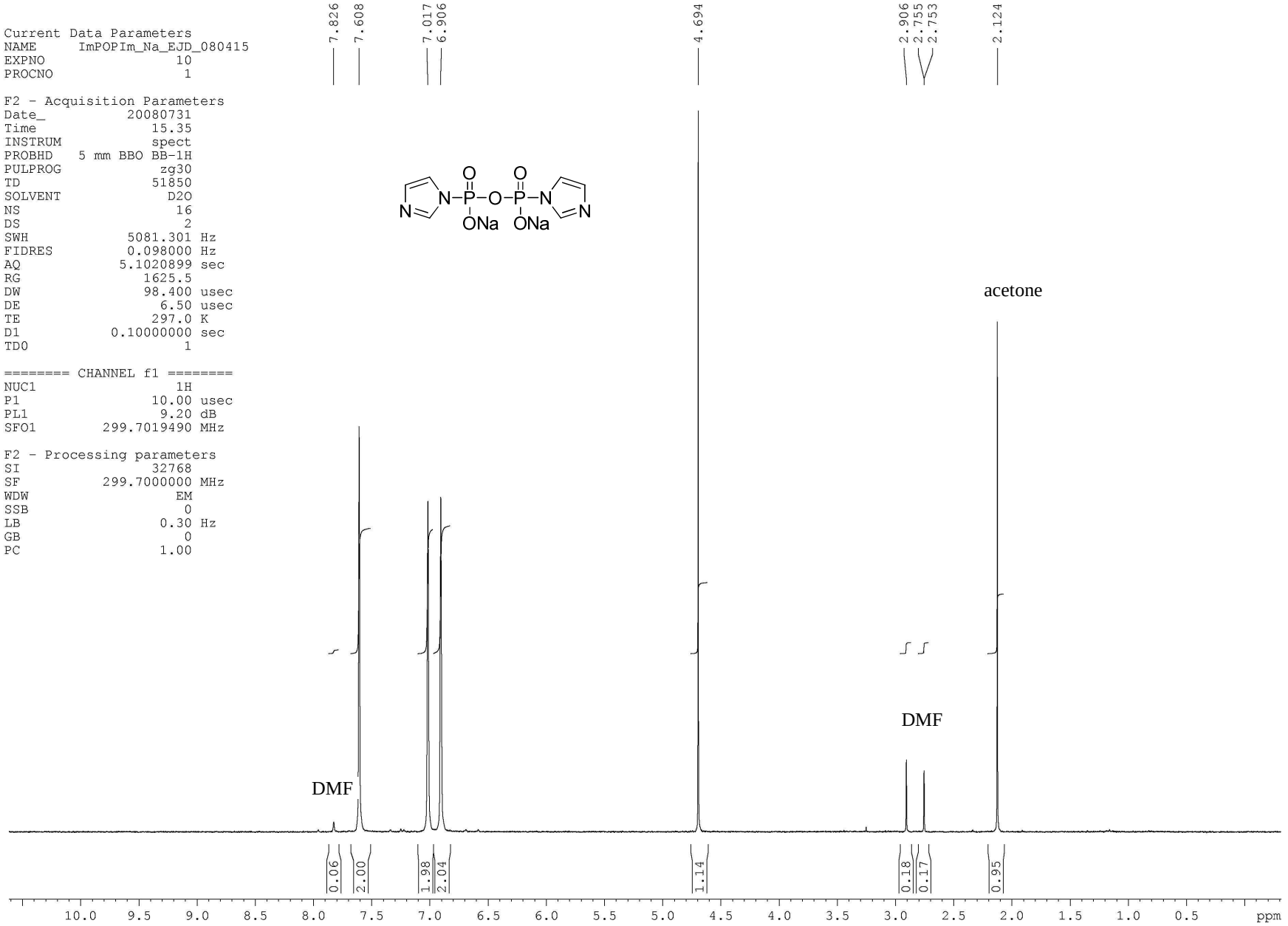
P^1,P^2-Diimidazolyl derivatives of pyrophosphate and *bis*-phosphonates – Synthesis, Properties, and Use in Preparation of Dinucleoside Tetraphosphates and Analogs

Ivan B. Yanachkov, Edward J. Dix, Milka I. Yanachkova, George E. Wright

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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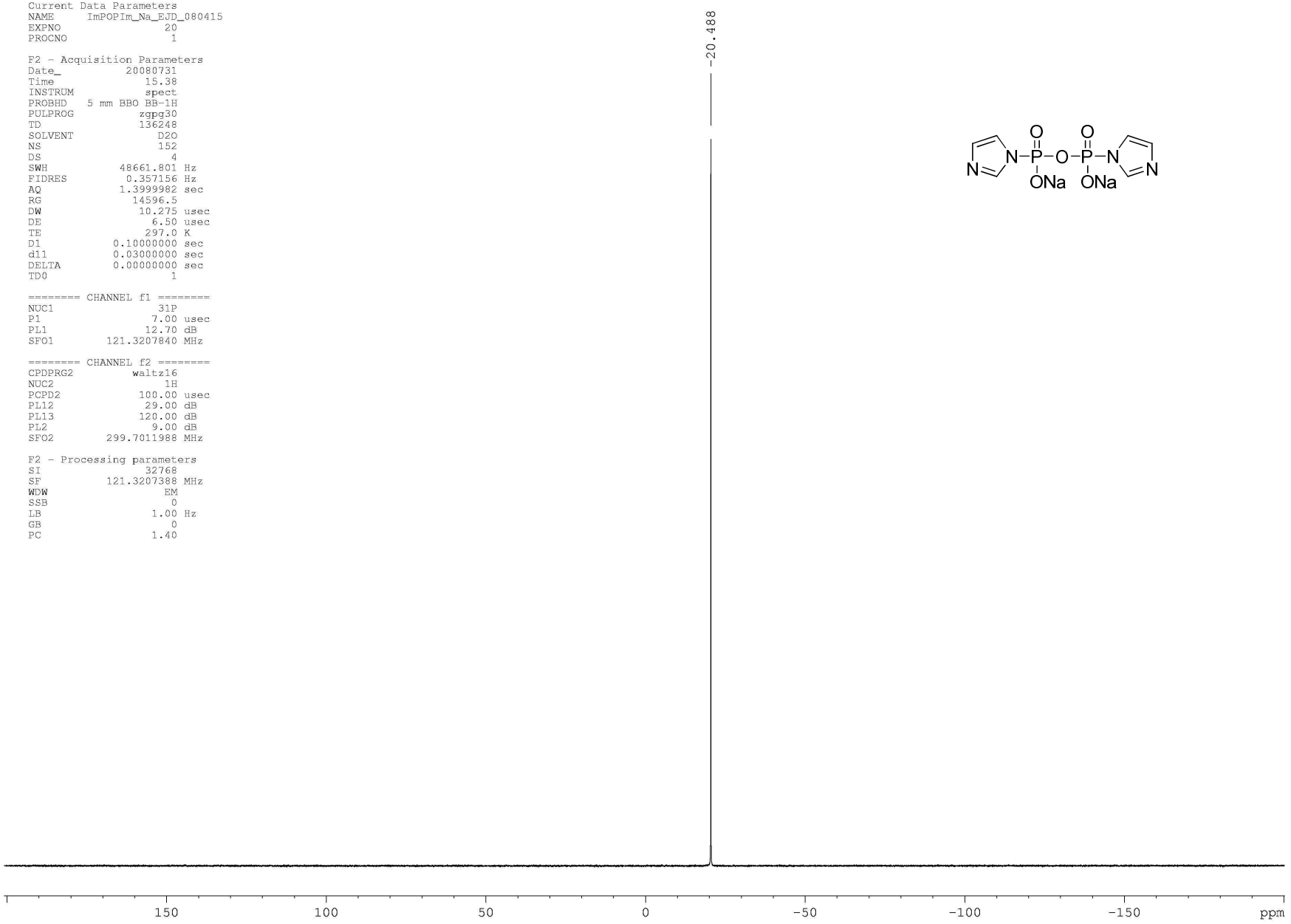

^{31}P NMR (proton coupled) of the di-sodium salt of compound **7a** in D_2O

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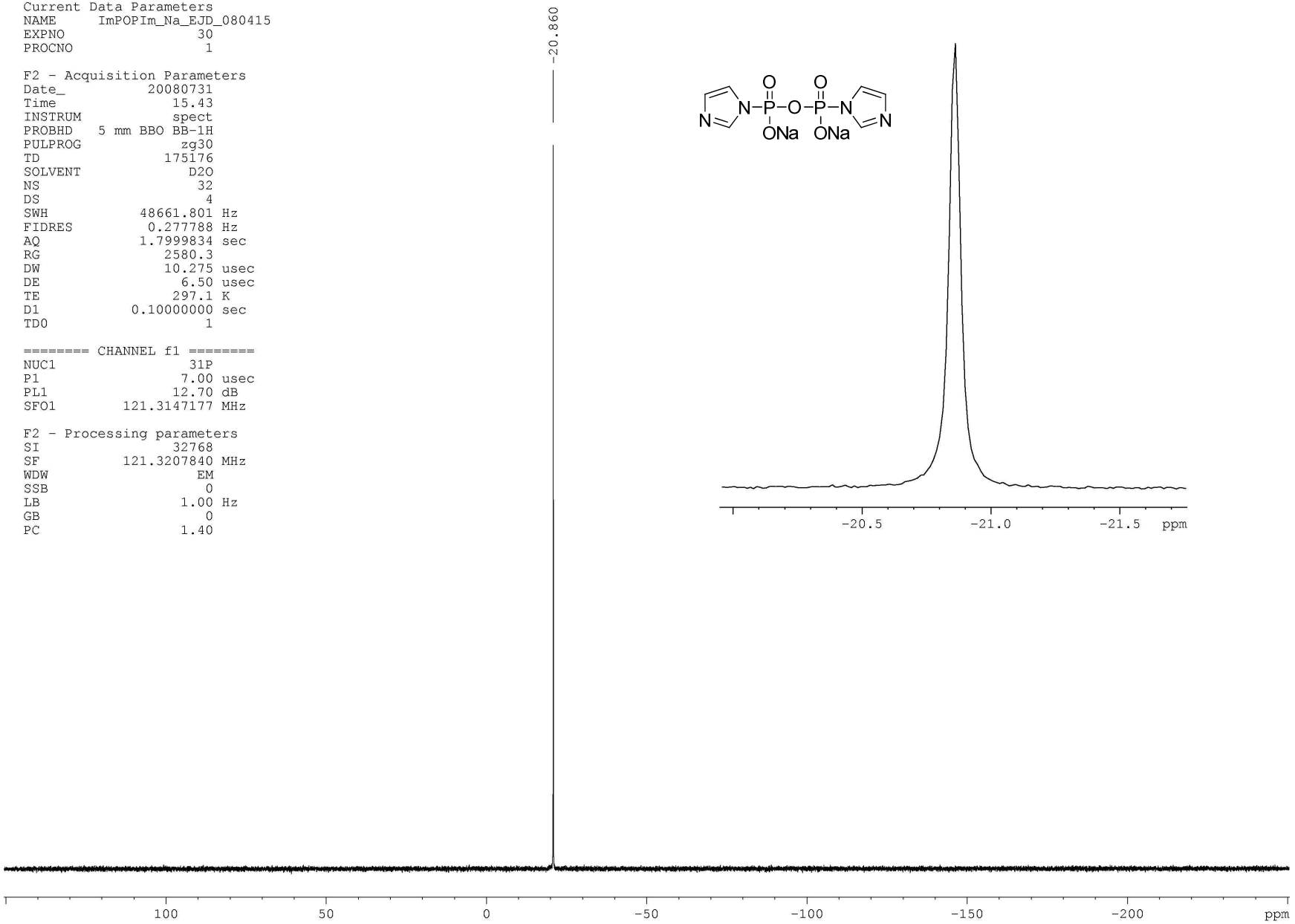
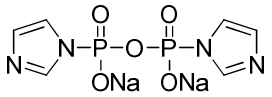
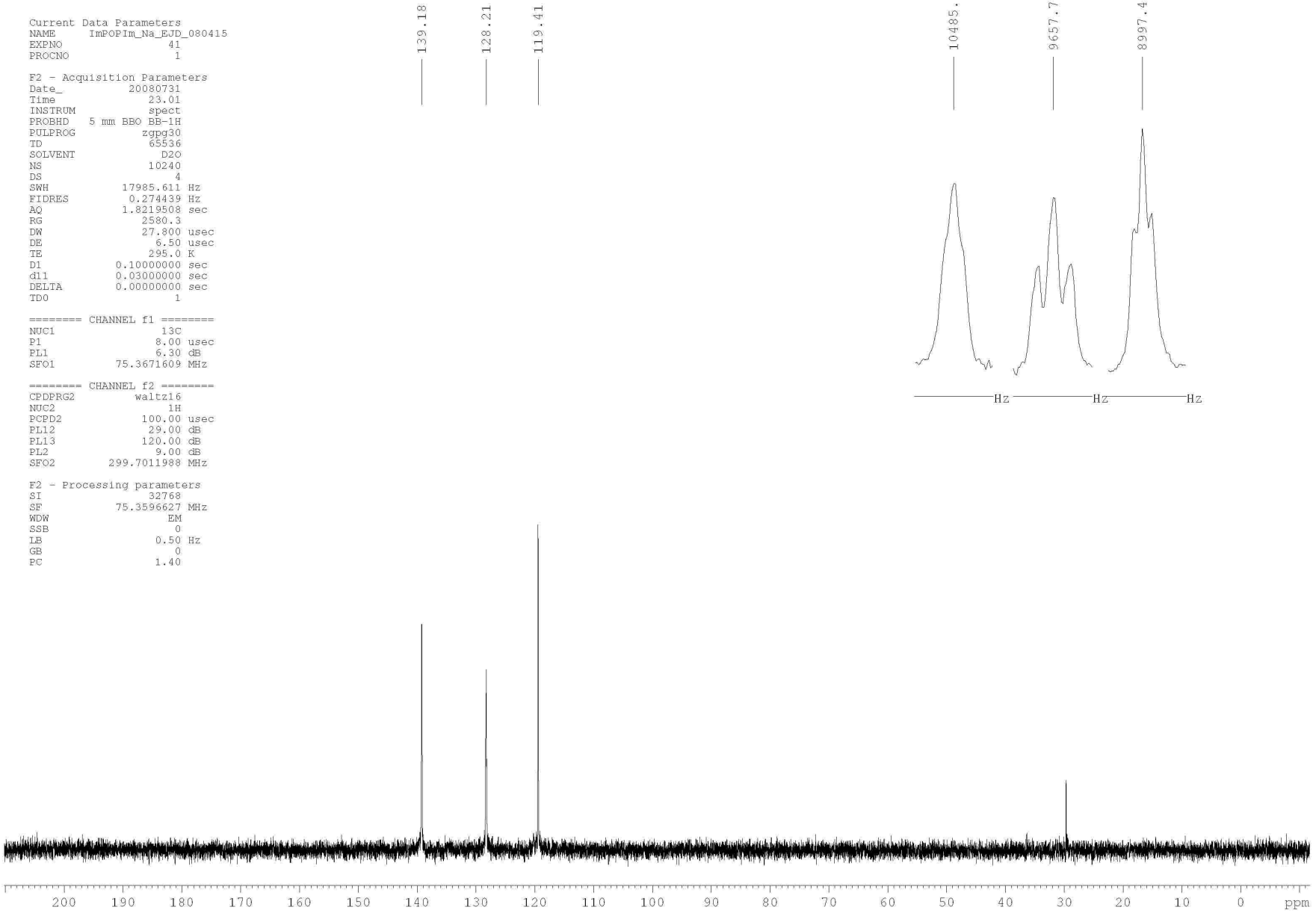
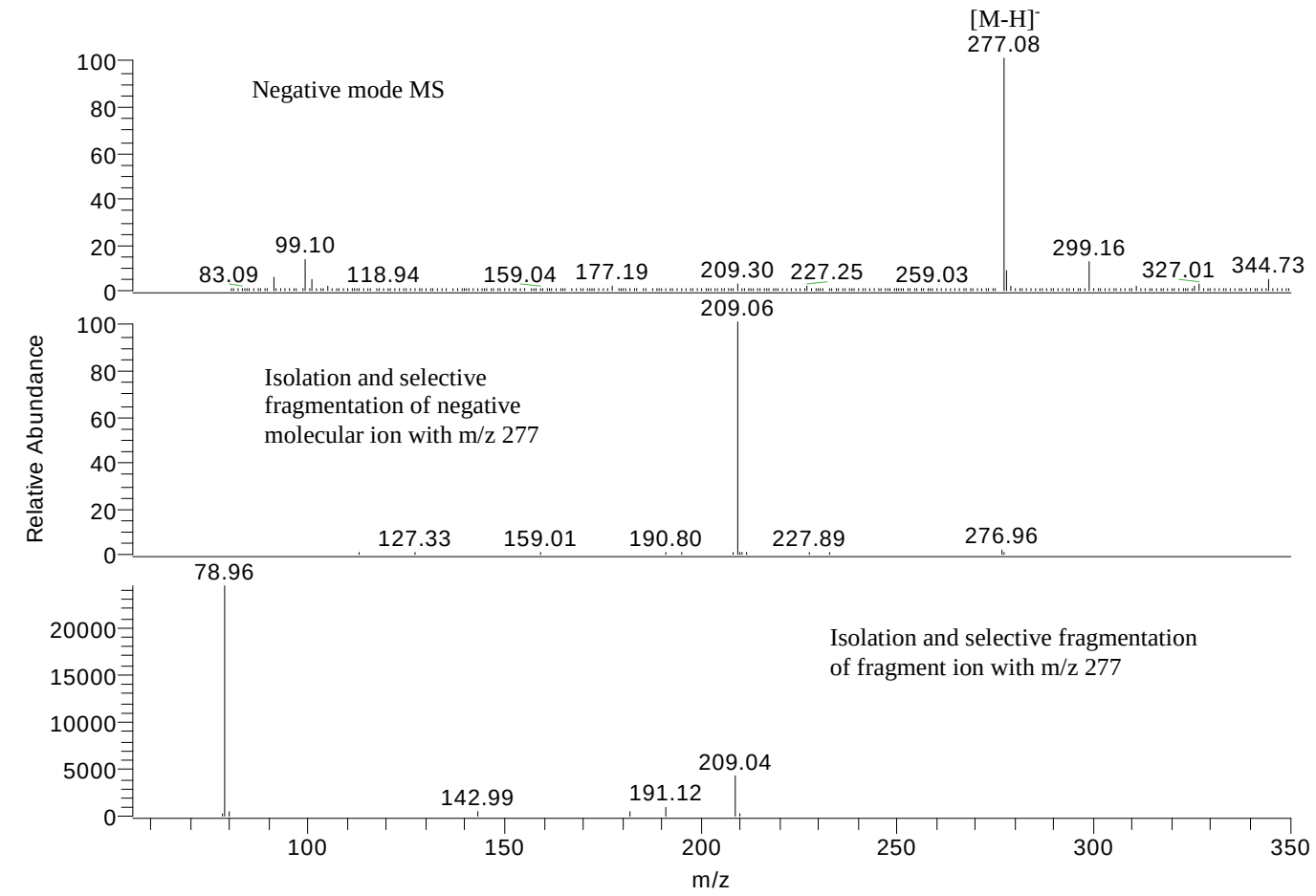


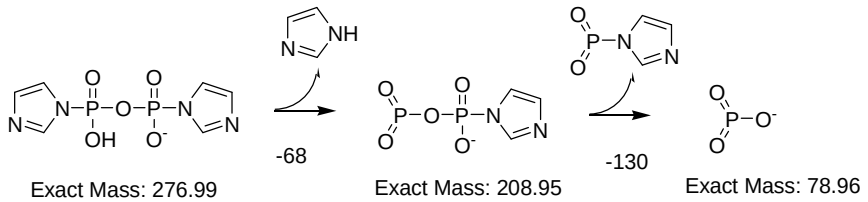
^{13}C NMR of the di-sodium salt of compound **7a** in D_2O





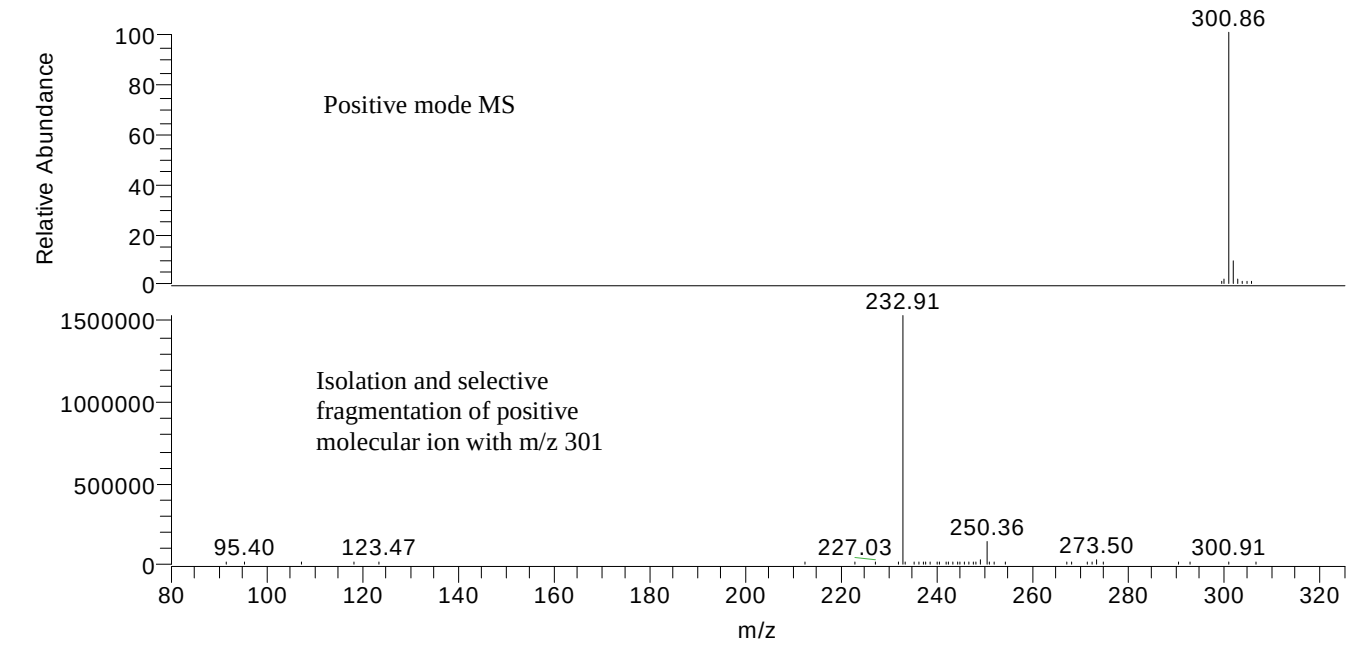
ESI Negative mode MS and selective MS^2 and MS^3 fragmentation of compound **7a**

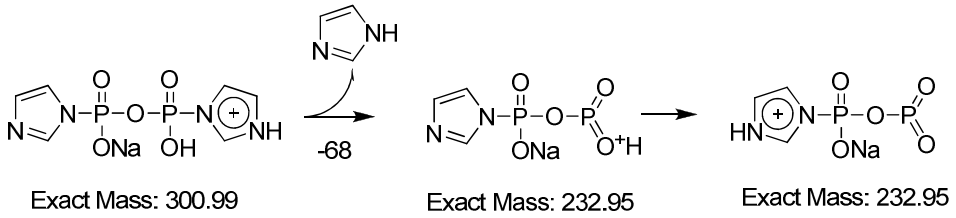




Negative mode fragmentation scheme

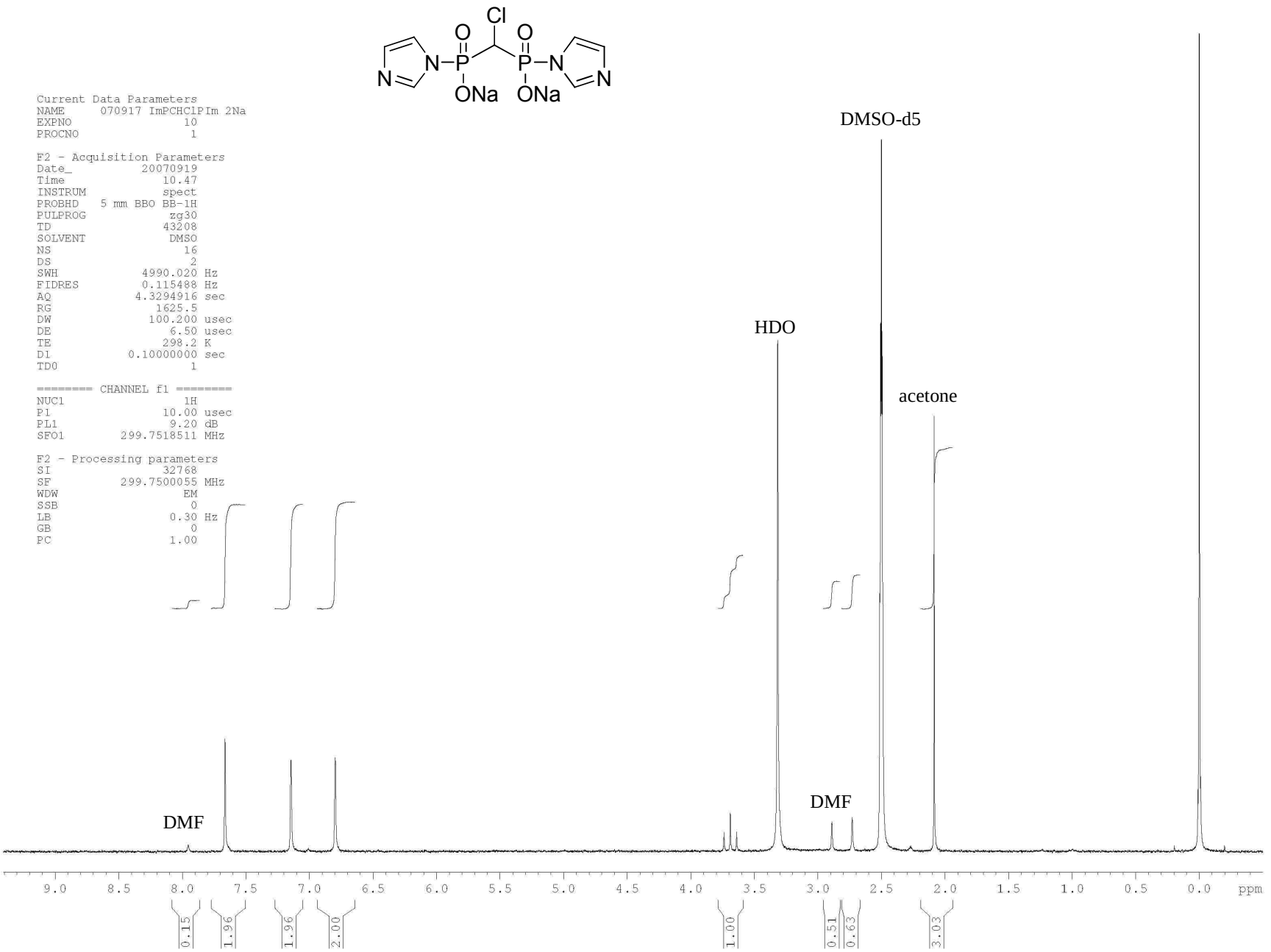
ESI Positive mode MS and selective MS^2 fragmentation of compound **7a**



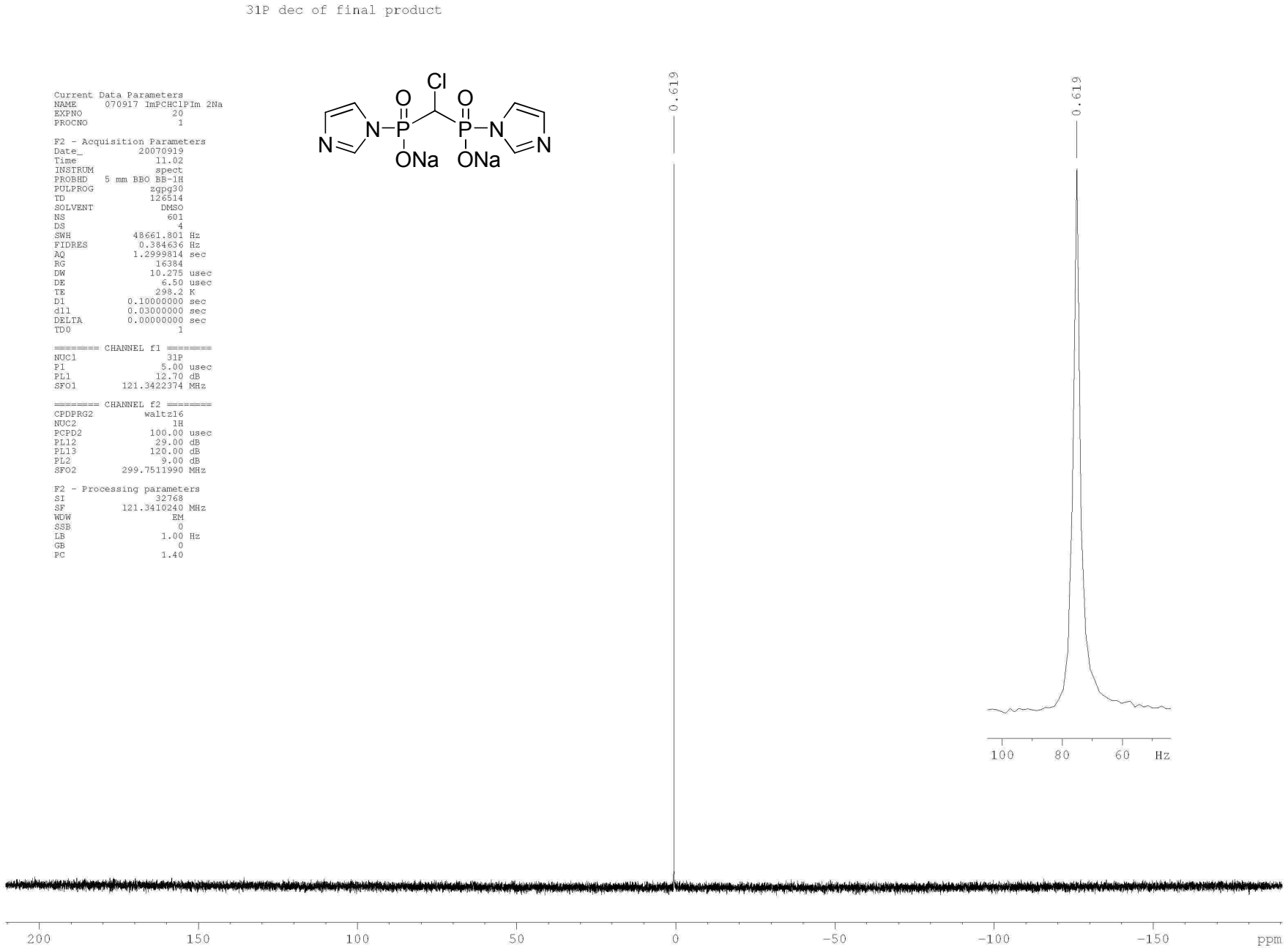


Positive mode fragmentation scheme

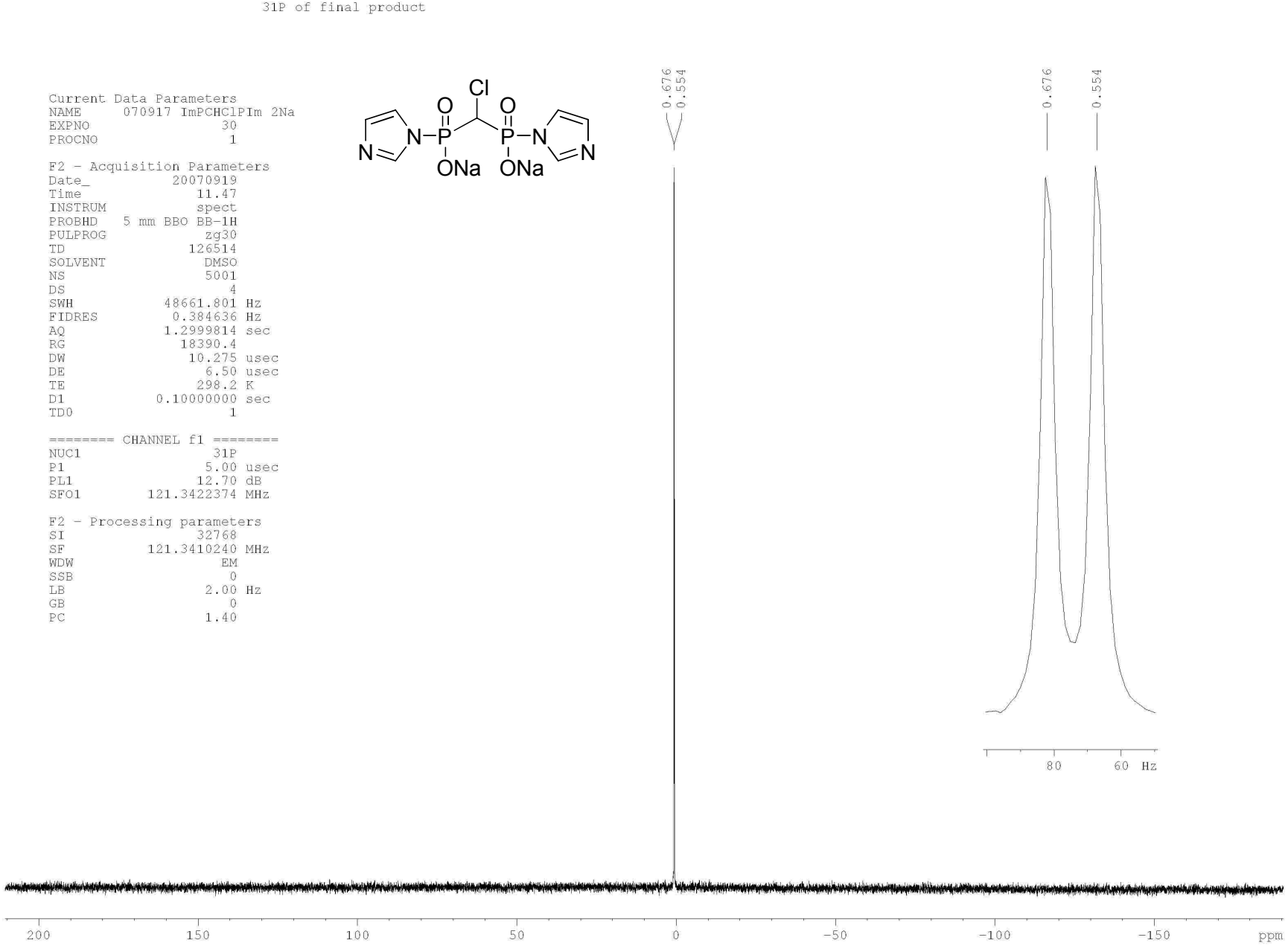
^{1}H NMR of the di-sodium salt of compound **7b** in DMSO-d6/D_2O



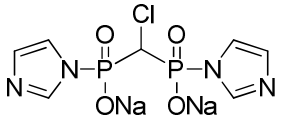
^{31}P NMR (proton decoupled) of the di-sodium salt of compound **7b** in DMSO-d6/D_2O



^{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**



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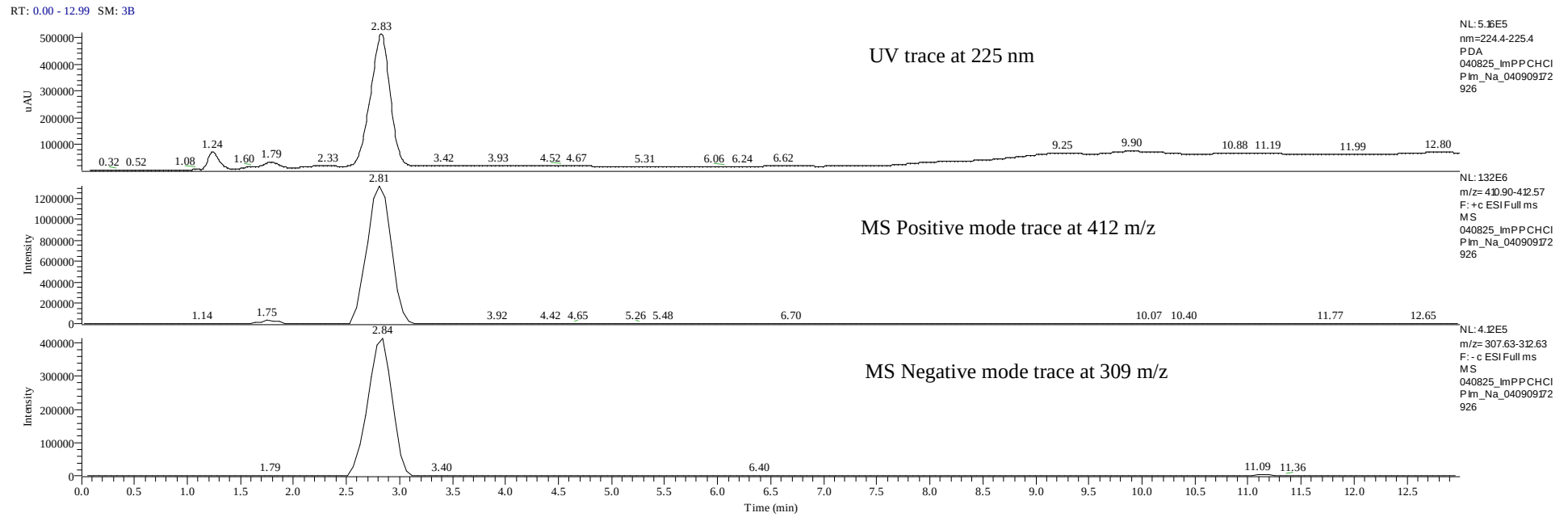
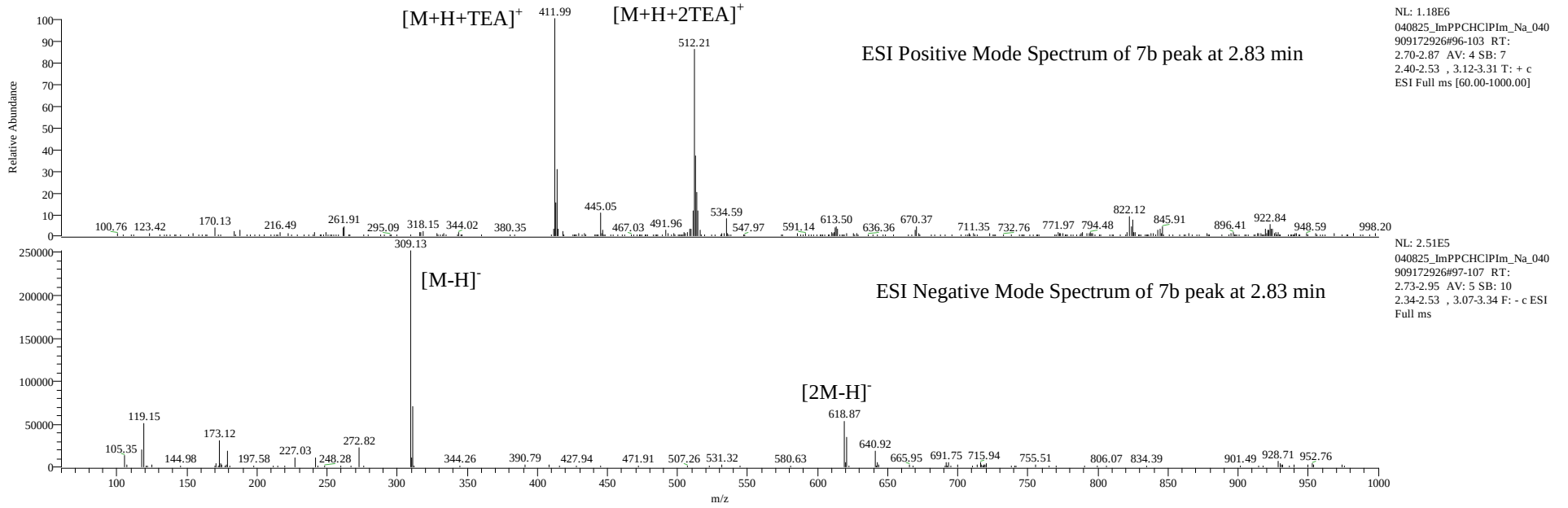
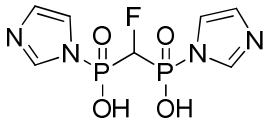




LCMS of the di-sodium salt of compound **7c**



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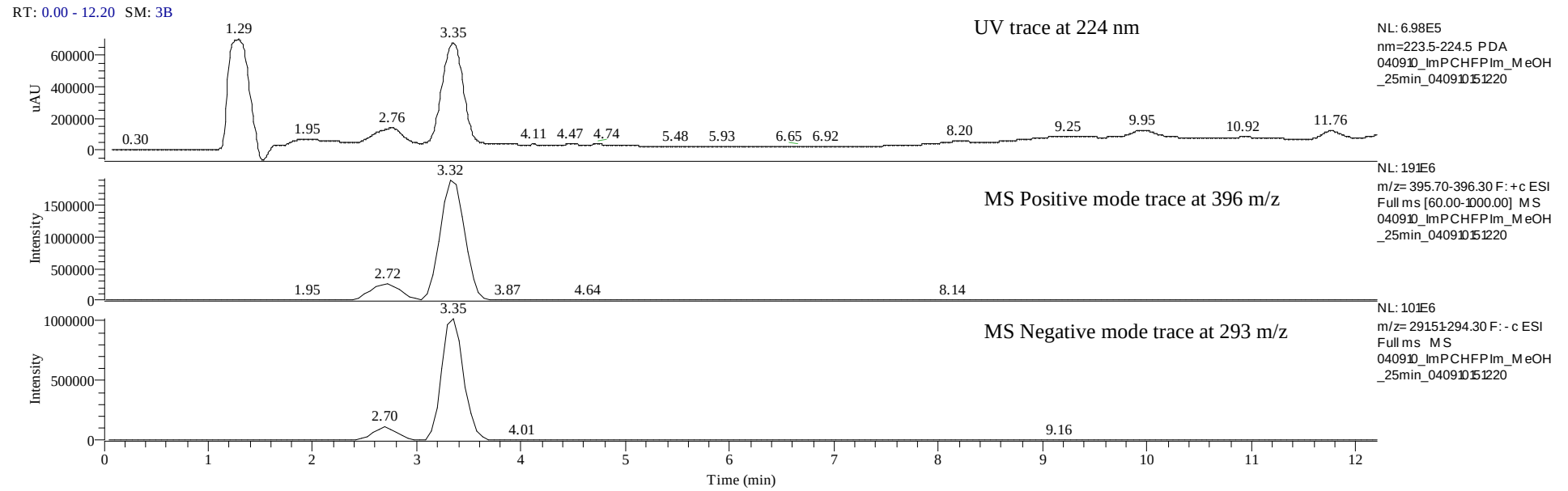
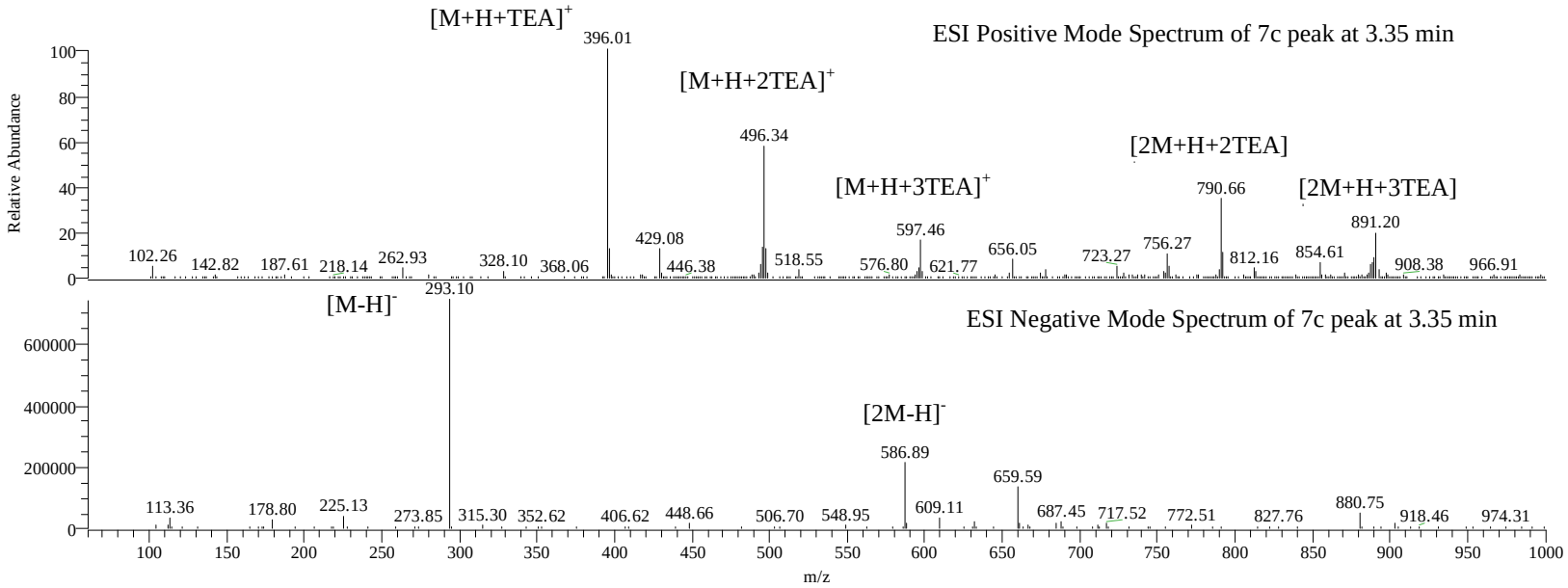
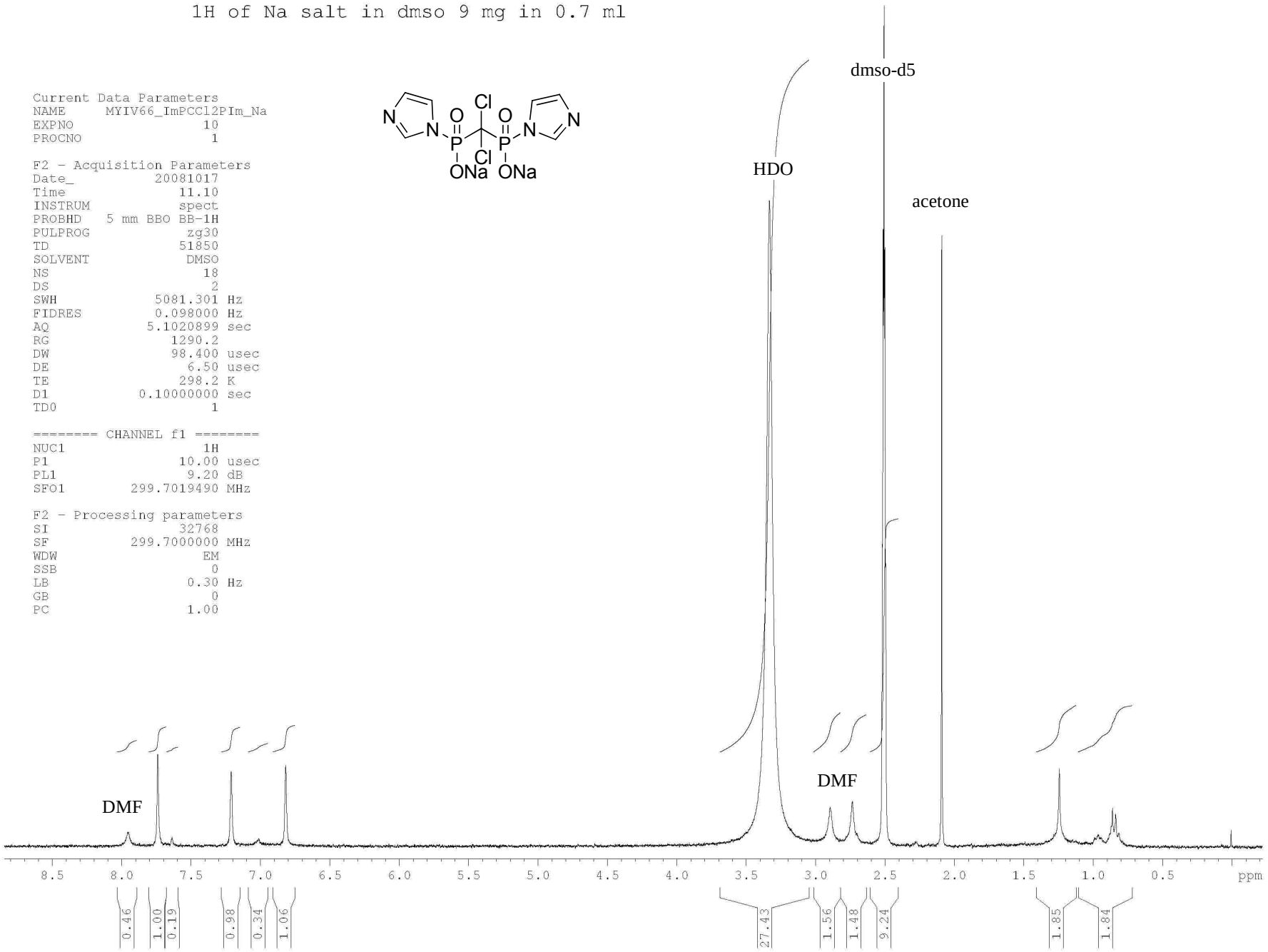
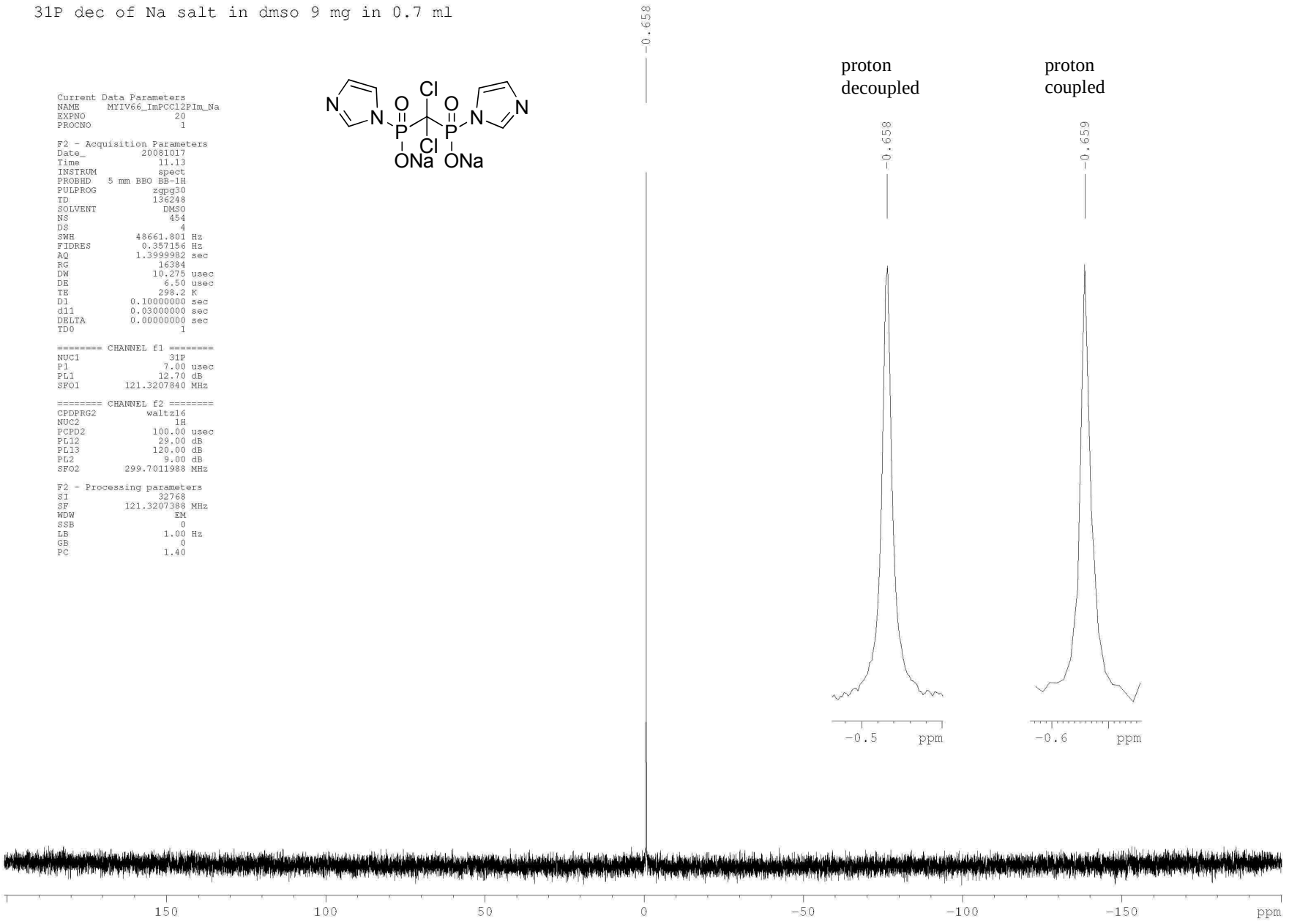




[1]H NMR of the di-sodium salt of compound **7d** in DMSO-d6

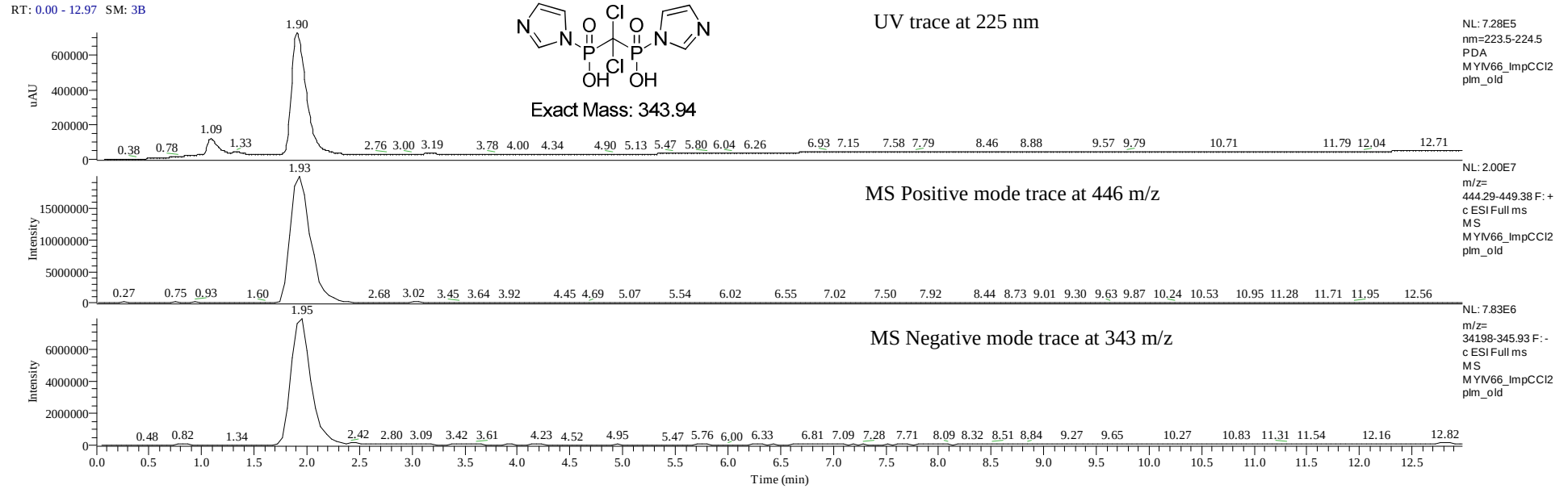


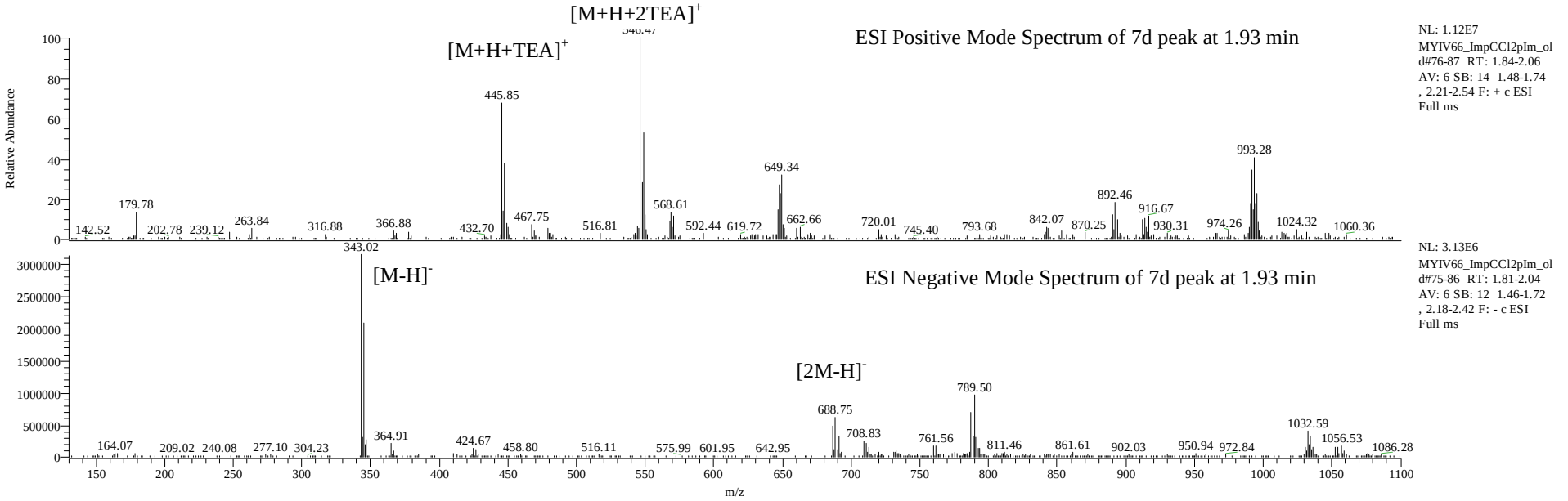
^{31}P NMR (proton decoupled) of the di-sodium salt of compound **7d** in DMSO-d6



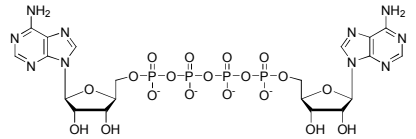
LCMS of the di-sodium salt of compound **7d**

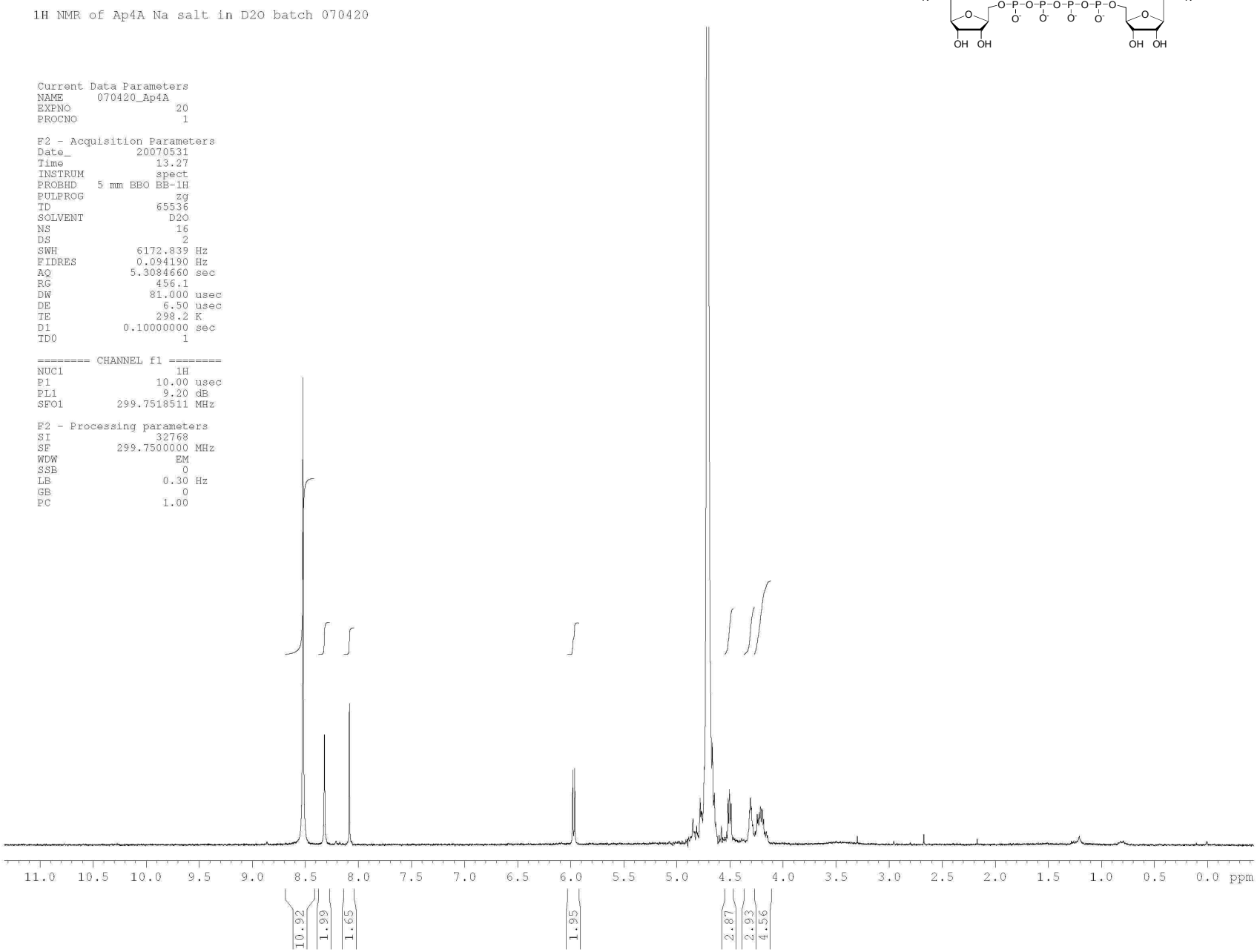
\\90.0.0.63\c$\...\MYIV66_ImpCCl2pIm_old 5/27/2009 6:00:40 PM
5 mg/ml in 20 mM TEAA



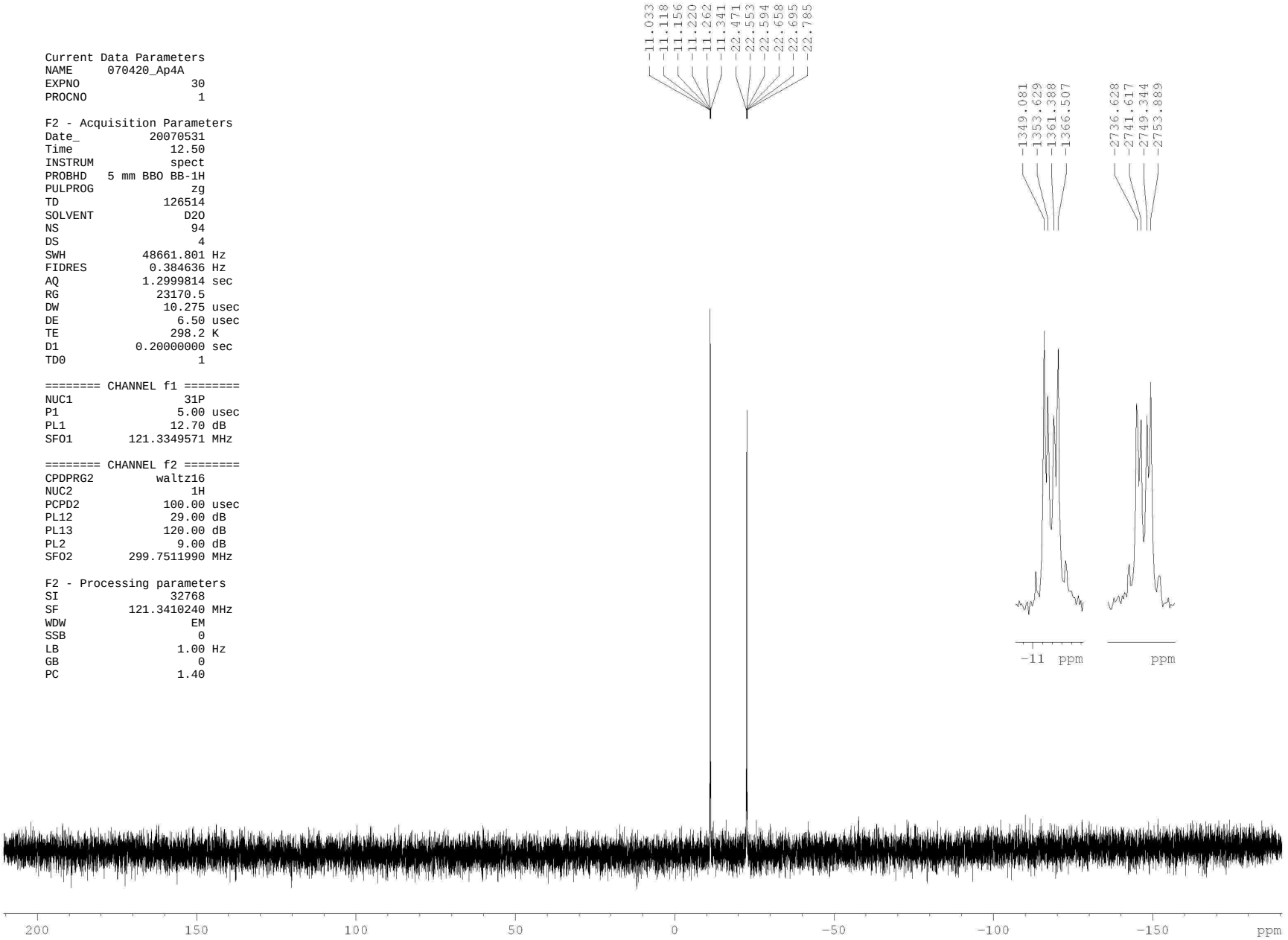


1H NMR spectrum of Ap_4A sodium salt, **3a** in D_2O

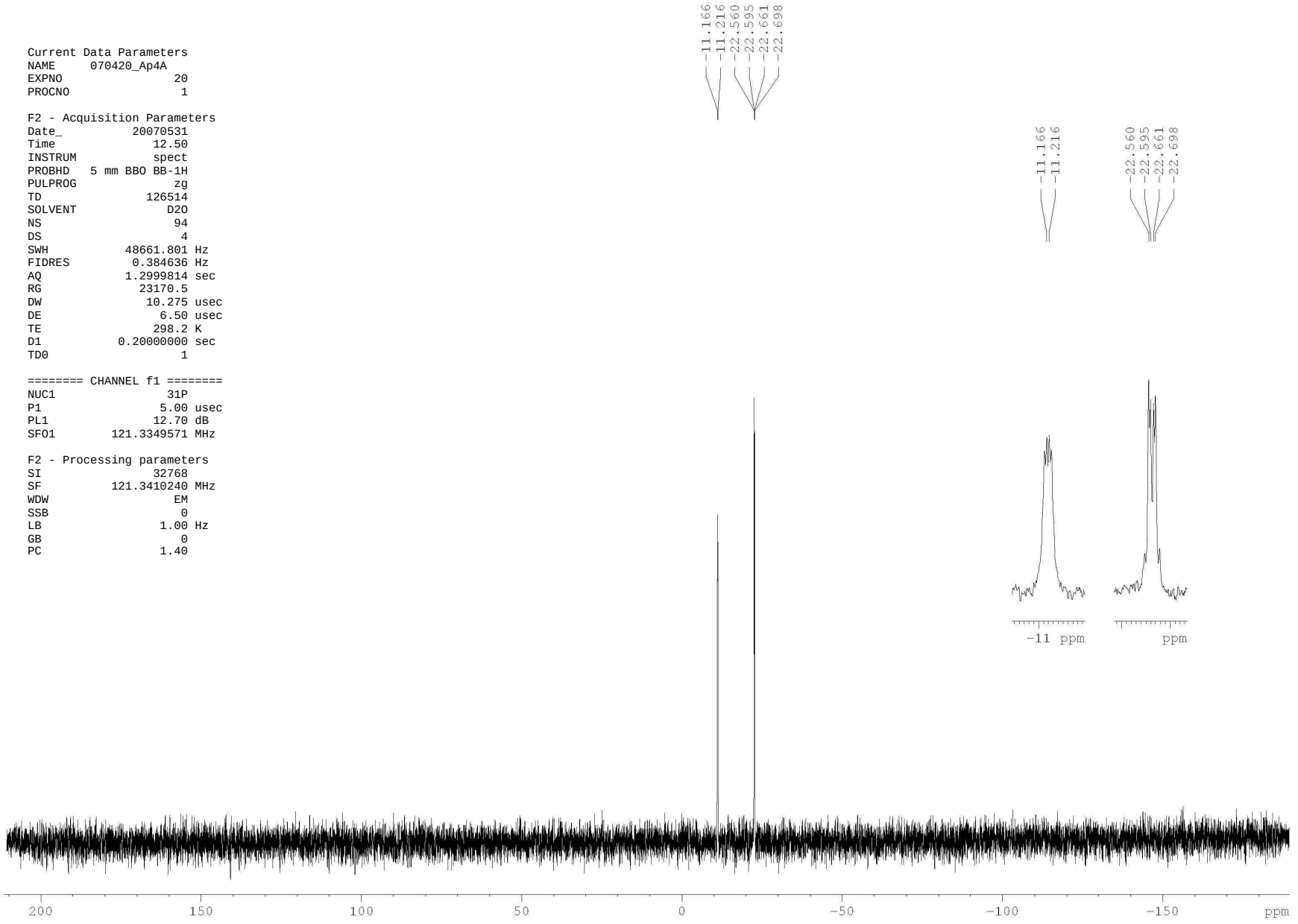




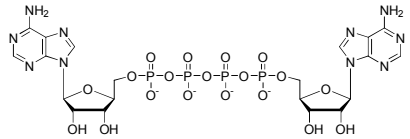
^{31}P (proton decoupled) NMR spectrum of Ap_4A sodium salt, **3a** in D_2O



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



LCMS of Ap_4A, **3a**



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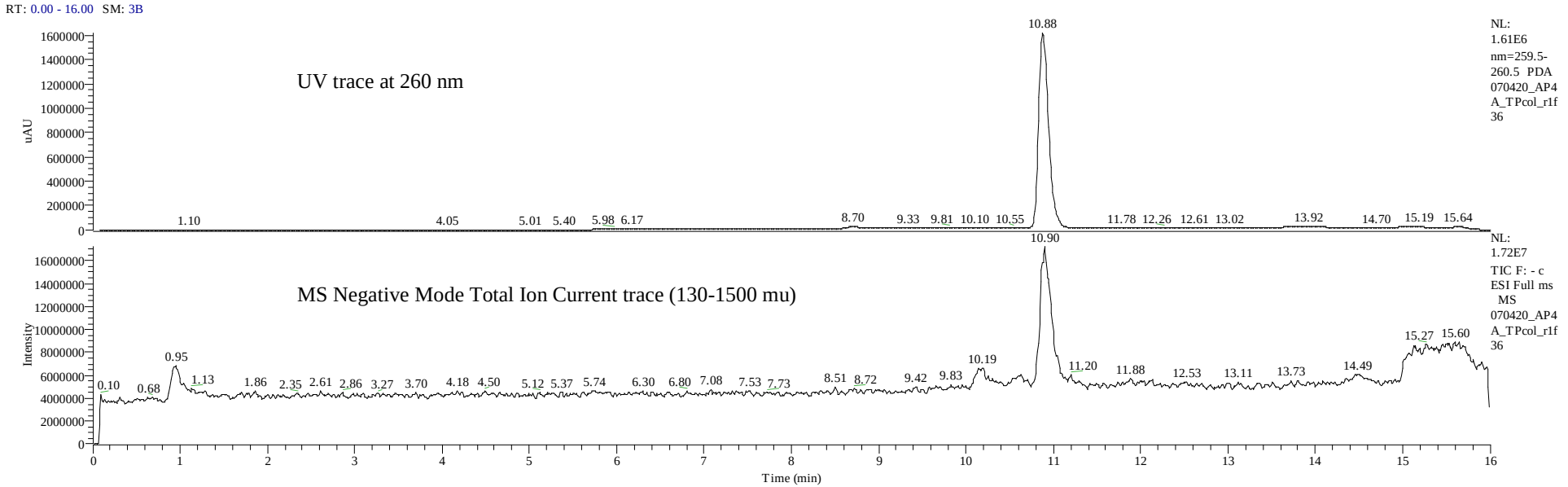

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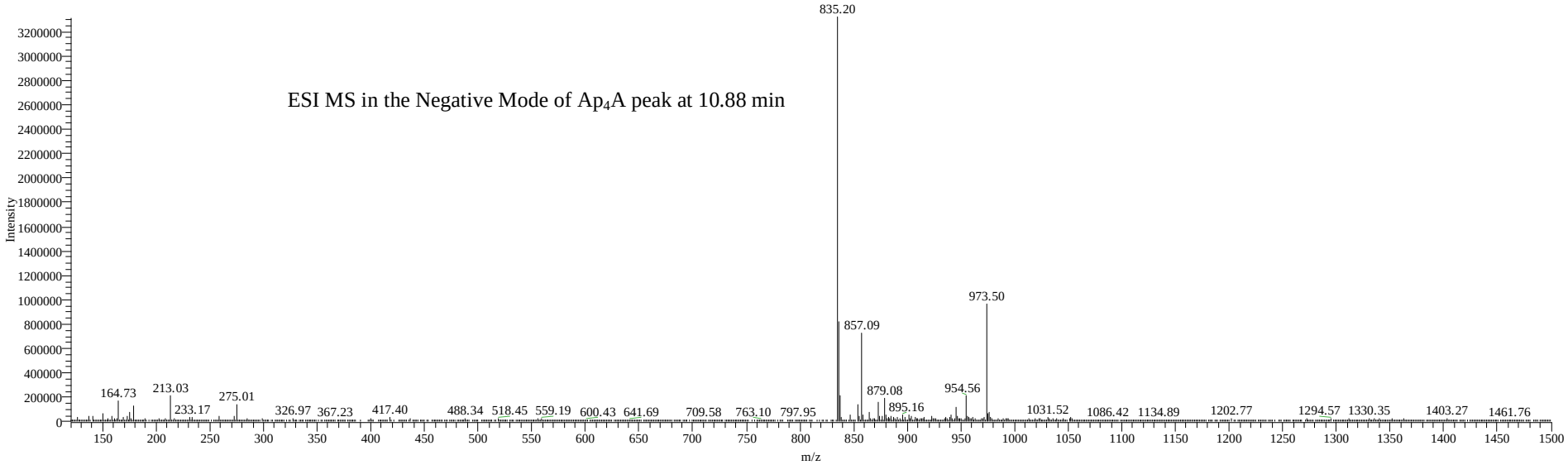
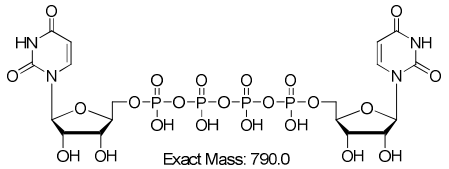
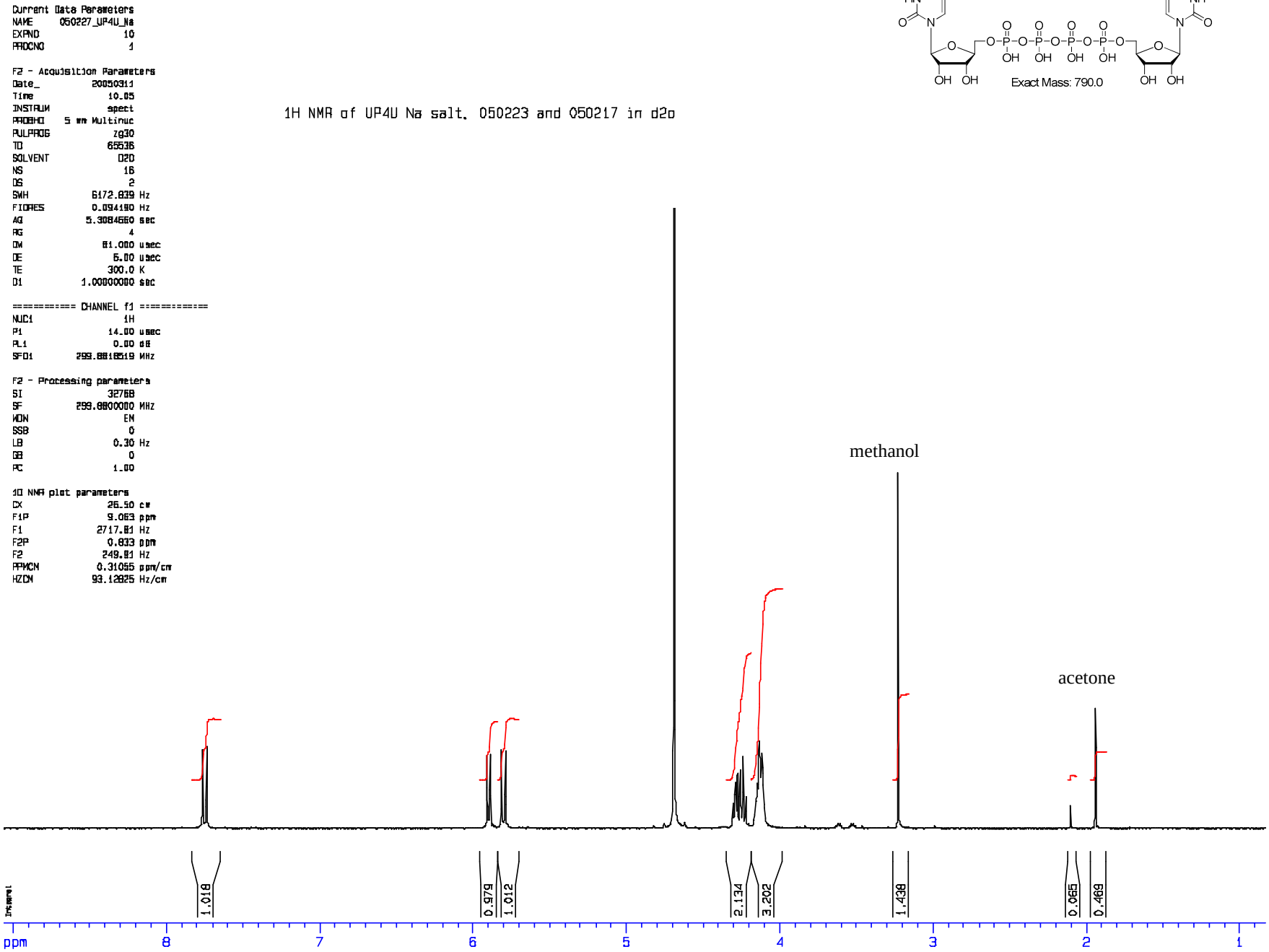
F: - c ESI Full ms

[M-H]

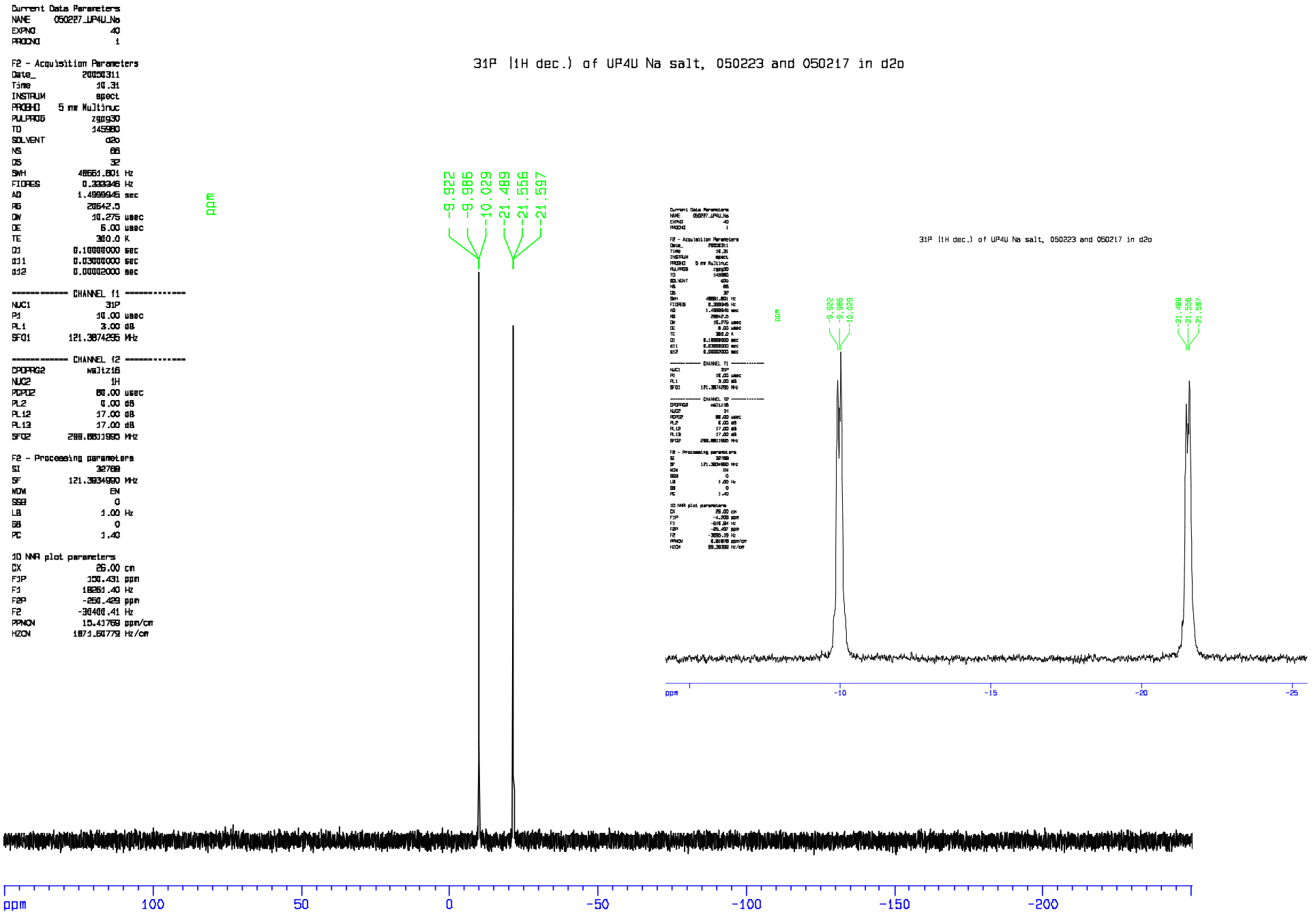


1H NMR spectrum of Up_4U sodium salt, **3f** in D_2O

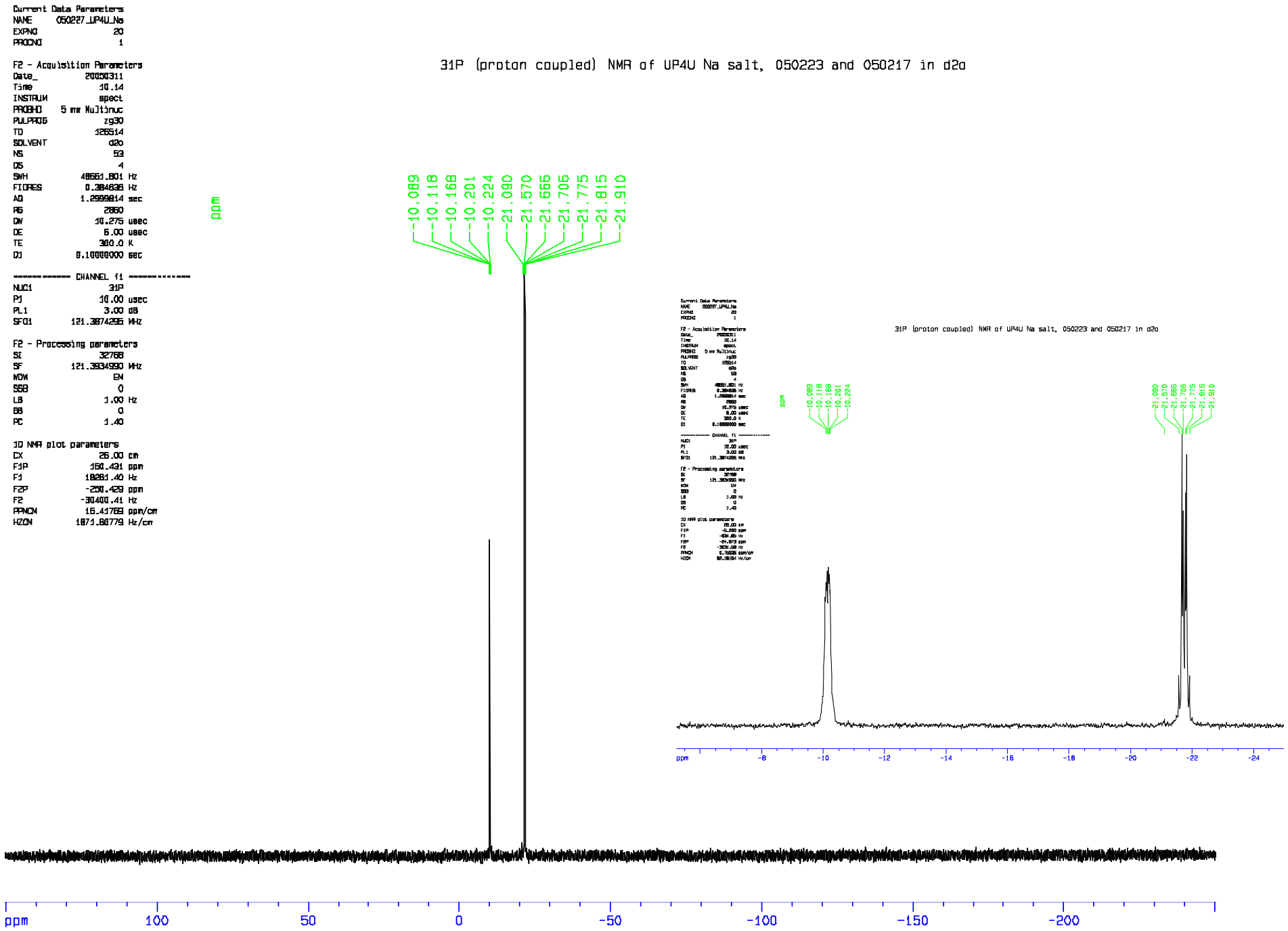




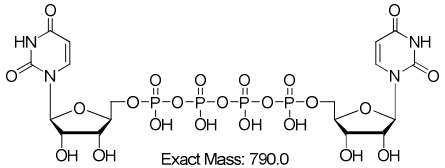
^{31}P (proton decoupled) NMR spectrum of Up_4U sodium salt, **3f** in D_2O



^{31}P (proton coupled) NMR spectrum of Up_4U sodium salt, **3f** in D_2O



LCMS of Up_4U, **3f**



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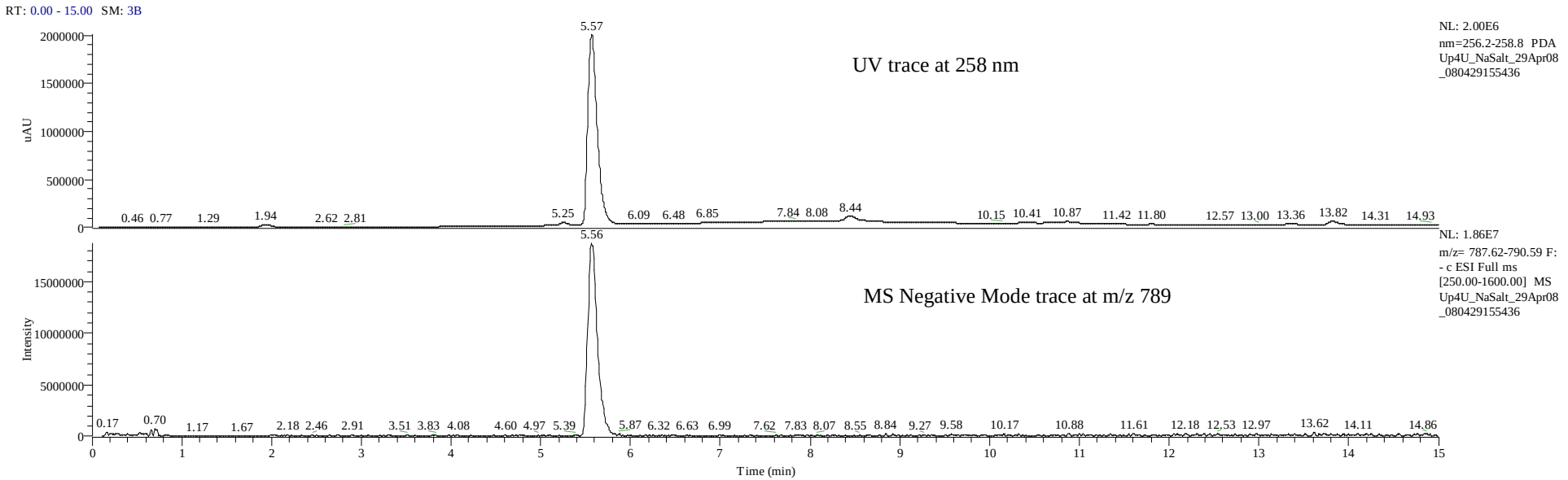

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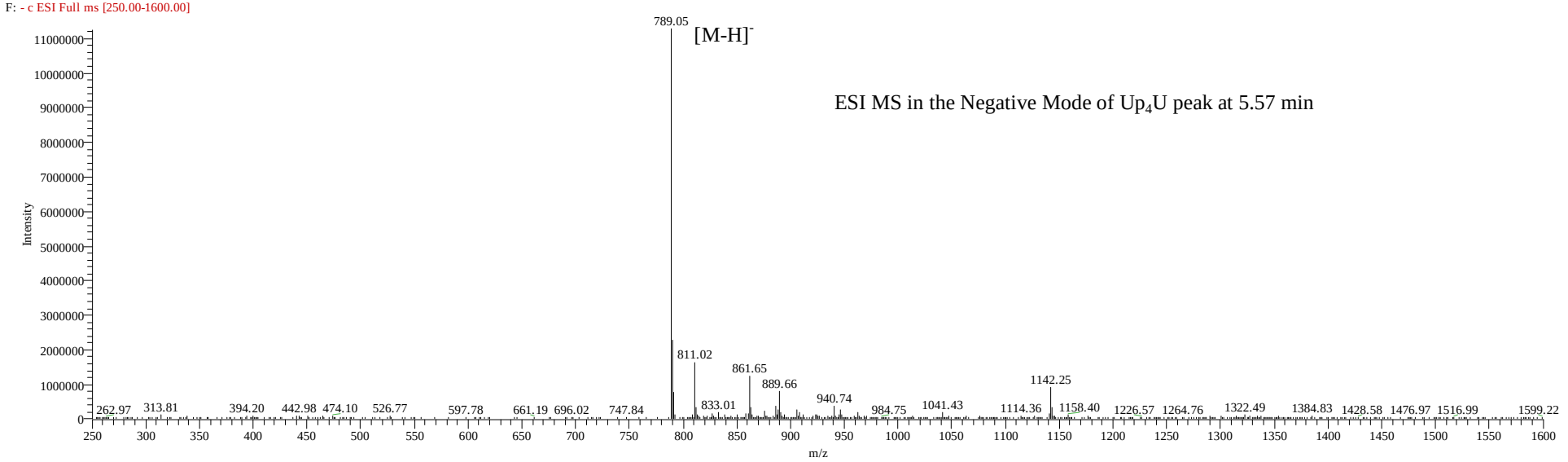
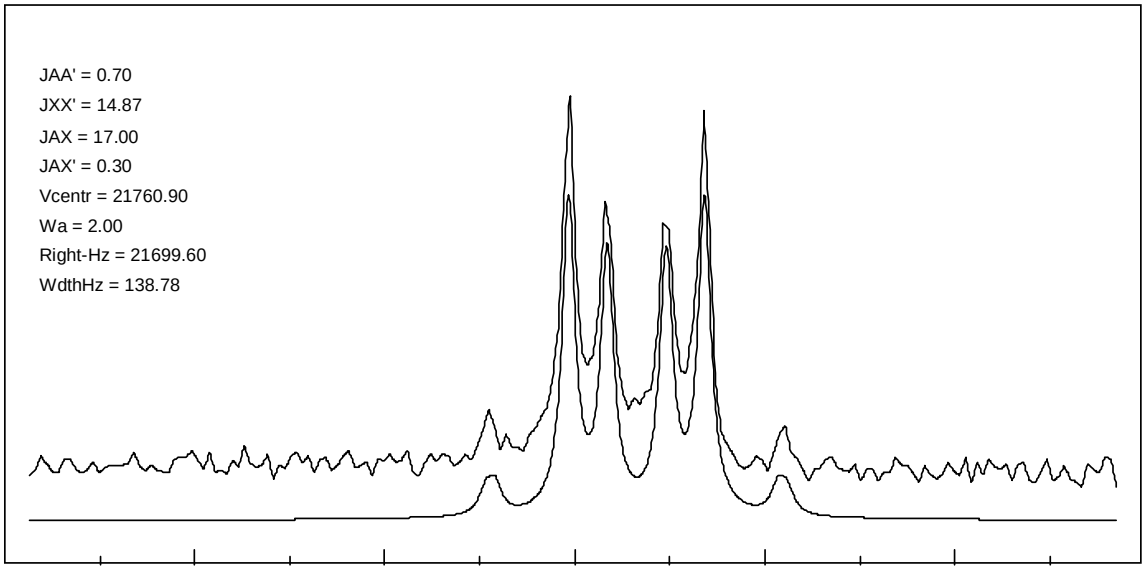
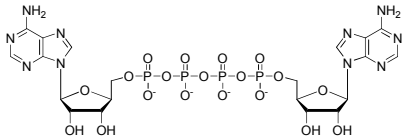
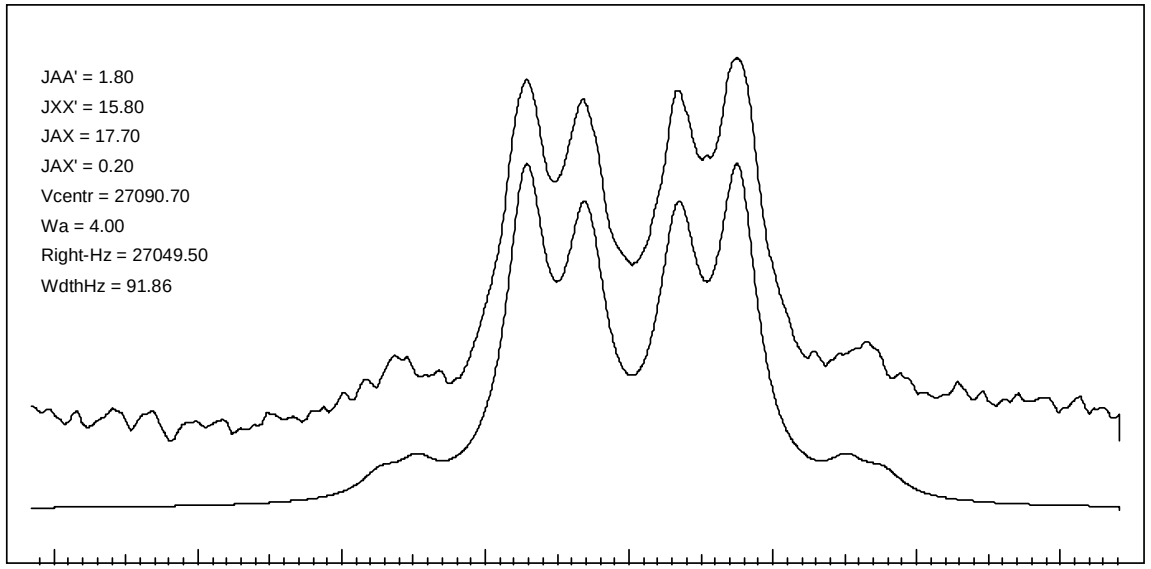
F: - c ESI Full ms [250.00-1600.00]

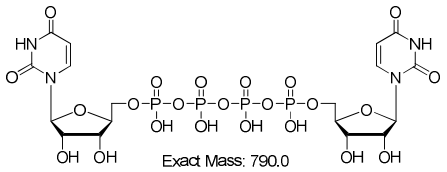


Simulated fitting of an AA'XX' spin system to the experimental ^{31}P NMR spectra of Ap_4A and Up_4U (**3a** and **3f**, resp.):

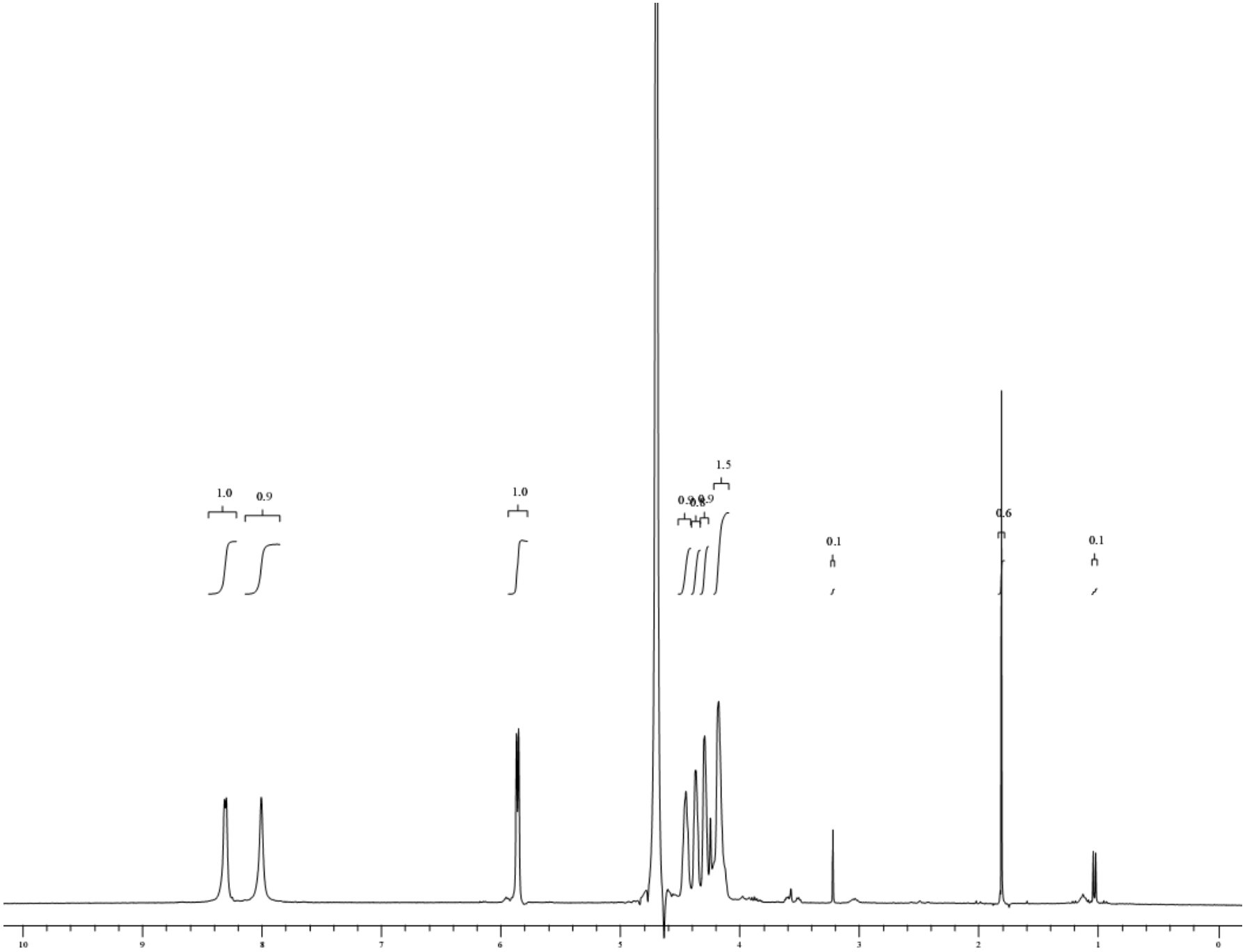




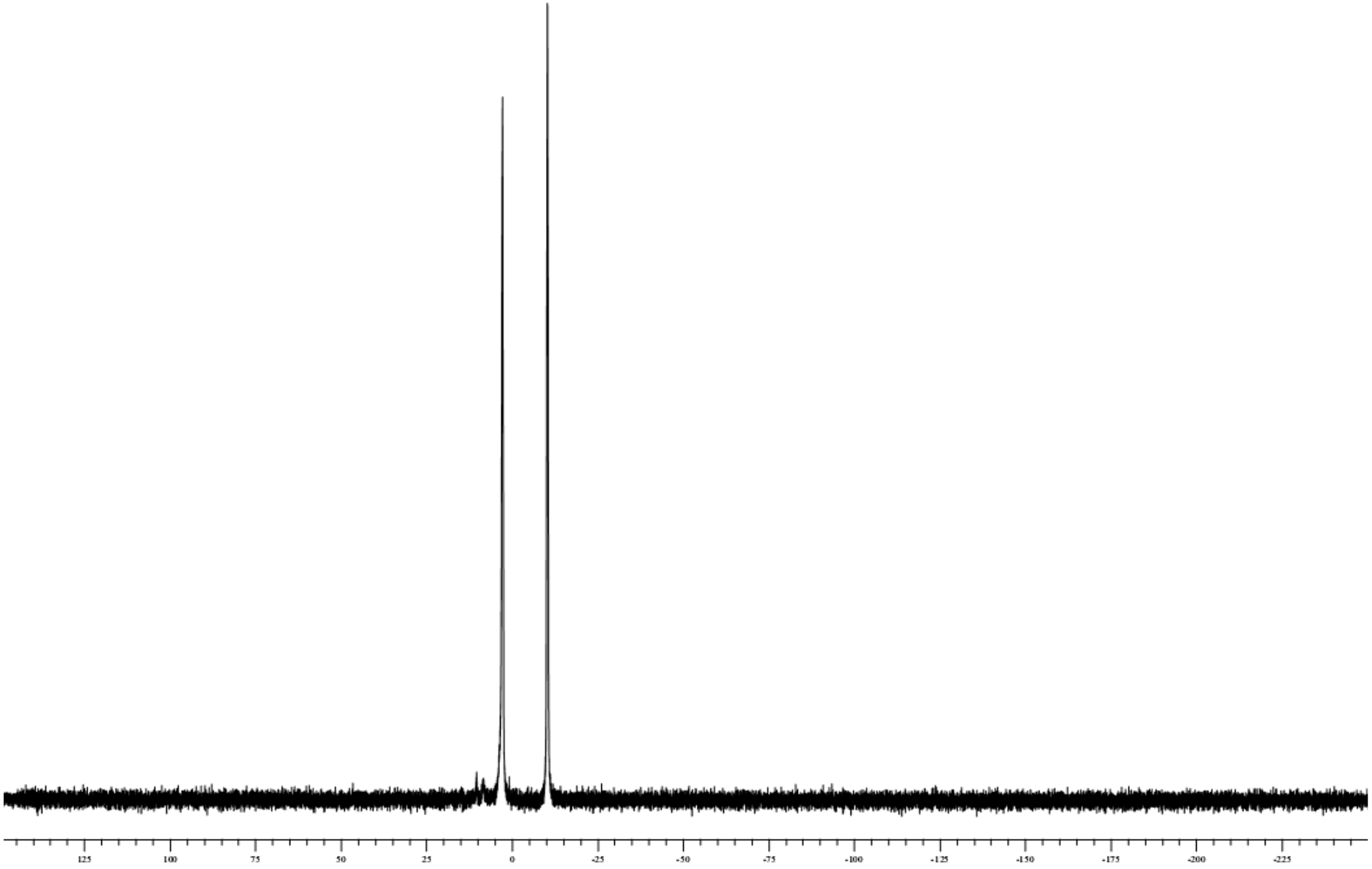




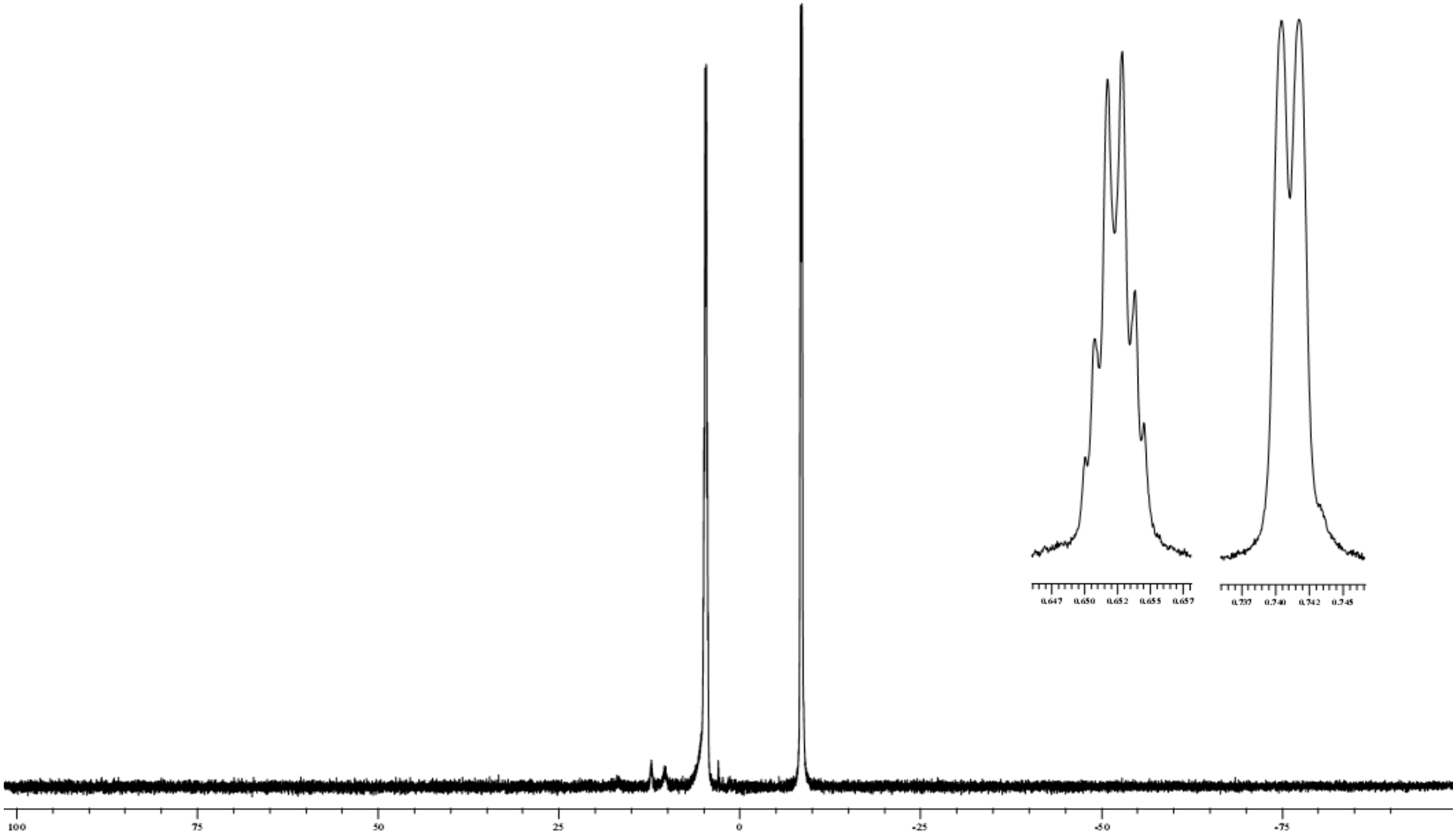
^{1}H NMR of APPCHClPPA sodium salt, **3c** in D_2O:



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

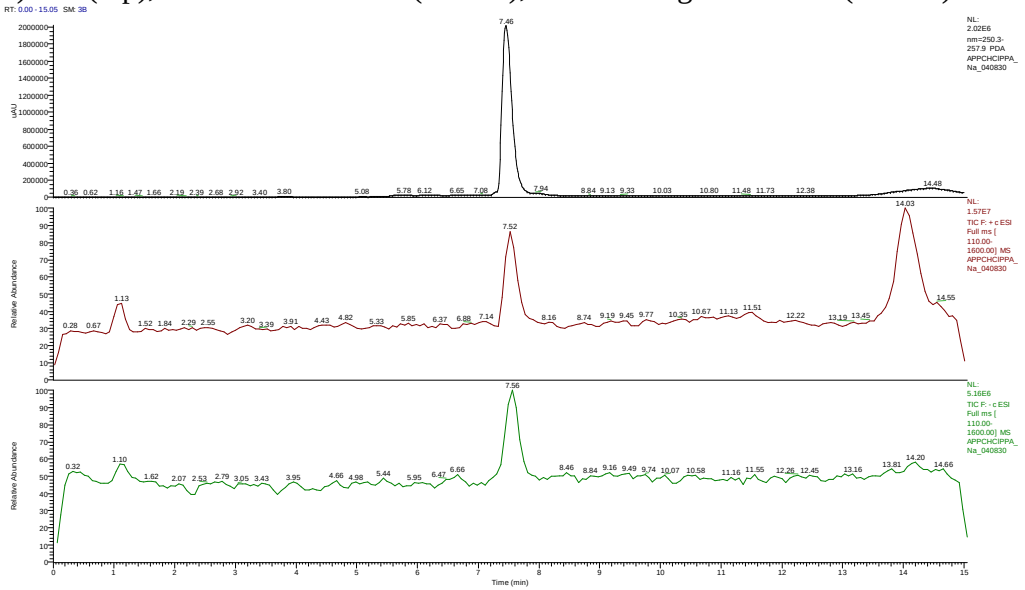


^{31}P NMR (proton coupled) of APPCHClPPA sodium salt, **3c** in D_2O:

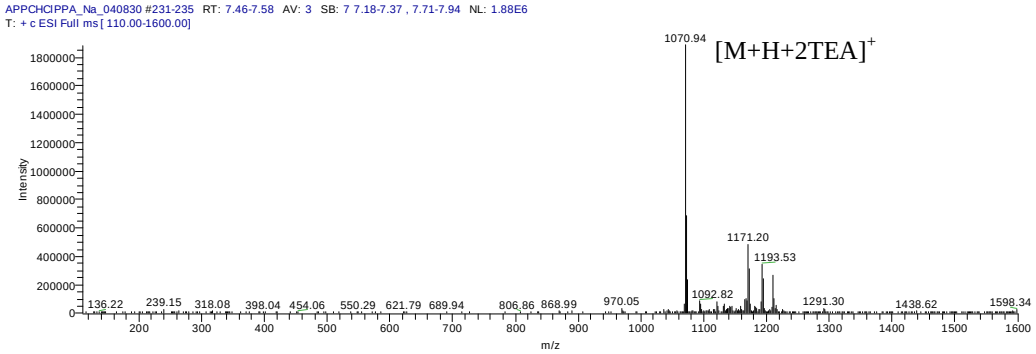


LCMS analysis of APPCHClPPA sodium salt, **3c**:

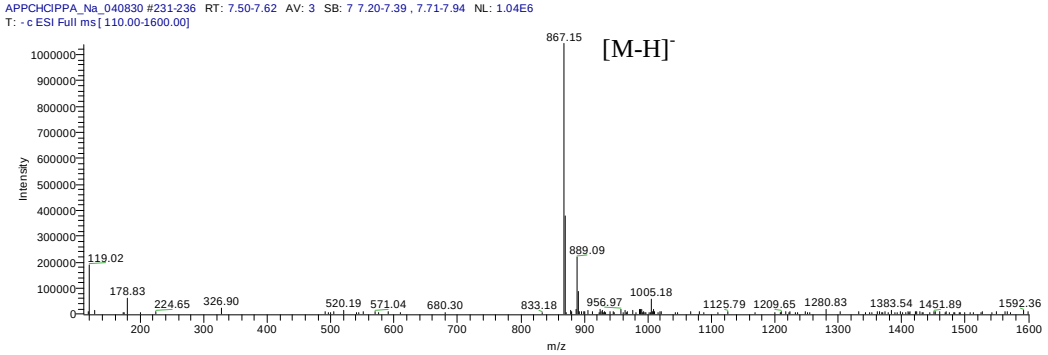
a) UV (top), MS Pos. mode TIC (middle), and MS Neg. mode TIC (bottom) chromatograms:



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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PROCNO                1

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TD                74072
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NS                    8
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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
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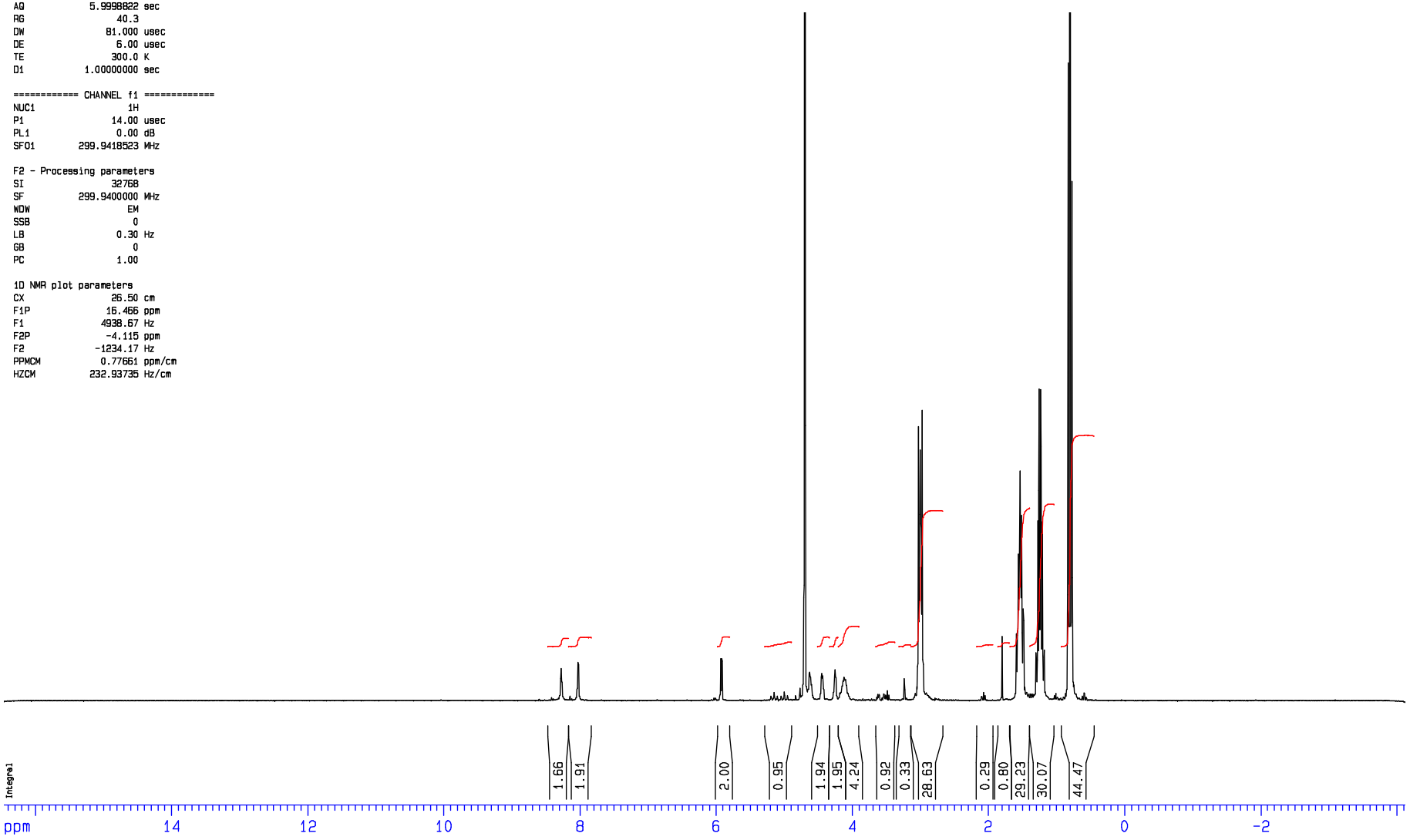
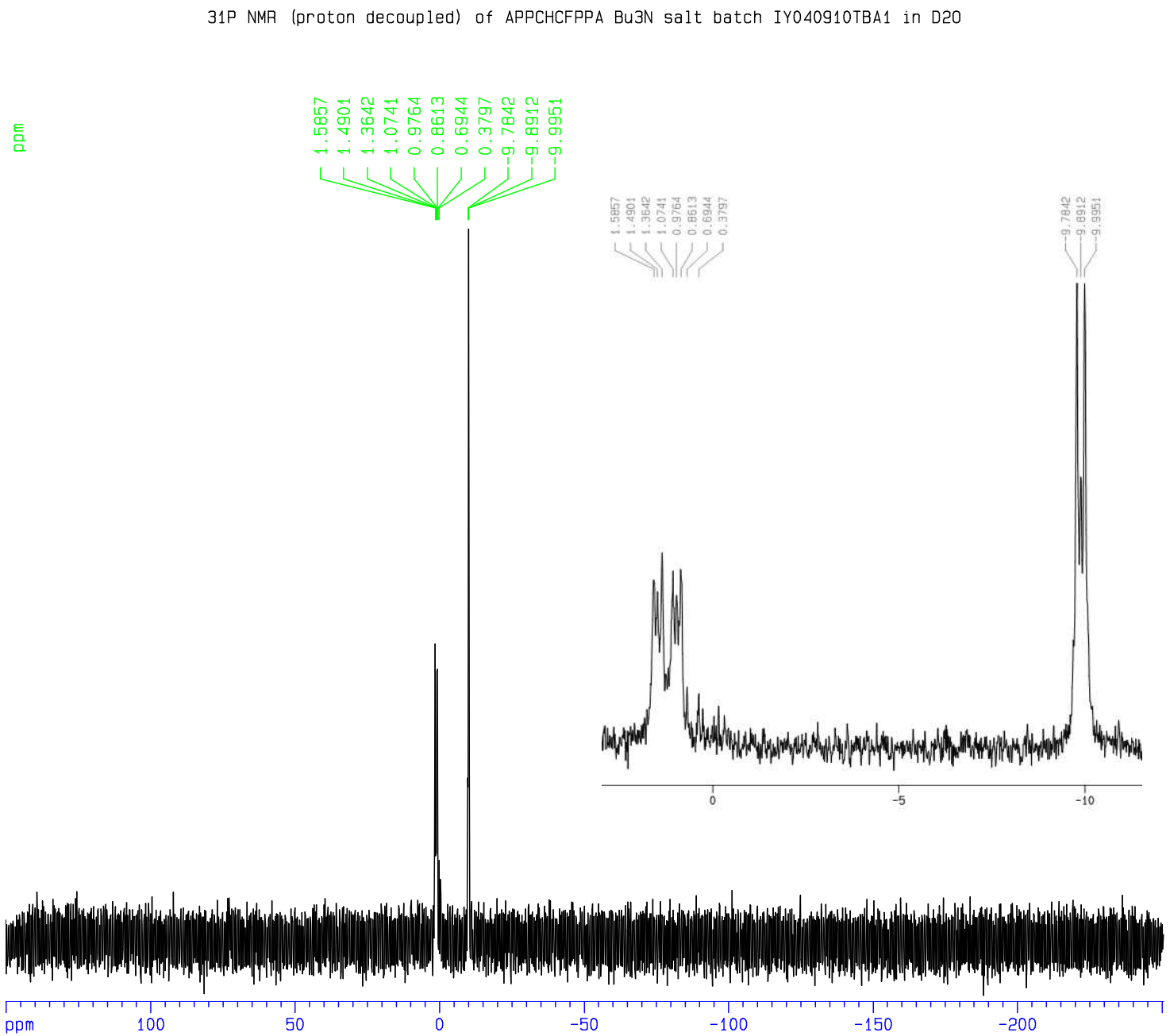
1H NMR of APPCHFPPA Bu3N salt batch IY040910TBA1 in D2O



^{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

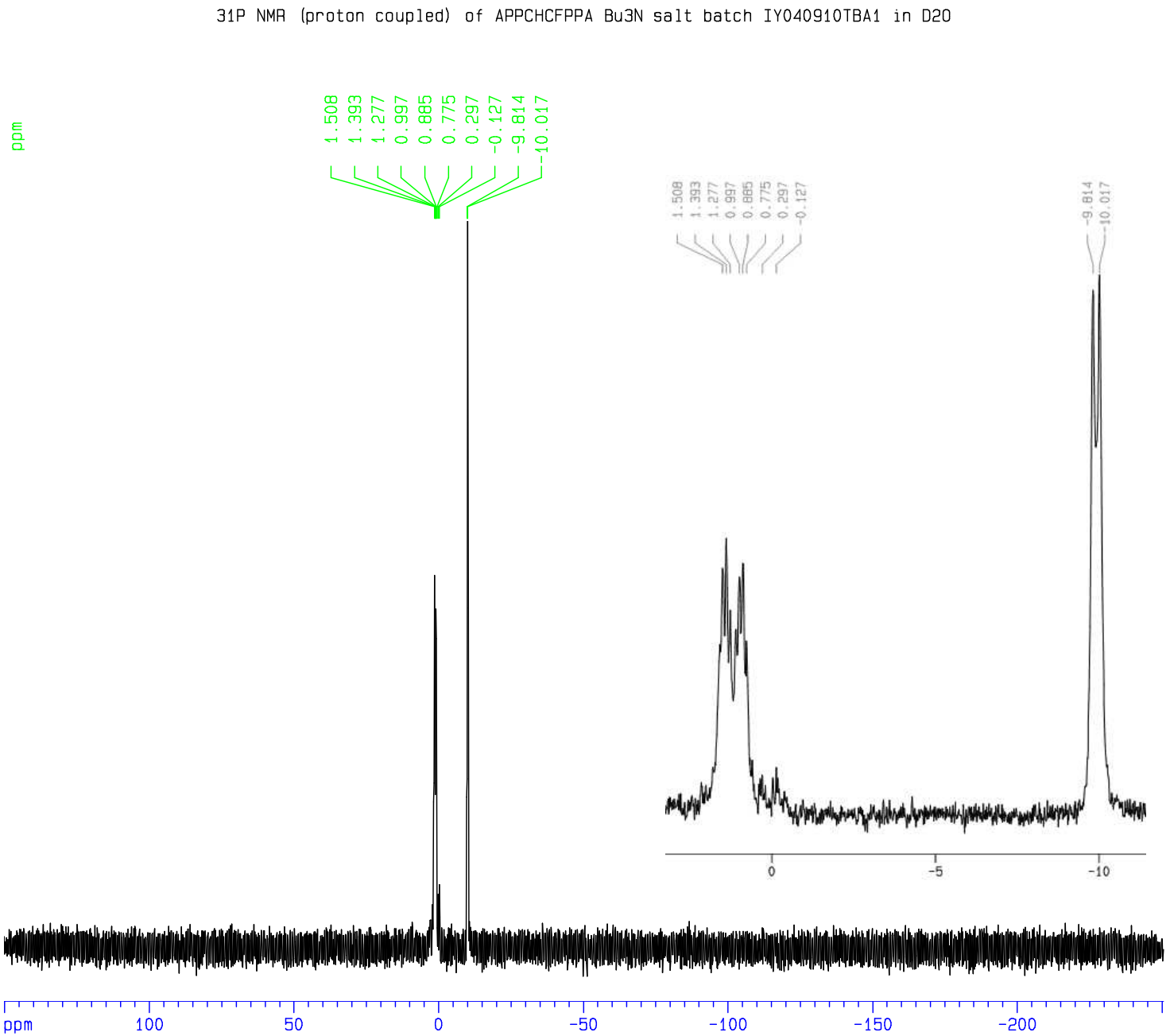
============ CHANNEL f1 =============
NUC1                31P
P1                10.00 usec
PL1                3.00 dB
SFO1         121.4117167 MHz

============ 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:

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Current Data Parameters
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EXPNO                 30
PROCNO                 1

F2 - Acquisition Parameters
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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
```
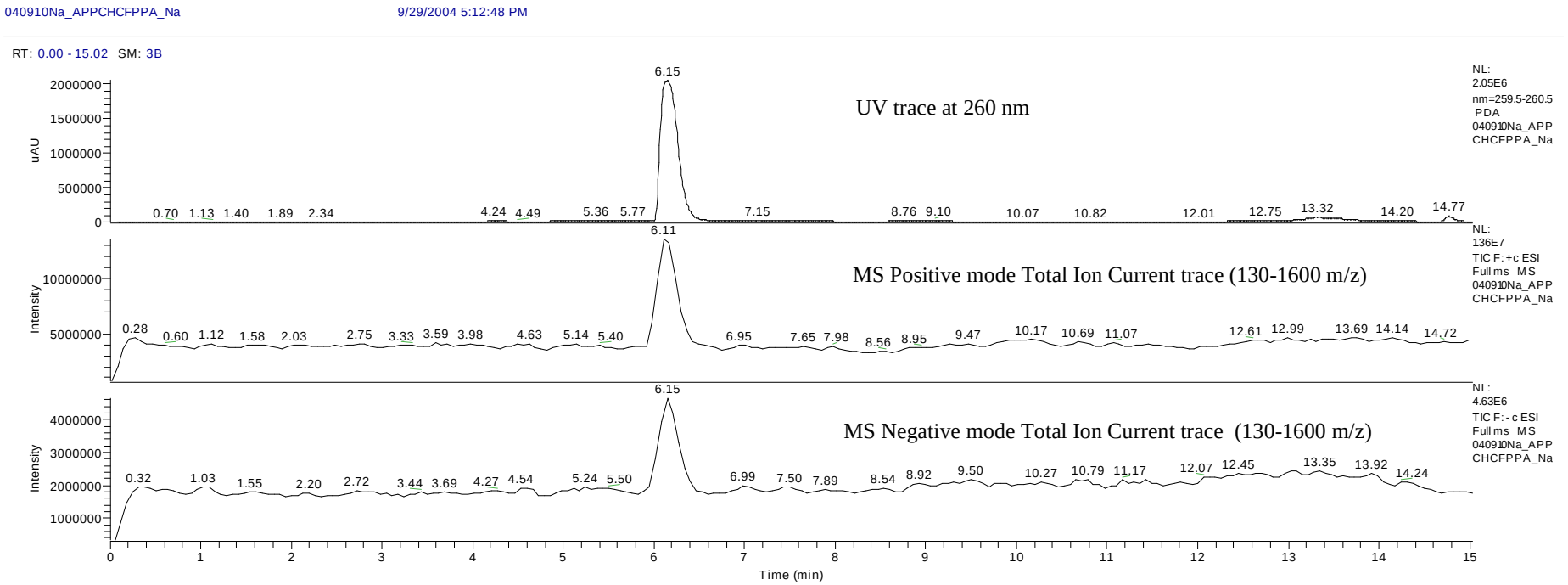
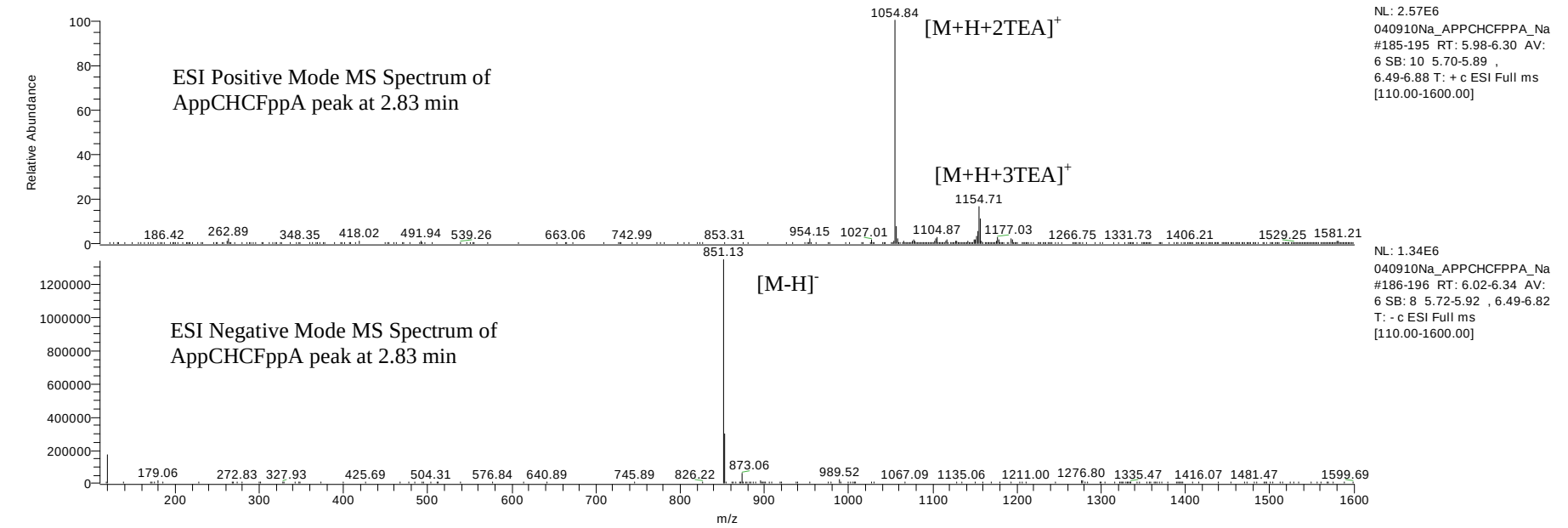
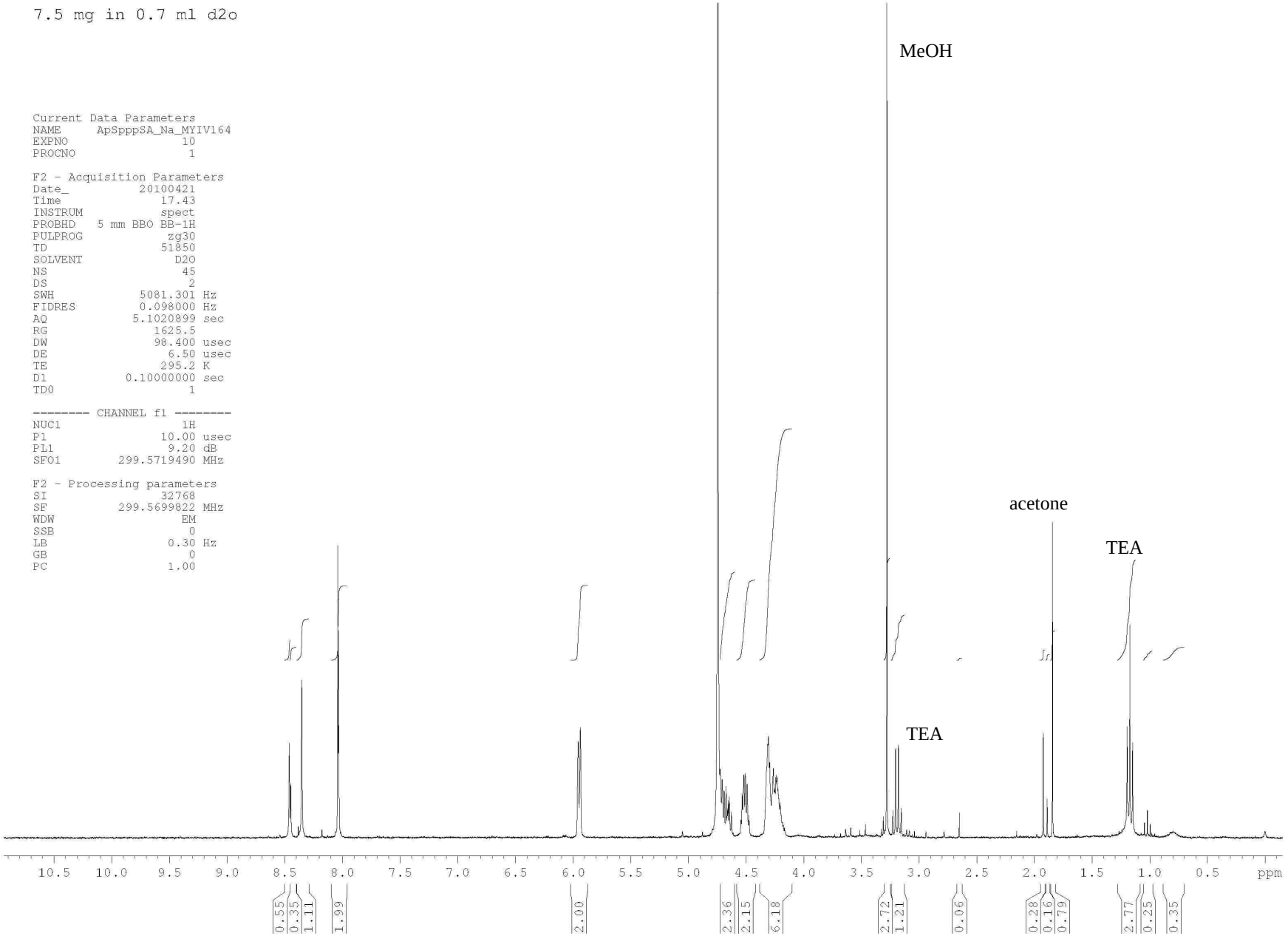

LCMS of APPCHFPPA sodium salt, **3e**:

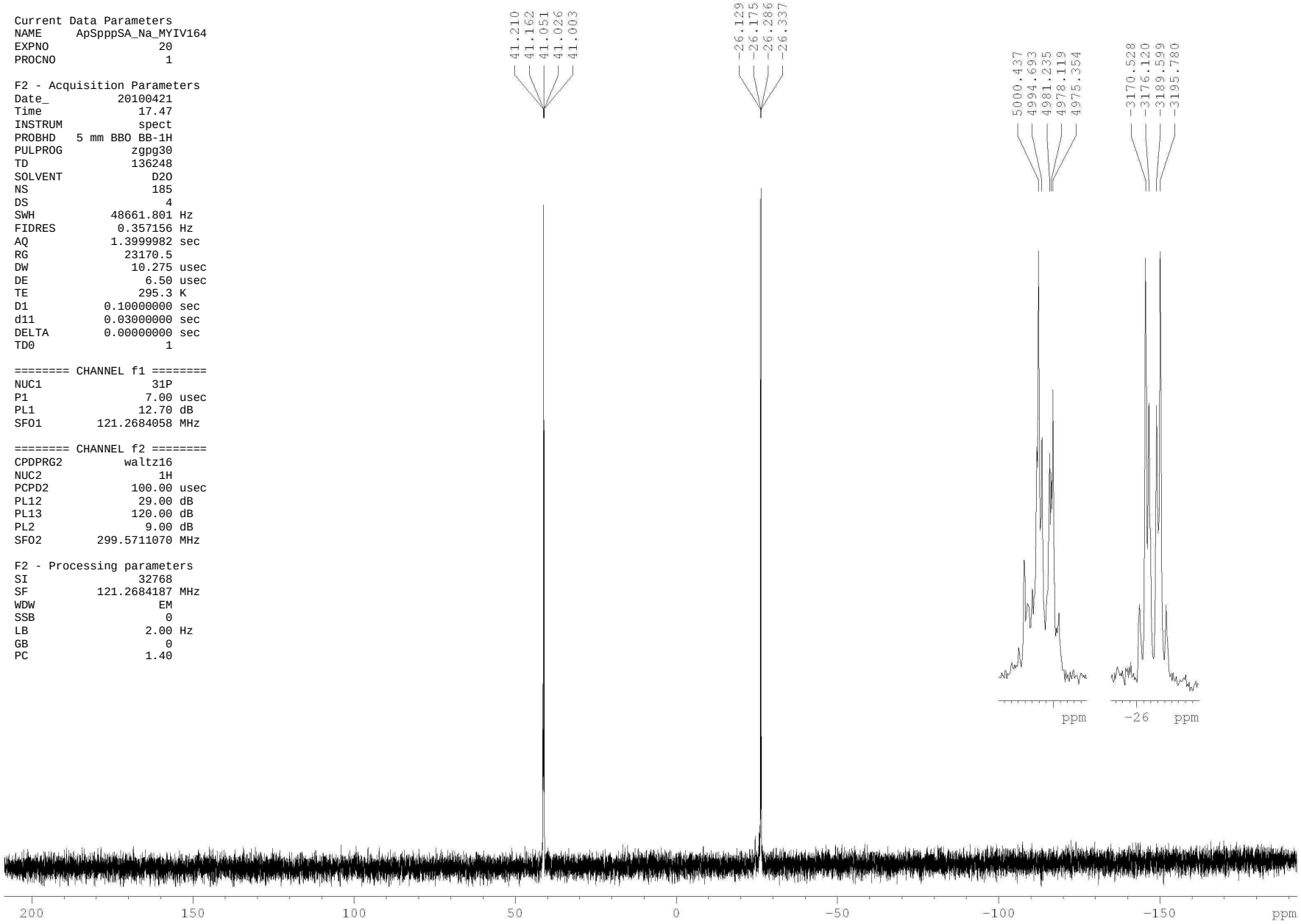




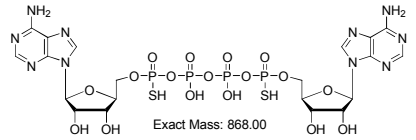
^{1}H NMR of AP(S)PPP(S)A sodium salt, **3b** in D_2O:

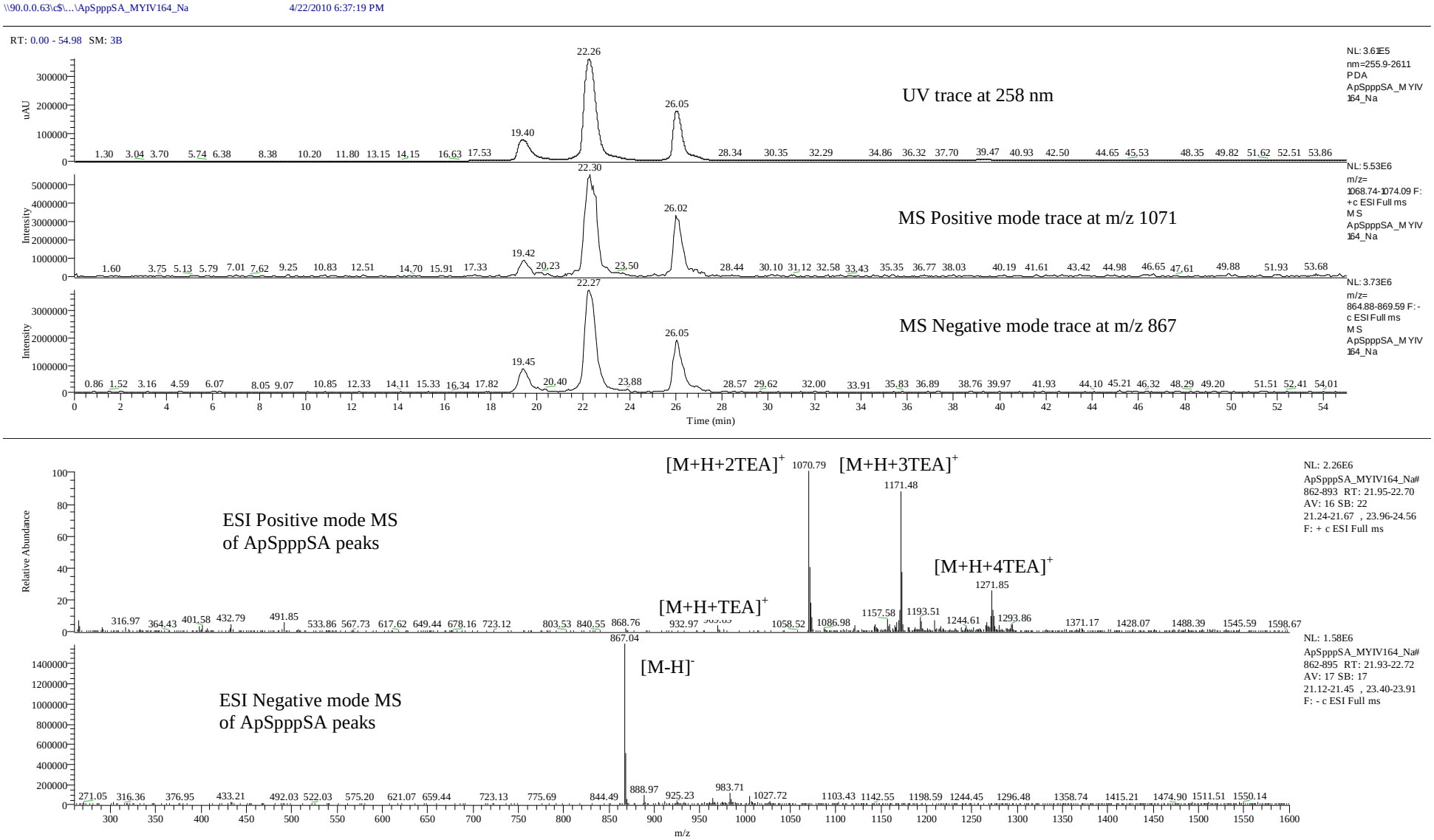


^{31}P (proton decoupled) NMR of AP(S)PPP(S)A sodium salt, **3b** in D_2O:

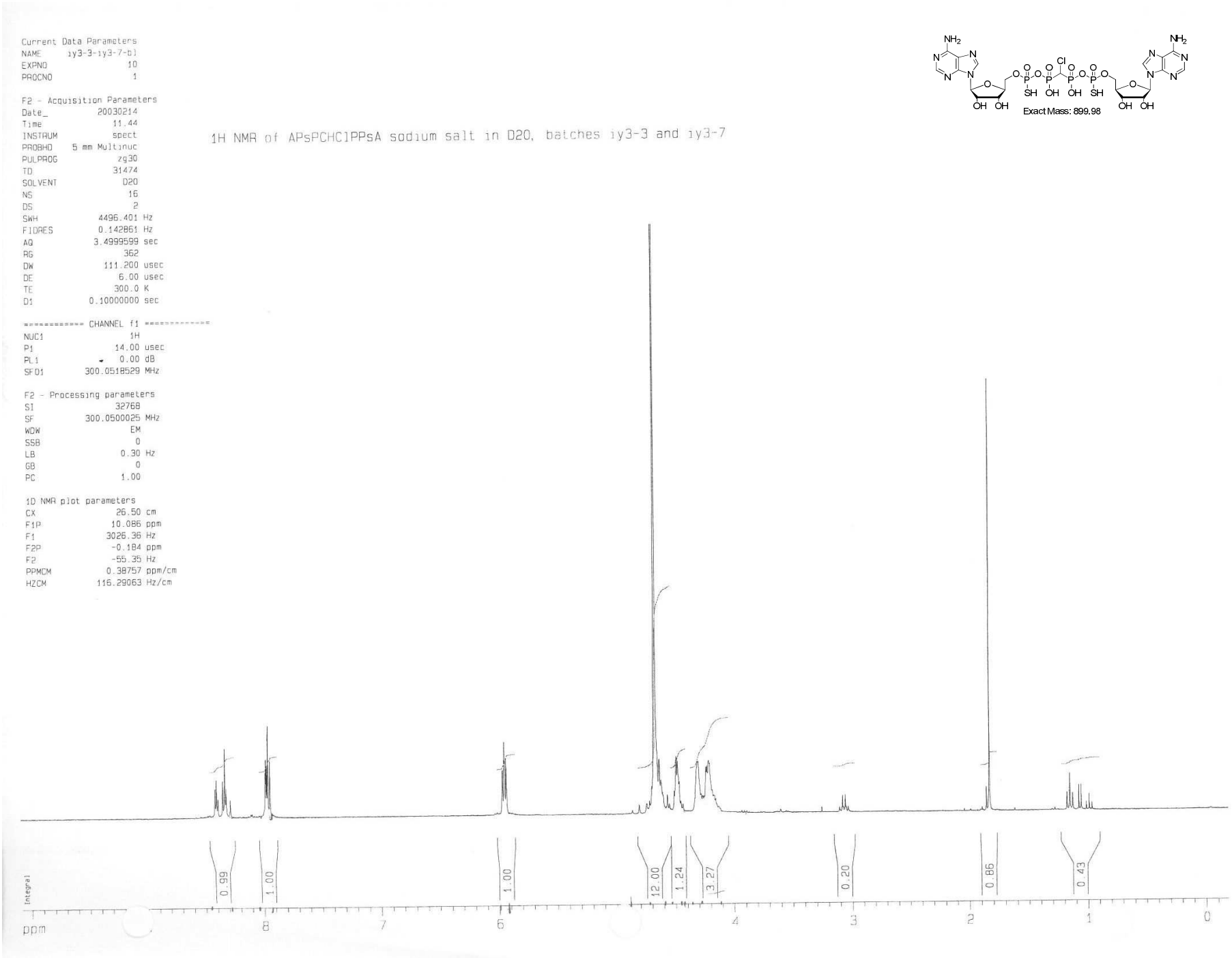


Separation of the three diastereomers of AP(S)PPP(S)A, **3b** by reverse phase ion-pairing chromatography:

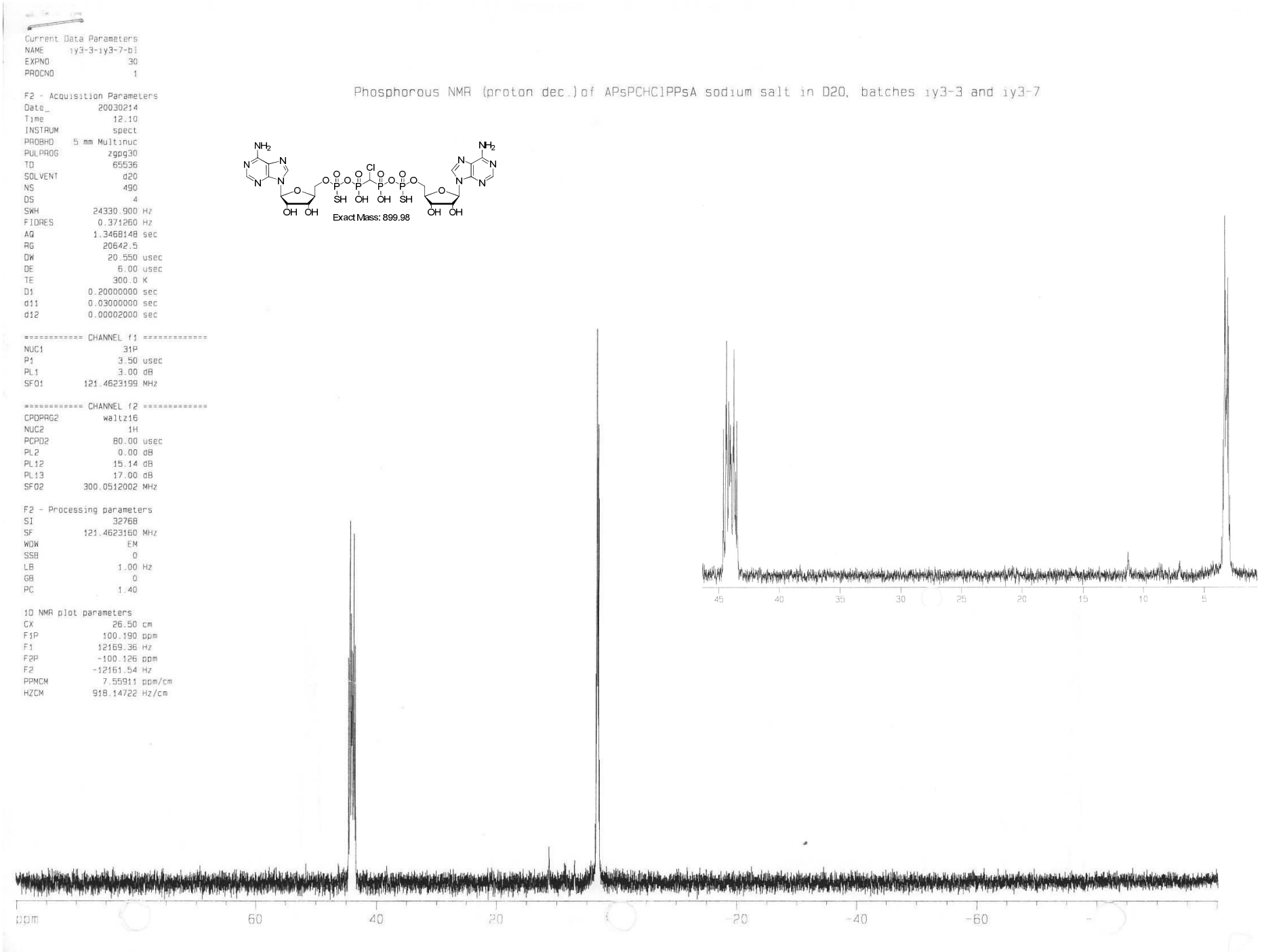




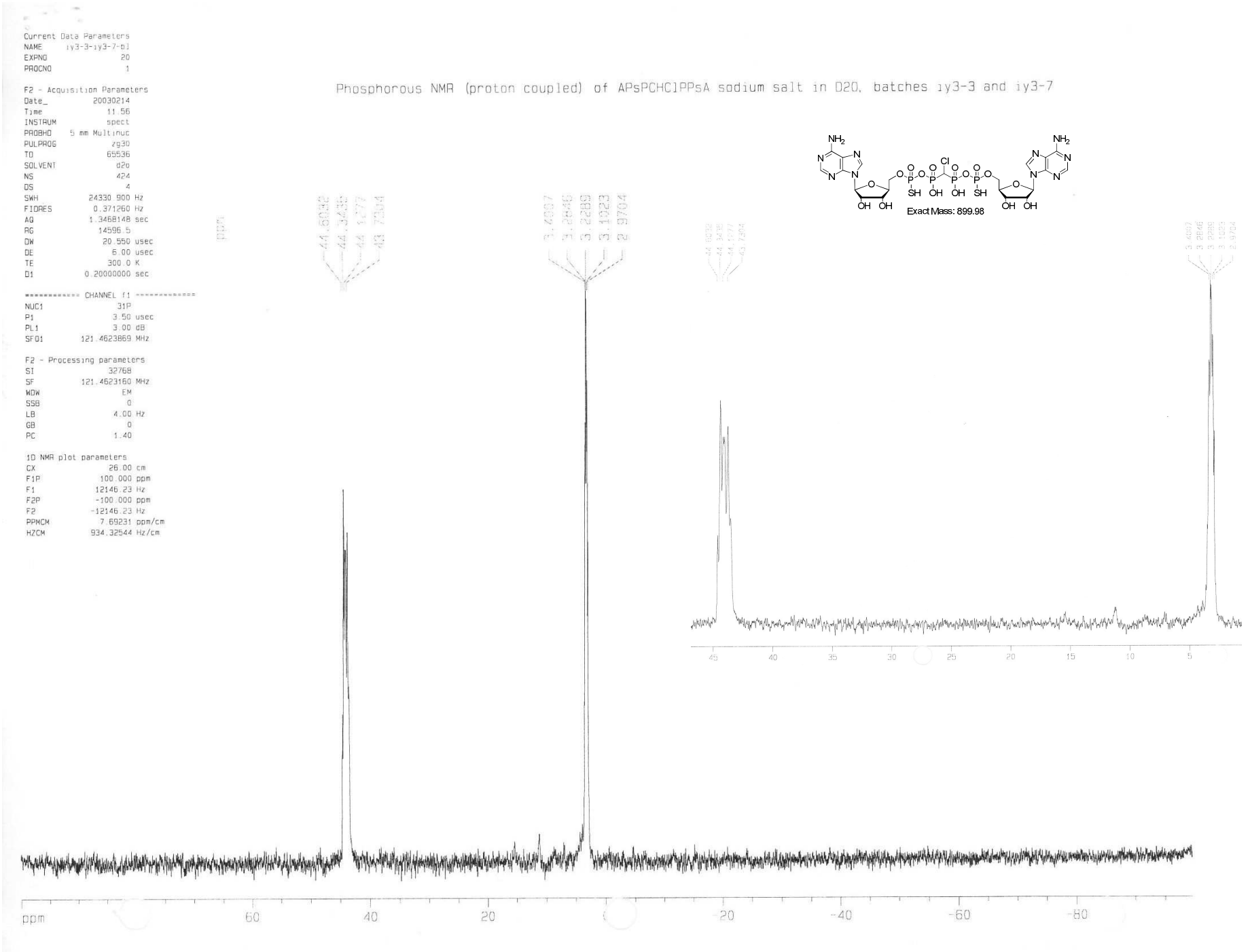
^{1}H NMR spectrum of AP(S)PCHClPP(S)A sodium salt, **3d** in D_2O:



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



^{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: