Supplementary Materials

3-Ketone-4,6-diene ceramide analogs exclusively induce apoptosis in chemo-resistant cancer cells

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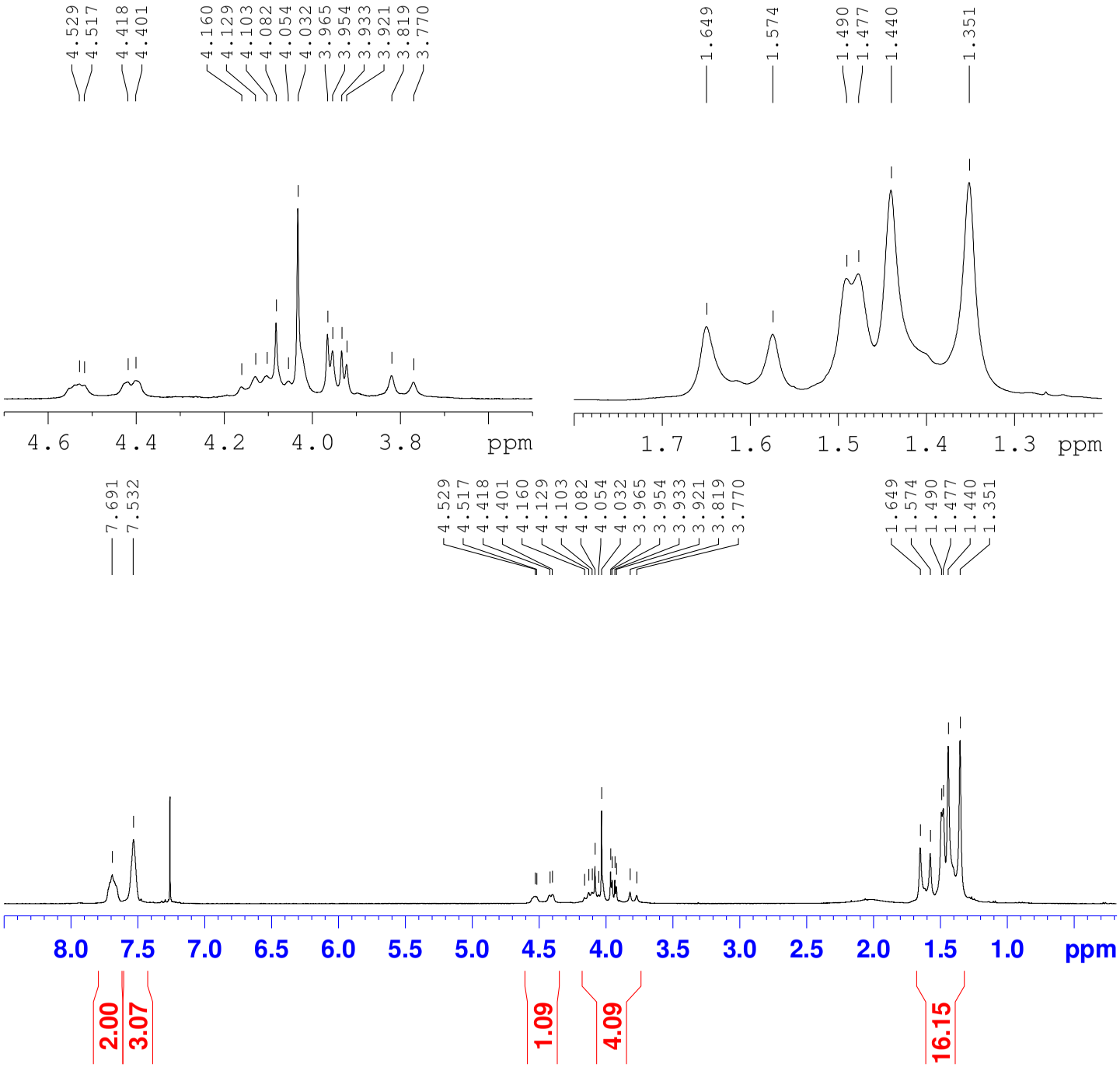
Contents

3_up



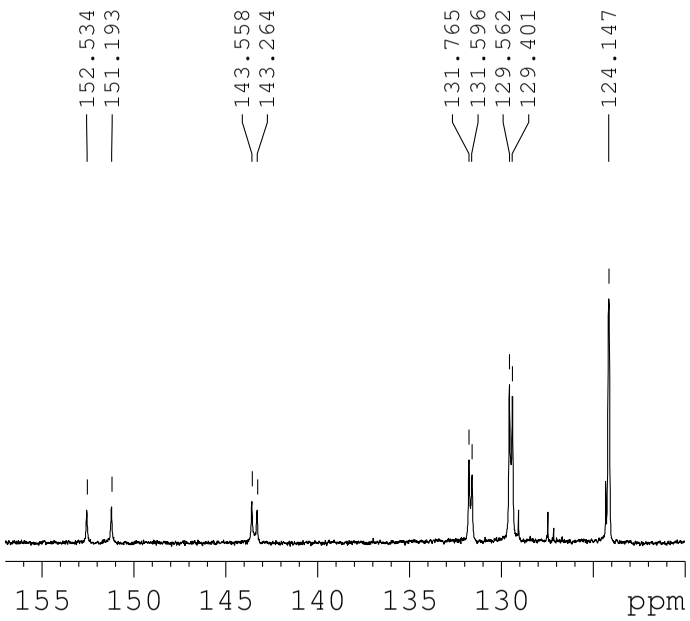
Current Data Parameters
NAME 3_up
EXPNO 1
PROCNO 1

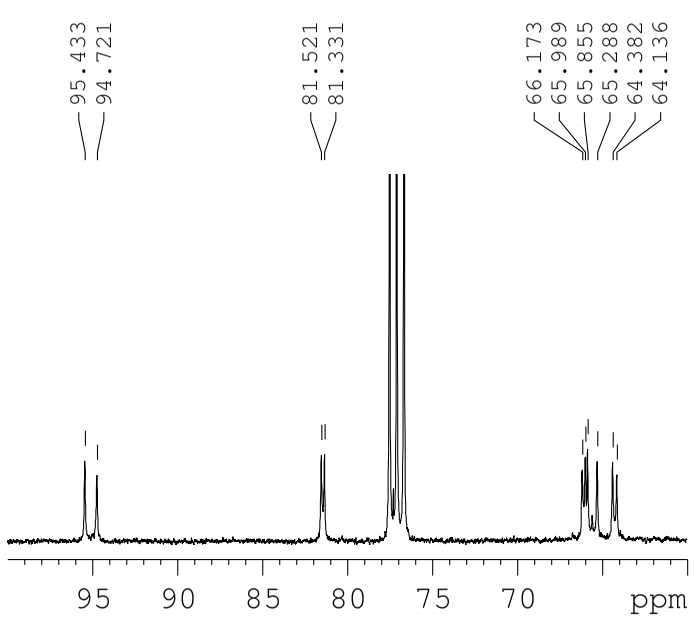
F2 - Acquisition Parameters
Date_ 20120411
Time 17.54
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6103.516 Hz
FIDRES 0.093132 Hz
AQ 5.3687091 sec
RG 33.7582
DW 81.920 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1

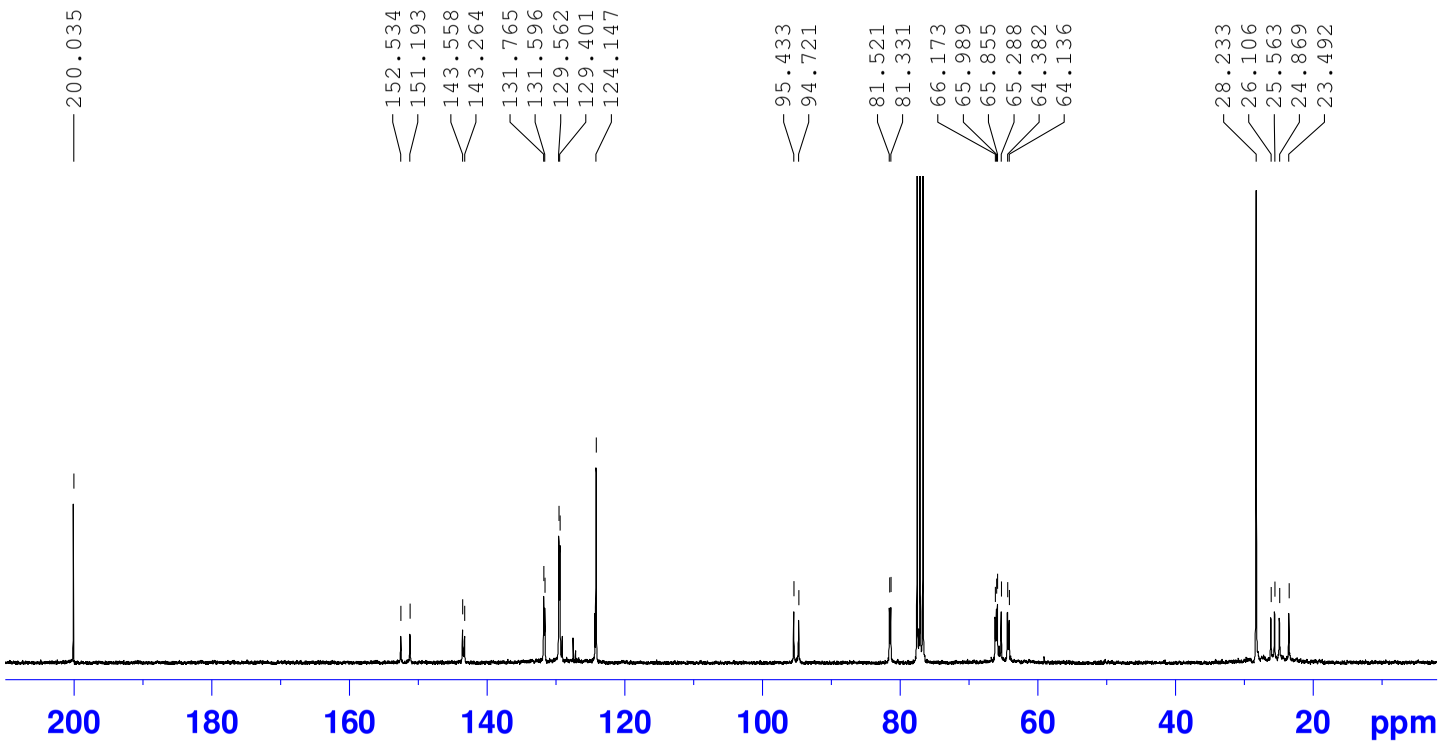
======== CHANNEL f1 ========
SFO1 300.1818537 MHz
NUC1 1H
P1 8.75 usec
PLW1 25.00000000 W

F2 - Processing parameters
SI 65536
SF 300.1800050 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

3_up





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Current Data Parameters
NAME                   3_up
EXPNO                     2
PROCNO                    1

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PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT             CDCl3
NS                  20000
DS                      4
SWH            24414.063 Hz
FIDRES             0.372529 Hz
AQ              1.3421773 sec
RG                501.187
DW                 20.480 usec
DE                  6.50 usec
TE                296.7 K
D1             2.00000000 sec
D11            0.03000000 sec
D31            0.00001600 sec
D40            0.02432300 sec
L4                     34
L5                     49
P32                105.00 usec
TD0                     1

======== CHANNEL f1 ========
SFO1          75.4878687 MHz
NUC1                 13C
P1                 16.00 usec
PLW1         60.00000000 W

======== CHANNEL f2 ========
SFO2         300.1812007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              105.00 usec
PLW2         10.00000000 W
PLW12         0.36280999 W
PLW13         0.40000001 W

F2 - Processing parameters
SI                  32768
SF            75.4803210 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
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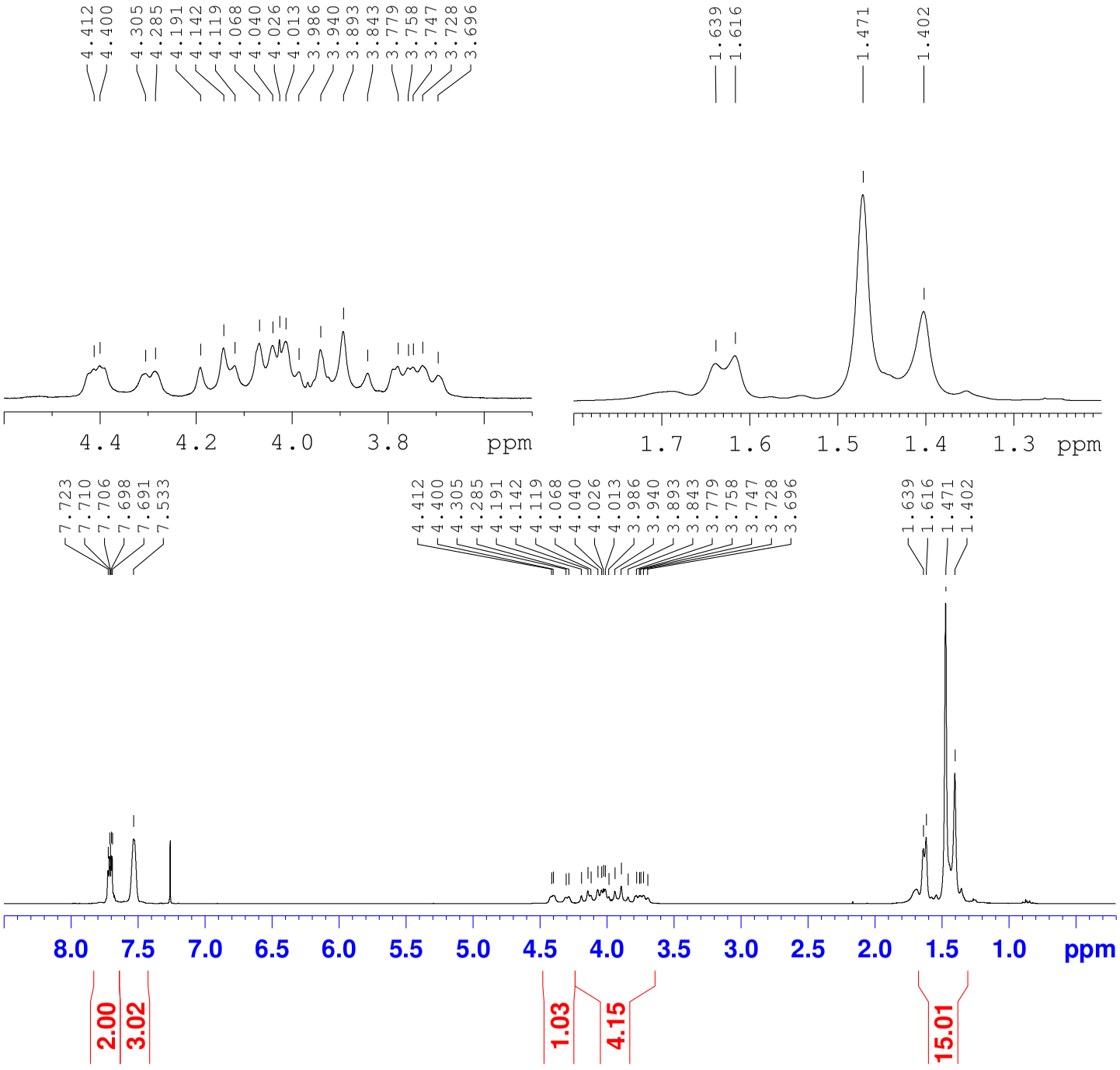
3_down



Current Data Parameters

NAME	3_down
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20120411
Time	15.28
INSTRUM	FOURIER300
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	32
DS	2
SWH	6103.516 Hz
FIDRES	0.093132 Hz
AQ	5.3687091 sec
RG	32
DW	81.920 usec
DE	6.50 usec
TE	297.1 K
D1	1.00000000 sec
TD0	1

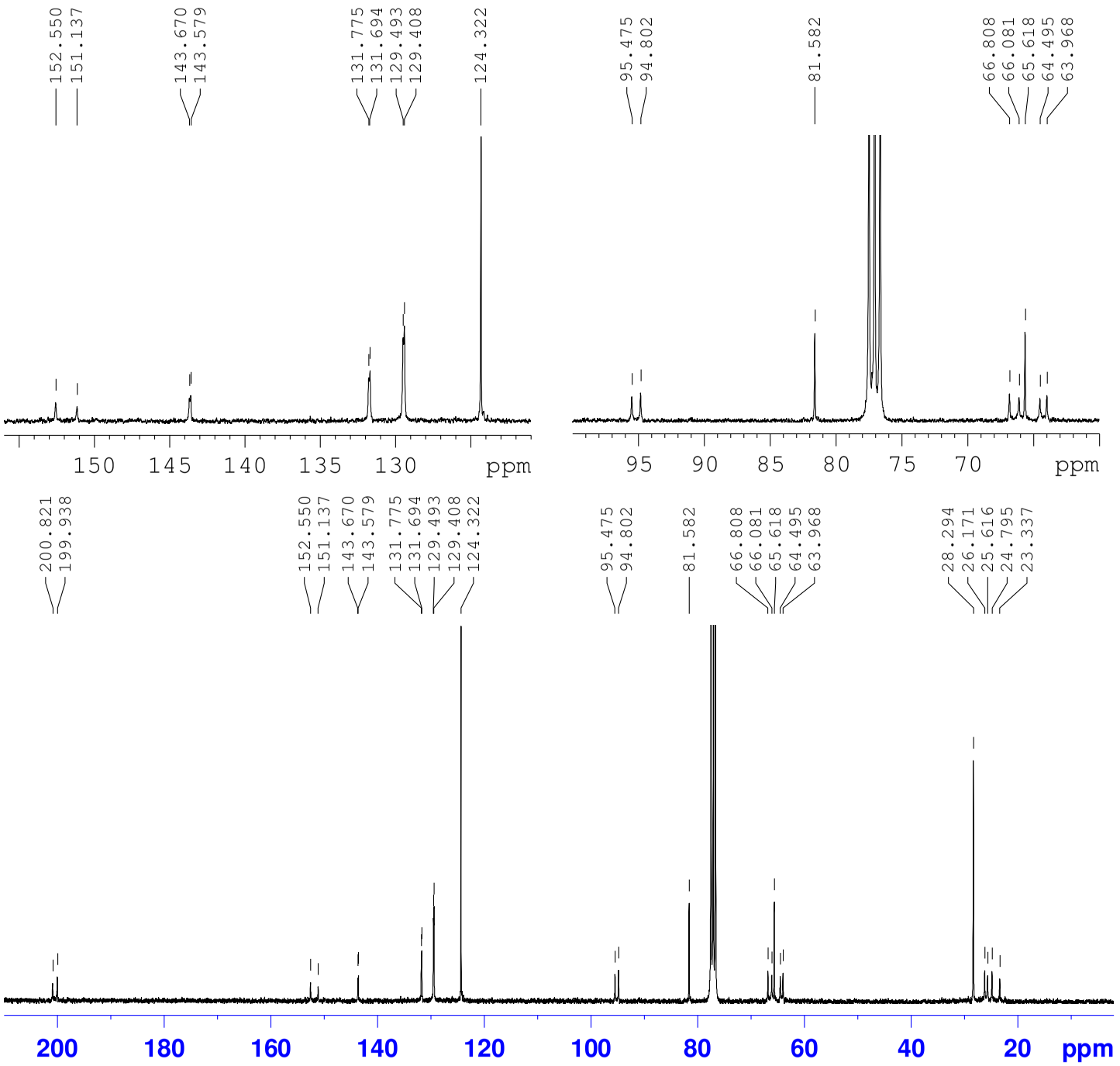
======== CHANNEL f1 ========

SFO1	300.1818537 MHz
NUC1	1H
P1	8.75 usec
PLW1	25.00000000 W

F2 - Processing parameters

SI	65536
SF	300.1800049 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

3_down



Current Data Parameters

NAME	3_down
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20120413	
Time	15.44	
INSTRUM	FOURIER300	
PROBHD	5 mm DUL 13C-1	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	4375	
DS	4	
SWH	24414.063	Hz
FIDRES	0.372529	Hz
AQ	1.3421773	sec
RG	501.187	
DW	20.480	usec
DE	6.50	usec
TE	297.3	K
D1	2.00000000	sec
D11	0.03000000	sec
D31	0.00001600	sec
D40	0.02432300	sec
L4	34	
L5	49	
P32	105.00	usec
TD0	1	

======== CHANNEL f1 ========

SFO1	75.4878687	MHz
NUC1	13C	
P1	16.00	usec
PLW1	60.00000000	W

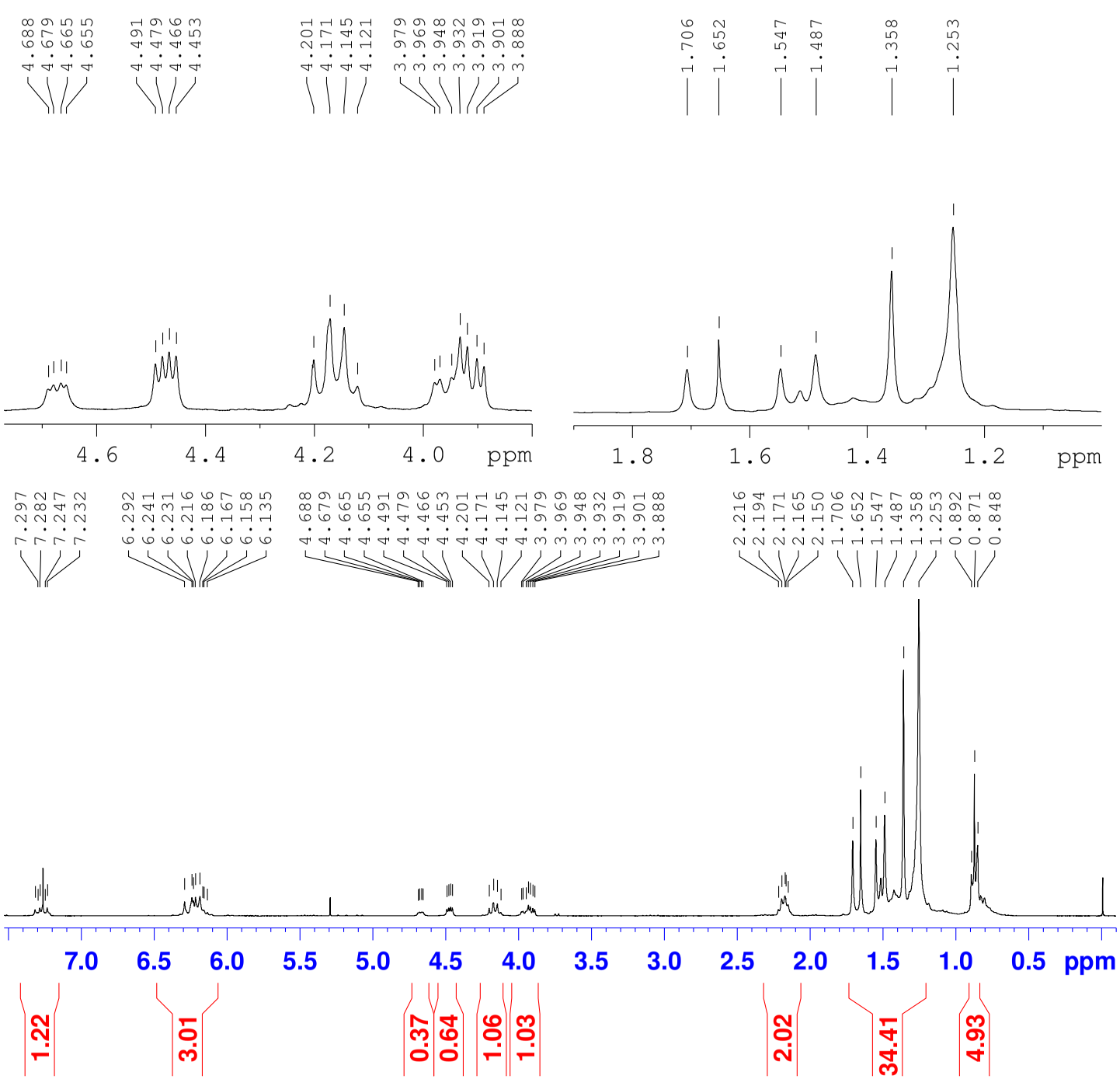
======== CHANNEL f2 ========

SFO2	300.1812007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	105.00	usec
PLW2	10.00000000	W
PLW12	0.36280999	W
PLW13	0.40000001	W

F2 - Processing parameters

SI	32768	
SF	75.4803210	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

401

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NAME                  401
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
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INSTRUM       FOURIER300
PROBHD   5 mm DUL 13C-1
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              6103.516 Hz
FIDRES           0.093132 Hz
AQ               5.3687091 sec
RG                 17.8865
DW                 81.920 usec
DE                  6.50 usec
TE                 296.3 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          300.1818537 MHz
NUC1                    1H
P1                   8.75 usec
PLW1          25.00000000 W

F2 - Processing parameters
SI                  65536
SF            300.1800050 MHz
WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
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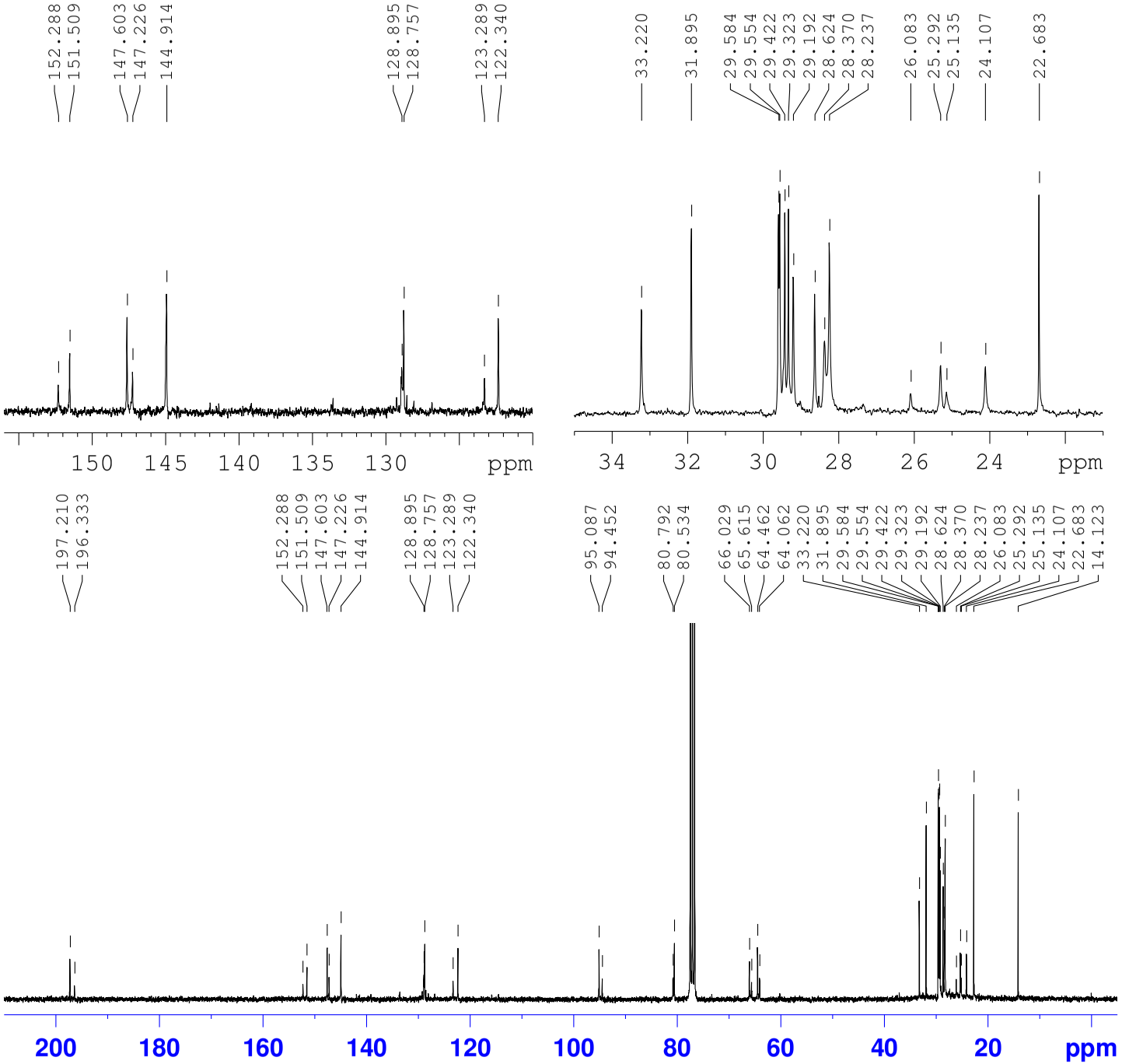
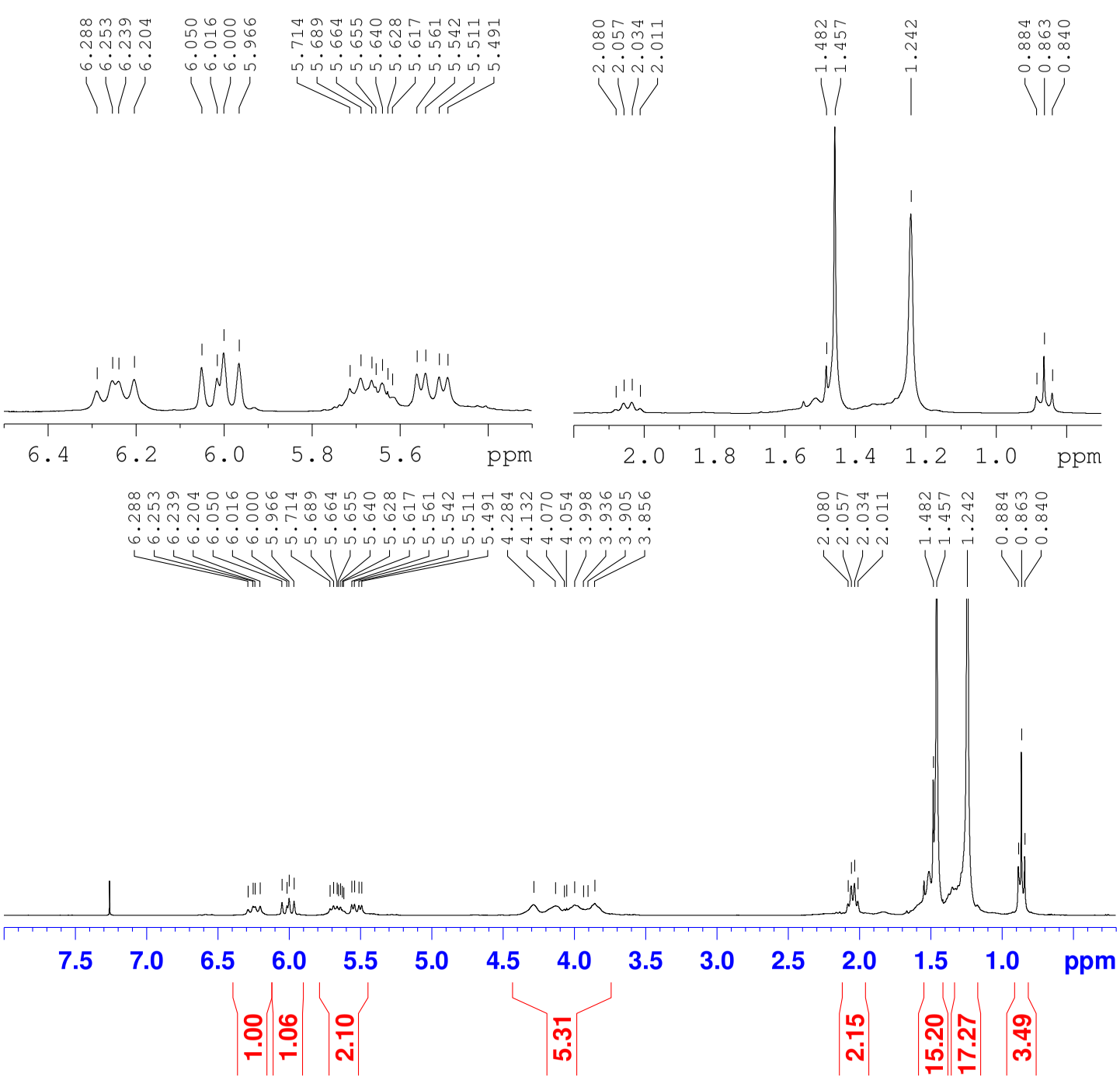
401



Current Data Parameters

NAME	401	
EXPNO	2	
PROCNO	1	
F2 - Acquisition Parameters		
Date_	20120423	
Time	18.08	
INSTRUM	FOURIER300	
PROBHD	5 mm DUL 13C-1	
PULPROG	zgpg30	
TD	65536	
SOLVENT	CDCl3	
NS	16000	
DS	4	
SWH	24414.063	Hz
FIDRES	0.372529	Hz
AQ	1.3421773	sec
RG	501.187	
DW	20.480	usec
DE	6.50	usec
TE	296.5	K
D1	2.00000000	sec
D11	0.03000000	sec
D31	0.00001600	sec
D40	0.02432300	sec
L4	34	
L5	49	
P32	105.00	usec
TD0	1	
======== CHANNEL f1 ========		
SFO1	75.4878687	MHz
NUC1	13C	
P1	16.00	usec
PLW1	60.00000000	W
======== CHANNEL f2 ========		
SFO2	300.1812007	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	105.00	usec
PLW2	10.00000000	W
PLW12	0.36280999	W
PLW13	0.40000001	W
F2 - Processing parameters		
SI	32768	
SF	75.4803210	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

402



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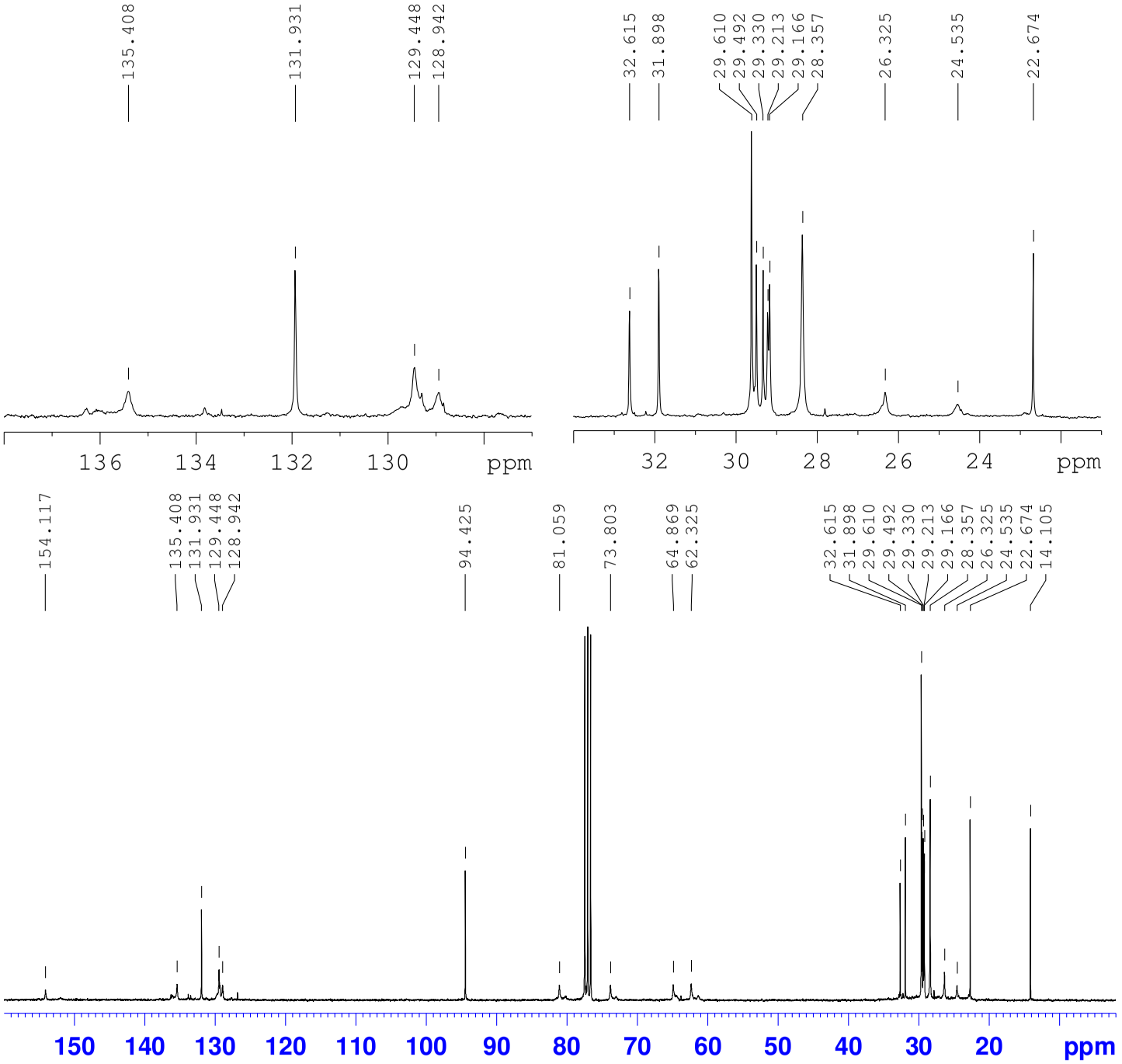
NAME 402
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20120512
Time 19.15
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 32
DS 2
SWH 6103.516 Hz
FIDRES 0.093132 Hz
AQ 5.3687091 sec
RG 7.24528
DW 81.920 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 300.1818537 MHz
NUC1 1H
P1 8.75 usec
PLW1 25.00000000 W

F2 - Processing parameters
SI 65536
SF 300.1800049 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

402



Current Data Parameters
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EXPNO 2
PROCNO 1

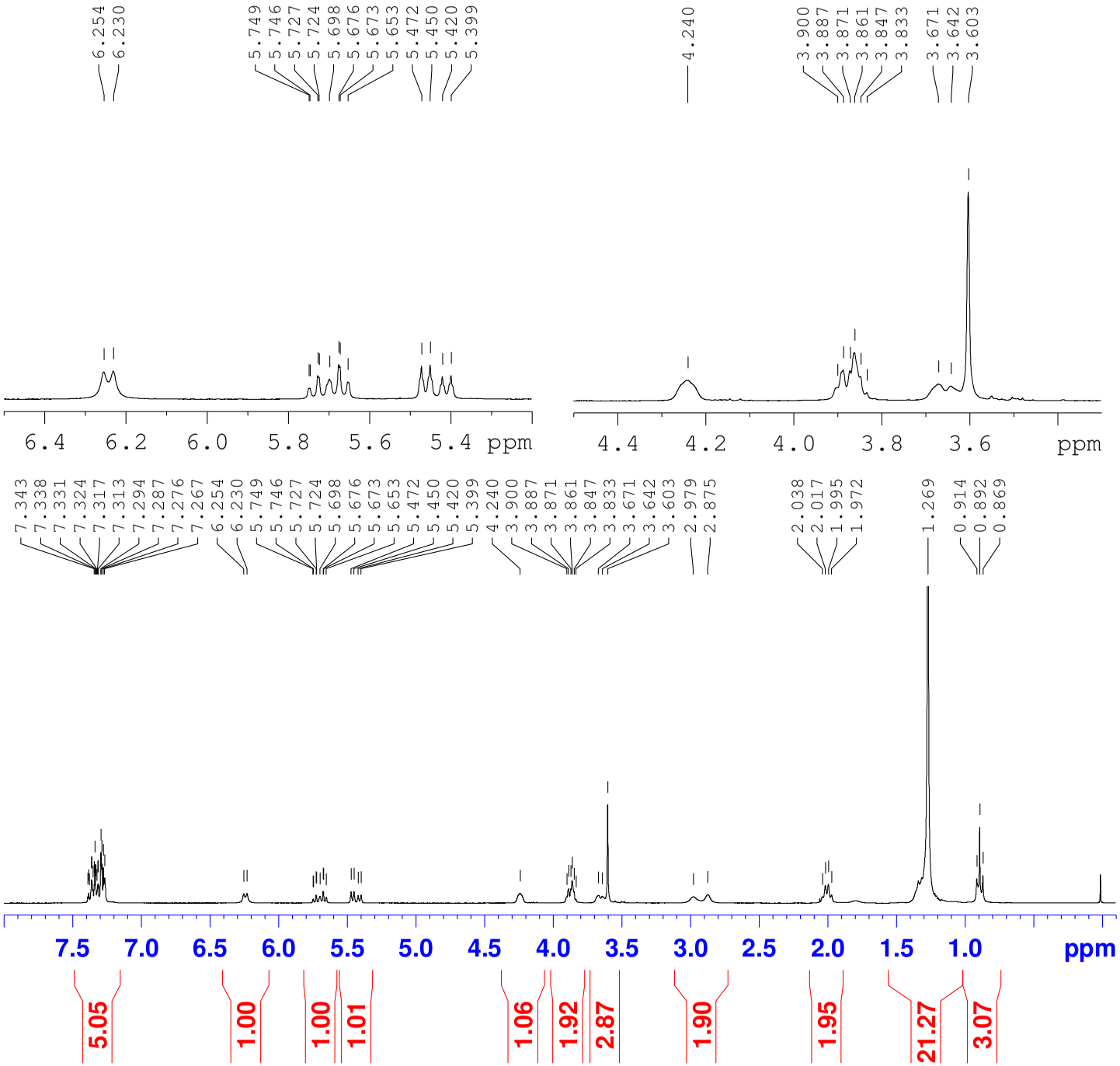
F2 - Acquisition Parameters
Date_ 20120515
Time 19.21
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg30
TD 65536
SOLVENT CDC13
NS 15000
DS 4
SWH 24414.063 Hz
FIDRES 0.372529 Hz
AQ 1.3421773 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D31 0.00001600 sec
D40 0.02432300 sec
L4 34
L5 49
P32 105.00 usec
TD0 1

======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 16.00 usec
PLW1 60.00000000 W

======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 105.00 usec
PLW2 10.00000000 W
PLW12 0.36280999 W
PLW13 0.40000001 W

F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

403

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Current Data Parameters
NAME                  403
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
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Time                14.05
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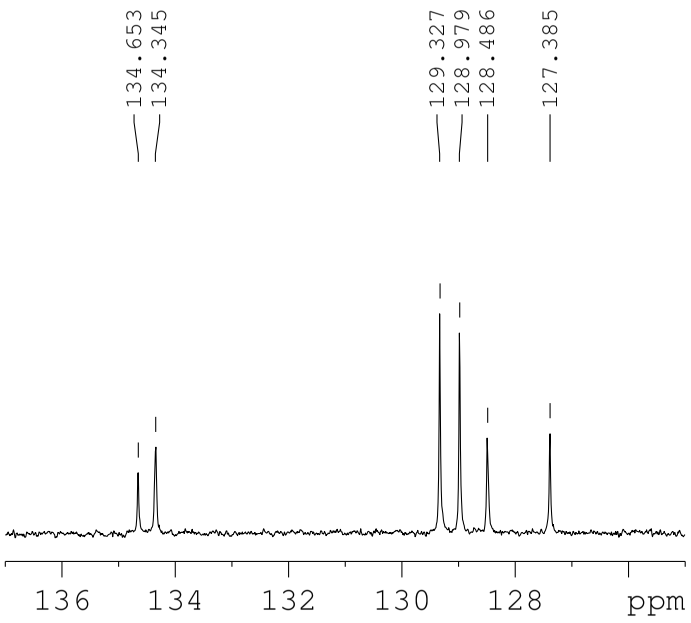
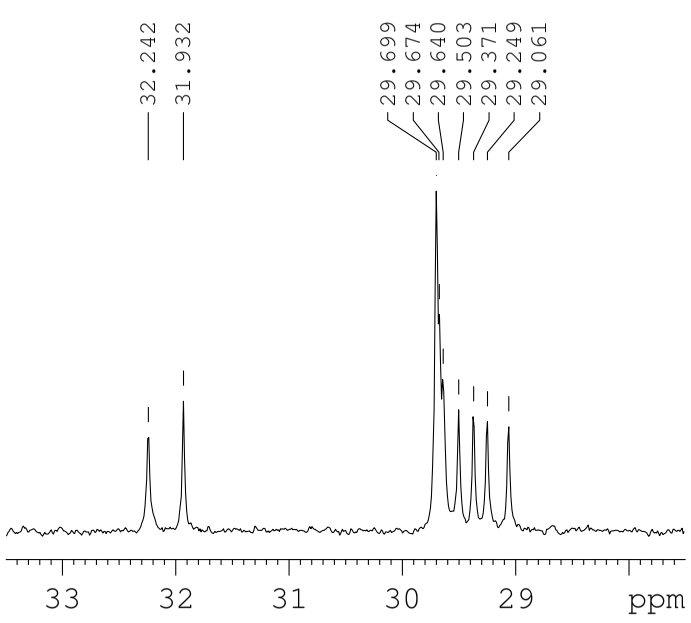
PROBHD    5 mm DUL 13C-1
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              6103.516 Hz
FIDRES           0.093132 Hz
AQ               5.3687091 sec
RG                 22.5468
DW                 81.920 usec
DE                  6.50 usec
TE                 296.4 K
D1              1.00000000 sec
TD0                     1

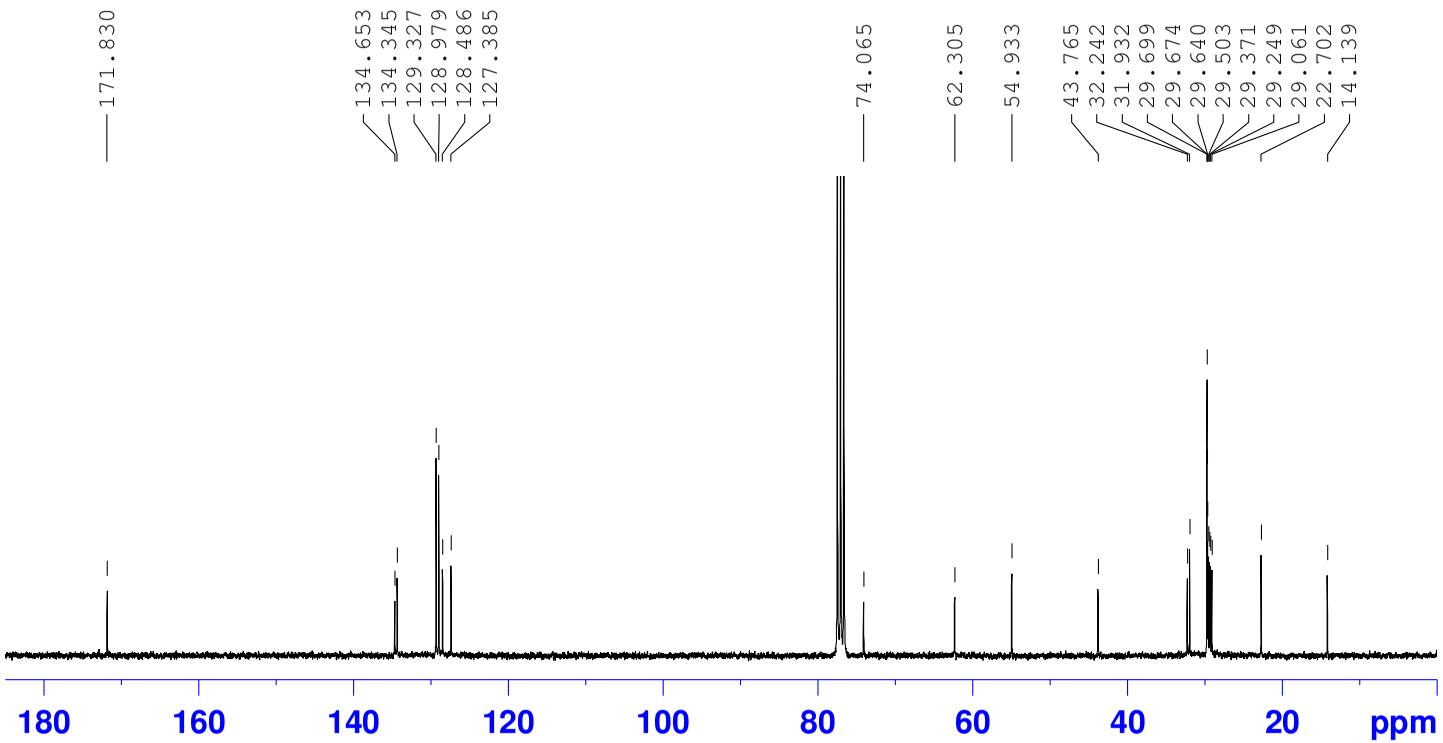
======== CHANNEL f1 ========
SFO1          300.1818537 MHz
NUC1                    1H
P1                   8.75 usec
PLW1          25.00000000 W

F2 - Processing parameters
SI                  65536
SF            300.1800000 MHz
WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

403





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Current Data Parameters
NAME                  403
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
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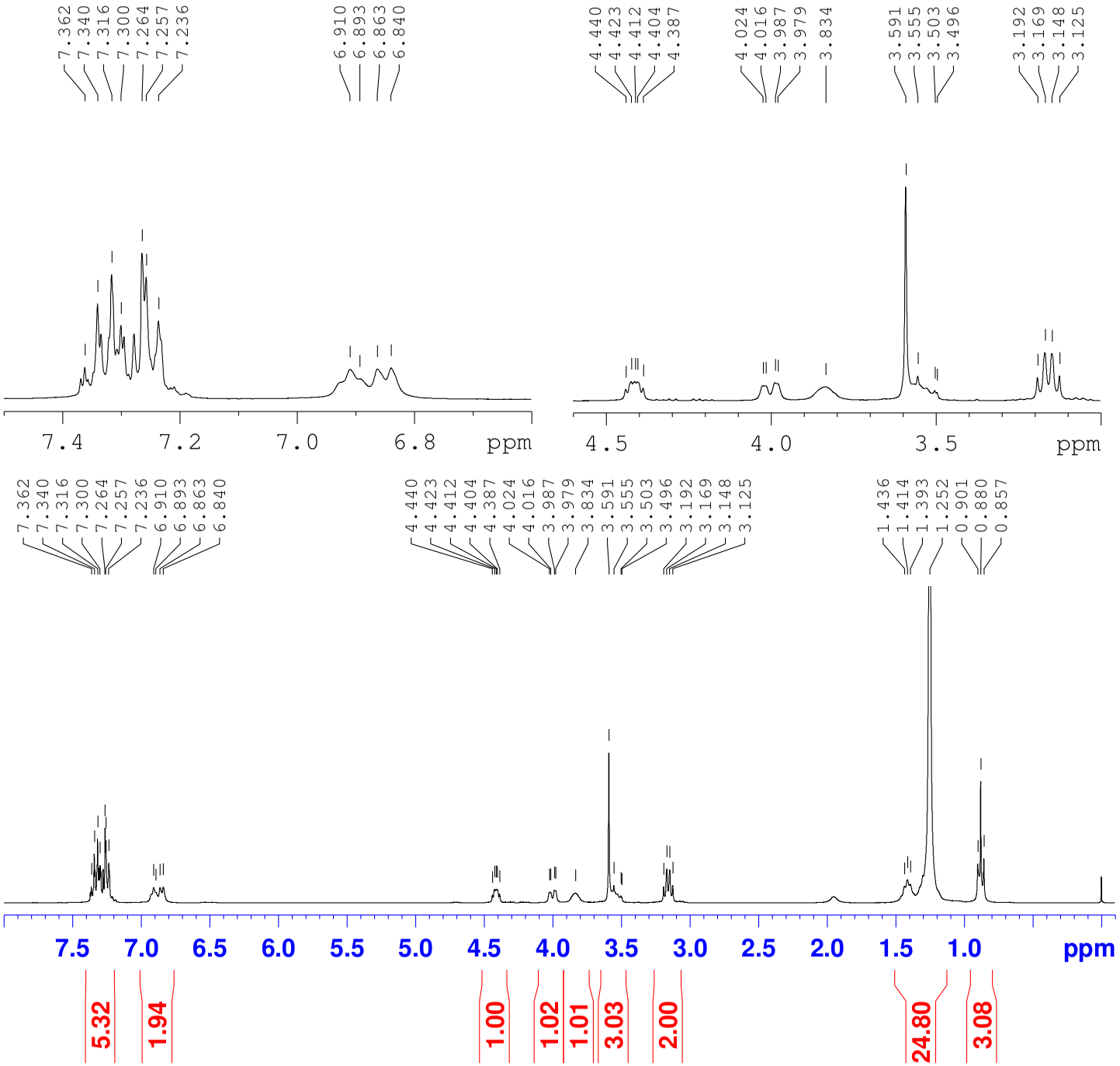
Time                16.28
INSTRUM       FOURIER300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  6000
DS                     4
SWH           24414.063 Hz
FIDRES         0.372529 Hz
AQ             1.3421773 sec
RG               501.187
DW                20.480 usec
DE                 6.50 usec
TE                296.7 K
D1             2.00000000 sec
D11            0.03000000 sec
D31            0.00001600 sec
D40            0.02432300 sec
L4                    34
L5                    49
P32              105.00 usec
TD0                    1

======== CHANNEL f1 ========
SFO1          75.4878687 MHz
NUC1                 13C
P1                16.00 usec
PLW1         60.00000000 W

======== CHANNEL f2 ========
SFO2         300.1812007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             105.00 usec
PLW2         10.00000000 W
PLW12         0.36280999 W
PLW13         0.40000001 W

F2 - Processing parameters
SI                 32768
SF            75.4803210 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

404



Current Data Parameters

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EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20120610	
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INSTRUM	FOURIER300	
PROBHD	5 mm DUL 13C-1	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	6103.516	Hz
FIDRES	0.093132	Hz
AQ	5.3687091	sec
RG	16.949	
DW	81.920	usec
DE	6.50	usec
TE	296.4	K
D1	1.00000000	sec
TD0	1	

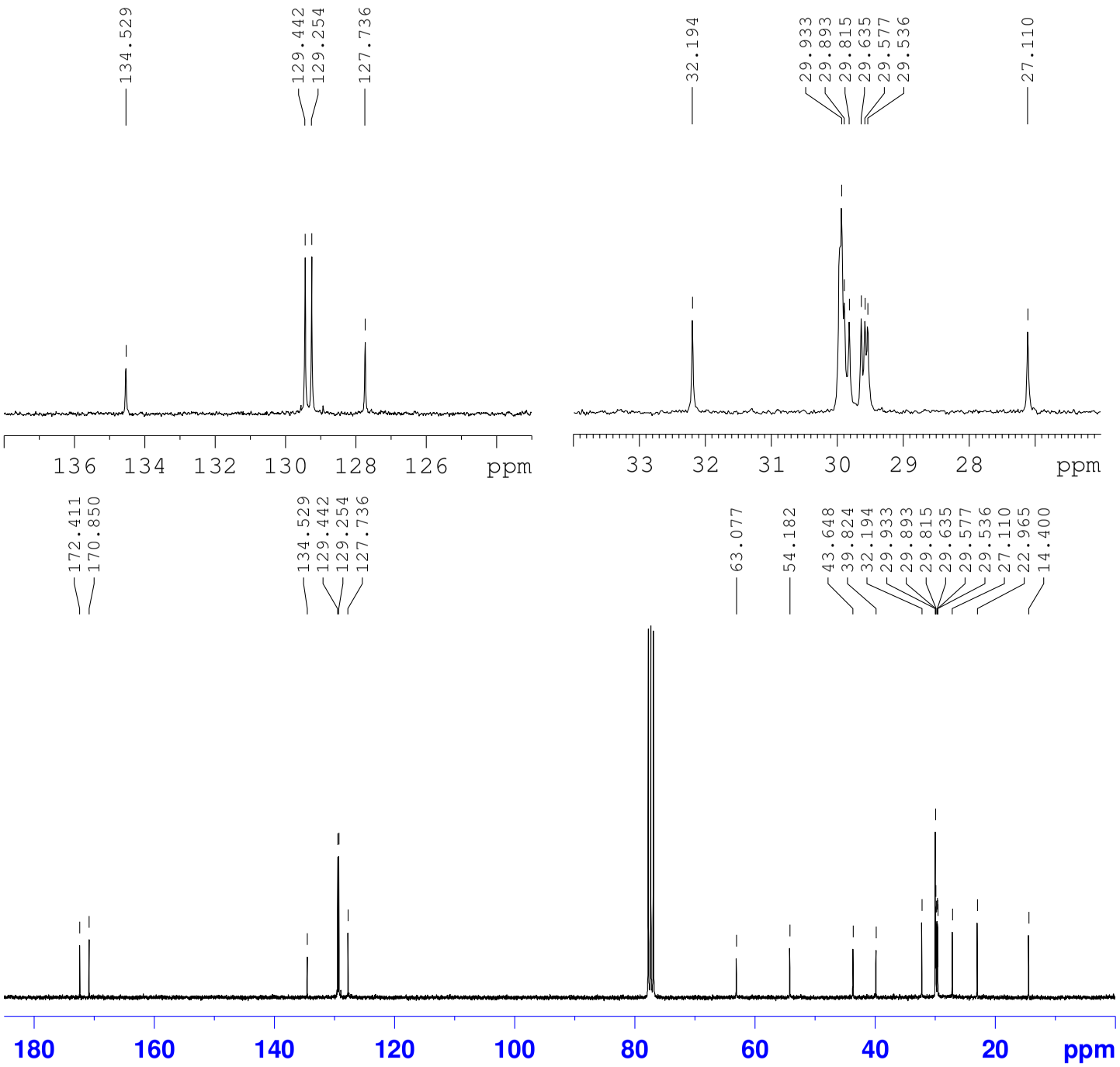
======== CHANNEL f1 ========

SFO1	300.1818537	MHz
NUC1	1H	
P1	8.75	usec
PLW1	25.00000000	W

F2 - Processing parameters

SI	65536	
SF	300.1800035	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

404




```
Current Data Parameters
NAME                  404
EXPNO                   2
PROCNO                  1

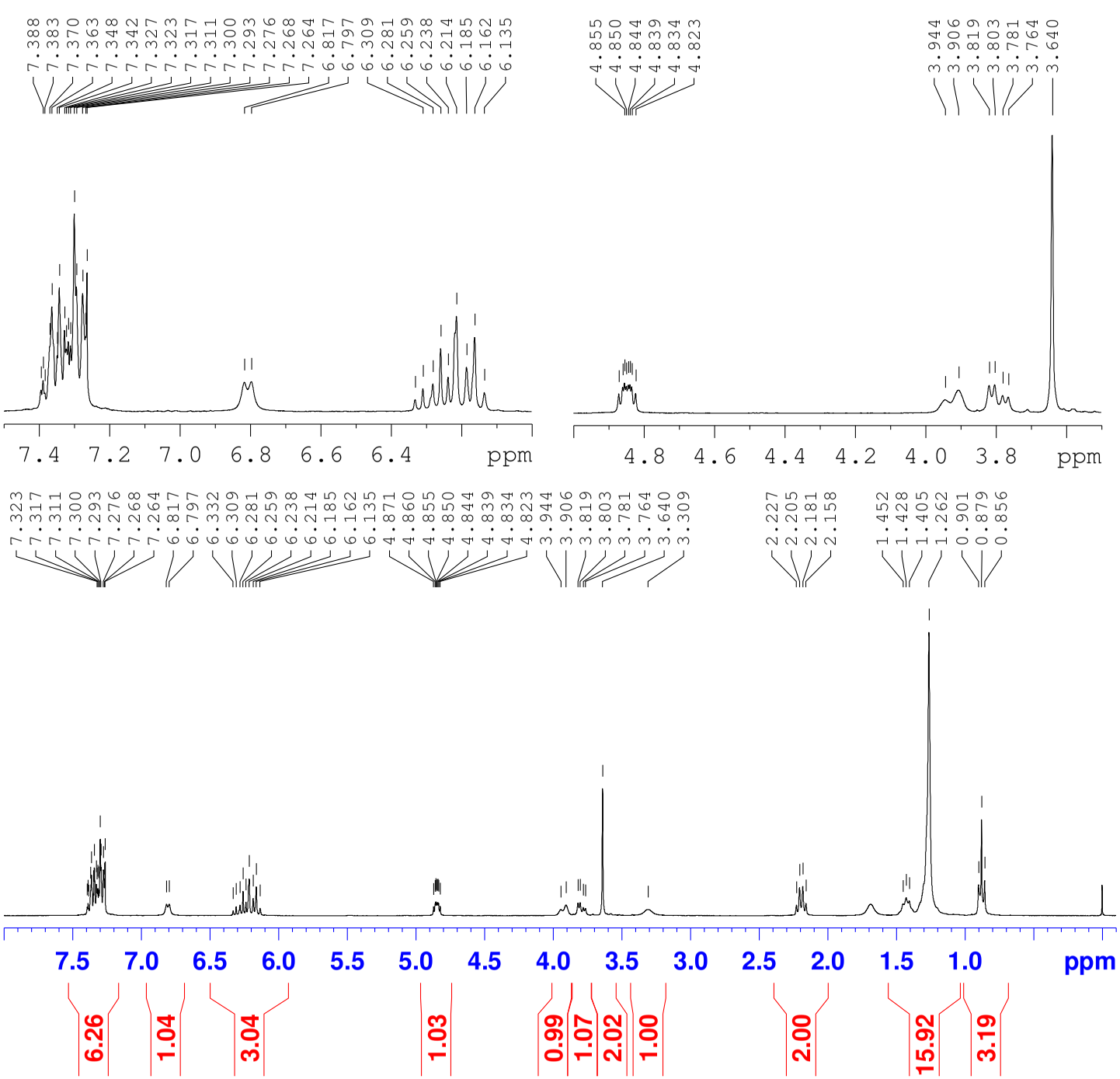
F2 - Acquisition Parameters
Date_            20120611
Time                18.18
INSTRUM       FOURIER300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  4000
DS                     4
SWH           24414.063 Hz
FIDRES            0.372529 Hz
AQ             1.3421773 sec
RG               501.187
DW                20.480 usec
DE                 6.50 usec
TE                296.8 K
D1           2.00000000 sec
D11          0.03000000 sec
D31          0.00001600 sec
D40          0.02432300 sec
L4                    34
L5                    49
P32              105.00 usec
TD0                    1

======== CHANNEL f1 ========
SFO1          75.4878687 MHz
NUC1                 13C
P1                16.00 usec
PLW1         60.00000000 W

======== CHANNEL f2 ========
SFO2         300.1812007 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             105.00 usec
PLW2         10.00000000 W
PLW12         0.36280999 W
PLW13         0.40000001 W

F2 - Processing parameters
SI                 32768
SF            75.4803012 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

406



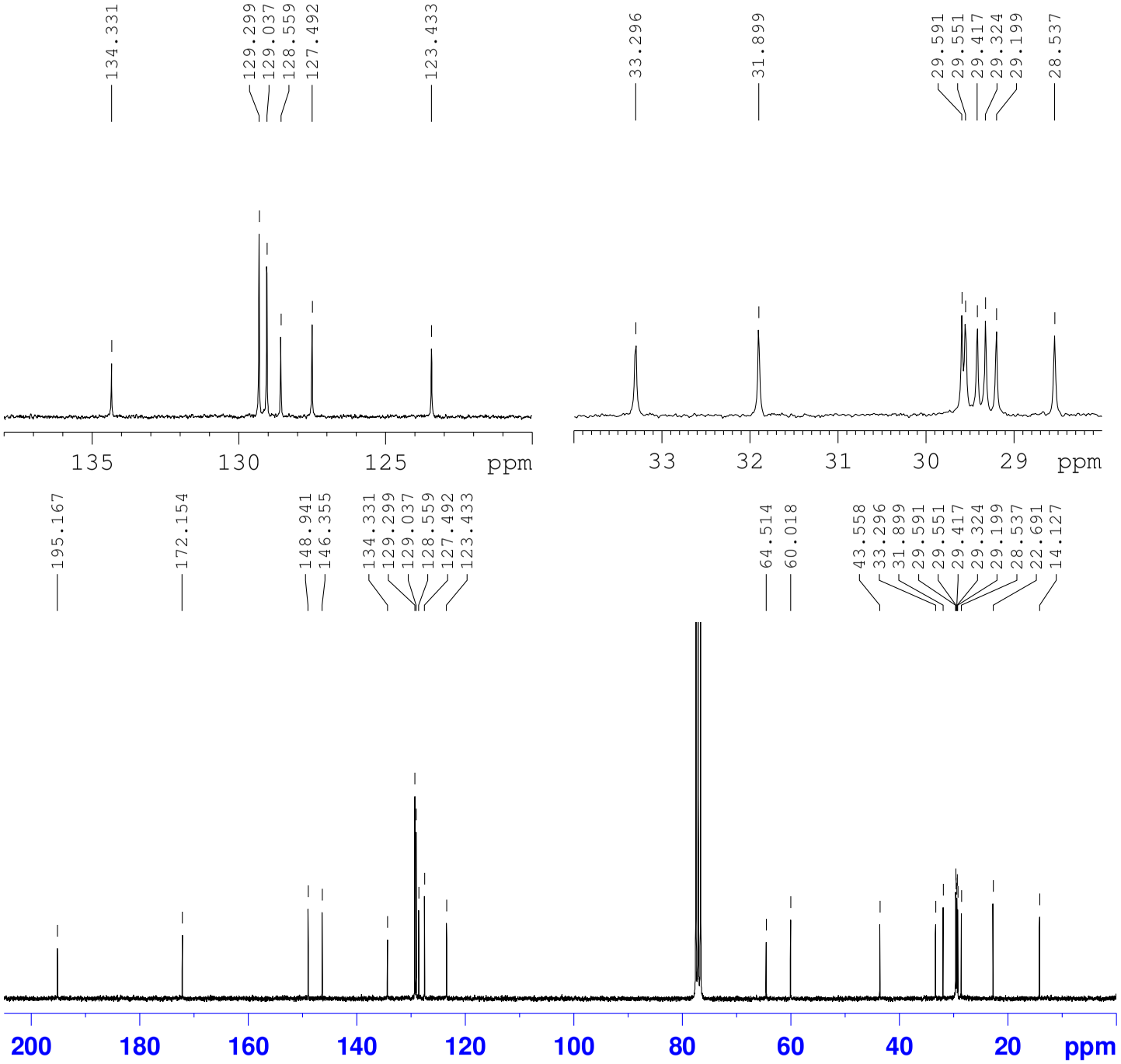

```
Current Data Parameters
NAME                  406
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20120714
Time                18.10
INSTRUM       FOURIER300
PROBHD   5 mm DUL 13C-1
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              6103.516 Hz
FIDRES           0.093132 Hz
AQ               5.3687091 sec
RG                 39.1338
DW                 81.920 usec
DE                  6.50 usec
TE                 296.4 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          300.1818537 MHz
NUC1                    1H
P1                   8.75 usec
PLW1          25.00000000 W

F2 - Processing parameters
SI                  65536
SF            300.1800037 MHz
WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```

406

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Current Data Parameters
NAME                  406
EXPNO                   2
PROCNO                  1

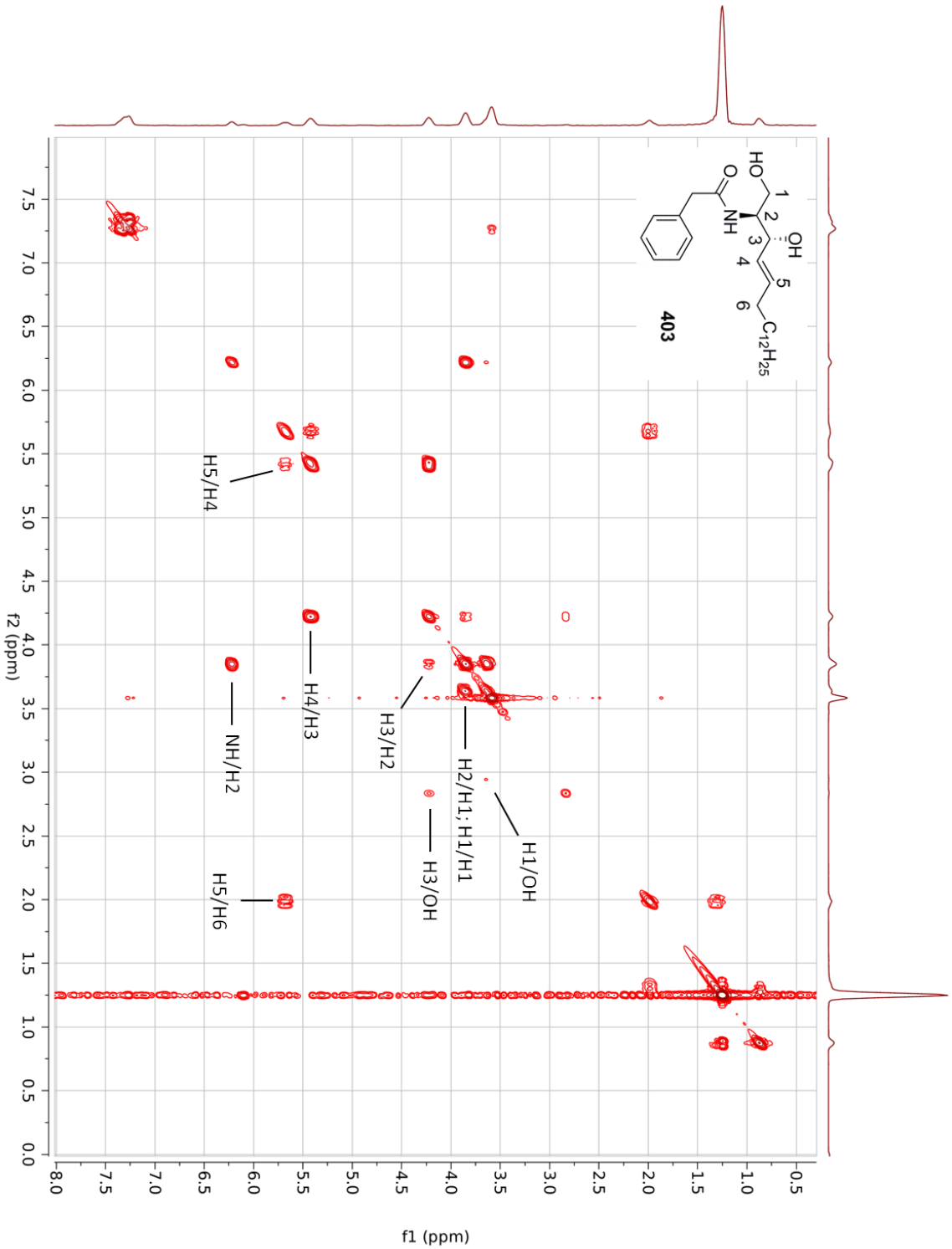
F2 - Acquisition Parameters
Date_            20120714
Time                18.23
INSTRUM       FOURIER300
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT             CDCl3
NS                   2000
DS                      4
SWH            24414.063 Hz
FIDRES             0.372529 Hz
AQ                 1.3421773 sec
RG                   501.187
DW                  20.480 usec
DE                   6.50 usec
TE                  296.5 K
D1              2.00000000 sec
D11             0.03000000 sec
D31             0.00001600 sec
D40             0.02432300 sec
L4                     34
L5                     49
P32                105.00 usec
TD0                     1

======== CHANNEL f1 ========
SFO1           75.4878687 MHz
NUC1                  13C
P1                  16.00 usec
PLW1          60.00000000 W

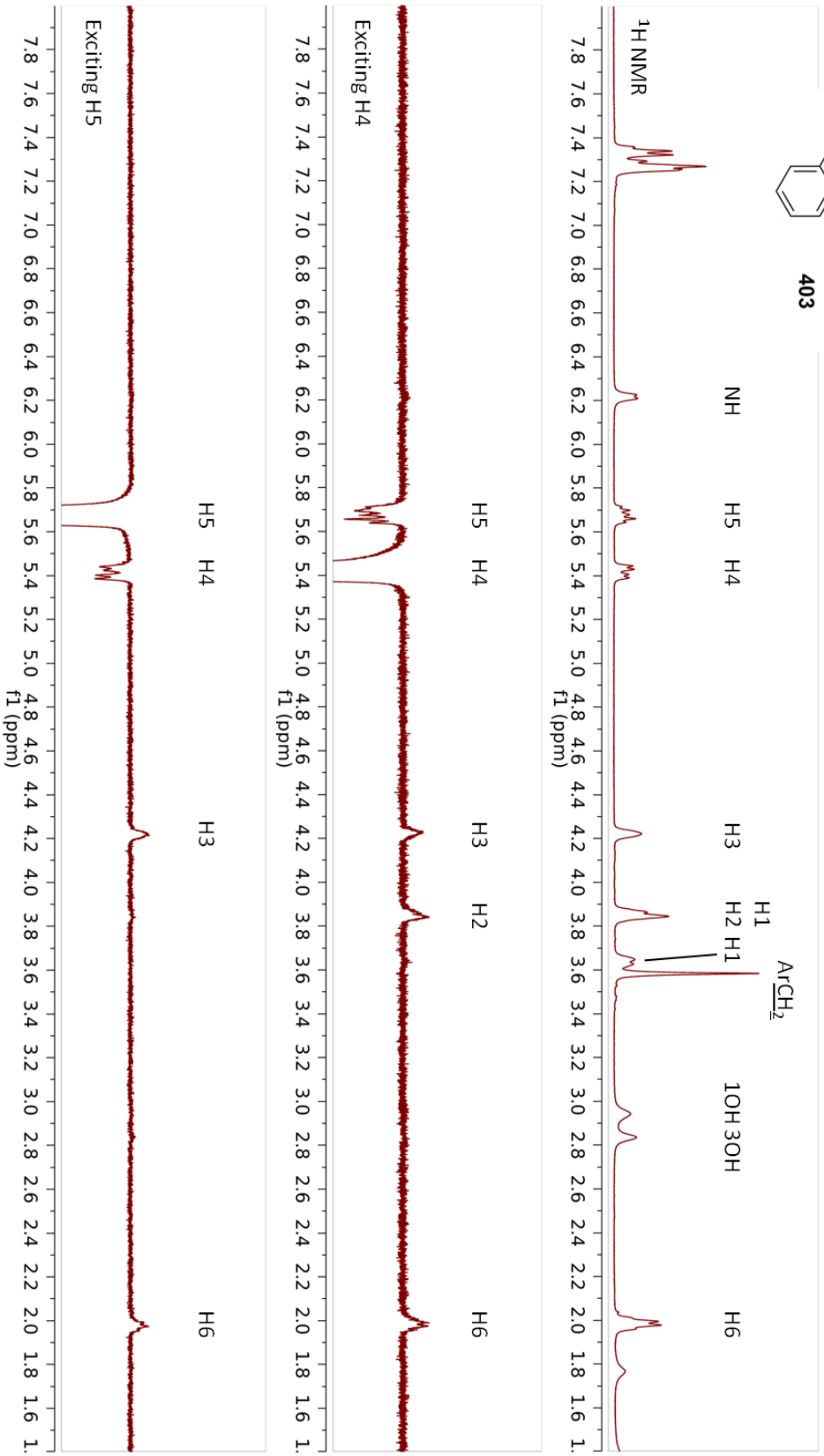
======== CHANNEL f2 ========
SFO2          300.1812007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              105.00 usec
PLW2          10.00000000 W
PLW12          0.36280999 W
PLW13          0.40000001 W

F2 - Processing parameters
SI                 32768
SF            75.4803210 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

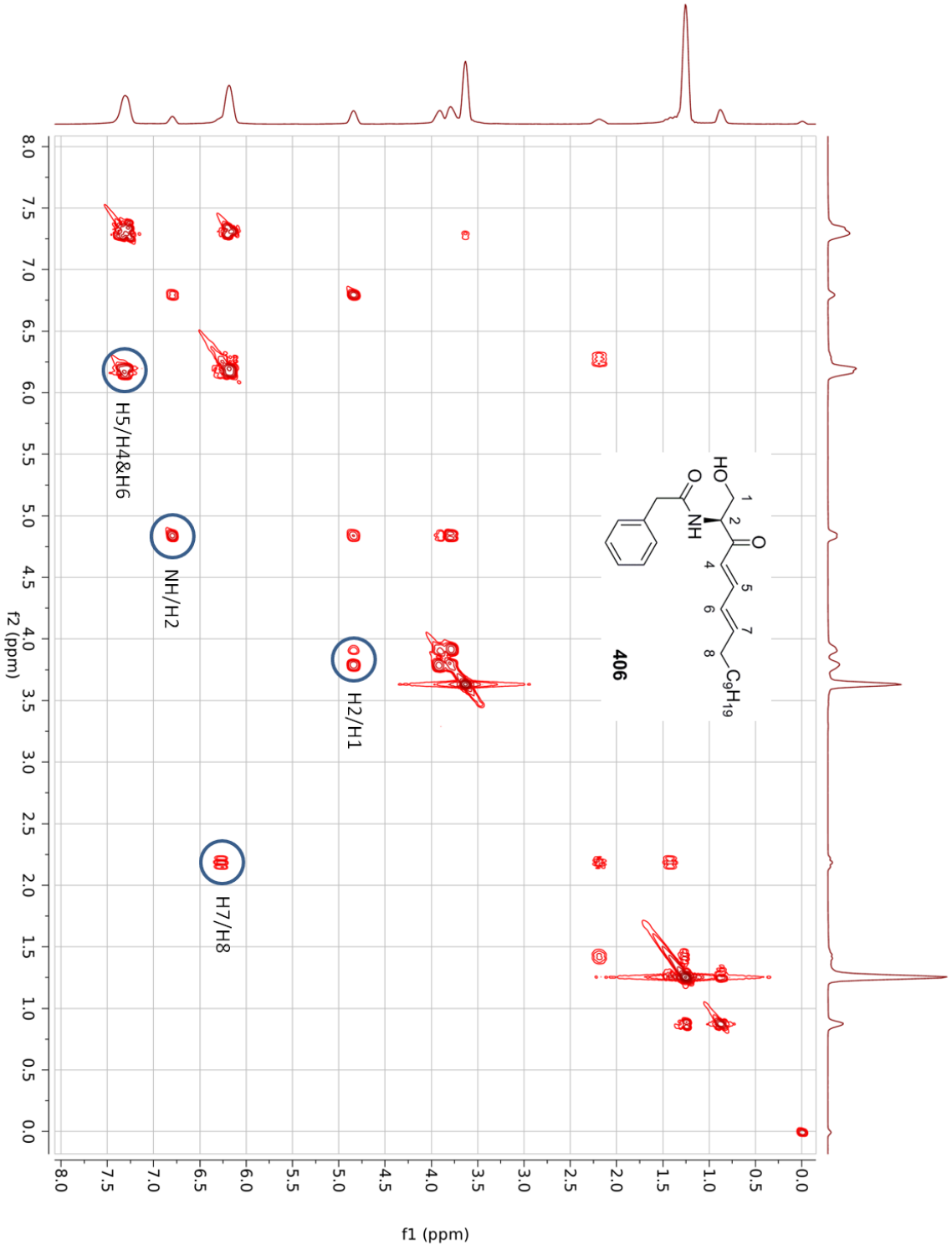
COSY spectrum of analog 403



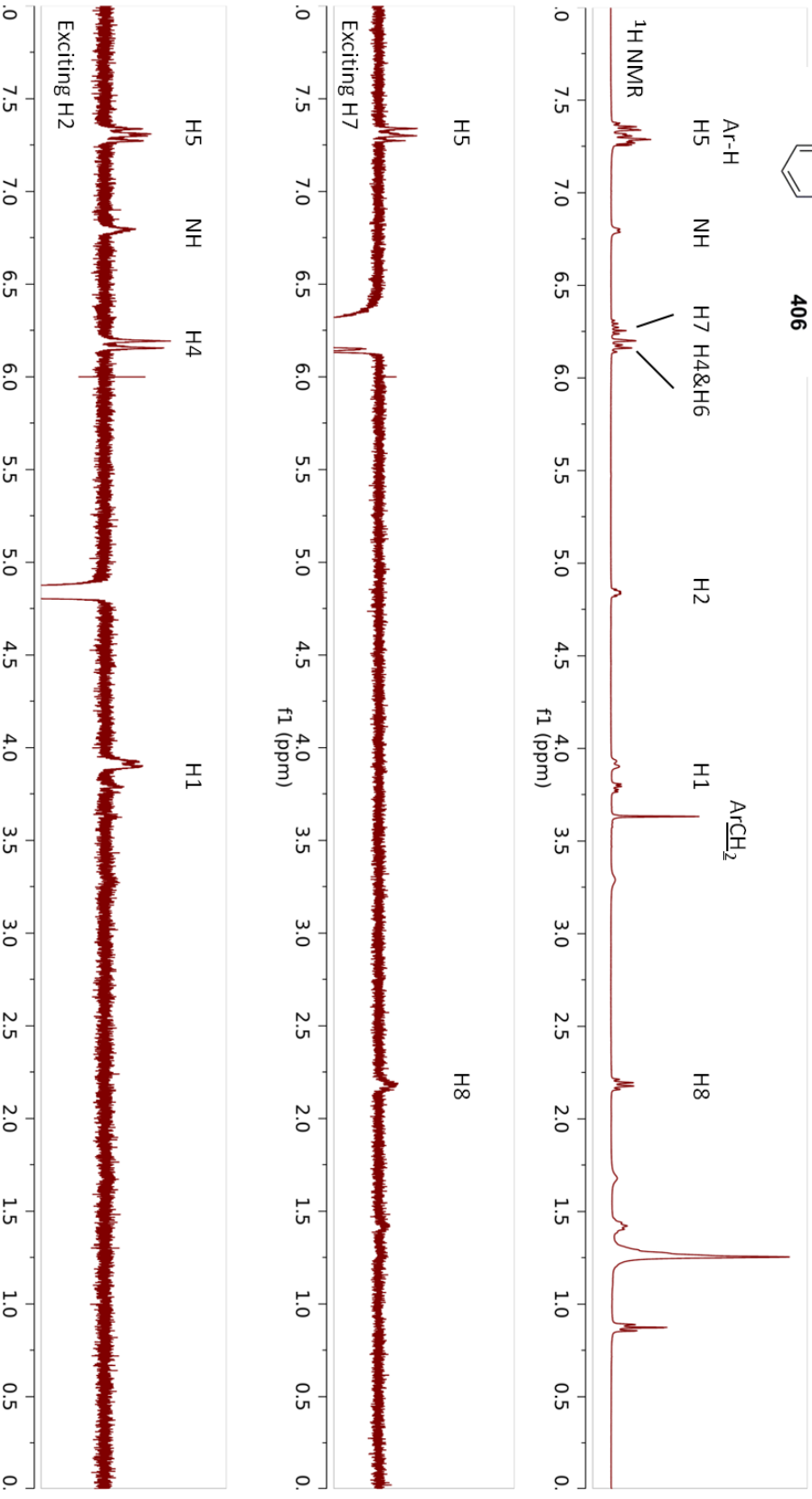
1D NOE spectra of analog 403



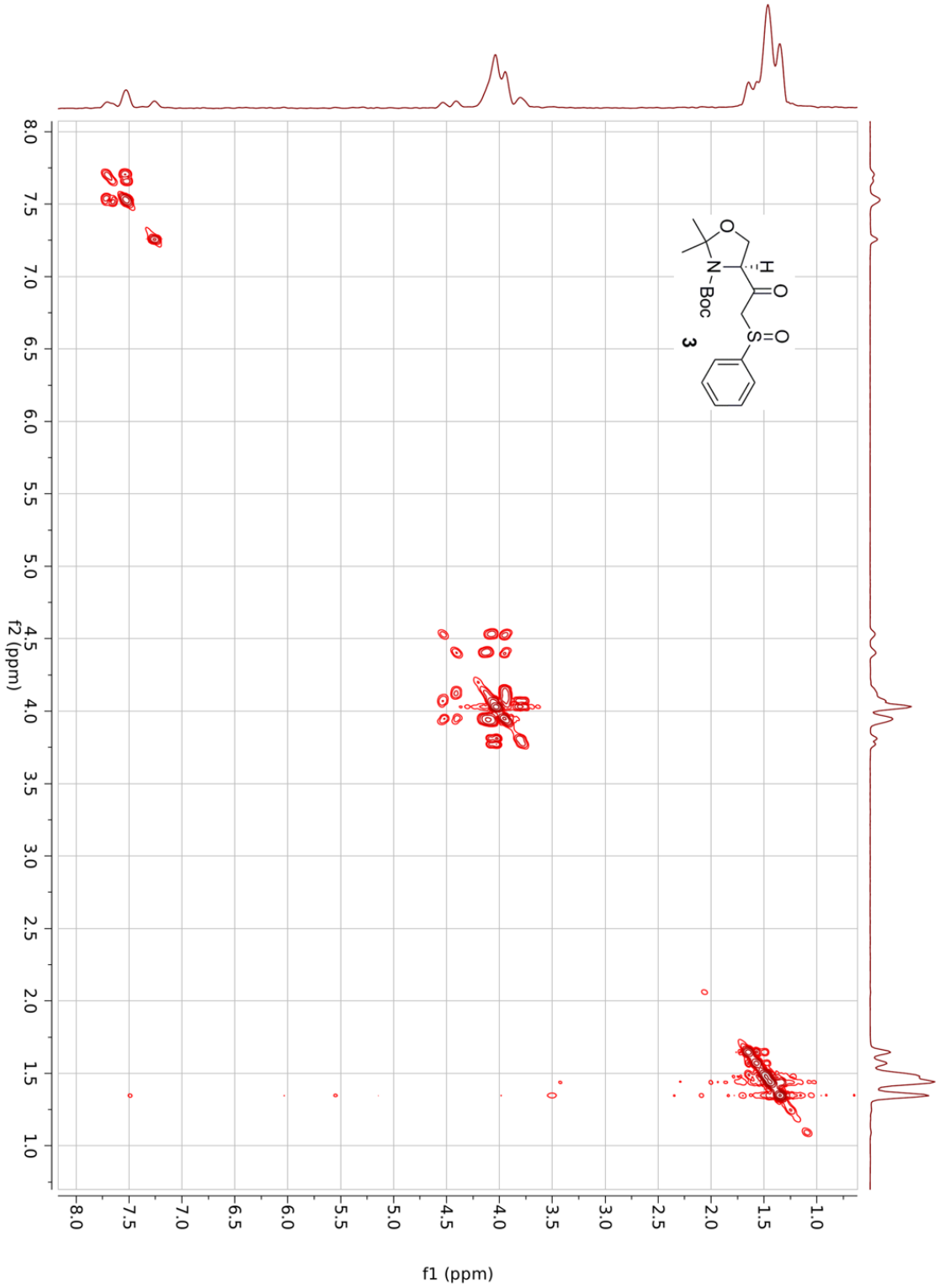
COSY spectrum of analog 406



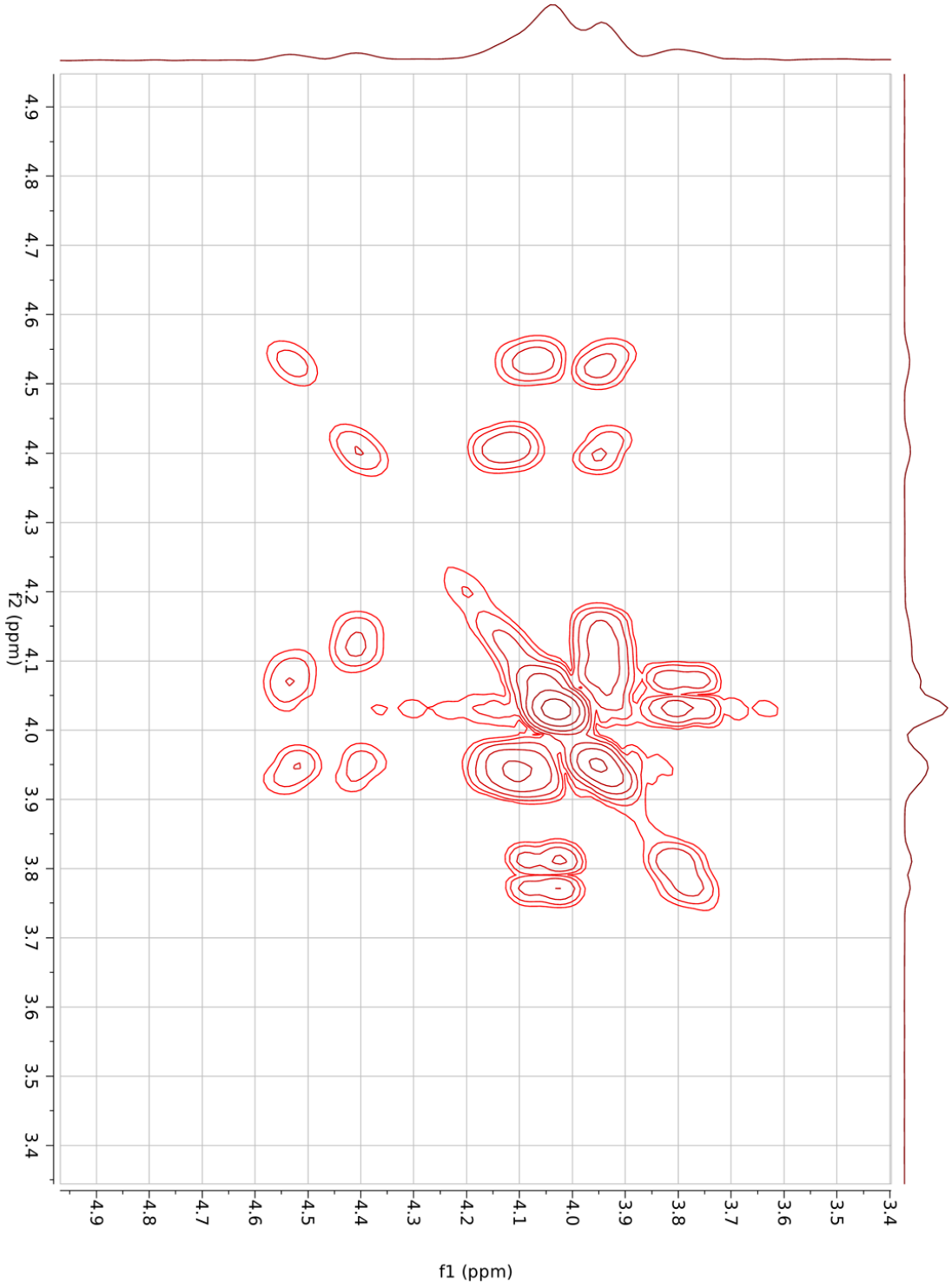
1D NOE spectra of analog 406



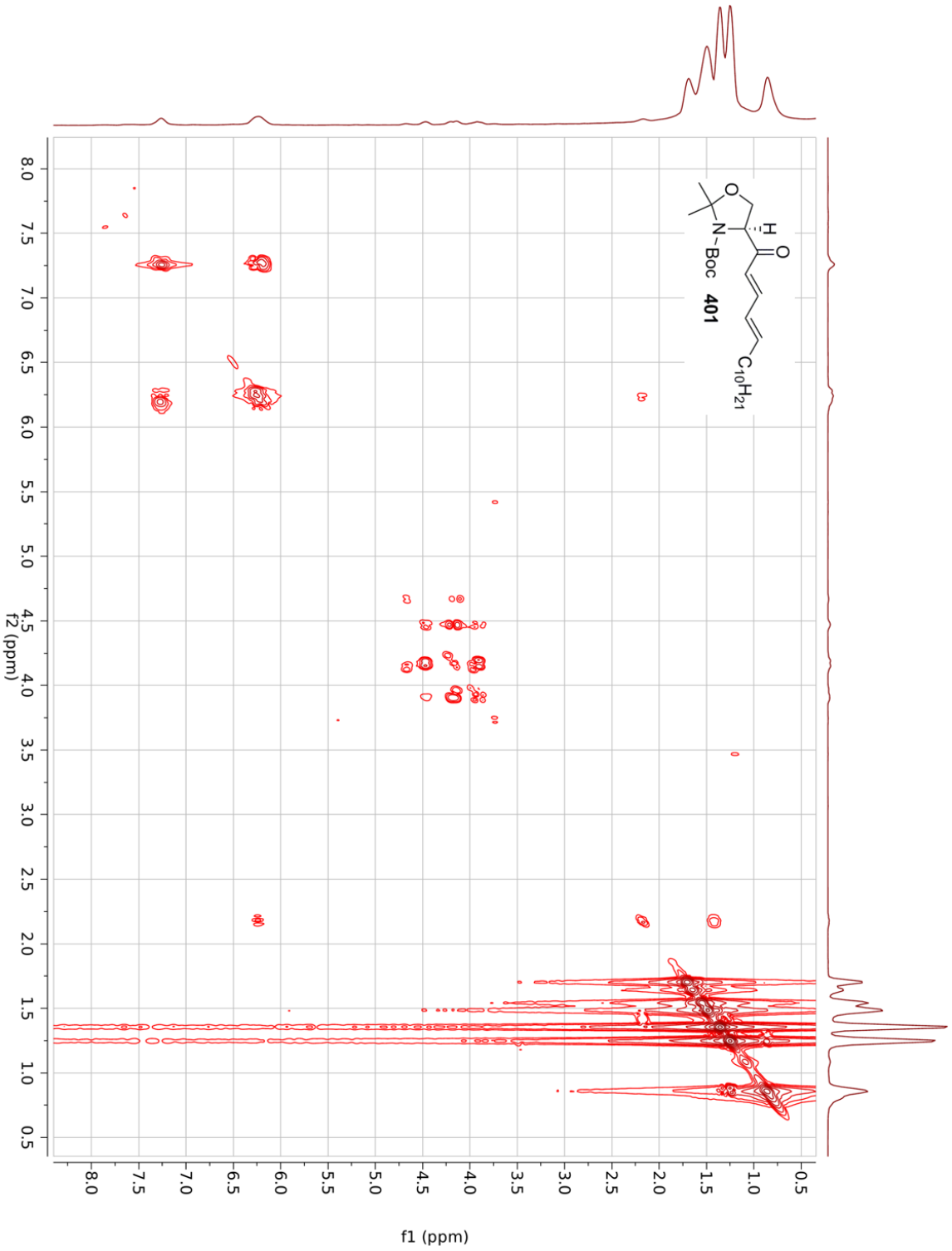
COSY spectrum of compound 3-up



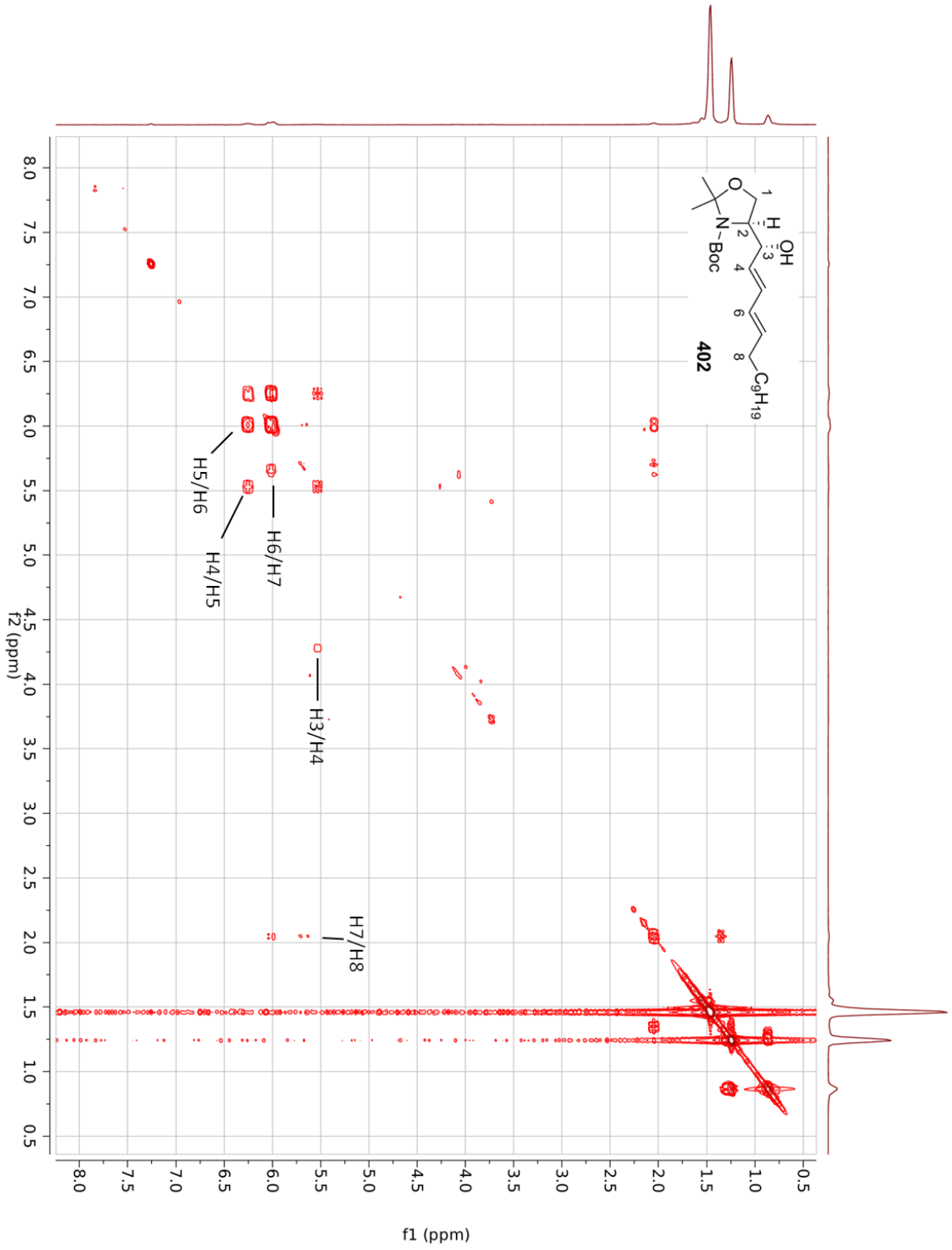
Zoom-in image of the COSY spectrum of compound 3-up (4.9 - 3.4 ppm)



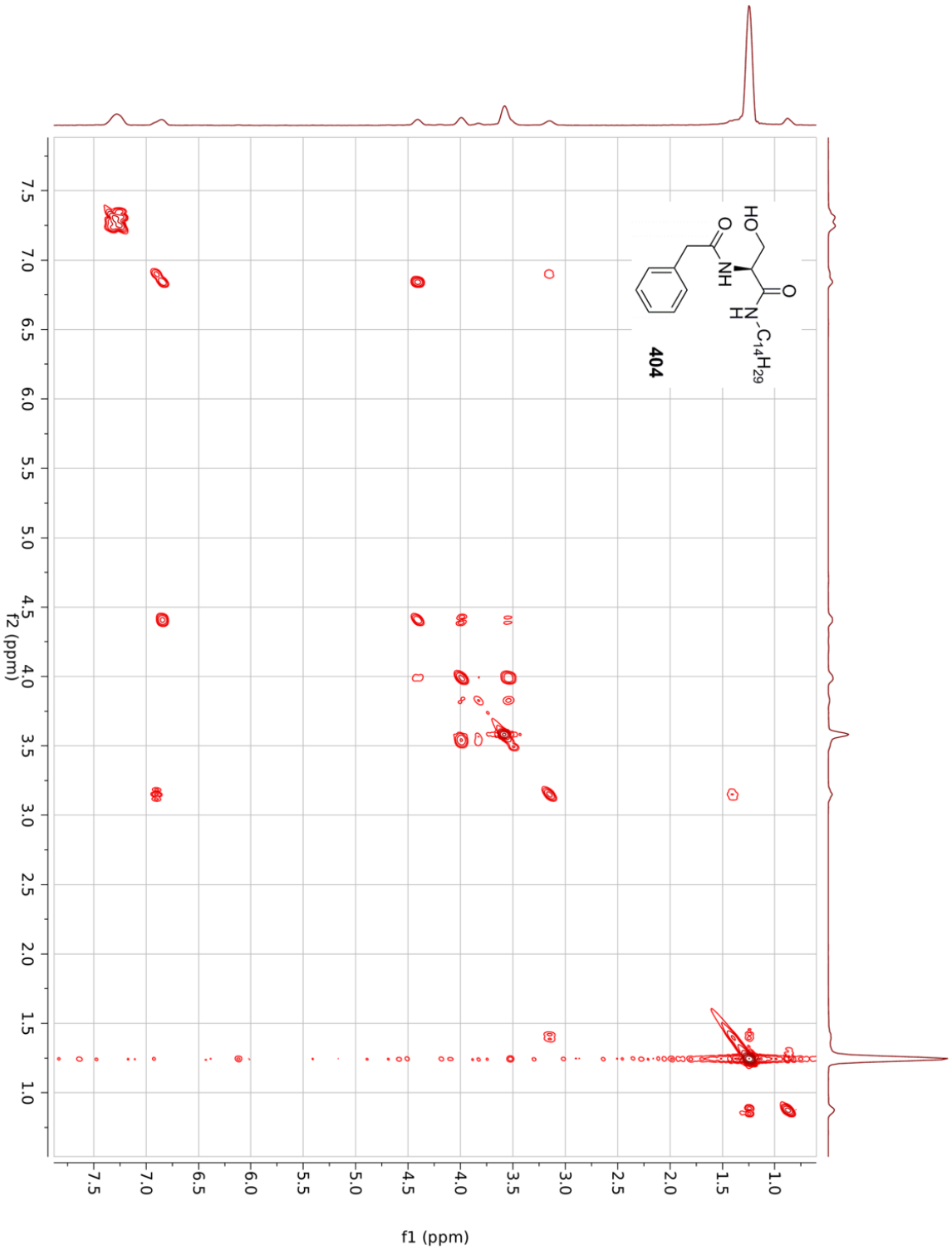
COSY spectrum of analog 401



COSY spectrum of analog 402



COSY spectrum of analog 404



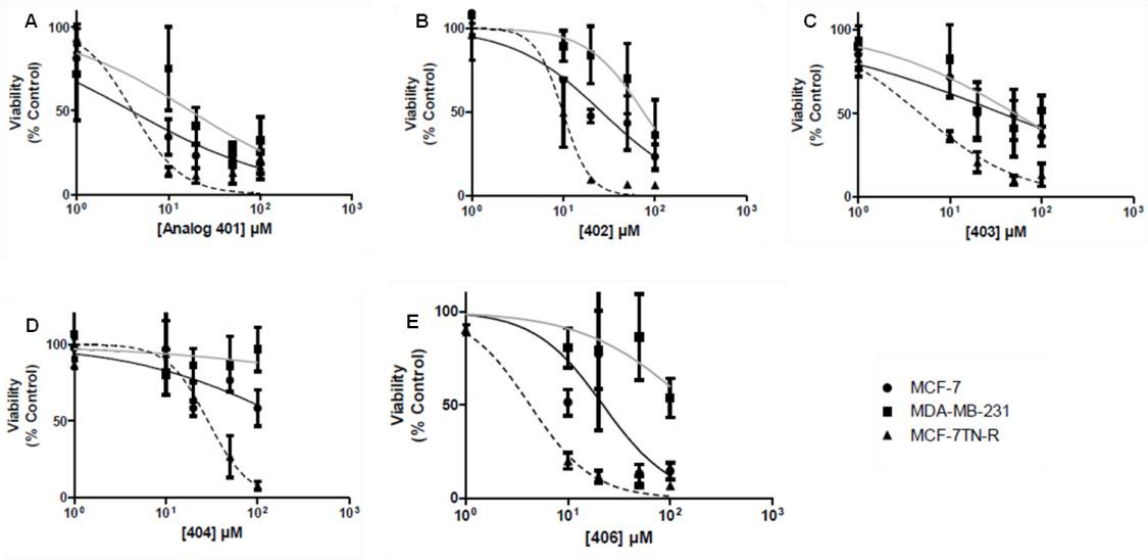


Figure S1. Effect of ceramide analogs on cell viability of breast cancers. MCF-7**,** MDA-MB-231, and MCF-7TN-R cells were treated with increasing concentrations of analogs for 24 h. The values are the mean ± SE of three independent experiments.

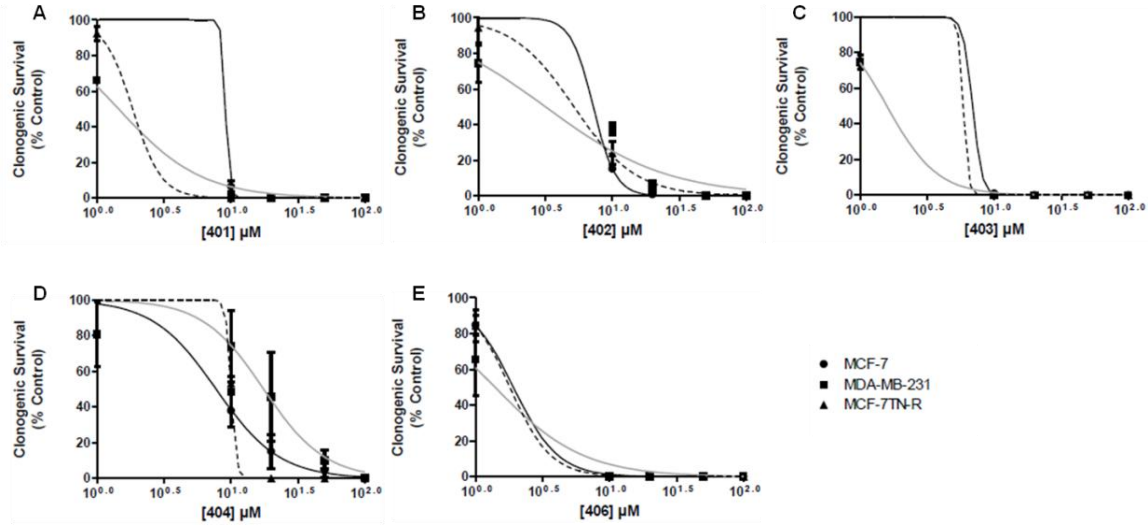


Figure S2. Effect of ceramide analogs on breast cancer clonogenic survival. MCF-7, MDA-MB-231, and MCF-7TN-R cells were treated with increasing concentrations of analogs and allowed to grow until colony formation was noted (generally 10-12 days). The values are the mean ± SE of three independent experiments.