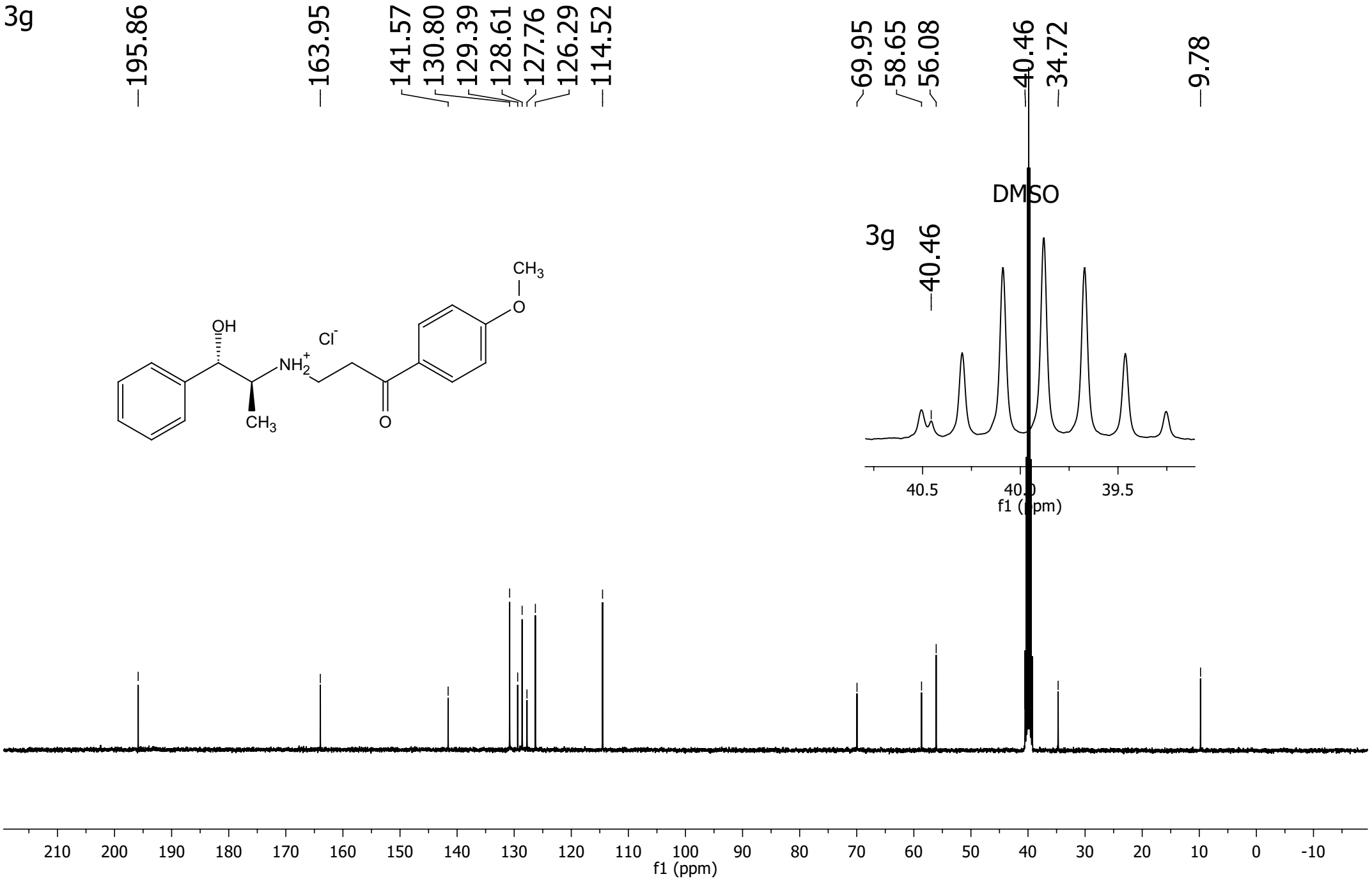



**Figure 27**: $^{13}$C-NMR Spectrum of Compound 3g

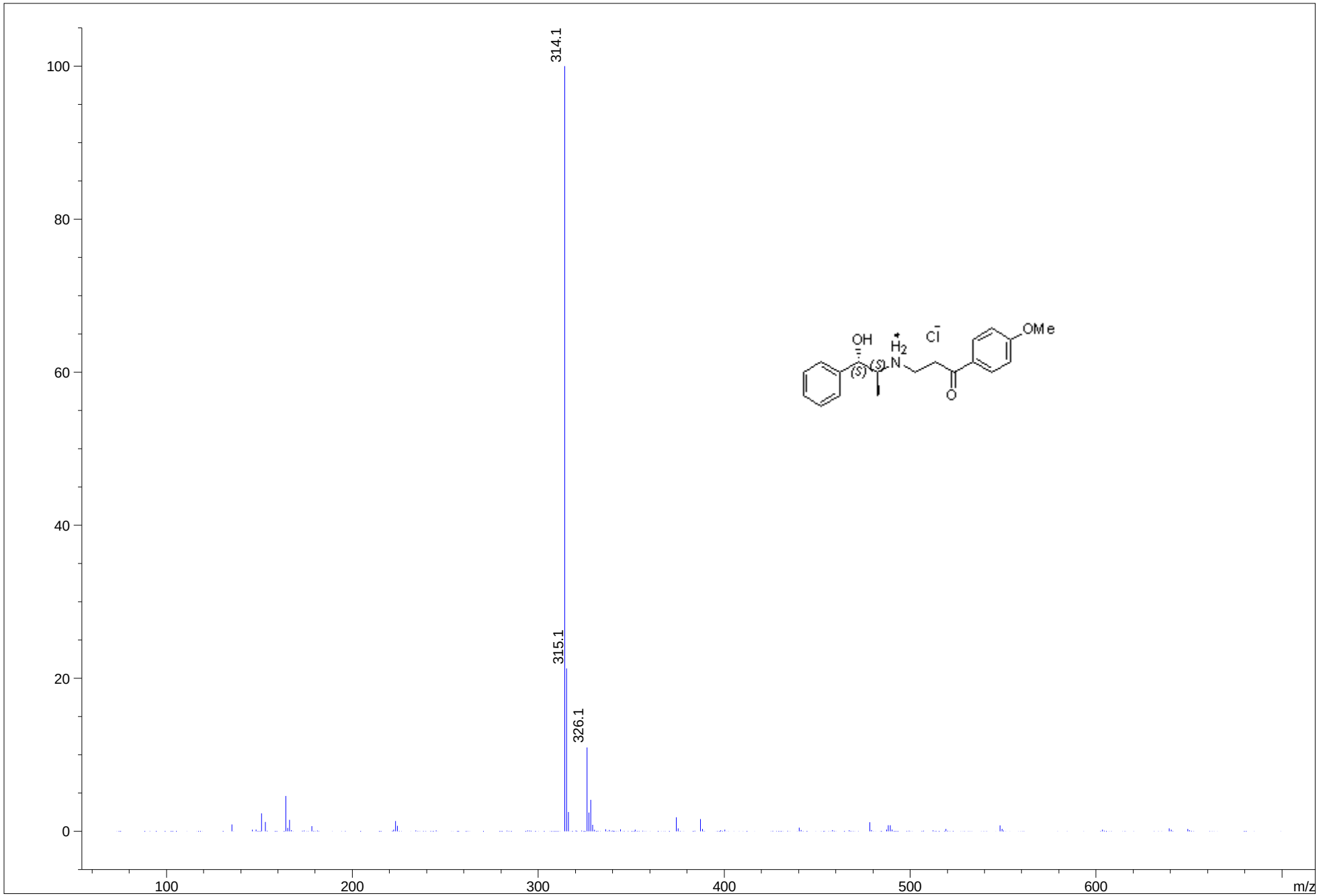



**Figure 28:** MassR Spectrum of Compound 3g

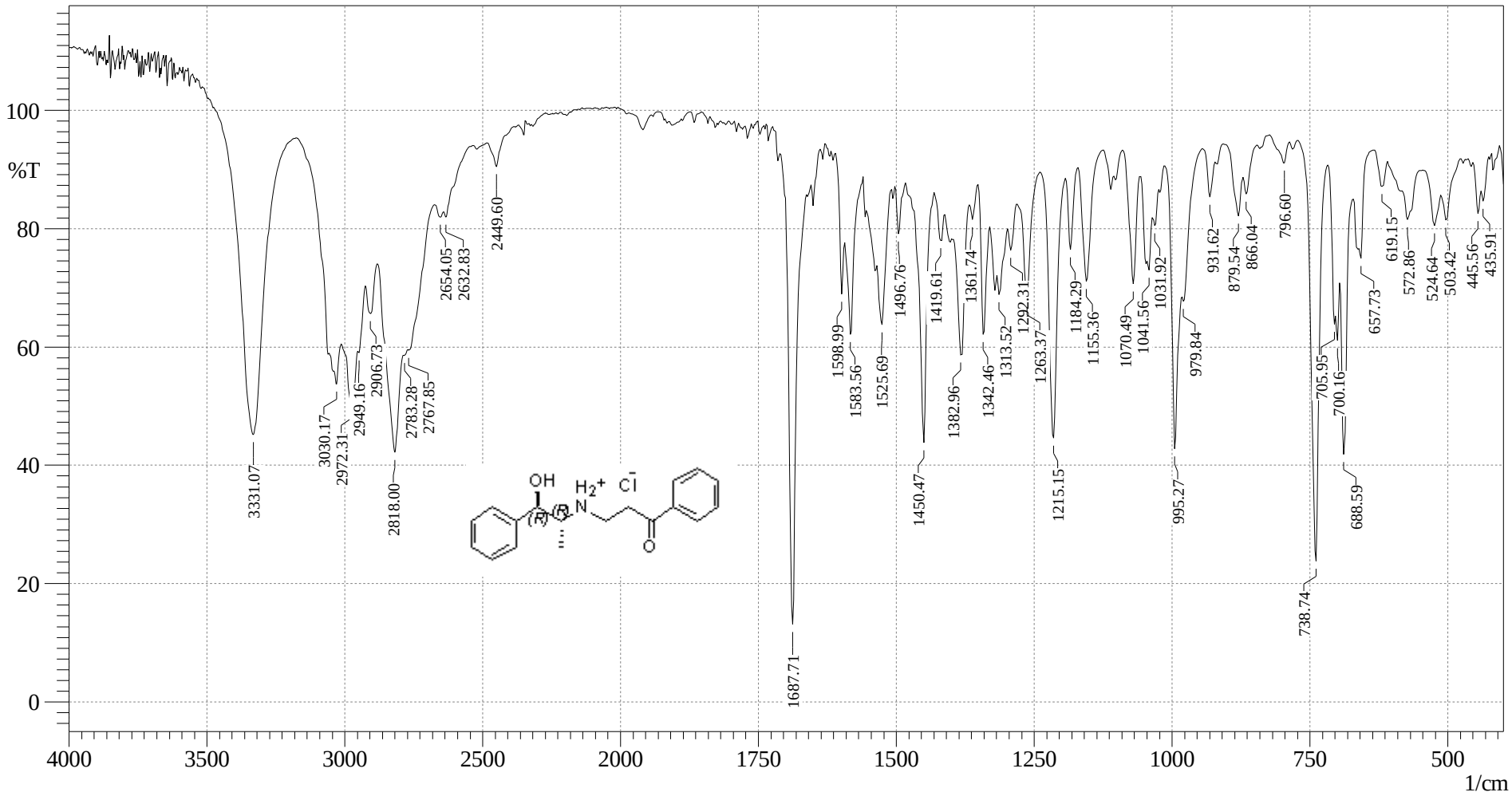



**Figure 29:** IR Spectrum of Compound 3a'

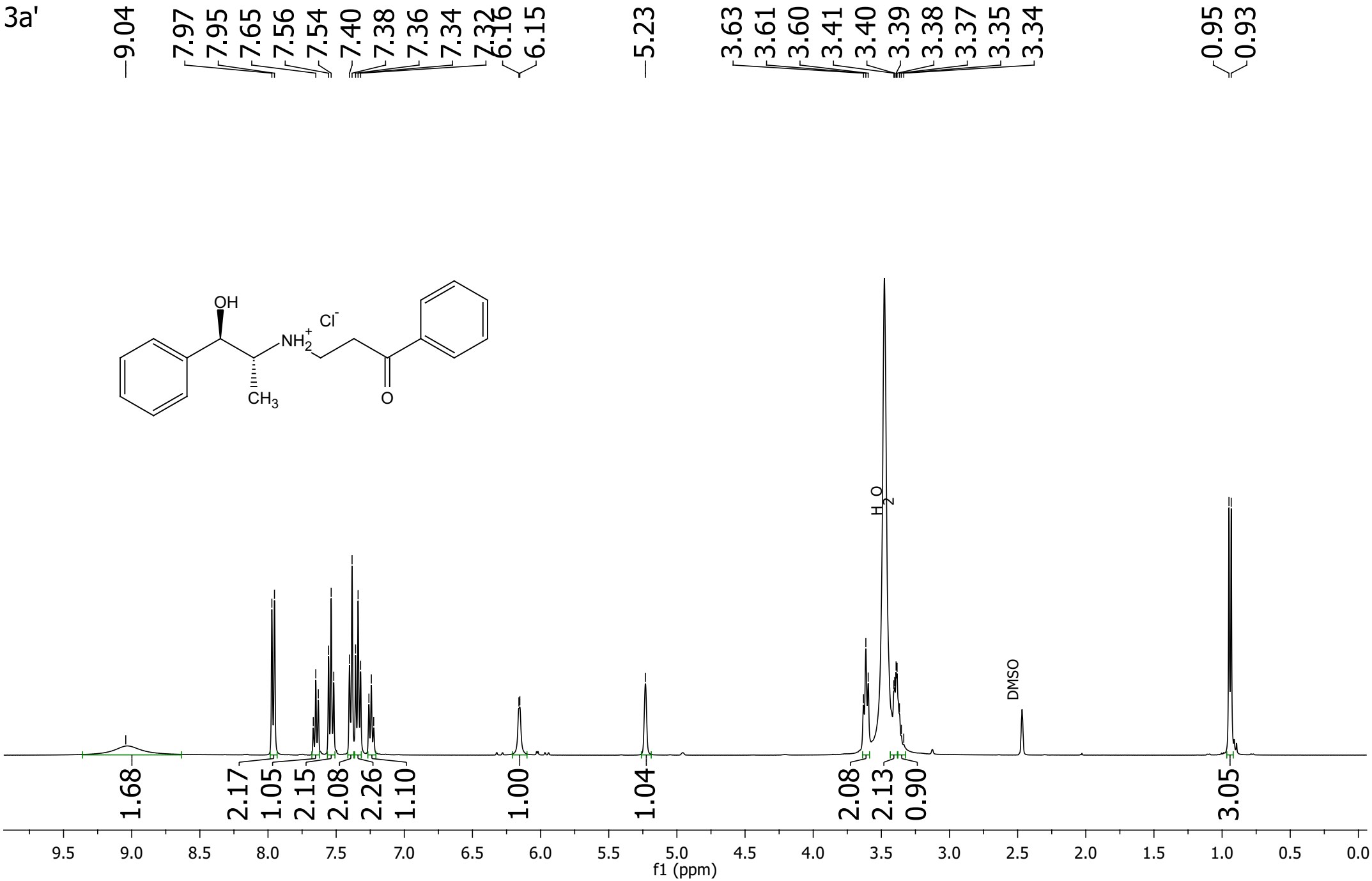



**Figure 30**: $^{1}$H NMR spectrum of Compound 3a'

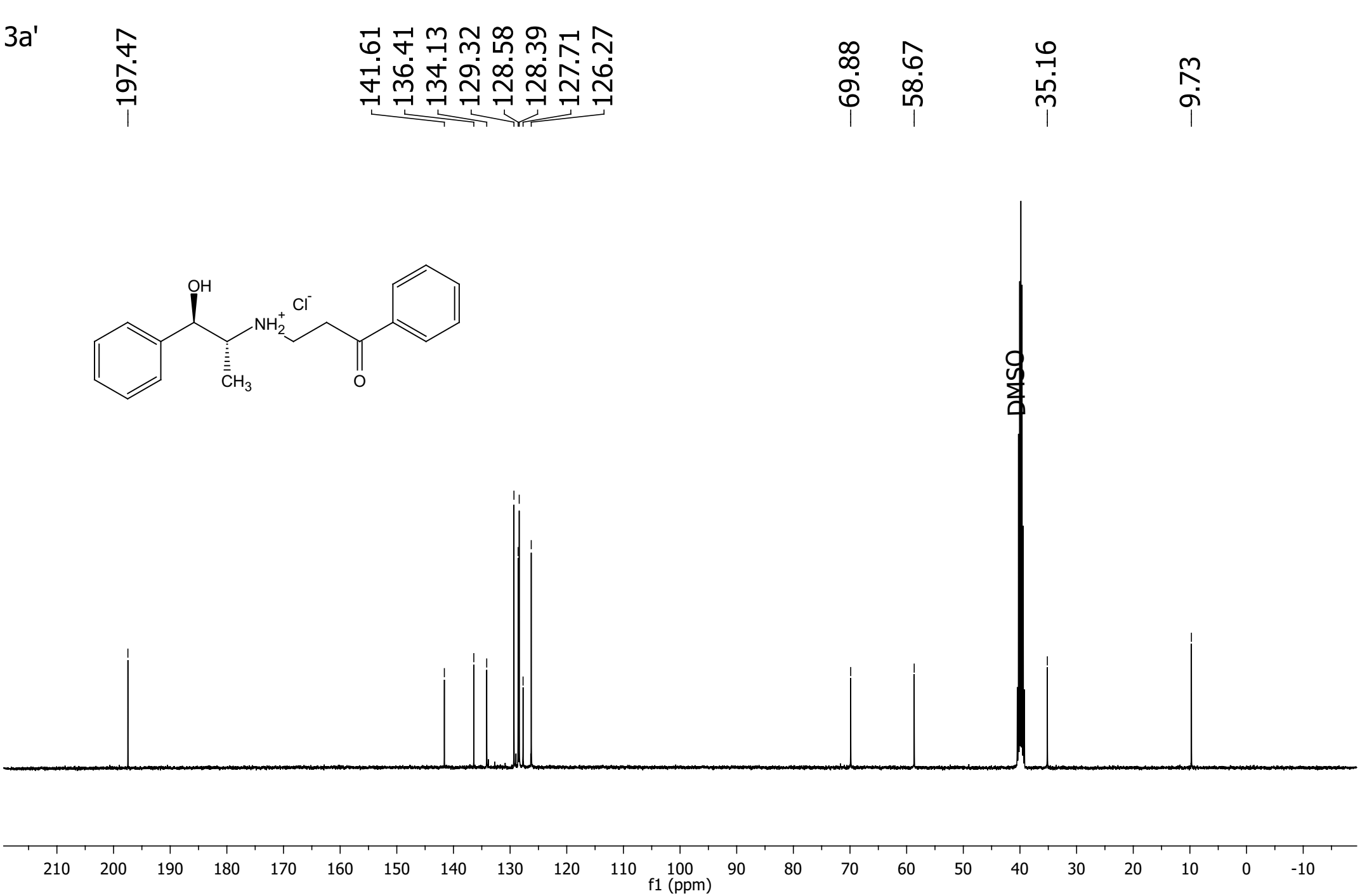



**Figure 31**: $^{13}C$ NMR spectrum of Compound 3a'

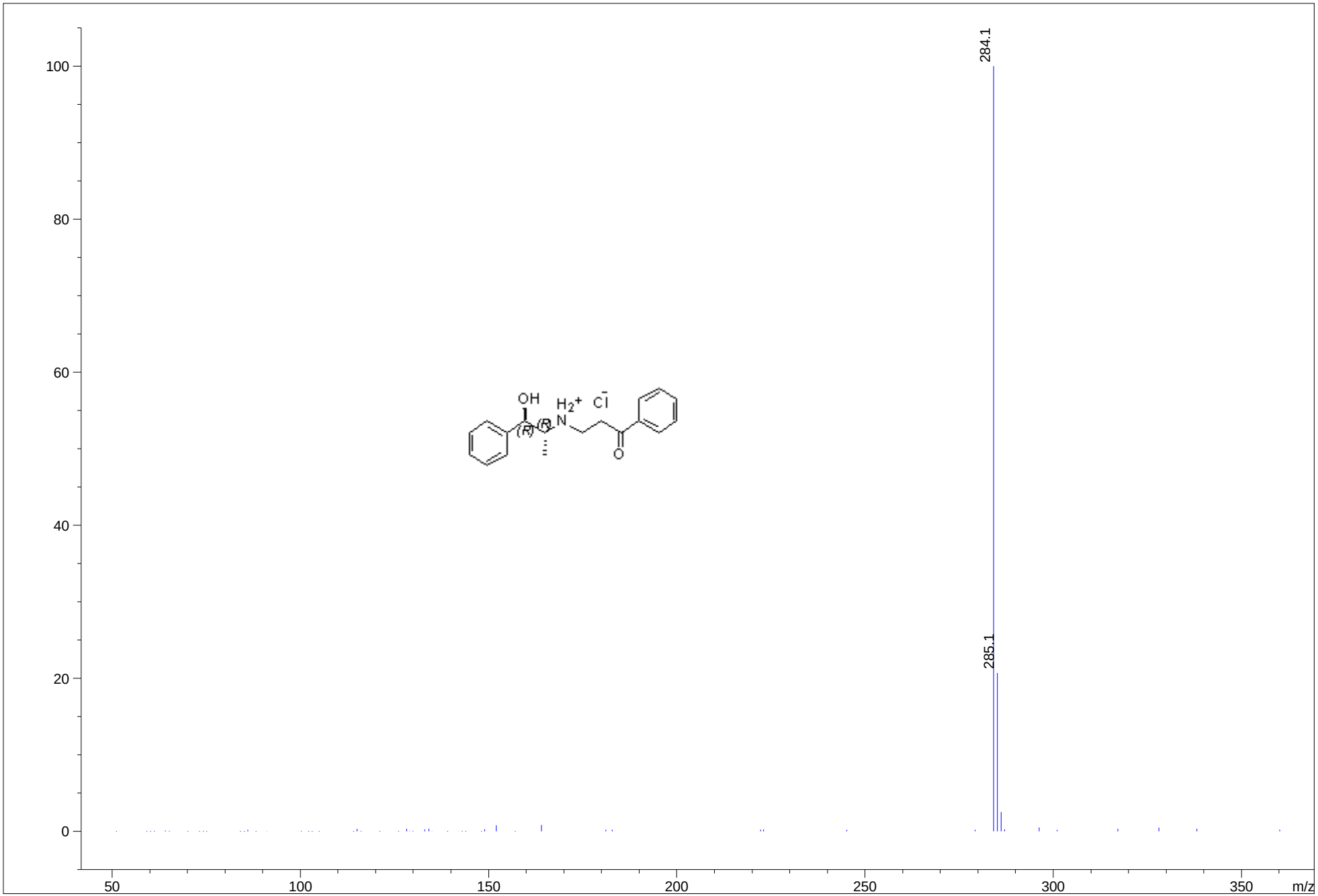



**Figure 32:** Mass Spectrum Compound 3a'

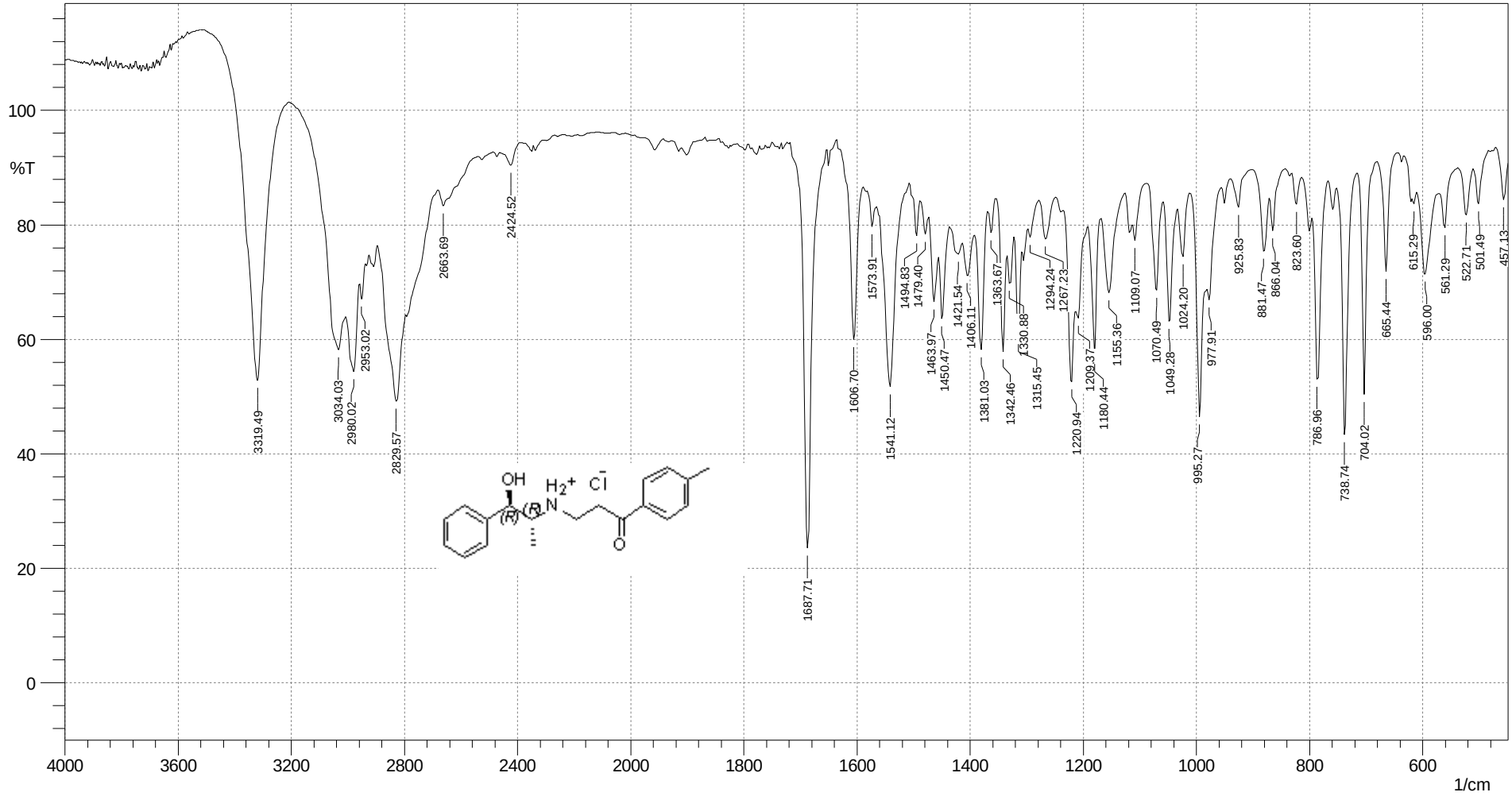



**Figure 33:** IR Spectrum of Compound 3b'

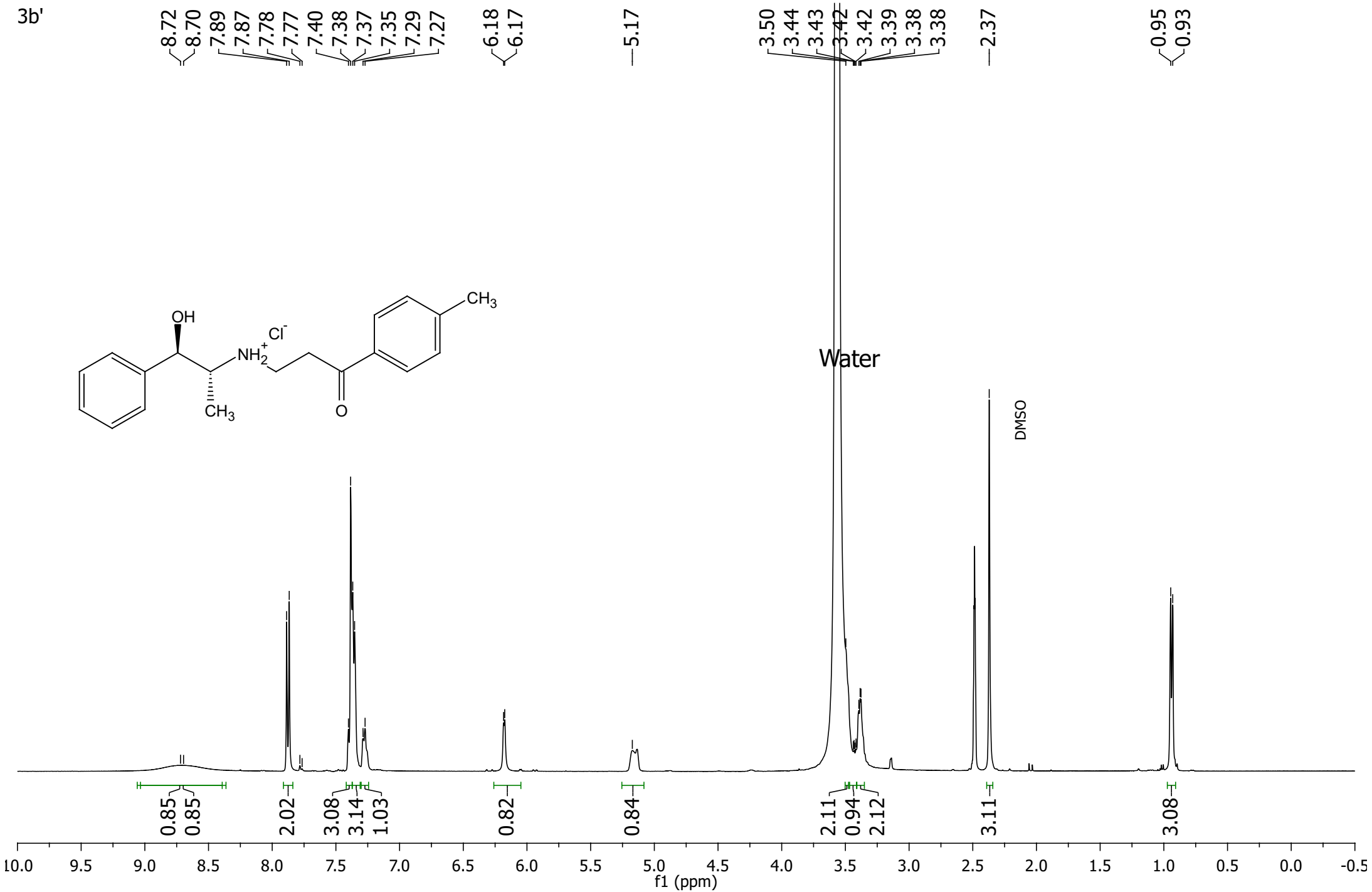



**Figure 34:** $^{1}$H-NMR Spectrum of Compound 3b'

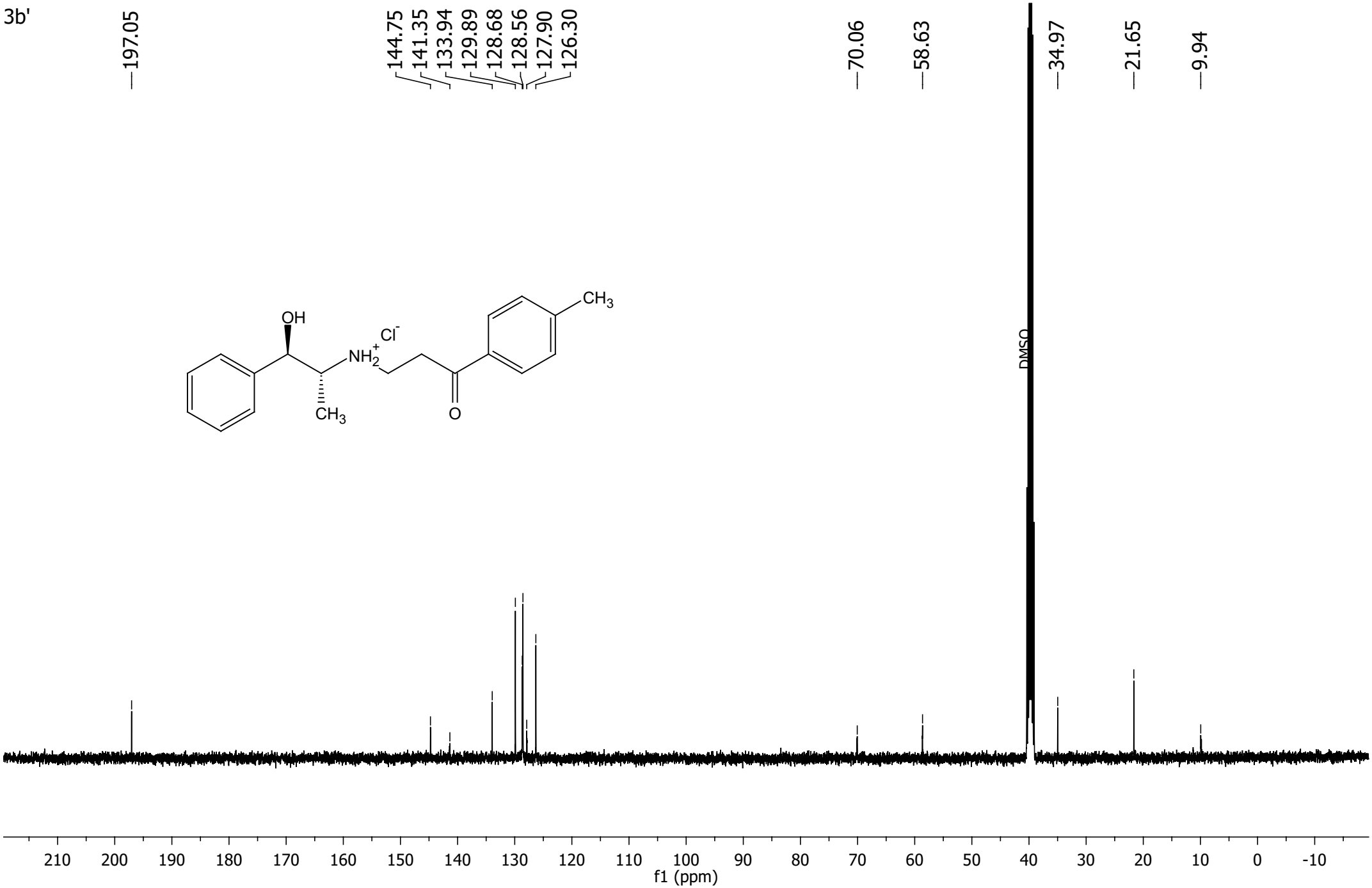



**Figure 35:** $^{13}$C-NMR Spectrum of Compound 3b'

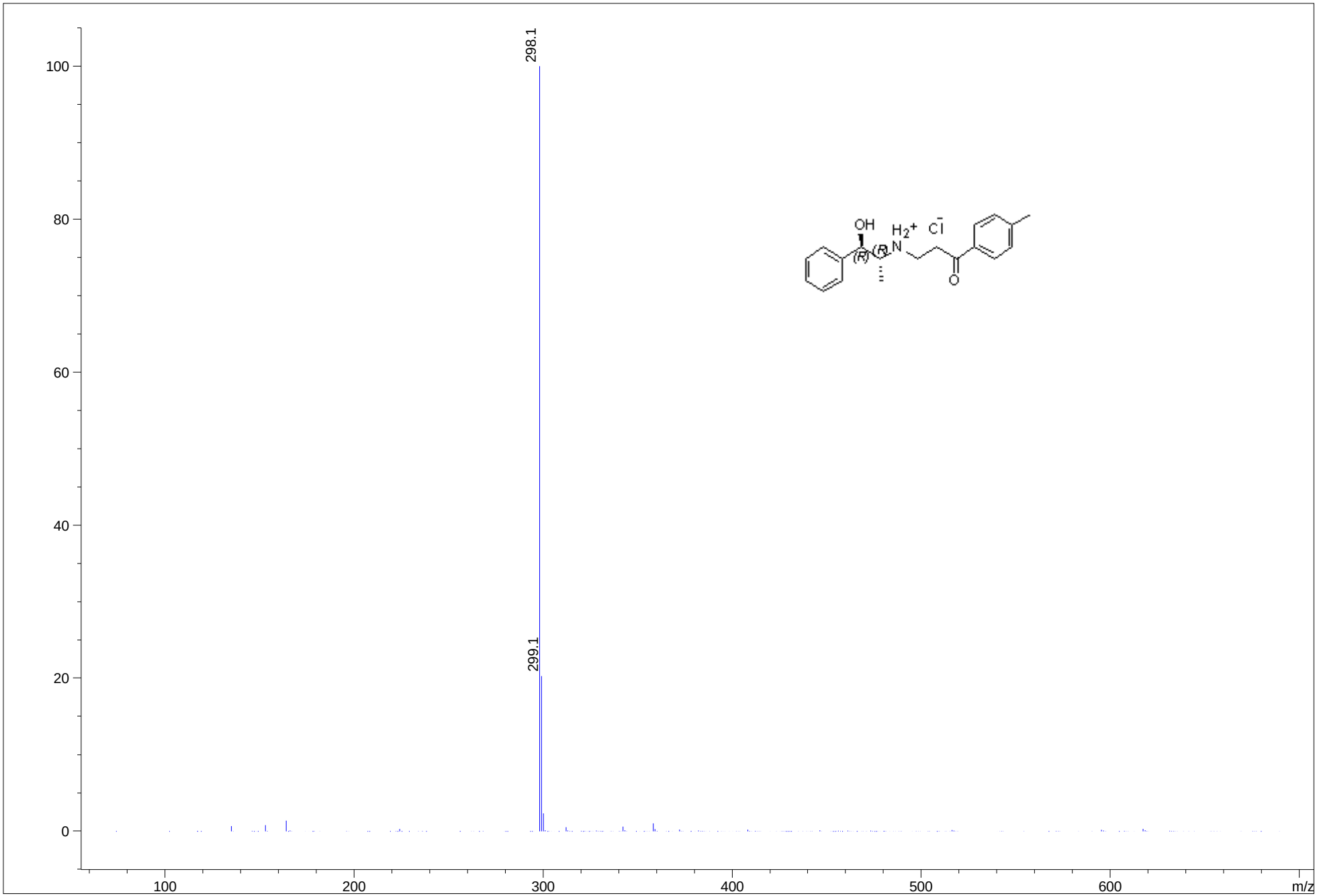



**Figure 36:** Mass Spectrum of Compound 3b'

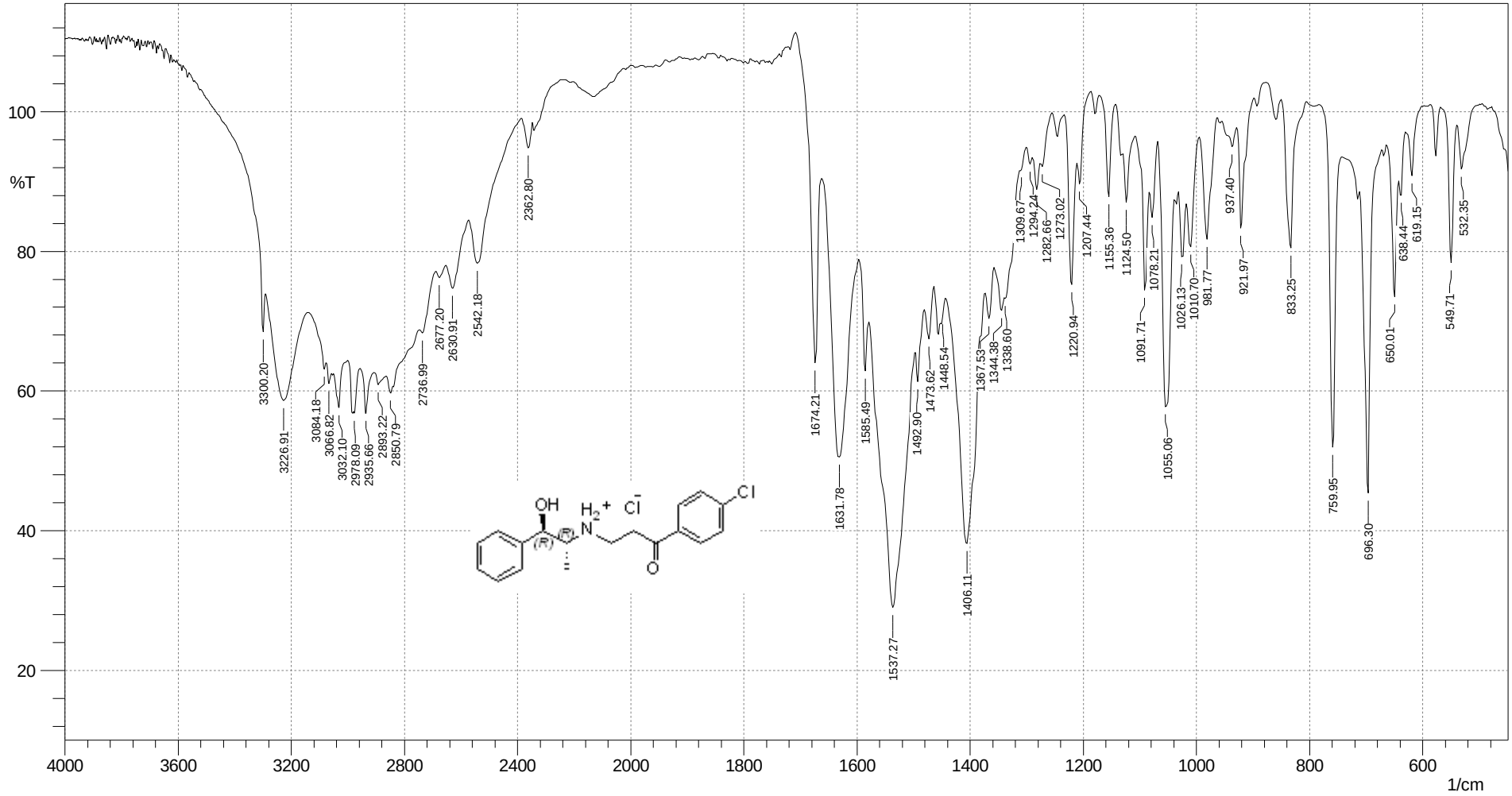



**Figure 37:** IR Spectrum of Compound 3c'

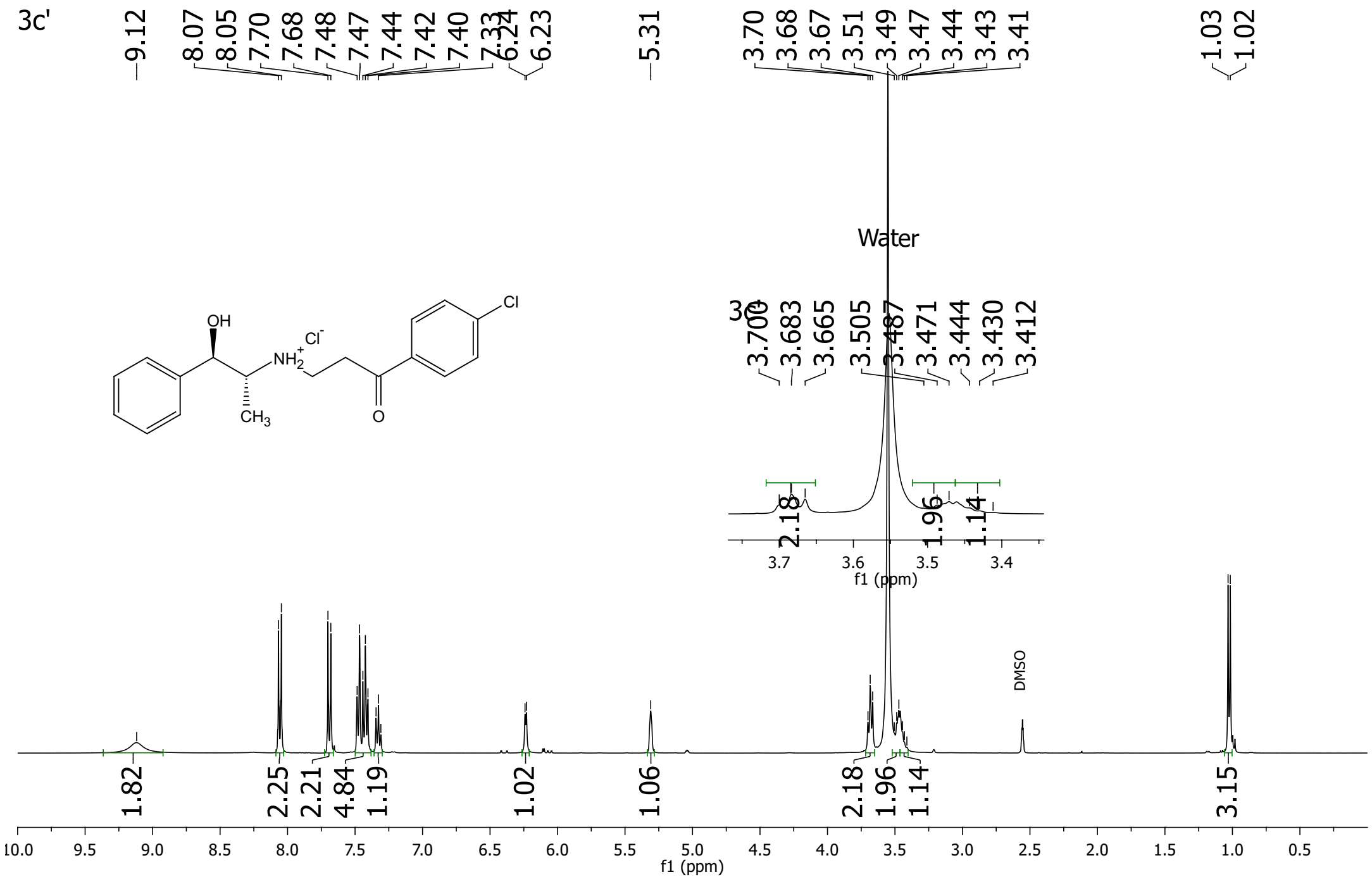



**Figure 38:** $^{1}$H-NMR Spectrum of Compound 3c'

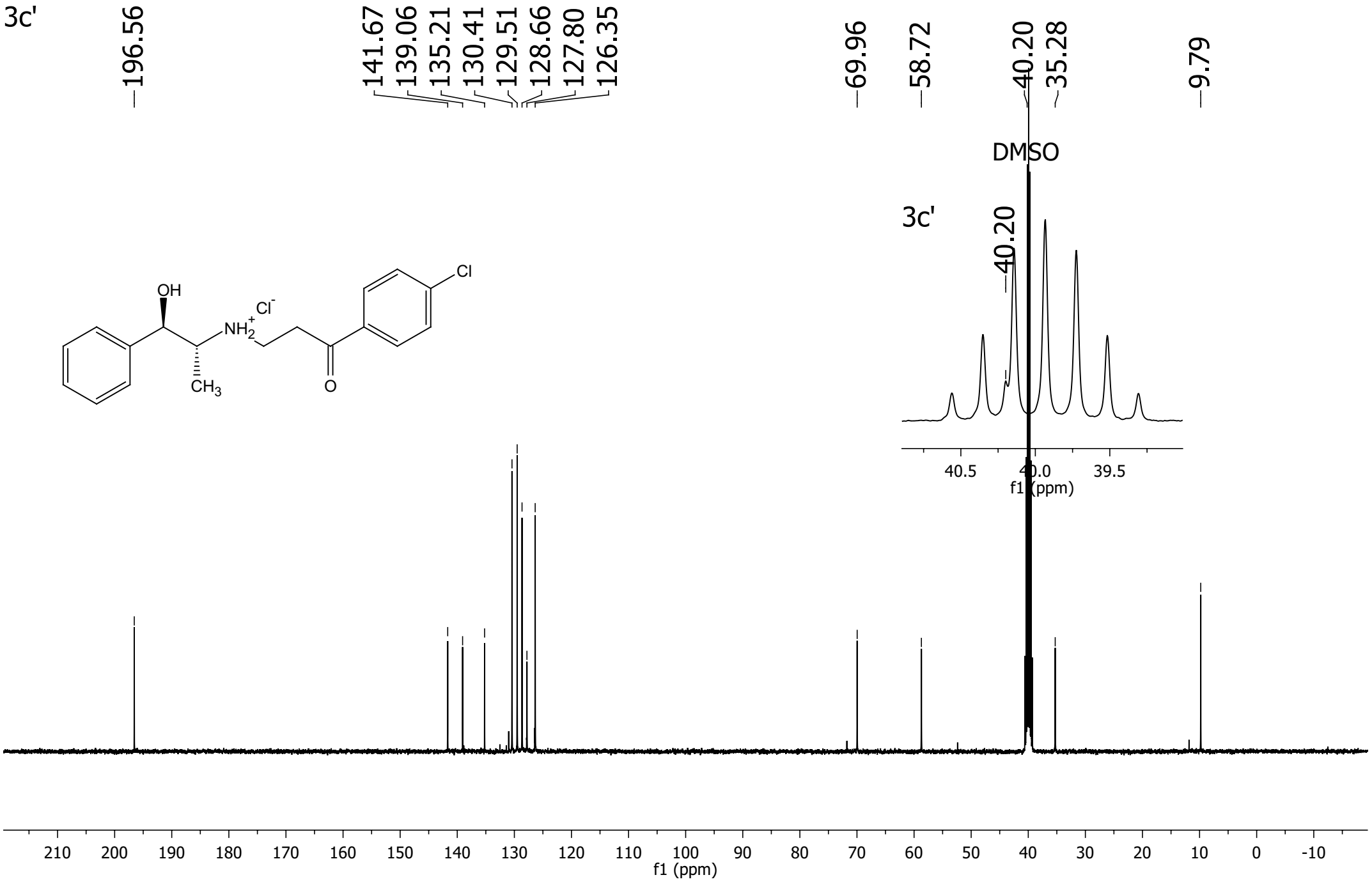



**Figure 39:** $^{13}$C-NMR Spectrum of Compound 3c'

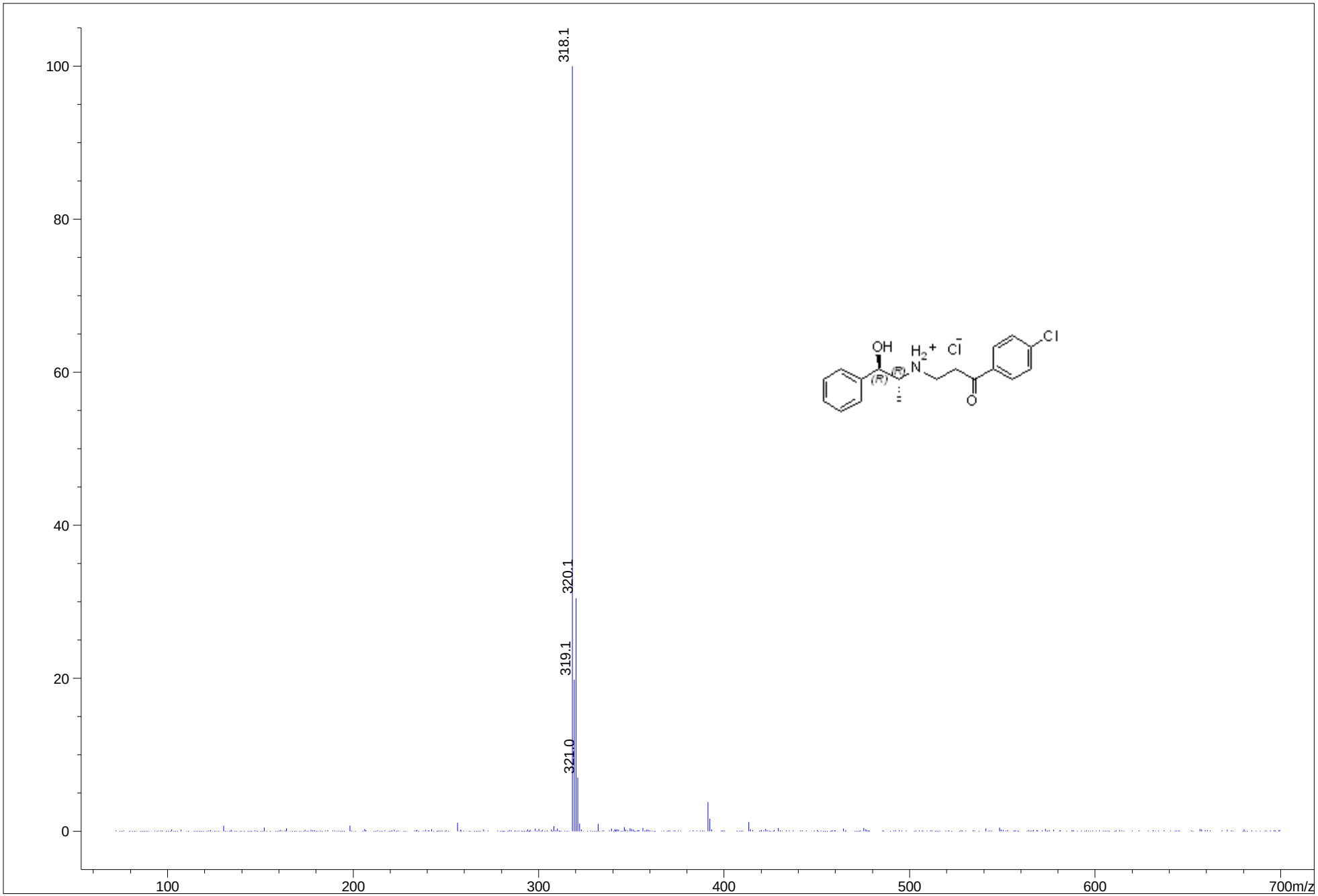



**Figure 40:** Mass Spectrum of Compound 3c'

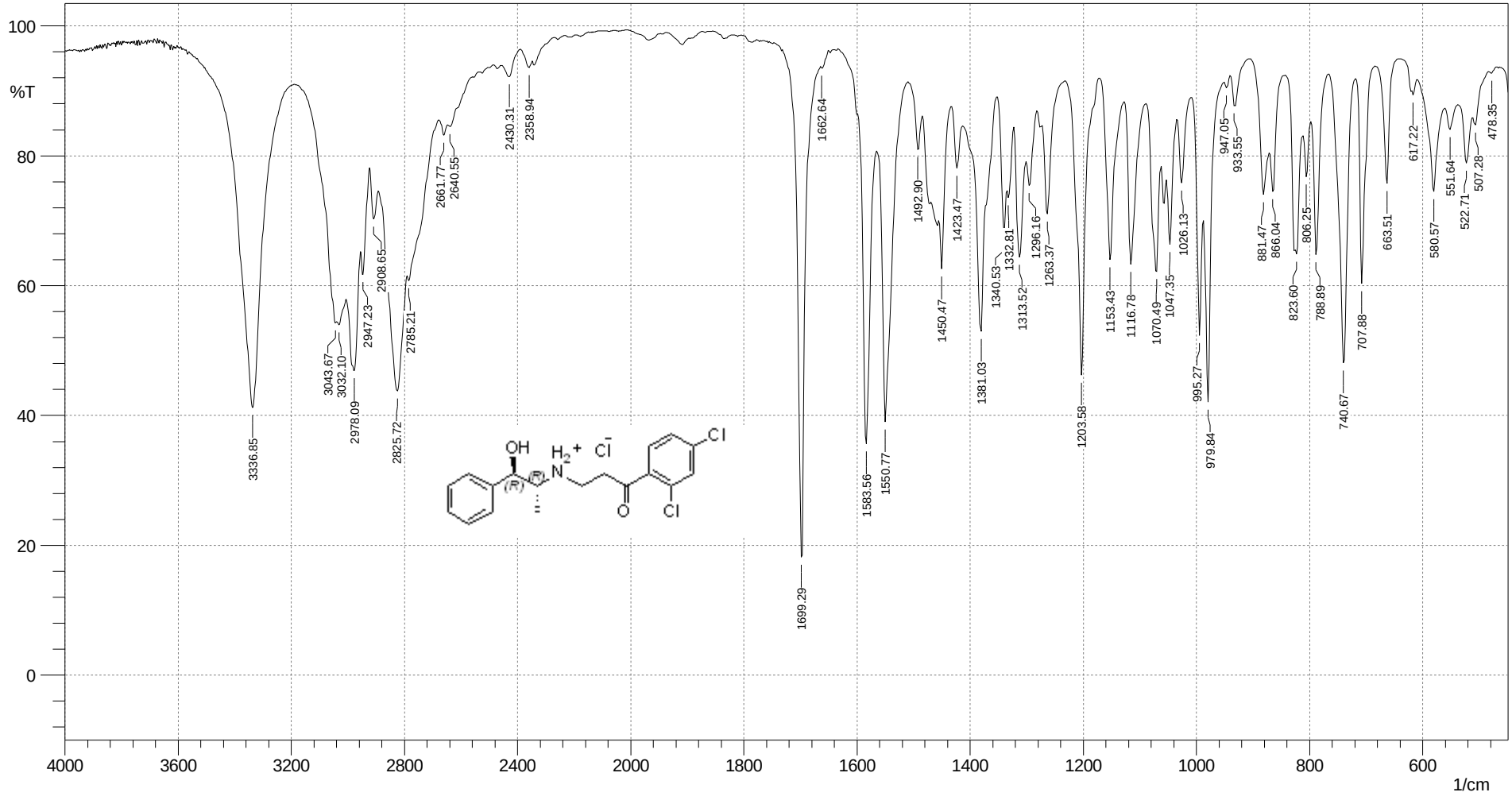



**Figure 41:** IR Spectrum of Compound 3d'

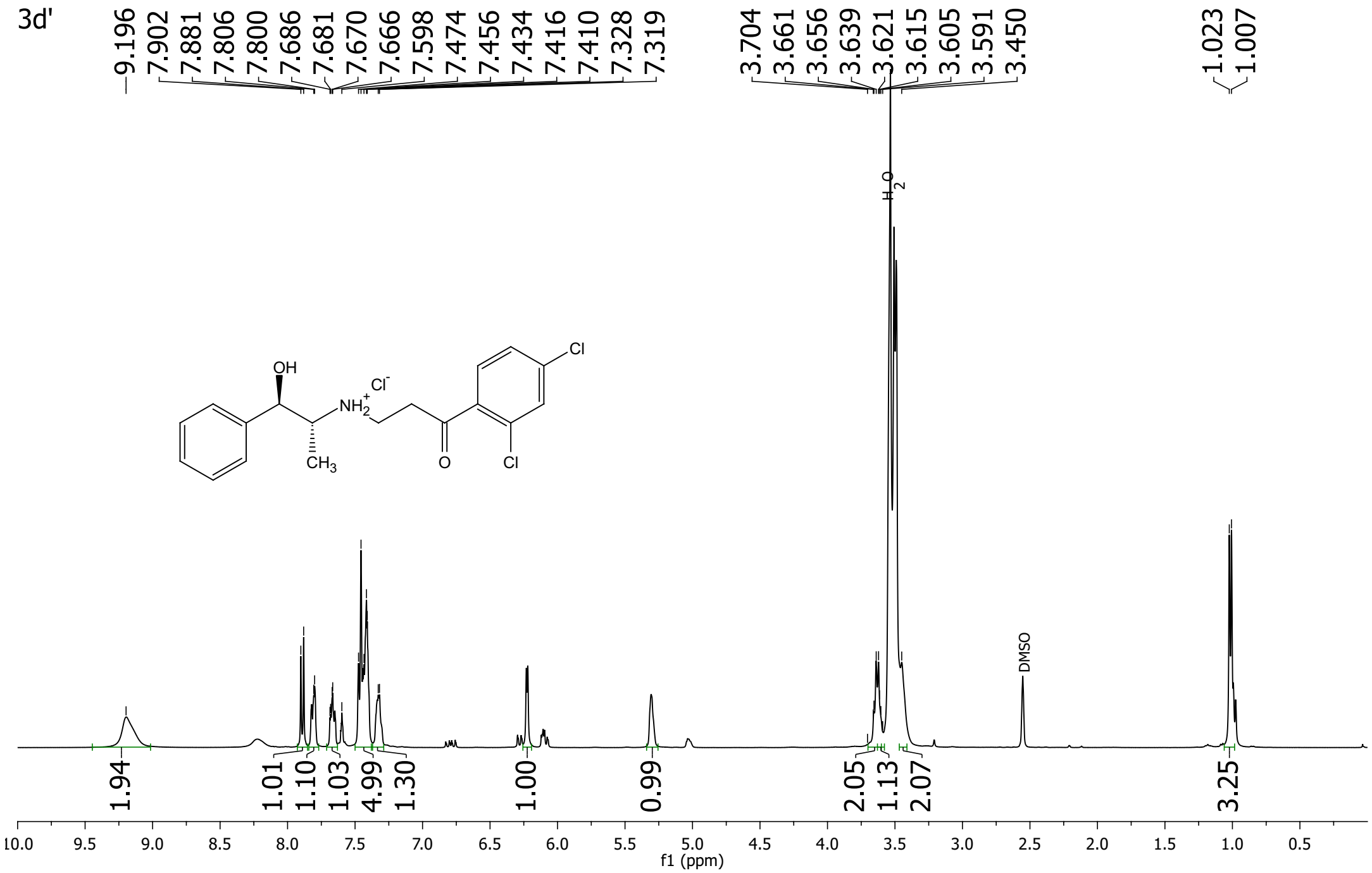



**Figure 42:** $^{1}$H-NMR Spectrum of Compound 3d'

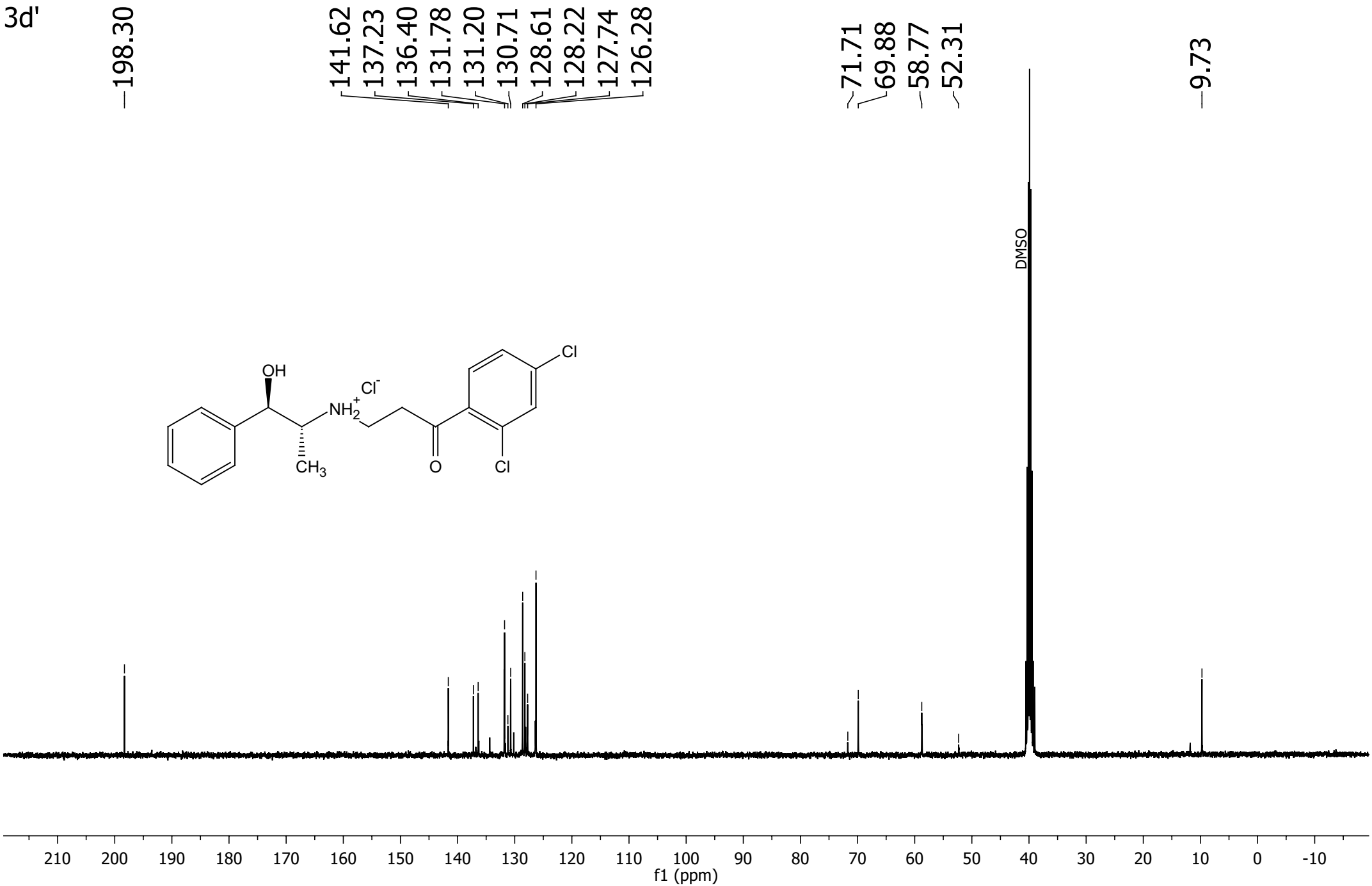



**Figure 43:** $^{13}$C-NMR Spectrum of Compound 3d'

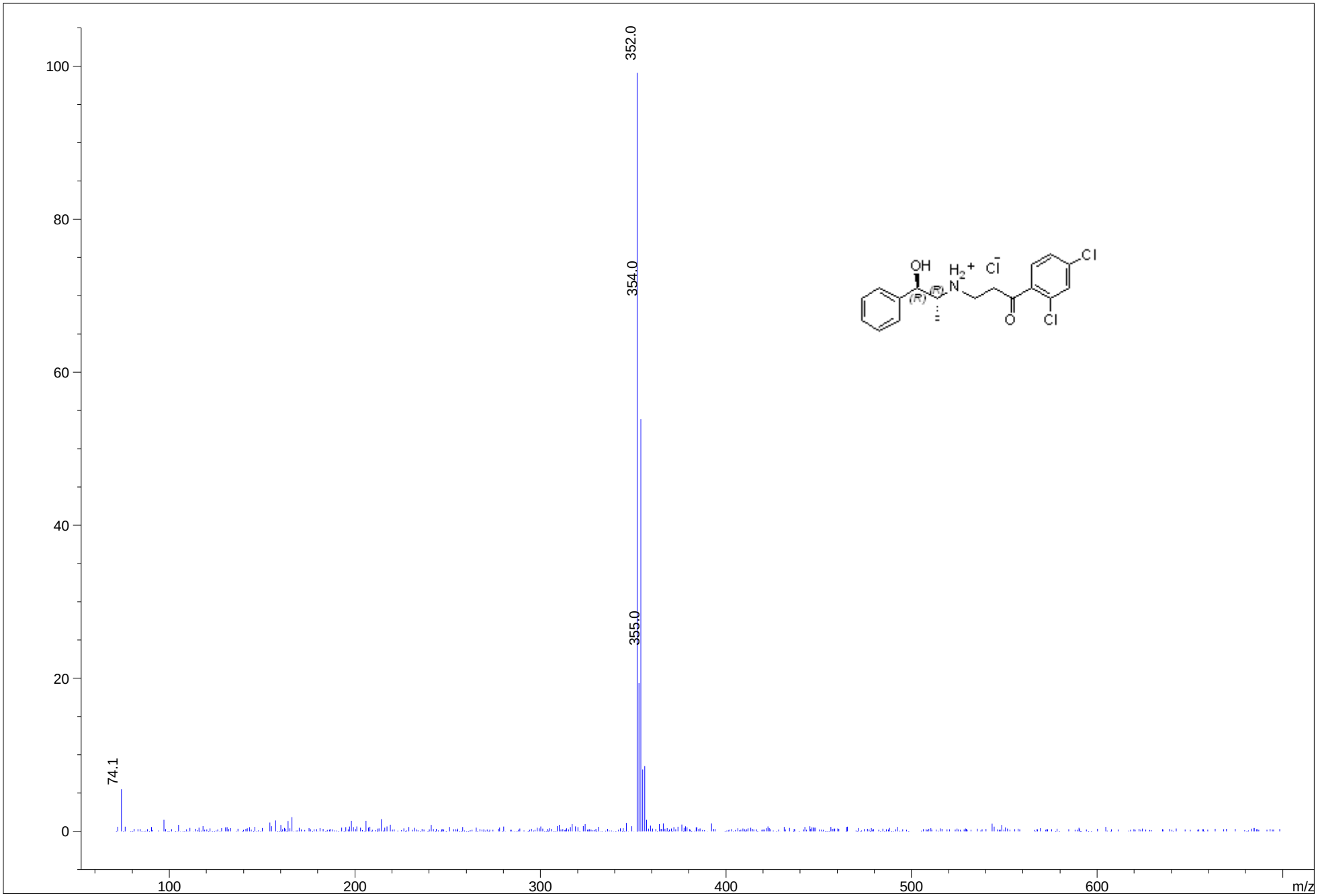



**Figure 44:** Mass Spectrum of Compound 3d'

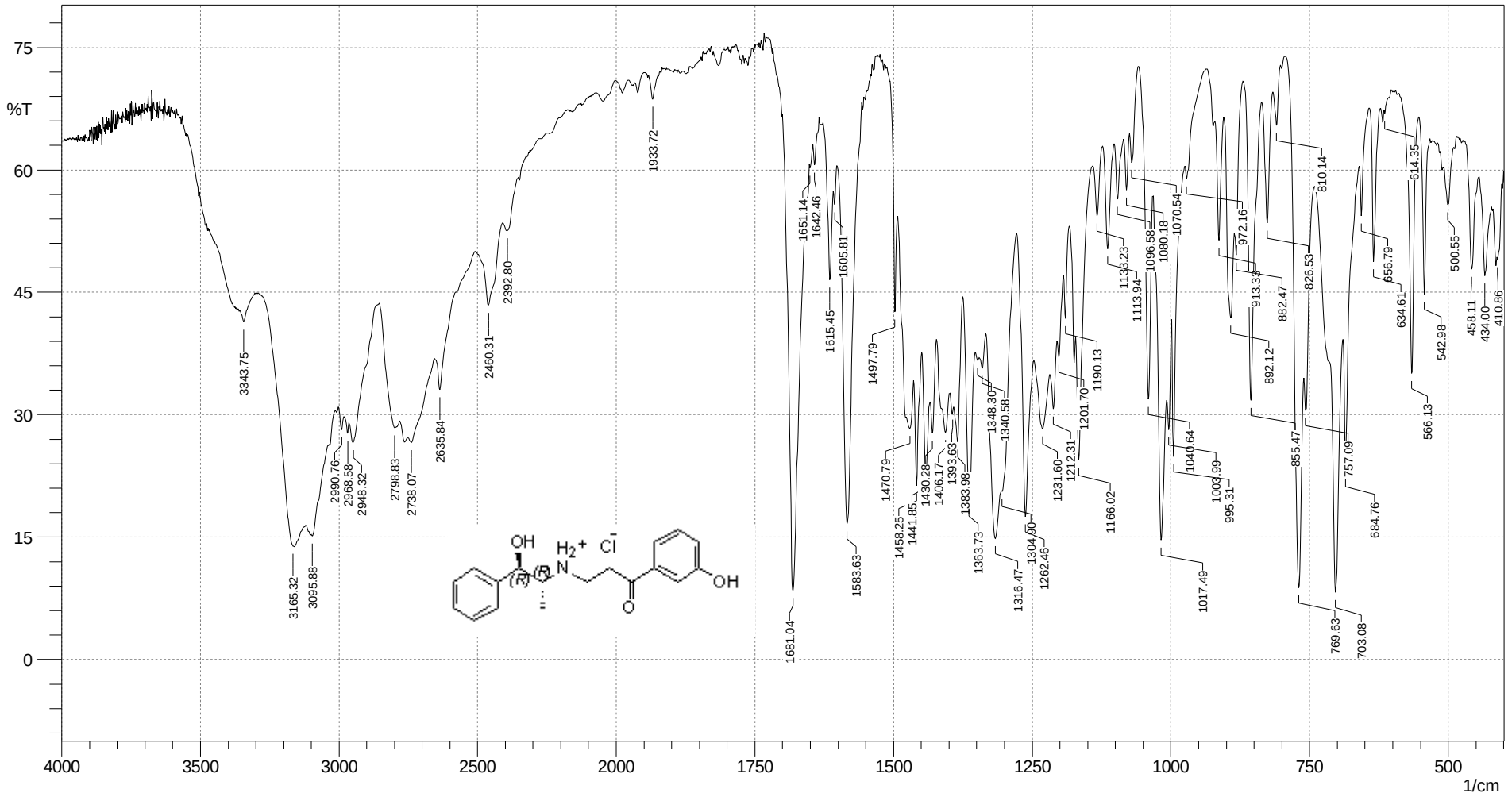



**Figure 45: IR** Spectrum of Compound 3e'

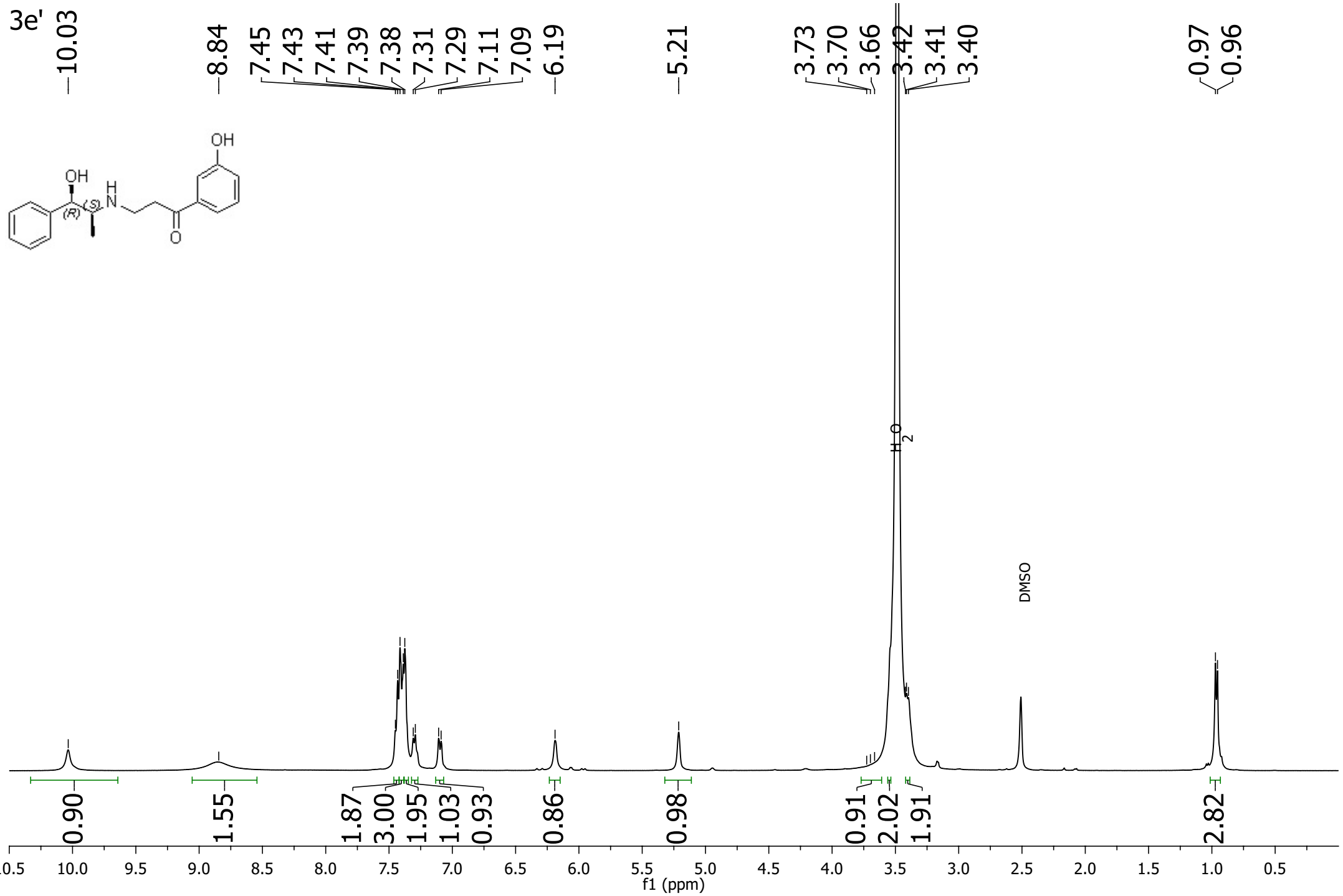



**Figure 46**: 1H-NMR Spectrum of Compound 3e'

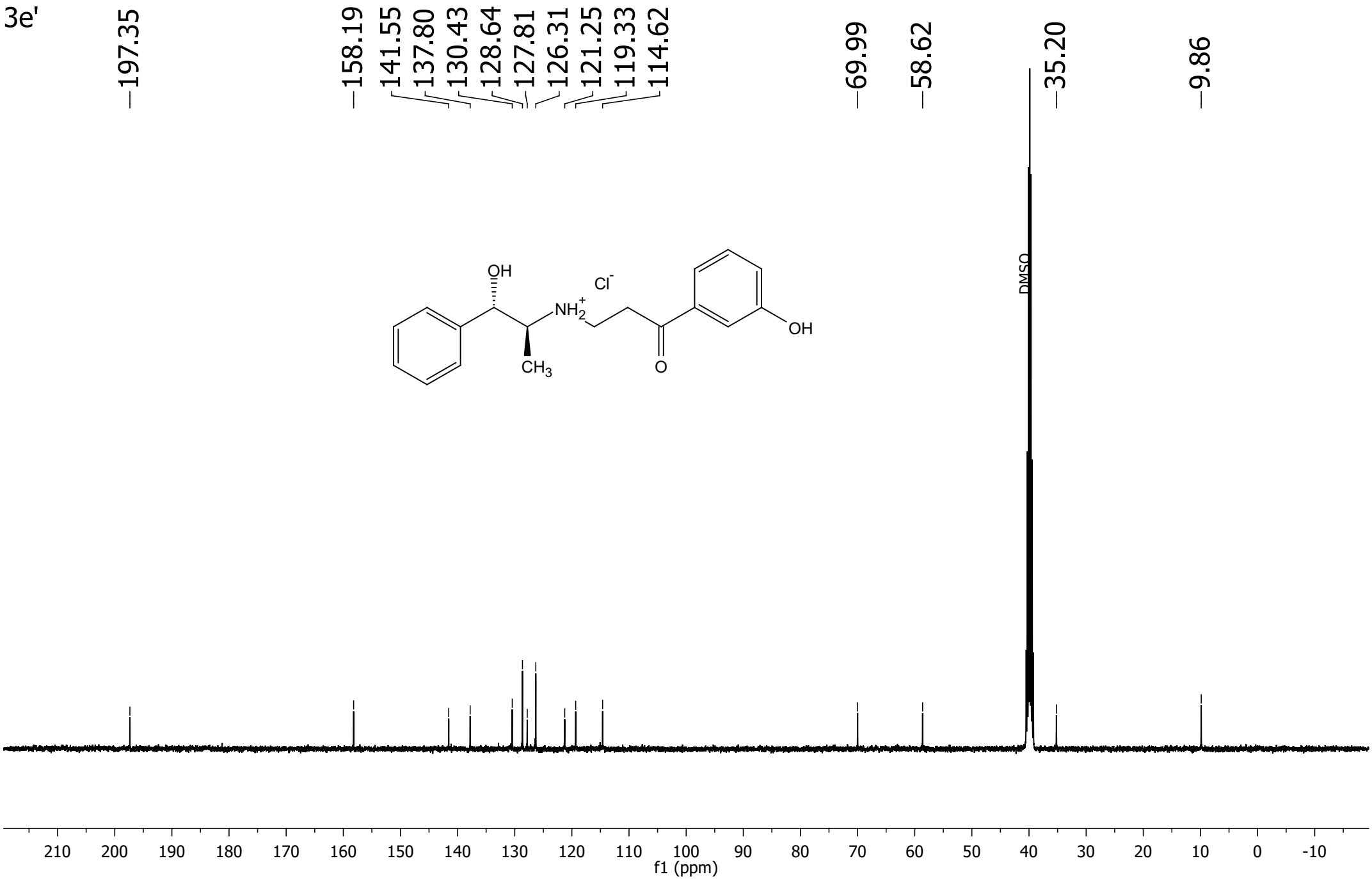



**Figure 47**: $^{13}$C-NMR Spectrum of Compound 3e'

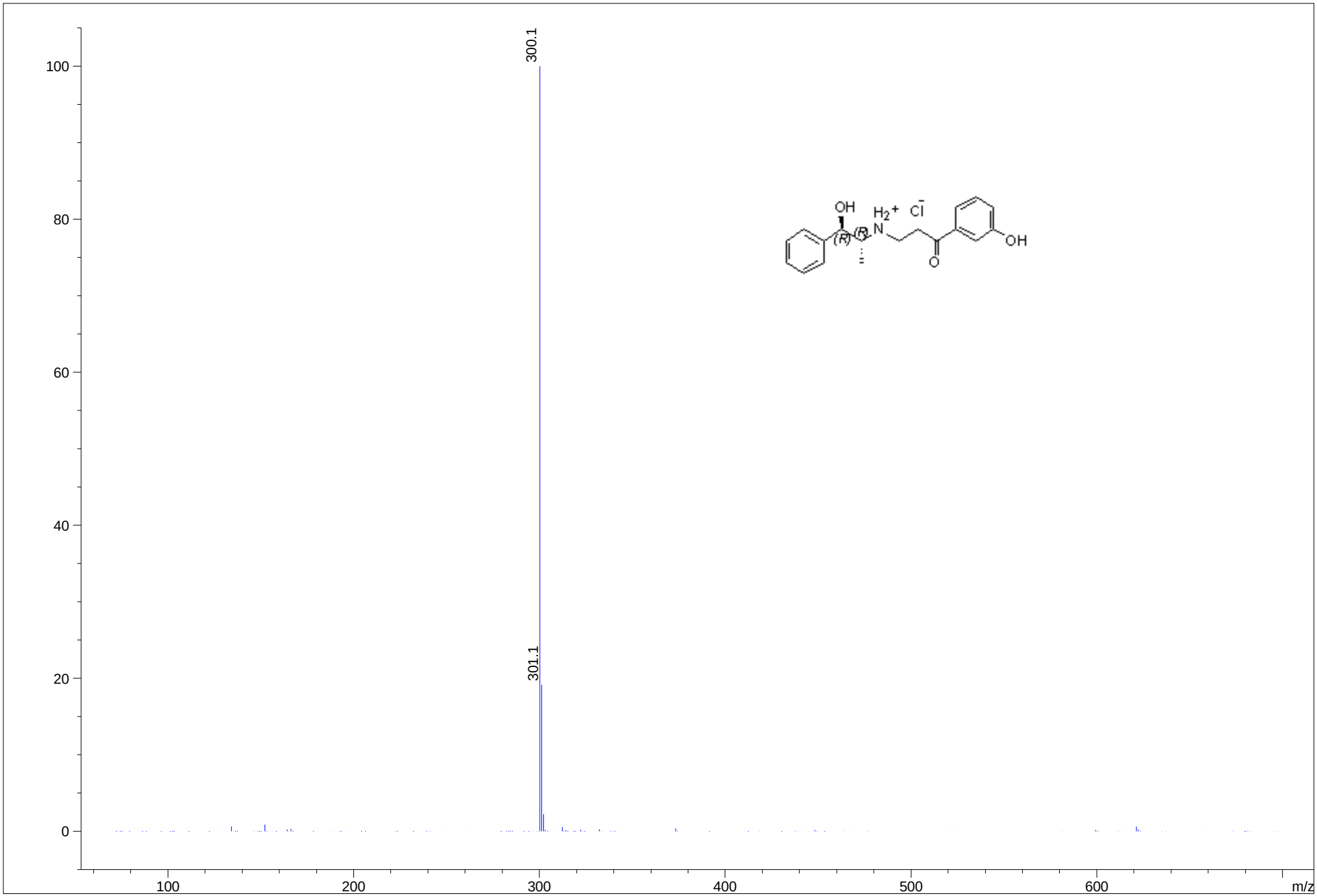



**Figure 48: Mass** Spectrum of Compound 3e'

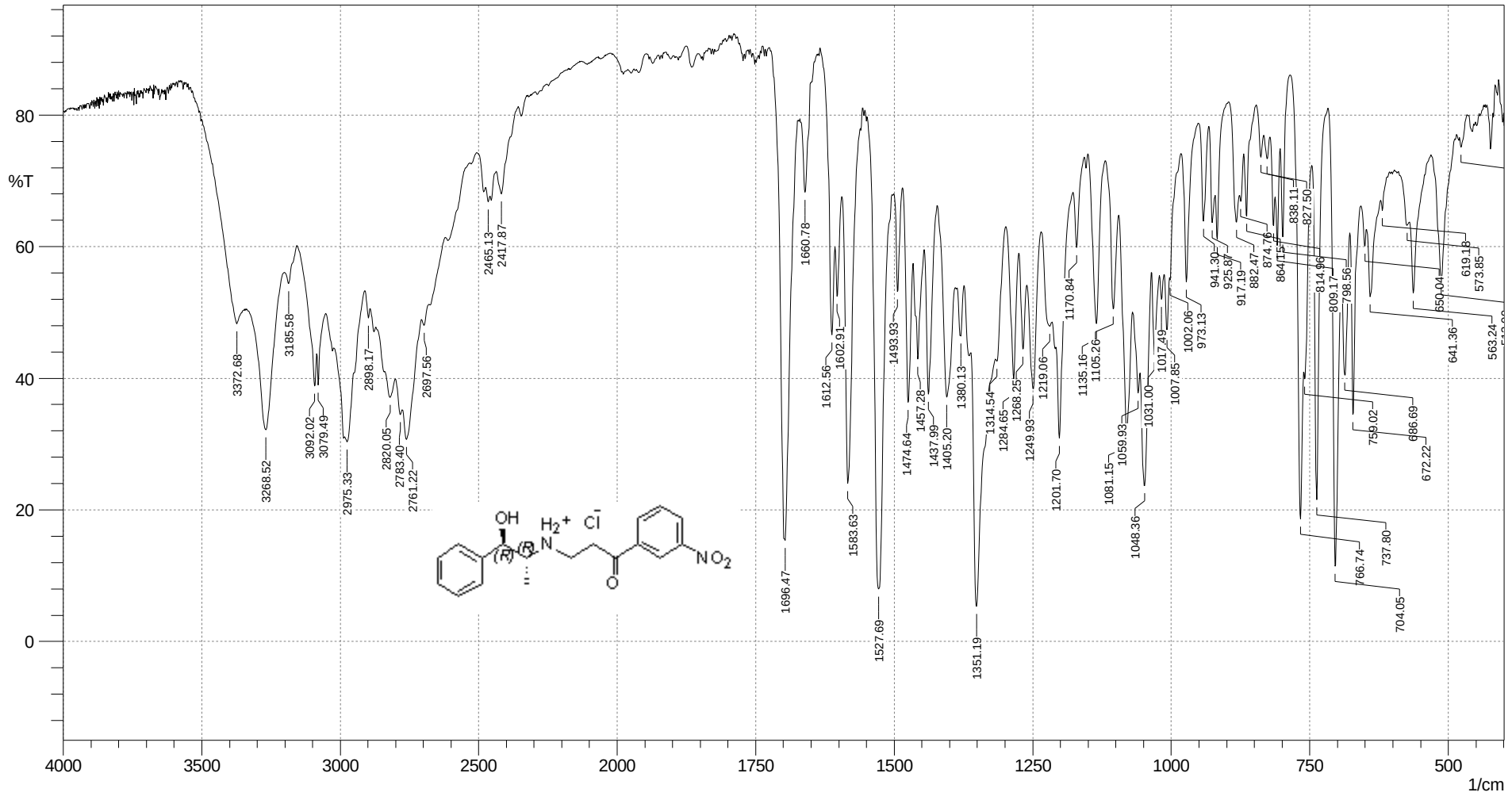



**Figure 49:** IR Spectrum of Compound 3f'

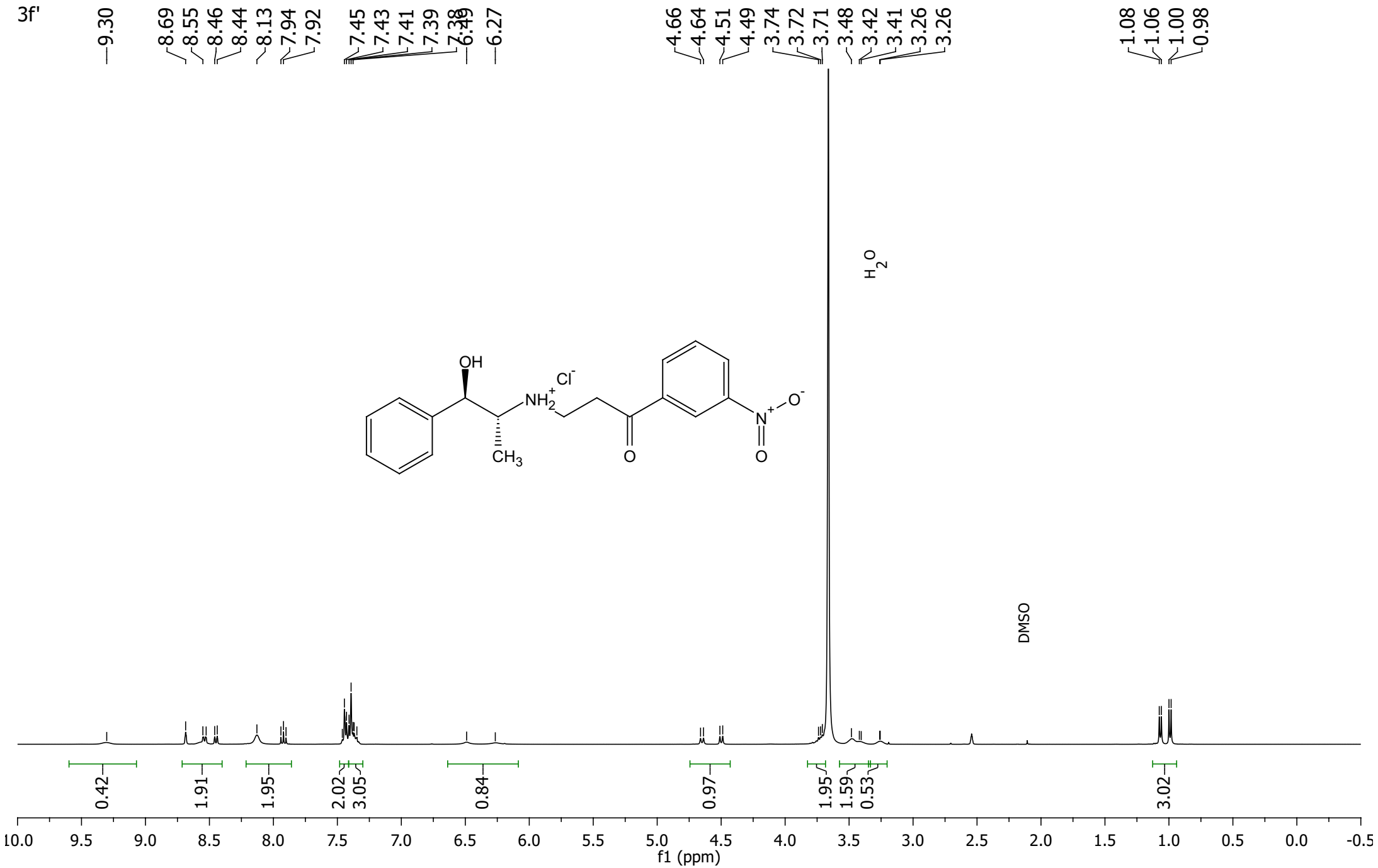



**Figure 50**: $^{1}$H-NMR Spectrum of Compound 3f'

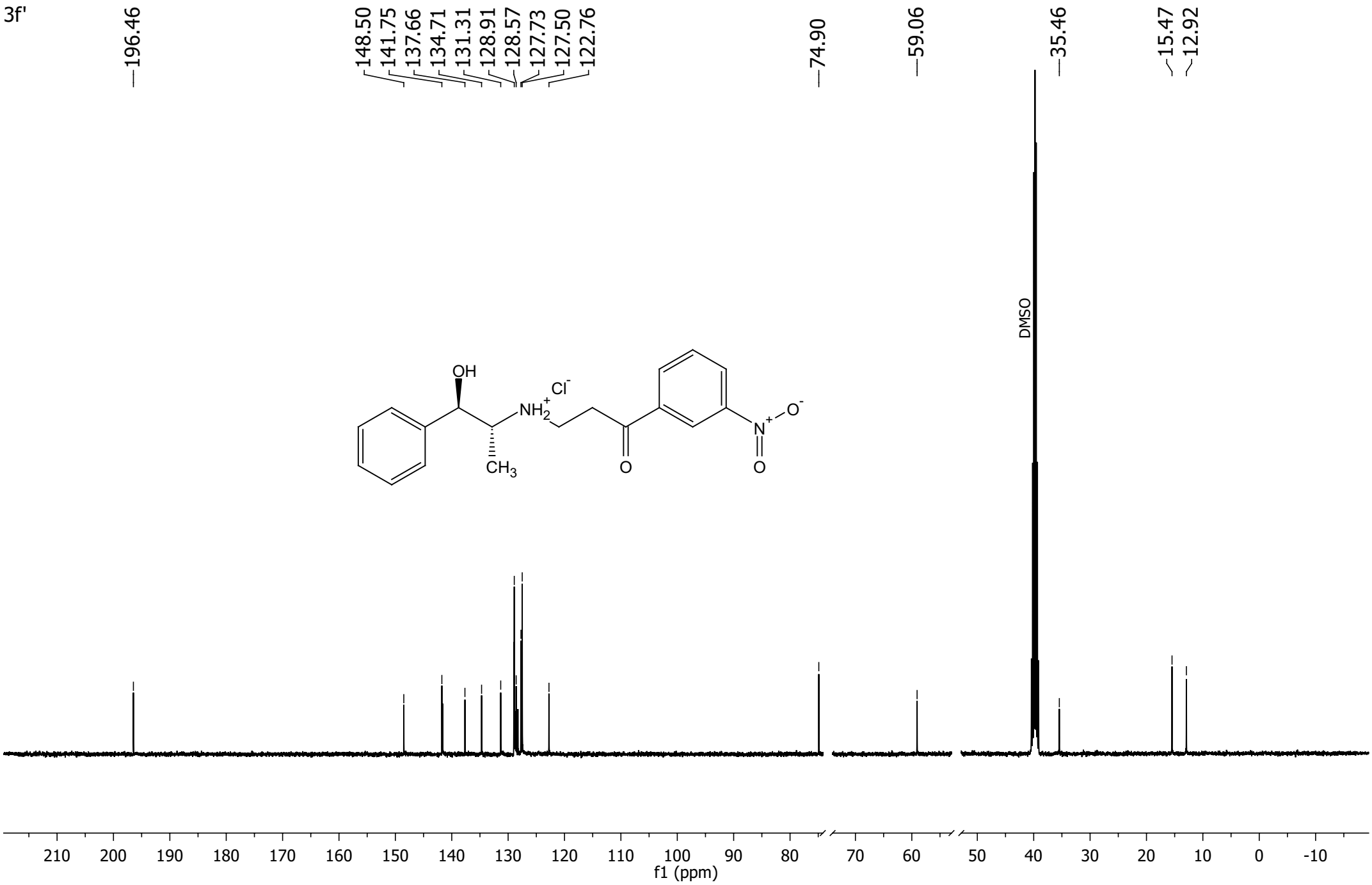



**Figure 51**: $^{13}$C-NMR Spectrum of Compound 3f'

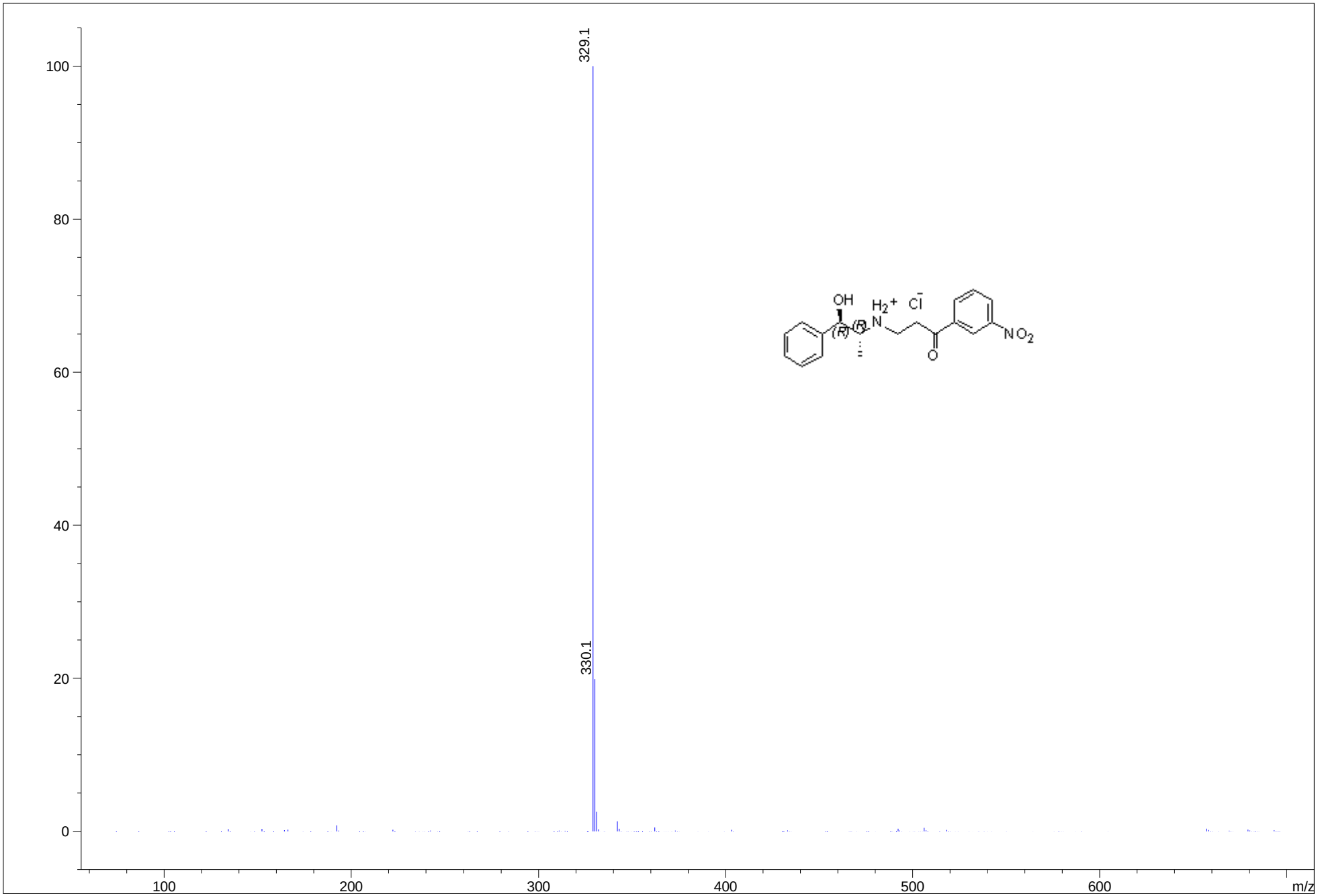



**Figure 52:** Mass Spectrum of Compound 3f'

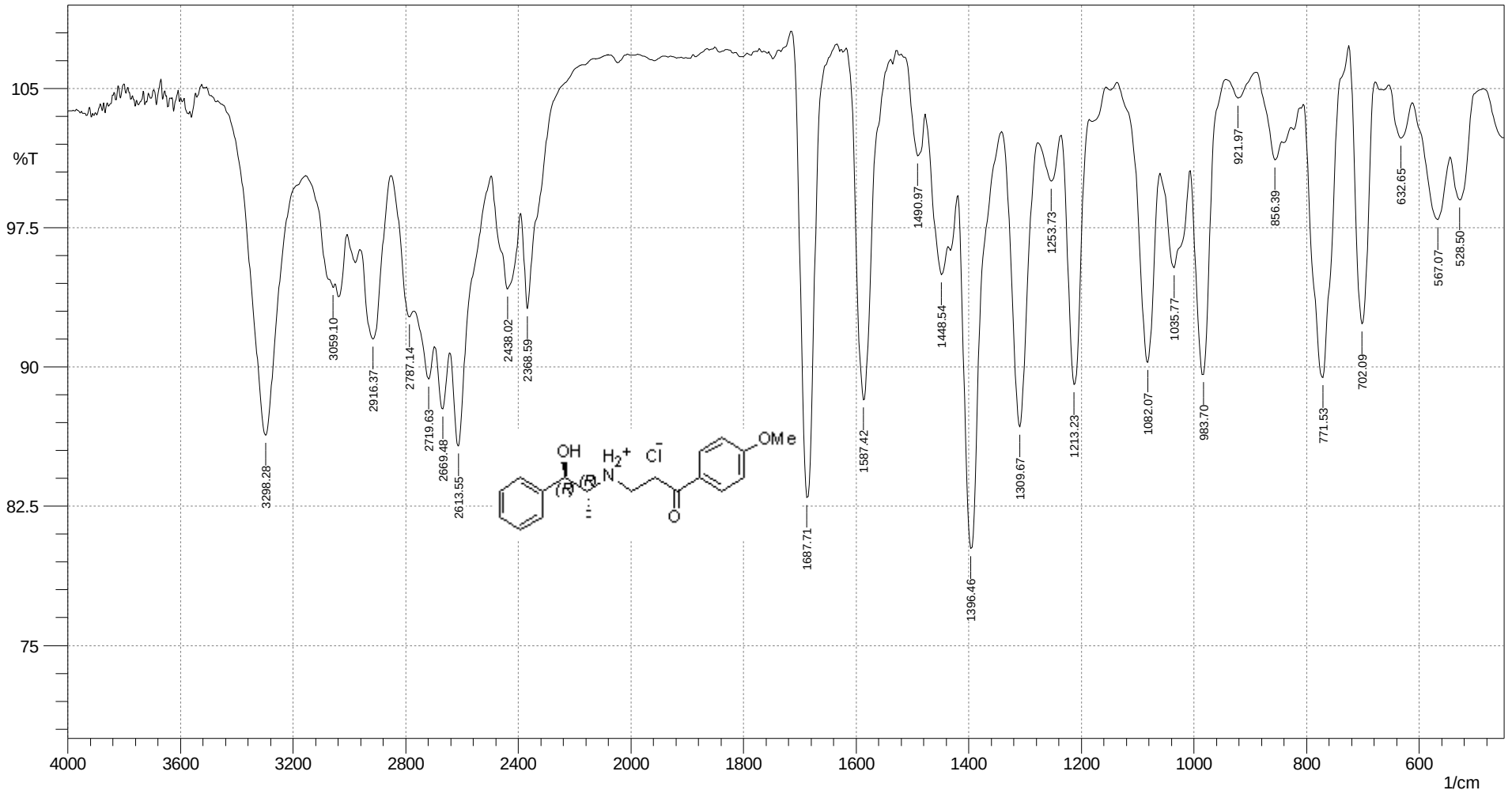



**Figure 53:** IR Spectrum of Compound 3g'

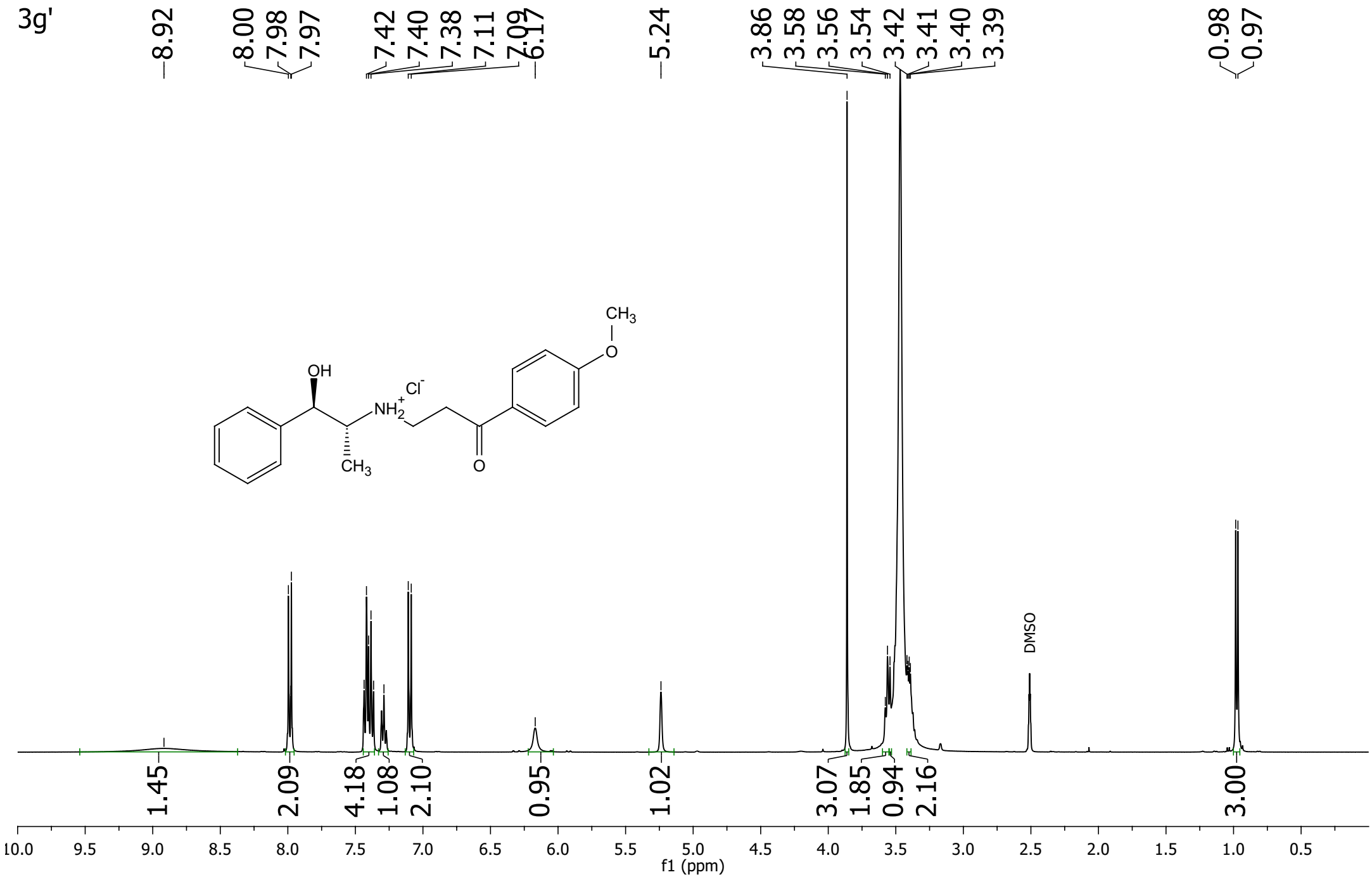



**Figure 54**: $^{1}$H-NMR Spectrum of Compound 3g'

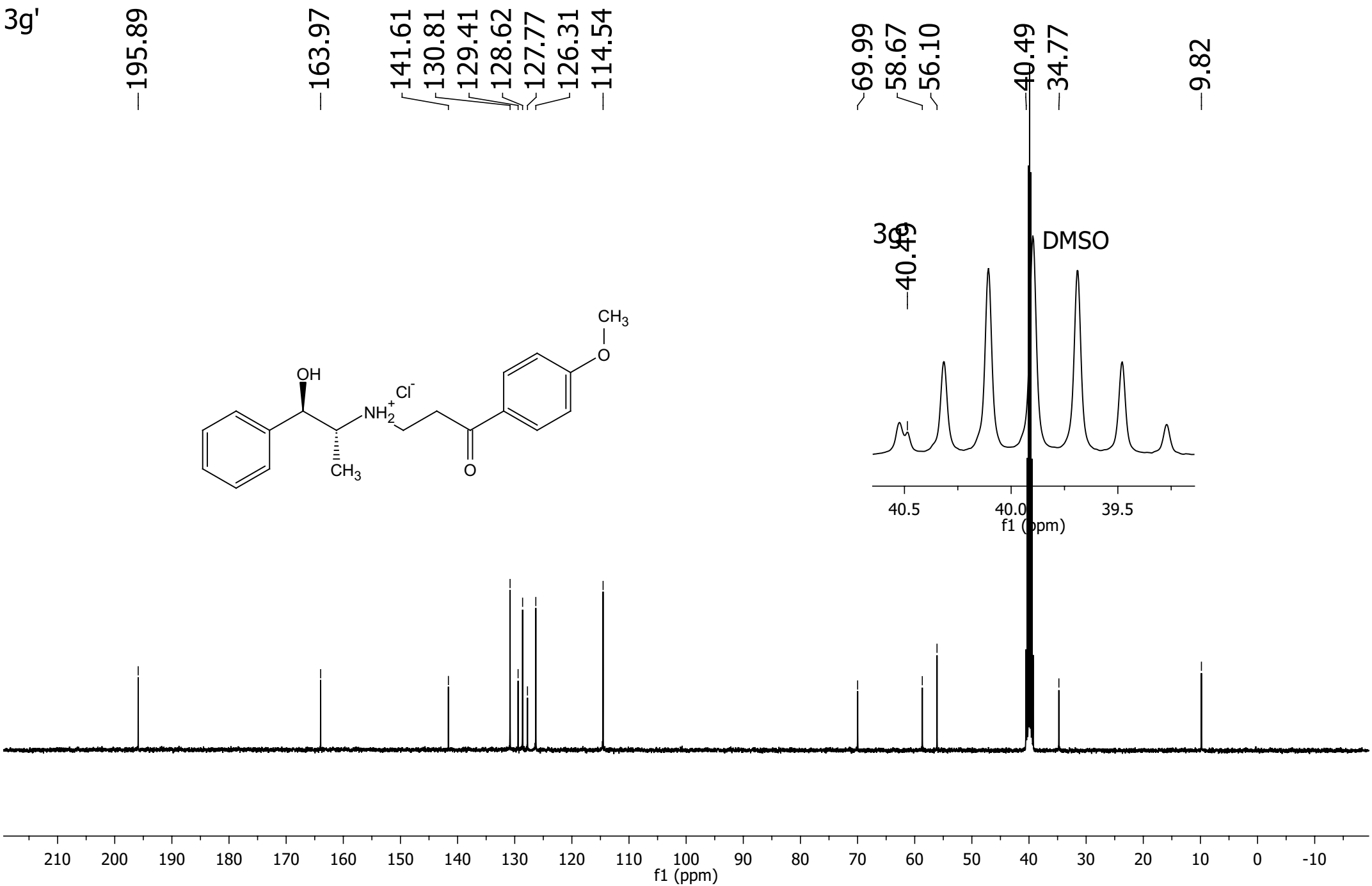



**Figure 55**: $^{13}$C-NMR Spectrum of Compound 3g'

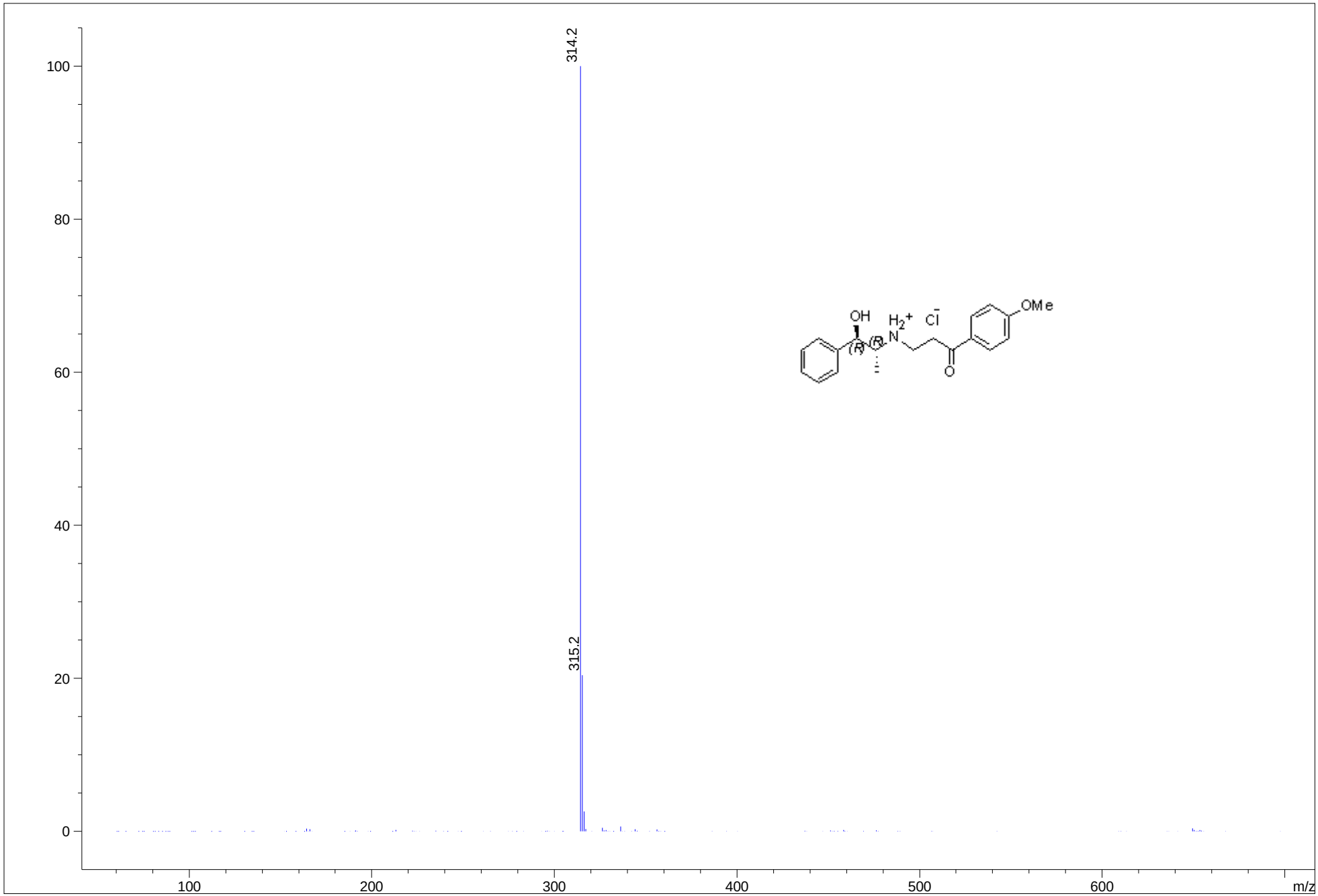
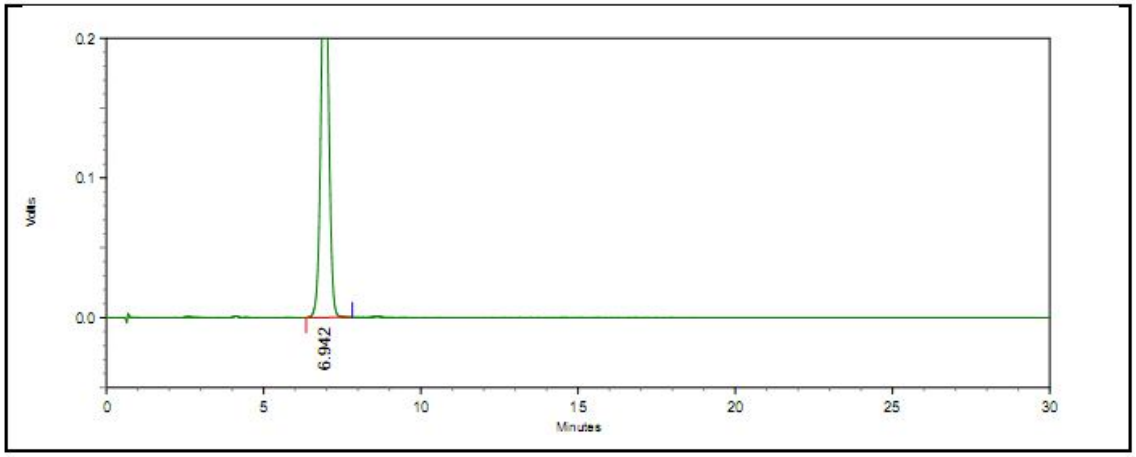



**Figure 56:** Mass Spectrum of Compound 3g'

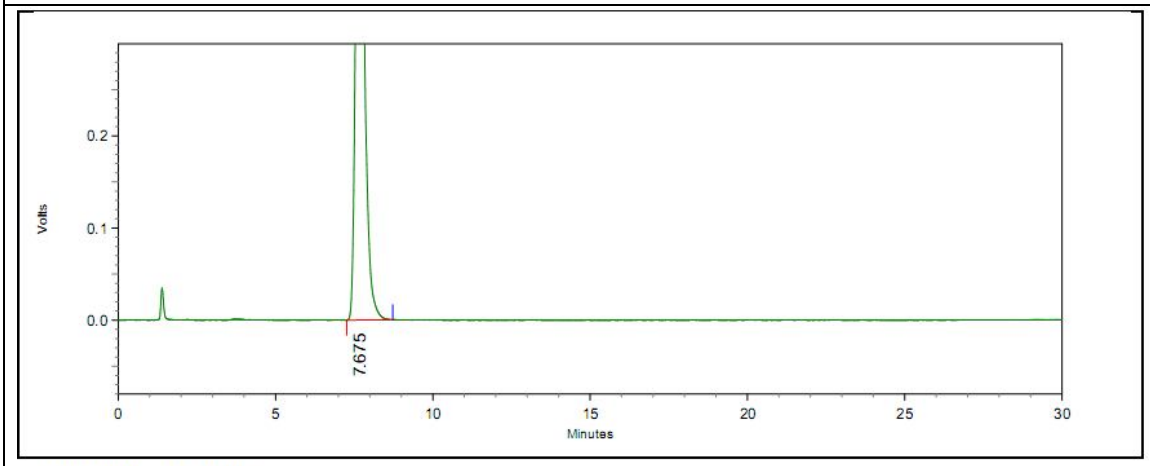
## 4.0 Chira HPLC chromatograms




Detector A - 1 (215nm)

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 6.942 | 4861294 | 100.00 |
| Totals | | 4861294 | 100.00 |

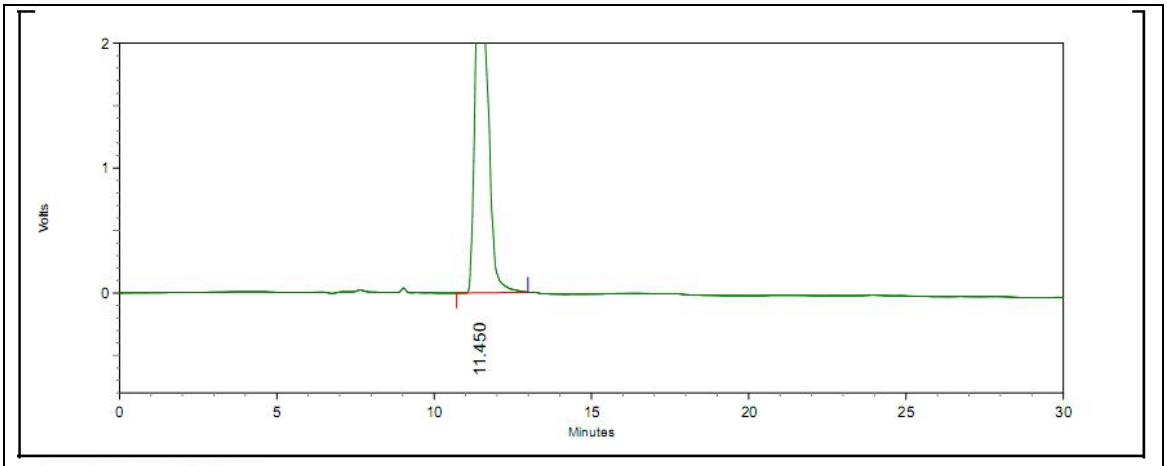
**Figure 57. Chiral HPLC chromatogram of 3a**




Detector A - 1 (210nm)

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 7.675 | 13686801 | 100.00 |
| Totals | | 13686801 | 100.00 |

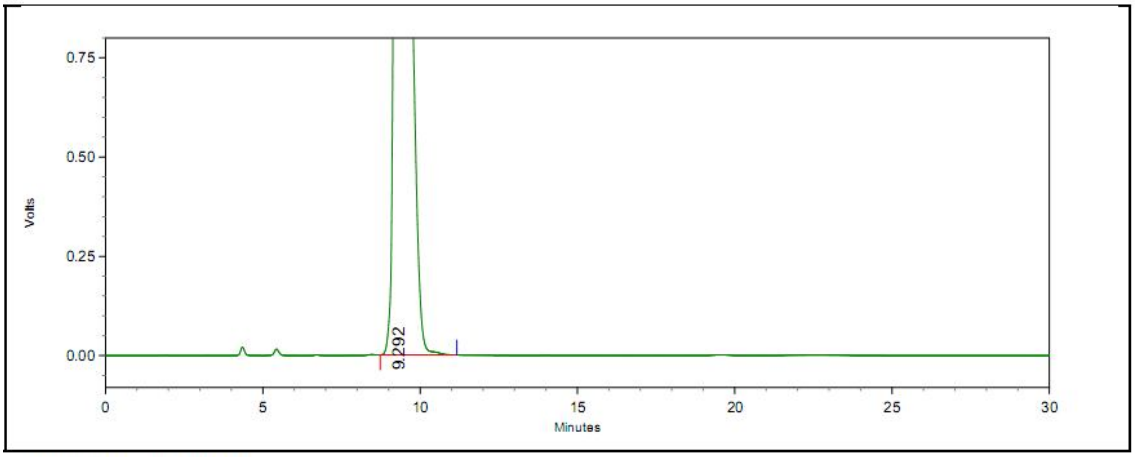
**Figure 58. Chiral HPLC chromatogram of 3a′**




**Detector A - 1 (210nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 11.450 | 74310521 | 100.00 |
| Totals | | 74310521 | 100.00 |

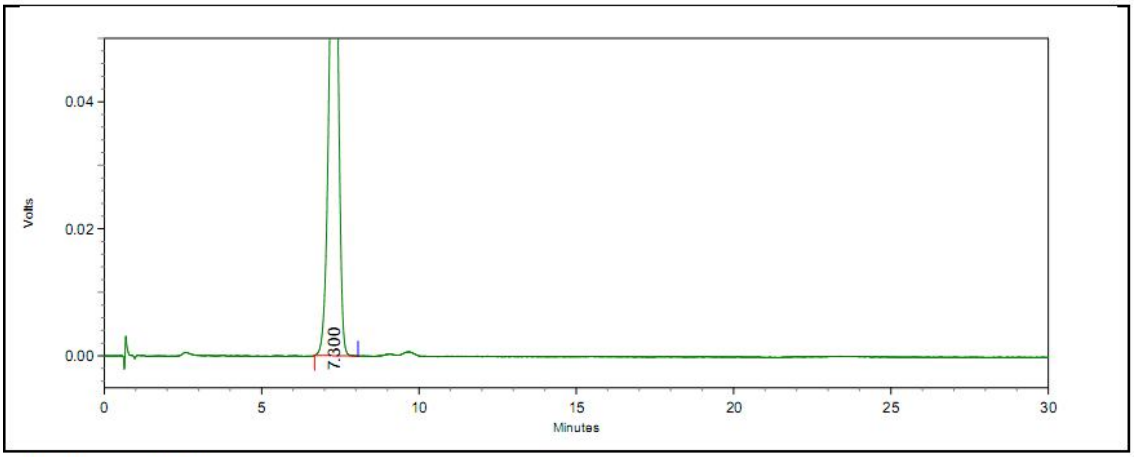
**Figure 59. Chiral HPLC chromatogram of 3b**




**Detector A - 1 (278nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 9.292 | 69893043 | 100.00 |
| Totals | | 69893043 | 100.00 |

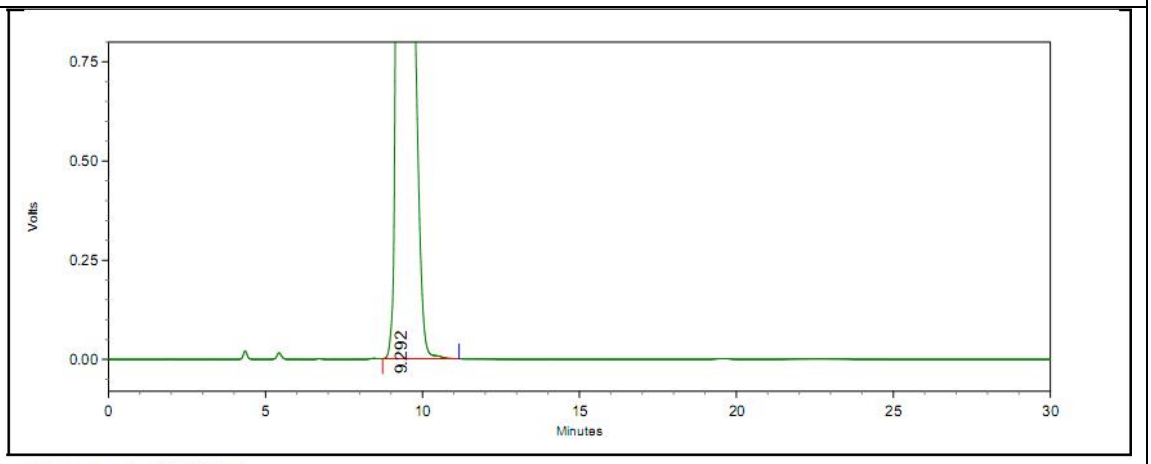
**Figure 60. Chiral HPLC chromatogram of 3b′**




**Detector A - 1 (210nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 7.300 | 1720488 | 100.00 |
| Totals | | 1720488 | 100.00 |

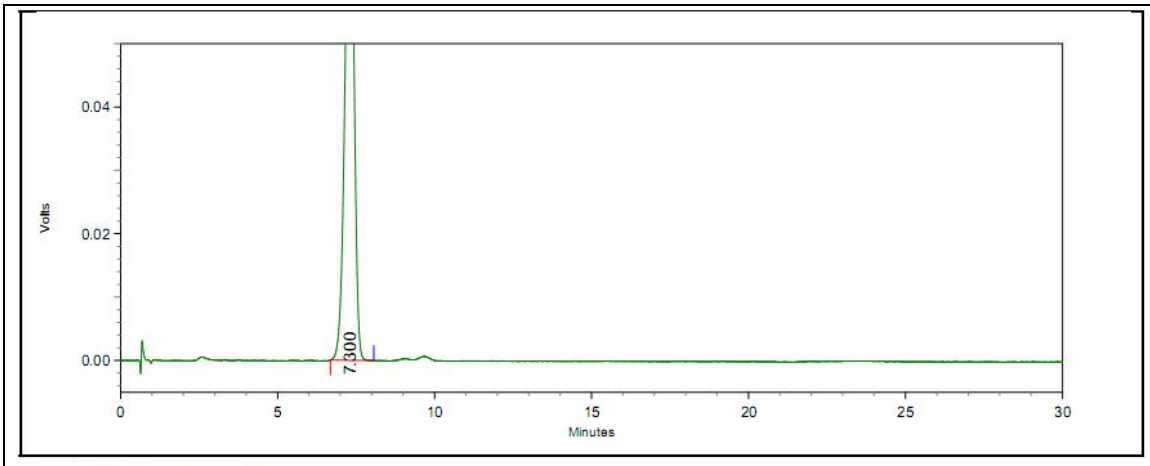
**Figure 61. Chiral HPLC chromatogram of 3c**




**Detector A - 1 (278nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 9.292 | 69893043 | 100.00 |
| Totals | | 69893043 | 100.00 |

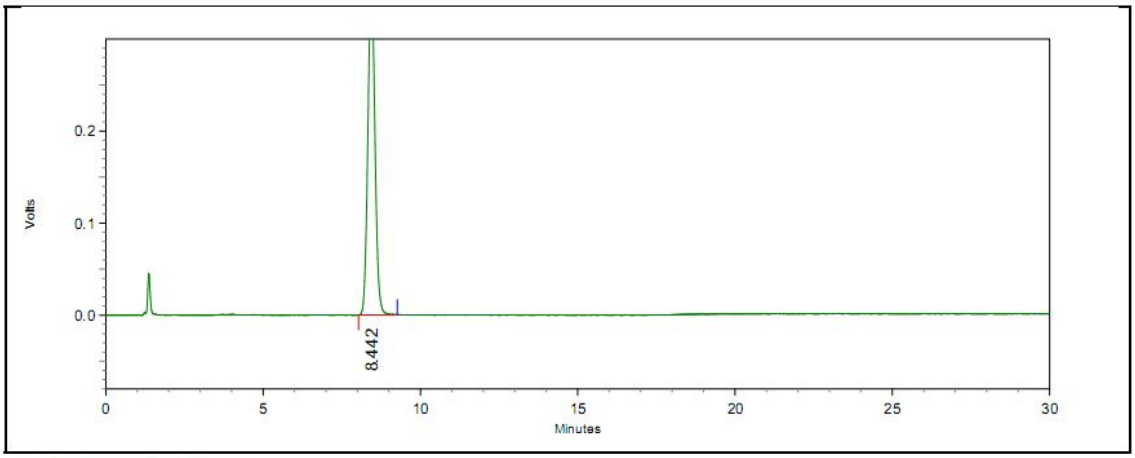
**Figure 62. Chiral HPLC chromatogram of 3′c**




Detector A - 1 (210nm)

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 7.300 | 1720488 | 100.00 |
| Totals | | 1720488 | 100.00 |

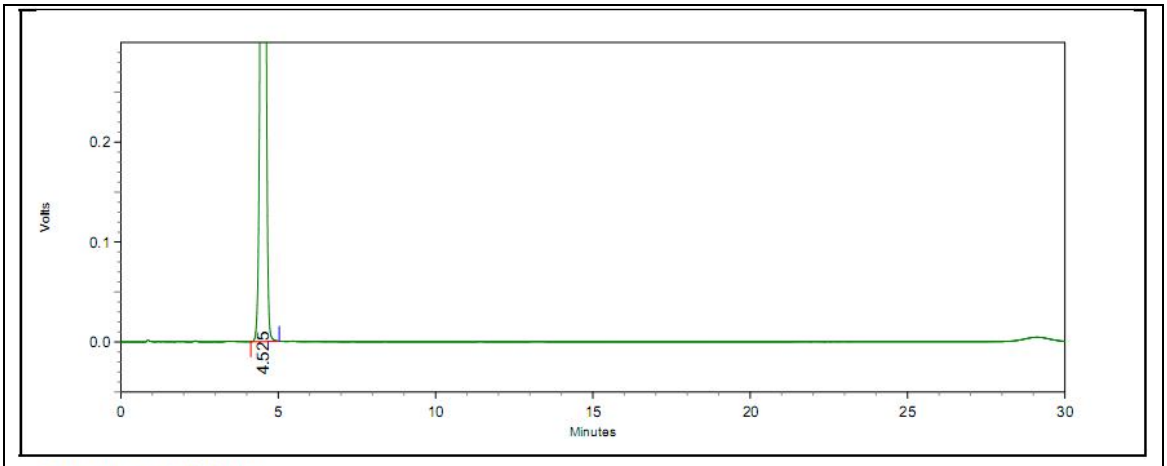
**Figure 63. Chiral HPLC chromatogram of 3d**




Detector A - 1 (210nm)

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 8.442 | 5663350 | 100.00 |
| Totals | | 5663350 | 100.00 |

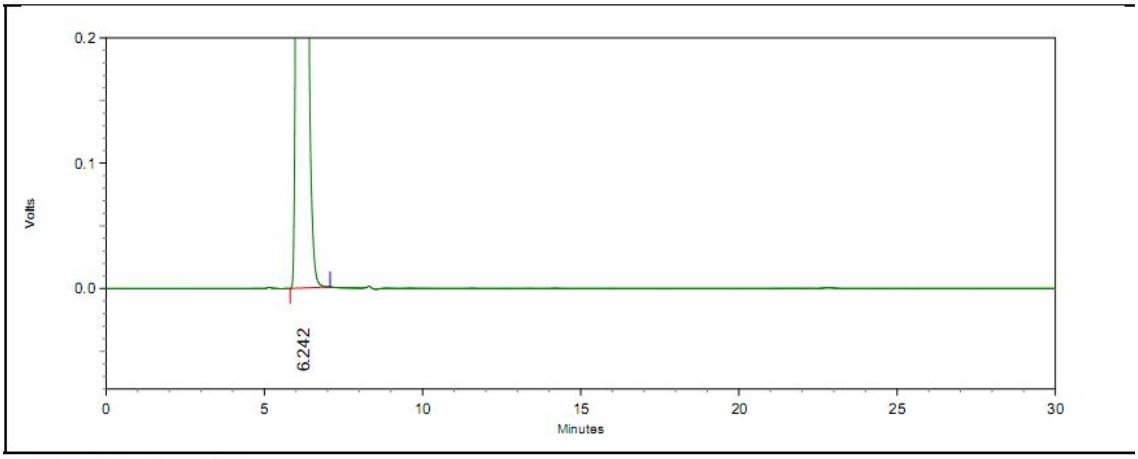
**Figure 64. Chiral HPLC chromatogram of 3d′**




**Detector A - 1 (210nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 4.525 | 11013553 | 100.00 |
| Totals | | 11013553 | 100.00 |

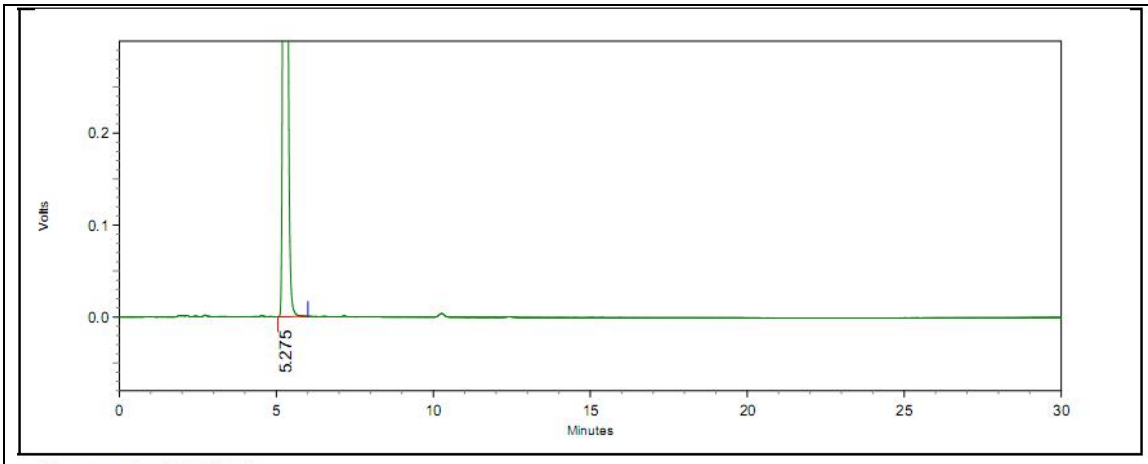
**Figure 65. Chiral HPLC chromatogram of 3e**




**Detector A - 1 (210nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 6.242 | 27998880 | 100.00 |
| Totals | | 27998880 | 100.00 |

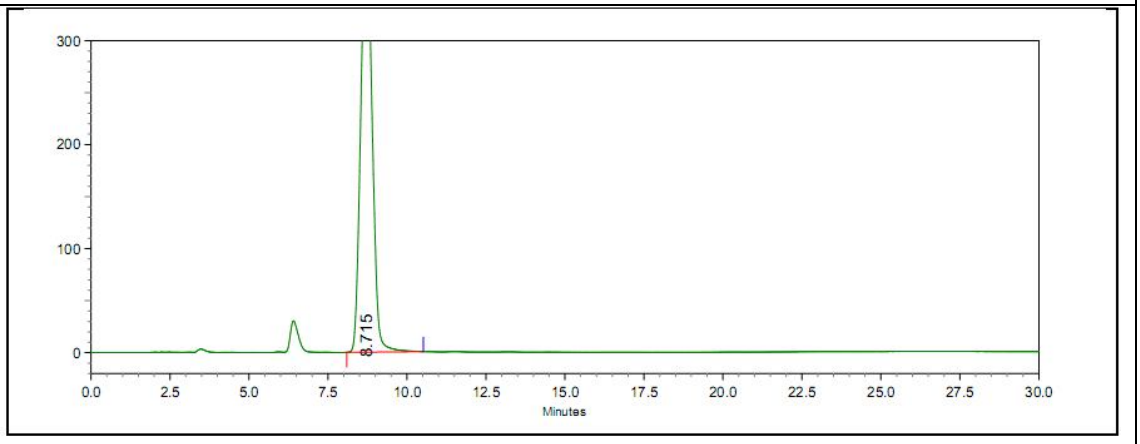
**Figure 66. Chiral HPLC chromatogram of 3e′**




**Detector A - 1 (210nm)**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 5.275 | 13218734 | 100.00 |
| Totals | | 13218734 | 100.00 |

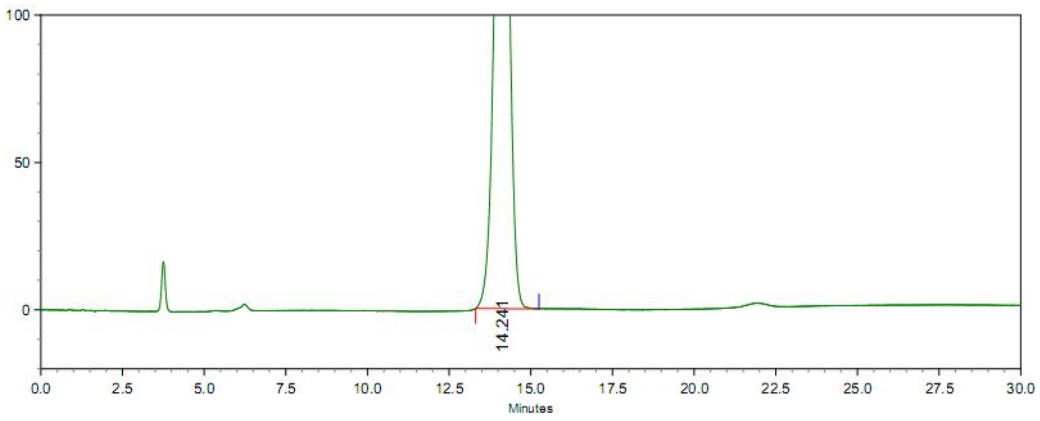
**Figure 67. Chiral HPLC chromatogram of 3f**




**1: 220 nm, 8 nm**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 8.715 | 10769595 | 100.00 |
| Totals | | 10769595 | 100.00 |

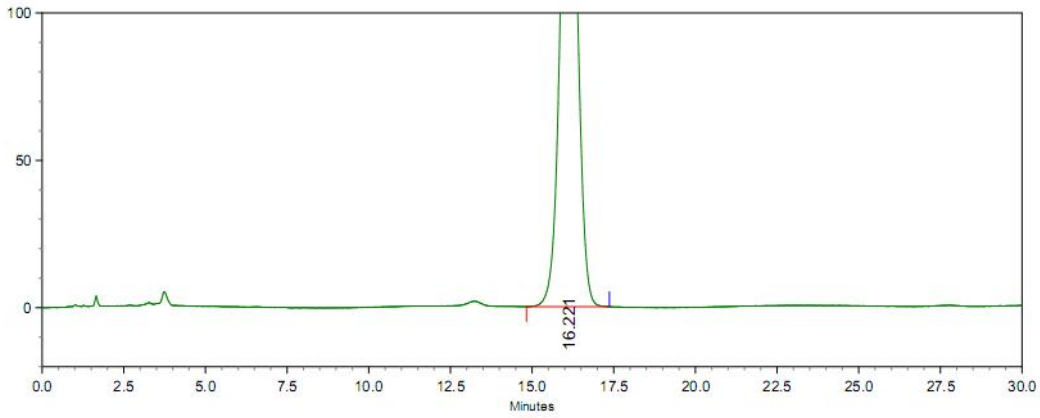
**Figure 68. Chiral HPLC chromatogram of 3f′**




**1: 210 nm, 8 nm**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 14.241 | 7940567 | 100.00 |
| Totals | | 7940567 | 100.00 |

**Figure 69. Chiral HPLC chromatogram of 3g**




**1: 210 nm, 8 nm**

| Pk # | Retention Time | Area | Area Percent |
|---|---|---|---|
| 1 | 16.221 | 7019055 | 100.00 |
| Totals | | 7019055 | 100.00 |

**Figure 70. Chiral HPLC chromatogram of 3g′**

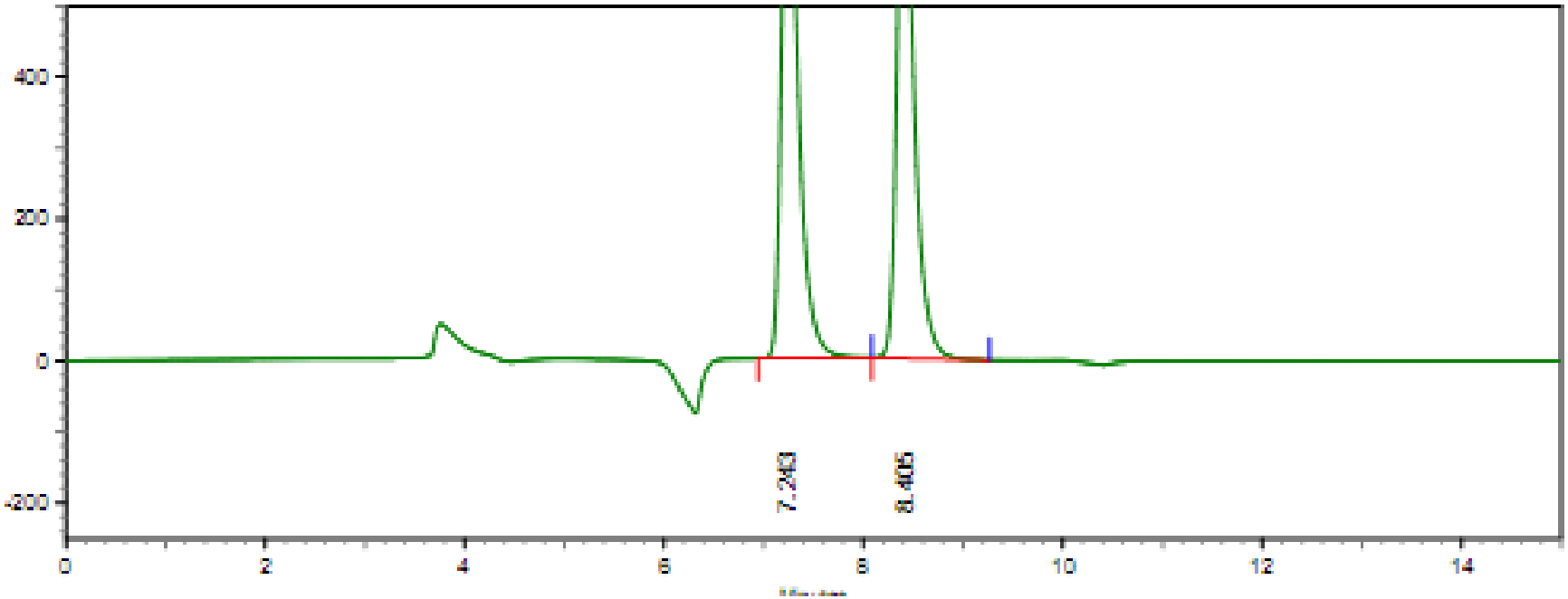



**Figure 71. Chiral HPLC chromatogram of racemic Mannich derivatives (3-(2,4-Dichlorophenyl)-N-(1-hydroxy-1-phenylpropan-2-yl)-3-oxopropan-1-amine HCl).**

## 5.0. Cytotoxic properties of synthesized compounds

**Table 2.** Cytotoxic properties of synthesised compounds against A549 cells at 24 h incubation. Data are mean ± SD of three independent experiments with each experiment conducted in triplicate.

| **Conc uM** | **3a** | | **3b** | | **3c** | | **3d** | | **3e** | | **3f** | | **3g** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D |
| 10 | 1.1 | 1.762±0.00490 | 5.2 | 1.688±0.00937 | 1.5 | 1.755±0.00388 | 4 | 1.71±0.00308 | 2.8 | 1.731±0.00881 | 7.1 | 1.654±0.00359 | 3 | 1.728±0.00498 |
| 25 | 4.1 | 1.708±0.00613 | 12.6 | 1.556±0.00890 | 5.7 | 1.679±0.00563 | 8.1 | 1.637±0.00411 | 9 | 1.621±0.00711 | 13.8 | 1.536±0.00415 | 4.6 | 1.699±0.00682 |
| 50 | 6.8 | 1.66±0.00507 | 19.1 | 1.44±0.00636 | 15.6 | 1.503±0.00717 | 13.1 | 1.547±0.00667 | 13.5 | 1.541±0.00668 | 23.3 | 1.366±0.00329 | 6.6 | 1.663±0.00635 |
| 75 | 18.7 | 1.448±0.00315 | 25.8 | 1.322±0.00630 | 16.1 | 1.494±0.00809 | 19.5 | 1.433±0.00862 | 20.2 | 1.445±0.00564 | 38.6 | 1.094±0.00787 | 15.7 | 1.502±0.00508 |
| 100 | 51 | 0.869±0.00794 | 37.1 | 1.121±0.00561 | 22.6 | 1.378±0.00733 | 65.2 | 0.619±0.01071 | 25.9 | 1.319±0.00437 | 46.3 | 0.957±0.00491 | 27.8 | 1.285±0.00662 |
| 150 | 89.5 | 0.188±0.00586 | 42.2 | 1.030±0.00240 | 31.6 | 1.219±0.00833 | 93.5 | 0.115±0.00491 | 57.6 | 0.755±0.00578 | 69.8 | 0.538±0.00841 | 38.7 | 1.091±0.00709 |

**Table 3.** Cytotoxic properties of synthesised compounds against A549 cells at 24 h incubation. Data are mean ± SD of three independent experiments with each experiment conducted in triplicate.

| **Con uM** | **3a′** | | **3b′** | | **3c′** | | **3d′** | | **3e′** | | **3f′** | | **3g′** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D |
| 10 | 6.9 | 1.658±0.00296 | 6.8 | 1.659±0.00665 | 1.5 | 1.755±0.00754 | 4.6 | 1.699±0.00655 | 4.3 | 1.705±0.00641 | 2.1 | 1.743±0.00 | 4.7 | 1.697±0.00738 |
| 25 | 18.6 | 1.450±0.00369 | 14.6 | 1.521±0.00939 | 3.9 | 1.712±0.00564 | 13.6 | 1.539±0.00856 | 6.5 | 1.665±0.00689 | 13.5 | 1.541±0.00534 | 8.3 | 1.633±0.00513 |
| 50 | 24.1 | 1.351±0.00409 | 25.2 | 1.332±0.00985 | 5.6 | 1.681±0.00664 | 27.6 | 1.289±0.00904 | 18.2 | 1.456±0.00363 | 25.8 | 1.322±0.00281 | 11.8 | 1.577±0.00616 |
| 75 | 45.3 | 0.975±0.00491 | 33.2 | 1.189±0.00929 | 7.6 | 1.645±0.00767 | 57.9 | 0.749±0.00908 | 25.6 | 1.347±0.00838 | 32.2 | 1.207±0.00967 | 18.7 | 1.448±0.00387 |
| 100 | 57.3 | 0.761±0.00888 | 46.3 | 0.956±0.00581 | 10.3 | 1.597±0.00463 | 64.2 | 0.638±0.00896 | 80.7 | 0.343±0.00768 | 65.3 | 0.618±0.00996 | 80 | 0.356±0.00608 |
| 150 | 80.5 | 0.347±0.00462 | 68.1 | 0.567±0.00244 | 12.7 | 1.554±0.00390 | 71.1 | 0.514±0.00437 | 93.1 | 0.122±0.00722 | 74.5 | 0.454±0.00520 | 93.7 | 0.112±0.00153 |

**Table 4**. Cytotoxic properties of synthesised compounds against HepG-2 cells at 24 h incubation. Data are mean ± SD of three independent experiments with each experiment conducted in triplicate.

| Conc. uM | 3a | | 3b | | 3c | | 3d | | 3e | | 3f | | 3g | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D |
| 10 | 19.9 | 1.857±0.00551 | 15.4 | 1.962±0.00929 | 17.7 | 1.907±0.00208 | 3.3 | 2.241±0.00784 | 2.4 | 2.263±0.00473 | 4.1 | 2.223±0.00504 | 1.8 | 2.277±0.00693 |
| 25 | 56.5 | 1.009±0.00289 | 19.5 | 1.865±0.00503 | 18.6 | 1.886±0.00361 | 8.8 | 2.113±0.00644 | 16.9 | 1.926±0.00896 | 18.5 | 1.889±0.00145 | 7.4 | 2.146±0.00629 |
| 50 | 75.9 | 0.557±0.00410 | 33.8 | 1.534±0.00250 | 20.4 | 1.845±0.00536 | 19.7 | 1.861±0.00872 | 22.9 | 1.787±0.00929 | 27.2 | 1.688±0.001212 | 11.4 | 2.053±0.00794 |
| 75 | 79.3 | 0.442±0.00726 | 73 | 0.626±0.00858 | 22 | 1.808±0.00252 | 33 | 1.552±0.00994 | 28.9 | 1.653±0.00503 | 41.8 | 1.348±0.00546 | 14.3 | 1.987±0.00520 |
| 100 | 83 | 0.393±0.00716 | 82.5 | 0.405±0.00895 | 24.2 | 1.758±0.00784 | 46.6 | 1.237±0.00462 | 81.7 | 0.425±0.00250 | 76.1 | 0.555±0.00649 | 19.9 | 1.856±0.00649 |
| 150 | 87.5 | 0.289±0.00681 | 83.4 | 0.384±0.00441 | 28.6 | 1.654±0.00321 | 70.9 | 0.674±0.00833 | 83.1 | 0.392±0.00858 | 81.9 | 0.419±0.00731 | 28.8 | 1.651±0.00876 |

**Table 5.** Cytotoxic properties of synthesised compounds against HepG-2 cells at 24 h incubation. Data are mean ± SD of three independent experiments with each experiment conducted in triplicate.

| Conc. uM | 3a′ | | 3b′ | | 3c′ | | 3d′ | | 3e′ | | 3f′ | | 3g′ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D | % | Mean±S.D |
| 10 | 17.8 | 1.906±0.00404 | 7.9 | 2.133±0.00895 | 1 | 2.304±0.00379 | 10.6 | 2.072±0.00749 | 3.6 | 2.234±0.00433 | 3.1 | 2.247±0.00463 | 3.4 | 2.239±0.00242 |
| 25 | 22.1 | 1.806±0.00406 | 14.2 | 1.990±0.00441 | 2.9 | 2.249±0.00721 | 27.4 | 1.682±0.00966 | 15.7 | 1.954±0.00361 | 23.7 | 1.769±0.00203 | 8.6 | 2.119±0.00745 |
| 50 | 28.3 | 1.661±0.00376 | 21.3 | 1.824±0.00749 | 7 | 2.156±0.00404 | 48.7 | 1.188±0.00636 | 41.2 | 1.363±0.00555 | 32.7 | 1.561±0.00348 | 15.2 | 1.966±0.00780 |
| 75 | 33.3 | 1.545±0.00330 | 32 | 1.577±0.00966 | 9 | 2.109±0.00384 | 59.6 | 0.937±0.00353 | 46.8 | 1.234±0.00481 | 58.7 | 0.958±0.00551 | 43 | 1.321±0.00249 |
| 100 | 63.9 | 0.837±0.00379 | 63.8 | 0.838±0.00636 | 10.6 | 2.072±0.00504 | 80.3 | 0.457±0.00529 | 70.1 | 0.692±0.00384 | 79.2 | 0.483±0.00593 | 86 | 0.323±0.00332 |
| 150 | 76.3 | 0.575±0.00470 | 73 | 0.627±0.00353 | 22.6 | 1.793±0.00410 | 90.3 | 0.223±0.00601 | 74.5 | 0.591±0.00636 | 83 | 0.394±0.00624 | 89.8 | 0.236±0.00769 |