Deuterated squalene and sterols from modified *Saccharomyces cerevisiae*

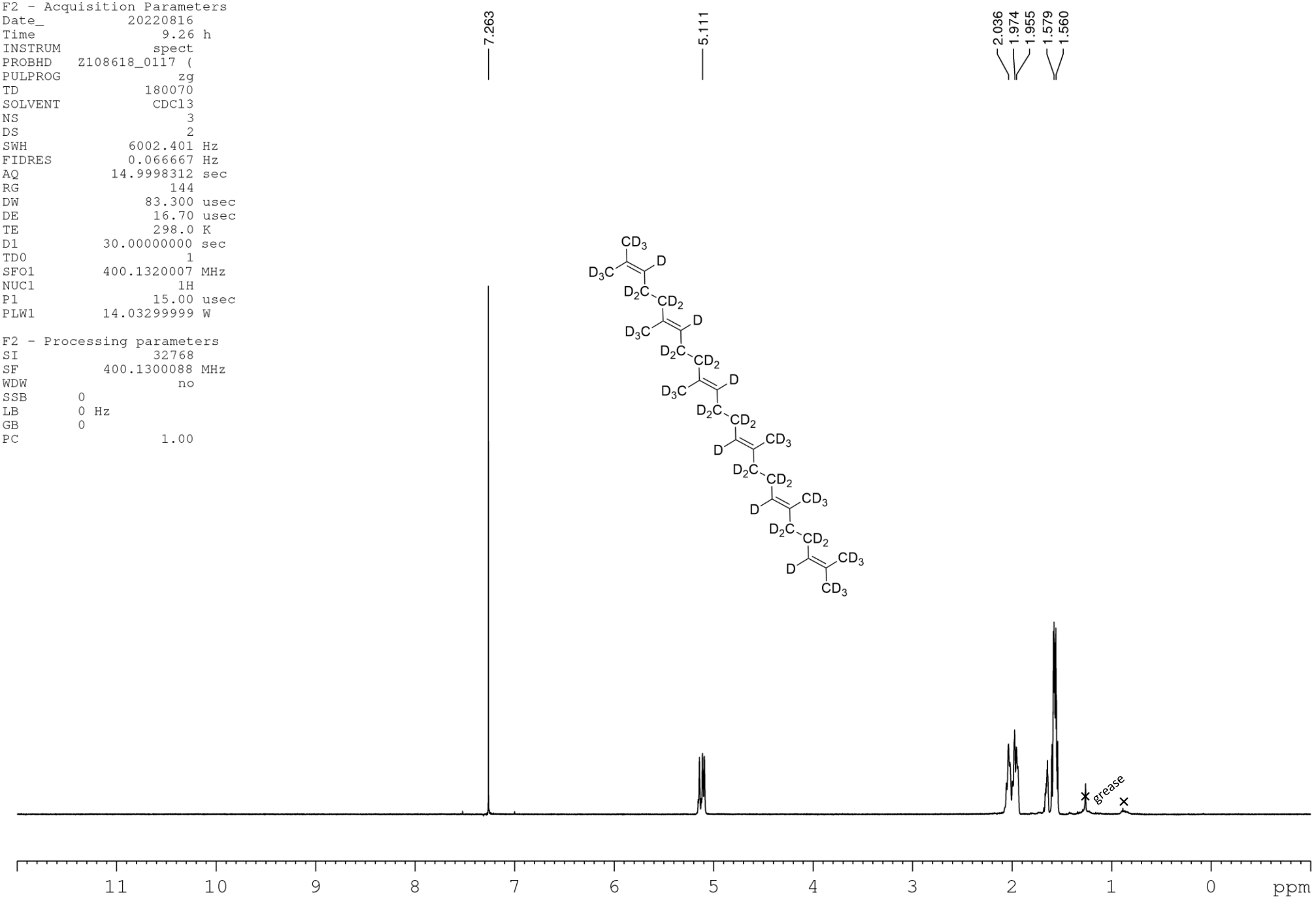
SUPPORTING INFORMATION: COPIES OF NMR SPECTRA

Carl Recsei,[†] Robert Russell, [†] Marina Cagnes[†] & Tamim Darwish[†]

[†]Australian Nuclear Science and Technology Organisation
National Deuteration Facility
New Illawarra Rd
Lucas Heights, New South Wales, Australia 2234

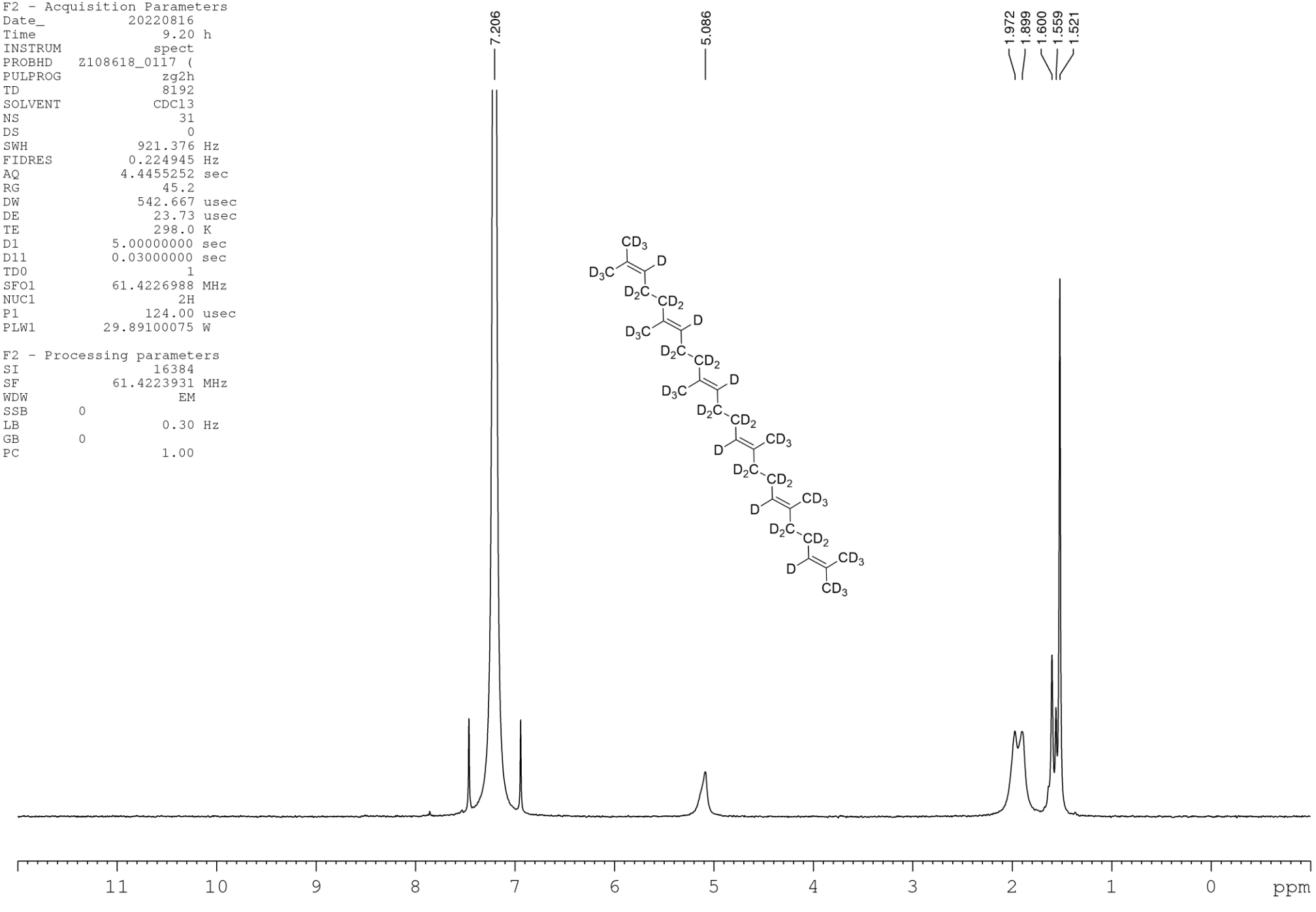
squalene-d_{50} (81%-*d*) ^{1}H NMR

```
F2 - Acquisition Parameters
Date_           20220816
Time               9.26 h
INSTRUM           spect
PROBHD   Z108618_0117 (
PULPROG             zg
TD              180070
SOLVENT           CDCl3
NS                    3
DS                    2
SWH            6002.401 Hz
FIDRES        0.066667 Hz
AQ           14.9998312 sec
RG                  144
DW               83.300 usec
DE               16.70 usec
TE               298.0 K
D1           30.00000000 sec
TD0                   1
SFO1         400.1320007 MHz
NUC1                 1H
P1                15.00 usec
PLW1         14.03299999 W

F2 - Processing parameters
SI                32768
SF           400.1300088 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                1.00
```



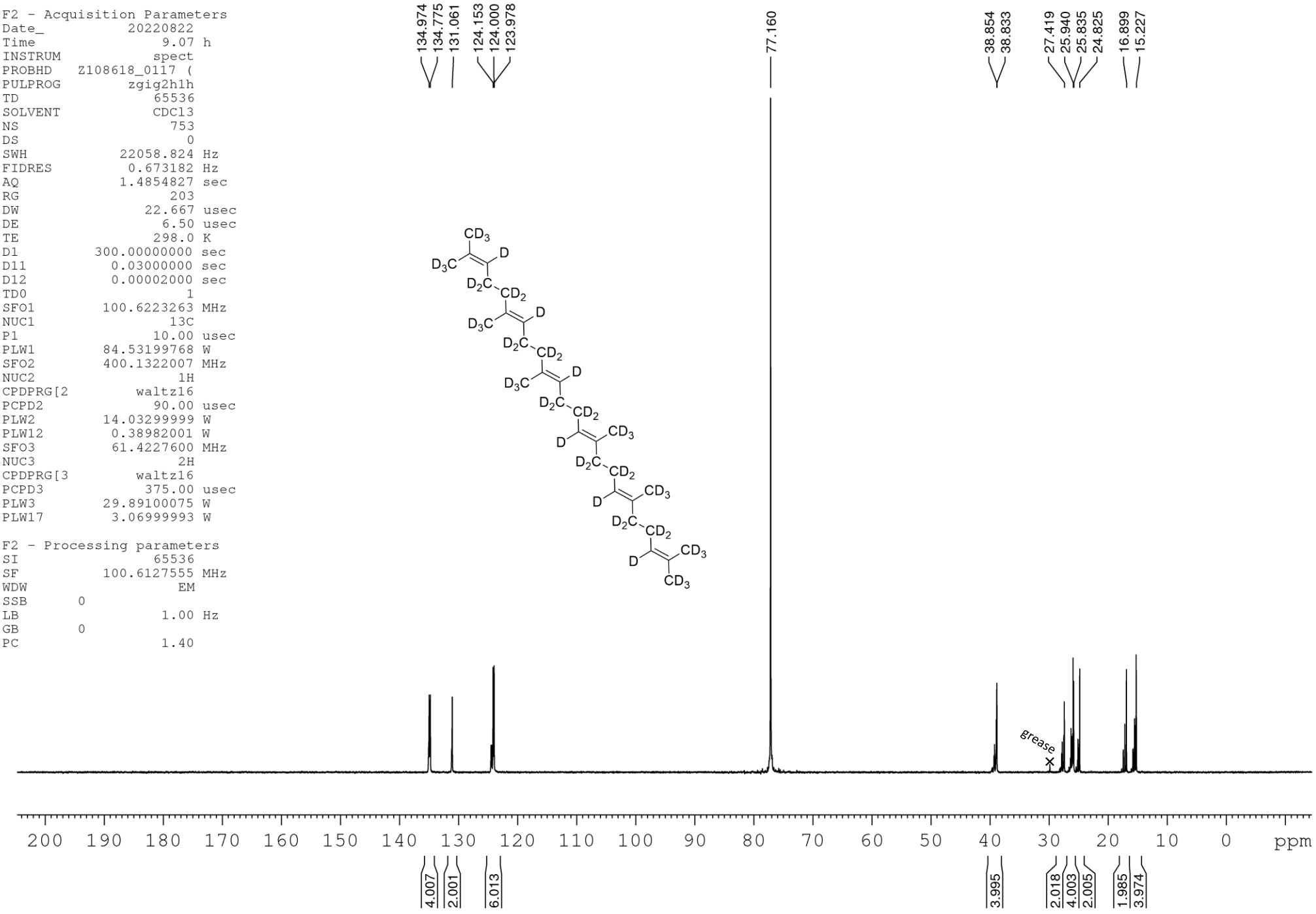
squalene-d_{50} (81%-*d*) ^{2}H NMR

```
F2 - Acquisition Parameters
Date_           20220816
Time                9.20 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG             zg2h
TD                  8192
SOLVENT            CDCl3
NS                    31
DS                     0
SWH              921.376 Hz
FIDRES          0.224945 Hz
AQ             4.4455252 sec
RG                  45.2
DW               542.667 usec
DE                 23.73 usec
TE                 298.0 K
D1            5.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          61.4226988 MHz
NUC1                  2H
P1                124.00 usec
PLW1         29.89100075 W

F2 - Processing parameters
SI                 16384
SF            61.4223931 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

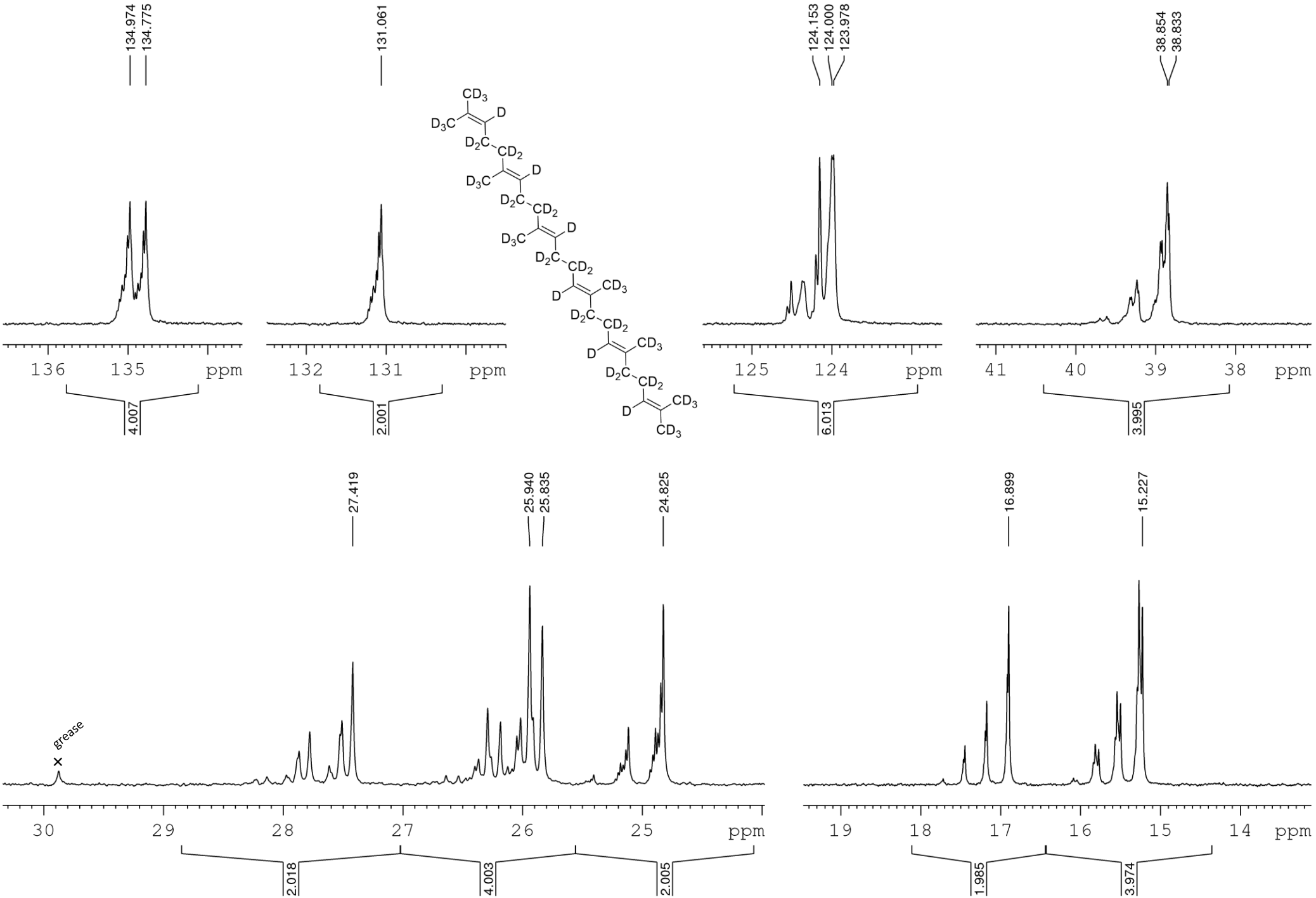


squalene-d_{50} (81%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20220822
Time                9.07 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG          zgig2h1h
TD                  65536
SOLVENT             CDCl3
NS                    753
DS                      0
SWH             22058.824 Hz
FIDRES           0.673182 Hz
AQ              1.4854827 sec
RG                    203
DW                 22.667 usec
DE                   6.50 usec
TE                  298.0 K
D1          300.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                     1
SFO1          100.6223263 MHz
NUC1                  13C
P1                  10.00 usec
PLW1          84.53199768 W
SFO2          400.1322007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2          14.03299999 W
PLW12          0.38982001 W
SFO3           61.4227600 MHz
NUC3                  2H
CPDPRG[3          waltz16
PCPD3              375.00 usec
PLW3          29.89100075 W
PLW17          3.06999993 W

F2 - Processing parameters
SI                  65536
SF            100.6127555 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

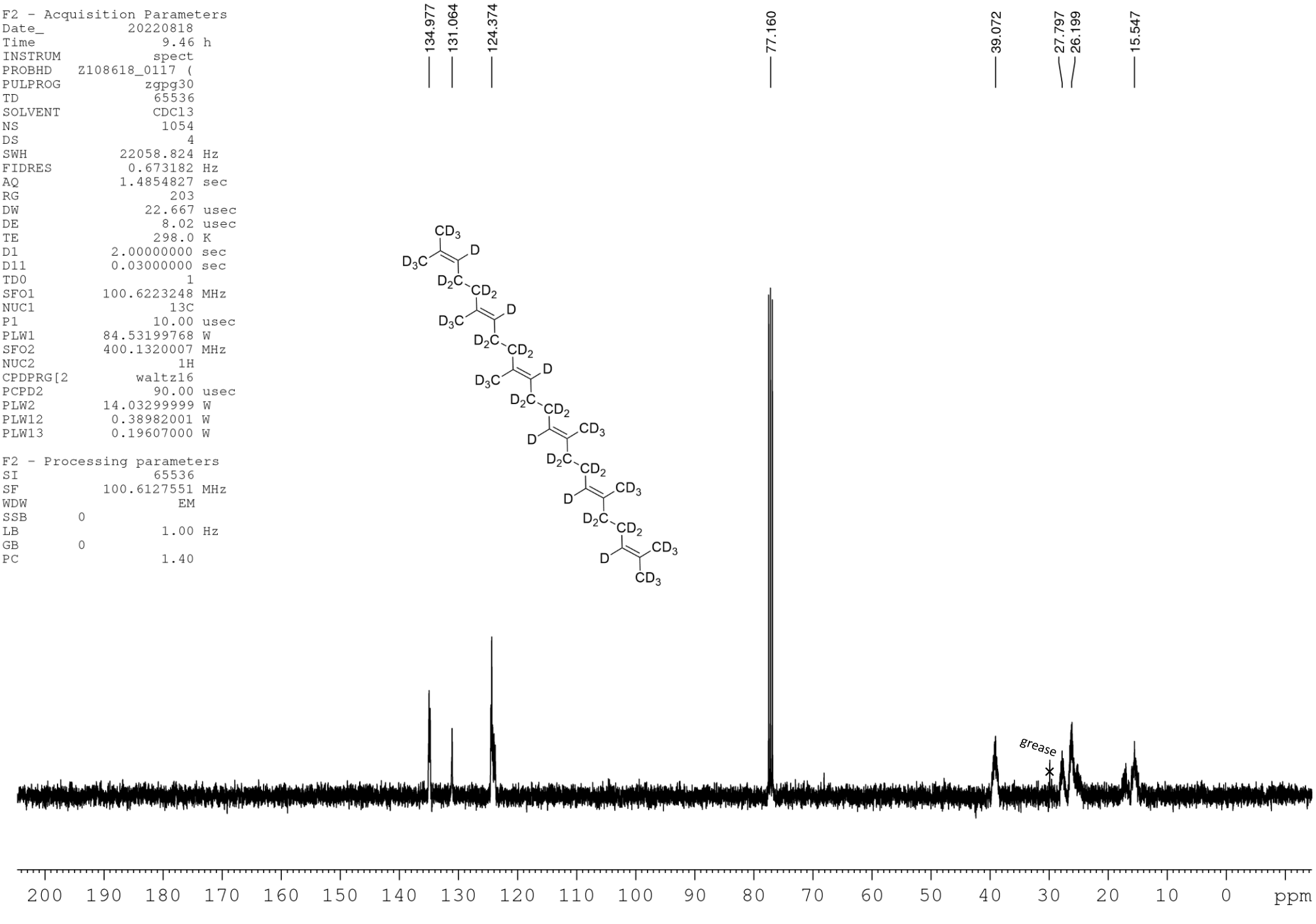


squalene-d_{50} (81%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions



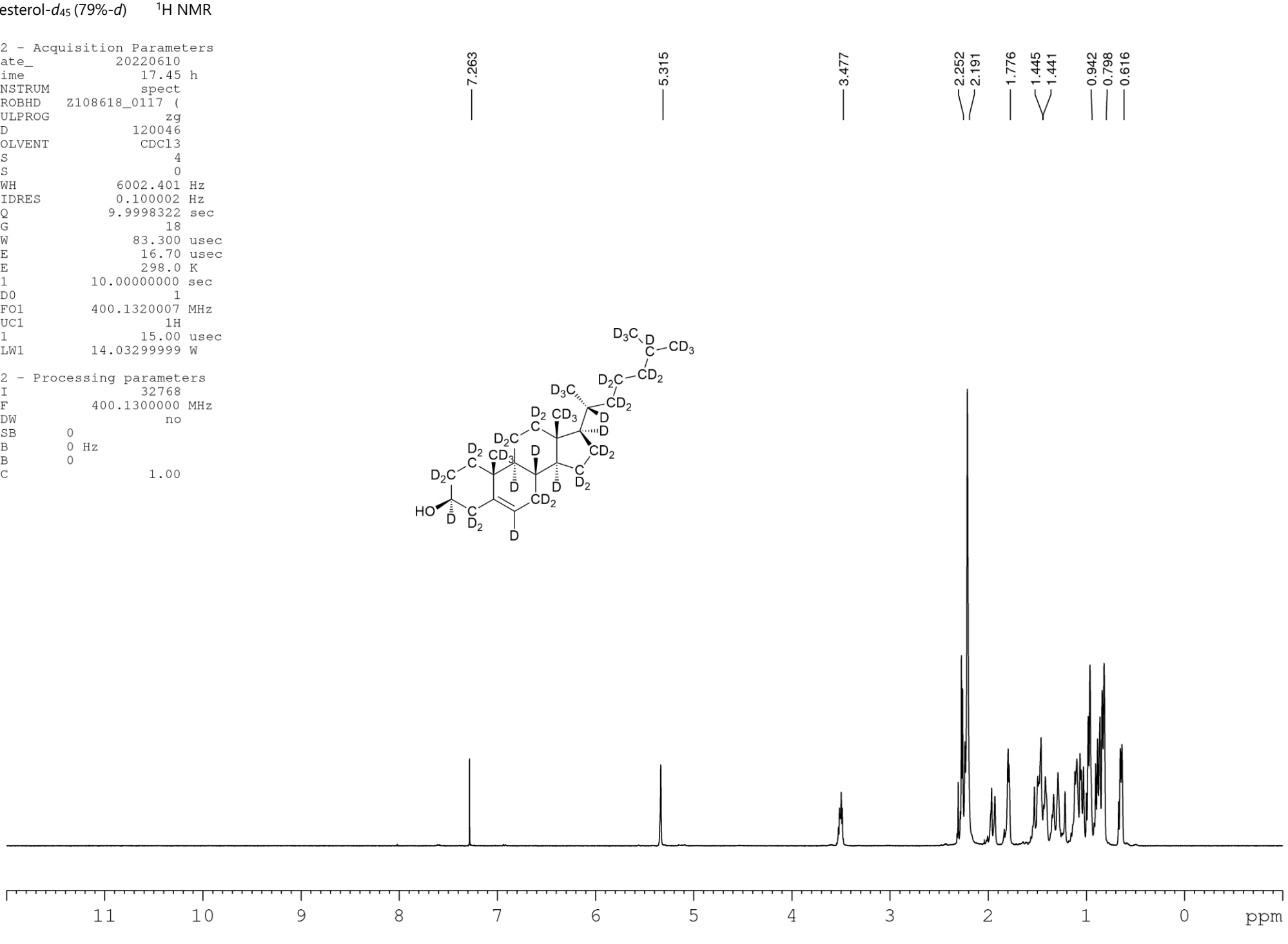
squalene-d_{50} (81%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20220818
Time                9.46 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   1054
DS                      4
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                    203
DW                 22.667 usec
DE                   8.02 usec
TE                  298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                     1
SFO1         100.6223248 MHz
NUC1                 13C
P1                 10.00 usec
PLW1         84.53199768 W
SFO2         400.1320007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
PLW13         0.19607000 W

F2 - Processing parameters
SI                  65536
SF           100.6127551 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

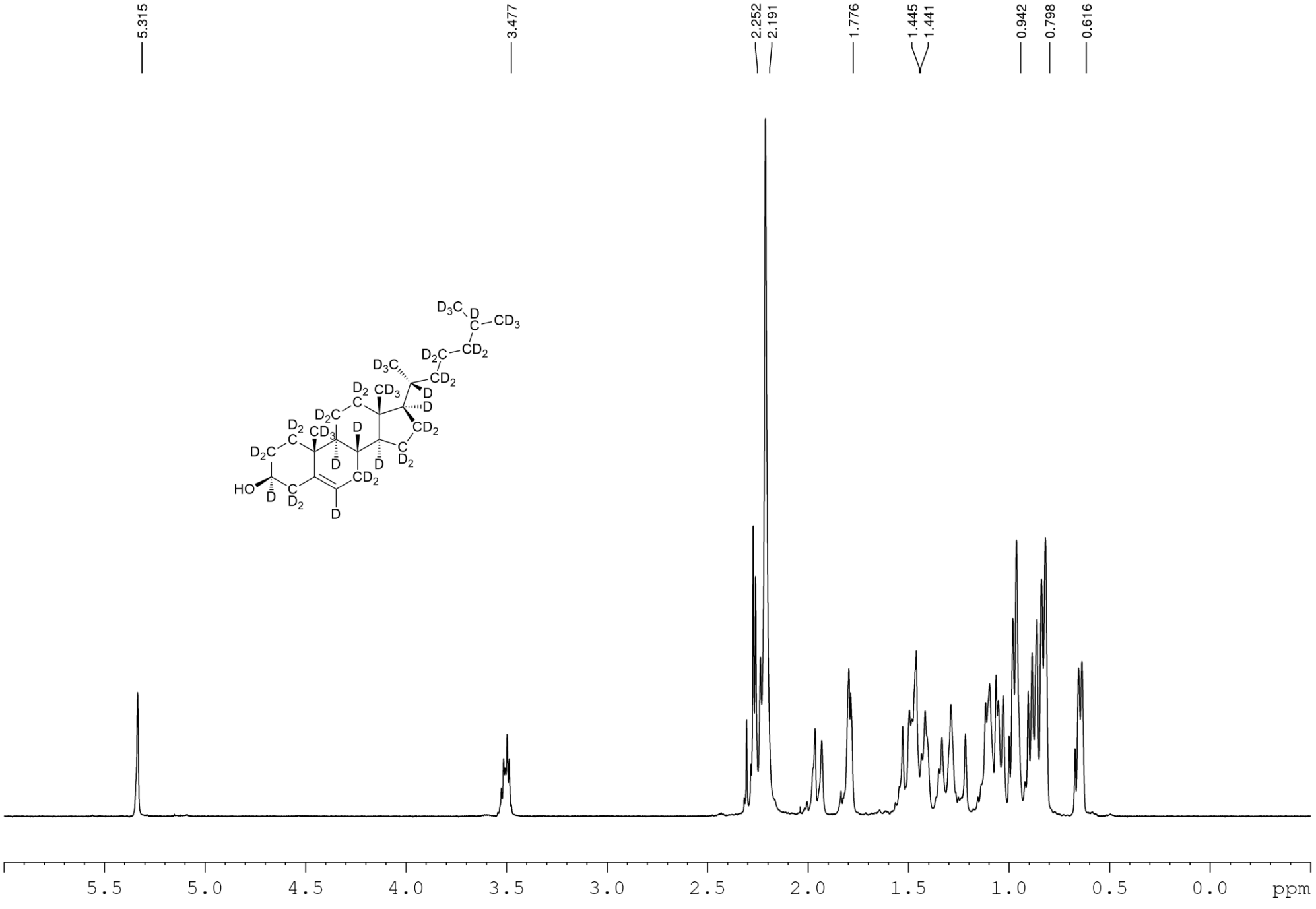


cholesterol-d_{45} (79%-*d*) ^{1}H NMR

```
F2 - Acquisition Parameters
Date_            20220610
Time               17.45 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG               zg
TD                120046
SOLVENT             CDCl3
NS                      4
DS                      0
SWH             6002.401 Hz
FIDRES          0.100002 Hz
AQ             9.9998322 sec
RG                     18
DW                83.300 usec
DE                16.70 usec
TE                298.0 K
D1           10.00000000 sec
TD0                     1
SFO1          400.1320007 MHz
NUC1                  1H
P1                 15.00 usec
PLW1          14.03299999 W

F2 - Processing parameters
SI                  32768
SF            400.1300000 MHz
WDW                    no
SSB       0
LB        0 Hz
GB        0
PC                   1.00
```

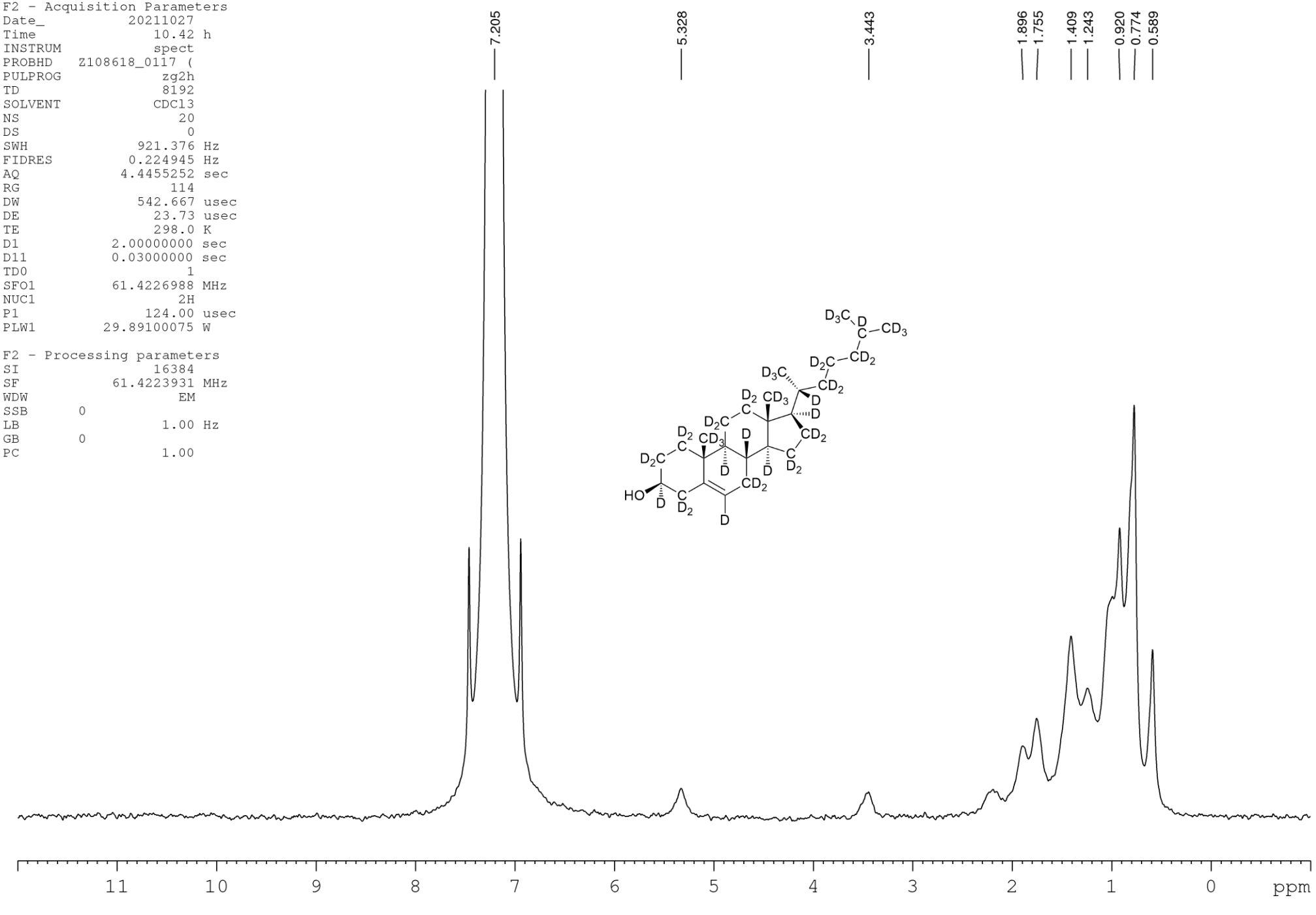


cholesterol-d_{45} (79%-*d*) ^{1}H NMR expansion



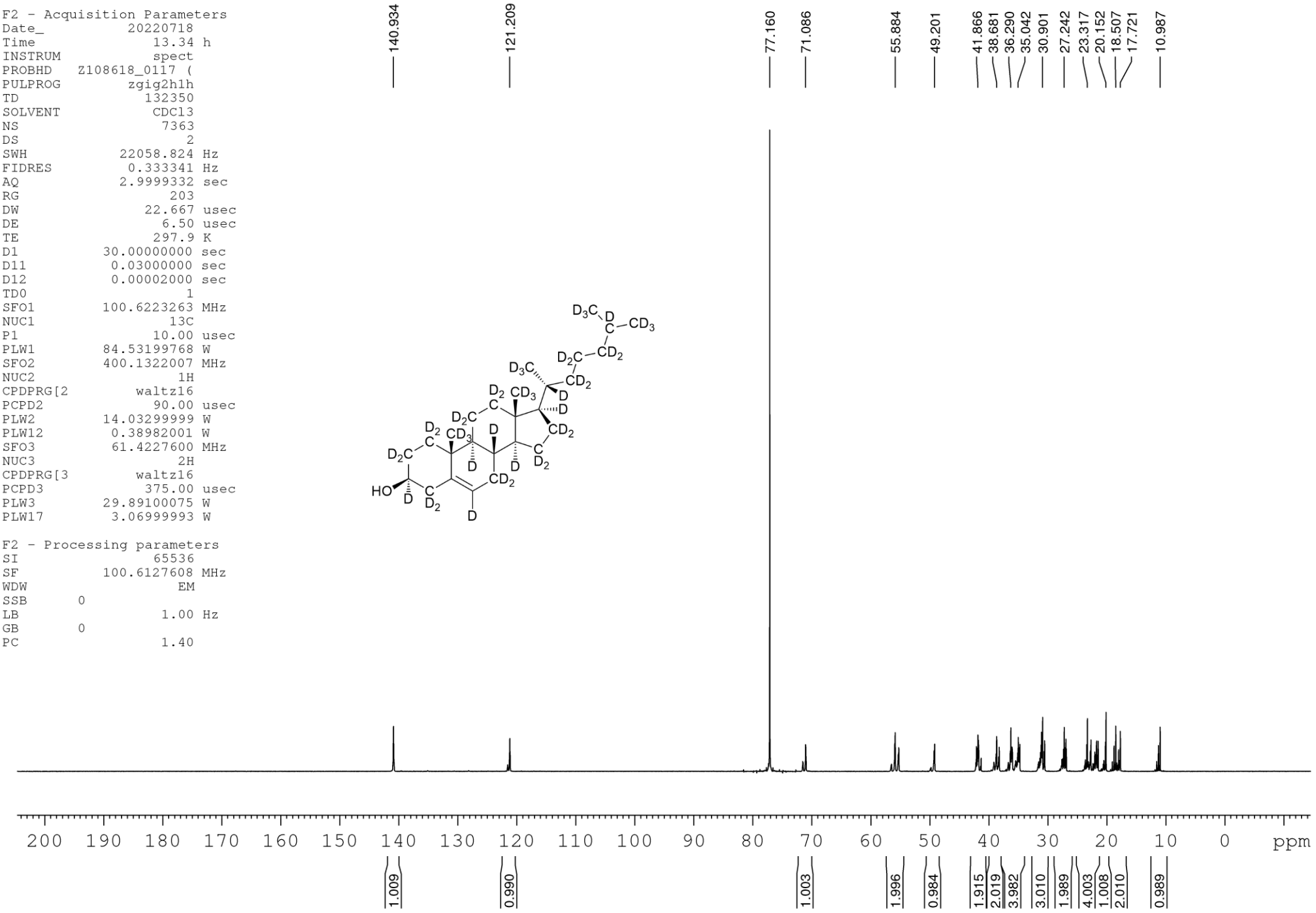
cholesterol-d_{45} (79%-*d*) ^{2}H NMR

```
F2 - Acquisition Parameters
Date_           20211027
Time              10.42 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG            zg2h
TD                 8192
SOLVENT           CDCl3
NS                   20
DS                    0
SWH             921.376 Hz
FIDRES         0.224945 Hz
AQ             4.4455252 sec
RG                   114
DW               542.667 usec
DE                23.73 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1
SFO1         61.4226988 MHz
NUC1                2H
P1               124.00 usec
PLW1        29.89100075 W

F2 - Processing parameters
SI                16384
SF           61.4223931 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.00
```



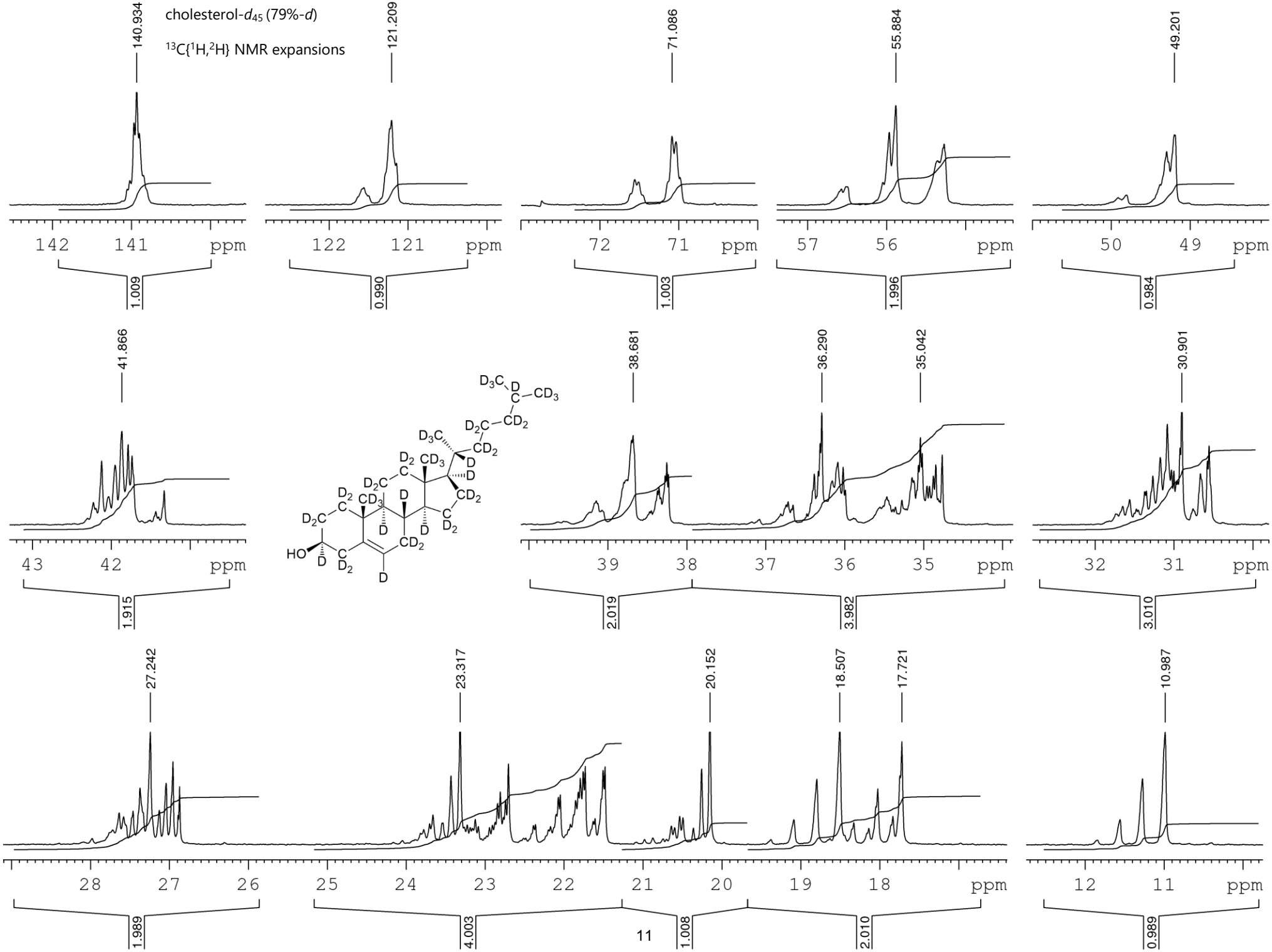
cholesterol-d_{45} (79%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20220718
Time               13.34 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG          zgig2h1h
TD                 132350
SOLVENT             CDCl3
NS                   7363
DS                      2
SWH            22058.824 Hz
FIDRES          0.333341 Hz
AQ             2.9999332 sec
RG                    203
DW                 22.667 usec
DE                   6.50 usec
TE                  297.9 K
D1           30.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1
SFO1         100.6223263 MHz
NUC1                 13C
P1                 10.00 usec
PLW1         84.53199768 W
SFO2         400.1322007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
SFO3          61.4227600 MHz
NUC3                 2H
CPDPRG[3          waltz16
PCPD3             375.00 usec
PLW3         29.89100075 W
PLW17         3.06999993 W

F2 - Processing parameters
SI                 65536
SF           100.6127608 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



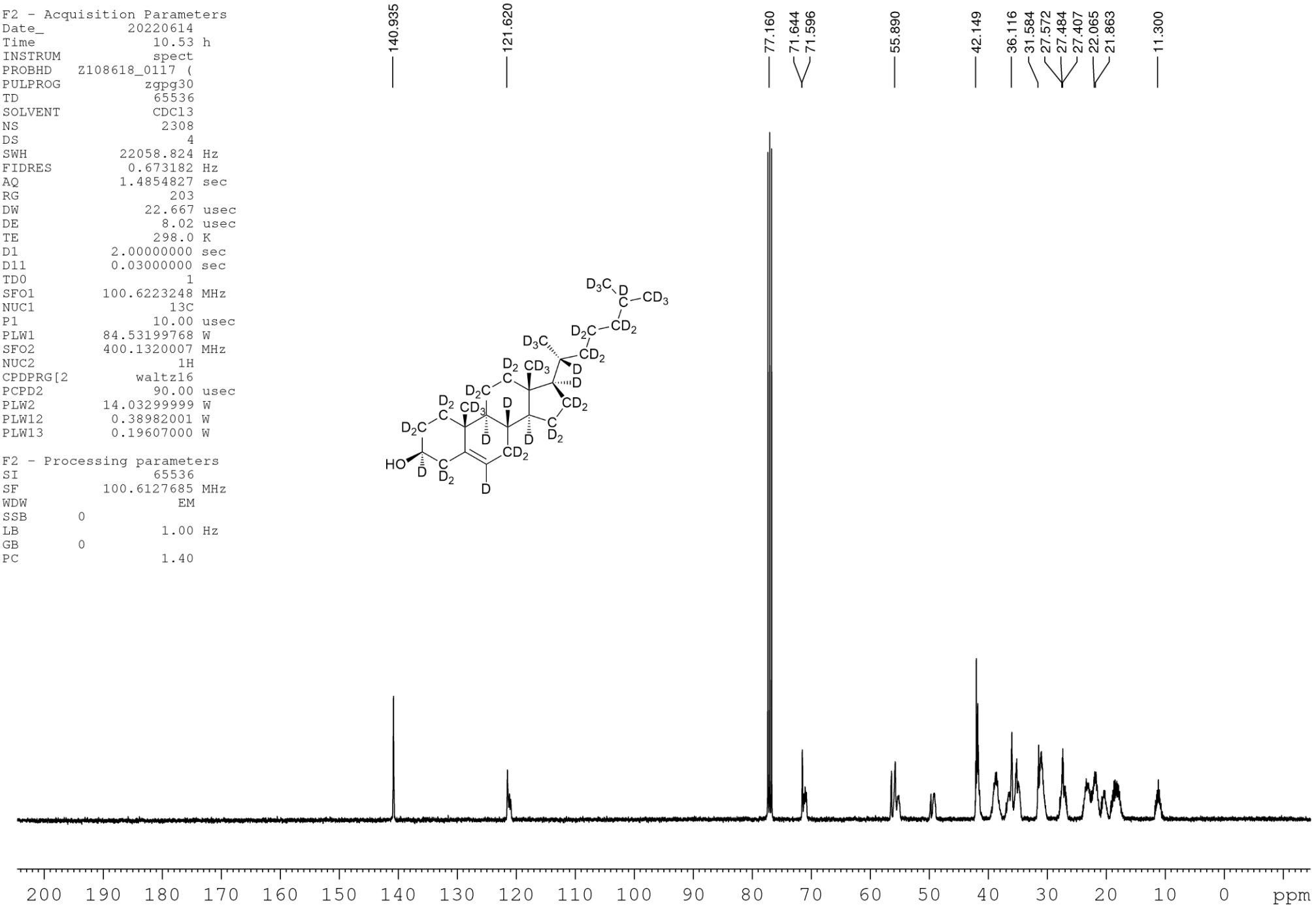
cholesterol-d_{45} (79%-*d*)

$^{13}C\{^{1}H,^{2}H\}$ NMR expansions

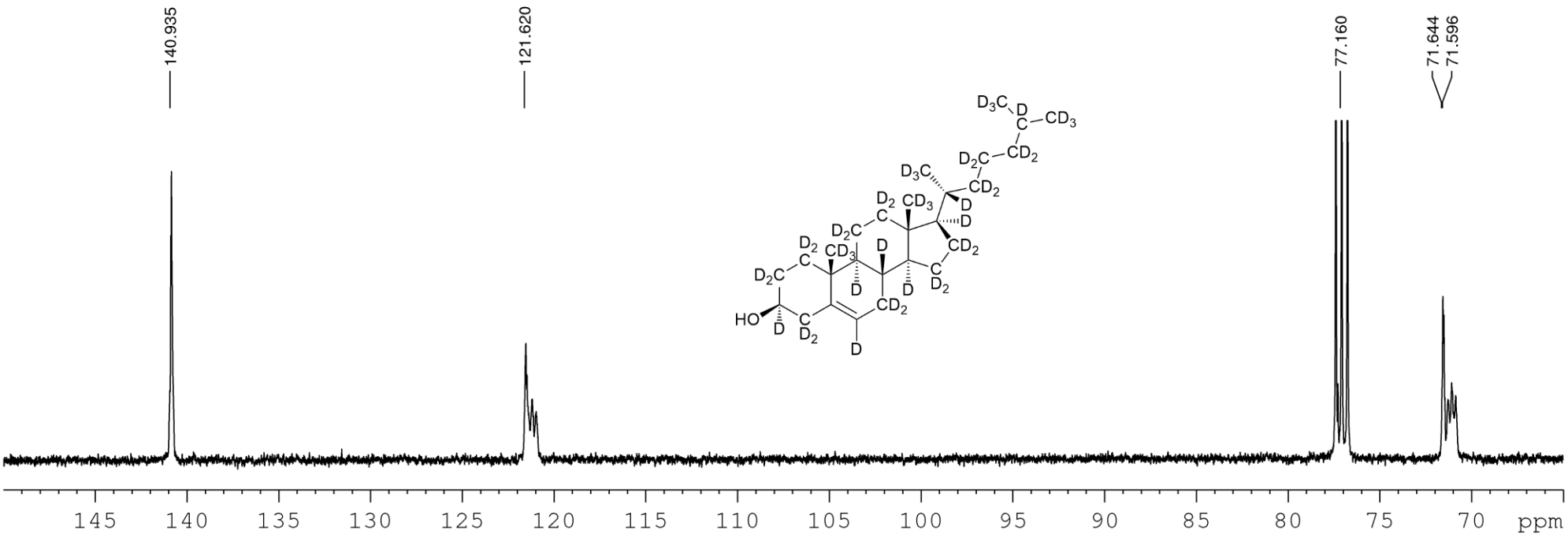


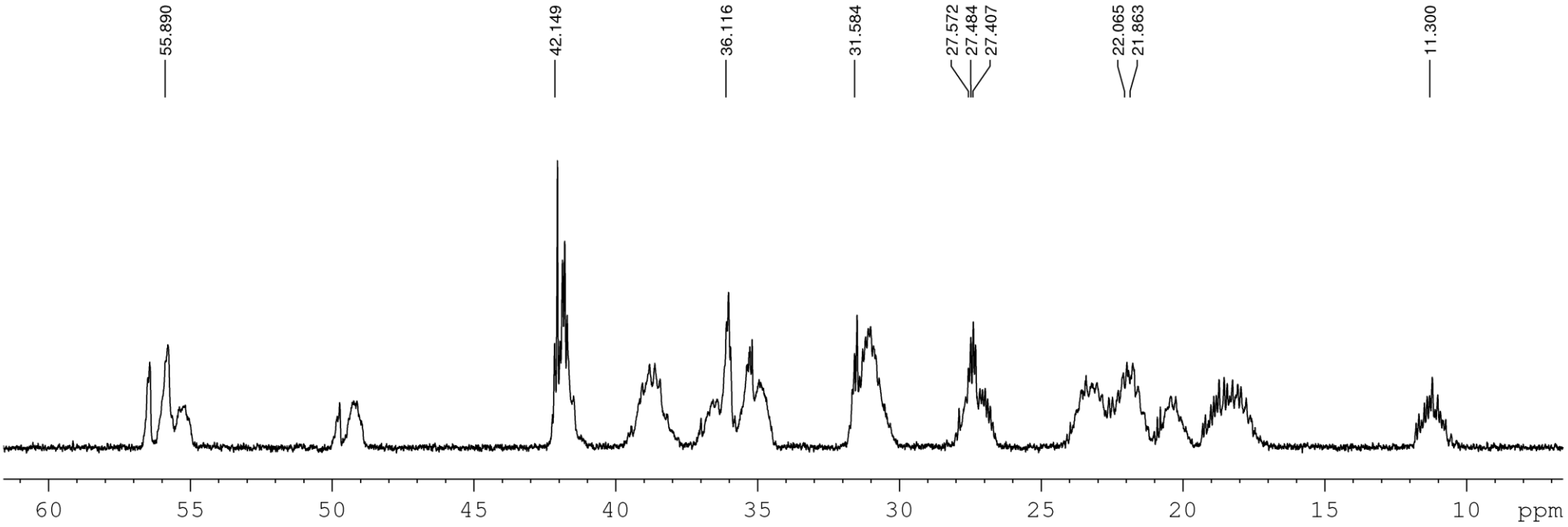
cholesterol-d_{45} (79%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20220614
Time               10.53 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   2308
DS                      4
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                    203
DW                 22.667 usec
DE                   8.02 usec
TE                  298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                     1
SFO1          100.6223248 MHz
NUC1                  13C
P1                  10.00 usec
PLW1          84.53199768 W
SFO2          400.1320007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2          14.03299999 W
PLW12          0.38982001 W
PLW13          0.19607000 W

F2 - Processing parameters
SI                  65536
SF            100.6127685 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```



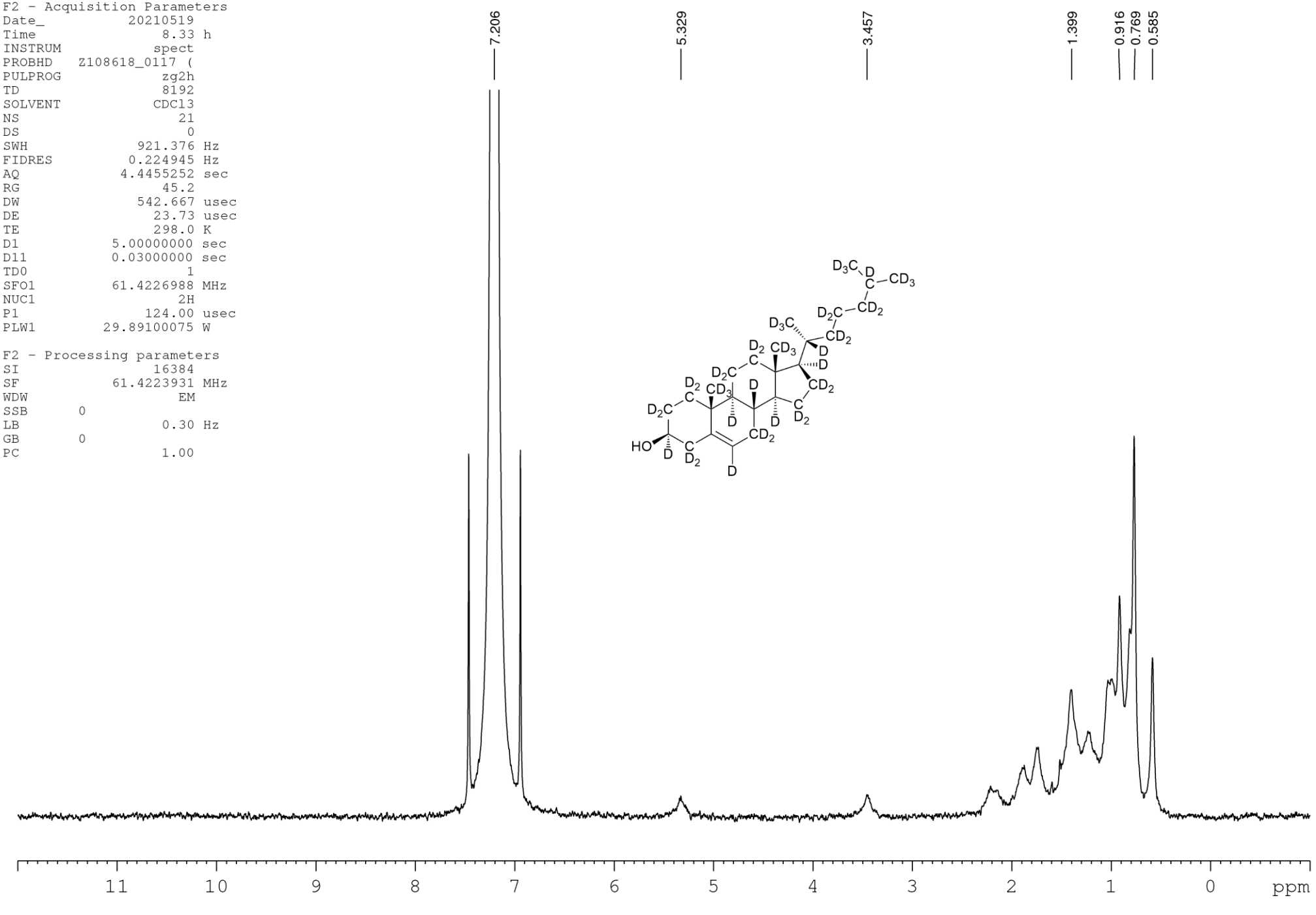
cholesterol-d_{45} (79%-d) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions





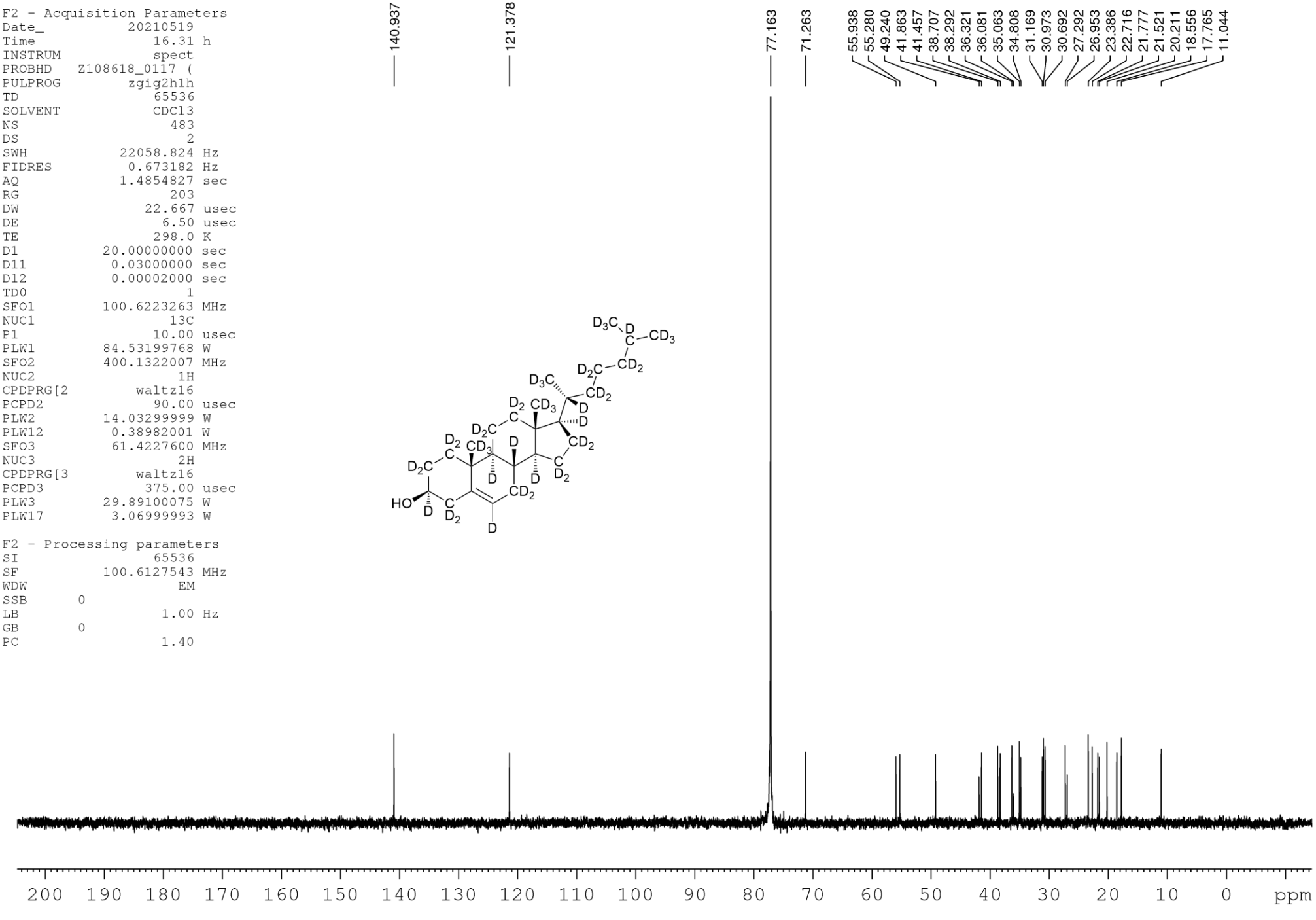
cholesterol-d_{45} (98%-*d*) ^{2}H NMR

```
F2 - Acquisition Parameters
Date_           20210519
Time                8.33 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG             zg2h
TD                  8192
SOLVENT             CDCl3
NS                    21
DS                     0
SWH               921.376 Hz
FIDRES           0.224945 Hz
AQ              4.4455252 sec
RG                   45.2
DW                542.667 usec
DE                 23.73 usec
TE                 298.0 K
D1            5.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          61.4226988 MHz
NUC1                  2H
P1                 124.00 usec
PLW1         29.89100075 W

F2 - Processing parameters
SI                 16384
SF            61.4223931 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

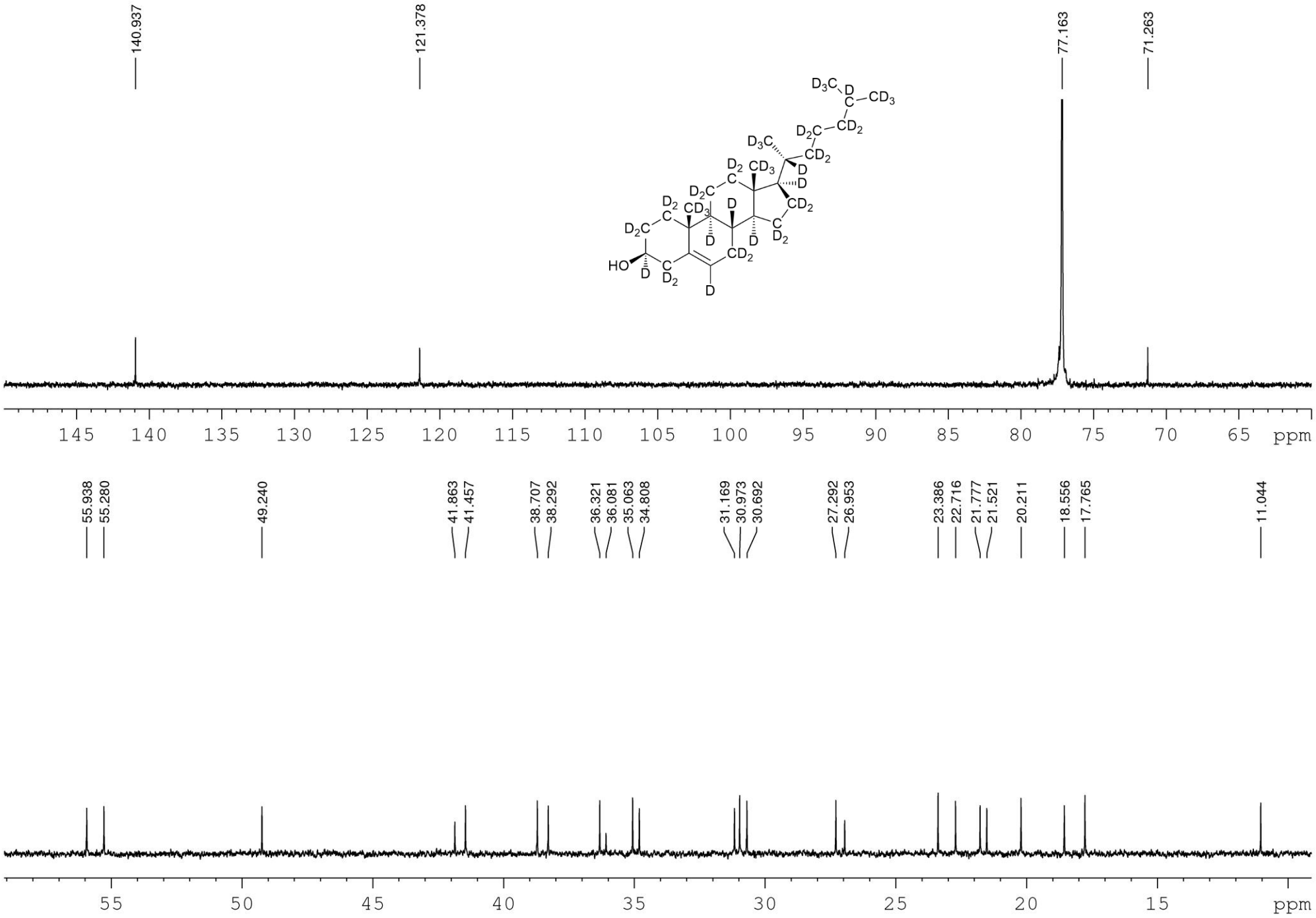


cholesterol-d_{45} (98%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20210519
Time               16.31 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG          zgig2h1h
TD                  65536
SOLVENT             CDCl3
NS                    483
DS                      2
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                    203
DW                 22.667 usec
DE                   6.50 usec
TE                  298.0 K
D1           20.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1
SFO1         100.6223263 MHz
NUC1                 13C
P1                 10.00 usec
PLW1         84.53199768 W
SFO2         400.1322007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
SFO3          61.4227600 MHz
NUC3                 2H
CPDPRG[3          waltz16
PCPD3             375.00 usec
PLW3         29.89100075 W
PLW17         3.06999993 W

F2 - Processing parameters
SI                  65536
SF           100.6127543 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

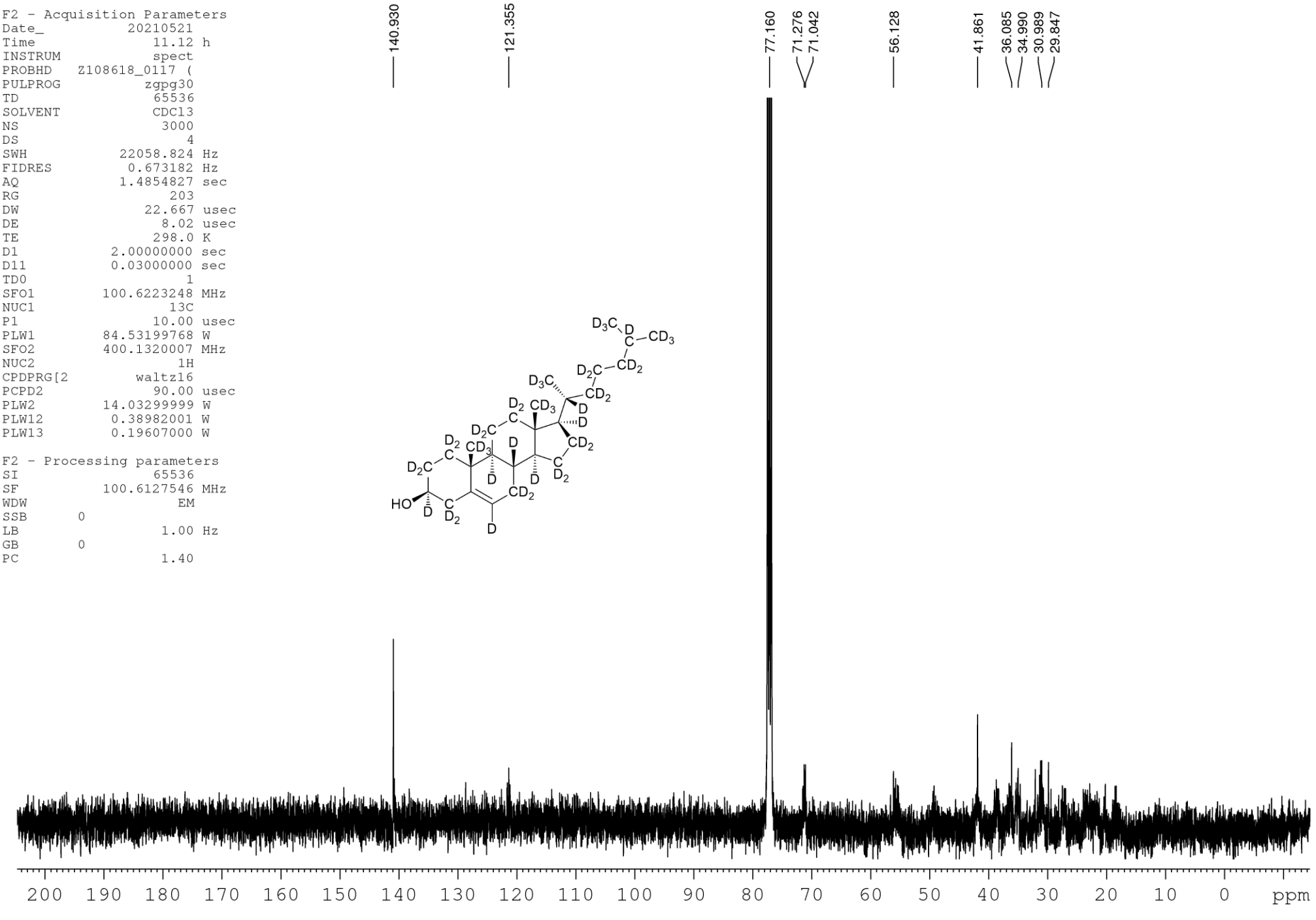


cholesterol-d_{45} (98%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions



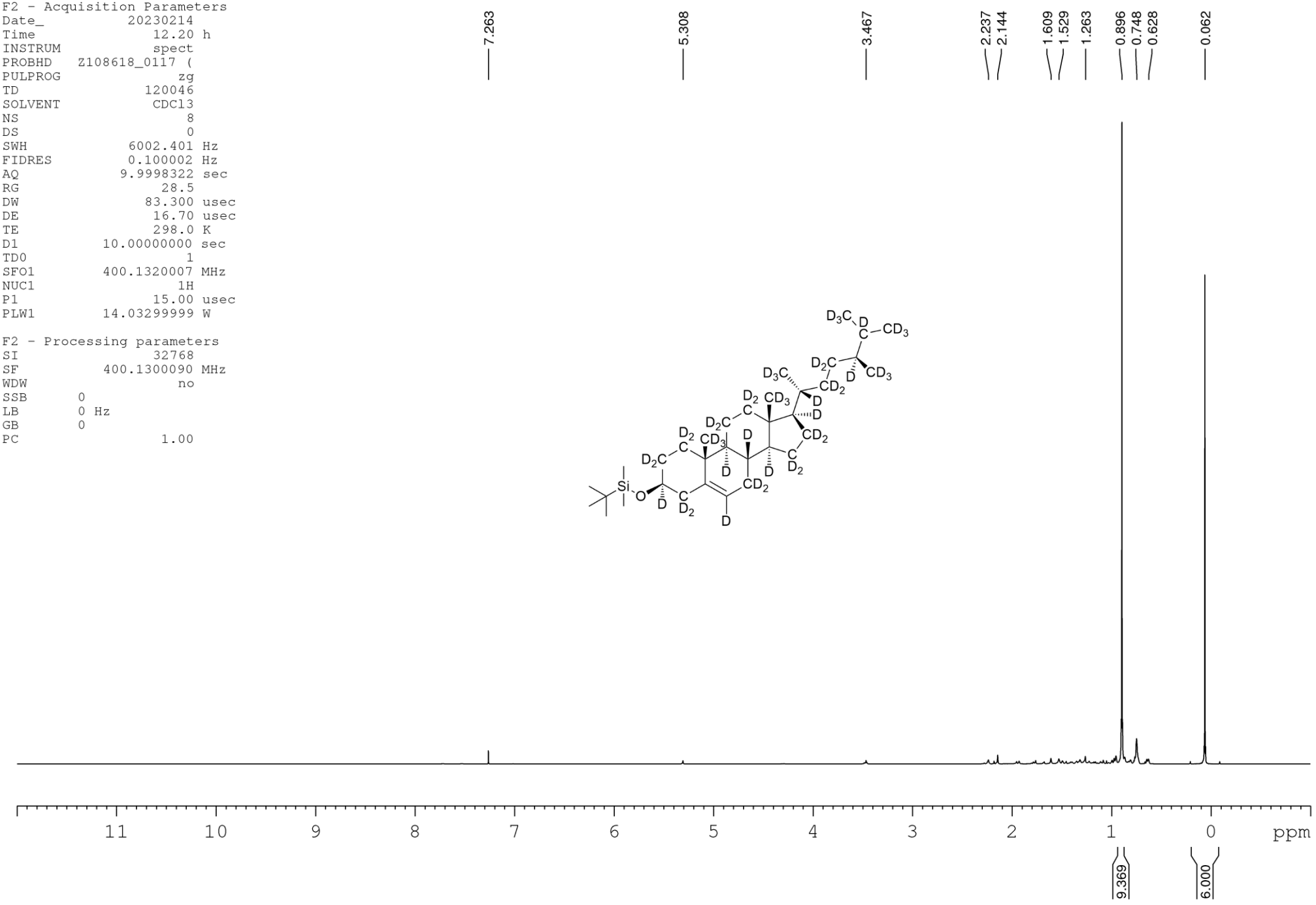
cholesterol-d_{45} (98%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20210521
Time               11.12 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   3000
DS                      4
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                    203
DW                 22.667 usec
DE                   8.02 usec
TE                  298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          100.6223248 MHz
NUC1                 13C
P1                 10.00 usec
PLW1          84.53199768 W
SFO2          400.1320007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2          14.03299999 W
PLW12          0.38982001 W
PLW13          0.19607000 W

F2 - Processing parameters
SI                  65536
SF            100.6127546 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

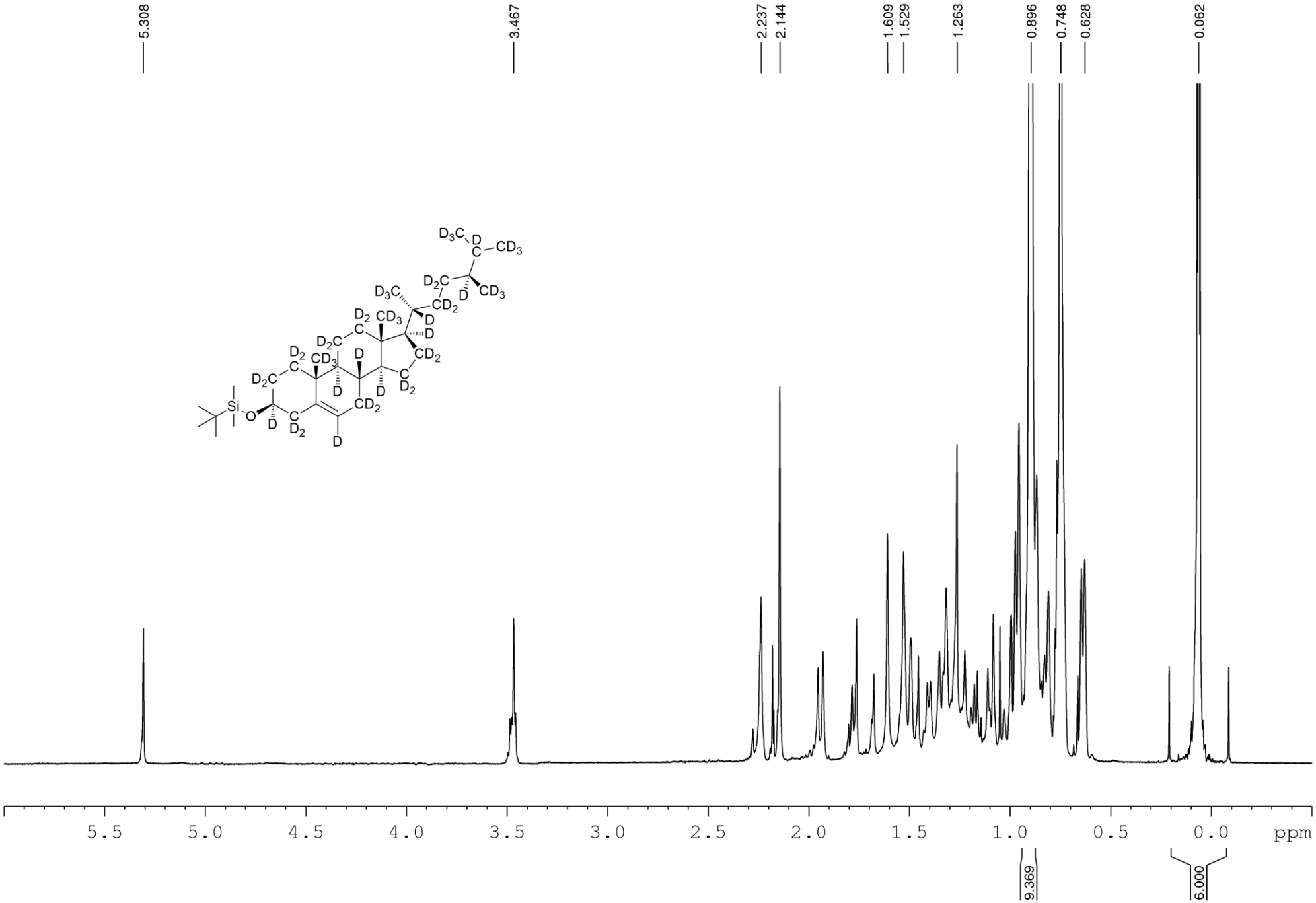


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) ^{1}H NMR

```
F2 - Acquisition Parameters
Date_           20230214
Time              12.20 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG              zg
TD               120046
SOLVENT            CDCl3
NS                     8
DS                     0
SWH             6002.401 Hz
FIDRES          0.100002 Hz
AQ             9.9998322 sec
RG                  28.5
DW                83.300 usec
DE                16.70 usec
TE                298.0 K
D1           10.00000000 sec
TD0                    1
SFO1         400.1320007 MHz
NUC1                  1H
P1                15.00 usec
PLW1         14.03299999 W

F2 - Processing parameters
SI                 32768
SF           400.1300090 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```

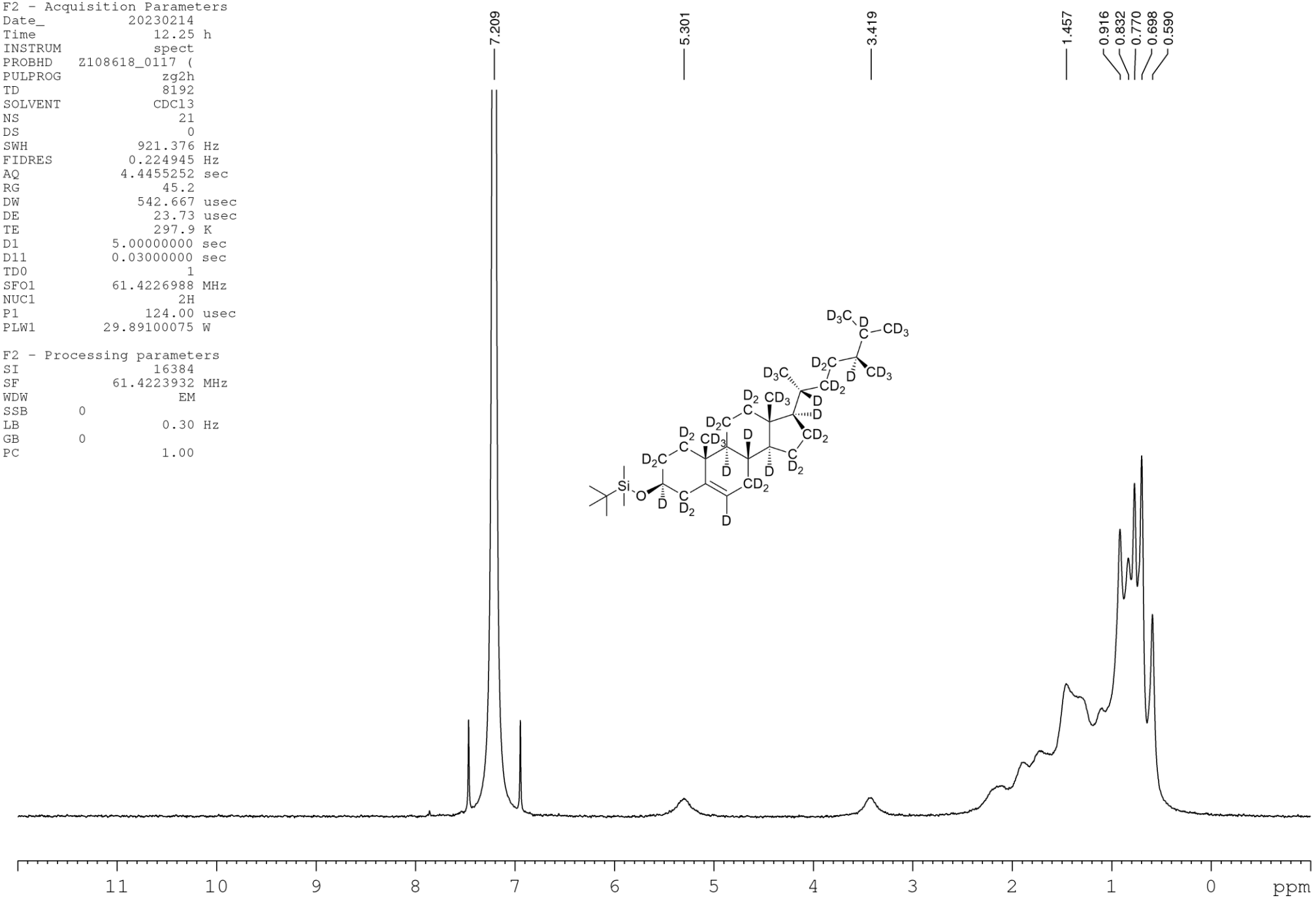


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) ^{1}H NMR expansion



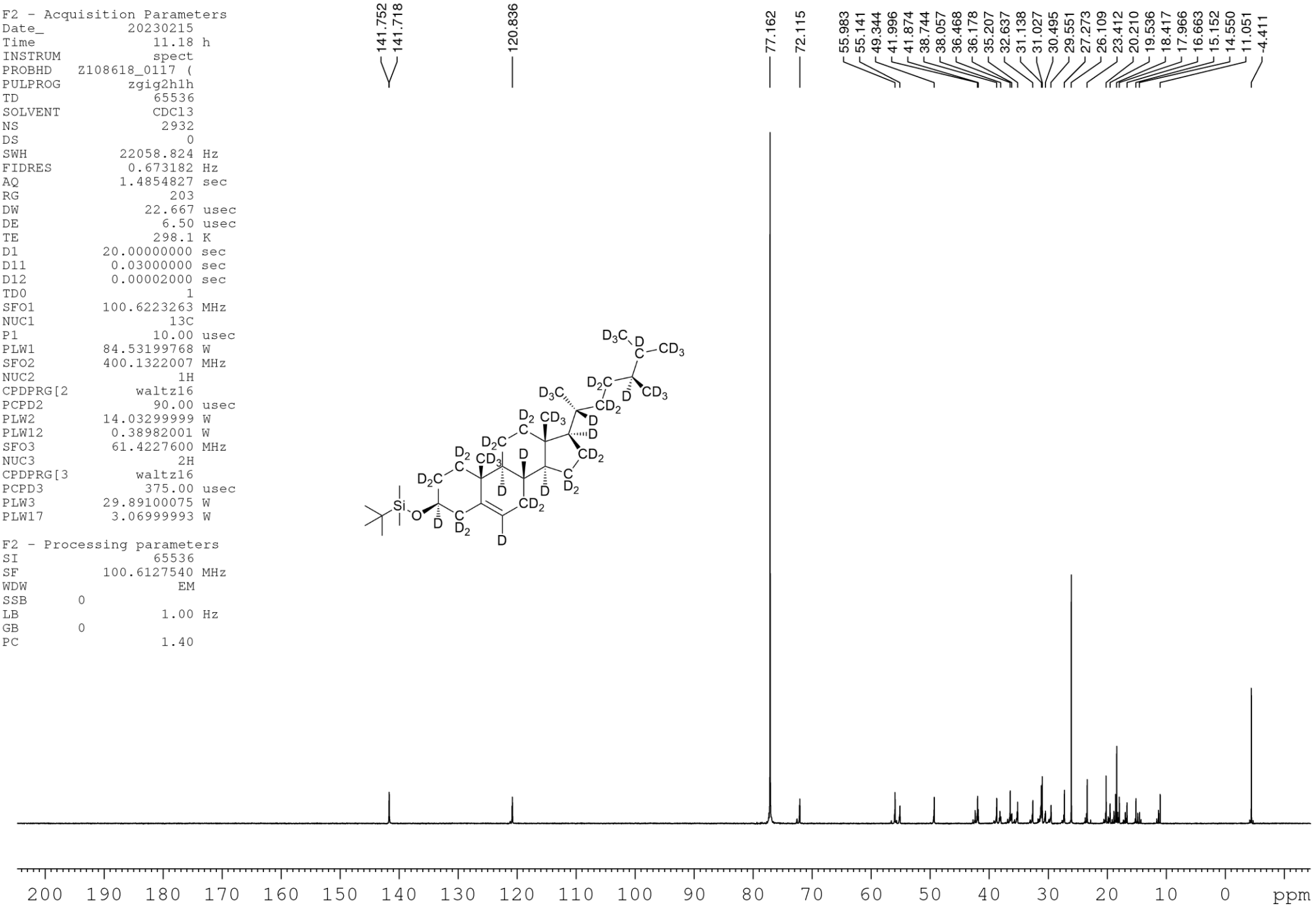
O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) ^{2}H NMR

```
F2 - Acquisition Parameters
Date_            20230214
Time                12.25 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG              zg2h
TD                   8192
SOLVENT             CDCl3
NS                     21
DS                      0
SWH               921.376 Hz
FIDRES           0.224945 Hz
AQ              4.4455252 sec
RG                   45.2
DW                542.667 usec
DE                  23.73 usec
TE                  297.9 K
D1             5.00000000 sec
D11            0.03000000 sec
TD0                     1
SFO1           61.4226988 MHz
NUC1                   2H
P1                 124.00 usec
PLW1          29.89100075 W

F2 - Processing parameters
SI                  16384
SF             61.4223932 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

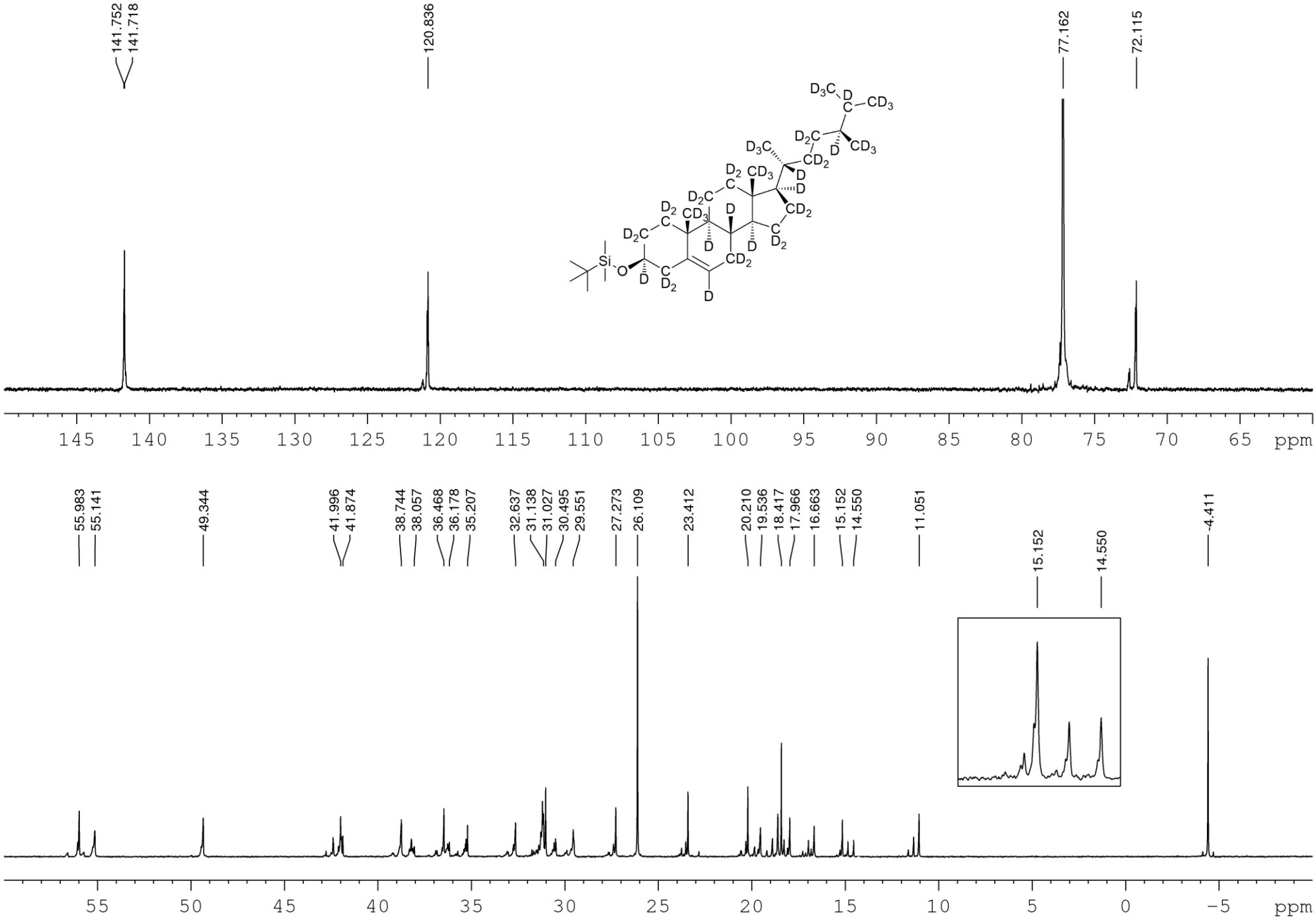


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230215
Time                11.18 h
INSTRUM              spect
PROBHD    Z108618_0117 (
PULPROG           zgig2h1h
TD                  65536
SOLVENT              CDCl3
NS                    2932
DS                       0
SWH             22058.824 Hz
FIDRES           0.673182 Hz
AQ              1.4854827 sec
RG                     203
DW                22.667 usec
DE                  6.50 usec
TE                 298.1 K
D1           20.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1
SFO1          100.6223263 MHz
NUC1                 13C
P1                  10.00 usec
PLW1          84.53199768 W
SFO2          400.1322007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2          14.03299999 W
PLW12          0.38982001 W
SFO3           61.4227600 MHz
NUC3                 2H
CPDPRG[3          waltz16
PCPD3              375.00 usec
PLW3          29.89100075 W
PLW17          3.06999993 W

F2 - Processing parameters
SI                  65536
SF            100.6127540 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

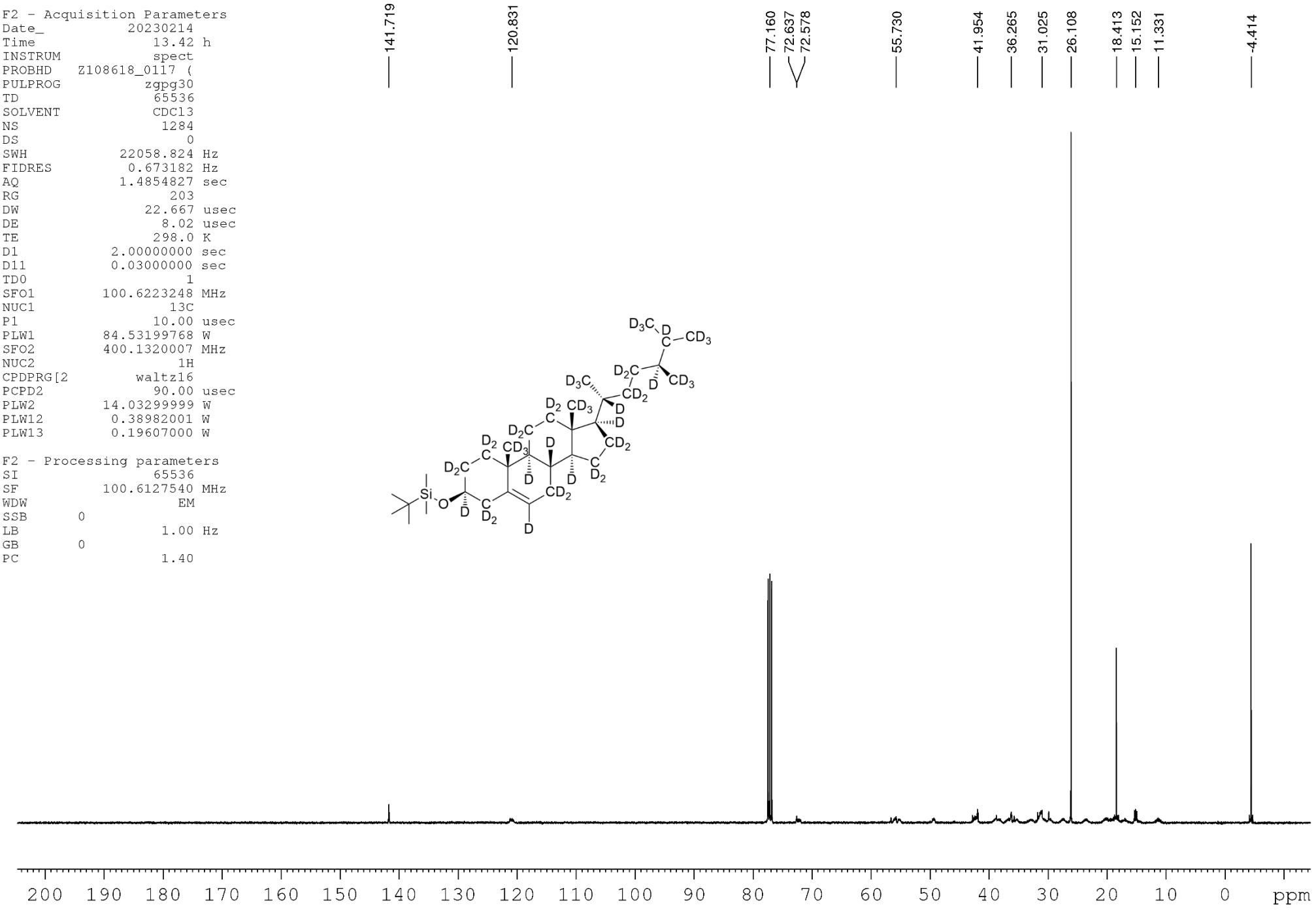


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions

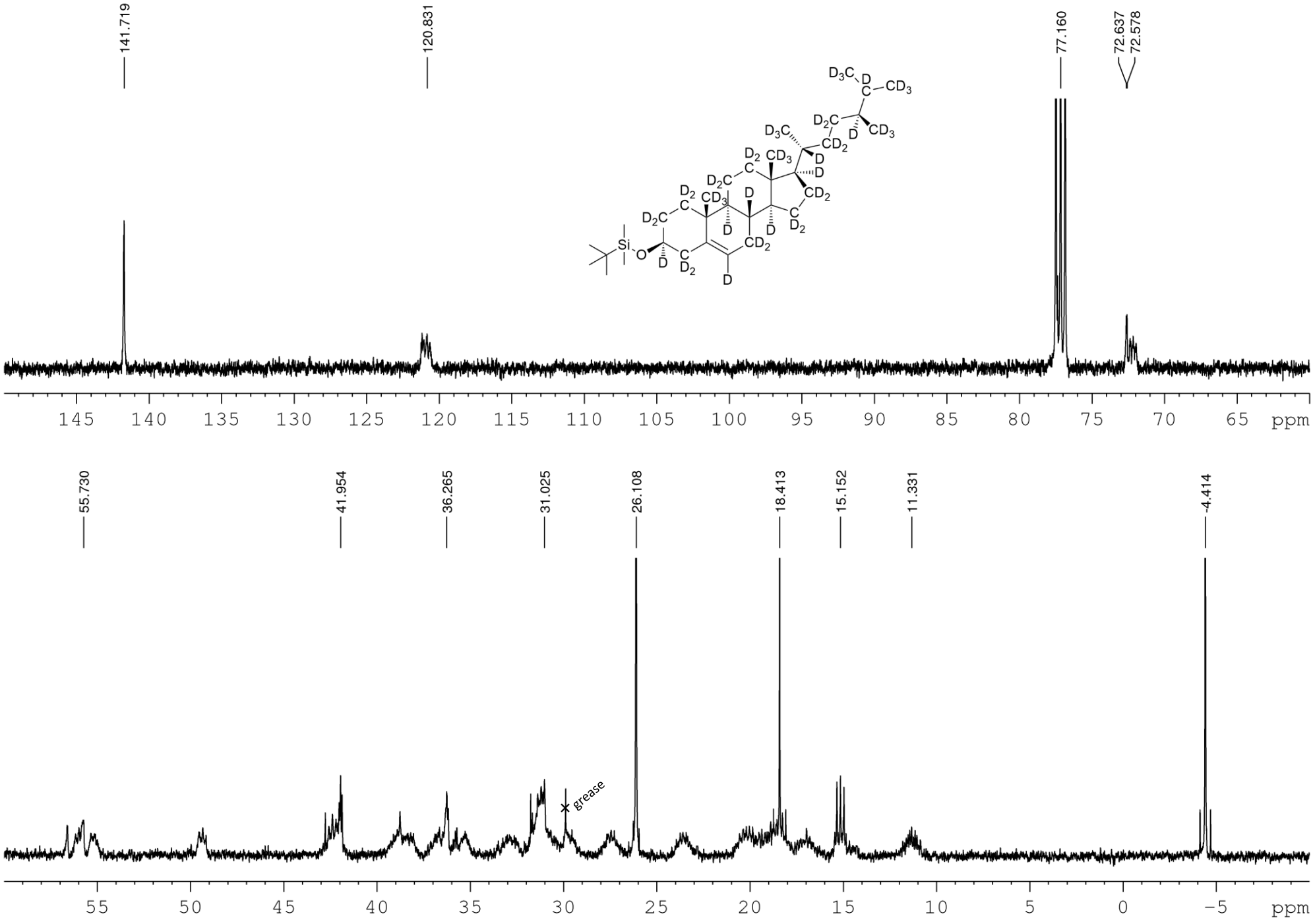


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230214
Time               13.42 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1284
DS                     0
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                   203
DW                22.667 usec
DE                 8.02 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1
SFO1         100.6223248 MHz
NUC1                13C
P1                10.00 usec
PLW1         84.53199768 W
SFO2         400.1320007 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
PLW13         0.19607000 W

F2 - Processing parameters
SI                 65536
SF           100.6127540 MHz
WDW                  EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40
```

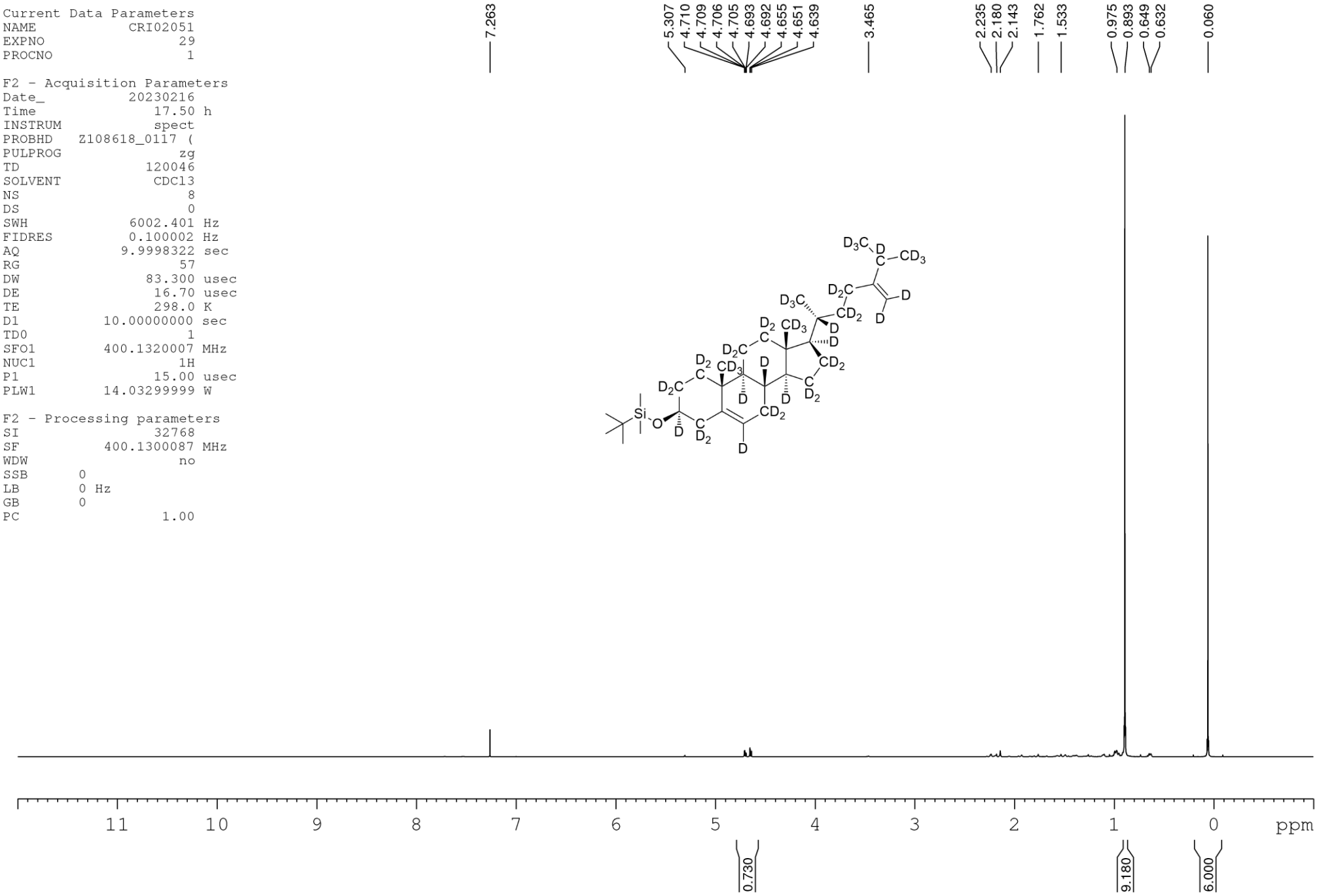


O-TBS-22,23-dihydrobrassicasterol-d_{47} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions

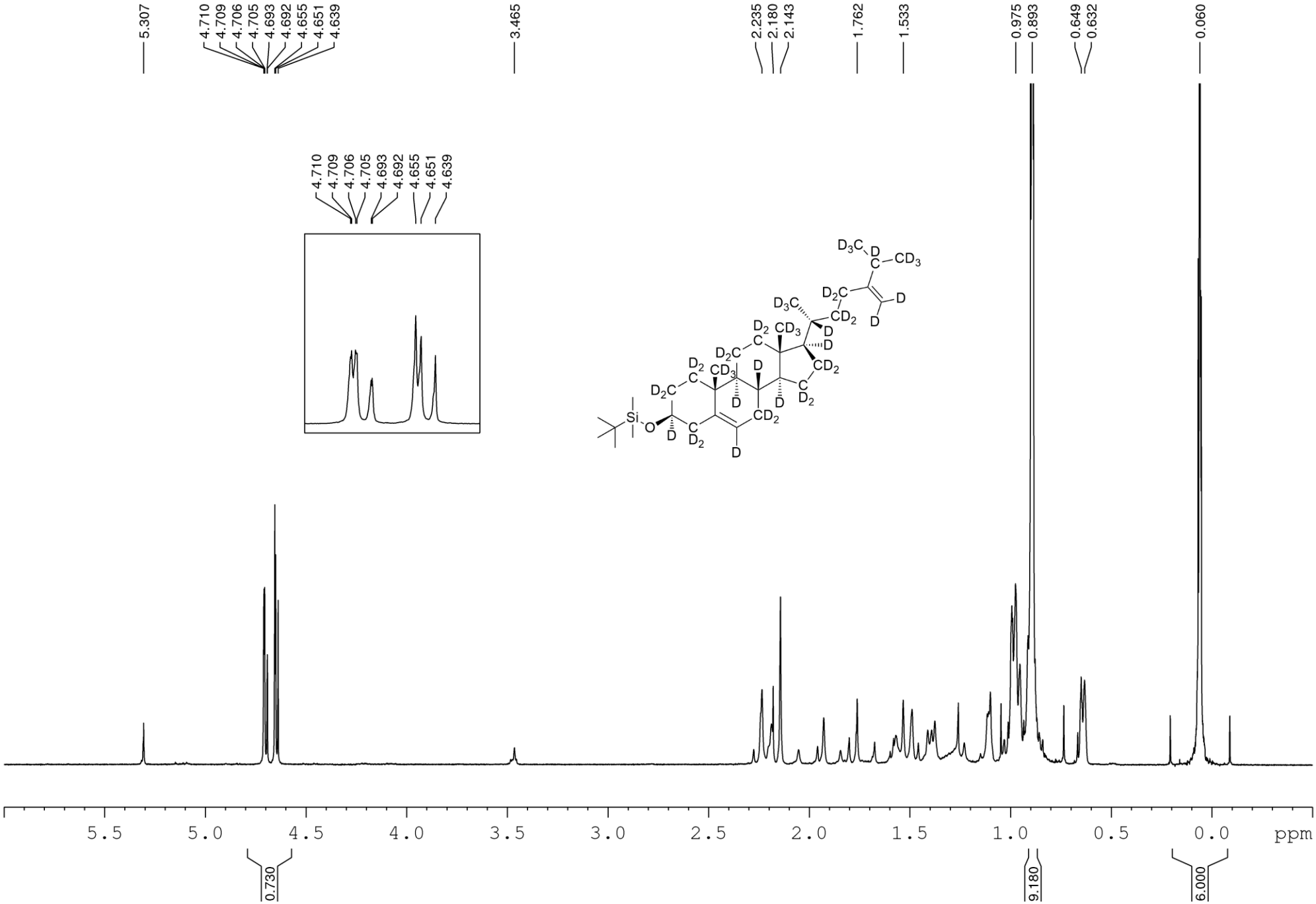


O-TBS-24-methylenecholesterol-d_{45} (87%-*d*) ^{1}H NMR

```
Current Data Parameters
NAME           CRI02051
EXPNO                29
PROCNO                1

F2 - Acquisition Parameters
Date_          20230216
Time              17.50 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG               zg
TD                120046
SOLVENT             CDCl3
NS                      8
DS                      0
SWH             6002.401 Hz
FIDRES          0.100002 Hz
AQ              9.9998322 sec
RG                     57
DW                83.300 usec
DE                16.70 usec
TE                298.0 K
D1           10.00000000 sec
TD0                     1
SFO1          400.1320007 MHz
NUC1                   1H
P1                15.00 usec
PLW1          14.03299999 W

F2 - Processing parameters
SI                  32768
SF            400.1300087 MHz
WDW                    no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```

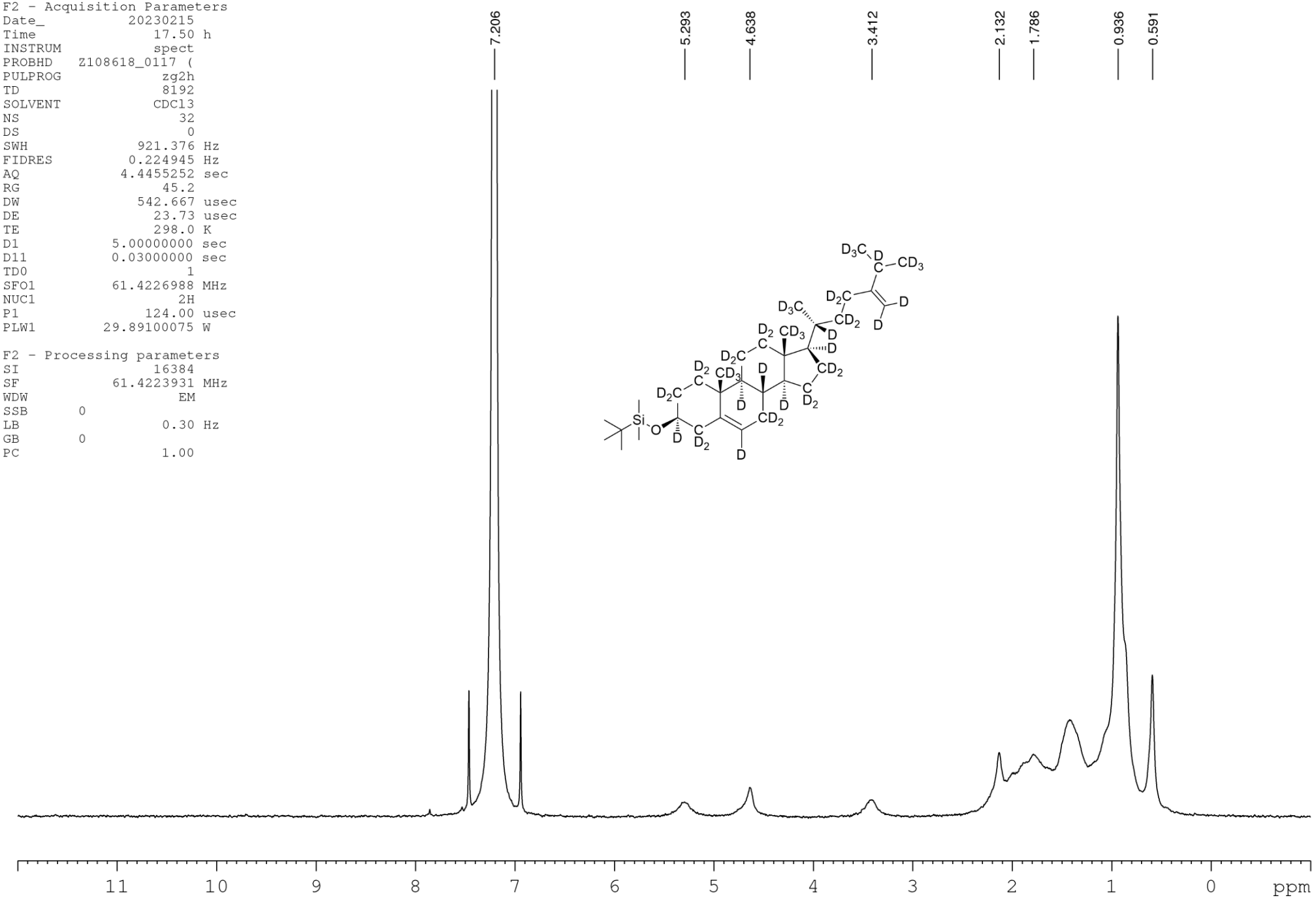


O-TBS-24-methylenecholesterol-d_{45} (87%-*d*) ^{1}H NMR expansion



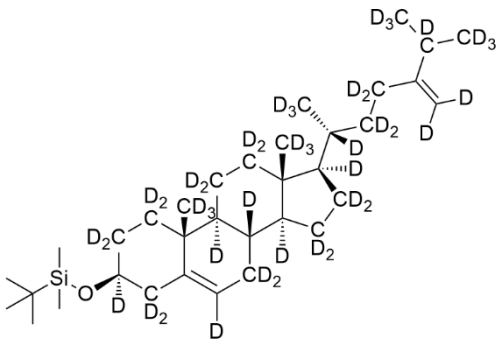
O-TBS-24-methylenecholesterol-d_{45} (87%-*d*) ^{2}H NMR

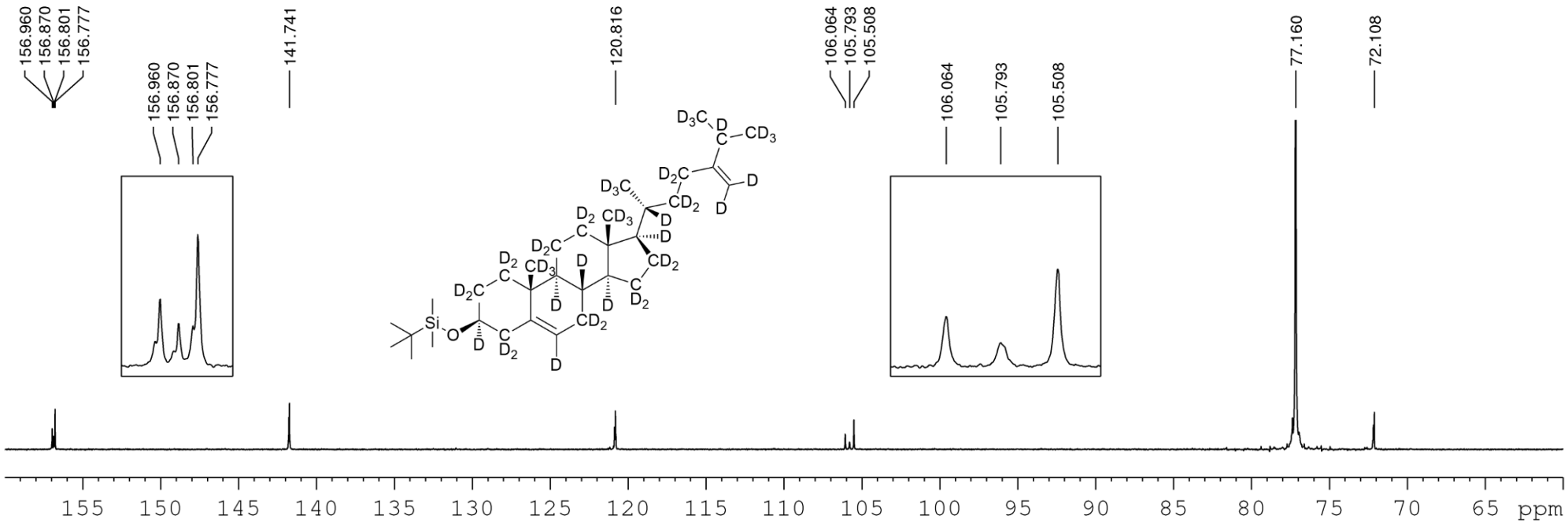
```
F2 - Acquisition Parameters
Date_           20230215
Time              17.50 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG             zg2h
TD                  8192
SOLVENT            CDCl3
NS                    32
DS                     0
SWH              921.376 Hz
FIDRES          0.224945 Hz
AQ             4.4455252 sec
RG                  45.2
DW               542.667 usec
DE                 23.73 usec
TE                 298.0 K
D1            5.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          61.4226988 MHz
NUC1                  2H
P1                124.00 usec
PLW1         29.89100075 W

F2 - Processing parameters
SI                 16384
SF            61.4223931 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

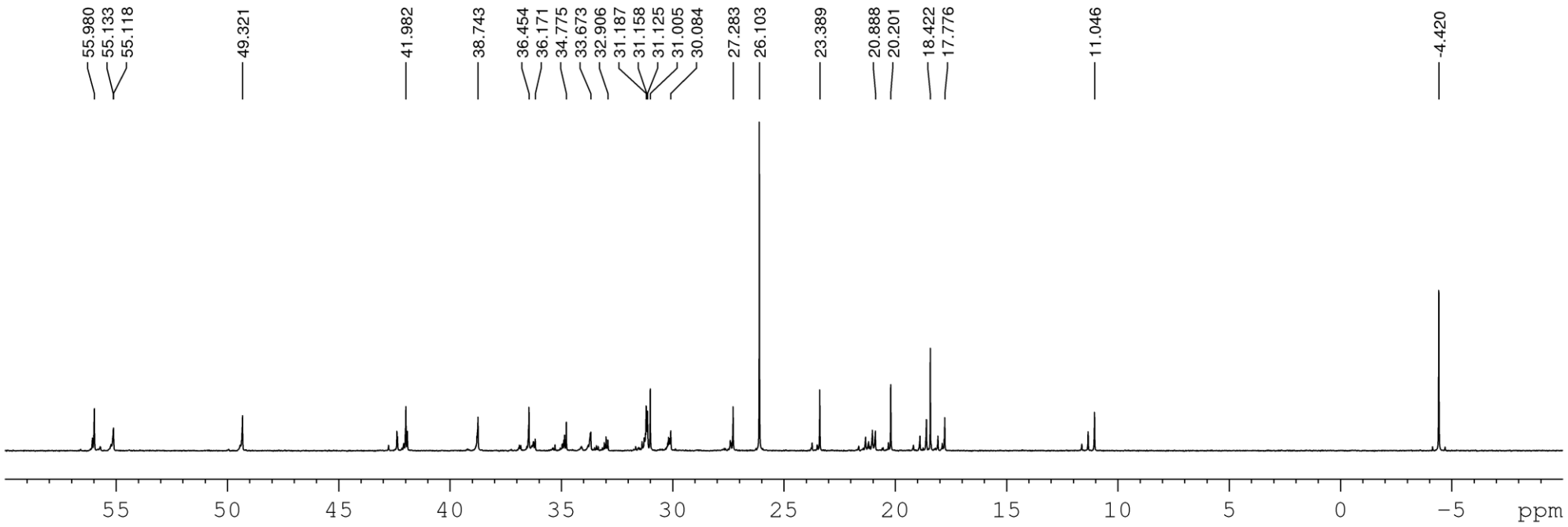


O-TBS-24-methylenecholesterol-d_{45} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230220
Time                8.31 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG          zgig2h1h
TD                  65536
SOLVENT             CDCl3
NS                  10522
DS                      0
SWH           22058.824 Hz
FIDRES         0.673182 Hz
AQ            1.4854827 sec
RG                    203
DW               22.667 usec
DE                6.50 usec
TE                298.0 K
D1           20.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                     1
SFO1         100.6223263 MHz
NUC1                 13C
P1                10.00 usec
PLW1         84.53199768 W
SFO2         400.1322007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
SFO3          61.4227600 MHz
NUC3                 2H
CPDPRG[3          waltz16
PCPD3            375.00 usec
PLW3         29.89100075 W
PLW17         3.06999993 W

F2 - Processing parameters
SI                  65536
SF           100.6127542 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

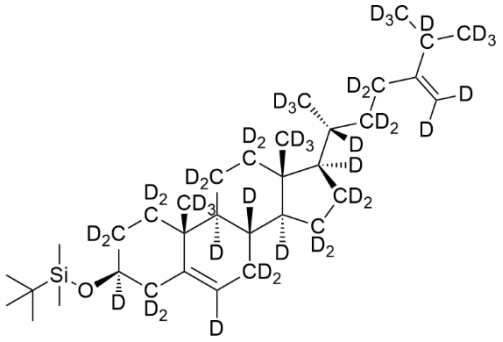


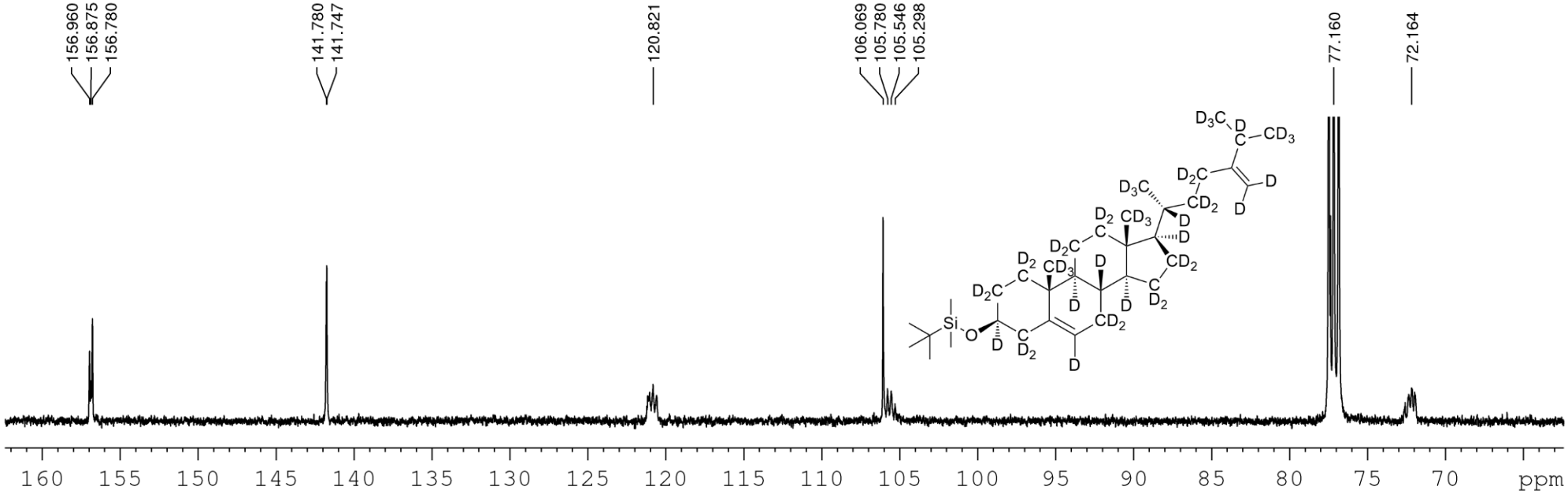

156.960
156.870
156.801
156.777
141.741
120.816
106.064
105.793
105.508
77.160
72.108
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 ppm

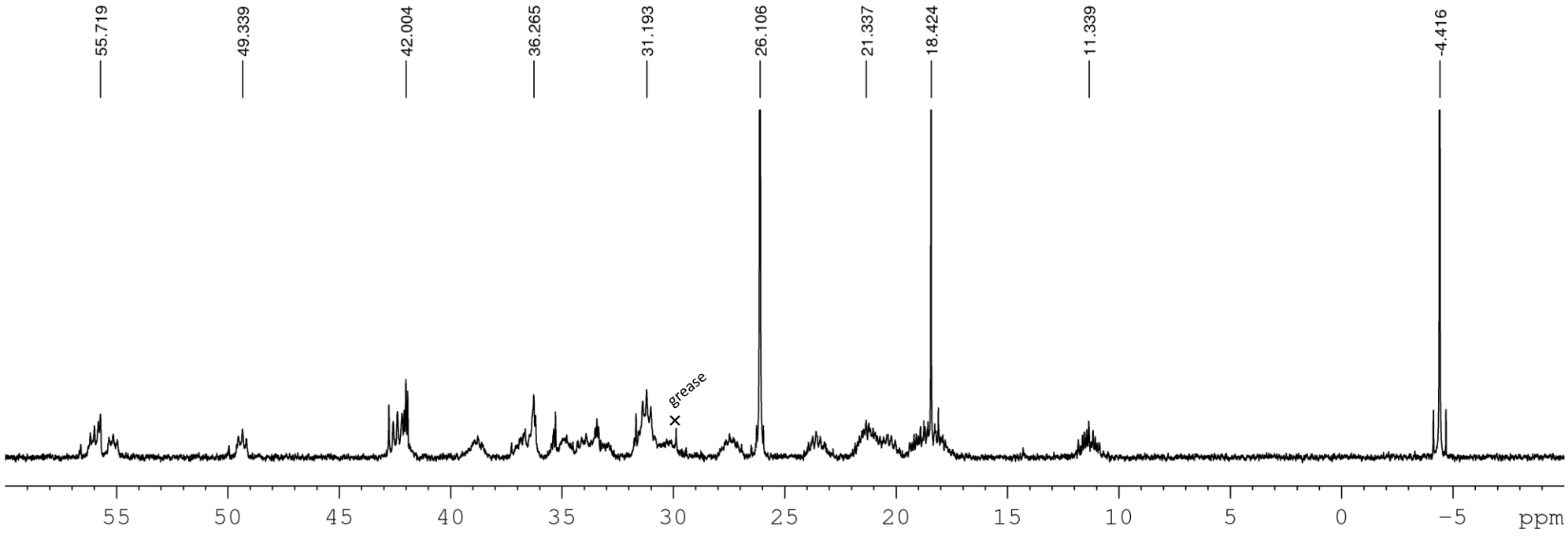

55.980
55.133
55.118
49.321
41.982
38.743
36.454
36.171
34.775
33.673
32.906
31.187
31.158
31.125
31.005
30.084
27.283
26.103
23.389
20.888
20.201
18.422
17.776
11.046
-4.420
55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

O-TBS-24-methylenecholesterol-d_{45} (87%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230217
Time                10.35 h
INSTRUM              spect
PROBHD    Z108618_0117 (
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                   16975
DS                       0
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                     203
DW                22.667 usec
DE                  8.02 usec
TE                 298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          100.6223248 MHz
NUC1                 13C
P1                 10.00 usec
PLW1          84.53199768 W
SFO2          400.1320007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2          14.03299999 W
PLW12          0.38982001 W
PLW13          0.19607000 W

F2 - Processing parameters
SI                   65536
SF            100.6127538 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

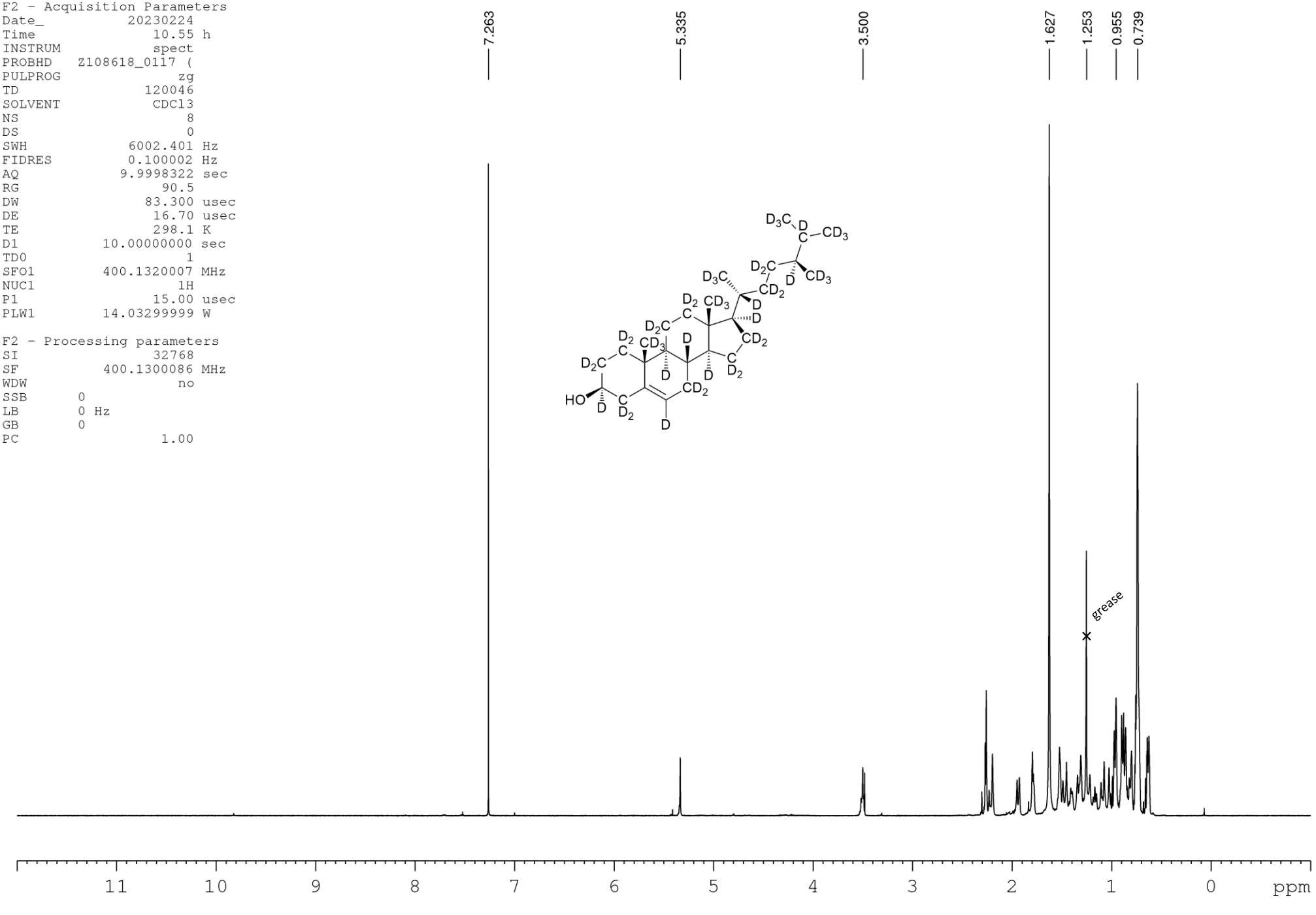



156.960
156.875
156.780
141.780
141.747
120.821
106.069
105.780
105.546
105.298
77.160
72.164
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 ppm


55.719
49.339
42.004
36.265
31.193
26.106
21.337
18.424
11.339
-4.416
grease
55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

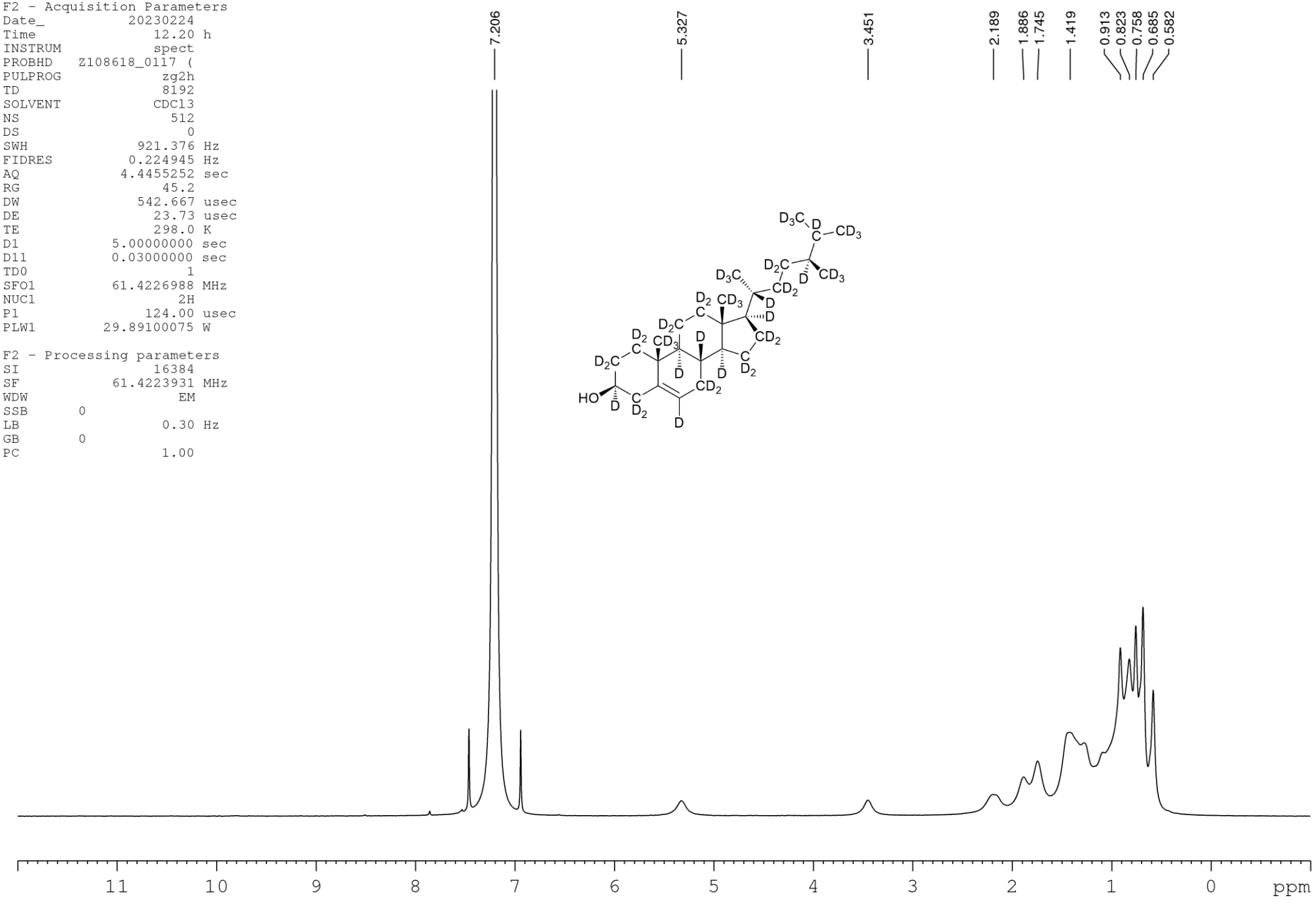
22,23-dihydrobrassicasterol-d_{50} (87%-d) ^{1}H NMR

```
F2 - Acquisition Parameters
Date_           20230224
Time              10.55 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG              zg
TD               120046
SOLVENT            CDCl3
NS                     8
DS                     0
SWH            6002.401 Hz
FIDRES        0.100002 Hz
AQ            9.9998322 sec
RG                  90.5
DW                83.300 usec
DE                16.70 usec
TE                298.1 K
D1           10.00000000 sec
TD0                    1
SFO1         400.1320007 MHz
NUC1                  1H
P1                15.00 usec
PLW1         14.03299999 W

F2 - Processing parameters
SI                 32768
SF           400.1300086 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```



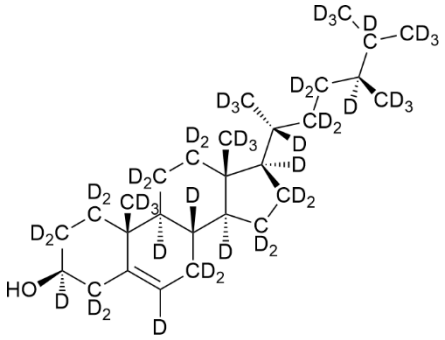
22,23-dihydrobrassicasterol-d_{50} (87%-d) ^{2}H NMR

```
F2 - Acquisition Parameters
Date_           20230224
Time              12.20 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG             zg2h
TD                  8192
SOLVENT            CDCl3
NS                    512
DS                      0
SWH               921.376 Hz
FIDRES           0.224945 Hz
AQ              4.4455252 sec
RG                   45.2
DW                542.667 usec
DE                 23.73 usec
TE                 298.0 K
D1            5.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          61.4226988 MHz
NUC1                  2H
P1                 124.00 usec
PLW1         29.89100075 W

F2 - Processing parameters
SI                 16384
SF            61.4223931 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

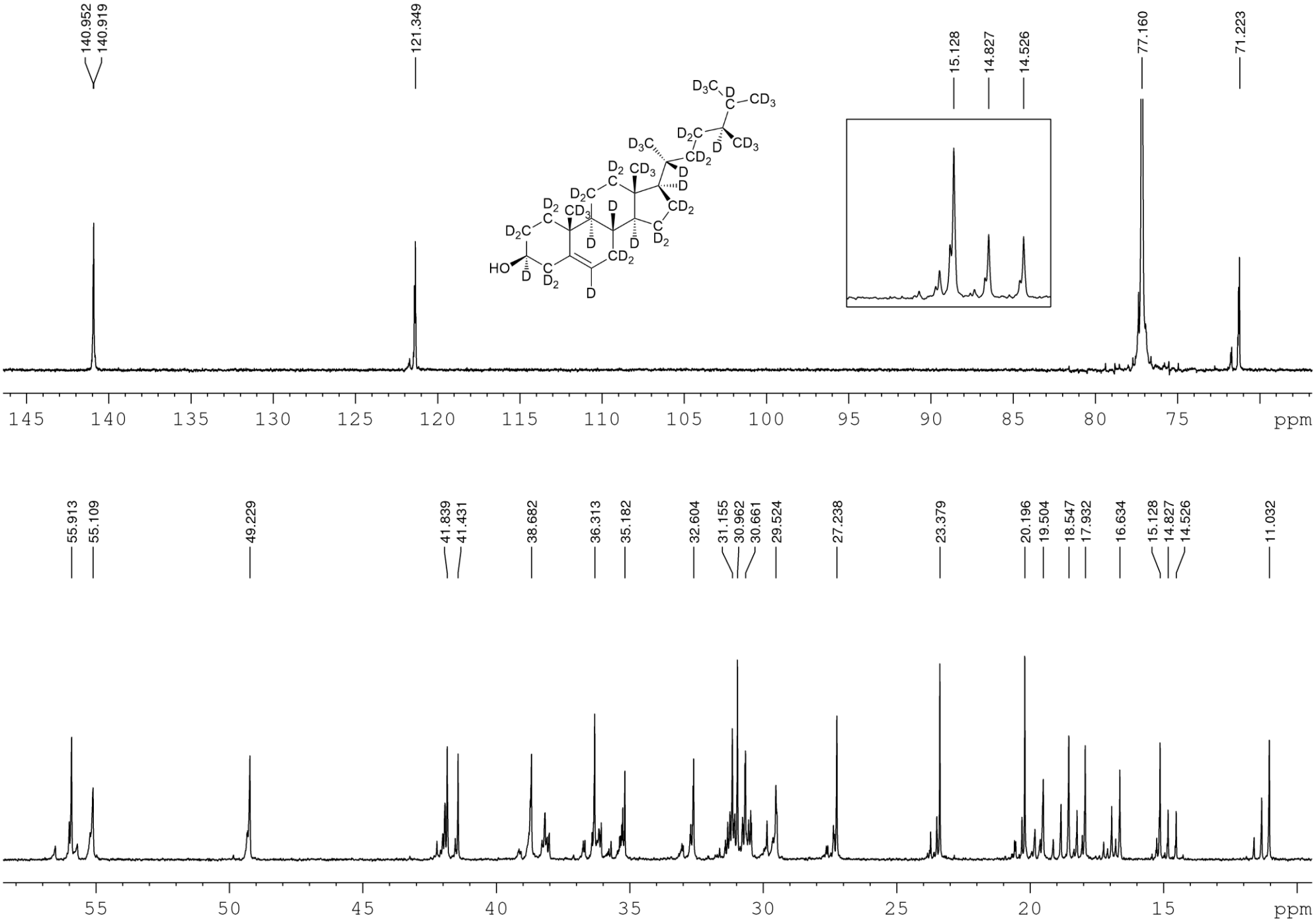


22,23-dihydrobrassicasterol-d_{50} (87%-d) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230227
Time                8.44 h
INSTRUM             spect
PROBHD    Z108618_0117 (
PULPROG          zgig2h1h
TD                  65536
SOLVENT             CDCl3
NS                  10724
DS                      0
SWH           22058.824 Hz
FIDRES         0.673182 Hz
AQ             1.4854827 sec
RG                    203
DW                22.667 usec
DE                 6.50 usec
TE                298.0 K
D1           20.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1
SFO1         100.6223263 MHz
NUC1                13C
P1                 10.00 usec
PLW1         84.53199768 W
SFO2         400.1322007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
SFO3          61.4227600 MHz
NUC3                2H
CPDPRG[3         waltz16
PCPD3             375.00 usec
PLW3         29.89100075 W
PLW17         3.06999993 W

F2 - Processing parameters
SI                  65536
SF           100.6127557 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

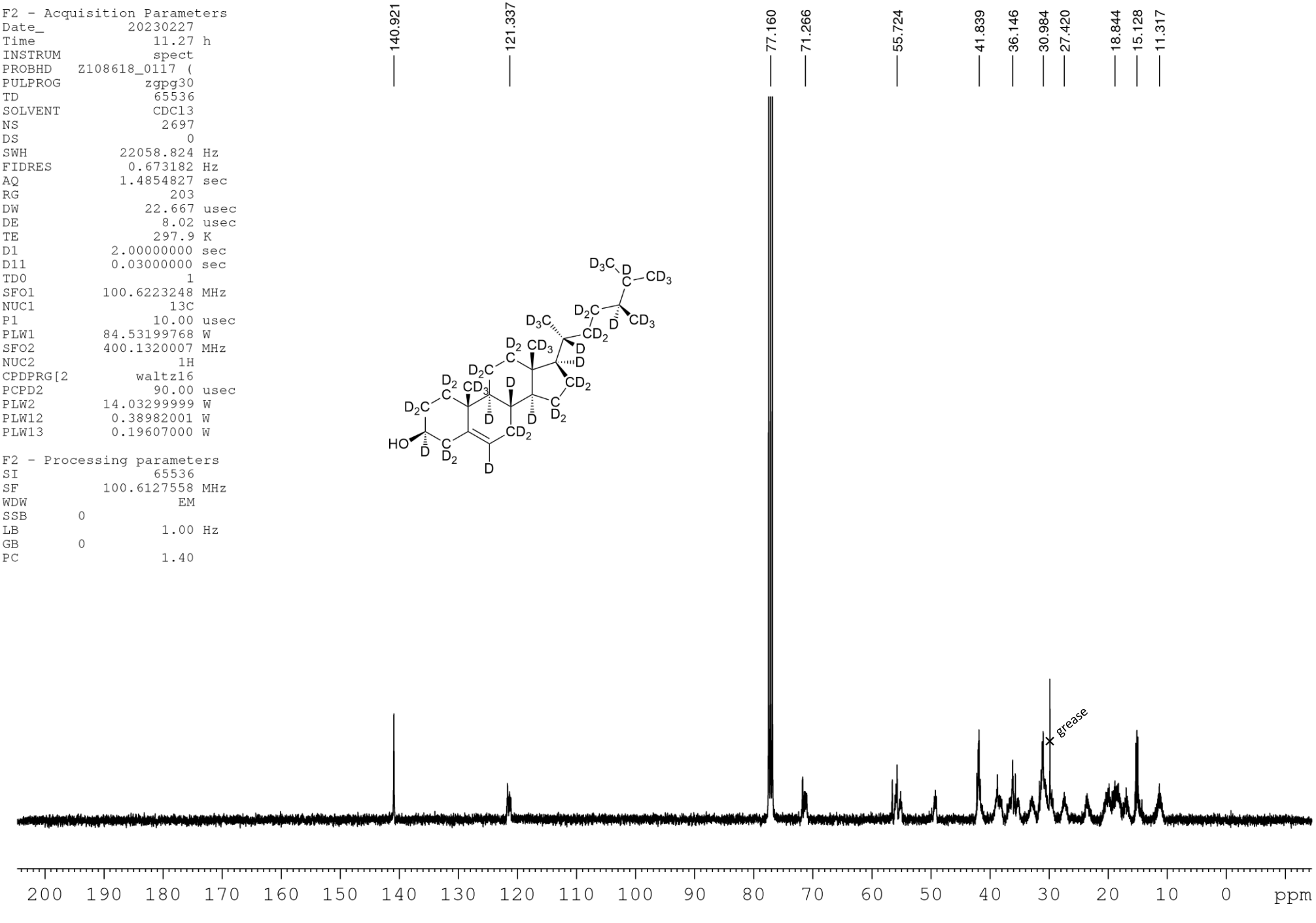


22,23-dihydrobrassicasterol-d_{50} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR expansions



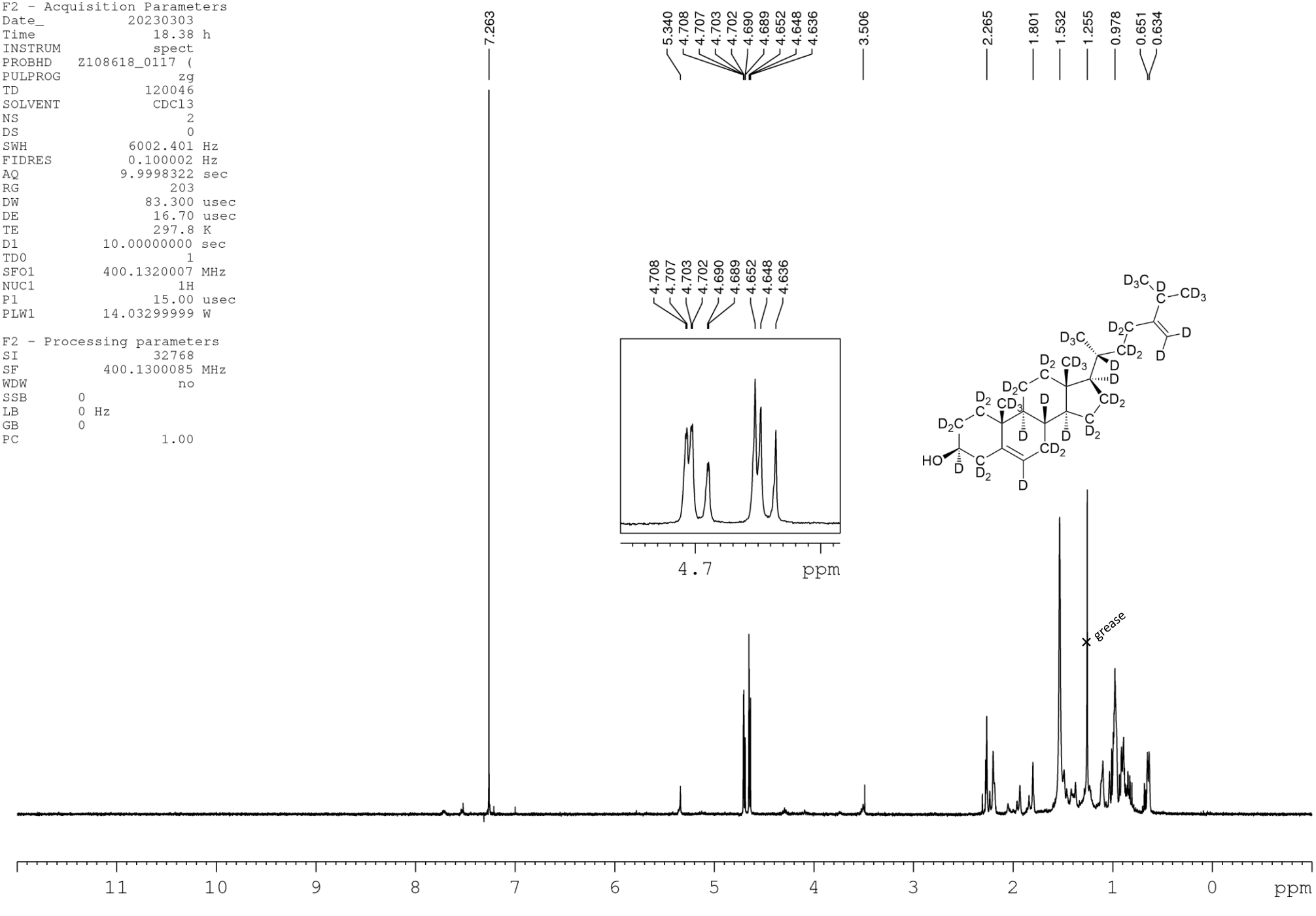
22,23-dihydrobrassicasterol-d_{50} (87%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230227
Time               11.27 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   2697
DS                      0
SWH           22058.824 Hz
FIDRES         0.673182 Hz
AQ            1.4854827 sec
RG                    203
DW                22.667 usec
DE                  8.02 usec
TE                 297.9 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6223248 MHz
NUC1                13C
P1                10.00 usec
PLW1         84.53199768 W
SFO2         400.1320007 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
PLW13         0.19607000 W

F2 - Processing parameters
SI                  65536
SF          100.6127558 MHz
WDW                    EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```



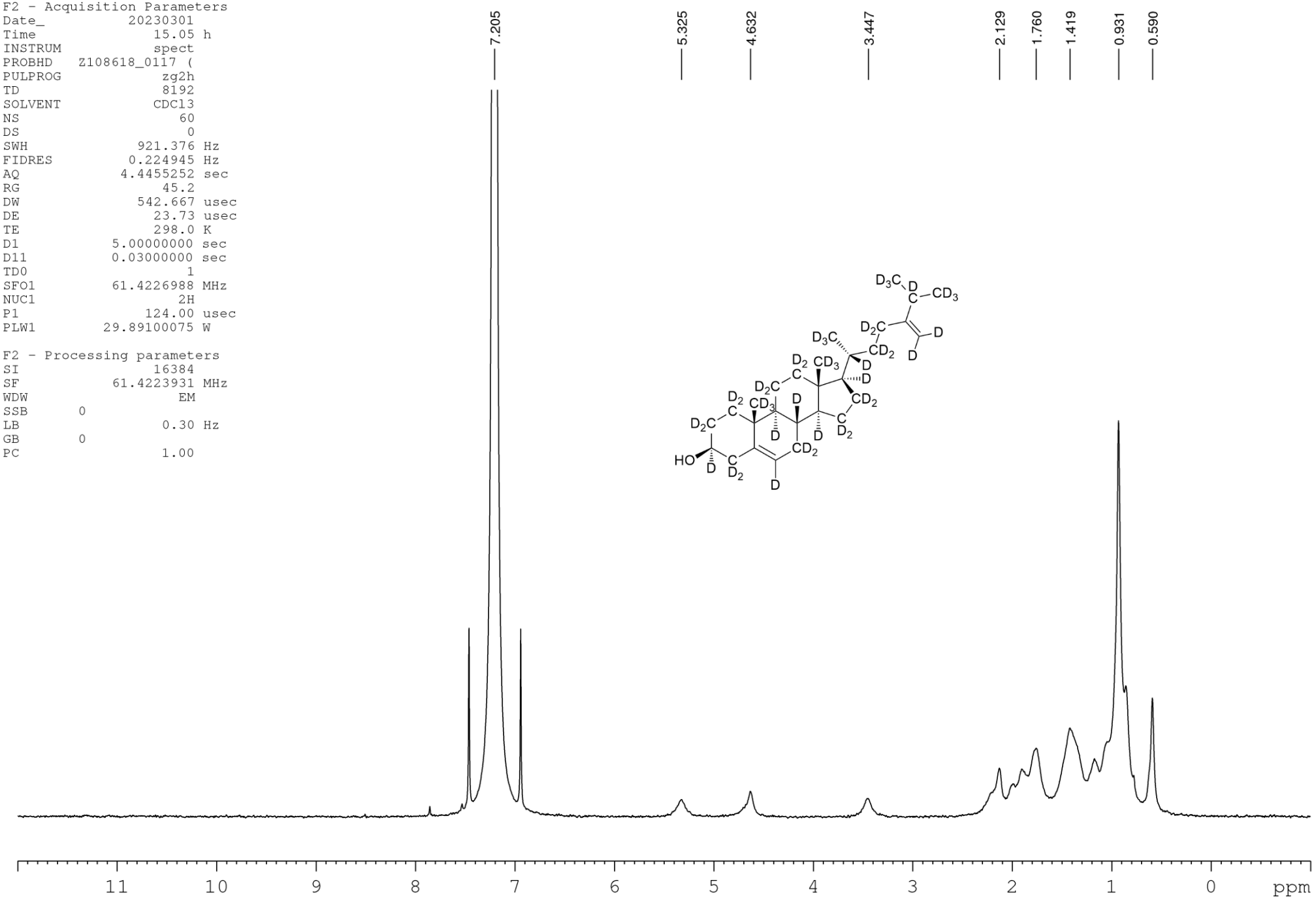
24-methylenecholesterol-d_{45} (87%-d) ^{1}H NMR

```
F2 - Acquisition Parameters
Date_            20230303
Time                18.38 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG               zg
TD                120046
SOLVENT             CDCl3
NS                      2
DS                      0
SWH              6002.401 Hz
FIDRES           0.100002 Hz
AQ               9.9998322 sec
RG                    203
DW                 83.300 usec
DE                 16.70 usec
TE                 297.8 K
D1           10.00000000 sec
TD0                     1
SFO1          400.1320007 MHz
NUC1                  1H
P1                 15.00 usec
PLW1          14.03299999 W

F2 - Processing parameters
SI                  32768
SF            400.1300085 MHz
WDW                    no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```



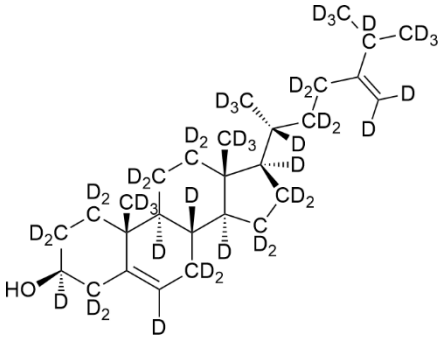
24-methylenecholesterol-d_{45} (87%-*d*) ^{2}H NMR

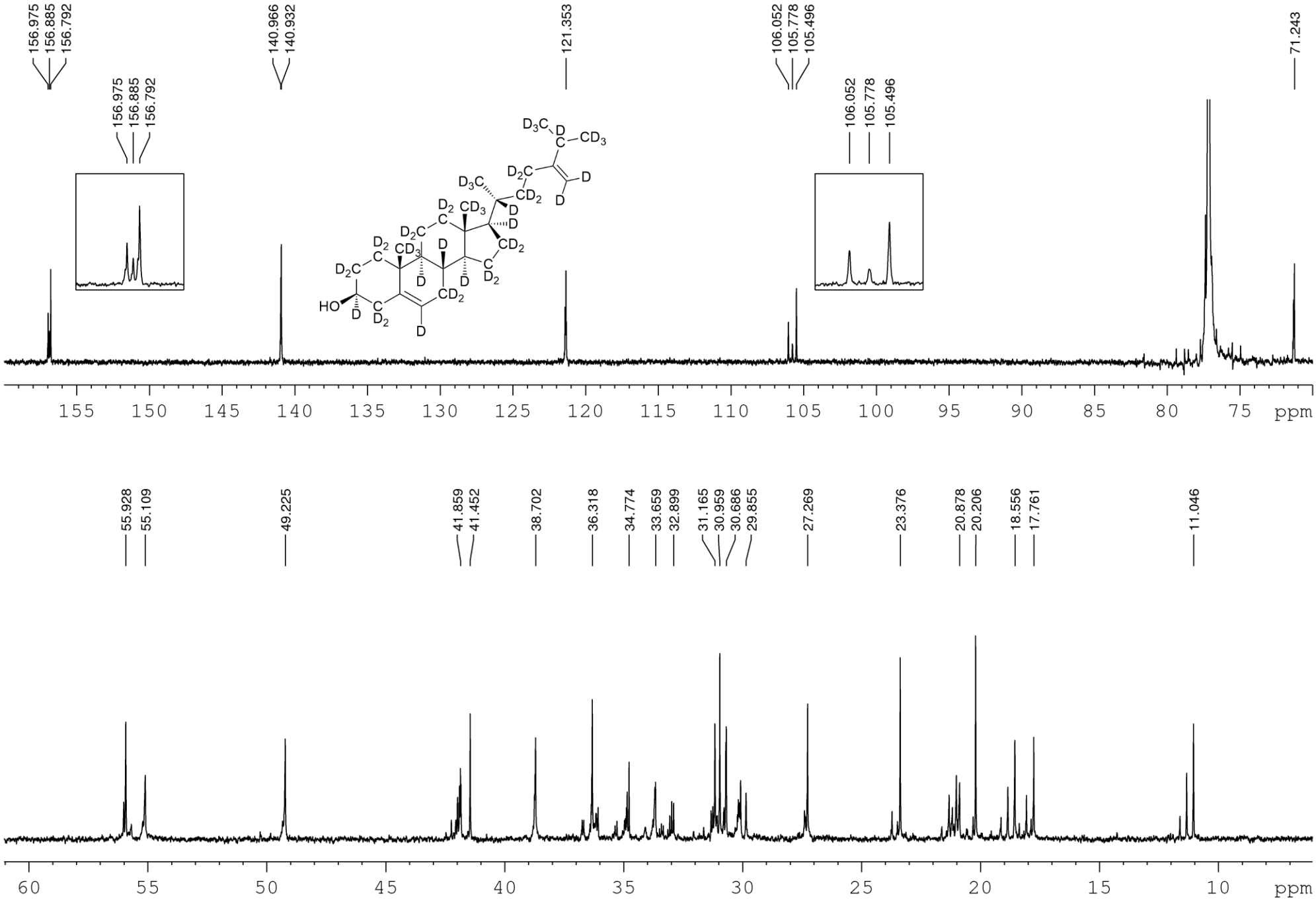
```
F2 - Acquisition Parameters
Date_           20230301
Time              15.05 h
INSTRUM            spect
PROBHD   Z108618_0117 (
PULPROG            zg2h
TD                 8192
SOLVENT           CDCl3
NS                   60
DS                    0
SWH             921.376 Hz
FIDRES         0.224945 Hz
AQ             4.4455252 sec
RG                  45.2
DW               542.667 usec
DE                23.73 usec
TE                298.0 K
D1           5.00000000 sec
D11          0.03000000 sec
TD0                  1
SFO1         61.4226988 MHz
NUC1                2H
P1               124.00 usec
PLW1        29.89100075 W

F2 - Processing parameters
SI                16384
SF           61.4223931 MHz
WDW                  EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```



24-methylenecholesterol-d_{45} (87%-*d*) $^{13}C\{^{1}H,^{2}H\}$ NMR

```
F2 - Acquisition Parameters
Date_           20230306
Time                8.39 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG          zgig2h1h
TD                 65536
SOLVENT            CDCl3
NS                 10357
DS                     0
SWH            22058.824 Hz
FIDRES          0.673182 Hz
AQ             1.4854827 sec
RG                   203
DW                22.667 usec
DE                 6.50 usec
TE                298.1 K
D1           20.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1
SFO1         100.6223263 MHz
NUC1                13C
P1                10.00 usec
PLW1         84.53199768 W
SFO2         400.1322007 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
SFO3          61.4227600 MHz
NUC3                2H
CPDPRG[3         waltz16
PCPD3             375.00 usec
PLW3         29.89100075 W
PLW17         3.06999993 W

F2 - Processing parameters
SI                 65536
SF           100.6127548 MHz
WDW                  EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40
```




156.975
156.885
156.792
140.966
140.932
121.353
106.052
105.778
105.496
71.243
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 ppm
55.928
55.109
49.225
41.859
41.452
38.702
36.318
34.774
33.659
32.899
31.165
30.959
30.686
29.855
27.269
23.376
20.878
20.206
18.556
17.761
11.046
60 55 50 45 40 35 30 25 20 15 10 ppm

24-methylenecholesterol-d_{45} (87%-*d*) $^{13}C\{^{1}H\}$ NMR

```
F2 - Acquisition Parameters
Date_            20230308
Time                8.43 h
INSTRUM             spect
PROBHD   Z108618_0117 (
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                  15424
DS                      0
SWH           22058.824 Hz
FIDRES         0.673182 Hz
AQ            1.4854827 sec
RG                    203
DW               22.667 usec
DE                8.02 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        100.6223248 MHz
NUC1                13C
P1                10.00 usec
PLW1         84.53199768 W
SFO2         400.1320007 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         14.03299999 W
PLW12         0.38982001 W
PLW13         0.19607000 W

F2 - Processing parameters
SI                  65536
SF          100.6127547 MHz
WDW                 EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```