

NMR Spectroscopy

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Exposing the Origins of Irreproducibility in Fluorine NMR Spectroscopy

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Abstract: Fluorine chemistry has taken a pivotal role in chemical reaction discovery, drug development, and chemical biology. NMR spectroscopy, arguably the most important technique for the characterization of fluorinated compounds, is rife with highly inconsistent referencing of fluorine NMR chemical shifts, producing deviations larger than 1 ppm. Herein, we provide unprecedented evidence that both spectrometer design and the current unified scale system underpinning the calibration of heteronuclear NMR spectra have unintentionally led to widespread variation in the standardization of ^{19}F NMR spectral data. We demonstrate that internal referencing provides the most robust, practical, and reproducible method whereby chemical shifts can be consistently measured and confirmed between institutions to less than 30 ppb deviation. Finally, we provide a comprehensive table of appropriately calibrated chemical shifts of reference compounds that will serve to calibrate ^{19}F spectra correctly.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful and convenient methods to elucidate the structure, interactions, and reactivity of molecules.^[1] In particular, the excellent sensitivity of fluorine nuclei and the advent of mild fluorination reagents and procedures^[2] over the past decades have resulted in an exponential and interdisciplinary use of ^{19}F NMR spectroscopy spanning the fields of synthetic and medicinal chemistry,^[3] drug screening,^[4] and RNA and protein function analysis^[5] (Figure 1).

Given the prominence of ^{19}F NMR spectroscopy, we were confounded by the relatively large discrepancies in ^{19}F NMR chemical shifts reported even for simple compounds.^[6] In ^1H and ^{13}C NMR spectroscopy, distinctive patterns of multiple signals can be analyzed, and residual solvent signals are present.^[7] In contrast, the ^{19}F NMR chemical shifts of partially fluorinated compounds often provide the sole information about the nature of a fluorine environment and are thus of utmost significance.^[8]

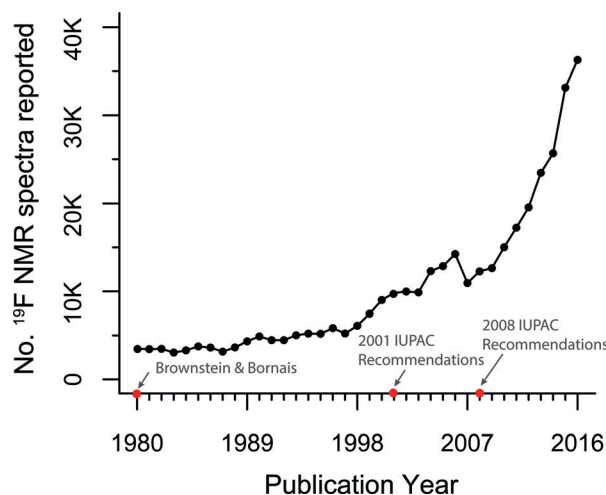


Figure 1. The popularity of ^{19}F NMR spectroscopy has risen exponentially with more than 35 000 new spectra published in 2016.^[13]

The basic tenets of science are reproducibility and effective communication of experimental results.^[9] This can only occur if there is an agreed and used basis for universally recognized conventions.^[10] Thus the lack of a reproducible protocol for reporting experimental spectra not only hinders structural assignment but also mechanistic labeling and machine-learning possibilities.^[11] To the best of our knowledge, the origins of these deviations in ^{19}F NMR chemical shifts have never been fully examined.^[12]

In this report, we analyze the basis for these inconsistencies and delineate a robust and practical method primarily for experimentalists to precisely calibrate ^{19}F NMR spectra. We illustrate that deploying a set of carefully determined internal reference compounds improves both the accuracy and precision by a factor of 50.

To understand the source of variation in ^{19}F NMR spectra, it was imperative to examine the principles of chemical shift referencing. In 2001, IUPAC endorsed a new, indirect referencing system in which the NMR chemical shifts of all nuclei are referenced with a unified scale (Figure 2, method A).^[10a,c] In this referencing regime, internal chemical species are no longer directly used to calibrate spectra. Rather, the resonance frequency of a given nuclide is calibrated to a universal primary reference by a predetermined ratio, Ξ [Eq. (1)].^[10a,14] These de facto Ξ values established by IUPAC,

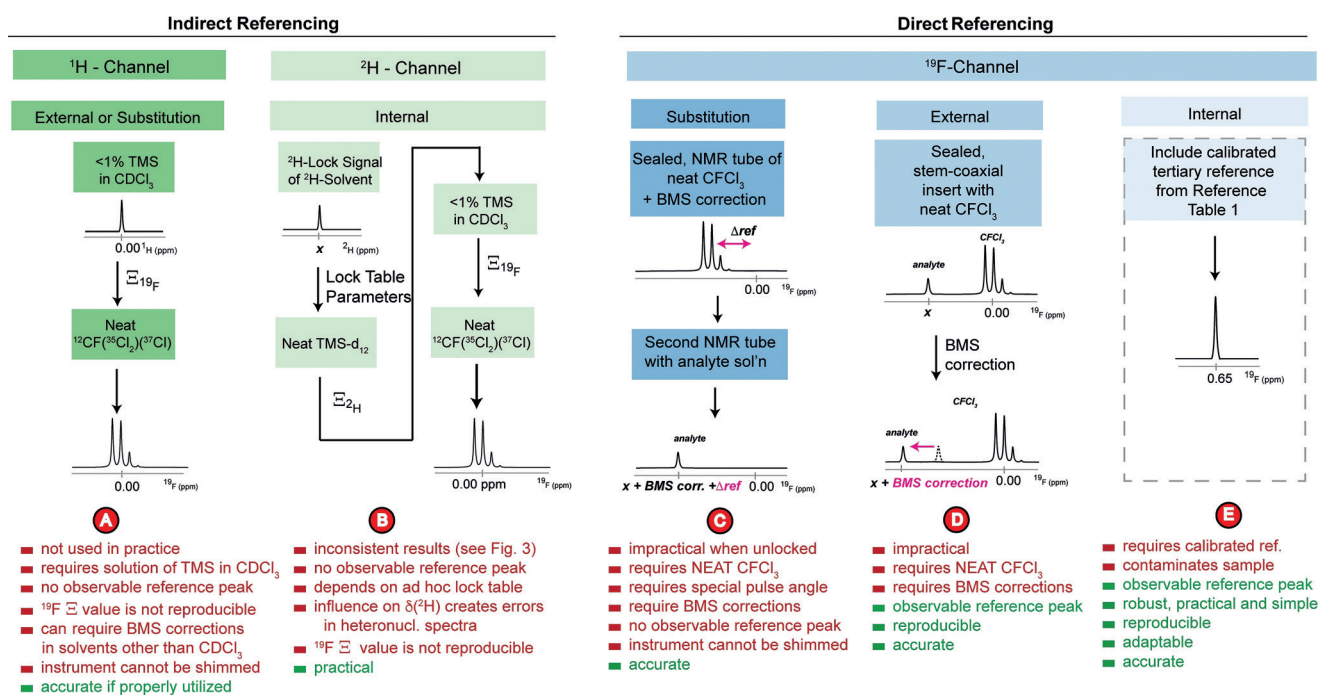
$$\Xi/\% = 100(\nu_x^{\text{obs}}/\nu_{\text{TMS}}^{\text{obs}}) \quad (1)$$

relate the ^1H resonance frequency ($\nu_{\text{TMS}}^{\text{obs}}$), of the universal primary reference (tetramethylsilane in chloroform- d ; 1%

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Current Manufacturer's Approach

This Work

Figure 2. Overarching methods for referencing heteronuclear NMR spectra, specifically exemplified here for ^{19}F NMR spectroscopy. Spectra can be referenced indirectly with a secondary NMR channel (A or B) or directly with a heteronuclear reference via the observe channel (C–E).^[14d] Contemporary spectra are routinely referenced by Method B. In general, BMS corrections are required if the solvents are changed between reference and analyte measurement. BMS = bulk magnetic susceptibility).

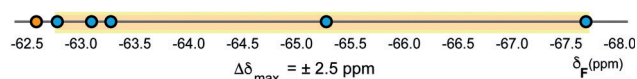
v/v) to the resonance frequency of a secondary heteronucleide (ν_x^{obs} , see the Supporting Information, Section S1.1).^[10a,15]

This system is particularly suitable for heteronuclear 2D experiments: It enables the calibration of indirect dimensions from a calibrated ^1H dimension even if the heteronuclear-specific reference signal is not observed directly. For 1D spectra prevalent in ^{19}F NMR spectroscopy, however, this system has shortcomings.

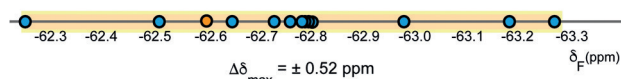
To evaluate the precision of current NMR referencing, four identically prepared samples were sent to eleven institutions worldwide for ^{19}F NMR measurements (Figure 3). The samples were prepared containing both CFCl_3 (1, 2.5% w/w) in chloroform-*d* and 0.1M of a fluorinated analyte ($\text{C}_6\text{H}_5\text{F}$, 2; $\text{C}_6\text{H}_5\text{CF}_3$, 3; C_6F_6 , 4; or $\text{CF}_3\text{CO}_2\text{H}$, 5) in hermetically sealed NMR tubes. The spectra that we received exhibited two troubling aspects. First, there was a chemical shift difference of > 1 ppm between institutions, and second, no institution reported spectra with the correct chemical shift with respect to neat CFCl_3 , the IUPAC specified ^{19}F secondary reference (Figure 3). However, when all spectra were calibrated to the signal of internal, dissolved CFCl_3 , the average difference in the chemical shifts of the measured analytes was less than ± 0.03 ppm, which is within the acceptable error based on peak linewidth (see Section S2.1).

The current technique of NMR referencing therefore faces two problems for heteronuclear NMR spectroscopy: First, modern NMR spectrometers circumvent either the IUPAC-recommended inclusion of tetramethylsilane (TMS) as a reference compound or the compulsory acquisition of a ^1H NMR calibration spectrum by making undiscerning use

of the deuterium (^2H) NMR lock signal as a surrogate reference signal (Figure 2, method B). In this regard, it is not TMS directly, but rather deuterium chemical shifts that provide the basis upon which universal calibration relies on to interconvert between the deuterium signal of the solvent and the heteronucleide of choice via the respective Ξ value. In principle, this process is more proficient but it relies on 1) the presence of one deuterated solvent and 2) a universally accepted table of precisely measured deuterium chemical shifts for each deuterated solvent.

A. Current Literature:^[6]

B. Identical Sample at 11 Institutions:



C. Identical Sample at 11 Institutions with a 3° Reference:

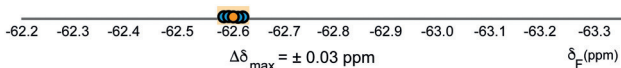


Figure 3. Chemical shifts of PhCF_3 (3) reported in the current chemical literature (all in chloroform-*d*, A).^[6] Analyzing ^{19}F NMR samples at eleven institutions gave a distribution of results (B), which converged upon inclusion of a calibrated tertiary reference (C). The orange bullet point represents the “true” chemical shift of PhCF_3 in chloroform-*d* with respect to neat CFCl_3 .

Currently, however, these lock tables often contain imprecise values specified by the manufacturer or edited by the end user. Furthermore, to meet the additional IUPAC criterium that dissolved TMS must resonate at 0.00 ppm in all solvents, an additional TMS-specific correction (accounting for solvent interactions of TMS) is also included in the lock chemical shift values.^[10c] This correction adds a solvent-specific systematic error to all heteronuclear NMR spectra referenced via the lock in solvents other than chloroform-*d* (see Section S3.3). Whereas a universally accepted deuterium lock table would ameliorate these specific inconsistencies, calibration of the spectrometer at the low ^2H frequency and propagating the rather low precision to six- to sevenfold higher ^1H or ^{19}F frequencies is generally not ideal. Furthermore, the influences of analyte(s) on the chemical shift of the deuterated solvent cannot be readily accounted for. These issues undermine the essence of the unified scale system, and thus heteronuclear spectra are usually not calibrated correctly. Additionally, the lack of an identifiable reference signal in the unified scale system makes it unclear whether heteronuclear NMR spectra have been appropriately calibrated.

In light of these NMR deficiencies, we set out to establish a practical method for referencing ^{19}F NMR spectra that yields precise and reproducible chemical shifts while espousing current IUPAC ideals. As evidenced by the consistency in measurements between institutions after simple calibration to an internal standard, we examined the anachronistic method of referencing to internal fluorinated reference compounds.

For ^{19}F NMR spectroscopy, IUPAC has defined trichlorofluoromethane (CFCl_3 , **1**), or more precisely, the specific isotopologue $^{12}\text{CF}(^{35}\text{Cl})_2(^{37}\text{Cl})$, as the reference substance for ^{19}F chemical shift calibration.^[10a,b,14a,c] This compound serves as a secondary reference in the unified scale system defining the fluorine Ξ value (Figure 4A). Whereas CFCl_3 has some assets as a reference substance as it is silent in routine ^1H and ^{13}C NMR spectra, can be used in low concentrations, resonates in an isolated region, and is easily removed from the bulk sample, many of its physical, spectral, and environmental aspects are concerning and render CFCl_3 impractical for routine measurements.^[16] As the ^{19}F NMR reference compound, we were surprised that the most recent published ^{19}F NMR spectrum for neat CFCl_3 was acquired in 1969 (see Figure 4B). Furthermore, the physical state of CFCl_3 for which the ^{19}F Ξ value has been established is not reported. The 2001 IUPAC recommendations cite the literature ^{19}F Ξ value reported by Brownstein and Bornais in 1980 who list a value for both neat CFCl_3 and a chloroform-*d* solution (of unknown concentration).^[10a,14a,b] In summary, there is no clear IUPAC specification of the correct isotopologue, the measurement sample conditions, or an explanation for the universally used ^{19}F Ξ value.

By interrogating CFCl_3 by high-field NMR spectroscopy and mass spectrometry, we confirmed the correct isotopologue to be $^{12}\text{CF}(^{35}\text{Cl})_2(^{37}\text{Cl})$ (Figure 4B).^[18] With this knowledge, 13 identical, stem-coaxial NMR inserts suitable for external referencing containing neat CFCl_3 were flame-sealed under vacuum and inserted into a 2.5% w/w solution of CFCl_3 in chloroform-*d* (Section S3.2). Based on all 13 samples and

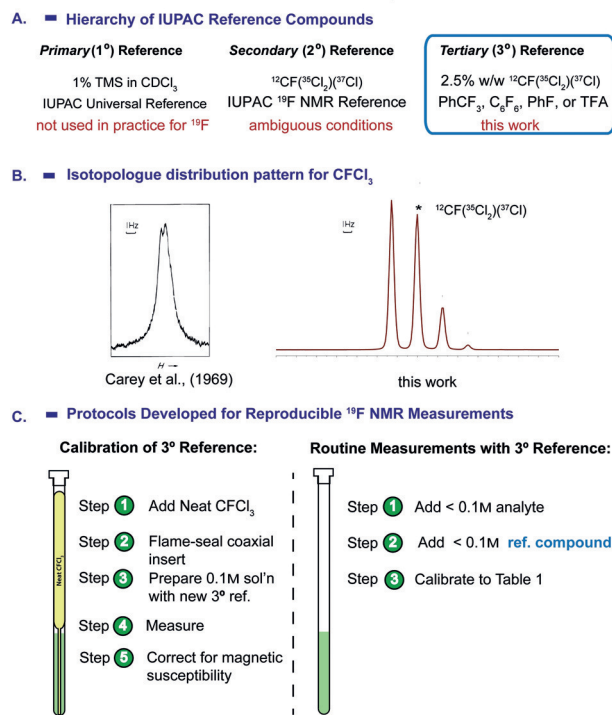


Figure 4. Evolution of ^{19}F NMR reference compounds.

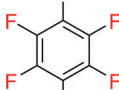
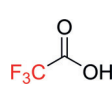
using the spectrometer-intrinsic indirect referencing based on locking on chloroform-*d*, the resonance for the specified isotopologue of neat CFCl_3 was located at 0.22 ± 0.0025 ppm and that of the solution at 0.50 ± 0.002 ppm.

Correcting for the difference in magnetic susceptibility of the neat solution did not yield a value of 0 ppm (-0.14 ppm for chloroform-*d*), suggesting that the current ^{19}F Ξ value or the deuterium-lock chemical shifts are likely incorrect (see Section S2.2).^[19]

Although operationally challenging, we reasoned that referencing external neat CFCl_3 with appropriate geometric magnetic susceptibility corrections is preferable over a solution-based internal reference as it limits solvent and concentration dependence for the CFCl_3 signal and can be more widely implemented (Figure 2, method D).^[14c] Furthermore, referencing neat CFCl_3 via a substitution method can pose additional complications (Figure 2, method C).^[14d]

As external referencing with the IUPAC secondary reference of neat CFCl_3 is impractical for routine measurements (Figure 2, method D), five of the most commonly used fluorinated analytes were selected as tertiary internal reference compounds (see Section S5).^[20] Each species was calibrated to neat CFCl_3 in eleven different deuterated solvents. This matrix thus provides a convenient method to appropriately calibrate ^{19}F NMR spectra without necessarily requiring the use of CFCl_3 or the presence of a deuterated solvent but yet comports with the general IUPAC specifications (see Table 1). In situations where these species are not satisfactory, a detailed method to calibrate new tertiary analytes to neat CFCl_3 can be employed, which is comprehensively described in Section S3 (see also Figure 4C).

Table 1: CFCl_3 -calibrated tertiary references suitable for ^{19}F NMR referencing in various deuterated solvents.

Solvent	$\delta(1)$ [ppm]	$\delta(2)$ [ppm]	$\delta(3)$ [ppm]	$\delta(4)$ [ppm]	$\delta(5)$ [ppm]
					
					
					
					
					
CDCl_3	0.65 ^[a]	−112.96	−62.61	−161.64	−75.39
CD_2Cl_2	0.02	−113.78	−62.93	−162.61	−75.76
C_6D_6	−0.19	−113.11	−62.74	−163.16	−75.87
acetone- d_6	−1.09	−114.72	−63.22	−164.67	−76.87
CD_3CN	−1.14	−114.81	−63.10	−164.38	−76.72
CD_3OD	−1.67	−115.42	−64.24	−165.37	−77.77
DMSO- d_6	−0.24	−112.83	−60.94	−162.45	−74.95
D_2O	−0.97 ^[b]	−113.73 ^[b]	— ^[c]	−163.01 ^[b]	−75.51
$\text{H}_2\text{O}/\text{D}_2\text{O}^{[d]}$	−0.82 ^[b]	—	—	—	−75.25
DMF- d_7	−1.16	−114.51	−62.66	−164.46	−76.46
toluene- d_8	−0.21	−113.09	−62.88	−163.33	−75.94
THF- d_8	−0.93	−114.31	−63.47	−164.51	−76.93

[a] Medians of 13 measurements with 13 sealed coaxial inserts containing neat CFCl_3 , $\Delta\delta_{\text{max}} = 5$ ppb (see Section S3.2.2 for details). [b] Low analyte solubility, saturated solution. [c] Low analyte solubility prevented a meaningful measurement. [d] Tested in six buffer solutions (90% $\text{H}_2\text{O}/10\%$ D_2O) at different pH values: $\Delta\delta_{\text{max}} = 4$ ppb (see Section S5.2). For work with biomolecules, the “locked substitution” method in the same buffer is recommended (see Refs. [10a, b, 14d]).

As laboratory environments are not uniform, we wondered to what extent various physical factors influence the chemical shift. We surmised that the solvent, analyte concentration, temperature, frequency, solute interactions, spectrometer stability, and spectrometer software could all impact the chemical shift and therefore reproducibility between institutions (see Table 2 and Section S3.4). Upon interrogation of each physical variable, we highlight the three effects observed to have the greatest impact.

First, the effect of solvent on chemical shift was analyzed by using five tertiary references with respect to CFCl_3 (see Table 1). Not surprisingly, the solvent has the largest influence on the chemical shift, with up to $\Delta\delta = 3.9$ ppm in the case of C_6F_6 and $\Delta\delta = 2.3$ ppm for CFCl_3 , which supports the necessity to report the solvent used for all ^{19}F NMR spectra, consistent with most publishing guidelines (see Table 1).^[21]

Second, to examine the influence of solute interactions and the concentration on the chemical shift (δ), two mixtures of 16 compounds each were analyzed by ^{19}F NMR spectroscopy at 0.1M and 0.01M.^[22] All compounds could be clearly discriminated based on chemical shift at 0.01M in both mixtures as they are in excellent agreement ($\Delta\delta_{\text{max}} =$

Table 2: Physical influences on the ^{19}F NMR chemical shifts in this study.

Factor	Parameter	Influence on $\delta(^{19}\text{F})$
solvent		± 1.95 ppm
concentration	0.1 M	< 0.01 ppm
compound mixture	0.1 M	0.15 ppm (average)
	0.01 M	≤ 0.01 ppm
temperature		6.2 ppb/K (average)
frequency		< 0.01 ppm

0.01 ppm) with the values reported in Tables S1 and S2. At the higher concentration (0.1M), all compounds experienced a general downfield shift by nearly +0.15 ppm, which in certain situations could prevent a clear structural assignment based on chemical shift. Based on these results, we recommend a concentration of $\leq 0.1\text{M}$ for single-compound spectra and $\leq 0.01\text{M}$ for the analysis of mixtures by ^{19}F NMR spectroscopy to exclude any concentration influences.

Third, the temperature dependence was found to be largely insignificant for the chemical shift of internal CFCl_3 with an average of 6.2 ppb K^{-1} . The effect is highest in DMF- d_7 with 11.8 ppb K^{-1} and lowest in chloroform- d with 1.5 ppb K^{-1} (see Table 2 and Section S4.4).^[23]

For any referencing technique, the inherent robustness and the accuracy are of paramount impor-

tance. This validation was achieved in two sets of experiments: 1) Internal and external referencing to CFCl_3 was compared for four analytes in eleven solvents (see Section S5.4). The results unequivocally illustrate excellent agreement with an average $\Delta\delta$ of 0.02 ppm. 2) Internal referencing with five different tertiary references (CFCl_3 , **1**; PhF, **2**; PhCF_3 , **3**; C_6F_6 , **4**; $\text{CF}_3\text{CO}_2\text{H}$, **5**) was examined for three analytes in three solvents (see Section S5.5). All tertiary references yielded analyte chemical shifts in excellent agreement with each other ($\Delta\delta = 0.01\text{--}0.07$ ppm).

Owing to the widespread use of fluorinated compounds in chemical synthesis and the need for spectral consistency, we present the appropriately referenced ^{19}F NMR chemical shifts of more than 240 partially fluorinated building blocks, reagents, and common side products (see Section S6.2). Each compound was referenced to neat CFCl_3 via a tertiary fluorinated reference under standard conditions for which the purity of each species was determined by an orthogonal spectroscopic technique.

In summary, a critical evaluation of contemporary referencing methods in ^{19}F NMR spectroscopy has revealed a number of serious inaccuracies in the current fluorine referencing system. These were attributed to vague definitions, inadequate implementation of indirect ^2H methods by instrument manufacturers, variable instrument setups between institutions, and a lack of an identifiable and validating reference signal. By deploying calibrated tertiary reference compounds comporting with established IUPAC practices, the disparity in fluorine chemical shifts across institutions can be reduced from the current 1 ppm to below 0.03 ppm. Aside from the solvent dependence, the chemical shifts are largely immune to typical laboratory conditions. The

improved reproducibility reported herein will therefore enable practicing scientists to use ^{19}F NMR spectroscopy to its full potential, allow for a suitable guideline for data reporting, and should serve as a blueprint for other hetero-nuclear NMR referencing techniques.

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Conflict of interest

The authors declare no conflict of interest.

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