Communication

IFSERF, an isotope-filtered SERF experiment for the precise measurement of proton–proton coupling constants between chemically equivalent protons

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Abstract

An isotope-filtered selective refocusing (IFSERF) experiment is presented for the sensitive and precise measurement of the proton–proton coupling constant between chemically equivalent protons. The 2D NMR method combines an initial doubly selective isotope filter based on heteronuclear cross-polarization followed by a selective *J*-resolved block. The coupling topologies obtained from several 2D variants of the IFSERF experiment are described for the simultaneous measurement of both proton–proton and proton–carbon coupling constants in the involved AA′XX′ spin system. Application on the determination of the relative configuration of double bonds in symmetrical molecules is illustrated.
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1. Introduction

Frequently, solution-state NMR studies on symmetrical molecules present a great interest due to their non-evident spectral analysis. Thus, the measurement of the mutual proton–proton coupling constant, *J*(HH), or the detection of the corresponding NOE between two chemically equivalent protons can not be performed by routine NMR methods. This is mainly due to the degeneracy of the NMR resonances that provides an apparent spectral simplicity, even in the case of smallest and simplest molecular structures but having some characteristic symmetry element such as a C_n axis or a symmetry plane. For instance, fundamental but not obvious structural issues such as the relative configuration of 1, 2-equally disubstituted olefinic or ethylenic oxides, the structural determination of symmetric polycyclic

aromatic hydrocarbon derivatives or of symmetric organometallic compounds could be some examples of these challenging topics already discussed in the past. So far, the measurement of *J*(HH) on these equivalent systems has been often carried out by direct analysis of 1D ^{1}H-coupled ^{13}C spectra or the corresponding ^{13}C satellites in ^{1}H spectra by optional aid of selective CW decoupling [1] or by simulation of the resulting multiplets in more complicated cases and, most recently, by analyzing the satellite lines in X-coupled inverse correlation 2D HMQC or 2D HSQC experiments [2]. However, all these classical approaches suffer of improper accuracy and precision and they are only relatively helpful in very simple spin systems. The successful application of these traditional methods is generally hampered when applied on resonances displaying complex multiplet patterns, when many different and small proton–proton coupling constants are involved, or when other overlapped resonances interfere the analysis. More recently, an innovative way to measure these coupling constants from the

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indirect F1 dimension of a 2D X-filtered TOCSY–HSQC map has been reported [3]. The authors proposed a variable t_1 period consisting of a selective TOCSY process between the involved protons followed by an isotope filter. The desired $J(HH)$ coupling value can be directly extracted from the doublet obtained in the indirect dimension of the 2D spectrum.

In this communication we report an alternative doubly selective NMR pulse scheme for the simple, sensitive, and precise measurement of $J(HH)$ between chemically equivalent protons. The coupling constant value is extracted directly from the doublet originated in the indirect dimension of a 2D J-resolved spectrum and without need of any post-processing protocol. The pulse scheme can be easily turn into other interesting applications, such as the measurement of the one-bond and two-bond proton–carbon coupling constants of the involved proton and carbon nuclei or the design of carbon-edited NOE experiments that can afford additional and complementary data to resolve structural question on such symmetric spin systems and, in addition, can extent its application to other molecules that do not exhibit symmetrical properties.

2. NMR experiments

The isotope-filtered selective refocusing (IFSERF) pulse sequence presented here (Fig. 1) to measure $J(HH)$ in symmetrical molecules of the general type $R_1CH{=}CHR_1$ and $R_1R_2CH{-}CHR_1R_2$, can be under-
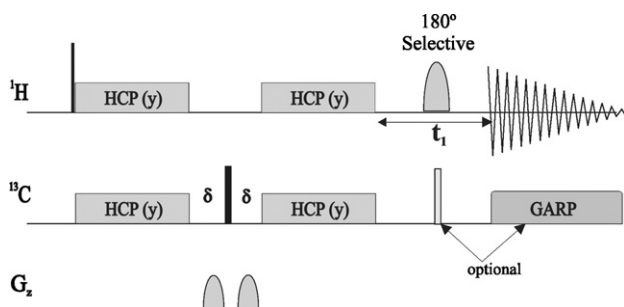

Fig. 1. General pulse scheme of the 2D ^1H–^{13}C IFSERF experiment to measure proton–proton and proton–carbon coupling constants in symmetrical AA′XX′ spin systems. A minimum four-step exorcycle phase cycle is applied on the 180° X pulse ($x, y, -x, -y$) and the receiver ($x, -x$). For selective transfer, the HCP mixing scheme is executed as selective continuous-wave CW RF-fields simultaneously applied from the y axis in both channels during a Δ period. Pulsed-field gradients of duration δ applied with a 1:1 ratio are indicated by shaded shapes on the line G_z. ^1H and ^{13}C offsets are set on resonance of the selected resonances through out the experiment. An optional non-selective 180° X pulse can be also applied simultaneously to the selective 180° ^1H pulse to refocus the large heteronuclear coupling constant in the indirect dimension. Experimental aspects such as the length of the selective 180° pulse, the heteronuclear X decoupling during acquisition or the application of this 180° X pulse in the middle of the t_1 period are illustrated in Fig. 2 and discussed along the text.

stood as an isotope-filtered version of the SERF experiment [4]. The pulse scheme consists of two independent and well differentiated parts. The first part is a selective isotope filter designed to breakdown the molecular symmetry by selecting the corresponding ^{13}C-bound proton signal. This filter is made by a two-way doubly selective heteronuclear cross-polarization (HCP) scheme that offers excellent selective excitation and sensitivity properties [5–7]. Briefly, after the initial 90° (^1H) pulse applied from the x axis, in-phase coherence transfer takes place during the doubly selective HCP(y) period, optimized to $\tau = 1/^1J(^1H–^{13}C)$, where cross-polarization, and selective excitation is performed simultaneously by applying weak RF continuous-wave fields under HCP conditions on-resonance to both ^1H and ^{13}C spins from the parallel y axis. After a dephasing period in which a gradient carbon echo purgues undesired magnetization, a second HCP(y) block, identical to the first HCP block, transfers back the selected in-phase ^{13}C magnetization to its directly attached ^1H nucleus also via $^1J(^1H–^{13}C)$. The result of this initial step is a clean in-phase proton excitation of the ^{13}C-bound proton multiplet from which the direct one-bond proton–carbon, $^1J(CH)$, and the vicinal proton–proton, $^3J(HH)$, coupling constants could be directly extracted in a 1D spectrum only for signals displaying simple satellite multiplet patterns with clearly resolved splittings.

However, in the general case of more complex multiplet shapes it is essentially impossible the direct extraction of $J(HH)$ from these 1D spectra with some reasonable accuracy and, therefore, improved NMR methods to be applied on a wide range of situations are strongly required. Thereby, in these cases the addition of a doubly selective spin–echo building block after the described isotope filter can greatly facilitate such measurements. The general idea we propose is quite similar to the SERF experiment which was reported to measure specifically the coupling constant value between two previously chosen proton resonances using a J-resolved type experiment [4]. However, as a major feature of our IFSERF experiment, the selective 180° proton pulse applied in the middle of the variable t_1 period does not need to be double-frequency selective. Thus, a standard gaussian shaped 180° pulse with a duration around 3 ms will invert simultaneously both ^{13}C- and ^{12}C-bound protons and, therefore, only this mutual coupling constant will be modulated during the t_1 period whereas all other homonuclear couplings will be efficiently refocused. At the end, proton detection with ^{13}C GARP decoupling recovers the magnetic equivalence and the multiplet structure is conveniently collapsed in the detected F2 dimension, resulting in an optimum gain in sensitivity. Thus, the active $J(HH)$ coupling constant value can be directly extracted with good precision from the doublet originated in the indirect F1 dimension of the 2D J-resolved map. The starting isotope filter offers

excellent ^{1}H–^{12}C suppression and therefore the use of gradients for coherence selection is not really necessary.

3. Results and discussion

To demonstrate the main features of the proposed IFSERF experiment, we have chosen a mixture of the

Table 1
Experimental chemical shifts and coupling constants involving the olefinic protons of **1** and **2**

H		CO$_2$Et	H	H
EtO$_2$C		H	EtO$_2$C	CO$_2$Et
1			**2**	

NMR parameter	Ethyl fumarate (1)	Ethyl maleate (2)
δ	6.82	6.27
$^1J(CH)$	+169.1	+167.6
$^2J(CH)$	−4.5	−2.0
$^3J(HH)$	+15.9	+12.2

two symmetrical diethyl fumarate, **1**, and diethyl maleate, **2**, isomers. The conventional proton spectrum shows two singlets resonating at 6.82δ and 6.27δ ppm, respectively. Although that *cis/trans* configuration in double bonds could be tentatively ascertained from proton chemical shift data, it can be dangerous to apply this trend to other systems and therefore it is highly advisable to get additional and most conclusive NMR data to confirm such verification (Table 1).

Fig. 2 shows several coupling topologies obtained after applying several versions of the 2D IFSERF sequence on the ^{1}H–^{13}C olefinic pair of **2**. The non-symmetrical multiplet structure consists of a large one-bond proton–carbon-coupling constant and a smaller vicinal proton–proton-coupling constant. The experimental variables used on these spectra has been the length/selectivity of the selective 180° proton pulse, the application of an optional hard 180° carbon pulse simultaneously to this selective pulse (an heteronuclear echo) in order to refocus heteronuclear coupling evolution in the F1 dimension and the use of optional ^{13}C decoupling during proton acquisition. When applying a very selective ^{1}H 180° pulse (with a gaussian shape of 48 ms, effective
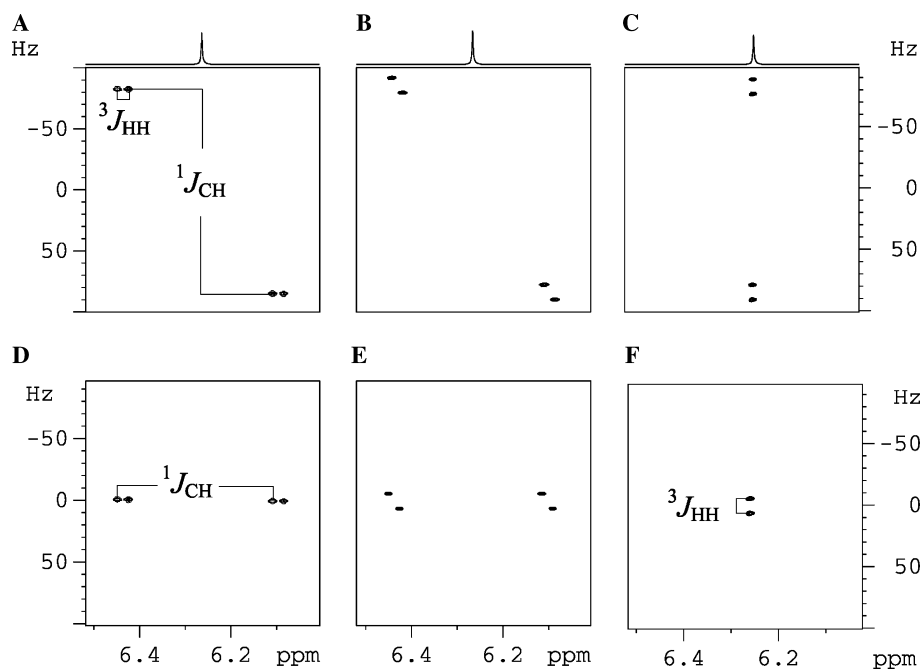

Fig. 2. Experimental spin coupling topology of the ^1H_A spin belonging to a ^1H_A–$^{13}C_A$–$^{12}C_B$–^1H_B moiety after applying the IFSERF experiment of Fig. 1 under different conditions: (A) Selective 180° ^{1}H pulse of 48 ms and no heteronuclear decoupling during acquisition; (B) Selective 180° ^{1}H pulse of 3 ms applied simultaneously to a hard ^{13}C 180 pulse and without heteronuclear decoupling during acquisition; (C) as B but with decoupling during acquisition; (D) as A but applying a hard 180° ^{13}C pulse simultaneously to the selective pulse; (E) as B but without the 180° ^{13}C pulse; and (F) as E with heteronuclear decoupling during acquisition. All 2D spectra have been recorded on a BRUKER AVANCE 500 MHz spectrometer equipped with a triple-resonance inverse probe head incorporating a *z*-gradient coil. The sample used consists of a mixture of 30 mg of **1** and 12 mg of **2** dissolved in 0.7 ml CDCl$_3$. The amplitudes of the selective CW RF-fields have been matched to $\gamma^I B_1^I/2\pi = \gamma^S B_1^S/2\pi = 65$ Hz and placed on-resonance to the desired ^1H_A–$^{13}C_A$ pair of compound **2**. The following general experimental conditions were applied: pre-scan delay of 1 s, HCP mixing delay of $\Delta = 1/J(CH) = 7$ ms. Eight scans were acquired for each one of the 32 increments (spectral width of 250 Hz) in the F1 dimension with 1K data points in the F2 dimension. For convenience, data were processed in magnitude mode using an unshifted sinusoidal window function in both dimension and zero filling in F1 to 128 real data points but there is no inconvenient to present data in a phase sensitive mode. See text for more details.

bandwidth of 25 Hz) only the ^{12}C-bound ^{1}H signal is inverted and the satellites ^{13}C-bound signals are not affected during t_1, therefore, a typical E.COSY coupling pattern results showing the one-bond $J(CH)$ as a passive spin (Fig. 2A). If the proton pulse (with a gaussian shape of 3 ms, effective bandwidth of 400 Hz) affects both ^{12}C- and ^{13}C-bound proton resonances, the mutual $J(HH)$ evolves during t_1 whereas the heteronuclear $J(CH)$ is refocused (Fig. 2E). Simultaneously to this selective proton pulse, it is also possible to apply an inversion carbon 180° pulse that modifies the coupling topology accordingly to the refocusing of the heteronuclear coupling during t_1 (compare Figs. 2A and E with D and B, respectively). Finally, if heteronuclear decoupling is applied during acquisition (Figs. 2C and F, with and without the 180° carbon pulse, respectively), the double–doublet splitting is collapsed to the original singlet multiplet structure in the detected F2 dimension affording an important sensitivity gain and allowing the direct extraction of the homonuclear coupling from the doublet displayed in the high-resolved indirect F1 dimension and without further multiplet component analysis (Fig. 2F).

Similar conclusions can be extracted from the analysis of the relayed ^{1}H$_B$ spin (belonging to the ^{1}H$_A$–^{13}C$_A$–^{12}C$_B$–^{1}H$_B$ moiety) in a IFSERF–TOCSY experiment in which a homonuclear ^{1}H–^{1}H TOCSY mixing period is added after the isotope filter to transfer the in-phase magnetization from the selected ^{13}C-bound ^{1}H to the ^{12}C-bound ^{1}H. In this case, a different modulation for the corresponding geminal $^2J(CH)$ and the vicinal $J(HH)$ coupling constants take place (data not shown) and, in cases of small $^2J(CH)$ values, spin-state selective editing properties could be also incorporated obtaining two separate spin-edited multiplets from which the sign and the magnitude of $^2J(CH)$ can be easily measured [6,7].

For simplest spin systems lacking of additional proton–proton coupling constants, such as those found in both **1** and **2** compounds, the IFSERF pulse sequence can be also applied under broadband conditions that means the application of a non-selective isotope filter consisting of a DIPSI-2 pulse scheme simultaneously to both ^{1}H and ^{13}C channels as a HCP scheme and a conventional hard proton 180° pulse in the middle of the t_1 period as the non-selective inversion element. This approach enables the simultaneous measurement of several $J(HH)$ at the same time from a single spectrum and would extend the application of the IFSERF methodology to mixture analysis of isomers. The broadband IFSERF spectrum for the mixture of **1** and **2** shows well-resolved doublet splittings for each resonance (Fig. 3) allowing the quick distinction and unambiguous assignment of the relative *cis/trans* configuration in basis of the general trend that *trans* isomers always exhibit larger $J(HH)$ values than *cis* isomers.

We have also make use of the IFSERF experiment to differentiate and to characterize two symmetrical nitro-

gen-containing 15-membered macrocycle isomers, the *trans* isomer **3** showing a C$_2$ axis and the *cis* isomer **4** showing a symmetry plane [8]. The classical analysis of their ^{13}C satellites by 1D or 2D spectra is highly complicated because of the neighbouring methylene groups rendering the $J(HH)$ determination practically impossible. Individual IFSERF spectra for each isomer (Fig. 4)
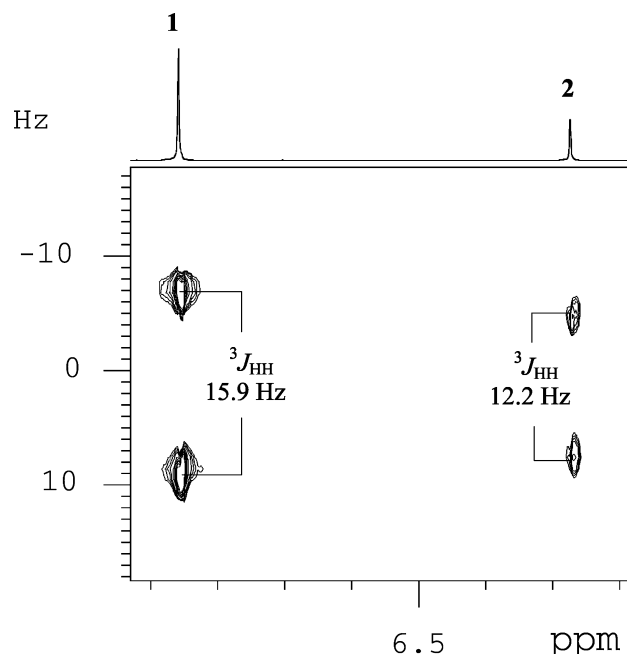

Fig. 3. 2D broadband IFSERF spectrum of a mixture of **1** and **2** using a 5.5 KHz DIPSI-2 pulse train as a non-selective HCP mixing in the initial isotope filter. Eight scans were acquired for each one of the 32 increments (spectral width of 50 Hz) in the F1 dimension with 1K data points in the F2 dimension. All other experimental conditions as described in legend of Fig. 2.
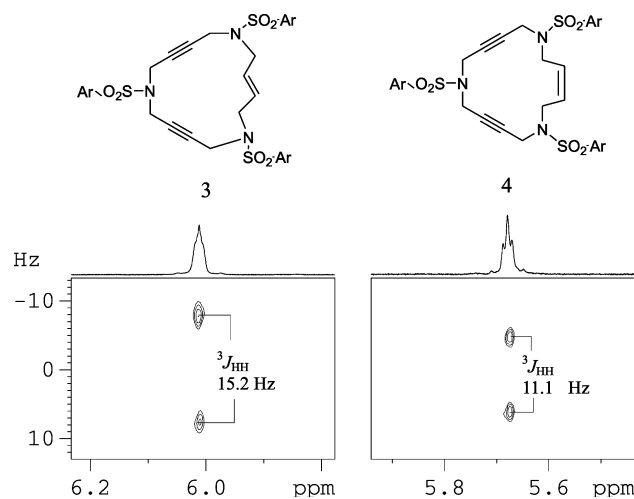

Fig. 4. Separate 2D IFSERF spectra of **3** and **4** acquired as described in Fig. 2. One hundred and twenty-eight scans were acquired for each one of the 32 increments (spectral width of 50 Hz) in the F1 dimension with 1K data points in the F2 dimension.

show clearly the usefulness and the simplicity of the proposed experiment.

Because of the resulting in-phase magnetization after the HCP block, the isotope-filter scheme can be also amenable to be combined with other mixing blocks providing, for instance, useful isotope-filtered NOE-type experiments (IF-NOESY or IF-ROESY experiments) (see [5b]) that could be also found enormous interest in the NOE measurements in other symmetric spin systems lacking for protons, such as tri- and tetra-substituted olefinic systems, in a similar way as reported using a variety of NMR methods such as direct heteronuclear NOE or HOESY experiment [9], HSQC–NOESY-type experiments [10], NOESY–HSQC experiments [11], and selective 1D NOE applied on the satellite lines by double-frequency excitation [12].

4. Conclusions

In summary, a remarkable sensitive and selective method has been developed to yield accurate measurement of the proton–proton coupling constant between chemically equivalent protons. The benefits of the proposed IFSERF experiment has been highlighted in the determination of the relative configuration of symmetrical double bonds and also could be exemplified to many other structural studies involving other different symmetrical spin systems as well as to non-equivalent mutually coupled protons but having the same or similar proton chemical shifts. Due to the highly selective properties of the initial isotope-filtered building block, the experiment can be applicable even in overcrowded proton and carbon spectral regions. As demonstrated for related versions of the SERF experiment, the proposed sequence could be also suitable to other applications as, for instance, in the spectral analysis of enantiomers dissolved in weakly oriented chiral liquid crystals or the measurement of enantiomeric excess [13].

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