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Enhanced ^{13}C resolution in semi-selective HMBC: a band-selective, constant-time HMBC for complex organic structure elucidation by NMR †

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Received 25th June 2003, Accepted 15th July 2003
First published as an Advance Article on the web 24th July 2003

A semi-selective 2D HMBC experiment is described which yields high-resolution in the indirect carbon-13 dimension by suppressing homonuclear proton coupling modulations and so provides an NMR technique suitable for the structure elucidation of organic compounds which exhibit particularly crowded carbon-13 spectra.

The Heteronuclear Multiple-Bond Correlation (HMBC) experiment is one of the most powerful NMR techniques available to chemists for the structure elucidation of natural or synthetic products. It enables the detection of long-range ^{1}H–^{13}C correlations over, most frequently, 2- or 3-bonds and can thus provide a wealth of data on molecular connectivity. Over the years the original sequence[1] has been enhanced through a variety of modifications ranging from the incorporation of pulsed field gradients[2,3] to improvements for sampling over a wider range of heteronuclear coupling constants[4–7] and more recently to techniques aimed at differentiating $^{2}J_{CH}$ and $^{3}J_{CH}$ correlations,[8,9] an otherwise troublesome feature of interpreting HMBC data.

One further limitation of HMBC when studying complex structures or those with especially crowded spectra is the difficulty in resolving correlations to closely neighbouring ^{13}C resonances, resulting in ambiguity in structure elucidation. The root of the problem lies in the potentially large ^{13}C shift range, possibly in excess of 200 ppm, that must be sampled indirectly to provide the ^{13}C (F_1) frequency dimension of the final 2D spectrum. Since a larger shift range demands more t_1-increments for characterisation and thus greater experiment time, many routine HMBC spectra are collected with relatively low F_1 digital resolution (typically only 50–100 Hz per pt) to keep experiment times acceptably short. In crowded regions of ^{13}C spectra, such conditions may be insufficient to resolve correlations to neighbouring carbons. When F_1-resolution is limiting in this way it is more time efficient to excite selectively only the crowded ^{13}C region and sample this at higher resolution than it is to collect the whole ^{13}C range with more increments, that is, with finer digitisation. Thus, so-called "semi-selective" or "band-selective" HMBC experiments have been proposed[10–14] which incorporate selective pulses on the ^{13}C channel to excite only a subset of all carbon centres and so focus the 2D correlation spectrum on these alone.

However, when recorded at high digital resolution a feature of the HMBC experiment that is usually unresolved in routine low-resolution spectra is revealed and this can itself limit the applicability of the high-resolution data set by contributing to peak overlap. This is the appearance of homonuclear proton *J*-couplings *in the indirect ^{13}C dimension*, as will be illustrated below. In this communication we demonstrate that the incorporation of a "constant-time" approach in the band-selective HMBC can further enhance F_1 resolution by suppressing these unwelcome proton *J*-coupling modulations, thus providing a useful NMR technique for the investigation of complex structures and/or crowded spectra.

The benefits and limitations of the band-selective HMBC techniques will be illustrated by reference to the assignment of the hexameric oligomer **1** (Fig. 1a). A range of such amide-linked carbohydrates has been developed by the Fleet group[15–18] to investigate their potential as templates for secondary structure generation, many of which have been subject to extensive NMR analysis. A key part of these NMR studies is the sequential assignment of the sugar ring protons. Assignments within each sugar residue are typically derived from COSY and TOCSY spectra whilst the sequential placement of each ring may be made through the observation of NOEs between adjacent sugars or, with potentially less ambiguity, through the observation of long-range through-bond heteronuclear correlations from protons on adjacent sugars to the intervening carbonyl carbon (Fig. 1b).

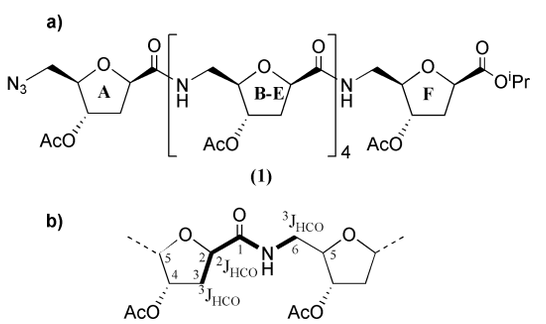

Fig. 1 a) The carbohydrate amino acid oligomer **1** and b) the key long-range heteronuclear couplings to the intervening carbonyl used to link neighbouring sugar residues and hence sequentially assign the oligomer.

Fig. 2a depicts part of the conventional, low-resolution gradient-selected HMBC spectrum collected over a 180 ppm carbon window. This region shows correlations to the amide carbonyls from which it is impossible to resolve correlations to all five intervening groups which fall within a narrow 2.5 ppm window. Fig. 2b again shows the carbonyl region but now of the band-selective HMBC collected over a 6 ppm window only, with selective carbon excitation centred at 172 ppm. Whilst the greater peak resolution is apparent due to the 15-fold improvement in F_1 digital resolution (see Experimental) the strong F_1-"spread" arising from the now resolved ^{1}H–^{1}H *J*-couplings is also revealed. ‡ The undesirable skew is especially pronounced for those correlations from protons with complex multiplet structures, such as those of the geminal pairs H6/6′ and H3/3′ in **1**, and leads to an effective broadening of each cross-peak in the ^{13}C dimension which may still contribute to peak overlap in heavily crowded spectra. Fig. 2c illustrates the effect of

† This is one of a number of contributions from the current members of the Dyson Perrins Laboratory to mark the end of almost 90 years of organic chemistry research in that building, as all its current academic staff move across South Parks Road to a new purpose-built laboratory.

DOI: 10.1039/b307122g

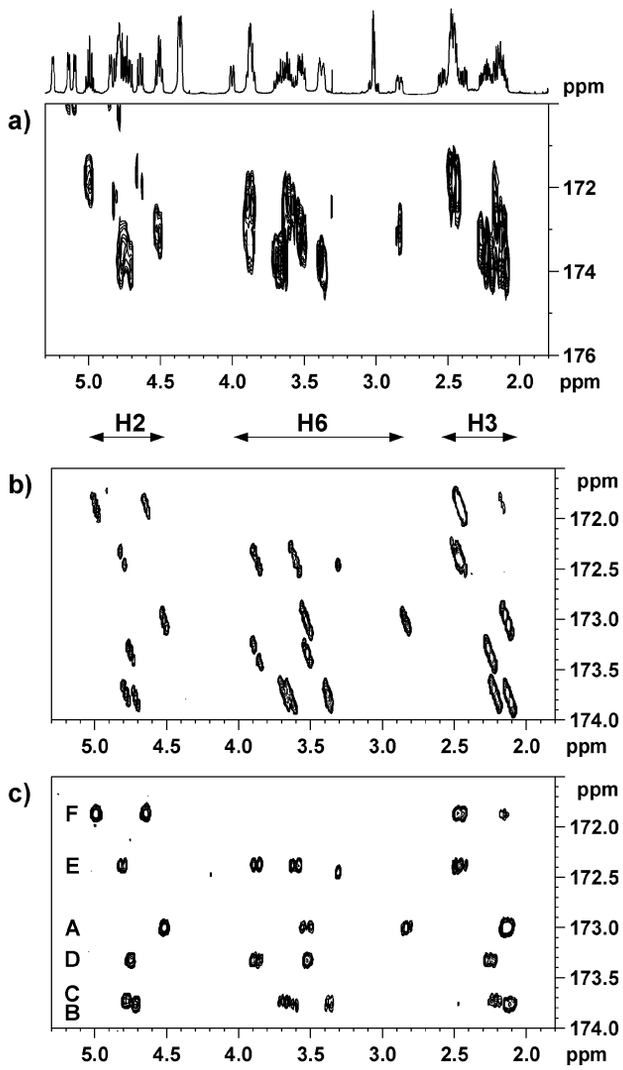

Fig. 2 a) The region from the conventional non-selective HMBC spectrum of **1** acquired with a 180 ppm ^{13}C window showing only correlations to the amide carbonyls, b) part of the band-selective HMBC acquired with a 6 ppm ^{13}C window centred at 172 ppm, and c) the band-selective, constant-time HMBC acquired as for b). The labelling in c) indicates the carbonyl carbon to which the long-range correlations are observed. For all spectra the weak $^{2}J_{CH}$ correlations from the amide protons are not shown.

incorporating the constant-time approach in the band-selective experiment, as described below. These data were collected with identical digital resolution as Fig. 2b and clearly illustrate the improved peak dispersion arising from the complete suppression of ^{1}H *J*-modulations. It is thus possible to differentiate with confidence the correlations to carbonyls of rings B and C at 173.72 and 173.69 ppm respectively, a shift difference of a mere 0.03 ppm. Such resolution is comparable to that attainable in routine 1D ^{13}C NMR spectra and allows for the unambiguous sequential placement of all sugar proton assignments for **1**.

The band-selective, constant-time HMBC sequence is illustrated in Fig. 3. The sequence is derived from the band-selective HMBC experiment described by Nuzillard *et al.*[14] and again makes use of a single pulsed field gradient spin echo (SPFGSE) on the ^{13}C channel to achieve restricted excitation in the F_1 domain. In addition, the t_1 evolution and SPFGSE periods are bracketed by variable time delays, $(\Delta_2 - t_1/2)$, that are systematically decremented in accordance with the stepwise incrementation of t_1 so as to define a constant time period for the proton coupling evolution, Δ_{CT}. Thus, regardless of the value of t_1, the period $(\Delta_1 + \Delta_{CT})$ remains constant and no net modulation of the data by J_{HH} coupling evolution takes place and hence homonuclear proton coupling fine structure does not appear in F_1.

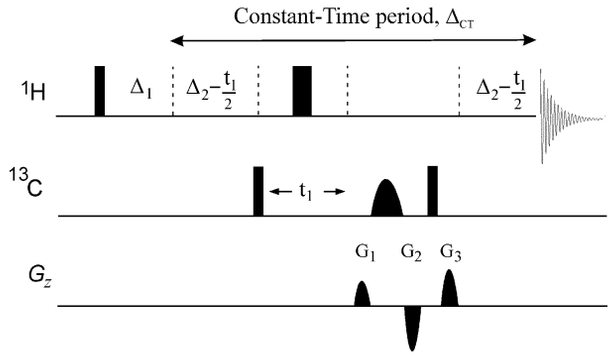

Fig. 3 The band-selective, constant-time HMBC sequence. Narrow solid bars indicate 90° pulses and the wide bar indicates a 180° pulse at the midpoint of t_1. The selective 180° pulse on the ^{13}C channel is shown as a half-sine shape. All pulse phases are *x*. The delay Δ_1 is for the evolution of $^{n}J_{CH}$ and Δ_2 is equal to $t_{1(max)}/2$. As t_1 is incremented the periods $(\Delta_2 - t_1/2)$ are decremented such that the period Δ_{CT} remains constant. Gradient pulses are applied in the ratio 3 : −5 : 4.01 for ^{13}C.

This constant-time approach parallels that suggested by Furihata and Seto for the conventional HMBC[19] although the benefits provided are considerably more pronounced when employed in a high-resolution, band-selective context than with the conventional non-selective HMBC.

As also described by Furihata and Seto, the step-wise variation in the total long-range heteronuclear coupling evolution period prior to the generation of heteronuclear multiple-quantum coherence $((\Delta_1 + (\Delta_2 - t_1/2))$ provides for sampling of a wider range of $^{n}J_{CH}$ values. This so-called "accordion" sampling has been used more deliberately in a number of recent HMBC variants[4–7] to enhance the total number of correlations observed in the spectra. In the context described herein this could also cause some slight residual broadening of correlation peaks in the F_1 dimension due to scaling of the heteronuclear couplings. However, in our experience such peak broadening is markedly less than the F_1 multiplet spread caused by homonuclear proton couplings and has not been problematic even in high-resolution spectra. The fact that only moderate broadening is observed from the accordion sampling of heteronuclear couplings may be attributed to the often small size of the $^{n}J_{CH}$ couplings being sampled combined with the fact that each proton in a single molecule will couple to only a single carbon-13 nucleus (to the limit of ^{13}C natural abundance). In contrast, a proton may potentially couple to many neighbouring protons and hence the homonuclear couplings and associated wide multiplet structure in F_1 are a potentially greater problem, which the constant-time approach suppresses completely.

The potential disadvantage of the constant-time variant is the lower sensitivity of the technique. This is due to the longer duration of the sequence for all values of t_1, as defined by the fixed period $(\Delta_1 + \Delta_{CT})$, and hence relaxation losses prior to collection of each FID. These losses must therefore be balanced against the benefits of the improved peak dispersion when choosing between the band-selective and the constant-time, band selective variants. The spectra presented in Fig. 2 illustrate, however, that good quality, high-resolution data can be collected on relatively "large" organic molecules since **1** has a molecular mass in excess of 1200 Da.

In conclusion, we have described a variation of the semi-selective HMBC method able to provide very high resolution in the ^{13}C dimension. The technique may find application in the structure elucidation of complex organic systems and especially those with very crowded carbon-13 NMR spectra.

Experimental

A sample of **1** was prepared as a 23 mM solution in C_6D_6 in a 5 mm NMR tube. All data were collected on a Bruker DRX500

equipped with a $^{1}H\{^{13}C$, X-broadband$\}$ triple resonance probe with the sample temperature regulated at 298 K.

Spectrum 2a was collected with the standard, non-selective pulsed field gradient HMBC sequence over 9 ppm (^{1}H) × 180 ppm (^{13}C) as 2K × 256 data points, corresponding to a $t_{1(max)}$ of 11 ms. Data were zero-filled to 2K × 512 points providing a final digital resolution of 2 × 45 Hz per pt for ^{1}H and ^{13}C respectively. The Δ_1 $^{n}J_{CH}$ coupling evolution delay was set to 60 ms. Spectrum 2b was collected over 9 ppm (^{1}H) × 6 ppm (^{13}C; encompassing the full carbonyl region for **1** of 169–175 ppm) as 2K × 128 data points, corresponding to a $t_{1(max)}$ of 170 ms. Data were zero-filled to 2K × 256 points providing a final digital resolution of 2 × 3 Hz per pt for ^{1}H and ^{13}C respectively and again the Δ_1 delay was set to 60 ms. The ^{13}C digital resolution was thus improved 15-fold over that of spectrum 2a. Selective excitation was achieved with a ^{13}C 0.5 ms 180° Gaussian pulse. Spectrum 2c was collected and processed as for 2b except that the Δ_1 delay was set to a shorter value of 40 ms. This is beneficial to reduce the total duration of the sequence and thus to lessen relaxation losses. This does not lead to a substantial loss of correlations because the additional ($\Delta_2 - t_1/2$) delay effectively extends the total coupling evolution time in the constant-time version. Pulsed field gradients were applied as 1 ms half-sine shaped pulses. All spectra were processed with squared sine-bell apodisation in both dimensions and are presented in magnitude-mode. No further enhancement through linear prediction has been employed so that the effects of the sequence modifications remain clear.

Low-pass filtration of one-bond heteronuclear couplings has not been used in the semi-selective work presented here as this was not relevent. However, such filtration schemes have been incorporated into these sequences and shown to be effective with other molecules, as for the conventional HMBC sequence.

Acknowledgements

We thank Alison Edwards for providing a sample of oligomer (**1**) and I. P-V. would like to thank Puleva Biotech, S. A. for funding.

Notes and references

‡ This homonuclear proton coupling arises in the carbon-13 dimension due to the existence of ^{1}H–^{13}C multiple-quantum coherence during the t_1 evolution period.

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