

Supporting Information (S-2)

A Complete Structure Investigation of an Organic Molecule for Chemistry Students; An Analysis of Aspartame

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A Complete Spectral Analysis of Aspartame (instructor only)

UV analysis

The UV spectrum of aspartame is presented in Figure 3 in the article. Table S3 gives an overview of the main findings from the UV experiment.

Table S3. Main findings from the UV spectrum of aspartame

Parameter		Comments
Wavelength maximum, λ_{max}	258.30 nm	Low intensity
Absorption at λ_{max}	0.007	Intensity is increasing close to the "cut-off" wavelength of the cuvette
Concentration	$3,54 \cdot 10^{-5}$ mole/L	Diluted stock solution
Molar absorption coefficient (ϵ) at λ_{max}	$197.7 \text{ M}^{-1}\text{cm}^{-1}$	Forbidden transition

The analysis showed poor absorption of UV light, giving a low ϵ at λ_{max} . The main chromophore in aspartame is aromatic moiety. It is not further conjugated, nor does it contain an activating substituent bonded to the aromatic ring. The effect of an activating versus a non-activating substituent on the aromatic ring is demonstrated by comparing the aspartame spectrum to the spectra of molecules with strong a UV chromophore like salicylic acid, and with the spectrum of ibuprofen, which also has an aromatic ring without further conjugation, as seen in Figure 3a and 3b in the article.

IR analysis

Table S4 summarizes the most important findings from the IR analysis of aspartame. The fingerprint area was not further investigated. The FTIR spectrum is presented in Figure 4 in the article.

Table S4. Main findings from the IR spectrum of aspartame

Functional group	Vibration absorbing IR	Expected absorption ¹ (cm^{-1})	Observed absorption (cm^{-1})	Comments
Amine	N-H stretch	3300-3500 (m)	3323.64 (m)	1° amine
Carboxylic acid	O-H stretch	2500-3600 (s)	3600-2200 (s)	Broad peak
Aromatic region	Aryl-H stretch	3010-3040 (m)	3063.14 3029.88 (m)	Overlapping carboxylic acid O-H stretch
Aliphatic region	C-H stretch in CH_2 - CH_2 and CH_3 groups	2850-2960 (s)	2950.19 (m)	Overlapping carboxylic acid O-H stretch
Ester	C=O stretch	1735-1750 (s)	1736.95 (s)	
Carboxylic acid	C=O stretch	1700-1725 (s)	1665.90 (s)	Overlapping with amide C=O peak
Amide	C=O stretch	1670-1700 (s)	1665.90 (s)	Overlapping with acid C=O peak
Amine	NH_2 bending	1560-1650 (m)	1545.41 (m)	

NMR analysis

The ^1H -NMR spectrum was simplified when the acidic hydrogens of aspartame were replaced by deuterium atoms from the NMR solvent, as shown in Figure S1.



Figure S1. Dissolving aspartame in deuterated NMR solvent replaced acidic hydrogens with deuterium atoms.

The resonances in the ^1H -NMR spectrum, presented in Figure 5 in the article, are summarized in Table S5. The water signal (4.80 ppm) was set as the reference shift². The positions of the resonances are due to the inductive and anisotropic effects. The non-equivalent splitting giving rise to the double-doublet at 4.30 ppm, for the hydrogen at carbon number 2, is most likely due to the large benzyl substituent reducing the free rotation of the neighboring CH_2 group.

Table S5. ^1H -NMR signals of aspartame

Hydrogen	Expected signal (ppm)	Observed signal (ppm)	Splitting	Area
Aromatic H (5-7)	7.25 ¹	6.88	Multiplet	5H
H_2O	4.80 ²	4.80	Singlet	-
CH (2)	4.55 ^a	4.30	Double doublet ($^3J=6.0$ Hz and 7.8 Hz)	1H
CH (9)	3.86 ^a	3.90	Triplet ($^3J=5,4$ Hz)	1H
Methyl ester CH_3 (12)	3.7 ¹	3.28	Singlet	3H
2 x CH_2 (3 and 10)	2.7-2.22 ¹	2.91-2.55	Multiplet	4H

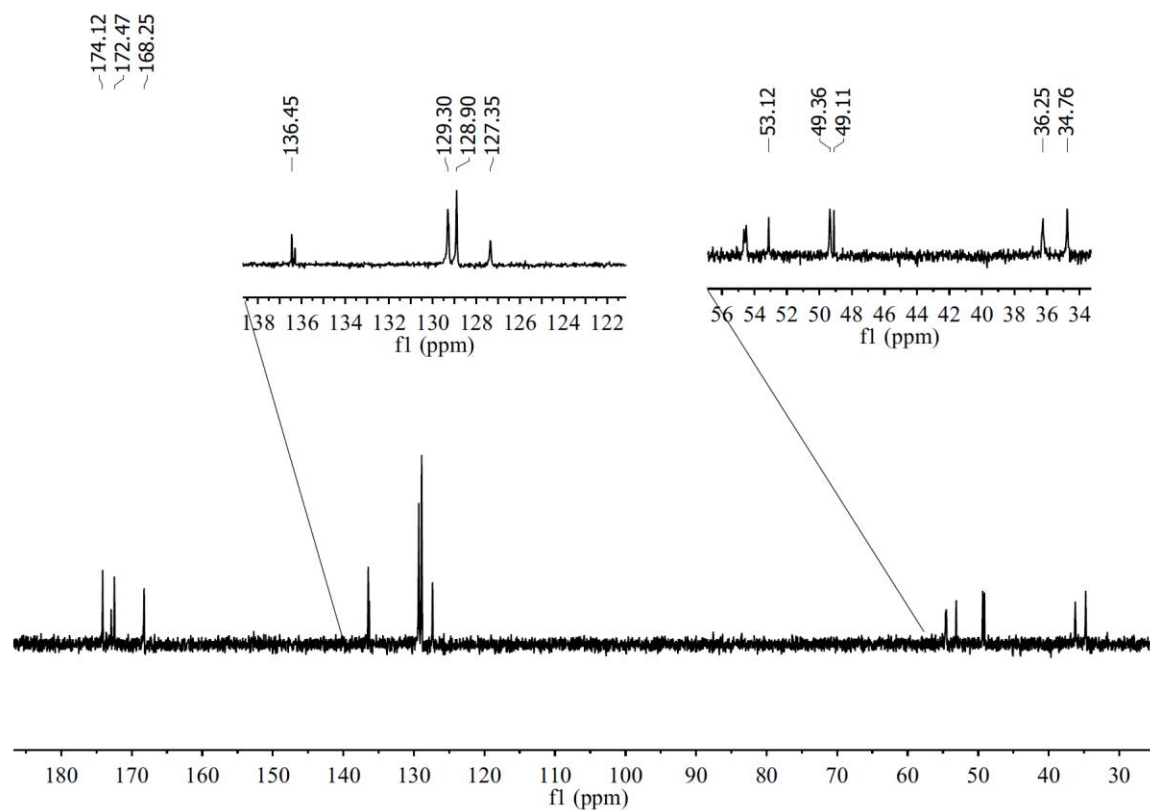
^aCalculated using Chemdraw Professional, Version 23.1.2.7. Revvity Signals Software, Inc.

For the ^{13}C -NMR, presented in Figure 6 in the article, a drop of concentrated HCl was necessary to achieve a concentration that allowed the experiment to be run within an acceptable duration. Aspartame is poorly soluble in neutral water, but by protonation, its solubility is increased, and a spectrum can be obtained after 24 hours. However, in the spectrum, several signals originate from the decomposition products due to the hydrolysis of the ester in the acidic solution. The students were told to focus only on the resonances assigned with ppm values. By comparing the ^{13}C -NMR (Figure S2) to the DEPT spectra (Figure S3) and literature data³, the carbon signals could be designated, as seen in Table S6.

Table S6. ^{13}C -NMR signals of aspartame

Carbon	Expected signal ³ (ppm)	Observed signal (ppm)	Comments
Acid carbonyl (11)	177.0	174.12	No signal in DEPT
Ester carbonyl (1)	174.0	172.48	No signal in DEPT
Amide carbonyl (8)	170.8	168.25	No signal in DEPT
Aromatic ring (4)	137.3	136.45	No signal in DEPT
Aromatic ring (o, 5)	130.1	129.30	Positive signal in DEPT
Aromatic ring (m, 6)	129.8	128.90	Positive signal in DEPT
Aromatic ring (p, 7)	128.2	127.35	Positive signal in DEPT
CH (2)	55.4	53.12	Positive signal DEPT

Methyl ester CH ₃ (12)	53.9	49.35	Positive signal DEPT
CH (9)	51.5	49.11	Positive signal DEPT
CH ₂ (10)	38.2	36.25	Negative signal DEPT 135
CH ₂ (3)	37.3	34.76	Negative signal DEPT 135



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Figure S2. The full ¹³C-NMR spectrum of aspartame in D₂O with one drop of concentrated HCl.

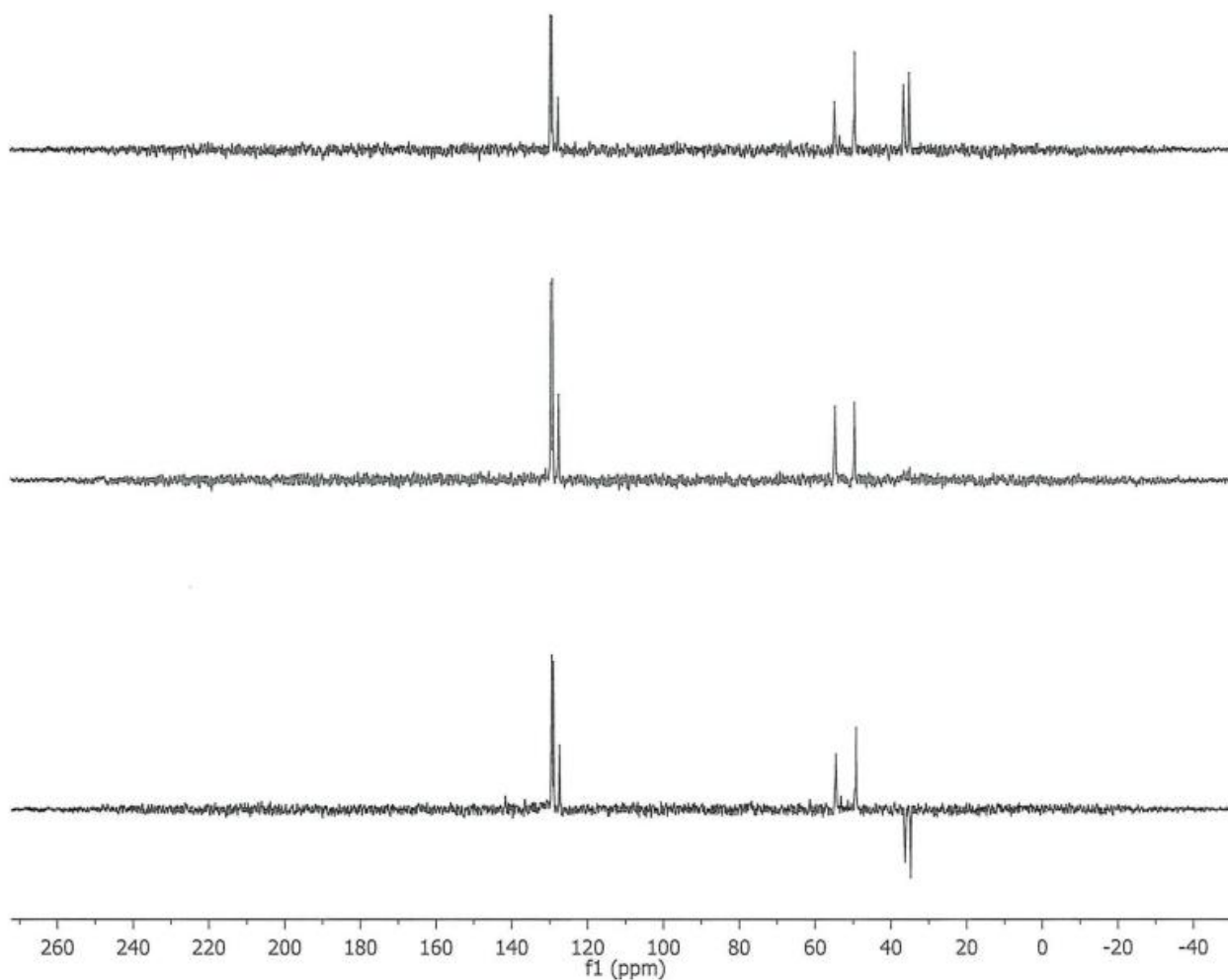


Figure S3. NMR DEPT 45 ° (upper), 90° (middle) and 135° (lower) spectra of aspartame in D₂O with one drop of concentrated HCl.

MS analysis

In the library MS spectrum of aspartame⁴, only two peaks dominated. It was the peak at m/z 262, which was a result of a loss of methanol, a common loss for methyl esters, and the “base peak” at m/z 91, the tropylium ion. This spectrum can be used if the result from the MS instrument is hard to interpret.

The GC-chromatogram acquired by the students is shown in Figure S4. Decomposition of the sample was explained to the students, and the peak at 8.45 was analyzed because we could identify many of the fragment ions, resulting from an intact aspartame.

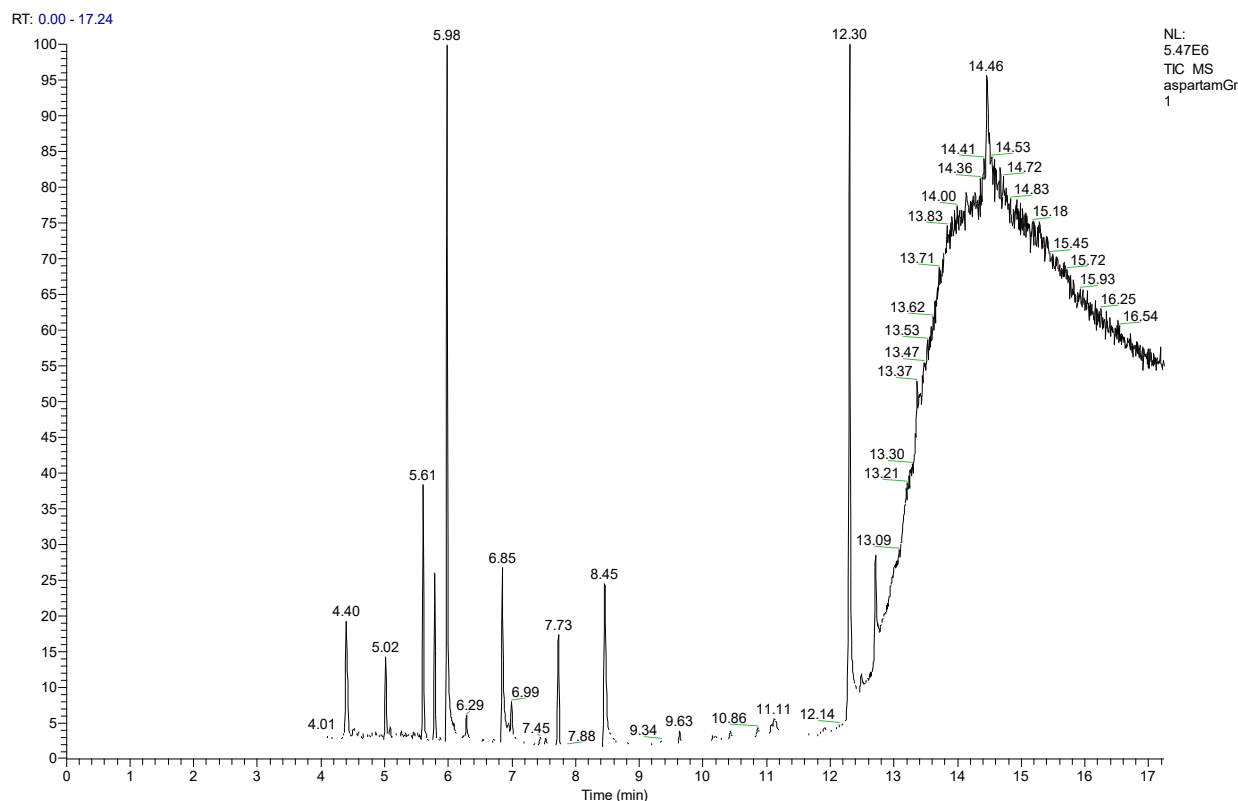


Figure S4. GC chromatogram of aspartame in methanol, where decomposition in the inlet of the GC-instrument is clearly visualized.

The typical fragmentations were identified¹ by the students, as shown in Figure 7 in the article. The result is summarized in Table S7. We decided to use the spectrum obtained by the students to demonstrate that real-life samples might not always be as easily interpreted as textbook examples.

Table S7. Spectral data from the MS spectrum

Fragment (m/z)	Comments
294	Molecular radical ion (M^+) is not visible
205.99	Ion formed from β -cleavage by the carbonyl amide
163.00	Product from McLafferty rearrangement at the carbonyl amine
161.14	Base peak: a product from McLafferty rearrangement at the carbonyl amine
91.12	The tropylium ion, a stable cation
77.04	Phenyl carbocation

References

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