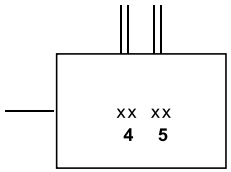
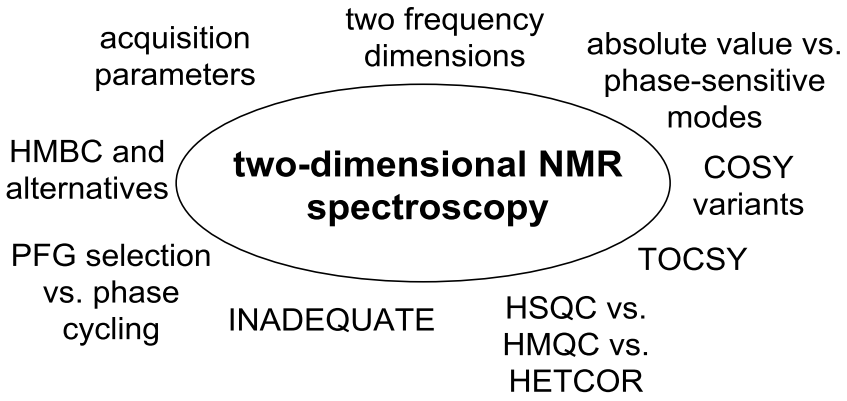
Nuclear Magnetic Resonance II

Eugene E. Kwan



Scope of Lecture



Key References

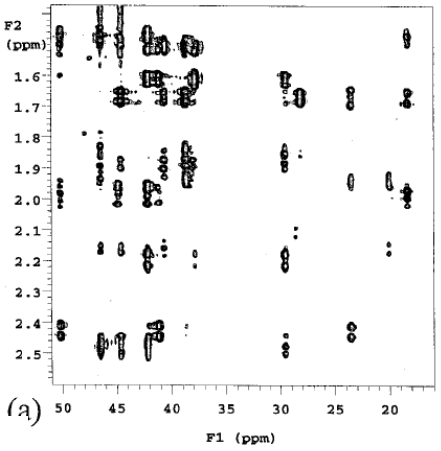
1. "Choosing the Best Pulse Sequences, Acquisition Parameters, Postacquisition Processing Strategies, and Probes for Natural Product Structure Elucidation by NMR Spectroscopy." Reynolds, W.F.; Enriquez, R.G. *J. Nat. Prod.* **2002**, *65*, 221-244. (advantages and disadvantages of various pulse sequences)

2. "Structural Elucidation with NMR Spectroscopy: Practical Strategies for Organic Chemists." Kwan, E.E.; Huang, S.G. *Eur. J. Org. Chem.* **2008**, *16*, 2671-2688. (solving structural elucidation problems with 2D NMR methods)

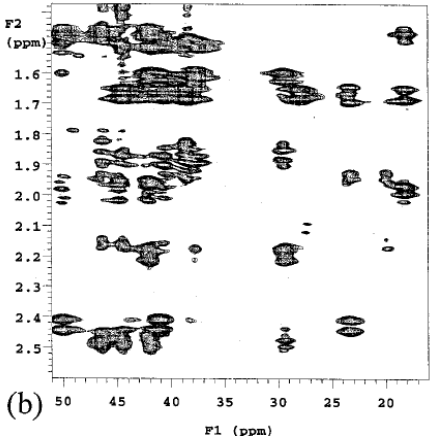
3. High-Resolution NMR Techniques in Organic Chemistry (2nd Ed.) Claridge, T.D.W. Elsevier, **2009**. (Chapters 5 & 6)

Key Questions

(1) What is the basic format in a 2D NMR experiment?
(2) What do all the different experiments do?
(3) What are the best parameters to use?
(4) What should be done with all this information?



(a) CIGAR spectrum taken with optimal parameters.



(b) CIGAR spectrum taken with default parameters.

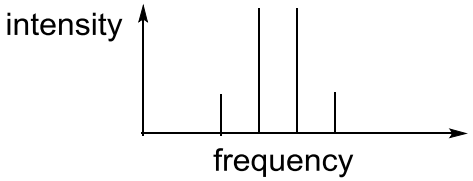
source: ref 1

I thank Professor William F. Reynolds (Toronto) for providing me with some useful material and guidance for this lecture.

Lecture notes edited by Richard Liu

Two Frequency Dimensions

1D NMR spectra are called "1D" because they have one *frequency* dimension, but actually have an additional dimension, intensity:



Q: 1D NMR spectra are pretty complicated already and I'm happy with the number of dimensions I have. Why should I add *another* level of complexity?

A: To figure out what the relationships between peaks are.

1D peaks tell you something about a particular chemical site: what it's chemical environment is like, how many nuclei are present, how many nuclei are near the site, etc. But there's no mechanism for telling you anything about the the connections between sites, which is very useful if you want to know the structure of a molecule.

Here are some questions we'll look at:

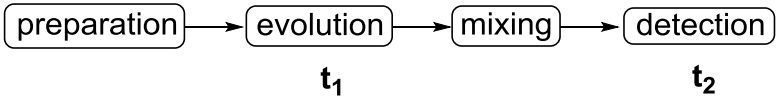
(1) Is proton/carbon A coupled to proton/carbon B (through bond)?

(2) Is proton A near proton B (through space)?

(3) How can we determine relative or absolute configuration?

In today's lecture, we'll just look at question 1. We first ask how two frequency dimensions are generated.

Every 2D NMR experiment has the same general format:



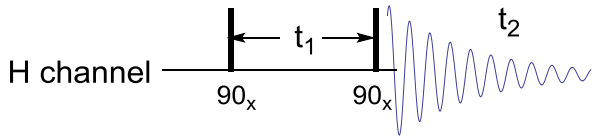
(1) **Preparation:** Some sequence of pulses is used to generate states that are poised to interact in a useful way. This is typically a 90° pulse that generates transverse magnetization.

(2) **Evolution:** The resonances precess in the rotating frame according to their offsets. This means that magnetization is "frequency-labeled" as a function of t_1.

(3) **Mixing:** Magnetization is transfered through bond (or (through space or chemical exchange).

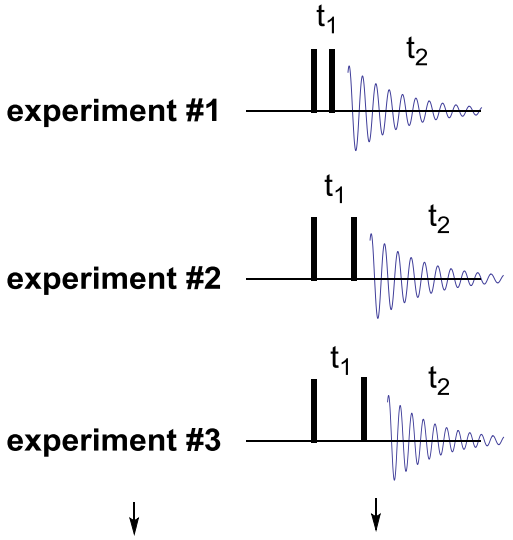
(4) **Detection:** The magnetization that did not get transferred during the mixing period will appear at the same frequency during the detection period. These are *diagonal peaks* of frequency (Ω_A, Ω_A). Magnetization that was at frequency A but moved to frequency B during the mixing period will precess at an *off-diagonal* frequency (Ω_A, Ω_B).

This is best illustrated by the basic **COSY-90 sequence**:

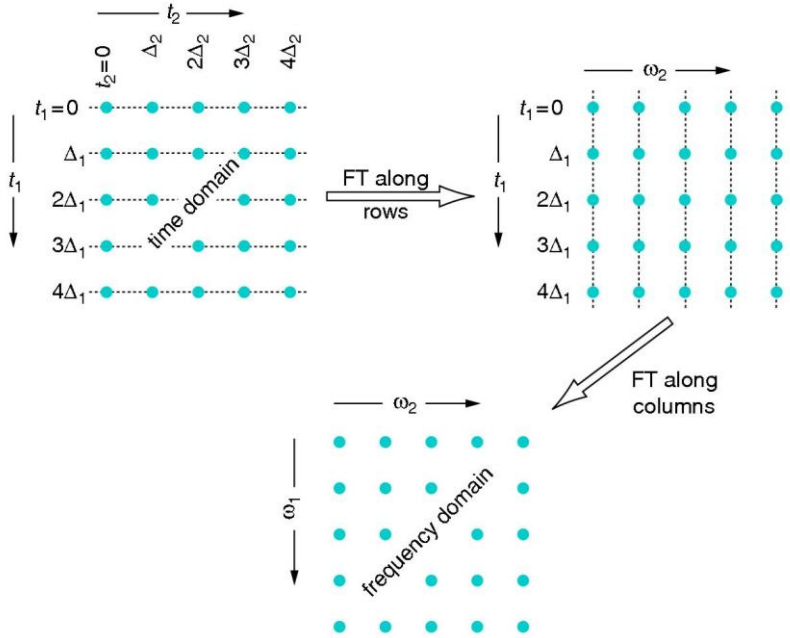


COSY stands for **CO**rrelations **SP**ectroscopy and is a method for finding **homonuclear, through-bond correlations.** (There are other methods for finding *hetero*nuclear, through-bond correlations.) Implicitly, the above diagram means that we run a series of experiments, with a fixed values of t_1 every time. On Varian machines, the parameter **ni** tells us the **number of increments.** Thus, doubling **ni** doubles the experimental time.

This is all illustrated here:

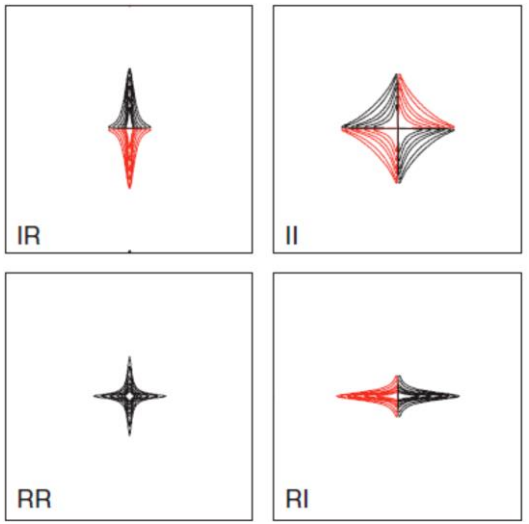


The entire experiment generates a 2D data matrix. Fourier transformation of the rows, followed by the columns gives the final 2D spectrum:



(1) Quadrature detection, i.e., the discrimination of positive vs. negative frequencies is possible and necessary, but is complicated and will not be considered in this lecture.

(2) For 1D spectra, we have a real and imaginary part and that phase correction ensures that the real part has the usual *absorptive* lineshape. In 2D spectra, there are two real parts and two imaginary parts (one for each dimension). In an ideal world, the real part of both would be absorptive as well:

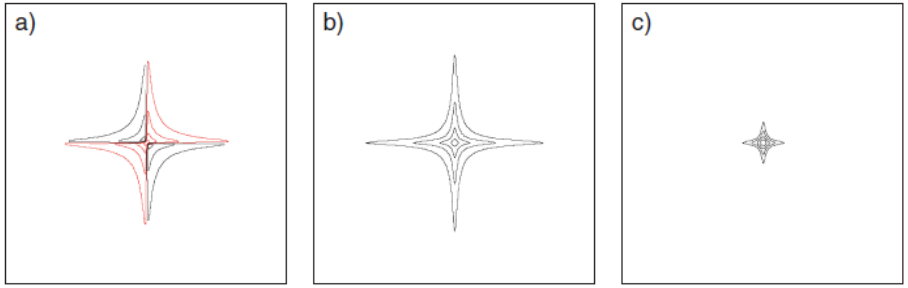


(Red = negative contour; black = positive contour)

If this is the case, we can present the data in a **phase-sensitive** format. However, in some experiments, this is impossible, and it is necessary to mix the real and imaginary parts to give an **absolute value** format:

$$\text{absolute value} = \text{Sqrt}[\text{real}^2 + \text{imaginary}^2]$$

These partially absorptive/dispersive peaks do *not* have a standard Lorentzian shape and instead appear as broader phase-twisted shapes:



As with any NMR experiment, 2D NMR experiments suffer from various instrumental imperfections which mean that signal averaging, along with other schemes for removing artifacts are necessary.

In **phase cycling**, the phases of the pulses and receiver are incremented so as to constructively add the desired signals and destructively cancel the artifacts. The disadvantage is that a minimum number of scans (usually 4 or 16) is required to complete the phase cycle.

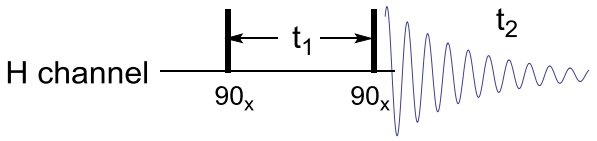
In **pulsed field gradient (PFG) selection**, a magnetic field whose strength varies as a function of position in the sample is applied. Without going into any specifics for now, this removes undesired signals in a more selective way than phase cycling. One says that the "supression ratio" of PFGs is higher.

This is particularly useful for removing intense background signals like ^{12}C-^{1}H peaks in the presence of ^{13}C-^{1}H peaks or solvent resonances. Because the undesired signals are *removed in each scan*, there is no need to complete a full phase cycle. However, there is no free lunch, and PFGs inherently reduce the sensitivity of an experiment (typically by a factor of 2 or Sqrt[2]).

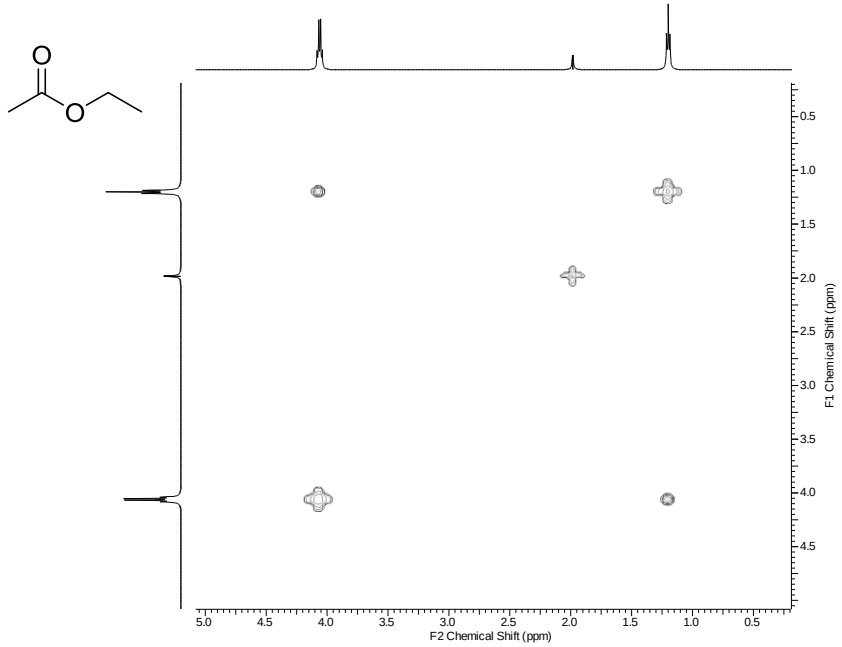
Flavors of COSY

Unfortunately, as Professor Roberts frequently complains in his book, there are a lot of acronyms in 2D NMR. In this lecture, I try to restrict the discussion to only the most useful NMR experiments.

As I mentioned, the basic COSY-90 sequence is:



In VNMR, this is requested with the "COSY" command. Most people use the "gCOSY" command, which requests a PFG-selected variant of it. (By default, phase cycles are also included in PFG-selected experiments.) This may be the most popular COSY variant because it is the simplest. Let's look at a simulated COSY-90 spectrum for ethyl acetate:



Let's look at this spectrum in detail.

The COSY-90 Spectrum of Ethyl Acetate

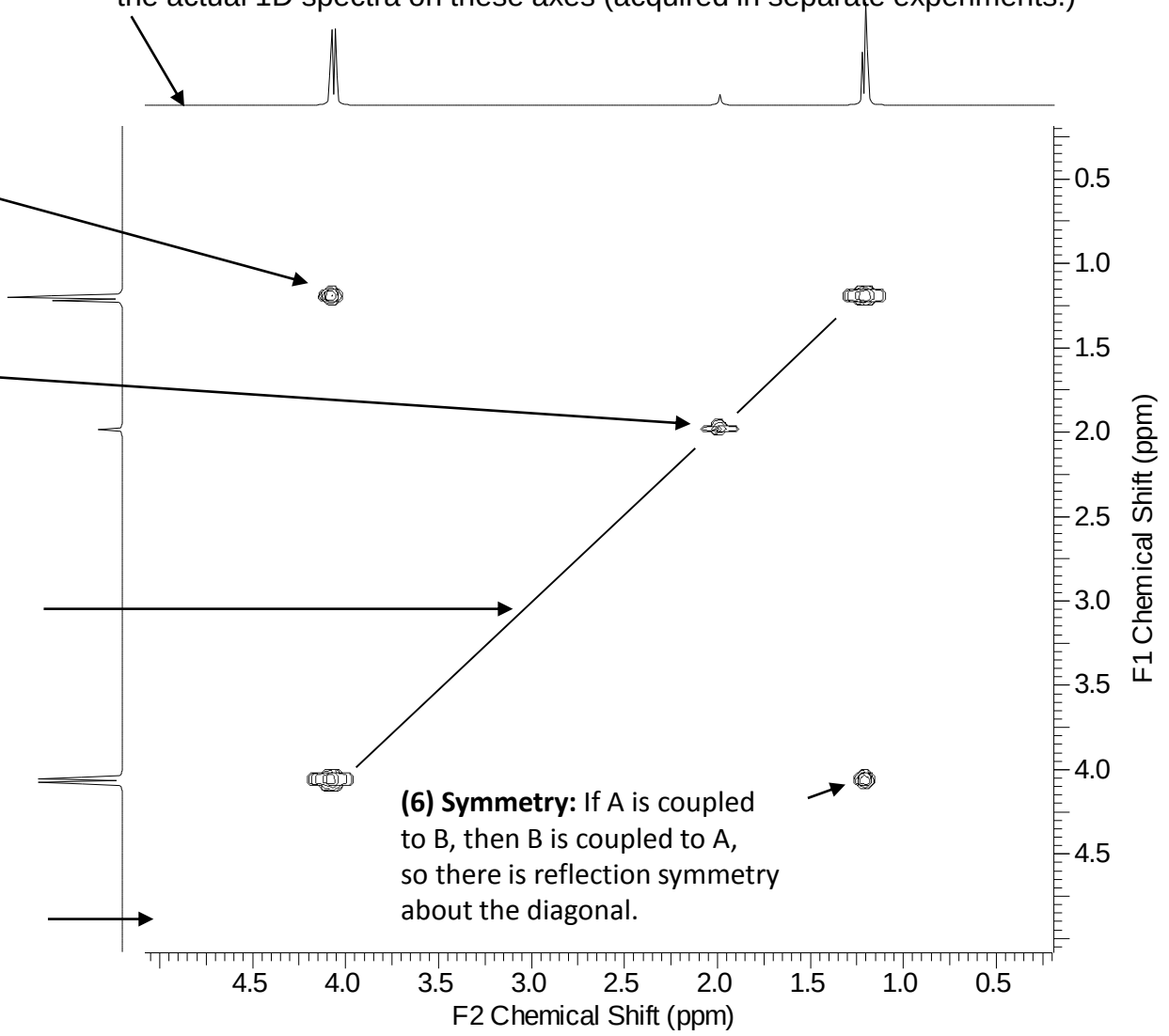
(5) 1D Curves: These are *not* part of the 2D experiment. They do not have to be drawn at all, but it is customary to add them to for reference. The curves shown below are "projections" of the peaks onto the axes. Because increasing **ni** is very costly, resolution is poor. A better alternative is to place the actual 1D spectra on these axes (acquired in separate experiments.)

(1) Cross-peaks: In ethyl acetate, there's an ethyl group, which means that a methyl and a methylene share a vicinal coupling.

(2) Isolated Peaks: Not everything has large enough couplings to give off-diagonal peaks (for example, this acetate). In general, COSY *mostly* shows ^{2}J and ^{3}J couplings.

(3) Diagonal: Of course, every proton is coupled to itself. There's nothing interesting to see here.

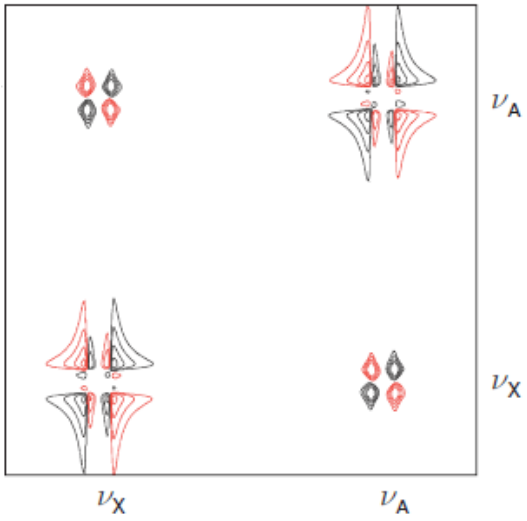
(4) Shape: The spectrum is a square, since we are correlating a spectrum to itself.



(6) Symmetry: If A is coupled to B, then B is coupled to A, so there is reflection symmetry about the diagonal.

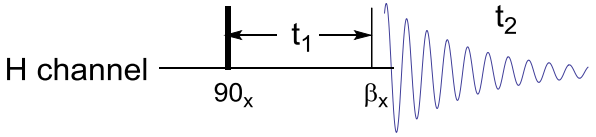
Flavors of COSY

Using COSY-90, if we phase the crosspeaks to have doubly absorptive lineshapes, the diagonal peaks will have doubly dispersive lineshapes:



(source: Claridge, pg 140) The long tails from the diagonal peaks can interfere with off-diagonal peaks. Therefore, COSY-90 data are presented in absolute-value mode with phase-twist lineshapes.

Just because something is *popular* doesn't necessarily mean it's the *best*. A popular absolute-value version of COSY is called COSY-β. Here, the second 90° pulse is replaced by a smaller pulse of tip angle β. Typically, β is 45° or 60°.



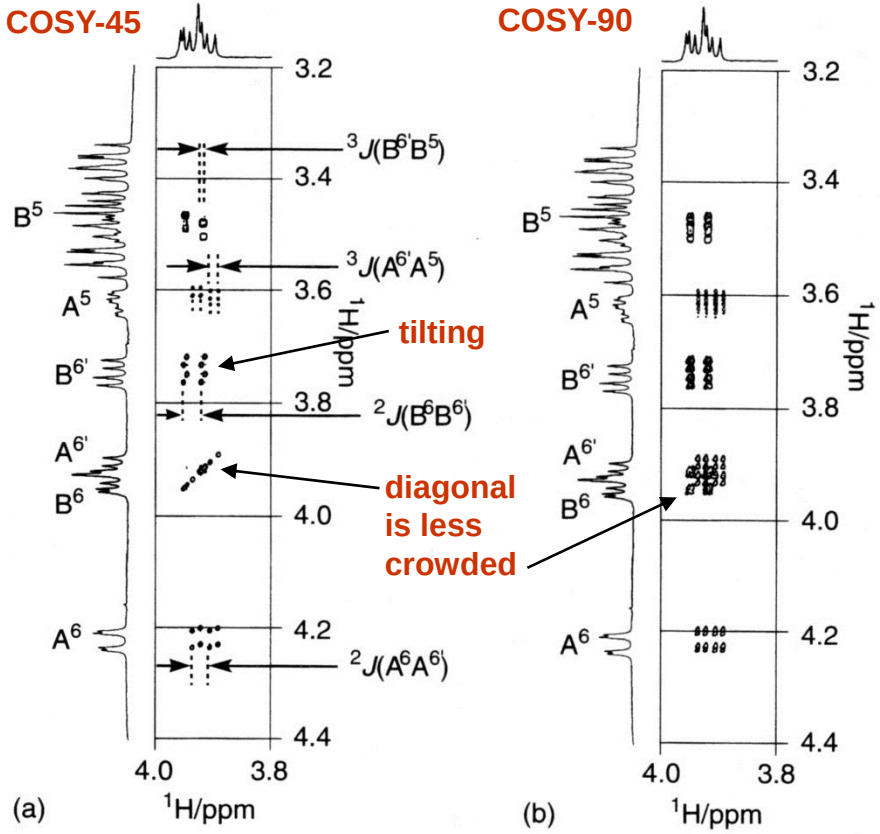
This has several important consequences:

(1) The diagonal is compressed. Since we don't care about the diagonal, and it can interfere with nearby cross-peaks, this is good.

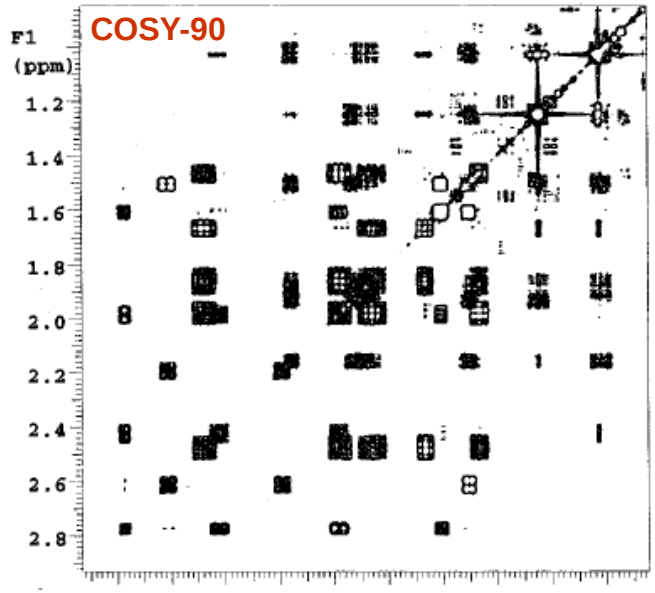
(2) At sufficiently high resolution, the tilt of the crosspeaks can indicate the sign of the couplings. Typically, geminal couplings will appear with positive slope, while vicinal couplings will appear with negative slope. However, due to the variations in *J*, this is not a hard and fast rule.

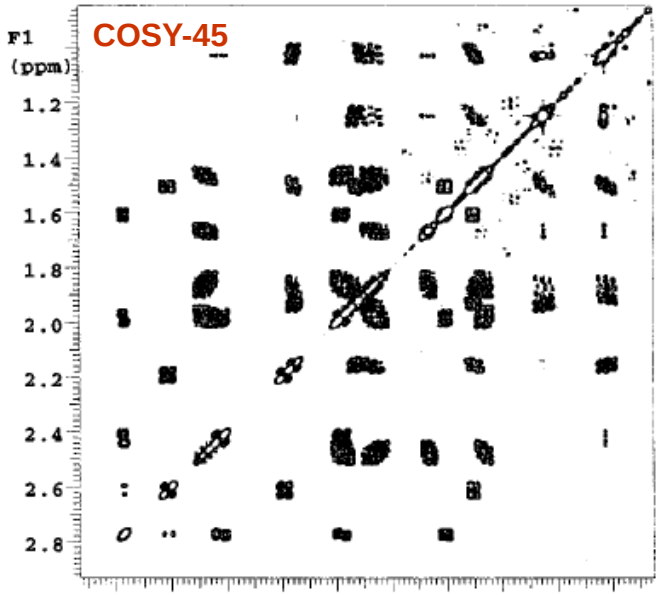
(3) COSY-45 is slightly less sensitive than COSY-90 (by about 15%), but since COSY-90 is already a very sensitive experiment, this is of no consequence. COSY-45 gives more simplification than COSY-60, and is therefore preferred.

COSY-45 is the best absolute value COSY experiment for routine use and should be used instead of COSY-90. The PFG version can be requested in VNMR with "gCOSY45."

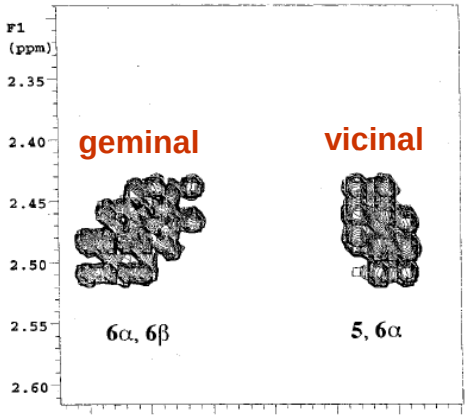


The difference between the two COSY spectra is particularly evident in this example from Reynolds and Enriquez:

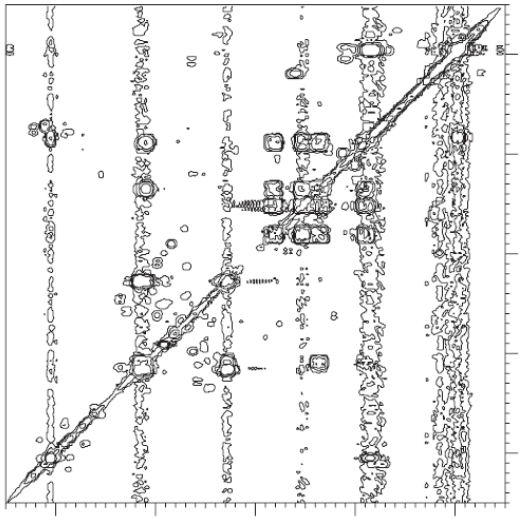




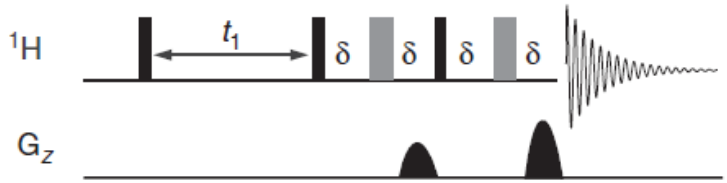
Expansions clearly show the tilting effect:



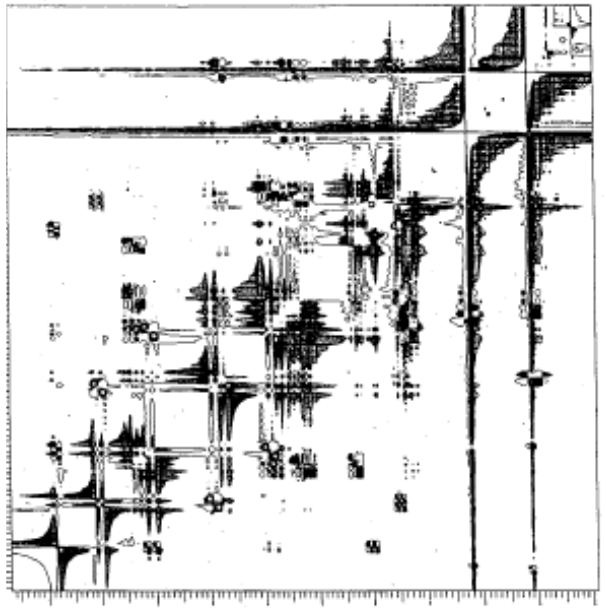
One post-acquisition strategy that is sometimes used to enhance S/N is called "symmetrization" and is based on the idea that the spectrum should have reflection symmetry about $f_1=f_2$ (VNMR: **foldt**). However, because f_1 is usually digitized better than f_2, crosspeaks are usually more resolved on one side of the diagonal. Therefore, peaks that appear more strongly on one side of the diagonal may be long-range peaks. In addition to losing this information, symmetrization may generate false crosspeaks from the coincidental symmetry of t_1 noise:



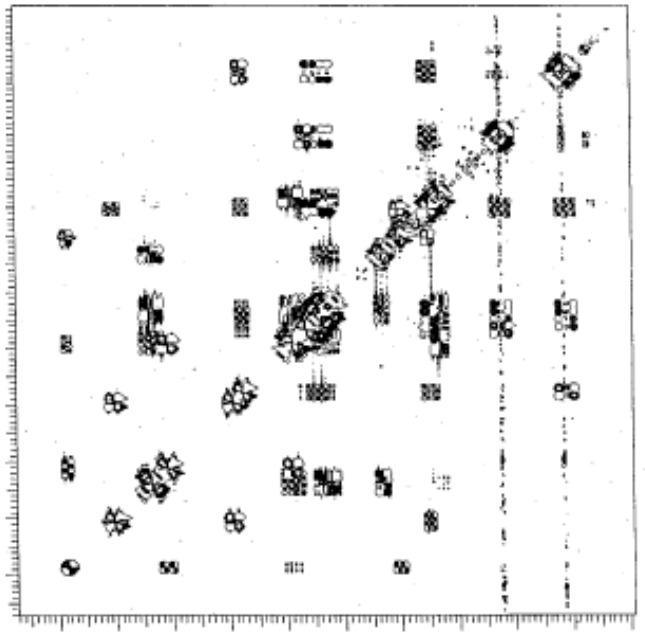
What about phase-sensitive COSY? As we have already seen, regular COSY cannot be displayed in phase-sensitive mode because the diagonal and the crosspeaks will appear with opposite lineshapes. However, a variant called **DQF-COSY** (double quantum filtered COSY) is useful. This is a schematic of the sequence.



Gradients, denoted by G_z are used to create the double-quantum filter. The idea is that only protons which share significant *J* couplings with other protons appear in the spectrum. Intense peaks, like the *tert*-butyl resonances of TBS groups, methyl singlets, and solvent singlets, are suppressed with ratios of up to 10 000:1. Here is a phase sensitive COSY spectrum showing long dispersive tails:

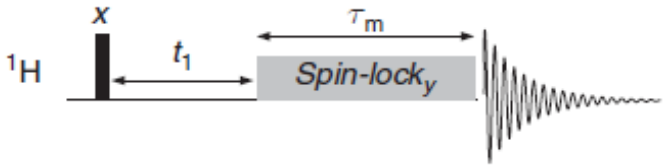


By contrast, the DQF-COSY is much cleaner (Reynolds and Enriquez):



TOCSY

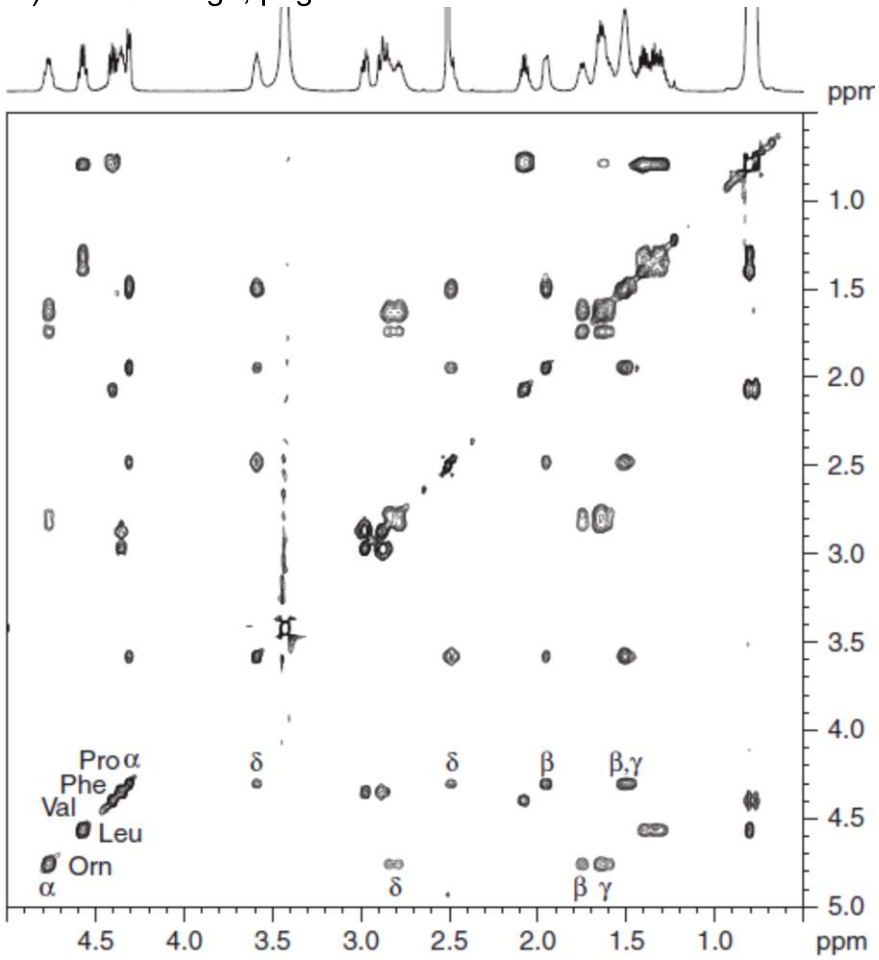
In **TOCSY (TOtal Correlation SpectroscopY)**, the mixing period is a continuous pulse called a spin-lock. Under these conditions, coherence is transferred very efficiently through *J*-couplings. With increasing mixing times, more and more of the spin system gets traced out:

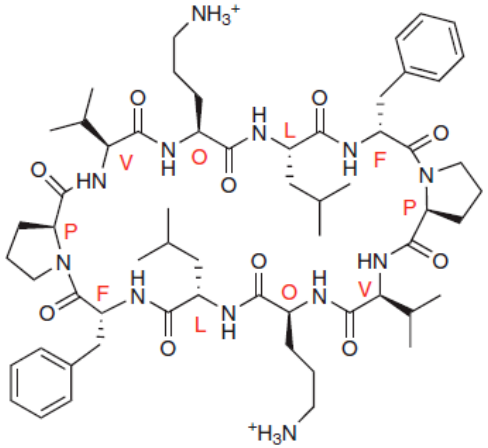


The Hartmann-Hahn Condition: TOCSY

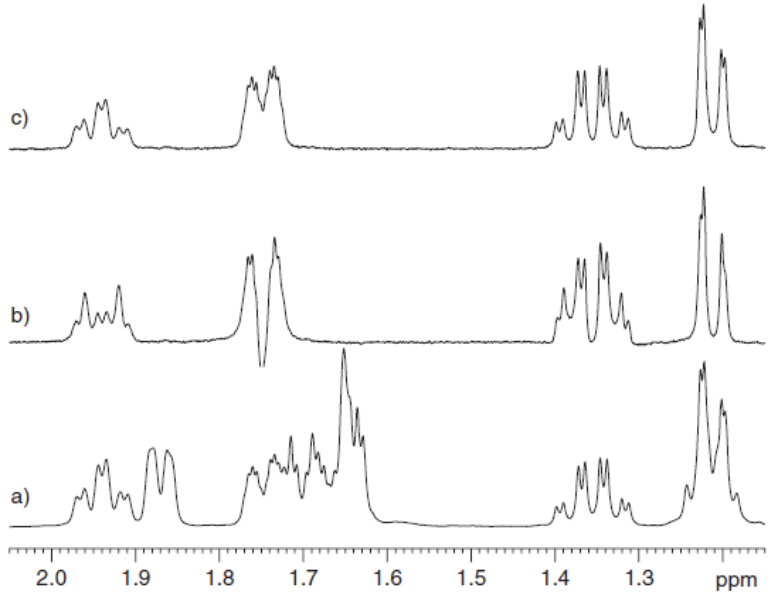
2D-TOCSY is useful because it can trace out entire spin systems in peptides even when only some of the protons in the spin system are clearly resolved due to spectral overlap. The transfer of magnetization over more bonds than is possible in COSY spreads out the correlations over more space, reducing the effects of spectral overlap.

Here is a 2D-TOCSY spectrum of gramicidin-S (80 ms mixing time) from Claridge, page 171:





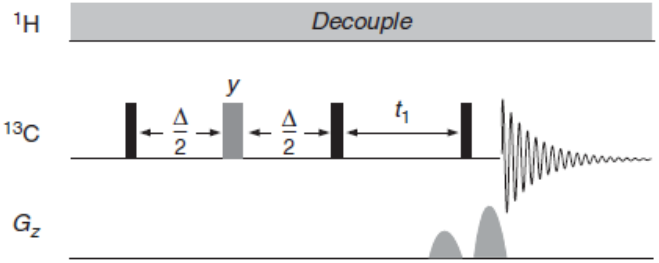
Of course, the overly profligate transfer of magnetization can be undesirable as well. In molecules without discrete spin systems, TOCSY can be used in conjunction with COSY to identify adjacent protons. Occasionally, rotating frame nOe (ROESY) peaks can appear in TOCSY spectra, but these are usually not problematic. A more common problem is the appearance of artifacts arising from zero-quantum coherence:



COSY for Carbons: INADEQUATE

"Problems, Artifacts and Solutions in the INADEQUATE NMR Experiment." Bain, A.D. et al. *Mag. Res. Chem.* **2010**, *48*, 630-641. (best parameters and strategies for INADEQUATE)

Sadly, the experiment that provides the best structural information is also the least sensitive of all the common NMR experiments. In the **INADEQUATE (Incredible Natural Abundance DoubIE QUAntum Transfer Experiment)**, *carbon-carbon connectivity* is traced out:



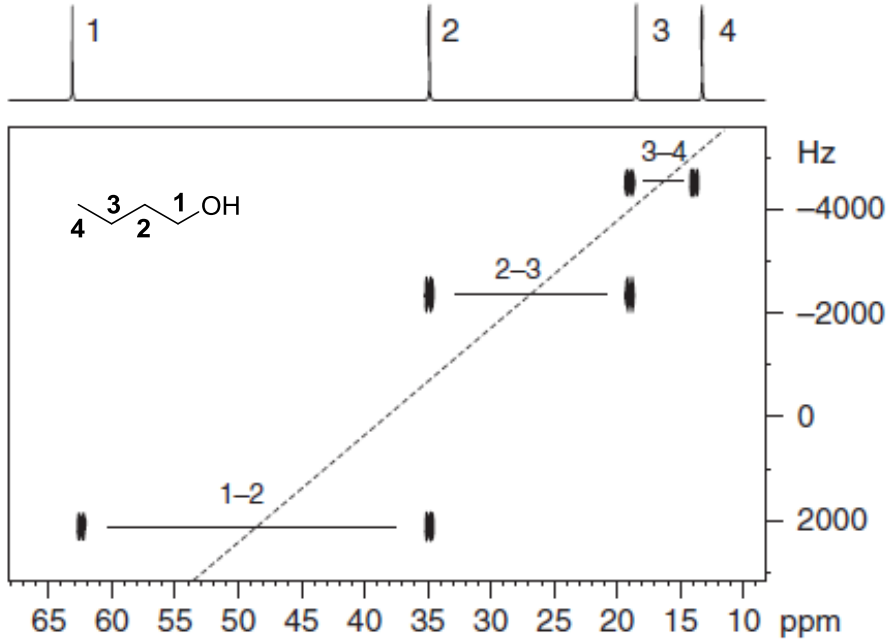
This is essentially a double-quantum filtered COSY experiment that is carbon-detected. The 2D-INADEQUATE spectrum of *n*-butanol is shown on the facing panel (Claridge, page 179).

(1) As usual, f_2 is the carbon dimension, but f_1 is the *double-quantum frequency*--the sum of the frequencies of the two carbons being connected.

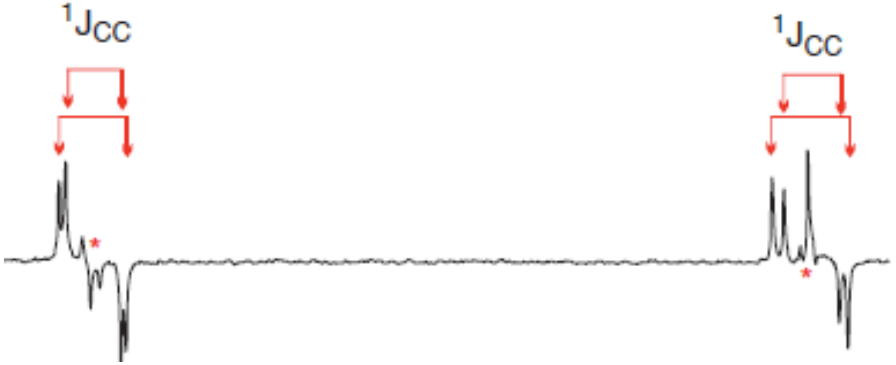
(2) The dashed line is the pseudo-diagonal. Why is there no diagonal? Because f_1 is the double-quantum frequency, not the single-quantum carbon frequency (regular chemical shift).

(3) Because this is double-quantum filtered, carbons with no couplings to other carbons do not appear.

(4) The experiment requires isotopomers with two adjacent ^{13}Cs. This is very unlikely (1 in 10^4), so the experiment is not very sensitive (requiring dozens of mg in an overnight acquisition with a direct detection cryoprobe). Automated processing routines can also help.



1D-INADEQUATE is also available, and sometimes used to measure $^1J_{CC}$, but can have very complicated lineshapes (residual signal from incompletely suppressed lone ^{13}C centers are marked with asterisks):



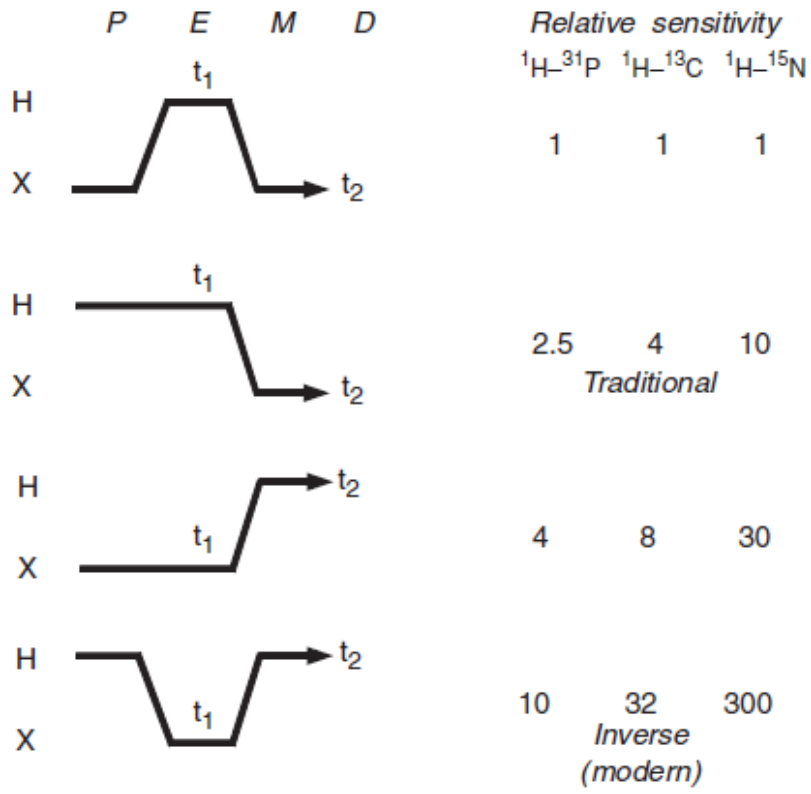
Experiments that transfer coherence to proton (INEPT-style) boldly called ADEQUATE are available, but are also not sensitive enough for routine use yet.

Heteronuclear Correlation Spectroscopy

For *heteronuclear* experiments, one has the option to prepare, evolve, or mix the magnetization on either proton or carbon (the X-nucleus).

old strategy: "direct" detection of X-nucleus (less sensitive)
new strategy: "indirect" detection of H-nucleus (better)

From Claridge, page 191:



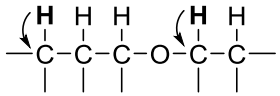
In reality, various experimental considerations mean that the advantage is less than 32/4=8. In general,

$$\frac{S}{N} \alpha \gamma_{exc} \gamma_{obs}^{3/2}$$

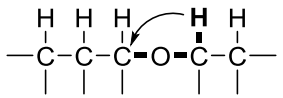
where *exc* is the initially excited spin and *obs* is the observed nucleus. In dual-band probes, there is always a coil on the inside (more sensitive) and a coil on the outside (less sensitive). For inverse-detection probes, the proton coil is on the inside; direct-detection probes have the carbon coil on the inside.

Regardless of the detection scheme, the goal of all of these experiments is to **connect protons with carbons**. Unlike proton-proton correlation experiments (COSY, TOCSY), we have the possibility of protons being directly (one-bond) or remotely (multiple-bond) connected.

For an **inverse-detected experiment**, we see carbons that are directly or remotely attached to protons:



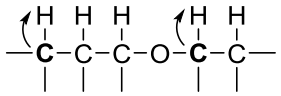
one-bond (direct)



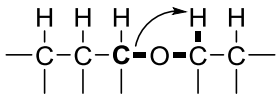
multiple-bond (remote)

(This means that quaternary carbons, which do not have any attached protons, do not appear in inverse-detection experiments.) Remote correlations can be transmitted through heteroatoms, but this is by no means required.

Conversely, for a **direct-detection experiment,** we see protons that are directly or remotely attached to carbons:

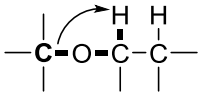


one-bond (direct)



multiple-bond (remote)

In this case, quaternary carbons can appear:



However, directly-detected multiple-bond correlation experiments are insensitive and not in routine use.

The alphabet soup is even worse for heteronuclear correlation experiments, and here, I only mention the most widely used experiments. For a much more comprehensive treatment, see Chapter 6 of Claridge. Here is a summary, with the preferred experiments in **bold** (other common or useful alternative experiments are mentioned):

	one-bond	**multiple-bond**
inverse	**HSQC**, HMQC	**HMBC**, CIGAR
direct	**HETCOR**	--

Exactly *how* these experiments work is beyond the scope of this lecture, but this understanding is not necessary successfully use the techniques in your research.

HSQC vs. HMBC

HSQC - heteronuclear single quantum correlation
HMQC - heteronuclear multiple quantum correlation

Ref: Reynolds, W.F et al *Magn. Reson. Chem.* **1997**, *35*, 614.

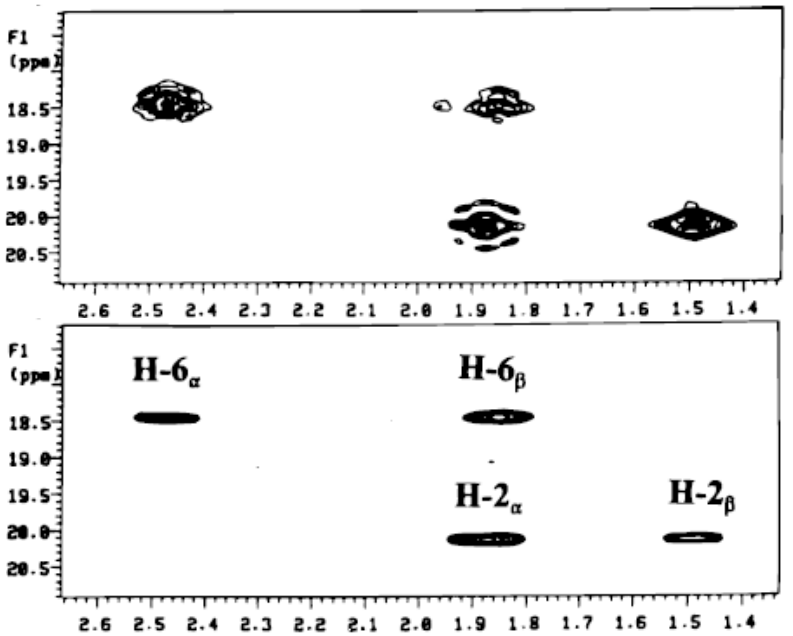
What they do: inverse single-bond heteronuclear correlation

Difference: HSQC has ^{1}H multiplet structure along f_1 only, while HMQC has ^{1}H multiplet structure along f_1 and f_2

Which is better: HSQC. The HSQC sequence does involve more pulses, so the sequence is more sensitive to incorrectly calibrated pulses. For methylenes, the S/N for is ~2x higher for HSQC than HMQC. However, HMQC remains popular.

Notes: Good HSQC spectra require correctly calibrated pulse widths and freshly tuned probes for good results. However, this only takes about ten minutes, and is well worth it for overnight experiments (since other experiments also benefit from this).

The fact that multiplets are collapsed to a smaller area in HSQC means that they have a greater height. Here are spectra taken under identical conditions (top: HMQC; bottom: HSQC):



Which is better, PFGs or phase-cycling?

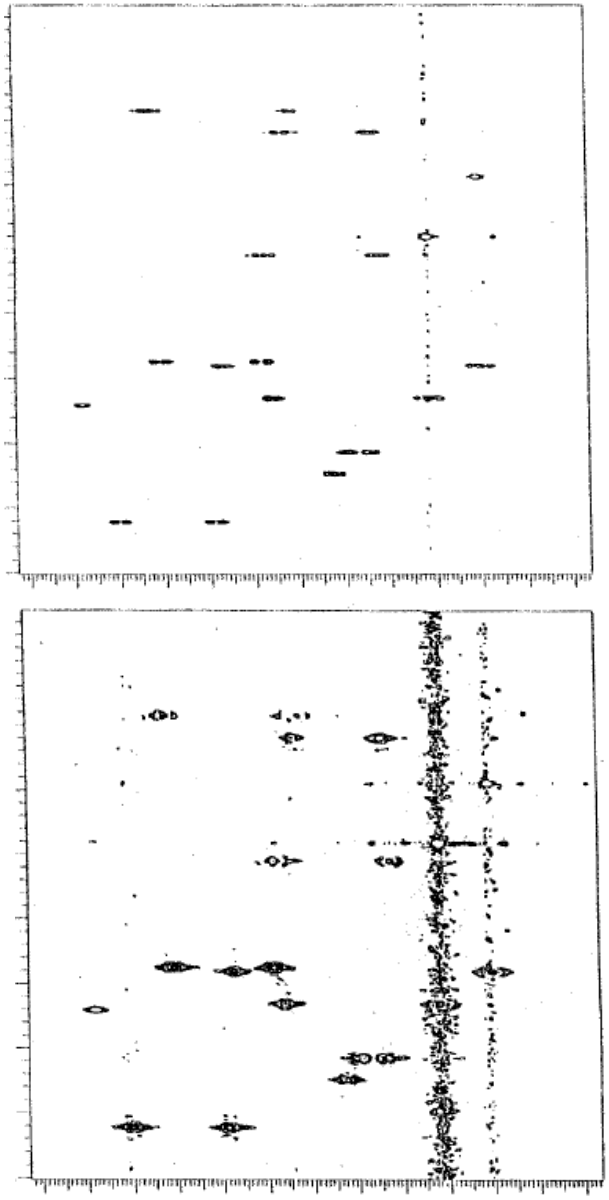
Note that, in general, there are two kinds of noise:

(1) **t_1 ridges**: related to instrumental instabilities and incomplete supression; intensity proportional to signal causing them (particularly intense for methyl singlets)

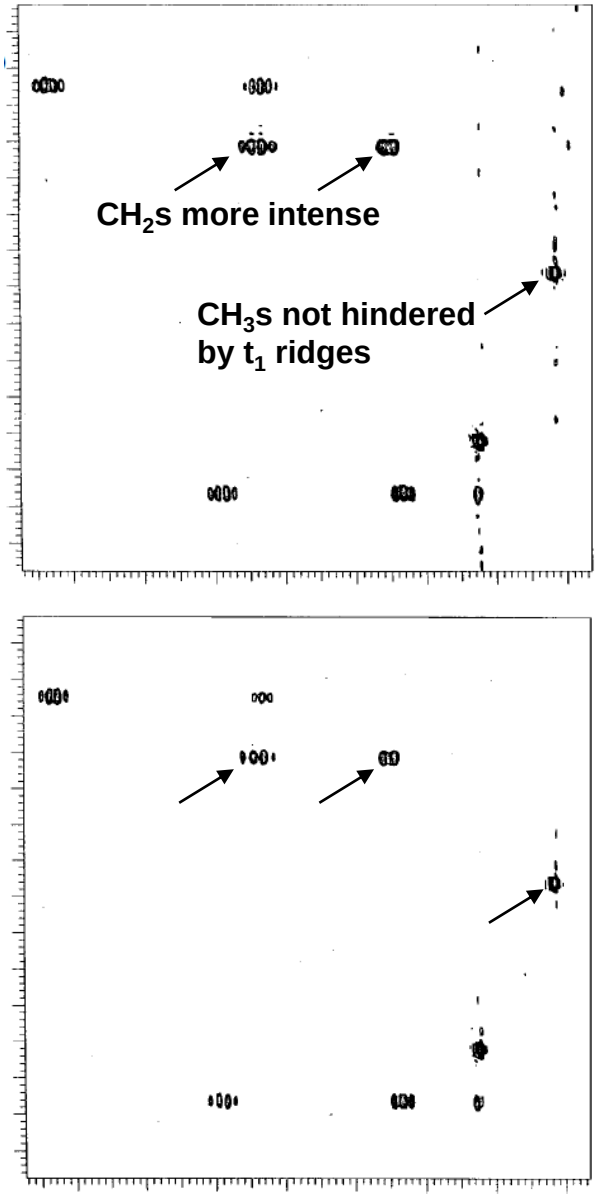
(2) **background noise:** intensity independent of concentration

Thus, the answer depends on concentration. In concentrated solutions, PFGs are much better at suppressing artifacts, and the spectra look better and require fewer scans (since there's no need to complete a full phase cycle which usually takes 8 or more steps). In dilute solution, however, phase-cycling is better because one does not suffer from the Sqrt[2] sensitivity loss associated with gradient selection.

Here are some pictures to illustrate what these points.
These are HSQC (top) and HMQC (bottom) taken under identical conditions:

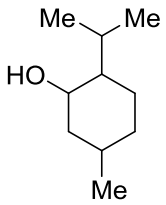


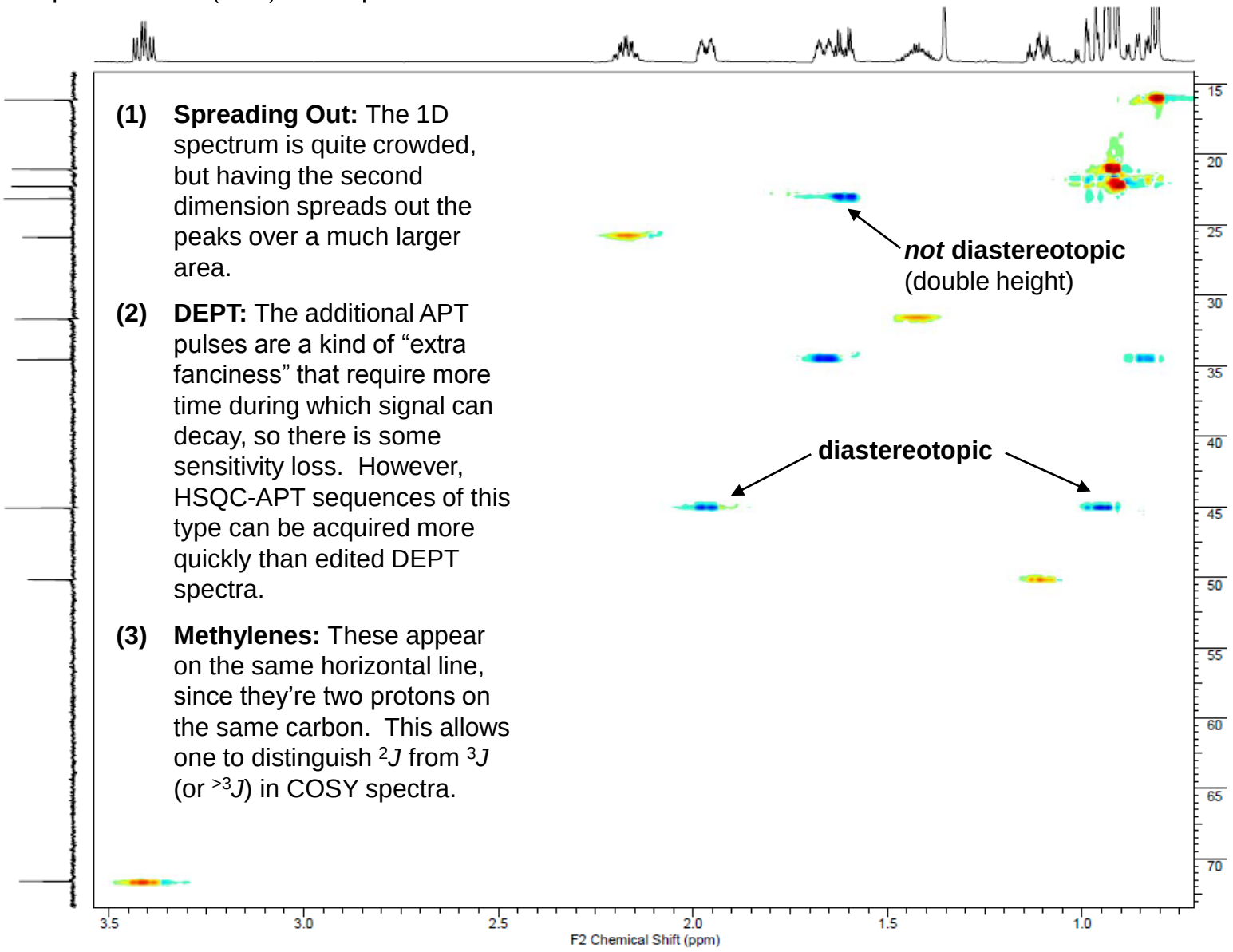
These are phase-cycled (top) and gradient-selected (bottom) HSQCs taken in dilute solution (Reynolds and Enriquez, *Magn. Reson. Chem.* **2001**, *39*, 531-538):



Spectral Editing

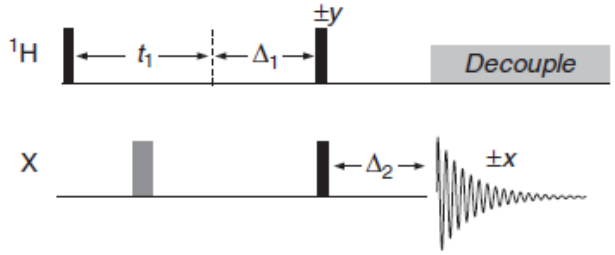
APT-type multiplicity editing is also available for HSQC (**mult=2** in VNMR). By convention, CH and CH_3 peaks are phased up (red) while CH_2 peaks are phased down (blue). The spectrum shown below is for menthol.





Direct Detection: HETCOR

Of the X-detected sequences, the HETCOR (heteronuclear correlation) experiment is the most useful:



Q: If inverse detection has an advantage of $(\gamma_H/\gamma_C)^{3/2}=8$, then why would anyone want to use HETCOR?

A: Better resolution for closely crowded carbons.

Because the resolution in t_1 depends on the number of time increments **ni**, inverse-detected experiments have good resolution in f_2 (proton) and relatively poor resolution in f_1 (carbon). In directly-detected experiments like HETCOR, the narrow range of chemical shifts, proton, is placed on the relatively poorly resolved f_1 axis, while the broad range of chemical shifts, carbon, is placed on the well resolved f_2 axis. Therefore, better resolution is achieved.

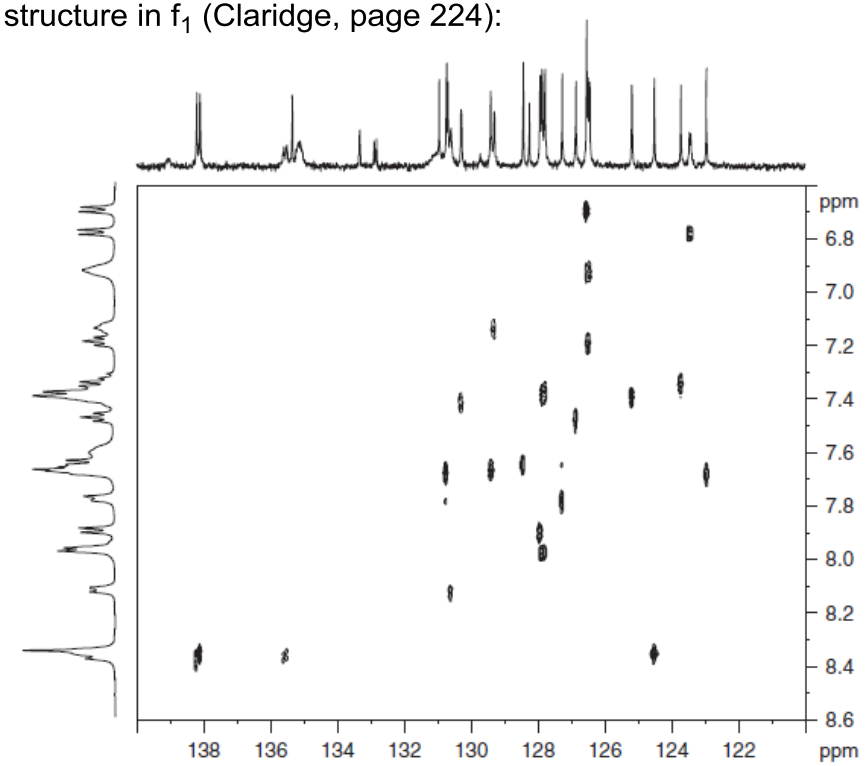
A better version incorporates a BIRD-nulling pulse at the midpoint of t_1 (Bax, A. *J. Magn. Reson.* **1983**, *53*, 517-520). When this is done, multiplet structure collapses:

CH_3, CH, equivalent CH_2: appear as ^{1}H singlets

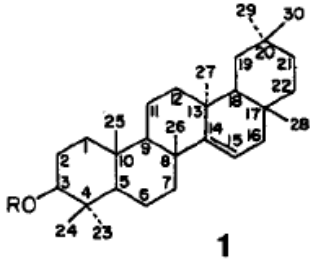
nonequivalent CH_2: appear as AB doublets

With this modification, carbons separated by 0.01 ppm can be resolved. Because this is phase-cycled, there is not Sqrt[2] loss in sensitivity, so the actual loss in sensitivity compared to HSQC is more like 3:1 or 4:1 instead of the theoretical 8:1. Thus, **BIRD-HETCOR is useful for very crowded natural products.**

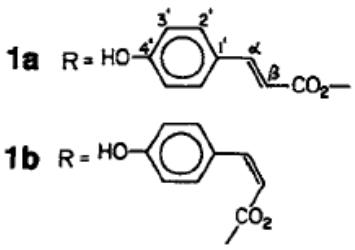
Here is a standard HETCOR spectrum that shows multiplet structure in f_1 (Claridge, page 224):



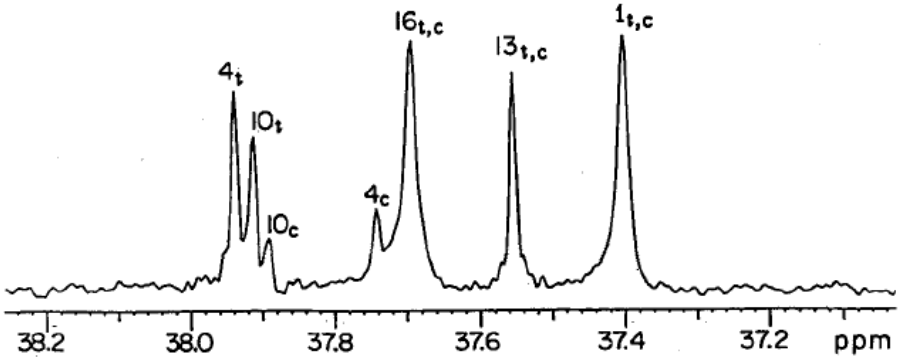
In contrast, BIRD-HETCOR has much sharper signals (Reynolds et al. *Magn. Reson. Chem.* **1994**, *32*, 422-428) and resolves carbons that are very close together. On the facing page, BIRD-HETCOR spectra are shown for a slowly inter-converting mixture of the *cis*- and *trans*-*p*-hydroxycinnamoyl esters of taraxerol:



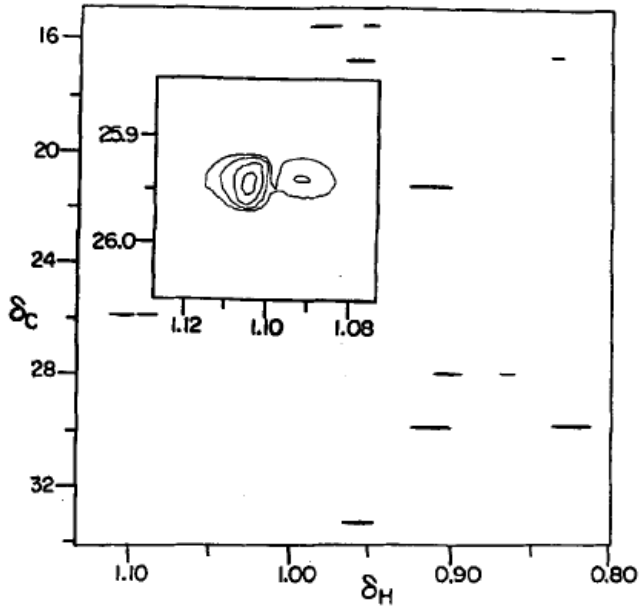
1



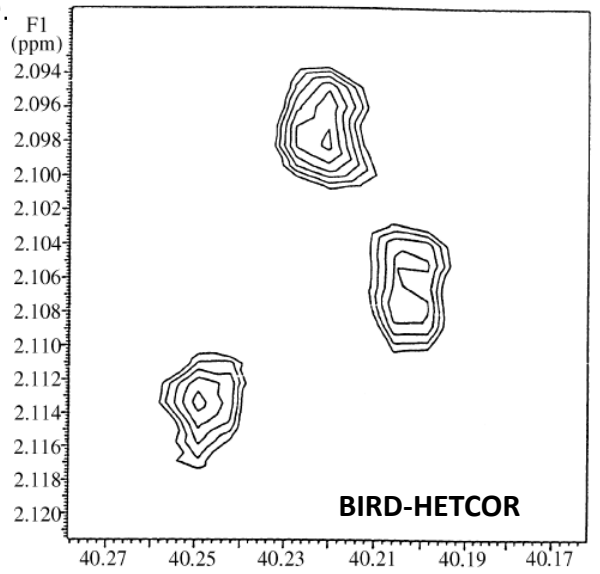
The carbon spectrum has a number of overlapping resonances from the cis (c) and trans (t) isomers:

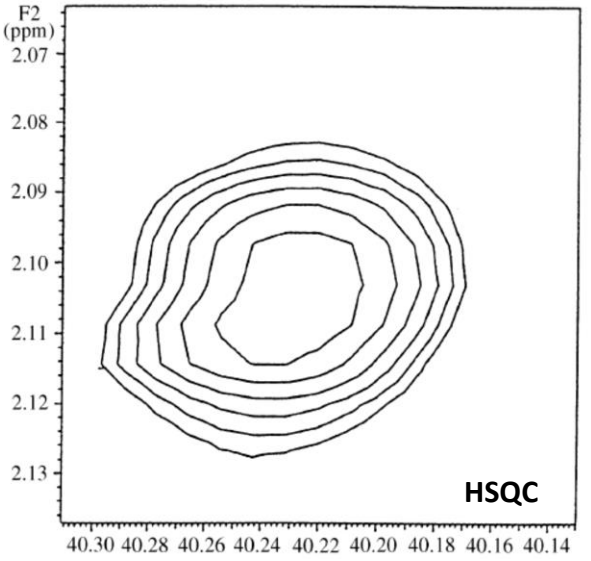


However, the HETCOR spectrum is able to resolve even very closely separated carbons:



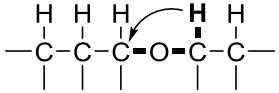
Polyprenol-12 has 12 isoprene units and consequently many overlapping signals. HETCOR (top) performs much better than HSQC (bottom). Ref: Reynolds et al. *Can. J. Chem.* **1999**, *77*, 1922-1930.





Heteronuclear Correlations over Multiple Bonds

Recall that these experiments are typically **inverse-detected.** In most cases, correlations are observed over two to three bonds, although longer-range couplings can be observed:

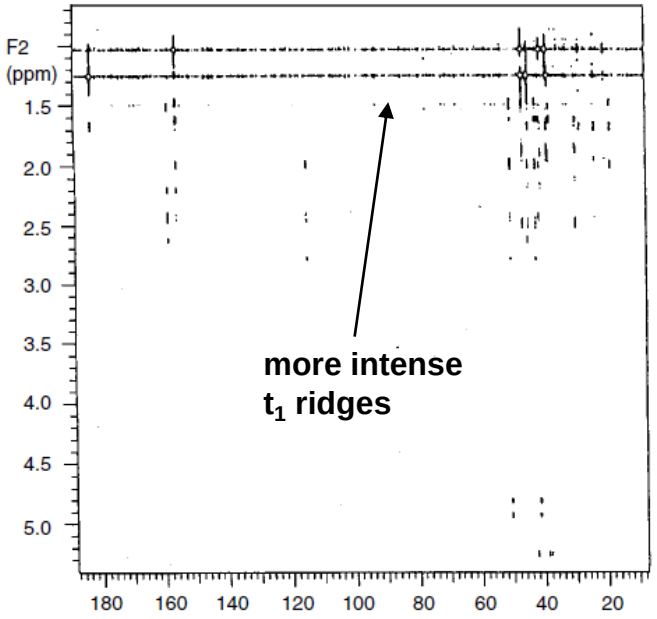


multiple-bond (remote)

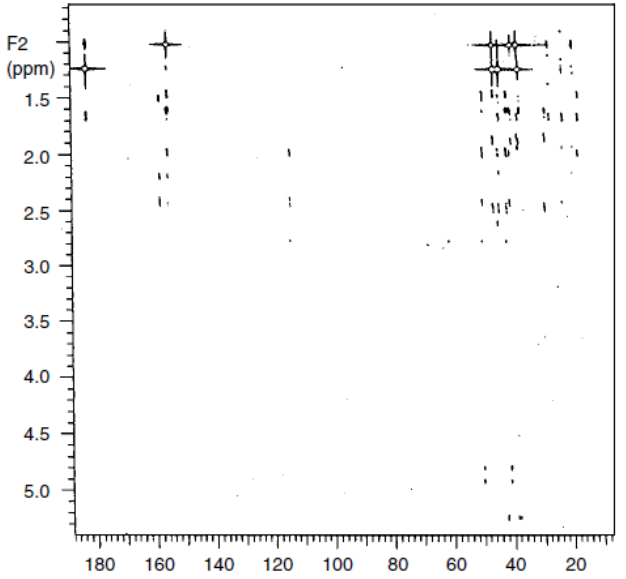
The most common experiment is called **HMBC** - heteronuclear multiple bond correlation. For technical reasons, phase-cycled HMBC is not compatible with a BIRD-nulling pulse, and therefore show large t_1 ridges. In contrast, the gradient-selected version is much more effective at signal supression.

Here are phase-cycled and gradient-selected HMBC spectra of kauradienoic acid plotted at the same scale for a sample at 20 mM (Reynolds et al, *Mag. Res. Chem.* **2001**, *39*, 531-538):

phase-cycled HMBC

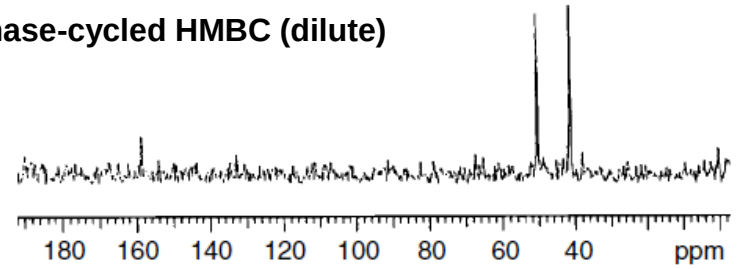


gradient-selected HMBC

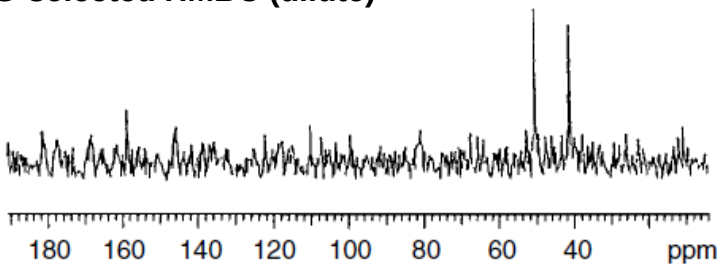


However, in dilute solution (1 mM), there are significant advantages to the phase-cycled version. These are cross-sections plotted at the same scale:

phase-cycled HMBC (dilute)



PFG-selected HMBC (dilute)



Q: Why is phase-cycling better for dilute samples?

(1) **Loss of signal:** Gradients discard some of the signal, but phase-cycling doesn't. The penalty for gradient-selection is Sqrt[2] in HMBC.

(2) **Phasing:** For technical reasons, gradient-selected HMBC is run in absolute-value mode, while phase-cycled HMBC is run in phase-sensitive mode with "mixed mode" processing. That means that f_1 (the carbon axis) is presented in phase-sensitive mode while f_2 (the proton axis) is presented in absolute-value mode. This is done because ^1H-^1H couplings evolve in f_2 and result in phase distortions which are different for every peak. All this results in an additional improvement in sensitivity of Sqrt[2], as well as better carbon resolution.

(3) **Linear Prediction:** This is a mathematical procedure for extending the fid by extrapolating from the earlier portion of the signal. Linear prediction is not very useful for standard 1D spectra, because every FID is made up of many oscillations, each of which is small in intensity. Using a lot of coefficients takes a lot of computer time and may introduce as much noise as additional signal. By contrast, t_1 FIDs in 2D spectra usually only involve a few resonances, so linear prediction provides significant gains.

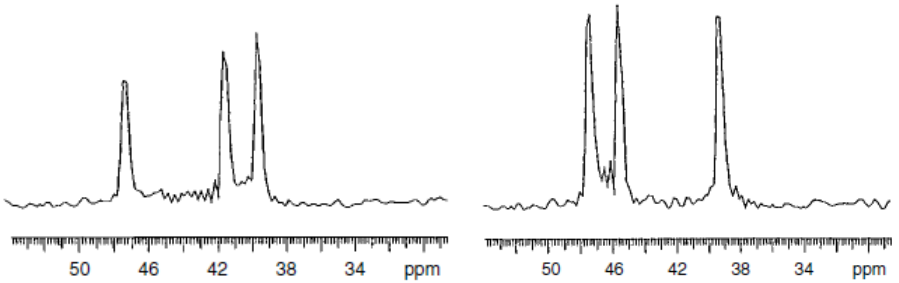
Absolute-value spectra only seem to benefit from two-fold linear prediction, while the phase-sensitive spectra seem to benefit from four-fold prediction. Thus, the phase-cycled version benefits more from linear prediction.

What about acquiring gradient-selected spectra in phase-sensitive mode? That's possible, but it doesn't seem to be widely available at the moment.

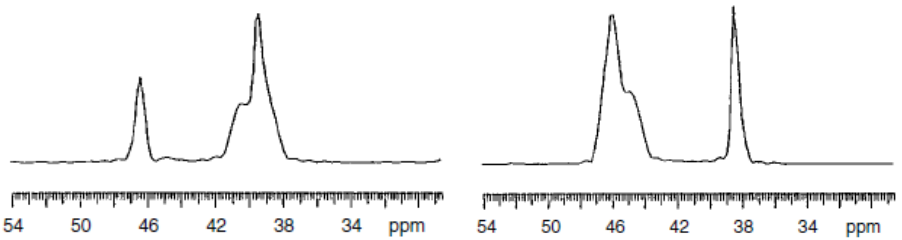
This difference is demonstrated on the facing panel. These are methyl groups as they appear in phase-cycled and gradient-selected HMBC with fourfold linear prediction:

(**Zero-filling** is simply extending the fid with a length of zero signal. **Apodization** is the process of weighting the fid by a function to enhance S/N or resolution.)

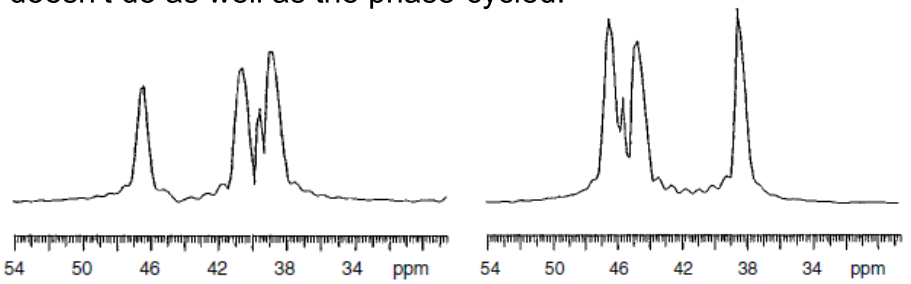
These are phase-cycled HMBC cross-sections with four-fold linear prediction, two-fold zero-filling, and sine-bell apodization. This works very well:



These are gradient-selected HMBC cross-sections with four-fold linear prediction, two-fold zero-filling, and sine-bell apodization. It doesn't really work at all:



Gaussian weighting is less severe and works a bit better, but still doesn't do as well as the phase-cycled:



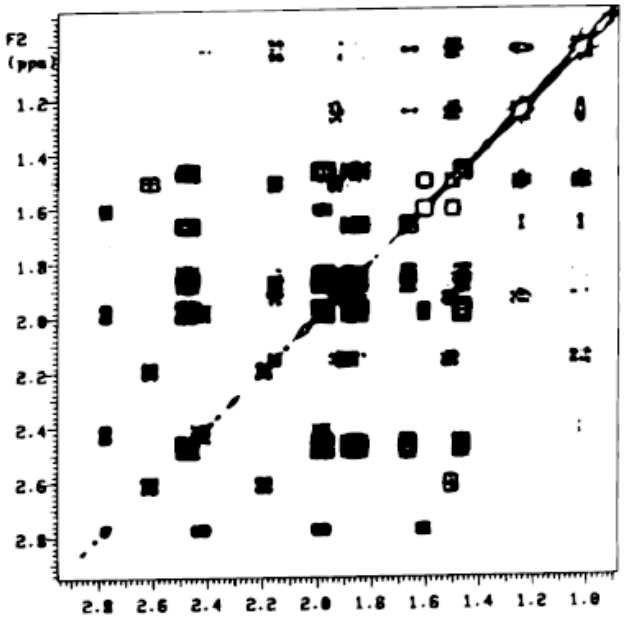
Linear Prediction

"Investigation of the Advantages and Limitations of Forward Linear Prediction for Processing 2D Data Sets." Reynolds et al. *Magn. Reson. Chem.* **1997**, *35*, 505-519. In general:

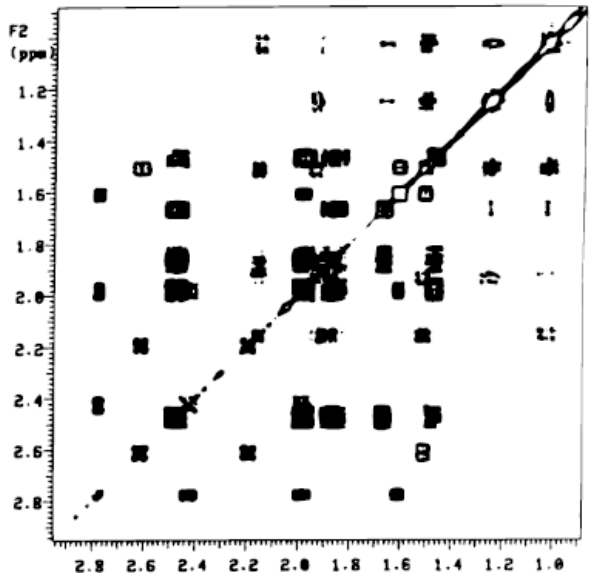
absolute value spectra: use two-fold linear prediction

phase-sensitive spectra: use four-fold or higher LP

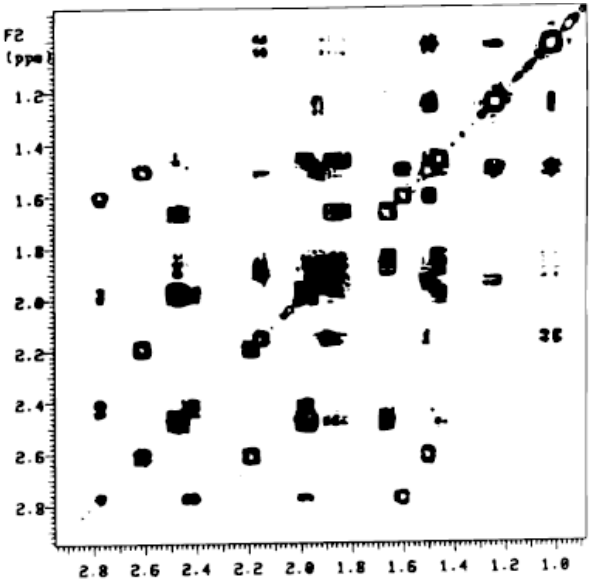
16 coefficients are sufficient for 2D spectra. Here is what happens in COSY spectra (figures from above reference):



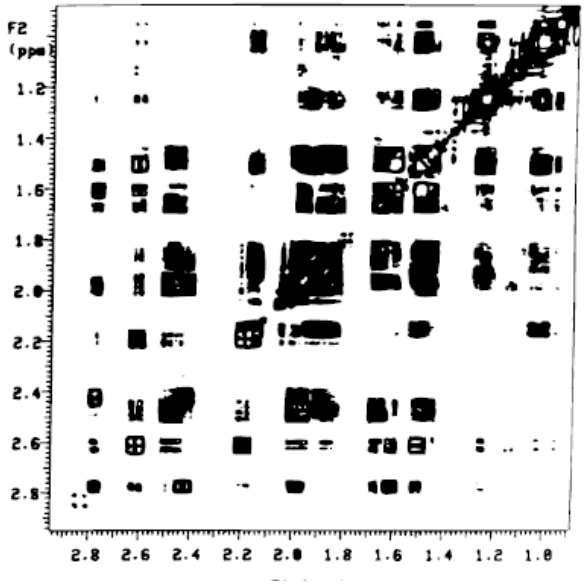
This is a COSY-90 taken, of course, in absolute-value mode. This has a lot of increments: ni=1024. Two-fold linear prediction was carried out. In the next spectrum, ni=512, and once again twofold linear prediction (with two-fold zero filling to make the total number of points in f1 the same (2048)) is carried out:



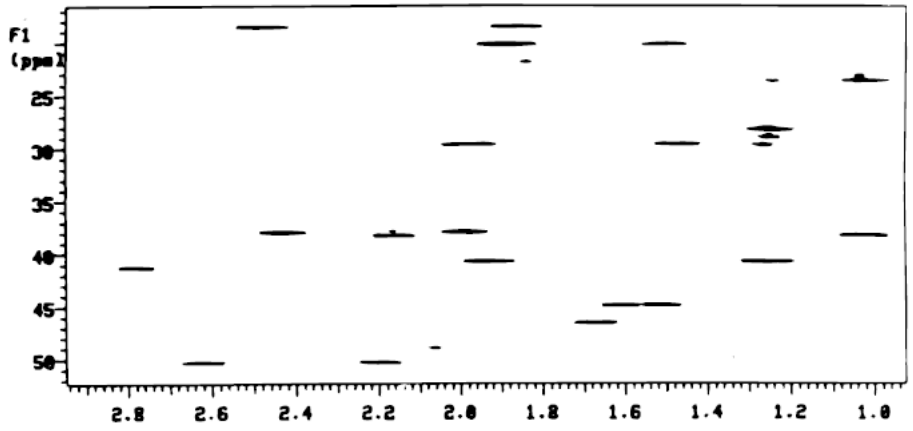
The experiment took half the time, but gets results that are almost as good! However, four-fold linear prediction from ni=256 does *not* work:



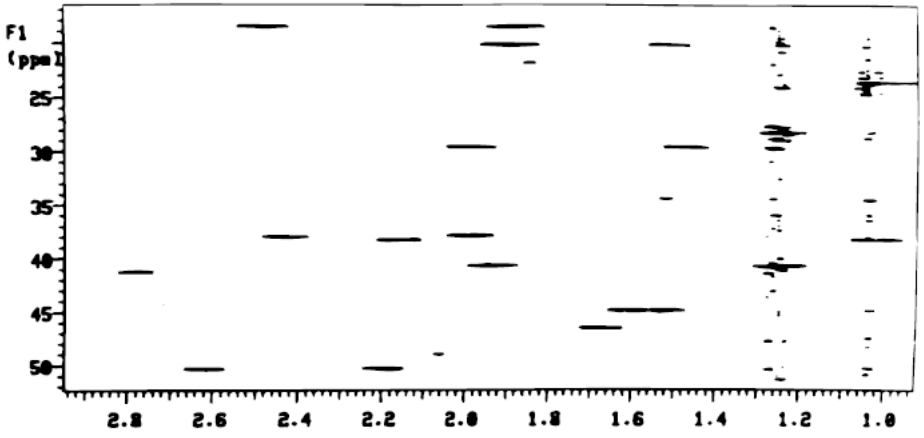
Excessive zero-filling (from **ni**=256 to a total number of f_1 points of 2048) is a very bad idea:



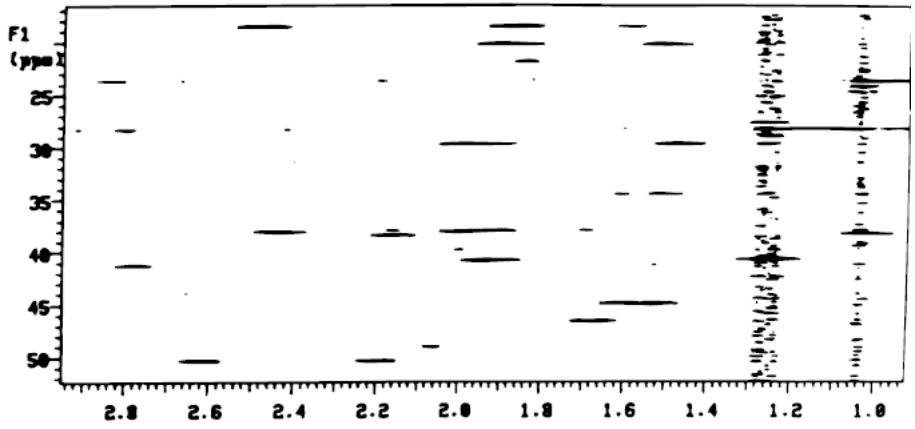
HSQC benefits tremendously from zero-filling. In this spectrum, **nt=16**, **ni=256**, four-fold linear prediction to 1024 points and zero-filling to 2048 points was carried out. The average S/N of the methylenes is **237:1**.



Next, we have **nt=4** and **ni=256** with four-fold linear prediction. This is one quarter the number of scans, and therefore the S/N is halved (observed S/N, **116:1**).



Finally, we have **nt=4** and **ni=1024** and no linear prediction. The average S/N is now **117:1**, which means that without linear prediction, achieving the same resolution and S/N takes quadruple the time!



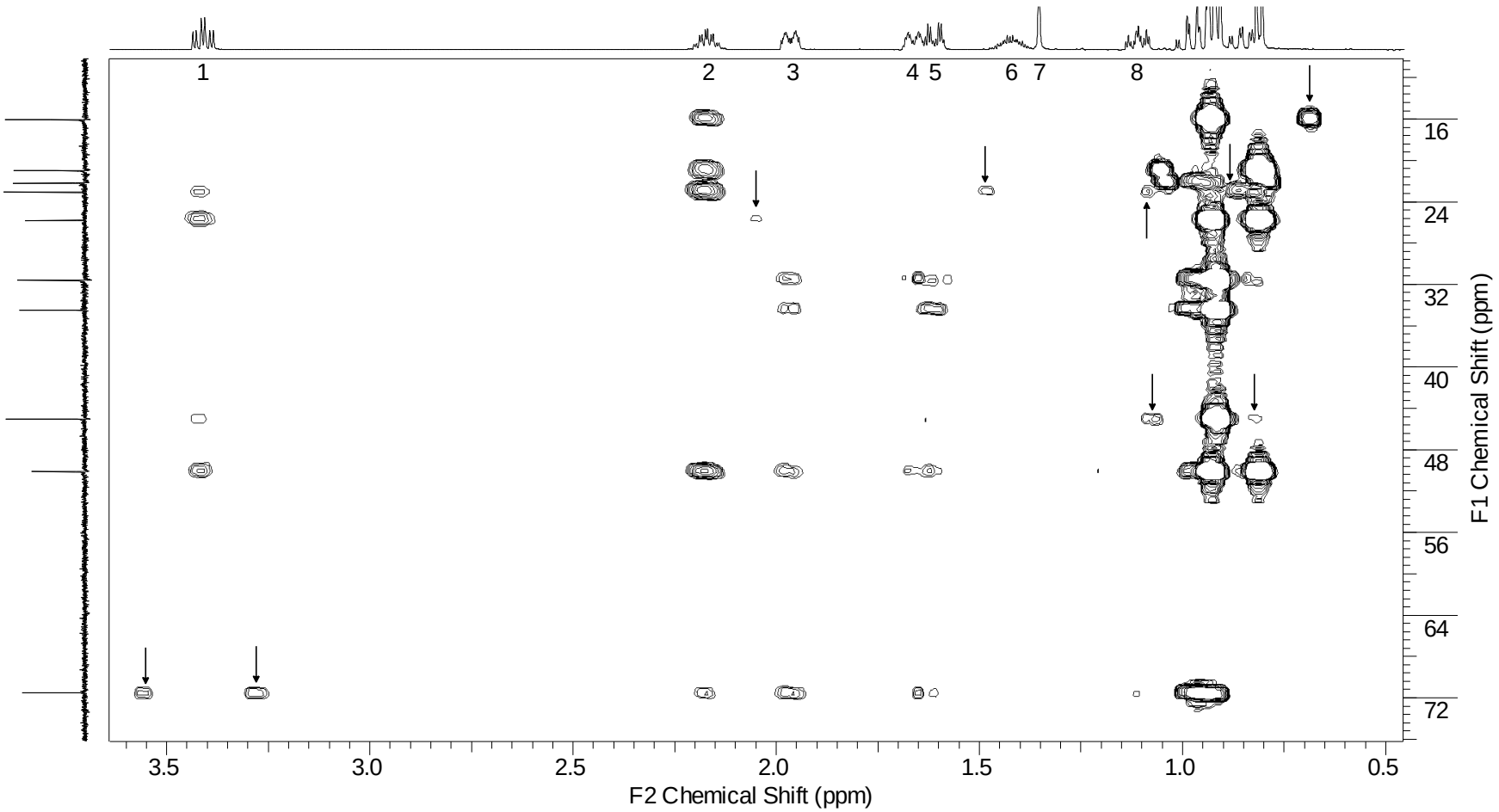
Interestingly, the intensity of the t_1 ridges is also reduced. Thus, **always use linear prediction for 2D spectra.**

HMBC Spectra

Returning to HMBC, there are two important points to note (below is the gradient-selected HMBC spectrum of menthol):

(1) A common artifact is the appearance of one-bond correlations. HMBC is "tuned" to detect the small couplings arising from long-range interactions, but this is not perfect. These one-bond artifacts appear as doublets in f_2, with $J={}^1J_{CH}$. Occasionally, this splitting is itself useful information. However, most of the time, one should be wary of these artifacts.

(2) HMBC incorporates a delay which under ideal circumstances is $1/2J_{CH}$. Since long-range couplings occupy a relatively wide range of 5-25 Hz, this delay is often set at a compromise value of 60 ms (8 Hz). This means that not all of the correlations will appear, and certainly not with equal intensity. Additionally, note that three-bond couplings are often larger than two-bond ones.



Alternatives to HMBC

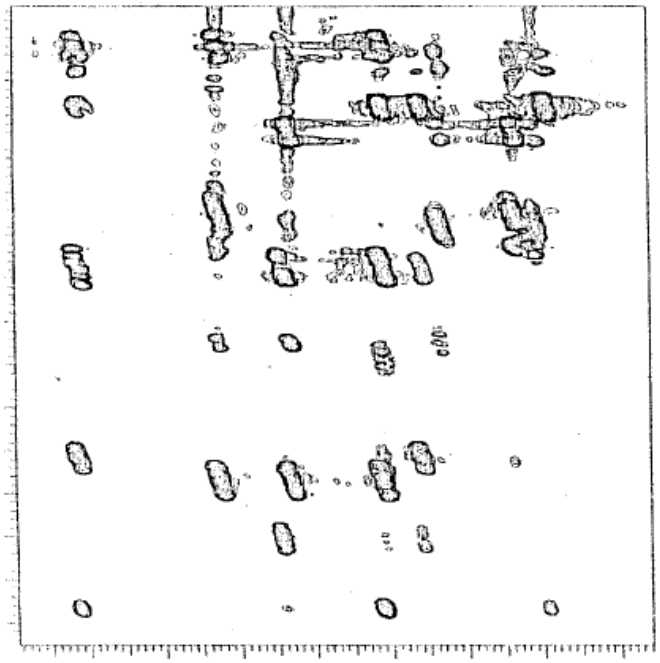
In an effort to deal with the ranges of long-range carbon-proton couplings that exist in natural products, a number of new sequences have developed which incorporate a "constant-time" or "accordion" delay. This allows a *range* of couplings to be sampled. Of these, the CIGAR sequence seems to be quite good (Hadden et al. *Magn. Reson. Chem.* **2000**, *38*, 143-157). A comparison of HMBC and CIGAR shows that:

(1) Some signals are stronger in CIGAR, but others are weaker. The CIGAR experiment is a "fancier" experiment that uses more pulses, so it sacrifices some sensitivity.

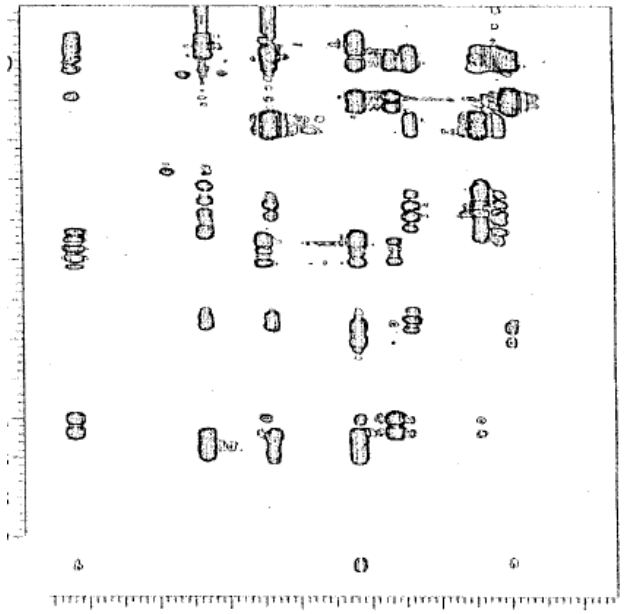
(2) However, f_1 (carbon) modulation is removed, so there is better carbon resolution for CIGAR.

(3) For larger molecules, HMBC is probably preferable.

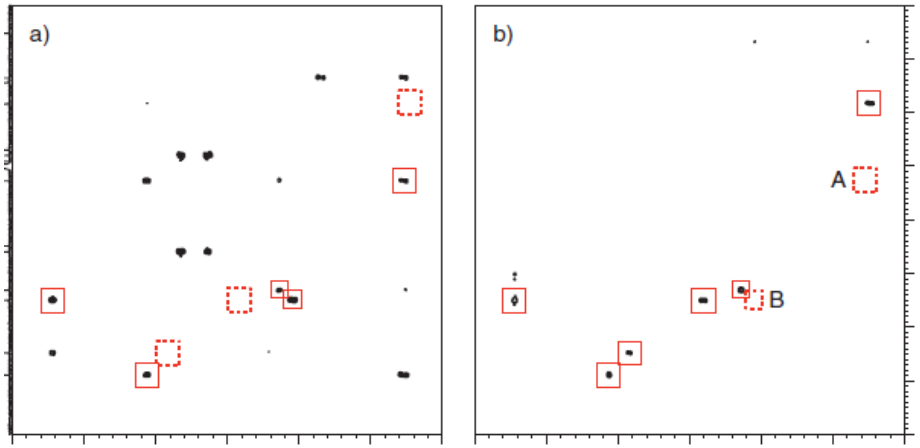
HMBC



CIGAR



Additionally, the inability to distinguish two-bond couplings from three-bond couplings in HMBC spectra is a general problem. The H2BC experiment is an alternative that, in principle, shows only the two-bond correlations (Claridge, page 220):



(a) HMBC spectrum; (b) H2BC spectrum. Notice that H2BC finds additional two-bond correlations.

Choice of Acquisition and Processing Parameters

The appropriate choice of data acquisition and processing parameters is absolutely essential. Time on NMR spectrometers is expensive and judicious choices mean the difference between noisy, unintelligible spectra, and crisp spectra that provide the answers you require.

Professor Reynolds and co-workers make the following recommendations:

(1) **Recycle Delay:** This is **at+d1**, *not* the repetition delay **d1**, since magnetization relaxes during acquisition. For inverse-detection experiments, a delay of **1.3 x T_1** is recommended. Since T_1 varies, this will have to be a compromise value, perhaps for methylenes, since they have a long relaxation time. For NOESY and t-ROESY experiments (to be discussed in Lecture 9) a longer delay of at least 2 T_1 is desirable. T_1 can be measured from inversion-recovery experiments and is generally on the order of one second.

(2) **Decoupler Heating:** In HSQC and HMQC experiments, decoupling is applied during acquisition. This heats up the sample and probe in an undetectable way, since the thermocouple measures air temperature. Too much heating will damage the probe. This is more of a problem with high dielectric solvents. Thus, one should collect as many data points as is practicable to observe the decay of T_2*, but minimize the fraction of time spent with the decoupler on (**at** / **d1**).

(3) **Number of Increments (ni):** This determines the resolution in f_1, the indirectly detected dimension. Unlike resolution in f_2, which is basically free because one can simply collect more data points, doubling **ni** slightly more than doubles total experiment time (VNMR: **time** command). More crowded spectra require more increments. Some suggested values are given on the next page.

(4) **Linear Prediction:** This is essential and allows the recycle delay and the number of increments to be minimized while still maintaining high S/N.

(5) **Apodization:** For various complicated reasons, different experiments need different windowing functions. Suggested functions are given on the following page.

(6) **Coupling Constants:** Heteronuclear correlation experiments depend critically on the choice of $^1J_{CH}$ (HSQC) and $^nJ_{CH}$ (HMBC).

(7) **HMBC:** Since the publication of the table on the following page, new parameters for HMBC spectra have been suggested by Reynolds et al. (*Magn. Reson. Chem.* **2009**, *47*, 1086-1094). For the gradient-selected version of HMBC, the recommendations are:

MW 200-600: at = 0.2 to 0.4 s; longer times for smaller molecules, shorter times for more viscous solvents like DMSO

- sensitivity losses for complex multiplets is observed with at < 0.2 s

- ni = 512 with two-fold linear prediction if better than 1 ppm ^{13}C resolution is desired

- sine bell or hybrid sine bell/Gaussian weighting along f_2
- use of exponential multiplication (**lb**) or gaussian multiplication alone (**gb**) give significant losses in S/N
- sine bell of Gaussian multiplication along f_1 are fine

- data sets acquired with short 0.1 s acquisition times can be somewhat salvaged with hybrid weighting (sine bell multiplied by negative line broadening of ca. 8 Hz)

- compromise $^nJ_{CH}$ of 8 Hz is acceptable

Name[a]	Comments	Situation[b]	Acquisition Parameters[c]	Calibrations[d]	Processing Parameters[e]
COSY-45 (very high)	- off-diagonal peaks indicate coupled protons - gradient-selected	short survey	at=0.3, d1=0.8, ni=128, 10 min	90° (^{1}H) tune (^{1}H)	absolute-value; 2x LP in F1; sine-bell squared
		typical/dilute	at=0.3, d1=0.8, ni=256, 30 min higher resolution: ni=512, 1 h		
HSQC (high)	- peaks are protons directly attached to carbons - phases indicate whether CH_2 or CH/CH_3 - avoid sample heating: d1 > 4at ($CDCl_3$, CD_3OD) d1 > 10at ([D_6]DMSO) - probe tuning is essential	short survey	gradient-selected, at=0.1, d1=0.5, ni=32, j1xh=140, 30 min	90° (^{1}**H**, ^{13}C) tune (^{1}**H**, ^{13}C)	phase sensitive; 2x LP in F1; Gaussian
		typical	<500 Da: gradient-selected, at=0.2, d1=0.9, ni=128, j1xh=140, 2 h; >500 Da: gradient-selected, at=0.1, d1=0.5, ni=128, j1xh=140, 4 h		phase sensitive; 4x LP in F1; Gaussian
		dilute	- as above; use phase-cycled mode - turn spin-echo off (all peaks will have the same phase)	90° (^{1}**H**, ^{13}**C**) tune (^{1}**H**, ^{13}**C**)	
HMBC (low)	- peaks are protons within three bonds of carbon - one-bond doublets are common artifacts (J=$^{1}J_{C,H}$)	short survey	- use gradient-selected mode at=0.1, d1=0.5, ni=32, j1xh=140, jnxh=8, 30 min	90° (^{1}**H**, ^{13}C) tune (^{1}**H**, ^{13}C)	absolute value; 2x LP in F1; Gaussian
		typical	- use gradient-selected mode <500 Da: at=0.2,d1=0.9, ni=256, j1xh=140, jnxh=8, 4 h >500 Da: at=0.1, d1=0.5, ni=256, j1xh=140, jnxh=8, 8h		absolute value; 4x LP in F1; sine-bell (F1 and F2)
		dilute	- use phase-cycled mode	90° (^{1}**H**, ^{13}**C**) tune (^{1}**H**, ^{13}**C**)	F1 (phase), F2 (absolute); 4x LP in F1; Gaussian
1D DPFGSE NOESY/ tROESY (high) **2D NOESY/ tROESY** (moderate)	- off-diagonal peaks are protons close in space - irradiated signal/ diagonal have opposite phase - artifacts: COSY (alternating phase), exchange (OH, NH) (inverted)	short survey	0-750 Da: 1D NOESY, at=2.0, d1=1.0, mix=0.5, 64 scans, 5 min 750-2000 Da: 1D ROESY, at=1.0, d1=1.0, mix=0.4, 64 scans, 5 min	90° (^{1}H) tune (^{1}H)	standard 1D processing
		typical	- use gradient-selected mode 200-750 Da: 2D NOESY, at=0.2, d1=1.3, ni=128, mix=0.6, 3 h 750-2000 Da: 2D ROESY: at=0.2, d1=0.8, ni=256, mix=0.4, 10 h	90° (^{1}**H**) tune (^{1}H)	phase sensitive; 4x LP in F1; Gaussian
1D TOCSY (very high)	- gives 1D ^{1}H subspectra for isolated spin systems	short survey	at=2.0, d1=2.0, mix=0.01 to 0.08 s, 64 scans, 5 min	90° (^{1}H) tune (^{1}H)	-standard 1D processing -use absolute value mode for significant phase distortions
		finding $^{n}J_{C,H}$	as above; at least 128 scans		

[a] Experiment (relative sensitivity). [b] Typical: 25 mM. Dilute: 1–5 mM. [c] Abbreviations: at (acquisition time, s), d1 (delay between scans, s), ni (number of increments), j1xh (estimated $^{1}J_{C,H}$, Hz), jnxh (estimated $^{n>1}J_{C,H}$, Hz), mix (mixing time, s). [d] Bold operations required; others recommended. [e] LP = linear prediction.

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