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ELECTRONIC SUPPLEMENTARY INFORMATION to the paper entitled:

Metabolic pathway of flonicamid in oranges using an orthogonal approach based on high resolution mass spectrometry and nuclear magnetic resonance

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Table S1. UHPLC-MS-Orbitrap parameters of flonicamid and metabolites

	<u>Precursor ion</u>		<u>Fragments</u>			Retention Time (min)
	Exact mass	Mass error (ppm)	Exact mass	Molecular formula	Mass error (ppm)	
Flonicamid (F)	230.05357	-0.013	203.04267	C ₈ H ₆ ON ₂ F ₃	-2.630	4.62
			174.01612	C ₇ H ₃ ONF ₃	-0.086	
			148.03686	C ₆ H ₅ NF ₃	-1.016	
TFNA	192.02669	0.002	174.01612	C ₇ H ₃ ONF ₃	-1.810	4.69
			148.03686	C ₆ H ₅ NF ₃	-0.678	
TFNG	249.04815	-0.013	203.04267	C ₈ H ₆ ON ₂ F ₃	-0.512	4.52
			174.01612	C ₇ H ₅ ONF ₃	-0.937	
			148.03686	C ₆ H ₅ NF ₃	-1.448	
TFNA-AM	191.04267	-0.021	174.01612	C ₇ H ₅ ONF ₃	3.880	4.15
			148.03686	C ₆ H ₅ NF ₃	-0.250	

NMR Experimental. NMR spectra were measured on a Bruker Avance III 300 (^1H , 300.13 MHz; ^2H , 46.10 MHz; ^{13}C , 75.47 MHz; ^{19}F , 282.40 MHz), Bruker Avance III 500 (^1H , 500.13 MHz; ^{13}C , 125.76 MHz; ^{19}F , 564.63 MHz), and a Bruker Avance III 600 (^1H , 600.13 MHz; ^{13}C , 150.92 MHz) spectrometers (Bruker, Bremen, Germany), using a 5 mm BBFO $^1\text{H}/\text{BB}(^{19}\text{F})$ probe, an indirect 5 mm TBI $^1\text{H}/^{31}\text{P}/\text{BB}$ triple probe, and a quadruple QCI (^1H , ^{13}C , ^{15}N , ^{31}P) cryoprobe, respectively. The spectral references used were internal tetramethylsilane for ^1H and ^{13}C and an external reference of $\text{CF}_3\text{CO}_2\text{H}$ for ^{19}F . Coupling constants (J) are given in Hertz as positive values regardless of their real individual signs. ^2H NMR spectra were recorded without proton decoupling, although in most cases splitting of the signals could not be observed due to quadrupolar line broadening. All reactions were monitored by ^1H or ^{19}F NMR spectroscopy using Topspin 3.2 on a Bruker Avance III operating at 500 MHz. The experimental temperature was set to the temperature described further below (25, 40, 60 or 80 °C) and maintained by a Bruker BCU II -80/60 temperature control unit. Initially, the temperature was equilibrated with a standard sample containing pure flonicamid in the same concentration as the actual sample.

Sample preparation for NMR analysis. In a scintillation vial, 80 mg of Teppeki® and 0.5 mL of D_2O are added. The vial is stirred and sonicated over 2 minutes. The resulting slurry is filtered off through a Millipore filter (pore size 0.45 μm) into an oven-dried 5 mm NMR tube. An extra volume of 0.2 mL is used to rinse the vial and after that, it is also filtered and then added into the same NMR tube. In order to know the amount of flonicamid (F), 50 μL of a 0.1% D_2O solution of the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid (TSP) as internal standard was added. By relative integration of their corresponding signals in the ^1H NMR spectrum (Figure S3),

4.1 mg of flonicamid (F) was calculated inside the NMR sample. For the hydroxide-catalyzed reactions the same D₂O extract of Teppeki® was employed and 40 µL of a 5M solution of NaOH was added into the same NMR tube. For the acid-catalyzed reaction, an extract of Teppeki® was prepared but using a mixture of ascorbic and citric acids in a ratio 0.7:1 instead of pure D₂O.

Flonicamid-*d*₃. It was obtained as a white solid. ¹H NMR (300.13 MHz, D₂O) δ (ppm): 8.84 (d, 1H, *J* = 4.1 Hz), 8.76 (s, 1H), 7.80 (d, 1H, *J* = 4.1 Hz). ²H NMR (46.10 MHz, THF-*d*₈) δ (ppm): 8.56 (s, ND, 1D), 4.41 (s, CD₂, 2D). ¹⁹F NMR (282.40 MHz, D₂O) δ (ppm): -61.90 (s).

TFNG-*d*₄. It was obtained as a white solid. ¹H NMR (300.13 MHz, D₂O) δ (ppm): 8.73 (s, 1H), 8.72 (d, 1H, *J* = 5.4 Hz), 7.71 (d, 1H, *J* = 5.4 Hz). ¹⁹F NMR (282.40 MHz, D₂O) δ (ppm): -61.71 (s).

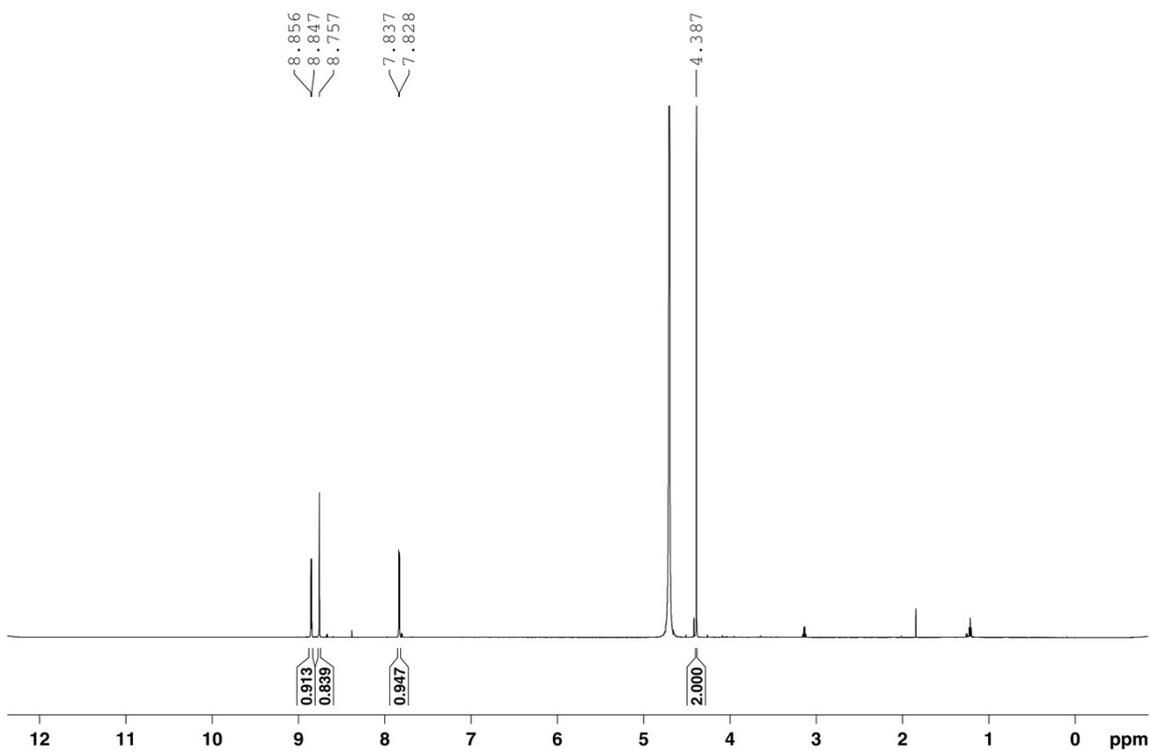


Figure S1. ^1H NMR (600.13 MHz, D_2O) spectrum of flonicamid (F) in D_2O .

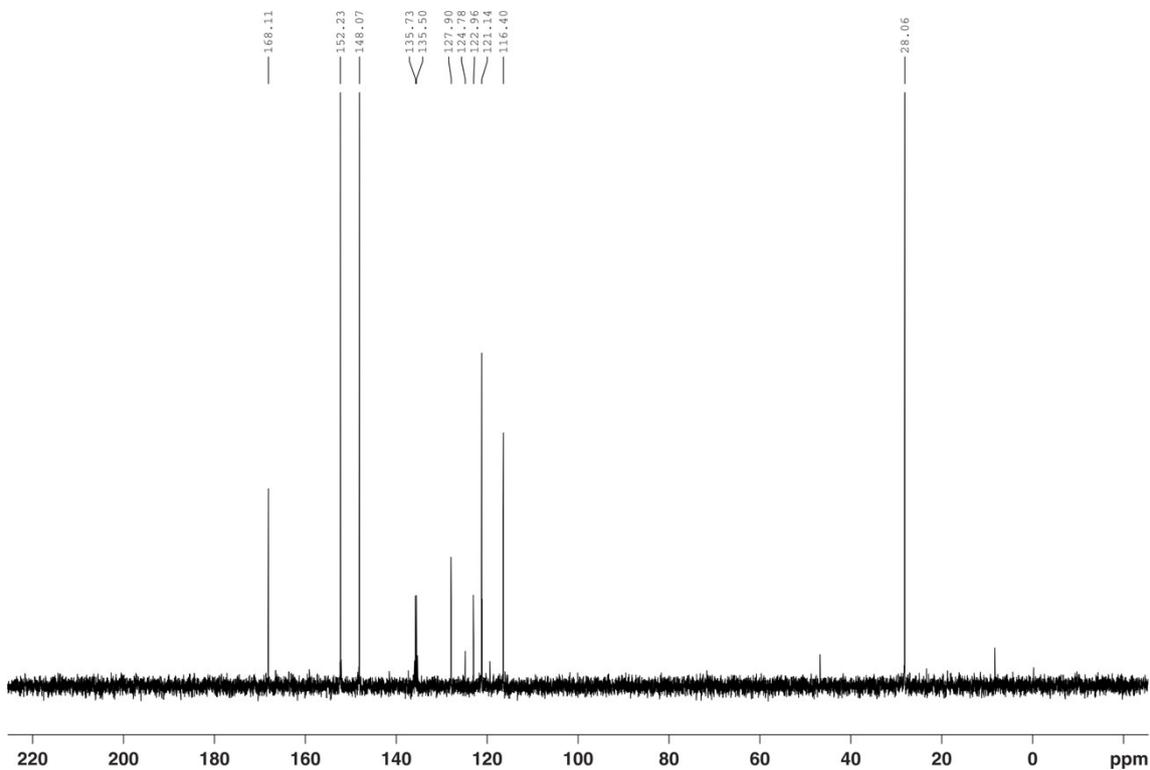


Figure S2. ^{13}C NMR (150.9 MHz, D_2O) spectrum of flonicamid (F) in D_2O .

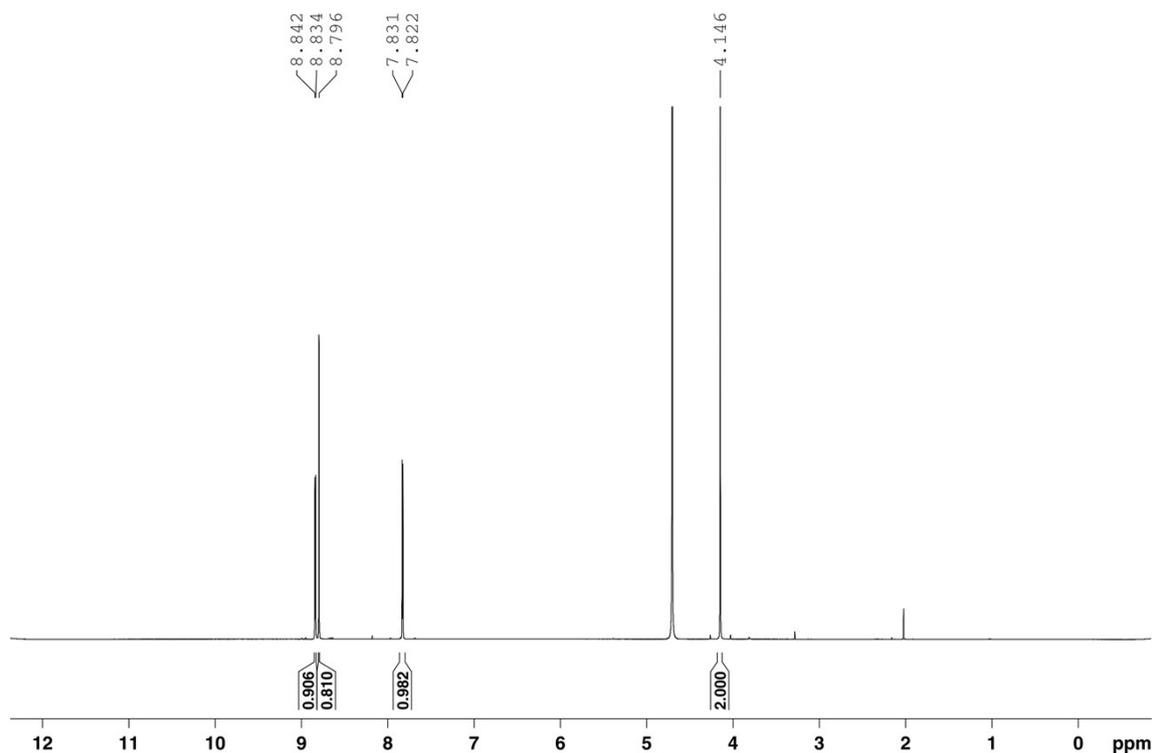


Figure S3. ^1H NMR (600.13 MHz, D_2O) spectrum of 4-(trifluoromethyl)nicotinol glycine (TFNG) in D_2O .

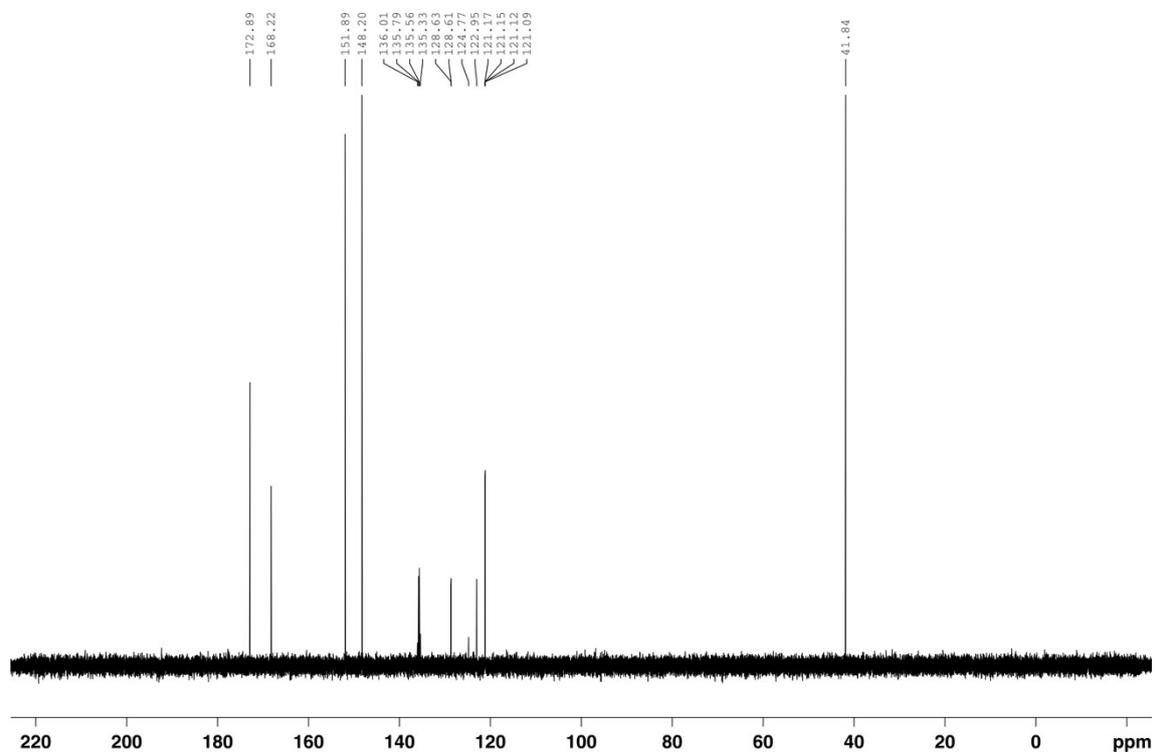


Figure S4. ^{13}C NMR (150.9 MHz, D_2O) spectrum of 4-(trifluoromethyl)nicotinol glycine (TFNG) in D_2O .

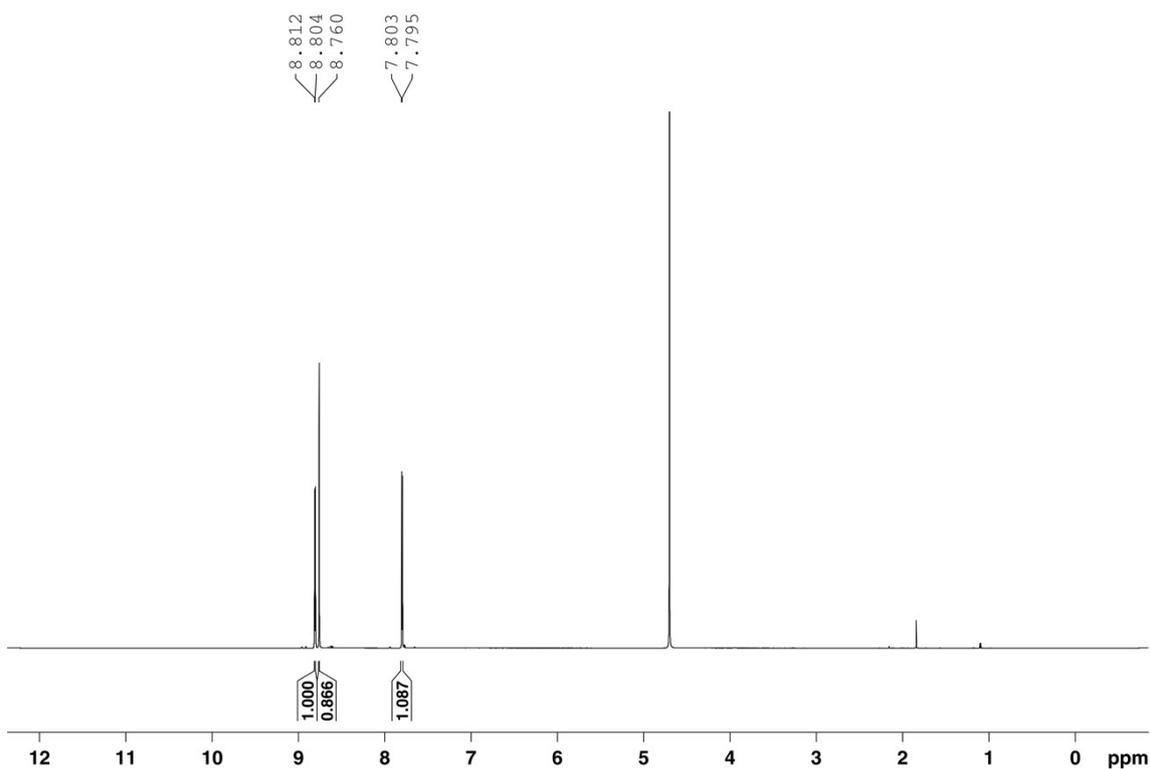


Figure S5. ^1H NMR (600.13 MHz, D_2O) spectrum of 4-trifluoromethylnicotinamide (TFNA-AM) in D_2O .

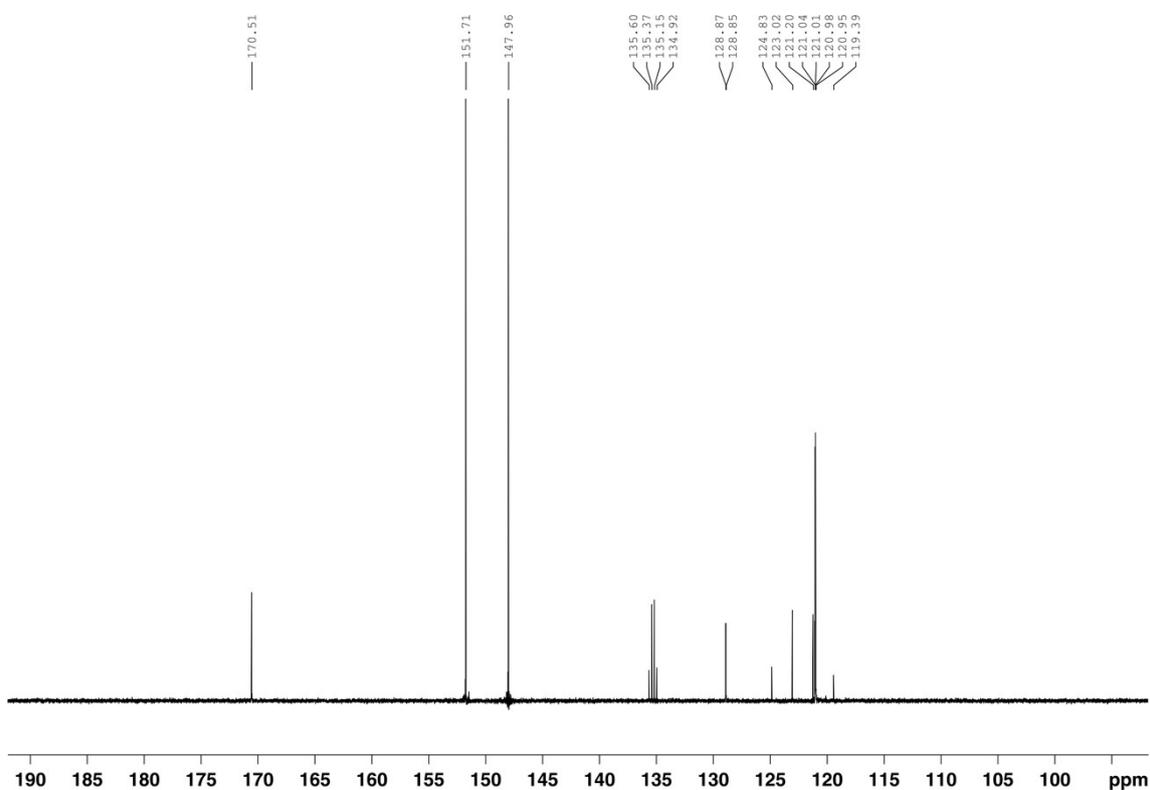


Figure S6. ^{13}C NMR (150.9 MHz, D_2O) spectrum of 4-trifluoromethylnicotinamide (TFNA-AM) in D_2O .

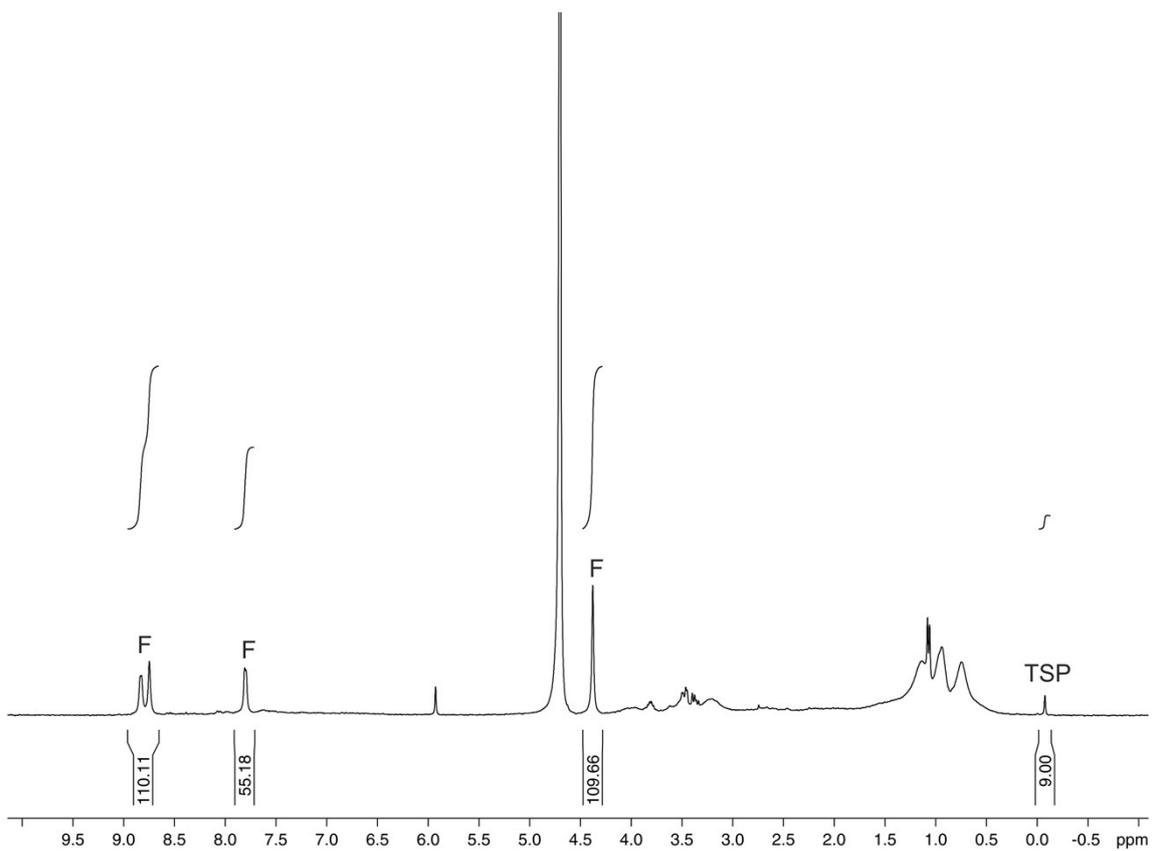


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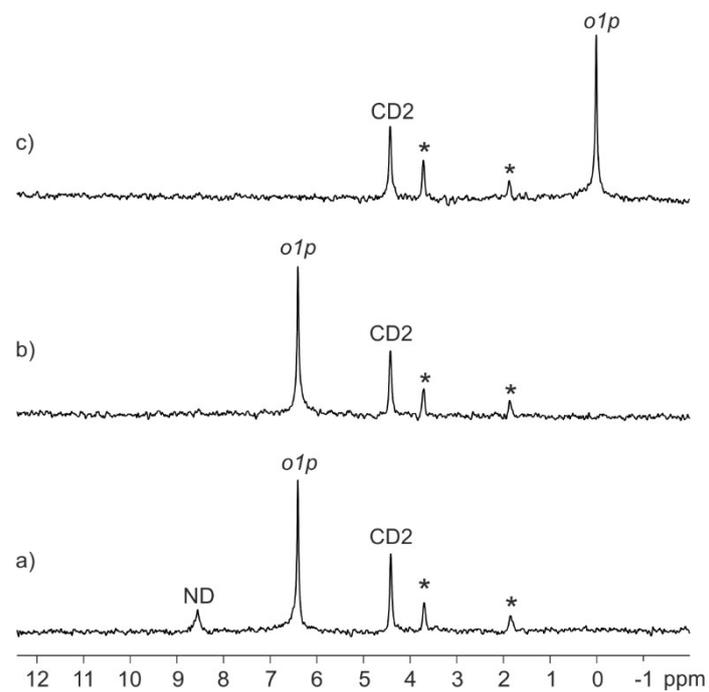


Figure S8. ^2H NMR (46.10 MHz, THF) spectra of a) Flonicamid- d_3 and b-c) Flonicamid- d_2 . The asterisks correspond to partially deuterated THF, and o1p represents the center of the radiofrequency transmitter.

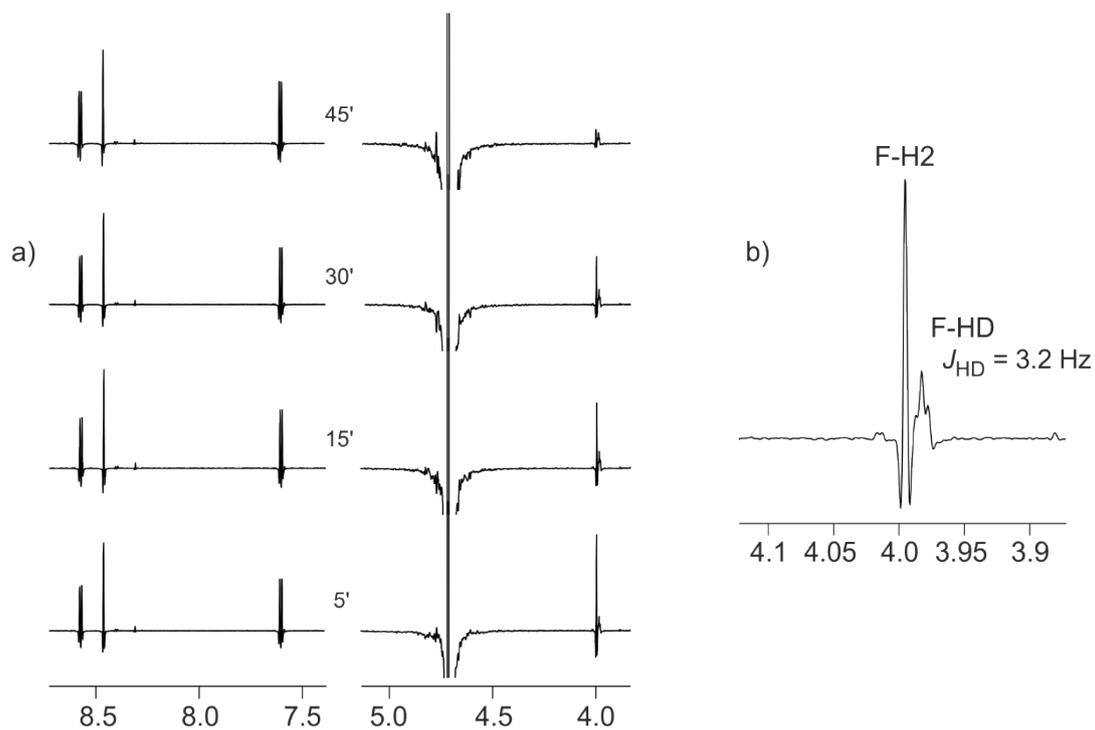


Figure S9. a) ¹H NMR monitoring (500 MHz, D₂O) of the treatment of flonicamid extract with 10 equiv. of NaOH 5 M at 25 °C. In almost one hour of reaction the deuteration of the methylenic carbon is almost complete. b) Expansion of the methylenic region at 30 minutes of reaction. From the up-field shifted signal one can deduce a two bond coupling constant of 3.2 Hz between proton and deuterium.

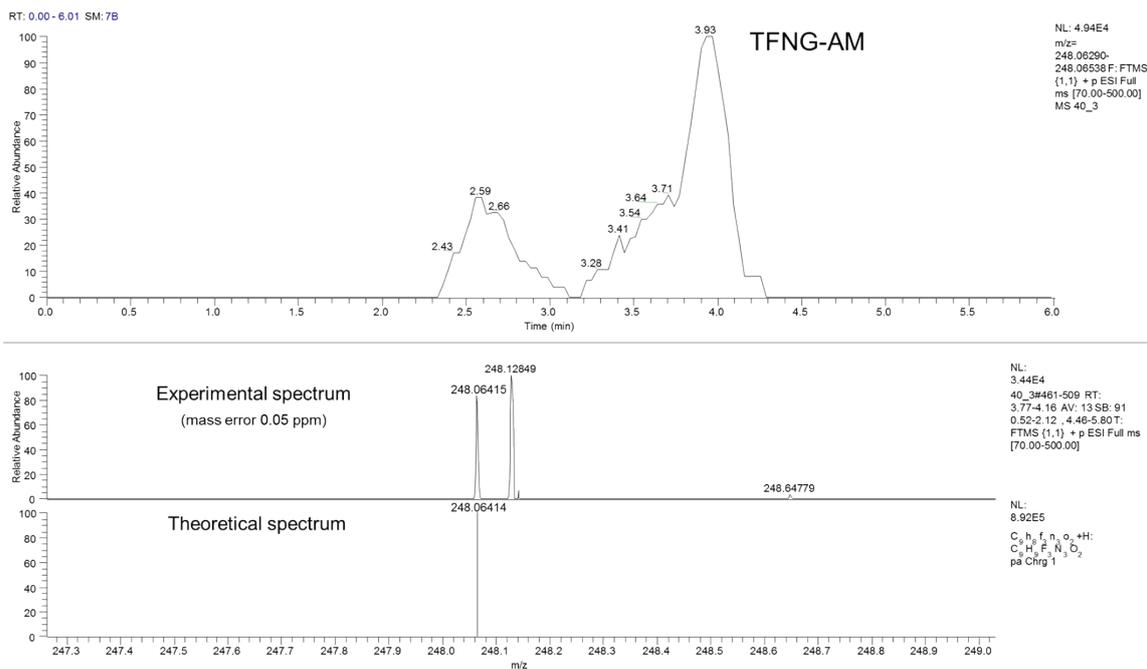


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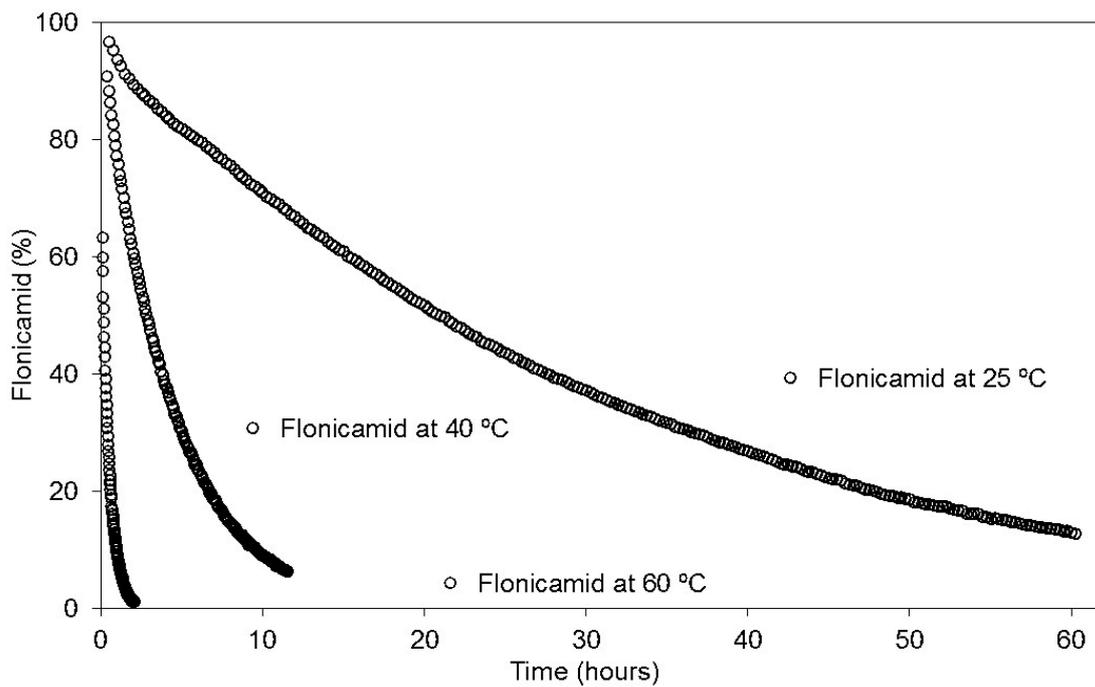


Figure S11. ^{19}F NMR (300 MHz, D_2O) monitoring of the transformation of flonicamid (F) after its treatment with 10 equiv. of NaOH 5M at 25, 40 and 60 °C.

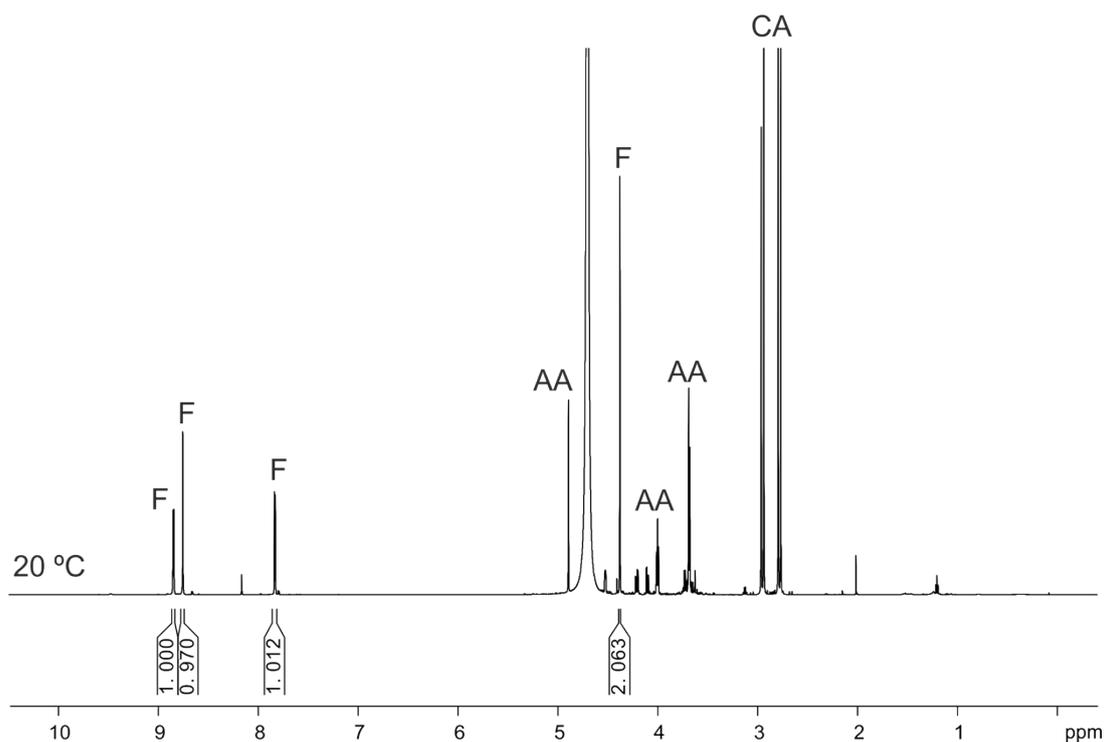


Figure S12. ^1H NMR (500.13 MHz, D_2O) spectrum of flonicamid measured in a mixture of ascorbic and citric acids (0.7:1, respectively) after 72 hours at room temperature (AA = ascorbic acid; CA = citric acid).

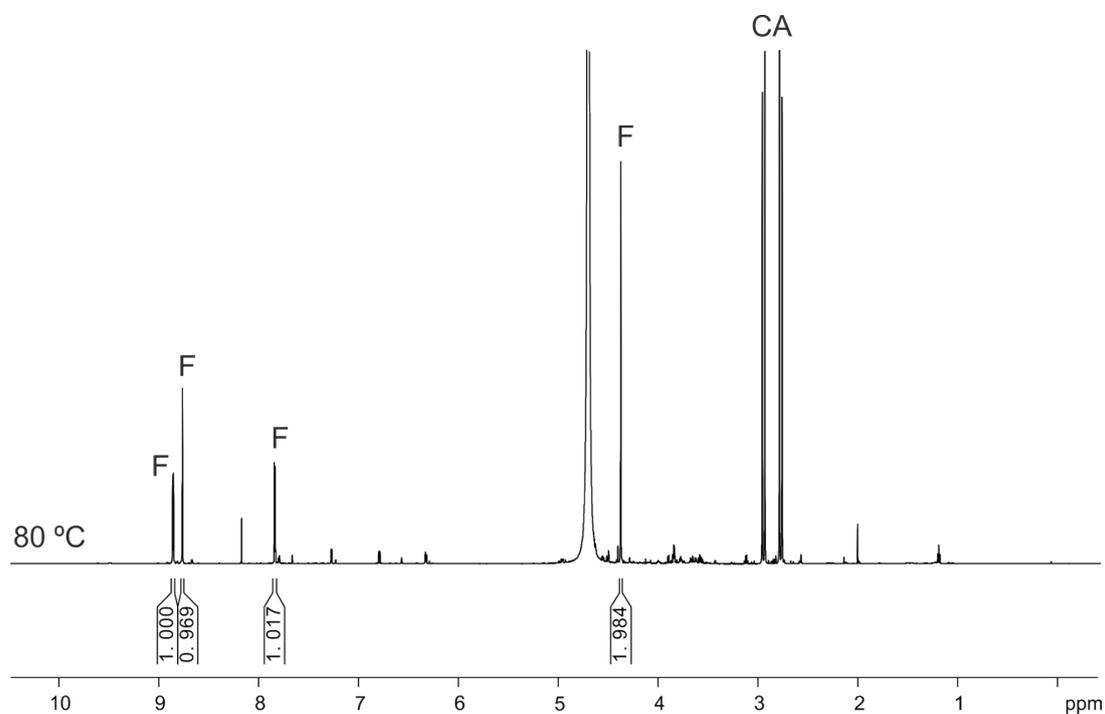


Figure S13. ^1H NMR (500.13 MHz, D_2O) spectrum of flonicamid measured in a mixture of ascorbic and citric acids (0.7:1, respectively) after 72 hours at 80 °C (AA = ascorbic acid; CA = citric acid).