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Utilization of SABRE-Derived Hyperpolarization To Detect Low-Concentration Analytes via 1D and 2D NMR Methods

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Supporting Information

ABSTRACT: The characterization of materials by the inherently insensitive method of NMR spectroscopy plays a vital role in chemistry. Increasingly, hyperpolarization is being used to address the sensitivity limitation. Here, by reference to quinoline, we illustrate that the SABRE hyperpolarization technique, which uses *para*-hydrogen as the source of polarization, enables the rapid completion of a range of NMR measurements. These include the collection of ^{13}C, $^{13}C\{^1H\}$, and NOE data in addition to more complex 2D COSY, ultrafast 2D COSY and 2D HMBC spectra. The observations are made possible by the use of a flow probe and external sample preparation cell to re-hyperpolarize the substrate between transients, allowing repeat measurements to be made within seconds. The potential benefit of the combination of SABRE and 2D NMR methods for rapid characterization of low-concentration analytes is therefore established.

The rate at which structurally informative two-dimensional (2D) NMR spectra, such as correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-quantum correlation (HMQC) spectra, and spatially instructive 1D nuclear Overhauser effect (NOE) spectra can be collected was increased remarkably by the introduction of pulsed field gradients to select desirable coherence transfer pathways. NMR experiments have since been limited by the signal available in a single transient rather than by the necessity to phase-cycle pulses to produce clean, artifact-free results.[1,2] In standard experiments, the signal produced is directly linked to the concentration of the sample, meaning that low-concentration samples require significant signal averaging to generate useful information.[3] Methods have been developed to reduce the associated long scan times substantially, offering significant opportunities for the characterization of materials by NMR spectroscopy.[4−6]

The non-Boltzmann populations produced through the use of hyperpolarization methods can be exploited to generate NMR signals that can be several orders of magnitude larger than those achievable in standard experiments. The formation of nonequilibrium populations of nuclear spin states has been achieved using techniques as diverse as dynamic nuclear polarization (DNP), optical pumping, and hydrogenative *para*-hydrogen (p-H_2)-induced polarization (PHIP).[7−12] These methods typically deliver a burst of hyperpolarized substrate molecules that must be rapidly monitored. Like PHIP, signal amplification by reversible exchange (SABRE)[13] is able to produce a hyperpolarized substrate in a few seconds. However, unlike PHIP, SABRE does not result in incorporation of p-H_2 into the substrate. Instead, it uses a labile complex that possesses two hydride ligands and a weakly bound substrate to facilitate polarization transfer. Polarization is transferred at low magnetic field through the temporary scalar coupling network between what was previously p-H_2 and the substrate. Equilibration of the free and bound substrate molecules over a period of a few seconds produces hyperpolarized material in solution. Unlike other hyperpolarization methods, SABRE allows rapid and repeatable production of a hyperpolarized sample without chemical modification of the contained analytes.[14−16]

SABRE was first demonstrated using [Ir(COD)(PCy_3)(Py)]-[BF_4] (Crabtree's catalyst; COD = cyclooctadiene, Cy = cyclohexyl),[17] and pyridine (Py). Under these conditions, >100-fold signal enhancements were observed for the ^1H, ^{13}C, and ^{15}N NMR signals of free Py. Modification of the polarization transfer catalyst used in SABRE to incorporate a carbene in place of the phosphine produced a >1000-fold ^1H NMR signal enhancement for free Py.[18] This change in ligand gives the polarization transfer catalyst precursor used in this study: [Ir(COD)(IMes)Cl] (**1**) [IMes =1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene]. It has been postulated that the increase in signal is a consequence of a higher exchange rate that results from the greater electron-donating capability of IMes[19] over PCy_3.[20] The effect is therefore very simple in concept, but the detailed rationalization of the polarization transfer process is actually highly complex and requires the inclusion of a number of variables.[21]

Herein we illustrate how inorganic chemistry and p-H_2 can be combined through SABRE to provide reproducible levels of hyperpolarized material consistently, thereby allowing the rapid

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acquisition of 2D NMR spectra. This approach is shown to address the NMR insensitivity issue and enable "single shot" hyperpolarization methods to be used. An apparatus consisting of an integrated reaction cell and flow probe was employed to achieve this aim. This apparatus was used previously to enable the study of sample hyperpolarization levels and SABRE-created nuclear spin states.[18] Briefly, the apparatus has a polarization chamber that contains the sample solution (located in a predefined but variable magnetic field) and can be purged with p-H_2. For measurement, a series of gas valves shuttle the hyperpolarized sample solution to and from the flow probe, which is connected to the polarization chamber via a transfer line. This aspect of operation is controlled from within the spectrometer software. The polarization transfer catalyst **1** is activated by the addition of hydrogen in the presence of quinoline (20-fold excess) and acetonitrile (1-fold excess). First, acetonitrile displaces the chloride ligand of **1**, thereby activating the newly formed adduct for subsequent hydrogenation of COD. Quinoline then enters the coordination sphere of the metal, forming the active polarization transfer catalyst, $[Ir(IMes)(quinoline)_3(H)_2]Cl$. When such a sample was purged with H_2 gas enriched to 93% p-H_2 in a magnetic field of 50 G for 6 s prior to transfer into the flow probe, the ^1H NMR trace shown in Figure 1 was obtained. All seven ^1H

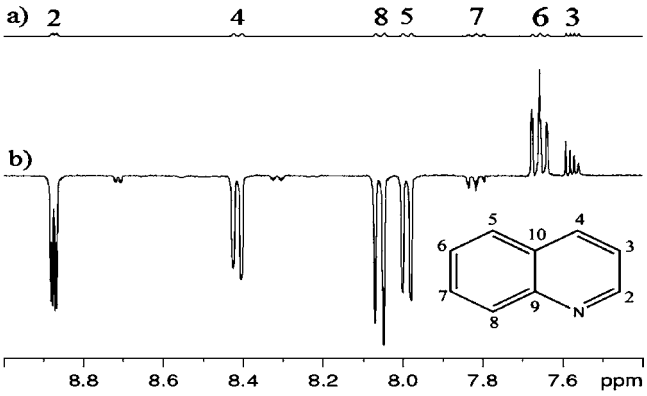

Figure 1. ^1H NMR spectra showing (a) the thermally polarized trace of quinoline and (b) the corresponding SABRE-hyperpolarized trace in methanol-d_4 achieved with the catalyst [Ir(IMes)(COD)Cl] (**1**) in a 50 G polarization transfer field.

resonances of quinoline were enhanced well beyond their thermally polarized level. The level of enhancement was maximized for the H(2) proton, which showed >60-fold enhancement when 2 μmol of quinoline was detected. This single response resulted in a signal-to-noise (S/N) ratio of 730:1 for the H(2) signal. When the sample detected amount was reduced to the 0.2 μmol level, the corresponding S/N ratio was 15:1. These data therefore serve to illustrate the potential of this method for detection of low-concentration analytes; when nicotinamide was used as the substrate, a 20 nmol sample produced a S/N ratio of 60:1.

When the 2 μmol quinoline sample was purged with p-H_2 for the increased time of 20 s, the level of signal enhancement increased by a further factor of 2.6, thereby illustrating how even lower concentrations of this analyte could be examined with SABRE.

Such samples can easily be returned to the polarization chamber and exposed to fresh p-H_2 prior to a second NMR measurement. The total time for this process is dominated by the purge duration. This approach produces hyperpolarized signals with an intensity that is reproducible to within a 5% tolerance when the measurement interval is 30 s and the purge time 6 s. This approach of shuttling the sample between the hyperpolarization chamber and the probe enables signal averaging, a key requirement of many NMR experiments. The completion of a range of multiscan NMR measurements are now shown to benefit from hyperpolarization achieved by SABRE.

A hyperpolarized quinoline sample was probed using the 1D NOE sequence of Stott, Keeler, and Shaka[22] with four polarization transfer steps. Examination of the resulting four-scan-hyperpolarized H(4) resonance, in conjunction with a 1.5 s NOE contact time, led to a cross-peak with an intensity of 0.9% at the H(5) resonance position. In contrast, when thermal polarization was used, the NOE transfer peak achieved a similar S/N value only after a potentially prohibitive 15 000 scans. Consequently, the normal measurement, which takes 20 h to implement at this 2 μmol sample loading, was completed in just 81 s. This multiscan observation establishes the utility of SABRE in examining low-concentration analytes by such 1D methods.

The same flow and replenishment approach was used to record a 2D ^1H OPSY–COSY spectrum for the 2 μmol sample (Figure 2); OPSY denotes the only *para*-hydrogen spectrosco-

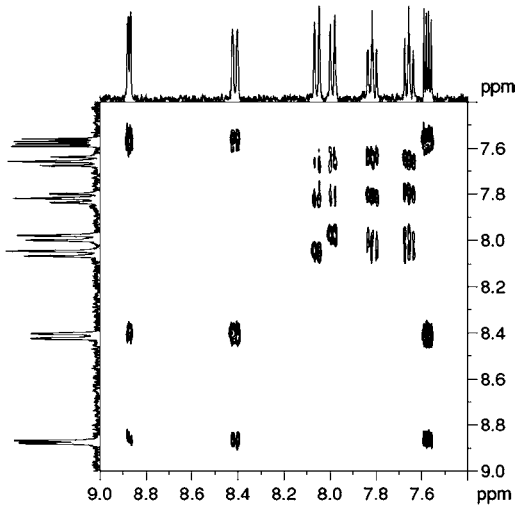

Figure 2. ^1H 2D OPSY–COSY NMR spectrum of hyperpolarized quinoline in methanol-d_4 achieved using **1** and p-H_2 with an applied polarization transfer field of 0 G.[27]

py protocol,[23] which was set for use as a double-quantum filter.[24] In this case, the p-H_2 purge time was 2 s and 64 increments were completed using the sample-shuttling process, giving a total experiment time of 16 min. This NMR spectrum could be recorded against a strong proton background because the OPSY filter removed any signals that did not originate from polarization transferred under SABRE. Unusually, cross-peak encoding was optimized at the start of the 2D data cascade for this SABRE-derived polarization, causing the cross-peaks to appear before the diagonal. Hence, far fewer increments were necessary for cross-peak mapping, with the diagonal peaks building up according to $\sin(\pi J_{HH} t)$; normally this term corresponds to the cross-peak buildup.[25] In this experiment, $^4J_{HH}$ couplings within the rings, such as those between protons

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H(5) and H(7), were readily visible. For them to be visible in a normal experiment, a very good S/N ratio would be required.

We also note that the increased sensitivity provided by hyperpolarization enables the completion of a 2D COSY measurement using ultrafast methods.[4] In this case, a single purge of *p*-H_2 yields sufficient hyperpolarization to allow the complete assignment of the coupling framework within quinoline in the 6 μmol sample in under 1 s.[4,5] A typical trace resulting from polarization transfer at 65 G is shown in Figure 3; a plot obtained at the 0.6 μmol level is reproduced in the Supporting Information.

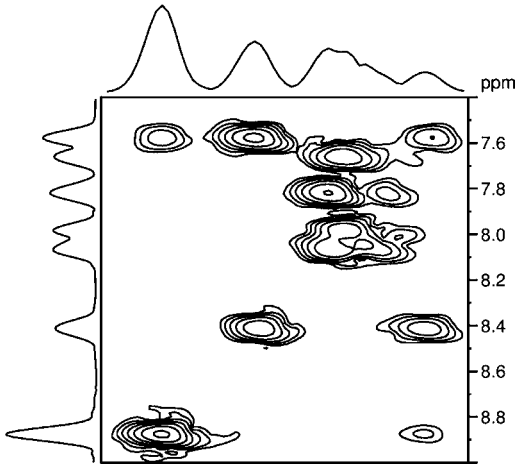

Figure 3. Ultrafast ^{1}H COSY spectrum of a 6 μmol sample of hyperpolarized quinoline in methanol-d_4 collected using a single transient.

The collection of ^{13}C data is routinely even harder to achieve than a COSY measurement because of the inherent 6400-fold drop in sensitivity associated with the direct detection of a ^{13}C signal relative to that of ^{1}H. Figure 4a shows the resulting single-scan, fully coupled ^{13}C signal acquired for a 60 μmol sample that was monitored after a 90° read pulse and polarization transfer at 50 G. While all nine of the expected signals for quinoline can be identified, the intensity of the signal corresponding to the C(2) position at δ 150.8 is significantly reduced.

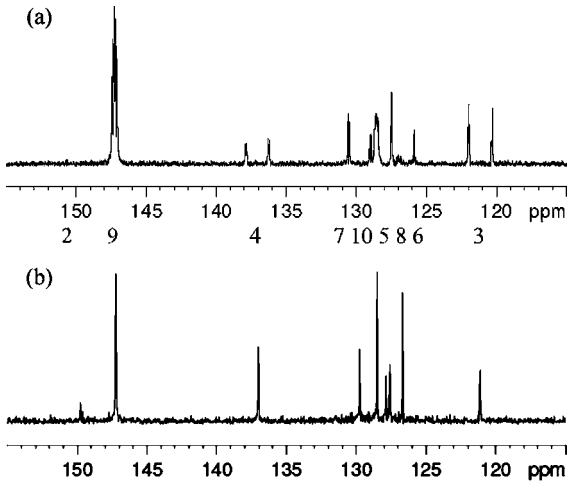

Figure 4. (a) Single-scan ^{13}C NMR spectrum of SABRE-hyperpolarized quinoline presented in magnitude mode after polarization transfer at 0 G with **1** in methanol-d_4 acquired using a 90° excitation pulse. (b) Corresponding refocused ^{13}C{^{1}H} INEPT NMR spectrum of a similar sample hyperpolarized at 50 G.

The detected resonances appear with a degree of antiphase character (which can be ascribed to the detection of signals associated with the combinations of the product operators $2I_{1z}S_x$ and $4I_{1z}I_{2z}S_x$ at each ^{13}C location); this effect has been overcome in Figure 4 by presenting these data in magnitude mode. The populations of the magnetic states created with SABRE vary with the magnitude of the magnetic field at which polarization transfer occurs. This affects both ^{1}H−^{1}H and ^{1}H−^{13}C spin pairs.[18] Consequently, if these ^{13}C−^{1}H terms are to be observed via a ^{1}H-decoupled low-sensitivity ^{13}C response, the antiphase character must be refocused. This was readily achieved by using an insensitive nuclei enhanced by polarization transfer (INEPT) refocusing scheme,[26] which allowed all nine ^{13}C signals for quinoline to be readily detected and resulted in a S/N ratio of 40:1 for the C(9) resonance of the 60 μmol sample after a single scan (Figure 4B). When a traditional ^{13}C{^{1}H} measurement was completed using an equivalent nonhyperpolarized sample, a recycle time of 3.6 s, and a 30° flip angle, an equivalent S/N ratio was obtained after 4096 scans.

Since the SABRE approach simultaneously hyperpolarizes both ^{13}C and ^{1}H nuclei, a new approach to data collection can be envisaged. First, it is possible to use the multiple-receive facility of a modern NMR spectrometer to collect sequential/simultaneous SABRE-hyperpolarized ^{13}C and ^{1}H data. This enables the rapid observation of both types of nuclei after a single hyperpolarization transfer step. A refinement of this approach enables the collection of two hyperpolarized ^{13}C NMR traces from a single polarization transfer cycle. The first of these corresponds to the measurement of a fully proton-coupled ^{13}C acquisition, thereby utilizing the hyperpolarized ^{13}C magnetization. The second acquisition also records ^{13}C data, but here a refocused and decoupled INEPT transfer protocol is employed to record the ^{13}C{^{1}H} NMR spectrum. This second measurement uses unaffected ^{1}H hyperpolarization to sensitize the second ^{13}C NMR spectrum. Figure 4b presents a typical hyperpolarized ^{13}C INEPT (refocused) trace.

It is also possible to use the flow method to collect a hyperpolarized ^{1}H−^{13}C heteronuclear 2D NMR spectrum. The result of this for a 2 μmol sample loading is shown in Figure 5. This HMBC spectrum of quinoline was collected as a 32-increment data set after polarization transfer at 60 G, with a single scan per increment. The total experiment time was 16 min. Cross-peaks now locate all nine of the ^{13}C signals and arise primarily through long-range rather than direct coupling pathways. When the polarization transfer step is completed with an applied field of 0 G, the corresponding cross-peaks are derived from short-range couplings.

The results presented here demonstrate that the SABRE technique enables rapid completion of a wide range of structurally informative 1D and 2D NMR experiments on low-concentration analytes. By variation of the strength of the polarization transfer field, it is possible to control the type of magnetization created in the hyperpolarized analyte. If the field strength is set to maximize the formation of correlated longitudinal two-spin order terms, the optimum characteristics for creating cross-peaks in both 2D OPSY−COSY and heteronuclear correlation experiments result. Consequently, both long- and short-range couplings and hence structural connections within molecules can be probed both rapidly and

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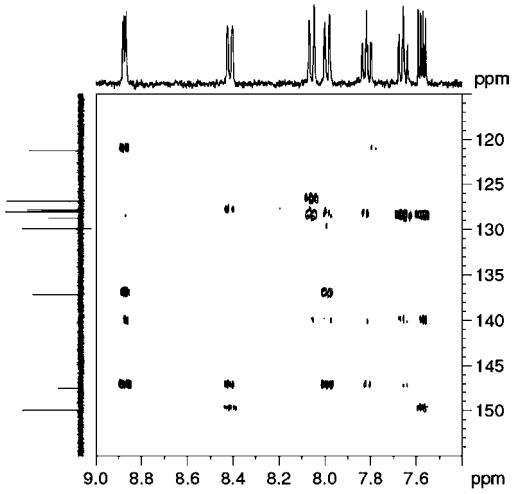

Figure 5. 2D $^{1}H-^{13}C$ HMBC NMR spectrum collected with quinoline that was hyperpolarized at 60 G in methanol-d_4 using **1** and p-H_2.[27]

at high sensitivity. This means that the user can select the type of magnetization required and hence tailor the measurement to the specific information element that is required. In view of the fact that SABRE has previously been reported to provide an 8600-fold ^{1}H signal enhancement for pyridine,[18] its potential to allow rapid NMR analysis of very low concentration species is an exciting future prospect for this method. Research is ongoing to demonstrate that mixtures can be examined using this method and that absolute concentrations can be determined using suitable calibration curves.

■ ASSOCIATED CONTENT

Supporting Information

Details of the samples and NMR procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(27) The external projections employed in Figures 2 and 5 were taken from experiments recorded with a high concentration of quinoline and are intended to enable the reader to see how the hyperpolarized cross-peaks shown in the 2D plots map to the expected correlations.