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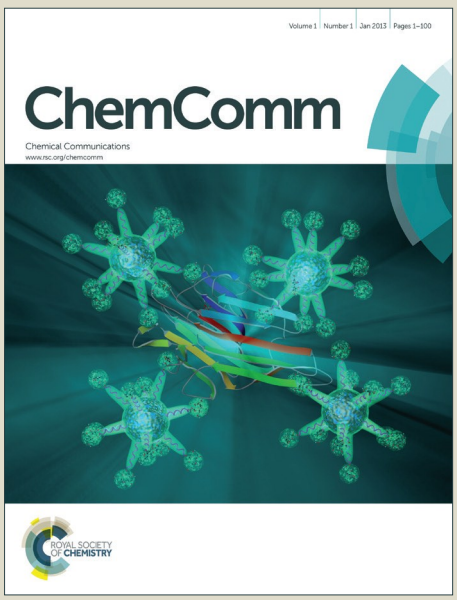

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Very Broadband Diffusion-Ordered NMR Spectroscopy: ^{19}F DOSY

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A new pulse sequence, CHORUS Oneshot, allows measurements of diffusion-ordered spectroscopy (DOSY) spectra over the full chemical shift range of ^{19}F for the first time. Swept-frequency pulses are used to give very broadband excitation; the sequence is a prototype for a large family of very broadband liquid state NMR methods.

DOSY is a powerful analytical tool, dispersing the NMR signals of a mixture according to the diffusion coefficients of the individual species involved.[1-4] Most DOSY spectra to date have been of ^{1}H, but the method is most powerful for nuclei with wide chemical shift ranges such as ^{19}F, for which signal overlap is rare. ^{19}F DOSY is of particular interest because of the increasing use of fluorine in pharmaceuticals: a quarter of drugs currently on the market contain fluorine.[5] Unfortunately, off-resonance effects mean that only a small fraction of the ^{19}F chemical shift range can be excited at any one time using existing DOSY experiments; even experiments on narrow chemical shift ranges show significant anomalies.[6] Similar problems affect all multiple pulse methods, and will increase, and extend to more nuclei, as the availability of very high magnetic field spectrometers improves. Here it is shown that the frequency range of DOSY can be extended by almost an order of magnitude using swept-frequency "chirp" pulses[7-17] with standard hardware. The resultant CHORUS Oneshot pulse sequence allows the full ^{19}F shift range at 470 MHz to be covered in a single acquisition. The principles used are equally applicable to a wide range of other experiments, and could be used as the basis for very broadband NOESY, MQF COSY and other methods.

Fluorine-19 is particularly suitable for DOSY because of its wide shift range, high abundance and high magnetogyric ratio γ. The high abundance and γ give excellent signal-to-noise ratio, and the high γ allows efficient diffusion encoding, but above all the exquisite sensitivity of the ^{19}F chemical shift to environment makes overlap between signals far rarer than in ^{1}H NMR. Because of the difficulty of disentangling superimposed exponential decays, individual signals with similar diffusion coefficients can only be distinguished in DOSY if they are resolved in the NMR spectrum. Much effort has therefore gone into designing DOSY experiments that reduce signal overlap, e.g. pure shift[18,19] or multidimensional[20-22] methods, and into heteronuclear experiments that exploit the much better resolution of ^{13}C spectra.[23-25] Fluorine-19 DOSY avoids such complications; individual signals can be completely resolved even when their chemical environments differ only in features ten or more bonds away, as in the example shown below.

In principle the problem of off-resonance effects in DOSY pulse sequences could be solved by composite radiofrequency (RF) pulses, but at present the bandwidths available fall far short of those required.[26-32] "Chirp" pulses with a linear frequency sweep cause large frequency-dependent phase shifts but are very wideband, and are used here to extend the bandwidth of the widely-used Oneshot[33] pulse sequence.

The calculated and the experimental performance of the resultant CHORUS Oneshot sequence are illustrated in Fig. 1, using ^{1}H measurements on a doped water sample for speed, along with results for the parent sequence using conventional hard pulses. In contrast to Oneshot, which gives uniform excitation over less than 10 kHz bandwidth and negligible excitation more than 50 kHz from resonance, CHORUS Oneshot gives uniform excitation over more than 250 kHz. This is sufficient to cover more than 500 ppm for ^{19}F at 470 MHz, well beyond the width of most ^{19}F spectra.

A practical illustration of the use of CHORUS Oneshot for ^{19}F DOSY is shown in Fig. 2, which compares the ^{1}H (a) and ^{19}F (b) spectra of a solution containing the pharmaceuticals **1** to **4** shown in Scheme 1 and the reference materials C_6F_6 (**5**, a common chemical shift reference) and SF_6 (**6**, a good choice for reference deconvolution[34]). Most of the ^{1}H signals in (a) are

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Electronic Supplementary Information (ESI) available: full experimental details; further experimental results; pulse sequence code. See DOI: 10.1039/x0xx00000x

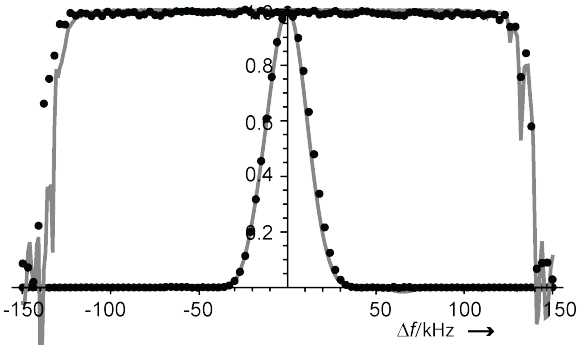
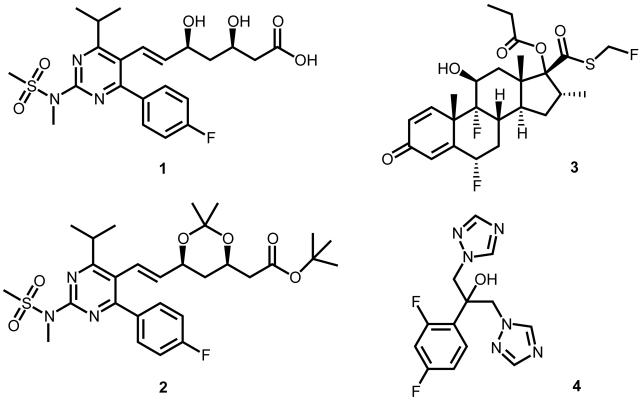


Fig. 1. Experimental (dots) and calculated (solid lines) ^{1}H excitation profiles for CHORUS Oneshot (upper trace) and conventional Oneshot (lower) for a sample of doped water (0.1 mg/mL $GdCl_3.6H_2O$ in 99.8% D_2O).

overlapped and appear at compromise diffusion coefficients (an effect particularly marked for signals overlapping with the water signal around 3.4 ppm), as the diffusion spectrum at the right shows. In contrast the ^{19}F spectrum (b) is completely resolved in both dimensions, cleanly separating the signals of the six solutes. The ^{19}F signals in (b) span 250 ppm (118 kHz at 470 MHz), making it impossible to excite the full spectrum with conventional Oneshot, as shown by Fig. S5 in the ESI. In contrast, CHORUS Oneshot gives full excitation of the complete spectrum allowing the diffusion coefficients of all the mixture ingredients to be distinguished.

The very high resolving power of ^{19}F NMR is illustrated by the separation of the parafluorophenyl signals of rosuvastatin and BEM (see inset), which show a 0.05 ppm difference in chemical shift despite the remoteness of the fluorine atom from the structurally different parts of these two species. Further expansion of (b) reveals three mM level impurities (see ESI, Fig. S6).

It is rare for homonuclear ^{19}F-^{19}F couplings to be seen in compounds of pharmaceutical interest, because of the sparsity of fluorine sites, but multiplet structure can be extensive in perfluorinated species. The relatively long durations of the chirp pulses in the CHORUS Oneshot sequence mean that large couplings can undergo significant evolution over the course of the sequence, leading to phase modulation within multiplets that can cause problems where multiplets are close in frequency. In such systems, modifications to the sequence to reduce its duration, and the use of a hard 45° pulse centred exactly at the at the echo maximum,[35] offer potential ways to minimize the effects of J modulation. It is also possible to reduce the durations of CHORUS sequences by adjusting the relative amplitudes and durations of the chirp pulses.



Scheme 1. Rosuvastatin (1), its precursor BEM (2), fluticasone propionate (3) and fluconazole (4).

The task of adapting the Oneshot pulse sequence for very wide spectra can be split into two: generating spatially-encoded *z*-magnetization, and converting that *z*-magnetization into refocused transverse magnetization. Perhaps surprisingly, it is the former that is easier. Replacing the two 90° pulses by counter-sweeping 90° chirp pulses, which cause complementary phase shifts, and the 180° pulse by a triple 180° chirp sandwich[7] allows uniform, high-bandwidth spatial encoding (see ESI, Figs. S2 and S3).

The problem of converting the encoded *z*-magnetization into observable signal is more complicated, because the simple combination of a chirp 90° and a chirp 180° pulse produces a

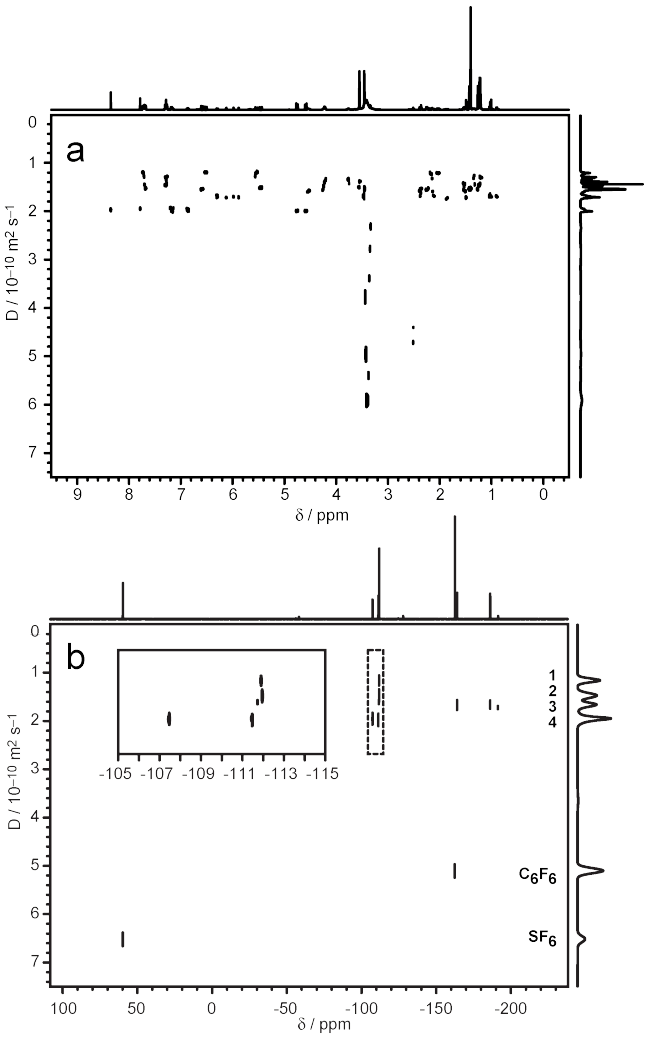


Fig. 2 (a) 500 MHz ^{1}H Oneshot and (b) 470 MHz ^{1}H decoupled CHORUS Oneshot ^{19}F DOSY spectrum of a solution of (**1**) to (**4**) in DMSO-d_6 with SF_6 and C_6F_6. Sample details are given in the Electronic Supplementary Information. Traces at top and side, show first spectrum and projection onto the diffusion axis respectively. The inset in (b) shows an expansion of the area around −111 ppm with signals from **1**, **2** and **4**.

very strong B_1-dependent phase shift that can lead to significant signal loss, and only partially refocuses the frequency dependence of the phase of excitation. The solution to this problem was described some time ago in a different context, that of pure phase band-selective excitation. In the ABSTRUSE pulse sequence,[16] adding a third pulse, of 180° flip angle, to a 90° – 180° hyperbolic secant ("HS", "sech/tanh") pulse pair refocuses the B_1-dependent phase shift. If the three hyperbolic secant pulses of this double echo sequence are replaced by chirp pulses the bandwidth increases almost fivefold, but the relatively small frequency-dependent phase shifts generated by ABSTRUSE become much larger and have to be corrected. Fortunately, this is relatively straightforward: because each time point within a chirp pulses corresponds to a particular frequency, the frequency-dependent phase shift required to correct the calculated phase dependence of the triple echo sequence can be implemented very simply by adding the corresponding time-dependence of phase to one or more of the chirp pulse shapes. The net result is the recently developed CHORUS sequence,[17] which gives efficient, broadband conversion of longitudinal into transverse magnetization of constant phase. Combining this with the initial spatial encoding gives the CHORUS Oneshot pulse sequence of Fig. 3, which incorporates diffusion-encoding and -decoding field gradient pulses into the two halves of the sequence.

Off-resonance effects pose very significant challenges in ^{19}F NMR - and that of many other nuclei - often preventing excitation of more than a small part of the chemical shift range where multiple pulse sequences are needed. Here it is shown that one such pulse sequence, for diffusion-ordered spectroscopy, can be adapted by the use of swept-frequency pulses to allow excitation over very high bandwidths, sufficient for almost all practical spectra at the magnetic fields currently available. The building blocks of the new sequence can be adapted for a wide range of other uses, including NOESY, MQF COSY, INADEQUATE and INEPT. The approach used is equally applicable to a range of other nuclei, including those with the difficult combination of wide chemical shift range and low γ.

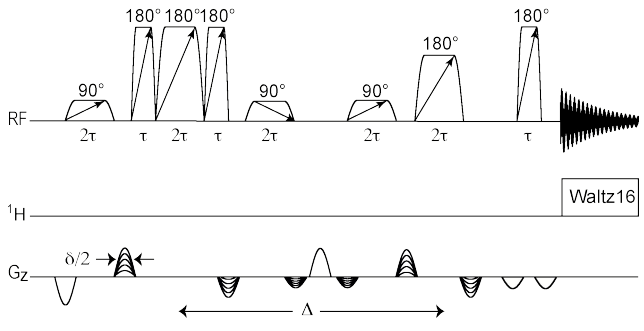


Fig. 3. Pulse sequence for CHORUS (CHirped, ORdered pulses for Ultra-broadband Spectroscopy) Oneshot. In the first half of the sequence pulse amplitudes for the 90° and 180° chirp pulses are in the ratio 0.21 : 1, and in the second half (the CHORUS element) 0.21 : 0.71 : 1.

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Notes and references

1 C.S. Johnson, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1999, **34**, 203-256.
2 B. Antalek, *Concepts Magn. Reson.*, 2002, **14**, 225-258.
3 G.A. Morris, in *Encyclopedia of Nuclear Magnetic Resonance*, *eMagRes*, ed. D. M. Grant and R. K. Harris, John Wiley & Sons Ltd, Chichester, 2009. DOI: 10.1002/9780470034590.emrstm0119.pub2.
4 H. Barjat, G.A. Morris, S. Smart, A.G. Swanson, S.C.R. Williams, *J. Magn. Reson.*, Series B, 1995, **108**, 170-172.
5 J. Wang, M. Sánchez-Roselló, J.L. Aceña, C. del Pozo, A.E. Sorochinsky, S. Fustero, V.A. Soloshonok, H. Liu, *Chem. Rev.*, 2014, **114**, 2432-2506.
6 G. Dal Poggetto, D.C. Favaro, M. Nilsson, G.A. Morris, C.F. Tormena, *Magn. Reson. Chem.*, 2014, **52**, 172-177.
7 T. L. Hwang, P. C. M. van Zijl and M. Garwood, *J. Magn. Reson.*, 1997, **124**, 250-254.
8 A. Tannús and M. Garwood, *NMR in Biomed.*, 1997, **10**, 423-434.
9 Ē. Kupče, R. Freeman, *J. Magn. Reson., Series A*, 1995, **117**, 246-256.
10 G. Garwood, L. DelaBarre, *J. Magn. Reson.*, 2001, **153**, 155-177.
11 J.M. Bőhlen, M. Rey, G. Bodenhausen, *J. Magn. Reson.*, 1989, **84**, 191-197.
12 J.M. Bőhlen, I. Burghardt, M. Rey and G. Bodenhausen, *J. Magn. Reson.*, 1990, **90**, 183-191.
13 J.M. Bőhlen, G. Bodenhausen, *J. Magn. Reson., Series A*, 1993, **102**, 293-301.
14 V.L. Ermakov, J.M. Bőhlen, G. Bodenhausen, *J. Magn. Reson., Series A*, 1993, **103**, 226-229.
15 V.L. Ermakov, G. Bodenhausen, *Chem. Phys. Lett.*, 1993, **204**, 375-380.
16 K.E. Cano, M.A. Smith, A.J. Shaka, *J. Magn. Reson.*, 2002, **155**, 131-139.
17 J.E. Power, M. Foroozandeh, R.W. Adams, M. Nilsson, S.R. Coombes, A.R. Phillips, G.A. Morris, *Chem. Commun.*, 2016, **52,** 2916 – 2919.
18 M. Nilsson, G.A. Morris, *Chem. Commun.*, 2007, 933-935.
19 J.A. Aguilar, S. Faulkner, M. Nilsson, G.A. Morris, *Angew Chem. Int. Ed.*, 2010, **49**, 3901-3903.
20 H. Barjat, G.A. Morris, A.G. Swanson, *J. Magn. Reson.*,1998, **131**, 131-138.
21 M. Nilsson, A. M. Gil, I. Delgadillo, G. A Morris, *Anal. Chem.*, 2004, **76**, 5418-5422.
22 J.M. Newman, A. Jerschow, *Anal. Chem.*, 2007, **79**, 2957-2960.
23 D. Wu, A. Chen, C.S. Johnson, *J. Magn. Reson., Series A*, 1996 **123**, 215-218.
24 D. Li, R. Hopson, W. Li, J. Liu., P.G. Willard, *Org. Lett.*, 2008, **10**, 909-911.
25 A. Botana, P.A. Howe, V. Caër, G.A. Morris, M. Nilsson, *J. Magn. Reson.*, 2011, **211**, 25-29.
26 R. Freeman, S.P. Kempsell, M.H. Levitt, *J. Magn. Reson.*, 1980, **38**, 453-479.
27 R. Tycko, H.M. Cho, E. Schneider, A. Pines, *J. Magn. Reson.*, 1985, **61**, 90-101.
28 E. Kupče, R. Freeman, *J. Magn. Reson., Series A*, 1994, **108**, 268-273.
29 T. E. Skinner, T. O. Reiss, B. Luy, N. Khaneja, S. J. Glaser, *J. Magn. Reson.*, 2004, **167**, 68-74.

30 T. E. Skinner, K. Kobzar, B. Luy, M. R. Bendall, W. Bermel, N. Khaneja, S. J. Glaser, *J. Magn. Reson.*, 2006, **179**, 241-249.
31 S.M. Odedra, M.J. Thrippleton, S. Wimperis, *J. Magn. Reson.*, 2012, **225**, 81-92.
32 K. Kobzar, S. Ehni, T. E. Skinner, S. J. Glaser, B. Luy, *J. Magn. Reson.*, 2012, **225**, 142-160.
33 M.D. Pelta, G.A. Morris, M.J. Stchedroff, S.J. Hammond, *Magn. Reson. Chem.*, 2002, **40**, S147-S152.
34 G.A. Morris, H. Barjat, T.J. Horne, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1997, **31**, 197-257.
35 A. Botana, J.A. Aguilar, M. Nilsson, G.A. Morris, *J. Magn. Reson*., 2011, 208, 270-278.