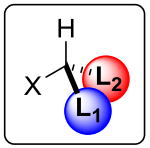
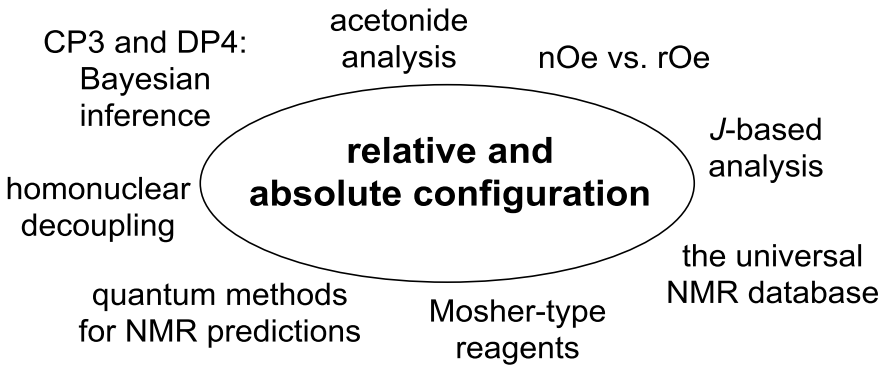
Nuclear Magnetic Resonance V

Eugene E. Kwan



Scope of Lecture



Key References

1. "Structural Elucidation with NMR Spectroscopy..." *Eur. J. Org. Chem.* **2008**, *16*, 2671-2688.

2. "Choosing the Best Pulse Sequences..." Reynolds, W.F.; Enriquez, R.G. *J. Nat. Prod.* **2002**, *65*, 221-244.

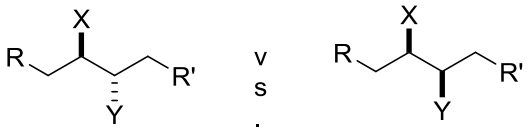
3. "Determination of Relative Configuration in Organic..." Bifulco, G. et al. *Chem. Rev.* **2007**, *107*, 3744-3779.

4. "The Assignment of Absolute Configuration by NMR." Seco, J.M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17-117.

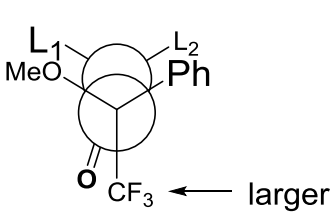
5. "Quantum Mechanical Calculation of NMR Parameters..." Bifulco, G. et al. *Eur. J. Org. Chem.* **2010**, 1411-1434.

Key Questions

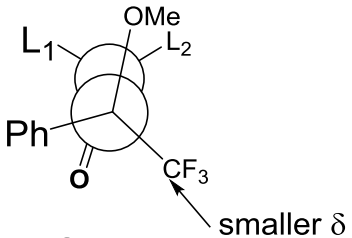
(1) How can coupling constant data be translated into an assignment of relative configuration in a flexible chain?



(2) How can the absolute configuration of a secondary alcohol be determined?

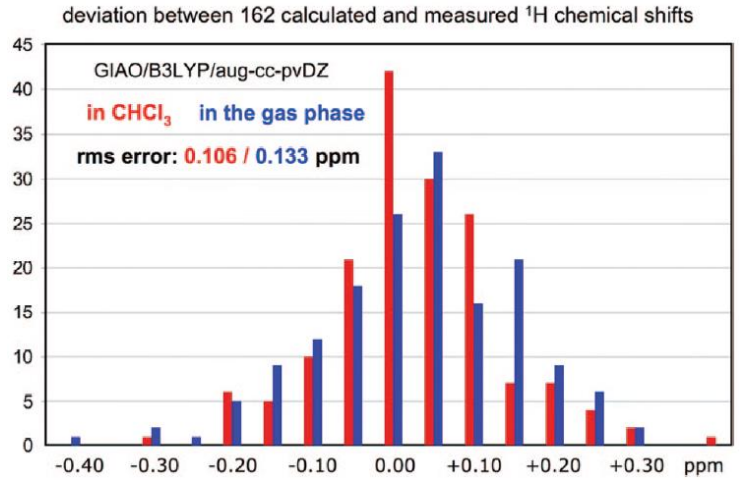


R ester



S ester

(3) How can NMR parameters be predicted?



I thank Professor Reynolds (Toronto) for providing some useful figures and discussions for this lecture.

Lecture notes edited by Richard Liu

Relative Stereochemistry

In general, the procedure for characterizing an unknown with NMR should be split into three stages:

(1) determine the 2D connectivity of the molecule (flat structure)
(2) determine the 3D structure of the molecule (relative stereochemistry)
(3) absolute stereochemistry determination

The rationale is that it is hard to understand the through-space interactions that underlie dipolar (nOe) and scalar (*J*) couplings if you don't know which peaks corresponds to which nucleus. With respect to step 3, absolute stereochemistry is typically determined for one particular polar group at a time (e.g., a secondary alcohol with Mosher ester analysis). The absolute configuration of neighboring stereocenters is then inferred from the relative configuration data.

Q: What can be used to determine relative stereochemistry?

We'll consider four options:

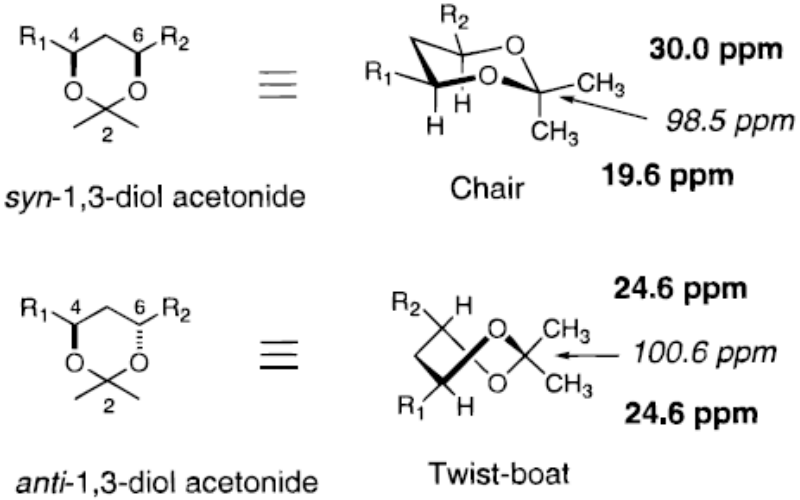
(1) empirical chemical shift correlations (e.g. acetonides)
(2) through-space correlations (NOESY vs. ROESY)
(3) homonuclear coupling constants (proton-proton)
(4) heteronuclear coupling constants (proton-carbon)

Empirical Chemical Shift Correlations

This is the NMR version of the functional group concept. The idea is that similar functional groups have similar conformations and magnetic shieldings, so they have reliable chemical shifts. If the relative configuration of a few test compounds can be determined with other, more sophisticated or time-consuming methods, then correlations can be made to related compounds.

A great example is: "Configurational Assignment of Polyene Macrolide Antibiotics Using the [^{13}C]Acetonide Analysis." Rychnovsky *Acc. Chem. Res.* **1998** *31* 9-17.

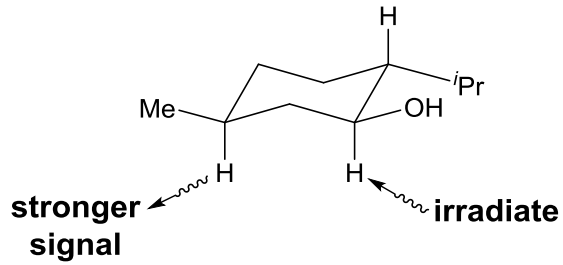
The idea is pretty simple: *syn*-1,3-diol acetnoides exist in chair conformations and have different methyl group chemical shifts, while *anti*-1,3-diol acetonides exist in twist boat conformations (due to 1,3-diaxial strain) and have pseudo C_2-symmetry and similar methyl group chemical shifts. Note that we are looking at the ^{13}C chemical shifts here. Therefore, it is useful to use 2D NMR data to assign the acetonides properly:



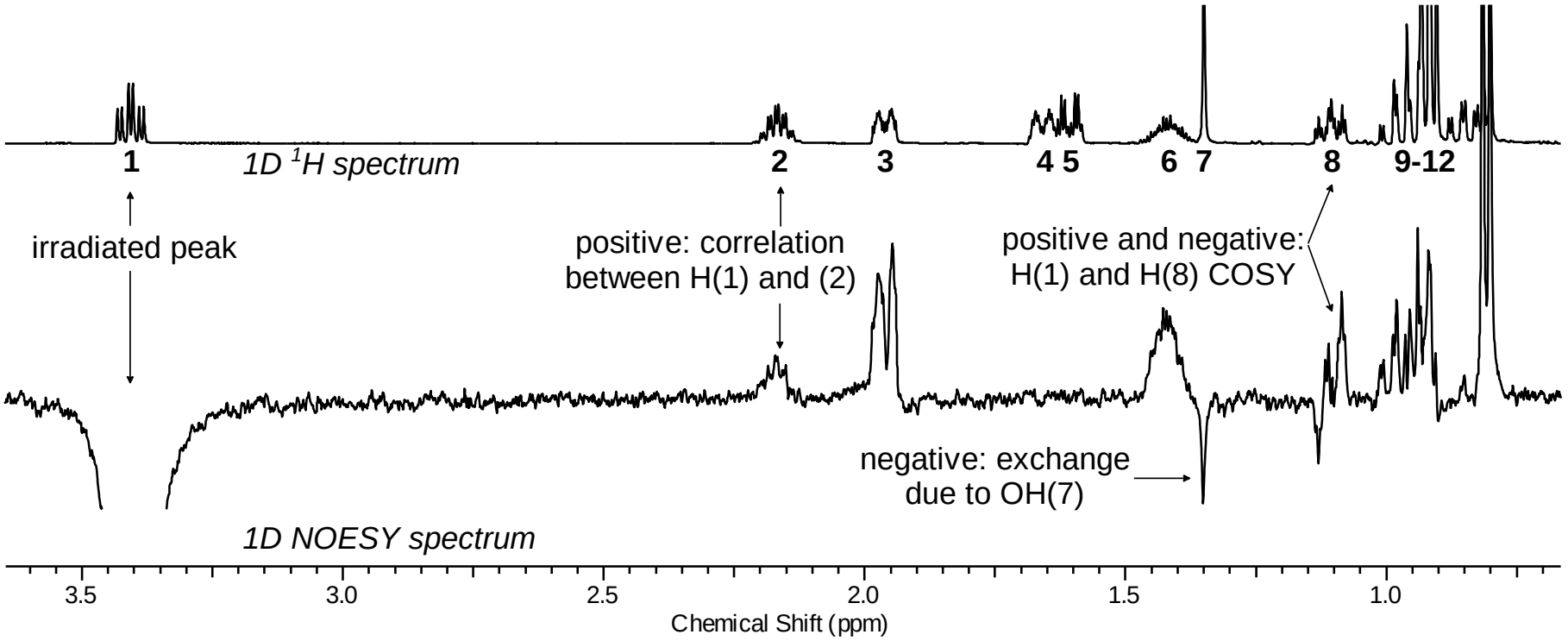
This is great if you if someone has been nice enough to figure out what the chemical shifts look like in your system. If not...

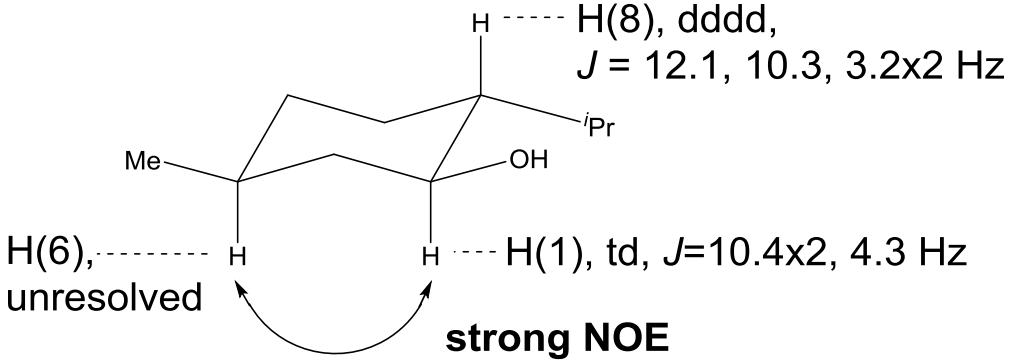
NOESY vs. ROESY

At this point, we are still at the "lightning bolt" stage. We imagine a garish cartoon of a particular proton resonance being somehow "zapped" and increased signal appearing at other protons that are close in space:



Unless you have a very complicated molecule, 2D-NOESY is probably unnecessary. It's important to remember the various features of a 1D-NOESY spectrum. Here is one I took on menthol:



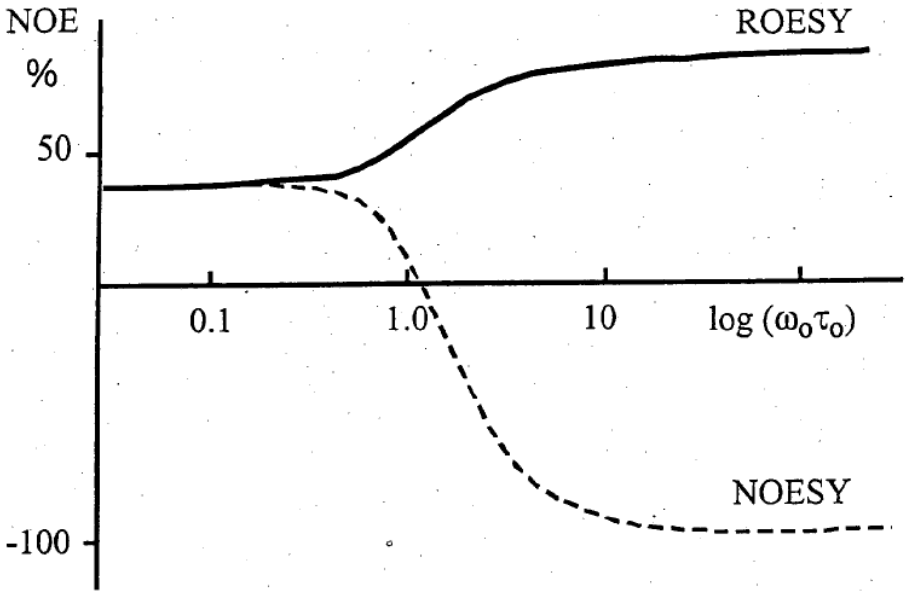


(1) Irradiated peak: negative; nOe correlation: positive; antiphase: COSY artifact; exchange: negative.

(2) Integrals of correlations are not quantitative; only qualitative observations like strong, medium, and weak can be made.

(3) In cyclohexanes, nOe gives you 1,3-relationships, while coupling constants give you 1,2-relationships.

It is important to note the dependence of correlation strength with the correlation time (molecular weight):

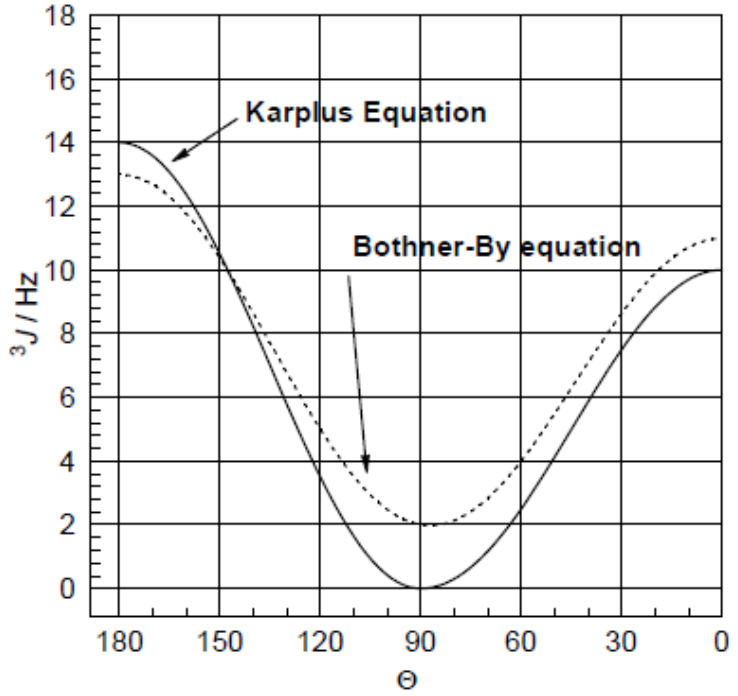


(This graph is for proton-proton through-space correlations only.) NOESY correlations start out moderately positive, but end up intensely negative as the compounds get larger. This is good news if you are working with a protein, but bad news if you are working with anything in the crossover region (in practice, 750-2000 Da, depending on the field strength, the molecular geometry, etc.). As it turns out, there is another experiment called ROESY (rotating frame nOe spectroscopy) that has a different effective correlation time, and gives positive signals for molecules of any weight. ROESY spectra have TOCSY, rather than COSY, artifacts. However, these are generally avoided by using the "transverse ROESY" (t-ROESY) experiment.

Coupling Constants

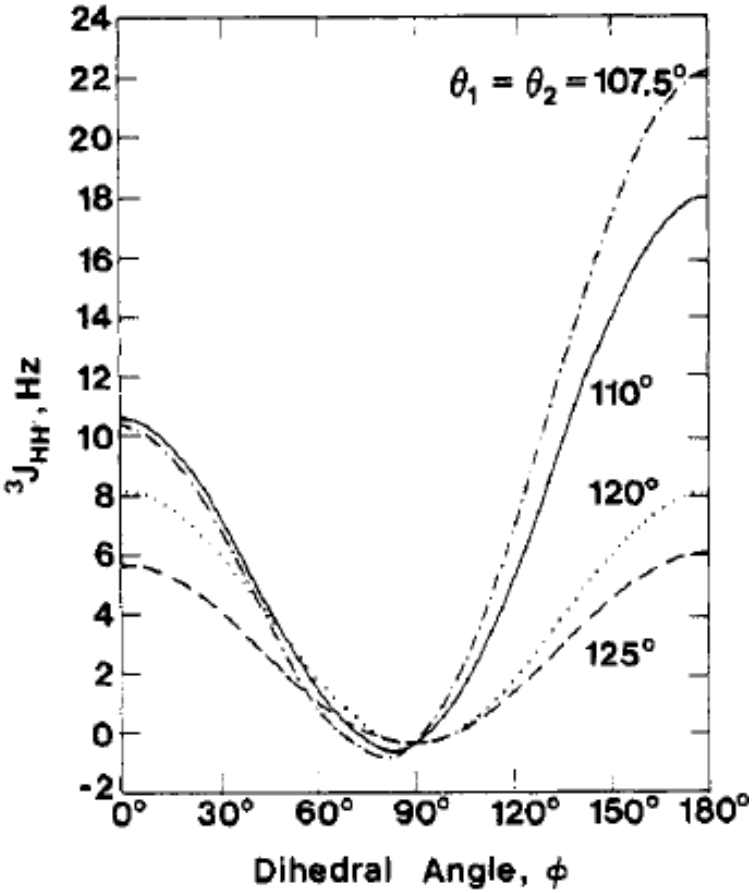
"Unravelling Molecular Structure and Conformation--the Modern Role of Coupling Constants." Thomas, W.A. *Prog. NMR Spectroscopy* **1997**, *30*, 183-207 (review).

As you well know, vicinal coupling constants are generally described by Karplus-like curves, with the exact shape of the curve depending on the exact molecular system:

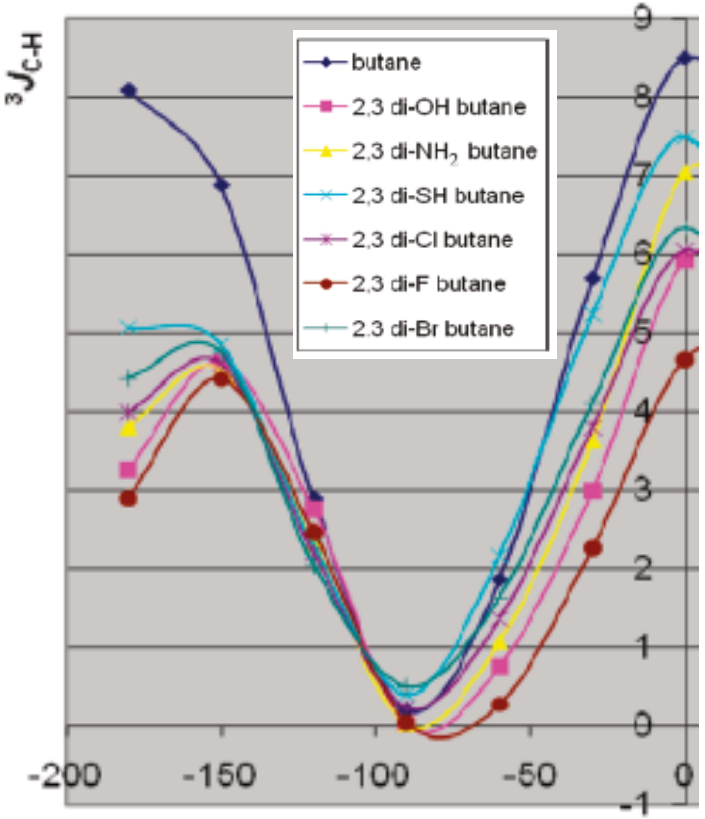


However, one soon realizes that *trans*-diaxial couplings in cyclohexanes are almost never as large as 14 Hz. Over the years, there have been many attempts to improve the Karplus relationship. The most widely is used is that by Altona and co-workers (*Tetrahedron* **1980** *36* 2783). Suffice it to say that it's a detailed analysis that produces a good fit, and software packages use equations like this to account for electronegativity, hybridization, etc. Statistical methods are used to generate these equations. For example, Osawa used 11 independent structural terms and 22 adjustable parameters! (*Magn. Res. Chem.* **1990**, *28*, 668.) Unfortunately, there seems to be little chemical insight to be gained by looking at these curves. One exception is the dependence on the H-C-C bond angle (Barfield *JACS* **1992** *114* 1574):

Although these are computed values at a very low level of theory (a form of molecular orbital theory), the results are quite good. They show that as the H-C-C bond angle widens out from tetrahedral, the overlap gets worse, and the couplings go down:



Work on proton-*carbon* coupling constants is more recent. Bifulco and co-workers have devoted considerable attention to this and have developed computed (MPW1PW91/6-31g(d,p)) coupling constant curves for a variety of substituent patterns (*JOC* **2010** *75* 1982):



Q: How are coupling constants determined?

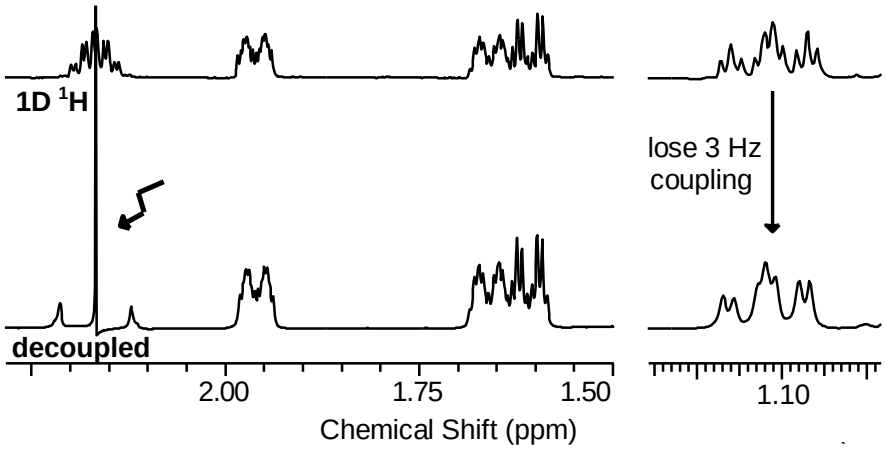
Here are some options:

(1) Read it off a 1D spectrum.
(2) Use homonuclear decoupling to simplify the 1D spectrum.
(3) Read it off a 1D slice of a 2D spectrum.
(4) Use 2D spectra to measure the couplings directly.

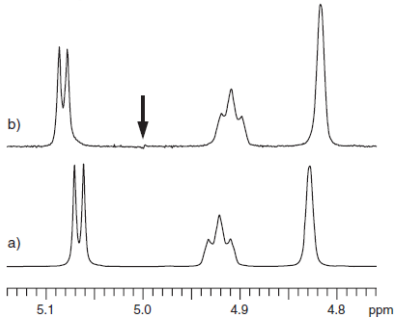
You are already familiar with option (1). For first-order spectra, this means Hoye's method; for second-order spectra, this means simulating the strong coupling with a computer program and adjusting parameters for a good fit.

Homonuclear Decoupling

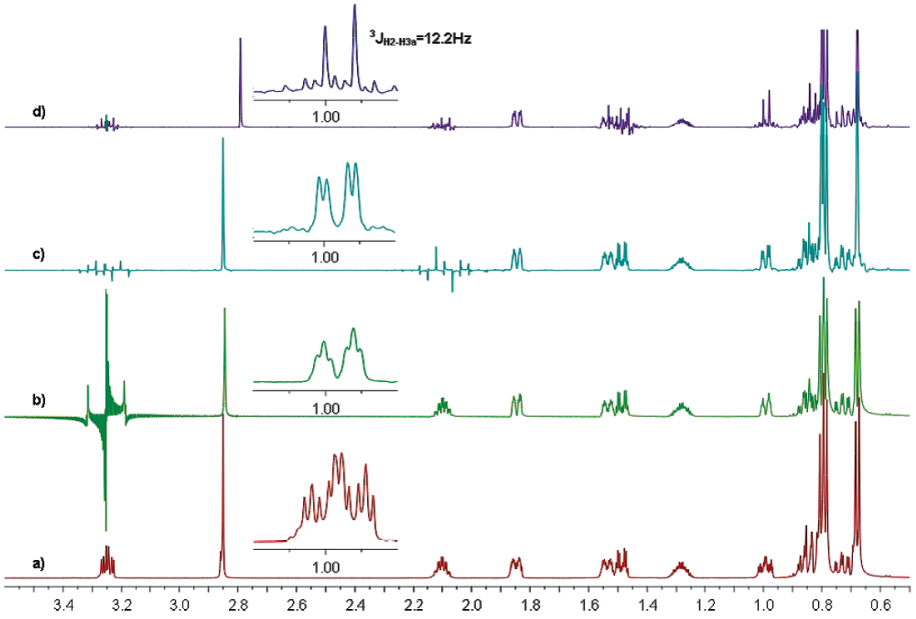
In homonuclear decoupling, you selectively irradiate a particular resonance. The spins flip up and down very quickly, so that other spins that are coupled to it see an averaged coupling of 0 (since the parallel and antiparallel states average their stabilization/destabilization to zero). The effect is to simplify multiplets. Here it is for menthol:



In some cases, the multiplet will still be too complicated to interpret. In others, distortions will appear. However, one can still get some information from the width of the multiplet: the amount it shrinks is presumably the sum of the lost couplings. Occasionally, one gets **Bloch-Siegert shifts**, which are small changes in resonance frequency casued by the decoupling field B_2. These are generally small, and of little concern unless you are doing a difference experiment. From Claridge, page 106:



More recently, MacMillan and co-workers have shown (*JACS* **2009** *131* 15994) that decoupling can be applied at multiple sites simultaneously to achieve greater simplification:



(Apparently, menthol is their favorite test compound, too.)

(d) MDEC (mult-frequency homonuclear decoupling) applied at H-1, H-3a, and H-8.

(c) MDEC irradiation at H-1 and H-8.

(b) MDEC irradiation at H-1.

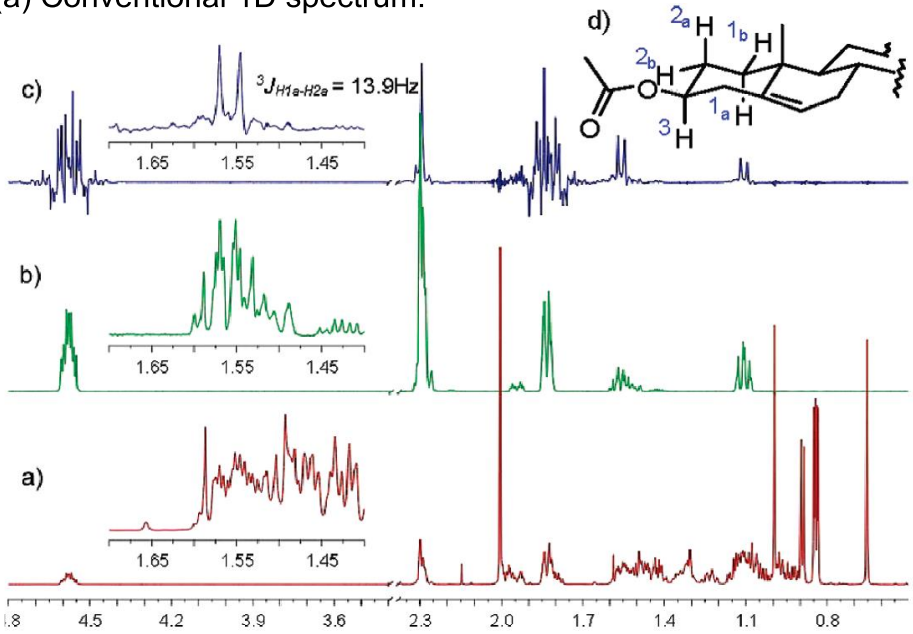
(a) Regular 1D proton spectrum.

If this is combined with a 1D-TOCSY experiment, then the advantages of peak separation can be combined with those of multiplet simplification. Here it is for cholesteryl acetate:

(c) 1D-TOCSY-MDEC at H-1b, H-2b, and H-3.

(b) 1D-TOCSY irradiation at H-3.

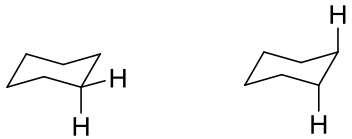
(a) Conventional 1D spectrum.



Clearly, this is a powerful technique.

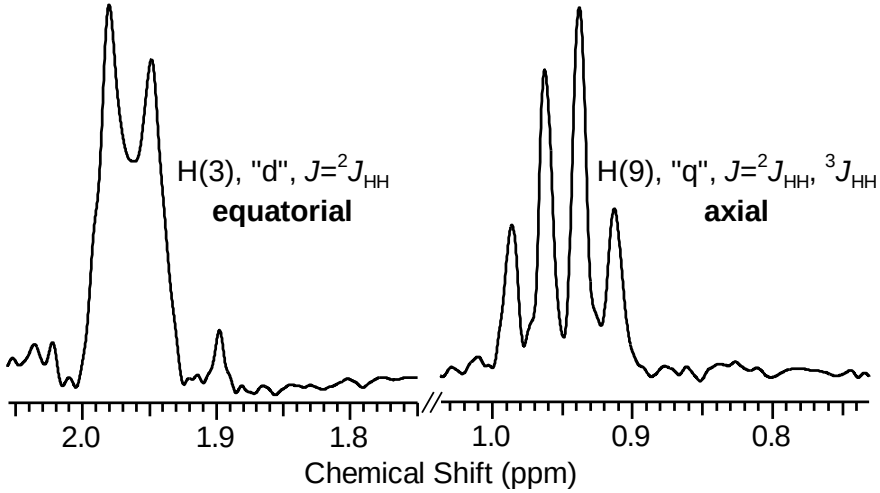
HSQC Slices

If you are too lazy to measure the couplings using new experiments, there is one "quick and dirty" method that applies to cyclohexane-type systems. The idea is that the largest couplings in cyclohexanes are geminal couplings and *trans*-diaxial couplings:



Now, suppose you want to know which proton on a methylene pair is axial and which is equatorial. The equatorial proton must have exactly one large coupling; viz., its geminal coupling. On the other hand, axial protons, which generally contain one or two large *trans* diaxial couplings in addition to a large geminal coupling will have more complicated patterns.

Recall that f_1 resolution in 2D spectra is time-limited. Thus, f_1 slices from HSQC spectra will have poor resolution and will only show the largest couplings. Equatorial protons will therefore look like doublets, while axial protons will look like triplets (one axial neighbor) or quartets (two axial neighbors):



Of course, this is by no means quantitative. This is just a way for you to make an intelligent guess, which can then be backed up at some later point by other data.

Direct 2D Measurement

There are two orthogonal ways to measure the couplings with 2D spectra directly. In *J*-resolved spectra, the couplings form the f_1 axis. In COSY-related methods, distances between the peaks inside correlations are measured. This is a complex area and I can't talk about it all here. Please see: "Scalar Coupling Constants--Their Analysis and Their Application for the Elucidation of Structures" Kessler, H. et al. *ACIE* **1995** *34* 1671.

Couplings from DQF-COSY

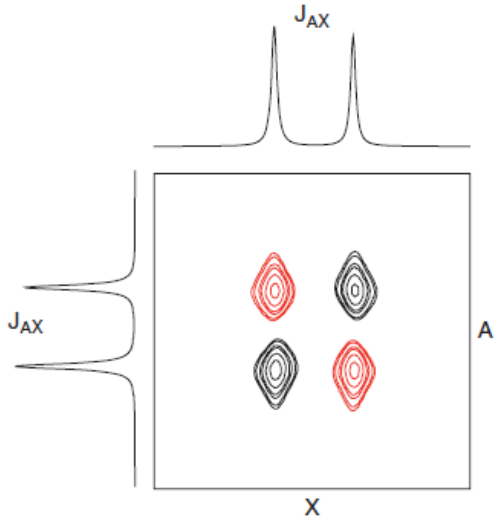
For a full discussion, see Claridge, pp. 161-165.

In DQF-COSY, only double quantum coherence arising from coherence transfer through *J* couplings appears (so no crosspeaks from singlets should appear). The convention is to define, for any particular crosspeak:

the active coupling as the one that gives rise to the crosspeak correlating the two spins and

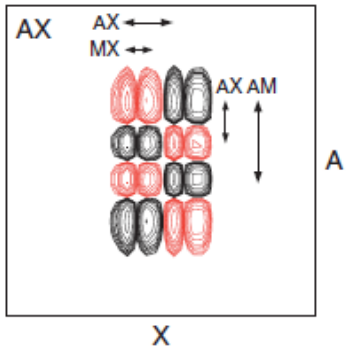
the passive couplings as the other couplings.

DQF-COSY is a phase-sensitive experiment. The crosspeak for an AX system shows an antiphase apperance, with the spacing corresponding to the coupling J_{AX}.



This antiphase character is actually an annoyance, because insufficient resolution in f_1 means that the peaks can start to lie on top of each other, causing cancellation. For this reason, measuring couplings with DQF-COSY takes a long time, even though COSY is inherently quite a sensitive experiment.

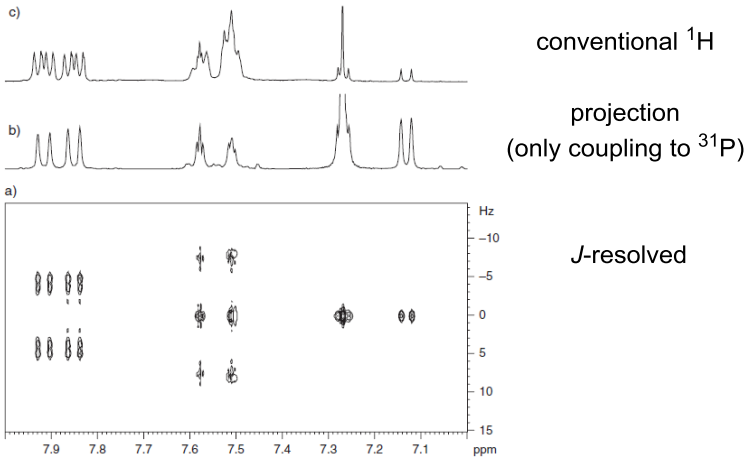
In an AMX system, the AX crosspeak will have an up-down phase for the active AX coupling, but up-up or down-down phases for the passive AM and MX couplings:



As you can see, life gets very complicated, very quickly here. I recommend that you consider this experiment a last resort.

Couplings from 2D *J*-Resolved Spectra

This sequence allows to get the coupling constant along the f_I axis and the chemical shift of each uncoupled proton along the f_2 axis.



For this to work, the splitting will have to be first order. In this case, the compound contains ^{31}P, so the horizontal couplings represent proton-phosphorus couplings while the vertical couplings represent proton-proton couplings.

Carbon-Proton Coupling Constants

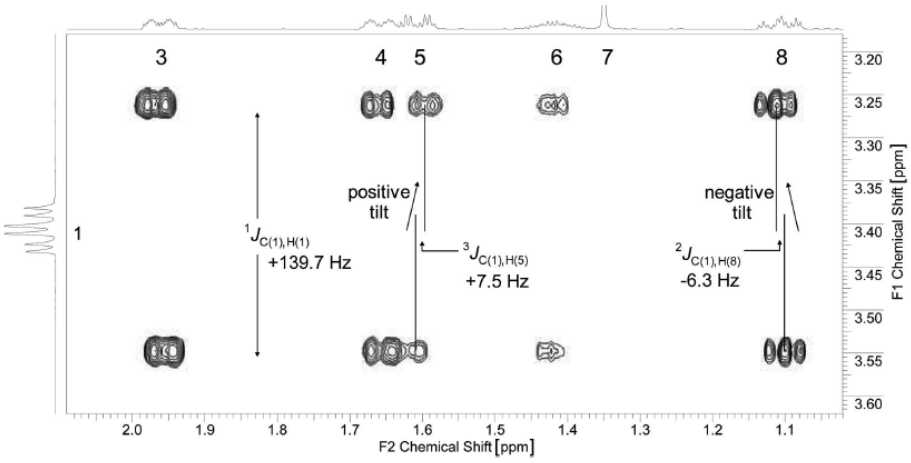
"Survey of NMR Experiments for the Determination of $^{n}J_{CH}$ Heteronuclear Coupling Constants in Small Molecules." Marquez, B.L.; Gerwick, W.H.; Williamson, R.T. *Magn. Reson. Chem.* **2001**, *39*, 499-530. (comprehensive review)

Q: Why measure carbon-proton coupling constants?

- Difficult cyclic stereochemistry problems where other data give ambiguous interpretations.
- More common: determine acylic stereochemistry within flexible chains.

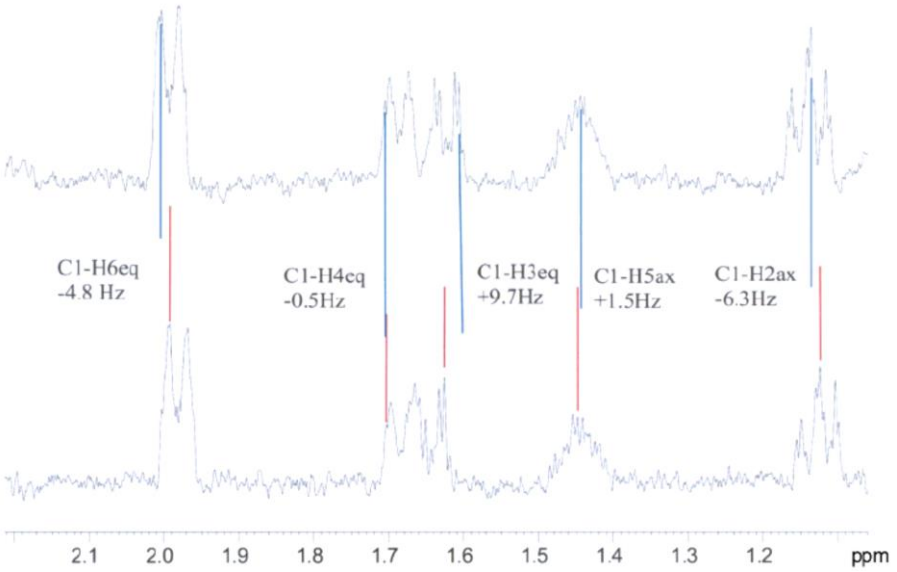
Usually, when one finds that a thousand different solutions to the same problem are in use, it means that there is no one solution that is particularly good. This is just such a case. C-H couplings are inherently hard to measure because we are generally trying to pick out small *long-range* couplings. The couplings are also associated with the low-sensitivity carbon-13 nucleus. Here are two solutions: HETLOC and 1D-TOCSY. Both rely on TOCSY transfer, and so cannot measure carbon-proton couplings to quaternary carbons.

HETLOC stands for heteronuclear long-range coupling:



Both axes are proton frequencies. Every crosspeak is a TOCSY correlation, but split by $^{1}J_{CH}$ in f_1 and $^{n}J_{CH}$ in f_2. This means wide separations in f_1 and narrow ones in f_2. The tilt of the peaks gives information on the sign of the coupling constants. Of course, things can get pretty crowded if a TOCSY spectrum is essentially getting another offset copy of itself overlaid on it.

A different method that partially gets around this problem has been reported by Espinosa and co-workers (*JOC* **2007** *72* 3166). It requires that the carbon-13 satellites of a proton be clearly visible for irradiation:



The spectra are then overlaid, and the shifts between them correspond to the couplings. How does it work? Consider an H-C-C-H fragment. We can ignore the dominant ^{1}H-^{12}C-^{12}C-^{1}H isotopomer. What about the ^{1}H-^{13}C-^{12}C-^{1}H isotopomer? Suppose we irradiate the underlined proton. Then, we expect to see TOCSY transfer to the other proton, which will then give a doublet at its chemical shift, split by $^{2}J_{CH}$ (ignoring isotope effects). By irradiating a ^{13}C satellite, we are selectively irradiating one isotopomer. By irradiating one satellite at a time, we are getting one half of the doublet in each experiment.

Determining Acyclic Stereochemistry

Q: How can stereochemistry be determined in a flexible chain?

This area has been reviewed extensively by Riccio et al (2007). There are two main methods:

(1) *J*-based configurational analysis, as developed by Murata in 1999 (*JOC* **1999** *64* 866): based on expected proton-proton and proton-coupling constants

(2) empirical shift correlations using a "universal NMR database" as introduced by Hoye (*JACS* **1987** *109* 4402) and developed by Kishi (*OL* **1999** *1* 2177): based on comparisons between observed shifts and those already known for model compounds

J-Based Configurational Analysis

There are three ideas here:

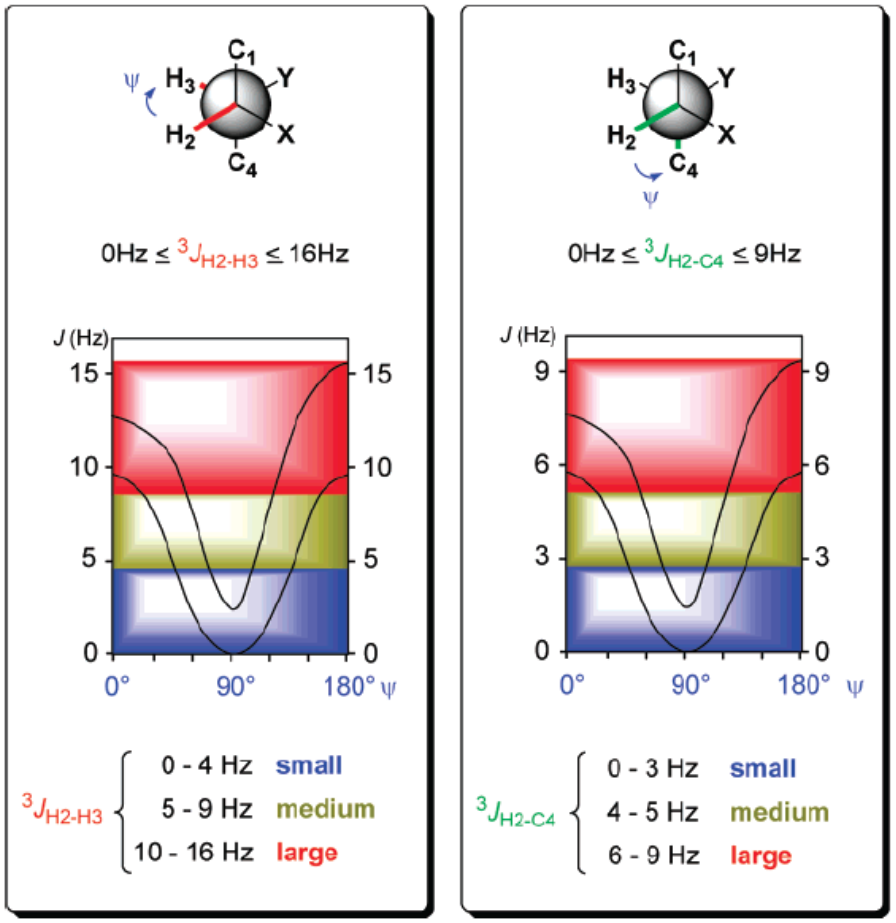
(1) Proton-carbon coupling constants follow a Karplus-type relationship, just like proton-proton ones do. The shape of the curve depends on the substituents and has been worked out for a wide variety of functional group patterns.

(2) Suppose one wants to determine if two adjacent stereocenters is related by a syn or anti relationship. One draws all the staggered conformers of both possibilities. Based on the Karplus relationship, one can decide if the couplings should be large, medium, or small.

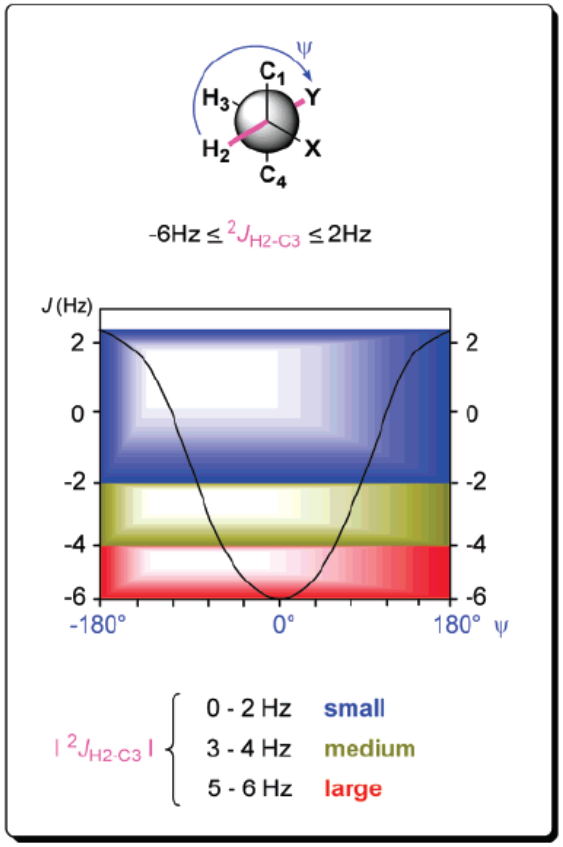
(3) The observed couplings can be compared to the expected couplings for the various rotamers and a prediction of the assignment made.

Here are some graphics from the Riccio review:

These are semi-quantitative values for 1,2-dioxygenated systems. The two curves indicate the ranges that are empirically found for the couplings as a function of H-C-C-H or H-C-C-C dihedral angle.



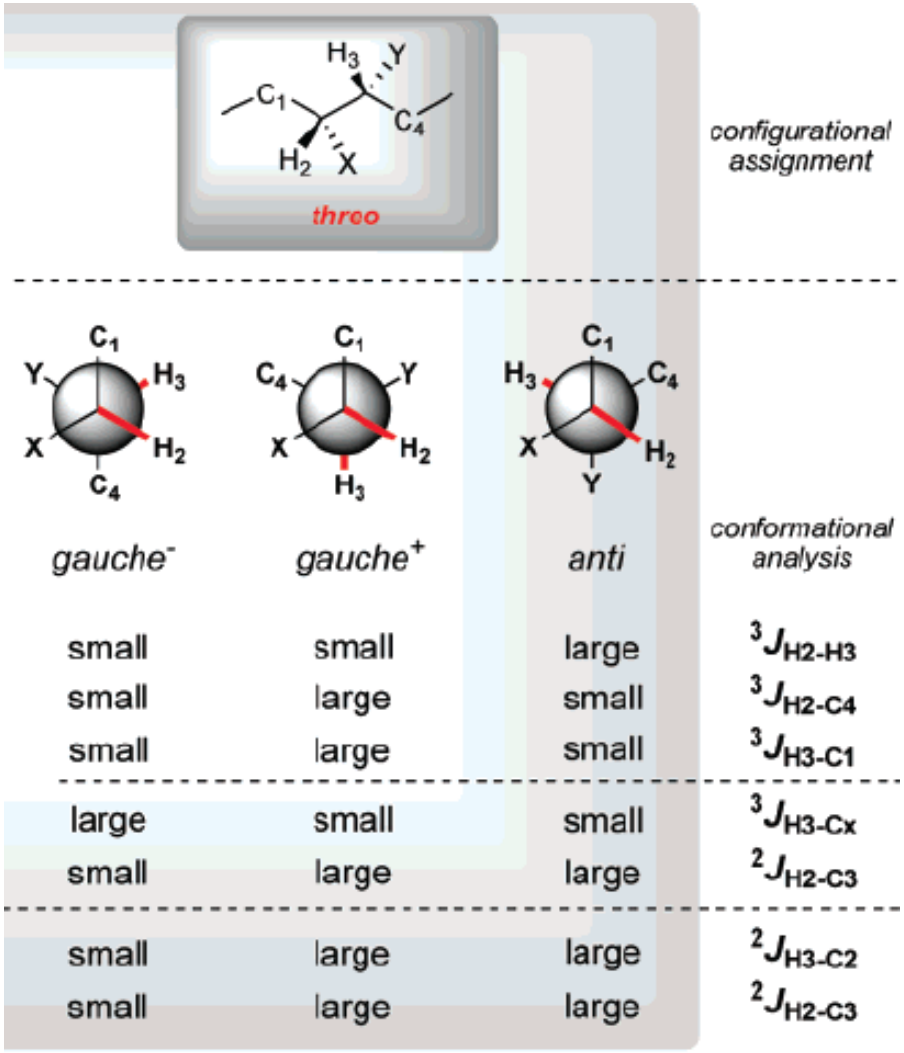
The above are vicinal relationships. As it turns out, *geminal* proton-carbon couplings can tell us something about the dihedral angle between a proton and an adjacent heteroatom as well. The corresponding Karplus-type curve is given on the next page:



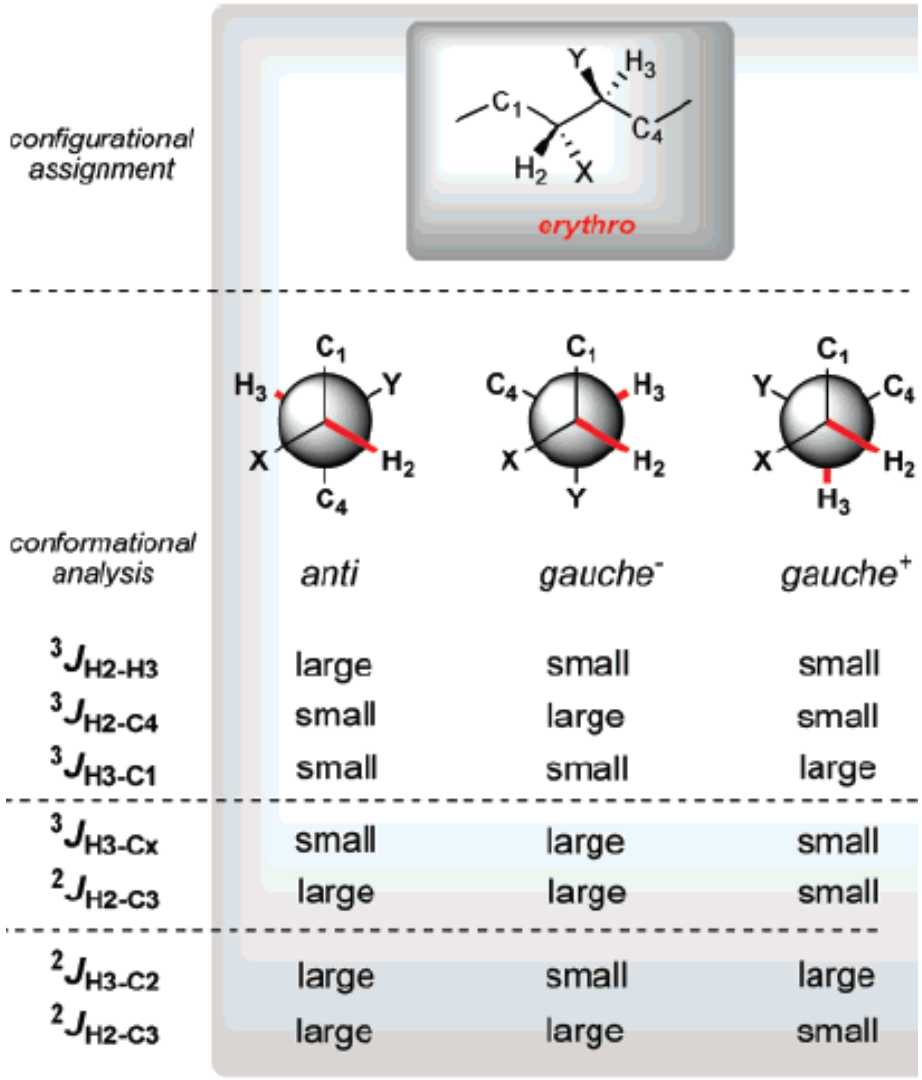
This means we are looking at the coupling between the proton in the front, marked H_2, and the carbon along the axis of the Newman projection, C_3. C_3 is behind C_2, so you can't see it.

Let us now consider a 1,2-methine system. It can have a threo or erythro relative stereochemical relationship (we don't want to use words like syn or anti, since they would depend on how the chain was drawn). One forms three staggered rotamers for both diastereomers and assigns expected couplings to them. Then, results can be compared against the experimental data. Note that there is no way to tell between the two anti conformers of the *threo* and *erythro* compounds. Typically, additional information, available through dipolar couplings (nOe) is needed to distinguish those two possibilities.

For X=OR, Y=OR and the threo stereoisomer:



For the *erythro*:

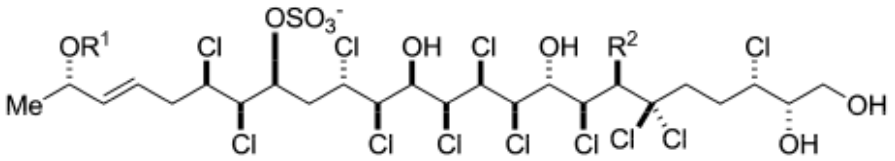


	anti	gauche⁻	gauche⁺
$^3J_{H2-H3}$	large	small	small
$^3J_{H2-C4}$	small	large	small
$^3J_{H3-C1}$	small	small	large
$^3J_{H3-Cx}$	small	large	small
$^2J_{H2-C3}$	large	large	small
$^2J_{H3-C2}$	large	small	large
$^2J_{H2-C3}$	large	large	small

(I copied these charts from page 3747 of the review. Note that there seems to be an error in the annotation on the right hand side of Figure 3: this is X=Y=OR, not X=Me, Y=OR.)

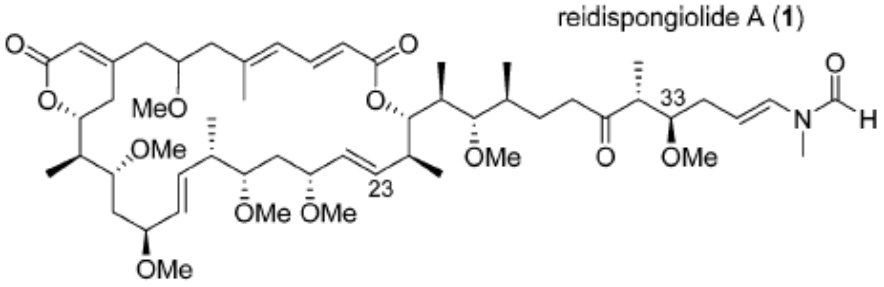
Charts for other substitution patterns are available in the review. The presence of *medium* sized couplings is a sign that there are multiple conformers whose couplings are being Boltzmann-averaged. This is a complicated situation and I will not get into it here.

Computations are helping in this arena, too. What if you have a functional group pattern which is not covered by this? For example, Carreira (*JACS* **2009**, *131*, 15866) recently dealt with polychlorinated natural products:



In this case, they chose to do something that is more or less a kind of universal NMR database method: they synthesized fragments and obtained some characteristic chemical shift and coupling constant data. A comparison of the natural product and these data allowed an assignment by analogy.

The other approach is to use computations to generate this set of characteristic data. For example, Bifulco recently looked at this polyketide (*OL* **2004** *6* 1025):



Bifulco noted that:

"…test calculations of ours have shown that such an intensive computational task is seldom necessary, since magnitudes of coupling constants are affected mainly by the local atomic environment and effects extending further than two atoms away from the nuclei involved in the coupling are usually not relevant…"

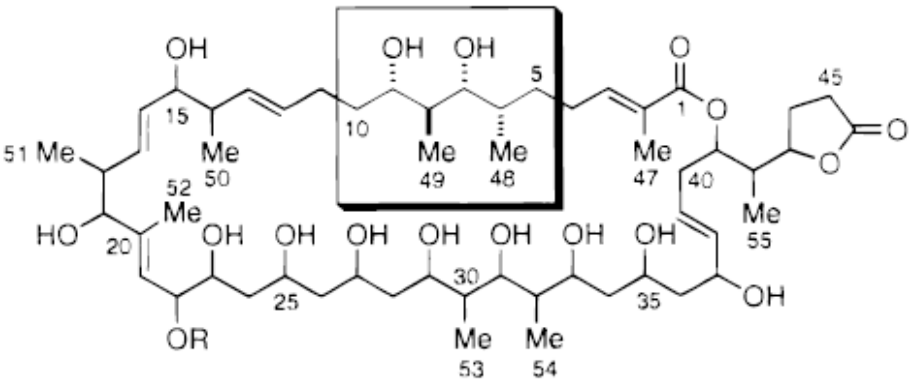
Therefore, they took two carbon fragments from the molecule and performed constrained optimizations for all of the staggered rotamers at mPW1PW91/6-31g(d). Then, they took these geometries and obtained GIAO predictions of the coupling constants with a larger 6-31g(d,p) basis set. Finally, they compared the expected and observed coupling constants by mean absolute error (MAE):

	calcd						exptl
	erythro			threo			
	g^+	anti	g^-	g^+	anti	g^-	
	C32–C33						
$^3J_{H32-H33}$	2.4	9.6	2.9	0.7	9.1	4.5	2.6
$^2J_{H32-C33}$	−4.7	−3.5	0.9	0.4	−3.4	−4.2	−5.4
$^3J_{H32-C34}$	4.4	1.5	1.1	2.9	1.4	5.1	4.8
$^3J_{H33-C31}$	1.9	1.7	6.1	3.7	0.8	6.1	0.5
$^3J_{H33-Me32}$	4.8	2.2	3.6	3.7	3.1	1.9	4.4
$\Sigma\lvert J_{calc}-J_{exp}\rvert$	3.1	15.6	16.7	13.5	13.5	11.5	

Evidently, the agreement is particularly good for the *gauche*+ rotamer of the *erythro* stereoisomer. Another advantage of this approach is that one no longer needs to rely on semi-quantitative descriptors like "large" or "small."

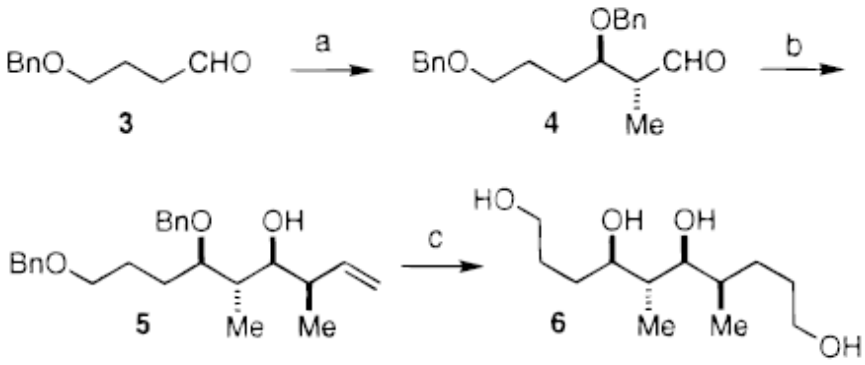
The NMR Database Method

One of the original demonstrations of this technique was done on the oasomycins by Kishi (*OL* **1999** *1* 2181):



Oasomycin A: R = H
Oasomycin B: R = α-D-mannosyl

The flat structure was known, but the relative configuration was not. In this particular study, they looked at the region highlighted above. They synthesized all the possible diastereomers of that region:



The chemical shifts of these diastereomeric fragments were compared to the chemical shifts of the natural product. For this to work, one assumes that (Kishi's words):

the structural properties of a compound are (1) inherent to the specific stereochemical arrangements of (small) substituents on its carbon backbone and (2) independent from the rest of the molecule.

This latter assumption is called the "self contained box" approach. Evidently the former assumption is quite reasonable:

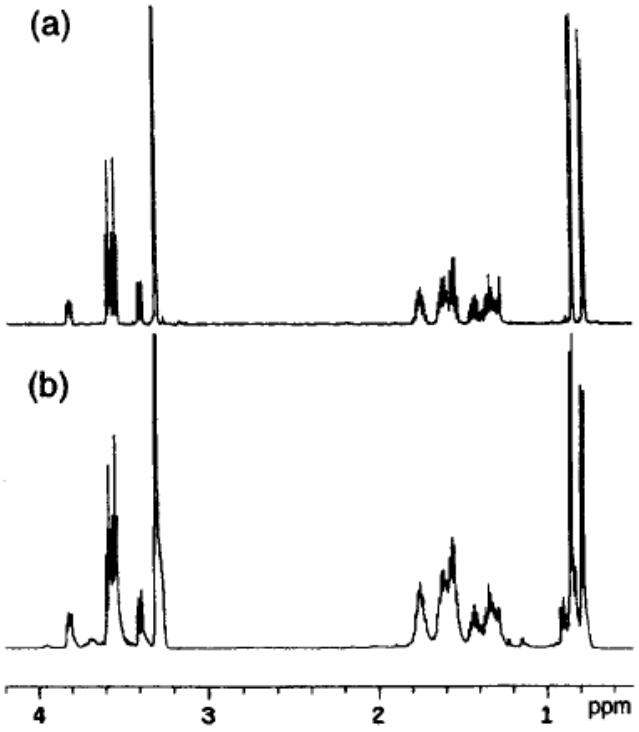


Figure 4. ^{1}H NMR spectra (500 MHz, CD_3OD). (a) Synthetic tetraol **6**. (b) Tetraol derived from oasomycin B.

One then compares the empirical data with the observed data. In this case, the carbon chemical shifts were used (but other metrics have also been used). According to Kishi's convention, the deviations are presented in bar chart form:

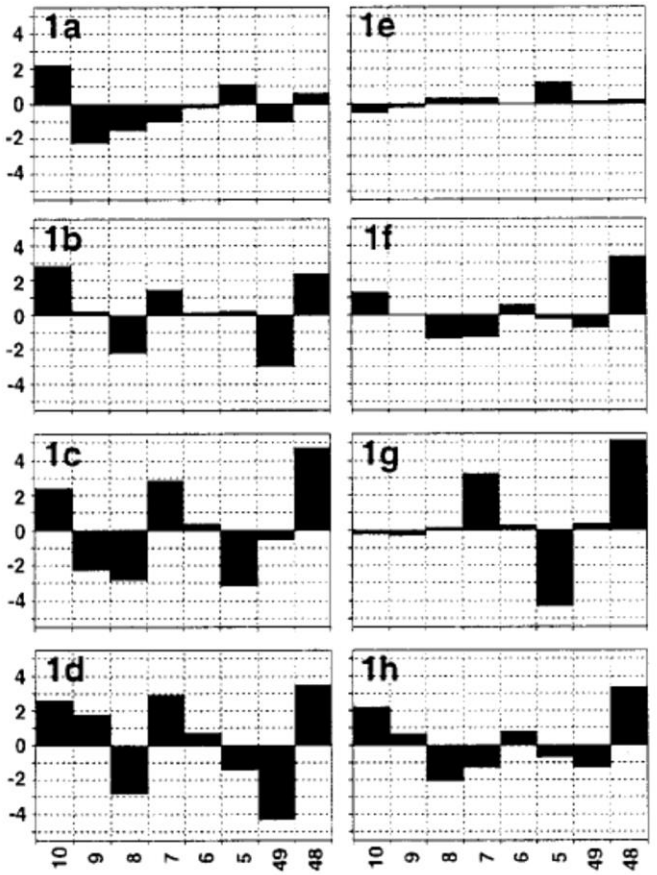


Figure 3. Difference between adjusted carbon chemical shifts of oasomycin B and those of each of **1a**−**h** (100 MHz, ppm, $(CD_3)_2$-SO).

In this case, one would conclude that the deviations in **1e** are the smallest, and therefore the relative stereochemistry of the natural product matches that of **1e**.

Chiral Derivatizing Agents

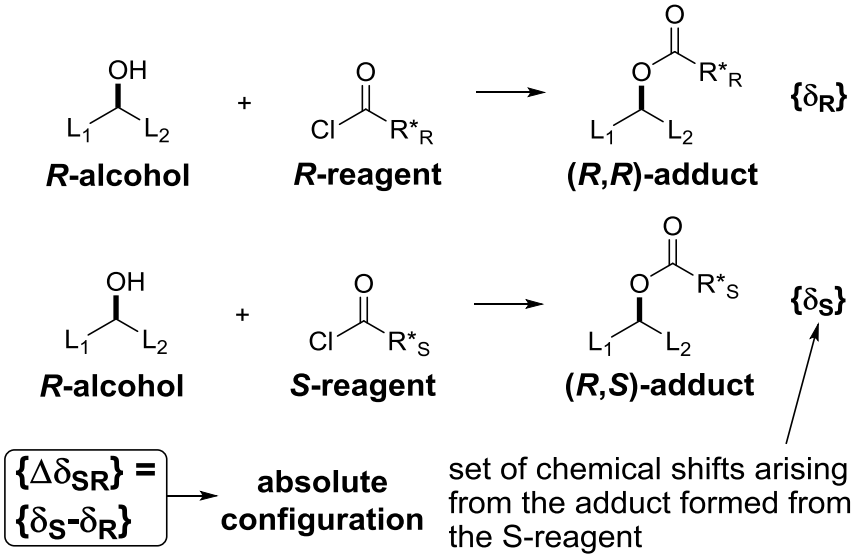
"The Assignment of Absolute Configuration by NMR." Seco, J.M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17-117. (comprehensive review on Mosher-like reagents).

Q: How can NMR help us determine the absolute configuration of a chiral alcohol or amine?

Since enantiomers have exactly the same NMR properties, it is impossible for NMR to distinguish between antipodes directly. Instead, one must form diastereomeric adducts with a **chiral derivatizing agent (CDA).** There are two strategies for this.

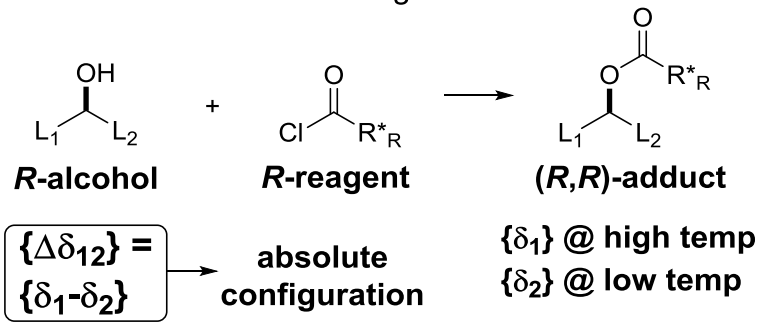
(1) Double Derivatization

The alcohol or amine is derivatized twice: once for each antipode of a chiral reagent. The NMR spectra of the resulting diastereomers are compared. The differences in the chemical shifts are translated into a stereochemical assignment of the absolute configuration with reference to some model for the conformations of the adducts and their shielding/deshielding effects.



(2) Single Derivatization

The other approach is to make *one* derivative, and then get *two* NMR spectra: either at different temperatures or in the presence or absence of some coordinating ion. Again, the differences in chemical shift are translated into a stereochemical assignment via some conformational/shielding model.



The Mosher Method for Secondary Alcohols

"Mosher Ester Analysis for the Determination of Absolute Configuration of Stereogenic (Chiral) Carbinol Carbons." Hoye, T.R. et al. *Nature Protocols*, **2007**, *2*, 2451-2458.

What it's for: A single, chiral secondary alcohol. (It can work for multiple alcohols simultaneously as well, but it's more complicated.) It does *not* work for tertiary alcohols.

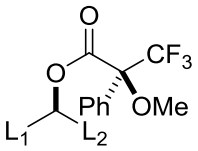
Mosher introduced this method in 1973. He used α-methoxy-α-trifluoromethyl-α-phenylacetic acid (MTPA) as the reagent:

X=OH: (*R*)-MTPA-OH
X=Cl: (*S*)-MTPA-Cl

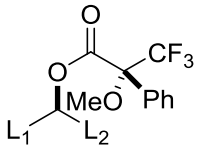
X=OH: (*S*)-MTPA-OH
X=Cl: (*R*)-MTPA-Cl

Note that due to the peculiarities of the Cahn-Ingold-Prelog priority system, changing from the carboxylic acid to acyl chloride inverts the *apparent* configuration.

Details on how to form the esters are in the Hoye paper. One uses DCC coupling with the acid, or standard acylation conditions with pyridine as base with the acid chloride. This results in two Mosher ester derivatives. For example, if we start with an *R* secondary alcohol, we get:



R alcohol, S Mosher ester

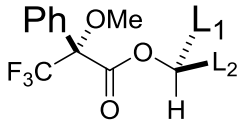


R alcohol, R Mosher ester

traditional method: look at whether the ^{19}F shift of the CF_3 group is larger in the *S* Mosher ester or the *R* Mosher ester

modified method: according to the modification proposed by Kakisawa, look at *groups* of proton chemical shifts instead

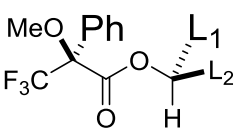
Conformational Analysis. Here is how it works in detail according to the original model proposed by Mosher. Without loss of generality, suppose L_1 is <u>bulkier</u> than L_2. We *assume* that the best conformation is where the CF_3 is in plane with the carbonyl group. We also assume the C-H bond of the carbinol is also in plane with the carbonyl group. (I'll talk about whether this is justified shortly.) Thus, we have this conformation:



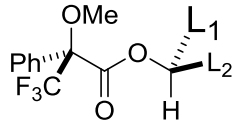
R ester

I have drawn L_1 bigger to remind you that it's the bulkier group. Everything is nice here because the bulky phenyl group and the bulky L_1 group are on opposite sides.

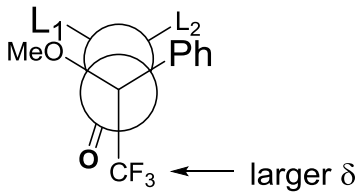
Of course, if we switch to the other antipode of the reagent, this will no longer be the case. Drawing the same conformation, we have:



S ester

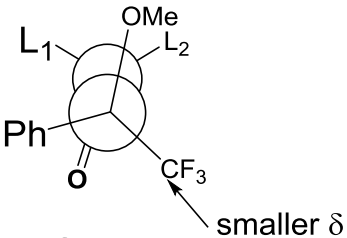


This time, the bulky groups are on the same side. Thus, it is reasonable to imagine that the CF_3 gets rotated out of plane a bit. Here are double Newman projections:



larger δ

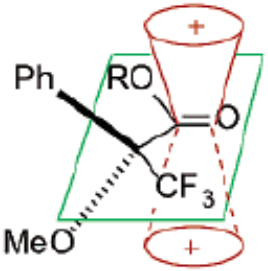
R ester



smaller δ

S ester

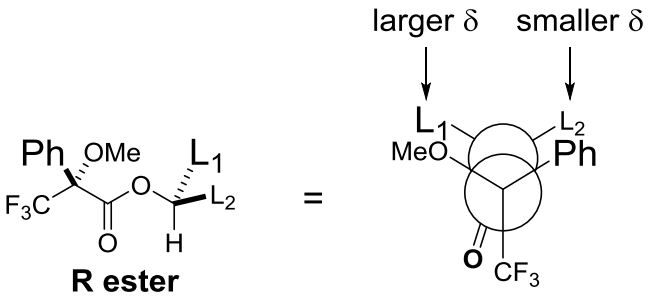
Now, remember the anisotropic behavior of carbonyl groups. When protons are in the same plane as the σ-framework of the carbonyl they get deshielded; when they are out of plane, they get deshielded:



We measure the chemical shift of the CF_3 group and compute a difference $\Delta\delta_{SR} = \delta_S - \delta_R$. In this case, we expect a negative value. From looking at what L_1 and L_2 are, we can then assign an absolute configuration to the carbinol.

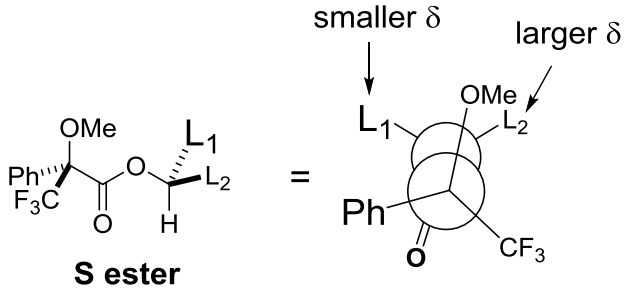
This procedure is advantageous because molecules usually have very few fluorines in them, so we don't have to worry about overlap. However, there's only one data point, so if something changes the conformational equilibria, we won't know about it. In the modified method, we collect a series of points from the proton chemical shifts.

Once again, we have to consider Mosher's canonical conformations for both esters. However, this time, we need to look at the shielding/deshielding effects on L_1 and L_2:



R ester

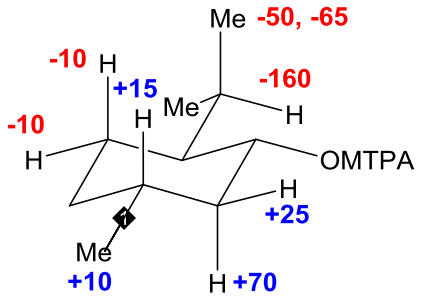
L_2 is in the shielding cone of the phenyl group, so it has a smaller chemical shift than L_2. For the *S* Mosher ester, the trends are reversed:



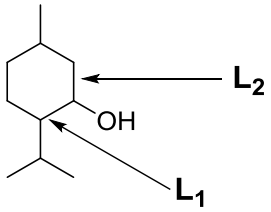
S ester

Thus, we expect $\Delta\delta_{SR}$ to be negative for L_1 and positive for L_2. So the procedure is to identify which side is L_1 by seeing that all the protons on that side have a negative $\Delta\delta_{SR}$ value. Then, one can identify the configuration by looking at the diagrams above.

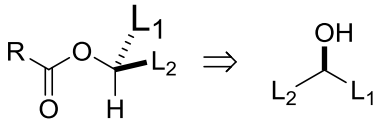
Here is a concrete example, menthol. Both the S and R Mosher derivatives were made, and the $\Delta\delta_{SR}$ values for each proton were derived. Here are the results (values are given in Hz):



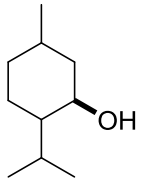
Note that protons along the center line have been ignored, including the methine at the secondary alcohol. Thus, we can identify L_1 and L_2. L_1 corresponds to the negative side, while L_2 corresponds to the positive side:



Regardless of which Mosher ester we used, we can write:



Therefore, the carbinol of menthol is up:



Then, relative configuration methods like coupling constants and nOe can be used to assign the absolute configuration of the other stereocenters.

Conformational Landscape

Mosher developed this conformational model based on the behavior of the esters of various alcohols with known conformations. Although this method worked, he realized that this is not a very sensitive probe for the actual conformational landscape and that the reality might be that there are multiple conformers that are important.

The criteria for a successful modified Mosher ester analysis are:

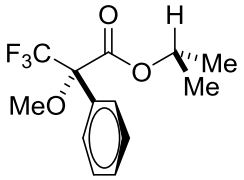
(1) Significant Δ_{SR} values (beyond the errors expected for slight changes in concentration, spectrometer frequency, etc.)

(2) Positive Δ_{SR} values on one side, negative Δ_{SR} values on the other.

(3) All nuclei have been unambiguously assigned. (Obviously, if you haven't assigned the nuclei, it's hard to say whether all the positive values are on one side or not.)

The fact that **these criteria are frequently not met**, leading to erroneous or ambiguous assignments, suggests that Mosher's model is, in fact, too simplistic. (Problems are often encountered when there are other anisotropic groups near the carbinol.) Detailed computational and spectroscopic studies now indicate that there are, in fact, three major conformers.

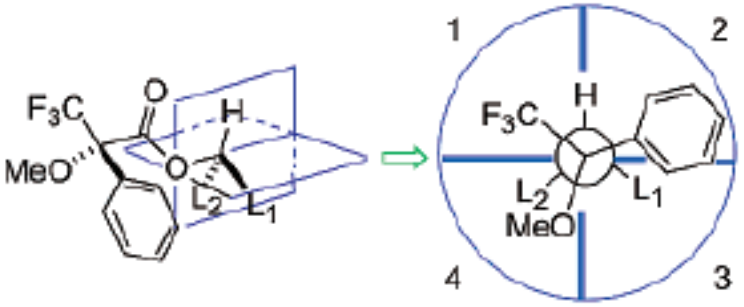
The first is called **sp1** and is the canonical Mosher conformation. Studies suggest it is near the global minimum:



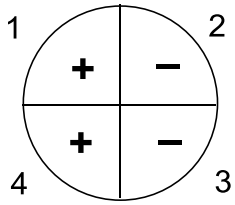
sp1 (canonical)
+0.4 kcal/mol

Choosing an enantiomer at random:

The shielding/deshielding effects area quantified by a "quadrant" model, with positive signs to indicate positive values of $\Delta\delta_{SR}$ and negative signs to indicate negative values of $\Delta\delta_{SR}$:

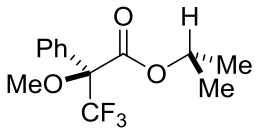


In this case, the phenyl group floats over quadrants 2 and 3, so they are shielded. Shielding means smaller chemical shifts, so those quadrants get a negative sign:

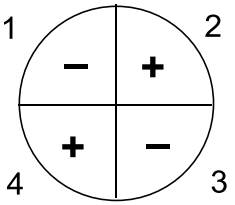


sp1 quadrant model

This is the *S* Mosher ester, so L_1 should sit in quadrant 3, while L_2 should sit in quadrant 4. The global minimum is called **ap1**:

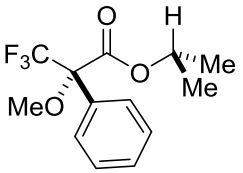


Its quadrants are:



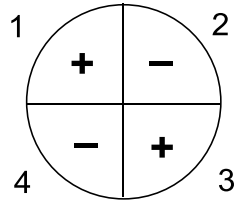
ap1 quadrant model

If L_1 and L_2 remain where they are, the presence of these two conformers will not affect the analysis. However, there is a third conformation that is 0.6 kcal/mol above the global minimum (**ap1**) called the **sp2** conformer:



sp2 (canonical)
+0.6 kcal/mol

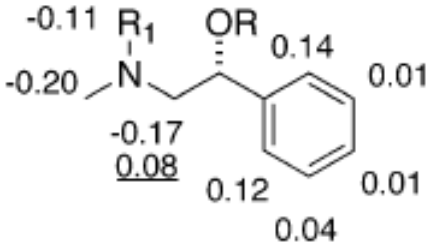
Note that one of the carbinol groups will now be placed in the *deshielding* plane of the benzene ring! (The only difference between this and the canonical conformation is a rotation of the phenyl ring.) The corresponding quadrant model is:



sp2 quadrant model

Now, we have a problem. If this conformer gets substantially populated, then it will work against the canonical trends in $\Delta\delta_{SR}$! This is the origin of "problematic" Mosher ester assignments. Additionally, substrate conformational biases can cause some parts of the molecule to stray out of quadrants 3 or 4 or cross between them, leading to apparently anomalous $\Delta\delta_{SR}$ values.

Here is an example of an "irregular" sign distribution:

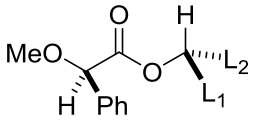


Methoxyphenylacetic Acid/Mandelate Derivatives

According to Riguera and co-workers, who are some of the world's experts in this area:

Overall, Mosher's reagent...is <u>not</u> recommended for the determination of absolute configuation of secondary alcohols by NMR. Such assignments should be made using other, more reliable reagents.

What are these improved reagents? In 1973, Mosher also described the use of mandelic acid derivatives:

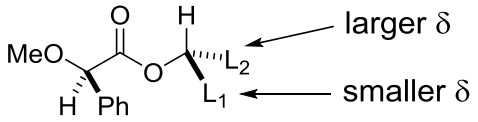


***R*-mandelate**

This was not used for some time because of problems with racemization, but Trost has diclosed reaction conditions that do not suffer from this (*JOC* **1981** *51* 2370; *TL* **1981** *22* 4929; *JACS* **1980** *102* 7595). These are advantageous for several reasons:

- mandeleic acid derivatives are much cheaper to prepare
- the conformational distribution is more favorable
- the magnitude of $\Delta\delta_{RS}$ is larger (for some reason, the convention is reversed for these "MPA" esters)
- much more reliable results overall

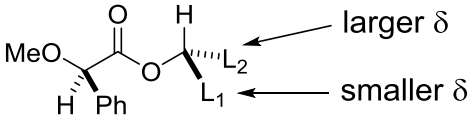
The conformational analysis is simple. The preferred conformation places the methoxy group in plane with the ester, taking the place of the CF_3 group in the MTPA esters:



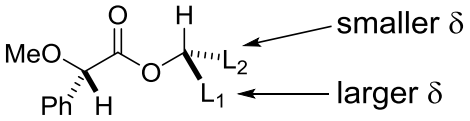
***R*-mandelate**

Once again, the phenyl group shields L_1.

Thus, we have:



***R*-mandelate**

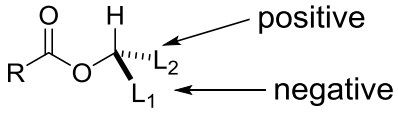


***S*-mandelate**

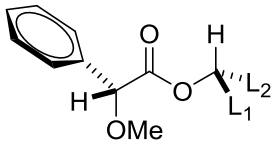
This means that:

$\Delta\delta_{RS}(L_1) < 0$ and

$\Delta\delta_{RS}(L_2) > 0$.



Conformational studies have also been done on MPA esters. They reveal that the **sp** conformation shown above is, in fact, the global energy minimum by about 1 kcal/mol. The next highest energy conformation is the **ap** conformer:



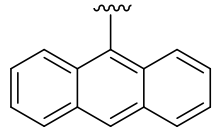
The orientation of the phenyl group is such that it is not particularly well suited to transmit any shielding or deshielding effects to L_1 or L_2. Therefore, we don't have to worry about reversing the sign of $\Delta\delta_{RS}$ if the conformational equilibrium is somehow disturbed from its canonical distribution. This is why MPA esters are much more reliable. **If you have to determine the absolute configuration of a secondary alcohol, make the MPA ester and not the MTPA ester!**

Improved Procedures

The mandelates are great, but researchers have used their knowledge of these conformational equilibria to develop better agents. For example, if the phenyl group in the mandelate is replaced by an 9-anthryl group, one obtains an "9-arylmethoxy-acetic acid" (9-AMA) derivative. The analysis is the same as for the MPA derivatives shown above.

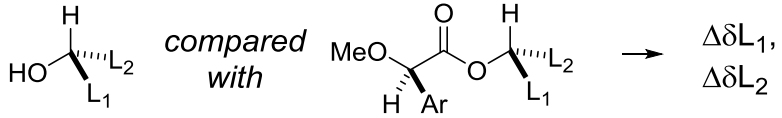
Advantages:

Ar =



- more conformationally rigid
- stronger anisotropic effects
- give larger $\Delta\delta_{RS}$ values, and therefore more reliable answers

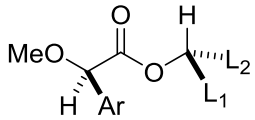
These benefits allow one to characterize alcohols by making *only one derivative*! The idea is to compare the NMR spectra of the free alcohol and one of the derivatives. For example:



free alcohol ***R*-9-AMA ester**

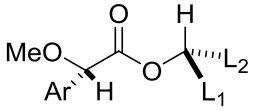
$\Delta\delta L_1$ is defined as the chemical shift of L_1 in the derivative minus the shift of L_1 in the free alcohol. The idea is that when the free alcohol is converted to its 9-AMA ester, only L_1 will change a lot. L_2 is pretty far away from the aryl group, so its chemical shifts don't change a lot. Thus, we have:

***R*-9-AMA ester**



$\Delta\delta L_1 >> \Delta\delta L_2$

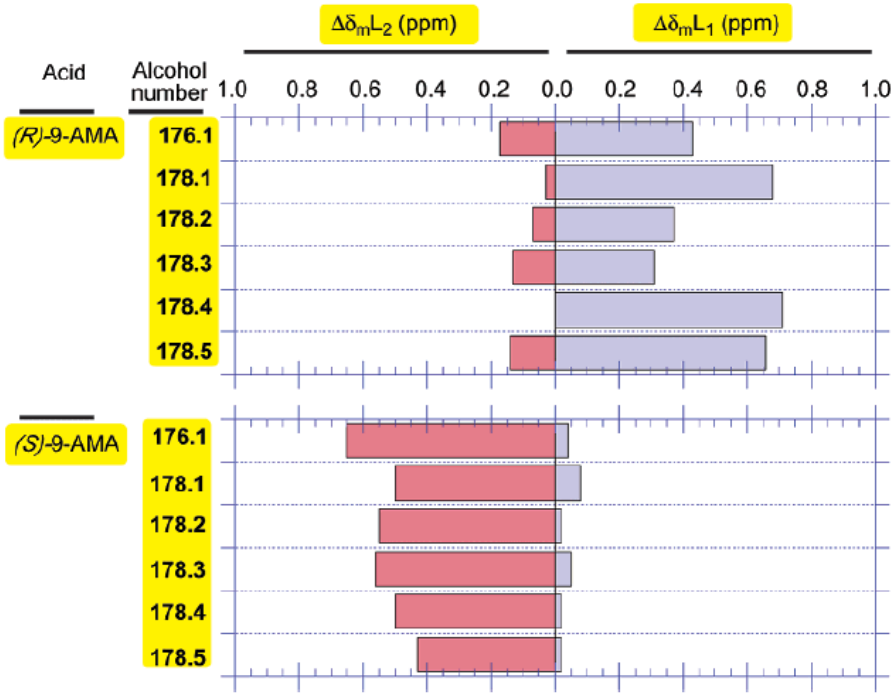
***S*-9-AMA ester**



$\Delta\delta L_2 >> \Delta\delta L_1$

Experiments on a variety of alcohols with known configuration have verified that this procedure works (Riguera et al. *Tetrahedron* **1999** *55* 569). In my opinion, this is a much more straightforward way to do things (and saves time). However, it has yet to see wide adoption. Note that MTPA and MPA esters don't give large enough chemical shift changes to make this single derivatization procedure work.

Here are some mean $\Delta\delta L_1$ vs. $\Delta\delta L_2$ values for some different alcohols:



The fact that the values are all larger on one side, and that the bottom numbers are essentially a mirror image of the ones on top is a nice verification of the method.

For secondary *amines*, there is no obvious advantage to using MPA over MTPA derivatives. The magnitudes of the shift differences are similar, despite the better conformer distribution in the former. This seems to be due to a better disposition of the phenyl ring in the MTPA amides, which allows them to shield groups more effectively.

Single-derivitization procedures involving the complexation of barium(III) salts to MPA esters are also known. Chelation shifts the conformational distribution to favor the **sp** over the **ap** forms:

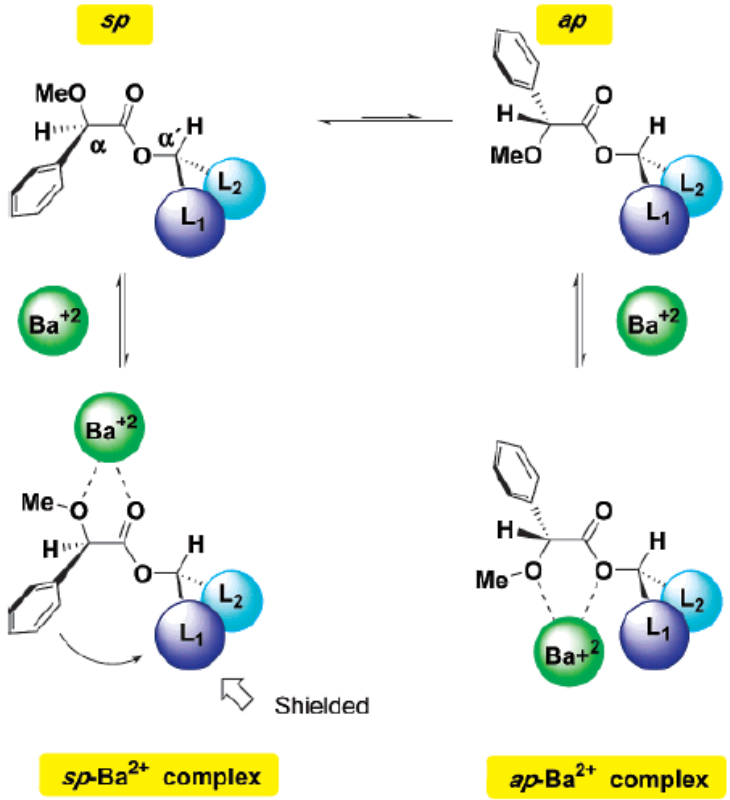
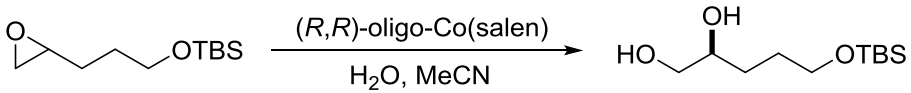


Figure 193. Formation of Ba^{2+} complexes of the (*R*)-MPA ester of a chiral secondary alcohol. The population of the sp form increases by formation of the most stable sp-Ba^{2+} complex, so the signals for substituent L_1 shift to higher field.

Q: If MTPA, MPA, 9-AMA, etc. derivatives can be used to determine absolute configuration, can they be used to determine enantiomeric excess?

Yes. This has been extensively reviewed (Parker, D. *Chem. Rev.* **1991** *91* 1441; Rothchild, R. *Enantiomer* **2000** *5* 457; Finn, M.G. *Chirality* **2002** *14* 534) I won't say much about it here, except that one can have a problem with kinetic resolution. For example, the reaction of the *R*-alcohol with the *R*-reagent may be faster than the reaction of the *S*-alcohol with the *R*-reagent.

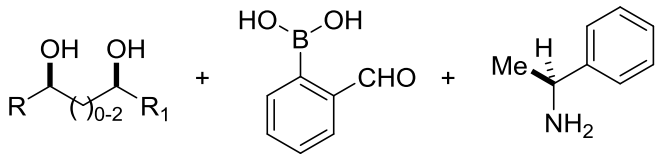
This sort of problem does come up in real life. For example, I did this reaction during my Ph.D.:



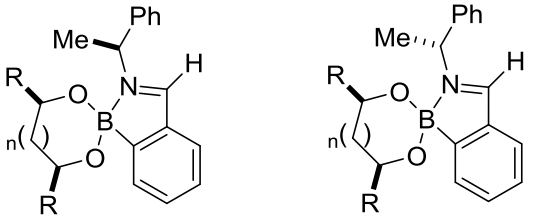
This is a Jacobsen hydrolytic kinetic resolution (you add just less than half an equivalent of water; I got a 46% yield). This reaction works fantastically well and can be run in virtually neat epoxide. The catalyst is very large, but also very efficient. I used a catalyst loading of 0.02 mol%. Unfortunately, this also means the epoxide must be very pure (requires distillation). I did not want to bother fiddling with a chiral GC (I would have had to go to another group), so I chose to follow this protocol by James and co-workers:

"Simple Protocols for NMR Analysis of the Enantiomeric Purity of Diols." James, T.D. et al. *Nature Protocols* **2008** *3* 215-219."

This involves a three component coupling:

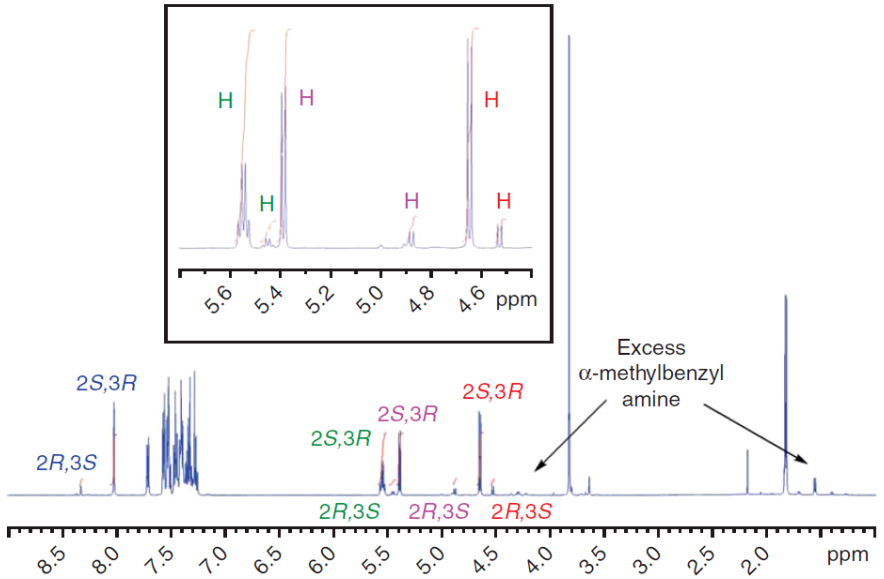


(Both of the reagents are commercially available. Optically pure α-methylbenzylamine is somewhat expensive, but we don't need much.) Coupling to a racemic diol results in a pair of diastereomeric iminoboronate esters:



They have pretty different chemical shifts, and taking an NMR spectrum with accurate integrals allows you to determine the ee of the diol in your reaction. (I got around 99% ee.)

The reactions occur at room temperature and are done in about ten minutes. They are done directly in $CDCl_3$, so no extraction is required. (To be absolutely sure of the assignments, it is probably wise to run two reactions in parallel: one with the *R* amine and one with the *S* amine.) Here is a 9:1 mixture:



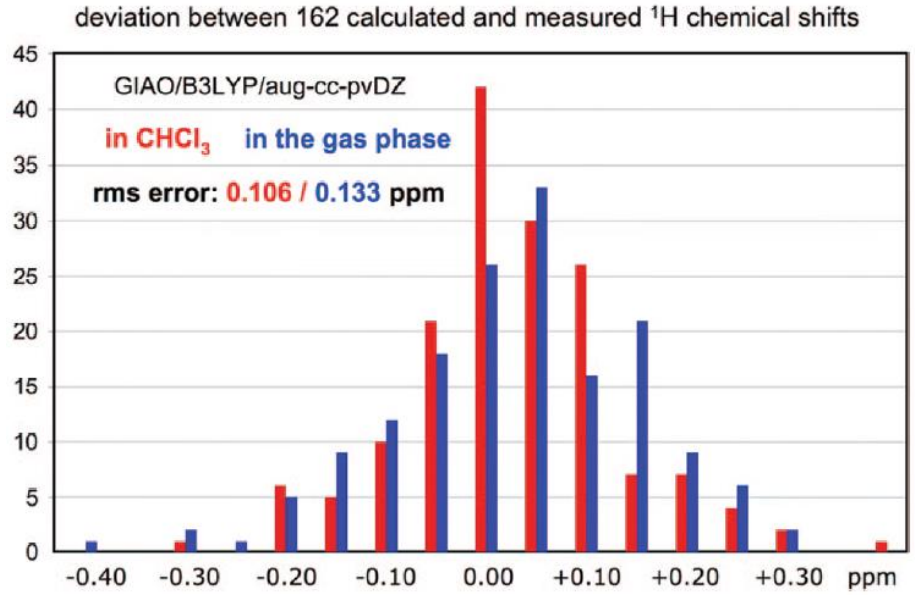
Predicting Chemical Shifts and Coupling Constants

As I mentioned already, you have two choices:

(1) Use an empirical, fragment-based approach, like that implemented in ChemDraw. It's fast, but may not be accurate for "weird" structures. Most of these programs have a fairly rudimentary understanding (if any at all) of stereochemistry. In complex systems, you're on your own.

(2) Use *ab initio* or density functional theory methods to predict shifts. It's more accurate, but it takes longer and you have to know what you're doing.

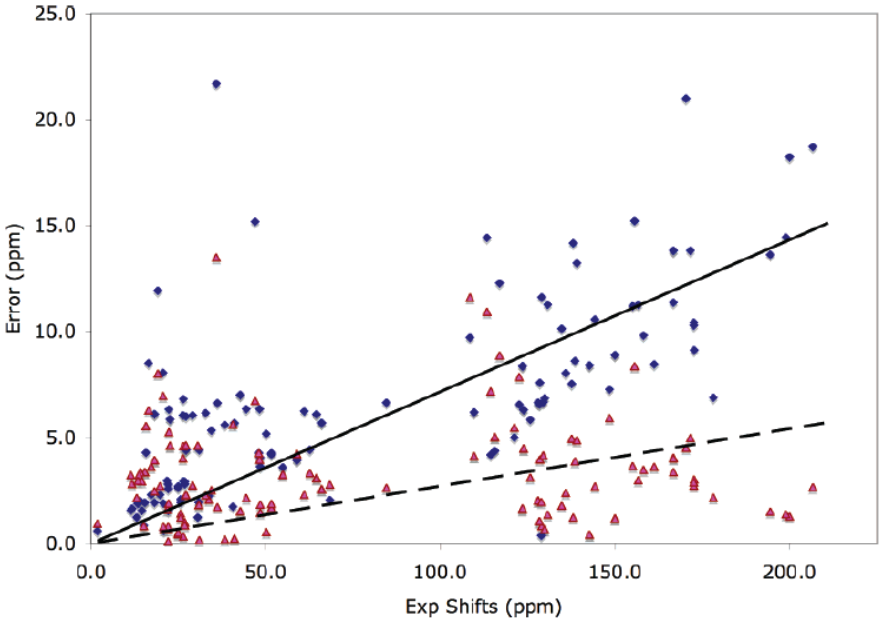
I'll focus on the latter here. There are a lot of different methods to do this, but the most popular method is called "gauge-including atomic orbitals" (GIAO). We won't worry about how it works.



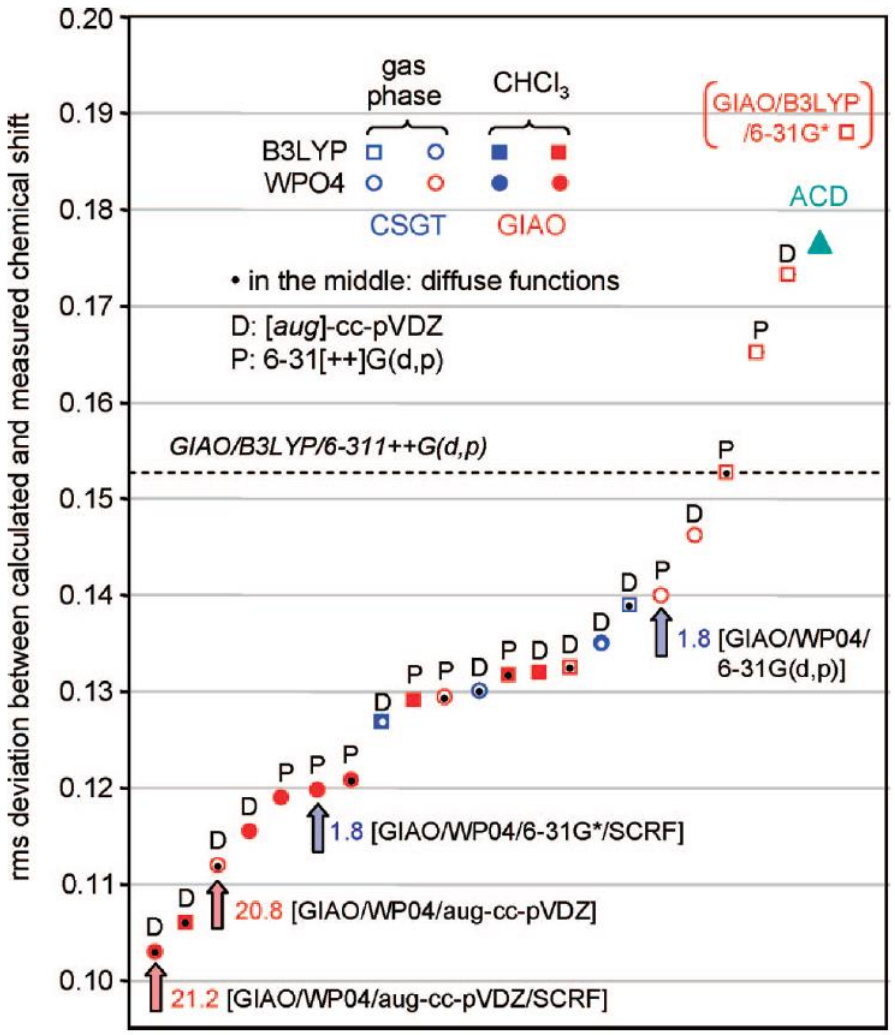
The above histogram is from Rablen and co-workers (*JOC* **2009** *74* 4017). He is using aug-cc-pVDZ, which is similar to 6-31+g(d,p). As you can see the errors are pretty small. The fact that DFT can predict something as complicated as NMR chemical shifts very well gives me real confidence that computations actually mean something. There are slightly smaller errors when solvent is included in the calculation, but it doesn't seem to be essential. (Adding solvation adds about 10% to the computational time.)

Cramer and co-workers have developed the "WP04" GGA DFT functional specifically to predict proton and carbon chemical shifts (*JCTC* **2006** *2* 1085-1092). It seems to do a bit better than B3LYP, PBE1, and mPW1PW91, but applying linear corrections to the other functionals brings their performance into line with WP04. So it's a modest improvement.

This is a benchmark for carbon shifts (blue diamonds=B3LYP; magenta triangles=WP04):



Rablen has looked at the accuracy of a variety of methods and basis sets and summarizes them on this convenient chart:

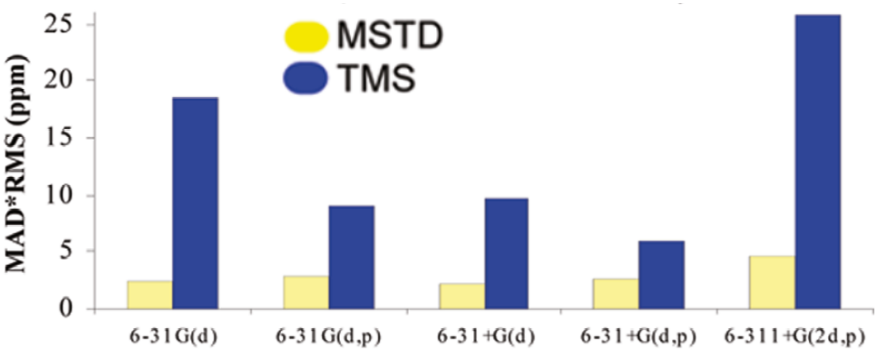


- double-zeta basis sets are good enough
- implicit solvent modeling is helpful
- GIAO is better than CSGT (another method for computing NMR shifts), but both do well with double-zeta bases
- ACD is much better than ChemDraw (RMSE 0.185 vs 0.329) but linearly scaled calculations with WP04 or B3LYP with the cc-pVDZ or 6-31G** basis sets are inexpensive and better (RMSE 0.12)
- scaling factors are detailed in the reference

"A Multi-Standard Approach for GIAO ^{13}C NMR Calculations." Sarotti, A.M.; Pellegrinet, S.C. *JOC* **2009** *74* 7254-7260.

As with NMR spectra in real life, predicted NMR shifts have to be referenced. The obvious choice is to predict the shift of tetramethylsilane (TMS) and use that as the reference. This turns out not to be entirely ideal. **A better approach is to use methanol as the reference standard for sp^3 carbons and benzene as the standard for sp^2 carbons.** Since we are dealing with *in silico* compounds, we don't need to worry about overlapping signals, reactivity, etc.

The errors are clearly higher with TMS than this multi-standard (MSTD) one, regardless of basis set. Here, they used the mPW1PW91 DFT and conclude that the results with the 6-31g(d) basis set are acceptable:

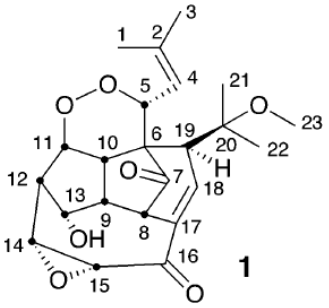


The bottom line: B3LYP/6-31g(d,p) is probably good enough. You will get reasonably accurate chemical shifts.

Coupling constants take a lot longer to calculate than chemical shifts. They are also more prone to errors.

Hexacyclinol

As an example, we turn our attention to the case of hexacyclinol, a compound that generated some controversy some years ago. The story begins in 2002, when Grafe and co-workers isolated a molecule from *Panus rudis* strain HKI 0254 (whatever that is). This original isolation paper proposed this structure (*J. Antibiot.* **2002**, *55*, 814):



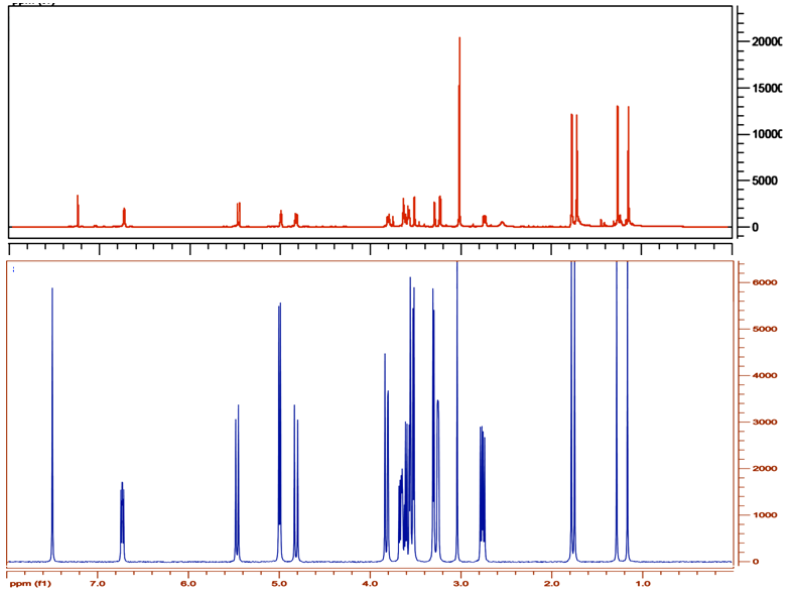
1

This drew little attention until 2006, when La Clair published a total synthesis of this molecule (*ACIE* **2006** *45* 2769). It had an eyebrow-raising Mitsunobu inversion of a tertiary alcohol and a bizarre singlet oxygen cycloaddition to install an endo-peroxide, which is itself quite unusual for a natural product. But more odd was this statement in footnote #22:

> Note added in proof: The ^{1}H NMR spectra for this Communication were determined by contract services. The spectra provided in the Supporting Information were collected by N. Voss (Berlin, Germany). The operator added the peak for $CDCl_3$ to the spectrum of synthetic hexacyclinol (1), however, this was done incorrectly at δ 7.5 ppm and against the request of the author. Additionally, one spectrum was duplicated and a copy of the spectra for natural 5-*epi*-hexacyclinol was not provided.

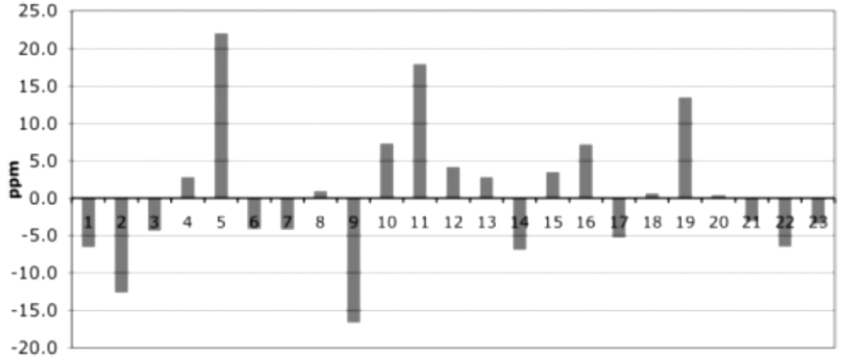
In total synthesis, it is common to provide a comparison between the spectra of the natural and synthetic materials. According to the La Clair report, a whopping 3.6 g was prepared from 1 mol of starting material in 37 linear steps. If that's the case, then the spectra should be gorgeous.

Here are the spectra of natural (top) and synthetic (bottom) hexacyclinol (I thank Professor William F. Reynolds for providing me with some of the spectra that follow and in-depth analysis):

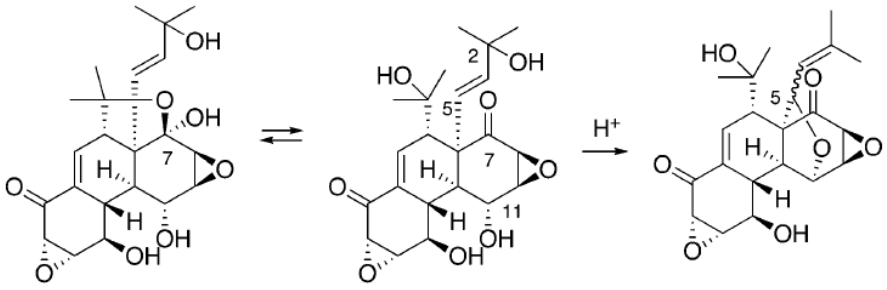


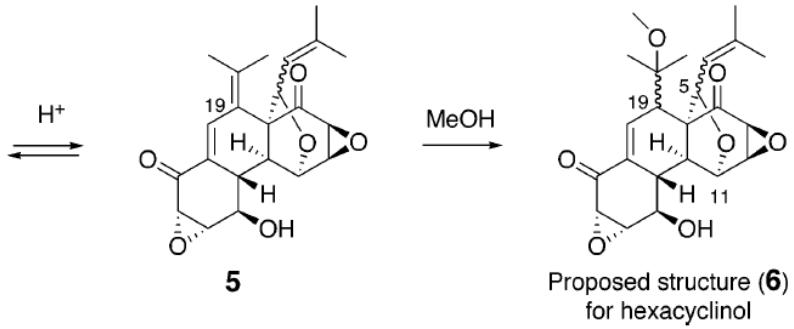
Note the following: (1) residual C**H**Cl_3 is in an unusual place; (2) the spectrum is whisker-clean after a 37-step synthesis; (3) there are no ^{13}C-satellites; (4) the linewidths are all very similar, lacking the expected broadenings due to unresolved couplings or differential T_2 relaxation; and (5) some of the simpler multiplets look like the tabulated data, rather than the actual spectrum(!). If you think this is suspicious, then you are evidently not alone. In 2006, Rychnovsky computed the carbon NMR spectrum and compared it to the actual data (*OL* **2006** *8* 2896). The results were striking!

The computed shifts of the Grafe structure and the experimental structure did not agree very well (MAE 6.8 ppm):

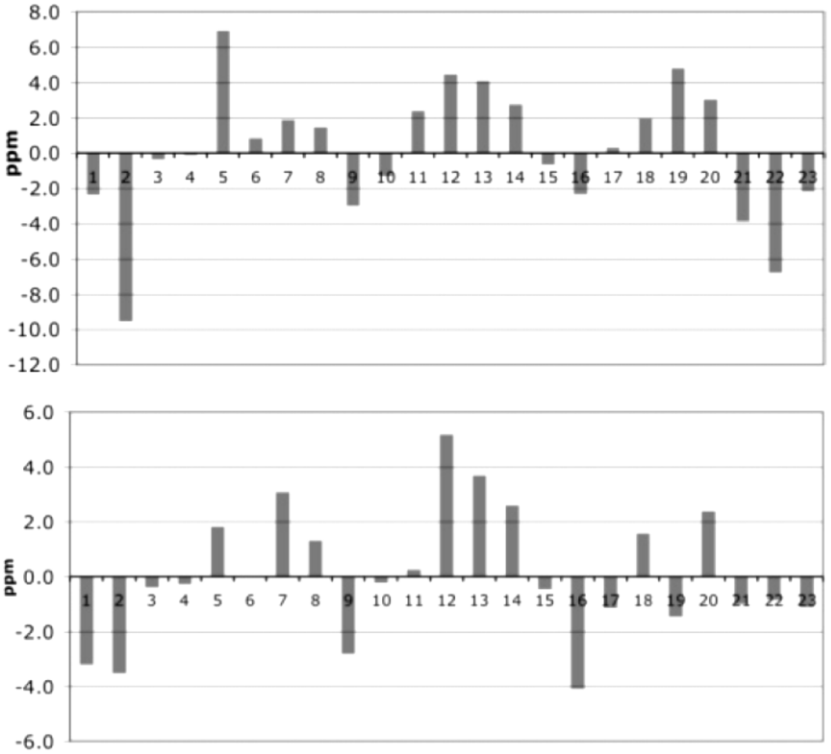


If that isn't hexacyclinol, then what was isolated/synthesized? Rychnovsky proposed that the actual structure is an isolation byproduct of an acidic workup in methanol of another known compound, panepophenanthrin, from the same source:

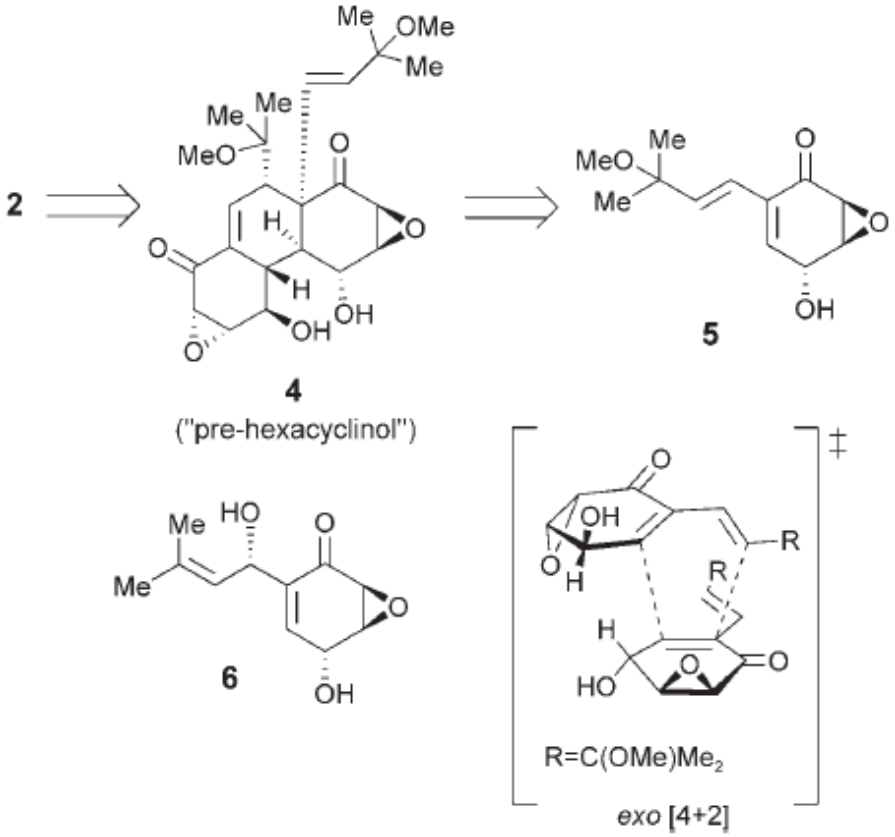




Do these structures agree better? Initially, they do not. There are some clear outliers, which Rychnovsky identifies as probably being due to misassignment of peaks that are close in the HMQC spectrum. Once this is corrected for, the results make much more sense. Hexacyclinol apparently has two low energy conformations (MM energies only, so the real ranking of these conformations is unknown):

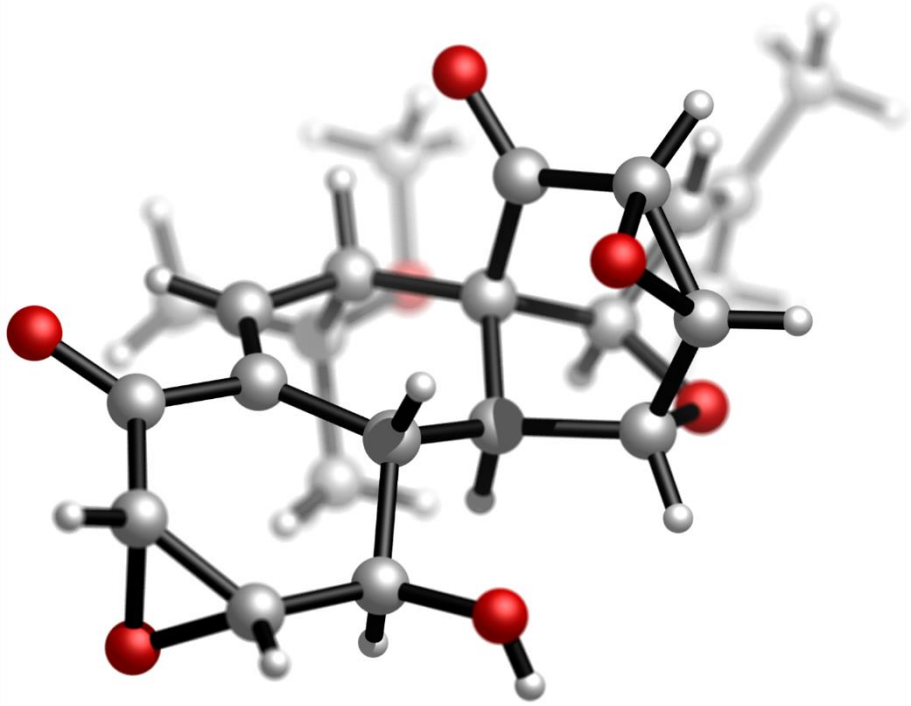


The average MAE is now only 1.8 ppm, which is much better. Now, the cynic would say that computations, after all, are all well and good, but that a real resolution would either come from (a) a high quality spectrum from the 3.6 g of synthesized material or (b) synthesis that shows that this isolation byproduct cascade does, in fact, occur. As it turns out, the latter was disclosed by Porco and co-workers later in 2006 (*ACIE* **2006** *45* 5790). Their synthesis is remarkably simple:



(Compound **2** is the proposed structure of hexacyclinol shown on the previous slide.) The proton and carbon NMRs of the product of this synthesis match very well, as do the optical rotations. An X-ray structure was also obtained (CSD: DUNFEY).

In fairness to Dr. La Clair, his reply is that it's possible that, unlikely as it sounds, the two molecules simply have the same NMR spectrum. This question was examined by Bagno



(*OL* **2009** *11* 1409) at B97-2/cc-pVTZ. They concluded that in this particular case, the spectra should be quite different. An orthogonal analysis by Williams (*J. Nat. Prod.* **2008** *71* 581) using computer aided structural elucidation reaches the same conclusion. They developed a computer algorithm that would automatically analyze the spectral data and come up with possible candidates that fit the data. Reacquisition of the spectra at 900 MHz confirmed Rychnovsky's suspicion that there were some misassignments made in the initial structural determination.

Higher quality computational data from Bagno for the chemical shifts:

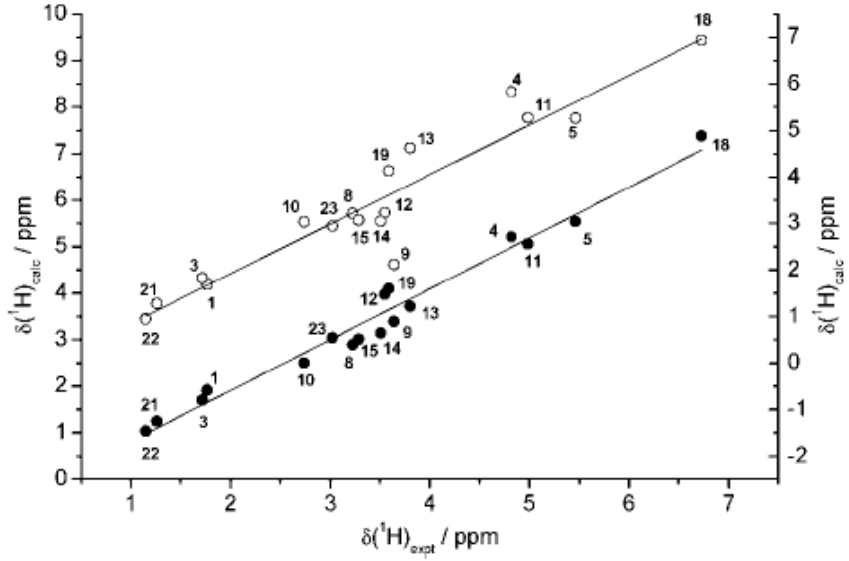


Figure 1. Correlation between calculated and experimental ^{1}H chemical shifts. ○, right axis (shifted by 2.5 ppm for clarity: **1**. ●, left axis: average of structures **2a** and **2b**. Solid lines are the best fit to $\delta_{calc} = a + b\,\delta_{exp}$. **1**: $a = -0.22$ ppm, $b = 1.066$, $r^2 = 0.89$; **2**: $a = -0.28$ ppm, $b = 1.093$, $r^2 = 0.97$. See Scheme 1 for label assignments.

Here, open circles mean the original structure (**1** in the notation of this paper) shaded circles mean the averaged of the revised structure's conformers (**2a** and **2b**, ΔE=0.6 kcal/mol). Note that the r^2 value is high for both, but much higher for the latter two structures. The couplings, however, match much better for the revised structure:

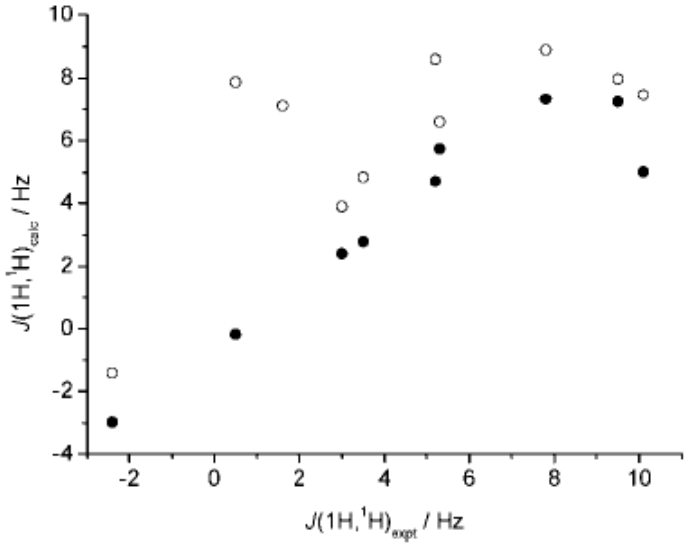
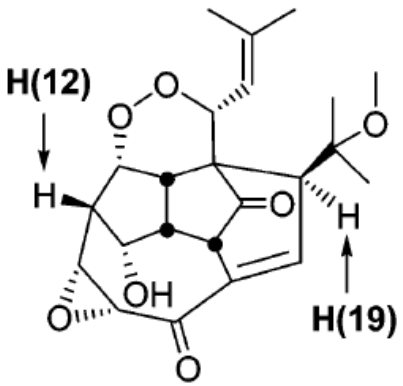
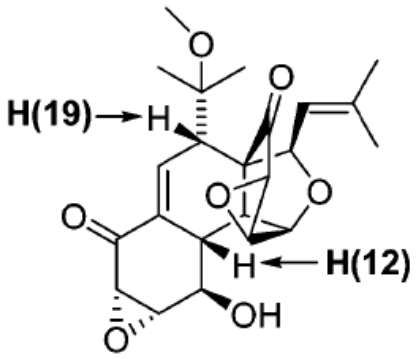


Figure 2. Correlation between calculated and experimental ^{1}H,^{1}H coupling constants. ○: **1**; ●: average of structures **2a** and **2b**. The negative sign of the experimental ^4J(H18,H12) is deduced from the calculated results.

In retrospect, a simple consideration of the shifts and couplings should have raised the alarm: H-12 (3.62 ppm, s); H-19 (3.59 ppm, d, J=5.3 Hz):



12 - original structure



13 - corrected structure