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Determination of Magnitudes and Relative Signs of $^{1}H-^{19}F$ Coupling Constants through 1D- and 2D-TOCSY Experiments

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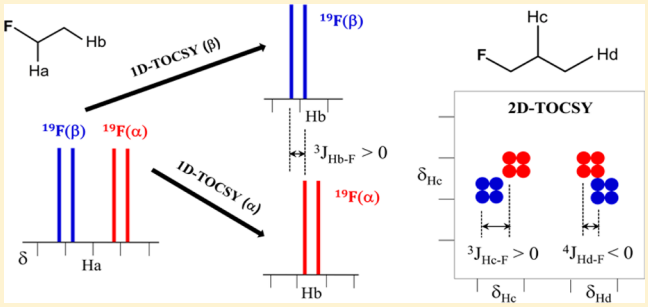
Supporting Information

ABSTRACT: A novel methodology based on 1D- and 2D-TOCSY experiments is described for a quick and accurate measurement of proton-fluorine coupling constants in fluorinated organic compounds. The magnitude of the $^{1}H-^{19}F$ coupling was measured from the displacement between the relayed peaks associated with the α or β spin state of the fluorine, and its relative sign was derived from the sense of the displacement.



Fluorinated compounds are widely used in organic chemistry, medicinal chemistry, and biochemistry.[1] Because of fluorine's unique characteristics, the incorporation of fluorine into an organic molecule may alter its physical and chemical properties, making fluorinated compounds an attractive option to modulate biological activity and enhance ADME properties.[2] In fluorinated compounds structural and conformational information can be derived from $^{1}H-^{19}F$ couplings, which display some differences relative to $^{1}H-^{1}H$ couplings.[3] Geminal $^{1}H-^{19}F$ coupling constants are much larger than $^{1}H-^{1}H$ couplings, and vicinal $^{1}H-^{19}F$ coupling constants exhibit more variation than do $^{1}H-^{1}H$ couplings; longer range $^{1}H-^{19}F$ couplings are also frequently observed. The sign of the coupling is another aspect to consider, and in certain cases, it can be used as a diagnostic tool for structural characterization. For instance, the sign of the $^{1}H-^{19}F$ coupling constant was diagnostic to distinguish between two fluorines coupled to the same proton with a $^{1}H-^{19}F$ coupling of similar magnitude but opposite sign.[4] In contrast to the well-known negative sign of geminal $^{1}H-^{1}H$ couplings, geminal $^{1}H-^{19}F$ couplings are typically positive,[5] and vicinal $^{1}H-^{19}F$ coupling constants can be of either sign.

$^{1}H-^{19}F$ coupling constants are usually measured in terms of peak separation on the multiplets of protons coupled to fluorine in a ^{1}H spectrum. In the case of complex multiplets, the extraction of the $^{1}H-^{19}F$ couplings is not as simple, and if the NMR probehead has a fluorine channel, the multiplet can be simplified by fluorine decoupling or, alternatively, the coupling constants can be derived from the fluorine multiplet in the ^{19}F spectrum with the aid of proton decoupling. The combination of ^{1}H and ^{19}F experiments, however, does not suffice for unresolved peaks or when the proton-fluorine coupling constant is of small magnitude, which is a common situation for $^{1}H-^{19}F$ couplings over three bonds. In addition, no information about the sign of the coupling can be determined in a ^{1}H spectrum. 2D *J*-Resolved ^{1}H NMR can be used to separate $^{1}H-^{1}H$ couplings from $^{1}H-^{19}F$ couplings that are displayed on different axes of the 2D spectrum.[6] The $^{1}H-^{19}F$ couplings are easily detected on the F2-axis, although values smaller that the signal line width cannot be measured and the experiment only provides the magnitude but not the sign of the coupling.

The relative sign of the $^{1}H-^{19}F$ coupling constant has been traditionally derived from 2D $^{1}H-^{13}C$ NMR spectra as described by Bax and Freeman.[7] A three spin HCF system shows two correlations in the H,C 2D spectrum, corresponding to the α or β spin states of the passive ^{19}F nucleus, which are separated by J_{HF} in the proton dimension and by J_{CF} in the carbon dimension. If the high-frequency cross-peak in the ^{1}H dimension correlates to the high-frequency cross-peak in the ^{13}C dimension, then the H−F and C−F couplings are of the same sign, whereas if the high-frequency cross-peak in the ^{1}H dimension correlates to the low-frequency cross-peak in the ^{13}C dimension, the signs are opposite. More recently, other low-sensitivity heteronuclear 2D-methods based on $^{13}C-^{19}F$ and $^{1}H-^{15}N$ experiments have been developed.[8,9] Lastly, 2D $^{1}H-^{19}F$ experiments represent an attractive option for higher sensitivity,[10] although the major limitation is the requirement of an NMR probe equipped with both proton and fluorine channels.

In this work, it is shown that an accurate measurement of the magnitude and sign of $^{1}H-^{19}F$ coupling constants, including small long-range couplings, can be achieved through high-sensitivity homonuclear TOCSY experiments irrespective of the complexity or broadening of the proton multiplet. The TOCSY experiment is a standard NMR pulse sequence that yields 1D subspectra of protons that belong to the same spin system as a

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proton selectively excited (1D-TOCSY) or 2D-spectra in which cross-peaks of protons of the same spin system are correlated (2D-TOCSY). The use of a 1D- or 2D-NMR approach depends on the existence of a large $^{1}H-^{19}F$ fluorine coupling constant (see below).

Fluorinated Compounds with a CHF Group. In a CH_aF-CH_b spin system, H_a appears as a doublet of doublets with a geminal H_a-F coupling constant and a vicinal H_a-H_b coupling constant. If we take into account that geminal $^{1}H-^{19}F$ couplings are positive,[5] the two high-frequency peaks of the H_a multiplet are associated with the β spin state and the two low-frequency peaks are associated with the α spin state of the ^{19}F nucleus (Figure 1). The peaks associated with each spin state of

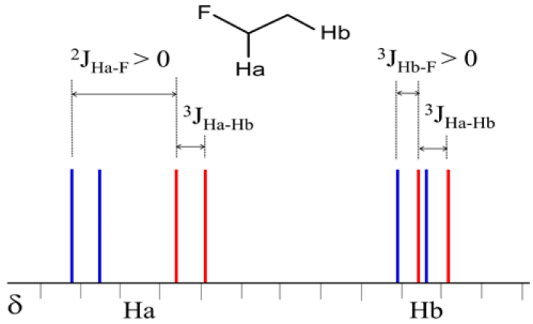
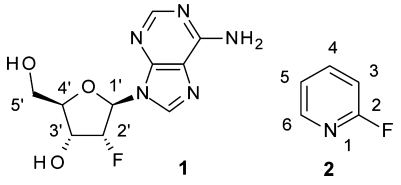


Figure 1. Schematic representation of the ^{1}H spectrum for the model system CH_aF-CH_b. The blue and red lines are associated with the β and α spin states of the ^{19}F nucleus, respectively. A positive sign for the geminal H_a-F coupling is taken into account for this analysis.

fluorine are well-separated by the large geminal H_a-F coupling, and selective excitation using a shaped-pulse can be achieved. If the H_a peaks showing the β state of ^{19}F are selectively excited, magnetization transfer to the coupled H_b using a spin-lock block in a 1D-TOCSY experiment would give rise to the peaks of the H_b multiplet associated with the β spin state of ^{19}F, because the spin state of the passive fluorine nucleus is preserved during the homonuclear spin-lock period (no fluorine pulses are applied). Similarly, the peaks of the H_b multiplet associated with the α spin state of ^{19}F would be obtained if the two low-frequency peaks of the H_a multiplet are selectively excited in a 1D-TOCSY experiment. The relative displacement of the H_b peaks in the two separate 1D-TOCSY spectra would provide the magnitude of the three-bond $^{1}H-^{19}F$ coupling constant, and the sense of the displacement would indicate the sign of the coupling. The use of soft pulses to select specific spin states was previously applied to the determination of the magnitudes and signs of $^{1}H-^{13}C$ coupling constants in heteronuclear 1D-SELINCOR-TOCSY experiments, in which two shaped carbon pulses were used, the first one to select a carbon resonance and the second one to select the spin state.[11]

This approach is demonstrated for the fluorinated adenosine analogue **1** (Scheme 1). In this compound, H2′ is geminal to a

Scheme 1. Structures and Atom Numbering for 1 and 2



fluorine and its signal is composed of two group of peaks separated by a large geminal $^{1}H-^{19}F$ coupling constant (52.8 Hz), exhibiting the β and α spin state of the fluorine nucleus, respectively. When an 80 ms Gaussian-shaped pulse was set to the high-frequency component, the resulting 1D-TOCSY spectrum featured the peaks of ribose protons (H1′, H3′, H4′) that are part of the same spin system as H2′ and are associated with the β spin state of ^{19}F, while excitation of the low-frequency component resulted in a TOCSY spectrum with the peaks corresponding to the α spin state of ^{19}F. The values of the H*i*-F2′ couplings were easily derived from the relative displacement between peaks when the two spin-state selective TOCSY subspectra were compared (Figure 2). The values of

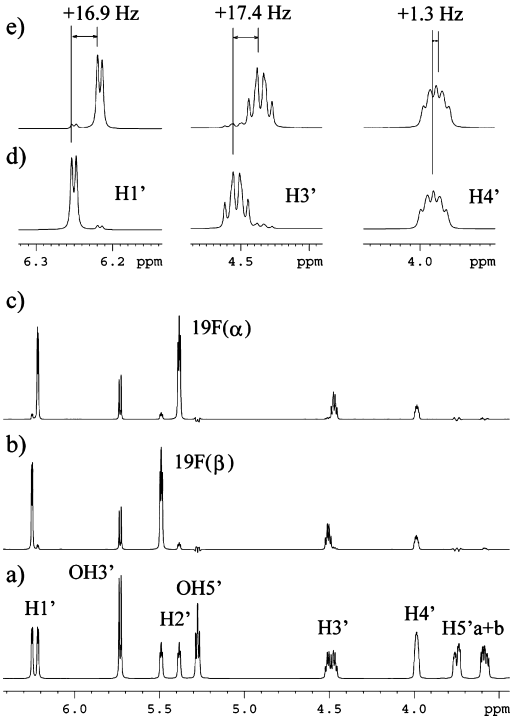


Figure 2. (a) ^{1}H spectrum of **1** in DMSO; (b–c) 1D-TOCSY spectra in which a Gaussian-shaped proton pulse was set to the H2′ peaks associated with the β (b) or α (c) spin states of F2′; (d–e) Expansions of the $^{19}F(\beta)$ 1D-TOCSY (d) and $^{19}F(\alpha)$1D-TOCSY (e) subspectra.

the vicinal H1′–F2′ and H3′–F2′ coupling constants obtained with this approach (16.9 and 17.4 Hz) were the same as those measured from the ^{1}H spectrum to an accuracy of ±0.1 Hz. However, the four-bond H4′–F2′ coupling constant (1.3 Hz) was too small to be accurately measured or even inferred from the unresolved H4′ signal in the ^{1}H spectrum. In addition, the fact that excitation of the high-frequency peaks of H2′ resulted in relayed peaks at a higher frequency than those arising from the excitation of the low-frequency peaks revealed that the signs of the H*i*–F2′ couplings are the same as the sign of the geminal H2′–F2′ coupling and, therefore, all the $^{1}H-^{19}F$ coupling constants of **1** are positive.

Fluorinated Compounds without a CHF Group. In the absence of a large geminal $^{1}H-^{19}F$ coupling constant, selective excitation in a 1D-TOCSY experiment was not feasible and a selective variant of the conventional 2D-TOCSY experiment with improved F_1 resolution was utilized. The modified pulse sequence includes a selective pulsed field gradient spin–echo after the initial 90° proton pulse to select a specific proton

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coupled to the fluorine that evolves during t_1 and then transfer magnetization to other protons of the same spin system during the spin-lock period (see the Supporting Information). The final 2D-spectrum exhibits cross-peaks between the selected proton that appears in the F_1 dimension and the coupled protons in the direct F_2 dimension. Analogous to the 1D-based approach, the spin state of the fluorine nucleus is preserved in the course of the experiment and, in the resulting 2D-TOCSY spectrum, the cross-peaks exhibiting a particular ^{19}F spin state would appear at a different F_2-frequency than the cross-peaks with the opposite spin state.

This second approach was exemplified for 2-fluoropyridine (**2**). A selective 2D-TOCSY experiment was performed in which the offset of the Gaussian-pulse was set to H4 (Figure 3a). Identification of the peaks corresponding to different spin

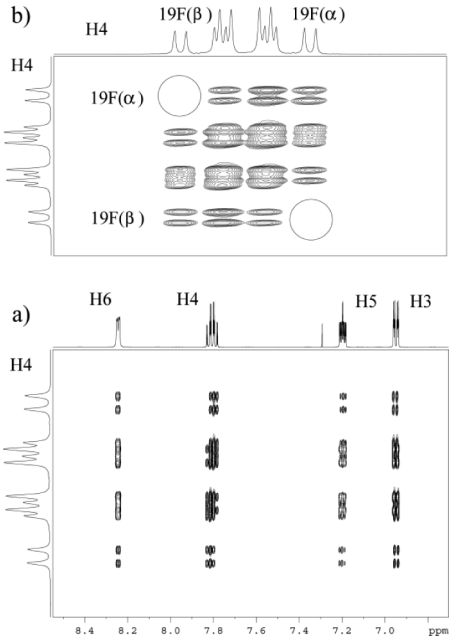

Figure 3. (a) Proton-selective 2D-TOCSY experiment in which a Gaussian-shaped pulse was set to H4. (b) Expansion of the H4/H4 region illustrating how to identify peaks associated with different spin states of ^{19}F (see text). A positive sign was assumed for the H4–F2 coupling constant.

states of fluorine was easily achieved through the analysis of the H4 multiplet, since the peaks associated with the $^{19}F(\beta)$ spin state do not correlate to those associated with the $^{19}F(\alpha)$ spin state. The absence of correlation between the two high-frequency peaks and the two low-frequency peaks of H4 revealed that they correspond to opposite spin states of ^{19}F (Figure 3b). Then, the magnitude of the $^1H-^{19}F$ coupling for each proton coupled to H4 was derived from the F2-displacement between peaks showing opposite spin states of ^{19}F, and the relative sign from the sense of the displacement (Figure 4), in a similar way to the 1D-TOCSY-based approach. It is worth noting that extraction of accurate values from the ^1H spectrum was not possible for $^3J_{H3-F2}$ and $^4J_{H6-F2}$ because the relevant peaks were unresolved on the H3 and H6 multiplets. Furthermore, it was deduced that the *meta* H4–F2 and *para* H5–F2 coupling constants are of opposite sign to the *ortho* H3–F2 and *meta* H6–F2 couplings. The coupling constants are collected in Table 1 together with the values previously reported for this compound using lower sensitivity 2D $^1H-^{13}C$

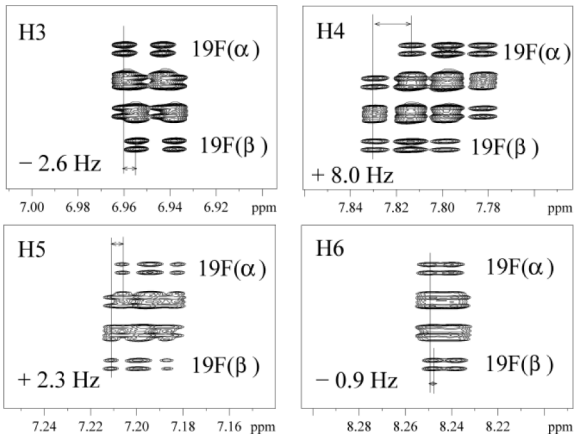

Figure 4. Expansions of the proton-selective 2D-TOCSY experiment in which a Gaussian-shaped pulse was set to H4. The determination of the magnitudes and signs for $^1H-^{19}F$ couplings from the F2 displacement of peaks associated with opposite spin states of ^{19}F is shown. A positive sign was assumed for the H4–F2 coupling.

correlation experiments.[12] Good agreement between both sets of data is observed.

Table 1. Experimental $^1H-^{19}F$ Coupling Constants Determined through 2D-TOCSY (this work) and through $^1H-^{13}C$ Correlation Experiments[12]

$^1H-^{19}F$ pair	selective 2D-TOCSY	$^1H-^{13}C$ correlation
H3–F2	2.6 Hz, opposite sign	−2.4 Hz
H4–F2	8.0 Hz, same sign	+8.5 Hz
H5–F2	2.3 Hz, same sign	+1.9 Hz
H6–F2	0.9 Hz, opposite sign	−1.0 Hz

In summary, a novel NMR methodology based on selective 1D- or 2D-TOCSY experiments has been developed for the rapid and simple determination of $^1H-^{19}F$ coupling constants for monofluorinated compounds. In the presence of a large geminal $^1H-^{19}F$ coupling, selective excitation of a proton peak associated with a specific spin state of the fluorine nucleus followed by a homonuclear mixing step provides a 1D-spectrum of proton signals showing only one spin state of the fluorine. Comparison of the $^{19}F(\alpha)$ and $^{19}F(\beta)$ subspectra affords the magnitude and relative sign of the $^1H-^{19}F$ couplings. In the absence of a large germinal coupling, a proton-selective 2D-TOCSY experiment with enhanced F_1 resolution is proposed to determine the $^1H-^{19}F$ couplings in a similar manner. $^1H-^{19}F$ coupling constants can be measured with high accuracy for all types of signals, including broad, unresolved, or complex multiplets, and irrespective of the magnitude of the coupling constant because couplings smaller than the line width can be determined.

The 2D-TOCSY approach method could be potentially applied to polyfluorinated molecules in which a proton is coupled to several fluorines, although the analysis of the 2D-spectrum would be less straightforward than for monofluorinated compounds. For example, if a proton is coupled to two fluorines (F_a and F_b), the extraction of the magnitudes and signs of the two $^1H-^{19}F$ coupling constants would involve identification of the peaks corresponding to each combination of spin states for F_a and F_b [(α,α), (α,β), (β,α), and (β,β)]. The analysis of the TOCSY spectrum may become cumbersome if the number of coupled fluorines is too high and, therefore, the

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simplicity of the proposed approach may be lost. Another drawback is that the assignment of each $^{1}H-^{19}F$ coupling to a particular fluorine nucleus is not possible unless a separate ^{19}F experiment is recorded and an NMR probe with a fluorine channel would be required.

Previous NMR methods aimed at determining the magnitudes and signs of the $^{1}H-^{19}F$ couplings are based on heteronuclear 2D $^{1}H-^{13}C$ experiments of much lower sensitivity or involve 2D $^{1}H-^{19}F$ experiments that require NMR probeheads equipped with both proton and fluorine channels. However, the NMR scheme proposed here is much simpler because it exclusively involves high-sensitivity proton-based experiments that can be acquired with an NMR probehead equipped with a proton channel and gradient capabilities.

EXPERIMENTAL SECTION

Solutions (0.1 M) of 2′-deoxi-2′-fluoroadenosine (**1**) in DMSO-d_6 and 2-fluoropyridine (**2**) in $CDCl_3$ were chosen as model samples of fluorinated compounds. NMR experiments were carried out on a 500 MHz instrument equipped with a TCI cryoprobe incorporating a z-gradient coil. The pulse sequences used for the 1D- and 2D-TOCSY experiments are shown in the Supporting Information. Sine bell-shaped gradients of 1 ms followed by a recovery delay of 100 μs were used for the pulse field gradients. Gradient strengths (20%) were expressed as percentages of an absolute gradient strength of 54 G/cm. The duration of the Gaussian-shaped pulse was 80 ms in the 1D-TOCSY and 20 ms in the 2D-TOCSY experiments. A DIPSY pulse train with a total duration of 40 ms was used for the spin-lock, and the zero quantum filter developed by Keeler and co-workers was incorporated into the pulse sequence.[13] For the 1D-TOCSY experiment the spectral window was 8 ppm and the number of data points was 16K, while for the 2D-TOCSY experiment the spectral windows were 4 (F2) and 0.2 ppm (F1), the number of data points in t_2 was 4K, and the number of increments in t_1 was 256. Zero filling to 32K (1D-TOCSY) and 8K × 1K (2D-TOCSY) data points was applied prior to Fourier transformation.

ASSOCIATED CONTENT

Supporting Information

Schematic representation of the pulse sequences for the 1D- and 2D-TOCSY experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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