

## **Synthesis, Molecular Docking, and Biological Evaluation of Novel 2-Pyrazoline Derivatives as Multifunctional Agents for the Treatment of Alzheimer's Disease**

Oya Unsal-Tan<sup>a\*</sup>, Tuba Tüylü Küçükkılınç<sup>b</sup>, Beyza Ayazgök<sup>b</sup>, Ayla Balkan<sup>a</sup>, Keriman Ozadali-Sari<sup>a</sup>

<sup>a</sup> Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY.

<sup>b</sup> Hacettepe University, Faculty of Pharmacy, Department of Biochemistry, Ankara, TURKEY.

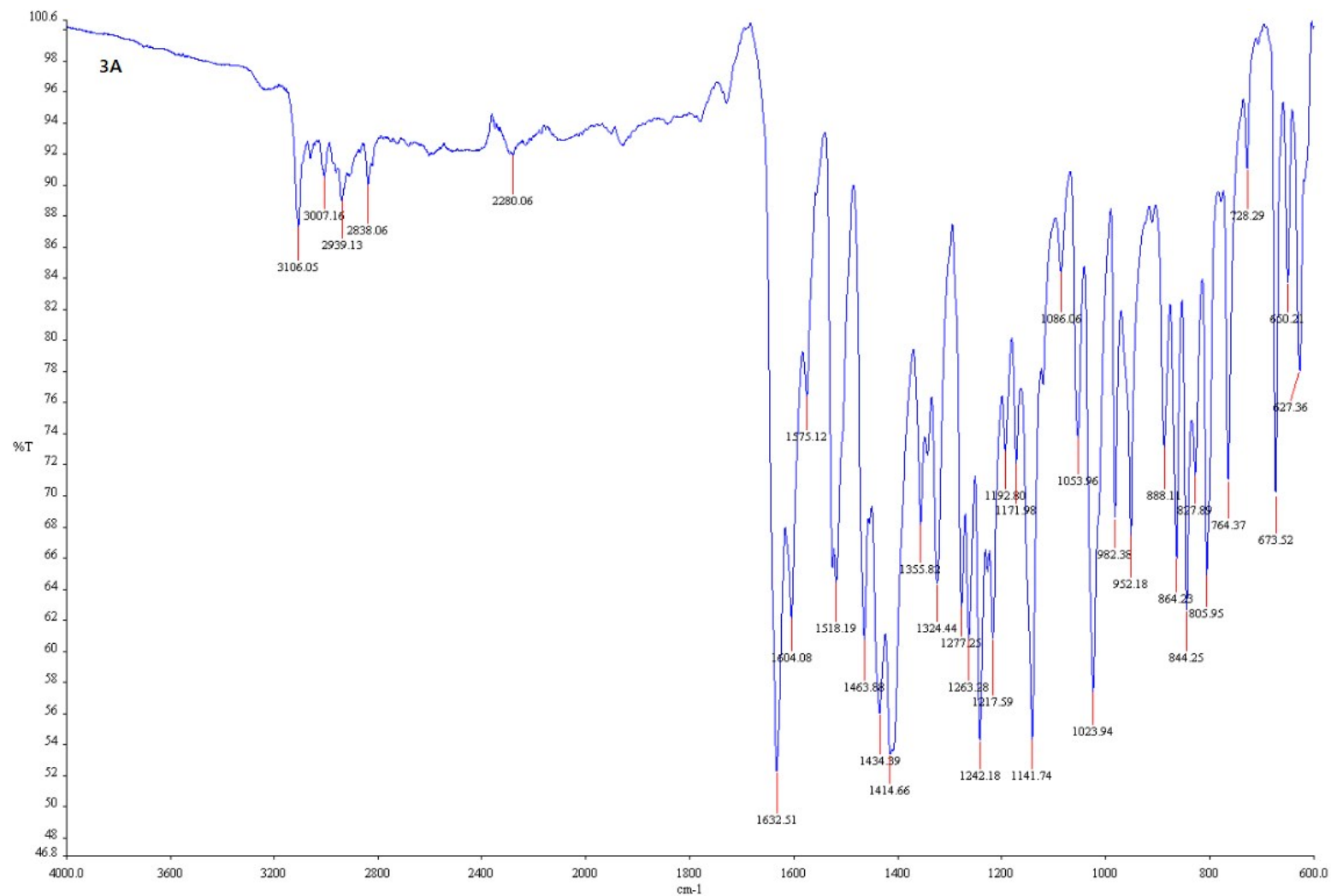
✉ Dr. Oya Unsal Tan  
oyaunsal@hacettepe.edu.tr

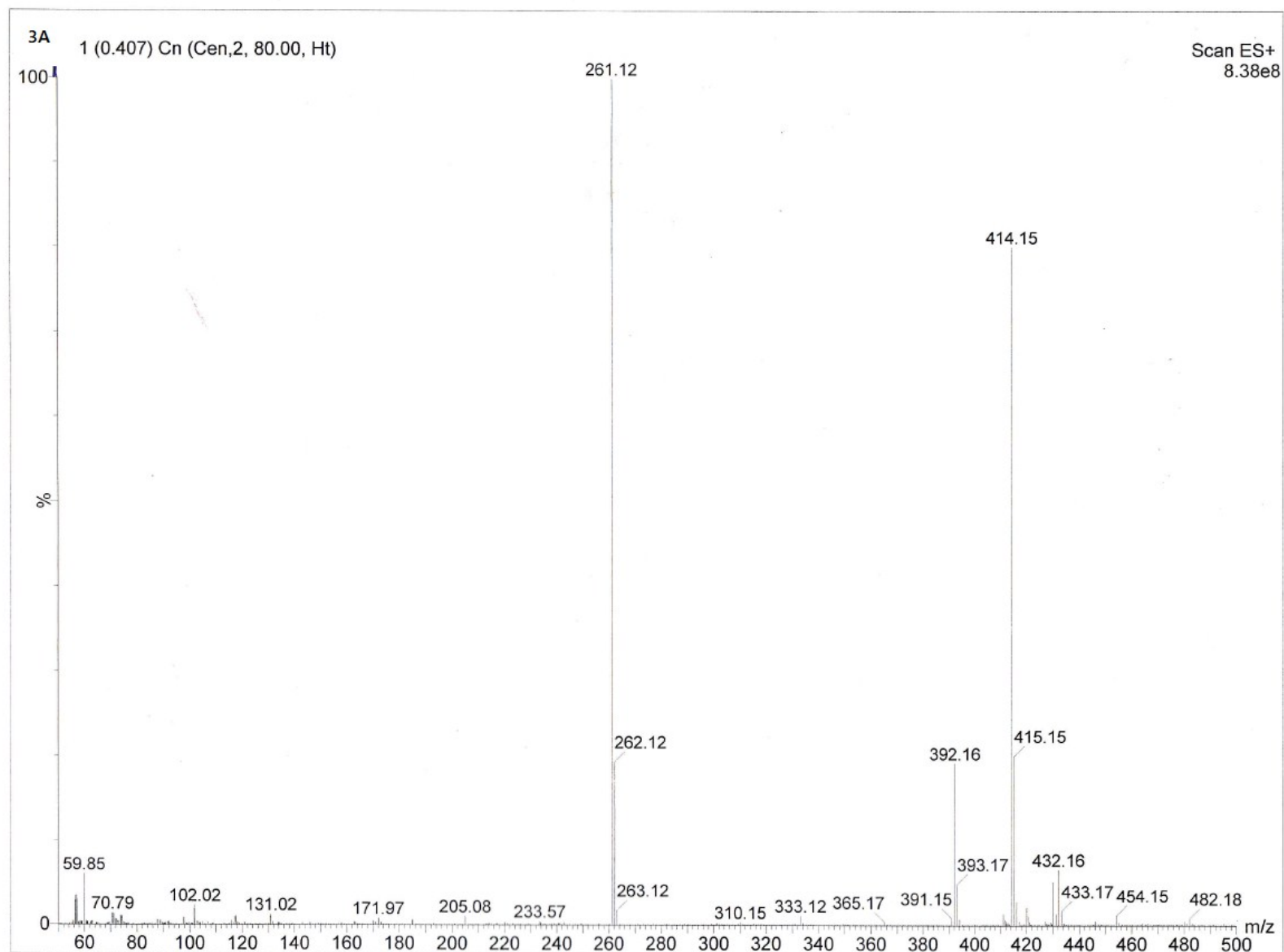
### **SUPPORTING FILE**

- 1) IR, ESI-MS, <sup>1</sup>H and <sup>13</sup>C- NMR SPECTRA OF THE COMPOUNDS (p 2-43)
- 2) DOSE-RESPONSE CURVES OF 3F AND 3G (p 44-45)
- 3) INHIBITION OF SELF-MEDIATED A $\beta$ <sub>1-42</sub> AGGREGATION at 10  $\mu$ M (p 46)

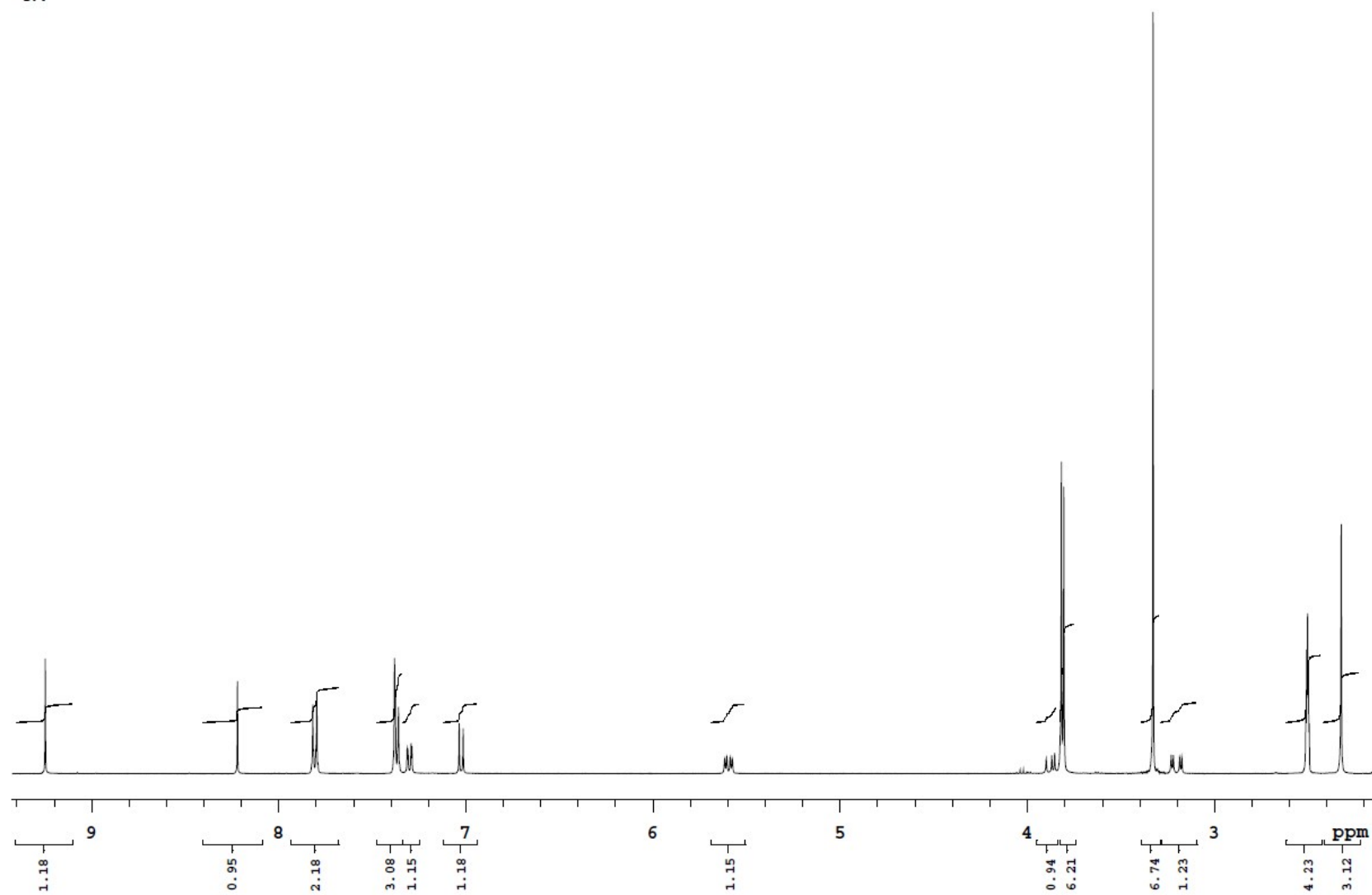
## 1) IR, ESI-MS, $^1\text{H}$ and $^{13}\text{C}$ - NMR SPECTRA OF THE COMPOUNDS

### 1-(5-(4-(1*H*-1,2,4-Triazol-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (3a)





3A



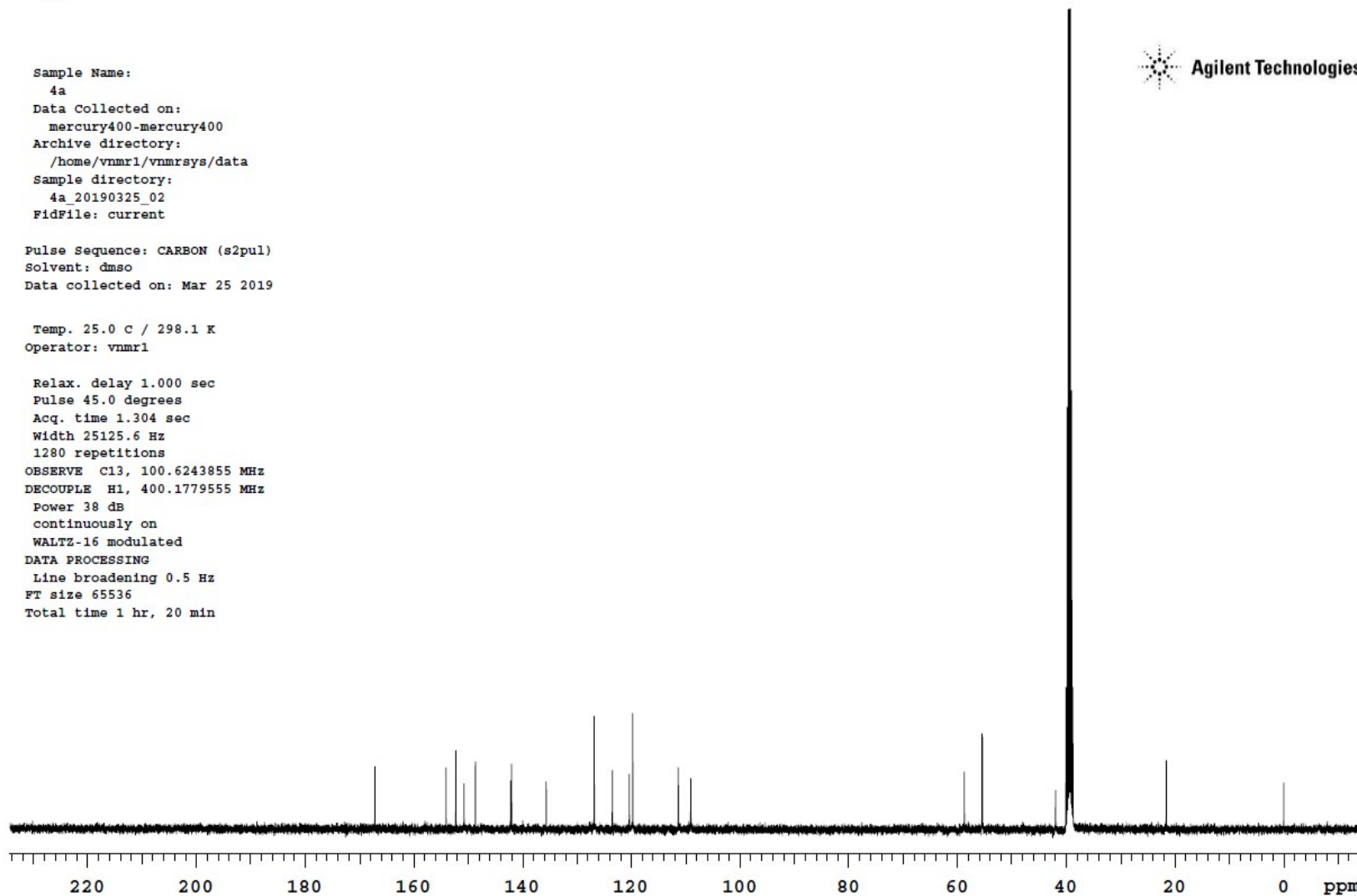
3A

Sample Name:  
4a  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4a\_20190325\_02  
FidFile: current

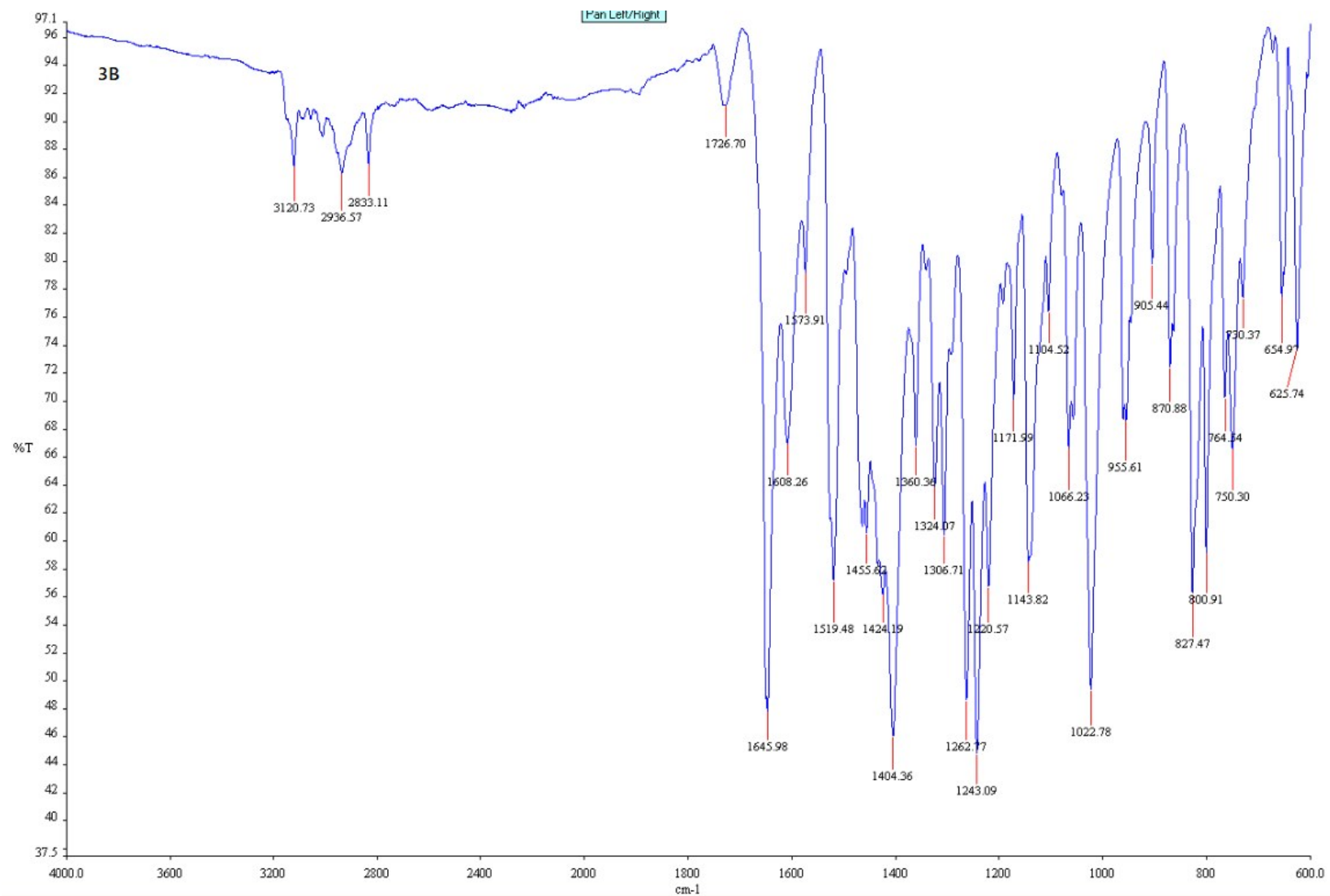
Pulse Sequence: CARBON (s2pul)  
Solvent: dmsc  
Data collected on: Mar 25 2019

Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
1280 repetitions  
OBSERVE C13, 100.6243855 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 1 hr, 20 min



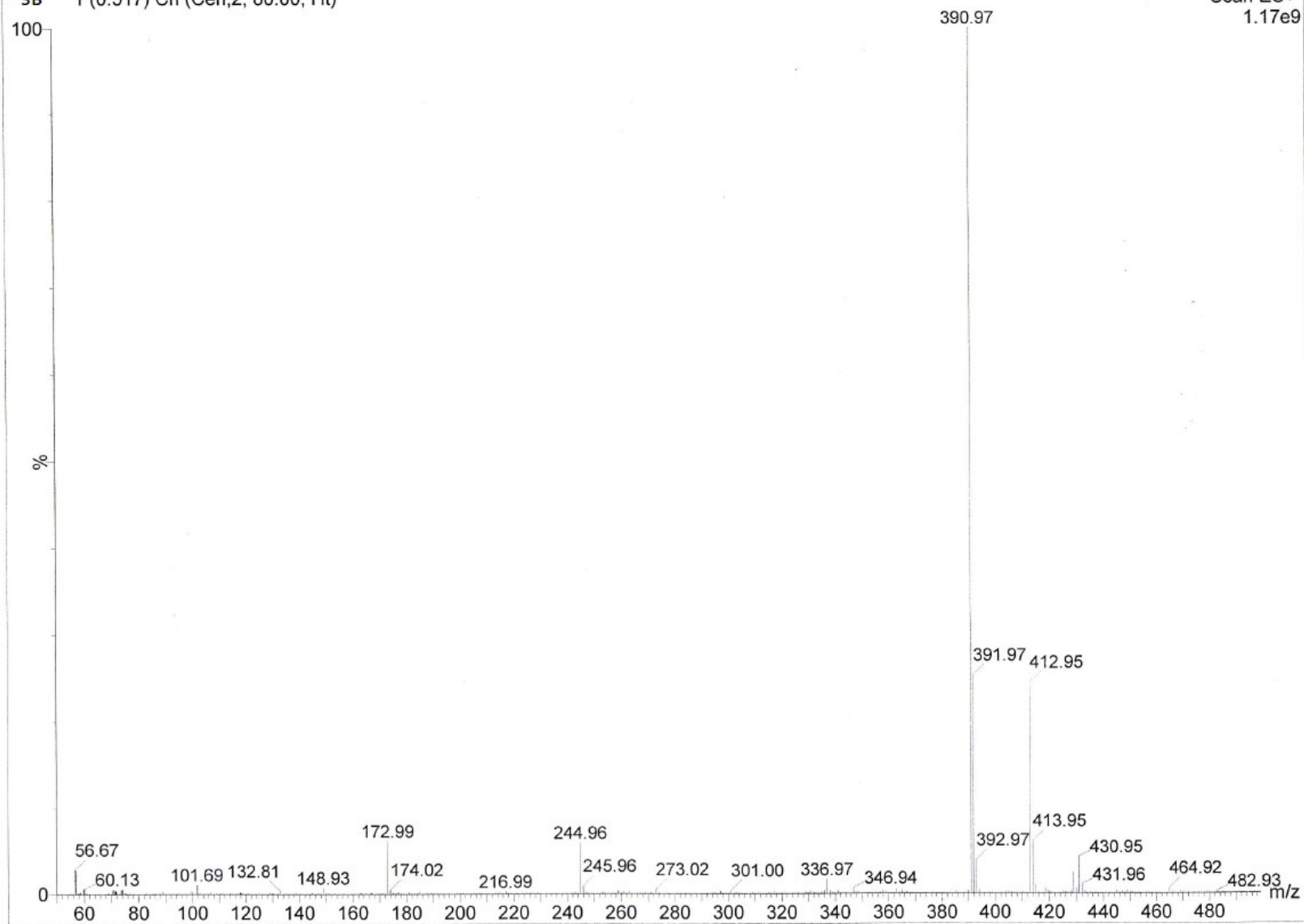
**1-(5-(4-(1*H*-Imidazol-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (3b)**



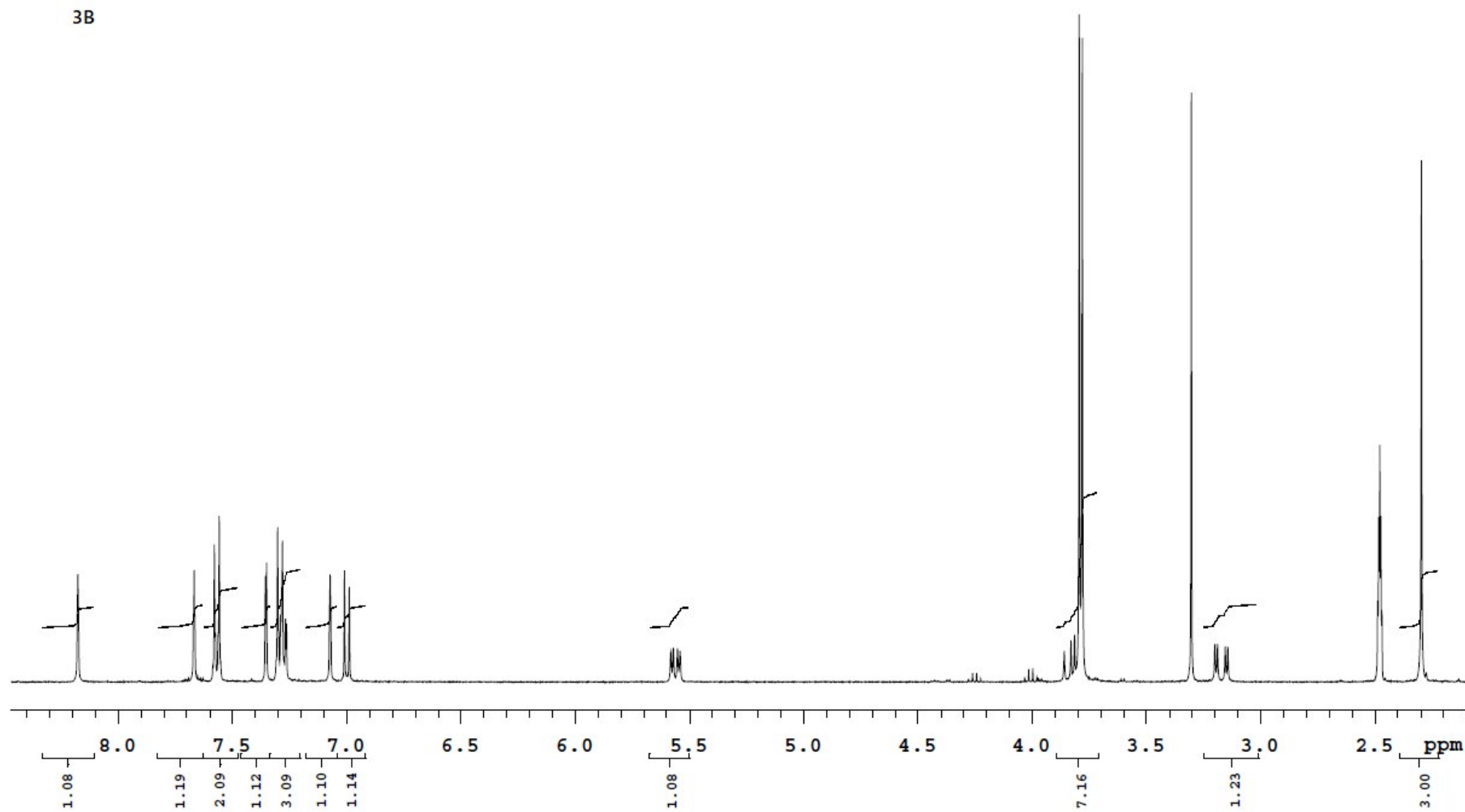
**Synthesis Product**

3B 1 (0.517) Cn (Cen,2, 80.00, Ht)

Scan ES+  
1.17e9



3B





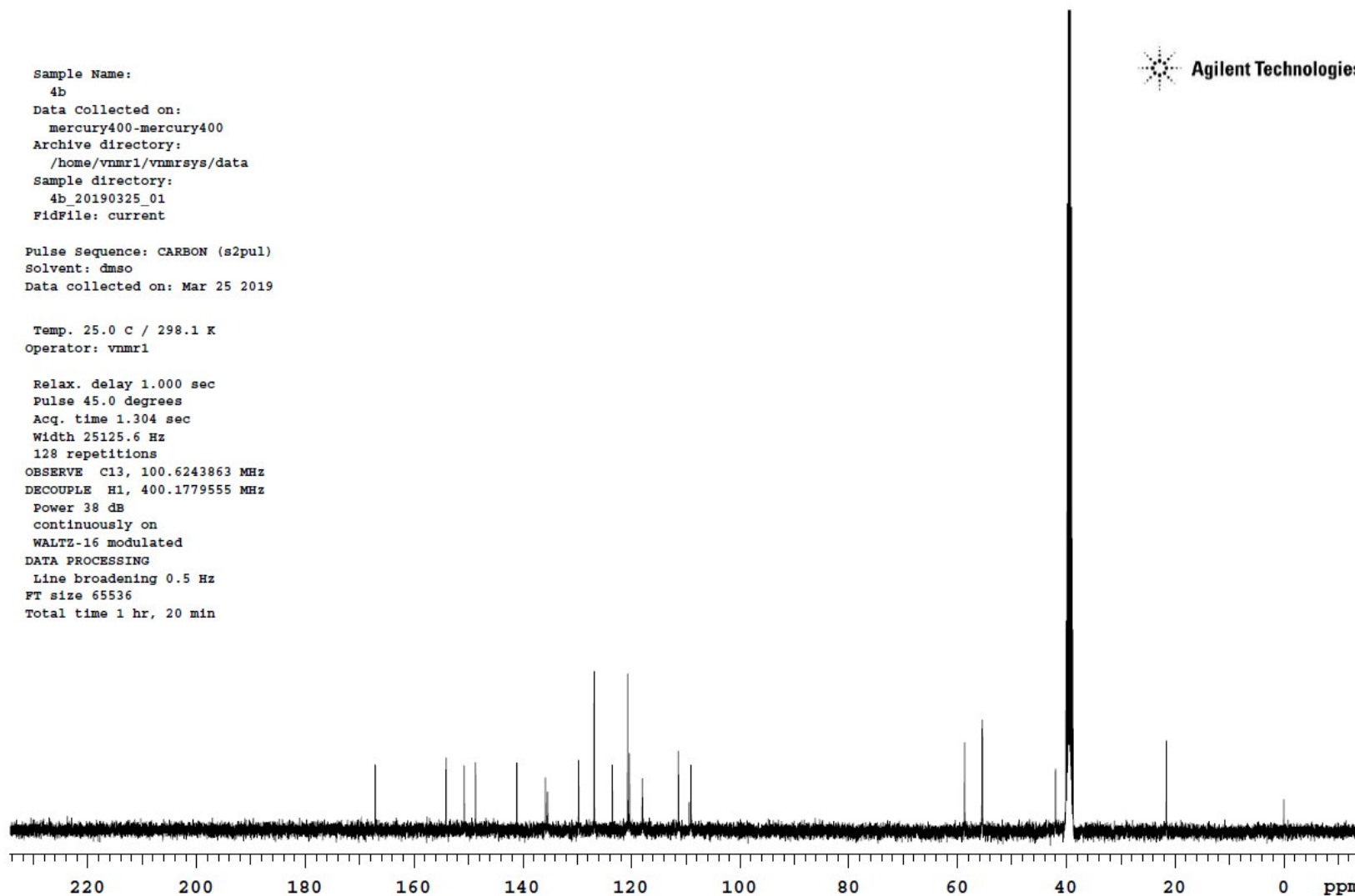
3B

Sample Name:  
4b  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4b\_20190325\_01  
FidFile: current

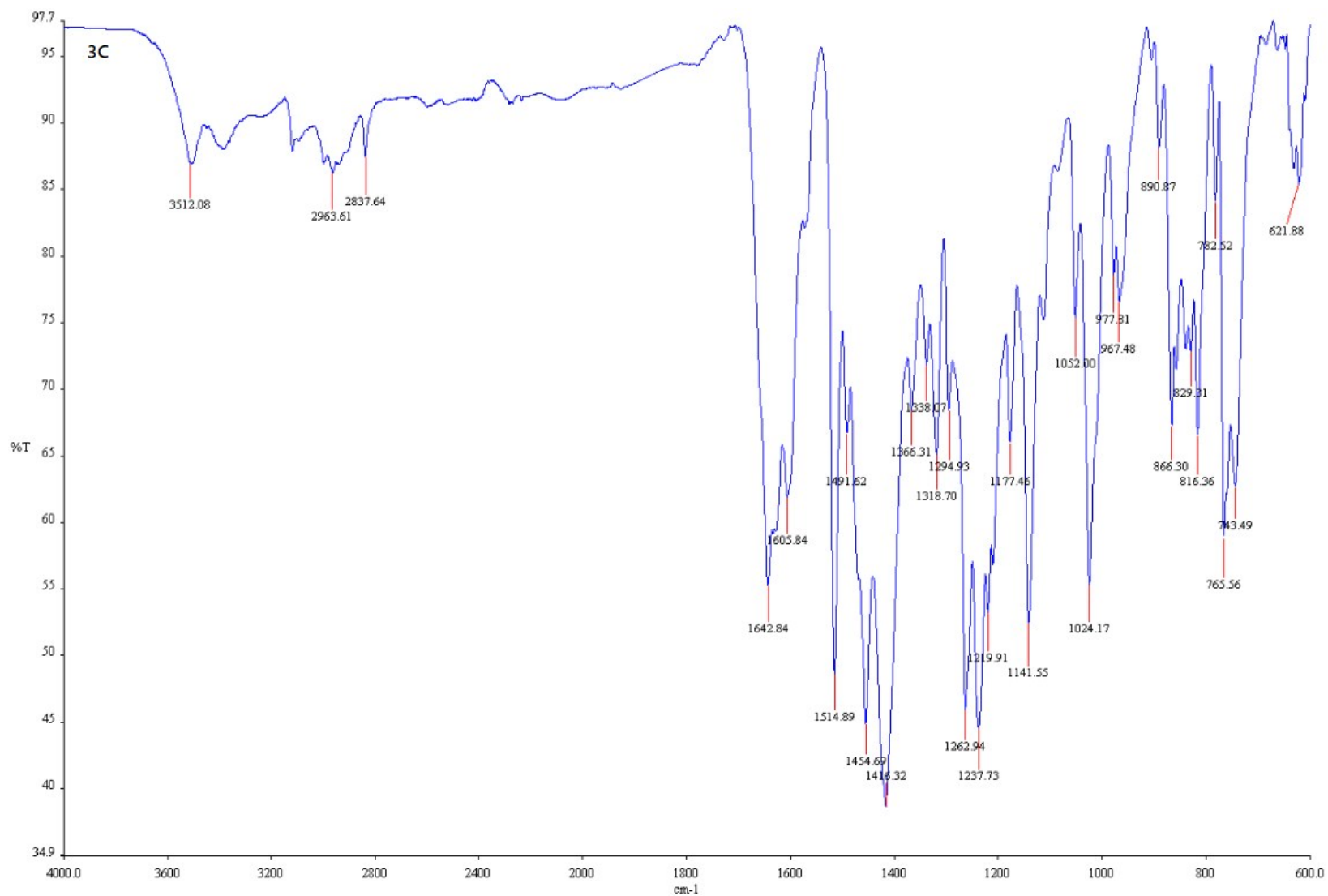
Pulse Sequence: CARBON (s2pul)  
Solvent: dmsc  
Data collected on: Mar 25 2019

Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
128 repetitions  
OBSERVE C13, 100.6243863 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 1 hr, 20 min



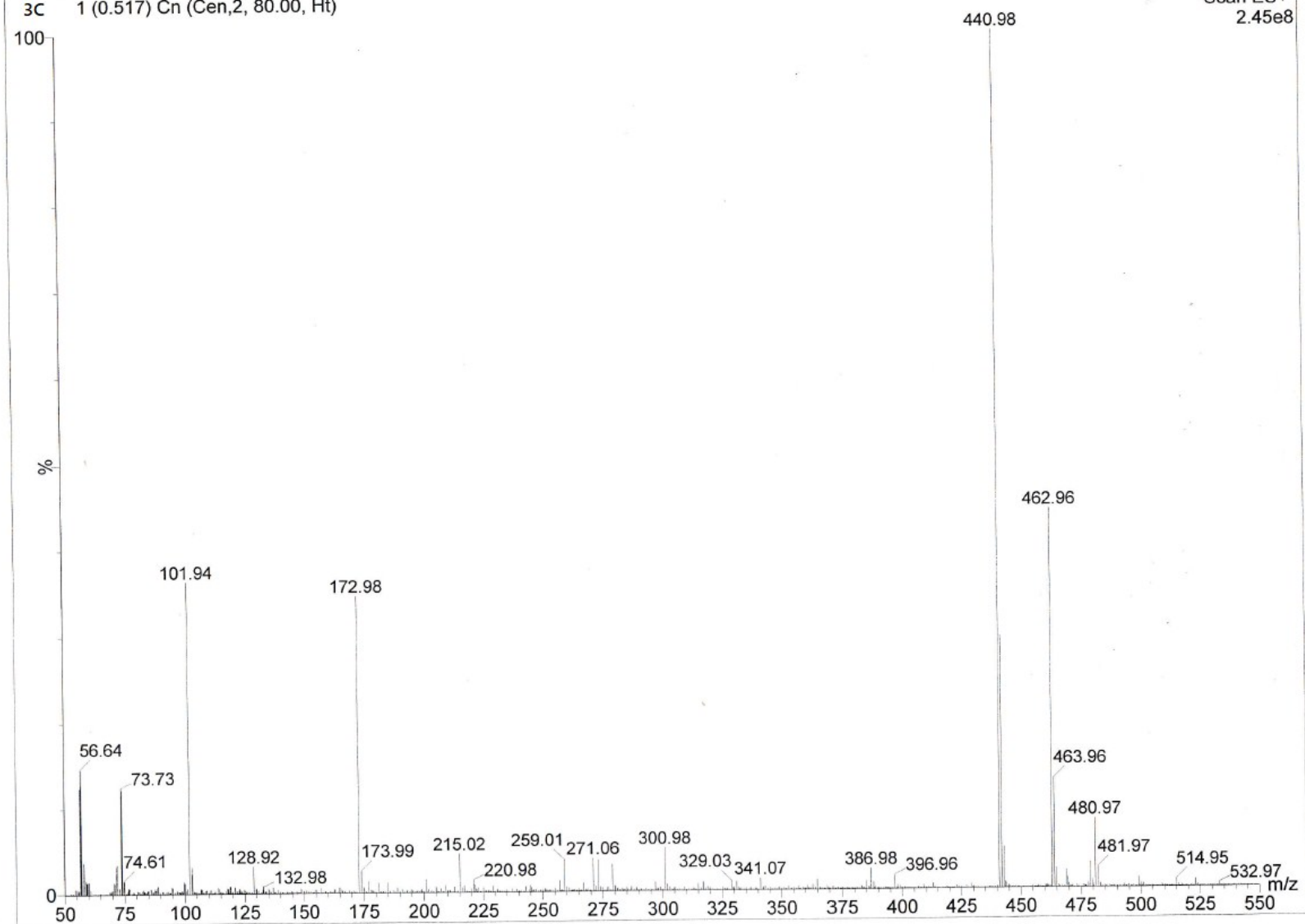
**1-(5-(4-(1*H*-Benzo[*d*]imidazol-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (3c)**



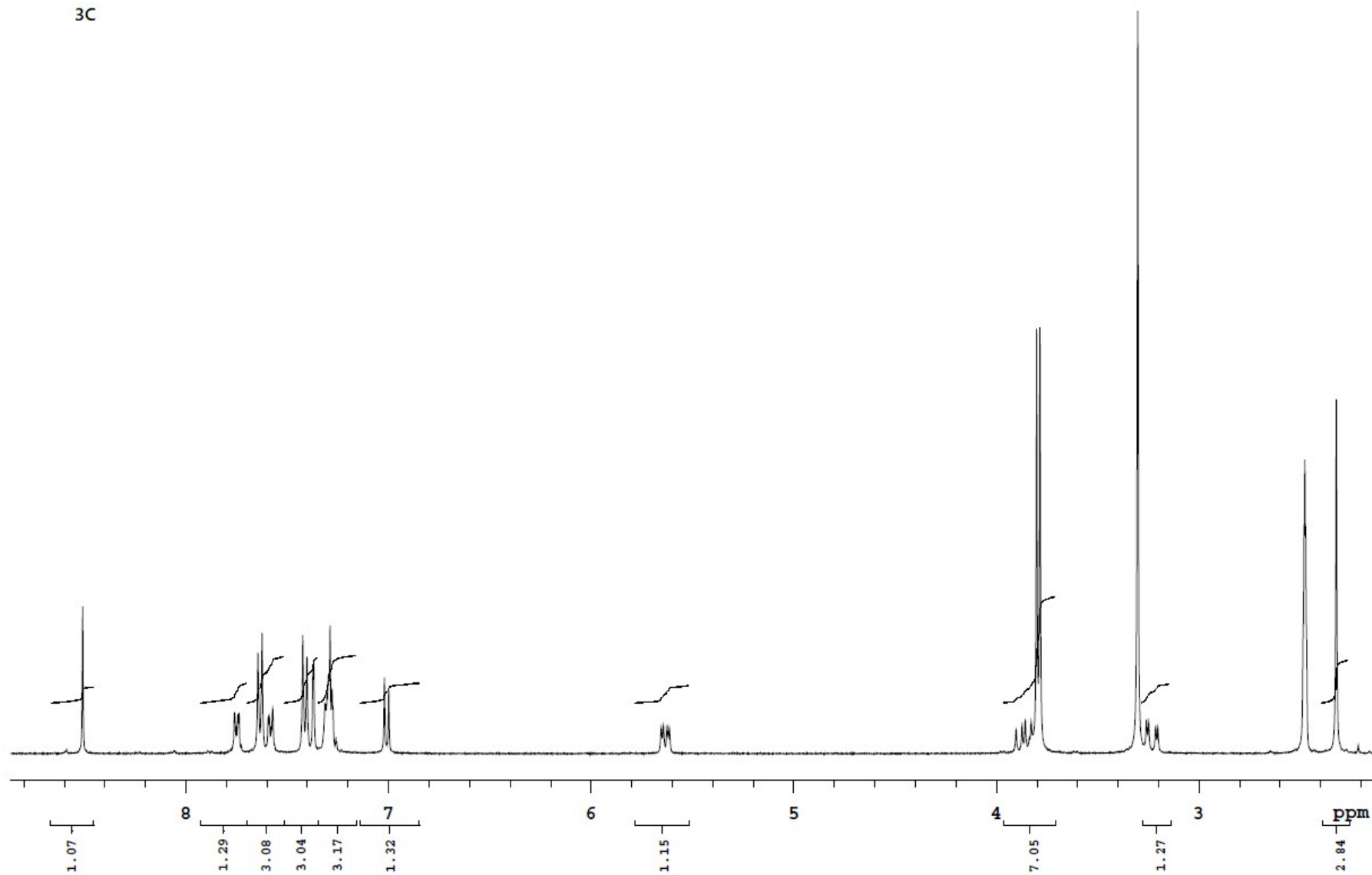
**Synthesis Product**

3C 1 (0.517) Cn (Cen,2, 80.00, Ht)

Scan ES+  
2.45e8



3C



3C

Sample Name:

4c

Data Collected on:

mercury400-mercury400

Archive directory:

/home/vnmr1/vnmrsys/data

Sample directory:

4c\_20190326\_01

FidFile: CARBON\_01

Pulse Sequence: CARBON (s2pul)

Solvent: dmsd

Data collected on: Mar 26 2019

Temp. 25.0 c / 298.1 K

Operator: vnmr1

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.304 sec

Width 25125.6 Hz

2000 repetitions

OBSERVE C13, 100.6243855 MHz

DECOUPLE H1, 400.1779555 MHz

Power 38 dB

continuously on

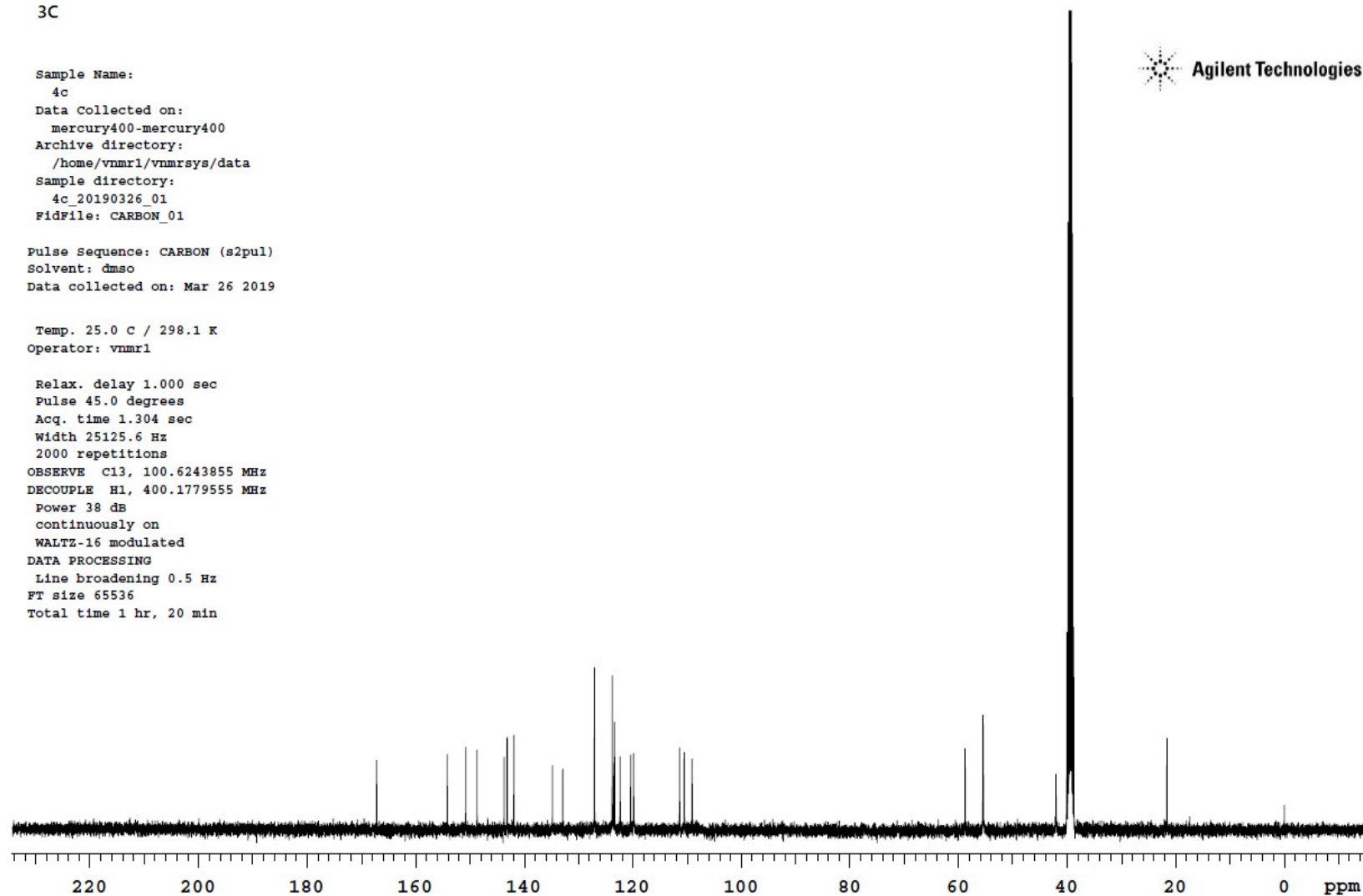
WALTZ-16 modulated

DATA PROCESSING

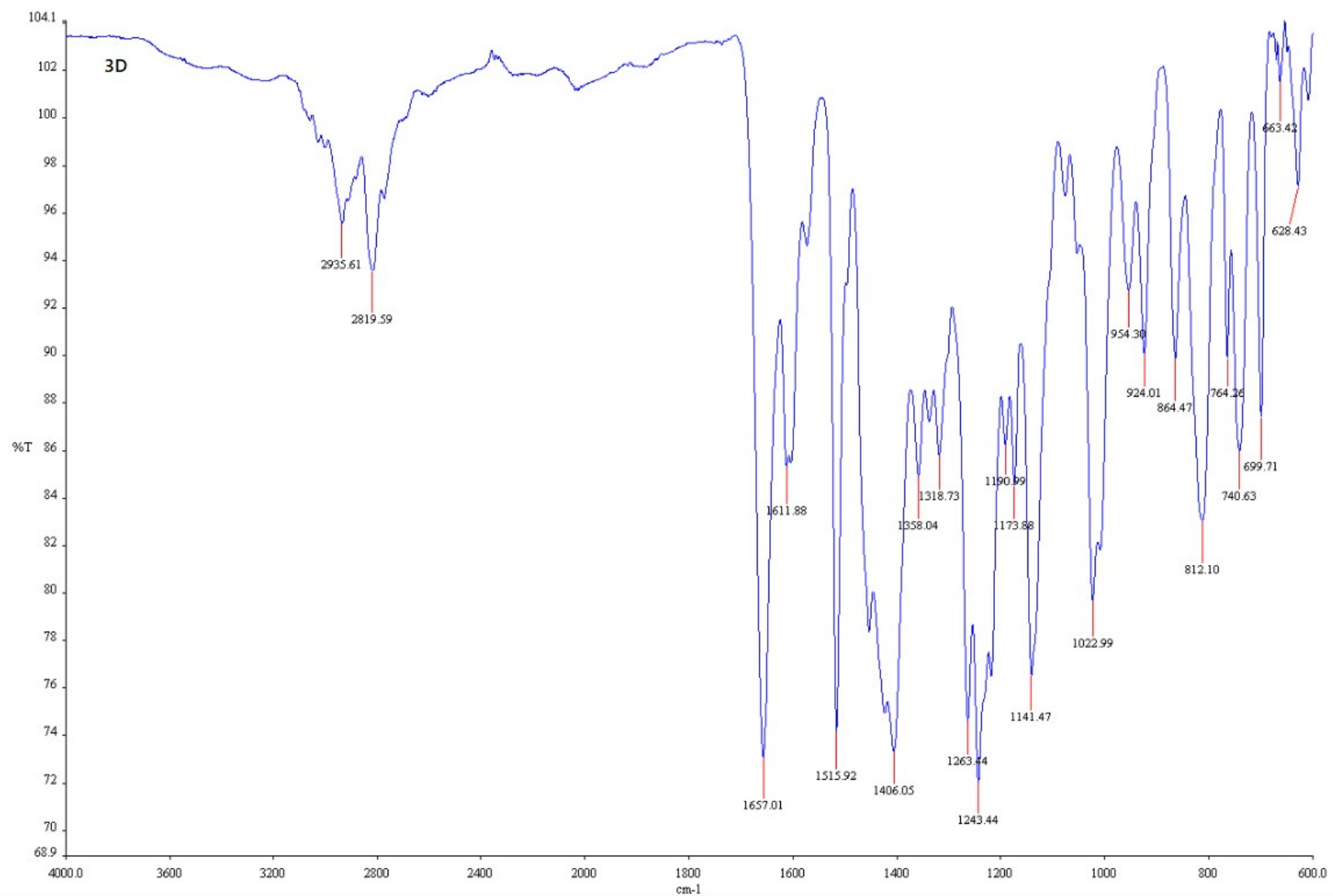
Line broadening 0.5 Hz

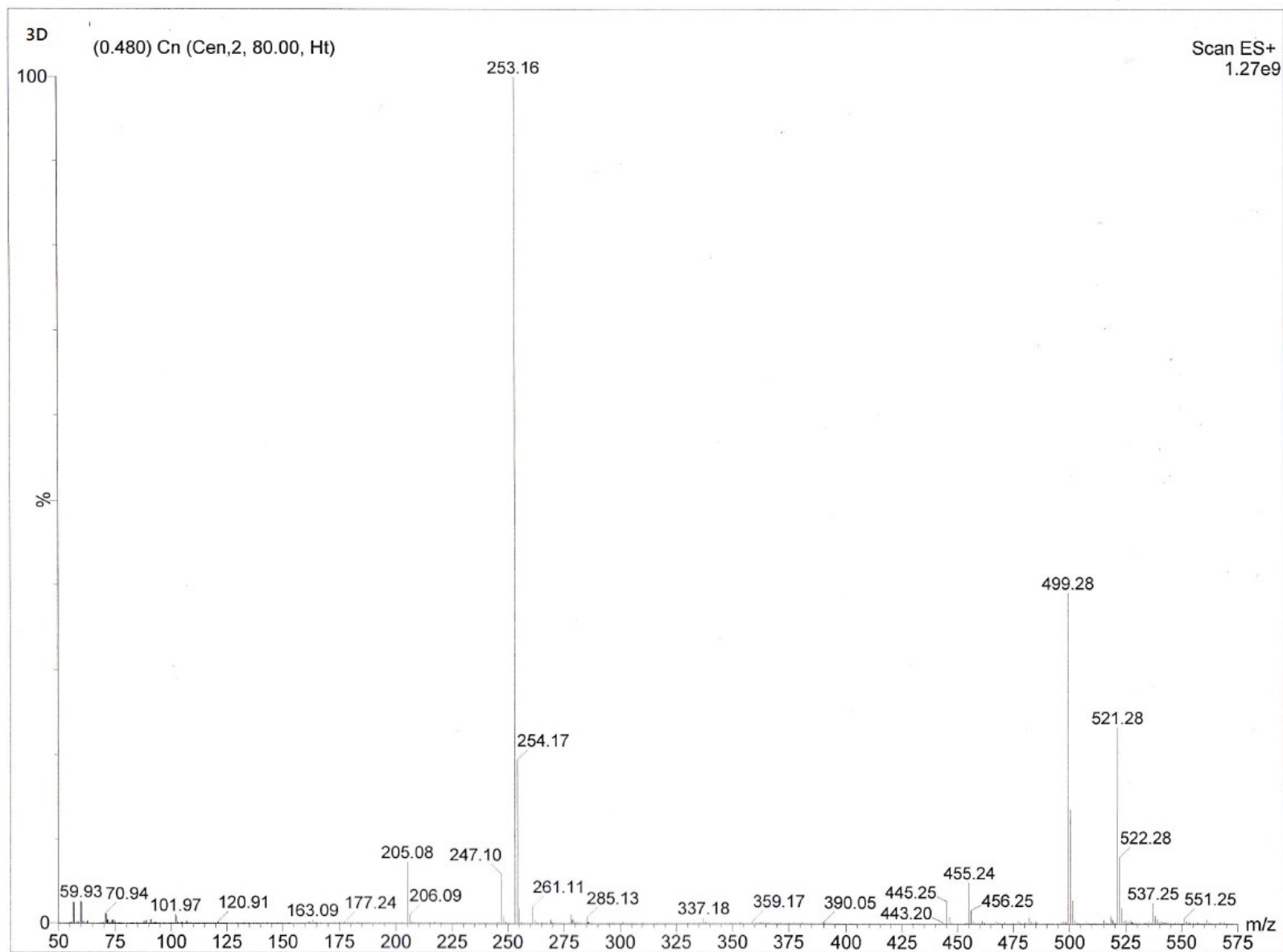
FT size 65536

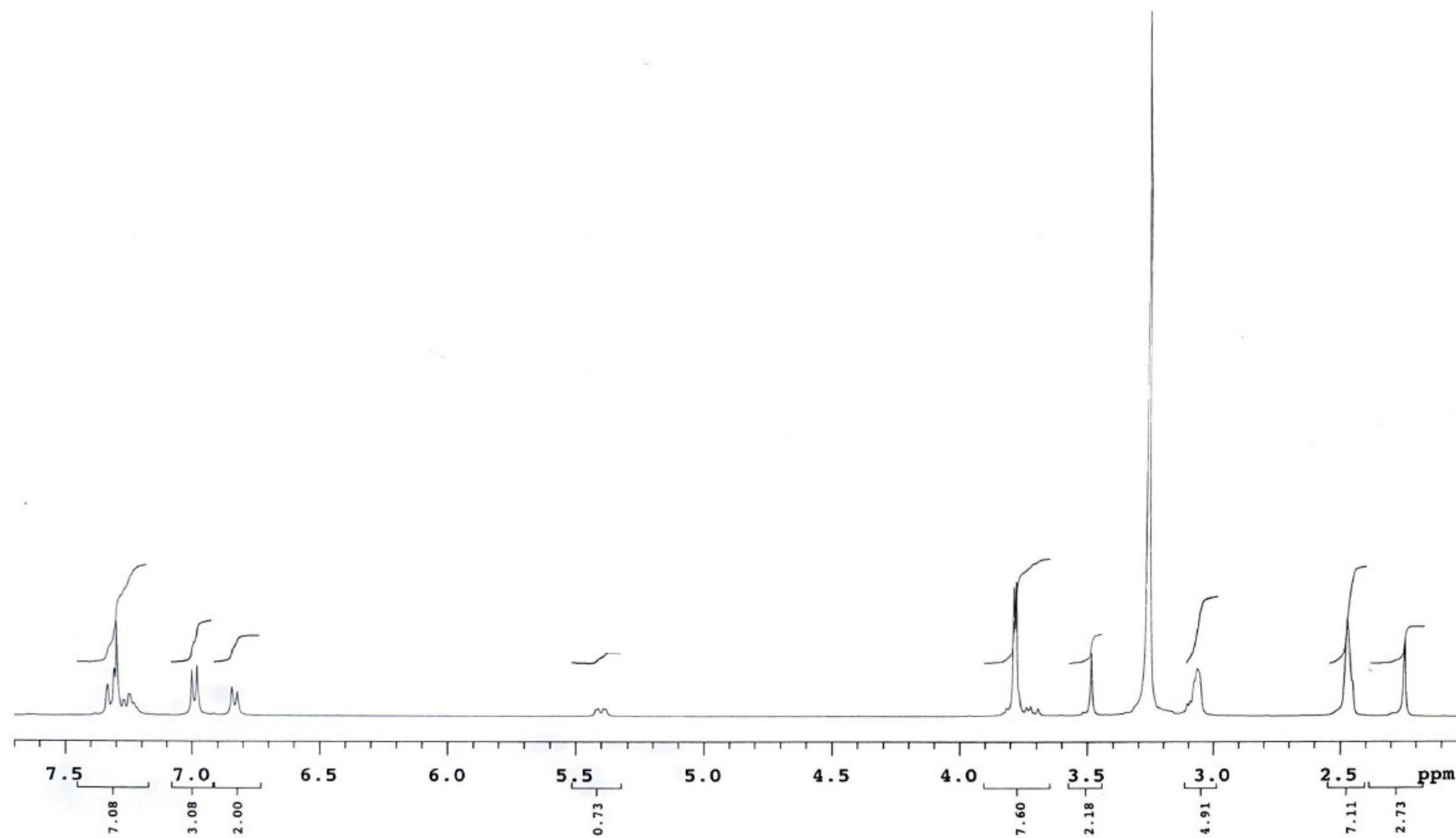
Total time 1 hr, 20 min



**1-(5-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3d)**









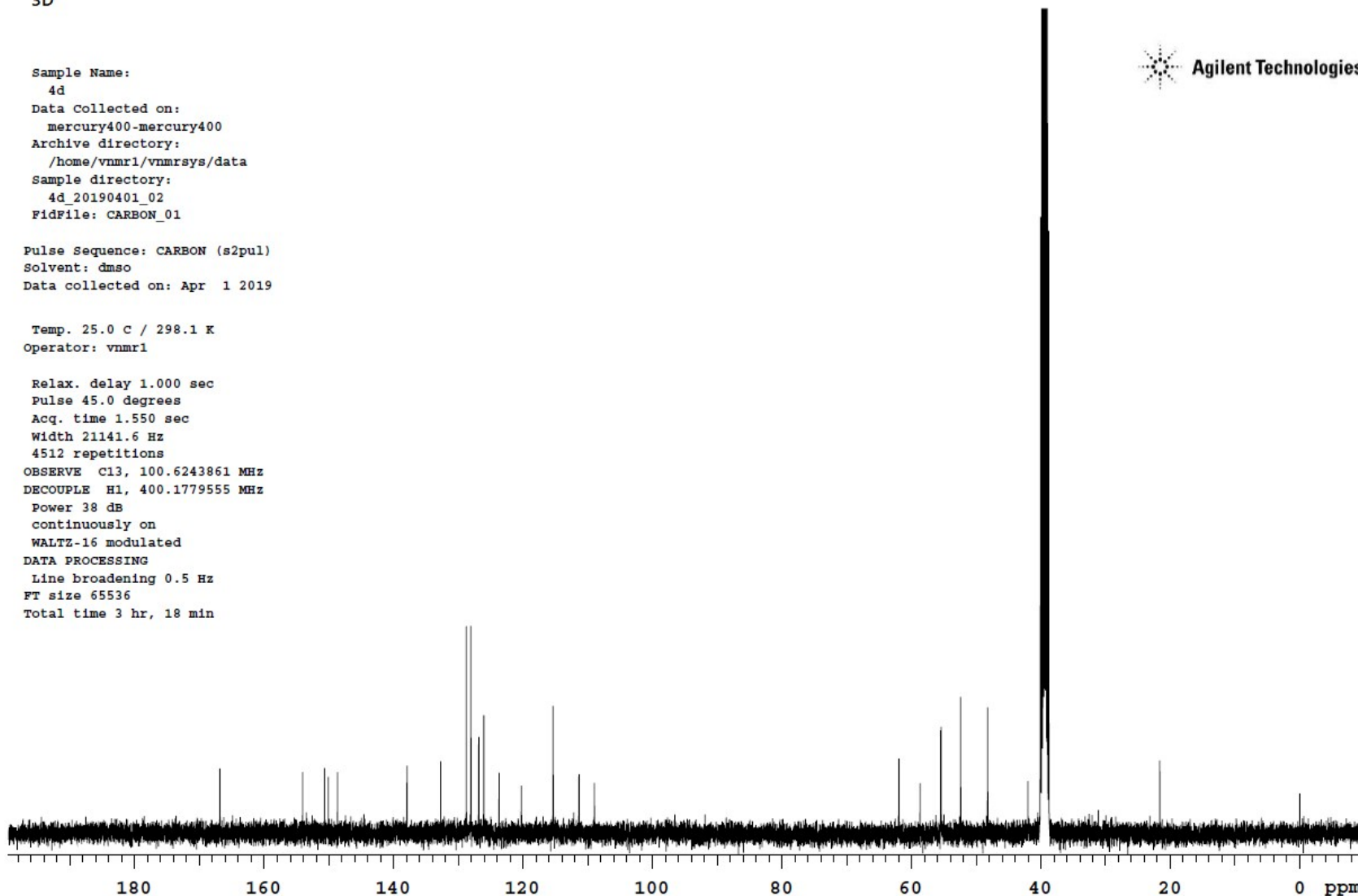
3D

Sample Name:  
4d  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4d\_20190401\_02  
FidFile: CARBON\_01

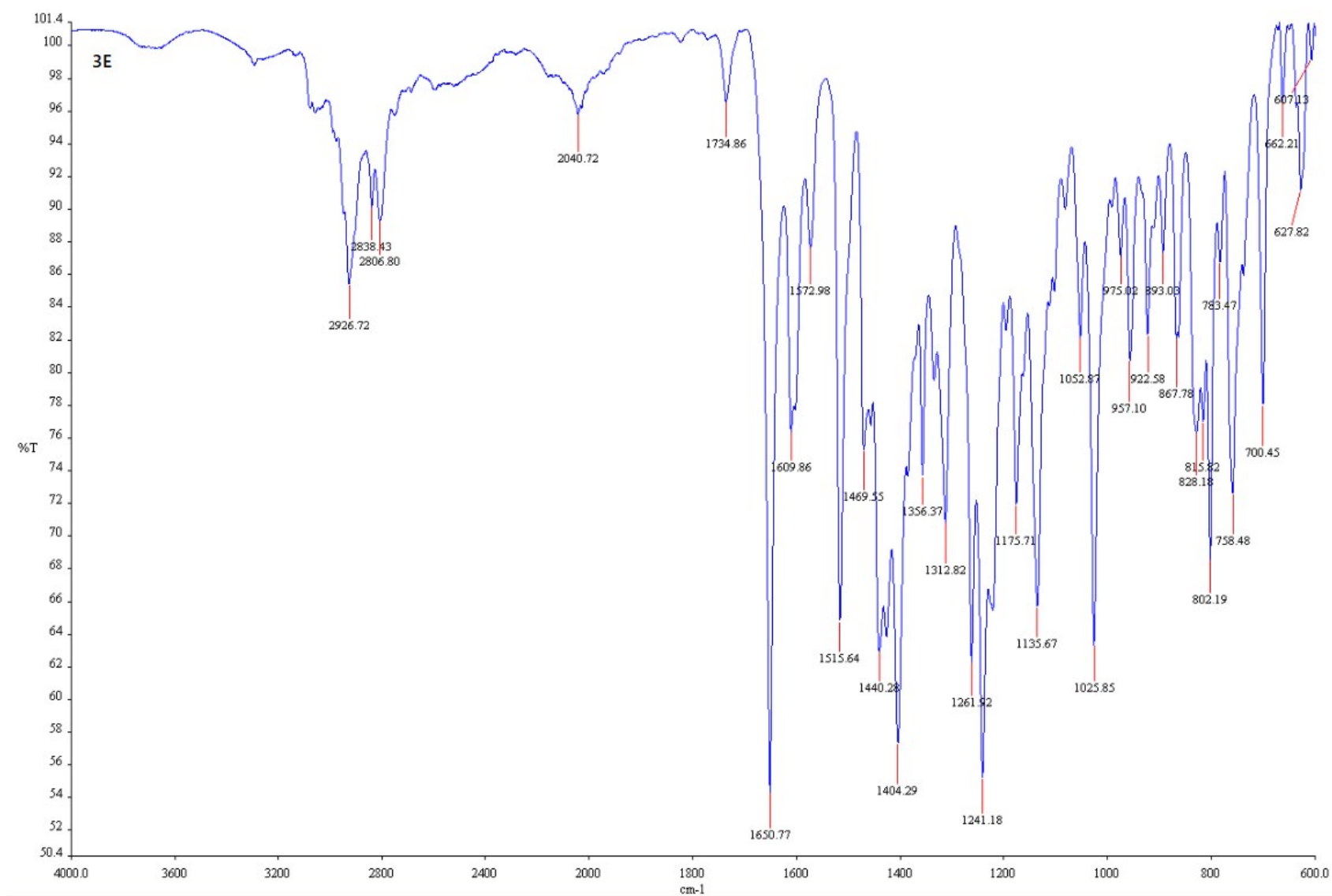
Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Apr 1 2019

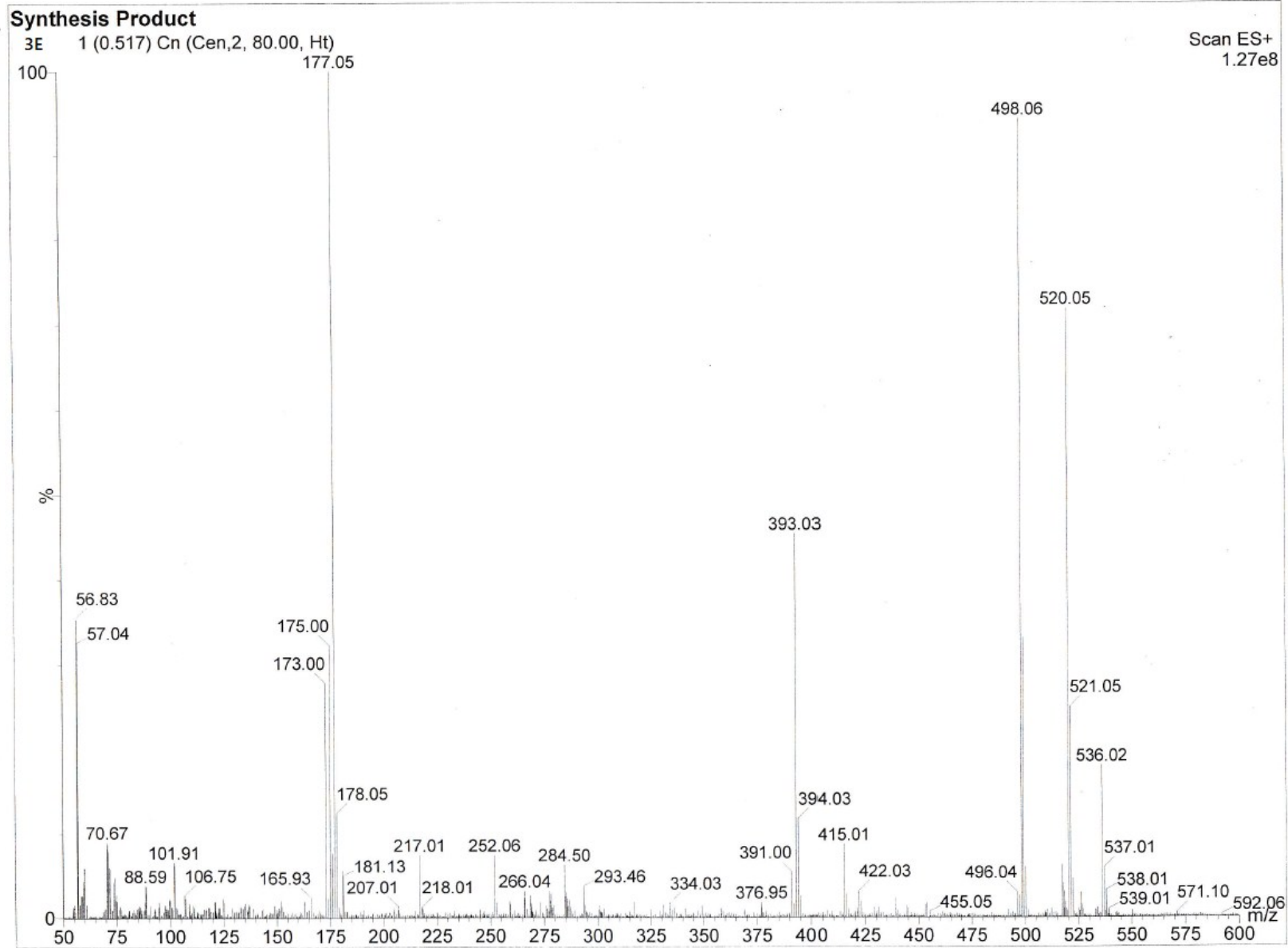
Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.550 sec  
Width 21141.6 Hz  
4512 repetitions  
OBSERVE c13, 100.6243861 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 3 hr, 18 min

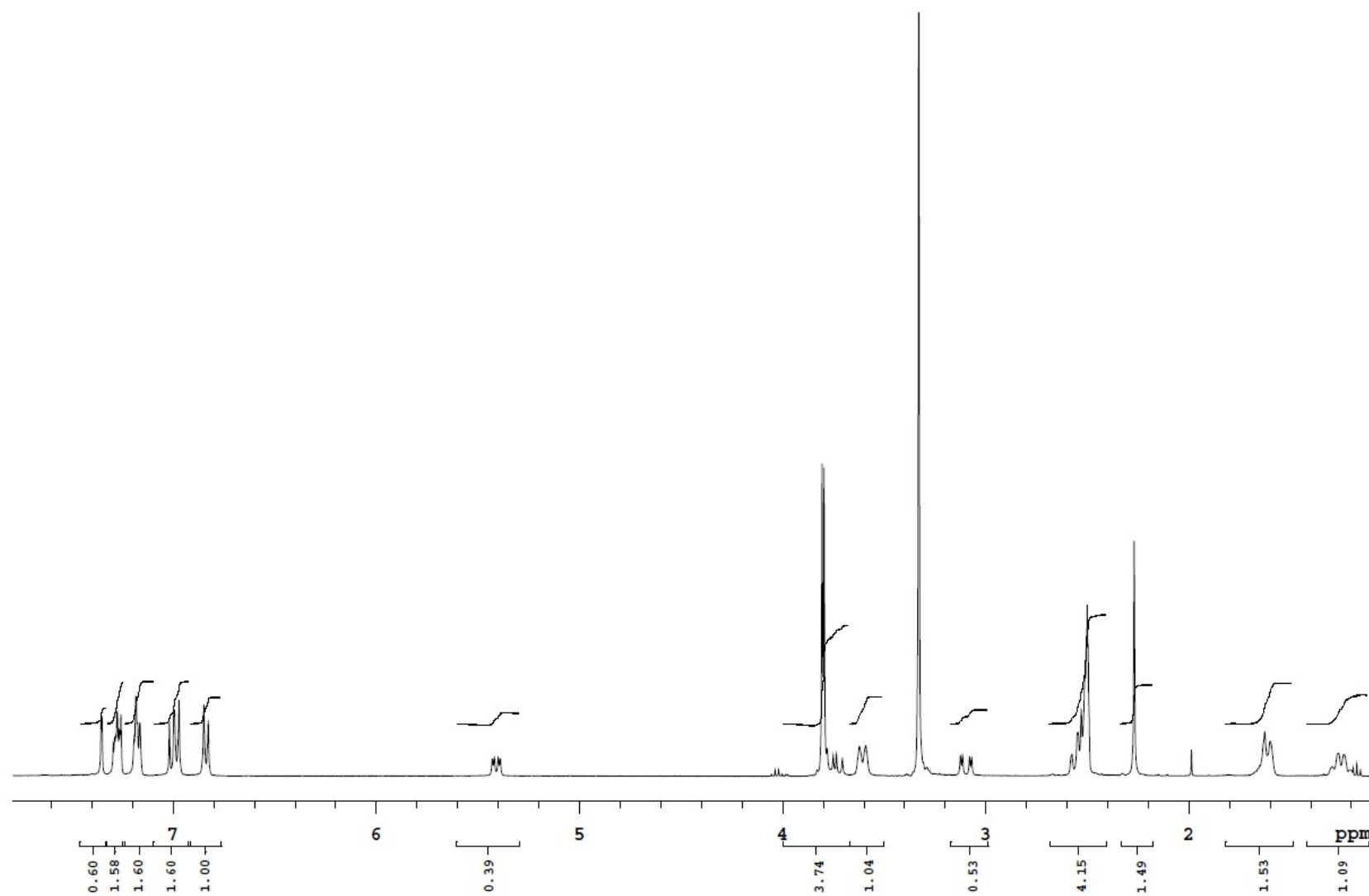


**1-(5-(4-(4-Benzylpiperidin-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3e)**

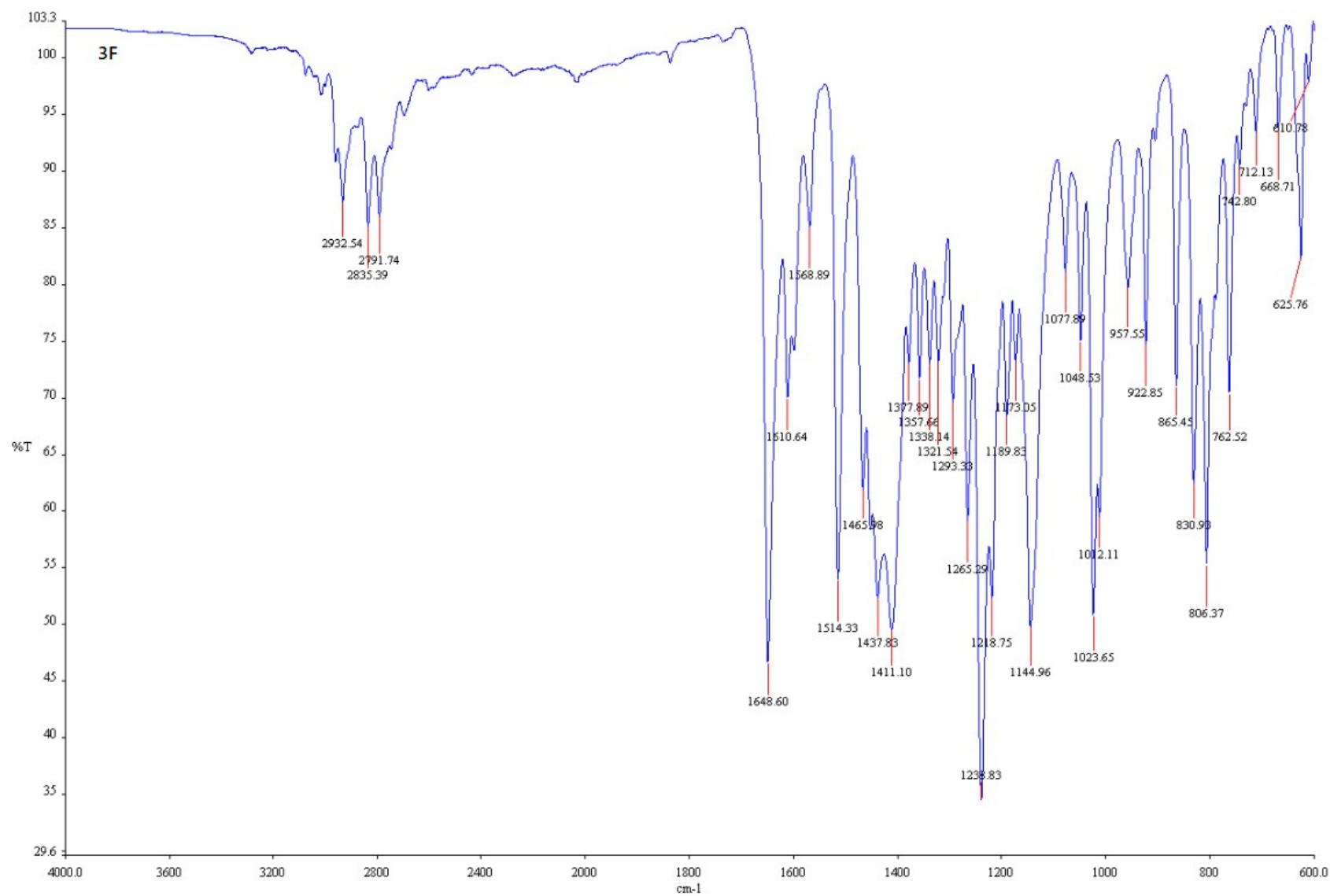


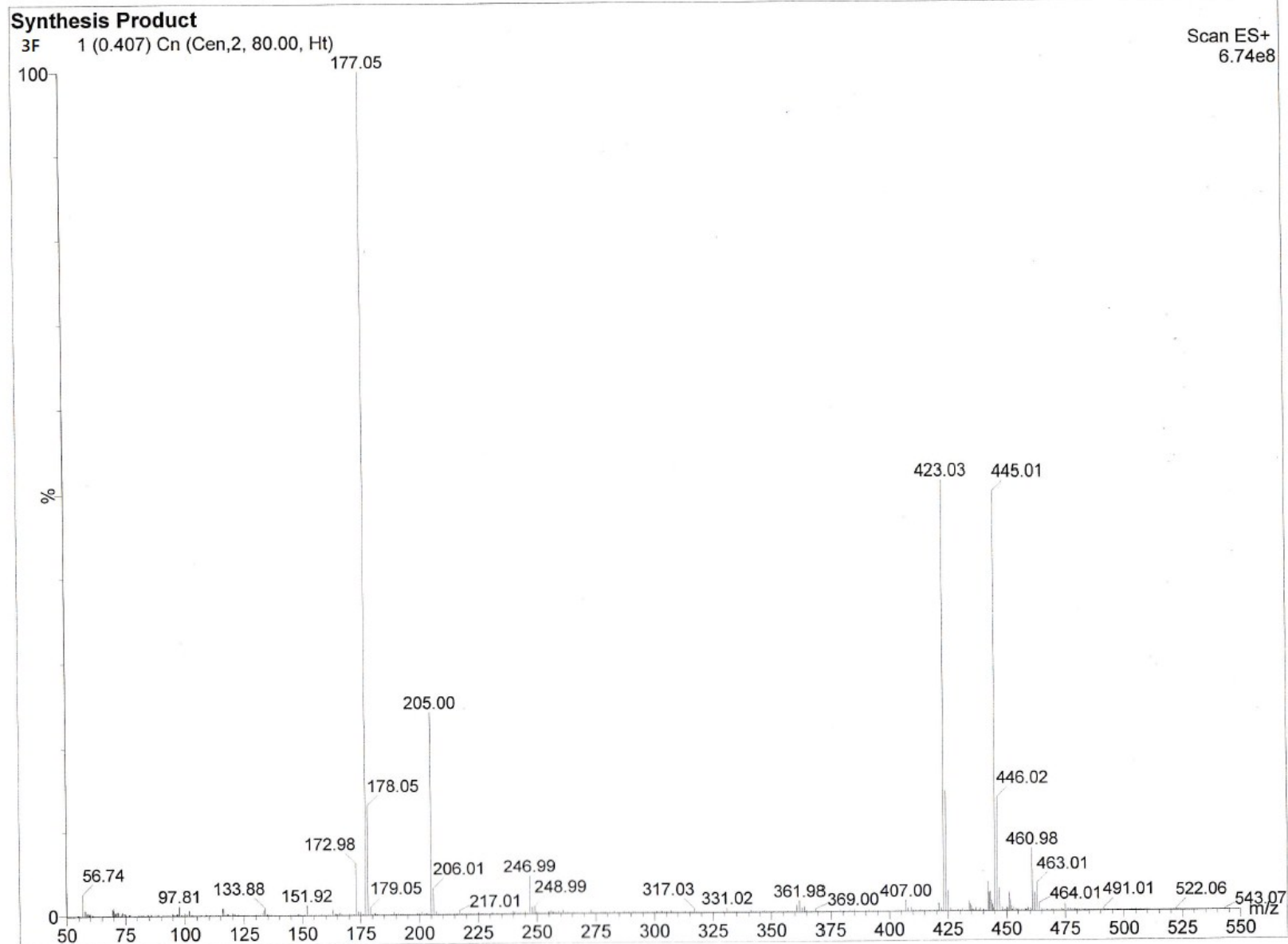


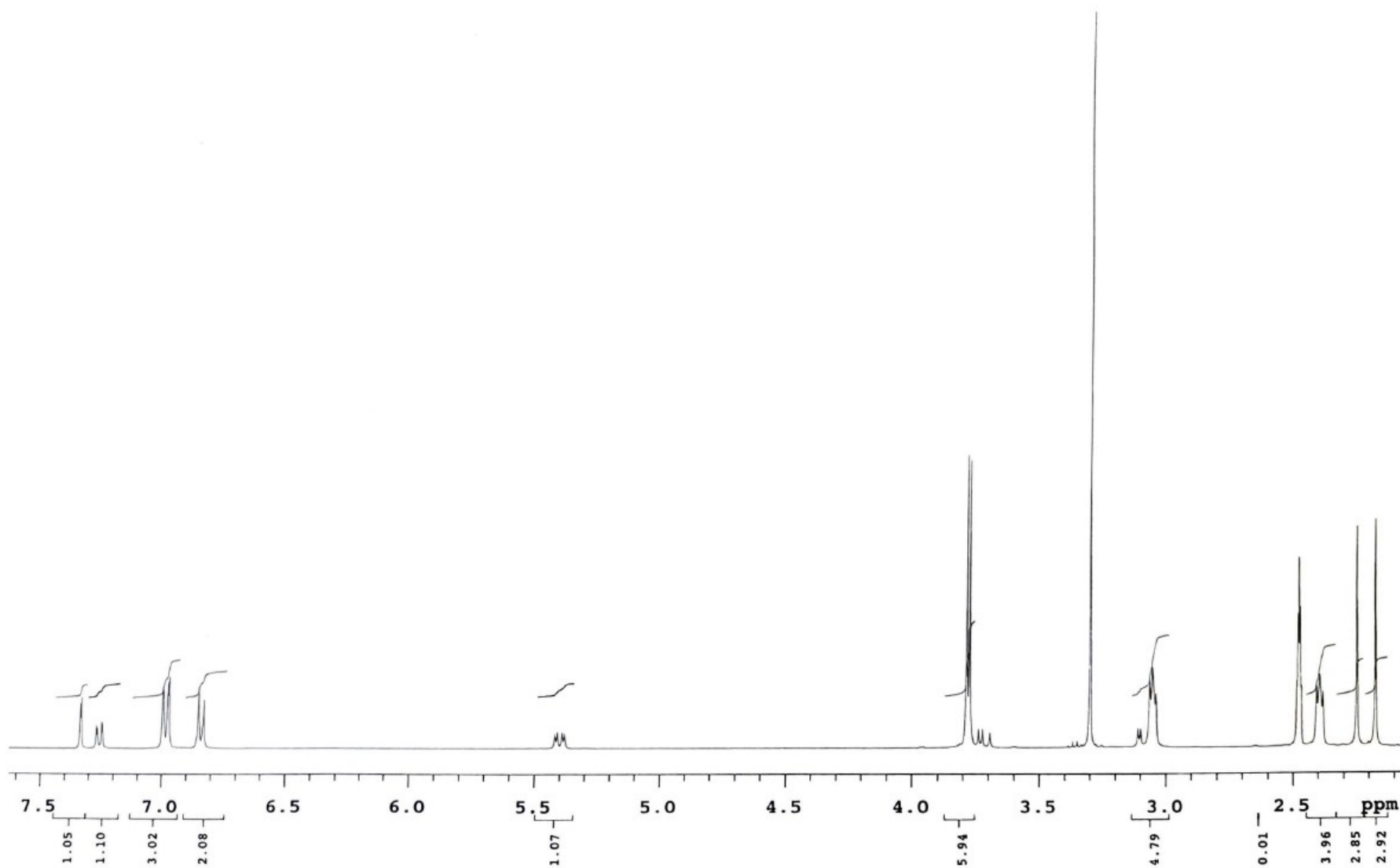
3E



**1-(5-(4-(4-Methylpiperazin-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3f)**







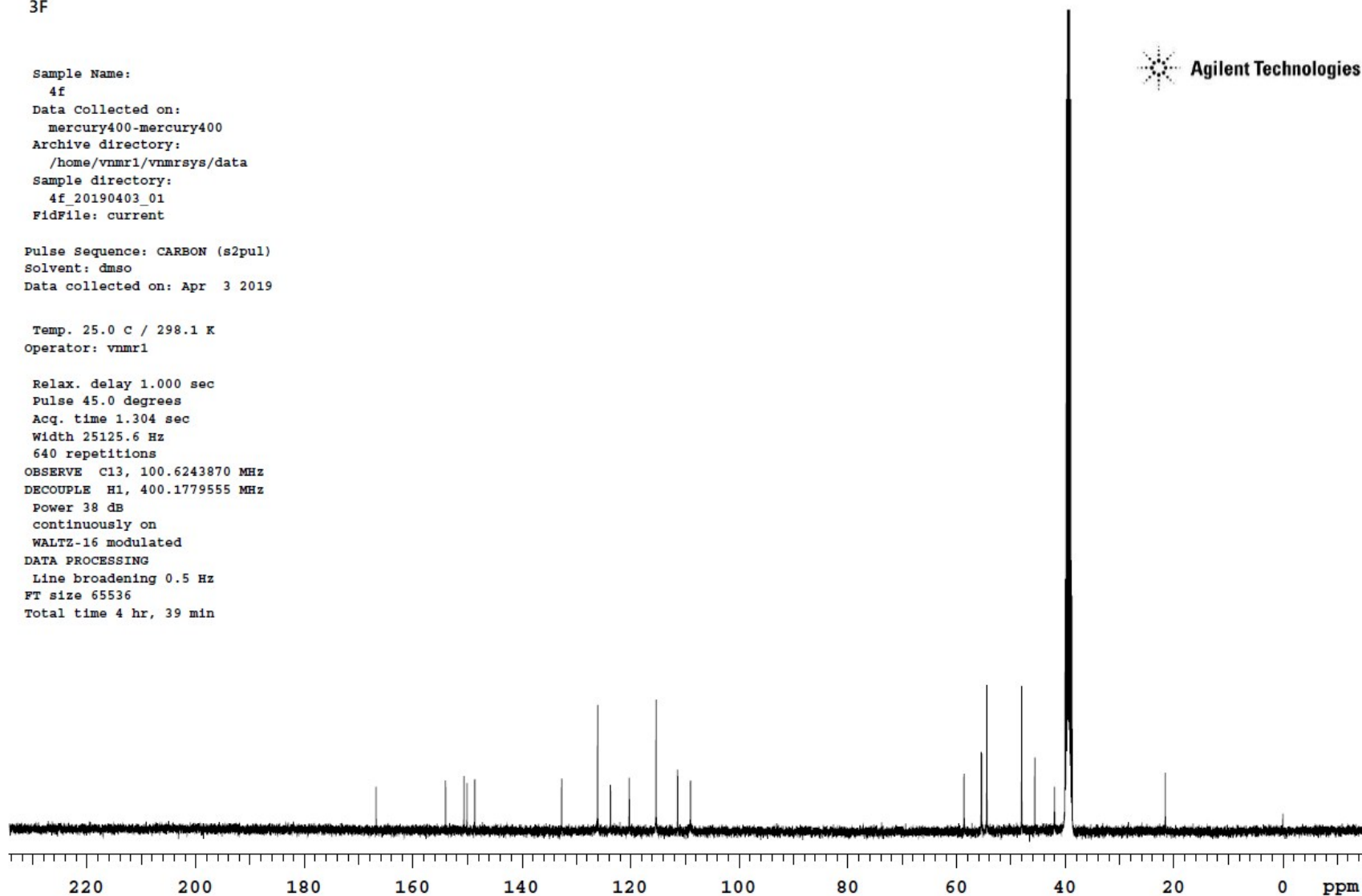
3F

Sample Name:  
4f  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4f\_20190403\_01  
FidFile: current

Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Apr 3 2019

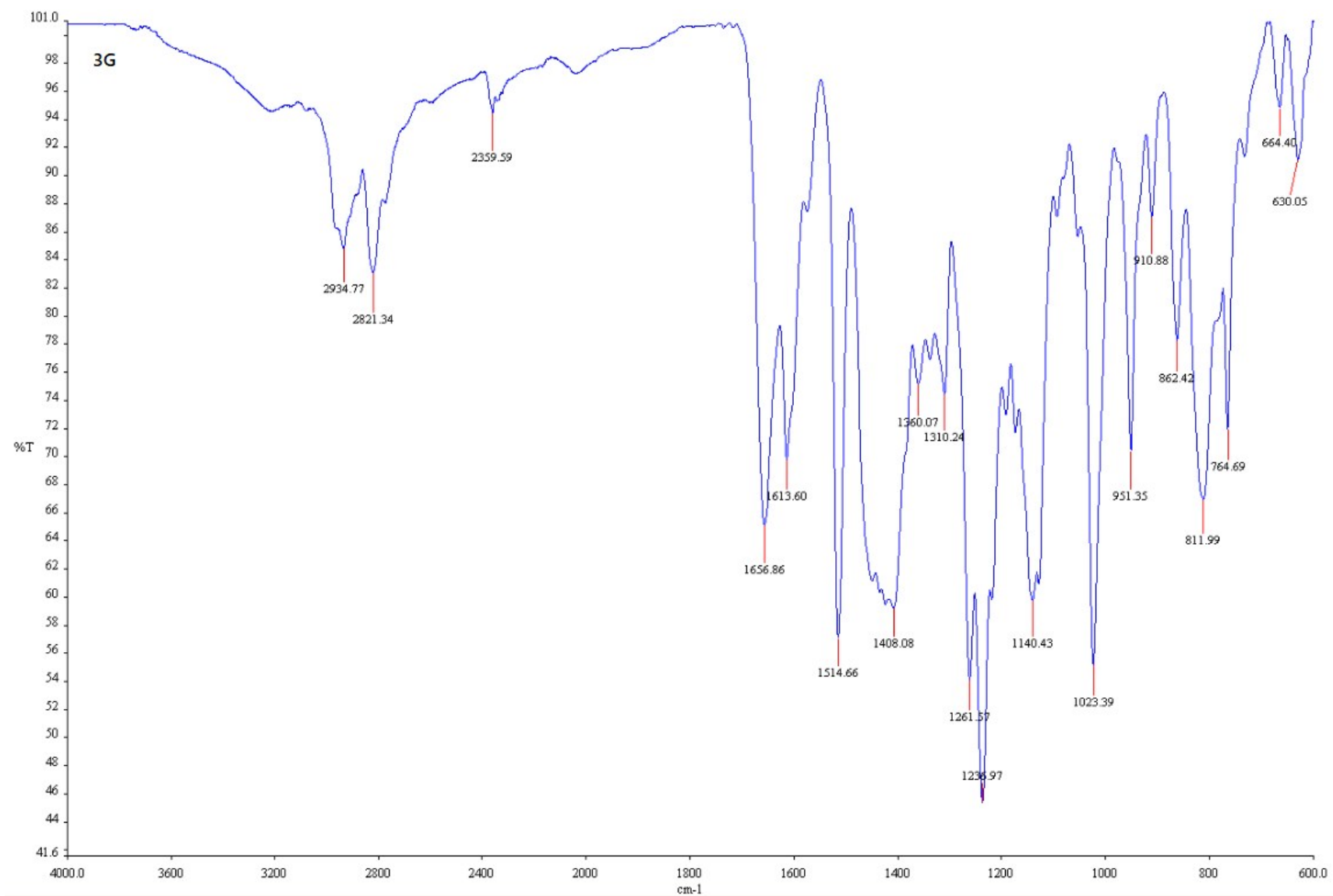
Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
640 repetitions  
OBSERVE C13, 100.6243870 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 4 hr, 39 min





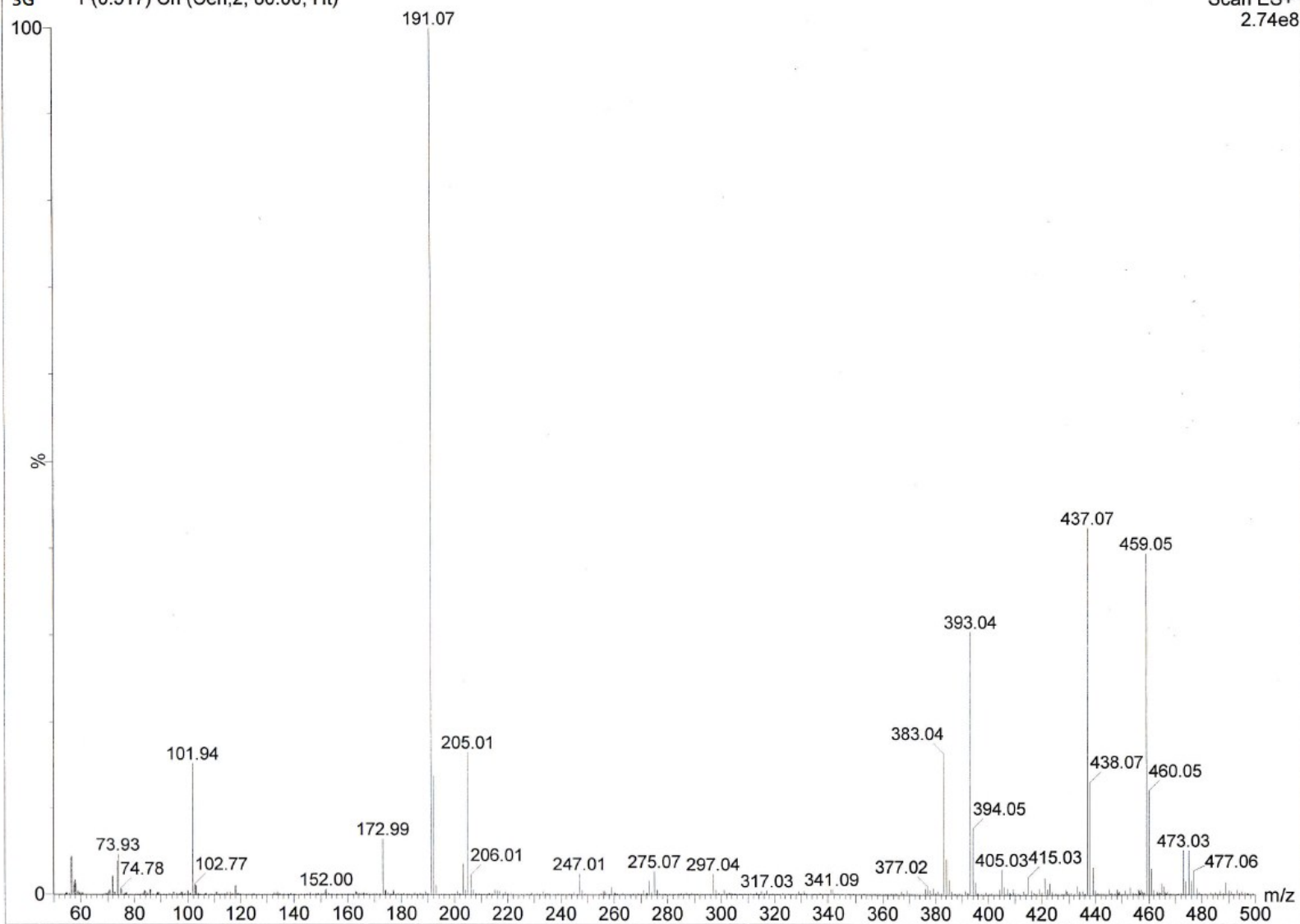
**1-(5-(4-(4-Ethylpiperazin-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3g)**



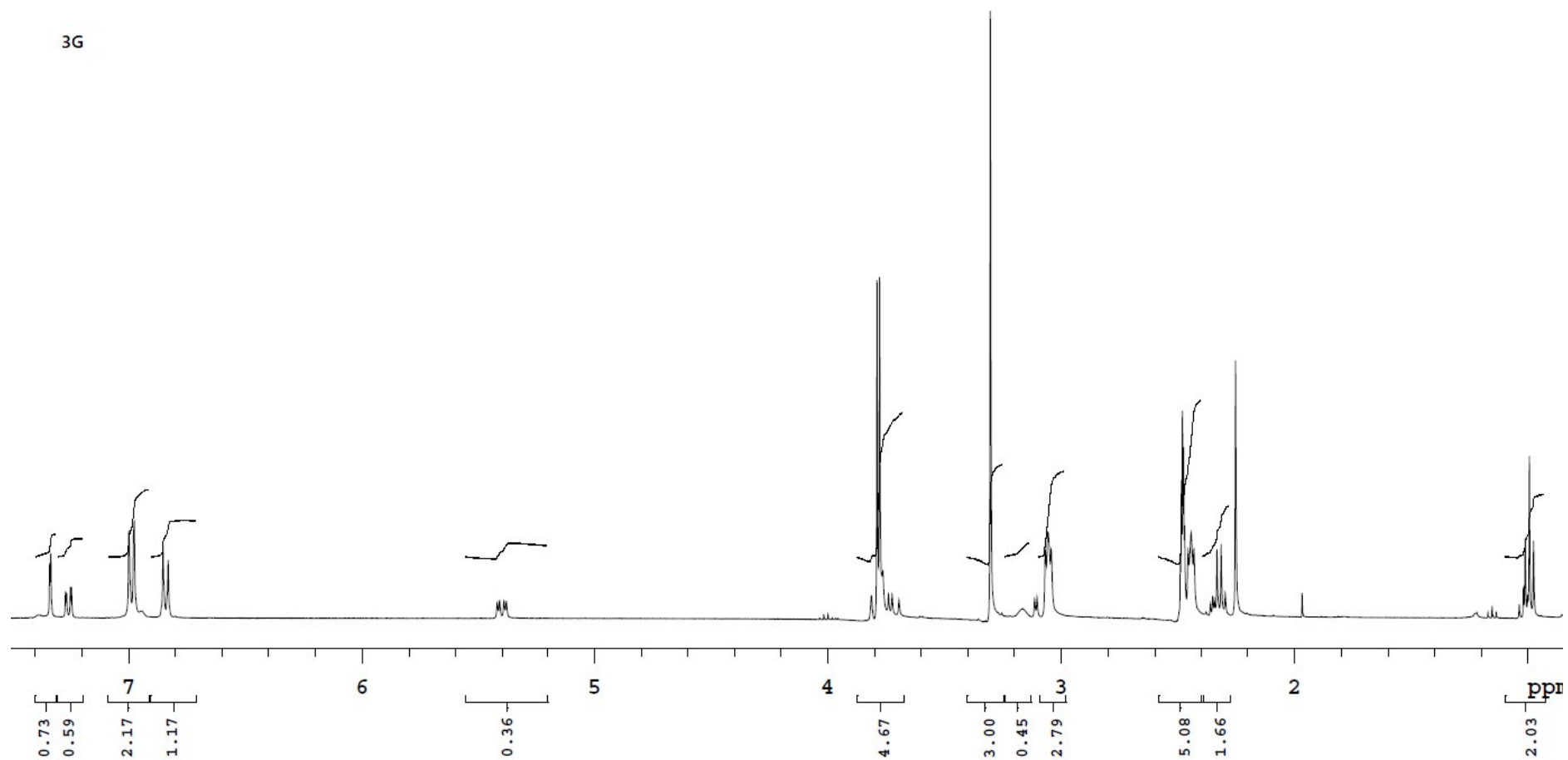
**Synthesis Product**

3G 1 (0.517) Cn (Cen,2, 80.00, Ht)

Scan ES+  
2.74e8



3G



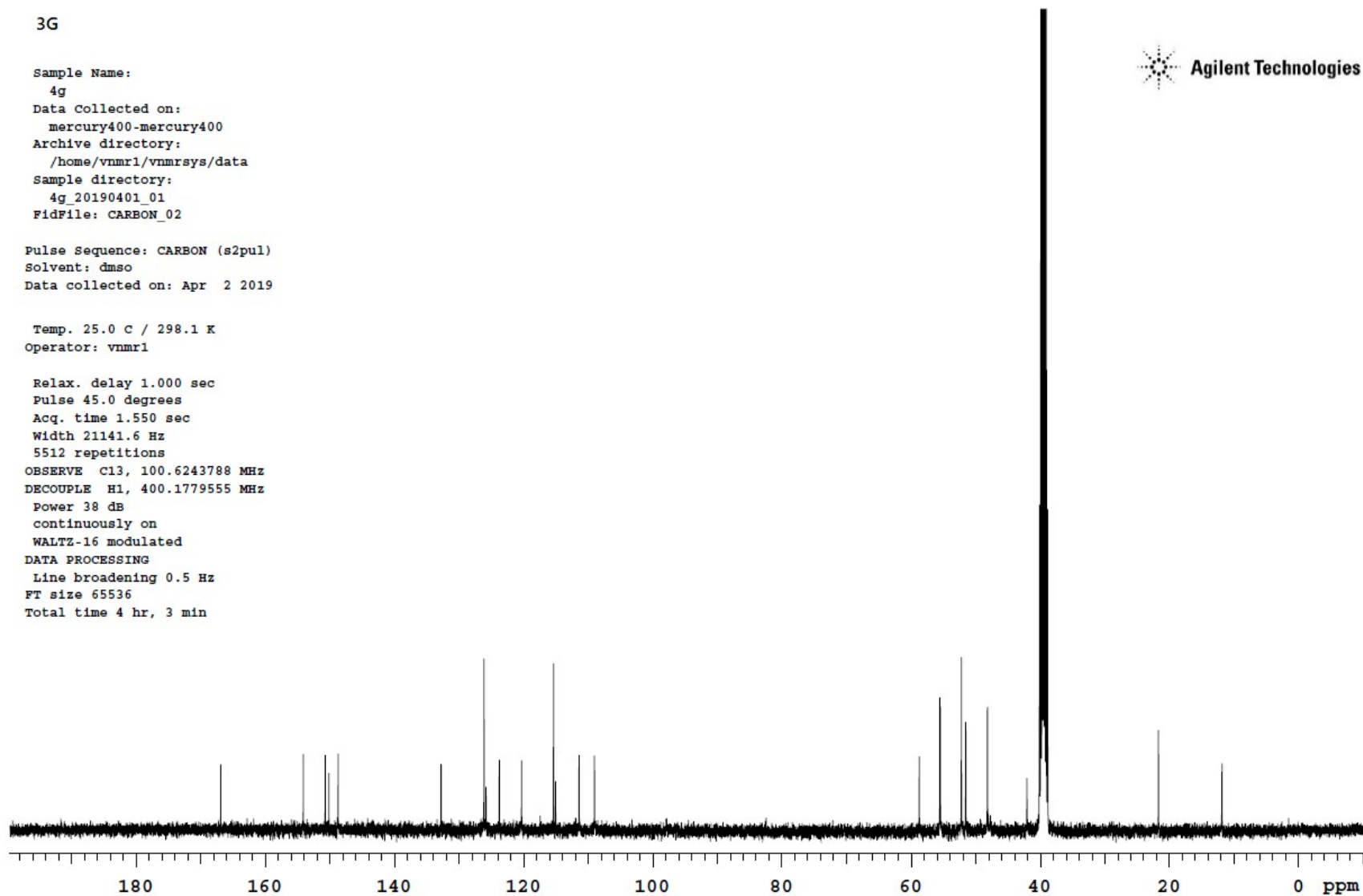
3G

Sample Name:  
4g  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4g\_20190401\_01  
FidFile: CARBON\_02

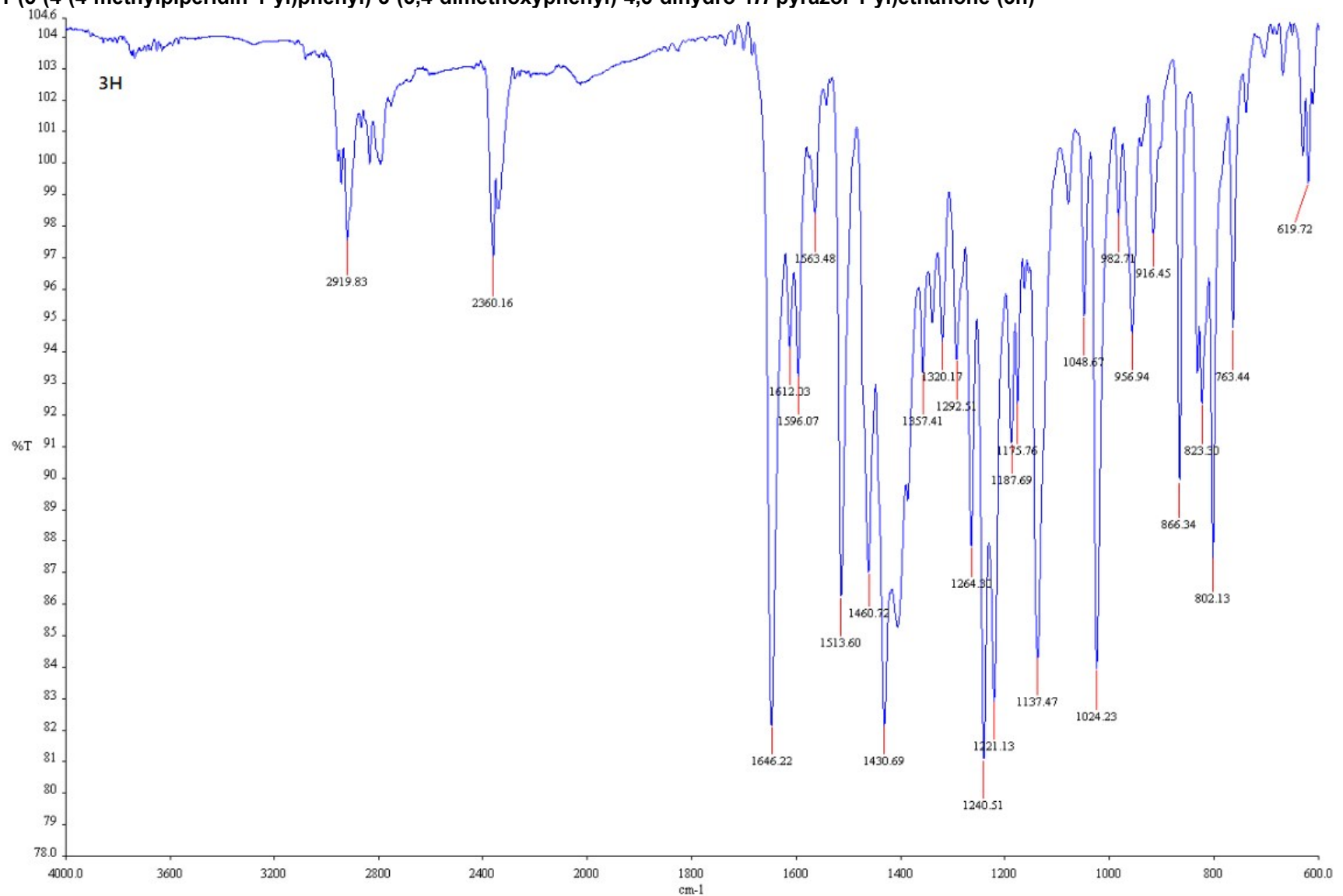
Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Apr 2 2019

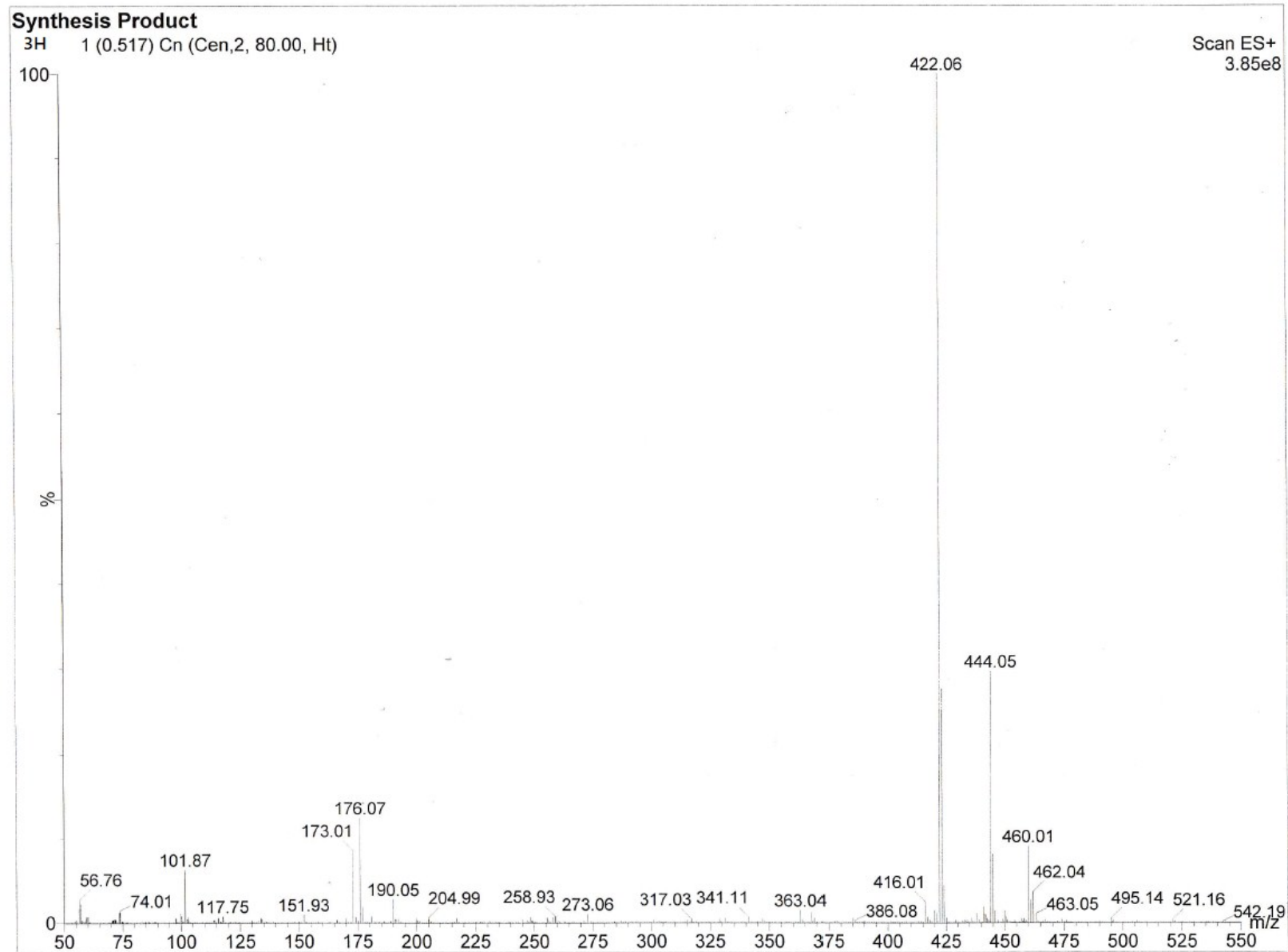
Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.550 sec  
Width 21141.6 Hz  
5512 repetitions  
OBSERVE C13, 100.6243788 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 4 hr, 3 min

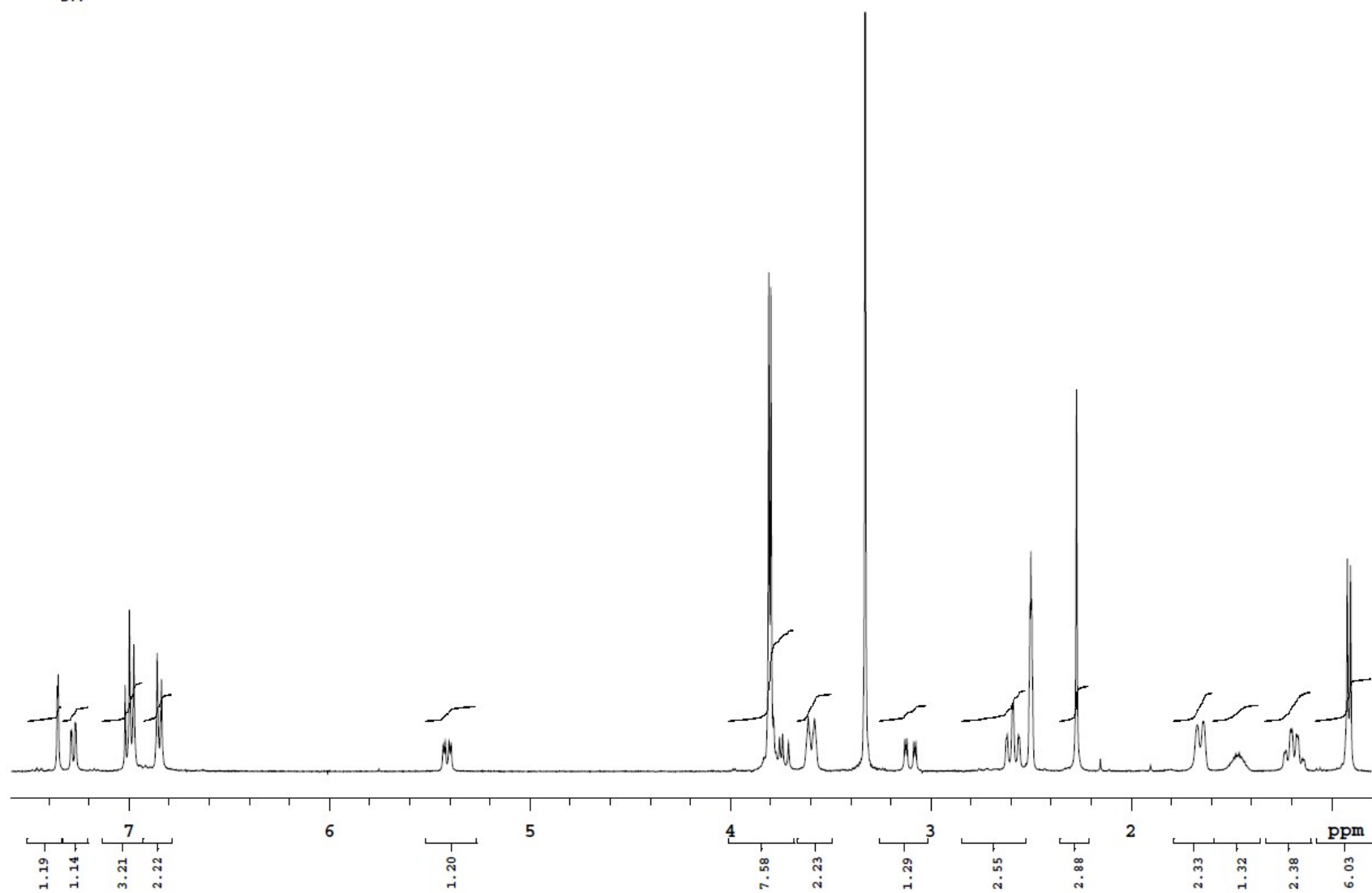


**1-(5-(4-(4-methylpiperidin-1-yl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3h)**





3H



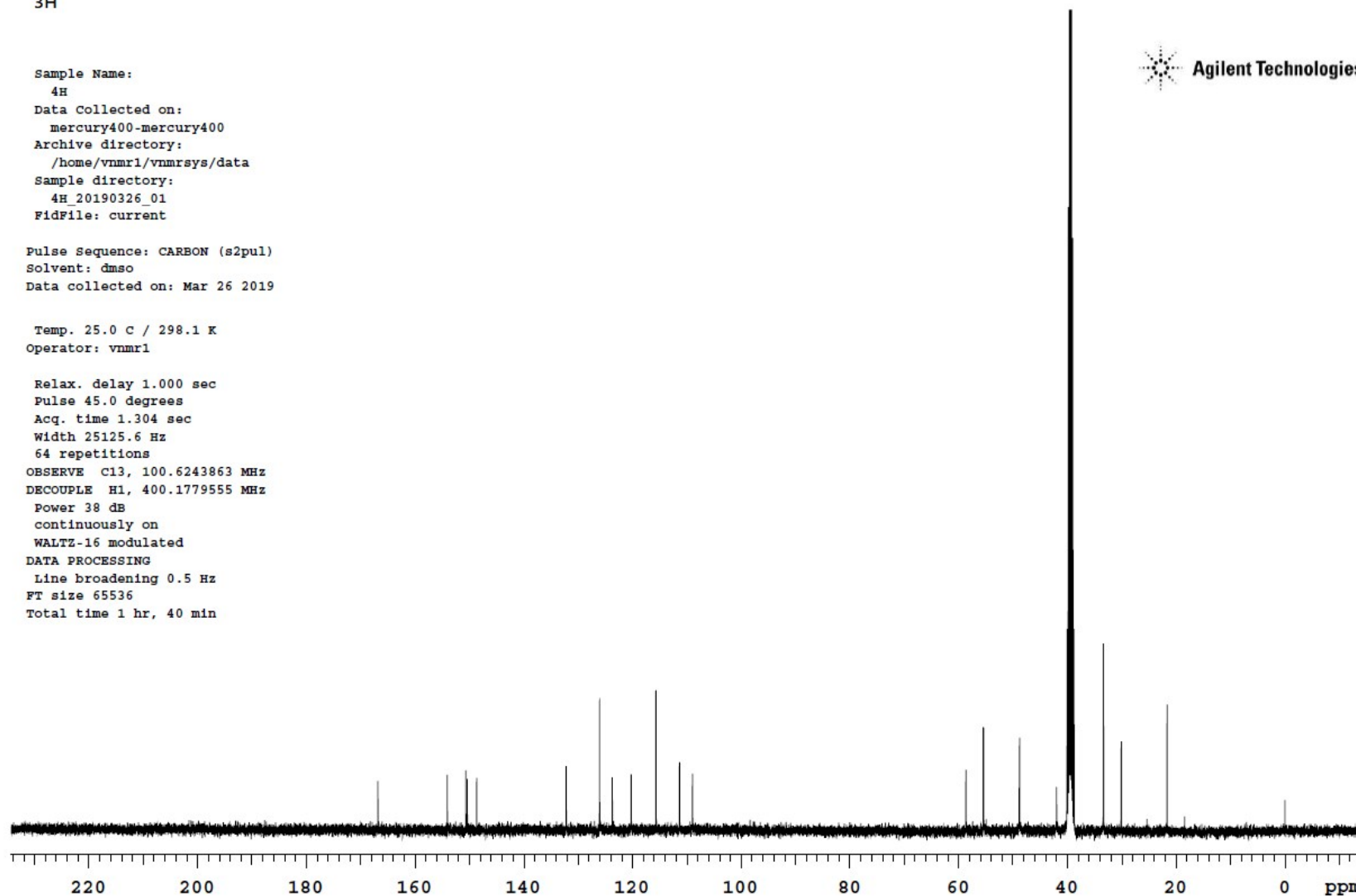
3H

Sample Name:  
4H  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
4H\_20190326\_01  
FidFile: current

Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Mar 26 2019

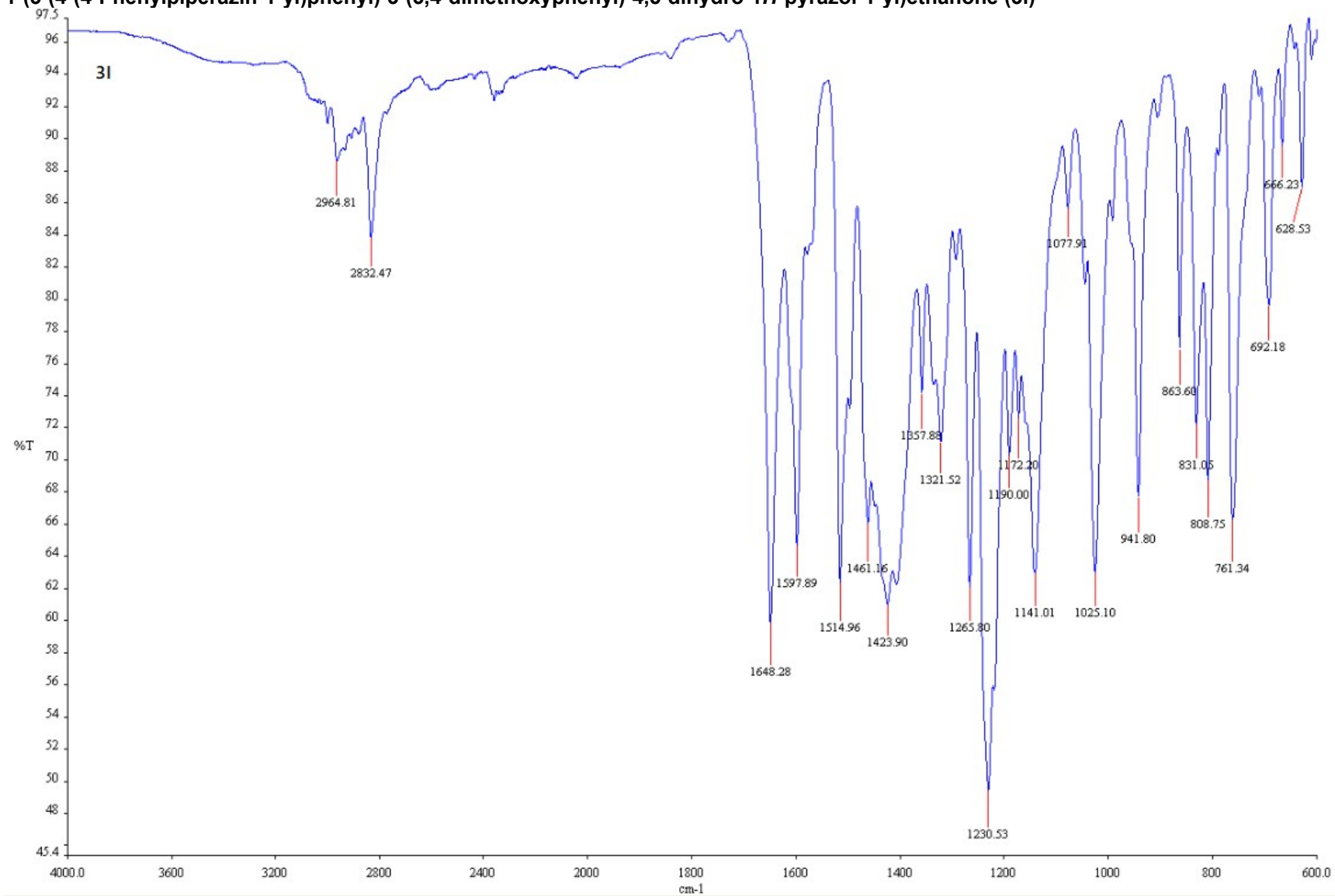
Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
64 repetitions  
OBSERVE C13, 100.6243863 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 1 hr, 40 min





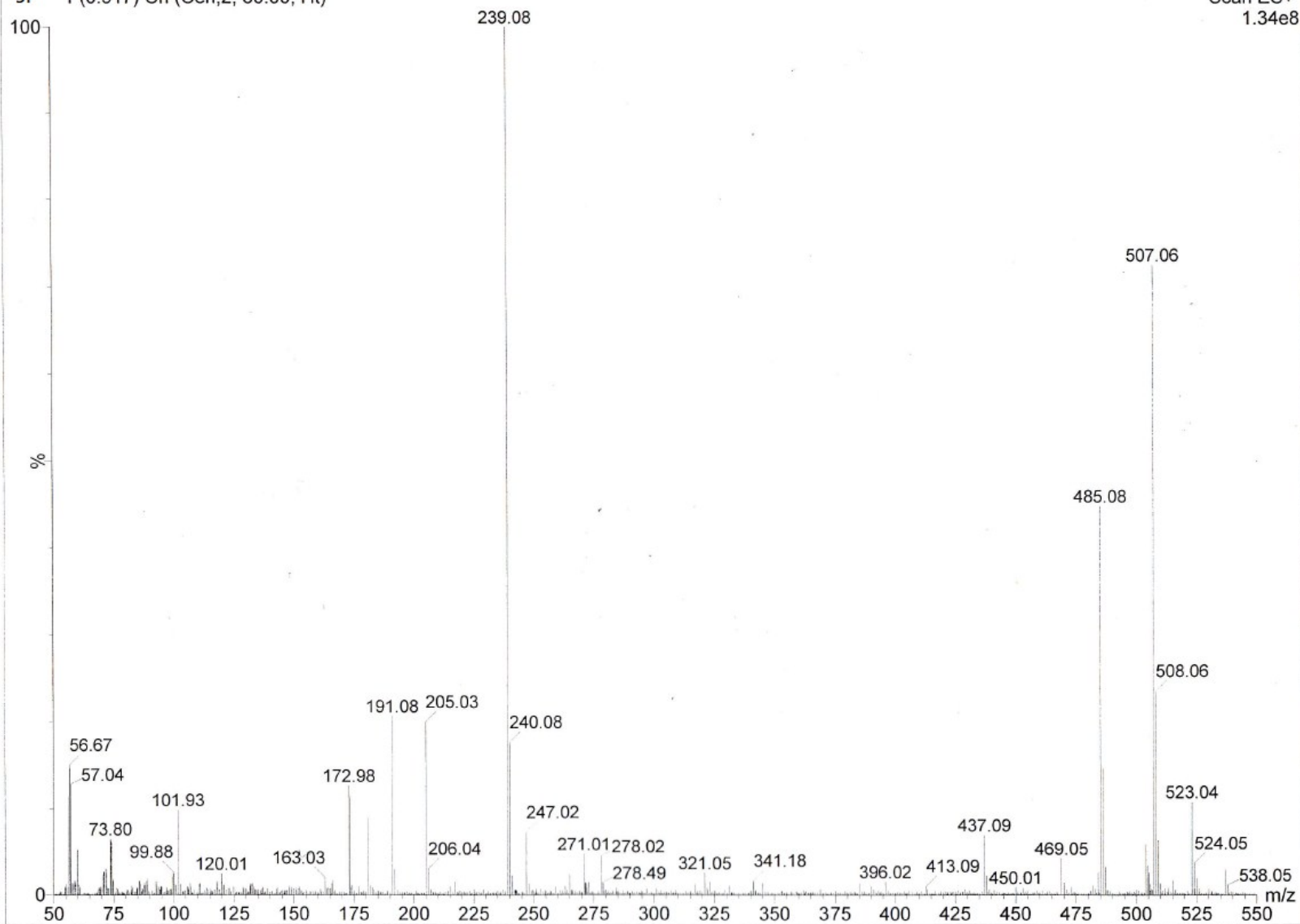
1-(5-(4-(4-Phenylpiperazin-1-yl)phenyl)phenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3i)

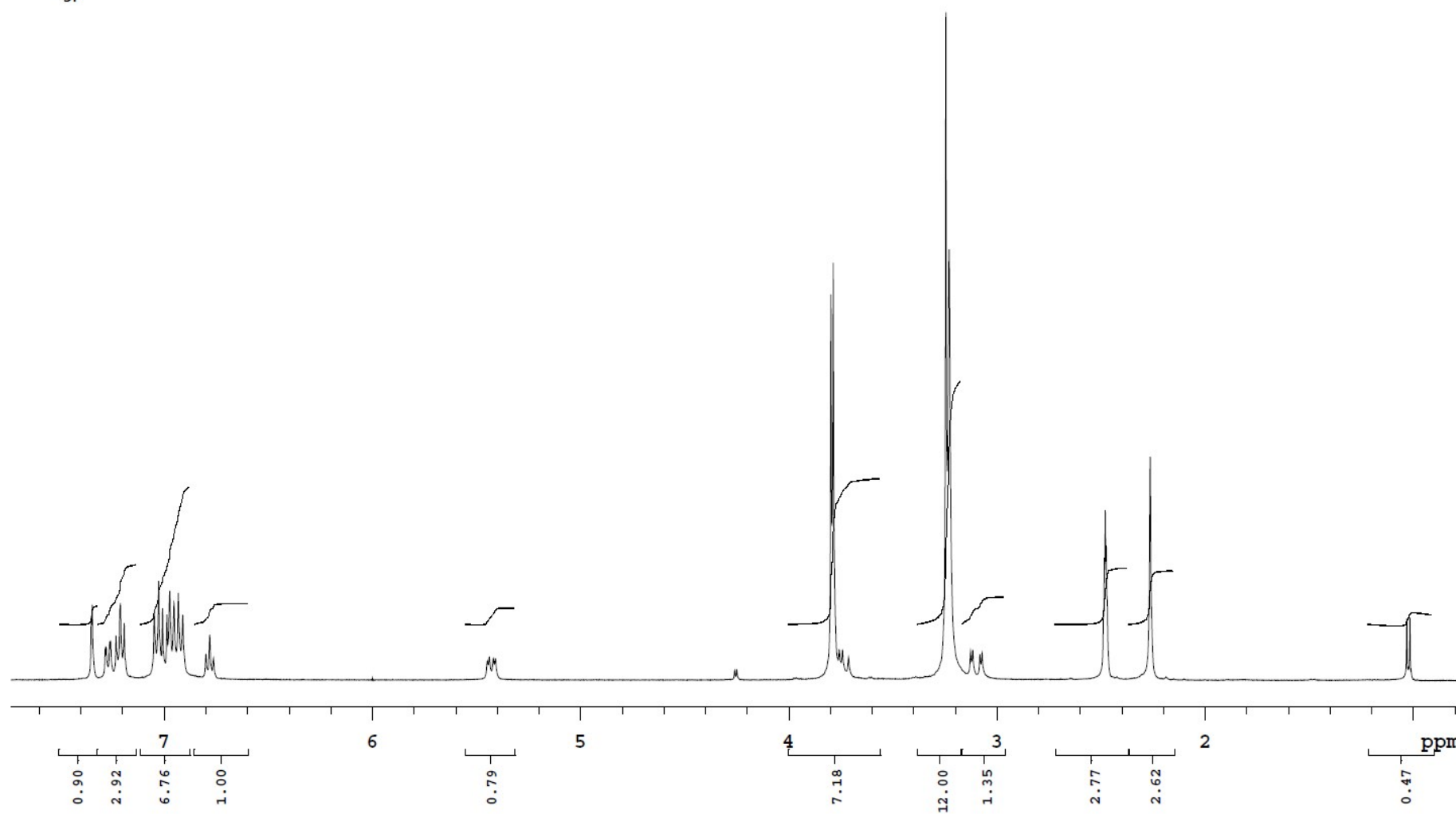


Synthesis Product

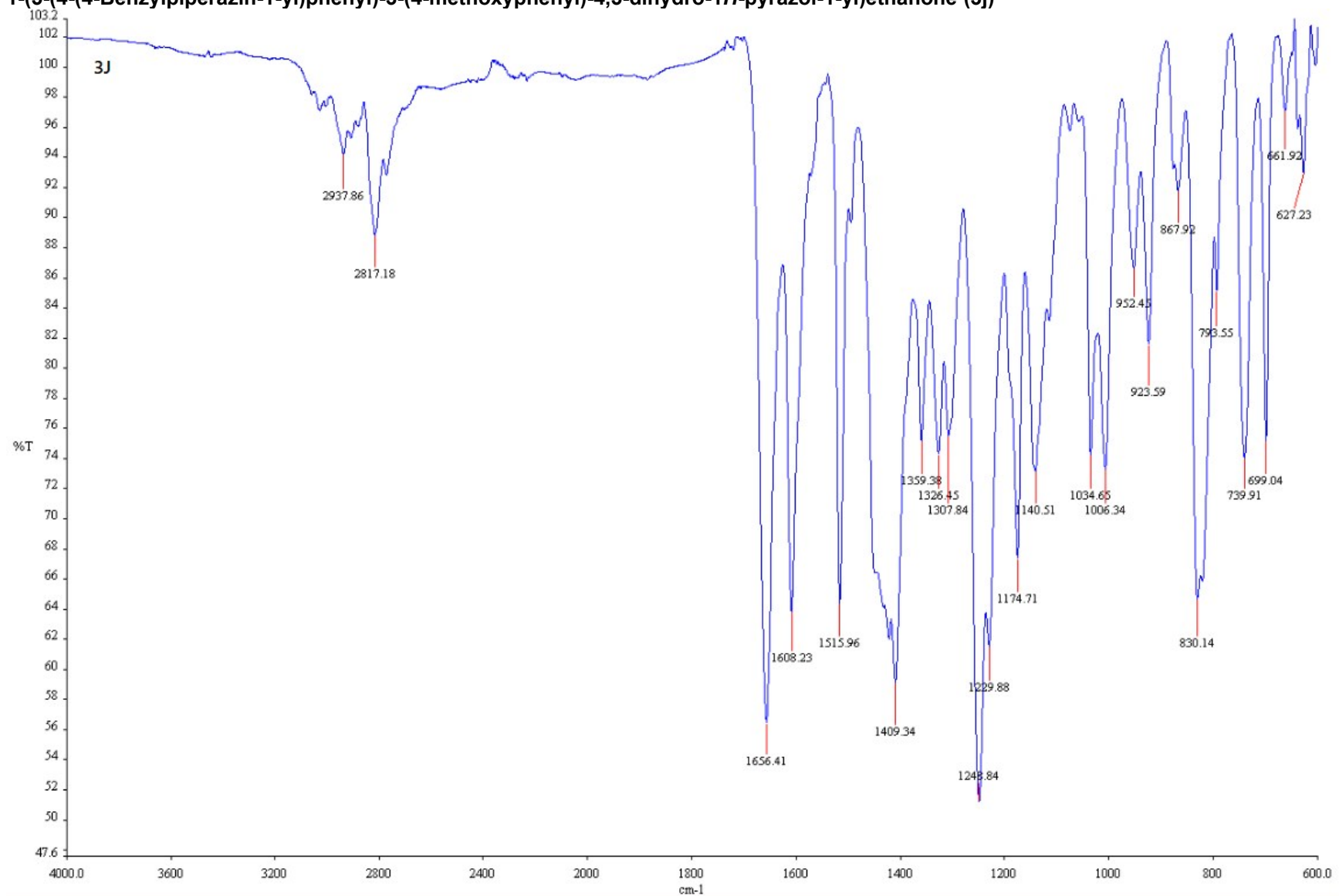
3I 1 (0.517) Cn (Cen,2, 80.00, Ht)

Scan ES+  
1.34e8





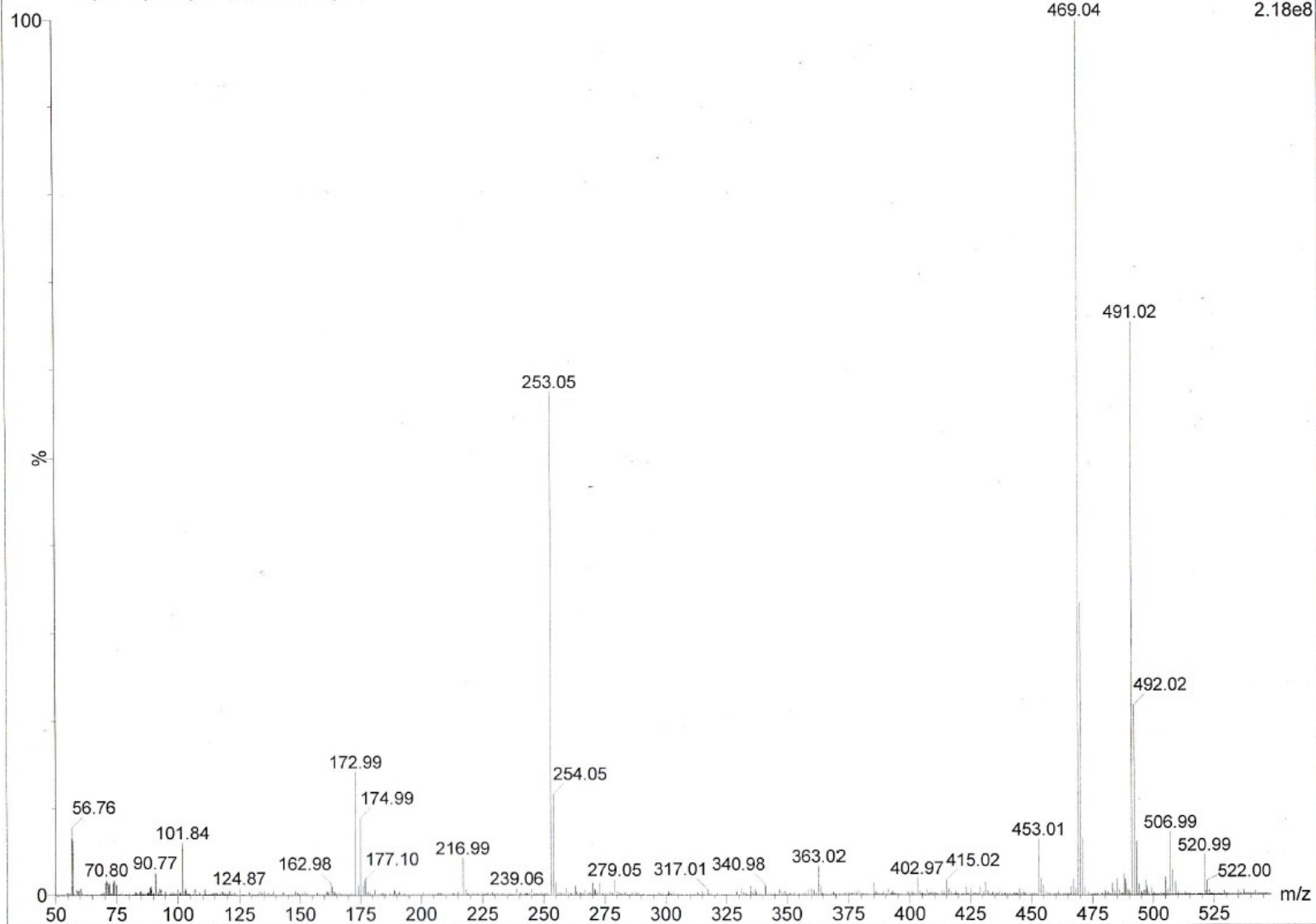
**1-(5-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3j)**



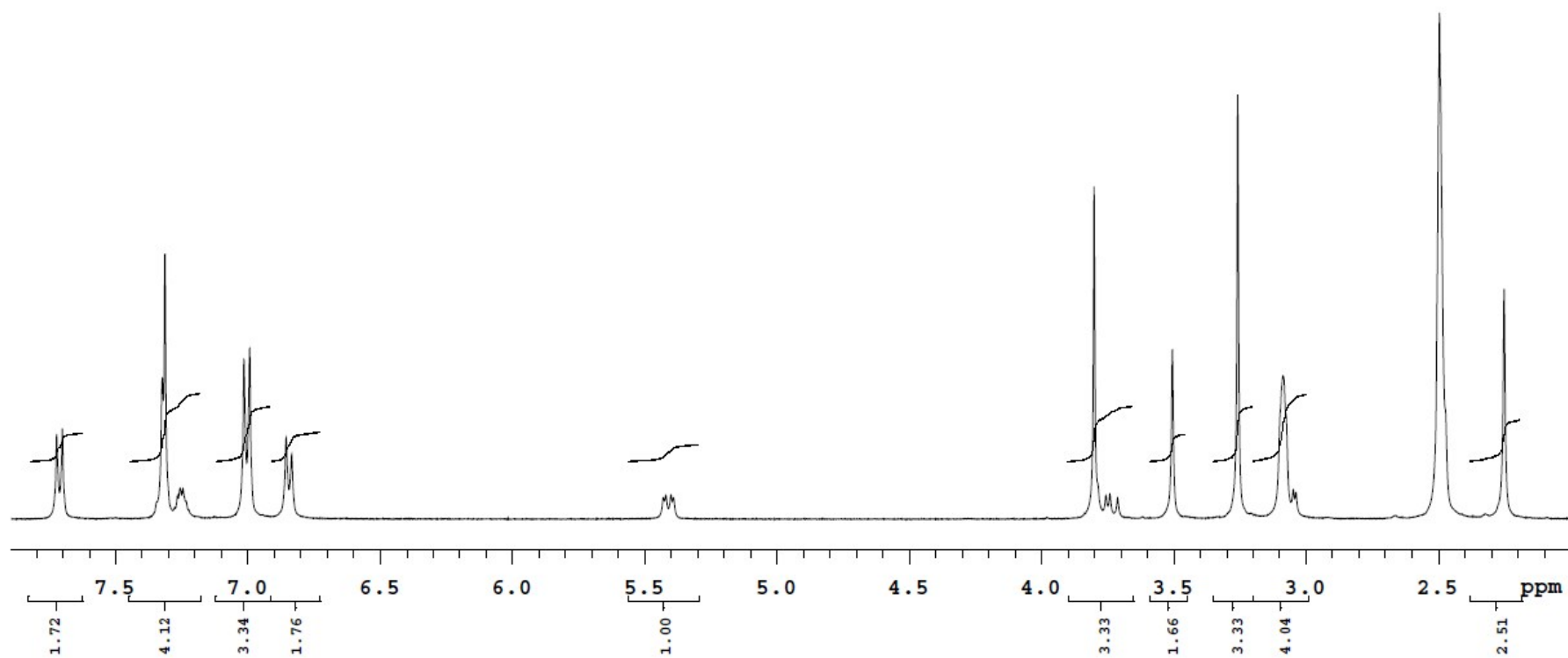
**Synthesis Product**

3J 1 (0.517) Cn (Cen,2, 80.00, Ht)

Scan ES+  
2.18e8



3J



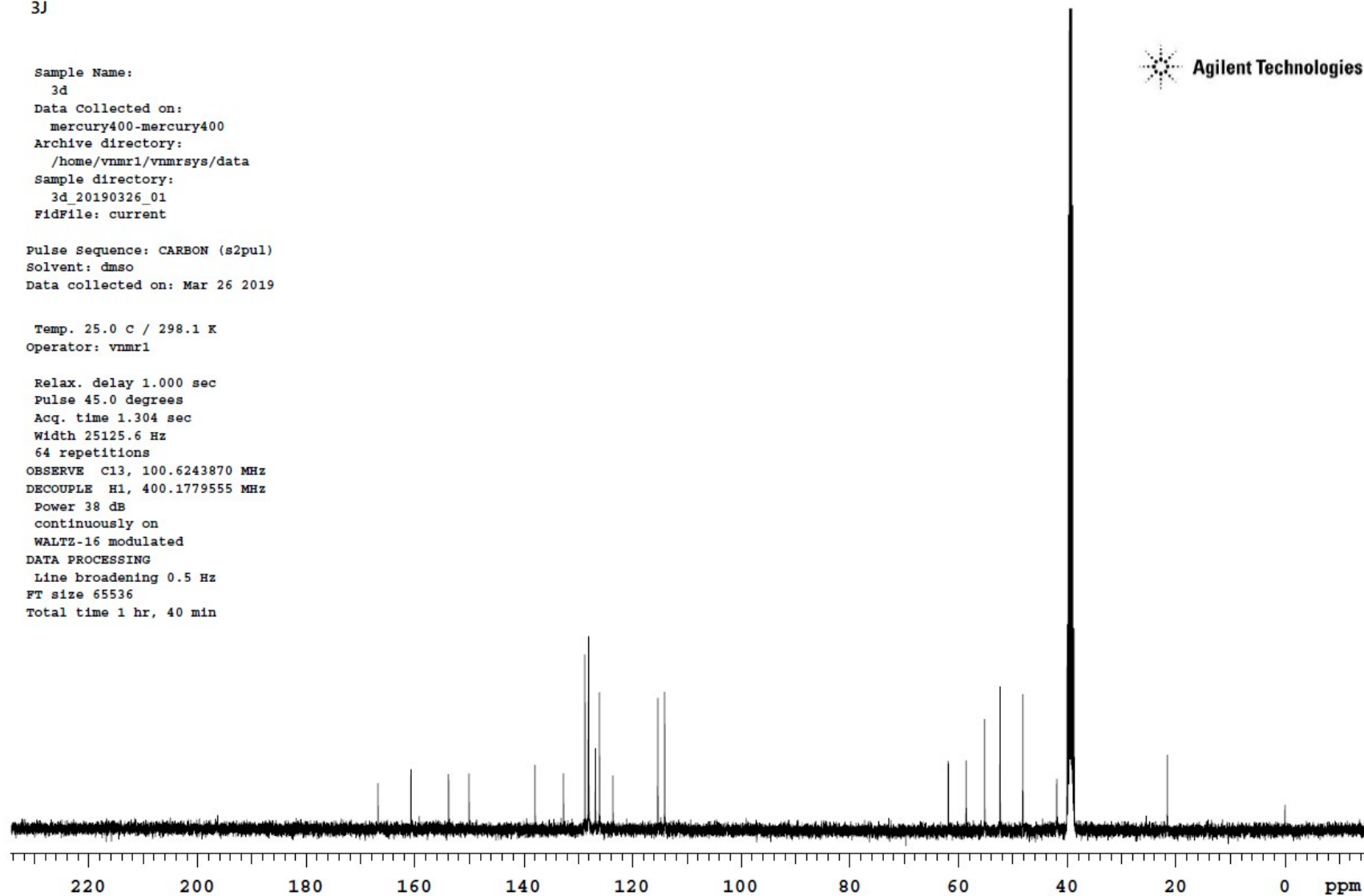
3J

Sample Name:  
3d  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
3d\_20190326\_01  
FidFile: current

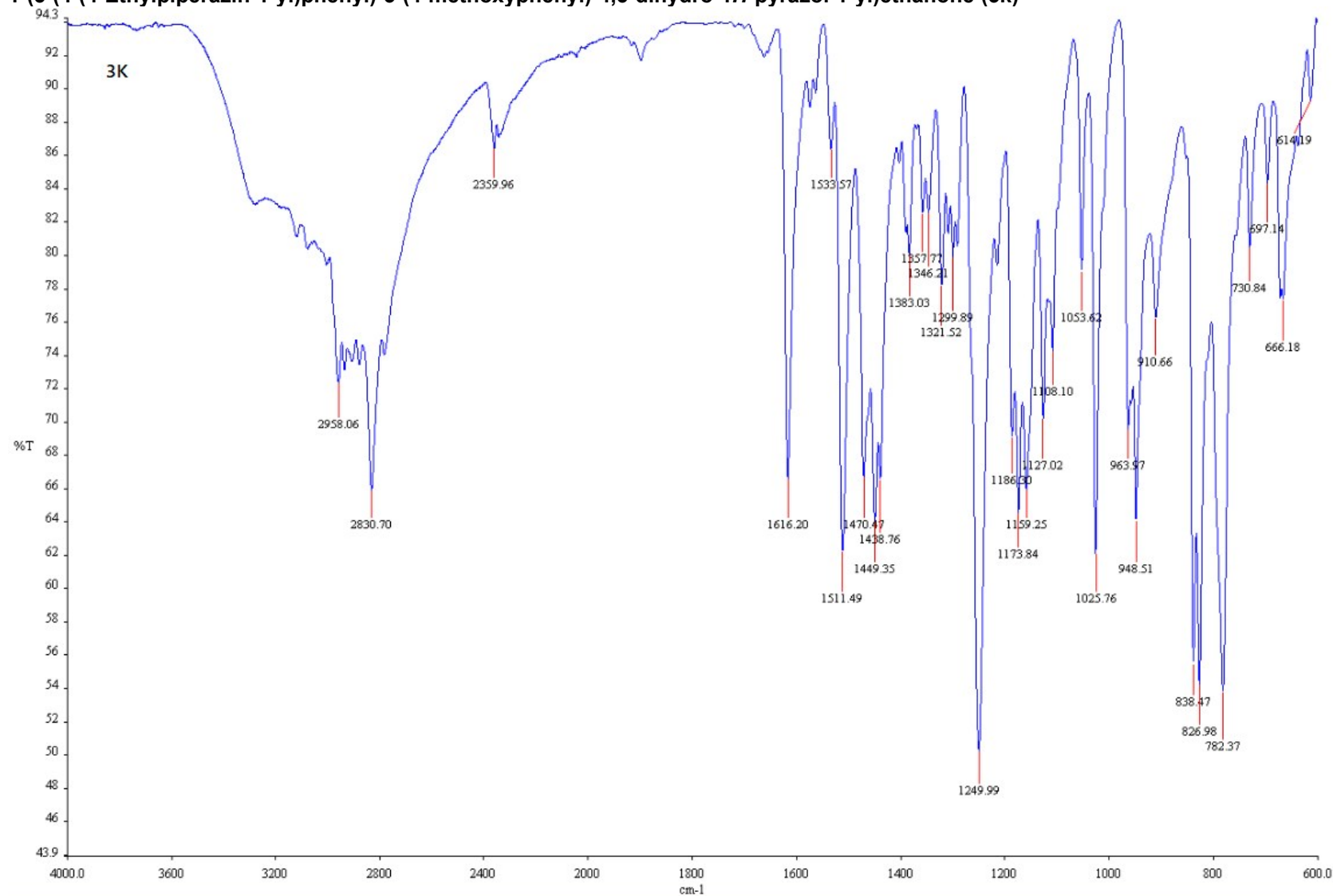
Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Mar 26 2019

Temp. 25.0 C / 298.1 K  
Operator: vnmr1

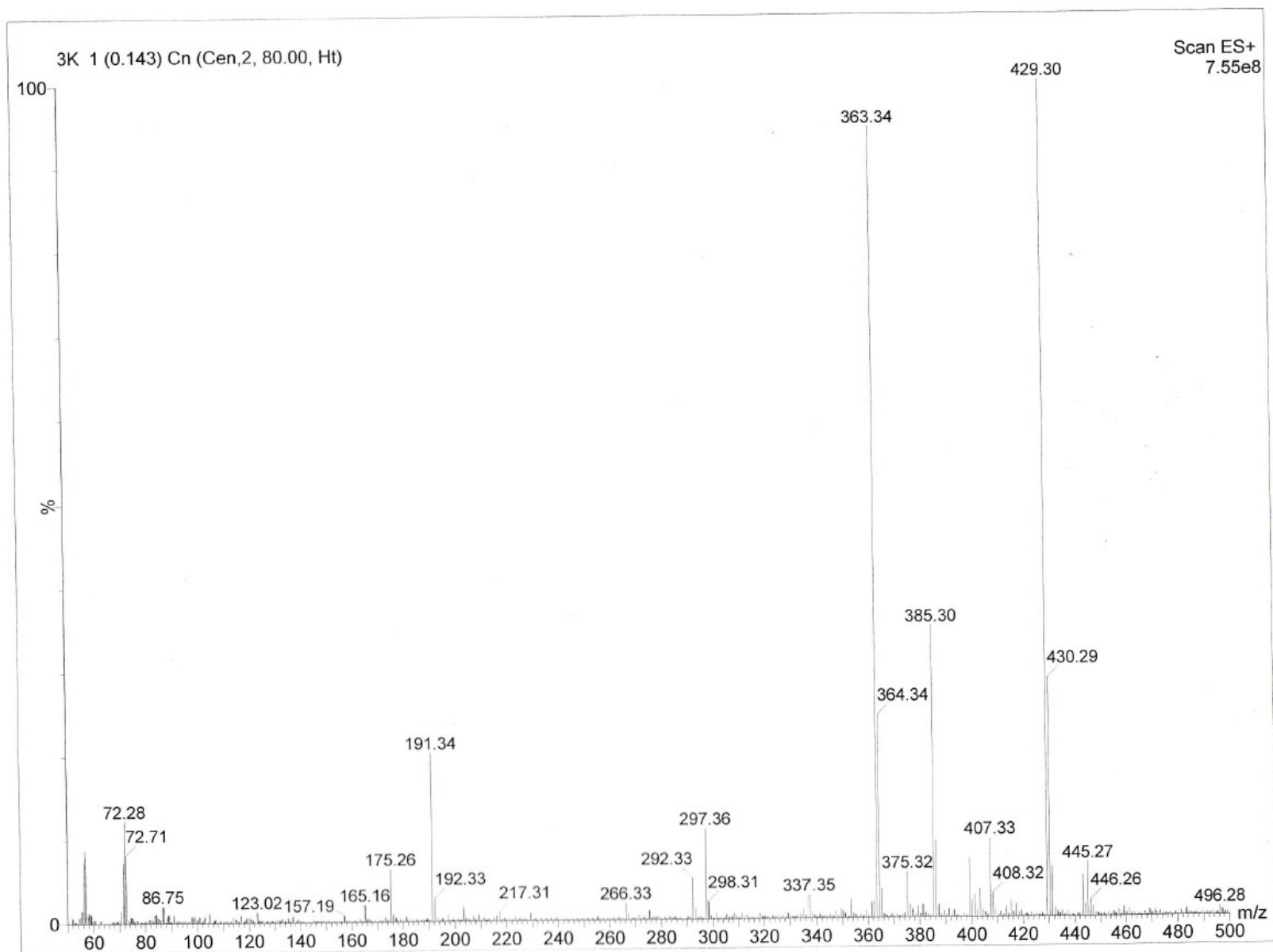
Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
64 repetitions  
OBSERVE C13, 100.6243870 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 1 hr, 40 min



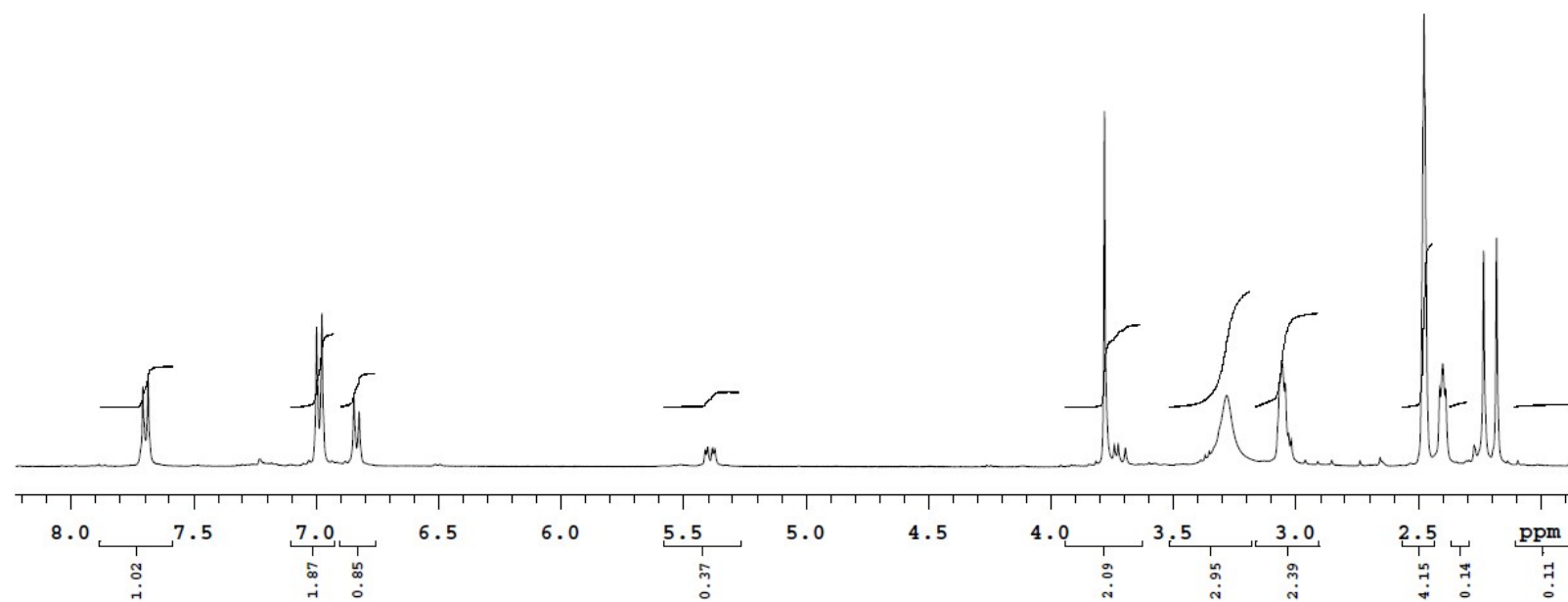
**1-(5-(4-(4-Ethylpiperazin-1-yl)phenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (3k)**







3K



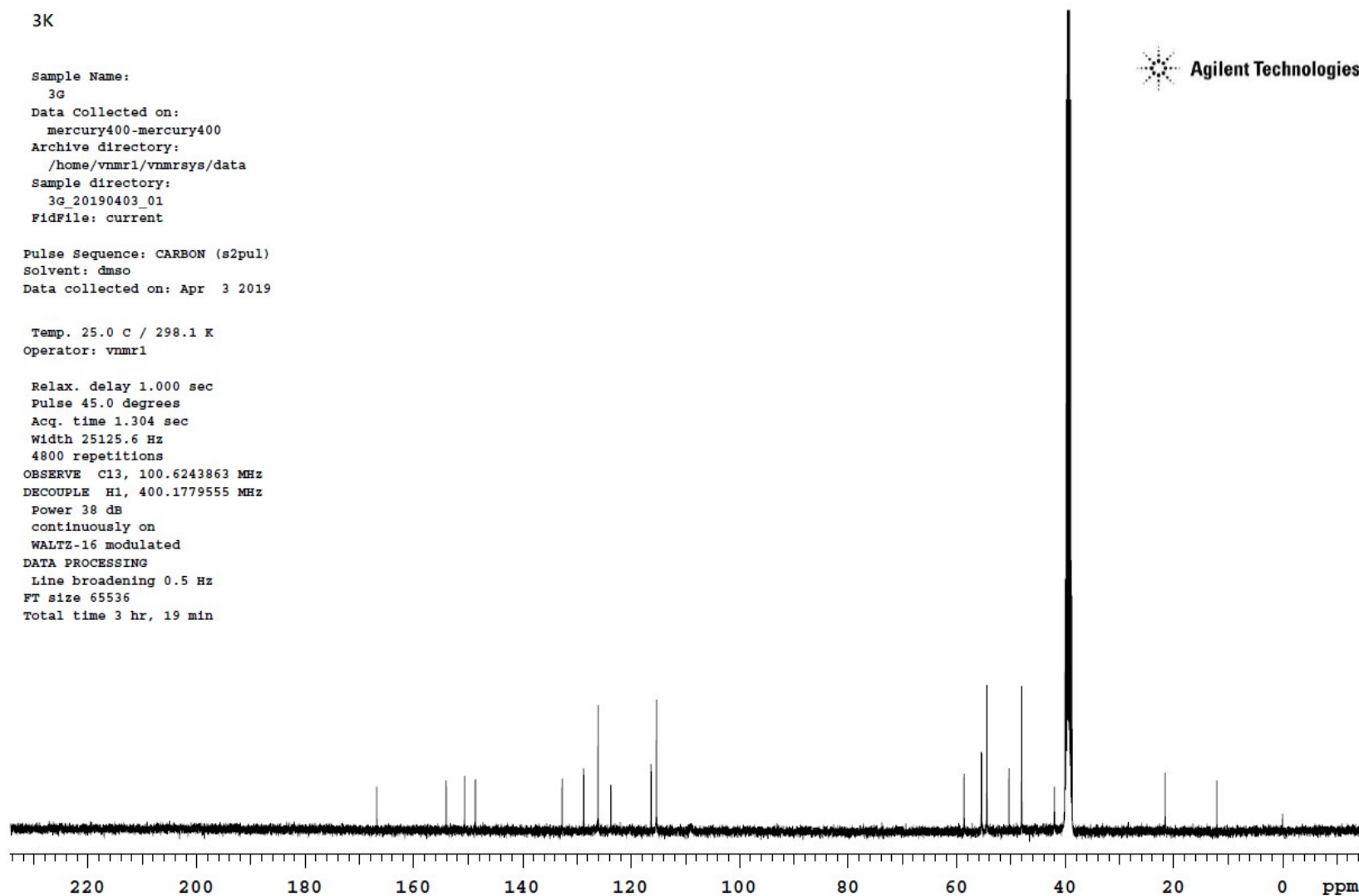
3K

Sample Name:  
3G  
Data Collected on:  
mercury400-mercury400  
Archive directory:  
/home/vnmr1/vnmrsys/data  
Sample directory:  
3G\_20190403\_01  
FidFile: current

Pulse Sequence: CARBON (s2pul)  
Solvent: dmsd  
Data collected on: Apr 3 2019

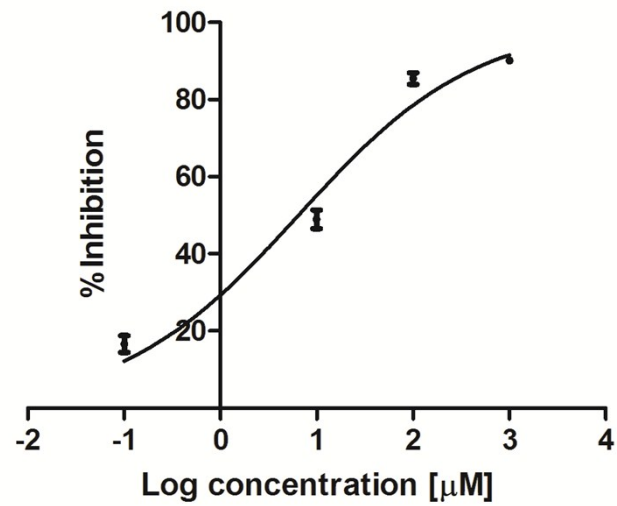
Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.304 sec  
Width 25125.6 Hz  
4800 repetitions  
OBSERVE C13, 100.6243863 MHz  
DECOUPLE H1, 400.1779555 MHz  
Power 38 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 3 hr, 19 min

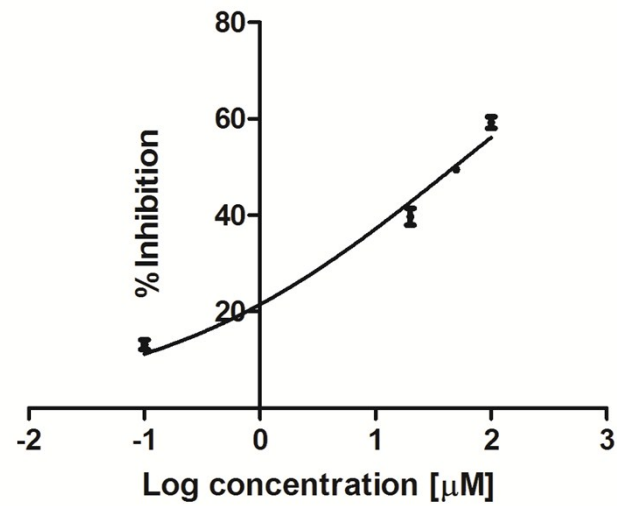


## 2) DOSE-RESPONSE CURVES OF 3F AND 3G

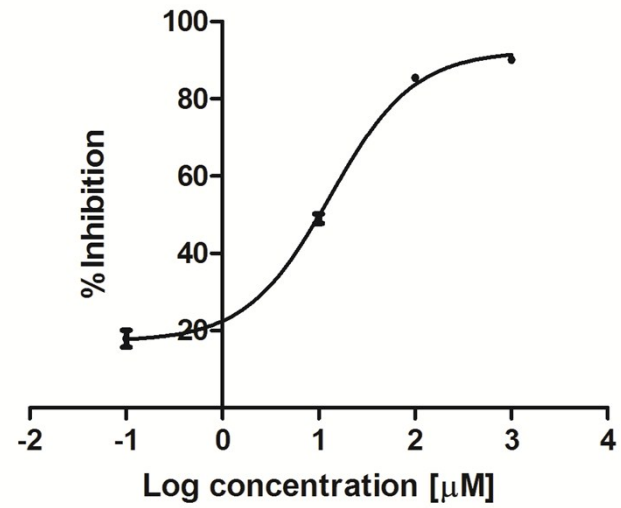
Dose-response curve of 3F for AChE



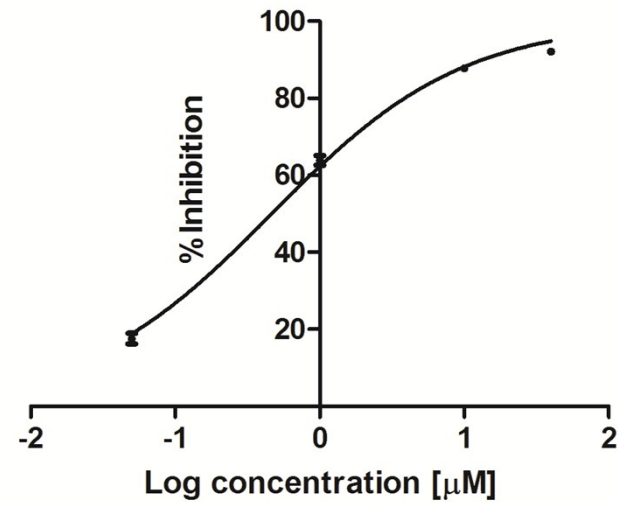
Dose-response curve of 3G for AChE



Dose-response curve of 3F for BChE



Dose-response curve of 3G for BChE



### 3) INHIBITION OF SELF-MEDIATED A $\beta_{1-42}$ AGGREGATION at 10 $\mu$ M

**Table S1.** *In vitro* effects on self-mediated A $\beta_{1-42}$  aggregation at 10  $\mu$ M

| Compound                   | A $\beta_{1-42}$<br>aggregation<br>(%) $\pm$ SEM |
|----------------------------|--------------------------------------------------|
| <b>3d</b>                  | 93.00 $\pm$ 2.96                                 |
| <b>3e</b>                  | 82.21 $\pm$ 6.69*                                |
| <b>3f</b>                  | 88.04 $\pm$ 9.69                                 |
| <b>3g</b>                  | 91.42 $\pm$ 4.01                                 |
| <b>3h</b>                  | 80.58 $\pm$ 1.70**                               |
| <b>Control<sup>a</sup></b> | 100                                              |
| <b>Donepezil</b>           | 64.75 $\pm$ 1.63***                              |

<sup>a</sup>) For each assay, the control was a non-treated sample.

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001