Supporting information for:

Variable-Temperature NMR Spectroscopy, Conformational Analysis, and Thermodynamic Parameters of cADPR Agonists and Antagonists.

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General Experimental

NMR spectra were acquired on an Agilent Technologies DD2 600 MHz NMR spectrometer outfitted with a variable temperature unit. The variable temperature unit had been calibrated within the last three months. Standard ^{1}H and {^{31}P}-^{1}H spectra were collected with a spectral width of 12 ppm, 32K time domain data points, and zero-filled to 128K points (spectral resolution of 0.05 Hz/pt). 1D TOCSY ^{1}H spectra were collected with a spectral width of 4 ppm, 8K time domain data points, and zero-filled to 64K points (spectral resolution of 0.03 Hz/pt). The mixing time was generally 100 ms. Spectra were converted to Bruker format and processed with TopSpin 3.5.7. Resolution enhancement was applied (lb ~-2 Hz and gb ~0.2) prior to transform. Ion-exchange chromatography was performed using a 20 mL HiPrep Q HP 16/10 column (Sepharose Q column, GE Healthcare Life Sciences) using a gradient of 0 to 125 mM triethylammonium actetate (TEAA), pH 7.

Synthesis of cADPR and NMR Sample Preparation

cADPR was synthesized as described previously (S. M. Graham, D. J. Macaya, R. N. Sengupta and K. B. Turner, *Org. Lett.*, 2003, **6**, 233–236.). A stock solution of ADP ribosyl cyclase (ADPRC, Sigma) was prepared by dissolving the contents of one vial of ADPRC (~250 units; 1 U of enzyme will convert 1 μmol of NAD to cADPR in 5 min) 1.0 mL of 10 mM Tris/Tris•AcOH, 100 mM NaOAc, pH 7.0. Next, ~50 μmol of NAD^{+} free acid (~33 mg) was dissolved in 25 mM HEPES buffer (15 mL, 375 μmol) and water (45 mL). After adjusting the pH to 7 with dilute KOH, ADPRC (40 μL, 10 U) was added. The solution was stirred for 1 – 3 h at room temperature, adjusted to pH 6 with dilute HCl, and purified via ion-exchange chromatography. cADPR eluted at ~75 mM TEAA and appropriate fractions were combined and lyophilized. At least two additional lyophilizations from water were performed, after which the product was dried under high vacuum. Yields ranged from 10 – 15 mg (15 – 23 μmol, ~50 – 80%) of cADPR triethylammonium salt. Approximately 5 μL (1 μmol) of a 200 mM D_2O stock solution of 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid (TMSP-d_4) was added, followed by 0.7 mL of >99.9% D_2O. The sample was lyophilized again, and, working under an atmosphere of dry nitrogen, dissolved in sufficient 99.96% D_2O to produce an ~15-20 mM cADPR concentration, and transferred to a dry 5 mm NMR tube.

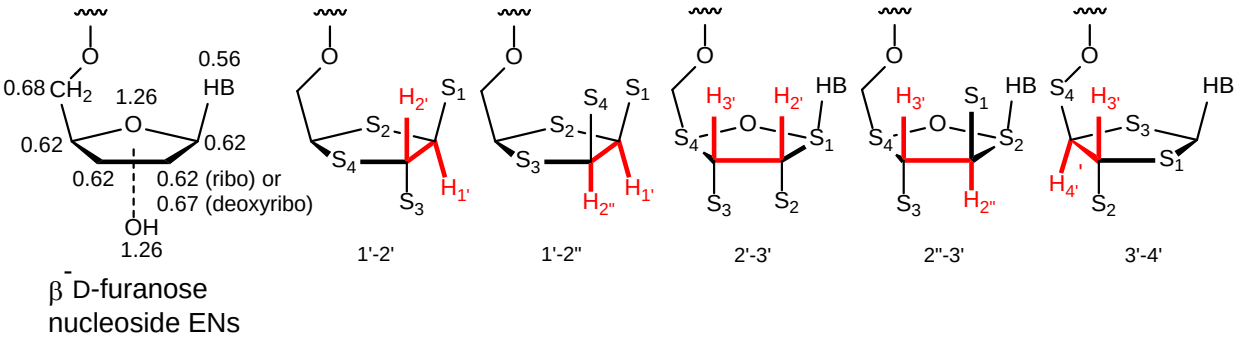
Determination of Coupling Constants

Furanose ring coupling constants ($J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$) were extracted from the standard 1D ^{1}H spectra or the 1D TOCSY spectra. Generally, $J_{4'5'}$ and $J_{4'5''}$ were extracted from the {^{31}P}^{1}H NMR spectrum. The 5'P couplings ($J_{5'P}$) were calculated by subtracting ($J_{4'5'}$ + $J_{5'5''}$) from $\Sigma J_{5'}$ from the normal 1D ^{1}H NMR spectrum; similarly $J_{5''P}$ values were calculated by subtracting ($J_{4'5''}$ + $J_{5'5''}$) from $\Sigma J_{5''}$. In the case of the $A_{5''}$ signal $J_{5''P}$ could often be extracted directly from the normal 1D ^{1}H NMR spectrum. The values of $J_{5'P}$ and $J_{5''P}$ are shown in Table 1.

PSEUROT Calculations and Input Files

Version 6.3, which utilizes the Donders form of the generalized Karplus equation (GKE), was used. Substituent electronegativities were as follows: heterocyclic base (0.56), O4' (1.26), 2'-OH (1.26), C3' (0.62), C1' (0.62), C4' (0.62), C2' (0.62), 3'-OH (1.26), C5' (0.68). The A and B parameters are used to convert the endocyclic ring torsion angles to the exocyclic H-C-C-H needed by the GKE to calculate expected *J* values. Standard values (see the input files below) appropriate for a ribofuranoses were used (reported as A,B): 1'-2' bond (1.102, 123.3), 2'-3' bond (1.090, 0.2), 3'-4' bond (1.095, -124.9).
Standard values (see the input files below) appropriate for a ribofuranoses were used (reported as A,B): 1'-2' bond (1.102, 123.3), 2'-3' bond (1.090, 0.2), 3'-4' bond (1.095, -124.9).

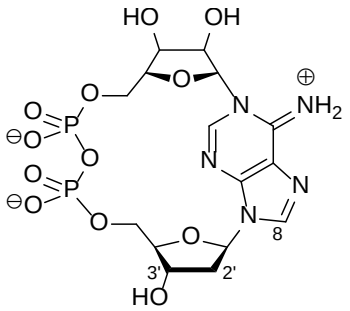
Shown below is a pictorial representation of the substituent electronegativities (S_1-S_4 in the input files) and their orientations, valid for any β-D-furanose. Full details can be found in an (updated 2013) pdf version of the PSEUROT 6.3 manual, available from the corresponding author upon request.



β-D-furanose
nucleoside ENs

On the following pages are the PSEUROT input files for the A- and R-rings for cADPR analogs 2'-deoxy-cADPR (**4b**), 7-deaza-cADPR (**4c**), and 8-Br-cADPR (**4d**). Note the <u>underlined</u> restrained ψ_m^N of the minor conformer.

PSEUROT Input files: 2'-deoxy-A-cADPR (**4b**)



2'-deoxy-A-cADPR
4b

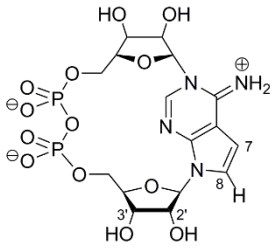
A-ring

```
SS 2'-dA cADPR A ring, 4 temp
CTRL  MAXIT 200  TRIM 0.1  RCNV 0.5  PRINT 2
data 5
1'-2'     -144.0    1.030    121.4    0.56    1.26    0.00    0.62
1'-2"     -144.0    1.020      0.9    0.56    1.26    0.62    0.00
2'-3'        0.0    1.060      2.4    0.62    0.00    1.26    0.62
2"-3'        0.0    1.060    122.9    0.00    0.62    1.26    0.62
3'-4'      144.0    1.090   -124.0    0.67    1.26    1.26    0.68
TSET 4
04 C      6.85     6.96     6.30     3.58     2.83
25 C      6.68     7.01     6.31     3.78     2.97
45 C      6.51     7.07     6.40     3.96     3.29
65 C      6.19     7.22     6.44     4.22     3.42
START     18.0     38.0     162.0    33.0     .50     .50     .50     .50
FITF 10111111
```

R-ring

```
400 MHz 2'-dA cADPR R ring, 4 temp, Phi N fixed
ctrl  maxit 200  trim 0.1  rcnv 0.5  print 2
data 3
1'-2'     -144.0    1.102    123.3    0.56    1.26    1.26    0.62
2'-3'        0.0    1.090      0.2    0.62    1.26    1.26    0.62
3'-4'      144.0    1.095   -124.9    0.62    1.26    1.26    0.68
tset 4
04 C      4.00     5.07     2.11
25 C      4.04     5.05     2.17
45 C      4.06     5.08     2.25
65 C      4.02     5.13     2.36
start     18.0     40.0     162.0    35.0     .50     .50     .50     .50
fitf      10111111
```

PSEUROT Input files: 7-deaza-cADPR (**4c**)



7-Deaza-cADPR
4c

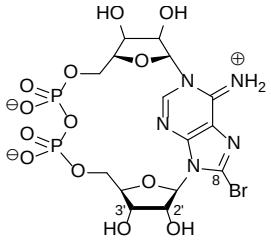
A-ring

```
400 MHz 7-deaza cADPR (A ring, phiN=35 phiS free)
ctrl  maxit  200  trim 0.1  rcnv 0.5  print 2
data 3
1'-2'    -144.0    1.102    123.3    0.56    1.26    1.26    0.62
2'-3'       0.0    1.090      0.2    0.62    1.26    1.26    0.62
3'-4'     144.0    1.095   -124.9    0.62    1.26    1.26    0.68
tset 5
04 C      6.38     5.06     2.65
25 C      6.18     5.12     2.81
45 C      6.13     5.18     2.95
65 C      5.97     5.28     3.09
80 C      5.84     5.28     3.20
start     18.0     35.0     162.0    35.0    .50     .50     .50     .50     .50
fitf      101111111
```

R-ring

```
400MHz 7-deaza cADPR (R ring, phi N 40, phi S free)
ctrl  maxit  200  trim 0.1  rcnv 0.5  print 2
data 3
1'-2'    -144.0    1.102    123.3    0.56    1.26    1.26    0.62
2'-3'       0.0    1.090      0.2    0.62    1.26    1.26    0.62
3'-4'     144.0    1.095   -124.9    0.62    1.26    1.26    0.68
tset 5
04 C      4.00     5.00     2.45
25 C      4.04     5.02     2.54
45 C      4.06     5.03     2.61
65 C      4.07     5.05     2.67
80 C      4.06     5.04     2.74
start     18.0     40.0     162.0    35.0    .50     .50     .50     .50     .50
fitf      101111111
```

PSEUROT Input files: 8-Bromo-cADPR (**4d**)



8-Bromo-cADPR
4d

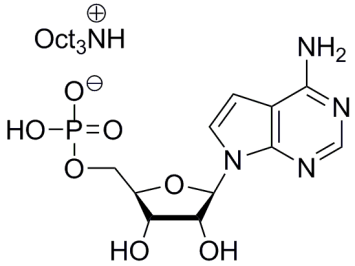
A-ring

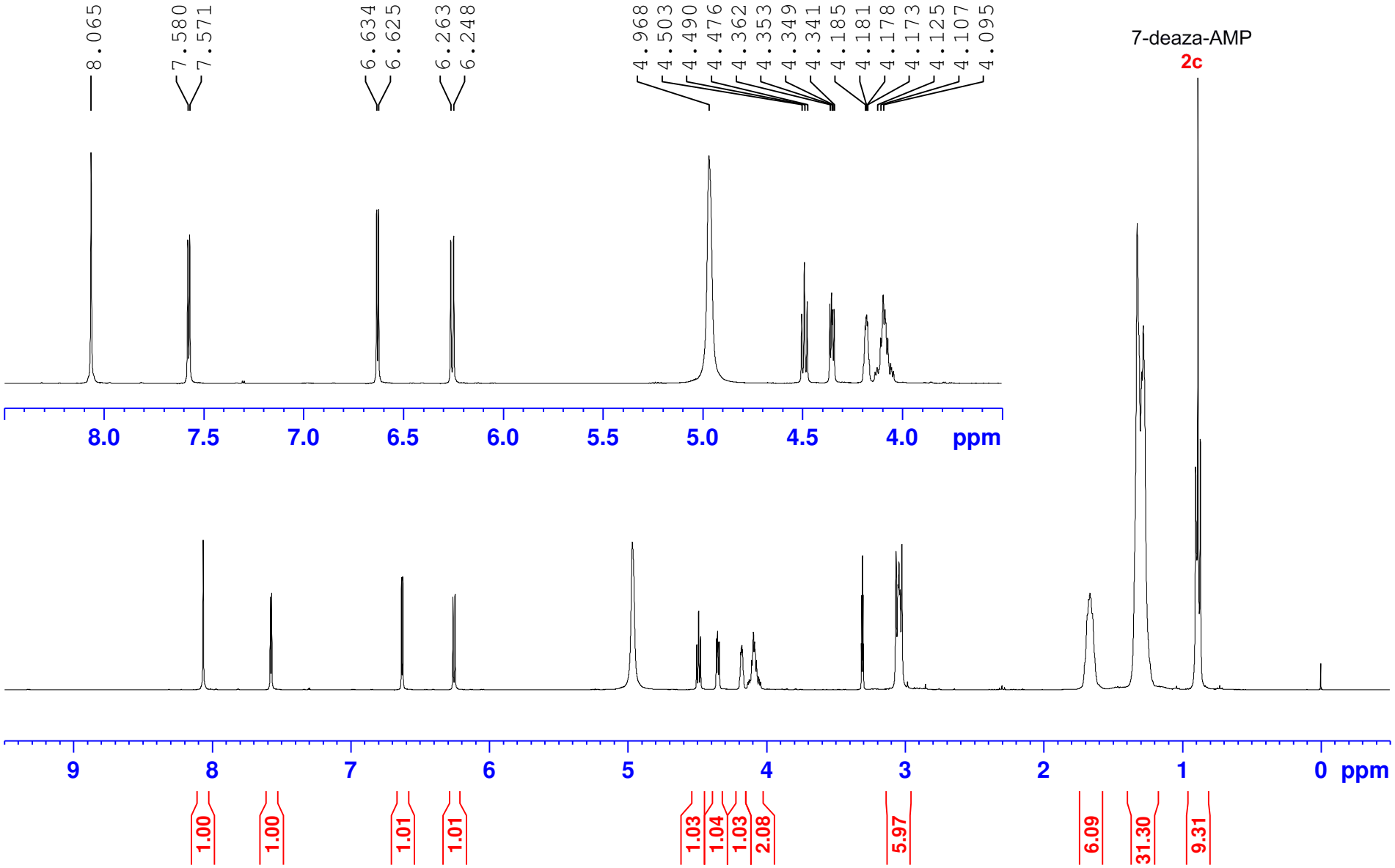
```
400MHz 8-Br cADPR (A ring, phiN=35 phiS free)
ctrl  maxit 200  trim 0.1  rcnv 0.5  print 2
tset 4
data 3
1'-2'    -144.0    1.102    123.3    0.56    1.26    1.26    0.62
2'-3'       0.0    1.090      0.2    0.62    1.26    1.26    0.62
3'-4'     144.0    1.095   -124.9    0.62    1.26    1.26    0.68
tset 4
04 C      5.41     5.13     3.44
25 C      5.37     5.20     3.50
45 C      5.27     5.24     3.53
65 C      5.15     5.28     3.57
start     18.0     35.0     162.0    35.0    .50     .50     .50     .50
fitf      10111111
```

R-ring

```
400MHz 8-bromo cADPR (R ring, phi N 40, phi S free)
ctrl  maxit  200  trim 0.1  rcnv 0.5  print 2
data 3
1'-2'    -144.0    1.102    123.3    0.56    1.26    1.26    0.62
2'-3'       0.0    1.090      0.2    0.62    1.26    1.26    0.62
3'-4'     144.0    1.095   -124.9    0.62    1.26    1.26    0.68
tset 4
04 C      3.98     5.06     2.23
25 C      4.03     5.08     2.23
45 C      4.07     5.10     2.28
65 C      4.10     5.09     2.28
start     18.0     40.0     162.0    35.0    .50     .50     .50     .50
fitf      10111111
```

~40mg 7-deaza-AMP TOA in 600uL CD3OD w/TMS



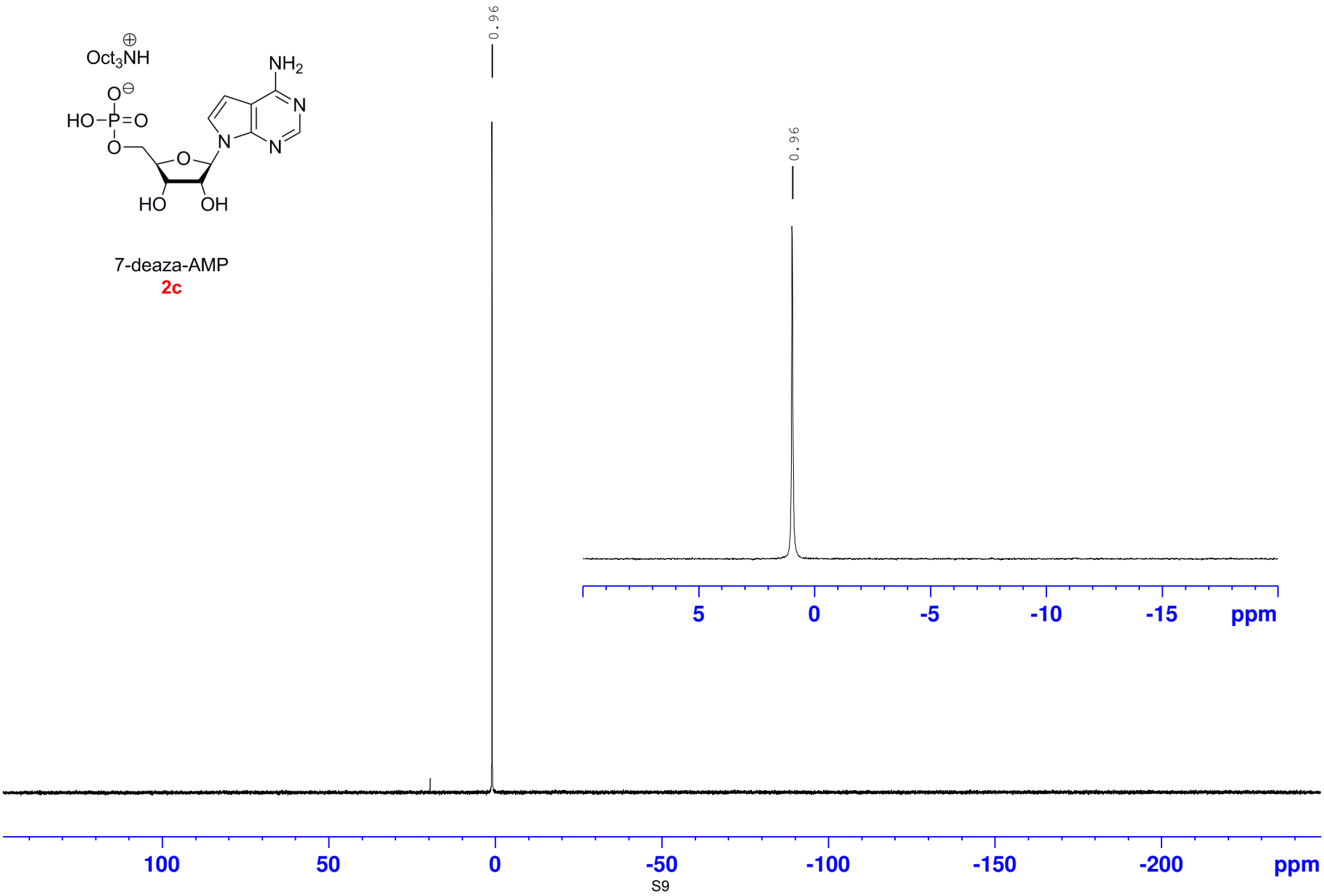


~40mg 7-deazaAMP TOA in 600uL CD3OD w/TMS

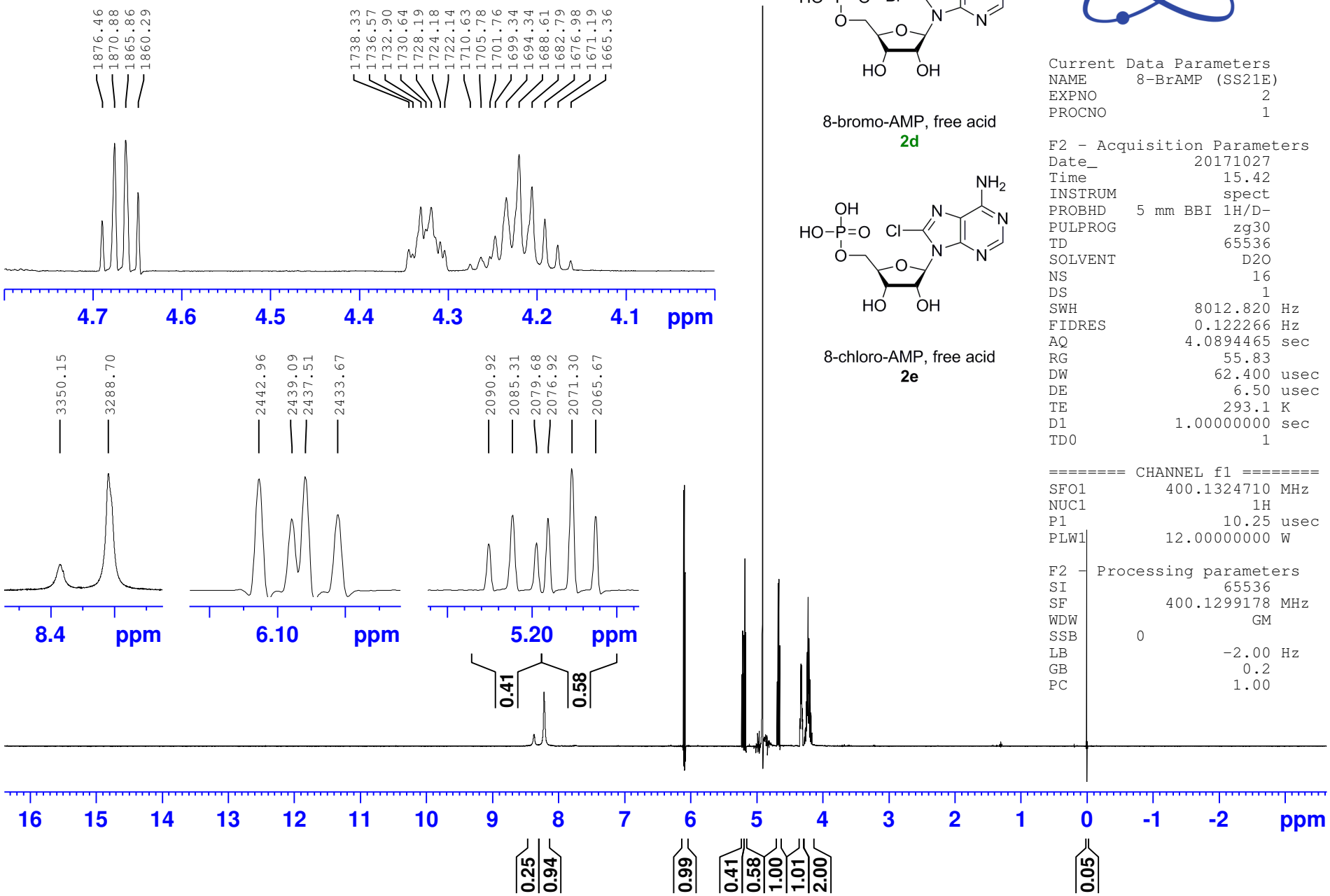
31P NMR

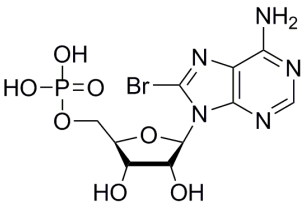
7-deaza-AMP

2c



Mixture of 8-Br- and 8-Cl-AMP produced from reaction of 8-Br-Ado and $POCl_3$





8-bromo-AMP, free acid
2d

8-chloro-AMP, free acid
2e

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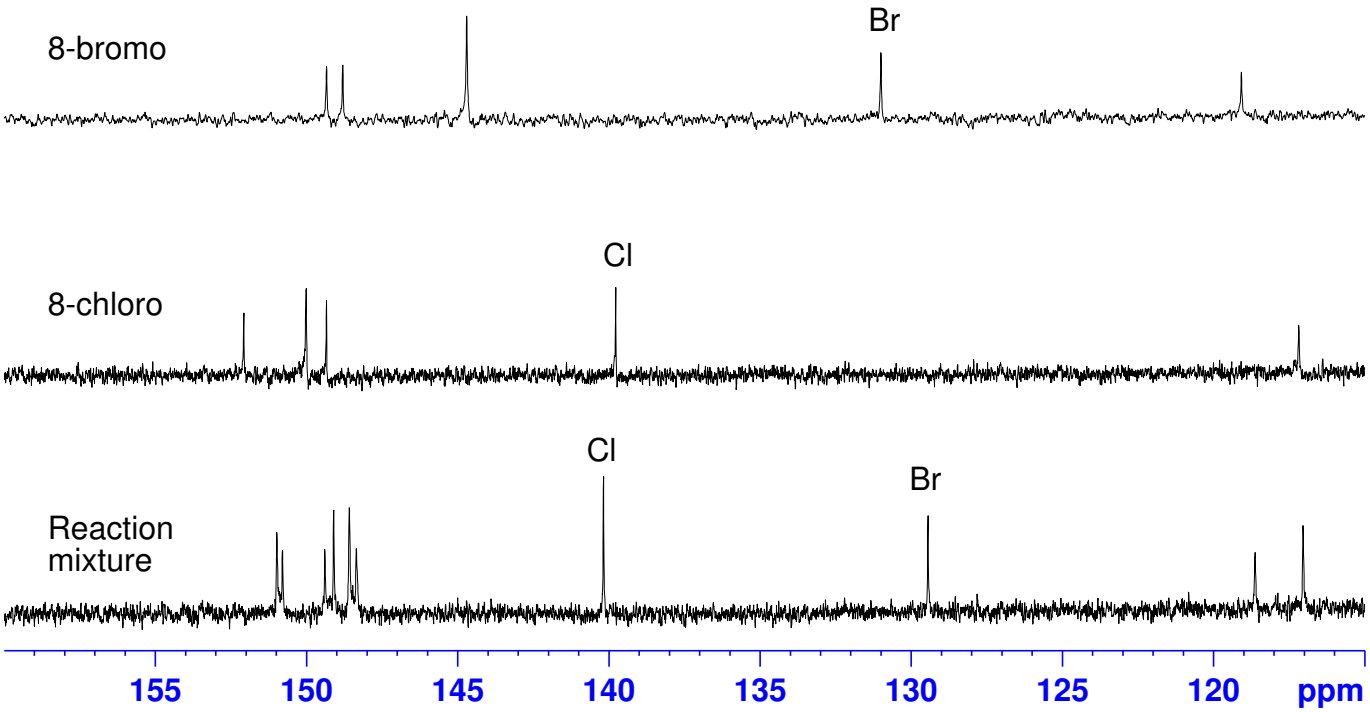
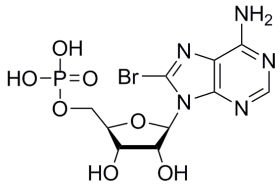
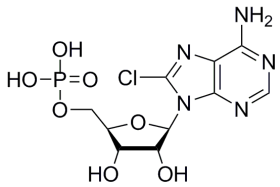
^{13}C NMR

Mixture of 8-Br- and 8-Cl-AMP produced from reaction of 8-Br-Ado and $POCl_3$

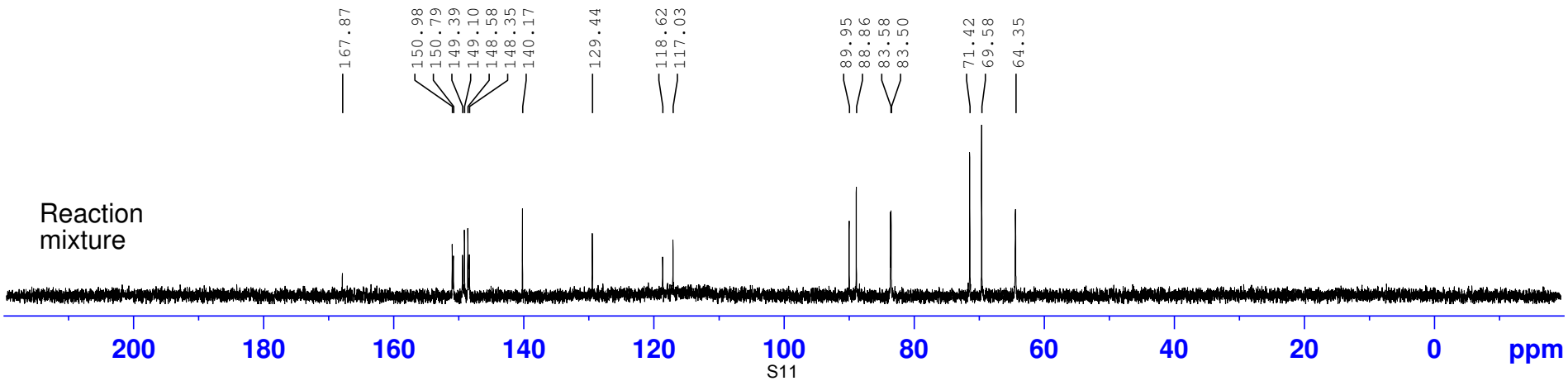




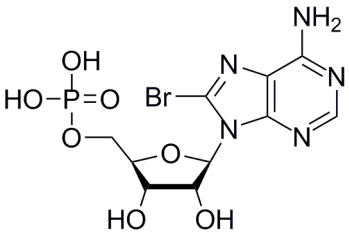
8-bromo-AMP, free acid
2d



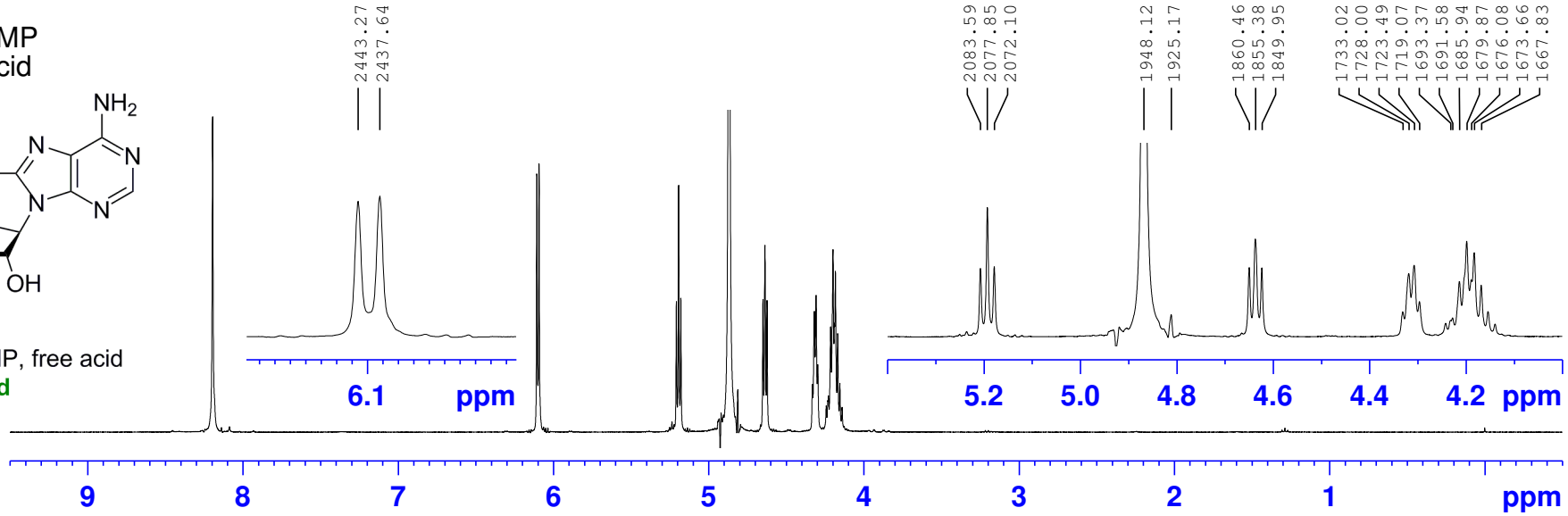
8-chloro-AMP, free acid
2e



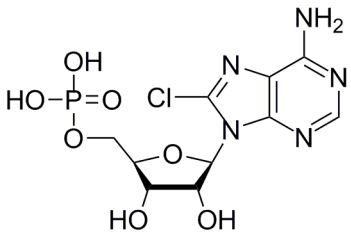
8-Br-AMP
Free acid



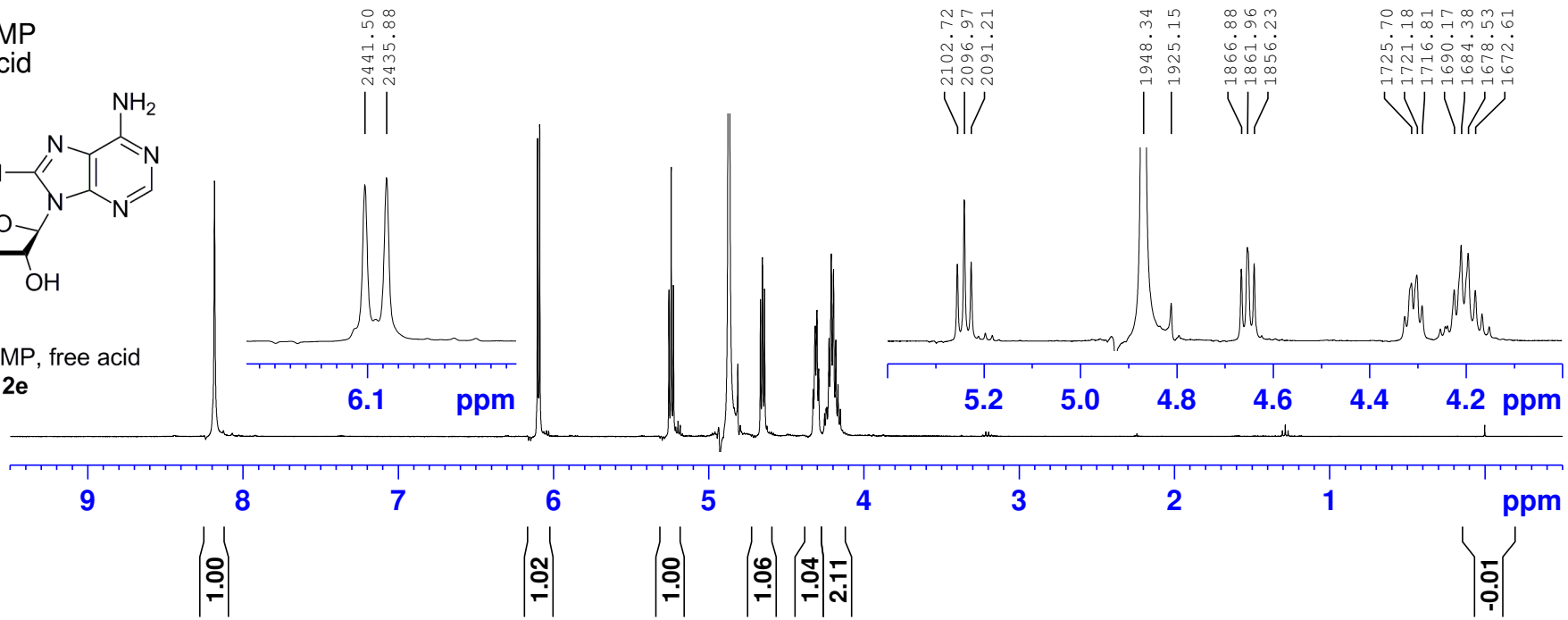
8-bromo-AMP, free acid
2d



8-Cl-AMP
Free acid

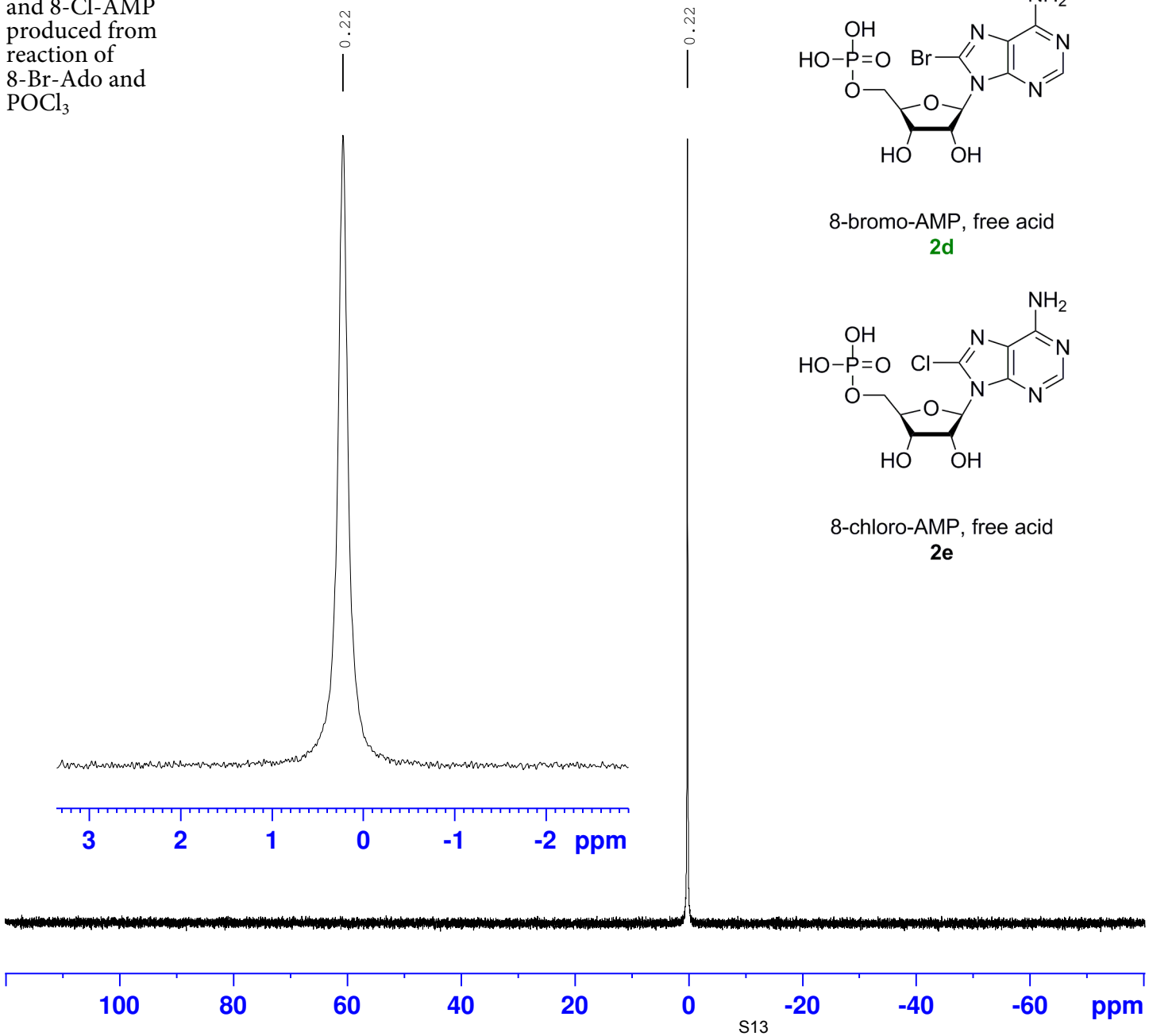


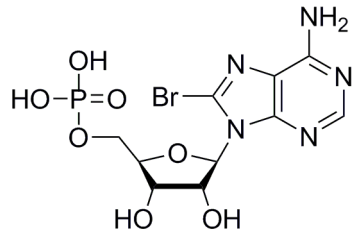
8-chloro-AMP, free acid
2e



^{31}P NMR

Mixture of 8-Br- and 8-Cl-AMP produced from reaction of 8-Br-Ado and $POCl_3$





8-bromo-AMP, free acid
2d

8-chloro-AMP, free acid
2e

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PROCNO                 1

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SOLVENT             D2O
NS                   38
DS                    2
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FIDRES          0.495415 Hz
AQ             1.0092545 sec
RG                199.73
DW                15.400 usec
DE                  6.50 usec
TE                295.0 K
D1            4.00000000 sec
D11           0.03000000 sec
TD0                    1

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NUC1                31P
P1                11.77 usec
PLW1         80.00000000 W

======== CHANNEL f2 ========
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NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         16.79999924 W
PLW12         0.10455000 W
PLW13         0.05259000 W

F2 - Processing parameters
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SF           161.9755930 MHz
WDW                  GM
SSB      0
LB                -2.00 Hz
GB                 0.08
PC                 1.40
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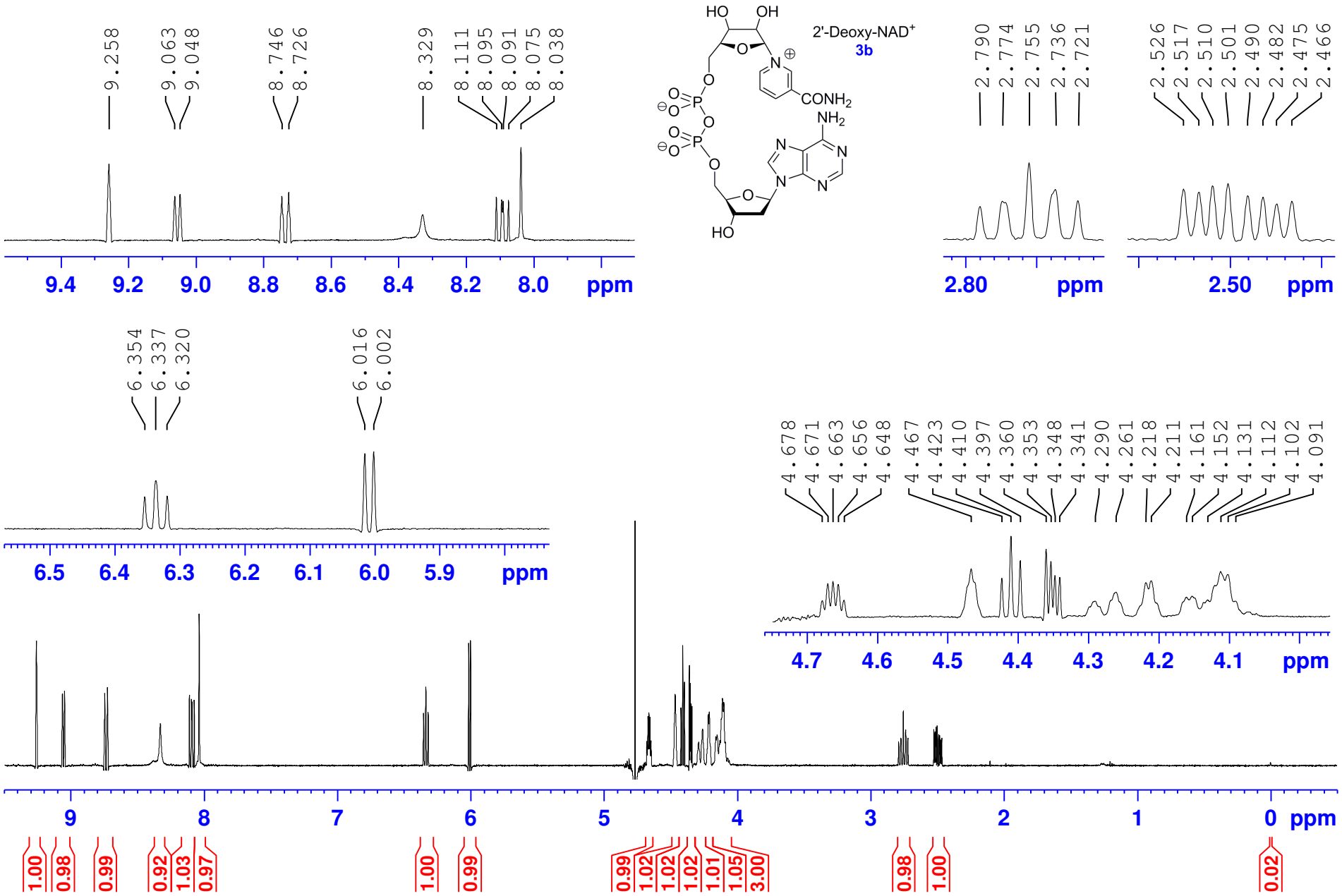
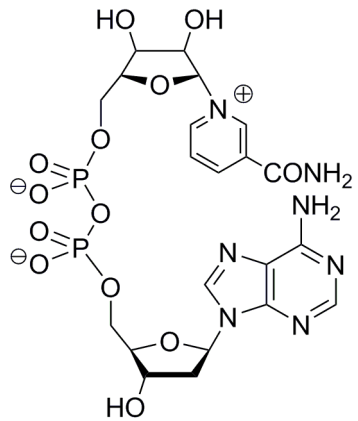
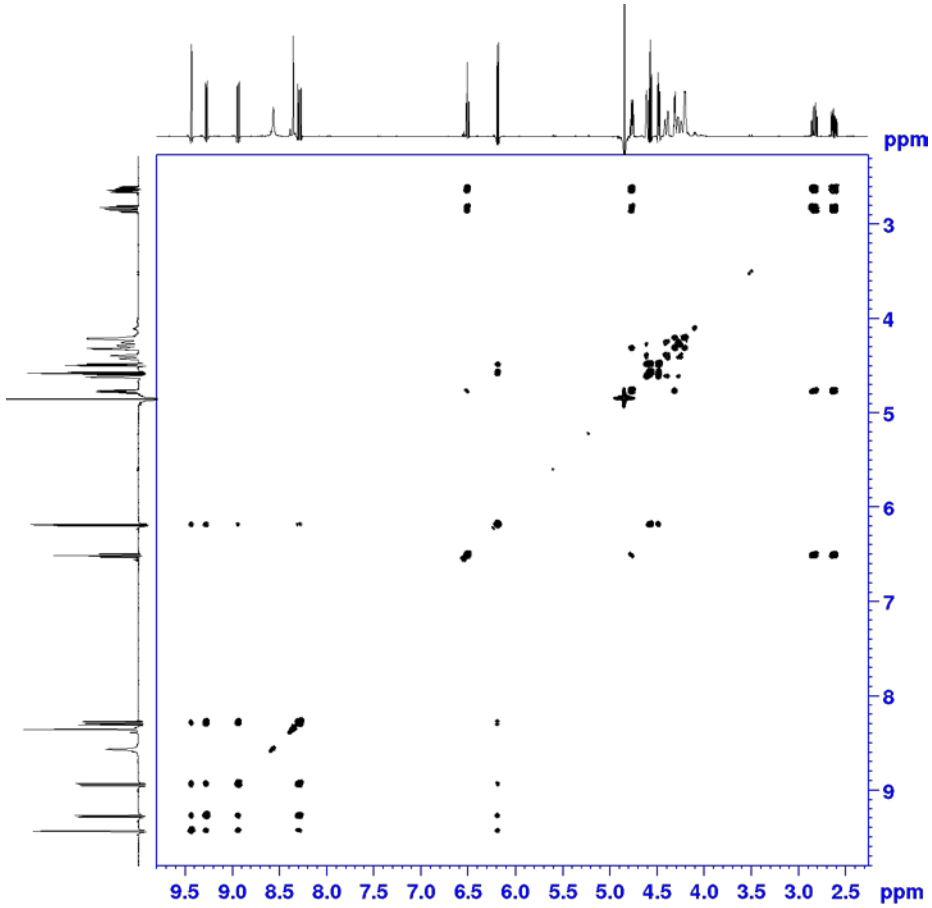
2'-deoxy-NAD+, ammonium salt - 7.8mg in 0.65mL D2O with TMSP
(after a THIRD purification)

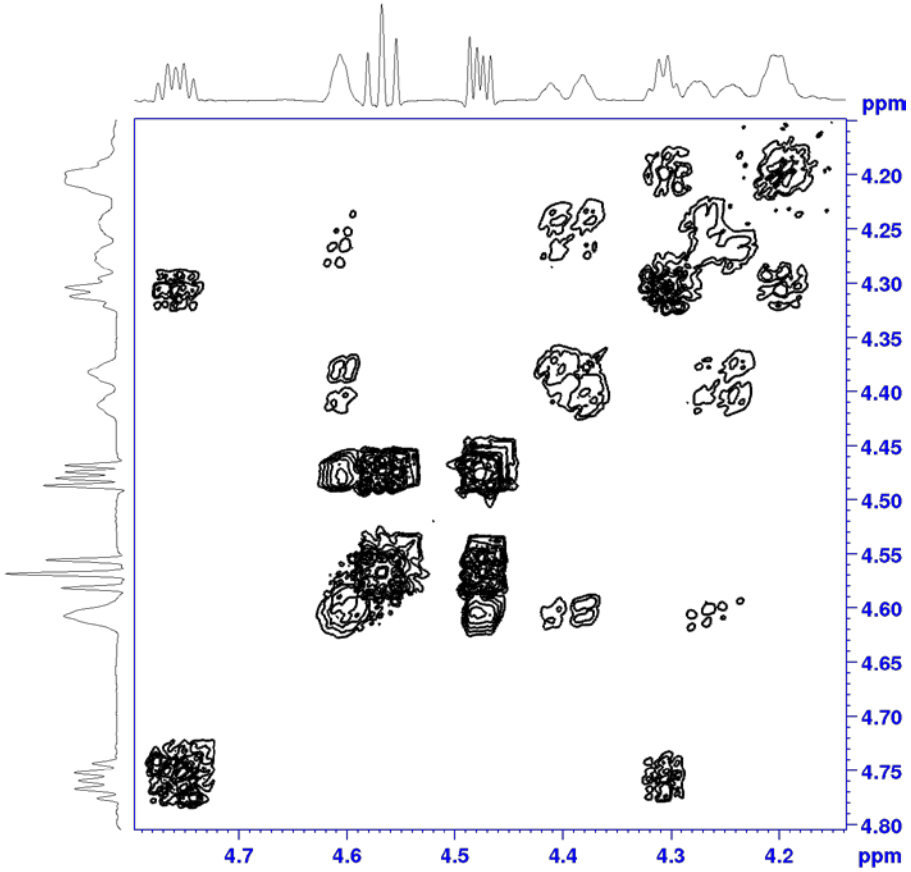


2'-dA NAD$^+$: COSY

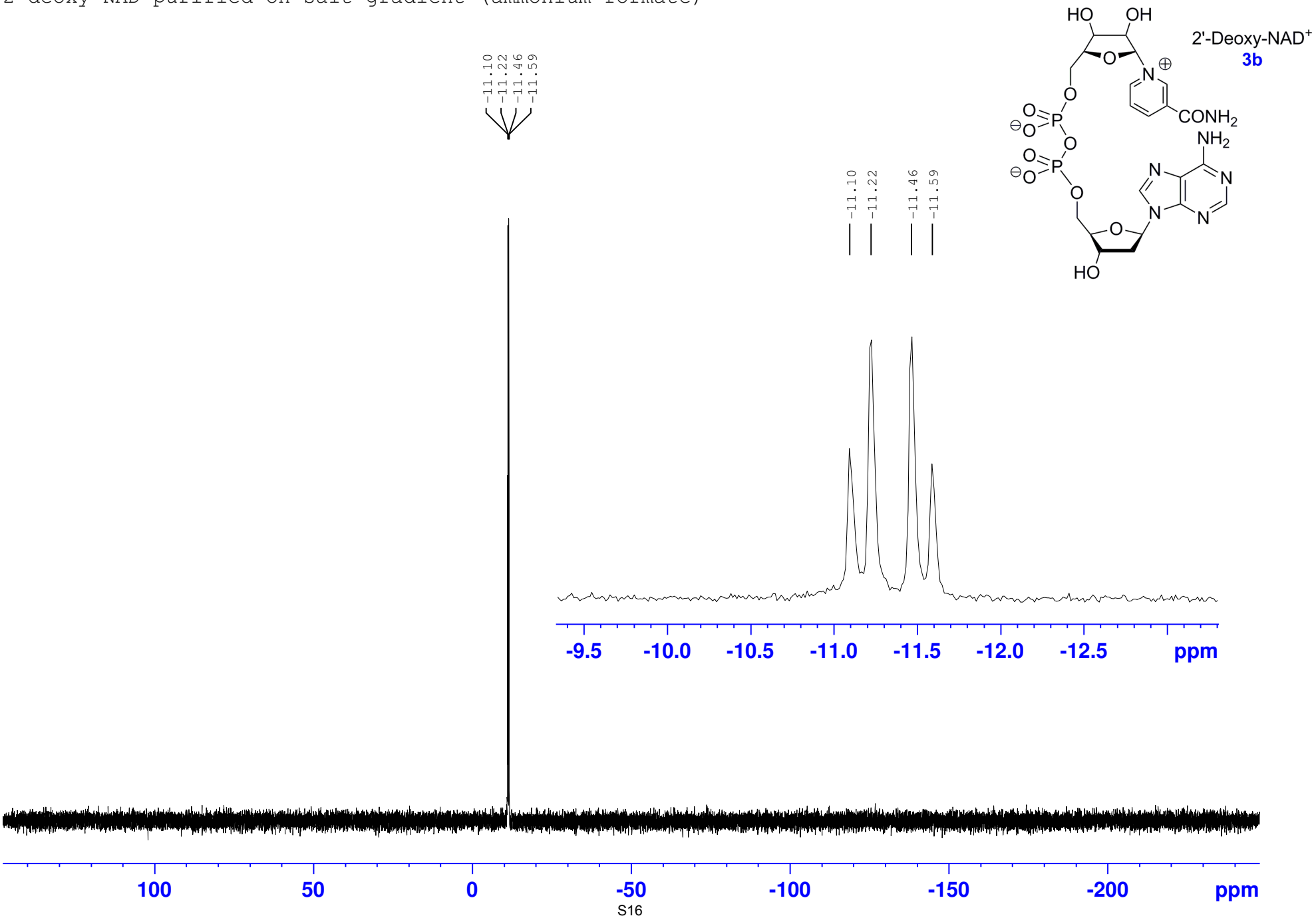


2'-dA-NAD$^+$
3b

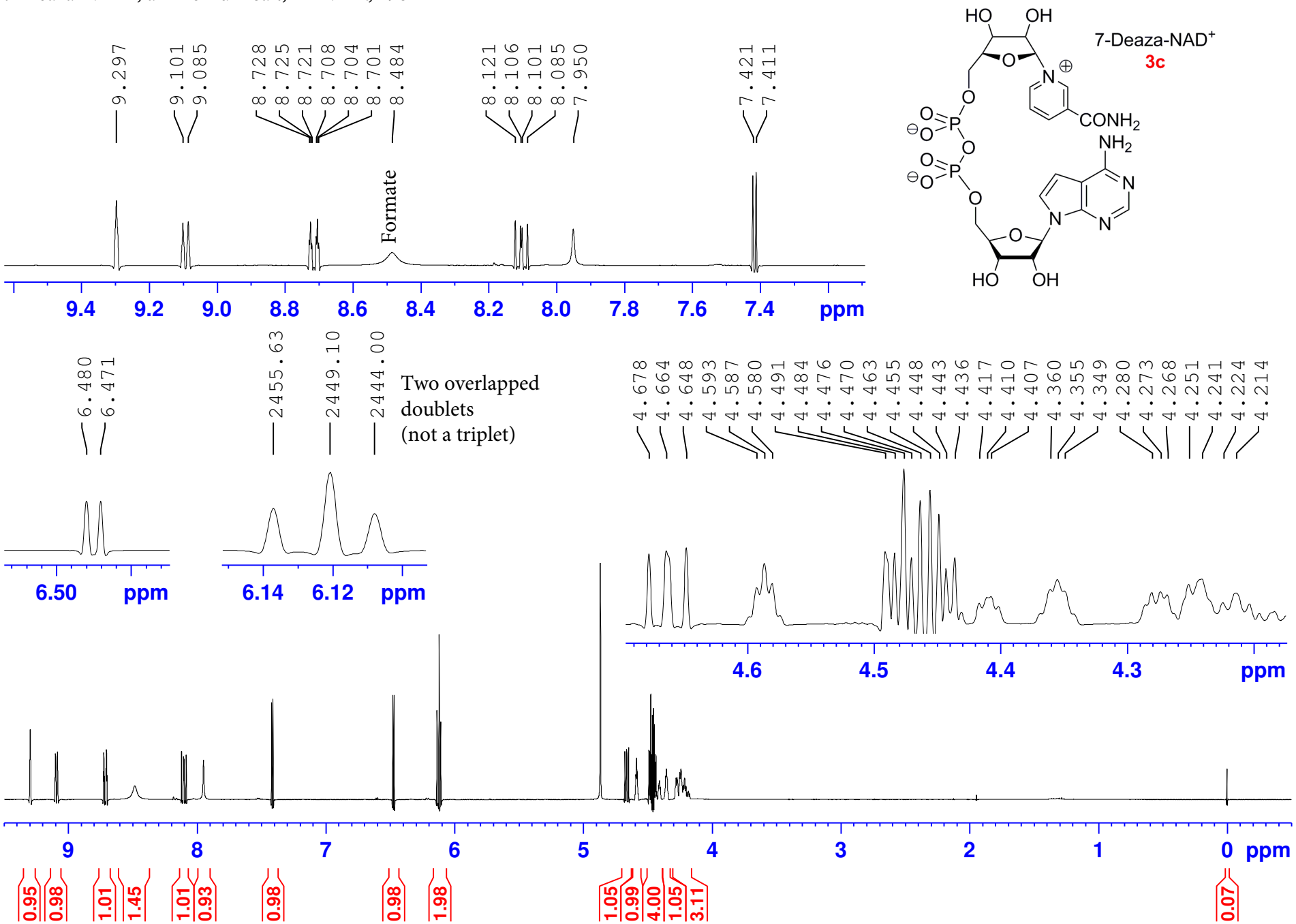




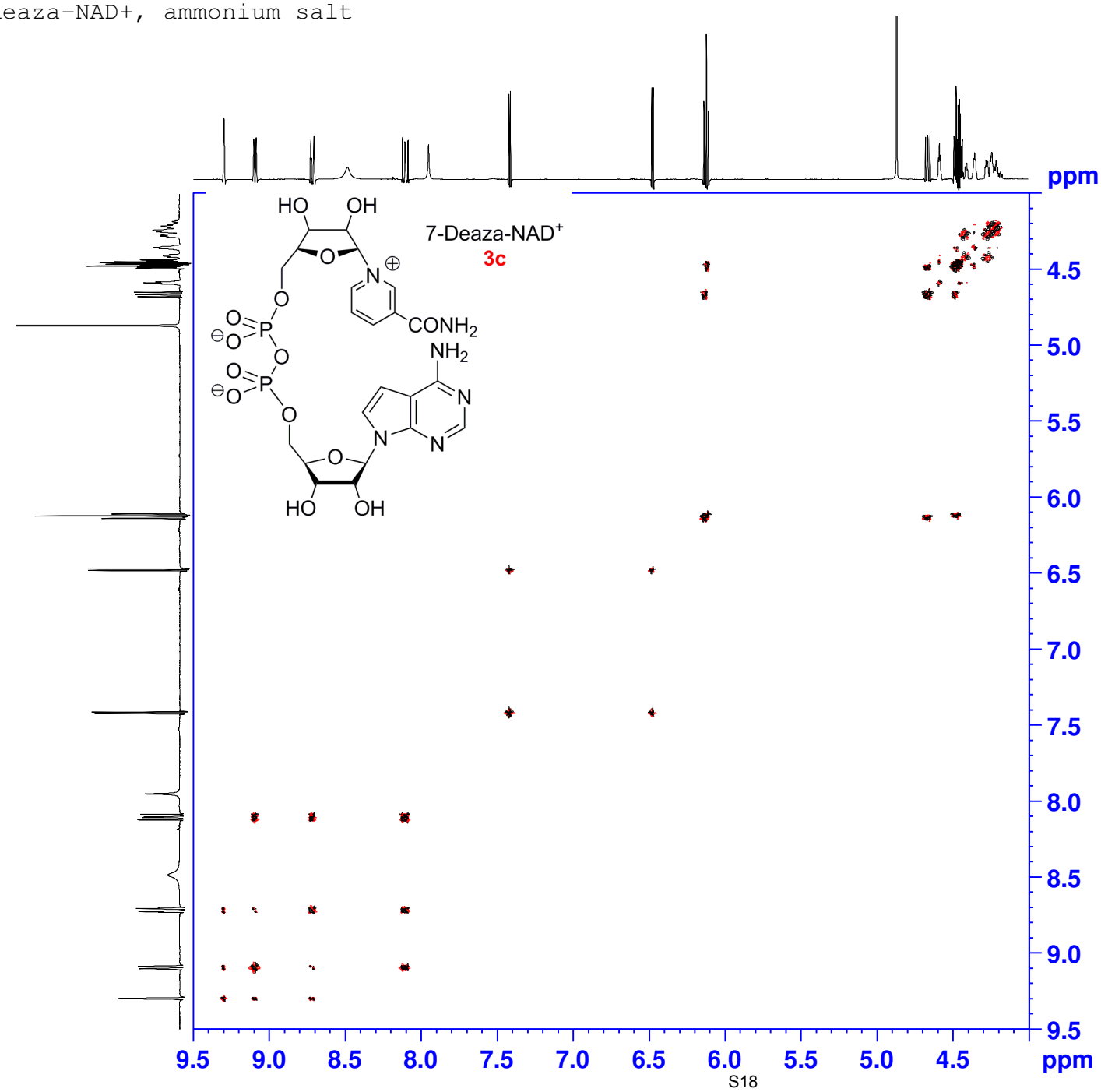
2'deoxy NAD purified on salt gradient (ammonium formate)



7-Deaza-NAD$^+$, ammonium salt, ^{1}H NMR, 298 K



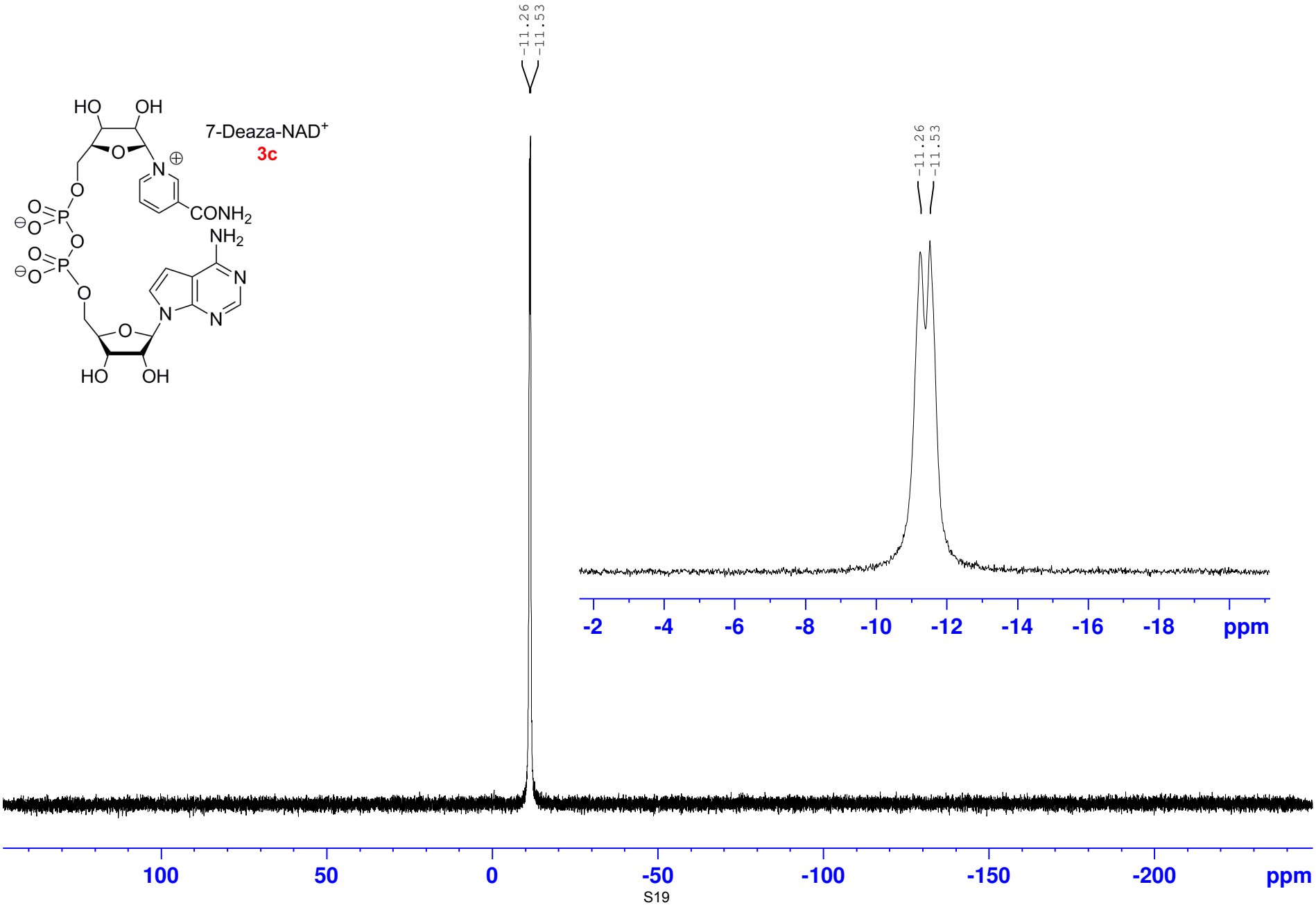
7-deaza-NAD+, ammonium salt

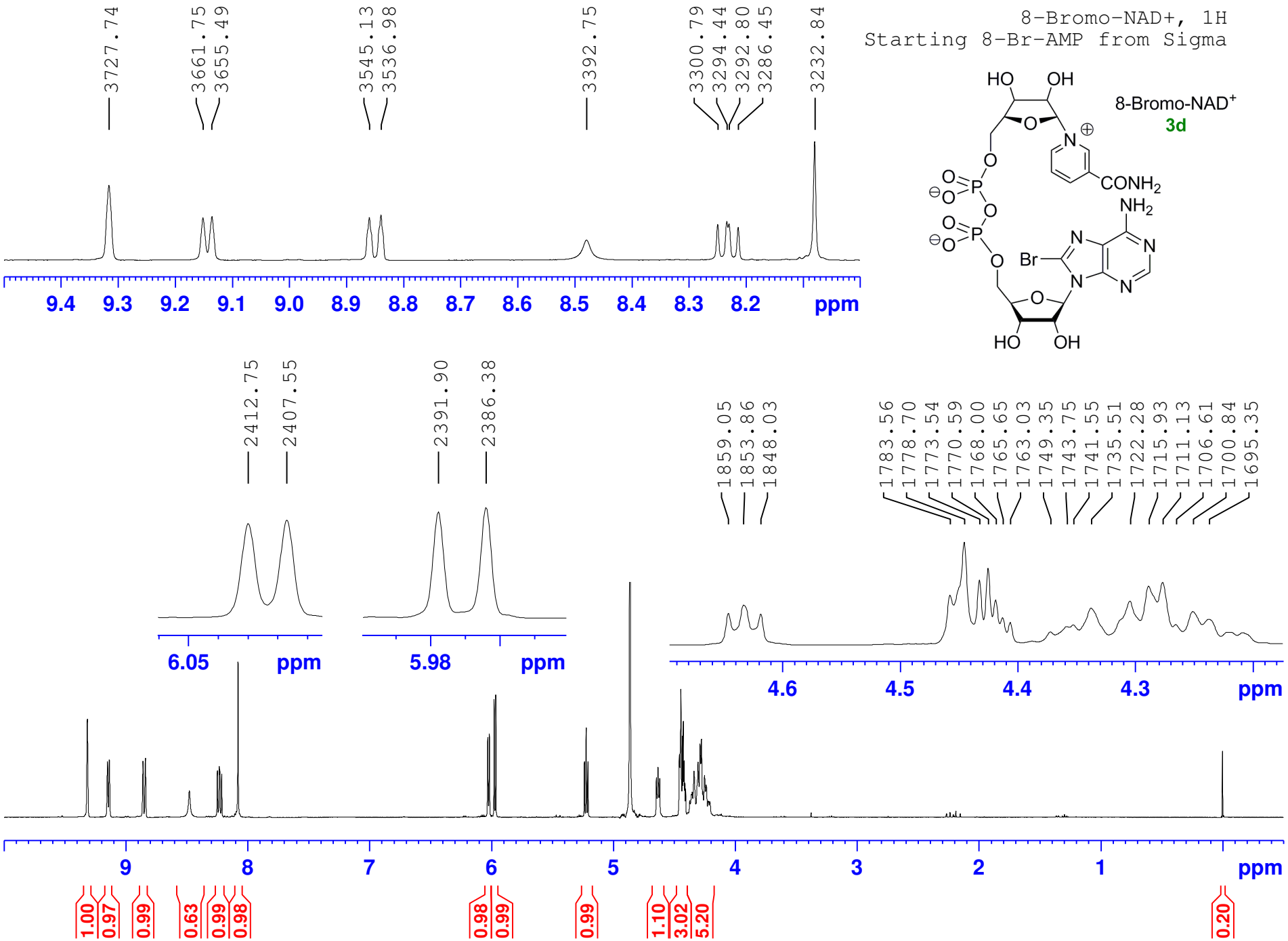



```
Current Data Parameters
NAME      7-deazaNAD_COSY
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_           20170530
Time               15.37
INSTRUM            spect
PROBHD   5 mm BBI 1H/D-
PULPROG    cosygpmfphpp
TD                 4096
SOLVENT             D2O
NS                    1
DS                    4
SWH            2600.555 Hz
FIDRES         0.634901 Hz
AQ            0.7875243 sec
RG               199.73
DW              192.267 usec
DE                 6.50 usec
TE                293.5 K
D0           0.00018326 sec
D1           2.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D16          0.00020000 sec
IN0          0.00038460 sec

======== CHANNEL f1 ========
SFO1        400.1327009 MHz
NUC1                 1H
P1                 7.10 usec
P2                14.20 usec
P17             2500.00 usec
PLW1        16.79999924 W
PLW10        0.94098997 W

====== GRADIENT CHANNEL =====
GPNAM[1]     SMSQ10.100
GPNAM[2]     SMSQ10.100
GPZ1              10.00 %
GPZ2              20.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                 1024
SFO1            400.1327 MHz
FIDRES          5.078328 Hz
SW                 6.498 ppm
FnMODE      States-TPPI

F2 - Processing parameters
SI                 8192
SF          400.1299343 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
PC                 1.00

F1 - Processing parameters
SI                 8192
MC2         States-TPPI
SF          400.1299343 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
```

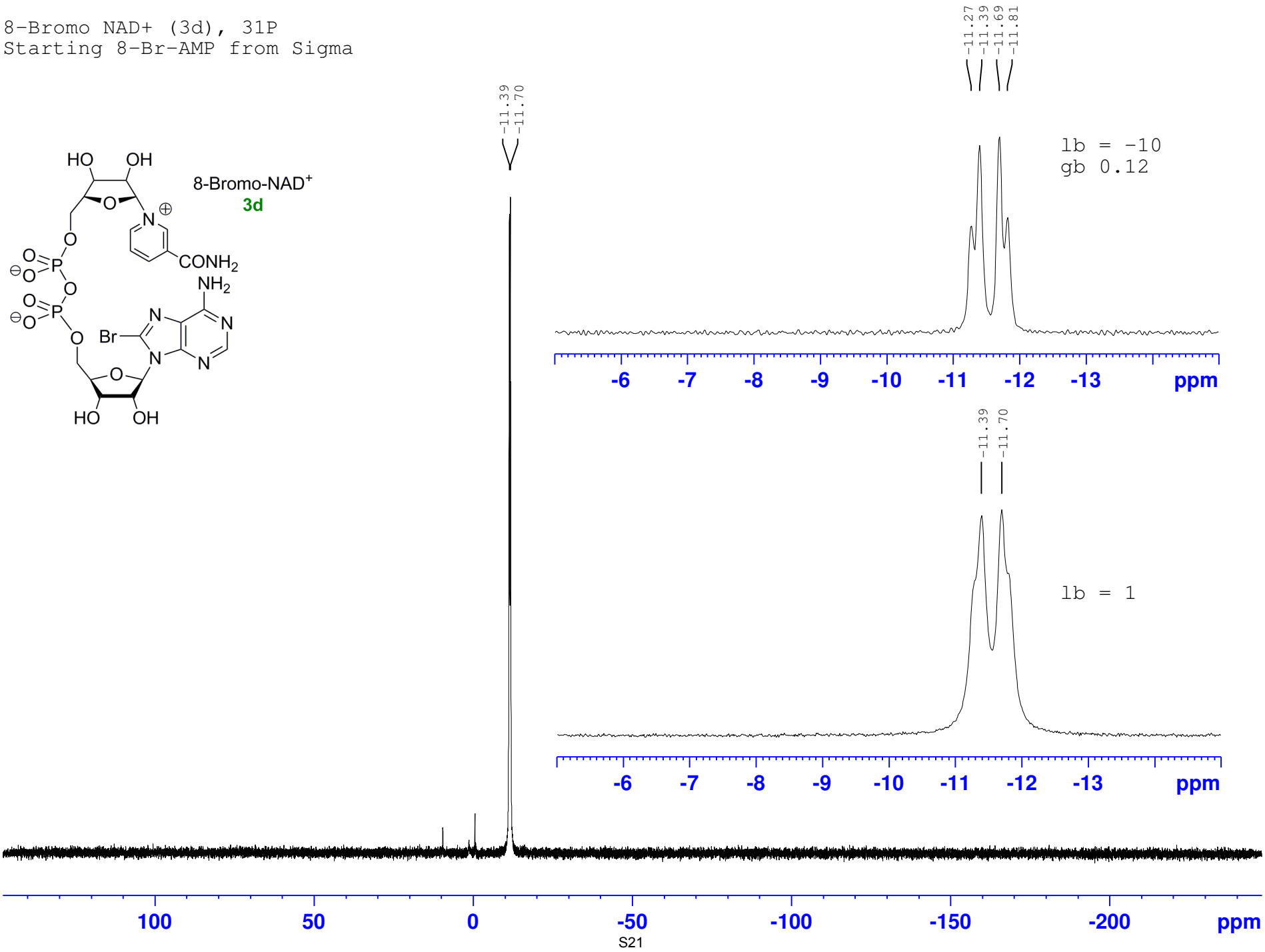
7-Deaza-NAD+ ammonium salt 500uL D_2O

^{31}P NMR

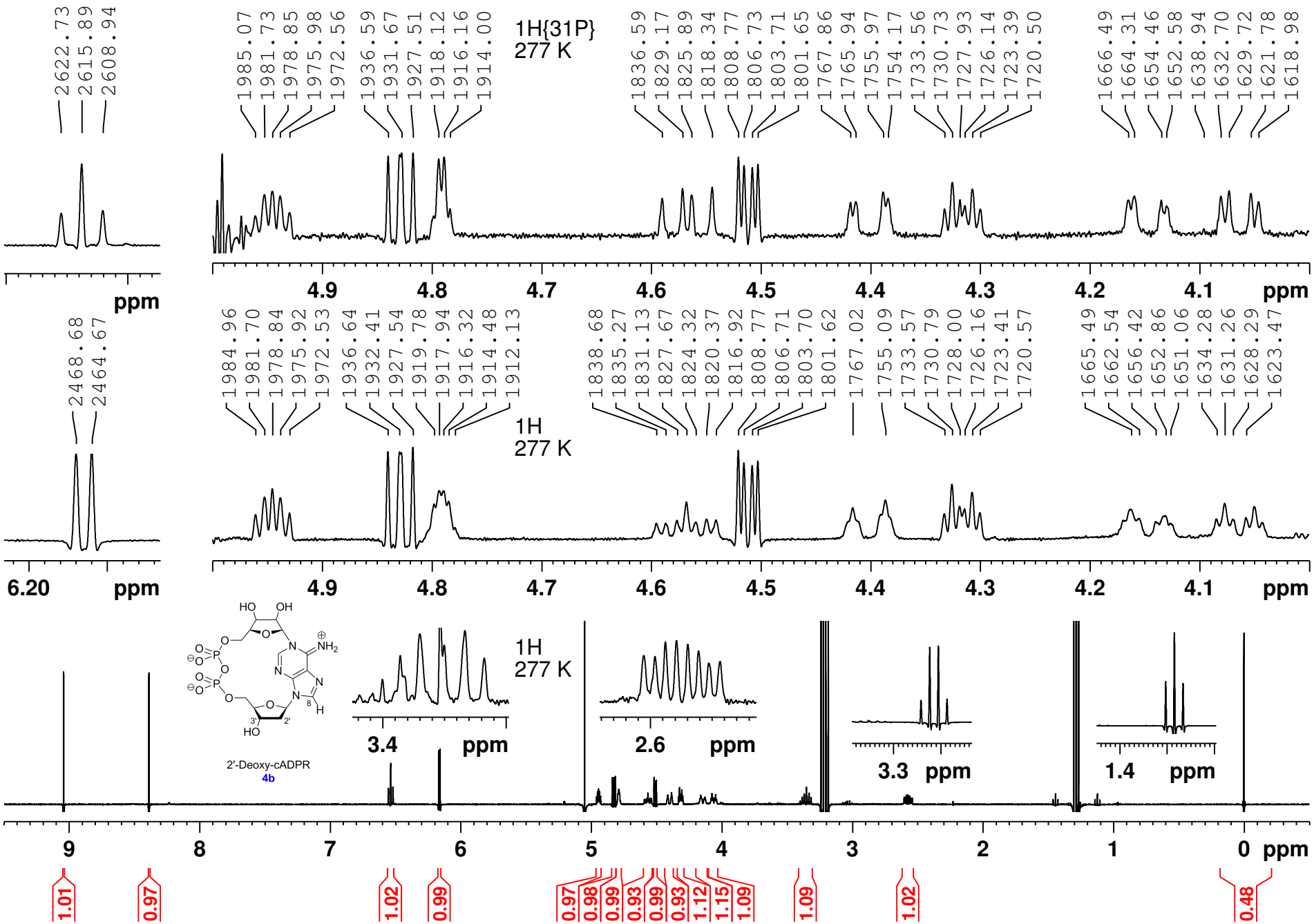


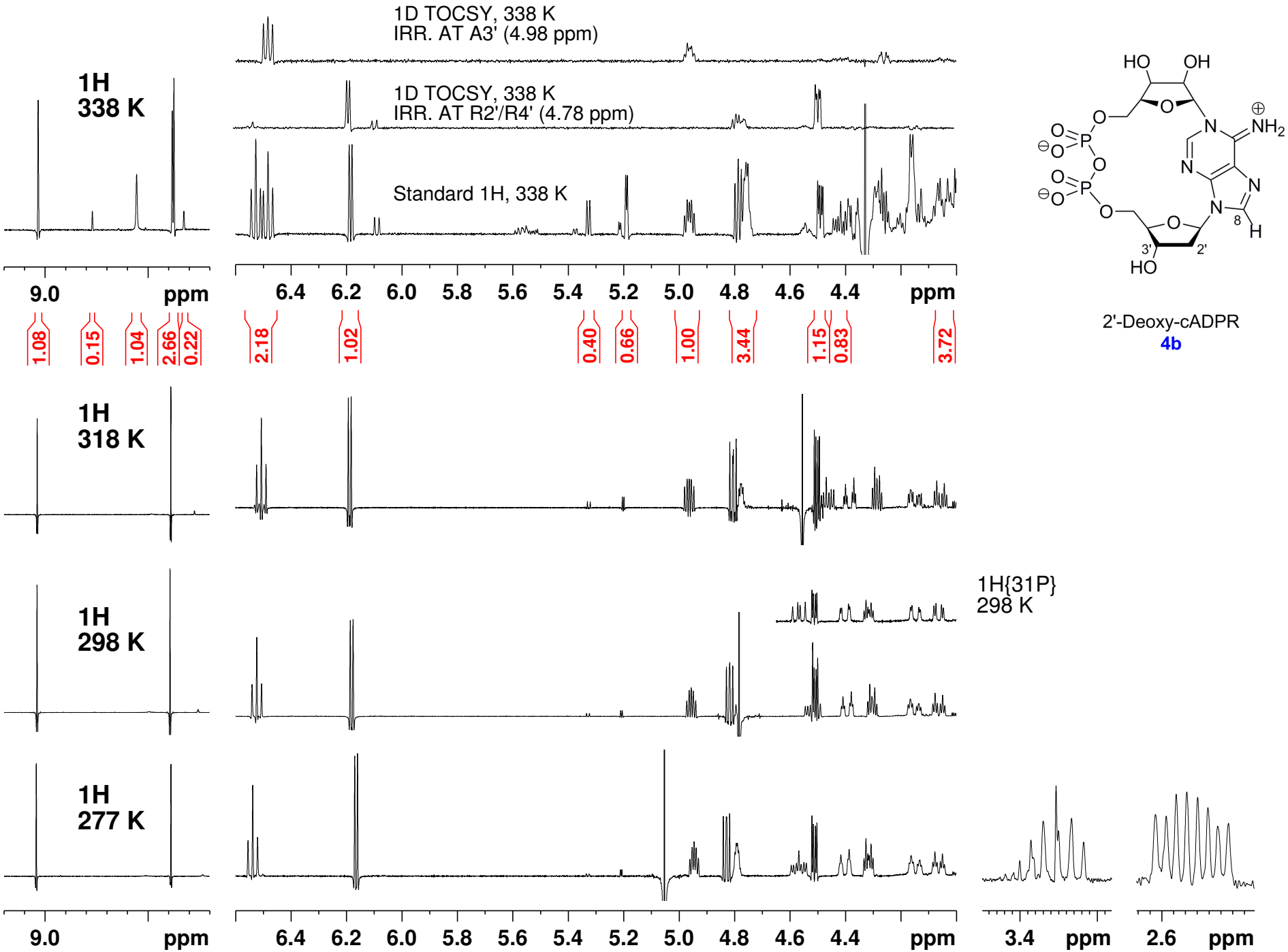

8-Bromo-NAD+, 1H
Starting 8-Br-AMP from Sigma
8-Bromo-NAD+
3d
3727.74
3661.75
3655.49
3545.13
3536.98
3392.75
3300.79
3294.44
3292.80
3286.45
3232.84
9.4 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 ppm
2412.75
2407.55
6.05 ppm
2391.90
2386.38
5.98 ppm
1859.05
1853.86
1848.03
1783.56
1778.70
1773.54
1770.59
1768.00
1765.65
1763.03
1749.35
1743.75
1741.55
1735.51
1722.28
1715.93
1711.13
1706.61
1700.84
1695.35
4.6 4.5 4.4 4.3 ppm
9 8 7 6 5 4 3 2 1 ppm
1.00 0.97 0.99 0.63 0.99 0.98 0.98 0.99 0.99 1.10 3.02 5.20 0.20

8-Bromo NAD+ (3d), 31P
Starting 8-Br-AMP from Sigma

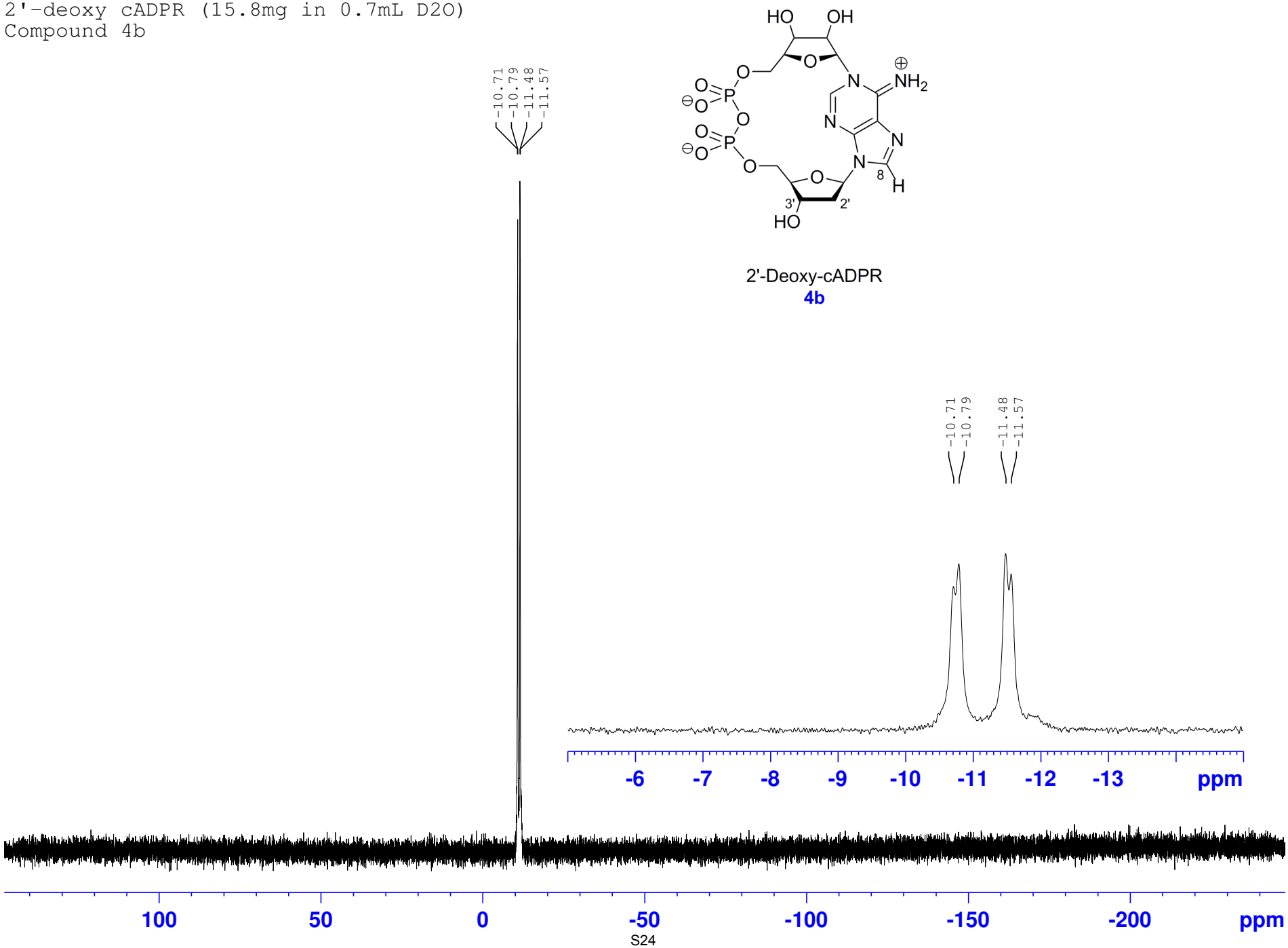


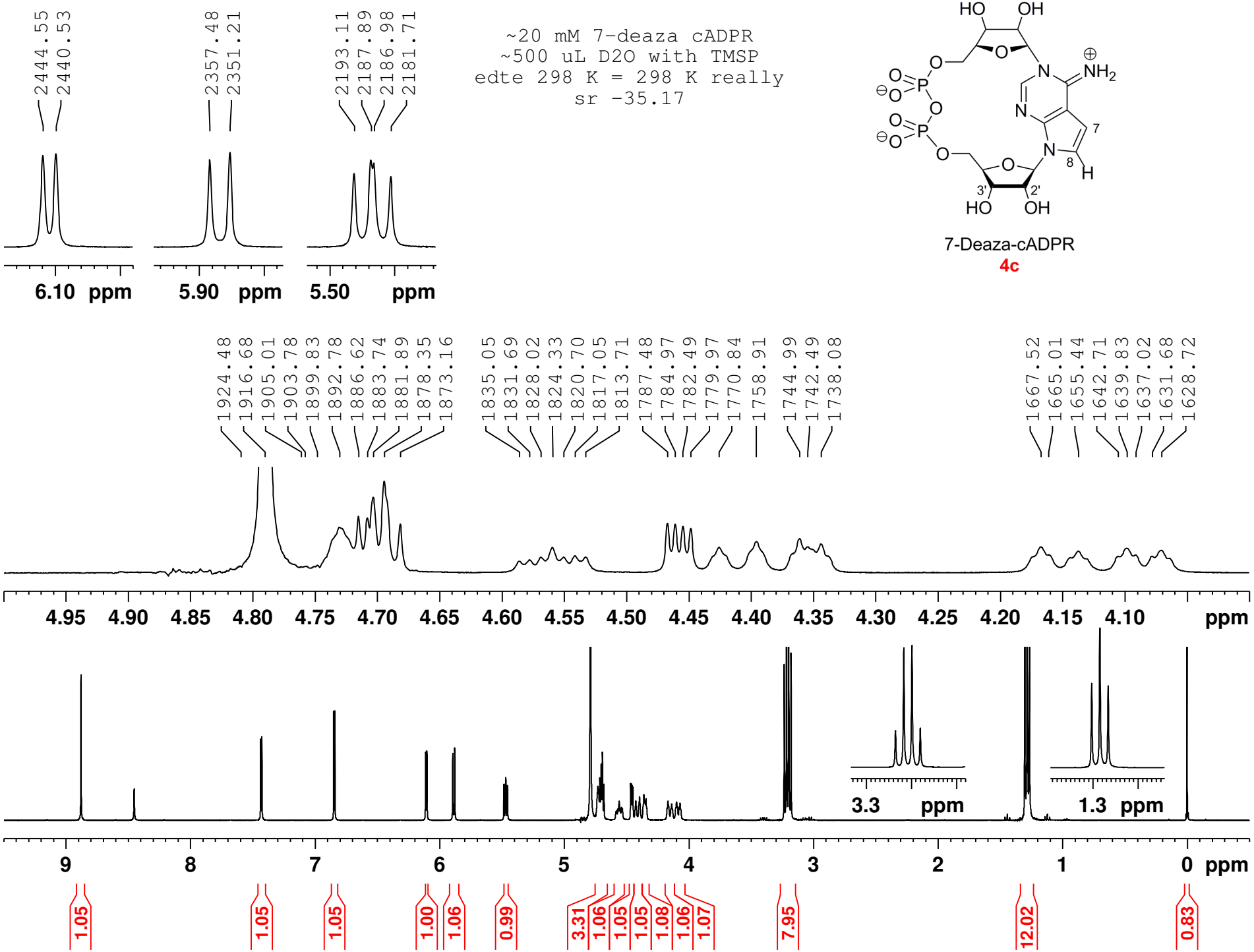
2'-deoxy cADPR, ~20 mM Standard 1H and 1H{31P}, 277 K (4 C)

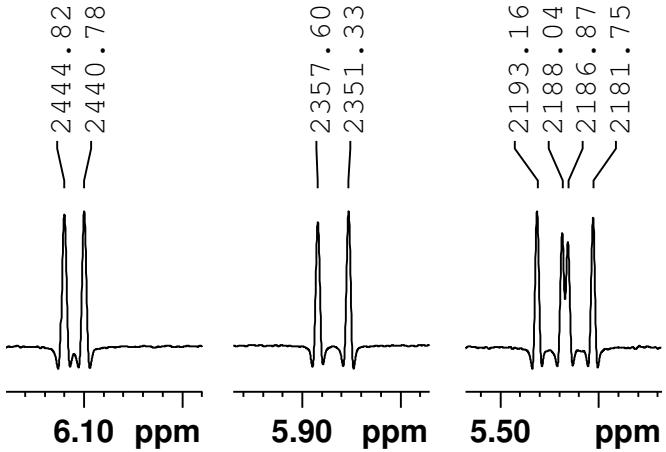



1H
338 K
1D TOCSY, 338 K
IRR. AT A3' (4.98 ppm)
1D TOCSY, 338 K
IRR. AT R2'/R4' (4.78 ppm)
Standard 1H, 338 K
9.0
ppm
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
ppm
1.08
0.15
1.04
2.66
0.22
2.18
1.02
0.40
0.66
1.00
3.44
1.15
0.83
3.72
1H
318 K
1H
298 K
1H
277 K
1H{31P}
298 K
3.4
ppm
2.6
ppm
HO
OH
NH2
N
H
8
3'
2'
HO
2'-Deoxy-cADPR
4b

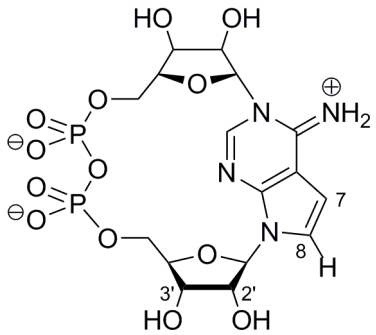
2'-deoxy cADPR (15.8mg in 0.7mL D2O)
Compound 4b



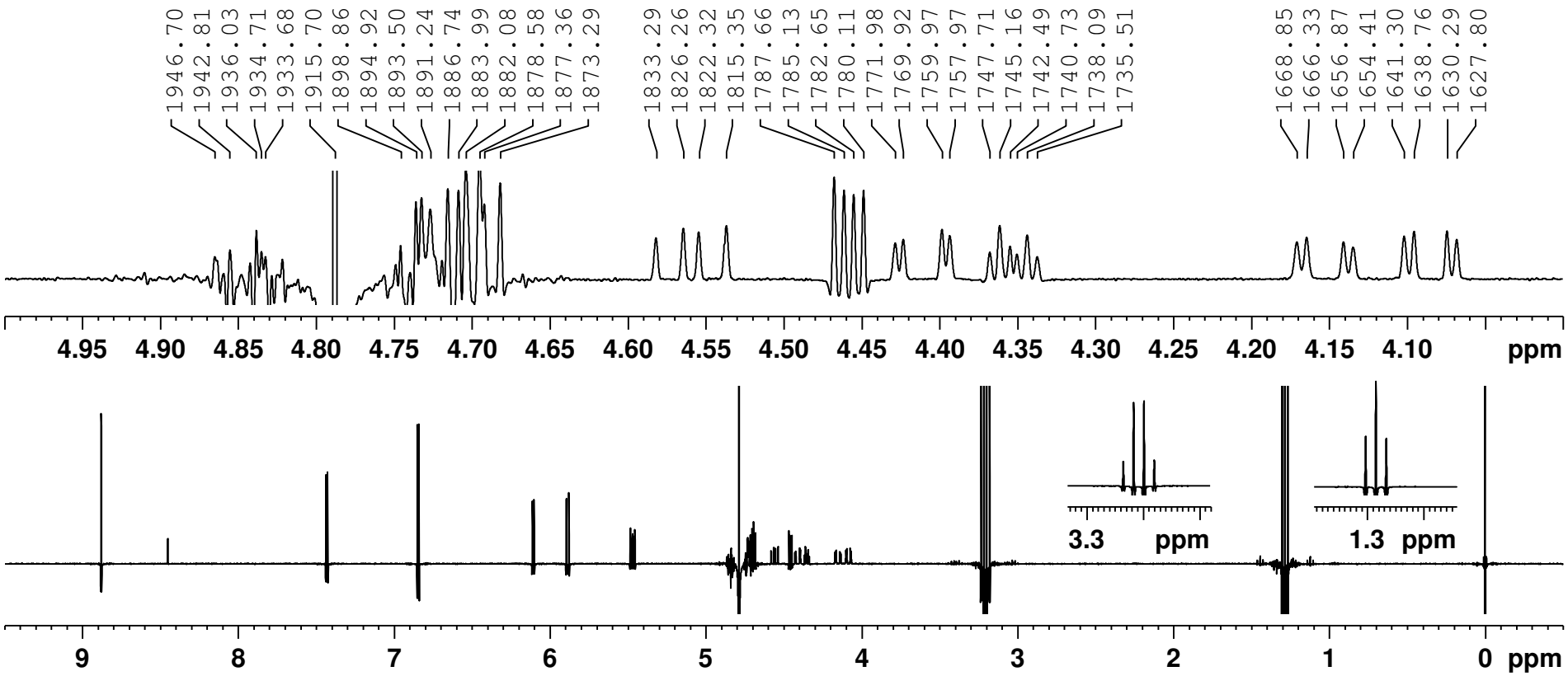

2444.55
2440.53
2357.48
2351.21
2193.11
2187.89
2186.98
2181.71
~20 mM 7-deaza cADPR
~500 uL D2O with TMSP
edte 298 K = 298 K really
sr -35.17
HO
OH
NH2
7
8
H
3'
2'
HO
OH
7-Deaza-cADPR
4c
6.10 ppm
5.90 ppm
5.50 ppm
1924.48
1916.68
1905.01
1903.78
1899.83
1892.78
1886.62
1883.74
1881.89
1878.35
1873.16
1835.05
1831.69
1828.02
1824.33
1820.70
1817.05
1813.71
1787.48
1784.97
1782.49
1779.97
1770.84
1758.91
1744.99
1742.49
1738.08
1667.52
1665.01
1655.44
1642.71
1639.83
1637.02
1631.68
1628.72
4.95 4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20 4.15 4.10 ppm
3.3 ppm
1.3 ppm
9 8 7 6 5 4 3 2 1 0 ppm
1.05
1.05
1.05
1.00
1.06
0.99
3.31
1.06
1.05
1.05
1.08
1.06
1.07
7.95
12.02
0.83



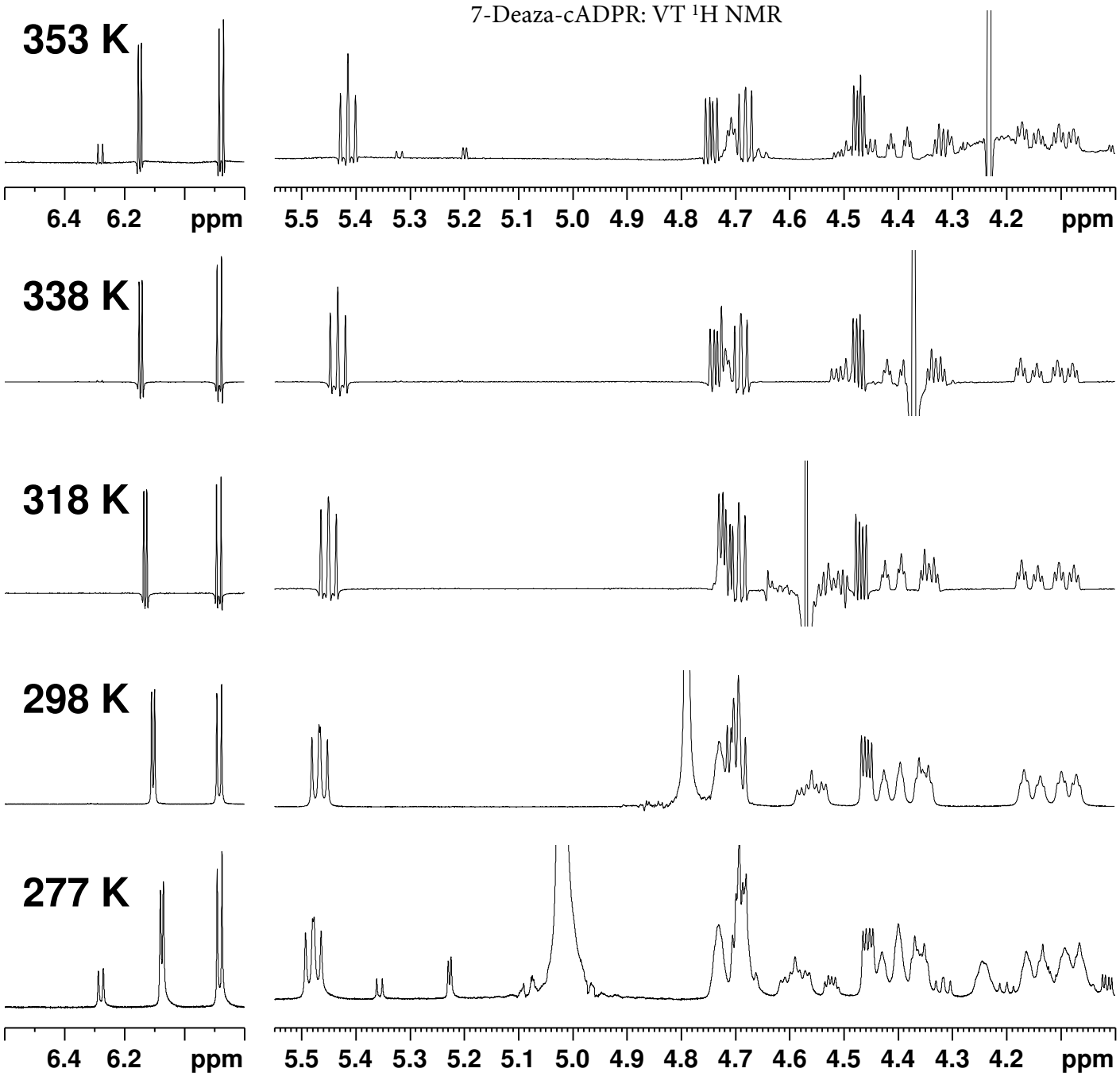
~20 mM 7-deaza cADPR
{31P} 1H std, 25 deg C
~500 uL D2O with TMSP
edte 298 K = 298 K really
sr -34.23

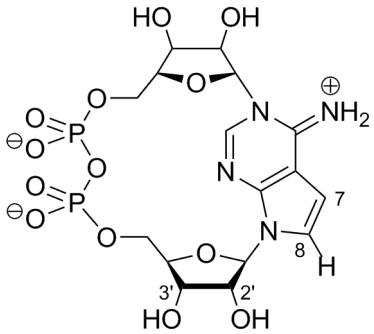


7-Deaza-cADPR
4c



7-Deaza-cADPR: VT ^{1}H NMR

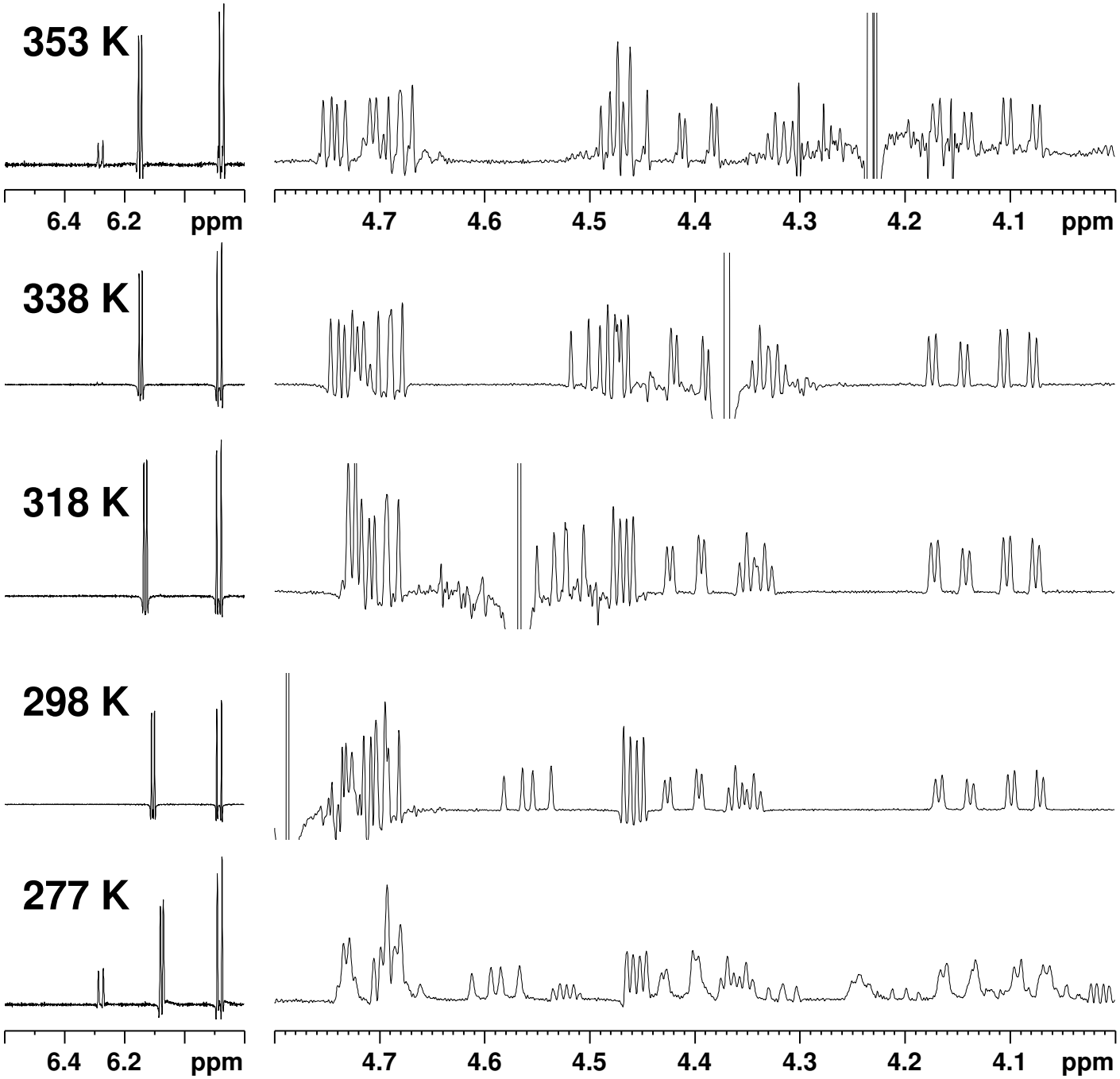


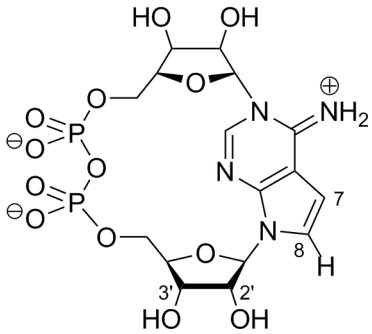


7-Deaza-cADPR
4c

277 K spectra acquired after 353 K spectra

7-Deaza-cADPR, VT $^{1}H\{^{31}P\}$ NMR



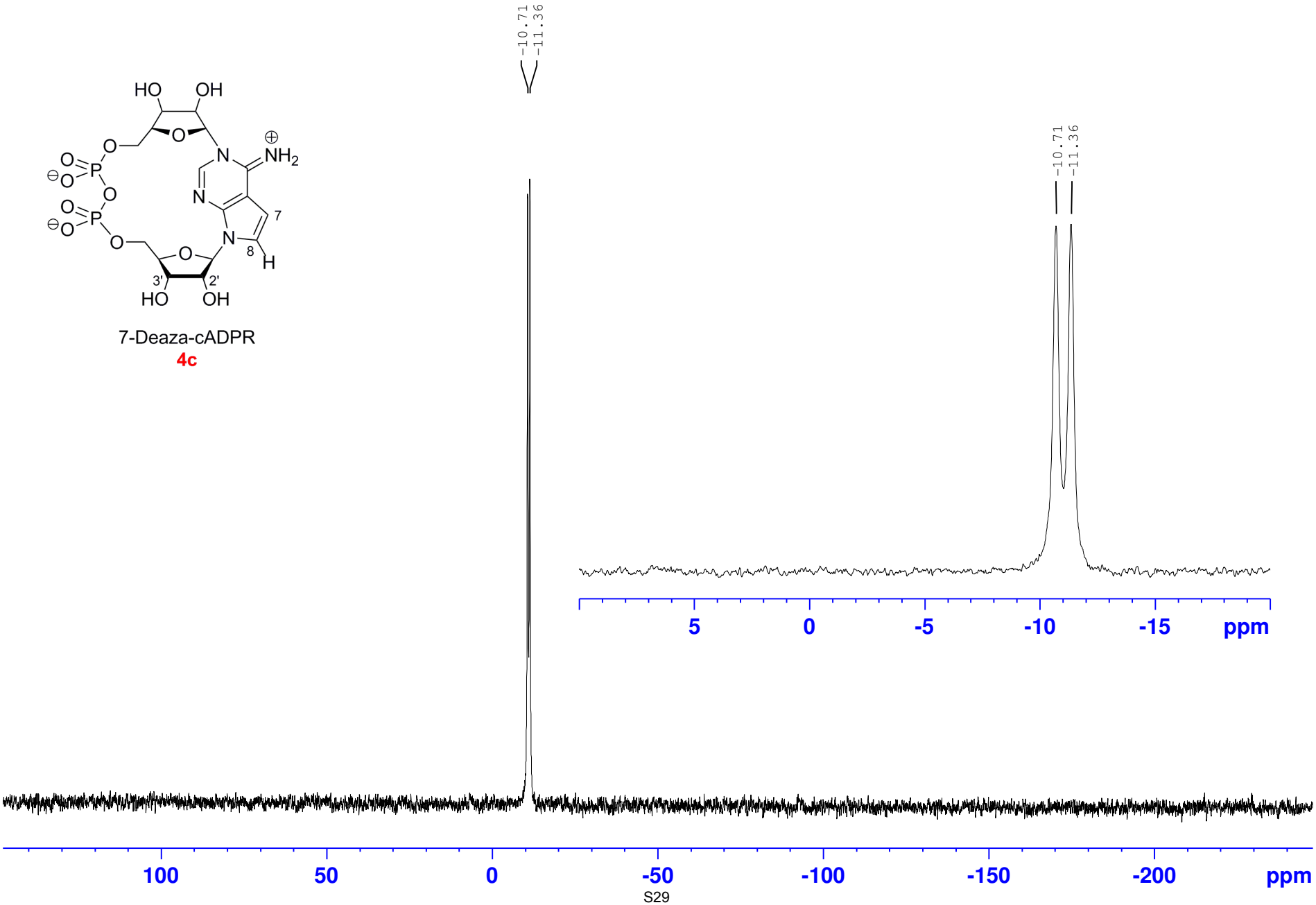


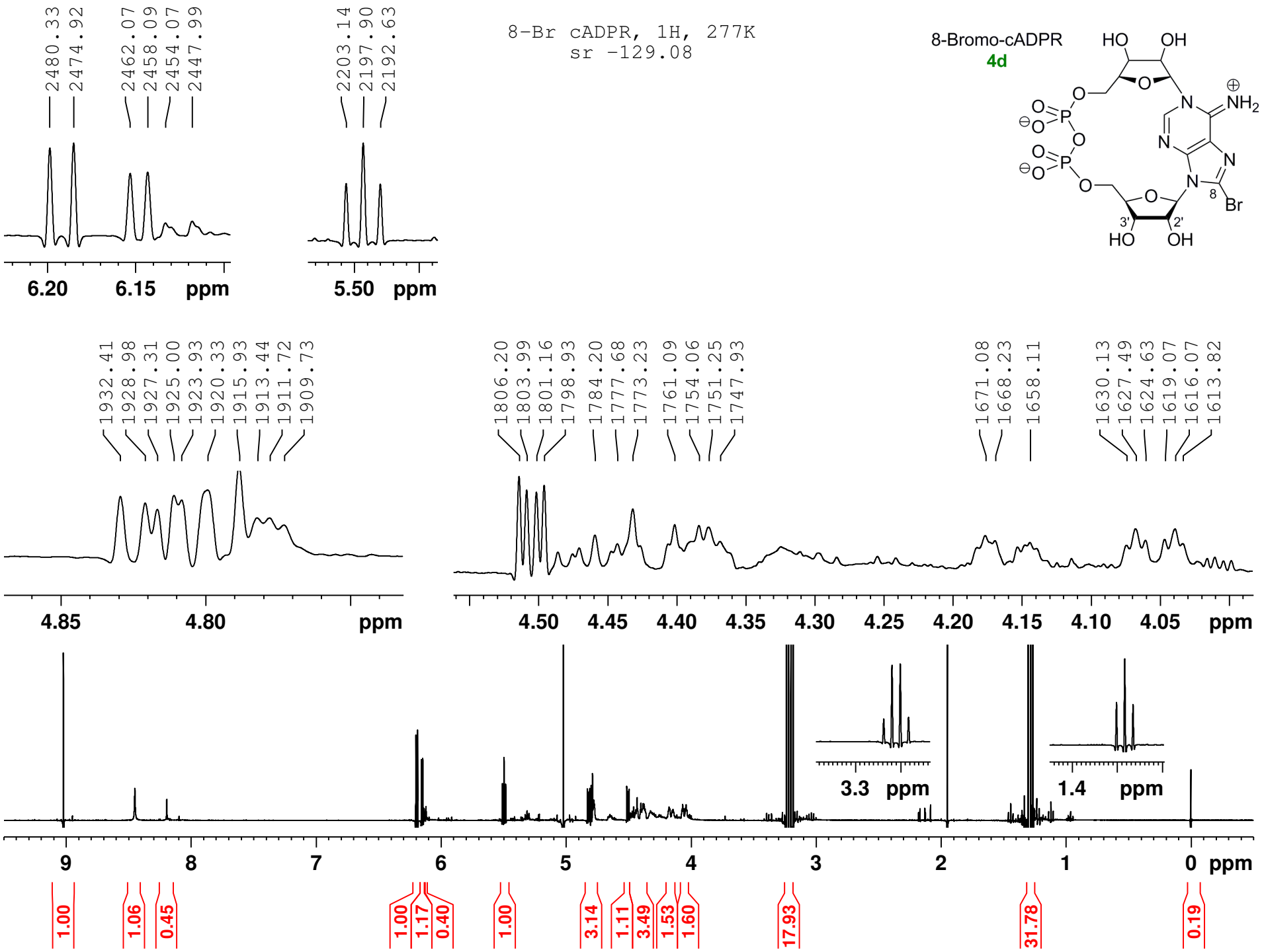
7-Deaza-cADPR
4c

277 K spectra acquired after 353 K spectra

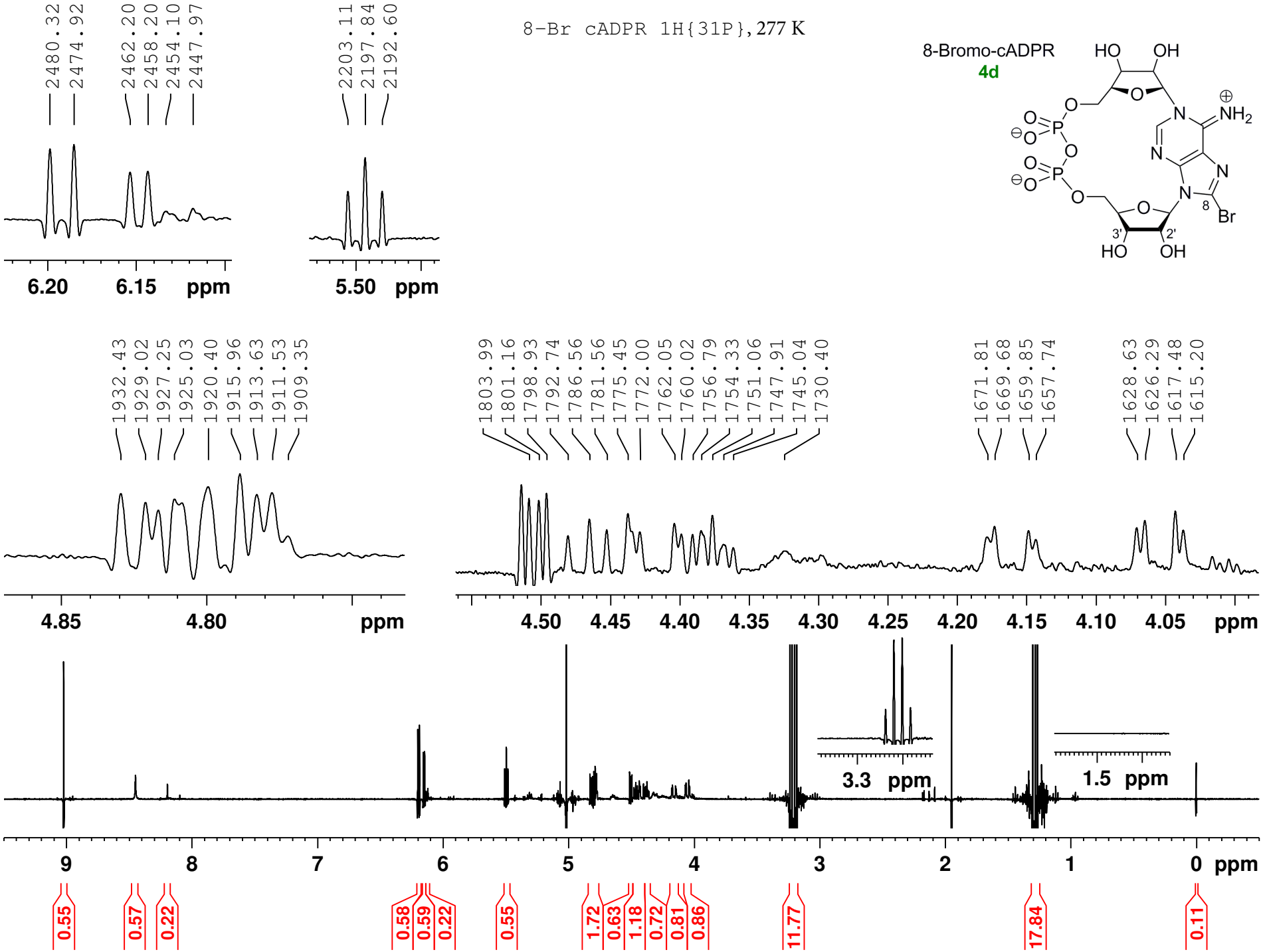
~20 mM 7-deaza cADPR, ^{31}P NMR
~500 uL D2O with TMSP edte
298 K = 298 K really

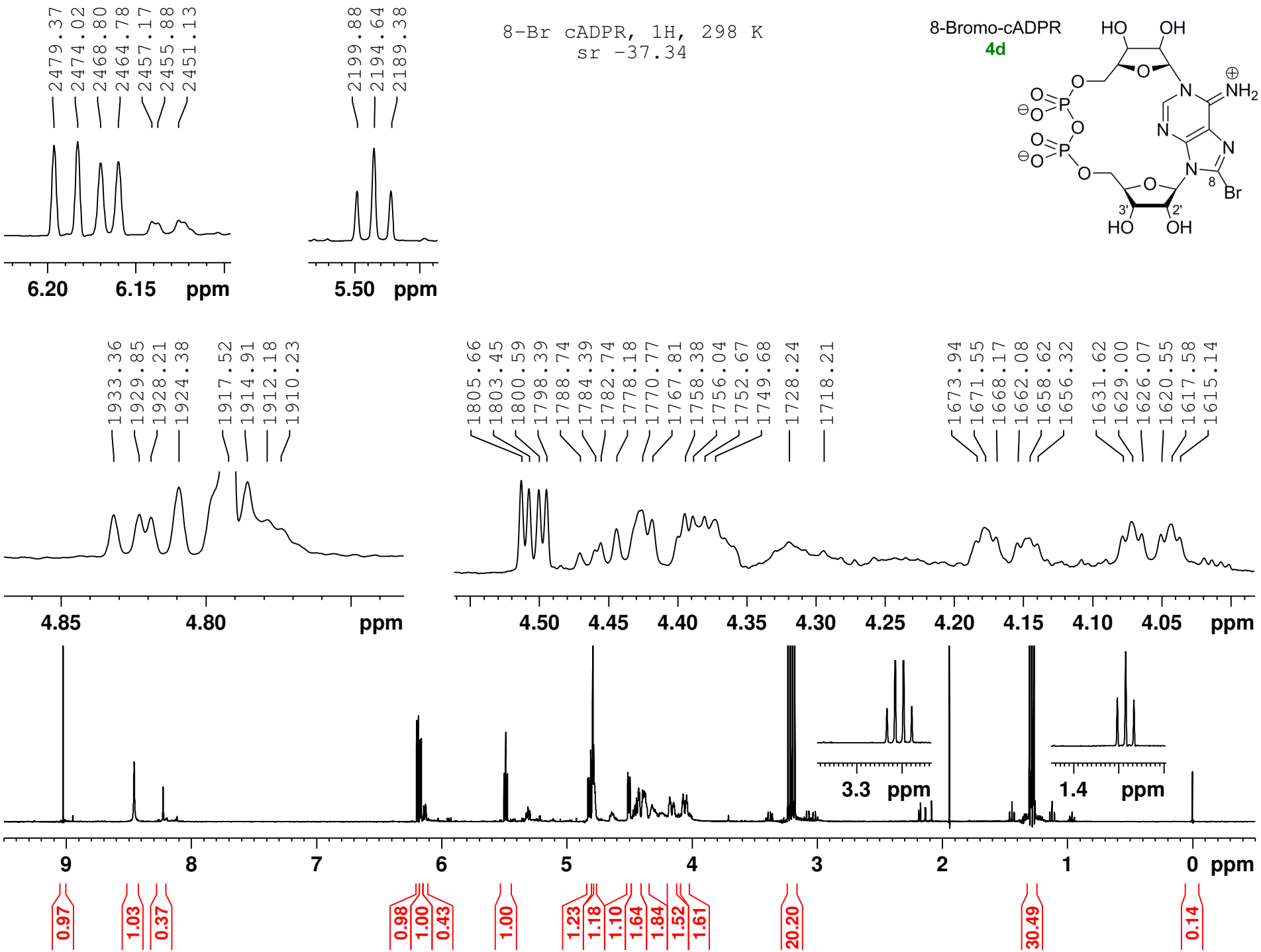
7-Deaza-cADPR
4c



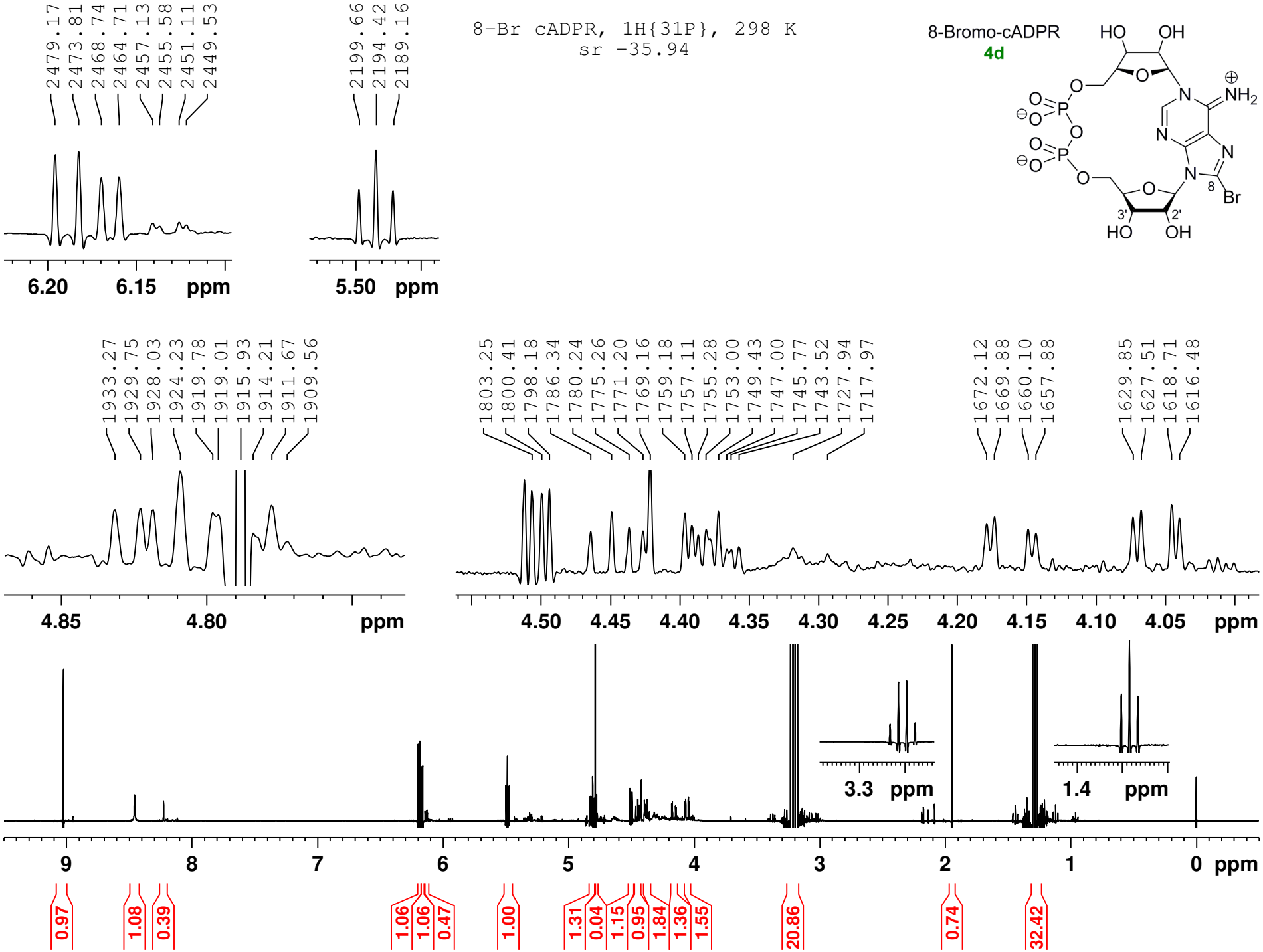

8-Br cADPR, 1H, 277K
sr -129.08
8-Bromo-cADPR
4d
2480.33
2474.92
2462.07
2458.09
2454.07
2447.99
2203.14
2197.90
2192.63
1932.41
1928.98
1927.31
1925.00
1923.93
1920.33
1915.93
1913.44
1911.72
1909.73
1806.20
1803.99
1801.16
1798.93
1784.20
1777.68
1773.23
1761.09
1754.06
1751.25
1747.93
1671.08
1668.23
1658.11
1630.13
1627.49
1624.63
1619.07
1616.07
1613.82
6.20
6.15
ppm
5.50
ppm
4.85
4.80
ppm
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
ppm
3.3
ppm
1.4
ppm
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.06
0.45
1.00
1.17
0.40
1.00
3.14
1.11
3.49
1.53
1.60
17.93
31.78
0.19

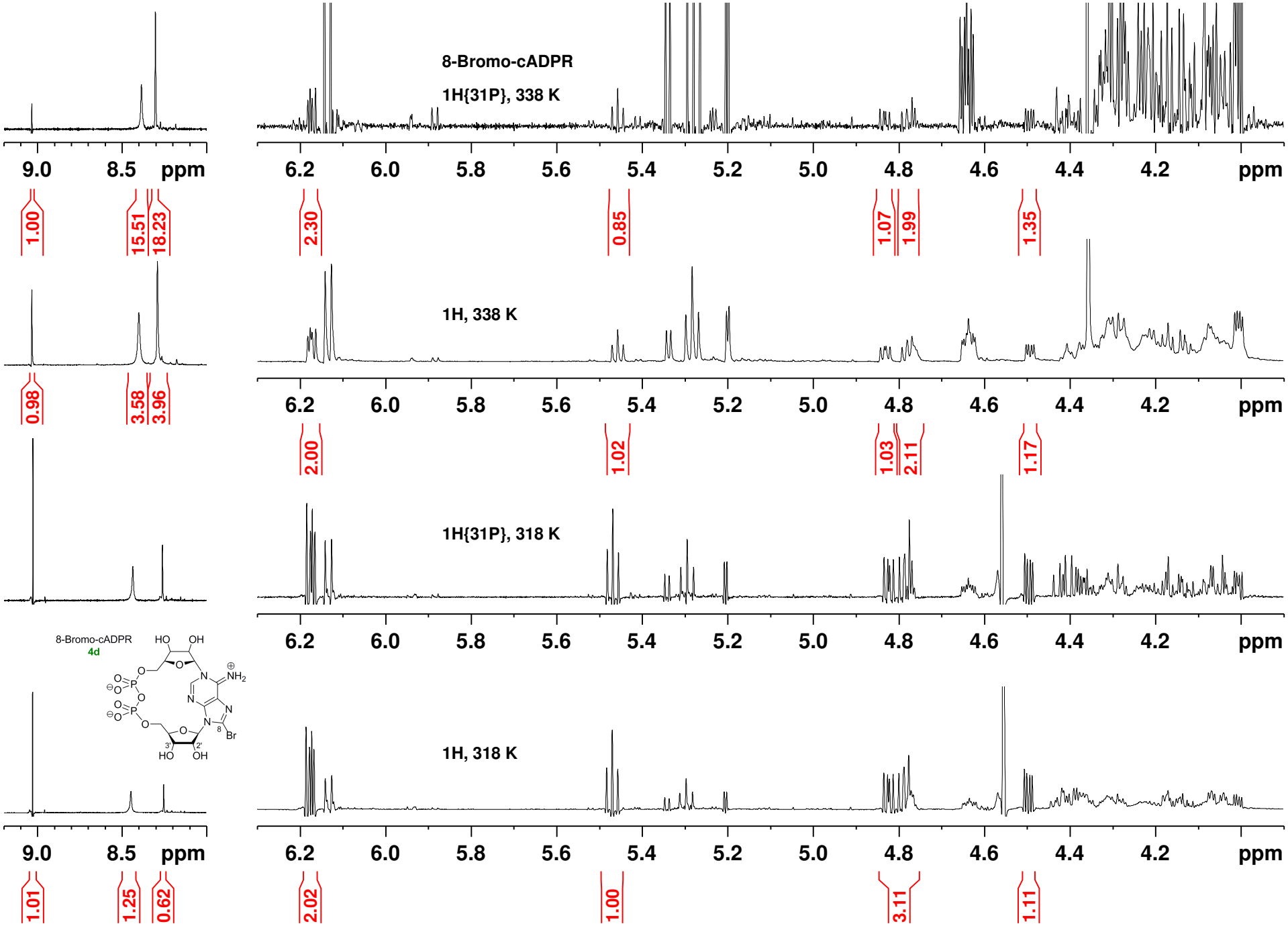
8-Br cADPR 1H{31P}, 277 K



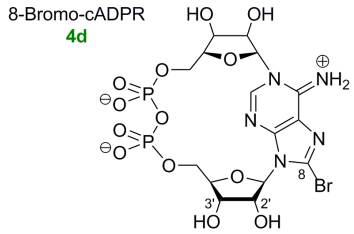

8-Br cADPR, 1H, 298 K
sr -37.34
8-Bromo-cADPR
4d
2479.37
2474.02
2468.80
2464.78
2457.17
2455.88
2451.13
2199.88
2194.64
2189.38
1933.36
1929.85
1928.21
1924.38
1917.52
1914.91
1912.18
1910.23
1805.66
1803.45
1800.59
1798.39
1788.74
1784.39
1782.74
1778.18
1770.77
1767.81
1758.38
1756.04
1752.67
1749.68
1728.24
1718.21
1673.94
1671.55
1668.17
1662.08
1658.62
1656.32
1631.62
1629.00
1626.07
1620.55
1617.58
1615.14
6.20
6.15
ppm
5.50
ppm
4.85
4.80
ppm
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
ppm
3.3
ppm
1.4
ppm
9
8
7
6
5
4
3
2
1
0
ppm
0.97
1.03
0.37
0.98
1.00
0.43
1.00
1.23
1.18
1.10
1.64
1.84
1.52
1.61
20.20
30.49
0.14

8-Br cADPR, 1H{31P}, 298 K
sr -35.94

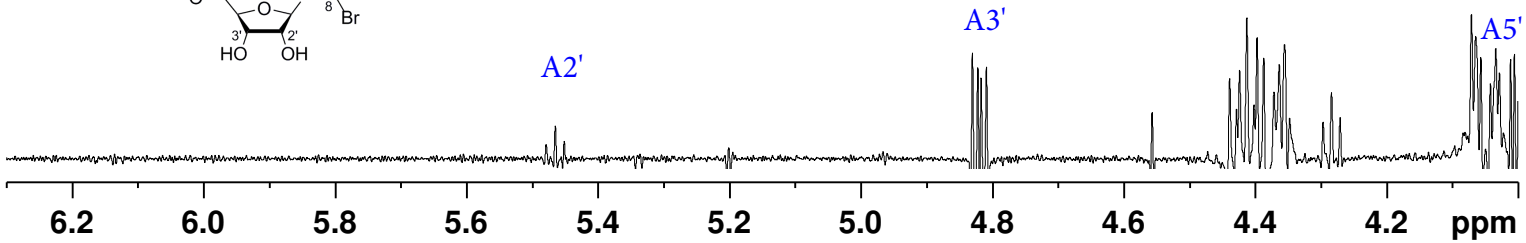



8-Bromo-cADPR
1H{31P}, 338 K
1H, 338 K
1H{31P}, 318 K
1H, 318 K
8-Bromo-cADPR
4d
9.0
8.5
ppm
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
1.00
15.51
18.23
2.30
0.85
1.07
1.99
1.35
0.98
3.58
3.96
2.00
1.02
1.03
2.11
1.17
1.01
1.25
0.62
2.02
1.00
3.11
1.11

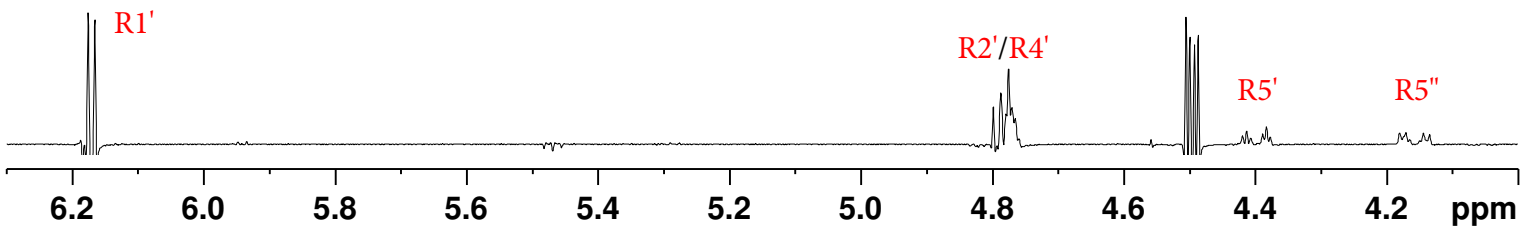
8-Bromo-cADPR (**4d**):
1D TOCSY at 318 K



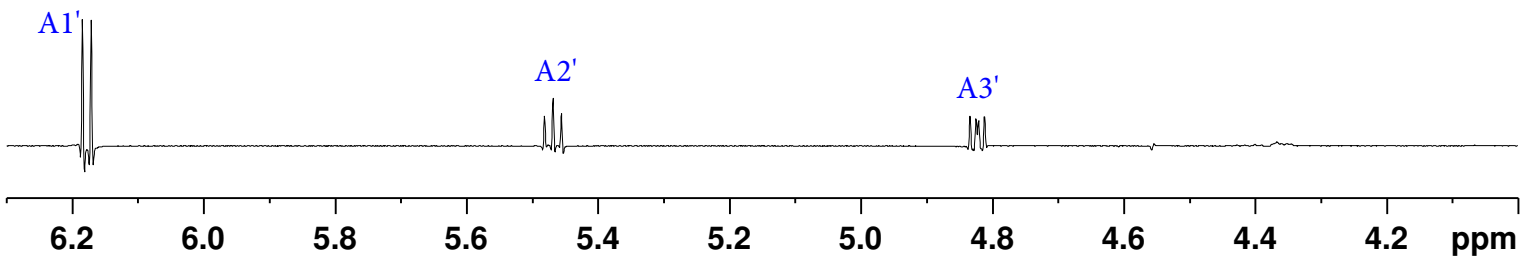
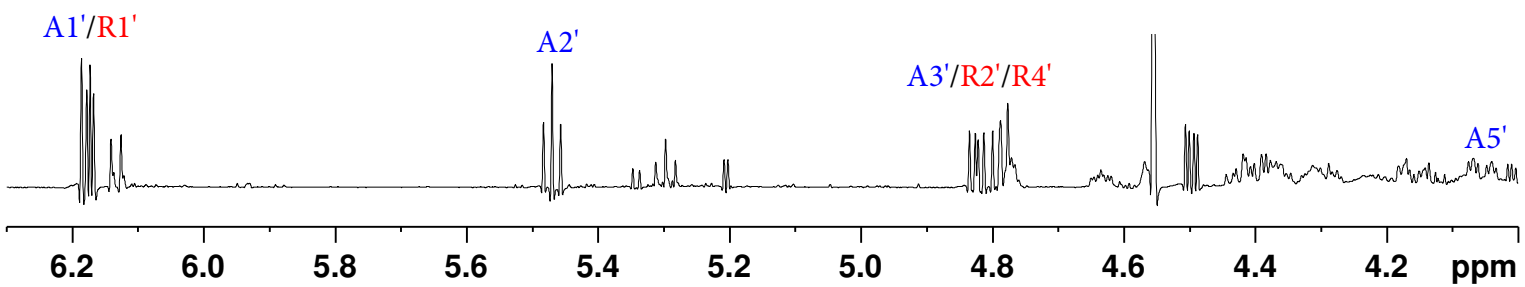
Irradiate at A5'



Irradiate at R4'/R2'



Irradiate at A2'



Standard 1H 318 K