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SHESSLOC: A selective variation of the FUCOUP sequence

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Abstract

In this paper we carried out a comparison between all the possible selective versions of the basic heteronuclear correlation experiment, the FUCOUP sequence. We concluded that the best experiment is that one in which the selective pulse is given in the carbon dimension, which we called SHESSLOC (Selective HEteronuclear Simultaneous Short and LOng-range Correlations). The sensitivity of the sequence was improved with the introduction of pulsed field gradients.

Keywords: FUCOUP; Heteronuclear correlation; Coupling constants; Magnetic field gradients; Selective excitation

1. Introduction

The importance of knowing correlations between protons and carbons is very well described in the Literature. The knowledge of direct connectivities is essential for the assignment of CH_n fragments, whereas the long-range correlations provide information about quaternary carbons, allowing their assignment and the establishment of the linking between different proton networks. In this sense, a large variety of different 2D and 3D techniques for measuring the values of ^1H–^{13}C coupling constants have been developed in the last years [1–17] and the great diversity of proposed solutions reflect the difficulty in obtaining the desired data. Many of those experiments use selective radio-frequency pulses which have been incorporated into several types of experiments in order to improve resolution.

Usually, only the heteronuclear correlations are enough for establishing the desired molecular connectivities, without the need for determining the corresponding coupling constant. Most of the recent heteronuclear correlation experiments use indirect detection to attain higher sensitivity [18–23] and pulsed-field gradients in order to suppress undesirable coherence pathways in a single transient, thereby eliminating or diminishing the need for time consuming phase cycling. However, most of those experiments do not allow for the reliable and simultaneous detection of all possible short and long range correlations or for the measurement of the corresponding heteronuclear coupling constants. All those experiments have fixed delays, thus being modulated by *J*-values. Moreover, the long-range correlation experiments need to be used and interpreted with care because not all the expected correlations are observed.

The only experiment that allows for the simultaneous determination of all those correlations and estimation of coupling constants in a single 2D correlation map is the basic heteronuclear pulse sequence known as FUCOUP (FUlly COUPled) [24], which consists of only three pulses $[d_1–90°\{^1H\}–t_1–90°(^1H)–90°(^{13}C)–t_2]$.

This experiment is usually not used due its low sensitivity, but it has the advantage of being a short sequence—

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which minimizes sample relaxation problems—with no fixed time periods—which allows for the simultaneous evolution of all short and long J couplings, thus leading to detection of all types of couplings and correlations. Also, for concentrated or isotopically enriched samples, it is possible to perform this experiment in a reasonable time with very good results [25].

Although we can observe long range correlations in a FUCOUP spectrum, it does not allow for the estimation of the corresponding coupling constants due to the usually low resolution in the carbon dimension (F2). For this purpose, Kessler et al. [26] proposed a slight modification of the FUCOUP sequence: a semiselective excitation one that they called soft-H,C-COSY, which makes use of selective excitation in the ^{1}H domain. That sequence was used for estimating $^{3}J_{\mathrm{CH}}$ coupling constants in peptides.

Altogether, Macheteau et al. [27] applied the FUCOUP sequence in its selective version [d_1–$sp\{^{19}\mathrm{F}\}$–t_1–$90°\{^{19}\mathrm{F}\}$–$90°\{^{1}\mathrm{H}\}$–t_2]–where *sp* is a selective pulse—for the observation of three and four bond ^{1}H–^{19}F coupling constants.

In this work we have tested different variations of the selective FUCOUP experiment for the detection of all types of correlations and estimation of heteronuclear coupling constants. We tested and compared the four combinations of hard and soft pulses, with two different samples as test compounds: *o*-ethoxybenzaldehyde and inosine (Fig. 1): (i) only the first proton pulse was selective; (ii) only the second proton pulse was selective (soft H,C-COSY); (iii) only the carbon pulse was selective; and (iv) all three pulses were selective.

2. Experimental

All experiments were carried out on a 300 MHz (7.05 T) Varian Mercury Plus NMR spectrometer. It was used a very concentrated solution of *o*-ethoxybenzaldehyde in DMSO-d_6 (2:1 v/v) and a saturated solution of inosine also in DMSO-d_6, in 5 mm NMR tubes. Soft pulses were obtained using the *e-burp* shape [28]. Pulse widths and power levels varied according to the selected spectral window.

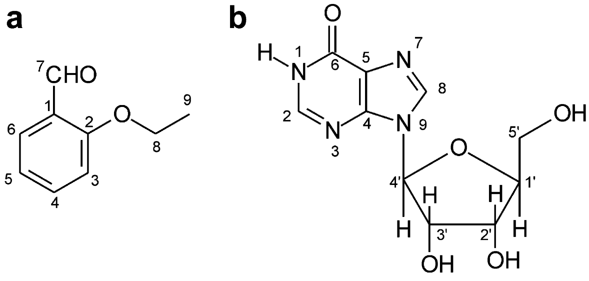

Fig. 1. Numbered structures of (a) *o*-ethoxybenzaldehyde and (b) inosine.

3. Results and discussion

3.1. Comparison among the different selective variations of the FUCOUP sequence

3.1.1. Data acquisition and basic observations

The analysis of the basic FUCOUP sequence using product operators in the spherical coordinate basis for a two-spin system showed that there are six detectable terms, as follows:

$$\begin{aligned}
&-0.35\mathrm{i}\,S_{+}\sin\lambda_1\sin\lambda_2\exp[-\mathrm{i}(\phi_1+\omega_{\mathrm{I}}t_1-\phi_2+\phi_3+\omega_{\mathrm{S}}t_2)]\\
&+0.35\mathrm{i}\,S_{+}\sin\lambda_1\sin\lambda_2\exp[-\mathrm{i}(-\phi_1-\omega_{\mathrm{I}}t_1+\phi_2+\phi_3+\omega_{\mathrm{S}}t_2)]\\
&-0.707\mathrm{i}\,S_{+}\cos\lambda_2\exp[-\mathrm{i}(\phi_3+\omega_{\mathrm{S}}t_2)]\\
&+0.35\mathrm{i}\,S_{-}\sin\lambda_1\sin\lambda_2\exp[-\mathrm{i}(\phi_1+\omega_{\mathrm{I}}t_1-\phi_2-\phi_3-\omega_{\mathrm{S}}t_2)]\\
&-0.35\mathrm{i}\,S_{-}\sin\lambda_1\sin\lambda_2\exp[-\mathrm{i}(-\phi_1-\omega_{\mathrm{I}}t_1+\phi_2-\phi_3-\omega_{\mathrm{S}}\mathrm{t}_2)]\\
&-0.707\mathrm{i}\,S_{-}\cos\lambda_2\exp[-\mathrm{i}(\phi_3-\omega_{\mathrm{S}}t_2)]
\end{aligned}$$

where ϕ is the axis about which the pulse field is applied, I and S are the ^{1}H and ^{13}C nucleus, respectively, ω is the precession frequency, λ_1 is $1/2J_{\mathrm{IS}}t_1$ and λ_2 is $1/2J_{\mathrm{IS}}t_2$, where J is the coupling constant between the I and S nucleus.

According to those terms, the chosen phase cycling was $\phi_1 = x$; $\phi_2 = x, y, -x, -y$; $\phi_3 = x$; $\phi_{\mathrm{detector}} = \phi_4 = x, y, -x, -y$.

When comparing the results from experiments (i) and (ii), we did not observe any significant differences on the spectra, all of which clearly showed the expected short and long range correlations, allowing for the direct estimation of the corresponding coupling constants. As discussed by Kessler and co-workers [26], the assignment of the heteronuclear coupling constants in these experiments is only possible if all but one of the homonuclear coupling constants is known. This has three consequences. First, it is necessary to know the homonuclear coupling constants for the assignment of the heteronuclear ones. Second, a problem emerges when homonuclear coupling constants are not different enough, thus leading to ambiguities in the assignment, this being a very common case for many organic and inorganic compounds. Finally, the very common superposition of signals in the proton spectrum can make difficult the selective excitation of the desired proton, as it was the case for the aromatic protons of *o*-ethoxybenzaldehyde. For this compound, the selective excitation of single aromatic hydrogen demanded the use of a very long soft pulse, making this a very time-consuming experiment even for concentrated samples. In fact, to accomplish this experiment in a reasonable time, it was necessary to increase the spectral window (thus decreasing selectivity).

For case (iii), the results were quite good, showing all correlations and coupling constants (direct and indirect). The spectra have the same appearance of the normal FUCOUP spectra, and the estimated coupling patterns and constants were confirmed using coupled ^{13}C spectra. In this experiment, only a small spectral window is observed in the carbon dimension due the selective pulse.