SUPPLEMENTARY MATERIALS

Title: The (bio)chemical diagnosis of nicotinamide-derived pyridones

Authors

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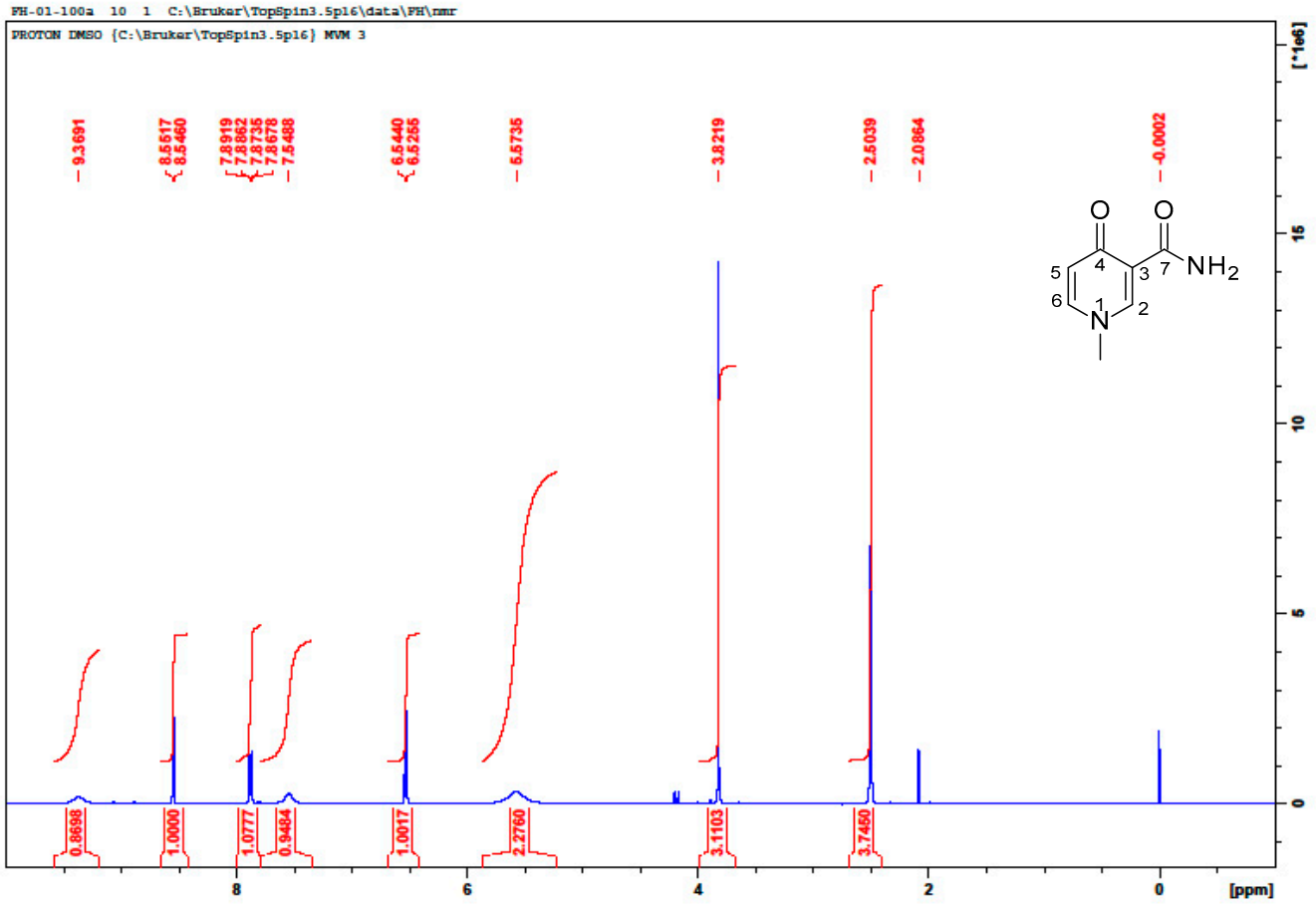
Table 1. Isotopic pattern with relative abundance of pyridones and their intermediates.

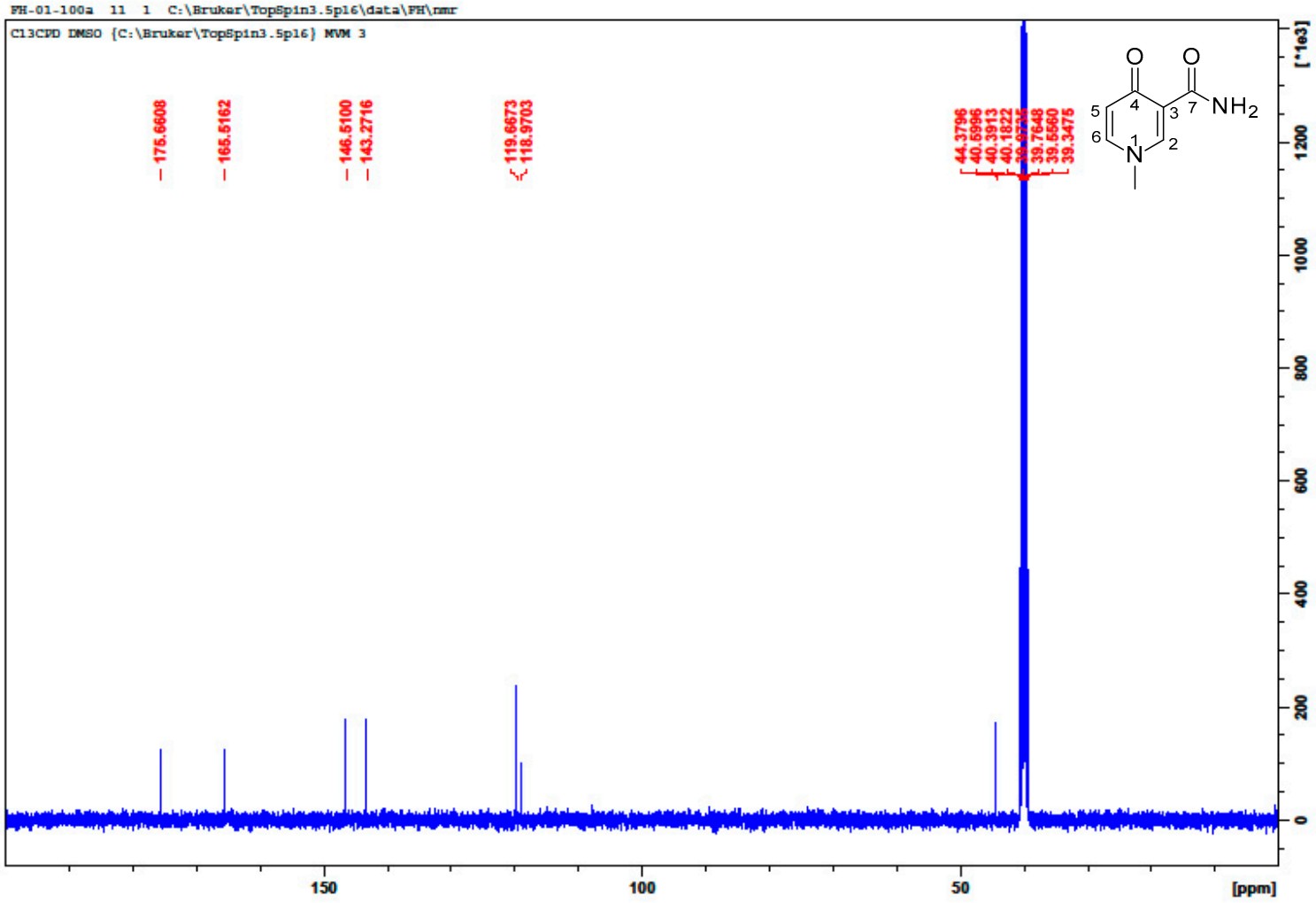
Compound name	Fragmentation Pattern [Relative Abundance (%)]							
		MW+0	MW+1	MW+2 from natural isotopic abundance [in source acylium products]	MW+3	MW+ Na	MW + CH_3CN	MW+Na +CH_3CN	[M-CH_3]$^+$
1-N-methyl-4-oxo-pyridine-3-carboxamide = 1-N-methyl-4-pyridone-3-carboxamide	HRMS $C_7H_8N_2O_2$		$[C_7H_9N_2O_2]^+$ 153.0652 (100%)	154.0491* (~10%) $[C_7H_8NO_3^+]$ (~10%)					
O-methyl 4-pyridone-3-carboxylate	HRMS $C_7H_7NO_3$		$[C_7H_8NO_3^+]$ 154.0490 (100%)	155.0522 (10%)					140.0335 (100%)
O-methyl, 1-N-methyl 4-pyridone-3-carboxylate	HRMS $C_8H_9NO_3$		$[C_8H_{10}NO_3^+]$ 168.0648 (100%)	169.0681 (10%)					154.0492 (100%)
O-methyl-1-methyl-6-oxo-pyridine-3-carboxylate	HRMS $C_8H_9NO_3$		$[C_8H_{10}NO_3^+]$ 168.0647 (100%)					209.69 (100%)	
1-N-methyl-6-pyridone-3-carboxamide	HRMS $C_7H_8N_2O_2$		$[C_7H_9N_2O_2]^+$ 153.0663 (100%)	154.0696 (5%)					
1-N-methyl-2-pyridone-3-carboxamide	HRMS		$[C_7H_9N_2O_2]^+$ 153.0648 (100%)	154.0487* (~10%) $[C_7H_8NO_3^+]$ (~10%)					
4-pyridone-3-carboxamide	HRMS $C_6H_6N_2O_2$		$[C_6H_7N_2O_2]^+$ 139.0495 (100%)	140.0334 (~10%) $[C_6H_6NO_3^+]$ (~10%)					
1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-4-pyridone-3-carboxamide	HRMS $C_{17}H_{20}N_2O_9$		$[C_{17}H_{21}N_2O_9]^+$ 397.1228 (100%)	398.1220 (20%)	399.1276 (1%)				

4-pyridone-3-carboxamide-1-β-D-ribofuranoside	HRMS $C_{11}H_{14}N_2O_6$	$[C_{11}H_{15}N_2O_6]^+$ 271.0918 (100%)	272.0951 (10%) $[C_{11}H_{14}NO_7]^+$ 272.0770 not selected			
6-pyridone 3-carboxamide	HRMS $C_6H_6N_2O_2$	$[C_6H_7N_2O_2]^+$ 139.0499 (100%)	140.0532 (5%)			
1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-6-pyridone-3-carboxamide	MS (ES)	397.63 (32%)			419.56 (55%)	460.67 (30%)
6-pyridone-3-carboxamide-1-β-D-ribofuranoside	HRMS $C_{11}H_{14}N_2O_6$	$[C_{11}H_{15}N_2O_6]^+$ 271.0938 (100%)	272.0971 (10%)			
1-(2′, 3′, 5′-tri-O-acetyl-β-D-ribofuranosyl)-4-pyridone-3-carboxylic methyl ester	HRMS $C_{17}H_{20}N_2O_9$	$[C_{17}H_{21}N_2O_9]^+$ 412.1244 (100%)	413.1275 (20%)	414.1292 (2%)		
O-methyl-4-pyridone-3-carboxylate ribonucleoside	HRMS $C_{12}H_{15}NO_7$	$[C_{12}H_{16}NO_7]^+$ 286.0929 (100%)	287.0960 (10%)			
4-pyridone-3-carboxylic acid ribonucleoside	HRMS $C_{11}H_{13}NO_7$	$[C_{11}H_{14}NO_7]^+$ 272.0768 (100%)	273.0801 (10%)			
2-pyridone-3-carboxamide	HRMS $C_6H_6N_2O_2$	$[C_6H_7N_2O_2]^+$ 139.0493 (100%)	140.0333 (~10%) $[C_6H_6NO_3^+]$ (~30%)			
N-methyl-2-pyridone-3-carboxamide	HRMS $C_7H_8N_2O_2$	$[C_7H_9N_2O_2]^+$ 153.0648 (100%)	154.0887 (~10%) $[C_7H_8NO_3]^+$ (~10%)			
2-pyridone-3-carboxamide riboside	HRMS $C_{11}H_{14}N_2O_6$	$[C_{11}H_{15}N_2O_6]^+$ 271.0909 (100%)	272.0941 (~10%) $[C_{11}H_{14}NO_7]^+$ 272.0770 not selected	273.0958 (~2%)		

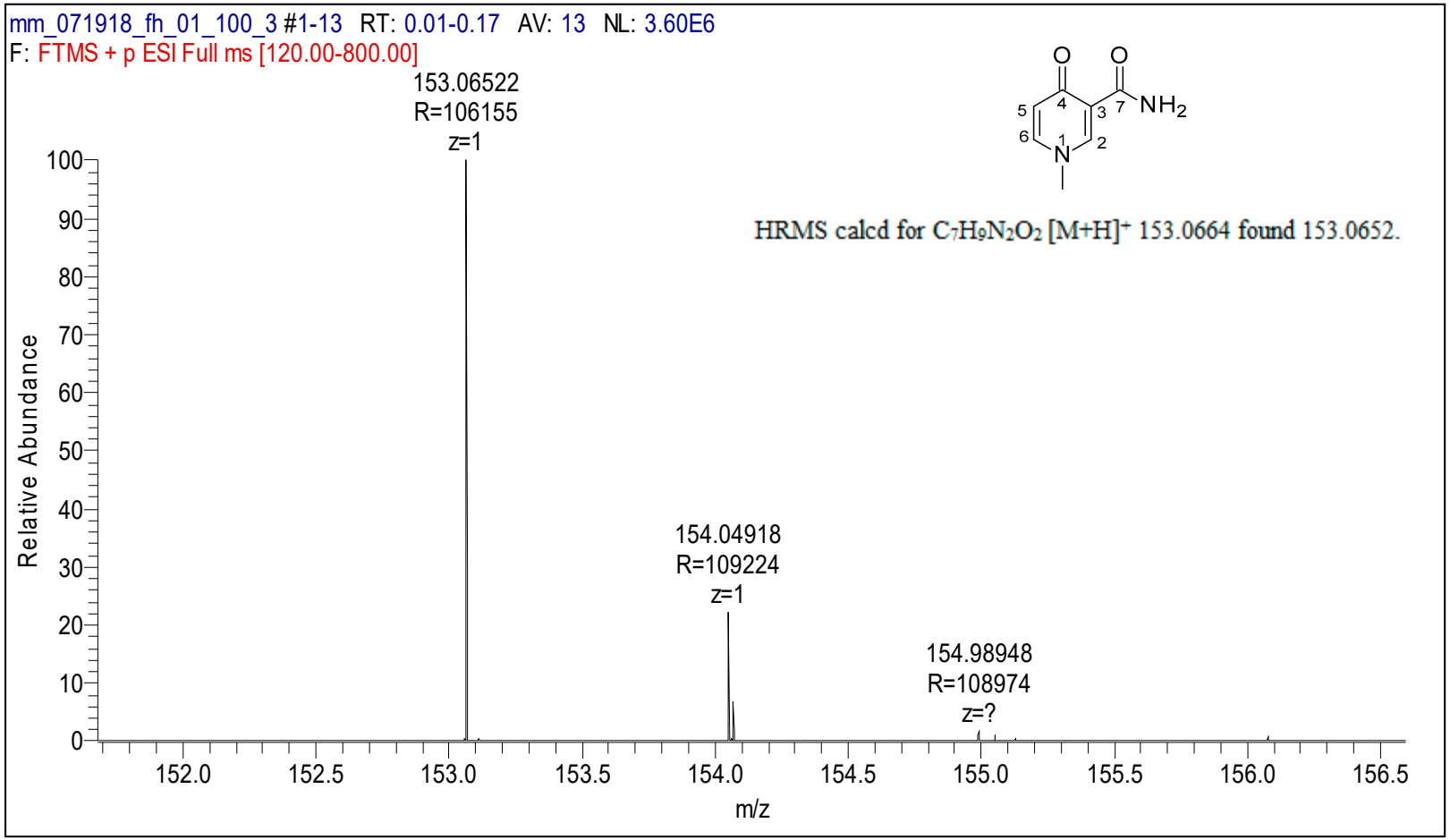
Figure S1: ^{1}H NMR, ^{13}C NMR and HRMS spectra



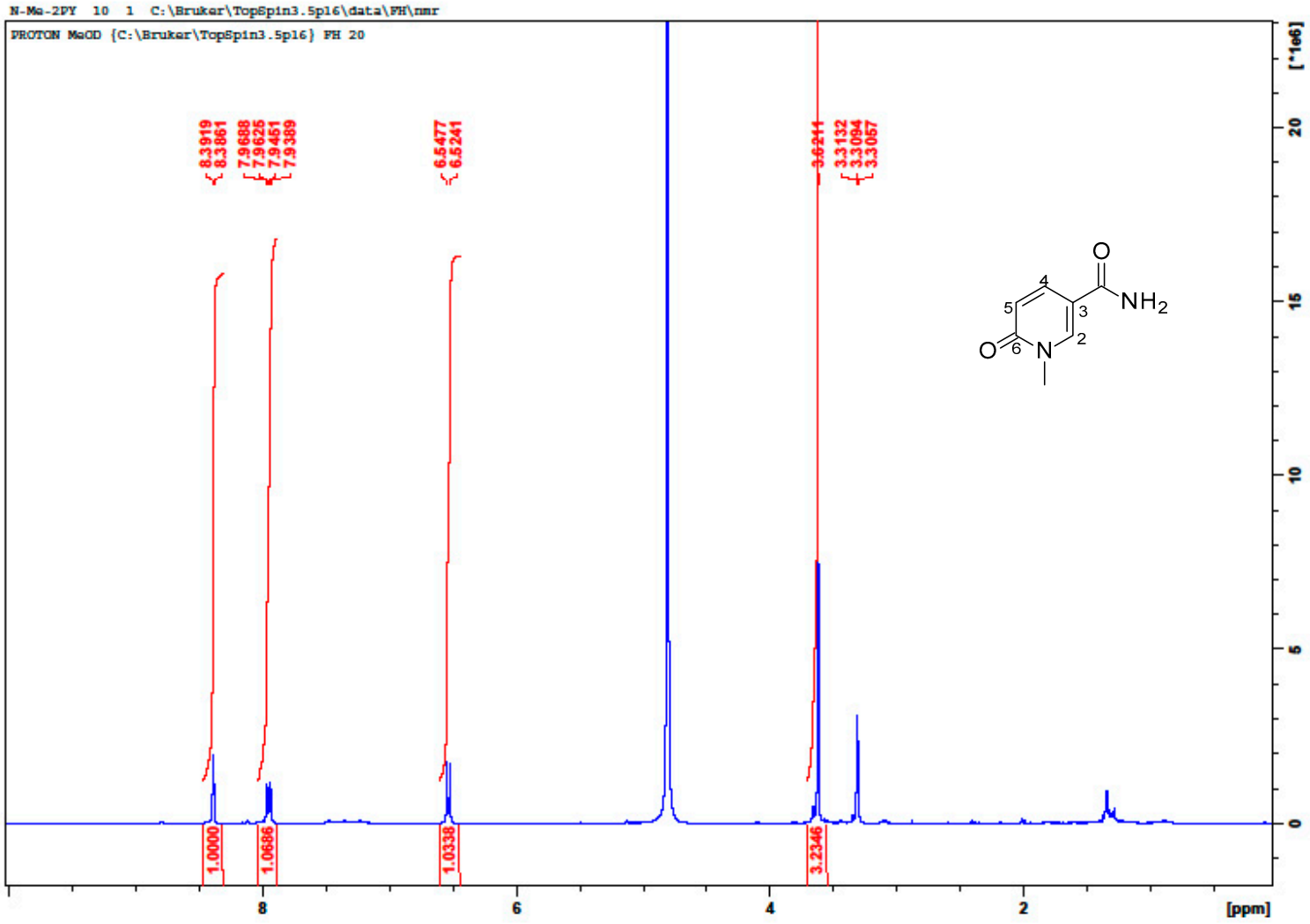
Compound 1. 400 MHz ^{1}H NMR spectrum in DMSO



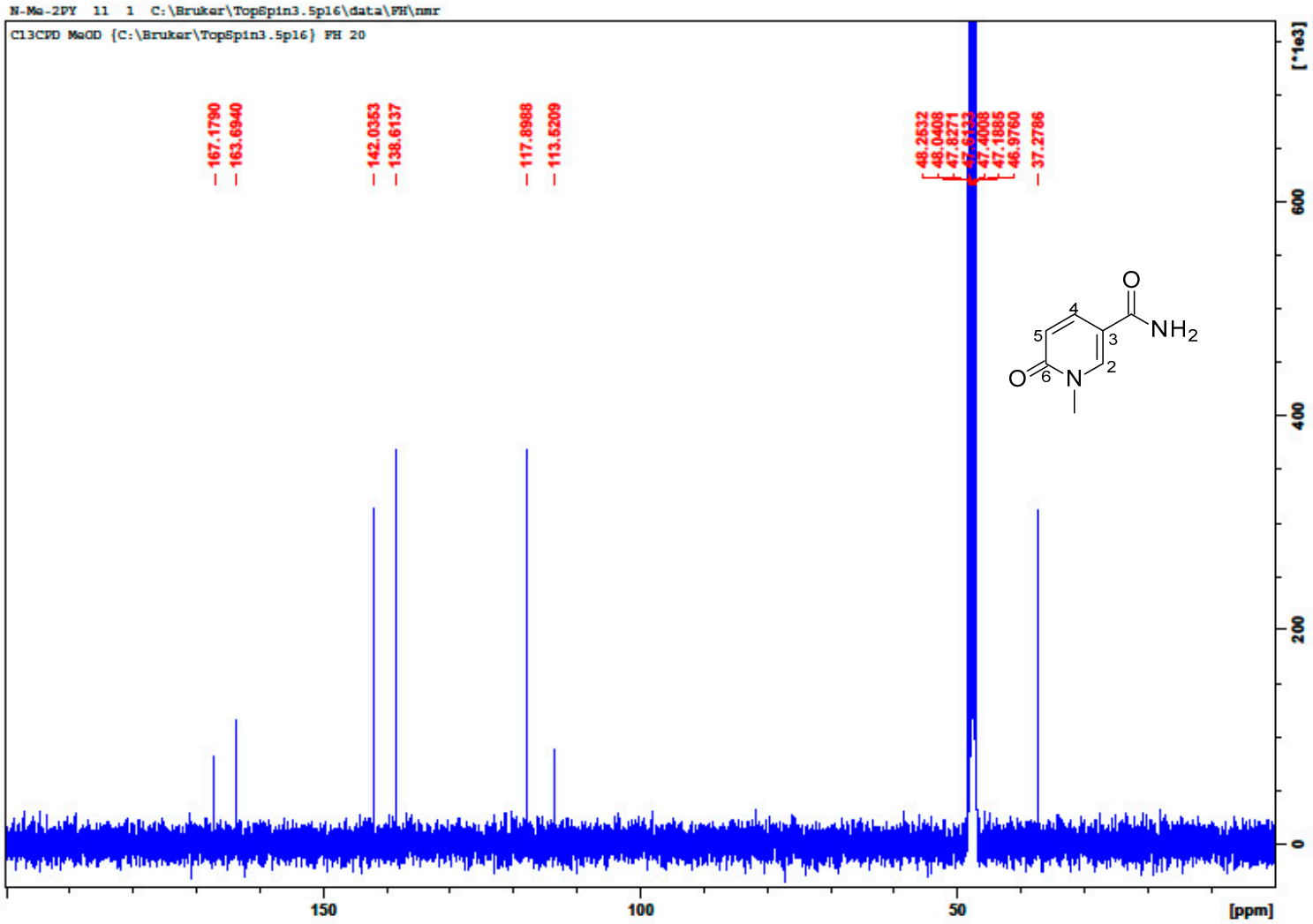
Compound 1. 400 MHz ^{13}C NMR spectrum in DMSO



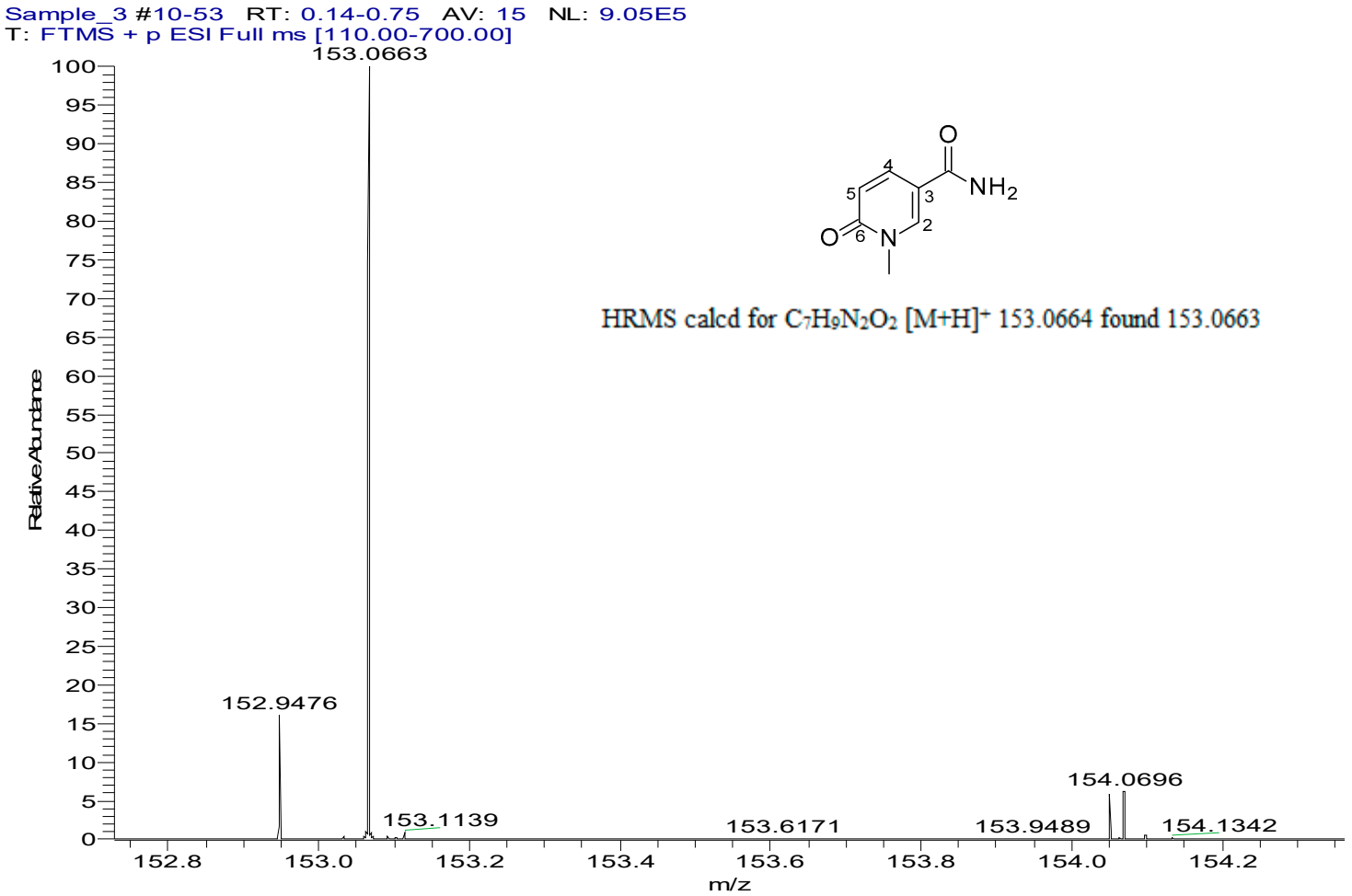
Compound 1. HRMS spectra



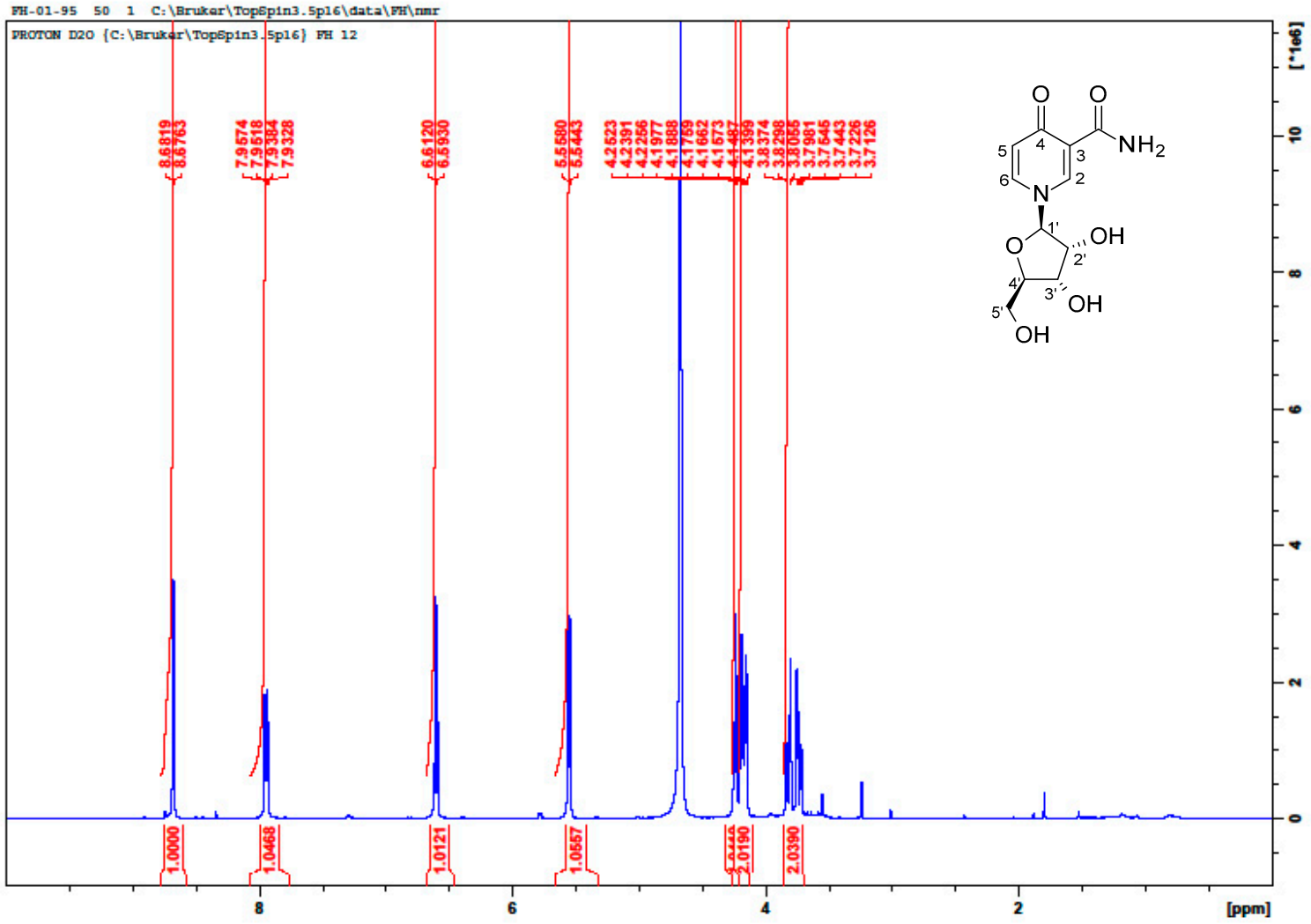
Compound 2. 400 MHz ^{1}H NMR spectrum in MeOD



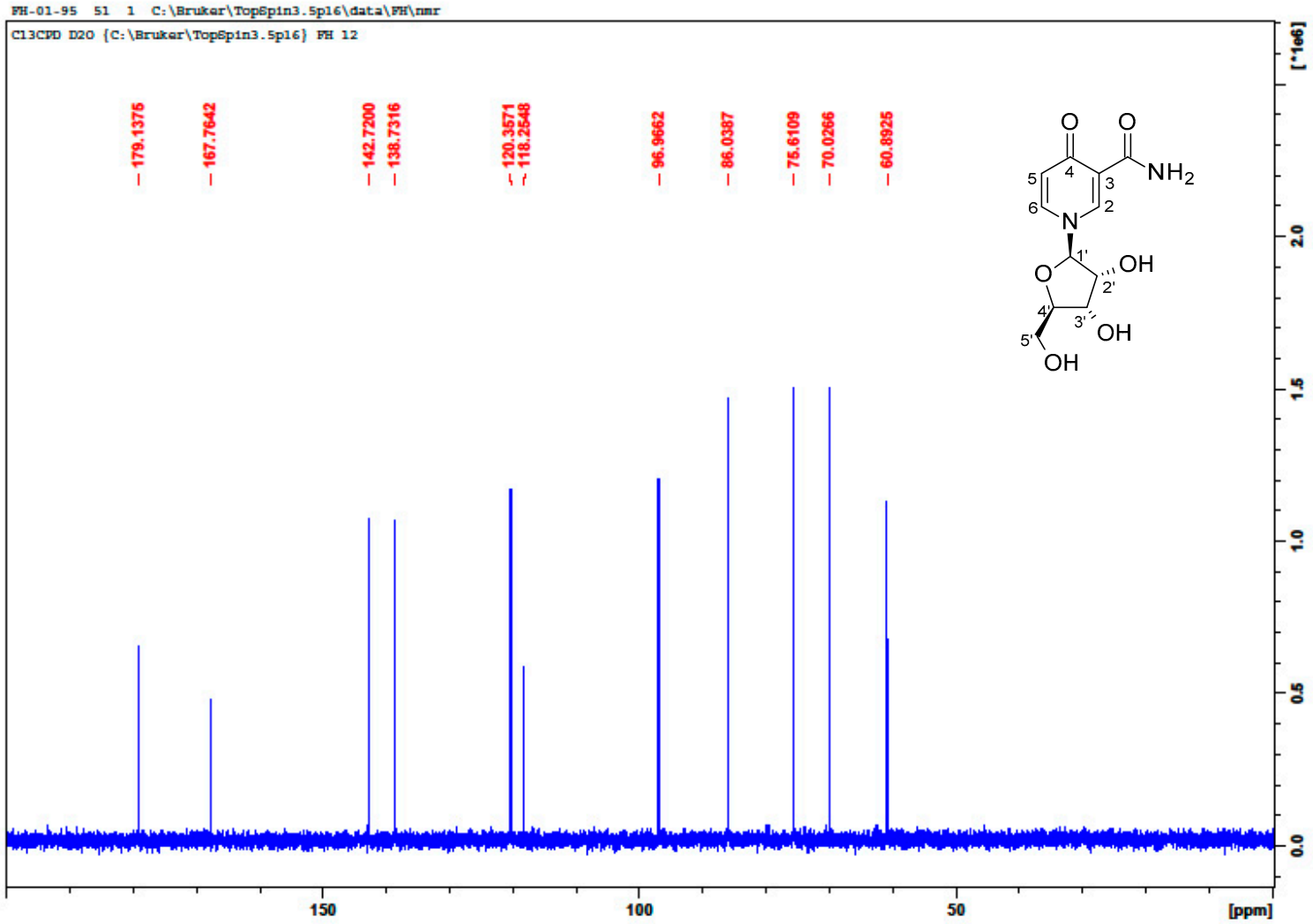
Compound 2. 400 MHz ^{13}C NMR spectrum in MeOD



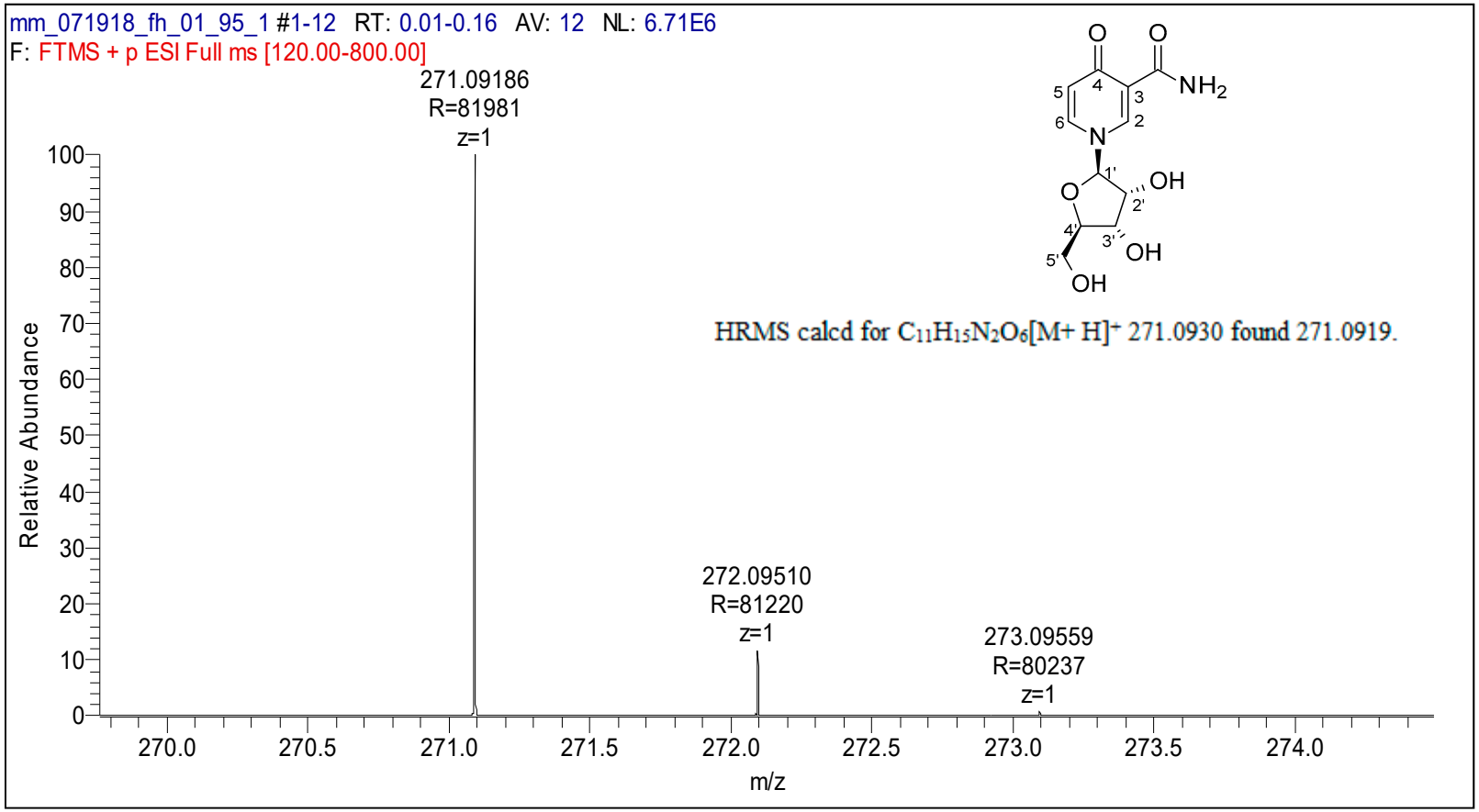
Compound 2. HRMS spectra



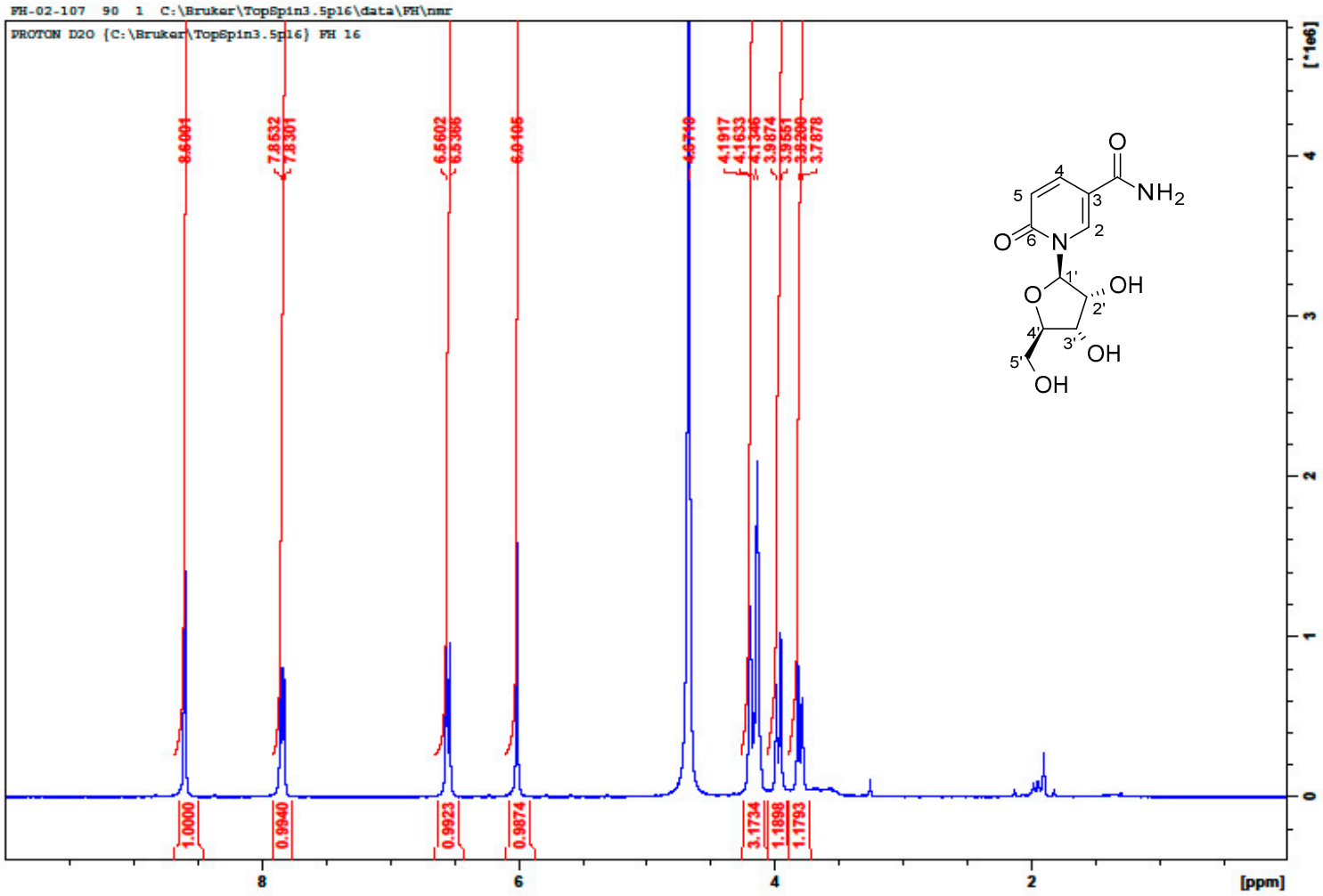
Compound 3. 400 MHz ^{1}H NMR spectrum in D_2O



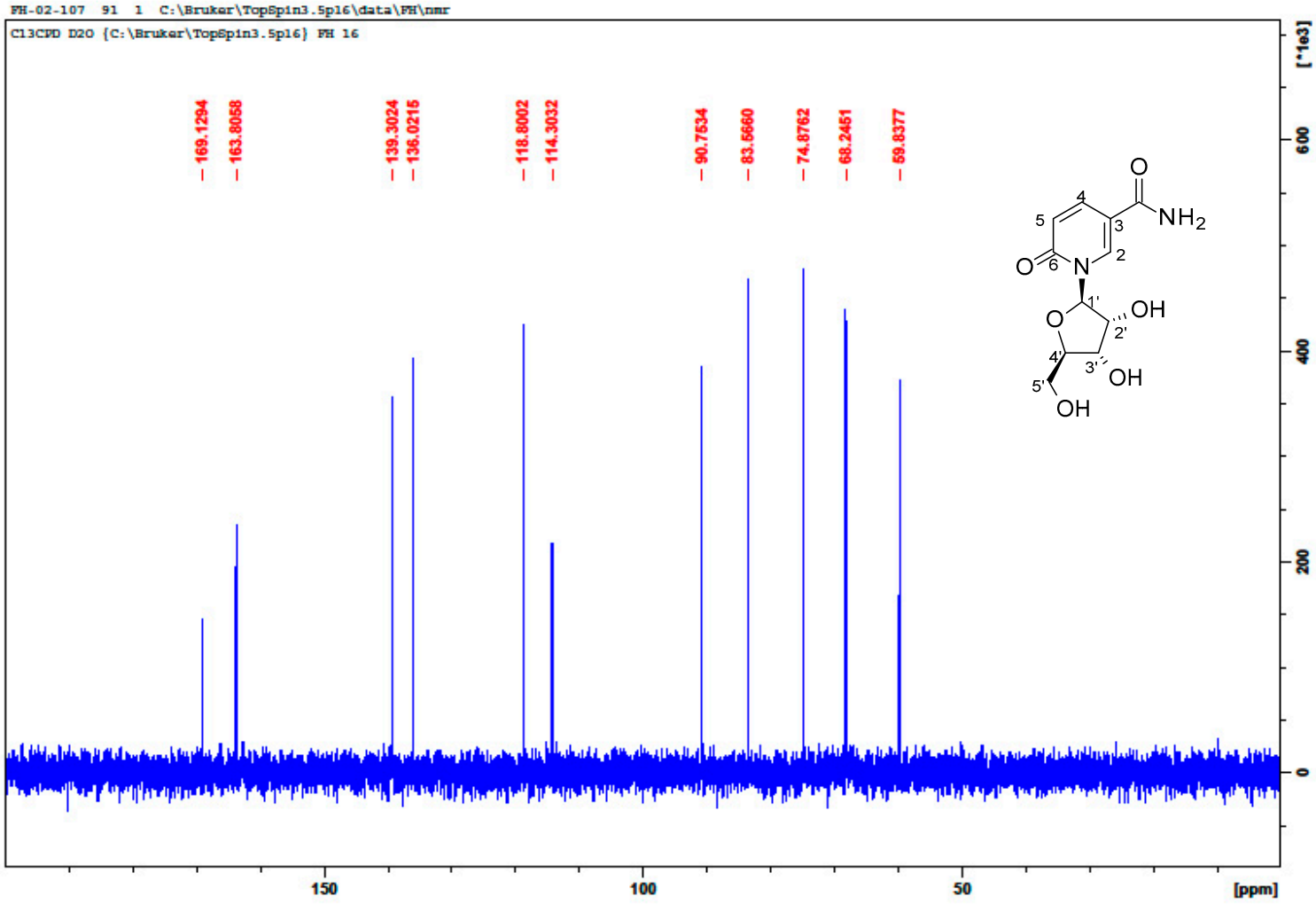
Compound 3. 400 MHz ^{13}C NMR spectrum in D_2O



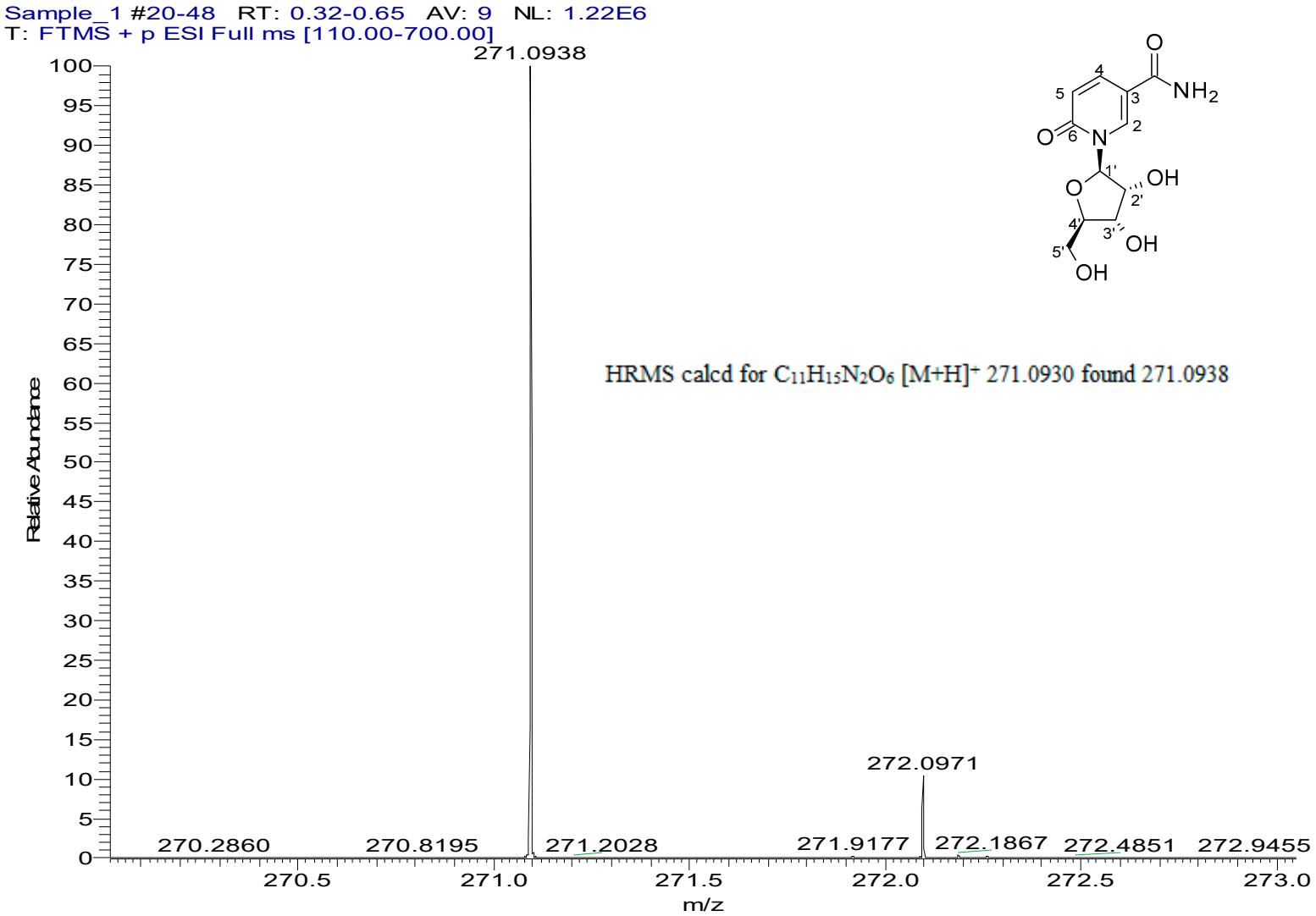
Compound 3. HRMS spectra



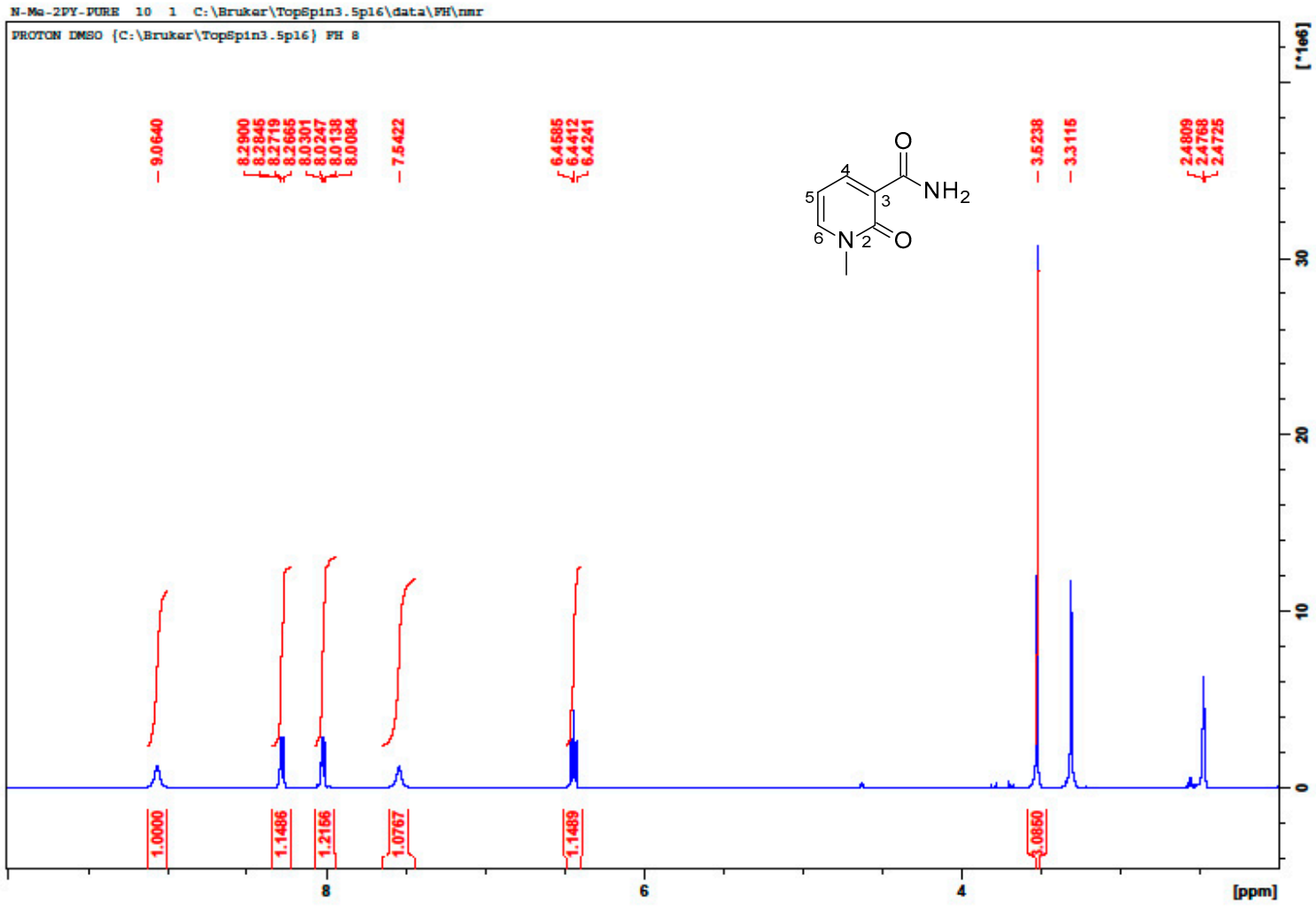
Compound 4. 400 MHz ^{1}H NMR spectrum in D_2O



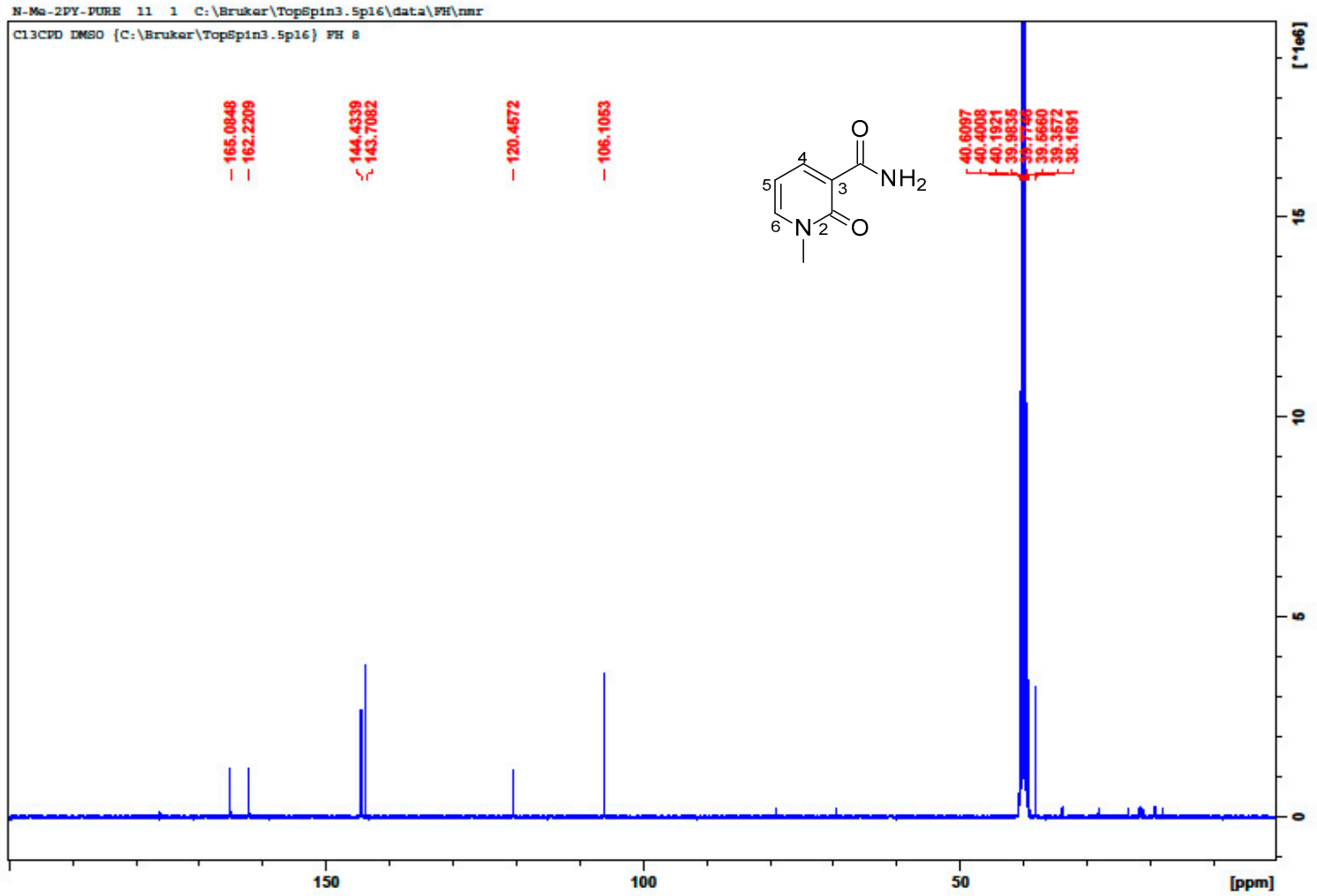
Compound 4. 400 MHz ^{13}C NMR spectrum in D_2O



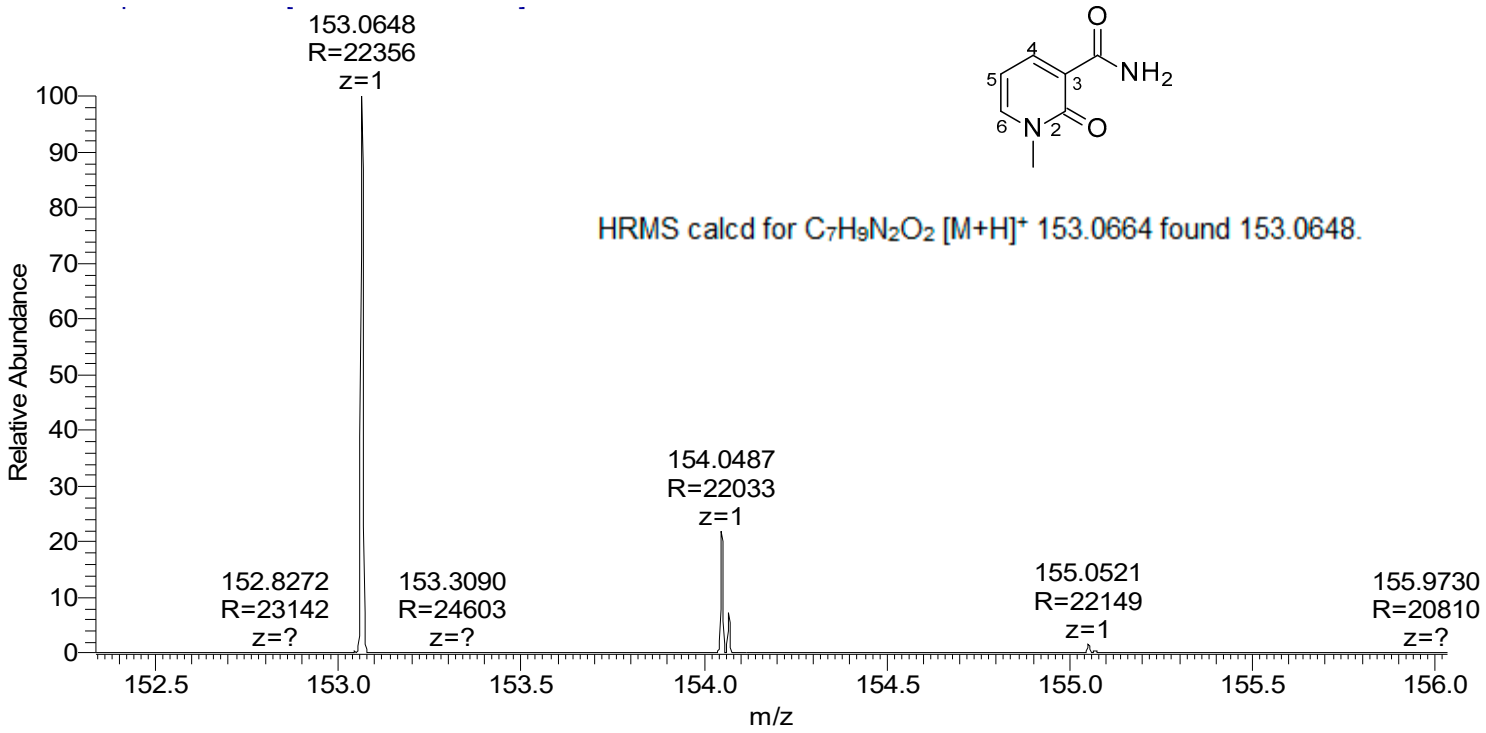
Compound 4. HRMS spectra



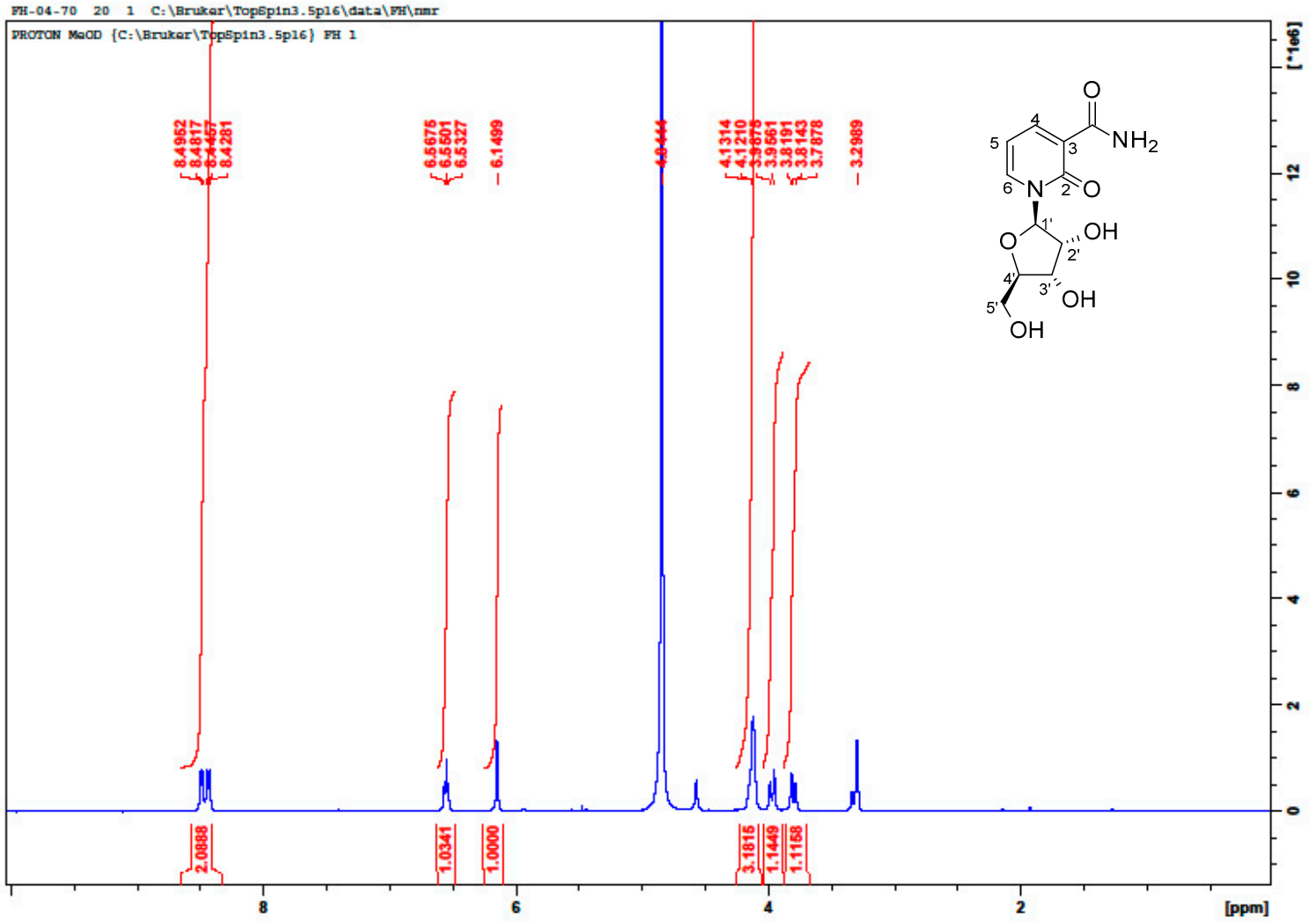
Compound 5. 400 MHz ^{1}H NMR spectrum in DMSO



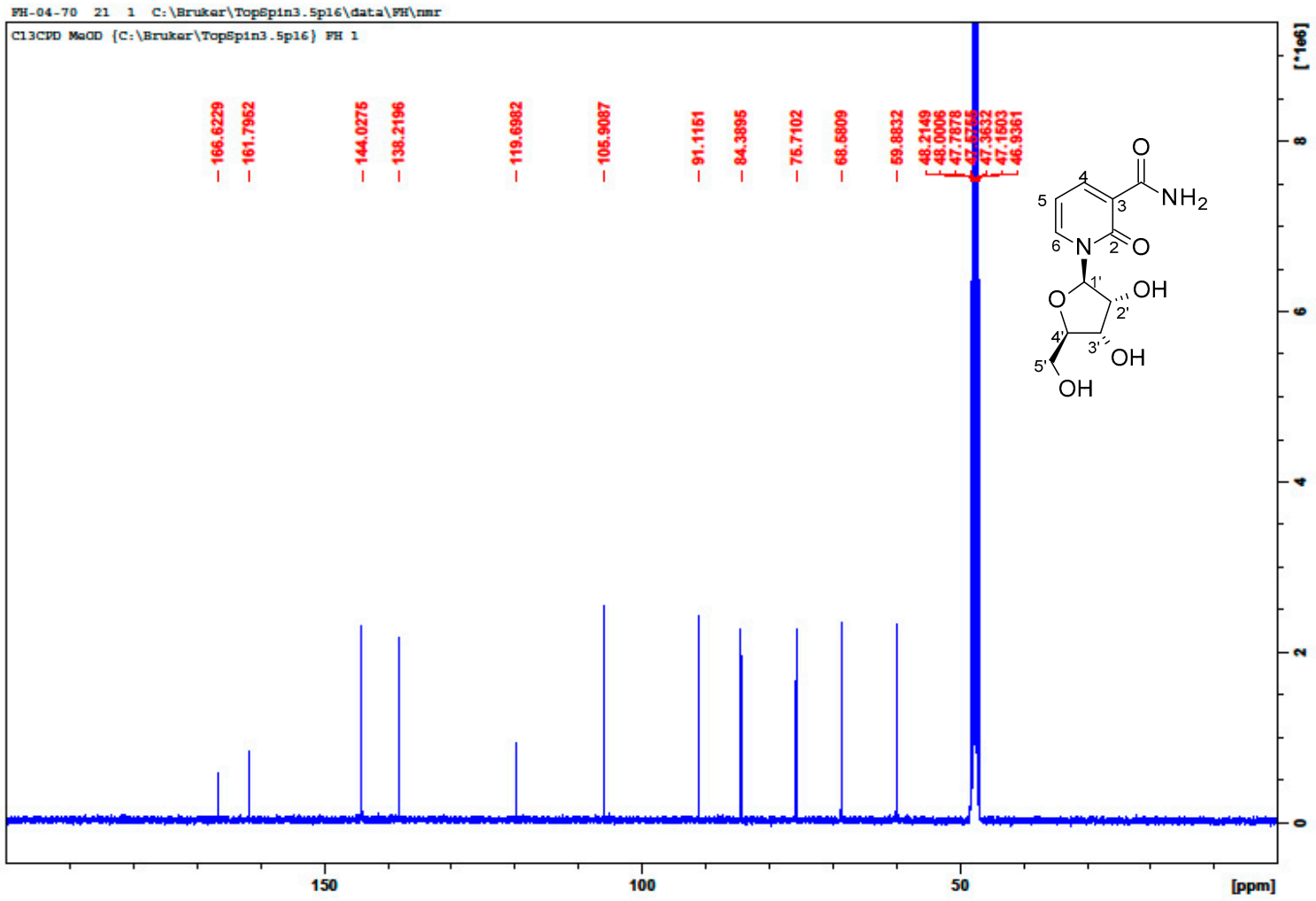
Compound 5. 400 MHz ^{13}C NMR spectrum in DMSO



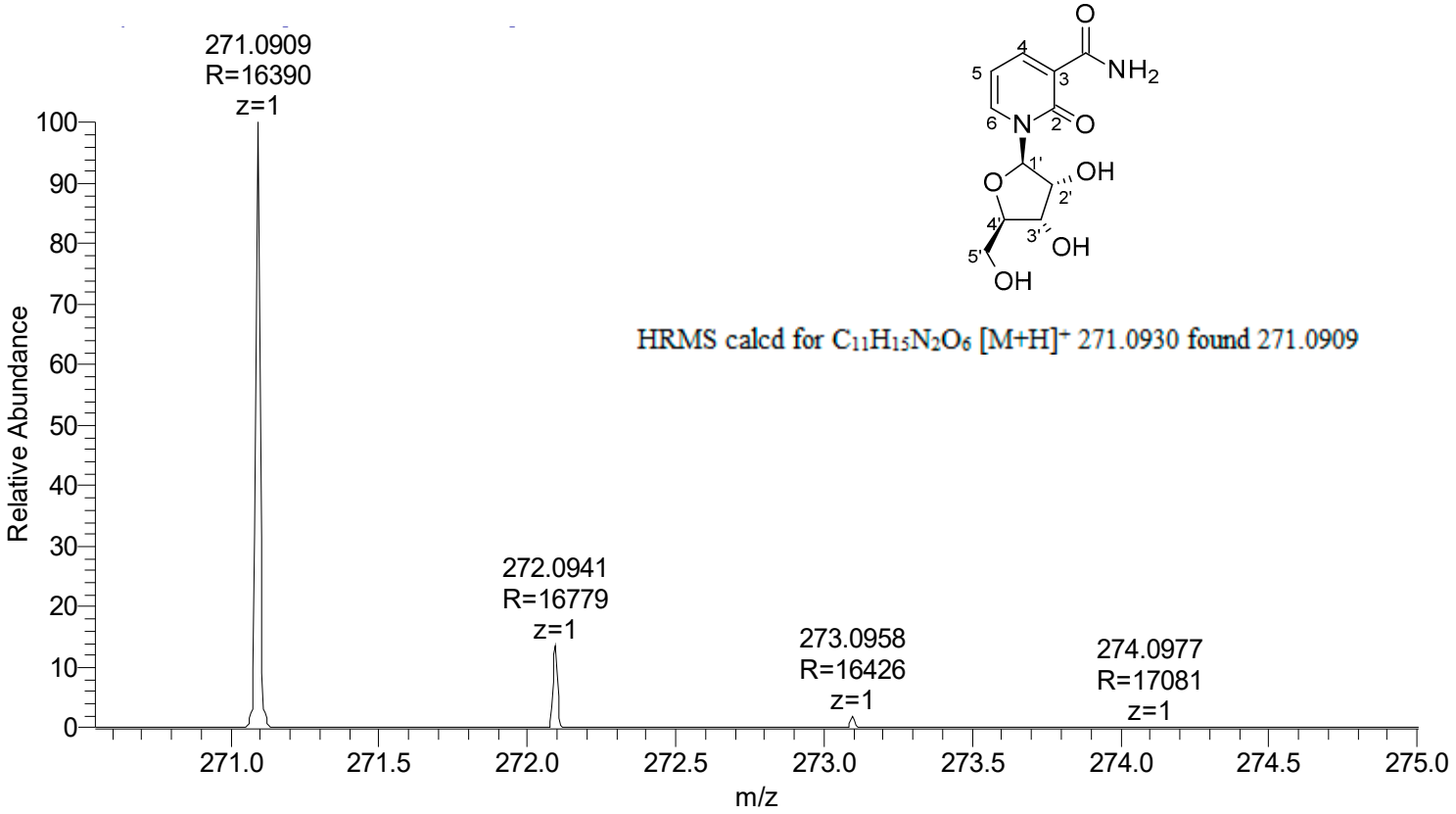
Compound 5. HRMS spectra



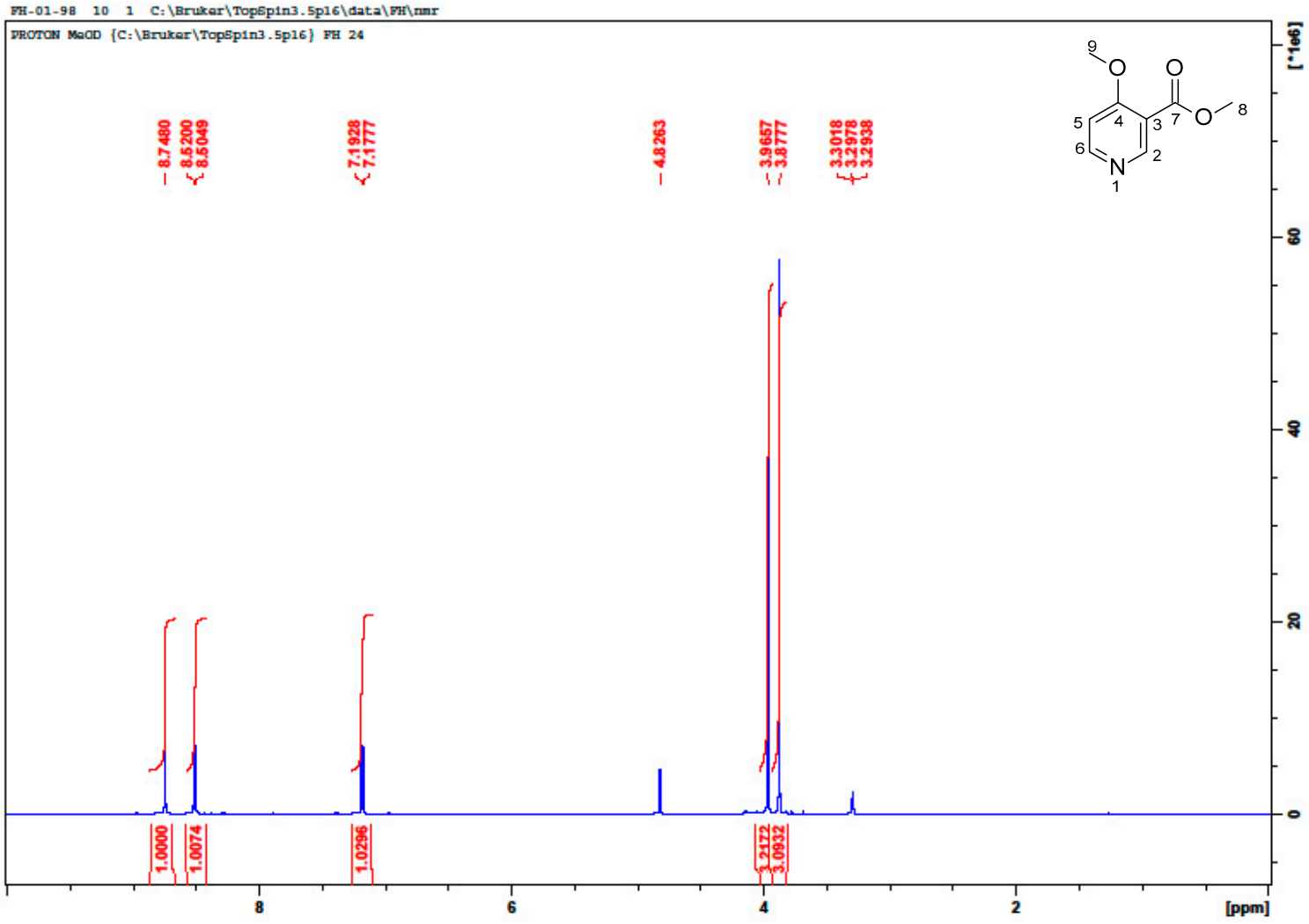
Compound 6. 400 MHz ^{1}H NMR spectrum in D_2O



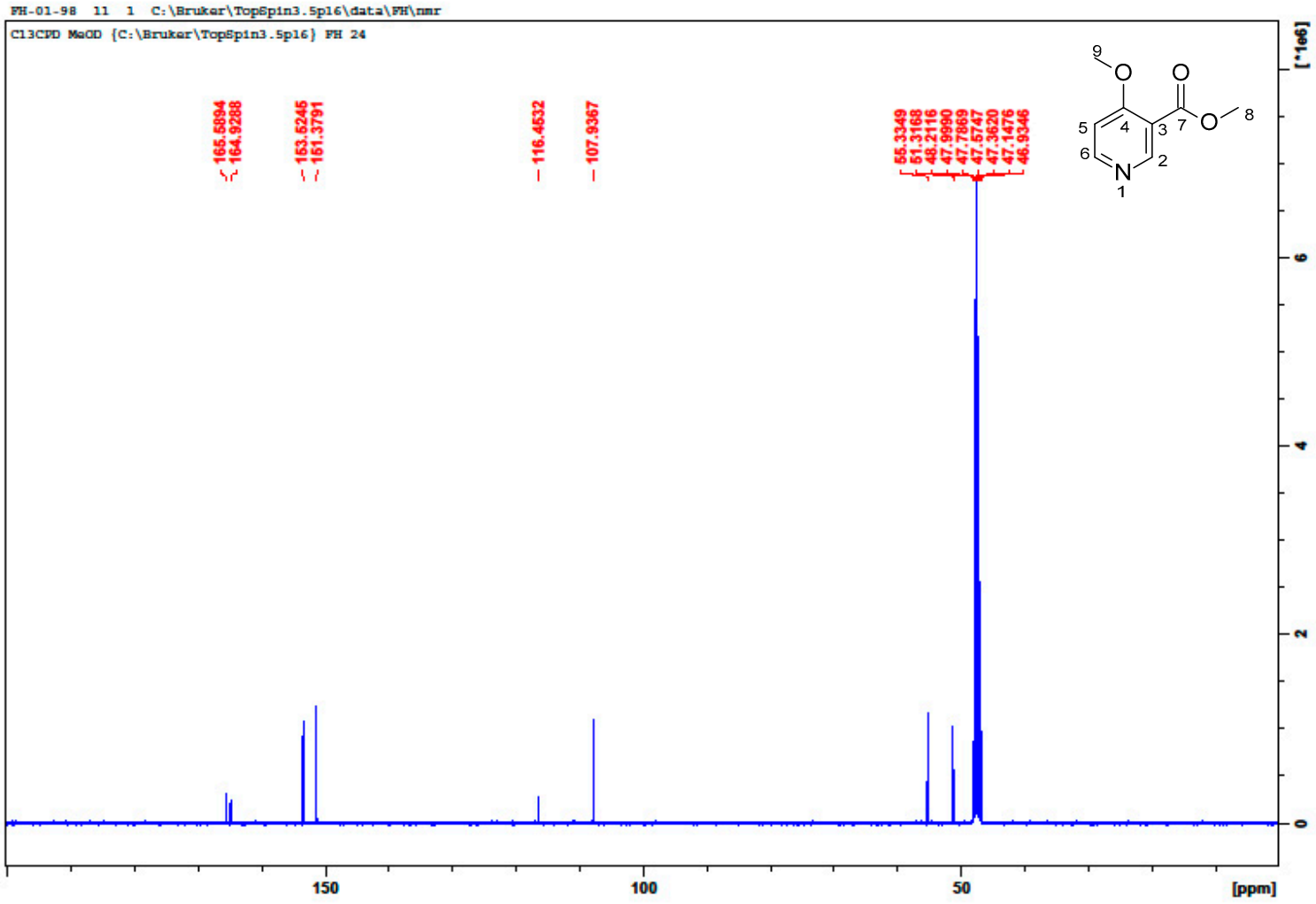
Compound 6. 400 MHz ^{13}C NMR spectrum in D_2O



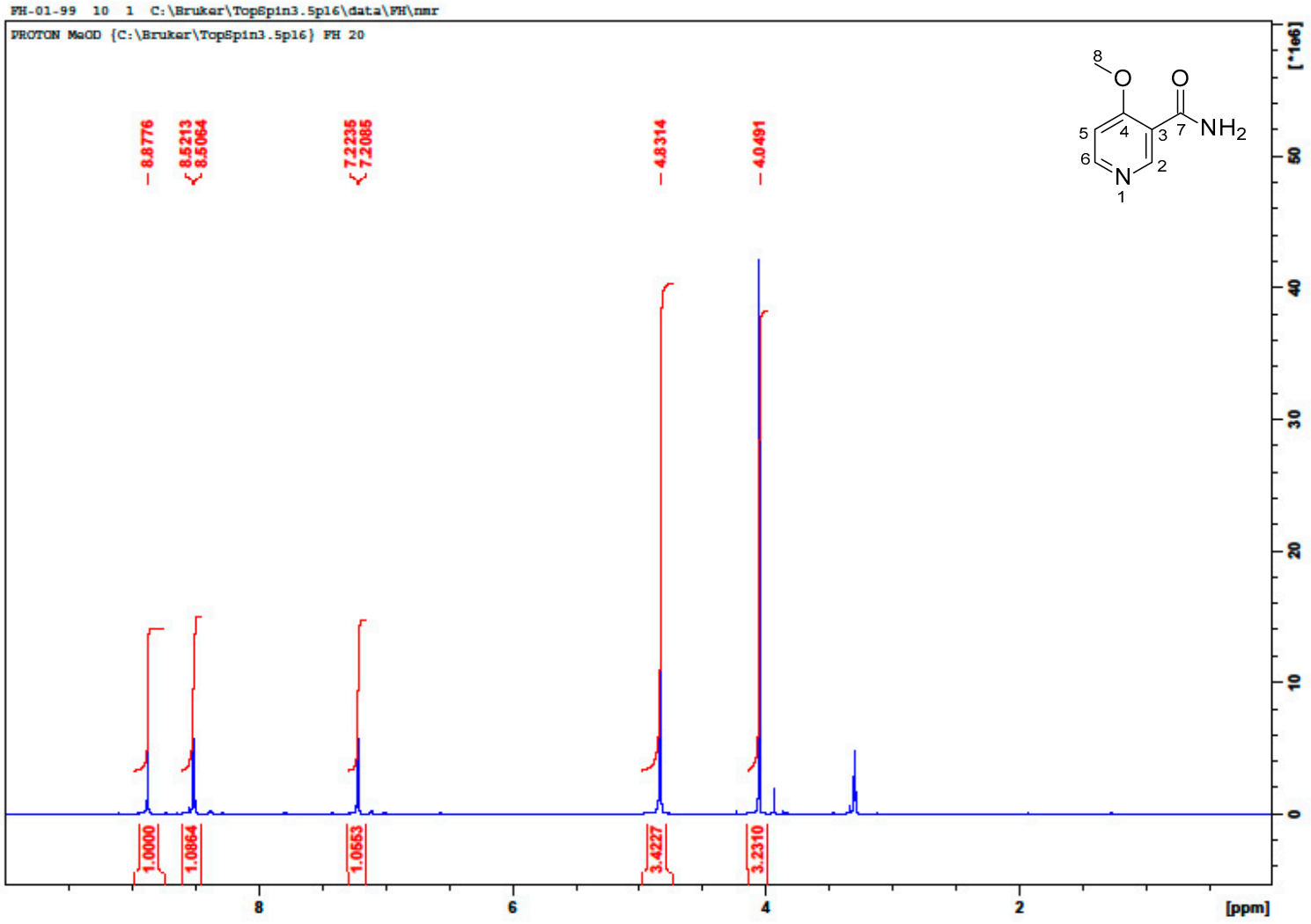
Compound 6. HRMS spectra



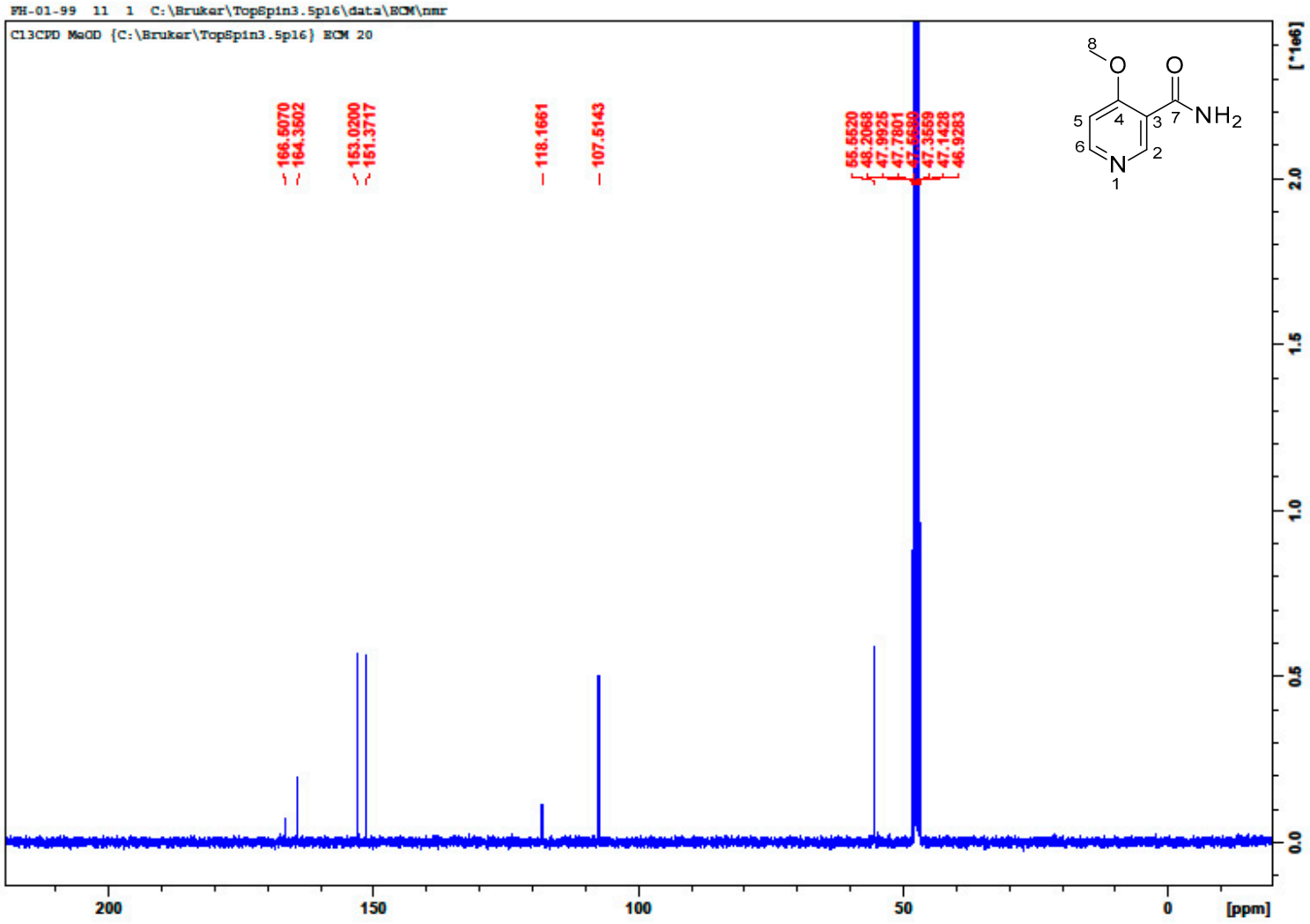
Compound 9. 400 MHz ^{1}H NMR spectrum in MeOD



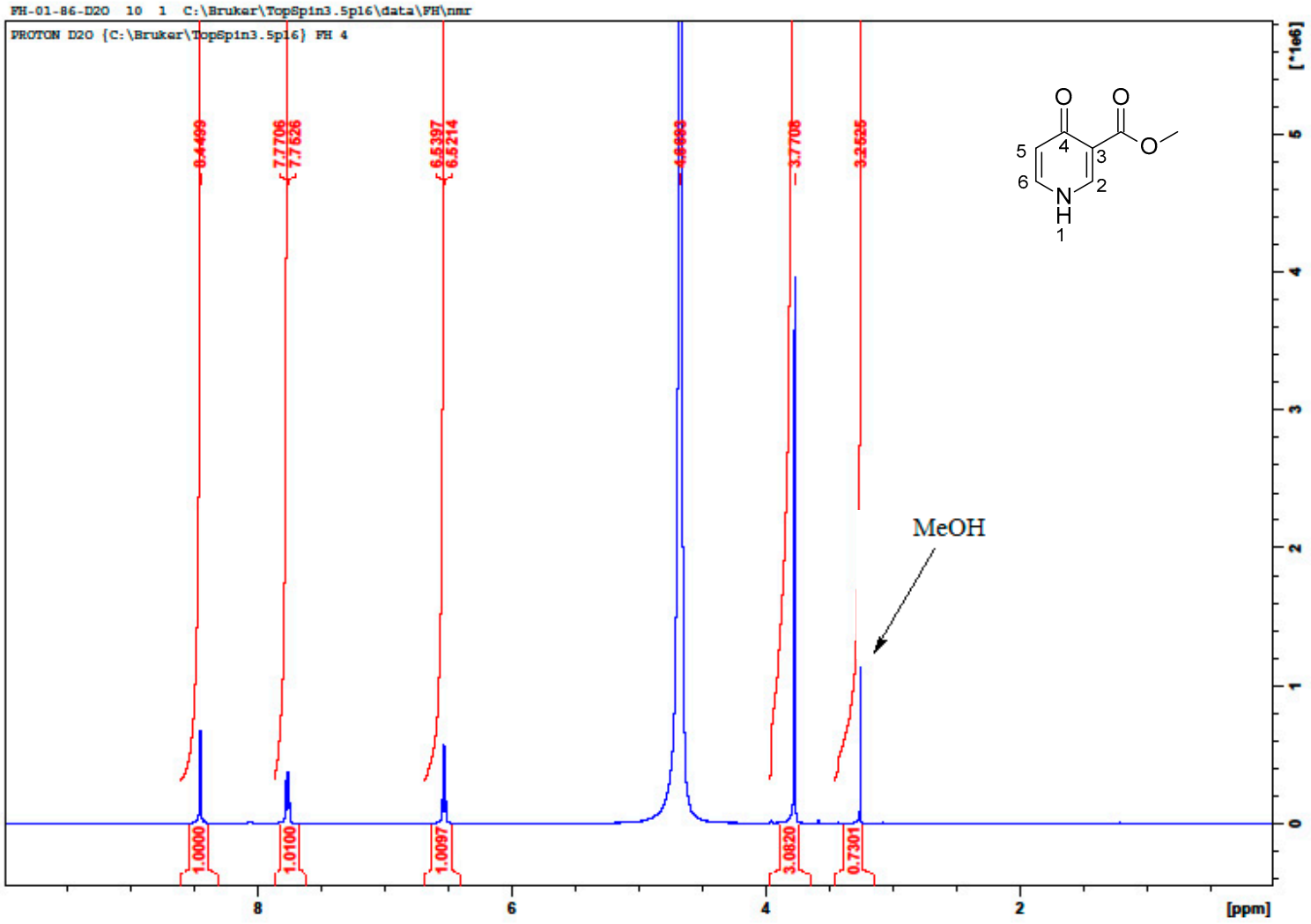
Compound 9. 400 MHz ^{13}C NMR spectrum in MeOD



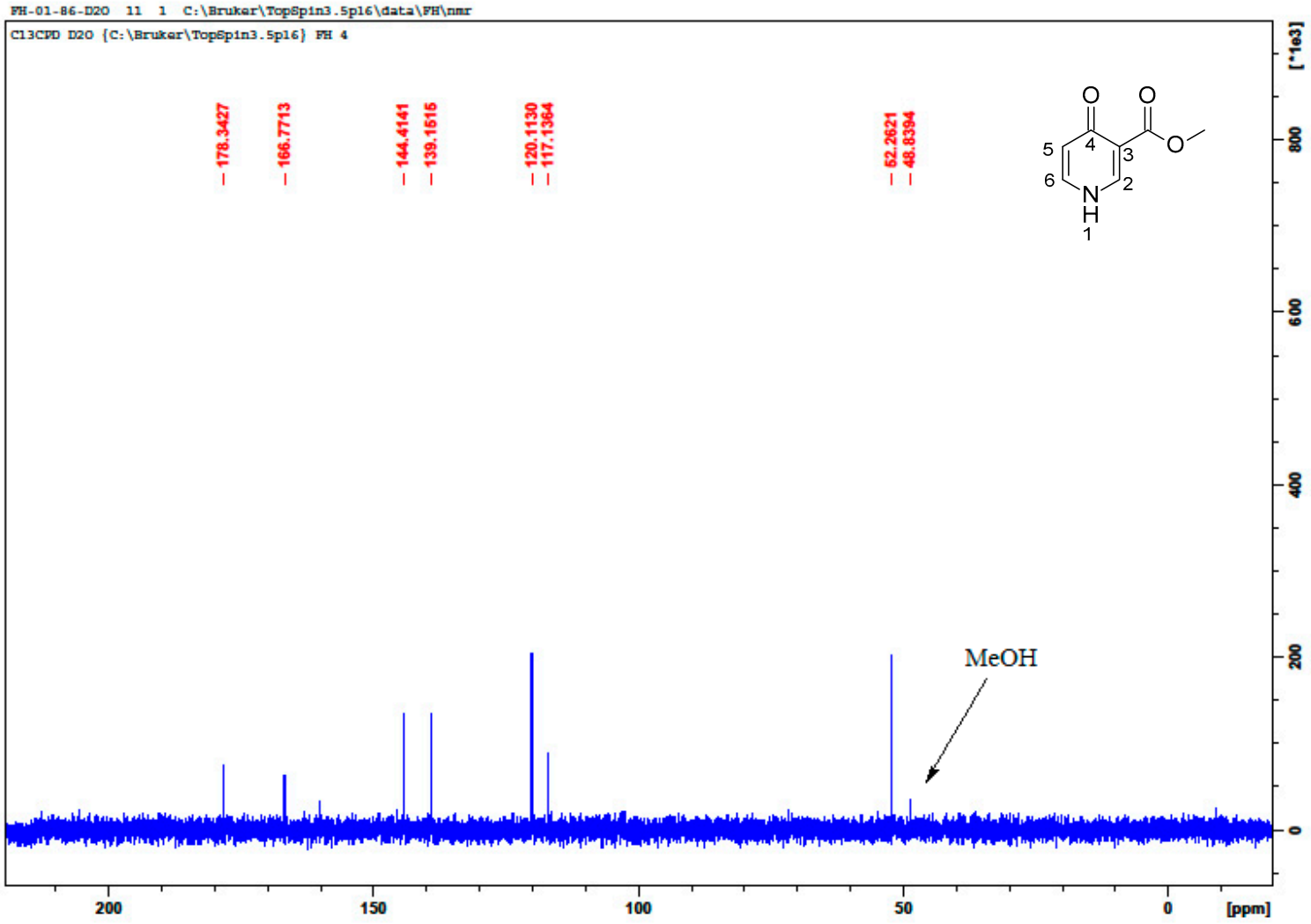
Compound 10. 400 MHz ^{1}H NMR spectrum in MeOD



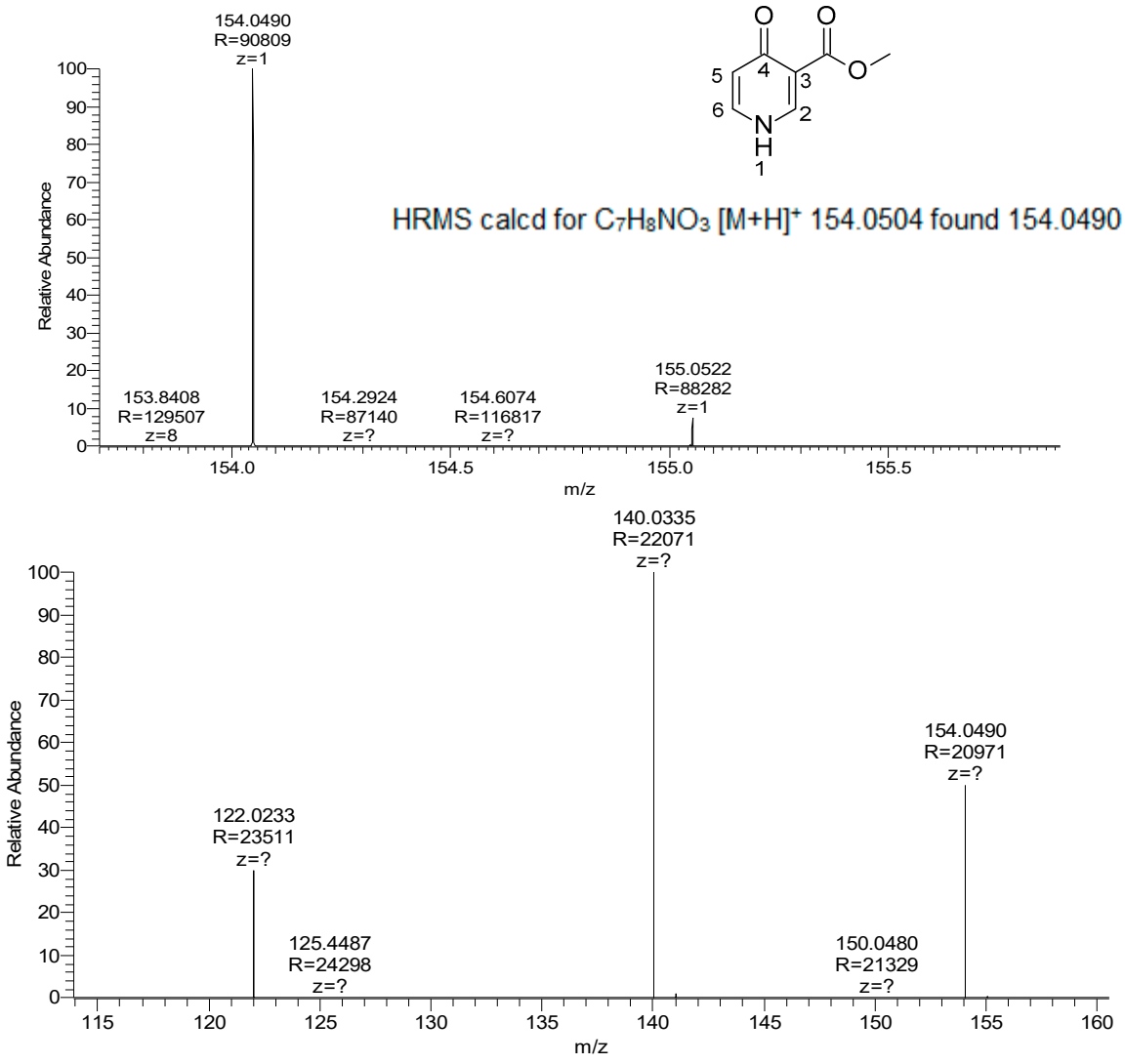
Compound 10. 400 MHz ^{13}C NMR spectrum in MeOD



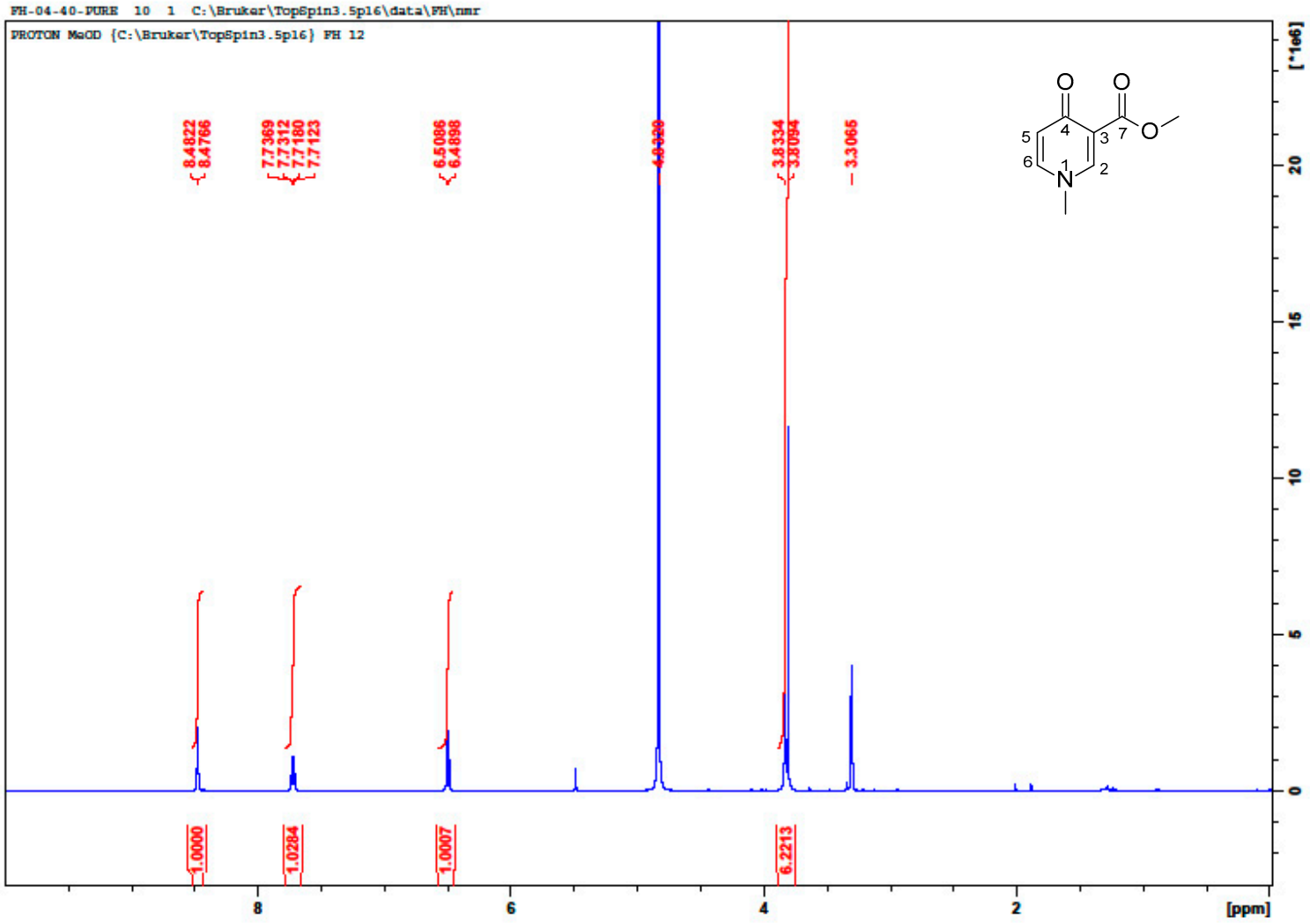
Compound 11. 400 MHz 1H NMR spectrum in D_2O



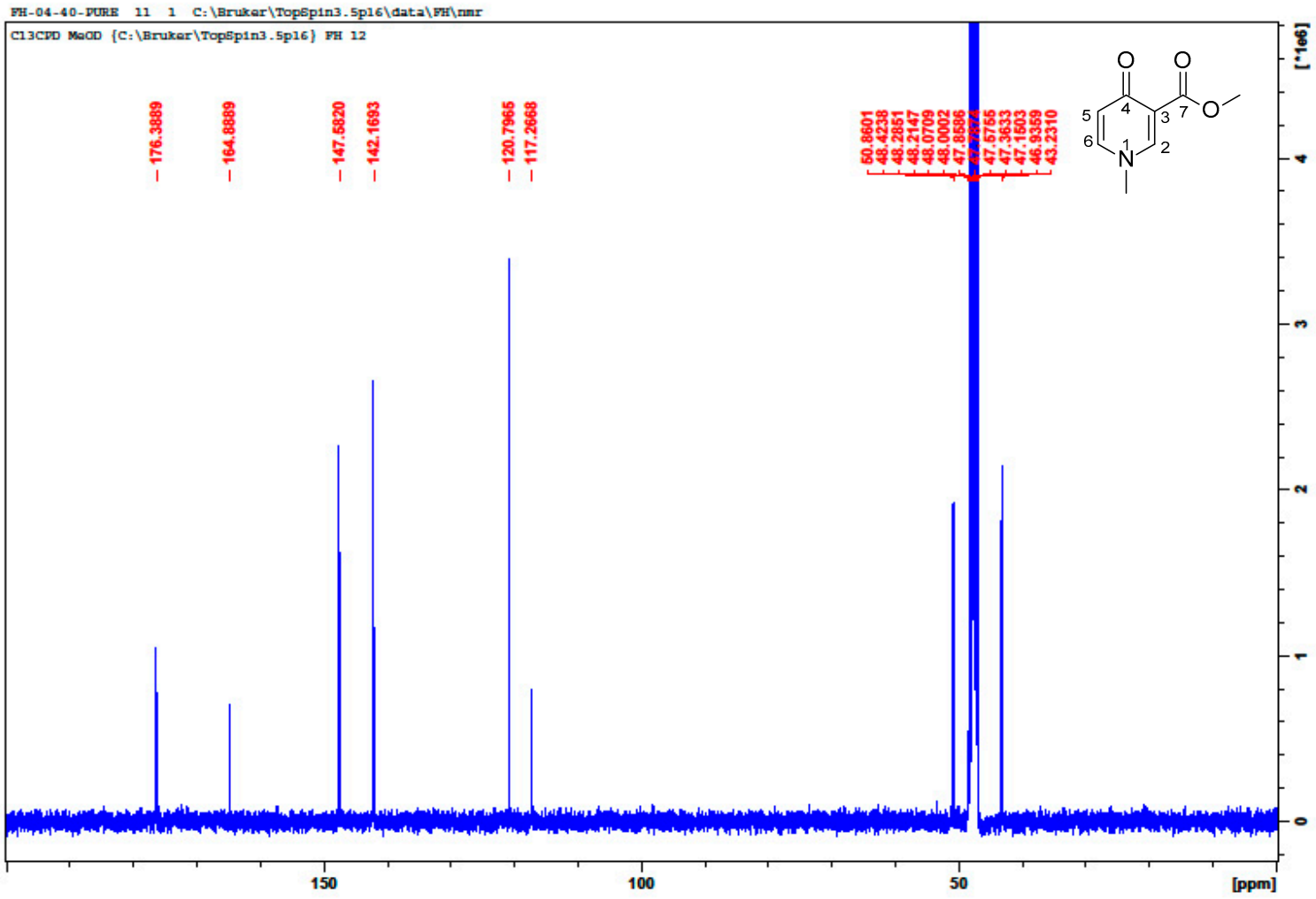
Compound 11. 100 MHz ^{13}C NMR spectrum in D_2O



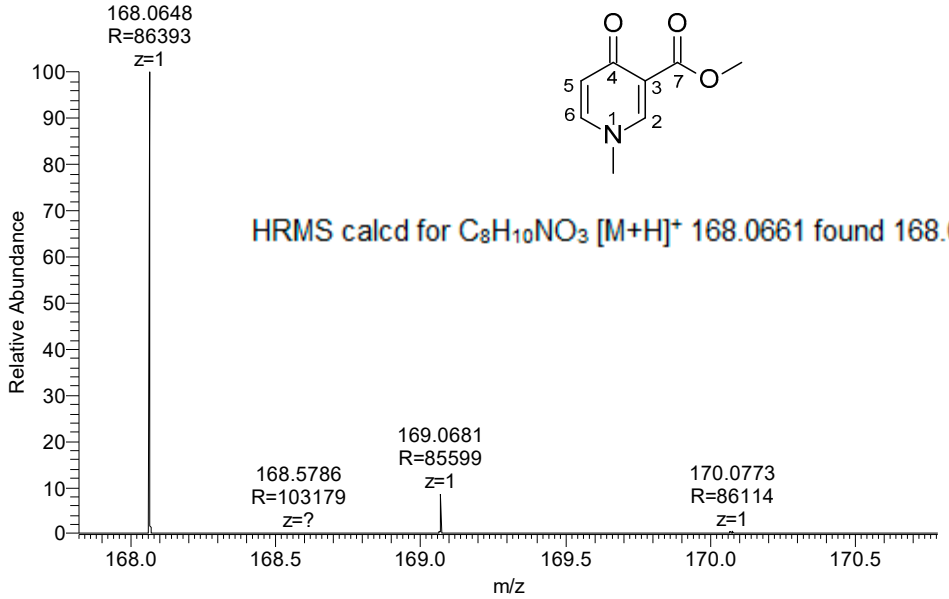
Compound 11. HRMS and MS/MS spectra

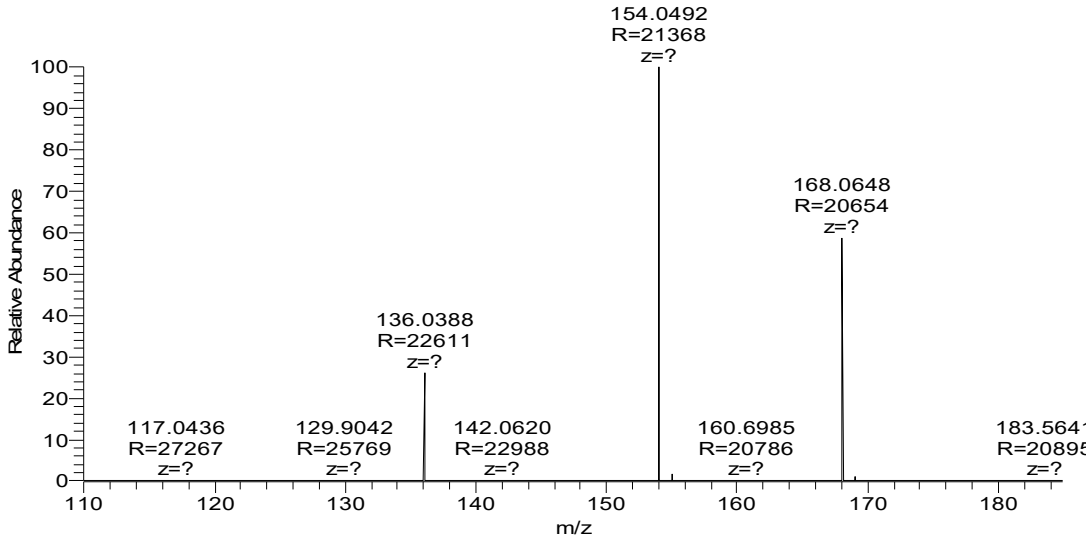


Compound 12. 400 MHz ^{1}H NMR spectrum in MeOD

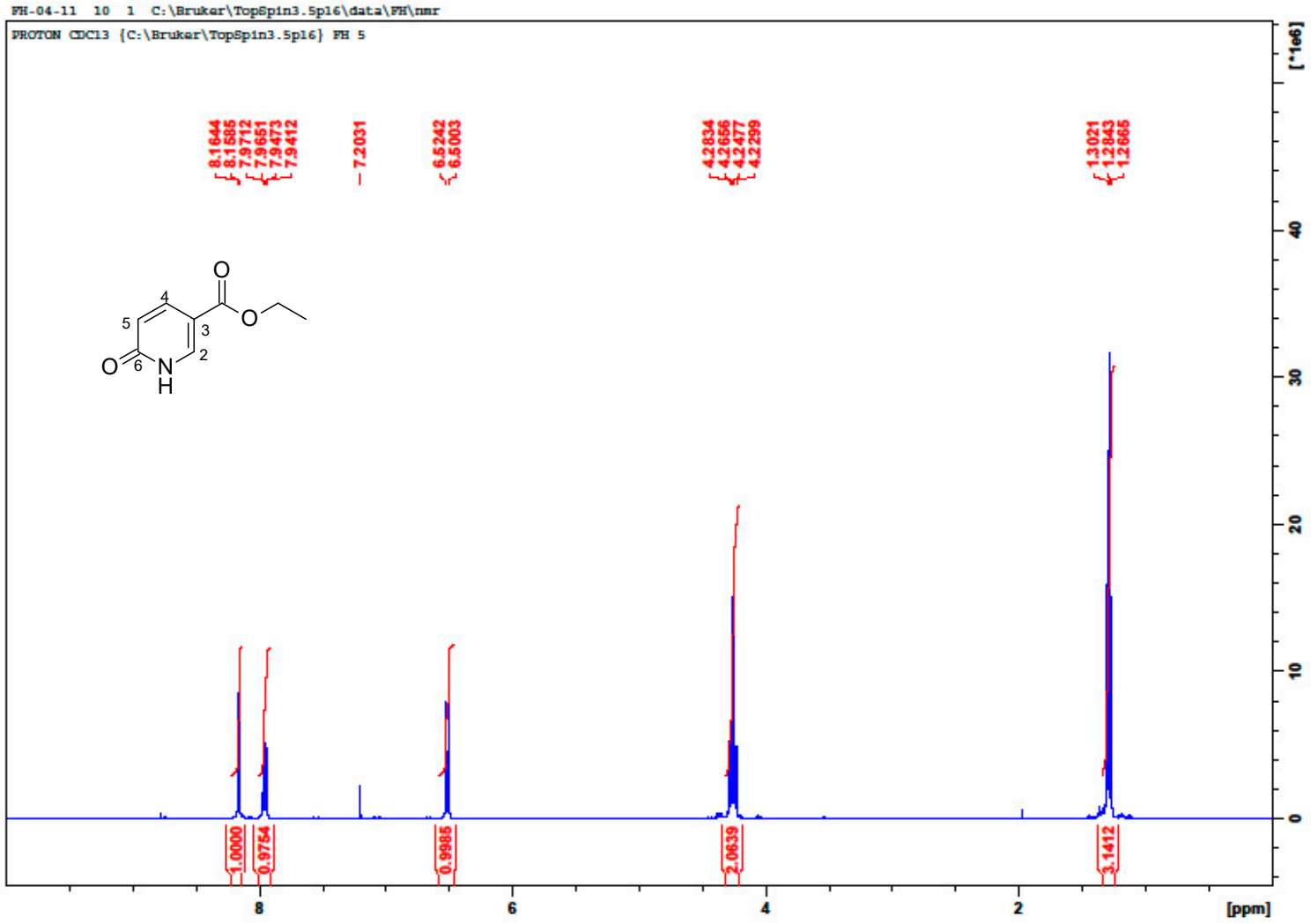


Compound 12. 400 MHz ^{13}C NMR spectrum in MeOD

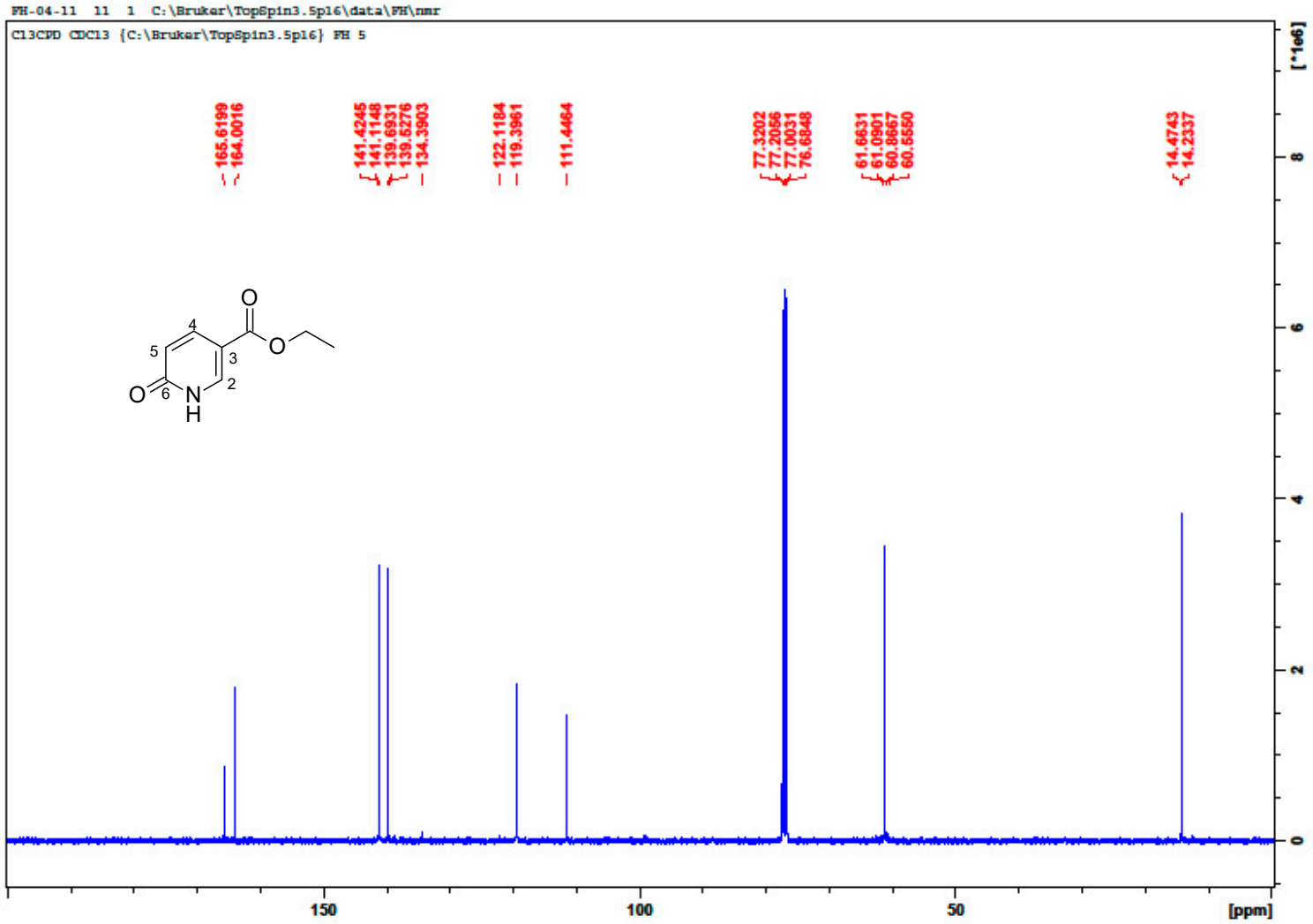




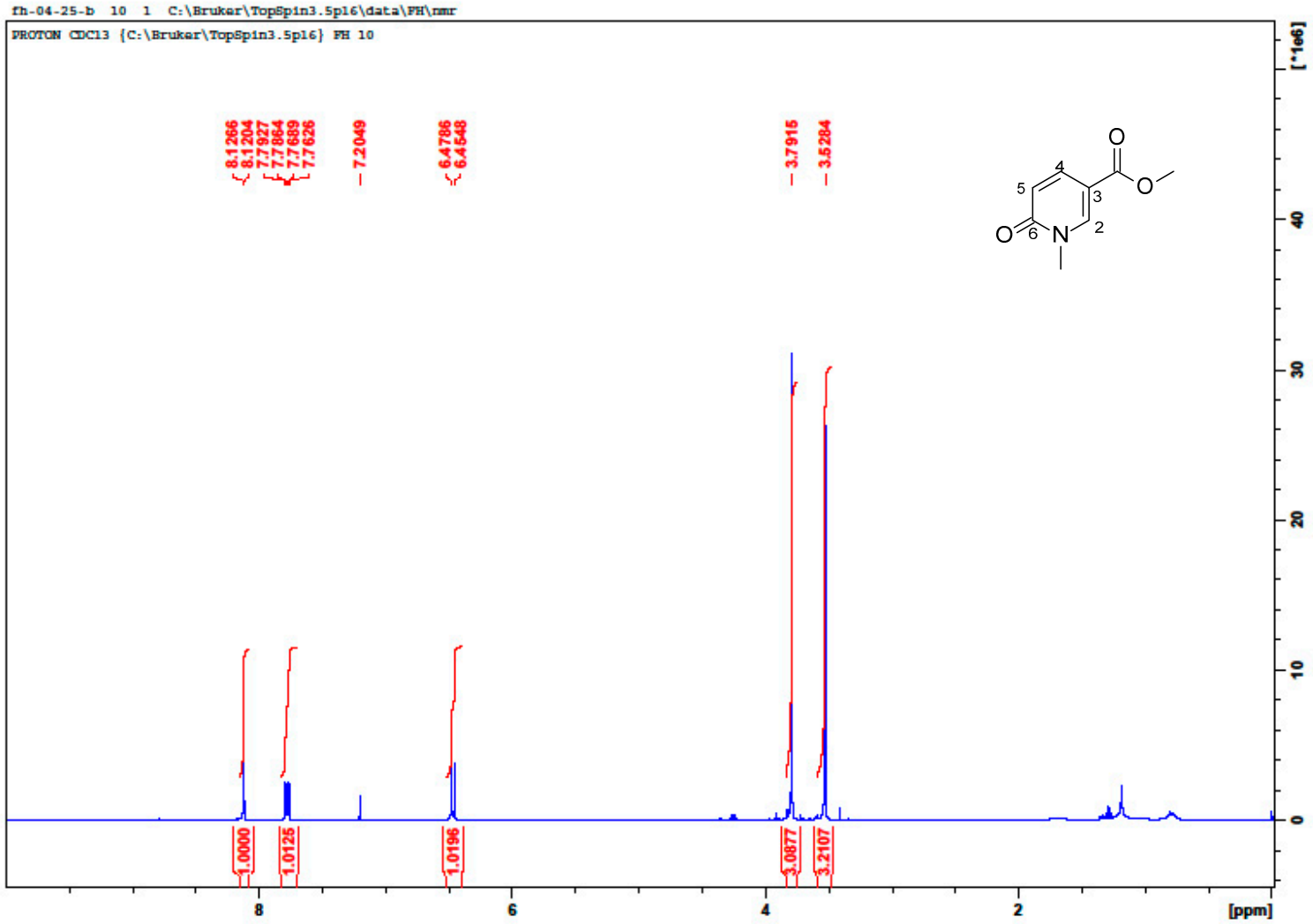
Compound 12. HRMS and MS/MS spectra



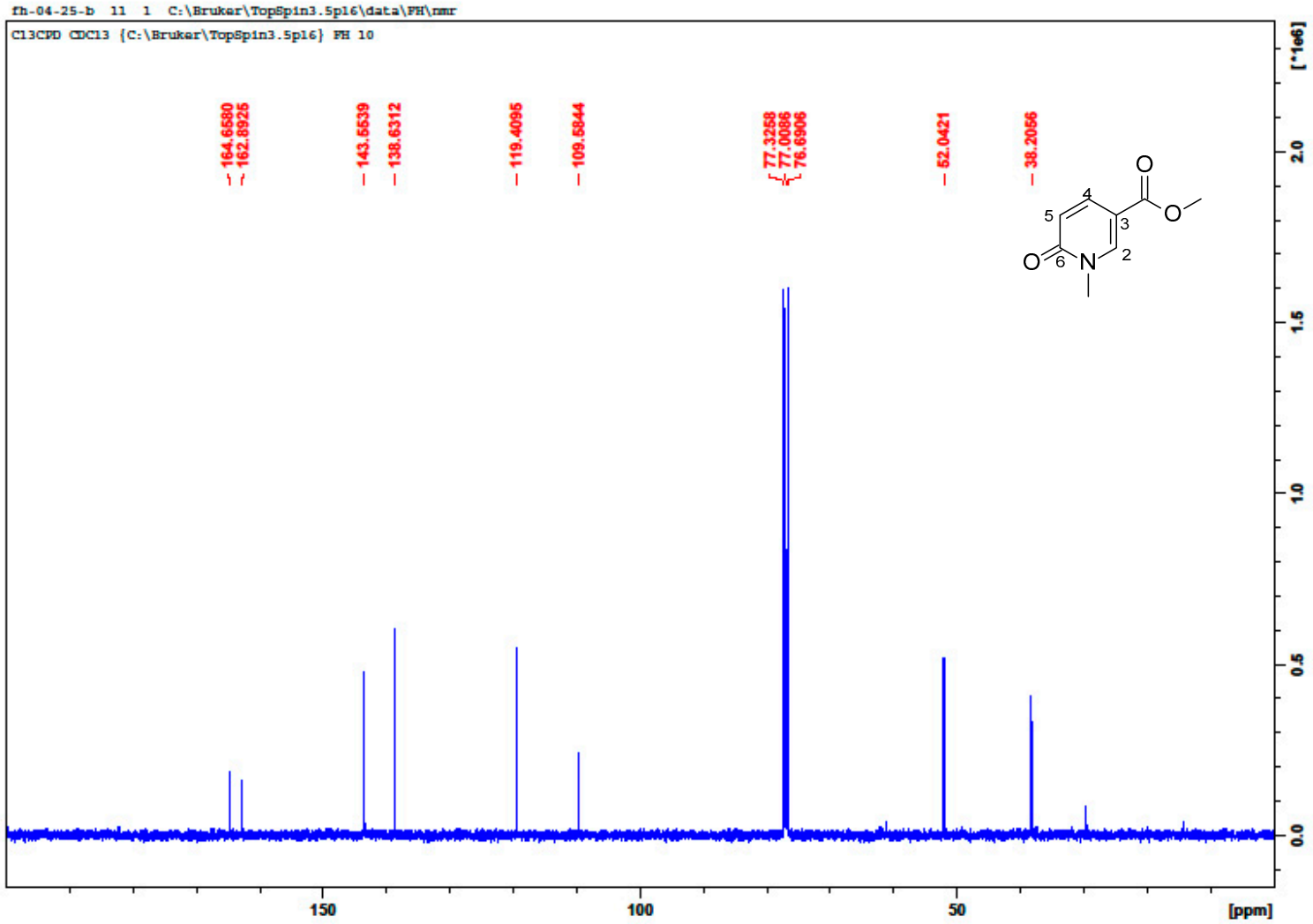
Compound 14. 400 MHz ^{1}H NMR spectrum in $CDCl_3$



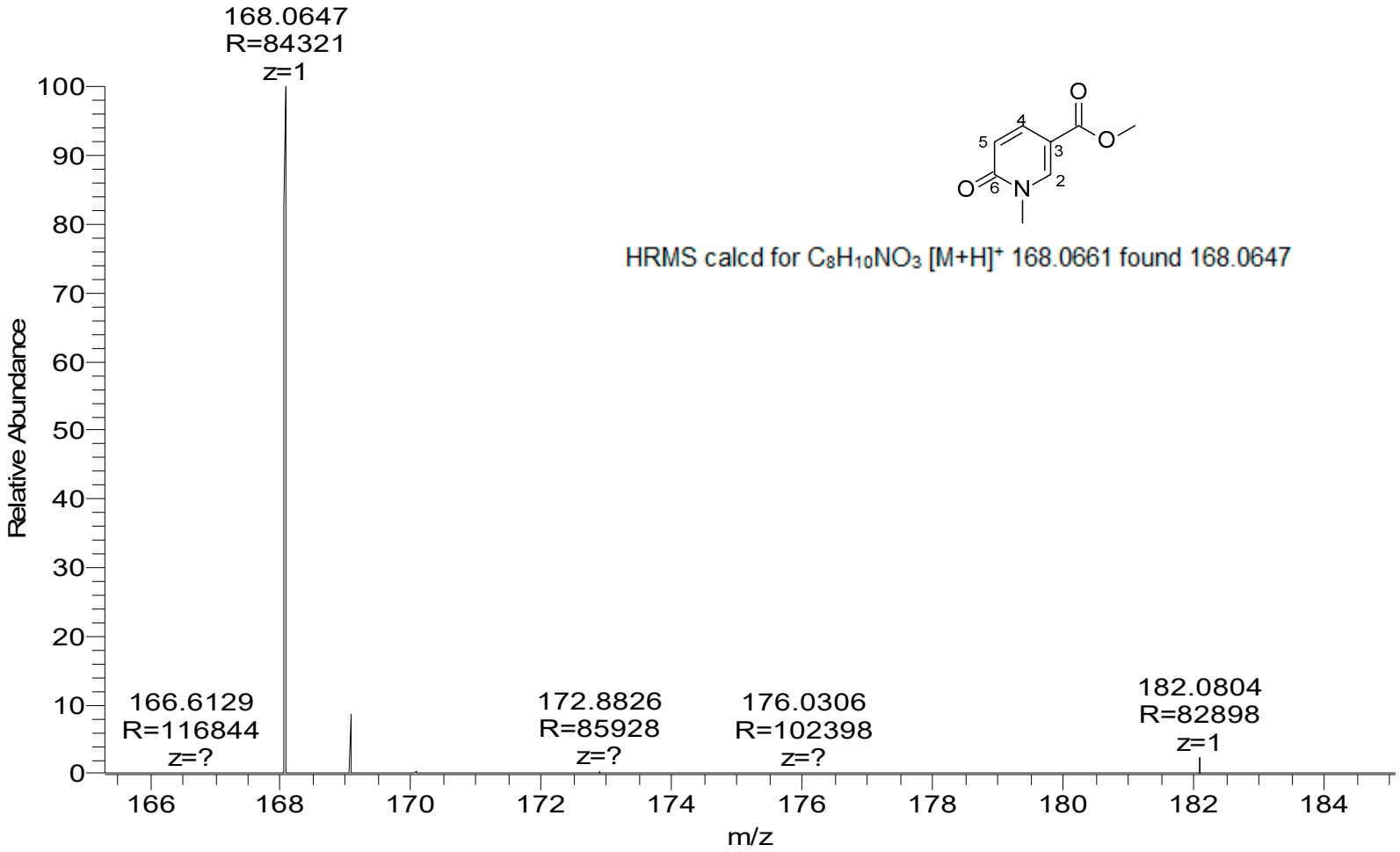
Compound 14. 400 MHz ^{13}C NMR spectrum in $CDCl_3$



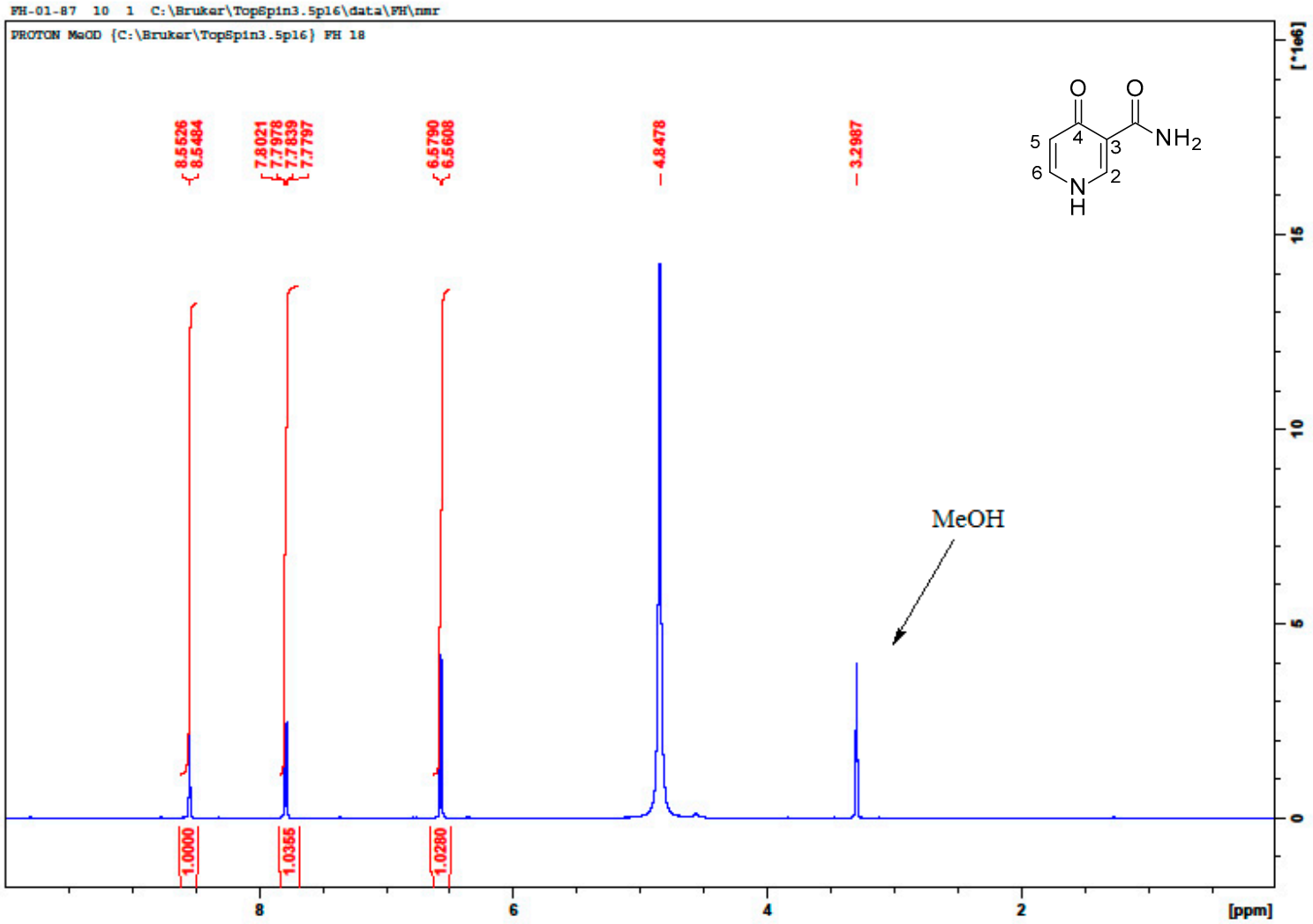
Compound 15. 400 MHz ^{1}H NMR spectrum in $CDCl_3$



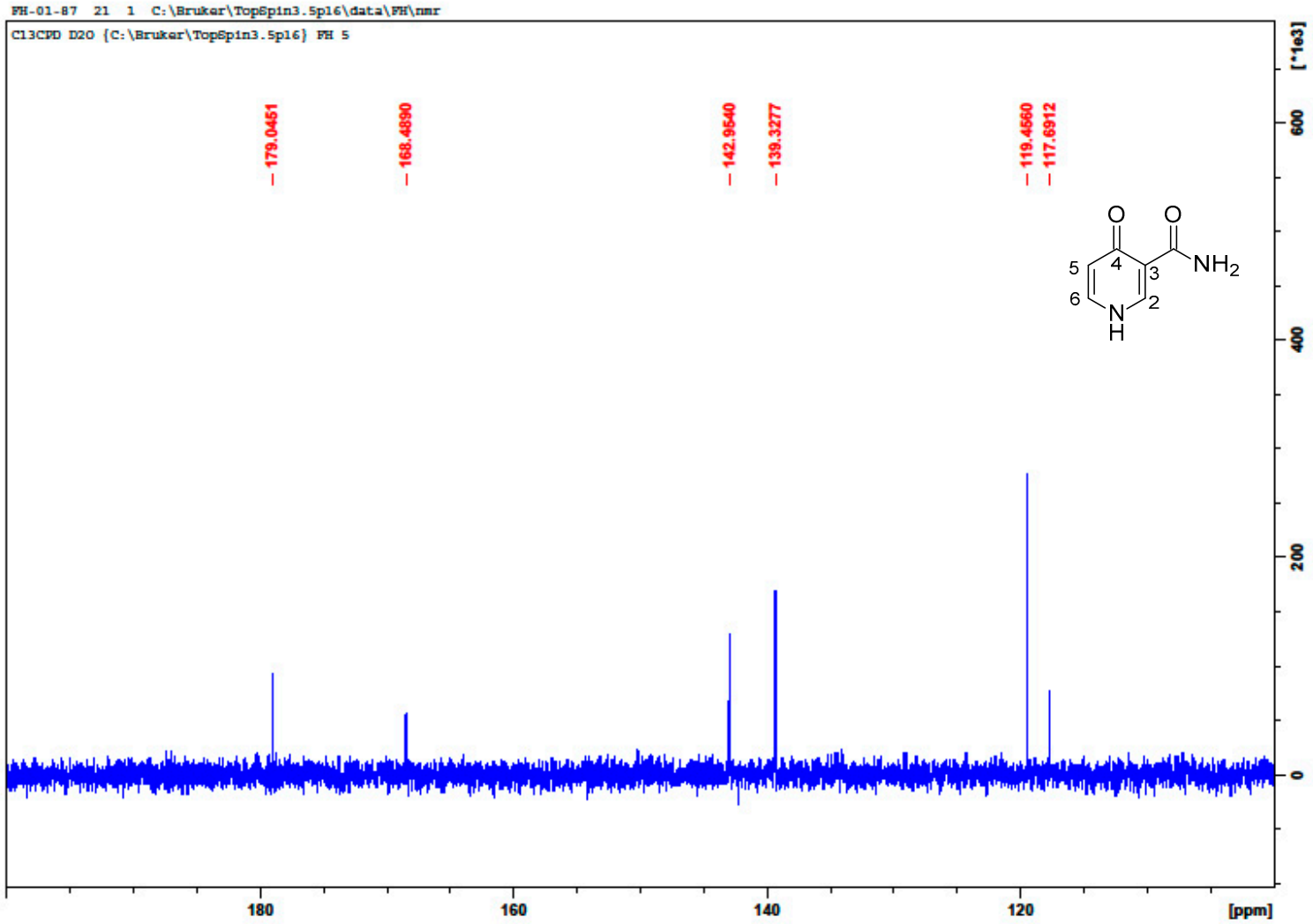
Compound 15. 400 MHz ^{13}C NMR spectrum in $CDCl_3$



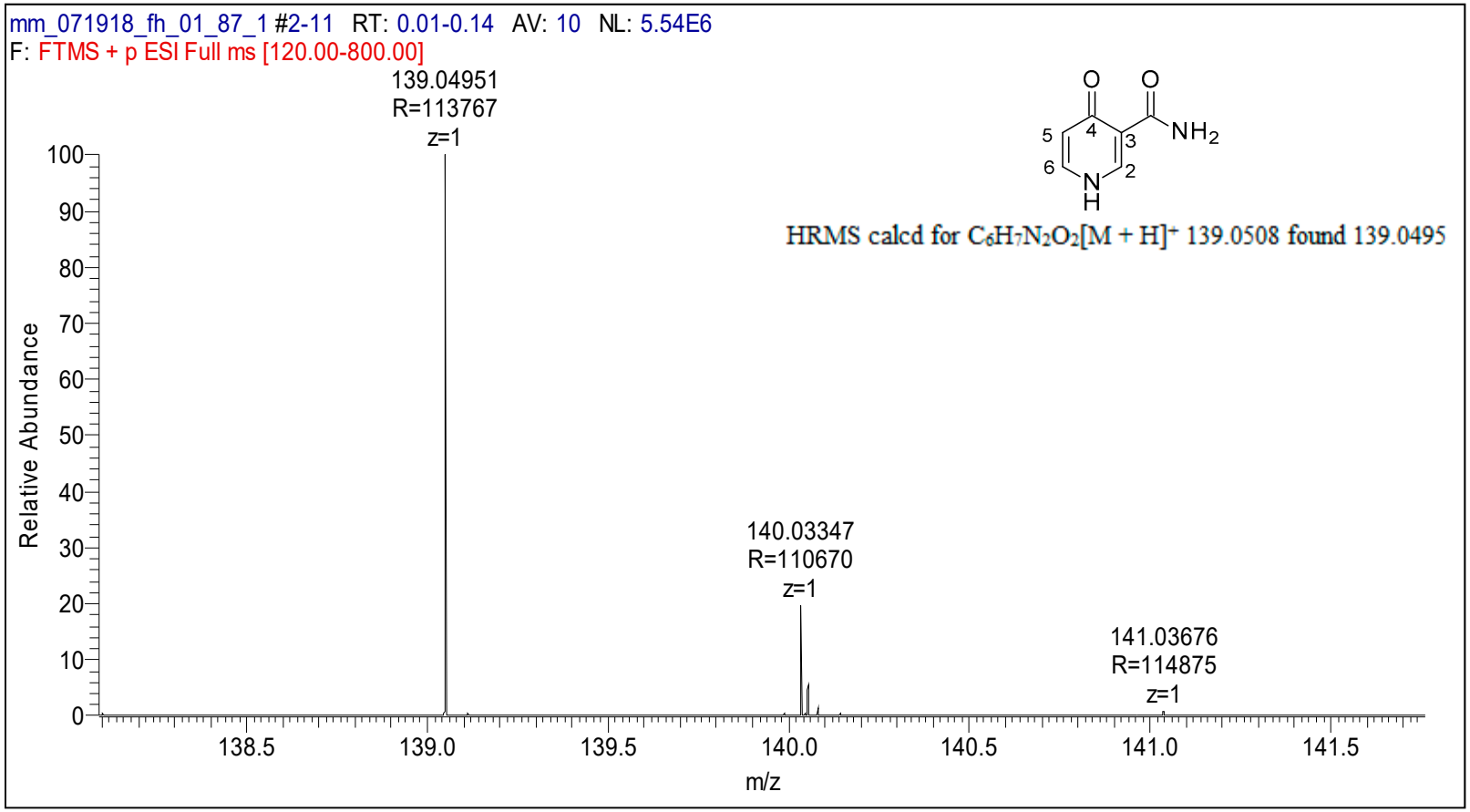
Compound 15. HRMS spectra



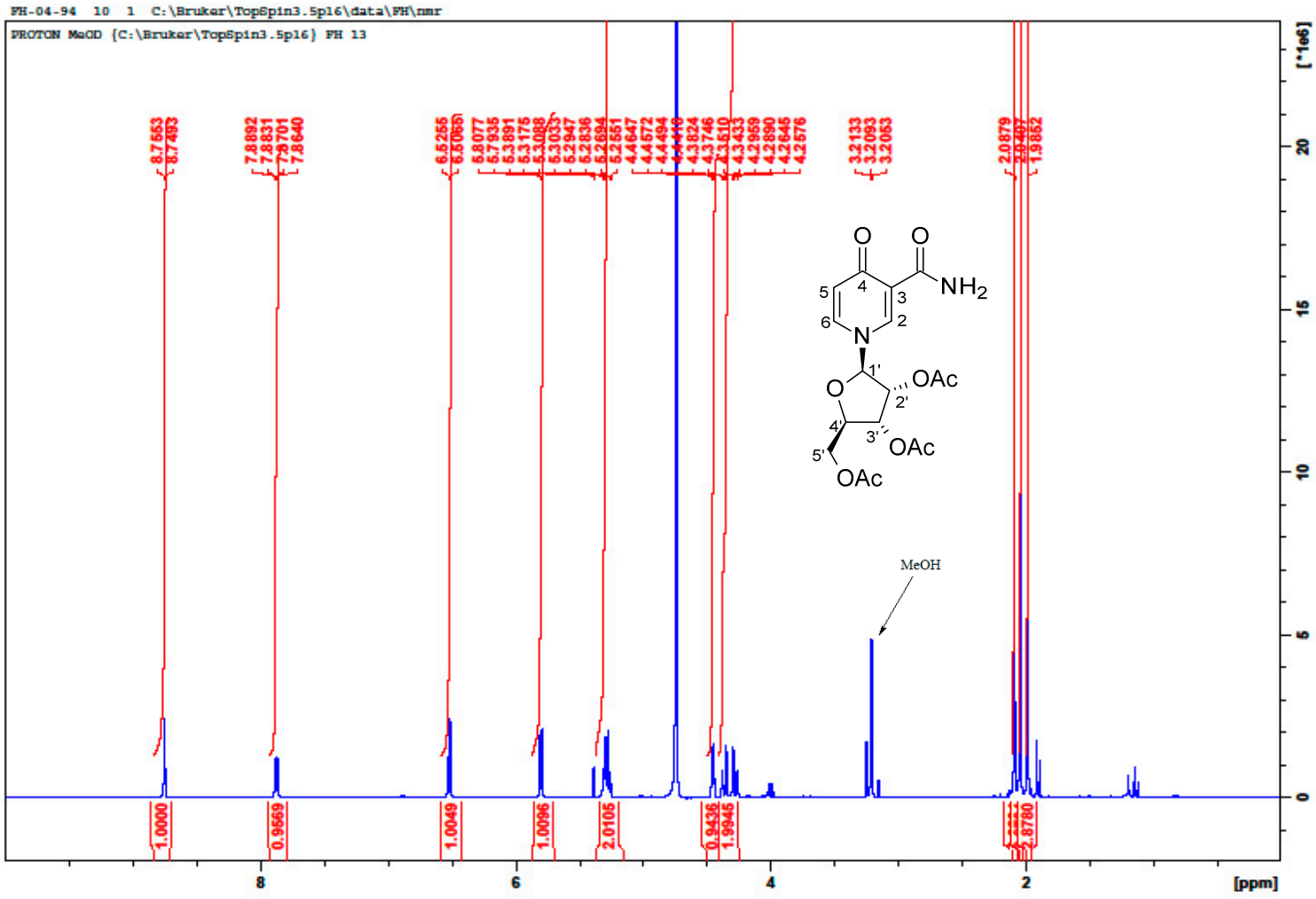
Compound 16. 400 MHz ^{1}H NMR spectrum in MeOD



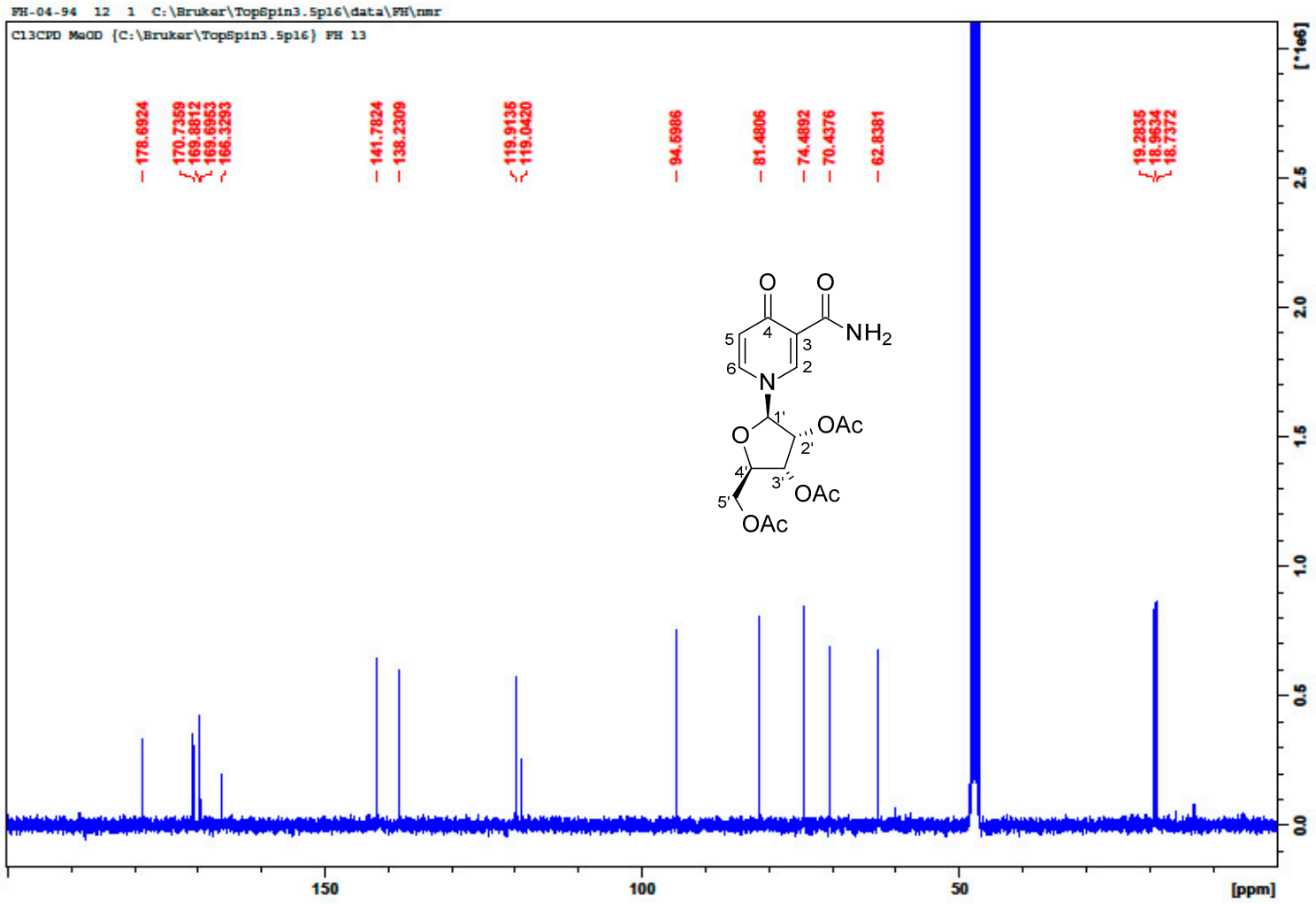
Compound 16. 100 MHz ^{13}C NMR spectrum in MeOD



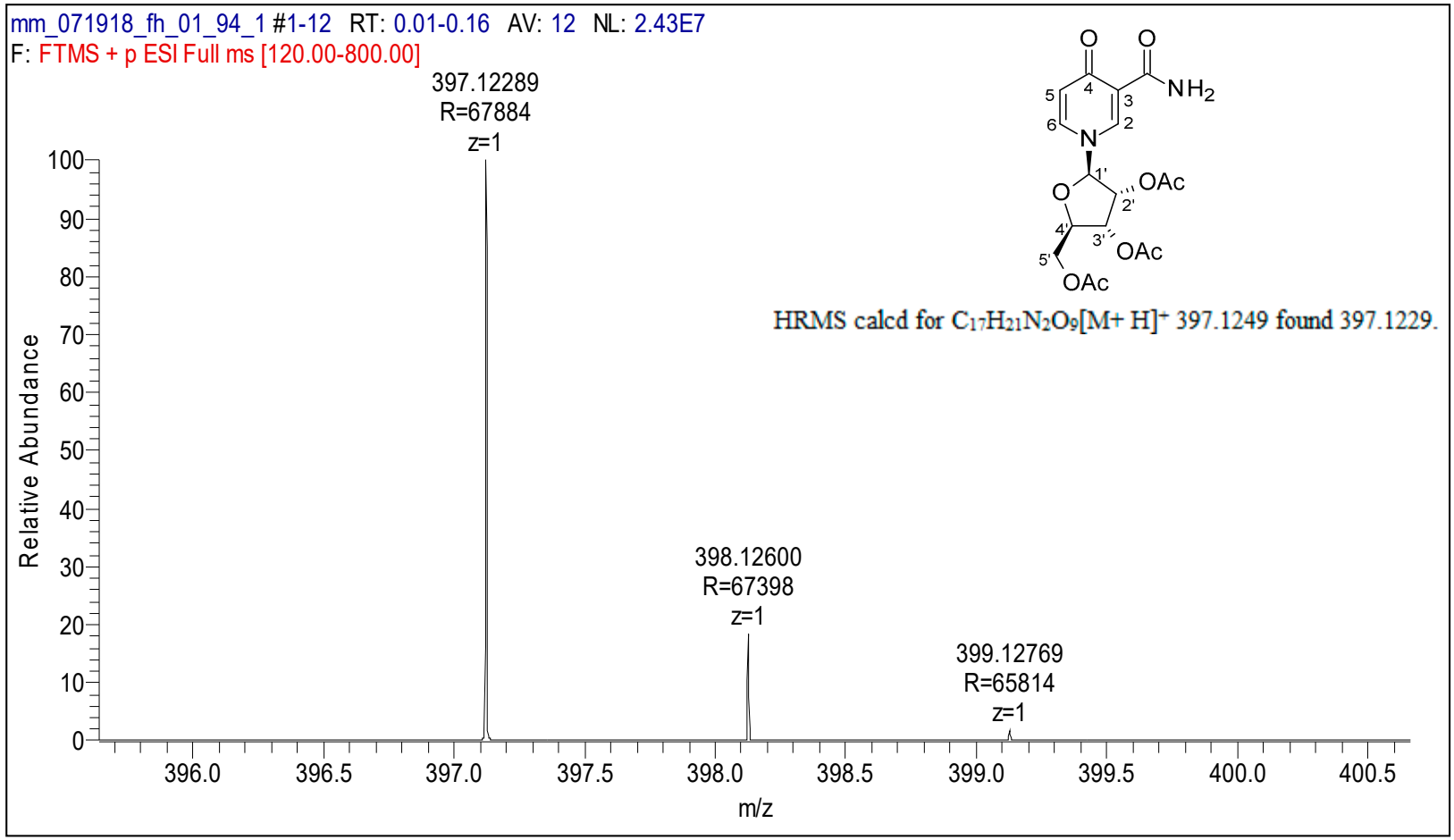
Compound 16. HRMS spectra



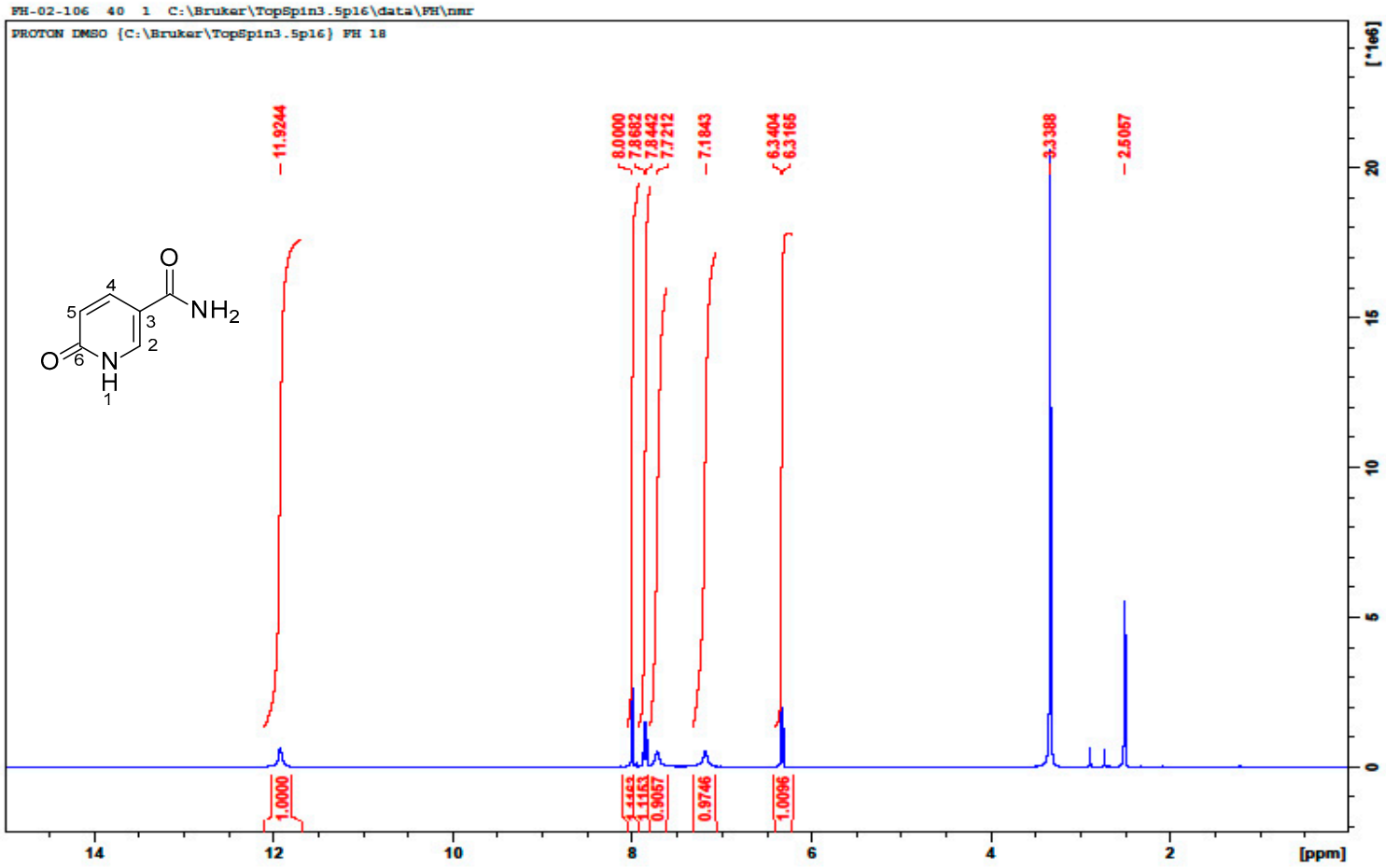
Compound 18. 400 MHz ^{1}H NMR spectrum in MeOD



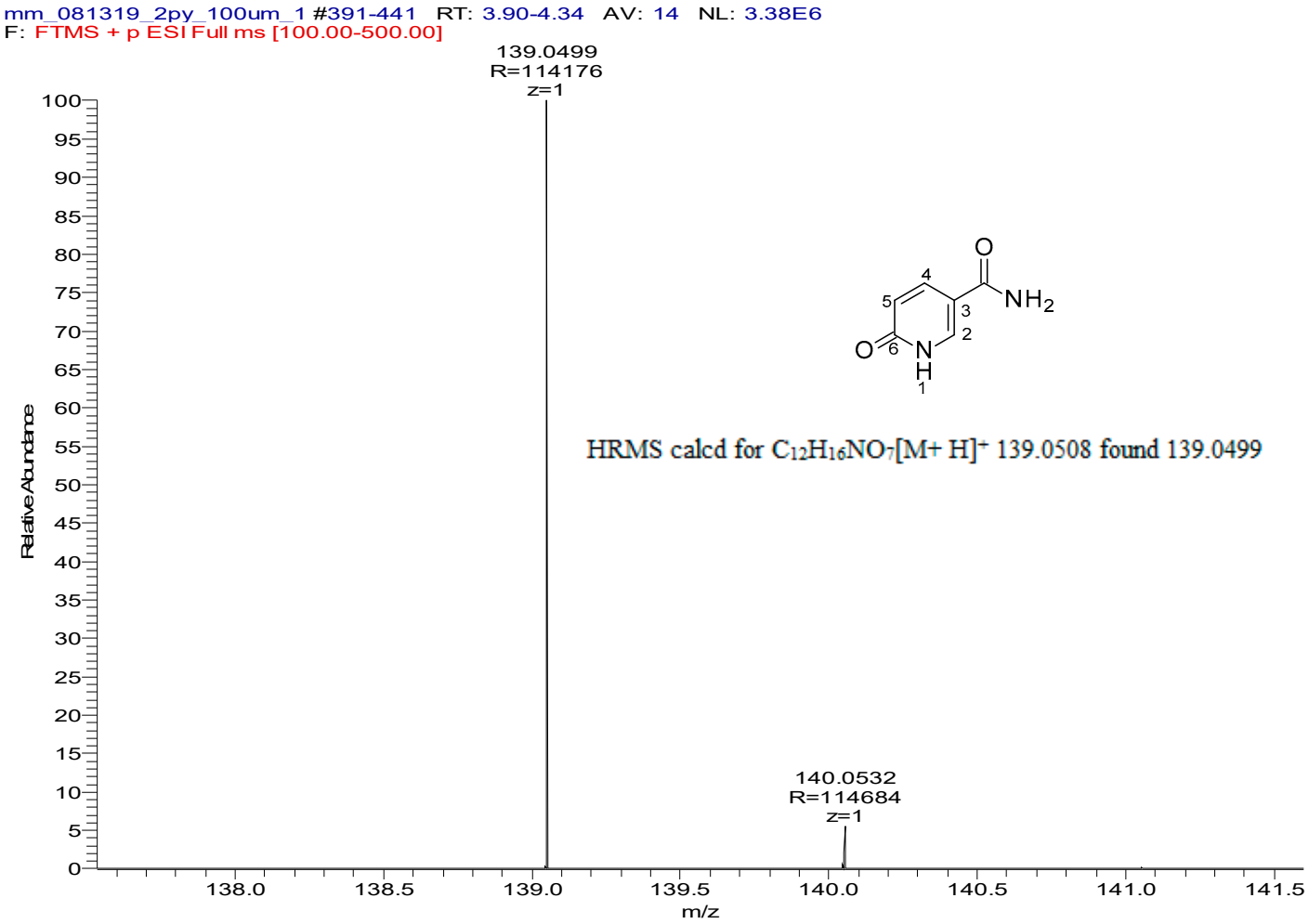
Compound 18. 100 MHz ^{13}C NMR spectrum in MeOD



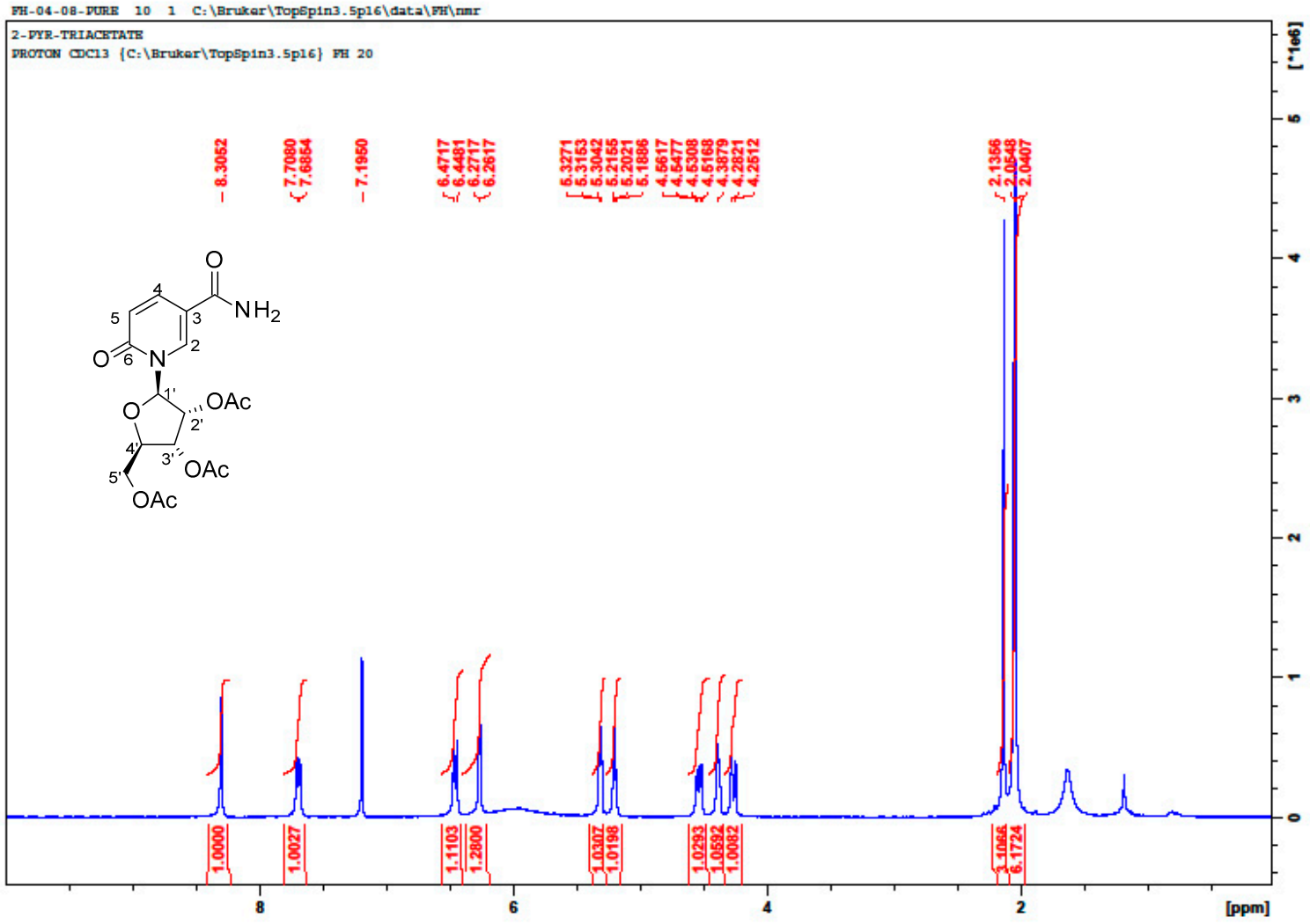
Compound 18. HRMS spectra



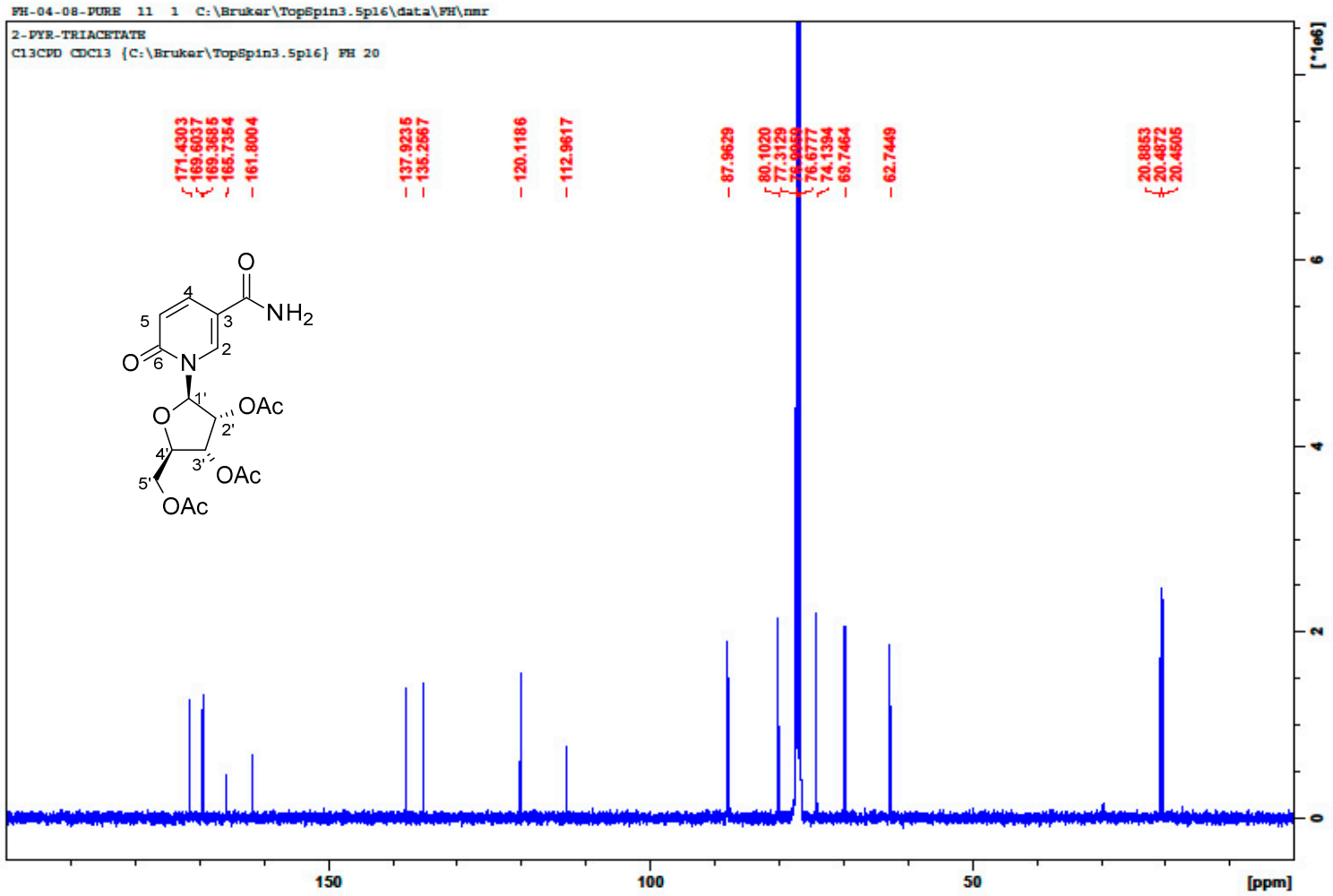
Compound 20. 400 MHz ^{1}H NMR spectrum in DMSO



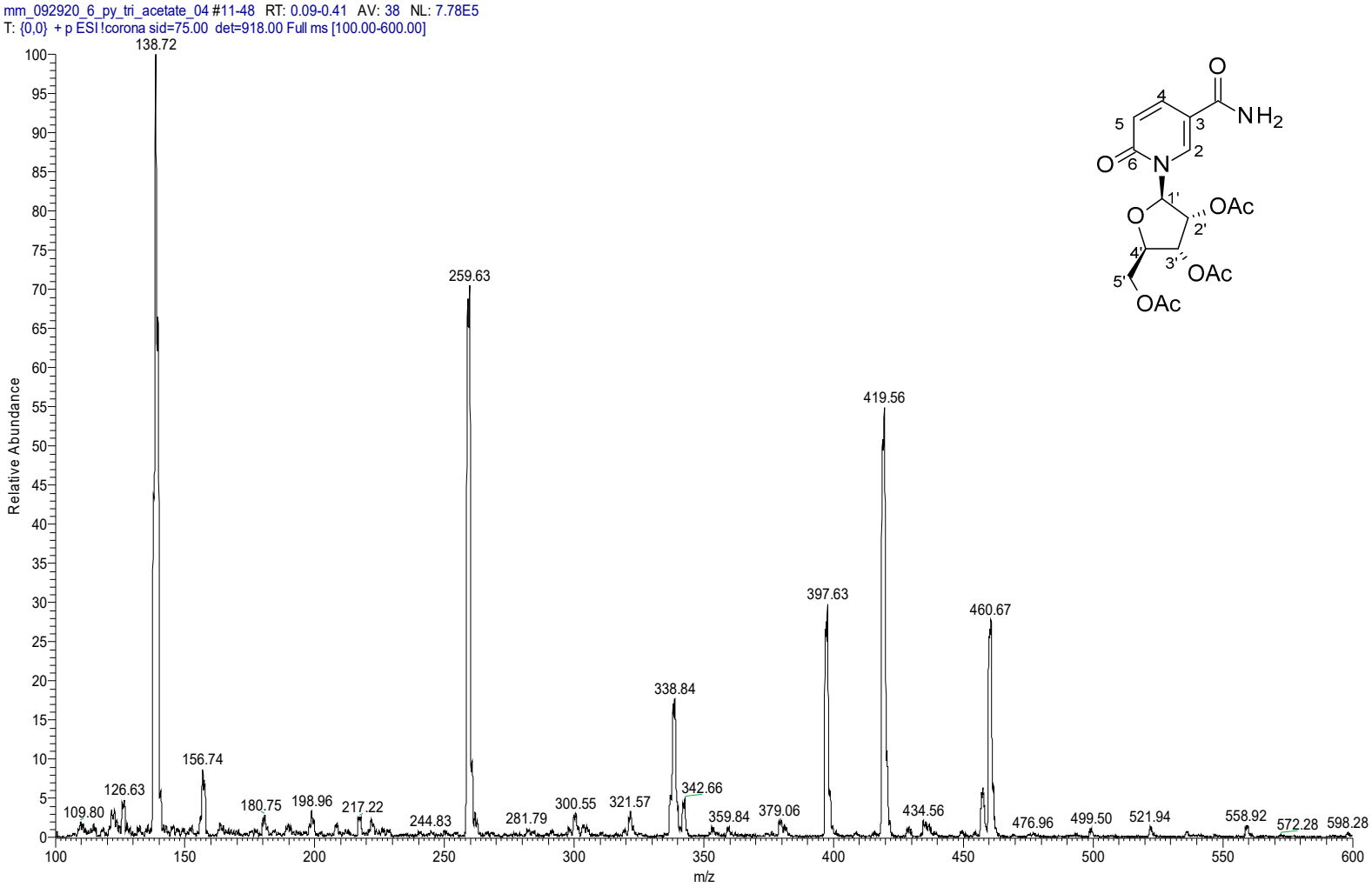
Compound 20. HRMS spectra



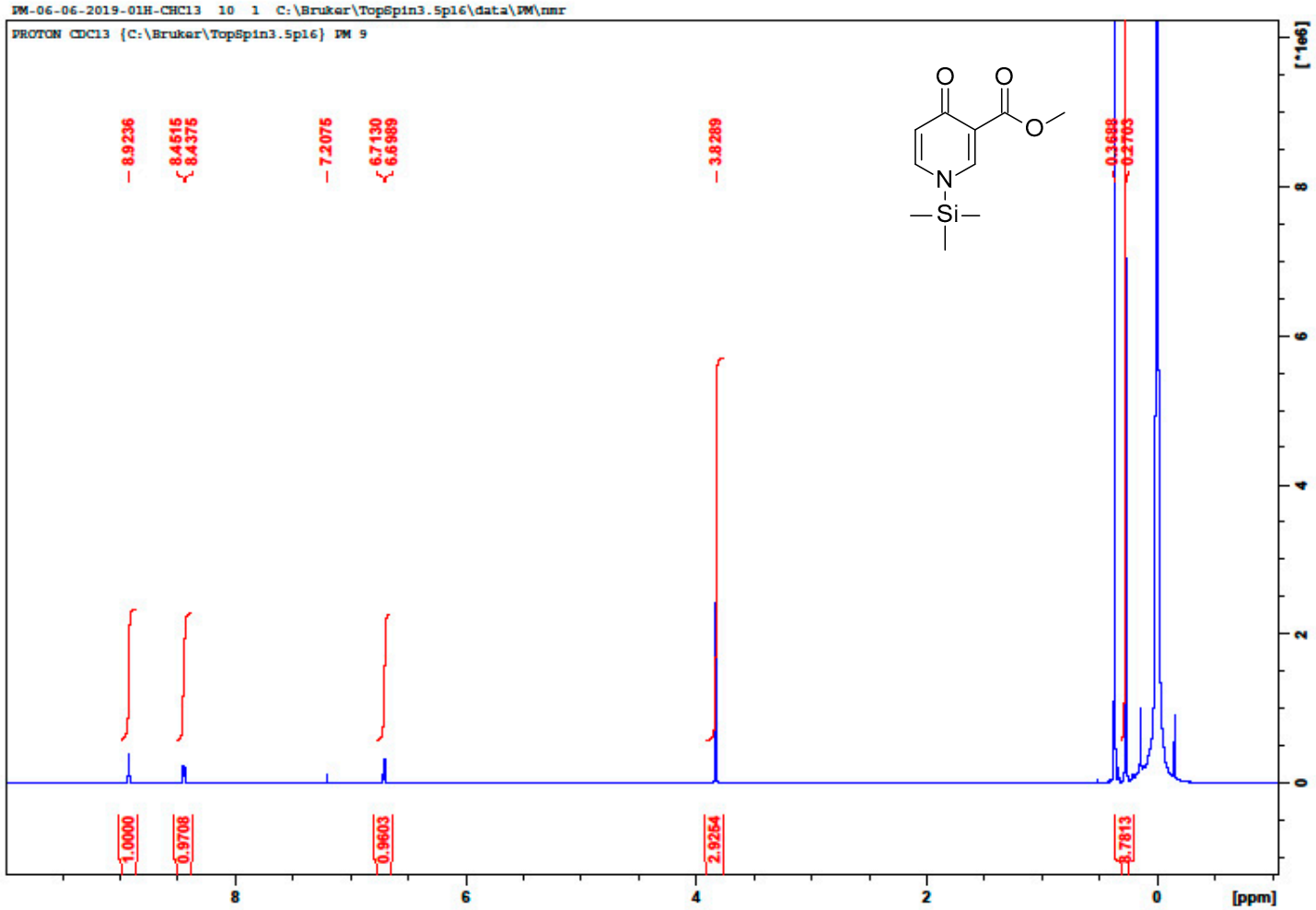
Compound 22. 400 MHz ^{1}H NMR spectrum in $CDCl_3$



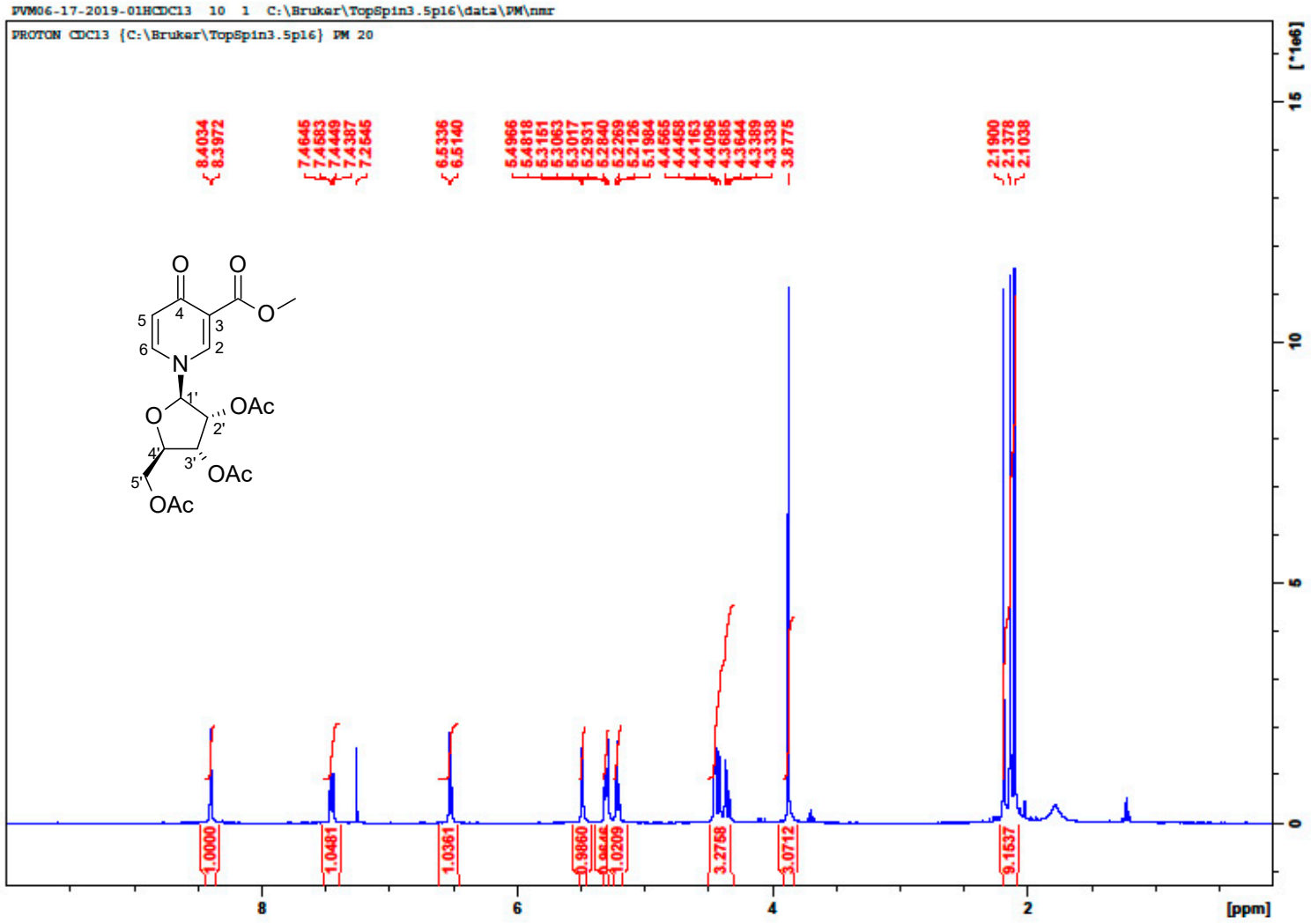
Compound 22. 400 MHz ^{13}C NMR spectrum in $CDCl_3$



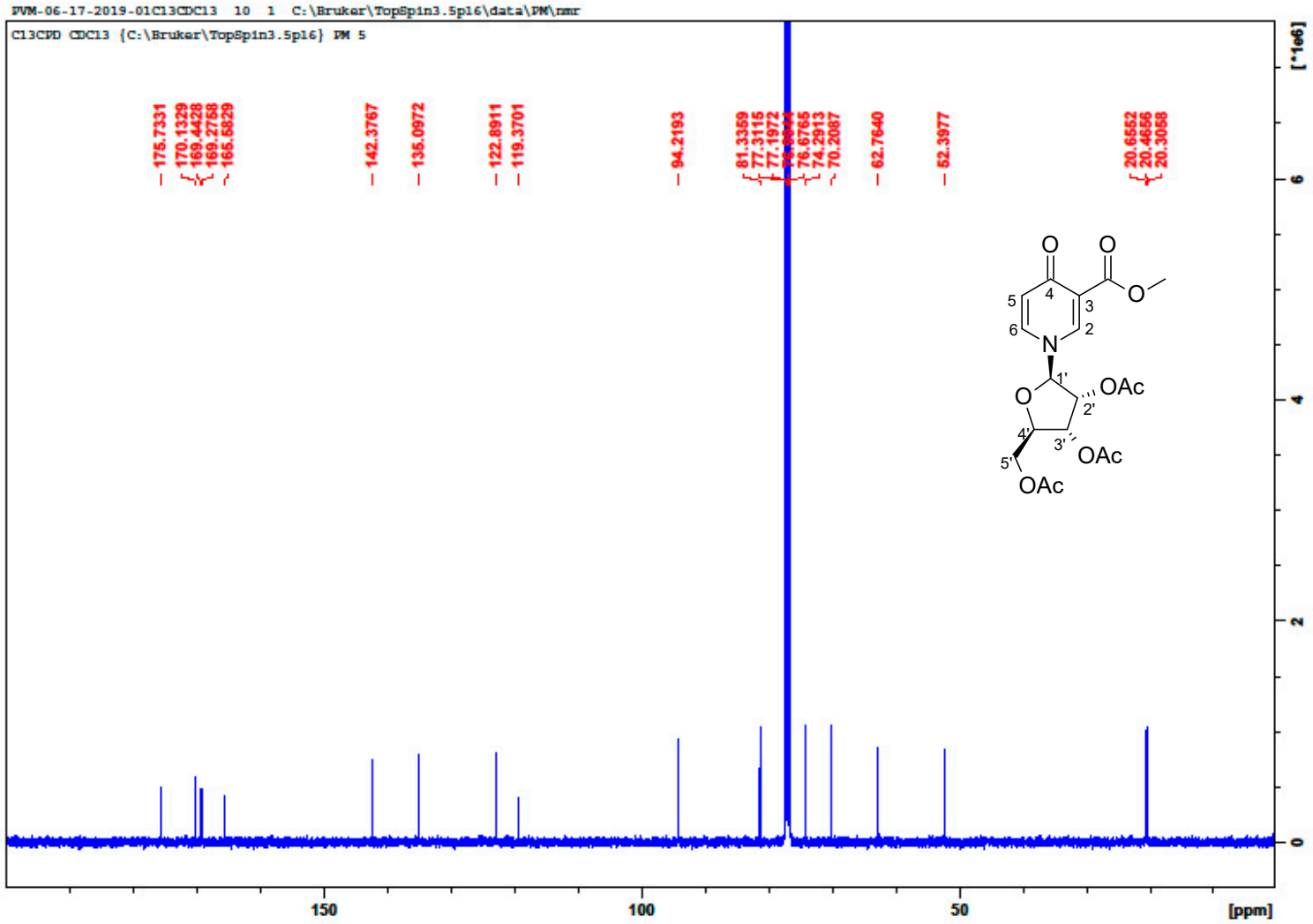
Compound 22. (ES)MS spectra



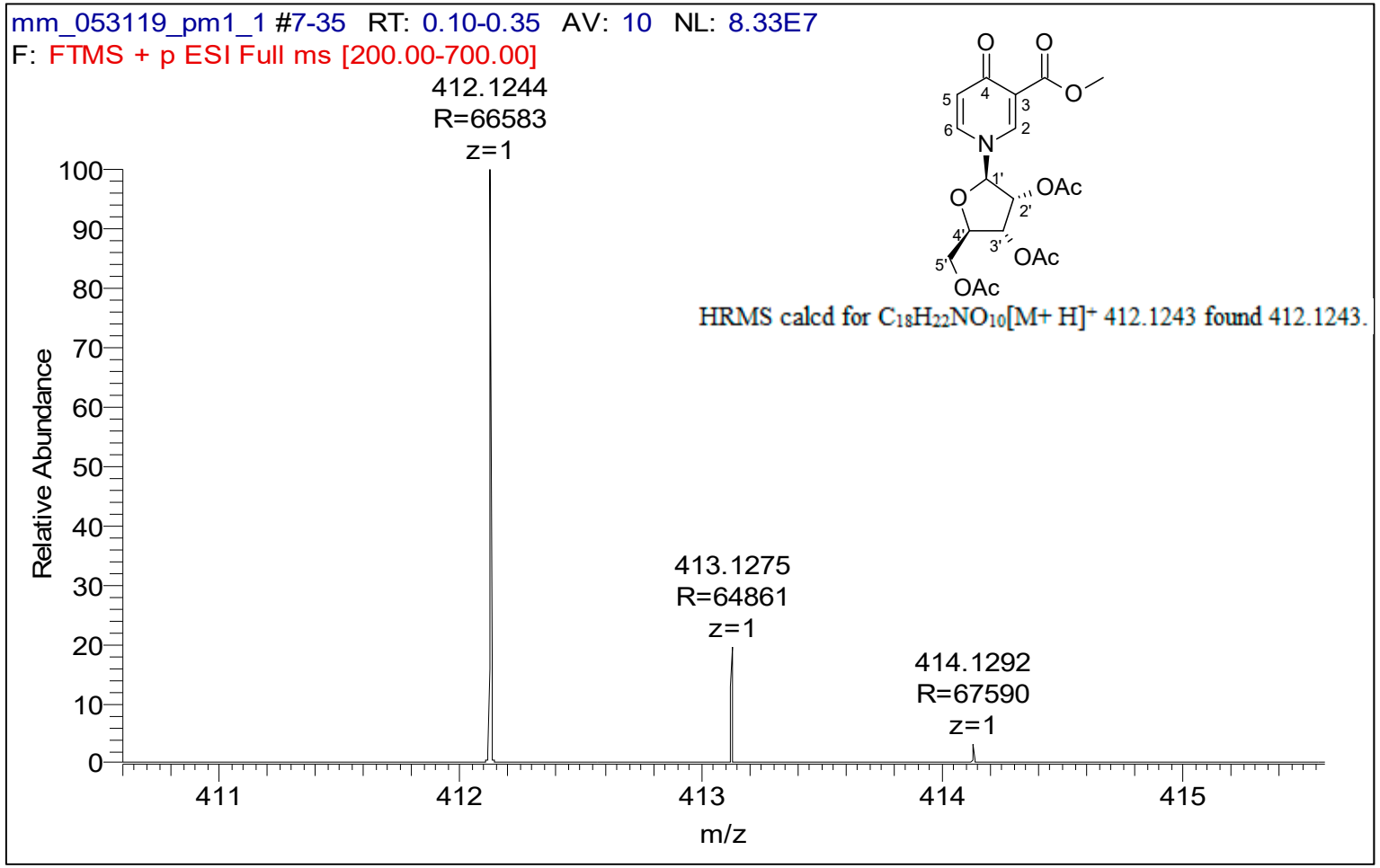
Compound 23. 400 MHz ^{1}H NMR spectrum in $CDCl_3$



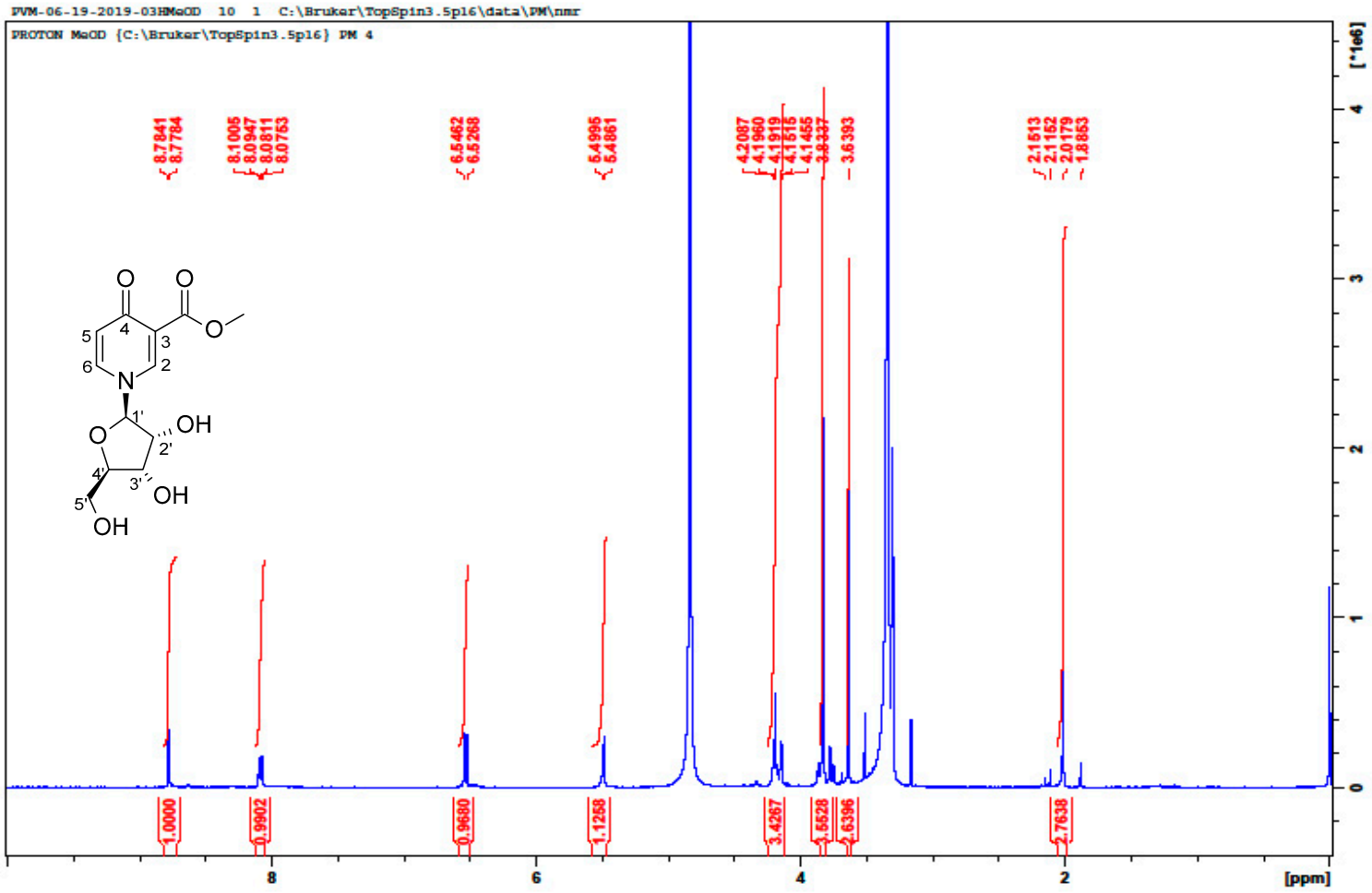
Compound 24. 400 MHz ^{1}H NMR spectrum in $CDCl_3$



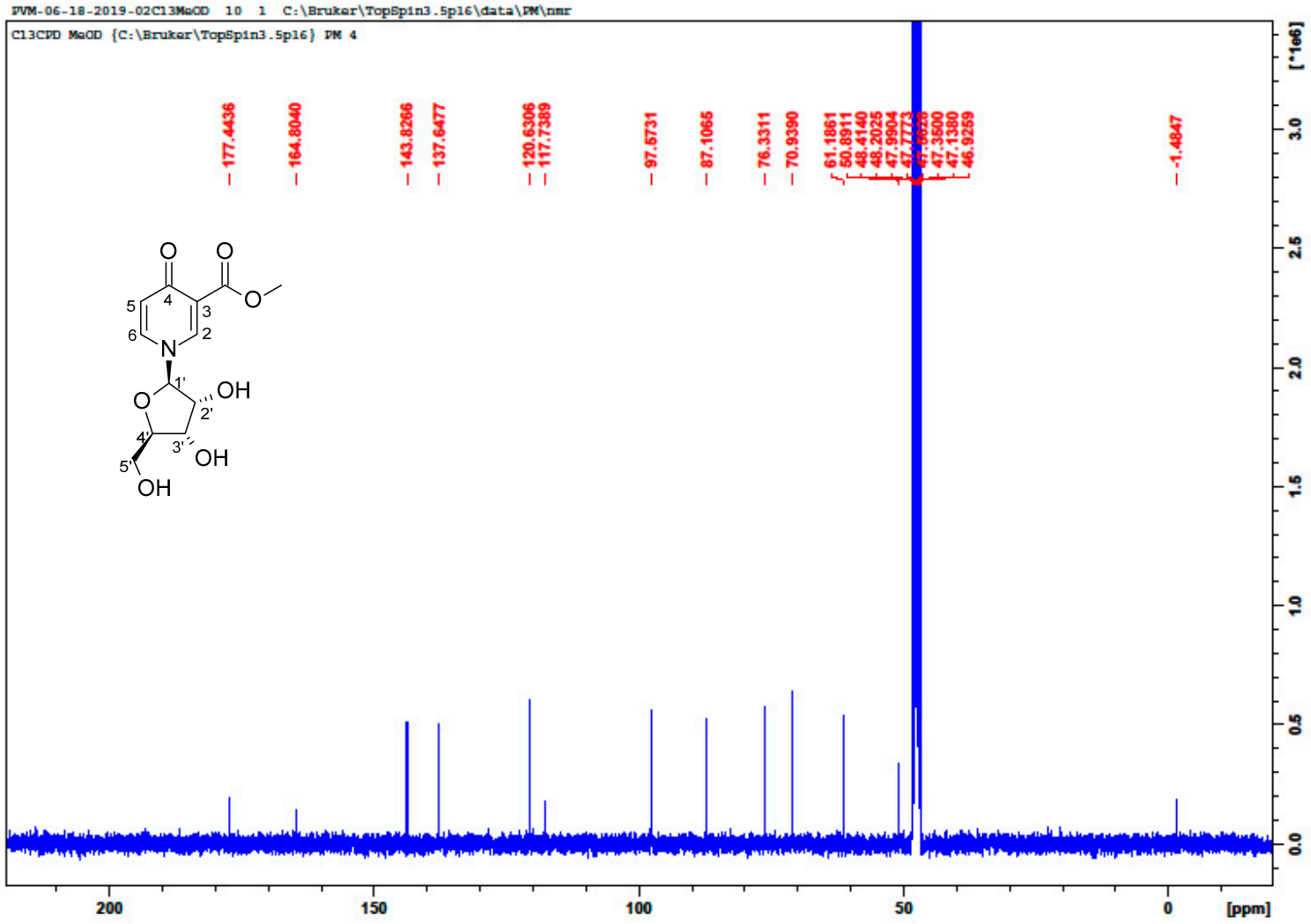
Compound 24. 400 MHz ^{13}C NMR spectrum in $CDCl_3$



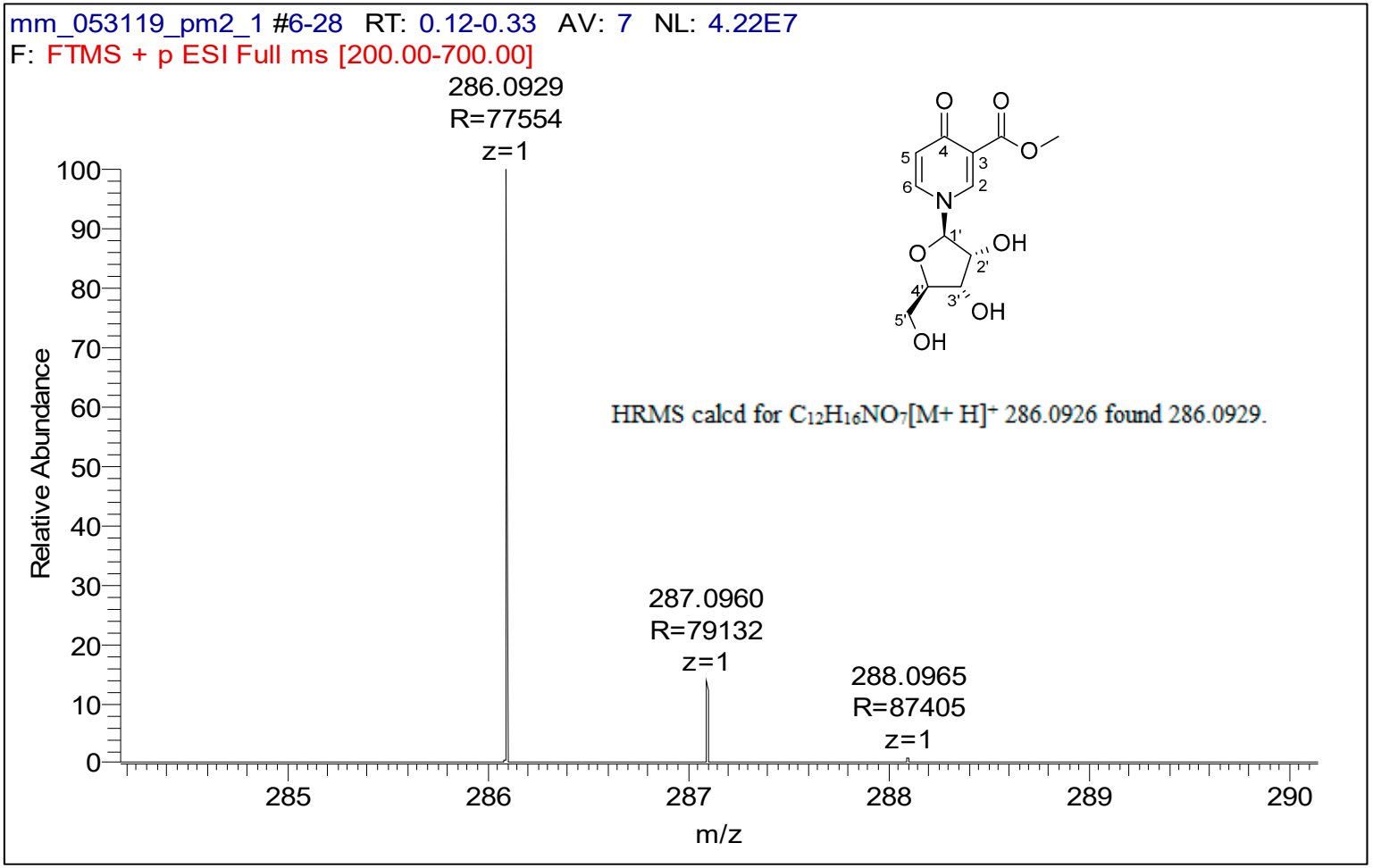
Compound 24. HRMS spectra



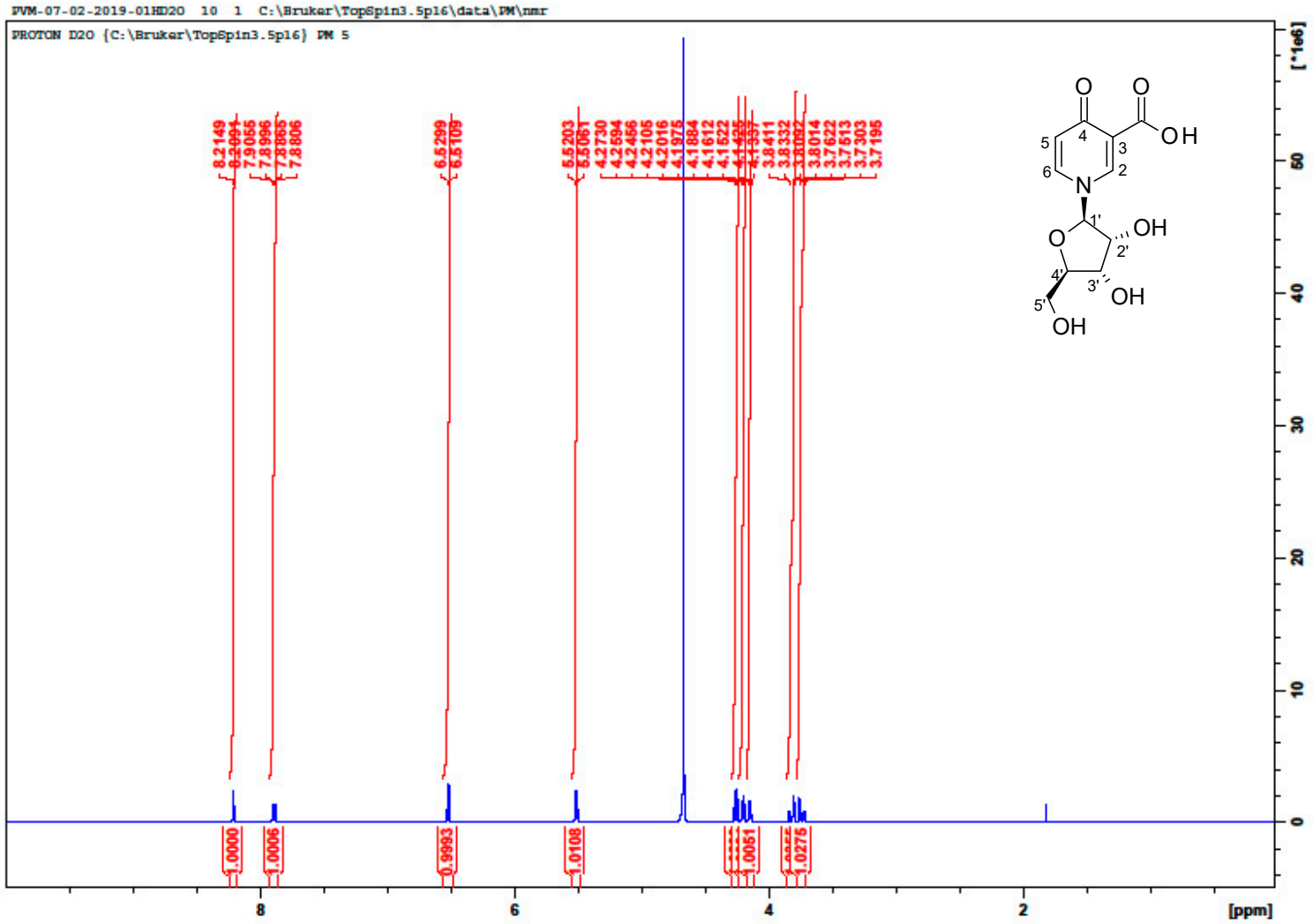
Compound 25. 400 MHz ^{1}H NMR spectrum in MeOD



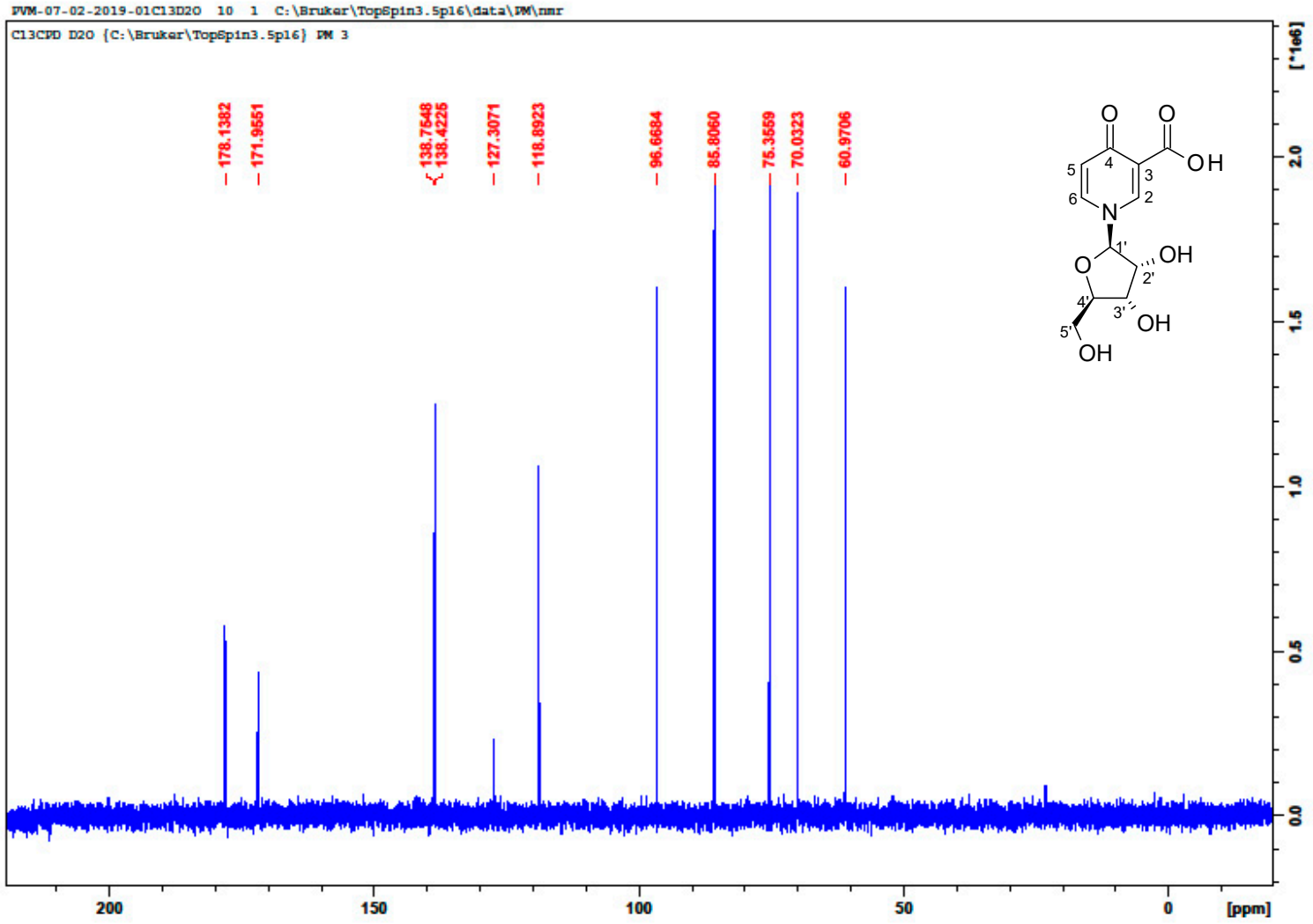
Compound 25. 400 MHz ^{13}C NMR spectrum in MeOD



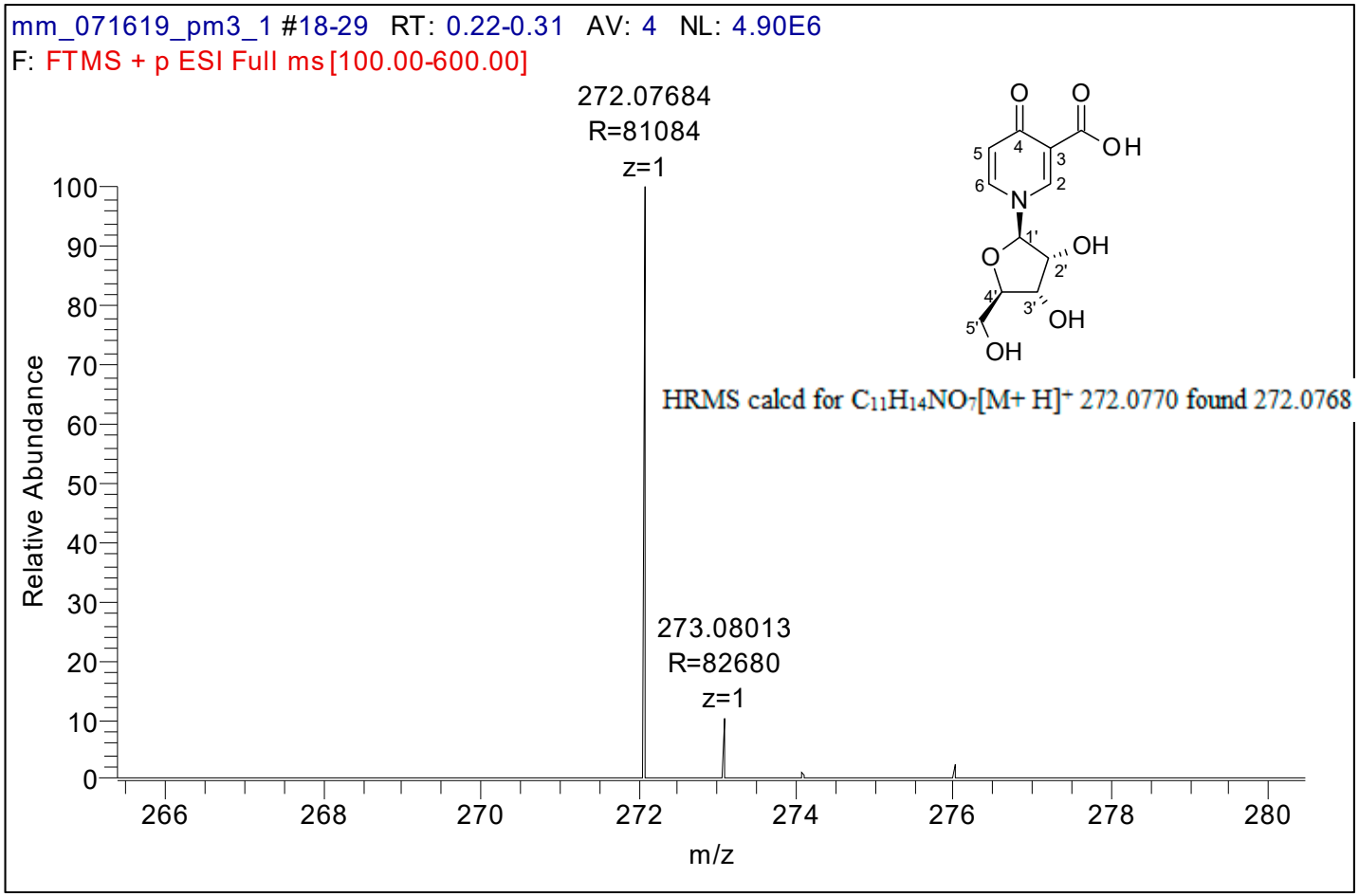
Compound 25. HRMS spectra



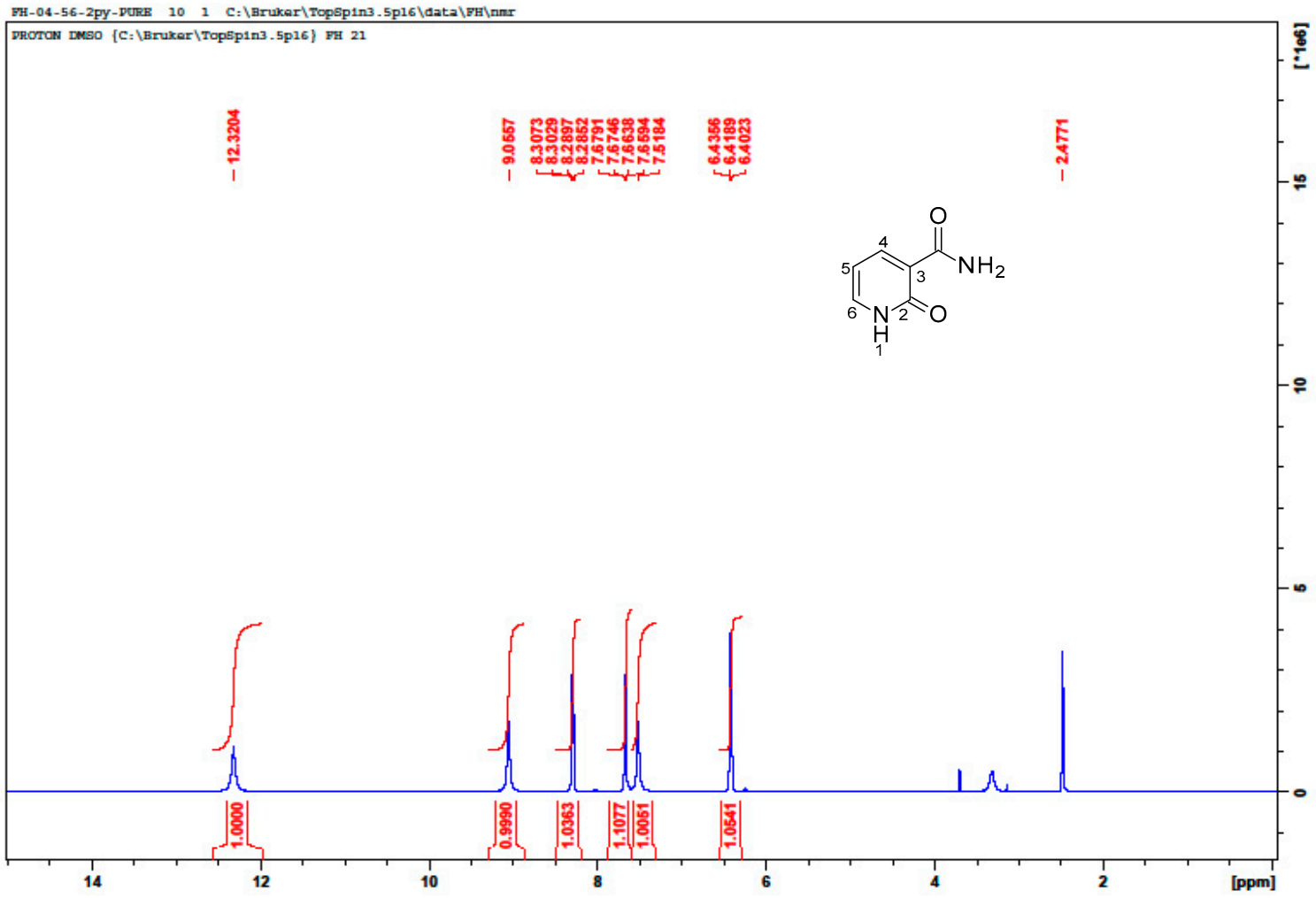
Compound 26. 400 MHz ^{1}H NMR spectrum in D_2O



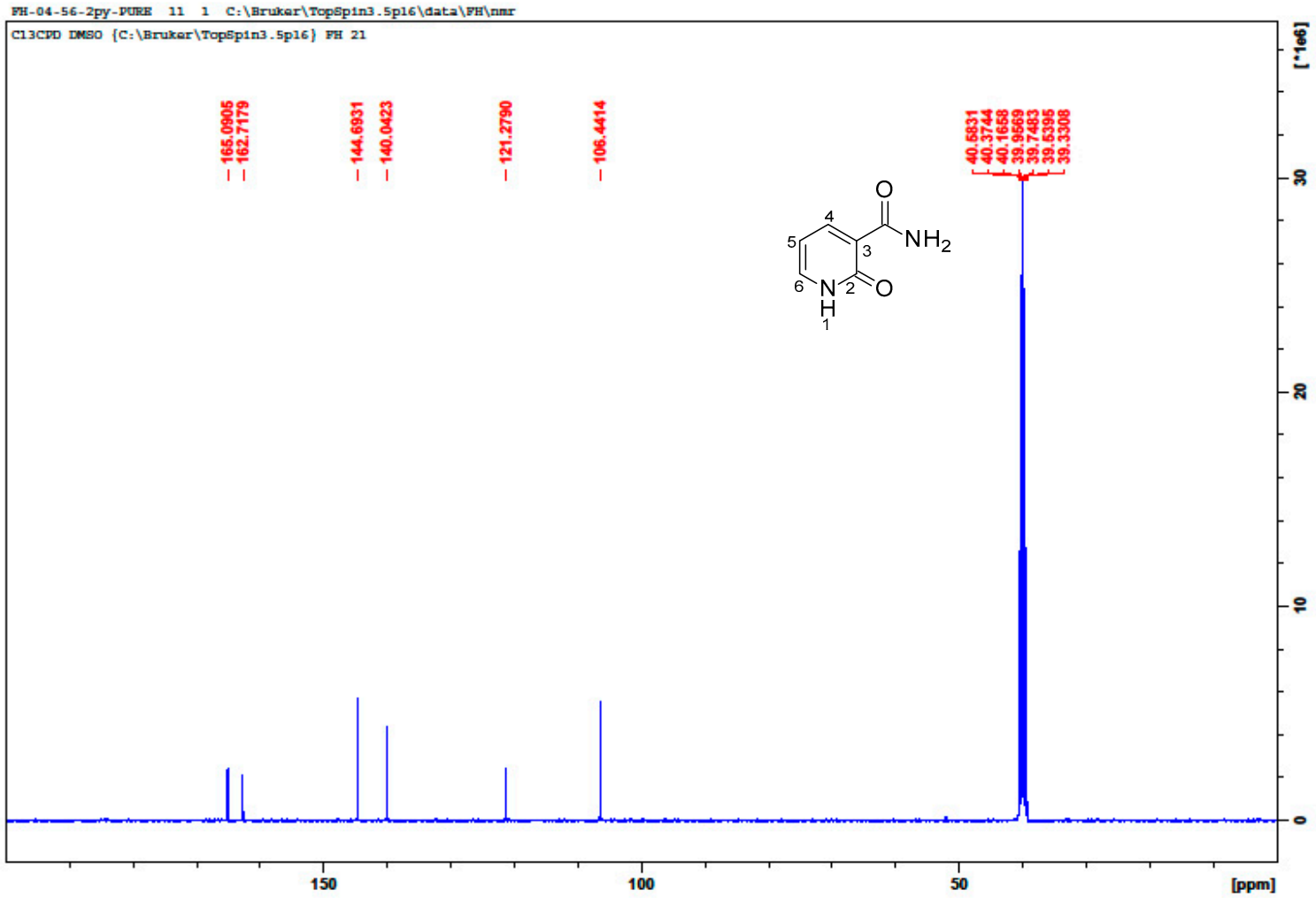
Compound 26. 400 MHz ^{13}C NMR spectrum in D_2O



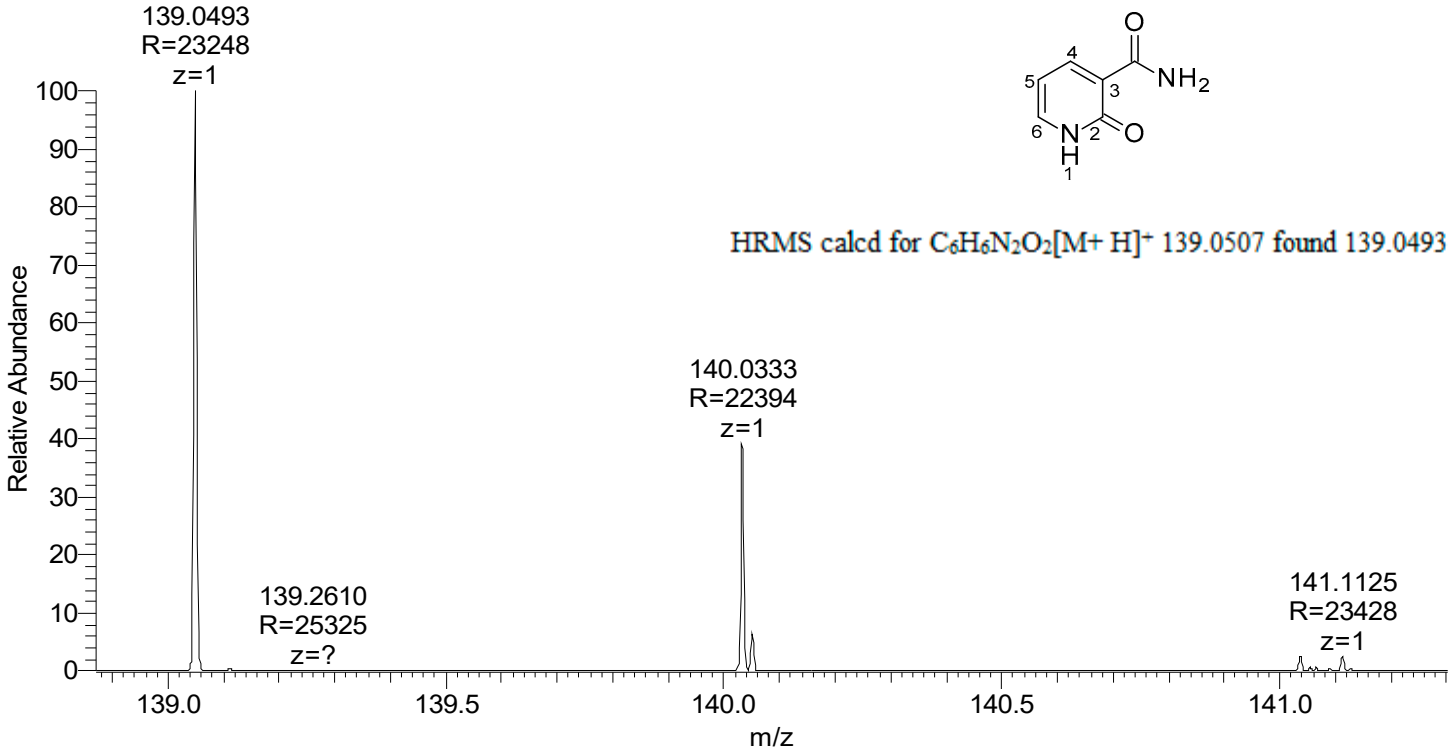
Compound 26. HRMS spectra



Compound 29. 400 MHz ^{1}H NMR spectrum in D_2O



Compound 29. 400 MHz ^{13}C NMR spectrum in D_2O



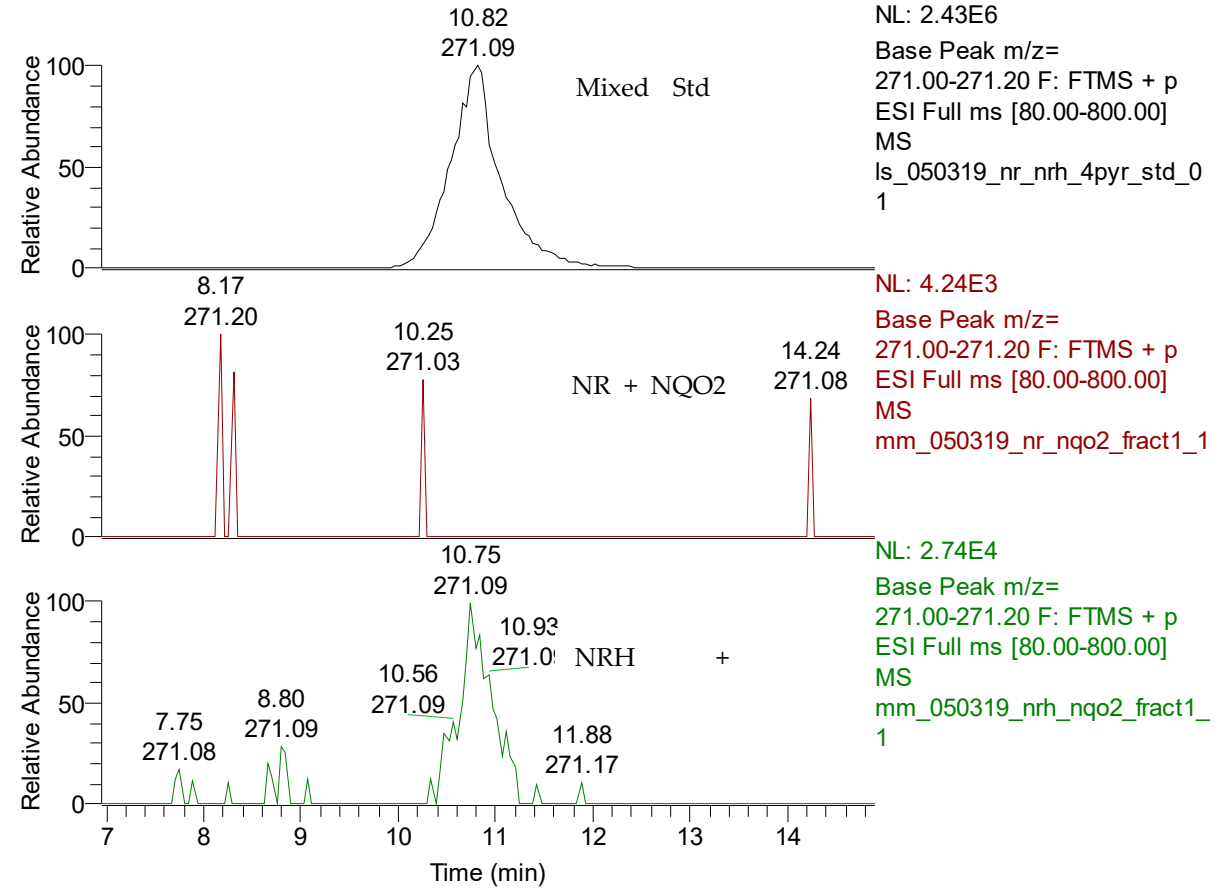
Compound 29. HRMS spectra

Figure 2: NQO2 catalyzed 4PYR formation

4PYR was observed from the reaction of NRH + NQO2, but not NR + NQO2.

Analyte	Parent Ion m/z	Major Fragment Ion	RT
NR	255.10	123.06	22.7
NRH	257.11	167.08	10.5
4PYR	271.09	139.05	10.8

XIC: 4PYR Observed in NRH + NQO2 Fraction 1



Targeted MS2 Spectrum of 4PYR

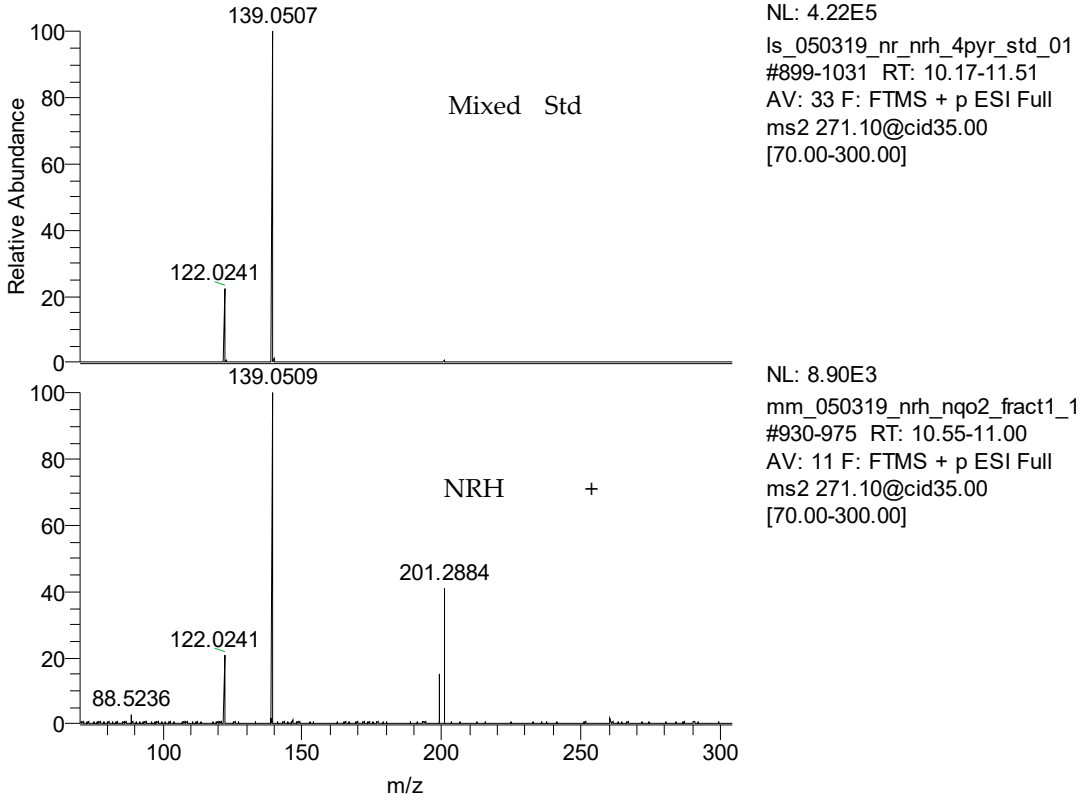


Figure S3: Fold change in human urinary NAD catabolites following 1gm administration of acetaminophen/APAP (average n=2, and technical replicates).