Bench-top, Indirect-detection Experiments for ^{13}C-NMR Acquisition: Utilizing low-field NMR at a Primarily Undergraduate Institution

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INTRODUCTION

In order to publish an article containing the synthesis of new molecules in a peer-reviewed research journal, accurate and reproducible NMR data must be obtained.[1] For many Primarily Undergraduate Institutions (PUIs), obtaining or allocating infrastructure and resources for the acquisition and maintenance of a high-field NMR is impractical.[2] Partnerships with local PhD-granting universities is the solution of choice for those research-oriented synthetic chemists working at PUIs. The challenge with these partnerships is often the time-cost of commuting to and from the location, the training of students, and the scheduling challenge associated with the walk-in nature of many instruments and monetary cost. In order to successfully utilize these partnerships, the principle investigator (PI) must often take time away from the high teaching load associated with PUIs. Any time spent at the distant NMR site without the students also subtracts from the valuable and essential mentoring time allotted to undergraduate researchers. Scheduling group field trips to the facility often complicate and lengthen the process further. Other obligations must also be rescheduled, reduced or eliminated altogether in order to arrive at the instrument in the early morning prior to graduate student use, or use the instrument on the weekend. These times are not ideal as troubleshooting is left up to the PI, with little help from the spectroscopist or instrumentation manager. These time-based challenges draw out the length of projects and often deny undergraduate students opportunities to perform synthesis-based research and the subsequent characterization adequate for undergraduate chemistry curriculum. Here we report the use of the MagriTek Spinsolve, a new, bench top, low-field NMR spectrometer, as it pertains to in-house, relatively low-concentration, ^{13}C-NMR acquisition of three different compounds, in conjunction with teaching advanced NMR techniques to undergraduate researchers for complex structure elucidation.

For many compounds that do not present a sufficiently resolved ^{1}H-NMR spectra due to low signal dispersion at low field instruments signal, a ^{13}C-NMR must be acquired for product verification. Due to the low natural abundance and hence concentration of ^{13}C nuclei (c) (see **Eq. 1**) in samples and low magetogyric ratio (γ) of the ^{13}C isotope (~4 times lower than ^{1}H), the direct detection of ^{13}C resonances is ~ 6000 time *less* sensitive than that of ^{1}H, and thus it's much more difficult to obtain a useful spectrum in a feasible amount of time.[3] As seen in Equation 1, and most well taught in undergraduate texts, the signal-to-noise ratio (S/N) is directly proportional to the cube root of the magnet strength, and square root of the number of scans (NS).

$$\frac{S}{N} = cT_2\gamma_{exc}\left(\frac{\sqrt[3]{B_0\gamma_{det}}\sqrt{ns}}{T}\right) \quad \textbf{Eq. 1}$$

The typical means of overcoming the poor S/N in ^{13}C acquisition is to increase the concentration of the sample (increase c), run the experiment for a longer time (increase ns), take the sample to a high-field instrument (increase B_0), or a combination of all these variables. However, if each variable is looked at individually, increasing the sample concentration is the simplest way to raise S/N, however this is not always possible when

dealing with costly chemicals or those with poor solubility, doubling *ns* only increases S/N by ~1.41 and doubling B_0 increases S/N by 1.25. Therefore, as the γ is a constant, the quickest way to increase S/N is by optimizing T_2 in the pulse sequence to match T_2 in the system, but this would require additional experiments and thus more time. However valuable, ultimately none of these factors affect the degree of dispersion.

Another option exists for the NMR-focused researcher: indirect detection (ID). In an indirect detection experiment, both the ^{13}C and ^{1}H nuclei are excited, but only the ^{1}H is detected. The observed free induction decay (FID) acquired allows observations of the neighboring ^{13}C nuclei. Thus, ^{13}C NMR data can be obtained through the ^{1}H nucleus. Consequently, the increase in S/N is greatly improved by detecting the more abundant ^{1}H nuclei, with the greater γ. This process is the basis for HSQC, HMQC, and HMBC NMR experiments. The direct detection HETCOR experiment also correlates the ^{13}C and ^{1}H nuclei, but is 30 times less sensitive due to limits on directly detecting the ^{13}C nucleus instead of the ^{1}H nucleus.[3] Therefore, until the recent availability of the MagriTek Spinsolve 2-channel NMR with ID protocols, no other option existed to increase S/N or overcome dispersion forces, and therefore obtain ^{13}C-NMR data of dilute samples at a PUI lacking a high-field instrument.

Indirect detection experiments work on the premise that the ^{13}C and ^{1}H are spin-coupled. It is the result of this coupling that is detected. One-bond coupling constants ($^{1}J_{CH}$) are ~145 Hz, and the two ($^{2}J_{CH}$) and three ($^{3}J_{CH}$) bond couplings are in the range of ~5-15Hz (See **Figure 1**). Karplus and others discovered $^{3}J_{CH}$ is dependent on cos(θ) and $\cos^2(\theta)$, both of which are largest at 0°, and 180°, and smallest at 90°, and 270°.[4,5]

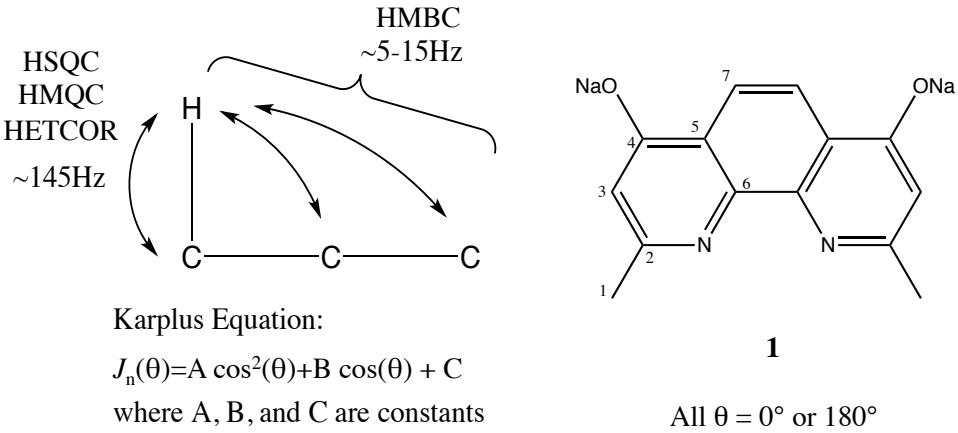


Figure 1 1D coupling constants for different experiments (left), a compound that should be highly sensitive to 1D experiments (right).

Therefore, planar compounds would have the highest sensitivity in ID NMR experiments as all θ would be 0° or 180°. As the range of the coupling constants is an order of magnitude larger, the ID experiments run to detect the ~145 Hz are called the HSQC or HMQC. The ID experiment for the smaller, 5-15 Hz coupling is the HMBC. Consequently, in HSQC and HMQC experiments, $^{2}J_{CH}$ and $^{3}J_{CH}$ are not typically observed, and in the HMBC experiment the single bond coupling ($^{1}J_{CH}$) is not typically observed. Thus, adequate reference for such experiments should contain both single-bond and multiple-bond coupling, such as acetonitrile or ethanol, allowing both spectra to be referenced to the same scale. However, such a reference is unnecessary if, in a single spin-system solvent such as benzene, the analyte

contains an easily identifiable $^{2}J_{CH}$ or $^{3}J_{CH}$ coupling such as in sodium 2,9-dimethyl-1,10-phenanthroline-4,7-bis(olate) (**1**).

METHODS AND RESULTS

In our work we aimed to synthesize the known compound sodium 2,9-dimethyl-1,10-phenanthroline-4,7-bis(olate) (**1**).[6] This molecule has only 3 distinct proton signals, but has 7 distinct carbon signals. **Figure 2** shows a comparison of the 45Hz and 300MHz, direct-detection, ^{13}C-broadband decoupled NMR of **1** (20mg), 0.147M in D_2O, CH_3CH_2OH (20:1). Only the ethanol reference is visible, as it is ~5.5 times more concentrated than the sample, after 16283 scans (a). When the number of scans is increased to 29304 scans, signals for **1** begin to appear, though are not will resolved (b). It is clear that at this low concentration, the higher-field instrument must be used to obtain a viable spectrum (c).

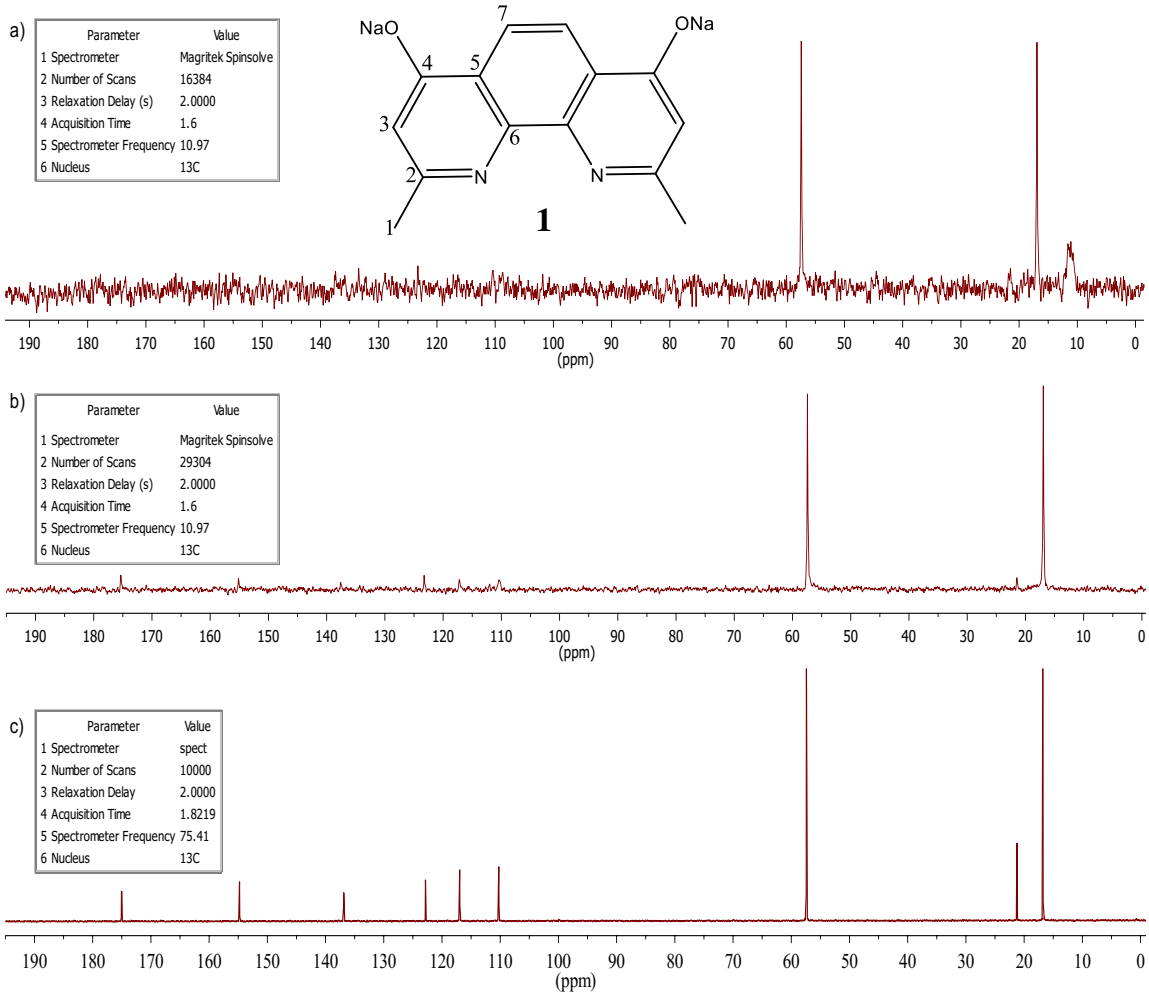


Figure 2 Comparison of broadband decoupled ^{13}C-NMR. A) 16384 scans on Bench-top NMR, B) 29304 scans on Bench-top NMR, c) 10000 scans on high-field (75MHz for ^{13}C) NMR.

When the HSQC and HMBC indirect protocols are used in sequence, referenced, and stacked (**Figure 3**), the ^{13}C data can be easily extracted with a high degree of confidence in two

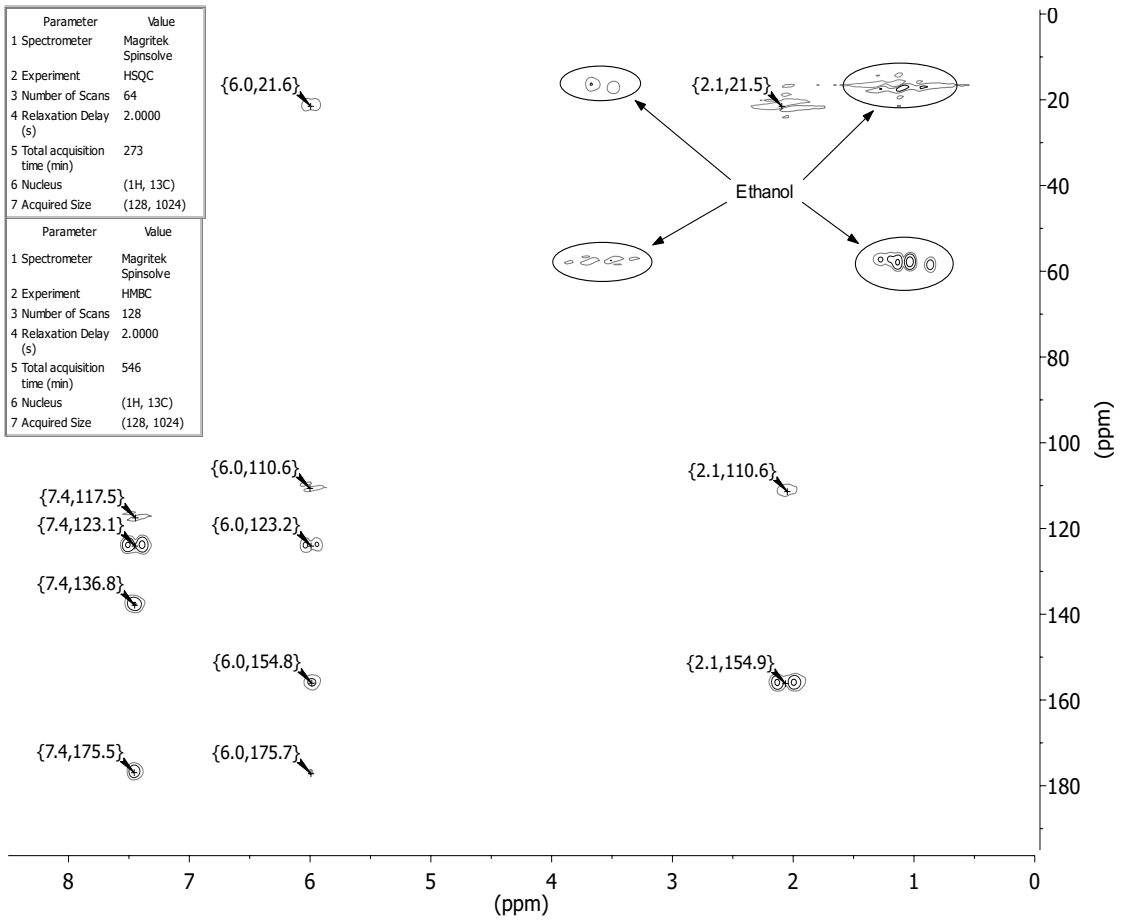


Figure 3 Stacked HSQC and HMBC of 1

sequential experiments totaling ~14hrs of instrument time. Additionally, as typical of such experiments, the carbons can now be assigned easily (**Table 1**), something not explicitly done in the literature.

When this technique is applied to another rigid system, where the dihedral angle are locked, but the system is non-planar, complications arise. We synthesized (1*R*,2*R*,5*S*,6*S*)-2,5-dimethyl-11-oxatricyclo[4.3.1.1^{2,5}]undec-3-en-10-one (**2**) via 3+4 cycloaddition reaction.[7,8,9] This tricyclic molecule has some interesting dihedral angle characteristics. The proton on C_1 is gauche to C_3 yet nearly perpendicular to C_4. We prepared a 30mg sample of this species in benzene-d_6 and performed the same consecutive HSQC+HMBC experiments. As expected, H_1 only shows correlation with C_3, not C_4 (**Figure 4**). However, in this instance, C_6 cannot be located at all (**Table 2**).

Table 1 Extracted ^{13}C chemical shifts for 1

Position	δ (ppm)	
	^{1}H	^{13}C
1	2.1	21.5
2	-	154.85
3	6	110.6
4	-	175.5
5	-	123.15
6	-	136.85
7	7.4	117.5

We performed numerous experiments changing the relaxation delay and the NS in the hopes of locating C6 to no avail. The absence of the C6 signal can be rationalized in two ways

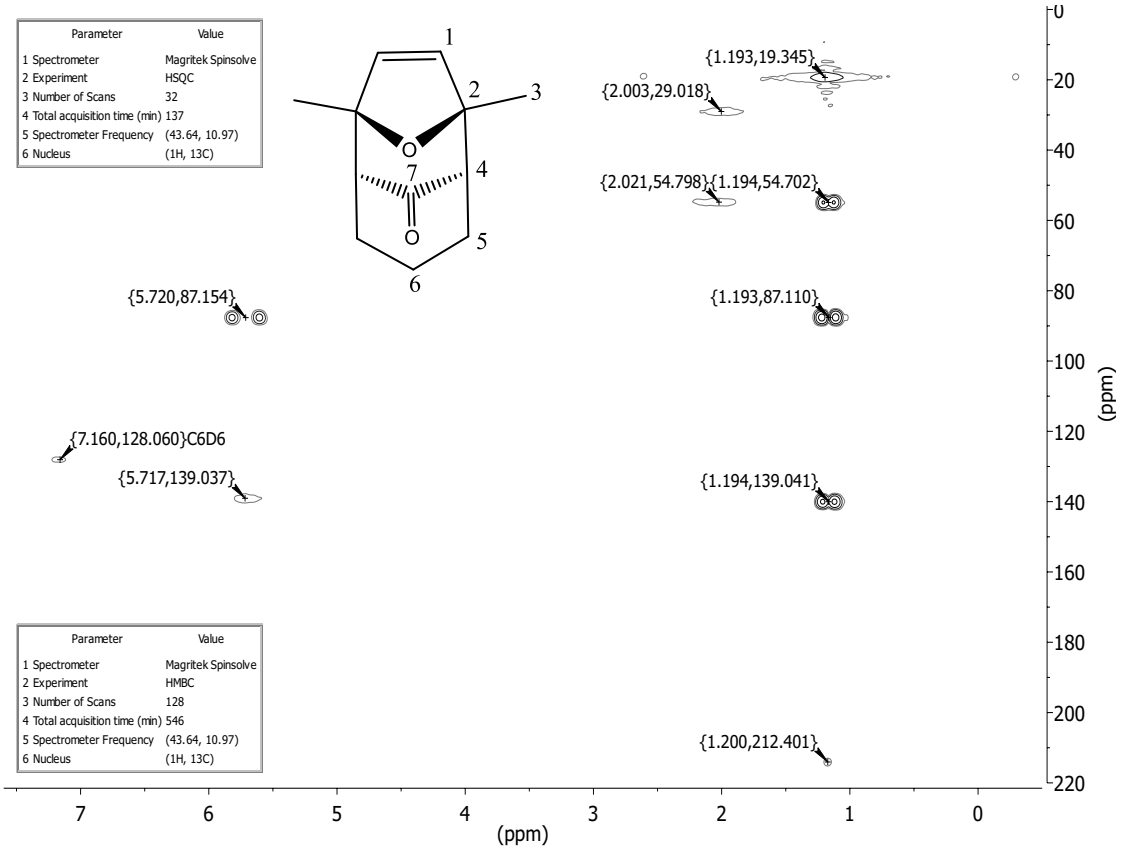


Figure 3 Stacked HSQC and HMBC of 2. Structure of 2 (inset)

1) the resonances for both ^{1}H and ^{13}C at positions 3 and 6 are overlapping in both 2D spectra, or 2) the coupling constants for C_6 are too small to transfer magnetization (whether due to field-strength issues or, perhaps due to the quality of the pulse sequence). After contacting MagriTek for a potential solution, we were invited to use a pre-release phase-sensitive HSQC-ME experiment. It never occurred to us that the pulse sequence could be the culprit for the lack of signal at C6. Nowhere in Eq.1 is there a pulse sequence-specific term. Though skeptical, we pressed forward and obtained the spectrum in **Figure 5**. This phase-sensitive spectra indicates CH and CH3 signals as positive (in red) and CH2 signals as negative (in blue). There is data correlating to the 20.76 ppm ^{13}C resonance, but it is not in any clear pattern, and looks a bit like noise. However, using the 1D projection tool in MestreNova we were able to extract what is essentially an Attached Proton Test (APT) spectrum suggesting a new CH2 was present in the region expected for C_6. We performed a ^{1}H-broadband decoupled ^{13}C-NMR of **2**, and subsequent DEPT (**Figure 6**) after accumulating enough material (185mg). **Figure 5** shows the C_6 resonance of **2** at 20.8 ppm is remarkable close to the methyl group resonance at C_3. These spectra are in excellent agreement with the extracted data, and show conclusively that the pulse sequence was the primary issue.

Table 2 Extracted ^{13}C chemical shifts for 2

Position	δ (ppm)	
	^{1}H	^{13}C
1	5.72	139.0
2	-	87.1
3	1.19	19.6
4	2.02	54.7
5	2.03	29
6	?	?
7	-	212.4

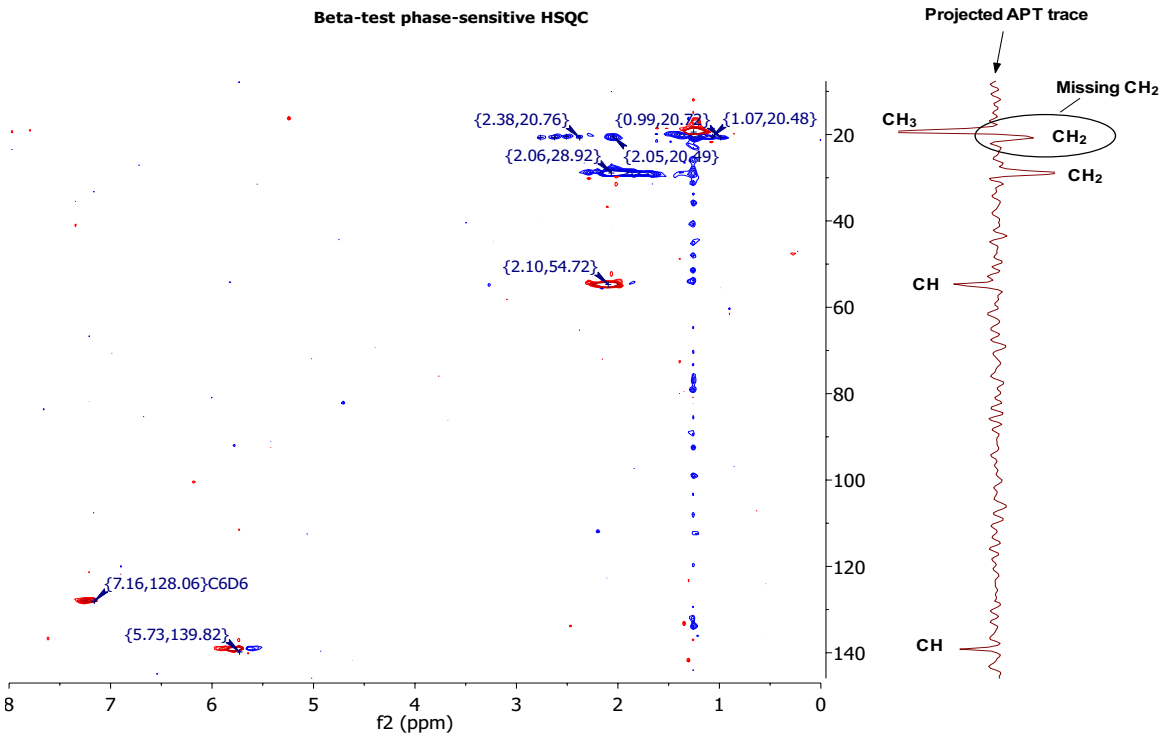


Figure 4 HSQC-ME (beta test courtesy of MagriTek) of 2.

We found another useful application of this technology during our attempts at

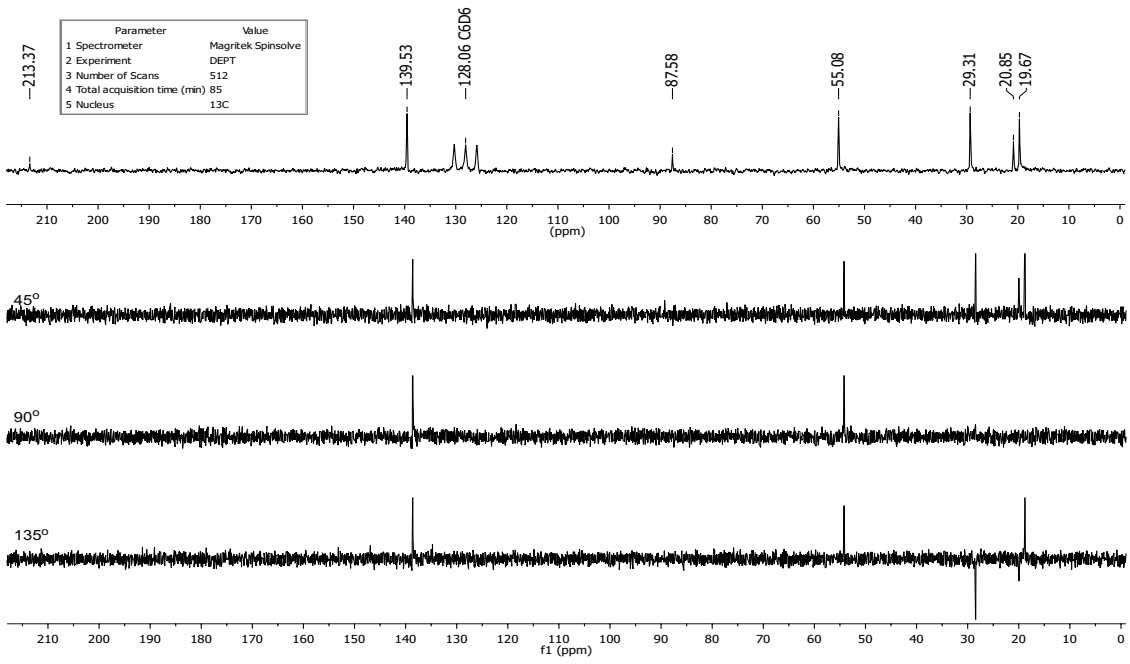


Figure 6 DEPT of pure 2

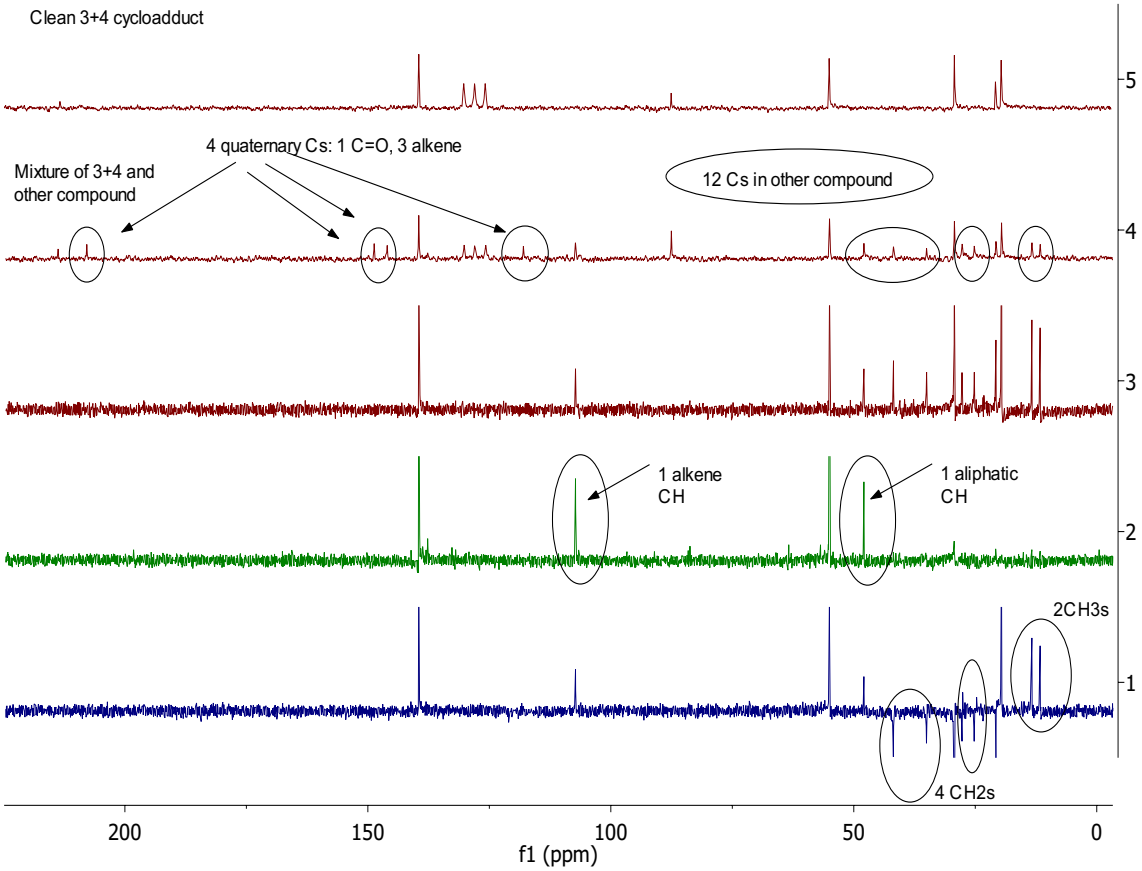
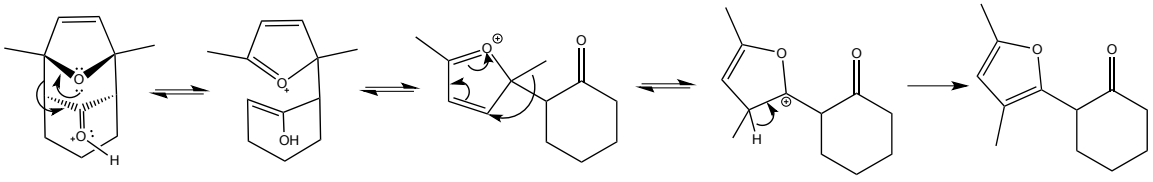


Figure 7. Pure 1 ^{13}C of (5), mixture of 2 and 2' ^{13}C (4), mixture of 2 and 2' DEPT 45° (3), 90° (2), 135° (1)

synthesizing this compound. We were able to use DEPT in order to determine the possible identity of impurities we were finding in our early attempts at this reaction. In similar reactions it was noted that the final tricyclic compound could decompose into a ring-open form.[7,8] We believed this to be the reason for the extra 12 peaks visible in our carbon NMR (**Figure 7**), but were unable to make this assertion using normal C-NMR scans. Through our proposed mechanism, (**Scheme 1**) we postulated a possible impurity, **2'**. We were then able to use DEPT (**Figure 7**) to rule out other possibilities and determine with reasonable certainty the identity of the impurity. This gave us valuable information about what might be causing our product to decompose.



Scheme 1 Proposed mechanism for the generation of 2'

Last, we analyzed the more flexible system in alkyne thioester **3** (**Figure 8** inset). This molecule showed indistinguishable resonances for C3 and C4, regardless of the experiment employed. The tandem 2D and stacking protocol used previously allowed for the assignment of all other carbons, but convoluted these two protonated resonances. Though we were confident we had synthesized the molecule *via* ^{1}H-NMR and GCMS analysis we still

wanted to see that we had all six of the proton-bearing carbon resonances. We were unable to obtain an adequate ^{13}C NMR even when a large concentration was employed. This suggested that C3 and C4 were isochronous in our spectrometer. When searching the literature for a means to change the chemical shift of resonances, and thereby deconvolute two signals, Lanthanide Shift Reagents (LSRs) were at the forefront. As we were not interested in any separation of stereoisomers, we settled on the cheapest, achiral LSR, Erbium(III) tris(2,2,6,6-tetramethyl-3,5-heptanedionate), $Er(TMHD)_3$. We found that in our spectrometer $Er(TMHD)_3$ had no 2D spectrum alone plausibly due to the typical line broadening associated with LSRs, and the low concentration present.[10] This line broadening also made the ^{1}H even more difficult to interpret (though improved ^{1}H signal dispersion wasn't the goal). Additionally, the HMBC had no data. However, when the HSQC was performed in the presence of $Er(TMHD)_3$, the proton-bearing resonances deconvoluted and all six carbon signals were visible (**Figure 8**).

CONCLUSION

We have shown that a benchtop NMR with 2D capabilities is adequate to determine ^{13}C framework of complex molecular structures, in particular when a tandem 2D and stacking approach is employed. When this approach fails, lanthanide shift reagents can be applied as a workaround. This method saved us countless hours traveling to and from an off-site high-field NMR. We discovered that the pulse sequence of the 2D protocol was just as important as the other system-specific factors. The improved dispersion and resolution of the HSQC-ME experiment allowed for viewing a ^{13}C resonance previously undetectable on our instrument, and allowed for assigning the resonances as C, CH, CH2 due to its phase-sensitivity. The CH3 resonances were then assigned by comparing the HSQC-ME scans to a standard ^{13}C

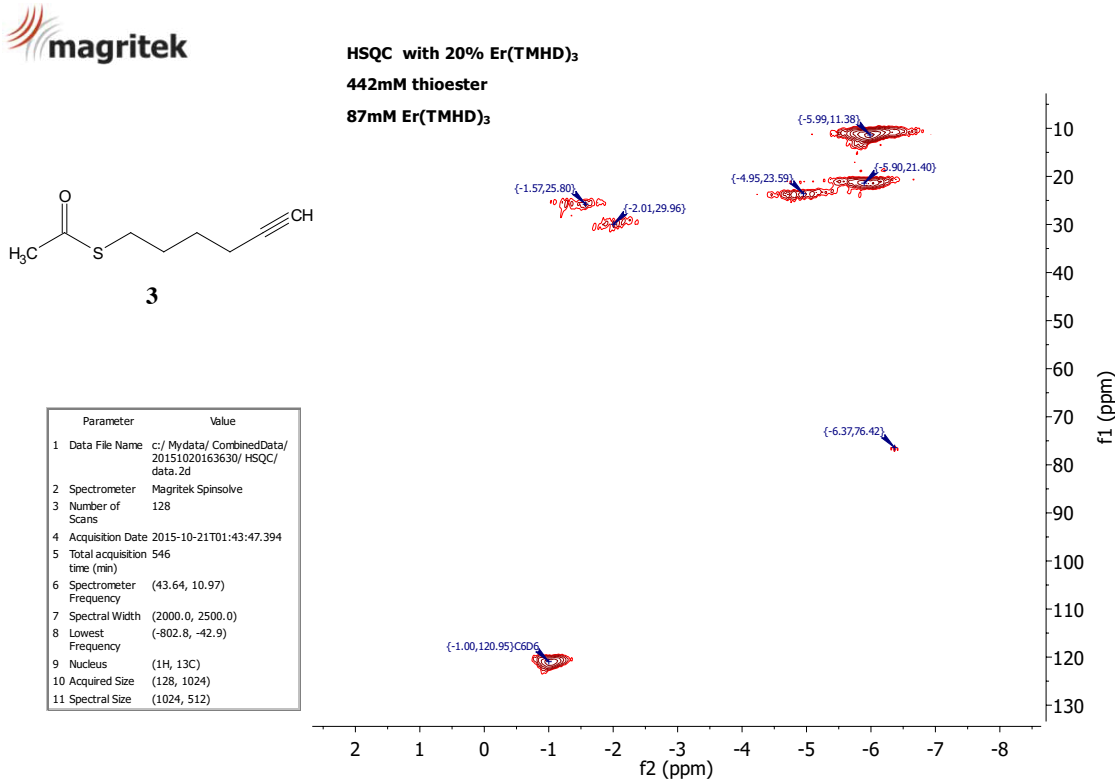


Figure 8 HSQC of 3 with 20% $Er(TMHD)_3$

experiment. The use of DEPT experiments also proved useful in elucidating the identities of compounds when presented with a mixture of products. All direct-detection methods, and their concentration or dispersion shortcomings were overcome using the methods described. There are two major experiments we would like to see these bench-top NMR companies include in the future. The first is called a PSYCHE[11] experiment that decouples the entire ^{1}H spectra while still allowing for accurate integration. This amazing experiment would elevate proton utility on low-field instruments by bypassing dispersion-based signal isochrony. Researchers would be able to simply count the resonances and evaluate their integration to gauge whether a reaction succeeded or failed. The second experiment is called Pure Shift HSQC NMR.[12] This experiment decouples the ^{1}H resonances in the 2D so that all resonances are presented as singlets, thereby increasing the strength of the signal that is correlated to any given ^{13}C. This experiment could have been useful when analyzing compound **2.** This experiment would have allowed for a much more robust and expeditious route toward ^{13}C NMR extraction from a dilute sample. As compound **3** showed that signal isochrony is still an issue, we encourage these companies to continue to strive for more powerful bench top magnets to decrease signal dispersion.

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CITATIONS

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