Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplement Information to

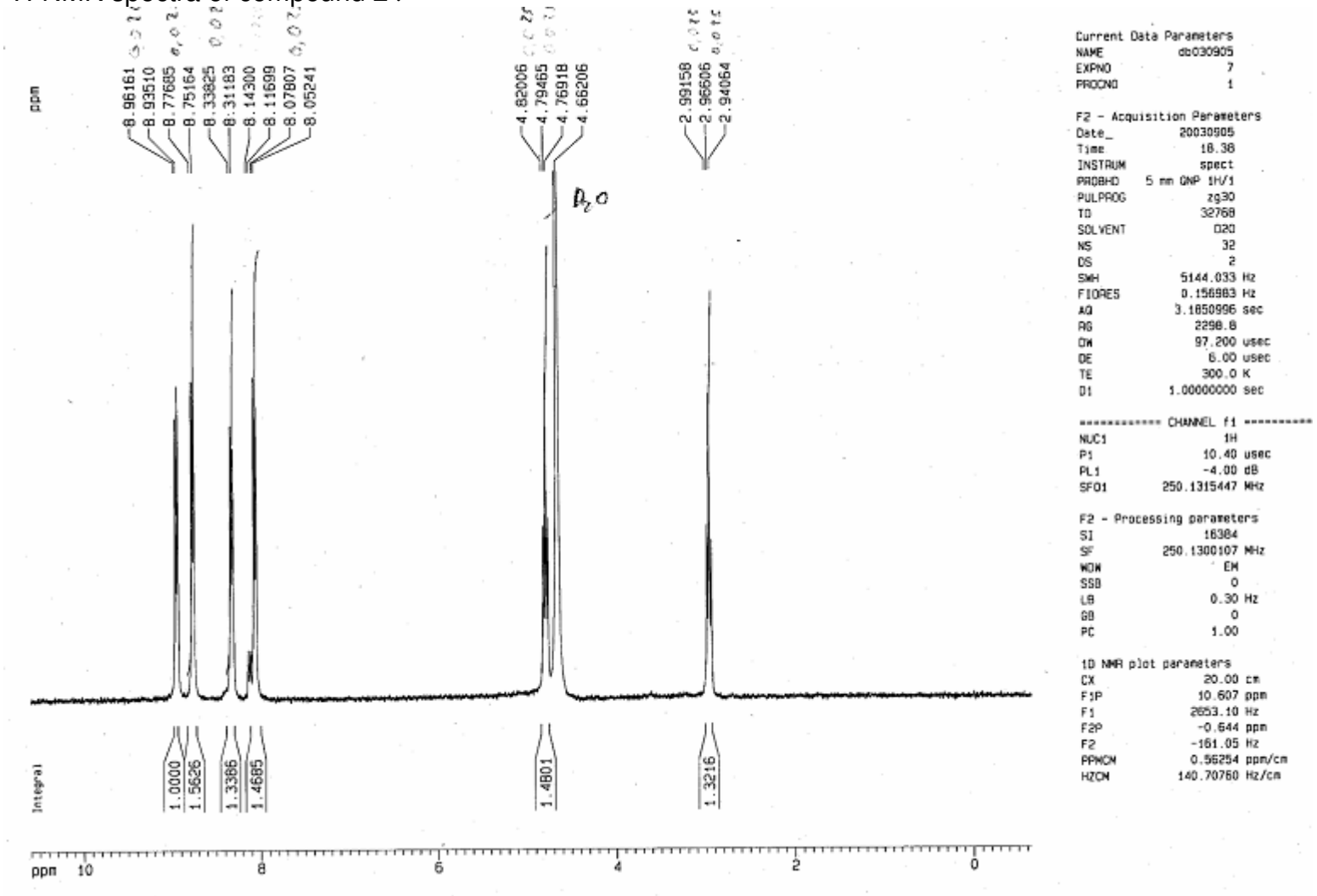
Alkylene-bridged Viologen Dendrimers: A Versatile Cell Delivery Tool With Biosensing Properties

by

D. Bongard*[1)], W. Bohr[2)], M. Swierczek[3)], T.H. Degefa[1)], L. Walder[1)], R. Brandt[3)]
[*1)] Institute of Chemistry of New Materials, University of Osnabrück
Barbarastr. 7, D-49076 Osnabrück, Germany

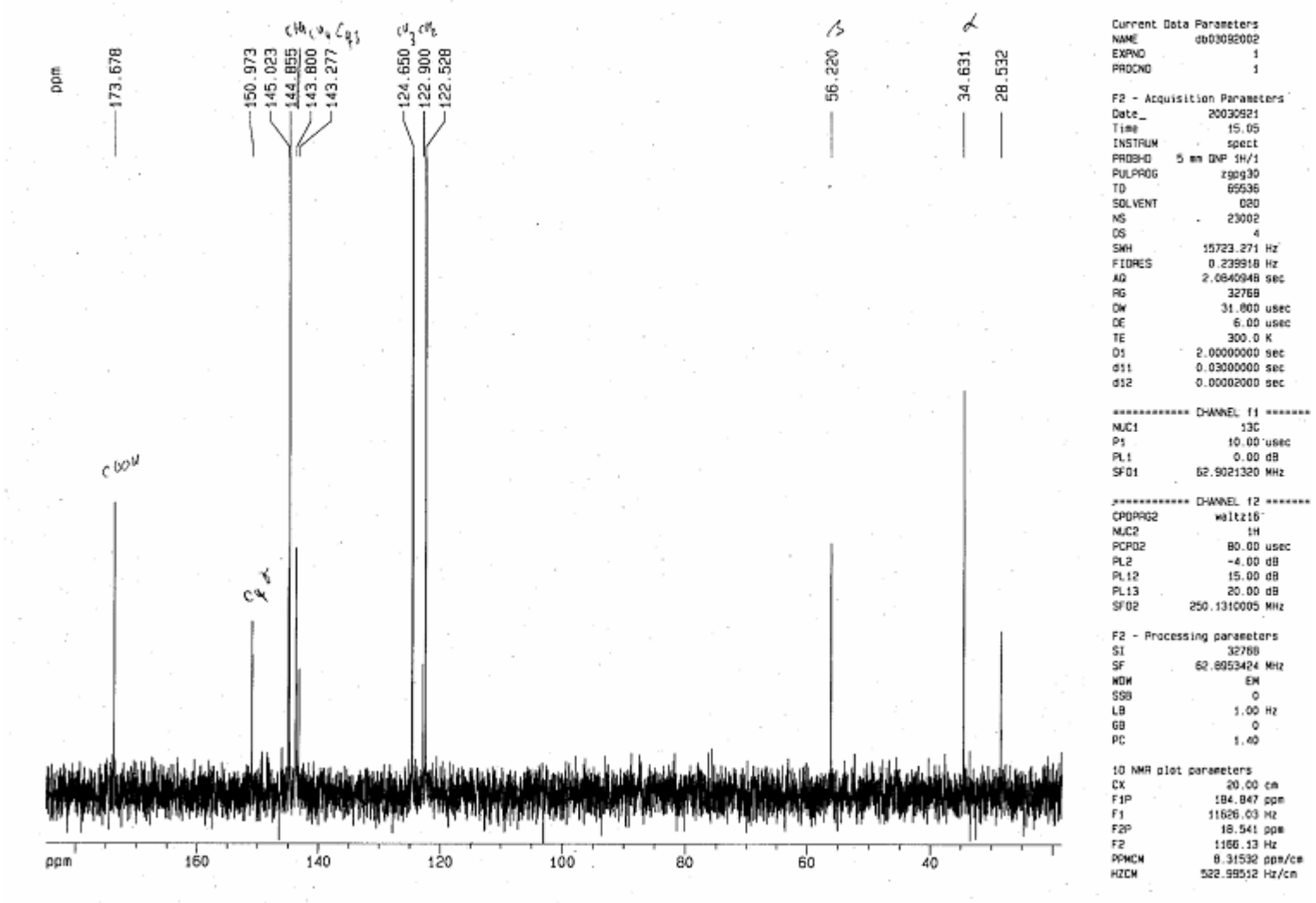
1. NMR spectra

^{1}H-NMR spectra of compound **2** :



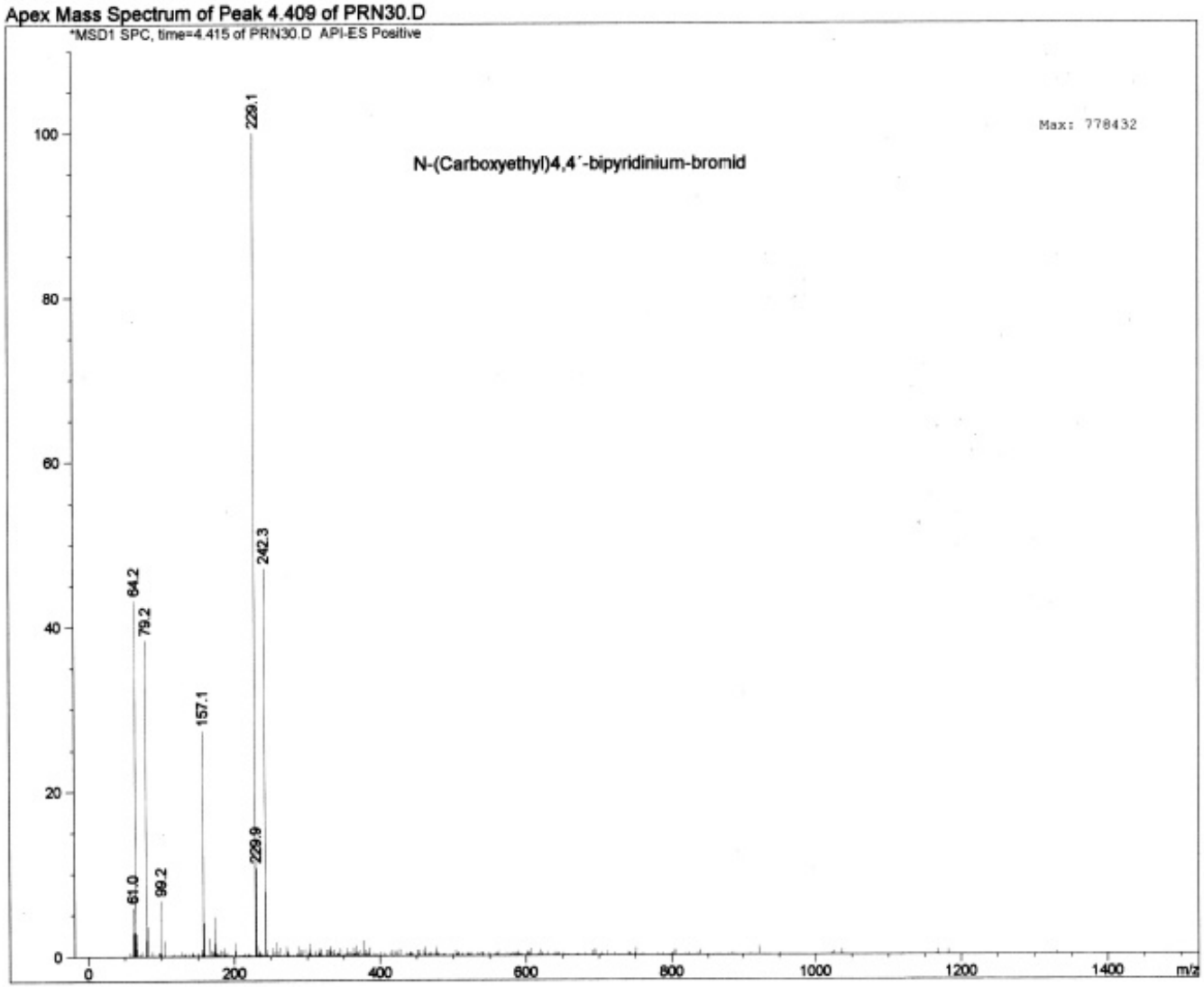

```
Current Data Parameters
NAME                  db030905
EXPNO                        7
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20030905
Time                     18.38
INSTRUM                  spect
PROBHD     5 mm QNP 1H/1
PULPROG                  zg30
TD                      32768
SOLVENT                   D2O
NS                           32
DS                            2
SWH                 5144.033 Hz
FIDRES              0.156983 Hz
AQ                  3.1850996 sec
RG                       2298.8
DW                     97.200 usec
DE                      6.00 usec
TE                      300.0 K
D1                  1.00000000 sec

============ CHANNEL f1 ==========
NUC1                         1H
P1                      10.40 usec
PL1                     -4.00 dB
SFO1               250.1315447 MHz

F2 - Processing parameters
SI                       16384
SF                 250.1300107 MHz
WDW                         EM
SSB                          0
LB                      0.30 Hz
GB                           0
PC                        1.00

1D NMR plot parameters
CX                      20.00 cm
F1P                    10.607 ppm
F1                     2653.10 Hz
F2P                    -0.644 ppm
F2                     -161.05 Hz
PPMCM                  0.56254 ppm/cm
HZCM                 140.70760 Hz/cm
```

^{13}C-NMR spectra of compound **2**

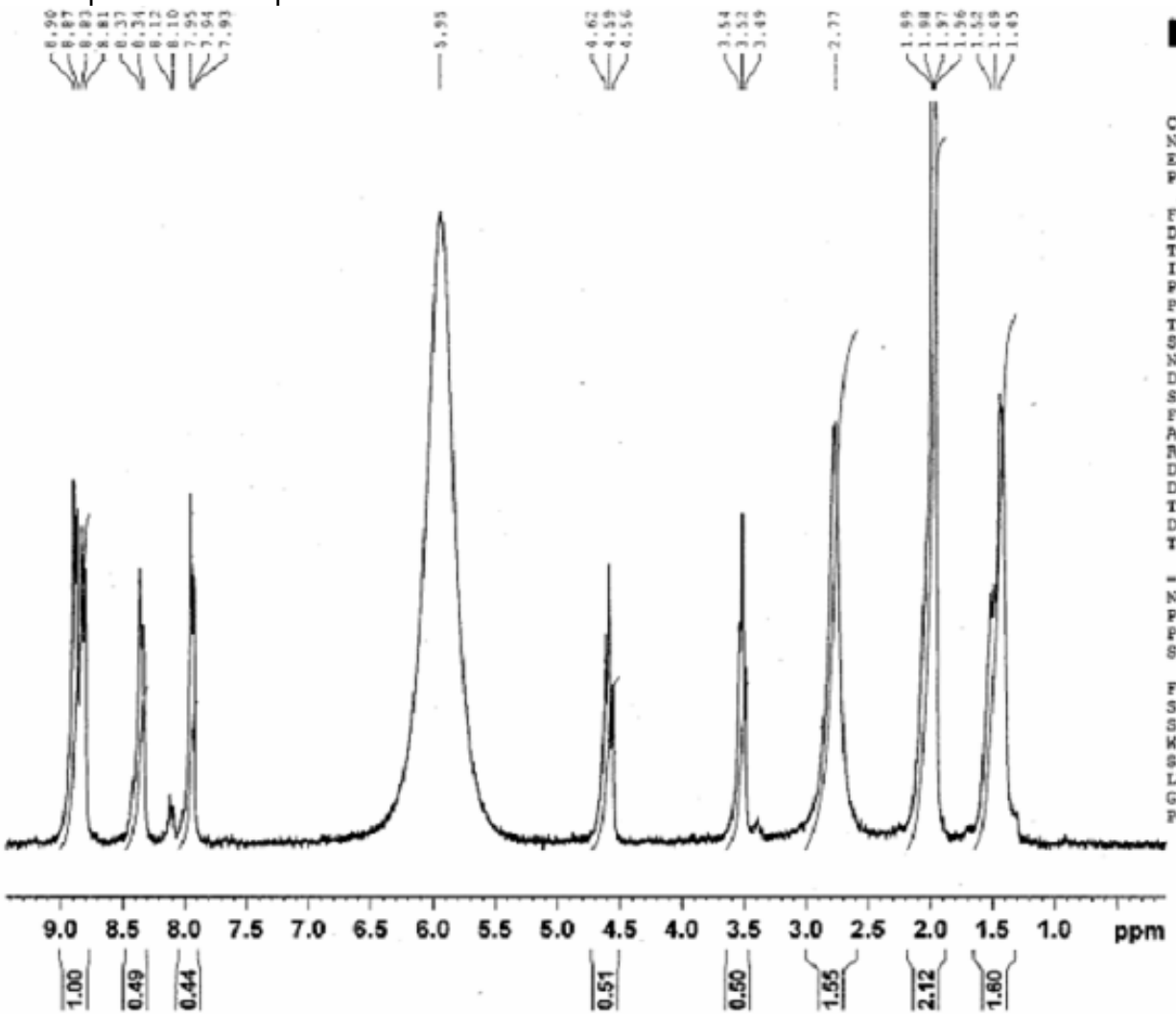



```
Current Data Parameters
NAME          db03092002
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20030921
Time               15.05
INSTRUM             spect
PROBHD    5 mm QNP 1H/1
PULPROG          zgpg30
TD                65536
SOLVENT             CDCl3
NS                 23002
DS                     4
SWH           15723.271 Hz
FIDRES         0.239918 Hz
AQ             2.0840948 sec
RG                 32768
DW                31.800 usec
DE                 6.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 =======
NUC1                  13C
P1                 10.00 usec
PL1                0.00 dB
SF01          62.9021320 MHz

========== CHANNEL f2 =======
CPDPRG2           waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2               -4.00 dB
PL12               15.00 dB
PL13               20.00 dB
SF02         250.1310005 MHz

F2 - Processing parameters
SI                 32768
SF            62.8953424 MHz
WDW                   EM
SSB                    0
LB                 1.00 Hz
GB                     0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              184.847 ppm
F1              11626.03 Hz
F2P               18.541 ppm
F2                1166.13 Hz
PPMCM             8.31532 ppm/cm
HZCM            522.99512 Hz/cm
```

ESI-MS spectra of compound **2** : 1-Carboxyethyl-4,4'-bipyridinium-hexafluorophosphat



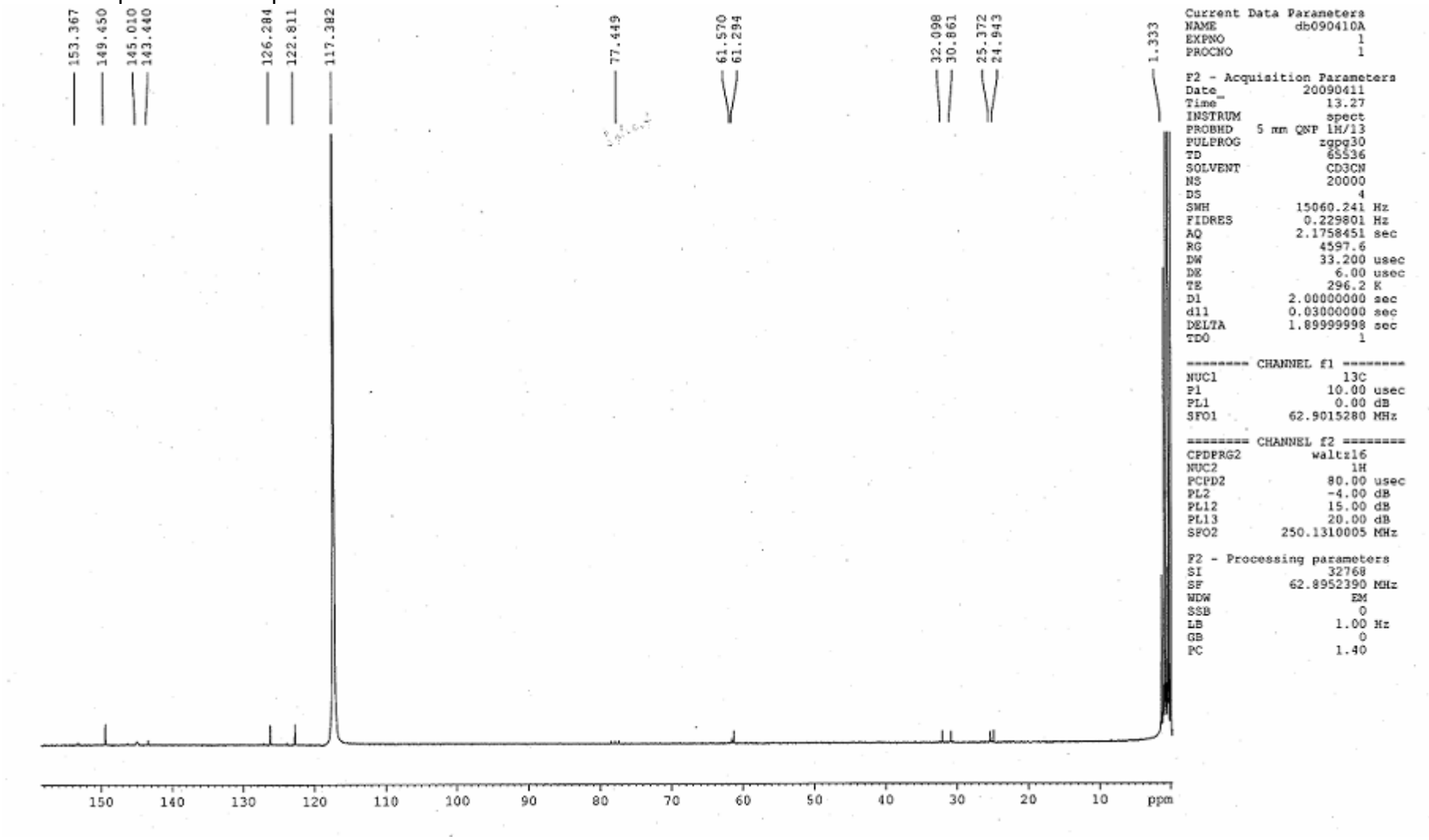
^{1}H-NMR spectra of compound **3** :



BRUKER

```
Current Data Parameters
NAME                  db092701
EXPNO                        2
PROCNO                       1

F2 - Acquisition Parameters
Date_              20090327
Time                   19.29
INSTRUM                spect
PROBHD    5 mm QNP 1H/13
PULPROG                 zg30
TD                     32768
SOLVENT                CD3CN
NS                        32
DS                         2
SWH                5144.033 Hz
FIDRES             0.156983 Hz
AQ                 3.1850996 sec
RG                      1024
DW                   97.200 usec
DE                    6.00 usec
TE                   295.2 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                     10.40 usec
PL1                    -4.00 dB
SFO1           250.1315447 MHz

F2 - Processing parameters
SI                        16384
SF             250.1300000 MHz
WDW                       EM
SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.00
```

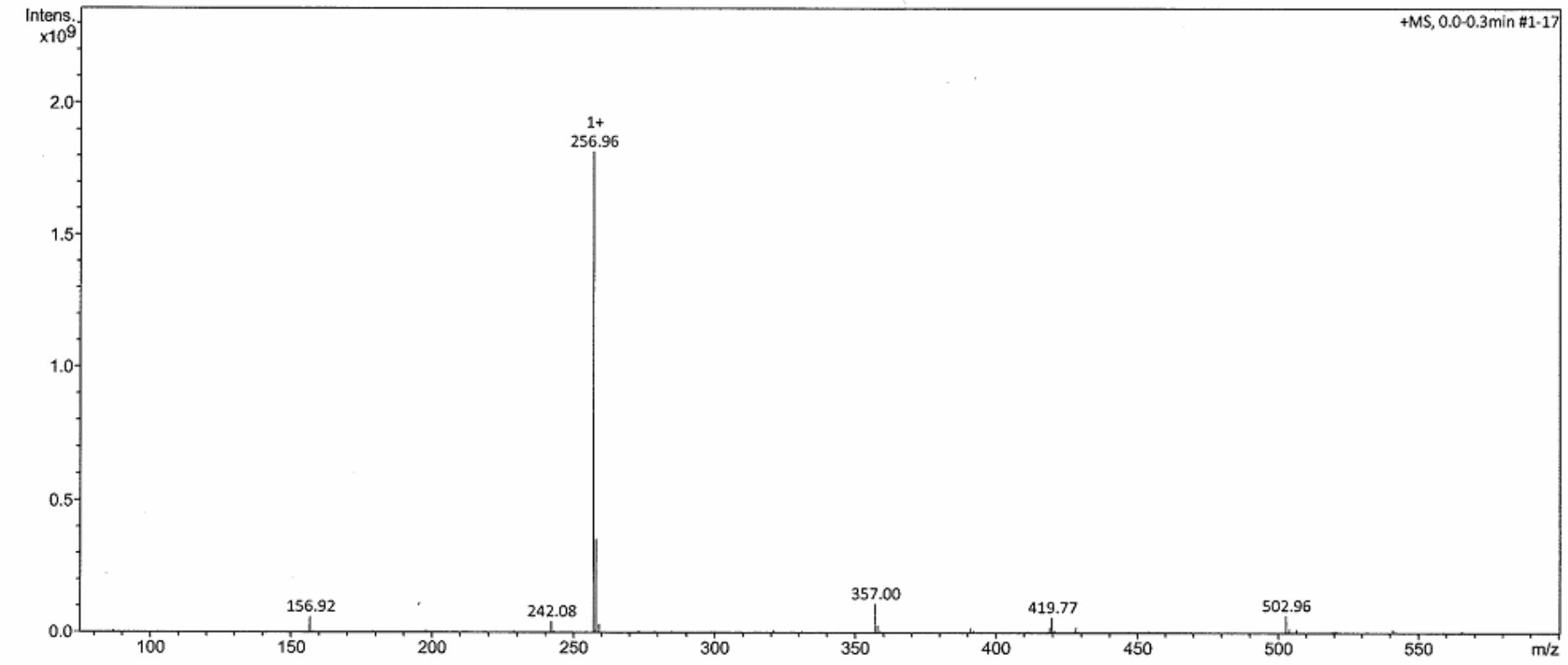
^{13}C-NMR spectra of compound **3** :



ESI-MS spectra of compound **3**: 1-(6-Hydroxyhexyl-)-4,4'-bipyridinium-hexafluorophosphate

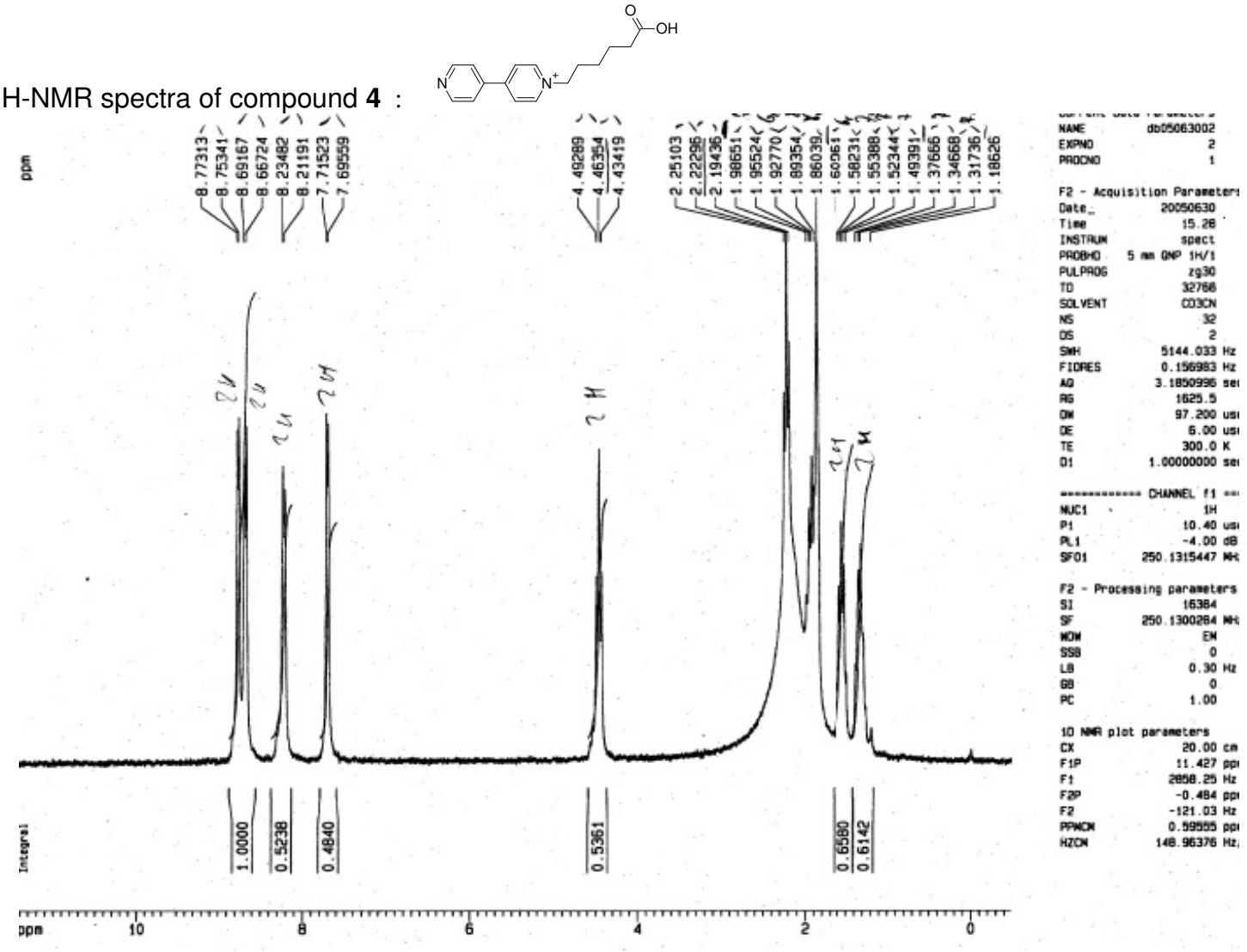
Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Negative	Alternating Ion Polarity	on
Mass Range Mode	Enhanced Resolution	Scan Begin	75 m/z	Scan End	600 m/z
Accumulation Time	143 µs	RF Level	54 %	Trap Drive	50.0
SPS Target Mass	287 m/z	Averages	10 Spectra	Auto MS/MS	off



Bruker Compass DataAnalysis 4.1 printed: 14.07.2014 15:01:56 by: Uni OS Page 1 of 1

^{1}H-NMR spectra of compound **4** :



NAME db05063002
EXPNO 2
PROCNO 1

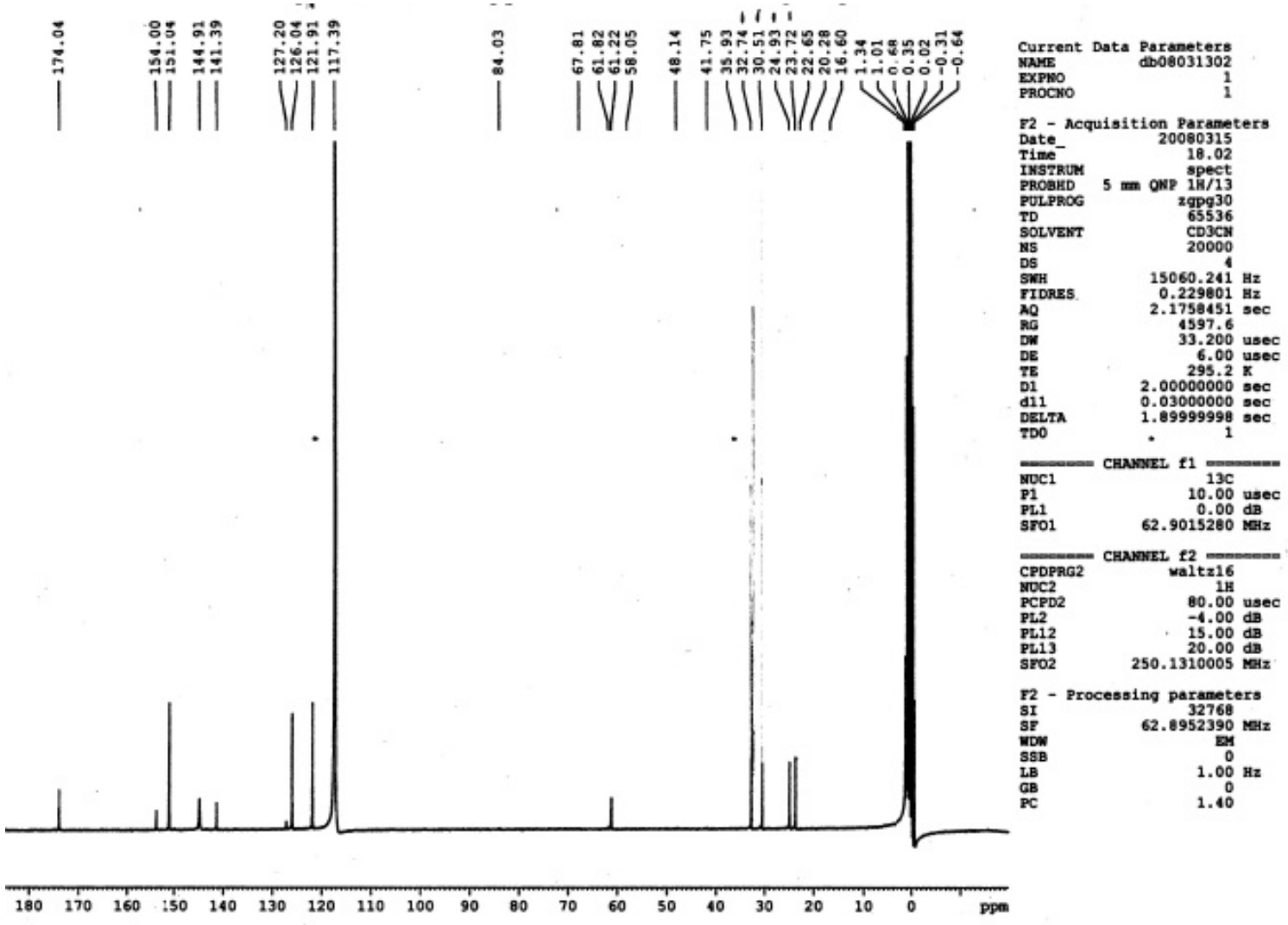
F2 - Acquisition Parameters
Date_ 20050630
Time 15.28
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CD3CN
NS 32
DS 2
SWH 5144.033 Hz
FIDRES 0.156983 Hz
AQ 3.1850996 se
RG 1625.5
DW 97.200 us
DE 6.00 us
TE 300.0 K
D1 1.00000000 se

========== CHANNEL f1 ==
NUC1 1H
P1 10.40 us
PL1 -4.00 dB
SFO1 250.1315447 MH

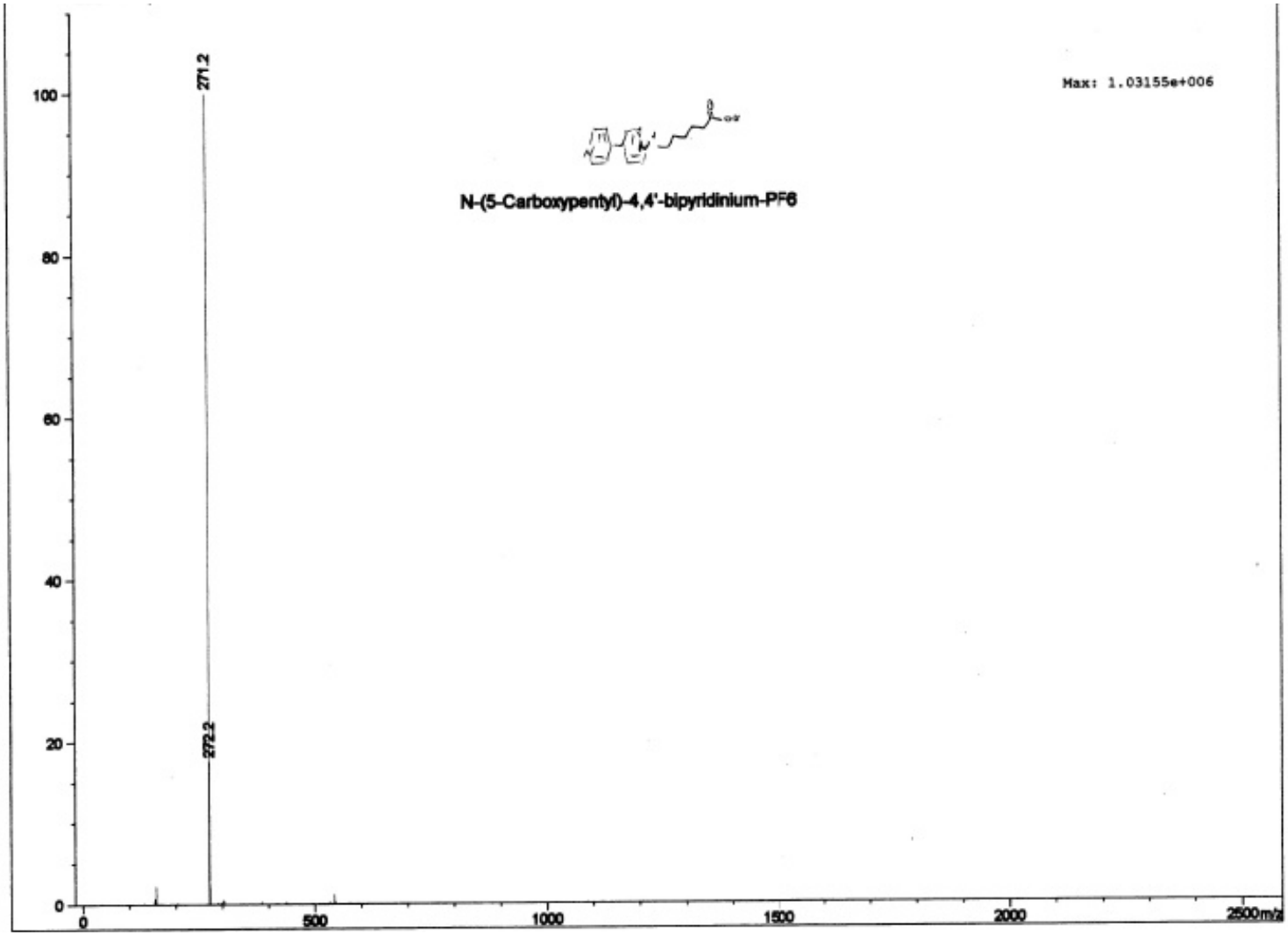
F2 - Processing parameters
SI 16384
SF 250.1300284 MH
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 11.427 pp
F1 2858.25 Hz
F2P -0.484 pp
F2 -121.03 Hz
PPMCM 0.59555 pp
HZCM 148.96376 Hz

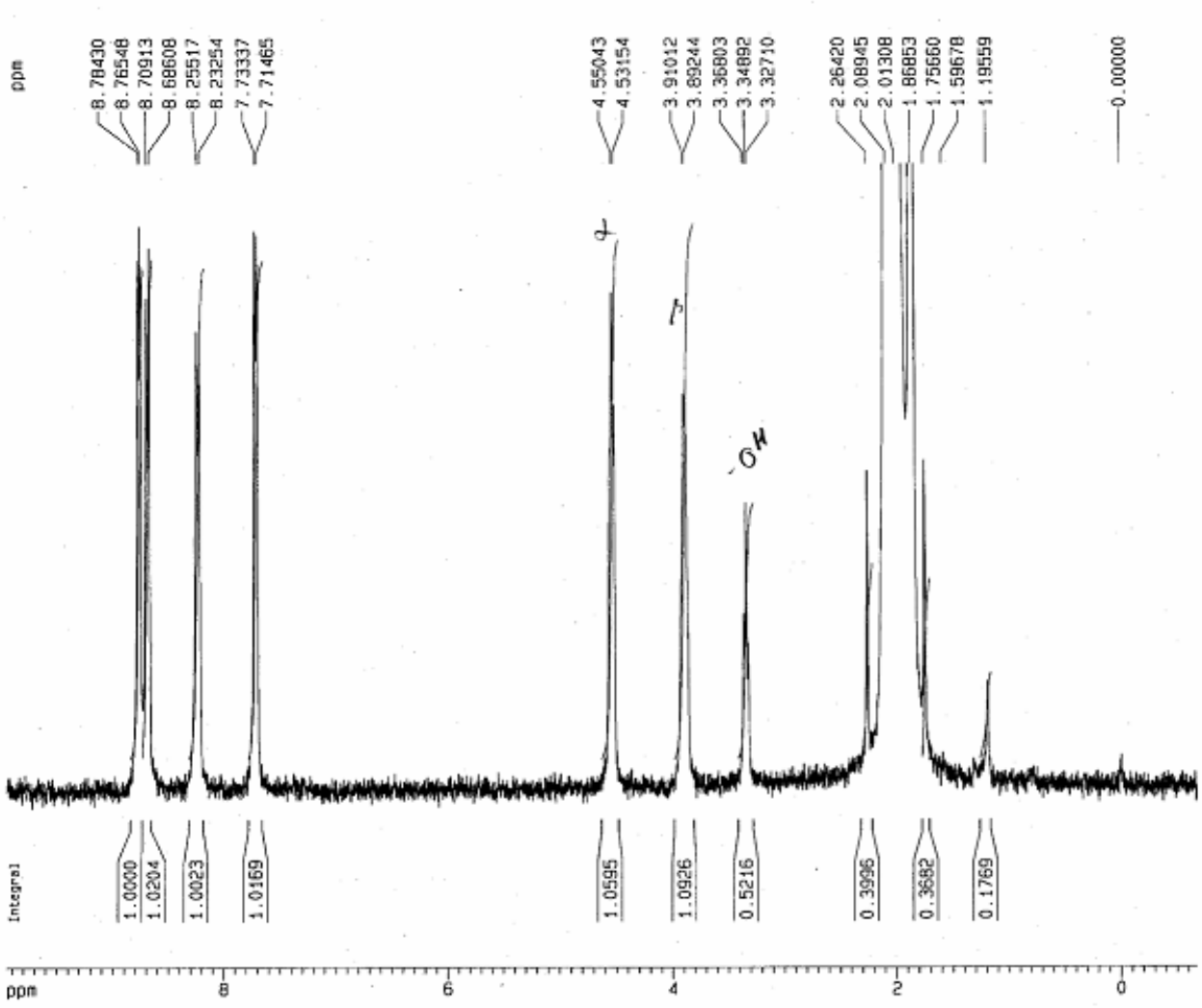
^{13}C-NMR spectra of compound **4** :



ESI-MS spectra of compound **4** :

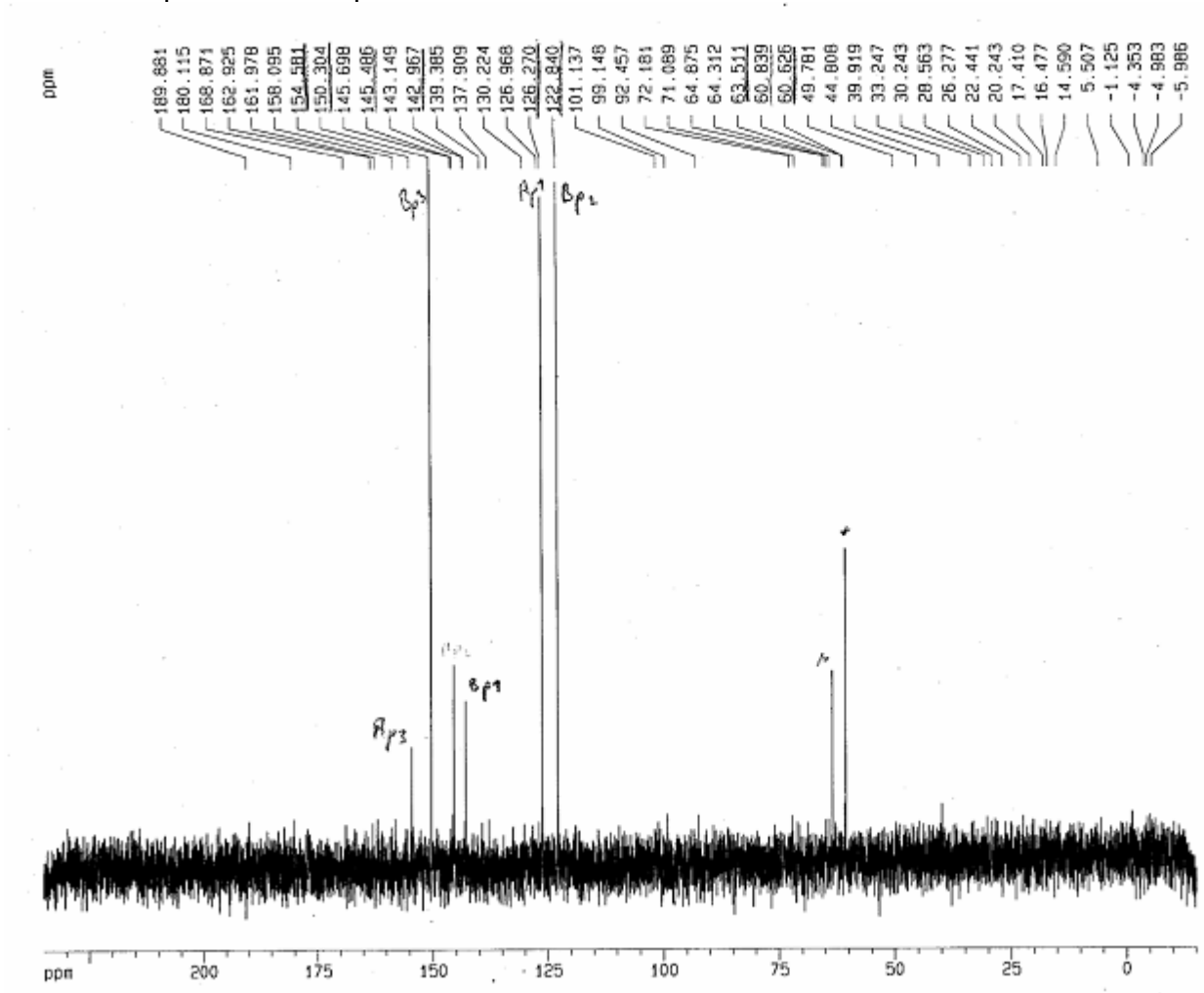


[1]H-NMR spectra of compound **5**




```
Current Data Parameters
NAME          db06051202
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20050512
Time                9.56
INSTRUM            spect
PROBHD     5 mm QNP 1H/1
PULPROG             zg30
TD                 32768
SOLVENT            CD3CN
NS                    32
DS                     2
SWH           5144.033 Hz
FIDRES        0.156983 Hz
AQ            3.1850996 sec
RG                1290.2
DW                97.200 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                10.40 usec
PL1               -4.00 dB
SF01         250.1315447 MHz

F2 - Processing parameters
SI                 16384
SF          250.1300265 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                1.00

1D NMR plot parameters
CX                20.00 cm
F1P               9.949 ppm
F1                2488.42 Hz
F2P               -0.673 ppm
F2                -168.32 Hz
PPMCM          0.53107 ppm/cm
HZCM          132.83740 Hz/cm
```

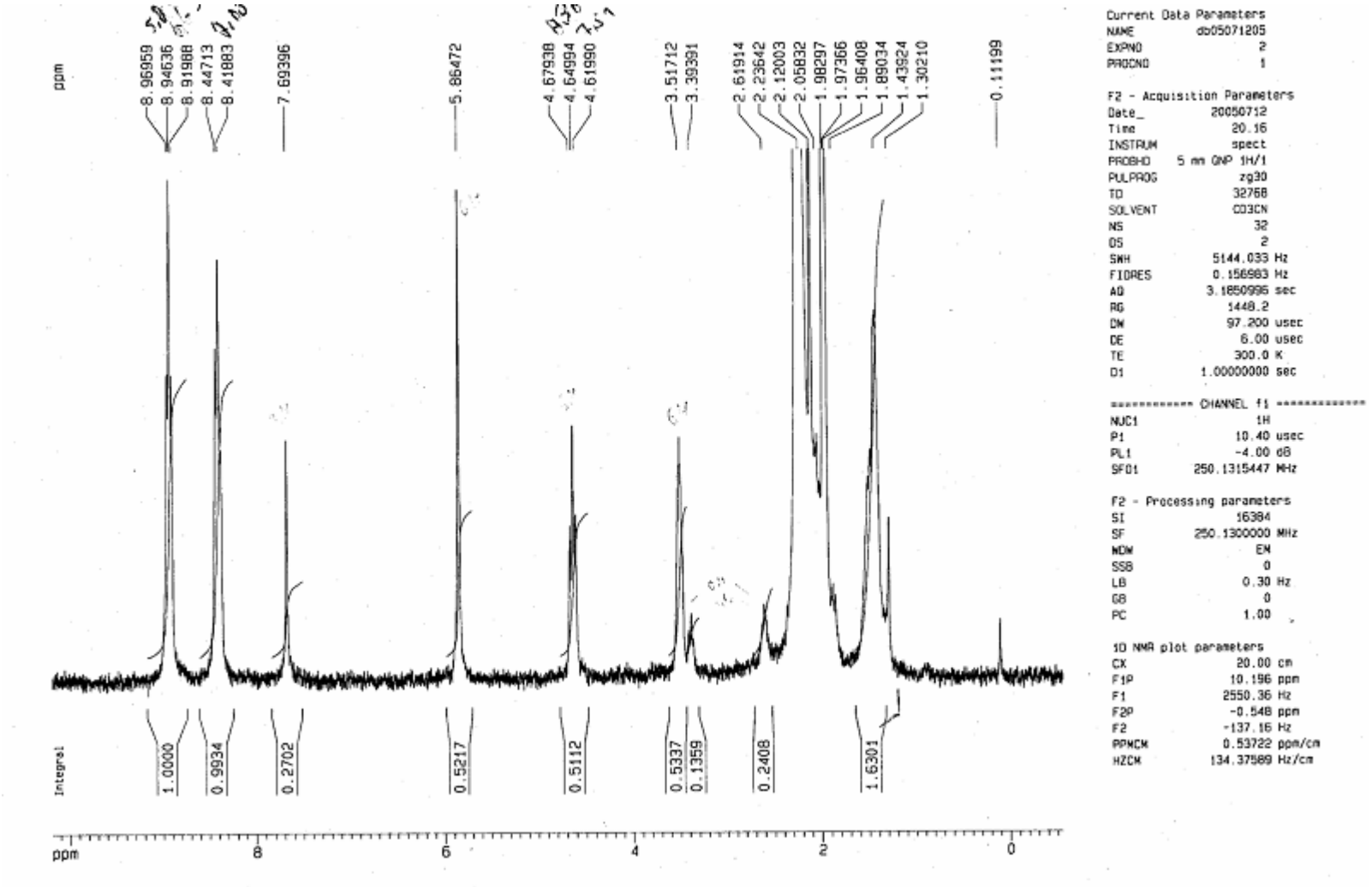
^{13}C-NMR spectra of compound **5**




```
Current Data Parameters
NAME            do030225
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20030226
Time                8.34
INSTRUM             spect
PROBHD    5 mm GNP 1H/1
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                 17922
DS                     4
SWH            15723.271 Hz
FIDRES         0.239918 Hz
AQ             2.0840948 sec
RG                  4096
DW                31.800 usec
DE                 6.00 usec
TE                300.0 K
D1             2.00000000 sec
d11            0.03000000 sec
d12            0.00002000 sec

============ CHANNEL f1 =============
NUC1                13C
P1                10.00 usec
PL1                0.00 dB
SF01          62.9021320 MHz

============ CHANNEL f2 =============
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -4.00 dB
PL12              15.00 dB
PL13              20.00 dB
SF02         250.1310005 MHz

F2 - Processing parameters
SI                 32768
SF            62.8952140 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                20.00 cm
F1P              234.988 ppm
F1               14779.62 Hz
F2P              -15.004 ppm
F2               -943.65 Hz
PPMCM            12.49958 ppm/cm
HZCM             786.16357 Hz/cm
```

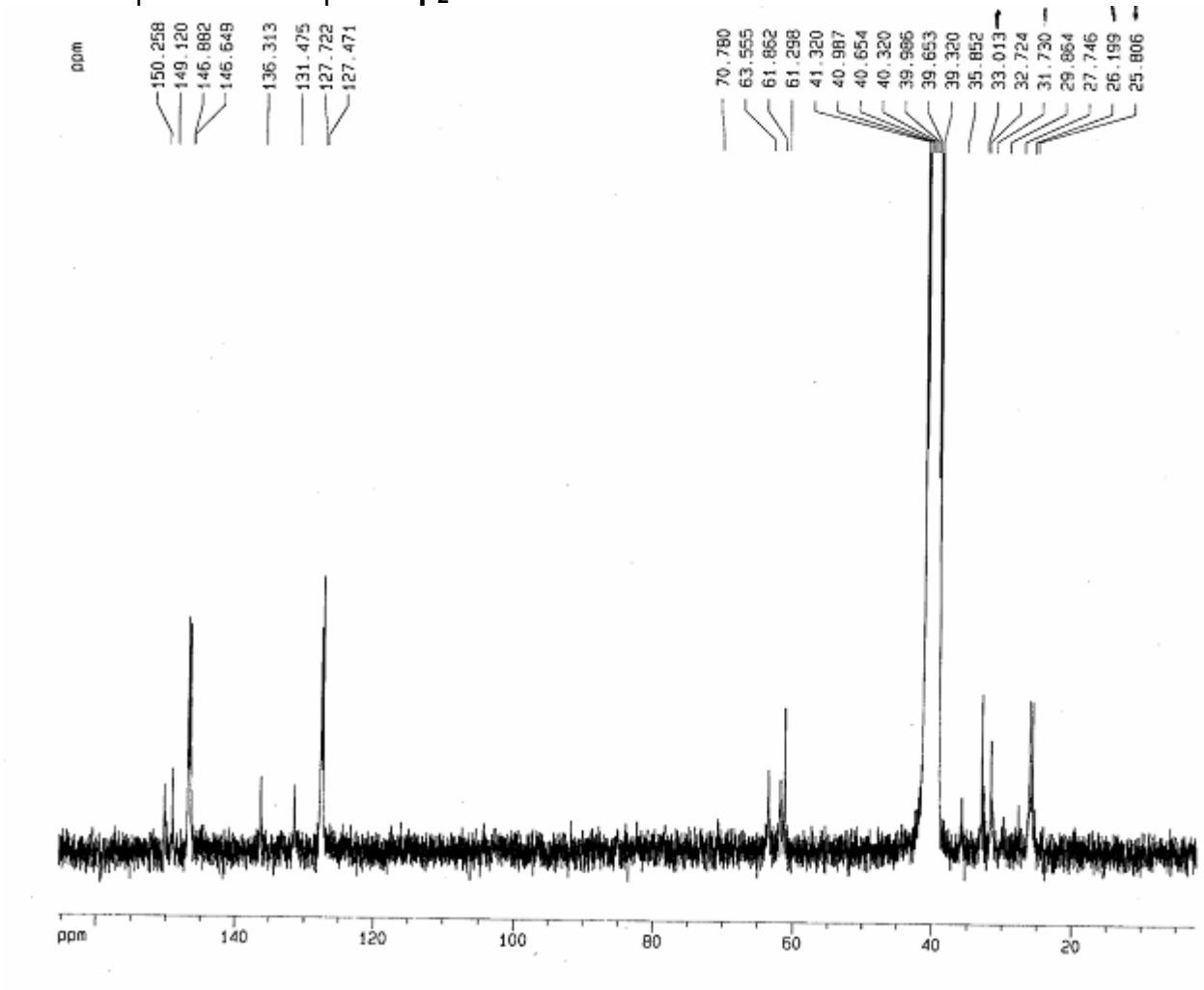
:

^{1}H-NMR spectra of compound **p_2OH** :



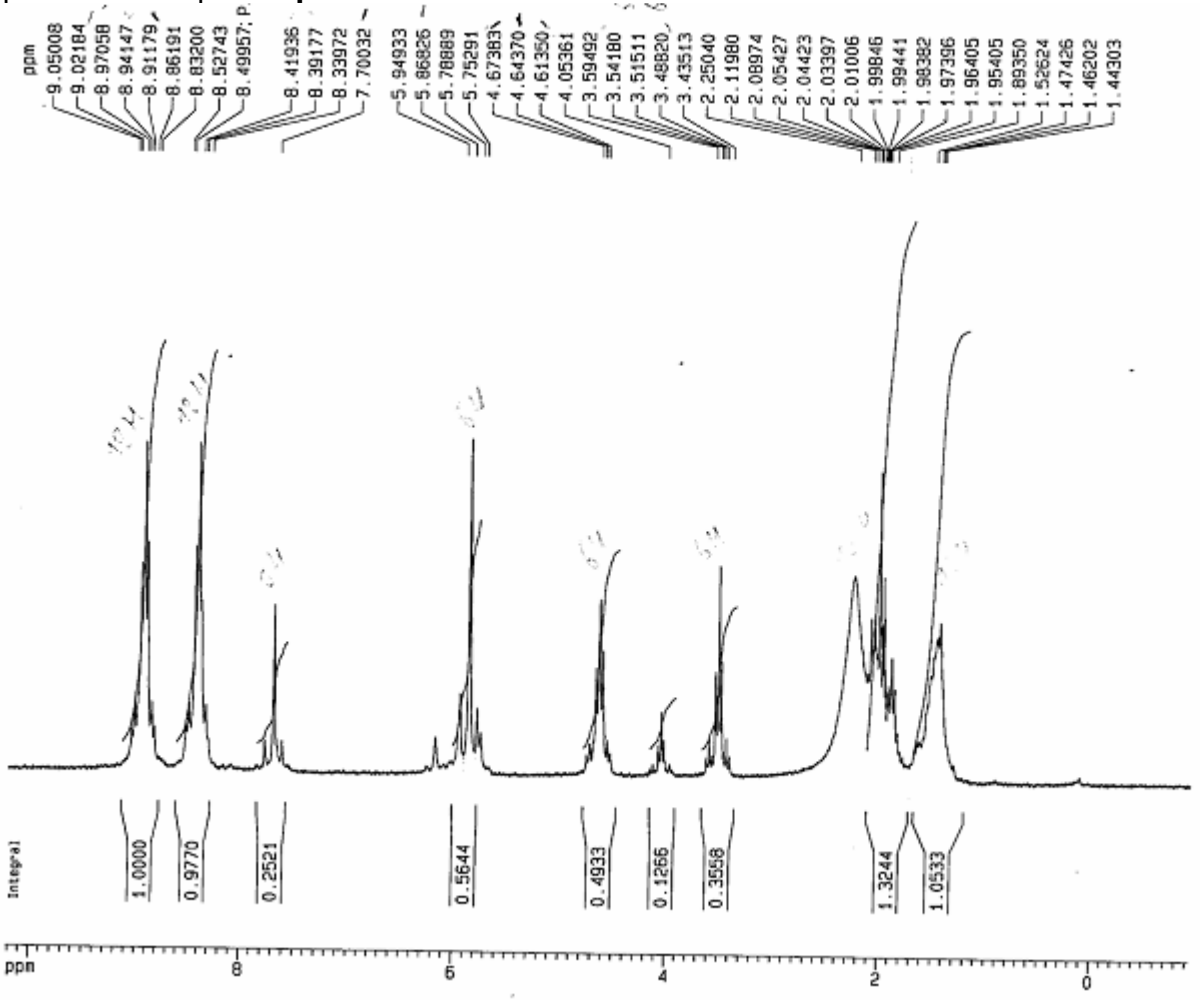

```
Current Data Parameters
NAME            db05071205
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20050712
Time                 20.16
INSTRUM              spect
PROBHD     5 mm QNP 1H/1
PULPROG              zg30
TD                  32768
SOLVENT             CD3CN
NS                     32
DS                      2
SWH              5144.033 Hz
FIDRES           0.156983 Hz
AQ               3.1850996 sec
RG                  1448.2
DW                 97.200 usec
DE                  6.00 usec
TE                 300.0 K
D1              1.00000000 sec

============ CHANNEL f1 ============
NUC1                   1H
P1                 10.40 usec
PL1                -4.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                    16384
SF              250.1300000 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
CX                   20.00 cm
F1P                 10.196 ppm
F1                 2550.36 Hz
F2P                 -0.548 ppm
F2                 -137.16 Hz
PPMCM              0.53722 ppm/cm
HZCM              134.37589 Hz/cm
```

^{13}C-NMR spectra of compound **p_2OH** :



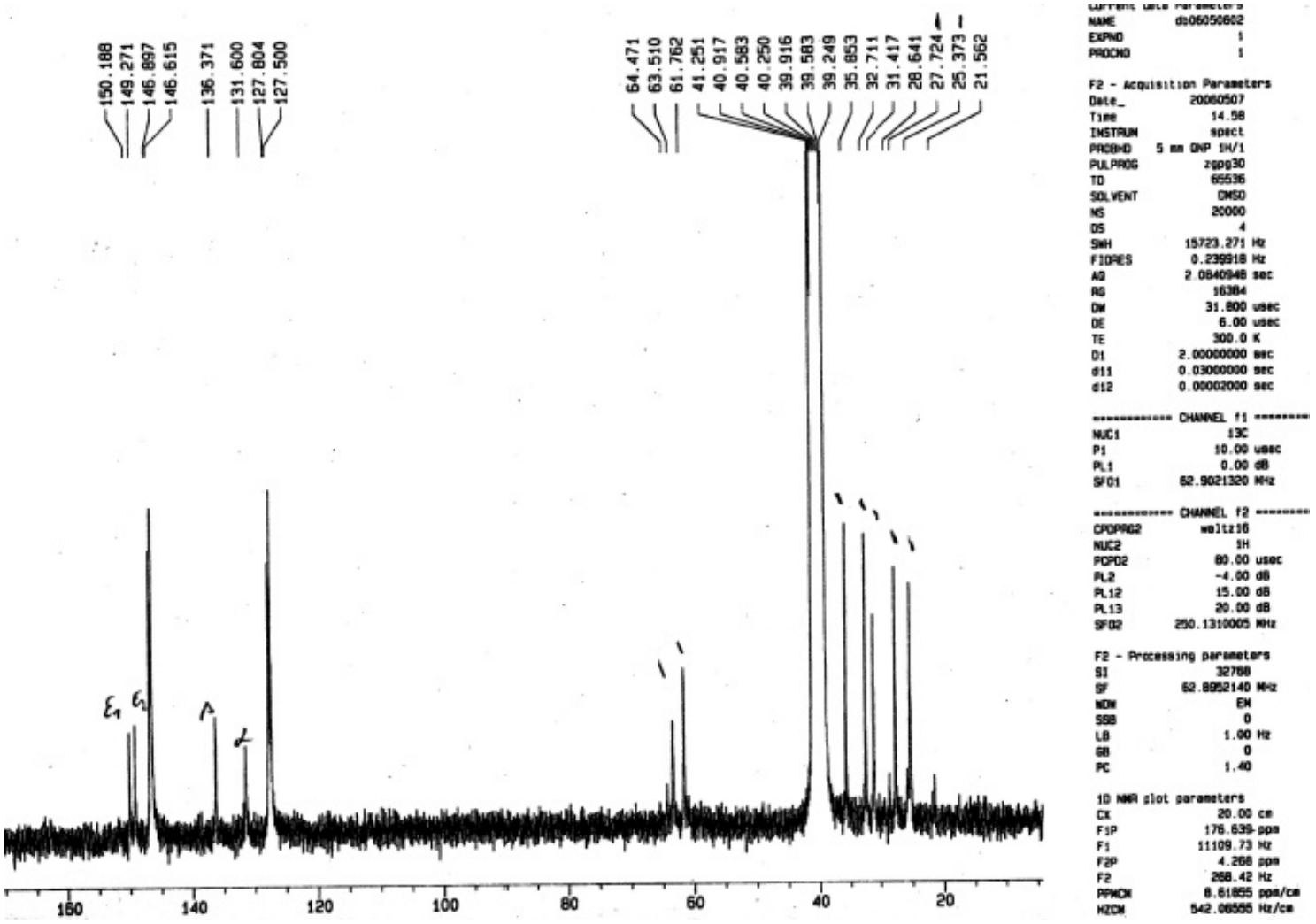

```
NAME            db06051002
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20060509
Time                 10.10
INSTRUM              spect
PROBHD     5 mm QNP 1H/1
PULPROG          zgpg30
TD                  65536
SOLVENT              DMSO
NS                   12177
DS                       4
SWH              15723.271 Hz
FIDRES           0.239918 Hz
AQ               2.0840948 sec
RG                    4096
DW                31.800 usec
DE                 6.00 usec
TE                 300.0 K
D1               2.00000000 sec
d11              0.03000000 sec
d12              0.00002000 sec

========== CHANNEL f1 ==========
NUC1                   13C
P1                  10.00 usec
PL1                  0.00 dB
SF01            62.9021320 MHz

========== CHANNEL f2 ==========
CPDPRG2             waltz16
NUC2                    1H
PCPD2               80.00 usec
PL2                 -4.00 dB
PL12                15.00 dB
PL13                20.00 dB
SF02           250.1310005 MHz

F2 - Processing parameters
SI                     32768
SF              62.8952140 MHz
WDW                       EM
SSB                        0
LB                  1.00 Hz
GB                         0
PC                  1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                165.397 ppm
F1                 10402.69 Hz
F2P                  2.127 ppm
F2                 133.75 Hz
PPMCM              8.16354 ppm/cm
HZCM              513.44733 Hz/cm
```

^{1}H-NMR spectra of compound **p_2Br** :

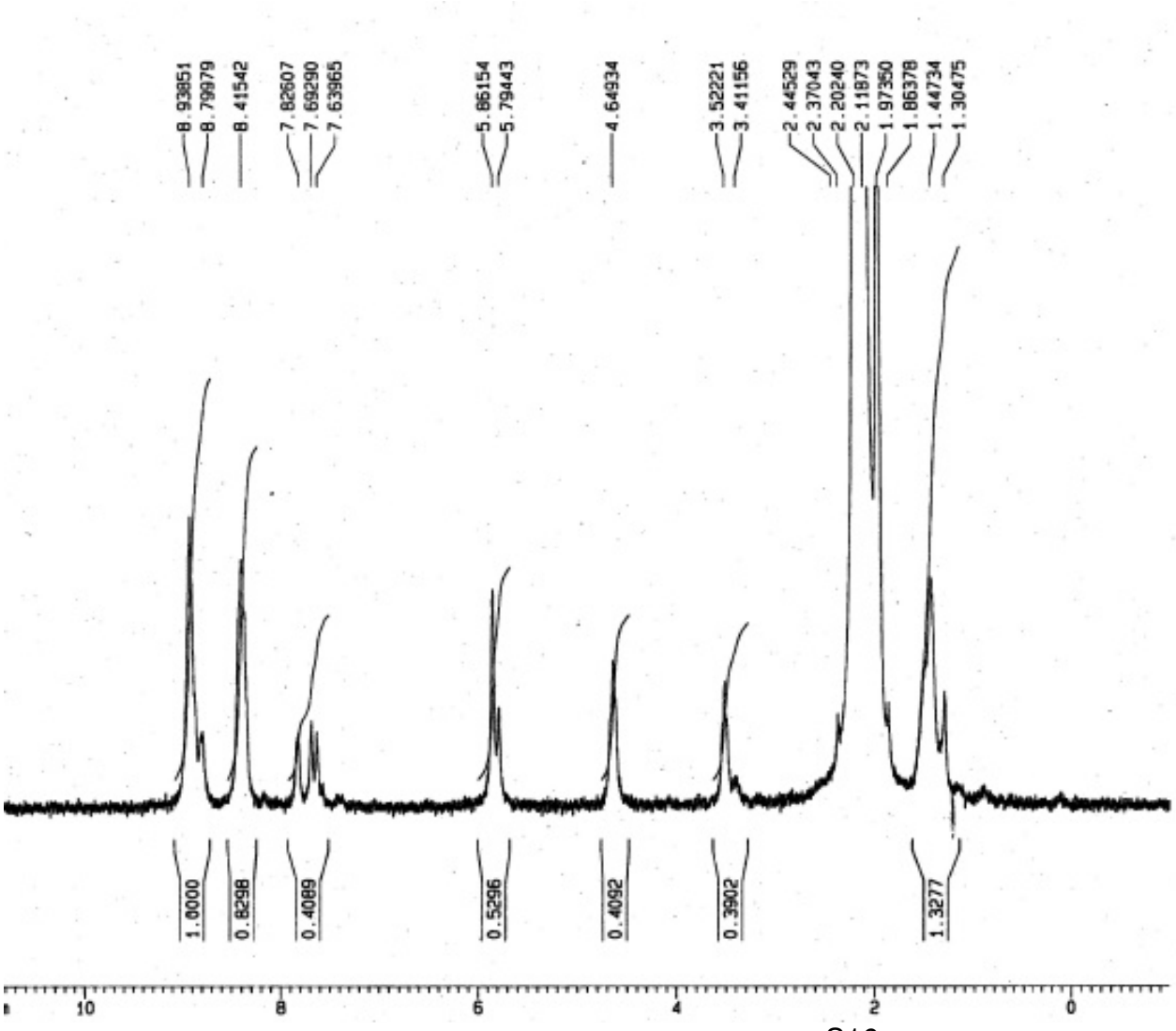



```
Current Data Parameters
NAME                  db07080601
EXPNO                          1
PROCNO                         1

F2 - Acquisition Parameters
Date_                   20070806
Time                       19.24
INSTRUM                    spect
PROBHD     5 mm QNP 1H/1
PULPROG                     zg30
TD                         32768
SOLVENT                    CD3CN
NS                            32
DS                             2
SWH                   5144.033 Hz
FIDRES                0.156983 Hz
AQ                    3.1850996 sec
RG                       1290.2
DW                      97.200 usec
DE                        6.00 usec
TE                       300.0 K
D1                    1.00000000 sec

========== CHANNEL f1 ==========
NUC1                         1H
P1                        10.40 usec
PL1                       -4.00 dB
SFO1                250.1315447 MHz

F2 - Processing parameters
SI                         16384
SF                  250.1300000 MHz
WDW                           EM
SSB                            0
LB                          0.30 Hz
GB                             0
PC                          1.00

1D NMR plot parameters
CX                         20.00 cm
F1P                        10.206 ppm
F1                       2553.25 Hz
F2P                       -0.936 ppm
F2                       -234.24 Hz
PPMCM                  0.55721 ppm/cm
HZCM                 139.37471 Hz/cm
```

^{13}C-NMR spectra of compound **p$_2$-Br**:

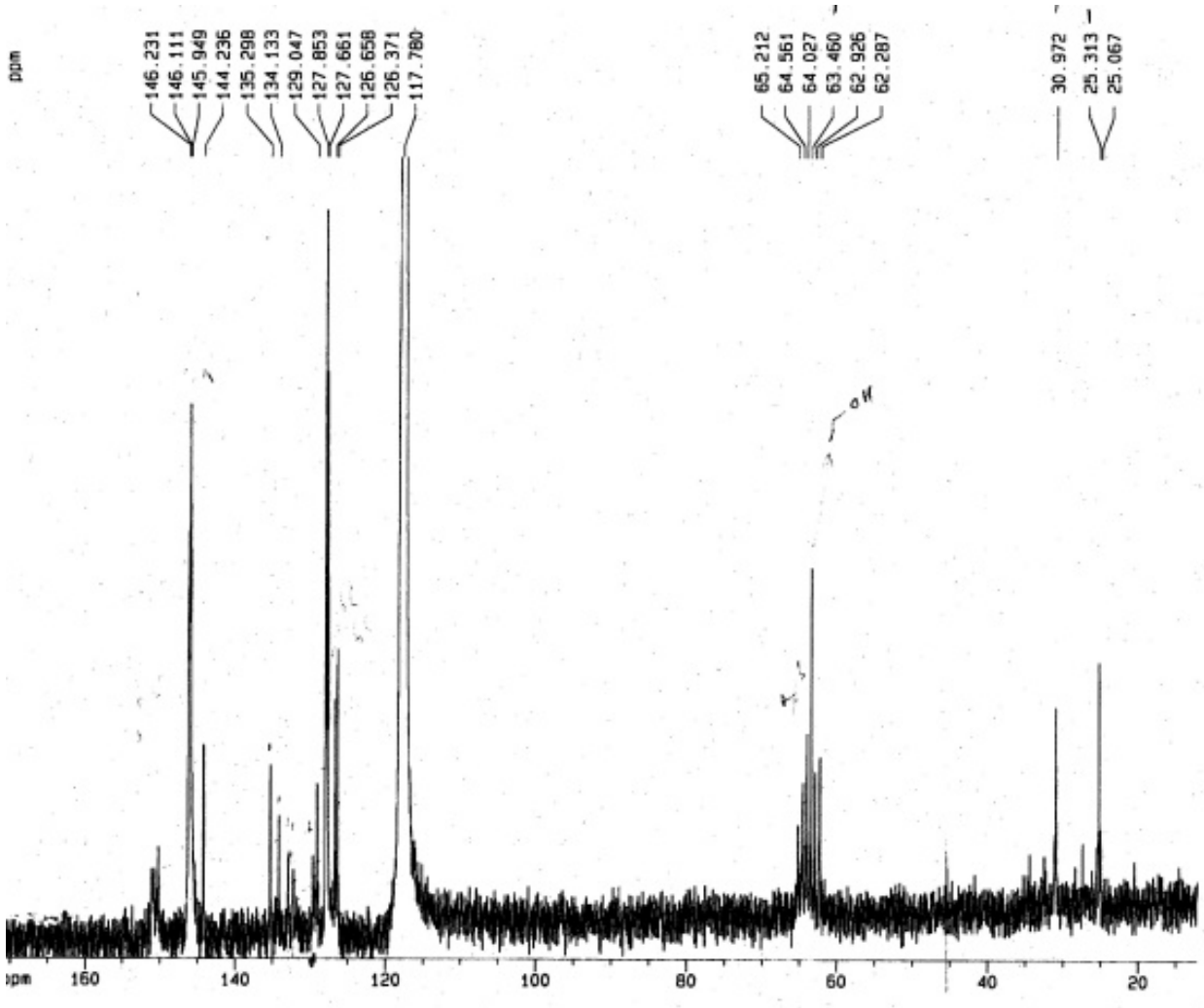


^{1}H-NMR spectra of compound **p_3OH** : Hexahydroxy-precursor



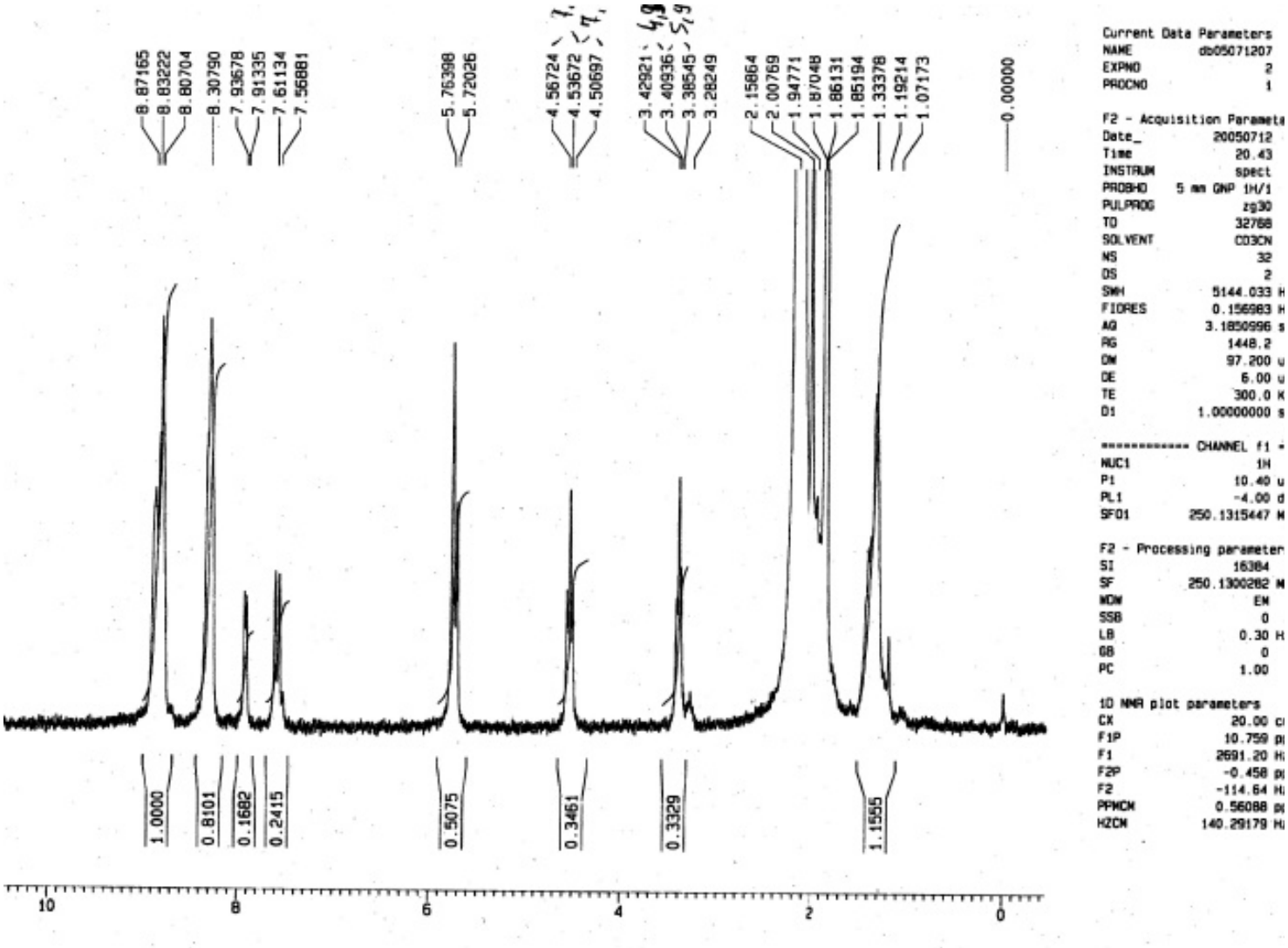

```
Current Data Parameters
NAME            db05071801
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20050718
Time                 14.15
INSTRUM              spect
PROBHD     5 mm QNP 1H/1
PULPROG              zg30
TD                  32768
SOLVENT             CD3CN
NS                      32
DS                       2
SWH              5144.033 Hz
FIDRES           0.156983 Hz
AQ               3.1850996 sec
RG                  1824.6
DW                 97.200 usec
DE                  6.00 usec
TE                 300.0 K
D1              1.00000000 sec

------------ CHANNEL f1 -------
NUC1                    1H
P1                   10.40 usec
PL1                  -4.00 dB
SFO1           250.1315447 MHz

F2 - Processing parameters
SI                      16384
SF             250.1300000 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
CX                   20.00 cm
F1P                 11.073 ppm
F1                2769.68 Hz
F2P                 -0.991 ppm
F2                -247.80 Hz
PPMCM             0.60318 ppm/cm
HZCM            150.87395 Hz/cm
```

^{13}C-NMR spectra of compound **p_3OH** :

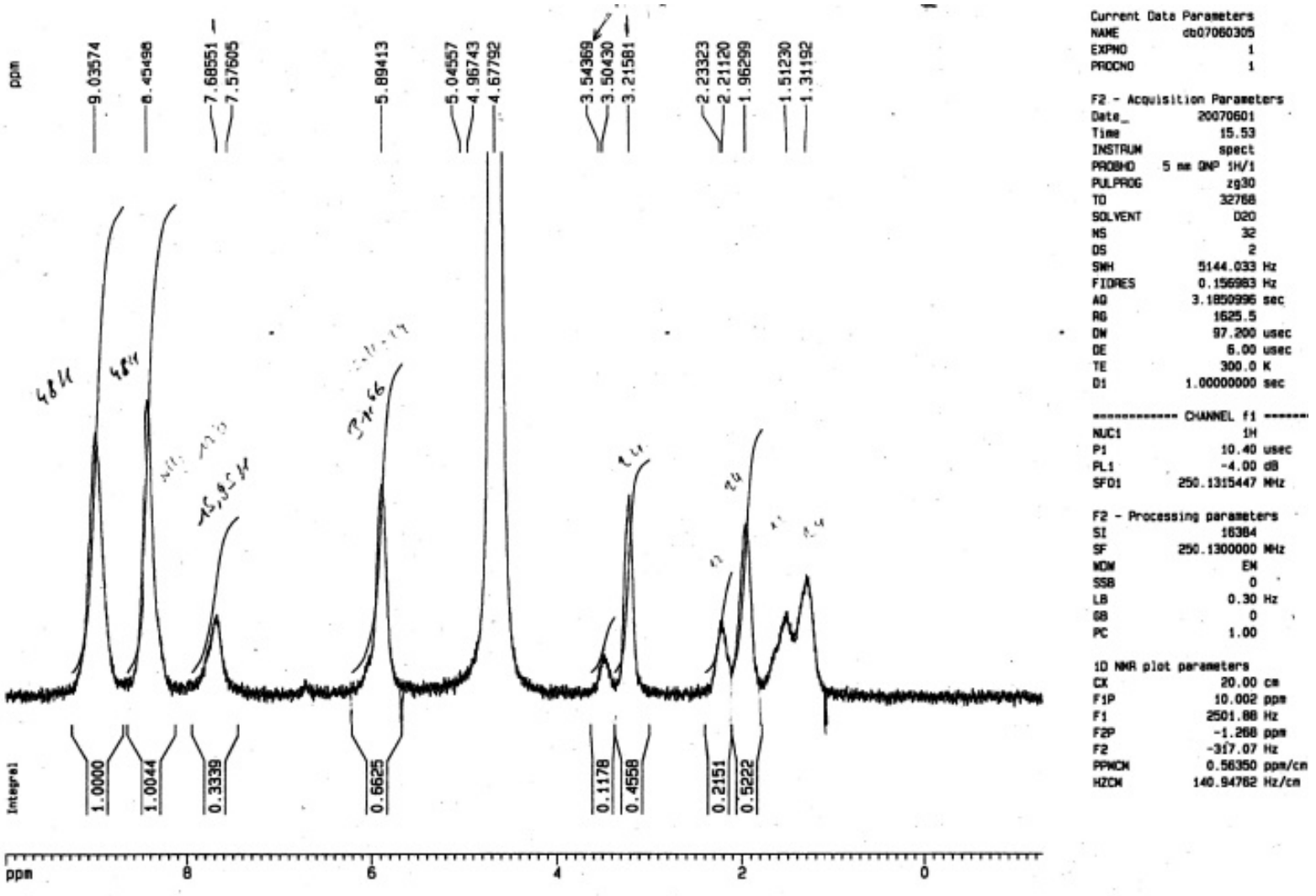



```
NAME            ds05080602
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_          20050808
Time                16.41
INSTRUM             spect
PROBHD   5 mm DNP 1H/1
PULPROG          zgpg30
TD                65536
SOLVENT            CD3CN
NS                 22258
DS                     4
SWH           15723.271 Hz
FIDRES        0.239918 Hz
AQ            2.0840948 sec
RG                  8192
DW                31.800 usec
DE                 6.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ======
NUC1                13C
P1                10.00 usec
PL1                0.00 dB
SFO1         62.9021320 MHz

========== CHANNEL f2 ======
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -4.00 dB
PL12              15.00 dB
PL13              20.00 dB
SFO2         250.1310005 MHz

F2 - Processing parameters
SI                 32768
SF            62.8952140 MHz
WDW                   EM
SSB                    0
LB                 1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                20.00 cm
F1P              171.243 ppm
F1              10770.37 Hz
F2P               12.220 ppm
F2                768.61 Hz
PPMCM           7.95113 ppm/cm
HZCM          500.08786 Hz/cm
```

^{1}H-NMR spectra of compound **p_3Br** : Hexa-bromo-precursor



^{1}H-NMR spectra of dendrimer : **G_1-Hexyl-COOH**



Current Data Parameters
NAME cb07060305
EXPNO 1
PROCNO 1

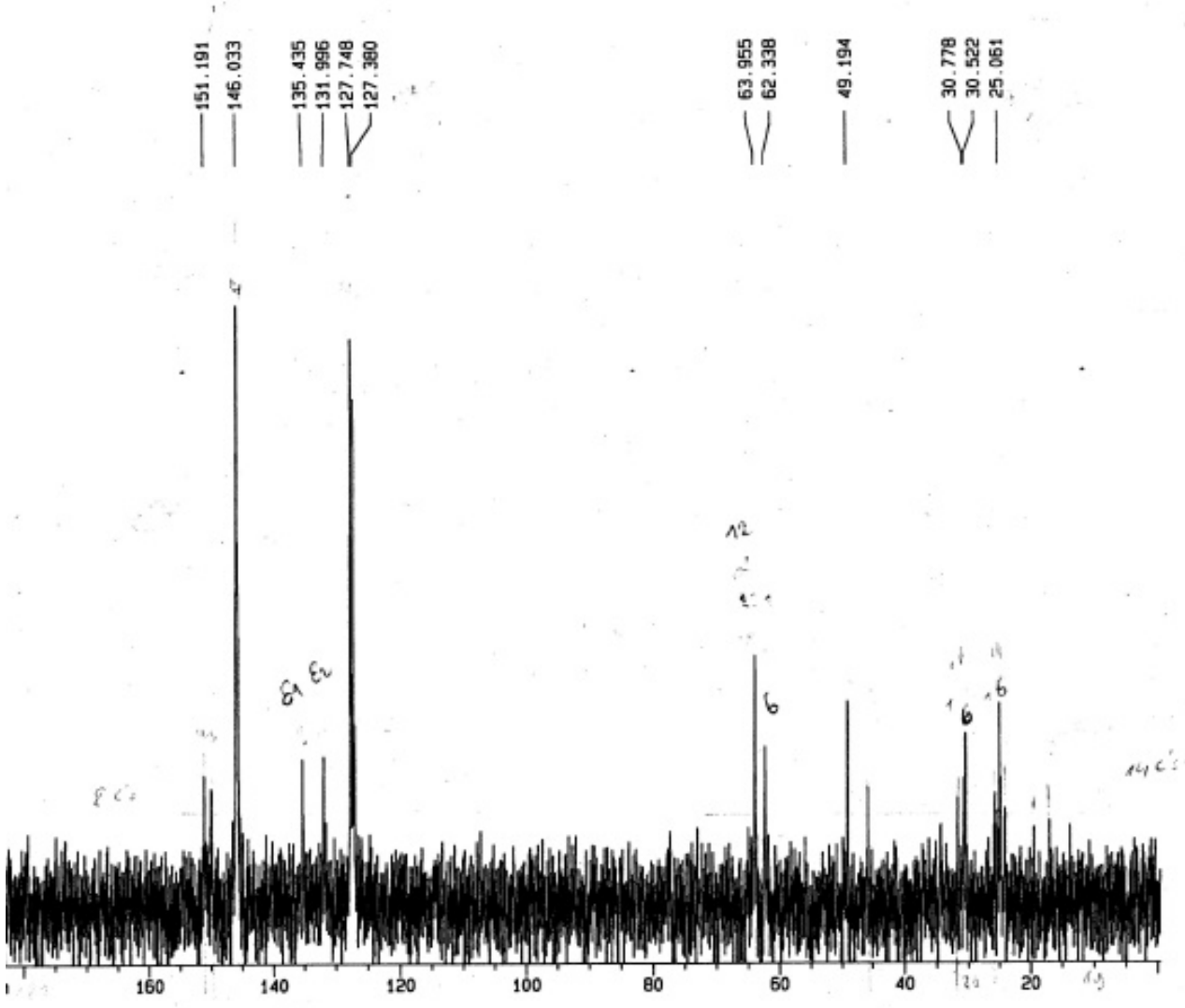
F2 - Acquisition Parameters
Date_ 20070601
Time 15.53
INSTRUM spect
PROBHD 5 mm BNP 1H/1
PULPROG zg30
TD 32768
SOLVENT D2O
NS 32
DS 2
SWH 5144.033 Hz
FIDRES 0.156983 Hz
AQ 3.1850996 sec
RG 1625.5
DW 97.200 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

---------- CHANNEL f1 ----------
NUC1 1H
P1 10.40 usec
PL1 -4.00 dB
SFO1 250.1315447 MHz

F2 - Processing parameters
SI 16384
SF 250.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

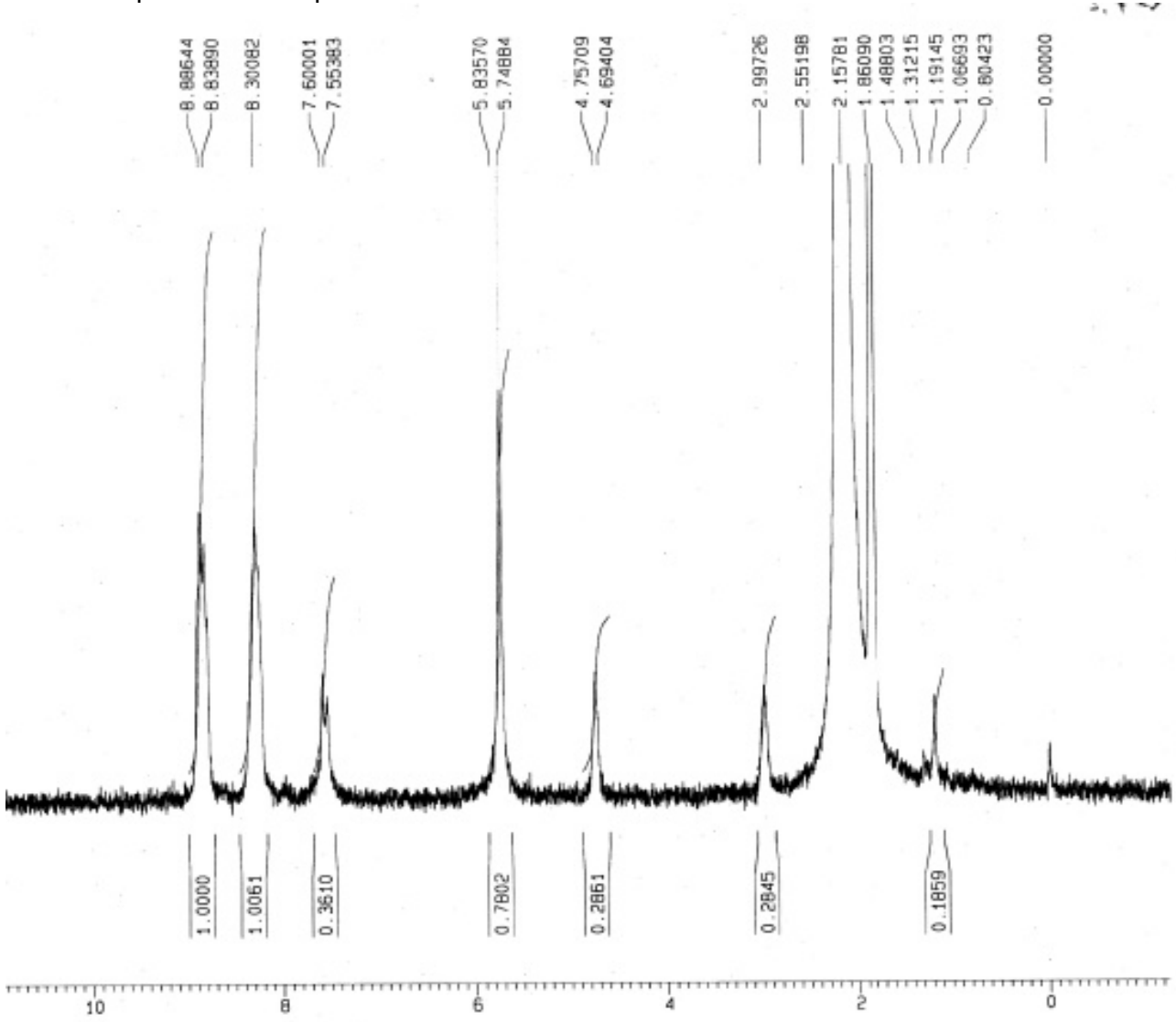
1D NMR plot parameters
CX 20.00 cm
F1P 10.002 ppm
F1 2501.88 Hz
F2P -1.268 ppm
F2 -317.07 Hz
PPMCM 0.56350 ppm/cm
HZCM 140.94762 Hz/cm

^{13}C-NMR spectra of dendrimer **G_1-Hexyl-COOH** :



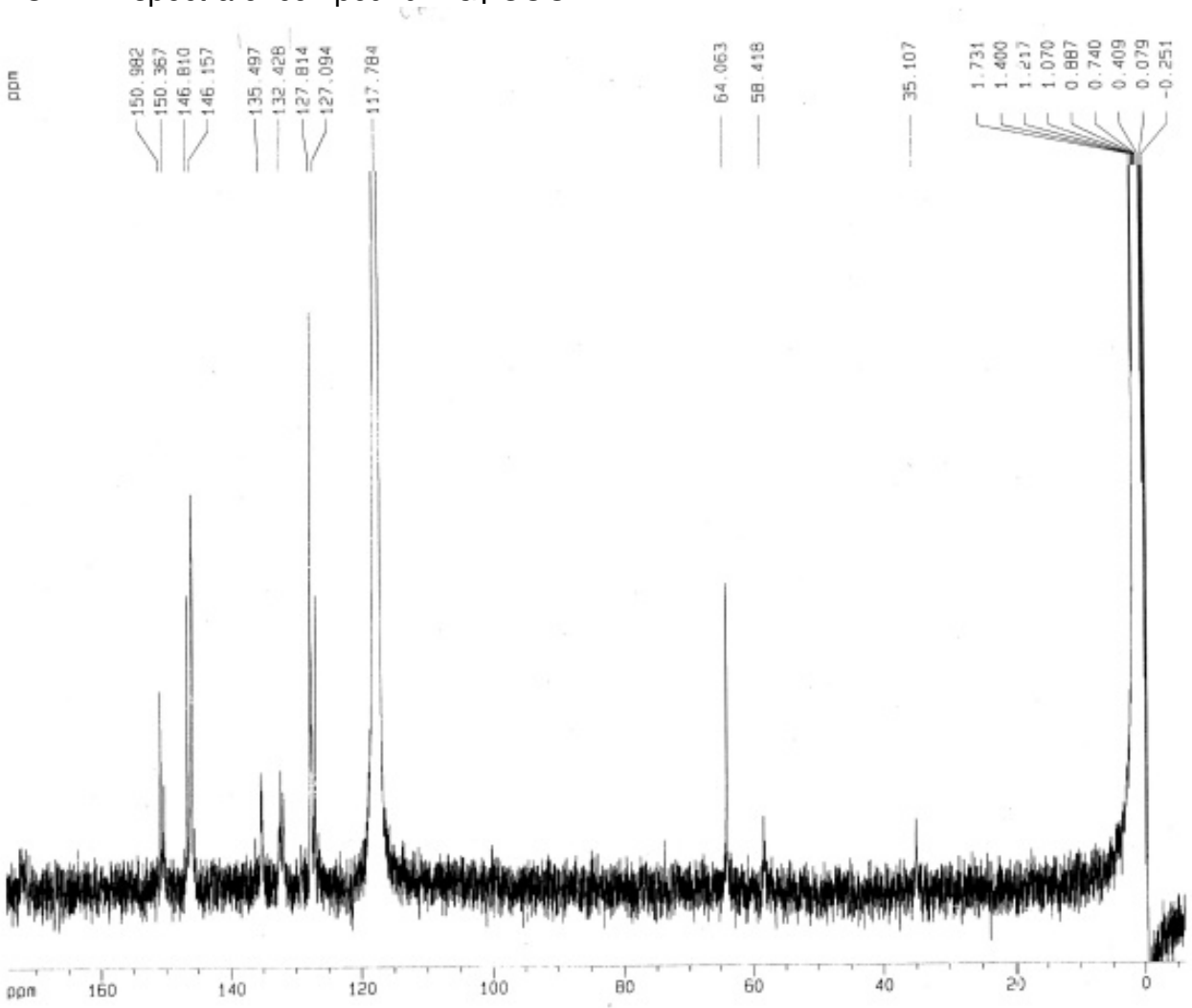

```
Current Data Parameters
NAME            db07060401
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20070604
Time                  9.04
INSTRUM               spect
PROBHD     5 mm BNP 1H/1
PULPROG           zgpg30
TD                 65536
SOLVENT              D2O
NS                   57297
DS                       4
SWH           15723.271 Hz
FIDRES        0.239918 Hz
AQ            2.0840948 sec
RG                    8192
DW               31.800 usec
DE                6.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

====== CHANNEL f1 =====
NUC1                 13C
P1                10.00 usec
PL1                0.00 dB
SFO1          62.9021320 MHz

====== CHANNEL f2 =====
CPDPRG2          waltz16
NUC2                  1H
PCPD2             80.00 usec
PL2               -4.00 dB
PL12              15.00 dB
PL13              20.00 dB
SFO2          250.1310005 MHz

F2 - Processing parameters
SI                   32768
SF            62.8952140 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                187.255 ppm
F1                 11777.44 Hz
F2P                -0.441 ppm
F2                 -27.73 Hz
PPMCM              9.38479 ppm/
HZCM             590.25867 Hz/
```

^{1}H-NMR spectra of compound : **G_1-COOH**



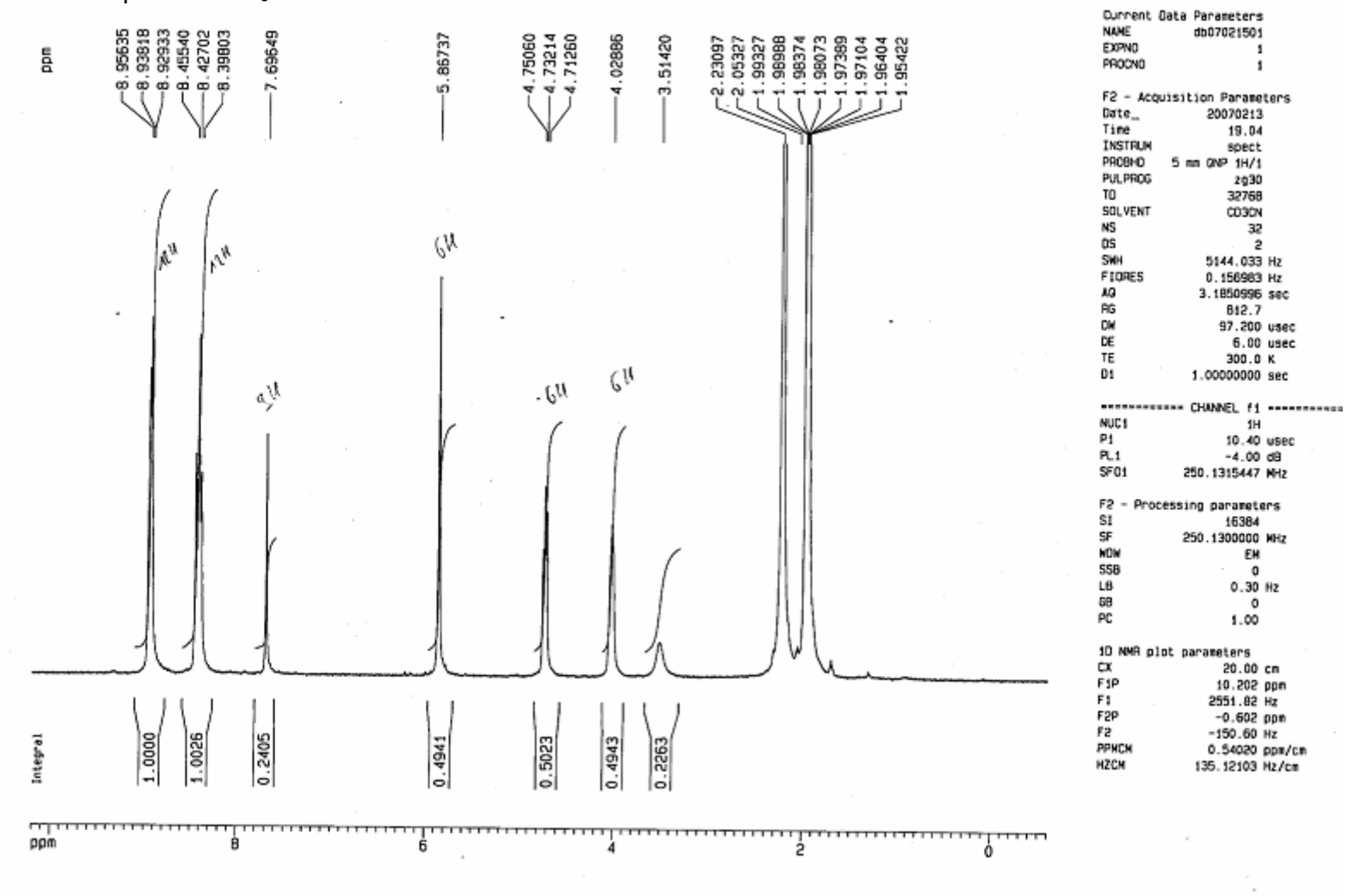

```
NAME            db05040503
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameter
Date_             20050405
Time                 15.37
INSTRUM              spect
PROBHD    5 mm QNP 1H/1
PULPROG              zg30
TD                  32768
SOLVENT              CD3CN
NS                      32
DS                       2
SWH              5144.033 Hz
FIDRES           0.156983 Hz
AQ               3.1850996 se
RG                    2048
DW                 97.200 us
DE                  6.00 us
TE                 300.0 K
D1              1.00000000 se

------------ CHANNEL f1 --
NUC1                    1H
P1                  10.40 us
PL1                 -4.00 dB
SFO1           250.1315447 MH

F2 - Processing parameters
SI                      16384
SF               250.1300279 MH
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
CX                   20.00 cm
F1P                 11.473 pp
F1                2869.75 Hz
F2P                -1.268 pp
F2                 -317.25 Hz
PPMCM             0.63707 pp
HZCM            159.35025 Hz
```

^{13}C-NMR spectra of compound : **G_1-COOH**



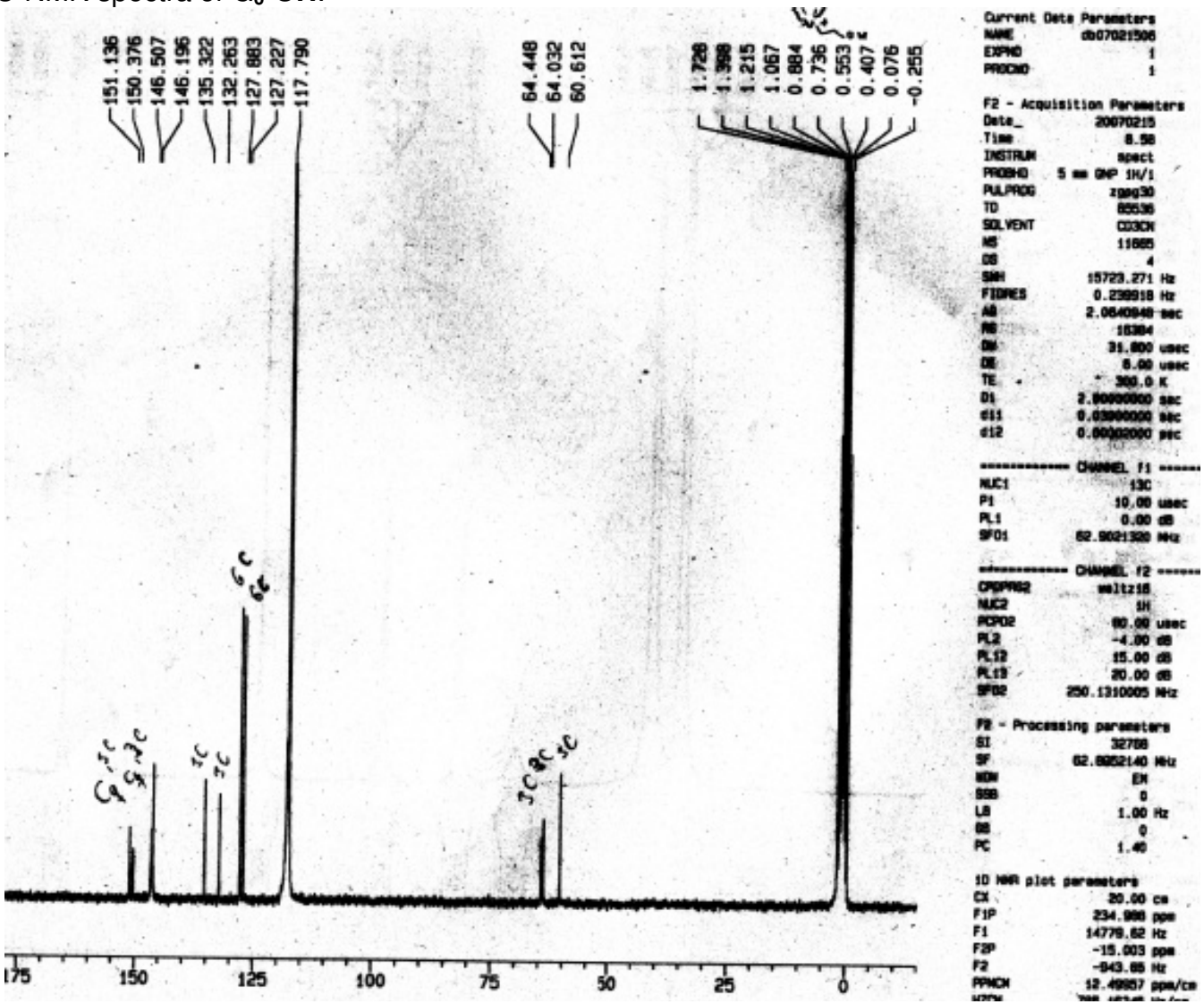
NAME	db06022205
EXPNO	2
PROCNO	1
F2 - Acquisition Parameters	
Date_	20060222
Time	14.12
INSTRUM	spect
PROBHD	5 mm BNP 1H/1
PULPROG	zgpg30
TD	65536
SOLVENT	CD3CN
NS	17948
DS	4
SWH	15723.271 Hz
FIDRES	0.239918 Hz
AQ	2.0840948 sec
RG	16384
DW	31.800 use
DE	6.00 use
TE	300.0 K
D1	2.00000000 sec
d11	0.03000000 sec
d12	0.00002000 sec
========== CHANNEL f1 ====	
NUC1	13C
P1	10.00 use
PL1	0.00 dB
SFO1	62.9021320 MHz
========== CHANNEL f2 ====	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 use
PL2	-4.00 dB
PL12	15.00 dB
PL13	20.00 dB
SFO2	250.1310005 MHz
F2 - Processing parameters	
SI	32768
SF	62.8952140 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40
1D NMR plot parameters	
CX	20.00 cm
F1P	174.600 pp
F1	10981.49 Hz
F2P	-6.038 pp
F2	-379.73 Hz
PPMCM	9.03187 pp
HZCM	568.06122 Hz

^{1}H-NMR spectra of **G_0-OH**:



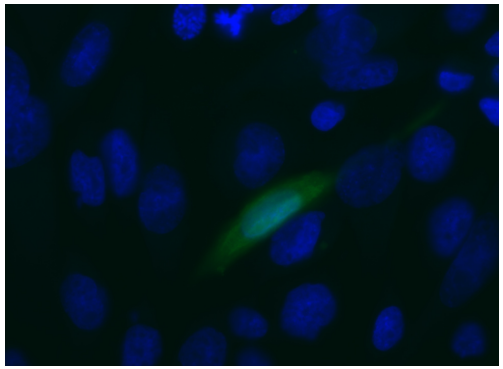

```
Current Data Parameters
NAME          db07021501
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20070213
Time               19.04
INSTRUM            spect
PROBHD  5 mm QNP 1H/1
PULPROG            zg30
TD                32768
SOLVENT            CD3CN
NS                    32
DS                     2
SWH           5144.033 Hz
FIDRES        0.156983 Hz
AQ            3.1850996 sec
RG                612.7
DW               97.200 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

------------ CHANNEL f1 ----------
NUC1                1H
P1                10.40 usec
PL1               -4.00 dB
SFO1         250.1315447 MHz

F2 - Processing parameters
SI                 16384
SF          250.1300000 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                20.00 cm
F1P               10.202 ppm
F1                2551.82 Hz
F2P               -0.602 ppm
F2                -150.60 Hz
PPMCM          0.54020 ppm/cm
HZCM          135.12103 Hz/cm
```

^{13}C-NMR spectra of **G_0-OH**:

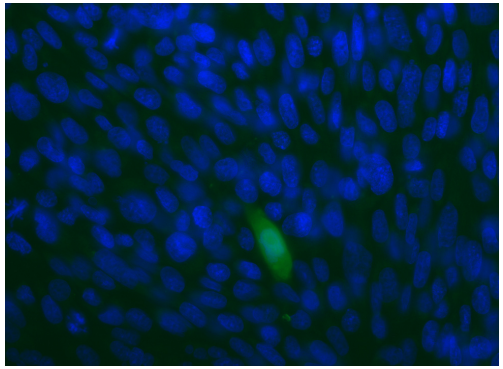



```
Current Data Parameters
NAME            db07021506
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_            20070215
Time                 8.58
INSTRUM             spect
PROBHD   5 mm GNP 1H/1
PULPROG             zgpg30
TD                   65536
SOLVENT              CD3CN
NS                   11665
DS                       4
SWH              15723.271 Hz
FIDRES           0.239918 Hz
AQ               2.0840948 sec
RG                   18384
DW                31.800 usec
DE                 6.00 usec
TE                 300.0 K
D1               2.00000000 sec
d11              0.03000000 sec
d12              0.00002000 sec

====== CHANNEL f1 ======
NUC1                    13C
P1                   10.00 usec
PL1                   0.00 dB
SFO1            62.9021320 MHz

====== CHANNEL f2 ======
CPDPRG2             waltz16
NUC2                    1H
PCPD2                90.00 usec
PL2                  -4.00 dB
PL12                 15.00 dB
PL13                 20.00 dB
SFO2           250.1310005 MHz

F2 - Processing parameters
SI                      32768
SF              62.8952140 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                234.998 ppm
F1                14779.62 Hz
F2P                -15.003 ppm
F2                 -943.85 Hz
PPMCM              12.49957 ppm/cm
```

2. Pictures of transfected CHO cells



6 µg Carboxy-term.,hexamethylene-bridged Dend. **G_1-Hexyl-COOH**
2 µg Plasmid-DNA, pC1-eGFP in 0.5 ml TE buffer



4 µg **G_1-Hexyl-COOH**
2 µg Plasmid-DNA, pC1-eGFP in 0.5 ml TE buffer