Supporting Information for

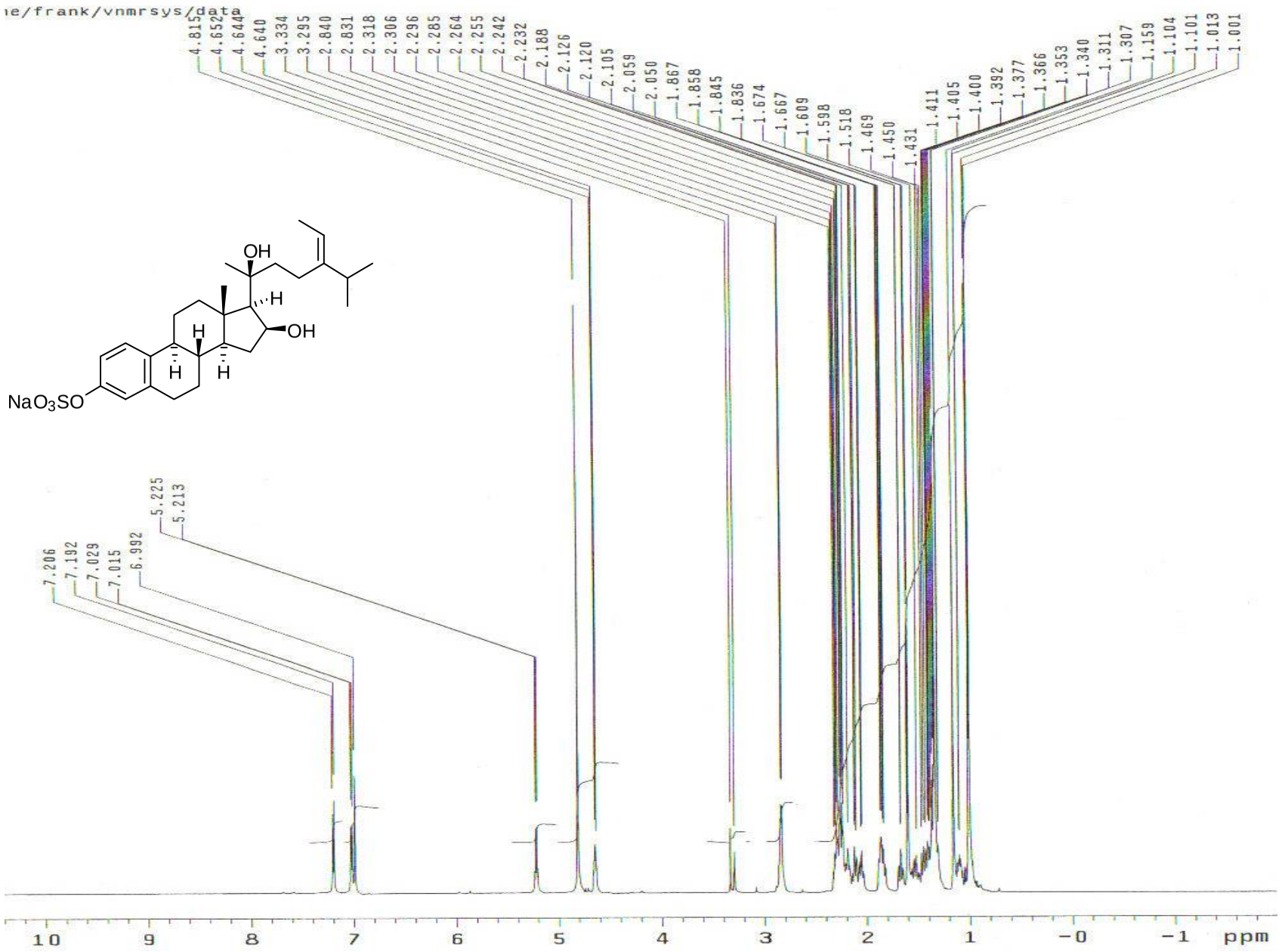
Reversal of Fluconazole Resistance by Sulfated Sterols from the Marine Sponge *Topsentia* sp.

Jessica A. DiGirolamo,[†] Xing-Cong Li,[†,‡,*] Melissa R. Jacob,[‡] Alice M. Clark,[†,‡] and Daneel Ferreira[†,‡,*]

Department of Pharmacognosy, National Center for Natural Products Research, and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi, 38677, USA

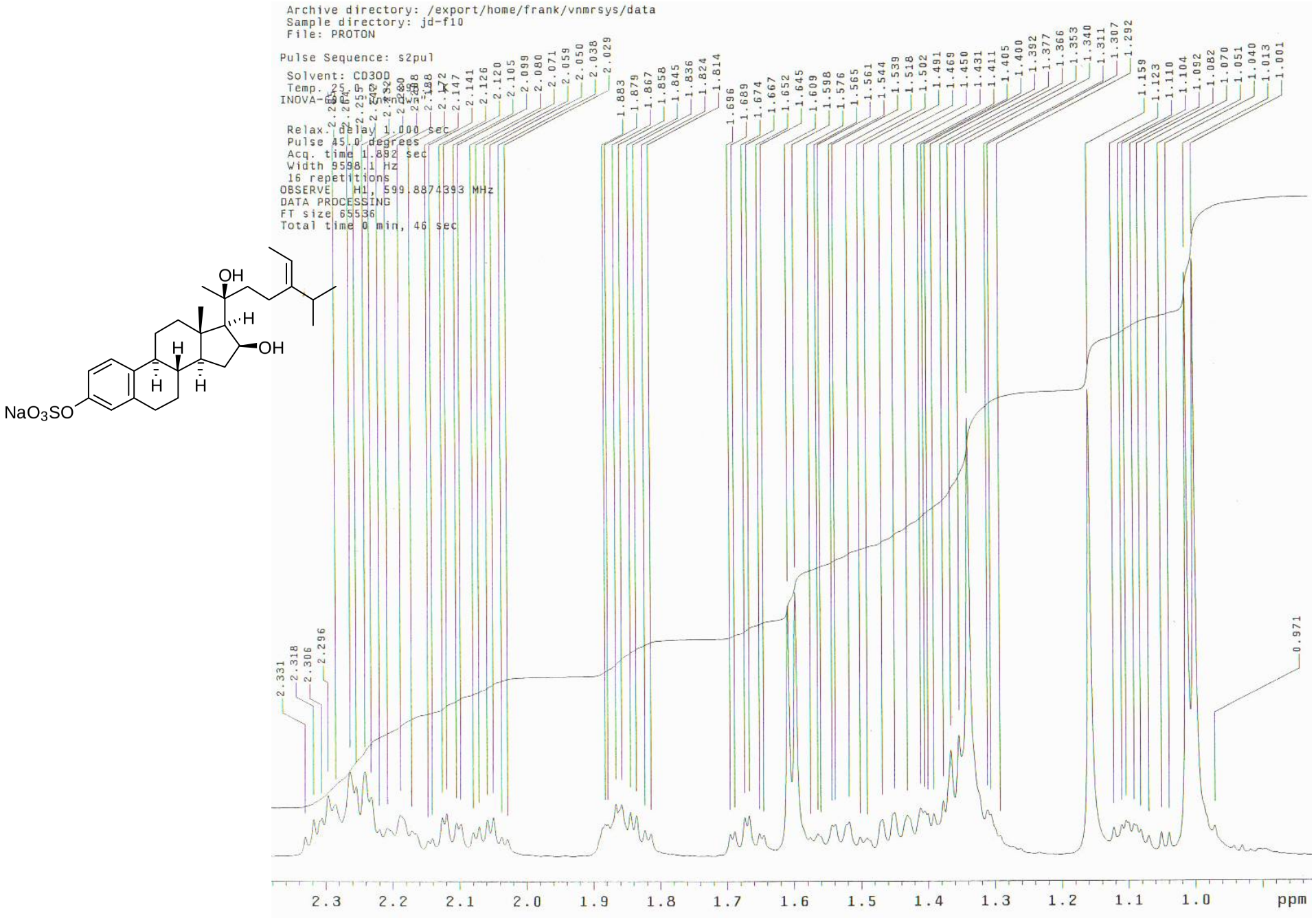
Table of Contents:

S1. 600 MHz ^{1}H NMR spectrum of compound 1 in methanol-d_4*

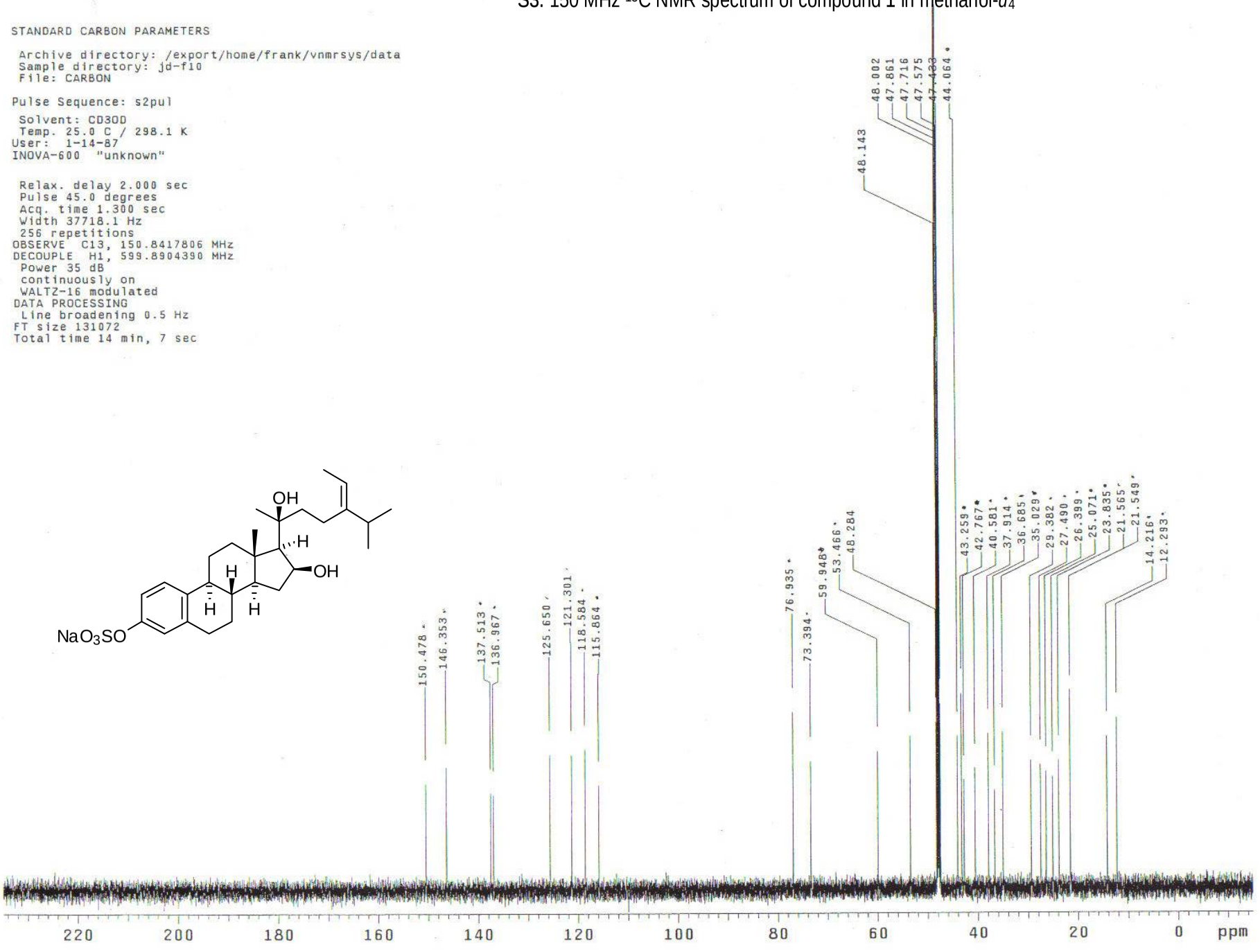


* $\delta_{H\ (methanol\text{-}d4)}$ 3.30; the data in the manuscript is calibrated by using the $\delta_{H\ (methanol\text{-}d4)}$ 3.33.

S2. 600 MHz ^{1}H NMR spectrum of compound **1** in methanol-d_4 (expansion)

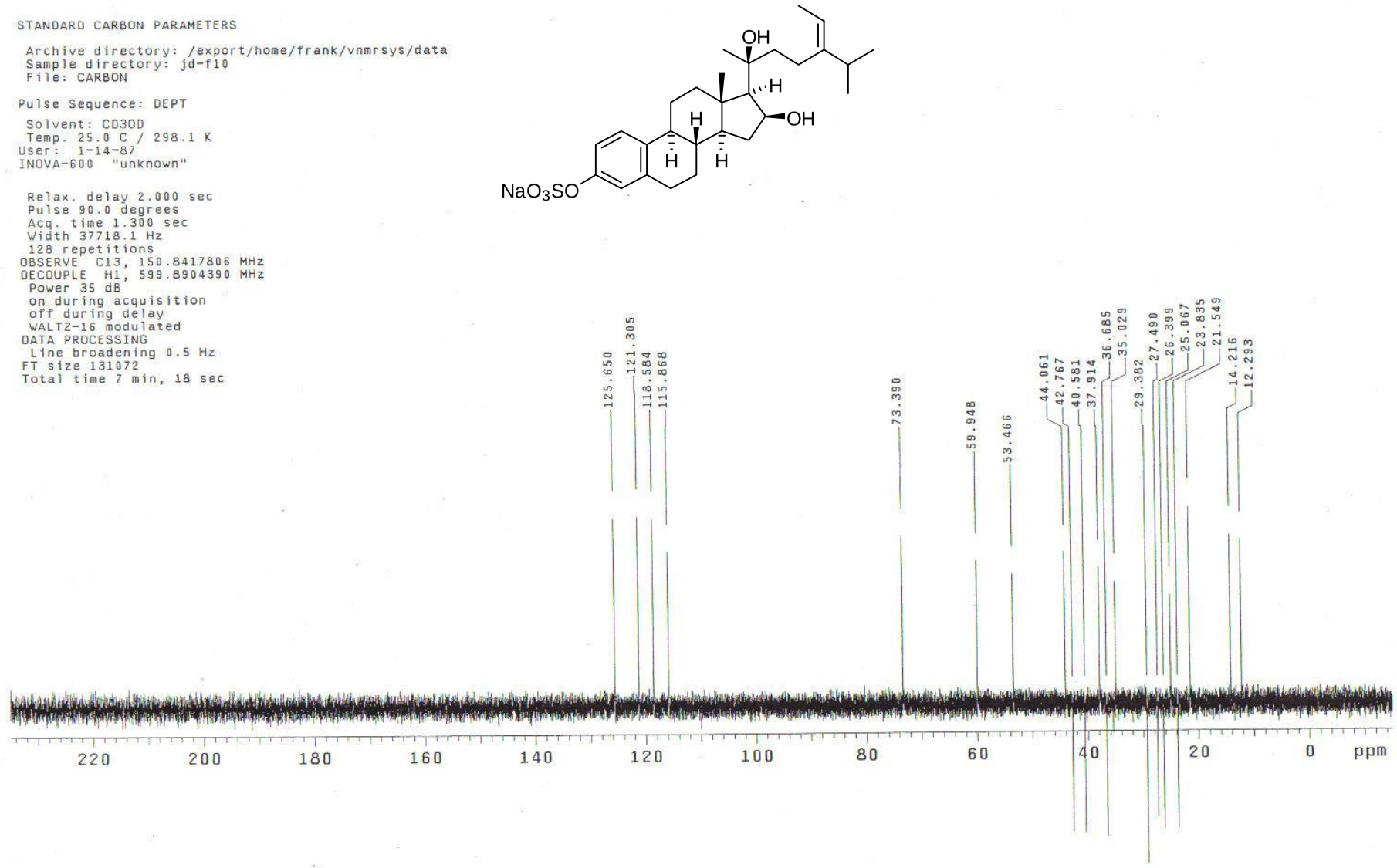


S3. 150 MHz ^{13}C NMR spectrum of compound 1 in methanol-d_4*

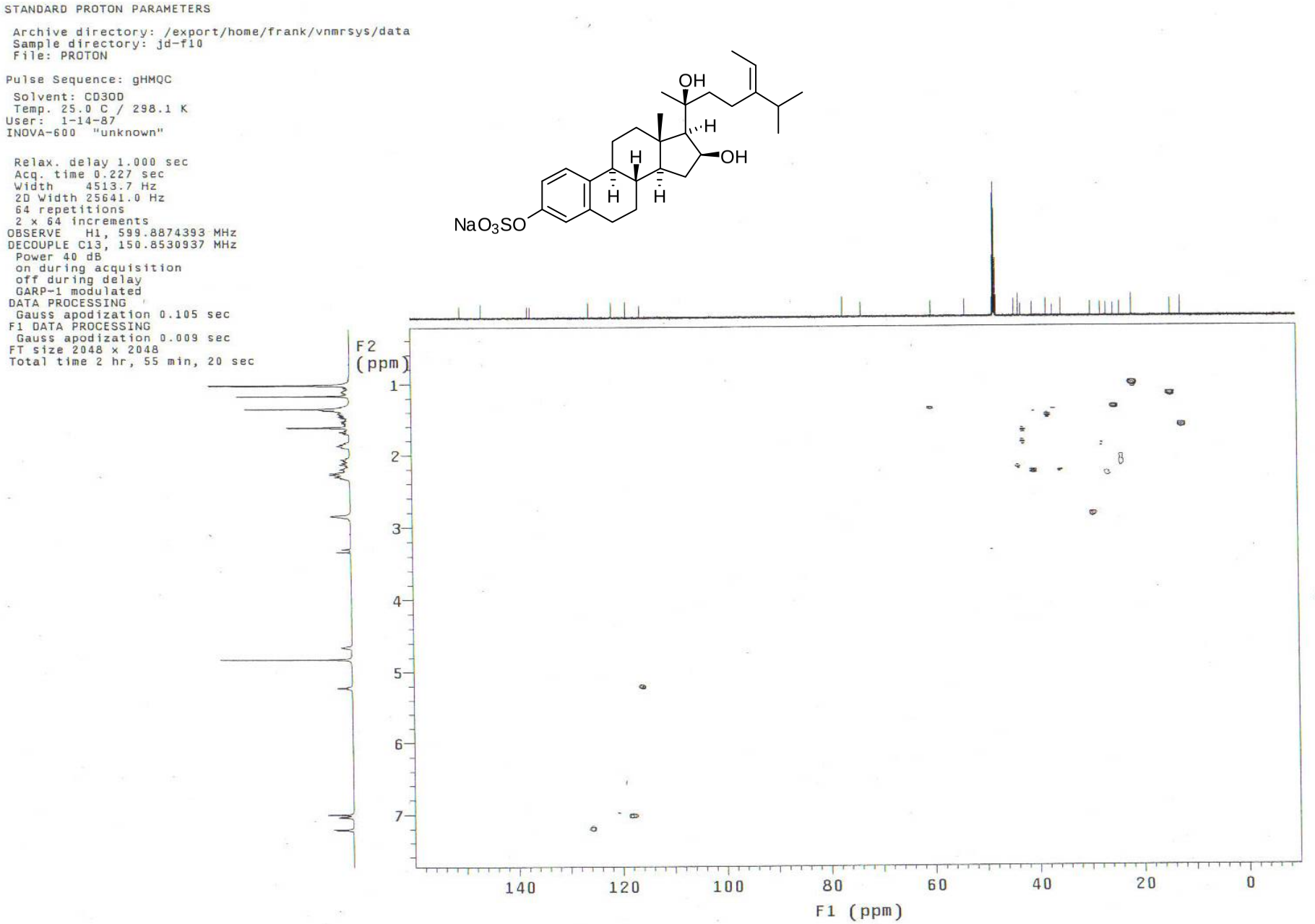


* $\delta_{C\ (methanol\text{-}d4)}$ 47.9; the data in the manuscript is calibrated by using the $\delta_{C\ (methanol\text{-}d4)}$ 49.0.

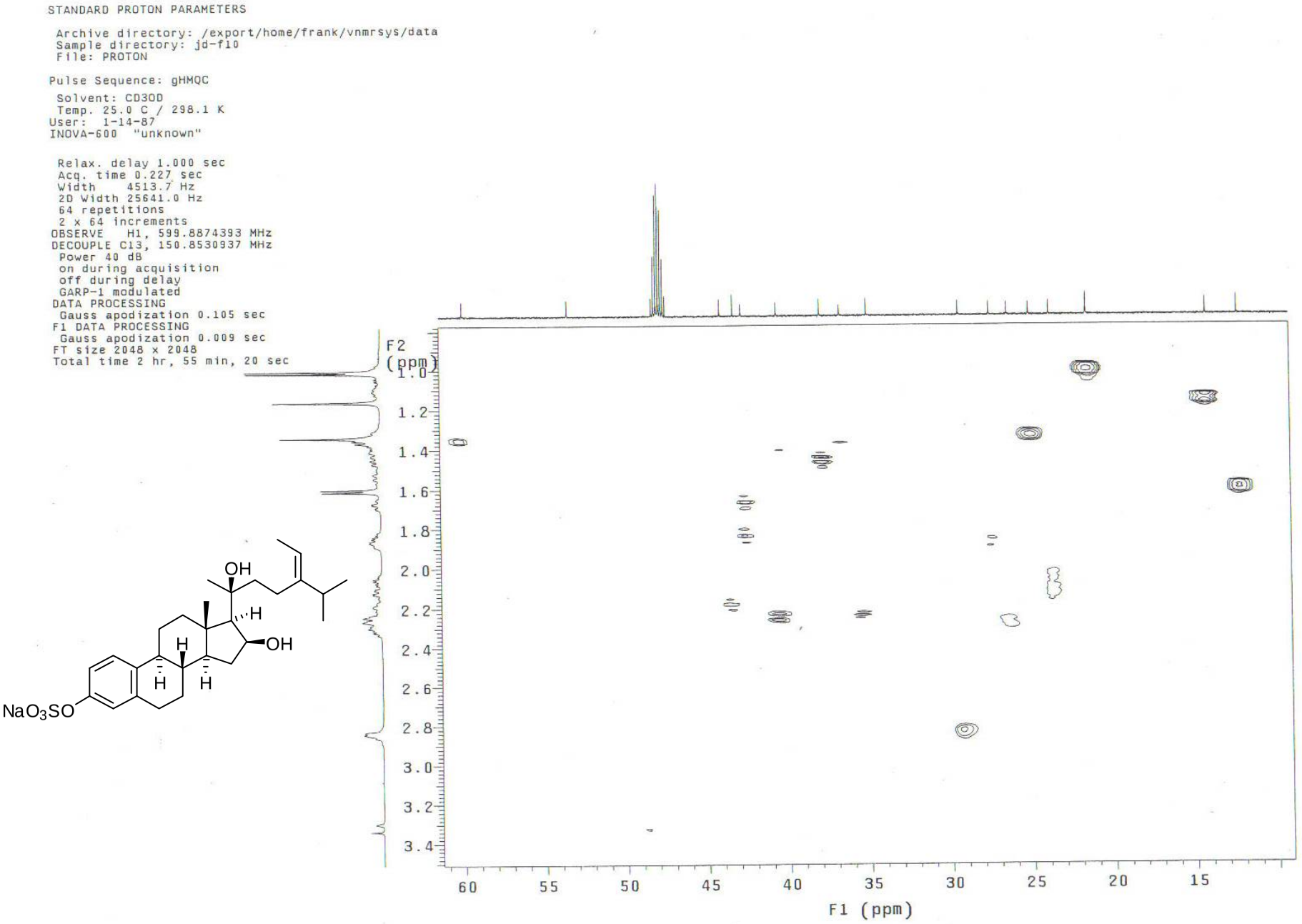
S4. 125 MHz DEPT-135 spectrum of compound **1** in methanol-d_4



S5. 600 MHz HMQC spectrum compound 1 in methanol-d_4

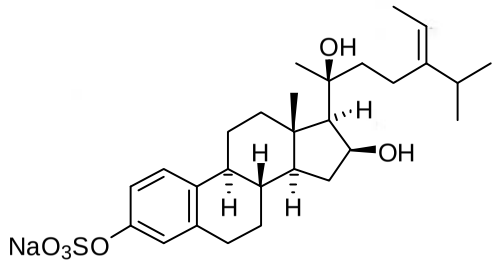


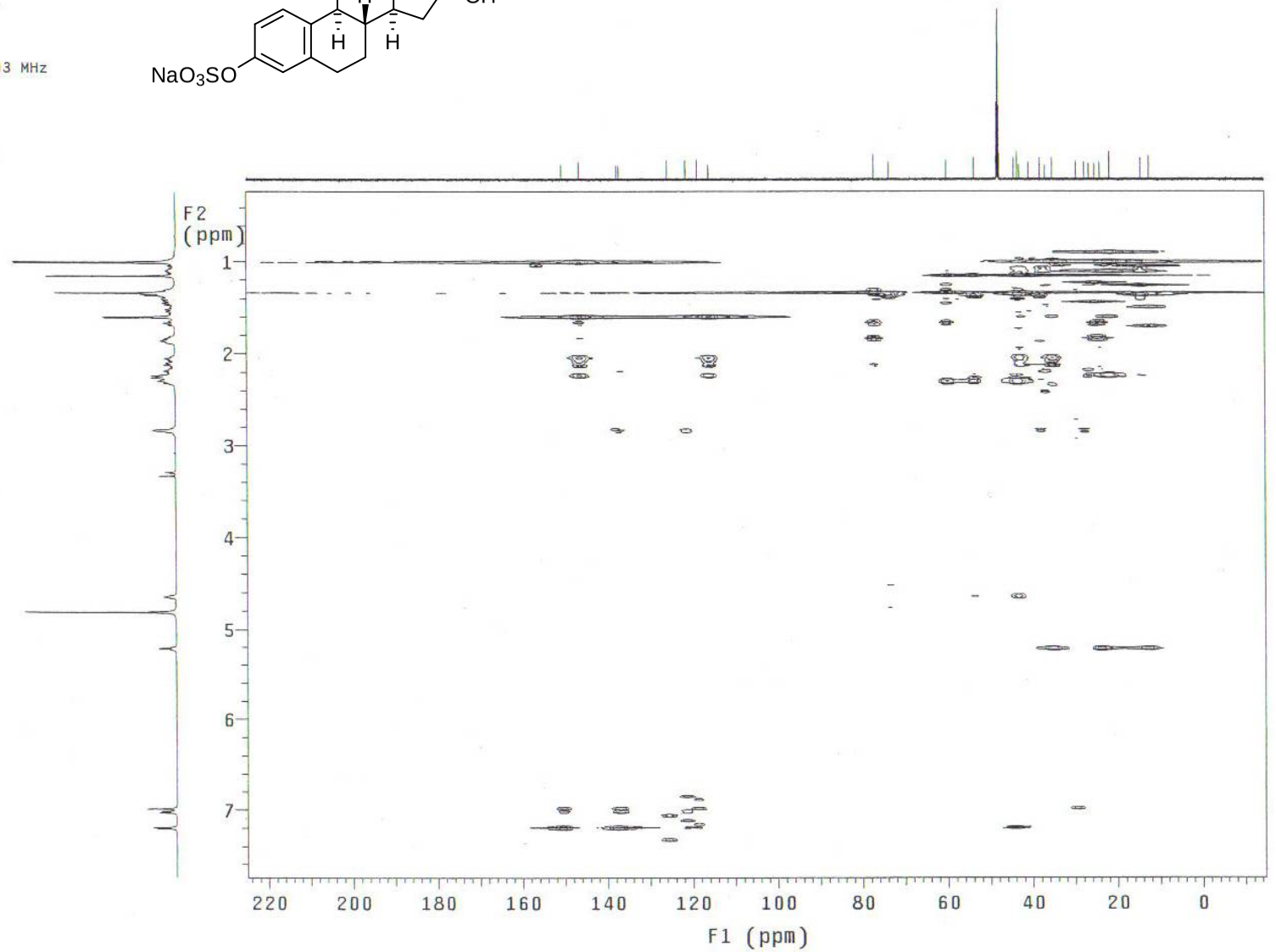
S6. 600 MHz HMQC spectrum of compound **1** in methanol-d_4 (expansion)



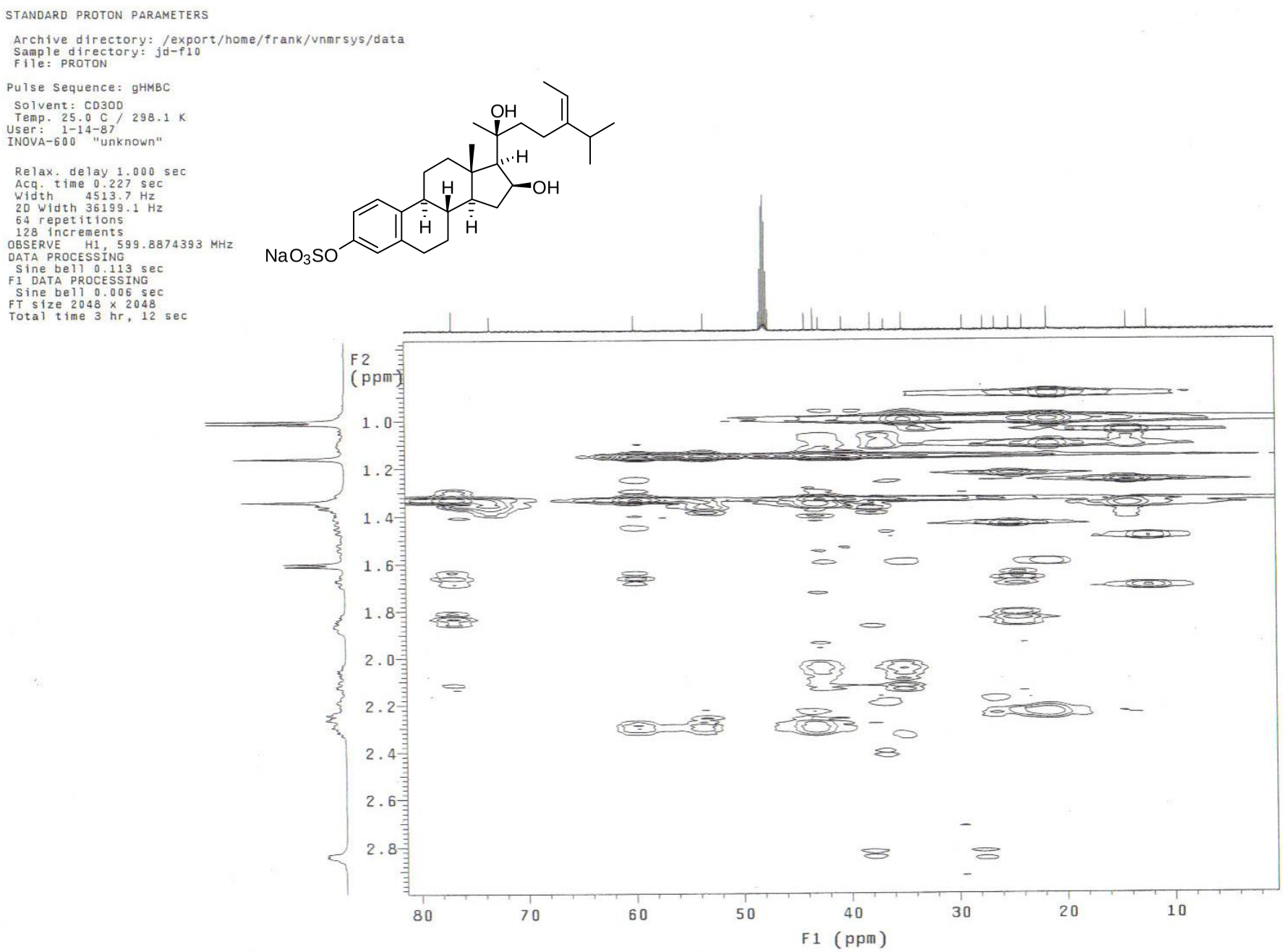
S7. 600 MHz HMBC spectrum of compound 1 in methanol-d_4

```
STANDARD PROTON PARAMETERS

 Archive directory: /export/home/frank/vnmrsys/data
 Sample directory: jd-f10
 File: PROTON

Pulse Sequence: gHMBC
 Solvent: CD3OD
 Temp. 25.0 C / 298.1 K
User:  1-14-87
INOVA-600  "unknown"

 Relax. delay 1.000 sec
 Acq. time 0.227 sec
 Width     4513.7 Hz
 2D Width 36199.1 Hz
 64 repetitions
 128 increments
OBSERVE   H1, 599.8874393 MHz
DATA PROCESSING
 Sine bell 0.113 sec
F1 DATA PROCESSING
 Sine bell 0.006 sec
FT size 2048 x 2048
Total time 3 hr, 12 sec
```



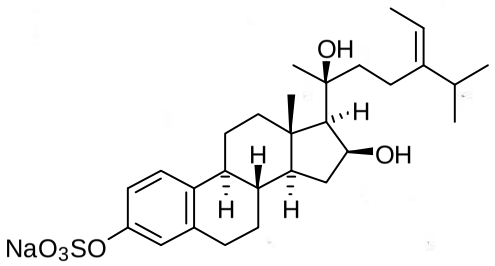


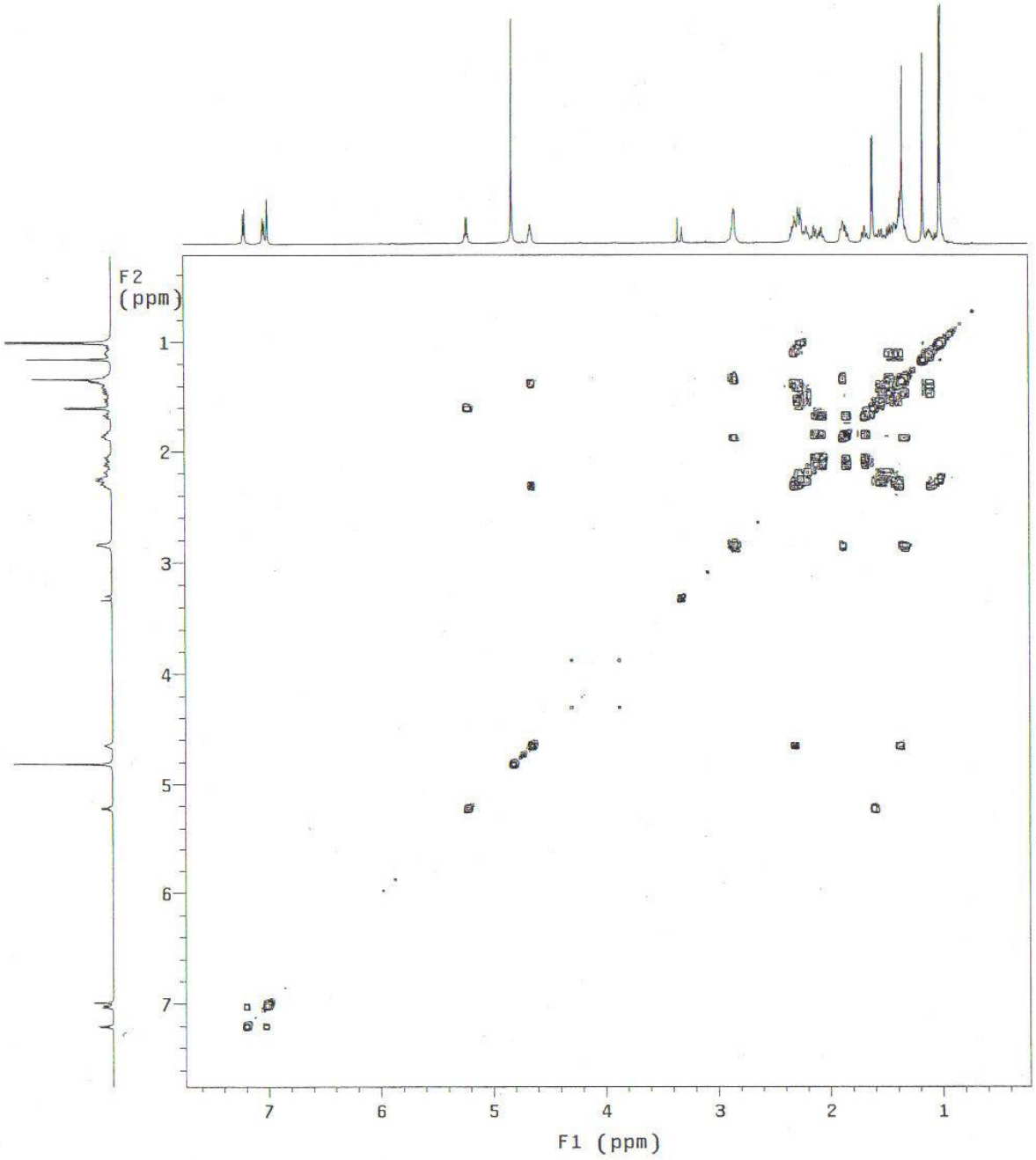
S8. 600 MHz HMBC sspectrum compound **1** in methanol-d_4 (expansion)



S9. 600 MHz COSY spectrum of compound 1 in methanol-d_4

```
STANDARD PROTON PARAMETERS

 Archive directory: /export/home/frank/vnmrsys/data
 Sample directory: jd-f10
 File: PROTON

Pulse Sequence: COSY
 Solvent: CD3OD
 Temp. 25.0 C / 298.1 K
INOVA-600  "unknown"

 Relax. delay 1.000 sec
 Acq. time 0.227 sec
 Width    4513.7 Hz
 2D Width 4513.7 Hz
 8 repetitions
 128 increments
OBSERVE   H1, 599.8874393 MHz
DATA PROCESSING
 Sq. sine bell 0.113 sec
F1 DATA PROCESSING
 Sq. sine bell 0.028 sec
FT size 2048 x 2048
Total time 22 min, 0 sec
```





S10. 600 MHz COSY spectrum of compound 1 in methanol-d_4 (expansion)

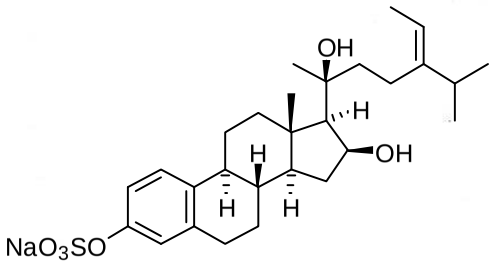
STANDARD PROTON PARAMETERS

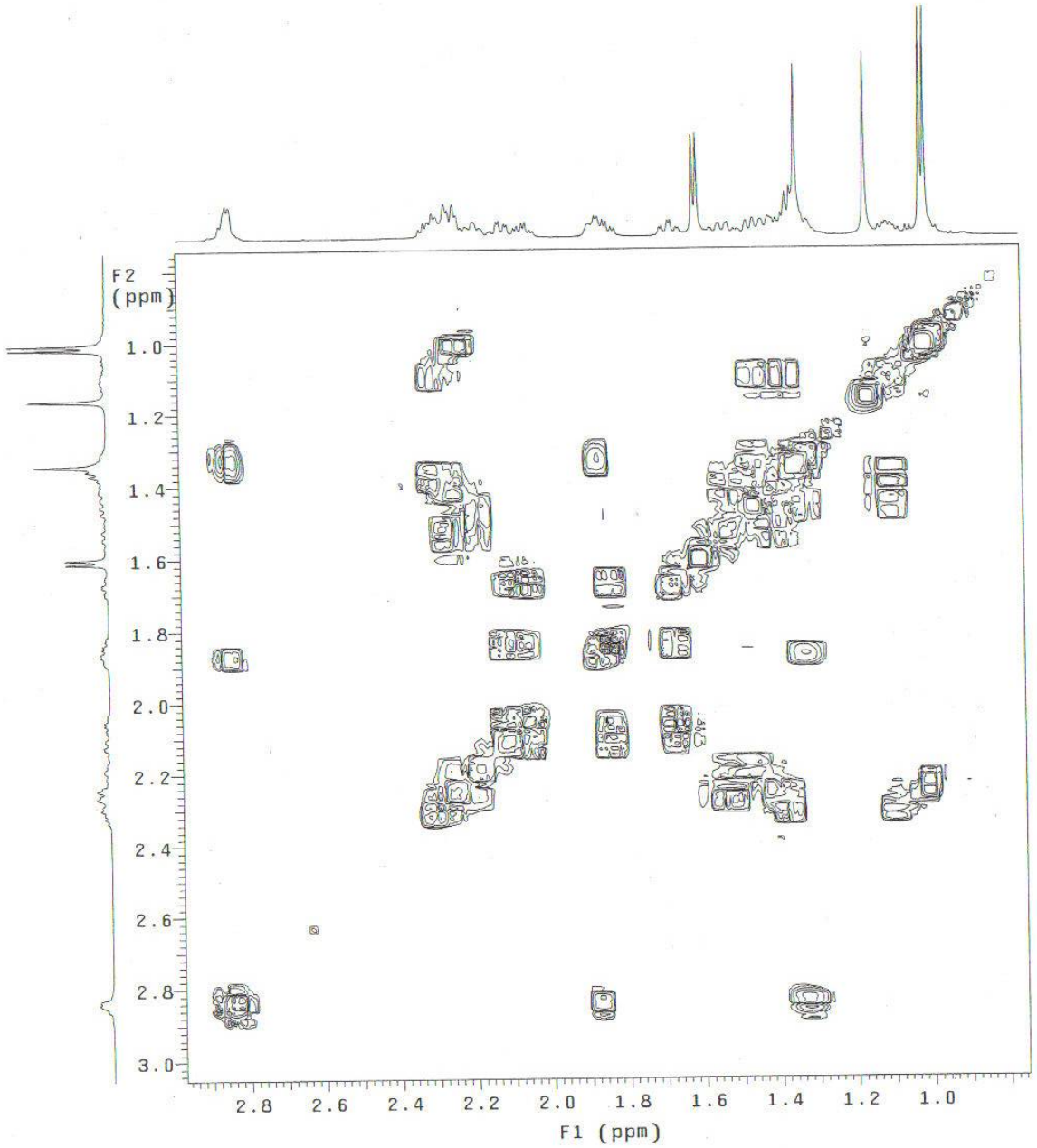
Archive directory: /export/home/frank/vnmrsys/data
Sample directory: jd-f10
File: PROTON

Pulse Sequence: COSY

Solvent: CD3OD
Temp. 25.0 C / 298.1 K
INOVA-600 "unknown"

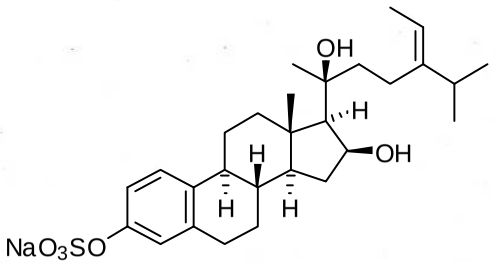
Relax. delay 1.000 sec
Acq. time 0.227 sec
Width 4513.7 Hz
2D Width 4513.7 Hz
8 repetitions
128 increments
OBSERVE H1, 599.8874393 MHz
DATA PROCESSING
Sq. sine bell 0.113 sec
F1 DATA PROCESSING
Sq. sine bell 0.028 sec
FT size 2048 x 2048
Total time 22 min, 0 sec

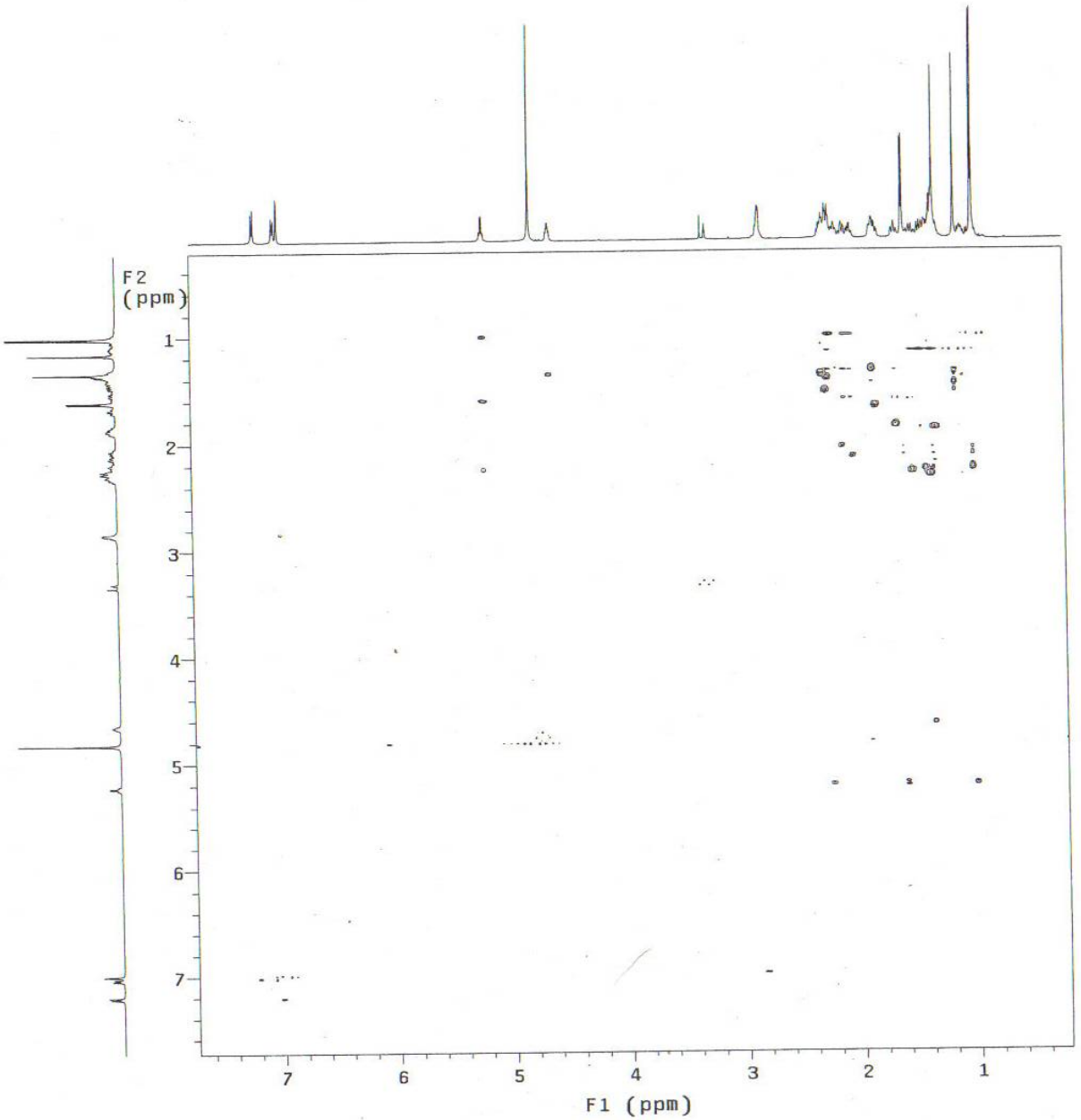




S11. 600 MHz NOESY spectrum of compound 1 in methanol-d_4

```
STANDARD PROTON PARAMETERS

 Archive directory: /export/home/frank/vnmrsys/data
 Sample directory: jd-f10
 File: PROTON

Pulse Sequence: NOESY

 Solvent: CD3OD
 Temp. 25.0 C / 298.1 K
INOVA-600  "unknown"

 Relax. delay 3.000 sec
 Mixing 0.800 sec
 Acq. time 0.227 sec
 Width     4513.7 Hz
 2D Width 4513.7 Hz
 8 repetitions
 2 x 128 increments
OBSERVE    H1, 599.8874393 MHz
DATA PROCESSING
 Gauss apodization 0.105 sec
F1 DATA PROCESSING
 Gauss apodization 0.041 sec
FT size 2048 x 2048
Total time 2 hr, 20 min, 24 sec
```





S12. 600 MHz NOESY spectrum of compound **1** in methanol-d_4 (expansion)

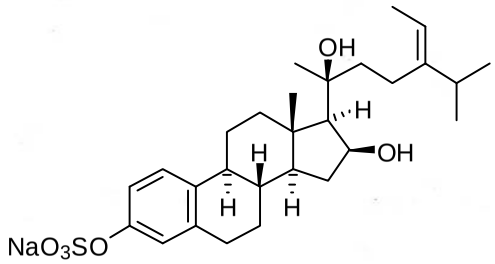
STANDARD PROTON PARAMETERS

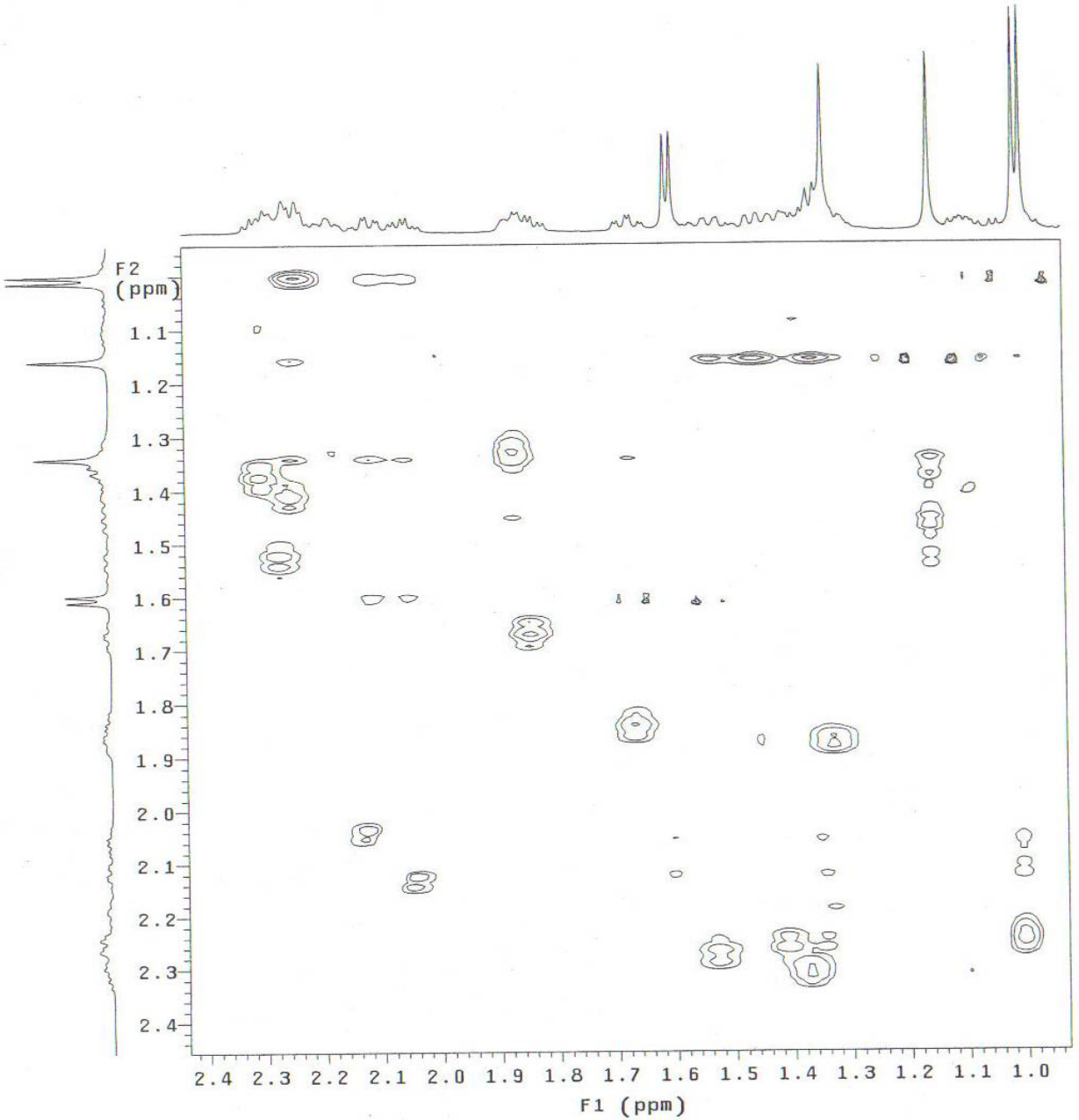
Archive directory: /export/home/frank/vnmrsys/data
Sample directory: jd-f10
File: PROTON

Pulse Sequence: NOESY

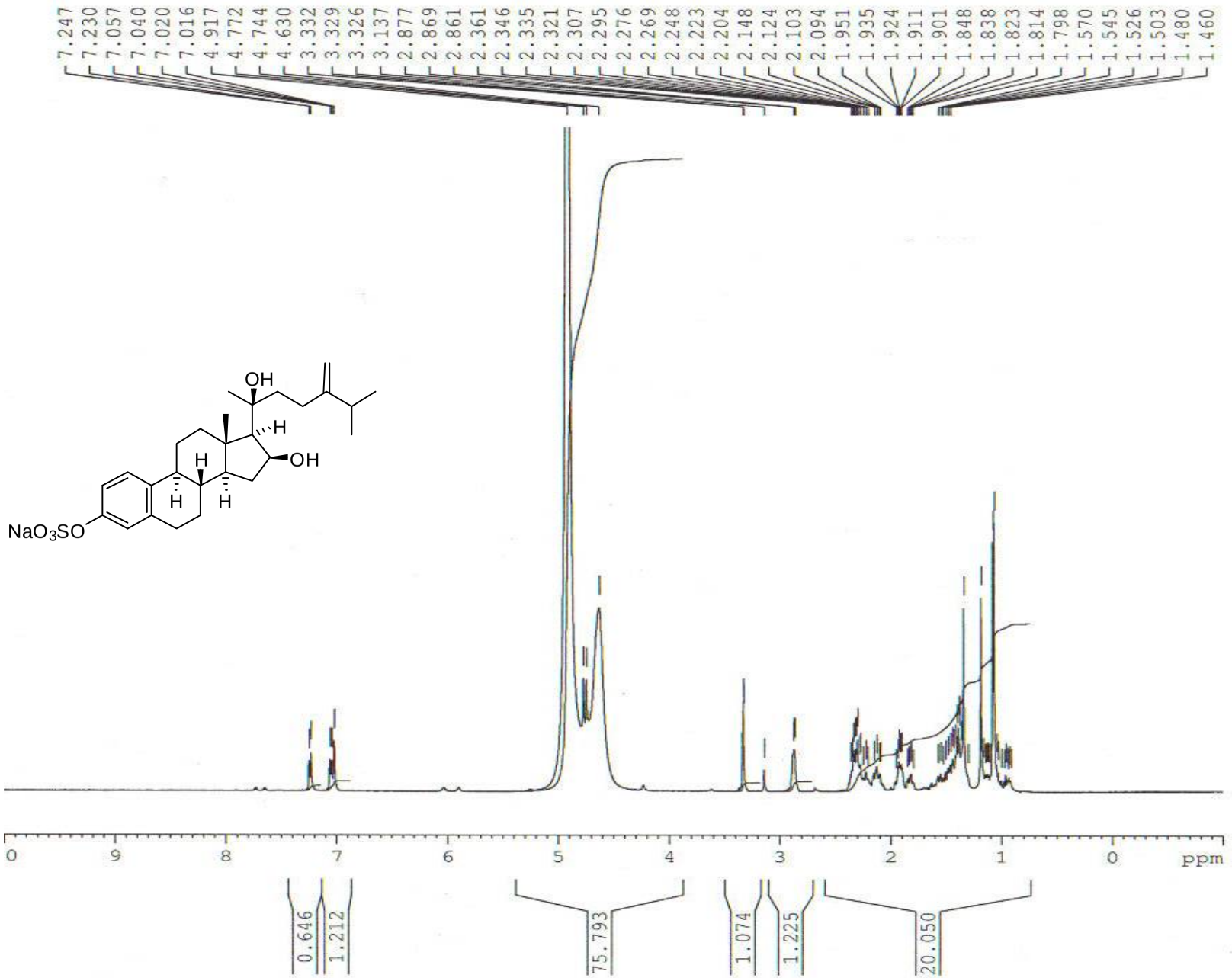
Solvent: CD3OD
Temp. 25.0 C / 298.1 K
INOVA-600 "unknown"

Relax. delay 3.000 sec
Mixing 0.800 sec
Acq. time 0.227 sec
Width 4513.7 Hz
2D Width 4513.7 Hz
8 repetitions
2 x 128 increments
OBSERVE H1, 599.8874393 MHz
DATA PROCESSING
Gauss apodization 0.105 sec
F1 DATA PROCESSING
Gauss apodization 0.041 sec
FT size 2048 x 2048
Total time 2 hr, 20 min, 24 sec



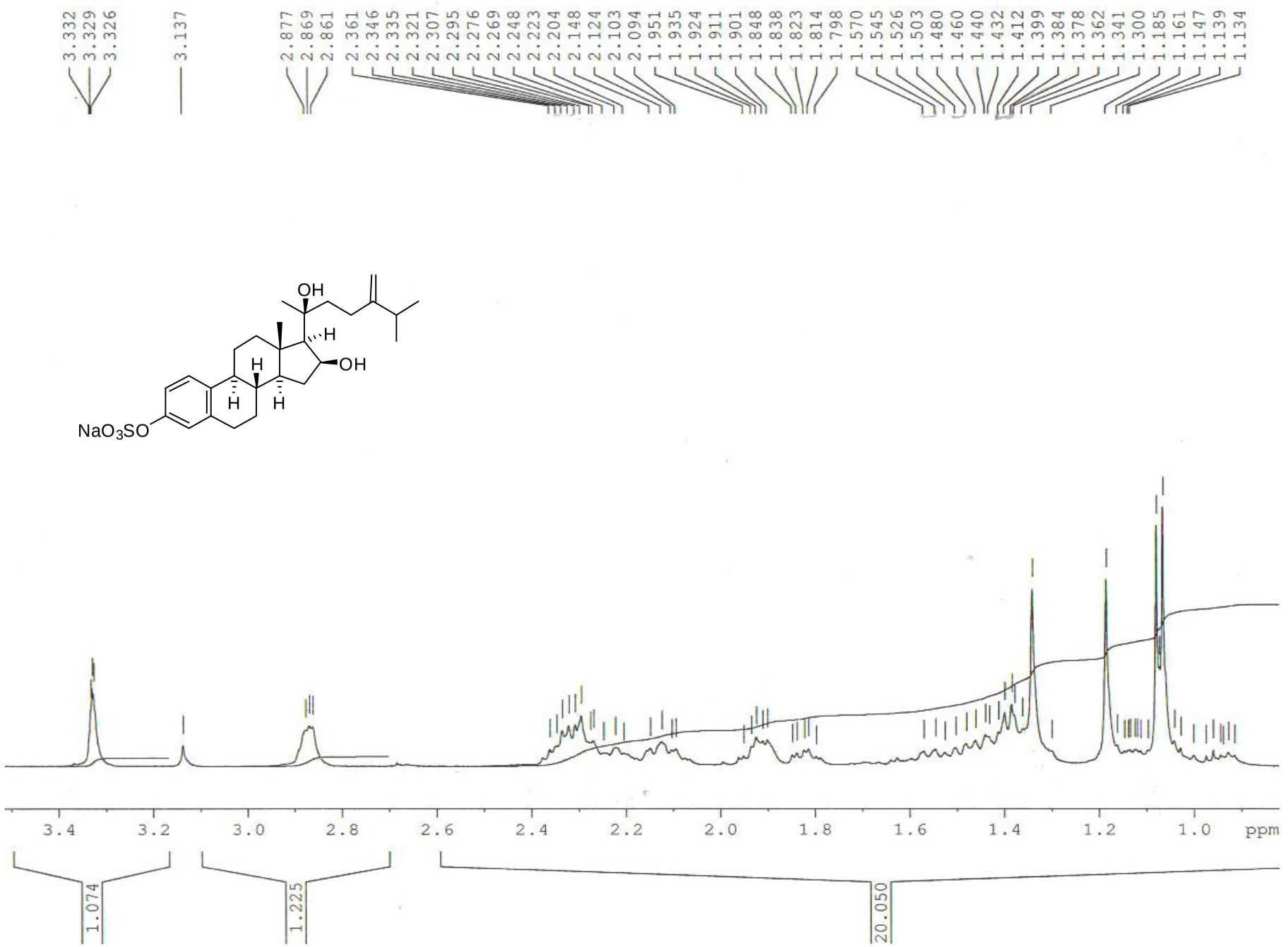


S13. 500 MHz ^{1}H NMR spectrum of compound **1** in methanol-d_4*

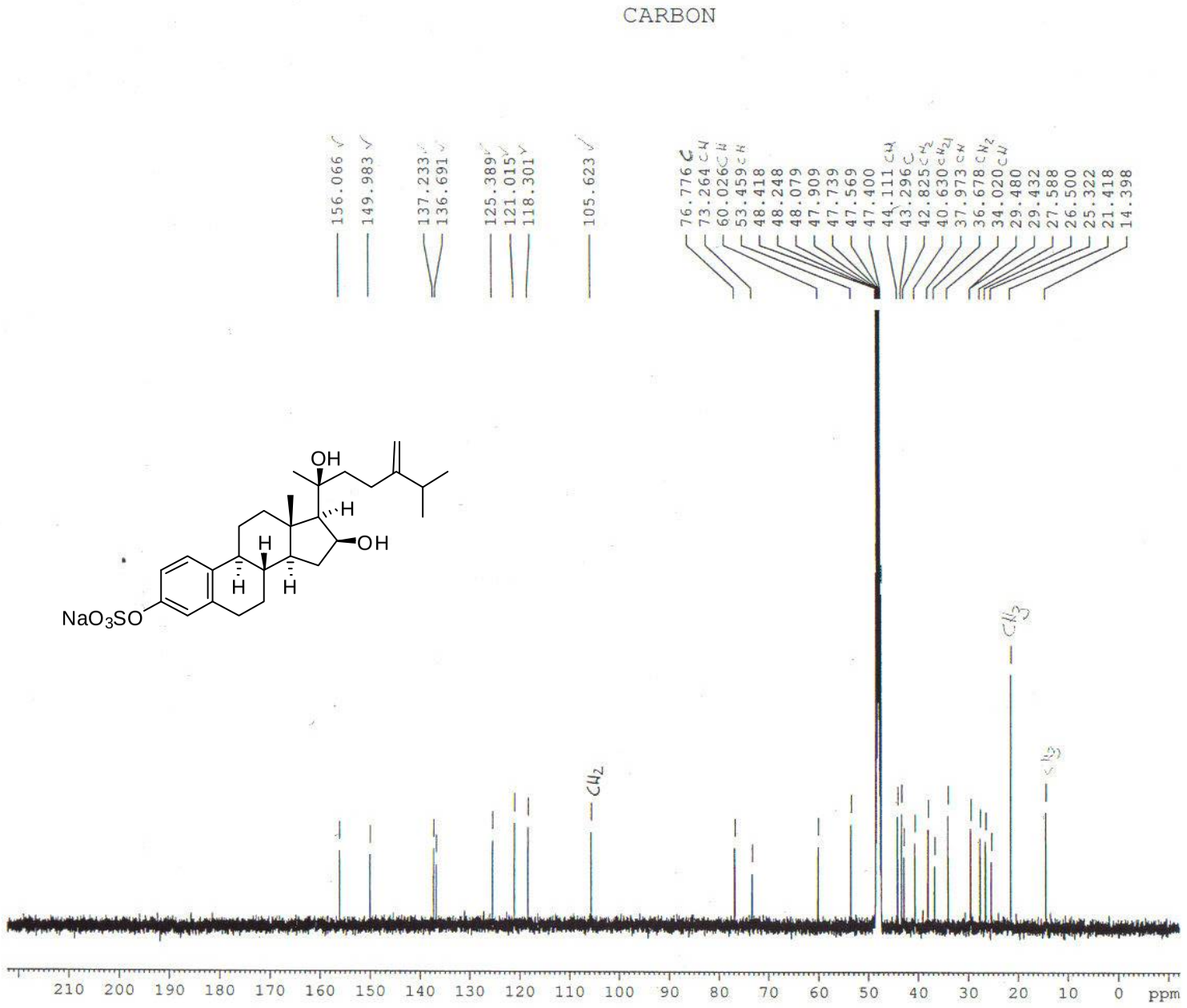


* $\delta_{H\ (methanol\text{-}d4)}$ 3.33; the data in the manuscript is calibrated by using the $\delta_{H\ (methanol\text{-}d4)}$ 3.33.

S14. 500 MHz ^{1}H NMR spectrum of compound 2 in CD_3OD (expansion)

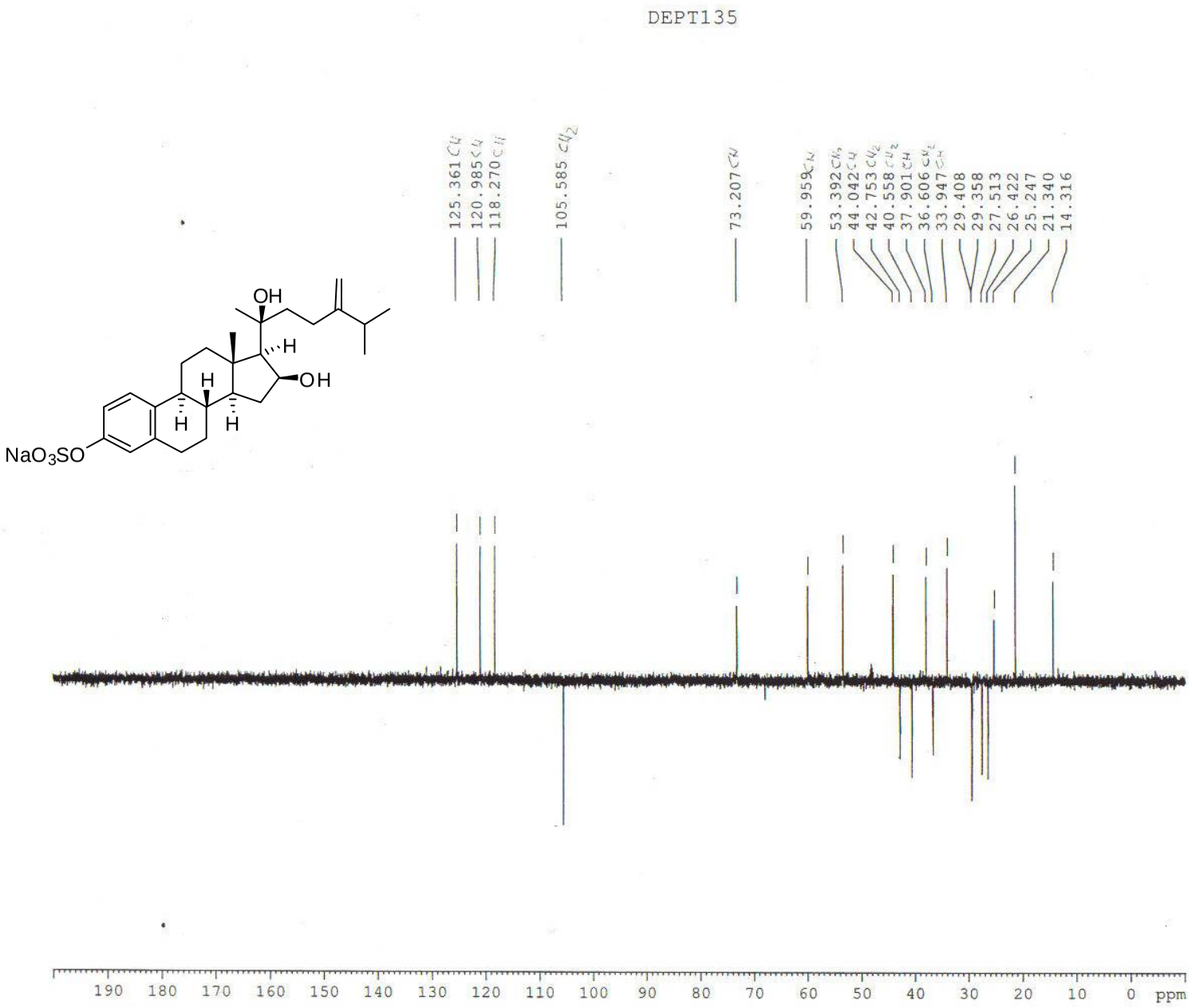


S15. 125 MHz ^{13}C NMR spectrum of compound 2 in methanol-d_4*



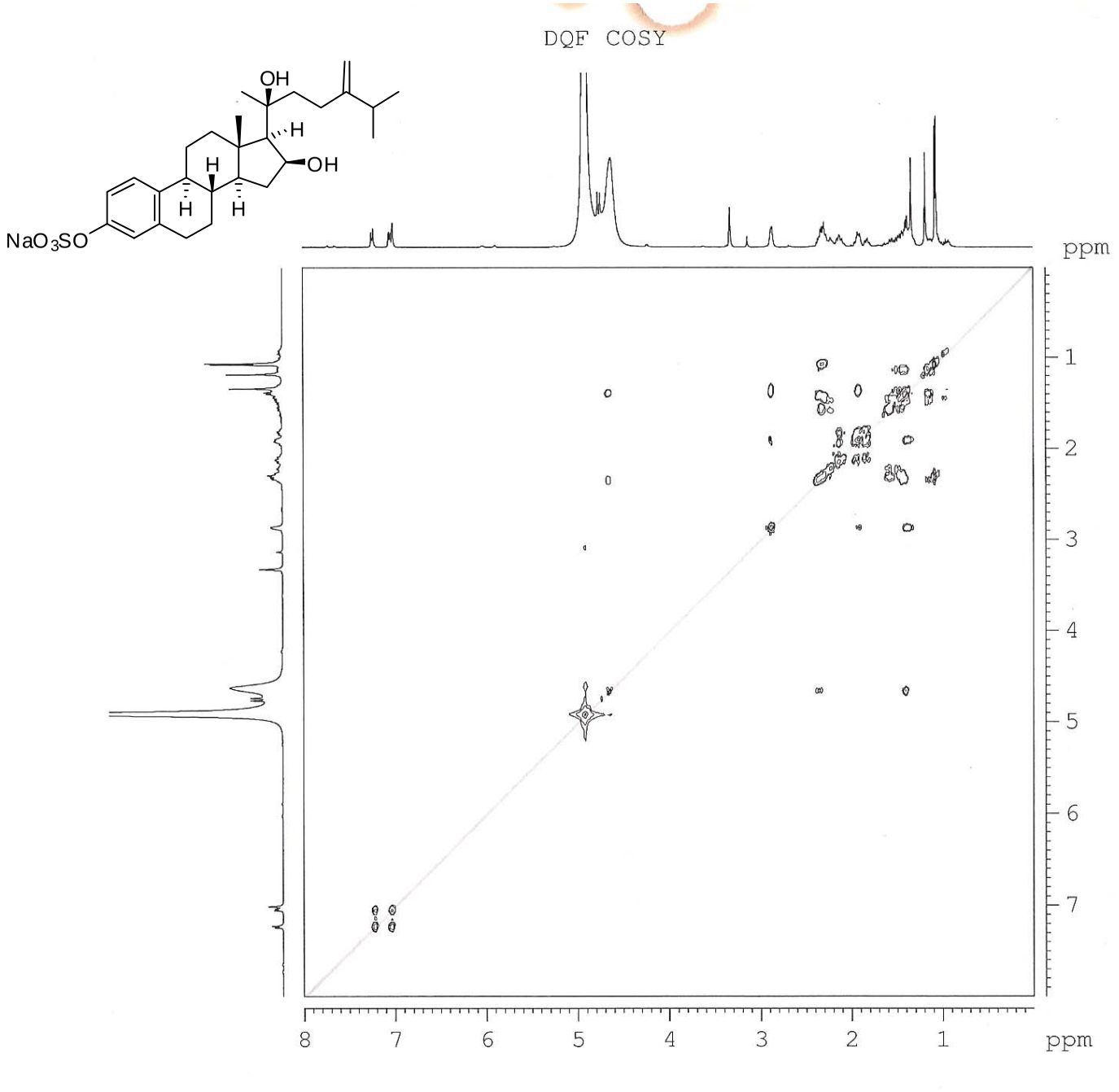

```
Current Data Parameters
NAME        jd-trach-6-7
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20050911
Time               19.25
INSTRUM            spect
PROBHD   5 mm Dual 13C/
PULPROG         zgdc30
TD               65536
SOLVENT           MeOD
NS                5000
DS                   4
SWH          29498.525 Hz
FIDRES        0.450112 Hz
AQ           1.1108851 sec
RG             11585.2
DW              16.950 usec
DE               6.00 usec
TE              300.0 K
D1          3.00000000 sec
d11         0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1                8.00 usec
PL1               3.00 dB
SFO1       125.7709936 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2               1H
PCPD2           100.00 usec
PL2               3.00 dB
PL12             22.00 dB
SFO2       500.1320005 MHz

F2 - Processing parameters
SI                32768
SF         125.7577890 MHz
WDW                EM
SSB                 0
LB                1.00 Hz
GB                  0
PC                1.40
```

S16. 125 MHz DEPT-135 spectrum of compound 2 in methanol-d_4



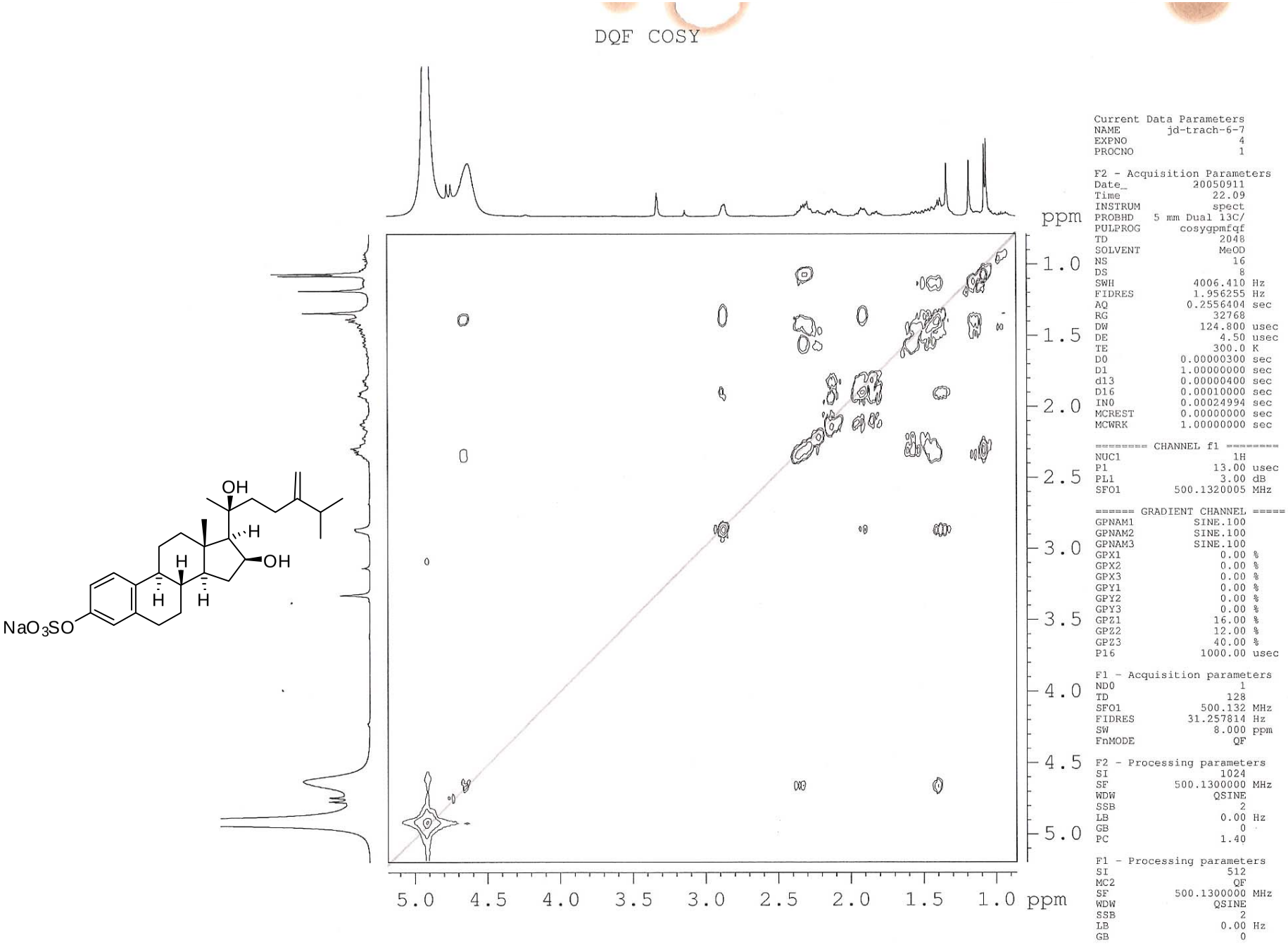

```
Current Data Parameters
NAME          jd-trach-6-7
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_              20050911
Time                  22.08
INSTRUM               spect
PROBHD    5 mm Dual 13C/
PULPROG           dept135
TD                  65536
SOLVENT              MeOD
NS                   3000
DS                      4
SWH            26455.027 Hz
FIDRES          0.403672 Hz
AQ              1.2386804 sec
RG                14596.5
DW                 18.900 usec
DE                  6.00 usec
TE                 300.0 K
CNST2         145.0000000
D1              2.00000000 sec
d2              0.00344828 sec
d12             0.00002000 sec
DELTA           0.00001019 sec

======== CHANNEL f1 ========
NUC1                  13C
P1                   8.00 usec
p2                  16.00 usec
PL1                  3.00 dB
SFO1         125.7697360 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
P3                  13.00 usec
p4                  26.00 usec
PCPD2              100.00 usec
PL2                  3.00 dB
PL12                22.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                   32768
SF           125.7577890 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

S17. 500 MHz COSY spectrum of compound **2** in methanol-d_4



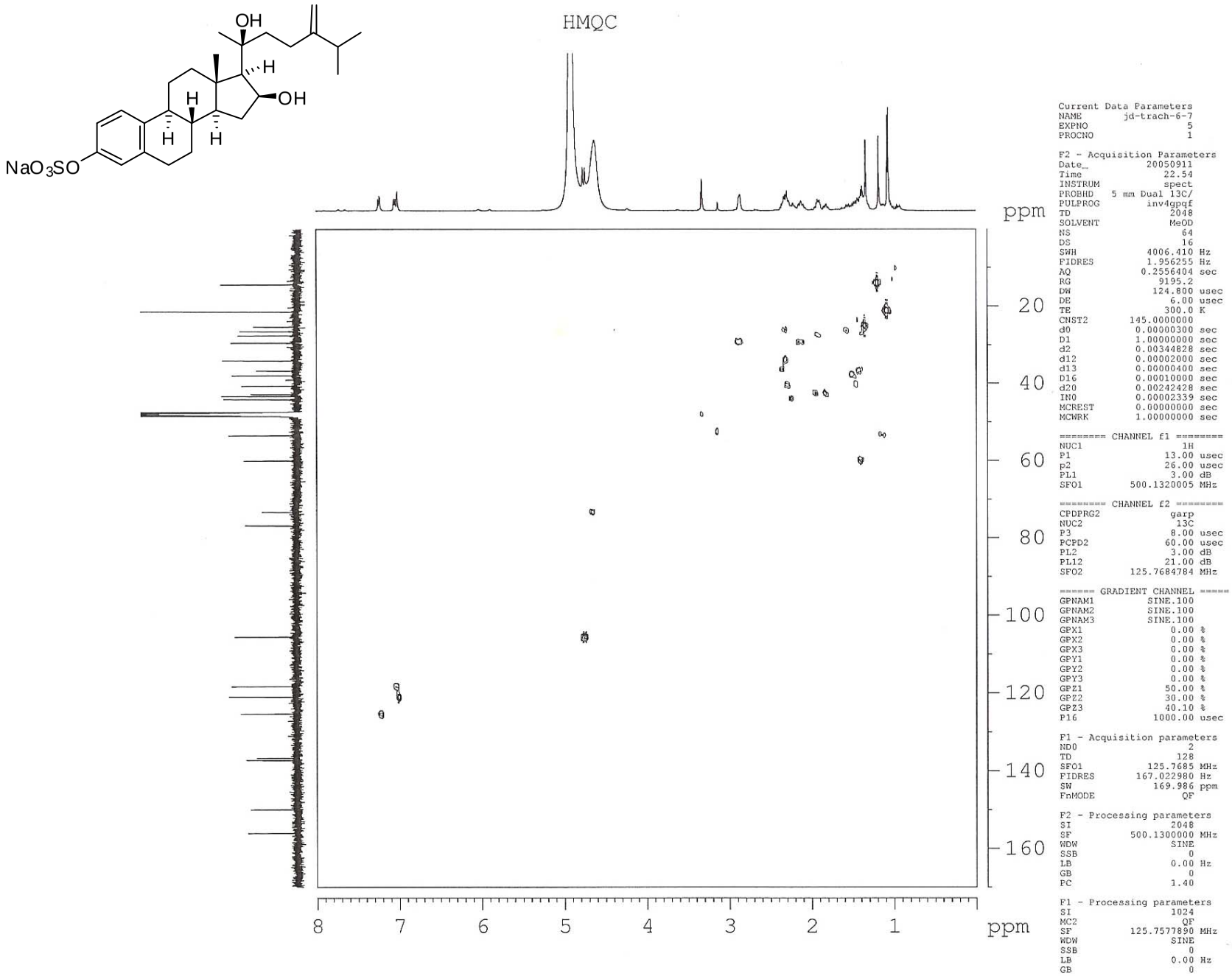

```
Current Data Parameters
NAME        jd-trach-6-7
EXPNO                   4
PROCNO                  1

F2 - Acquisition Parameters
Date_            20050911
Time                22.09
INSTRUM             spect
PROBHD   5 mm Dual 13C/
PULPROG       cosygpmfqf
TD                  2048
SOLVENT              MeOD
NS                     16
DS                      8
SWH              4006.410 Hz
FIDRES           1.956255 Hz
AQ               0.2556404 sec
RG                  32768
DW                124.800 usec
DE                  4.50 usec
TE                 300.0 K
D0           0.00000300 sec
D1           1.00000000 sec
d13          0.00000400 sec
D16          0.00010000 sec
IN0          0.00024994 sec
MCREST       0.00000000 sec
MCWRK        1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 13.00 usec
PL1                 3.00 dB
SFO1         500.1320005 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPNAM3          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPX3                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPY3                0.00 %
GPZ1               16.00 %
GPZ2               12.00 %
GPZ3               40.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                     1
TD                    128
SFO1           500.132 MHz
FIDRES        31.257814 Hz
SW                8.000 ppm
FnMODE                 QF

F2 - Processing parameters
SI                   1024
SF          500.1300000 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                    512
MC2                    QF
SF          500.1300000 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
```

S18. 500 MHz COSY spectrum of compound 2 in methanol-d_4 (expansion)



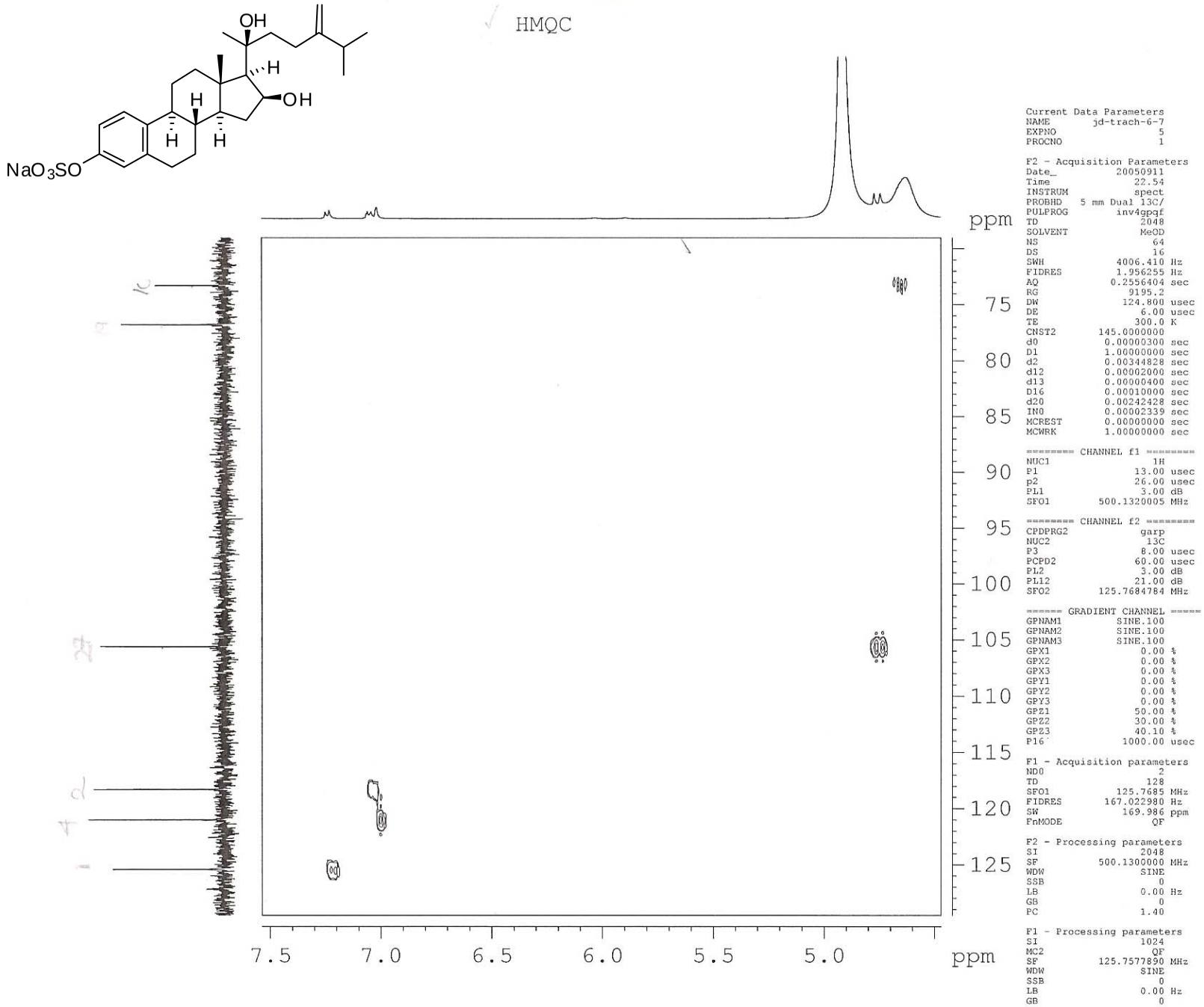

```
Current Data Parameters
NAME        jd-trach-6-7
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20050911
Time               22.09
INSTRUM            spect
PROBHD   5 mm Dual 13C/
PULPROG      cosygpmfqf
TD                 2048
SOLVENT            MeOD
NS                   16
DS                    8
SWH           4006.410 Hz
FIDRES        1.956255 Hz
AQ            0.2556404 sec
RG                32768
DW             124.800 usec
DE               4.50 usec
TE              300.0 K
D0          0.00000300 sec
D1          1.00000000 sec
d13         0.00000400 sec
D16         0.00010000 sec
IN0         0.00024994 sec
MCREST      0.00000000 sec
MCWRK       1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                13.00 usec
PL1                3.00 dB
SFO1        500.1320005 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPNAM3          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPX3                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPY3                0.00 %
GPZ1               16.00 %
GPZ2               12.00 %
GPZ3               40.00 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                    1
TD                   128
SFO1         500.132 MHz
FIDRES      31.257814 Hz
SW           8.000 ppm
FnMODE              QF

F2 - Processing parameters
SI                  1024
SF         500.1300000 MHz
WDW              QSINE
SSB                    2
LB              0.00 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                   512
MC2                   QF
SF         500.1300000 MHz
WDW              QSINE
SSB                    2
LB              0.00 Hz
GB                     0
```

S19. 500 MHz HMQC spectrum of compound 2 in methanol-d_4



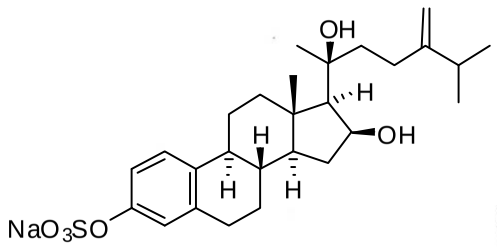

```
Current Data Parameters
NAME            jd-trach-6-7
EXPNO                      5
PROCNO                     1

F2 - Acquisition Parameters
Date_                20050911
Time                    22.54
INSTRUM                 spect
PROBHD   5 mm Dual 13C/
PULPROG             inv4gpqf
TD                      2048
SOLVENT                 MeOD
NS                        64
DS                        16
SWH                 4006.410 Hz
FIDRES              1.956255 Hz
AQ                  0.2556404 sec
RG                     9195.2
DW                   124.800 usec
DE                     6.00 usec
TE                     300.0 K
CNST2              145.0000000
d0                  0.00000300 sec
D1                  1.00000000 sec
d2                  0.00344828 sec
d12                 0.00002000 sec
d13                 0.00000400 sec
D16                 0.00010000 sec
d20                 0.00242428 sec
IN0                 0.00002339 sec
MCREST              0.00000000 sec
MCWRK               1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      13.00 usec
p2                      26.00 usec
PL1                      3.00 dB
SFO1               500.1320005 MHz

======== CHANNEL f2 ========
CPDPRG2                 garp
NUC2                     13C
P3                      8.00 usec
PCPD2                  60.00 usec
PL2                      3.00 dB
PL12                    21.00 dB
SFO2               125.7684784 MHz

====== GRADIENT CHANNEL =====
GPNAM1                SINE.100
GPNAM2                SINE.100
GPNAM3                SINE.100
GPX1                     0.00 %
GPX2                     0.00 %
GPX3                     0.00 %
GPY1                     0.00 %
GPY2                     0.00 %
GPY3                     0.00 %
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
ND0                        2
TD                       128
SFO1               125.7685 MHz
FIDRES             167.022980 Hz
SW                   169.986 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       2048
SF                 500.1300000 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                       QF
SF                 125.7577890 MHz
WDW                      SINE
SSB                         0
LB                       0.00 Hz
GB                          0
```

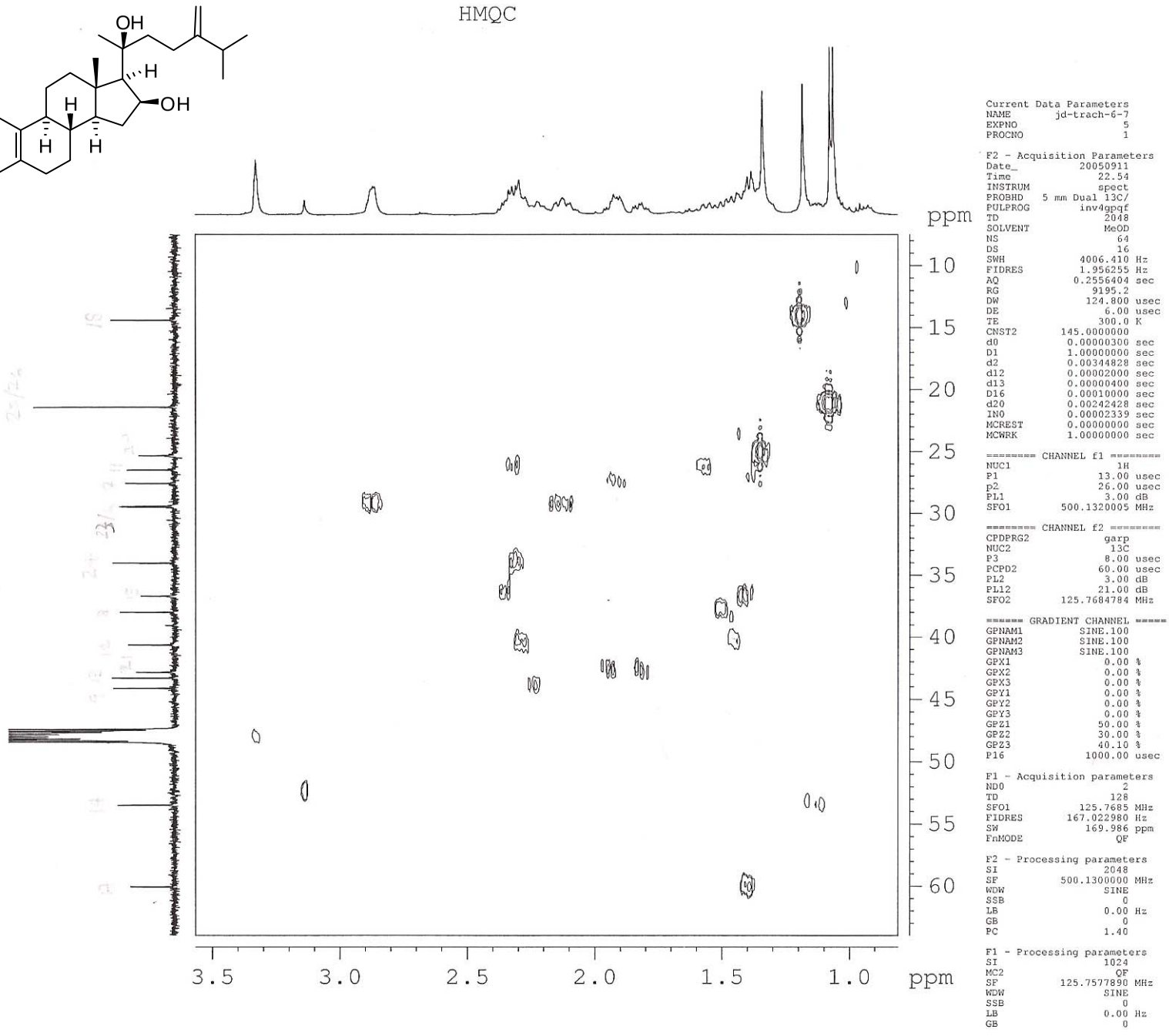
S20. 500 MHz HMQC spectrum of compound 2 in methanol-d_4 (expansion)




```
Current Data Parameters
NAME                  jd-trach-6-7
EXPNO                            5
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20050911
Time                         22.54
INSTRUM                      spect
PROBHD    5 mm Dual 13C/
PULPROG                  inv4gpqf
TD                           2048
SOLVENT                      MeOD
NS                             64
DS                             16
SWH                    4006.410 Hz
FIDRES                 1.956255 Hz
AQ                     0.2556404 sec
RG                        9195.2
DW                      124.800 usec
DE                        6.00 usec
TE                       300.0 K
CNST2                145.0000000
d0                     0.00000300 sec
D1                     1.00000000 sec
d2                     0.00344828 sec
d12                    0.00002000 sec
d13                    0.00000400 sec
D16                    0.00010000 sec
d20                    0.00242428 sec
IN0                    0.00002339 sec
MCREST                 0.00000000 sec
MCWRK                  1.00000000 sec

======== CHANNEL f1 ========
NUC1                         1H
P1                       13.00 usec
p2                       26.00 usec
PL1                       3.00 dB
SFO1                500.1320005 MHz

======== CHANNEL f2 ========
CPDPRG2                   garp
NUC2                        13C
P3                        8.00 usec
PCPD2                    60.00 usec
PL2                       3.00 dB
PL12                     21.00 dB
SFO2                125.7684784 MHz

====== GRADIENT CHANNEL =====
GPNAM1                SINE.100
GPNAM2                SINE.100
GPNAM3                SINE.100
GPX1                      0.00 %
GPX2                      0.00 %
GPX3                      0.00 %
GPY1                      0.00 %
GPY2                      0.00 %
GPY3                      0.00 %
GPZ1                     50.00 %
GPZ2                     30.00 %
GPZ3                     40.10 %
P16                    1000.00 usec

F1 - Acquisition parameters
ND0                           2
TD                          128
SFO1                125.7685 MHz
FIDRES              167.022980 Hz
SW                     169.986 ppm
FnMODE                      QF

F2 - Processing parameters
SI                         2048
SF                  500.1300000 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0
PC                         1.40

F1 - Processing parameters
SI                         1024
MC2                          QF
SF                  125.7577890 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0
```

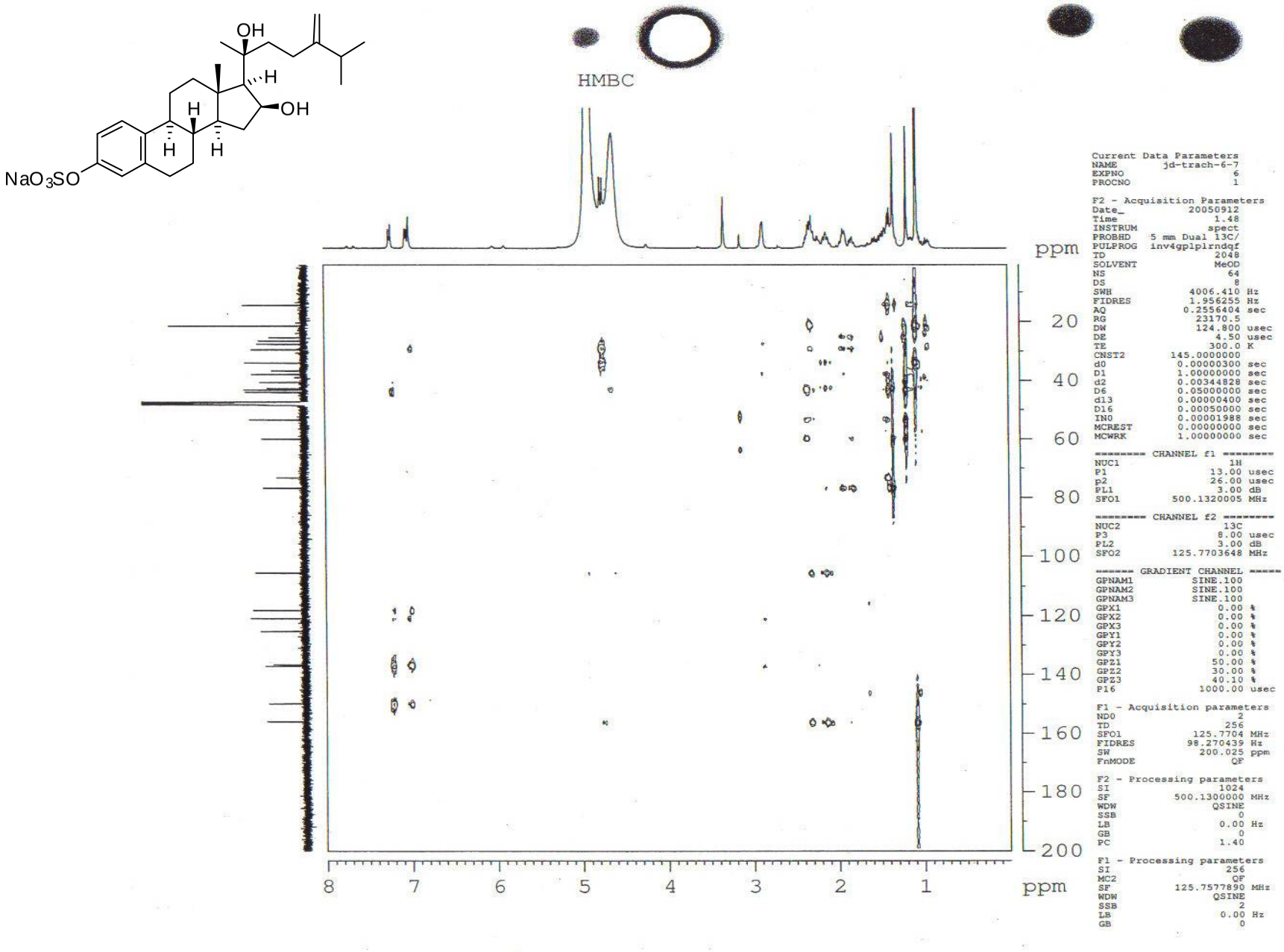
S21. 500 MHz HMQC spectrum of compound 2 in methanol-d_4 (expansion)



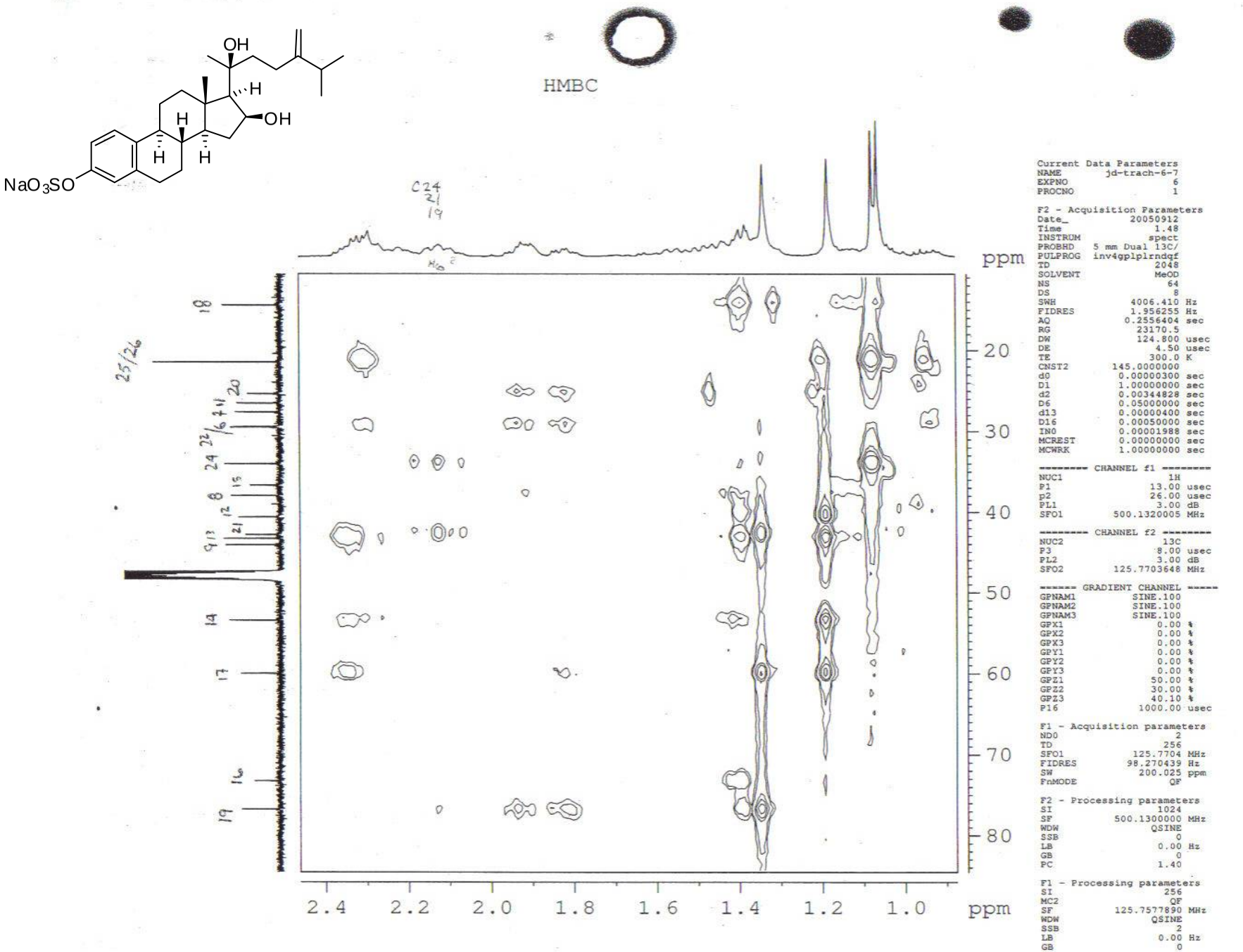



```
Current Data Parameters
NAME          jd-trach-6-7
EXPNO                    5
PROCNO                   1

F2 - Acquisition Parameters
Date_              20050911
Time                 22.54
INSTRUM              spect
PROBHD   5 mm Dual 13C/
PULPROG          inv4gpqf
TD                   2048
SOLVENT              MeOD
NS                     64
DS                     16
SWH             4006.410 Hz
FIDRES          1.956255 Hz
AQ              0.2556404 sec
RG                 9195.2
DW                124.800 usec
DE                  6.00 usec
TE                300.0 K
CNST2         145.0000000
d0              0.00000300 sec
D1              1.00000000 sec
d2              0.00344828 sec
d12             0.00002000 sec
d13             0.00000400 sec
D16             0.00010000 sec
d20             0.00242428 sec
IN0             0.00002339 sec
MCREST          0.00000000 sec
MCWRK           1.00000000 sec

========= CHANNEL f1 =========
NUC1                   1H
P1                  13.00 usec
p2                  26.00 usec
PL1                  3.00 dB
SFO1          500.1320005 MHz

========= CHANNEL f2 =========
CPDPRG2              garp
NUC2                  13C
P3                  8.00 usec
PCPD2              60.00 usec
PL2                  3.00 dB
PL12                21.00 dB
SFO2          125.7684784 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPNAM3          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPX3                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPY3                0.00 %
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
ND0                    2
TD                   128
SFO1          125.7685 MHz
FIDRES        167.022980 Hz
SW               169.986 ppm
FnMODE                QF

F2 - Processing parameters
SI                   2048
SF          500.1300000 MHz
WDW                  SINE
SSB                     0
LB                 0.00 Hz
GB                      0
PC                 1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF          125.7577890 MHz
WDW                  SINE
SSB                     0
LB                 0.00 Hz
GB                      0
```

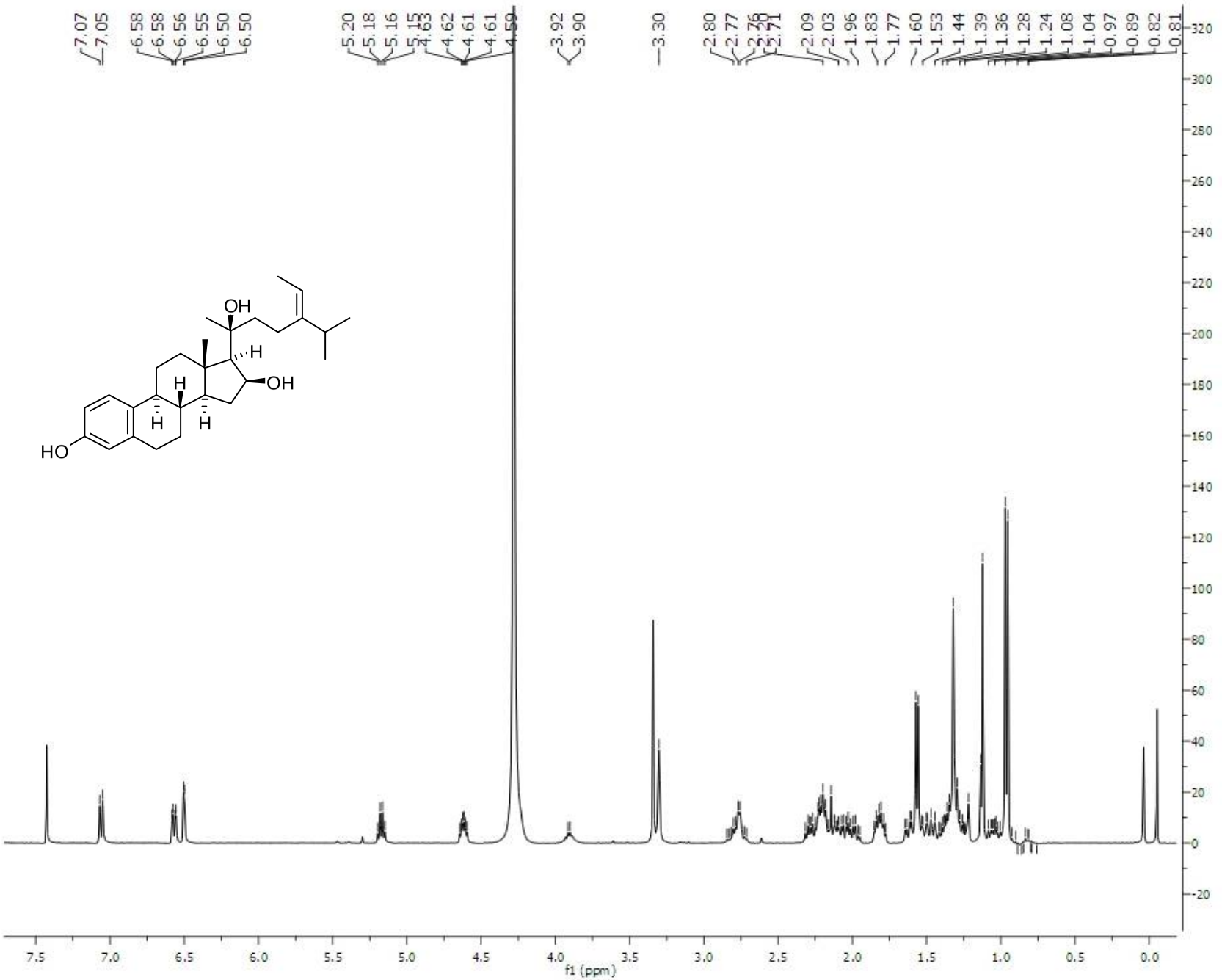
S22. 500 MHz HMBC spectrum of compound 2 in methanol-d_4



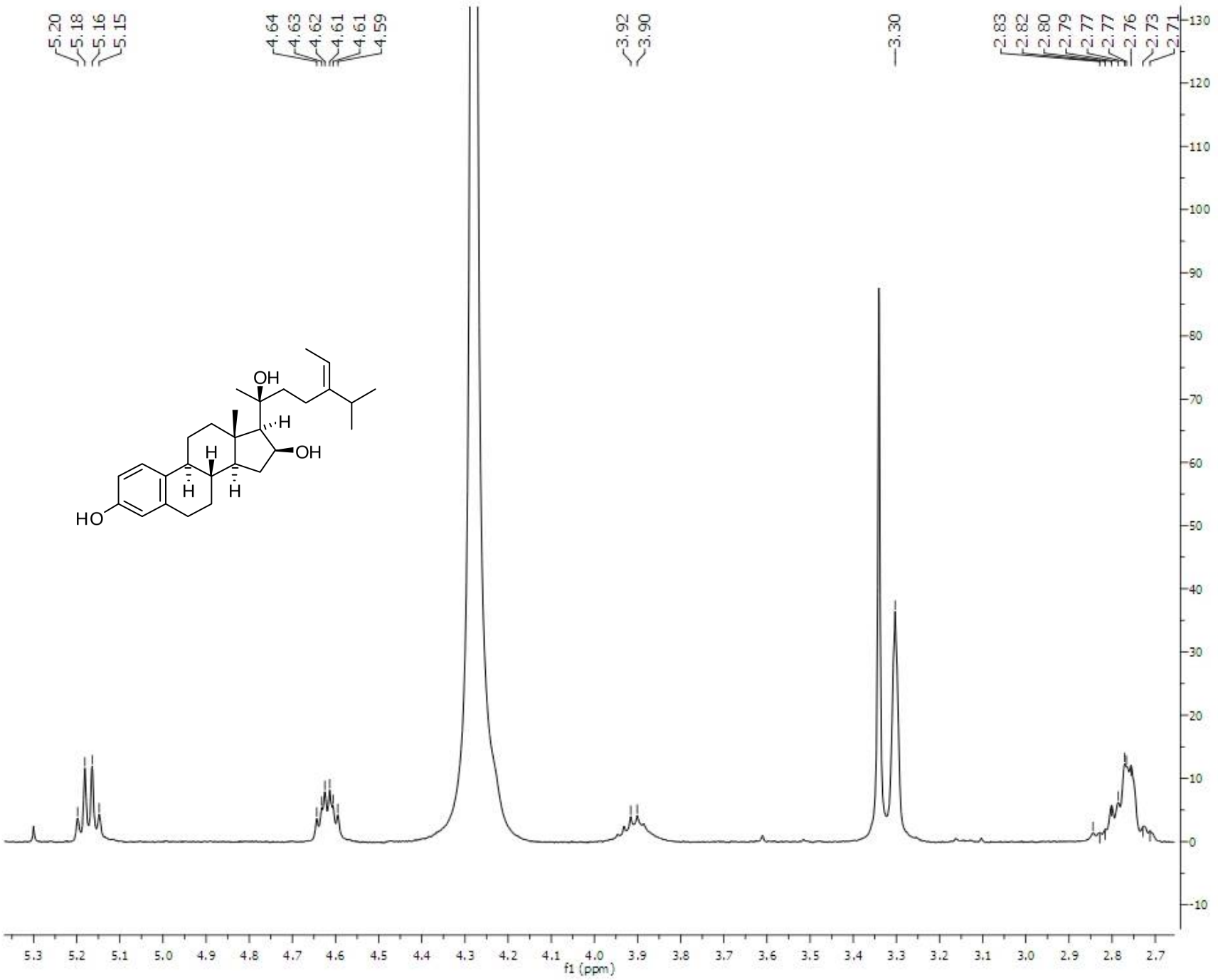
S23. 500 MHz HMBC spectrum of compound 2 in methanol-d_4 (expansion)



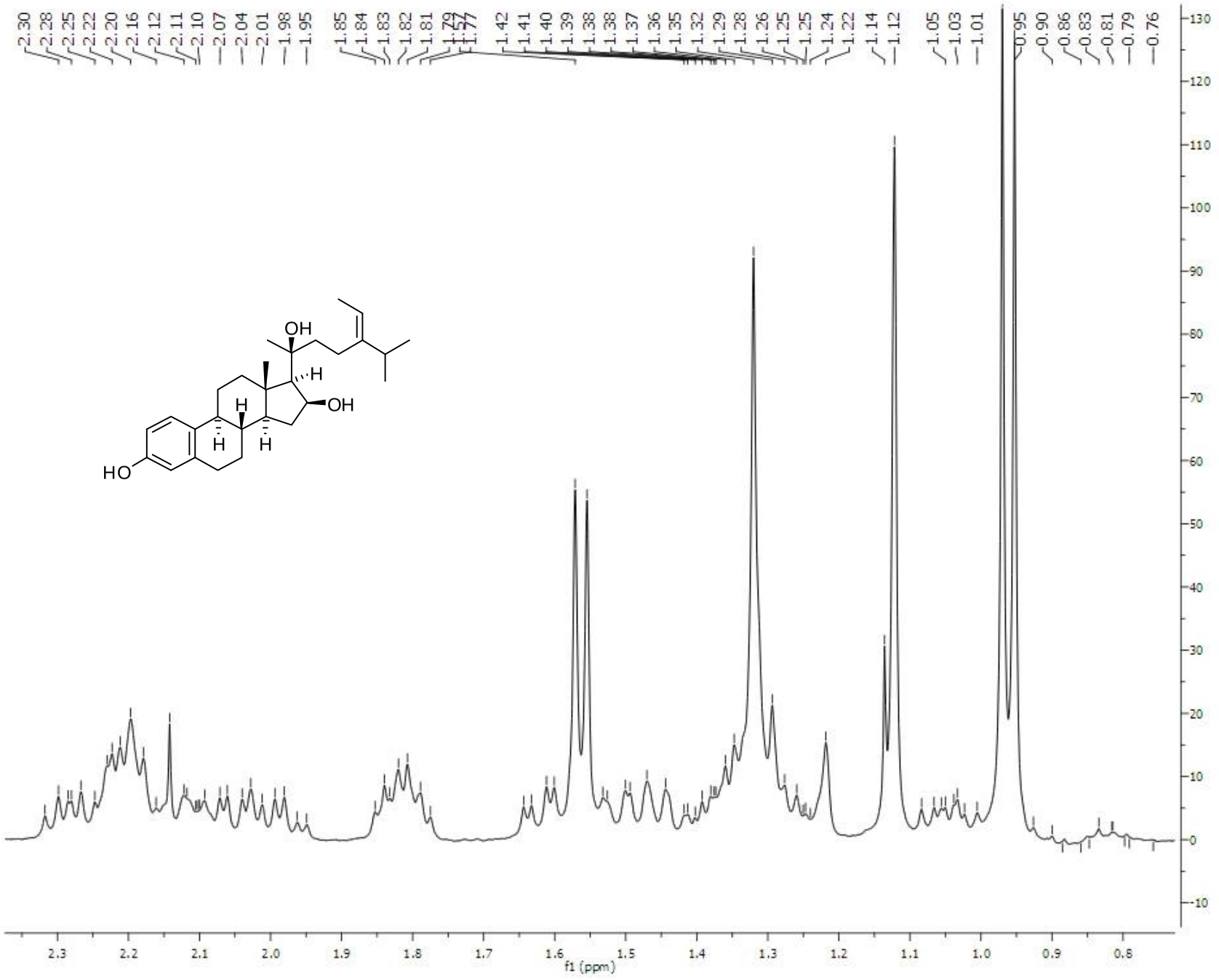
S24. 400 MHz ^{1}H NMR spectrum of compound **3** in 2:1 $CDCl_3$:methanol-d_4



S25. 400 MHz ^{1}H NMR spectrum of compound **3** in 2:1 $CDCl_3$:methanol-d_4 (expansion)



S26. 400 MHz ^{1}H NMR spectrum of compound 3 in 2:1 $CDCl_3$:methanol-d_4 (expansion)



S27. 100 MHz ^{13}C NMR spectrum of compound **3** in 2:1 $CDCl_3$:methanol-d_4