Available online at www.sciencedirect.com
ScienceDirect
Journal of Magnetic Resonance 190 (2008) 292–297
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Application of biselective refocusing soft pulses to the simplification of heteronuclear correlation spectra

Jean-Marc Nuzillard *

CNRS, Institut de Chimie Moléculaire de Reims, BP 1039, 51687 REIMS Cedex 2, France

Received 29 May 2007; revised 15 November 2007
Available online 22 November 2007

Abstract

The biselective spin echo technique allows the signals of coupled proton pairs to be extracted from crowded liquid state proton NMR spectra. Its use as a preparation sequence in heteronuclear chemical shift correlation experiments requires the removal of the heteronuclear coupling interaction during the biselective echo time. The discrimination between coupled and uncoupled protons signals is achieved by double quantum filtration, which delivers antiphase magnetization states. The latter are not directly compatible with the design of an HSQC-like pulse sequence. The conversion of antiphase to in-phase magnetization states by a second biselective echo sequence solves this problem. The optimization of spin echo delays is also discussed. Lastly, the article presents modified HSQC and HMBC pulses sequences in which information is obtained solely for the biselectively selected proton pairs. A peracetylated trisaccharide was used as a test molecule.

Keywords: Liquid state NMR; Selective pulses; Spin echo; 2D NMR; Heteronuclear correlation

1. Introduction

Multiplet selective radiofrequency pulses are generally used in liquid-state NMR to facilitate the analysis of complex samples through spectral simplication and dimensionality reduction.[1,2]. A proton multiplet selective pulse typically has an action band width in the 10–50 Hz range. It may concern more than one multiplet when applied to a crowded spectral region. Biselective experiments reduce the number of responding spins, as the recorded signals only arise from nuclei pairs whose chemical shifts fall within two given spectral regions, and only if their spins are scalarly coupled. [3,4]. The underlying concepts were first put in practice in the TSETSE and TSETSE–2 experiments, whose name was given after the so-called ''Twin Spin Effect'' [3].

Obtaining biselectivity involves either excitation [4,5], or refocusing pulses [6–9]. The latter may be placed advantageously within double pulsed field gradient spin echo sequences (DPFGSE), to benefit from their inherently high selectivity [10]. The discrimination between coupled and uncoupled spin pairs is achieved by double quantum filtration [3]. Biselective refocusing is obtained through either the ''simultaneous'' [5] or the ''sequential'' approach [8]. In the former, a single site selective pulse RF profile that operates at null offset is multiplied by a ν_0 frequency cosine function that produces modulation side bands at $+\nu_0$ and $-\nu_0$ offsets. The ''sequential'' approach is even simpler: the two-site refocusing pulse sequence is built by concatenation of two single site soft refocusing pulses. One of them operate at the $+\nu_0$ offset and the other one at the $-\nu_0$ offset. Although the ''sequential'' procedure is more flexible and easier to implement, the ''simultaneous'' approach was retained for the present work, mainly because a given selectivity is obtained in less time (one pulse length instead of two).

* Fax: +33 (0)3 26 91 35 96.
E-mail address: jm.nuzillard@univ-reims.fr

1090-7807/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2007.11.011

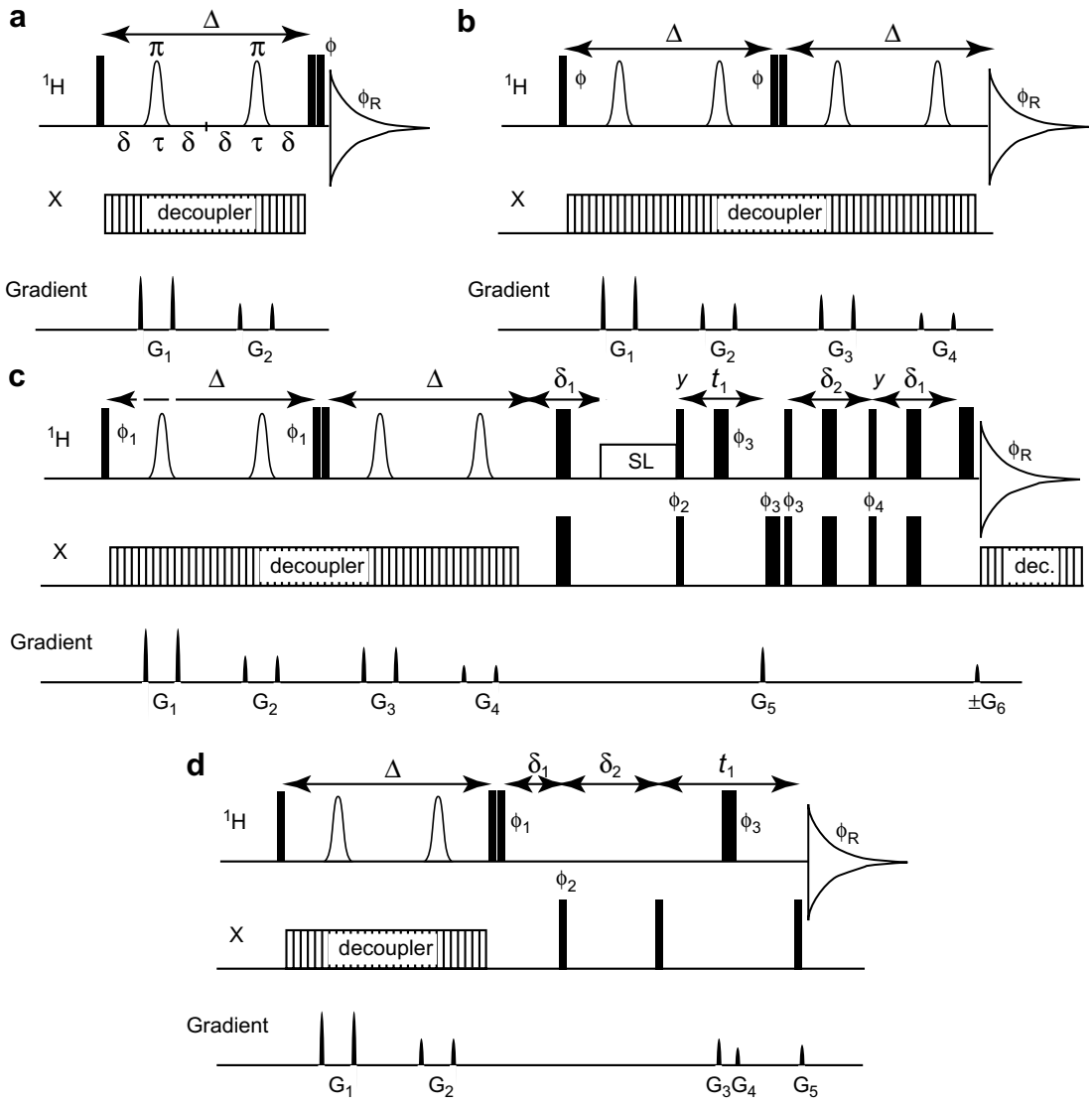

Fig. 1. Pulse sequences of the biselective experiments. Thin and thick black rectangular bars are 90° and 180° degree hard RF pulses, respectively. Their phase is x unless otherwise specified. All bell shaped open symbols are biselective refocusing pulses of x phase. (a) The basic biselective pulse sequence. $\phi = x, -y, -x, y$ and $\phi_R = x, y, -x, -y$. $G_1 : G_2 = 76 : 20$ (relative gradient intensities). (b) Biselective generation of in-phase transverse magnetization. $\phi = x, -y, -x, y$ and $\phi_R = 2(x, -x)$. $G_1 : G_2 : G_3 : G_4 = 76 : 20 : 51 : 27$. (c) The biselective HSQC experiment. $\phi_1 = x, -y, -x, y$, $\phi_2 = 4(x), 4(-x)$, $\phi_3 = 8(x), 8(-x)$, $\phi_4 = 8(y), 8(-y)$ and $\phi_R = 2(x, -x), 4(-x, x), 2(x, -x)$. $G_1 : G_2 : G_3 : G_4 : G_5 : G_6 = 76 : 20 : 51 : 27 : 80 : \pm 20$. $\delta_1 = 1/(4 \times {}^1J(\mathrm{XH}))$. $\delta_2 = 1/(4 \times {}^1J(\mathrm{XH}))$ for XH signals only and $1/(8 \times {}^1J(\mathrm{XH}))$ for all multiplicities. Two FIDs are recorded per t_1 value, with inverted G_6 values to produce an echo/anti-echo phase modulated data set. (d) The biselective HMBC experiment. $\phi_1 = x, -y, -x, y$, $\phi_2 = 4(x), 4(-x)$, $\phi_3 = 8(x), 8(-x)$, $\phi_4 = 8(y), 8(-y)$ and $\phi_R = x, y, 2(-x, -y), x, y$. $G_1 : G_2 : G_3 : G_4 : G_5 = 76 : 20 : 50 : 30 : 40$. $\delta_1 = 1/(2 \times {}^1J(\mathrm{XH}))$ (low pass J filter). δ_2 is the delay for XH long-range coupling evolution (70 ms). The phase modulated data set is transformed in magnitude mode after pseudo-echo filtering.

The present article shows how the module in Fig. 1a that is formed by a biselective DPFGSE sequence followed by a double quantum filter can be used as a spin excitation sequence in gradient enhanced HSQC and HMBC experiments.

2. Results and discussion

The ^{1}H NMR spectrum in Fig. 2a is that of cinnamic acid in $CDCl_3$. The spectrum in Fig. 2b is obtained by selection of the ethylenic protons at 6.54 and 7.86 ppm by the pulse sequence in Fig. 1a in which the ^{13}C decoupler is switched off, as in a purely homonuclear experiment [7]. A high vertical scaling factor was applied to look for ^{13}C satellite signals, that were clearly absent. This observation is consistent with a soft pulse excitation band width of 30 Hz and a ^1J ^{1}H–^{13}C coupling constant of about 160 Hz: the satellite peaks are out of reach of the soft pulses. In these conditions, there is no way to correlate the chemical shift of the protons with those of the attached X atoms. This problem is simply solved by switching the decoupler on. Such a method was already proposed in

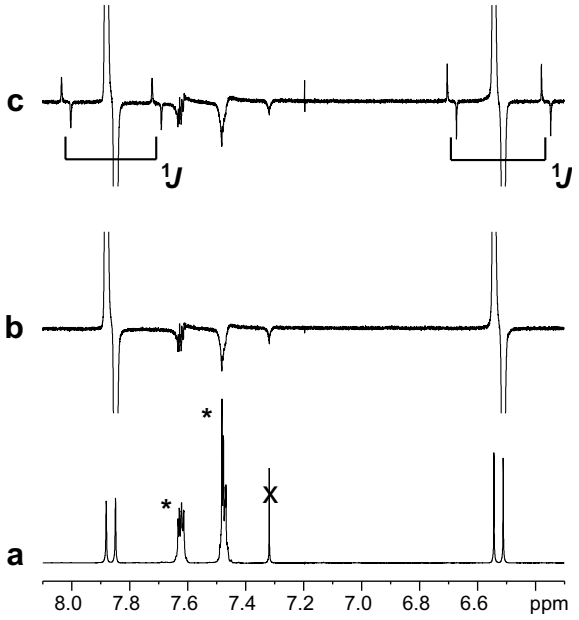

Fig. 2. (a) The standard ^{1}H NMR spectrum of cinnamic acid. The stars indicate the aromatic proton signals and the striked out peak correspond to the $CHCl_3$ solvent residue. (b) The spectrum of cinnamic acid from the pulse sequence in Fig. 1a in which only the olefinic proton magnetization is refocused. No ^{13}C decoupling is applied. The high vertical scaling makes it possible to check for the absence of ^{13}C satellites around the main antiphase doublets (clipped). (c) As in (b), but with the X decoupling on. The antiphase satellite peak pairs become visible.

the reverse context of the HETGOESY experiment, in which selective excitation concerns X nuclei that are directly coupled to protons [11]. The desired X satellite peaks are clearly visible in Fig. 2c.

For all pulse sequences in Fig. 1, the Δ delay value is $4\delta + 2\tau$. The pulse length τ is adjusted to obtain the desired selectivity while δ rules the $f(\delta)$ amount of antiphase magnetization that is delivered by the double quantum filter:

$$f(\delta) = F_0^2 \sin(\pi J(4\delta) + 2\theta_0) + 2F_0F_e \cos(\pi \nu_0 \Delta) \sin(\pi J(4\delta) + \theta_0 + \theta_e) + F_e^2 \cos(2\pi \nu_0 \Delta) \sin(\pi J(4\delta) + 2\theta_e). \quad (1)$$

The F_0, θ_0, F_e and θ_e parameters depend on the initially chosen one site RF intensity profile (before its multiplication by the cosine modulation function), on ν_0, and on J, in a way that is reported in [12], using the theoretical results and methods in [13–15]. The biselective spin echo behavior in Eq. (1) is significantly different from the one obtained in [16]. A free computer program, named Bisel, calculates a table of f values within a given interval of δ values. It is available at http://www.univ-reims.fr/LSD/JmnSoft/Bisel. The shortest δ value that maximises $|f(\delta)|$ is best obtained by plotting f as a function of δ.

Starting a HSQC pulse sequence with a proton antiphase magnetization state does not produce the desired effects (see below). However, the application of a second biselective double echo (pulse sequence in Fig. 1b) back converts antiphase states to the required in-phase states.

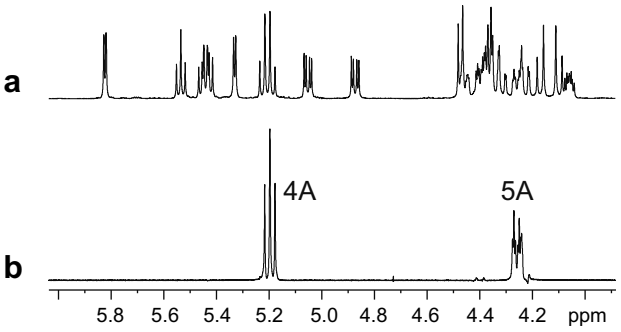

Fig. 3. (a) The ^{1}H NMR spectrum of melezitose peractate (structure in Fig. 4), drawn in the sugar proton domain. (b) The spectrum that is obtained using the pulse sequence in Fig. 1b. Only protons H-4A and H-5A are selected and appear as mostly in-phase multiplets.

This is visible in Fig. 3b, that is recorded for protons H-4 and H-5 in the A sugar unit of melezitose peracetate. The structure of this compound is in Fig. 4 and its standard ^{1}H NMR spectrum in Fig. 3a. The antiphase to in-phase magnetization conversion factor is also $f(\delta)$, as given by Eq. (1). The amount of remaining antiphase states g before signal acquisition must be as small as possible. Like f, g depends on δ, but according to an even more complicated law that is practically approximated by:

$$g(\delta) = F_0^2 \sin(\pi J(4\delta) + 2\theta_0) + 2F_0F_e \cos(\pi \nu_0 \Delta) \cos(\pi J(4\delta) + \theta_0 + \theta_e) + F_e^2 \cos(2\pi \nu_0 \Delta) \cos(\pi J(4\delta) + 2\theta_e). \quad (2)$$

A table of g values is also produced by the Bisel program, so that the user can chose the shortest δ value that maximizes $|f|$ and minimizes $|g|$. Alternatively, the choice of the optimum δ delay may be assisted by any NMR simulation software that handles field gradient pulses.

The pulse sequence in Fig. 1b achieves the in-phase excitation of the X satellites of two protons on the conditions that their offset frequencies are $+\nu_0$ and $-\nu_0$ and that they are coupled together. Their transverse magnetization can thus be engaged in the sensitivity enhanced version of an HSQC experiment [17]. The regular (non selective) HSQC spectrum of melezitose peracetate is presented in Fig. 5a. The H-4A and H-5A biselective HSQC spectrum in

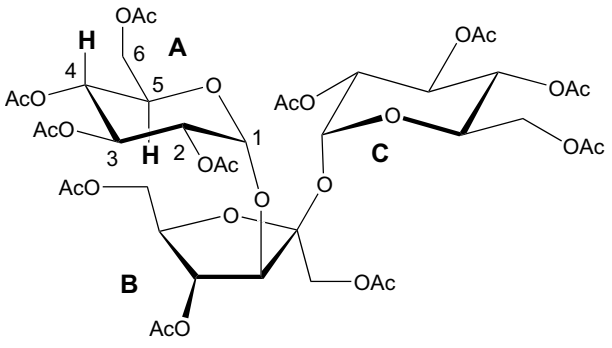

Fig. 4. The structure of melezitose peracetate.

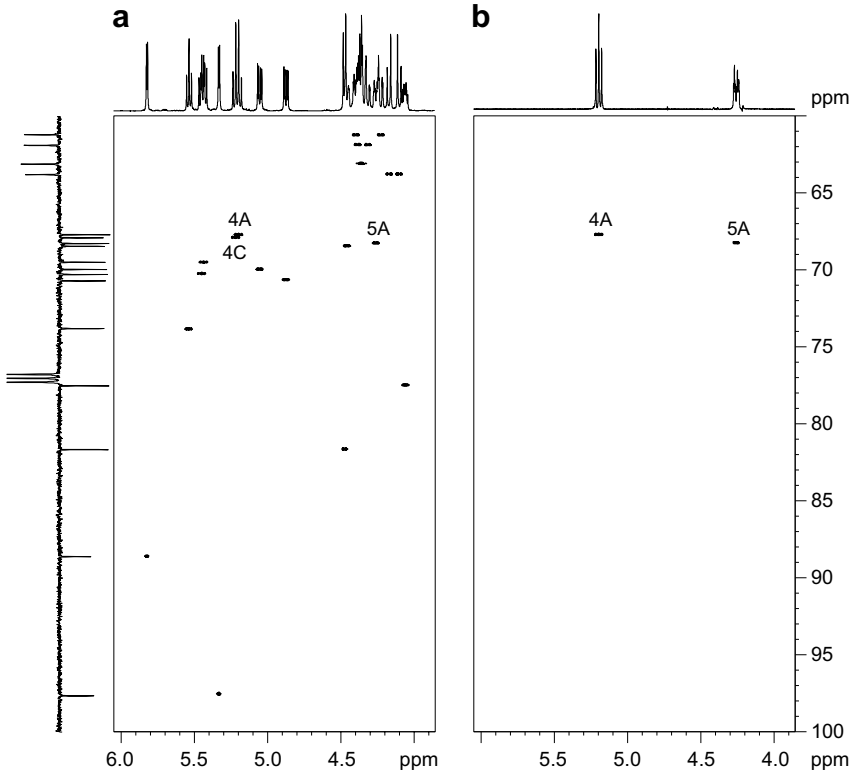

Fig. 5. (a) The ^{1}H–^{13}C HSQC spectrum of melezitose peracetate, drawn in the sugar resonance frequency domains. (b) The biselective spectrum, obtained from the pulse sequence in Fig. 1c.

Fig. 5b shows the degree of spectral simplification that is obtained. The correlation spots of H-4A and H-4C are very close, as shown by Fig. 5a, and the partial superposition of both H-4A and H-5A signals with other ones does not facilitate spectrum interpretation. Any remaining ambiguity is removed by the spectrum in Fig. 5b.

The biselective HSQC sequence (Fig. 1c) must include a proton refocusing double echo because of the presence of the first ^{1}H 90° pulse of phase y. It causes a magnetization transfer between protons that would lead to the correlation of the chemical shift of C-4A with those of both H-4A and H-5A. This undesired transfer is avoided by means of the biselective refocusing double echo. Such a problem does not exist with the HMBC experiment, because it does not involve any heteronuclear magnetization transfer step. The pulse sequence of the biselective HMBC experiment in Fig. 1d is therefore made of the biselective device in Fig. 1a, followed by the gradient-enhanced HMBC pulse sequence [18]. It produces the spectrum in Fig. 6b when protons H-4A and H-4B of melezitose peracetate are selected. The correlation spots of H-5A are clearly visible, even though their intensity is less than those of H-4A. It must be noted that the former are scarcely visible in the non-selective HMBC spectrum in Fig. 6a and that both spectra in Fig. 6 were acquired in equal times (4 h).

Obviously, the presence of double echoes and of quadruple echoes has a non-negligible impact of signal losses due to transverse relaxation. As already stated in [7], single echoes may also be used, at the price of a reduced quality of the pulse selectivity. The magnetization transmission factors f and g then become f_1 and g_1:

$$f_1(\delta) = F_0 \sin(\pi J(2\delta) + \theta_0) + F_e \cos(\pi \nu_0 \Delta) \sin(\pi J(2\delta) + \theta_e) \quad (3)$$

$$g_1(\delta) \approx F_0 \cos(\pi J(2\delta) + \theta_0) + F_e \cos(\pi \nu_0 \Delta) \cos(\pi J(2\delta) + \theta_e) \quad (4)$$

in which $\Delta = 2\delta + \tau$ is still the full echo time. Special care must then be taken to ensure the phase of all soft pulses in their middle point is an exact multiple of 90°. Any phase error would result in an efficiency reduction of the double quantum filter and therefore to signal attenuation.

Protons H-4A and H-5A in melezitose peracetate are good candidates for this study because their resonances are partially superposed with ones from other spin systems. In a real problem, in which the molecular structure is unknown, each COSY correlation peak between such protons provides the information that is required in order to set up a ^{1}H–^{1}H biselective experiment: a pair of chemical shifts and the existence of a scalar coupling. A simple spectrum such as the one provided by the pulse sequence in Fig. 1a, with the X decoupler off, provides an estimation the scalar coupling value of interest and thus allows the spectroscopist to easily set up and optimize a biselective 2D experiment.

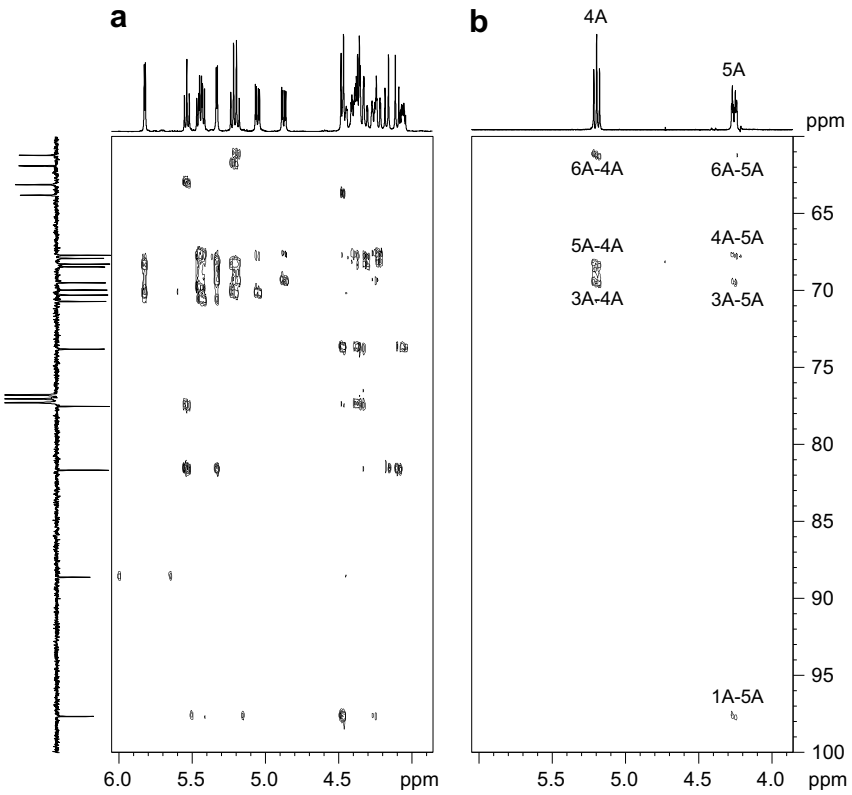

Fig. 6. (a) The ^{1}H–^{13}C HMBC spectrum of melezitose peracetate, drawn in the sugar resonance frequency domains. (b) The biselective spectrum, obtained from the pulse sequence in Fig. 1d.

The experiments that are presented here, like any other selective experiment, are designed for the extraction of a particular piece of information that would otherwise be difficult to obtain in non-selective ways. The investigation of a high number of spin pairs by means of HSQC or HMBC bislective spectra requires the recording of the same number of 2D spectra, a task that can be difficult to perform within the available spectrometer time.

3. Experimental

Spectra are recorded on a Bruker DRX spectrometer operating at 500.13 MHz for the ^{1}H nucleus and fitted with a z gradient, inverse detection probehead. All field gradient pulses are sine bell shaped, of 1 ms duration, and are followed by a 100 μs recovery delay. The minimum δ delay is therefore 1.1 ms long. A gradient pulse relative intensity of 100 gives a 5 mT cm^{-1} field gradient strength. All shaped pulses are derived from a 8 k point, 1% truncated Gaussian pulse (the ''basic'' shape, see below). Broadband heteronucelar decoupling is achieved using GARP.

The sample that is used for the spectra in Fig. 2 is a solution of 10 mg of cinnamic dissolved in 0.7 mL of $CDCl_3$. A 50 ms biselective pulse is obtained by modulation of the basic shape at $\nu_0 = 334.6$ Hz by means of the Bruker shape tool, in order to simultaneously refocus the magnetization of the ethylenic protons. The δ value is 1.1 ms. It allows a theoretical signal transmission factor f equal to 0.91, computed with $J = 15.96$ Hz.

For the spectra in Figs. 3, 5 and 6, the sample is made of 15 mg of melezitose peracetate (structure in Fig. 4) dissolved in 0.7 mL of $CDCl_3$. A 30 ms biselective pulse is obtained by modulation of the basic shape at $\nu_0 = 235.7$ Hz, as required by protons H-4A and H-5A ($J = 10.21$ Hz) A 2.12 ms δ delay ensures a 0.98 theoretical signal transmission factor f and a negligible antiphase to antiphase magnetization preservation during the second double echo (pulse sequences 1b and 1c).

The calibration of a biselective soft pulse is achieved by the searching for the RF power that is required to obtain a 180° degree pulse by means of the corresponding basic RF profile. A 6 dB is then added to take into account the effect of the modulation operation, as a two–fold RF intensity increase is required to manipulate the magnetization of two nuclei instead of one at the same time.

4. Conclusion

The biselective TSETSE–2 experiment was initially designed to probe for the existence of a scalar coupling between two proton signals in crowded spectra [6]. It was then improved by the introduction of the DPFGSE scheme, and extended for the production of in–phase homonuclear magnetization states. This work led to 1D TSETSE–2–TOCSY and 2D TSETSE–2–TOCSY–COSY homonuclear experiments [7]. Adjusting the spin echo δ delays was empirical at that time, as no exact theoretical treatment of biselective refocusing was available. This

now has changed as a consequence of the study of artefacts in the SERF spectra [12]. The present article proposes a practical application of all of these briefly summarized achievements to heteronuclear 2D NMR spectroscopy. The resulting TSETSE–2–HSQC and TSETSE–2–HMBC experiments should find a place in the wide arsenal of techniques that are dedicated to the structure elucidation of complex molecules.

Acknowledgments

I thank Dr. K. Plé for linguistic advice and Dr. C. Bliard for the use of his melezitose peracetate sample.

References

[1] T. Parella, High quality 1D spectra by implementing pulsed-field gradients as the coherence patway selection procedure, Magn. Reson. Chem. 34 (1996) 329–347.
[2] H. Kessler, S. Mronga, G. Geemecker, Multi-dimensional NMR experiments using selective pulses, Magn. Reson. Chem. 29 (1991) 527–557.
[3] Ē. Kupče, J.-M. Nuzillard, V.-S. Dimitrov, R. Freeman, A new form of correlation spectroscopy, J. Magn. Reson. A 107 (1994) 246–250.
[4] J.-M. Nuzillard, R. Freeman, Correlation spectroscopy with two simultaneous soft pulses (TSETSE), J. Magn. Reson. A 112 (1995) 72–82.
[5] L. Emsley, I. Burghardt, G. Bodenhausen, Double selective inversion in NMR and multiple quantum effects in coupled spin systems, J. Magn. Reson. 90 (1990) 214–220.
[6] X. Miao, R. Freeman, a spin-echo technique for separation of multiplets in crowded spectra, J. Magn. Reson. A 116 (1995) 273–276.
[7] S. Bourg, J.-M. Nuzillard, In phase double selective exitation of coupled spin systems using excitation sculpting, J. Magn. Reson. 133 (1998) 173–176.
[8] T. Parella, F. Sánchez-Ferrando, A. Virgili, A simple approach for ultraclean multisite excitation using excitation sculpting, J. Magn. Reson. 135 (1998) 50–53.
[9] F. Rastrelli, A. Bagno, Selective *J*-resolved spectra: a double pulsed field gradient spin-echo approach, J. Magn. Reson. 182 (2006) 29–37.
[10] T.-L. Hwang, A.J. Shaka, Water suppression that works. Excitation sculpting using arbitrary waveforms and pulsed field gradients, J. Magn. Reson. A (1995) 275–279.
[11] K. Scott, J. Keeler, Gradient-enhanced one-dimensional heteronuclear nOe experiment with ^{1}H detection, Magn. Reson. Chem. (1996) 554–558.
[12] J.-M. Nuzillard, Biselective refocusing pulses and the SERF experiment, J. Magn. Reson. 187 (2007) 193–198.
[13] V. Blechta, J. Schraml, Selective on-resonance double pulses applied to an IS spin system, J. Magn. Reson. A 112 (1995) 30–35.
[14] V. Zhou, C. Ye, B.C. Sanctuary, Response of a coupled two-spin system to on-resonance amplitude modulated RF pulses, Molecular Phys. 87 (1996) 679–689.
[15] J. Cavanagh, W.J. Fairbrother, A.G. Palmer III, N.J. Skelton, Protein NMR Spectroscopy: Principles and Practice, Academic Press, San Diego, 1996, pp. 39–41.
[16] X. Miao, R. Freeman, Spin-echo modulation experiments with soft gaussian pulses, J. Magn. Reson. A 119 (1996) 90–100.
[17] L.E. Kay, P. Keifer, T. Saarinen, Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity, J. Am. Chem. Soc. 114 (1992) 10663–10665.
[18] R.E. Hurd, B.K. John, Gradient-enhanced proton-detected heteronuclear multiple-quantum coherence spectroscopy, J. Magn. Reson. 91 (1991) 648–653.