Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

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

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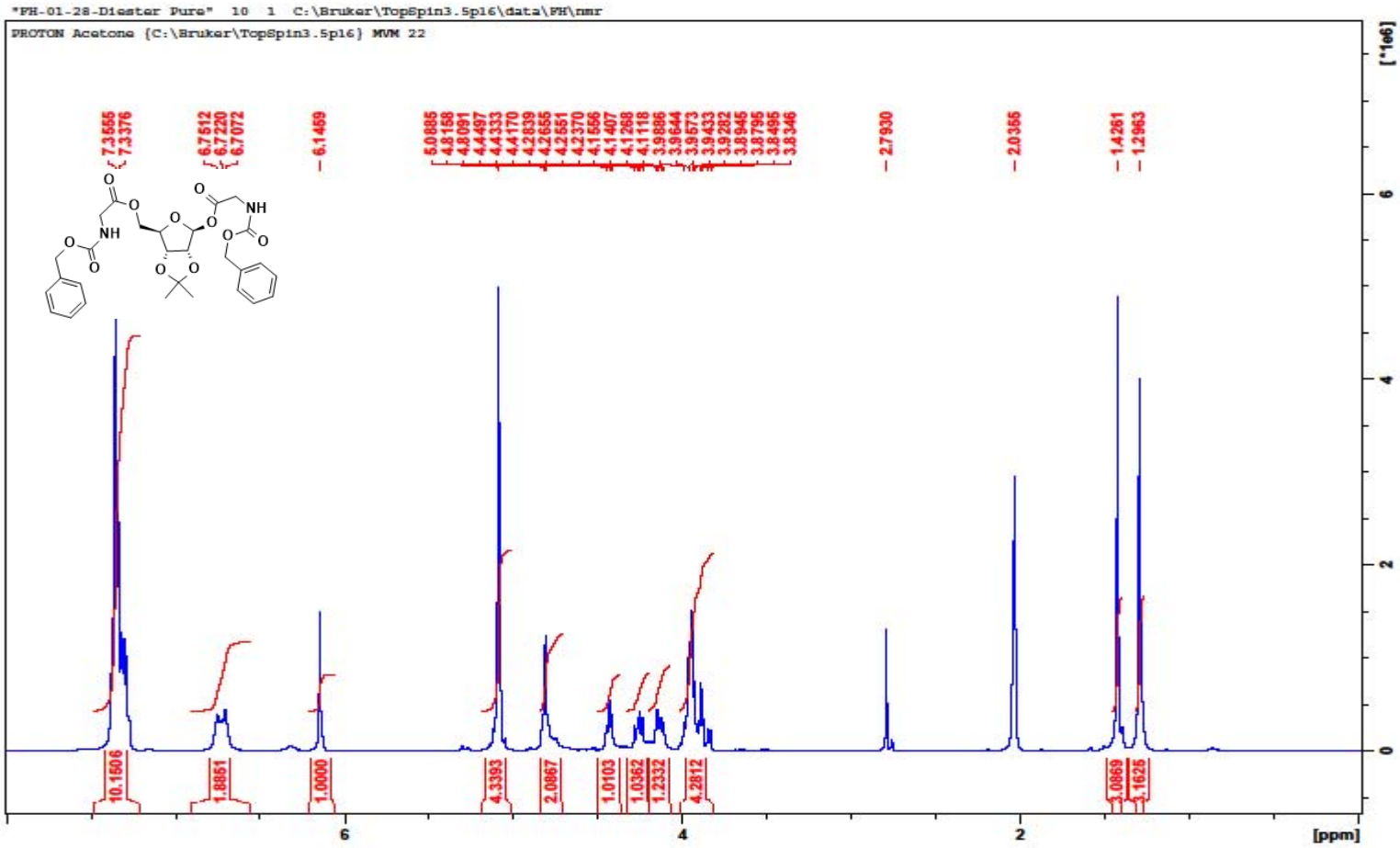
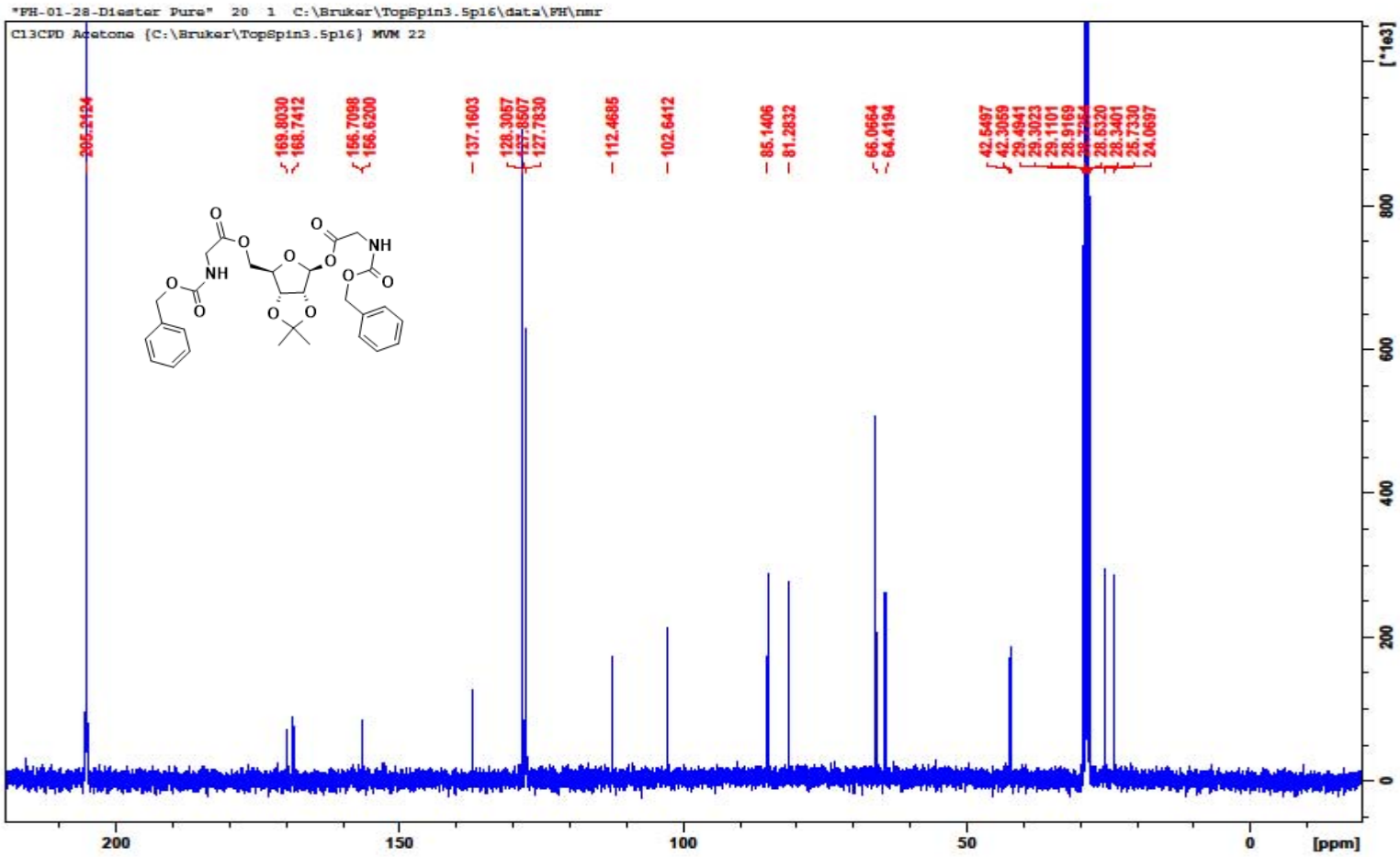
Mitchell Cancer Institute, University of South Alabama, 1660 Springhill Ave., Mobile, AL 36604, USA

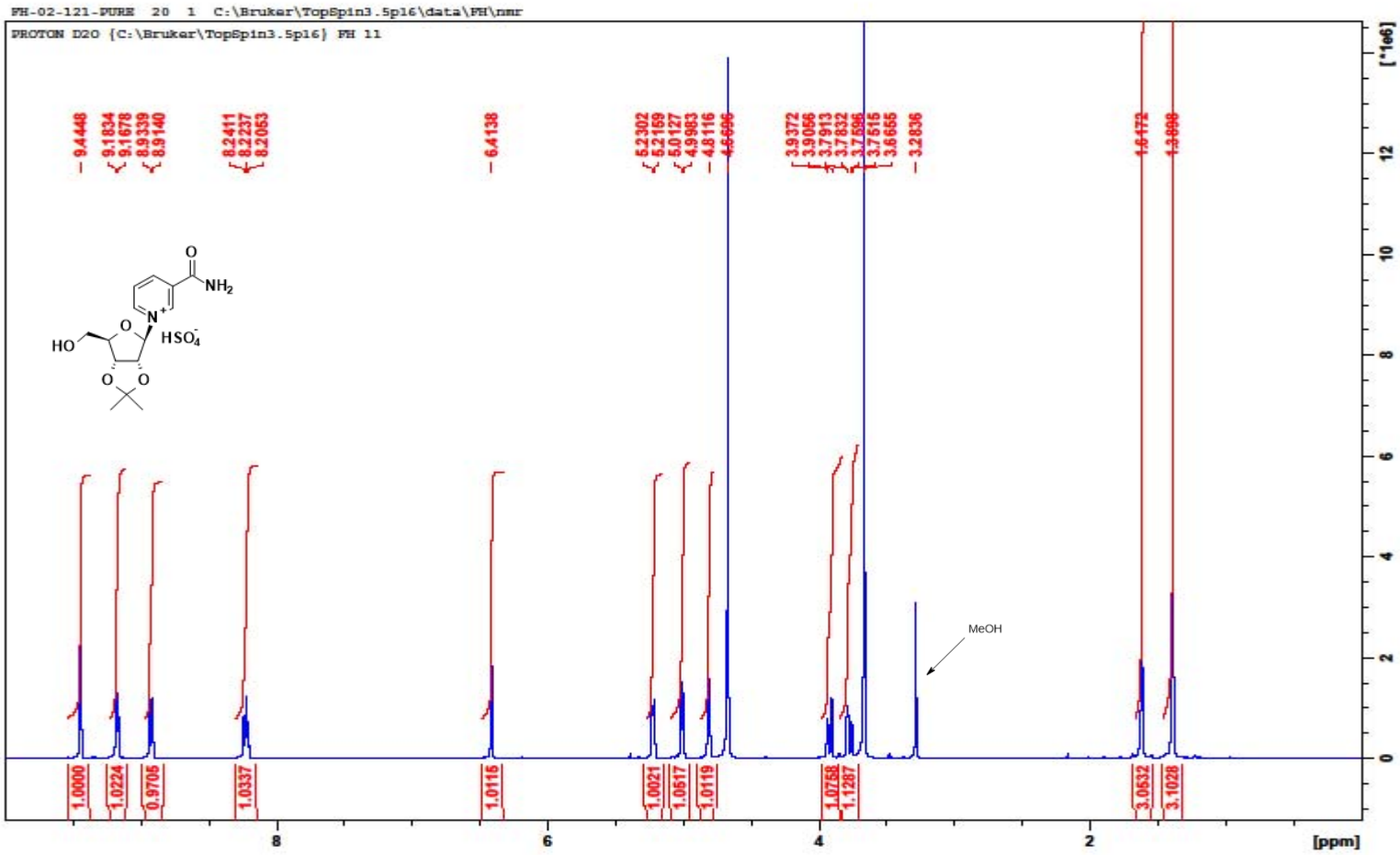
Supplementary



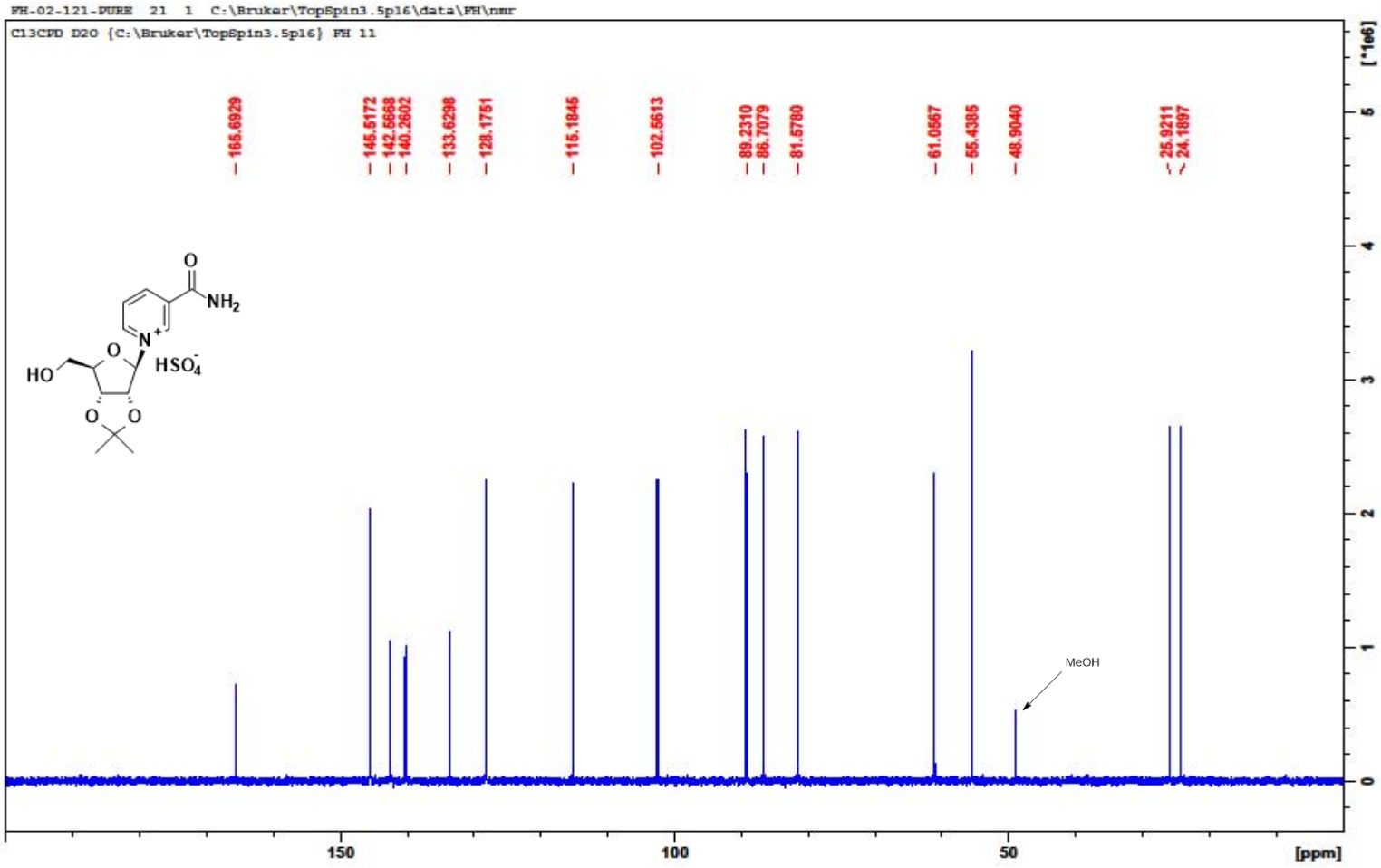
Compound 4. 400 MHz ^{1}H NMR spectrum in Acetone-d_6



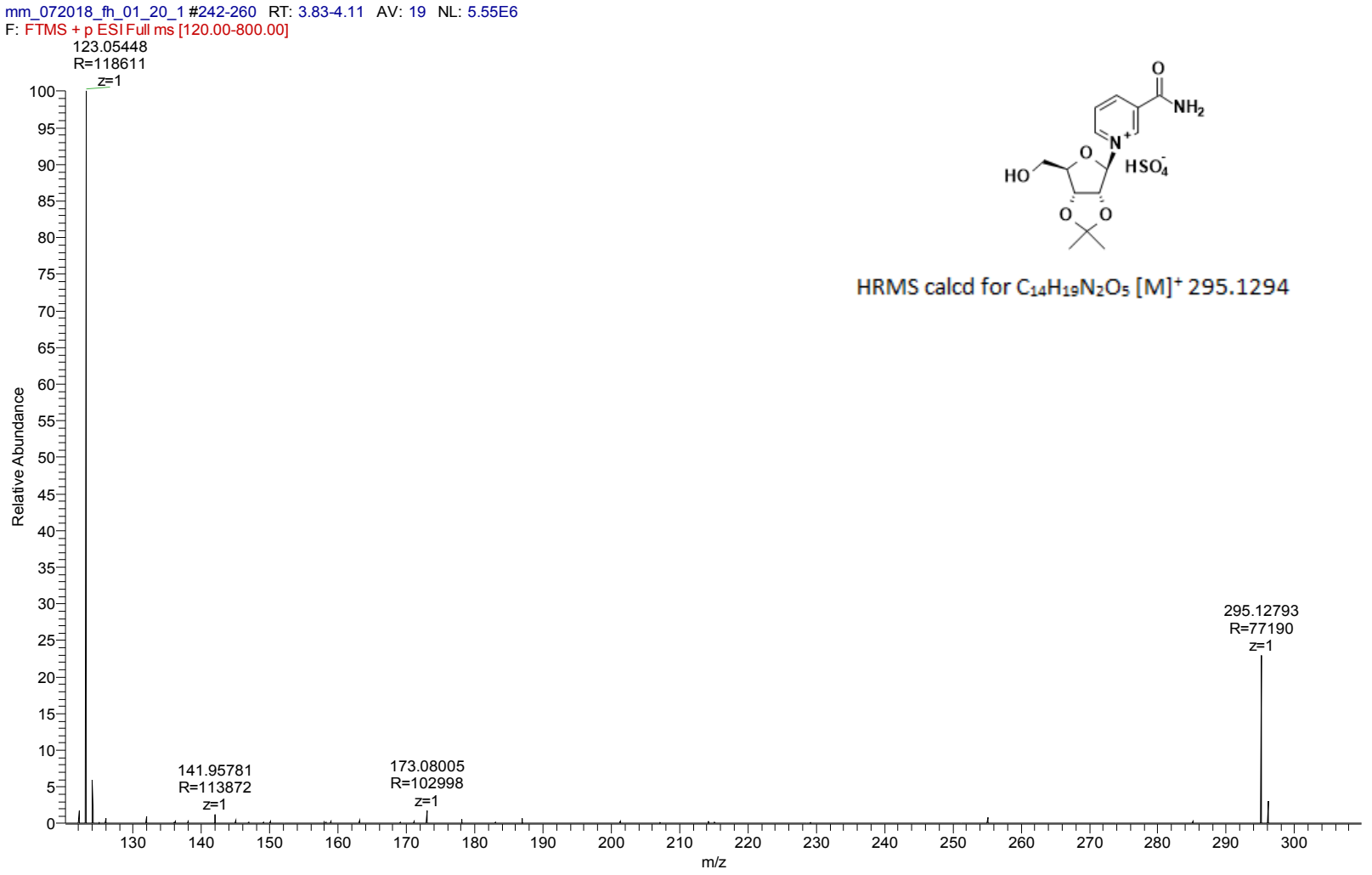
Compound 4. 100 MHz ^{13}C NMR spectrum in Acetone-*d*$_6$



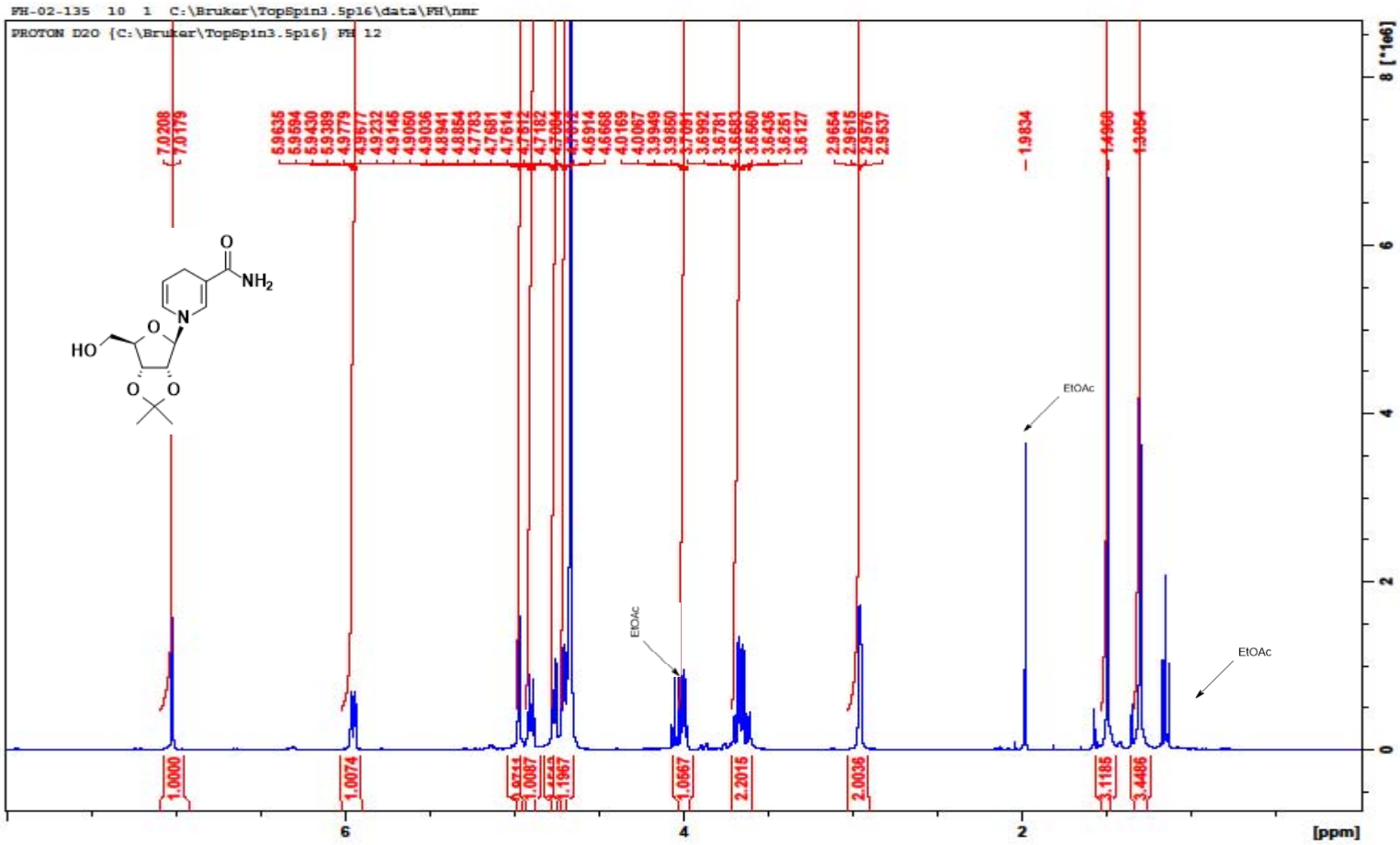
Compound 7. 400 MHz ^{1}H NMR spectrum in D_2O



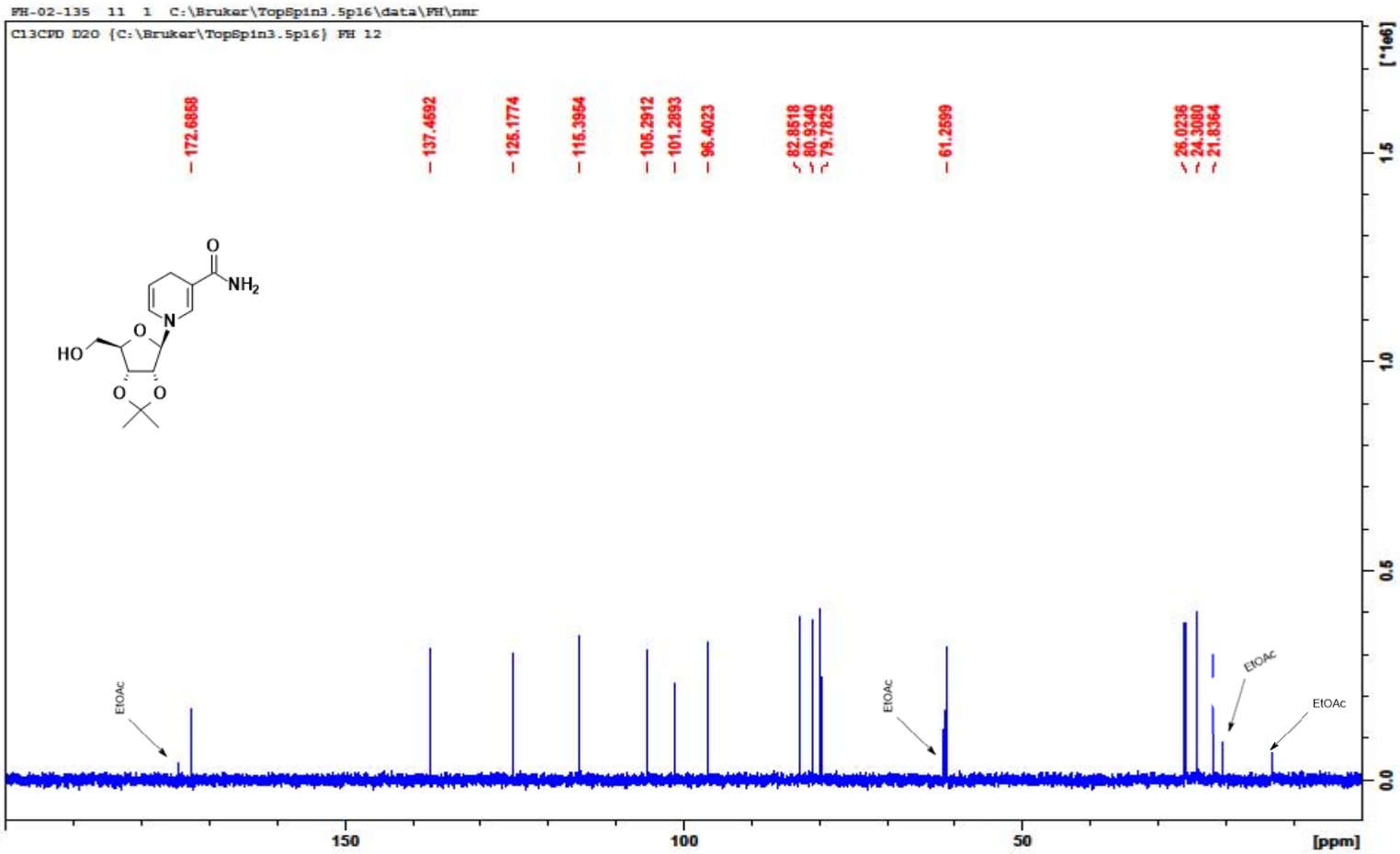
Compound 7. 100 MHz ^{13}C NMR spectrum in D_2O



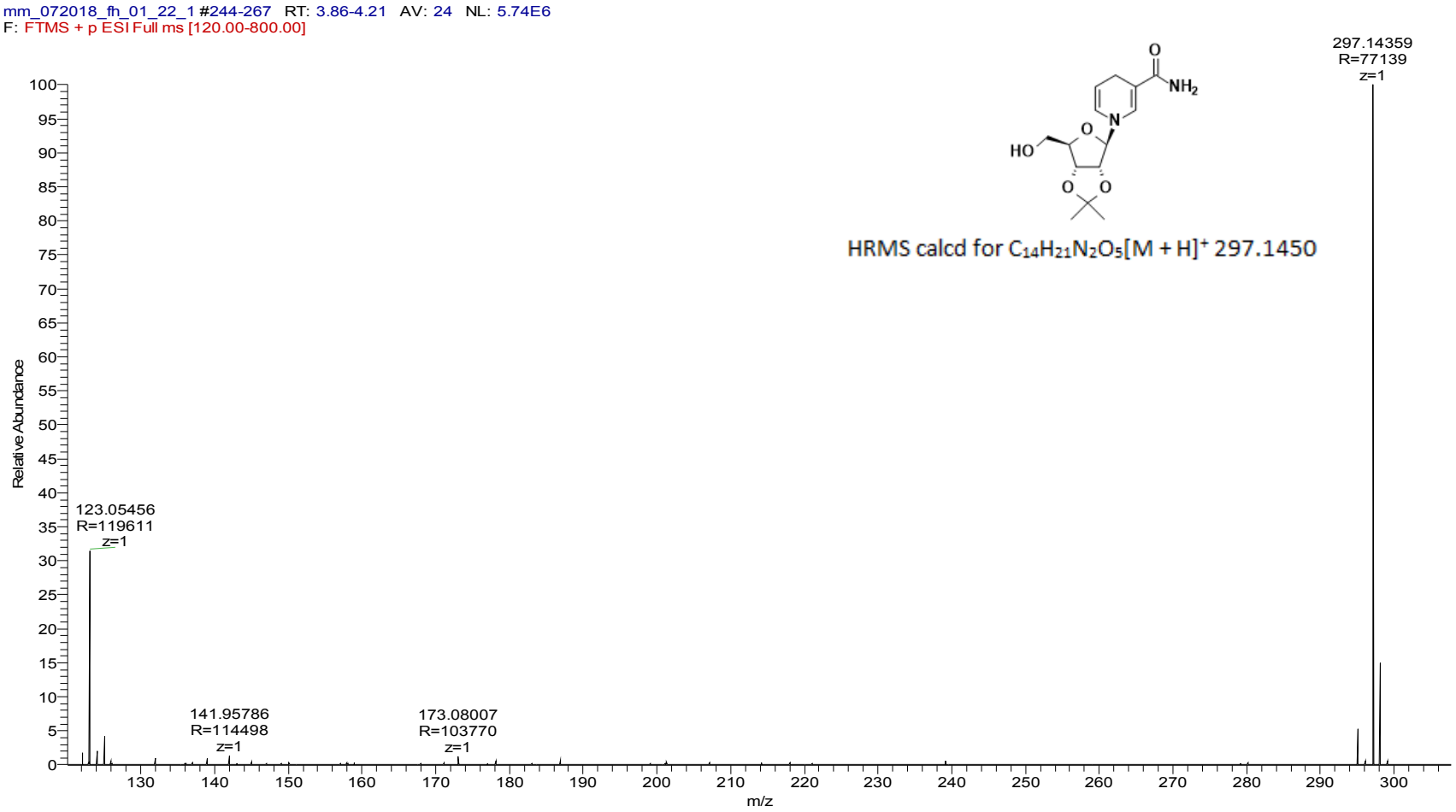
Compound 7. HRMS spectra



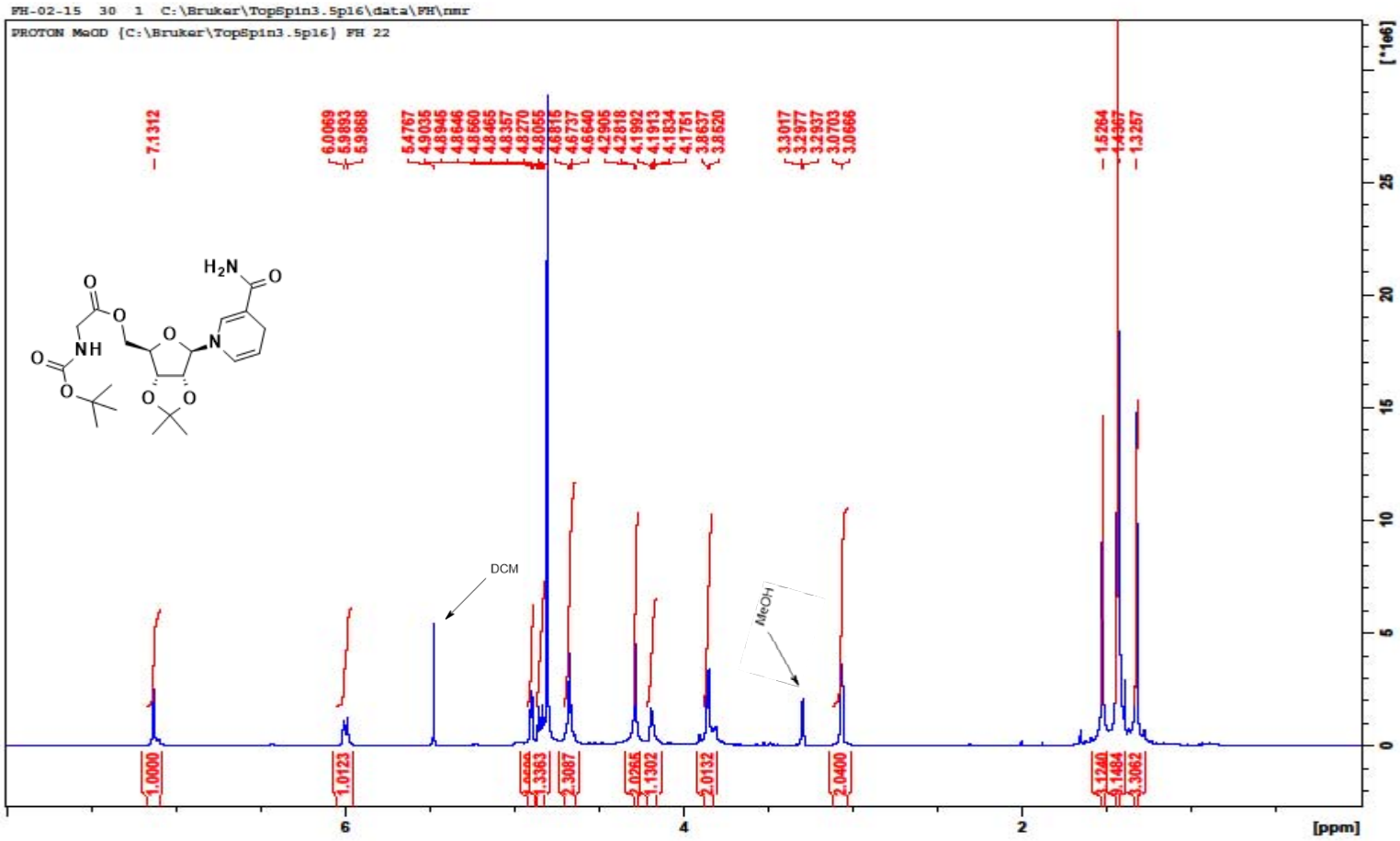
Compound 8. 400 MHz ^{1}H NMR spectrum in D_2O



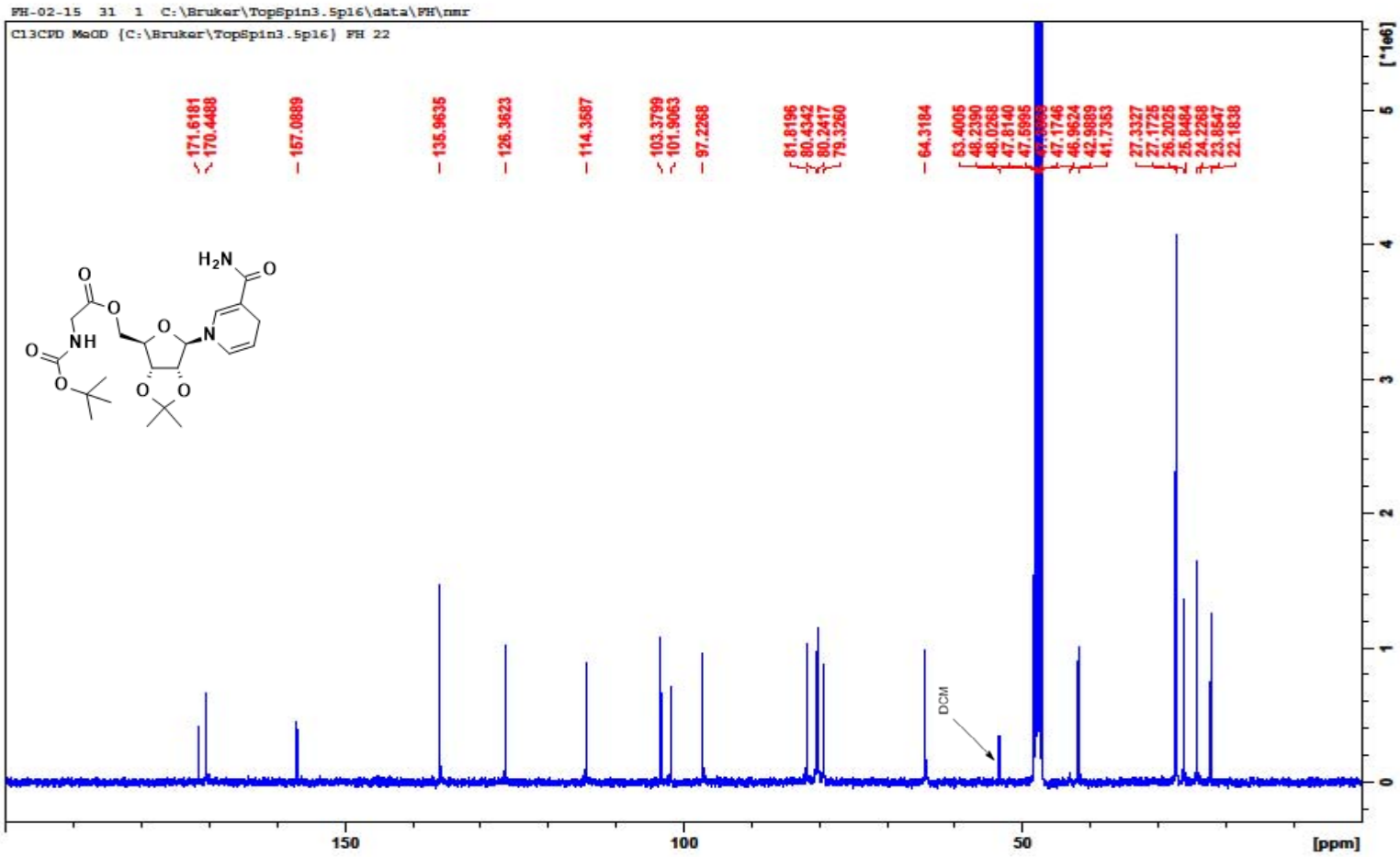
Compound 8. 100 MHz ^{13}C NMR spectrum in D_2O



Compound 8. HRMS spectra



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



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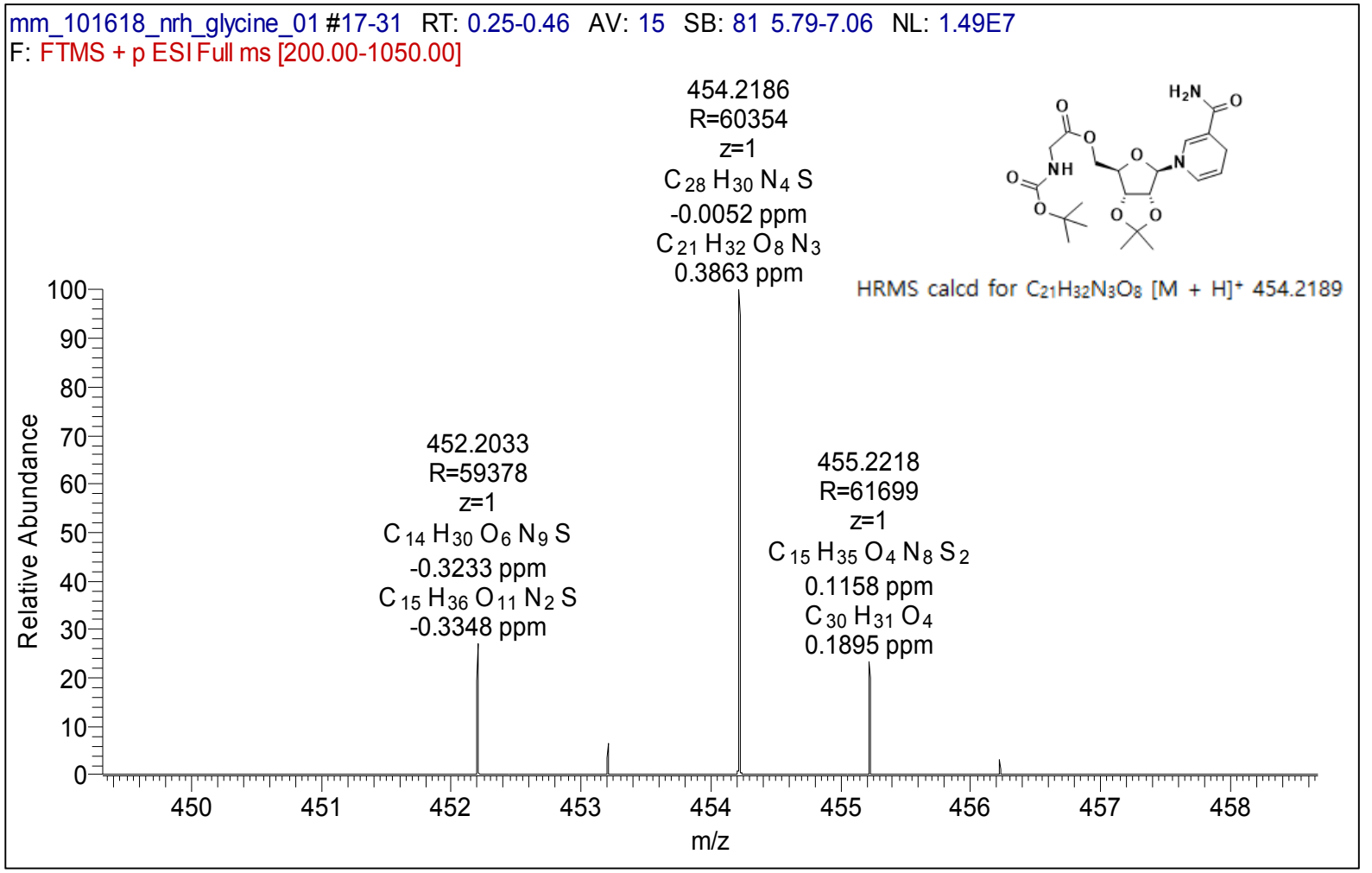
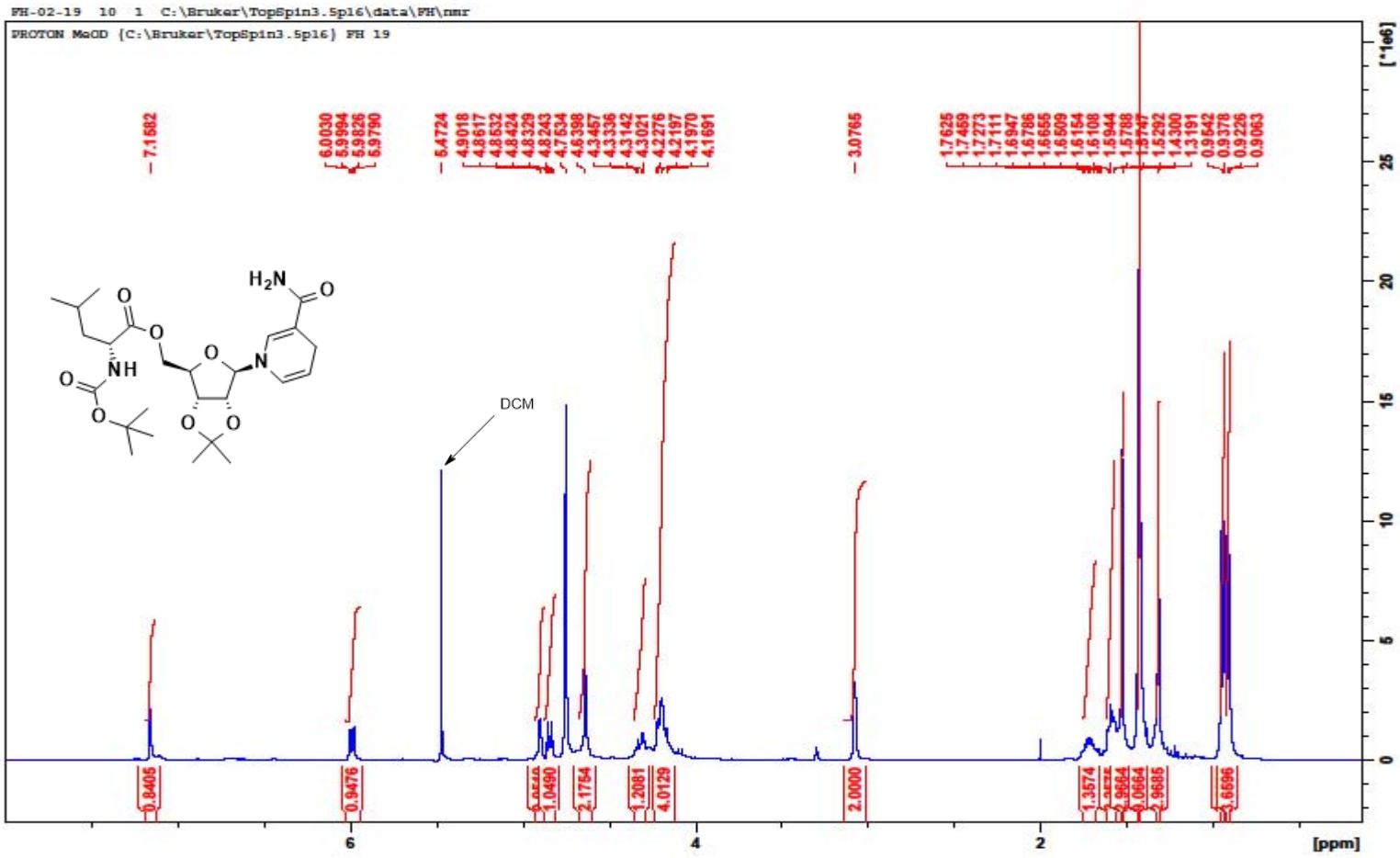


Compound 10a. HRMS spectra



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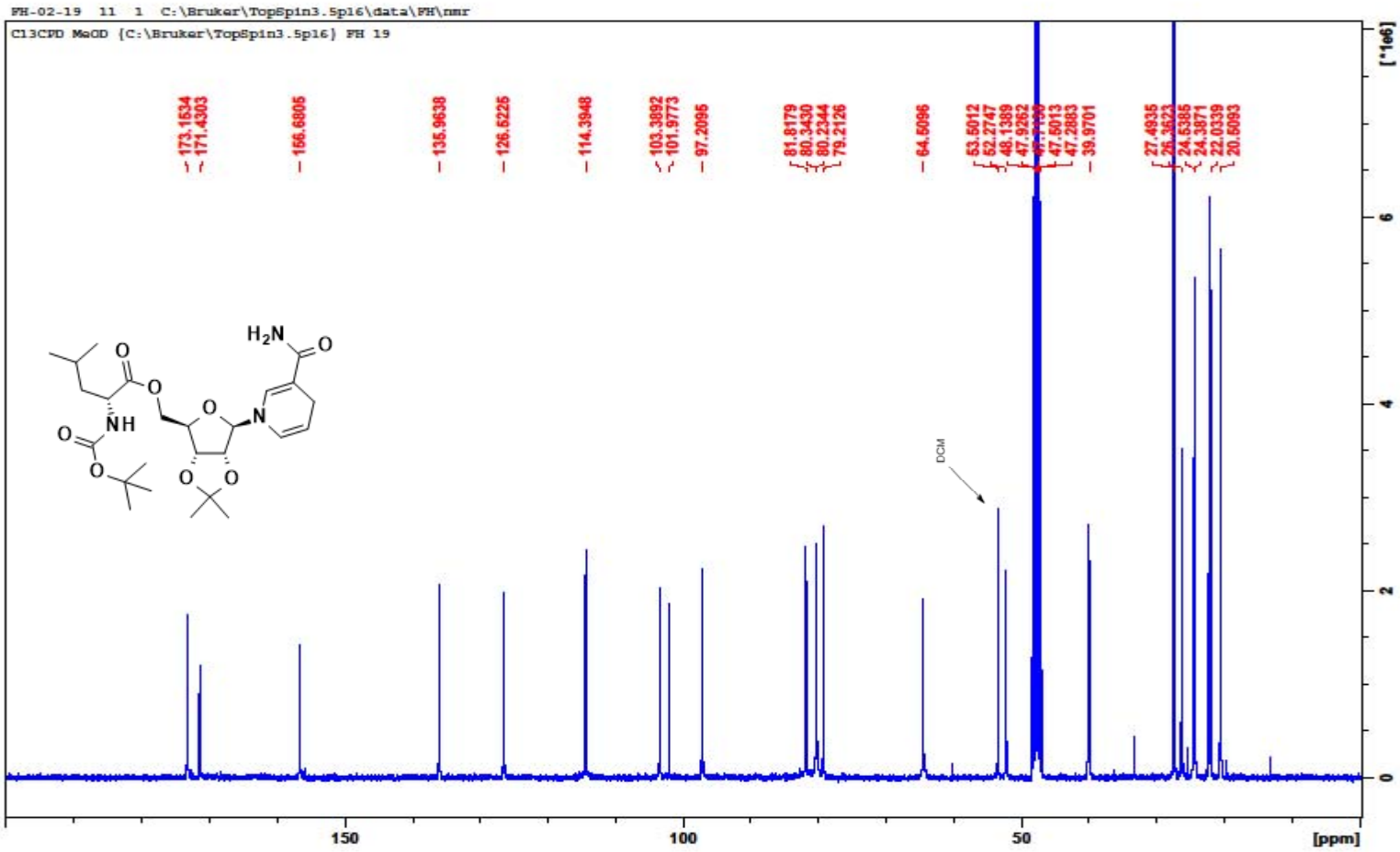
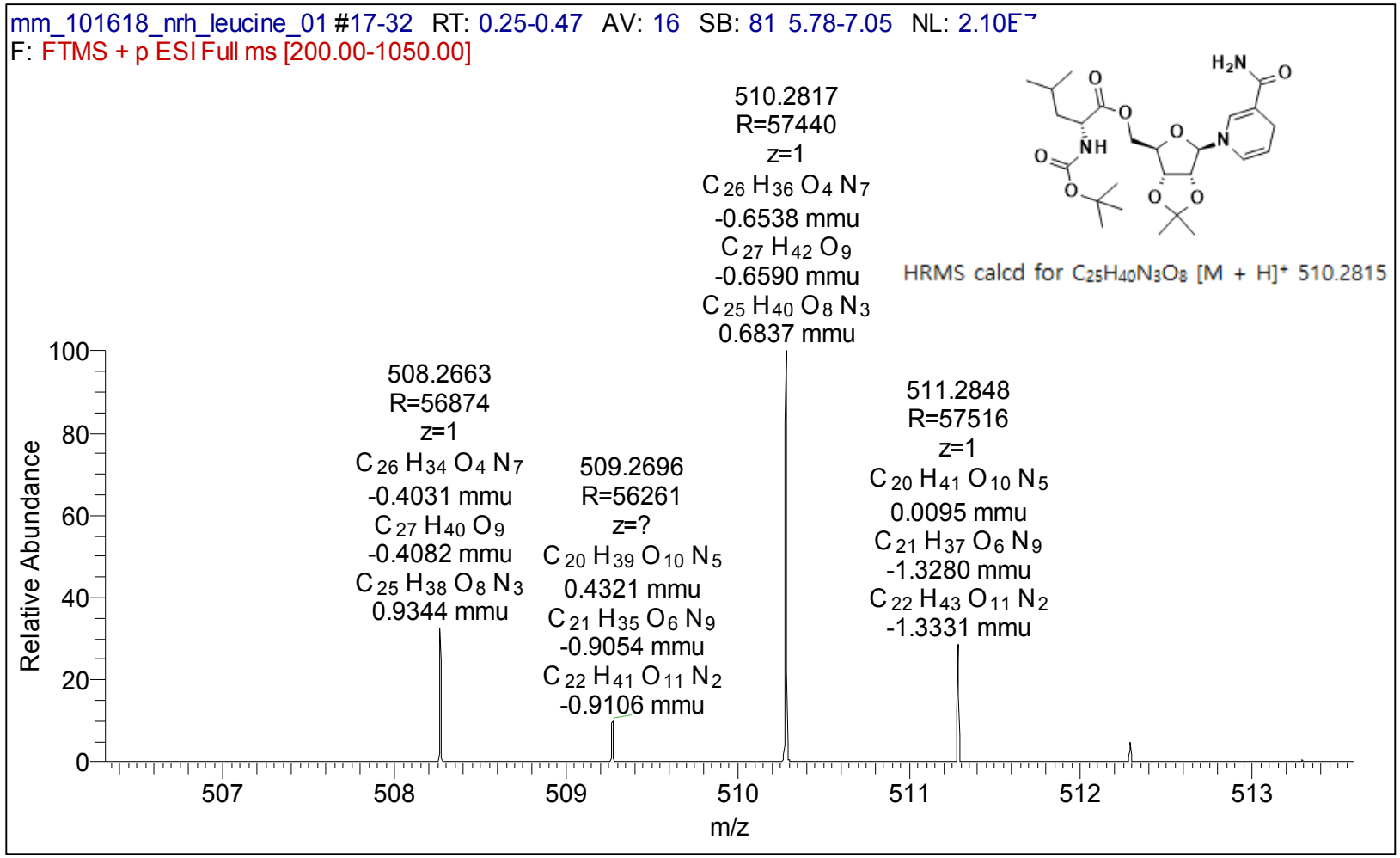


Compound 10b. 100 MHz ^{13}C NMR spectrum in MeOD



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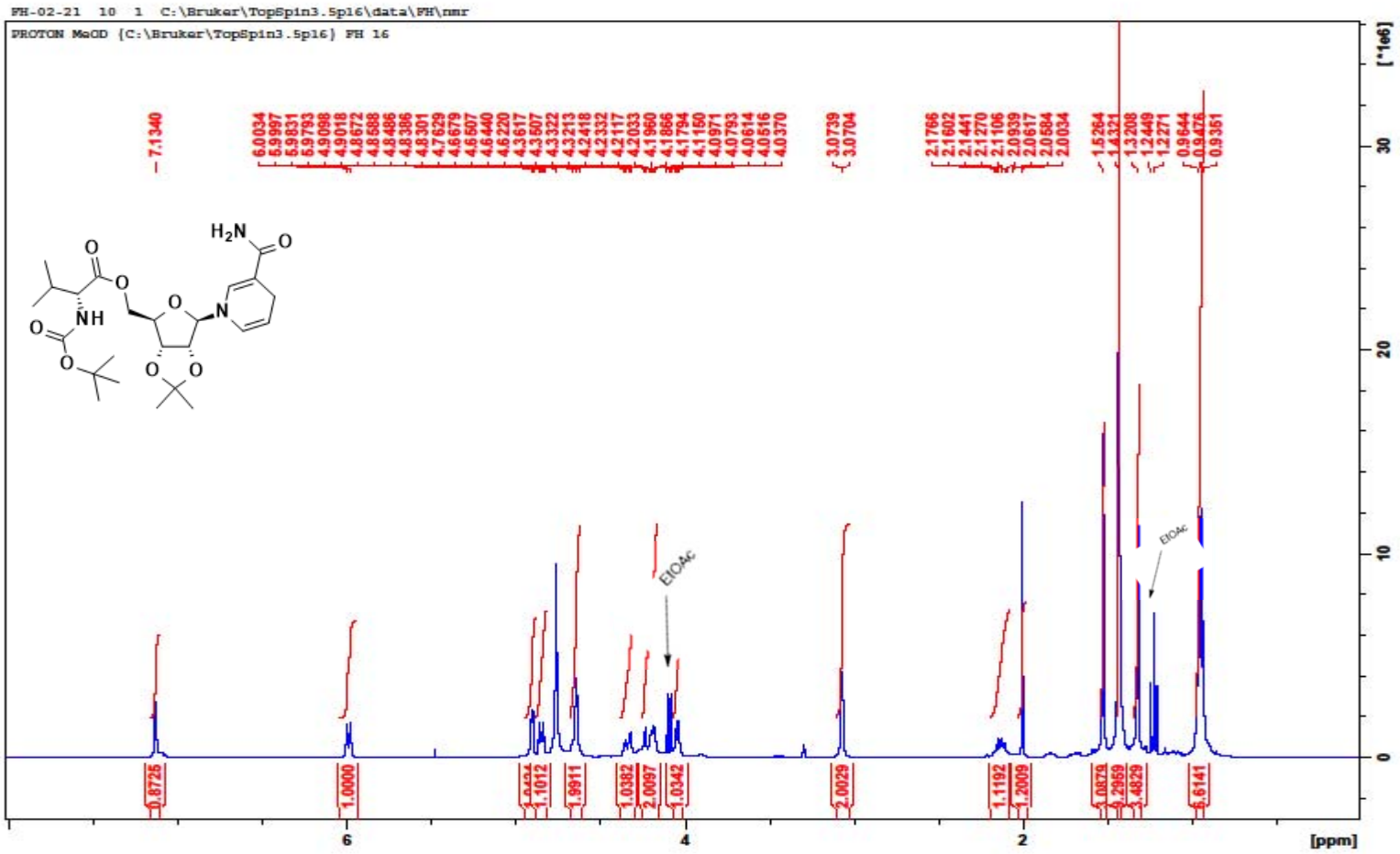

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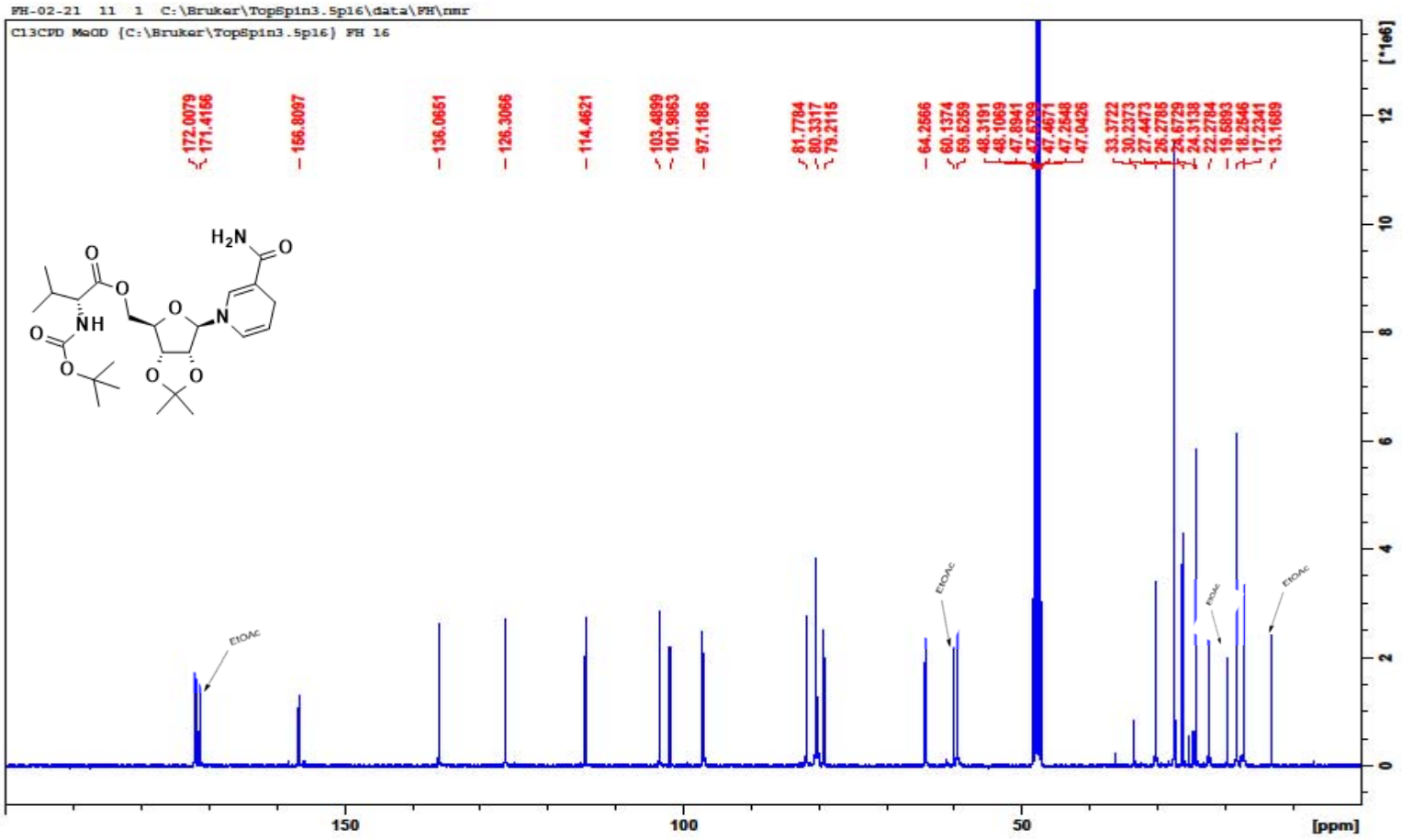

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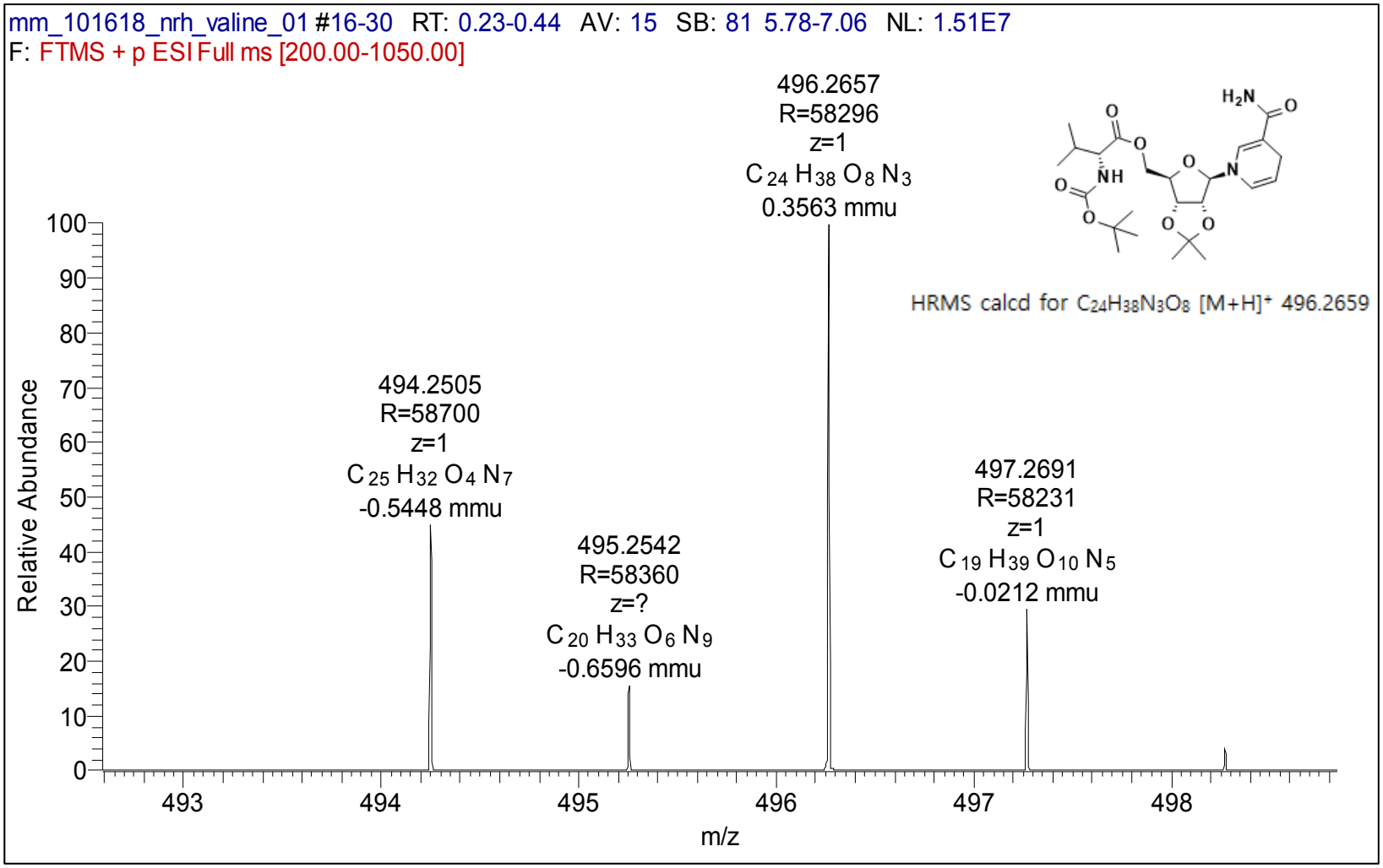

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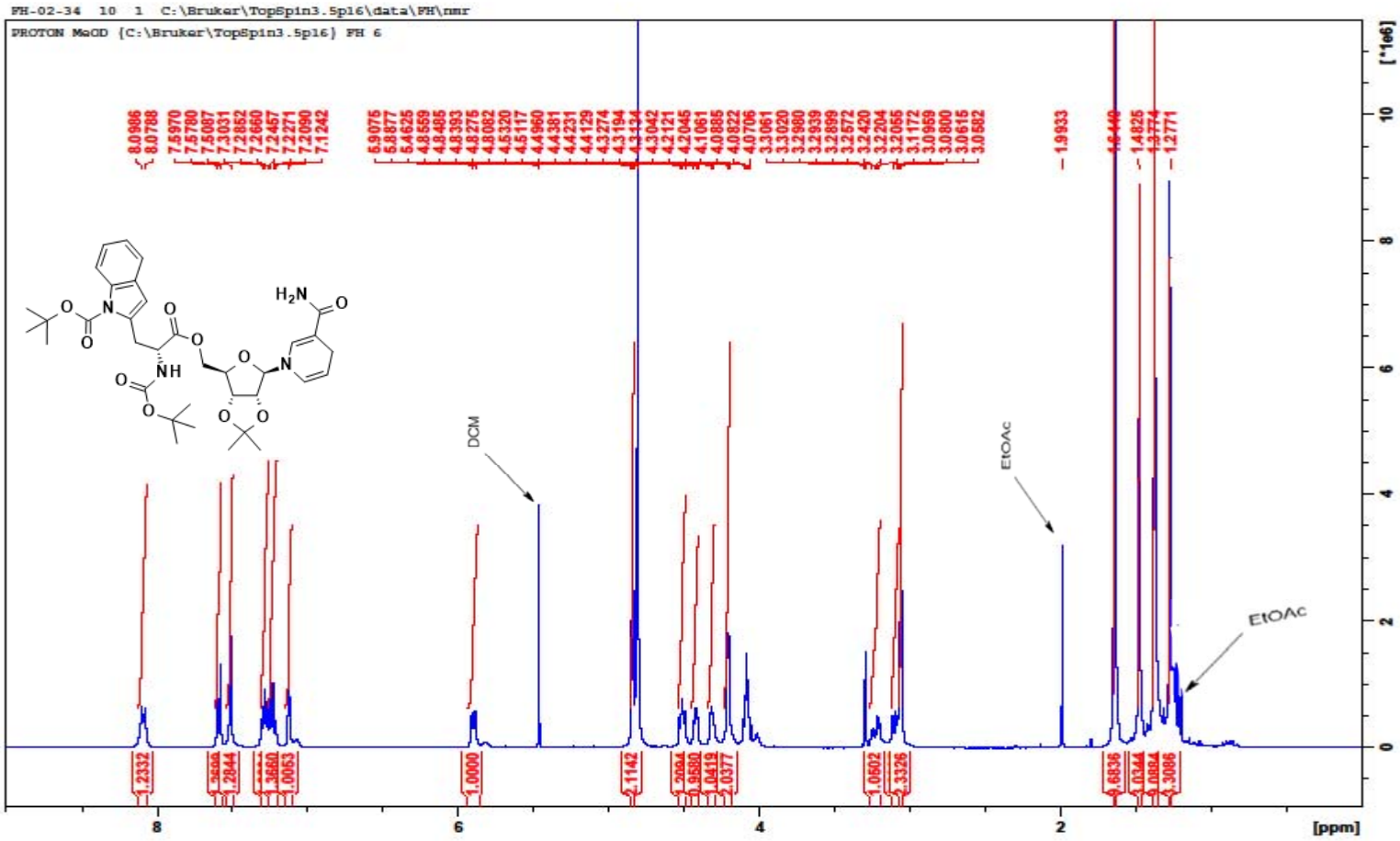

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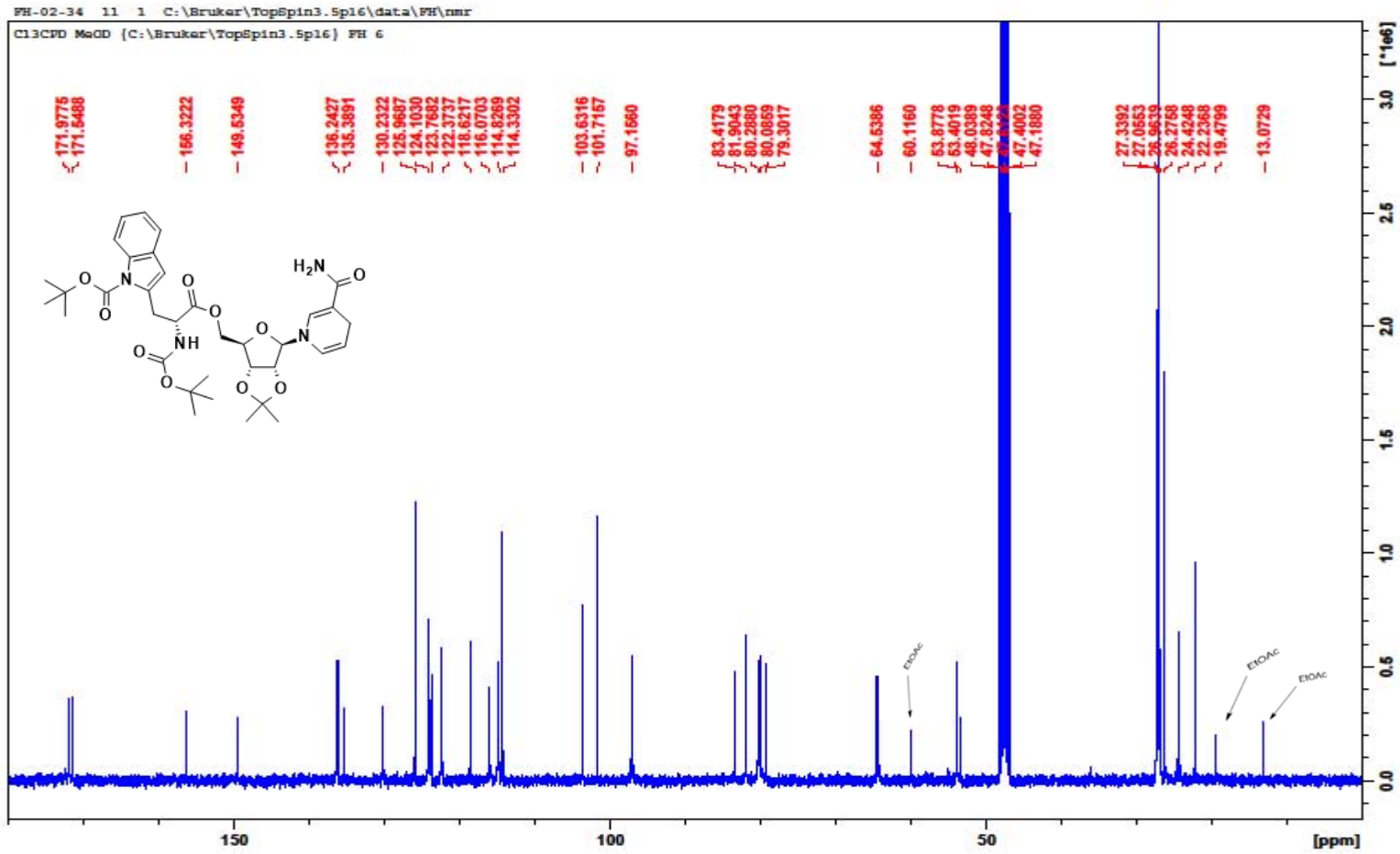

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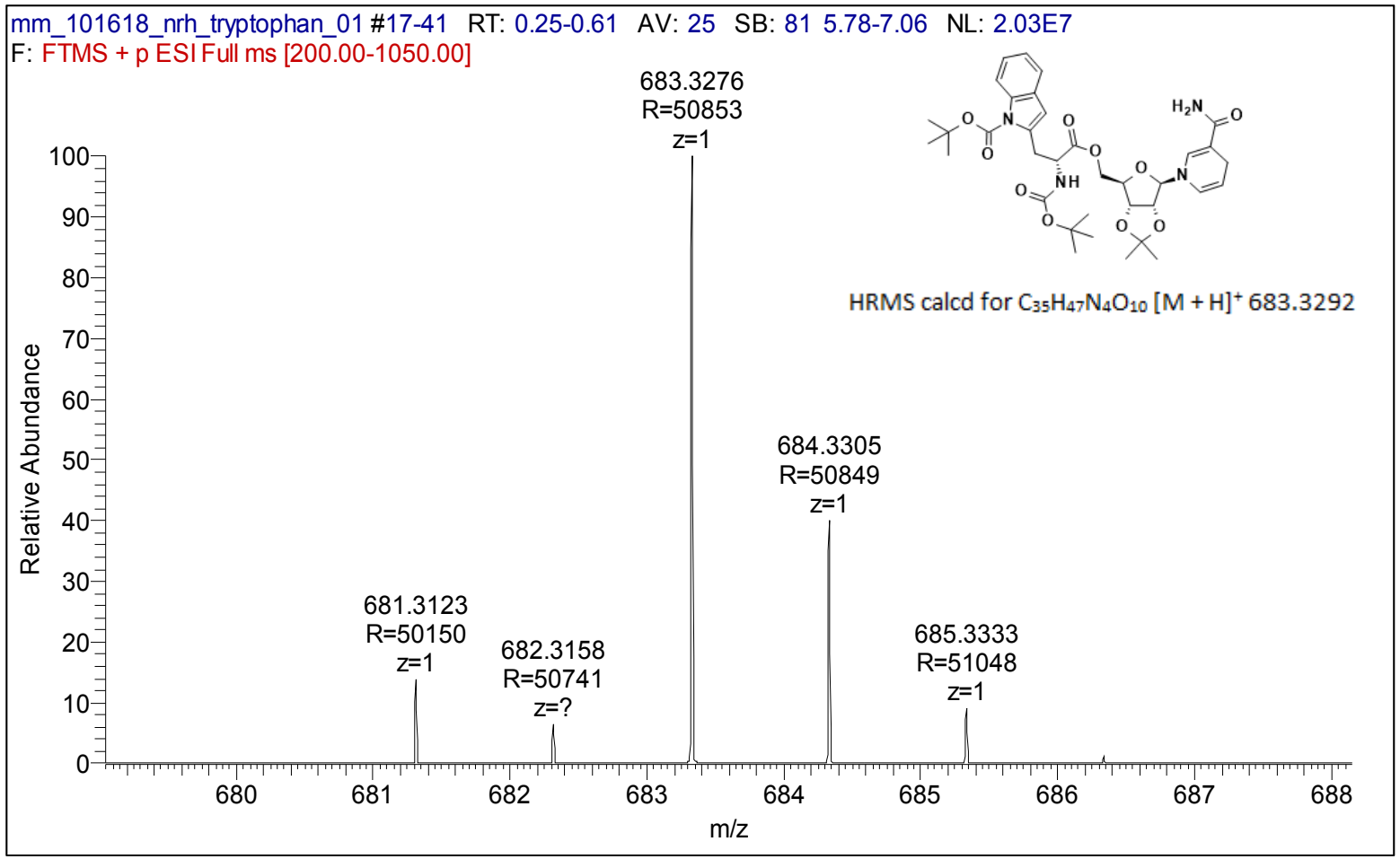

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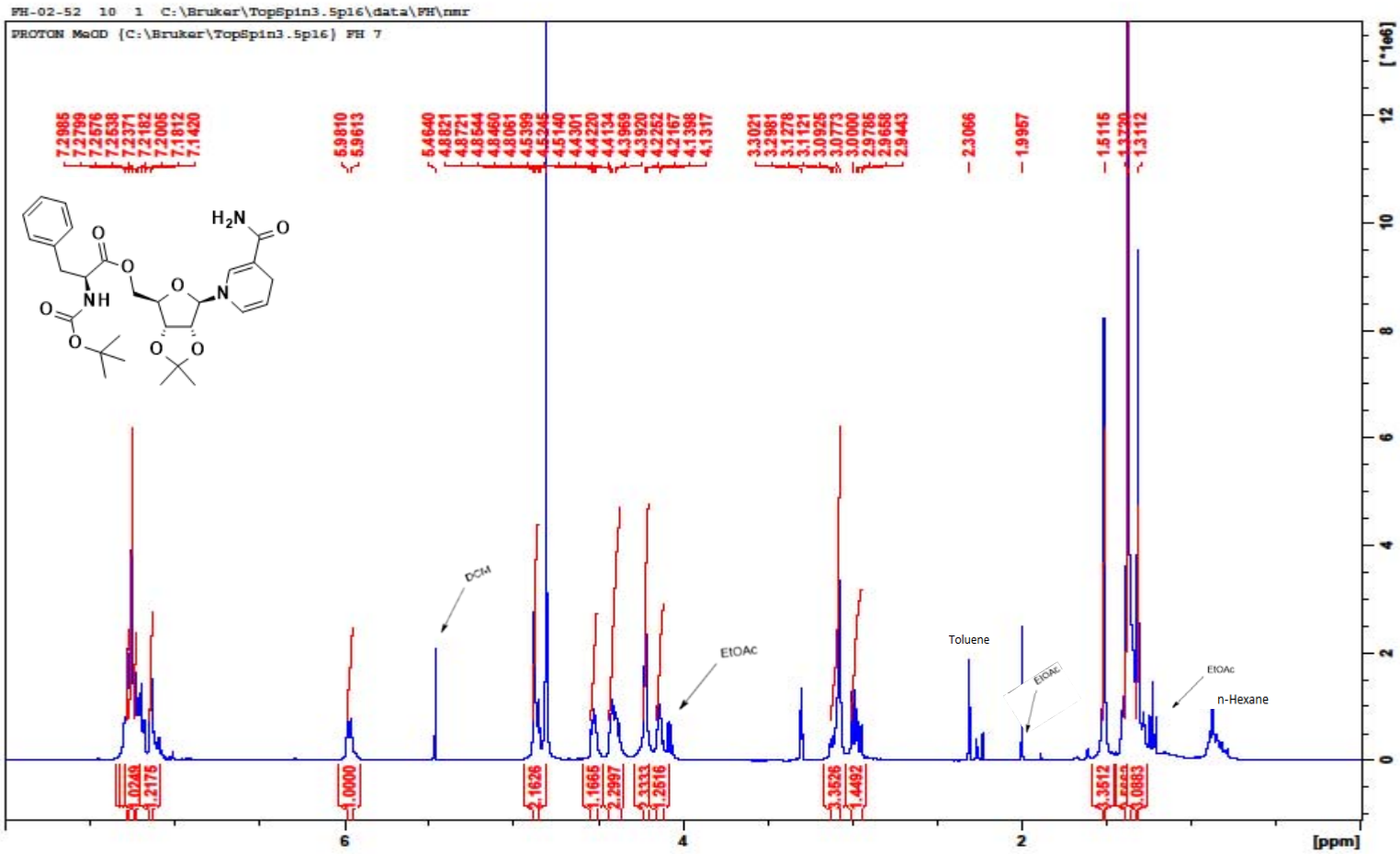

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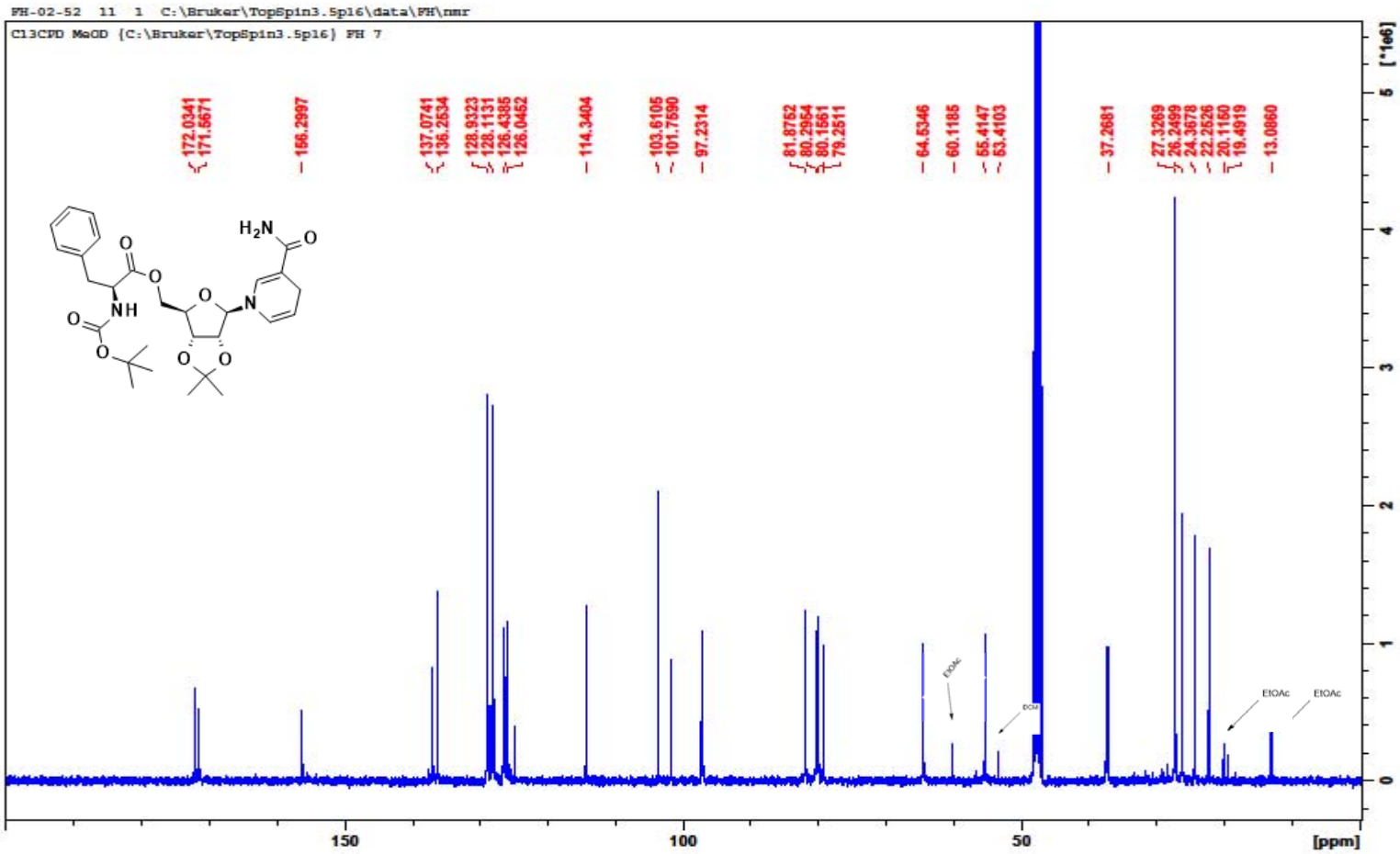

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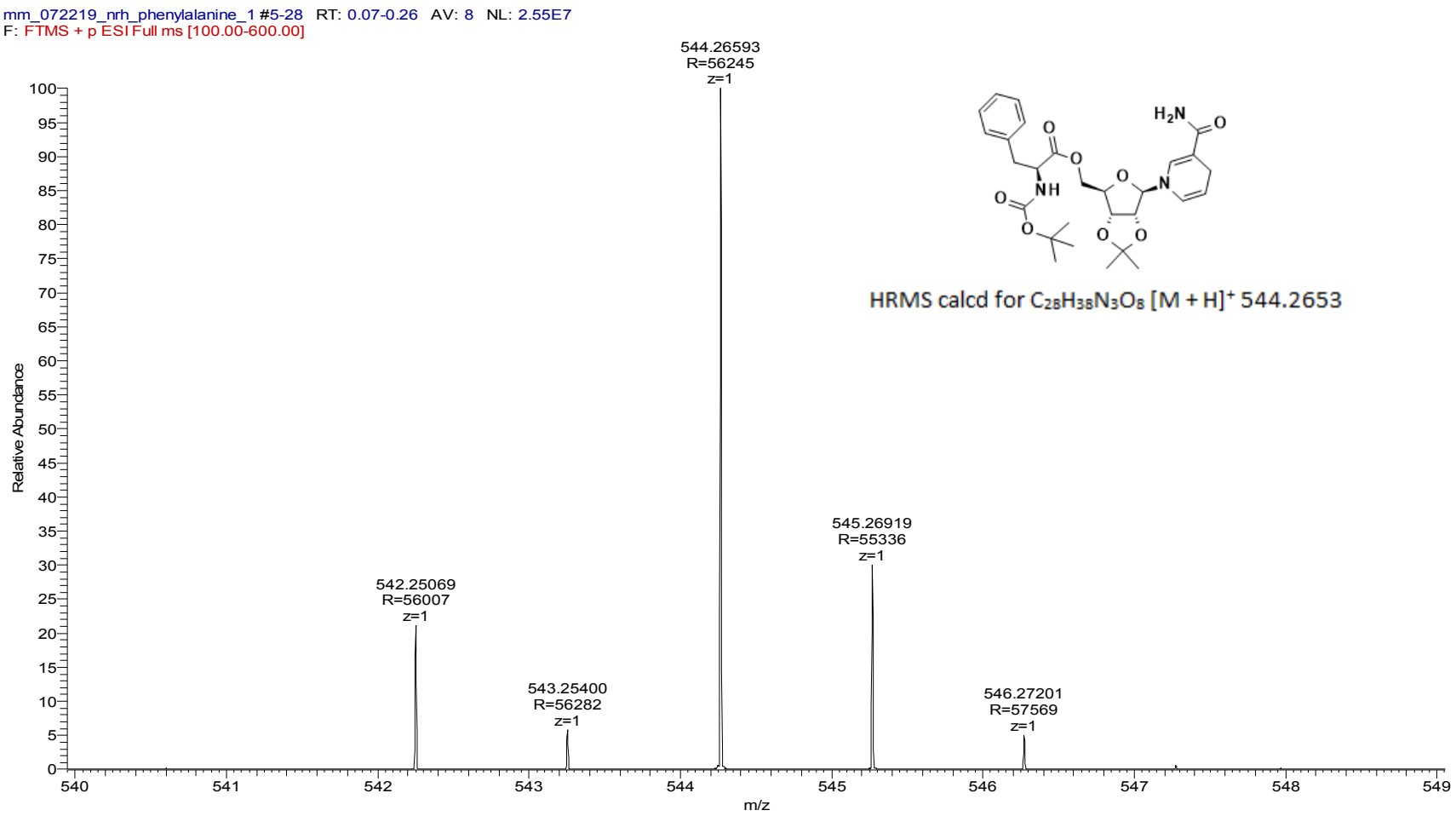

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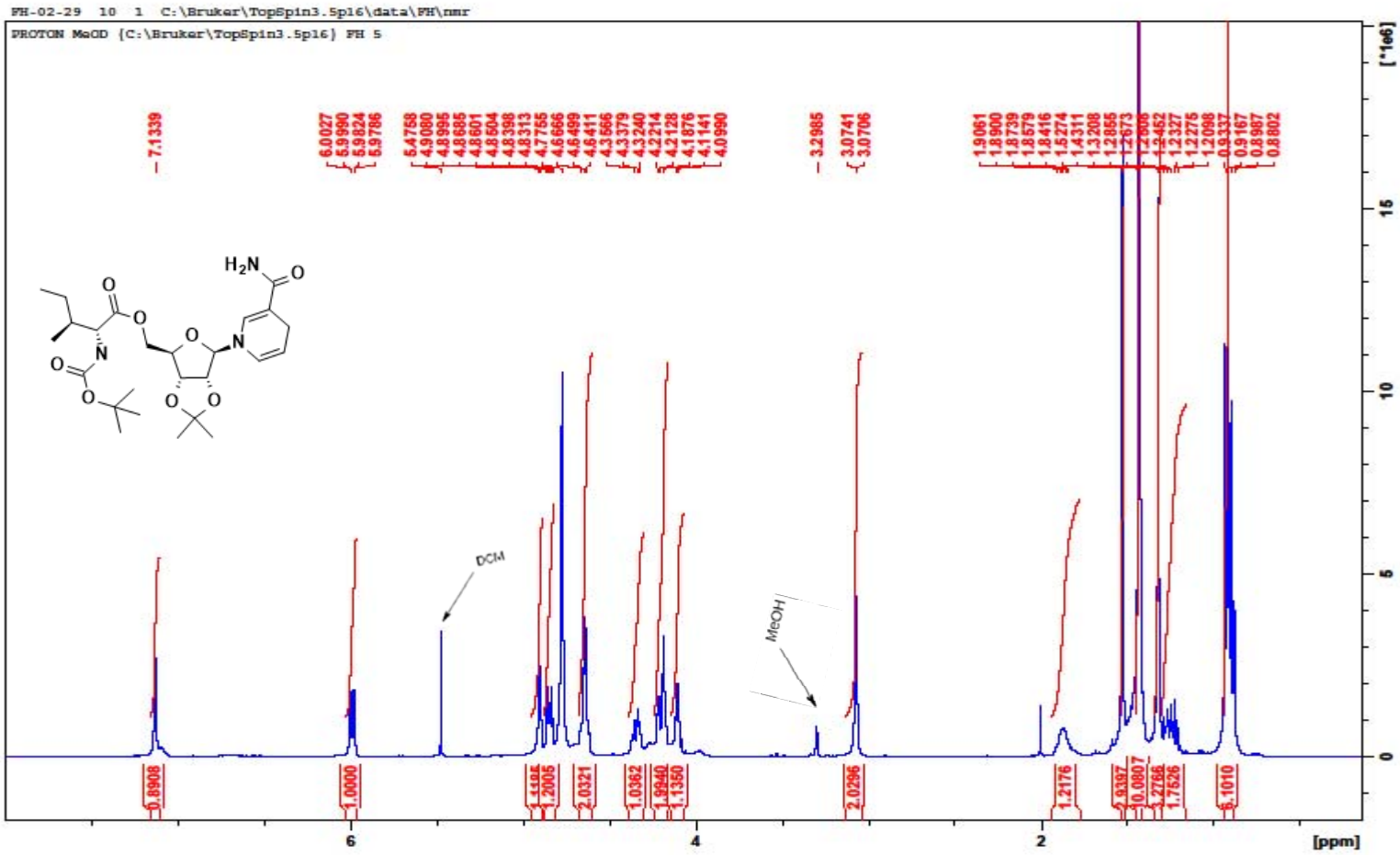
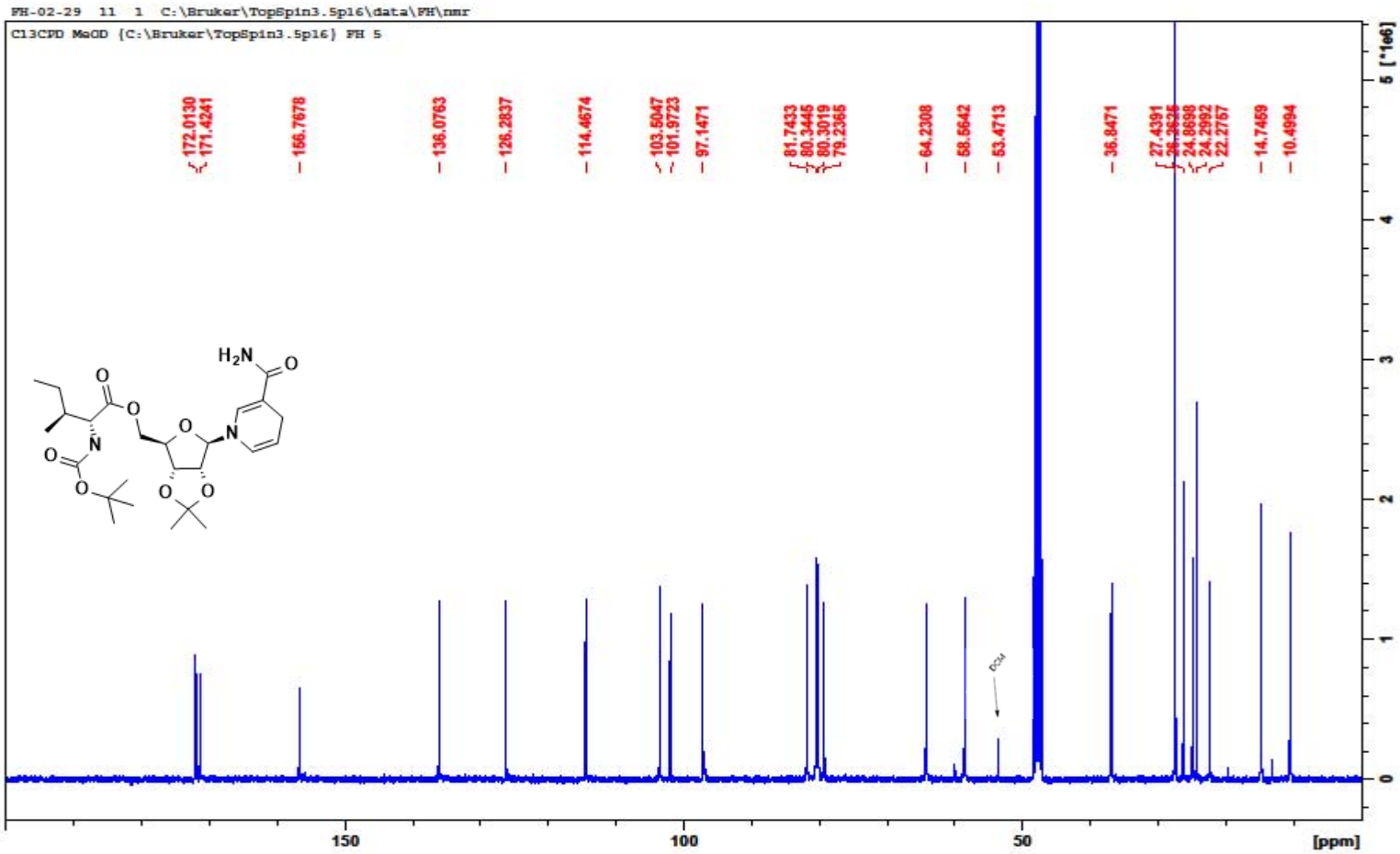


Compound 10f. 400 MHz ^{1}H NMR spectrum in MeOD



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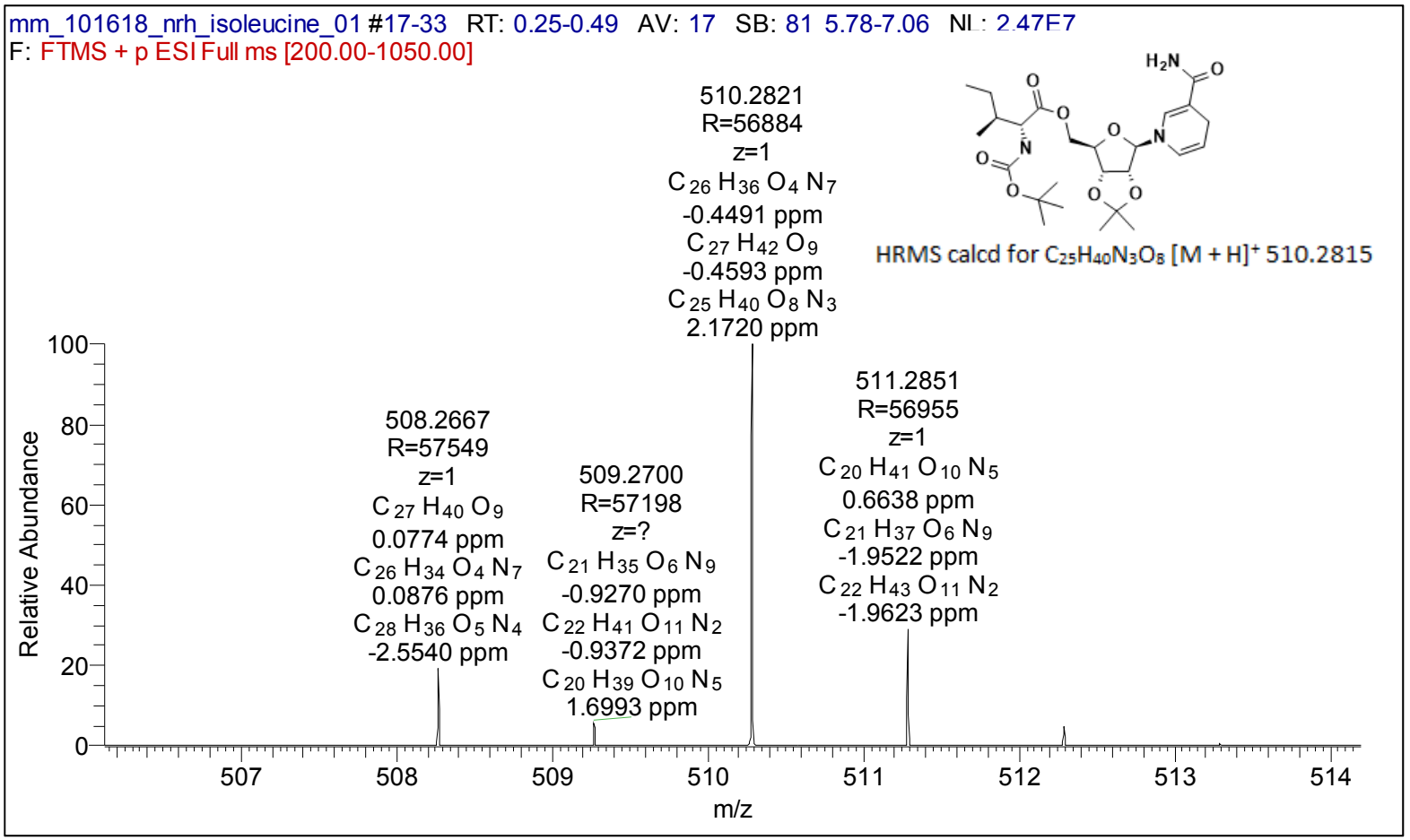

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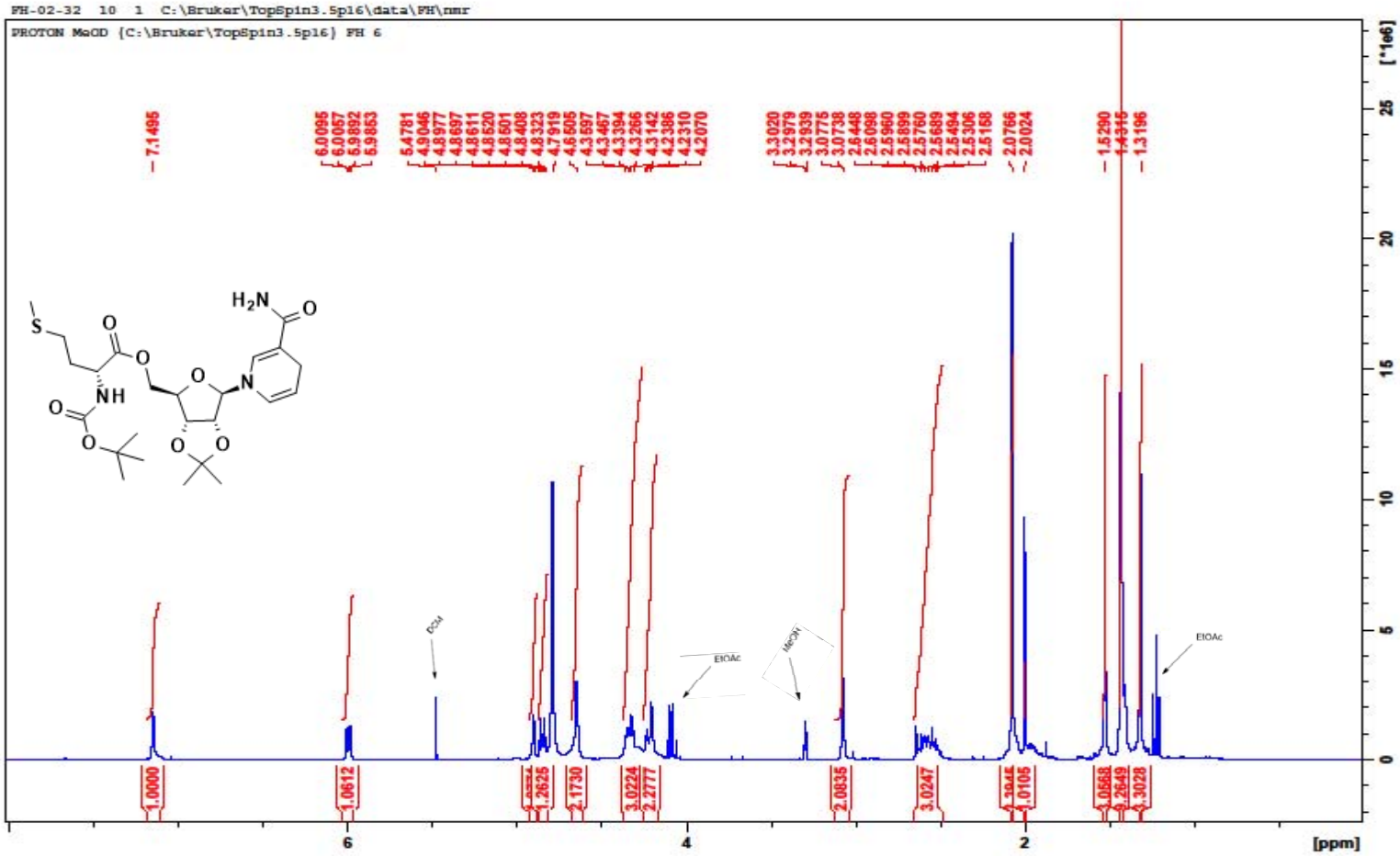
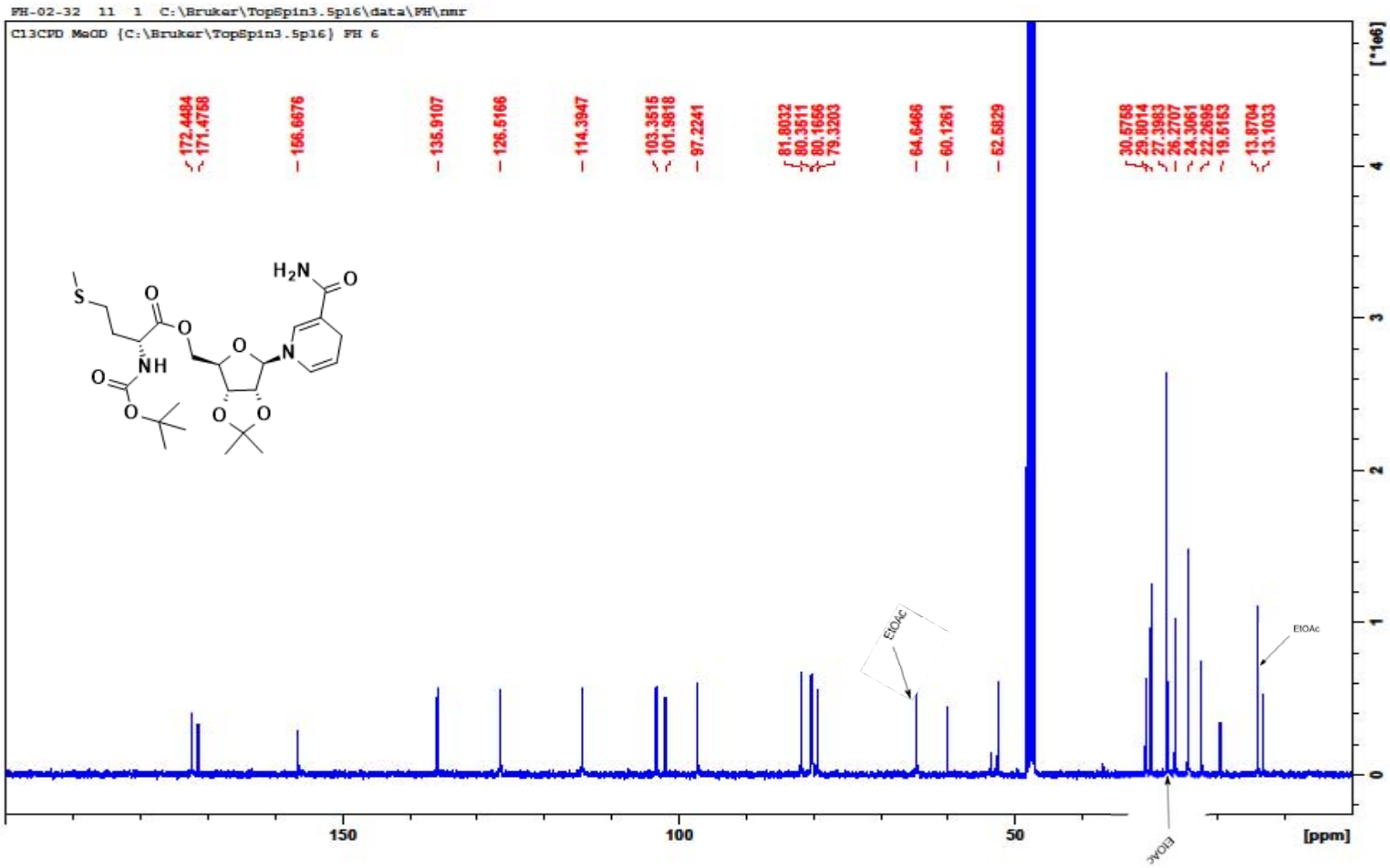


Compound 10g. 400 MHz ^{1}H NMR spectrum in MeOD



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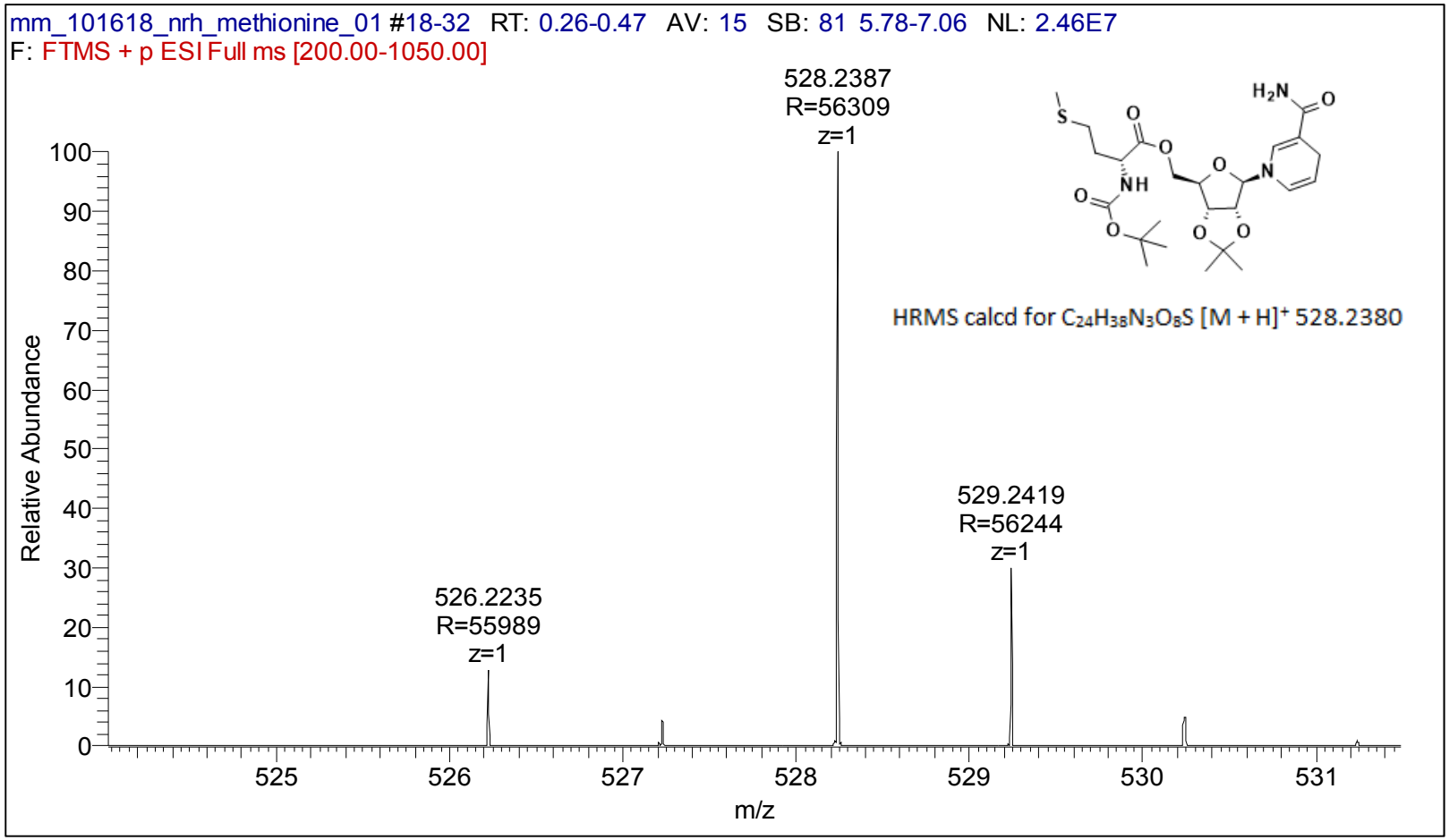

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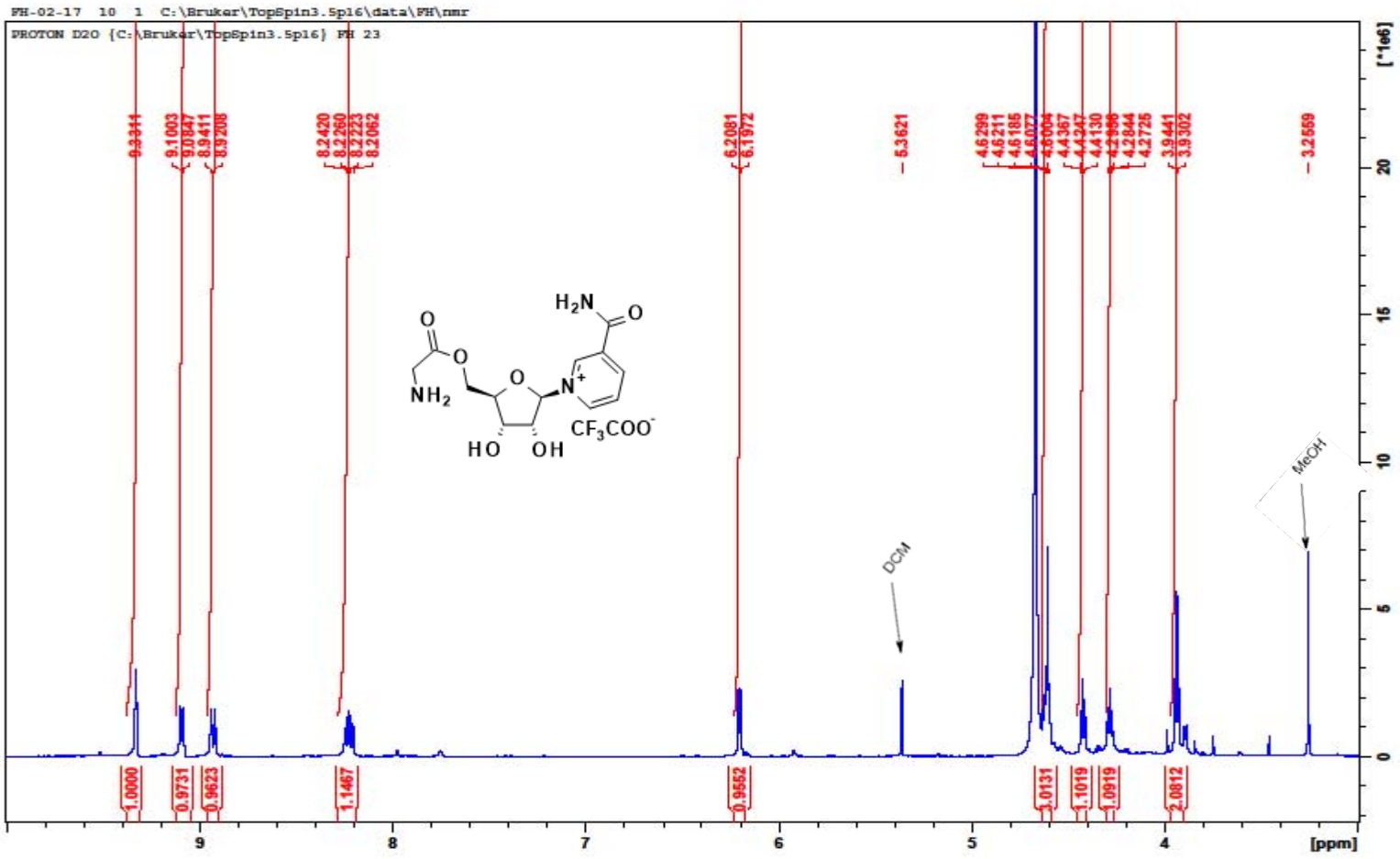
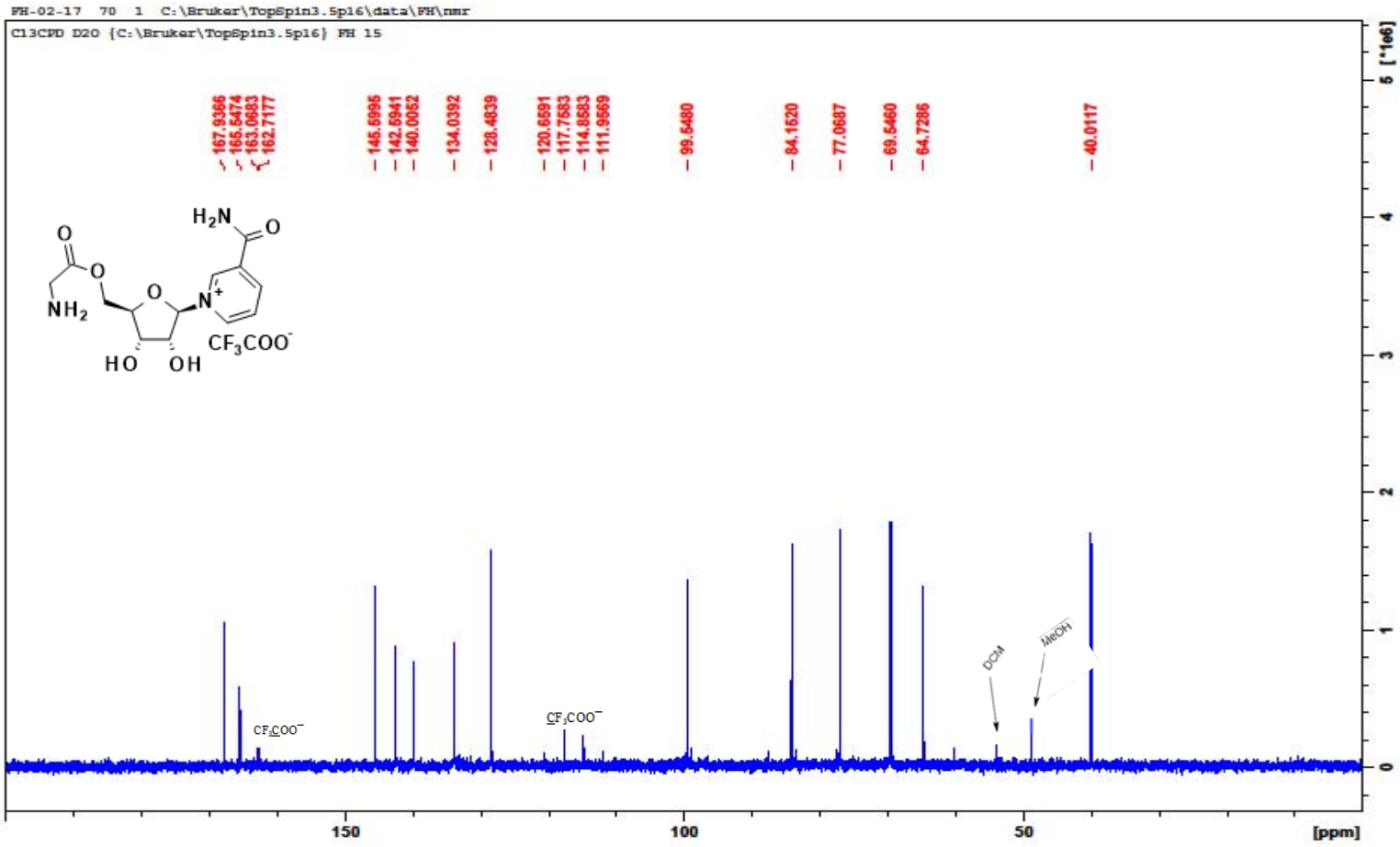


Compound 12a. 400 MHz ^{1}H NMR spectrum in D_2O



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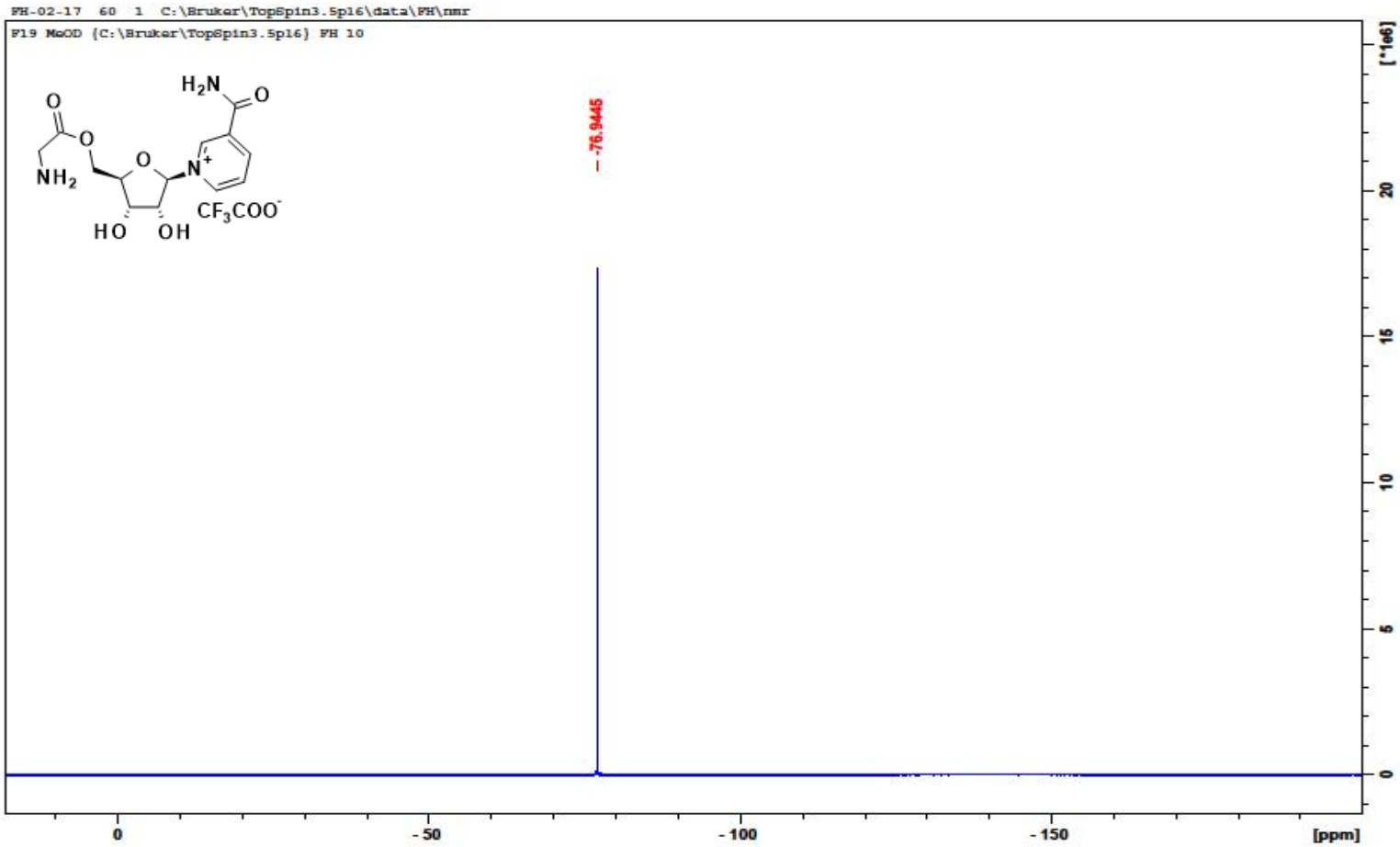
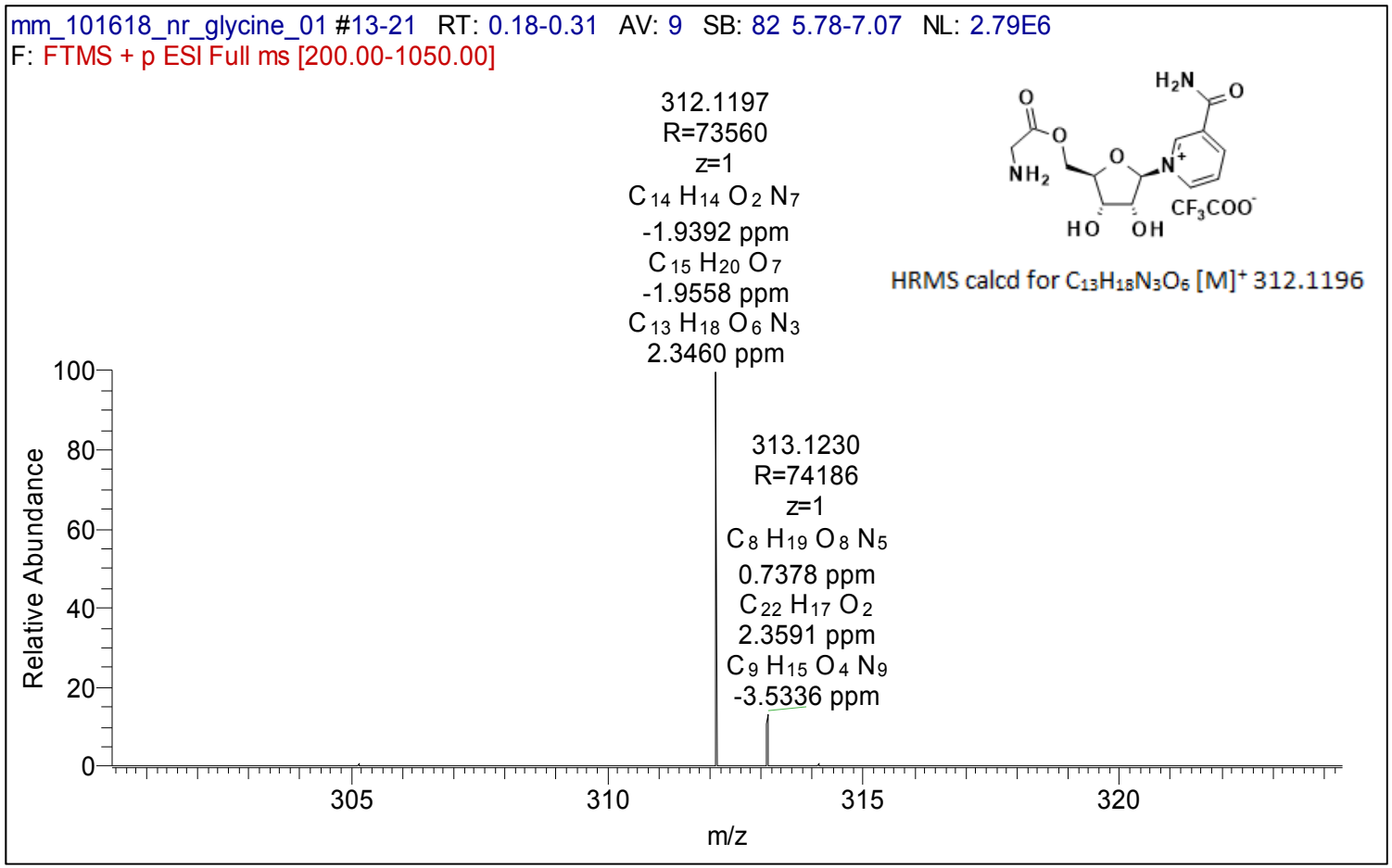
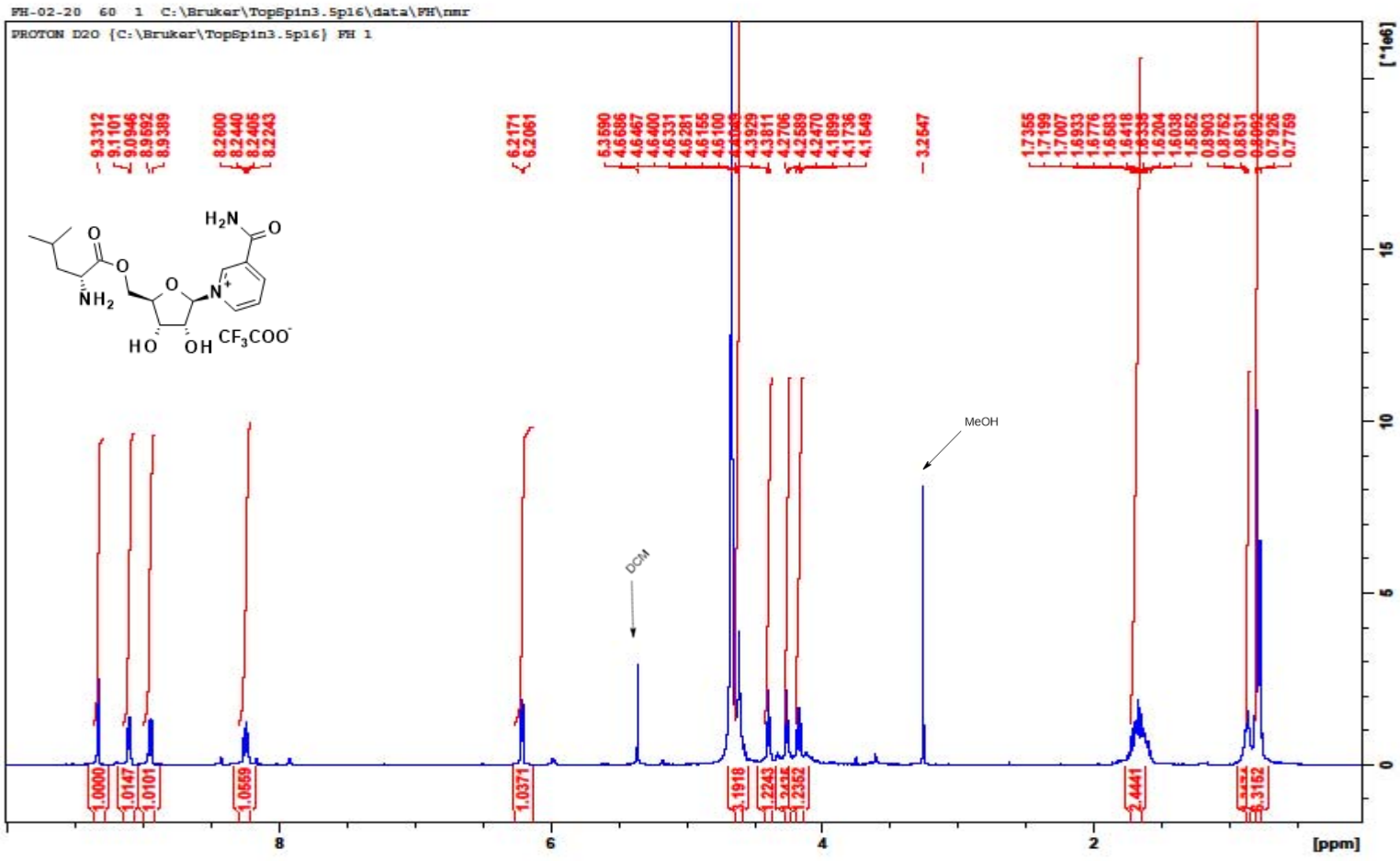


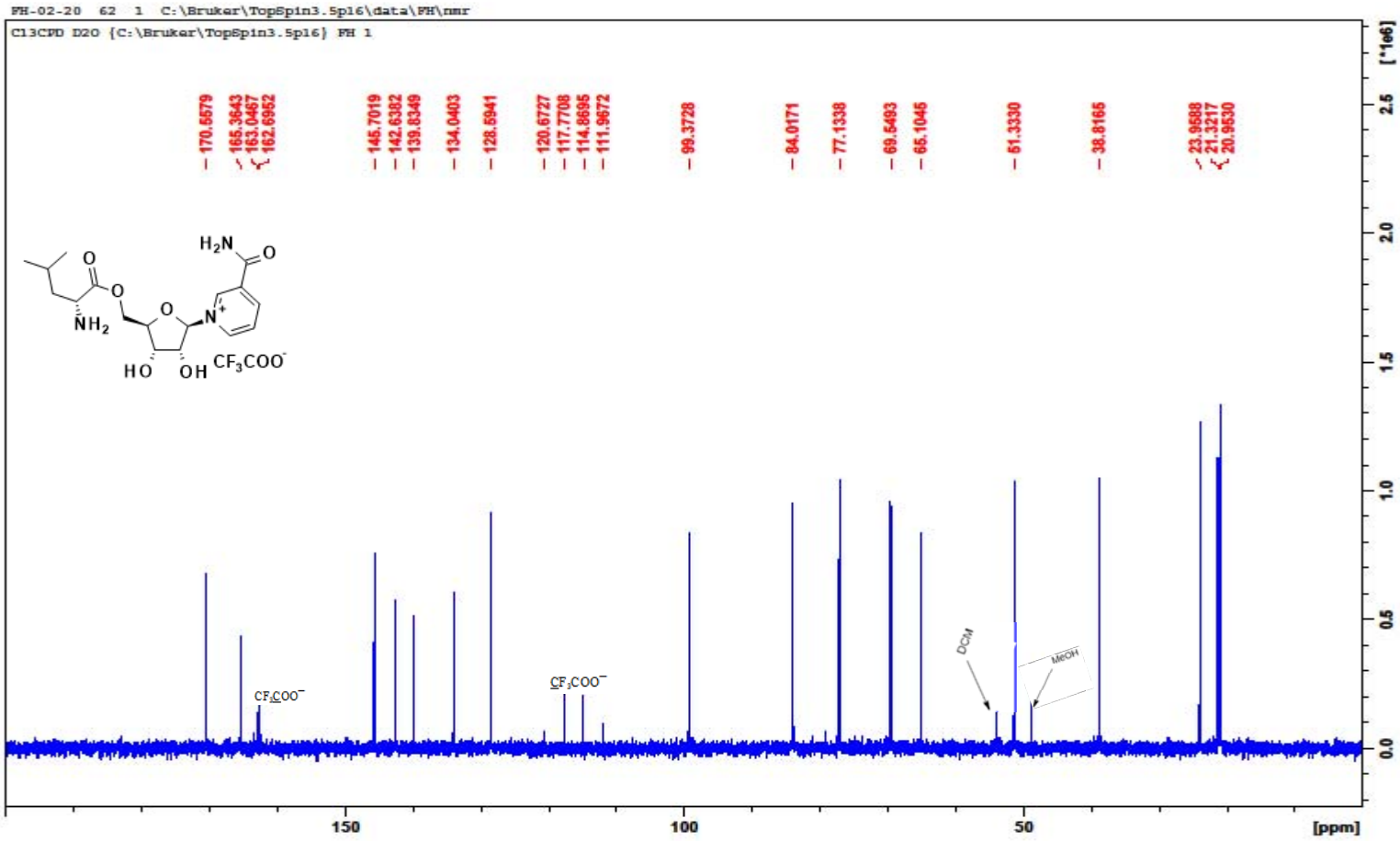
Compound 12a. 377 MHz ^{19}F NMR spectrum in MeOD



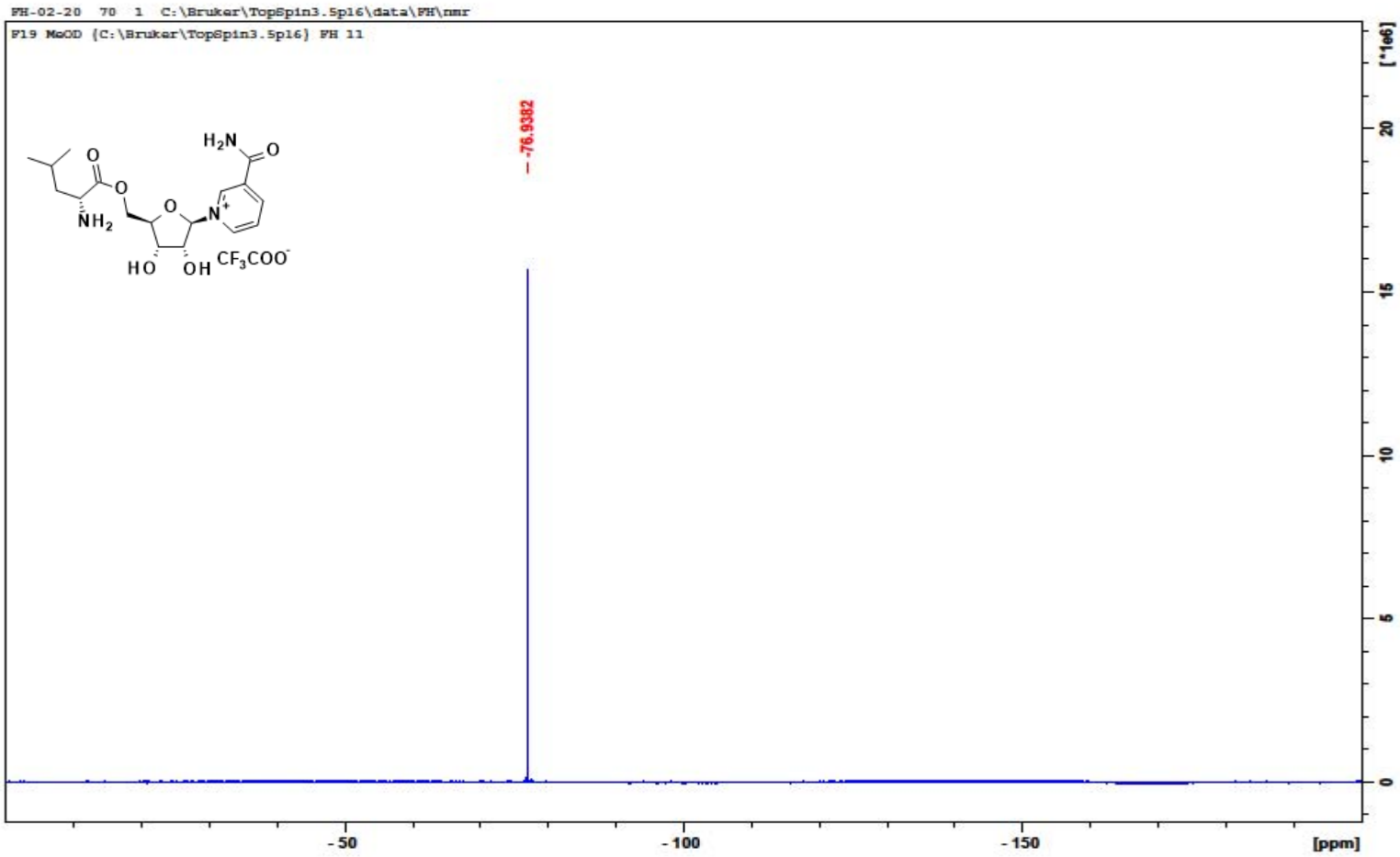
Compound 12a. HRMS spectra



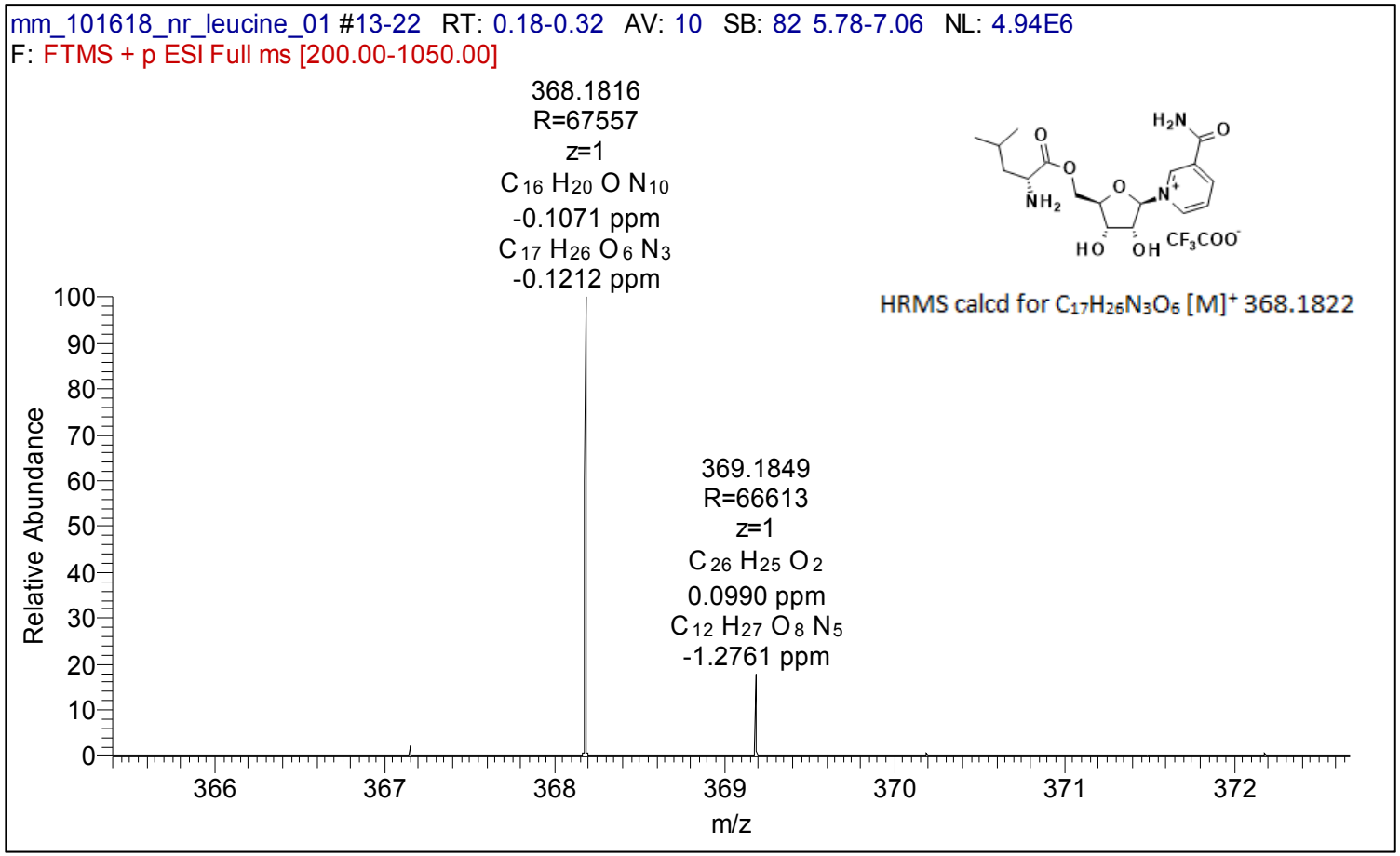
Compound 12b. 400 MHz ^{1}H NMR spectrum in D_2O



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



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



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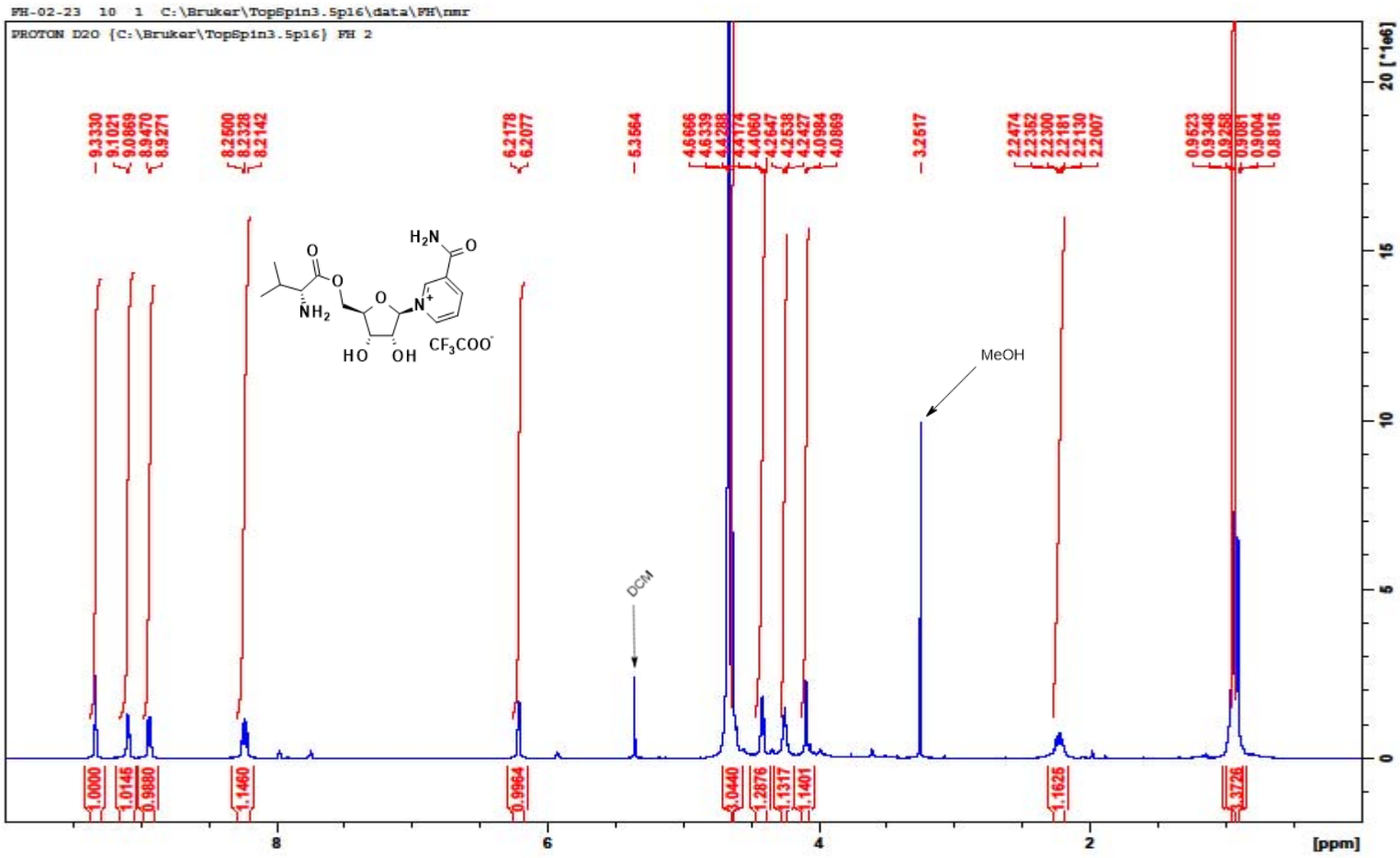

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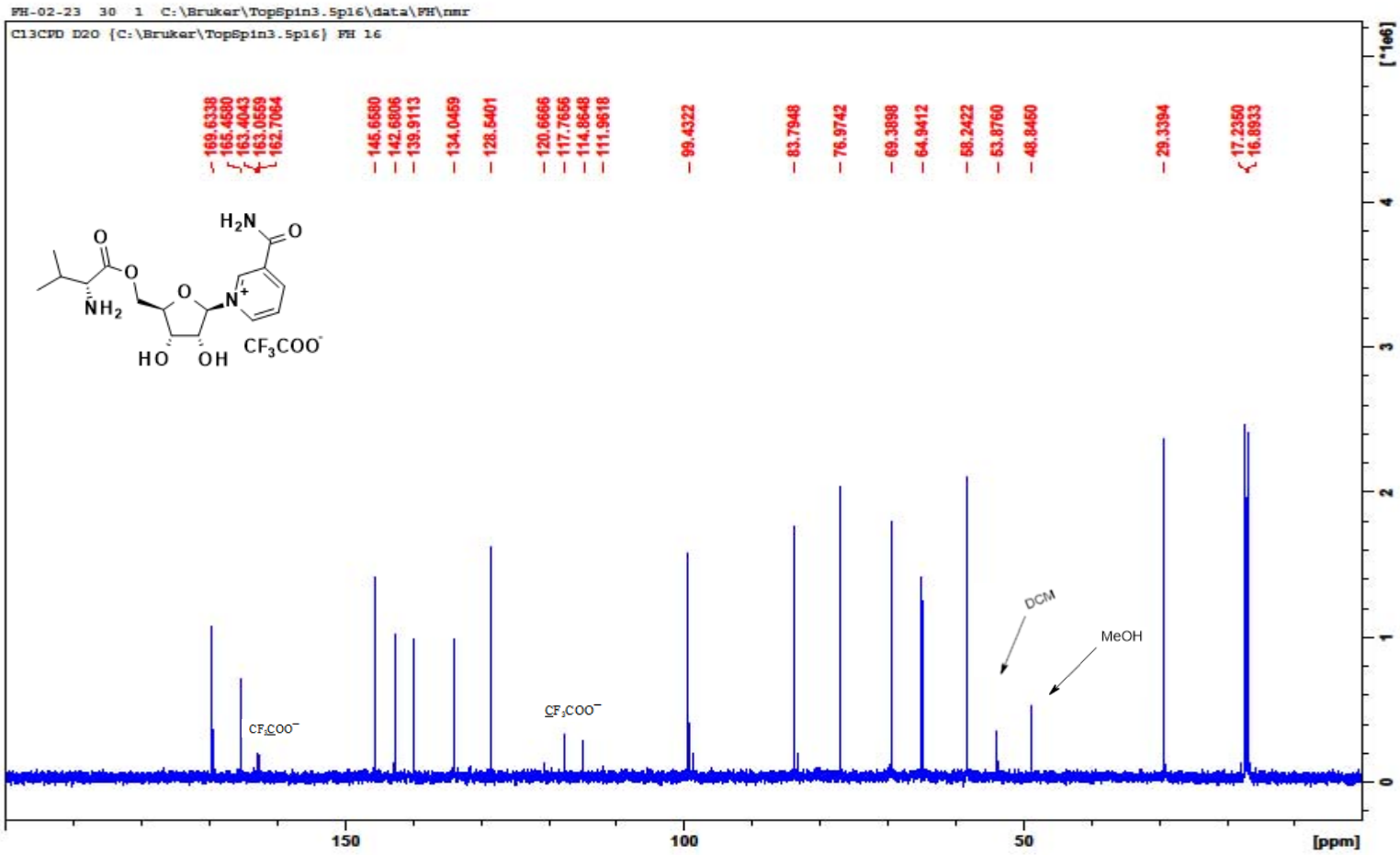

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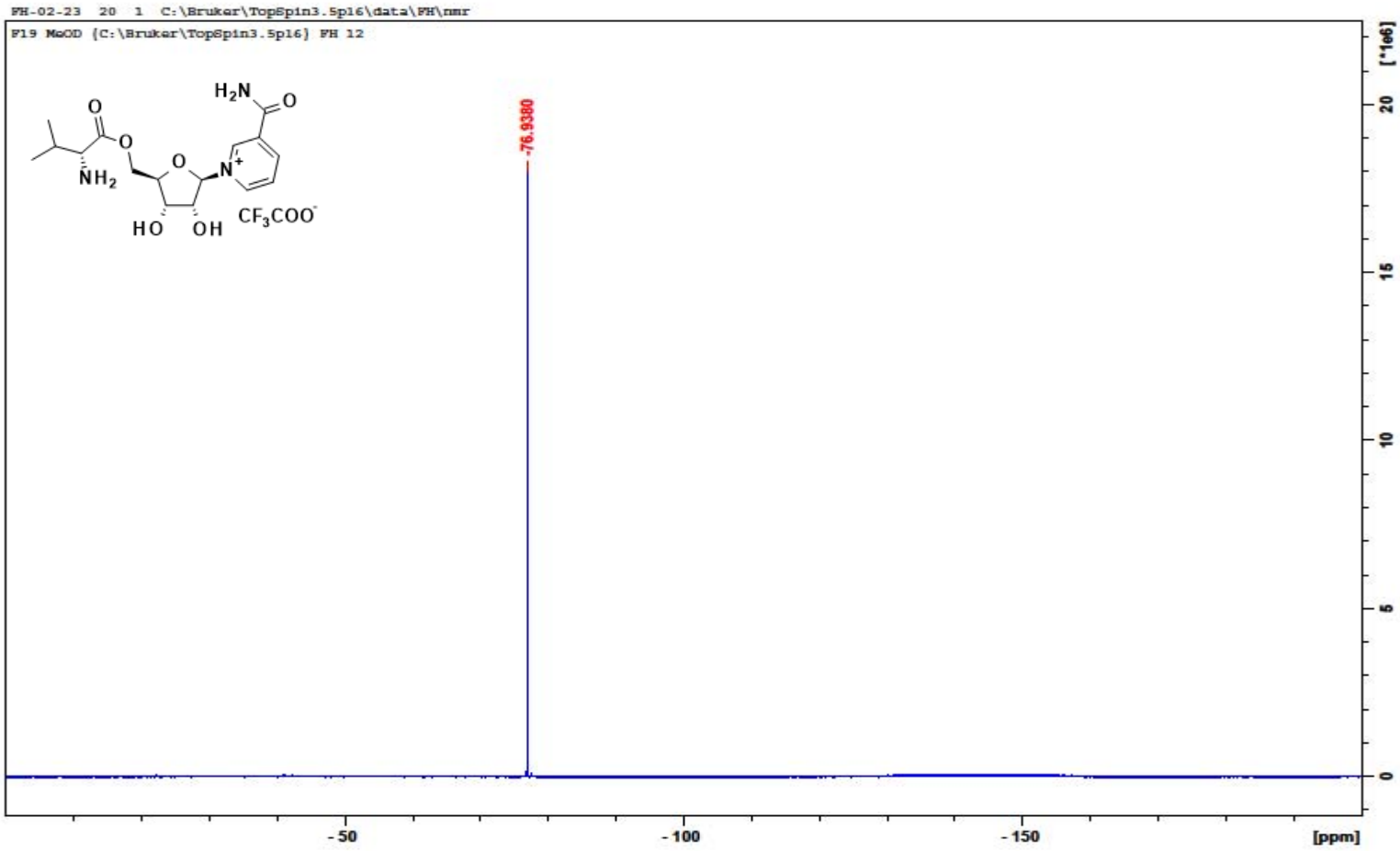
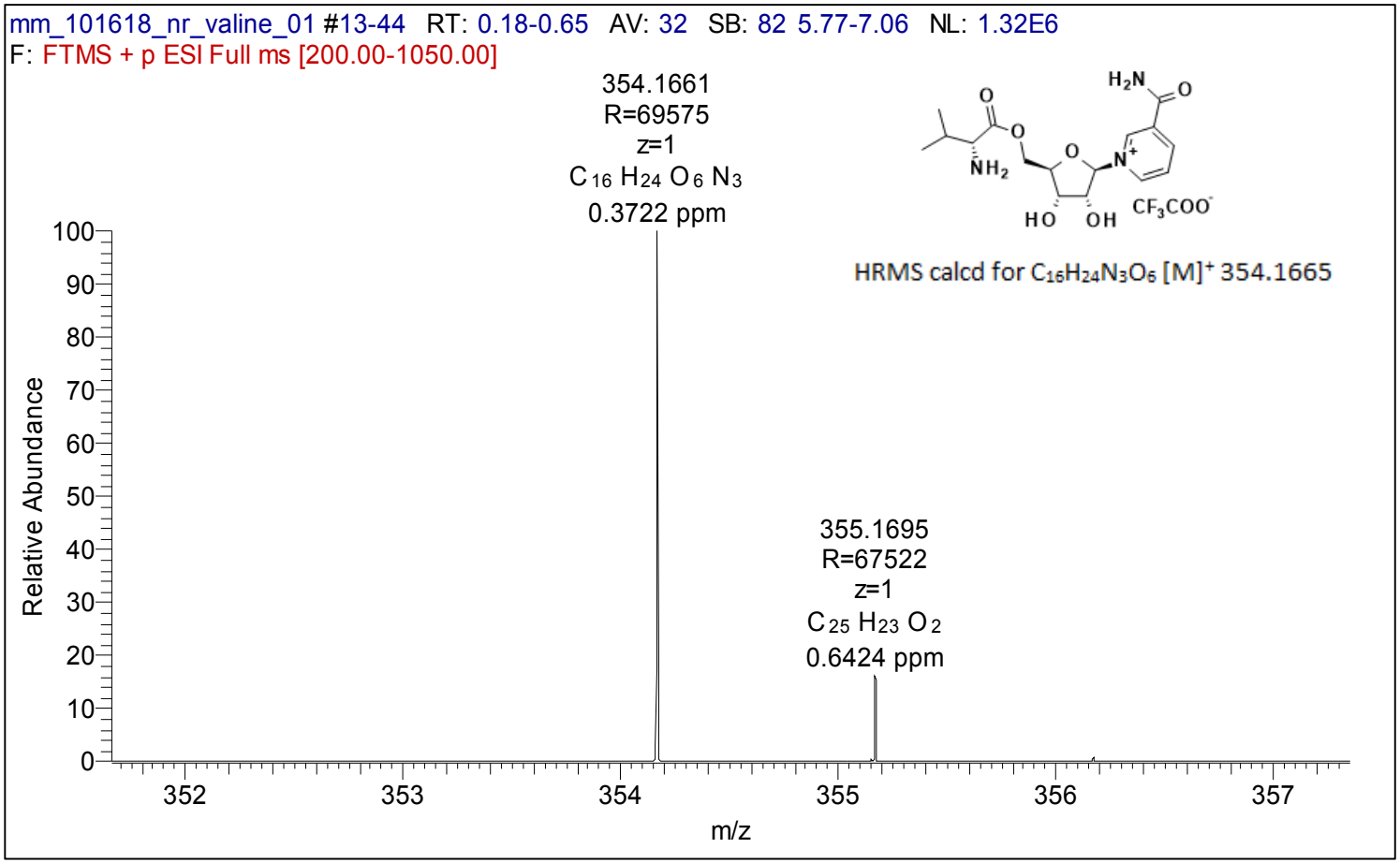


Compound 12c. 377 MHz ^{19}F NMR spectrum in MeOD



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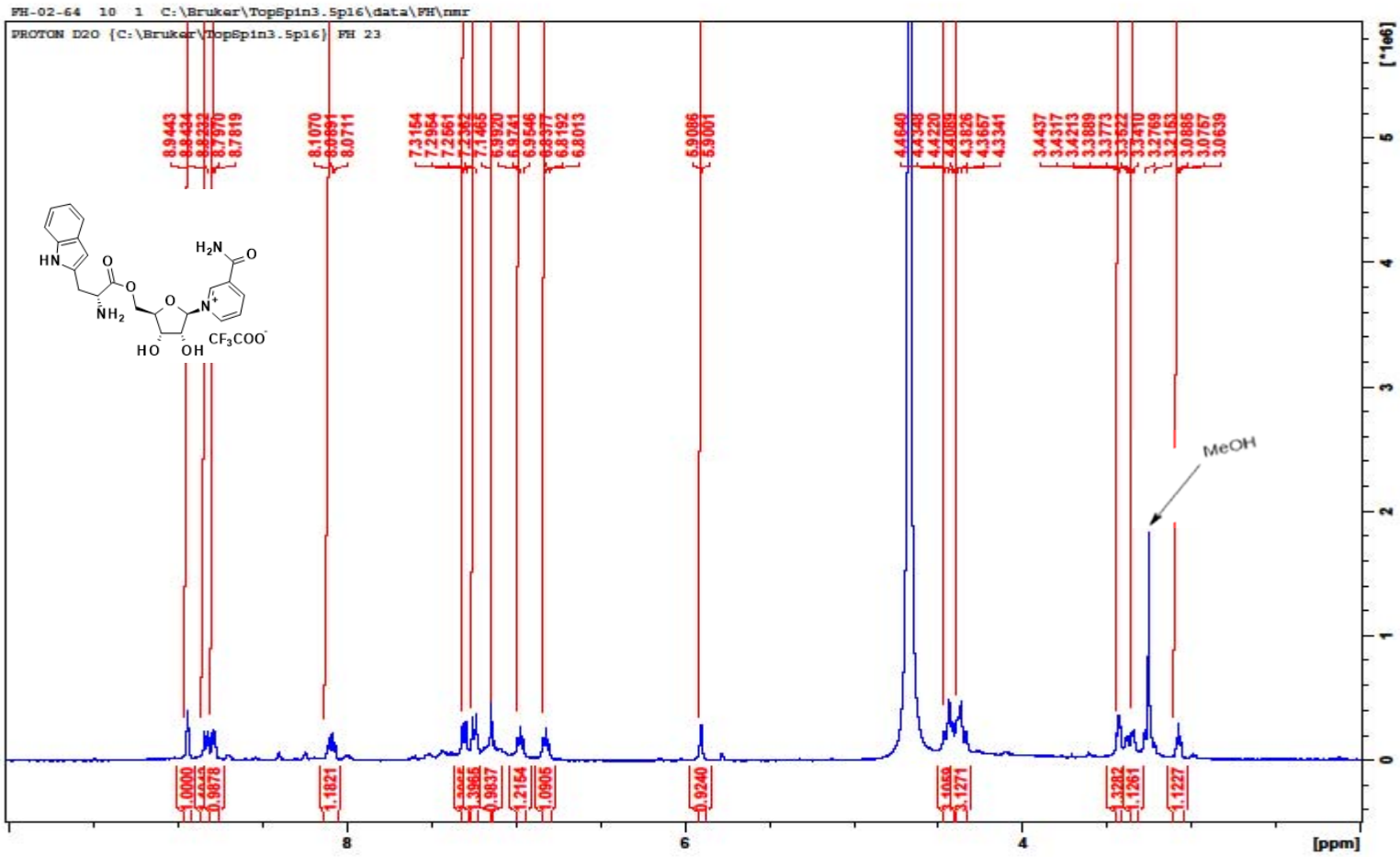
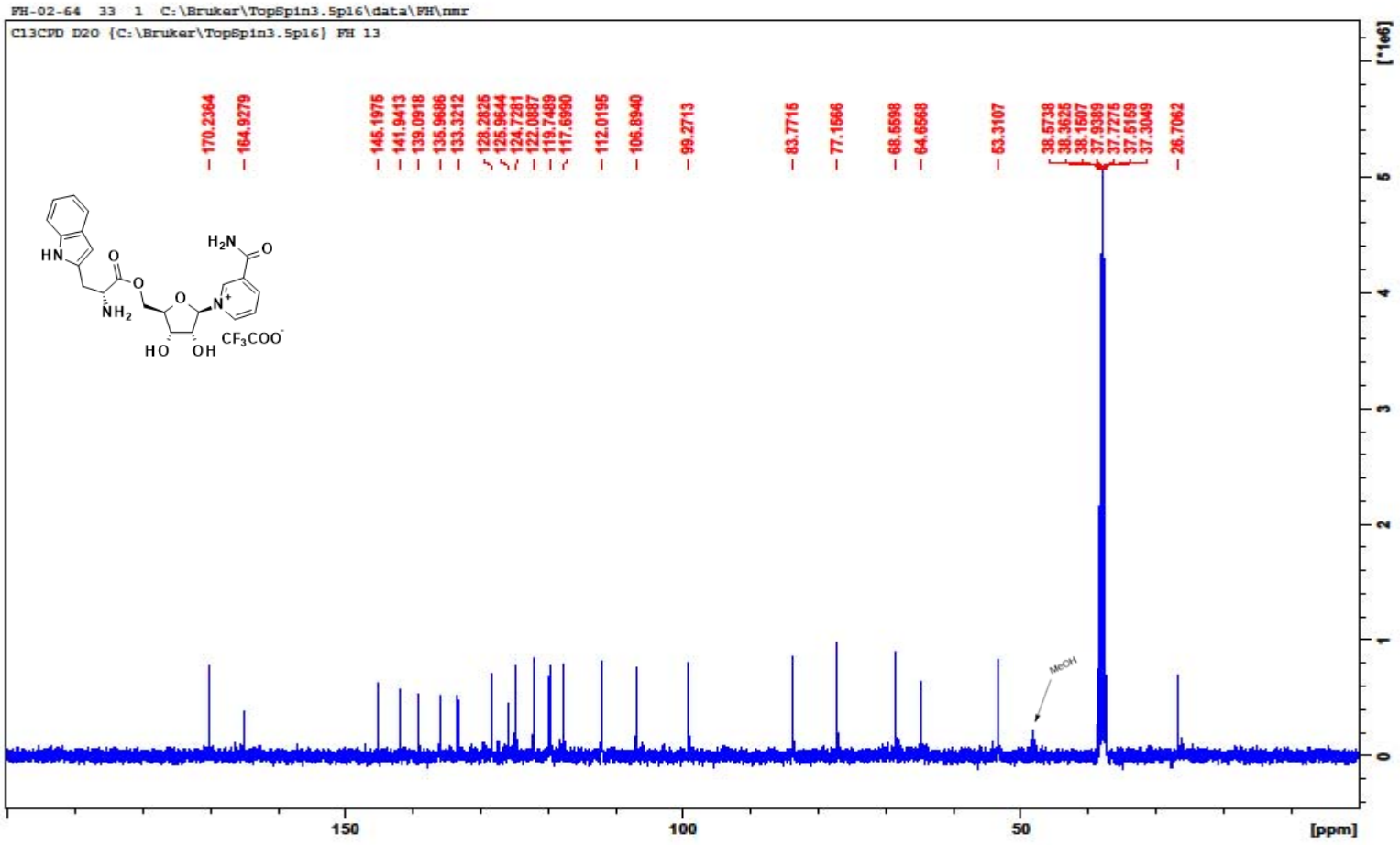
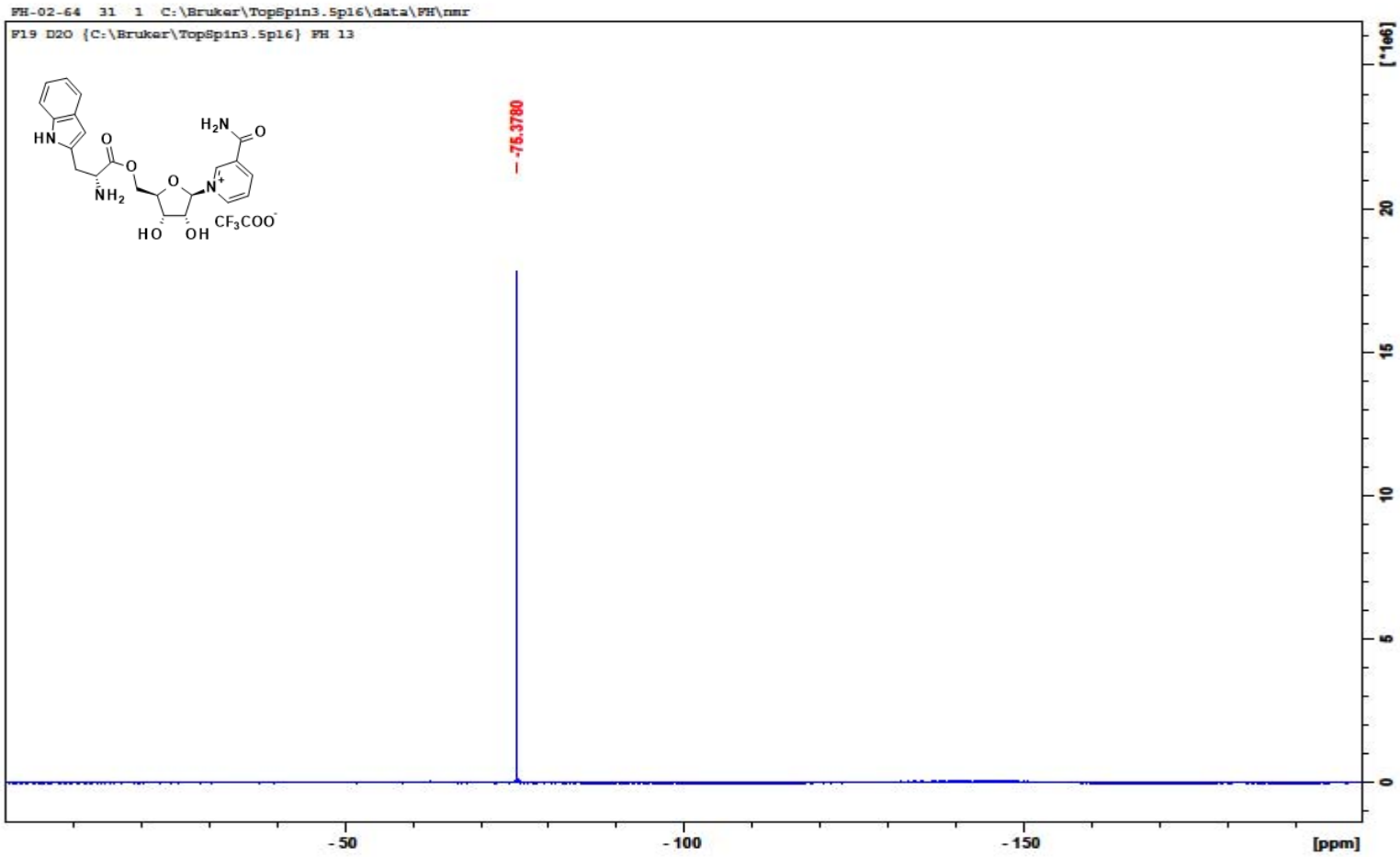


Compound 12d. 400 MHz ^{1}H NMR spectrum in D_2O



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



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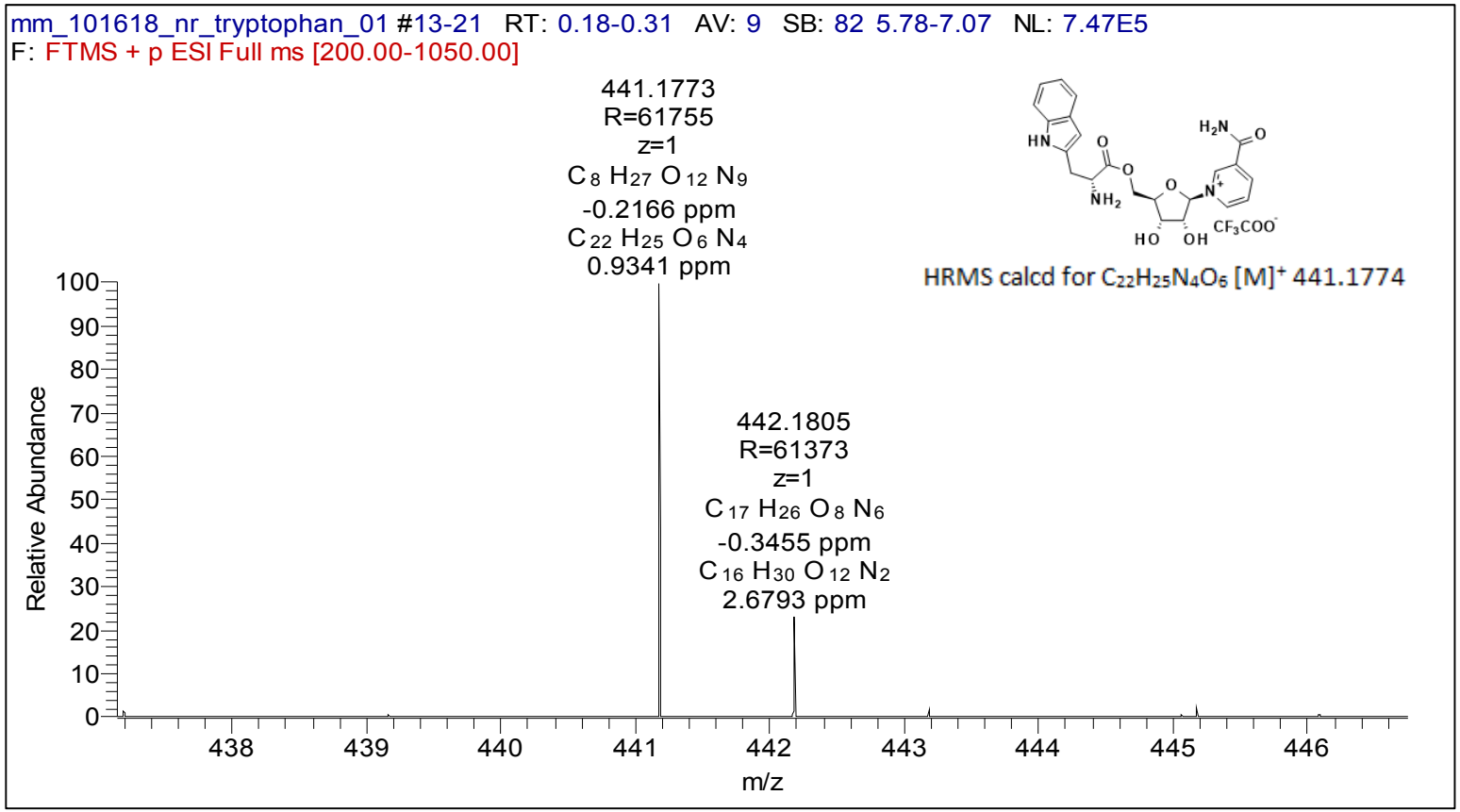

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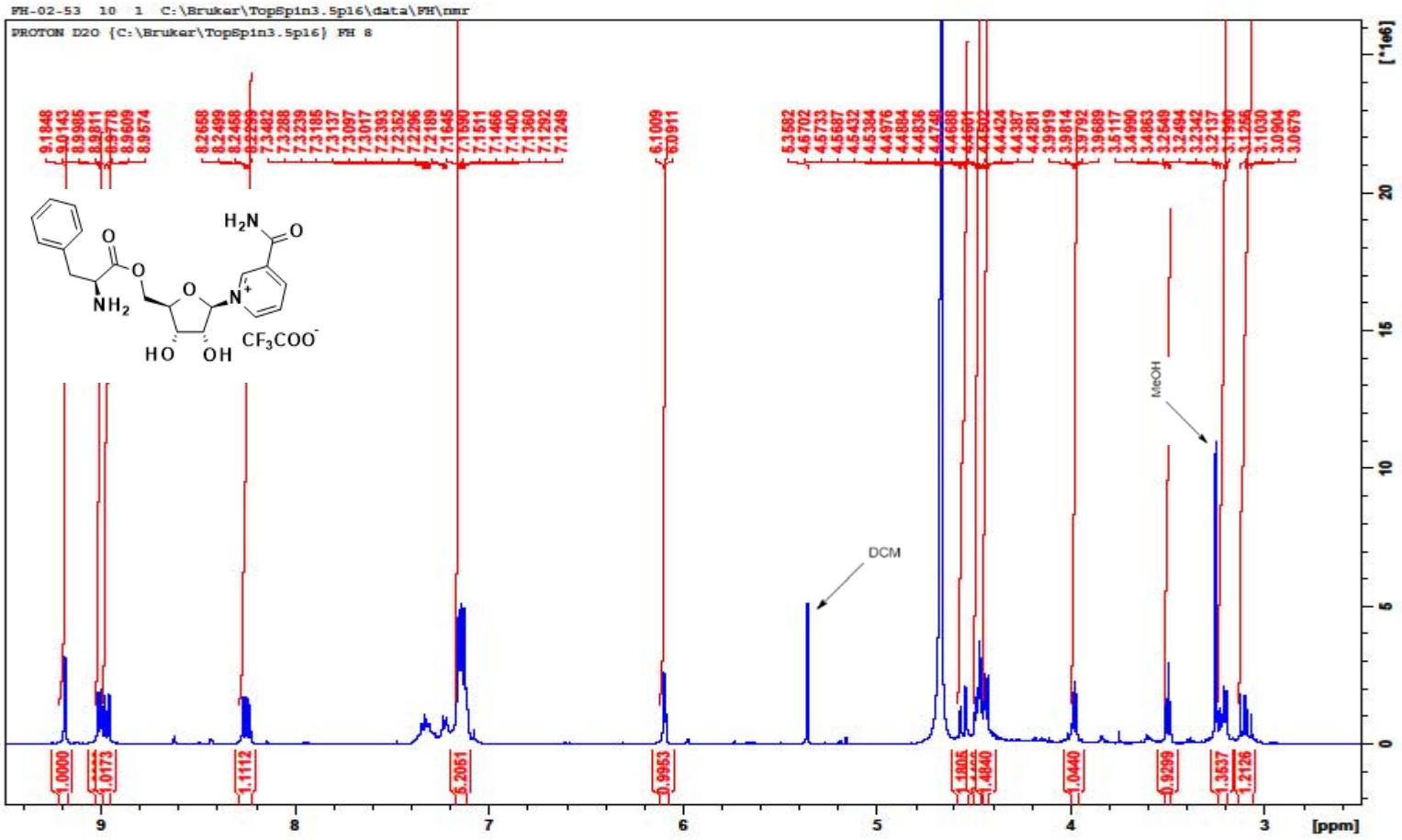
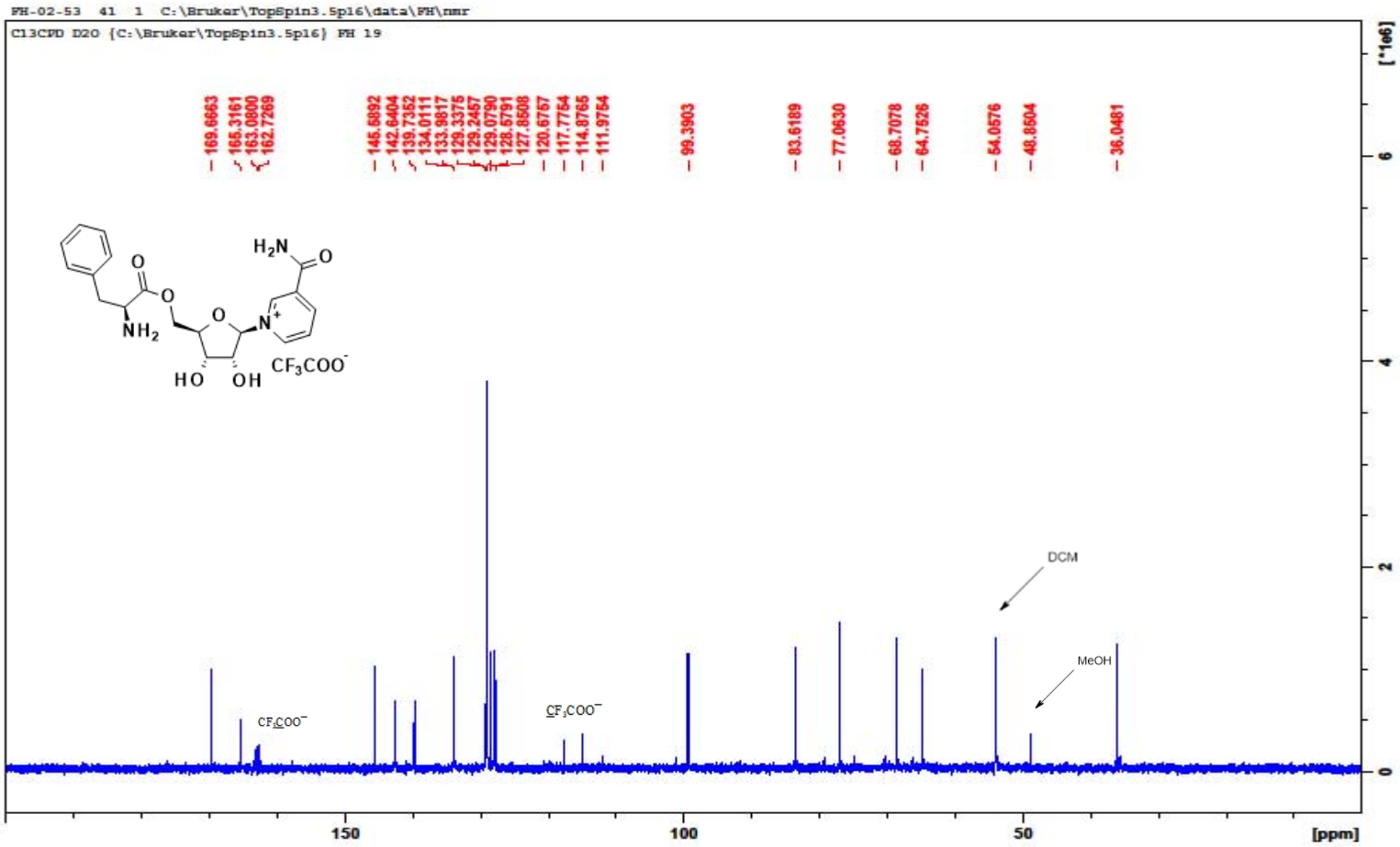


Compound 12e. 400 MHz ^{1}H NMR spectrum in D_2O



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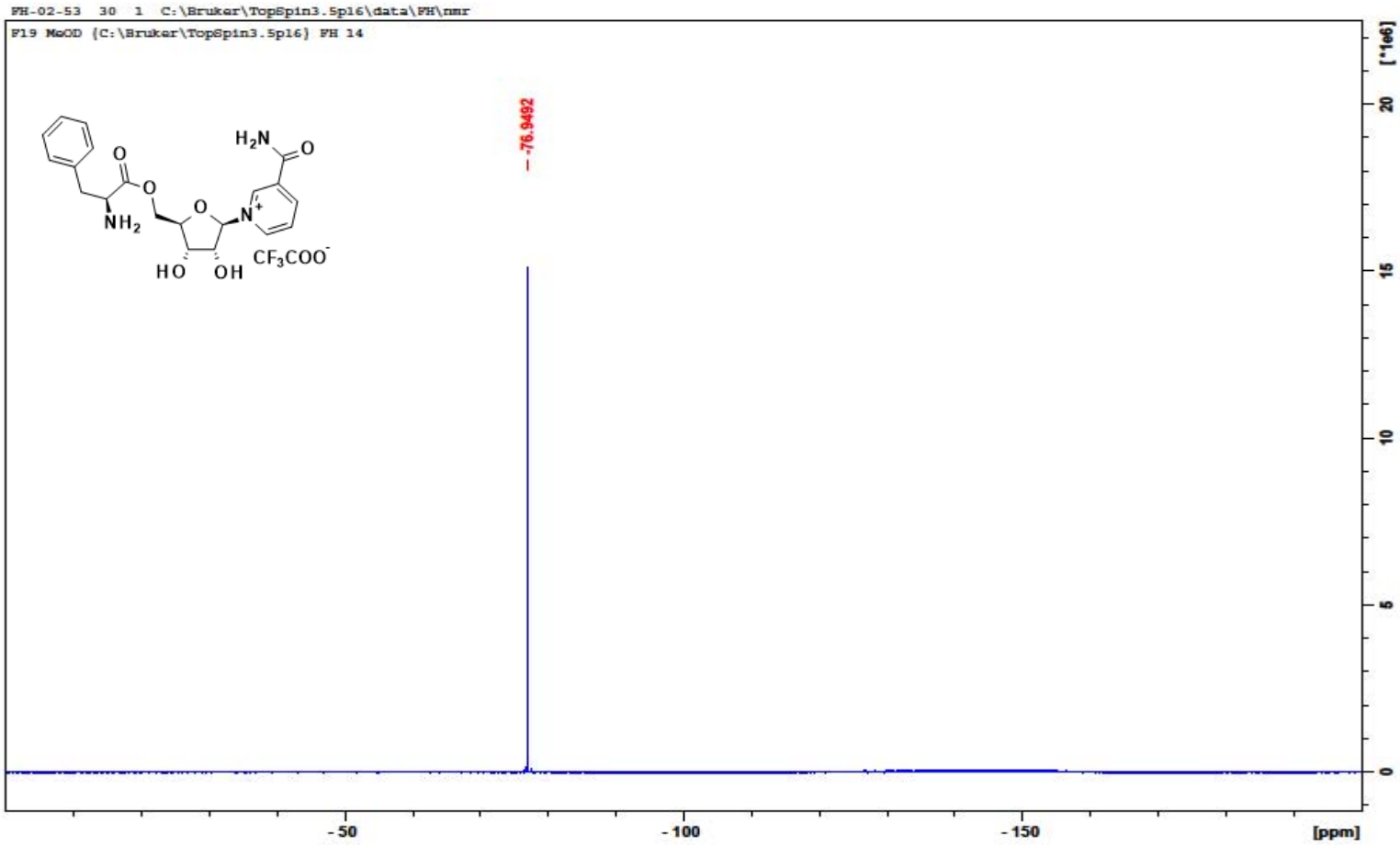
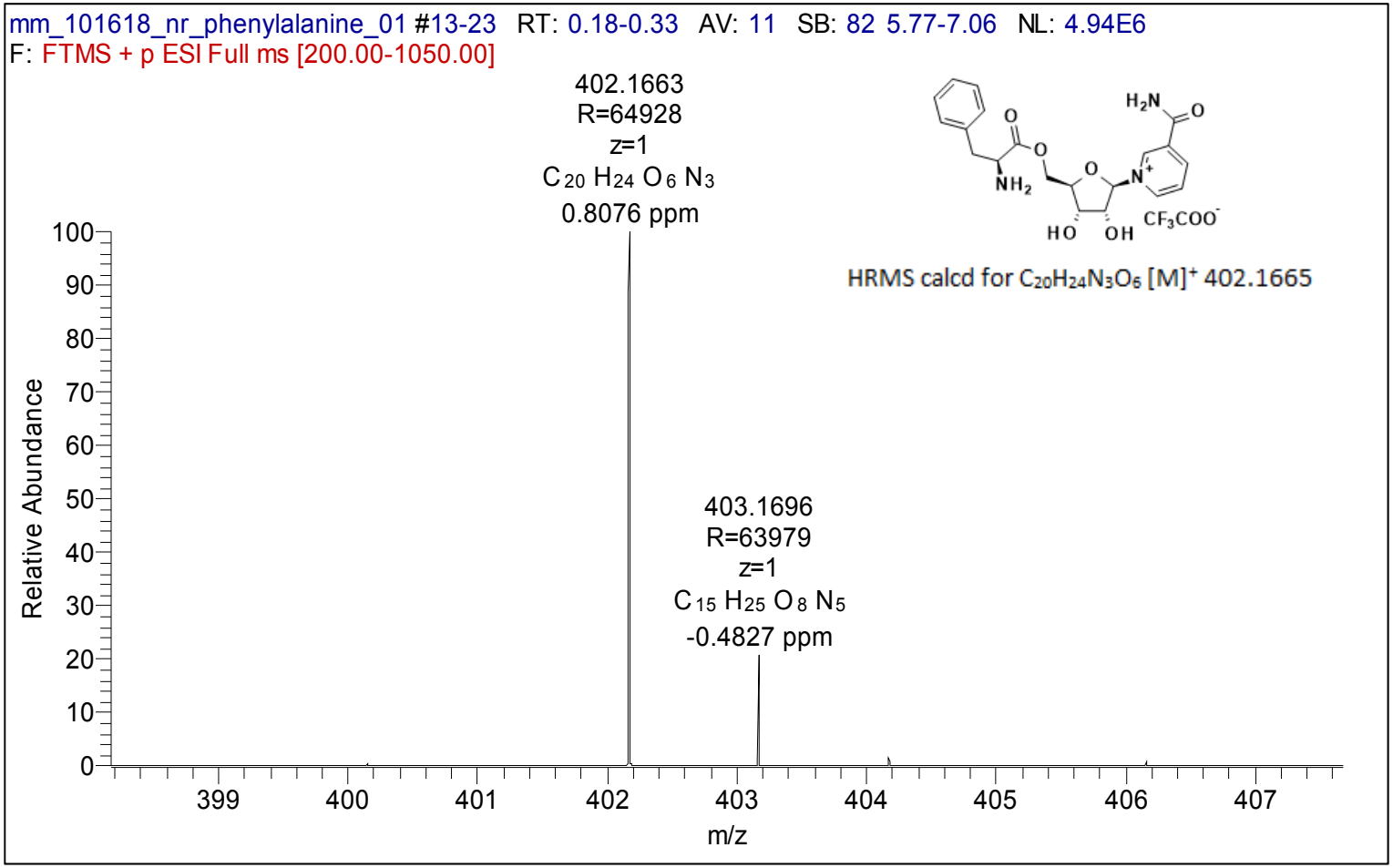


Compound 12e. 377 MHz ^{19}F NMR spectrum in MeOD



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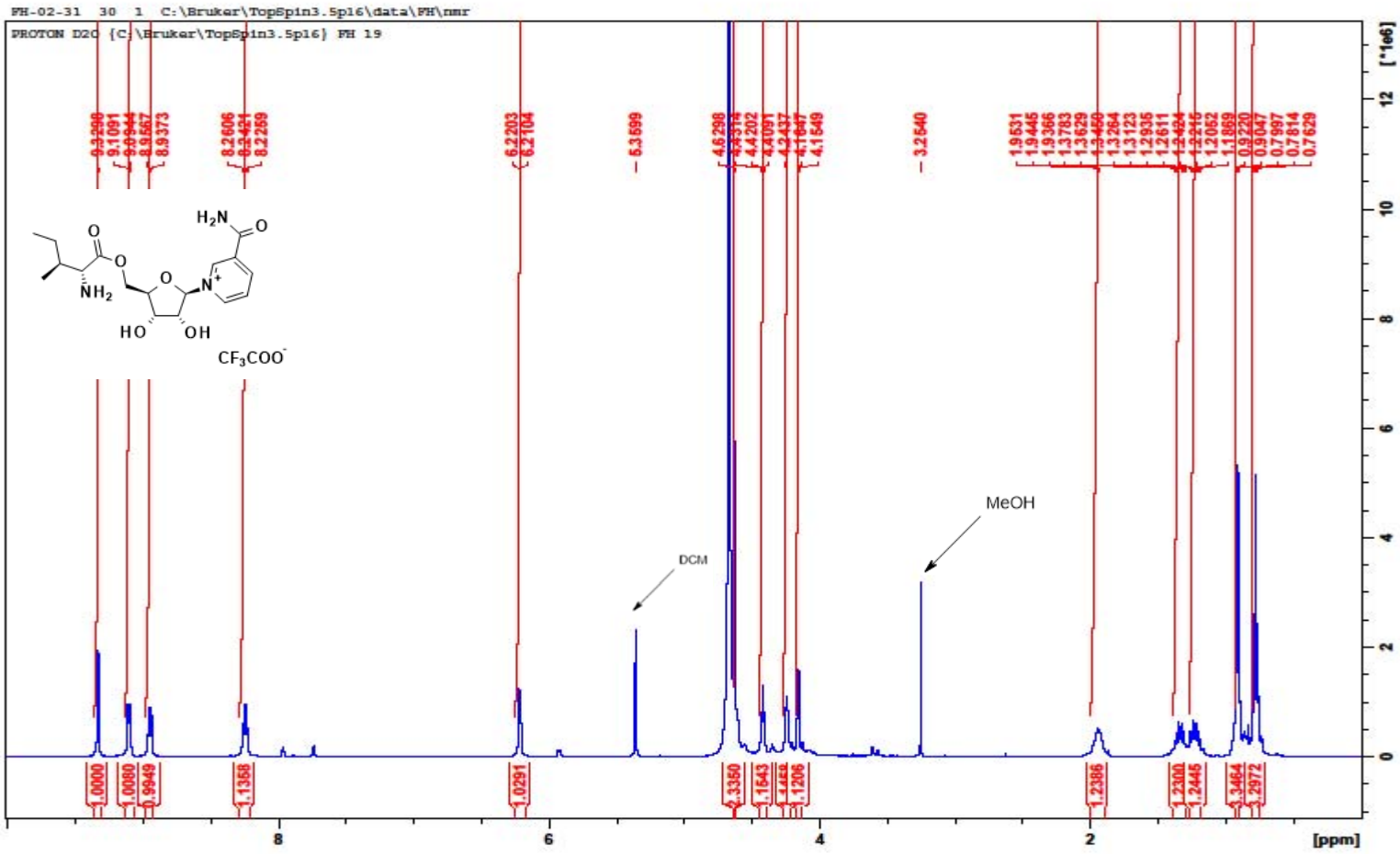
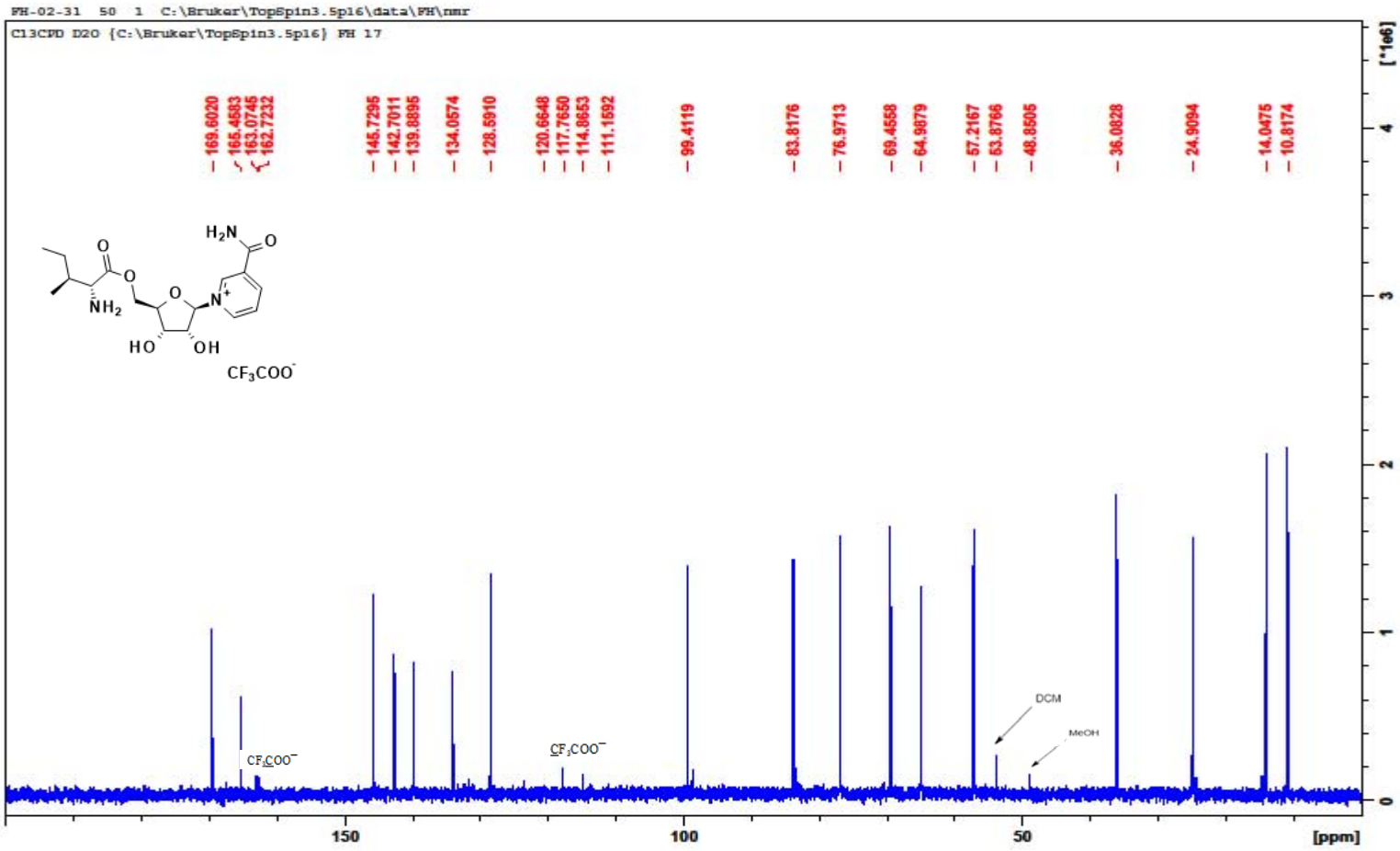


Compound 12f. 400 MHz ^{1}H NMR spectrum in D_2O



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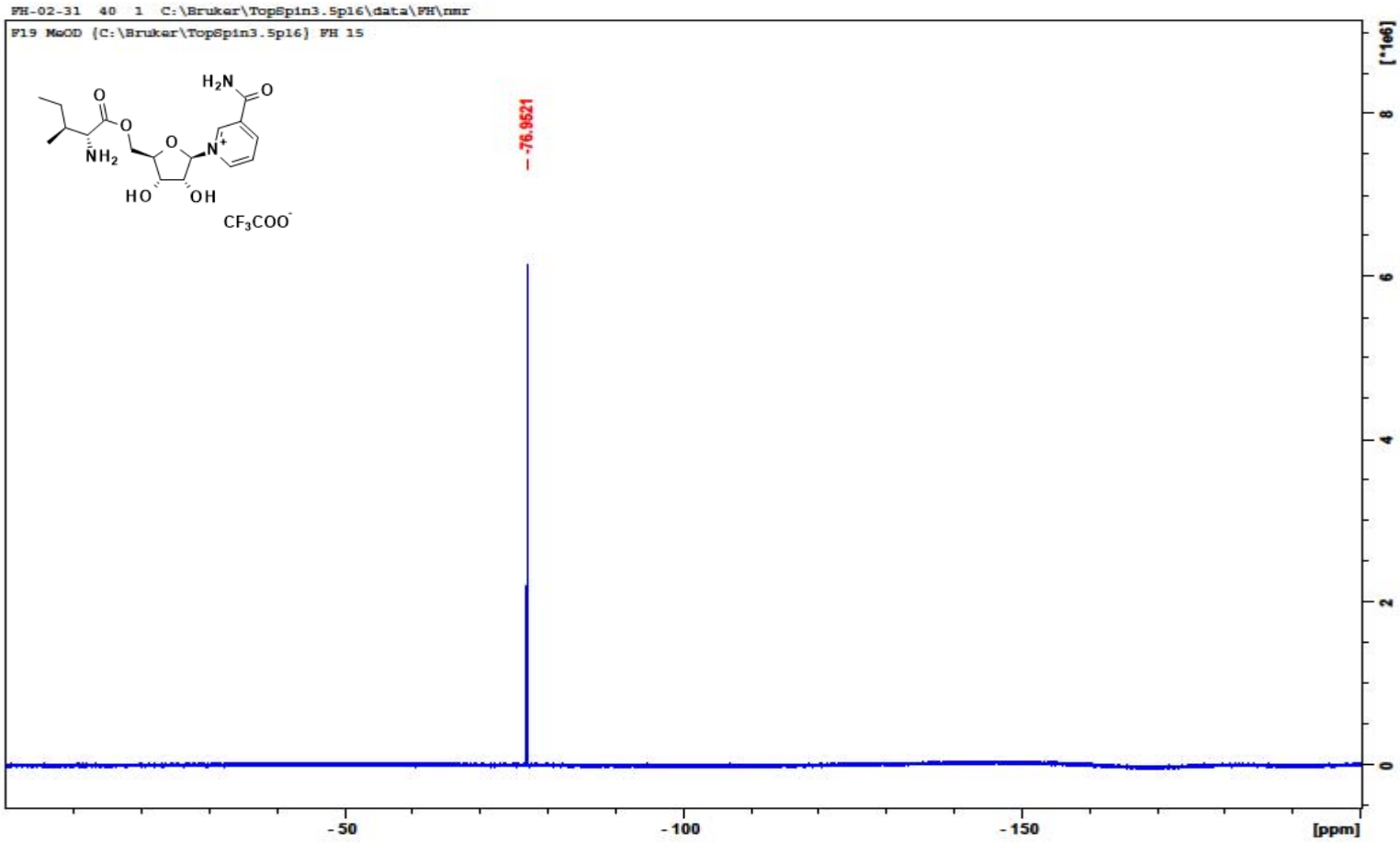
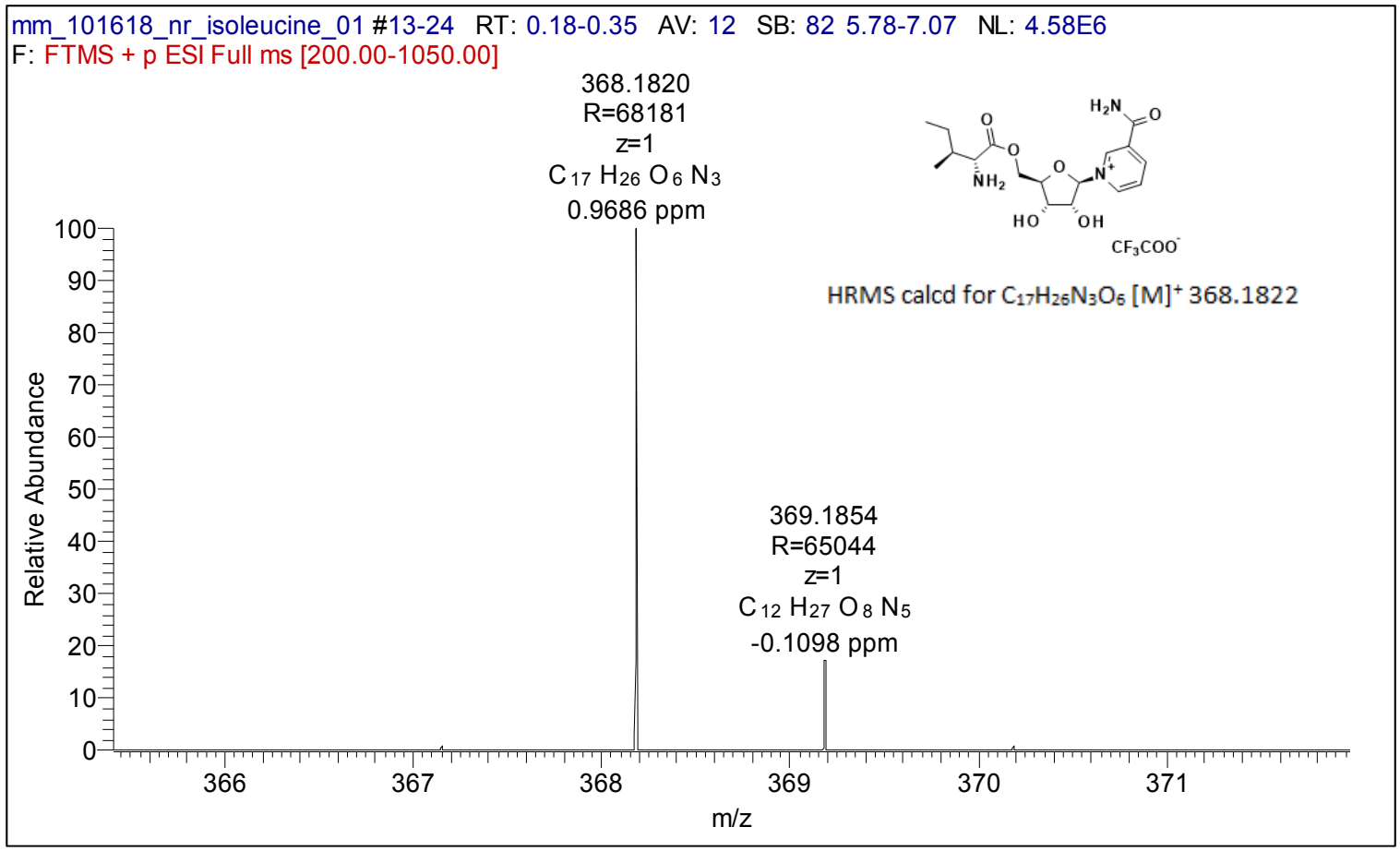


Compound 12f. 377 MHz ^{19}F NMR spectrum in MeOD



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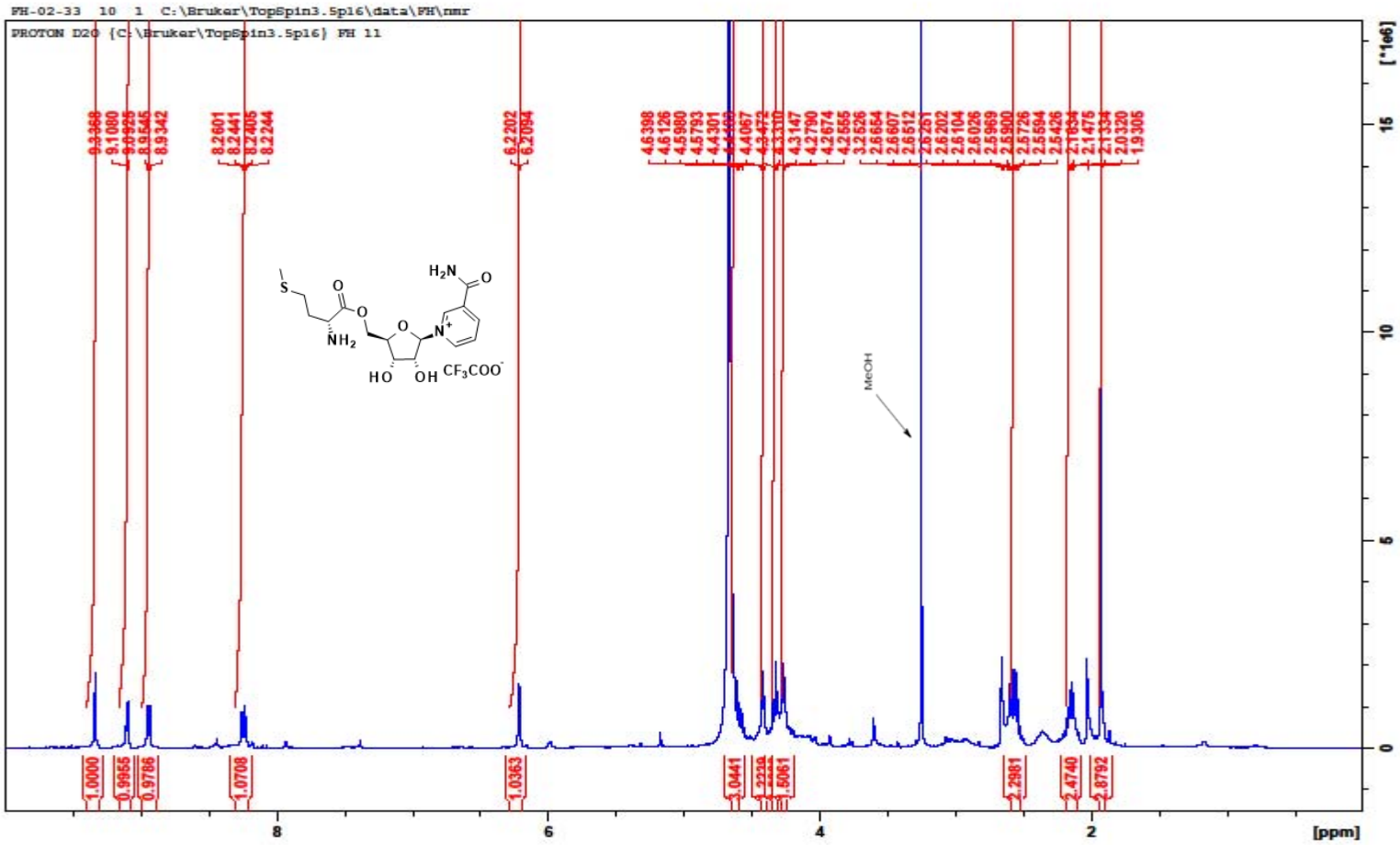
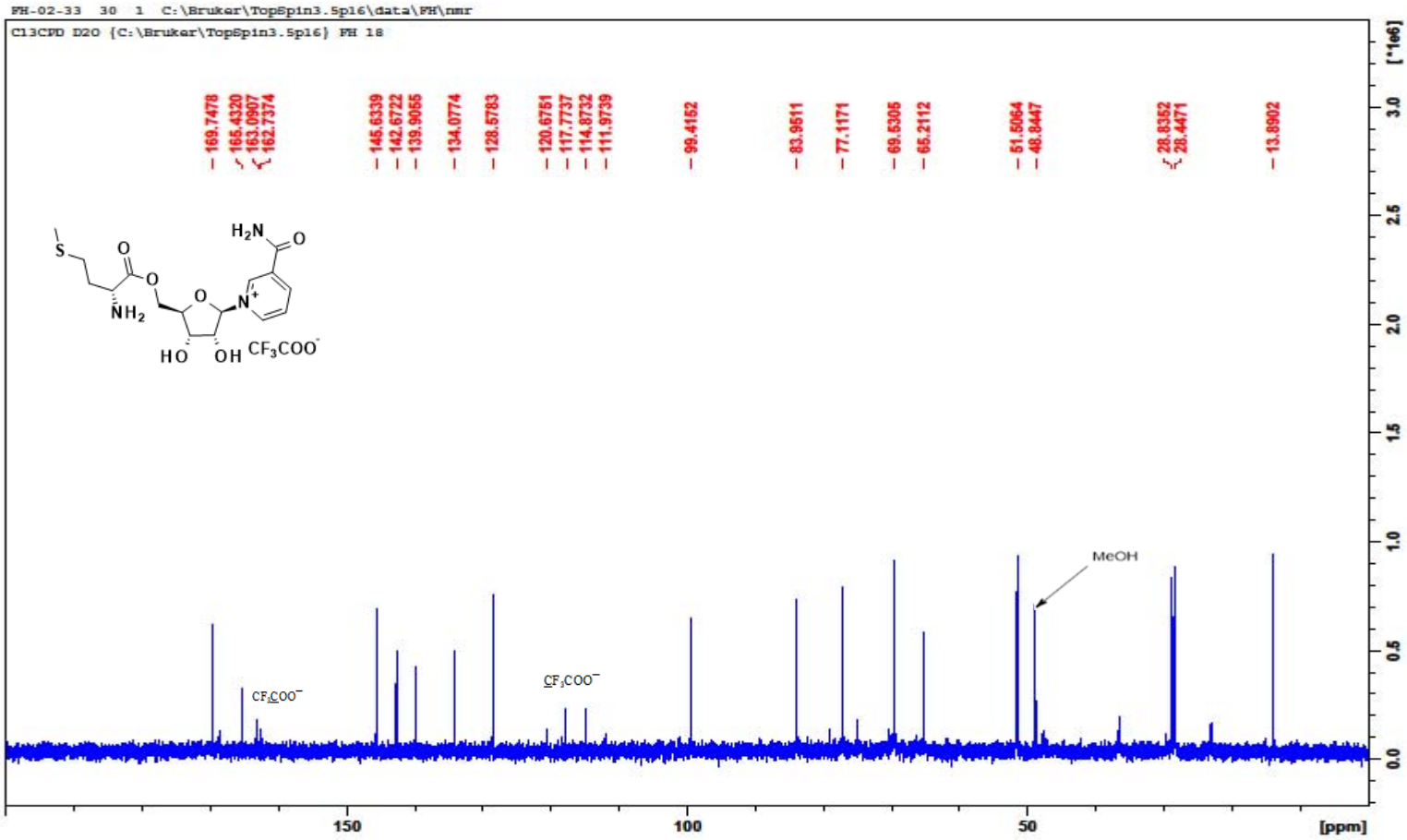


Compound 12g. 400 MHz ^{1}H NMR spectrum in D_2O



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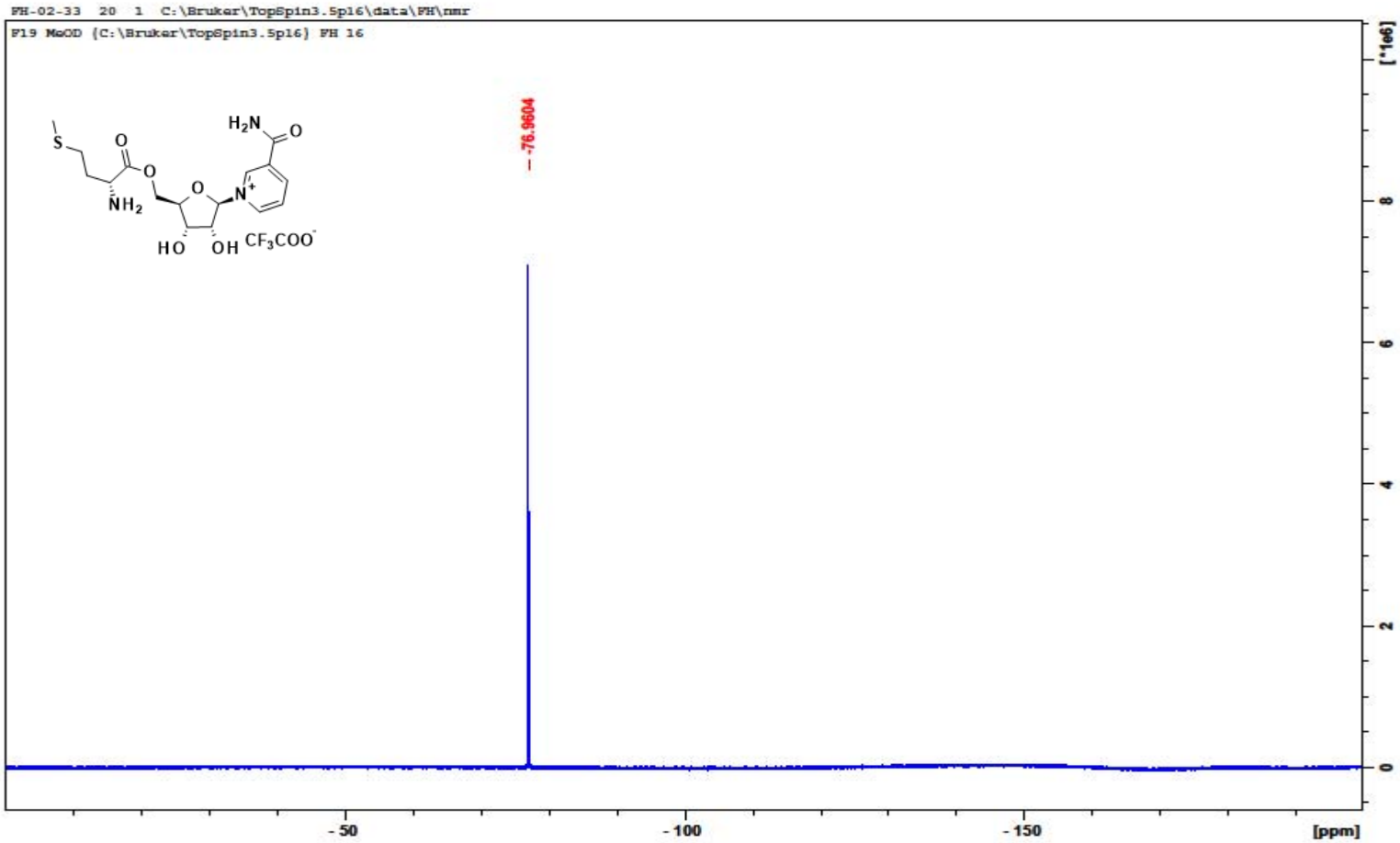
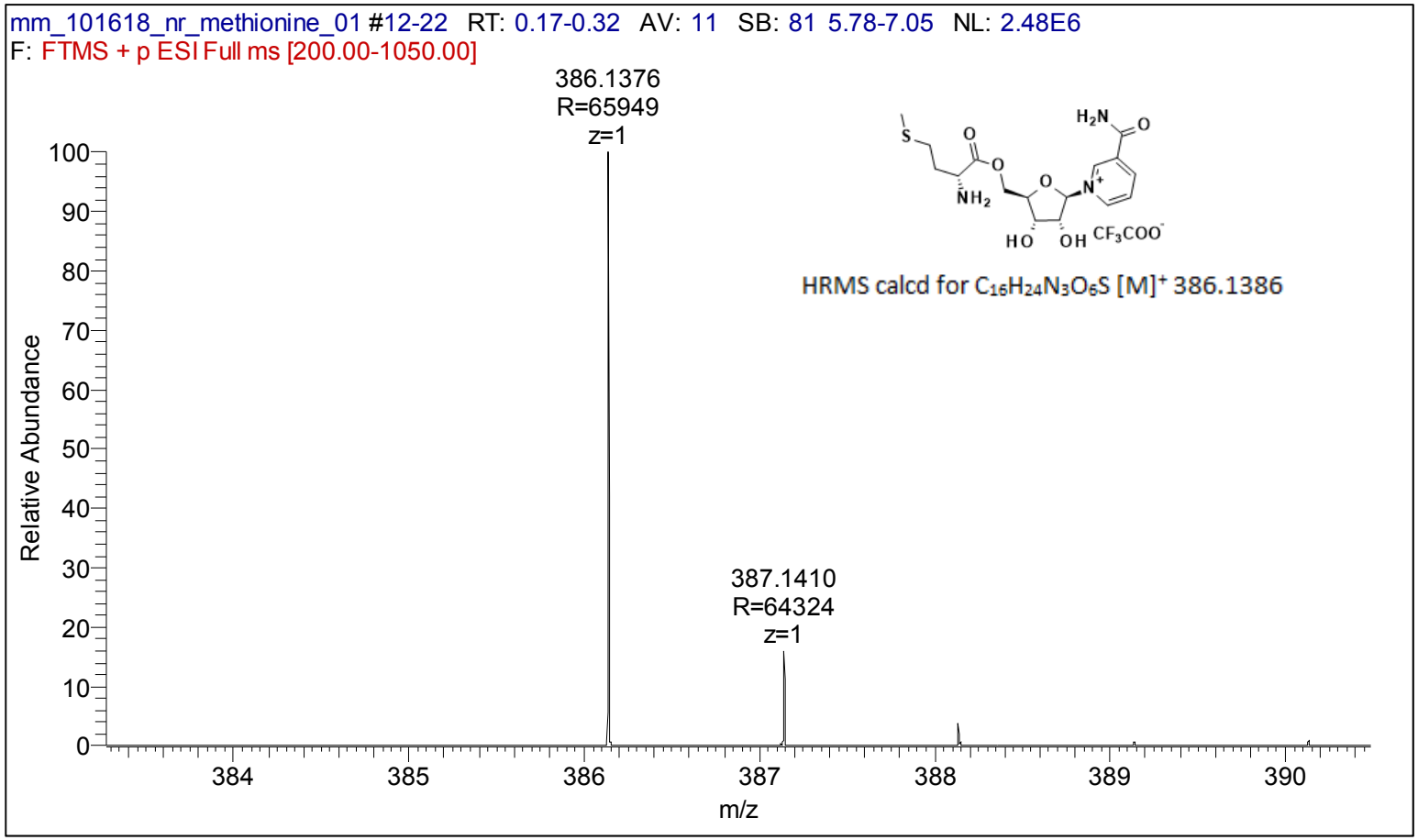
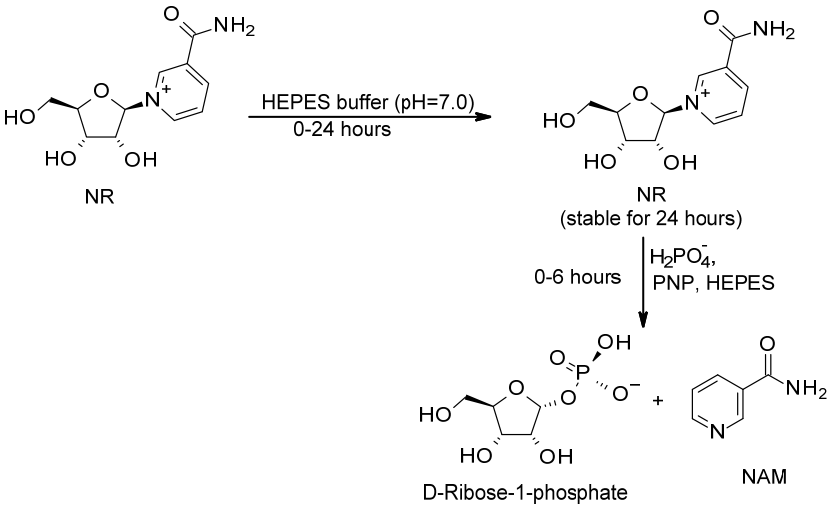
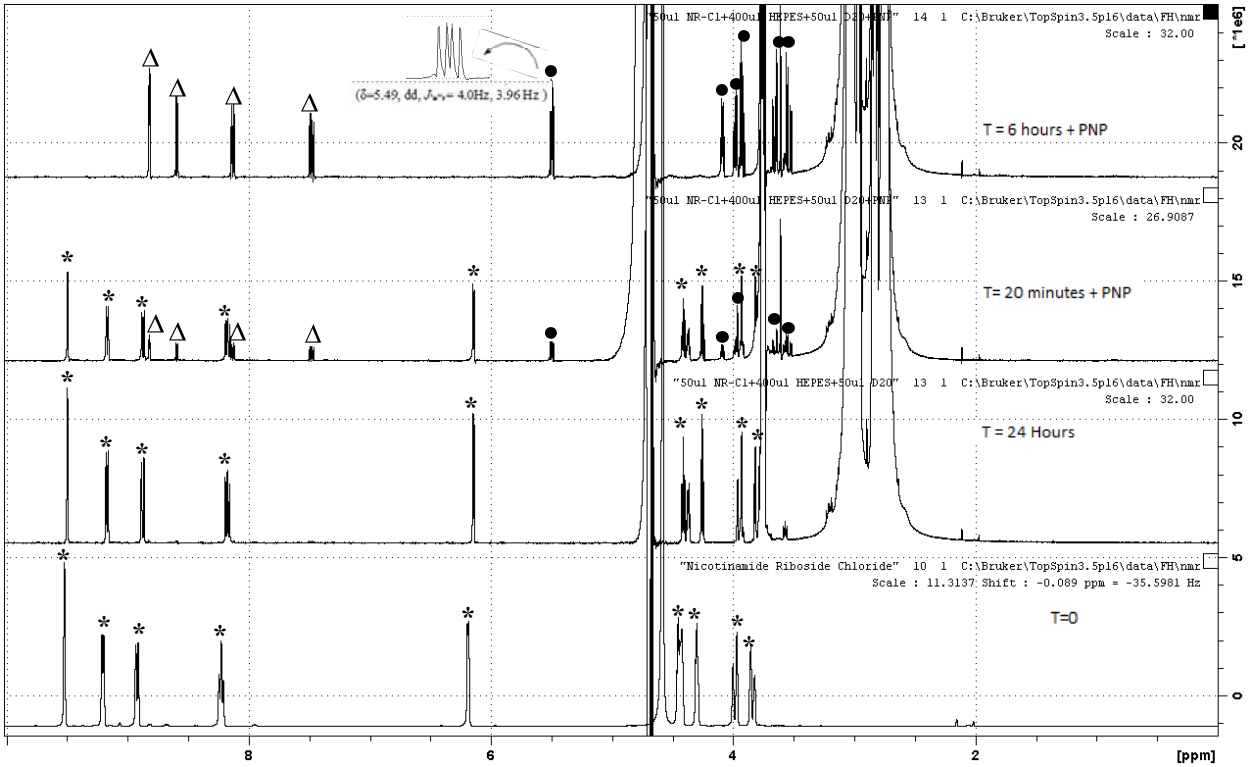


Compound 12g. 377 MHz ^{19}F NMR spectrum in MeOD



Compound 12g. HRMS spectra

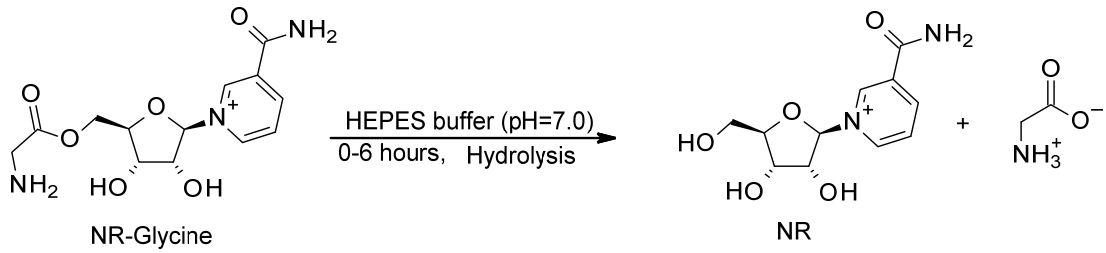


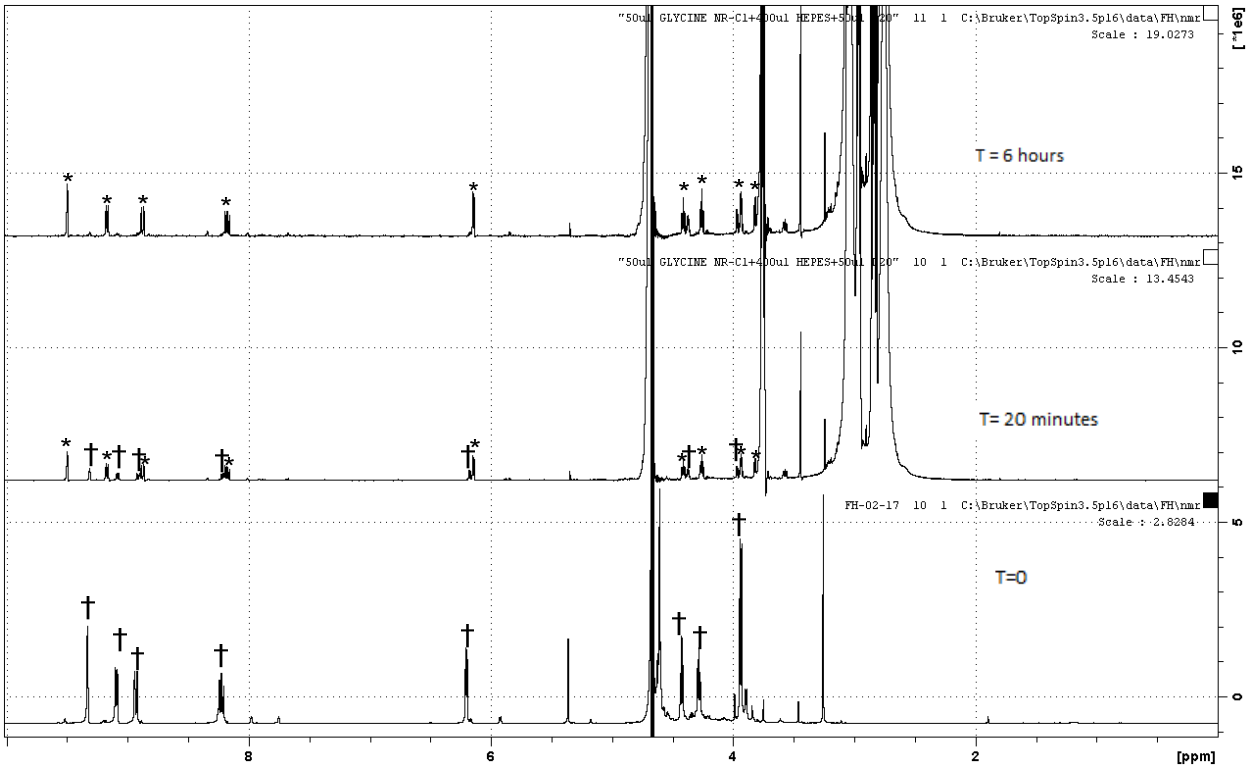


* **Nicotinamide riboside**

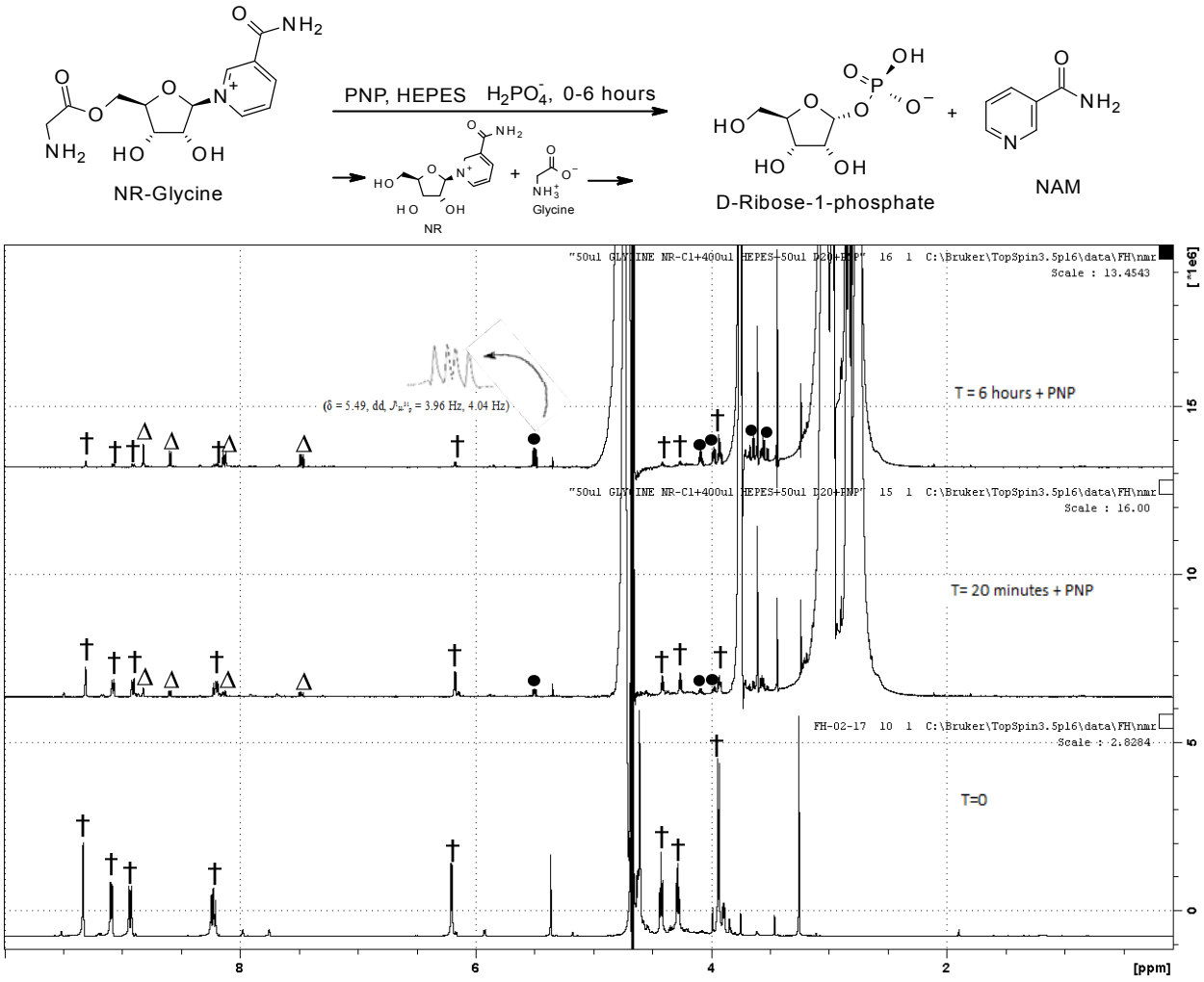
Δ **Nicotinamide**

● **D-Ribose-1-phosphate**





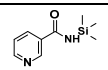
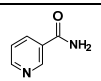
† NR-glycine
*** Nicotinamide riboside**



† 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 ^{1}H NMR at t = 0, 20 min and 6 h. All one dimensional spectra were obtained at 300 K on a Bruker Ascend^{TM} 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 (ns=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 (ns=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 50µl 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_3CN (1 eqv)	trace
5	1 eqv		2 eqv	CH_3CN (3 eqv)	trace
6	1 eqv		3 eqv	CH_3CN (5 eqv)	trace

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