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Determination of Heteronuclear Coupling Constants via Semiselective Two-Dimensional *J* Spectroscopy

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The earliest types of two-dimensional NMR experiments that received widespread attention were the proton-flip and the gated-decoupler experiments (*1–8*). Conceptually those early experiments are rather simple to understand, but the use of those experiments in practical cases often is complicated by data storage requirements and sensitivity considerations. Only in the case where the spectroscopist is interested in determining the multiplicity of a certain carbon-13 site is the use of the two-dimensional experiment straightforward; in such cases only a very low resolution of the carbon-13 multiplet structure along the F_1 axis is necessary, and hence the data matrix can be kept small. The sensitivity will be fairly good in this application because the carbon-13 intensity is spread over a maximum number of only four multiplet components. However, this application of two-dimensional *J* spectroscopy has been outdated by even more sensitive and experimentally more convenient "spectral editing" experiments (*9–11*).

The use of the original 2D *J*-spectroscopy experiments for the exact determination of coupling constants requires a very fine digitization along the F_1 frequency axis; this leads to a very large size of the required data matrix, unless the acquisition time along the t_2 axis is made very short, which in turn decreases the sensitivity of the experiment dramatically. The intensity of a certain carbon-13 nucleus in this high-F_1-resolution application will generally be spread over a large number of multiplet components, which decreases the sensitivity even further. All problems mentioned above can be overcome by using a modified version of the proton-flip experiment in which only one proton is "flipped" by the proton pulse (which in this case is selective) in the center of the evolution period (*12*). This allows the accurate determination of all heteronuclear long-range couplings with this particular proton.

In this communication we describe two experiments which rely on the same principle as the selective proton-flip experiment and which allow the accurate determination of either all direct ^1H–^{13}C coupling constants by removal of the long-range splittings or the determination of all long-range couplings while removing all the direct ^1H–^{13}C splittings.

The experimental pulse scheme is set out in Fig. 1. As in the original proton-flip experiment (*3, 4*), broadband low power ^1H irradiation during the preparation period provides a NOE effect, enhancing the sensitivity, and high power proton noise decoupling is employed during the detection period. A carbon-13 180° pulse is applied

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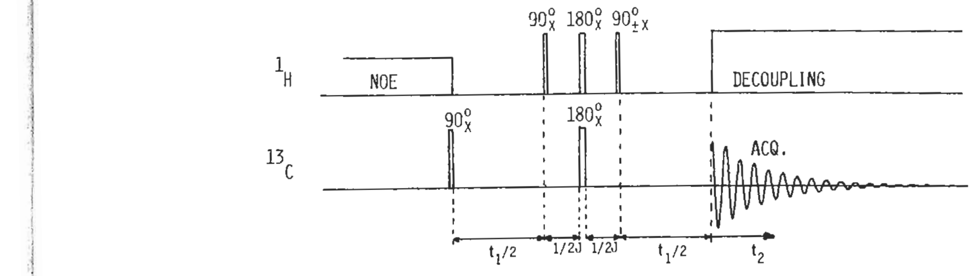

FIG. 1. Pulse scheme for the semiselective proton-flip experiments. In the case where the final proton 90° pulse is applied along the positive x axis, only direct couplings will be observed. In the case where the final 90° pulse is applied along the negative x axis, only long-range couplings will be observed.

in the center of the evolution period. However, the proton 180° pulse in the center of the evolution period of the original proton-flip experiment is now replaced by the 90°_x–½J–180°_x–½J–$90^\circ_{\pm x}$ sequence, where J is the magnitude of the direct ^{1}H–^{13}C coupling constant in hertz. For an isolated ^{13}C–^{1}H$_n$ set it is readily seen that in the case where the final pulse is applied along the positive x axis, the spin state of the proton(s) will be inverted, i.e., experience a flip. However, magnetization vectors from protons not directly coupled to the ^{13}C will undergo only small dephasing during the short intervals, ½J, at a rate determined by the homonuclear proton–proton couplings and the long-range proton–carbon couplings. The final 90°_x pulse brings those magnetization vectors back along the positive z axis, and therefore the sequence has the effect of not changing the spin state of those protons.

The picture sketched above is a simplified one, but it can be shown with simple density matrix calculations that it is valid, given the assumption that the homonuclear proton–proton and the heteronuclear long-range proton–carbon couplings are very much smaller than the direct proton–carbon coupling constant. Only protons with a directly coupled carbon-13 nucleus will be flipped, and the detected magnetization will be modulated in amplitude by the frequency of the direct coupling only. The multiplets along the F_1 axis of the two-dimensional spectrum will all be simple singlets, doublets, triplets, or quartets, depending on the number of protons directly coupled to the carbon-13 considered. This makes it possible to allow multiple folding along the F_1 axis without introducing overlap, and therefore reducing the size of the data matrix considerably.

In a similar way it can be seen that in the case where the final 90° proton pulse is applied along the negative x axis, only protons which are not directly coupled to a carbon-13 nucleus will have their spin state flipped, and hence the detected magnetization will be modulated by the heteronuclear long-range couplings only. In this case a sampling frequency along the t_1 axis equal to about 40 Hz, giving a spectral width of ± 20 Hz in the F_1 dimension, will usually be sufficient, and no fold-over will occur. The number of resonances in the F_1 dimension is again reduced compared with the original proton-flip experiment, and hence sensitivity is improved.

The method is demonstrated here on pyridine, a compound for which the heteronuclear coupling constants have been extensively investigated in the past (*7, 13, 14*). Experiments were performed on a Nicolet-360 spectrometer controlled by a Nicolet

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