## *Supplementary Materials*

# Synthesis of Carvone derived 1,2,3-Triazoles. Study of their Antioxidant Properties and Interaction with Bovine Serum Albumin

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## 1. *Fluorescence Spectroscopy Studies*

BSA purchased from the Sigma Chemical. Co. USA was used without further purification. The concentration of BSA (0.4 mg/ml) was determined spectrophotometrically using a molar extinction coefficient 39080M$^{-1}$ cm$^{-1}$ at 280 nm. The concentration of **4e** was varied in the range of 4.00x10$^{-5}$ - 2.00x10$^{-4}$M. The protein solutions were prepared in saline solution. The fluorescence spectra were recorded on a Cary Eclipse (Varian) spectrofluorometer at 298 and 308K, in the range $\lambda$ = 300-500 nm at the excitation wavelength $\lambda$ = 280 nm. The temperature of the samples was maintained constant by circulating the thermostated water connected to the spectrophotometer of the LAUDA Alpha 100 thermostat (Germany). $l$ = 1 cm cuvettes were used. The graphs were constructed and analyzed using the ORIGIN 8.0 software.

### *1.1. Determination of the thermodynamic binding parameters of BSA -* ***4e***

The character of intermolecular interactions between the biomolecule and the ligand was determined by the relationship of thermodynamic parameters (Δ*H*, Δ*S* and Δ*G*). Δ*G* shows the possibility of spontaneous rate of the reaction, and Δ*H* and Δ*S* are the main criteria for confirming the character of interactions. The thermodynamic parameters of binding with BSA are determined by the following equations:

$$\Delta G = -RT \ln K_b \quad (1),$$

$$\ln \frac{K_{b_1}}{K_{b_2}} = \left(\frac{1}{T_1} - \frac{1}{T_2}\right)\frac{\Delta H}{R} \quad (2),$$

$$\Delta G = \Delta H - T\Delta S \quad (3),$$

where $K_{b_1}$ and $K_{b_2}$ are the binding constants at temperatures of 298 and 303K, and *R* is the gas constant. The obtained results are presented in Table 4.

The binding constant and the number of binding sites (*n*) with BSA were determined based on the data of quenching of BSA fluorescence according to the well-known [Lakowicz, J. Principles of Fluorescence Spectroscopy, 3rd ed., New York: Springer, 2006, 529.] equation:

$$\lg[(F_0 - F)/F] = \lg K_b + n\lg[Q] \qquad (4),$$

where $F_0$ and $F$ are the fluorescence intensities of BSA in the absence and presence of **4e**, respectively; $K_b$ is the binding constant, $n$ – is the number if binding sites. *[Q]* is the concentration of quencher-**4e**. $K_b$ and $n$ are determined from the dependence $\lg(F_0 - F)/F$ vs lg[Q]. Figure 7 shows a plot of log $(F_0 - F)/F$ vs log [Q] at two different temperatures.

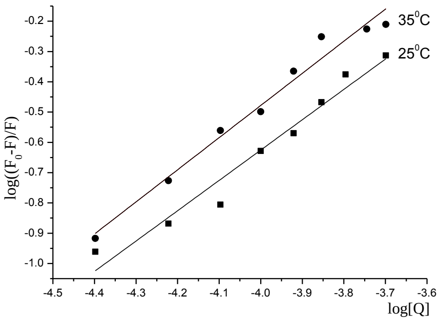


Figure 7. The plot of log $(F_0 - F)$ / $F$ versus log [Q] at two different temperatures.

### *1.2. Determination of the distance between the protein and ligand -* ***4e***

The distance between the protein and the ligand can be calculated using the theory of resonance energy transfer (Foerster theory) [Lakowicz, J. Principles of Fluorescence Spectroscopy, 3rd ed., New York: Springer, 2006, 529.]. The efficiency of energy transfer *E* is calculated using the equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \qquad (5),$$

where *E* is the energy transfer efficiency, $F_0$ and *F* are the fluorescence intensities of BSA in the absence and presence of **4e**, respectively; *r* is the distance between the donor and the acceptor, $R_0$ is the Foerster radius, when the energy transfer efficiency is 50% and can be calculated by the following equation:

$$R_0^6 = 8.79\times10^{-25}\kappa^2 n^{-4}\Phi J \qquad (6),$$

where $\kappa^2$ - is the spatial orientation factor ($\kappa^2$ = 2/3); $n$ – is the refractive index (1.336); $\Phi$ is the fluorescence quantum yield of the donor, $J$ is the overlap integral of the donor fluorescence spectrum and the acceptor absorption spectrum.

$$J = \sum F(\lambda)\ \varepsilon(\lambda)\ \lambda^4\Delta\lambda) / \sum F(\lambda)\Delta\lambda \qquad (7)$$

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength $\lambda$, $\varepsilon(\lambda)$ is the molar extinction coefficient of acceptor at wavelength $\lambda$. Using equations (5) - (7), the overlap integral, the energy transfer efficiency, the Foerster radius, and the distance between the donor and the acceptor were calculated and given in Table.

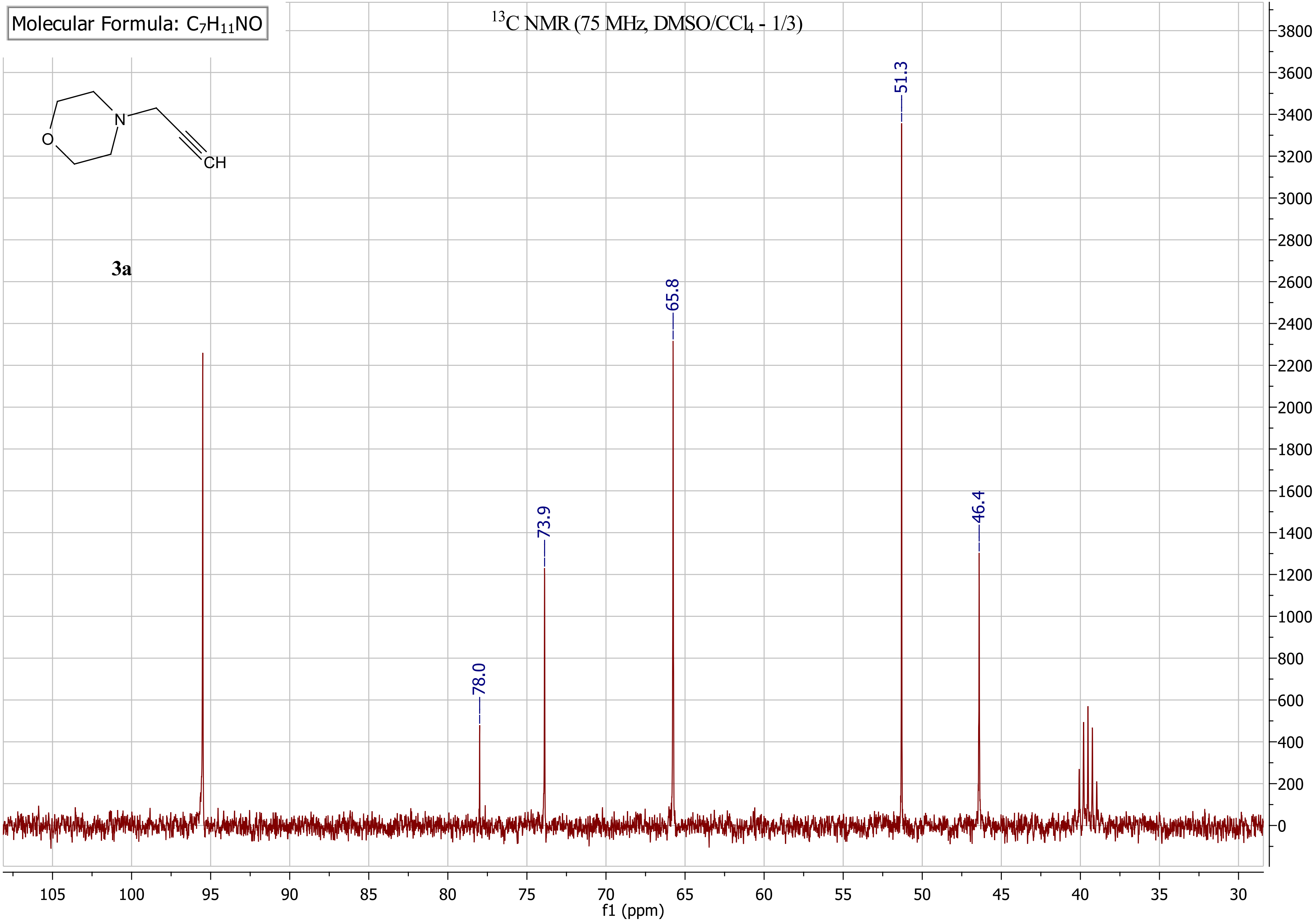
**Table.** The values of the overlap integral, the energy transfer efficiency, the Forster radius and the distance between the BSA and **4e**.

| T, K | *I*, $cm^3 \cdot l \cdot mol^{-1}$ | *E* | $R_0$, nm | *r, nm* |
|---|---|---|---|---|
| 298 | $1.79\times10^{-14}$ | 0.019 | 3.778 | 7.27 |
| 308 | $1.80\times10^{-14}$ | 0.043 | 3.782 | 7.06 |

## 2. NMR Spectrum of Compounds **3a-i**, **10-$N_3$-Car**. and **4a-i**

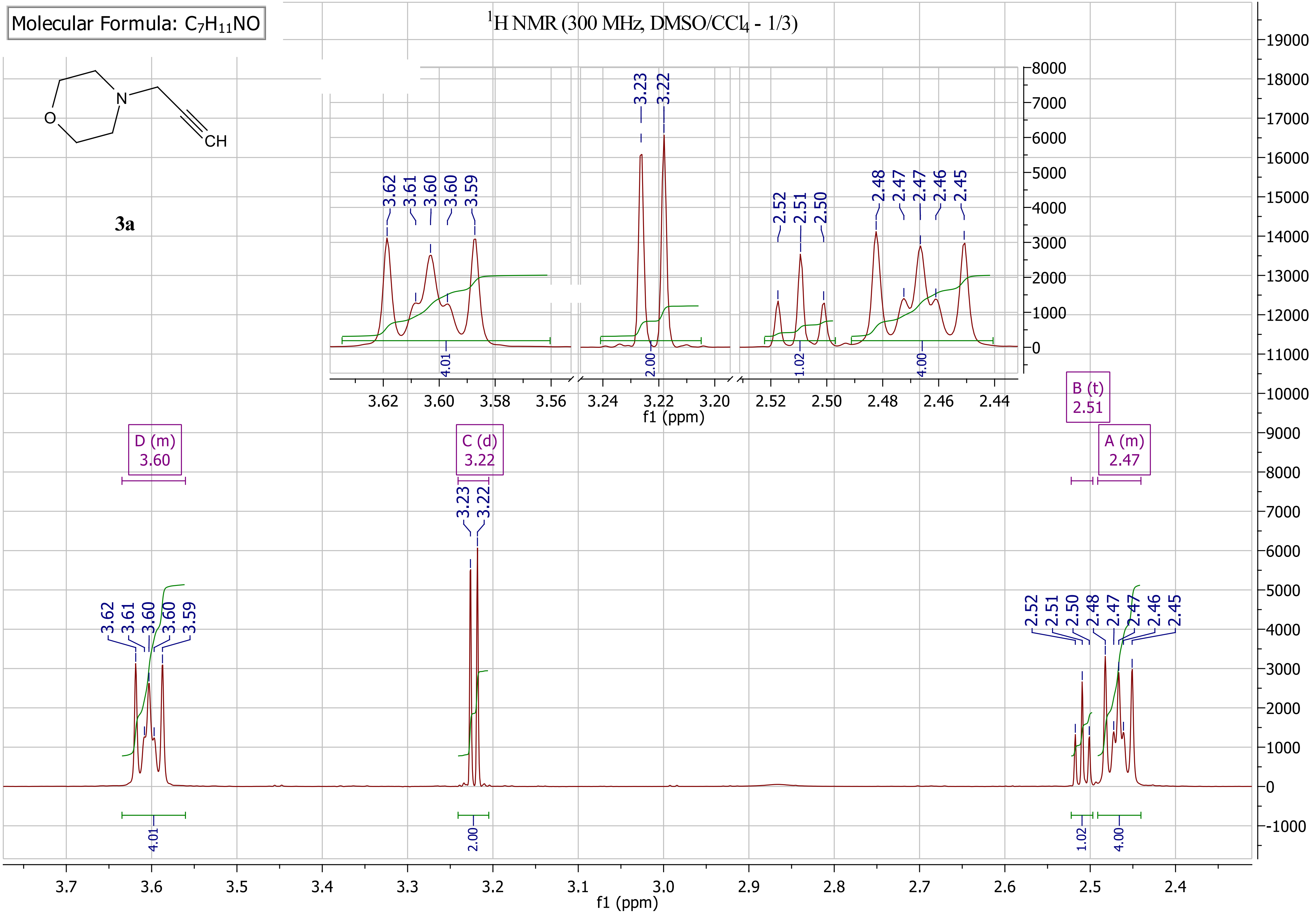
Molecular Formula: $C_7H_{11}NO$

$^{13}$C NMR (75 MHz, DMSO/CCl$_4$ - 1/3)



Molecular Formula: $C_7H_{11}NO$

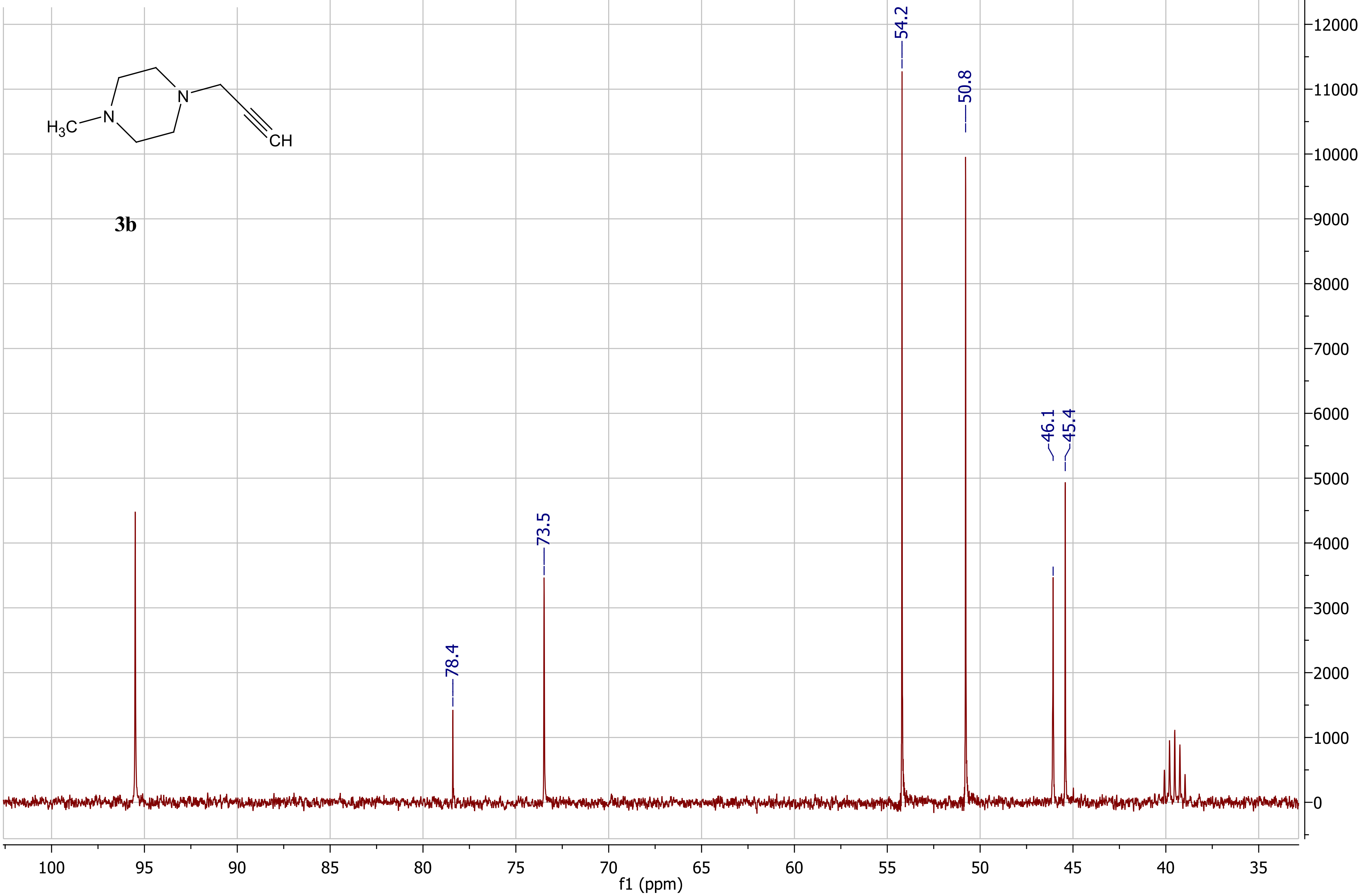
$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)



**3a**

Molecular Formula: $C_8H_{14}N_2$

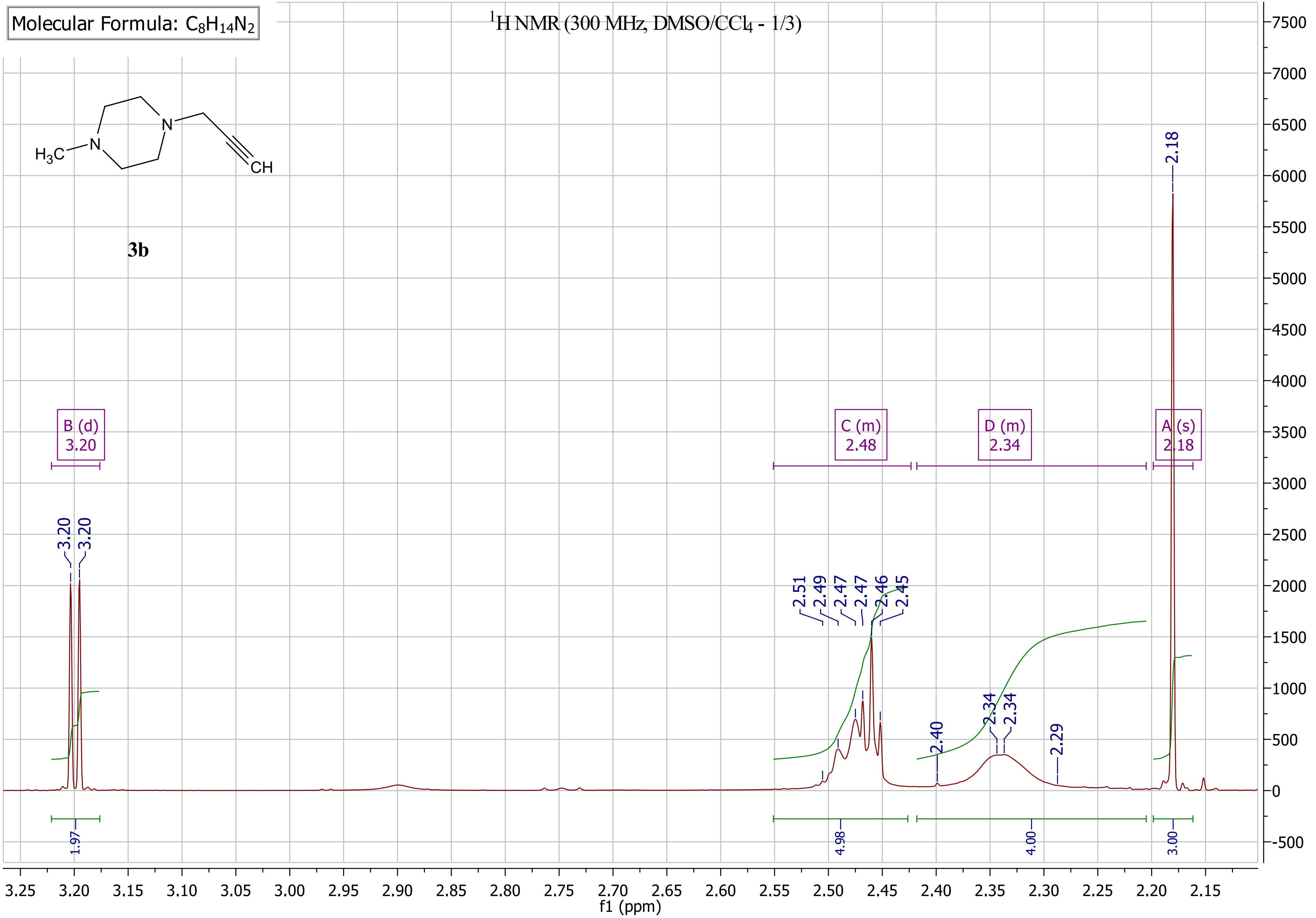
$^{13}$C NMR (75 MHz, DMSO/$CCl_4$ - 1/3)



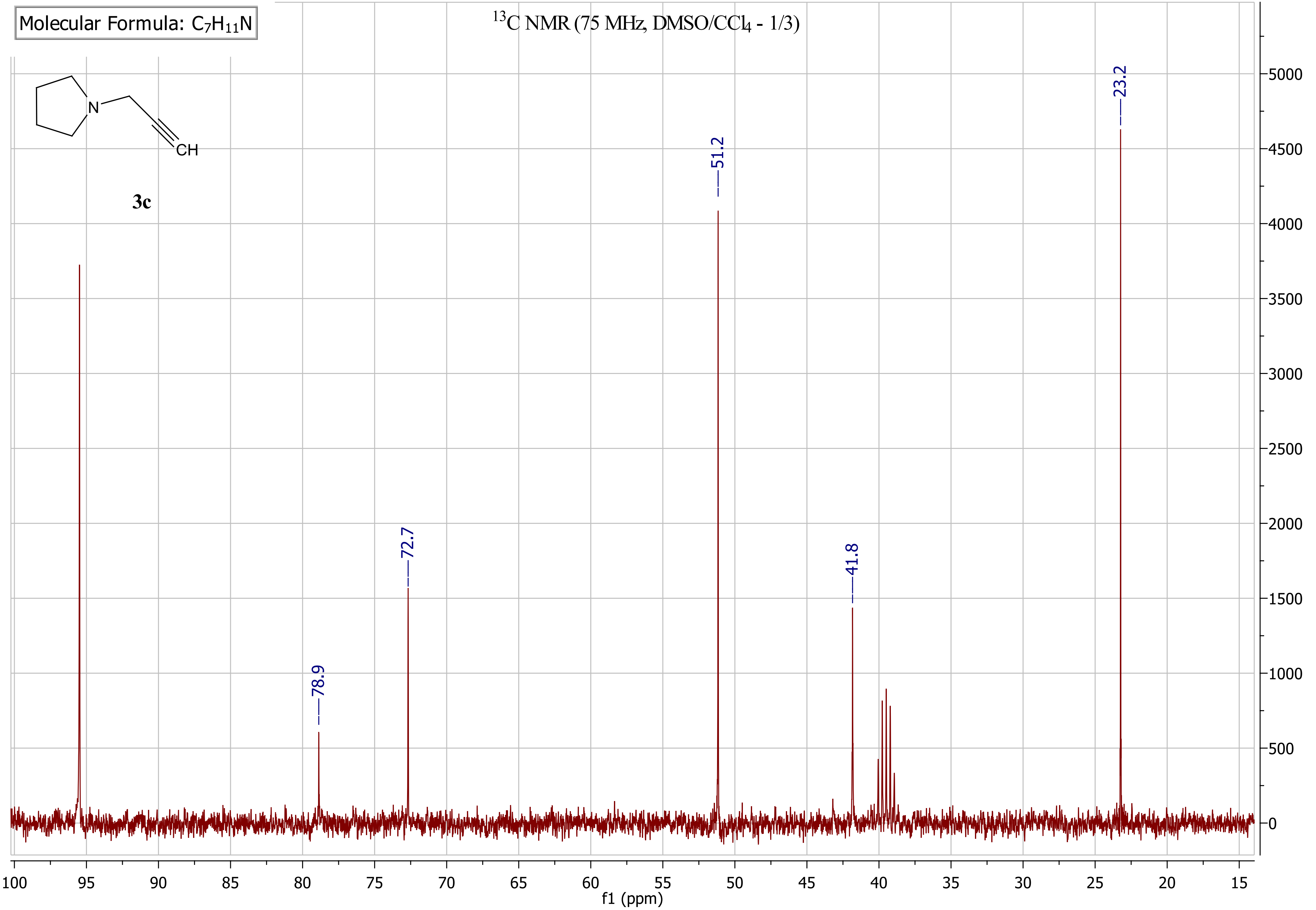
**3b**

Molecular Formula: $C_8H_{14}N_2$

$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)

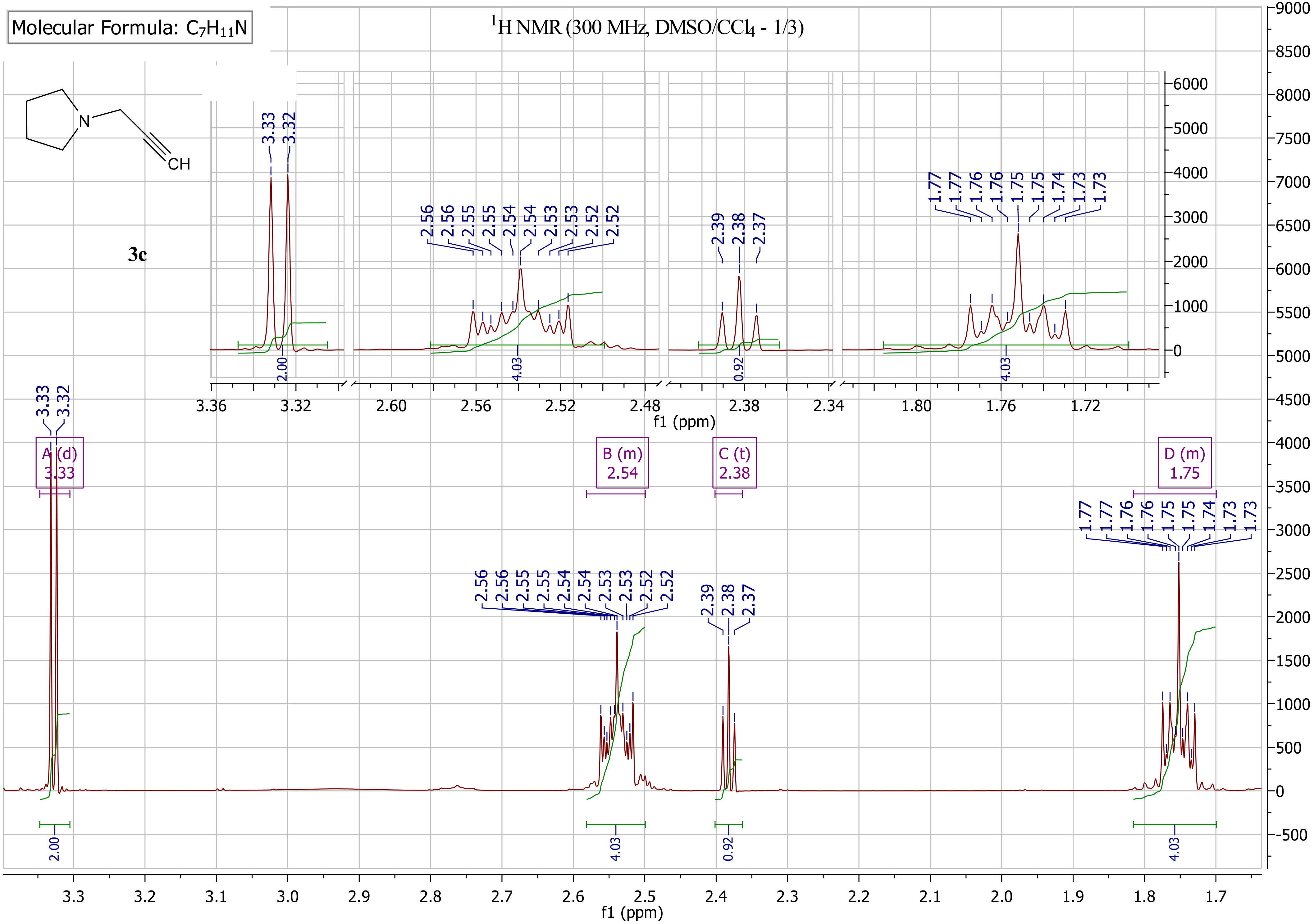


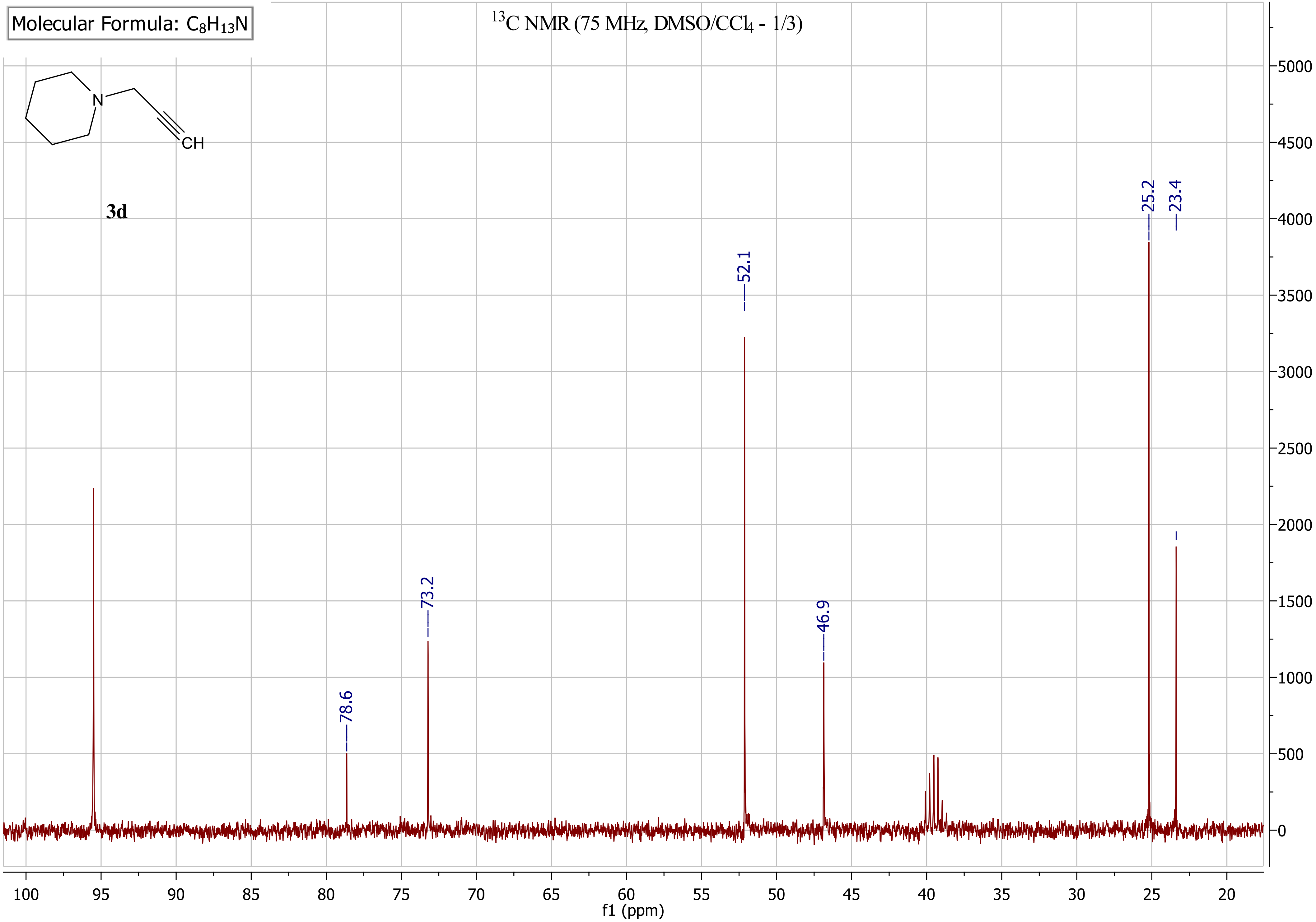
Molecular Formula: $C_7H_{11}N$

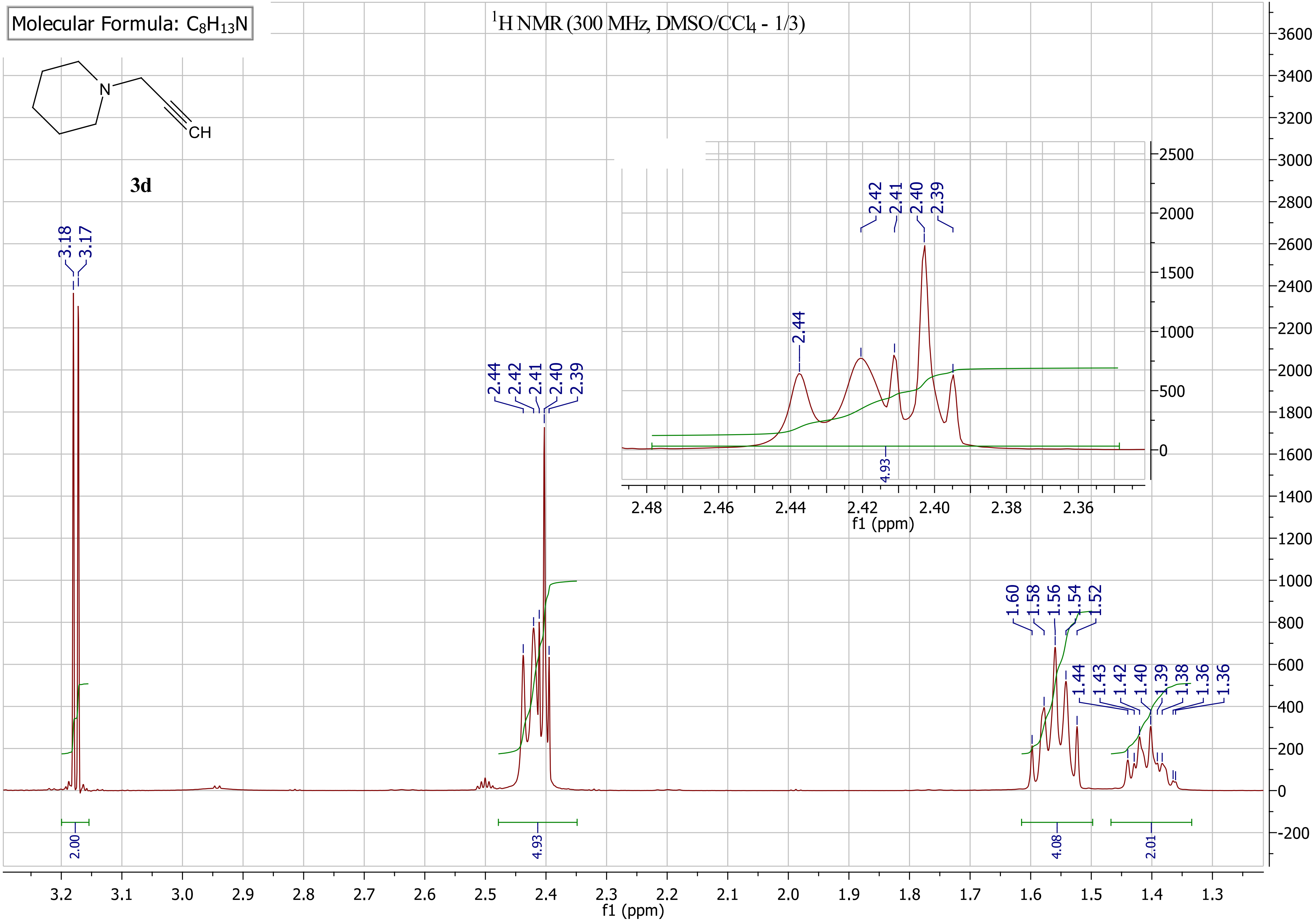


Molecular Formula: $C_7H_{11}N$

$^1H$ NMR (300 MHz, DMSO/$CCl_4$ - 1/3)



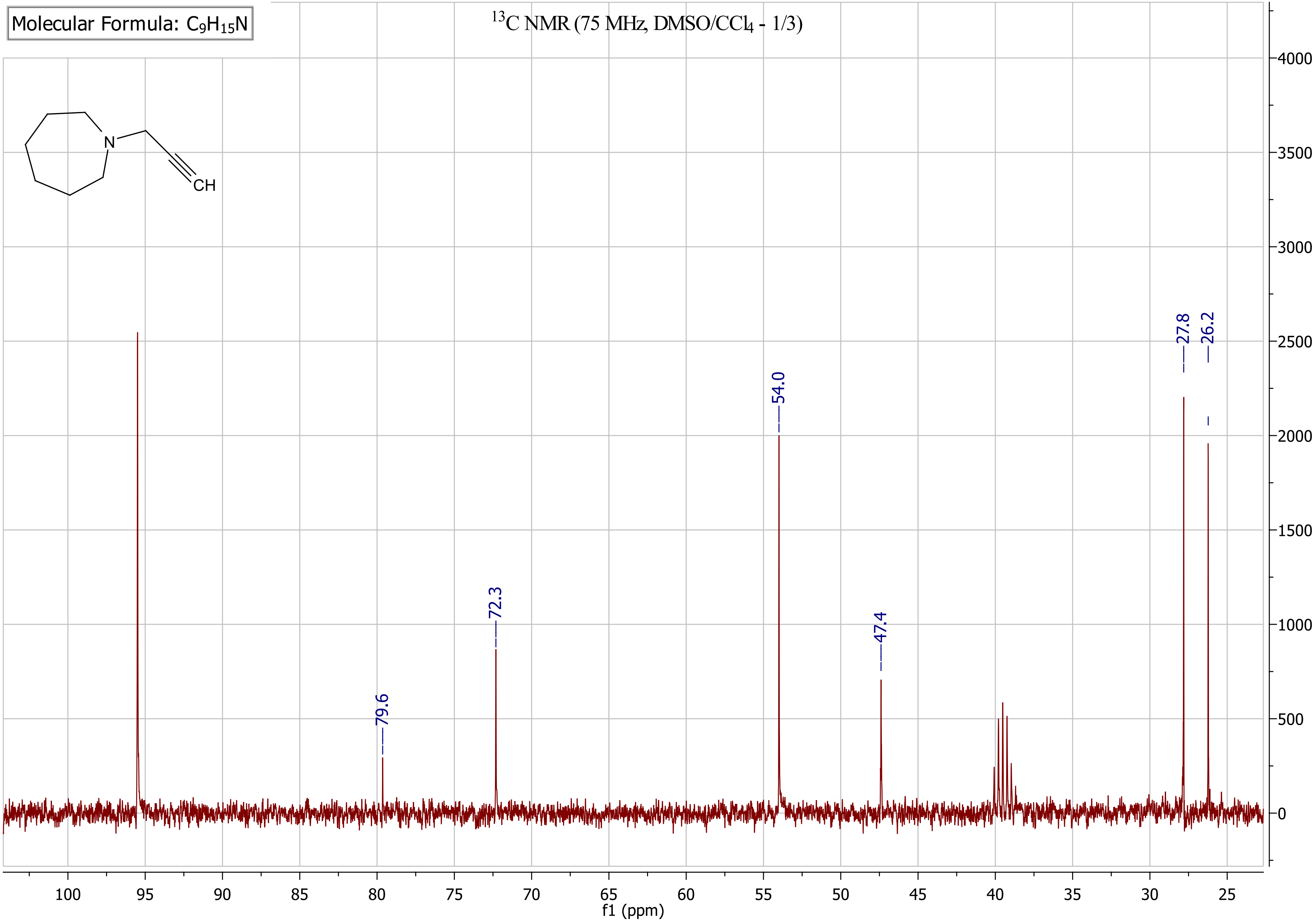

Molecular Formula: $C_8H_{13}N$
$^{13}$C NMR (75 MHz, DMSO/$CCl_4$ - 1/3)
N
CH
3d
78.6
73.2
52.1
46.9
25.2
23.4
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
f1 (ppm)



Molecular Formula: $C_8H_{13}N$

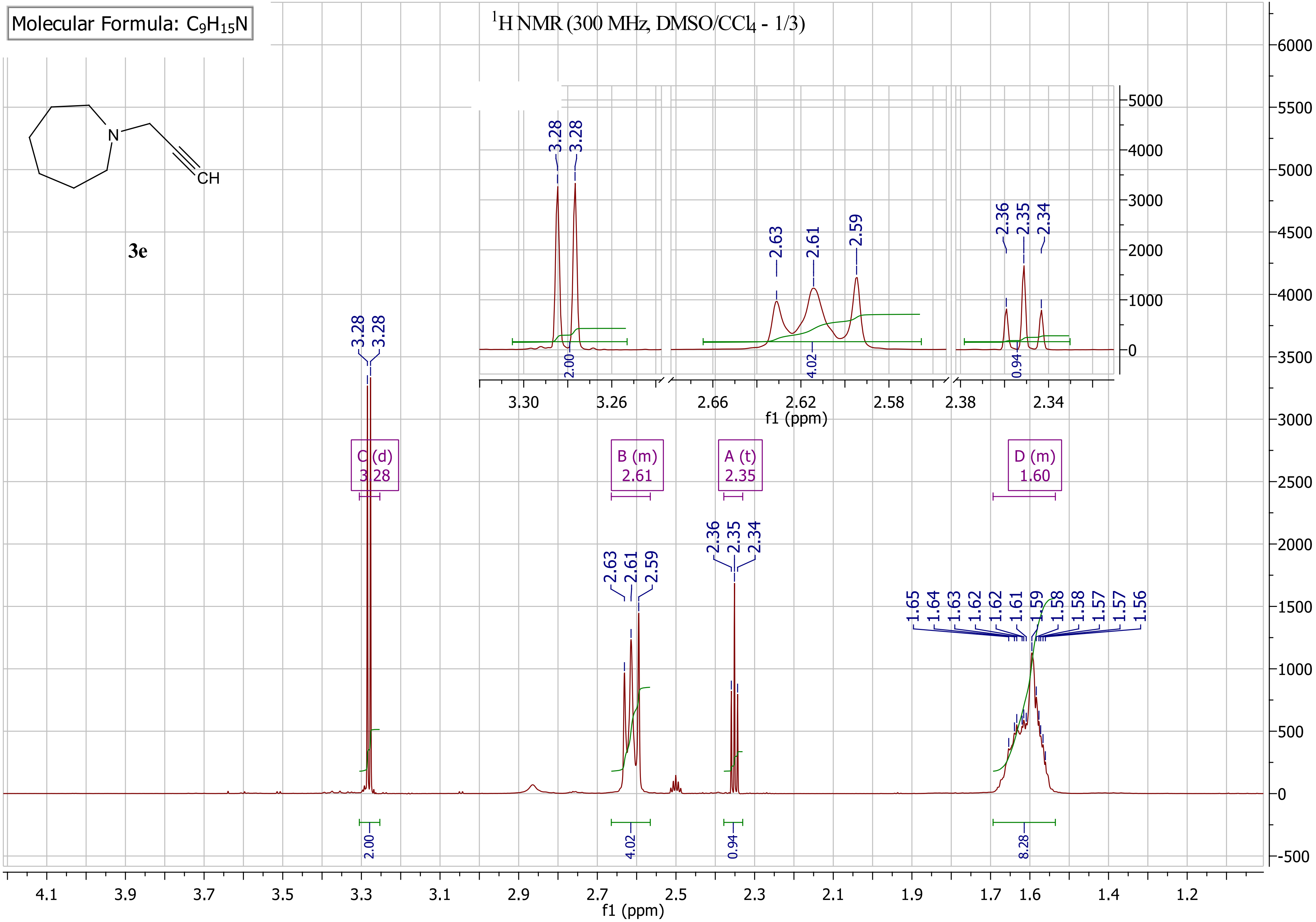
$^1H$ NMR (300 MHz, DMSO/$CCl_4$ - 1/3)

**3d**


Molecular Formula: $C_9H_{15}N$
$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)
N
CH
79.6
72.3
54.0
47.4
27.8
26.2
4000
3500
3000
2500
2000
1500
1000
500
0
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
f1 (ppm)

Molecular Formula: $C_9H_{15}N$

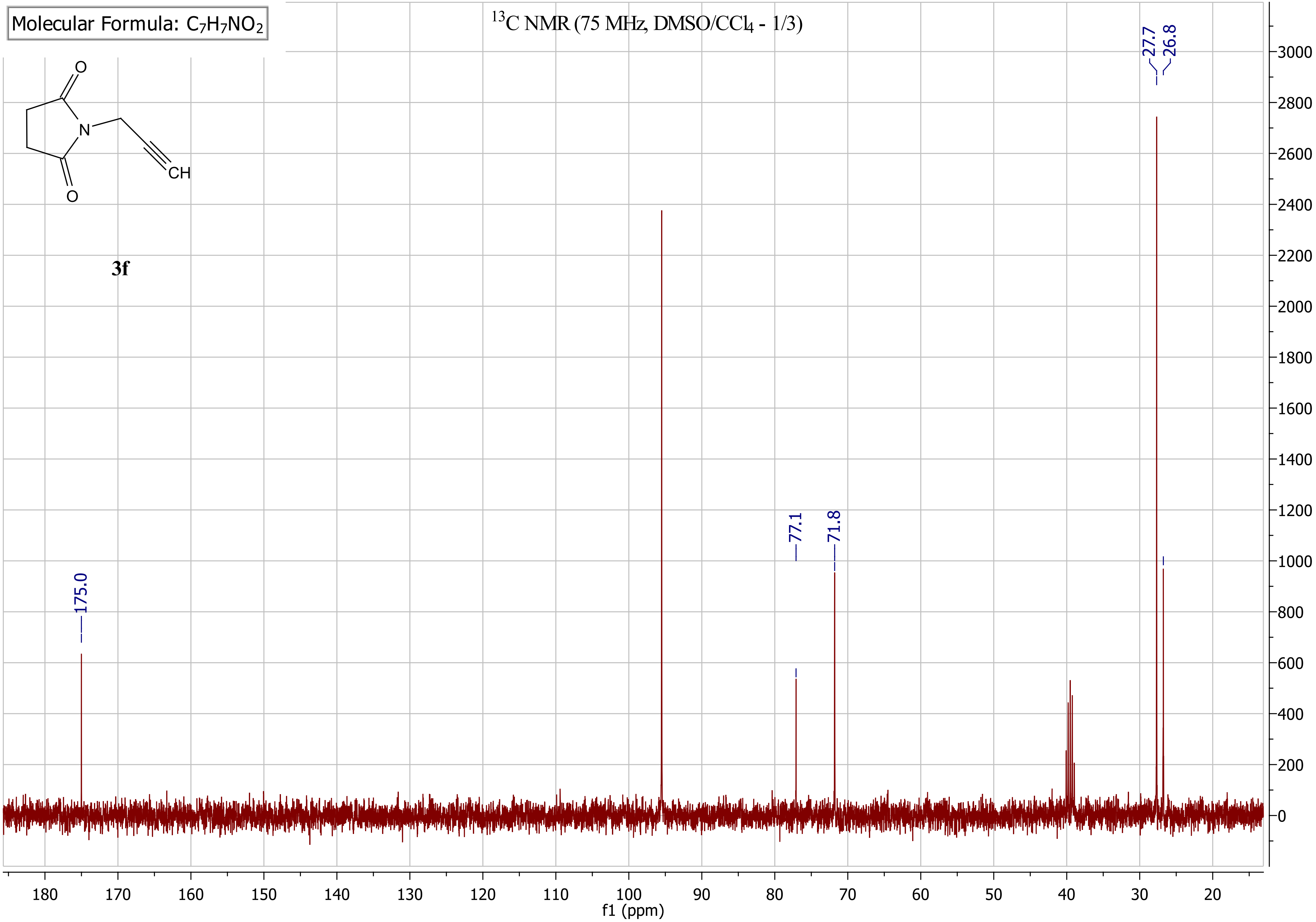
$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)



**3e**

Molecular Formula: $C_7H_7NO_2$

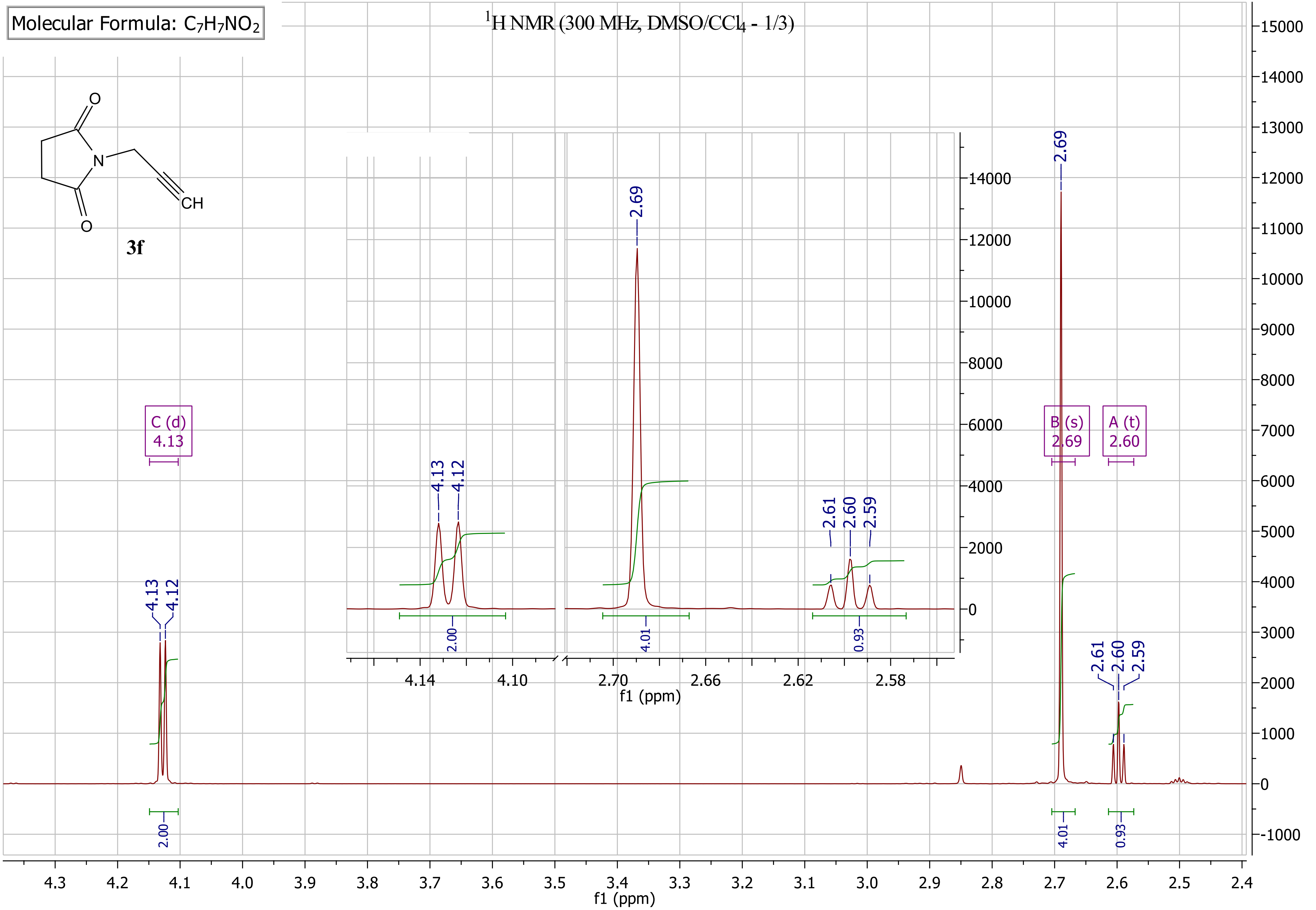
$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)

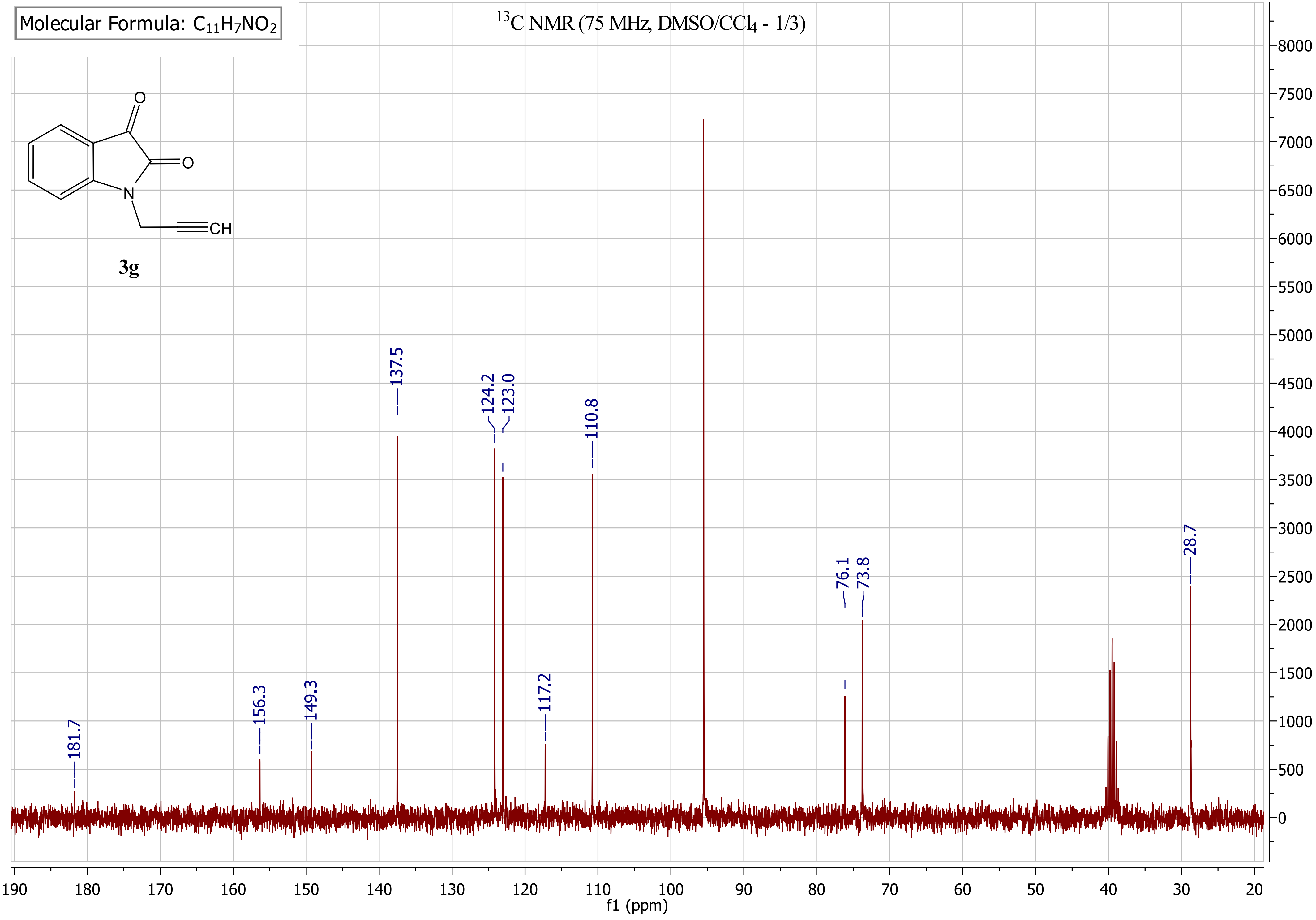


**3f**

Molecular Formula: $C_7H_7NO_2$

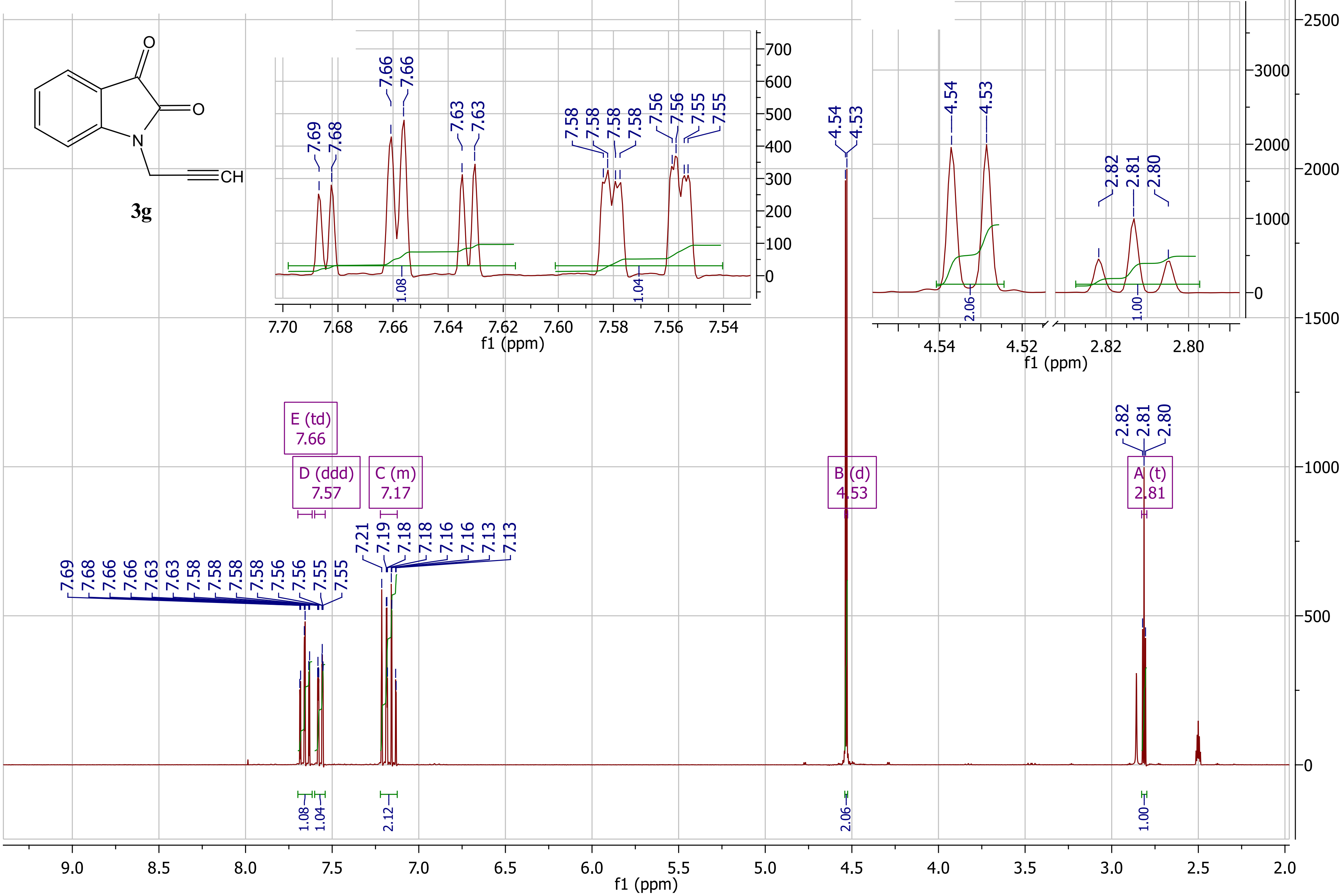
$^1H$ NMR (300 MHz, DMSO/$CCl_4$ - 1/3)




Molecular Formula: $C_{11}H_7NO_2$
$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)
O
O
N
CH
3g
181.7
156.3
149.3
137.5
124.2
123.0
117.2
110.8
76.1
73.8
28.7
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

Molecular Formula: $C_{11}H_7NO_2$

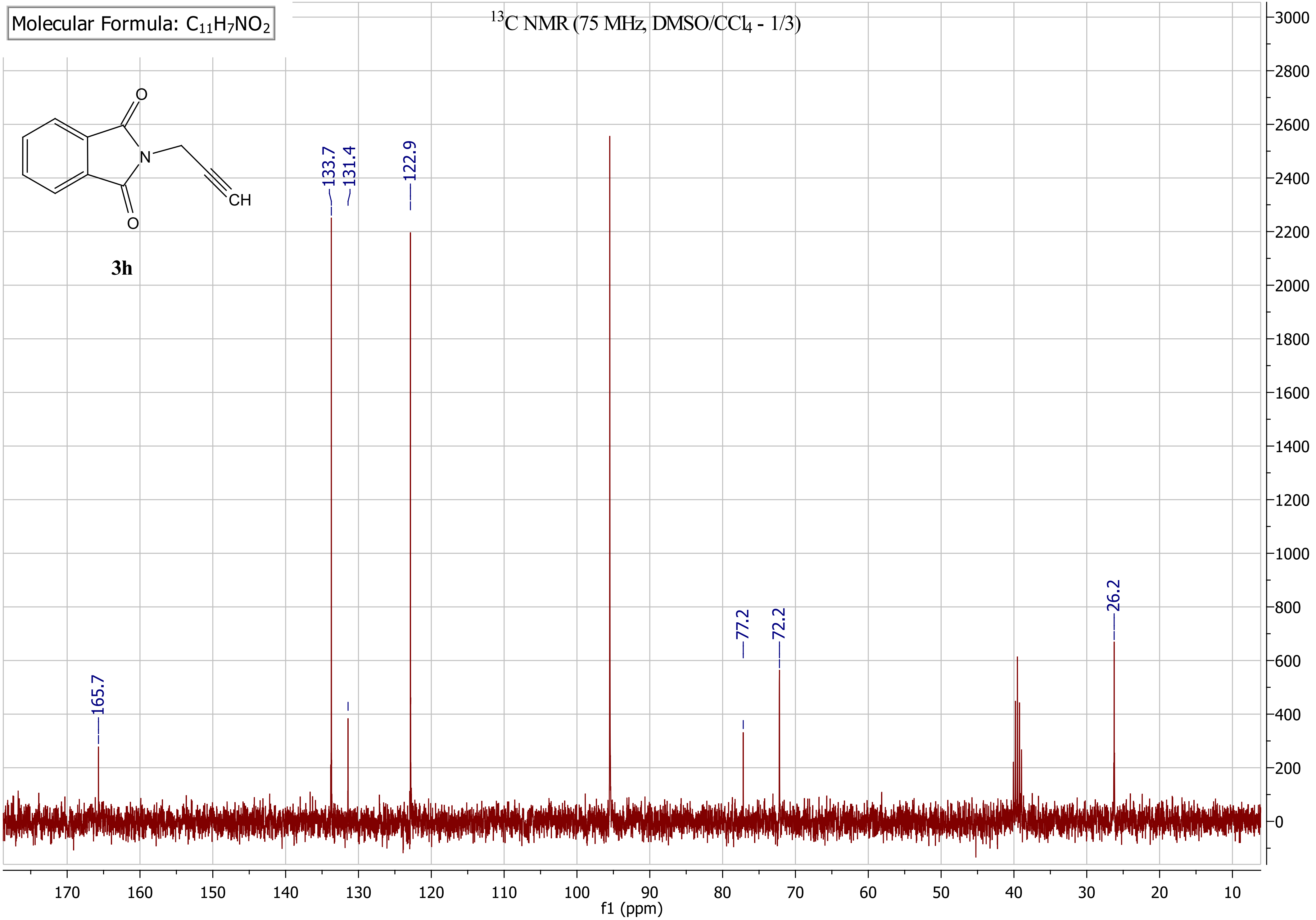
$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)



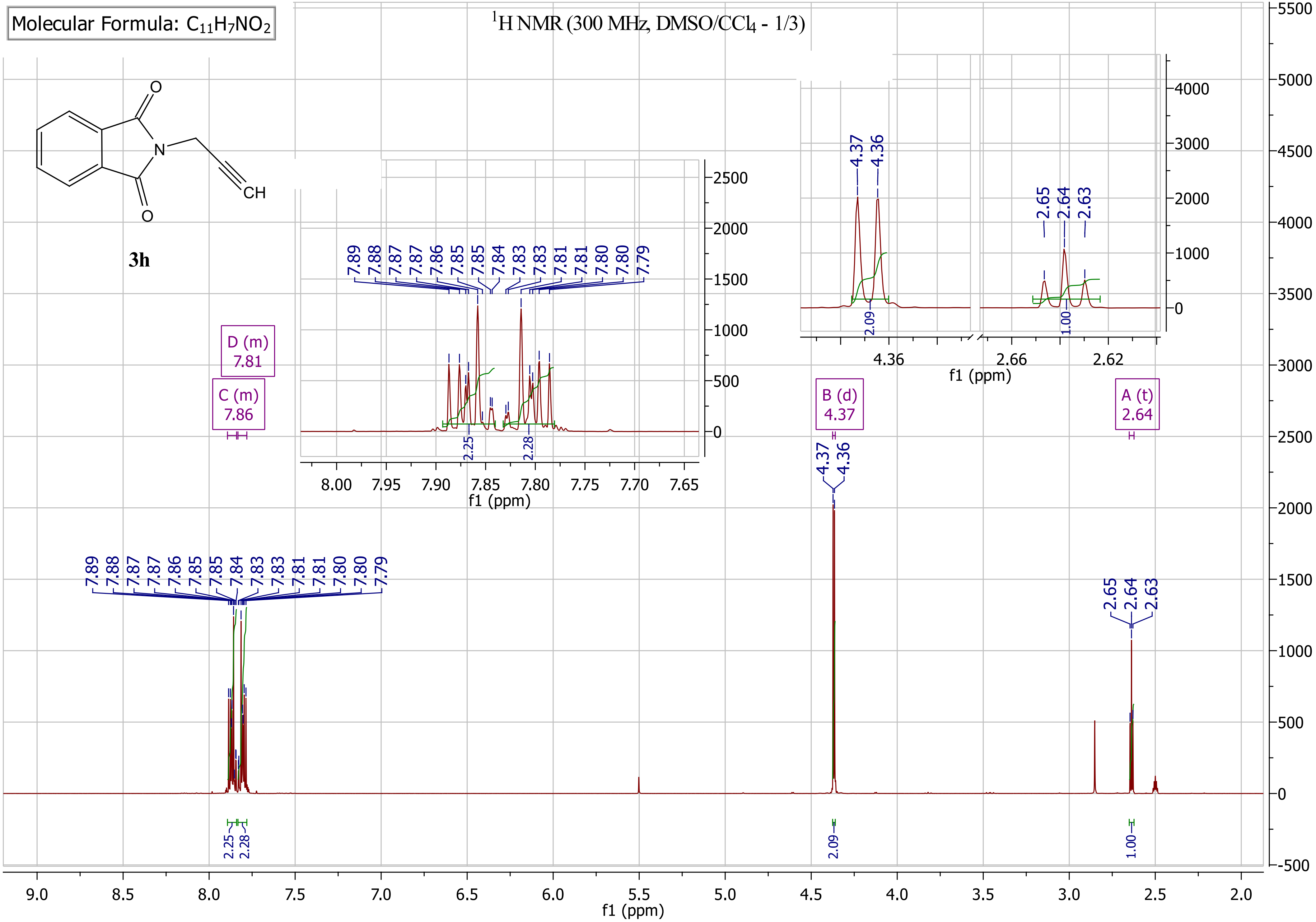
**3g**

Molecular Formula: $C_{11}H_7NO_2$

$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)

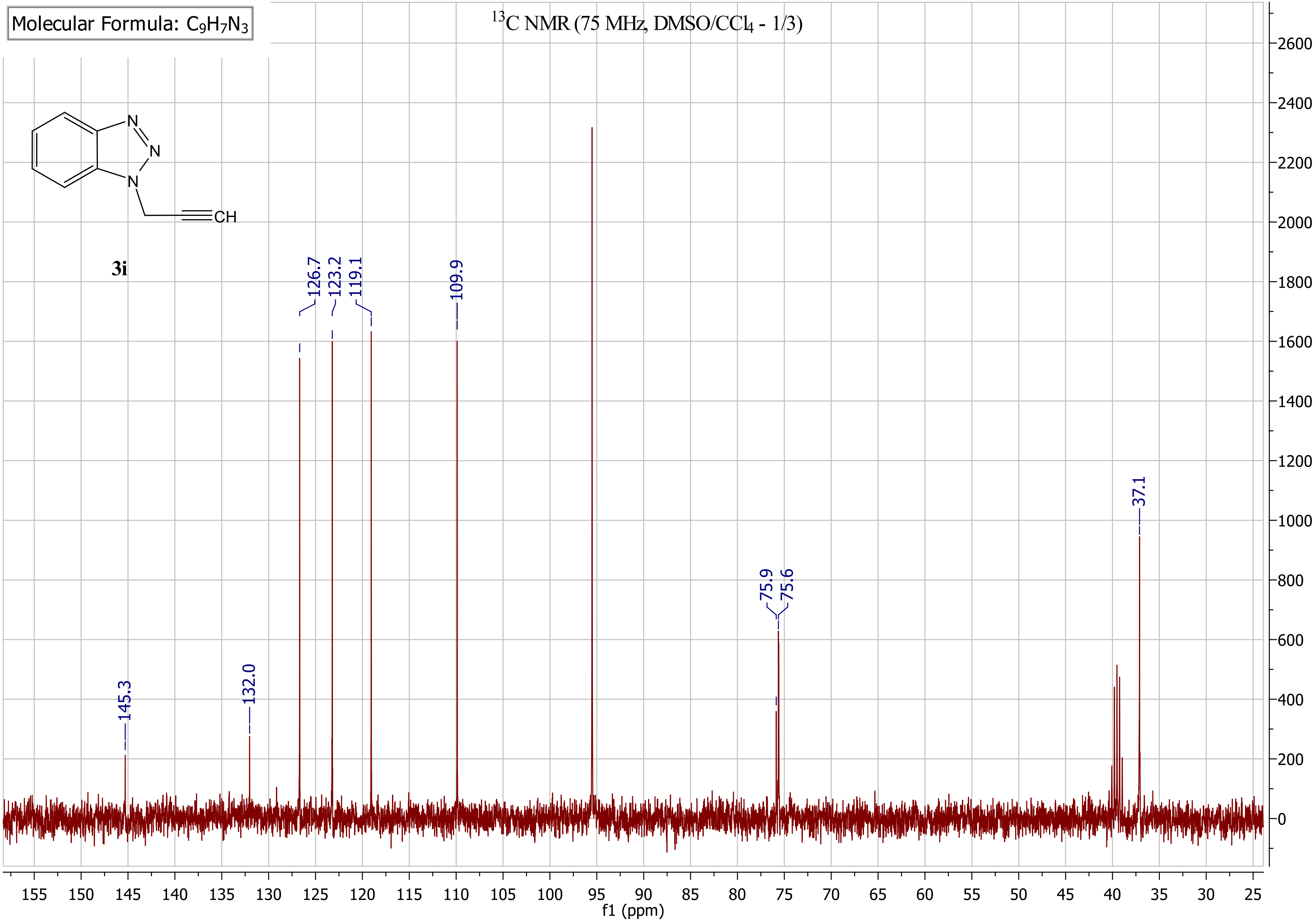


**3h**


Molecular Formula: $C_{11}H_7NO_2$
$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)
O
N
CH
O
3h
D (m) 7.81
C (m) 7.86
B (d) 4.37
A (t) 2.64
7.89 7.88 7.87 7.87 7.86 7.85 7.85 7.84 7.83 7.83 7.81 7.81 7.80 7.80 7.79
4.37 4.36
2.65 2.64 2.63
2.25
2.28
2.09
1.00
8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65
4.36
2.66 2.62
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f1 (ppm)
5500 5000 4500 4000 3500 3000 2500 2000 1500 1000 500 0 -500
2500 2000 1500 1000 500 0
4000 3000 2000 1000 0

Molecular Formula: $C_9H_7N_3$

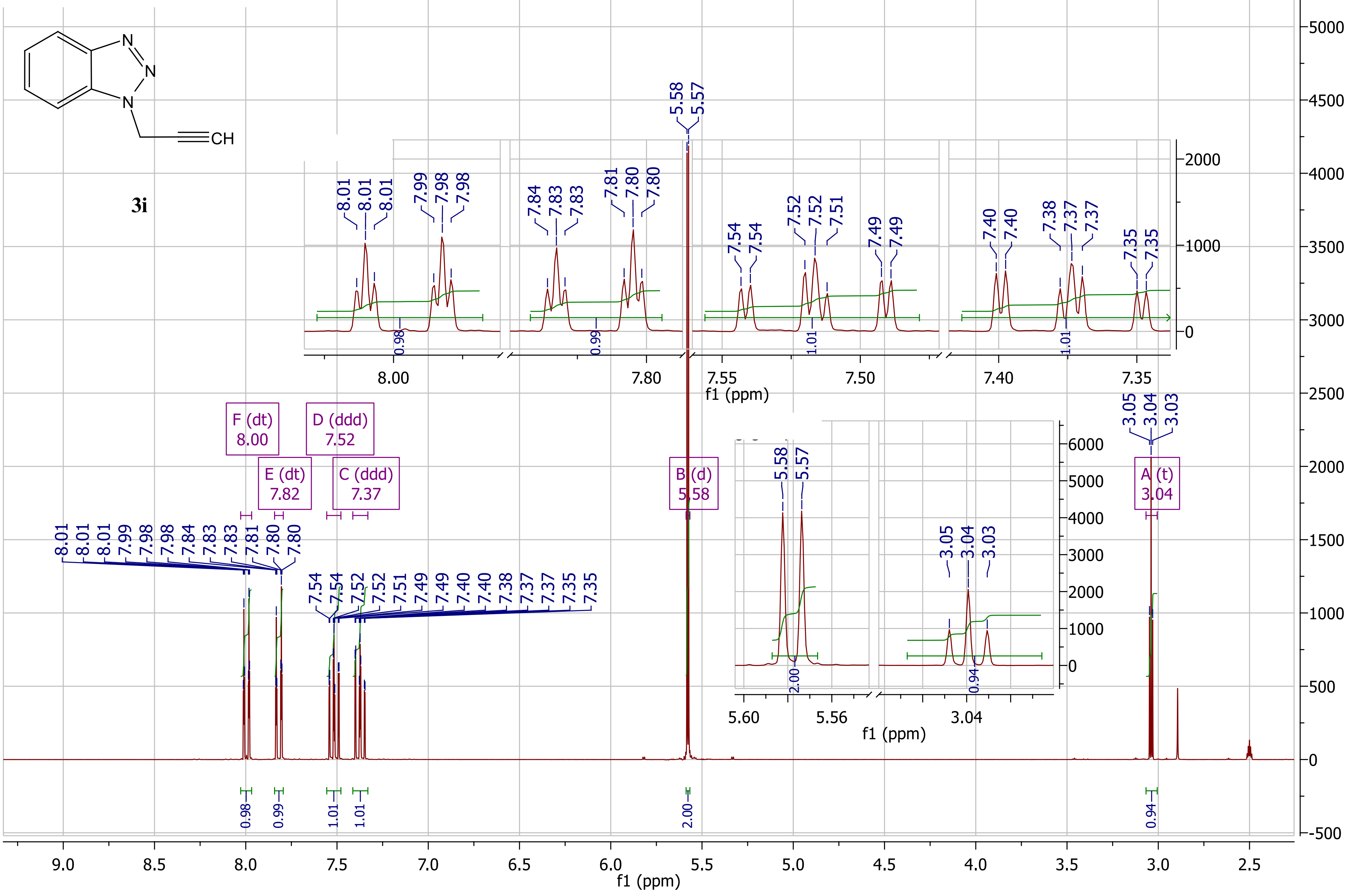
$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)



3i

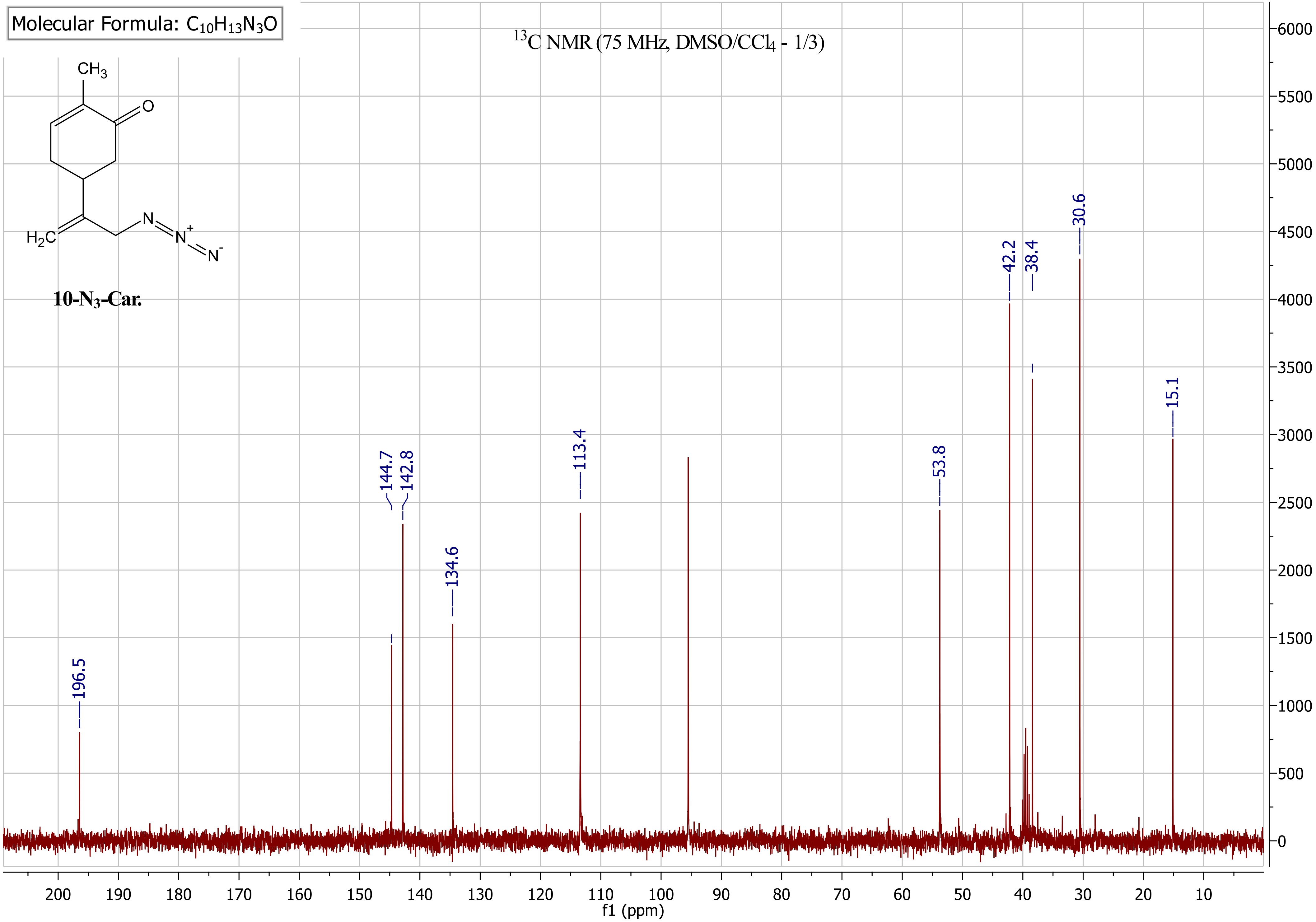
Molecular Formula: $C_9H_7N_3$

$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/3)



**3i**

Molecular Formula: $C_{10}H_{13}N_3O$

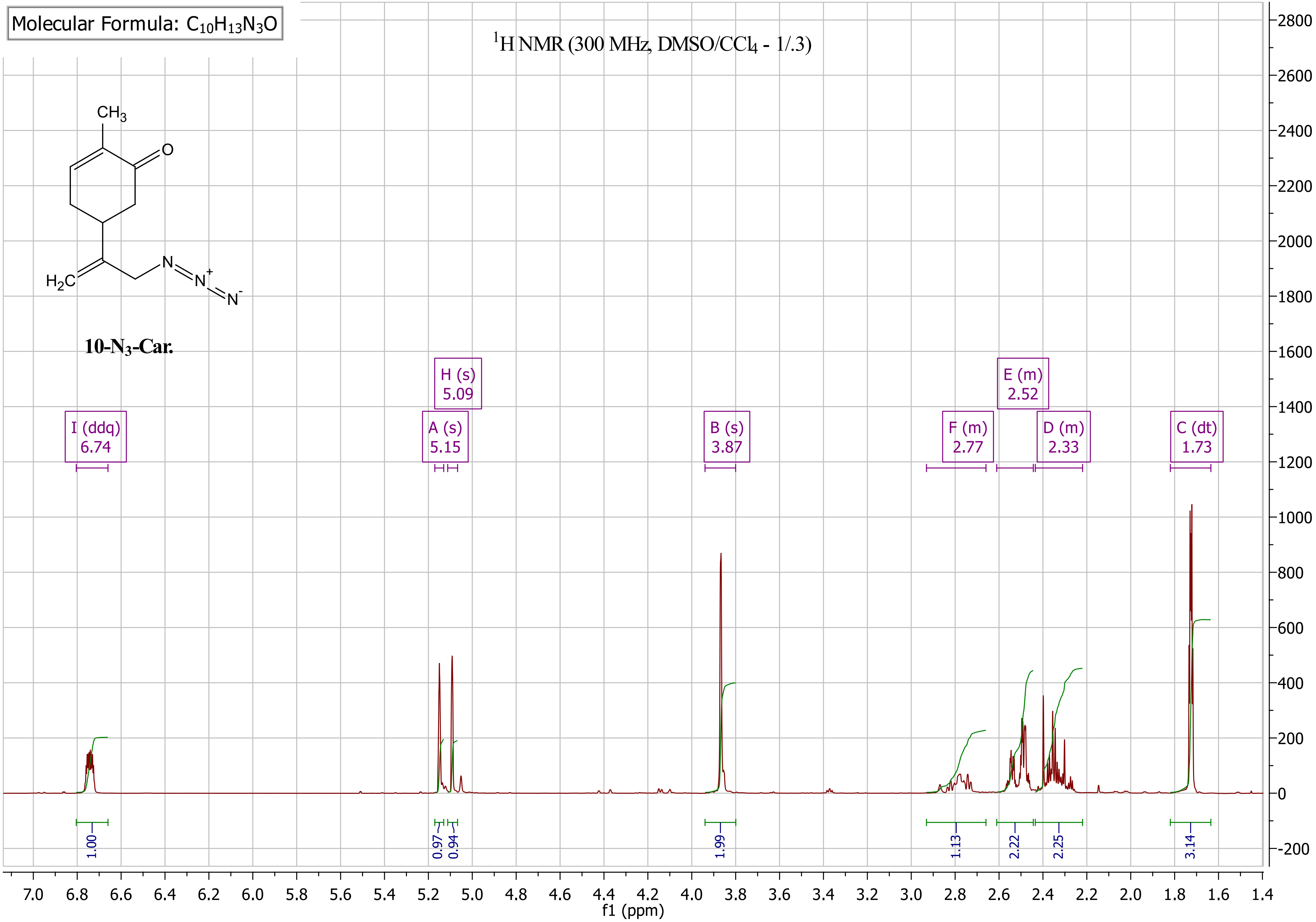


**10-$N_3$-Car.**

$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)

Molecular Formula: $C_{10}H_{13}N_3O$

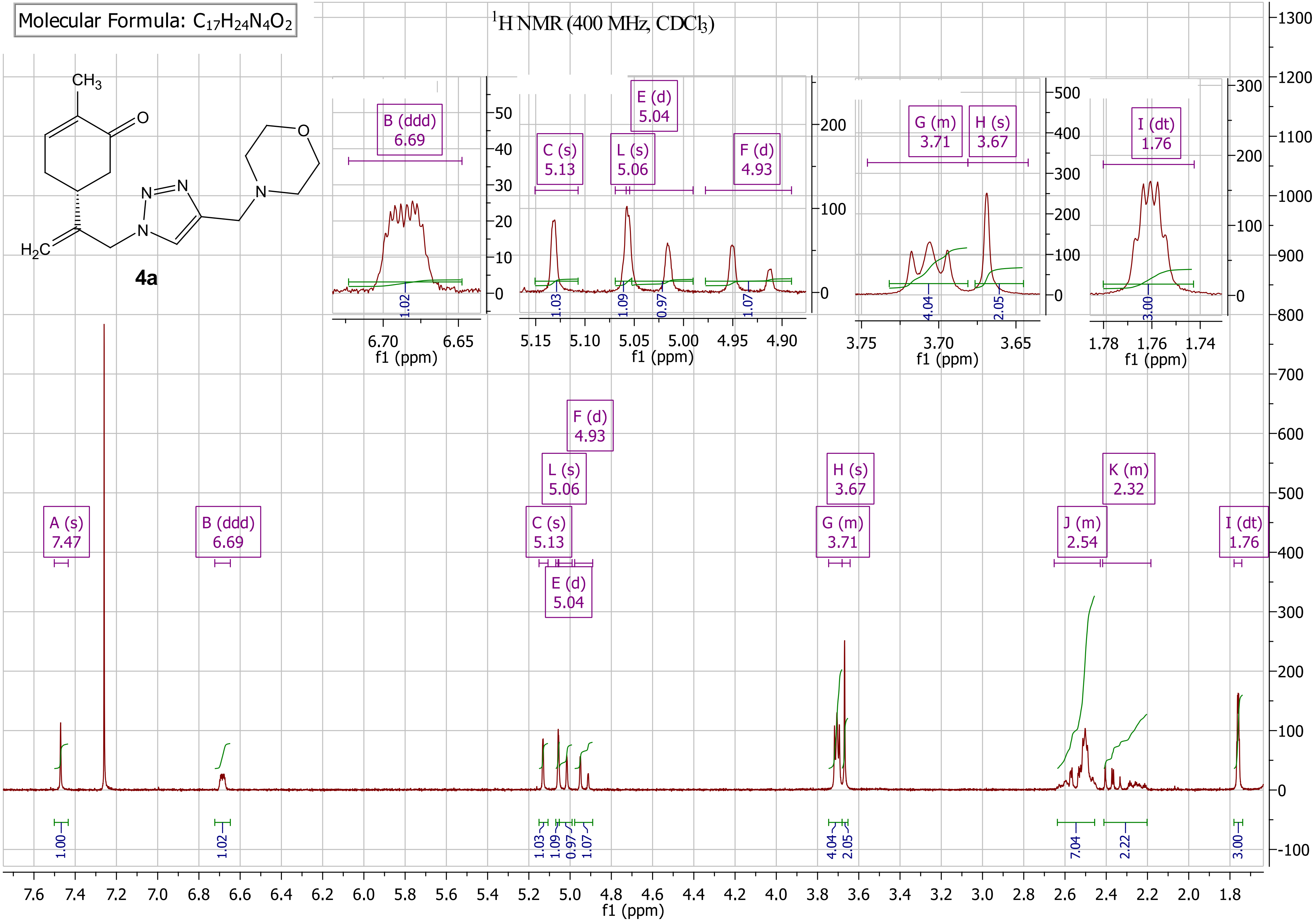
$^1$H NMR (300 MHz, DMSO/$CCl_4$ - 1/.3)



**10-$N_3$-Car.**

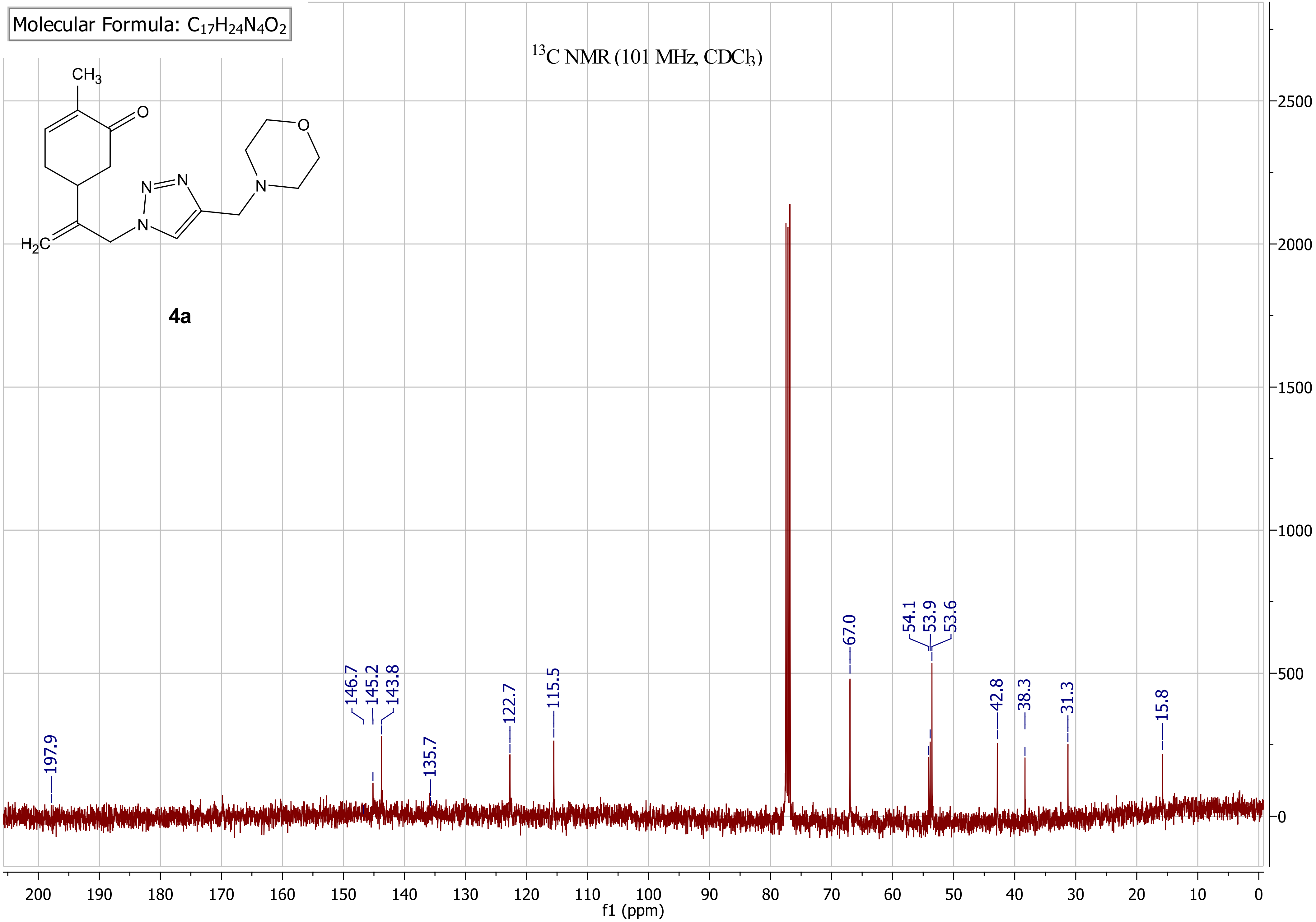
Molecular Formula: $C_{17}H_{24}N_4O_2$

$^1$H NMR (400 MHz, $CDCl_3$)

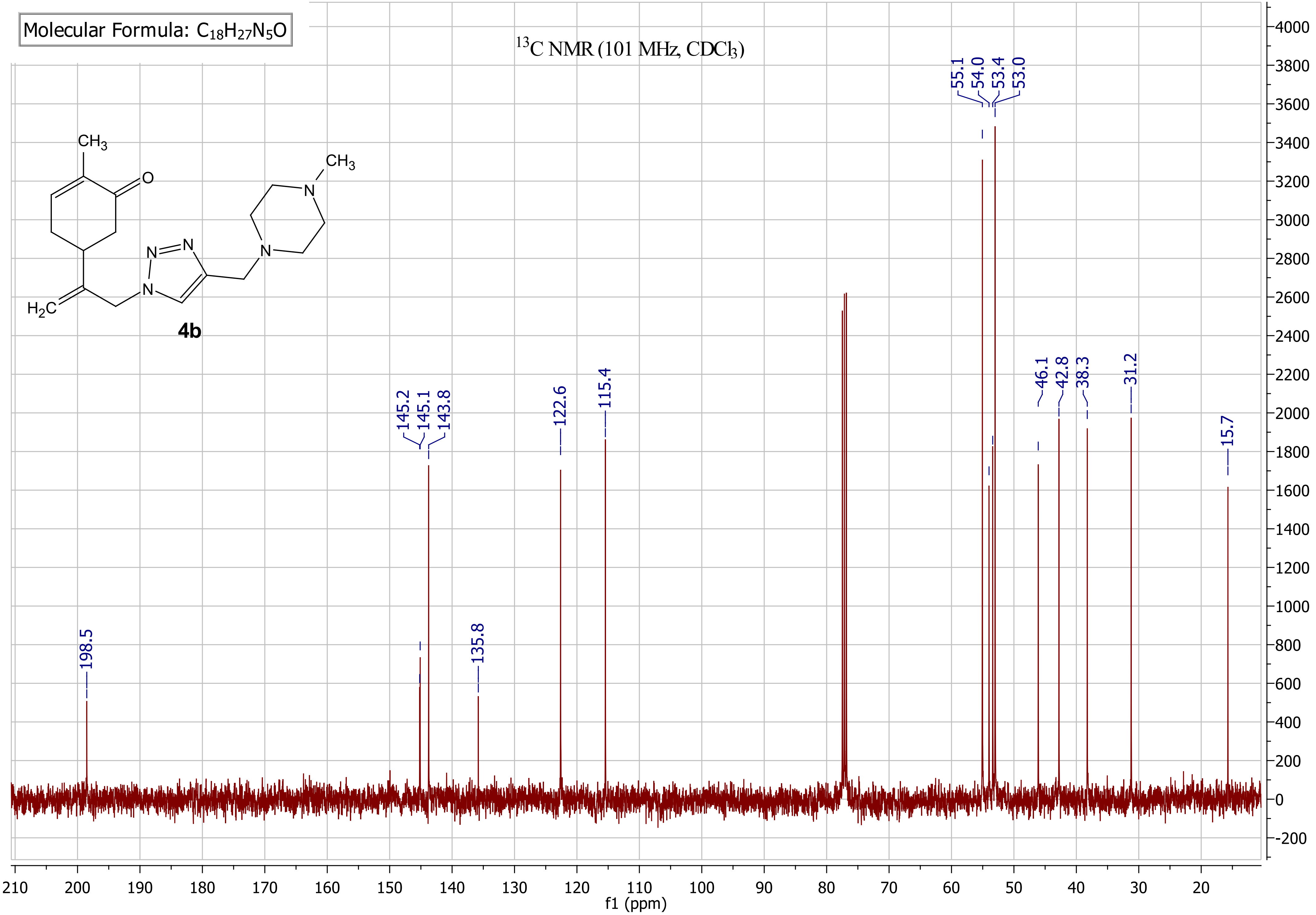


**4a**

Molecular Formula: $C_{17}H_{24}N_4O_2$



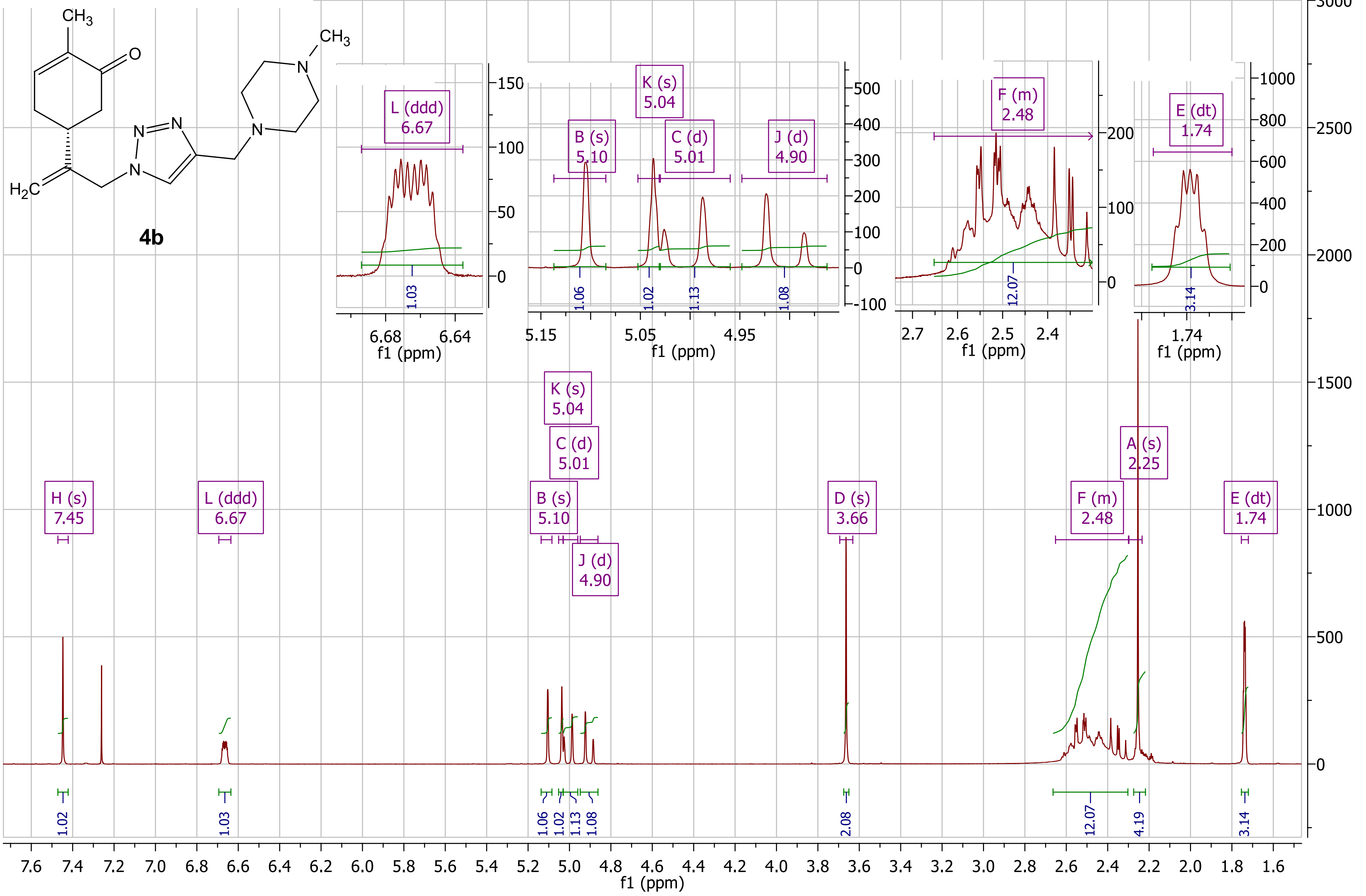
Molecular Formula: $C_{18}H_{27}N_5O$



**4b**

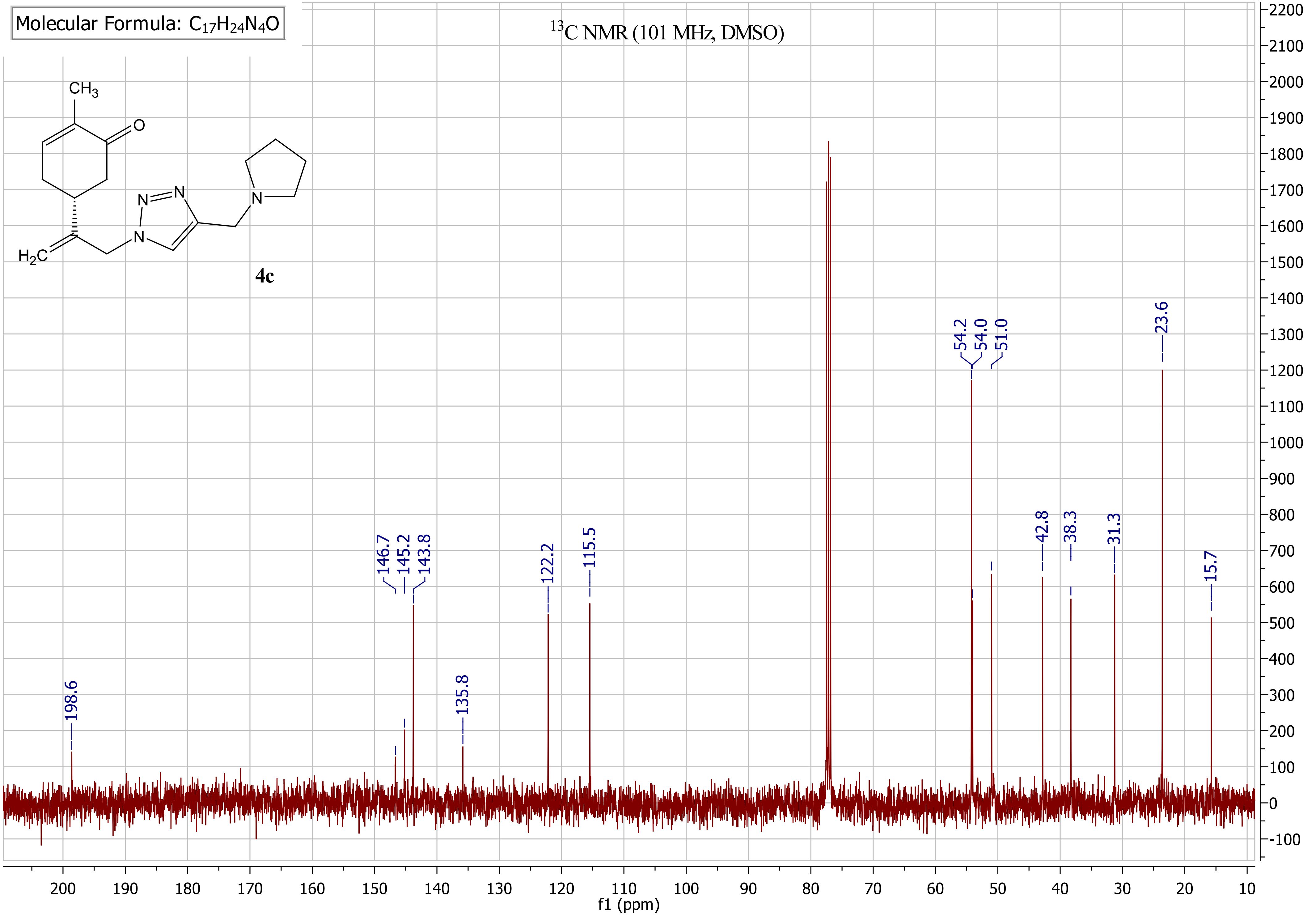
Molecular Formula: $C_{18}H_{27}N_5O$

$^1H$ NMR (400 MHz, $CDCl_3$)



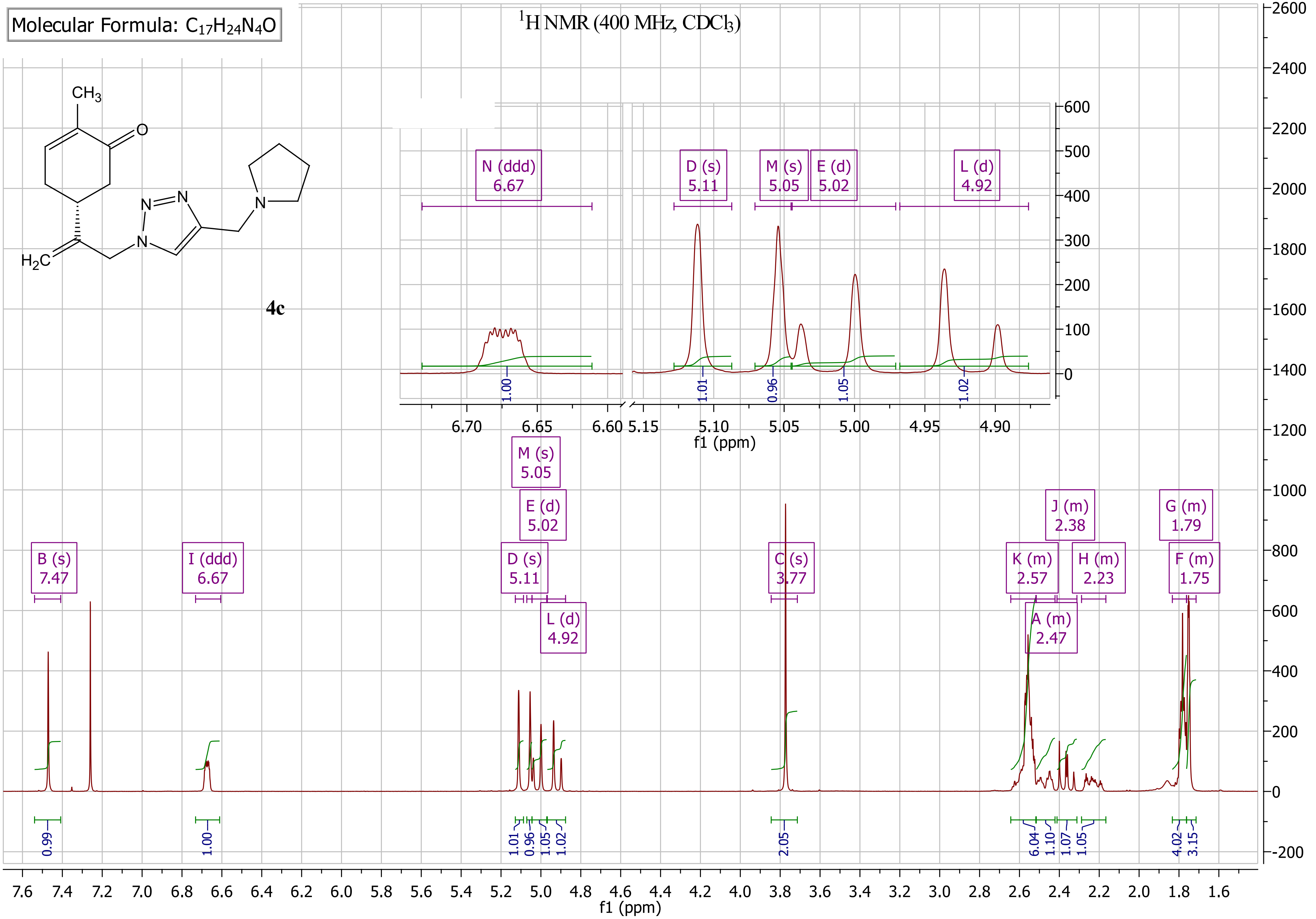
**4b**

Molecular Formula: $C_{17}H_{24}N_4O$



**4c**

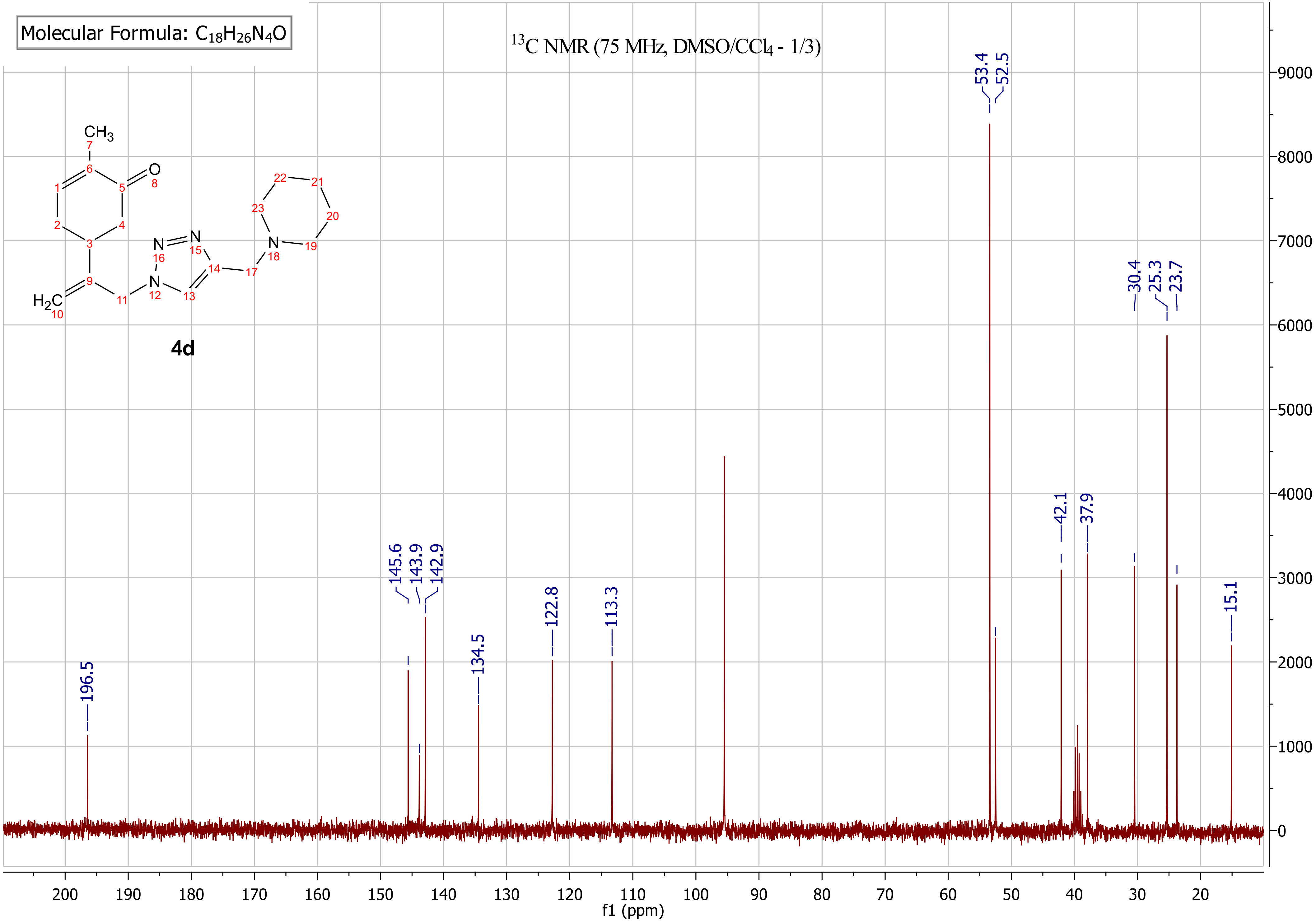
Molecular Formula: $C_{17}H_{24}N_4O$



**4c**

Molecular Formula: $C_{18}H_{26}N_4O$

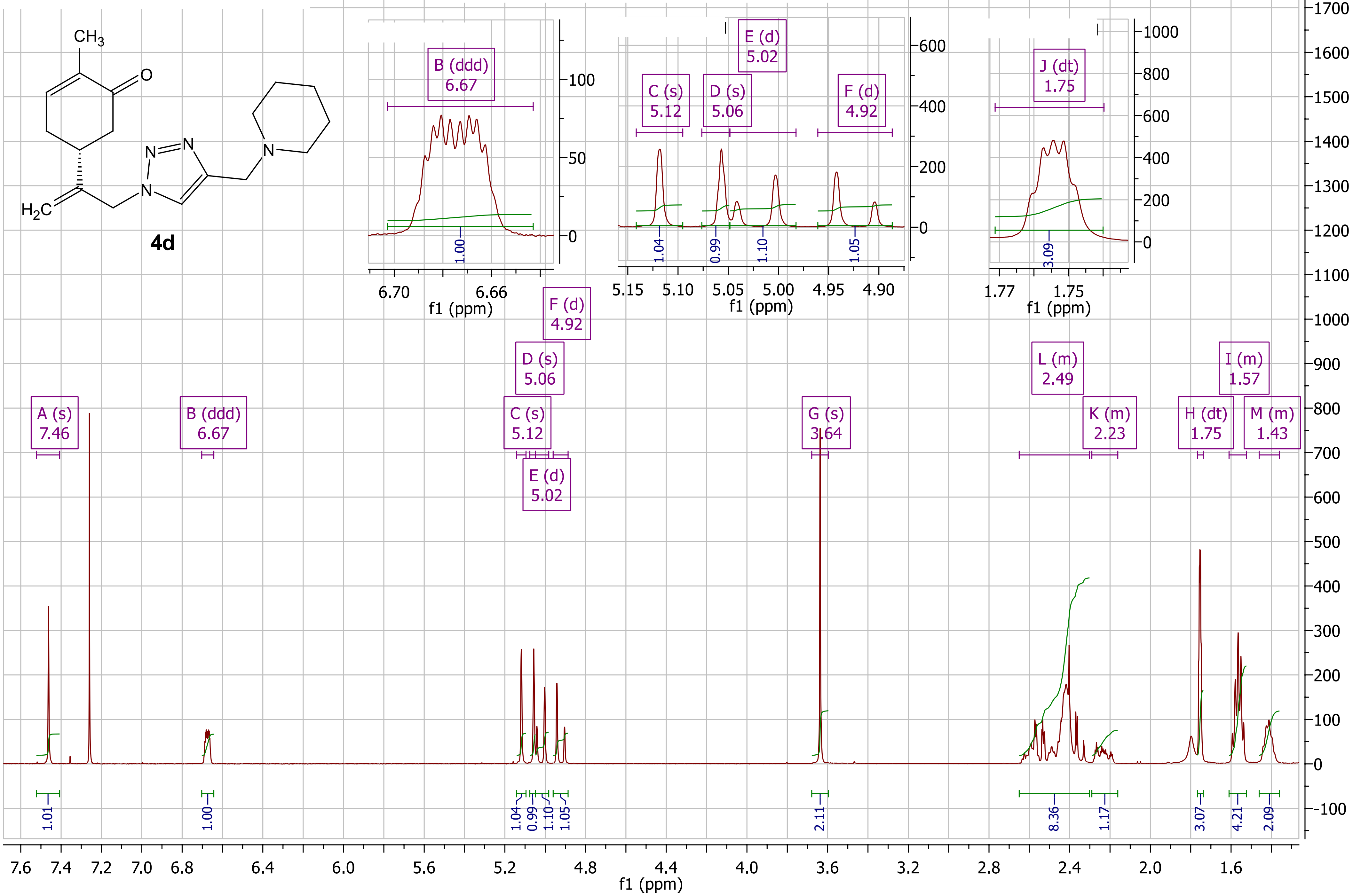
$^{13}C$ NMR (75 MHz, DMSO/$CCl_4$ - 1/3)



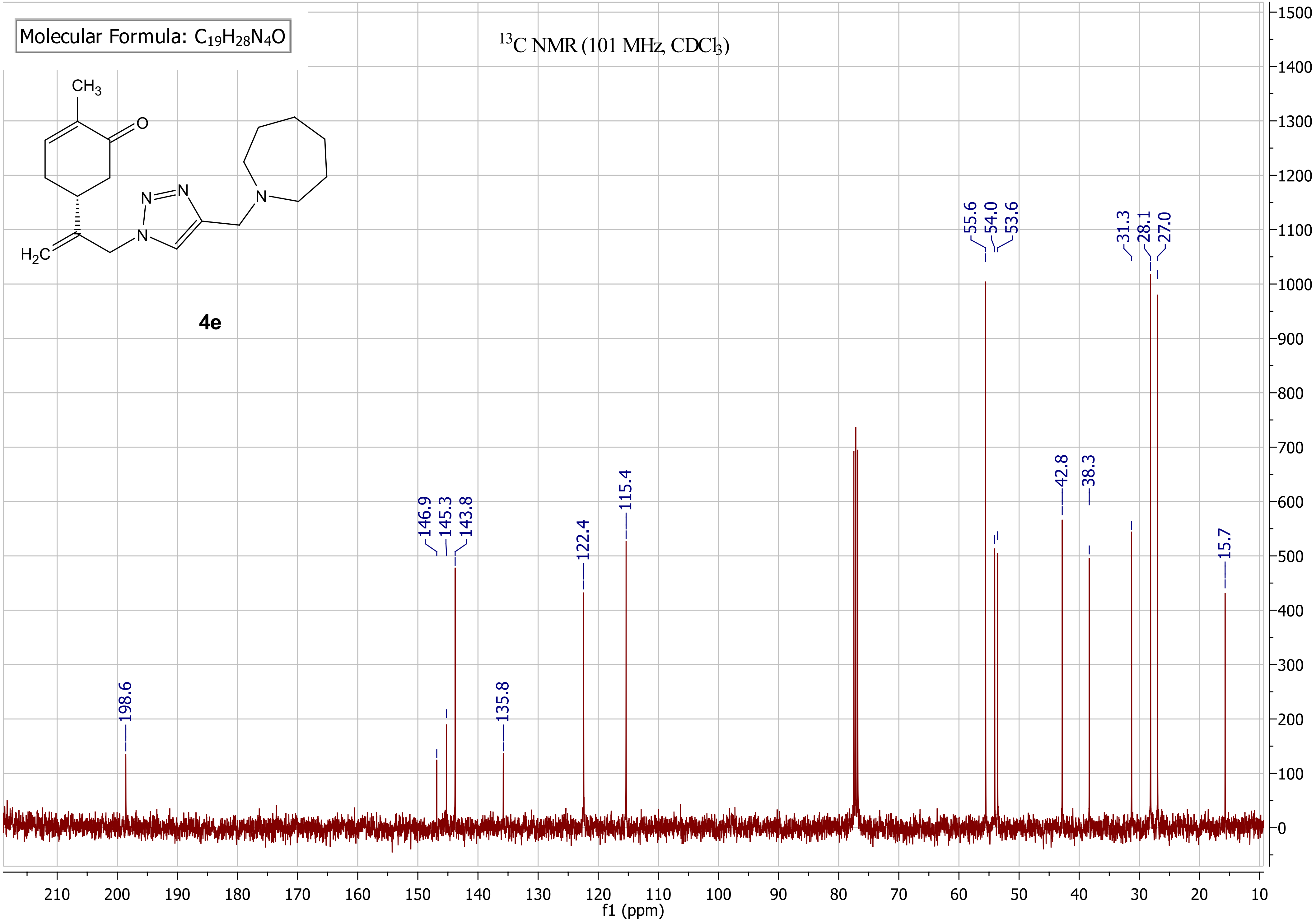
**4d**

Molecular Formula: $C_{18}H_{26}N_4O$

$^1H$ NMR (400 MHz, $CDCl_3$)

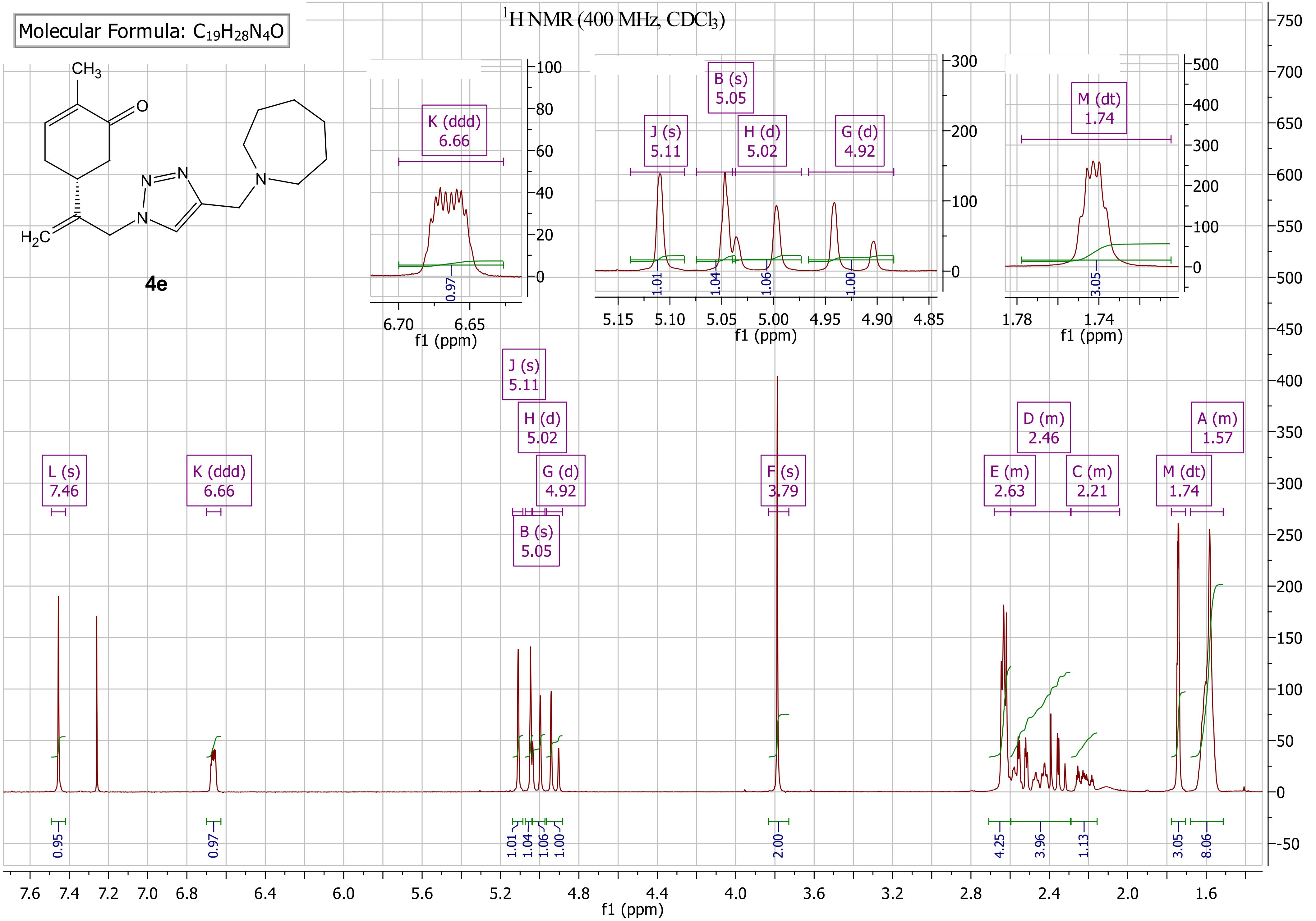


**4d**


Molecular Formula: $C_{19}H_{28}N_4O$
$^{13}$C NMR (101 MHz, CDCl$_3$)
CH$_3$
O
N
N
N
N
H$_2$C
4e
198.6
146.9
145.3
143.8
135.8
122.4
115.4
55.6
54.0
53.6
42.8
38.3
31.3
28.1
27.0
15.7
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
1500 1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0

Molecular Formula: $C_{19}H_{28}N_4O$

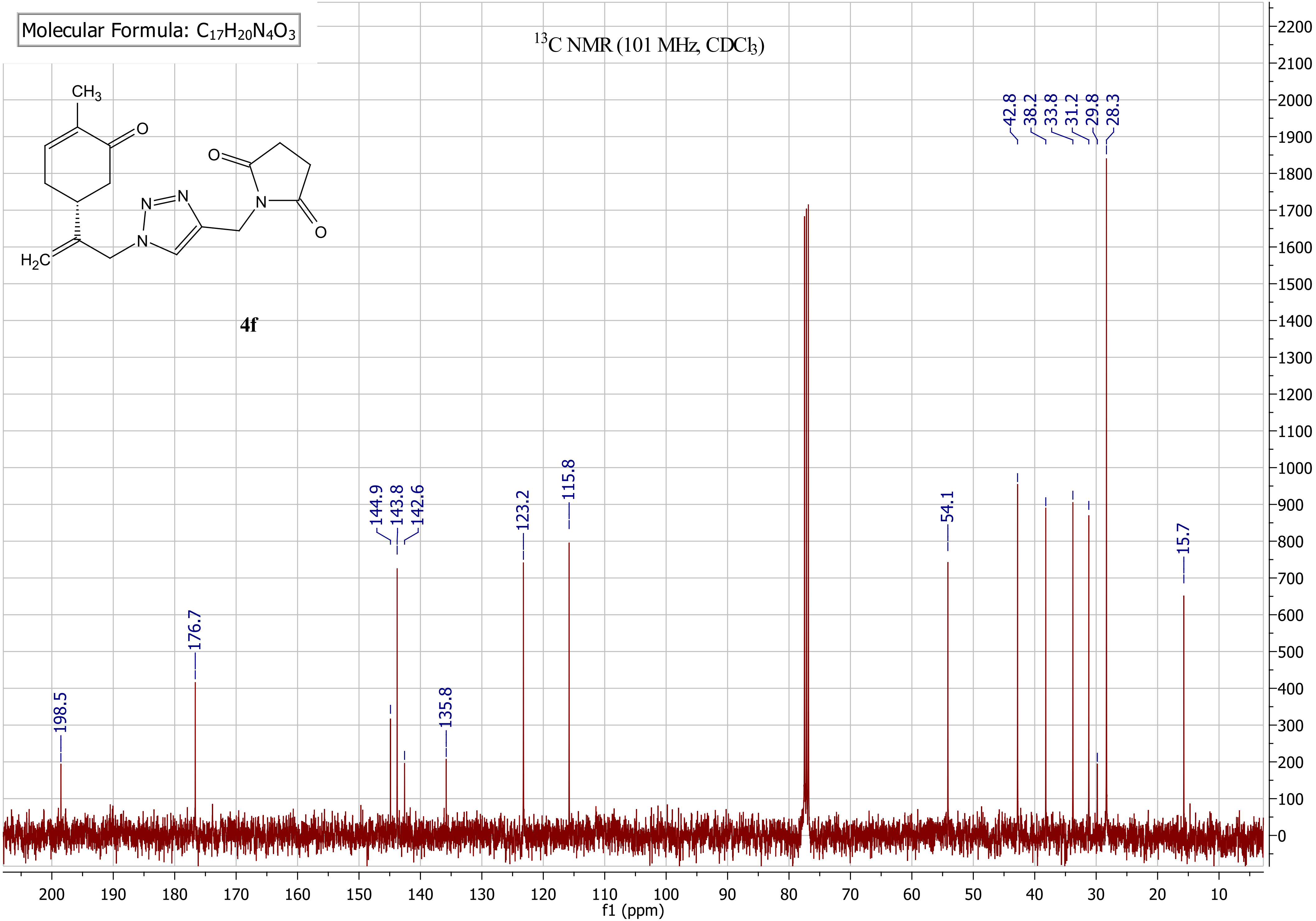
$^1$H NMR (400 MHz, $CDCl_3$)



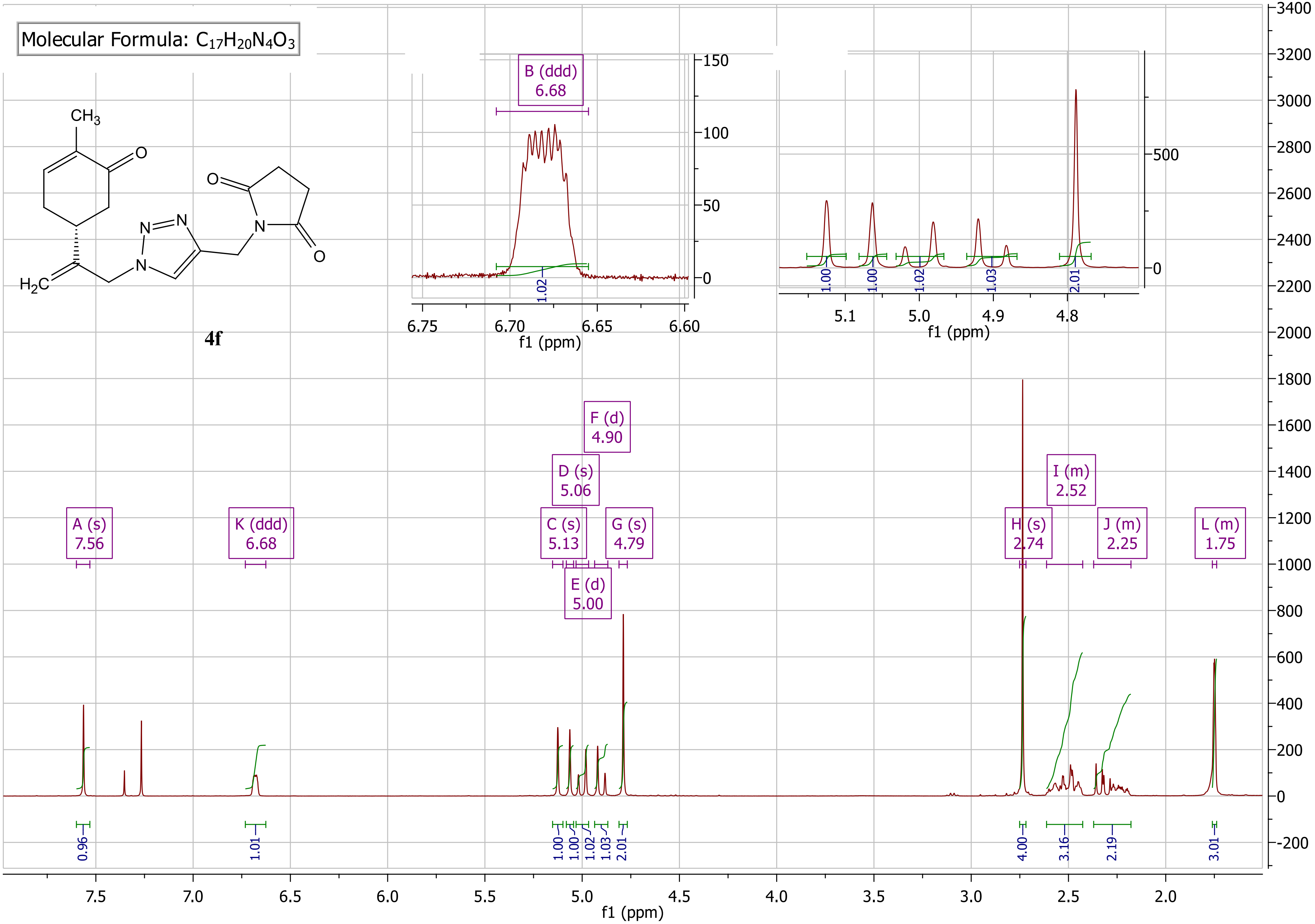
**4e**

Molecular Formula: $C_{17}H_{20}N_4O_3$

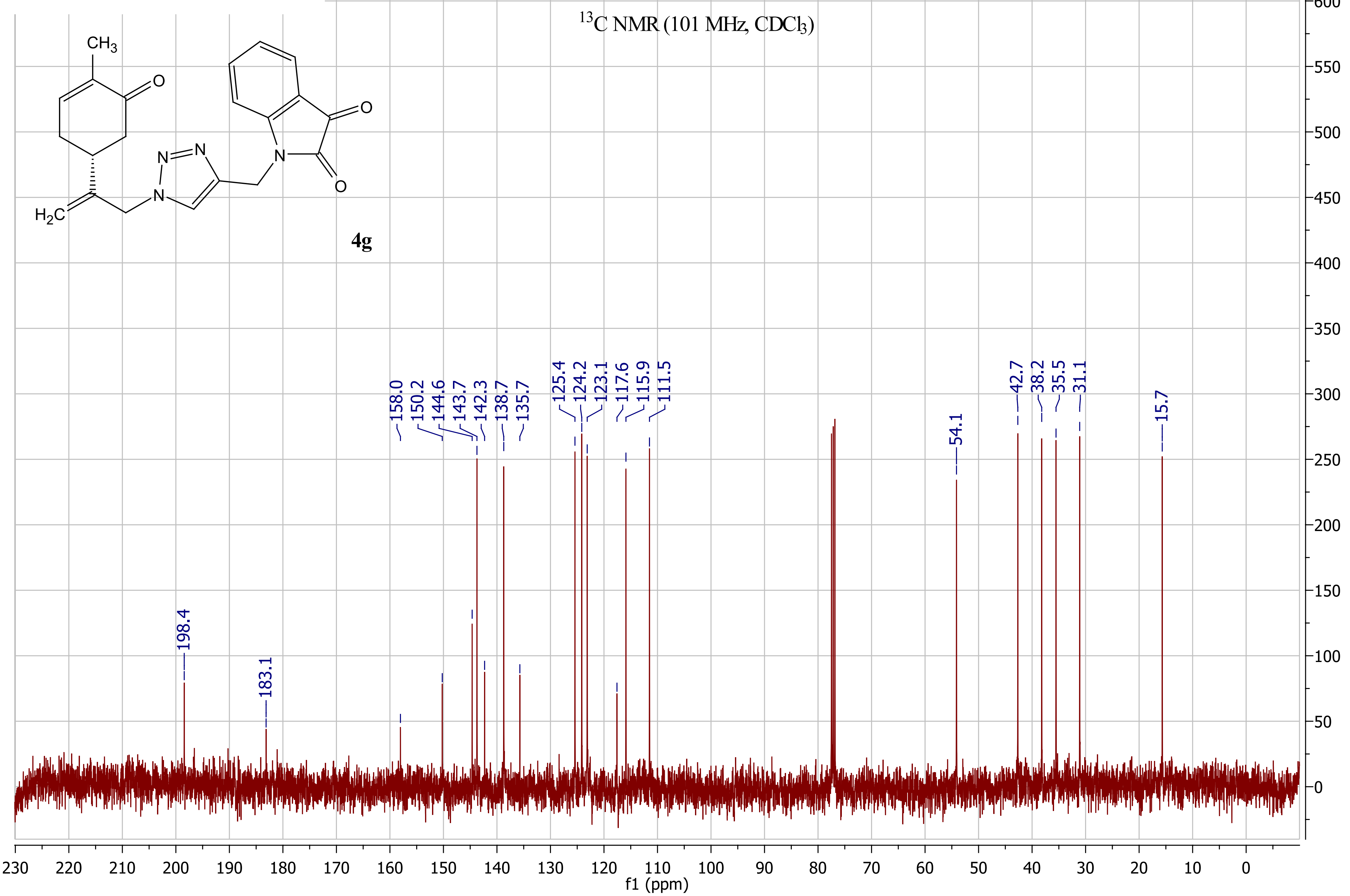
$^{13}$C NMR (101 MHz, $CDCl_3$)



**4f**

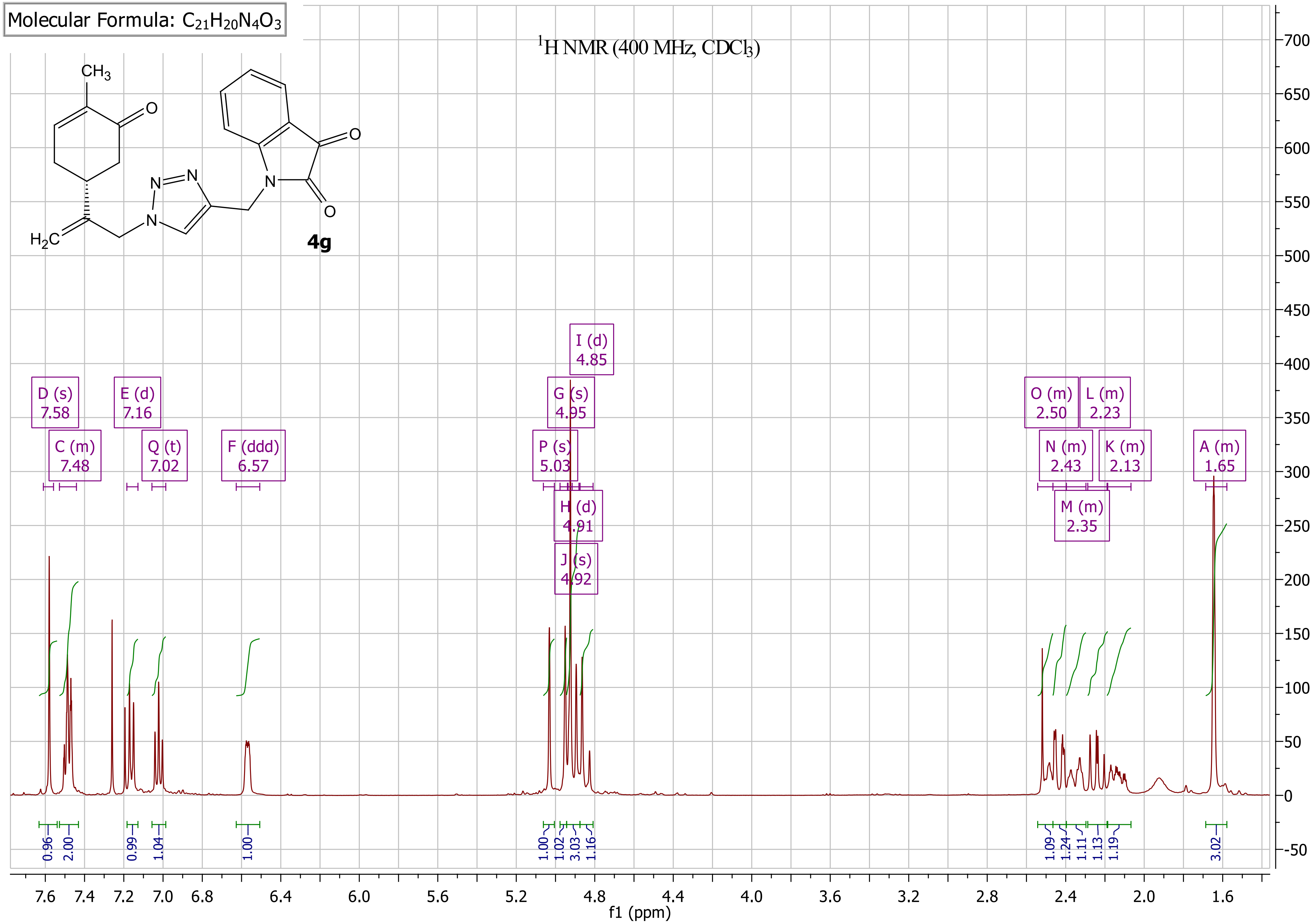

Molecular Formula: $C_{17}H_{20}N_4O_3$
$CH_3$
O
O
O
N
N=N
N
$H_2C$
4f
B (ddd)
6.68
1.02
6.75
6.70
6.65
6.60
f1 (ppm)
150
100
50
0
1.00
1.00
1.02
1.03
2.01
5.1
5.0
4.9
4.8
f1 (ppm)
500
0
F (d)
4.90
D (s)
5.06
I (m)
2.52
A (s)
7.56
K (ddd)
6.68
C (s)
5.13
G (s)
4.79
H (s)
2.74
J (m)
2.25
L (m)
1.75
E (d)
5.00
0.96
1.01
1.00
1.00
1.02
1.03
2.01
4.00
3.16
2.19
3.01
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

Molecular Formula: $C_{21}H_{20}N_4O_3$



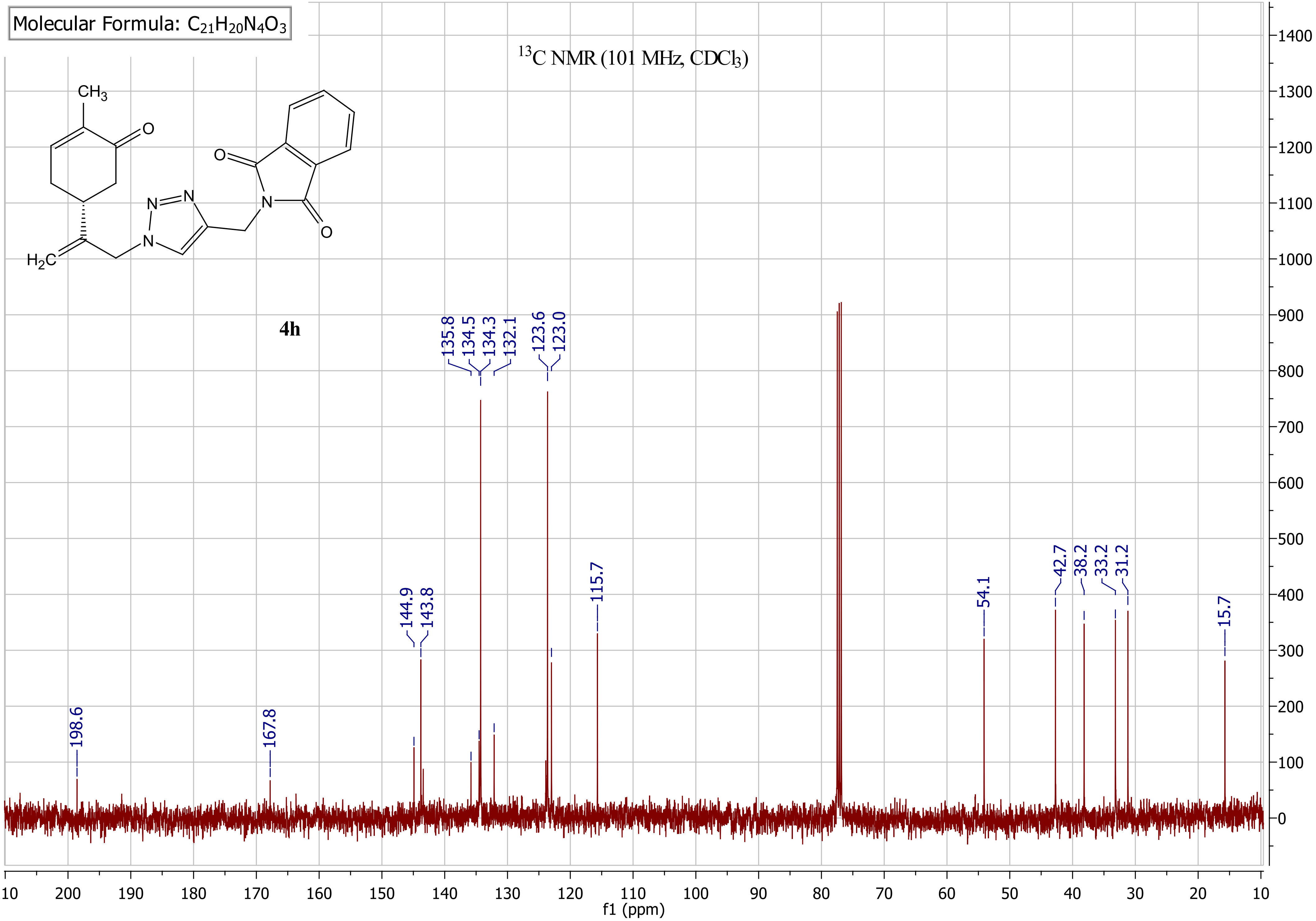
**4g**

Molecular Formula: $C_{21}H_{20}N_4O_3$



Molecular Formula: $C_{21}H_{20}N_4O_3$

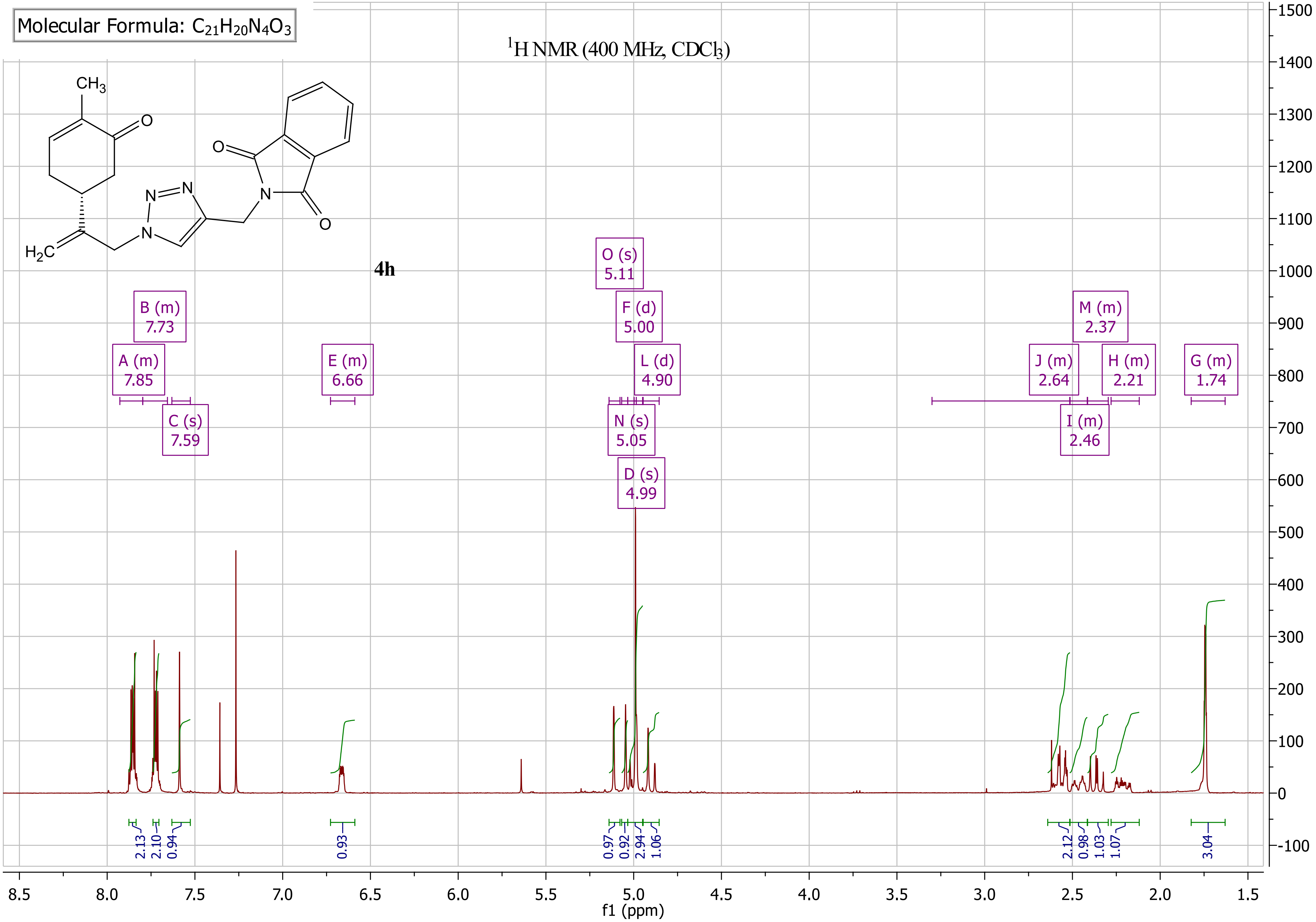
$^{13}C$ NMR (101 MHz, $CDCl_3$)



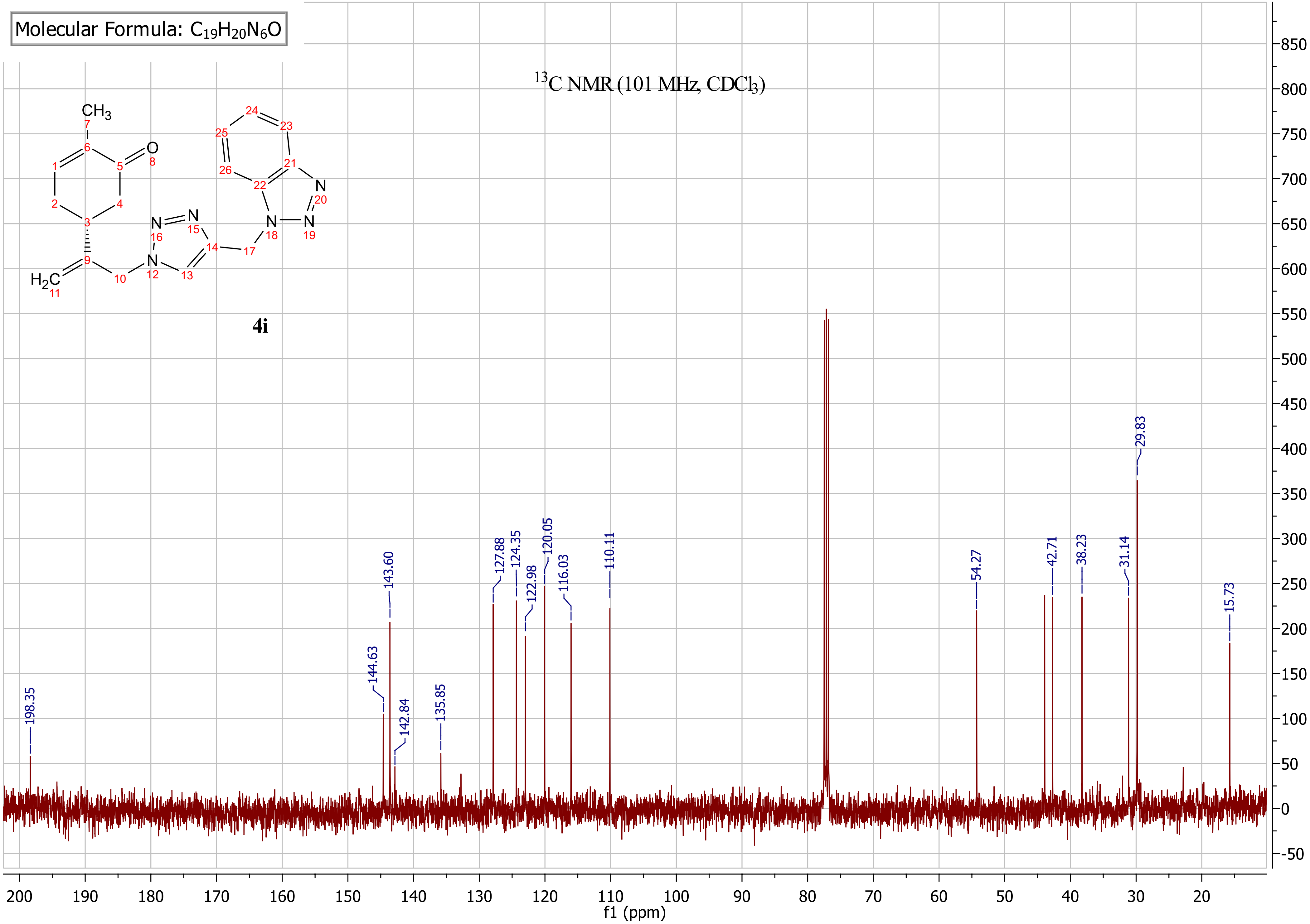
**4h**

Molecular Formula: $C_{21}H_{20}N_4O_3$

$^1H$ NMR (400 MHz, $CDCl_3$)



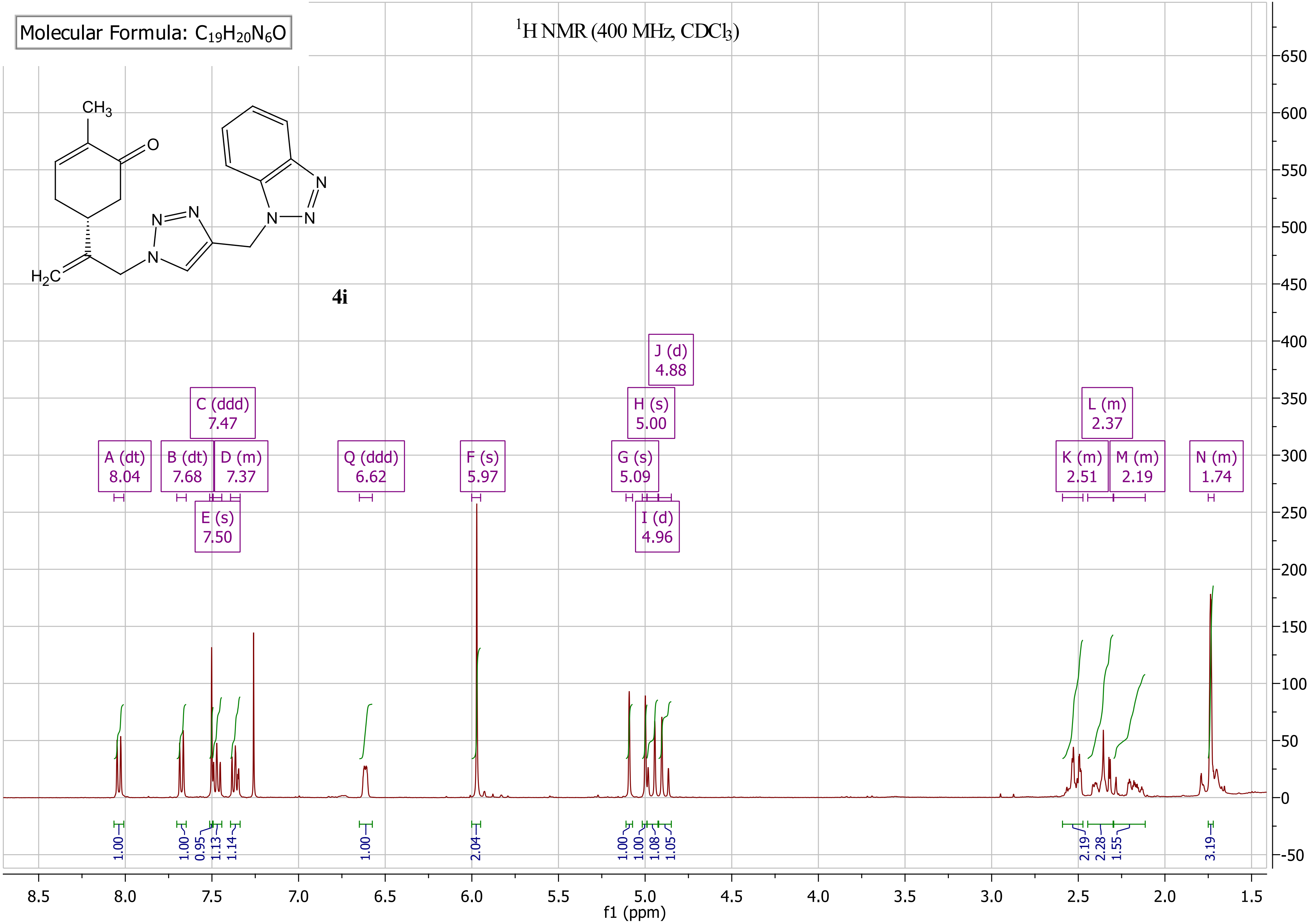
Molecular Formula: $C_{19}H_{20}N_6O$



**4i**

Molecular Formula: $C_{19}H_{20}N_6O$

$^1H$ NMR (400 MHz, $CDCl_3$)



**4i**