Improving the accuracy of $^{1}\text{H}$–$^{19}\text{F}$ internuclear distance measurement using 2D $^{1}\text{H}$–$^{19}\text{F}$ HOESY

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Abstract

With the rise in fluorinated pharmaceuticals, it is becoming increasingly important to develop new $^{19}\text{F}$ NMR-based methods to assist in their analysis. Crucially, obtaining information regarding the conformational dynamics of a molecule in solution can aid the design of strongly binding therapeutics. Herein, we report the development of a 2D $^{1}\text{H}$–$^{19}\text{F}$ Heteronuclear Overhauser Spectroscopy (HOESY) experiment to measure $^{1}\text{H}$–$^{19}\text{F}$ internuclear distances, with accuracies of $\sim 5\%$ when compared with $^{1}\text{H}$–$^{19}\text{F}$ internuclear distances calculated by quantum chemical methods. We demonstrate that correcting for cross-relaxation of $^{1}\text{H}$, using the diagonal peaks from the 2D $^{1}\text{H}$–$^{1}\text{H}$ Nuclear Overhauser Enhancement Spectroscopy (NOESY), is critical in obtaining accurate values for $^{1}\text{H}$–$^{19}\text{F}$ internuclear distances. Finally, we show that by using the proposed method to measure $^{1}\text{H}$–$^{19}\text{F}$ internuclear distances, we are able to determine the relative stereochemistry of two fluorinated pharmaceuticals.

KEYWORDS

$^{19}\text{F}$, $^{1}\text{H}$, HOESY, NMR, PANIC, quantitative $^{1}\text{H}$–$^{19}\text{F}$ distances

1 | INTRODUCTION

The development of new $^{19}\text{F}$ NMR-based methods is being driven by the growth in newly approved small molecule drugs containing at least one fluorine atom arising from the beneficial pharmaceutical effects when a hydrogen is replaced with a fluorine including improvements to conformation, potency, and metabolic stability.$^{[2]}$ $^{19}\text{F}$ NMR is also highly attractive as a tool for these systems with 100% natural abundance, $I = \frac{1}{2}$, high gyromagnetic ratio, and minimal spectral overlap (spectral width of $^{19}\text{F}$ is $\gg 400$ ppm, with typically only a handful of $^{19}\text{F}$ environments in a given molecule).$^{[3]}$

Recently, there has been growing emphasis on understanding the conformational bias of both the unbound and bound state of a drug molecule in the design of strongly binding therapeutics.$^{[4]}$ The combination of chemical shift, scalar coupling, and internuclear distance information from NMR spectroscopy is key to this understanding, and maximizing the accuracy of such measurements increases the precision of any such study. Of particular relevance, here is the use of through-space interproton contacts from $^{1}\text{H}$–$^{1}\text{H}$ NOESY experiments, helping to elucidate molecular structure, conformation, and relative stereochemistry.$^{[5–10]}$ Arguably, this technique is the most valuable experimental technique for measuring the crucial conformational dynamics of drug molecules in solution, due to the strong nonlinear distance dependence, which can make it sensitive to vanishingly low levels of minor conformers.$^{[11]}$

We have recently demonstrated that $^{1}\text{H}$–$^{1}\text{H}$ NOESY analysis of small molecules can give accurate quantitative interproton distances, building on improvements in NMR hardware, NOE experimental methods, and data analysis.$^{[11,12]}$ Specifically, the measurement of interproton distances involves a comparison between the relative NOE intensities for spin pairs in transient NOESY experiments. Provided that the molecule is in the fast tumbling regime.
(ωT < <1) and that measurements are made within the initial rate approximation limits, then the ratio of a pair of NOE intensities is proportional to the ratio of their internuclear distances (vide infra).13,14 The key weakness of this approach is the assumption of the initial rate approximation, that is, the NOE build-ups of the spins are not affected by external relaxation. There are numerous approaches to addressing this assumption, but probably the simplest is the use of Peak Amplitude Normalization for Improved Cross Relaxation (PANIC).15,16 PANIC corrects the experimental NOE intensities by standardization against the irradiated or diagonal peak in the one-dimensional or two-dimensional NOESY experiments, respectively—effectively assuming that the external relaxation rates that perturb the NOE signal are dominated by the external relaxation of the irradiated/diagonal peak. Using PANIC, 1H–1H distances can be measured with accuracies of ±3% in ideal systems. With an increasing number of fluorinated pharmaceuticals being developed, measuring the 1H–1H distances of a molecule is not always sufficient to understand the conformation of a drug molecule. Measurement of 1H–19F distances instead offers a complimentary and useful approach. In 2012, Claridge et al. reported the measurement of 1H–19F distances using a 1D 19F–1H Heteronuclear Overhauser Spectroscopy (HOESY) experiment17 but did not apply PANIC—presumably for the simple reason that the irradiated 19F signal does not appear in the measured 1H spectrum. Consequently, heteronuclear NOE build-up curves were required to identify the region in which the initial rate approximation holds true.

This work demonstrates how PANIC can be applied to the measurement of 1H–19F internuclear distances, avoiding the need for NOE build-up curves. Instead, we propose using the diagonal peaks of the 2D 1H–1H NOESY to correct the intensities in a 2D 1H–19F HOESY experiment. Although this requires the acquisition of both a 2D 1H–19F HOESY and a 2D 1H–1H NOESY, all possible 1H–19F internuclear distances that can be observed by NOE will be extracted.

2 | RESULTS AND DISCUSSION

Voriconazole, a commercially available antifungal medicine featuring three aromatic fluorine nuclei, was used to develop the proposed method.18 We measured a 1H-observed 2D 19F–1H HOESY (Figure 1b), putting 19F in the low digital resolution “indirect” dimension, which revealed a total of 12 measurable 1H–19F NOE correlations within the molecule. The pulse sequence used was that reported by Bauer in 1996.19 Full experimental details are provided in the supporting information. A 2D 1H–1H NOESY (Figure 1a) was also acquired with equal mixing time, to provide the diagonal peaks needed to correct for relaxation differences of the observed nucleus (both spectra are shown in Supporting Information).

By analogy with our previous 1H–1H internuclear distance measurements, assuming that the initial rate approximation is true, and that the molecule is tumbling isotropically in solution, the internuclear 1H–19F distances should be measurable by comparing the PANIC-corrected HOESY intensities (η), via Equation (1)

$$r_{HF(target)} = r_{HF(ref)} \left( \frac{\eta_{HF(target)}}{\eta_{HF(ref)}} \right)^{-1/6},$$

where $r_{HF(target)}$ is the desired internuclear distance between the target 1H–19F pair, $r_{HF(ref)}$ is the distance between a “reference” 1H–19F pair with a known internuclear separation (best chosen for a pair where the internuclear distance is not affected by conformational dynamics), and $\eta_{HF(target)}$ and $\eta_{HF(ref)}$ are the corresponding PANIC-corrected HOESY intensities. The PANIC-corrected intensities are obtained from the experimental spectra by multiplying the target 1H–19F NOE intensity by a scaling factor, $f$, obtained from the ratio of the corresponding target protons 1H–1H diagonal peak intensity against a chosen (arbitrary) standard diagonal peak intensity.

For example, to PANIC-correct the H12”–F9 NOE intensity (Figure 1b, η = 0.06), we first calculate the PANIC scaling factor for H12” by comparing the absolute intensity of the 1H–1H–NOESY diagonal peak for H12” (Figure 1a, D = 2.93) to that of (arbitrarily chosen) H14 (Figure 1a, D = 4.74). The ratio of these intensities, 2.93/4.74, gives the corresponding target protons 1H–1H diagonal peak intensity against a chosen (arbitrary) standard diagonal peak intensity.

To obtain the H12”–F9 internuclear distance, we simply now apply Equation (1), using a reference NOE intensity and distance from another 1H–19F pair. As the H21–F23 NOE arises from a fixed internuclear distance (2.55 Å), which is independent of the molecular conformation, it can be used as a sensible reference pair. NOTE: To provide a best fit for each dataset, the experimentally determined reference NOE-distance H21–F23 is subsequently incremented to maximize the quality of fit between experimental and computed distances (see SI for full details). The PANIC-correction for the
experimental intensity of the reference H21–F23 NOE (1.26) is obtained from the absolute intensity of the H23 $^1$H–$^1$H NOESY diagonal peak (4.857) compared with that of H14, which gives $f_{H21} = 1.024$, and thus a PANIC-corrected intensity, $\eta_{H21-F23}$ of 1.23. Plotting the NOE build-up curves for these PANIC-corrected intensities versus their corresponding uncorrected intensities shows substantial improvement in linearity (illustrative plots are shown in Section 5.4 in Supporting Information), highlighting a key source of NOE-distance errors in uncorrected data. Now applying the values to Equation 1 (i.e., $r_{HF(ref)} = 2.55 \text{ Å}$, $\eta_{HF(ref)} = 1.23$, $\eta_{H12'-F9} = 0.10$), we thus calculate the H12″–F9 distance to be 3.89 Å (see highlighted row in Table 1). Extending this approach to all other $^1$H–$^{19}$F NOE pairs in the 2D $^1$H–$^{19}$F HOESY spectrum (using the H14 $^1$H–$^1$H diagonal intensity and H21–F23 reference NOE intensity in all cases), a full set of $^1$H–$^{19}$F internuclear distances are obtained for Voriconazole.

In order to determine the accuracy of the PANIC-corrected internuclear distance measurements, we compared the experimentally derived NOE distances with
TABLE 1  The determination of the $^1$H–$^{19}$F distances in Voriconazole using PANIC-corrected 2D $^1$H–$^{19}$F HOE SY

| 2D $^1$H–$^{19}$F HOE SY | 2D $^1$H–$^{19}$F HOE SY | DFT calc. |
|-------------------------|-------------------------|-----------|
| H  | Diagonal peak intensity | F  | Uncorrected | PANIC-corrected | PANIC-corrected | PANIC-corrected |
| 1  | 9.08                    | 9  | 0.11        | 3.84          | 0.06          | 4.26          | 3.99          |
| 2  | 4.39                    | 9  | 0.88        | 2.71          | 0.95          | 2.66          | 2.43          |
| 7  | 4.34                    | 9  | 0.98        | 2.66          | 1.08          | 2.61          | 2.61          |
| 12' | 2.83                   | 9  | 0.07        | 4.09          | 0.12          | 3.74          | 3.89          |
| 12'' | 2.93                  | 9  | 0.06       | 4.23          | 0.10          | 3.89          | 4.02          |
| 1  | 9.08                    | 20 | 0.10        | 3.88          | 0.05          | 4.31          | 4.20          |
| 2  | 4.39                    | 20 | 0.70        | 2.81          | 0.76          | 2.76          | 2.32          |
| 12' | 2.83                   | 20 | 0.45    | 3.03          | 0.75          | 2.77          | 2.35          |
| 12'' | 2.93                 | 20 | 0.04     | 4.57          | 0.06          | 4.20          | 3.81          |
| 21 | 4.86                    | 20 | 1.35    | 2.52          | 1.32          | 2.52          | 2.56          |
| 21 | 4.86                    | 23a | 1.26   | 2.55          | 1.23          | 2.55          | 2.60          |
| 24 | 4.93                    | 23 | 1.27   | 2.55          | 1.22          | 2.56          | 2.59          |
| 14a | 4.74                   |    |          |              |              |              |

Mean absolute deviation, MAD (\%) 8.02 5.91

Standard deviation, SD (\%) 9.67 7.15

Abbreviations: HOE SY, Heteronuclear Overhauser Spectroscopy; PANIC, Peak Amplitude Normalization for Improved Cross-Relaxation.

*Denotes a reference peak or distance.

TABLE 2  A comparison of the MADs obtained when applying the possible PANIC corrections to the $^1$H–$^{19}$F internuclear distances

| $^1$H-observed $^1$H–$^{19}$F HOE SY | $^1$H-observed $^1$H–$^{19}$F HOE SY |
|----------------------------------|----------------------------------|
| MAD before PANIC                 | MAD after PANIC                  | MAD after PANIC                  |
| (\%)                             | (\%)                             | (\%)                             |
| 8.02                             | 5.91                             | 7.72                             |

$^{19}$F-observed $^1$H–$^{19}$F HOE SY

| MAD before PANIC                 | MAD after PANIC                  | MAD after PANIC                  |
|----------------------------------|----------------------------------|----------------------------------|
| (\%)                             | (\%)                             | (\%)                             |
| 8.52                             | 7.36                             | 8.28                             |

Abbreviations: HOE SY, Heteronuclear Overhauser Spectroscopy; MAD, mean absolute deviation; PANIC, Peak Amplitude Normalization for Improved Cross-Relaxation.

Those predicted for Voriconazole by DFT calculations. An initial conformational search of Voriconazole, using molecular mechanics, found a total of 21 non-redundant conformations under 21 kJmol$^{-1}$. All 21 conformations were geometry optimized at the mpw1pw91/6-31 g(d) level of theory, and frequency calculations were performed for each. A subsequent geometry optimization and single-point energy calculations were performed at higher level (mpw1pw91/6-31 g(d,p)). The lower level frequency corrections were then applied to the higher level single-point energies to establish the free energy for each conformer. The contribution to each NOE arising from each conformer (derived the negative sixth power of the corresponding $^1$H–$^{19}$F distance, $\eta_{HF} \propto r_{HF}^{-6}$, in each conformer) were then population-averaged using estimated Boltzmann populations from the free energies of the conformer ensemble, before conversion back to a single effective internuclear NOE distance averaged across all conformers ($r_{HF} \propto \eta_{HF}^{1/6}$). Full computational details are provided in the supporting information.

The calculated NOE distances, $r_{HF}$, were then compared with the experimentally derived values, and it was found that using the PANIC-corrected HOE SY intensities (Column 6, Table 1) leads to a good fit between experimental and DFT-determined NOE distances (5.9% MAD, 7.2% SD) with a maximum deviation of 16.1% for the distance between H2 and F20. These deviations, although not as good as the ~3% values obtained for $^1$H–$^{19}$F distances in rigid molecules, are in line with those we have observed previously for conformationally flexible systems with errors arising from DFT energy/population estimations and non-$r^{-6}$ NOE kinetics of dynamic systems.[5,7] Crucially, ignoring PANIC and using the uncorrected HOE SY intensities (Column 4, Table 1) gave a substantially less good fit (MAD 8.0%, SD 9.7%) with maximum deviation of 22.5% for H12' to F20.

The PANIC correction applied here ($^1$H-PANIC to the $^1$H-observed 2D $^1$H–$^{19}$F HOE SY) is only one of a number of possible combinations that could be applied to $^1$H–$^{19}$F interproton distance measurements using NOE. Consequently, we compared the four combinations with each other:

- $^1$H-PANIC correction (from the $^1$H–$^{19}$F NOE SY diagonal) to the $^1$H-observed HOE SY (described above)
- $^{19}$F-PANIC correction (from the $^{19}$F–$^{19}$F NOE SY diagonal) to the $^1$H-observed HOE SY
- $^1$H-PANIC correction (from the $^1$H–$^{19}$F NOE SY diagonal) to the $^{19}$F-observed HOE SY
- $^{19}$F-PANIC correction (from the $^{19}$F–$^{19}$F NOE SY diagonal) to the $^{19}$F-observed HOE SY
The $^{19}$F-PANIC correction based on the diagonal peak of the 2D $^{19}$F–$^{19}$F NOESY to correct for cross-relaxation of the $^1$H-observed 2D $^1$H–$^{19}$F HOESY resulted in a negligible improvement to the deviations between experimental and calculated internuclear distances (Table 2, Column 3). This suggests that correcting for variations between cross relaxation of the $^1$H spins is more helpful than correcting for these between the $^{19}$F spins. Supporting this, $T_1$ measurements (see Supporting Information) show a much greater variability in $T_1$ for $^1$H (from 0.38 s for H11 to 7.39 s for H5) than for $^{19}$F (from 0.32 s for F20 to 0.78 s for F23).
The same general trend (\(^1\)H-\(^{19}\)F-PANIC is more helpful than \(^1\)H-\(^{19}\)F-HOESY) was observed for NOE distances extracted from the \(^{19}\)F-observed HOESY (Table 2, Columns 4 and 5); however in this case, the improvement over the uncorrected NOE distances was relatively negligible in either case, with a best MAD of 7.36% obtained for \(^1\)H-PANIC corrected distances compared with DFT.

We then applied \(^1\)H-PANIC for \(^1\)H-\(^{19}\)F HOESY NOE-distance measurements to a number of other fluorinated drug-like molecules 2-5 of varying flexibility and complexity (Figure 2). There were significant improvements in the MAD/SD upon \(^1\)H-PANIC correction 3, 4, and 5, although only marginal changes were observed for the Flumethasone pivalate 2, where the fit was already very good without PANIC.

A key application of internuclear distance measurements are the determination of relative stereochemistry and/or conformation. To examine this, we calculated the \(^1\)H-\(^{19}\)F NOE distances for alternative diastereomers of both Voriconazole and 4. In both cases (Figure 3), the calculated NOE distances for the alternative diastereomers give substantially worse fits to the PANIC-corrected experimental NOE distances (voriconazole_RR: MAD 10.7%, SD 15.6%; 4_S: MAD 14.1%, SD 18.3%). Importantly, the discrimination between the correct and incorrect diastereomers for both voriconazole and 4 is <1-fold without PANIC correction and improves to ≥2-fold when using the PANIC-corrected distances. Although in both cases the discrimination might still be made without PANIC correction, the level of confidence in this discrimination is substantially lower. This better quality of fit for the correct diastereomers validates the value of maximizing the accuracy and sensitivity to molecular structure with PANIC-corrected analysis.

3 | CONCLUSIONS
In summary, the accuracy of \(^1\)H-\(^{19}\)F NOE-distance measurements can be maximized by the \(^1\)H-PANIC correction of NOE intensities in \(^1\)H-observed 2D \(^1\)H-\(^{19}\)F HOESY experiments, demonstrated here on several different fluorinated drug-like molecules of varying complexity and flexibility. We show the importance of accounting for the cross-relaxation of \(^1\)H in order to determine accurate values for \(^1\)H-\(^{19}\)F internuclear distances and that the diagonal peaks of the 2D \(^1\)H-\(^{19}\)F NOESY can be used to correct for this \(^1\)H cross-relaxation. In these systems, correcting for \(^{19}\)F relaxation is less helpful, presumably due to the more homogeneous \(T_1\) times of these nuclei, at least in the examples examined here. The benefit of this increased accuracy is demonstrated in stereochemical elucidations of two of the test cases, where PANIC correction offers a clear and superior discrimination between the rival structures.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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