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Simple Proton Spectra from Complex Spin Systems: Pure Shift NMR Spectroscopy Using BIRD**

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Resolution in proton NMR spectra can be greatly enhanced by collapsing all homonuclear coupling structure to give a "pure shift" spectrum. Where protons are strongly coupled, greatly improved results can be obtained by ^{13}C isotope filtration using a modified bilinear rotation decoupling[1] (BIRD) method. Here it is shown for the first time that a pure shift spectrum can be obtained even in the notoriously difficult case of the methylene envelope of a long alkyl chain.

Proton NMR spectroscopy is the single most important structural tool in chemistry; it is so familiar that its limitations are often taken for granted. Chief among these is the problem of resolution: the limited range of chemical shifts means that multiplets overlap in all but the simplest spectra, complicating analysis and obscuring information. There has been a recent revival of interest in pure shift NMR spectroscopy, in which broadband homonuclear decoupling is used to collapse multiplets to singlets, greatly improving spectral resolution.[1–10] The largest family of techniques derives from the experiment of Zangger and Sterk[2] (ZS), in which a 180° pulse that is selective both for chemical shift and for spatial position causes a subset of protons to evolve as though they lack homonuclear couplings. Such methods are effective and versatile, but suffer one severe disadvantage. The narrow range of proton chemical shifts, as well as placing a premium on resolution, increases the likelihood of strong coupling, which is responsible for many of the most intractable problems in spectral assignment. Unfortunately such effects can severely complicate or indeed vitiate ZS pure shift methods.

There is, however, a close analogy between ZS methods and the BIRD[1] method, which uses the dilute ^{13}C isotope to allow the subset of protons directly bonded to ^{13}C to be manipulated. Where a signal in the normal proton spectrum is strongly coupled, the ^{13}C satellites will generally be weakly coupled, so a pure shift experiment based on BIRD should circumvent the problem. Paradoxically, although BIRD was originally proposed as a pure shift method, it seems never to have been used as such, instead becoming a standard building block in heteronuclear multidimensional NMR spectroscopy; only very recently has it formed the basis of a fully decoupled pure shift HSQC experiment.[8]

Both ZS and BIRD pure shift methods rely on applying a selective perturbation to a subset of spins, to refocus the effects of homonuclear J modulation while retaining that of the chemical shift. In the ZS case the perturbation uses a selective pulse in the presence of a magnetic field gradient, so that the active spins are restricted to those in a thin slice close to resonance. In BIRD, the net effect of the four pulses is to apply a 180° rotation only to those protons bound directly to ^{13}C. In each case the price of simplification is a loss in sensitivity, in ZS because only a thin slice of sample is used for any given chemical shift, and in BIRD because of the low (1.1%) natural abundance of ^{13}C.

The main reason for the lack of interest shown in BIRD pure shift NMR hitherto is that the original experiment was extremely slow, typically requiring several thousand t_1 increments. The solution to this problem is implicit in the ZS experiment.[2] In the original BIRD experiment, a single data point is acquired for each value of t_1 and t_1 is incremented in small steps $1/sw$, where sw is the width of the spectrum required. In the ZS method, a chunk of data lasting $1/sw_1$ is acquired, where sw_1 is an integer submultiple of sw, and t_1 is incremented in larger steps $1/sw_1$, typically of several tens of ms. The result is a time saving of a factor sw/sw_1, typically 1–2 orders of magnitude. Because homonuclear couplings evolve much more slowly than chemical shifts, the data acquired in chunks should differ negligibly from those acquired individually if $sw_1 \gg J$. The improvement in speed reduces the minimum time required from hours to minutes.

Figure 1 shows a practical BIRD sequence using an improved version[3] of the ZS data chunking. The initial modulated echo uses difference spectroscopy to restrict observation to those protons directly coupled to ^{13}C. These then evolve for a time $t_1/2$ under both chemical shifts and

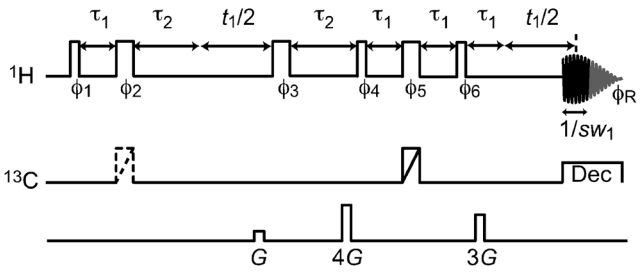

Figure 1. Pulse sequence for the measurement of pure shift ^1H spectra by the modified BIRD method, with $\tau_1 = 1/(2\,{}^1J_{CH})$ and $\tau_2 = 1/(2sw_1)$. Narrow RF pulses are 90° and wide 180°; BIP[11] composite 180° pulses are indicated by a diagonal line; the dotted line indicates pulse omitted on alternate transients. Phase cycling: $\phi_1 = 0$, $\phi_2 = [0\ 1\ 2\ 3]_8$, $\phi_3 = [0\ 1\ 2\ 3]_{32}$, $\phi_4 = \phi_5 = \phi_6 = [0\ 1\ 2\ 3]_2$, $\phi_R = [0\ 2] + 2(\phi_2 + \phi_3 - \phi_4) + \phi_1$.

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couplings (homo- and heteronuclear). The combined effect of the proton 180° pulse and the BIRD pulse sequence element ($90°_H$–τ–$180°_H$, $180°_C$–τ–$90°_H$) is then to apply a 180° rotation to the passive spins (those not coupled to ^{13}C) only, so that J evolution in the remaining delay $t_1/2$ reverses that experienced in the first $t_{1/2}$. The net result is to ensure that the heteronuclear coupling is refocused at the start of acquisition ($t_2 = 0$), the homonuclear couplings refocus $1/(2sw_1)$ later, and the chemical shift evolves for a net time t_1. Broadband ^{13}C decoupling is used during acquisition.

Figure 2 illustrates the application of pure shift methods to a mixture of aromatic species showing different degrees of strong coupling. The ZS method (Figure 2b) shows very

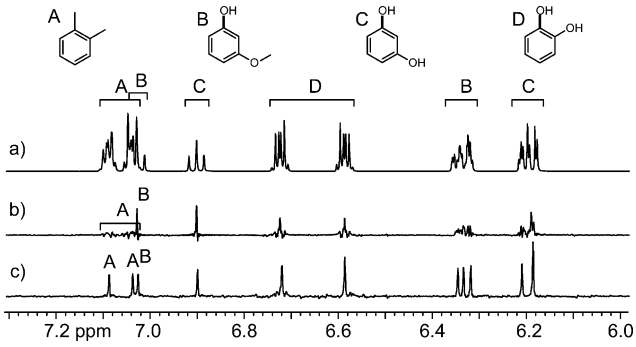

Figure 2. a) Normal ^1H spectrum, b) Zangger–Sterk pure shift spectrum measured in 1.25 min using the pulse sequence of reference [9], and c) BIRD pure shift spectrum measured in 1.5 min using the sequence of Figure 1, for a solution of *o*-xylene (A, 243 mM), *m*-methoxyphenol (B, 181 mM), resorcinol (C, 191 mM), and catechol (D, 175 mM) in [D_6]DMSO.

mixed results, with two triplets successfully decoupled, the mildly strongly coupled AA′BB′ system of catechol giving some decoupling but strong residual sidebands, and all the remaining signals being severely distorted. The BIRD spectrum (Figure 2c) in contrast gives essentially perfect decoupling, with a single peak for each distinct chemical shift.

One of the commonest and most problematic instances of strong coupling is that of the inner methylene resonances of an extended alkyl chain. Here, the very narrow shift range means that the spin states of protons are inextricably mixed throughout the inner chain, even at the highest magnetic fields available. It is therefore not generally possible to distinguish individual protons without either perturbing the chemical shifts (e.g. with a lanthanide shift reagent) or using indirect methods (e.g. HMQC). Using BIRD, however, the degeneracies are lifted for the individual methylene groups and distinct singlet signals can be seen. Figure 3c illustrates the method for a solution of *n*-hexanol, showing one singlet for each chemical shift and revealing the pattern of shifts that underlies the methylene envelope in the conventional spectrum of Figure 3a. Once again the ZS method (Figure 3b) works well for the comparatively weakly coupled multiplets, but it fails for the inner methylenes.

Pure shift NMR spectroscopy has a wide range of potential uses. The simplification it offers can greatly facilitate spectral analysis, especially in the current case where the effects of strong coupling are circumvented. The

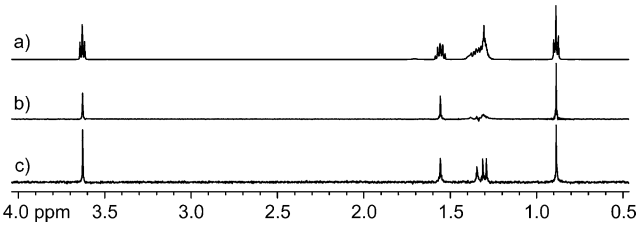

Figure 3. a) Normal ^1H spectrum, b) Zangger–Sterk pure shift spectrum measured using the pulse sequence of reference [9] in 1.25 min, and c) BIRD pure shift spectrum measured using the sequence of Figure 1 in 1.5 min, for a solution of *n*-hexanol (127 mM) in $CDCl_3$.

extra resolution is particularly useful[3,9] in diffusion-ordered spectroscopy (DOSY), where signal overlap is a critical limiting factor. Collapsing multiplet structure pays extra dividends in multidimensional NMR spectroscopy;[10] it should be straightforward to adapt existing ZS-based experiments to use BIRD.

Experimental Section

Experiments were run at room temperature on a Varian VNMRS 500 spectrometer with a triple resonance gradient probe of maximum gradient 66 G cm^{-1}. Spectrum 2b used 32 increments of 2 transients of 68 complex points each with $sw1 = 46.95$ Hz, $sw = 1502.4$ Hz and a reburp selective 180° pulse of duration 48.8 ms and bandwidth 100 Hz under a slice-select gradient G_{sl} of 0.5 G cm^{-1}. Coherence transfer selection gradient pulses were rectangular with width δ 0.5 ms and unit amplitude 15 G cm^{-1}. Spectrum 3b used the same parameters except for sw (2500 Hz), $sw1$ (52.08 Hz), δ (1 ms) and the number of complex points (256). Spectra 2c and 3c were acquired using similar parameters to spectra 2b and 2c, respectively; the $^1J_{CH}$ values used were 160 and 135 Hz, respectively.

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[1] J. R. Garbow, D. P. Weitekamp, A. Pines, *Chem. Phys. Lett.* **1982**, *93*, 504 – 509.
[2] K. Zangger, H. Sterk, *J. Magn. Reson.* **1997**, *124*, 486 – 489.
[3] M. Nilsson, G. A. Morris, *Chem. Commun.* **2007**, 933 – 935.
[4] S. Simova, H. Sengstschmid, R. Freeman, *J. Magn. Reson.* **1997**, *124*, 104 – 121.
[5] A. J. Pell, R. A. Edden, J. Keeler, *Magn. Reson. Chem.* **2007**, *45*, 296 – 316.
[6] A. J. Pell, J. Keeler, *J. Magn. Reson.* **2007**, *189*, 293 – 299.
[7] N. Giraud, M. Joos, J. Courtieu, D. Merlet, *Magn. Reson. Chem.* **2009**, *47*, 300 – 306.
[8] P. Sakhaii, B. Haase, W. Bermel, *J. Magn. Reson.* **2009**, *199*, 192 – 198.
[9] J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, *Angew. Chem.* **2010**, *122*, 3993 – 3995; *Angew. Chem. Int. Ed.* **2010**, *49*, 3901 – 3903.
[10] G. A. Morris, J. A. Aguilar, R. Evans, S. Haiber, M. Nilsson, *J. Am. Chem. Soc.* **2010**, *132*, 12770 – 12772.
[11] M. A. Smith, H. Hu, A. J. Shaka, *J. Magn. Reson.* **2001**, *151*, 269 – 283.

