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Multiple Ultrafast, Broadband 2D NMR Spectra of Hyperpolarized Natural Products

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Liquid-state NMR exhibits a wide range of chemical and biochemical applications. NMR is also highly insensitive, largely due to the small spin polarizations that under normal conditions contribute to the observable signals. Compensation for this handicap is traditionally sought in NMR hardware improvements.[1] Recent years have seen the emergence of a different approach to enhancing NMR's sensitivity, based on replacing the weak polarizations arising at room-temperature, with metastable spin states reaching nearly unity alignments. A promising and generally applicable hyperpolarization technique is *ex situ* dynamic nuclear polarization (DNP),[2] which can lead to $\sim 10^4$ per-scan signal enhancements compared to conventional NMR. *Ex situ* DNP achieves hyperpolarization by cryogenic cooling and microwave irradiation of a comixed, stable electron radical.[3] This is followed by an irreversible melting and transporting of the sample into the spectrometer, where an otherwise conventional liquid-state NMR acquisition takes place. Given the nonequilibrium state on which the experiment relies, *ex situ* DNP NMR is best suited to collect a single or at most a small number of scans. This makes it a poor starting point for acquiring arrayed transients involving complex pulse sequences, of the kind needed to complete contemporary 2D NMR acquisitions.[4] Alternatives have been recently suggested for overcoming this difficulty, including 2D sequences optimized using small-angle pulses, repeated meltings/freezings of the sample, and combinations of *ex situ* DNP with spatially encoded methods delivering arbitrary 2D correlations within a single scan.[5] Particularly promising appears to be the advent of inverse-detected 2D "ultrafast" methods,[6] exploiting the hyperpolarization of slowly relaxing low-γ nuclei (withstanding well the polarizer→spectrometer transfer) with the sensitivity and information afforded by a direct-domain ^{1}H-based detection. Using this approach we have recently collected 2D ^{13}C−^{1}H correlation spectra within 0.2 s acquisition times, on 0.1 mM mixtures of self-glassing chemicals at natural abundance. Still, a limitation affecting these single-scan 2D NMR experiments, rests in their inability to cover spectral ranges exceeding ∼10−20 ppm. This is a consequence of the strong acquisition gradients G_a that would otherwise be required, making these methods ill-suited to cover the large bandwidths and achieve the high resolutions expected from indirectly detected ^{13}C NMR.[7] To deal with this challenge, we discuss here new spatial/spectral encoding strategies capable of "shifting" ^{13}C resonances into arbitrary positions of a 2D single-scan spectrum. By virtue of being spectrally selective, these new approaches greatly ease the gradient demands; they also allow one to obtain—following a single hyperpolarization process—multiple 2D heteronuclear correlations arising from different ^{13}C regions. It is here shown that, in combination with *ex situ* DNP, these principles enable the consecutive acquisition of heteronuclear multiple-bond and single-quantum (HMBC and HSQC) 2D NMR spectra[8] on ∼1 mM mixtures of natural products, characterizing with high resolution sites spread over ∼70 ppm spectral bandwidths.

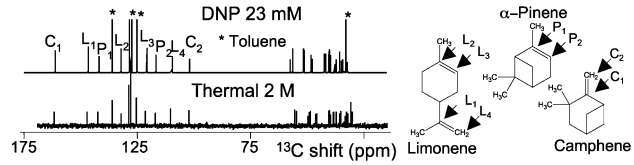

Figure 1. Comparison between single-scan {^{1}H}^{13}C NMR spectra of limonene, α-pinene, camphene dissolved in toluene (whose signal was vertically truncated). Labels indicate resonances targeted in this work. (Top) Spectrum obtained after freezing a 50 μL 1 M terpene solution at 1.4 K and irradiating it for 90 min in a Hypersense DNP. Sudden dissolution by preheated methanol led to a 23 mM final concentration in the NMR tube, as determined by spectrophotometric and ^{1}H NMR measurements. (Bottom) Thermal NMR spectrum arising from a 2 M terpene solution. All NMR experiments were conducted on a 11.7 T Varian Inova spectrometer equipped with a 5 mm indirect-detect probe.

Figure 1 summarizes 1D ^{13}C studies of the system chosen to illustrate these new capabilities of hyperpolarized 2D ultrafast NMR. This involved a mix of limonene, camphene and α-pinene, dissolved at natural abundance in toluene as glassing solvent, and comixed with 20 mM α,γ-bisdiphenylene-β-phenylallyl (BDPA) as the source of unpaired electrons. The single-pulse ^{13}C NMR spectrum obtained on this system after ca. 90 min of microwave irradiation (100 mW) and sudden melting, revealed signal enhancements of up to 32 000 vis-à-vis a reference, thermally polarized counterpart. This result underlines the efficiency of BDPA/C_7H_8 as DNP medium for hydrophobic organic molecules, leading to solutes endowed with ca. 30% liquid-state spin polarization. Unfortunately toluene itself also undergoes a very efficient hyperpolarization, originating strong aromatic signals that hamper the observation of certain terpene-based ^{13}C−^{1}H correlations. Employing toluene-d_8 compensates for this drawback; tests, however, reveal much poorer polarizing properties for deuterated toluene vis-à-vis its protonated counterpart. A compromise was sought by carrying out the DNP using 1:5 toluene/toluene-d_8 as solvent.

The ∼150 ppm ^{13}C shift range in Figure 1 exemplifies the challenges arising when collecting hyperpolarized ultrafast 2D heteronuclear correlation spectra. A new peak-shifting encoding procedure was employed to deal with this limitation; while a full account of this approach will be described elsewhere, the HMBC pulse sequence in Figure 2A illustrates its main components. This sequence begins with a series of selective excitation pulses on the hyperpolarized ^{13}C nonprotonated sites, followed by magnetic field gradients imparting site-specific spatial windings. If suitably chosen these gradients will shift the indirect-domain ^{13}C resonances into arbitrary positions within the ultrafast 2D spectrum; these spatial/spectral manipulations are done so as to place all peaks in a reduced final window, compatible with the minimal G_a acquisition gradients desired. This initial selective excitation train is followed by a constant-time spatial encoding based on a pair of adiabatic π sweeps;[6c] additional evolution delays, pulses and coherence-selective gradients, serve for filtering out the sought J^2-correlations

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and for removing undesirable $^{1}H-^{12}C$ background signals.[8b] Further details on the principles of these experiments, on their timings and their gradients, are given as Supplement. Figure 2B shows single-scan 2D results arising from this HMBC scheme, characterizing the nonprotonated olefinic ^{13}C's of the hyperpolarized terpenes at 1−2 mM final concentrations in the NMR tube. The cross sections show a resolution and a signal-to-noise ratio comparable to that of a conventional 2D counterpart (Figure 2C)—provided the latter is signal averaged for 90 min at ca. an order of magnitude higher concentrations.

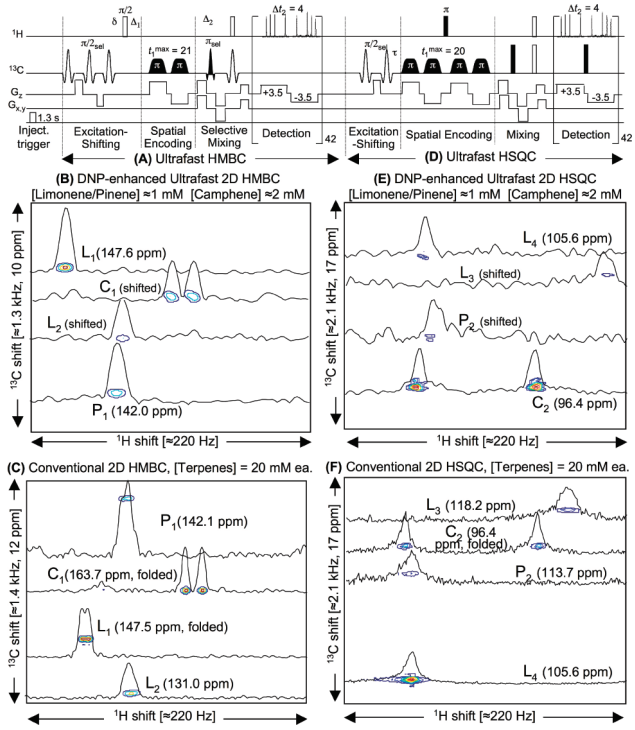

Figure 2. (A,D) Successive 2D HMBC and HSQC $^{13}C-^{1}H$ ultrafast pulse sequences, including gradient and timing parameters (in G/cm and ms) and spectral/spatial manipulations bringing the relevant signals inside the targeted spectral window. In these sequences $\delta = 0.4$ ms, $\Delta_1 = 9$ ms, $\Delta_2 = 12$ ms, $\tau = 0.4$ ms, and data were digitized with a 2 μs dwell time. (B,E) Ultrafast HMBC and HSQC 2D spectra with relevant cross sections, obtained after polarizing 4.4 μL of a 0.5 M limonene/α-pinene/camphene 1:1:2 solution in a toluene/toluene-d_8 1:5 mixture with 20 mM BDPA. Sudden dissolution in methanol-d_4 led to a final concentration of 1 mM for limonene and α-pinene, and 2 mM for camphene. (C,F) Conventional HMBC and HSQC 2D NMR spectra collected on a 20 mM equimolar terpene mixture in $CDCl_3$, obtained in 90 min using 32 scans, 64 t_1 increments, a 2 s recycle delay and a 0.5 s acquisition time. These conventional data were acquired with the same ^{13}C spectral widths as the ultrafast spectra, leading to the aliasing of some of the peaks. Also shown are the unfolded positions of the ^{13}C resonances characterized in each experiment (in ppm's from TMS). Both HSQC and HMBC conventional pulse sequences were made spectrally selective to avoid the appearance of unwanted ^{13}C resonances (for further details on these experiments, see Supporting Information).

A noteworthy aspect of the HMBC scheme introduced in Figure 2A stems from the fact that, except for the ^{13}C sites that were excited, all remaining ^{13}C positions remain in their initial hyperpolarized state. This is a consequence of DNP being a process that affects the full spin reservoir, and of the fact that the only broadbanded pulses in the spatial encoding process were two adiabatic π sweeps -amounting to a 0° rotation for all but the targeted carbons. This opens the possibility of running, immediately after the completion of the ultrafast 2D HMBC acquisition, additional 2D NMR experiments that exploit the remaining low-γ sites' hyperpolarization. In the present case we chose 2D HSQC[8a] as second experiment to assay, leading to an efficient way of characterizing directly bonded $^{13}C-^{1}H$ spin pairs without having to repolarize the sample. The sequence used to demonstrate this concept (Figure 2D) was carried out immediately following the conclusion of the preceding HMBC acquisition. Like in the latter experiment, spectral/spatial encoding schemes were used to fit the targeted indirect-domain ^{13}C region. Overall these HMBC/HSQC experiments managed to characterize ^{13}C bandwidths exceeding 70 ppm with high indirect- and direct-domain resolutions, while keeping $G_a \leq 4$ G/cm.

These examples highlight the efficiency with which pulse sequencing improvements and the identification of good hyperpolarizing conditions, may allow one to target realistic organic samples at 1 mM concentrations using DNP-enhanced ultrafast 2D NMR. Naturally many options besides the ones here discussed could be considered -involving other kinds of single-scan 2D heteronuclear concatenations (e.g., HMBC/HSQC/HMQC), acquisitions on aliphatic and on aromatic regions, $^{15}N-^{1}H$ and $^{13}C-^{1}H$ experiments, etc. Judicious choices of selective spectral/spatial manipulations should allow one to implement all such variations starting from a singly hyperpolarized sample. We trust to report on these possibilities in upcoming studies.

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Supporting Information Available: Explanations of how spectral/spatial manipulations alleviate ultrafast NMR limitations; further details on Figure 2 sequences and experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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