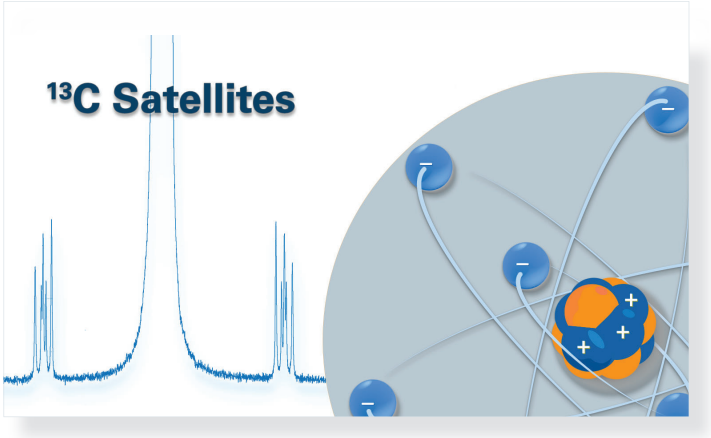

^{13}C Satellites in Proton NMR Spectroscopy

Analyzing the ^{13}C satellites in proton spectra enables interesting experimental strategies. In this application note, we give examples which illustrate the vast amount of information that can be obtained from ^{13}C satellites.

Typical high-resolution NMR spectrometers, such as the AvanceCore, can directly record carbon spectra which provide high spectral dispersion over a range of approximately 0 to 200 ppm. In addition, ^{13}C nuclei in the sample also affect the proton spectrum and manifest themselves as "^{13}C satellites". The ^{13}C satellites are observed to the right and left of hydrogen signals that originate from carbon-containing moieties such as CH, CH_2 and CH_3 groups.

Analyzing ^{13}C satellites offers the possibility to obtain "carbon information" combined with the superior sensitivity of the ^{1}H nucleus, enabling interesting experimental strategies. Some of these strategies are outlined in this application note.

^{13}C Satellites for the Quick Classification of ^{1}H Signals

NMR is a popular analytical method in chemistry, mostly due to its ease of use and due to the wealth of structural and quantitative information it yields. For instance, a ^{1}H NMR spectrum can be used to assign the molecular structure. This has briefly been described in the application note "How to Read and Interpret NMR Spectra in 15 Minutes". When working with organic molecules, an even quicker overview can be gained from analyzing the presence of the ^{13}C satellites. Assuming that a sufficiently high signal-to-noise (S/N) ratio is achieved, carbon satellites mark hydrogen atoms that are bound to a carbon atom. Other signals, such as signals from residual water, hydroxy groups, or amines that are not bound to a carbon nucleus, do not have ^{13}C satellites. For example, the ^{1}H spectrum of the small organic molecule isopropyl alcohol contains two signals which are accompanied by satellites (from the CH_3 and CH groups), while the hydroxy signal merges with the signal of the residual water and has no satellites (Figure 1B).

Figure 1

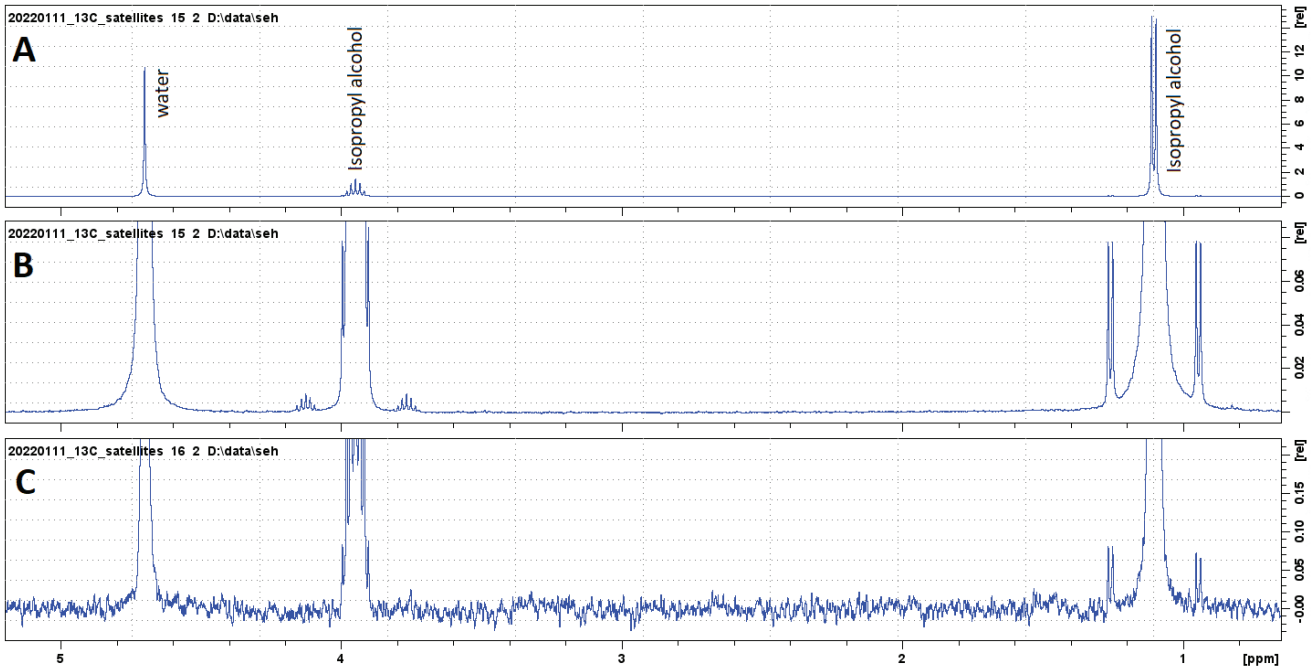

Figure 1: ^{1}H NMR spectrum of isopropyl alcohol in D_2O. **A:** Overview **B:** Zoomed to the intensity of the ^{13}C satellites and acquired with 16 scans. ^{13}C satellites are observed symmetrically around the central signals at 1.1 and 3.9 ppm, **C:** Zoomed to the intensity of the ^{13}C satellites acquired with 1 scan.

^{13}C Satellites as an Error Estimate for Integration and Quantitation

In addition to the structural assignment, the quantitative composition of a reaction mixture is often of interest. With NMR, the gravimetric share of a compound in a mixture (% weight/weight) can be deduced from a quantitative proton spectrum.

Other common analytical techniques, such as HPLC and GC, quantify single compounds against individual reference standards. Such reference standards are either purchased or specifically synthesized and qualified as reference material. When using NMR, several compounds can be quantified against a single reference standard. Several suitable reference standards are commercially available and can be bought for a variety of solvents. Consequently, NMR is a straight-forward method to accurately and quickly establish the gravimetric sample composition of mixtures.

When dealing with a larger number of samples, it is often not time-efficient to establish the entire gravimetric composition of each analytical sample. Instead, the mass fraction of the main compounds (the assay values) can be determined with quantitative NMR. Successively subtracting the assay values from 100% reveals which gravimetric share can be allotted to the remaining components or impurities, assuming the experimental error of each assay value is known.

The error of an assay determination can be roughly estimated from the ^{13}C satellites. If the ^{13}C satellites are clearly visible (sufficient S/N, such as the isopropyl alcohol signal at 1.1 ppm in Figure 1B), the integral error of the central signal is likely below 0.55% (0.5% = 1.10%/2, where 1.10% is the natural abundance of ^{13}C). Note that this simple procedure does not account for errors due to spectral distortions such as baseline deviations or overlain peaks.

In case of complex mixtures or if the available sample amount is limited, the S/N can be reduced (e.g., as shown in Figure 1C). The isopropyl alcohol signal at 1.1 ppm has clearly discernible ^{13}C satellites and the related integral error is thereby assumed on the order of 0.55% or better. However, the S/N ratio is not sufficient to detect the satellites of the signal at 4.0 ppm. The noise of the baseline is larger than the ^{13}C satellites so that the integral error is likely larger than 0.55%. The noise also limits the accuracy and precision of the baseline correction, which can lead to large errors in the integrals that are not obvious and often stay unnoticed.

Increasing Spectral Dispersion by Decoupling ^{13}C Satellites

The ^{1}H NMR experiment is one of the most sensitive NMR experiments which makes it possible to acquire signals from diluted compounds or traces in the presence of the concentrated sample matrix. However, its spectral dispersion is limited. For example, the spectral dispersion of the AvanceCore NMR spectrometer is 400 Hz per 1 ppm (since the AvanceCore is a 400 MHz NMR spectrometer). At a given dispersion, signals are increasingly likely to overlap as the number of signals in the spectrum goes up. To alleviate this, a higher field strength can be chosen (for example an Avance NEO 600 MHz).

The spectrum can also be simplified by decoupling the ^{13}C satellites. An example is shown in Figure 2.

Figure 2

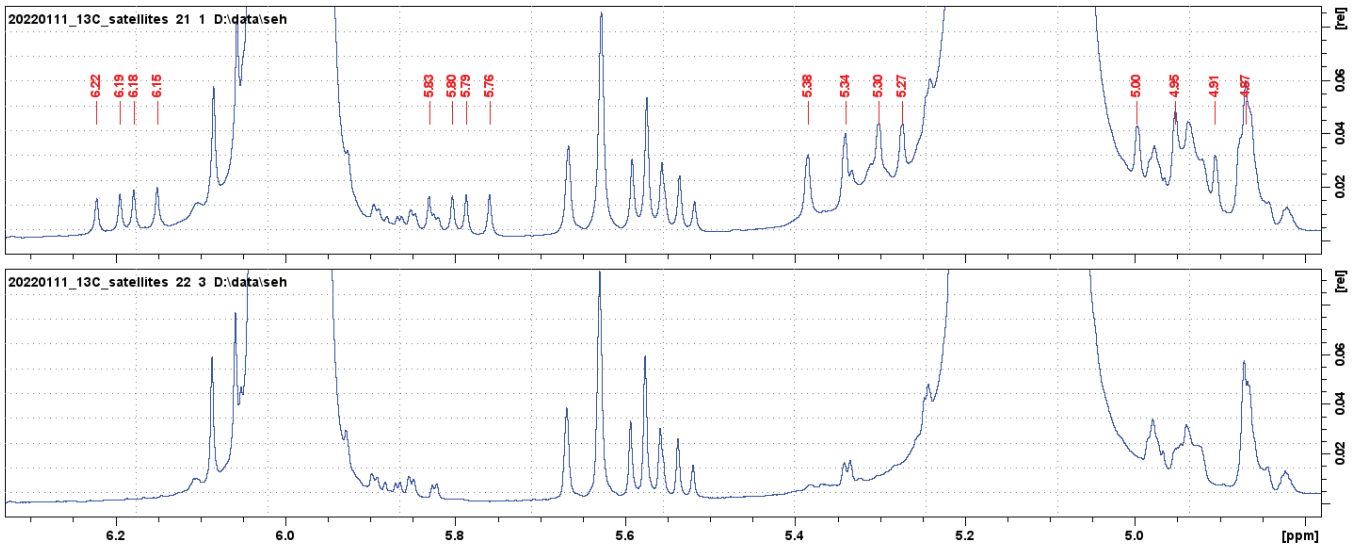

Figure 2: NMR spectrum of linalyl acetate in DMSO. **Top:** Standard ^{1}H experiment, **Bottom:** ^{1}H experiment with carbon decoupling. The carbon decoupling collapses the ^{13}C satellites until they merge with the central peaks they are originating from. By comparison, the ^{13}C satellites are marked with red labels **(top)**. Decoupling the satellites simplifies the spectrum and enables the identification of smaller signals which were previously partly overlain by the satellites (e.g., at 4.95 ppm, 5.33 ppm and 5.83 ppm).

Typical ^{1}H NMR spectra, such as those from the small organic molecule linalyl acetate, contain many ^{13}C satellites (Figure 2 **top**) that can be unwanted as they occupy significant amounts of spectral space which increases the likelihood of overlap, can obscure other signals, and complicates the assignment of trace compounds. The ^{13}C satellites can be suppressed by "decoupling", which causes the ^{13}C satellites to largely merge with the central signal so that they are effectively removed from the spectrum (Figure 2 **bottom**). Decoupling is accomplished by ^{13}C-irradiation using a decoupling sequence such as "garp", "waltz" or adiabatic shapes, which aim to facilitate uniform decoupling over a certain spectral range of carbon frequencies. For ^{13}C decoupling, an AvanceCore Select or Convenience is required.

In most cases, the ^{13}C satellites exhibit the same multiplet structure as the central signal and have the same spectral width. Removing one ^{13}C satellite from the spectrum doubles the resolving power of the instrument; removing the second satellite increases the overall resolution power by a factor of four. ^{13}C decoupling is thus a very straightforward way to increase dispersion and in contrast to many other experimental strategies which also increase dispersion but trade in S/N (e.g., multidimensional experiments or heteronuclear correlation), ^{13}C decoupling leaves the sensitivity of the NMR experiment unchanged. Employing ^{13}C decoupling leverages the full potential of ^{1}H NMR spectroscopy to sensitively detect trace compounds and boosts the sensitivity.

Starting materials, reaction products and by-products are often closely related by the similarity of their structures, causing NMR signals to cluster in certain regions of the spectrum. ^{13}C decoupling specifically increases the resolution where it is needed and thereby helps to reduce the likelihood of spectral overlap and unleashes NMR's full potential to sensitively determine reaction products, mixtures, and traces.

How to Set Up ^{13}C Decoupling

Using an AvanceCore spectrometer (with Select or Convenience configuration), carbon-decoupled proton spectra can be obtained by starting from a standard ^{1}H experiment and performing the following steps:

- Go to the ACQUPARS tab and enter the following parameters
- PULPROG = zgig
- In the row NUC1 click "Edit" and activate ^{1}H for channel F1 and ^{13}C for channel F2. Click "Default" and "Save and Close" to establish the default routing.
- In the line CPDPRG 2 select the decoupling sequence "garp". (For "narrow-band" decoupling "waltz" can be used as well).
- Type "getprosol" in the TopSpin command line to set the pulse parameters.
- In the line O2, define the center of the carbon signals in ppm (e.g., 100 ppm) or type o2p in the TopSpin command line to define the center. In case carbon signals group in a certain spectral region (e.g., around 60 ppm for aliphatic compounds), the carbon frequency can be chosen accordingly to obtain the optimal decoupling performance.
- Start the experiment with "zg".

An alternative approach to setup carbon-decoupled proton experiments employs adiabatic decoupling that allows to freely configure the decoupling bandwidth:

- Create a new experiment and/or read the parameter set P_PROTON_IG by typing "rpar P_PROTON_IG" in the TopSpin command line.
- Type "getprosol" in the TopSpin command line to set the pulse parameters.
- Start the experiment by typing "xaua" in the TopSpin command line. Use "xaua". In contrast to "zg", "xaua" launches the WaveMaker to setup adiabatic decoupling.
- To optimize the experiment, follow the instructions given in the TITLE tab.

Isomers, Symmetric Structures and ^{13}C Satellites

NMR is particularly powerful as an analytical method as it can discriminate between different molecular isomers which have the same molecular mass.

Isomers can complicate mixture analysis by HPLC/GC/MS: After resolving the mixture components using column chromatography, the mass spectroscopical analysis reveals the associated mass. In case of isomers, identical masses are obtained for several HPLC peaks, which can lead to an ambiguous assignment.

NMR complements the mass spectroscopical analysis as it is nicely suited to study the structural similarities of isomers, even in the case of regio-isomers. Regio-isomers don't only have identical molecular formulae and hence the same molecular mass, but in addition, they also feature similar molecular structures and often only differ in the location of a few functional groups. Such symmetric structures can be challenging to assign as they show a reduced number of signals in the NMR spectrum. For example, the ring-structure of benzene contains six hydrogen atoms. Due to its high symmetry, it is observed as a single singlet in the ^{1}H NMR spectrum as shown in Figure 3.

Figure 3

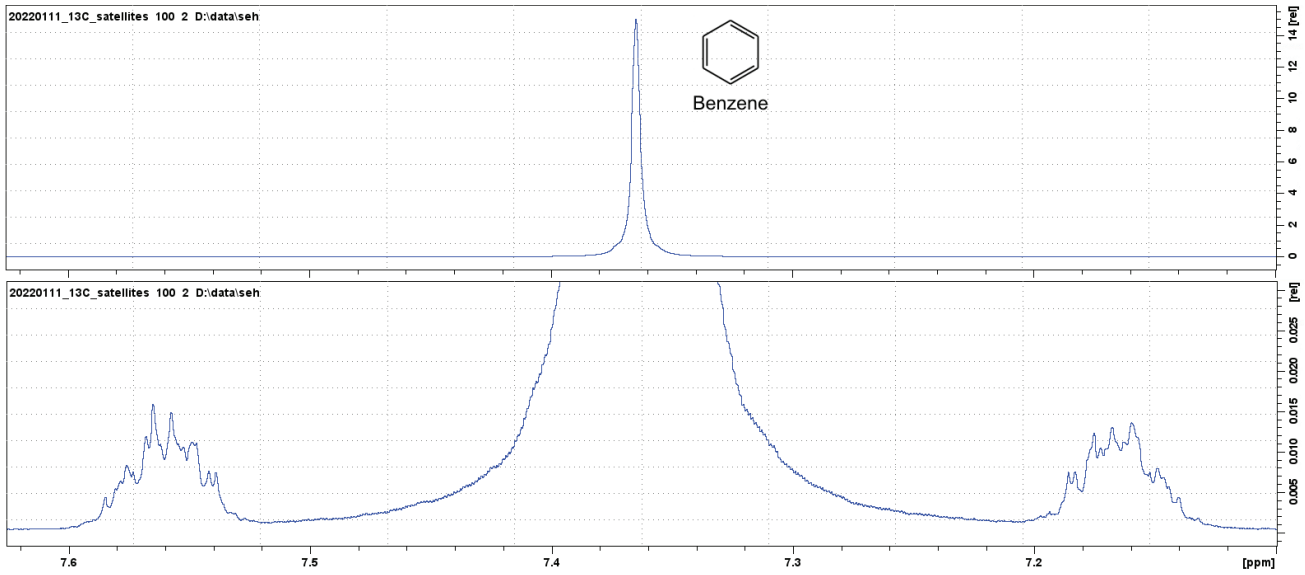

Figure 3: ^{1}H NMR spectrum of the symmetric compound benzene. **Top:** Overview spectrum **Bottom:** Zoomed to the intensity of the ^{13}C satellites. In benzene, the multiplicity of the satellites differs from the multiplicity of the central signal which indicates that the spectrum must be from a substance with a symmetric structure.

^{13}C satellites break the symmetry and reveal additional information.

Figure 4

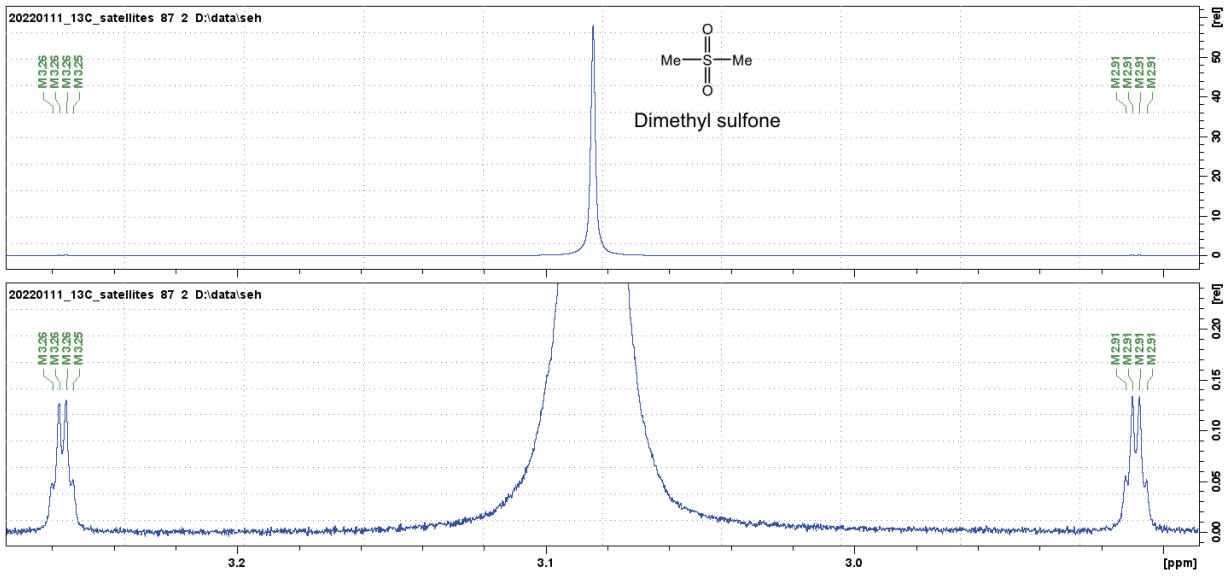

Figure 4: ^{1}H NMR spectrum of the symmetric compound dimethyl sulfone. **Top:** Overview spectrum **Bottom:** Zoomed to the intensity of the ^{13}C satellites. In dimethyl sulfone, the multiplicity of the satellites (quartet, green peak assignment) differs from the multiplicity of the central signal (singlet) which indicates that the spectrum must be from a substance with a symmetric structure.

Another example is dimethyl sulfone. The symmetric molecule contains two methyl groups (Figure 4) that contribute to a single singlet at 3.1 ppm. As the signals of both methyl groups have the exact same chemical shift, spin-coupling is not observed (isochrone nuclei, i.e. nuclei with the same chemical shift, don't couple).

Usually, the ^{13}C satellites have the same multiplicity as the central signal. However, in dimethyl sulfone this is not the case: the central signal is a singlet, while the ^{13}C satellites are quartets. The chemical shift of the satellite is not isochrone with the central signal, which is why spin coupling occurs and the ^{13}C satellites are observed as quartets. Being quartets, the satellites indicate coupling to a neighboring CH_3 group in accordance with the symmetric structure of dimethyl sulfone.

Figure 5

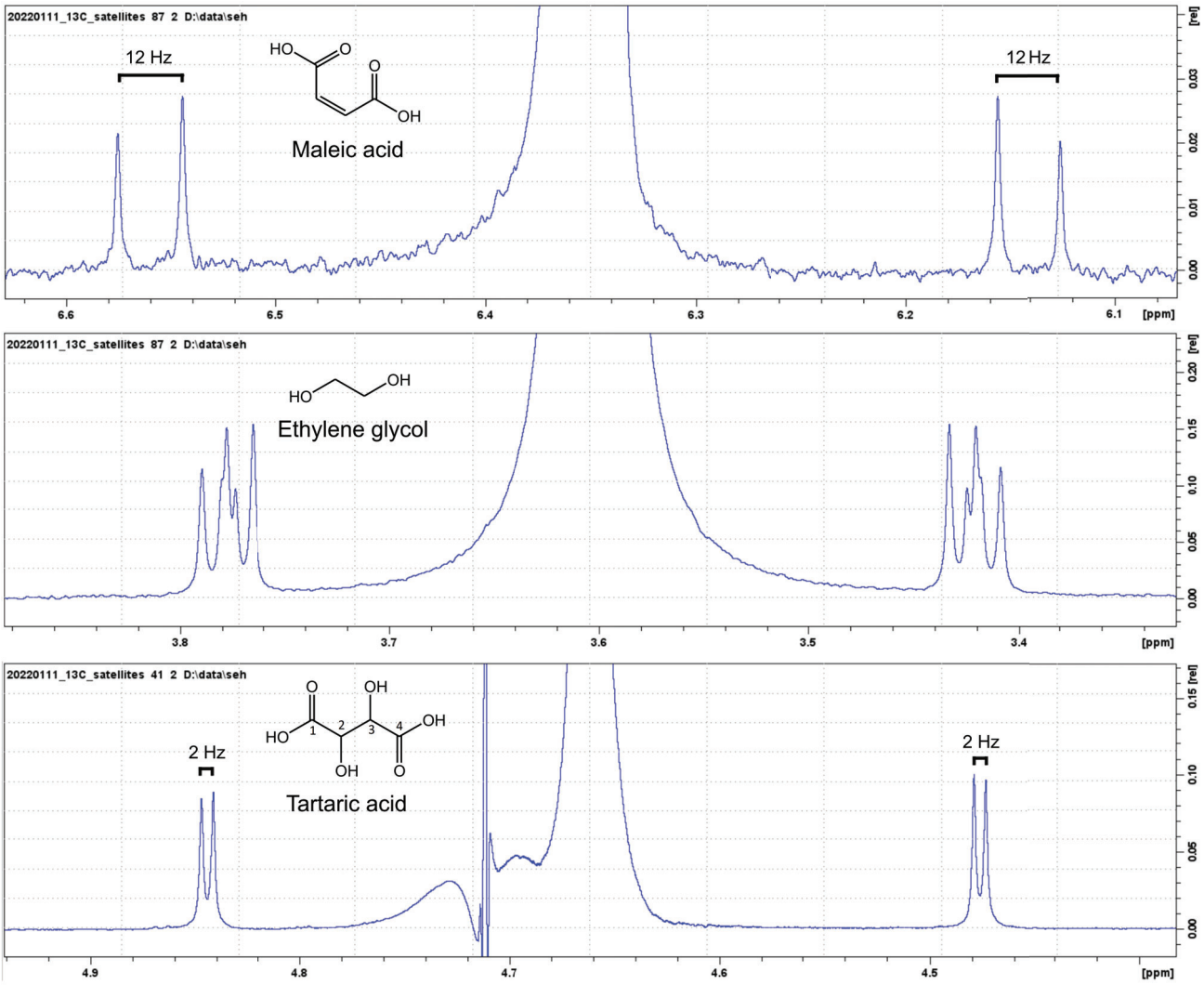

Figure 5: ^{1}H NMR spectra of the symmetric molecule maleic acid, ethylene glycol and tartaric acid zoomed to the intensity of the ^{13}C satellites. While all spectra have a singlet as a central signal, the multiplicities of the ^{13}C satellites differ as they are influenced by spin-coupling across the symmetry center.

^{13}C Satellites, Karplus' Relation and the Gauche Effect

Many small organic molecules are symmetric. Figure 5 shows three examples of such symmetric molecules. The $^{3}J_{HH}$ coupling constants (i.e., the distance between the individual peaks) within the ^{13}C satellites can be used in Karplus' relation to deduce the dihedral angle between the hydrogen atoms. According to Karplus' relation, the size of $^{3}J_{HH}$ couplings is grouped:

- Small coupling constants on the order of 2 Hz are observed if hydrogens are arranged in *syn*-conformation (dihedral angle of approx. 60°).
- Large coupling constants on the order of 12 Hz are observed if hydrogens are arranged with a dihedral angle of 0° or 180° (*anti*-conformation).
- Intermediate coupling constants on the order of 7 Hz are observed if the orientation between hydrogen atoms is subject to fast rotation (e.g., around a single bond).

For example, maleic acid contains a C=C double bond and is a planar molecule where the hydrogen atoms enclose a dihedral angle of 0° and exhibit a large $^{3}J_{HH}$ coupling of 12 Hz (Figure 5).

Ethylene glycol is another widespread symmetric molecule that is used in pharmaceutical preparations and in synthetic chemistry, that can be identified by its characteristic ^{13}C satellites.

Yet another example is tartaric acid which shows an interesting behavior (Figure 5, **bottom**). The carbon atoms two and three carry four different substituents (one of them being the hydroxy functions). These carbon atoms are sp^3 hybridized causing these carbons to bond to neighbor groups with single bonds. A fast rotation around the single bond would result in $^3J_{HH}$ couplings on the order of 7 Hz. However, in case of tartaric acid, 2 Hz are observed, indicating

- the absence of rotation around the single bond and
- the *syn*-orientation of the hydrogen residues (dihedral angle of 60°).

The *syn*-orientation of the hydrogen atoms implies that the larger residues (the hydroxy functions and the carboxylic acid functions) are oriented to the same side. The resulting steric hindrance is energetically unfavorable. This somewhat unexpected observation is known as the "Gauche effect". It results from a special electronic situation caused from vicinal electronegative atoms (in this case the two hydroxy functions). These electronegative groups induce changes in the molecular orbitals that allow for the formation of a partial double bond between the carbon atoms two and three. This partial double bond is energetically favorable and only possible in the *syn*-conformation and prevents rotation around the single bond. This example illustrates the amount of detailed information that can be obtained from the study of the ^{13}C satellites.

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