# Application of the BIRD Sandwich for the Rapid and Accurate Determination of $^1$H−$^1$H NMR Coupling Constants in Higher Order Spin Systems

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A method is presented that allows for the convenient and reliable determination of $^1$H−$^1$H NMR coupling constants in higher order or symmetrically coupled spin systems. The method can be applied on any programmable FT−NMR spectrometer and is demonstrated here on micromole quantities of sample in a standard 5-mm NMR tube.

$^1$H−$^1$H NMR coupling constants are used extensively for establishing configurations and conformations of natural products. Unfortunately, these values are not readily attainable for resonances that are isochronous, or nearly isochronous, and scalar-coupled to each other, due to the resulting complex higher-order coupling patterns. The classical approach for determining coupling constants under these circumstances is to observe the $^{13}$C satellite peaks in a normal $^1$H NMR spectrum.[1] At natural isotopic abundance, the satellite peaks contain coupling information of protons attached to $^{13}$C as they are coupled to other protons not attached to $^{13}$C (e.g., $^{12}$C atoms). Therefore, as a simple example, an $A_2$ system for the $^1$H's on $^{12}$C will appear as an AMX system in the satellites, with $^{13}$C as the X component. However, this procedure has a severe dynamic-range problem because only 1.1% ($^{13}$C natural abundance) of the $^1$H's in the molecule are being observed. The large dynamic range precludes the use of a high receiver gain, and, as a result, only concentrated samples can be analyzed without extremely long acquisition times to obtain an acceptable signal-to-noise ratio (S/N) for the determination. Additionally, the coupling patterns in the satellite peaks can still be quite complex due to other couplings. These factors generally make the method impractical for natural product chemists because of the small amounts of sample that are typically isolated for characterization.

As alternatives to the classical method, other approaches based on HMQC- or HSQC-type experiments have been proposed for analysis of these complicated spin systems. Although these experiments are valuable for analyzing systems that are simply isochronous or symmetrical, they are not capable of deconvoluting multiplets that contain more complex splittings.[2] One other interesting method, termed SAPHIR HSQC−TOCSY, was proposed, which requires the acquisition of a full 2D HSQC−TOCSY experiment and employs a complicated selective composite pulse decoupling scheme along with band-selective $^{13}$C excitation.[3] Although useful when there are many overlapped olefinic proton signals present in a spectrum, this experiment is not practical in terms of S/N and lacks simplicity for routine use by non-NMR specialists. In this note, we propose a simple method to measure $^1$H−$^1$H couplings that circumvents these problems.

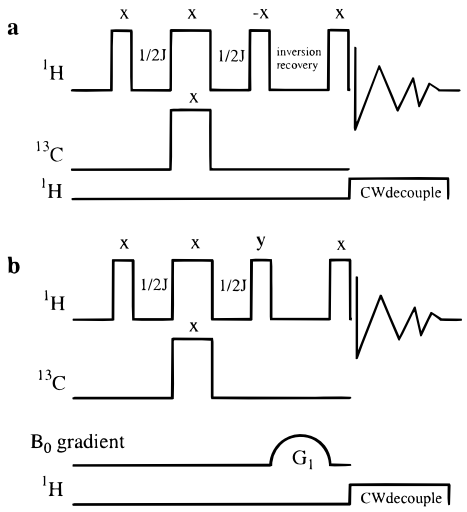


**Figure 1.** Pulse programs for the BIRD experiment: (a) standard BIRD pulse sequence with the inclusion of selective homonuclear decoupling, and (b) heteronuclear *z,z* filter with selective homonuclear decoupling.

The method that we propose utilizes a simple bilinear rotation decoupling (BIRD) pulse sandwich (Figure 1).[4] This commonly used pulse-sequence module selects magnetization from only those $^1$H's that are bound to $^{13}$C nuclei. By selecting for only these protons, the dynamic range problem is removed, allowing a much higher receiver gain to be used, and, as a result, a much higher S/N can be obtained in a shorter amount of time. The experiment also incorporates selective homonuclear $^1$H decoupling to further simplify spectral interpretation. The decoupling can also increase the S/N because signals that would normally be observed as complicated multiplets can usually be observed as a simple doublet of doublets as long as there is no extensive overlap in the region of interest.

We first demonstrate the utility of this approach by determining the *cis* and *trans* coupling constants for two fatty acids. Establishing the cis versus trans configuration in fatty acids has remained a formidable problem, and several approaches have been utilized with varying degrees of success. Among these is the use of $^{13}$C chemical shift,[5] and the observation of an absorption at 930−990 and 1300 cm$^{-1}$ in IR for *trans* fatty acids and at 715−725 cm$^{-1}$ for *cis* fatty olefins.[6] Other methods include derivatization followed by GC−MS analysis,[7] and finally GC analysis using specialized columns.[8] All of these methods can be useful but suffer from some serious shortcomings. The first

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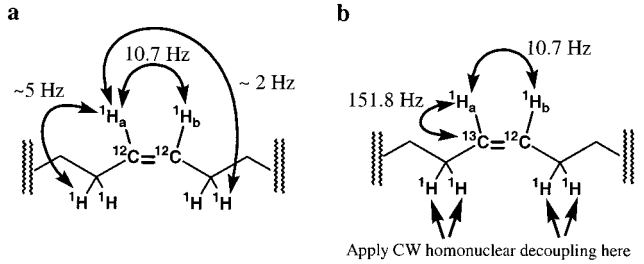


**Figure 2.** Diagrams showing (a) normal couplings in an olefinic system, and (b) couplings observed with the BIRD experiment presented in this note.

suffers from the fact that the chemical shift difference of *cis* vs *trans* fatty acids is often quite small, less than 1 ppm in many cases.[5] Analysis of a chemical shift difference this small without having authentic standards is frequently equivocal. The use of IR suffers from the fact that the absorption for *trans* or *cis* double bonds is generally quite small in magnitude and resides in the "fingerprint region" of the spectrum, which is often obscured by other absorption bands and could easily lead to incorrect assignments. Moreover, if both *cis* and *trans* double bonds are present in the fatty acid, then this method proves useless. Derivatization followed by GC−MS analysis can be very useful to the trained practitioner but requires careful interpretation on unusually unsaturated systems to prevent misassignment of the double-bond configuration. Finally, GC analysis on specialized columns can be very reliable but requires the availability of authentic standards.

The coupling constants of olefinic $^1$H's in fatty acids or similar systems cannot be accurately measured by direct analysis of the standard 1D $^1$H NMR spectrum. This is because the protons are almost isochronous and are coupled to each other, which leads to coupling patterns of higher order. However, the actual coupling constant can be extracted with our technique. This new experiment isolates the individual olefinic protons that are coupled to the attached $^{13}$C atoms and that are also coupled to the vicinal olefinic proton as well as to the two vicinal methylene protons (Figure 2). A small allylic coupling may also be observed, which unfortunately can further complicate the observed coupling pattern. The coupling pattern observed for oleic acid is a higher order ddtt with coupling constants of 151.8, 10.7, ~4.8, and <2 Hz, while for elaidic acid the same pattern is observed with values of 149.5, 15.1, ~5, and <2 Hz. To simplify these patterns and facilitate spectral interpretation, the vicinal methylene protons can be simultaneously decoupled during acquisition. This will reduce the coupling pattern to a simple double doublet with coupling constants of 151.8 and 10.7 Hz for oleic acid and 149.5 and 15.1 Hz for elaidic acid (Figure 3b,e). It should be noted that by decoupling the vicinal methylene protons, the allylic coupling is also removed from the spectrum, inasmuch as these two sets of protons possess the same chemical shift. The apparent ddd pattern shown in Figure 3b,e arises from the small chemical-shift difference of the two olefinic protons ($H_a$ and $H_b$). This small difference in

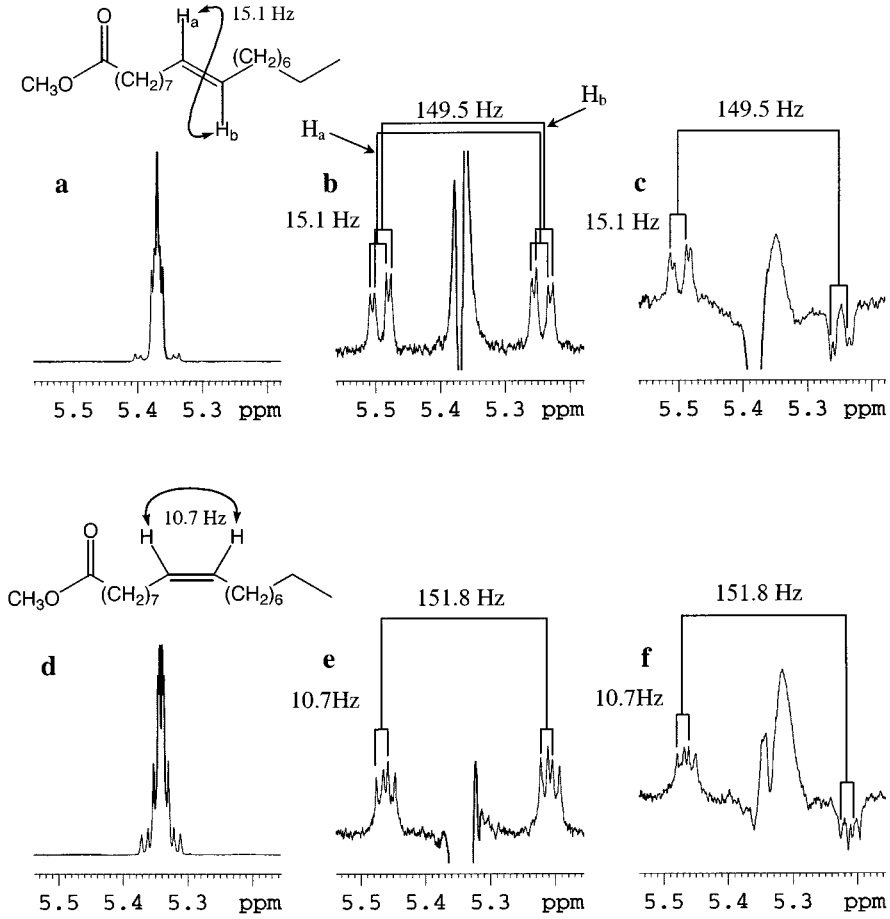


**Figure 3.** NMR data for the fatty acid methyl ester examples: **(a)** normal $^1$H NMR spectrum of elaidic acid methyl ester; **(b)** data for elaidic acid methyl ester obtained with the pulse sequence shown in Figure 1a showing observed couplings for both olefinic protons ($H_a$ and $H_b$); **(c)** data for elaidic acid obtained using the pulse sequence shown in Figure 1b; **(d)** normal $^1$H NMR spectra of oleic acid methyl ester; **(e)** data for oleic acid methyl ester obtained with the pulse sequence shown in Figure 1a, and **(f)** data for oleic acid obtained using the pulse sequence shown in Figure 1b.

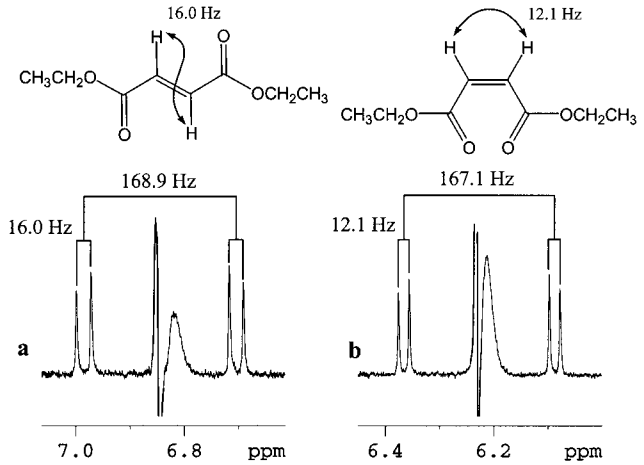


**Figure 4.** $^1$H NMR data obtained for **(**a**)** diethyl fumarate and **(**b**)** diethyl maleate using the pulse sequence shown in Figure 1a.

chemical shift is not observed in the 1D $^1$H spectrum because of higher-order coupling effects.

This approach can also be utilized to elucidate the configuration of symmetric or degenerate molecules. Diethyl fumarate and diethyl maleate are shown here as an example. As can be seen in Figure 4, diethyl fumarate exhibits the expected 16.0 Hz homonuclear coupling for the *trans* configuration, and diethyl maleate exhibits the expected 12.1 Hz homonuclear coupling for the *cis* configuration.

We have recently used the BIRD method to determine the double bond geometries of a number of natural products. A trans relationship of the olefinic double bond of the fatty acid portion was established for the cyanobacterial metabolite malyngamide R (Milligan, K. E., III; Marquez, B. L.; Williamson, R. T.; Gerwick, W. H., unpublished data). We have also used this method in the structure elucidation of the bruchins, several of which are esters containing long-chain unsaturated diols (Doss, R. P.; Oliver, J. E.; Proebsting, W. M.; Potter, S. W.; Kuy, S.; Clement, S. L.; Williamson, R. T.; Carney, J. R.; DeVilbiss, E. D., unpublished data). The cis double-bond geometries of these compounds were subsequently confirmed by total synthesis (Oliver, J. E.; Doss, R. P.; Williamson, R. T.; Carney, J. R., unpublished data).

The data shown in Figures 3b,e and 4 were acquired with the BIRD sandwich pulse sequence performed without the use of pulsed-field gradients. This was done in an effort to show the practicality of the experiment and that it can be performed on any programmable FT−NMR instrument. Comparable data to those shown in Figures 3b and 3e can be acquired with the simple heteronuclear *z*,*z* filter shown in Figure 1b (Figure 3c,f).[9] When used in conjunction with a moderate pulsed field gradient strength of 20−30 G/cm$^2$, this sequence alleviates the need for an inversion recovery delay, since all transverse magnetization is dephased after the second 90 degree $^1$H pulse, and the desired antiphase magnetization evolves during acquisition.

The approach demonstrated here is superior to the $^{13}$C shift approach in that it is 11 times more sensitive and can be performed in just a fraction of the time it would take to acquire 1D $^{13}$C data on micromole amounts of compound. It also allows for a more convenient analysis of complicated spin systems than has been possible with the previously reported HMQC- and HSQC-based experiments. As can be seen, some residual signal from the protons attached to $^{12}$C remains. If one has pulsed-field gradient capability, slightly better suppression of the signal from protons attached to $^{12}$C can be obtained using the Quad G-BIRD sequence developed by Shaka and co-workers.[10] However, in our experience with this latter experiment, a decrease in S/N due to the larger number of 180 degree pulses and the extended length of the sequence is realized with only a slightly better suppression of $^1$H's attached to $^{12}$C. We are presently developing methods utilizing an HSQC-based approach that will provide both adequate sensitivity and superior suppression of any $^1$H's bound to $^{13}$C.

## Experimental Section

One milligram (3.5 $\mu$mol) samples of elaidic acid and oleic acid and 1-mg (5.8 $\mu$mol) samples of diethyl fumarate and diethyl maleate were each dissolved in 500 $\mu$L $CDCl_3$. All spectra were recorded at 600.077 MHz on a Bruker DRX600 NMR spectrometer equipped with an inverse-detected 5-mm $^1$H, $^{13}$C, $^{15}$N triple resonance probe with actively shielded triple axis gradients. The $1/2J_{C,H}$ delays for all experiments were set to 3.45 ms (optimized for a 145 Hz coupling). The inversion recovery delay ranged from 1 to 2 s and was optimized for each molecule under analysis. For the heteronuclear *z*,*z*-filter, a sinusoidal gradient of 20 G cm$^{-1}$ (72 G cm$^{-1}$ maximum strength) was used in the place of the inversion recovery delay. Ninety degree pulse widths were 9.2 $\mu$s for $^1$H and 13.2 $\mu$s for $^{13}$C, at a power level of 0.0 dB. Relaxation delays were 1.5 s for all experiments. The data for elaidic acid and oleic acid were acquired in 1024 scans, and the data for diethyl maleate and diethyl fumarate were acquired with 128 scans.

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