^{19}F DOSY NMR analysis for spin systems with $^{n}J_{FF}$ couplings

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Abstract

NMR is powerful method for identification and quantification of drug components and contaminations. These problems present themselves as mixtures, and here one of the most powerful tools is diffusion-ordered spectroscopy (DOSY). DOSY works best when there is no spectral overlap between components, so drugs containing fluorine substituents are well-suited for DOSY analysis as ^{19}F spectra are typically very sparse. Here we demonstrate the use of a modified ^{19}F DOSY experiment (based on the Oneshot sequences) for various fluorinated benzenes. For compounds with significant $^{n}J_{FF}$ coupling constants, as is common, the undesirable J-modulation can be efficiently suppressed using the Oneshot45 pulse sequence. This investigation highlights ^{19}F DOSY as a valuable and robust method for analysis of molecular systems containing fluorine atoms even where there are large fluorine-fluorine couplings.

Introduction

The importance of fluorine in medicinal chemistry is widely recognized. An increasing number of drugs (antidepressant, immunosuppressant, antibacterial and antiviral drugs, etc.) contain fluorine atoms, often within a fluorobenzene system, where the presence of the fluorine atoms is vital for the drug action.[1-3]

NMR spectroscopy is by far the most popular technique for structure elucidation in solution and its application in quality control of medicines is steadily growing.[4] The most informative nuclei are generally ^{1}H and ^{13}C, but sometimes the sheer abundance of signals makes spectra very difficult to interpret. 1D ^{19}F NMR has recently been shown to reinforce ^{1}H NMR analysis of commercial formulations containing fluorine as an active ingredient.[5,6] Using a less common nucleus such as ^{19}F certainly helps resolution, but in a mixture it is often difficult to assign signals to specific mixture components. This sometimes necessitates the use of costly and time-consuming purification procedures before NMR analysis.

However, the advent of DOSY (diffusion-ordered spectroscopy) experiments[7-9] has given the spectroscopist a powerful tool for separating the signals from different compounds based on their diffusion behaviour (i.e. according to size in most instances), by spreading out the signals in a second, diffusion, dimension. A recent example is for the analysis of admixtures of the antibiotic ciprofloxacin[5] where ^{1}H DOSY was used to obtain fingerprints for several formulations, allowing characterization of some of the excipients present in the formulations studied. DOSY is at its most powerful when the spectral resonances are well separated. In this situation differences in diffusion coefficients of less that 1% are distinguishable, for high quality data.[10]

When signals do overlap, more advanced processing can be used but the underlying mathematical difficulties only allow the separation of signals with a much larger difference in diffusion coefficients, and only for a limited, typically 3-4, number

of chemical components.[11-27] The advantages of avoiding overlap have spurred the development of several NMR experiments to simplify spectra[28,29] or to reduce the overlap by using multidimensional experiments.[30-33] The overlap problem is very common in ^{1}H DOSY, so the use of alternative nuclei can be advantageous.[34-36] DOSY is most commonly used to assess relative diffusion coefficients (i.e. sizes), but with some care a reasonable estimate of absolute molecule weight can also be obtained.[37]

The direct combination of ^{19}F NMR and DOSY in a ^{19}F DOSY experiment has the potential to be very useful for studying drug formulations with fluorine-containing compounds that are part of a complex mixture. ^{19}F acquisition ensures that only the compounds of interest are seen (so long as they retain a fluorine atom), that the chances of overlap are small, and DOSY enables the relative sizes of the species to be assessed. Very recently a^{19}F DOSY experiment[38] using a spin-echo scheme was used to separate the (singlet) fluorine signals of four molecules containing CF_3 groups.

Here we present an alternative ^{19}F DOSY pulse sequence, based on the Oneshot sequences,[14,39] with ^{1}H-^{19}F decoupling. This stimulated echo sequence allows better lock stability, has more flexibility in setting the diffusion encoding, and avoids problems with long periods of transverse magnetisation (e.g. T_2 relaxation and J-modulation), but at a cost of up to a factor of two in signal-to-noise ratio (depending on the values of T_1 and T_2). For coupled spins a spin-echo based sequence, as used previously, will suffer from very severe J-modulation, and is rarely an appropriate choice for such systems. The Oneshot sequences advocated here should work well in most situations.

Even in a stimulated echo based sequence, there are problems, although much less pronounced, with J-modulation. The effects of J-modulation due to homonuclear proton-proton couplings can be efficiently suppressed using the Oneshot45 sequence.[14]

However, ^{1}H-^{1}H couplings constants are relatively small (typically up to 20 Hz), while ^{19}F-^{19}Fcan be significantly larger (up to 200 Hz). Therefore the problems posed by J-modulation are expected to be significantly greater for many fluorine systems. Here we illustrate the use of ^{19}F Oneshot and Oneshot45 pulse sequences with ^{1}H-^{19}F decoupling and evaluate their use for ^{19}F DOSY. The sequences are demonstrated using a set of model fluorinated compounds (Fig. 1) with significant J_{FF} couplings.

The fluorinated aromatic compounds of Fig. 1 were chosen as model systems because a number of fluorine-containing pharmaceuticals,[40] such as sitagliptin, viroconazole, ezetimibe, and fluconazole, contain aromatic rings with similar substitution patterns to those present in compounds **1** to **4**.

Experimental Section

All compounds (**1-4**) were obtained commercially (Aldrich) and were used without further purification; samples were prepared using 10 mg of compound in 0.8 mL of DMSO-d_6. ^{19}F{^{1}H} 1D NMR and ^{19}F{^{1}H} DOSY measurements were carried out non-spinning on a 11.74 Tesla Bruker spectrometer equipped with a 5 mm BBO SmartProbe equipped with a z-gradient coil producing a nominal maximum gradient of 50 G cm^{-1}, operating at 499.87 MHz and 470.29 MHz for ^{1}H and ^{19}F respectively.

^{19}F{^{1}H} DOSY data were acquired using the Oneshot[39] and Oneshot45[14] sequences, modified to add broadband (waltz16) ^{1}H decoupling (Fig. 2). The total diffusion-encoding pulse duration δ (p30) was 2.0 ms, the delay for gradient recovery (d16) 1.0 ms, and the diffusion delay Δ (d20) 40 ms for the Oneshot and Oneshot45 sequences, and ten nominal gradient amplitudes were use ranging from 4.8 to 38.4 G cm^{-1}. The experiments were carried out at a nominal probe temperature of 25 °C with standard VT (variable temperature) regulation. DOSY spectra were constructed using

the DOSY Toolbox[15] using a line broadening of 3 Hz and without zero filling. The errors given are the standard errors estimated by the fitting procedure.

Results and Discussion

The Oneshot and Oneshot45 DOSY pulse sequences were modified (Fig. 2) to be suitable for ^{19}F DOSY experiments by incorporating broadband ^{1}H decoupling and switching of the quadruple nucleus probe (QNP) unit to change radiofrequency channel between ^{1}H to ^{19}F. The Bruker pulse programs are included in the supporting information.

The results of ^{1}H and ^{19}F DOSY experiments using the Oneshot sequence of Fig. 2 (without the 45° pulse) for a solution containing 2-fluorophenol (**1**) and 2-fluoroanisole (**2**) are shown in Figure 3.

Judging by the Oneshot ^{1}H DOSY spectrum alone (Fig. 3a) there would appear to be several components in the aromatic region. This is not the case: the peaks at intermediate diffusion coefficients are attributable to resonance overlap in the ^{1}H spectrum for hydrogen atoms from 2-fluorophenol and 2-fluoroanisole (Fig. 1). Due to this overlap and its effect on apparent diffusion coefficients is not possible to determine unambiguously how many chemical species are present in the sample.

However, in the ^{1}H decoupled Oneshot ^{19}F DOSY spectrum of the same sample, the two resonances for fluorine in compounds **1** and **2** (Fig 1) are decoupled from the protons and are well resolved, so two diffusion coefficients are clearly observed, showing that two different fluorinated chemical species are present. These results (Fig. 3) highlight the utility of ^{19}F DOSY for rapidly and easily determining the number of different fluorinated species that are present in an unknown sample (provided that they diffuse at different rates).

To demonstrate some of the challenges of ^{19}F DOSY, the standard Oneshot ^{19}F experiment was also performed for a solution of 1-bromo-2,3-difluorobenzene (**3**) in DMSO-d$_6$ (Fig. 4). The two resonances for fluorine atoms present in benzene ring (Fig. 1) are perfectly decoupled from the protons. However, the *J*-modulation due to homonuclear vicinal fluorine couplings distorts the doublets (Fig. 4a). This distortion is due to the $^3J_{FF}$ coupling, which for compound **3** is 22.6 Hz. During the echo time the coupling evolves to produce *J*-modulation, evident even when the echo time was reduced as much as possible. It is well-known[14] that this effect can significantly affect the diffusion coefficients measured when signals overlap; this is not the case here, but is the norm for more complex samples.

For ^{1}H DOSY the effects of *J*-modulation have been shown to be efficiently suppressed by the Oneshot45 sequence[14], but this sequence has not hitherto been evaluated for ^{19}F DOSY, where the problems are expected to be much worse due to the larger coupling constants. To evaluate the utility of the Oneshot45 sequence it was compared with the Oneshot sequence for compound **3** using identical conditions. As can be seen (Fig. 4b) the *J*-modulation distortion was completely eliminated using Oneshot45 sequence.

The ^{19}F DOSY spectrum from the Oneshot45 experiment shows perfect agreement between the signals in the diffusion dimension (Fig. 5), with a diffusion coefficient of $5.11 \pm 0.02 \times 10^{-10} \text{m}^2 \text{s}^{-1}$.

To demonstrate the potential of the Oneshot45 sequence for ^{19}F-^{19}F coupled systems, a more complex mixture sample containing compounds **3** and **4** was chosen. Compound **4** was chosen due to the range ($^5J_{FF} = 4.4$ Hz, $^4J_{FF} = 13.6$ Hz and $^3J_{FF} = 22.4$ Hz) of the homonuclear coupling constants between fluorine atoms, and because this

fluorine substitution pattern (2,4,5-) is common in fluorine-containing pharmaceuticals, for example in sitagliptin.[40]

The Oneshot45 ^{19}F DOSY spectrum for the mixture of compounds **3** and **4** can be seen in Figure 6. The signals from the two compounds line up as expected in the diffusion dimension, thanks to the well-resolved signals in the ^{19}F dimension, clearly showing that there are two different fluorine-containing compounds present. The diffusion coefficients were determined with good statistical confidence as $4.58\pm0.03\times 10^{-10}$m^2 s^{-1}for compound **4** and $5.04\pm0.01\times 10^{-10}$ m^2 s^{-1}for compound **3**.

A closer inspection of the least attenuated 1D ^{19}F spectrum (Fig. 7a) from the Oneshot45 ^{19}F DOSY present (Figure 6), shows a slight unexpected distortion in the relative amplitude of multiplet components, but there is no corresponding distortion in the diffusion dimension. For the Oneshot sequence the distortion is less pronounced (but of course the *J*-modulation is obvious).

This amplitude distortion observed in the ^{19}F DOSY spectra for compounds **3** and **4** (Fig. 7a) can be explained by off-resonance effects of the radio frequency pulses, and therefore related to the large (compared to proton) spectral width (22 kHz). Here the excitation is not uniform for all resonances, introducing distortion in the relative amplitudes of the signals. The effect is more pronounced when using the Oneshot45 sequence because when the 45° pulse is far from its nominal value some antiphase character is added to the signals, leading to unequal multiplet intensities, although happily the signal phases remain in pure absorption mode. Off-resonance effects are always a concern in ^{19}F NMR, and can where necessary be countered by appropriate use of composite pulses, but it is comforting that even with these amplitude distortions the Oneshot45 sequence provides reliable ^{19}F DOSY spectra.

Conclusion

In the present study it has been demonstrated that ^{19}F DOSY NMR can be used as a powerful, simple, rapid, and versatile technique for fluorinated compounds exhibiting fluorine-fluorine couplings (J_{FF}). The J-modulation due to homonuclear J_{FF} couplings observed with the Oneshot sequence can be completely removed using the Oneshot45 sequence. Examples of potential uses of ^{19}F DOSY of both coupled and uncoupled systems include the analysis of mixtures formed during degradation processes, and the characterization of fluorinated contaminants in pharmaceutical formulations.

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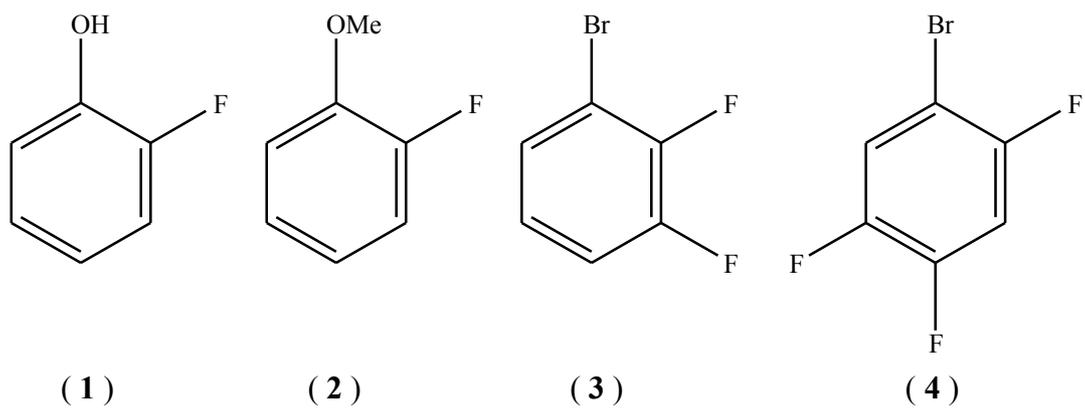
Figure 1. Studied fluorinated compounds: 2-fluorophenol (**1**), 2-fluoroanisole (**2**), 1-bromo-2,3-difluorobenzene (**3**) and1-bromo-2,4,5-trifluorobenzene (**4**).
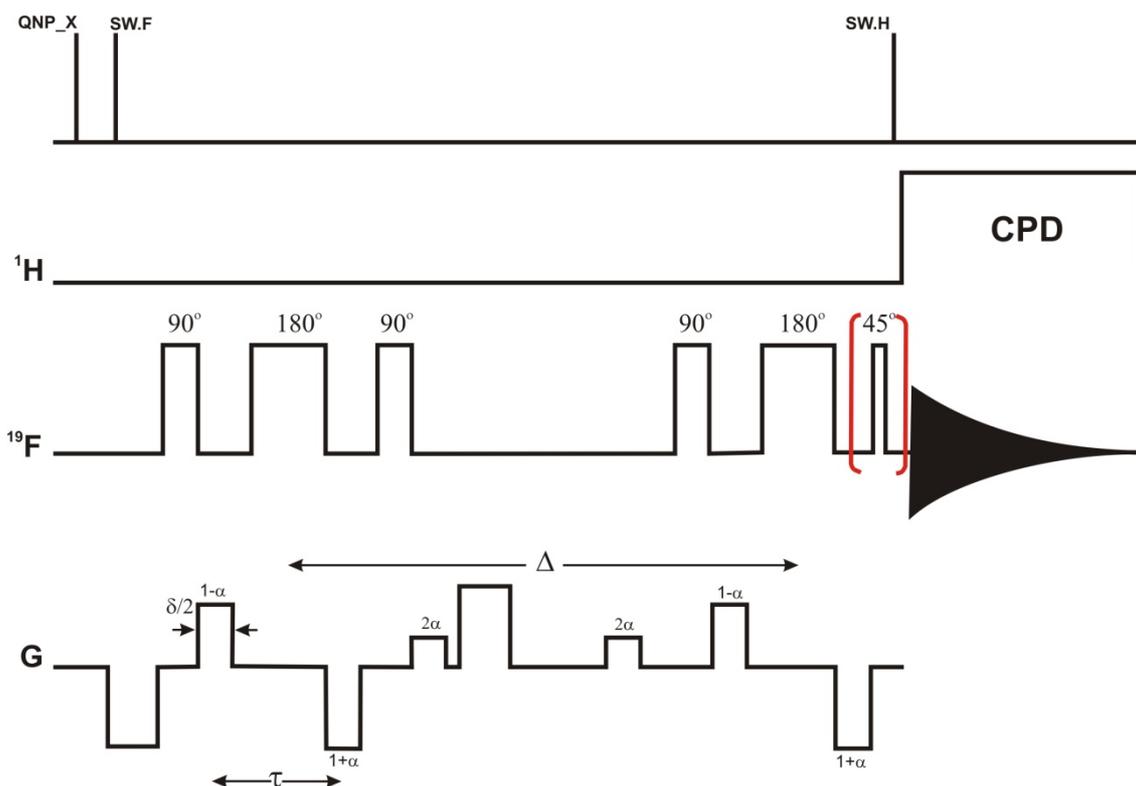
Figure 2. Stimulated echo Oneshot[39] and Oneshot45[14] pulse sequences (with the extra 45° pulse for the latter experiment shown in red brackets), adapted for ^{19}F DOSY by the inclusion of broadband ^1H decoupling and by the addition (top line) of explicit gating between ^1H and ^{19}F channels. The diffusion delay Δ is the time between the midpoints of the two diffusion-encoding periods, τ is the time between the midpoints of the antiphase field gradient pulses within a given diffusion-encoding period, $\delta/2$ is the total diffusion-encoding gradient pulse width, and the amplitudes of the diffusion-encoding gradient pulses are unbalanced by a factor α to suppress unwanted coherence transfer pathways.
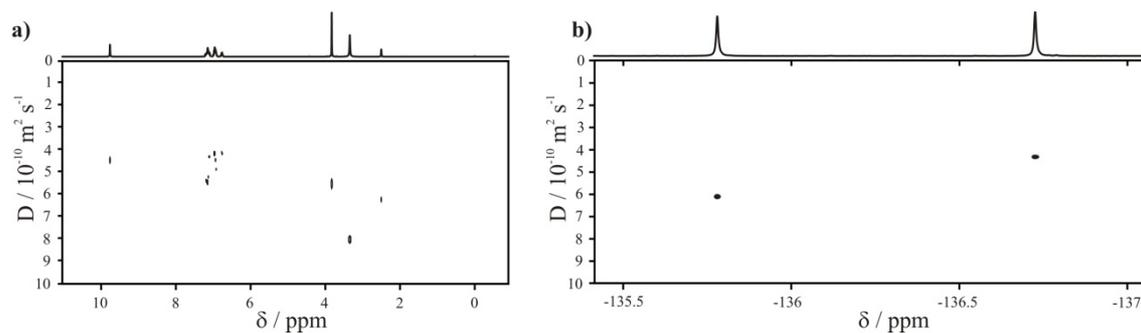
Figure 3. DOSY spectra using Oneshot sequences (Fig. 2) for a sample containing 2-fluorophenol (**1**) and 2-fluoroanisole (**2**) in DMSO-d_6. **a**) 499.87 MHz ^{1}H DOSY spectrum, with the least attenuated 1D spectrum shown at the top; **b**) 470.29 MHz ^{19}F DOSY spectrum with ^{1}H decoupling, again, with the least attenuated 1D spectrum shown at the top.
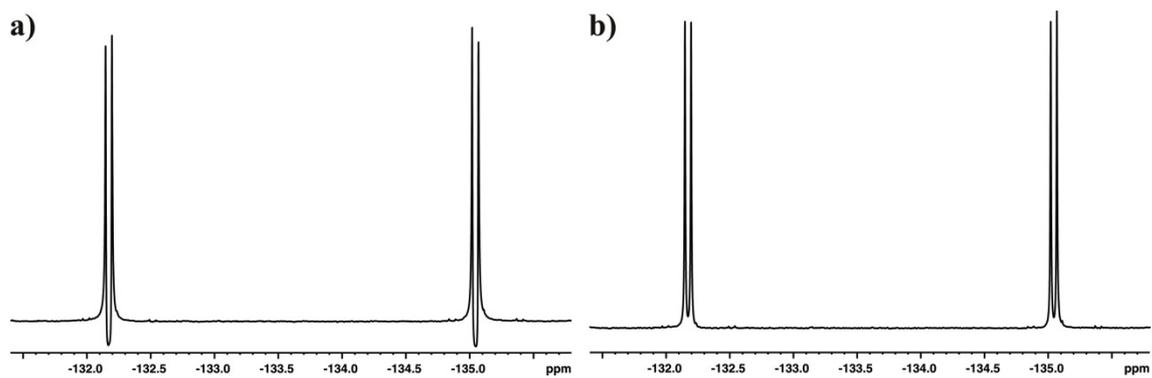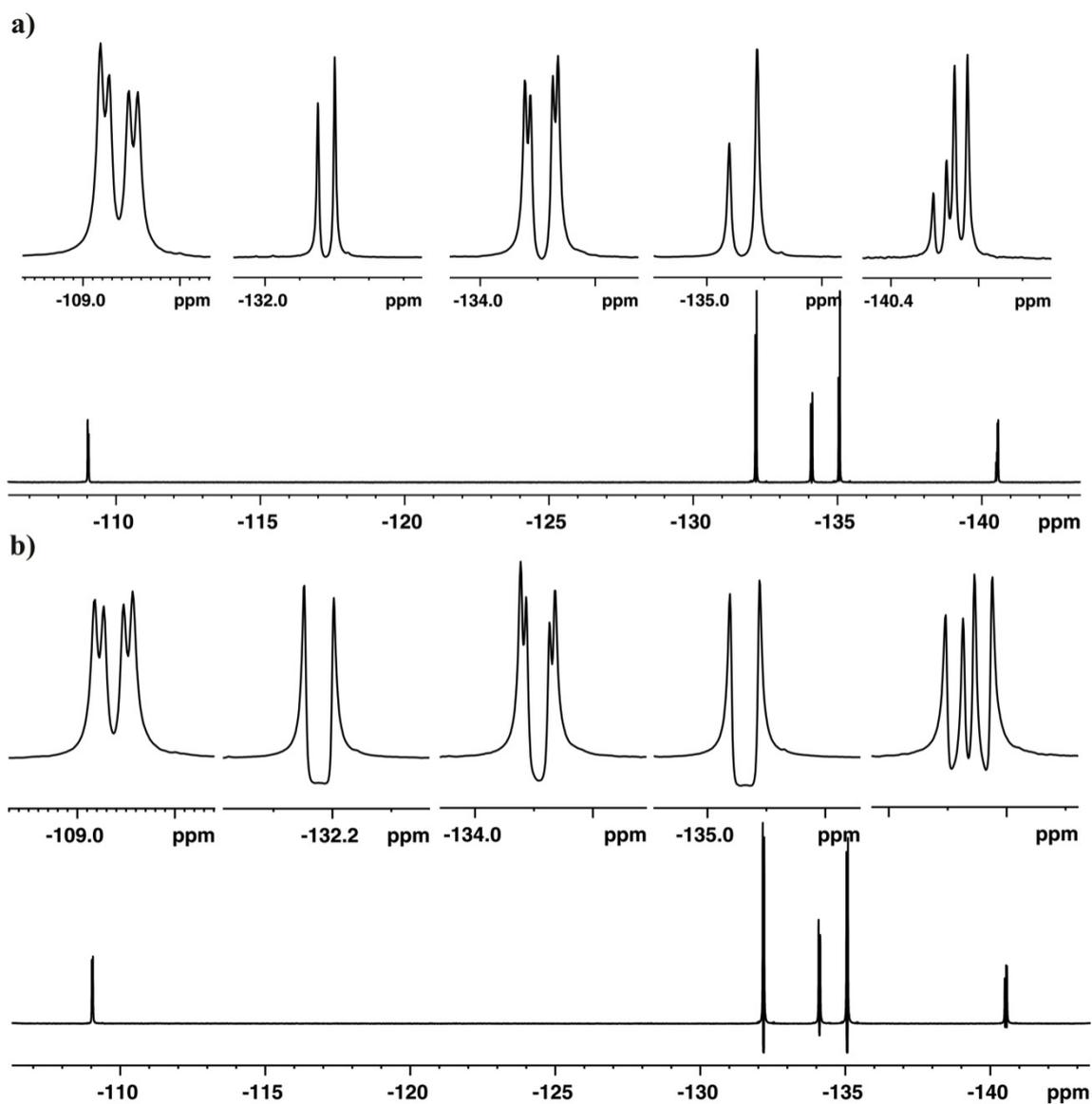
Figure 4. Least attenuated signals from ^{19}F DOSY (470.29 MHz) experiments on 1-bromo-2,3-difluorobenzene (**3**) in DMSO-d$_6$ at 25 °C, using the two pulse sequences from Fig. 2; **a)** Oneshot; **b)** Oneshot45.
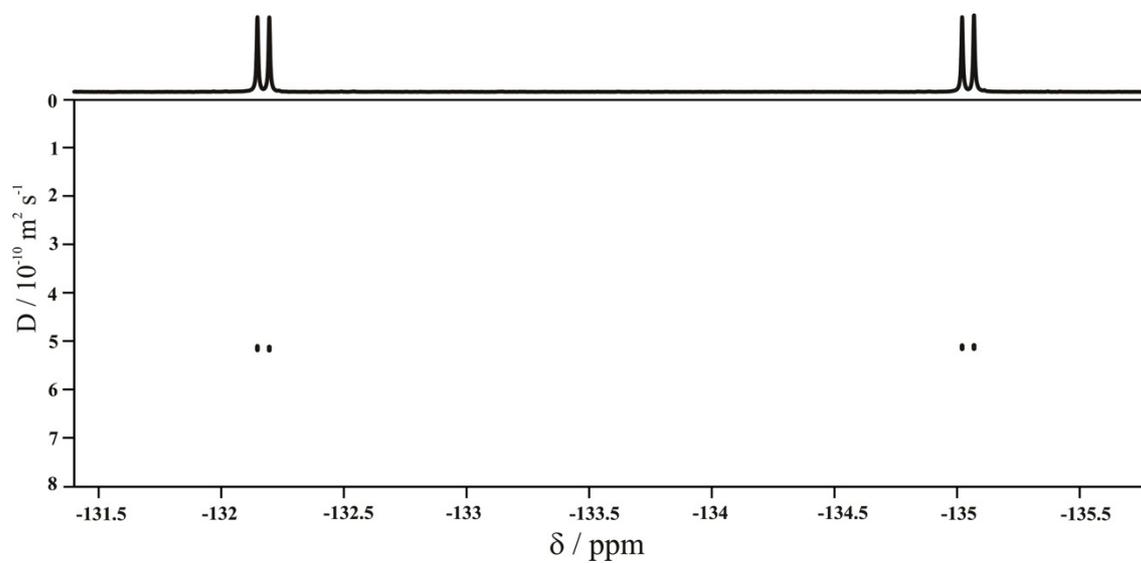
Figure 5. 470.29 MHz ^{19}F DOSY spectrum, with the least attenuated 1D spectrum shown at the top, for 1-bromo-2,3-difluorobenzene (**3**) in DMSO-d$_6$ using the Oneshot45 sequence (Fig 2).
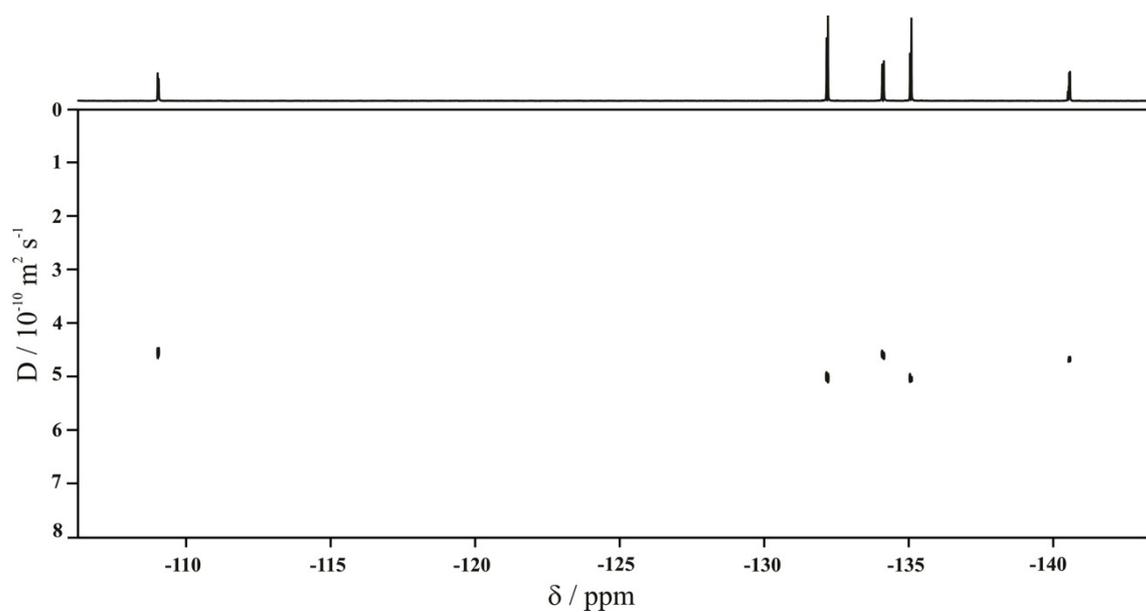
Figure 6. 470.29 MHz ^{19}F DOSY spectrum, with the least attenuated 1D spectrum shown at the top, for a mixture containing compounds **3** and **4** in DMSO-d$_6$ using the Oneshot45 sequence.

Figure 7. Least attenuated signals from ^{19}F DOSY (470.29 MHz) experiments on a mixture containing compounds **3** and **4** in DMSO-d_6 at 25 °C; **a)** using the Oneshot45 pulse sequence; **b)** using the Oneshot sequence.