

## Supplementary material

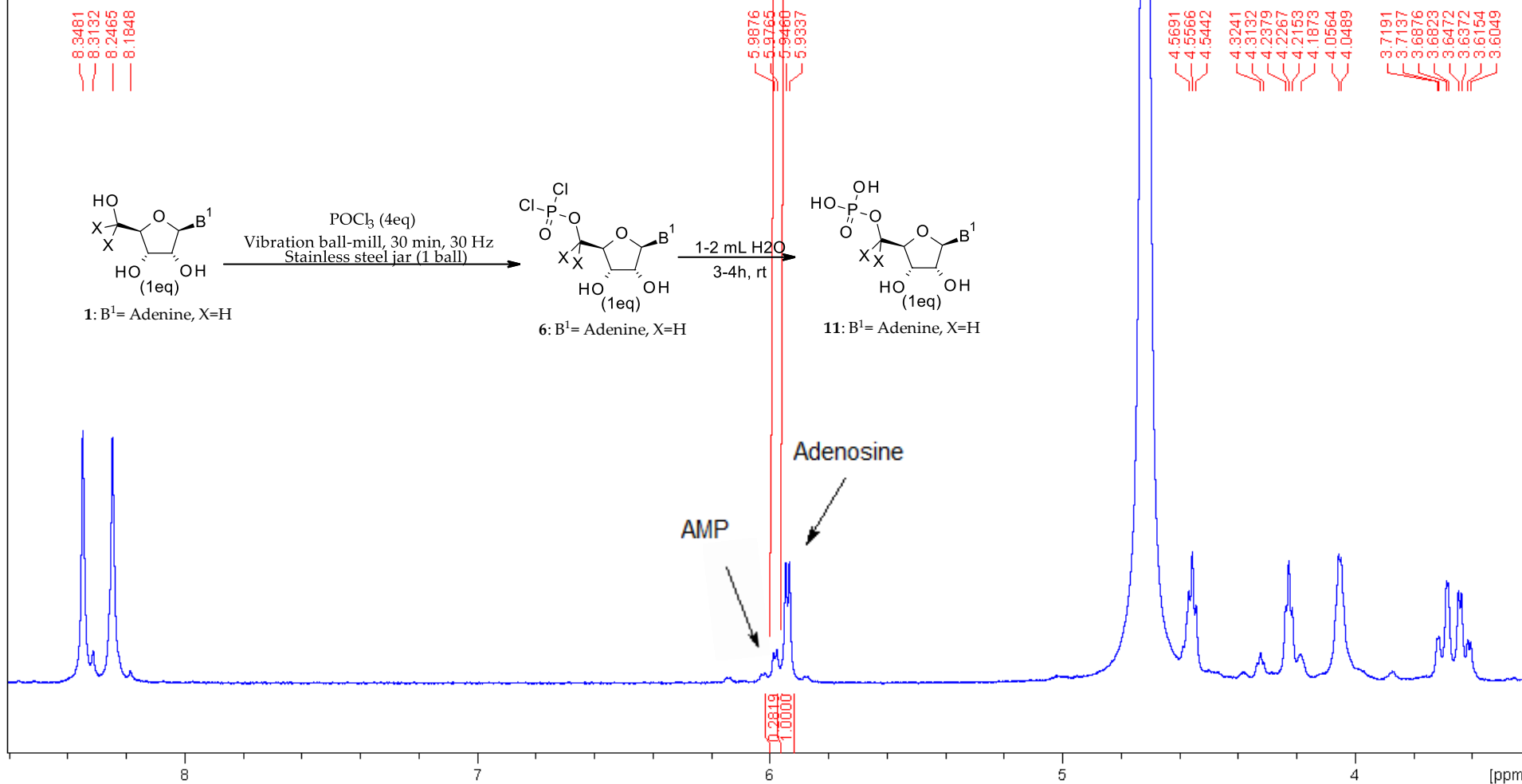
### Synthesis of mixed dinucleotides by mechanochemistry

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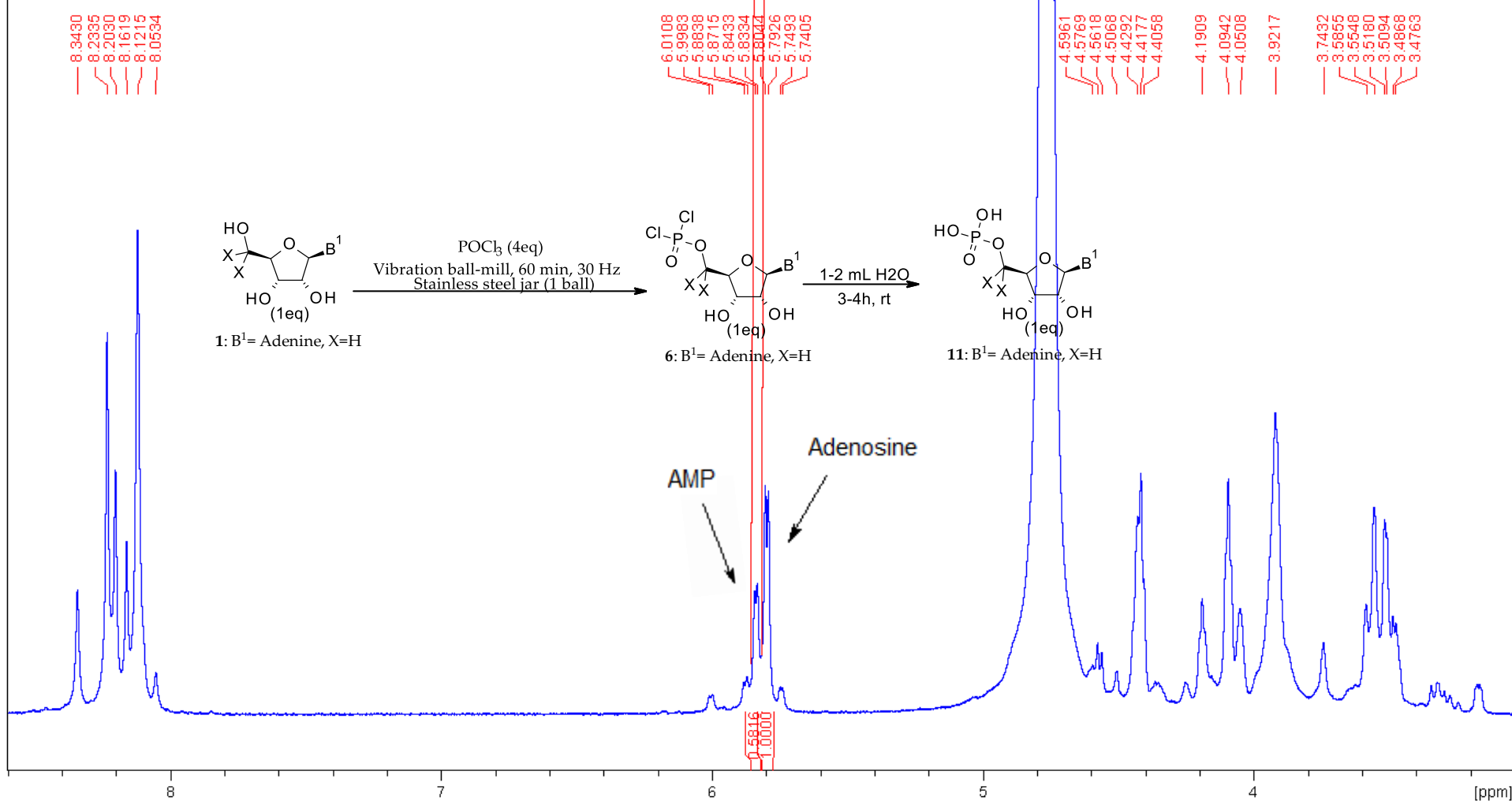
\* Correspondence: [mmigaud@southalabama.edu](mailto:mmigaud@southalabama.edu);

ADENOSINE-POCl<sub>3</sub>-30MIN BALLMILLING  
 PROTON D<sub>2</sub>O {C:\Bruker\TopSpin3.5pl6} FH 2

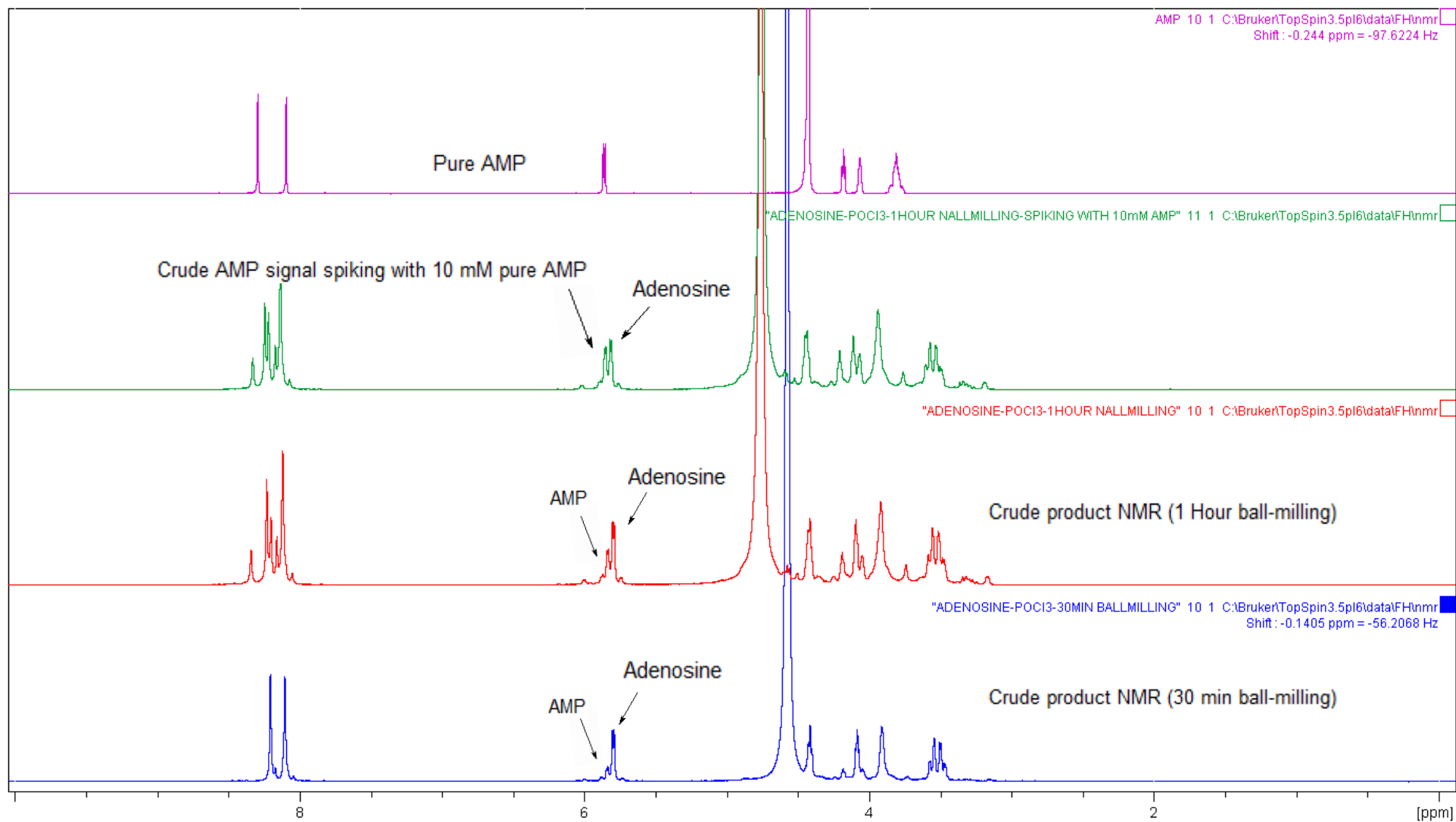


**Compound 11:** <sup>1</sup>H-NMR of crude product after 30 minutes of ball-milling (D<sub>2</sub>O)

PROTON D2O {C:\Bruker\TopSpin3.5pl6} FH 3

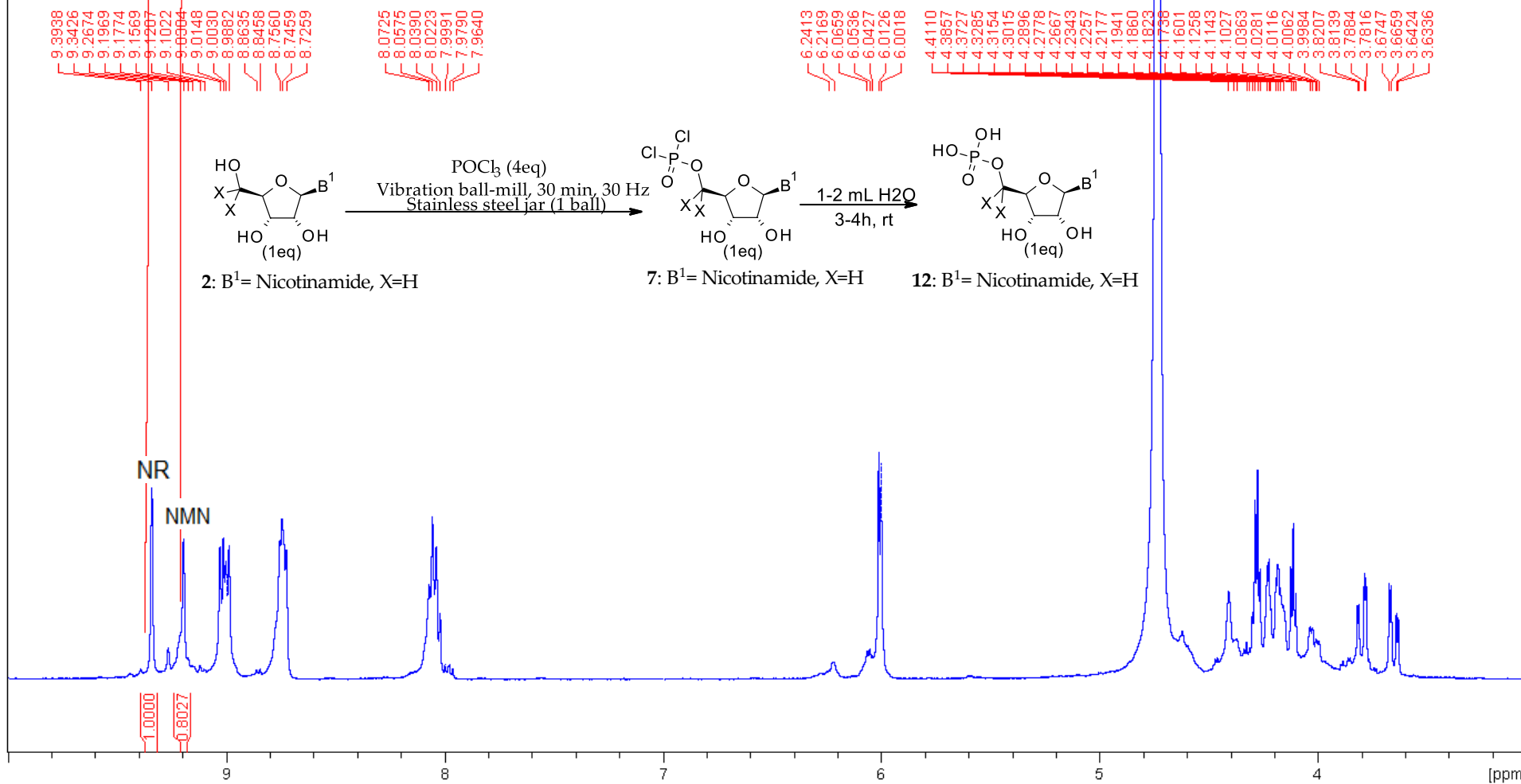


Compound 11: <sup>1</sup>H-NMR of crude product after 1 hour of ball-milling (D<sub>2</sub>O)



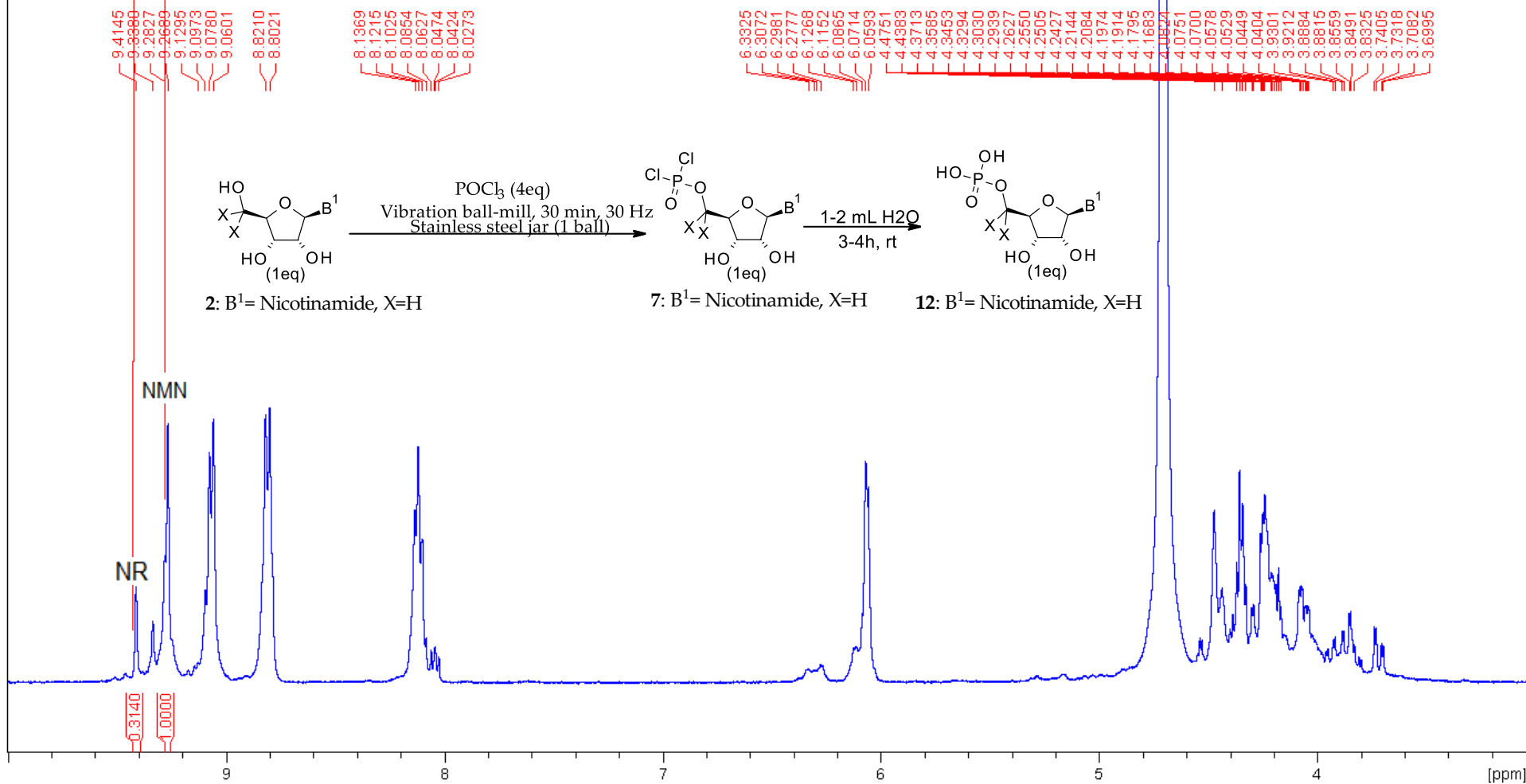
**Compound 11:** AMP Signals from a one-hour ball-milling crude product spiked with 10 mM pure AMP ( $\text{D}_2\text{O}$ )

NR-POCl3-30MIN BALLMILLING  
 PROTON D2O {C:\Bruker\TopSpin3.5\pl6} FH 4

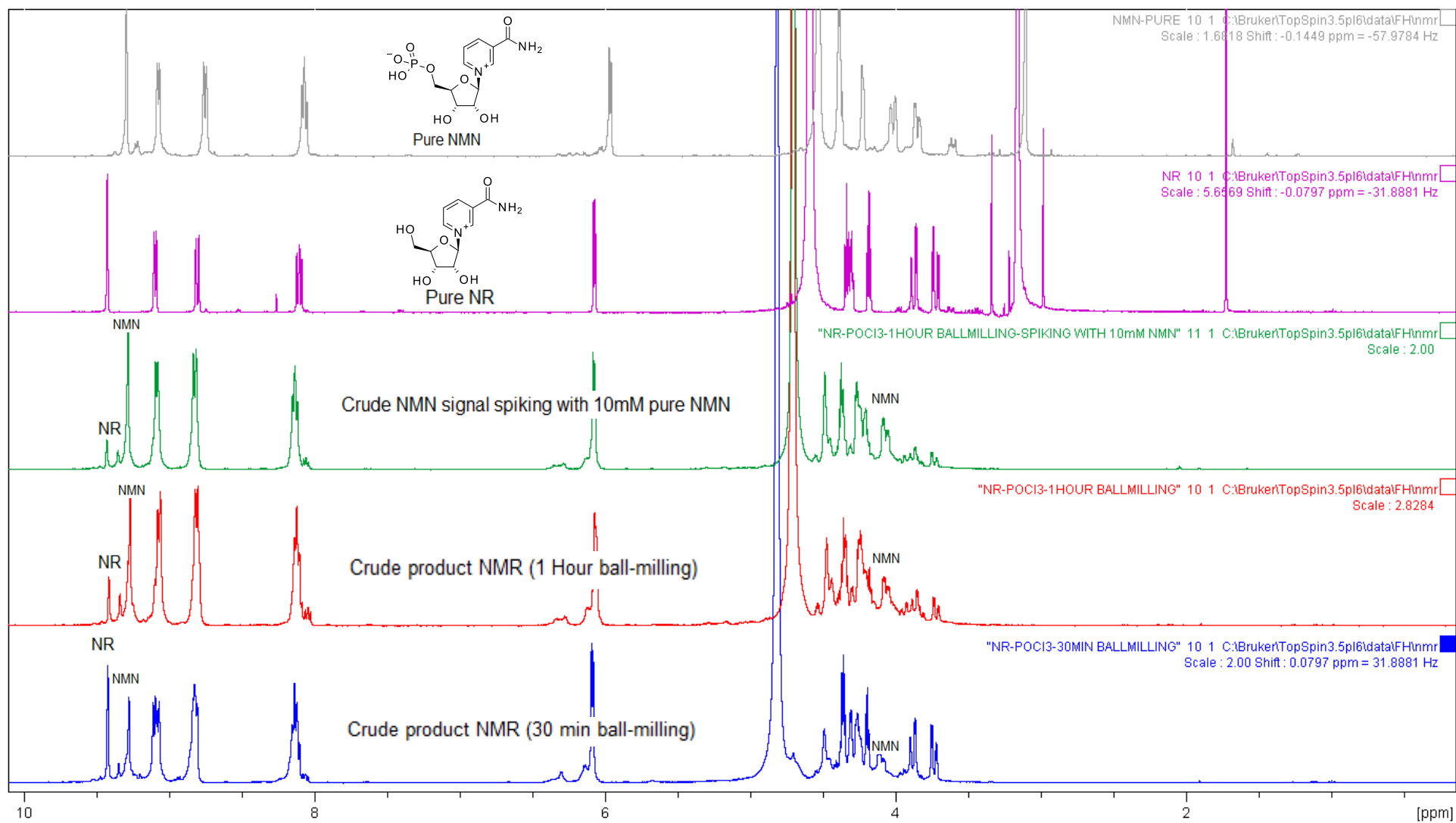


**Compound 12:** <sup>1</sup>H-NMR of crude product after 30 minutes of ball-milling (D<sub>2</sub>O)

NR-POCl<sub>3</sub>-1HOUR BALLMILLING  
 PROTON D2O {C:\Bruker\TopSpin3.5pl6} FH 5

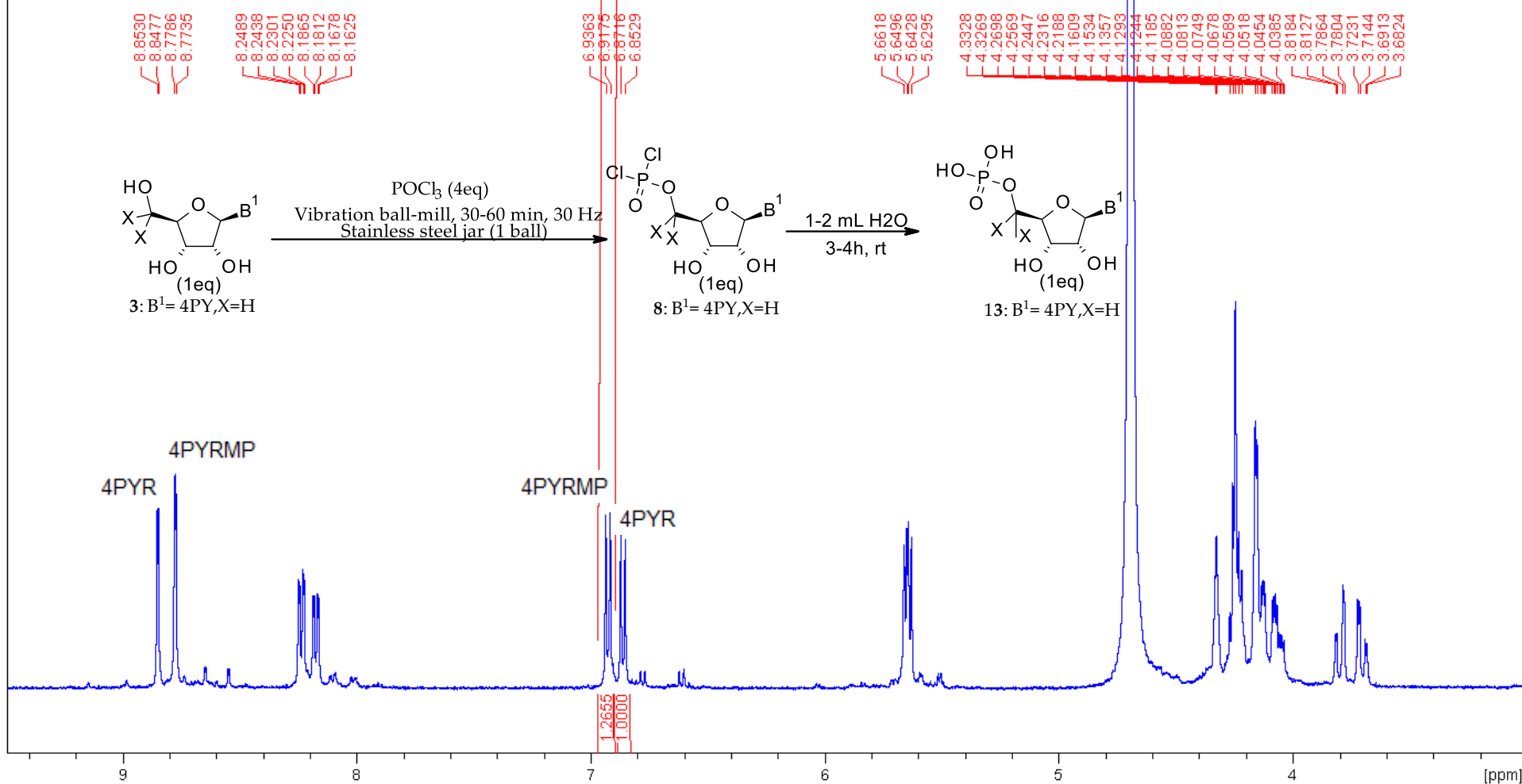


Compound 12: <sup>1</sup>H-NMR of crude product after 1 hour of ball-milling (D<sub>2</sub>O)



**Compound 12:** NMN Signals from a one-hour ball-milling crude product spiked with 10 mM pure NMN (D<sub>2</sub>O)

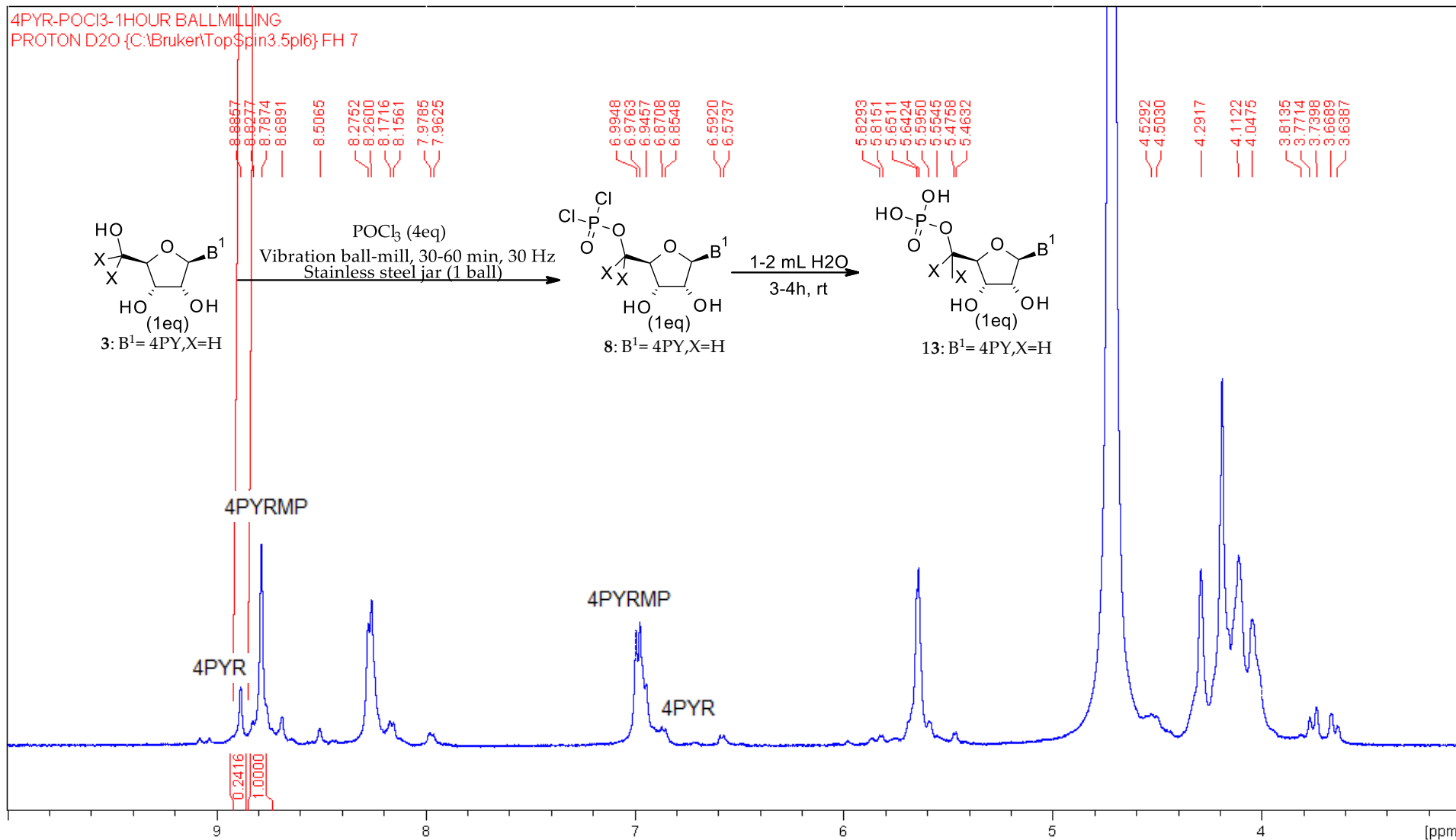
4PYR-POCl<sub>3</sub>-30MIN BALLMILLING  
 PROTON D2O {C:\Bruker\TopSpin3.5pl6} FH 6



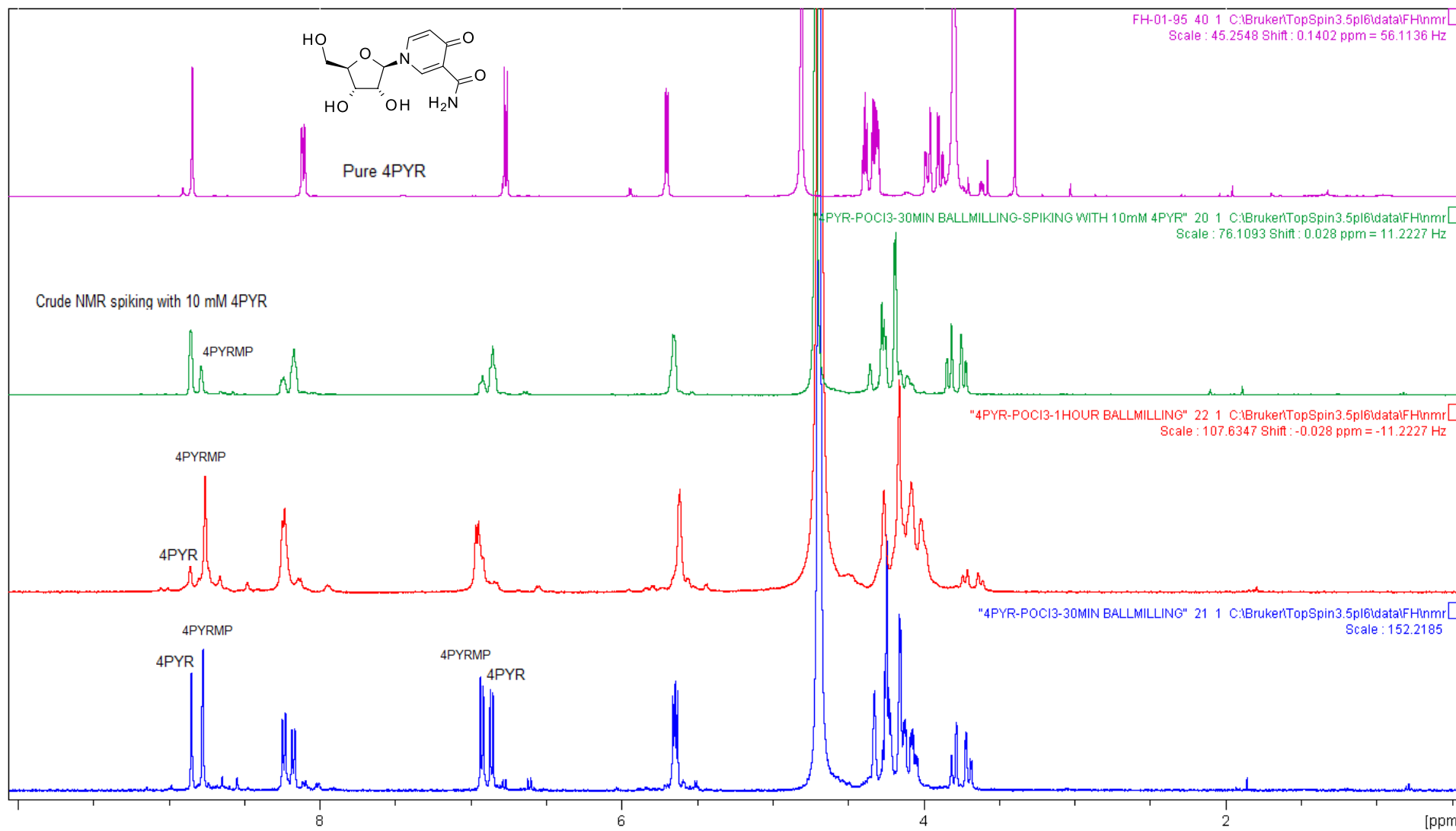
**Compound 13:** <sup>1</sup>H-NMR of crude product after 30 minutes of ball-milling (D<sub>2</sub>O)



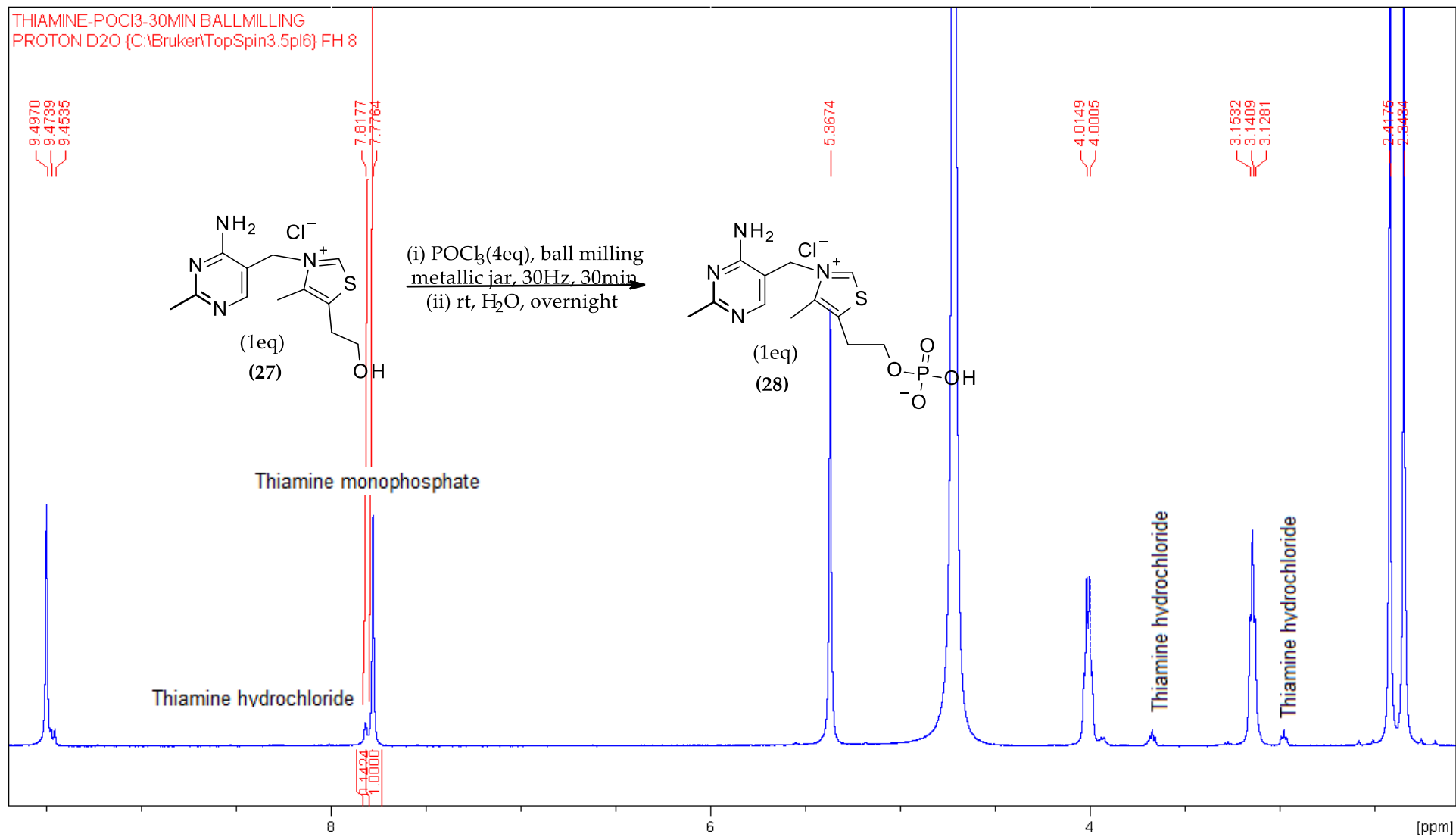
4PYR-POCl<sub>3</sub>-1HOUR BALLMILLING  
 PROTON D<sub>2</sub>O {C:\Bruker\TopSpin3.5pl6} FH 7



**Compound 13:** <sup>1</sup>H-NMR of crude product after 1 hour of ball-milling (D<sub>2</sub>O)

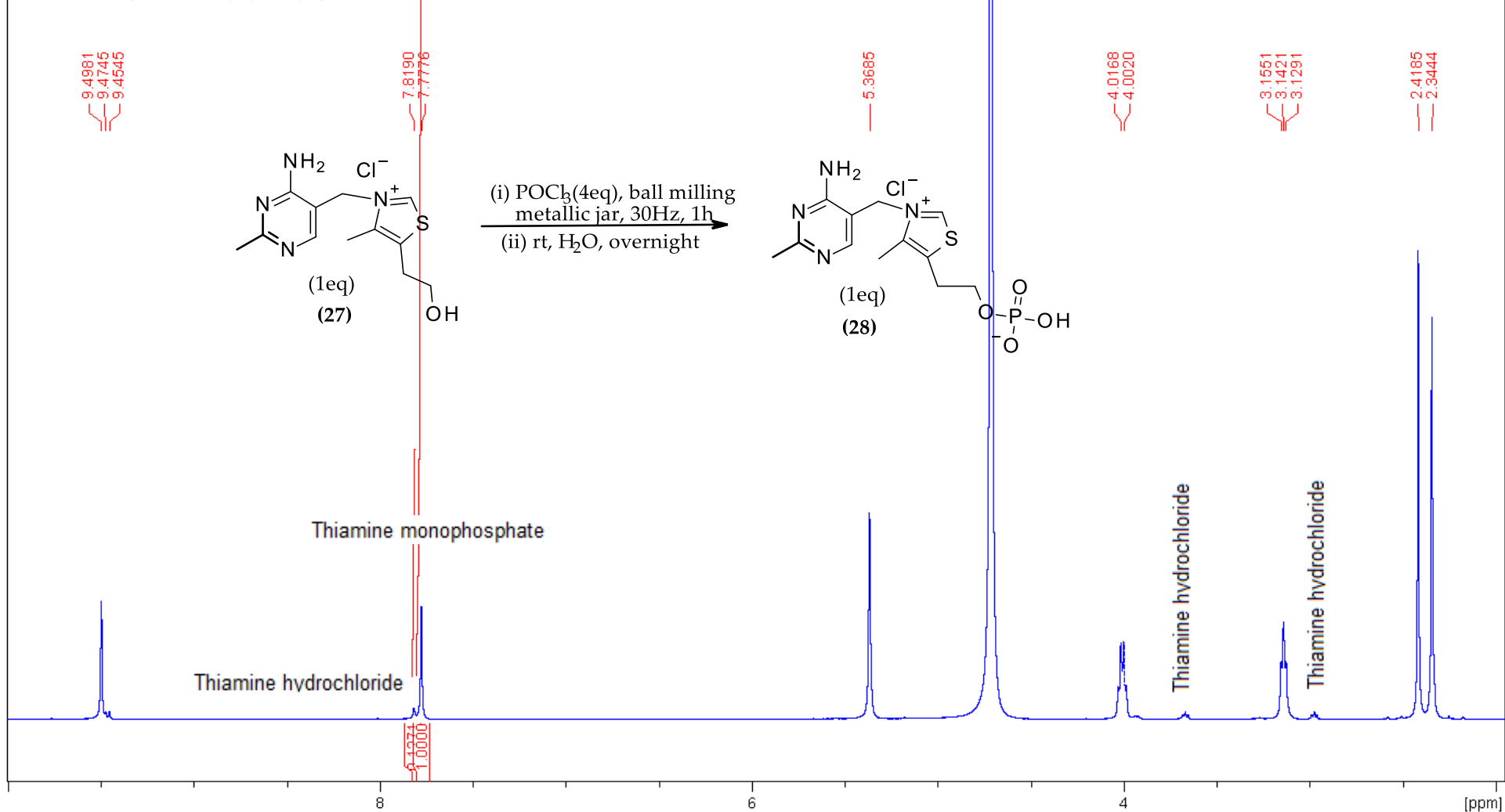


**Compound 13:** 4PYR Signals from a one-hour ball-milling crude product spiked with 10 mM pure 4PYR ( $\text{D}_2\text{O}$ )

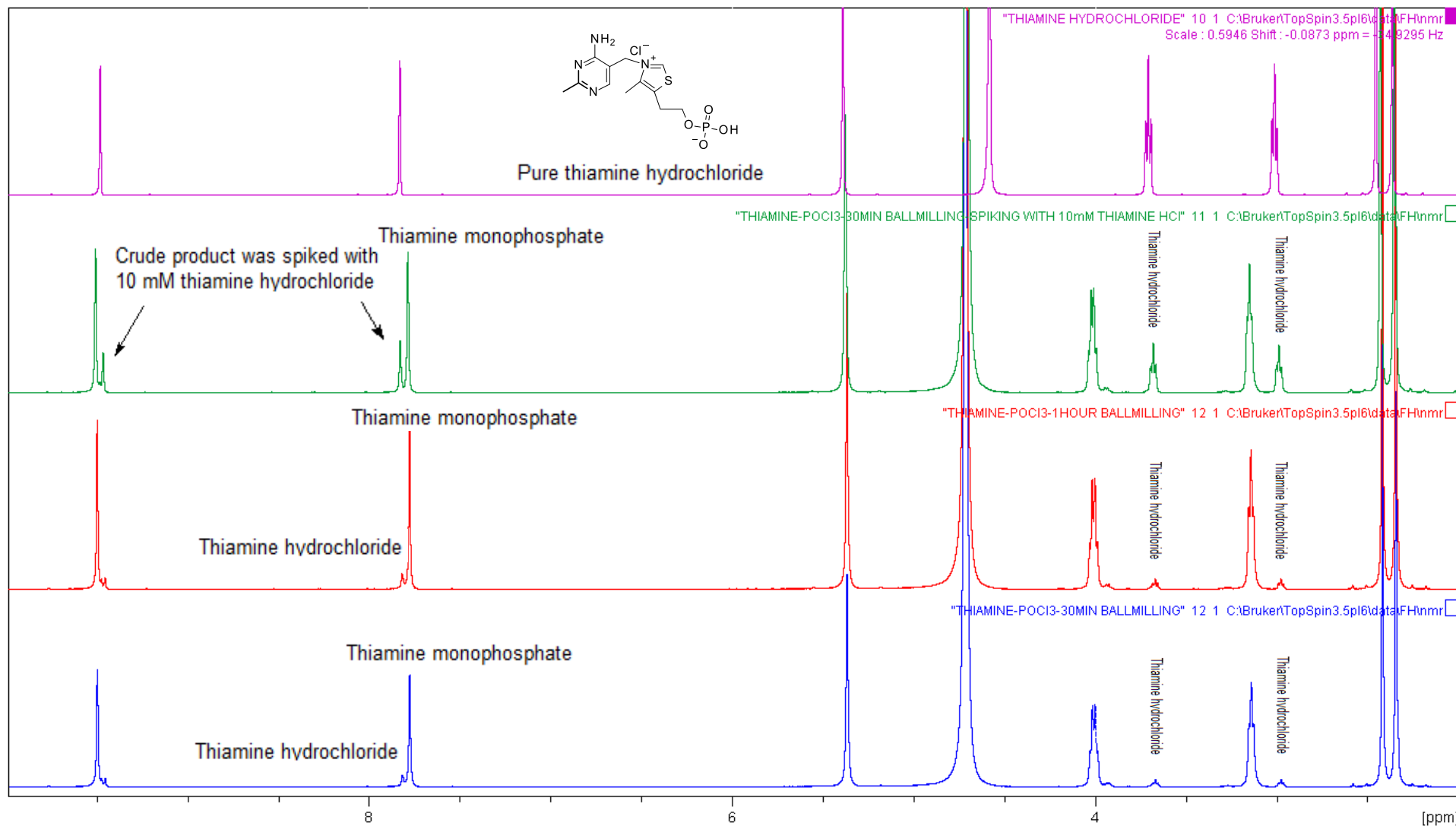


Compound 28: <sup>1</sup>H-NMR of crude product after 30 minutes of ball-milling (D<sub>2</sub>O)

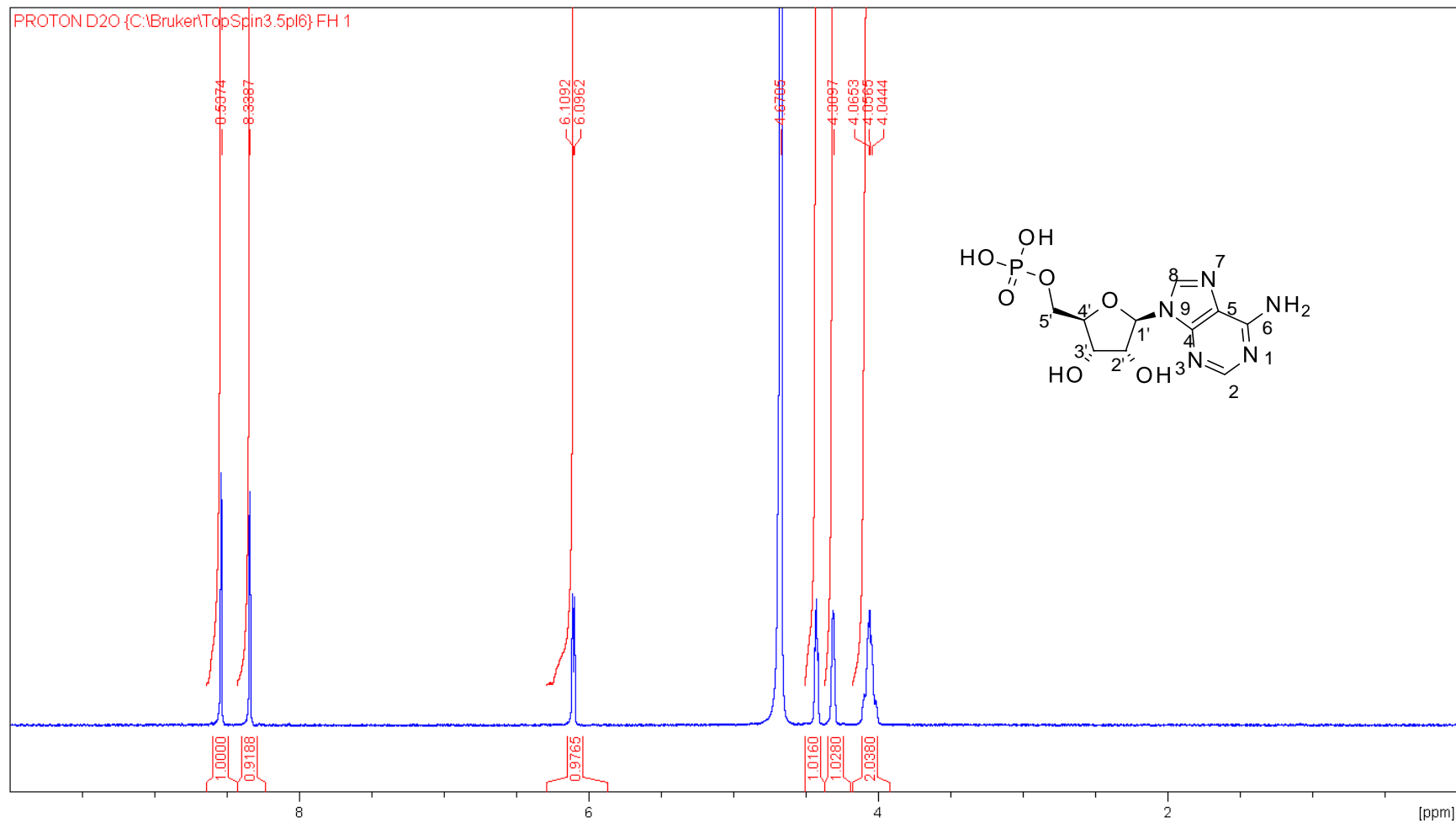
THIAMINE-POCl<sub>3</sub>-1HOUR BALLMILLING  
 PROTON D<sub>2</sub>O {C:\Bruker\TopSpin3.5\pl6} FH 9



Compound 28: <sup>1</sup>H-NMR of crude product after 1 hour of ball-milling (D<sub>2</sub>O)



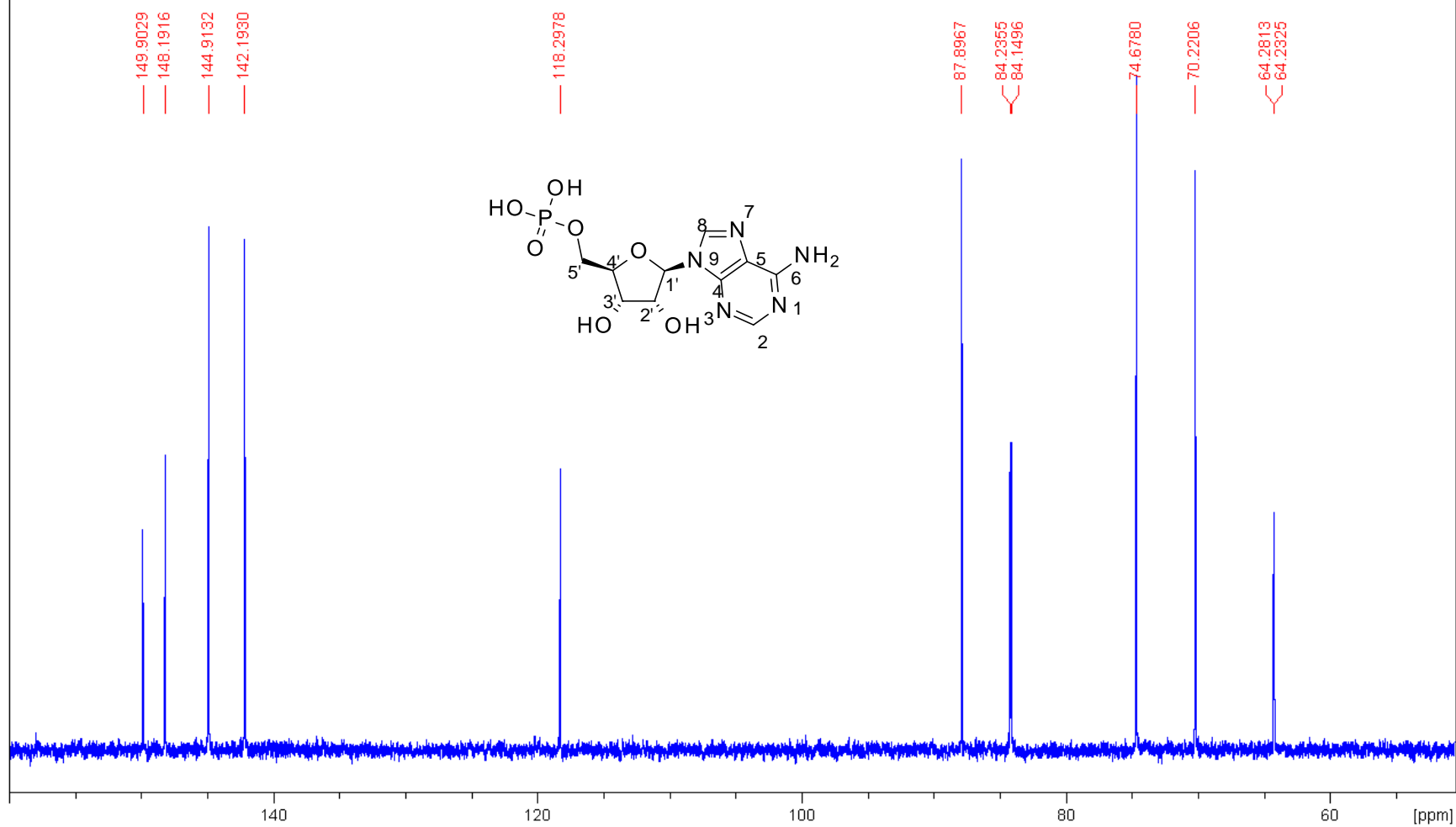
**Compound 28:** Thiamine Signals from a one-hour ball-milling crude product spiked with 10 mM pure thiamine hydrochloride ( $\text{D}_2\text{O}$ )



**Compound 11.** 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

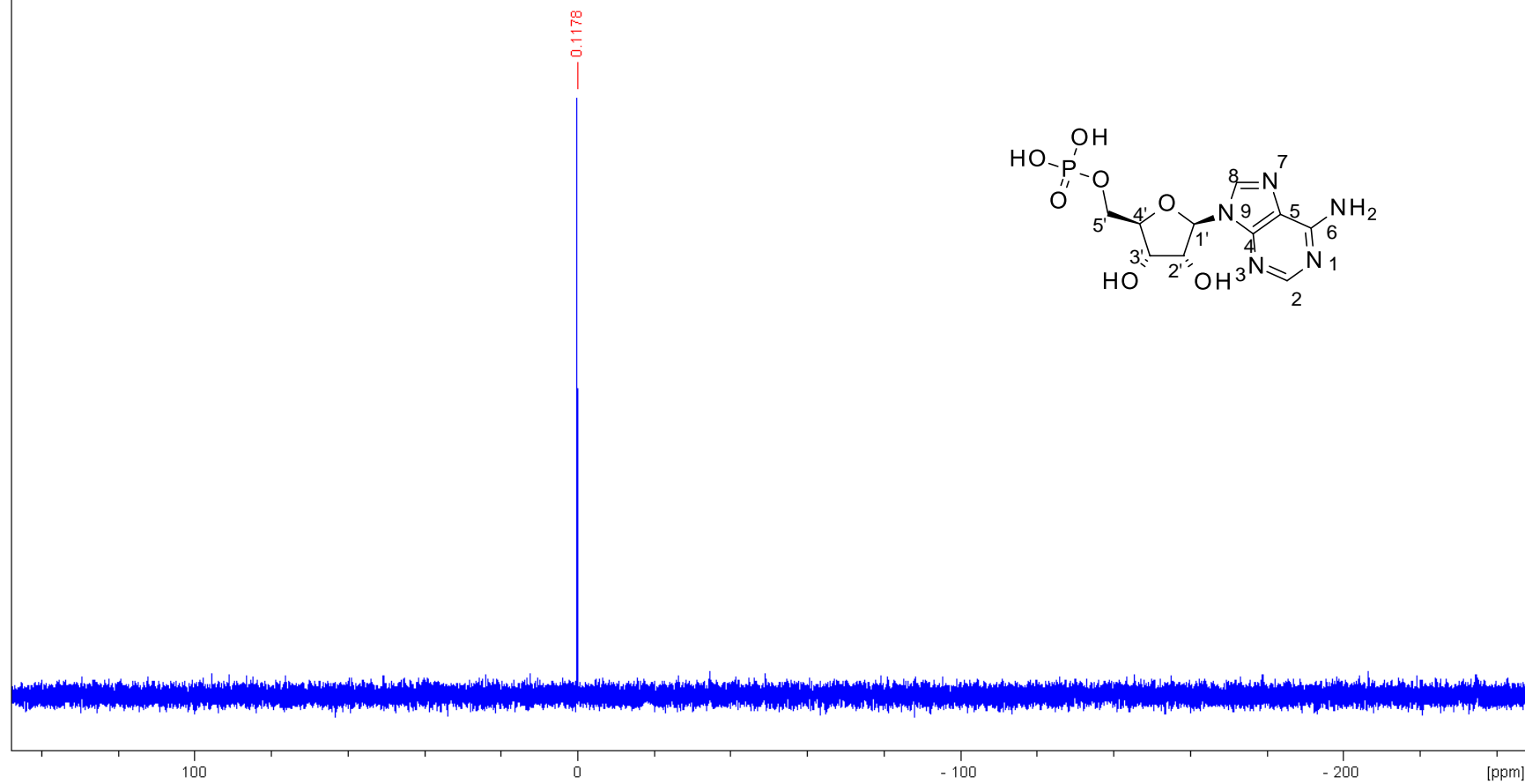
ADENOSINE MONOPHOSPHATE

C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 1



Compound 11. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$

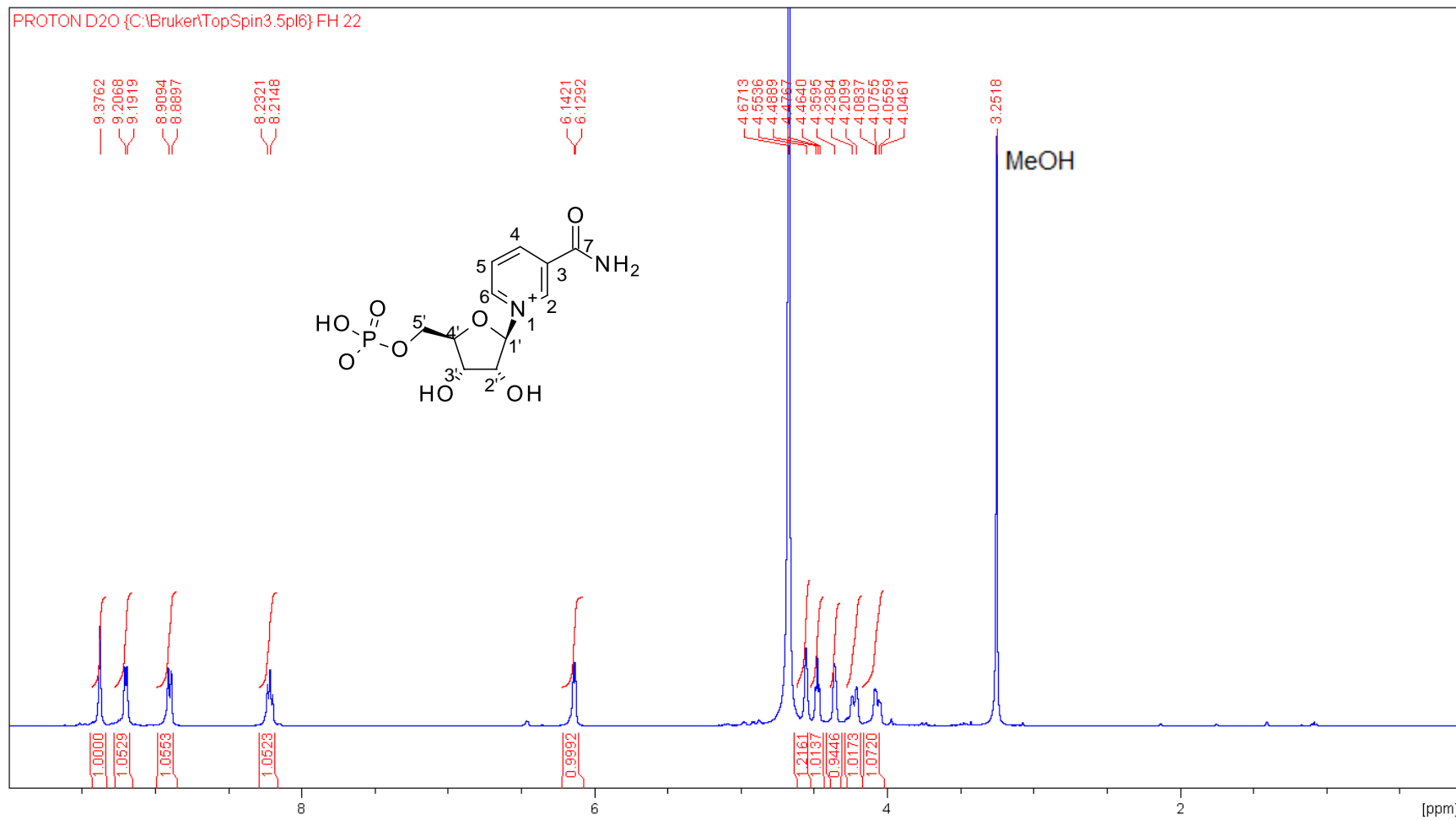
P31 D2O {C:\Bruker\TopSpin3.5\pl6} FH 1



**Compound 11.** 162 MHz  $^{31}\text{P}$ NMR spectrum in  $\text{D}_2\text{O}$

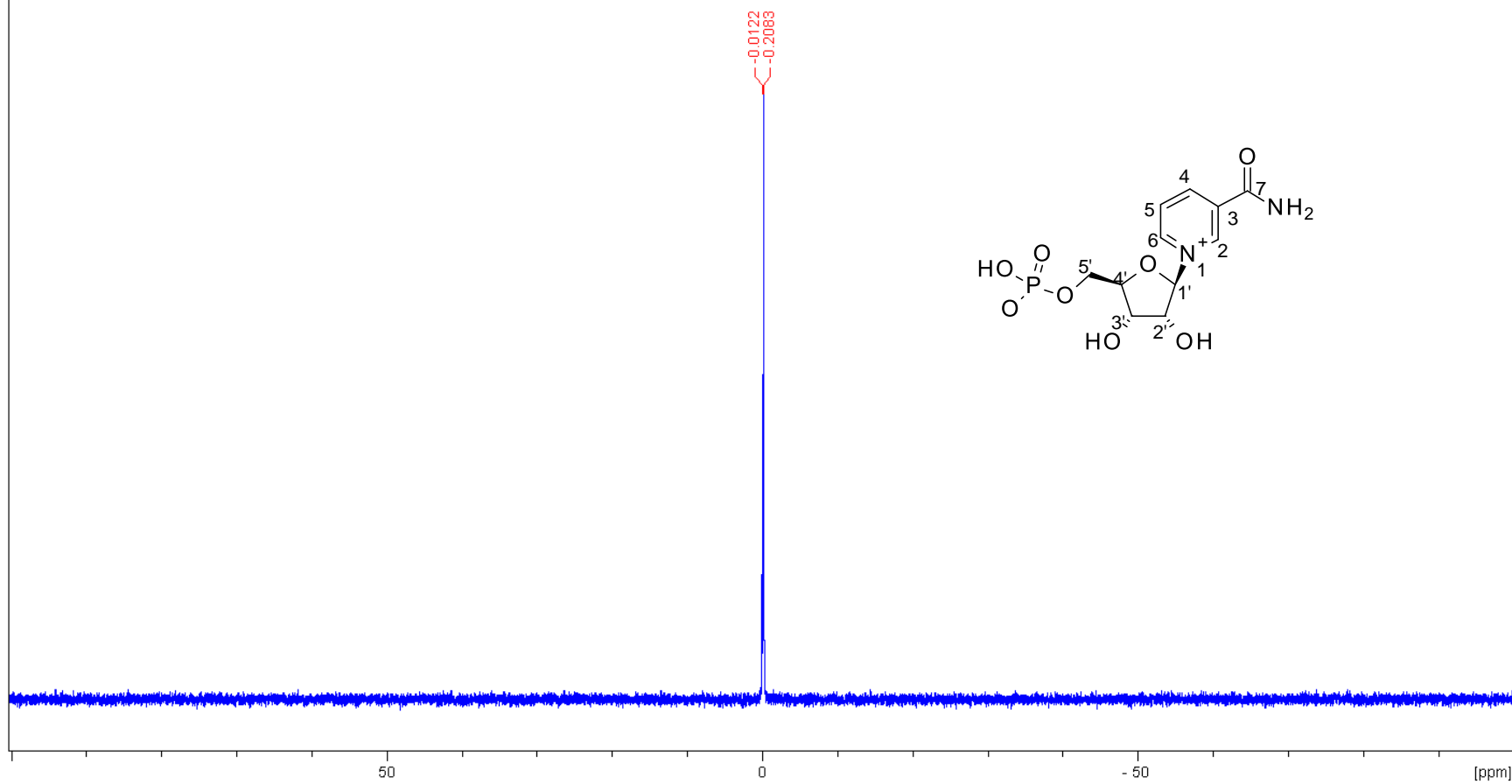


PROTON D2O {C:\Bruker\TopSpin3.5pl6} FH 22



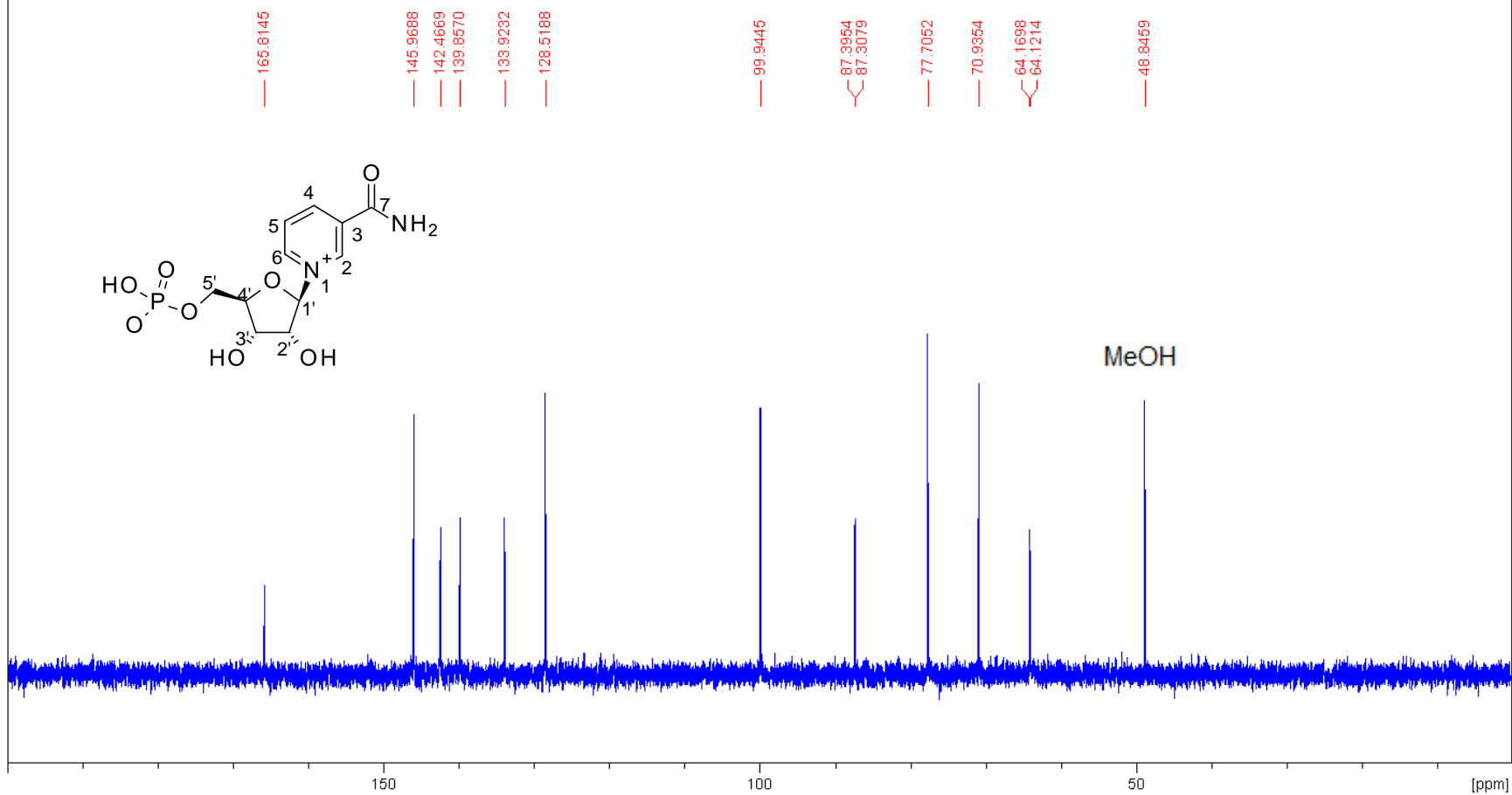
Compound 12. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

P31 D2O {C:\Bruker\TopSpin3.5pl6} FH 22

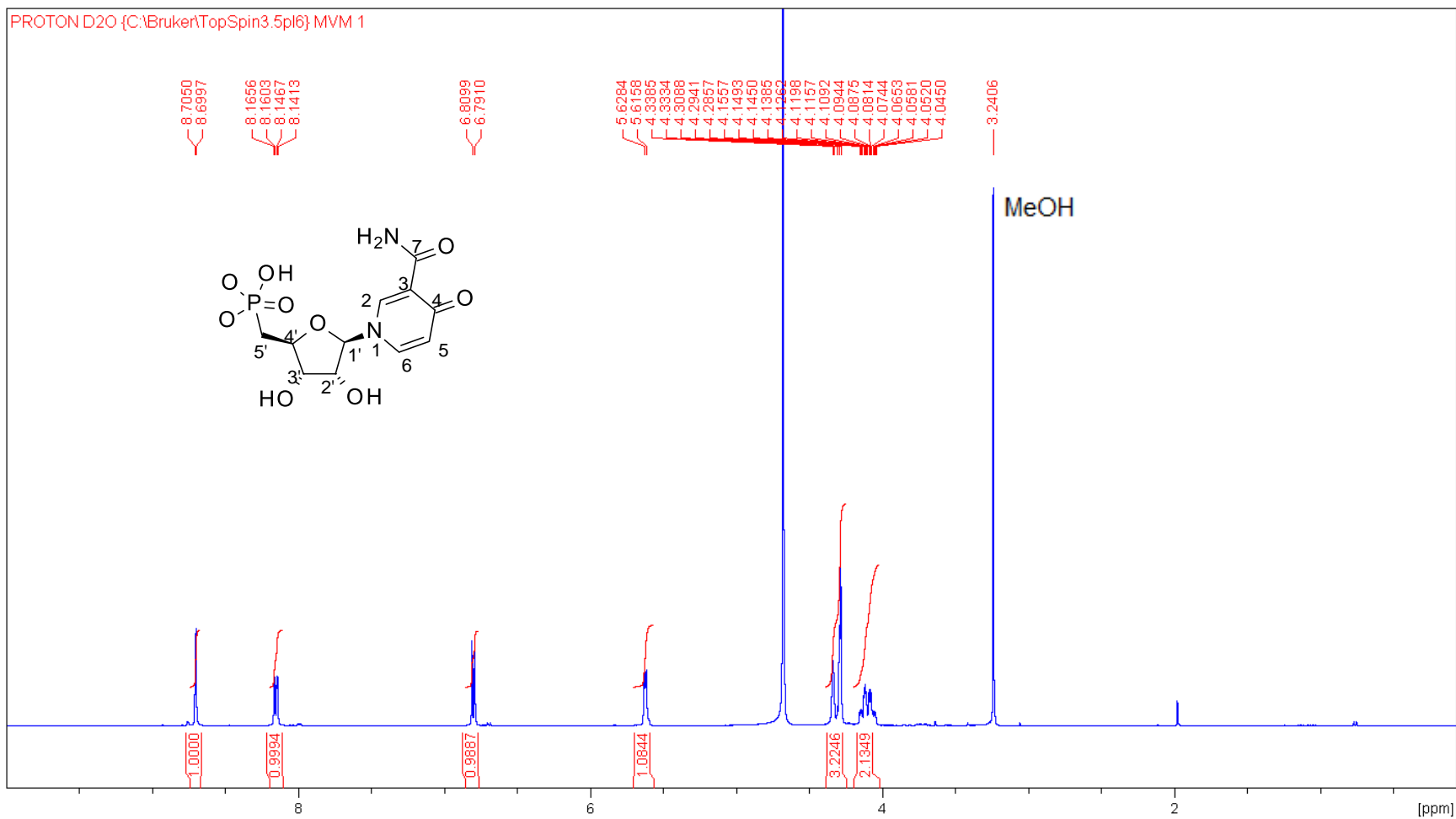


**Compound12.** 162 MHz  $^{31}\text{P}$ NMR spectrum in  $\text{D}_2\text{O}$

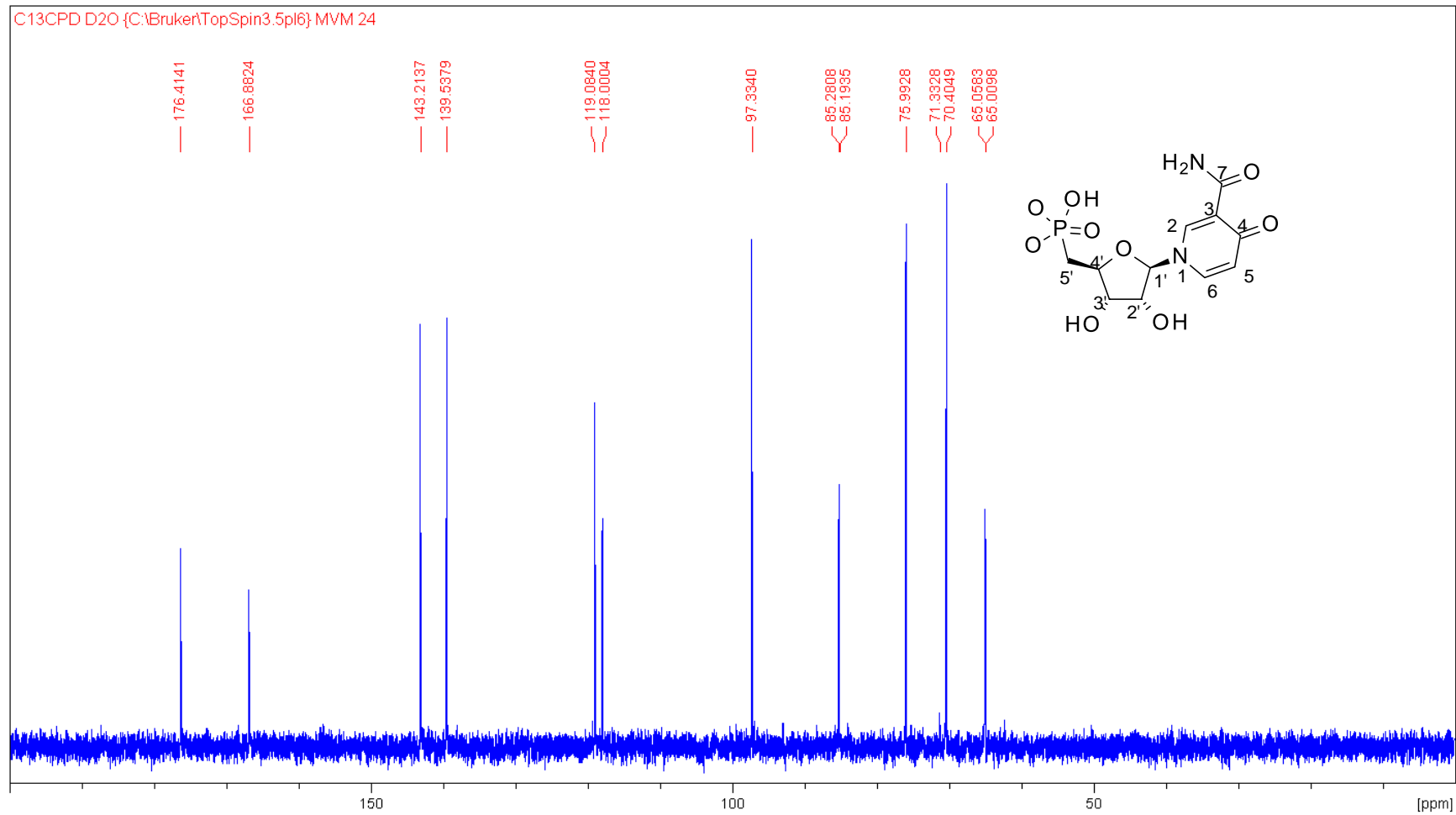
C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 22



Compound 12. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O



Compound 13. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

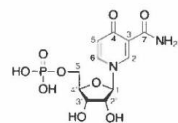


Compound 13. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

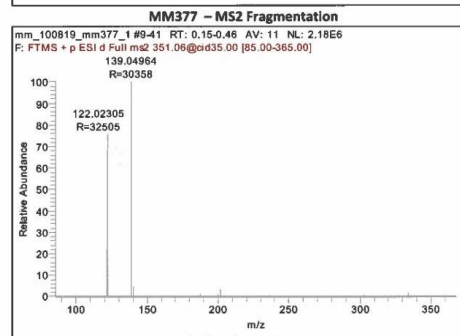
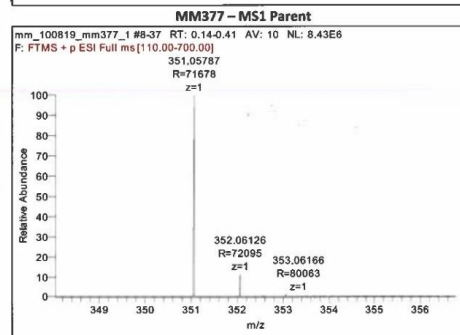
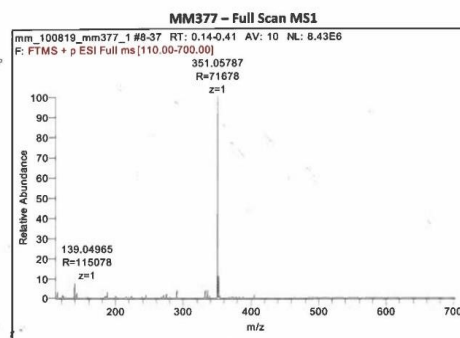
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} MVM 1



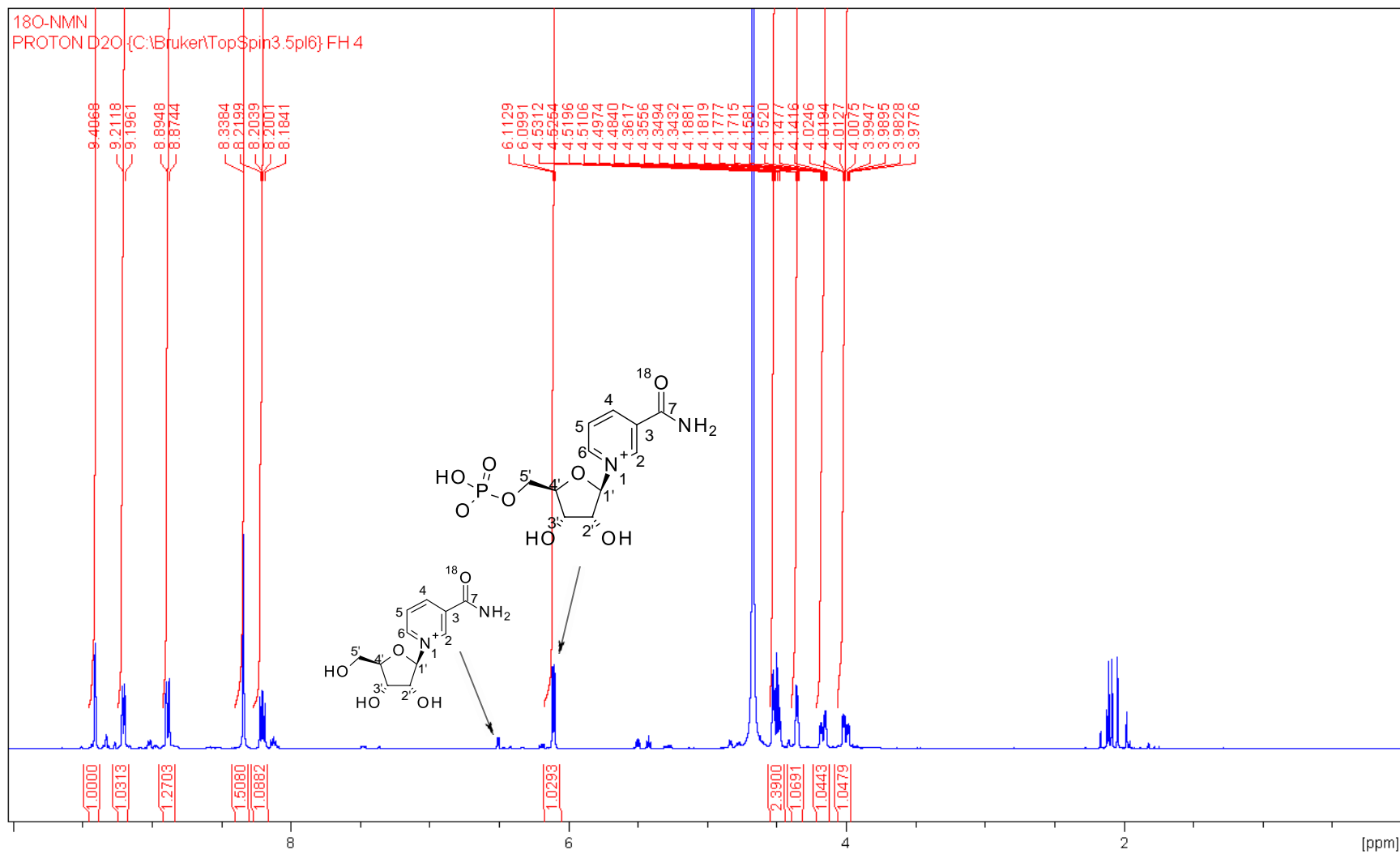
**Compound 13.** 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



Theoretical  $M/Z =$   
351.059793

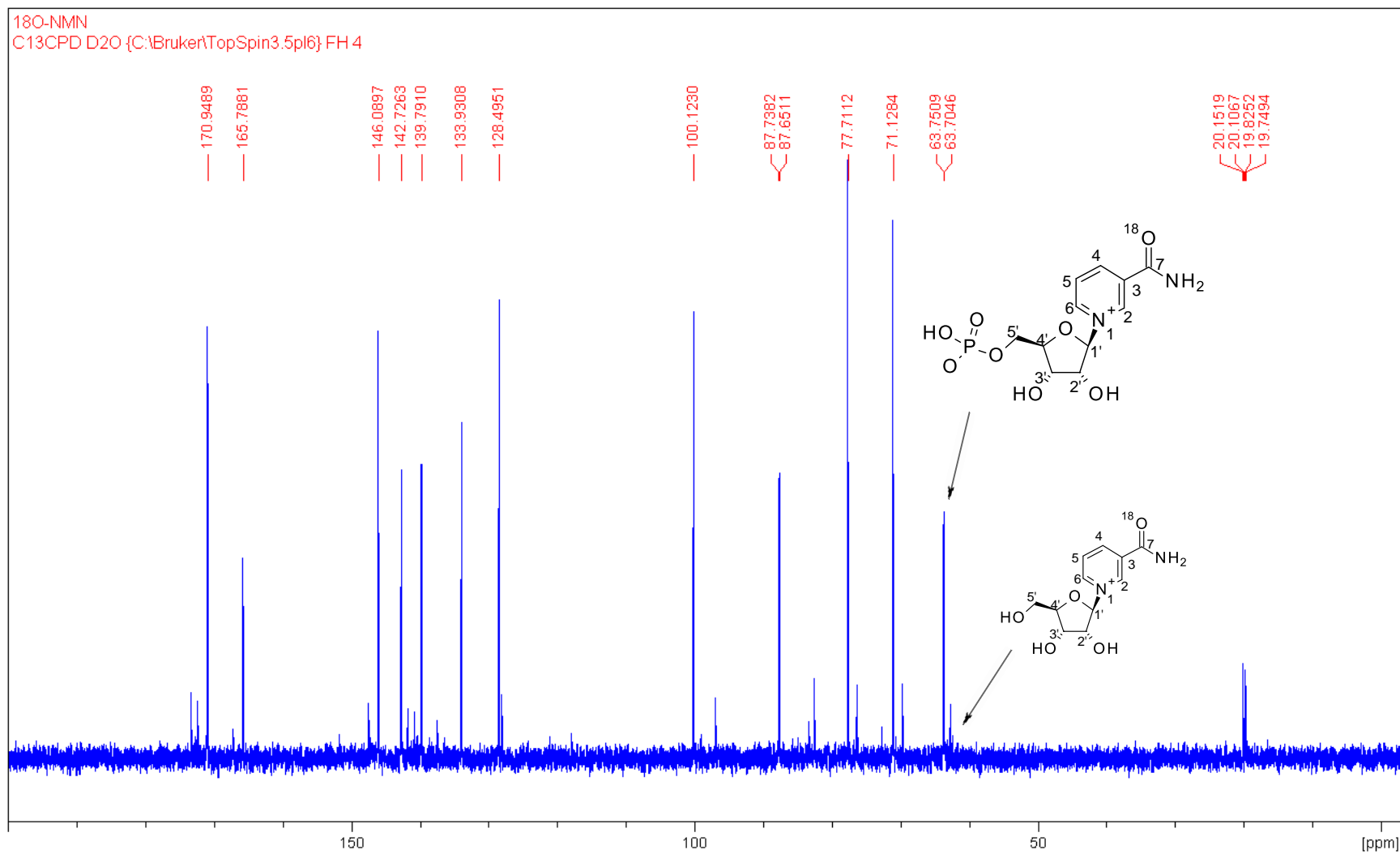


**Compound 13.** HRMS spectra



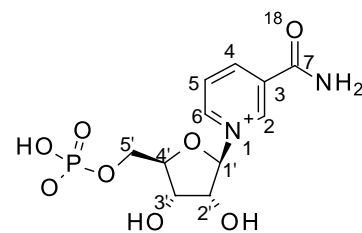
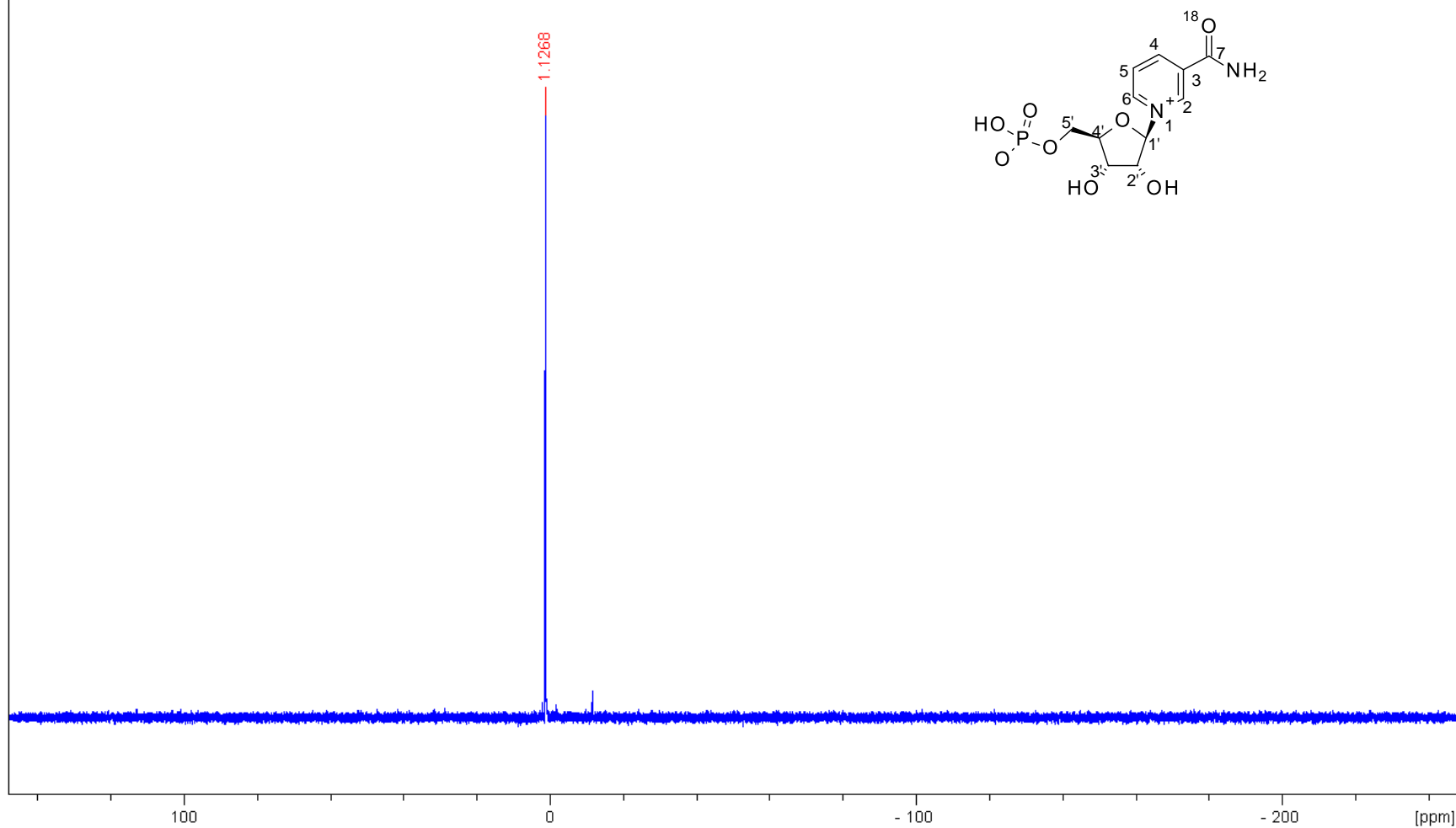
Compound 15. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$





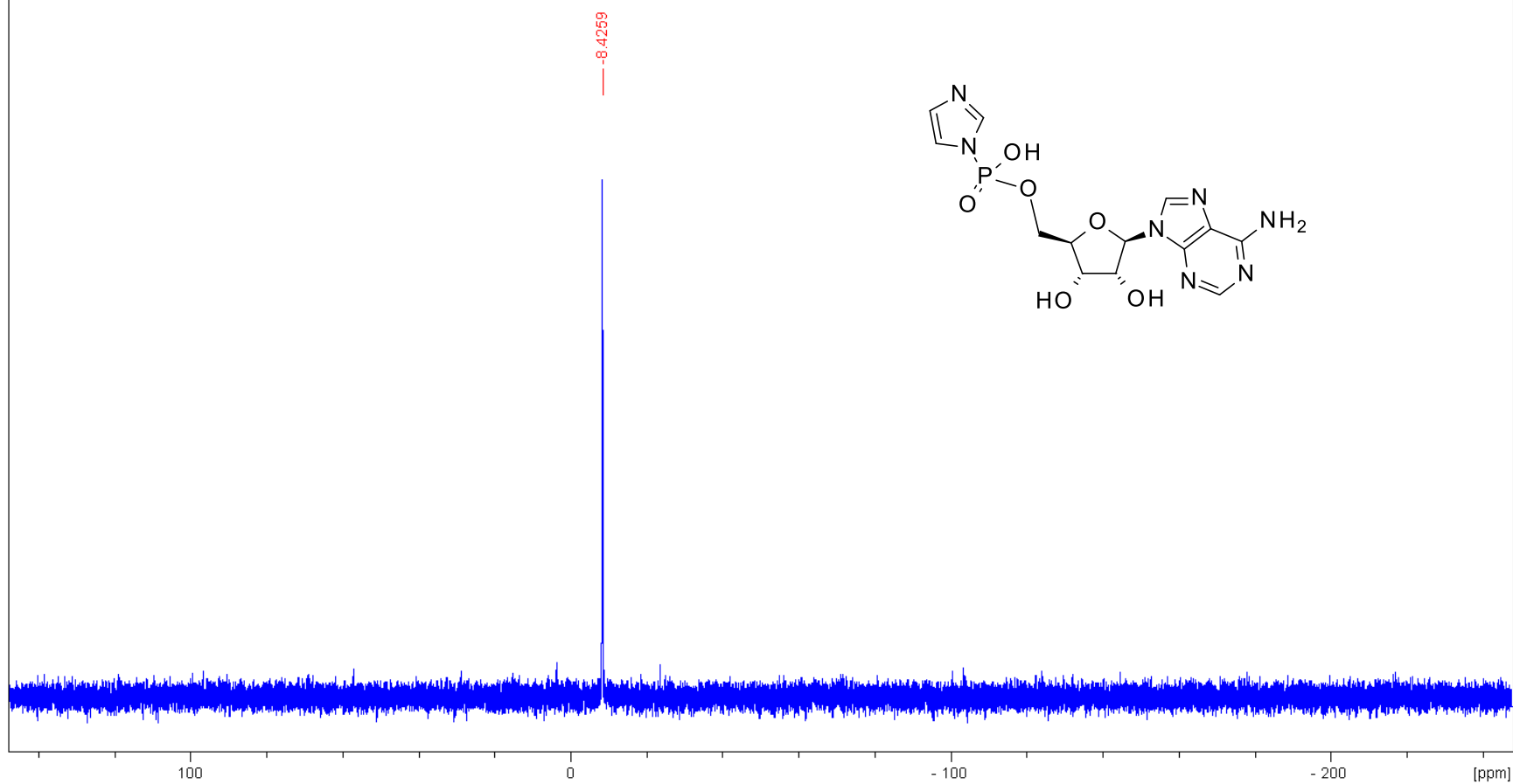
Compound 15. 100 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

18O-NMN  
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 4



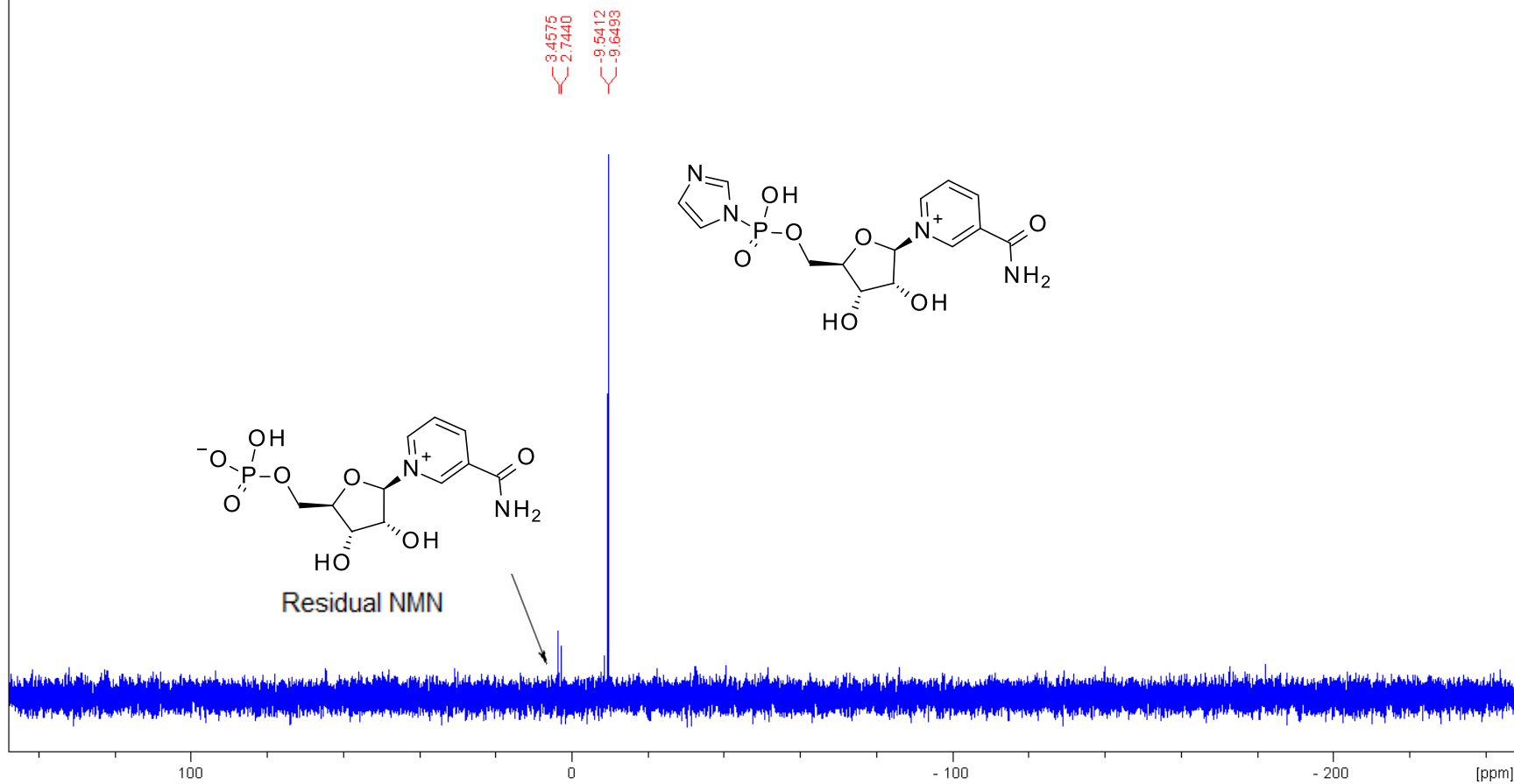
Compound 15. 162 MHz <sup>31</sup>P NMR spectrum in D<sub>2</sub>O

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 4



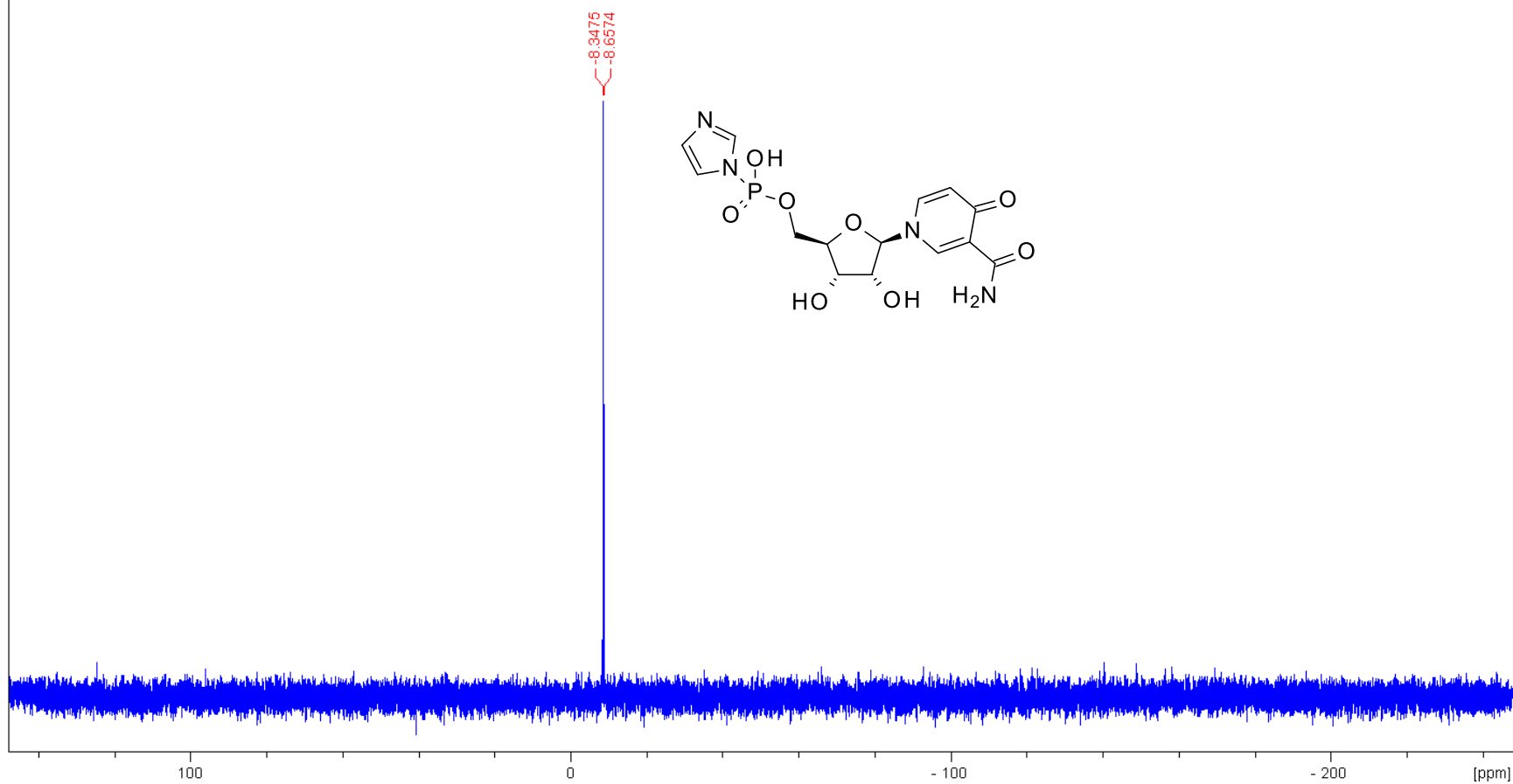
Compound 16. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 14



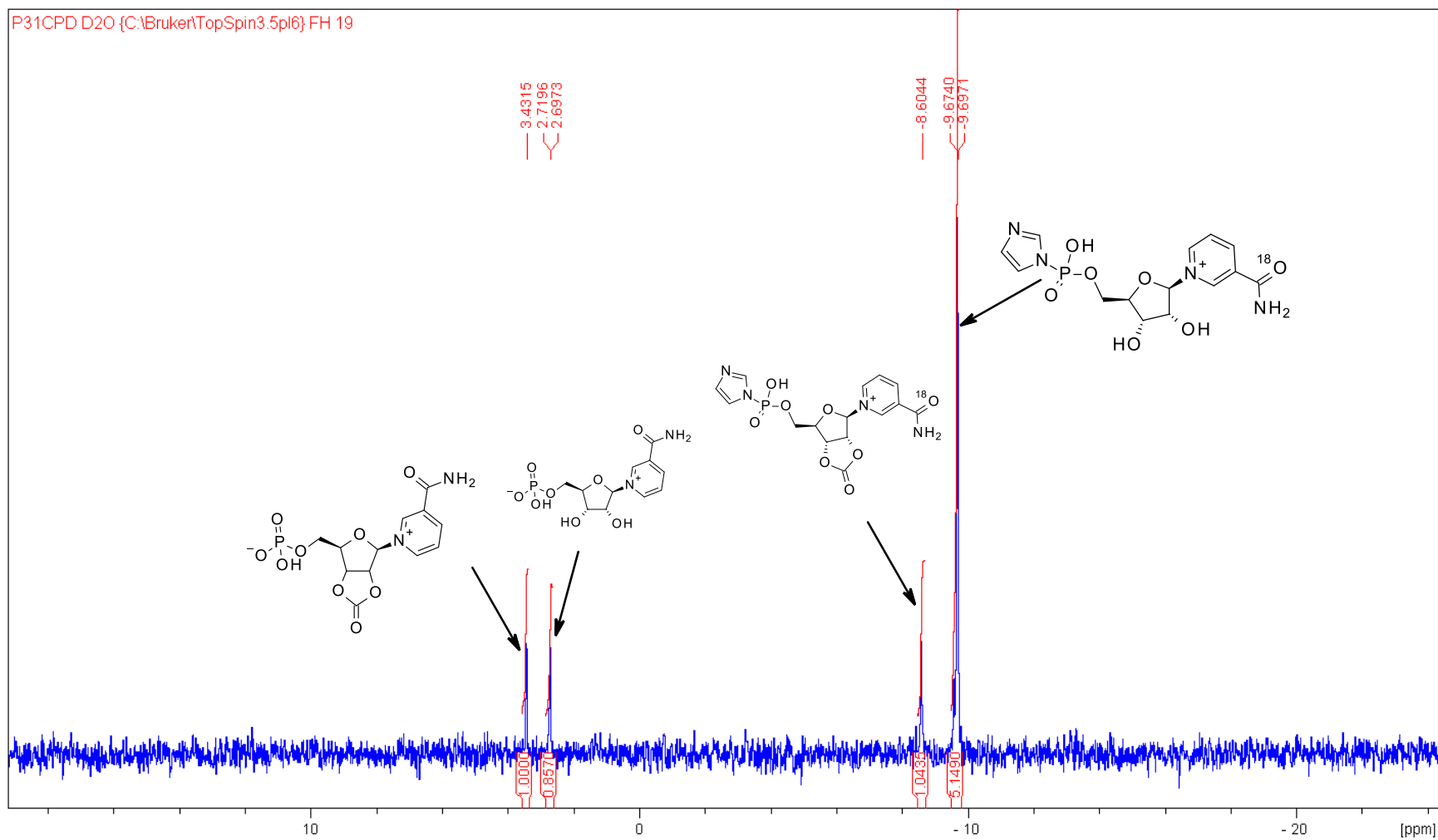
Compound 17. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 4

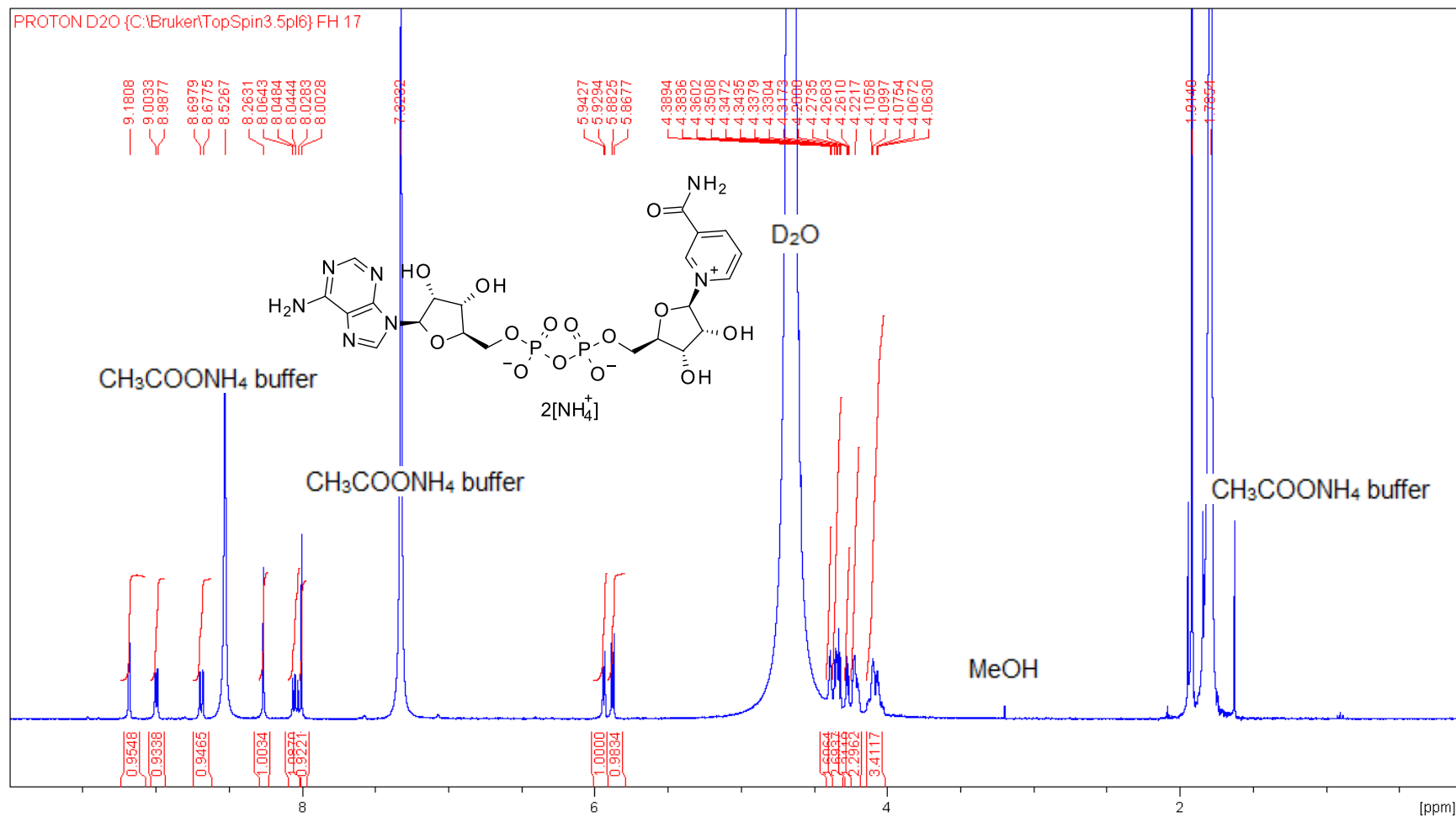


Compound 18. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

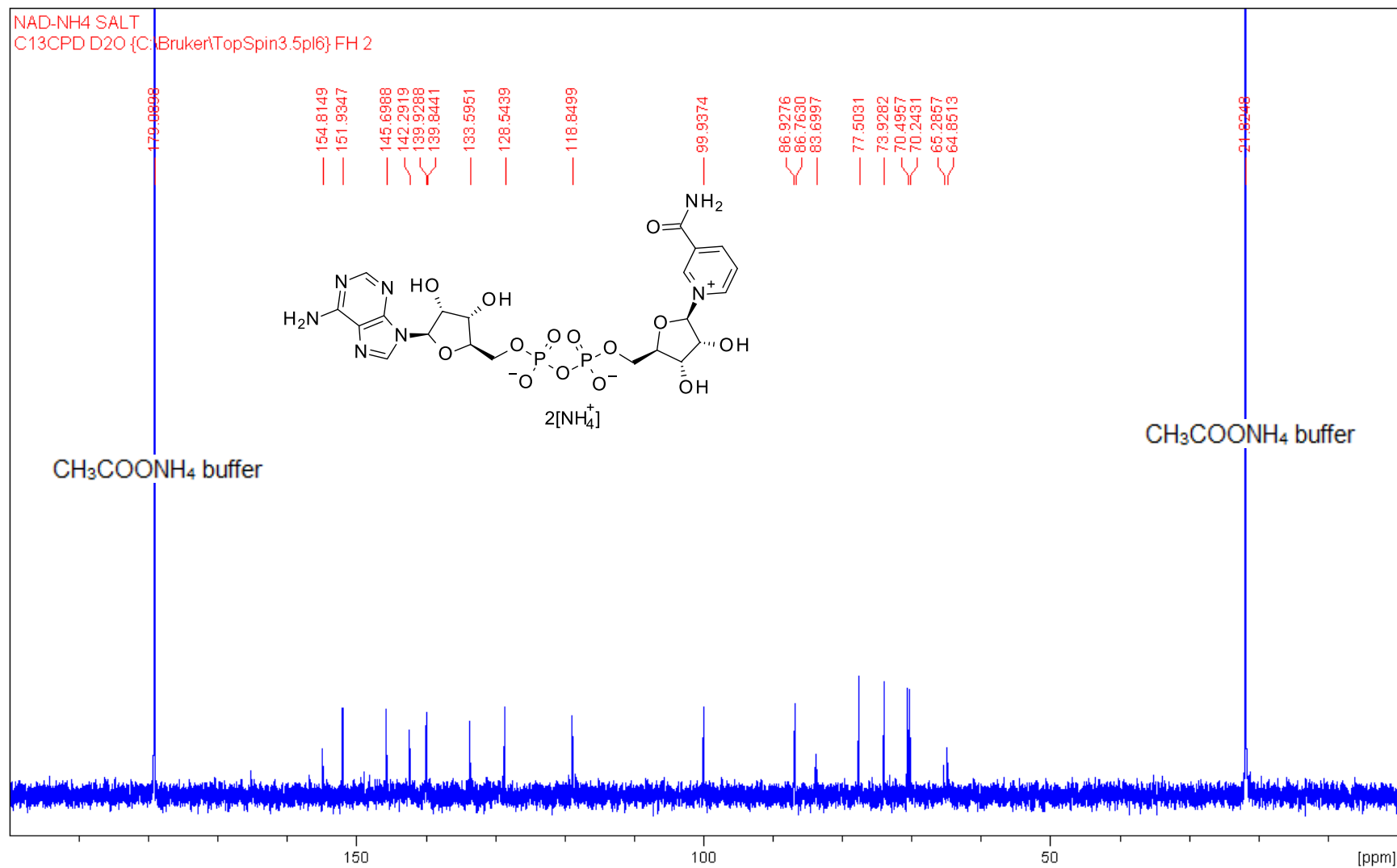
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 19



Compound 19. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



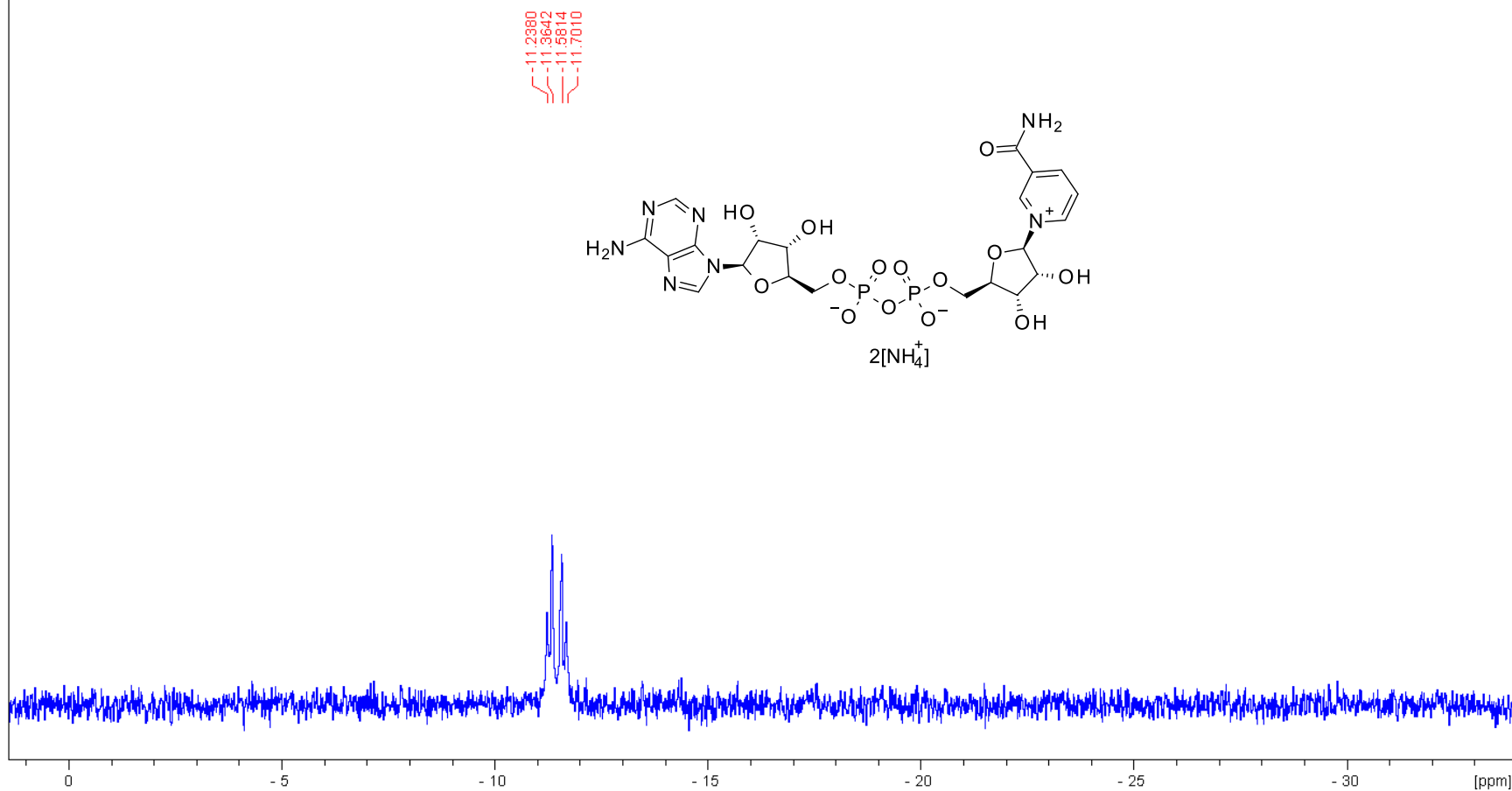
Compound 20. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O



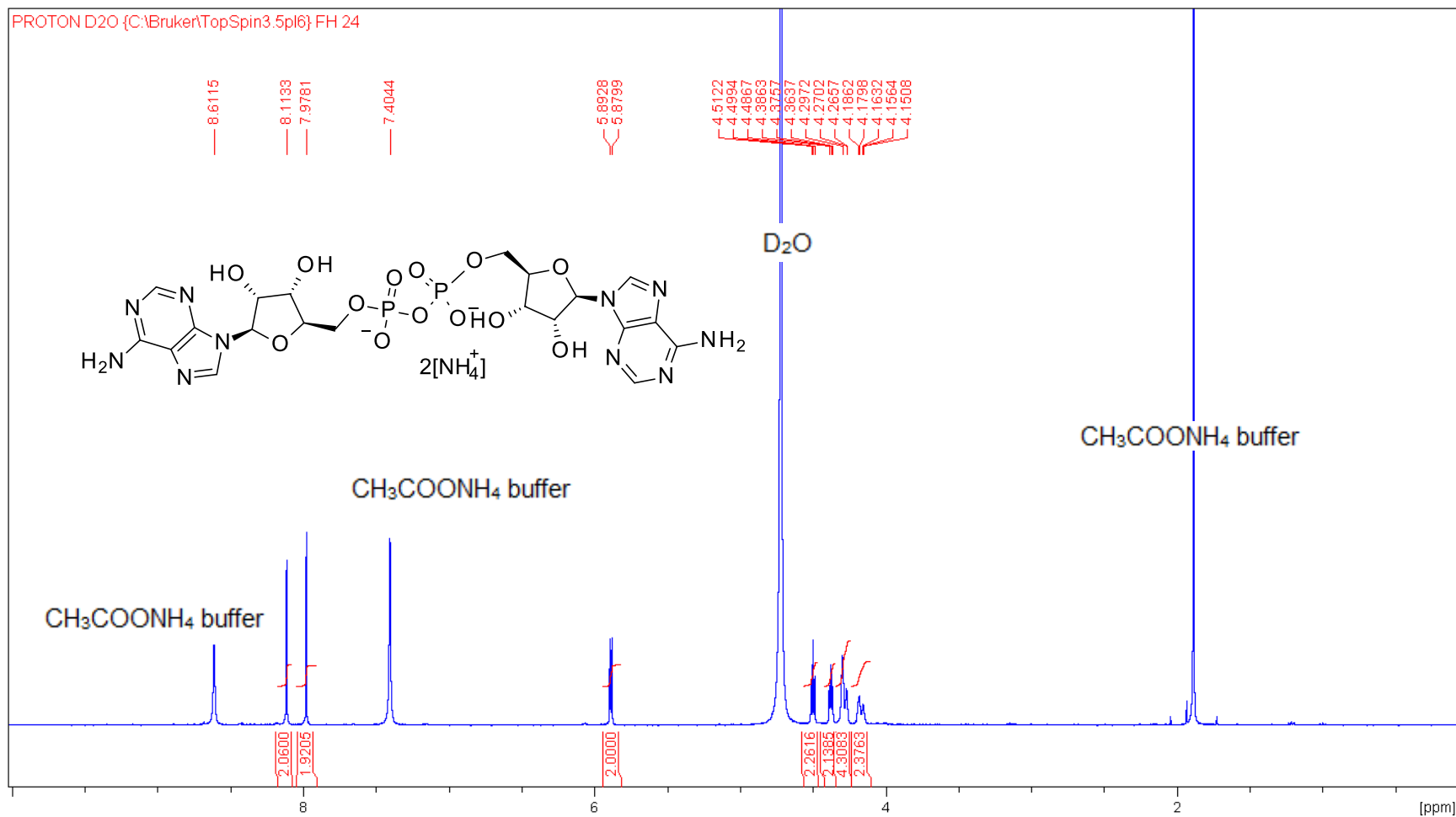
Compound 20. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O



P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 17

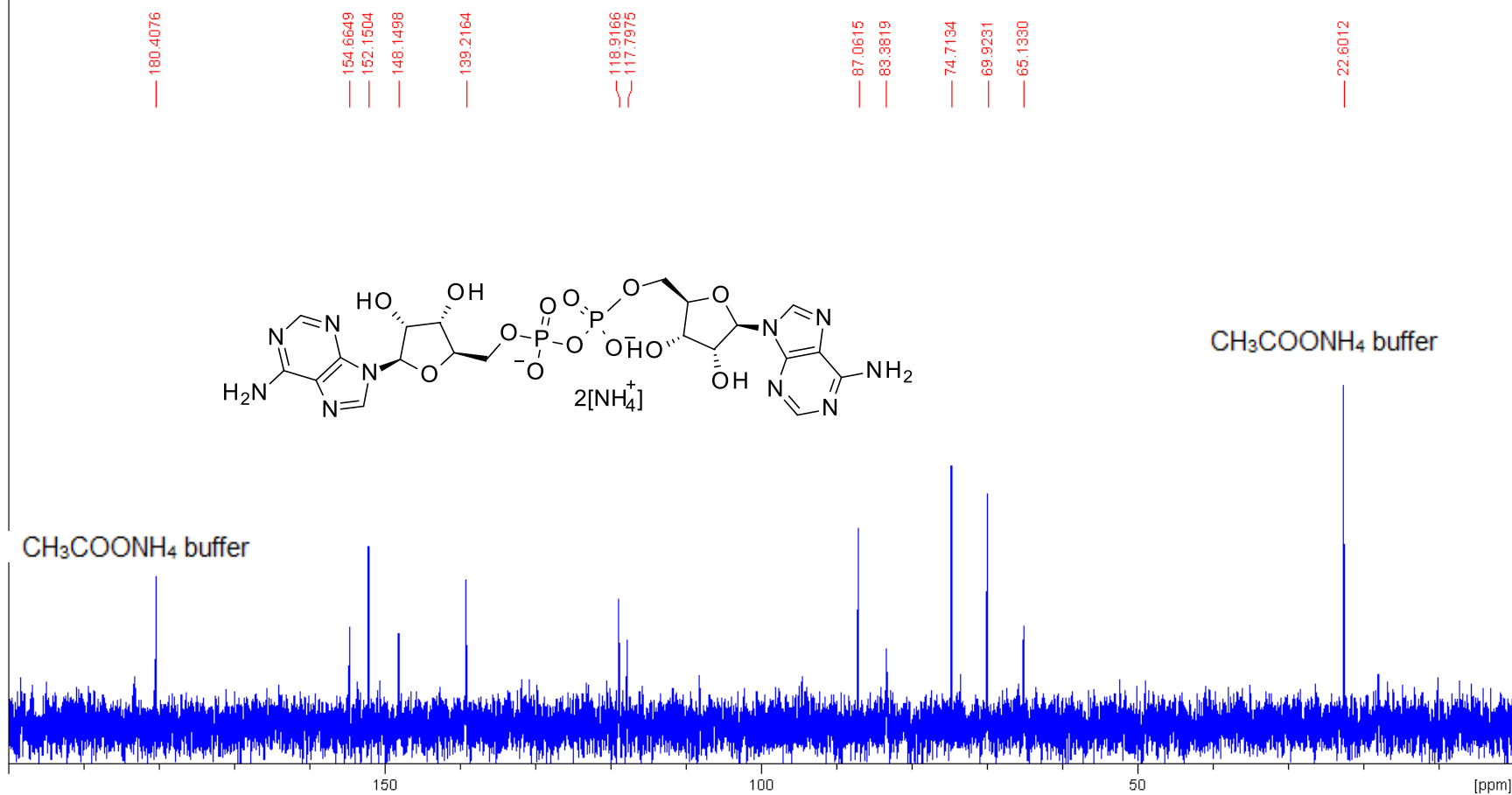


**Compound 20.** 162MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



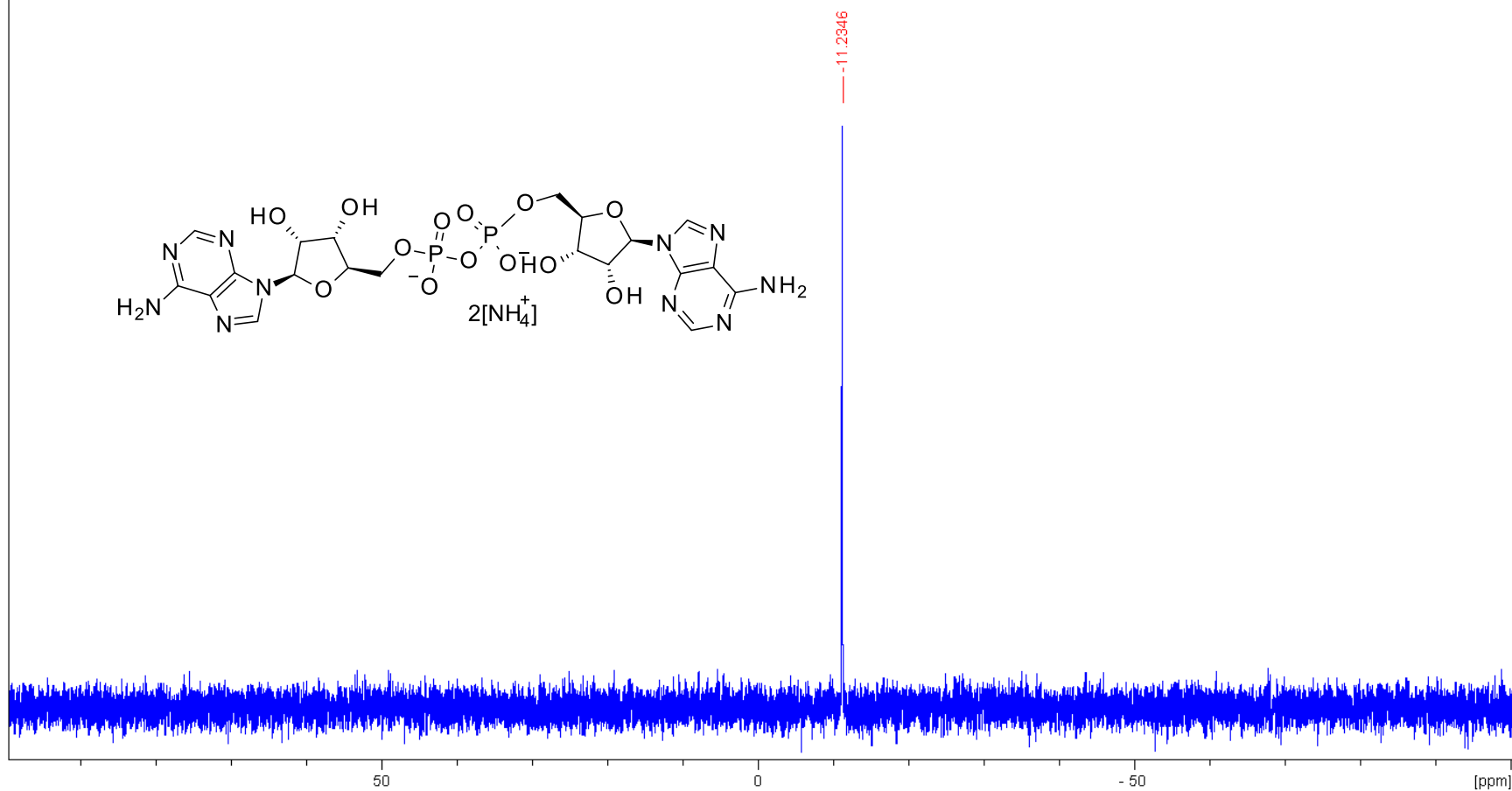
Compound 21. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O

C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 24

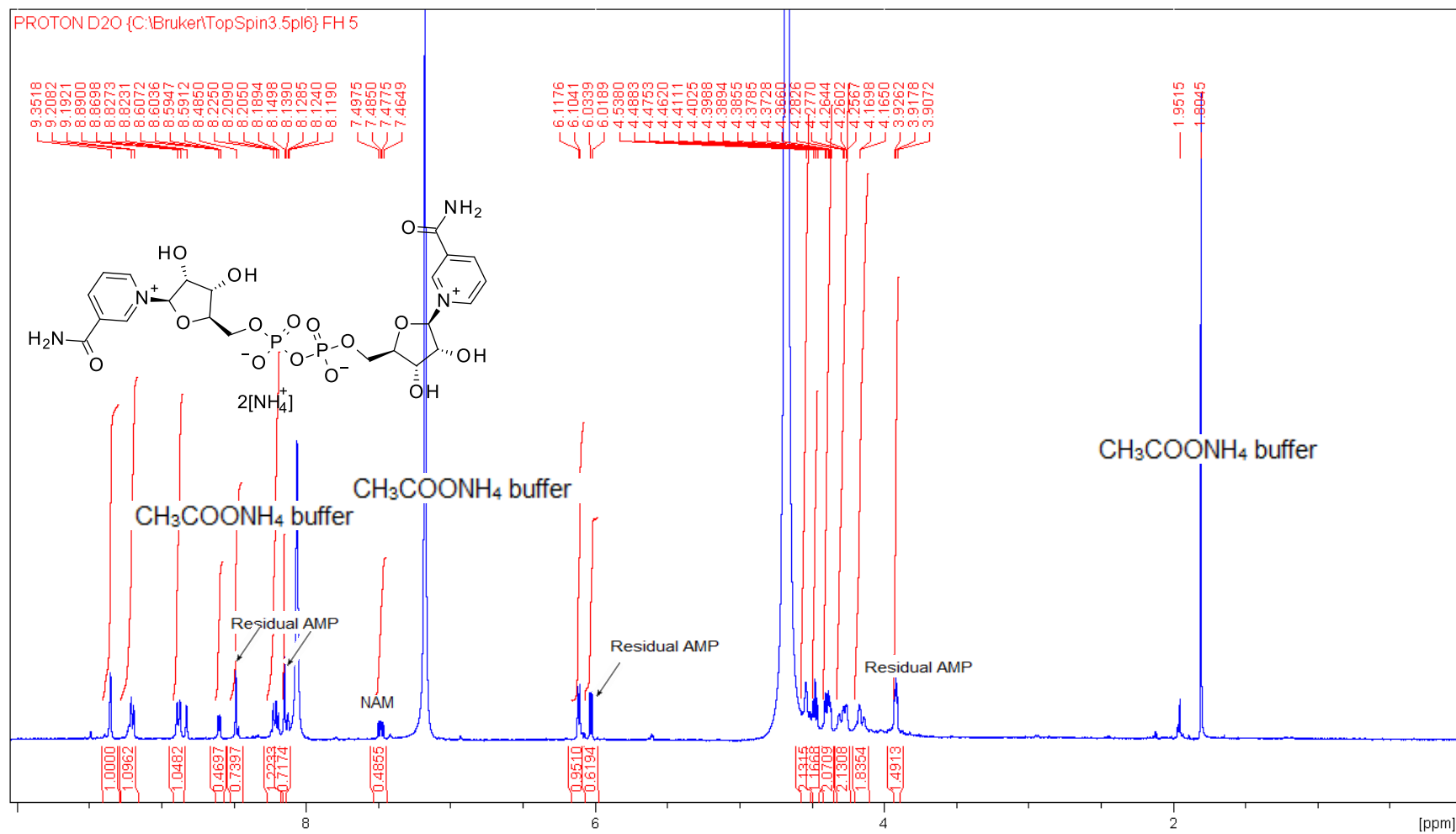


Compound 21. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 24

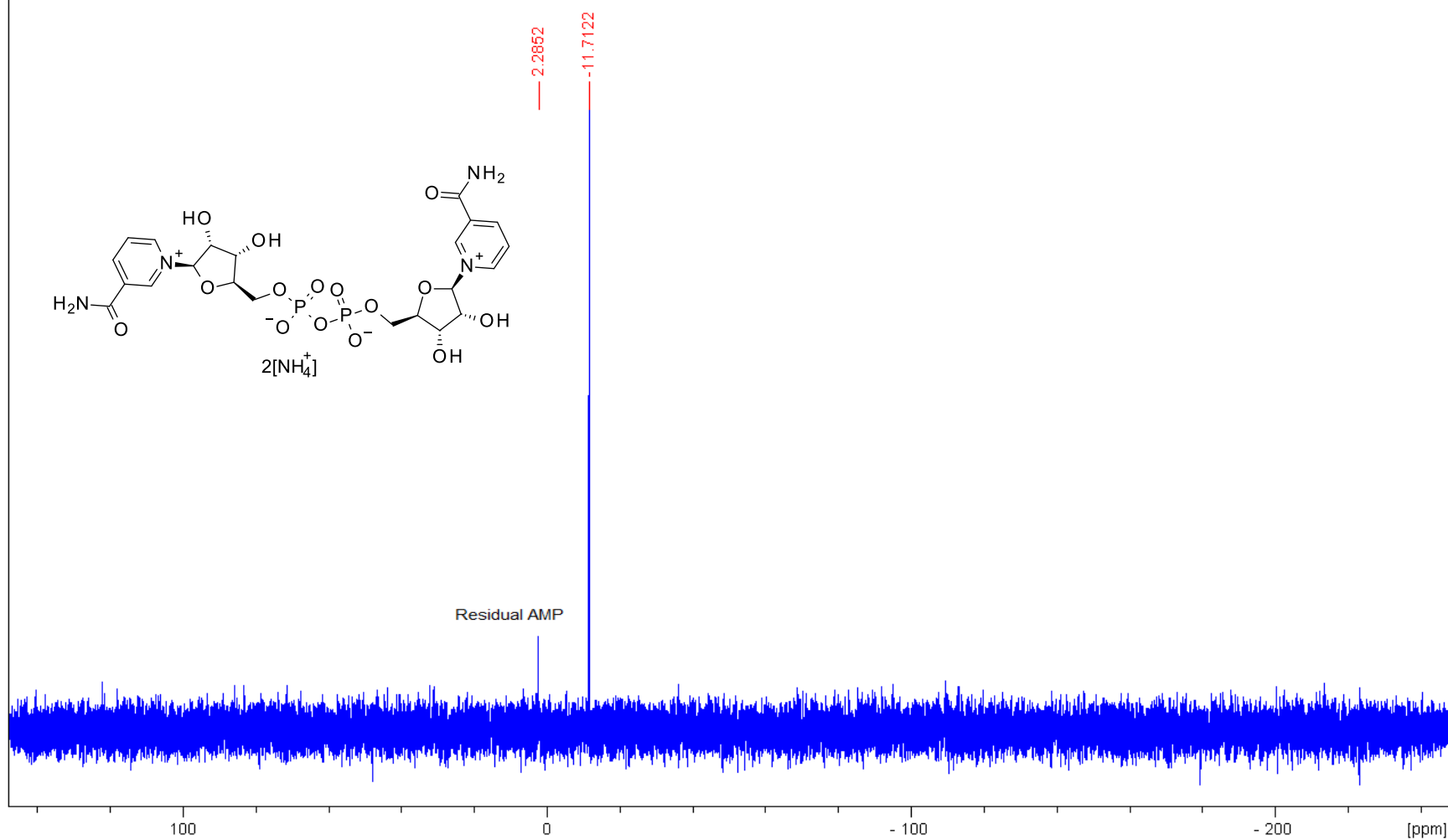


**Compound 21.** 162MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



Compound 22. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O

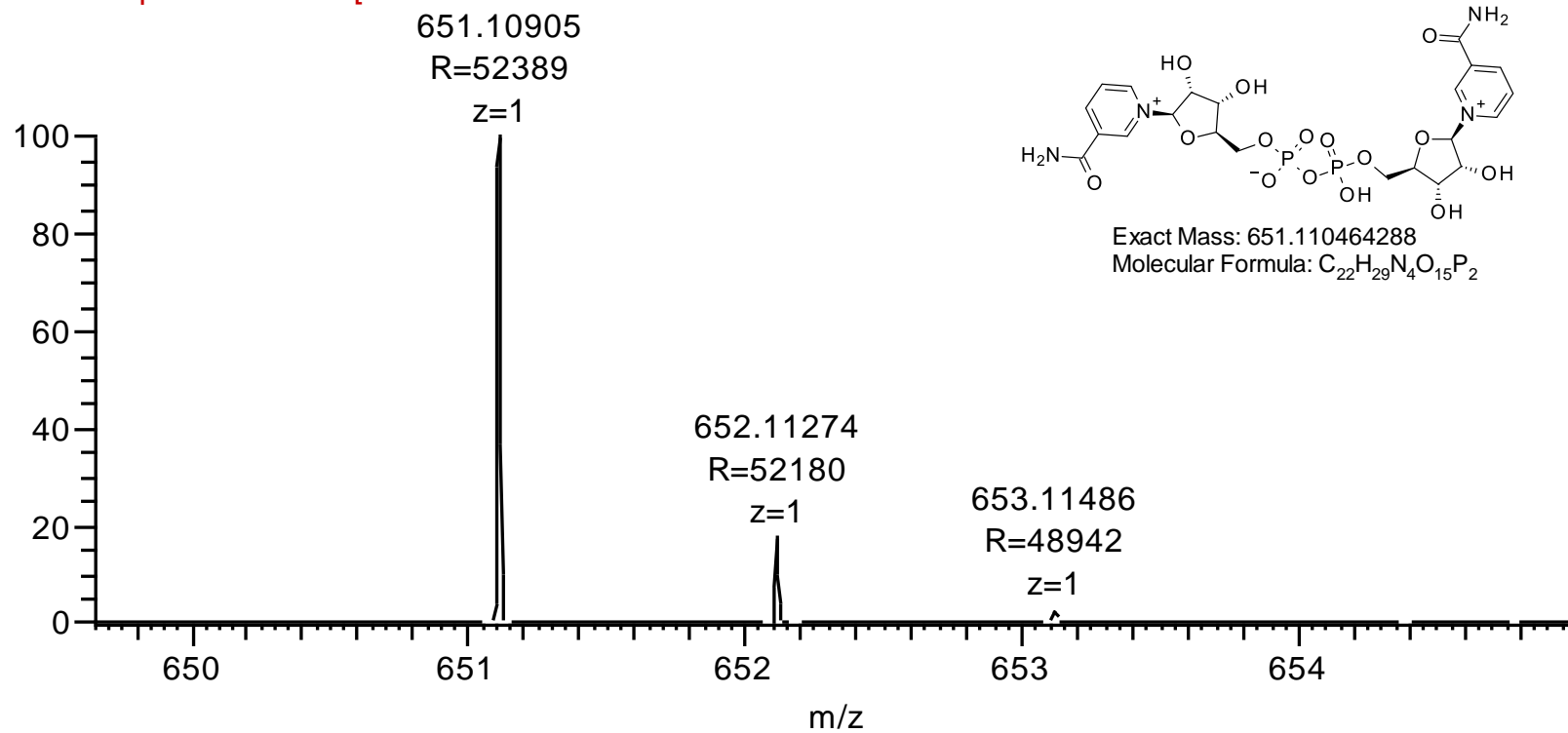
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 24



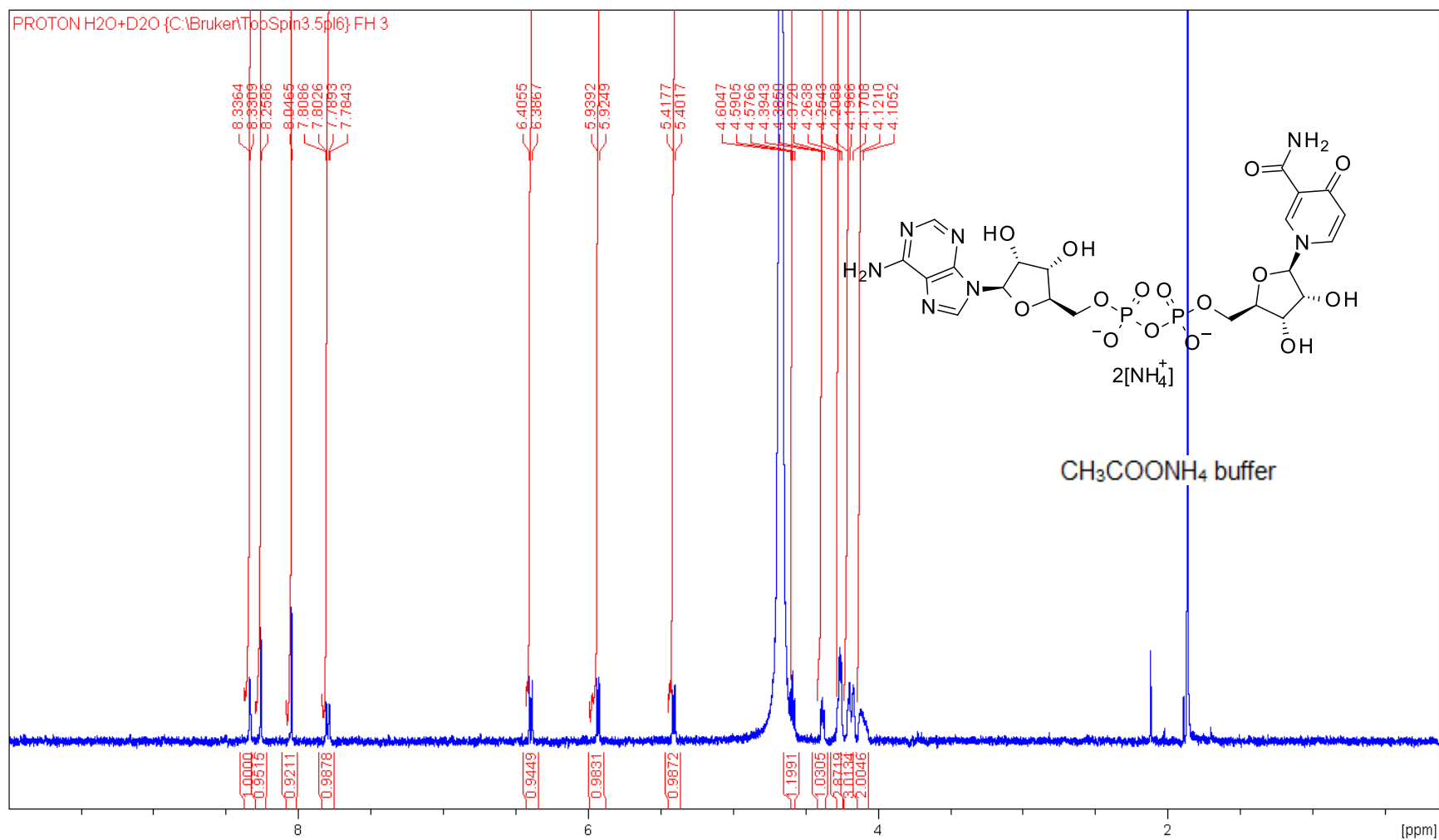
Compound 22. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

mm\_1119121\_2\_double\_nmn\_02 #21 15 RT: 0.200.60 AU: 3 NL: 4.12E5

F: FTMS + p ESI Full ms[50.00-900.0]



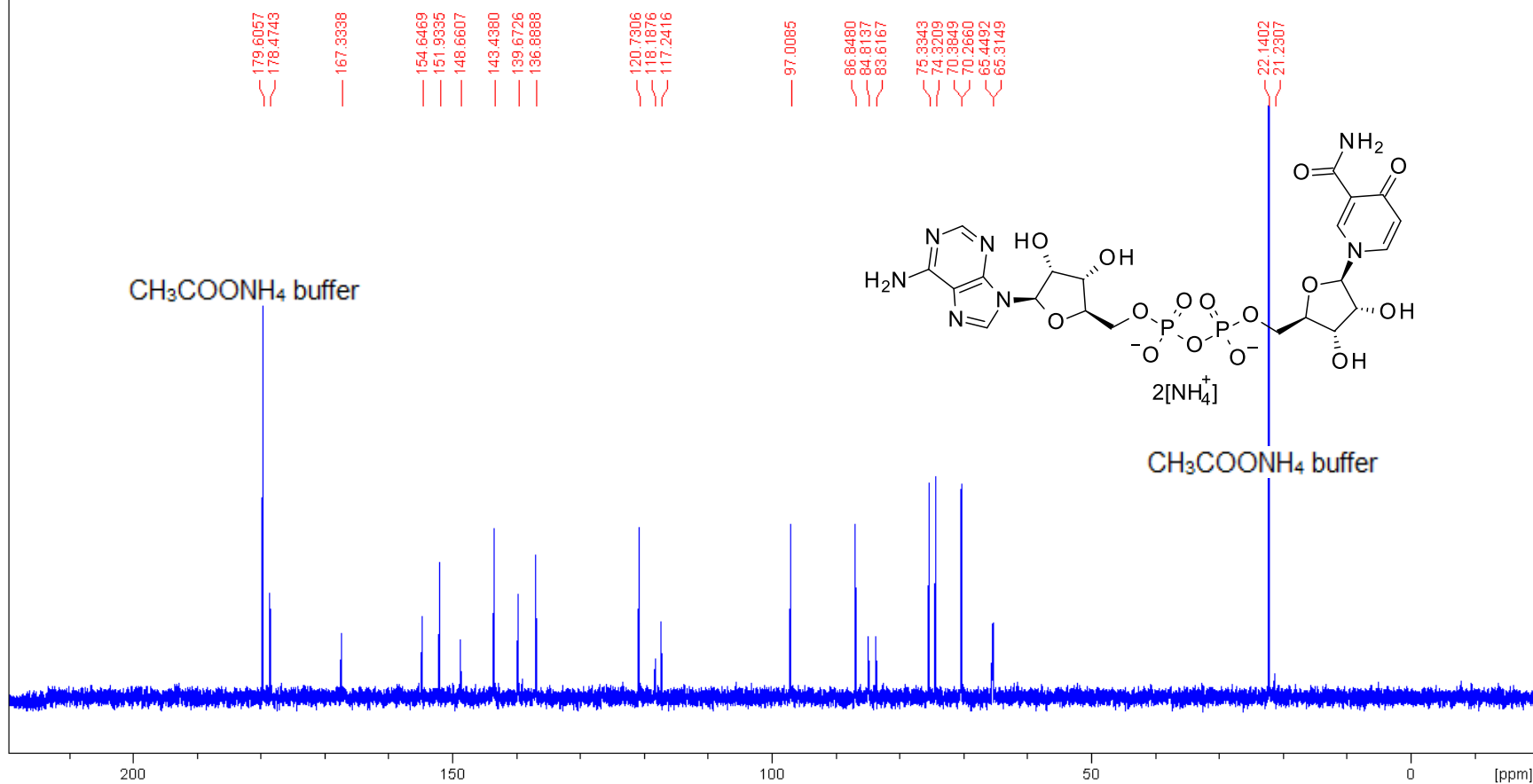
Compound 22. HRMS spectra



Compound 23. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

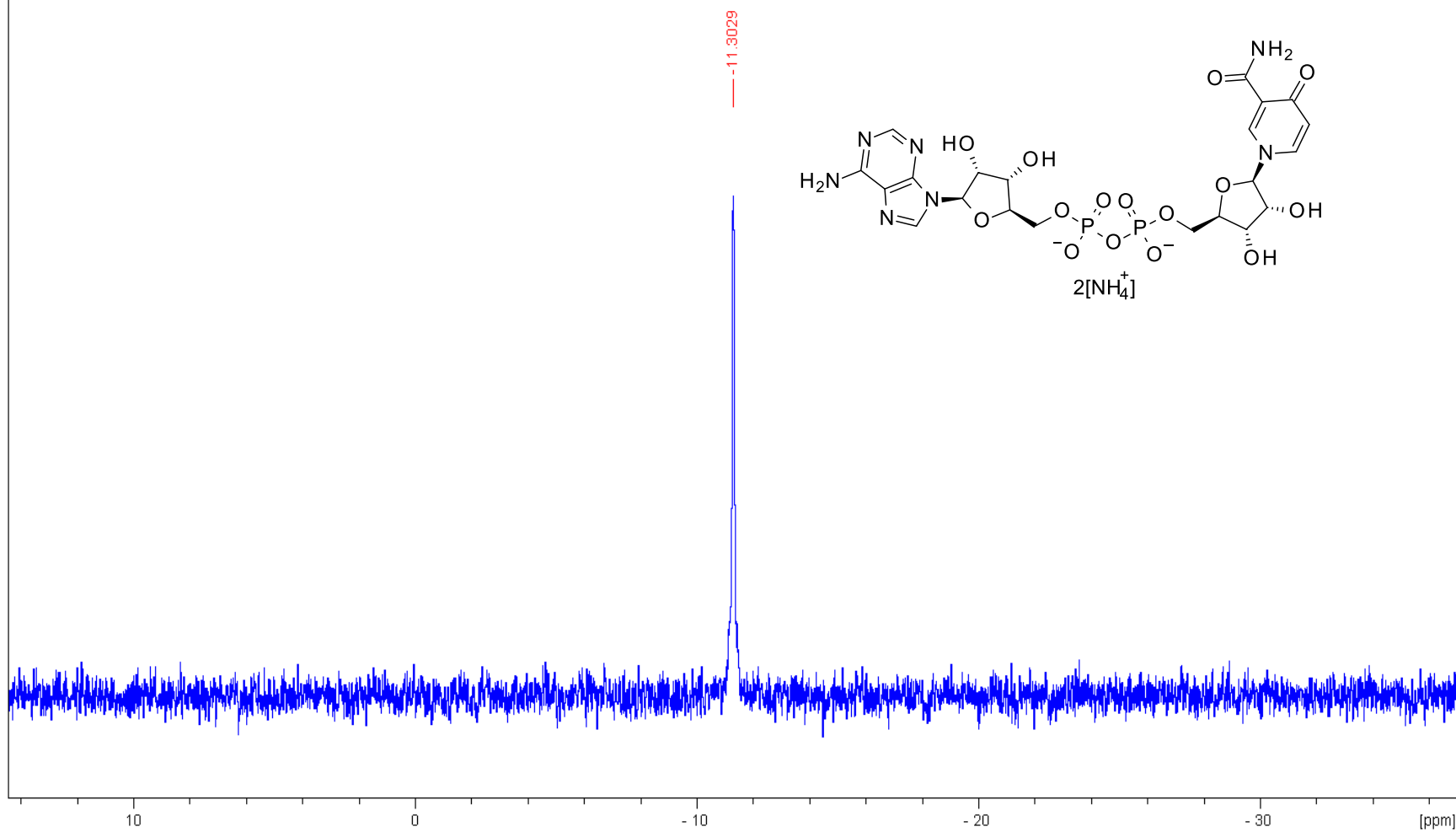


C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 14



Compound 23. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

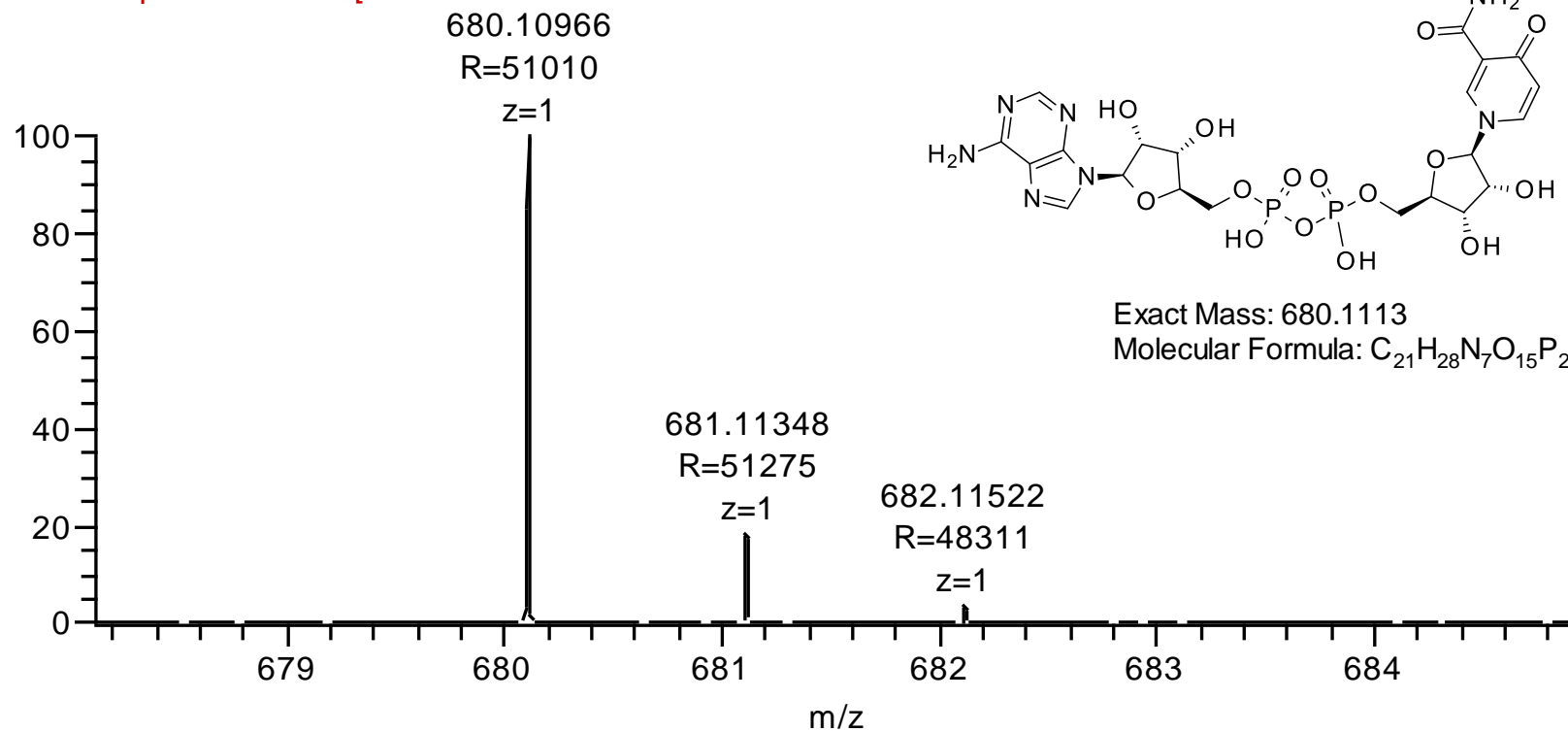
P31CPD H2O+D2O {C:\Bruker\TopSpin3.5pl6} FH 3



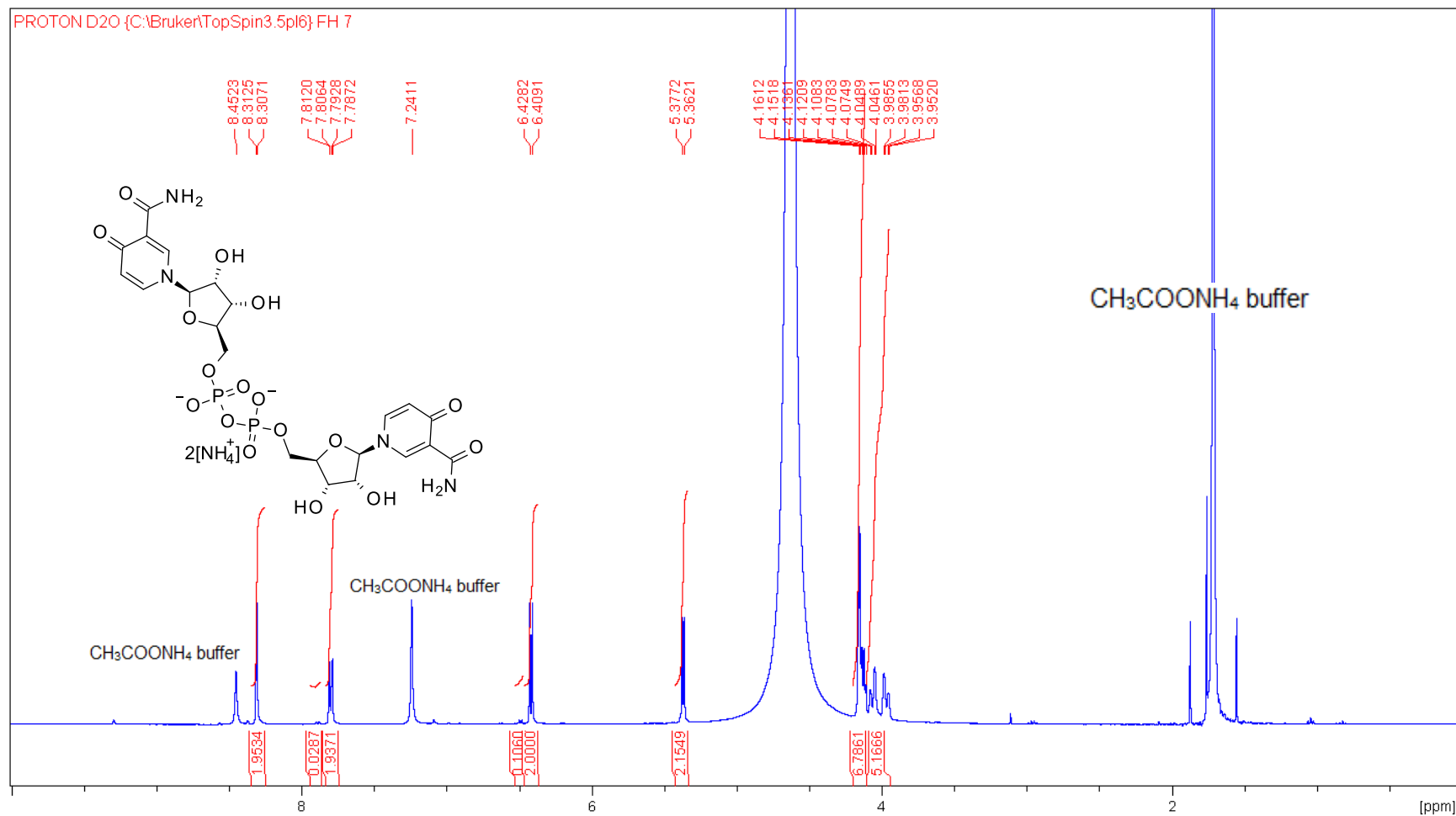
Compound 23. 162 MHz <sup>31</sup>P NMR spectrum in D<sub>2</sub>O

mm\_1119121\_4ndao\_02 #21-46 RT: 0.000000 min. 12E5

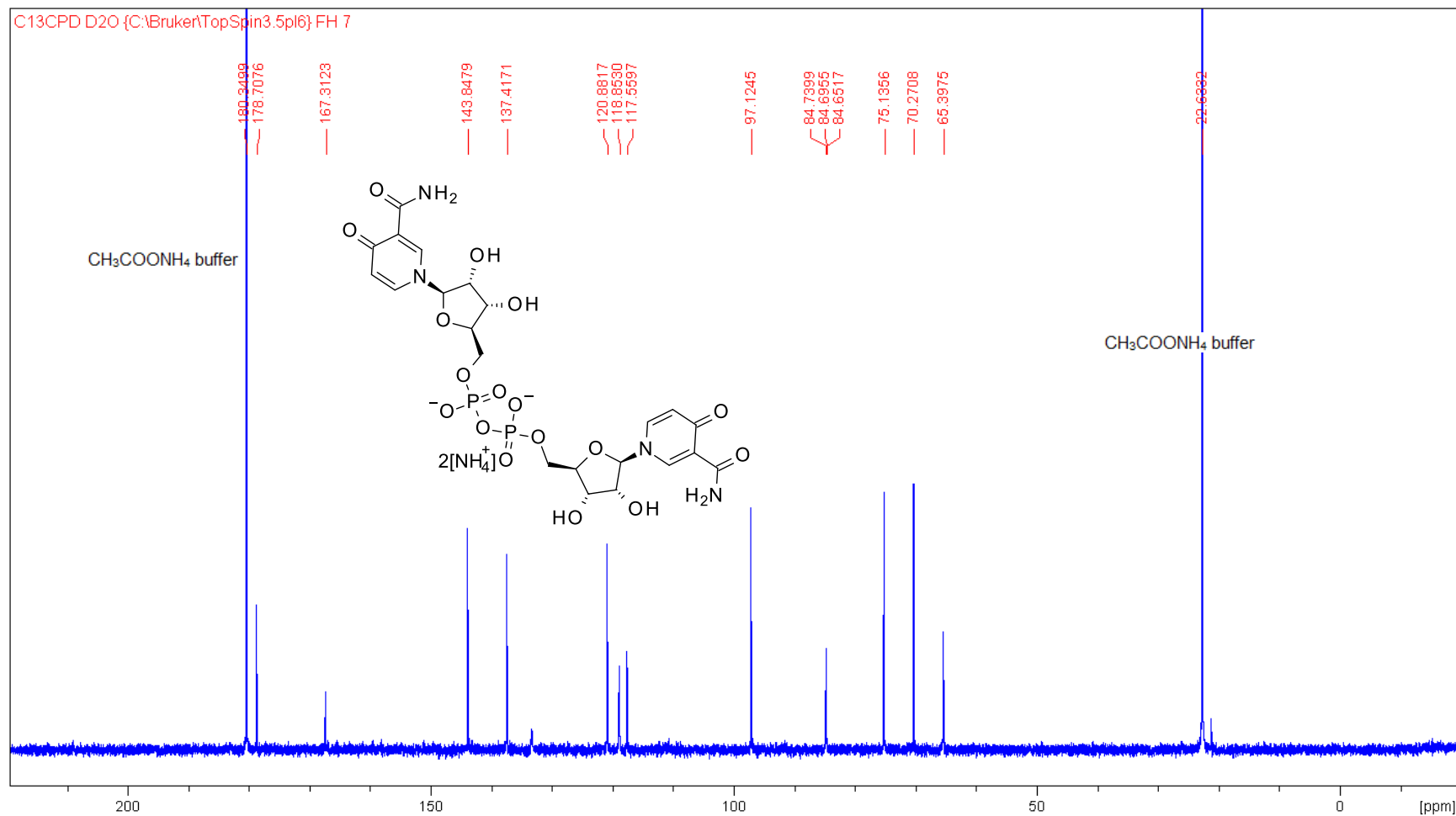
F: FTMS + p ESI Full ms [50.00-900.0]



Compound 23. HRMS Spectra

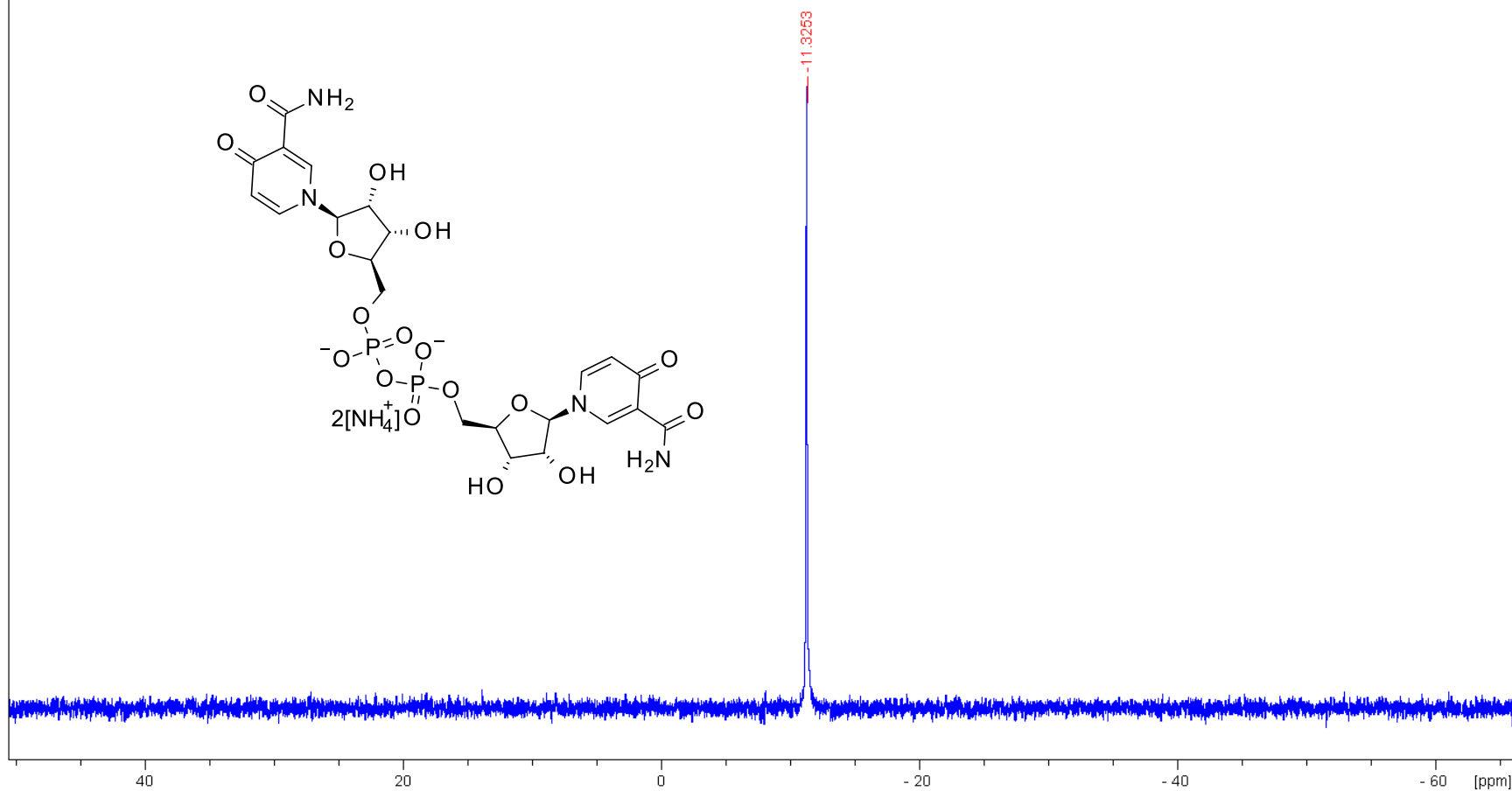


**Compound 24.** 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O



Compound 24. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

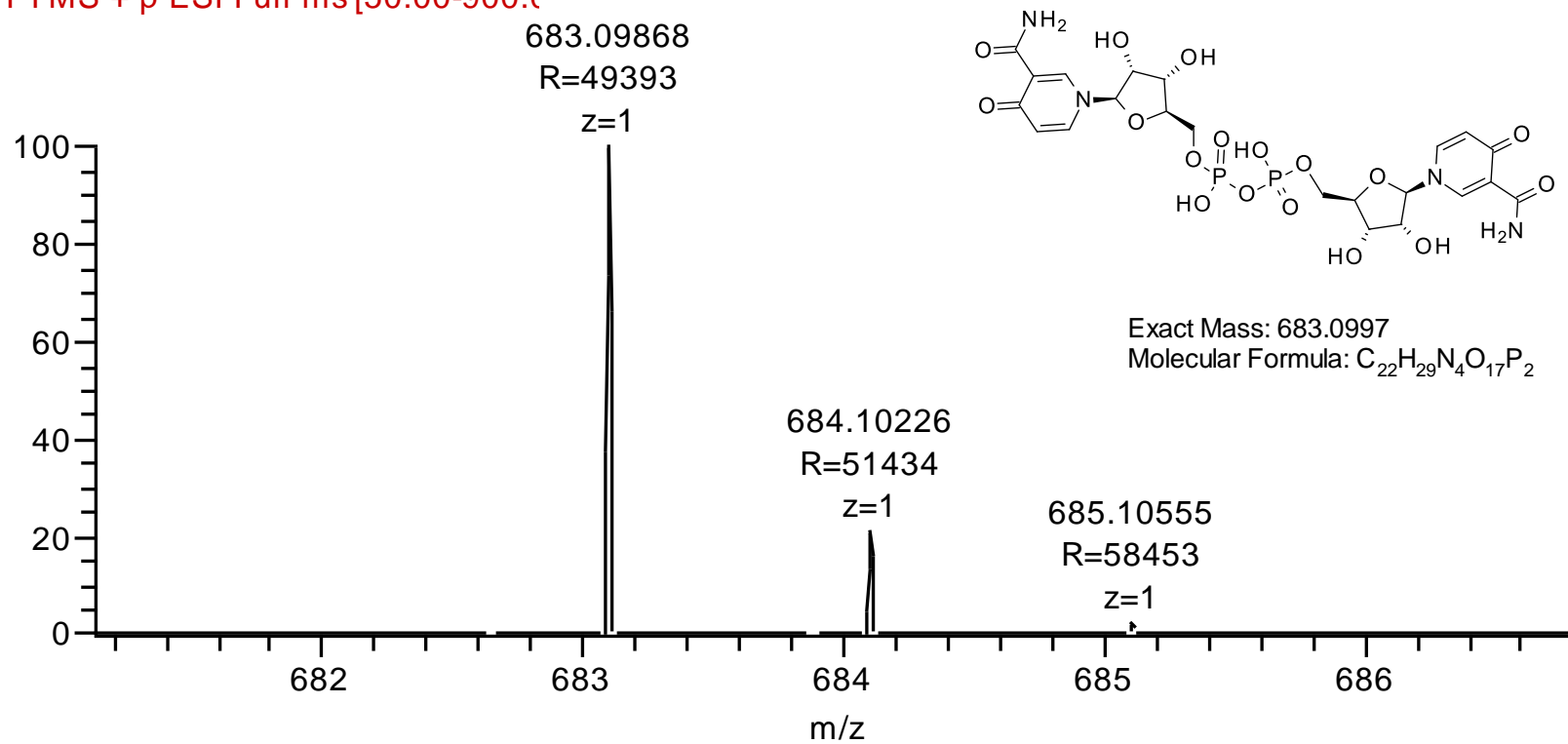
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 7



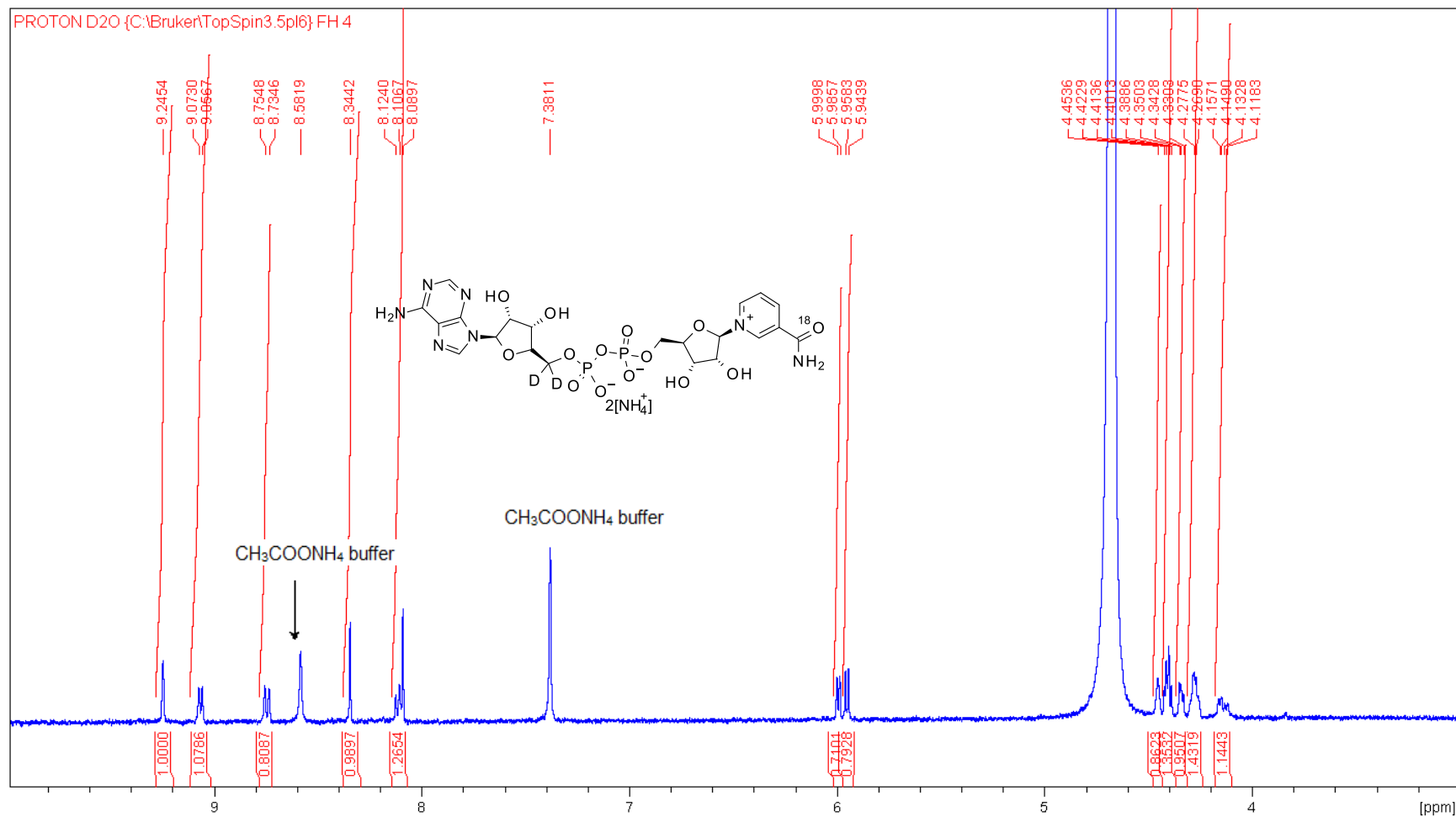
Compound 24. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

mm\_1119121\_3\_double\_nmno\_02 #2042 RT: 0.20052 Min: 7 NL: 1.12E5

F: FTMS + p ESI Full ms [50.00-900.0]

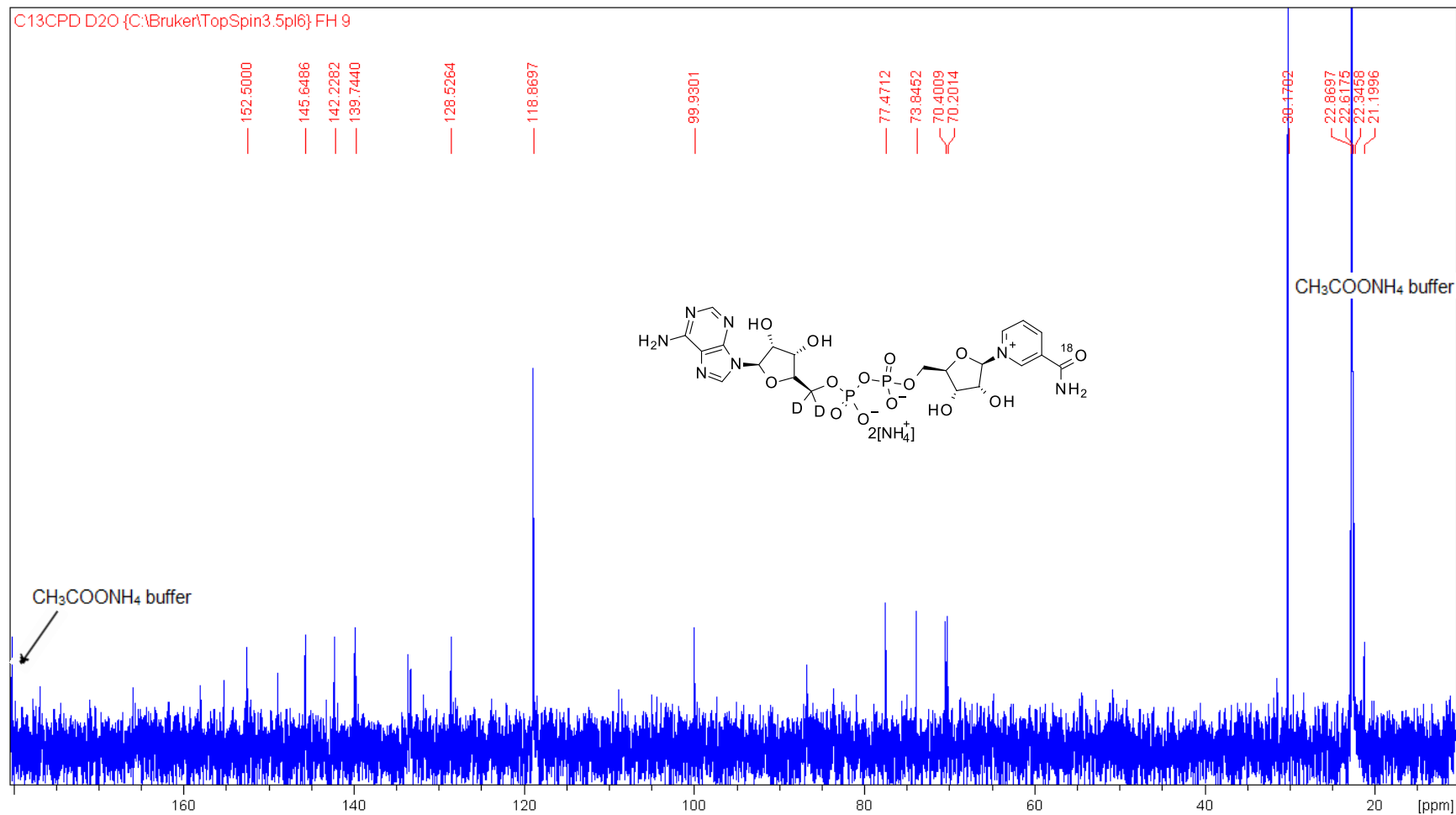


Compound 24. HRMS Sprctra



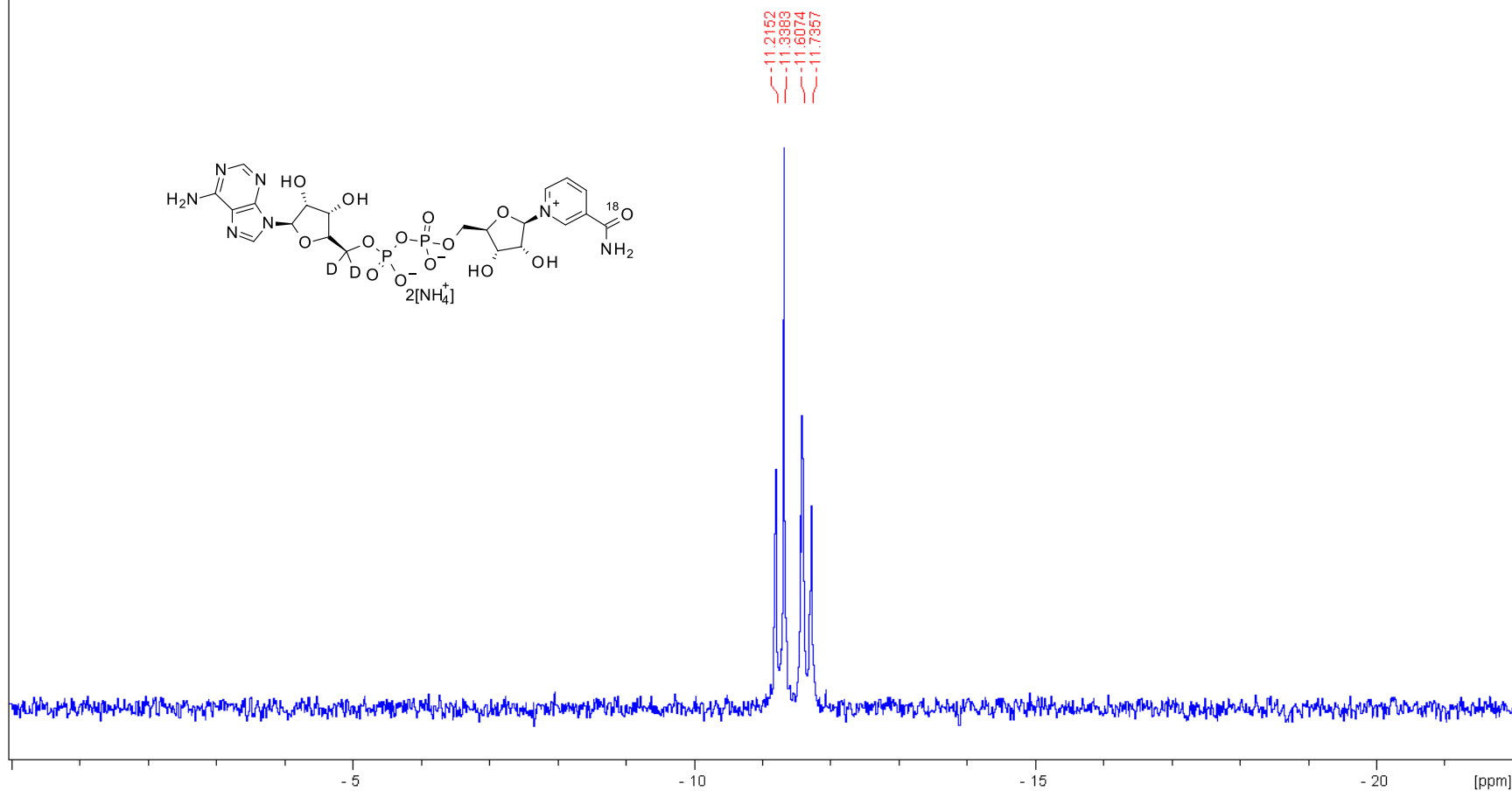
Compound 25. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O





Compound 25. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

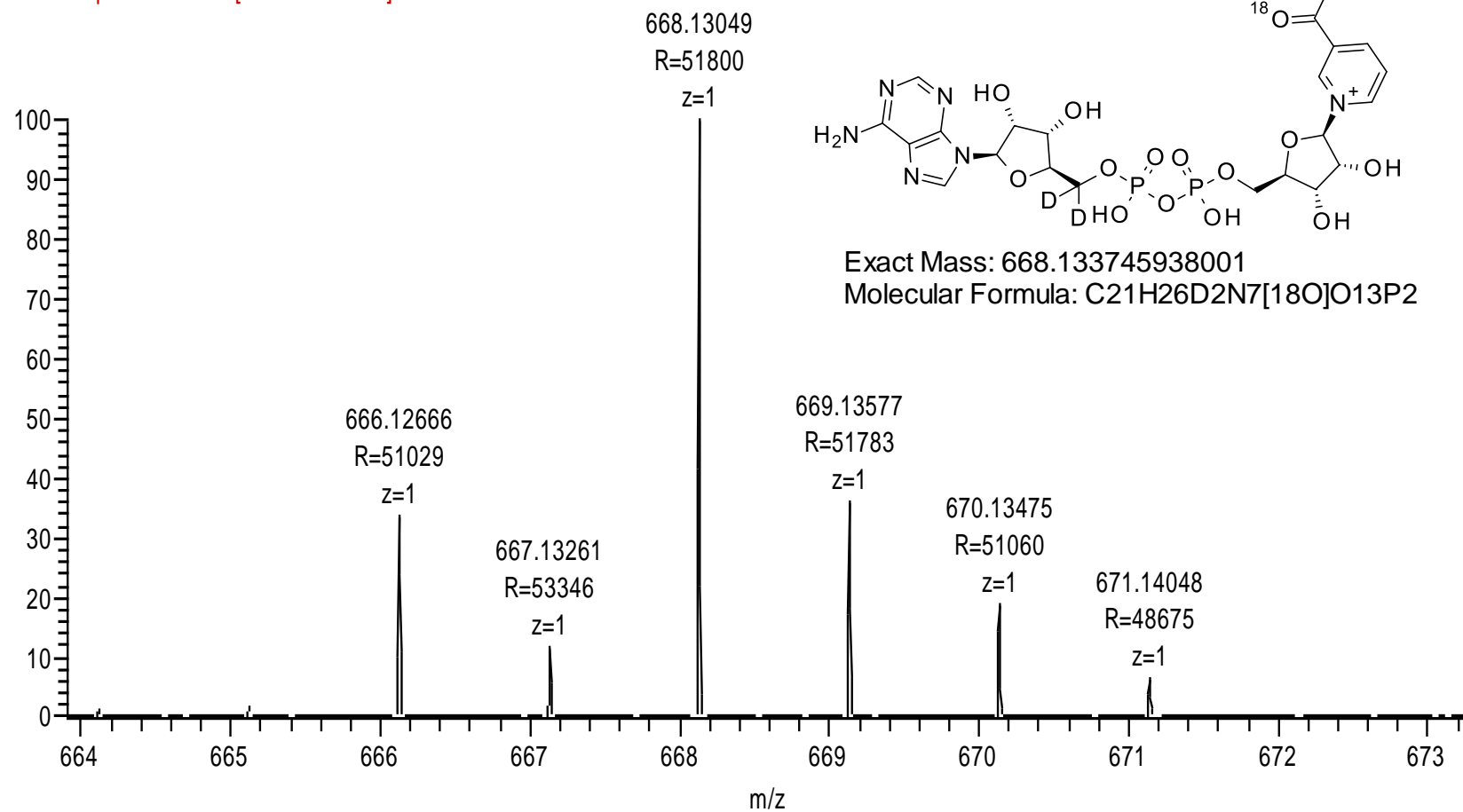
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 1



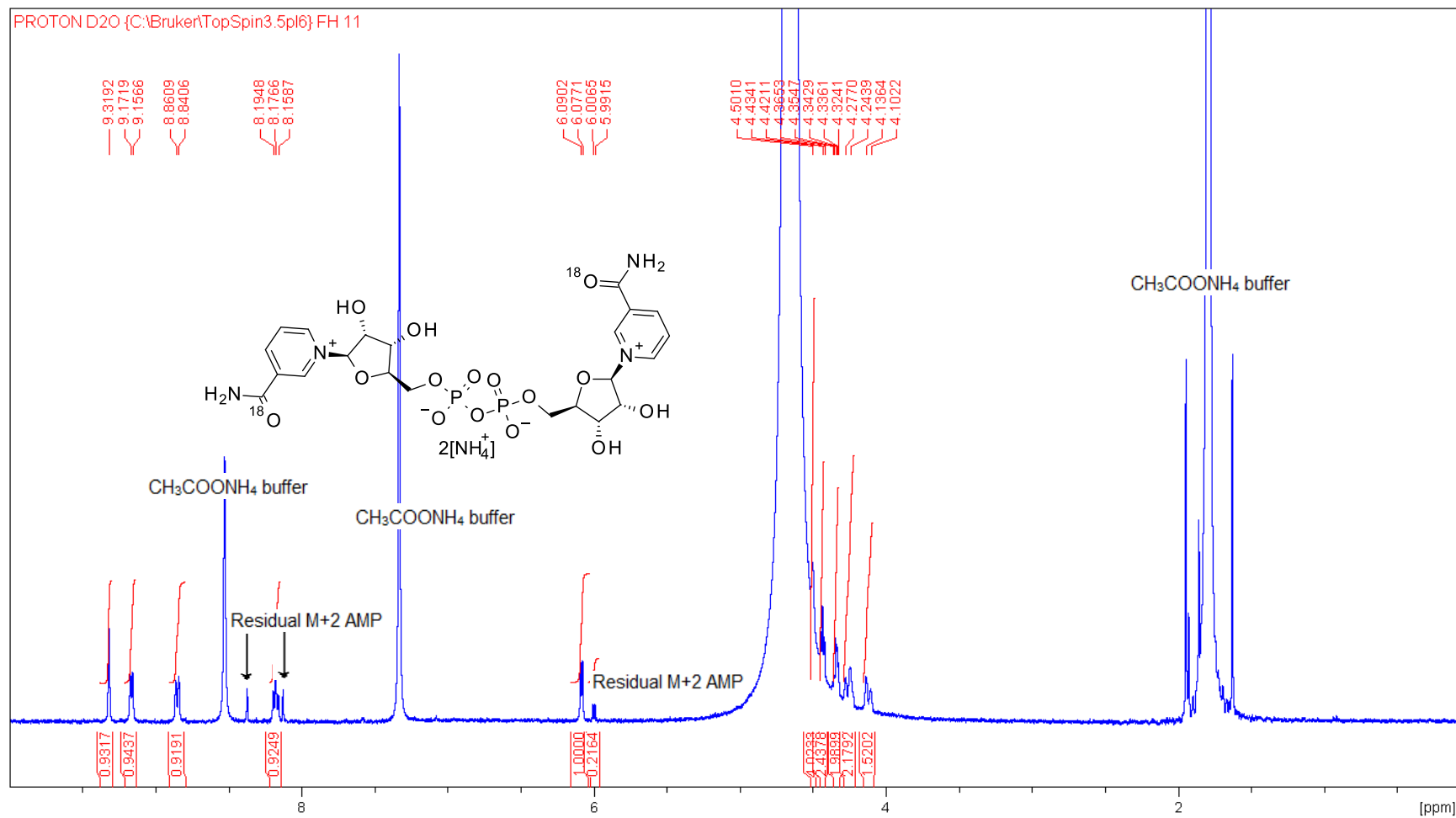
Compound 25. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

Fisal-NAD\_direct\_infusion #15-59 RT: 0.23 min

F: FTMS + p ESI Full ms[110.00-700.00]

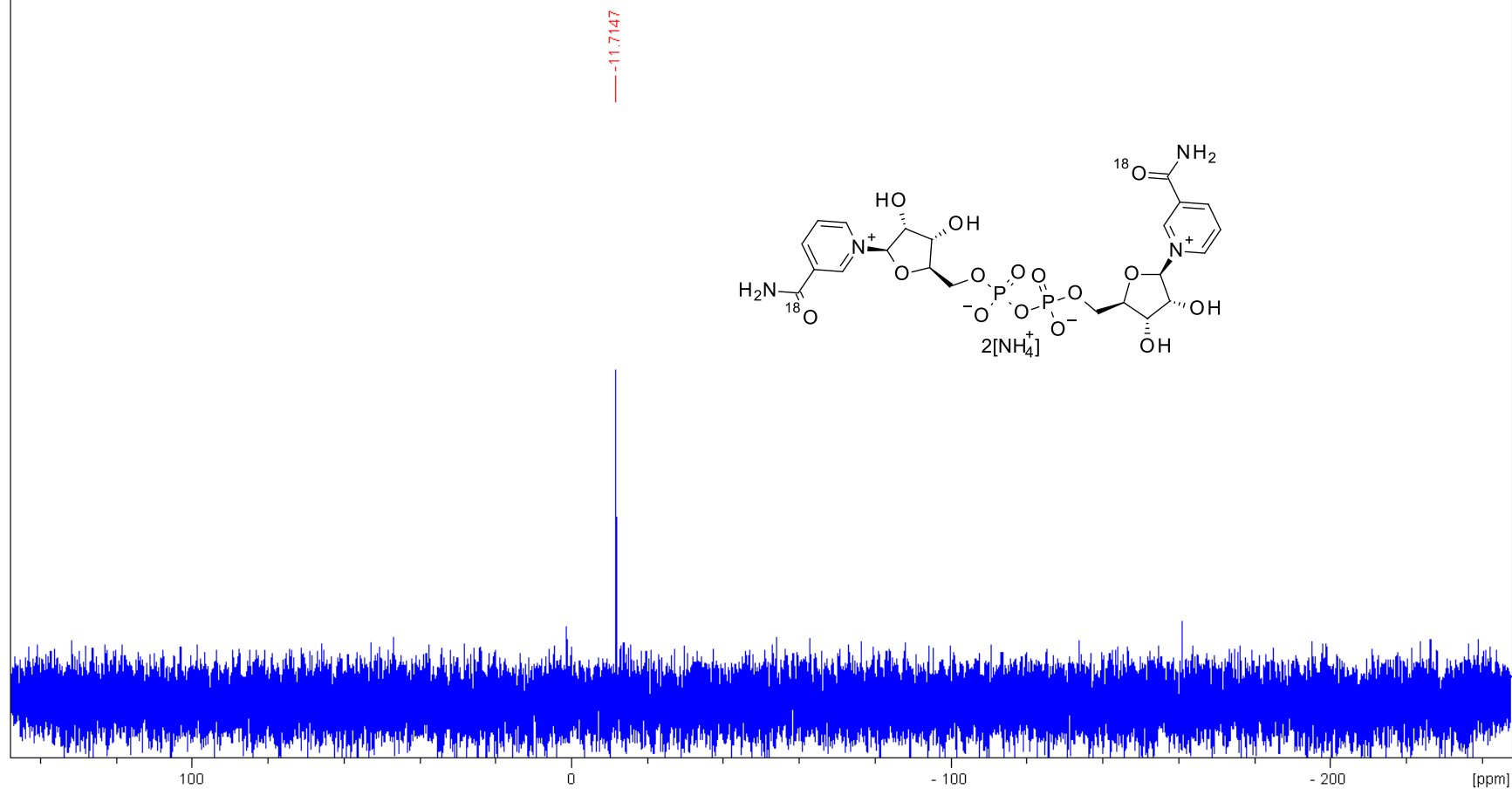


Compound 25. HRMS Spectra



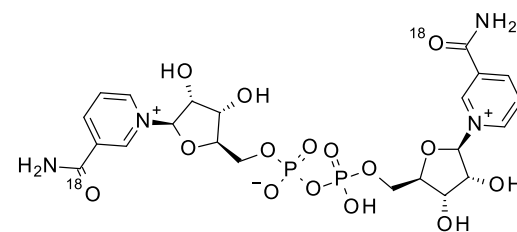
Compound 26. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 11

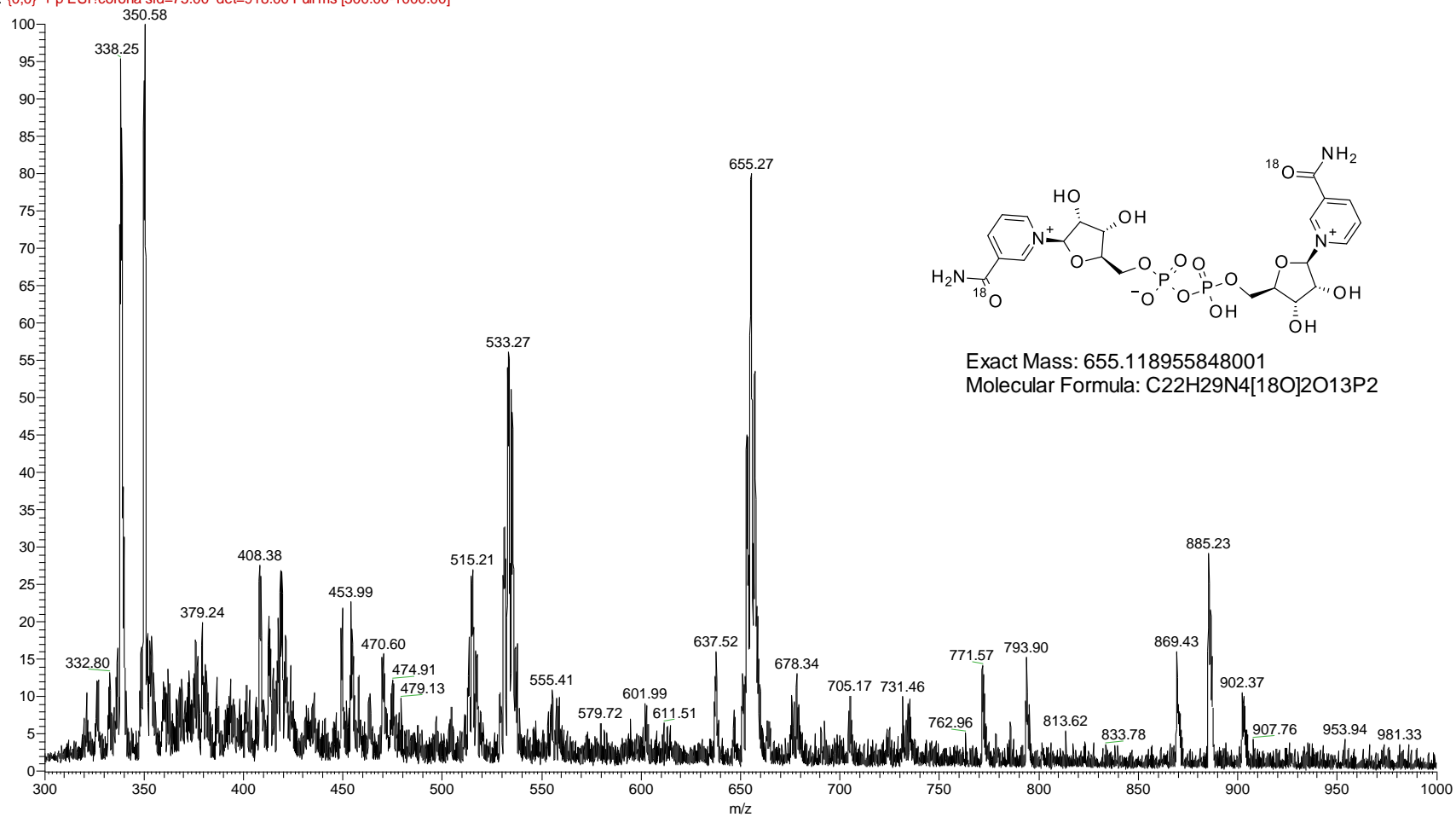


Compound 26. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

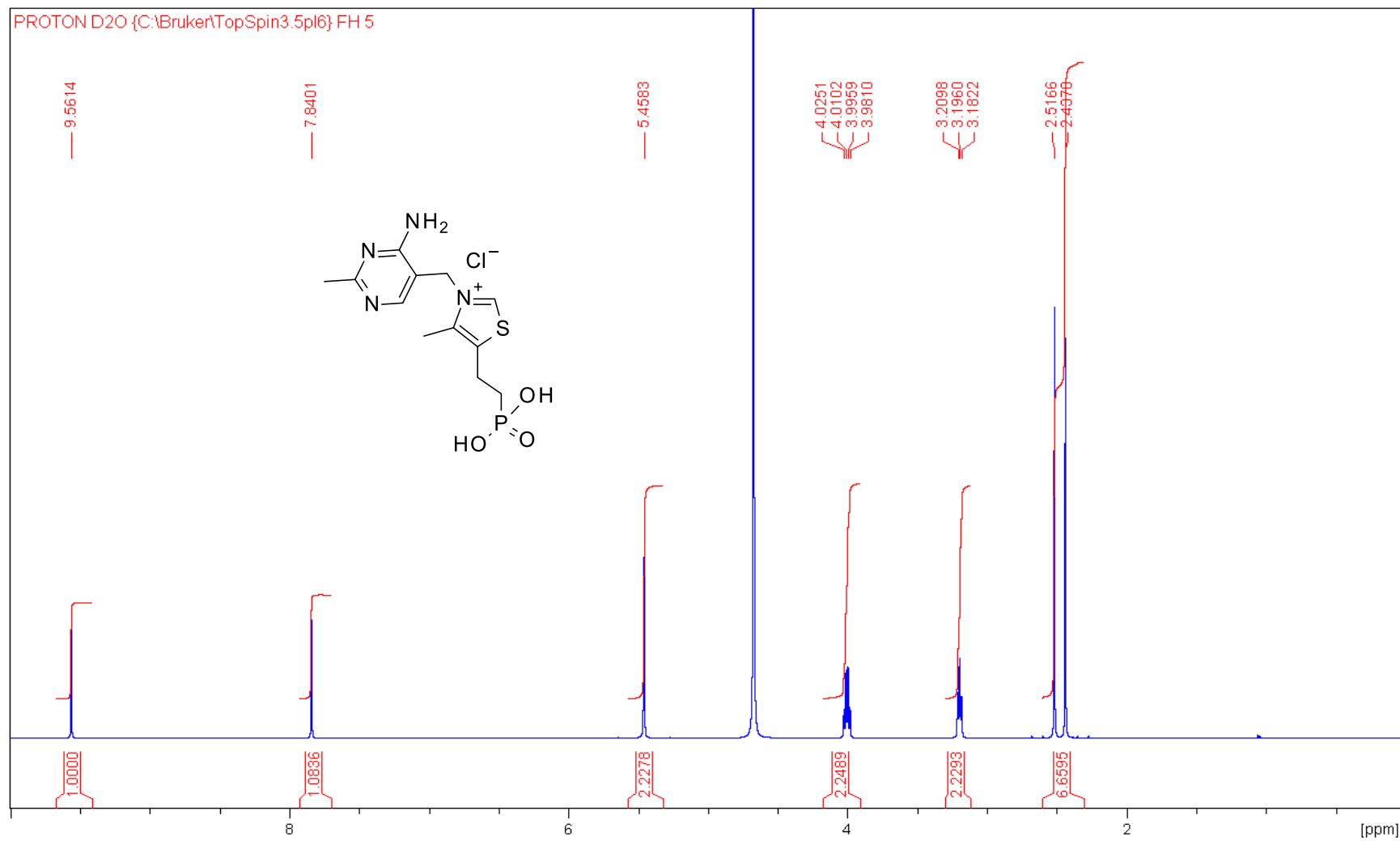
mm\_061920\_double\_nmn\_pure\_04 #7-29 RT: 0.05-0.25 AV: 23 NL: 3.98E4  
F: (0.0) +p ESI!corona sid=75.00 det=918.00 Full ms [300.00-1000.00]



Exact Mass: 655.118955848001  
Molecular Formula: C<sub>22</sub>H<sub>29</sub>N<sub>4</sub>[<sup>18</sup>O]<sub>2</sub>O<sub>13</sub>P<sub>2</sub>

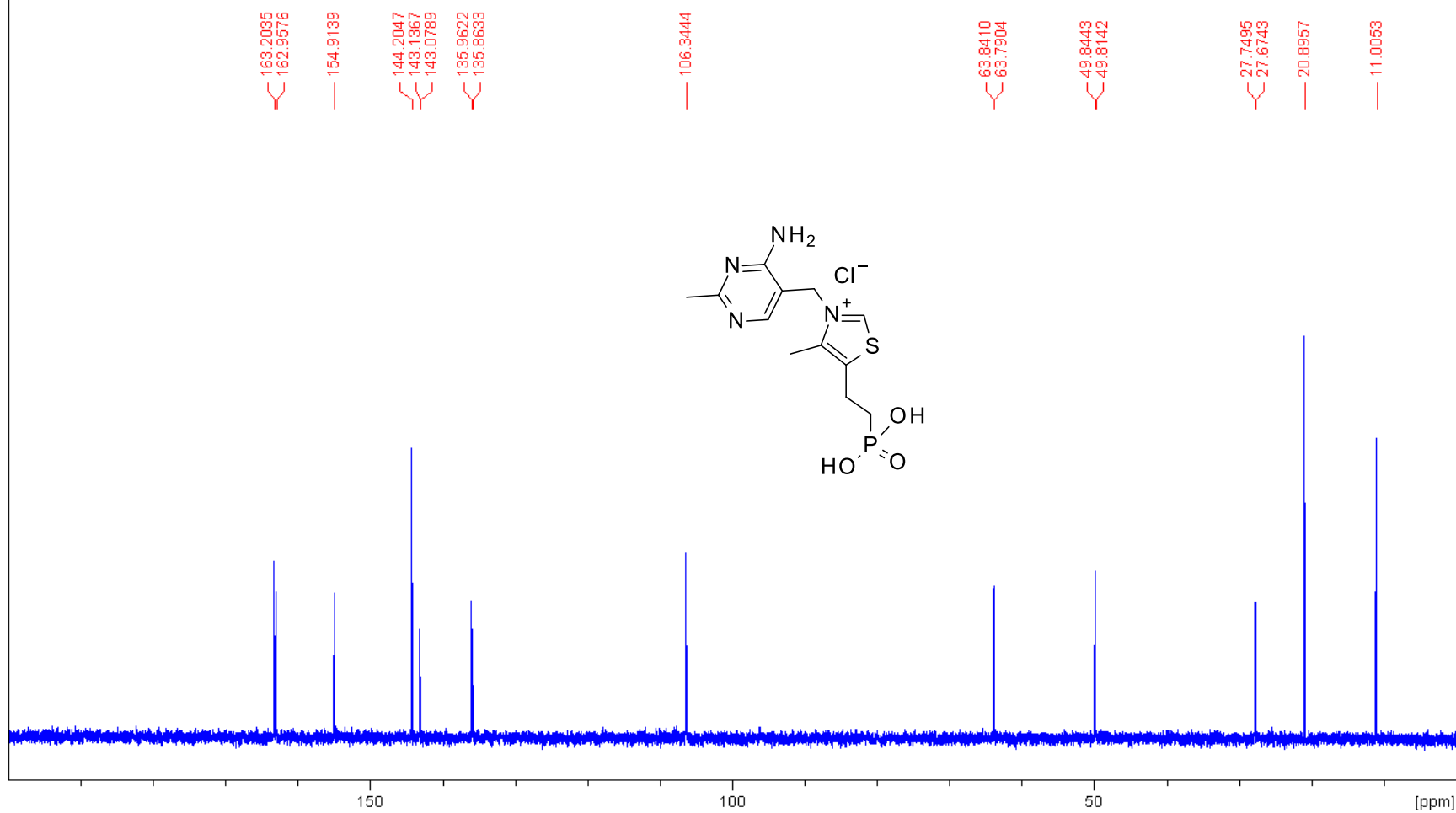


Compound 26. ESI-MS spectra



Compound 28. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

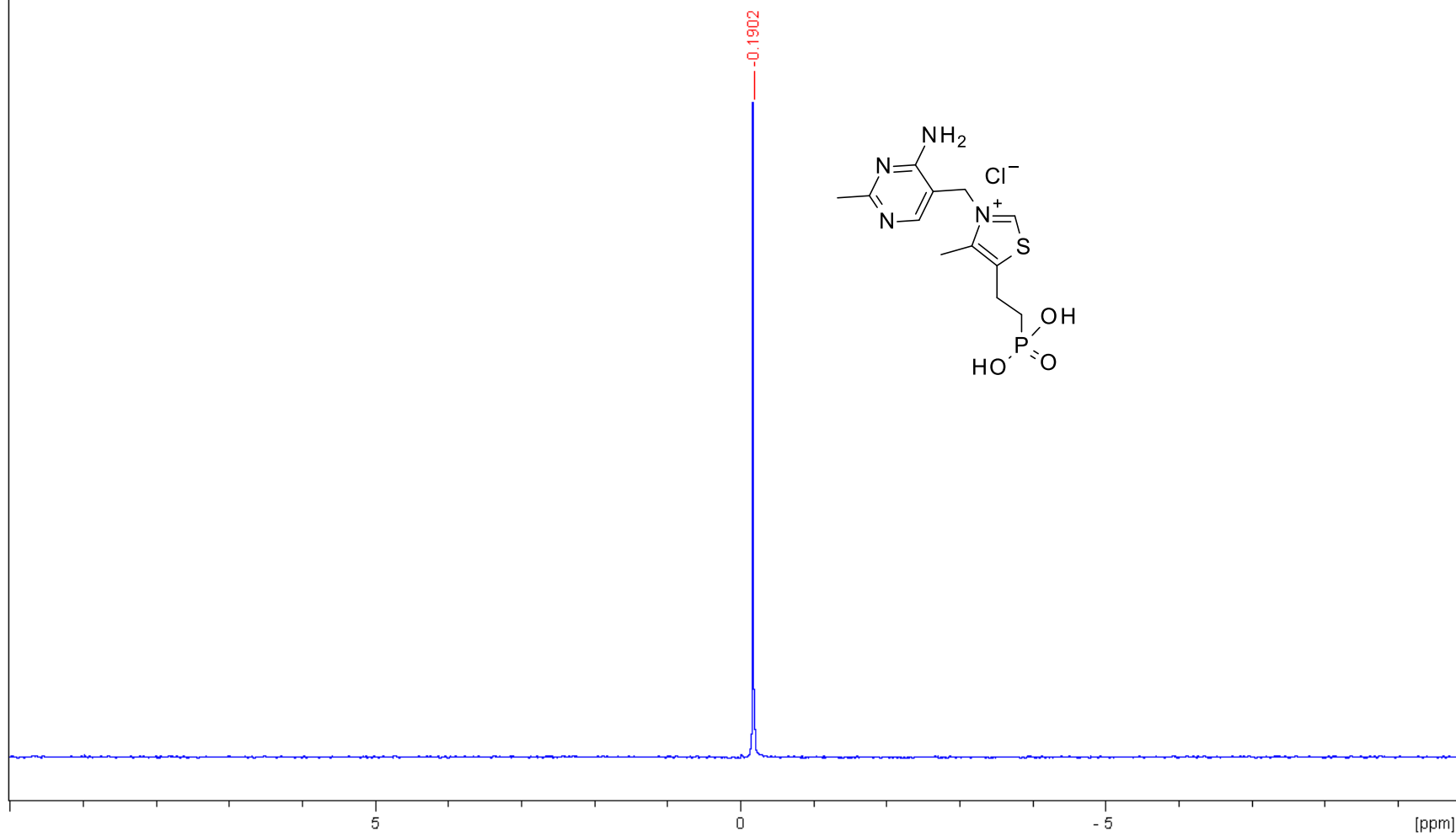
C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 5



Compound 28. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$

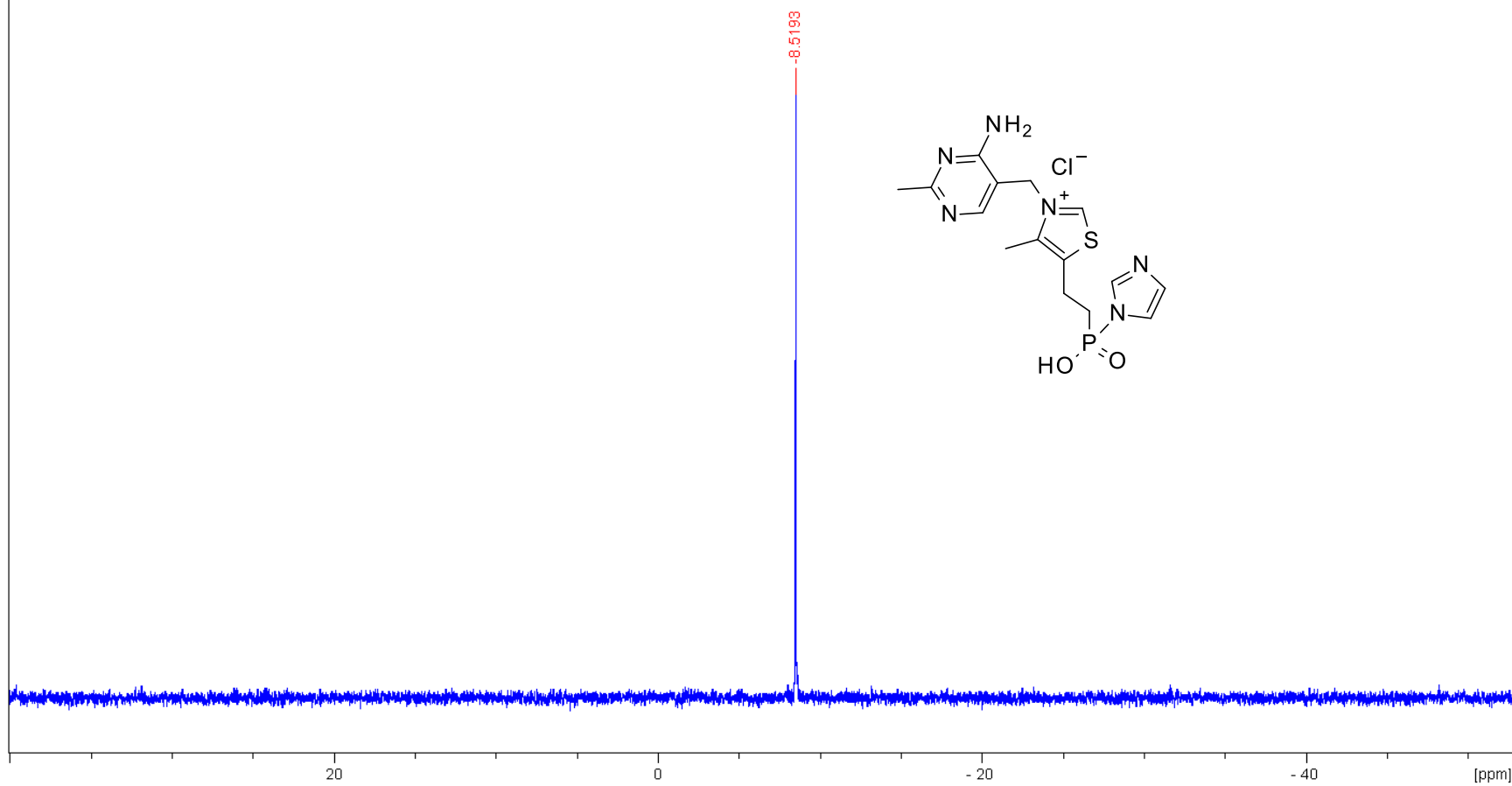


P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 5

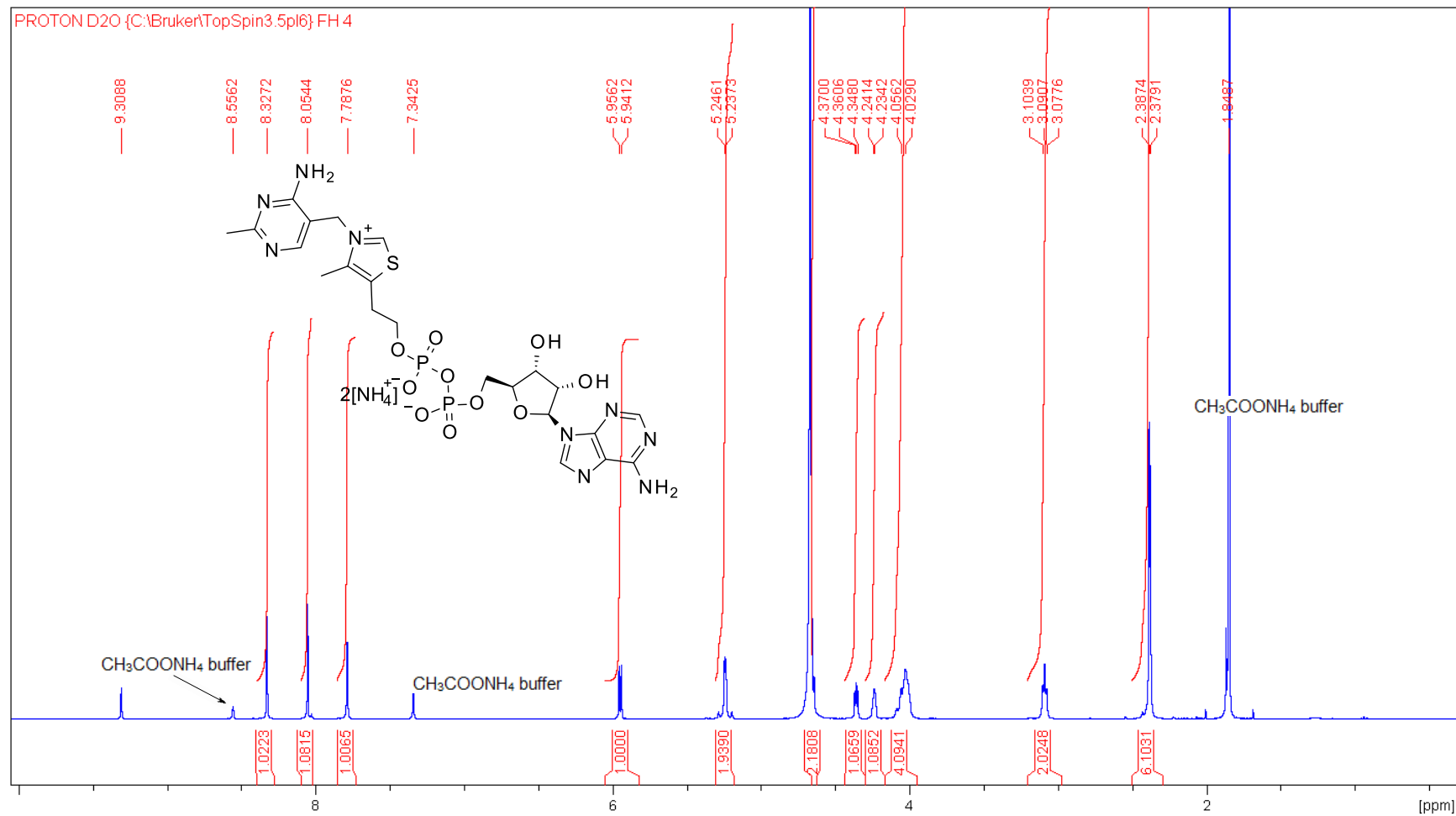


Compound 28. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 11

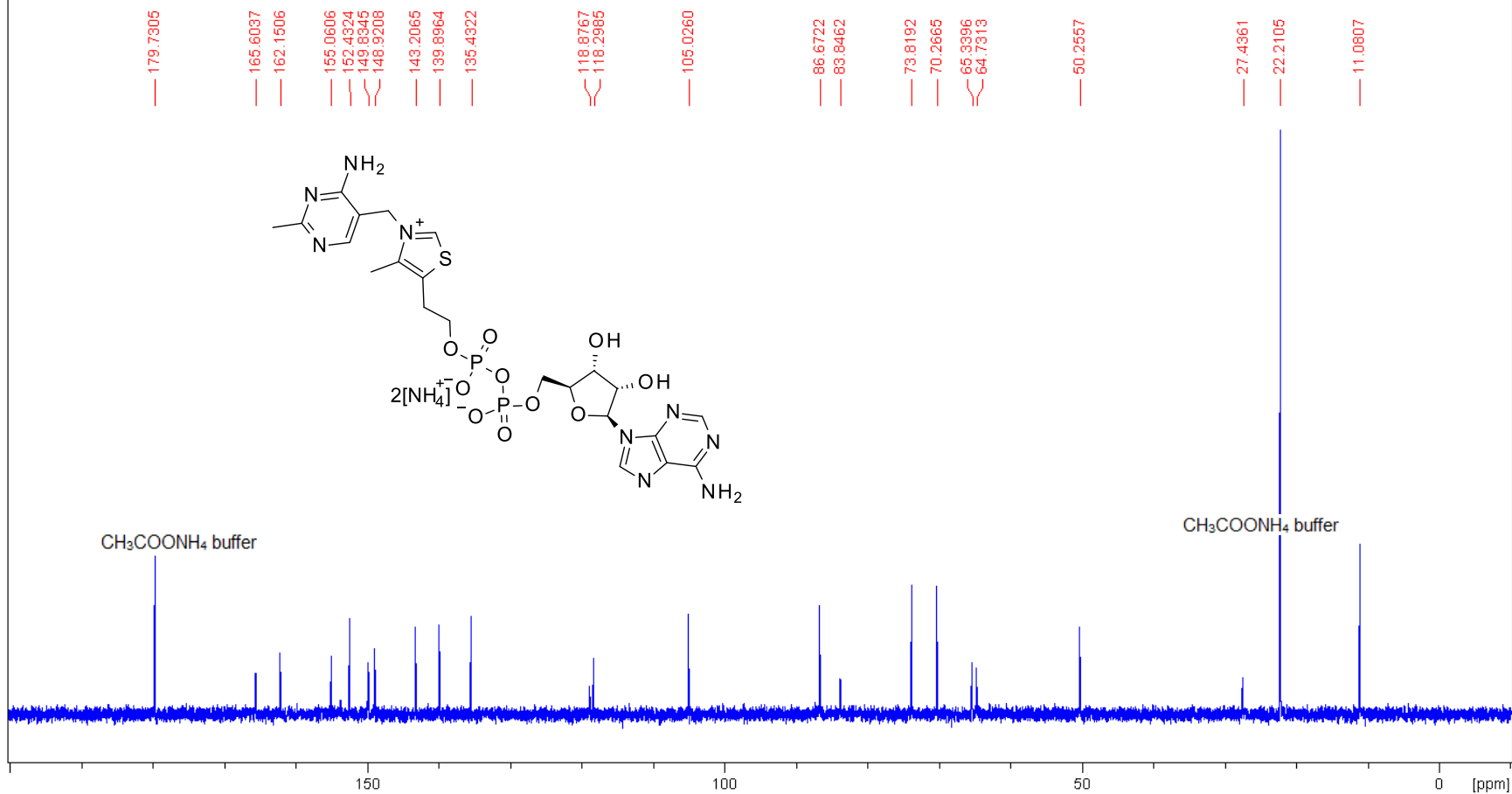


Compound 29. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

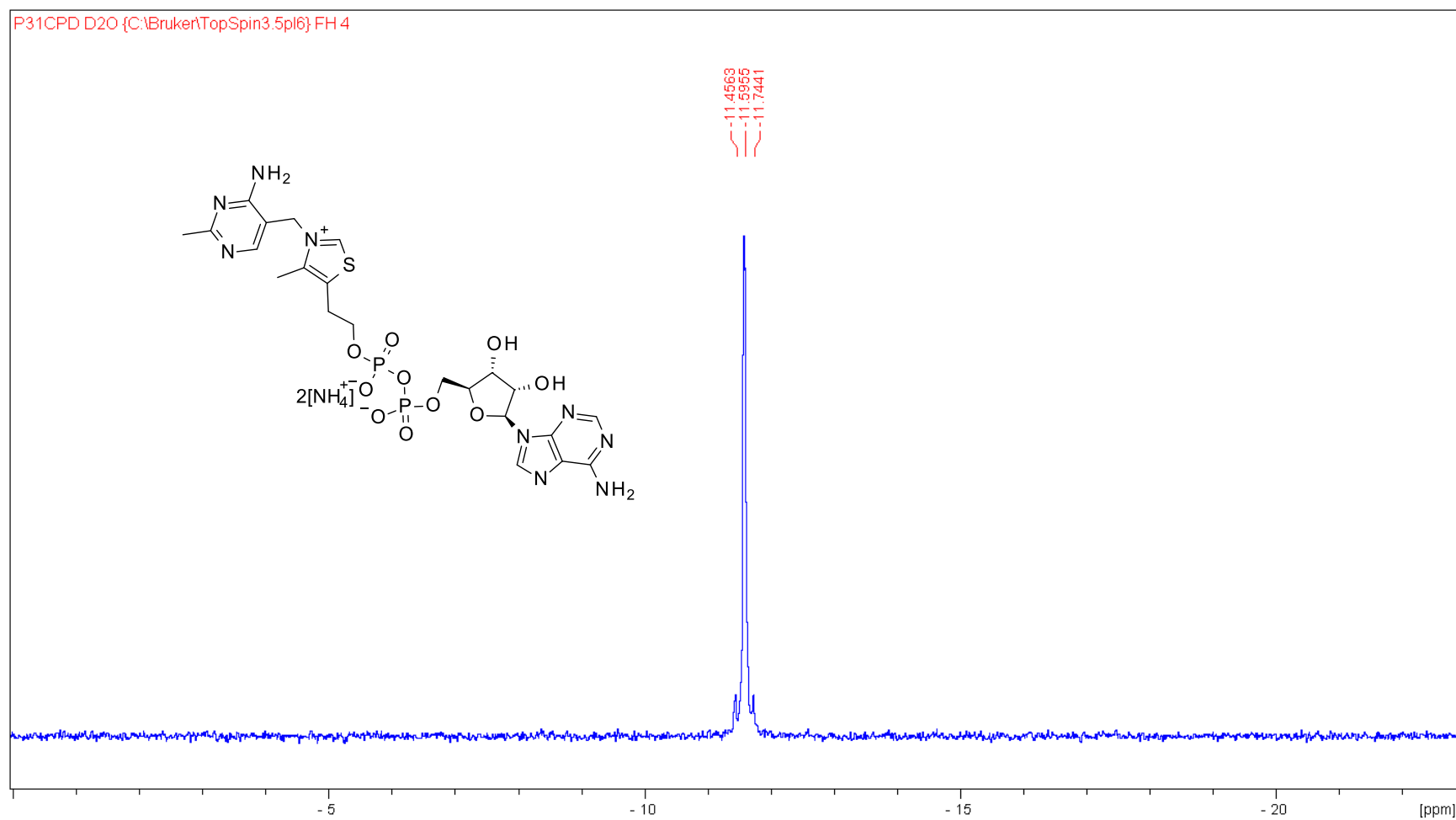


Compound 30a. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O

C13CPD D2O-{C:\Bruker\TopSpin3.5pl6}-FH 4



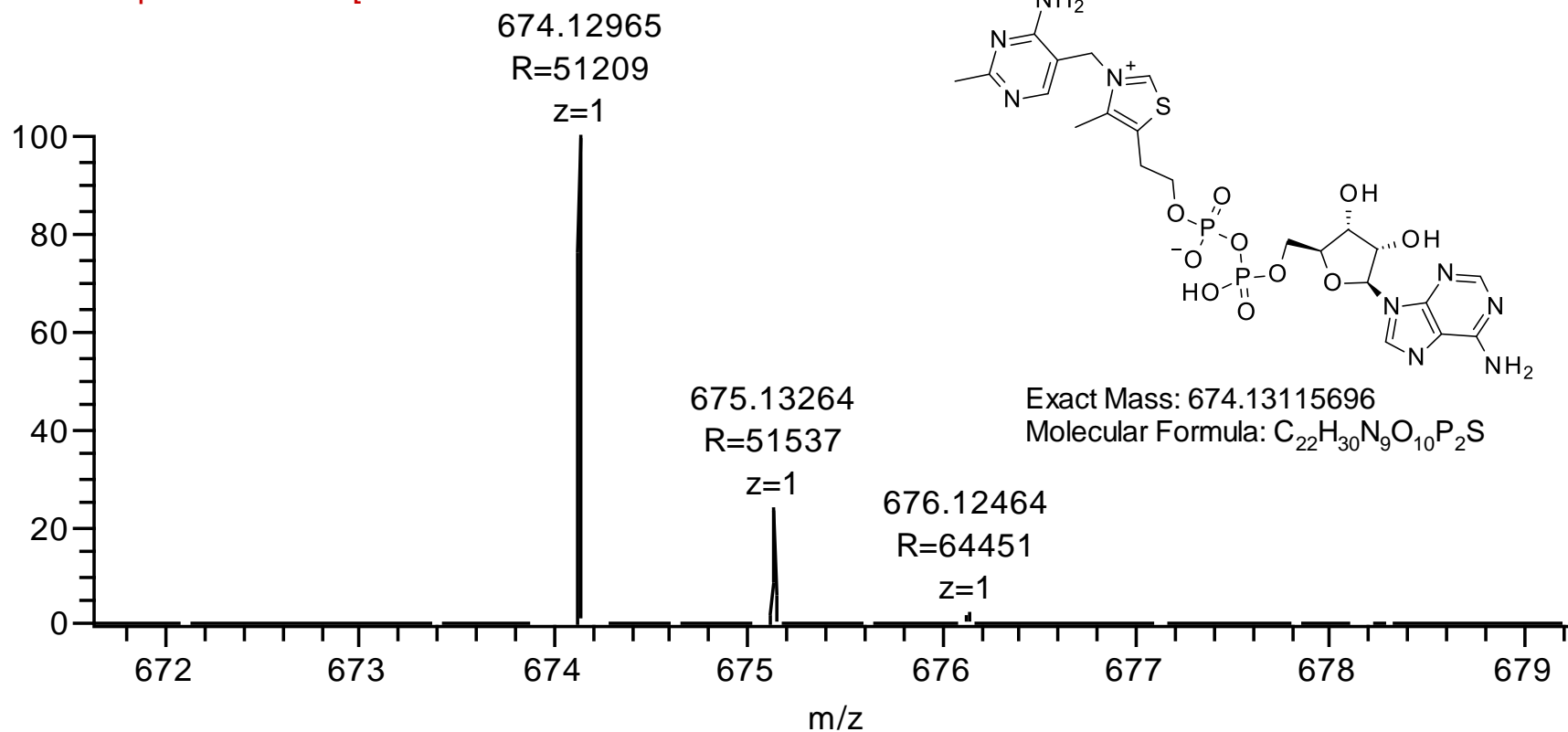
Compound 30a. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$



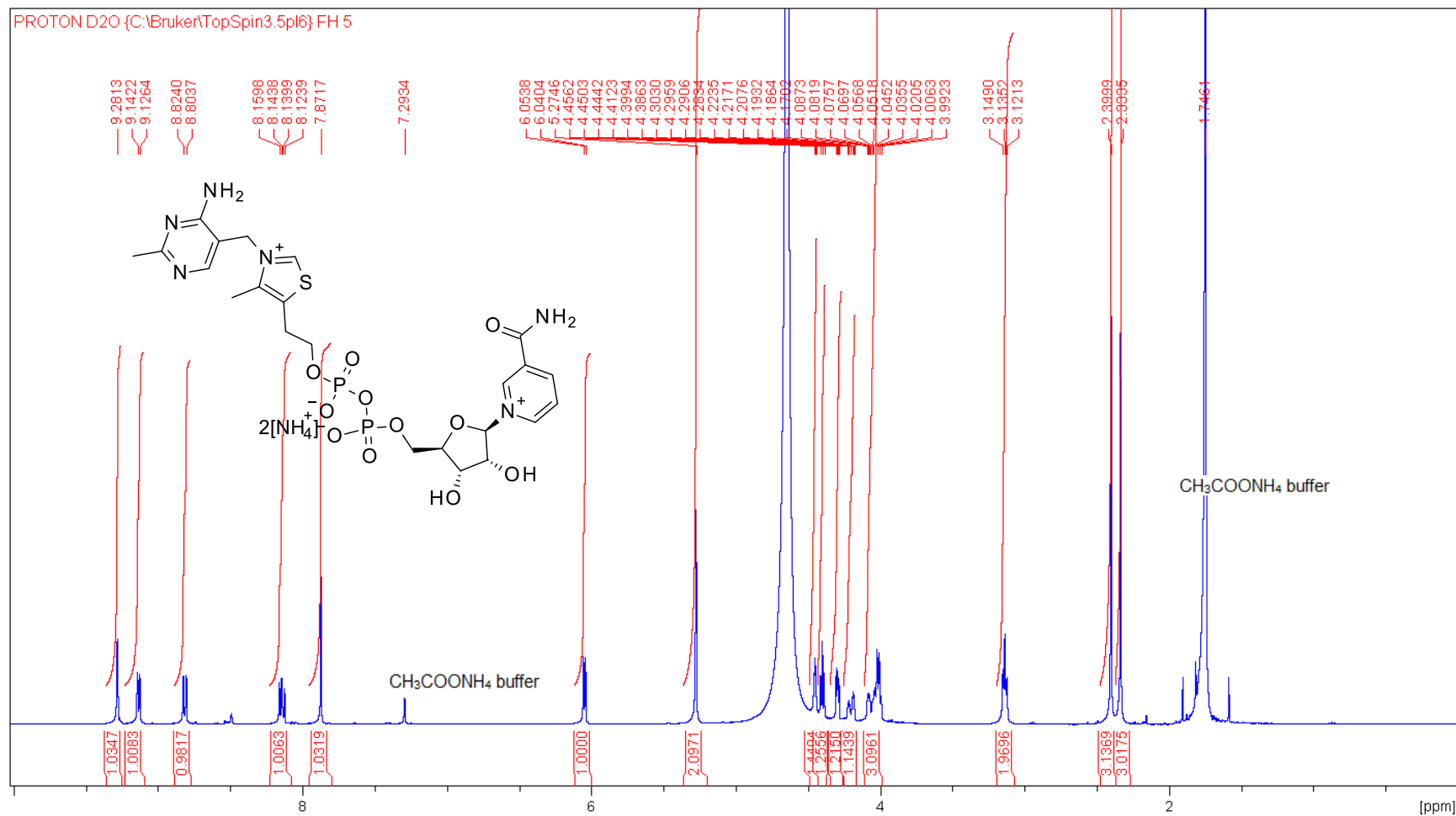
Compound 30a. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

mm\_1119121\_4\_thiamine\_adenosine ... #1652 RT: 0.21-0.65 AV: 13 NL: 9.85E6

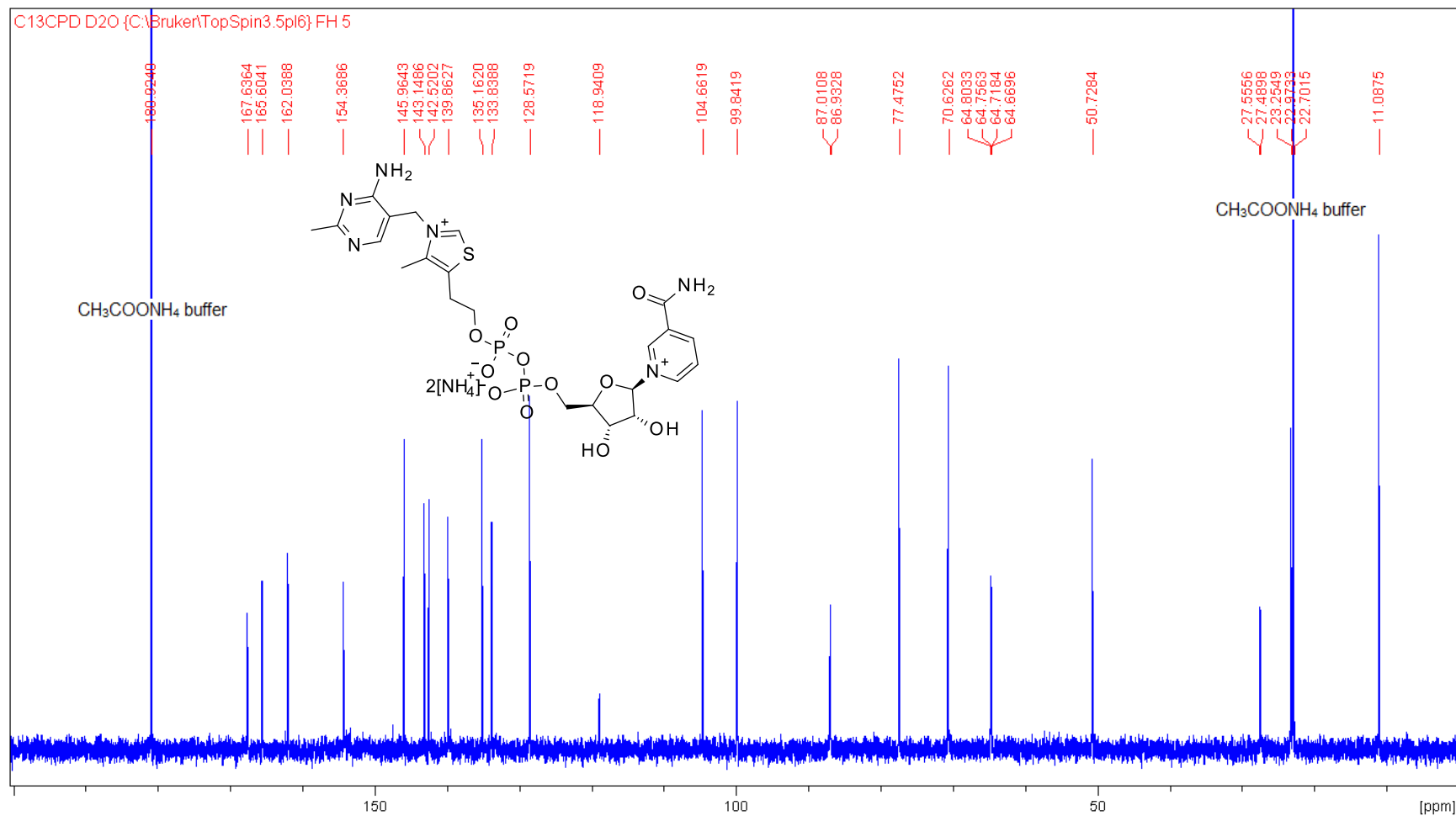
F: FTMS + p ESI Full ms [50.00-900.0]



Compound 30a. HRMS spectra



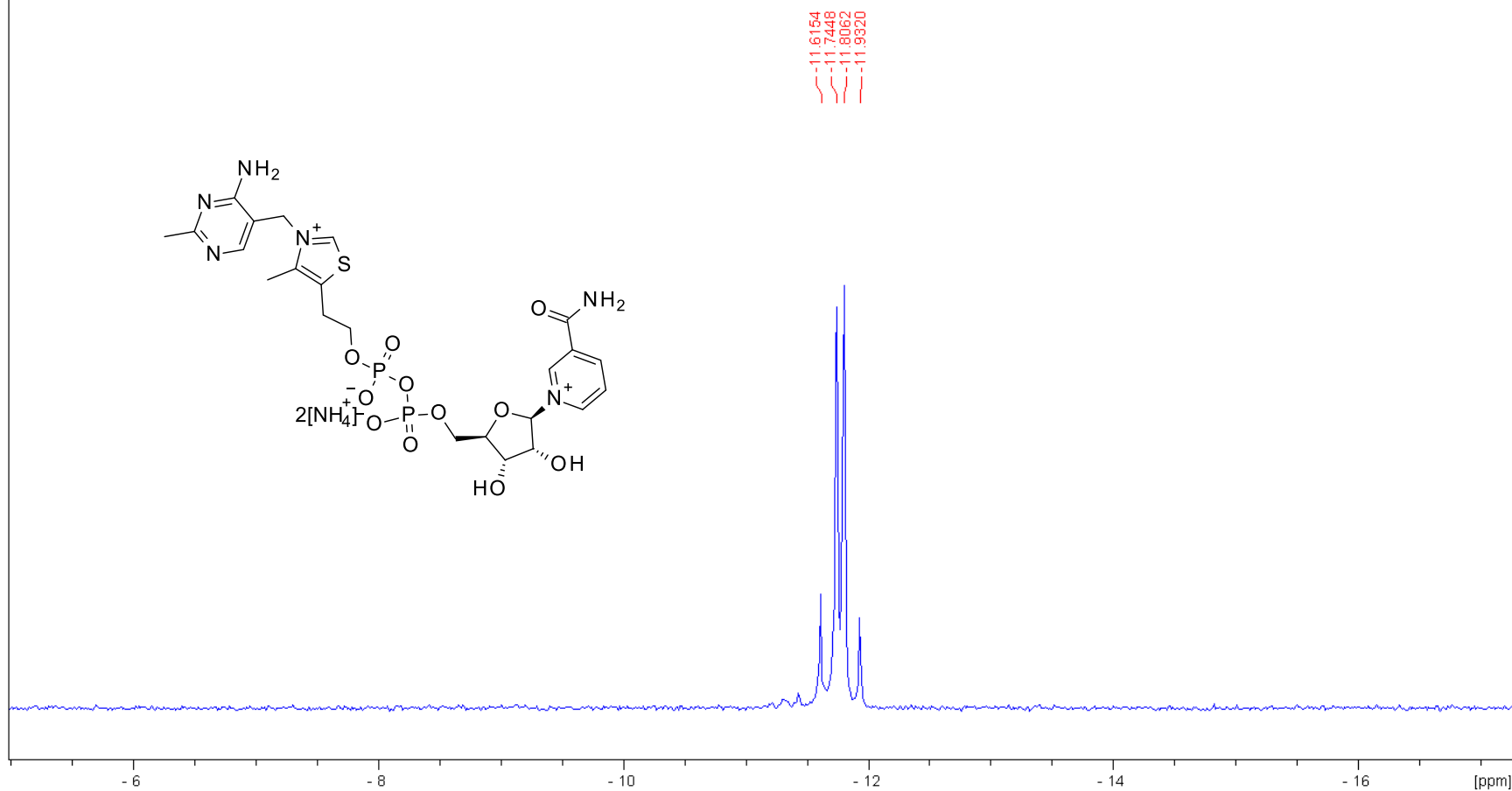
Compound 31. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O



Compound 31. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O



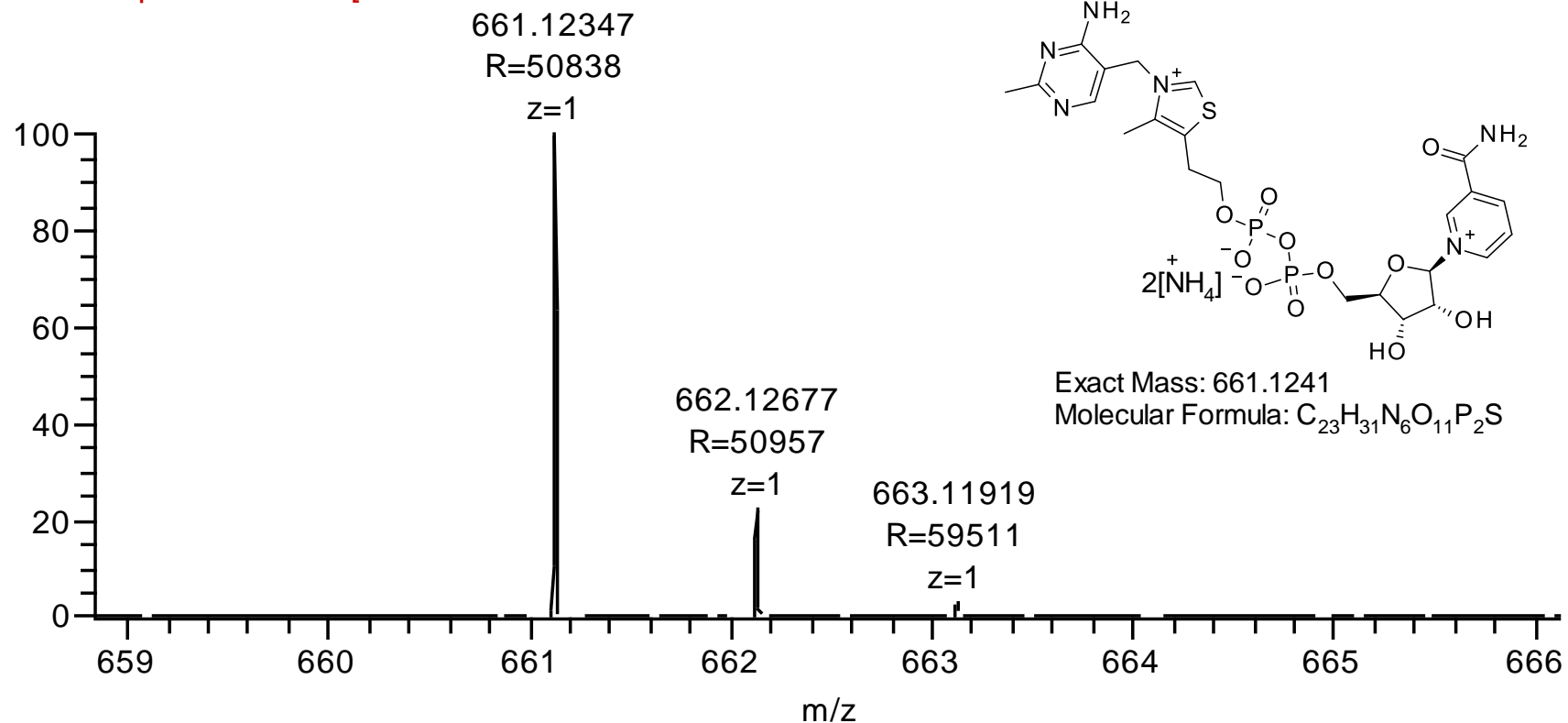
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 5



**Compound 31.** 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

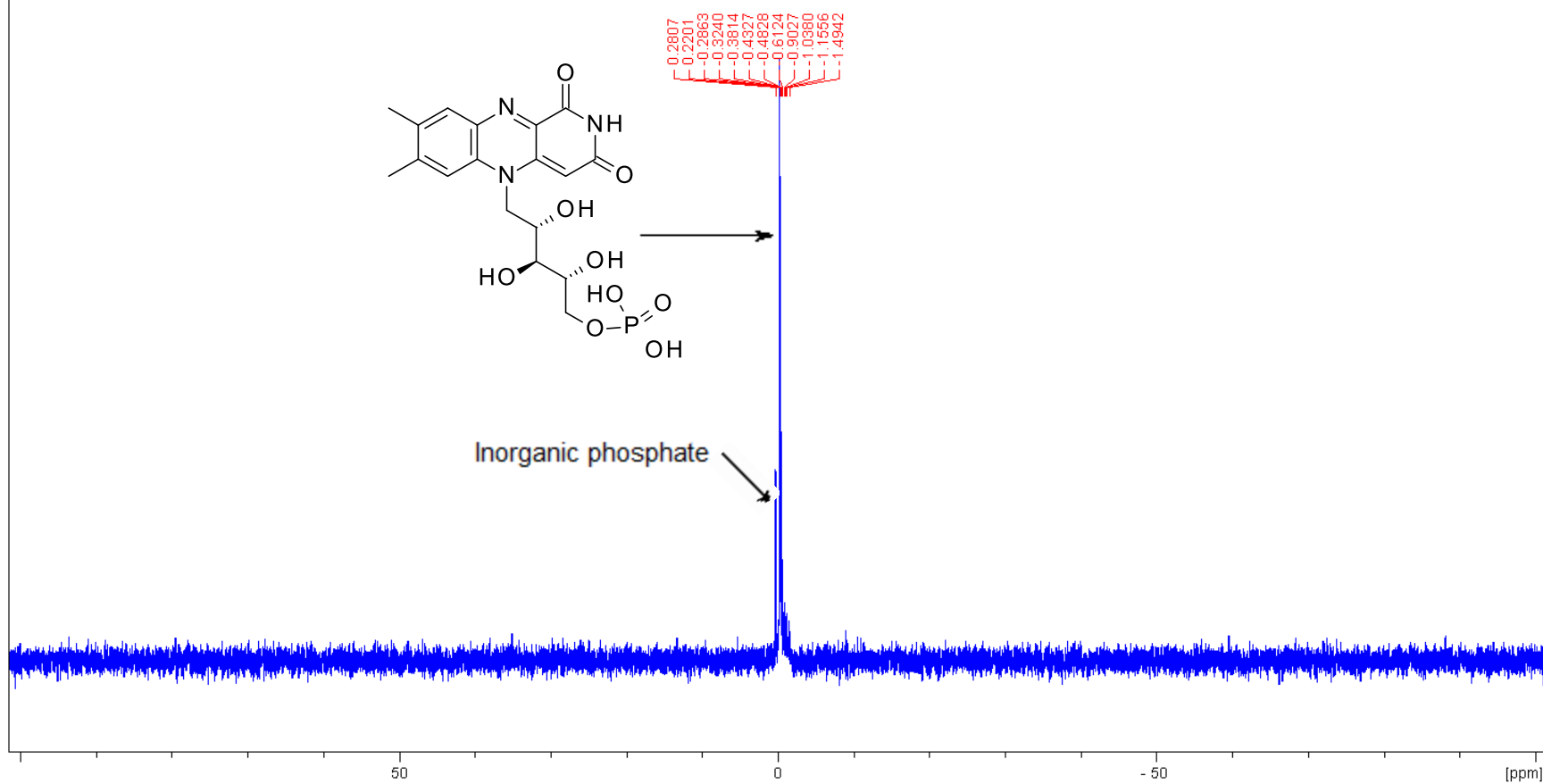
mm\_1119121\_5\_thiamine\_nmn\_pop 02 #19 19 RT: 0.35 0.57 AV: 10 NL: 6.64E6

F: FTMS + p ESI Full ms[50.00-900.0]

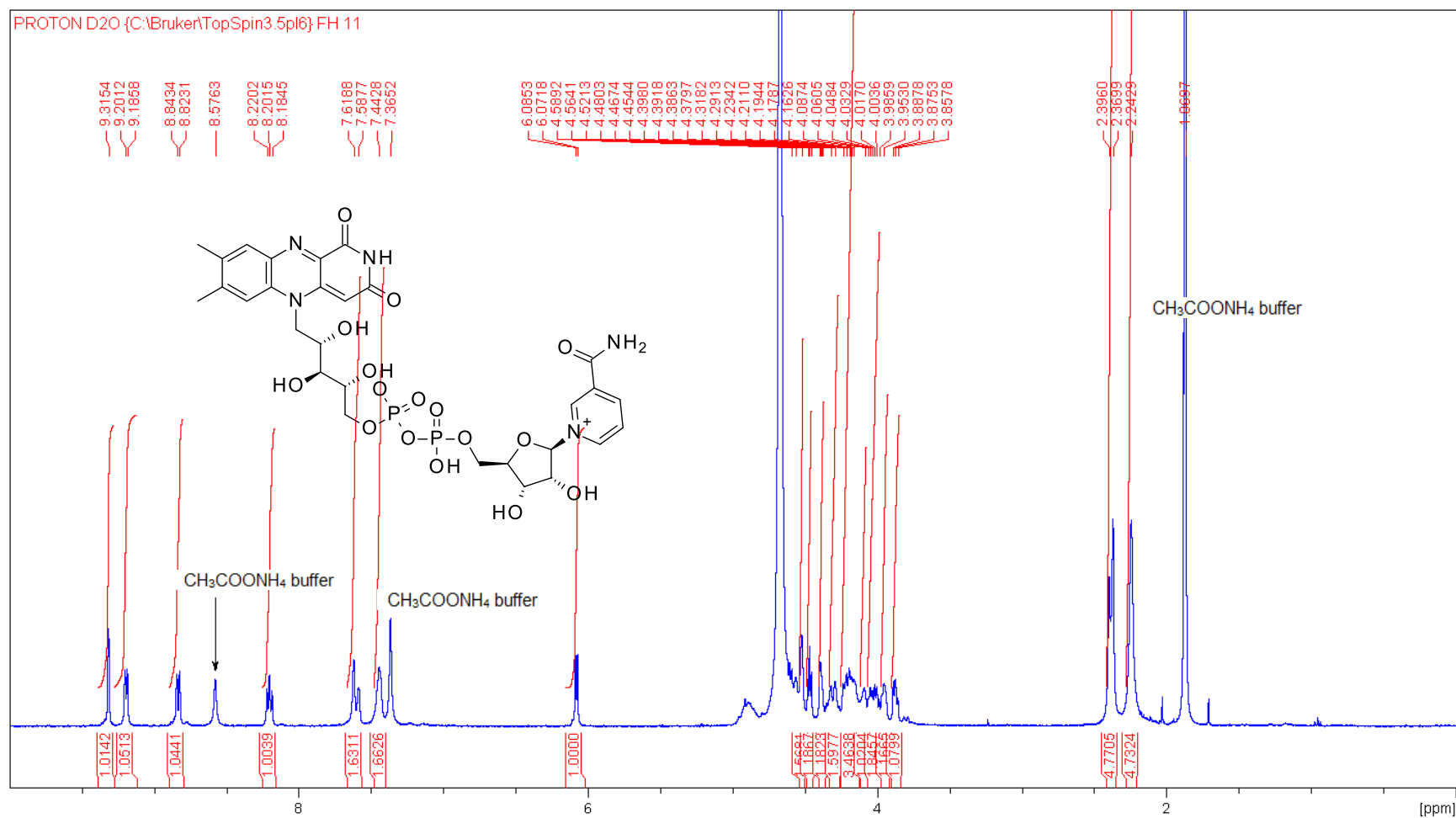


Compound 31. HRMS spectra

P31 DMSO {C:\Bruker\TopSpin3.5pl6} FH 2

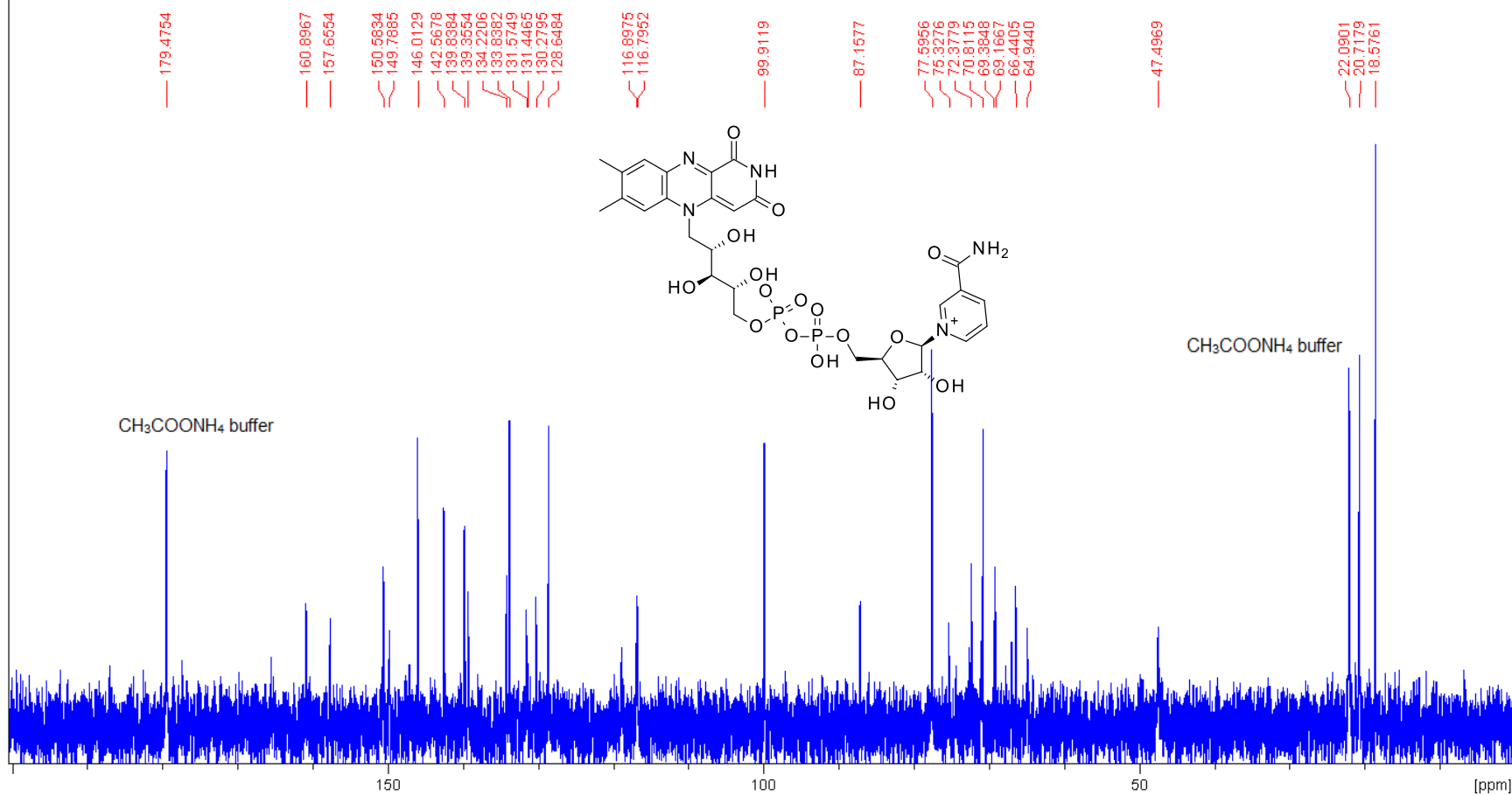


Compound 32. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



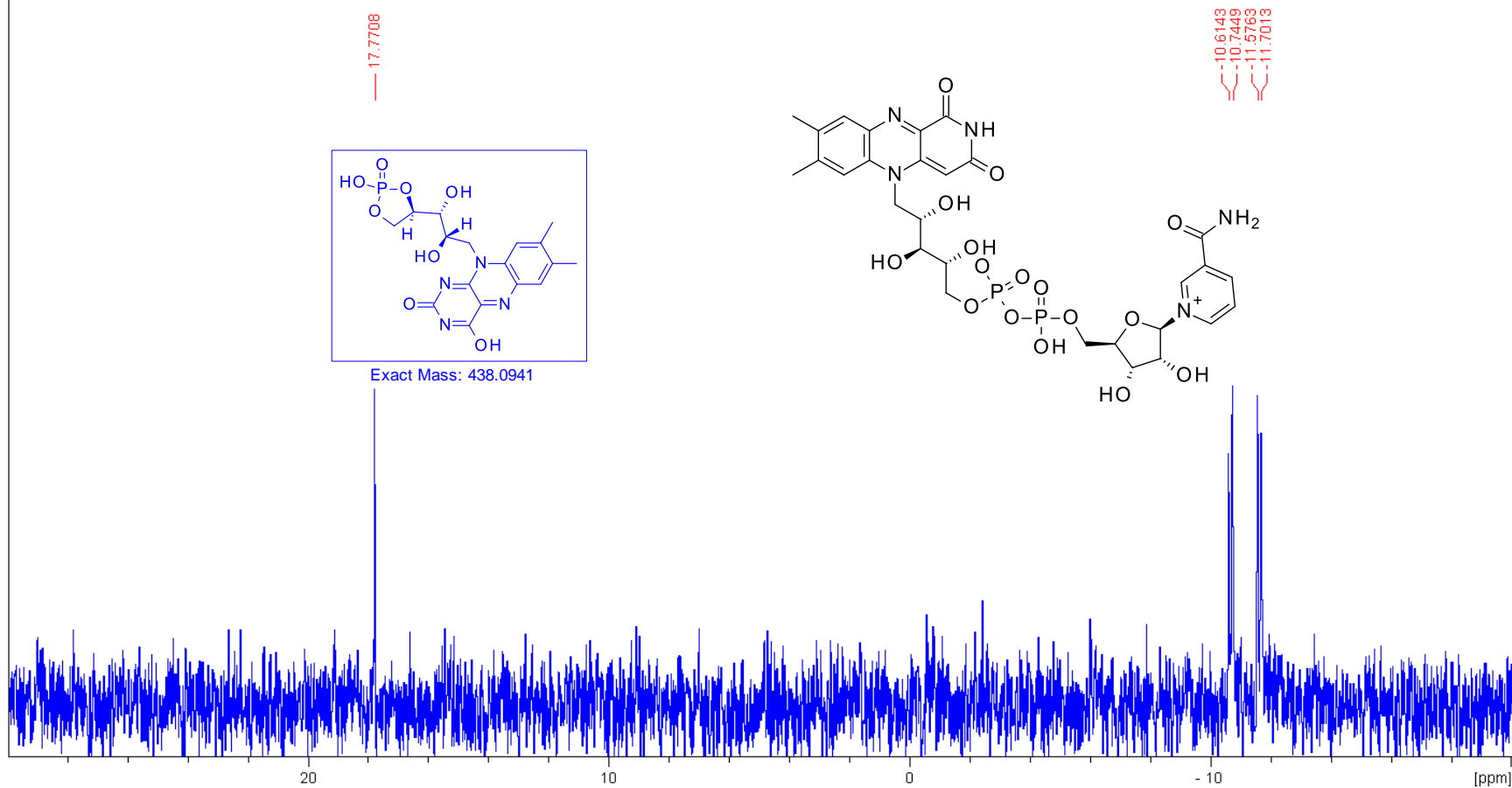
Compound33. 400 MHz <sup>1</sup>H NMR spectrum in D<sub>2</sub>O

C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 11



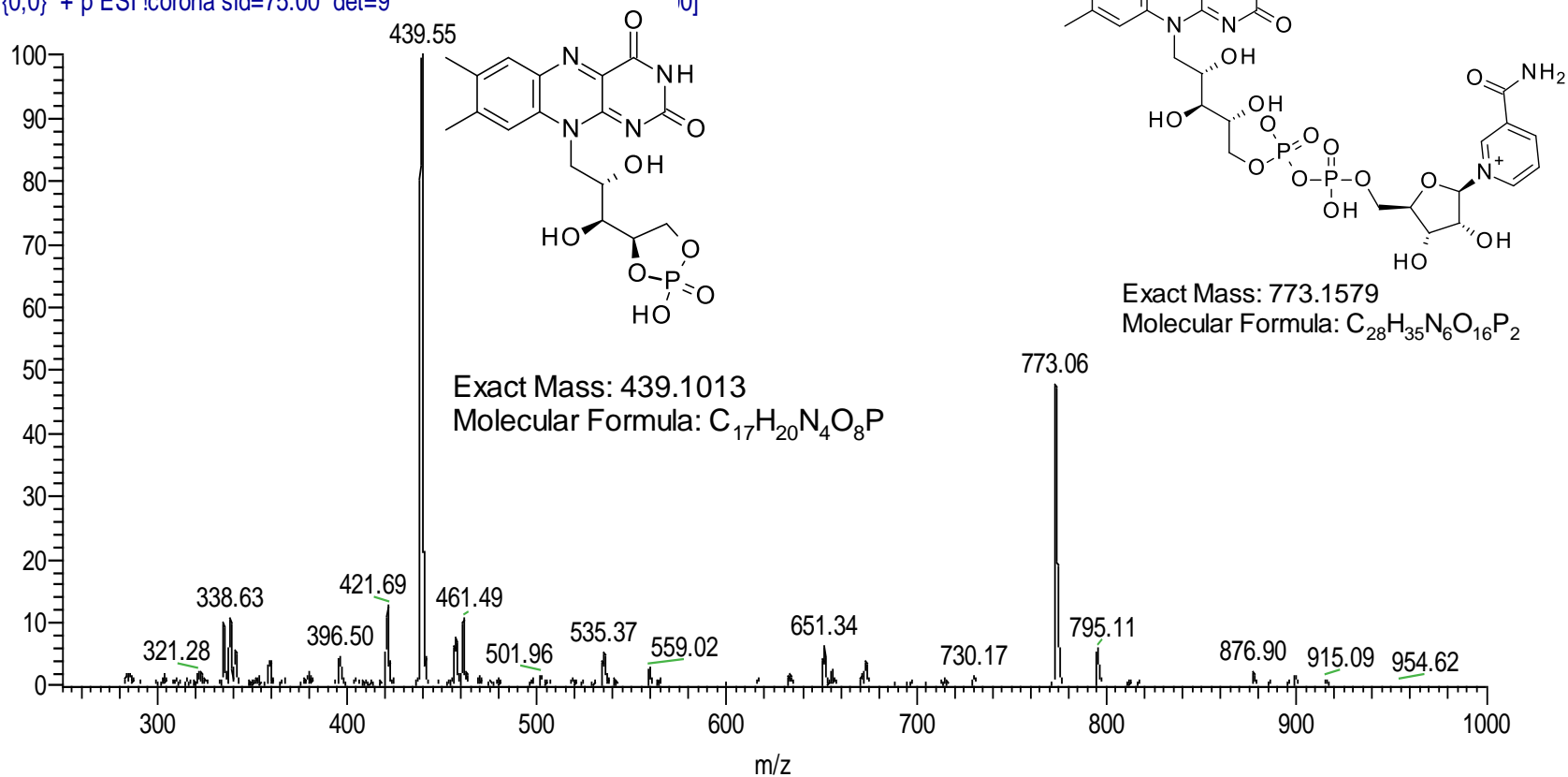
Compound 33. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$

P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 2



Compound 33. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

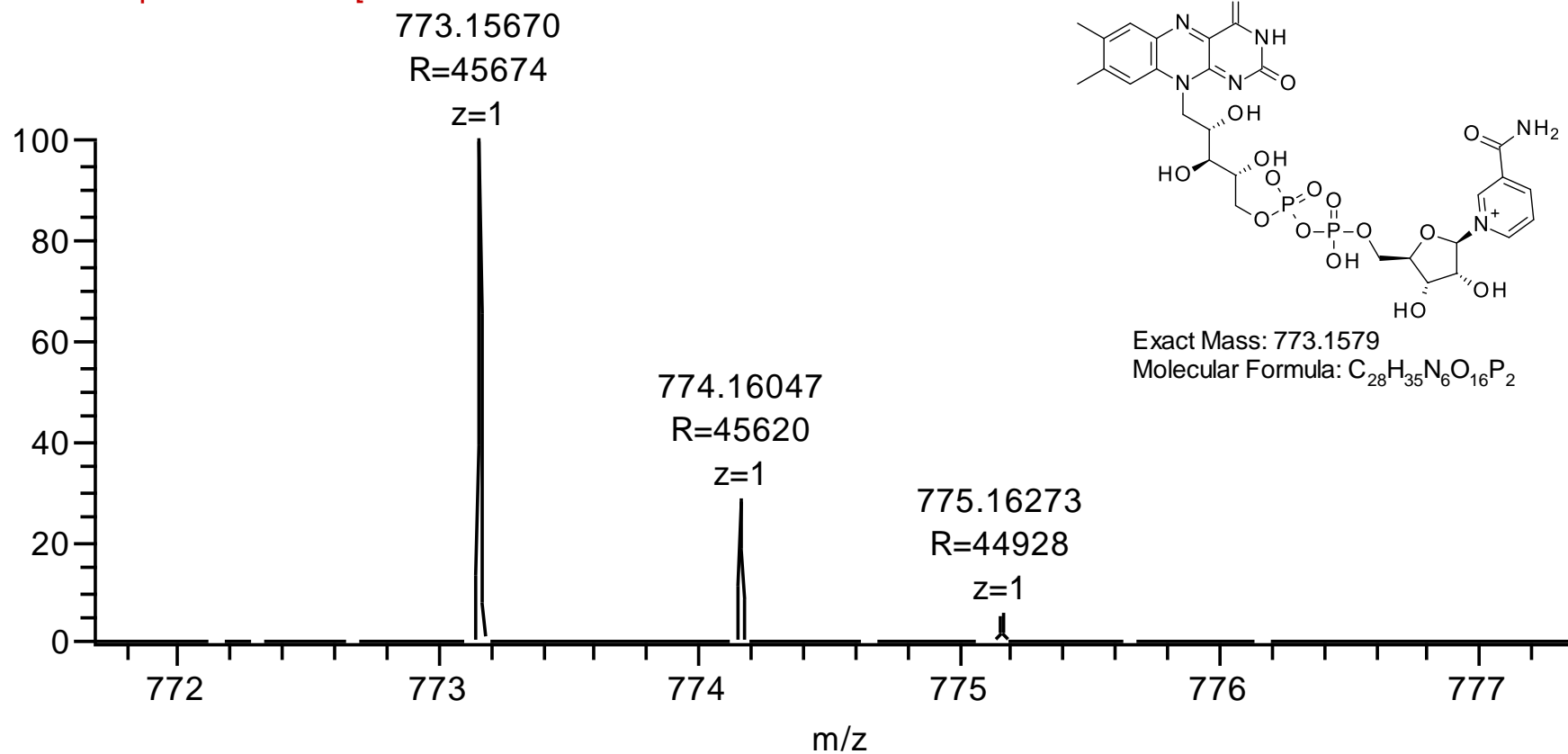
mm\_092720\_fh\_04\_53\_1000mass\_02 #1-33 RT: 0.00-0.28 AV: 33 NL: 3.20E5  
T: {0,0} + p ESI !corona sid=75.00 det=9



**Compound 33&34.** ESI-MS spectra

mm\_1119121\_6\_riboflavin\_nmn\_pop 02 #00 40 RT: 0.42 0.63 AV: 7 NL: 4.93E5

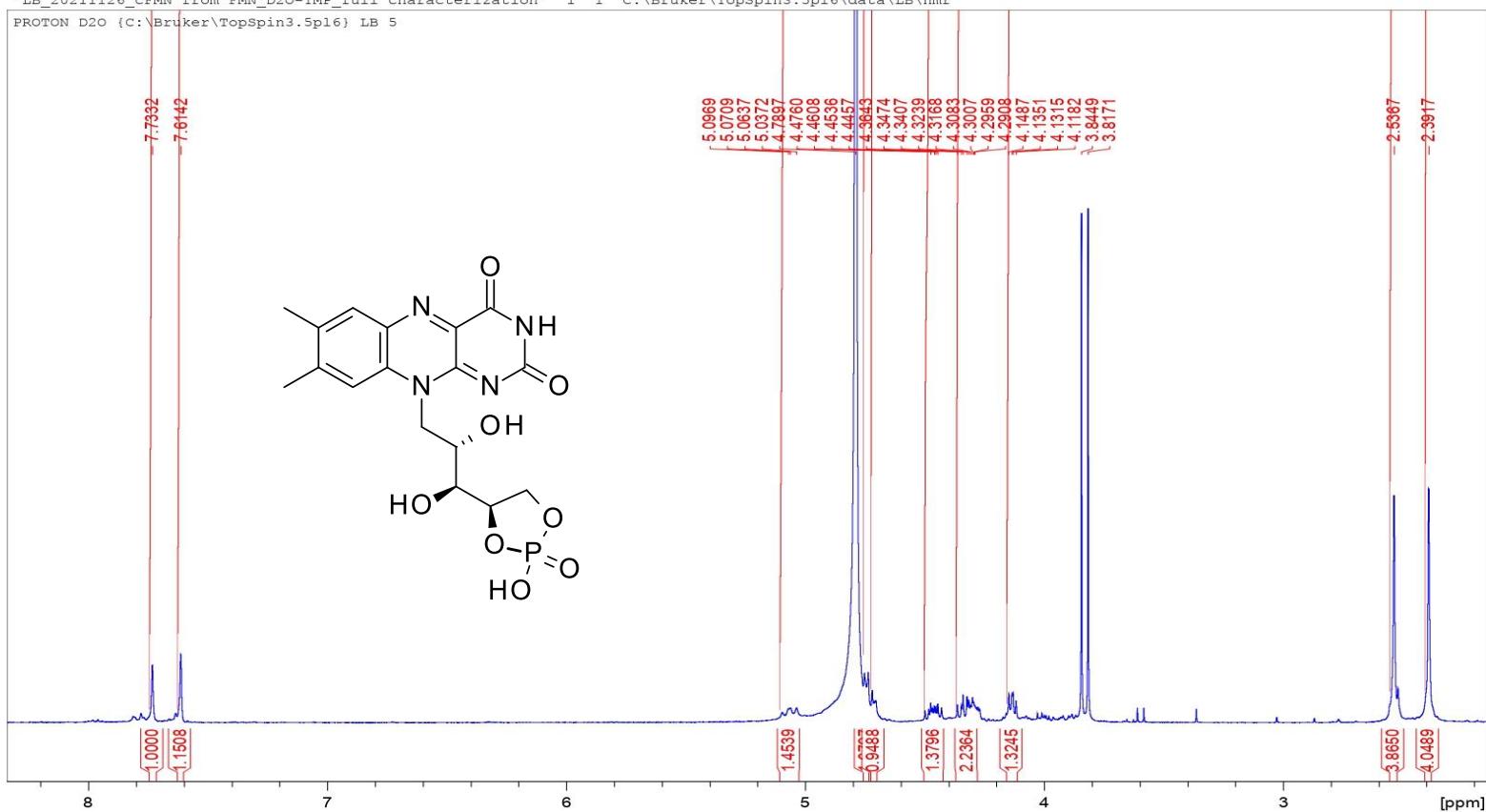
F: FTMS + p ESI Full ms [50.00-900.0]



Compound 33. HRMS spectra

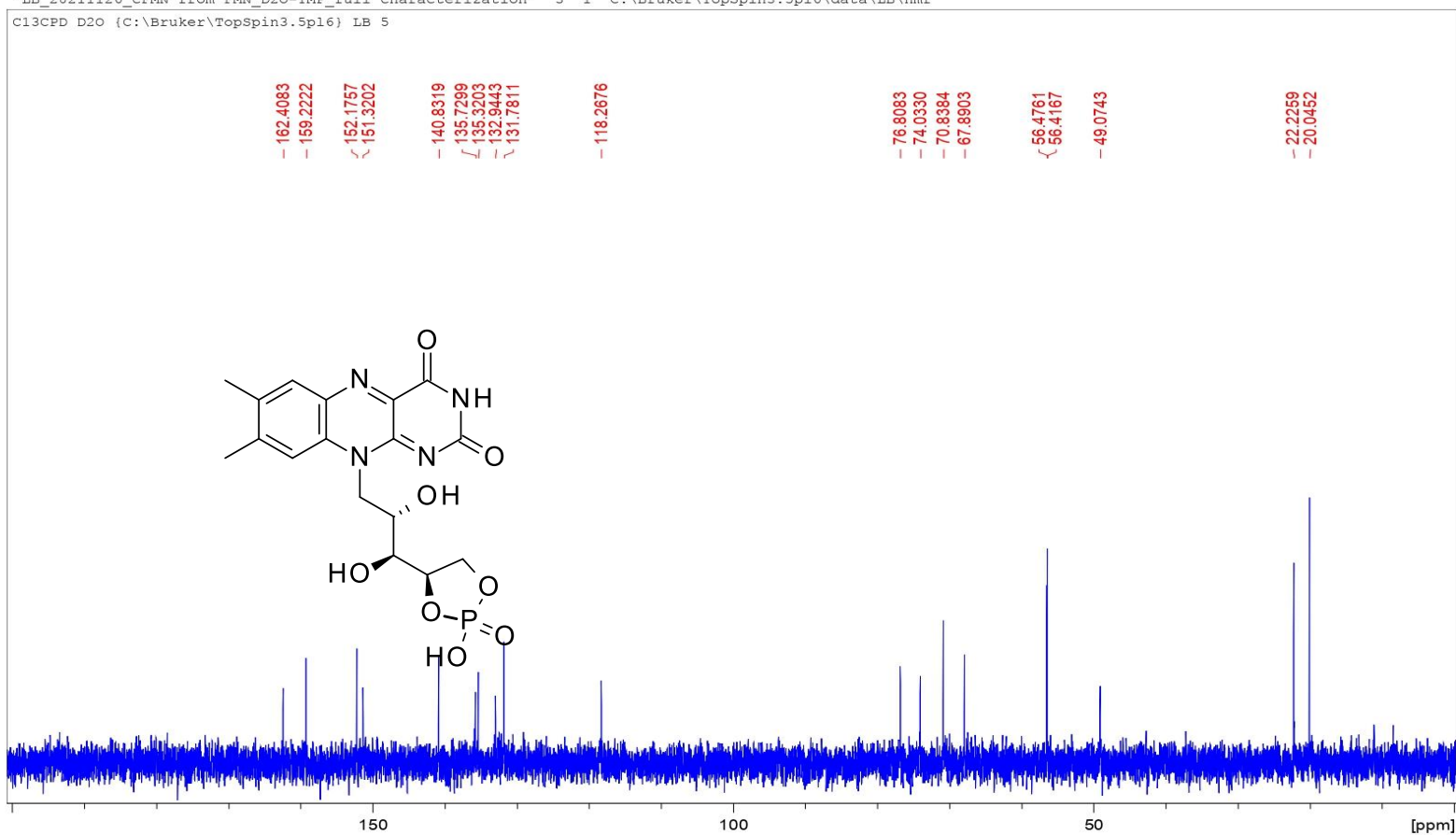


"LB\_20211126\_cFMN from FMN\_D2O-TMP\_full characterization" 1 1 C:\Bruker\TopSpin3.5pl6\data\LB\nmr  
 PROTON D2O {C:\Bruker\TopSpin3.5pl6} LB 5



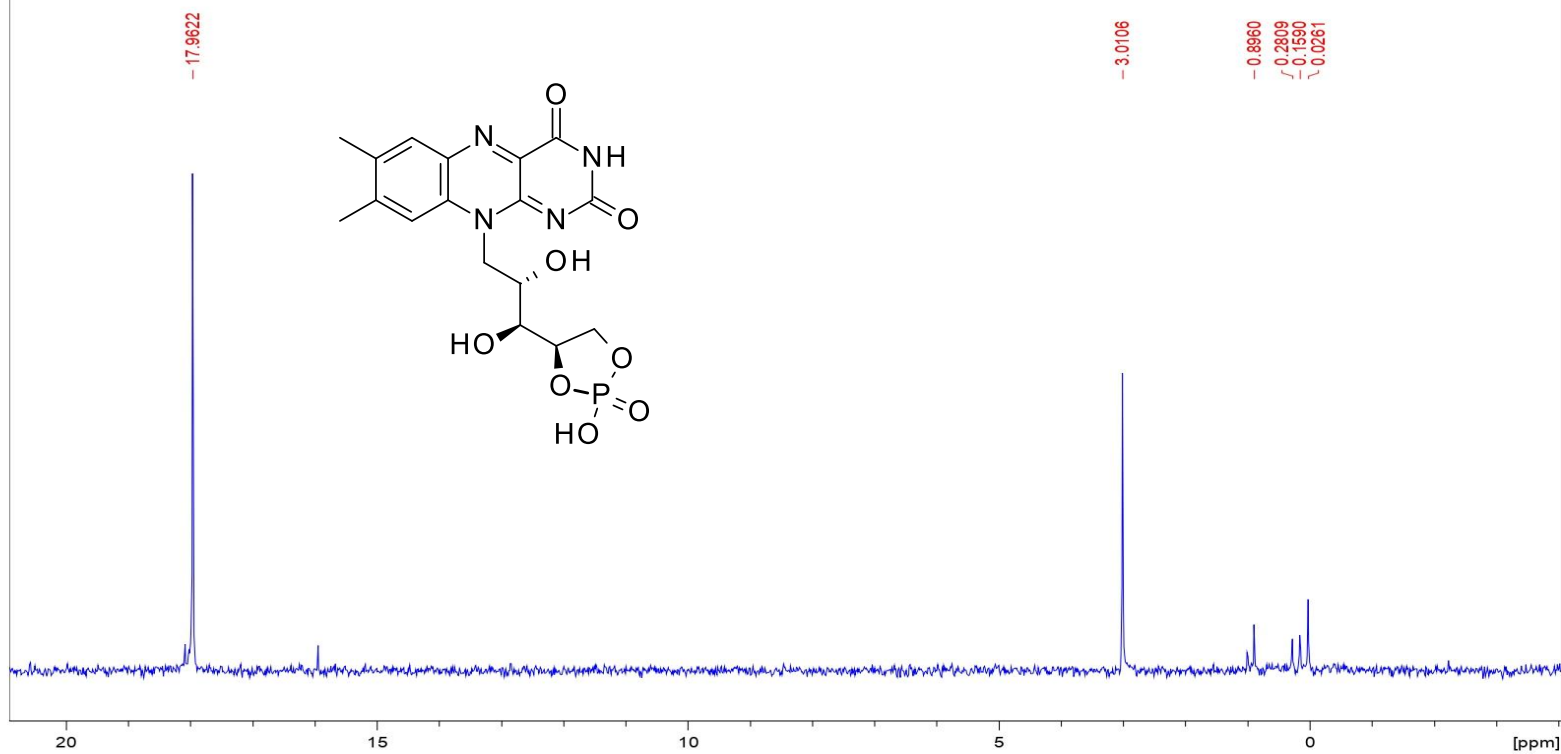
**Compound 34.** 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

"LB\_20211126\_cFMN from FMN\_D2O-TMP\_full characterization" 3 1 C:\Bruker\TopSpin3.5pl6\data\LB\nmr  
 C13CPD D2O {C:\Bruker\TopSpin3.5pl6} LB 5



Compound 34. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$

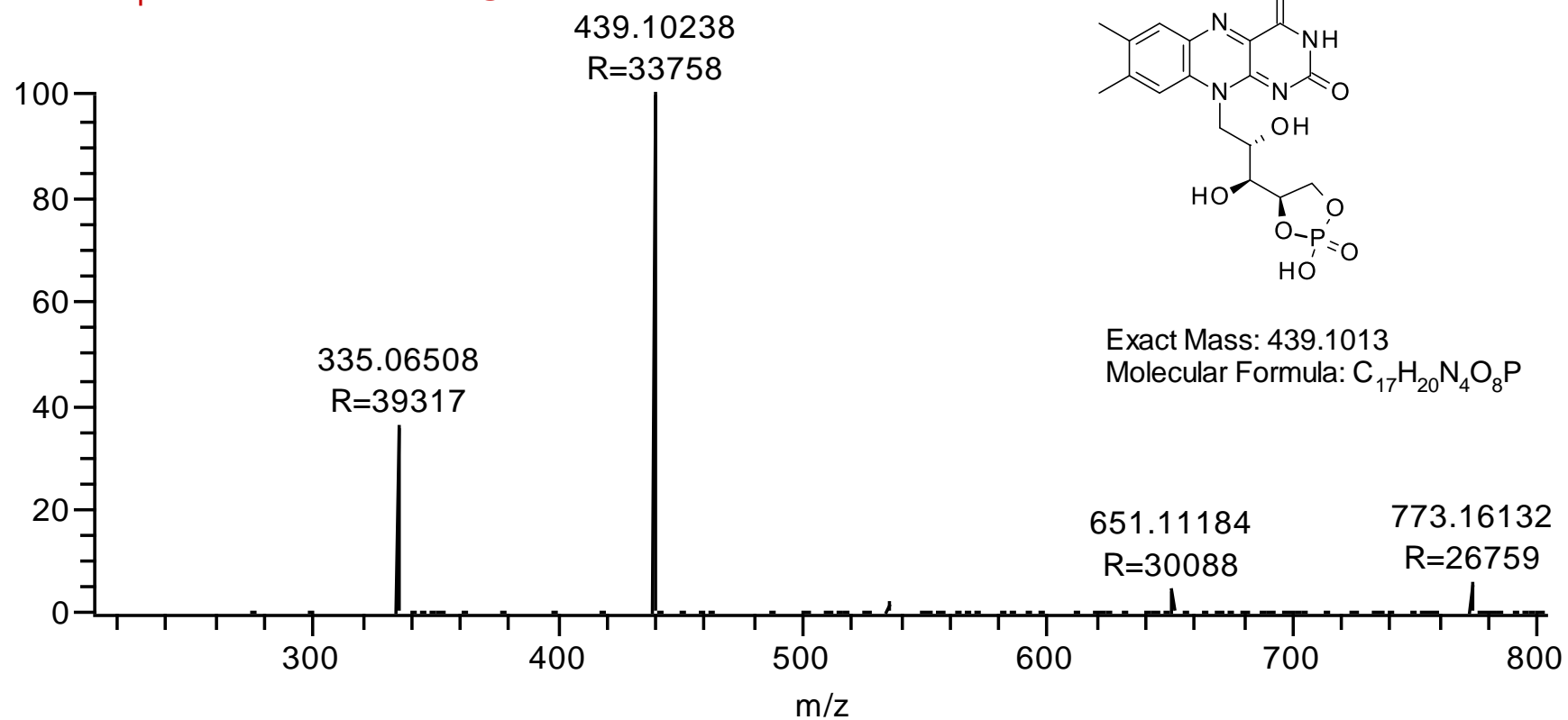
"LB\_20211126\_cFMN from FMN\_D2O-TMP\_full characterization" 2 1 C:\Bruker\TopSpin3.5pl6\data\LB\nmr  
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} LB 5



**Compound 34.** 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

mm\_112221\_riboflavin\_nmn\_pop #244 227 RT: 2.58202 ^V: 27 NL: 1.53E4

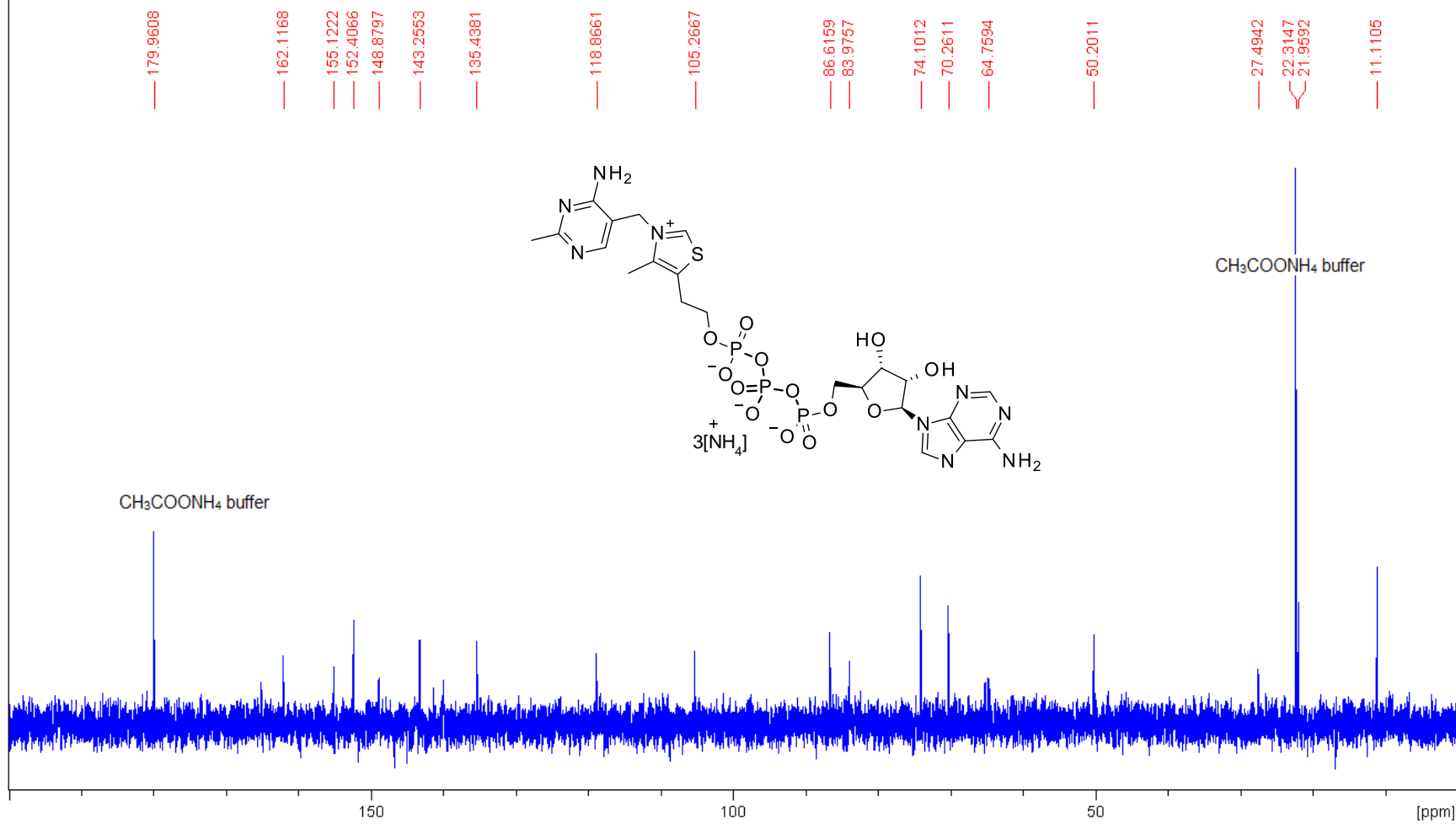
F: FTMS + p ESI Full ms2 773.16@



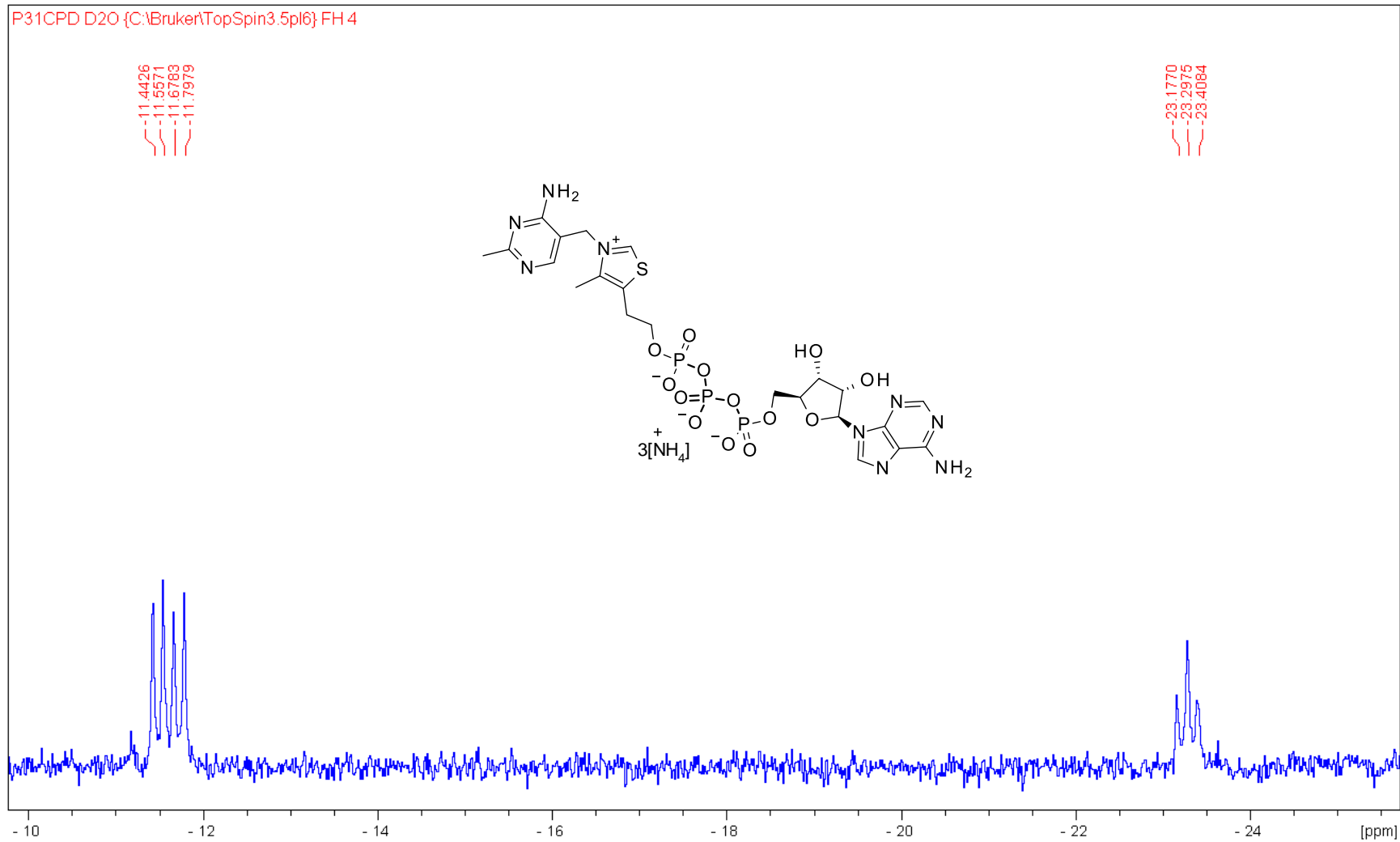
Compound 34. HRMS spectra



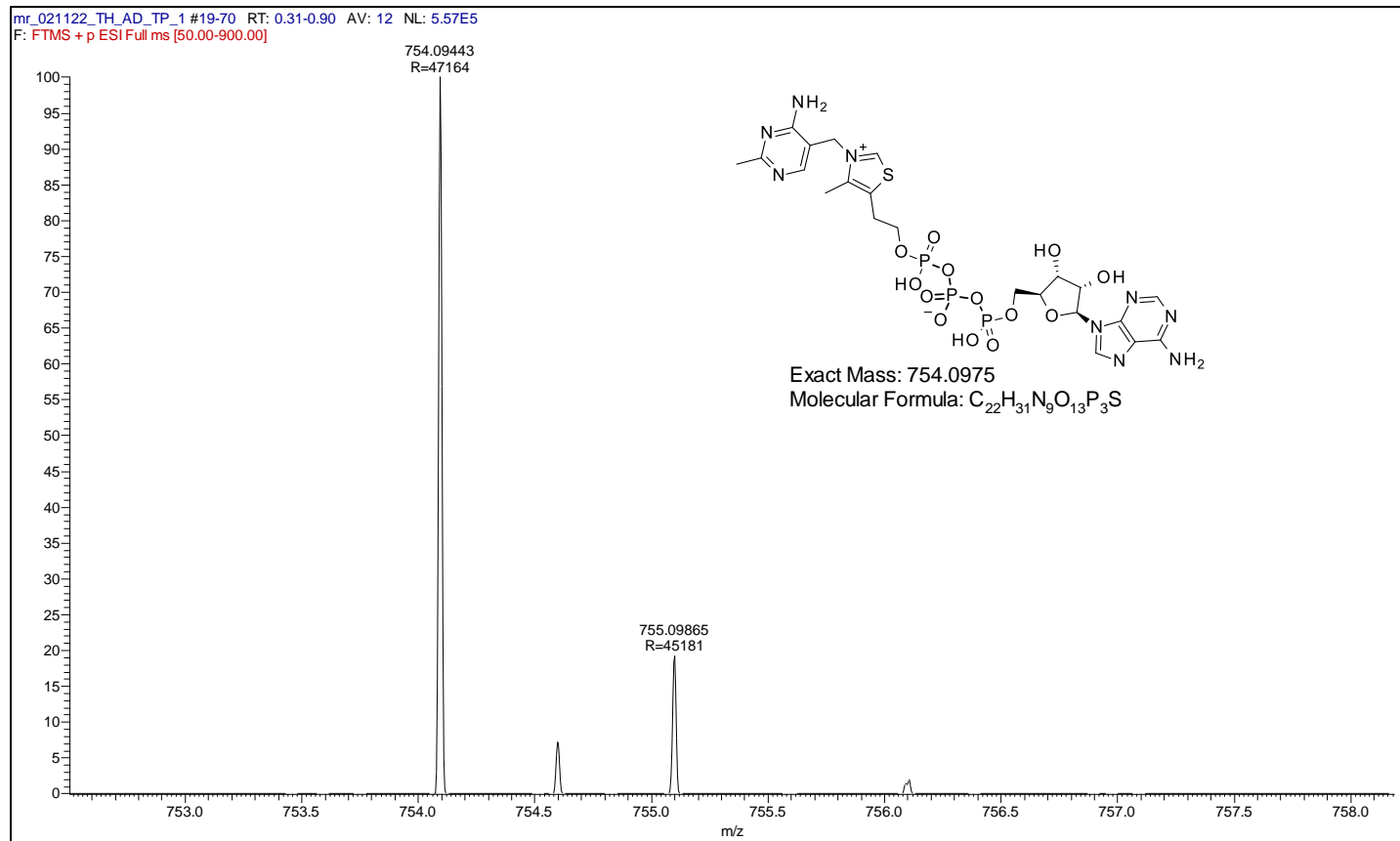
C13CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 24



Compound 37. 100 MHz <sup>13</sup>C NMR spectrum in D<sub>2</sub>O

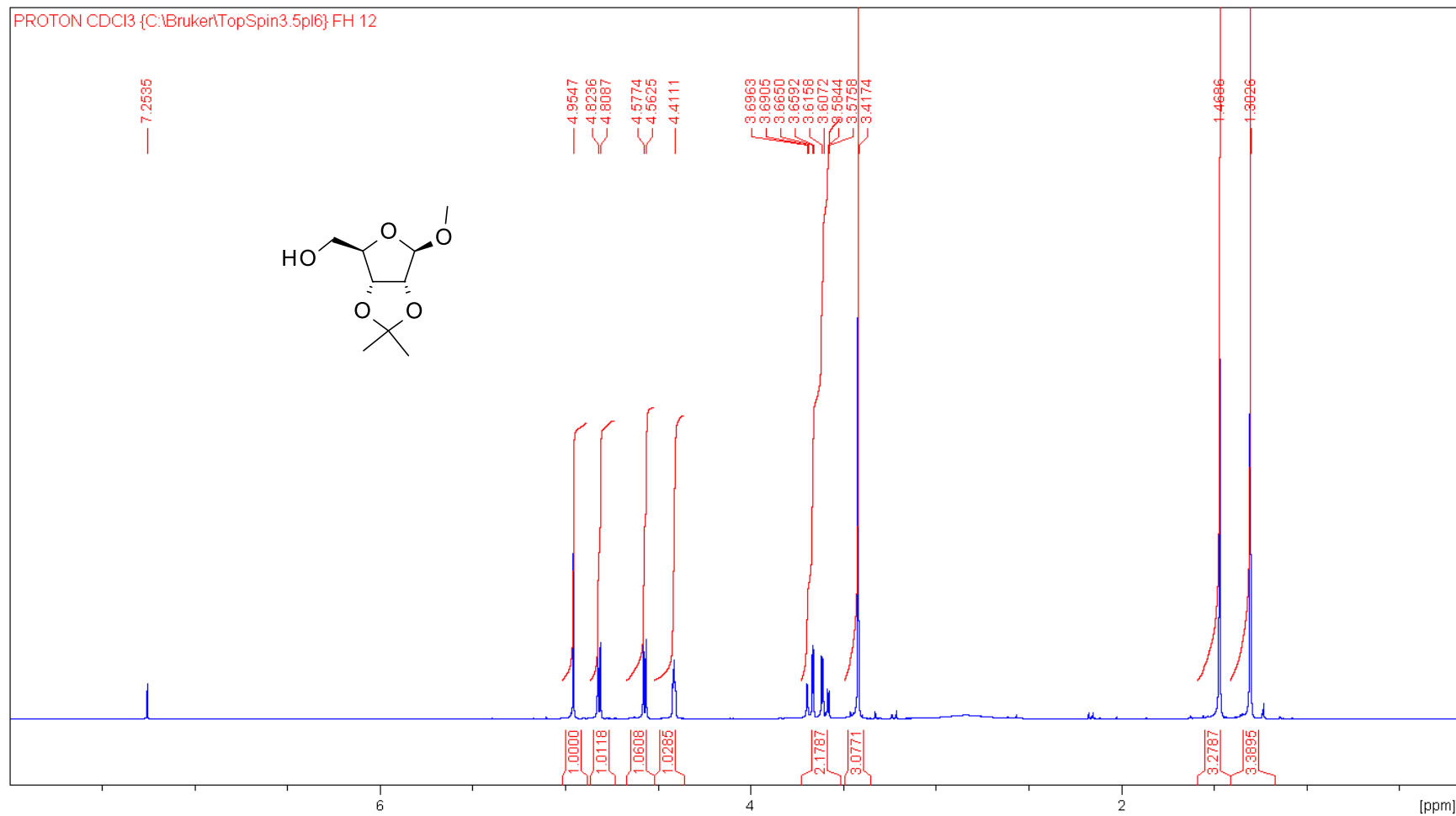


**Compound 37.** 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$



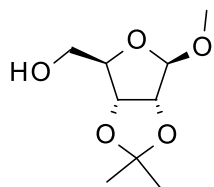
Compound 37. HRMS spectra





Compound 39. 400 MHz <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub>

C13CPD CDCl3 {C:\Bruker\TopSpin3.5pl6} FH 20



112.0738  
109.9401

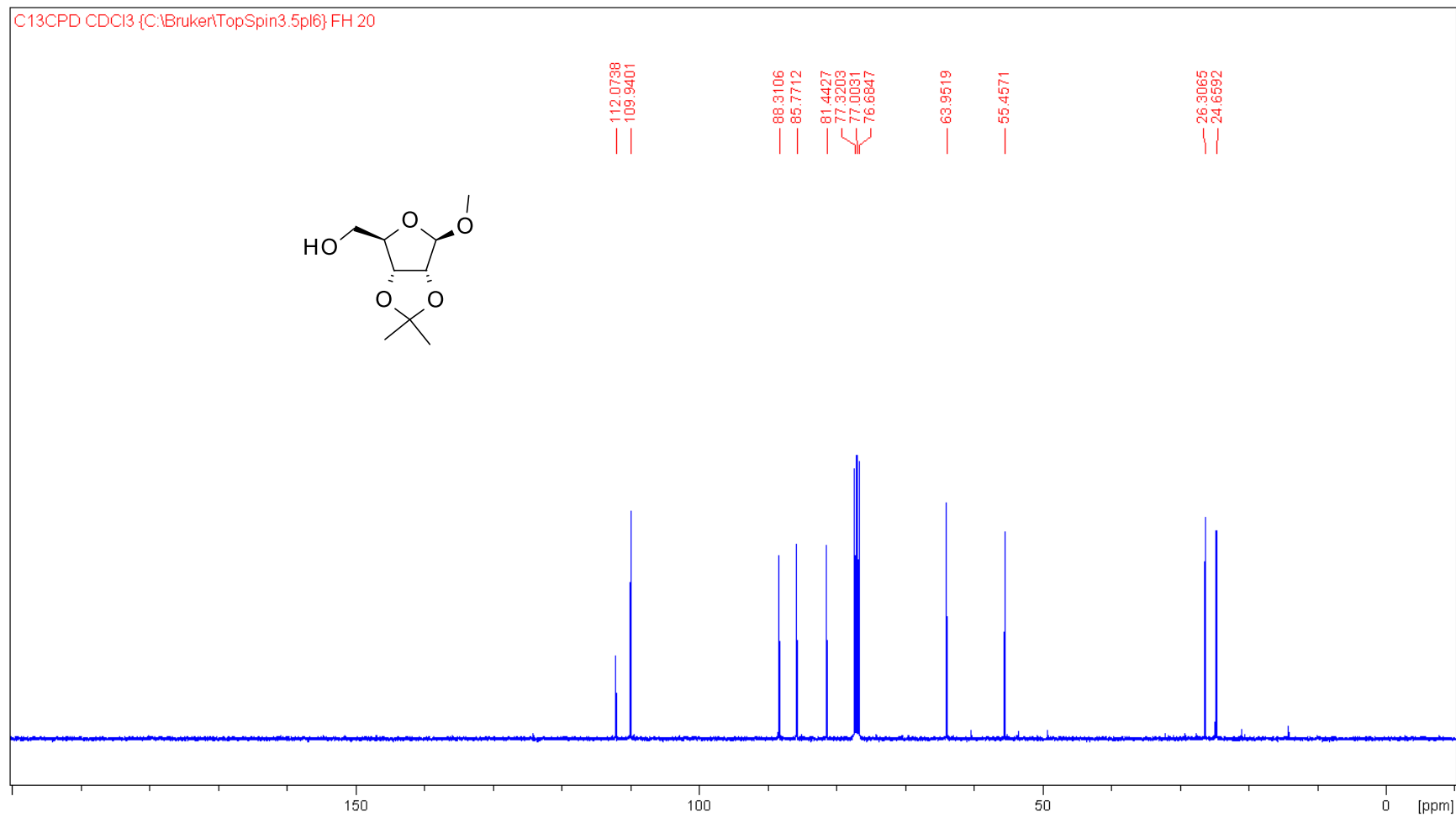
88.3106  
85.7712

81.4427  
77.3203  
77.0081  
76.6847

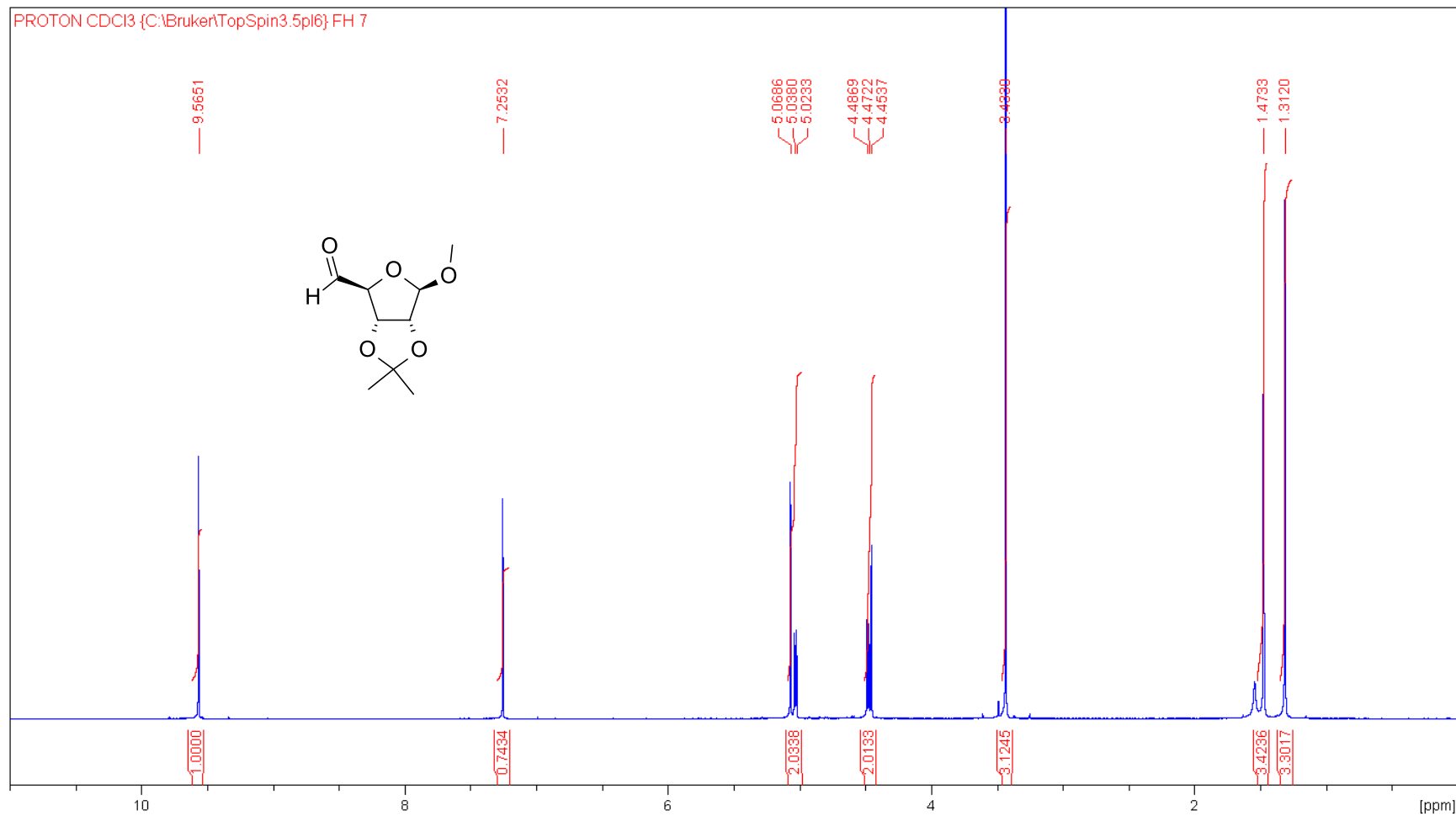
63.9519

55.4571

26.3065  
24.6592

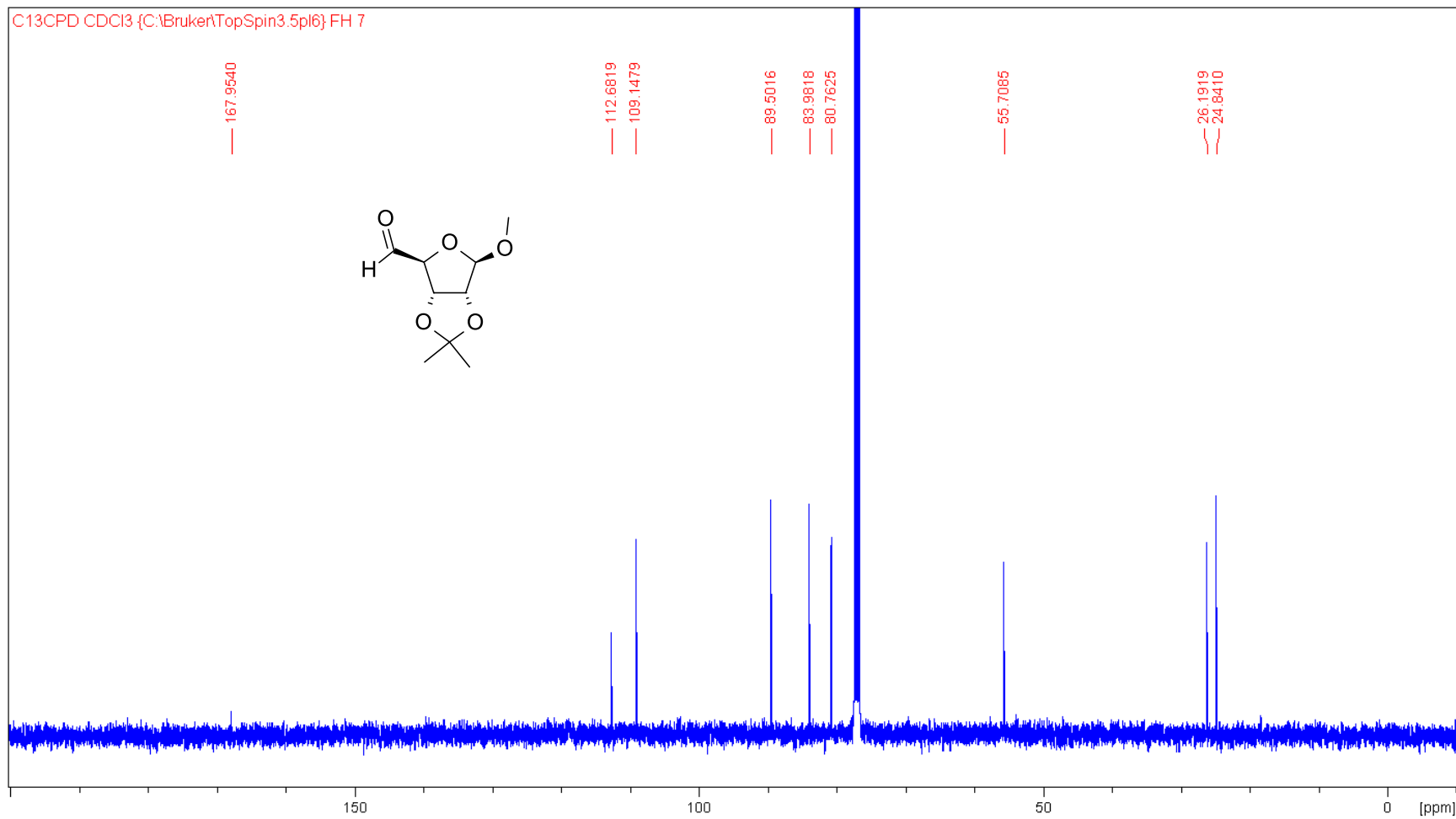
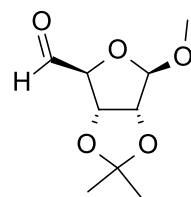


Compound 39. 100 MHz  $^{31}\text{C}$  NMR spectrum in  $\text{CDCl}_3$

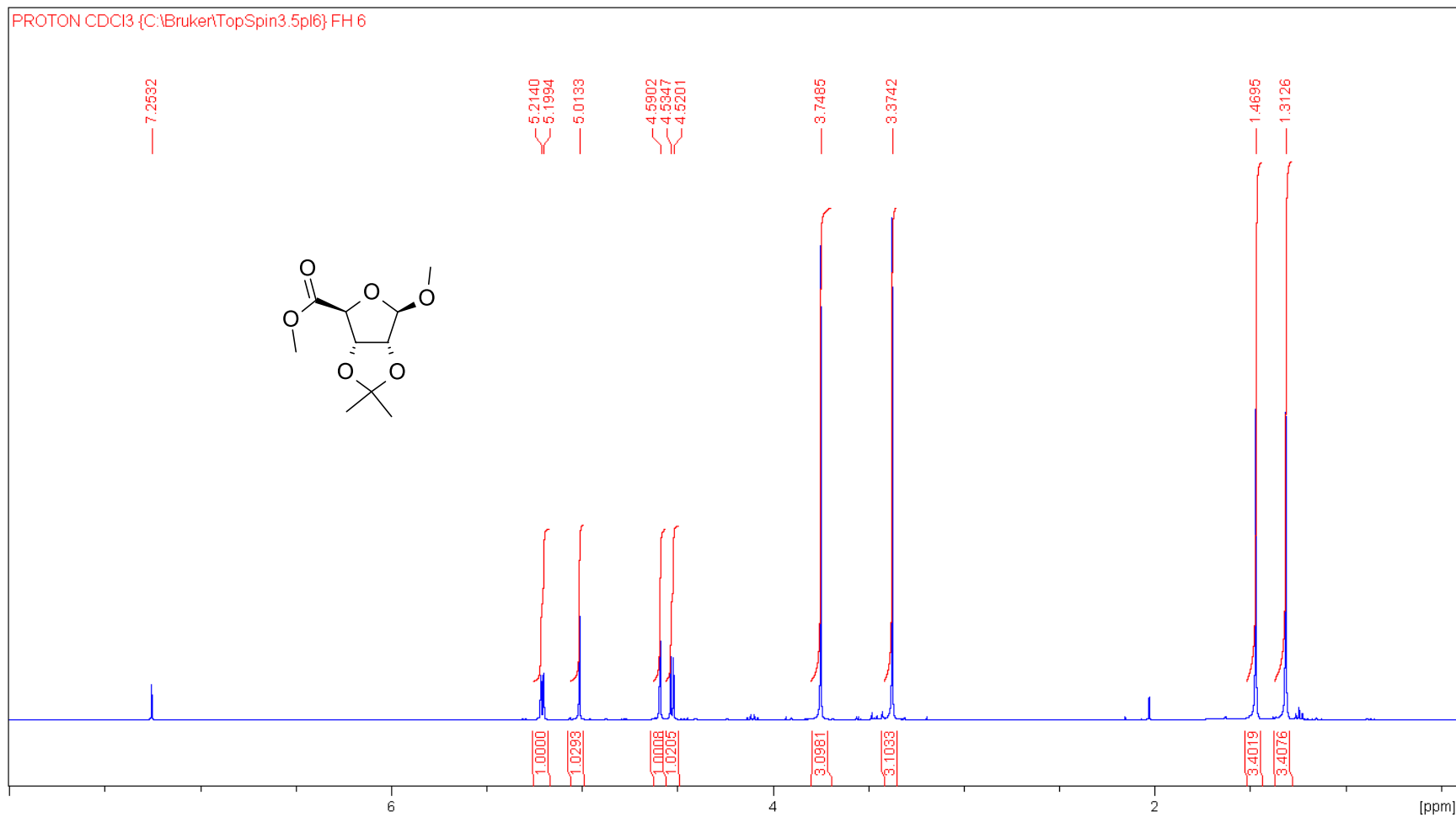


Compound 39. 400 MHz <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub>

C13CPD CDCl3 {C:\Bruker\TopSpin3.5pl6} FH 7

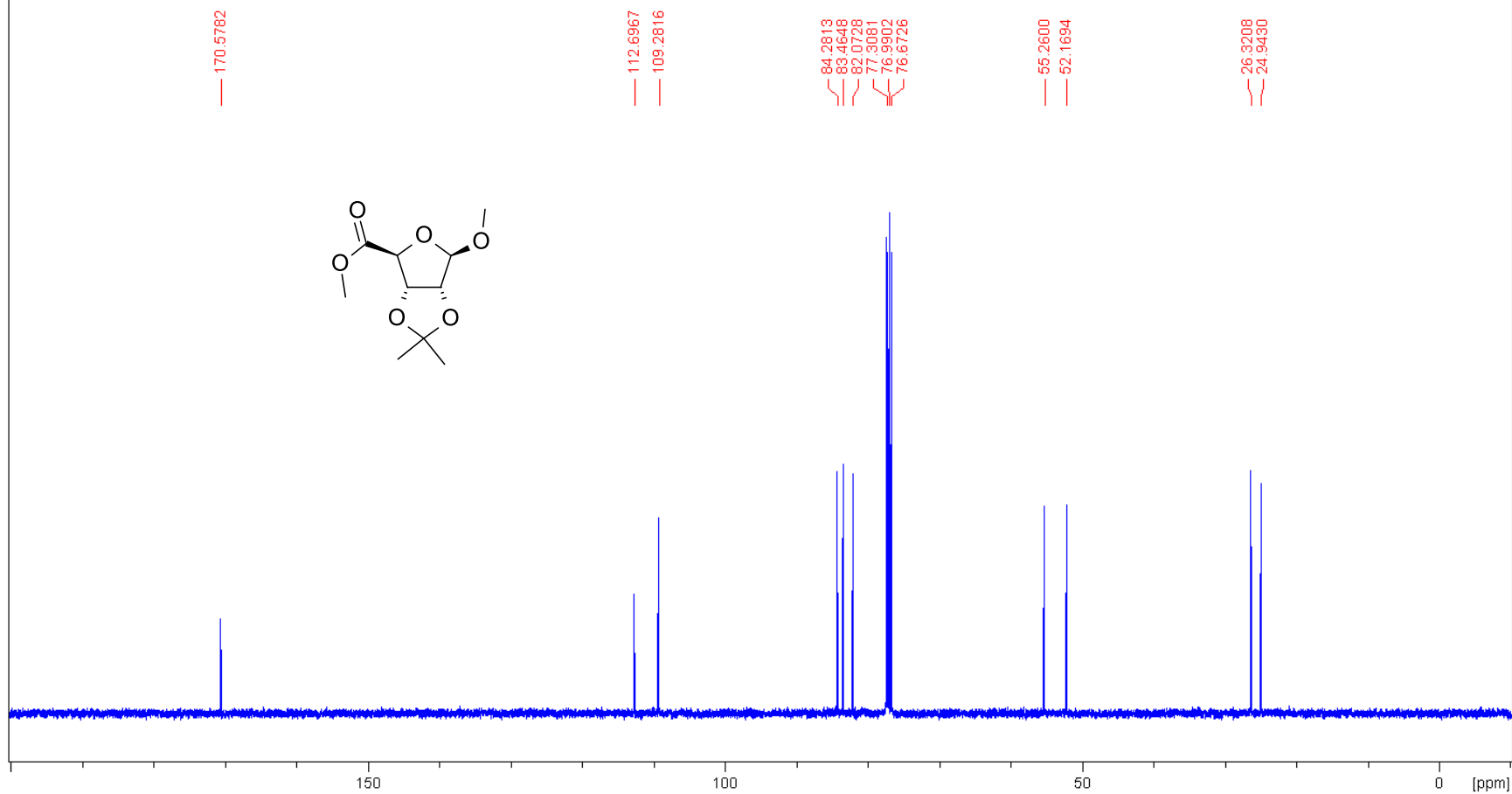


Compound 40. 100 MHz  $^{31}\text{C}$  NMR spectrum in  $\text{CDCl}_3$



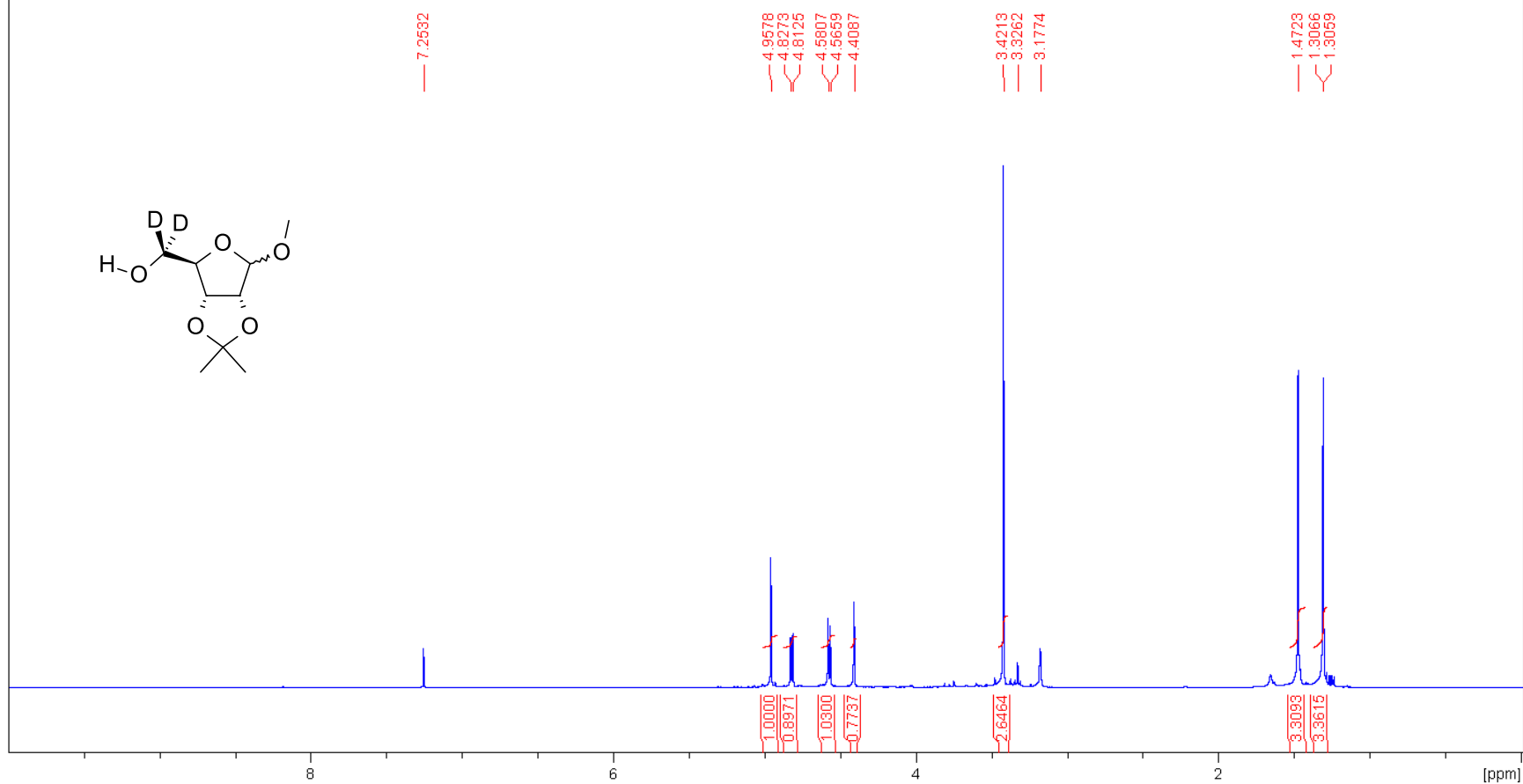
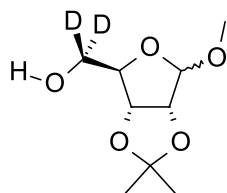
Compound 41. 400 MHz <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub>

C13CPD CDCl3 {C:\Bruker\TopSpin3.5pl6} FH 6

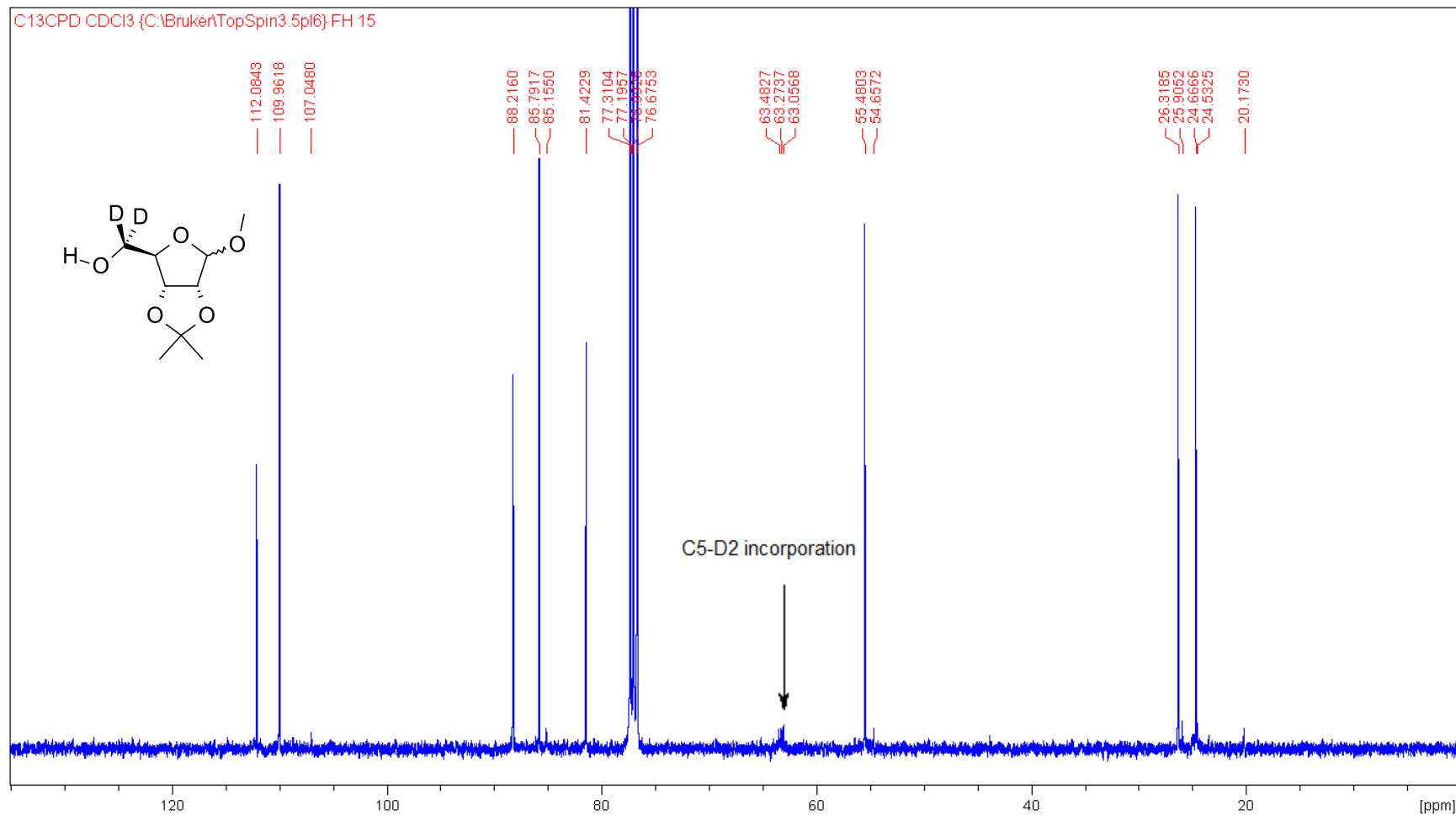


Compound 41. 100 MHz  $^{31}\text{C}$  NMR spectrum in  $\text{CDCl}_3$

PROTON CDCl3 {C:\Bruker\TopSpin3.5pl6} FH 15

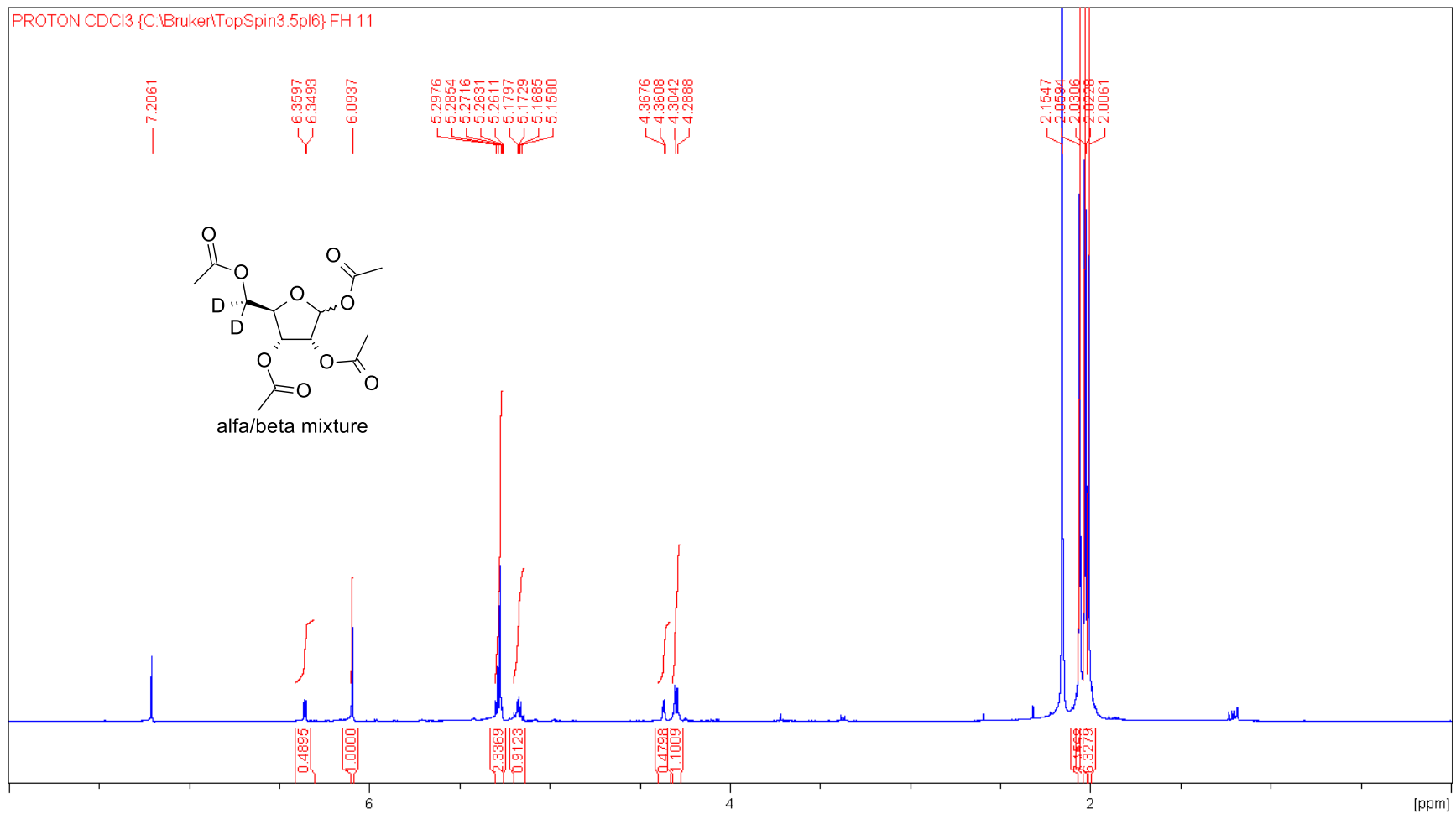


Compound 42. 400 MHz <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub>

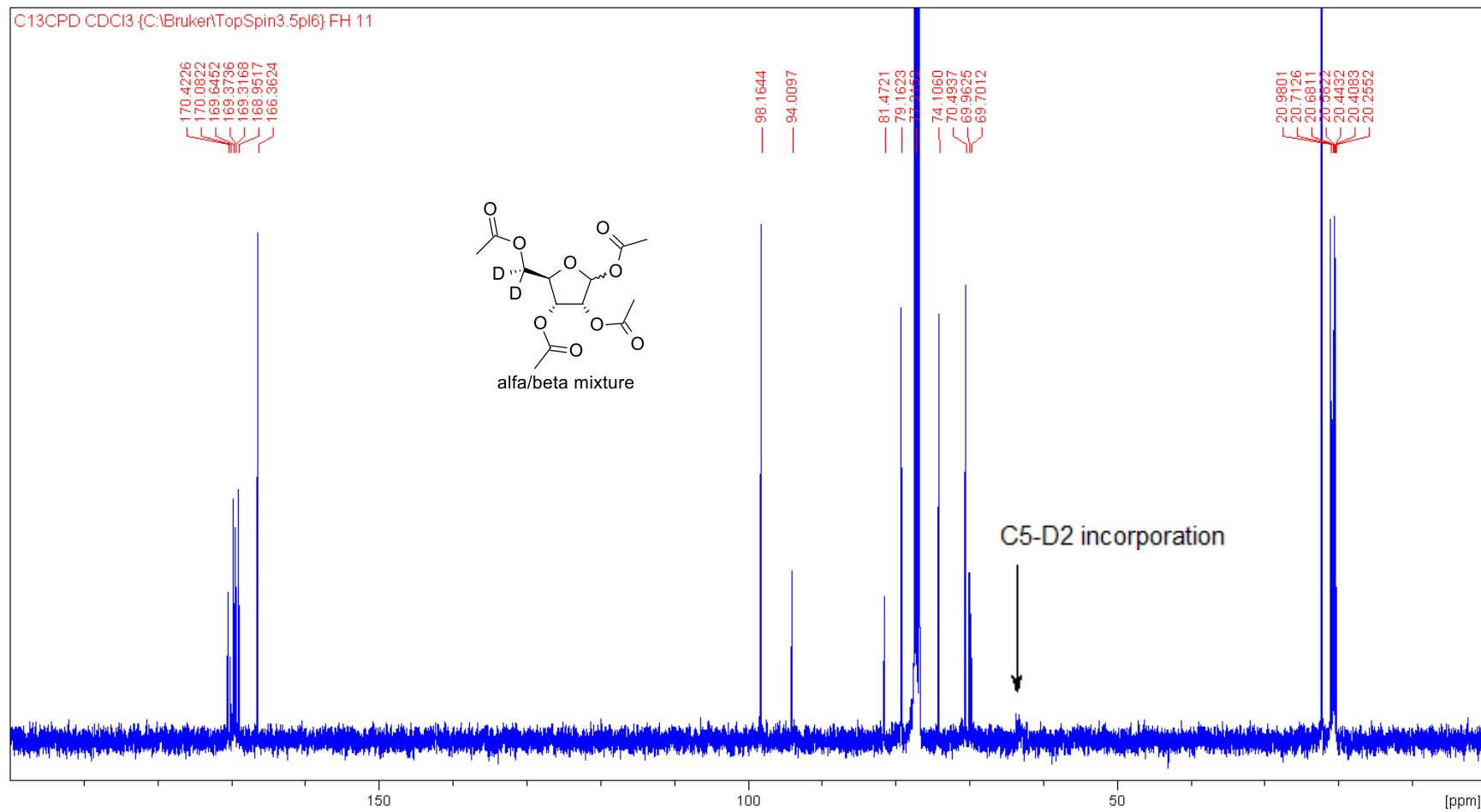


Compound 42. 100 MHz  $^{31}\text{C}$  NMR spectrum in  $\text{CDCl}_3$

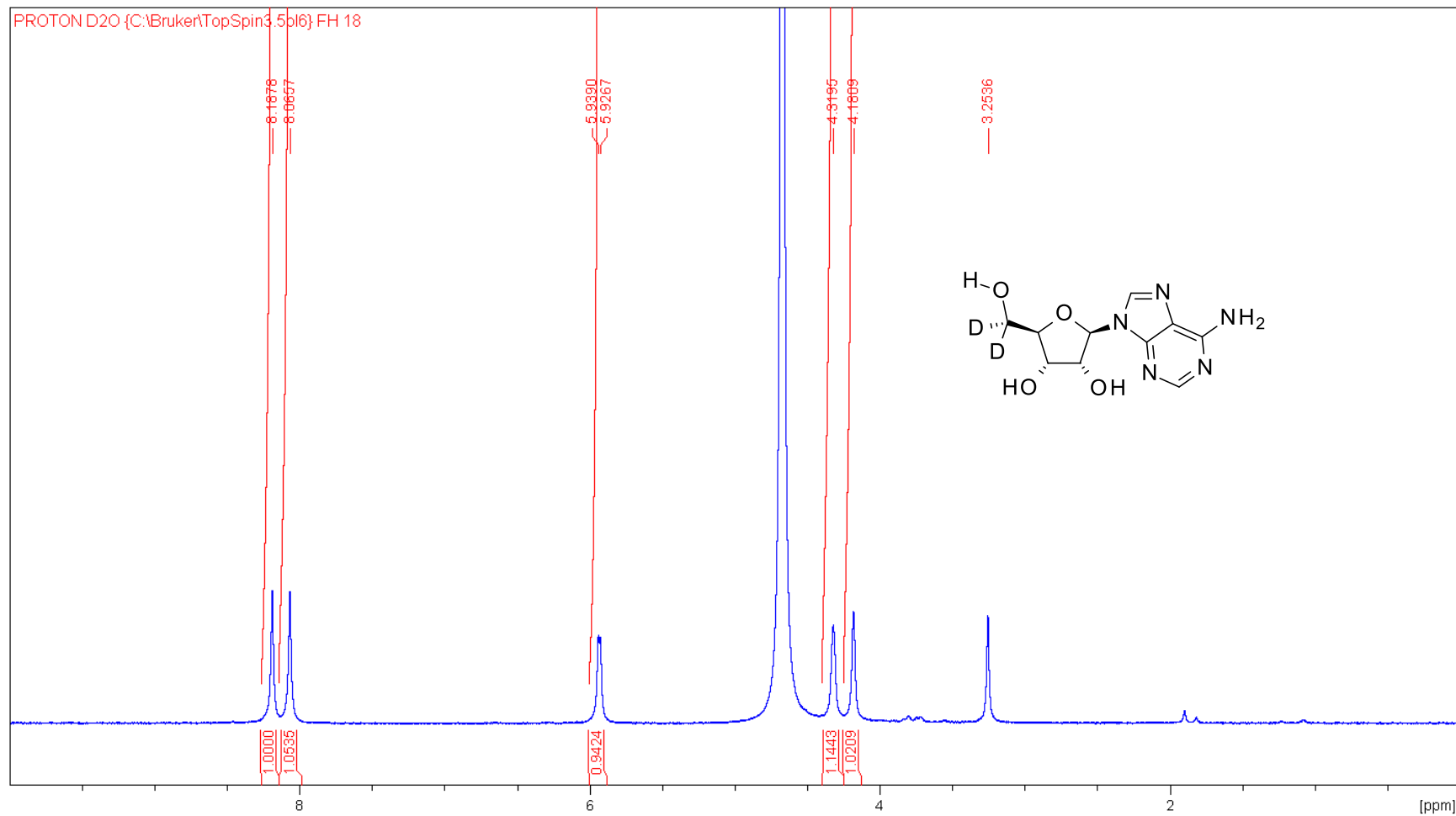




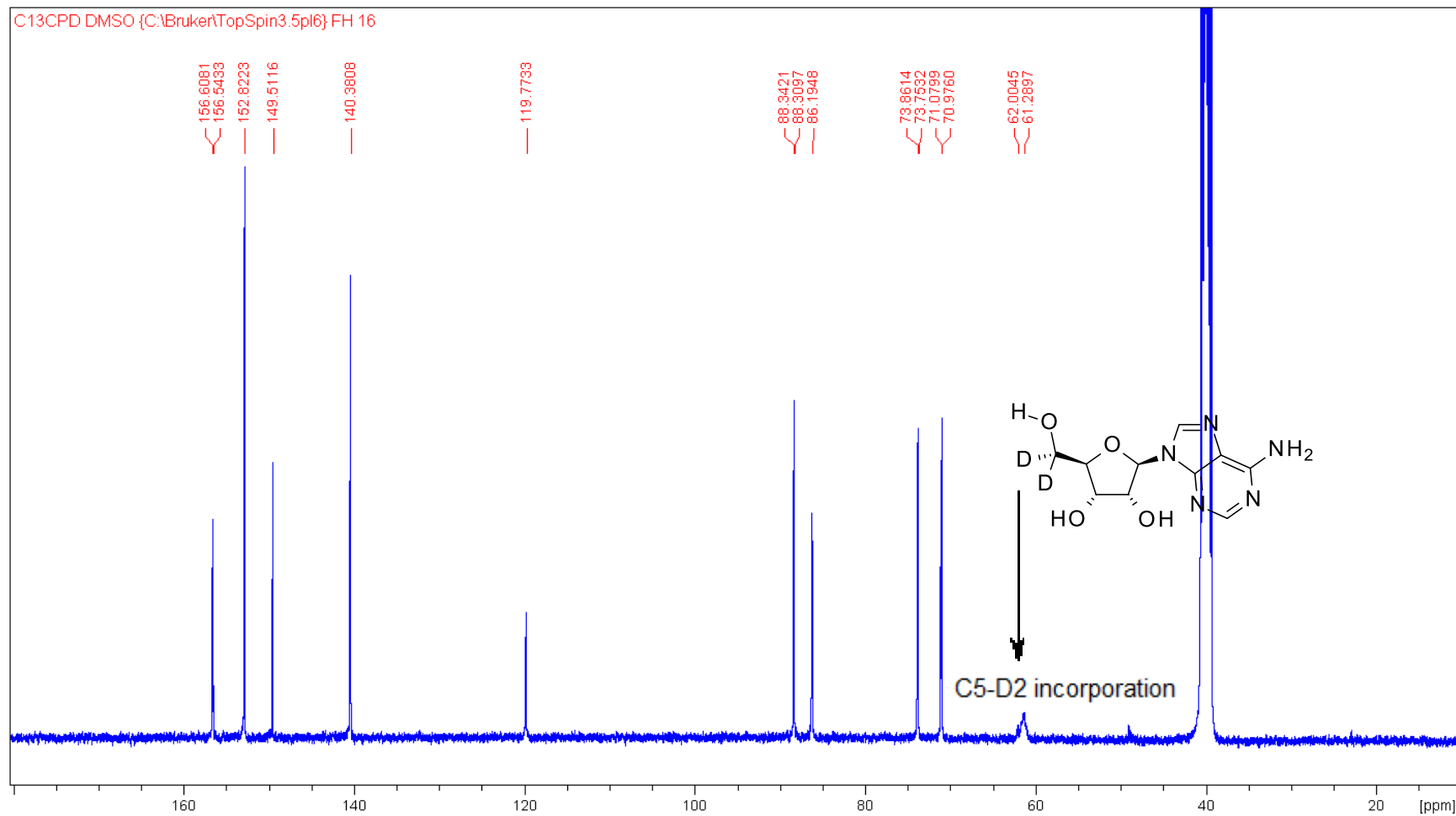
Compound 46. 400 MHz <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub>



Compound 46. 100 MHz  $^{31}\text{C}$  NMR spectrum in  $\text{CDCl}_3$



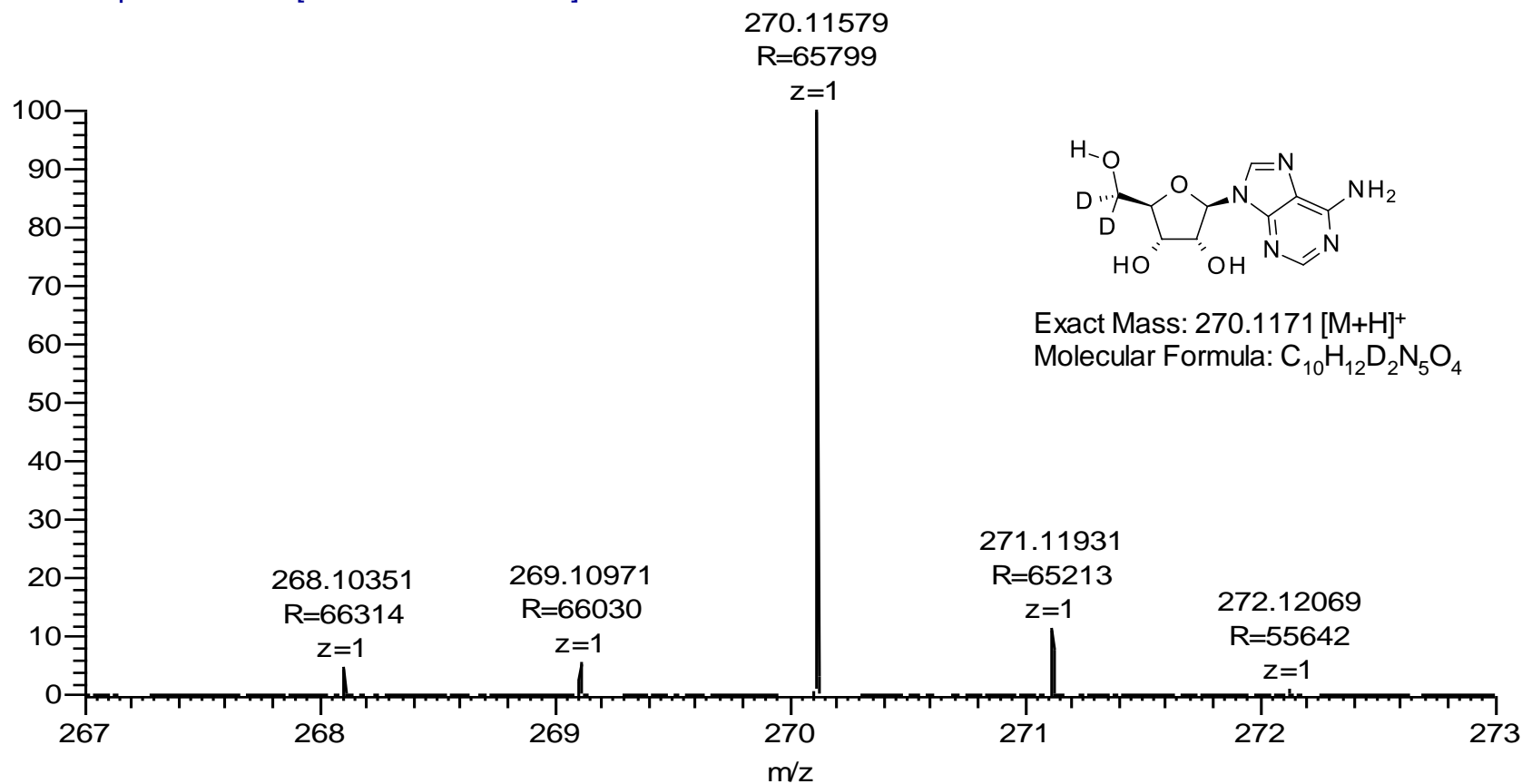
Compound 48. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$



Compound 48. 100 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$

mm\_031320\_c\_5\_deuterated\_adenosine\_1 #107-377 RT: 0.47-1.65 AV: 271 NL: 8.27E8

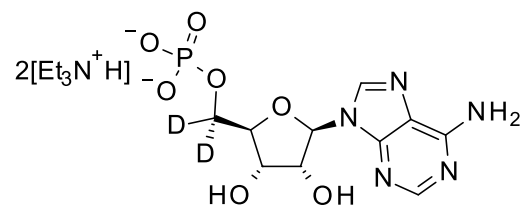
T: FTMS + p ESI Full ms [100.0000-600.0000]



Compound 48. HRMS Spectra

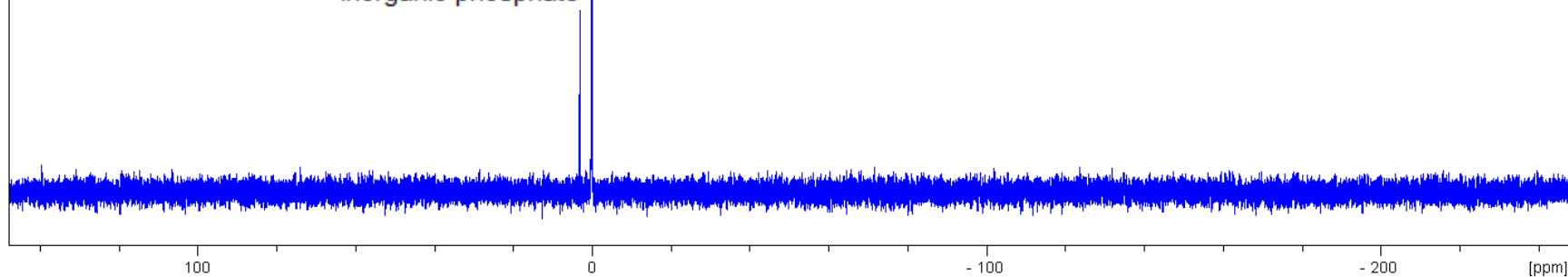
P31CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 16

3.0013  
0.1826  
-0.0176

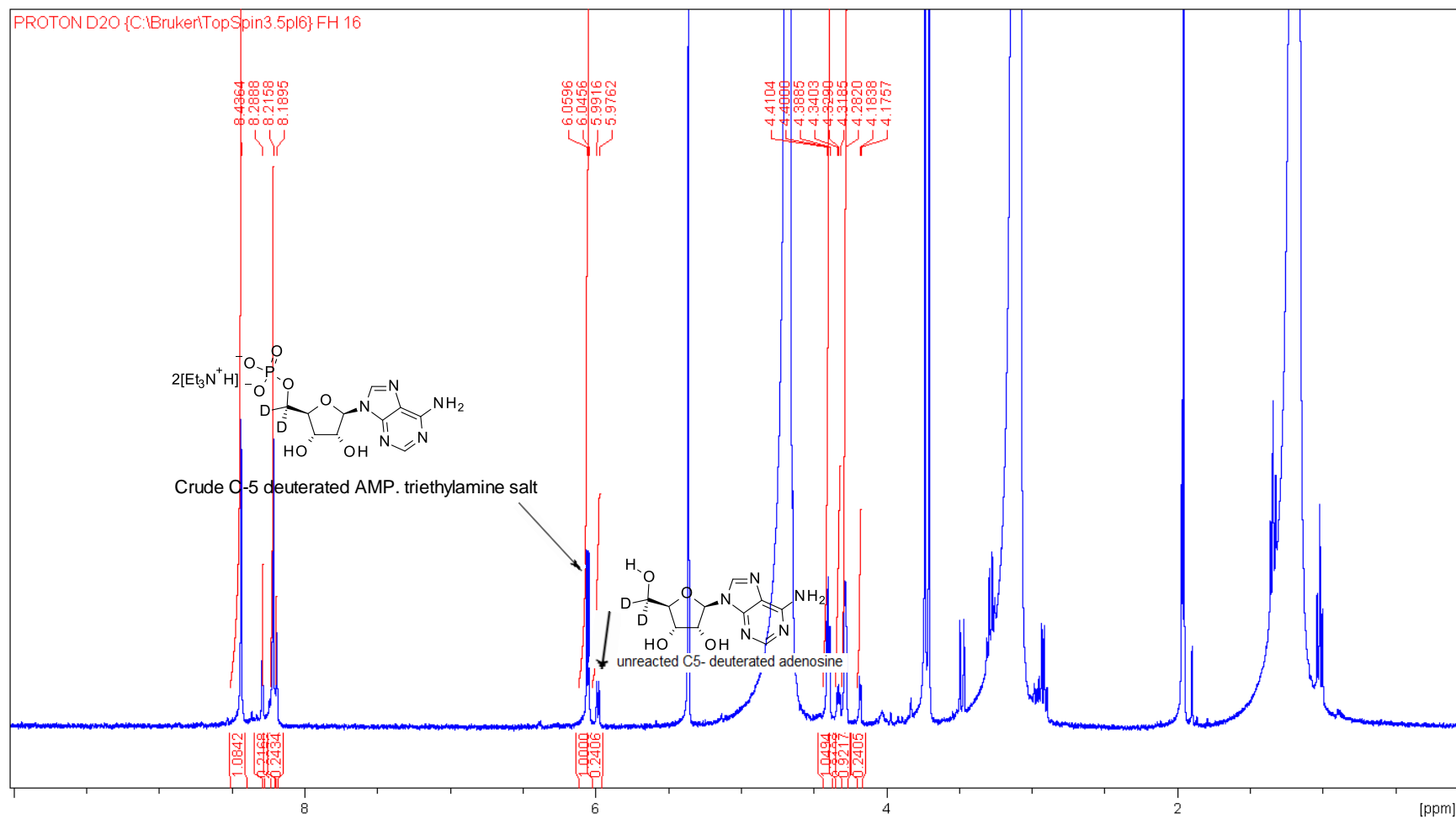


Crude C-5 deuterated AMP. triethylamine salt

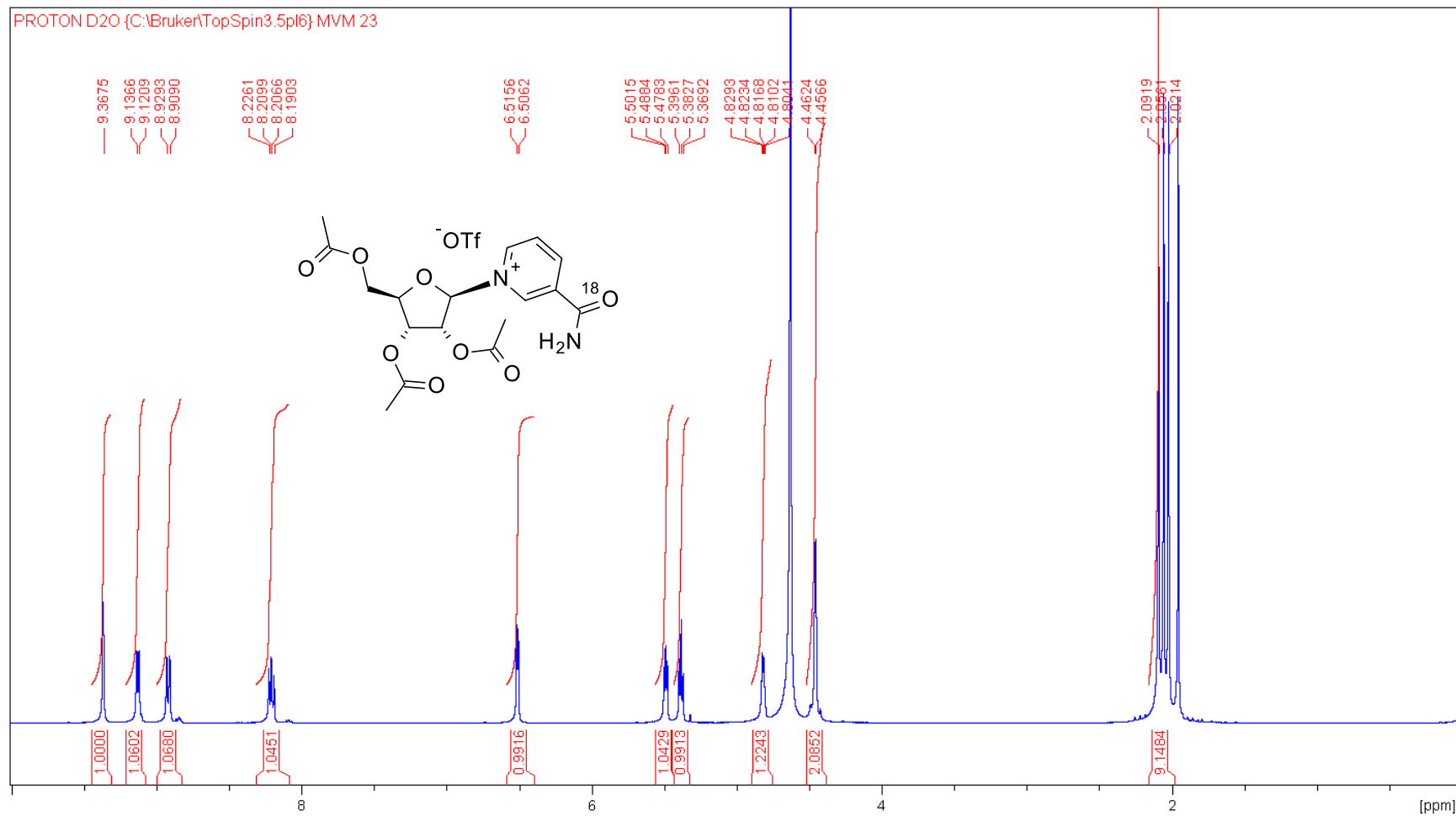
Inorganic phosphate



Compound 49. 162 MHz  $^{31}\text{P}$  NMR spectrum in  $\text{D}_2\text{O}$

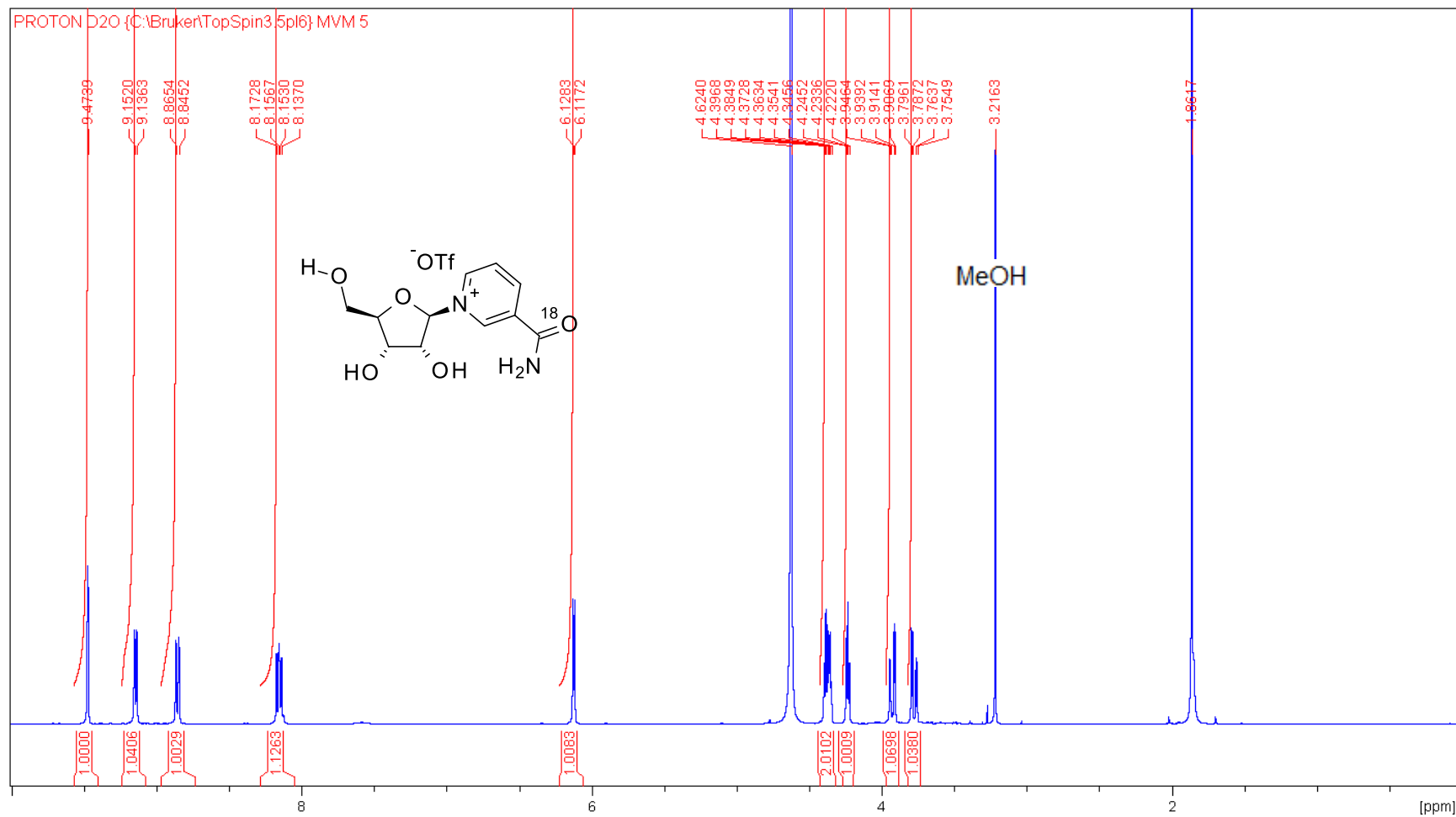


Compound 49. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$



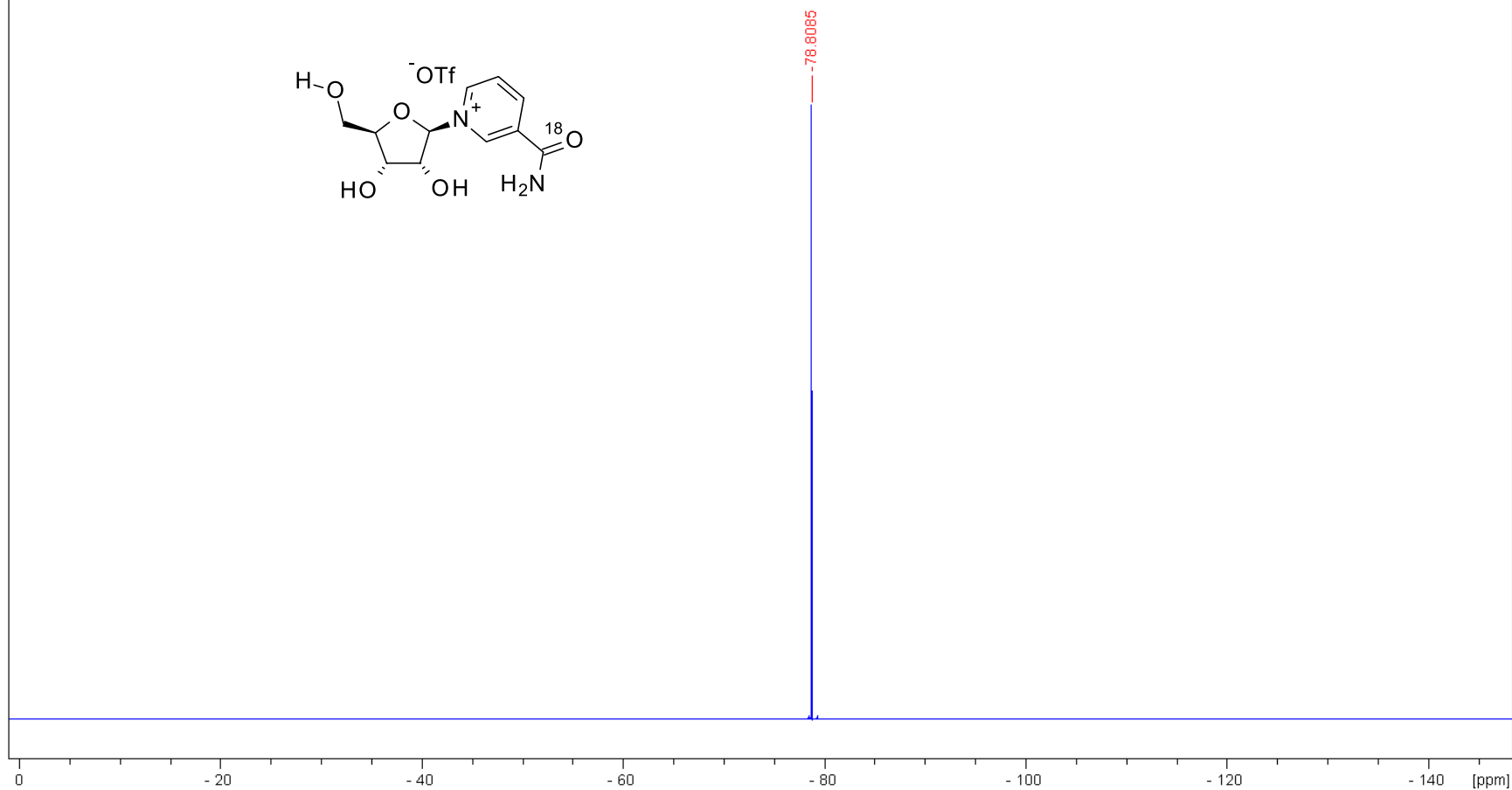
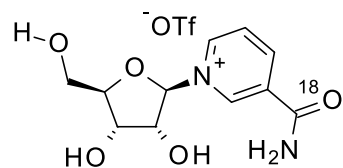
Compound 53. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$





Compound 54. 400 MHz  $^1\text{H}$  NMR spectrum in  $\text{D}_2\text{O}$

F19CPD D2O {C:\Bruker\TopSpin3.5pl6} FH 1



Compound 54. 377 MHz <sup>19</sup>F NMR spectrum in D<sub>2</sub>O