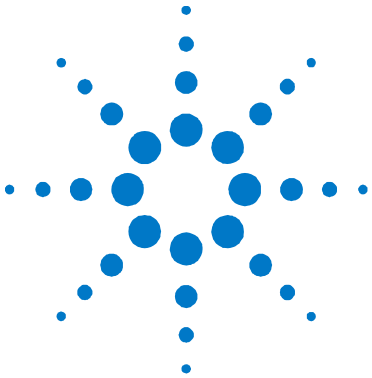

Agilent

Basic NMR Experiments

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

G7446-90559

Edition

Rev. C, Nov. 2013

Printed in USA

Agilent Technologies, Inc.

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1 Introduction

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Content and Goals

This manual has been created for the benefit of organic chemists and novice NMR spectroscopists.

The purpose of this document is to provide a more detailed discussion about some of the most commonly used pulse sequences available in the VnmrJ Experiment Selector. The focus is on how to use NMR data to either elucidate or confirm the structures of small molecules. For more specific information about setting up the acquisition parameters for a given experiment, or about processing the resulting NMR data, please consult the Experiment Guide manual for the experiment in question.

Organization

This Basic Experiment Guide begins with a discussion in Chapter 2 of the 1D PROTON experiment, a description of the types of information contained within the data, using ethylindanone as an example, and suggestions of experiments to acquire additional data for structure problems. The subsequent four chapters cover the basic types of 2D experiments, subdivided by information type, e.g., through-bond, heteronuclear one- and multiple-bond, and through-space experiments. Each of these chapters has an overview of the experiment, data interpretation using spectra acquired on ethylindanone, limitations of the experiment, and a list of pulse-sequence variants. Finally, chapter 7 describes carbon 1D spectra, and Chapter 8 contains brief descriptions of some specialized experiments to consider for more advanced applications. If you are trying to learn these experiments by yourself, we encourage you to first learn chapters 2, 7, and 4, in order, ranked by a combination of their simplicity, ease of data collection, and facile interpretation. If you want to learn them instead in order of decreasing NMR sensitivity, follow the numerical order (chapters 2, 3, 4, 5, 6, 7, and then 8). What will not be covered is the theory behind the experiments or NMR itself; references for further reading are included at the end of each chapter.

NMR and Small Molecules

NMR is one of the most important analytical tools for characterizing small molecules. The technique is fully capable of providing both qualitative and quantitative information about structures. Qualitatively, NMR is often used either to verify a proposed structure or to determine the identity of an unknown compound. Quantitatively, NMR can ascertain the amounts of compounds in solution, even if the structure of the compound is not fully known or if the material is present in a mixture. Any NMR analysis, however, may require the use of multiple experiments and data sets. This guide will explore the use of a variety of common NMR experiments to solve small molecule structure problems.

Structure Confirmation and Determination

Many small molecule NMR problems fall into one of two broad categories: structure confirmation or structure determination. The distinction is an important one, as it may determine the optimal kind(s) of NMR data to collect. Another important consideration is the level of certainty needed for the proposed structure, given the circumstances of the problem. For example, identifying a minor impurity in a multiple-step, research-type chemical synthesis requires one level of confidence, but determining the structure of an important human metabolite to be reported to the FDA would require far more certainty.

The simpler of the two problem types is structure confirmation, i.e. when the origin of the sample is reasonably well known, and there is a proposed structure. Often the best approach to such a problem is to assign the proton and carbon resonances, and then verify that the chemical shifts and proton coupling patterns are consistent with the proposed structure. The most efficient way to obtain this information is almost always the combination of a proton spectrum and a one-bond proton-carbon correlation experiment. In fact, several software-based structure verification algorithms are based on using the data from this powerful set of experiments. This is due in large part to the typically unambiguous interpretation of the one-bond proton-carbon correlation data. Once these data have been analyzed, additional experiments may be acquired to target any remaining informational gaps. Chapters 2 and 4 cover proton 1D and heteronuclear 2D one-bond correlation experiments, respectively.

The second type of structural problem, one of structure elucidation, can be much more challenging to solve. The level of difficulty will usually depend on the complexity of the molecule, and how much background knowledge one has regarding the sample origin. In the case of a metabolite, one may know a lot about its identity, as the structure of the parent drug is available, as well as a likely list of potential metabolic transformations. If the sample is a natural product isolate, however, the problem may be a completely *de novo* structure elucidation. In either case, the best approach, depending on the amount of sample available, is often to begin with the proton/HSQC-type experiment combination again. Here the data will typically be used to construct molecular fragments from the protonated parts of the molecule. Connecting these fragments together to form a structure, however, will often require additional data such as homonuclear 2D - through bond and/or heteronuclear 2D multiple-bond correlation experiments (Chapters 3 and 5). Finally, relative stereochemical information is, in many cases, best obtained from homonuclear 2D - through space experiments such as a NOESY or ROESY (Chapter 6). The interpretation of some of the latter experiments is sometimes more ambiguous and subjective, in contrast to the HSQC-type data.

Further Reading

General NMR references:

A. E. Derome, “Modern NMR Techniques for Chemistry Research”, Pergamon Press, New York, 1987.

E. Breitmaier, “Structure Elucidation by NMR in Organic Chemistry, 3rd ed.”, John Wiley & Sons, New York, 2002.

T. D. W. Claridge, “High-Resolution NMR Techniques in Organic Chemistry, Volume 27, 2nd edition”, Elsevier, New York, 2008. (This book is effectively the second edition of Derome.)

J. K. M. Sanders, B. K. Hunter, “Modern NMR Spectroscopy: A Guide for Chemists, 2nd ed.”, Oxford University Press, New York, 1993.

J. Keeler, “Understanding NMR Spectroscopy, 2rd ed.”, John Wiley & Sons, New York, 2012.

2 Proton 1D Spectra

Overview

The one-dimensional (1D) proton experiment is the most sensitive NMR experiment that can be obtained on an unlabelled molecule. As such, it is almost always the first piece of data acquired for a small molecule structure elucidation or confirmation problem, and is sometimes the only piece needed. Indeed, there is a wealth of structural information that can be gleaned from the 1D proton spectrum; however, a great deal of knowledge and experience is often required to be able to extract it. Typically, a more complicated 2D experiment can provide the same information, but in a clearer, more easily interpretable manner. In some cases, the information obtained from a 1D proton is suggestive, or perhaps consistent with a particular structure, but a user may need additional data for confirmation. We will discuss here the types of information that can be obtained from the proton spectrum, and discuss other types of experiments that might provide complimentary or supplementary information.

Chemical Shift

The chemical shift of a proton is dependent upon the functional groups around it, and is thus an indicator of local structure. The broadest chemical shift classification tends to divide protons into aliphatics, which occur lower chemical shift (lower ppm) in the NMR spectrum, - typically <5 ppm, and the higher chemical shift (higher ppm) aromatic protons, - typically >6.5 ppm. Many tables of common NMR chemical shifts exist that subdivide these two categories further, e.g., the aliphatics can be subdivided into methyl, methylene, and methine-type protons. In addition, software programs exist that can, given a structure input, predict proton chemical shifts. The output from some of these programs can serve as a very good reference tool for structure confirmation/elucidation, particularly with databases that have been trained by the user. In general, however, structures are merely inferred from chemical shift data, not implied. In other words, the chemical shift may be suggestive of, or consistent with, a proposed structure, but it does not constitute a proof of structure. If the structural problem requires a high degree of certainty, additional 2D data, such as a TOCSY, HSQC, or HMBC will usually be necessary.

Coupling

Proton-proton coupling can often be used to provide the first explicit information regarding chemical structure. The couplings observed in 1D proton spectra are usually either geminal (i.e. 2-bond, $^{2}J_{H,H}$) or vicinal (i.e. 3-bond, $^{3}J_{H,H}$) in nature. Geminal coupling constants are typically large (>10 Hz), unless they are attenuated by nearby electronegative atoms. Vicinal couplings provide direct information about how many protons are attached to the neighboring carbon(s), and which of these are NMR-distinct. For example, a triplet tells us that there are two, NMR-identical proton neighbors, whereas a doublet of doublet pattern indicates the presence of two neighboring protons that are non-identical. By carefully analyzing the couplings, one can begin to sketch the backbone of the chemical structure. Coupling patterns can rapidly become complex, although software programs do exist to help with the deconvolution and analysis of multiplets.

Vicinal couplings can also provide information about relative configuration, especially with regard to the Karplus relation, which describes the interaction between the size of the coupling and the dihedral angle between the two protons. In particular, vicinal coupling constants are often used to distinguish between *E* and *Z* alkenes, and can also provide insights into axial vs. equatorial orientations for protons in rigid ring systems. The coupling constant dependencies have been well established, especially for these two conformational systems, and are usually easy to interpret.

There are other, more atypical, proton-proton coupling tendencies that can also provide information for structure elucidation/confirmation. One example is the presence of strong coupling. Strong coupling occurs when the chemical shift distance between two J-coupled signals is small and begins to approach the size of the coupling constant in Hz. The population of spins occupying the coupling states is then influenced, such that the multiplets appear to lean toward one another. In this way, it can sometimes be directly apparent from the proton spectrum which protons are coupled to one another, even without the benefit of 2D COSY or TOCSY data or without measuring coupling constants. Another bit of information can come from the occasionally visible long range (>3-bond) couplings. A fairly common instance of this is meta-coupling in aromatic systems, which is often on the order of 1-1.5 Hz, and thus resolved in a high resolution proton spectrum. With this information, one can often sort out the aromatic proton assignments easily from the 1D data.

If the proton coupling becomes too complex, however, the typical next step is to acquire homonuclear through-bond 2D data, e.g., a COSY or TOCSY.

Integration

Integration of the proton signals is a simple concept that provides straightforward chemical information, namely the number of protons present under a certain signal and finally in the whole molecule. Integral values can be rendered inaccurate for a number of reasons, many of which are under user control, and care must be taken if the most reliable quantitative data is to be obtained. First, the proton spectrum must have a sufficient signal-to-noise (S/N) ratio. Next, one should ensure that the baseline is sufficiently flat. Large or broad signals near the protons being integrated (such as water) can decrease the integral accuracy, and so such signals may need to be suppressed (see Solvent Suppression in Chapter 8 for details). Finally, the total interscan delay (length of the acquisition time plus the relaxation delay) must be sufficiently long so as to allow the protons of interest to relax back to equilibrium. The NMR signal is generated by tipping the sample magnetization into the xy-plane with a radiofrequency pulse. This magnetization returns to the z axis (which is aligned with the NMR magnetic field) through a process called T_1, spin-lattice, or longitudinal relaxation. A typical interscan delay of 3 s (2 s acquisition time plus a 1 s relaxation delay) and pulse angle of 45° should provide integration values with ~90% accuracy for most protons, however longer times may be required for some protons, or to achieve improved accuracy. T_1 values can be measured using the T1_MEASURE experiment, and the typical rule of thumb for the best integration is to use a 90° tip angle and wait $5*T_1$ between scans. For further information about measuring T_1 values, see the Experiment Guide, Chapter 2.

Dynamics and Lineshape

Molecules in solution are not static, and can undergo a number of dynamic processes that can affect the NMR spectrum. In the last section, we touched on the process of T_1 relaxation. NMR signals also decay through a second mechanism called T_2, spin-spin, or transverse relaxation. With this process, the magnetization in the xy-plane gradually loses phase coherence, meaning that it spreads out and eventually becomes undetectable. For small molecules, T_1 and T_2 are approximately the same, but for larger molecules, T_2 can become quite short, which creates broad lines in the NMR spectrum. This is also true for small molecules that aggregate in solution, so T_2 rates can provide insight into the solution behavior of a molecule. The T2_MEASURE experiment can be used to measure T_2 values; further information can be obtained in the Experiment Guide, Chapter 2.

Additionally, there are other processes that can affect the linewidths in proton spectra. Some can be influenced by the user, such as magnetic field inhomogeneity from poor shimming, while others can give structural insights, such as chemical exchange. An example of the latter is a conformational change, such as a *cis-trans* amide bond flip. When a molecule has two interconverting solution conformations, one of several things can happen to the NMR spectrum. If the rate of conversion is slow relative to the frequency difference between the two proton resonances of the conformers, the NMR will show two separate sharp peaks for each of the resonances. When the interconversion rate becomes faster on the NMR timescale, the peaks first begin to broaden, then appear to move together, and finally coalesce at the weighted average chemical shift. When confronted with two sets of resonances for what one believes to be a pure sample, the existence of multiple solution conformations can often be verified by acquiring NMR data at increasing sample temperatures, to see if the peaks broaden and eventually coalesce at some elevated sample temperature. As an alternative, if the peaks are broad at room temperature, one can acquire NMR data at lower sample temperatures in an effort to resolve each single broad signal into two discrete sharper signals. The NOESY/ROESY experiment can also be used to check for conformers (see Chapter 6).

Another type of chemical exchange can occur between protons attached to heteroatoms (e.g., nitrogen and oxygen). This can often be observed when using solvents such as water and methanol. This also means that if the sample is dissolved in entirely deuterated water or methanol, the exchangeable protons in the sample will usually not be observed at all. When using solvents such as $CDCl_3$ or d_6-DMSO, -OH and -NH protons in the solute are sometimes broadened due to exchange if the solvent and/or sample is not completely free of dissolved water. This phenomenon leads to a very simple procedure that can used to identify protons attached to heteroatoms. First, acquire the proton spectrum for the sample dissolved in a relatively water-free solvent, such as $CDCl_3$ or anhydrous d_6-DMSO. Then add a drop or two of D_2O or CD_3OD to the sample, mix the sample, and re-acquire the data. Any protons that disappear are exchangeable protons, which are typically attached to heteroatoms. Although a small amount of added D_2O to an NMR sample will usually not affect the proton spectrum in a detrimental way, significant amounts of protonated water will degrade the spectral quality. This will necessitate the use of solvent suppression techniques, which are discussed in Chapter 8.

Ethylindanone Example

To illustrate the types of information the 1D proton spectrum can provide, let's examine a spectrum of 2-ethyl-1-indanone in $CDCl_3$ from the VnmrJ fidlib (located in /vnmr/fidlib; see Figure 1).

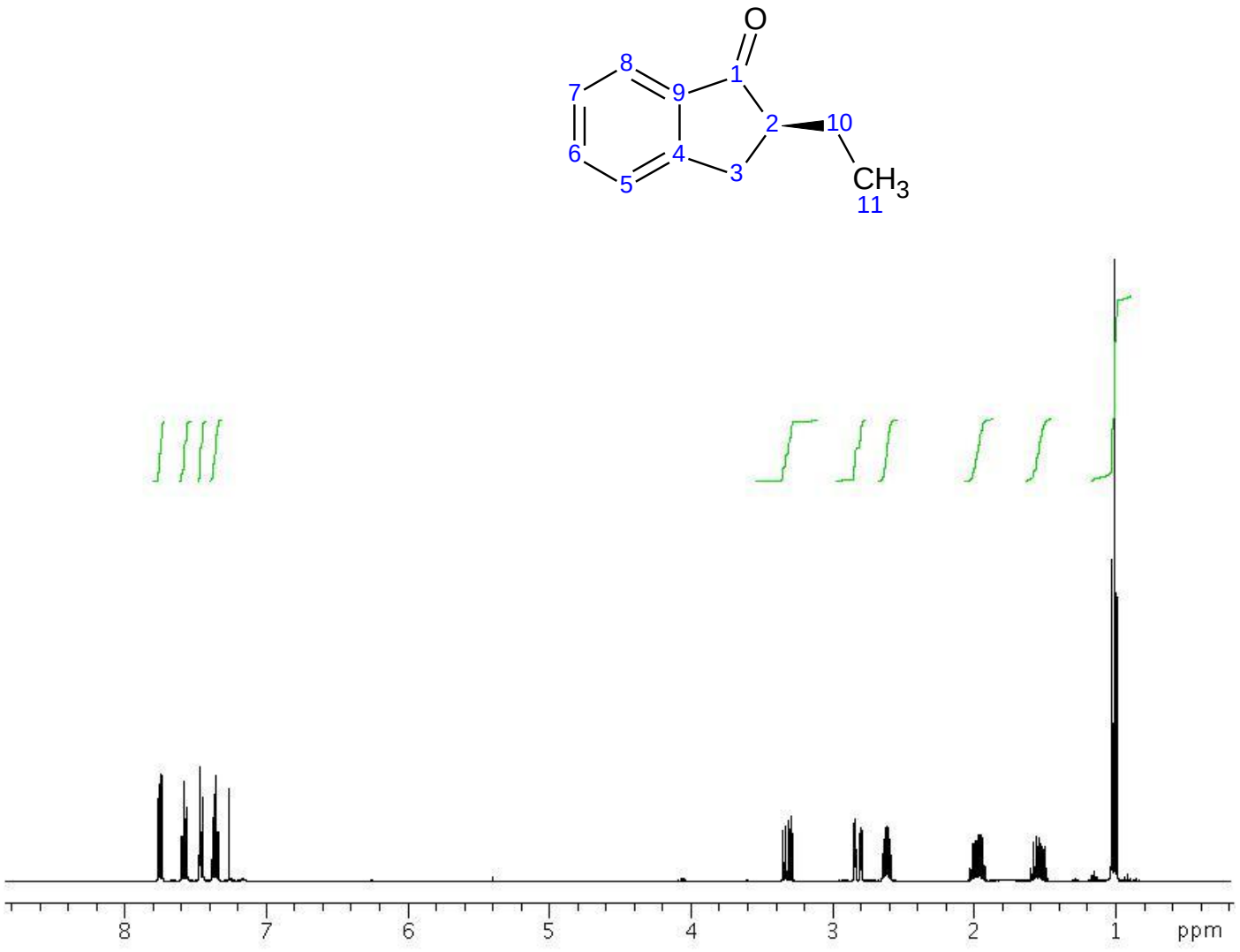


Figure 1

The spectrum has two groups of peaks- the aromatic protons located between 7-8 ppm and the aliphatic protons located between 1-4 ppm. Often it is best to begin a structural elucidation/confirmation problem from a peak with a distinctive chemical shift or other unique property. In the ethylindanone spectrum, the peak that best fits this description is the triplet at ~1.0 ppm. Both the chemical shift and relative integral value (3 protons) suggest that this signal belongs to a methyl group. Moreover, the splitting (into a triplet) indicates that this methyl group is adjacent to a methylene, giving the structural fragment CH_3CH_2-. Now let's examine an expansion of the aliphatic region (Figure 2).

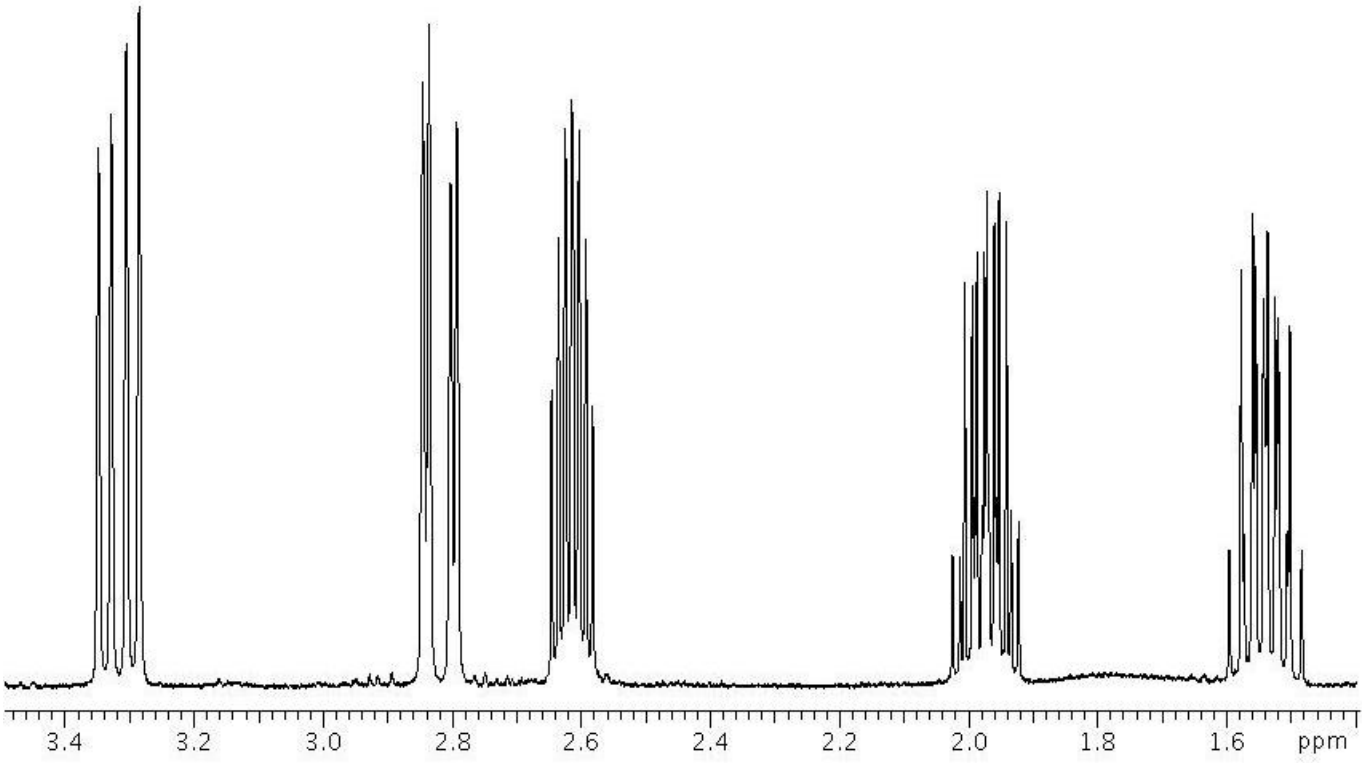


Figure 2

There are five other signals in the aliphatic region in addition to the putative methyl, three of which are complex multiplets, and the other two appear to be a strongly coupled pair (notice the “lean”) of doublet of doublets at 3.32 and 2.28 ppm. The size of the largest coupling (~17 Hz) indicates that these two signals belong to a geminally coupled non-magnetically equivalent methylene pair. The second, smaller coupling for each doublet of doublets shows that there is a single proton on the neighboring carbon (a methine). As such, this methylene cannot be the one next to the methyl group, but must be a second CH_2. Mostly likely the signal at ~2.6 ppm belongs to the methine resonance. That would leave the two remaining signals at 1.96 and 1.53 ppm for the methylene next to the methyl group. If the structure of ethylindanone was known, we have thus been able to make reasonable assignments for aliphatic protons 2, 3, 10, and 11. Definitive assignments would require additional data. If the structure was an unknown, we could say that we likely have 2 aliphatic fragments of CH_3CH_2- and –CH_2CHX-, which may or may not be connected.

Finally, let’s consider the aromatic proton region shown in Figure 3.

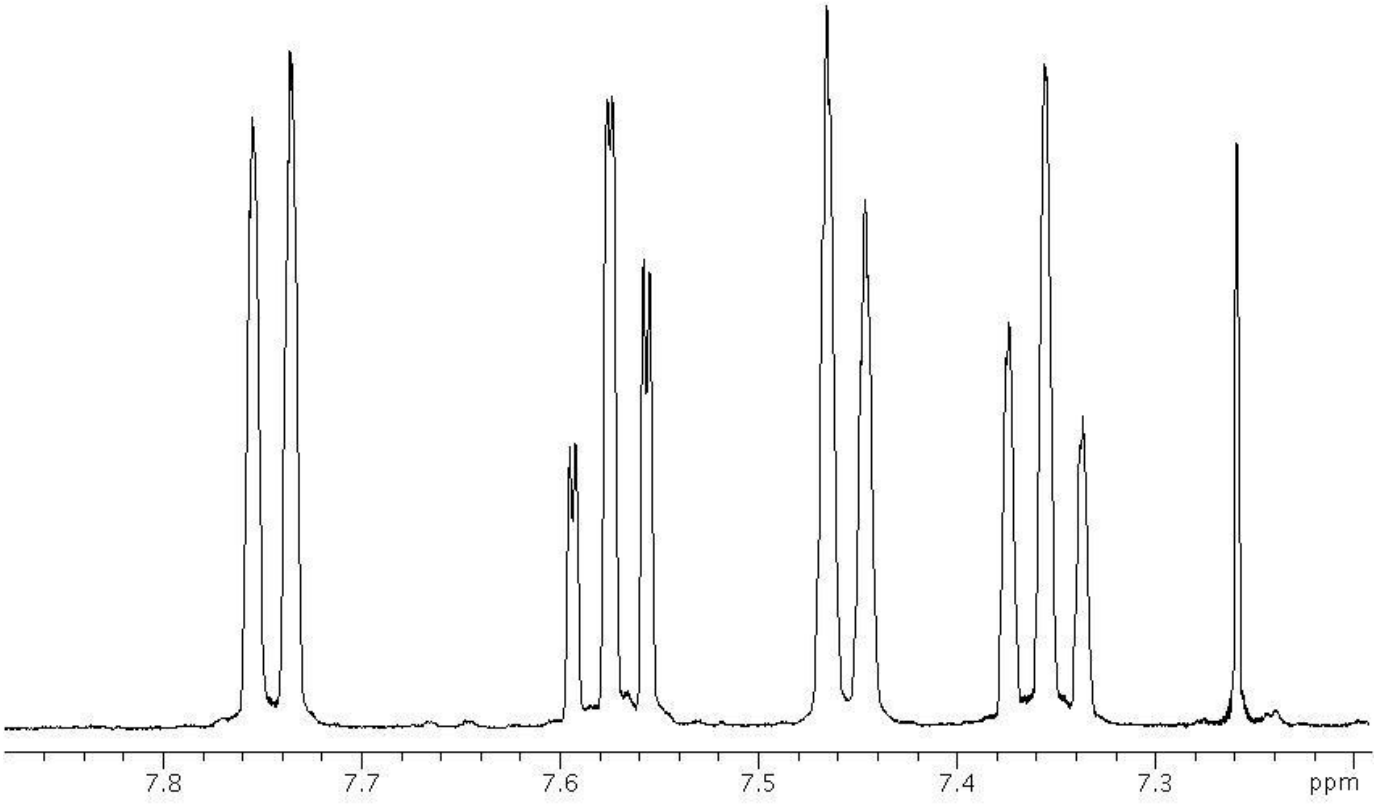


Figure 3

The peak at 7.26 ppm can be disregarded as it is from residual protonated chloroform solvent. There are four aromatic protons present, two doublets, and two triplets, which indicates a 1,2-disubstituted aromatic ring. Note that the triplet at 7.58 ppm has a visible, small meta-coupling as well. This triplet and the doublet at 7.46 are "leaning" toward one another, which shows that they are strongly coupled. So, the likely order of proton resonances, as one moves around the aromatic ring, is 7.75-7.36-7.58-7.46 ppm, although the direction is uncertain. Again, if the structure of ethylindanone was known, a chemical shift argument could be made that proton 8, which is ortho to the benzylic carbonyl, would be the most higher chemical shift proton at 7.75 ppm, rendering the order of assignments for the listed chemical shifts as 8-7-6-5. If the structure is not known, however, the main useful bit of information gleaned is the presence of a 1,2-disubstituted aromatic ring.

Limitations and Problems

For a simple molecule such as ethylindanone, we were able to infer reasonable assignments from the 1D proton spectrum; however, complete assignments may not be possible for a more complicated structure. More complex molecules may have overlapping multiplets and more intricate spin systems that require the additional information and resolution available from 2D experiments to sort out the ambiguities. Moreover, while one can often make good inferences regarding the assignments of the protons in the 1D spectrum if the structure is known, a *de novo* structure elucidation will nearly always require additional NMR data. Finally, some of the information in the 1D proton spectrum requires further knowledge and experience with chemical shifts and coupling behavior for interpretation.

Experimental Variants

The "Std 1D" tab of the experiment selector contains the following variants of the 1D proton experiment (for more information on any of these, see the Experiment Guide).

- (H)PRESAT - This experiment acquires a 1D proton with presaturation-type solvent suppression.
- (H)wet1D - This experiment acquires a 1D proton with WET-type solvent suppression.

- T1_MEASURE - This experiment is used to measure T_1 relaxation rate constants.
- T2_MEASURE- This experiment is used to measure T_2 relaxation rate constants.
- PureShift 1D - The PureShift 1D experiment is used to simplify proton spectra by removing multiplet patterns. It produces the equivalent of a broad-band decoupled proton spectrum, but has much lower signal-to-noise.
- (H)HomoDec - This experiment uses narrow-band irradiation to decouple a 1D-^{1}H dataset at a single or multiple frequencies. This can be used to determine which multiplets are coupled to each other.
- BilevelDec - This experiment acquires a 1D proton spectrum with heteronuclear decoupling. The bi-level style decoupling collapses ^{13}C satellites into the main signal for more accurate integrations. This also makes it useful for uniformly ^{13}C-labelled samples, which may have very complex, difficult to interpret 1D PROTON spectra from ^{13}C-^{1}H coupling. This experiment can also be used to remove cyclic decoupling sidebands associated with large ^{1}H-X couplings by varying the length of the higher power decoupling at the beginning of the acquisition. These sidebands are often a problem when decoupling a nucleus of high abundance like ^{31}P.

Further Reading

R. J. Abraham, P. Loftus, “Proton and Carbon-13 NMR Spectroscopy: An Integrated Approach”, Heyden, London, 1978. (General reference)

E. Pretsch, T. Clerc, J. Seibl, W. Simon, “Tables of Spectral Data for Structure Determination of Organic Compounds, 2nd ed.” Springer-Verlag, Heidelberg, 1989. (Chemical shift)

T. R. Hoye, P. R. Hanson, J. R. Vyvyan, J. Org. Chem. 59 (1994) 4096-4103. (Multiplet analysis)

V. F. Bystrov, Prog. NMR Spectroscopy 10 (1976) 41-81. (Karplus curve)

D. D. Traficante, Concepts Magn. Reson. 4 (1992) 153-160. (Quantitative analysis)

H. Y. Carr, E. M. Purcell, Phys. Rev. 94 (1954) 630-638. (T_2)

S. Meiboom, D. Gill, Rev. Sci. Instrum. 29 (1958) 688-691. (T_2)

3 Homonuclear 2D Through-Bond Spectra

Overview

The homonuclear 2D through-bond experiments, namely COSY and TOCSY, are often the first auxiliary pieces of data collected beyond the 1D proton spectrum for structure elucidation/confirmation. Because these experiments are proton-proton correlations, they are the most sensitive and have the best chance of providing additional structural data beyond the PROTON spectrum if the sample quantity is limited.

Homonuclear 2D spectra have a diagonal, which is effectively the 1D proton spectrum displayed as contours, from a “topographical” perspective. The signals that are not located on the diagonal are called crosspeaks, which occur between protons that share through-bond connectivity, also called scalar coupling. The information obtained from these data indicates which protons are connected to which other protons in the 1D spectrum. While we can infer some of this molecular connectivity from the 1D proton spectrum, the TOCSY and COSY data lay this information out in a more obvious, easy-to-interpret, manner, and also often help resolving signals which overlap in the 1D proton.

The TOCSY experiment is usually acquired so as to provide correlations among an entire spin system. For example, if proton A is coupled to B, B coupled to C, and C to D, the default parameters of the TOCSY should give correlations between all protons A-D. The COSY data, however, is step-wise in nature, which means that it shows a crosspeak between A and B, B and C, and C and D, but none between A and C or D, or B and D. Both approaches provide highly useful structural information and can be complimentary. When a molecule has multiple spin systems that are isolated in the molecule but overlapping in the 1D spectrum (such as a peptide), the TOCSY data is very useful for sorting out which signals belong to which structural fragments. The COSY data, on the other hand, can distinguish which protons are directly coupled to each other within the spin system.

Whichever experiment is used, 2D homonuclear through-bond data should be considered essential data for small molecule structure elucidation to supplement the 1D PROTON data. With reasonable amounts of material, the J-coupling experiments can often be acquired in 5 minutes or less. To illustrate the utility and differences of these data, we will examine both a zTOCSY and a gCOSY experiment acquired from a sample of 2-ethyl-1-indanone in $CDCl_3$.

zTOCSY of Ethylindanone

To obtain a zTOCSY dataset, the experiment should be chosen from the Experiment Selector under the “(HH)Homo2D” tab. If the zTOCSY parameters are set up in an experiment containing a previously acquired proton spectrum, any customizations regarding the proton chemical shift range will be maintained. Only a few simple parameters need to be adjusted; such as the number of scans, the number of t1 increments, and the spinlock duration. For now, we will leave the spinlock duration at the default value of 80 ms, which will provide “typical” TOCSY-like data. Later we will discuss why and how this parameter might be modified. For further details on setting up and processing the experiment, please refer to the Experiment Guide, Chapter 3.

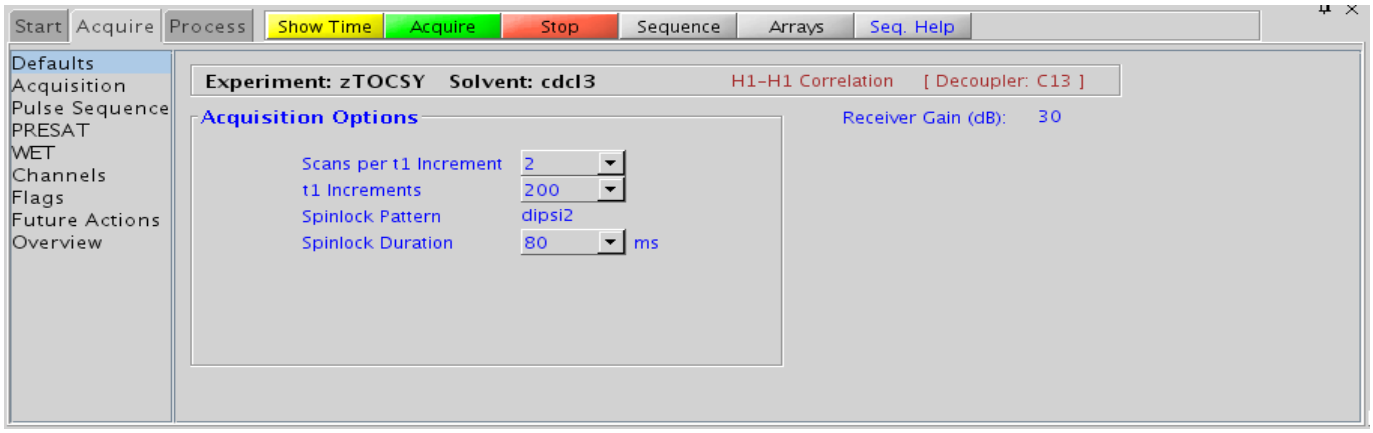
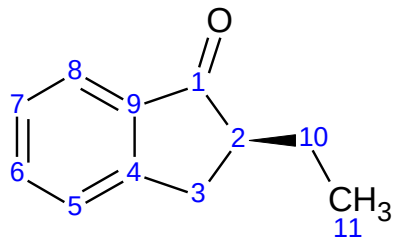


Figure 4

Figure 5 shows the zTOCSY spectrum of ethylindanone found in the VnmrJ fidlib.



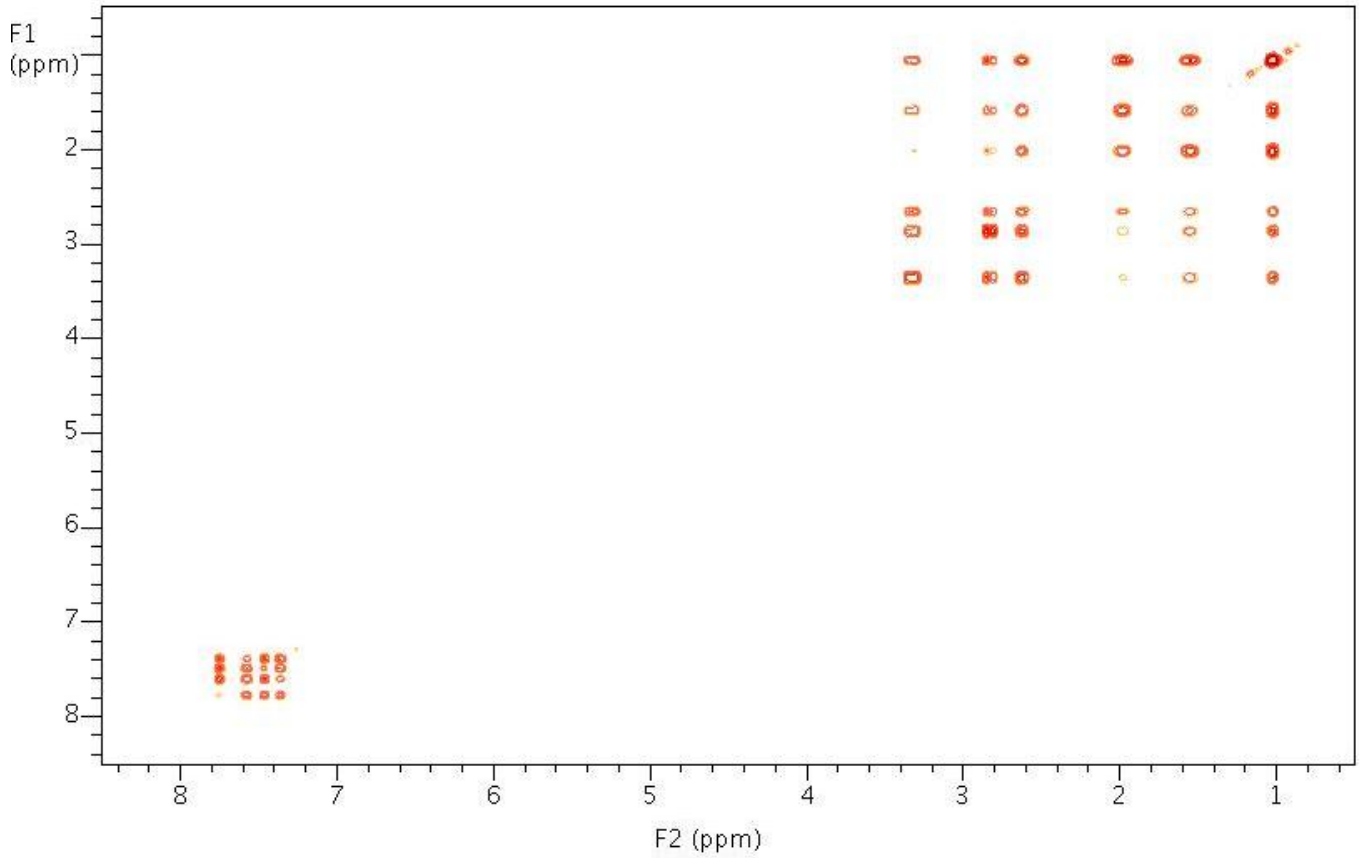


Figure 5

Within this spectrum, one can see the diagonal, which runs from the lower chemical shift, aliphatic region (top right corner) to the higher chemical shift, aromatic region (lower left corner), and a large number of off-diagonal crosspeaks. Note that there are no crosspeaks between the aliphatic protons and the aromatic protons. This tells us that (as would be expected) there is no coupling connectivity between the aromatics and aliphatics and that they are separate, isolated spin systems. Next let's examine an expansion of the aliphatic region (Figure 6).

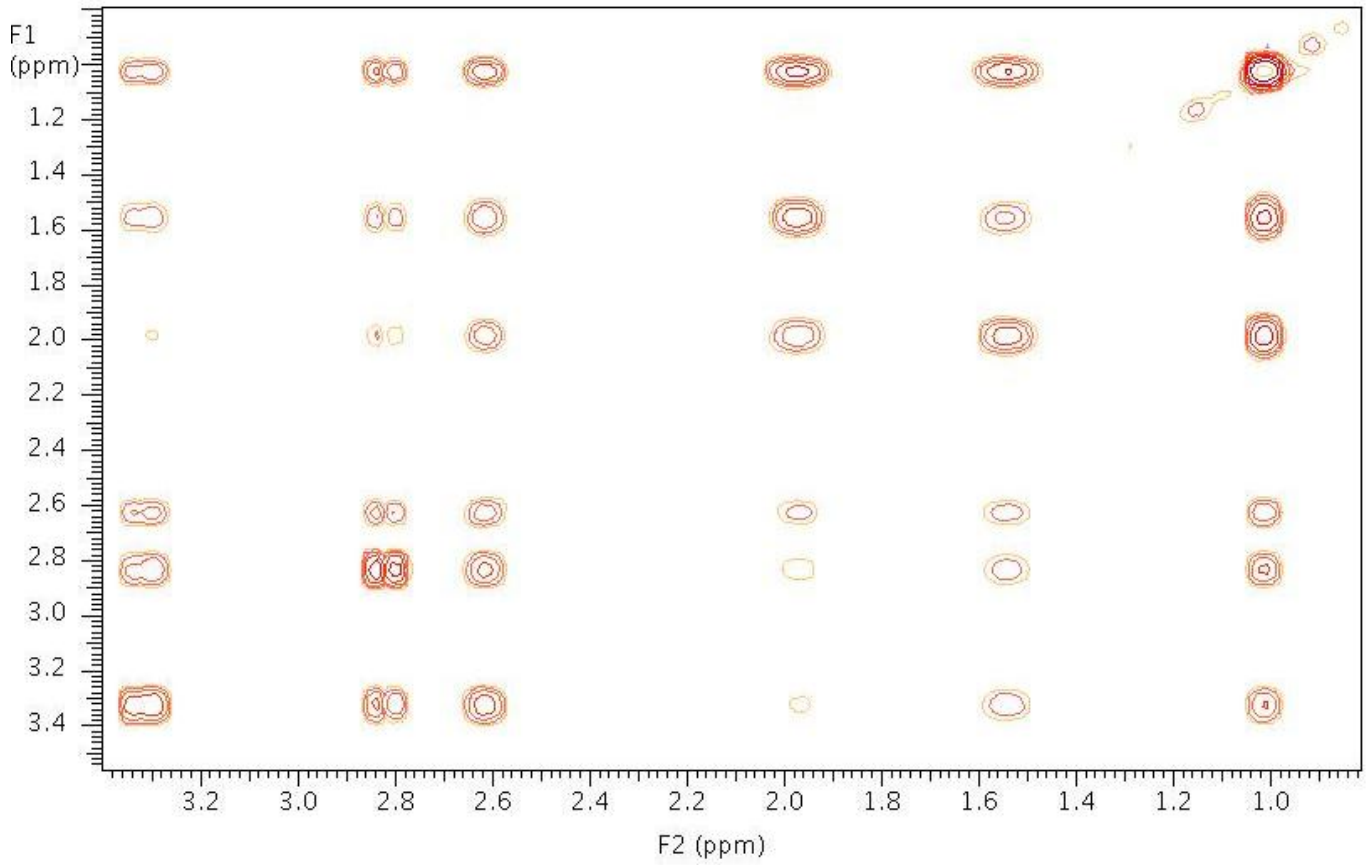


Figure 6

Crosspeaks are visible among all six aliphatic protons, which indicates that there is a single aliphatic spin system with all the protons connected in a through-bond coupling network. There are, however, variabilities in the intensities of the crosspeaks. Weaker crosspeaks are sometimes assigned as belonging to protons that are further apart in the spin system (i.e. not directly coupled), however, COSY data are better suited for definitively identifying which protons are directly coupled and which are not.

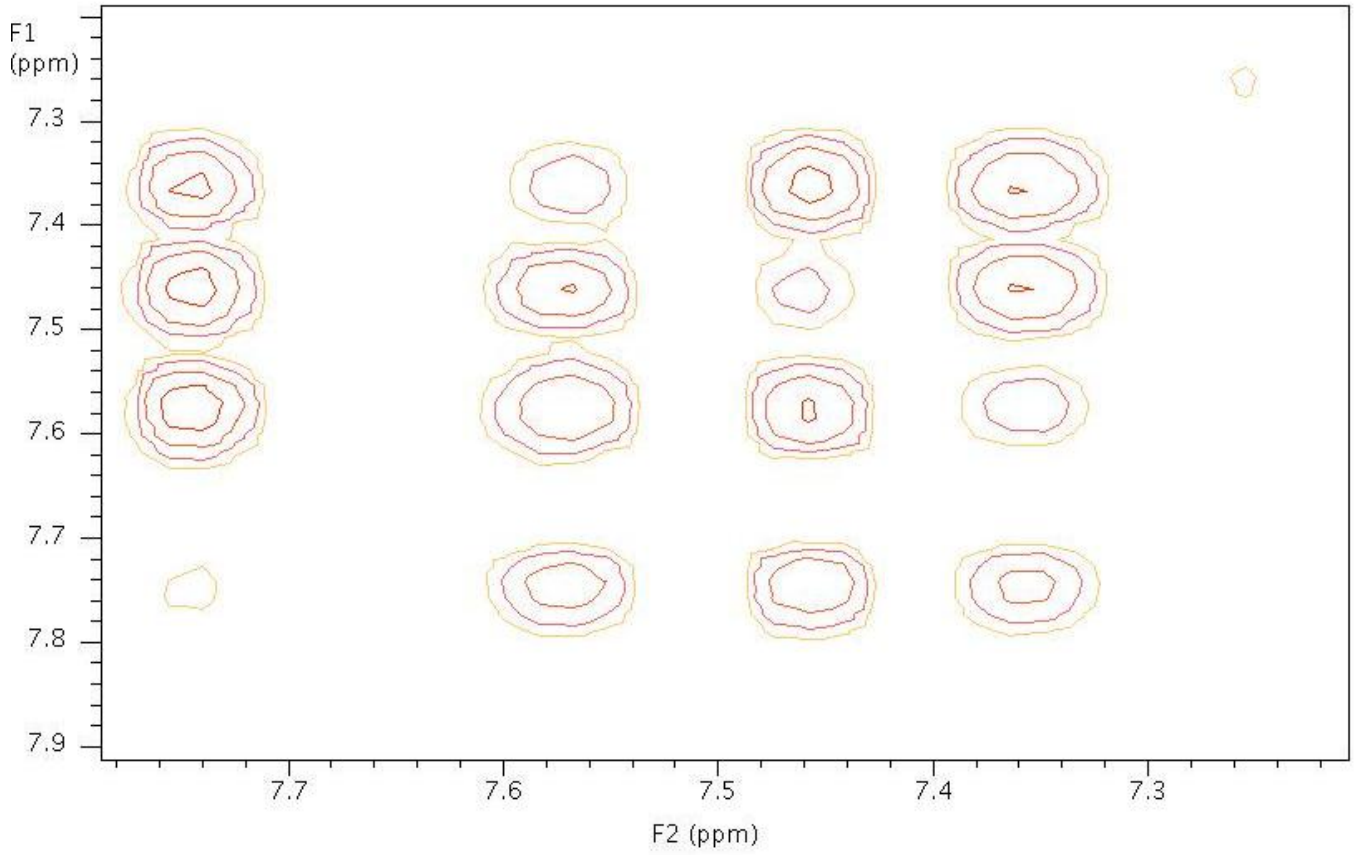


Figure 7

An expansion of the aromatic region (Figure 7) tells a similar story as the aliphatic region, namely that there is only aromatic spin system.

Limitations and Problems

While the data are of very good quality and artifact-free, ethylindanone provides a good example of a molecule where the typical TOCSY-type data is of limited value for assignment or structure elucidation. Beyond the information that there are two separate spin systems in the molecule, one aliphatic and one aromatic, there is very little other information in the zTOCSY spectrum shown. The problem stems from the nature of the TOCSY experiment to transfer magnetization along the entire spin system. This feature is tunable, however, by simply modifying the spinlock duration. The data shown for ethylindanone was acquired with the standard spinlock duration of 80 ms. This spinlock duration will usually show complete correlations among protons for spin systems of ~4 carbons in length (e.g., CH_3-CH_2-CH_2-CH_2X), depending on the size of the couplings constants, and larger $J_{H,H}$ will result in more efficient TOCSY magnetization transfer. If the spinlock duration is shortened to ~20-30 ms, the resulting data will appear much more like a COSY experiment, where the correlations will appear primarily between geminal and vicinal protons only. The occasional 4-bond correlation, such as with W-type coupling, may appear when the coupling constants are large, but this would be true of the COSY experiment as well. It is also possible to lengthen the TOCSY spinlock duration to attempt to see correlations for longer spin systems; however, it is not advisable to exceed 100-150 ms as it may damage the probe, or warm up the sample.

Experimental Variants

The “(HH)Homo2D” tab of the experiment selector contains an experimental variant called the standard TOCSY, which does not have the z-filter. In general, it is recommended to use the z-filtered version of the experiment as it produces fewer artifacts. (For more information, see the Experiment Guide.)

gCOSY of Ethylindanone

To obtain a gCOSY dataset, the experiment should be chosen from the Experiment Selector under the "(HH)Homo2D" tab. If the gCOSY parameters are set up in an experiment containing a previously acquired proton spectrum, any customizations regarding the proton chemical shift range will be maintained. Only a few simple parameters such as the number of scans and the number of t1 increments need to be adjusted. For further details on setting up and processing this experiment, please refer to the Experiment Guide, Chapter 3.

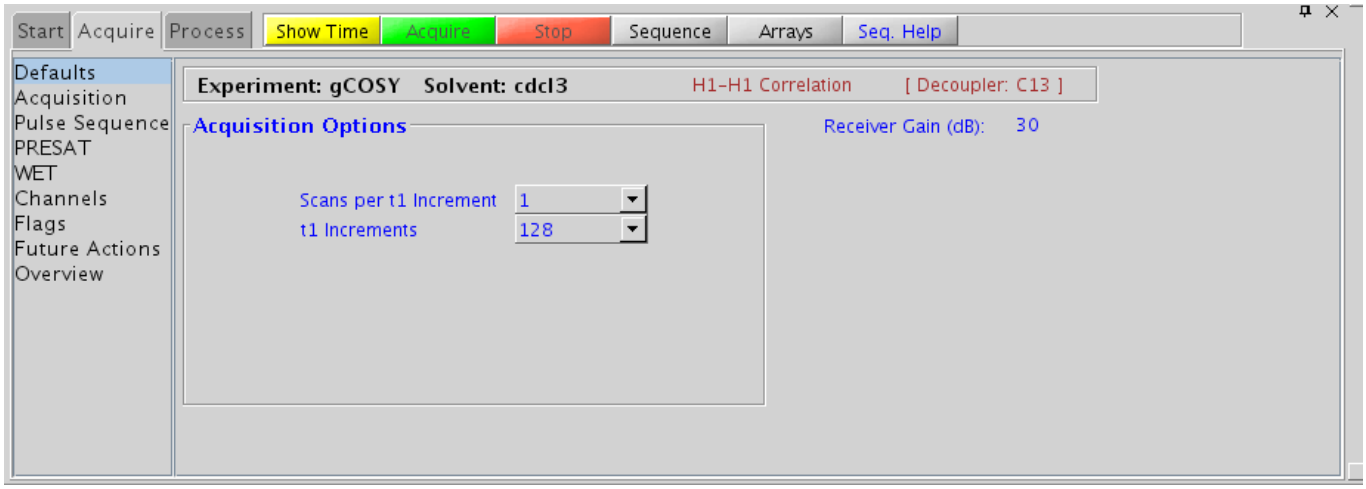


Figure 8

Figure 9 shows the gCOSY spectrum of ethylindanone that can be found in the VnmrJ fidlib.

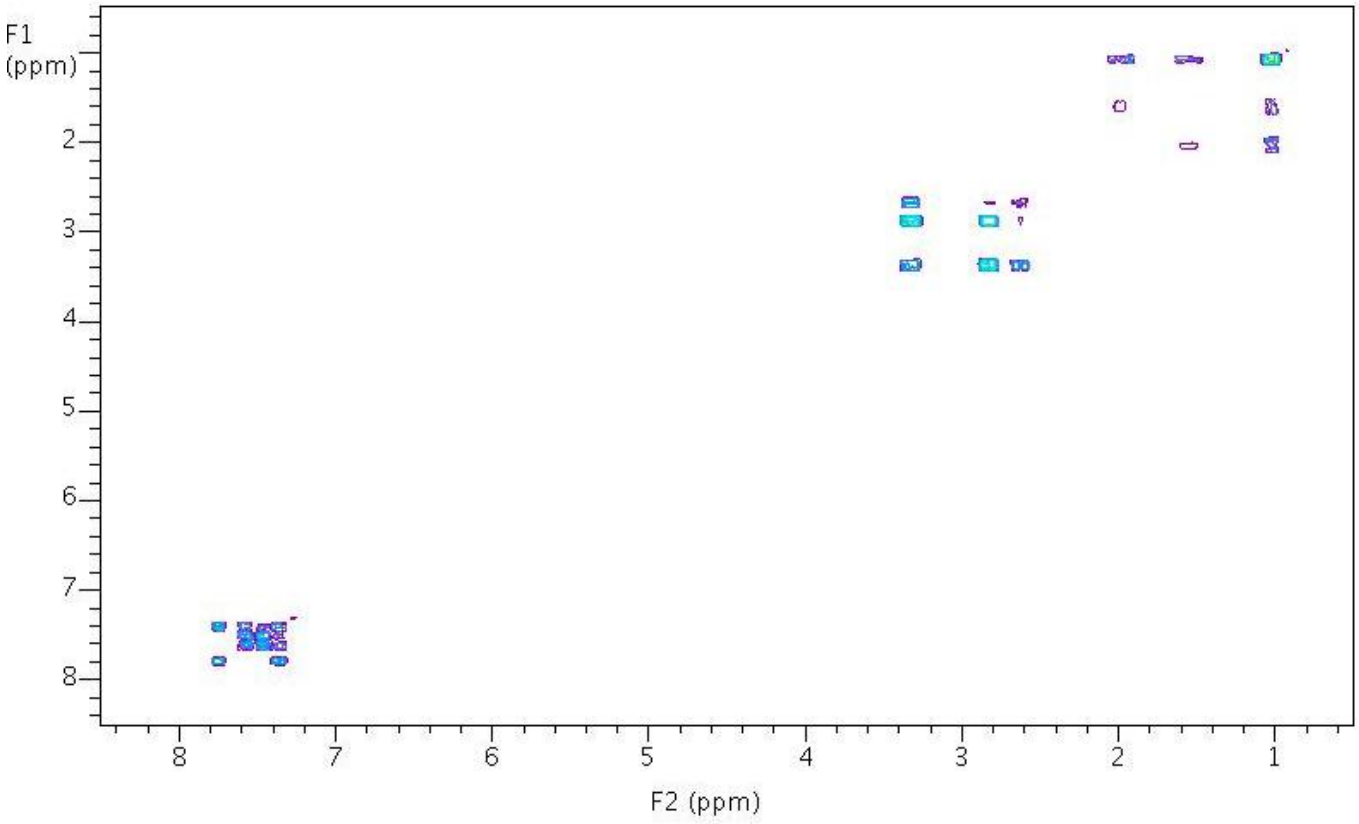


Figure 9

Similar to the zTOCSY, one can again see that there are no crosspeaks between the aliphatic protons and the aromatic protons and thus no coupling connectivity. There are, however significant differences from the zTOCSY that can be seen in the expansions of the two regions.

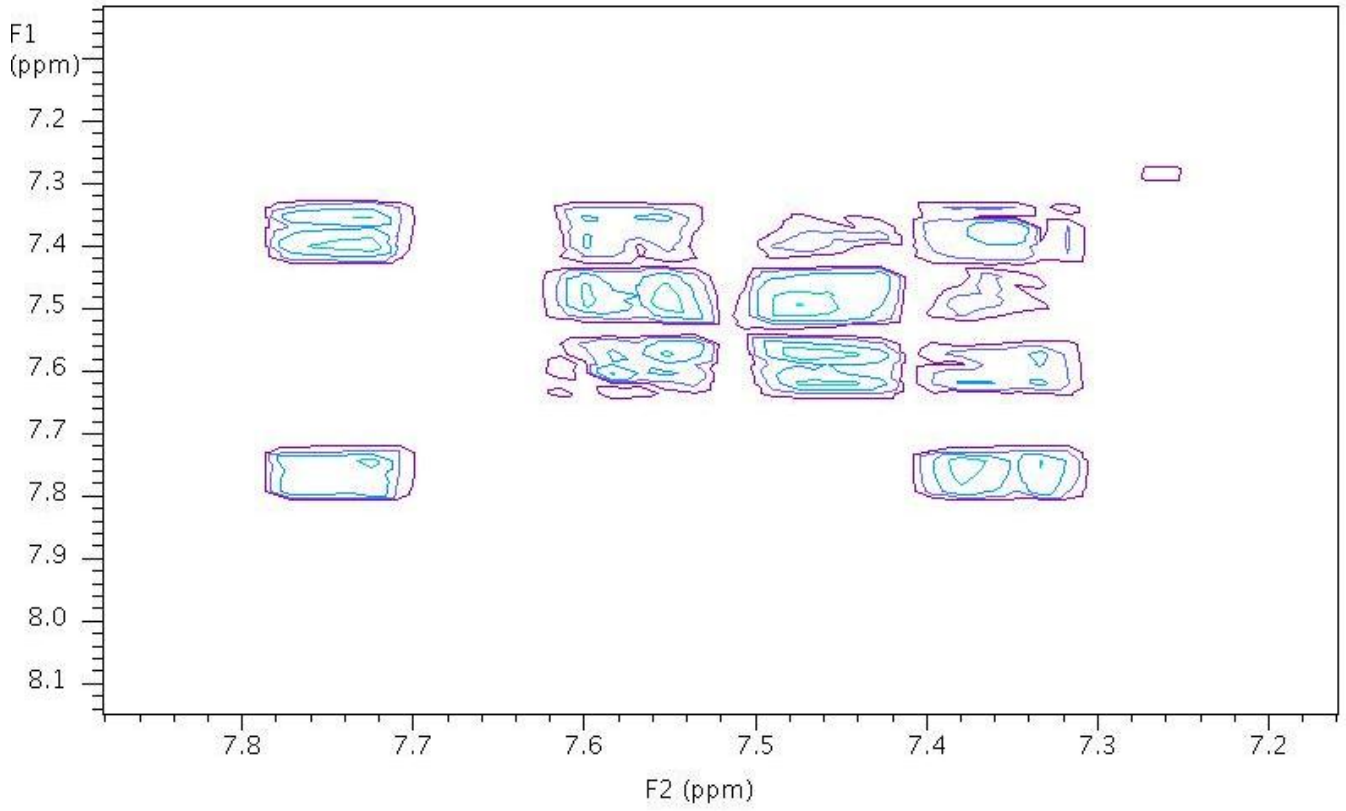


Figure 10

First let's look at the expansion of the aromatic region (Figure 10). Recall from our analysis of the proton data that the doublets at 7.46 and 7.75 ppm belong to the 3- and 6-position protons of a 1,2-disubstituted aromatic ring (e.g., the protons 5 and 8 of ethylindanone), and the triplets at 7.36 and 7.58 ppm belong to the 4- and 5-position protons.

We also surmised that proton producing the doublet at 7.46 ppm was adjacent to the triplet at 7.58 ppm because of the "lean" of the doublet towards the triplet, indicating strong coupling. Indeed there is a crosspeak observed in the COSY spectrum between these two protons, confirming that they are directly coupled to one another. The other doublet and triplet pair at 7.36 and 7.75 ppm also has a visible crosspeak in the COSY spectrum, as do the two triplets, which should also be directly coupled.

Finally, there is a weak crosspeak between the triplet at 7.36 ppm and the doublet at 7.46 ppm. The smaller intensity of this crosspeak indicates that it arises from the smaller, typically ~1-2 Hz $^{4}J_{H,H}$, which is often observed in aromatic rings. So, our aromatic proton assignments would be, sequentially around the ring, 7.75, 7.36, 7.58, and 7.46 ppm, but we are not certain whether the order would be 8-7-6-5 or 5-6-7-8 for our ethylindanone structure. If the structure is known, however, the chemical shifts are more consistent for the former rather than the latter, especially for proton 8 at 7.75 ppm. Next we will look at the aliphatics in Figure 11.

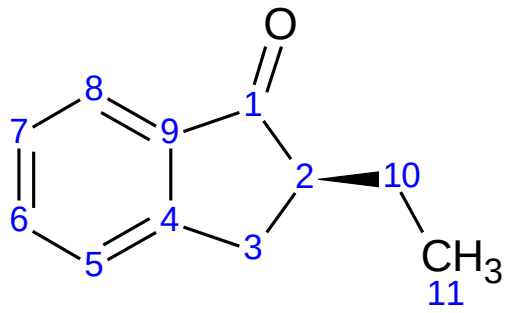
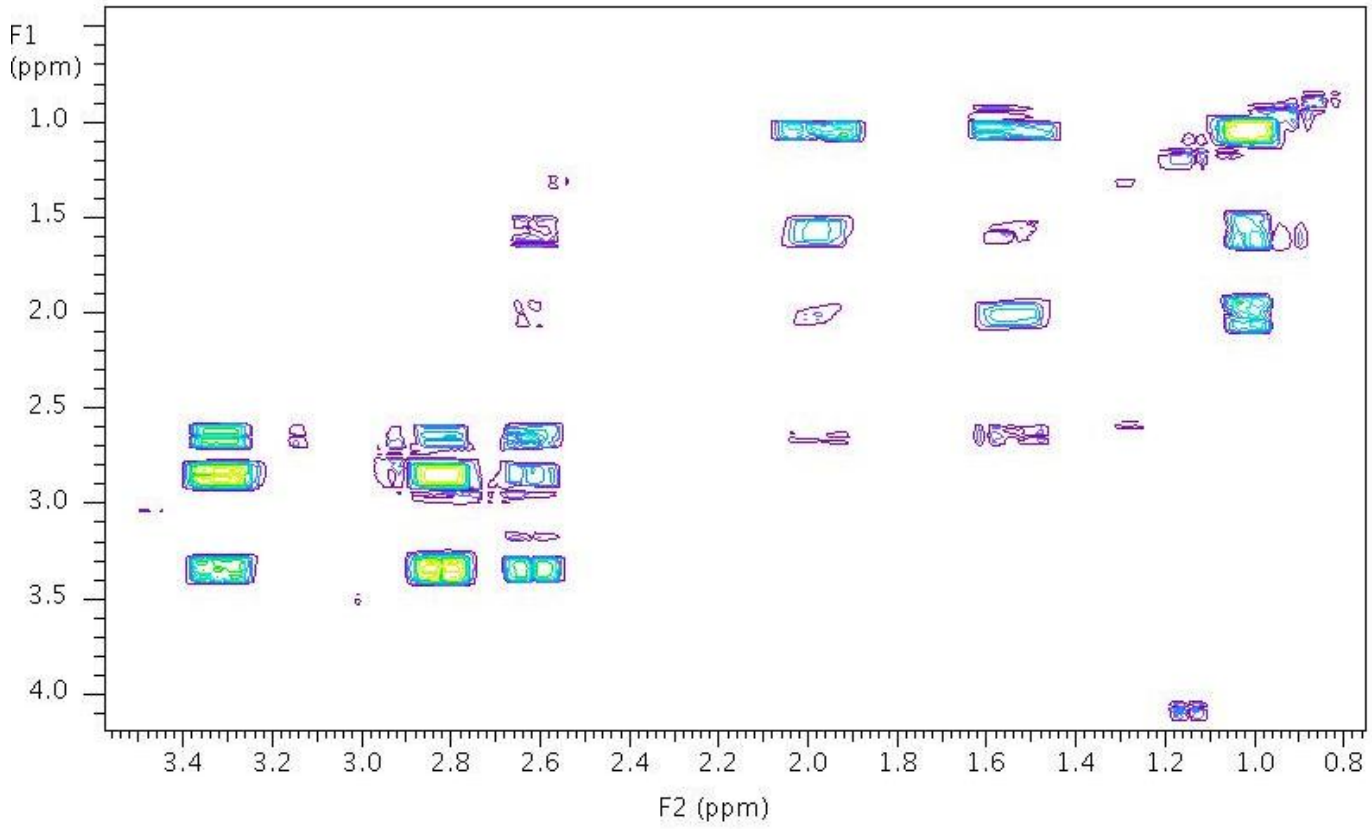


Figure 11



To start assigning protons for a structure elucidation/confirmation, it is usually advisable to use, as an entry point, the resonance with the least ambiguity as to its identity. From the proton spectrum, we decided that the signal at 1.0 ppm was most likely a methyl group, due to its integration and chemical shift. This signal makes an excellent starting point for interpreting the gCOSY data.

This methyl resonance shows crosspeaks to two other signals at 1.53 and 1.96 ppm, both of which integrate to 1 proton in the 1D spectrum. These two peaks, therefore, belong to the adjacent diastereotopic methylene protons. In turn, these methylene protons both have crosspeaks (the proton at 1.96 has a weaker correlation due to a smaller coupling constant) to the signal at 2.6 ppm, which can be assigned as the methine proton. Finally, this methine has correlations to both adjacent methylene protons at 2.82 and 3.32 ppm. The COSY has updated our hypothesis from the proton spectrum to an aliphatic spin systems of CH_3CH_2- and –CH_2-CHX- to a single spin system of CH_3-CH_2-CHX-CH_2- with definitive corresponding proton assignments as 1.0, 1.53 and 1.96, 2.6, 2.82 and 3.32, respectively.

Limitations and Problems

The examination of the gCOSY data has illuminated an additional shortcoming of the 2D through-bond correlation experiments in general, namely that, where there is no proton-proton coupling, there will be no structural information. Looking at our ethylindanone example, we have a good idea of what the aromatic and aliphatic spin systems look like and their assignments, which is probably sufficient if one is simply looking for confirmation of the proposed structure. If the problem is a *de novo* structure elucidation, however, we don't have an unambiguous picture of how the pieces fit together. We know that we have a 1,2-disubstituted aromatic ring, and could make the argument that the chemical shifts of the methylene at 2.82 and 3.32 ppm are consistent for benzylic protons, but definitive data would require additional experiments. We are also uncertain as to what else is connected to the methine, but we can be sure that it doesn't involve protons. To obtain further structural information at this juncture, most NMR spectroscopists will turn to the proton-carbon correlation experiments described in the next two chapters. These types of experiments can help fill in the missing regiochemical gaps in a structure problem and provide additional molecular information.

Experimental Variants

The "(HH)Homo2D" tab of the experiment selector contains the following variants of the 2D COSY-type correlation experiment. (For more information on any of these, see the Experiment Guide.)

- gCOSY - This is the most commonly used version of the COSY experiment gradient coherence selection. The "g" in the name indicates that it uses gradients (e.g., pulsed field gradients; PFG). It provides better artifact suppression at the expense of a loss of sensitivity by a factor of ~1.4. The gradient coherence selection also allows the experiment to be acquired with nt = 1, such that with a sufficiently concentrated sample, a reasonable spectrum may be obtained in 5 minutes or less.

- COSY - This is the classic homonuclear through-bond correlation spectroscopy experiment. It uses phase cycling to select the data rather than PFG, which implies that it requires a minimum number of transients for each increment, often nt=4. The gCOSY version is typically the preferred experiment unless sensitivity is an issue.
- gDQCOSY - A homonuclear double-quantum filtered through-bond correlation spectroscopy experiment with gradient coherence selection. The main advantages of the gDQCOSY versus the COSY are better resolution because it is a phase-sensitive experiment, and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a gDQCOSY spectrum. It should be noted that the default ni=200 may miss a significant number of correlations, especially for the gDQCOSY family, because the signals build up with time in t1 as a function of 1/J. (Values of ni=350 to ni=512 can often be useful.)
- DQCOSY - This is the standard two-dimensional, homonuclear double-quantum filtered through-bond correlation spectroscopy experiment. It uses phase cycling to select the data instead of PFG. The gDQCOSY version is typically preferred unless sensitivity is an issue.
- zCOSY - This version of the COSY experiment has a z-filter and is the modern replacement for the traditional E.COSY. This experiment is mostly used to determine homonuclear coupling constants, especially smaller long-range couplings. These are typically obstructed by the complex coupling pattern in a traditional COSY but are accessible in zCOSY because of its E.COSY (Exclusive COSY) pattern.

TOCSY versus COSY

If you compare the zTOCSY and gCOSY data presented for ethylindanone, you can see that the peak shape of the zTOCSY data is superior and exhibits higher resolution. The zTOCSY data also has fewer artifacts and is thus of higher quality. This is due to how the correlations are generated experimentally. Overall, this makes the zTOCSY experiment more sensitive and a better choice than the gCOSY experiment, although the gCOSY experiment is much faster to acquire and easier to setup and process. If COSY-type data is desired for the structural problem, the optimal choice is often to acquire the zTOCSY with a 20-30 ms spinlock duration.

Further Reading

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M. J. Thrippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

4 Heteronuclear 2D One-Bond Correlation Spectra

- Overview
- gHSQCAD of Ethylindanone
- Limitations and Problems
- Experimental Variants
- Further Reading

Overview

After ^{1}H, ^{13}C is the second most important nucleus for small molecule NMR. Carbon has a much broader chemical shift range than proton (~220 ppm vs. ~14 ppm), because it is more sensitive to local chemical structure and functional groups. Unfortunately, carbon NMR is naturally less sensitive, due to its smaller gyromagnetic ratio and reduced abundance. The most abundant carbon isotope is ^{12}C, which does not have an NMR signal; in contrast, the detectable carbon isotope, ^{13}C, has a natural abundance of only 1.1%. This means that carbon NMR information is simply more difficult to obtain and one usually has to resort to slightly more complex experiments to obtain it efficiently.

The most sensitive experiments for mining this information are the family of heteronuclear 2D one-bond correlation experiments. These experiments can be found on the Experiment Selector under the “J1(CH)corr” tab. Although the experiments themselves can be quite sophisticated, the data obtained is very easily interpreted. The resulting 2D spectrum has proton chemical shifts along one axis and carbon chemical shifts along the other. Crosspeaks indicate that “the proton at chemical shift X is attached to a carbon at chemical shift Y”. The absence of a crosspeak for a particular proton indicates that the proton is not attached to a carbon, which usually means that it is attached to an oxygen or nitrogen atom instead.

This proton-carbon correlation can be extremely useful for structure confirmation and elucidation. Peak assignments are easily made from this data, and the results are unambiguous; if a particular carbon assignment is known, its attached proton can be assigned, and vice versa. Knowledge gained from carbon chemical shift prediction can also be applied to the proton assignments with this type of data. It should be noted that proton-carbon correlation datasets have no diagonal, which is in contrast to the common homonuclear 2D experiments.

Of the varieties of proton-carbon correlation experiments provided in VnmrJ, the gHSQCAD (gradient-enhanced heteronuclear single quantum coherence with adiabatic pulses) is a good choice. It is a robust experiment, with good sensitivity, and produces spectra that are relatively free of artifacts.

gHSQCAD of Ethylindanone

To obtain a gHSQCAD spectrum, the experiment should be chosen from the Experiment Selector under the "J1(CH)corr" tab. If the gHSQCAD parameters are set up in the StudyQ, or in an experiment that contains a previously acquired proton spectrum, customizations such as the proton chemical shift range will be maintained. Only a few simple parameters such as the number of scans, the number of t1 increments, and the carbon spectral width need to be adjusted. For further details on setting up and processing the experiment, please refer to the Experiment Guide, Chapter 4. Please note that the following data has been obtained with the multiplicity-editing function turned on, using the checkbox on the "Acquire→Defaults" tab (see Figure 12). We will discuss the effects of this feature later.

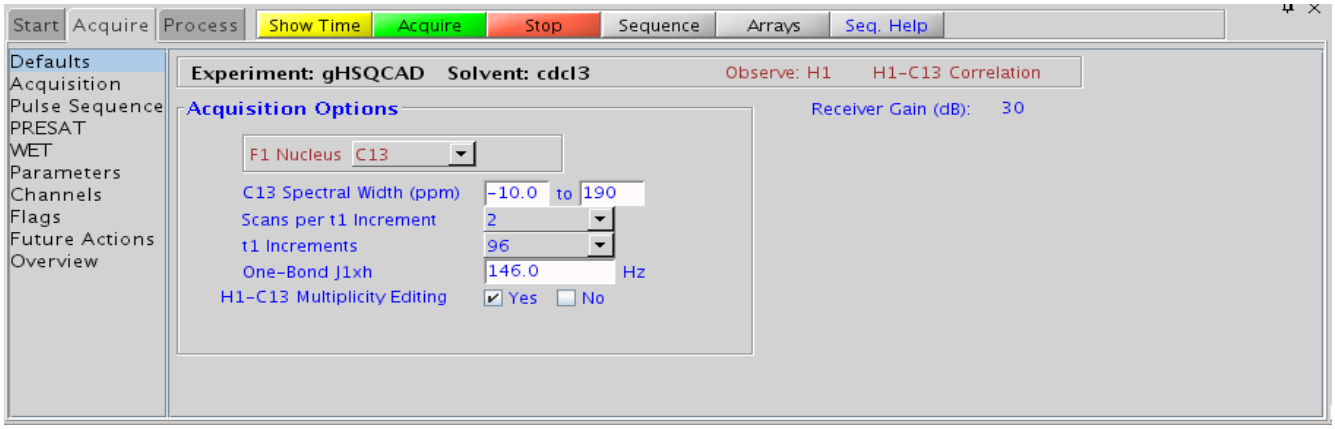
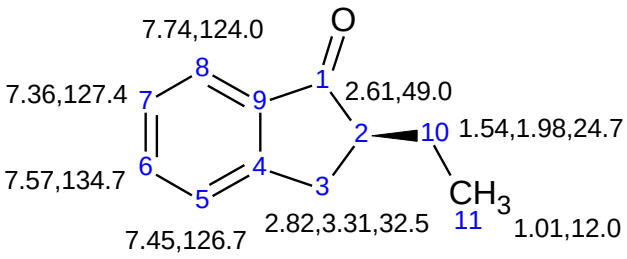


Figure 12

Figure 13 shows the gHSQCAD spectrum of ethylindanone that can be found in the VnmrJ fidlib. At the level of vertical scale chosen, four peaks can be seen in the aromatic region and six peaks in the aliphatic region, which gives us some immediate structural information. Moreover, there appear to be two peaks (proton chemical shifts of ~2 and 1.5 ppm), both at the carbon chemical shift of ~24 ppm. This suggests that these two protons are attached to the same carbon, as would be the case for either carbon 3 or 10. Another carbon at ~32 ppm also appears to have two distinct protons attached to it.



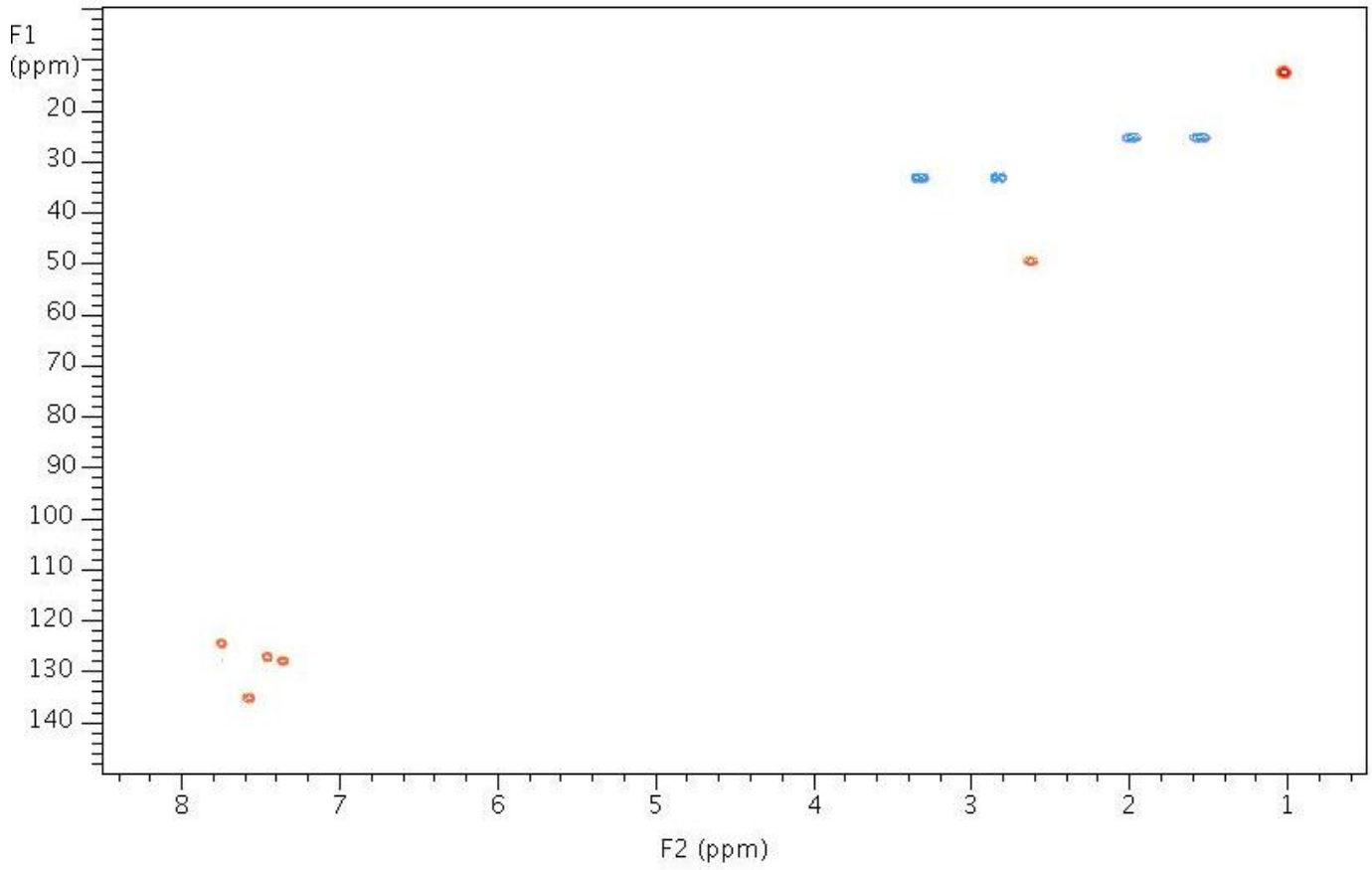


Figure 13

Notice also that this spectrum has both positive (red) and negative (blue) peaks. This is because the spectrum was acquired with the multiplicity-editing option. When this option is selected, methylene peaks will have an opposite phase as compared to methyl and methine peaks. In the spectrum below, the phase has been adjusted such that the methyl and methine peaks are positive and the methylene negative. (The relative signs matter, but the absolute sign is arbitrary.) Ethylindanone contains 1 methyl and 5 methine carbons, and correspondingly, the spectrum contains six red peaks. The two methylene carbons each have two diastereotopic protons attached, each with distinct chemical shifts, hence four peaks are observed. With this single gHSQCAD spectrum we have assigned the carbon chemical shifts and determined the carbon "multiplicities" for every carbon that has an attached proton. We have also determined which pairs of protons belong to each methylene carbon. With some knowledge of carbon chemical shifts and good chemical shift prediction software, we could also verify that the shifts observed are reasonable for the structure proposed.

Limitations and Problems

So, what are we missing? One glaringly obvious deficiency is the lack of information for quaternary carbons 1, 4, and 9. Recall that the proton spectrum suggested the presence of a 1,2-disubstituted aromatic ring. From this we can infer the presence of carbons 4 and 9, but we don't have any direct information concerning the carbonyl at carbon 1. The chemical shift of ~49 ppm for methine carbon 2 may be suggestive or perhaps consistent with a neighboring carbonyl, but it is not definitive. Additional data beyond the gHSQCAD experiment is required if either a higher level of certainty is required for a structural confirmation or if the problem is a structure elucidation. Another piece of information missing is where the ethyl group is attached. We know from the COSY coupling data that it is attached to an aliphatic carbon, but we don't really know whether it is attached to carbon 2 or carbon 3. Again, the proton and carbon chemical shifts are suggestive of the structure drawn, but we may need stronger evidence. To obtain regiochemical information like this, we can use multiple-bond proton-carbon correlation data.

Although the gHSQCAD experiment is slightly less sensitive than the typical homonuclear 2D TOCSY/COSY type experiment, most modern spectrometers are able to provide good data in just a few minutes of experiment time on small molecule samples at ~10 mM concentrations.

Experimental Variants

The "J1(CH)corr" tab of the experiment selector contains the following variants of the 2D 1-bond proton-carbon correlation experiment. (For more information on any of these, see the Experiment Guide.)

- HSQCAD - This version contains adiabatic 180° X-nuclei pulses to compensate for carbon pulse imperfections and provide more uniform performance over wide carbon spectral widths. The gHSQCAD version is typically preferred unless sensitivity is an issue.
- gHSQCAD - This version is similar to the HSQCAD, but includes gradient coherence selection, which provides better artifact suppression at the expense of a loss of sensitivity by a factor of ~1.4. This version is usually the preferred HSQC-type pulse sequence.
- HSQC- This is the standard heteronuclear single-quantum one-bond J-correlation experiment. The adiabatic version of the HSQC is typically preferred as it usually gives superior results.
- gHSQC - This version is similar to the HSQC, but includes gradient coherence selection. The adiabatic version is typically preferred as it usually gives superior results.
- HMQC - This is the standard heteronuclear multiple-quantum one-bond J-correlation experiment. In general, the HSQC-type experiments produce narrower peaks shapes than the HMQC-type experiments, which typically leads to better S/N and resolution. The HSQC experiments also have the added ability to do multiplicity editing. HMQC is useful for older and special types of probes that have lower RF homogeneity, or for samples with very broad resonances.
- gHMQC - Similar to the HMQC, but also includes gradient coherence selection. This version would typically be preferred over HMQC unless sensitivity is an issue.

- HETCOR - This is a carbon observe version of the heteronuclear one-bond shift correlation experiment, e.g., ^{13}C is in the F2 dimension. This makes the HETCOR much less sensitive than the proton-detected HSQC/HMQC (approximately 8-fold less sensitive), particularly with inverse-type probes, although a direct-observe (carbon) probe can partially mitigate this. The HETCOR is useful for special circumstances, such as where higher resolution is desired in the carbon dimension, as compared to the proton dimension. The decoupling power used during acquisition is also usually less, which causes less sample heating (because the proton chemical shift range of proton is smaller than it is for carbon, in Hz).
- gHETCOR - Similar to the HETCOR, but also includes gradient coherence selection. This version would typically be preferred over HETCOR unless sensitivity is an issue.
- ASAPHMQC - This is a heteronuclear multiple-quantum one-bond J-correlation experiment with adiabatic 180° X-nuclei pulses and an "ASAP" feature for rapid recycle times. ASAPHMQC can be used as a fast survey experiment as it can provide a complete HX correlation spectrum with reasonable resolution in about a minute or less for samples of at least ~40mM concentration.

Other more advanced variants of the HSQC-type family of experiments are available under the "(HC)Crisis2" tab of the Experiment Selector. These experiments might provide the best signal-to-noise available for modern spectrometers. (The (HC)Crisis2 experiments are a suite of commonly-used heteronuclear 2D experiments that differ by having bip (broadband inversion pulse) pulses or adiabatic pulses in both the low-band and high-band channels. These experiments can be particularly useful when acquiring a collection of samples in automation that may adversely and variably affect the probe tuning/calibrations because of variations in salt concentrations, different solvents, etc. The bip pulses can compensate for imperfect calibrations, resulting in good data quality under these difficult conditions. Another prominent use for Crisis2 experiments are 19F-X correlations, where the much wider (compared to 1H) spectral width of fluorine would be difficult to cover with standard rectangular 180° pulses. For more information on any of these, see the Experiment Guide.)

- c2hsqcse - The c2hsqcse is of HSQC type but has an additional sensitivity enhancement feature which, in theory, can result in an increase of approximately a factor of 1.4x in sensitivity – optimized for CH protons - over c2hsqc (and hence of 2x over gc2hsqc). Minor COSY-type transfer crosspeaks may be visible though in sensitivity enhanced spectra because of the additional delays employed in the sequence.
  This sensitivity enhanced version of the c2hsqc is typically preferred as it usually gives superior results, except possibly for molecules with rapid diffusion or relaxation (because the sequence contains more delays).
- c2hsqc - This is the standard Crisis2 heteronuclear single-quantum one-bond J-correlation experiment.
- gc2hsqcse - This version is similar to the c2hsqcse, but includes gradient coherence selection, which provides better artifact suppression (and gives approximately the same signal-to-noise). This version is usually the preferred Crisis2 HSQC pulse sequence.
- gc2hsqc - This version is similar to the c2hsqc, but includes gradient coherence selection. The sensitivity enhanced version of the gc2hsqc is typically preferred instead, as it usually gives superior results, except possibly for molecules with rapid diffusion or relaxation.

Further Reading

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5 Heteronuclear 2D Multiple-Bond Correlation Spectra

- Overview
- gHMBCAD of Ethylindanone
- Limitations and Problems
- Experimental Variants
- Further Reading

Overview

The long-range or multiple-bond proton-carbon correlation experiments give crosspeaks between protons and carbons that are separated by two or more bonds. This data is typically used in small molecule NMR to answer the often essential structural gaps remaining after the COSY/TOCSY and HSQC-type data have been analyzed. In particular, the HMBC-type experiment can provide key connectivities either between separated spin systems or to difficult-to-detect quaternary carbons. Usually the HMBC-type experiments are more sensitive than 1D CARBON spectra for detecting quaternary carbons that are coupled to a proton less than 4 bonds away. The sensitivities of these experiments vary widely, however, for different correlations within a dataset. Although HMBC might not show all possible correlations when used on less concentrated samples, even a partial long-range correlation data set can be highly valuable for structure elucidation.

Most of these experiments rely on obtaining ^{1}H-^{13}C correlations via long-range coupling constants. In general 3-bond coupling constants (~8 Hz) are larger than 2-bond coupling constants (~2-3 Hz) and typically tend to give stronger crosspeaks when the experimental parameters are optimized for 8 Hz. However, caution must be exercised in assigning these weaker crosspeaks as 2-bond correlations solely from their lowered intensity, as they can only be definitively identified as ‘crosspeaks that arise from smaller coupling constants’. Modified experiments do exist, however, for distinguishing between 2- and 3-bond correlations.

Of the varieties of multiple-bond proton-carbon correlation experiments provided in VnmrJ, the gHMBCAD (gradient-enhanced heteronuclear multiple bond correlation with adiabatic pulses) is often a good choice. It is a robust experiment, with good sensitivity, and produces spectra relatively clean of artifacts.

gHMBCAD of Ethylindanone

To obtain a gHMBCAD spectrum, the experiment should be chosen from the Experiment Selector under the “Jn(CH)corr” tab. If the gHMBCAD parameters are set up in the StudyQ, or in an experiment that contains a previously acquired proton spectrum, customizations such as the proton chemical shift range will be maintained. Only a few simple parameters such as the number of scans, the number of t1 increments, and the carbon spectral width need to be adjusted. At the bottom is a pull-down menu to select an optimal long-range proton-carbon coupling constant. The default of 8 Hz should work for most standard organic molecules and problems. For further details on setting up and processing the experiment, please refer to the Experiment Guide, Chapter 5.

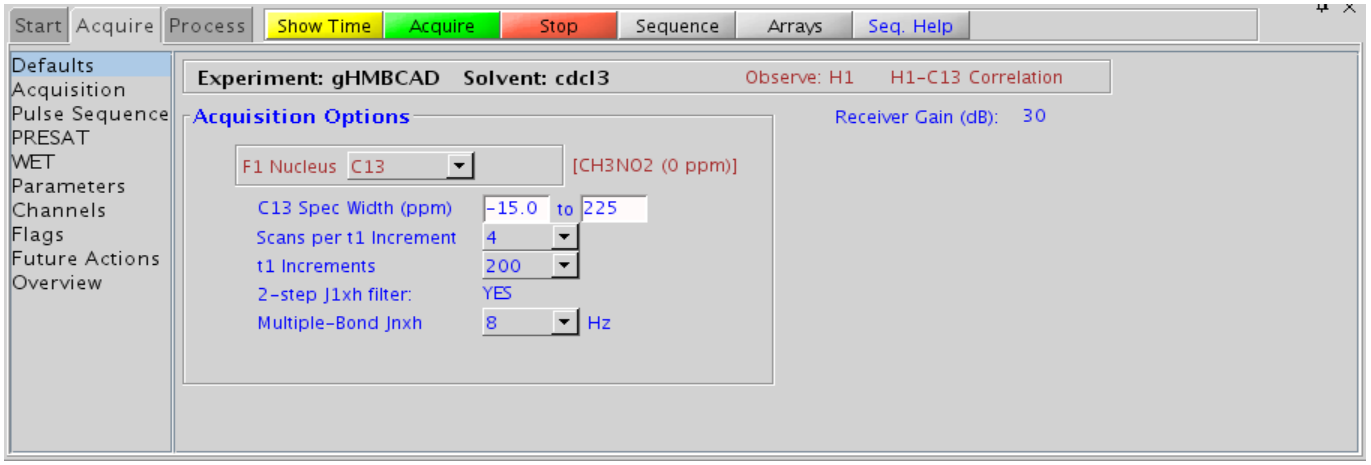


Figure 14

Figure 15 shows the full-scale gHMBCAD spectrum of ethylindanone that can be found in the VnmrJ fidlib. At this vertical scale, the correlations for the aromatic protons are primarily to the aromatic carbons, whereas the aliphatic protons show correlations to a wide range of carbon chemical shifts. This illustrates the power of HMBC-type data to provide connectivity information.

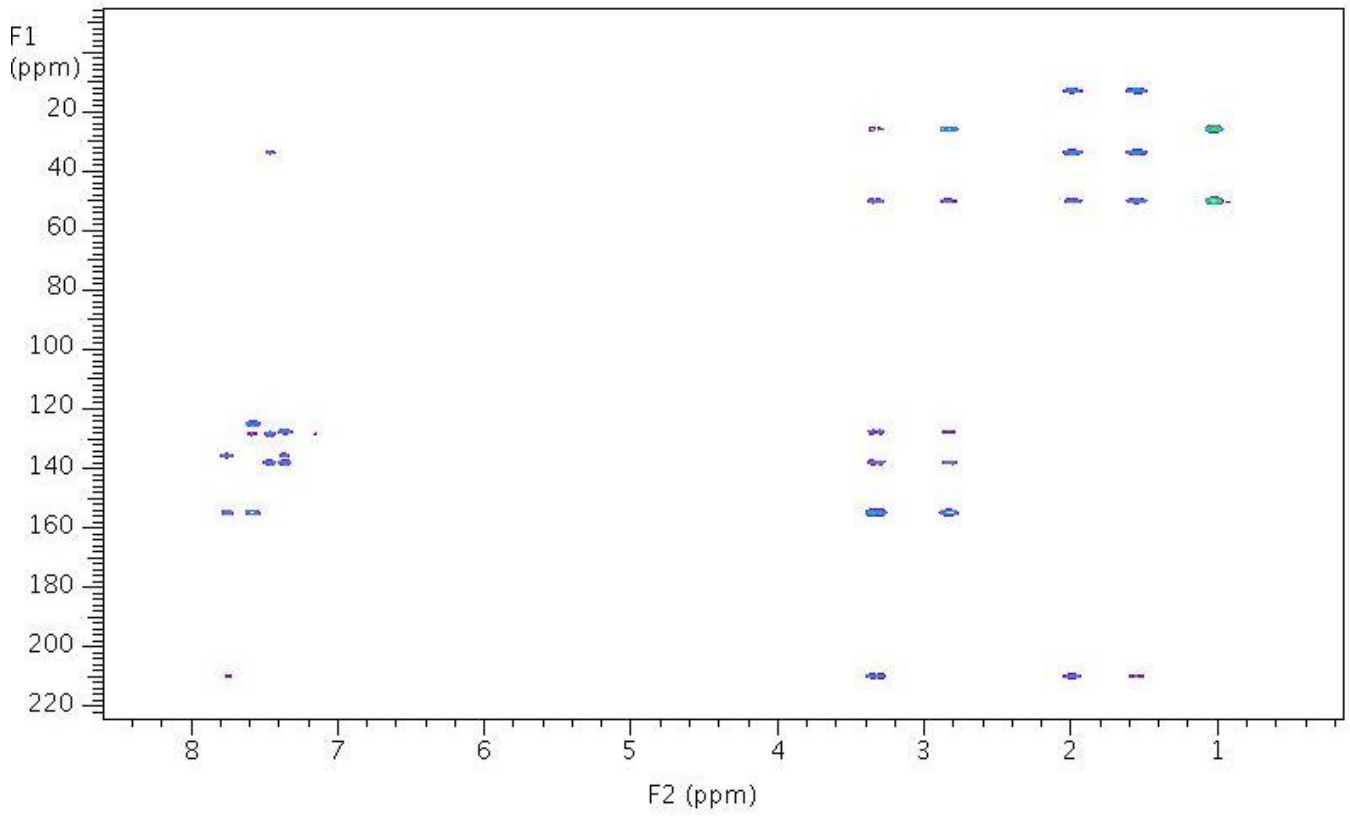
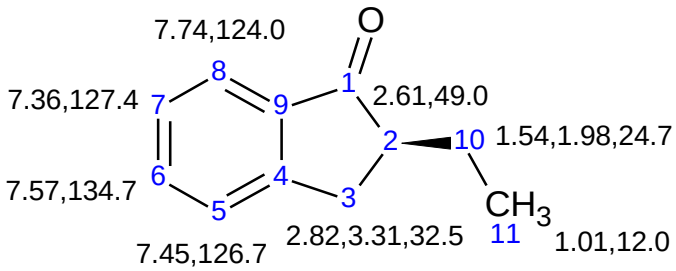


Figure 15

Recall from Chapter 4 that after analysis of the gHSQCAD experiment, information was lacking regarding quaternary aromatic carbons 4 and 9, carbonyl carbon 1, and regiochemistry of the ethyl attachment. Figure 16 is an expansion of the aromatic carbon/proton region that shows how the gHMBCAD data can be used to fill this informational gap.



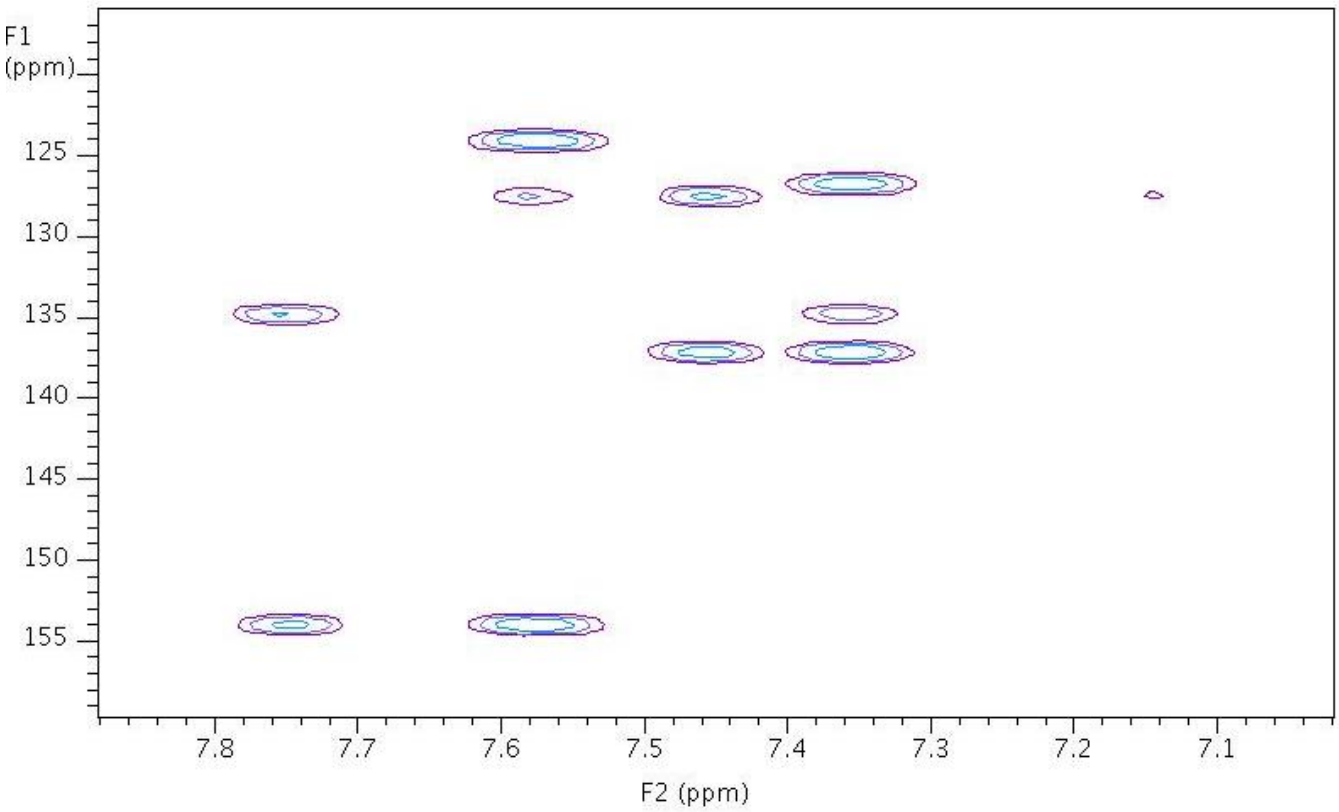


Figure 16

In this expansion, proton 8 at 7.74 ppm shows two correlations: one to a carbon at ~135 ppm and another to a carbon at ~154 ppm. When the gHMBCAD data is acquired with the standard 8 Hz $^{n}J_{C,H}$, the 3-bond proton-carbon correlations are typically stronger than the 2-bond correlations, so these signals likely arise from correlations between proton 8 and carbons 4 and 6. The carbon chemical shift of the top correlation is also consistent with the chemical shift of 134.7 seen for carbon 6 in the gHSQCAD experiment. This suggests that the correlation at ~154 arises from coupling to carbon 4. There is a second aromatic proton that shows a correlation to this carbon, proton 6, which is also three bonds from carbon 4, providing additional evidence that carbon 4 is at ~154 ppm. Proton 6 has two other correlations to carbons at ~124 and ~127 ppm. The stronger peak at 124 ppm is the three-bond correlation to carbon 8, while the weaker peak at 127 ppm is the two-bond correlation to carbon 7. Once again, these chemical shifts are consistent with those observed in the gHSQCAD. The next proton, H-8 at 7.45 ppm, shows peaks to two aromatic carbons at ~127 and ~137 ppm. The first is the correlation to carbon 7, while the second is most likely the 3-bond correlation to carbon 9. Proton 7 at 7.36 ppm also shows this correlation to carbon 9, in addition to two other correlations to carbon 5 (stronger 3-bond) and carbon 6 (weaker 2-bond) at ~127 and ~135 ppm, respectively. So, after analysis of this portion of the gHMBCAD data, we now have evidence of, and plausible assignments for, quaternary aromatic carbons 4 and 9.

Next, let's look at an expansion of the aliphatic protons for evidence of carbonyl carbon 1 in Figure 17.

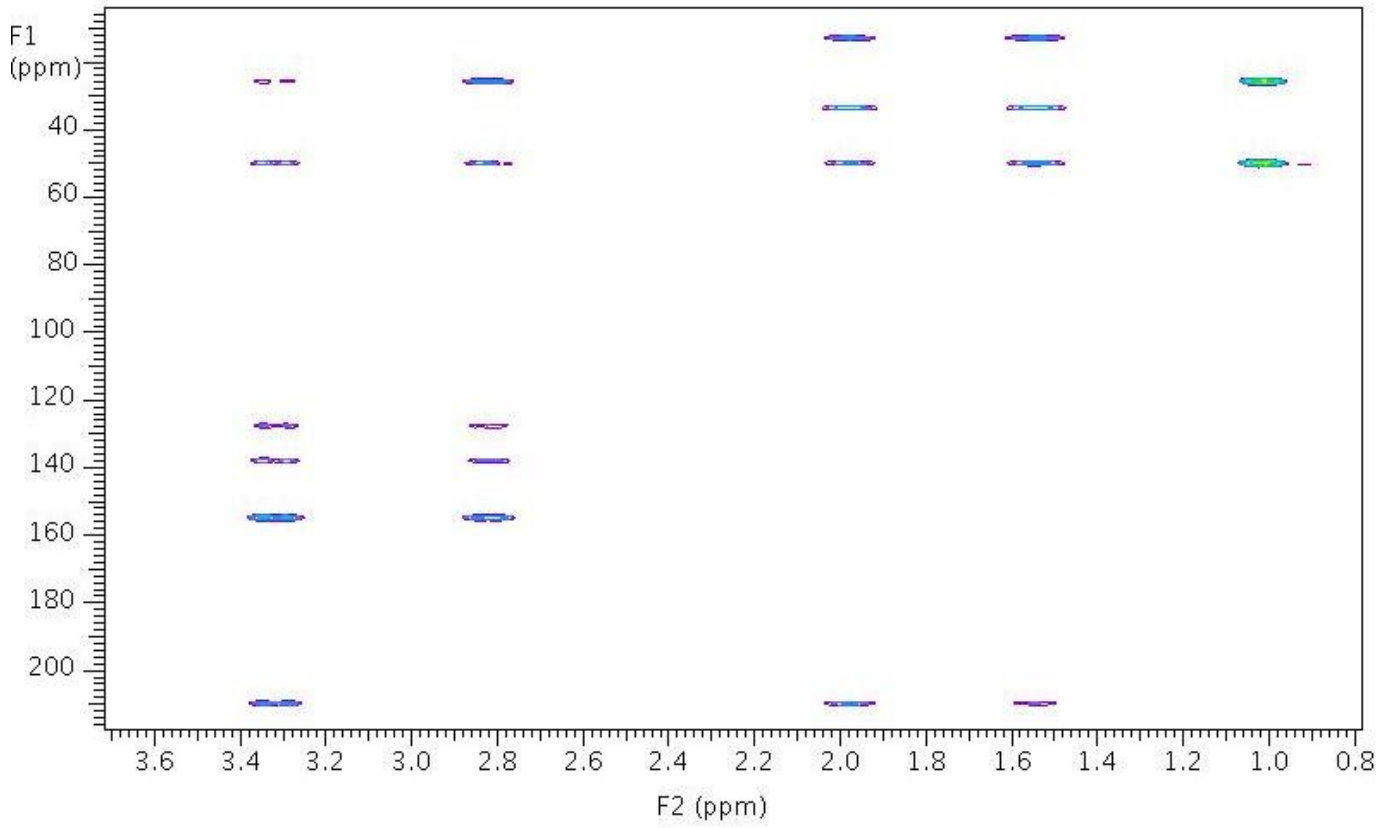


Figure 17

Figure 17 indicates that three aliphatic protons show correlations to a carbon at 209 ppm: one methylene proton from H-3 at 3.31 ppm, and both H-10 methylene protons at 1.54 and 1.98 ppm. These protons, being three bonds away, could be expected to show a correlation to carbon 1, and the chemical shift of 209 ppm is consistent with a carbonyl carbon. What about the other methylene H-3 proton at 2.82 ppm? The dihedral angle between this proton and carbon is likely such that the coupling constant is much smaller and hence the correlation correspondingly weaker. In fact, if the vertical scale is increased, a very weak correlation can be seen near the noise level.

Finally, we will look for evidence of the regiochemistry of the ethyl attachment, e.g., is the molecule 2-ethylindanone or 3- ethylindanone?

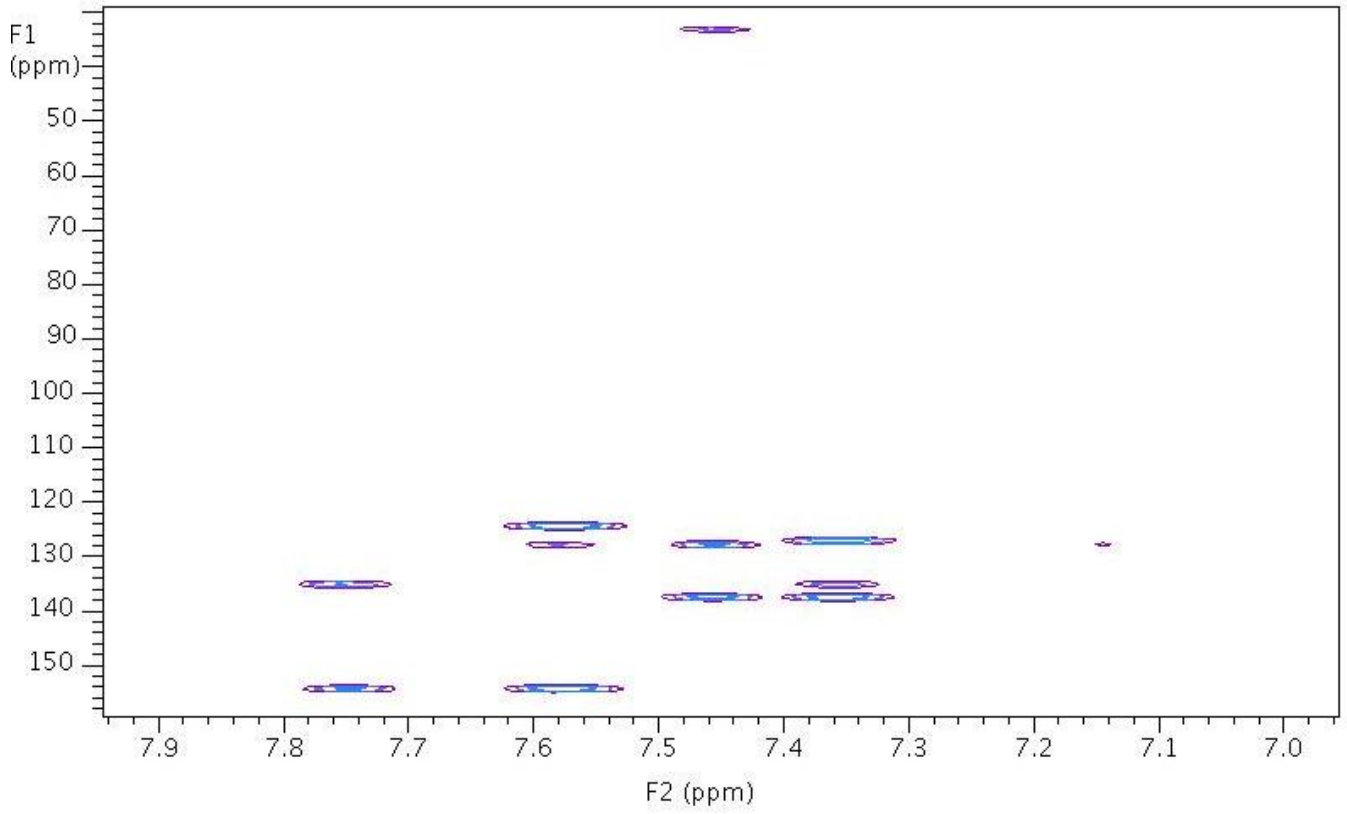


Figure 18

To answer this question, the most straightforward piece of information is a correlation between aromatic proton 5 at 7.45 ppm and aliphatic methylene carbon 3 at ~33 ppm. From this correlation we can discern that proton 5 and carbon 3 are most likely 3 bonds apart, confirming that the structure is, indeed, 2-ethylindanone.

Limitations and Problems

The multiple-bond proton-carbon correlation experiments are powerful and provide useful data, but they have limitations, such as their low sensitivity and the dependency of the magnitude of the correlation upon the proton-carbon coupling constant. The sensitivity problem can only be overcome with additional acquisition time or an increased amount of sample. The optimal coupling constant for the experiment can be fine tuned to see a specific correlation, or reduced from the default 8 Hz to see longer-range correlations, although this still does not always let you see missing correlations. If the coupling constant is too small, or the correlation is too long-range, or the signals too broad, a 1D carbon experiment may be a better way to detect a carbon signal of interest.

HMBC-type experiments are clearly rich with regiochemical information for structure determination; however, they don't let you obtain stereochemical information. For instance, in the ethylindanone example, the stereochemistry (*R* or *S*) at the 2-position cannot be determined with NMR (except perhaps with chiral shift reagents), even though we might want stereospecific assignments for the methylene protons at position 3. This type of information is best obtained with a through-space correlation experiment, e.g., a NOESY or ROESY.

Experimental Variants

The "Jn(CH)corr" tab of the experiment selector contains the following variants of the 2D heteronuclear multiple-bond J-correlation experiments, some of which provide unique structural information. (For more information on any of these, see the Experiment Guide.)

- gHMBCAD - This version contains adiabatic 180° X-nuclei pulses to compensate for carbon pulse imperfections and provide more uniform experimental performance over wide carbon spectral widths. This experiment also uses gradient coherence selection, which provides better artifact suppression at the expense of a loss of sensitivity by a factor of ~1.4.

- CIGAR - The CIGAR experiment is used to correlate protons to carbons over multiple bonds. Although less sensitive than its HMBC counterpart, this experiment is useful when there is a need to see longer range correlations (>3 bonds and/or with small coupling constants), or when there is a large range of $^{n}J_{X,H}$ coupling constants, such as with proton-nitrogen.
- CIGARAD - This version of the CIGAR experiment includes the adiabatic pulses and will typically give better results than CIGAR.
- gHMBCmeAD- The gHMBCmeAD experiment is a multiplicity-edited version of gHMBCAD. It is used to distinguish between methyl/methine and methylene/quaternary carbons. This experiment is acquired as an array of 2 spectra, which are processed two different ways to generate the edited 2D spectra, and, as such, will take twice as long to acquire a spectrum of comparable quality.
- gHMBCRELAY - Although less sensitive than the gHMBCAD, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations, as the 2-bond correlations are generated through a separate pathway and may be separated into a subspectrum.

Other more advanced variants of the HMBC-type family of experiments are available under the "(HC)Crisis2" tab of the Experiment Selector. These experiments might provide the best signal-to-noise available for modern spectrometers. (For more information on any of these, see the Experiment Guide.)

- gc2hmbc - This is the standard Crisis2 heteronuclear multiple-bond J-correlation experiment. The (HC)Crisis2 experiments are a suite of commonly-used heteronuclear 2D experiments that differ by having bip (broadband inversion pulse) pulses or adiabatic pulses in both the ^{13}C and ^{1}H channels. These experiments can be particularly useful when acquiring a collection of samples in automation that may adversely and variably affect the probe tuning/calibrations because of variations in salt concentrations, different solvents, etc. The bip pulses can compensate for imperfect calibrations, resulting in good data quality under these difficult conditions.

- gc2hmbcme - The gc2hmbcme experiment is a multiplicity-edited version of the gc2hmbc. It is used to distinguish between methyl/methine and methylene/quaternary carbons. This experiment is acquired as an array of 2 spectra, which are processed two different ways to generate the edited 2D spectra, and, as such, will take twice as long to acquire a spectrum of comparable quality.
- gc2h2bc - The gc2h2bc experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over 2 bonds. Although the gc2h2bc is less sensitive than the gc2hmbc, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra.
- gc2h2bcme - The gc2h2bcme experiment is a multiplicity-edited version of the gc2h2bc. It is used to distinguish between methyl/methine and methylene/quaternary carbons. This experiment is acquired as an array of 2 spectra, which are processed two different ways to generate the edited 2D spectra, and, as such, will take twice as long to acquire a spectrum of comparable quality.

Further Reading

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

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C. E. Hadden, Magn. Reson. Chem. 43 (2005) 330-333. (Adiabatic Pulses)

6 Homonuclear 2D Through-Space Spectra

Overview

The NOESY (nuclear Overhauser effect spectroscopy) experiment is a uniquely useful tool for structure elucidation as it looks at interactions *through-space*, as opposed to the experiments in the previous chapters that were all *through-bond*. The experiment is used to determine if any pair of protons are close in space, and generates a cross peak if they approach within ca. < 5 Angstroms. This provides information for things such as relative stereochemistry, or connectivity through heteronuclei such as oxygen or nitrogen. The NOE is a signal enhancement that is generated through dipole-dipole spin relaxation, analogous to the T_1 and T_2 relaxation discussed in Chapter 2. The NOE affects the spins during a delay in the NOESY pulse sequence called the mixing time. The size of the NOE, e.g., how much it affects peak heights, is dependent upon the distance between the two nuclei of interest. Longer distances cause the NOE to build up more slowly, which in turn requires the user to use longer mixing times in the experiment in order to see the effect.

The intensity and sign of the NOE is also dependent upon the molecule's correlation time, which can be thought of as a molecular tumbling rate. It depends upon a number of variables, including the size and shape of the molecule, the spectrometer frequency, solvent viscosity, and sample temperature. Smaller molecules are in what is called the extreme narrowing limit, which means they have higher correlation frequencies, and exhibit positive NOEs, with a maximum value +0.5 for ^{1}H-^{1}H interactions. (This means that their NOEs can change signal intensities by a maximum of +50%.) Larger molecules tumble more slowly, which means they have lower correlation frequencies, and they exhibit negative NOEs, with a maximum effect of -1.0 for ^{1}H-^{1}H interactions). The change in sign of the NOE as a function of molecular weight creates an issue for medium-sized molecules, as it means that their measured NOEs can be zero, even if two protons are close in space. Therefore, the lack of an NOE effect implies nothing when performing structure elucidations - only the presence of an NOE effect can be interpreted and be meaningful. For these intermediate-sized molecules, which are usually ~500-2000 MW, one should use the ROESY experiment to circumvent the problem of near-zero NOEs. The ROESY experiment uses a spin-lock during the mixing time to move the system within the extreme narrowing limit and generate the ROE (rotating-frame Overhauser enhancement). The ROE is always positive, regardless of the correlation time, so the magnitude of the ROE never crosses through zero like the NOE. The downside of ROESY is that, for large molecules, the magnitude of the ROE effect is normally smaller than the NOE effect, so for molecular weights>5000, NOESY is normally used. Figure 19 shows a comparison of how the NOE and ROE intensities vary with the product of the spectrometer frequency and the molecular correlation time ($\omega\tau_c$).

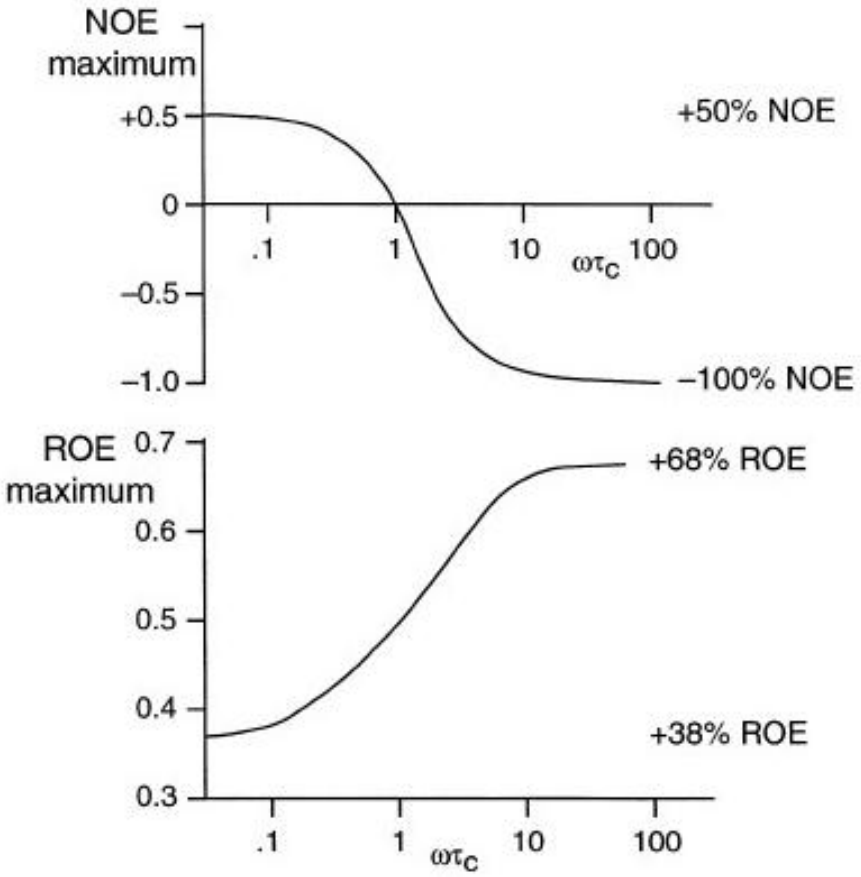


Figure 19

If you acquire both ROESY and NOESY-type data, you should have all available through-space correlation information.

ROESYAD of Ethylindanone

To obtain a ROESYAD spectrum, the experiment should be chosen from the Experiment Selector under the “(HH)Homo2D” tab. If the ROESYAD parameters are set up in the StudyQ, or in an experiment that contains a previously acquired proton spectrum, customizations such as the proton chemical shift range will be maintained. Only a few simple parameters such as the number of scans, the number of t1 increments, and the relaxation time need to be adjusted. At the bottom is a pull-down menu to select a spinlock mixing time for the experiment. A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but these longer mixing times can cause sample heating. Do not to exceed a 500ms spinlock as this can damage the spectrometer hardware. For further details on setting up and processing the experiment, please refer to the Experiment Guide, Chapter 3.

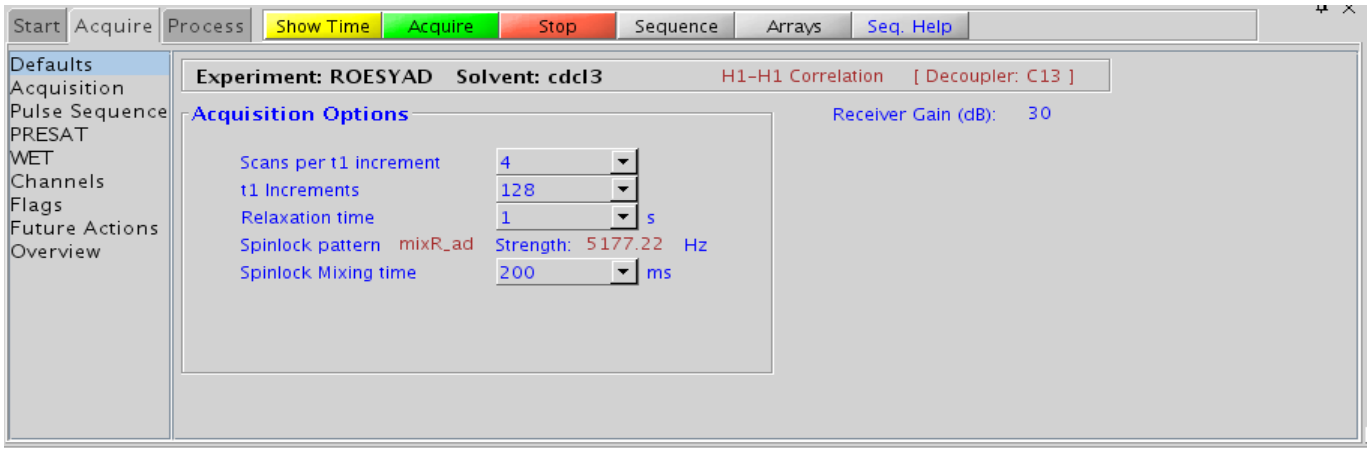
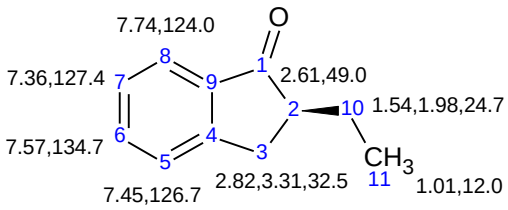


Figure 20

Figure 21 shows the full-scale ROESYAD spectrum of ethylindanone that can be found in the VnmrJ fidlib. Note that for this data set, there do not appear to be any crosspeaks between the aliphatic and aromatic protons. Possible weak correlations appear at much higher vertical scale, but it is difficult to distinguish them from T1 noise in this spectrum. It should also be noted that the default processing in VnmrJ is to phase the diagonal peaks negative (blue), however, this choice is arbitrary and can easily be changed by the user. True ROESY crosspeaks should always appear with a sign opposite to the diagonal phase; in this spectrum they should be positive (red).



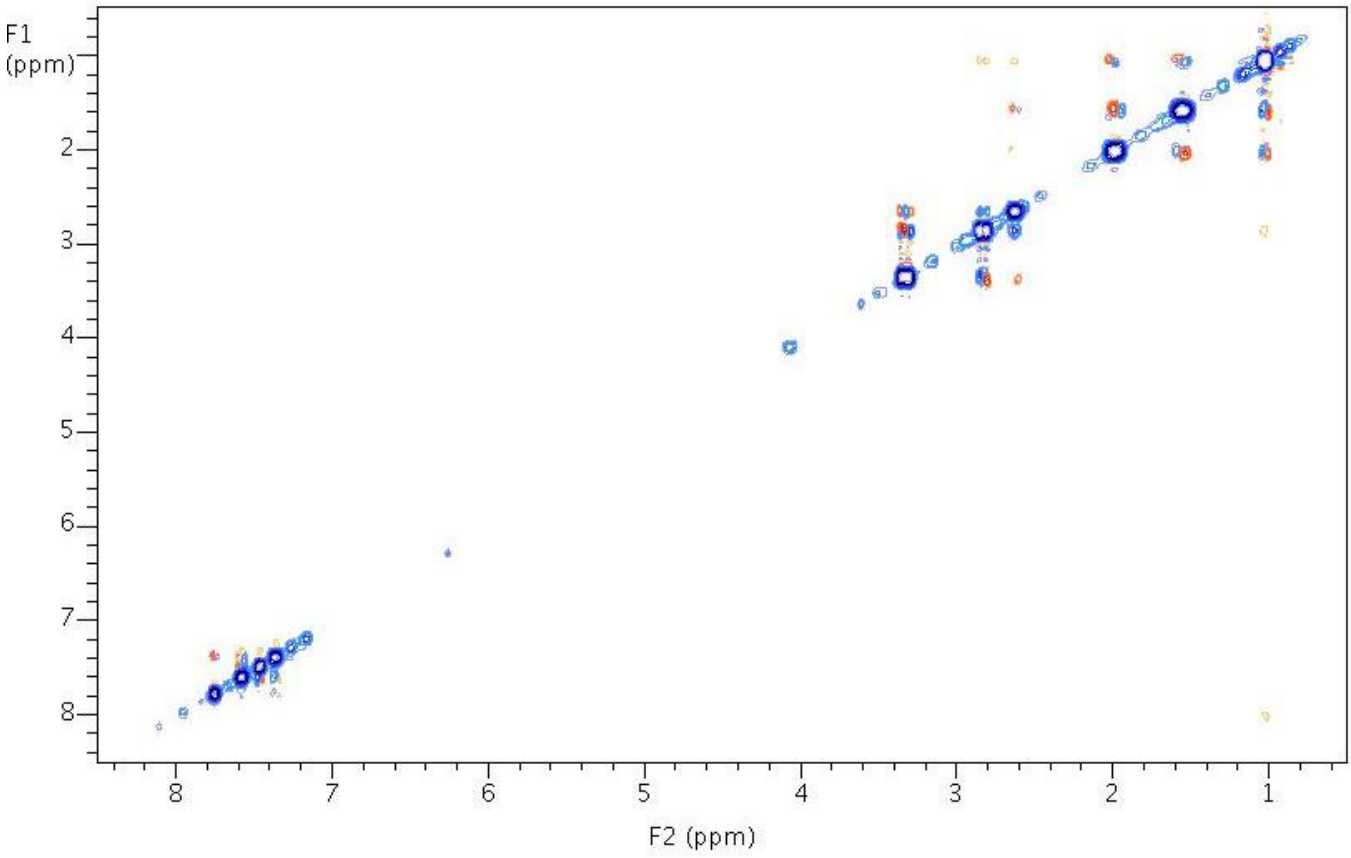


Figure 21

To examine the relative stereochemistry of the 2-ethyl group and the methylene protons at position 3, let's examine an expansion of the aliphatic region of the ROESYAD (Figure 22).

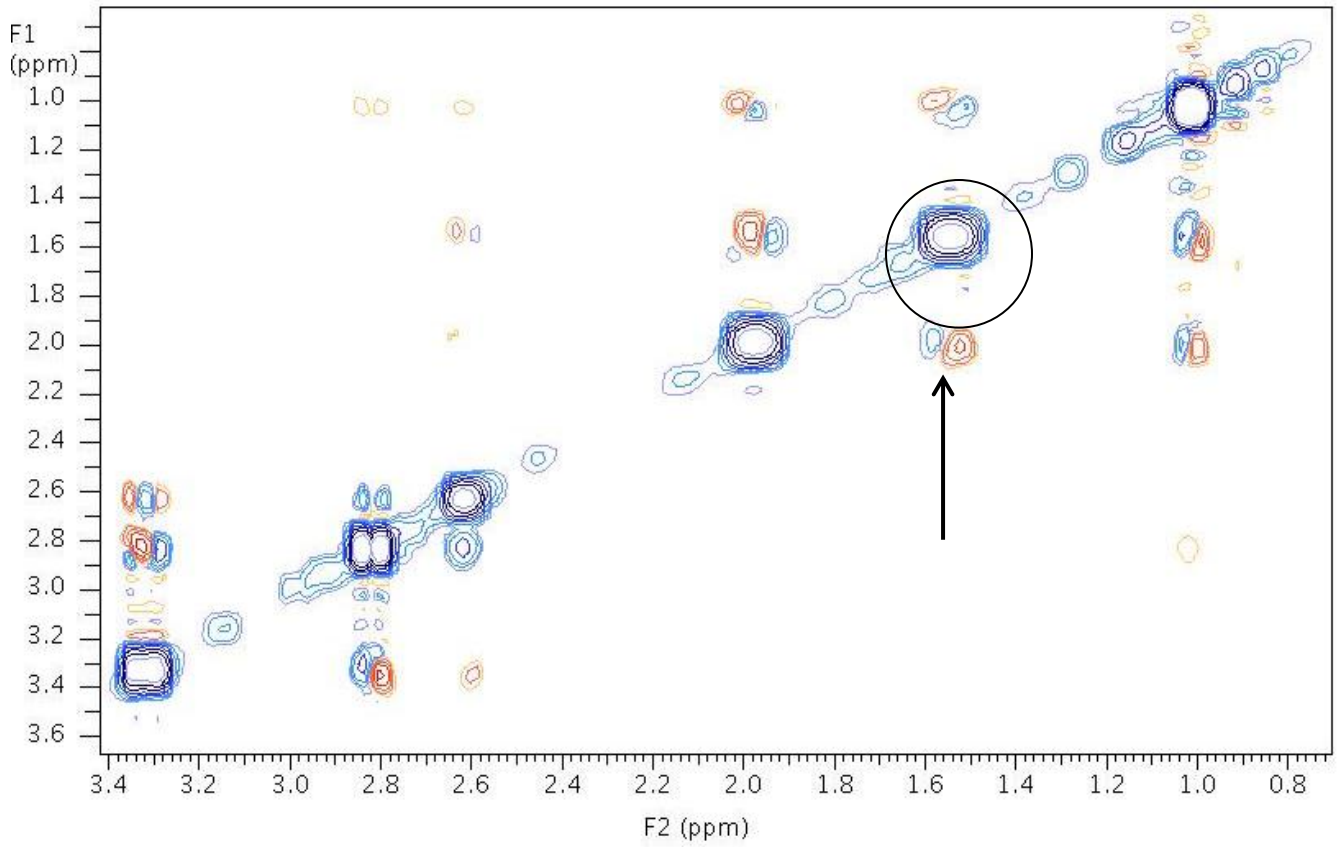


Figure 22

In this expansion, we can see that while the diagonal has been phased as pure negative peaks, some of the crosspeaks appear to have a mixture of both positive and negative phases. This is because the crosspeaks come from a mixture of TOCSY-type through-bond magnetization transfer, which has the same sign as the diagonal, and ROESY-type through-space magnetization transfer, which has a sign opposite to the diagonal peaks. For example, consider the crosspeaks circled on the above spectrum. These are correlations from the methyl (11) and the neighboring methylene protons (10). These protons are both scalar coupled and close in space, so the crosspeaks have both a positive (TOCSY) and negative (ROESY) component.

There are, however, a few crosspeaks that appear to have relatively pure phase, for example two of the crosspeaks to methine 2 at 2.61 ppm. Methine 2 has a correlation the same sign as the diagonal to the methylene 3 protons at 2.82 ppm, but an opposite sign correlation to the methylene at 3.31 ppm. The different signs provide information regarding the nature of these two correlations. The positive sign of the crosspeak to the proton at 2.82 indicates that this correlation is primarily coupling in nature, while the peak to the other methylene arises from a through-space interaction. For 5 and 6-membered organic ring systems, protons that are *anti* to one another are typically more strongly coupled, while protons with a *syn* stereochemical orientation are close enough in space to show NOE/ROE correlations. Given this, the logical stereochemical assignments for the 3 methylene protons are that the proton at 2.82 is *anti* to the 2-ethyl group and the proton at 3.31 is *syn*. There is, in fact, an additional piece of evidence to support this hypothesis; the 11 methyl shows an ROE crosspeak to the methylene proton at 2.82, which shows that they are *syn*.

NOESY of Ethylindanone

To obtain a NOESY spectrum, select the experiment from the Experiment Selector under the “(HH)Homo2D” tab. If the NOESY parameters are set up in the StudyQ, or in an experiment that contains a previously acquired proton spectrum, customizations such as the proton chemical shift range will be maintained. Only a few simple parameters such as the number of scans, the number of t1 increments, and the relaxation time need to be adjusted. At the bottom is a pull-down menu to select an NOE mixing time for the experiment. A mixing time of 500ms is a good starting place for small molecules.

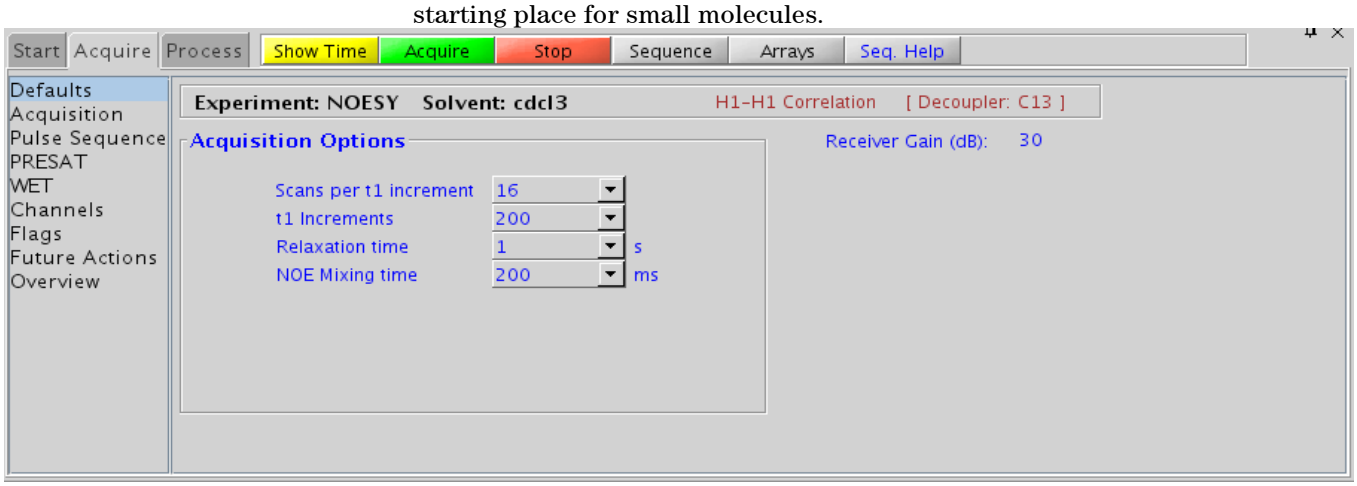
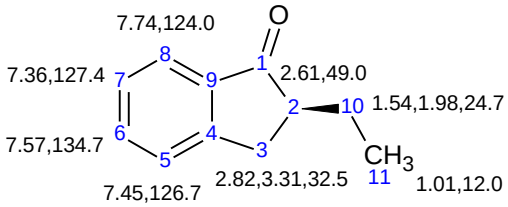


Figure 23

Very weak NOE interactions may require longer mixing times (up to ~1s), but the intensity of some correlations may begin to fall off with longer mixing times. Moreover, longer mixing times increases the risk of generating potentially misleading peaks from a mechanism called spin diffusion. With this process, we can have a situation where proton A is close in space to proton B, and proton B is close to proton C. Protons A and B and protons B and C are expected to show NOE correlations, but a crosspeak may also arise between A and C, although they are not close enough for a direct NOE interaction due to spin diffusion. Thus, caution is required, especially when using longer mixing times. It may, in fact, be necessary to acquire the data with several different mixing times to see and properly interpret all correlations. For further details on setting up and processing the experiment, please refer to the Experiment Guide.

First let's take a look at the same expansion of the aliphatic region as the NOESY (Figure 24).



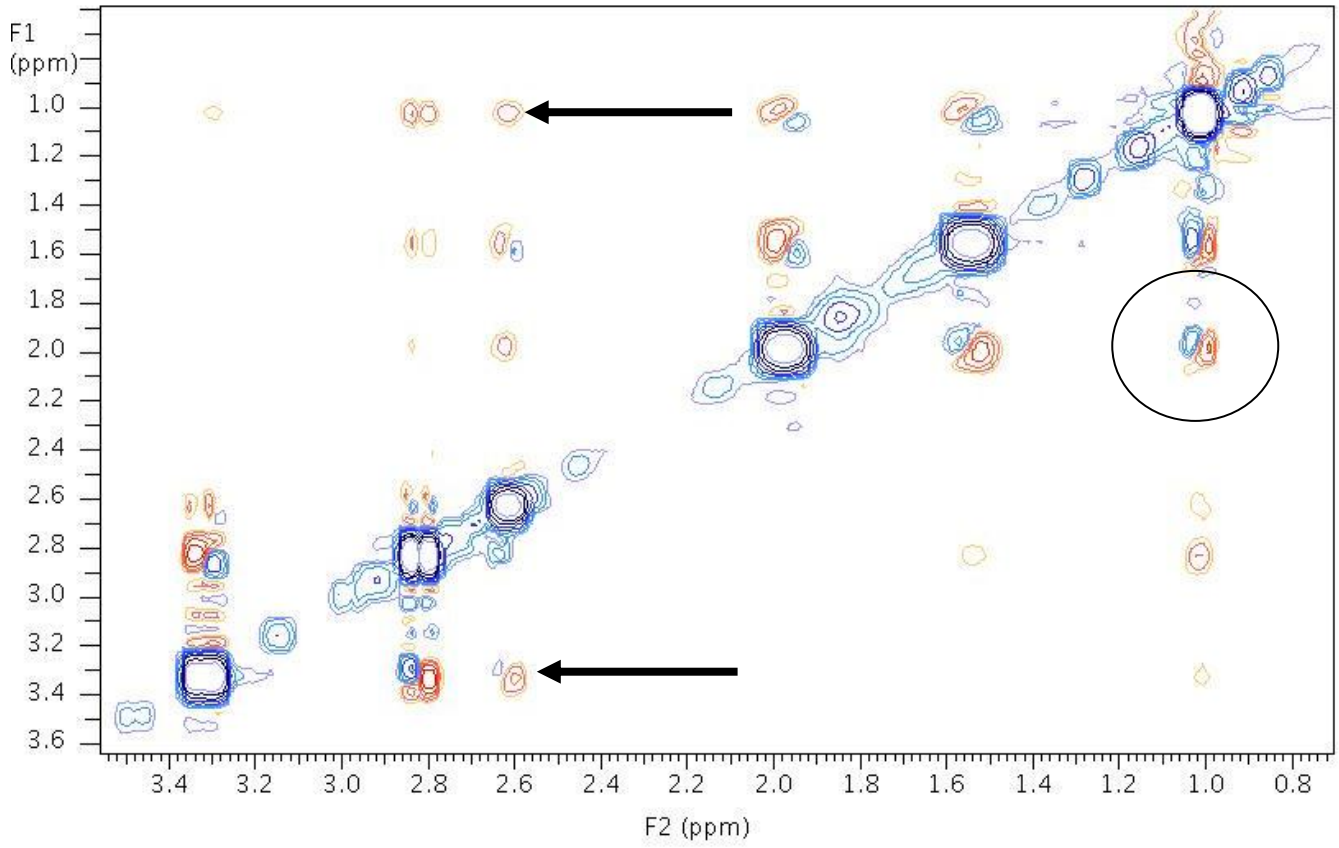


Figure 24

Again the appearance is similar to the ROESYAD, as expected, with positively phased crosspeaks from NOE interactions (red) and negatively (blue, same sign as the diagonal) phased arising from zero-quantum (coupling-type) correlations. In Figure 24, the circled crosspeaks between the methyl (11) and the neighboring methylene protons (10) also show a positive and negative phase component, indicating both an NOE and a scalar coupling interaction. In addition, the 3 proton at 3.31 ppm shows an NOE correlation to methine 2, while the methyl 11 has a crosspeak to the methylene at 2.82 ppm, confirming the respective *syn* stereochemical relationships.

Looking at a larger view of the NOESY spectrum (Figure 25), however, we can see a difference between the NOESY and ROESYAD spectra.

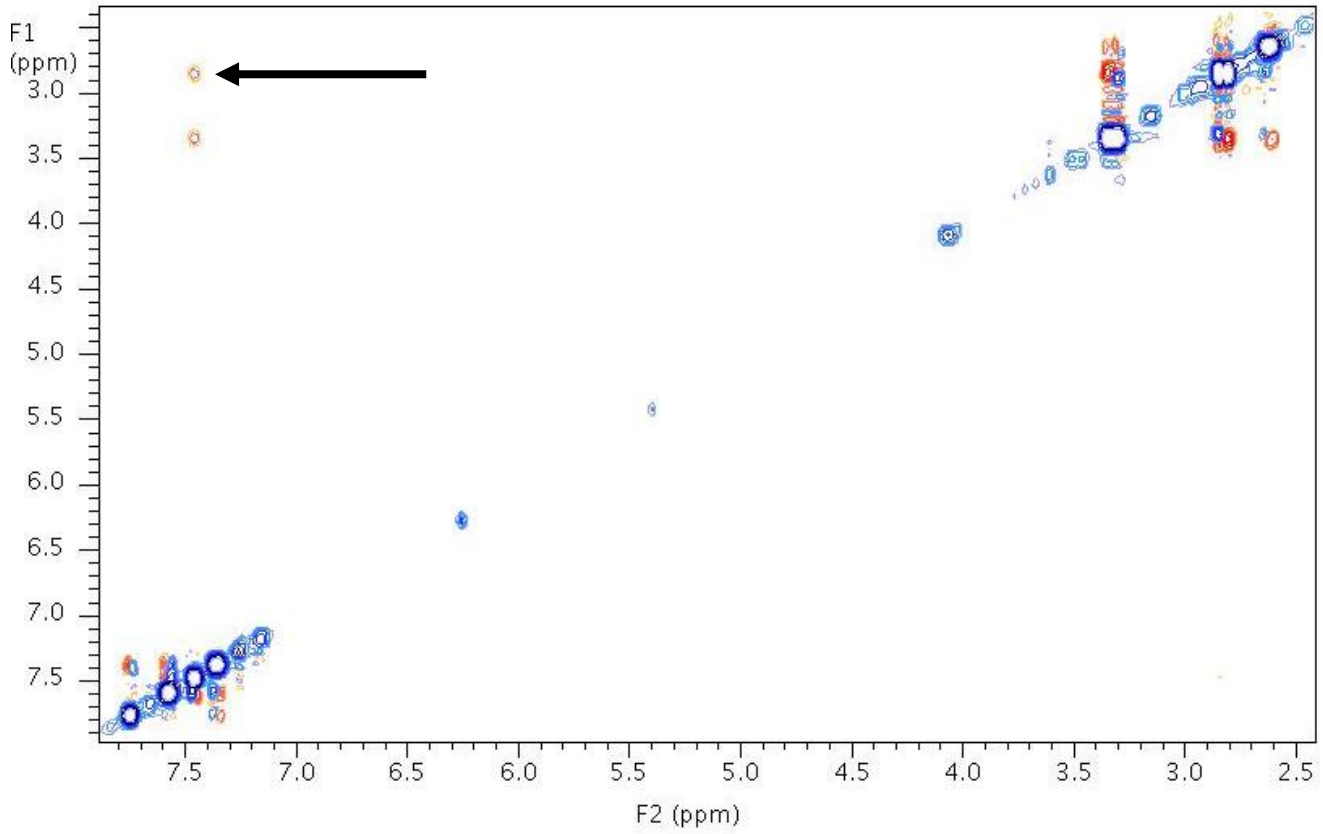


Figure 25

In the NOESY, we see two, clear correlations from the 3 methylene protons to aromatic proton 5. This is useful structural information, as it confirms the regiochemistry of the ethyl attachment (2 vs. 3-position) in much the same way as the proton-carbon correlation between aromatic proton 5 at aliphatic methylene carbon 3 did in the gHMBCAD. In fact, NOE information can be often be used to determine/confirm regiochemistry where the amount of material available is sufficient only for homonuclear experiments and not enough for HMBC-type.

NOESY vs. ROESY

Although the NOESY and ROESY experiments may seem roughly equivalent and the ROESY the obvious choice for medium-sized molecules, there are some important distinctions between them. Both suffer from unwanted coupling-based artifacts. For the NOESY, these are usually readily removed with the use of a ZQ (zero-quantum) filter, but for the ROESY, they often prove more recalcitrant. In the next section, we will discuss a methodology to help improve the ROESY data.

Another key difference between the NOESY and ROESY experiments is the maximum interproton distance for which one might expect to be able to see and NOE/ROE correlation. The maximum NOE distance is typically stated as < 5 Å, while the ROE is less, approximately < 4 Å. There are several reasons for this difference. The first reason relates to hardware limitations for the demanding ROESY spinlock. There are limits to the length of the ROESY mixing time, the main limits being eventual signal loss from decay and experiment time, although longer ROESY mixing times will cause sample heating and eventual hardware (probe) damage. The adiabatic spinlock used in the ROESYAD helps considerably, but it is still not advisable to exceed 500 ms for the mixing time. Given this, one may not be able to see weaker, longer distance correlations in the ROESY as they may not have enough time to build up.

The second reason the ROESY distance limit is less than the NOESY stems is that the ROE always arises from within the extreme narrowing limit and not from the larger molecule range with longer correlation times. Recall that not only is the sign of the NOE different for small vs. large molecules (positive vs. negative), but also the *magnitude* of the maximum intensity, namely 0.5 vs. -1. Therefore, the relative correlation intensities may be larger for larger molecules with negative NOEs, versus smaller molecules with positive NOEs, and ROEs are always positive. This raises some interesting strategy issues to consider when faced with a medium-sized molecule with near-zero NOEs. One solution is to simply use the ROESY experiment, but some weaker long-distance correlations may not be observed. Another possibility is that the correlation time of the system may be able to be adjusted to the large molecule range such that the sample will now yield negative NOE peaks in the NOESY experiment, with potentially larger relative intensities. One way to achieve this is to perform the experiment at a higher field strength, however this option may not always be available. A simpler solution may be to simply use a more viscous solvent. For example, if one is using $CDCl_3$ or CD_3OD, switching to d_6-DMSO may do the trick. If one is already using d_6-DMSO, addition of a small amount of D_2O (up to ~10-20%) will usually not affect the sample solubility or lineshape much, but will significantly increase the solution viscosity (keep in mind, however, the exchangeable protons will disappear). One final option is to simply lower the temperature, which will both increase the solution viscosity and slow the molecular tumbling rate. Any or a combination of these techniques can be used to push the system into the desirable large-molecule NOE range. Keep in mind that NOEs may build up more quickly for molecules with longer correlation times, so a shorter mixing time may be optimal.

Whether the NOESY or ROESY sequence is selected in the end, it is important to be aware that although the data obtained may be similar, there are important experimental differences and an informed decision must be made between the two. Some users avoid the problem by acquiring data with both experiments.

Limitations and Problems

Most of the limitations and problems of the through-space correlation experiments have already been touched upon in one way or another, but we will briefly summarize them. It is clear that the intensities of both the NOE and ROE correlations are variable and dependent on a large number of factors, some of which can be modified, some not. As such, it is important to realize the negative evidence in the NOESY/ROESY experiment is not conclusive. In other words, if one does not observe an NOE or ROE peak between protons, one cannot conclude that they are not close in space.

We have also mentioned a few confounding factors in interpreting the peaks that are present. One issue covered for longer mixing times is the possible appearance of spin diffusion peaks. Another type of peak that may show up in a NOESY/ROESY spectrum is called a chemical exchange crosspeak. These peaks occur when a molecule has two different NMR-distinguishable solution conformations that interconvert on a timescale favorable for observation by NMR. The crosspeaks will occur between the same proton in the two different conformations and will be the same sign as the diagonal. For ROESY and small molecule NOESY spectra, these peaks can easily be distinguished from true NOE/ROE correlations by their sign if one is alert to the possibility of multiple solution conformations. For large-molecule NOESYs, the chemical exchange peaks will be the same sign as the NOE crosspeaks, so it may be useful to acquire a ROESY to distinguish between the two.

One final limitation of the NOESY/ROESY data is the presence of artifacts related to coupling interactions. For the NOESY experiment, as mentioned previously, the typical fix is to apply a zero-quantum (ZQ) filter. The ZQ filter is set to be on by default on the “Acquire” tab/”Pulse Sequence” panel (see Figure 26) and should be used unless there is a specific reason not to.

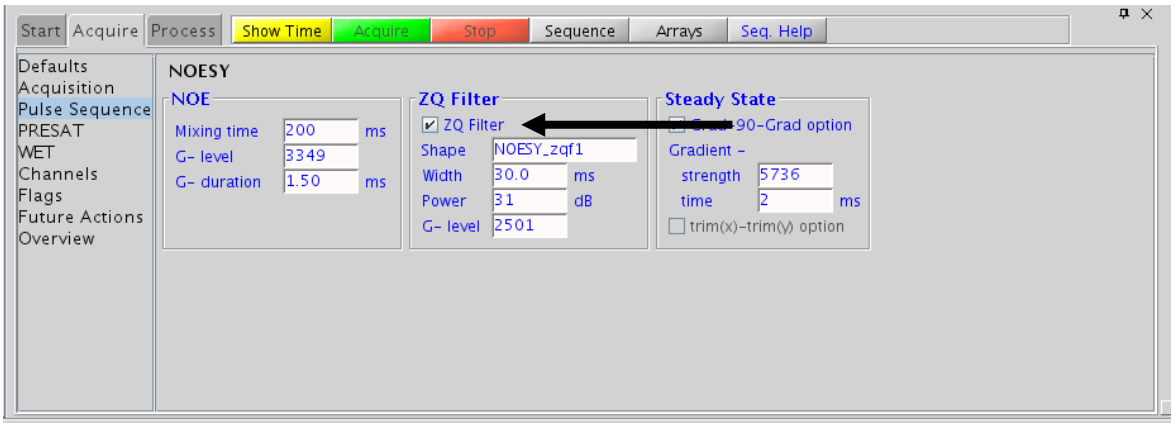
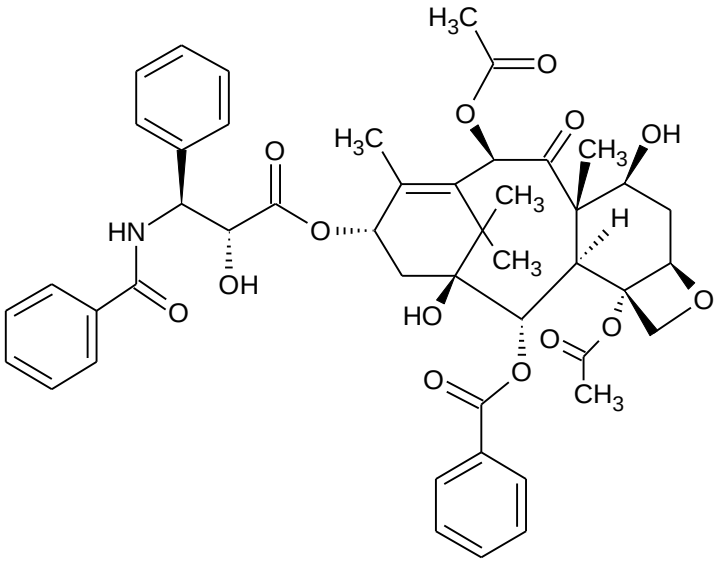


Figure 26

For the TOCSY-type artifacts in the ROESY spectrum, a different approach is needed. The ROESYAD experiment has the ability to rotate the spinlock axis away from Z. Effectively two spinlock periods are creating that are offset from one another. This tends to cancel or reduce the potential for TOCSY-type artifacts because the offset creates a sign change for these signals and not the ROESY signals. This new feature has been implemented in the latest version of VnmrJ and is, by default, turned on for the ROESYAD experiment. The sample ROESYAD data for ethylindanone in the fidlib was not acquired using this feature.

A final comment on the limitations of the NOESY and ROESYAD experiments is that some molecules, especially small rigid ones, may be ill-suited for these types of experiments simply because they have few protons that are close in space. The ethylindanone data presented in this chapter suffers from this to a certain extent, so these data should not necessarily be viewed as representative of the quality usually obtained from the NOESY and ROESYAD experiments.

As an example, Figure 27 shows the NOESY data for paclitaxel available in the fidlib. One can see that this spectrum contains many useful, easily interpreted, NOE correlations and very few visible artifacts.



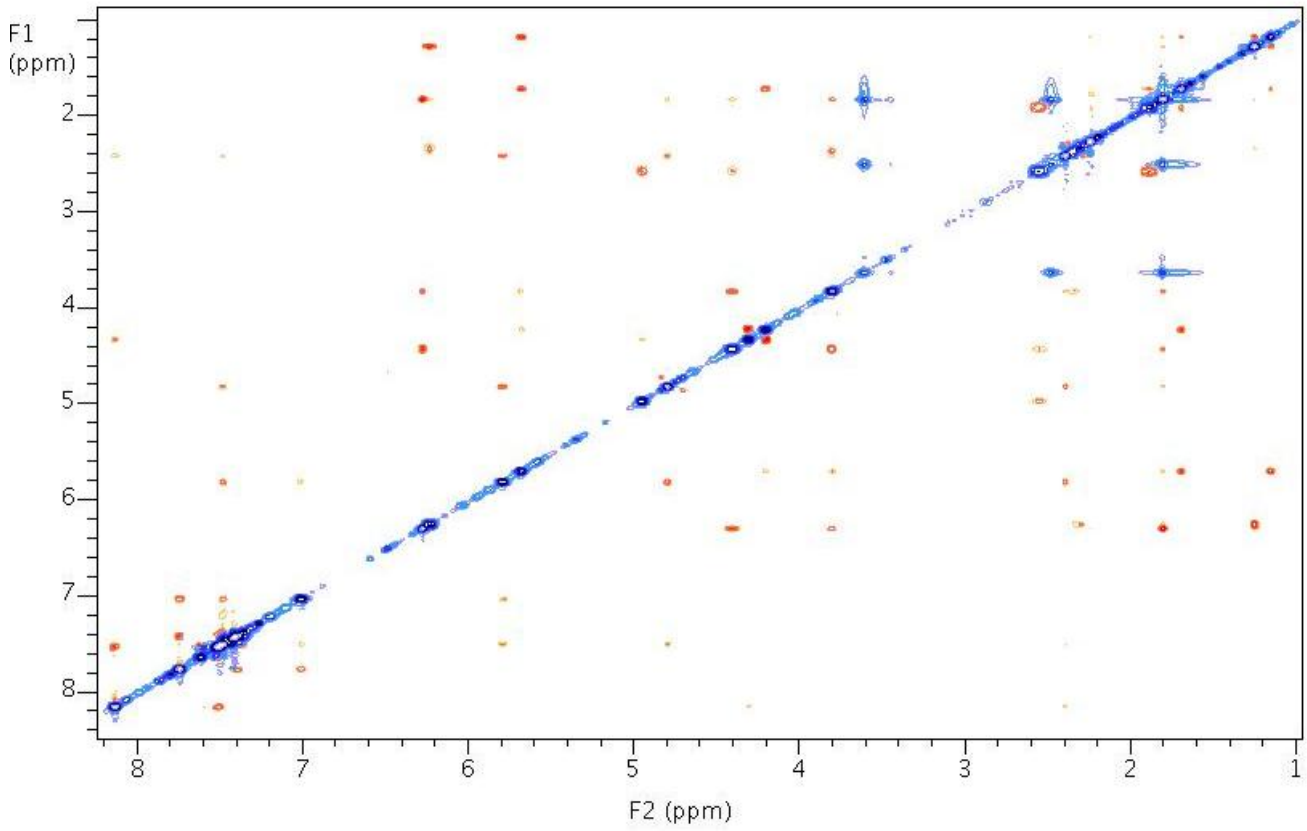


Figure 27

Experimental Variants

The “(HH)Homo2D” tab of the experiment selector contains the following variants of the 2D through-space correlation experiments. (For more information on any of these, see the Experiment Guide.)

- NOESY - This is the standard homonuclear through-space correlation experiment. It is useful for small molecules (<MW 350) and large molecules (MW > 5000), but it has limitations if used for intermediate-sized molecules. NOESY is a simpler and cleaner experiment than ROESY, so start with NOESY if you are unsure which one to use.
- ROESY - This is the standard homonuclear through-space correlation experiment in the rotating frame.
- ROESYAD - This is the preferred version of the ROESY experiment. It includes adiabatic pulses for the spin lock, which should give superior results as compared to the standard ROESY.

Further Reading

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M. J. Thrippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

7 Carbon 1D Spectra

- Overview
- CARBON of Ethylindanone
- APT and DEPT of Ethylindanone
- Limitations and problems
- Experimental Variants
- Further Reading

Overview

The 1D CARBON experiment is a tool commonly used by organic chemists for structure elucidation and confirmation, as the carbon chemical shift is highly sensitive to nearby functional groups. Carbon chemical shifts are also quite reliably calculated by prediction software from structural input. Proton decoupling is traditionally used to collapse the carbon signals into single lines, which has the dual benefit of effectively increasing the sensitivity of the experiment while simplifying the spectrum. The CARBON experiment, however, still requires a relatively large amount of sample or spectrometer time, as compared to proton-detected experiments, due to the lower relative natural abundance of the ^{13}C nuclide and its reduced receptivity when compared to ^{1}H. The detection of ^{13}C is ca. 6000 times less sensitive than ^{1}H for the same sample concentration, all else being equal.

For these reasons, this experiment – despite its simplicity and usefulness – has been relegated to a position in this manual following the proton-detected 2D experiments. This is to serve as a reminder that the HSQC-type experiments are a more sensitive and efficient way to obtain almost the same information regarding carbon chemical shifts.

There are times, however, when a 1D carbon is the appropriate experiment. For example, the HSQC does not contain information regarding quaternary carbons – only carbons with protons attached. The HMBC experiment is most likely the first choice to obtain quaternary carbon data, as one will also receive a wealth of connectivity information, but there will be circumstances when the $^{n}J_{H,C}$ will be too small to observe the correlation. In these instances, the 1D carbon spectrum may be the best choice to see these resonances.

Another time when the carbon might be a good choice is when quantitative information is needed. Although a great deal of care is needed to obtain quantitative carbon data, the 1D carbon spectrum may be the more straightforward choice. Finally, the 1D carbon spectrum has much more resolution in the carbon dimension than the 2D HSQC, which can be useful in some applications, such as when studying samples that have many carbons with similar chemical shifts, such as polymers or fatty acids.

CARBON of Ethylindanone

To obtain a CARBON spectrum, choose the CARBON protocol from the Experiment Selector under the “Std 1D” tab. At the top of the Default C13 page of acquisition options are the basic parameters for spectral width, number of scans, relaxation delay, and pulse angle. At the bottom are settings to turn the proton decoupling and NOE enhancement features on/off and a way to set up automatic S/N checking. For more information about setting up a CARBON experiment, see the Experiment Guide, Chapter 2.

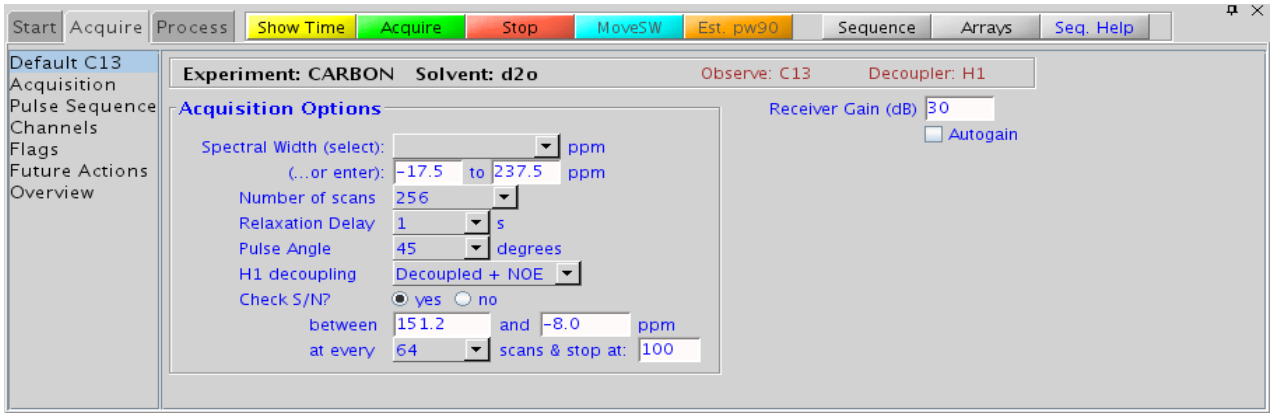

Figure 28

The 1D CARBON spectrum of ethylindanone is shown in Figure 29.

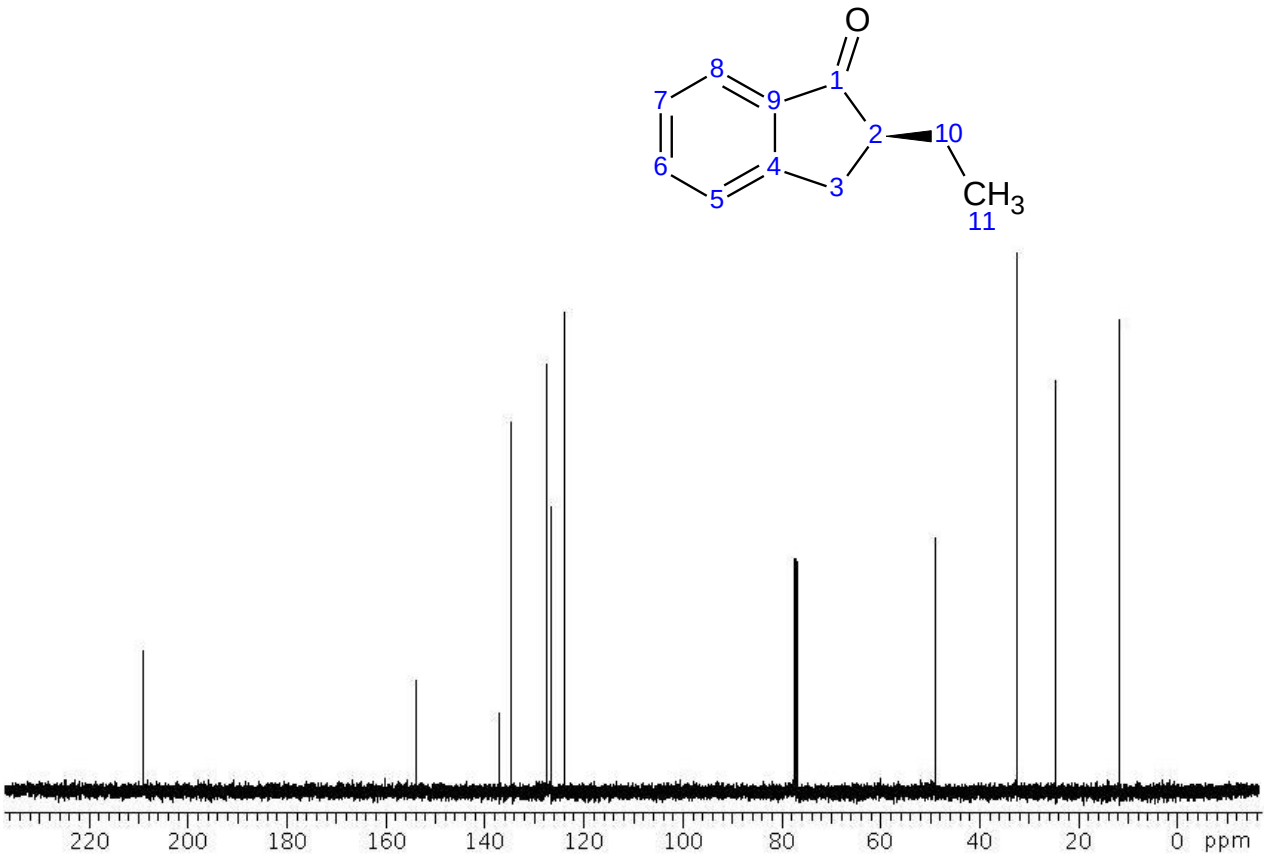

Figure 29

With the 1D carbon spectrum, we can easily obtain a count of the different types of carbons present in ethylindanone, namely 4 aliphatic carbons (~15-50 ppm), 6 aromatic carbons (~120-160 ppm), and one carbon at ~210 ppm that is likely a carbonyl. The three peaks at 77 ppm are from the solvent, $CDCl_3$. More detailed information could perhaps be obtained from carbon chemical shift analysis and/or prediction, but, in general, the structural inferences gleaned from the basic 1D carbon data are often initially minimal.

APT and DEPT of Ethylindanone

The carbon experiment can also be acquired with what is known as multiplicity editing. One of the most commonly used edited-carbon experiments is the DEPT (distortionless enhancement by polarization transfer) experiment. DEPT experiments are used to produce edited 1D carbon subspectra that contain carbon signals for only a certain multiplicity type. Choices include: (1) methine and methyl peaks positive, methylenes negative; (2) methines only, or; (3) methyls, methylenes, and methines (no quaternary carbons). Mathematical combinations of the three choices can be used to produce spectra that contain only a specific type of carbon, e.g., 'methylenes only' can be produced by the linear combination of choices 1-3. Additionally, quaternary carbons can be selected (using DEPTQ) to be in-phase or anti-phase with respect to the methyls, or not to be present at all (using traditional DEPT). With full DEPTQ editing, quaternary carbons only can also be displayed. The DEPT experiments tend to be slightly more sensitive than a carbon experiment, as protonated carbons are enhanced by magnetization transfer from the protons. However, obtaining the full set of multiplicity information requires the acquisition of multiple data sets, which ultimately increases the overall experiment time.

To obtain a DEPT spectrum, chose the DEPT protocol from the Experiment Selector under the "Std 1D" tab. At the top of the Default C13 page of acquisition options are the basic parameters for spectral width, number of scans, and relaxation delay. At the bottom are settings to control the type of multiplicity and quaternary carbon editing. For more information about setting up a CARBON experiment, see the Experiment Guide, Chapter 2.

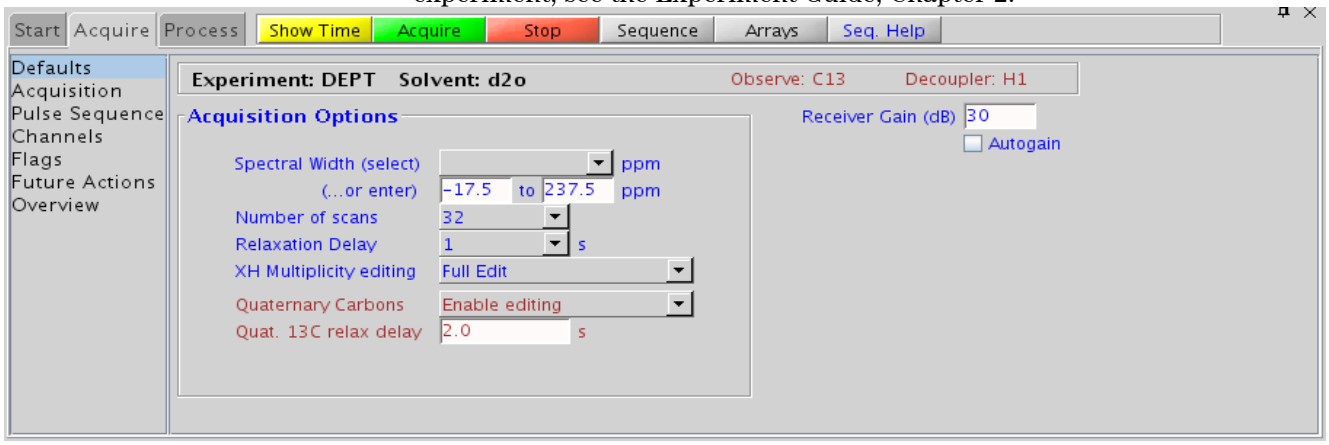

Figure 30

A DEPT series of spectra for ethylindanone is shown in Figure 31.

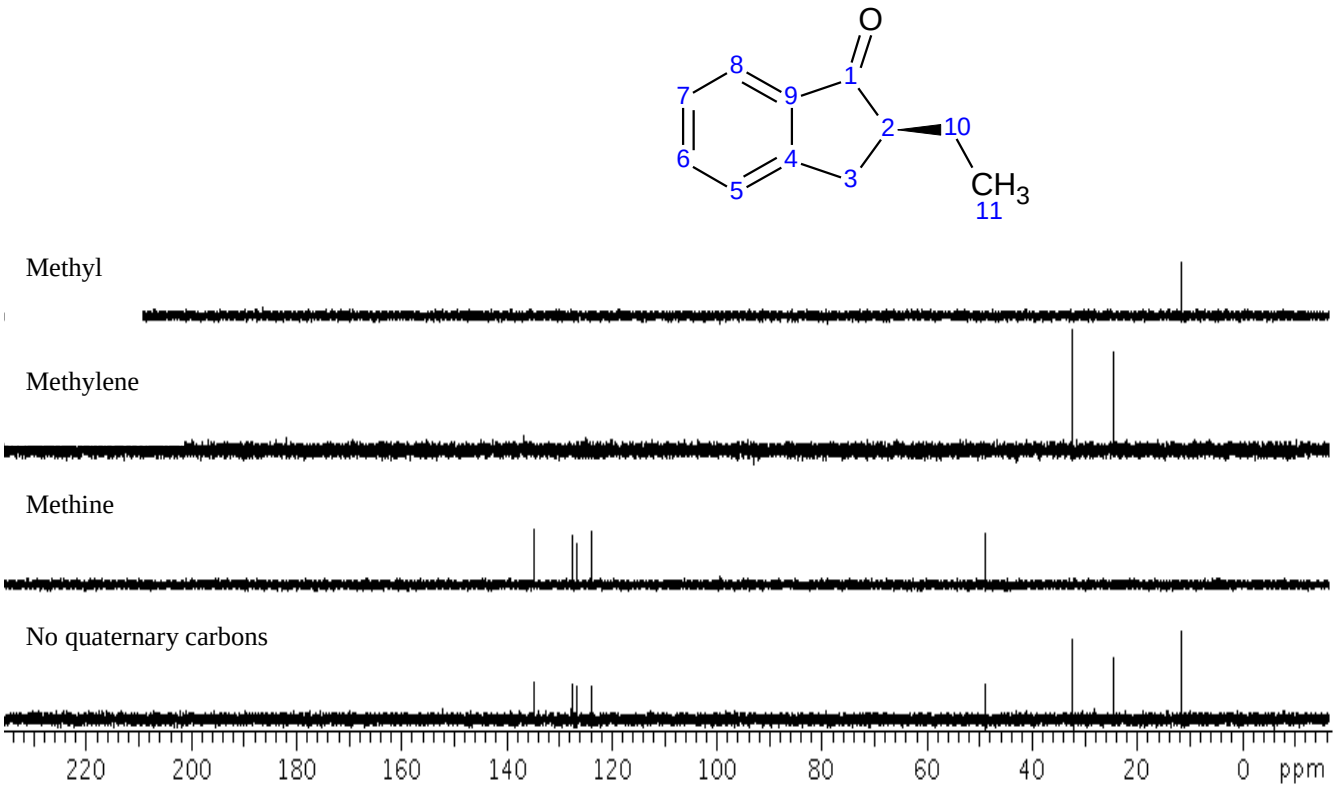

Figure 31

By having the DEPT spectra in addition to the carbon spectrum, we now have more structural information about the molecule. The bottom spectrum in the DEPT array is missing three signals that were in the 1D carbon spectrum. These three signals are from the quaternary carbons, e.g., the putative carbonyl, and two aromatic carbons. In the methine subspectrum, the other 4 aromatics carbon signals are observed, along with a single, aliphatic CH. There also appear to be two aliphatic methylene carbon signals. Finally, the top spectrum shows the presence of a single methyl group.

Another variant of multiplicity-edited carbon 1D data is the APT experiment. The DEPT experiment is primarily used to produce edited carbon sub-spectra containing a single type of carbon multiplicity for easy interpretation. In contrast, the APT experiment is often used as a replacement for a 1D carbon spectrum, as it is a single experiment, compared to multiple experiments for the full DEPT series. The default APT parameters should result in a spectrum with methyl and methine-type carbon signals having opposite phase with respect to that of methylenes and quaternary-type carbons. Quaternary carbon lines will typically be smaller than methylene signals, and can often be distinguished in this manner. Figure 32 shows an example of the APT spectrum of ethylindanone. Note that the quaternary aromatic carbons at 154 and 137 ppm, as well at the carbonyl at 209 ppm are smaller than the two methylene peaks at 24 and 32 ppm, as expected.

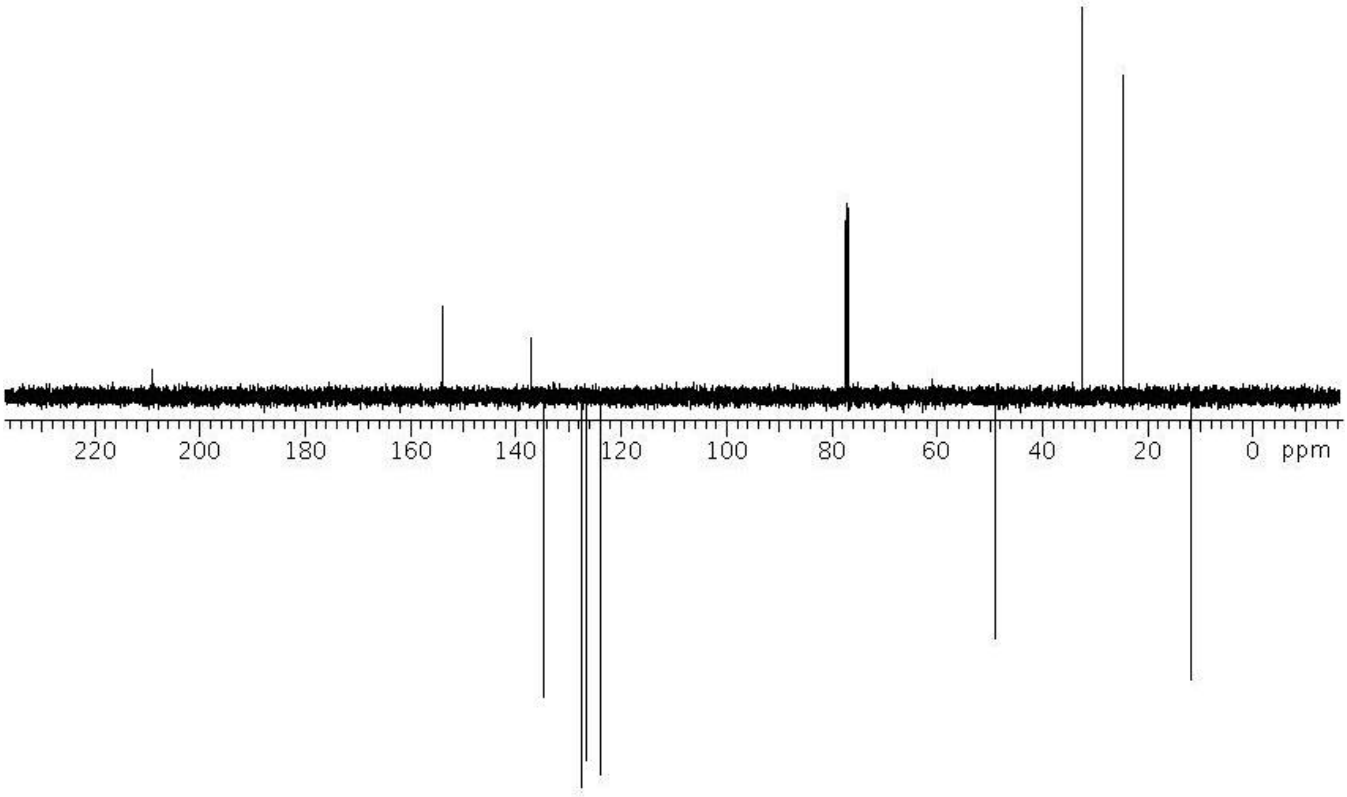

Figure 32

Limitations and Problems

Carbon 1D experiments are useful when you have very concentrated samples. If your samples are dilute, however, their decreased sensitivity can become a big issue. Users with dilute samples often never acquire carbon spectra, because for them it can consume a lot of instrument time to obtain a limited amount of structural information. Multiplicity edited experiments offer an increased amount of information, but they can require multiple datasets to be acquired and can suffer from interpretational ambiguities due to imperfect editing.

Experimental Variants

The “Std 1D” tab of the experiment selector contain the following variants of the 1D carbon experiment. (For more information on any of these, see the Experiment Guide.)

- CARBON - The basic 1D carbon experiment. This is a good place to start if you don’t know what to use.
- (C)DEPT - (C)DEPT experiments are used to produce edited subspectra of 1D carbon signals that contain only the carbon signals of a certain multiplicity type.
- (C)APT - The (C)APT experiment can be used as an alternative to DEPT or even the 1D CARBON spectrum. The default parameters will produce a spectrum in which methyl and methine-type carbon signals will have an opposite phase with respect to that of methylenes and quaternary-type carbons. The absolute phase you obtain is not meaningful, only the relative phases of the signals. Quaternary carbon lines will typically be smaller than methylene signals, and can often be distinguished in this manner.
- CARBONecho - This is a highly specialized experiment to acquire 1D carbon data on a high Q probe, such as a ^{13}C cold probe, which may have long pulse ringdown times. You should not try this experiment unless you are already familiar with it.

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8 Special Situations

- Overview
- Other Nuclei
- Nitrogen
- Phosphorus
- Fluorine
- Quantification
- Solvent Suppression
- Selective 1D
- Selective 2D
- Further Reading

Overview

This chapter is not meant to serve as an exhaustive guide for working with these more advanced techniques in NMR, but should be considered as primarily “food for thought”. We will cover some useful and hopefully inspiring NMR applications; for further experimental details, refer to the Experiment Guide. Included are some descriptions of how to obtain NMR spectra of nuclei other than proton and carbon, special considerations for quantification, an introduction to three varieties of spectral editing techniques, solvent suppression, and selective 1D- and 2D-spectroscopy.

Other Nuclei

Although ^{1}H and ^{13}C are the most commonly used nuclei for structure elucidation/confirmation, organic molecules often contain other NMR active nuclei, such as ^{15}N, ^{31}P, and ^{19}F. For complex problems, NMR experiments involving these nuclei can provide a unique handle for structure elucidation and peak assignment. These nuclei can also provide a means to sort out overlapping resonances that are unresolved with proton/carbon data alone. Finally, if these nuclei are unique to the molecule of interest, they can be used as a filter to selectively view only the resonances from this molecule when working with mixtures of compounds or impure samples. It should be noted that, although most NMR probes have the ability to acquire proton and carbon data, the observation of other nuclei may require specialized probes or hardware.

Nitrogen

Many organic molecules contain nitrogen, which would initially make it a logical nucleus to observe in NMR. Indeed, many interesting chemical reactions of organic nuclei involve nitrogen and it would be helpful to study the local structural changes around it. Unfortunately, nitrogen is ill-suited for direct observation. The most naturally abundant isotope, ^{14}N, has extremely broad lines in NMR, as it is quadrupolar. The only evidence one occasionally sees of ^{14}N in small molecule NMR is the $^{1}J_{N,H}$ for NH_4^+ in the proton spectrum, which sometimes appears as a higher chemical shift triplet in the aromatic region for very dry samples that contain ammonia. This coupling is observable because ammonia is a symmetrical molecule.

A second nitrogen isotope, ^{15}N, produces sharp NMR signals, however, it is not an abundant isotope. The absolute sensitivity of direct observe 1D ^{15}N relative to proton is 0.0004%! This makes it generally not feasible to directly observe ^{15}N. Another challenge for ^{15}N NMR is the wide chemical shift range, although the chemical shifts of many common nitrogen-containing functional groups are well-documented and fairly predictable. The best method to surmount the sensitivity challenge is to use proton-detected nitrogen-correlation experiments, especially HSQC-type experiments. These experiments are easily set up by changing the F1 nucleus from C13 to N15 via a pull-down menu on the “Defaults” page of the “Acquire” tab (see Figure 33).

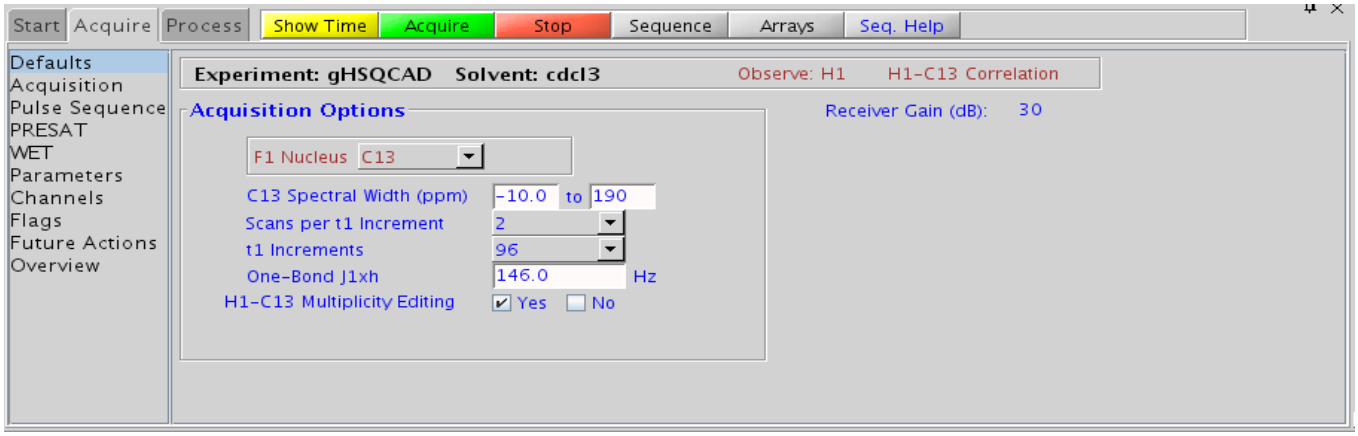

Figure 33

With ~1 mg of sample (MW ~500), one should be able to obtain a good quality ^{1}H-^{15}N HSQC spectrum easily and fairly rapidly. At slightly higher concentrations, the very useful long-range HMBC-type ^{1}H-^{15}N data are also within reach. For more information about setting up these experiments, please refer to the Experiment Guide, Chapters 4 and 5.

Phosphorus

Phosphorus is a much easier nucleus to observe. The NMR-active isotope, ^{31}P, occurs with 100% natural abundance, which gives it an absolute sensitivity compared to ^{1}H of 0.7%. Like nitrogen, phosphorus has a wide chemical shift range, however the ^{31}P chemical shift is primarily dependent upon the oxidation state of the phosphorus (e.g., the paramagnetic shielding tensor) rather than upon the nearby functional groups (e.g., diamagnetic shielding). As such, ^{31}P NMR is typically used to observe direct chemical changes at the phosphorus, or to verify the presence of phosphorus in the molecule. Because ^{31}P is 100% abundant, organic molecules that contain phosphorus will show $J_{P,H}$ couplings in 1D proton spectra. The coupling constants are variable, but typically quite large. One bond couplings can be hundreds of Hz, $^{3}J_{P,H}$ is often ~20 Hz, and even four-bond couplings are often large enough to be resolved. These extensive couplings mean that phosphorus direct-observe 1D spectra are normally acquired with proton decoupling, as is used for carbon 1D spectra. With the proper hardware, this experiment is easy to set up and is available in the “Std 1D” tab of the Experiment Selector (for more information, see the Experiment Guide, Chapter 2).

Fluorine

Fluorine is a high-frequency nucleus that has more in common with proton NMR than with the low-frequency nuclei carbon, nitrogen, and phosphorous. Their frequencies are similar (e.g., ^{19}F would be 470.4 MHz when ^{1}H is at 500 MHz) and ^{19}F also has a 100% natural abundance, which together gives ^{19}F a relative sensitivity compared to ^{1}H detection of ~83%. Fluorine has very strong couplings to neighboring nuclei, both proton and carbon, and its wide chemical shift range is very sensitive to local structure. For these reasons, fluorine NMR can serve as a very powerful tool for the structure elucidation and peak assignment of fluorinated compounds. When acquiring data on impure samples of fluorinated compounds, fluorine can also be used as a filter to select only those resonances that belong to the fluorinated compounds and reject the background signals from unfluorinated compounds. This is an especially powerful tool when analyzing complex samples that have a biological origin, such as metabolites of fluorinated pharmaceuticals. The basic 1D fluorine experiment is described in the Experiment Guide, Chapter 2.

To maximize the sensitivity of the 1D fluorine experiment, it is often desirable to acquire the spectrum with proton decoupling in order to collapse the fluorine signals into singlets. It is also useful to be able to acquire a 1D proton spectrum with fluorine decoupling, as the strong and long-range couplings can create complexity in the spectrum. This is a bit more challenging due to the wide chemical shift range of fluorine, however VnmrJ has pulse sequences available in the Experiment Selector to acquire either experiment. Proton-fluorine NMR experiments require a probe that can be simultaneously tuned to H and F and appropriate H-F calibrations in the probe file. There are unique calibration routines in the channel sharing mode. Please refer to the VnmrJ Installation and Administration manual, Chapter 9, for information on calibration. For more information about setting up the experiments, see the Experiment Guide, Chapter 11.

Quantification

Quantitative NMR is useful for many applications. For proton spectra, the integration values using standard parameters are reasonably accurate, say within +/-10% accuracy 90% of the the time. When higher levels of accuracy are needed, NMR is capable of accuracies within ~1% if reasonable care is taken. Quantification of other nuclei may not be as accurate unless even more precautions are taken. For example, standard carbon 1D spectra are typically acquired with proton decoupling constantly 'on', which gives an NOE enhancement from the attached protons. This increases the sensitivity of the carbon spectrum, but it also means that the integration values for each carbon peak will vary with the multiplicity. The solution to this problem is to acquire the data with a gated decoupling scheme, where the decoupling is turned on only during the acquisition time (see Experiment Guide Chapter 2 for details).

The most important parameter for accurate integration of all nuclei is the relaxation delay, d1. A pulse width of 90° is typically used to maximize the signal, but then adequate time must be allowed for the magnetization to relax back to equilibrium. The rate constant for relaxation is T_1, which was discussed in Chapter 2 for protons. Other nuclei, such as ^{13}C and ^{31}P may have even longer T_1 rates than proton. If you need accurate quantification, the important thing to remember is that you need to measure the T_1's for the molecule and allow ~5x the longest value for full (>99%) relaxation of the magnetization. If you guess at the T_1's, you will be guessing at the quantification results.

Another important factor is to consider the effects of other NMR-active nuclei on the signal to be quantified. In ^{1}H spectra, carbon, phosphorus, and fluorine will all show coupling to the proton signals. It is easy to see the ^{31}P and ^{19}F coupling, and thus remember to integrate the full multiplet structure, however one often forgets about the ^{13}C satellites. The ^{13}C satellites of a proton signal, however, account for 1.07% of the integration and must be integrated for best accuracy. If there is too much overlap, consider using a proton experiment that incorporates carbon decoupling, such as BilevelDec (see Experiment Guide Chapter 2). For a full discussion of all aspects of quantification (e.g., sample preparation, acquisition, and processing), please consult the references at the end of the chapter.

Solvent Suppression

If a drop or two of D_2O is added to an NMR sample, it will not usually deteriorate the proton spectrum; however, significant amounts of protonated water (H_2O) will deteriorate the spectral quality. Pure water has a natural proton concentration of 110 M and its signal tends to be broad, so its presence can easily swamp signals from less concentrated sample components, especially those with chemical shifts near the water. Likewise, signals from any other solvent or additives (i.e., ammonium acetate or formic acid), can negatively impact the quality of the NMR data. Ideally, these unwanted components are removed from the sample by processes such as drying, extraction, or chromatographic purification. Sometimes, however, this may not be feasible, such as for hygroscopic samples, or when the additive arises from the use of a mobile-phase modifier in a HPLC purification. There are also situations where it may be advantageous to dissolve NMR samples in protonated solvents. In these cases, it is usually necessary to use some form of solvent suppression when acquiring the proton NMR data.

Solvent suppression is a spectral editing technique that seeks to remove unwanted signals from the NMR spectrum by using a specialized pulse sequence. The experiment selector in VnmrJ contains two commonly used solvent suppression experiments, (H)PRESAT and (H)wet1D. Both experiments can provide good suppression, but the decision about which one to use depends upon the intended use of the data, and normally involves decisions about performance tradeoffs. PRESAT is the normal default choice if you don't know what else to use. It contains a variant called PURGE that is an alternative; it often produces a smaller residual water signal, but the resulting spectrum will have decreased integral accuracies. As an example, Figure 34 shows the proton spectrum of Vitamin B12 in D_2O, both with and without PURGE-type water suppression.

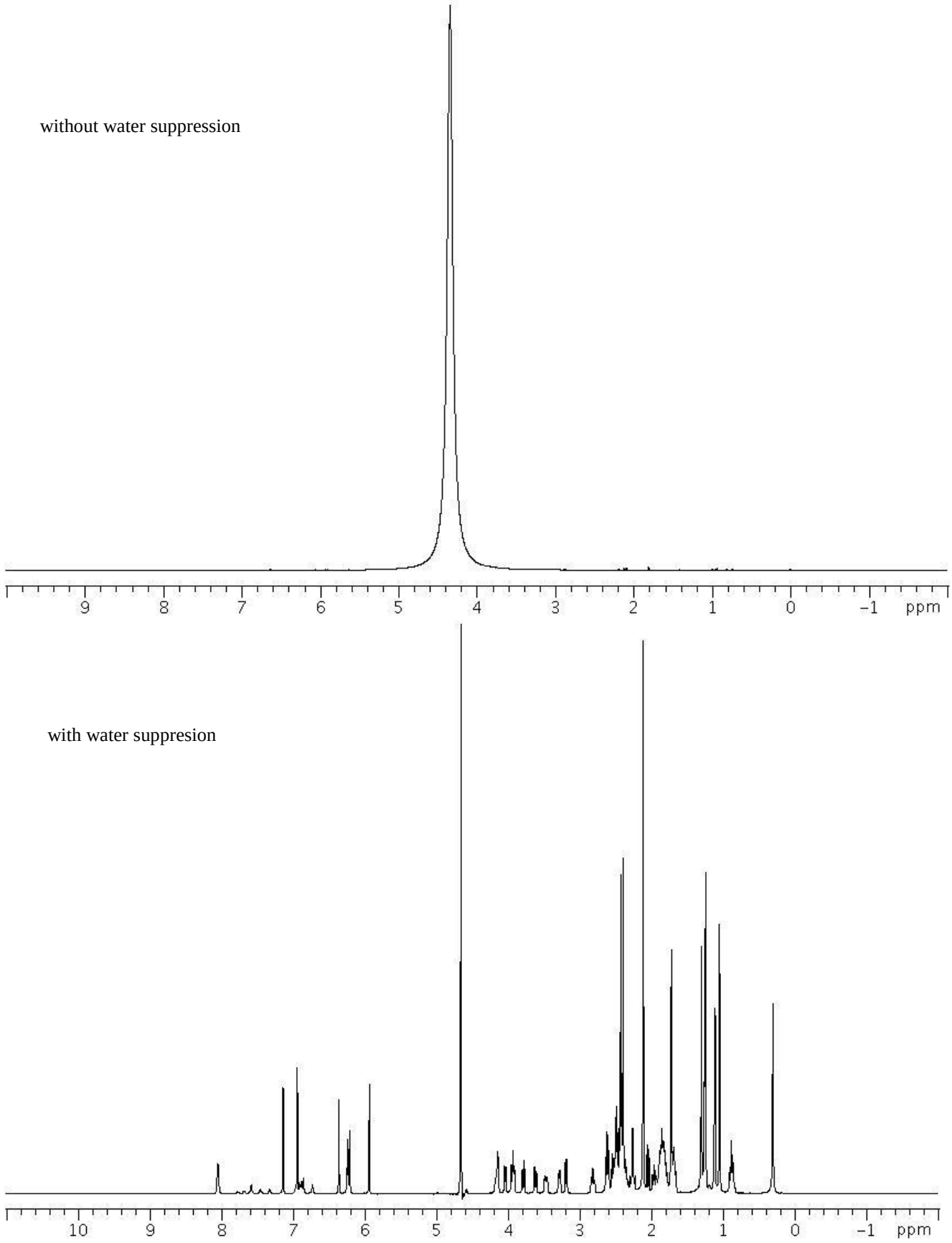
without water suppression
9
8
7
6
5
4
3
2
1
0
−1
ppm
with water suppresion
10
9
8
7
6
5
4
3
2
1
0
−1
ppm

Figure 34

For some applications that require statistical analyses, such as metabonomics, flat baselines are important, and different forms of solvent suppression may yield different qualities of flat baselines. In other applications, you may want to better observe exchangeable protons, and the WET experiment often proves to be better at this than PRESAT. Either experiment is capable of suppressing multiple signals, should that be necessary. For further information about setting up solvent suppression, please refer to the Experiment Guide, Chapter 2.

Selective 1D

Selective 1D experiments are useful for obtaining targeted information about either single resonances or small areas of complicated spectra. Their pulse sequences depend upon the use of selective rf pulses. When a structural problem requires investigation of correlations to only one or two resonances, these experiments may be more time efficient than a full 2D. The Experiment Selector in VnmrJ contains selective J-coupled experiments (TOCSY1D and zTOCSY1D), through-space experiments (NOESY1D, ROESY1D), and a simple selective-excitation experiment (selexcit). These experiments are usually set up by first acquiring a proton spectrum and then setting-up the appropriate selective 1D experiment. The proton spectrum is then displayed so that the desired peaks/regions for the experiment can be selected with cursors in the graphics window. Alternatively, the desired peaks/regions can simply be entered manually into the parameter panels (of the selective 1D experiment). For additional information about selective 1D experiments, please refer to the Experiment Guide, Chapter 6.

Selective 2D

One can also acquire 2D experiments with region selectivity. The experiments in the “Sel2D tab” of the Experiment Selector are F1 band-selected 2D experiments. If you are interested in the data from only a portion of the F1 dimension, these experiments will give you better resolution in less time than you will get from the corresponding conventional 2D experiment. This requires that you know exactly what data you are looking for before running the selective experiment. In the heteronuclear experiments this can be especially useful for resolving nearly-degenerate ^{1}H-^{13}C resonances into unambiguous correlations. With homonuclear spectra, it can, for example, better resolve a crowded aromatic region of a NOESY. To obtain equally high resolution with the traditional broadband experiment, you would need a very large number of increments and much more spectrometer time. The selective 2D versions of these experiments help you obtain the same information in much less time. These experiments do require the use of selective (shaped) pulses, but VnmrJ sets them up automatically, so these experiments are still quite easy to use. For more information, please see the Experiment Guide, Chapter 7.

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Rev. C, Nov. 2013

G7446-90559