

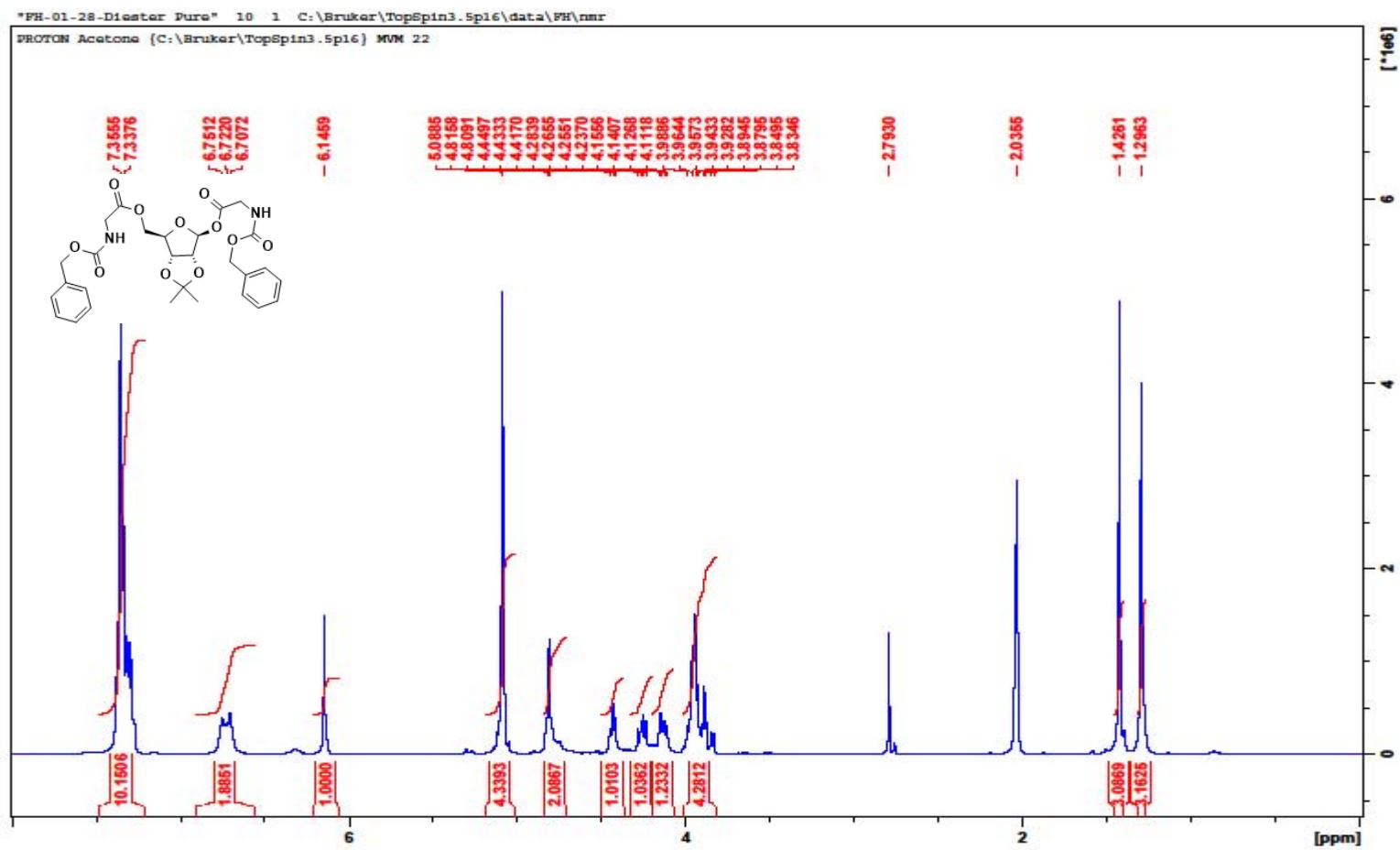
Nicotinamide riboside-amino acid conjugates that are stable to purine nucleoside phosphorylase

Faisal Hayat and Marie E. Migaud*

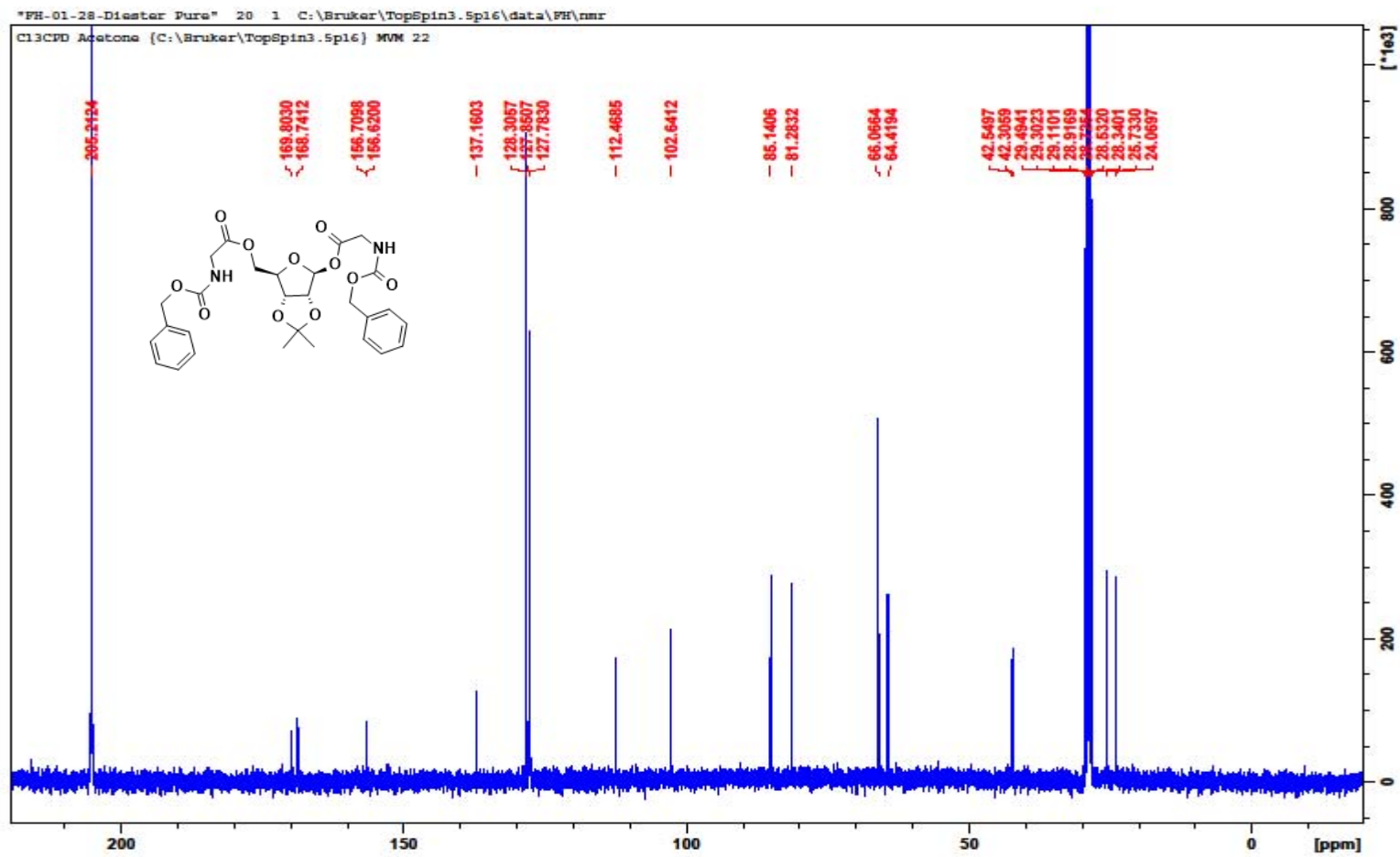
Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Ave., Mobile, AL 36604, USA

Supplementary

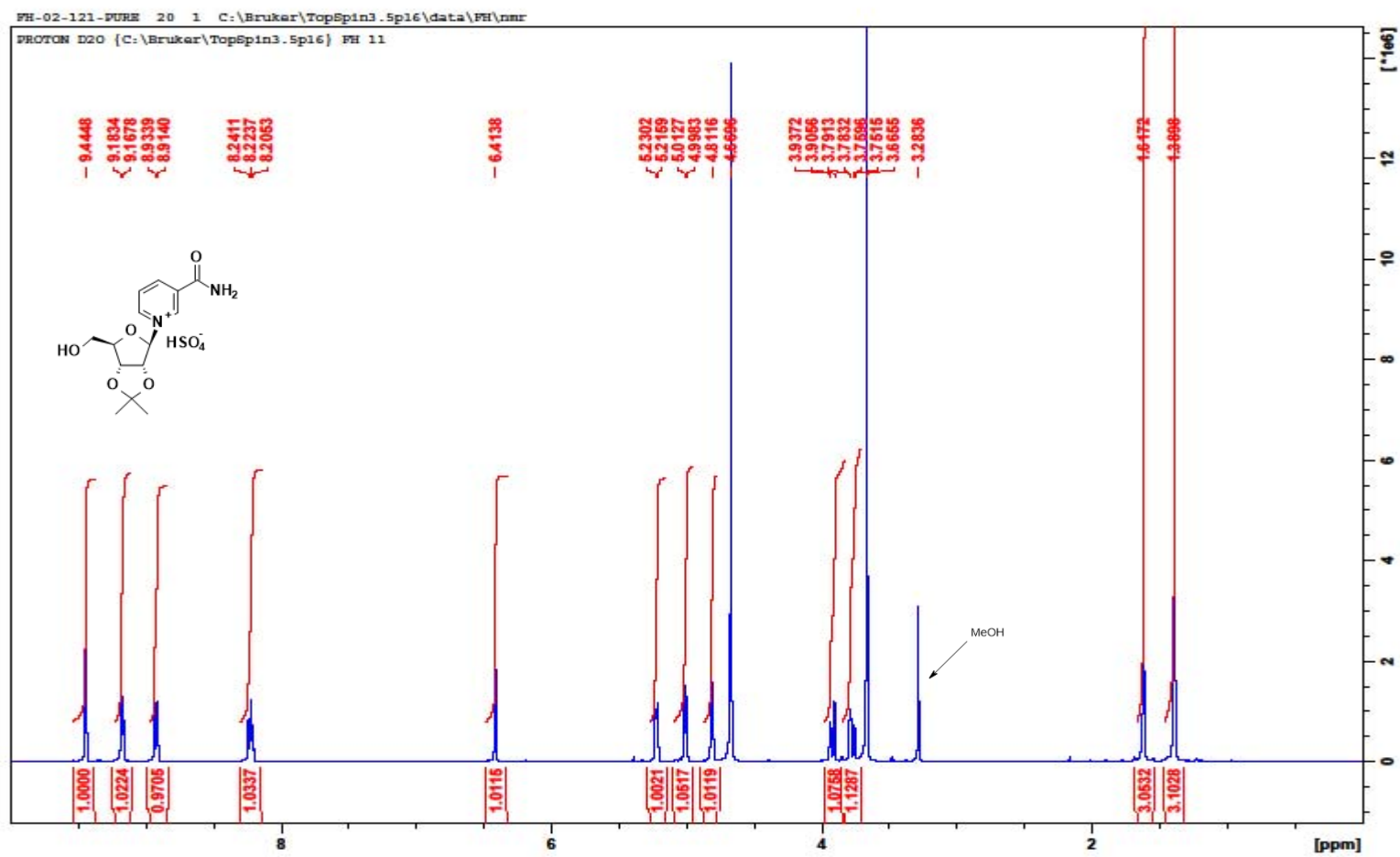
1. Copies of ^1H NMR, ^{13}C NMR, ^{19}F -NMR and HRMS Spectra.....2-58
2. Enzymatic assays by ^1H NMR.....59-61
3. Table of Vorbrüggen reaction outcomes.....62



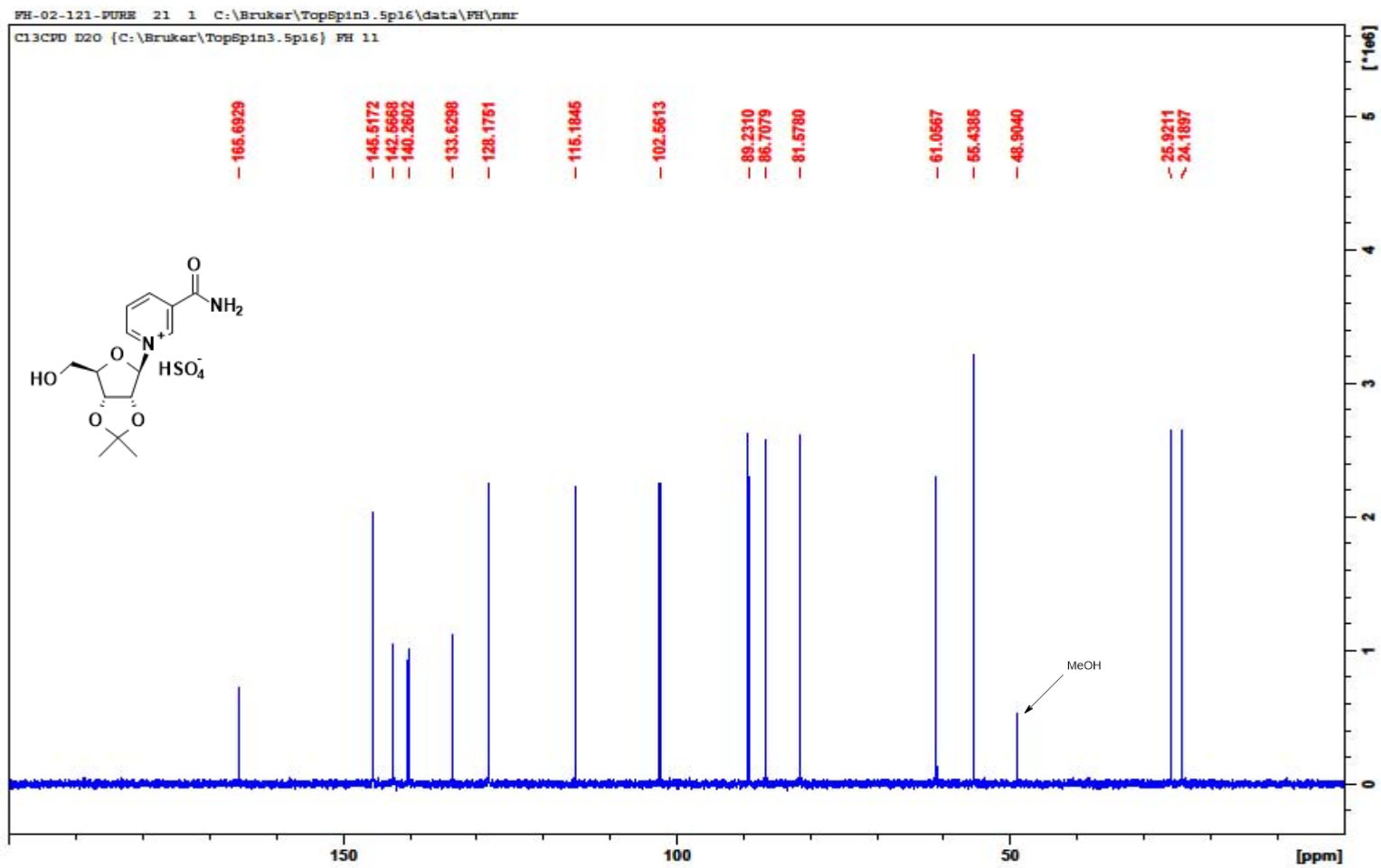
Compound 4. 400 MHz ¹H NMR spectrum in Acetone-*d*₆



Compound 4. 100 MHz ^{13}C NMR spectrum in Acetone- d_6



Compound 7. 400 MHz ¹H NMR spectrum in D₂O

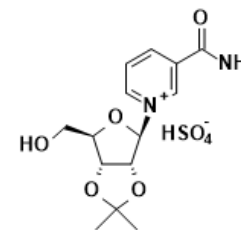
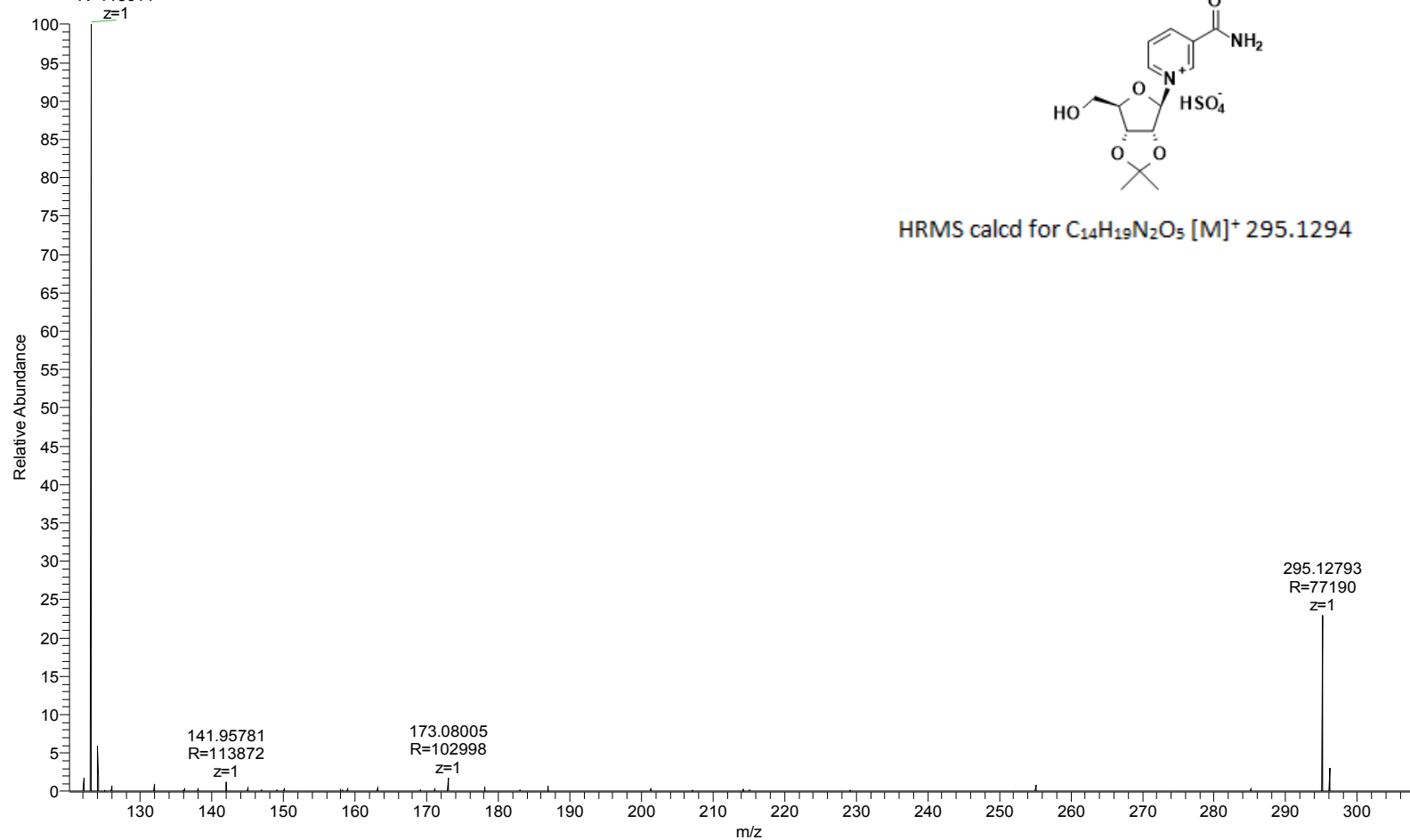


Compound 7. 100 MHz ^{13}C NMR spectrum in D_2O

mm_072018_fh_01_20_1 #242-260 RT: 3.83-4.11 AV: 19 NL: 5.55E6

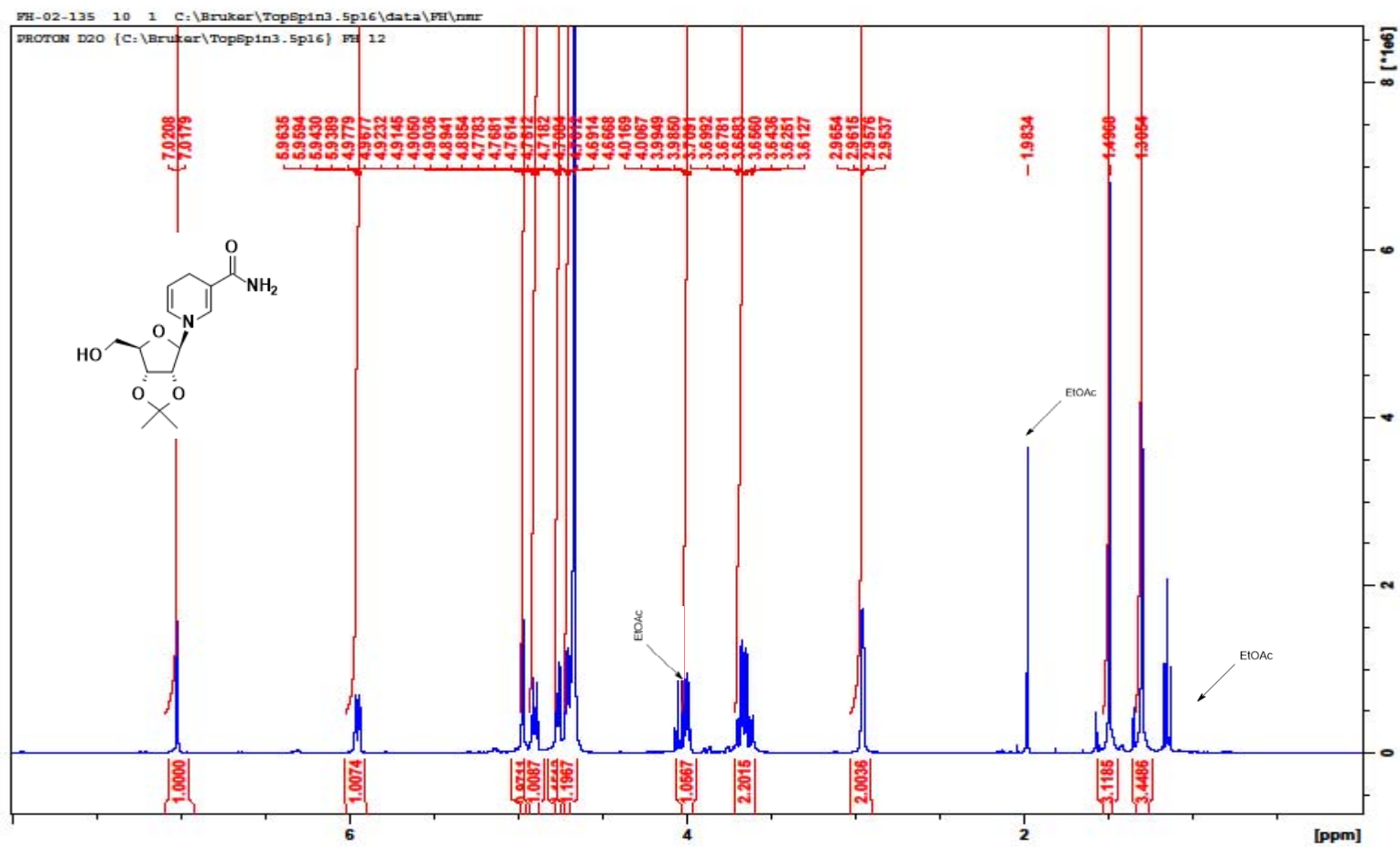
F: FTMS + p ESI Full ms [120.00-800.00]

123.05448
R=118611

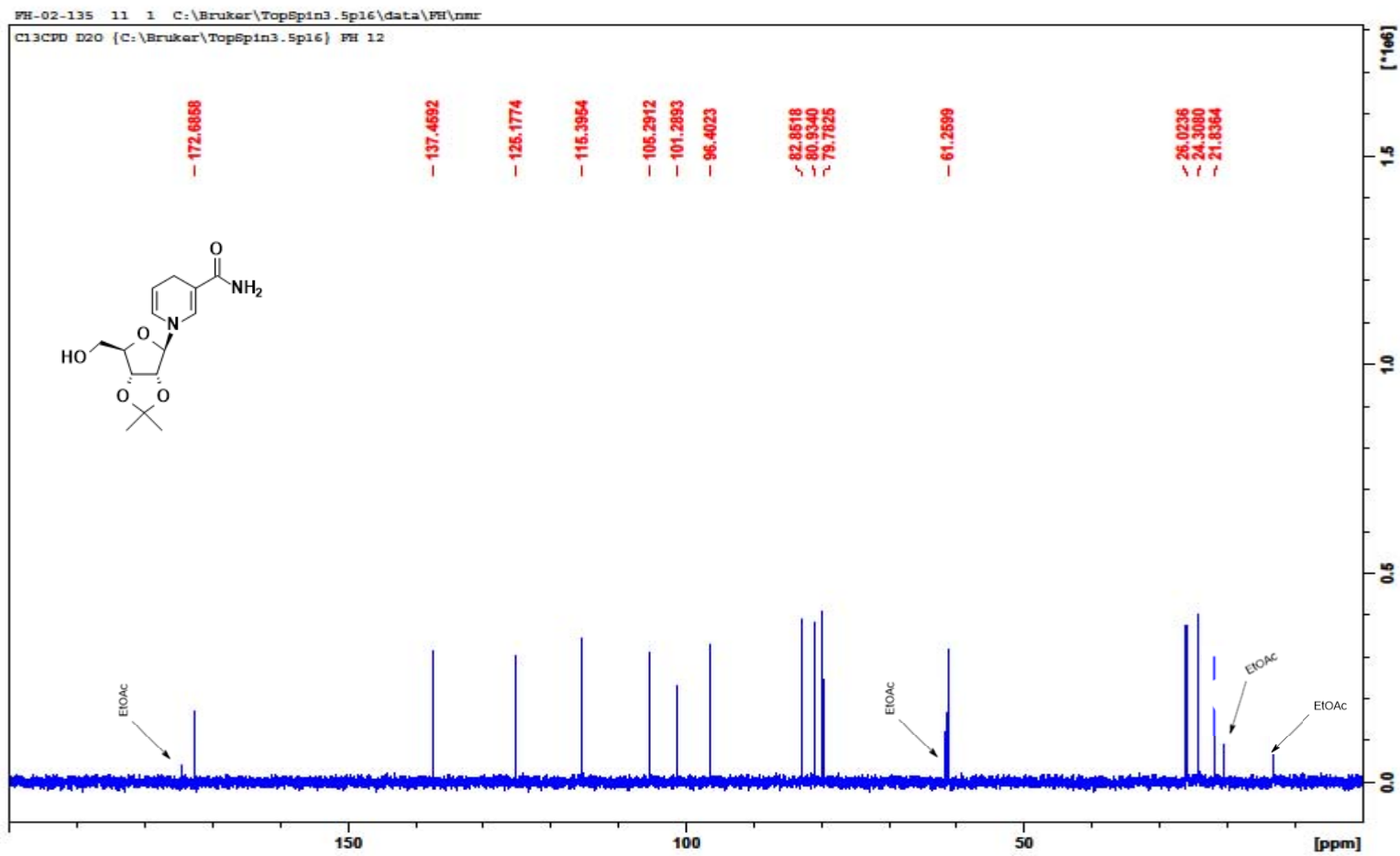


HRMS calcd for C₁₄H₁₉N₂O₅ [M]⁺ 295.1294

Compound 7. HRMS spectra

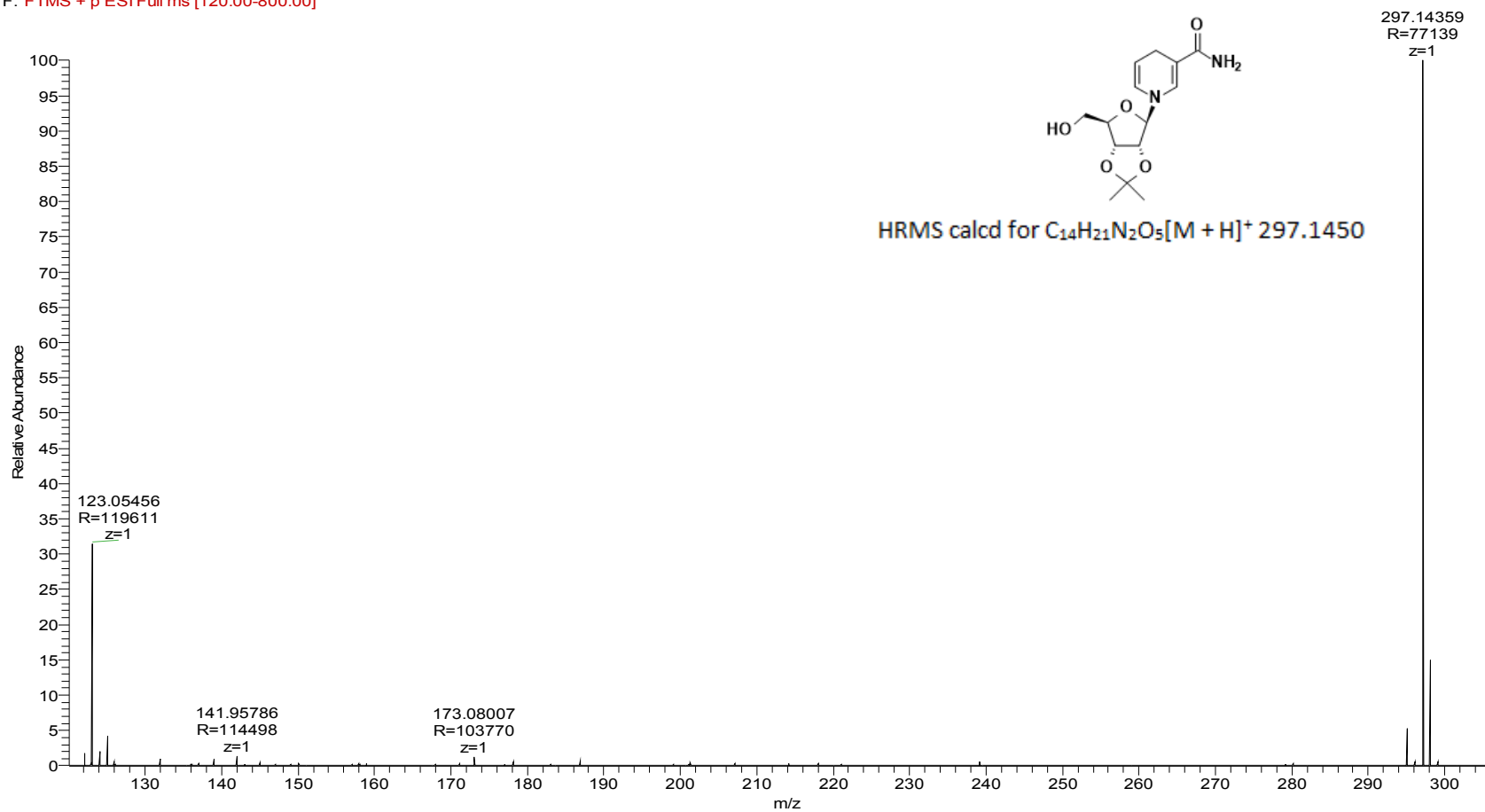


Compound 8. 400 MHz ¹H NMR spectrum in D₂O

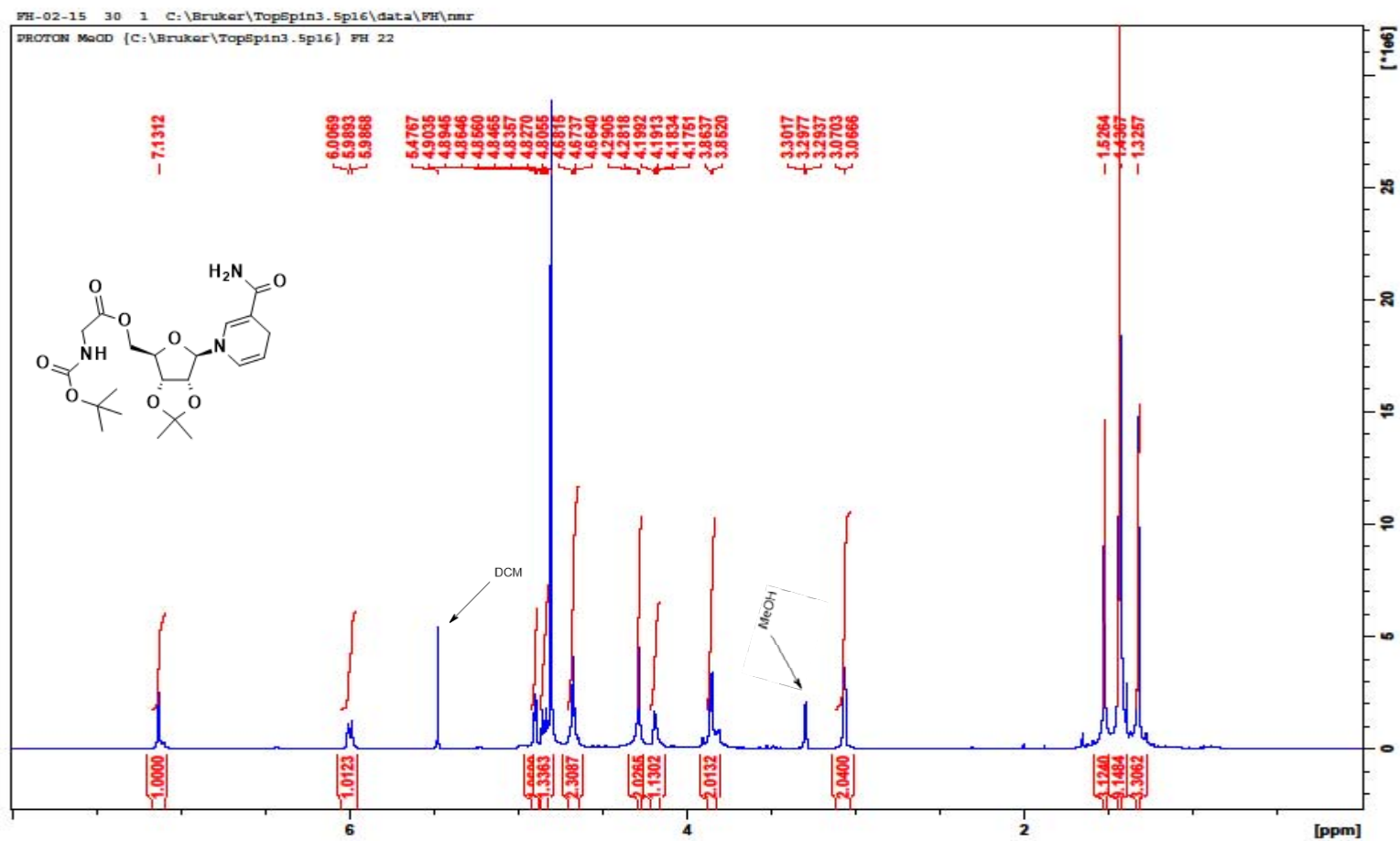


Compound 8. 100 MHz ¹³C NMR spectrum in D₂O

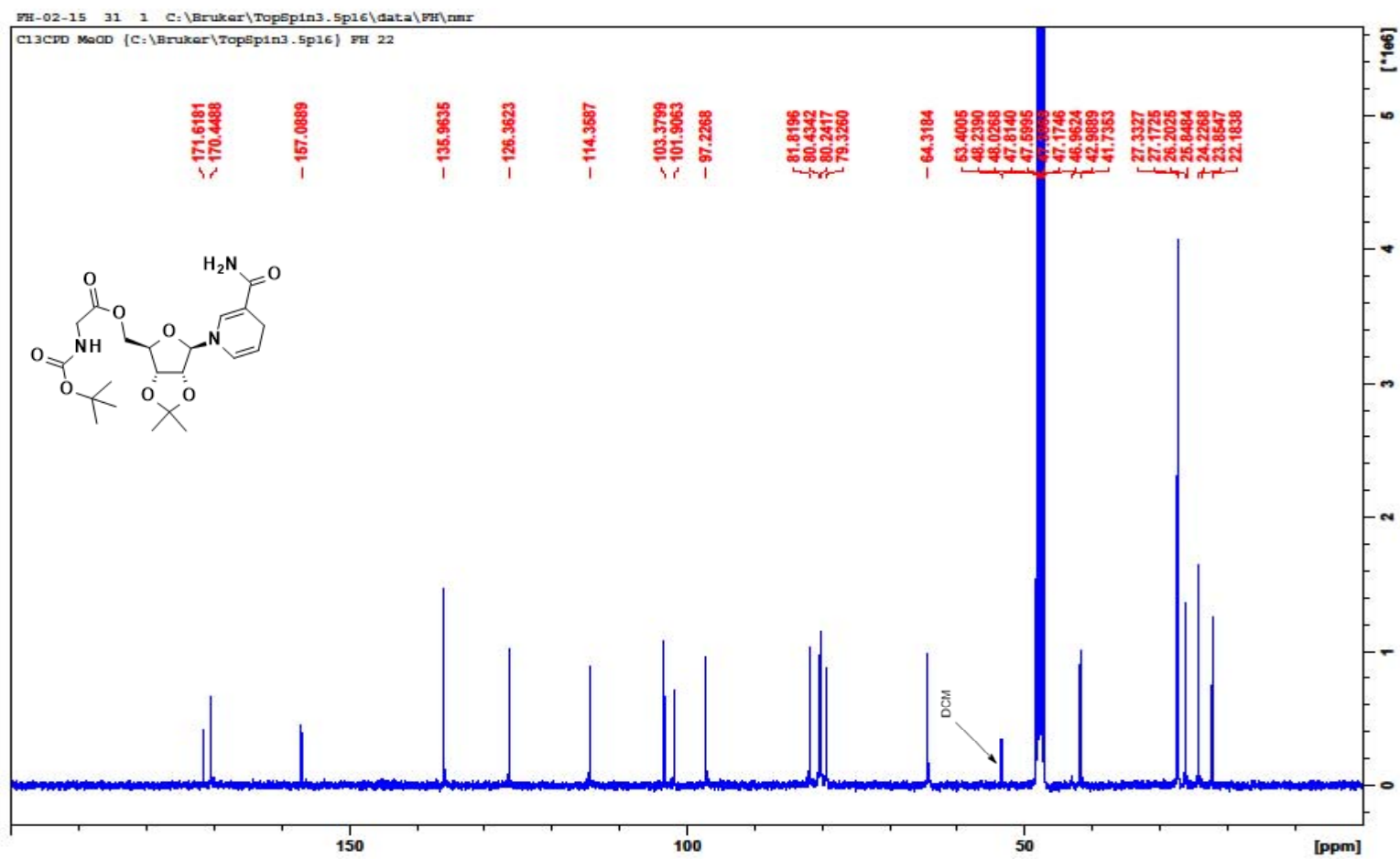
mm_072018_fh_01_22_1#244-267 RT: 3.86-4.21 AV: 24 NL: 5.74E6
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Compound 8. HRMS spectra



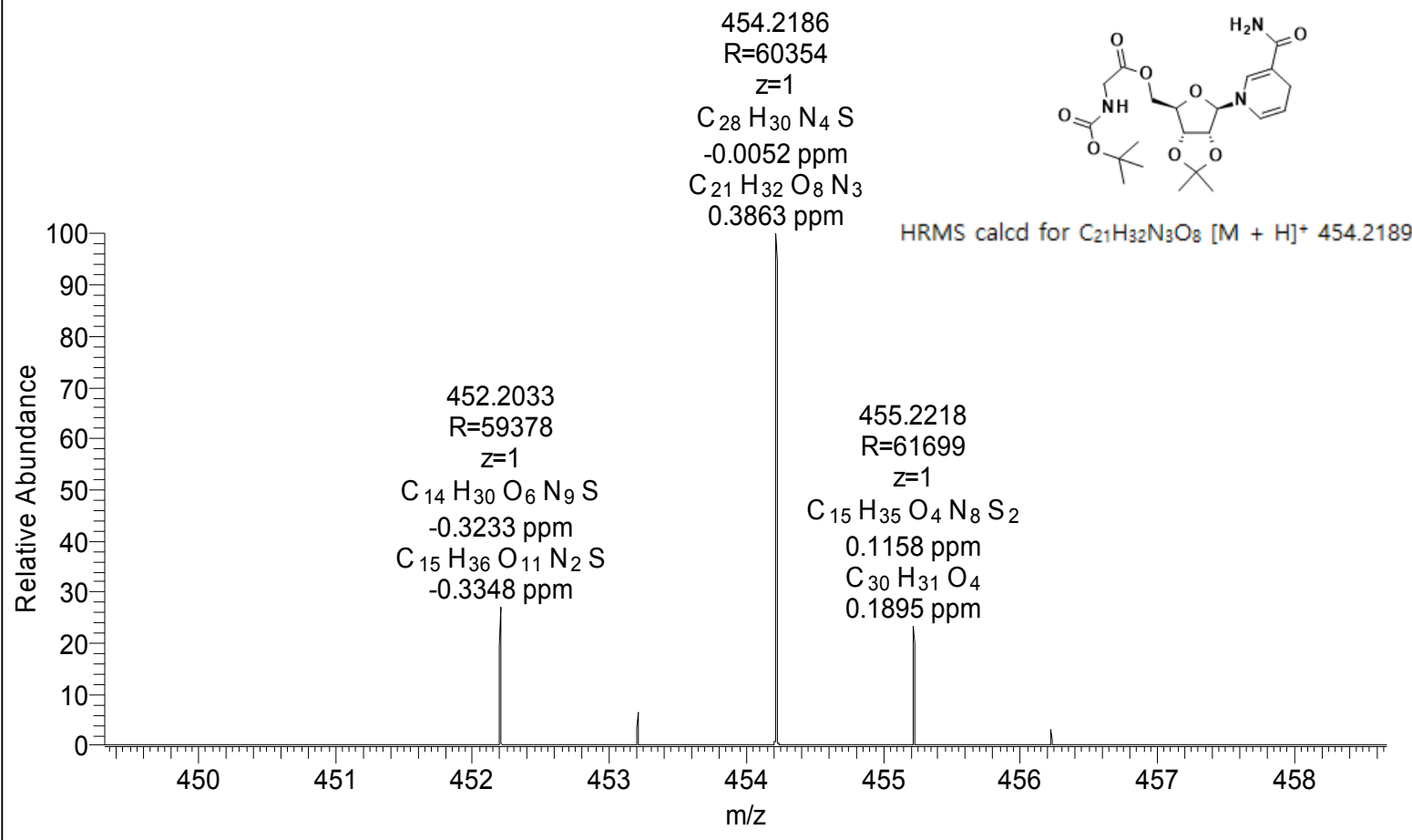
Compound 10a. 400 MHz ^1H NMR spectrum in MeOD



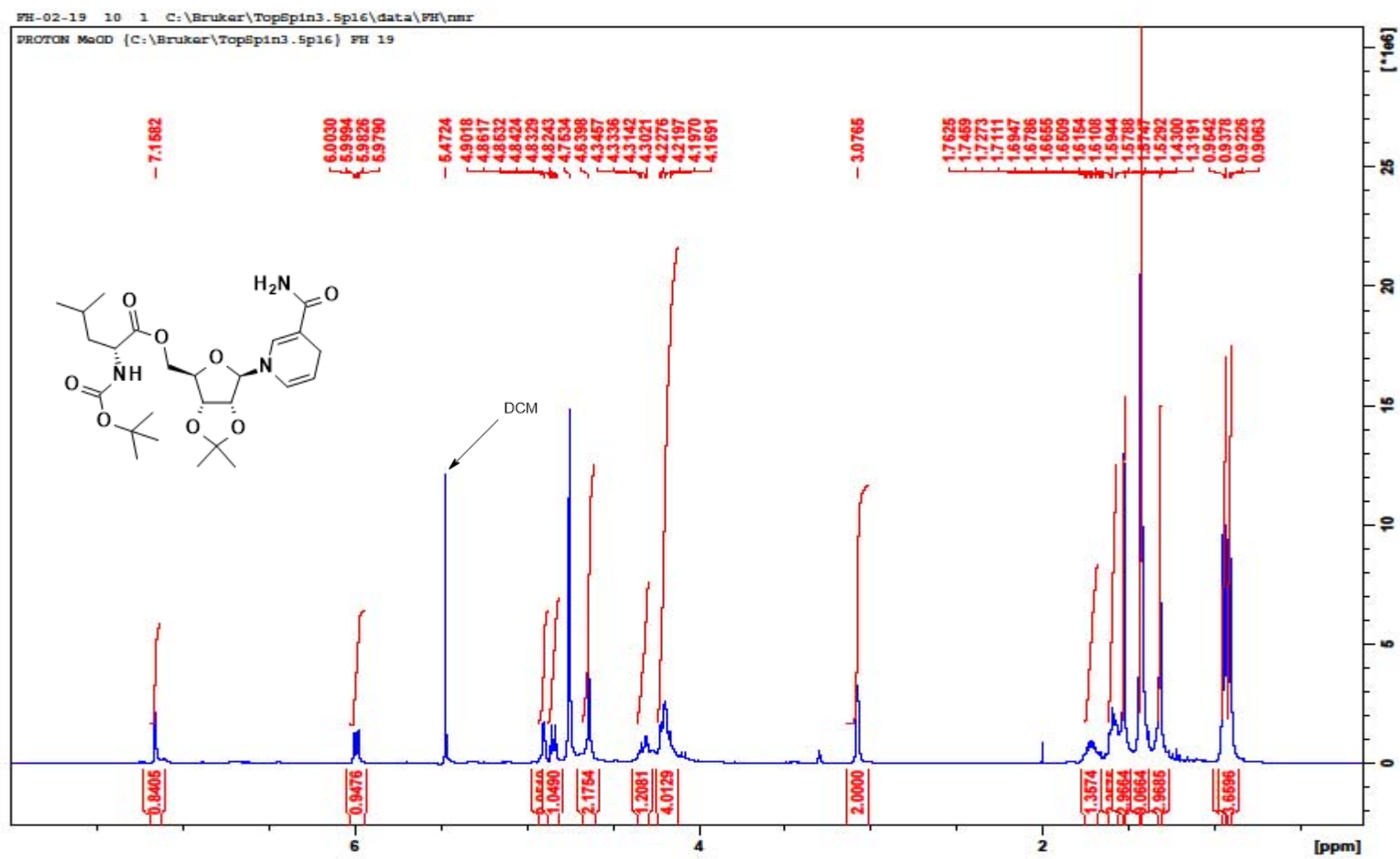
Compound 10a. 100 MHz ^{13}C NMR spectrum in MeOD

mm_101618_nrh_glycine_01 #17-31 RT: 0.25-0.46 AV: 15 SB: 81 5.79-7.06 NL: 1.49E7

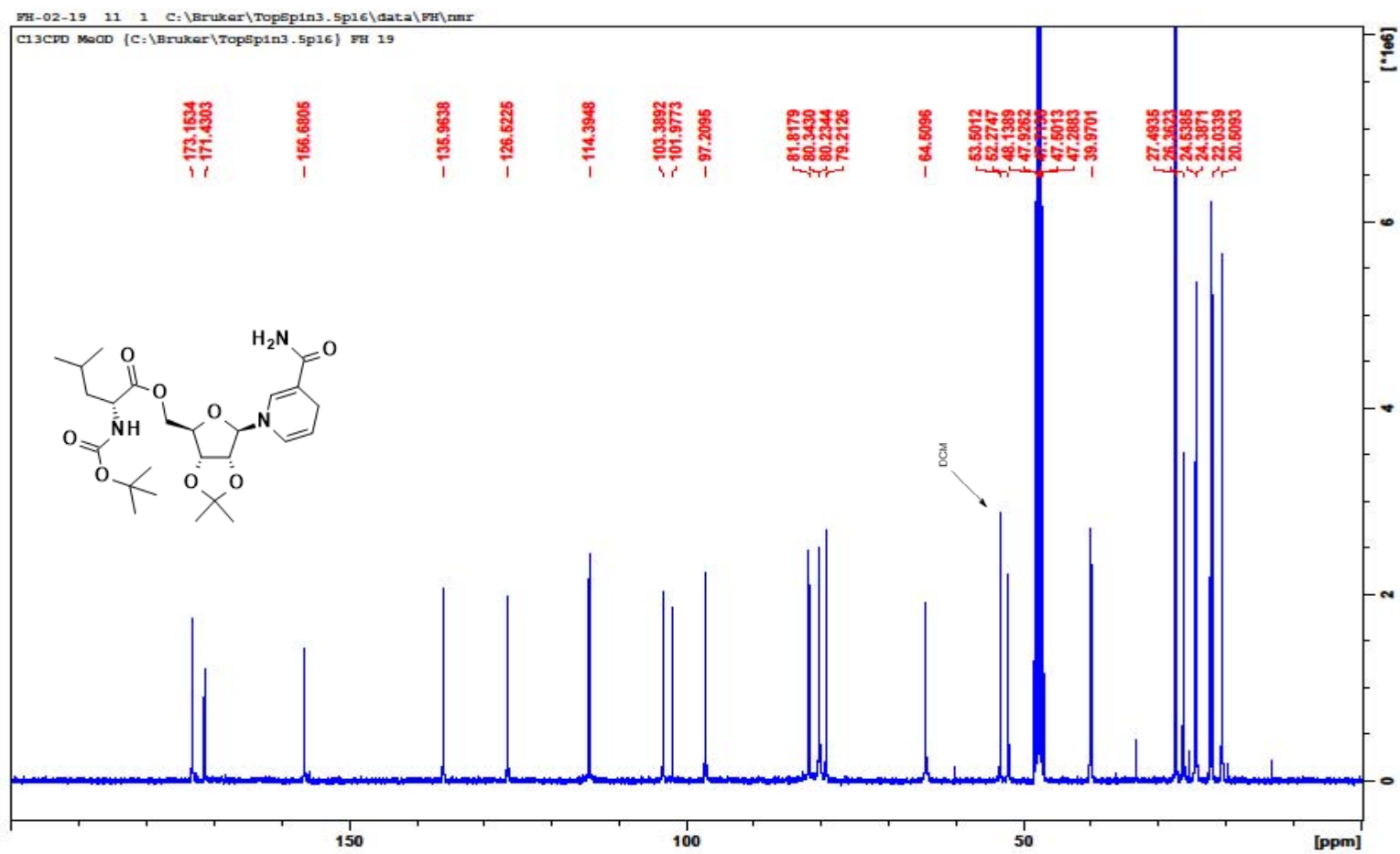
F: FTMS + p ESI Full ms [200.00-1050.00]



Compound 10a. HRMS spectra



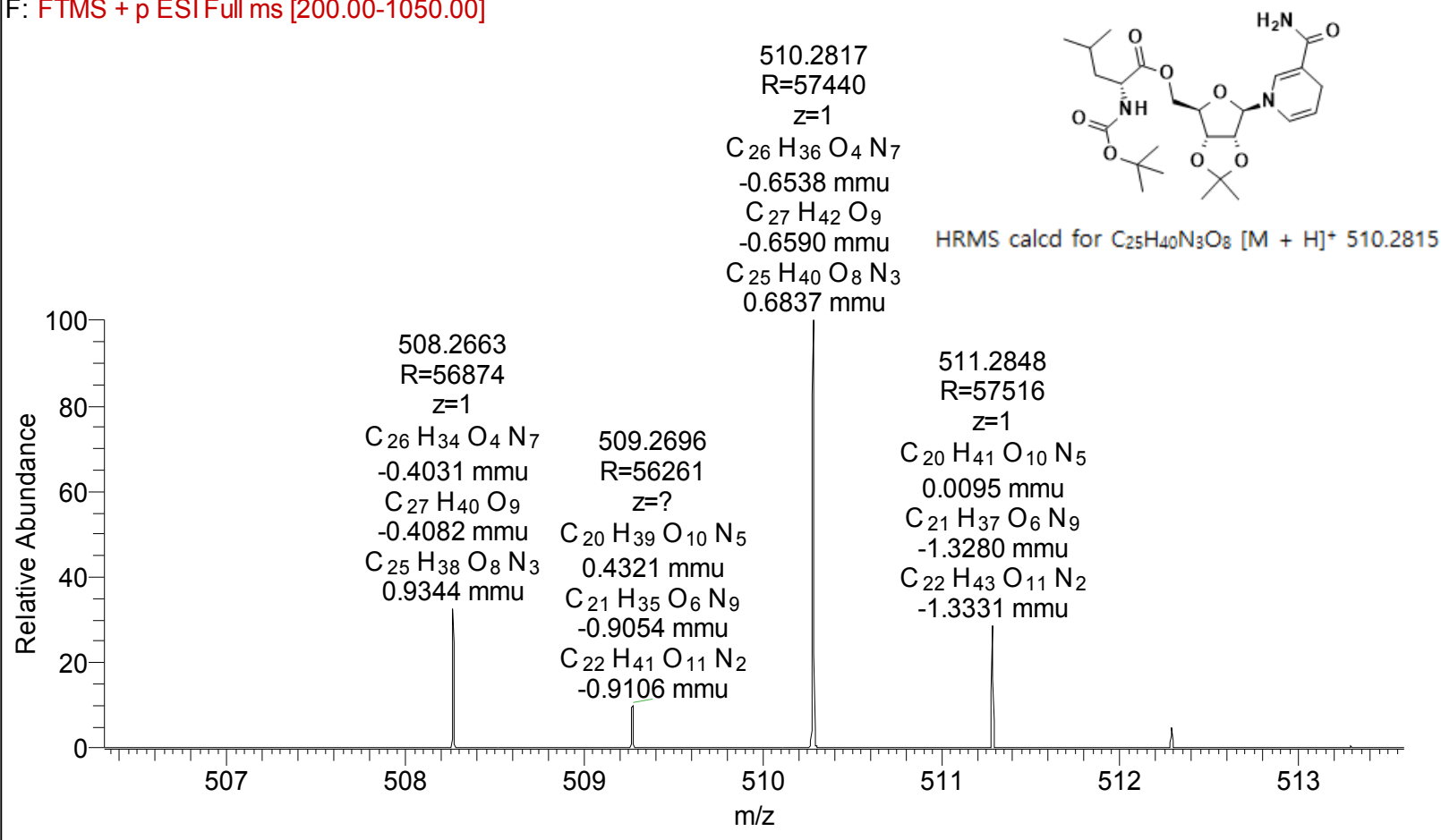
Compound 10b. 400 MHz ^1H NMR spectrum in MeOD



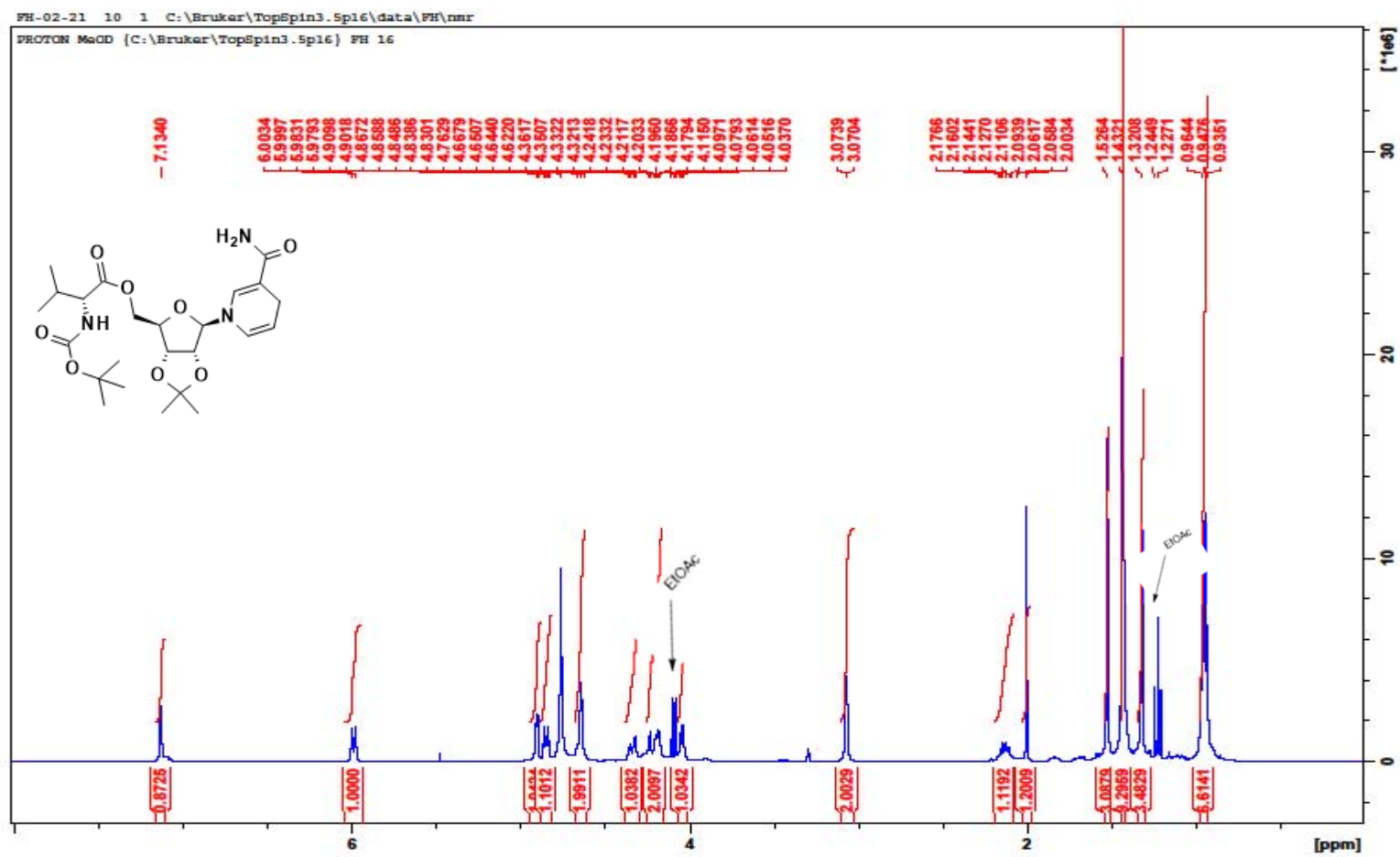
Compound 10b. 100 MHz ^{13}C NMR spectrum in MeOD

mm_101618_nrh_leucine_01 #17-32 RT: 0.25-0.47 AV: 16 SB: 81 5.78-7.05 NL: 2.10E⁷

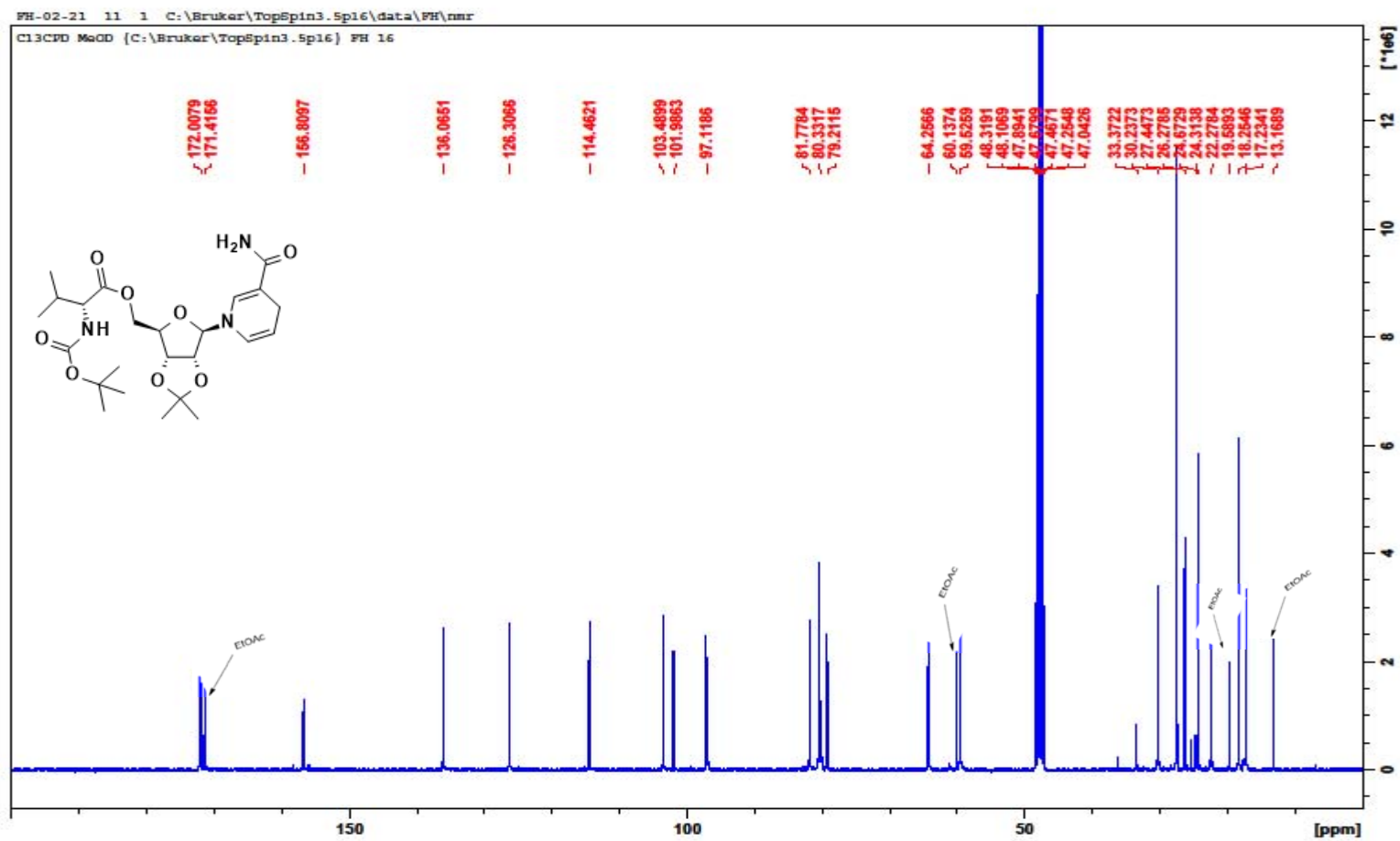
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Compound 10b. HRMS spectra



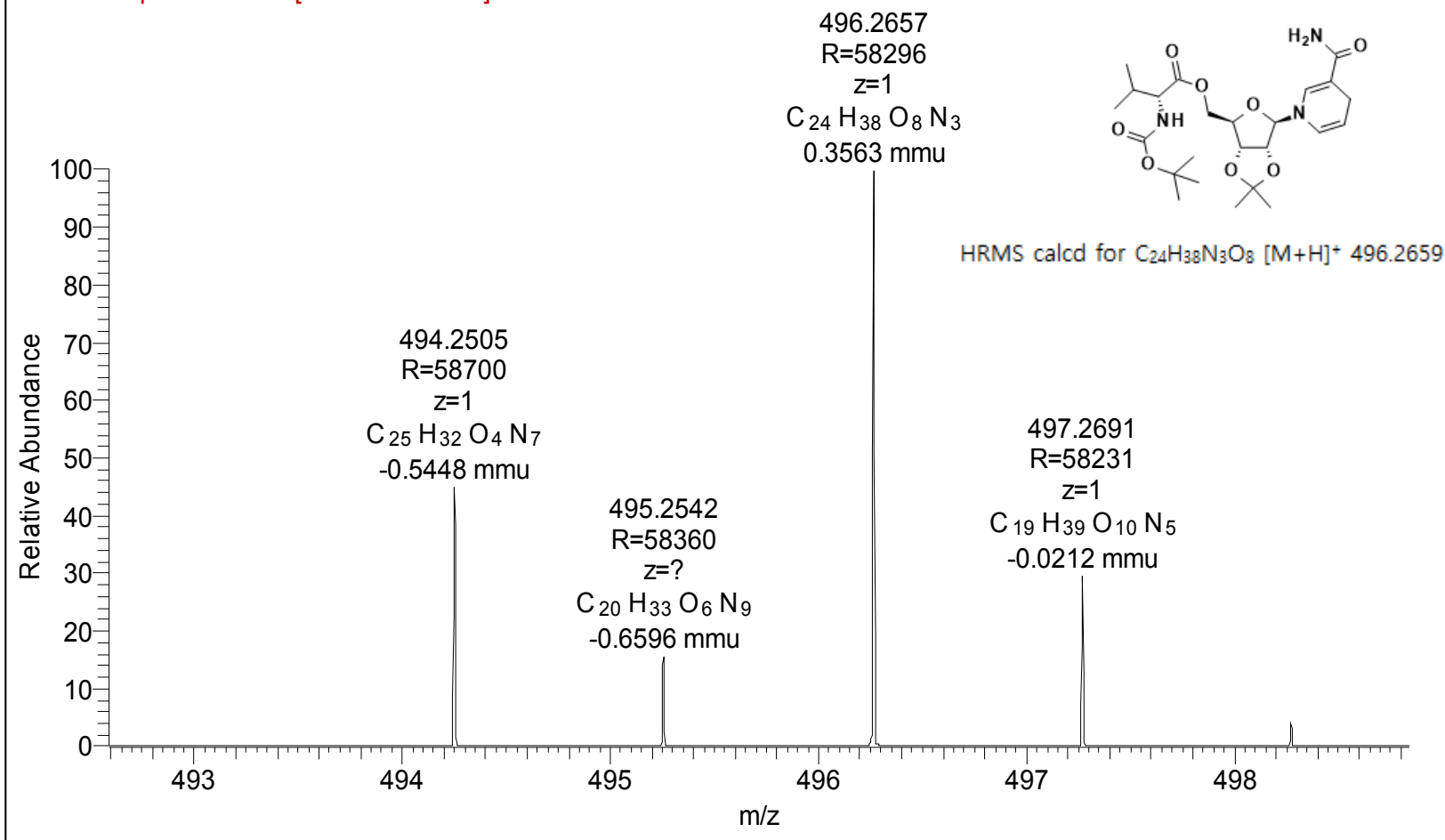
Compound 10c. 400 MHz ¹H NMR spectrum in MeOD



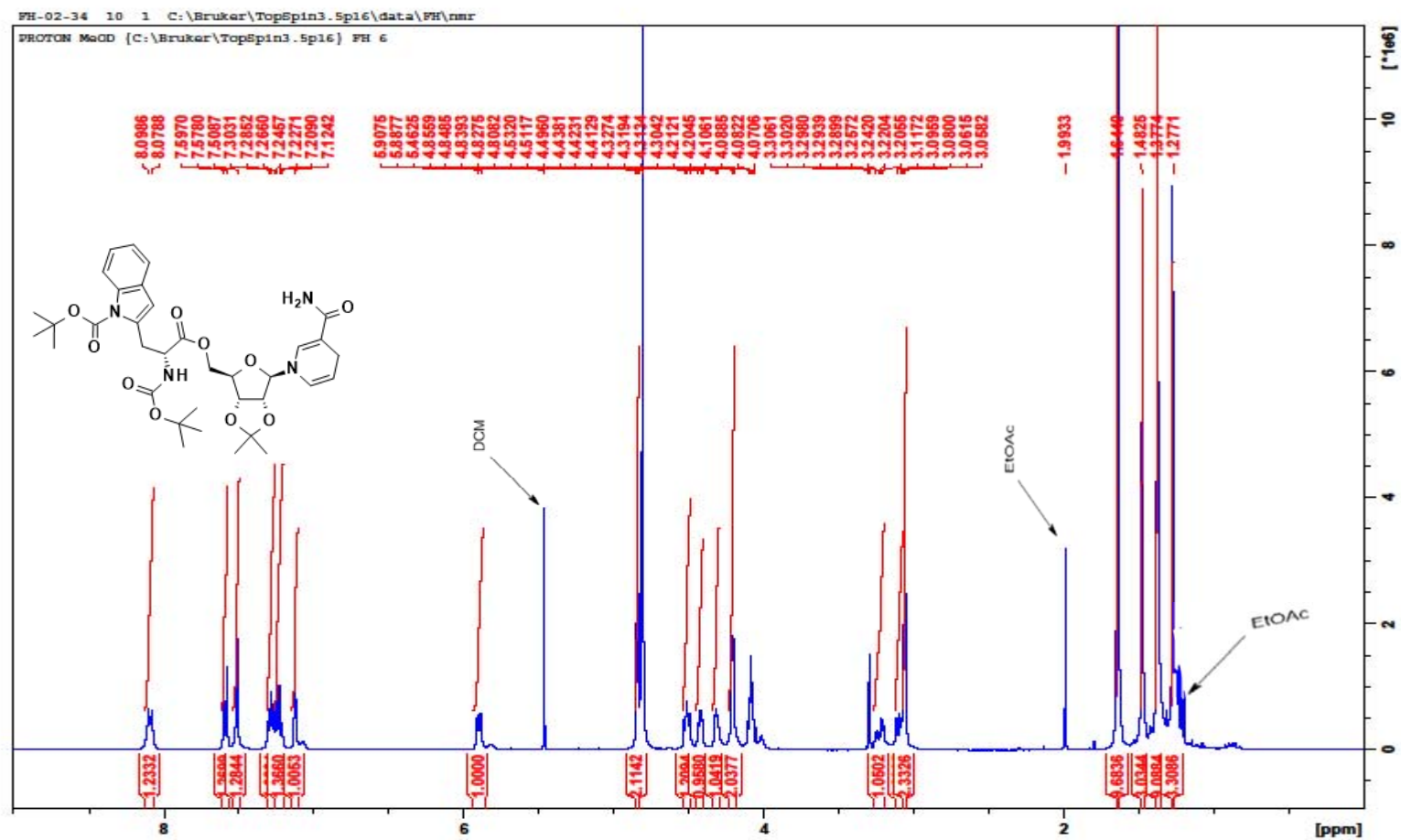
Compound 10c. 100 MHz ^{13}C NMR spectrum in MeOD

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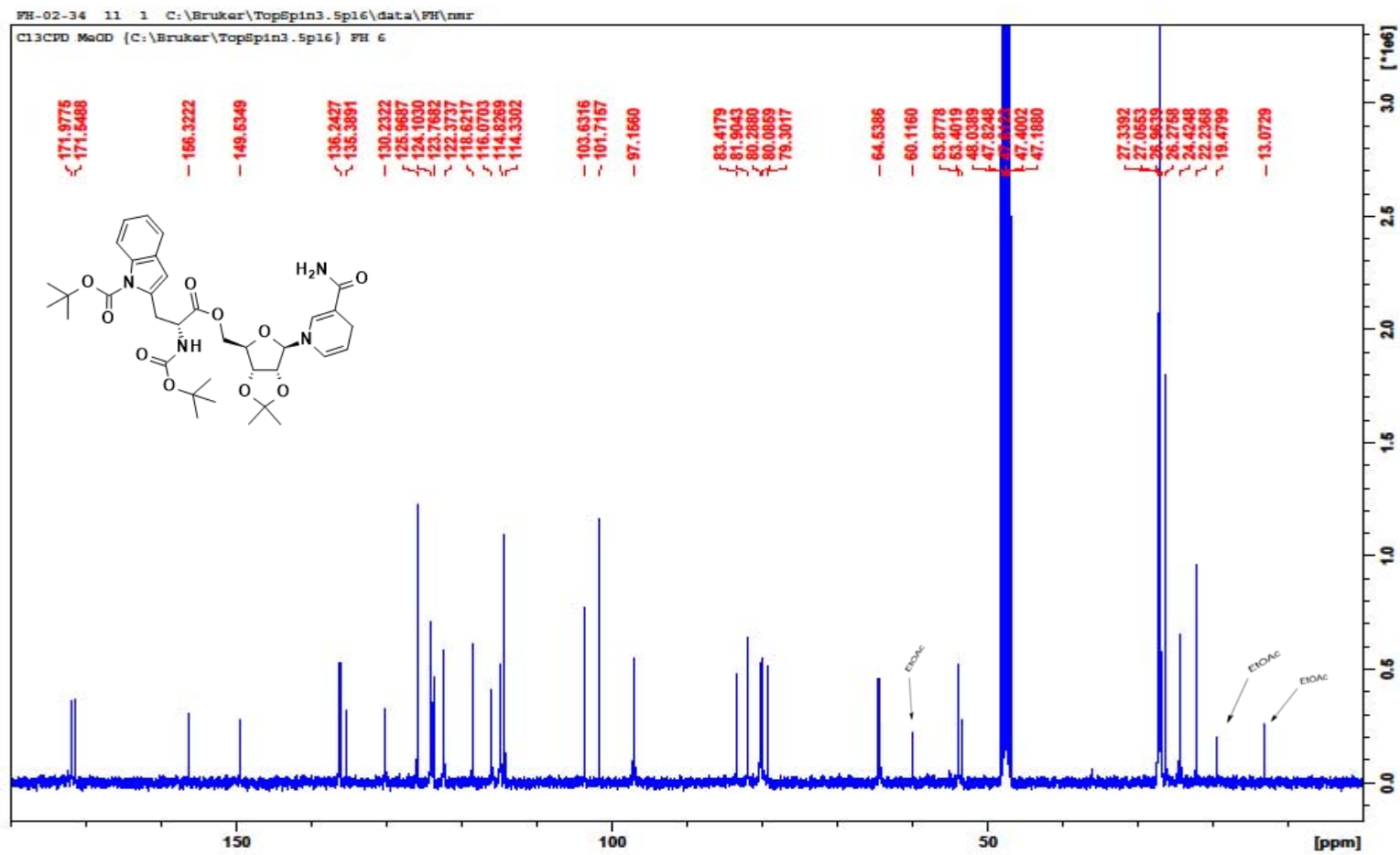
F: FTMS + p ESI Full ms [200.00-1050.00]



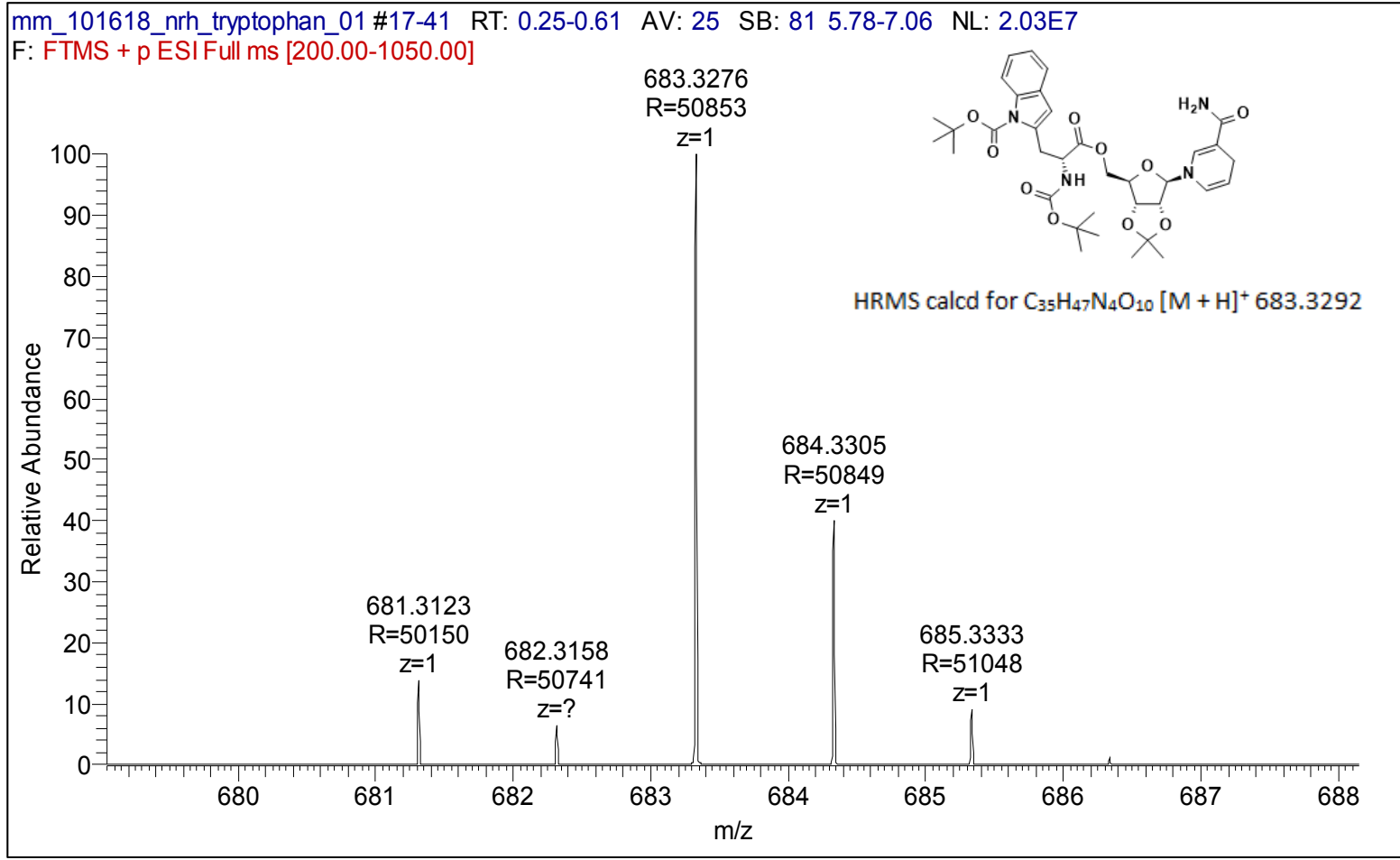
Compound 10c. HRMS spectra



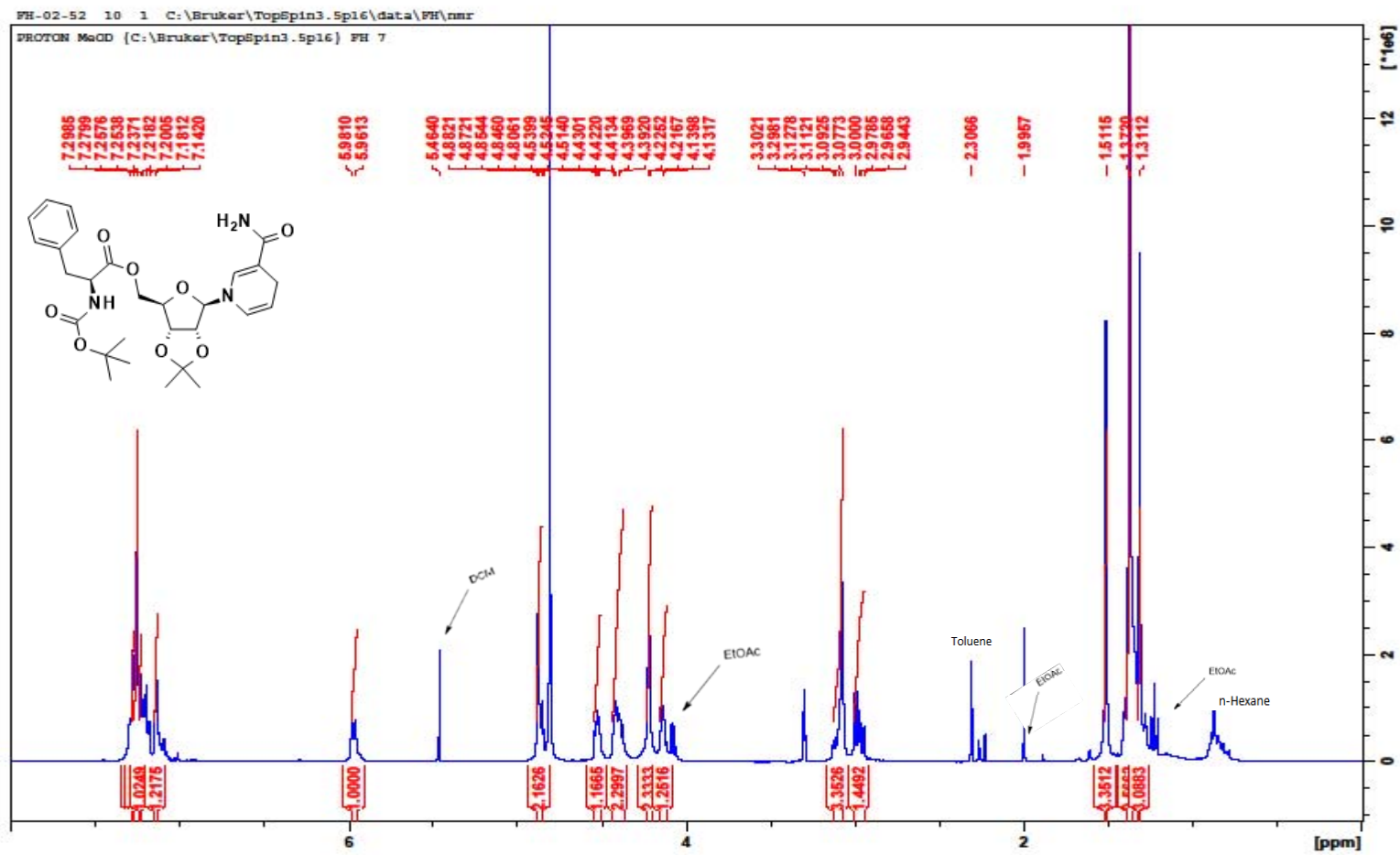
Compound 10d. 400 MHz ^1H NMR spectrum in MeOD



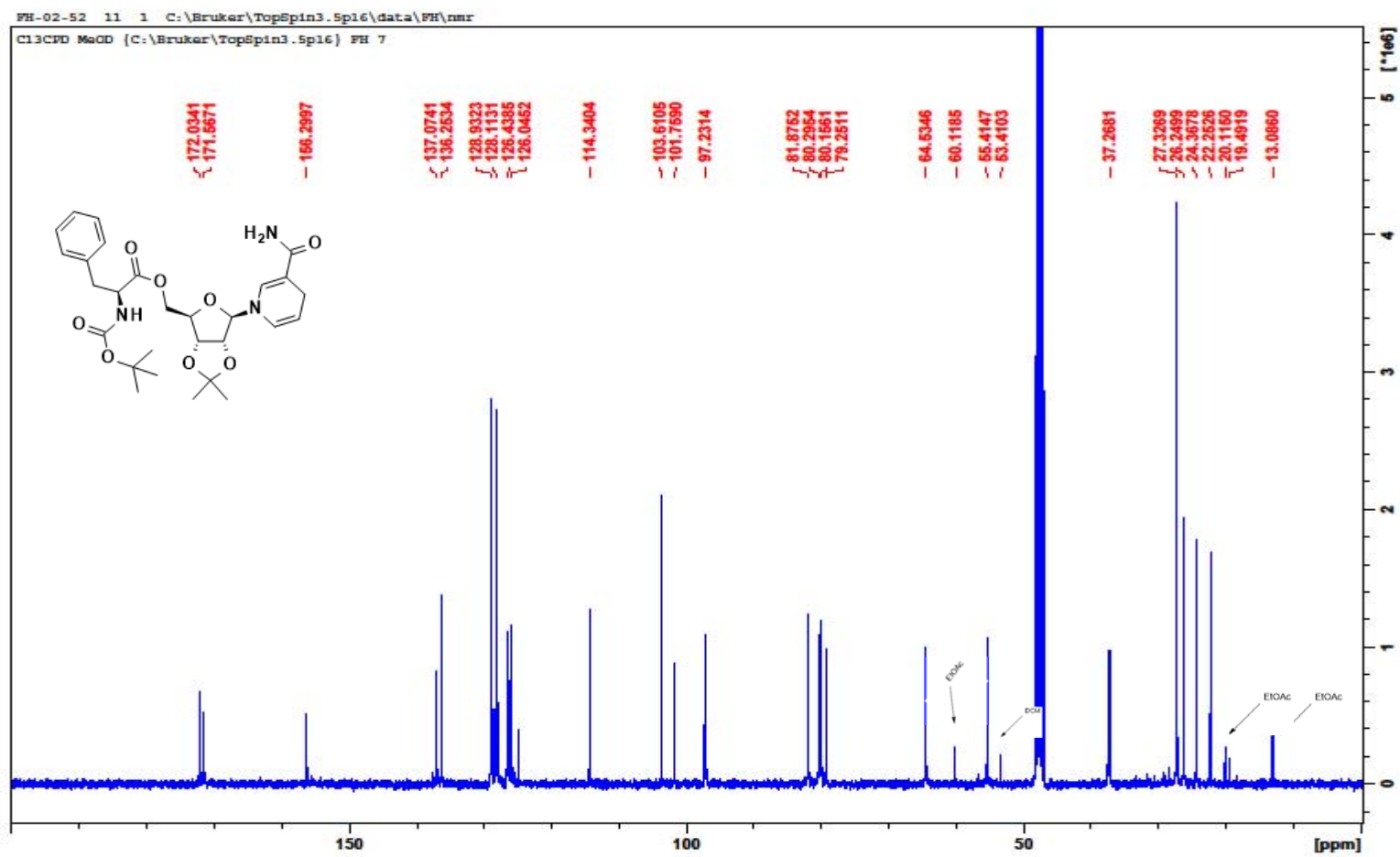
Compound 10d. 100 MHz ¹³C NMR spectrum in MeOD



Compound 10d. HRMS spectra

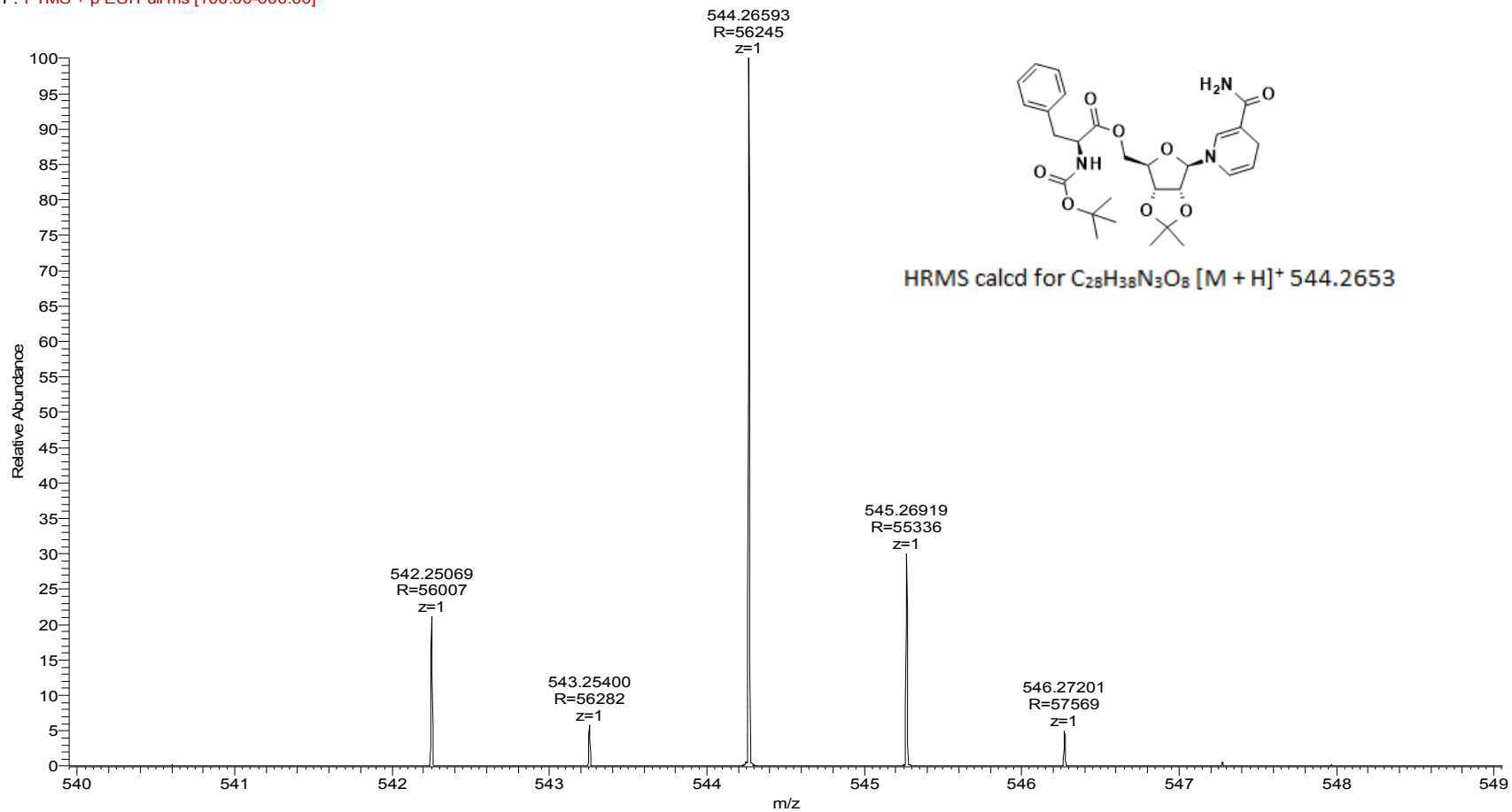


Compound 10e. 400 MHz ^1H NMR spectrum in MeOD

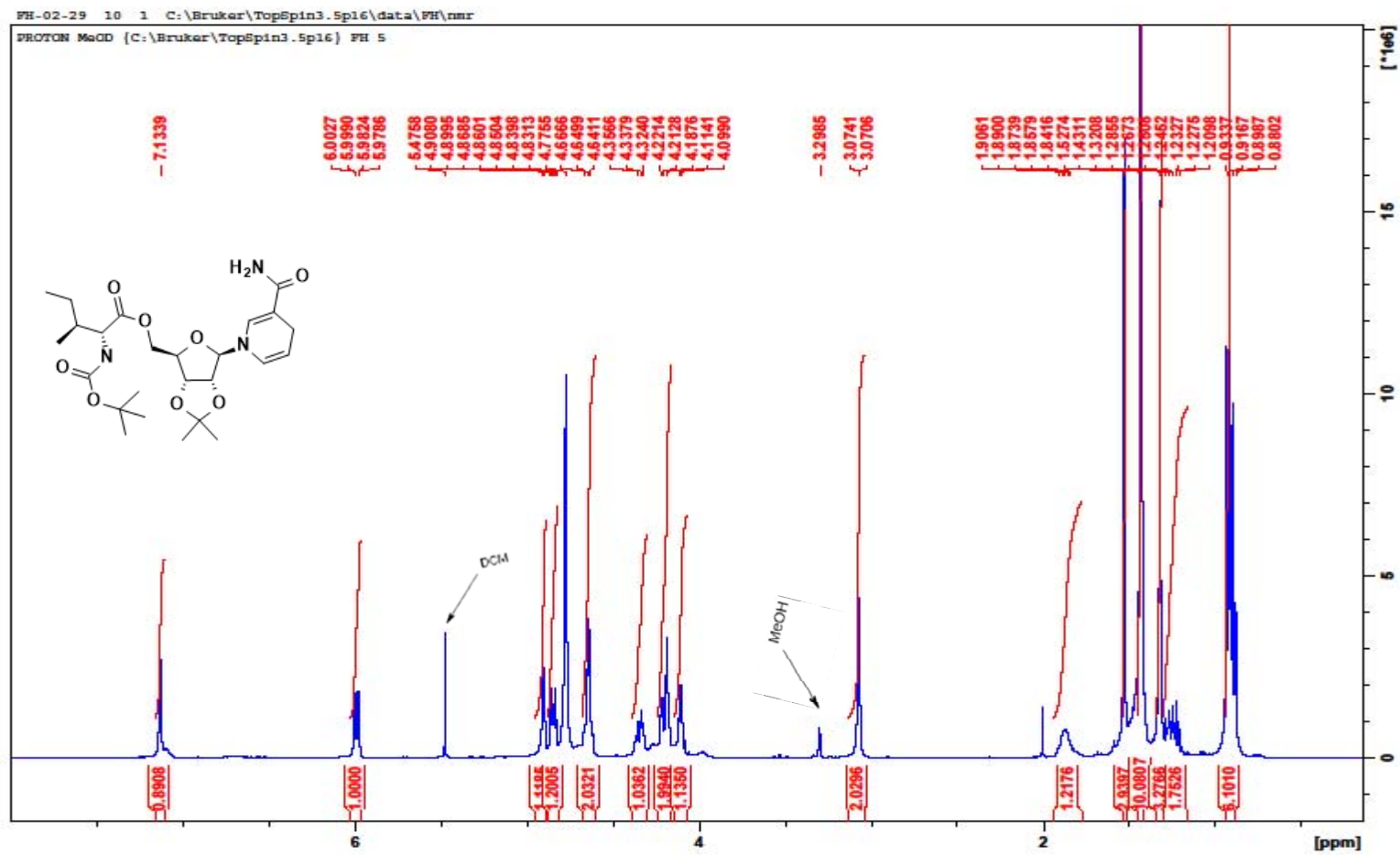


Compound 10e. 100 MHz ^{13}C NMR spectrum in MeOD

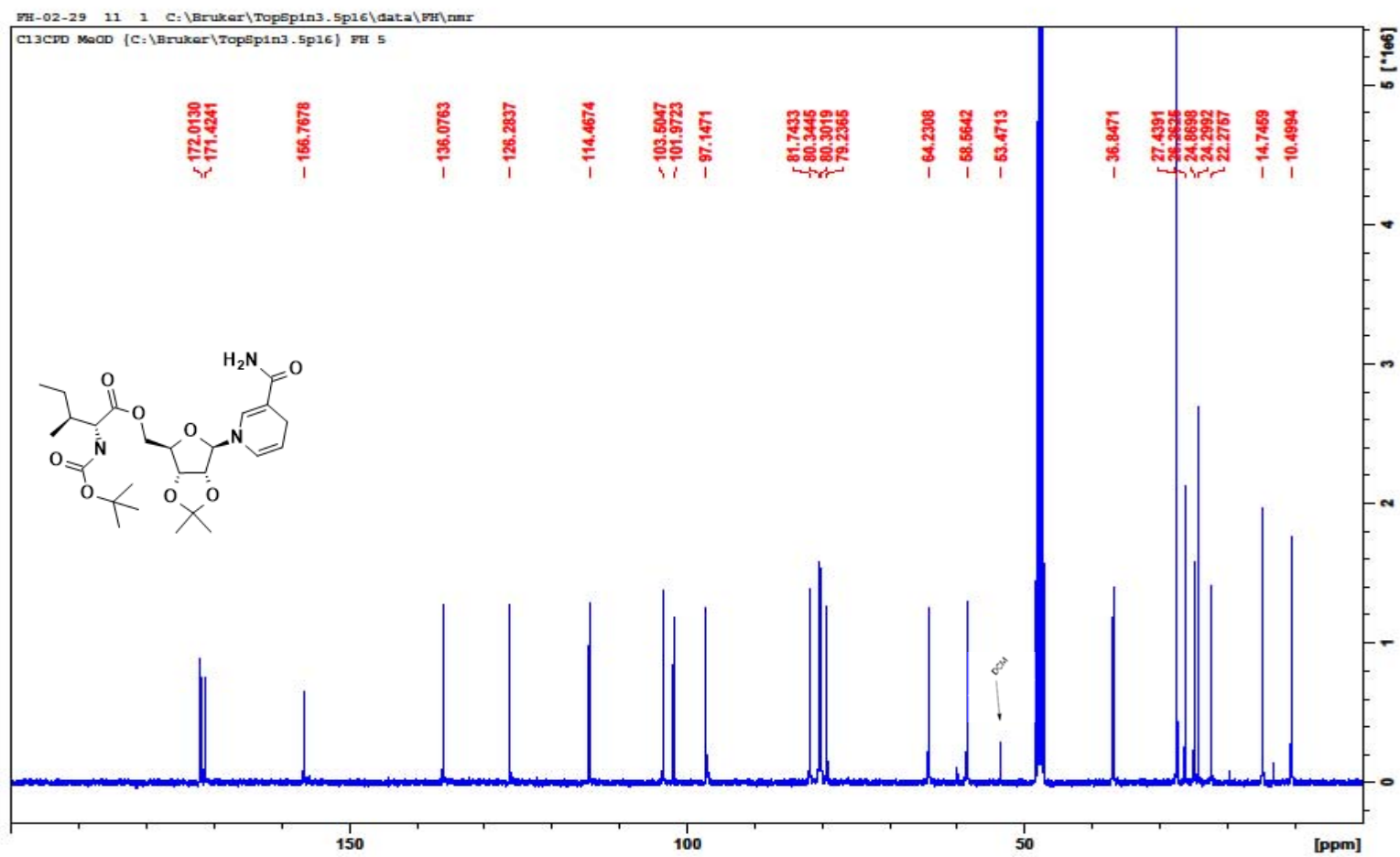
mm_072219_nrh_phenylalanine_1 #5-28 RT: 0.07-0.26 AV: 8 NL: 2.55E7
F: FTMS + p ESI Full ms [100.00-600.00]



Compound 10e. HRMS spectra

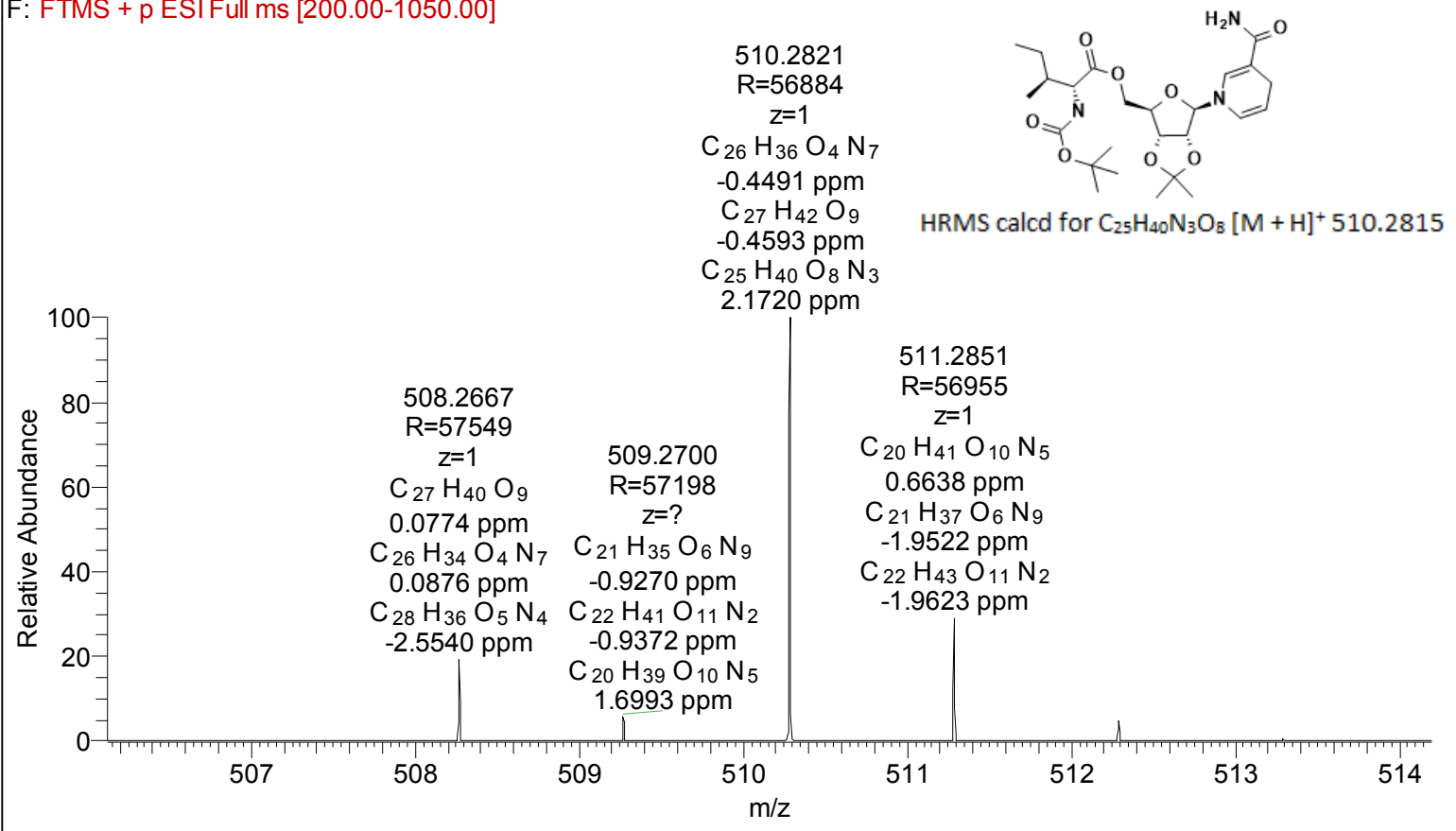


Compound 10f. 400 MHz ^1H NMR spectrum in MeOD

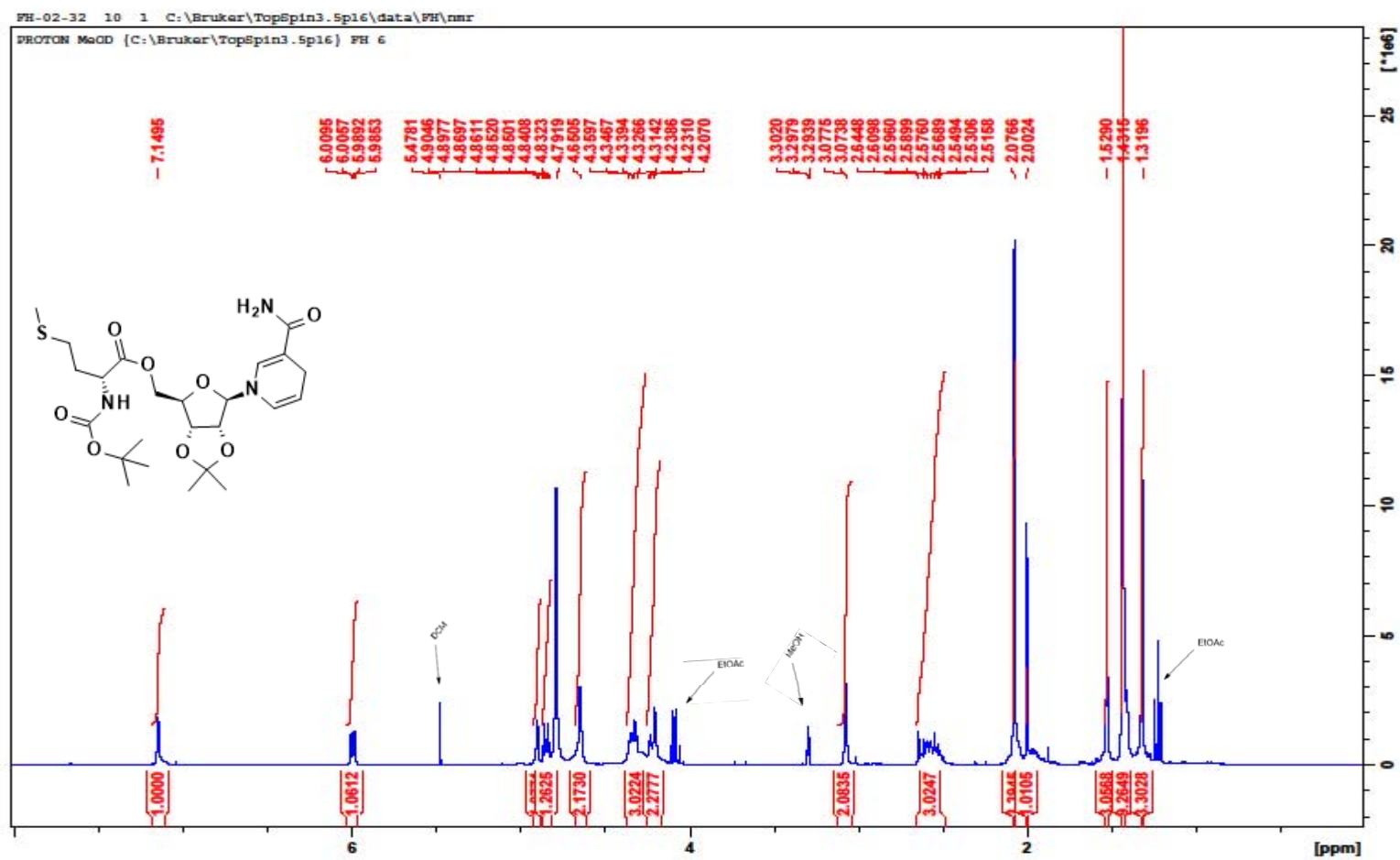


Compound 10f. 100 MHz ^{13}C NMR spectrum in MeOD

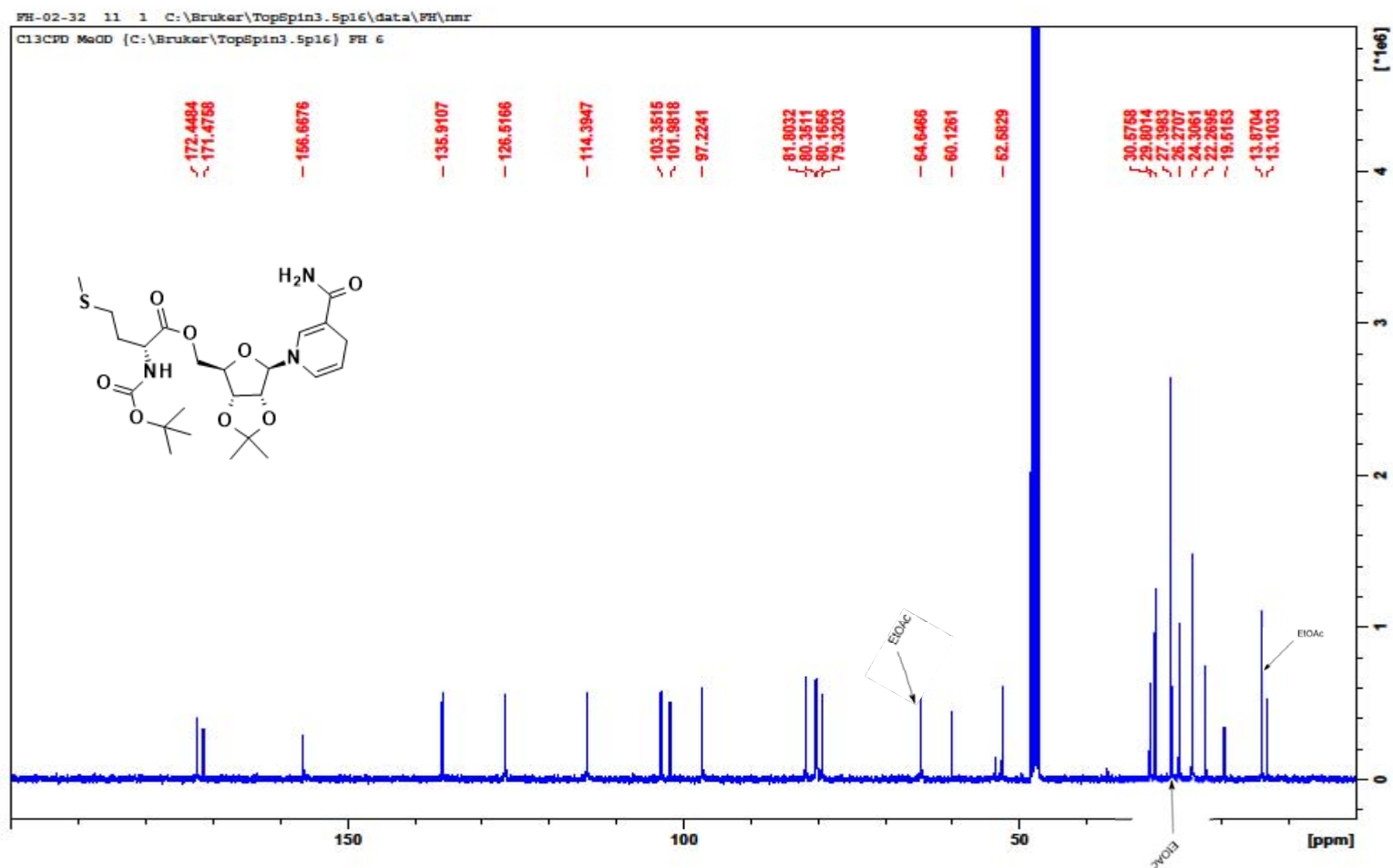
mm_101618_nrh_isoleucine_01 #17-33 RT: 0.25-0.49 AV: 17 SB: 81 5.78-7.06 NI: 2.47E7
F: FTMS + p ESI Full ms [200.00-1050.00]



Compound 10f. HRMS spectra



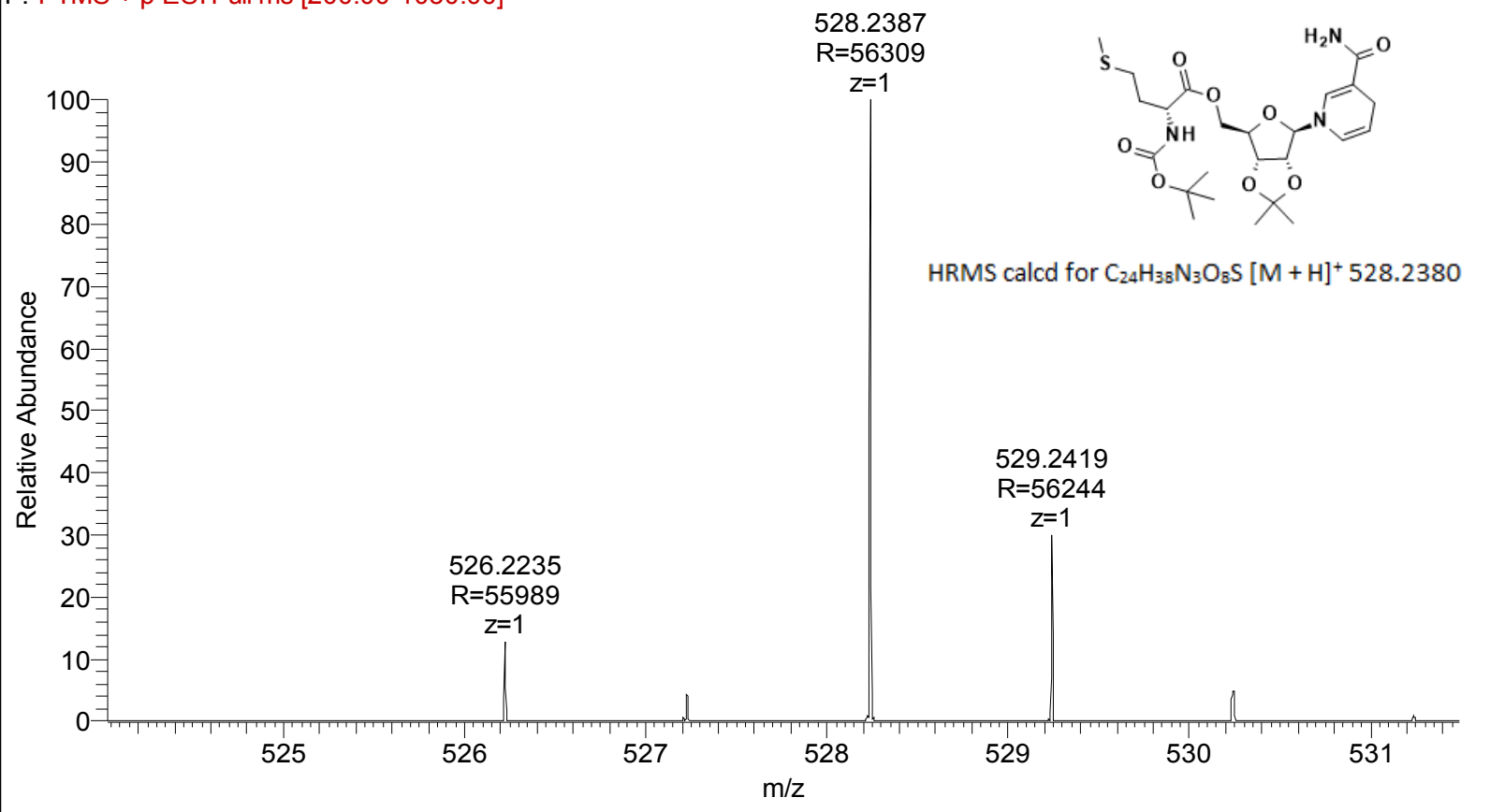
Compound 10g, 400 MHz ¹H NMR spectrum in MeOD



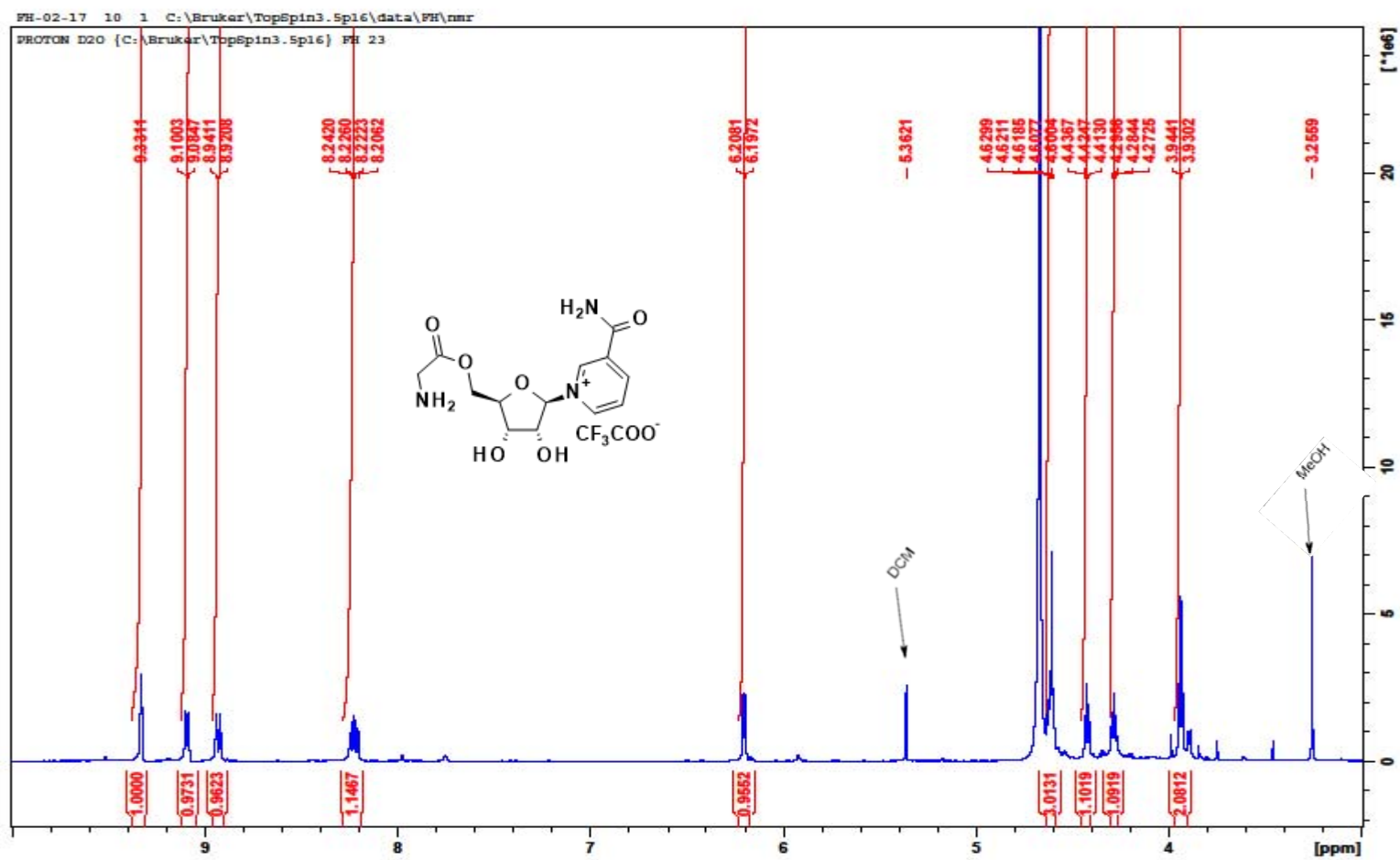
Compound 10g. 100 MHz ^{13}C NMR spectrum in MeOD

mm_101618_nrh_methionine_01 #18-32 RT: 0.26-0.47 AV: 15 SB: 81 5.78-7.06 NL: 2.46E7

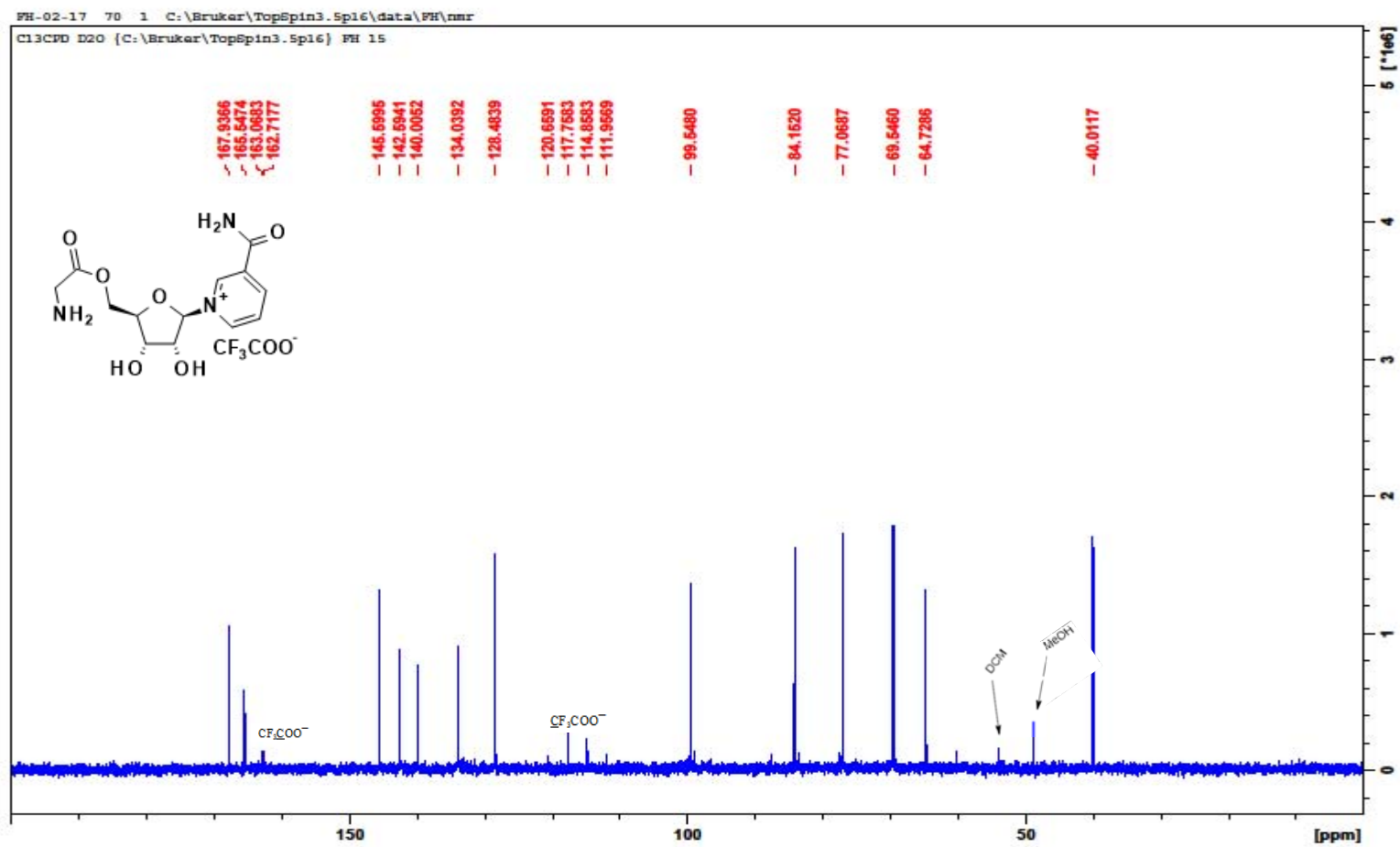
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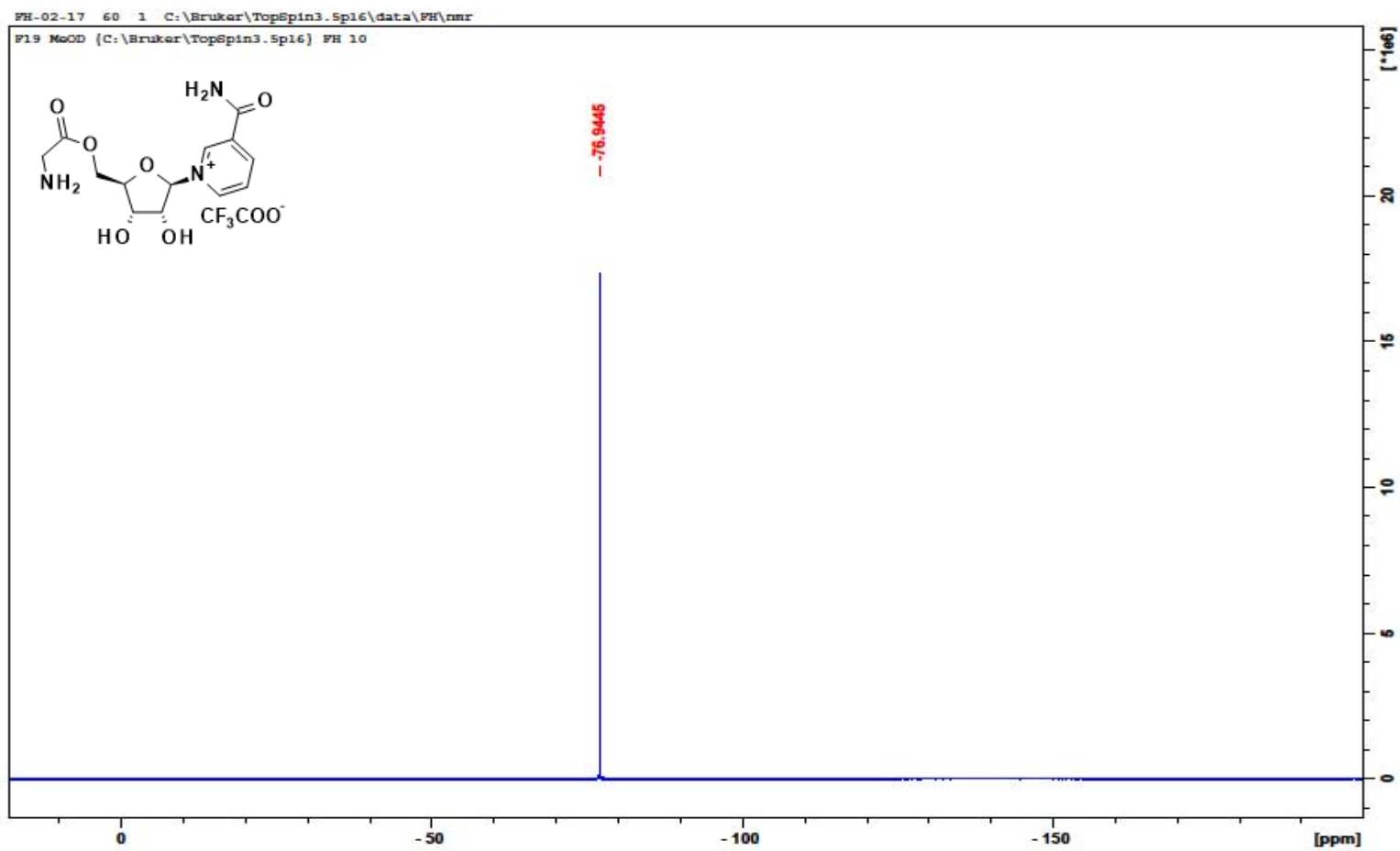
Compound 10g. HRMS spectra



Compound 12a. 400 MHz ^1H NMR spectrum in D_2O

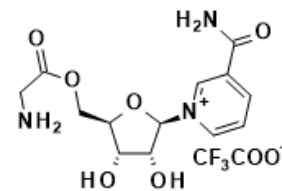
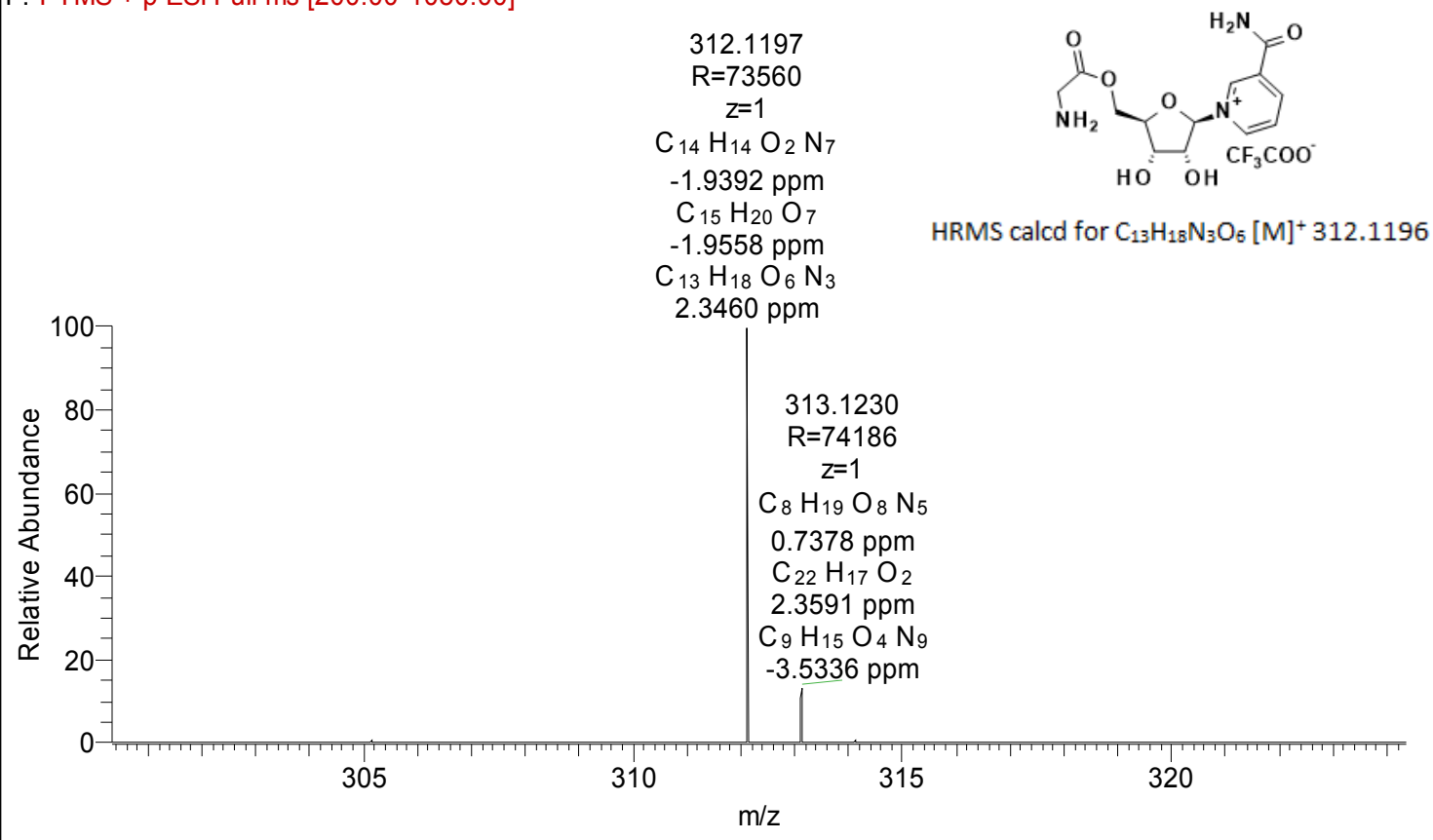


Compound 12a. 100 MHz ^{13}C NMR spectrum in D_2O

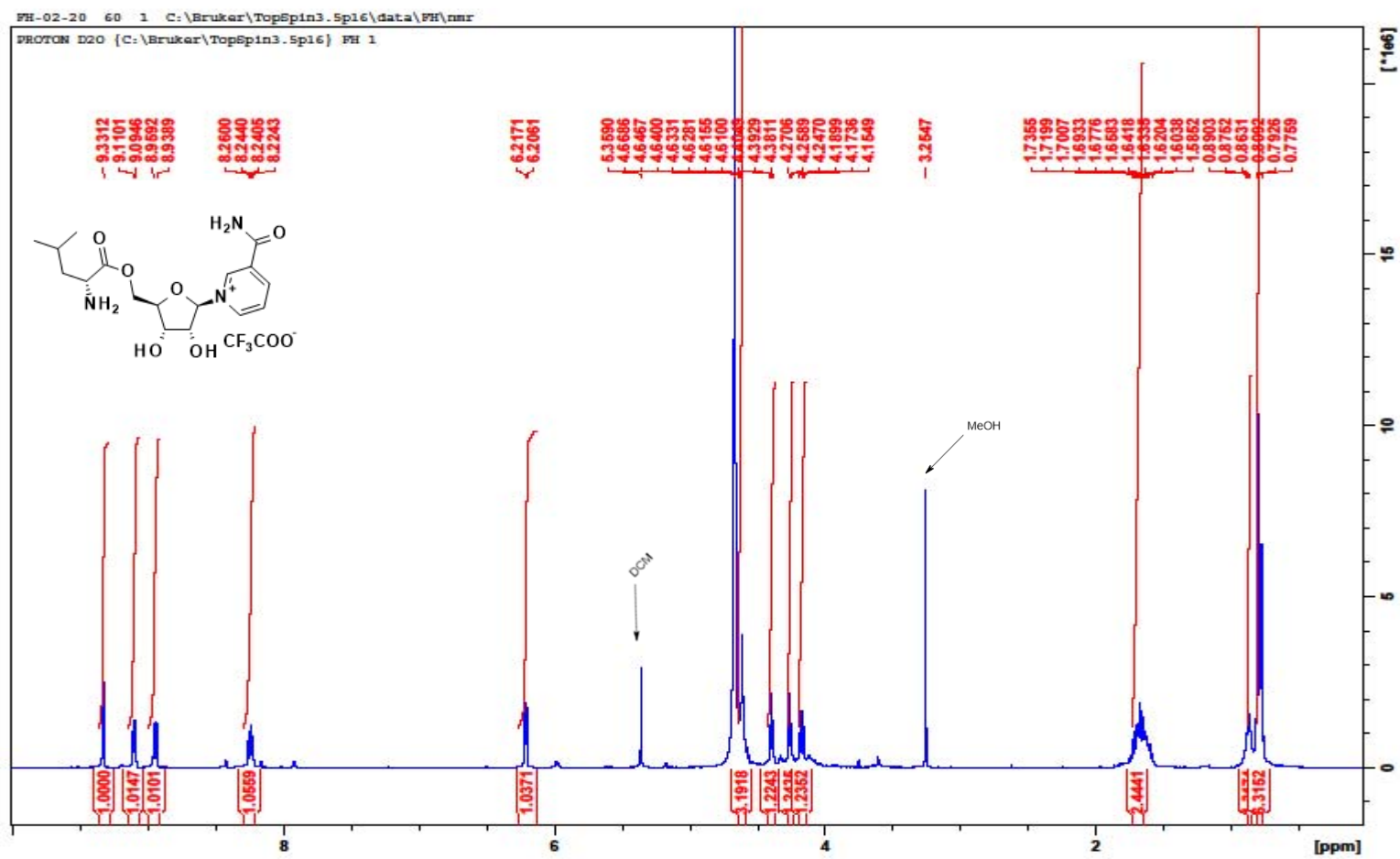


Compound 12a. 377 MHz ^{19}F NMR spectrum in MeOD

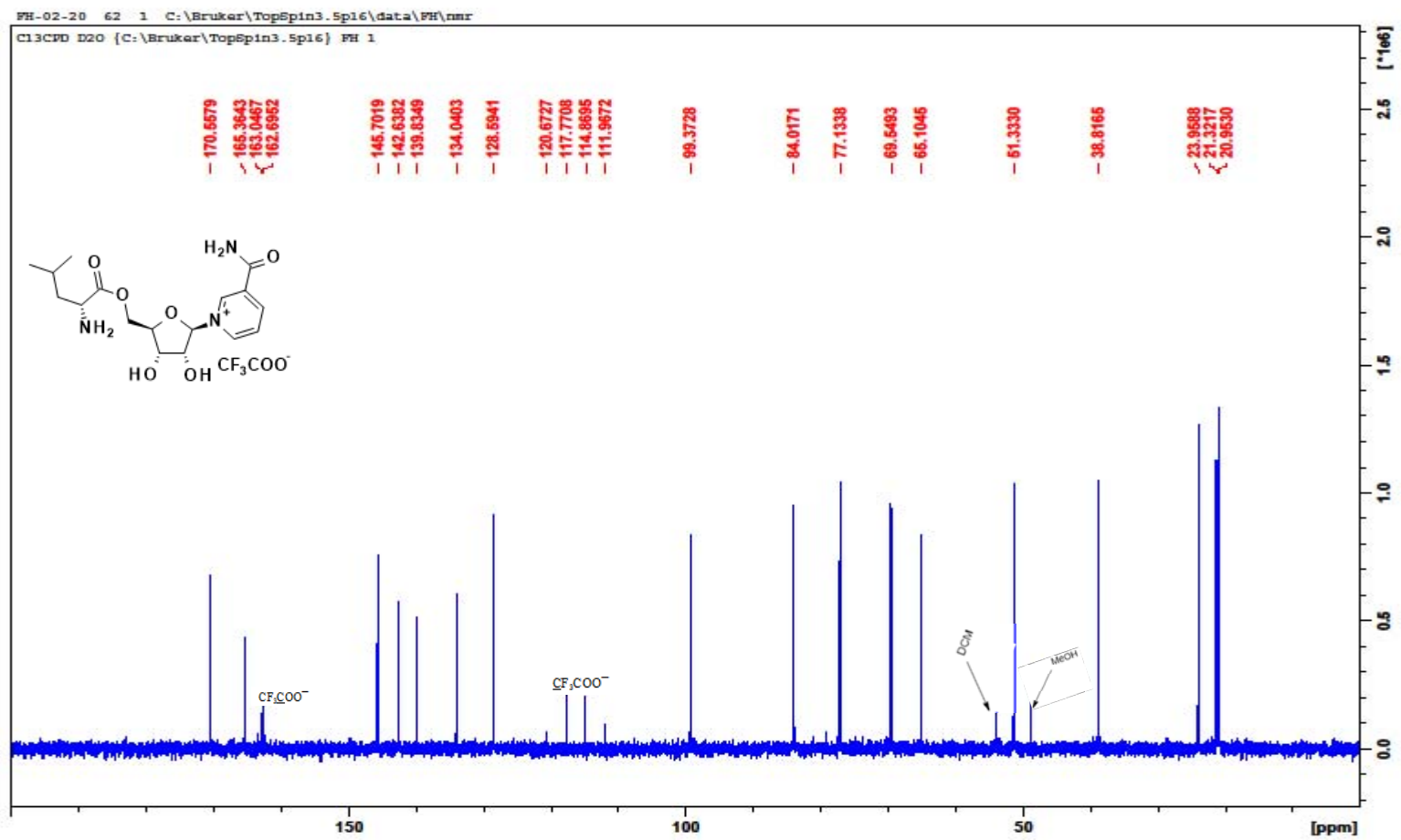
mm_101618_nr_glycine_01 #13-21 RT: 0.18-0.31 AV: 9 SB: 82 5.78-7.07 NL: 2.79E6
F: FTMS + p ESI Full ms [200.00-1050.00]



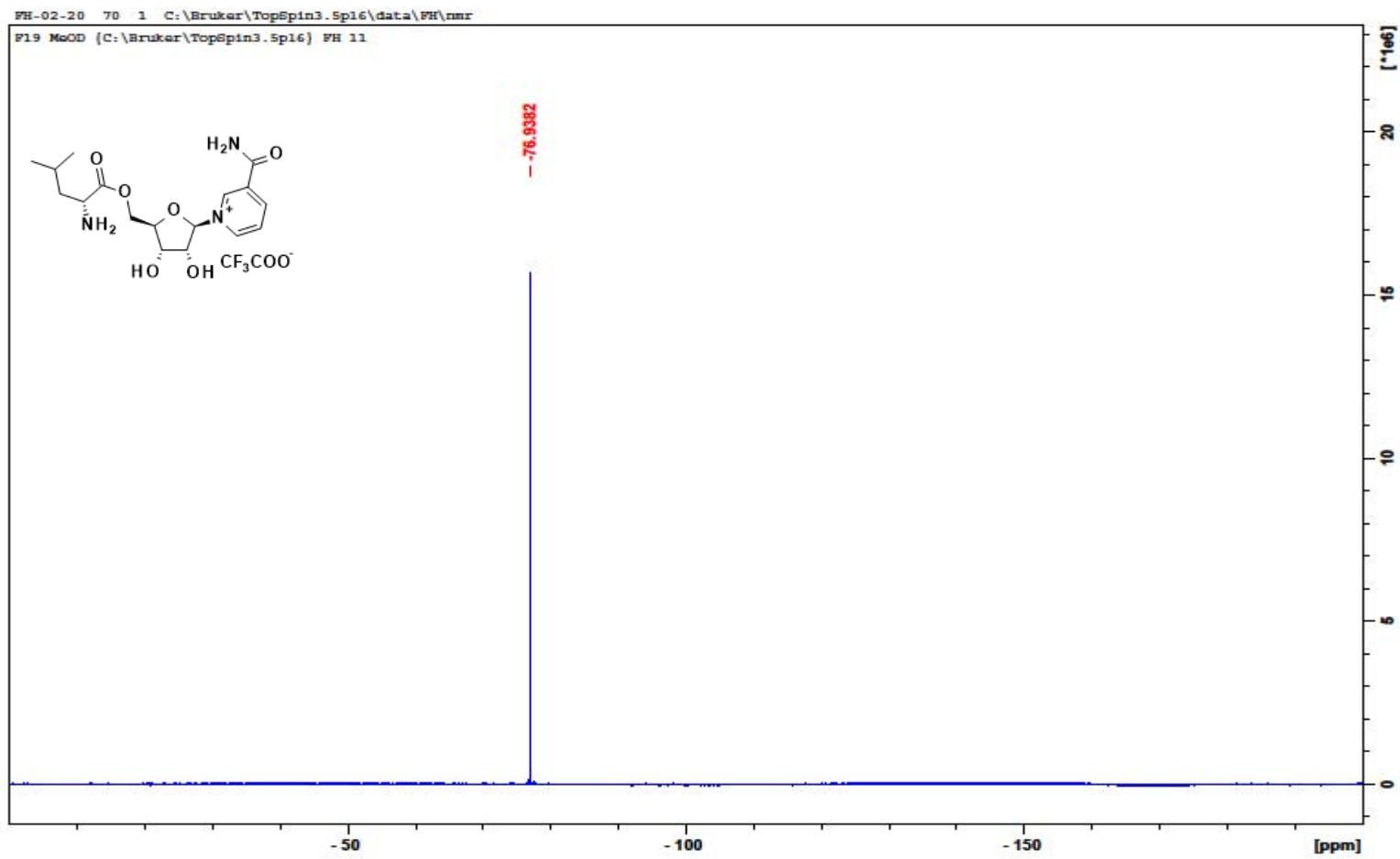
Compound 12a. HRMS spectra



Compound 12b. 400 MHz ^1H NMR spectrum in D_2O



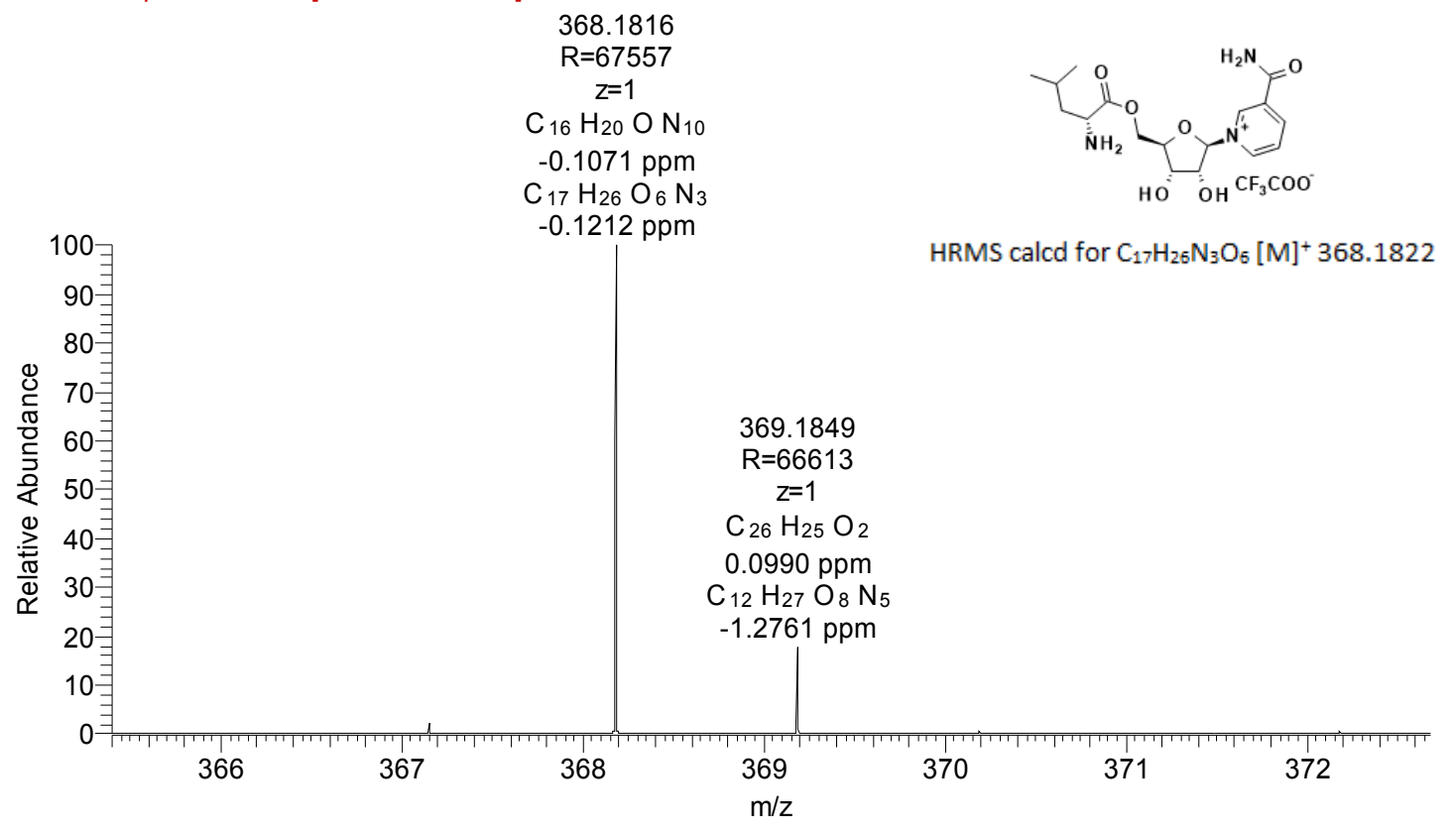
Compound 12b. 100 MHz ^{13}C NMR spectrum in D_2O



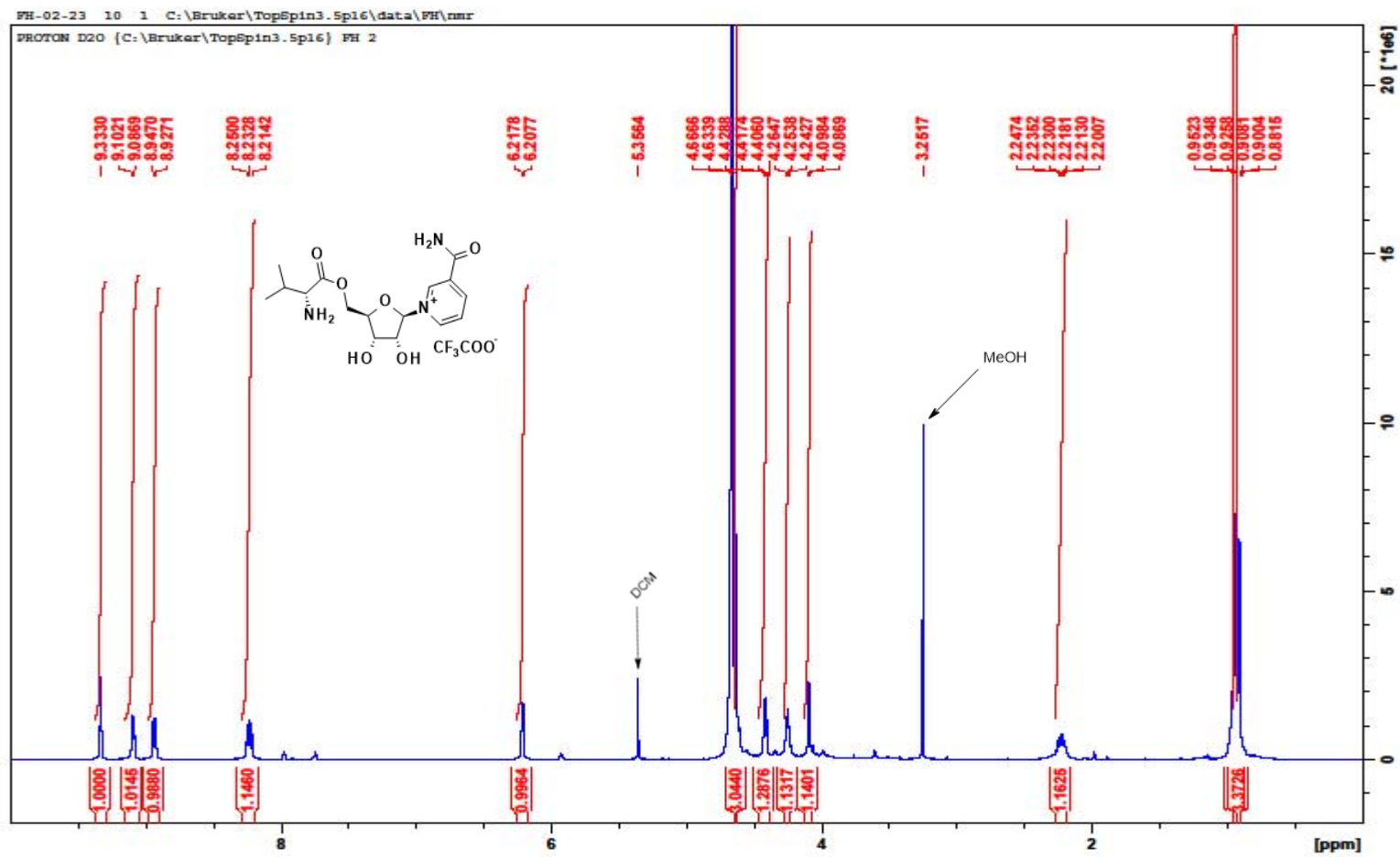
Compound 12b. 377 MHz ^{19}F NMR spectrum in MeOD

mm_101618_nr_leucine_01 #13-22 RT: 0.18-0.32 AV: 10 SB: 82 5.78-7.06 NL: 4.94E6

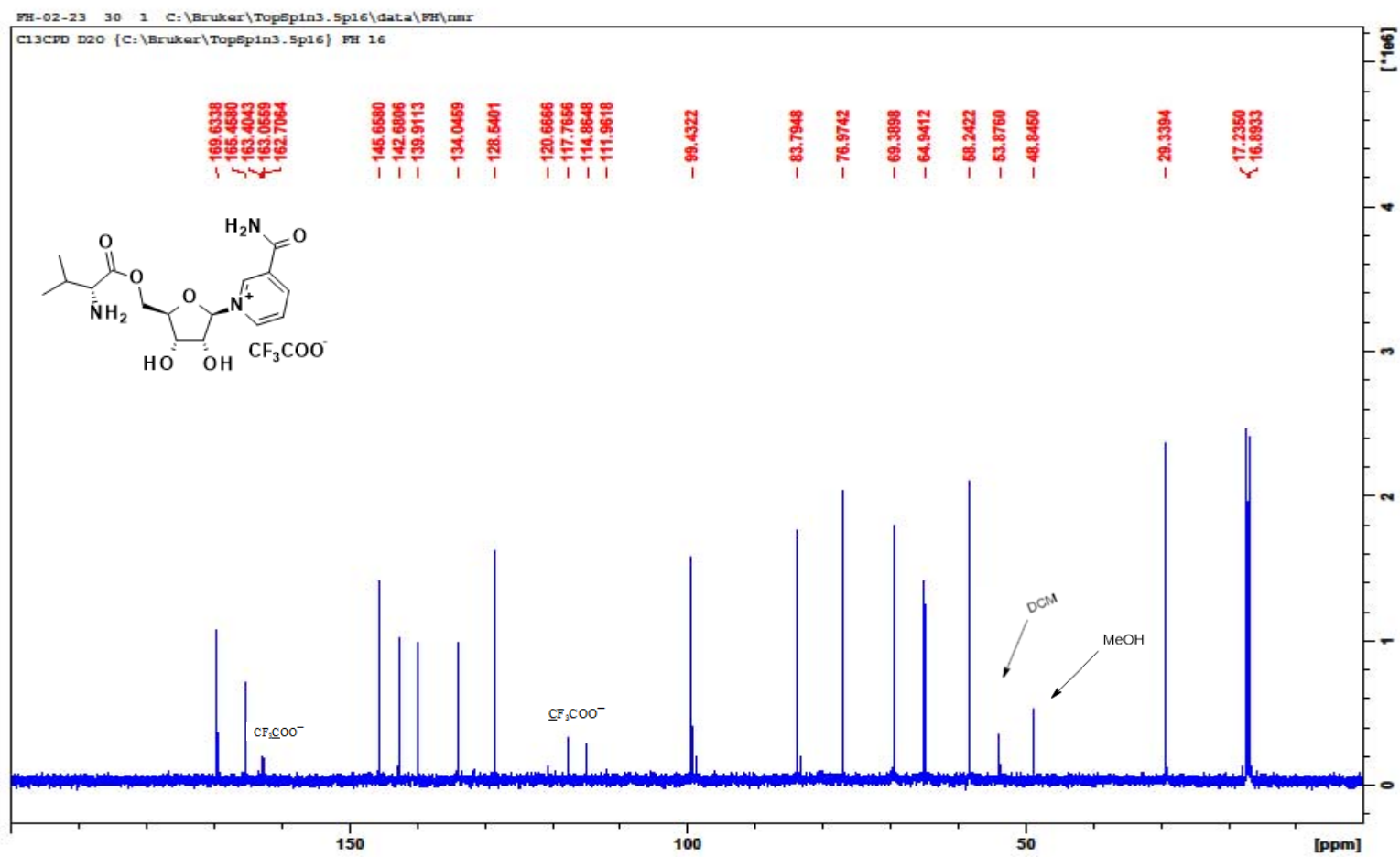
F: FTMS + p ESI Full ms [200.00-1050.00]



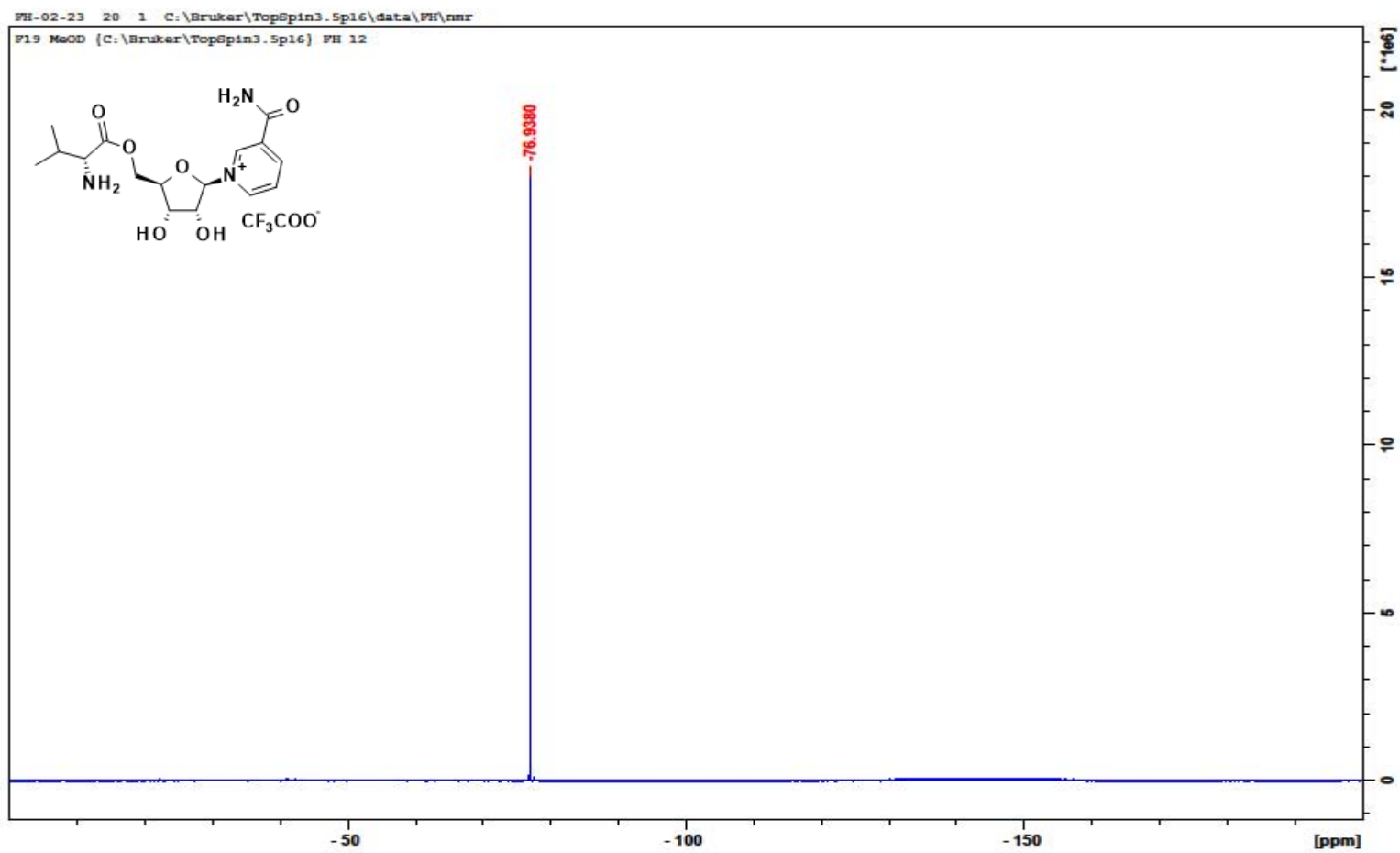
Compound 12b. HRMS spectra



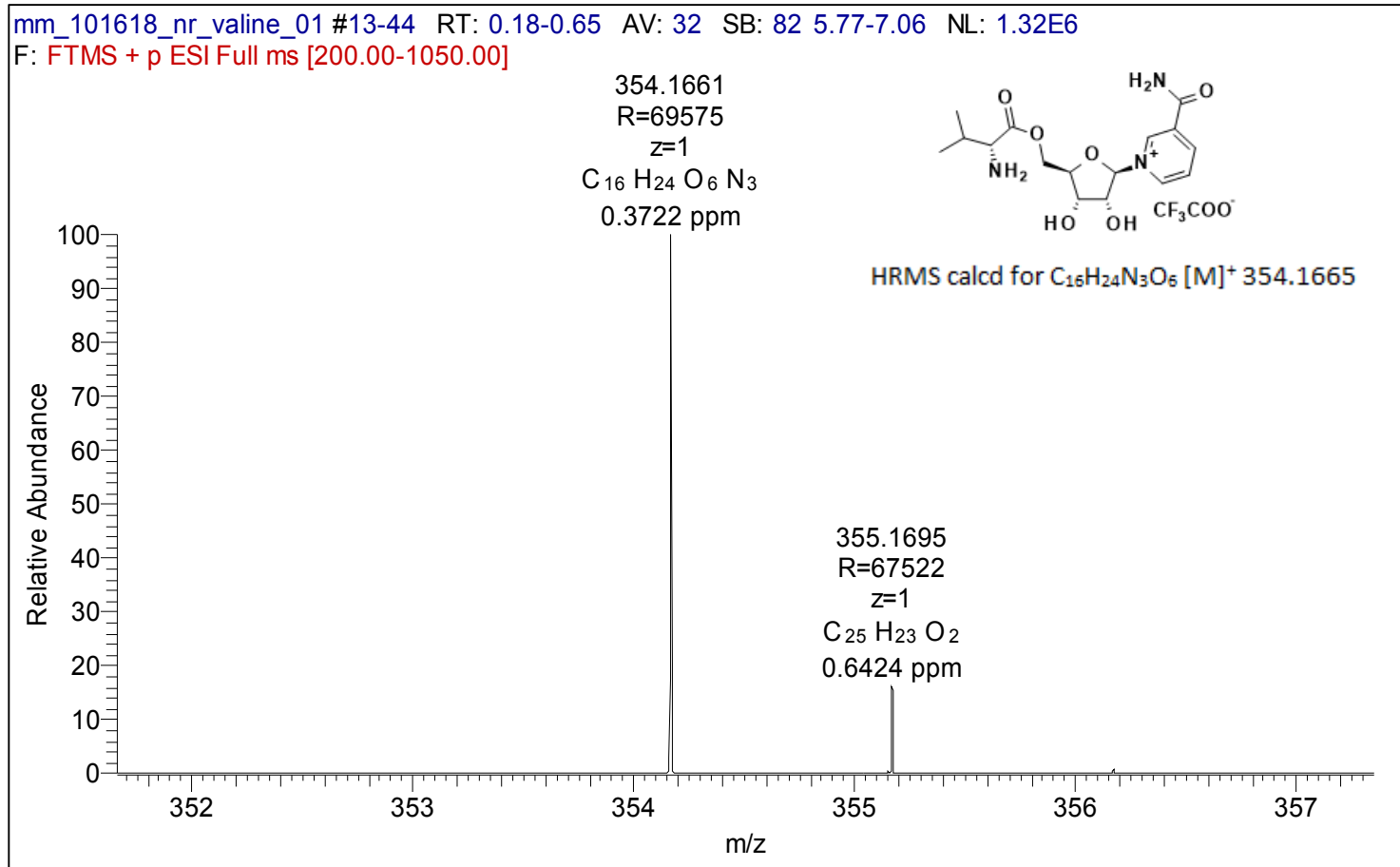
Compound 12c. 400 MHz ^1H NMR spectrum in D_2O



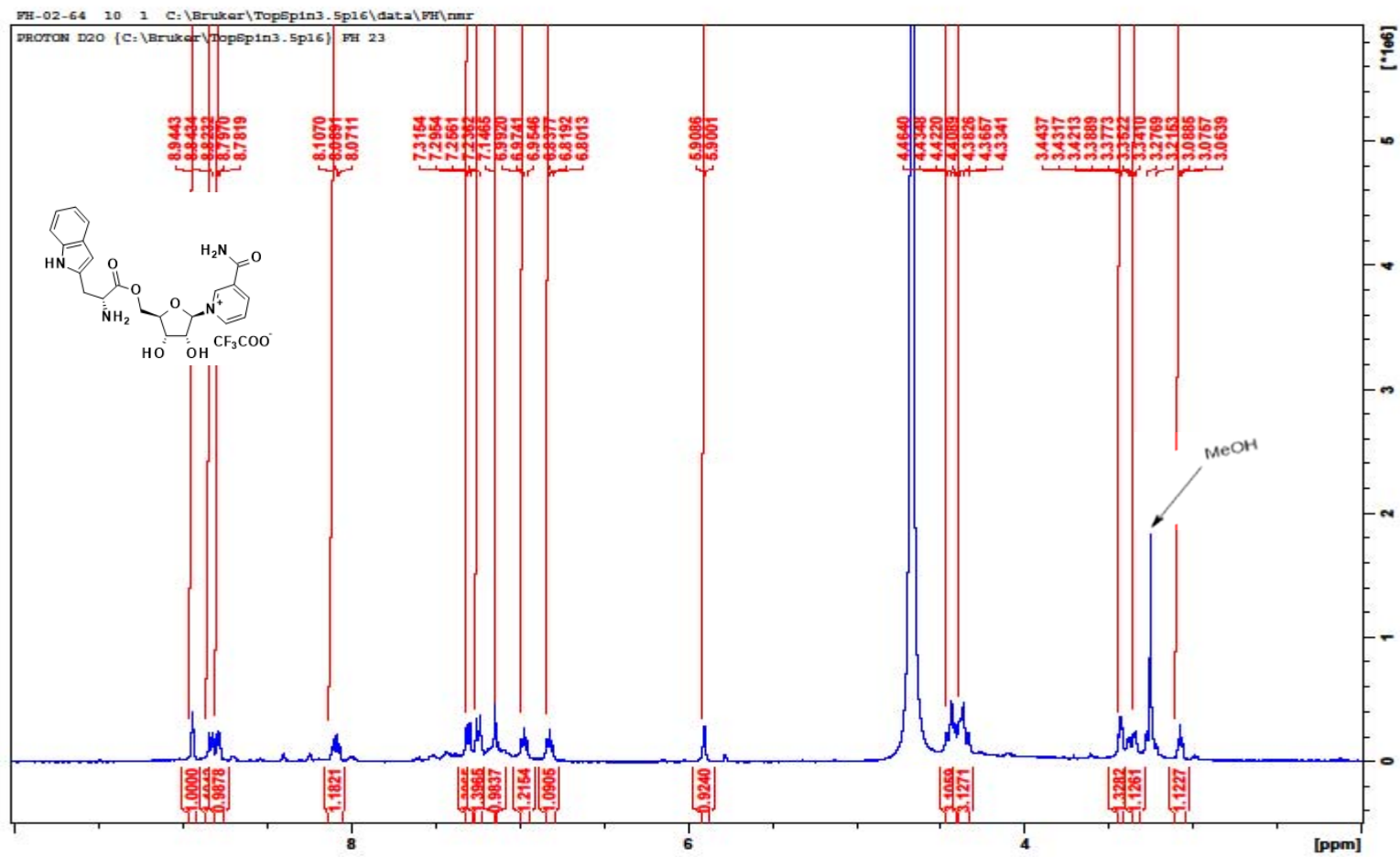
Compound 12c. 100 MHz ¹³C NMR spectrum in D₂O



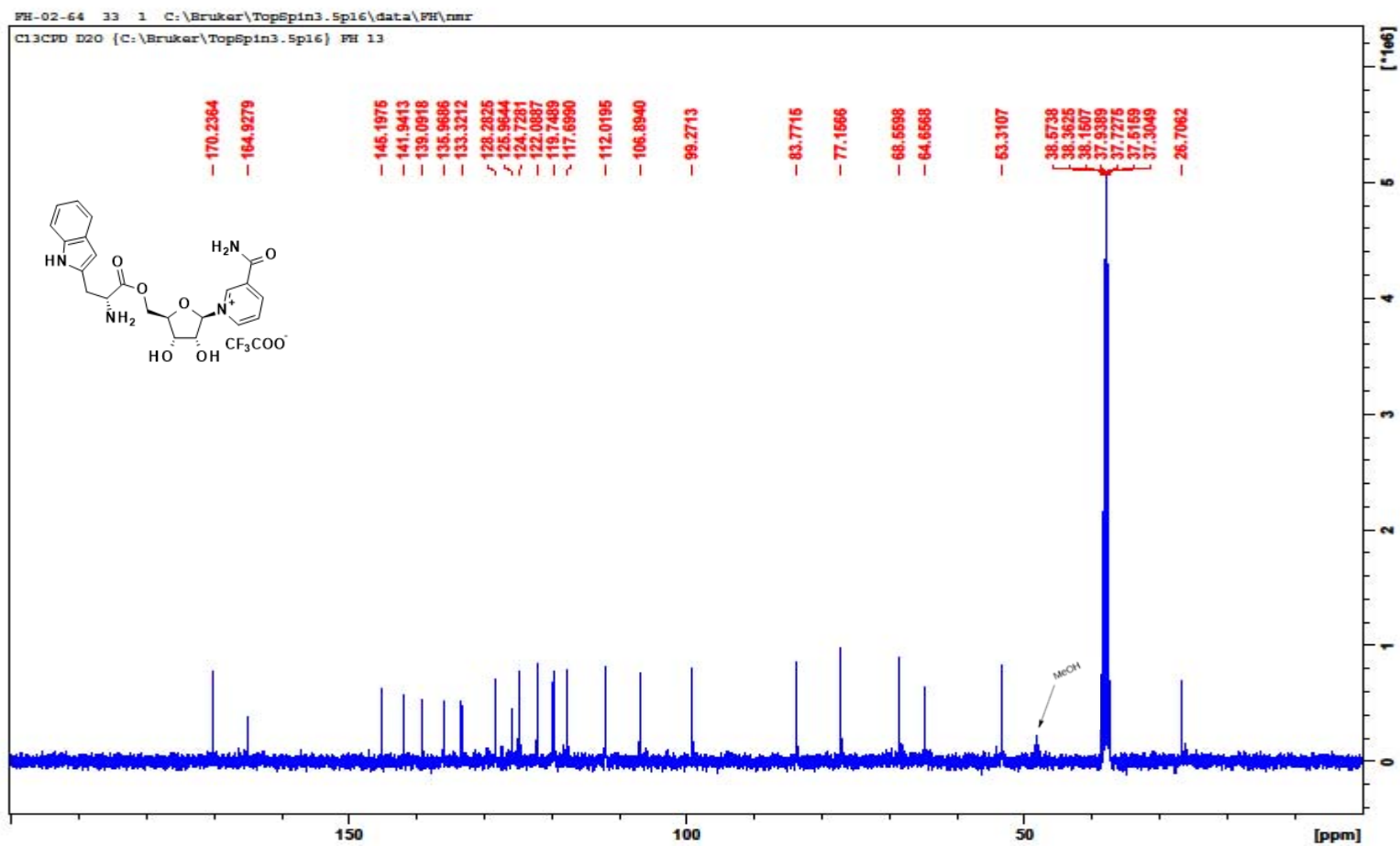
Compound 12c. 377 MHz ^{19}F NMR spectrum in MeOD



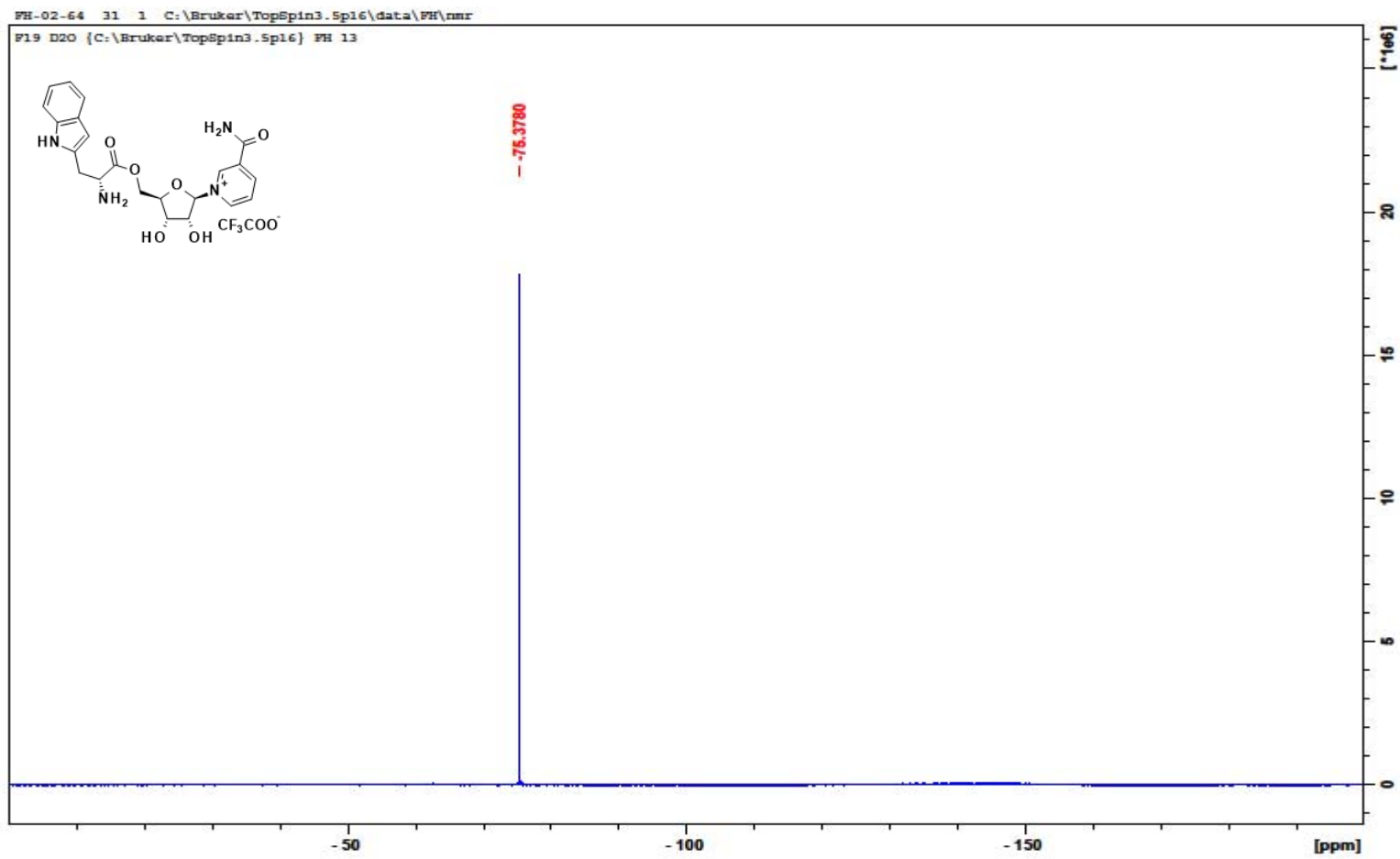
Compound 12c. HRMS spectra



Compound 12d. 400 MHz ^1H NMR spectrum in D_2O

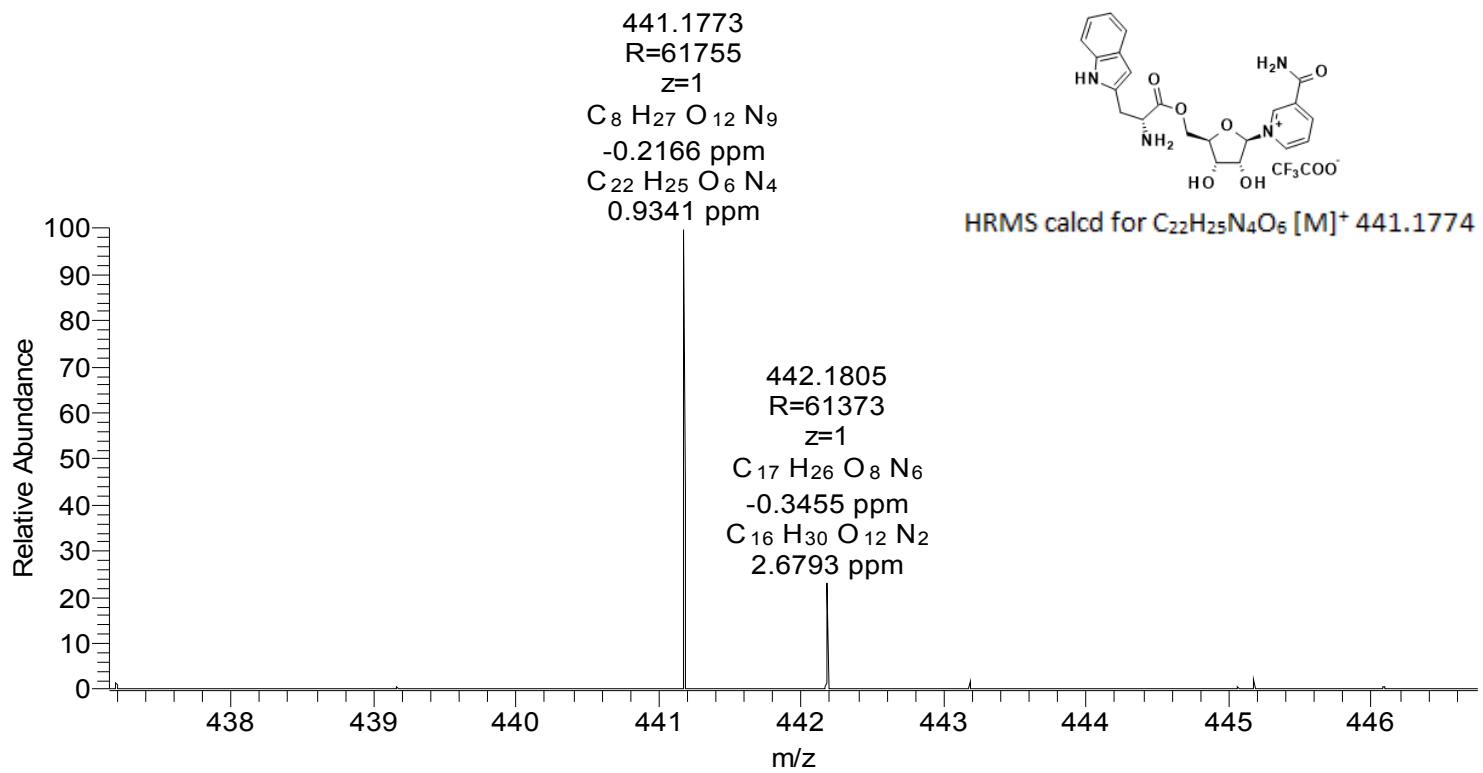


Compound 12d. 100 MHz ^{13}C NMR spectrum in D_2O

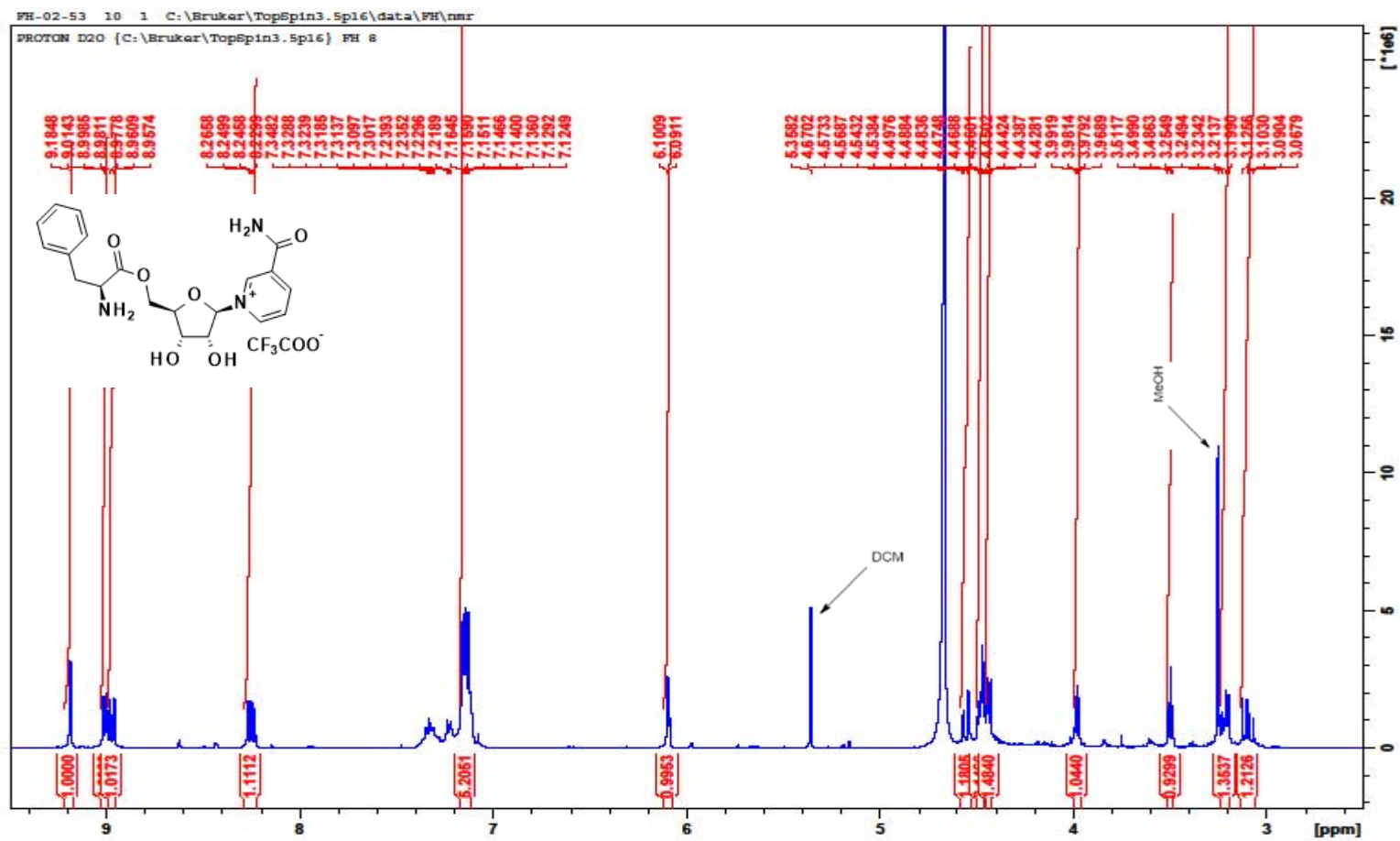


Compound 12d. 377 MHz ^{19}F NMR spectrum in MeOD

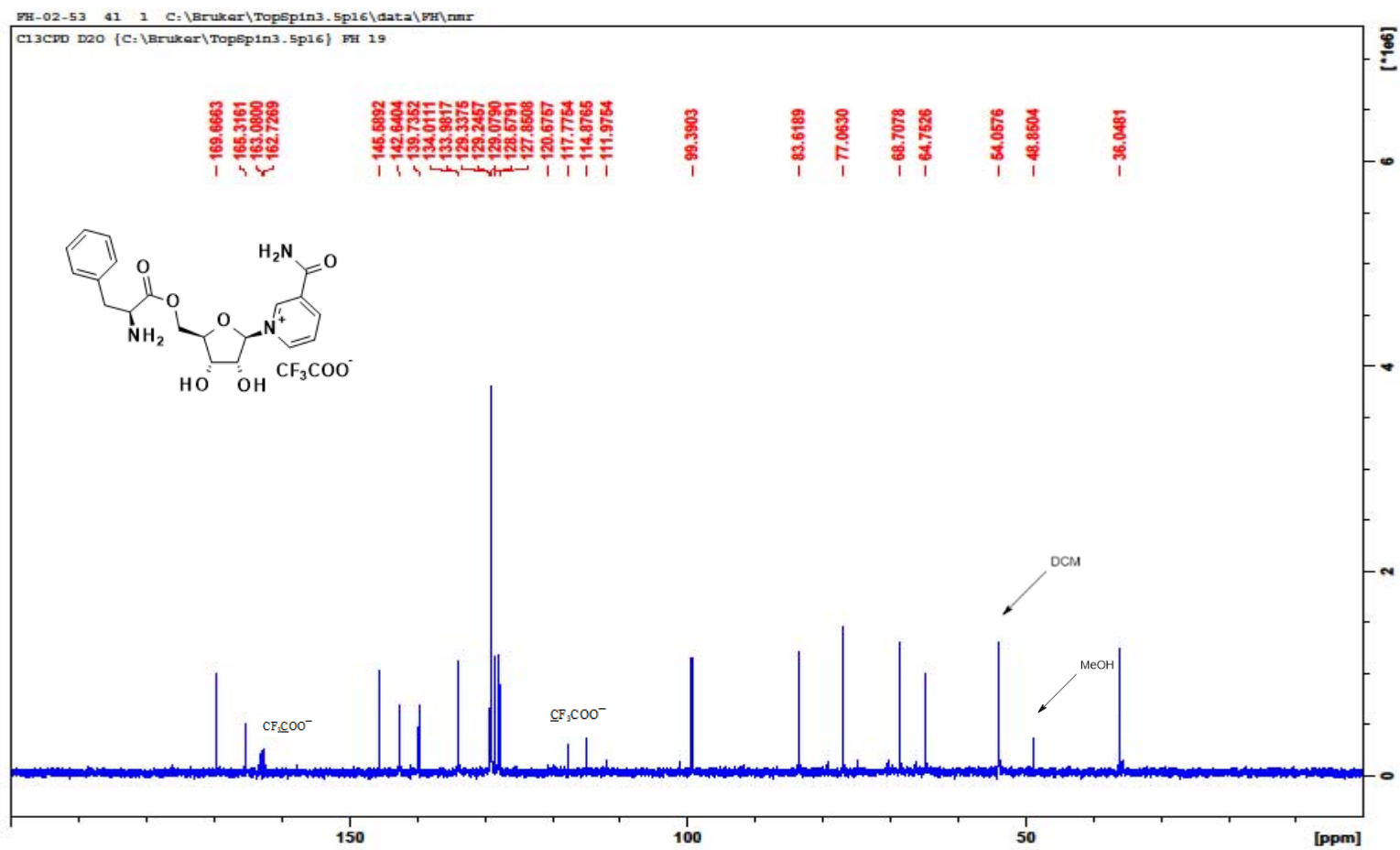
mm_101618_nr_tryptophan_01 #13-21 RT: 0.18-0.31 AV: 9 SB: 82 5.78-7.07 NL: 7.47E5
F: FTMS + p ESI Full ms [200.00-1050.00]



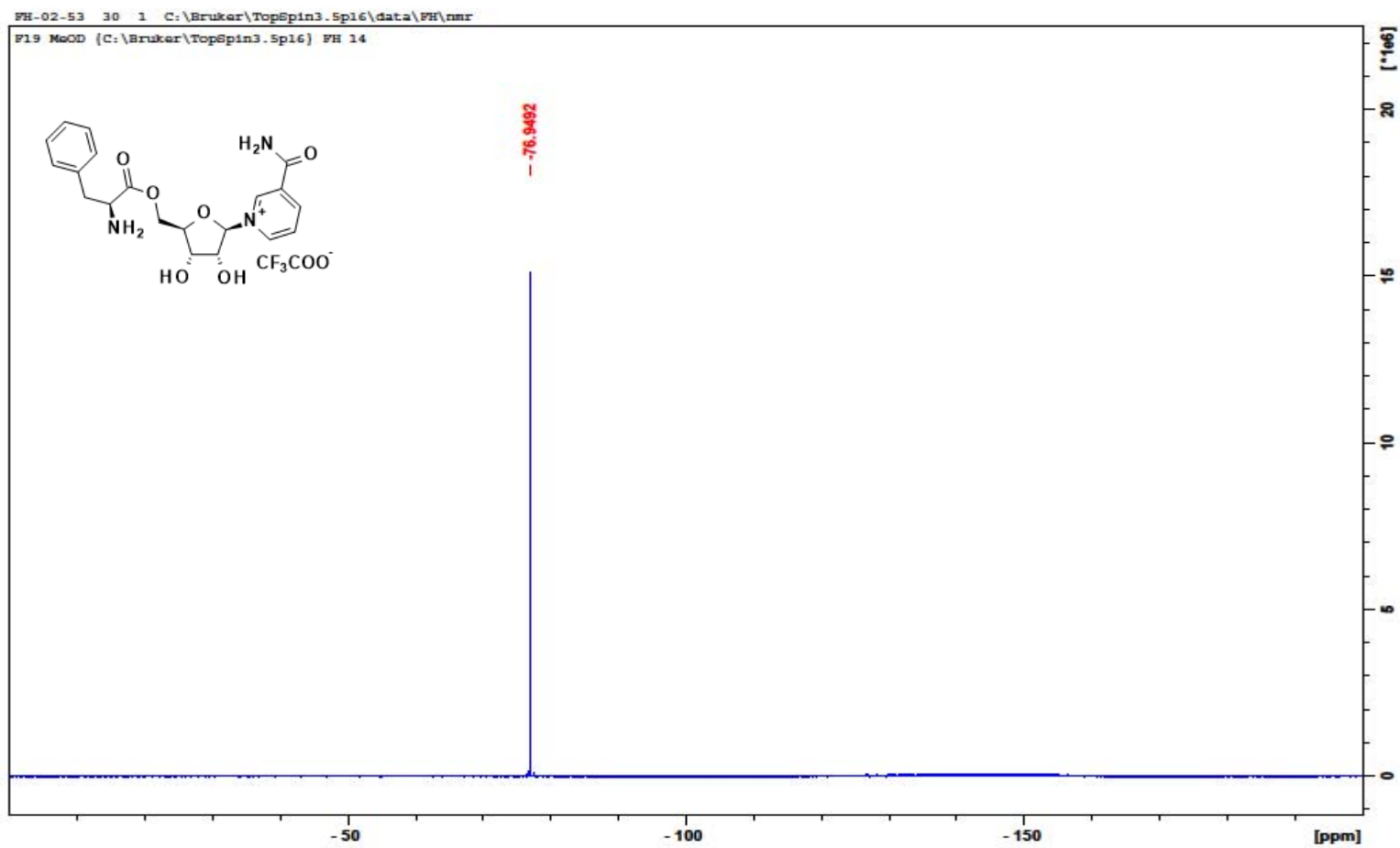
Compound 12d. HRMS spectra



Compound 12e. 400 MHz ^1H NMR spectrum in D_2O

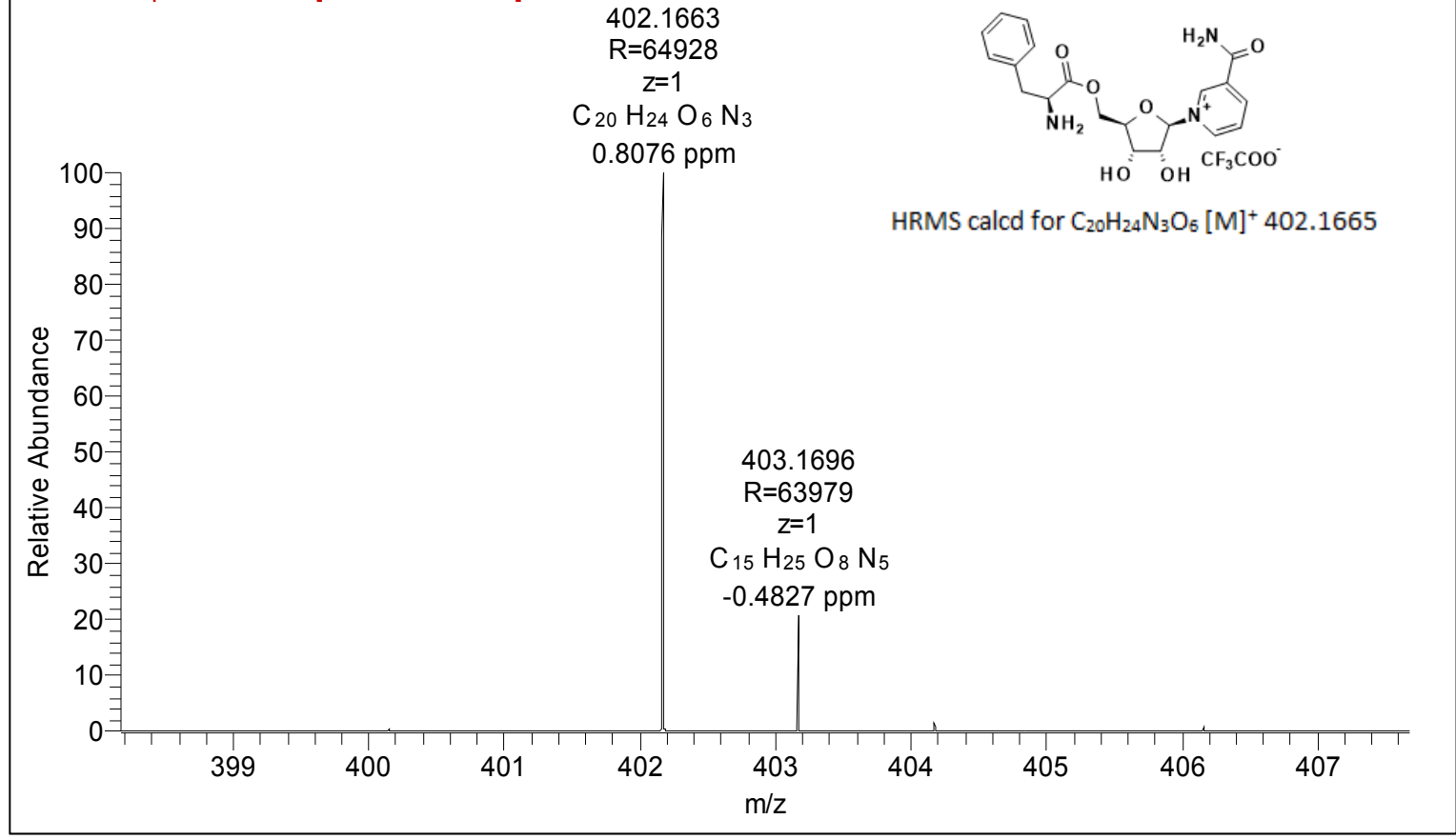


Compound 12e. 100 MHz ¹³C NMR spectrum in D₂O

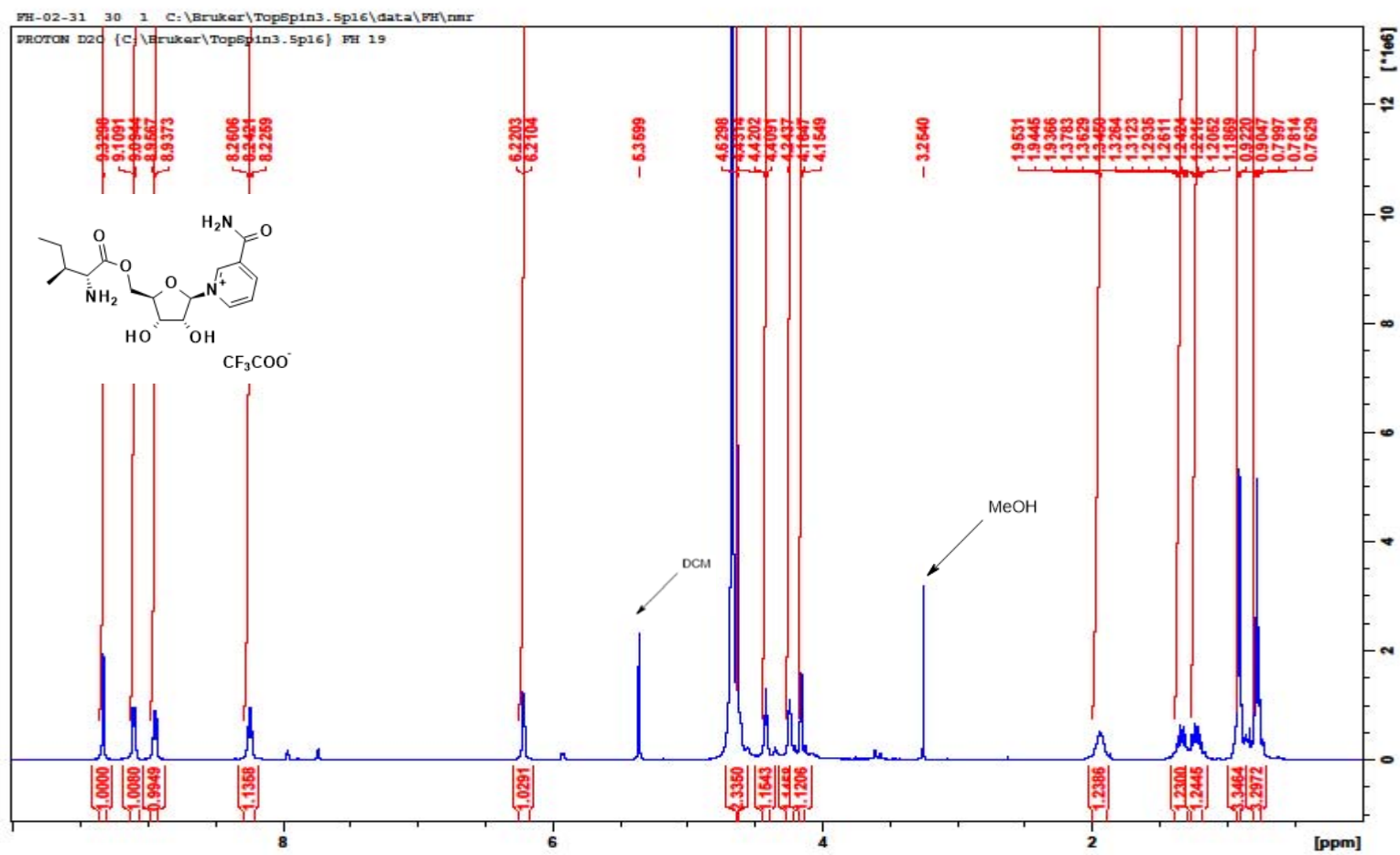


Compound 12e. 377 MHz ^{19}F NMR spectrum in MeOD

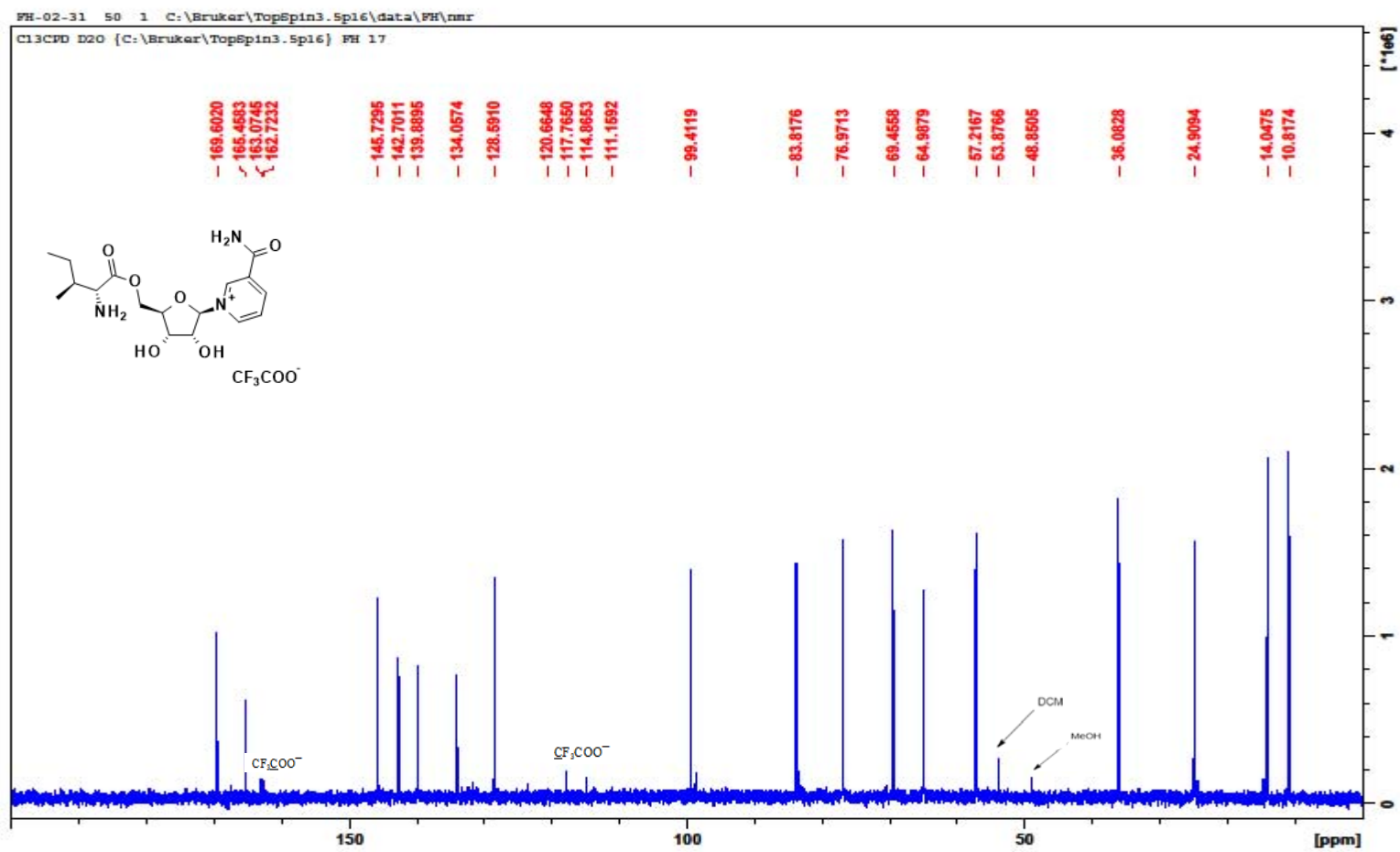
mm_101618_nr_phenylalanine_01 #13-23 RT: 0.18-0.33 AV: 11 SB: 82 5.77-7.06 NL: 4.94E6
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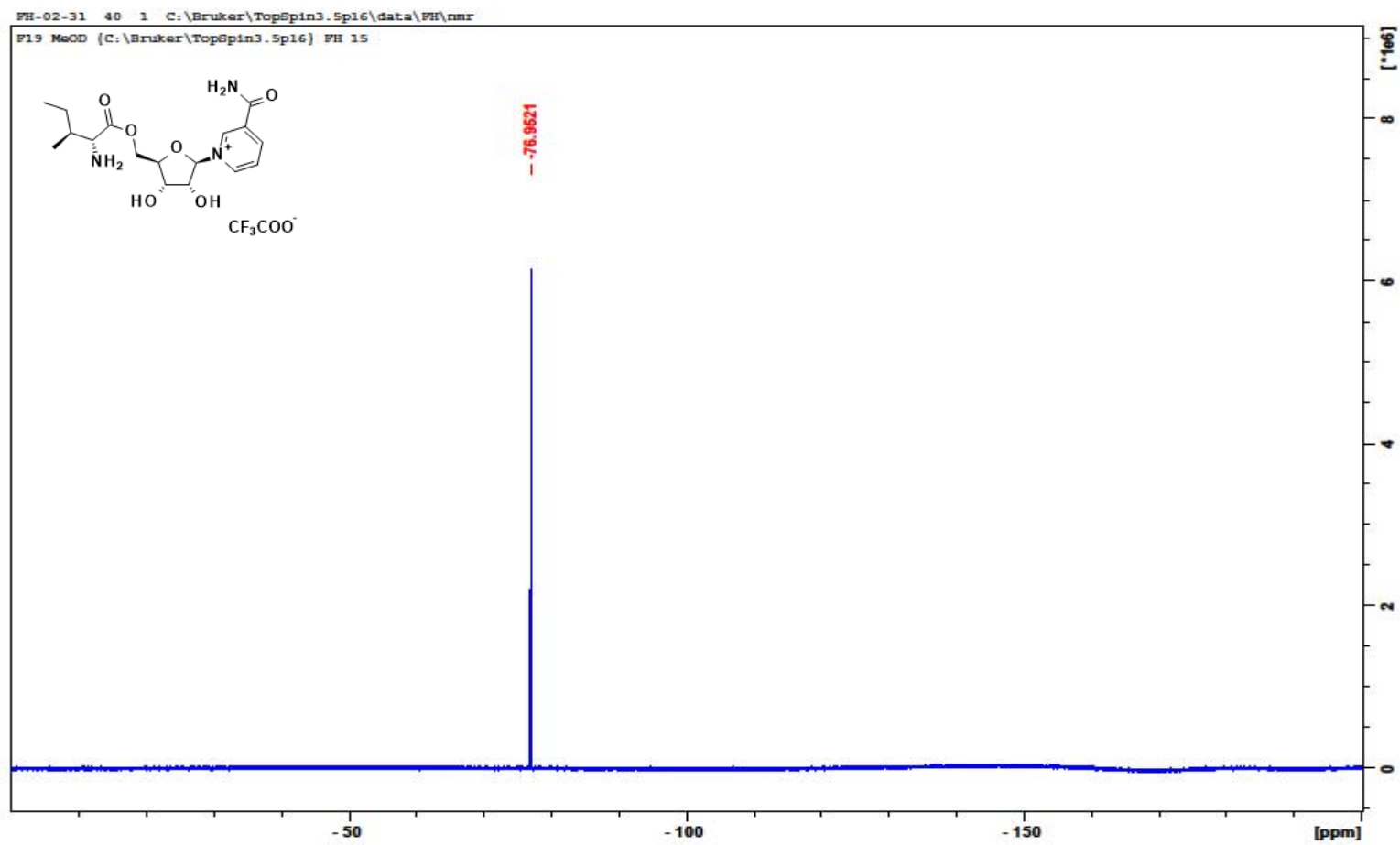
Compound 12e. HRMS spectra



Compound 12f. 400 MHz ¹H NMR spectrum in D₂O

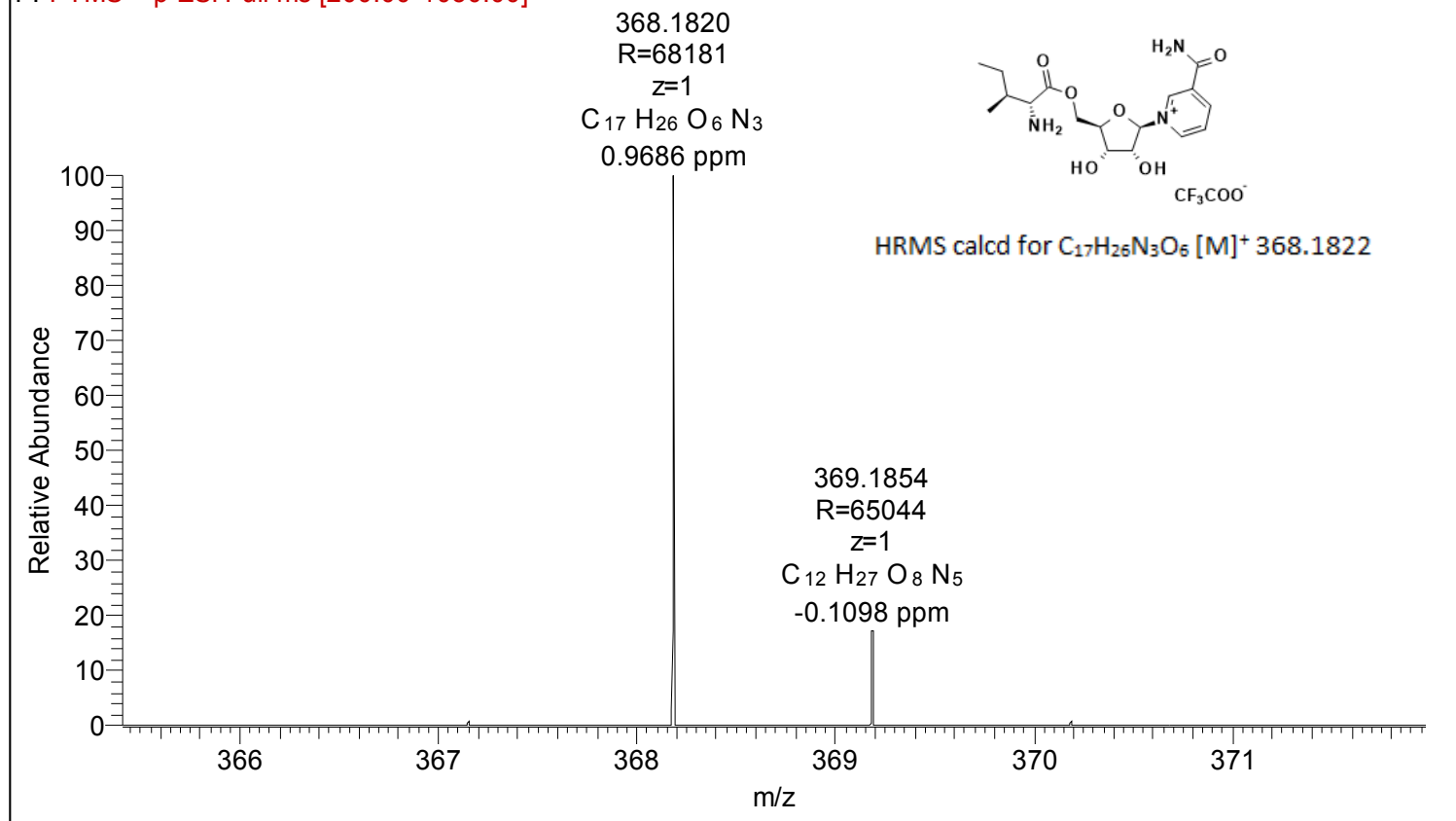


Compound 12f. 100 MHz ¹³C NMR spectrum in D₂O

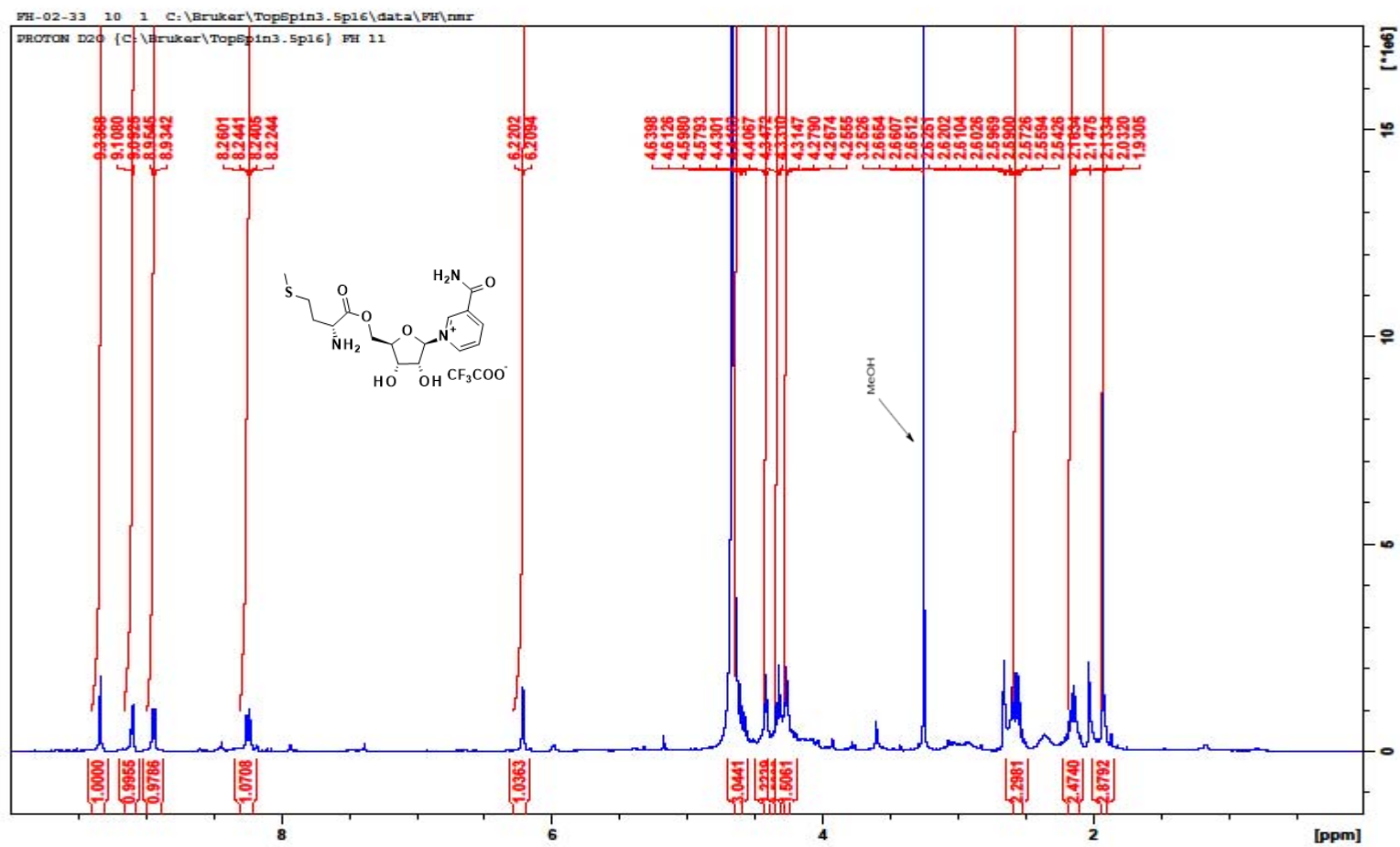


Compound 12f. 377 MHz ¹⁹F NMR spectrum in MeOD

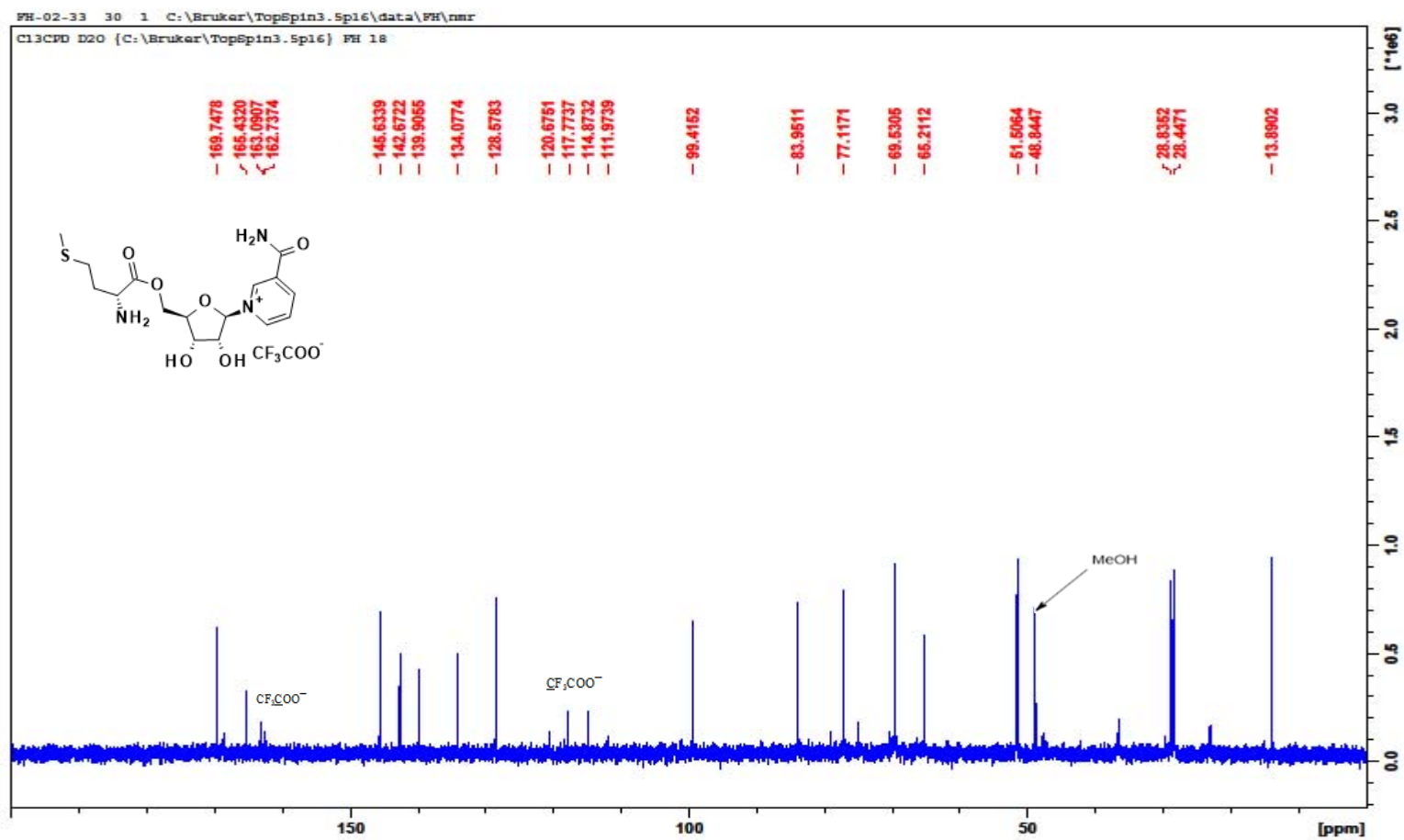
mm_101618_nr_isoleucine_01 #13-24 RT: 0.18-0.35 AV: 12 SB: 82 5.78-7.07 NL: 4.58E6
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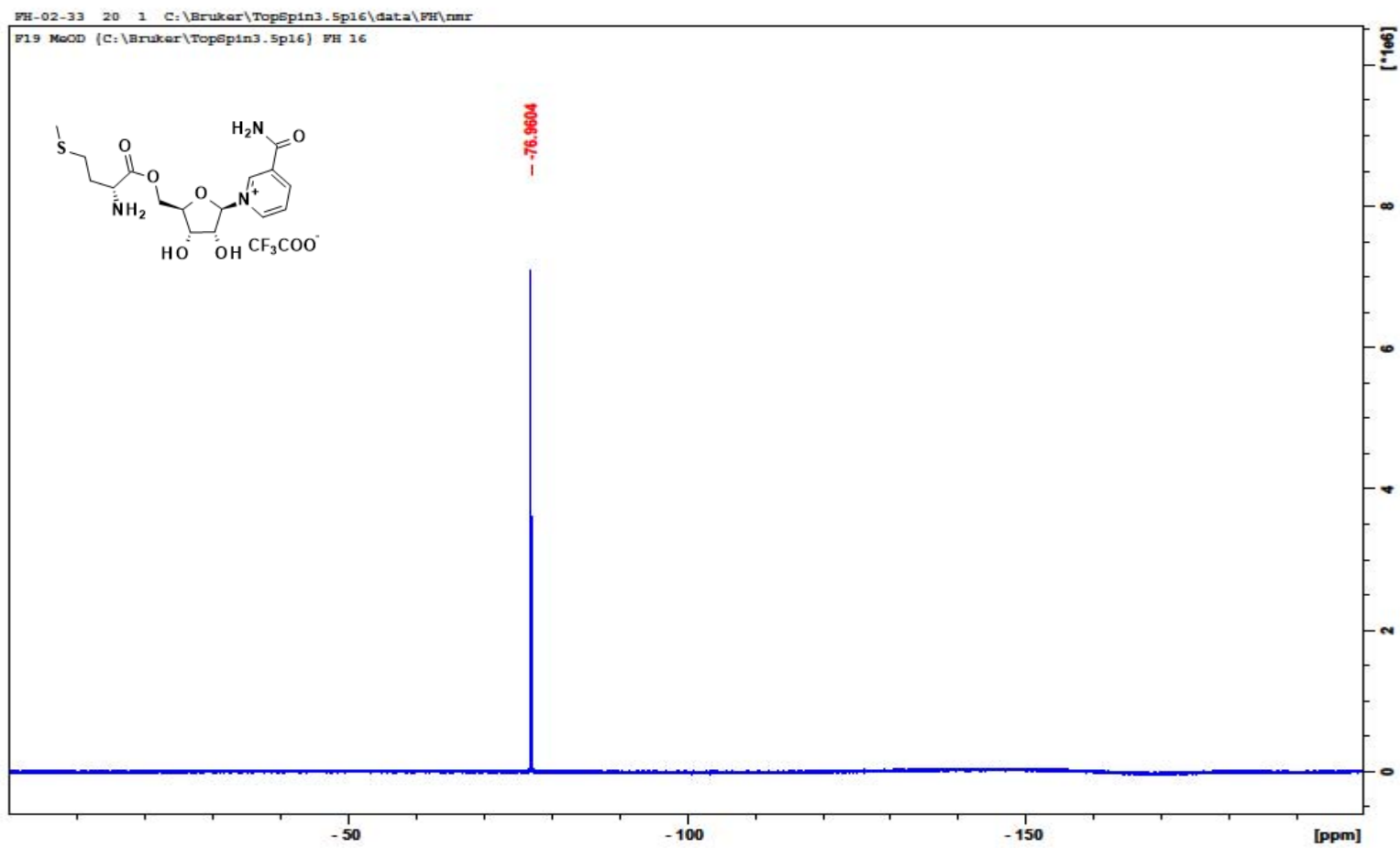
Compound 12f. HRMS spectra



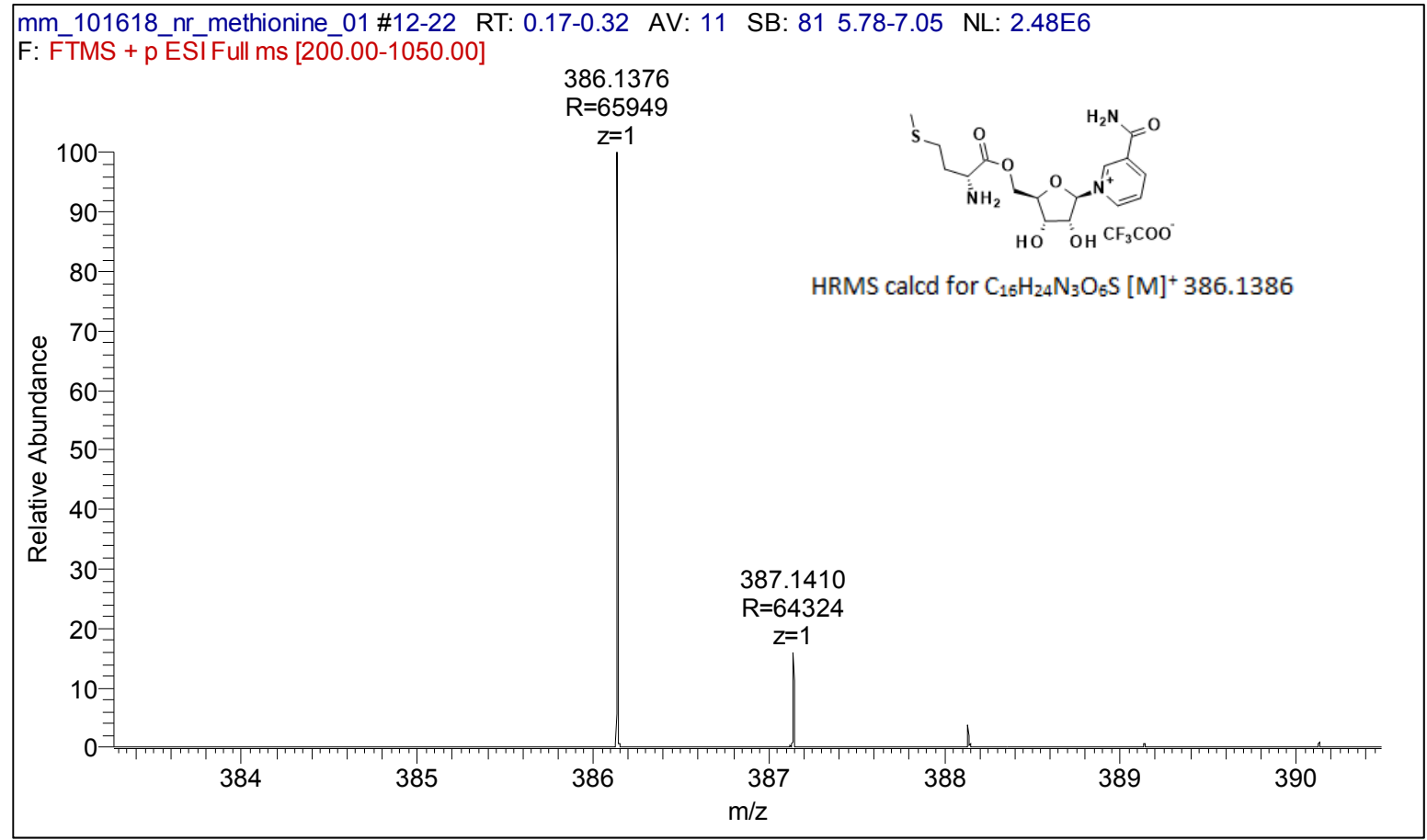
Compound 12g. 400 MHz ^1H NMR spectrum in D_2O



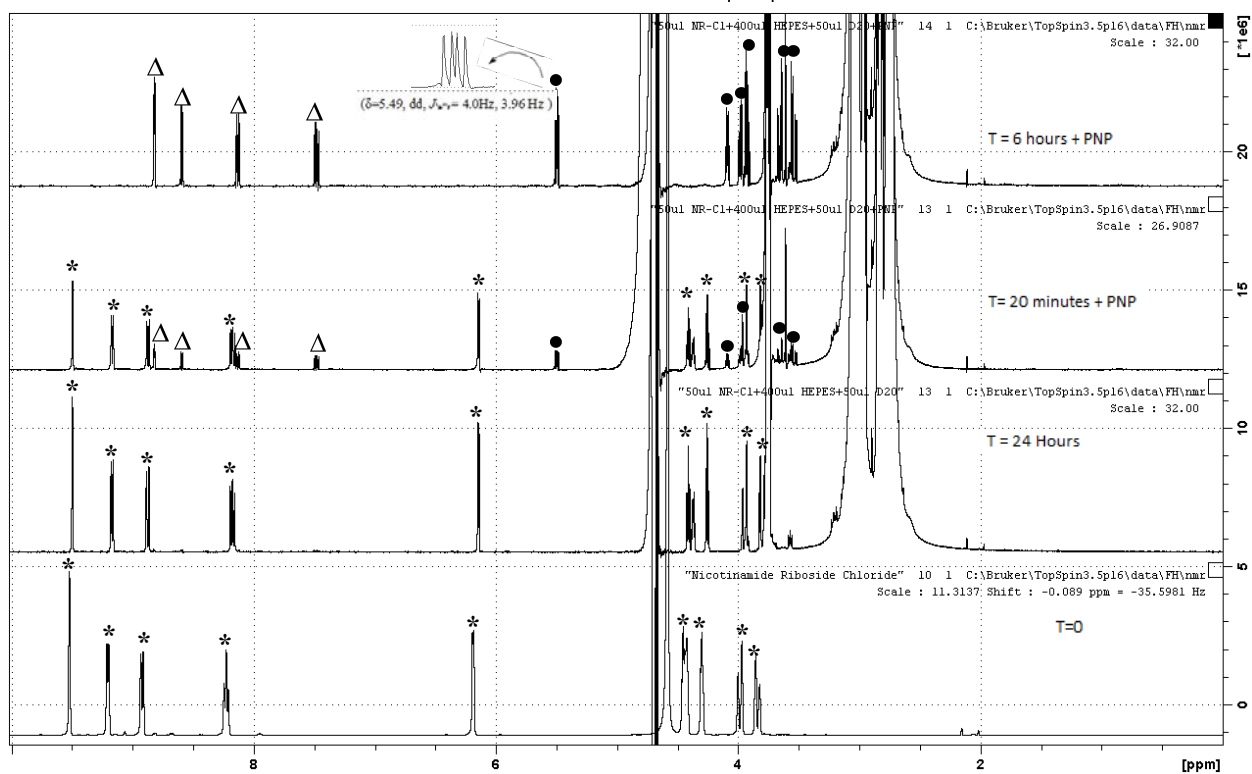
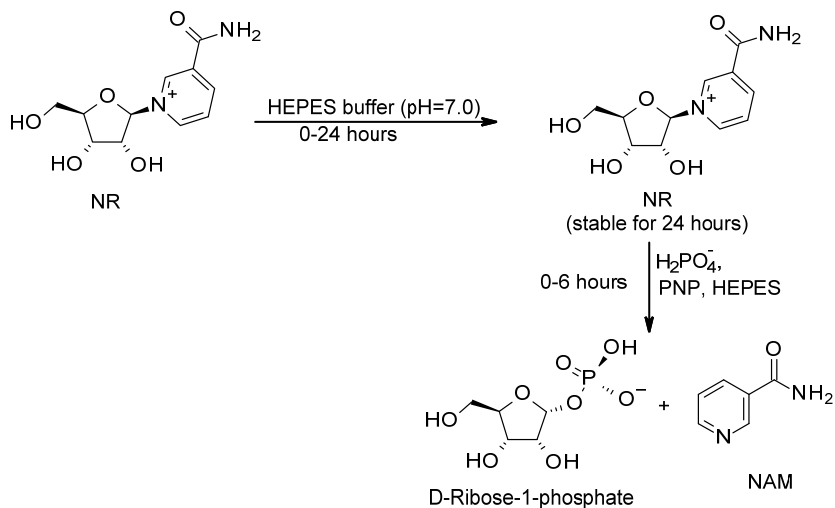
Compound 12g. 100 MHz ¹³C NMR spectrum in D₂O

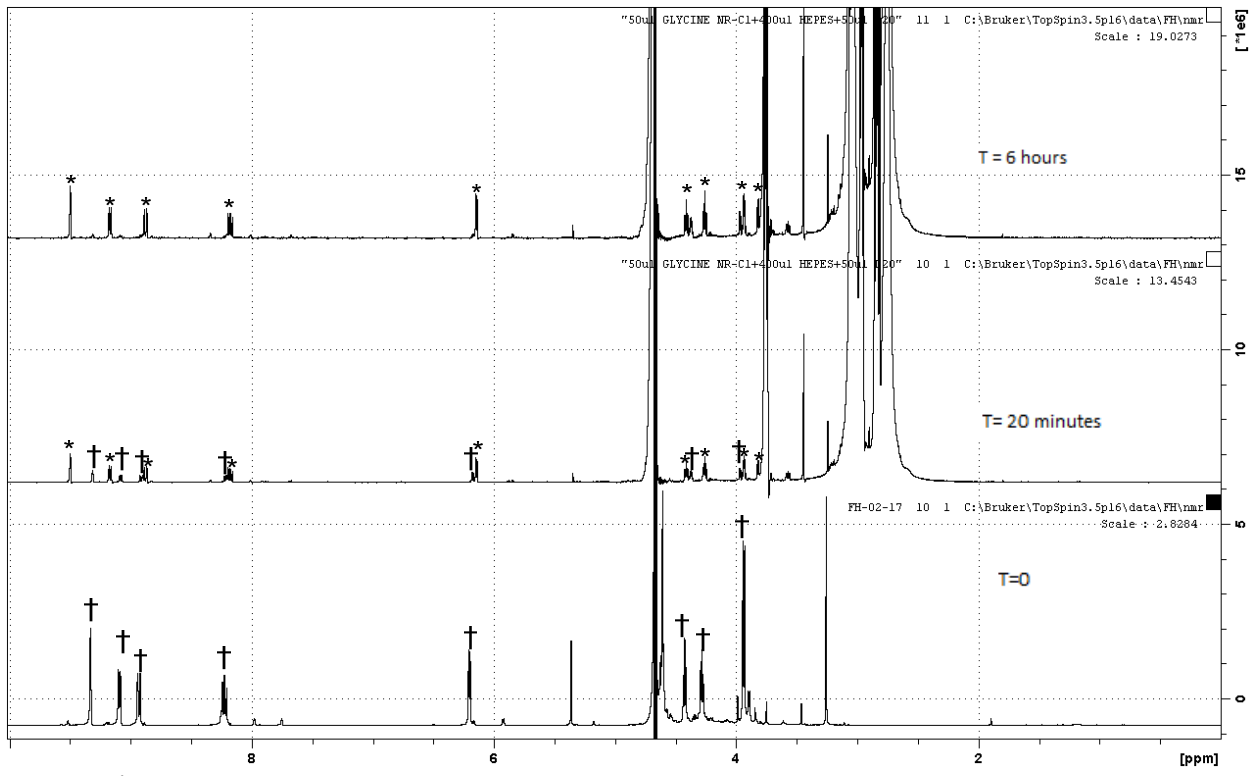
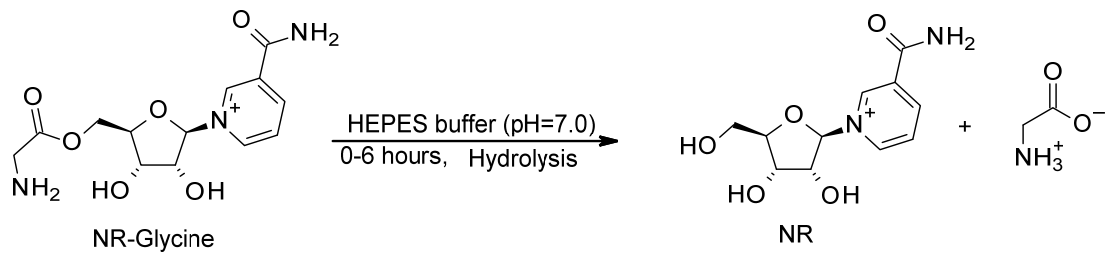


Compound 12g. 377 MHz ^{19}F NMR spectrum in MeOD

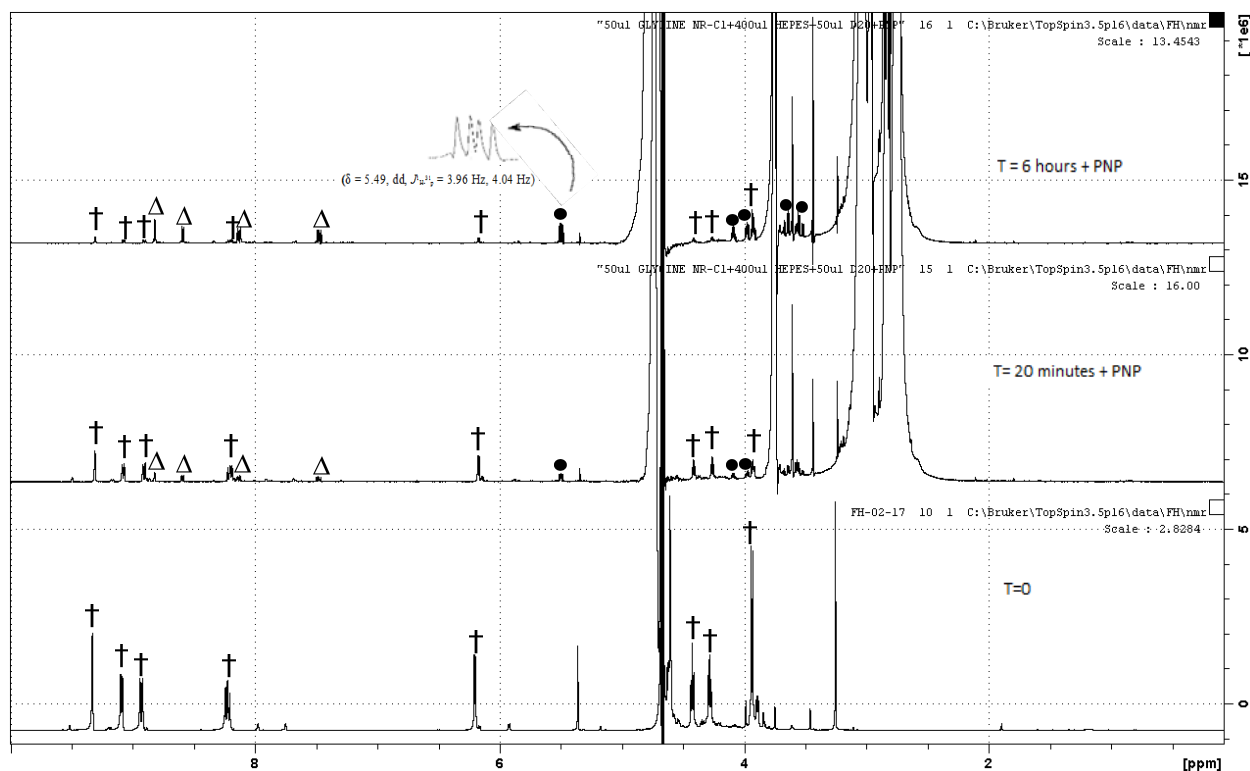
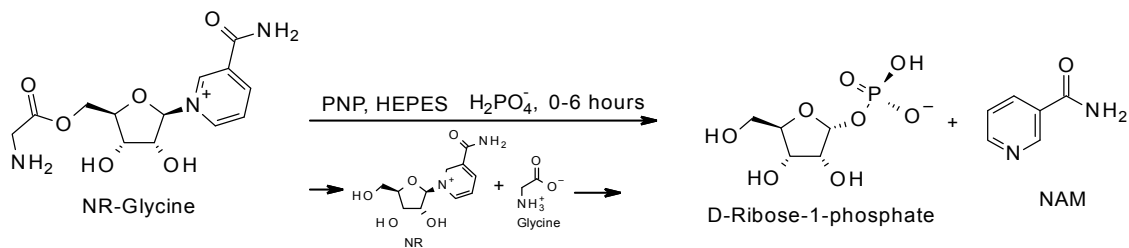


Compound 12g. HRMS spectra







† NR-glycine
 * Nicotinamide riboside



- † NR-glycine
- Δ Nicotinamide
- D-Ribose-1-phosphate

Detailed PNP enzyme activity assay. The phosphorolysis of nicotinamide riboside chloride (NR-Cl) and glycine nicotinamide riboside conjugate (Gly-NR) catalyzed by PNP (Purine Nucleoside Phosphorylase) was performed at room temperature and monitored by ^1H NMR at $t = 0$, 20 min and 6 h. All one dimensional spectra were obtained at 300 K on a Bruker Ascend™ 400 MHz ultrashielded spectrometer (Bruker Biospin) operating at 400.13 MHz for protons (9.39 Tesla). TopSpin 3.2 (Bruker BioSpin) was used for all NMR spectral acquisition ($n_s=128$) and pre-processing, and the automation of sample submission was performed using ICON-NMR (Bruker BioSpin). All samples were automatically shimmed, and the spectra acquisition time was 10 minutes 08 seconds ($n_s=128$). Each NMR tube (7inch x 5mm) contained 450 μl HEPES buffer (100.0 mM, pH 7.0) which included 100 mM KH_2PO_4 , 5 μl PNP (1mg dissolved in 50 μl HEPES buffer), 50.0 μl NR-Cl and NR glycine conjugate (50.0 mM in 1 mL D_2O). For each independent experiment used freshly prepared solutions of NR-Cl and NR glycine conjugate in HEPES buffer, containing 100 mM KH_2PO_4 (13.6 mg/mL) and 50 μl D_2O .

TABLE 1: exploratory conditions for the synthesis of NR amino acid conjugate 5 by ball-milling.

Entry			TMSOTf	Solvent	Reaction outcomes ^{a,b}
1	1 eqv		2 eqv	DCE (1 eqv)	trace
2		1 eqv	2 eqv	DCE (1eqv)	trace
3	1 eqv		0.5 eqv	DCM (1 eqv)	trace
4	1 eqv		1 eqv	CH ₃ CN (1 eqv)	trace
5	1 eqv		2 eqv	CH ₃ CN (3 eqv)	trace
6	1 eqv		3 eqv	CH ₃ CN (5 eqv)	trace

^a ball-milling (30Hz), 30 minutes; ^b crude ¹H-NMR indicated decomposition of the riboside and formation of trace quantities of the expected product, product formation was confirmed by MS.