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A band-selective composite gradient: Application to DQF-COSY

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Abstract

We describe a unique band-selective method that utilizes a selective composite gradient to simultaneously achieve band selection and coherence pathway selection. This element is similar to the composite gradient known as the CLUB sandwich except the original broadband pulses have been replaced with selective pulses and the strengths of the antipolar gradients have been unbalanced. In this way, only the signals within the inversion band will continue to dephase throughout the duration of the element and satisfy the proper encoding-to-decoding gradient ratio necessary for coherence selection. Apart from the inverted polarity and asymmetry of the gradients, the band-selective CLUB sandwich is identical to the DPFGSE sequence and provides many of its desirable characteristics. We have successfully incorporated the band-selective CLUB into the DQF-COSY pulse sequence to create a band-selective experiment that offers the selectivity desired for resolution enhancement while maintaining excellent phase behavior. This is demonstrated on the congested aliphatic region of the ionophorous antibiotic Lasalocid A.

Keywords: NMR; gDQF-COSY; BASE-gDQF-COSY; Composite gradient; CLUB sandwich; BASE-CLUB

1. Introduction

Band-selective 2D NMR experiments make it possible to reduce the spectral width in one or both dimensions without causing the unwanted peaks to appear aliased [1–3]. As a result, they can provide the resolution enhancement needed for certain tasks, such as the unambiguous assignment of crowded regions or the accurate determination of coupling constants. On the other hand, they can also be used to obtain the same resolution as a broadband experiment but in a fraction of the time. This utility, along with the availability of a number of excellent selective pulses, has led to their proliferation [1–16].

Band-selective pulse sequences are generally derived from their non-selective counterparts in one of two ways. In one approach, the rectangular 90° pulse prior to t_1 or t_2 (or both) is replaced with a selective 90° pulse so that excitation is limited to only a specific region [1–10]. This method requires additional phase cycling to efficiently suppress the unwanted signals and pure-phase pulses (i.e., self-refocusing pulses) to prevent phase errors from the divergence of the magnetization trajectories that occur throughout the duration of the pulse. The latter is critical if the resulting data are to be presented in phase-sensitive mode. In the other approach, the double pulse field gradient spin echo (DPFGSE) [17] is inserted next to the t_1 evolution period to rephase the transverse magnetization of only the desired signals. This method, also known as excitation sculpting, is particularly impressive because the resulting excitation profile depends only on the inversion profile of the chosen pulse, not on its phase properties, and is scaled only by the relaxation that occurs during the selective pulses. Furthermore, because it relies on gradients, no additional phase cycling is needed to eliminate the signals outside the desired band. Nevertheless, considerable

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phase errors can still occur in the final spectrum because the evolution of homonuclear scalar coupling during the DPFGSE is not refocused for spins within the inversion band of the selective pulse.

In this report, we describe a novel method for band selection that relies on a composite gradient to selectively encode coherences within the desired frequency window. The composite gradient, which is an adaptation of the CLUB sandwich (*c*omposite gradient *l*eaving an *u*ndisturbed B_0 field) [18], consists of two sets of antipolar gradient pairs encompassing selective inversion pulses. In this manner, only the spins inverted by the selective pulses will be completely dephased by the antipolar gradients and experience the proper encoding-to-decoding ratio required for detection. An ideal candidate for the application of this band-selective CLUB (BASE-CLUB) is the gDQF-COSY pulse sequence, as it relies on an encoding gradient located within the double-quantum filter to select the desired coherence pathway [19]. Key to the success of the proposed method in the DQF-COSY is the asymmetry of the antipolar gradients, which serves to eliminate ''pseudo'' double quantum coherence, leaving only ''true'' double quantum coherence from within the desired inversion band to survive the coherence selection. The superb phase properties of the BASE-CLUB combined with its strategic placement in the gDQF-COSY pulse sequence leads to spectra with excellent phase and lineshape.

2. Results and discussion

Fig. 1A shows the pulse sequence for a gDQF-COSY experiment. Coherence selection is accomplished with an encoding-to-decoding gradient ratio (G_1:G_2) of 1:2. This selects only the signals that exist as double quantum coherence (DQC) during the time between the last two 90° pulses, the so-called double quantum filter (DQF), while all other coherence pathways are destroyed. Chemical shift evolution that occurs during G_1, which would result in a loss of sensitivity, and G_2, which would lead to a large phase error, must be refocused with spin echoes. Alternatively, the spin echo for G_2 can be disregarded, as shown in Fig. 1A, and the phase error avoided by setting the sum of G_2 and the recovery delay to an integer multiple of the dwell time and right-shifting the FID post-acquisition by the integer. If desired, the ''missing data points'' in the initial part of the FID can be extrapolated by backward linear prediction. In practice, this is usually unnecessary due to the spin echo nature of the time-domain signal for the DQF-COSY experiment.

Fig. 1B shows the pulse sequence for the CLUB composite gradient. This element was originally designed as an ''ideal'' gradient for nearly instantaneous system recovery [18]. The layers in the CLUB sandwich were arranged in such a way that transverse magnetization continues to dephase throughout the element; thus, the total effective gradient area is the sum of all four gradients. Fig. 1C shows the pulse sequence for the BASE-CLUB, where the original broadband refocusing pulses have been replaced with selective pulses. In this manner, only the signals inverted by the selective pulse are dephased for the total gradient duration. Furthermore, for reasons to be discussed later, the strengths of the antipolar gradients have been slightly unbalanced (denoted by the unbalancing factor, κ). Apart from the inverted polarity and asymmetry of the gradients, the BASE-CLUB is identical to the DPFGSE; therefore, it provides a selective inversion profile independent of the phase property of the pulse, requires no additional phase cycling, and refocuses the evolution of chemical shift and scalar coupling to spins outside the inversion band. Note that unbalancing the gradients does reduce the fast recovery properties for which the element was originally designed.

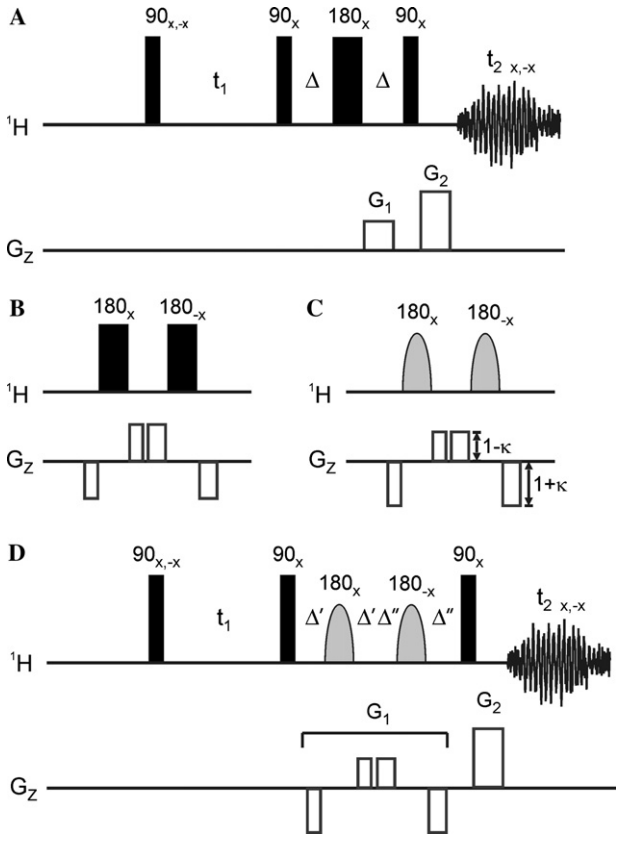

Fig. 1. The timing diagrams for the pulse sequences discussed in this report: (A) gDQF-COSY, (B) CLUB sandwich composite gradient, (C) BASE-CLUB composite gradient, and (D) BASE-gDQF-COSY. Δ is the spin echo delay and is set to the minimum time needed to accommodate the sum of the G_1 encoding gradient duration and the recovery delay. κ is the unbalancing factor.

Fig. 1D shows the pulse sequence for a band-selective gDQF-COSY that uses the BASE-CLUB as the encoding gradient. Coherence selection is still accomplished

with an encoding-to-decoding gradient ratio (G_1:G_2) of 1:2, but this will only occur for those signals refocused by the selective pulses of the BASE-CLUB. Sequence 1D is clearly distinct from previously reported band-selective COSY experiments [1–5,8–10] in that band selection and coherence selection are achieved simultaneously during the double-quantum filter by selective encoding rather than preceding t_1 or t_2 by selective excitation or the DPFGSE. As a result, our proposed method is free of the phase errors encountered in earlier methods due to homonuclear scalar coupling evolution during the selective excitation period. To distinguish our method from prior ones, as well as for the sake of simplicity, we will refer to the pulse sequence of Fig. 1D as BASE-gDQF-COSY hereinafter.

The capabilities of the BASE-gDQF-COSY were tested with a sample of the ionophorous antibiotic Lasalocid A (**1**). The ^{1}H 1D spectrum, which is shown in Fig. 2 along with the molecular structure, shows substantial overlap in the aliphatic region (0.5–2.3 ppm). Fig. 3 compares the broadband 2D DQF-COSY spectra of **1** acquired with Sequence 1A (A) to the band-selective spectra acquired with Sequence 1D using either a fully symmetric BASE-CLUB (B) or a slightly unbalanced

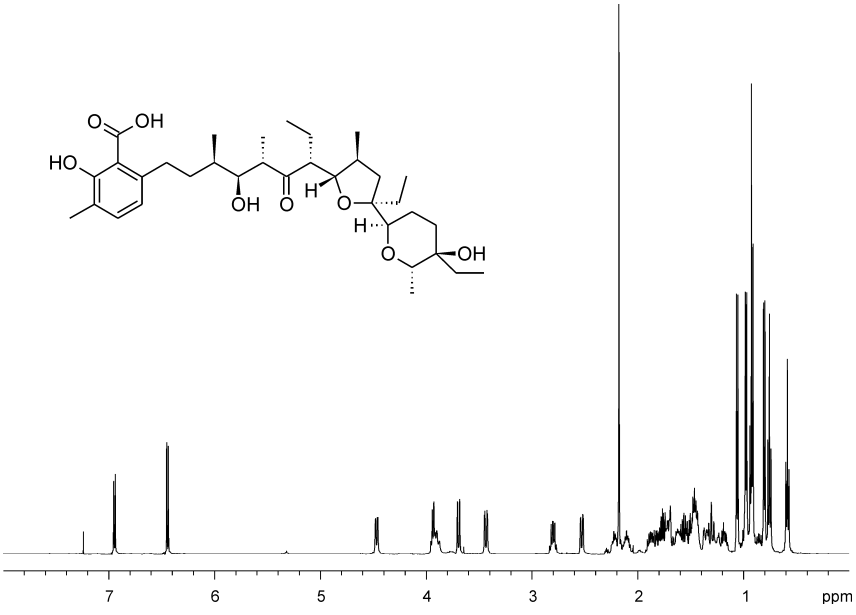

Fig. 2. The 1D ^{1}H spectrum and molecular structure of Lasalocid A (**1**).

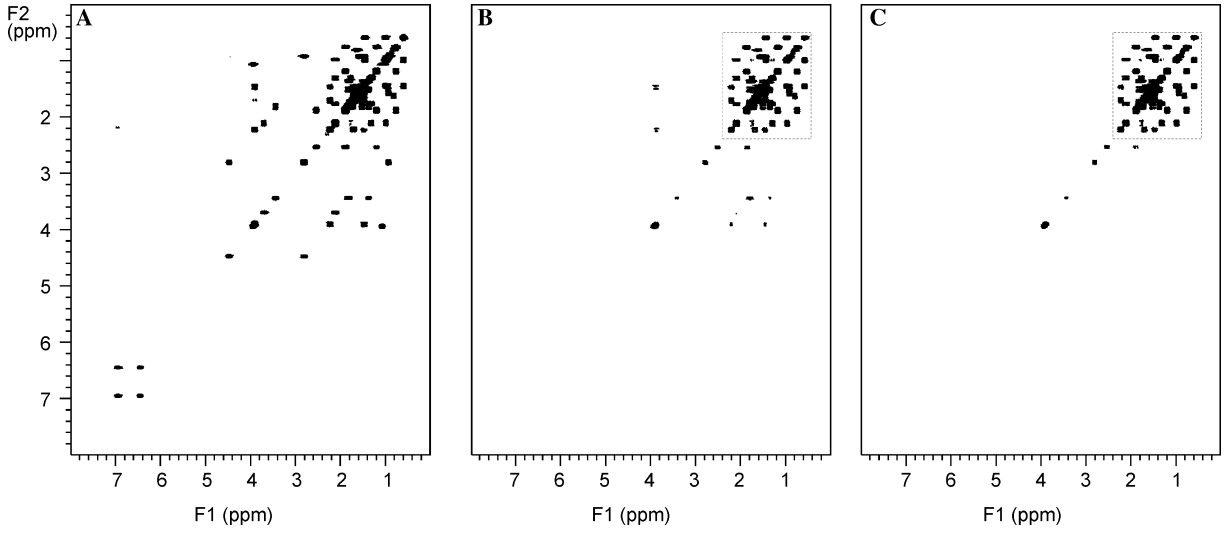

Fig. 3. Comparison of the broadband 2D spectrum of **1** acquired with gDQF-COSY (A) to the band-selective spectra acquired with BASE-gDQF-COSY using $\kappa = 0$ (B), and $\kappa = 0.45$ (C). All spectra were displayed with the vertical scaling factor adjusted such that the average measured noise of four F_2 traces is the same.

BASE-CLUB (C) to selectively encode the region of 0.5–2.3 ppm (indicated by the dashed box). For illustration purposes, the full F_1 spectral width was used in all three spectra. The selectivity of the fully symmetric BASE-CLUB was rather inadequate, as both diagonal and crosspeaks well outside the inversion band are observed (Fig. 3B). Because these peaks will appear folded when the F_1 spectral width is reduced to span only the region of interest, they will complicate the data analysis.

The origin of the unwanted diagonals can be understood using the product operator formalism. Since these peaks arise from spin systems involving resonances both inside and outside the inversion band, let us consider a three-spin system (I_1, I_2, I_3) where spins 2 and 3 are inside the inversion band and spin 1 is outside the desired band. The term I_{1z} present at the start of the sequence is subjected to an initial 90° pulse, t_1, and a second 90° pulse, which leads to the following term:

$$4I_{1z}I_{2y}I_{3y}\cos(\Omega_1 t_1)\sin(\pi J_{12}t_1)\sin(\pi J_{13}t_1). \quad (1)$$

This is a mixture of zero-quantum and double-quantum coherence for spins 2 and 3

$$\begin{aligned}4I_{1z}I_{2y}I_{3y} &= I_{1z}[2I_{2y}I_{3y} - 2I_{2x}I_{3x} + 2I_{2x}I_{3x} + 2I_{2y}I_{3y}] \\ &= -2I_{1z}[DQ_x^{23} - ZQ_x^{23}]. \quad (2)\end{aligned}$$

Because both spins 2 and 3 are inside the inversion band, the double-quantum portion of Eq. (2) will be properly encoded by the ensuing BASE-CLUB encoding gradient. Note that the symmetry of the BASE-CLUB is irrelevant here, because the gradients have no effects on the longitudinal magnetization of I_1. After the third 90° pulse and the decoding gradient, this term gives rise to

$$\begin{aligned}&1/2(4I_{1y}I_{2x}I_{3x} - 4I_{1y}I_{2z}I_{3z})[\cos(\Omega_1 t_1)\sin(\pi J_{12}t_1) \\ &\times \sin(\pi J_{13}t_1)]. \quad (3)\end{aligned}$$

While the first term in Eq. (3) is unobservable, the second term $4I_{1y}I_{2z}I_{3z}$ leads to an I_1 diagonal peak doubly antiphase with respect to I_2 and I_3.

The origin of the unwanted crosspeaks is somewhat more complicated. Presumably, they can originate from three-spin terms that are transverse for all three spins during the double-quantum filter and labeled by the offset frequency of I_1 during t_1 (e.g., $4I_{1y}I_{2x}I_{3y}$). Because I_1 is outside the inversion band, a fully symmetric BASE-CLUB will have no net effect on I_{1y} (neglecting chemical shift evolution), and the term $4I_{1y}I_{2x}I_{3y}$ behaves as if it were a simple mixture of zero- and double-quantum coherence for spins 2 and 3. A similar analysis shows that the double-quantum portion of this term will survive the coherence pathway selection and lead to cross peaks to I_2 and I_3. Interestingly, the crosspeaks seem to occur only for the spin systems involving geminal couplings, suggesting a role of strong coupling. However, further experiments or more detailed calculations are needed to confirm this hypothesis.

Fortunately, the unwanted crosspeaks can be suppressed simply by slightly unbalancing the gradients within the BASE-CLUB encoding gradient, as demonstrated in Fig. 3C. This ensures that resonances outside the inversion band will be partially dephased and only "true" DQ crosspeaks from within the inversion band will survive the coherence selection. The unwanted diagonal peaks, on the other hand, are not suppressed for reasons discussed above. Nevertheless, they will not complicate the analysis, as they fold outside the region of interest when the F_1 spectral width is reduced. As with any band-selective experiment, peaks close to the boundary might appear due to the finite width of the waveform's transition band. While they can be completely suppressed by using a pulse with steeper transition boundaries than the REBURP pulses used here, such a pulse will be inevitably longer and lead to a significant reduction in the signal-to-noise. From our experience, the actual value of κ does not appear to be important, although certain values should be avoided so as not to accidentally form higher order gradient echoes.

Fig. 4A shows an expansion around the congested region from the non-selective gDQF-COSY spectrum obtained with the pulse sequence of Fig. 1A. Fig. 4B is the band-selective spectrum obtained with the BASE-gDQF-COSY pulse sequence of Fig. 1D by selectively encoding only the region of 0.5–2.3 ppm and reducing the F_1 spectral width from 4000 to 1000 Hz. (The spectral width in F_2 remains at 4000 Hz and is simply shown expanded around this region.) In addition to the anticipated resolution enhancement, the spectrum from the BASE-gDQF-COSY is free of phase errors and contains peaks with excellent lineshape, as can be verified by a comparison with the broadband spectrum acquired with 4-fold the number of t_1 increments (Fig. 4C). The excellent phase behavior of the spectrum acquired with the BASE-gDQF-COSY results from the combination of the superb phase properties of the BASE-CLUB and its strategic placement within the pulse sequence.

3. Conclusion

We have described a band-selective CLUB sandwich that can be used to selectively encode the coherences within a desired frequency band. Like the DPFGSE, the BASE-CLUB offers an excitation profile that depends only on the inversion profile of the waveform and not on its phase properties, requires no additional phase cycling, and refocuses the evolution of chemical shift and coupling to spins outside the selected region. As an example, we have successfully incorporated the BASE-CLUB into the gDQF-COSY pulse sequence. This BASE-gDQF-COSY was found to provide the selectivity necessary for resolution enhancement while maintaining excellent phase behavior and lineshape.

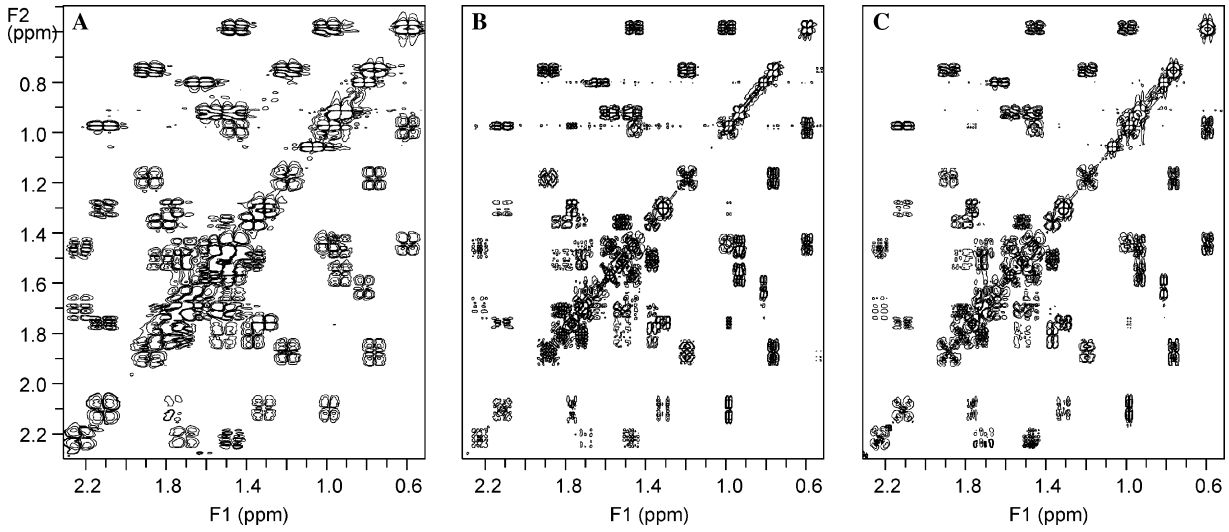

Fig. 4. Comparison of the broadband 2D spectra of **1** acquired with gDQF-COSY (A,C) to the band-selective spectrum acquired with BASE-gDQF-COSY (B). The spectra were acquired with the following parameters: (A) 180, (B) 180, and (C) 720 t_1 increments; (A) 4000 Hz, (B) 1000 Hz, and (C) 4000 Hz F_1 spectral width; and a 4000 Hz F_2 spectral width. The total acquisition times were (A) 2 h 5 min, (B) 2 h 12 min, and (C) 8 h 43 min. For (C), a +1.30 kHz frequency shift (difference between the carrier frequency and the center of desired region) was applied to the t_1 interferograms prior to transformation. The spectra are displayed with the vertical scaling factor adjusted to give equal peak intensity.

The BASE-CLUB element can be used, in principle, to convert any pulse sequence that relies on coherence selection with gradients into a band-selective variant.

4. Experimental

The sample of lasalocid was prepared as a slightly less than saturated solution in deuterochloroform and transferred to a 3 mm NMR tube. All spectra were acquired at 25.0 °C on a Varian INOVA 500 MHz NMR spectrometer equipped with a Varian 5 mm Cold Probe. The shaped pulses were generated using the Pandora's Box shaping program [20], which is part of the Varian software package. Typical acquisition parameters include a 1 sec relaxation delay, 6.0 μs ^{1}H 90° pulse length at 55 dB (63 dB max), 1024 acquired complex points, and 180 t_1 increments (16 scans each). The encoding and decoding gradients had a duration of 2.5 ms and amplitudes of 7.5 and 15 G/cm, respectively. For the BASE-CLUB encoding gradient, the 2.5 ms duration was divided into 0.5, 0.5, 0.75, and 0.75 ms. All gradients were rectangular and were followed by a 500 μs recovery delay. The recovery delay after G_2 was adjusted to 509 μs so that the sum of the gradient duration and recovery delay was an integer multiple of the dwell time. A gradient-90°-gradient element (2.0 ms and 10 G/cm apiece) was used to randomize the magnetization prior to the relaxation delay. REBURP pulses ($pw_{90} = 5.42$ ms, $B_{1(rms)} = 0.327$ kHz) [21] designed to refocus a 900 Hz bandwidth centered at 1.30 kHz upfield of the transmitter were used in the BASE-CLUB. All spectra were right-shifted 12 points, weighted with a mild Lorenztian-to-Gaussian function in both dimensions, and zero-filled to 2048×2048 complex points prior to Fourier transformation. To minimize the effect of post-acquisition processing tools on the observed resolution or sensitivity, all spectra were processed without linear prediction in either dimension.

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