P^1,P^2-Diimidazolyl derivatives of pyrophosphate and *bis*-phosphonates – Synthesis, Properties, and Use in Preparation of Dinucleoside Tetraphosphates and Analogs

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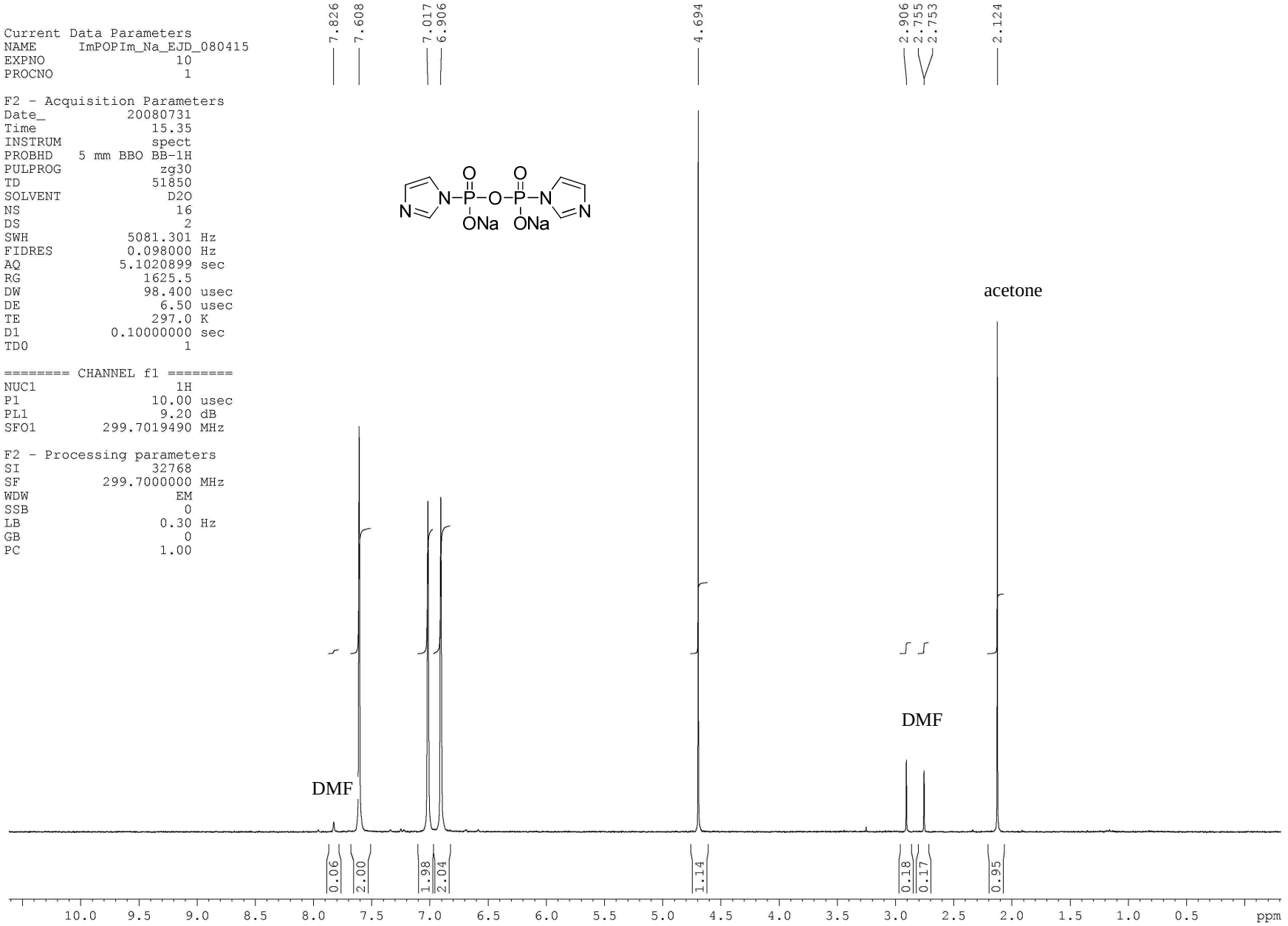
Supplementary Materials

^{1}H NMR of the di-sodium salt of compound **7a** in D_2O



^{31}P NMR (proton decoupled) of the di-sodium salt of compound **7a** in D_2O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PROCNO                       1

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FIDRES              0.357156 Hz
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DW                    10.275 usec
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d11               0.03000000 sec
DELTA             0.00000000 sec
TD0                          1

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PL1                      12.70 dB
SFO1             121.3207840 MHz

======== CHANNEL f2 ========
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NUC2                       1H
PCPD2                   100.00 usec
PL12                     29.00 dB
PL13                    120.00 dB
PL2                       9.00 dB
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F2 - Processing parameters
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PC                        1.40
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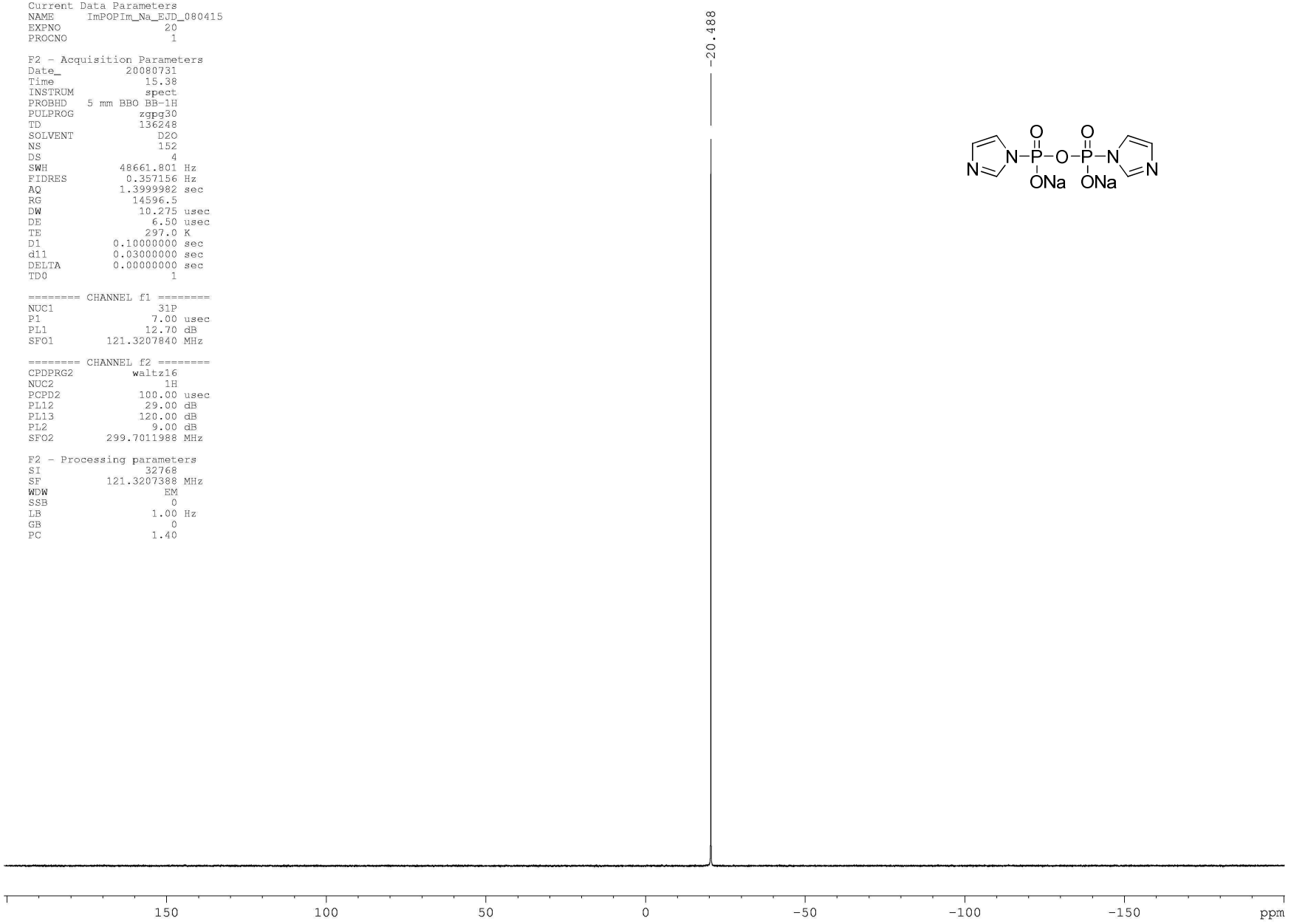

^{31}P NMR (proton coupled) of the di-sodium salt of compound **7a** in D_2O


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PROCNO                   1

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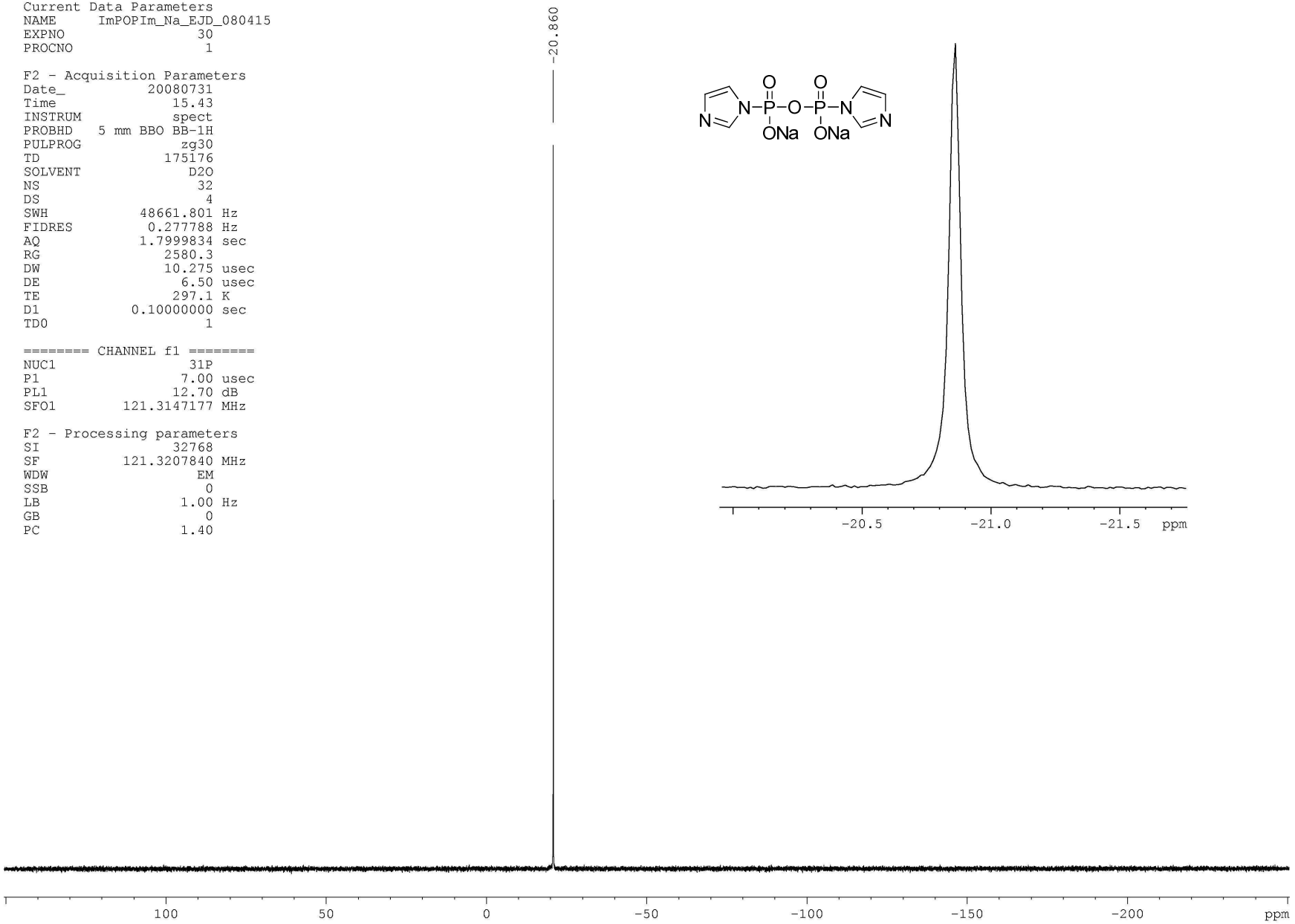
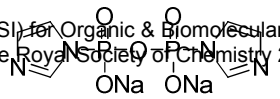
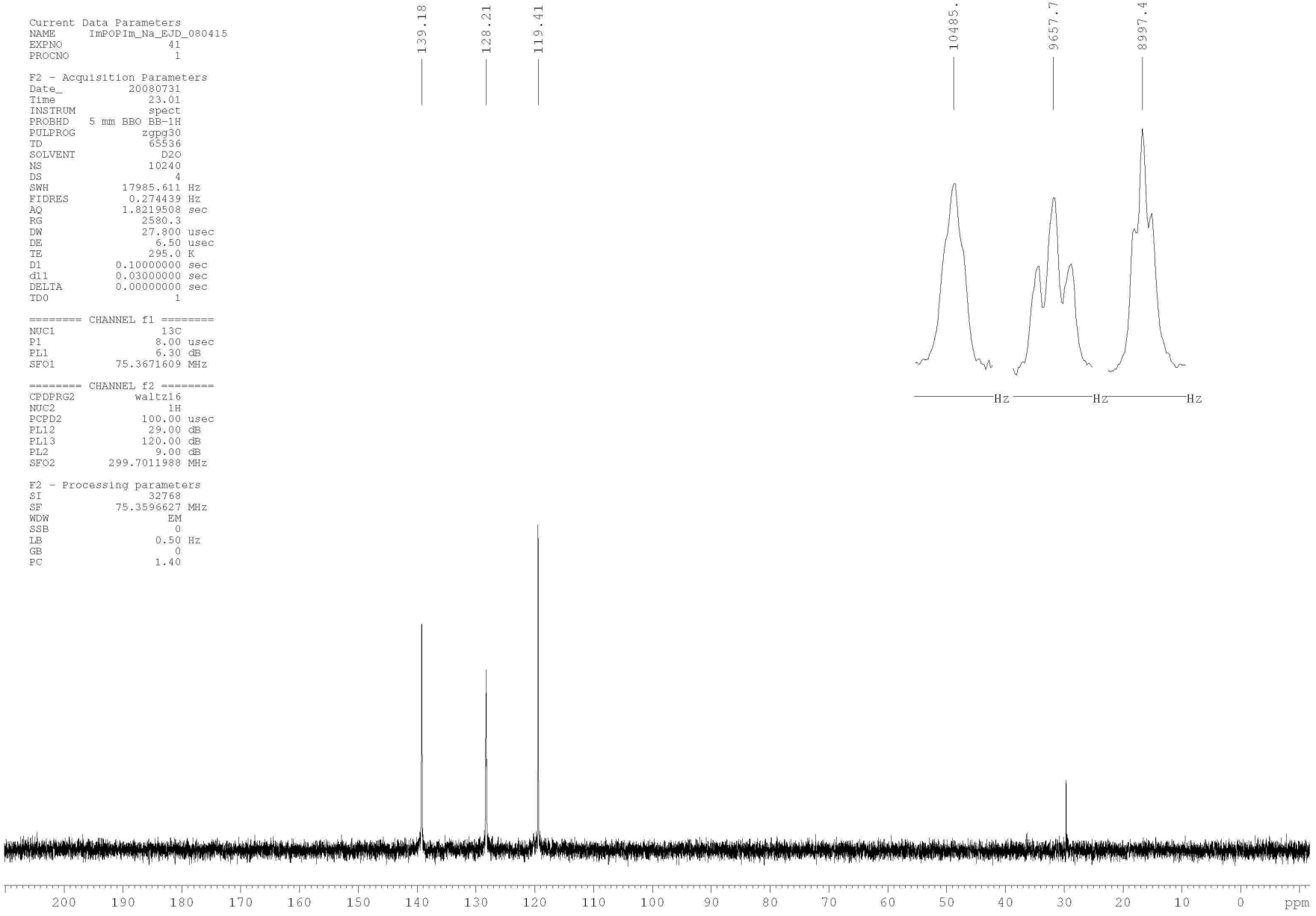
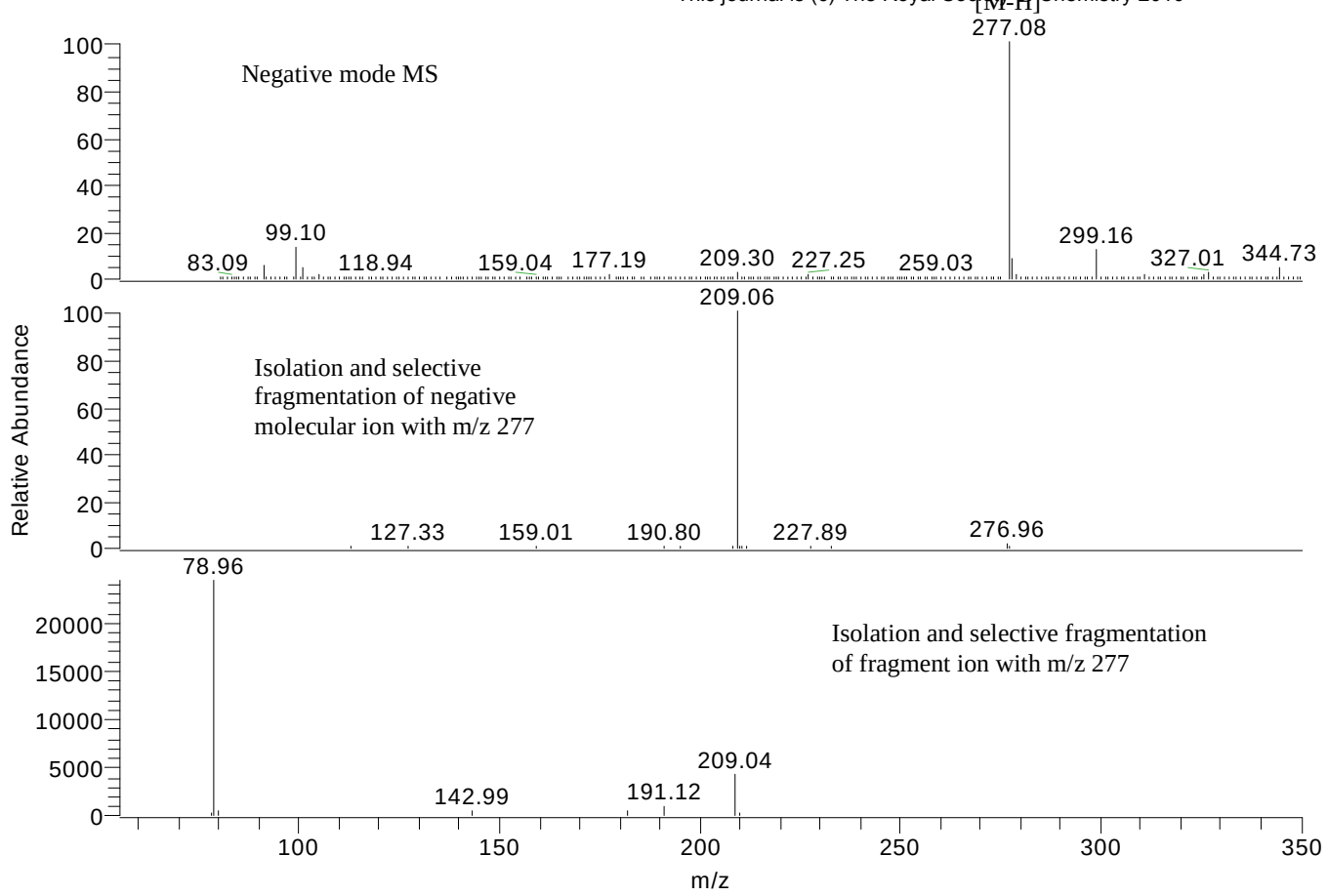


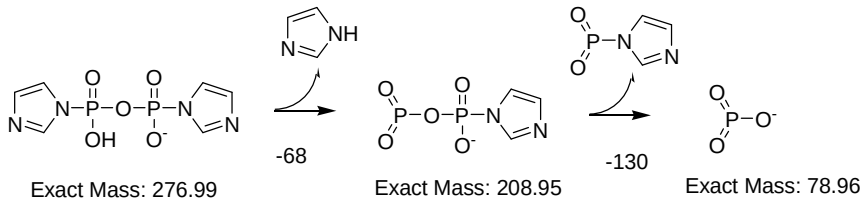
^{13}C NMR of the di-sodium salt of compound **7a** in D_2O





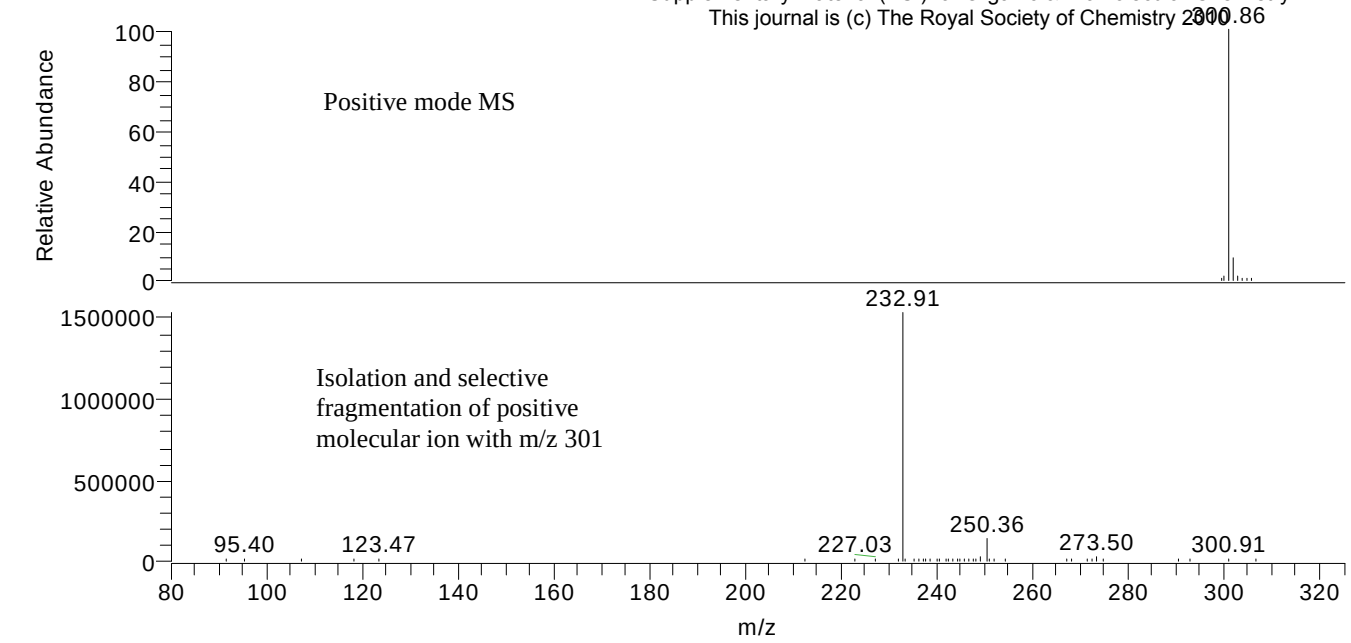
ESI Negative mode MS and selective MS^2 and MS^3 fragmentation of compound **7a**

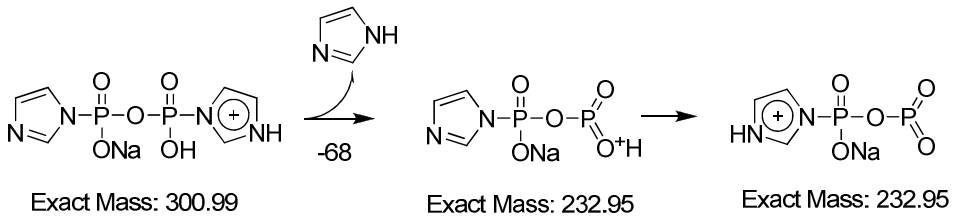




Negative mode fragmentation scheme

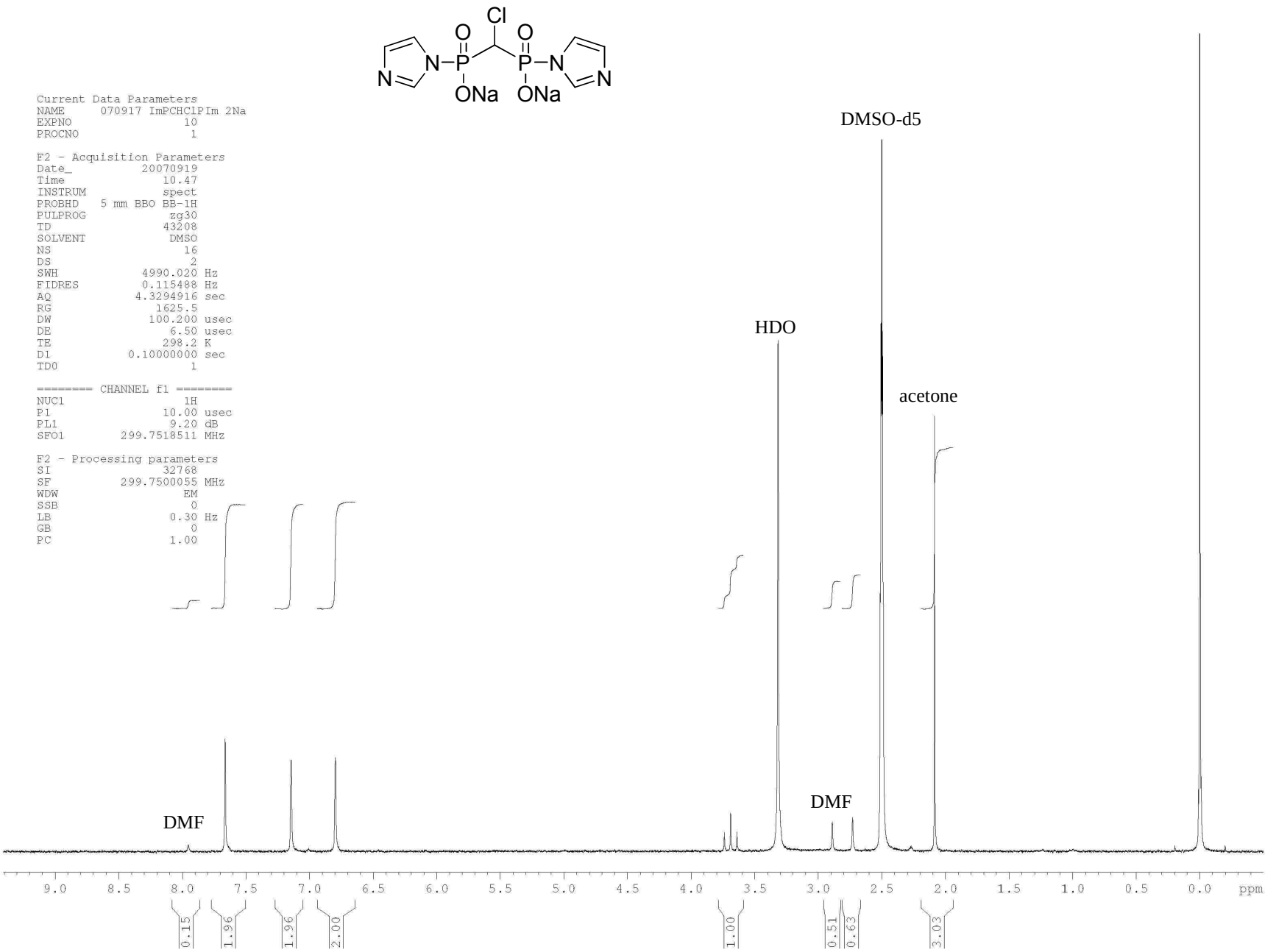
ESI Positive mode MS and selective MS^2 fragmentation of compound **7a**





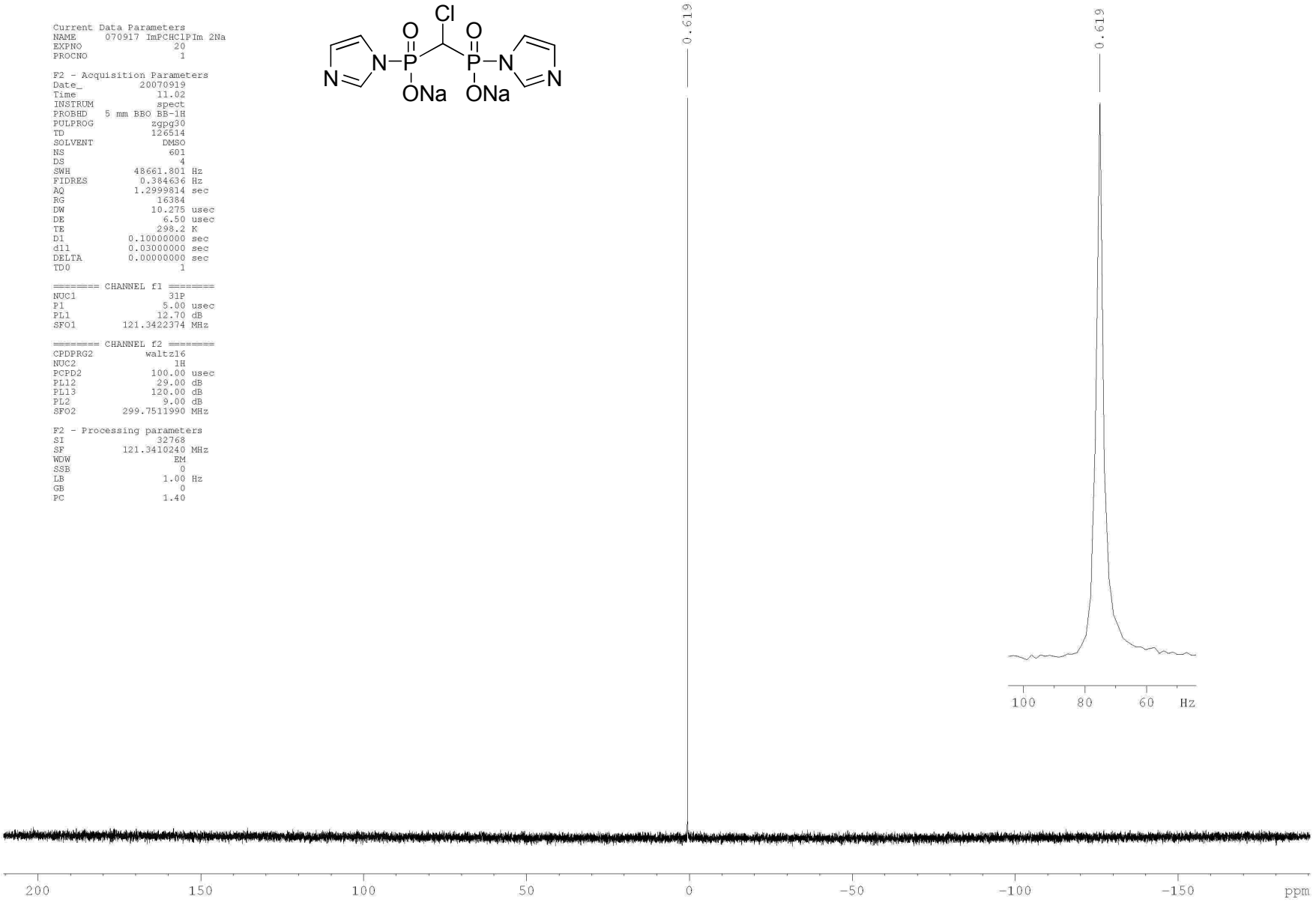
Positive mode fragmentation scheme

^{1}H NMR of the di-sodium salt of compound **7b** in DMSO-d6/D_2O

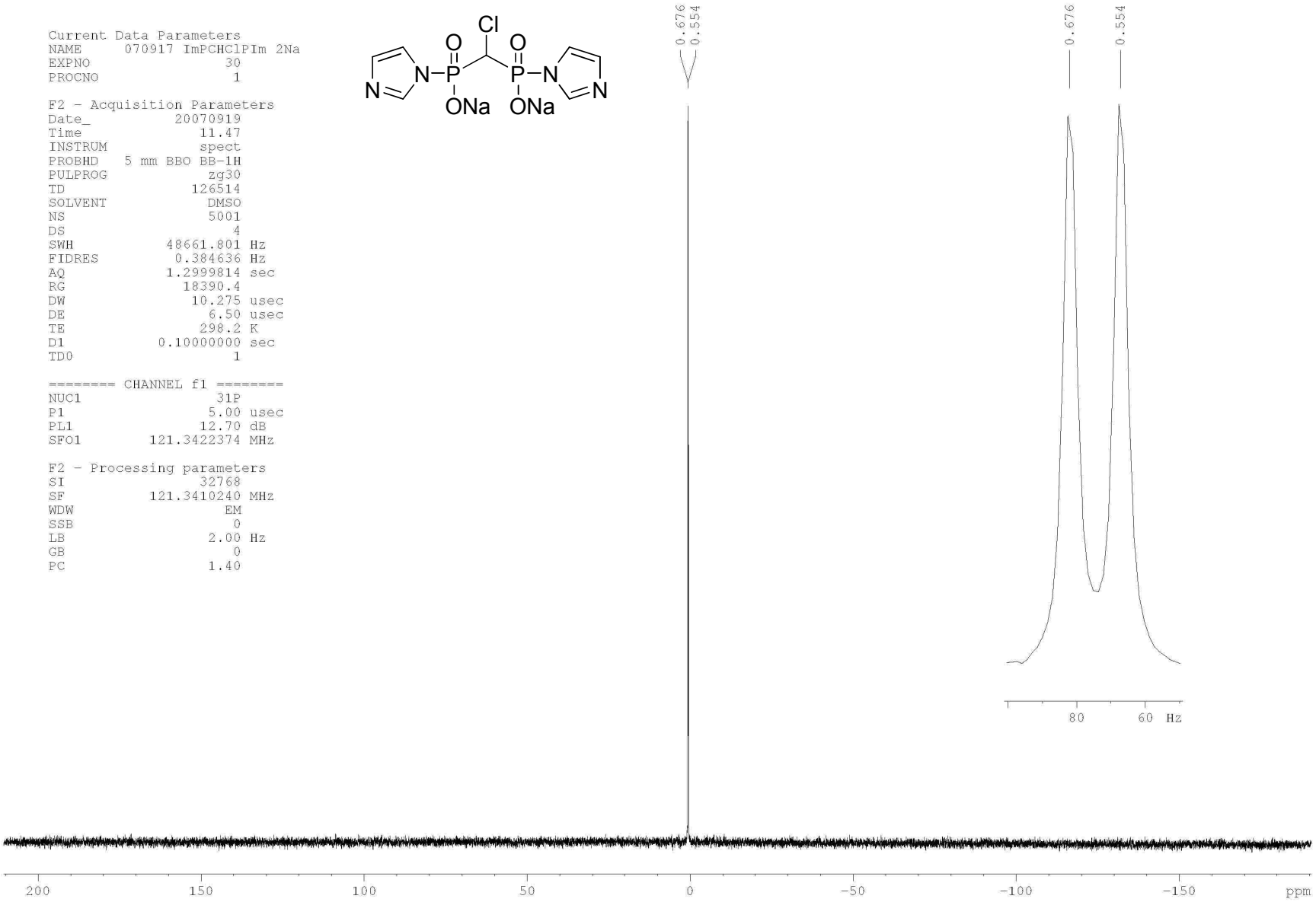


^{31}P NMR (proton decoupled) of the di-sodium salt of compound **7b** in DMSO-d6/D_2O

31P dec of final product

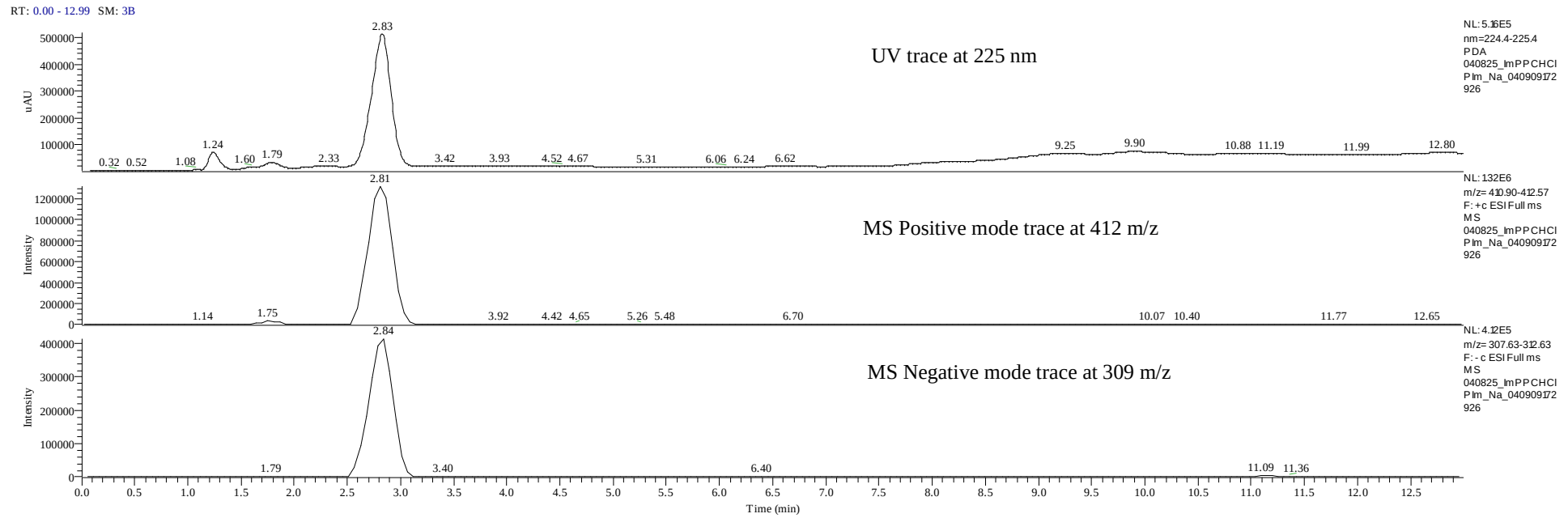


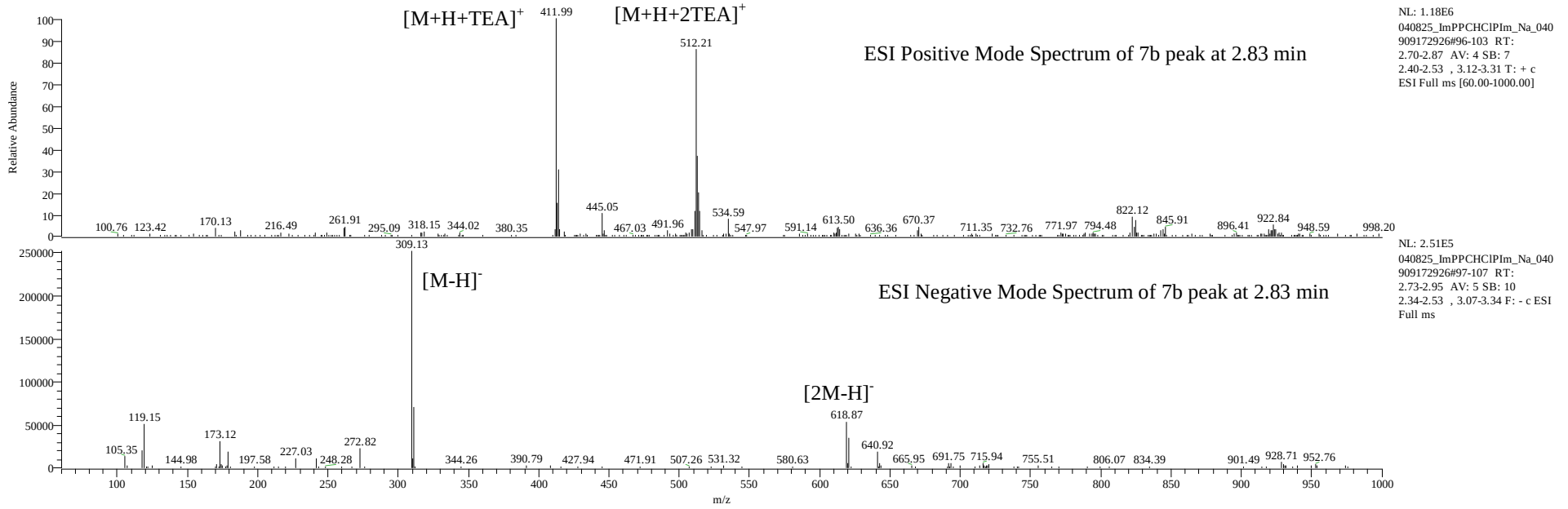
^{31}P NMR (proton coupled) of the di-sodium salt of compound **7b** in DMSO-d6/D_2O



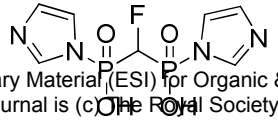
LCMS of the di-sodium salt of compound **7b**

040825_ImPPCHClPIm_Na_040909172926 9/9/2004 5:29:26 PM





LCMS of the di-sodium salt of compound **7c**



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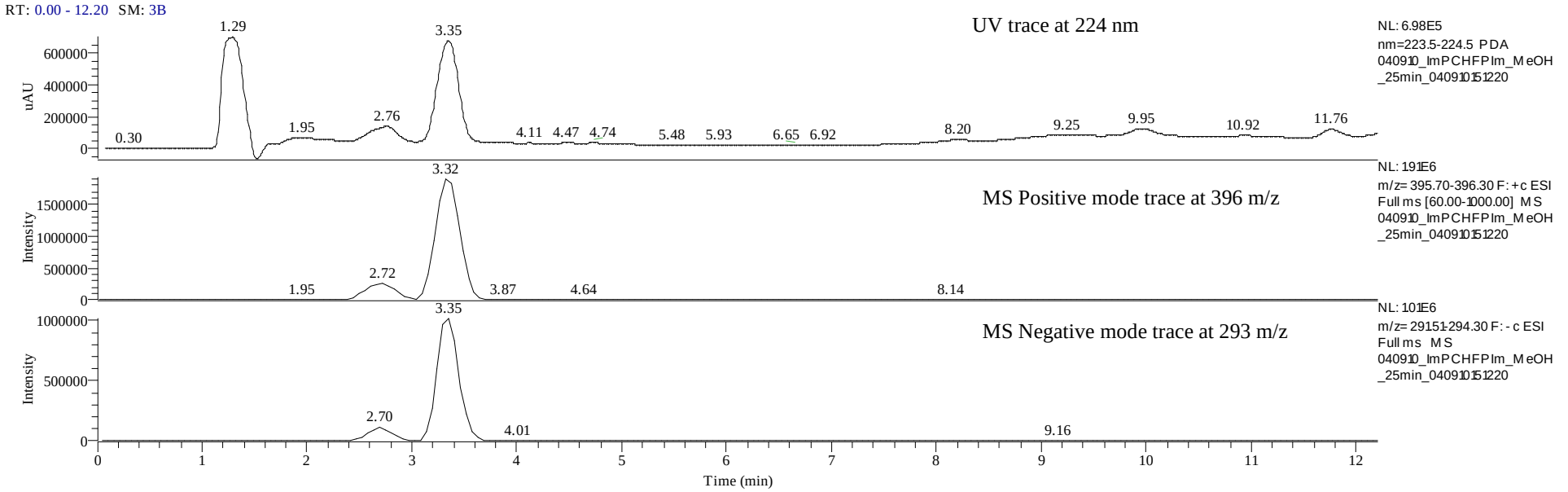
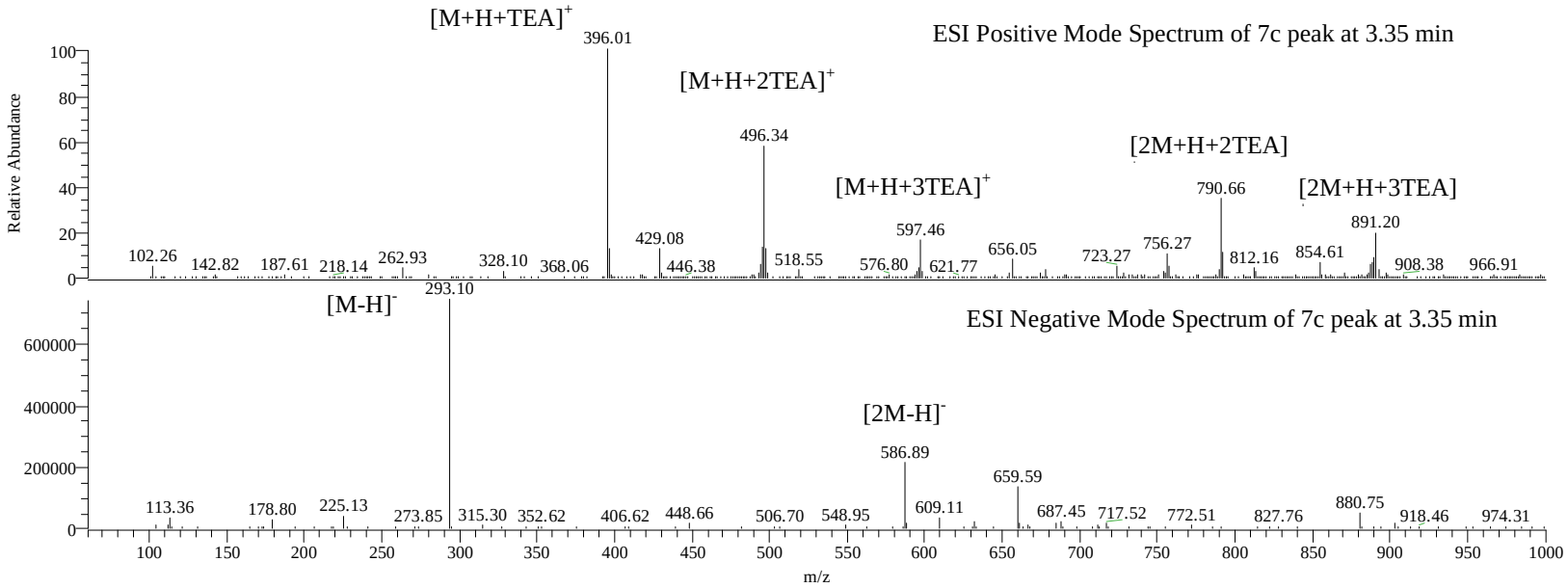
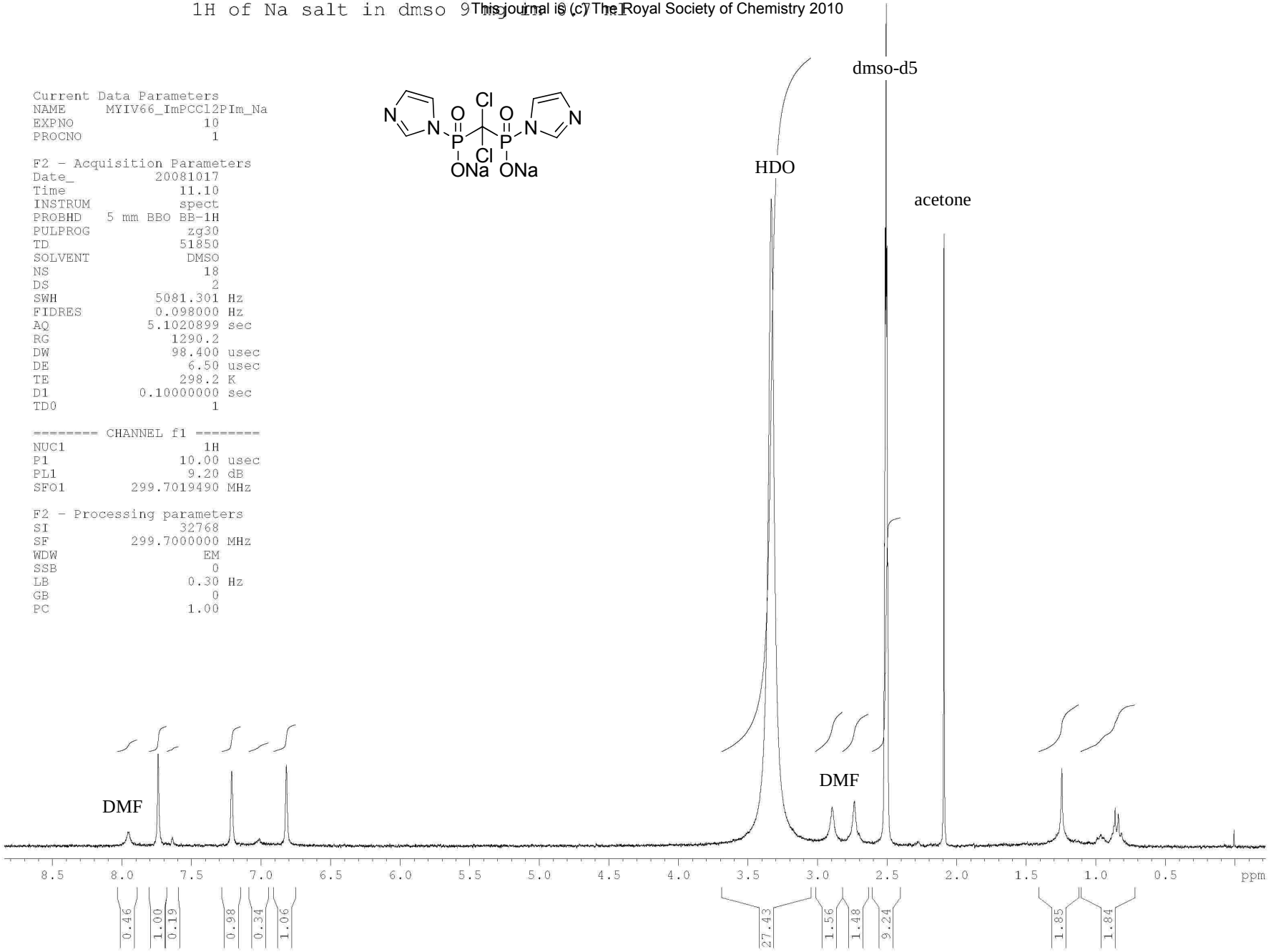
Exact Mass: 294.01

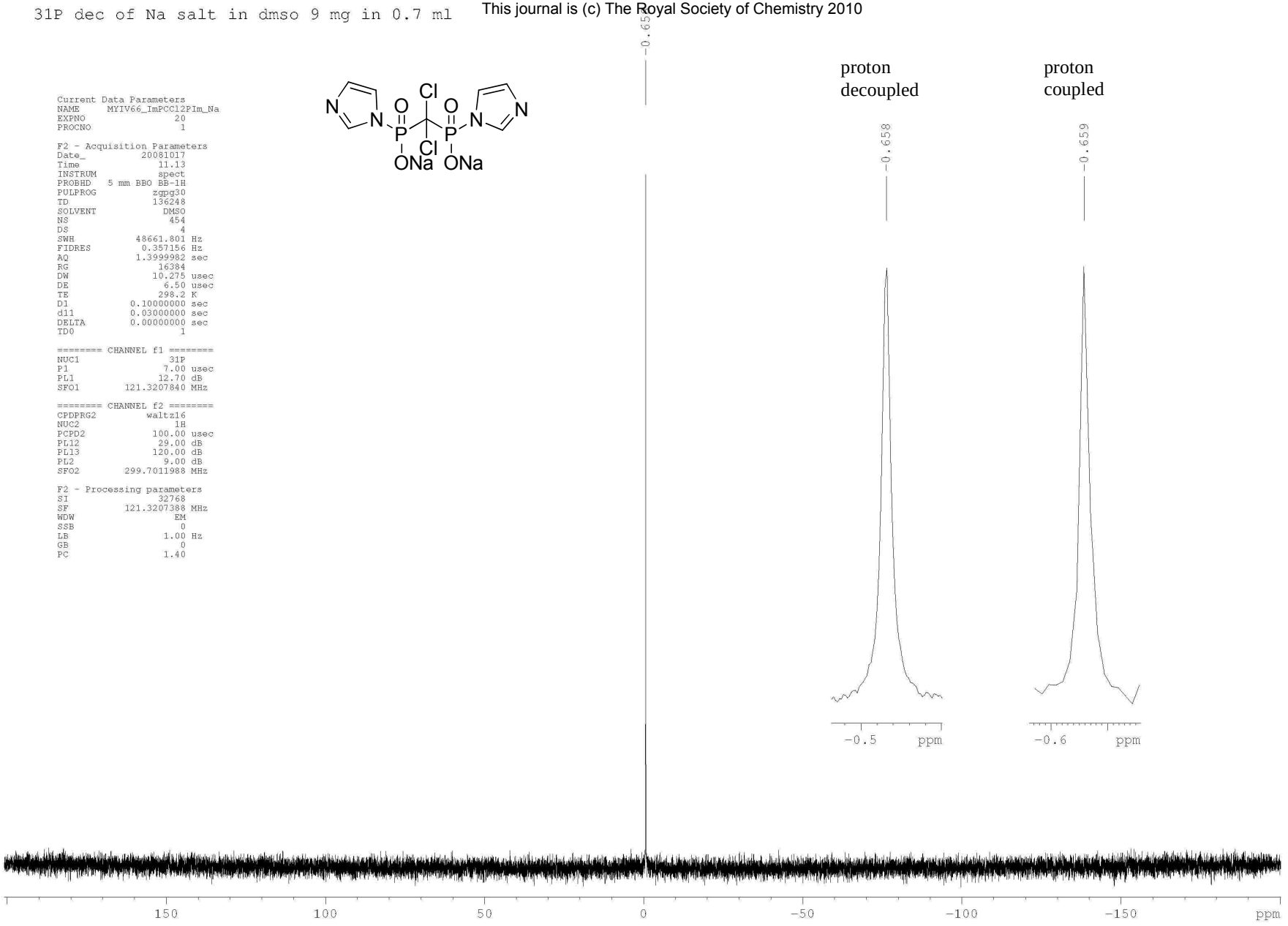




[1]H NMR of the di-sodium salt of compound **7d** in DMSO-d6



^{31}P NMR (proton decoupled) of the di-sodium salt of compound **7d** in DMSO-d6

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SOLVENT             DMSO
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SWH           48661.801 Hz
FIDRES         0.357156 Hz
AQ             1.3999982 sec
RG                 16384
DW                10.275 usec
DE                  6.50 usec
TE                298.2 K
D1           0.10000000 sec
d11          0.03000000 sec
DELTA        0.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  31P
P1                   7.00 usec
PL1                 12.70 dB
SFO1         121.3207840 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2              100.00 usec
PL12                29.00 dB
PL13               120.00 dB
PL2                 9.00 dB
SFO2         299.7011988 MHz

F2 - Processing parameters
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SF           121.3207388 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
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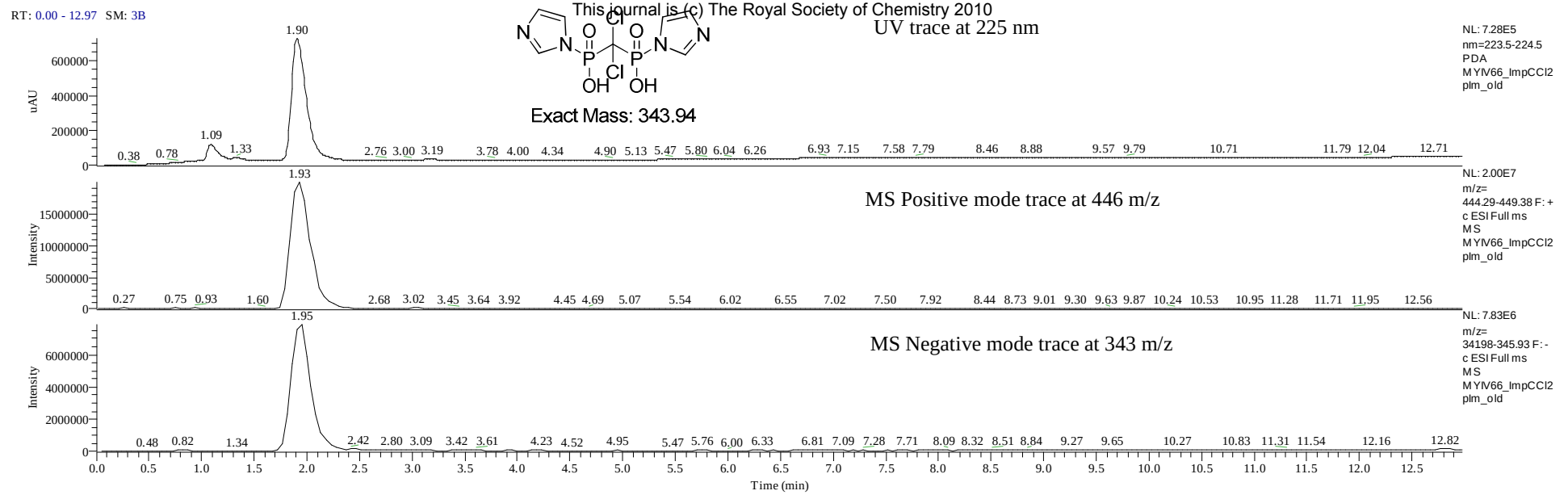
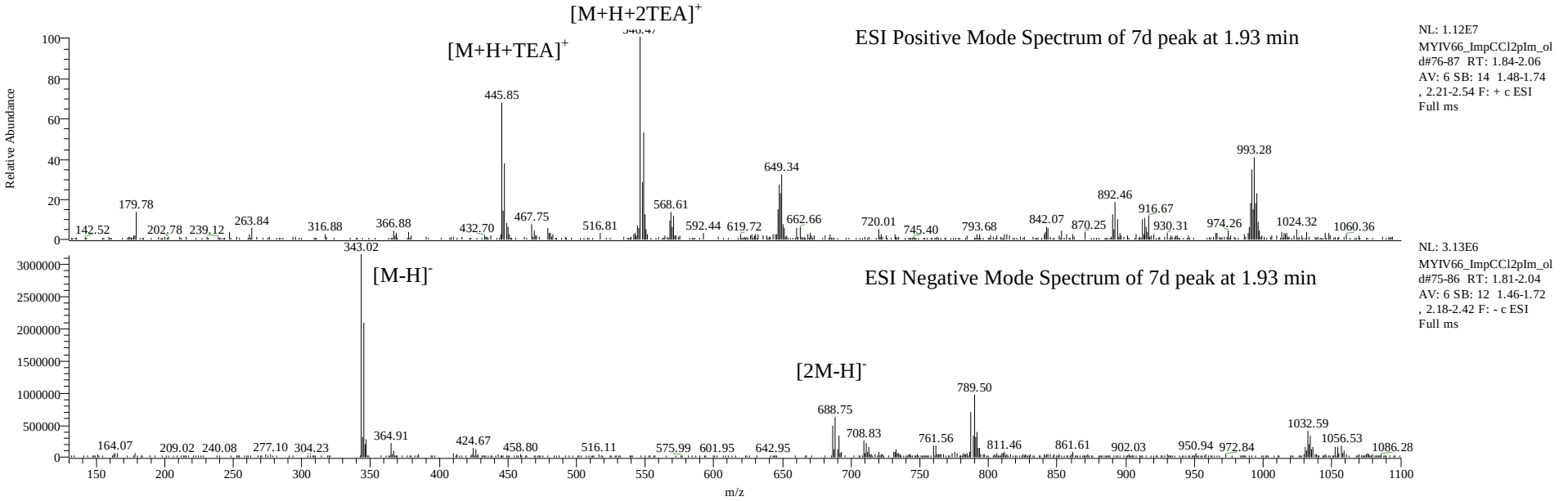
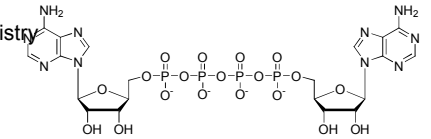
LCMS of the di-sodium salt of compound **7d**

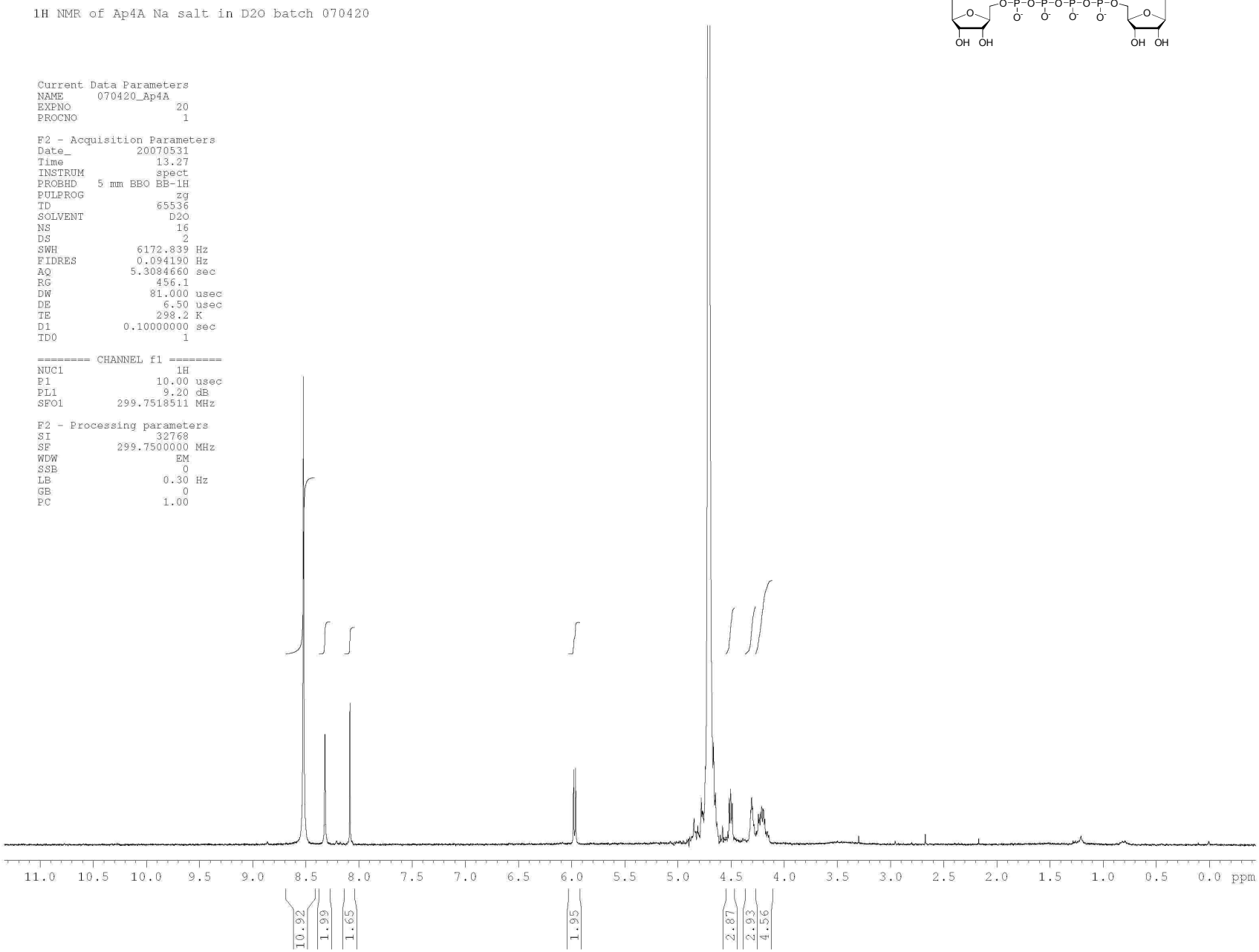
\\90.0.0.63\c$\...\MYIV66_ImpCCl2pIm_old 5/27/2009 6:00:40 PM
5 mg/ml in 20 mM TEAA



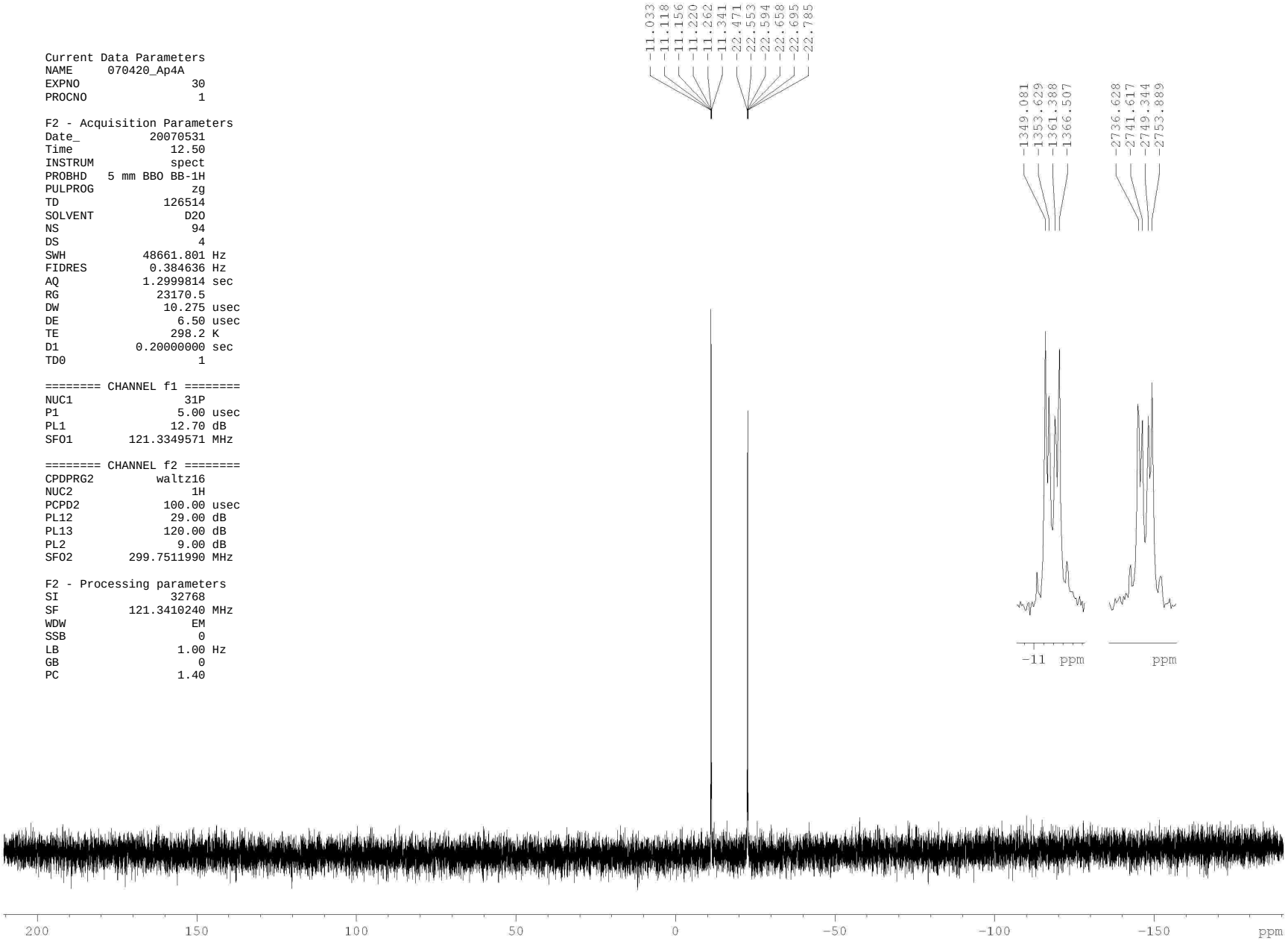


1H NMR spectrum of Ap_4A sodium salt, **3a** in D_2O





^{31}P (proton decoupled) NMR spectrum of Ap_4A sodium salt, **3a** in D_2O

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PROCNO                1

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DS                     4
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FIDRES         0.384636 Hz
AQ            1.2999814 sec
RG              23170.5
DW               10.275 usec
DE                6.50 usec
TE                298.2 K
D1           0.20000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 31P
P1                  5.00 usec
PL1                12.70 dB
SFO1        121.3349571 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL12               29.00 dB
PL13              120.00 dB
PL2                 9.00 dB
SFO2        299.7511990 MHz

F2 - Processing parameters
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WDW                  EM
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GB                    0
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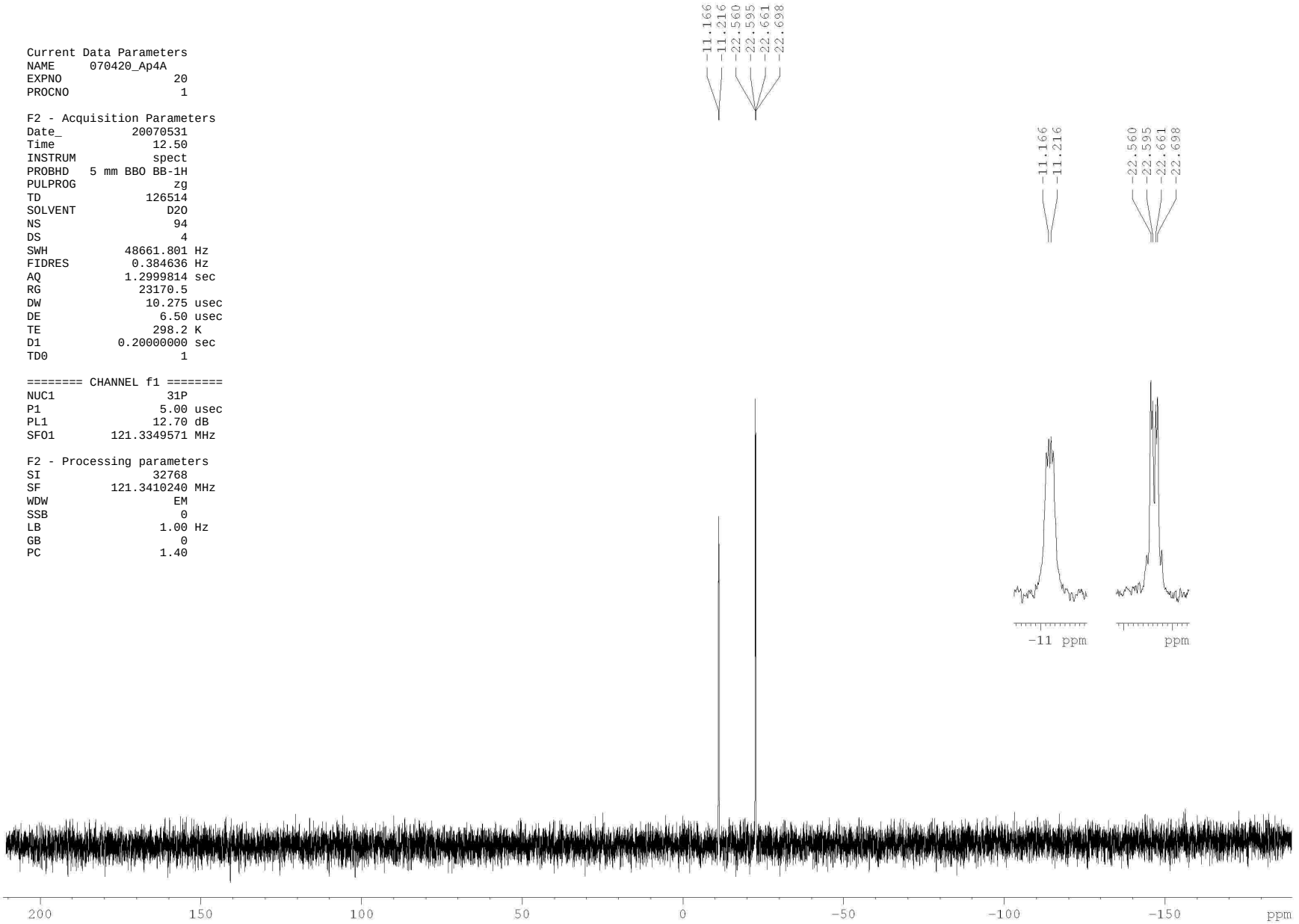
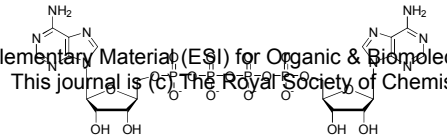
^{31}P (proton coupled) NMR spectrum of Ap_4A sodium salt, **3a** in D_2O



LCMS of Ap_4A, **3a**



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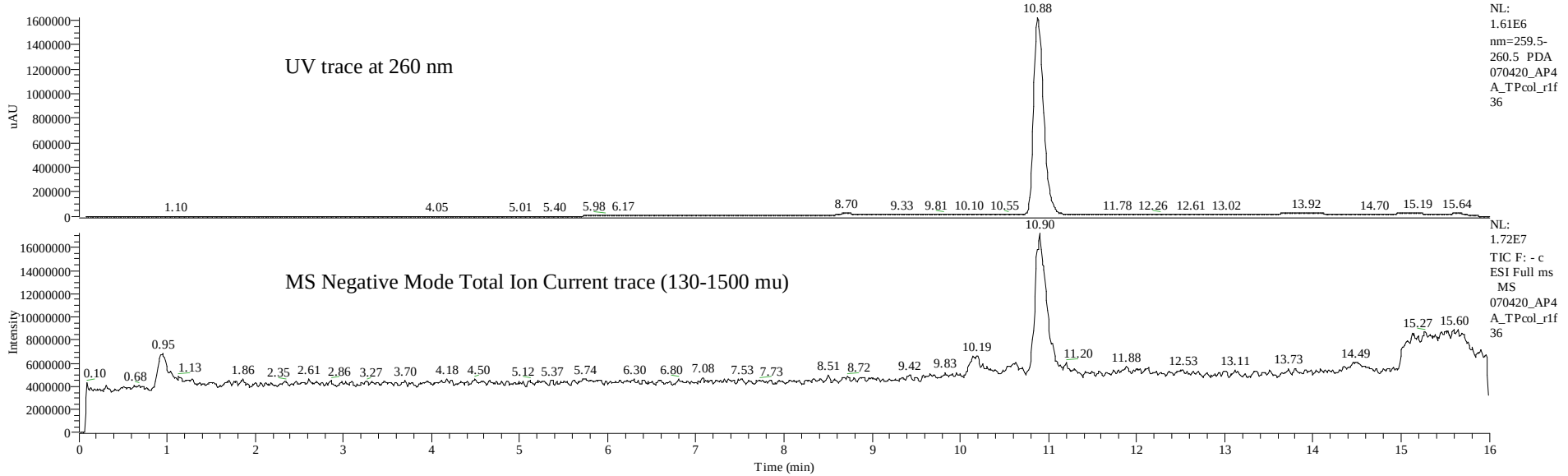
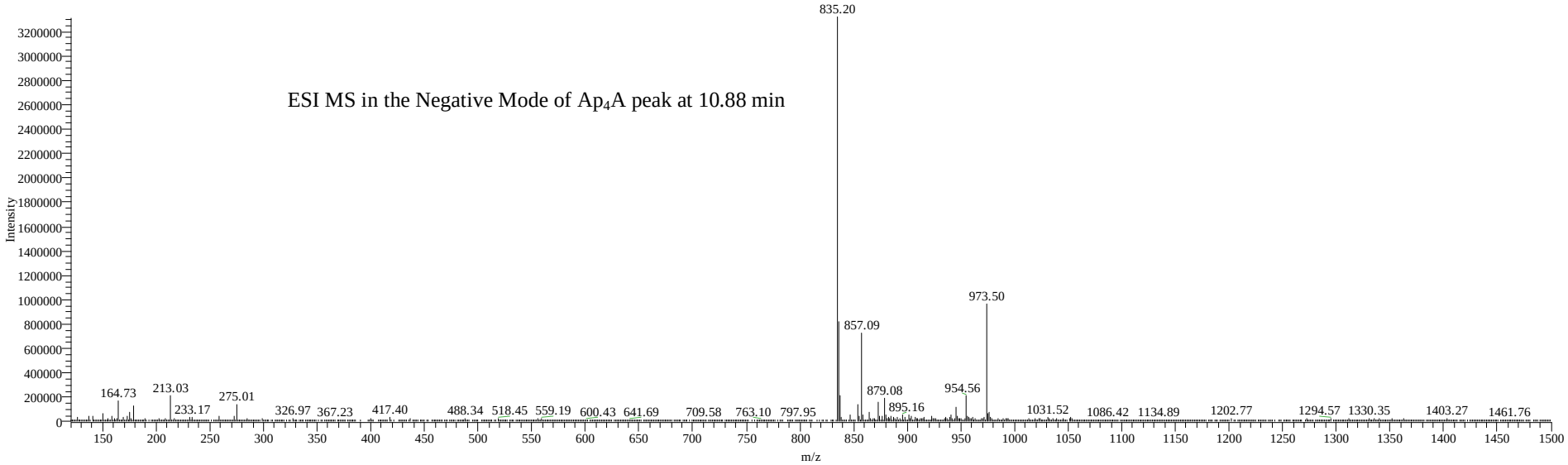
RT: 0.00 - 16.00 SM: 3B

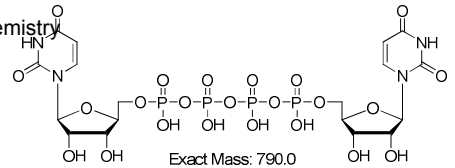


070420_AP4A_TPcol_r1f36 #1010-1028 RT: 10.84-11.00 AV: 19 NL: 3.31E6
F: - c ESI Full ms

[M-H]



1H NMR spectrum of Up_4U sodium salt, **3f** in D_2O



^{31}P (proton decoupled) NMR spectrum of Up$_4$U sodium salt, **3f** in D_2O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PROCNO               1

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NS                   88
DS                   32
SWH           48661.801 Hz
FIDRES        0.333348 Hz
AQ            1.4999945 sec
RG              20642.5
DW               10.275 usec
DE                6.00 usec
TE               300.0 K
D1            0.10000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ==========
NUC1                31P
P1                10.00 usec
PL1                3.00 dB
SFO1        121.3874295 MHz

========== CHANNEL f2 ==========
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                0.00 dB
PL12              17.00 dB
PL13              17.00 dB
SFO2        299.8611995 MHz

F2 - Processing parameters
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WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40

1D NMR plot parameters
CX               26.00 cm
F1P             150.431 ppm
F1            18251.40 Hz
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PPMCM         15.41769 ppm/cm
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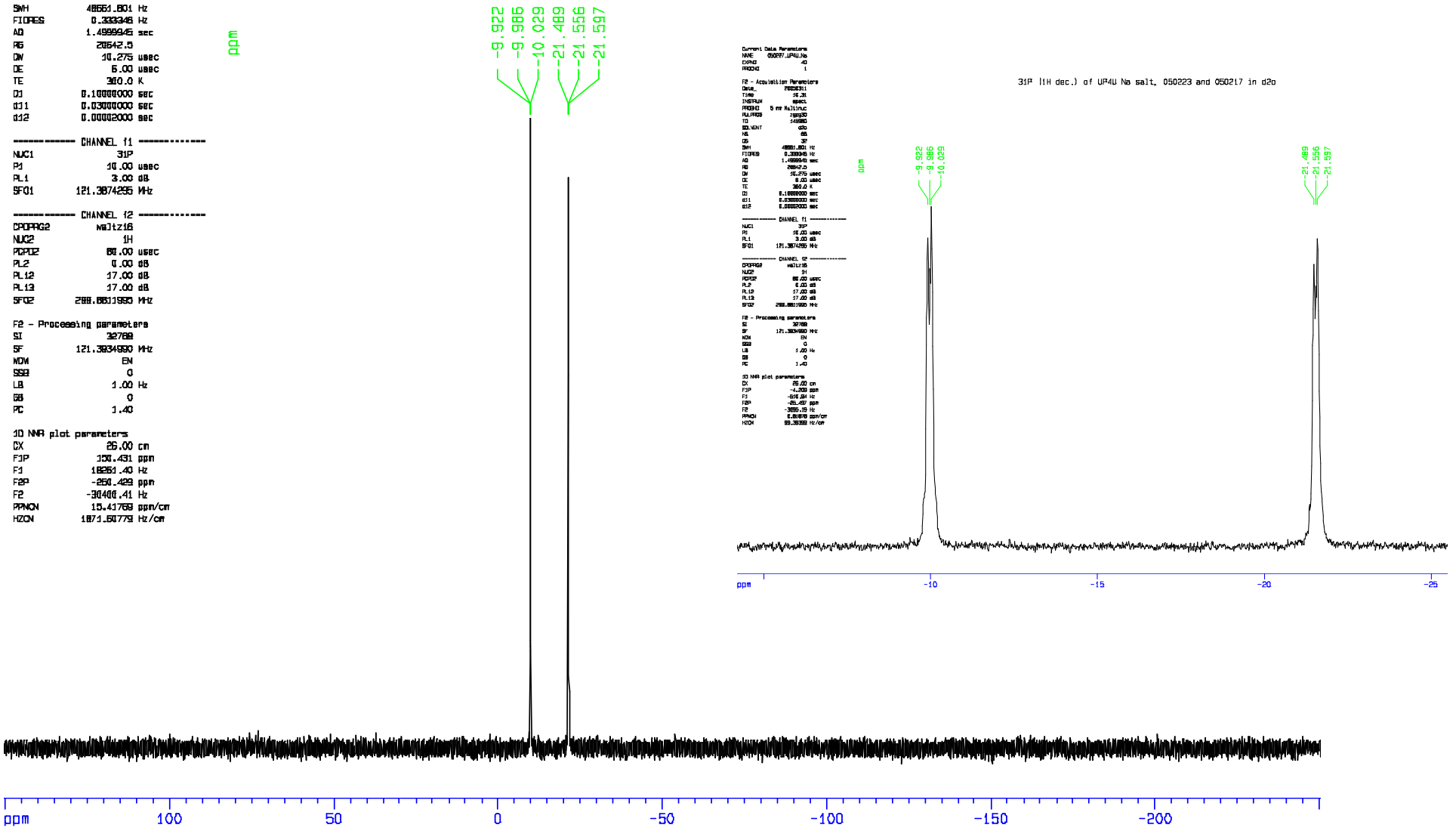
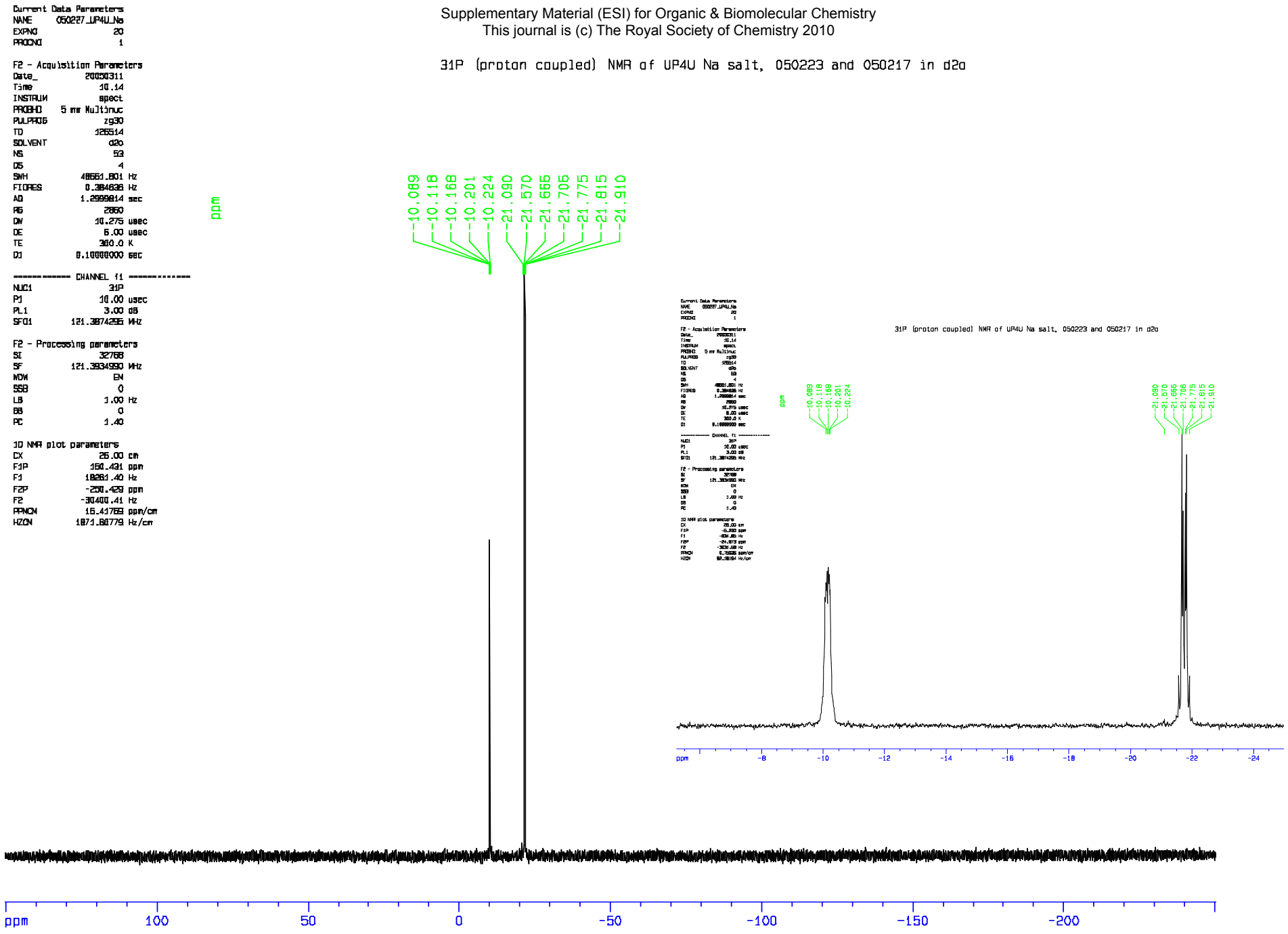
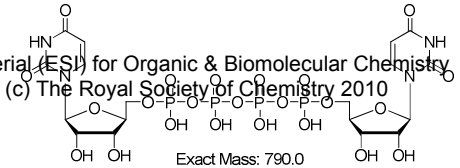


^{31}P (proton coupled) NMR spectrum of Up$_4$U sodium salt, **3f** in D_2O

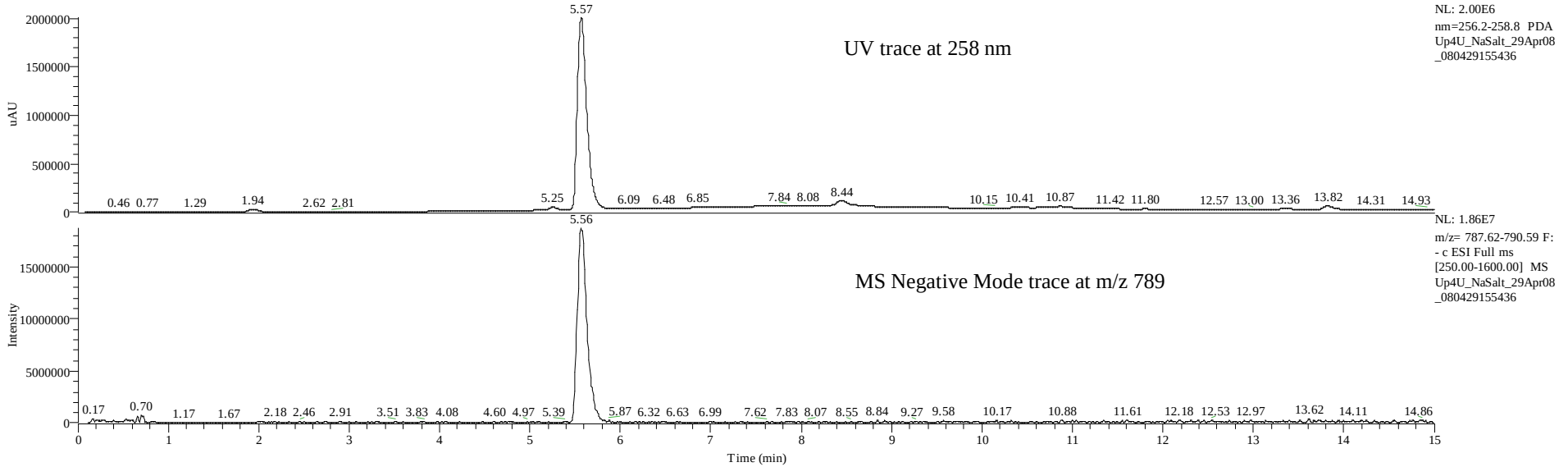


LCMS of Up_4U, **3f**

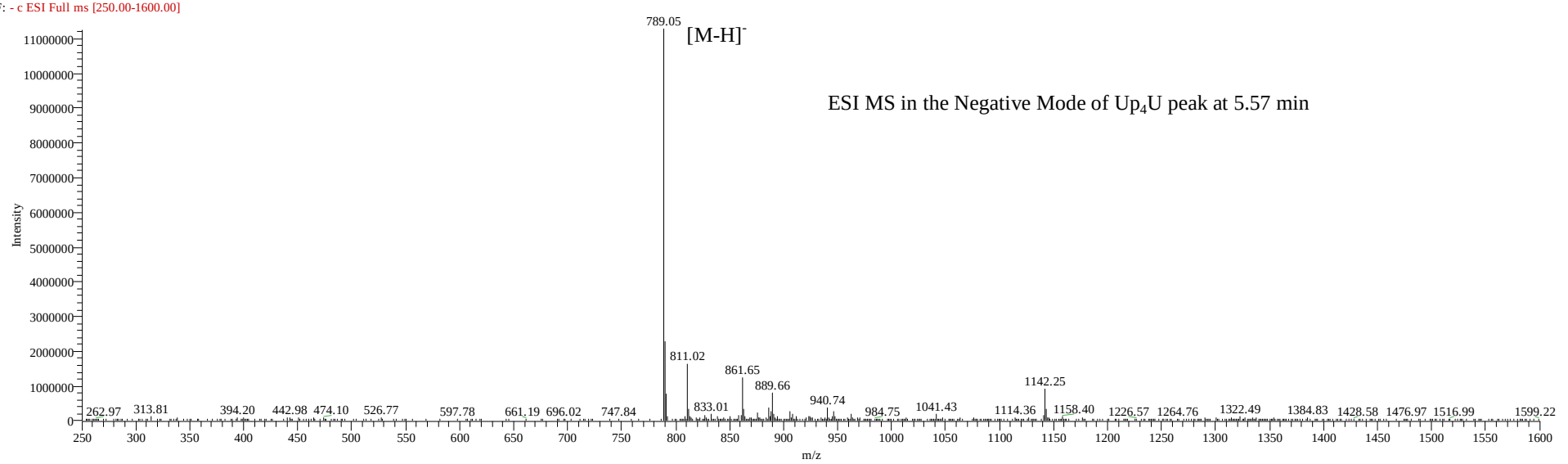


Up4U_NaSalt_29Apr08_080429155436 4/29/2008 3:54:36 PM

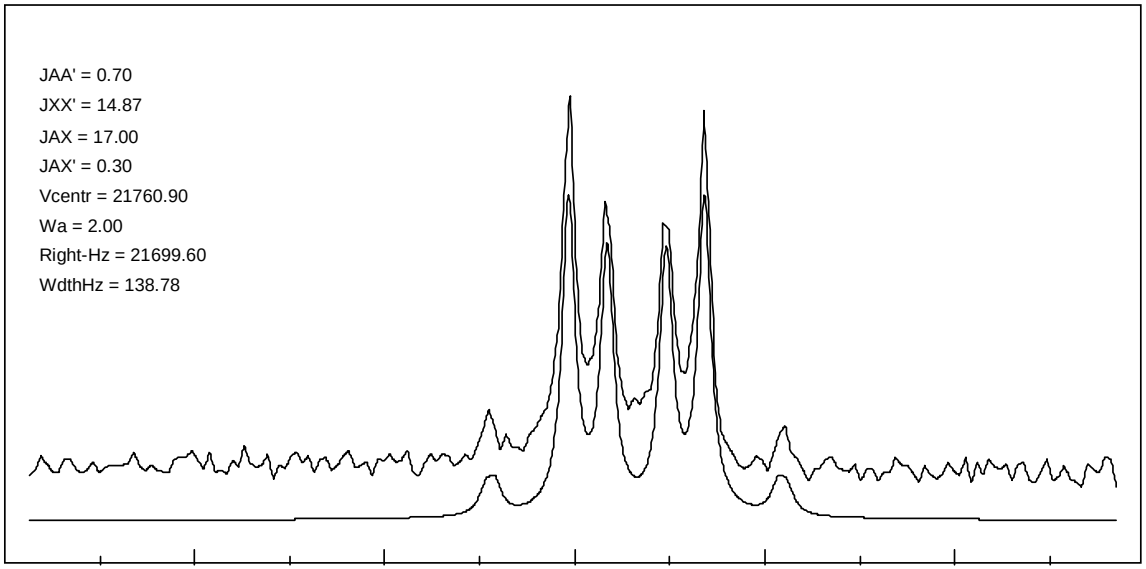
RT: 0.00 - 15.00 SM: 3B

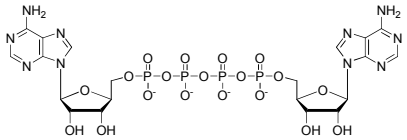


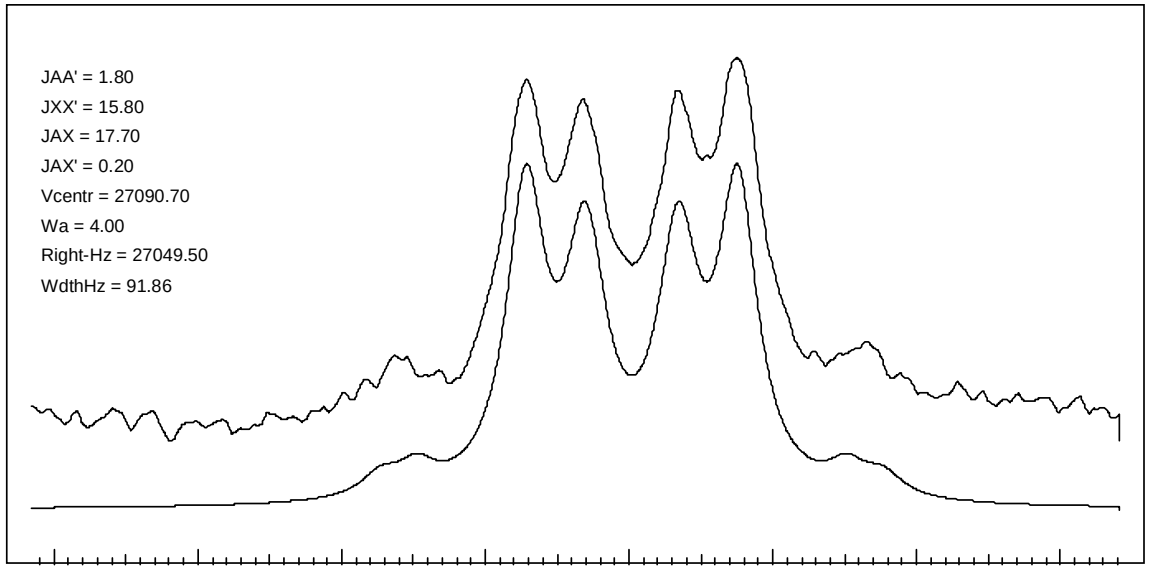
Up4U_NaSalt_29Apr08_080429155436 #674-691 RT: 5.51-5.63 AV: 18 SB: 39 5.36-5.48 , 5.67-5.84 NL: 1.12E7
F: - c ESI Full ms [250.00-1600.00]

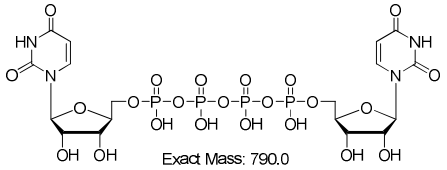


Simulated fitting of an AA'XX' spin system to the experimental ^{31}P NMR spectra of Ap_4A and Up_4U (**3a** and **3f**, resp.):

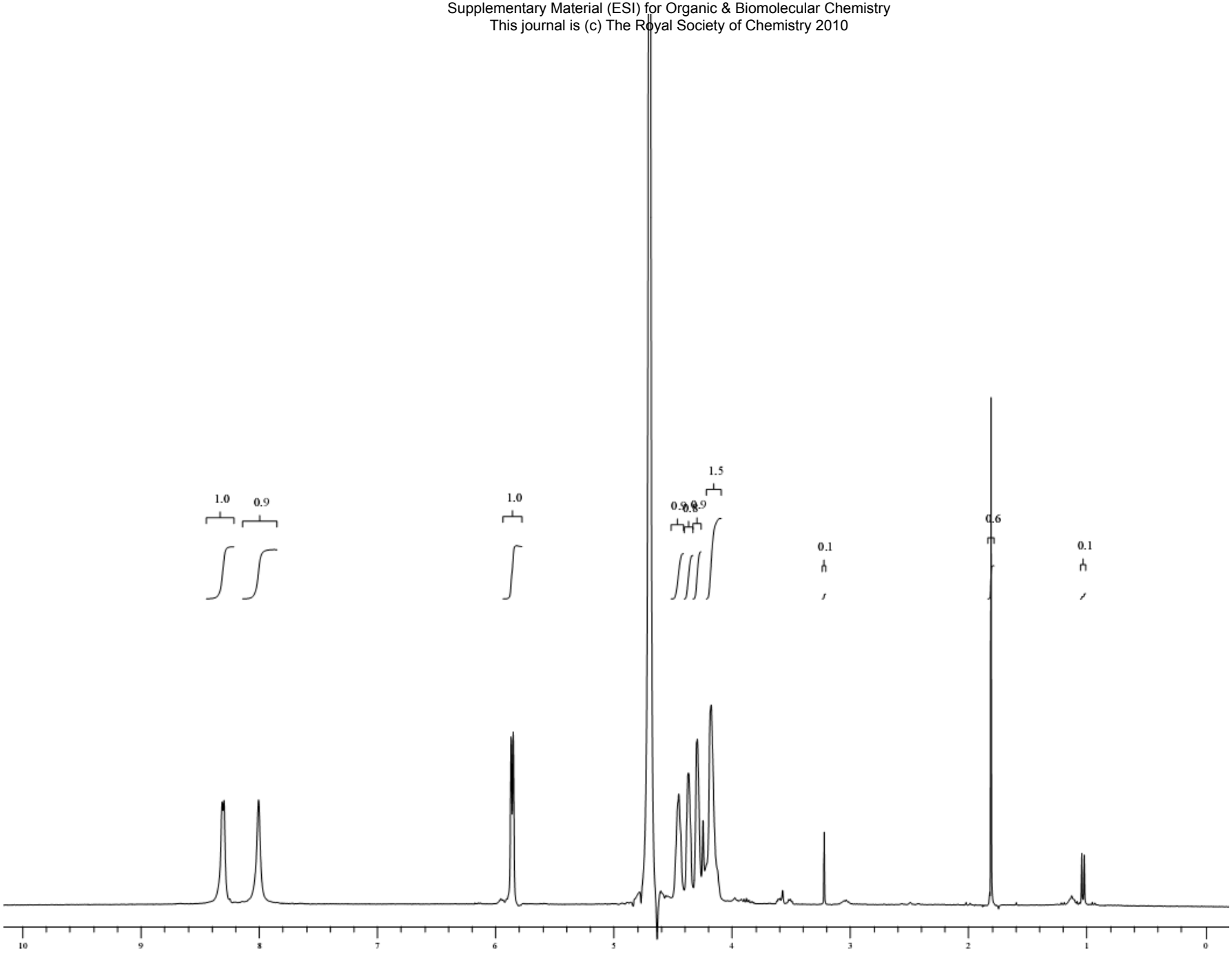




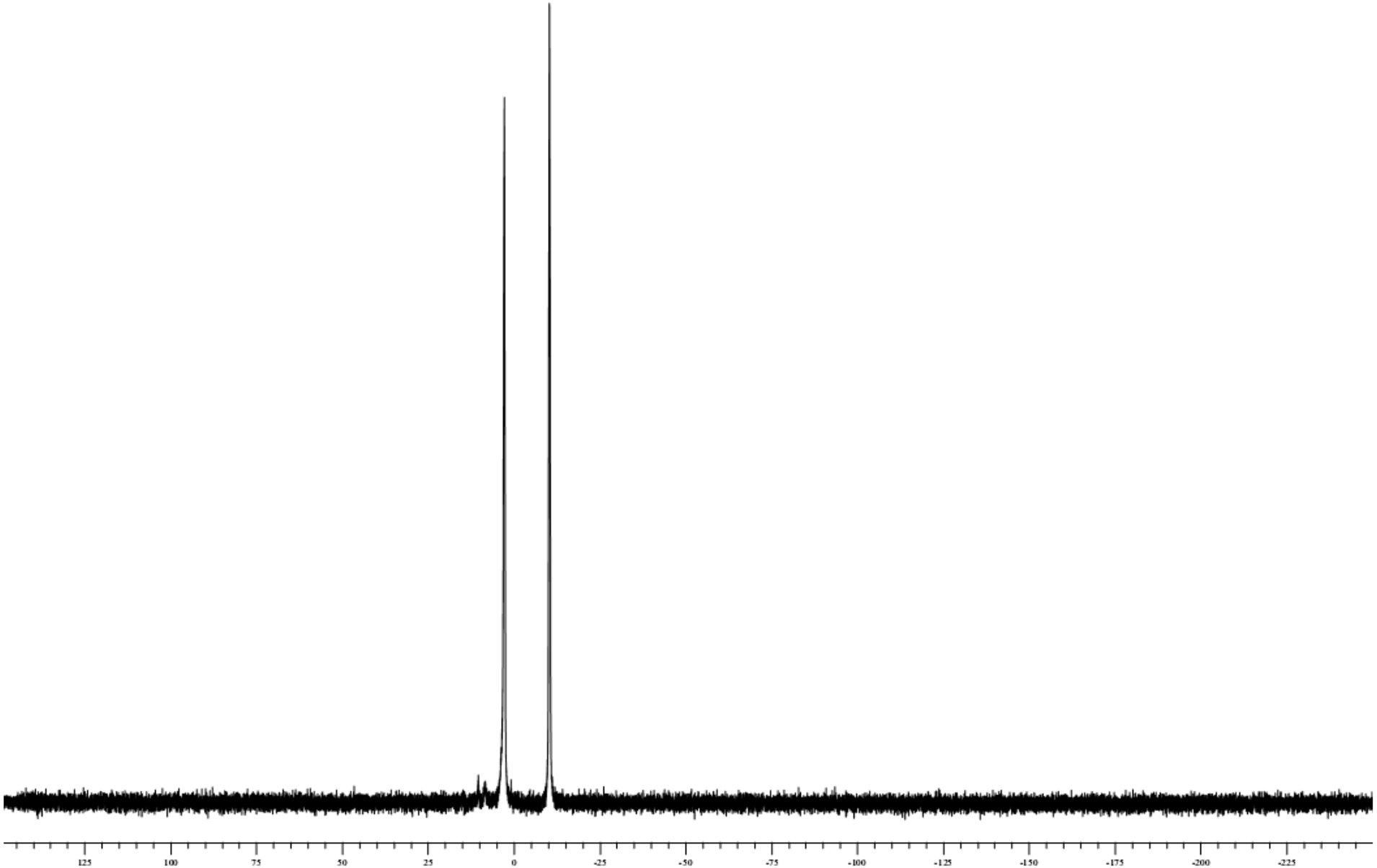




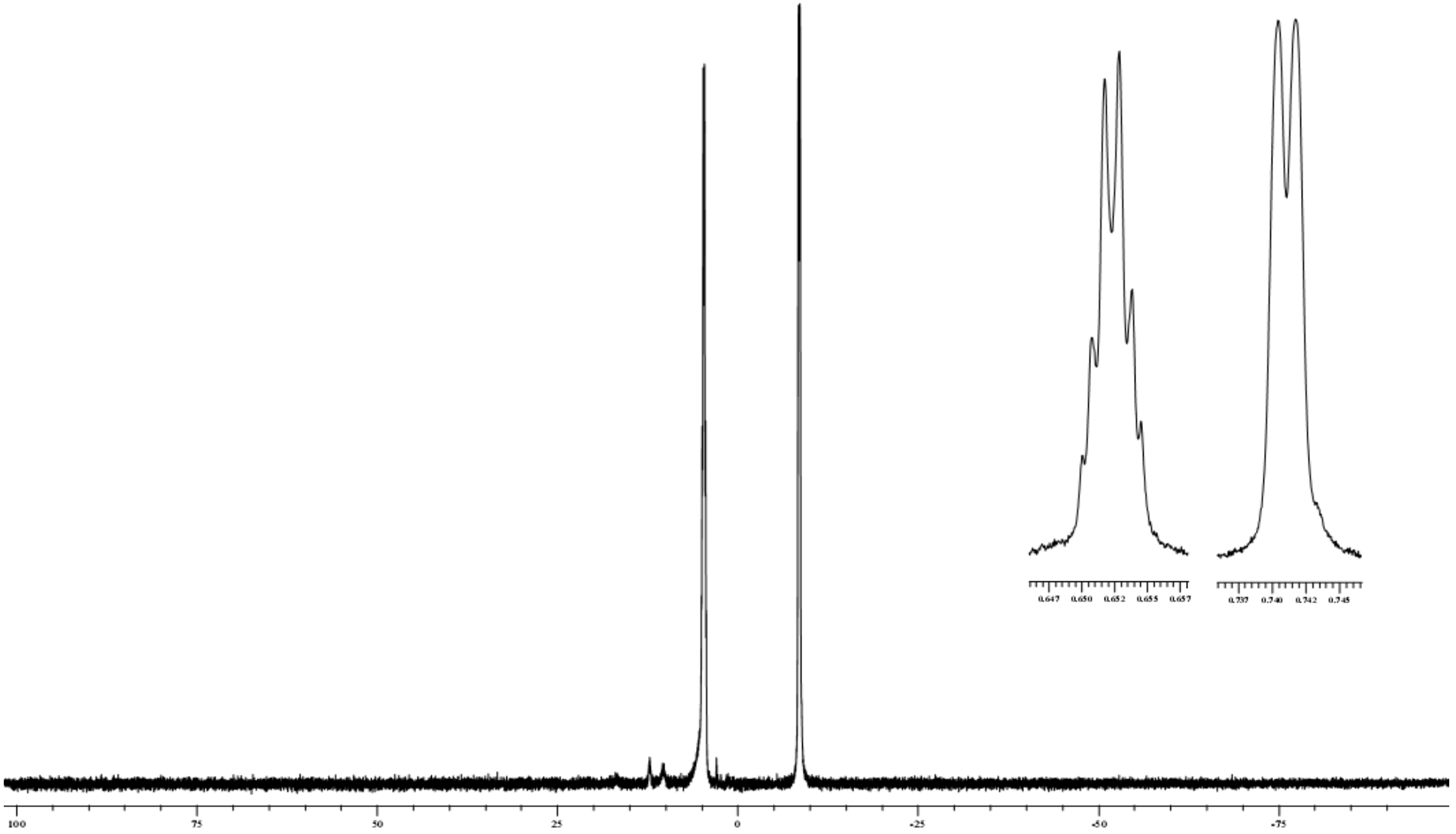
^{1}H NMR of APPCHClPPA sodium salt, **3c** in D_2O:



^{31}P NMR (proton decoupled) of APPCHClPPA sodium salt, **3c** in D_2O:

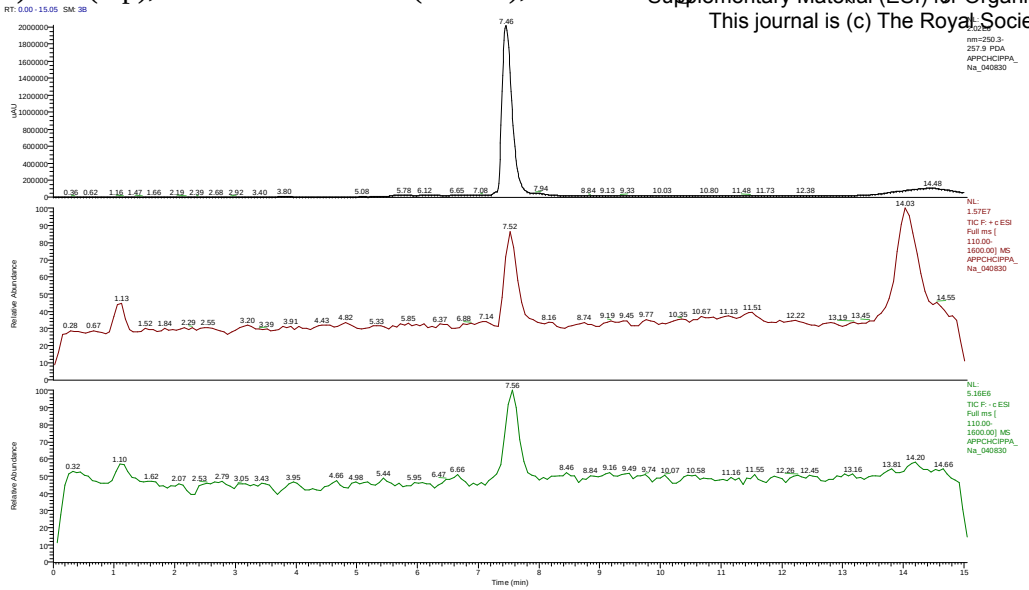


^{31}P NMR (proton coupled) of APPCHClPPA sodium salt, **3c** in D_2O:

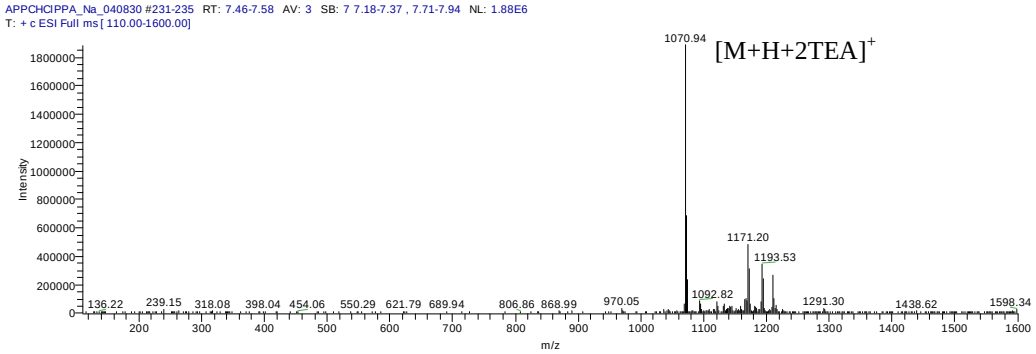


LCMS analysis of APPCHClPPA sodium salt, **3c**:

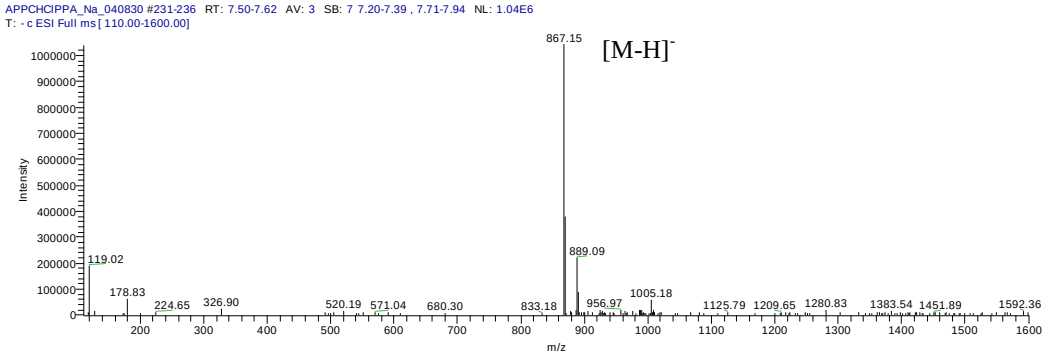
a) UV (top), MS Pos. mode TIC (middle), and MS Neg. mode TIC (bottom) chromatograms:



b) Positive mode MS of APPCHClPPA peak at 7.46 min



c) Negative mode MS of APPCHClPPA peak at 7.46 min



^{1}H NMR of APPCHFPPA, **3e** as the tetrabutylammonium salt in D_2O: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Current Data Parameters
NAME      040910TBA1_APPCHFPPA_TBA
EXPNO                  20
PROCNO                  1

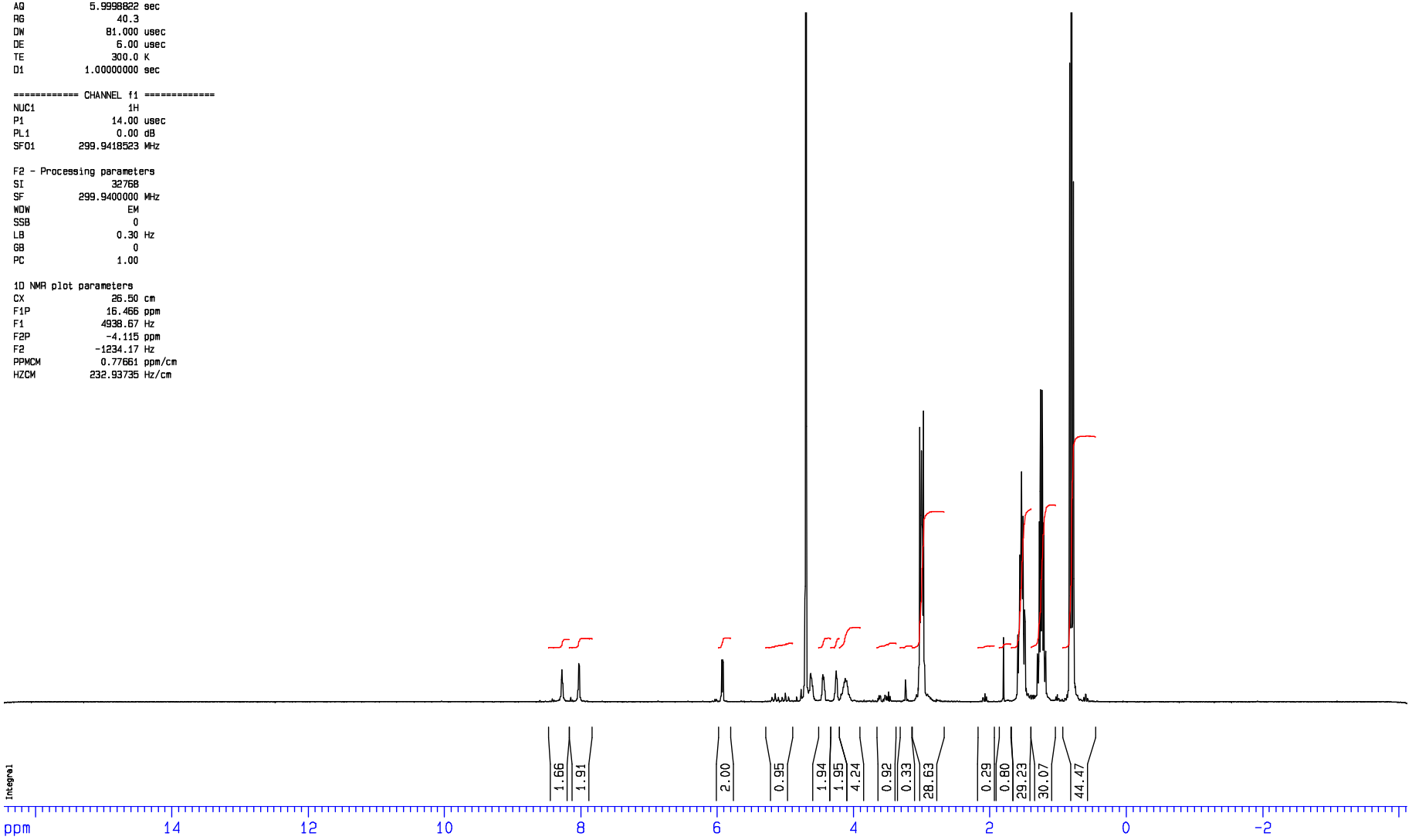
F2 - Acquisition Parameters
Date_          20041018
Time               14.17
INSTRUM            spect
PROBHD     5 mm Multinuc
PULPROG             zg30
TD                 74072
SOLVENT              D2O
NS                     8
DS                     2
SWH            6172.839 Hz
FIDRES         0.083336 Hz
AQ             5.9998822 sec
RG                  40.3
DW                81.000 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec

============ CHANNEL f1 =============
NUC1                   1H
P1                 14.00 usec
PL1                 0.00 dB
SFO1         299.9418523 MHz

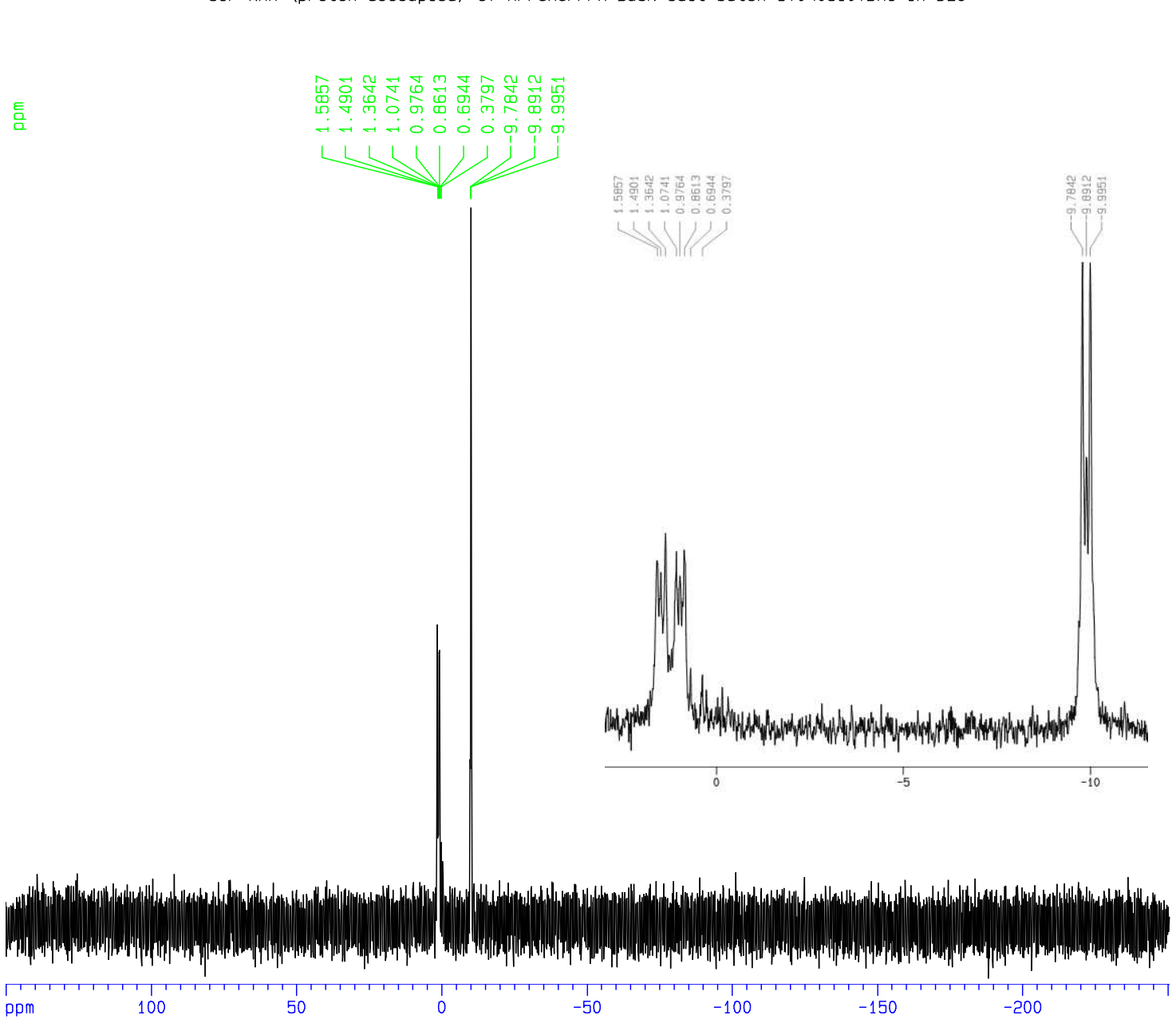
F2 - Processing parameters
SI                 32768
SF           299.9400000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 26.50 cm
F1P               16.466 ppm
F1              4938.67 Hz
F2P               -4.115 ppm
F2             -1234.17 Hz
PPMCM           0.77661 ppm/cm
HZCM          232.93735 Hz/cm
```

1H NMR of APPCHFPPA Bu3N salt batch IY040910TBA1 in D2O



^{31}P NMR (proton decoupled) of APPCHFPPA, **3e** as the tetrabutylammonium salt in D_2O:

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Current Data Parameters
NAME      040910TBA1_APPCHFPPA_TBA
EXPNO                  40
PROCNO                  1

F2 - Acquisition Parameters
Date_           20041018
Time                14.58
INSTRUM             spect
PROBHD     5 mm Multinuc
PULPROG           zgpg30
TD                126514
SOLVENT              d2o
NS                    24
DS                     4
SWH            48661.801 Hz
FIDRES         0.384636 Hz
AQ             1.2999814 sec
RG                 16384
DW                10.275 usec
DE                 6.00 usec
TE                300.0 K
D1            0.10000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

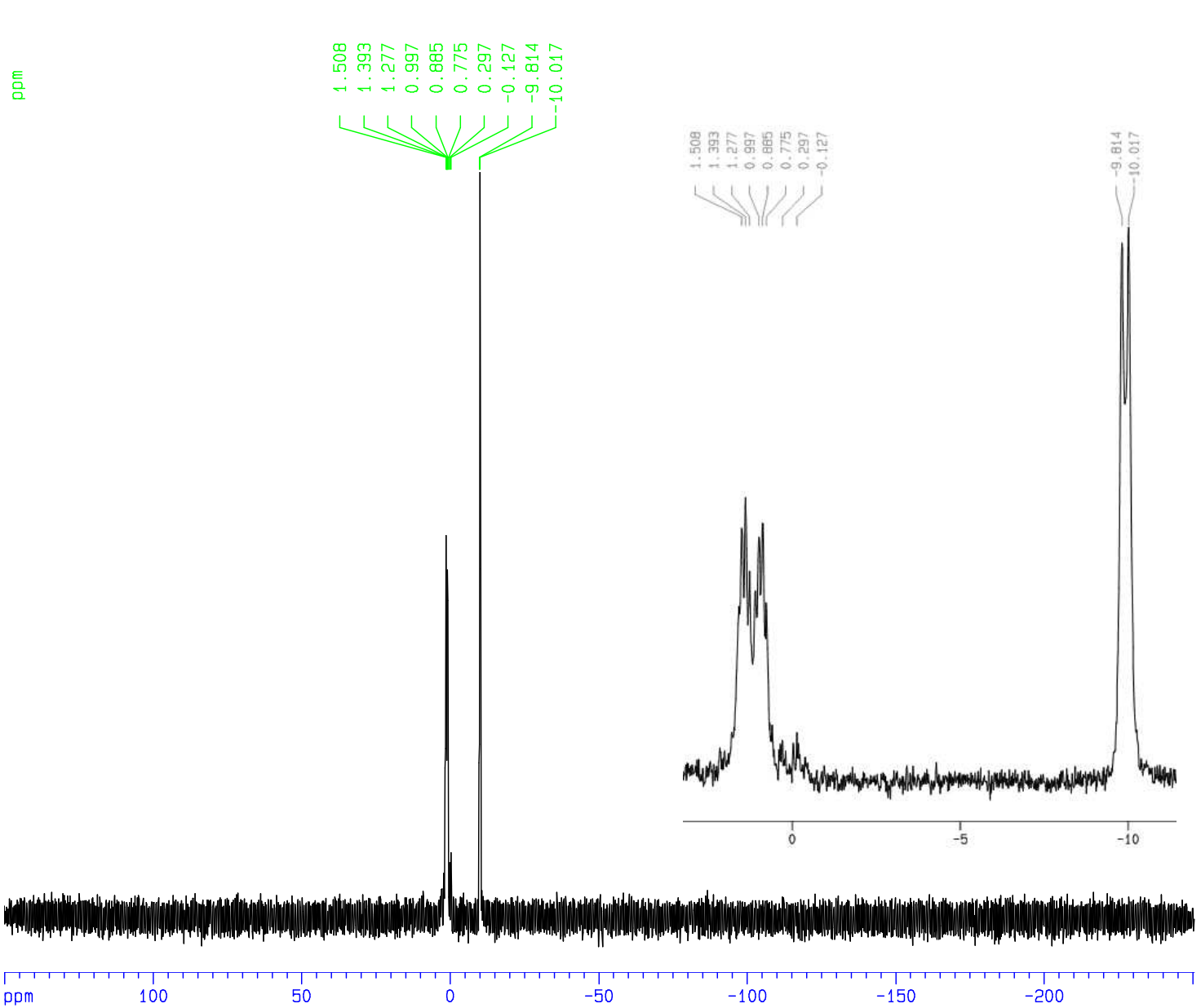
============ CHANNEL f1 =============
NUC1                31P
P1                10.00 usec
PL1                3.00 dB
SFO1         121.4117167 MHz

============ CHANNEL f2 =============
CPDPRG2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                0.00 dB
PL12              16.00 dB
PL13              17.00 dB
SFO2         299.9411998 MHz

F2 - Processing parameters
SI                 32768
SF           121.4177880 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              150.387 ppm
F1              18259.60 Hz
F2P             -250.393 ppm
F2             -30402.20 Hz
PPMCM           20.03899 ppm/cm
HZCM          2433.09009 Hz/cm
```

^{31}P NMR (proton coupled) of APPCHFPPA, **3e** as the tetrabutylammonium salt in D_2O:




```
Current Data Parameters
NAME      040910TBA1_APPCHFPPA_TBA
EXPNO                 30
PROCNO                 1

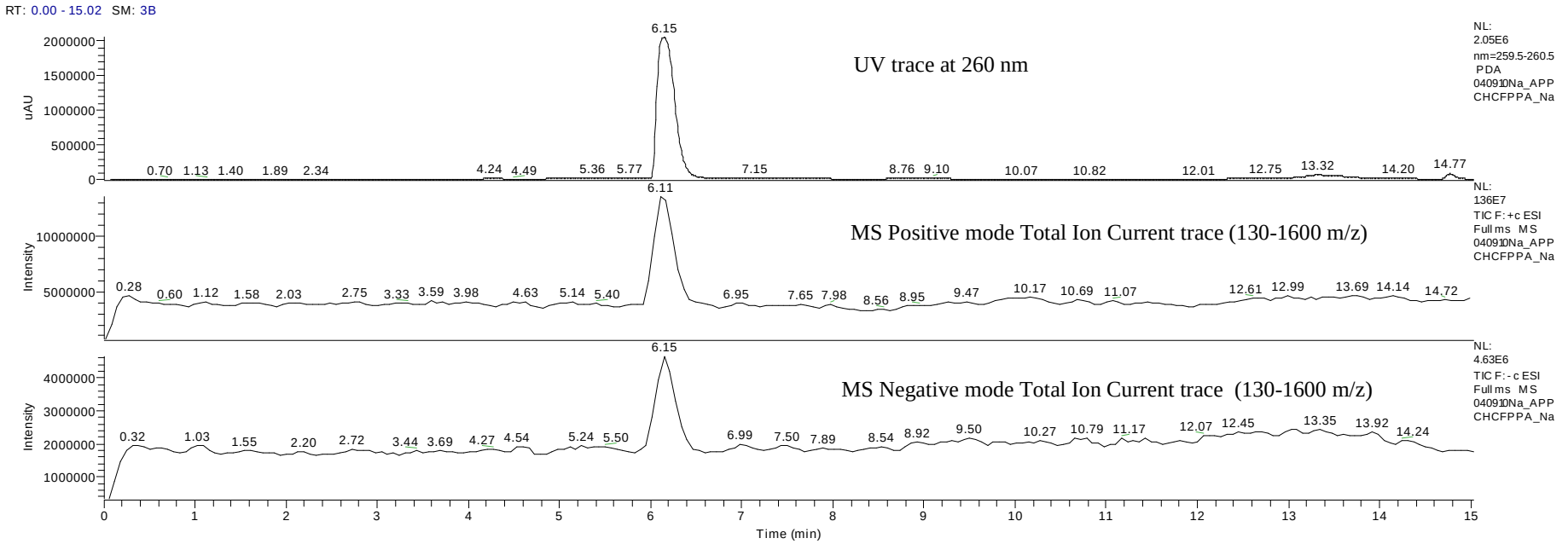
F2 - Acquisition Parameters
Date_           20041018
Time                14.44
INSTRUM             spect
PROBHD     5 mm Multinuc
PULPROG             zg30
TD                126514
SOLVENT              d2o
NS                   157
DS                     4
SWH            48661.801 Hz
FIDRES         0.384636 Hz
AQ             1.2999814 sec
RG                2580.3
DW                10.275 usec
DE                 6.00 usec
TE                300.0 K
D1           0.10000000 sec

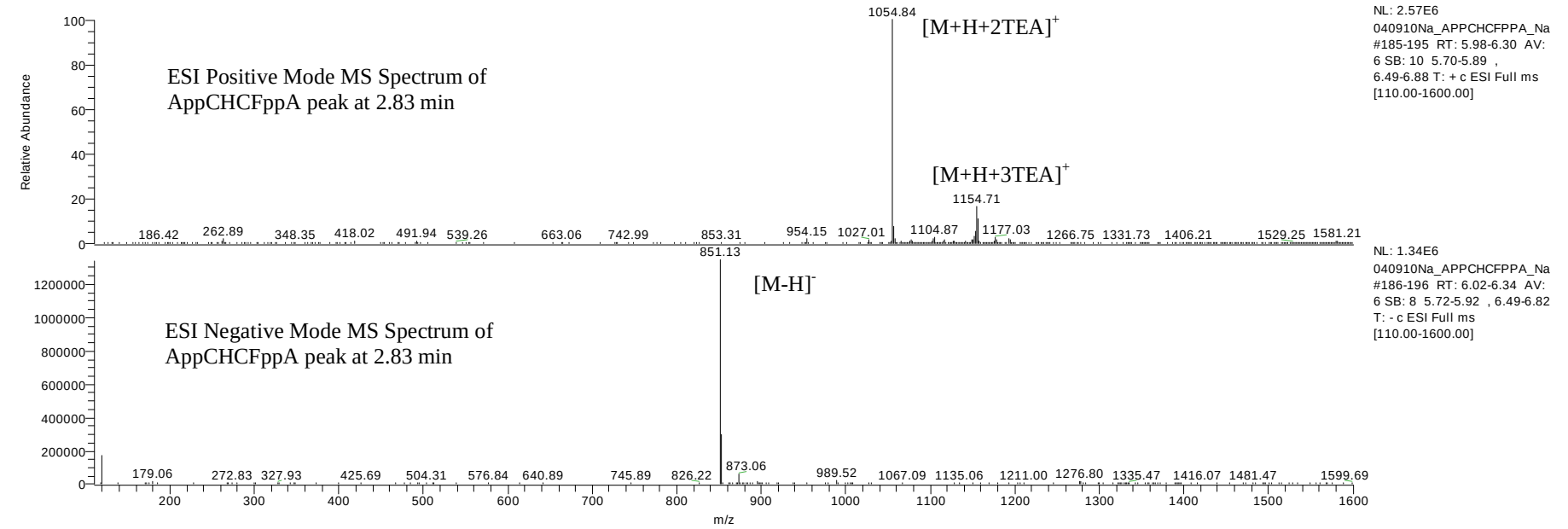
============ CHANNEL f1 =============
NUC1                31P
P1                10.00 usec
PL1                3.00 dB
SFO1         121.4117167 MHz

F2 - Processing parameters
SI                 32768
SF           121.4177880 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

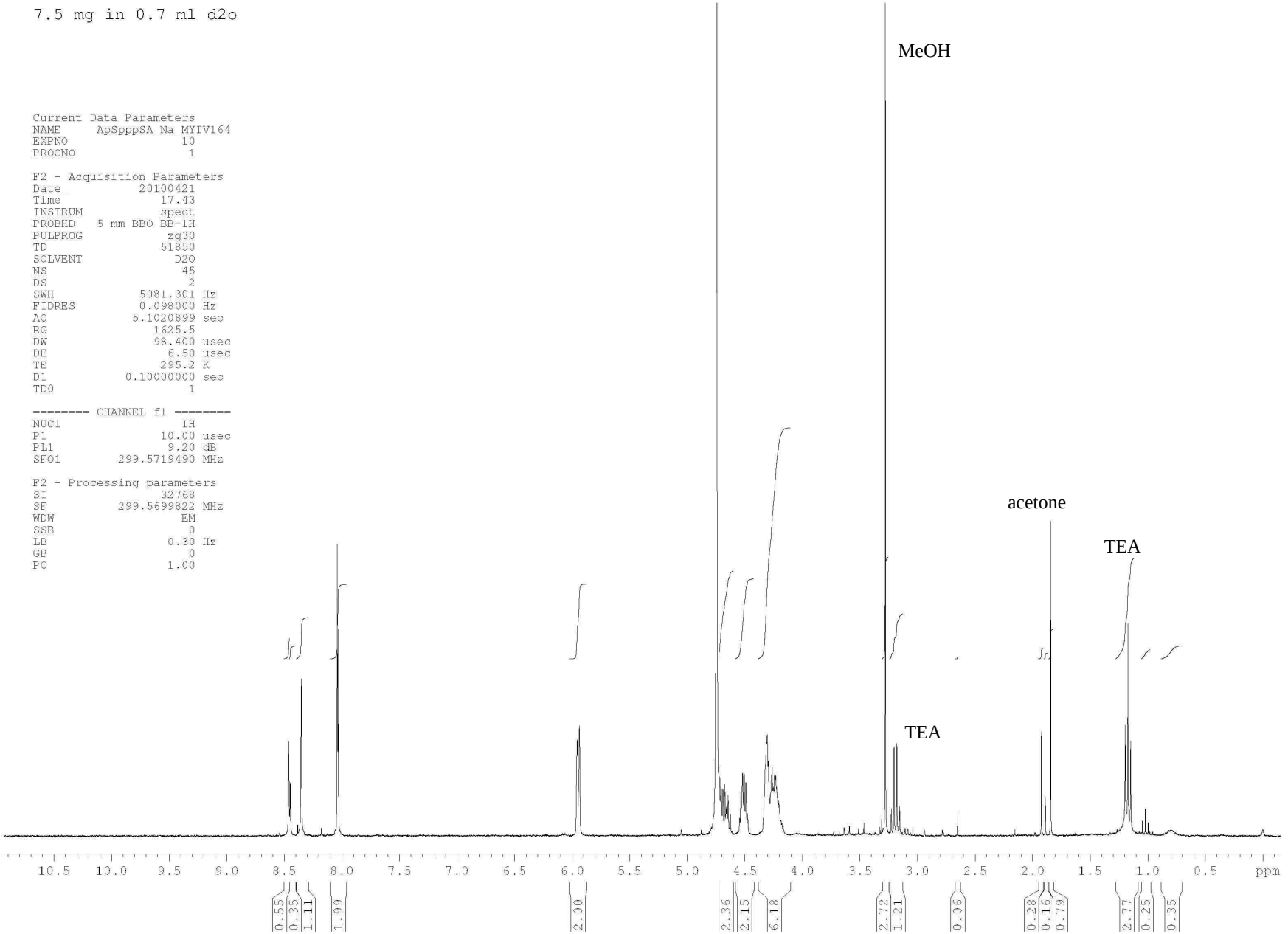
1D NMR plot parameters
CX                20.00 cm
F1P              150.387 ppm
F1              18259.60 Hz
F2P             -250.393 ppm
F2             -30402.20 Hz
PPMCM           20.03899 ppm/cm
HZCM          2433.09009 Hz/cm
```

LCMS of APPCHFPPA sodium salt, **3e**:

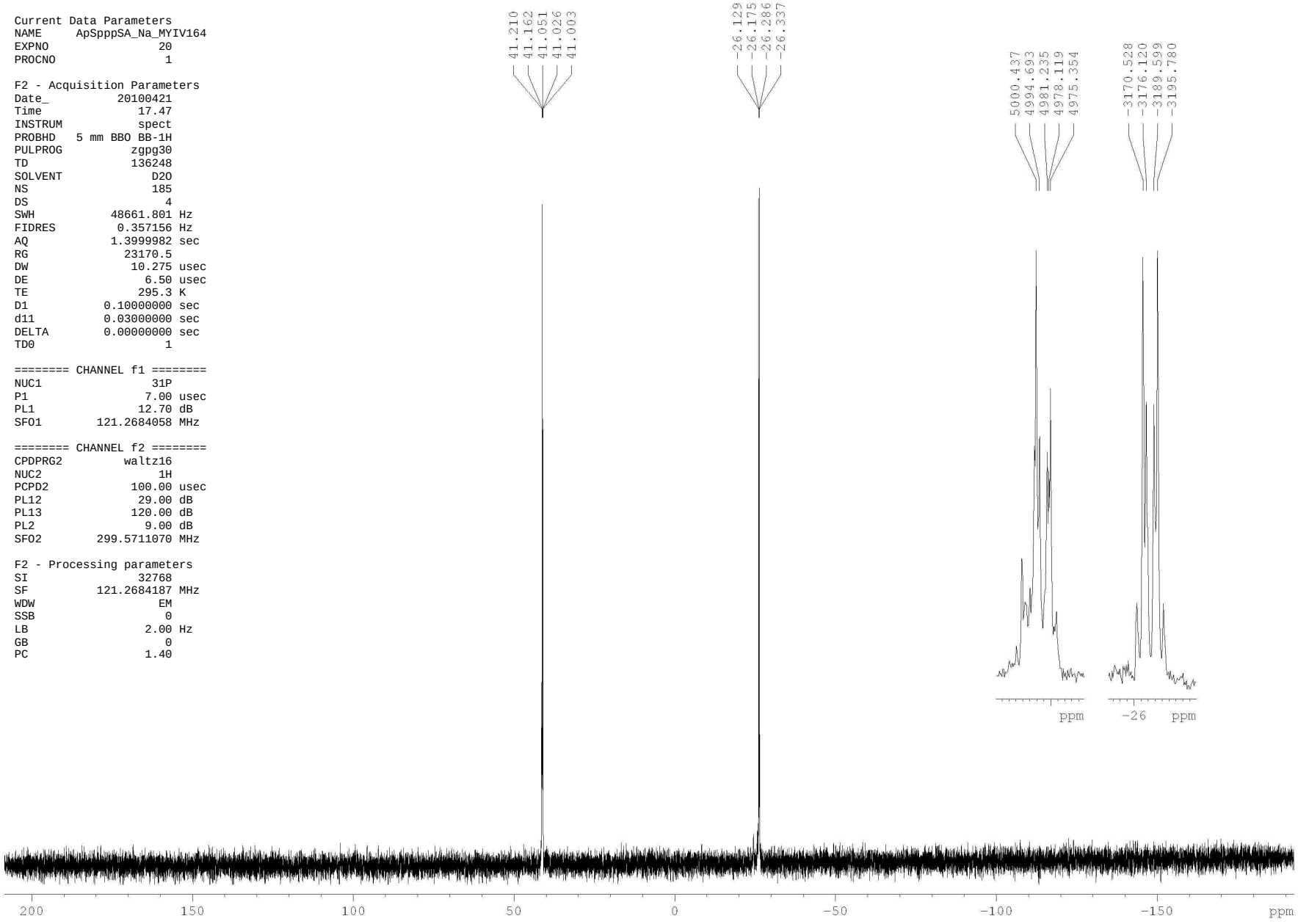




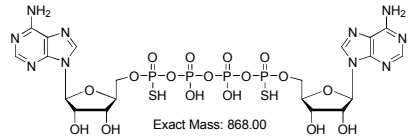
^{1}H NMR of AP(S)PPP(S)A sodium salt, **3b** in D_2O:

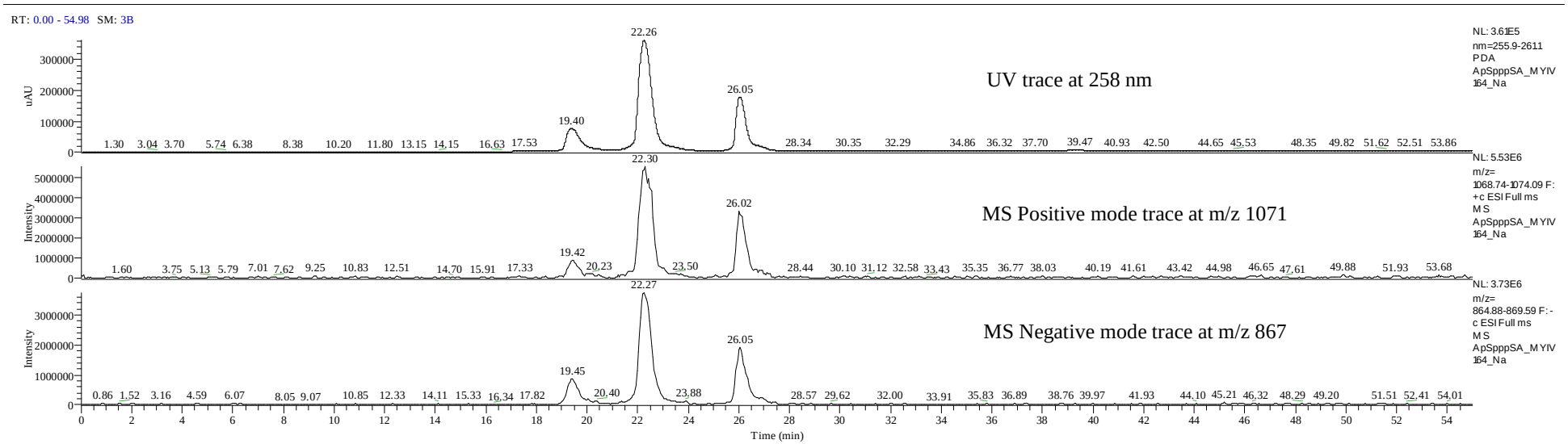


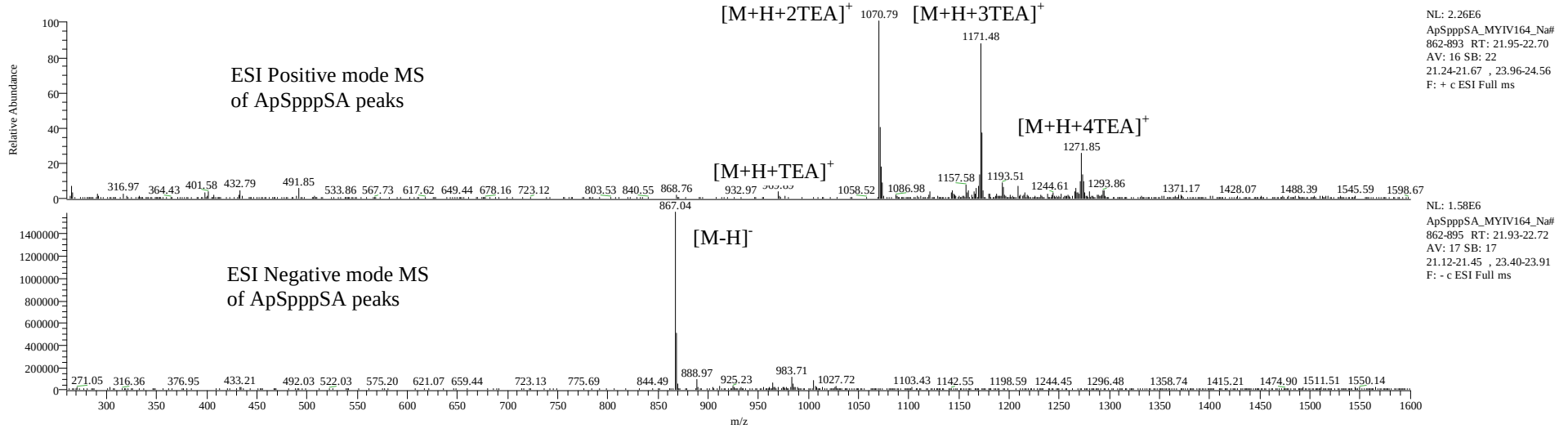
^{31}P (proton decoupled) NMR of AP(S)PPP(S)A sodium salt, **3b** in D_2O:



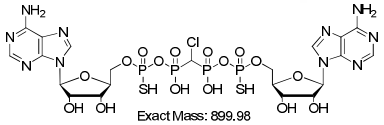
Separation of the three diastereomers of AP(S)PPP(S)A, **3b** by reverse phase ion-pairing chromatography:





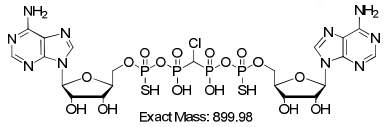


^{1}H NMR spectrum of AP(S)PCHClPP(S)A sodium salt, **3d** in D_2O:



^{31}P (proton decoupled) NMR spectrum of AP(S)PCHClPP(S)A sodium salt, **3d** in D_2O:

Phosphorous NMR (proton dec.) of APsPCHClPPsA sodium salt in D20, batches iy3-3 and iy3-7

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Current Data Parameters
NAME      iy3-3-iy3-7-b1
EXPNO                 30
PROCNO                 1

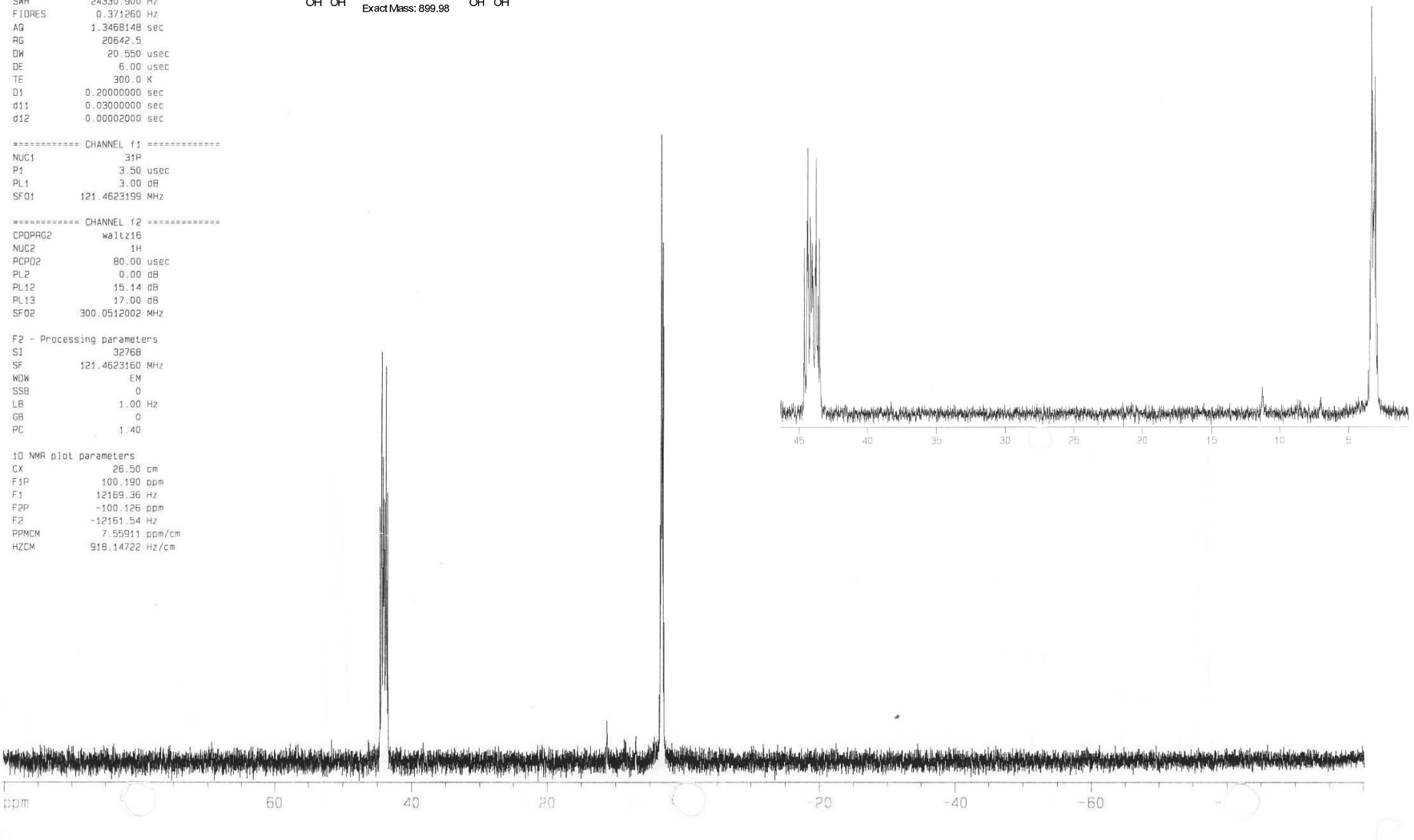
F2 - Acquisition Parameters
Date_           20030214
Time               12.10
INSTRUM            spect
PROBHD    5 mm Multinuc
PULPROG          zgpg30
TD                65536
SOLVENT             d20
NS                  490
DS                    4
SWH          24330.900 Hz
FIDRES        0.371260 Hz
AQ            1.346848 sec
RG              20642.5
DW             20.550 usec
DE              6.00 usec
TE             300.0 K
D1          0.20000000 sec
d11         0.03000000 sec
d12         0.00002000 sec

========== CHANNEL f1 =============
NUC1                31P
P1               3.50 usec
PL1              3.00 dB
SFO1       121.4623199 MHz

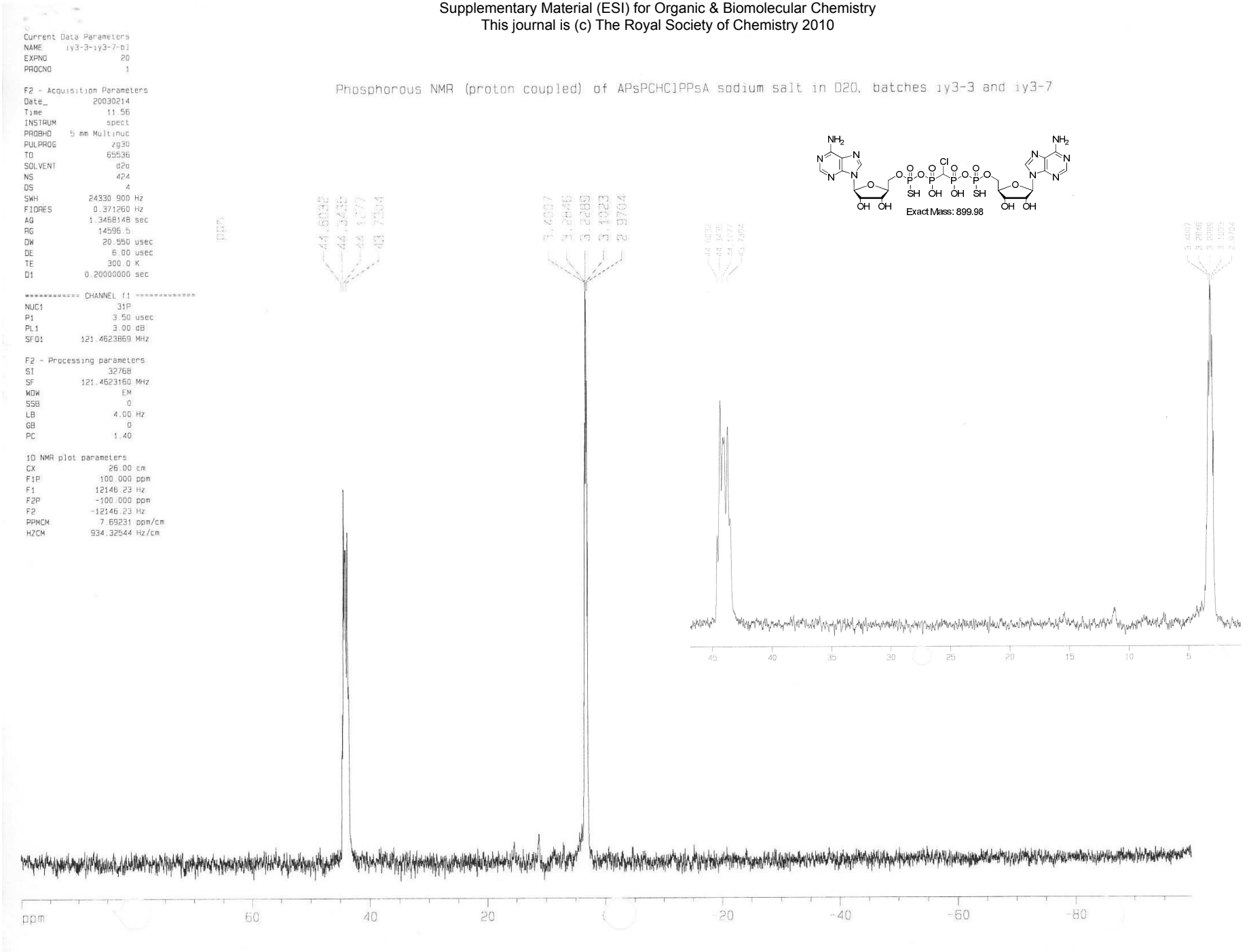
========== CHANNEL f2 =============
CPDPRG2         waltz16
NUC2                1H
PCPD2           80.00 usec
PL2              0.00 dB
PL12            15.14 dB
PL13            17.00 dB
SFO2       300.0512002 MHz

F2 - Processing parameters
SI                 32768
SF         121.4623160 MHz
WDW                   EM
SSB                    0
LB               1.00 Hz
GB                     0
PC               1.40

1D NMR plot parameters
CX              26.50 cm
F1P           100.190 ppm
F1          12169.36 Hz
F2P          -100.126 ppm
F2          -12161.54 Hz
PPMCM         7.55911 ppm/cm
HZCM        918.14722 Hz/cm
```



^{31}P (proton coupled) NMR spectrum of AP(S)PCHClPP(S)A sodium salt, **3d** in D_2O:



Separation of the four diastereomers of AP(S)PPCHClP(S)A, **3d** by reverse phase ion-pairing chromatography:

Supplementary Material (ESI) for Organic & Biomolecular Chemistry