

Toward single-shot pure-shift solution ^1H NMR by trains of BIRD-based homonuclear decoupling

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ABSTRACT

Achieving homonuclear ^1H decoupling remains one of the key challenges in liquid-state NMR. Such spectra would endow a variety of organic and analytical applications with an increased resolution, and would ideally do so even in a one-dimensional format. A number of parallel efforts aimed at achieving this goal using two-dimensional acquisitions have been proposed; approaches demonstrated over recent years include, among others, new modes for achieving purely-absorptive J spectroscopy, the use of spatially-selective manipulations, and exploiting the natural spin dilution afforded by heteronuclei. The present study relies on the latter approach, and explores the use of BIRD pulses distinguishing between protons bonded to ^{13}C from those bonded to ^{12}C , to achieve homonuclear decoupling in a continuous 1D scan. Studies on several representative compounds demonstrate that this goal can be implemented in a robust format, provided that suitable care is also taken to suppress unwanted coherences, of making all manipulations sufficiently broad-banded, and to provide adequate *heteronuclear* decoupling of the targeted protons. Dependable homonuclear decoupling performance can then be achieved, with minimal line width, fine-tuning, and sensitivity penalties.

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1. Introduction

High-resolution liquid state spectroscopy on protons is arguably the most mature and developed area in the field of NMR. Still, the one-site/one-peak correlation ideally sought in an NMR analysis – an ideal easily realized in the area of heteronuclear spectroscopy – has eluded ^1H acquisitions due to the ubiquitous presence of homonuclear J couplings. Unlike their dipolar counterparts these isotropic effects cannot be easily refocused by either spatial- or spin-space broadband manipulations, resulting in the presence of J -multiplets that have accompanied organic NMR analyses for the last half century. Solving this long standing problem has been the aim of numerous investigations, starting with resonance-specific selective irradiation [1,2] and culminating in a variety of two-dimensional (2D) approaches [3–19]. Worth remarking among the latter efforts are experiments focused on 2D J -spectroscopy [3] aimed at separating a pure J evolution along the indirect t_1 domain from a mixed J /shift evolution under t_2 , the use of bilinear rotation decoupling (BIRD) pulses [5,6] capable of distinguishing the evolution of protons that are bonded to ^{13}C from those that are ^{12}C -bound, and the Zangger/Sterk sequence [14] relying on spatially-selective ^1H spin inversions. In order to achieve high spectral resolution, all of these two dimensional approaches face a number of common

challenges – foremost among them the Nyquist-derived demand for relatively lengthy multi- t_1 acquisitions, regardless of sensitivity and according to criteria dictated solely by the targeted spectral widths and resolutions. Efforts to alleviate these demands have been recently reported by Morris and coworkers, in a series of studies [17,19] showing that lengthy 2D acquisitions just mentioned could be collapsed into minutes-long 2D experiments by exploiting the differences in size between the shifts being sought and the homonuclear couplings being refocused. The present study discusses a related BIRD-centered alternative, which permits collecting homonuclear J -decoupled proton spectra in a one-dimensional scan. In order to achieve this we explore the use of *trains* of BIRD rotations, executed along the lines normally used in multi-pulse decoupling. The conditions under which such a train can yield the same kind of homonuclear decoupling performance as observed in optimized 2D acquisitions employing the same BIRD element, were thoroughly explored. It was observed that under suitable setups single-scan BIRD-based approaches can offer a very competitive decoupling performance vis-à-vis their 2D counterparts, without suffering from pure-shift scaling factors, and without requiring extensive or customized precalibrations. Among the essential ingredients required to achieve such a robust performance, we found it important to use a clean selection of signals from protons bound to ^{13}C , as well as high-quality ^{13}C manipulations – including both broadband inversion and broadband decoupling of the heteronuclei. The following paragraphs describe the nature of the experiments assayed, the elements which we found were important for maximizing the

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performance of these 1D homonuclear-decoupled ^1H acquisitions, and illustrative results obtained in a number of representative compounds posing increasingly challenging decoupling scenarios.

2. Theoretical considerations

The basic element in this study is, as mentioned, the BIRD composite pulse. This is a four-pulse element

$$(90)_y^{\text{H}} - 0.5/J_{\text{CH}} - (180)_x^{\text{H}} \& (180)_x^{\text{C}} - 0.5/J_{\text{CH}} - (90)_y^{\text{H}} \quad (1)$$

proposed by Garbow et al. [5] to distinguish among the evolution of protons that are coupled to ^{13}C from those that are not. The scheme in Eq. (1) would impart a 180° pulse on the ^{12}C -bound protons, while leaving unchanged the ^{13}C -bound ones. Indeed if for the sake of simplicity one considers two protons H_1 and H_2 , a ^{13}C nucleus C coupled only to H_1 , and a heteronuclear coupling among these spins $J_{\text{CH}} = 0.25/\Delta$ that is much larger than the homonuclear J_{HH} interaction, the evolution imparted onto the ^{13}C -bound H_1 proton magnetization would be

$$\begin{aligned} \text{H}_{1x} &\xrightarrow{90_y^{\text{H}}} -\text{H}_{1z} \xrightarrow{2\Delta} -\text{H}_{1z} \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} \text{H}_{1z} \xrightarrow{2\Delta} \text{H}_{1z} \xrightarrow{90_y^{\text{H}}} \text{H}_{1x} \\ \text{H}_{1y} &\xrightarrow{90_y^{\text{H}}} \text{H}_{1z} \xrightarrow{2\Delta} -2\text{H}_{1x}\text{C}_z \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} 2\text{H}_{1x}\text{C}_z \xrightarrow{2\Delta} \text{H}_{1y} \xrightarrow{90_y^{\text{H}}} \text{H}_{1y} \\ \text{H}_{1x} &\xrightarrow{90_y^{\text{H}}} \text{H}_{1z} \xrightarrow{2\Delta} 2\text{H}_{1x}\text{C}_z \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} 2\text{H}_{1y}\text{C}_z \xrightarrow{2\Delta} \text{H}_{1x} \xrightarrow{90_y^{\text{H}}} \text{H}_{1z} \end{aligned} \quad (2a)$$

i.e., a unity operator. By contrast, for the H_2 (^{12}C -bound) proton, its magnetization would undergo

$$\begin{aligned} \text{H}_{2x} &\xrightarrow{90_y^{\text{H}}} -\text{H}_{2z} \xrightarrow{2\Delta} -\text{H}_{2z} \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} \text{H}_{2z} \xrightarrow{2\Delta} \text{H}_{2z} \xrightarrow{90_y^{\text{H}}} \text{H}_{2x} \\ \text{H}_{2y} &\xrightarrow{90_y^{\text{H}}} \text{H}_{2y} \xrightarrow{2\Delta} -2\text{H}_{2y} \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} -\text{H}_{2y} \xrightarrow{2\Delta} \text{H}_{2y} \xrightarrow{90_y^{\text{H}}} -\text{H}_{2y} \\ \text{H}_{2z} &\xrightarrow{90_y^{\text{H}}} \text{H}_{2x} \xrightarrow{2\Delta} \text{H}_{2x} \xrightarrow{180_x^{\text{H}}-180_x^{\text{C}}} \text{H}_{2x} \xrightarrow{2\Delta} \text{H}_{2x} \xrightarrow{90_y^{\text{H}}} \text{H}_{2z} \end{aligned} \quad (2b)$$

which is equivalent to the action of a 180_x pulse. This distinction lies at the heart of the 2D proposals aimed at decoupling one given proton from a neighboring partner: these are experiments of the form

$$\{(\text{excite solely } ^{13}\text{C-bound protons}) - t_{1/2} - \text{BIRD} - t_{1/2} - (\text{acquire } ^{13}\text{C-bound protons})\} \quad (3)$$

that exploit the high statistical dilution brought about by the ^{13}C , to refocus the homonuclear proton J couplings along the indirect dimension. A main price paid for this decoupling is that the observable spectrum is restricted only to those protons that are bound to ^{13}C ; under natural-abundance conditions this is an onerous 99% sensitivity price, but one which may be worth paying for the sake of achieving the desired measure of resolution.

As mentioned, most uses of BIRD-based homonuclear decoupling schemes have so far focused on 2D experiments, where high resolution is achieved along the indirect domain by incrementing a t_1 like that in Eq. (3) over a suitable support. In the present study we shall focus instead on the two 1D NMR variants illustrated in Fig. 1, which systematically provided good results for a wide range of samples and conditions. The main blocks worth stressing in these sequences include the following motifs:

$$\begin{aligned} &(\text{INEPT-based filtering of } ^{13}\text{C-bound protons}) \\ &- (\text{BIRD-based excitation of } ^{13}\text{C-bound protons}) - \{(^{13}\text{C} \\ &- \text{decoupled } ^1\text{H acquisition}) - (\text{BIRD } 180_x \text{ pulse on } ^{12}\text{C} \\ &- \text{bound } ^1\text{Hs}) - (^{13}\text{C-decoupled } ^1\text{H acquisition}) \\ &- (\text{BIRD } 180_y \text{ pulse on } ^{12}\text{C-bound } ^1\text{Hs})\}_{\text{loop}} \end{aligned} \quad (4)$$

This scheme incorporates a number of elements that we found important to ensure the sequence's robust operation. These include a thorough suppression of undesired ^{12}C -bound proton background via the purging of heteronuclear spin-ordered states $2\text{H}_2\text{C}_z$, a subsequent selective excitation of solely these states, and a supercycling of the BIRD blocks using an XY-type scheme. The resulting time domain signal will therefore be the result of concatenating $2n$ acquisition segments, each one lasting $at/2n$ – where at denotes the overall acquisition time. As long as $at/2n \ll 1/J_{\text{HH}}$, homonuclear J modulations occurring during these acquisition segments can be disregarded with no compromise in the final spectral resolution, leading to the potential collapse of all J coupling multiplets. Deviations from this condition lead to the rise of distinct “decoupling sidebands” flanking each purely-shifted resonance at a spacing $2n/at$. It follows from these considerations that satisfactory decoupling will have to arise in these 1D acquisitions from a compromise between high n -values – which will lead to a more accurate homonuclear J -refocusing at the expense of a reduced

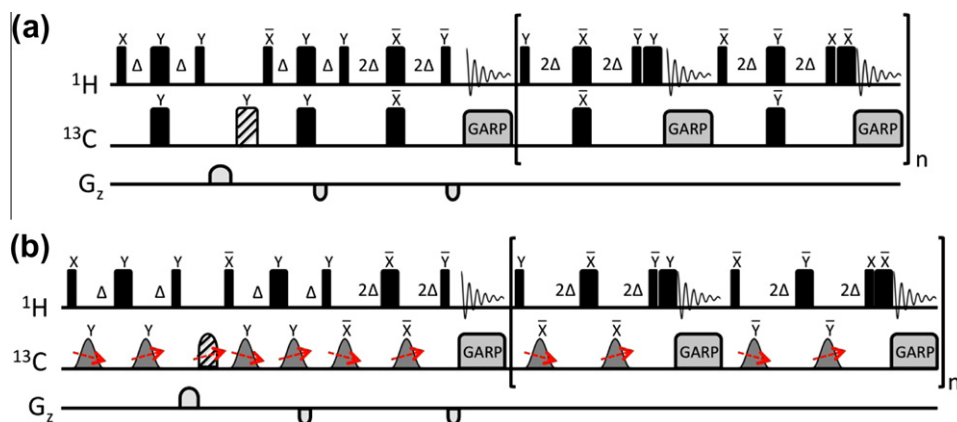


Fig. 1. BIRD-based homonuclear decoupling sequences assayed in this study, with narrow and wide lines indicating 90° and 180° pulses respectively. (a) Hard-pulse version. (b) Broadband swept-pulse version. Each sequence incorporates two filters to suppress the ^{12}C -bound proton background: an initial INEPT-type isotope filter to select ^{13}C -bound protons followed by a homospoil pulse, and a gradient-flanked BIRD element that also serves to decouple ^{13}C -bound protons during the first acquisition. All subsequent BIRD pulses and acquisitions are looped in a pairwise block, incorporating XY supercycling [22] to suppress effects of unequal J_{CH} couplings. For the sake of cycling out the residual ^{12}C -bound proton signals experiments were repeated twice, in the presence and absence of the 180° pulses shown dashed. As the presence of these ^{13}C 180° pulses effectively reverses the intermediate $2\text{H}_2\text{C}_z$ state but leaves unaffected ^{12}C -bound proton signals, a concomitant addition/subtraction of the resulting FIDs can remove the latter. In addition to this two-scan cycle, CYCLOPS on the full initial ^1H INEPT block and receiver phase was occasionally implemented. Notice that in the broadband version of the sequence shown in (b), all hard 180° ^{13}C pulses of (a) have been replaced by pairs of 180° chirped pulses, whose sweep directions are as indicated by the red arrows (i.e., from the low-frequency to the high-frequency ends of the spectrum or vice versa).

shift evolution within a given, T_2 -defined acquisition period at – and longer inter-BIRD acquisition segments associated with a more extensive homonuclear evolution. In practice we find that optimal results were obtained using piece-wise acquisition blocks lasting ca. 30 ms. Moreover, it is worth pointing out that although BIRDs involves effective 180° pulses on the directly-bonded ^{13}C and therefore provide some form of heteronuclear decoupling, the ensuing heteronuclear-driven “sidebands” can nevertheless be significantly strong – particularly if the acquisition windows are longer than the characteristic heteronuclear J -modulation times $1/J_{\text{CH}}$. We therefore found an efficient form of ^{13}C decoupling [20] to be essential for retrieving sharp, fully-decoupled proton singlets. GARP [21] typically applied with a ≥ 20 kHz bandwidth, was suitable for this end.

The ideal behavior expected for spins during the action of a BIRD-based train of decoupling pulses can be compromised in realistic situations by (i) the fact that a single delay Δ – ideally tuned to $0.25/J_{\text{CH}}$ – may not simultaneously satisfy the distribution in heteronuclear couplings arising for different sites in the molecules; and (ii) the challenges that the BIRD train will face in performing nearly perfect inversions for ^{13}C sites spanning up to ≈ 200 ppm in their chemical shift ranges. Either of these two deviations from ideality will affect the behaviors expected for the ^{13}C -bonded protons, leading to artifacts that are clearly visible in the ensuing ^1H spectra. In practice we found that (i) by using XY supercycling [22], a suitable compromise value of Δ can be found which minimizes the J_{CH} -derived artifacts; and (ii) by replacing all hard ^{13}C pulses in the sequence with suitably broad-banded manipulations, one can eliminate most artifacts that would otherwise arise from a broad distribution in carbon resonance frequencies. Replacement of all ^{13}C hard pulses by composite-pulse counterparts [23] was not sufficient to produce the required broadband BIRD rotations: none of the alternatives assayed ($\{90_y-180_x-90_y\}$, $\{90_x-225_x-315_x\}$, $\{90_x-240_y-90_y\}$, $\{90_x-240_x-90_x\}$) improved in any remarkable fashion the performance delivered by our maximum power pulses. Therefore their results are not presented here. Instead, an alternative sequence was developed in which all the hard-pulse ^{13}C rf manipulations were replaced by suitable *pairs* of chirped pulses, capable of compensating the large quadratic phase dispersions that would otherwise result in the involved coherences if the hard pulses were substituted by single chirps alone. Compensated refocusing schemes like the one shown in Fig. 1B, where each ^{13}C pulse is replaced by pairs of chirped pulses with appropriate sweep directions as discussed in Ref. [24], could successfully fulfill all the necessary phase and frequency demands.

3. Experimental methods

NMR experiments were performed on a Varian VNMRs[®] 600 MHz NMR spectrometer using a triple-resonance proton-optimized (HCN) single-gradient probe. Unless specified otherwise the following parameters were used: two- or eight-cycled scans; $T = 298$ K; 2 s recycling delays; 3.6 and $15 \mu\text{s}$ 90° pulse lengths for ^1H and ^{13}C ; 2.2 ms, 35 G/cm gradients for the initial $2\text{H}_2\text{C}_z$ purge; 1.0 ms, 25 G/cm gradients for the subsequent BIRD filter; total acquisition times $at = 0.8$ s; $n = 25$ concatenated acquisition segments. For every sample three complementary 1D NMR data sets (single-pulse and variants in Fig. 1) were recorded; so were 2D-based acquisitions [17] incorporating a single BIRD decoupling pulse over t_1 . Chirped pulses were generated using Varian's Pbox software, with bandwidth of 50 kHz and duration of 1 ms. Final time-domain signals were constructed by direct concatenation of the piece-wise acquisitions, with no manipulation other than a standard processing with 32 K points zero-filling and 0.2 Hz apodization.

4. Results

We have already addressed the key provisions and parameters that we found had to be optimized, for operating the multi-pulse homonuclear decoupling sequences illustrated in Fig. 1. These included a need to filter out interferences arising from the natural-abundance ^1H - ^{12}C background, optimizing the $at/2n$ durations to achieve sufficiently small decoupling sidebands, implementing an efficient heteronuclear decoupling during the piece-wise acquisitions, and the need to use an average ^{13}C - ^1H J -evolution in each BIRD block to account for dispersions in these J values. It is enlightening to examine the kind of resolution and sensitivity that, under these guidelines, was obtained from the proposed sequence for a number of model compounds. Fig. 2 shows this for the case of Pyridine, a sample with well-resolved sites whose couplings are also well characterized. Owing to a homonuclear J -derived fine structure made by 1–2 Hz wide individual components, the three peaks at 8.48, 7.53 and 7.14 ppm in Pyridine's standard one-pulse ^1H spectrum span full half-height widths (FWHH) of 9.5, 19.5 and 15.5 Hz respectively. ^{13}C -satellites also reveal one-bond heteronuclear couplings ranging from 162 to 177 Hz. All these homonuclear J -multiplets collapse to single peaks of FWHH ~ 2 –4 Hz in the fully J -decoupled spectra, upon applying a train of BIRD pulses ca. 30.3 ms apart and under the assumption of an average $J_{\text{CH}} = 167$ Hz. As these BIRD-based decoupling schemes select signals solely from protons bound to ^{13}C , natural abundance considerations suggest that under ideal conditions these single peaks' integrated areas should reach ca. 1% of those observed in a standard one-pulse spectrum. These area expectations are well reproduced by the experimentally decoupled singlets – in fact in most instances the latter intensities were larger than the 1/50th non-decoupled expectations, even if the corresponding integrals were always under the 1% threshold for the fully decoupled peaks vis-à-vis the standard one-pulse areas (at $\approx 75\%$ values). For both decoupling sequences the spectral shift axis is also remarkably well-preserved, with no scaling of the frequency axis being required for obtaining a one-to-one correspondence of all peak positions – at least none measurable within our error and isotope effect uncertainties. At first glance this may look unexpected, as multi-pulse trains like those illustrated in Fig. 1 usually require some measure of post-processing before segments collected using separate explicit acquisition blocks can be subject to a common processing. In the present case, however, we find that the refocusing of all shift evolutions – at least in the weak coupling the protons experience *within* each BIRD element – is robust enough to

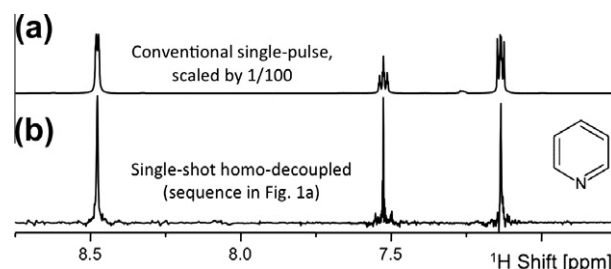


Fig. 2. Comparison of pyridine spectra (structure shown) acquired using: (a) a standard ^1H one-pulse sequence; (b) the one-shot hard-pulse BIRD decoupling sequence in Fig. 1a, with parameters as in the Experimental. To permit a direct comparison, both spectra were collected using identical spectrometer parameters (recycle delays, gain, pulse powers and durations, two cycled scans); the one-pulse proton spectrum (a) is shown with its vertical scale scaled down by 1/100 as noted in the text. For the hard-pulse experiment, BIRD delays used an average J_{CH} of 167 Hz, and the FID was constructed from concatenated 30.3 ms acquisition blocks with total acquisition time 0.66 s.

warrant a straightforward concatenation of all the digitized points into a common FID.

As mentioned, achieving broadband performance along both the ^1H and ^{13}C domains is an essential ingredient in the operation of these sequences. This is well illustrated by the 2-ethylindanone ^1H NMR spectra shown in Fig. 3. Like its counterpart in Fig. 2, indanone's standard one-pulse ^1H spectrum (Fig. 3a) is once again scaled by 1/100 as appropriate for a meaningful comparison. Also like the data in Fig. 2b, the data shown in panels 3b and 3c were obtained with a hard-pulse BIRD sequence. Unlike Pyridine, however, ethyl indanone's ^{13}C spectrum spans nearly 150 ppm, meaning that even under maximum ^{13}C rf irradiation powers the ^{13}C manipulations will be frequency selective. As seen in Fig. 3b and c, it is thus possible to obtain satisfactory results using separate applications of the hard-pulse BIRD version with the carbon transmitter positioned on either the aromatic or aliphatic regions of interest – but not on both simultaneously. By contrast, switching from the hard-pulse version in Fig. 1a to its frequency-swept counterpart in Fig. 1b, efficiently deals with this problem. The main difference among these sequences is the replacement of the hard 180° pulse manipulations by frequency-swept equivalents, capable of covering much wider bandwidths using identical rf peak powers. Direct substitution of the hard 180° s by considerably longer swept pulses would introduce substantial periods of undesired free J_{CH} evolution. As shown by Kupc̄e and Freeman [24], this can be avoided by replacing instead each hard pulse by paired sweeps performed in opposing directions, to refocus this J evolution. The ensuing results are quite satisfactory from the chemical shift bandwidth perspective, even if it is worth noting that for the individual regions, the optimized on-resonance, hard-pulse sequences lead to slightly sharper lines than their frequency-swept broadband counterpart.

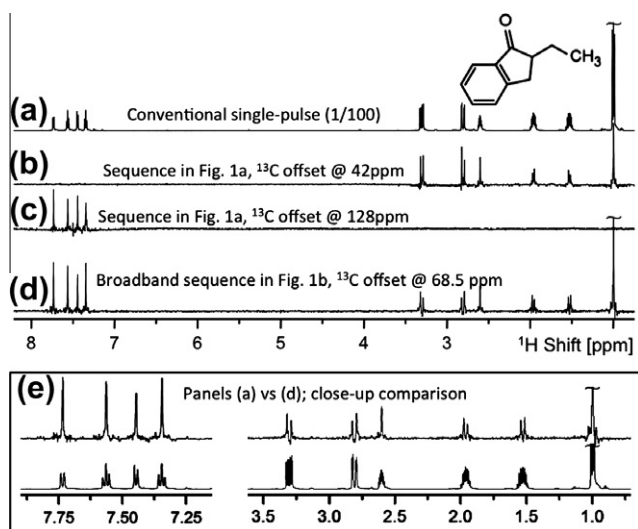


Fig. 3. Comparison of 2-ethylindanone's conventional ^1H NMR spectrum (a, vertically scaled by 1/100), against ^1H 1D spectra acquired using one-shot BIRD decoupling sequences with the carbon transmitter offset placed at the center of indanone's aromatic and aliphatic regions (128 in (b) and 42 ppm in (c)). (d) Spectrum acquired using the broadband one-shot BIRD decoupling sequence in Fig. 1b, with carbon irradiation at 68.5 ppm. BIRD J_{CH} -dependent Δ delays were set to 3.6 and 3.2 ms for the hard-pulse and chirped-RF experiments respectively, and the FIDs were constructed from 30.2 ms and 32.1 ms acquisition blocks. (e) Close-ups of the aromatic (left) and aliphatic (right) regions of 2-ethyl indanone spectra in panels (a) and (d), with the upper panel showing the spectrum acquired using the broadband sequence and the lower panel showing the corresponding conventional spectrum. All remaining parameters not specific to the BIRD sequences, including processing ones, are as described in the Experimental.

Fig. 4 illustrates another bandwidth consideration related to these single-scan homonuclear decoupling experiments, this time with a series of spectra collected for uridine in water. Once again, we found for this molecule that ^{13}C bandwidth limitations constrain the homonuclear decoupling performance if the latter is implemented with the hard ^{13}C pulse sequence introduced in Fig. 1a: even at maximum ^{13}C transmitting powers, sharp peaks can then solely be obtained for either the sugar or the base ^1H s, but not for both (data not shown). Fig. 4 highlights the importance of implementing a highly efficient heteronuclear decoupling over the course of the ^1H acquisition windows, if attempting to reach maximum resolution in this kind of homonuclear decoupling experiments. Indeed panels 4a and 4b illustrate the good homonuclear decoupling that can be obtained if the chirped RF version in Fig. 1b is executed with a large ^{13}C -decoupling bandwidth – at least in excess of 20 kHz. The same sequence but implemented with a narrower GARP decoupling bandwidth of 9.5 kHz (Fig. 4c), gives a clearly poorer performance. An added benefit of all these ^{13}C -based homonuclear decoupling schemes is also illustrated by the spectra in Fig. 4, which shows the efficiency of the solvent suppressing qualities of the BIRD-based procedure for this 90% $\text{H}_2\text{O}/10\%$ D_2O solution.

The ≈ 3.7 ppm resonance of uridine arising from this molecule's H5'/H5'' sugar sites (close-up in Fig. 4d) highlights an interesting limitation of the BIRD approach: although this method will efficiently decouple protons among themselves by relying on the dilution introduced by the ^{13}C natural abundance, the approach will fail if the homonuclear effects one is trying to eliminate involve a ^1H pair that is sure to be bound to a ^{13}C . Such will be the case for diastereotopic protons bonded to the same carbon atom and exhibiting geminal J_{HH} couplings; this effect is fairly common in sugar rings, and explains the splitting in uridine's H5'/H5''

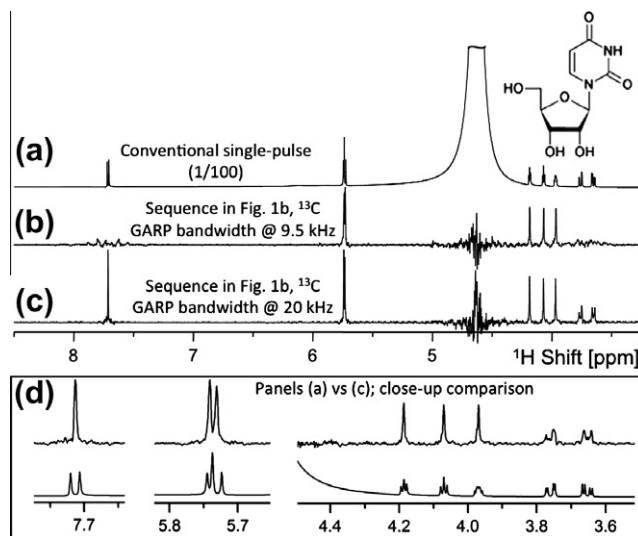


Fig. 4. Comparison of uridine spectra acquired using: (a) a conventional one-pulse ^1H acquisition performed without water suppression vs (b and c) one-shot BIRD-based acquisitions executed with the broadband (chirp-based) homonuclear decoupling sequence introduced in Fig. 1b with carbon irradiation centered at 121.5 ppm. (b) and (c) illustrate the key role of heteronuclear decoupling during acquisitions of these BIRD-based schemes: with the latter showing results obtained using 20 kHz GARP decoupling bandwidth over the ^1H acquisitions, and the former showing results upon holding all other parameters constant but using 9.5 kHz GARP instead. In all cases the sample was 0.5 M uridine in 90% $\text{H}_2\text{O}/10\%$ D_2O , BIRD J_{CH} delays were 2.8 ms, and 32.8 ms acquisition blocks were used to construct the FIDs as described in the Experimental. No explicit water suppression was used in any of the experiments. (d) Close-ups of the aromatic (H6) (left), anomeric and H5 (middle), and aliphatic (H2', 3', 4' and 5'/5'') (from left to right) (right) regions of the spectra shown in panels (a) and (b).

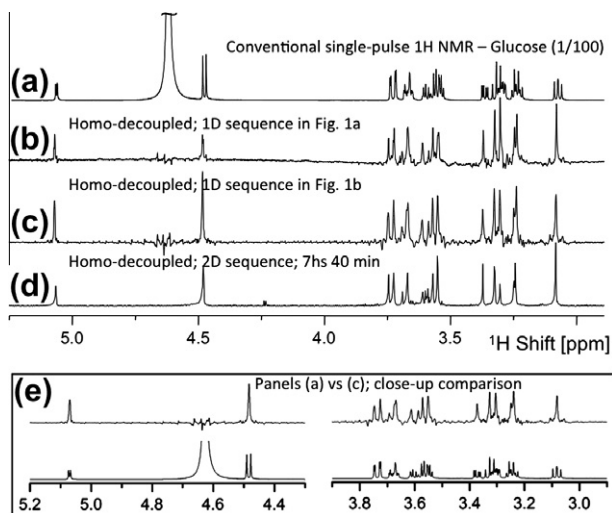


Fig. 5. Glucose spectra acquired in D_2O using: (a) a conventional one-pulse 1H acquisition performed without water suppression; (b) a 1D BIRD-based homo-decoupled acquisition executed with the hard-pulse sequence in Fig. 1a; (c) the broadband (chirp-based) homonuclear decoupling sequence introduced in Fig. 1b; (d) a 7.66-h two-dimensional acquisition incorporating BIRD-based decoupling in t_1 . The J_{CH} delay was set at 3.3 ms for all BIRD-derived experiments; FIDs in (b) and (c) were constructed from 30.3 ms and 30.8 ms acquisition blocks for the hard-pulse and chirped single-scan experiments, and processed as described in the Experimental. (e) Close-ups of the different regions displayed in panels (a) and (c).

(3.65/3.75 ppm) resonance. Fig. 5 illustrates the spectral complexity to which this kind of effect can lead, for a crowded 1H spectrum like that of glucose. As can be seen by comparing panels 5a and 5b, only a mild simplification of the conventional 1H NMR spectrum is introduced by the execution of the single-shot BIRD scheme. This time it is not ^{13}C offsets that can be blamed for the limited success, as for Glucose only a slight improvement in the BIRD's homonuclear decoupling performance is achieved upon assaying its ^{13}C -chirped pulse mode (Fig. 5b and c). That this complexity in the spectral pattern is not the result of limitations of the 1D BIRD-train approach, can be concluded by comparing these results with those arising from the more robust 2D version of the experiments incorporating a single BIRD refocusing element mid-way along t_1 (Fig. 5d). Despite the higher sensitivity afforded by this latter pulse sequence – and of the nearly four orders-of-magnitude longer acquisition times that this 2D acquisition requires over its 1D counterparts introduced here – the spectral complexity remains unchanged. In a case like Glucose the BIRD-derived patterns retain a fair deal of complexity that exceeds the one site/one peak expectation – even if considering the existence of anomeric pairs. We ascribe this to a combination of the multiplicities introduced by homonuclear geminal splittings, compounded by higher-order interference effects that set on in crowded spectral regions like this one.

5. Discussion and conclusions

The present study revisited the possibility of achieving total hetero- and homo-nuclear decoupling, based on the dilution and distinction introduced in naturally-occurring molecules by the ^{13}C isotope. Numerous studies had demonstrated that by combining this dilution with the selectivity introduced by BIRD pulses 2D sequences could be devised, whereby the proton indirect-domain evolution appeared effectively decoupled. In the present study, it was shown that this concept could be extended to single-shot acquisitions, thereby avoiding the costs of monitoring a high-resolution evolution along an NMR indirect domain. As described

herein, the experiments introduced a periodic toggling in the sign of the homonuclear J_{HH} coupling, in a fashion that leads to a full refocusing of all homonuclear effects at echo times $at/2n$. Moreover, it was found that if n was chosen such that these echo times were ca. 30 ms long, only very weak “decoupling sidebands” due to modulations of the relatively small homonuclear couplings resulted, and no compromises in resolution were incurred. Beyond the removal of vicinal homonuclear couplings, particularly attractive features of the ensuing approach included (i) a considerable reduction in the acquisition time needed to collect a homonuclear decoupled spectrum, (ii) a very high experimental robustness, (iii) a faithfulness in the position of the resulting 1H peaks, with no chemical shift scaling parameters, phasing difficulties, or other kinds of experimentally-derived “tricks” required, and (iv) a very efficient built-in water suppression. In order to reach this robustness of operation a number of additional precautions had to be taken; including ensuring a good quality of ^{13}C decoupling over the acquisition segments, introducing an efficient suppression of the natural-abundance 1H - ^{12}C background by relying on an INEPT-type filtration (aided as needed by homospoil gradients and/or phase cycling of the heteronuclear pulses), a judicious choice of BIRD delay that matched average one-bond (J_{CH}) values, and employment of broadband ^{13}C manipulations when the span of heteronuclear offsets could not be addressed using hard pulses.

A key feature of this form of decoupling is, naturally, to have a sparse ^{13}C enrichment. This in turn brings about a number of limitations, including sensitivity penalties for natural abundance samples and performance limitations in applying these sequences to highly ^{13}C -enriched samples. The former, however, are likely to be aided by the 1D nature of the method here proposed; moreover, further researches are in progress to exploit the unused 1H - ^{12}C polarization in order to at least partly offset this penalty [25]. The latter kind of complication on the other hand is most likely to arise in biomolecular contexts that have purposely ^{13}C -enriched, and hence liable to require two-dimensional measurements where the present development is less important in any case. An additional penalty worth stressing, arises when attempting to use BIRD-based homodecoupling schemes for dealing with samples containing diastereomeric sites. In these cases two inequivalent, geminal protons need to be decoupled from one another; as these are both bonded to a common ^{13}C , they are not amenable to decoupling by this scheme. On the other hand, strategies capable of delivering spectra with solely one component out of AX-type doublets [26–28] could prove useful for simplifying this well-defined limitation. Another challenge remaining to be dealt with concerns sites exhibiting homonuclear interactions in the strong coupling regime, where a 180° pulse on a ^{12}C -bound neighbor will not suffice to achieve full homonuclear decoupling on its ^{13}C -bearing partner. Also interesting to consider are further uses of the BIRD pulses hereby discussed, as ingredients in new multi-pulse spin-echo experiments, capable of probing dynamic and multi-quantum relaxation effects. These, as well as additional uses of these spin-selective manipulations, are currently being explored.

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