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NMR spectral analysis of strongly second-order 6-, 8-, 9- and 10- spin-systems (^1H - ^{19}F , ^{19}F - ^{19}F and ^{13}C - ^{19}F) in Perfluorotoluy- and Tetrafluoro-pyridyl-aromatics using the line-shape method ANATOLIA.

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Abstract.

A simple to use NMR analysis method has been tested on complex ^1H , ^{19}F and ^{13}C multiplets. This open-source line-shape analysis method ANATOLIA^[1] (**AN**ALYSIS of **T**OTAL **L**inesh**A**pe) provides some significant advantages over traditional assign-iterate methods of NMR spectral analysis by avoiding false minima and progressing optimisation to the global minimum. The target molecules are 1-perfluorotol-4-yl-2-perfluorotol-4-yl-oxymethyl-1H-benzimidazole (molecule-I) and 1-tetrafluoropyrid-4-yl,-2-tetrafluoropyrid-4-yl-thio-1H-benzimidazole (molecule-II) which were produced as part of a family of fluorinated drug scaffolds prepared for anti-cancer and anti-parasitic screening. Spectra display significant second-order effects with ^1H $\Delta\delta$ = 3.68 Hz and 4.67 Hz for the aromatic hydrogen "triplets", with ^{19}F $^4J_{\text{AA}'}$, $^4J_{\text{BB}'}$, $^4J_{\text{XX}'}$, and $^4J_{\text{YY}'}$ coupling constants range from +4.8 Hz to -14.0 Hz and for ^{13}C -isotopomers ^{19}F $\Delta\delta$ of up to 111.56 Hz. A spin-system of 6 coupling nuclei ($\text{H}_a\text{H}_b\text{H}_c\text{H}_d\text{F}_Y\text{F}_{Y'}$) was analysed in 12 seconds, a spin-system of 9 coupling fluorine nuclei ($\text{AA}'\text{BB}'\text{CCC}'\text{YY}'$) was analysed within 2 minutes and 10 coupling nuclei ($\text{XX}'\text{YY}'\text{ZZZ}'\text{BB}'\text{H}_d$) was optimised in 6 minutes using a laptop computer. ANATOLIA was also robust enough to be able to yield accurate spectral values from inaccurate input values. In both compounds a fluorine-fluorine coupling constant was identified between the two fluoro-aromatic rings ($\text{F}_{\text{BB}'}$ and $\text{F}_{\text{YY}'}$) of +4.05 Hz and +4.67 Hz and attributed to a through-space interaction. *Ab initio* structure optimisations and coupling constant calculations provided useful input data for spectral analysis. A modern ^{19}F NMR spectrum of perfluorotoluene (octafluorotoluene) and analysis from 1975 was used as a test data set to assess ANATOLIA.

1 Introduction.

More than 20% of new pharmaceutically active drugs contain a fluorine atom,^[2, 3] and there is renewed interest in understanding complex features that often appear in ^{19}F NMR spectra.^[4] However, the literature is rather sparsely populated with detailed and accurate analyses of ^{19}F multiplets, as extracting accurate chemical shift values and scalar coupling constants from liquid-state NMR spectra remains a significant challenge. In the 1950's pencil and paper calculations allowed chemical shifts and coupling constants to be extracted,^[5, 6] from the AA'BB' spin-system of thiophene,^[7] furan and pyrrole,^[8] and substituted fluoro-aromatics^[9] recorded at 30, 40 and 60 MHz. Pentafluorophenyl derivatives with X = H, CH₃, CF₃, Cl, Br, I, OCH₃, CN, and NH₂ were analysed in 1965.^[10] Recent interest in benchtop NMR spectrometers has led to a resurrection in spectral analysis at 40-80 MHz in order to help understand the complex structures that appear in low-field spectra,^[11] for chemical research,^[12] hyperpolarisation methods^[13] and educational purposes.^[14, 15, 16] Theoretical methods^[17] and computational efficient calculations have vastly improved our chances of solving such NMR problems, and has resulted in the employment of computational methods such as Laocoon,^[18] and PANIC,^[19] and in more recent decades, by graphic-based calculations such as those featured in WinDNMR,^[20] NMR-SIM,^[21] DAISY,^[22] DAVINS,^[23] NMRCON,^[24] PERCHit,^[25] ChemAdder,^[26] NUTS,^[27] gNMR,^[28] MestRenova,^[29] efficient code in SPINACH,^[30] iterative methods such as SpinWorks.^[31]

In 2018 a new line-shape algorithm approach ANATOLIA^[1] was made available as open-source free software,^[32, 33] that applies a series of line broadening effects (LB = 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.2,) to both the experimental and calculated spectra before parameters are optimised. This has the effect of avoiding thousands of local minima that provided good fits but are based on inaccurate parameters, and this enables a wider landscape of parameter values to be explored and thus increases the probability of locating the global minimum.

Here, ANATOLIA was applied to complex ^1H - ^{19}F and ^{19}F - ^{19}F spin-systems in the well-studied perfluorotoluene molecule and for the first time applied to novel biologically active molecule 1-perfluorotol-4-yl-2-perfluorotol-4-yl-oxymethyl-1H-benzimidazole (molecule-I) and yielded accurate chemical shifts and coupling constants more quickly, and without recourse to, assign-iterate methods. The complex second-order multiplets in the ^{13}C spectrum of 1-tetrafluoropyrid-4-yl,-2-tetrafluoropyrid-4-yl-thio-1H-benzimidazole (molecule-II) were readily analysed using ANATOLIA, a feat that was extremely challenging and time consuming for assign-iterate methods. It is envisaged that this software will be widely accessible to bench chemists.^[34]

2 Experimental

^1H , ^{13}C , DEPT, COSY and HMQC spectra were recorded using JEOL ECS-400 MHz, JEOL-ECZ-500 MHz, Bruker Avance-I 400 MHz and Bruker-Neo 500 MHz NMR spectrometers, using an auto-tune probe and 5 mm NMR tubes. Samples were prepared using ~20 mg of sample dissolved in a 0.80 ml volume of CDCl₃ obtained from Apollo-UK, i.e. ~ 100 mM solutions.

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High quality 5mm diameter NMR tubes purchased from Norell were used at both 400 and 500 MHz operating frequencies.

^1H NMR spectra were recorded using the small flip-angle pulse program 30° using a $5.8\ \mu\text{s}$ (Bruker) and 45° using a $3.8\ \mu\text{s}$ (JEOL) covering a sweep width of 20.7 ppm (8,278 Hz) with 64K time domain data points giving an acquisition time of 3.95 seconds, Fourier-transformed using 128K data points and referenced to an internal TMS standard at $\delta = 0.00$ ppm.

^{13}C NMR spectra were obtained on a spectrometer operating at 100.6 MHz (400 MHz equivalent for ^1H), also employing a 5 mm high-resolution broad-band auto-tune gradients probe. The pulse program employs a direct 30° pulse on carbon with WALTZ-16 decoupling during acquisition, and a relaxation delay of 1.00 second. Spectra were recorded over a sweep width of 250 ppm (25,252 Hz) with 64K time domain datapoints, giving an acquisition time of 1.30 seconds. A single exponential function of 1.00 was applied, and spectra were Fourier-transformed using 128K datapoints and referenced to an internal tetramethylsilane (TMS) standard at $\delta = 0.00$ ppm.

DEPT ^1H NMR spectra were obtained using the DEPT-135 method, using WALTZ-16 decoupling during acquisition, a delay of 3.45 ms (145 Hz), and a relaxation delay of 1 second. Spectra were recorded over a sweep width of 250 ppm (25,252 Hz) with 64K time domain datapoints, yielding an acquisition time of 1.30 seconds. A single exponential function of 1.00 was applied, and spectra were Fourier-transformed using 128K datapoints, and referenced to an internal TMS standard at $\delta = 0.00$ ppm.

2D gradient-enhanced COSY ^1H - ^1H NMR spectra were obtained using the multiple-quantum filtered COSY method, with typical $P_{90} = 15.25\ \mu\text{s}$. Spectra were typically recorded over a 12 ppm sweep-width with 2K data points in the f_2 domain, and 256 experiments in the f_1 domain; FID's were modified with a QSINE window function (single exponential for JEOL data sets) and linear prediction used to enhance the f_1 domain, and the data Fourier transformed using 4K and 1K datapoints in the f_2 and f_1 domains respectively.

2D gradient-enhanced HMQC ^1H - ^{13}C NMR spectra were obtained using the quantum-filtered HMQC method, with typical $P_{90} = 15.25\ \mu\text{s}$, $^1J_{\text{CH}}$ coupling of 145 Hz. WALTZ-16 decoupling of the ^{13}C channel was applied during acquisition, and spectra were typically recorded over a 12 ppm sweep-width with 2K data points in the f_2 domain, and 128 experiments in the f_1 domain. FID's were modified with a QSINE window function (single exponential for JEOL data sets) and linear prediction was used to enhance the f_1 domain; data were Fourier-transformed using 4K and 1K datapoints in the f_2 and f_1 domains respectively.

Non Uniform Sampling methods used TD = 256, and used NUS = 25%, providing 64 acquisition increments and multi dimensional decomposition processing.^[35]

^1H - ^{19}F HOSEY and selective nOe $^1\text{H}\{^{19}\text{F}\}$ and $^{19}\text{F}\{^1\text{H}\}$ data sets were acquired using the standard ^1H and ^{19}F parameters for observe pulses and decoupling sequences.

Gaussian-03 calculations were run on a i7 PC using 1-core and 8Gb of memory employing “nmr=spinspin” keyword. Gaussian-09 calculations were run on a iMac using 4-cores and 8Gb of memory employing “nmr=mixed” keyword.

3 Results & Discussion.

3.1 A re-investigation of perfluorotoluene (octafluorotoluene)

A useful starting point in this analysis was to re-investigate the complex multiplets produced in the ^{19}F NMR spectrum of perfluorotoluene (octafluorotoluene) which was analysed in 1975.^[36] The excellent resolution achieved for the experimental spectrum allowed detailed analysis, which yielded the coupling constants listed in Table 1.

In this study, a ^{19}F spectrum of dilute perfluorotoluene was acquired using a 400 MHz NMR spectrometer (376.498 MHz for ^{19}F) and analysed using SpinWorks, where lines in the multiplet were assigned to a calculated spectrum and the coupling constants modified in an iterative manner to improve the fit. The result shows an excellent agreement between calculation and experiment (Figure 1).

Figure 1. Experimental ^{19}F NMR multiplets (lower) and calculated (upper) of perfluorotoluene (octafluorotoluene) ~20 mg in 0.7 ml of CDCl_3 referenced to $\text{CFCl}_3 = 0$ ppm) recorded using a 400 MHz spectrometer (376 MHz for ^{19}F). Analysed using SpinWorks, WinDNMR and ANATOLIA.

The coupling constants published in 1975 (using a JEOL-PS-100 spectrometer) are very similar to the values obtained in this paper using Spinworks, WinDNMR and ANATOLIA (Table 1).

In general, the sign of $^3J_{\text{FF}}$ coupling constants in aromatic compounds is negative and for longer-range couplings $^4J_{\text{FF}}$ and $^5J_{\text{FF}}$ tends to be positive,^[37] however, in the case of perfluorotoluene $^4J_{\text{AA}'}$ is negative and $^4J_{\text{BB}'}$ is less than 0.5 Hz and can be optimised as positive or negative.

Table 1. ^{19}F coupling constants for perfluorotoluene (octafluorotoluene)

Coupling and Spin label	1975 *	SpinWorks	ANATOLIA
$^4J_{A-CF_3} = A'-CF_3$	+22.68	+22.12	+22.036
$^5J_{B-CF_3} = B'-CF_3$	+0.62	+0.59	+0.586
$^6J_{C-CF_3}$	+1.33	+1.34	+1.367
$^3J_{AB} = A'B'$	-20.01	-21.70	-21.984
$^4J_{AC} = A'C$	+5.54	+5.56	-5.501
$^5J_{AB'} = A'B$	+8.34	+8.37	+8.330
$^4J_{AA'}$	-7.52	-7.54	-7.624
$^3J_{BC} = B'C$	-18.97	-20.99	-21.006
$^4J_{BB'}$	-0.43	-0.56	+0.468

*analysis from 1975 *Ortho*-F (AA') $\delta = -140.28$ ppm, *meta*-F (BB') $\delta = -161.06$, *para*-F (C) $\delta = -148.37$ ppm, a 75% (v/v) in and referenced to degassed $CFCl_3$, recorded using a Varian HA 60 MHz spectrometer (56.4422 MHz for ^{19}F), a Varian XL-100 and a JEOL PS 100 spectrometer (94.6 MHz for ^{19}F).

3.2 Spectral Analysis using SpinWorks, WinD-NMR and ANATOLIA

Once the integrity of the data from 1975 had been confirmed and re-analysis achieved using a ^{19}F NMR spectra obtained at 400 MHz and 500 MHz (for 1H) using WinD-NMR and SpinWorks, then the ANATOLIA method was applied to the same spectrum using inaccurate input data. ANATOLIA has a useful feature that allows spin-symmetry and equivalence of the chemical shifts for AA', BB', C and CF_3 fluorine atoms in perfluorotoluene to be defined and linked, similarly the symmetry can be defined for the coupling constants too, such that $AB = A'B'$ too (see SI). Initially it is useful to optimise just a few parameters at a time, hence, the chemical shifts for AA', BB', C and CF_3 can be optimised, even although these values are readily accessible from the experimental spectrum and should be accurately known already. Thereafter, a series of coupling constants can be optimised, where some approximate values for the coupling constants can be estimated from the literature or extracted from the multiplets particularly from *para*-fluorine multiplet which has couplings of approximately 20 Hz, 5 Hz and 2 Hz, and the CF_3 group, which has couplings of approximately 20 Hz, 2 Hz and 1 Hz. The remaining coupling constants (AA', $AB' = A'B$, and BB') are very difficult to visually approximate from the multiplets and literature values vary significantly even for similar molecules (see molecule-II), and therefore must be optimised by the ANATOLIA program.

As optimisation of these parameters requires only 2 minutes on a laptop computer, a range of starting values can be used for AA', $AB' = A'B$, and BB', and once the estimate value is within few Hz of the actual value then the optimised fit between experimental multiplet and calculated multiplet is excellent. This is a significant advantage of the ANATOLIA method, as the appearance of the multiplets is very sensitive to the size of $^4J_{AA'}$ and $^4J_{BB'}$, thus this line-shape analysis method lends itself to this type of complex multiplet more than the traditional assign-iterate methods, which need a much closer approximation before parameter optimisation works well. The closeness of fit is provided in Supporting Information, as well as some inaccurate input data that yields accurate results and some wildly inaccurate input data that fails to optimise to an acceptable result. The robustness of the ANATOLIA method has been clearly proven on this test ^{19}F spectrum of perfluorotoluene (octafluorotoluene).

3.3 Investigation of 1-perfluorotol-4-yl-2-perfluorotol-4-yl-oxymethyl-1H-benzimidazole (molecule-I).

3.3.1 Proton NMR spectrum (six spin-system $H_A H_B H_C H_D - {}^{19}F_{YY'}$)

The 1H and ${}^{19}F$ NMR spectra display second-order closely coupled spin-systems, the hydrogen signals comprise of 4 aromatic multiplets and are labelled as H_A H_B H_C H_D and a CH_2 singlet (Figure. 2), here the aromatic signals do not appear as a traditional doublet-triplet-triplet-doublet pattern. The 1H - 1H COSY identifies the doublets as the largest and smallest chemical shifts, with the two triplets in almost the same place in the spectrum overlapping significantly. The distortion of all aromatic signals prevents extraction of coupling constants by direct measurement, as the “doublet” at 7.1 ppm is more complex than expected and is not similar in appearance to the “doublet” at 7.9 ppm. Selective decoupling of the ${}^{19}F$ multiplets using a dual-tuned 1H $\{ {}^{19}F \}$ probe setup, identified that the extra coupling was due to a long-range interaction with the YY' aromatic fluorine atoms. No coupling was observed between the aromatic hydrogen atoms and the CH_2 hydrogens, nor between the aromatic fluorine atoms and the CH_2 hydrogens. Traditional spectral analysis using WinD-NMR and SpinWorks required an hour to extract accurate coupling constant values, hindered by the poorly resolved H_d “doublet” at 7.1 ppm that required some trial-and-error fitting to determine the long-range H-F coupling to be 1.14 Hz. Analysis was further made more complex by the very small chemical shift difference between hydrogens B and C of only $\delta\Delta = 3.678$ Hz (~ 0.001 ppm) that had a significant effect on the appearance and intensities of all hydrogen signals.

Figure 2. Chemical structure and spin-labels of 1-perfluorotol-4-yl-2-perfluorotol-4-yl-oxymethyl-1H-benzimidazole (molecule-I).

Just a few minutes were required to setup the ANATOLIA “Input_Data” and “parameters_start” files for this spin-system and 16 seconds of calculation time to optimise and produce accurate results. The simplicity of setup and speed of result is extremely useful. To highlight how user-friendly this program is, a grossly inaccurate set of coupling constants was placed in the “Input_Data” file, with the same value for all ${}^3J_{HH}$, ${}^4J_{HH}$ and ${}^5J_{HH}$ coupling constants set to 7 Hz, and optimisation still produced an excellent set of coupling constant parameters.

The modern dual-tuned 1H - ${}^{19}F$ probes allowed proton spectra to be recorded with selective fluorine decoupling ${}^1H\{sel-{}^{19}F\}$, this enabled a simplified 1H spectrum to be obtained where

the aromatic ^1H signal H_d at 7.15 ppm, which shows a long-range coupling to $\text{F}_{\text{YY}'}$, collapsed to reveal well resolved complex “doublet”. Removing the $\text{F}_{\text{YY}'}$ coupled spins from the spectrum also made the input data file simpler to setup and the line-shape fitting calculation faster. Accurate input data of 8 Hz, 2 Hz and 0.5 Hz for $^3\text{J}_{\text{HH}}$, $^4\text{J}_{\text{HH}}$ and $^5\text{J}_{\text{HH}}$ produced an excellent fit within 10 seconds, and even inaccurate input data of 9, 3, 2 Hz also converged on an excellent fit. Results are shown in Table 2 and Figure 3.

Figure 3. Experimental (lower) and ANATOLIA calculated (upper) ^1H NMR multiplets of molecule-I ~20 mg in 0.7 ml of CDCl_3 referenced to TMS at 0.0 ppm, ^1H and $^1\text{H}\{\text{sel-}^{19}\text{F}\}$ recorded at 500 MHz.

Table 2. Chemical shifts and ^1H - ^1H and ^1H - ^{19}F coupling constants for molecule-I, ^1H and $^1\text{H}\{\text{sel-}^{19}\text{F}\}$ spectra analysed using SpinWorks and ANATOLIA.

Spin Label	SpinWorks	Input (Hz)	ANATOLIA	Input (Hz)*	ANATOLIA*
A		3592	7.909 ppm	3592	7.909 ppm
B		3727	7.463 ppm	3727	7.463 ppm
C		3733	7.453 ppm	3733	7.453 ppm
D		3955	7.184 ppm	3955	7.184 ppm
YY'		-40000	-40000	-40000	-40000
$^3\text{J}_{\text{AB}}$	8.25 Hz	8.0 Hz	8.27 Hz	8.0 Hz	8.23 Hz
$^4\text{J}_{\text{AC}}$	1.04 Hz	2.0 Hz	0.96 Hz	2.0 Hz	1.03 Hz
$^5\text{J}_{\text{AD}}$	0.71 Hz	0.5 Hz	0.67 Hz	0.5 Hz	0.70 Hz
$^3\text{J}_{\text{BC}}$	7.22 Hz	8.0 Hz	7.29 Hz	8.0 Hz	7.28 Hz
$^4\text{J}_{\text{BD}}$	1.08 Hz	2.0 Hz	1.02 Hz	2.0 Hz	1.04 Hz
$^3\text{J}_{\text{CD}}$	8.30 Hz	8.0 Hz	8.19 Hz	8.0 Hz	8.19 Hz
$^n\text{J}_{\text{H}_\text{D}-\text{F}_{\text{YY}'}}$	1.14 Hz	0.5 Hz	1.10 Hz	decoupled	decoupled
R-factor			8.53 %		7.66 %

*Proton NMR spectrum recorded with selective fluorine decoupling $^1\text{H}\{^{19}\text{F}_{\text{YY}'}\}$.

3.3.2 Fluorine NMR spectrum (nine spin-system AA'BB'CCC-YY')

The molecule of interest, molecule-I, has two perfluorotolu-4-yl groups that produce different signals in the ^{19}F spectrum (Figure. 4), not only are the chemical shifts different and distinct but the structure of the multiplets are significantly different too. It is this complexity of fluorine NMR spectra that often prevents detailed analysis of the multiplets from appearing in the literature. The influence of the long-range H_d - $\text{F}_{\text{YY'}}$ coupling was readily identified and a spin-system setup to enable correct analysis, however, the reasons for the significant differences in the perfluoro-4-yl multiplets was much less obvious. Spin systems labels are devised clearly to distinguish between two chemically similar moieties, hence, AA'BB'CCC and XX'YY'ZZZ- H_d were constructed, but initial attempts to analyse the spectrum were unable to accurately reproduce the ^{19}F experimental multiplets. The extra complexity observed in the experimental multiplets was eventually assigned to a very long-range ^{19}F coupling $\text{F}_{\text{YY'}} - \text{F}_{\text{BB'}}$ between the two fluorinated aromatic rings of 4.05 Hz (Table 3).

The original analysis was achieved using WinD-NMR and SpinWorks and was a significant challenge taking many days of time (in total). The validity of the extracted data was confirmed using state-of-the-art NMR probes from JEOL and Bruker, a dual-tuned ^1H - ^{19}F COSY using NUS provided evidence of the H-F coupling, a ^{19}F - ^{19}F COSY using NUS over the whole chemical shift range provided a detailed correlation map in just a 10 minutes (requires ~ 1 hour without NUS and is prohibitive in our automation setup), selective $^1\text{H}\{^{19}\text{F}\}$ decoupling identified the long-range H-F coupling, and returned the low-field hydrogen to the expected complex "doublet" pattern, $^1\text{H}\{^{19}\text{F}\}$ COSY using NUS identified the correlation between H_D and $\text{F}_{\text{YY'}}$, 2D and selective-1D ^1H - ^{19}F nOe identified the close approach of the fluoroaromatic groups and the CH_2 group.

Subsequent analysis using ANATOLIA had a significant advantage in that the correct 9 spin-system setup of AA'BB'CCC-YY' was already known, however, to prove that ANATOLIA was able to provide a suitable analysis of this complex spectrum, the accurate values from SpinWorks analysis were used as input and within a few minutes extremely similar values were obtained with an excellent fit factor of 12.58%. A more real-world scenario was to use inaccurate data extracted directly from the experimental ^{19}F spectrum (~ 21.7 Hz) and the long-range H-F coupling (~ 1 Hz) from the ^1H spectrum, thus, this partially blind analysis was a more realistic challenge. Here, changing the input couplings to inaccurate values by approximately 2 Hz variation on each coupling still allowed accurate data to be produced. Only when the $^4\text{J}_{\text{FF}}$ AA' coupling was changed from an accurate value of -7.67 Hz to an inaccurate value of $+2.0$ Hz, and $^4\text{J}_{\text{FF}}$ BB' changed from an accurate value of $+0.54$ Hz to an inaccurate value of $+4.0$ Hz did the ANATOLIA optimisation fail.

For such a complex set of multiplets, which only yielded its coupling constant values after significant investment of time using traditional assign-iterate methods, ANATOLIA analysis offers a significant improvement in speed.

3.3.3 Fluorine NMR spectrum (ten spin-system XX'YY'ZZZBB'- H_d)

Within each perfluorotoluyyl group, each multiplet can be clearly distinguished as the influence of the oxygen or benzimidazole nitrogen in this molecule produces subtly

environment differences that result unique chemical shifts. Also, the ^{19}F - ^{19}F coupling constants are significantly different, particularly for the couplings that show significant second-order effects $^4J_{\text{FF}} \text{ AA}'$ vs XX' (-7.67 Hz vs -5.7 Hz) and $^4J_{\text{FF}} \text{ BB}'$ vs YY' (0.54 Hz vs 4.8 Hz), which have a significant effect on the appearance of the multiplets. Here the traditional assign-iterate NMR analysis methods required a significant input of time and effort and many optimisation steps to extract accurate parameters. In contrast, ANATOLIA achieved an accurate result for a 10-spin-system in only 7 minutes. Faster analysis was achieved by using homo- and hetero- nuclei decoupling to simplify the spectrum to create a smaller spin-system, this was achieved by using the state-of-the-art H-F probes and selective $^1\text{H}\{^{19}\text{F}\}$, $^{19}\text{F}\{^1\text{H}\}$ and $^{19}\text{F}\{^{19}\text{F}\}$ methods (see supporting information).

Analysis using ANATOLIA required a 10 spin-system $\text{XX}'\text{YY}'\text{ZZZ}\text{-YY}'\text{-H}_\text{d}$ to be setup in the and within a few minutes accurate values were obtained with an excellent fit factor of 11.63%. Here, the only values that could be extracted directly from the experimental ^{19}F spectrum were the couplings to the CF_3 groups (~ 21.7 Hz) and the long-range H-F coupling (~ 1 Hz), hence the influence of inaccurate input data was unavoidable. It was shown that changing the input coupling constant values by approximately 4 Hz, still allowed accurate data to be produced. Only when coupling constants that show significant second-order effects, such as AA' , was changed from -5.7 Hz to +2.0 Hz, and BB' changed from +4.8 Hz to +3 Hz did the ANATOLIA optimisation fail.

Figure 4. Experimental ^{19}F NMR spectra (lower) and calculated (upper) of molecule-I ~ 20 mg in 0.7 ml of CDCl_3 referenced to $\text{CFCl}_3 = 0$ recorded using a JEOL ECZ 500 (470 MHz for ^{19}F). Analysed using data derived from SpinWorks and WinDNMR to obtain accurate parameters, then subsequently optimised using ANATOLIA from inaccurate input values.

Table 3. ANATOLIA optimisation $\text{AA}'\text{BB}'\text{CCC YY}'$ and $\text{XX}'\text{YY}'\text{ZZZ-BB}'\text{-H}_\text{d}$ using accurate starting values, molecule-I.

	$\text{AA}'\text{BB}'\text{CCCYY}'$		$\text{XX}'\text{YY}'\text{ZZZBB}'\text{H}_\text{d}$
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Spin label	ANATOLIA label		Spin label	ANATOLIA label	
AA'	Freq A	10739.16	XX' shift	Freq X	12060.1
BB'	Freq B	3737.33	YY' shift	Freq Y	9935.2
CF ₃	Freq C	50402.18	CF ₃ shift	Freq Z	50242.1
H	Freq H	9935.0	BB' shift	Freq BB	3744.0
AA'	J 1,2	-7.05	XX	J 1,2	-6.420
AB	J 1,3	-21.49	XY	J 1,3	-21.86
AB'	J 1,4	8.88	XY'	J 1,4	11.604
A-CF ₃	J 1,5	21.68	X-CF ₃	J 1,5	21.97
BB'	J 3,4	0.917	YY'	J 3,4	4.20
BB'-YY'	J 3,8	3.998	YY'-BB' *	J 3,8	4.05
			YY'-H _d *	J 9,10	1.14
Linewidth		1.59			
R-Factor		12.58 %			11.63 %

* Parameters not optimised. Time for calculation ~1 min for AA'BB'CCC-YY' and ~7 minutes for XX'YY'ZZZBB'-H_d using Toshiba laptop, Intel core i5 @ 2.3 GHz, 16Gb RAM, W10-64 bit.

3.4 Ab initio structure calculations.

Geometry optimised structures were readily obtained using Gaussian-03^[38] employing B3LYP/6-31G*, which provided three rotamers for which the global minimum (R1) was in agreement with experimental nOe data (Figure 5). A detailed description of the application of GIAO method^[39, 40] and calculated coupling constant values^[41] is provided in the supplementary information section.

Figure 5. Gaussian-09 calculated structures of molecule-I optimising the geometry using B3LYP/6-31G*. Relative energies of rotamer 1 = 0.00 kcal/mol, rotamer 2 = 3.46 kcal/mol and rotamer 3 = 2.32 kcal/mol. A coupling constant of 4.05 Hz between fluorine atoms YY' and BB' (2.26513 Å), is assigned to a through-space F...F interaction.

Detailed NMR analysis of the experimental multiplets identified the long-range ^{19}F - ^{19}F coupling between the different aromatic rings (BB'-YY') of 4.05 Hz, this is unlikely to be due to a pure through-bond interaction of " 9J " via σ and π bonds. It is prudent to consider a through-space F-F coupling [42,43, 44] as a distance relationship in difluorocyclophanes show J_{FF} couplings of up to 110 Hz at 2.419 Å, reducing to a few 10's of Hz at approximately 3.0 Å to 3.2 Å. [45] For a series of structurally rigid 1,8-difluoronaphthalene compounds the through-space J_{FF} coupling was between 85.2 Hz to 36.6 Hz corresponding to distances of 2.452 Å to 2.718 Å, [46] with the specific contributions to the measured experimental coupling being dissected, showing that the Fermi contact term of +56 Hz results from a combination of a through-space coupling (+75 Hz) and σ through-bond coupling (-19 Hz). [47] The contributions from the diamagnetic and paramagnetic parts of the ^{19}F nuclear magnetic shielding tensor, paramagnetic spin-orbit term and Fermi contact contributions to indirect nuclear spin-spin coupling constants have been rationalised. [48]

The fluxional nature of this compound at room temperature in dilute CDCl_3 solution is implicit in the equivalence of the AA', BB', XX' and YY' ^{19}F chemical shifts, thus the barrier to rotation of the perfluorotoluyll groups is expected to be sufficiently small to allow 'free' rotation. Also, the experimentally measured nOe $^1\text{H} \rightarrow ^{19}\text{F}$ shows that a structure exists that allows both F-YY' and F-BB' groups to have a close proximity to the CH_2 group, and this is supported by geometry optimisations that show a global minimum for rotamer R1 (F---H = 2.26 Å and H---F = 2.38 Å). However, the authors are not aware of literature examples of a through-space $\text{F}_{\text{YY'}}\cdots\text{H}-\text{C}-\text{H}\cdots\text{F}_{\text{BB'}}$ pathway to transmit the coupling, indeed the CH_2 carbon and hydrogen signals show no couplings to the near-neighbour fluorine atoms. A more likely pathway to allow coupling between the fluorine atoms exists within the same low-energy rotamer (R1) as a close approach between $\text{F}_{\text{YY'}}$ and $\text{F}_{\text{BB'}}$ of 2.265 Å exists which would equate to a large through-space coupling in the order of several hundred Hz, if this were a rigid structure.

The close approach of H_d and $\text{F}_{\text{YY'}}$ was confirmed experimentally by ^1H - ^{19}F nOe and NOESY experiments, and the potential energy map shows that multiple conformations exist that show a close proximity of these two atoms. The small H_d and $\text{F}_{\text{YY'}}$ coupling of 1.14 Hz may be due to a through-space interaction or a long-range $^6J_{\text{HF}}$ through bond interaction.

4.1 Investigation of 1-tetrafluoropyrid-4-yl,-2-tetrafluoropyrid-4-yl-thio-1H-benzimidazole (molecule-II).

4.1.2 Proton NMR spectrum (six spin-system $\text{H}_A\text{H}_B\text{H}_C\text{H}_D$ - $^{19}\text{F}_{\text{YY'}}$)

Figure 6. Chemical structure and spin-labels of 1-(4,4,4,4-tetrafluorophenyl)-2-(4,4,4,4-tetrafluorophenylthio)-1H-benzimidazole (molecule_II), Gaussian optimised structure and $^{19}\text{F}\cdots^{19}\text{F}$ interatomic distance for global minimum structure, barrier to rotation of thio-tetrafluoro-pyridyl group = 2.295 kcal/mol.

This molecule clearly illustrates the complexity of second-order couplings in what would appear to be similar systems. The general features of the ^1H spectrum are similar to the previous compound, displaying a second-order closely-coupled spin-system where the hydrogen signals comprise of 4 aromatic multiplets (H_A H_B H_C H_D) with a long-range H_D coupling to the YY' fluorine atoms (Figures 6 and 7). The H_b and H_c 'triplets' have almost the same chemical shift producing significant distortion and overlap. A selective dual-tuned ^1H $\{^{19}\text{F}\}$ probe setup, allowed identification of extra coupling constants, that were assigned to a long-range through-space interaction with the YY' aromatic fluorine atoms. The ANATOLIA input files were copied and pasted from the previous analysis and only required trivial modifications of specific chemical shifts, offering a convenient time saving. The very small chemical shift difference between hydrogen atoms B and C has a significant effect on the appearance and intensities of all hydrogen signals, ANATOLIA produced an excellent fit for the whole ^1H spectrum, and identified $\delta\Delta = 5.35$ Hz (~ 0.001 ppm) in just a few seconds (Table 4 and Figure 7).

Figure 7. Experimental(lower) and ANATOLIA calculated (upper) ^1H NMR multiplets of molecule-II ~20 mg in 0.7 ml of CDCl_3 referenced to TMS at 0.0 ppm, ^1H (left) and $^1\text{H}\{\text{sel-dec-}^{19}\text{F}\}$ (right) spectra recorded using a JEOL ECZ 500.

Table 4. ANATOLIA optimised ^1H and ^{19}F chemical shifts and ^{19}F - ^{19}F and ^1H - ^{19}F coupling constants for molecule-II, ^1H spectrum ($\text{H}_\text{A}\text{H}_\text{B}\text{H}_\text{C}\text{H}_\text{D}\text{-F}_{\text{YY}'}$), $^1\text{H}\{\text{sel-}^{19}\text{F}\}$ * ($\text{H}_\text{A}\text{H}_\text{B}\text{H}_\text{C}\text{H}_\text{D}$) and ^{19}F ($\text{AA}'\text{BB}'\text{XX}'\text{YY}'\text{-H}_\text{d}$ and $\text{AA}'\text{BB}'\text{XX}'\text{YY}'$) spectra. ~20 mg in 0.7 ml of CDCl_3 using a JEOL ECZ 500.

Spin labels	Input (Hz)	ANATOLIA	Input(Hz)*	ANATOLIA*
$^1\text{H } \delta \text{ H}_\text{A}$	3887	7.772 ppm	3887	7.773 ppm
$^1\text{H } \delta \text{ H}_\text{B}$	3710	7.417 ppm	3710	7.418 ppm
$^1\text{H } \delta \text{ H}_\text{C}$	3715	7.428 ppm	3715	7.429 ppm
$^1\text{H } \delta \text{ H}_\text{D}$	3589	7.177 ppm	3589	7.177 ppm
$^{19}\text{F } \delta \text{ YY}'$	-40000	-40000	-40000	-40000
$^3\text{J AB}$	8.0 Hz	8.23 Hz	8.0 Hz	8.23 Hz
$^4\text{J AC}$	2.0 Hz	1.12 Hz	2.0 Hz	1.11 Hz
$^5\text{J AD}$	0.5 Hz	0.74 Hz	0.5 Hz	0.70 Hz
$^3\text{J BC}$	8.0 Hz	7.36 Hz	8.0 Hz	7.35 Hz
$^4\text{J BD}$	2.0 Hz	1.09 Hz	2.0 Hz	1.09 Hz
^3JCD	8.0 Hz	8.27 Hz	8.0 Hz	8.28 Hz
$^n\text{J H}_\text{A}\text{-F}_{\text{YY}'}$	0.5 Hz	1.54 Hz	0	0
R-factor		11.04 %		11.91 %
Spin label		$\text{AA}'\text{BB}'\text{XX}'\text{YY}'\text{-H}_\text{d}$		$\text{AA}'\text{BB}'\text{XX}'\text{YY}'$ **
$^{19}\text{F } \delta \text{ AA}'$		-88.197 ppm		-88.199 ppm
$^{19}\text{F } \delta \text{ BB}'$		-135.208 ppm		-135.208 ppm
$^{19}\text{F } \delta \text{ XX}'$		-85.113 ppm		-85.115 ppm
$^{19}\text{F } \delta \text{ YY}'$		-142.992 ppm		-142.995 ppm
$^1\text{H } \delta \text{ H}_\text{d}$				
AA'		-13.33 Hz		-13.35 Hz

AB		-20.92 Hz		-20.92 Hz
AB'		28.98 Hz		28.97 Hz
BB'		-3.39 Hz		-3.39 Hz
BB'-YY'		4.63 Hz		4.67 Hz
YY'-H _d		1.46 Hz		¹ H decoupled
XX'		-14.00 Hz		-14.03 Hz
XY		-20.44 Hz		-20.45 Hz
XY'		29.28 Hz		29.28 Hz
YY'		-4.50 Hz		-4.50 Hz
R-Factor		11.47 %		20.75 %

*Proton NMR spectrum recorded with fluorine decoupling ¹H{¹⁹F}.

**Fluorine NMR spectrum recorded with proton decoupling ¹⁹F{¹H}.

4.1.3 Fluorine NMR spectrum (nine spin-system AA'BB'XX'YY'-H_d)

The previous spin-system input files were not suitable to copy and paste as they refer to a slightly different spin-system, but were useful as a starting point to speed up analysis. Here ANATOLIA was able to fit all four multiplets into the ¹⁹F spectrum to produce accurate data within 5 minutes (Table 5 and Figure 8). The molecule of interest, has two tetrafluoropyridine groups, one bonded to a sulfur and one bonded to nitrogen, these different environments cause each pyridyl group to produce distinct chemical shifts for each multiplet in the ¹⁹F spectrum (XX' chemical shift is similar to AA' and YY' chemical shift is similar to XX'). The relative simplicity of the XX' and AA' multiplets allowed identification of the key coupling constants that show strongly second-order effects, and these data served as input to analyse the more complex YY' and BB' multiplets. Part of the extra complexity observed in the experimental multiplets YY' and BB' was assigned to a very long-range ⁸J_{FF} coupling F_{YY'}-F_{BB'} between the two fluorinated aromatic rings of 4.55 Hz, with an additional long-range coupling between F_{YY'}-H_d.

Figure 8. Experimental ¹⁹F NMR spectra (lower) and calculated (upper) of molecule-II, analysed using data derived from *ab initio* GIAO which was then further optimised using ANATOLIA, ~20 mg in 0.7 ml of CDCl₃ referenced CFCl₃ = 0 ppm, recorded using a JEOL ECZ 500.

4.1.4 Analysis of ^{13}C NMR Isotopomers.

The existence of a single ^{13}C atom in the *ortho*-(AA'), *meta*-(BB'), or *ortho*-(XX'), or *meta*-(YY') positions of the pyridine ring changes the spin-system and breaks symmetry. This subtle isotope effect changes the charge density around the ^{13}C -F bond, thus the chemical shift of previously equivalent fluorine atoms can now be different, such that $\delta\text{A} \neq \delta\text{A}'$. Here, the isotope-shift effect is greatest for the directly attached fluorine, the influence of this isotope effect also propagates over several bonds allowing $\delta\text{B} \neq \delta\text{B}'$. Once symmetry of the pyridyl ring is broken by a single ^{13}C atom, there is no longer a strict requirement for coupling constants to be equivalent, such that $^3\text{J AB} \neq \text{A}'\text{B}'$ and/or $^5\text{J AB}' \neq \text{A}'\text{B}$, however, in this analysis good results were obtained by retaining equal values of "symmetry-related" FF coupling constants.

Therefore, each ^{13}C isotopomer is a distinct and different molecule and spin-system, which can have unique and distinct chemical shifts and coupling constants from one another (Table 5). Experimental evidence of this isotopomer effect is clearly visible in the carbon spectrum where *ortho*- ^{13}C -AA'BB' and *meta*- ^{13}C -AA'BB' have multiplets that are completely different in appearance (Figure 9). Each isotopomer sub-spectrum requires an individual analysis in order to extract $\Delta\delta$ ^{19}F shifts and $^n\text{J}_{\text{CF}}$ coupling constants, this has traditionally involved the investment of significant time such that these experimental values are not routinely reported, here the speed advantage of ANATOLIA helps make these analyses much more accessible.

Good signal-to-noise and digital resolution in the carbon spectrum is preferable as this allows some of the low-intensity second-order signals to be visible and well resolved. The appearance of the carbon-fluorine multiplets for *ortho*- and *para*- carbons are completely different, the isotopomer that is responsible for the *ortho*-carbon(AA') signal at 143 ppm approximates to a triplet-of-doublets, and the isotopomer that is responsible for the *meta*-carbon(BB') signal at 141 ppm is a deceptively complex multiplet. This significant difference in appearance of the multiplets is mainly as a result of the complex interplay of $\delta\text{A} \neq \delta\text{A}'$ and $\delta\text{B} \neq \delta\text{B}'$ for the fluorine chemical shifts and $^4\text{J AA}'$ and $^4\text{J BB}'$ couplings, but subtle differences in the spectrum are also linked to the size and sign of the $^1\text{J-}^4\text{J CF}$ couplings ($^1\text{J}_{\text{CF}}$ is negative). To confirm assignments of the $^n\text{J}_{\text{CF}}$ coupling constants, a $^{13}\text{C}\{\text{sel-}^{19}\text{F}\}$ carbon spectrum was acquired to selectively remove the large $^1\text{J}_{\text{CF}}$ coupling and the associated $^3\text{J}_{\text{CF}}$. This produced a much simplified ^{13}C multiplet and reduced the spin-systems to $^{13}\text{C-YY}'$ and $^{13}\text{C-BB}'$ comprising of $^2\text{J}_{\text{CF}}$, $^4\text{J}_{\text{CF}}$ coupling constants and $^{19}\text{F } \Delta\delta$. Once these values were known then the previously analysed ^{19}F coupling data was utilised as a starting point and ^{19}F chemical shift difference ($\delta\Delta$) explored and optimised, then the influence of the couplings and $\Delta\delta$ explored concurrently, and finally the influence of all $^1\text{J-}^4\text{J CF}$ couplings was investigated. Good agreement between experimental ^{13}C multiplets and the ANATOLIA optimisation was achieved in just a few minutes to obtain the final fit.

The coupling constants obtained using B3LYP/6-31G* were more accurate than for aug-pcJ-0 method, though aug-pcJ-2 with diffuse function is recommended, the chemical shifts obtained using aug-pcJ-0 were more accurate.

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Table 5. ANATOLIA optimised isotopomer ^{13}C multiplets for molecule-II, $^{13}\text{C-AA'}$, $^{13}\text{C-BB'}$, $^{13}\text{C-XX'}$ and $^{13}\text{C-YY'}$ employing some Gaussian derived starting values.

Spin labels	B3LYP/6-311 ⁺⁺ G ^{**} /nmr=mixed	B3LYP/aug_cc_pcJ_0	ANATOLIA			
			Ortho- ¹³ C AA'	Meta- ¹³ C BB'	Ortho- ¹³ C XX'	Meta- ¹³ C YY'
δ ¹⁹ F A	-102.7 ppm	-92.62 ppm	-88 ppm			
δ ¹⁹ F A'			Δδ = 35.53 Hz	Δδ = 10.12 Hz	Δδ = 0 Hz	
δ ¹⁹ F B	-159.0 ppm	-152.99 ppm	-135 ppm			
δ ¹⁹ F B'			Δδ = 10.12 Hz	Δδ = 73.26 Hz	Δδ = 0 Hz	
δ ¹⁹ F X	-99.3 ppm	-87.75 ppm	-85 ppm			
δ ¹⁹ F X'			Δδ = 0 Hz		Δδ = 37.3 Hz	Δδ = 9.55 Hz
δ ¹⁹ F Y	-159.9 ppm	-149.37 ppm	-142 ppm			
δ ¹⁹ F Y'			Δδ = 0 Hz		Δδ = 10.36 Hz	Δδ = 111.56 Hz
δ ¹³ C A <i>ortho</i>	150.7 ppm	153.27 ppm	143.515 ppm			
δ ¹³ C B <i>meta</i>	146.5 ppm	150.62 ppm		141.161 ppm		
δ ¹³ C X <i>ortho</i>	151.3 ppm	153.57 ppm			144.115 ppm	
δ ¹³ C Y <i>meta</i>	145.8	150.21 ppm				138.117 ppm
Meta-C-F A (² J _{CF})	28.6 Hz	14.12 Hz		29.08 Hz (25.26)		
Meta-C-F A' (⁴ J _{CF})	7.8 Hz	8.37 Hz		6.64 Hz (6.05)		
Meta-C-F B (¹ J _{CF})	-333.1 Hz	-347.10 Hz		-261.71 Hz		
Meta-C-F B' (³ J _{CF})	-4.9 Hz	-9.85 Hz		-1.83 Hz		
Ortho-C-F A (¹ J _{CF})	-321.6 Hz	-348.54 Hz	-247.50 Hz			
Ortho-C-F A' (³ J _{CF})	8.4 Hz	-3.13 Hz	12.21 Hz			
Ortho-C-F B (² J _{CF})	14.7 Hz	3.47 Hz	17.45 Hz			
Ortho-C-F B' (⁴ J _{CF})	3.8 Hz	3.99 Hz	3.27 Hz			
Meta-C-F X (² J _{CF})	27.3 Hz	12.91 Hz				29.24 Hz (27.46)
Meta-C-F X' (⁴ J _{CF})	9.0 Hz	9.46 Hz				7.28 Hz (7.40)
Meta-C-F Y (¹ J _{CF})	-345.0 Hz	-364.08 Hz				-268.19 Hz
Meta-C-F Y' (³ J _{CF})	-5.8 Hz	-9.83 Hz				-2.41 Hz
Ortho-C-F X (¹ J _{CF})	323.2 Hz	-351.88 Hz			-248.08 Hz	
Ortho-C-F X' (³ J _{CF})	8.9 Hz	-2.98 Hz			13.00 Hz	

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<i>Ortho</i> -C-F Y (² J _{CF})	12.5 Hz	1.56 Hz			15.11 Hz	
<i>Ortho</i> -C-F Y' (⁴ J _{CF})	4.4 Hz	4.42 Hz			3.56 Hz	
AA' (⁴ J _{FF})	-14.075 Hz	-9.52 Hz			-13.32 Hz	
AB (³ J _{FF})	-16.576 Hz	-16.15 Hz			-20.93 Hz	
AB' (⁵ J _{FF})	34.649 Hz	38.40 Hz			28.89 Hz	
A'B (⁵ J _{FF})	36.015 Hz	39.19 Hz			28.89 Hz	
A'B' (³ J _{FF})	-17.399 Hz	-14.27 Hz			-20.93 Hz	
BB' (⁴ J _{FF})	-5.135 Hz	-3.21 Hz			-3.312 Hz	
XX' (⁴ J _{FF})	-15.374 Hz	-11.03 Hz			-14.49 Hz	
XY (³ J _{FF})	-17.437 Hz	-15.18 Hz			-20.42 Hz	
XY' (⁵ J _{FF})	37.331 Hz	41.31 Hz			29.21 Hz	
X'Y (⁵ J _{FF})	37.831 Hz	40.82 Hz			29.21 Hz	
X'Y' (³ J _{FF})	-15.873 Hz	-14.41 Hz			-20.42 Hz	
YY' (⁴ J _{FF})	-8.267 Hz	-6.11 Hz			-4.00 Hz	

Calculated B3LYP/6-31G* and aug_cc-pcJ-0 : values are averages. Values in brackets from ¹³C{sel-¹⁹F}.

Symmetry retained for ⁿJ_{FF} couplings

AA' (⁴J_{FF}) = -13.32 Hz AB (³J_{FF}) = A'B' (³J_{FF}) = -20.93 Hz AB' (⁵J_{FF}) = A'B (⁵J_{FF}) = 28.89 Hz BB' (⁴J_{FF}) = -3.31 Hz

XX' (⁴J_{FF}) = -14.49 Hz XY (³J_{FF}) = X'Y' (³J_{FF}) = -20.42 Hz XY' (⁵J_{FF}) = X'Y (⁵J_{FF}) = 29.21 Hz YY' (⁴J_{FF}) = -4.00 Hz

Figure 9. Experimental $^{13}\text{C}\{^1\text{H}\}$ NMR spectra (lower) and calculated (upper) of molecule-II ~20 mg in 0.7 ml of CDCl_3 recorded using a JEOL ECZ 500. Experimental $^{13}\text{C}\{\text{sel-}^{19}\text{F}\}$ NMR spectra (lower) and calculated (upper) removed the large $^1J_{\text{CF}}$ coupling and the associated $^3J_{\text{CF}}$ couplings and produced the much simplified ^{13}C multiplet and reduced the spin-systems to $^{13}\text{C-YY'}$ and $^{13}\text{C-BB'}$ comprising of $^2J_{\text{CF}}$, $^4J_{\text{CF}}$ coupling constants only.

5 Conclusions

The ANATOLIA program is not only free but also simple to setup and readily accessible to bench chemists within Bruker TopSpin software. For the first time it has been applied to analyse complex second-order ^1H , ^{19}F and ^{13}C NMR spectra displaying unusual multiplets, it is shown that accurate coupling constants can be readily obtained. ANATOLIA was shown to be robust enough to be able to yield accurate spectral values from inaccurate input values, particularly for difficult to analyse couplings that display significant second-order effects like, $^4J_{\text{AA'}}$, $^4J_{\text{BB'}}$, $^4J_{\text{XX'}}$, and $^4J_{\text{YY'}}$. A significant challenge was to extract $^nJ_{\text{CF}}$ couplings and $\delta\Delta^{19}\text{F}$ chemical shifts from the ^{13}C -isotopomer multiplets in the carbon spectrum, but this was readily achieved in a timely manner. The *ab initio* coupling constants from Gaussian-09 were a useful starting point for ANATOLIA analysis, but were often less accurate than the semi-empirical method of Abraham, particularly for $^4J_{\text{FF}}$ AA', BB' XX' and YY'. The optimised geometries showed that molecules I and II are fluxional with 'free' rotation of the perfluoro-groups that allowed intra-molecular close approach of fluorine atoms, resulting in

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experimental measured through-space couplings of 4.05 Hz and 4.67 Hz that propagate between the aromatic fluoro-rings YY' and BB'.

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References

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- [1] D. A. Cheshkov, K. F. Sheberstov, D. O. Sinitsyn, V. A. Chertkov, ANATOLIA: NMR software for spectral analysis of total lineshape, *Magn. Reson. Chem.*, **2018**, *56*, 449-457.
- [2] Y. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J. L. Aceña, V. A. Soloshonok, K. Izawa, H. Liu, Next Generation of Fluorine-Containing Pharmaceuticals, Compounds Currently in Phase II–III Clinical Trials of Major Pharmaceutical Companies: New Structural Trends and Therapeutic Areas, *Chem. Rev.* **2016**, *116*, 422–518.
- [3] J. Wang, M. Sánchez-Roselló, J. L. Aceña, C. del Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok, H. Liu, Fluorine in pharmaceutical industry: fluorine-containing drugs introduced to the market in the last decade (2001–2011), *Chem. Rev.*, **2014**, *114*, 2432–506. doi: 10.1021/cr4002879
- [4] H. Chen, S. Viel, F. Ziarelli, L. Peng, ¹⁹F NMR: A Valuable Tool for Studying Biological Events. *Chem. Soc. Rev.* **2013**, *42*, 7971–7982.
- [5] R. E. Richards, T. P. Schaeffer, High Resolution Proton Resonance Spectra of some p-substituted Benzenes. *Trans. Faraday Soc.* **1958**, *54*, 1280.
- [6] P. L. Corio, *Structure of High-Resolution NMR-Spectra*. Academic Press. New York **1966**, 143.

- [7] R. J. Abraham, H. J. Bernstein, The Proton Magnetic Resonance Spectra of Thiophene, *Can. J. Chem.*, **1959**, 37, 2059.
- [8] R. J. Abraham, H. J. Bernstein, The Proton Resonance Spectra of Furan and Pyrrole. *Can. J. Chem.*, **1959**, 37, 1056.
- [9] H. S. Gutowsky C. H. Holm, A. Saika, G. A. Williams, Electron Coupling of Nuclear Spins. I. Proton and Fluorine Magnetic Resonance Spectra of Some Substituted Benzenes. *J. Am. Chem. Soc.*, **1957**, 79, 4596.
- [10] I. J. Lawrenson, High-resolution Fluorine Magnetic Resonance Spectra of Some Pentafluorophenyl-1-Derivatives, *J. Chem. Soc.*, **1965**, 1117-1120.
- [11] R. J. Abraham, *The Analysis of High Resolution NMR Spectra*, Elsevier Publishing Co., New York, **1971**. DOI: 10.1021/ed049pA603.
- [12] J. M. Granda, L. Donina, V. Dragone, D. L. Long, L. Cronin, Controlling an organic synthesis robot with machine learning to search for new reactivity, *Nature*, **2018**, 559, 377-381.
- [13] P. M. Richardson, A. J. Parrott, O. Semenova, A. Nordon, S. B. Duckett, M. E. Halse, SABRE hyperpolarization enables high-sensitivity ^1H and ^{13}C benchtop NMR spectroscopy, *Analyst*, **2018**, 143, 3442-3450.
- [14] M. Edgar, B. C. Percival, M. Gibson, J. Masania, K. Beresford, P. B. Wilson, M. Grootveld, Benchtop NMR Spectroscopy and Spectral Analysis of the cis- and trans-Stilbene Products of the Wittig Reaction, *J. Chem. Educ.*, **2019**, 96, 1938-1947. doi.org/10.1021/acs.jchemed.8b00657.
- [15] K. L. Yearty, J. T. Sharp, E. K. Meehan, D. R. Wallace, D. M. Jackson, R. W. Morrison, Implementation of PicoSpin Benchtop NMR Instruments into Organic Chemistry Teaching Laboratories through Spectral Analysis of Fischer Esterification Products, *J. Chem. Educ.*, **2017**, 94, 932-935.
- [16] M. F. Isaac-Lam, Analysis of Bromination of Ethylbenzene Using a 45 MHz NMR Spectrometer: An Undergraduate Organic Chemistry Laboratory Experiment, *J. Chem. Educ.*, **2014**, 91, 1264-1266.
- [17] J. S. Martin, A. R. Quirt, NMR spectra of symmetric molecules. I. The spin Hamiltonian for twofold symmetry, *J. Magn. Reson.*, **1971**, 5, 318-327.
- [18] M. Clark, J. S. Thrasher, LAOCOON PC: NMR simulation on a personal computer, *J. Chem. Educ.*, **1990**, 67, 235.
- [19] Bruker GmbH software available in 1970s onwards on the ASPECT computer.
- [20] H. J. Reich, WinDNMR: Dynamic NMR Spectra for Windows. *J. Chem. Educ.*, **1995**, 72, 1086.
- [21] NMRSIM Copyright (C) 1999 by Bruker Analytik GmbH. Part No. H9171.
- [22] G. Hagele, R. Fuhler, H. W. Hoffken, T. Lenzen, R. Spiske, U. Weber, Novel PC-Program-Systems for Efficient Analysis – Simulation and Iteration of High Resolution 1-D NMR-Spectra, *Comput. Chem.*, **1995**, 19, 283-285. doi.org/10.1016/0097-8485(95)00040-Y.
- [23] D. S. Stephenson, Analysis of Spectra: Automatic Methods, eMagRes, **2007**, doi.org/10.1002/9780470034590.emrstm0013

- [24] J. Heinzer, Iterative Least-Squares NMR Lineshape Fitting with Use of Symmetry and Magnetic Equivalence Factorization, *J. Magn. Reson.*, **1977**, *26*, 301-316. doi.org/10.1016/0022-2364(77)90176-7.
- [25] R. Laatikainen, M. Niemitz, U. Weber, J. Sundelin, T. Hassinen, J. Vepsäläinen, General Strategies for Total-Lineshape-Type Spectral Analysis of NMR Spectra Using Integral-Transform Iterator, *J. Magn. Reson. ser. A*, **1996**, *120*, 1-10. doi.org/10.1006/jmra.1996.0094.
- [26] ChemAdder software: <http://www.chemadder.com/index.html>
- [27] NUTS NMR data processing software, Acorn NMR Inc., <http://www.acornnmr.com/oldsite/nuts/>.
- [28] NMR simulation: gNMR, <https://home.cc.umanitoba.ca/~budzelaa/gNMR/gNMR.html>
- [29] Mnova NMR, Mestrelab Research, <http://mestrelab.com/software/mnova/nmr/>
- [30] H. Hogben, M. Krzystyniak, G. Charnock, P. Hore, I. Kuprov, Spinach – A software library for simulation of spin dynamics in large spin systems, *J. Magn. Reson.*, **2011**, *208*, 179. doi.org/10.1016/j.jmr.2010.11.008.
- [31] SpinWorks 3.1, Copyright © 2009, Kirk Marat, University of Manitoba.
- [32] <https://github.com/dcheshkov/ANATOLIA>
- [33] <http://anatolia.nmrclub.ru/>
- [34] M. Edgar, F. Zeinali, M. Mojally, C. Hughes, S. Riaz, G. W. Weaver, NMR Spectral Analysis of Second-Order ^{19}F - ^{19}F , ^{19}F - ^1H and ^{13}C - ^{19}F Coupling Constants in Pentafluorobenzene and Tetrafluoro-4-(morpholino)pyridine using ANATOLIA, *J. Fluorine Chem.*, **2019**, *224*, 35-44. doi.org/10.1016/j.jfluchem.2019.04.018.
- [35] V. Y. Orekhov, I. Ibraghimov, M. Billeter, Optimizing resolution in multidimensional NMR by three-way decomposition, *J. Biomol. NMR*, **2003**, *27*, 165-173.
- [36] C. W. Haich, J. Hilton, L. H. Sutcliffe, G. J. T. Tiddy, A Positive “Through-Space” Fluorine-Fluorine Coupling Constant in Perfluorotoluene, *J. Mag. Reson.*, **1975**, *18*, 241-247.
- [37] R. J. Abraham, M. Ashley Cooper, A re-investigation of $^4J_{\text{FF}}$ and $^5J_{\text{FF}}$ nuclear spin-spin couplings in substituted benzenes, a novel conformational tool, *Phys. Chem. Chem. Phys.*, **2016**, *18*, 15822-15827.
- [38] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

- [39] R. Ditchfield Self-consistent perturbation theory of diamagnetism, *Mol. Phys.*, **1974**, *27*, 789-807. <https://doi.org/10.1080/00268977400100711>.
- [40] K. Wolinski J. F. Hinton P. Pulay, Efficient Implementation of the Gauge-Independent Atomic Orbital Method for NMR Chemical Shift Calculations, *J. Am. Chem. Soc.*, **1990**, *112*, 8251-8260. DOI: 10.1021/ja00179a005
- [41] T. Helgaker, M. Watson, N. C. Handy, Analytical calculation of nuclear magnetic resonance indirect spin-spin coupling constants at the generalized gradient approximation and hybrid levels of density-functional theory, *J. Chem. Phys.*, **2000**, *113*, 9402-9409. DOI: 10.1063/1.1321296
- [42] W. J. E. Parr, T. Schaefer, Through-space or proximate ^{19}F - ^{19}F and ^{19}F - ^1H spin-spin coupling constants in some benzenesulfonyl fluoride derivatives, *Can. J. Chem.*, **1976**, *54*, 3564 – 3568.
- [43] W. D. Arnold, J. Mao, H. Sun, E. Oldfield, Computation of Through-Space ^{19}F - ^{19}F Scalar Couplings via Density Functional Theory, *J. Am. Chem. Soc.*, **2000**, *122*, 12164-12168.
- [44] T. Tuttle, J. Gräfenstein, D. Cremer, Analysis of the NMR through-space coupling mechanism between ^{19}F atoms, *Chem. Phys. Lett.*, **2004**, *394*, 5–13.
- [45] J. -C. Hierso, Indirect Nonbonded Nuclear Spin–Spin Coupling: A Guide for the Recognition and Understanding of “Through-Space” NMR J Constants in Small Organic, Organometallic, and Coordination Compounds, *Chem. Rev.*, **2014**, *114*, 4838–4867. [dx.doi.org/10.1021/cr400330g](https://doi.org/10.1021/cr400330g).
- [46] F. B. Mallory, C. W. Mallory, K. E. Butler, M. B. Lewis, A. Qian Xia, E. D. Luzik, Jr., L. E. Fredenburgh, M. M. Ramanjulu, Q. N. Van, M. M. Francl, D. A. Freed, C. C. Wray, C. Hann, M. Nerz-Stormes, P. J. Carroll, L. E. Chirlian, Nuclear Spin-Spin Coupling via Nonbonded Interactions. 8.1 The Distance Dependence of Through-Space Fluorine-Fluorine Coupling, *J. Am. Chem. Soc.*, **2000**, *122*, 4108-4116.
- [47] I. Ghiviriga, L. Zhang, H. Martinez, R. H. Contreras, C. F. Tormena, L. Nodina, W. R. Dolbier Jr, ^{19}F chemical shifts, coupling constants and conformational preferences in monosubstituted perfluoroparacyclophanes, *Magn. Reson. Chem.*, **2011**, *49*, 93–105.
- [48] R. H. Contreras, T. Llorente, L. C. Ducati, C. F. Tormena, Revisiting NMR Through-Space J_{FF} Spin–Spin Coupling Constants for Getting Insight into Proximate F–F Interactions, *J. Phys. Chem. A*, **2014**, *118*, 5068–5075. [dx.doi.org/10.1021/jp501929t](https://doi.org/10.1021/jp501929t).

NMR spectral analysis of strongly second-order 6-, 8-, 9- and 10- spin-systems (^1H - ^{19}F , ^{19}F - ^{19}F and ^{13}C - ^{19}F) in Perfluorotoluly- and Tetrafluoro-pyridyl- aromatics using the line-shape method ANATOLIA.

Mark Edgar*, Dee Hayward, Fatemeh Zeinali, Shahzad Riaz and George W. Weaver.

Abstract.

A simple to use NMR analysis method (ANATOLIA) has been tested on complex ^1H , ^{19}F and ^{13}C multiplets. Upto 10 coupling nuclei were analysed in only 6 minutes using a laptop computer, and ANATOLIA was robust enough to be able to excellent results from inaccurate input values. A fluorine-fluorine coupling constant was attributed to a through-space interaction and modelled using Gaussian-09.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 7

Figure 8

Figure 9