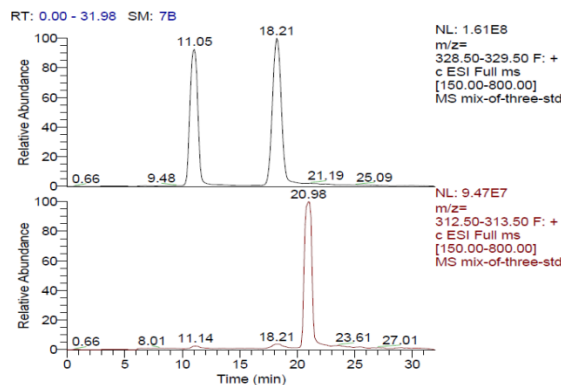


## Supporting Information

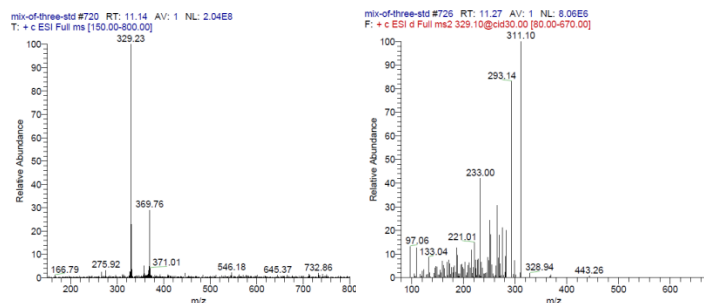
### Epoxidation Activities of Human Cytochromes P450c17 and P450c21

Francis K. Yoshimoto, Hwei-Ming Peng, Haoming Zhang, Sean M. Anderson, Richard J. Auchus

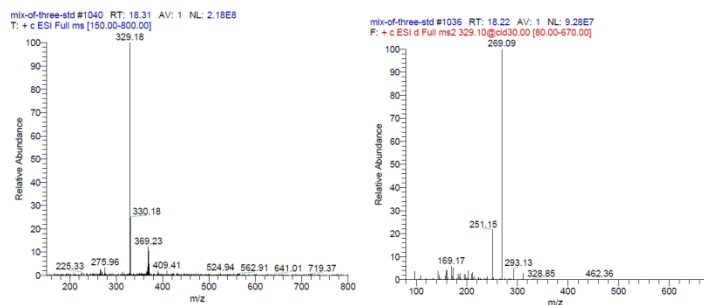
| <u>Table of contents</u>  | <u>Page</u> |
|---|-------------|
| Figure S1. Mass spectrometry of standards   | S2          |
| Figure S2. CYP17A1 incubation with compound <b>1</b>  | S3          |
| Figure S3. CYP17A1-A105L incubation with compound <b>1</b>  | S4          |
| Figure S4. CYP21A2 incubation with compound <b>1</b>  | S5          |
| Figure S5. CYP21A2-V359A incubation with compound <b>1</b>  | S6          |
| Figure S6. CYP17A1 incubations with compound <b>1</b> with and without ketoconazole or abiraterone      | S7          |
| Figure S7. Inhibition of CYP21A2 and metabolism of compound <b>1</b> by 3-keto- $\Delta^4$ -abiraterone | S8          |
| Figure S8. CYP17A1 activity after pre-incubation with progesterone or compounds <b>1</b> and <b>2</b>   | S9          |
| Figure S9. Proposed fragmentation of parent ions of compounds <b>1</b> , <b>2</b> , and <b>3</b>        | S10         |
| Figure S10. NMR spectra of new compounds in this study  | S11-27      |



### 21-hydroxy-16,17-dehydroprogesterone (3):



### 16,17-epoxyprogesterone (2):



### 16,17-dehydroprogesterone (1):

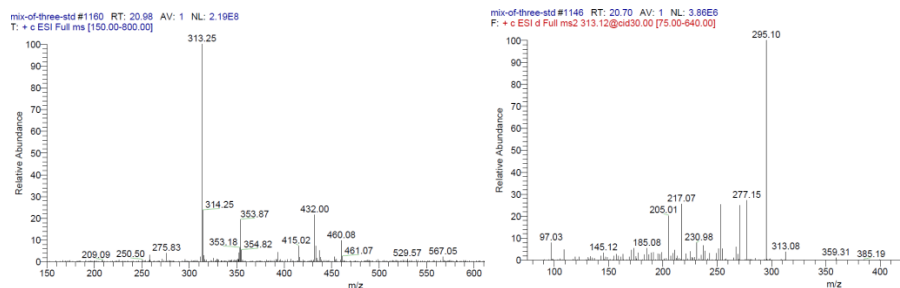
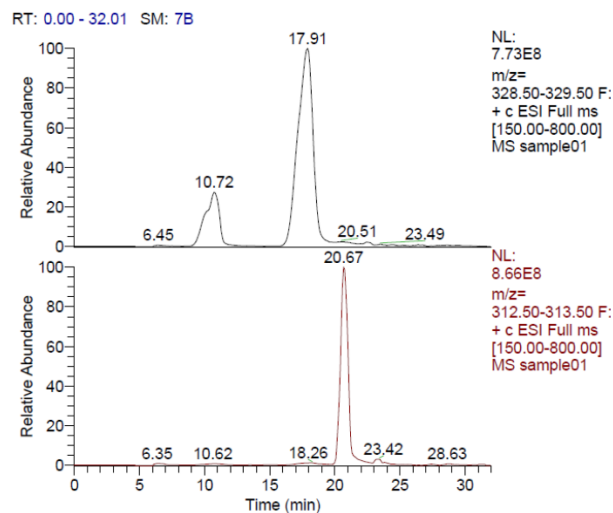
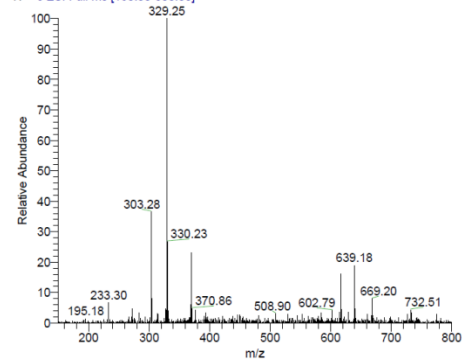


Figure S1. Extracted ion chromatograms, MS and MS<sup>2</sup> spectra for 21-hydroxy-16,17-dehydroprogesterone (3, 11.05 min), 16,17-epoxyprogesterone (2, 18.21 min) and 16,17-dehydroprogesterone (1, 20.98 min) standards. The [M+H]<sup>+</sup> ions of products 3 and 2 or of substrate 1 are m/z 329, 329 and 313 respectively. Proposed fragmentation pathways are shown in Figure S9.

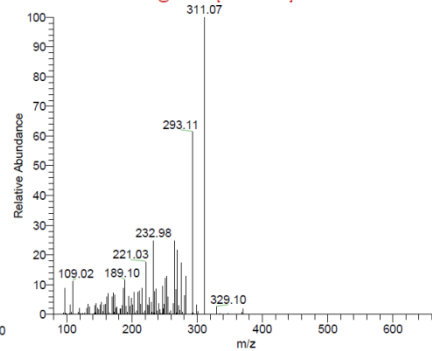


### Metabolite A:

sample01 #774 RT: 10.82 AV: 1 NL: 2.85E8  
T: + c ESI Full ms [150.00-800.00]

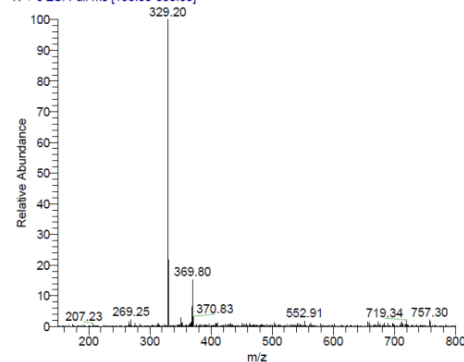


sample01 #760 RT: 10.55 AV: 1 NL: 2.43E7  
F: + c ESI d Full ms2 329.10@cid30.00 [80.00-670.00]



### Metabolite B:

sample01 #1129 RT: 17.80 AV: 1 NL: 8.10E8  
T: + c ESI Full ms [150.00-800.00]



sample01 #1145 RT: 18.10 AV: 1 NL: 2.78E8  
F: + c ESI d Full ms2 329.10@cid30.00 [80.00-670.00]

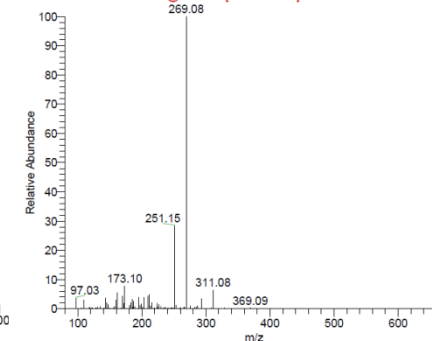
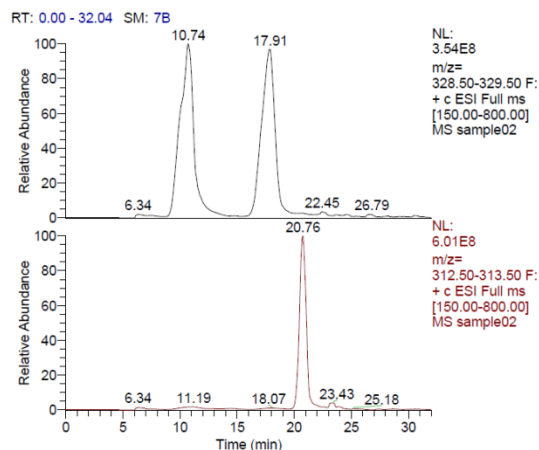
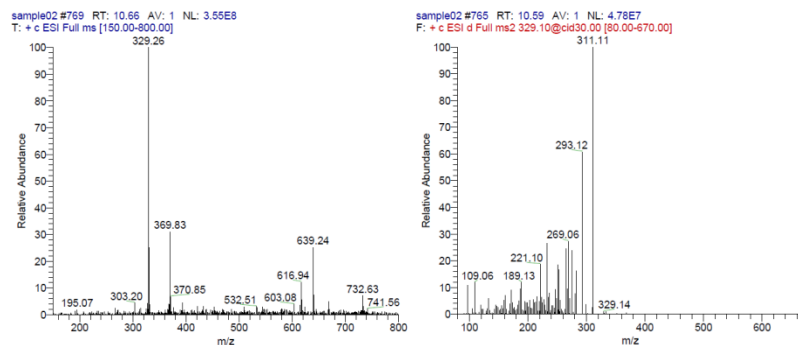


Figure S2. Extracted ion chromatograms, MS and MS<sup>2</sup> spectra of Metabolite A = 21-hydroxy-16,17-dehydroprogesterone (**3**) and Metabolite B = 16,17-epoxyprogesterone (**2**) obtained by LC-MS/MS analysis of products derived from incubations of wild-type CYP17A1 in yeast microsomes with compound **1** (16,17-dehydroprogesterone) in the presence of NADPH.



### Metabolite A:



### Metabolite B:

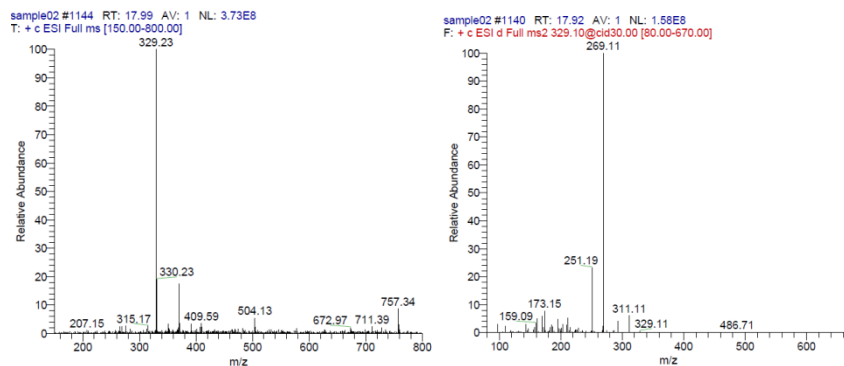
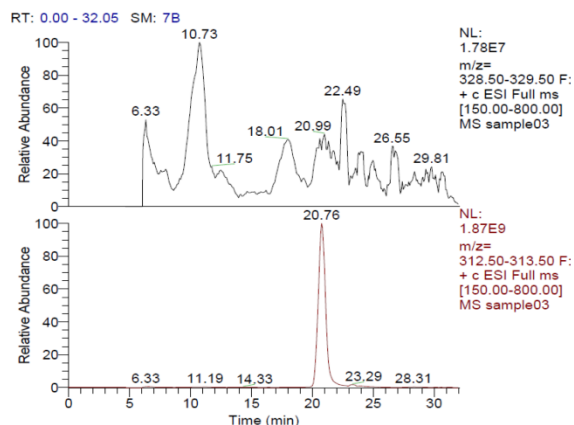
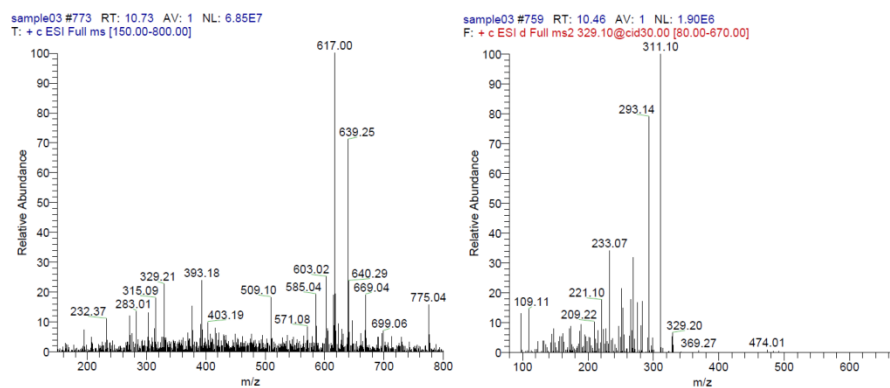


Figure S2. Extracted ion chromatograms, MS and MS<sup>2</sup> spectra of Metabolite A = 21-hydroxy-16,17-dehydroprogesterone (**3**) and Metabolite B = 16,17-epoxyprogesterone (**2**) obtained by LC-MS/MS analysis of products derived from incubations of CYP17A1 mutation A105L in yeast microsomes with compound **1** (16,17-dehydroprogesterone) in the presence of NADPH.



### Metabolite A:



### Metabolite B:

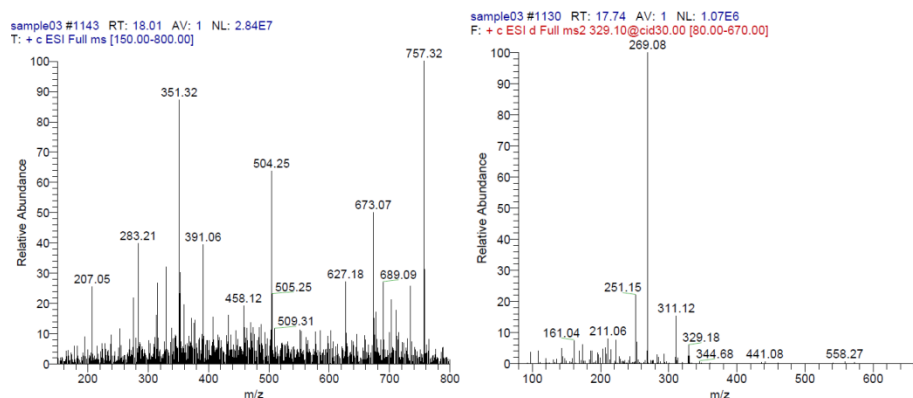
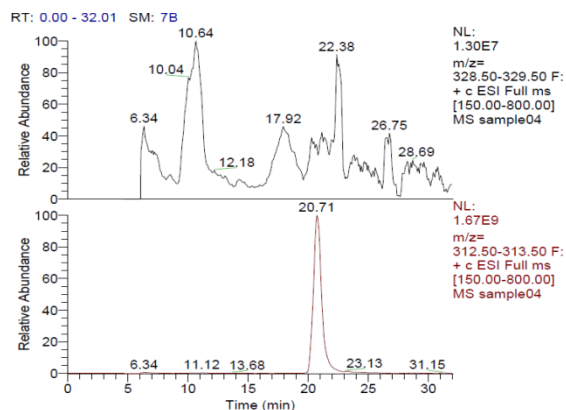
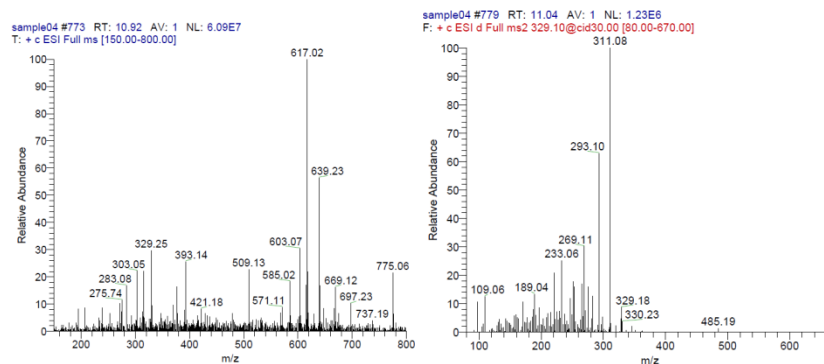


Figure S4. Extracted ion chromatograms, MS and MS<sup>2</sup> spectra of Metabolite A = 21-hydroxy-16,17-dehydroprogesterone (**3**) and Metabolite B = 16,17-epoxyprogesterone (**2**) obtained by LC-MS/MS analysis of products derived from incubations of wild-type CYP21A2 in yeast microsomes with compound **1** (16,17-dehydroprogesterone) in the presence of NADPH.



### Metabolite A:



### Metabolite B:

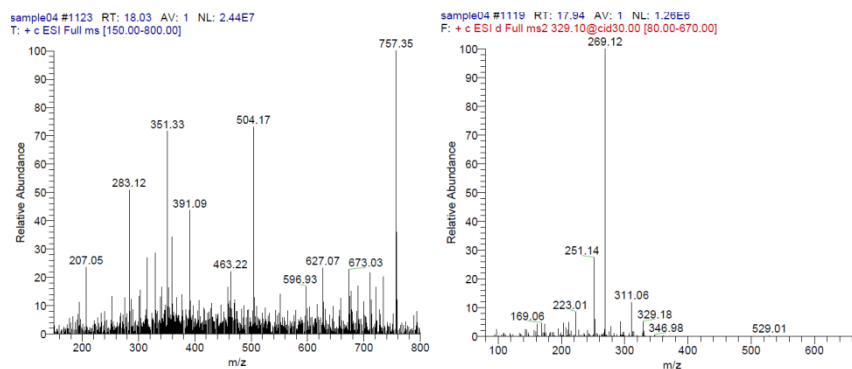


Figure S5. Extracted ion chromatograms, MS and MS<sup>2</sup> spectra of Metabolite A = 21-hydroxy-16,17-dehydroprogesterone (**3**) and Metabolite B = 16,17-epoxyprogesterone (**2**) obtained by LC-MS/MS analysis of products derived from incubations of CYP21A2 mutation V359A in yeast microsomes with compound **1** (16,17-dehydroprogesterone) in the presence of NADPH.

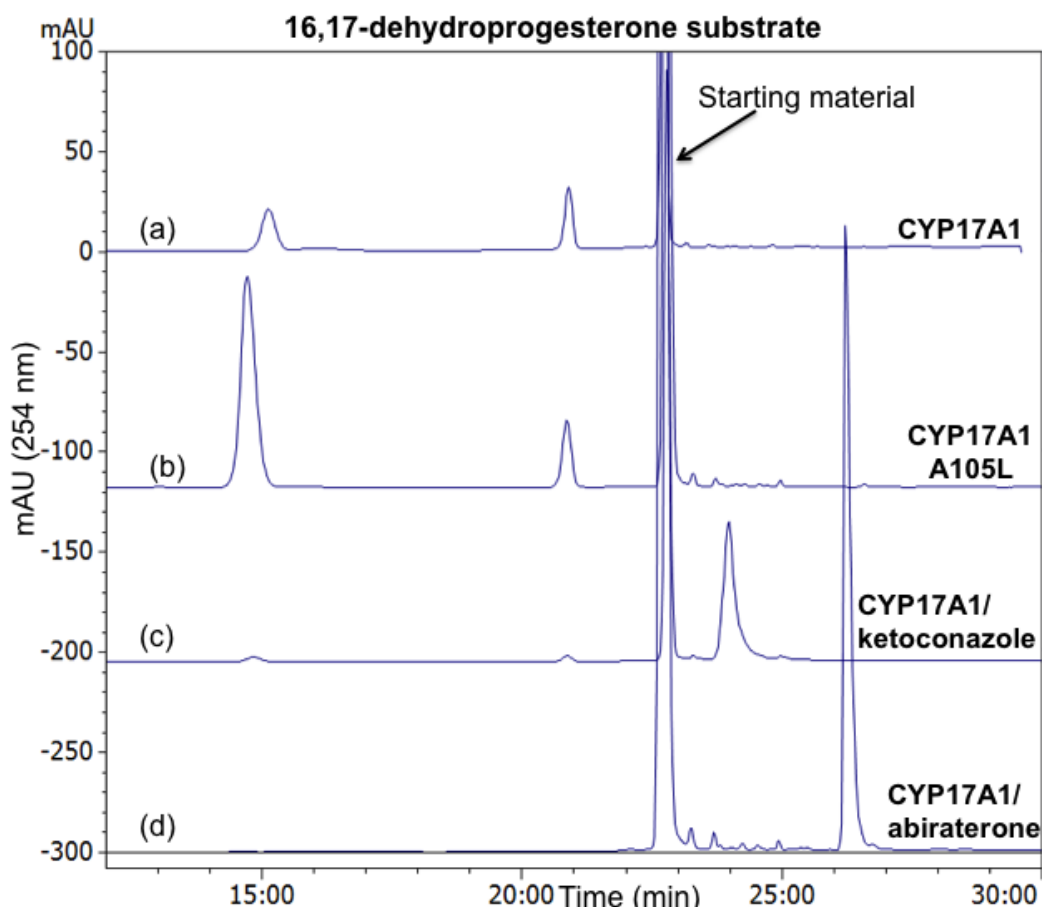
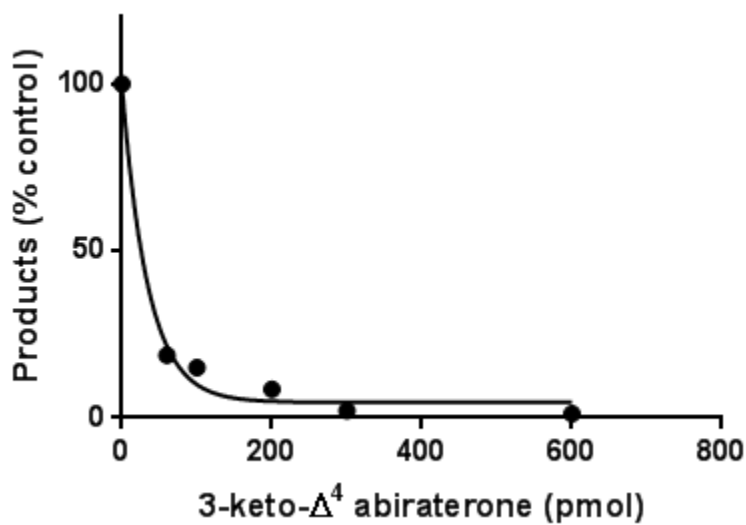


Figure S6. HPLC chromatograms of products from incubations of CYP17A1 with compound **1** (16,17-dehydroprogesterone) in the absence and presence of ketoconazole or abiraterone. (A) CYP17A1 incubation with compound **1**. (B) CYP17A1 A105L incubation with compound **1**. (C) CYP17A1 incubation with compound **1** and ketoconazole (50  $\mu$ M). (D) CYP17A1 incubation with compound **1** and abiraterone (20  $\mu$ M). Ketoconazole and abiraterone inhibit formation of both products.

A



B

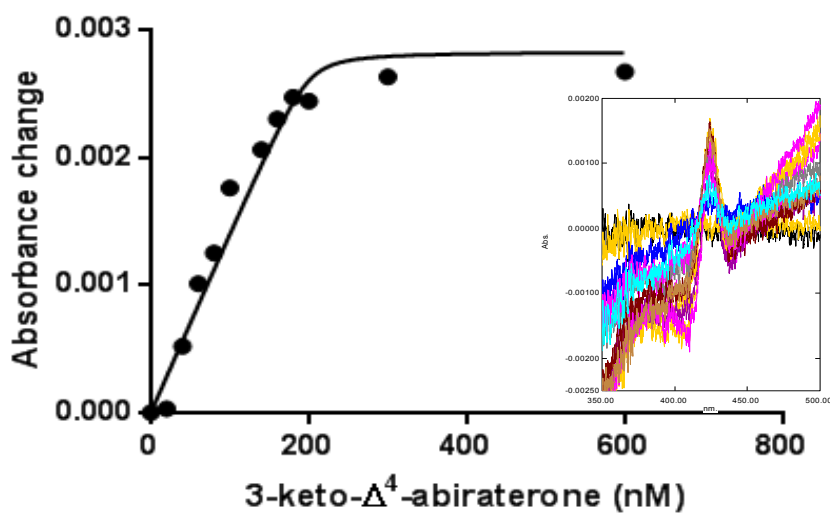


Figure S7. Inhibition of CYP21A2-catalyzed product formation by 3-keto- $\Delta^4$ -abiraterone. A, Total products formed (primarily compound **3**) from incubations of CYP21A2 (30 pmol) and POR with compound **1** (16,17-dehydroprogesterone, 20  $\mu$ M) are progressively reduced by increasing amounts of added 3-keto- $\Delta^4$ -abiraterone. B, Type 2 spectral changes from titration of purified CYP21A2 (200 pmol) with 3-keto- $\Delta^4$ -abiraterone and curve fitting (ref 23), yielding  $K_s = 1.1 \pm 0.5$  nM,  $\epsilon = 15 \pm 1$   $\text{mM}^{-1}\text{cm}^{-1}$ .



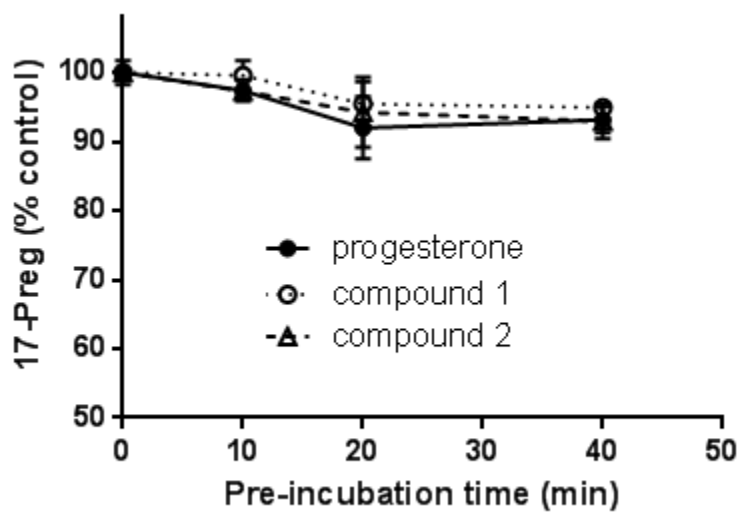


Figure S8. Lack of time-dependent CYP17A1 inhibition by compound **1** or epoxide **2**. Purified CYP17A1 and POR were pre-incubated with progesterone, compound **1**, or epoxide **2** for specified times, then the reaction was diluted and assayed for 17-hydroxylase activity with pregnenolone substrate.

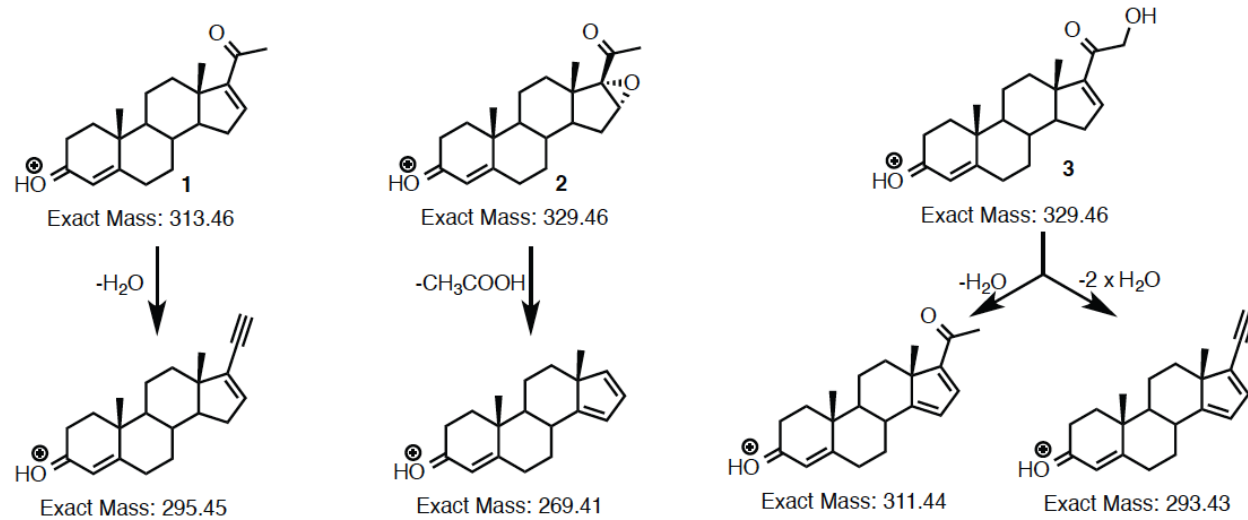
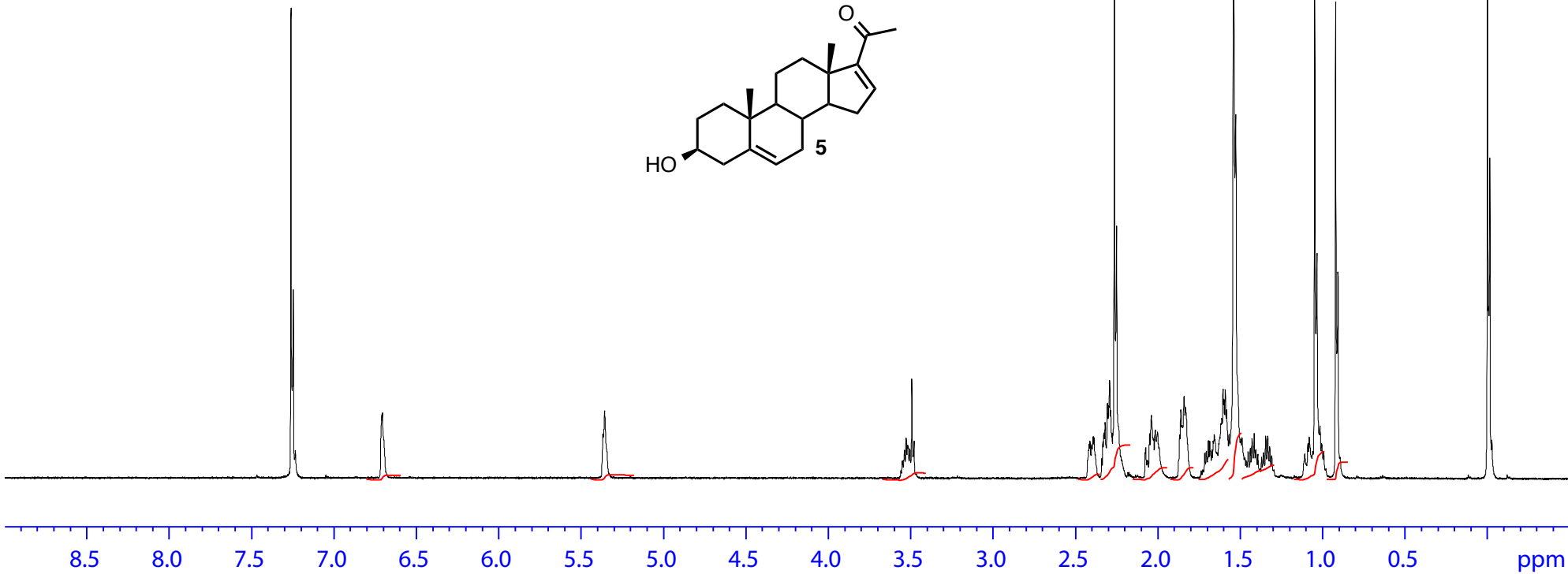
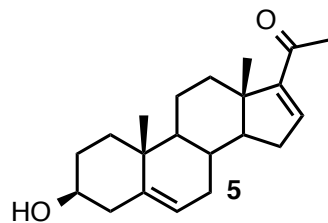


Figure S9. Proposed fragmentation pathways of parent ions in LC-MS/MS experiments.

delta.16.preg

7.261  
7.260  
7.246  
6.708  
6.705  
5.367  
5.356  
3.526  
3.492  
3.491  
2.391  
2.385  
2.326  
2.318  
2.304  
2.296  
2.291  
2.285  
2.277  
2.262  
2.248  
2.047  
2.037  
2.014  
2.000  
1.865  
1.859  
1.851  
1.838  
1.831  
1.655  
1.610  
1.602  
1.597  
1.589  
1.581  
1.565  
1.539  
1.525  
1.494  
1.487  
1.426  
1.413  
1.343  
1.332  
1.079  
1.046  
1.032  
1.013  
0.919  
0.905  
-0.002  
-0.003  
-0.009  
-0.010  
-0.012  
-0.017  
-0.030

Current Data Parameters  
NAME delta16preg  
EXPNO 1  
PROCNO 1  
  
F2 - Processing parameters  
SI 32768  
SF 499.7783801 MHz  
WDW no  
SSB 0  
LB 0 Hz  
GB 0  
PC 1.00



FYVII-044B.delta5.delta16.prog

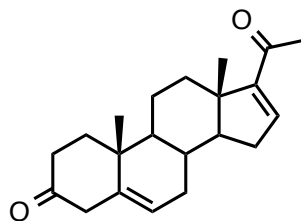
7.264  
7.261  
7.256

6.710

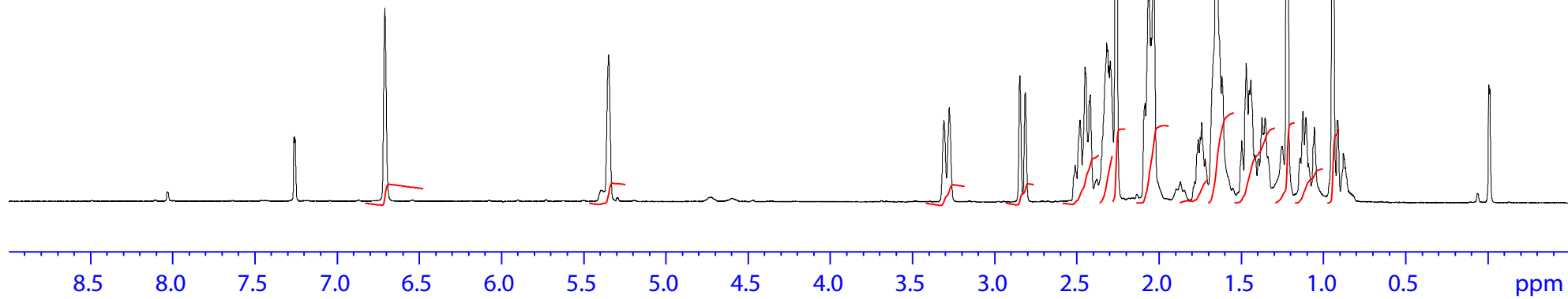
5.349  
3.309  
3.277  
2.847  
2.814  
2.480  
2.451  
2.445  
2.433  
2.424  
2.419  
2.319  
2.312  
2.305  
2.298  
2.268  
2.265  
2.260  
2.089  
2.085  
2.064  
2.061  
2.050  
2.033  
1.763  
1.750  
1.741  
1.652  
1.618  
1.497  
1.470  
1.453  
1.442  
1.418  
1.374  
1.365  
1.355

Current Data Parameters  
NAME delta5delta16progPROTON  
EXPNO 1  
PROCNO 1

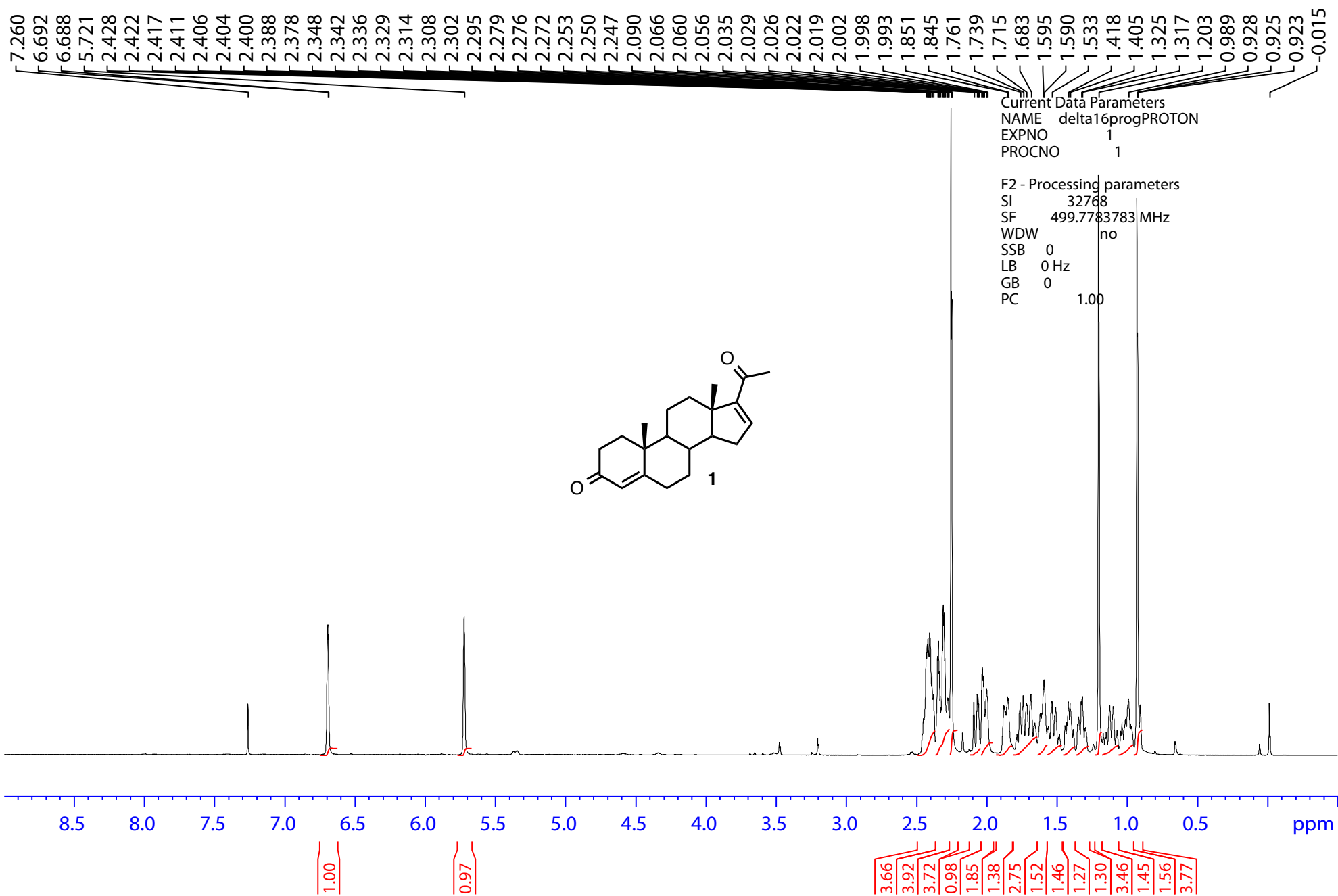
F2 - Processing parameters  
SI 32768  
SF 499.7783782 MHz  
WDW no  
SSB 0  
LB 0 Hz  
GB 0  
PC 1.00



pregna-5,16-diene-3,20-dione



1.00  
1.29  
1.16  
1.28  
3.33  
3.35  
5.14  
5.36  
1.65  
6.27  
5.23  
5.57  
2.37  
5.14



Standard Carbon

199.6782  
196.8531

171.0995

155.2108

144.3061

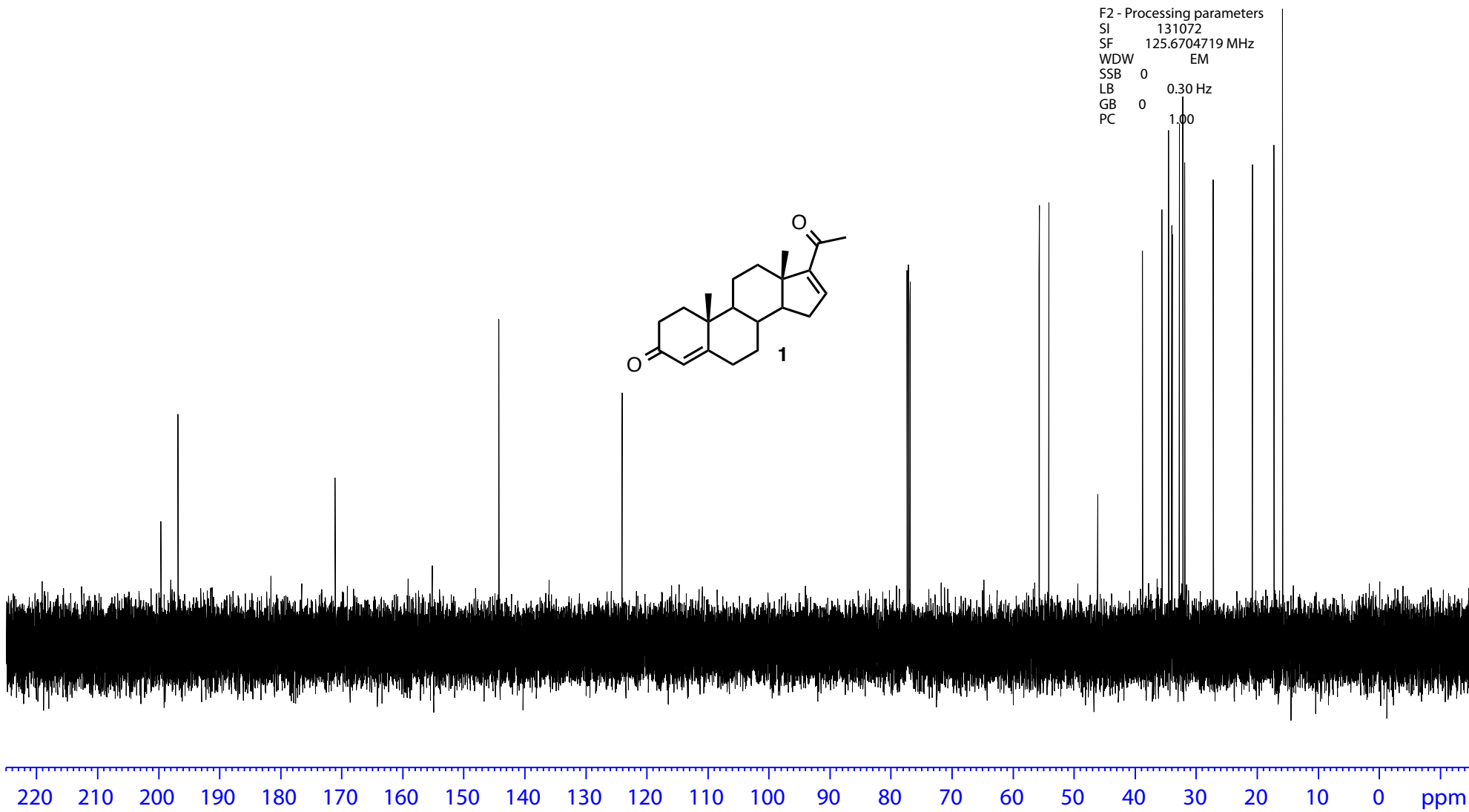
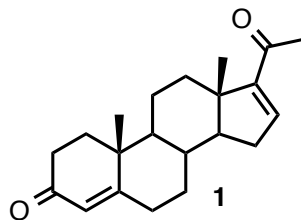
124.0936

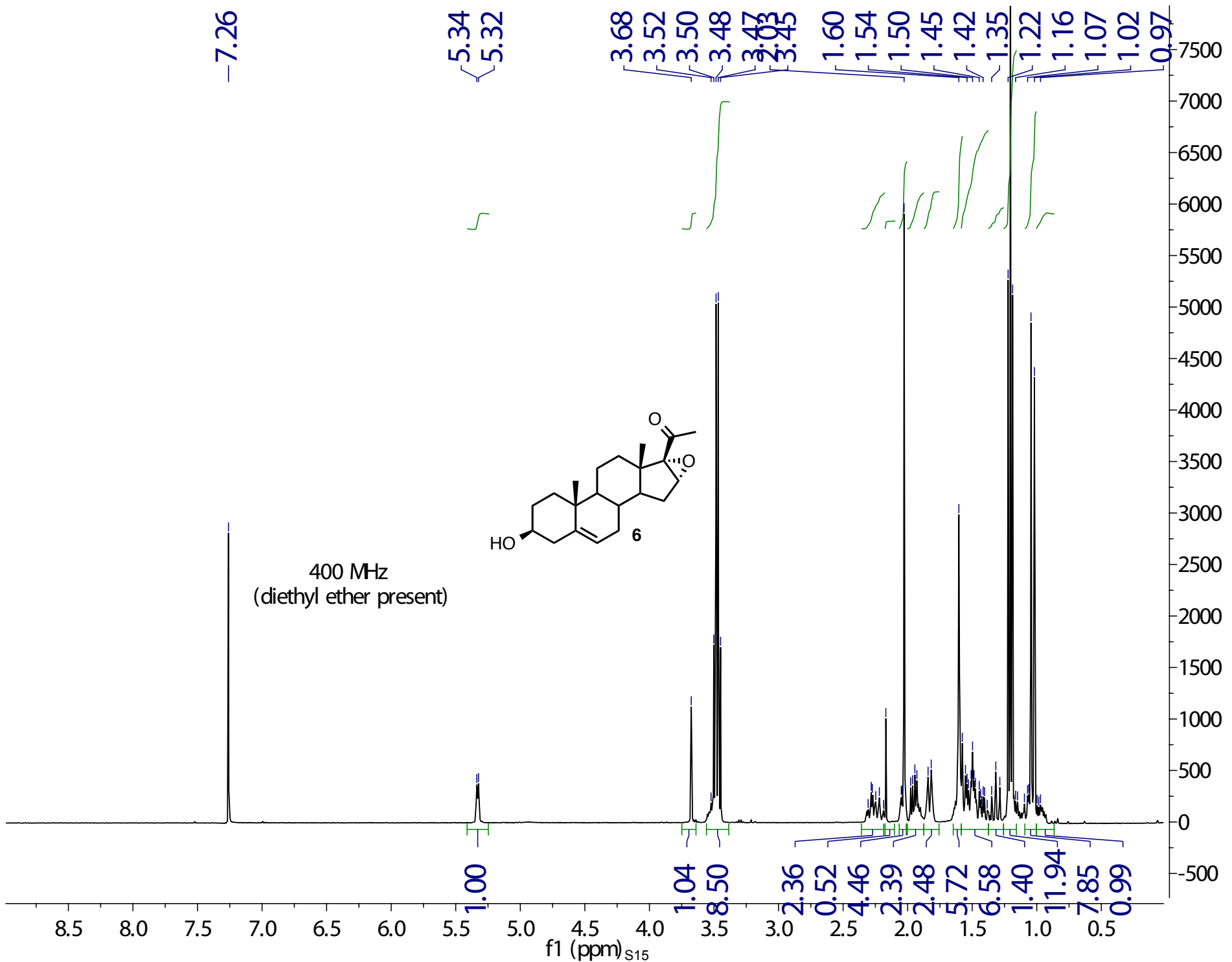
77.4181  
77.1593  
76.9045

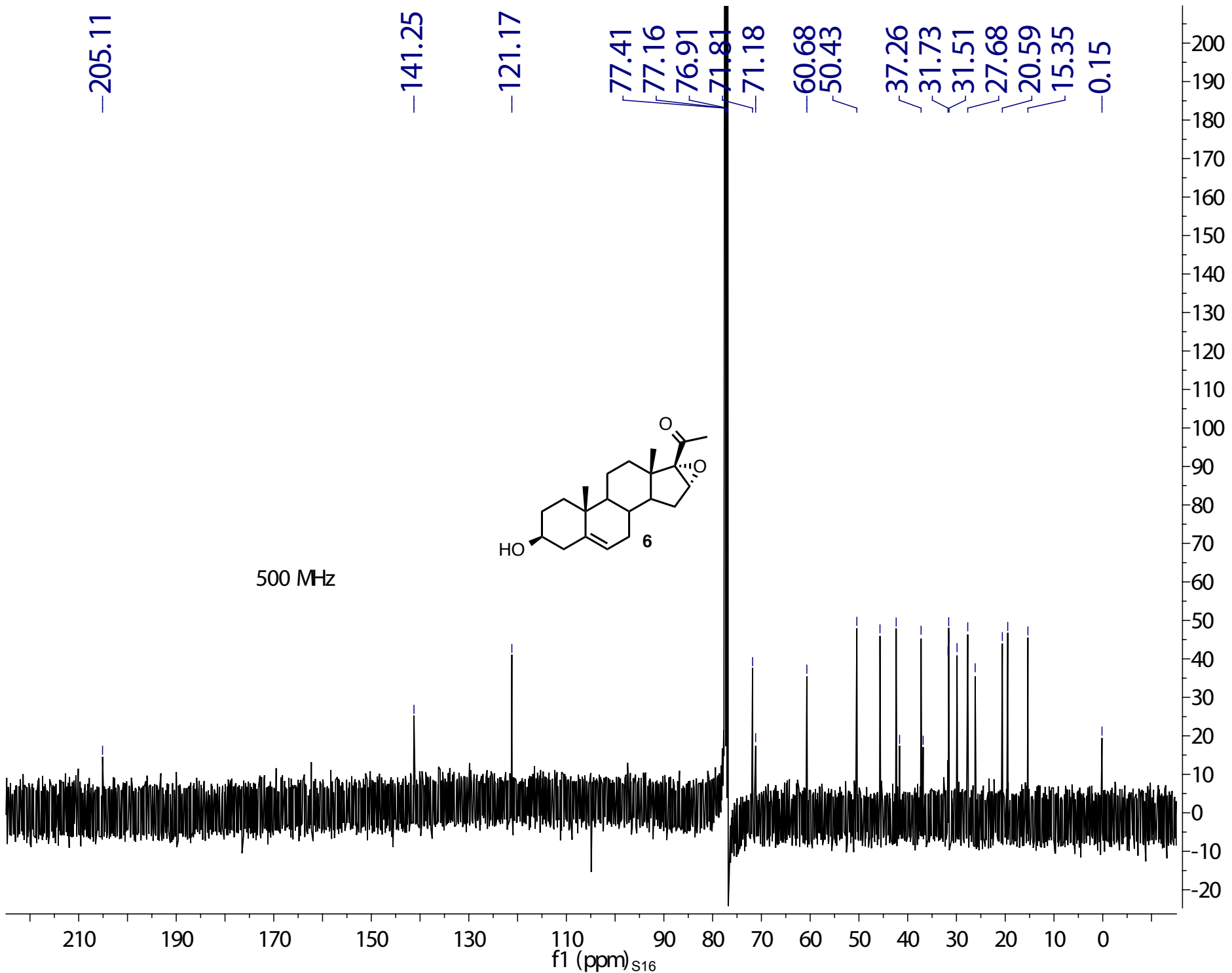
55.7530  
54.1624  
46.1511  
38.8188  
35.6288  
34.5364  
34.0468  
33.9254  
32.8009  
32.2047  
31.8916  
27.2284  
20.8244  
17.2908  
15.8963

Current Data Parameters  
NAME: 44110101toprdCARBON  
EXPNO: 1  
PROCNO: 1

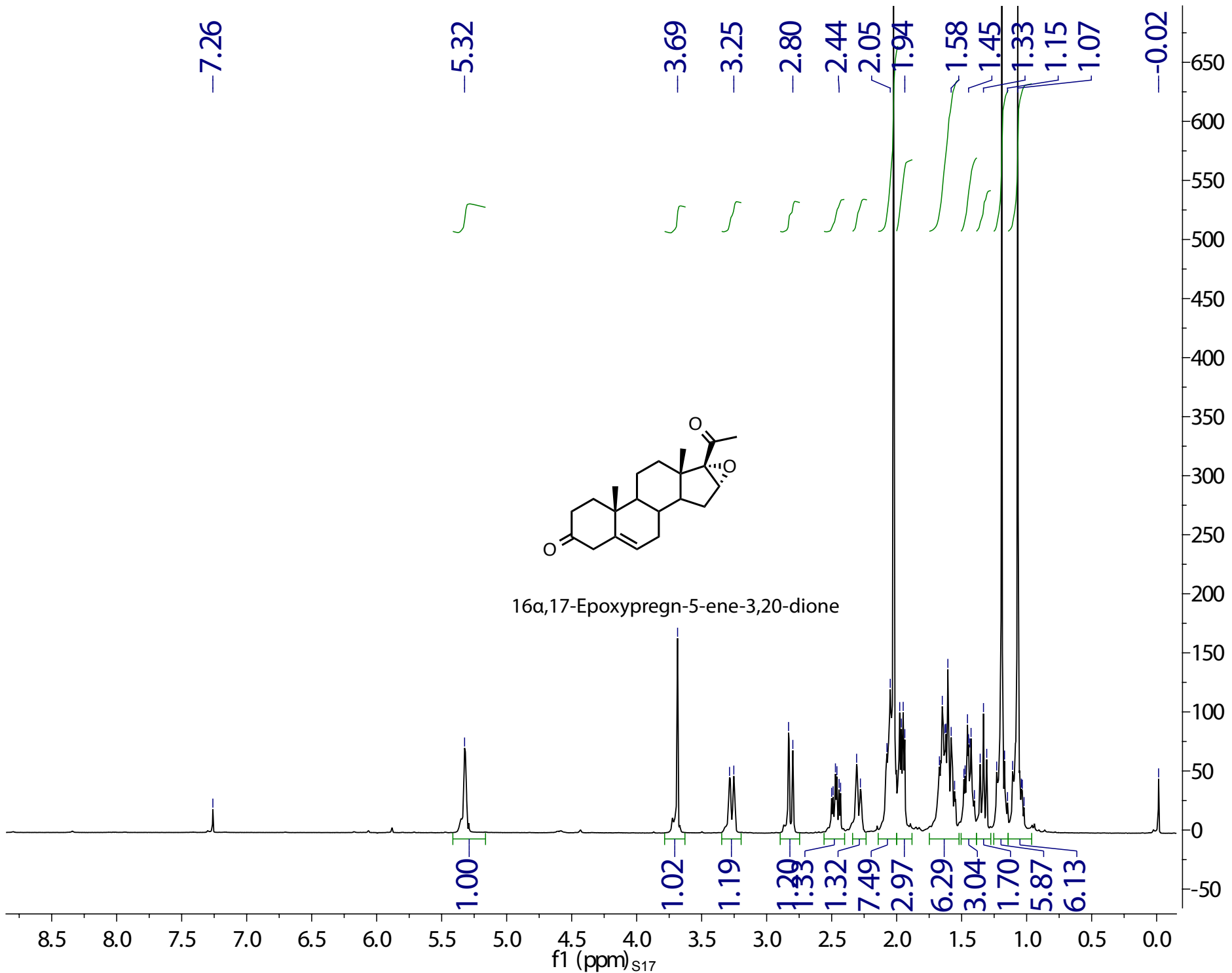
F2 - Processing parameters  
SI: 131072  
SF: 125.6704719 MHz  
WDW: EM  
SSB: 0  
LB: 0.30 Hz  
GB: 0  
PC: 1.00

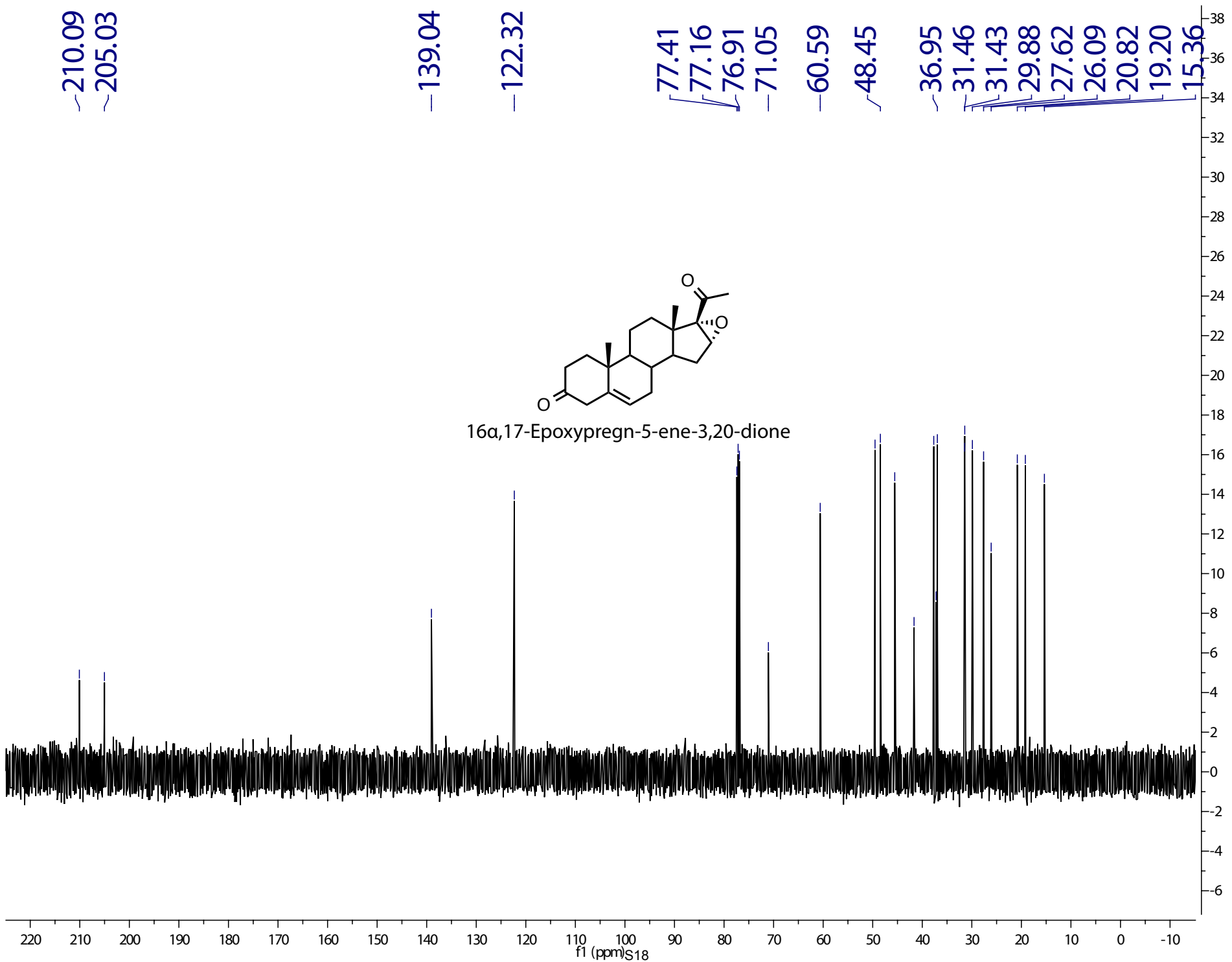


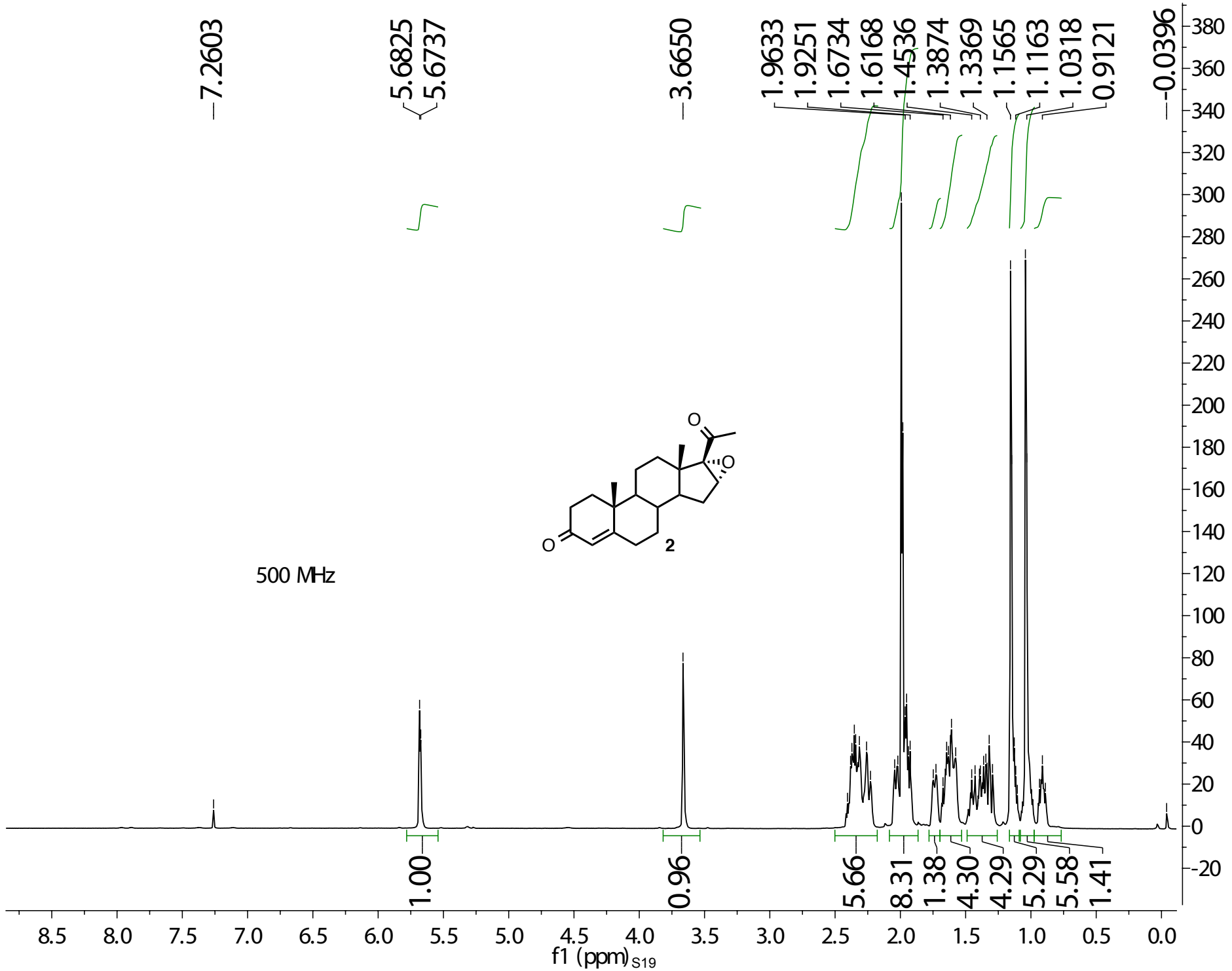


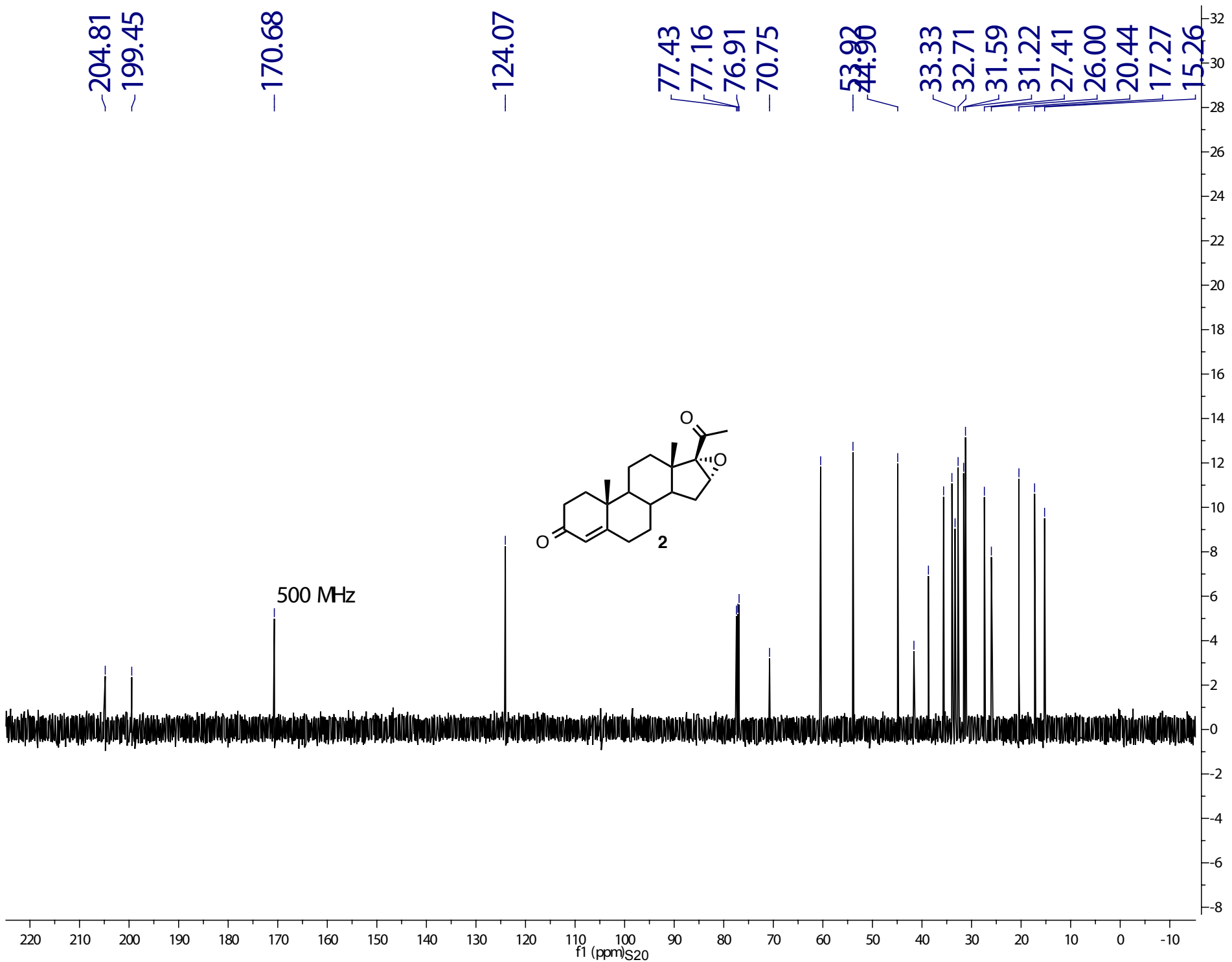












~204.81  
~199.45

-170.68

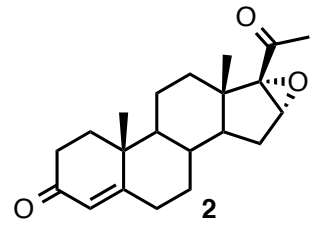
-124.07

77.43  
77.16  
76.91  
70.75

53.92  
44.96

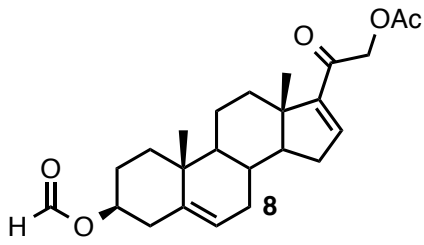
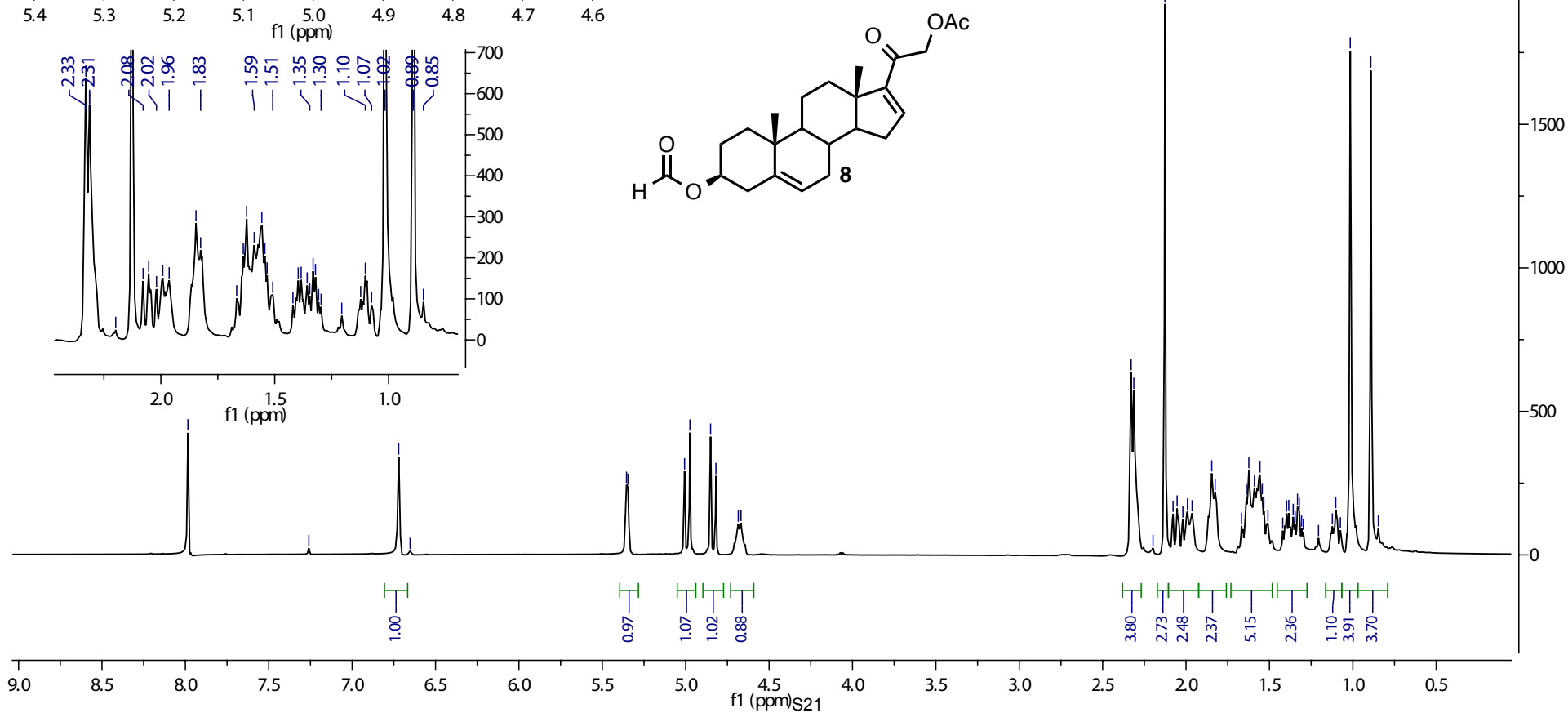
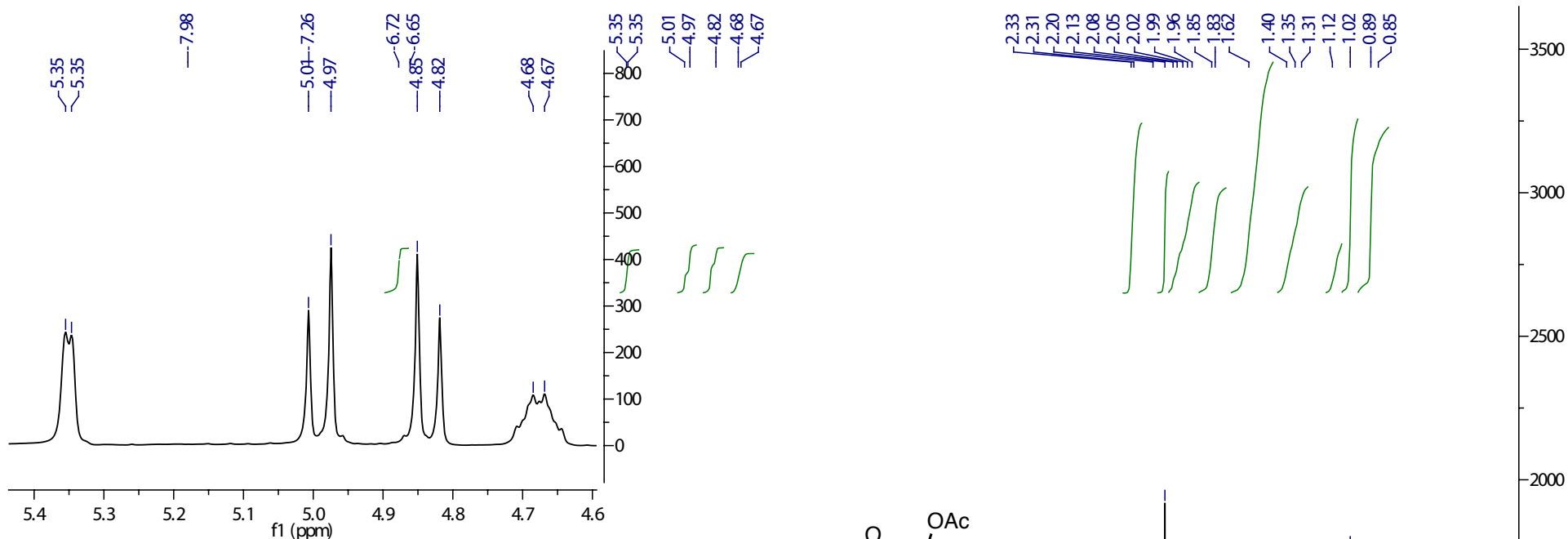
33.33  
32.71  
31.59  
31.22  
27.41  
26.00  
20.44  
17.27  
15.26

500 MHz



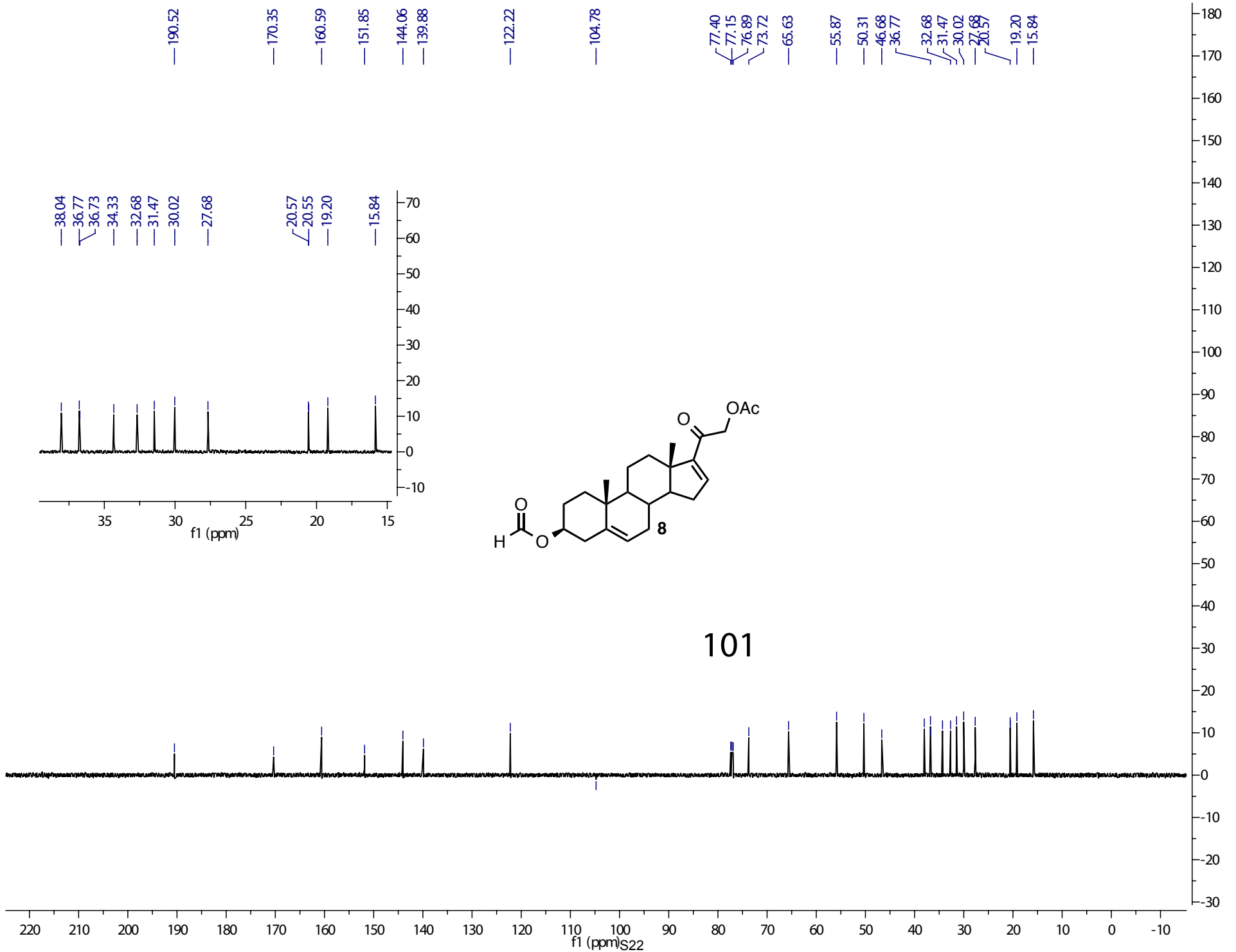
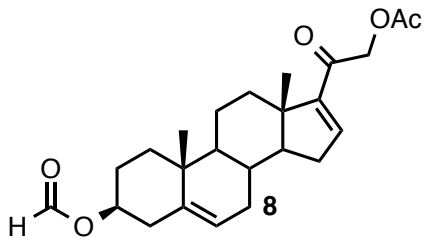
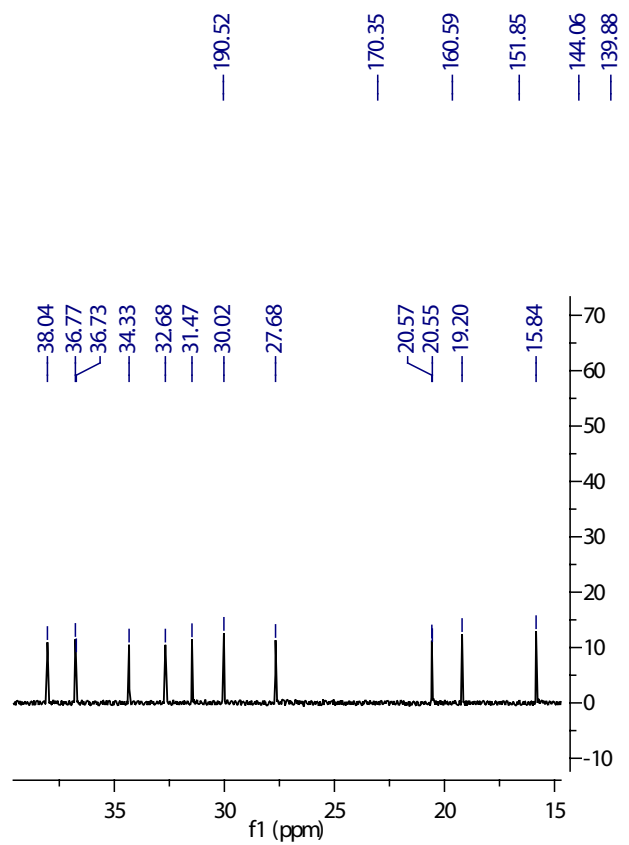
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

f1 (ppm)S20

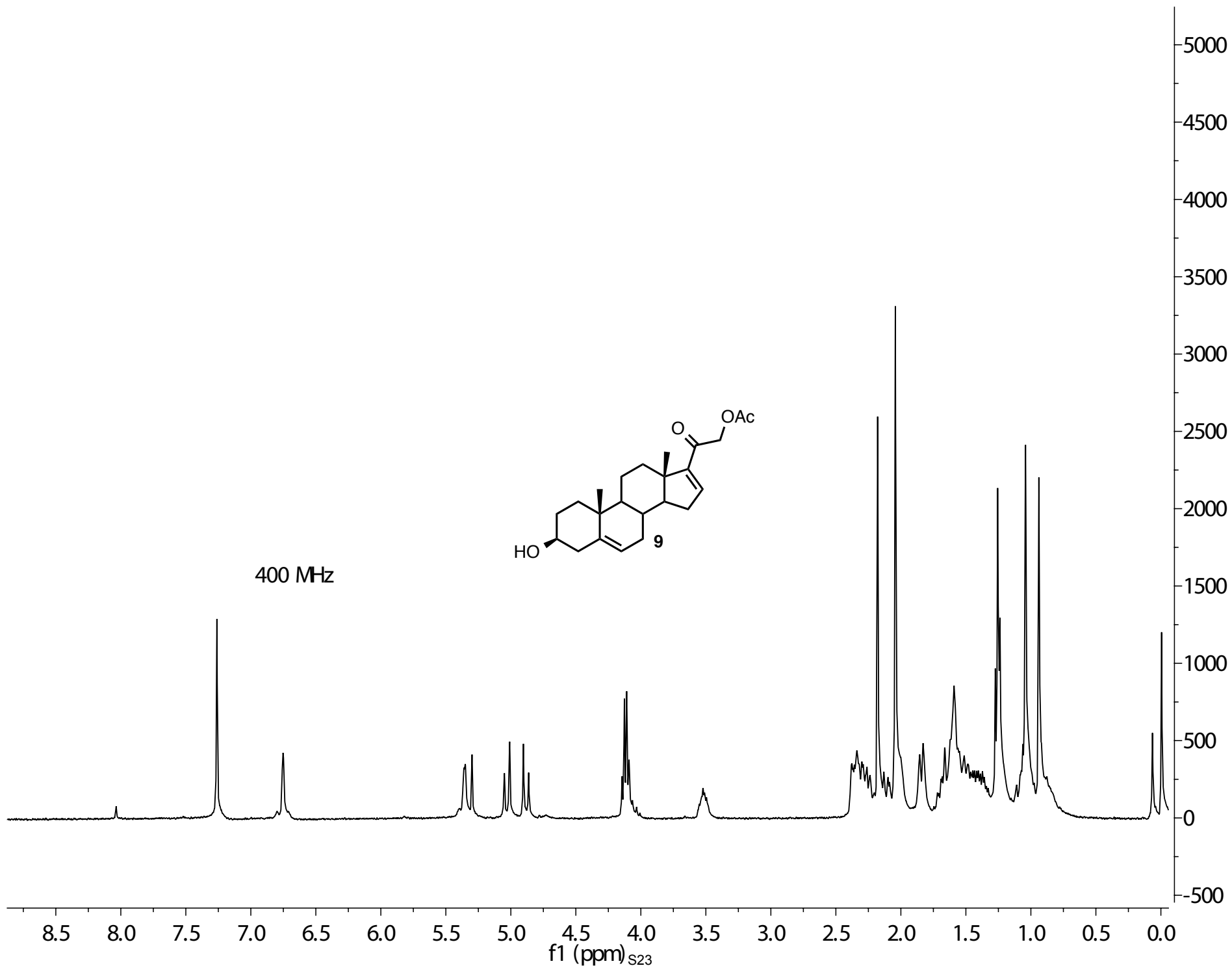


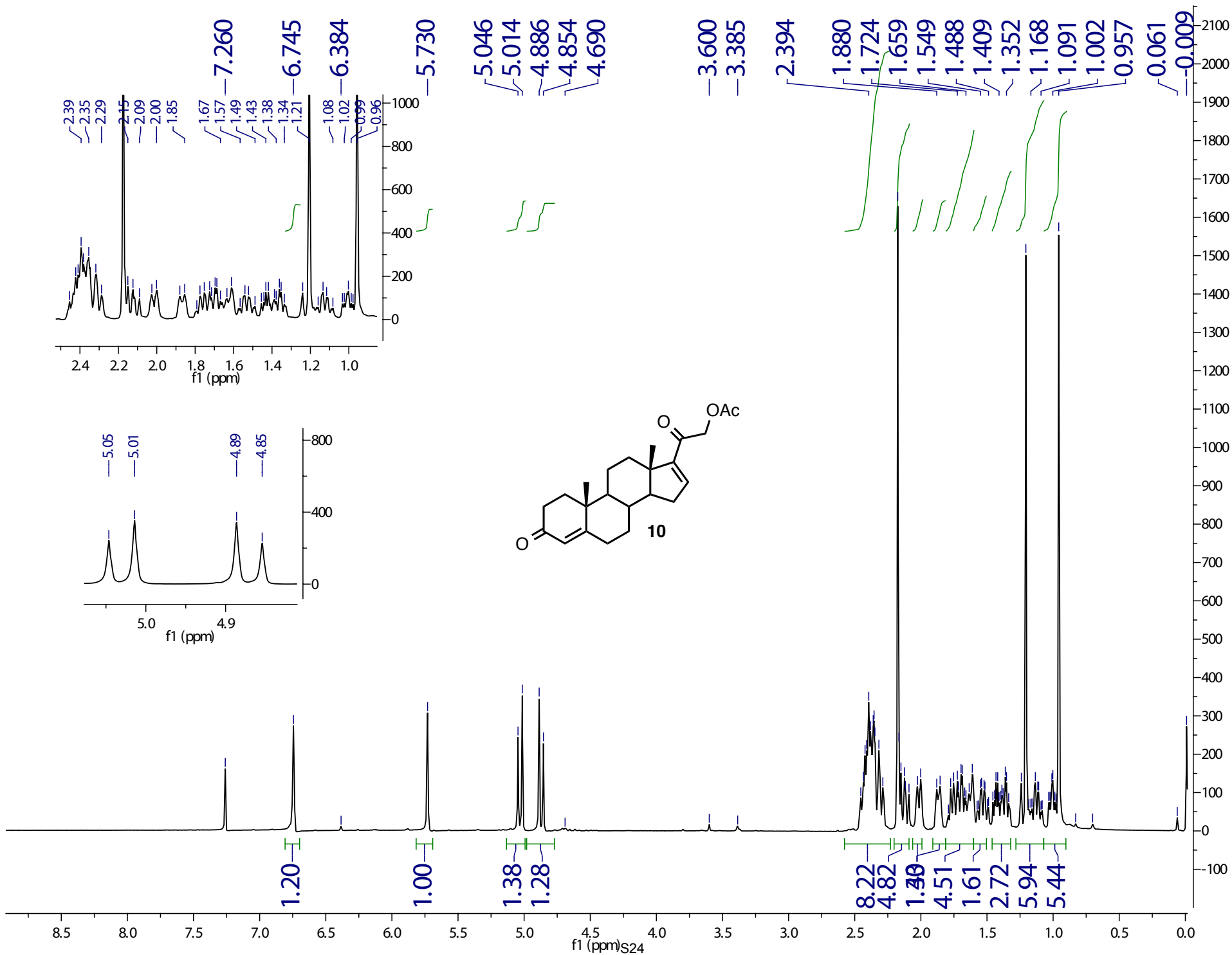
Integration values (green): 2.33, 2.31, 2.20, 2.13, 2.08, 2.05, 2.02, 1.99, 1.96, 1.85, 1.83, 1.62, 1.40, 1.35, 1.31, 1.12, 1.02, 0.89, 0.85.

Integration values (green): 1.00, 0.97, 1.07, 1.02, 0.88, 3.80, 2.73, 2.48, 2.37, 5.15, 2.36, 1.10, 3.91, 3.70.

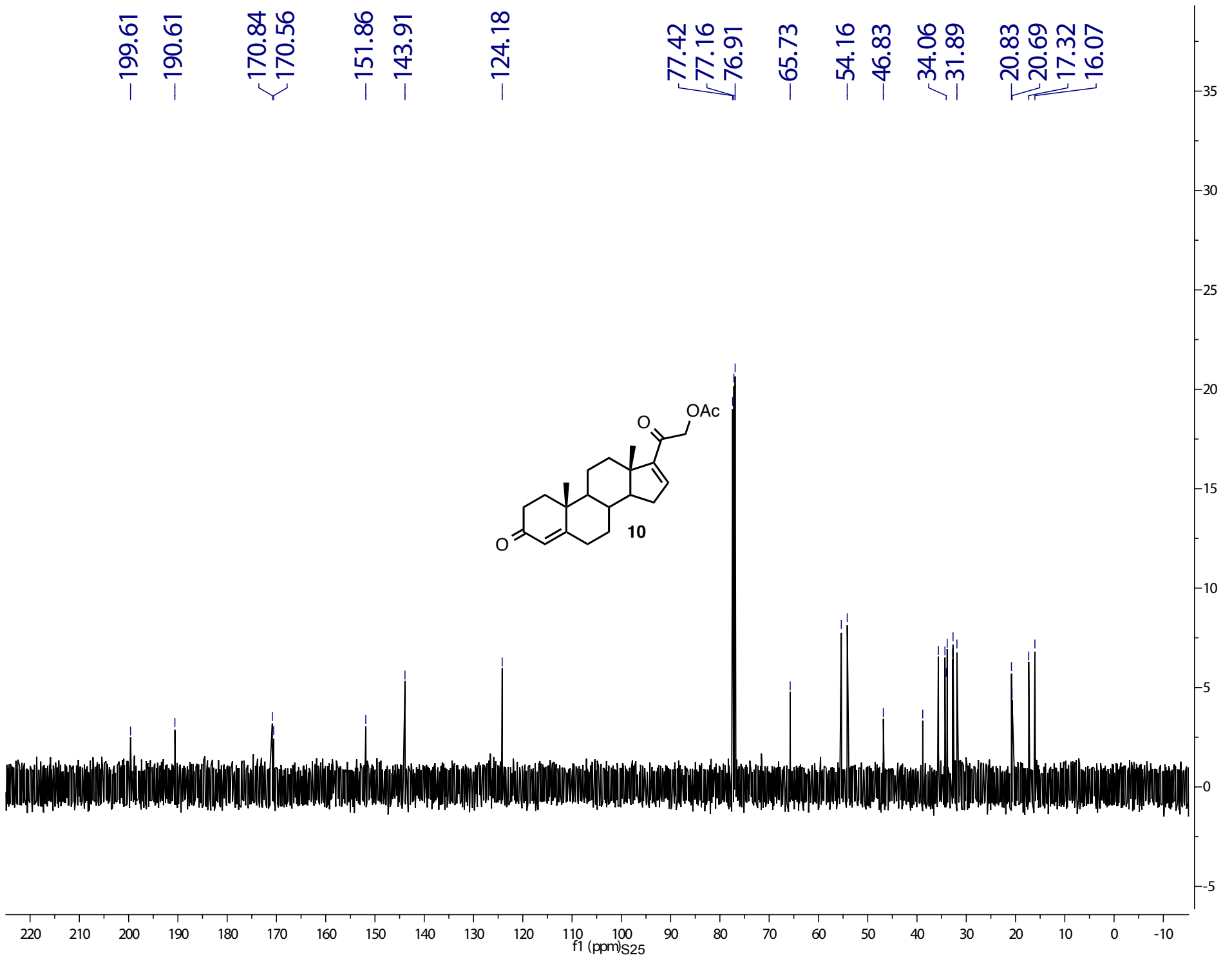
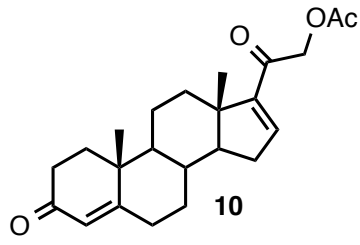


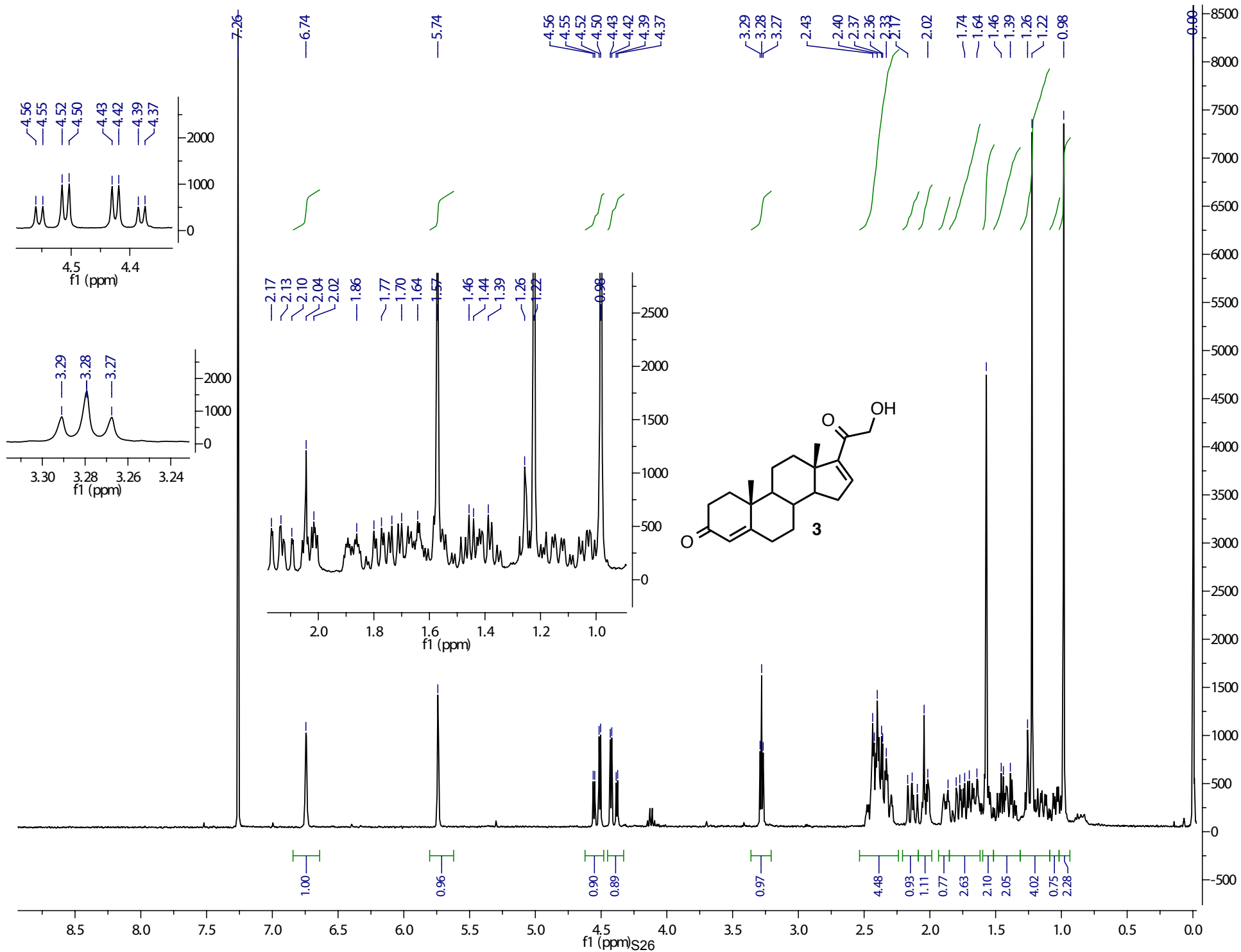
101

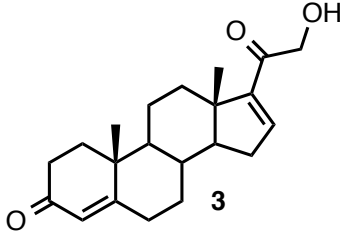












500 MHz

