



Paramagnetic Relaxation Enhancement to Improve Sensitivity of 1,1-ADEQUATE NMR

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Abstract. The 1,1-ADEQUATE NMR experiment provides easily interpretable spectra to identify two-bond proton-carbon correlations in the structural analysis of small molecules. Despite this, the low natural abundance of ¹³C drastically reduces the efficacy of this experiment due to its dependence on C-C magnetization transfers. A reduction of T_1 and T_2 relaxation times via paramagnetic relaxation enhancement (PRE) represents an opportunity to improve sensitivity and reduce experiment times by facilitating a reduction in recycle delays. In this work, we demonstrate Cr^{3+} at a concentration of 10 mM to be an excellent PRE agent. Paramagnetic relaxation enhancement was then applied in 1,1-ADEQUATE analysis of several compounds, limonene, camphor, amoxapine and quinidine, demonstrating signal-to-noise improvements up to 4.5-fold over diamagnetic conditions.

1 Introduction

1.1 Paramagnetic Relaxation Enhancement

Nuclear magnetic resonance (NMR) spectroscopy has long been the most effective and versatile analytical method for establishing through-bond and through-space connectivities between nuclei, allowing quantitation and identification of unknown molecules. NMR has also been widely used to study the structures and interactions of proteins (Bax and Clore, 2019), nucleic acids (Plavec, 2022) and carbohydrates (Speciale et al., 2022).

As recycle delays (d_1) typically encompass ~80% of total acquisition time in 2D NMR experiments (Cai et al., 2006), reducing T_1 represents a direct route to sensitivity enhancement. Reduction of T_1 can be achieved by paramagnetic reagents added to the sample to elicit Paramagnetic Relaxation Enhancement (PRE). Application of PRE has been used extensively in quantitative NMR/metabolomics and biomolecular NMR, with the aim of reducing lengthy experimental time (Luchinat et al., 2018; Mulder et al., 2019). Among the most frequently used PRE agents are Ni^{2+} (Cai et al., 2006), Cr^{3+} (Levy and Cargioli, 1973), Cu^{2+} (Murthy et al., 1997) and Gd^{3+} (Honrao et al., 2021; Kretsch et al., 2023), which can all be used with diverse counterions/chelators depending on the solvent matrix. While PRE can be used to reduce T_1 relaxation, it can also impact transverse relaxation times (T_2). A drastic increase in T_2 -dependent linewidth can lead to significant line broadening, which reduces signal-to-noise, necessitating careful selection of paramagnetic ion to favor T_1 over T_2 relaxation enhancement (Luchinat et al., 2018).



1.2 Establishing Carbon-Carbon Connectivity in Small Molecules

In the realm of small molecule NMR, a common issue arising from structure elucidation efforts is the differentiation of two- and three-bond correlations in HMBC experiments. Though methodologies like H2BC (Petersen et al., 2006), SEA XLOC (Gyöngyösi et al., 2018), and i-HMBC (Wang et al., 2023) have offered solutions to this problem in certain instances, the 1,1-ADEQUATE experiment maintains an advantage in terms of universality combined with simplicity of spectral interpretation (Köck et al., 1996, 2003; Sauri et al., 2015). It provides a complementary readout to HMBC with correlations mainly arising from $^2J_{CH}$ couplings. While both HMBC and 1,1-ADEQUATE are proton-detected, after the initial polarization transfer from ^1H to ^{13}C 1,1-ADEQUATE experiments utilize a J_{CC} carbon-carbon magnetization transfer to establish $^2J_{HC}$ coupling pathways. Due to the low natural abundance of ^{13}C (1.1%), the J_{CC} magnetization transfer is especially inefficient necessitating long acquisition times or large sample quantities (Buevich et al., 2014). Additionally, due to the absence of heteronuclear dipole-dipole relaxation pathways for quaternary carbons correlations arising from these nuclei are generally weaker due to significantly longer T_1 times relative to protonated carbons (Fujiwara, et al., 1989). As quaternary carbons are overrepresented in structure revisions, accurate determination of quaternary carbon connectivity is critical to the generation of high fidelity NMR assignments (Cohen et al., 2022; Khatri Chhetri et al., 2018).

While PRE has been used extensively for decades, we know of no case where this approach has been used to improve the sensitivity of 1,1-ADEQUATE NMR, which would benefit significantly from increased sensitivity. To test this, we applied PRE to 1,1-ADEQUATE to quantify improvements in S/N over diamagnetic conditions. We hypothesize two significant impacts on the 1,1-ADEQUATE spectra: the first is that through a reduction in ^1H and ^{13}C T_1 via PRE, the optimal d1 for 1,1-ADEQUATE can be reduced, thereby resulting in a decrease in the total acquisition time of the experiment. And the second is that S/N of $^2J_{CH}$ correlations to quaternary carbons will be significantly improved. The protocol we present here represents a simple and effective strategy that can be utilized in difficult structure elucidation efforts by increasing the efficiency of acquiring 1,1-ADEQUATE NMR. Furthermore, this methodology does not require intimate knowledge of pulse sequencing, nor complex experimental set-ups, and so can be readily applied by a broad spectrum of investigators.

2 Materials & Methods

2.1 Study Consumables

All materials used in this study were purchased from Sigma Aldrich and were of analytical grade. Diamagnetic samples were prepared with 100 mg of (*R*)-(+)-Limonene and $CDCl_3$ to a final volume of 550 μL . For paramagnetic samples, a stock solution of the respective PRE metal chelate [$Fe(acac)_3$, $Ni(acac)_2$, $VO(acac)_2$ and $Cr(acac)_3$] was prepared in $CDCl_3$ (99+%-*d*) to 100 mM. This stock was then diluted into 100 mg (*R*)-(+)-Limonene and $CDCl_3$ to a final concentration of 0.5, 1, 2, 5 and 10 mM and a final sample volume of 550 μL .



Camphor, amoxapine and quinidine samples were prepared with 20 mg of each respective analyte and Cr(acac)₃ (10 mM diluted from a 100 mM stock solution) in CDCl₃ to a final sample volume of 550 µL.

2.2 NMR Spectroscopy & Data Processing

- 65 NMR spectra were acquired on a Bruker Neo spectrometer operating at 599.81 MHz equipped with a 5 mm QCI-F CryoProbe. The following acquisition parameters were used with the 1,1-ADEQUATE pulse program (adeq11etgprdsp) for limonene, which corresponded to a total acquisition time of 11m4s:

Table 1: Acquisition parameters for the adeq11etgprdsp pulse program.

	F2	F1
Points	2048	128
Scans	4	
Transmitter Offset (ppm)	3.25	89.993
Spectral Width (Hz)	5882.35	28657.152
J(CH) (Hz)	145	
J(CC) (Hz)	45	
D1 (s)	1	
Acquisition Time (s)	0.17408	0.0022333

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For d1-dependent experiments, d1 was changed from 1 s to the value reported in the results. Spectra were processed with the following variables in TopSpin 4.3 and imported into MestReNova v. 14.2.1 for signal-to-noise calculations:

Table 2: Post-acquisition processing settings for 1,1-ADEQUATE spectra.

	F2	F1
Window Function	Squared Sine	Squared Sine
Line Broadening (Hz)	1.0	0.3
Gaussian	0	0.1
Sinebell Shift	2	2
Baseline Polynomial Degree	5	5



Linear Prediction	0	4x
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2.3 Limonene Signal-to-Noise Calculations

Individual F1 (^{13}C) slices were extracted and peaks in F2 (^1H) were picked manually at the chemical shifts indicated in Table 3.

80 **Table 3: Chemical shift values of peaks extracted for Signal-to-Noise calculations.** Peaks were selected based on spectral resolution derived from assigned limonene spectrum (Fig. 1).

F1 Shift (^{13}C ppm)	F2 Shift (^1H ppm)	Assigned Correlation
30.988	5.43	$\text{H}_4 \rightarrow \text{C}_5$
30.988	2.14	$\text{H}_6 \rightarrow \text{C}_5$
30.988	1.84	$\text{H}_{1'} \rightarrow \text{C}_2$
30.988	1.5	$\text{H}_{1''} \rightarrow \text{C}_2$
120.61	5.43	$\text{H}_4 \rightarrow \text{C}_4$
120.61	2.12	$\text{H}_{5'} \rightarrow \text{C}_4$
120.61	1.94	$\text{H}_{5''} \rightarrow \text{C}_4$
149.927	4.74	$\text{H}_9 \rightarrow \text{C}_7$
149.927	2.14	$\text{H}_6 \rightarrow \text{C}_7$
149.927	1.77	$\text{H}_8 \rightarrow \text{C}_7$

85 Signal-to-Noise ratios were then calculated for each peak with the default MestReNova SNR Calculation macro, using the region from 6.5-7.5ppm as the noise region. Spectra with exceptionally low S/N required an expansion of the noise region to 6.0-8.0 ppm. Peak S/N values were then imported into Microsoft Excel for graphical visualization and subsequent calculations. Signal-to-Noise ratios were calculated as the average of the paramagnetic:diamagnetic ratio of all peaks. Signal-to-Noise ratios per unit time were calculated as the ratio of S/N to total experiment time, in seconds. An identical method was employed for the test compound set, although different F1 slices were selected depending on the compound under investigation.



90 2.4 INEPT Sequence Optimization

Values for J_{CC} and J_{CH} were set manually and screened in a combinatorial fashion. Total acquisition times for different J_{opt} values were only decreased by 1-3 s, so number of scans was not changed. The S/N values reported are the ratios of the average S/N of all correlations reported in Table S3 under varying J_{opt} values to the average S/N values observed under default J_{opt} values. An outlier correlation (H_9-C_7) was not included in average calculations as this peak demonstrated significant S/N enhancement under all conditions and was not representative of the general S/N trend. We propose this effect is due to the large $^1J_{CC}$ of olefins (>70 Hz), and so under normal 1,1-ADEQUATE settings ($J_{CC} = 45$ Hz) this peak was absent but did appear with varying J_{CC} delays.

2.5 Model Compounds

100 Camphor, amoxapine and quinidine spectra were collected with the following settings:

Table 4: Acquisition parameters for camphor 1,1-ADEQUATE spectra

Camphor	F2	F1
Points	2048	128
Scans	64	
Transmitter Offset (ppm)	3.818	113.209
Spectral Width (Hz)	4854.369	34089.358
J(CH) (Hz)	145	
J(CC) (Hz)	45	
D1 (s)	1	
Acquisition Time (s)	0.210944	0.0018774

Table 5: Acquisition parameters for amoxapine 1,1-ADEQUATE spectra

Amoxapine	F2	F1
Points	2048	128
Scans	64	
Transmitter Offset (ppm)	4.515	84.208
Spectral Width (Hz)	5263.158	26872.137



J(CH) (Hz)	145	
J(CC) (Hz)	45	
D1 (s)	1	
Acquisition Time (s)	0.19456	0.0023816

105 **Table 6:** Acquisition parameters for quinidine 1,1-ADEQUATE spectra

Quinidine	F2	F1
Points	2048	128
Scans	64	
Transmitter Offset (ppm)	4.856	83.193
Spectral Width (Hz)	6250	25464.231
J(CH) (Hz)	145	
J(CC) (Hz)	45	
D1 (s)	1	
Acquisition Time (s)	0.16384	0.0025133

Model compound S/N ratios were calculated with the same methodology as limonene.

3 Results & Discussion

3.1 1,1-ADEQUATE NMR

- 110 To establish a robust model PRE system, we selected limonene as a standard compound. As limonene is a liquid at standard temperature and pressure, large quantities can be used in NMR samples without S/N reductions due to intermolecular interactions facilitating faster data acquisition. Furthermore, limonene contains sufficient structural diversity comprising sp^2 and sp^3 hybridization of primary, secondary, tertiary and quaternary carbons. Two-dimensional 1,1-ADEQUATE NMR data was collected with default Bruker acquisition parameters utilizing the `adeq11etgprdsp` pulse program (Table 1) and processed
- 115 with default TopSpin parameters (Table 2). Under these conditions, high quality 1,1-ADEQUATE NMR spectra were generated for limonene in $CDCl_3$ (Fig. 1). Peaks for S/N calculations were then selected based on sufficient resolution in F2 (Table 3).

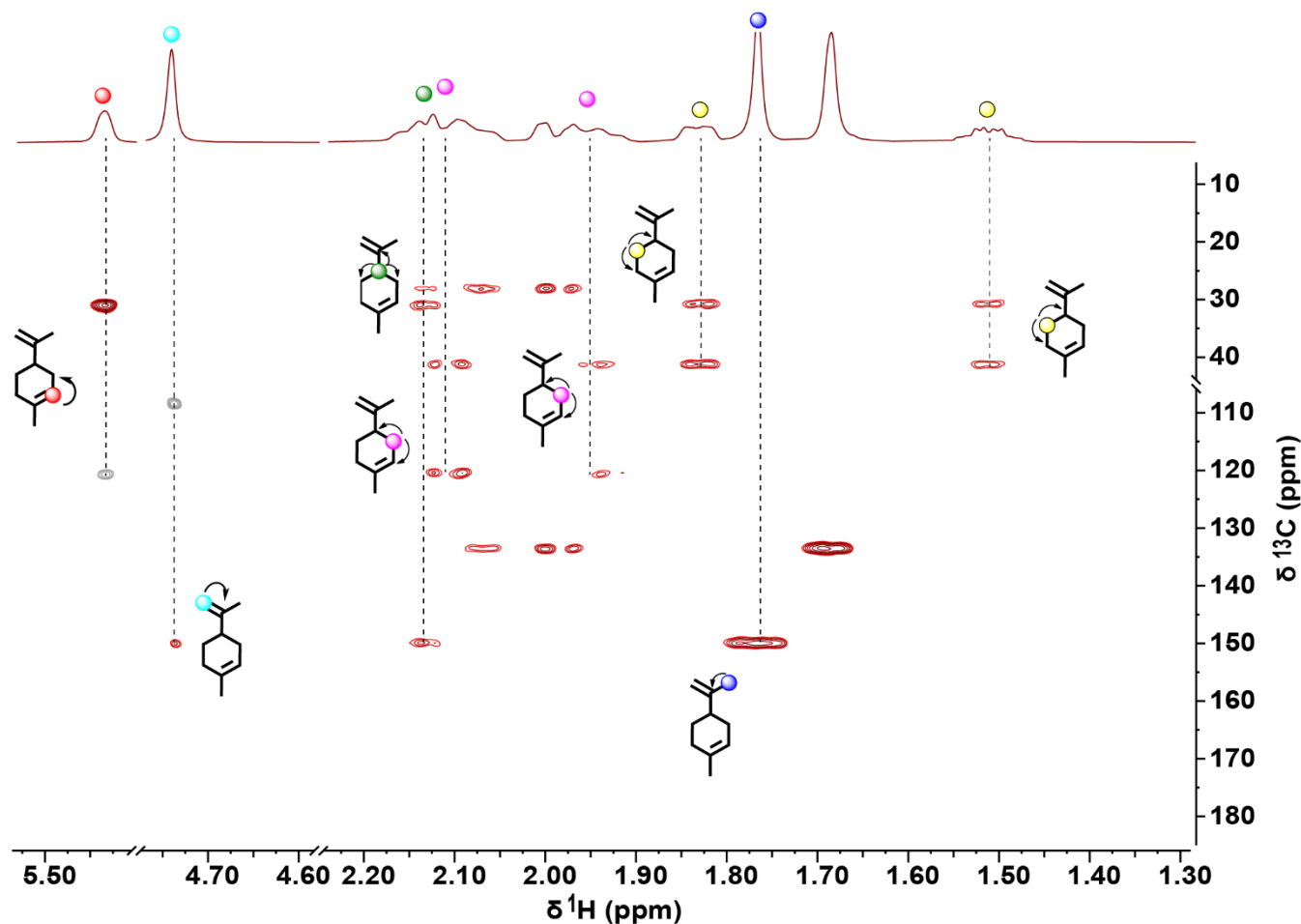


Figure 1: 1,1-ADEQUATE spectrum of limonene in CDCl_3 . Individual proton resonances used in S/N calculations are indicated by colored circles. Cross-peaks occurring on the dotted lines correspond to 2-bond HC correlations and are labelled on the limonene line-angle drawings. Artifacts arising from HSQC correlations are shown as grey cross-peaks. Empty spectral regions removed from the graphic are indicated by axis breaks. Only protons used in S/N calculations are labelled.

3.2 Selection of Paramagnetic Ions

Agents for PRE studies were selected based on two criteria: (1) readily available and inexpensive and (2) compatibility with organic solvents. In our objective to reduce T_1 with minimal impact on spectral quality, we selected four nuclei with minimal potential for pseudocontact shift effect (Luchinat et al., 2018), including Cr^{3+} (Levy and Cargioli, 1973), Fe^{3+} (La Mar and Walker, 1973), Ni^{2+} (Cai et al., 2006) and VO^{2+} (Lagostina et al., 2023). For all nuclei, the chelator, acetylacetonate, was used because it meets selection criteria (1) and (2).



3.3 Paramagnetic Ion Comparison

- 130 To analyze the impact of PRE on S/N, peaks of sufficient resolution identified in Section 2.1 were used to calculate S/N ratios (S.I. Materials & Methods: Limonene Signal-to-Noise Calculations). A marginal increase in S/N was observed for Ni^{2+} and VO^{2+} at low (< 1.0 mM) concentrations, while higher concentrations of Ni^{2+} demonstrated a reduction in S/N, which was due likely to T_2 -dependent line broadening (Fig. S1). At higher concentrations of VO^{2+} , S/N increased further overall, but only slightly (Fig. S2).
- 135 In contrast, both Cr^{3+} and Fe^{3+} demonstrated large increases in S/N relative to diamagnetic samples. In the case of Fe^{3+} , the optimum screened PRE concentration was found to be 2.0 mM, which corresponded to a S/N increase of 2.3-fold over diamagnetic conditions. At 5.0 mM, S/N subsequently decreased, though was still 1.8-fold greater than the diamagnetic S/N (Fig. S3). Similarly, Cr^{3+} greatly increased the S/N relative to the diamagnetic sample, reaching a maximum at 10.0 mM (the maximum concentration screened), which corresponded to a S/N increase of 2.9-fold over diamagnetic conditions (Fig. S4 &
- 140 S5). For purposes of visualization, optimal PRE concentrations were compared to diamagnetic conditions, demonstrating the general improvement of S/N due to PRE (Fig. 2).

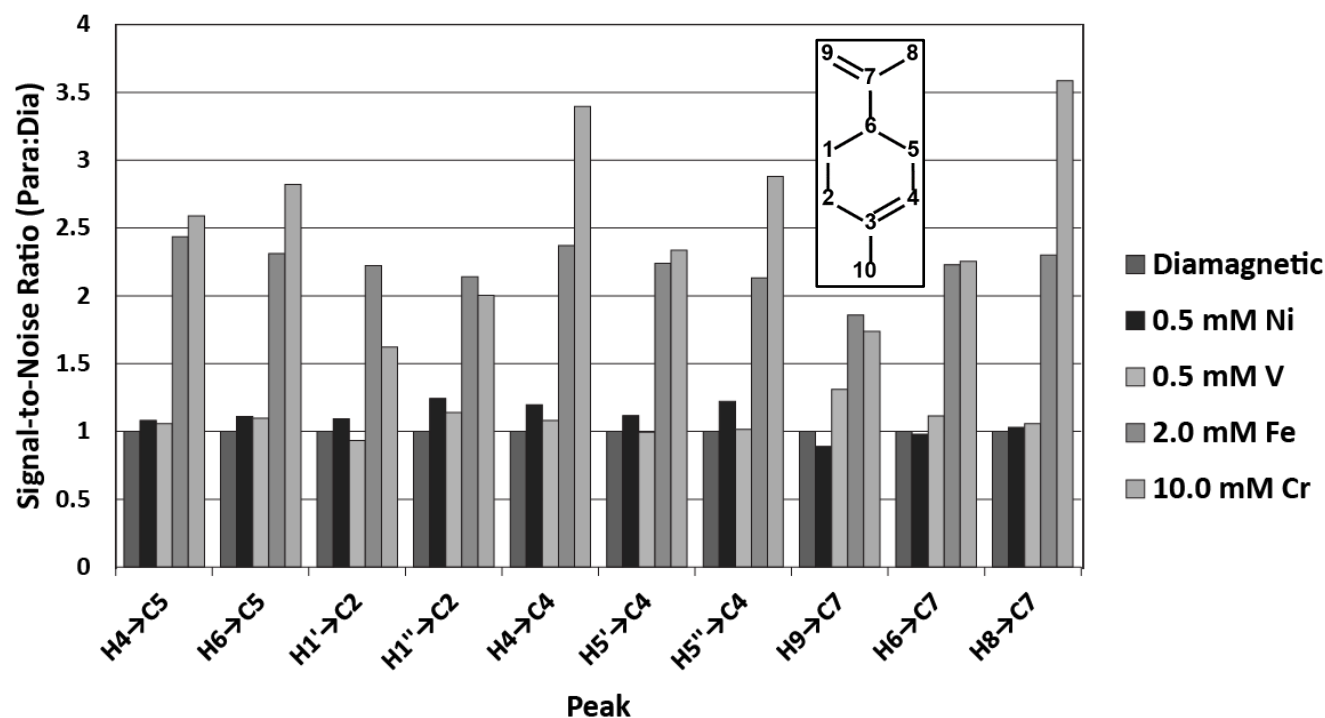


Figure 2: Comparison of paramagnetic ions based on signal-to-noise enhancement of 1,1-ADEQUATE correlations over diamagnetic conditions. Nickel and vanadium had minimal improvements in S/N ratios over diamagnetic experiments, in some cases even reduced S/N likely due to a significant decrease in T_2 . Both chromium and iron significantly enhanced S/N over diamagnetic conditions at 10.0 and 2.0 mM, respectively with the greatest increase occurring in samples prepared with



chromium. The $^2J_{CH}$ correlation H₄-C₄ was included in the analysis to determine the effect of the C-C magnetization transfer on S/N under paramagnetic conditions. Assignments are derived from Fig. 1.

150 In all cases tested, T_2 -dependent line broadening was observed in a concentration dependent manner (Fig. S6-S9). In the case of VO²⁺, the T_2 effects seemed to be relatively minor, though the marginal improvement in S/N over the concentration series also suggests a minimal impact on T_1 (Fig. S6). It is also worth noting that in contrast to the other metals, VO²⁺ exhibited significant instability, and began precipitating within 12 h of sample preparation. Similar T_1 and T_2 dependent observations were made for Ni²⁺ (Fig. S7). In contrast, both Cr³⁺ and Fe³⁺ demonstrated large increases in linewidth, with Fe³⁺ having a
155 greater impact at higher concentrations (Fig. S8 & S9). This suggests a PRE on both T_1 and T_2 , with Fe³⁺ having a larger impact on T_2 relative to Cr³⁺. This agrees with the S/N results, where data quality in 2D NMR suffers at higher Fe³⁺ concentrations due to increased transverse relaxation rates, which reduces cross-peak intensity.

Based on these results we determined Cr(acac)₃ to be the most effective PRE agent at concentrations of ~10 mM (Fig. 2) under conditions tested. We elected to avoid screening Cr(acac)₃ concentrations past 10 mM as the S/N of several correlations (H1'-
160 C2, H5'-C4, H9-C7, and H6-C7) decreased versus 5 mM Cr(acac)₃ (Fig. S4). In the interest of balancing PRE and data set completeness, we elected to run model compounds at 10 mM Cr(acac)₃ to achieve maximum average S/N enhancement as determined in the limonene standard system. Furthermore, none of the paramagnetic conditions from Fig. 2 drastically impacted the spectral quality or induced PCS (Fig. 3), supporting our method as generally applicable for structure elucidation efforts. Furthermore, S/N enhancements were demonstrated to be statistically significant by running diamagnetic and Cr³⁺
165 paramagnetic conditions in triplicate (Fig. S10). Due to instrument resource constraints, replicates could not be collected for all data sets.

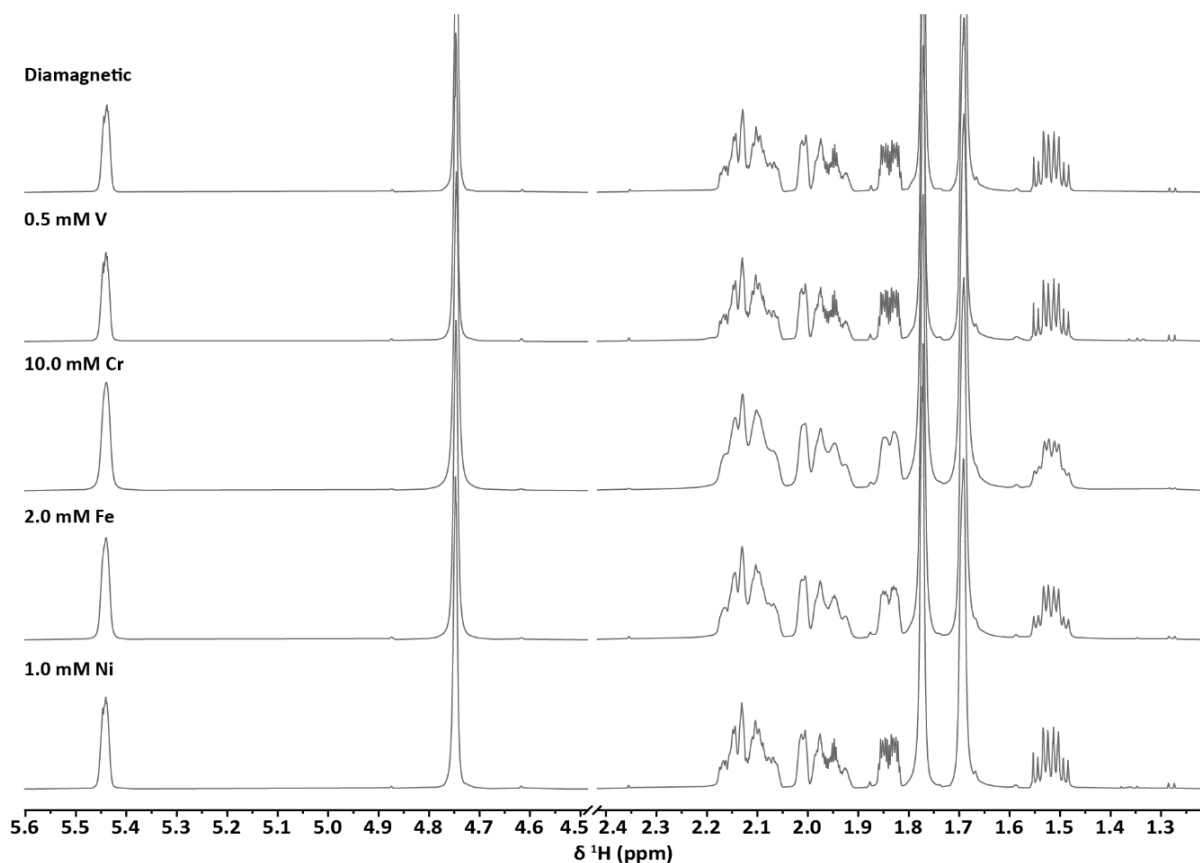


Figure 3: Comparison of spectral quality for limonene under diamagnetic and paramagnetic conditions. Under optimum PRE conditions for each tested metal ion, the impact on spectral quality was relatively minor, and J-couplings could still be observed for most peaks in nearly all samples. The greatest decrease in spectral quality was observed for 10 mM Cr, likely due to PRE of R_2 .

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To determine the impact of $\text{Cr}(\text{acac})_3$ S/N increases on acquisition time, a series of experiments were run with relaxation delays of 1, 0.5, 0.25, 0.1 and 0 seconds. Relative to diamagnetic experiments, the S/N ratio of paramagnetic spectra were greater for all delays (Table 7).

195 **Table 7.** 1,1-ADEQUATE signal-to-noise per unit time comparison of diamagnetic and paramagnetic samples with variable relaxation delays (d1). A comparison of S/N under dia- and paramagnetic conditions with variable d1 delays demonstrated a generalized decrease in S/N/min at shorter delays.

Condition	D1 (s)	Acquisition	S/N	S/N
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		Time	(average)	(per min)
Diamagnetic	1	11m04s	12.6	1.14
	0.5	6m31s	10.2	1.56
	0.25	4m15s	7.3	1.74
	0.1	2m53s	3.1	1.08
	0	1m59s	1.4	0.72
Paramagnetic	1	11m04s	30.9	2.82
	0.5	6m31s	27.2	4.2
	0.25	4m15s	21.9	5.16
	0.1	2m53s	14.8	5.1
	0	1m59s	7.5	3.78

200 At a delay of 0.1 seconds, the paramagnetic sample exhibited an average S/N of 14.8, compared to 12.6 for the diamagnetic
 sample at the longest delay of 1.0 s. Furthermore, on a S/N per min comparison, the paramagnetic sample at a delay of 0.1 s
 possessed a S/N per min of 5.1, more than 4-times greater than the S/N per min of 1.14 for the diamagnetic sample at d1=1 s.
 The most favorable experimental conditions for the diamagnetic sample were comparable to the paramagnetic sample run with
 a minimal d1 (Table 7). In summary, PRE via Cr(acac)₃ enabled the acquisition of data of higher S/N in 25% of the time as
 205 the same compound under diamagnetic conditions (Fig. 4).

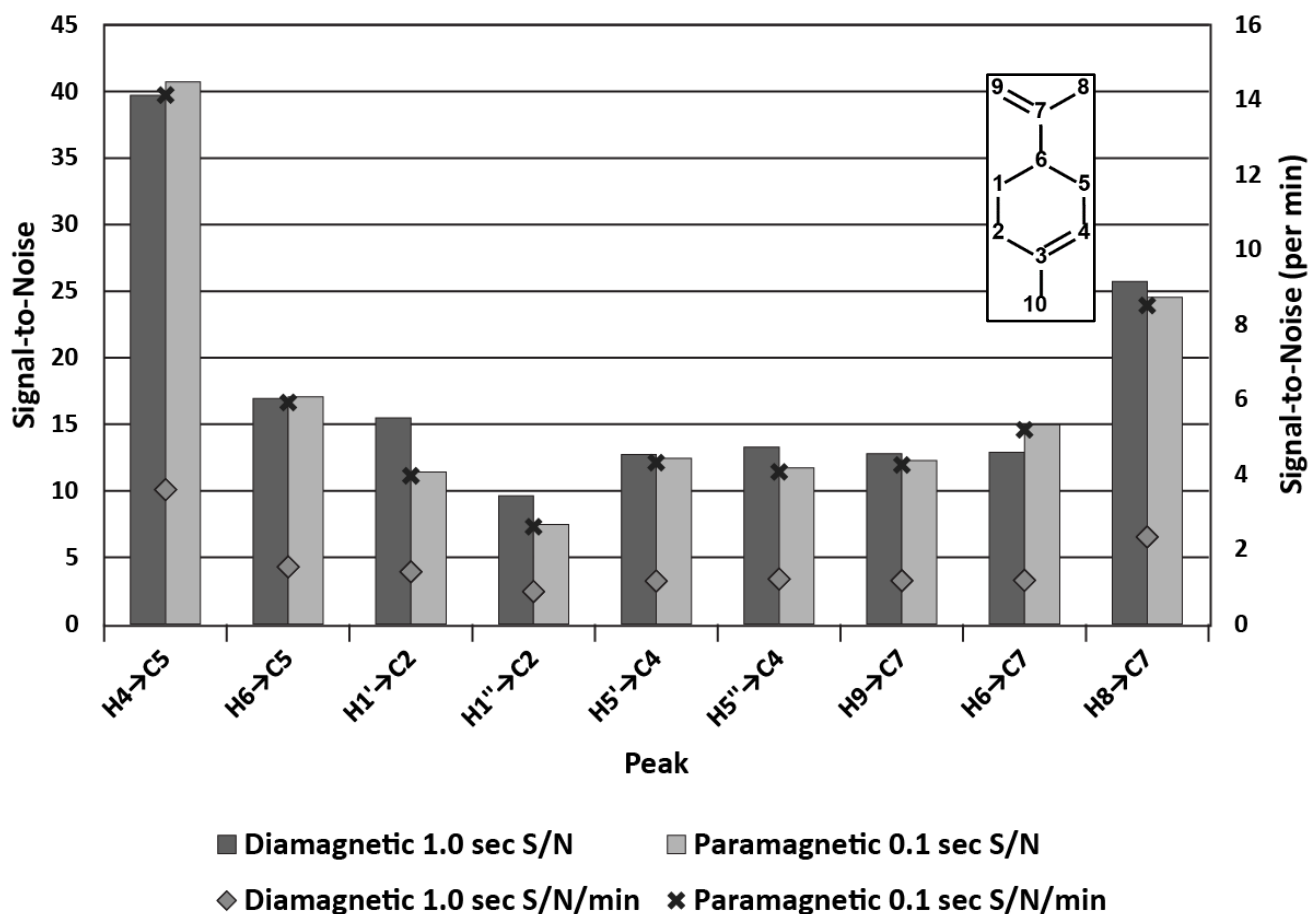


Figure 4: Comparison of diamagnetic and paramagnetic data under variable delays in the 1,1-ADEQUATE experiment. Signal-to-Noise ratios were approximately equivalent for diamagnetic spectra with a 1 s d1 and paramagnetic spectra with a 0.1 s d1 (secondary axis, black “X” and grey diamonds, respectively). As a d1 of 0.1 s required a total acquisition time 1/4th that of a d1 of 1 s, S/N per min were compared between these spectra, demonstrating a S/N improvement under paramagnetic conditions (dark grey bars, primary axis) of approximately 3-fold over diamagnetic conditions (light grey bars, primary axis).

In addition to S/N, we quantified the impact of PRE nuclei on the longitudinal relaxation rates of ^1H and ^{13}C . Despite the substantial increases in S/N in the presence of paramagnetic ions, decreases in T_1 times for limonene carbons were minimal and demonstrated a consistent decrease across all tested nuclei (Fig. S11). Such small decreases in T_1 were unexpected, though the greatest decrease in ^{13}C T_1 corresponded to C7 and C3, the two quaternary sp^2 nuclei in limonene. This reduction in quaternary carbon T_1 promises to have a significant impact on S/N, and therefore assignment accuracy, of peaks corresponding to proton-deficient regions of analytes.



220 Similarly to ^{13}C T_1 , the measured ^1H T_1 for Cr^{3+} and Fe^{3+} , the most potent PRE ions we tested, demonstrated similarly mixed results (Fig. S12). Limonene H8, H9 and H10 demonstrated small increases in T_1 , though based on the limonene structure analysis these protons represented methyl and terminal olefin groups which are fast relaxing due to their unrestrained bond rotation. In contrast, protons with limited conformational flexibility ($^1\text{H}_2$ and $^1\text{H}_5$) demonstrated large decreases in ^1H T_1 in the presence of paramagnetic ions (Fig. S12). These results suggest the principal mechanism by which paramagnetism
 225 increases S/N is through a marked reduction in the inflexible, i.e. slow-relaxing, ^1H T_1 in the analyte.

3.4 Optimization of INEPT Sequence

Given the significant increase in S/N observed under paramagnetic conditions, we sought to determine if additional sensitivity gains could be achieved by optimization of the Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) delays utilized in the 1,1-ADEQUATE experiment. In an effort to minimize signal losses to T_2 relaxation, we screened multiple, combinatorial
 230 J -optimization values for J_{CC} and J_{CH} to determine the impact of PRE on magnetization transfers (Fig. 5).

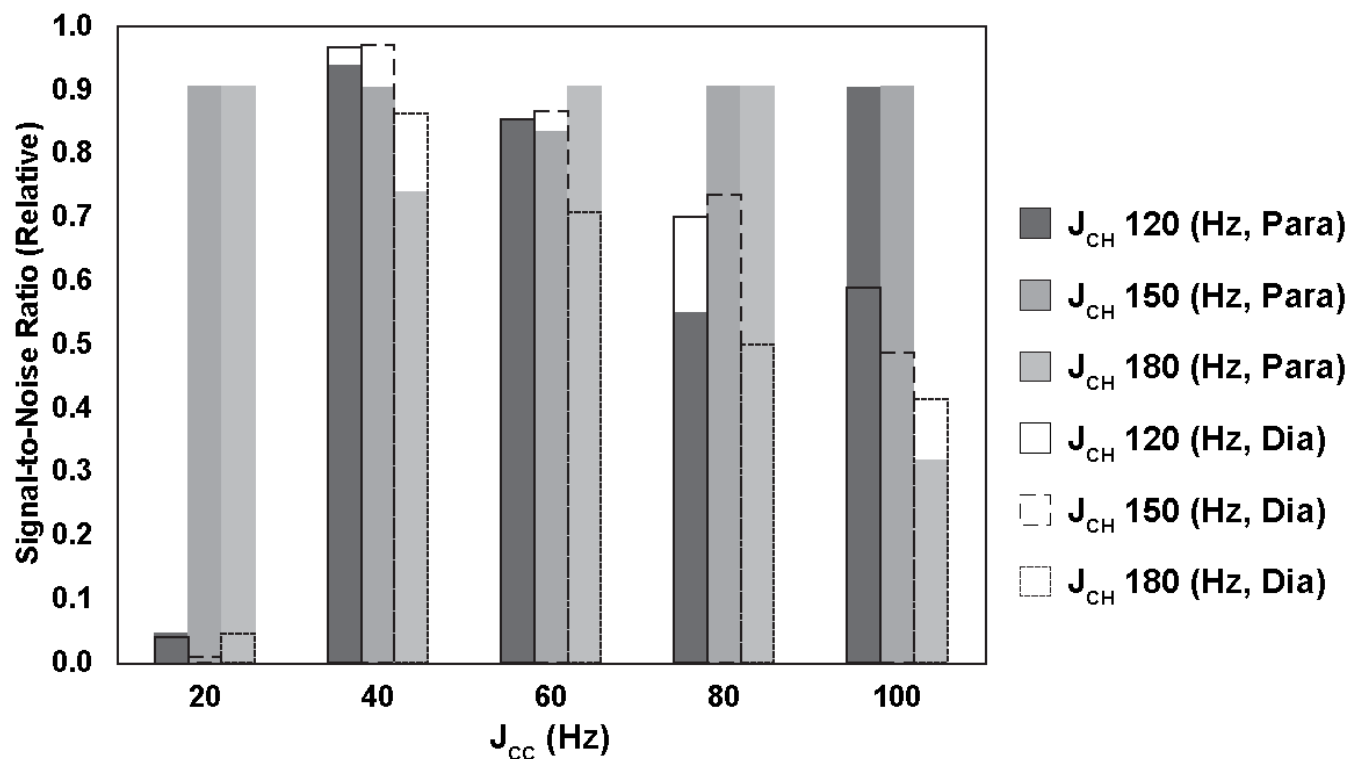


Figure 5: J-optimization screening conditions for INEPT sequence. Values for J_{CC} and J_{CH} were screened under diamagnetic (open, dashed bars) and paramagnetic (solid, grey bars) conditions and signal-to-noise ratios were calculated relative to S/N values for 1,1-ADEQUATE with default J_{opt} values ($J_{\text{CC}} = 45$ Hz and $J_{\text{CH}} = 145$ Hz) collected under
 235 paramagnetic and diamagnetic conditions. While all S/N values tested were lower than those of the default J_{opt} values,



paramagnetic samples demonstrated greater relative S/N at higher J_{opt} values (shorter INEPT delays), demonstrating PRE reduces the need for precise experimental optimization.

Overall, changing J_{CH} delays from the default value of 45 Hz did not have a significant impact on S/N relative to default 1,1-ADEQUATE settings for both diamagnetic and paramagnetic samples. At each J_{CC} value, S/N variations at different J_{CH} were low, except at the highest and lowest J_{CC} delay (Fig. 5). That is to say, changing J_{CH} delays had a minimal impact on S/N. For diamagnetic samples, there was a greater sensitivity to J_{CC} values, with the greatest S/N values observed for conditions near the default value of 45 Hz, with a subsequent decrease in S/N observed for shorter J_{CC} delays. Overall, paramagnetic samples demonstrated greater resistance to S/N decreases over all tested J_{CC} values relative to diamagnetic samples. While shorter INEPT delays utilized in this study did not lead to any significant S/N gains, utilization of a PRE agent dampened the effects of sub-optimal experimental settings, demonstrating that PRE reduces the need for extensive experimental parameter optimization, further increasing the efficiency of structure elucidation workflows.

3.5 Paramagnetic Relaxation Enhancement of Model Compounds

To study PRE in model systems, we sought a chemically diverse test set of compounds containing heteronuclei, aliphatic and aromatic groups and varying degrees of unsaturation. To this end we analyzed camphor, amoxapine and quinidine by 1,1-ADEQUATE in the presence and absence of Cr^{3+} at 10 mM (Table 4-6). We hypothesized that camphor, with its compact, rigid structure would demonstrate a significant increase in S/N under paramagnetic conditions. Comparison of the S/N ratio of camphor under diamagnetic and paramagnetic conditions demonstrated a substantial increase in S/N (Fig. 6). In line with our expectations, the greatest increase in S/N was observed for correlations involving the quaternary carbons C3, C2 and C9.



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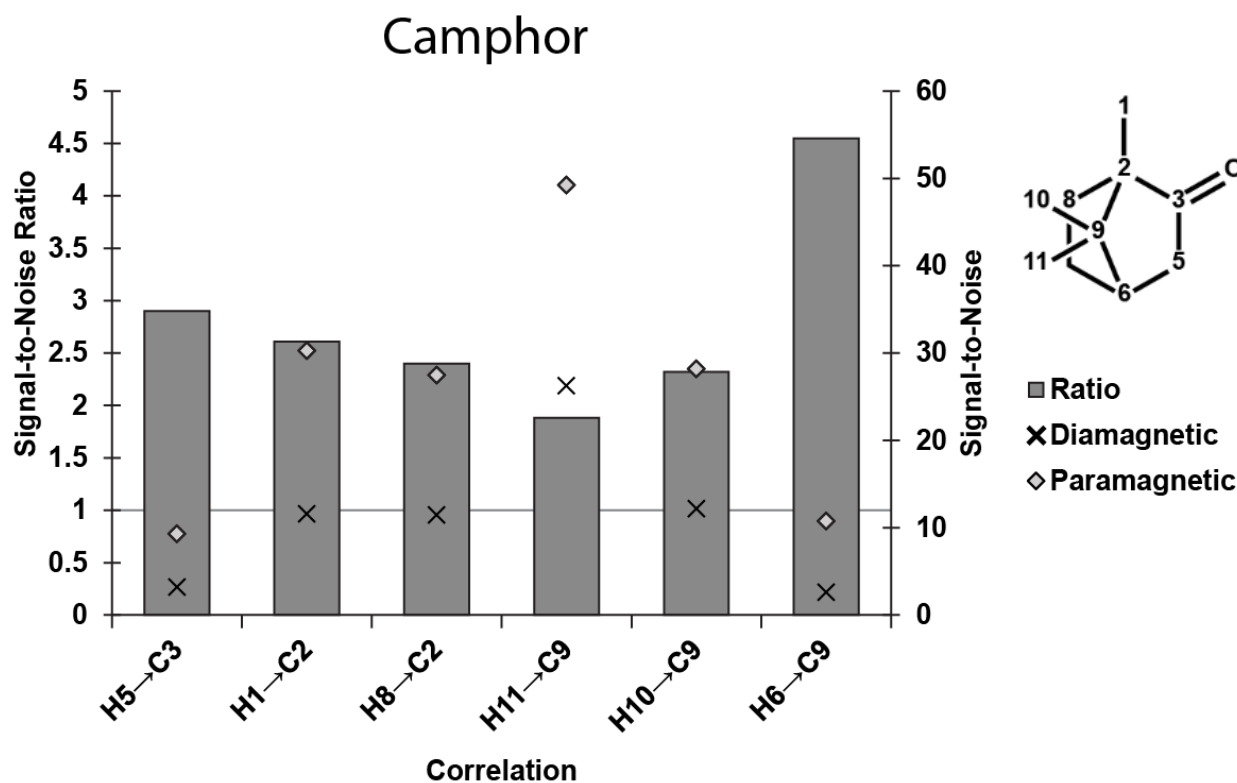


Figure 6: Comparison of signal-to-noise ratios of 1,1-ADEQUATE NMR of camphor under paramagnetic and diamagnetic conditions. Introduction of a paramagnetic nucleus substantially improved the S/N of camphor relative to diamagnetic conditions (grey diamonds and “X”, secondary axis). Increases in relative S/N under paramagnetic conditions ranged from ~2 to ~4.5-fold (grey bars, primary axis). A Diamagnetic:Paramagnetic ratio of 1.0 is indicated by the grey line. The d1 used was 1 s and total acquisition time was 2h52m. Correlations for diastereotopic protons H_{5'} and H_{5''} are reported as the average S/N of two peaks.

The compound amoxapine was also studied as it possessed multiple quaternary carbons and a greater proportion of sp² hybridized carbons and contrasted in structural complexity relative to camphor. In contrast to camphor, amoxapine underwent minimal increases in S/N under paramagnetic conditions, and in some cases exhibited a small decrease (Fig. 7). Despite this, and in agreement with previous observations, quaternary carbons C2, C4 and C12 represented the greatest increase in S/N ratios further confirming our hypothesis that PRE will have the greatest impact on quaternary carbon correlations.



Unexpectedly, ^1H spectra demonstrated significant line broadening of the piperazine moiety (Fig. S13), indicating amoxapine may possess a faster T_2 relaxation rate likely due to chemical exchange of nitrogen atoms, making it more susceptible to increased relaxation under paramagnetic conditions.

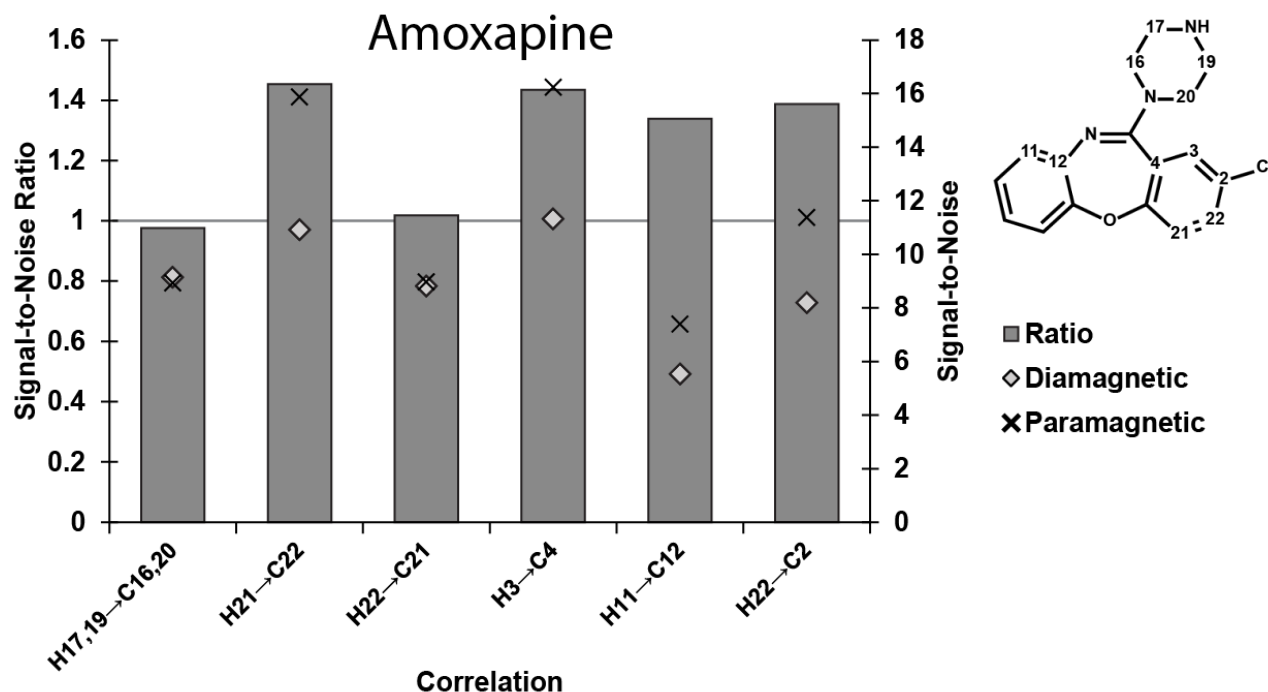


Figure 7: Comparison of signal-to-noise ratios of 1,1-ADEQUATE NMR of amoxapine under paramagnetic and diamagnetic conditions. Introduction of a paramagnetic nucleus induced minimal increases of S/N of amoxapine relative to diamagnetic conditions (grey diamonds and Xs, secondary axis). Increases in relative S/N under paramagnetic conditions ranged from ~1 to ~1.5-fold (grey bars, primary axis). A Diamagnetic:Paramagnetic ratio of 1.0 is indicated by the grey line. The d1 used was 1 s and the total acquisition time was 2h47m.

Finally, quinidine produced results demonstrating modest gains in S/N (Fig. 8), in agreement with the findings for camphor and amoxapine. Furthermore, correlations to C15, C16, C18 and C23 underwent a higher than average S/N increase due to PRE which was in agreement with our expectations and all previous model compounds.

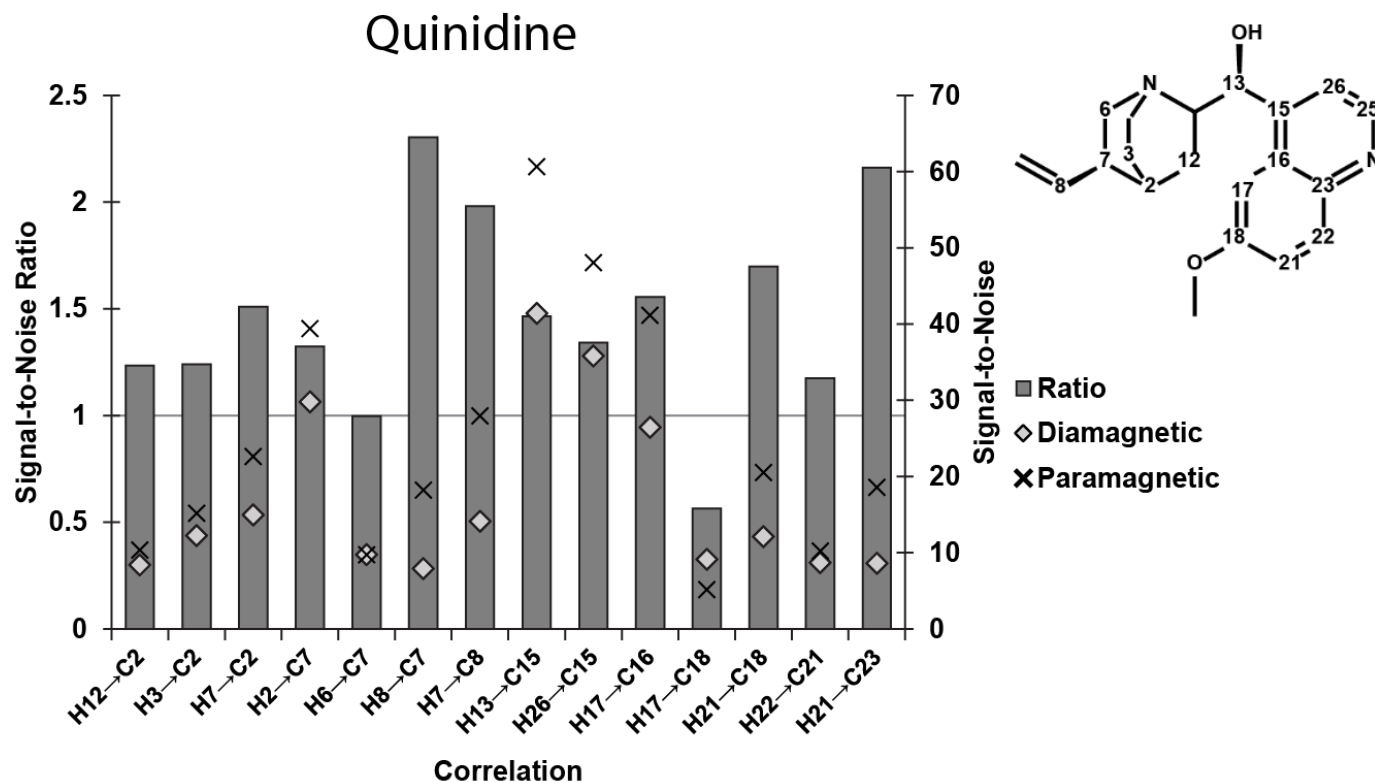


Figure 8: Comparison of signal-to-noise ratios of 1,1-ADEQUATE NMR of quinidine under paramagnetic and diamagnetic conditions. Introduction of a paramagnetic nucleus induced both increases and decreases in S/N of quinidine relative to diamagnetic conditions (grey diamonds and Xs, secondary axis). Increases in relative S/N under paramagnetic conditions ranged from ~0.5 to ~2.5-fold (grey bars, primary axis). A Diamagnetic:Paramagnetic ratio of 1.0 is indicated by the grey line. The d1 used was 1 s and total acquisition time was 2h45m.

4 Conclusion

Screening of paramagnetic ions demonstrated both Fe^{3+} and Cr^{3+} had the largest PRE effect at 2 and 10 mM, respectively, of the paramagnetic ions studied. Of these two, the largest PRE was attributed to Cr^{3+} , which was selected for additional studies on a series of pharmacologically active small molecules. Maximally observed S/N enhancement in our study was 4.55X, 1.45X and 2.3X for camphor, amoxapine and quinidine, respectively. While Cr^{3+} significantly increased the S/N of all 1,1-ADEQUATE correlations in camphor (Fig. 6), its effect on amoxapine and quinidine was mixed; S/N gains were generally modest, and in some cases decreased (Figs. 7 and 8). Overall, the varying impact of PRE on S/N is largely consistent with fundamental understanding of small molecule relaxation. Nuclei with long T_1 and T_2 relaxation times benefit most from PRE, whereas fast T_2 nuclei underwent S/N decreases due to T_2 -dependent line broadening. Most critically, PRE performance was



the best for correlations involving quaternary carbons, which generally possess the longest ^{13}C T_1 times. Additionally, samples
310 run under paramagnetic conditions demonstrated greater resistance to variations in J_{opt} for J_{CC} and J_{CH} INEPT delays, which
partially obviates the need for laborious experimental parameter optimization.

Application of this simple PRE method promises outsized impact on small molecule structure elucidation strategies. While
addition of a PRE agent contaminates the sample, we believe this method will prove especially valuable in cases where target
analyte quantities are restricted by low solubility and not sample availability. Given widely understood limitations in structure
315 elucidation of proton-deficient and quaternary carbon-rich molecules, PRE-dependent S/N enhancement provides a simple
strategy to ameliorate challenges in determining these structures. Furthermore, the large beneficial impact of PRE on the
collection of 1,1-ADEQUATE NMR data increases the utility of this method, which despite providing extremely valuable
structural information, is often overlooked due to significantly limited sensitivity. In combination with modern spectrometer
hardware like cryoprobes and data acquisition strategies like non-uniform sampling, a PRE strategy promises further,
320 significant S/N enhancement. While PRE can have a sizeable positive impact on S/N, it does not represent a panacea and must
be applied judiciously to compounds with inherently long relaxation times as collateral impacts on T_2 can negatively impact
S/N.

5 Appendices

Code and data availability

325 Processed spectral files annotated with S/N values were deposited to nmrXiv and can be accessed via
<https://doi.org/10.57992/nmrXiv.d4ffcd9b>

Supplement link

The supplement is available online.

Author contributions

330 AJD, RDC and MR designed this study and AJD performed experimental work. Manuscript was prepared by AJD and with
contributions from RDC and MR.

Competing interests

The authors declare no competing interests.



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