

SHESSLOC: A selective variation of the FUCOUP sequence

Claudia Jorge do Nascimento^{a,b,1}, José Daniel Figueroa-Villar^{b,*}

^a Instituto de Química, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Instituto Central de Ciências (ICC), Ala Sul, C.P. 04478 CEP: 70904-970, Brasília, D.F., Brazil

^b Departamento de Química, Instituto Militar de Engenharia (IME), Praça General Tibúrcio, 80. Praia Vermelha, Rio de Janeiro, CEP: 22290-270, R.J., Brazil

Received 18 December 2006; revised 15 March 2007

Available online 20 April 2007

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.

© 2007 Elsevier Inc. All rights reserved.

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–¹³C 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, with-

out 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°(¹³C)–*t*₂].

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

* Corresponding author. Fax: +55 21 2546 7059.

E-mail addresses: claudian@unb.br (C.J. do Nascimento), figueroa@ime.eb.br (J.D. Figueroa-Villar).

¹ Fax: +55 61 3273 4149.

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 ^1H domain. That sequence was used for estimating $^3J_{\text{CH}}$ coupling constants in peptides.

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

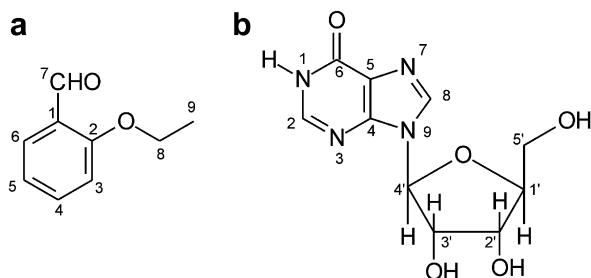


Fig. 1. Numbered structures of (a) *o*-ethoxybenzaldehyde and (b) inosine.

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:

$$\begin{aligned} & -0.35i S_+ \sin \lambda_1 \sin \lambda_2 \exp[-i(\phi_1 + \omega_1 t_1 - \phi_2 + \phi_3 + \omega_S t_2)] \\ & + 0.35i S_+ \sin \lambda_1 \sin \lambda_2 \exp[-i(-\phi_1 - \omega_1 t_1 + \phi_2 + \phi_3 + \omega_S t_2)] \\ & - 0.707i S_+ \cos \lambda_2 \exp[-i(\phi_3 + \omega_S t_2)] \\ & + 0.35i S_- \sin \lambda_1 \sin \lambda_2 \exp[-i(\phi_1 + \omega_1 t_1 - \phi_2 - \phi_3 - \omega_S t_2)] \\ & - 0.35i S_- \sin \lambda_1 \sin \lambda_2 \exp[-i(-\phi_1 - \omega_1 t_1 + \phi_2 - \phi_3 - \omega_S t_2)] \\ & - 0.707i S_- \cos \lambda_2 \exp[-i(\phi_3 - \omega_S t_2)] \end{aligned}$$

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

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

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.

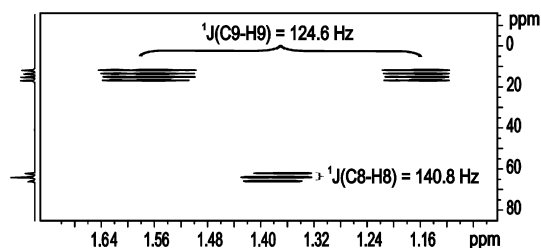


Fig. 2. Selective excitation of H9 (1.40 ppm) from *o*-ethoxybenzaldehyde using pulse sequence i. A 30.3 ms long soft pulse with 1.5 mW (15 dB; 0.2841 kHz) power level was used. Acquisition time = 0.054 s and spectral width in F1 dimension = 402.3 Hz. Thirtytwo transients were collected per increment (total of 192 increments) for a total experiment time of 2 h 20 min 54 s.

In this case, we increased the acquisition time in order to improve the spectral resolution, thus allowing for the use of $d_1 = 0$. These experimental conditions permit the obser-

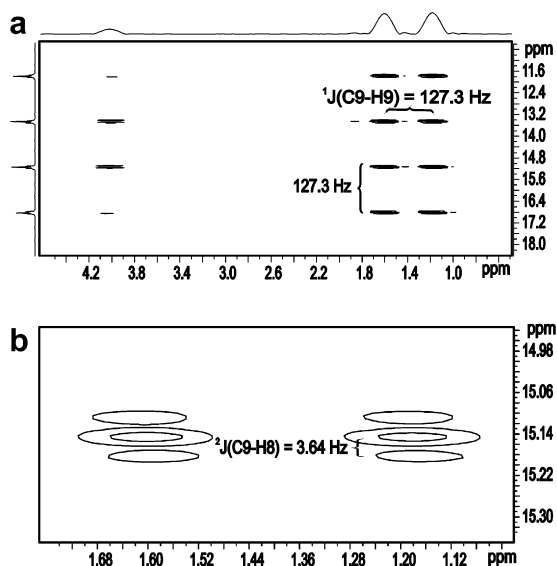


Fig. 3. (a) Selective excitation spectrum of C9 (14.4 ppm) from *o*-ethoxybenzaldehyde using experiment (iii). A 6.36 ms long soft pulse at 36.6 mW (24 dB; 1.3139 kHz) power level was used. Acquisition time = 1.067 s, $d_1 = 0$ and spectral width in F2 dimension = 831.9 Hz. Four transients were collected per increment (total of 128 increments) for a total experiment time of 10 min 24 s. (b) Expanded quartet from spectrum A, showing the triplets inside it. The long range coupling constant (not observed in FUCOUP using hard pulses) can be estimated from the F2 dimension.

vation of small coupling constants, which are not detectable in the non-selective FUCOUP spectrum, as mentioned before.

Finally, experiment (iv), where only one proton and one carbon are selected, allows for the estimation of their specific coupling. In this case both spectral windows are smaller. Also, because of the increase in the acquisition time, the delay d_1 can be eliminated, as it was done for experiment (iii). The observed sensitivity is lower than that obtained with sequences (i), (ii), (iii) and the non-selective FUCOUP experiment.

3.1.2. Comparison between the different spectra

Fig. 2 shows the spectrum obtained with selective excitation of the protons from the methyl group (H9, 1.40 ppm) of *o*-ethoxybenzaldehyde using experiment (i), which is the same as the spectra obtained with pulse sequence ii.

As mentioned before, we could not observe significant differences between experiments (i) and (ii). Comparison of the expanded spectrum shown in Fig. 2 with the spectrum in which carbon C9 (14.4 ppm) is selectively excited (experiment (iii)), shown in Fig. 3, indicates that heteronuclear coupling constants can be easily estimated from both spectra. The only difference in this case is that, due to the lower resolution in F1 for experiment (iii), homonuclear ^1H – ^1H coupling constants can be estimated only from the spectrum obtained with experiment (i) (Fig. 2).

However, by comparing the experiments, the selective excitation of carbons (experiment (iii)) shows some advantages which can be better understood looking at Table 1, which summarizes some results for long-range correlations for *o*-ethoxybenzaldehyde.

Although we can observe the correlations described in Table 1, it is important to note that, in those cases, we have a complex coupling pattern in the F2 dimension, which makes very difficult to estimate the corresponding coupling constants, as it can be observed in Fig. 4.

With inosine, the best example for choosing selective excitation on the ^{13}C domain can be seen in Fig. 5, where the 3J correlation between C8 (139.4 ppm) and H4' (5.96 ppm), which are located on different rings, can be easily seen with selective excitation on the carbon domain. When the selective excitation is performed on the proton domain, this correlation is not observed even after 12 h acquisition.

Table 1
Comparison between experiments for *o*-ethoxybenzaldehyde

Correlation	Selective pulse on proton (experiments (i) and (ii))	Selective pulse on carbon (experiment (iii))
C2–H8 (3J)	Observed only after 3 h of acquisition	Observed after 10 min, but clearly visible after 40 min of acquisition
C2–H5 (4J)	Bad selectivity (aromatic protons); observed after 14 h of acquisition, but it can not be distinguished from C2–H3	Observed in 40 min of acquisition; it can be distinguished from C2–H3 after 12 h of acquisition (Fig. 4)
C2–H3 (2J)	Same observations as for C2–H5	Same observations as for C2–H5
C3–H7 (4J)	Not observed even after 17 h of acquisition	Observed after 2 h 40 min of acquisition
C1–H4 (4J)	It can not be observed after 14 h of acquisition	Observed after 12 h of acquisition

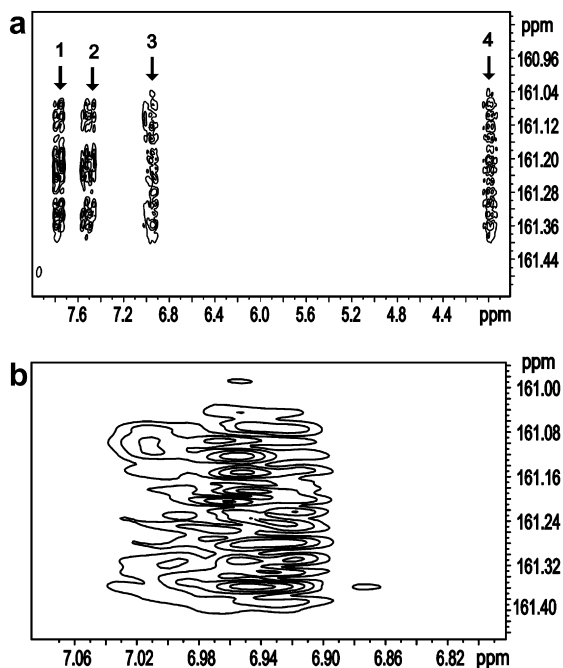


Fig. 4. Selective excitation of C2 (161.2 ppm) for *o*-ethoxybenzaldehyde. A 23.8 ms long soft pulse at 2.3 mW (12 dB; 0.3269 kHz) power level was used. Acquisition time = 1.067 s, $d_1 = 0$ and spectral width in F2 dimension = 306.4 Hz. Signal 1 is due to coupling between C2 and H6 (7.76 ppm) (3J); signal 2 is due to coupling between C2 and H4 (7.56 ppm) (3J); signal 3, coupling between C2 and H3 (6.96 ppm) (2J) and/or C2 and H5 (6.94 ppm) (4J), as discussed in the text; signal 4 is due to coupling between C2 and H8 (4.05 ppm) (3J). (a) Seventytwo transients were collected per increment (total of 512 increments) for a total experiment time of 12 h 39 min 52 s (b) Expansion of (a) showing correlation between C2–H3 (2J) and C2–H5 (4J).

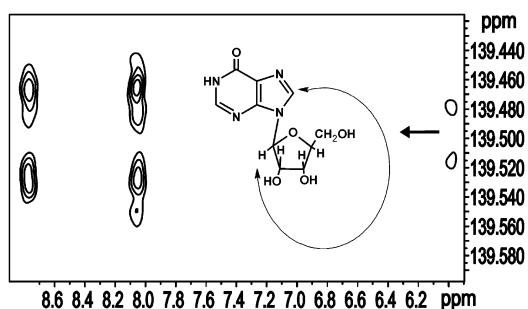


Fig. 5. Selective excitation of C8 from inosine. A 15.37 ms long soft pulse at 5.77 mW (16 dB; 0.5231 kHz) power level was used. Acquisition time = 1.086 s, $d_1 = 0$ and spectral width in F2 dimension = 510.2 Hz. 48 transients were collected per increment (total of 256 increments) for a total experiment time of 4 h 1 min 1 s. The atoms involved in this three-bond correlation between carbon and hydrogen is shown in the figure inside the spectrum.

Analyzing experiment (iv), in which all the pulses are selective; we can observe that it is the most insensitive of the tested experiments. Because it is doubly selective, this is a very specific experiment, but it is useful as it is the only experiment that was able to detect the coupling between C3–H5 (3J , Fig. 6) in *o*-ethoxybenzaldehyde.

Considering that the best results were obtained with sequence iii, this experiment was chosen for improving

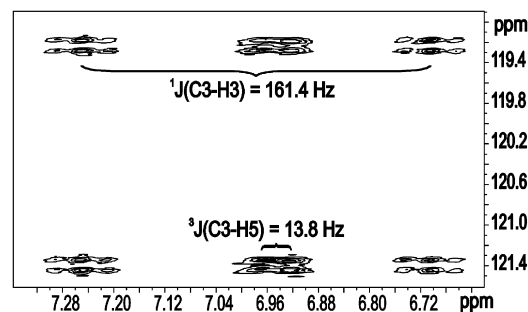


Fig. 6. Simultaneous selective excitation of C3 and the aromatic protons of *o*-ethoxybenzaldehyde. A 4.97 ms long soft pulse at 5.77 mW (16 dB; 0.5231 kHz) power level was used for carbon and a 9.67 ms long soft pulse at 19.37 mW (26 dB; 0.8524 kHz) power level for proton. Acquisition time = 1.072 s, $d_1 = 0$, spectral width in F2 dimension = 452.5 Hz and in F1 dimension = 973.0 Hz. Four transients were collected per increment (total of 128 increments) for a total experiment time of 11 min 21 s.

Table 2

Some of the possible gradient powers and lengths for the SHESLOC sequence

G_1 (G/cm)	t_1 (ms)	G_2 (G/cm)	t_2 (ms)
2.49	1.00	9.95	1.00
4.98	0.75	9.95	1.50
7.36	0.75	14.7	1.50

the acquisition time and the quality of the spectra with the insertion of gradients on the sequence before and after the polarization transfer step. For simplicity, we called this experiment SHESLOC (Selective Heteronuclear Simultaneous Short and Long range Correlations).

Considering the observable terms mentioned before and the magnetogyric constants ($\gamma_H = 4\gamma_C$), it can be shown that for the heteronuclear case the possible gradients are given by the relation $G_2 t_2 = \pm 4G_1 t_1$, where G is the gradient power and t its length. Table 2 summarizes some of the different gradient powers and lengths used for SHESLOC.

All the gradients shown in Table 2 were tested and afforded very similar results.

By comparing the spectra obtained using only phase cycling to those obtained using only gradients, it can be observed that the information using gradients is clearer and more complete. Only for quaternary carbons it is observed that the two experiments have about the same performance, especially when more transients and increments are used to observe some of the long-range correlations.

4. Conclusions

Although experiments (i) and (ii) show homo- and heteronuclear correlations and their corresponding coupling constants, by comparing the different results we concluded that SHESLOC is the best experiment for detecting all types of heteronuclear correlations using direct detection.

In fact, the spectra obtained using SHESSLOC contain all the observable heteronuclear information (correlations and coupling constants) found in experiments (i) and (ii), and it usually displays new information, even when using less scans. For example, couplings between C1–H4 and C3–H7 (4J couplings) in *o*-ethoxybenzaldehyde were observed only with SHESSLOC. Second, the success of this experiment does not depend on the values of the H–H couplings, thus making this sequence independent from homonuclear experiments. Moreover, peak selectivity problems due to signal superposition are very rare, as the selective pulse is in the more frequency dispersed ^{13}C dimension. This is an advantage even when considering that the selective pulses have to be applied to the carbon multiplets, which usually contain 1J couplings.

Despite its very low sensitivity when compared with the other pulse sequences, experiment (v) can be useful for estimating a specific coupling constant, as it was shown for the coupling between C1–H7 and C3–H5 in *o*-ethoxybenzaldehyde.

Insertion of gradients in the SHESSLOC sequence showed that we can reduce significantly the total time of the experiment without loss of quality and information.

The advantage of SHESSLOC over other heteronuclear correlation experiments is that, in principle, it is able to detect all the observable coupling information from a sample without the need for the optimization of any time periods. Also, despite its relatively low sensitivity, because this is a very short sequence it is less affected by relaxation; therefore it can be applied to bigger molecules.

Acknowledgment

The authors thank the Brazilian agencies FINEP, FAPERJ and CNPq for financial support.

References

- [1] R.E. Hoffman, A. Weitz, M. Rabinovitz, Improved pulse program for long-range heteronuclear correlation, *J. Magn. Reson. Ser. A* 102 (1993) 1–7.
- [2] J.-M. Nuzillard, R. Freeman, A new route to long-range ^{13}C –H couplings, *J. Magn. Reson. Ser. A* 110 (1994) 262–265.
- [3] G. Zhu, A. Bax, Measurement of long-range ^1H – ^{13}C coupling constants from quantitative 2D heteronuclear multiple-quantum correlation spectra, *J. Magn. Reson. Ser. A* 104 (1993) 353–357.
- [4] E.R.P. Zuiderweg, S.W. Fesik, Band-selective heteronuclear decoupling using shaped pulses as an aid in measuring long-range heteronuclear coupling constants, *J. Magn. Reson.* 93 (1991) 653–658.
- [5] G. Zhu, A. Renwick, A. Bax, Measurement of two- and three-bond ^1H – ^{13}C J couplings from quantitative heteronuclear J correlation for molecules with overlapping ^1H resonances, using t_1 noise reduction, *J. Magn. Reson. Ser. A* 110 (1994) 257–261.
- [6] D.G. Davis, Selective excitation of protons directly bonded to carbon-13, *J. Magn. Reson. Ser. B* 101 (1993) 229–239.
- [7] B. Adams, L. Lerner, Measurement of long-range ^1H – ^{13}C coupling constants using selective excitation of carbon-13, *J. Magn. Reson. Ser. A* 103 (1993) 97–102.
- [8] J.M. Richardson, J.J. Titman, J. Keeler, Assessment of a method for the measurement of long-range heteronuclear coupling constants, *J. Magn. Reson.* 93 (1991) 533–553.
- [9] U. Wollborn, D. Leibfritz, Measurements of heteronuclear long-range coupling constants from inverse homonuclear 2D NMR spectra, *J. Magn. Reson.* 98 (1992) 142–146.
- [10] W. Bermel, K. Wagner, C. Griesinger, Proton-detected C,H correlation via long-range couplings with soft pulses; determination of coupling constants, *J. Magn. Reson.* 83 (1989) 223–232.
- [11] J.J. Titman, D. Neuhaus, J. Keeler, Measurement of long-range heteronuclear coupling constants, *J. Magn. Reson.* 85 (1989) 111–131.
- [12] R.C. Crouch, G.E. Martin, Selective inverse multiple bond analysis. A simple 1D experiment for the measurement of long-range heteronuclear coupling constants, *J. Magn. Reson.* 92 (1991) 189–194.
- [13] A. Bax, G.A. Morris, An improved method for heteronuclear chemical shift correlation by two-dimensional NMR, *J. Magn. Reson.* 42 (1981) 501–505.
- [14] A.J. Shaka, R. Freeman, Selective excitation of proton spectra by polarization transfer from an adjacent carbon atom, *J. Magn. Reson.* 50 (1982) 502–507.
- [15] D. Neuhaus, J. Keeler, R. Freeman, Investigation of individual proton spin multiplets by C–H correlation spectroscopy, *J. Magn. Reson.* 61 (1985) 553–558.
- [16] T. Jippo, O. Kamo, K. Nagayama, Determination of long-range proton-carbon 13 coupling constants with selective two-dimensional INEPT, *J. Magn. Reson.* 66 (1986) 344–348.
- [17] S. Berger, Selective inverse correlation of ^{13}C and ^1H NMR signals, an alternative to 2D NMR, *J. Magn. Reson.* 81 (1989) 561–564.
- [18] L. Müller, Sensitivity enhanced detection of weak nuclei using heteronuclear multiple quantum coherence, *J. Am. Chem. Soc.* 101 (16) (1979) 4481–4484.
- [19] A. Bax, R.H. Griffey, B.L. Hawkins, Correlation of proton and nitrogen-15 chemical shifts by multiple quantum NMR, *J. Magn. Reson.* 55 (1983) 301–315.
- [20] G. Bodenhausen, D.J. Ruben, Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy, *Chem. Phys. Lett.* 69 (1980) 185–189.
- [21] A. Bax, M.F. Summers, ^1H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR, *J. Am. Chem. Soc.* 108 (1986) 2093–2094.
- [22] I.A. Palmer, J. Cavanagh, P. Wright, M. Rance, Sensitivity improvement in proton-detected two-dimensional heteronuclear correlation NMR spectroscopy, *J. Magn. Reson.* 93 (1991) 151–170.
- [23] J. Cavanagh, I.A. Palmer, P. Wright, M. Rance, Sensitivity improvement in proton-detected two-dimensional heteronuclear relay spectroscopy, *J. Magn. Reson.* 91 (1991) 429–436.
- [24] G. Bodenhausen, R. Freeman, Correlation of proton and carbon-13 NMR spectra by heteronuclear two-dimensional spectroscopy, *J. Magn. Reson.* 28 (1977) 471–476.
- [25] C.J. do Nascimento, E.R. Cruz, J.D. Figueroa-Villar, Complete H-1 and C-13 NMR spectral assignment of 6,7,8,9-tetrahydro-7,7-dimethylidioxolo [5,4-b]acridin-9-one using FUCOUP, *Magn. Reson. Chem.* 37 (1998) 239–242.
- [26] H. Kessler, U. Anders, G. Gemmecker, Determination of heteronuclear long-range coupling constants by soft-H,C-COSY, *J. Magn. Reson.* 78 (1988) 382–388.
- [27] J.-P. Macheteau, H. Oulyadi, B. Hemelryck, M. Bourdonneau, D. Davoust, 2D experiments for the characterization of fluorinated polymers: pulsed-field gradients ^1H – ^{19}F hetero-COSY and its selective version, *J. Fluorine Chem.* 104 (2000) 149–154.
- [28] H. Geen, R. Freeman, Band-selective radiofrequency pulses, *J. Magn. Reson.* 93 (1991) 93–141.