Journal of Magnetic Resonance **137,** 93–99 (1999)
Article ID jmre.1998.1638, available online at http://www.idealibrary.com on IDEAL®

Gradient $BIRD_R$: A Method to Select Uncoupled Magnetization

Sami Heikkinen and Ilkka Kilpeläinen[1]

Institute of Biotechnology, P.O. Box 56, FIN-00014, University of Helsinki, Finland

Received June 8, 1998; revised October 14, 1998

The gradient-BIRD-method is an effective way to select ^{13}C-bound protons. By changing the phase of the proton π-pulse, the same sequence can be used to select protons attached to ^{12}C atoms, i.e., for filtering out the ^{13}C-bound protons. These filters, consisting of single or double gradient-$BIRD_R$ clusters (BIRD inversion for remote protons) have considerable filtering bandwidth. Because of the efficient suppression of the coupled magnetization, we implemented the gradient-$BIRD_R$ filter in HMBC to replace the conventional low-pass filter. © 1999 Academic Press

Key Words: **NMR spectroscopy; isotope filters; BIRD; B_0 gradients; RF gradients; low-pass filter.**

INTRODUCTION

The gradient-BIRD cluster is an efficient way to select ^{13}C-bound protons and to filter out ^{12}C-bound ones (*1–5*). This isotope filtering is supreme when four clusters are placed in series. However, when the GBIRD method is used as an add-on in the HSQC sequence to suppress the ^{12}C-bound protons, two clusters are enough as rest of the unwanted signals are readily suppressed with phase cycling (*3, 4*). The GBIRD method can also be used as an isotope filter to *select* the noncoupled magnetization, i.e., ^{12}C-bound protons, simply by changing the phase of the proton π-pulse of the BIRD propagator from x to y (or by changing only the phase of the last $\pi/2$-pulse from x to $-x$) (*6*). Now the remote protons (*not* directly attached to ^{13}C) are inverted by this $BIRD_R$ (BIRD inversion for remote protons, or $BIRD_y$) cluster while the local protons (^{13}C-bound ones) experience 360° net rotation and are thus dephased by the gradients embracing the $BIRD_R$-cluster. The remote proton magnetization remains unaffected, as the dephasing caused by the first gradient is rephased by the second one (because of the inversion of the magnetization by the $BIRD_R$-cluster). The heteronuclear $^1J_{CH}$ evolution during B_0-gradient pulses is refocused by $BIRD_R$, when the tuning delay Δ $(= 1/^1J_{CH})$ is matched. This is because the $BIRD_R$ results in no inversion for ^{13}C-bound protons, whereas the carbon is affected by the 180° pulse. When RF gradients are used, the effects of $^1J_{CH}$ coupling are eliminated (provided that the RF field is strong enough) whether the delay Δ is matched or not. The $BIRD_R$ isotope filter is quite tolerant of the tuning mismatches that frequently occur when there is not a single value for $^1J_{CH}$. Simply by performing two similar filter steps in series, the efficiency of the filter can be further improved. Artifacts originating from leaking ^{1}H–^{13}C one-bond correlations are common in HMBC experiments. Here, the double-$GBIRD_R$ filter can be successfully utilized to replace the conventional low-pass filter that has a very J-sensitive filtering performance. The pulse sequences for single and double $GBIRD_R$ filters, and for double-$GBIRD_R$-filtered HMBC, are presented in Fig. 1.

THEORY

The product operator calculations (*7–9*) were performed for isolated ^{1}H–^{13}C fragments to evaluate the leakage of the coupled magnetization. The evolution of the heteronuclear coupling during the field gradient pulses was neglected. The intensities of the leaking magnetization for single and double $GBIRD_R$ filters are presented in Eq. [1] ($-H_Y$ magnetization is considered positive).

$$\begin{aligned} I(\text{single}) &\propto -0.5\{1 + \cos(\pi\Delta\,^1J_{CH})\}H_Y \\ &= \{-\cos^2(\pi\Delta\,^1J_{CH}/2)\}H_Y \\ I(\text{double}) &\propto -0.25\{1 + \cos(\pi\Delta\,^1J_{CH})\}^2H_Y \\ &= \{-\cos^4(\pi\Delta\,^1J_{CH}/2)\}H_Y \end{aligned} \qquad [1]$$

As can be seen, complete suppression of ^{1}H-magnetization directly attached to ^{13}C is impossible because of the variety of the $^1J_{CH}$:s in real molecules. In Fig. 2 the calculated leaking intensities for both single and double filters are presented as a function of $^1J_{CH}$. The delay Δ was set to 1/160 s (optimal filtering for $^1J_{CH}$ = 160 Hz, $\Delta = 1/J$) to calculate the intensities in Fig. 2. Obviously, the filtering efficiency of a single $GBIRD_R$ filter is relatively good (leaking intensity < 10%) for $^1J_{CH}$:s 130–190 Hz, i.e., a single $GBIRD_R$ filter can tolerate ±30 Hz mismatch and still have reasonably good filtering properties. If the $GBIRD_R$ filter is tuned for $^1J_{CH}$=145 Hz, good suppression can be obtained for both aliphatic and aromatic regions. A purging spin-lock pulse in the proton channel (phase y) can be used prior to acquisition to clean up the phase distortion due to homonuclear couplings that are active throughout the pulse sequence. In addition, the spin-lock pulse

[1] To whom correspondence should be addressed.

1090-7807/99 $30.00

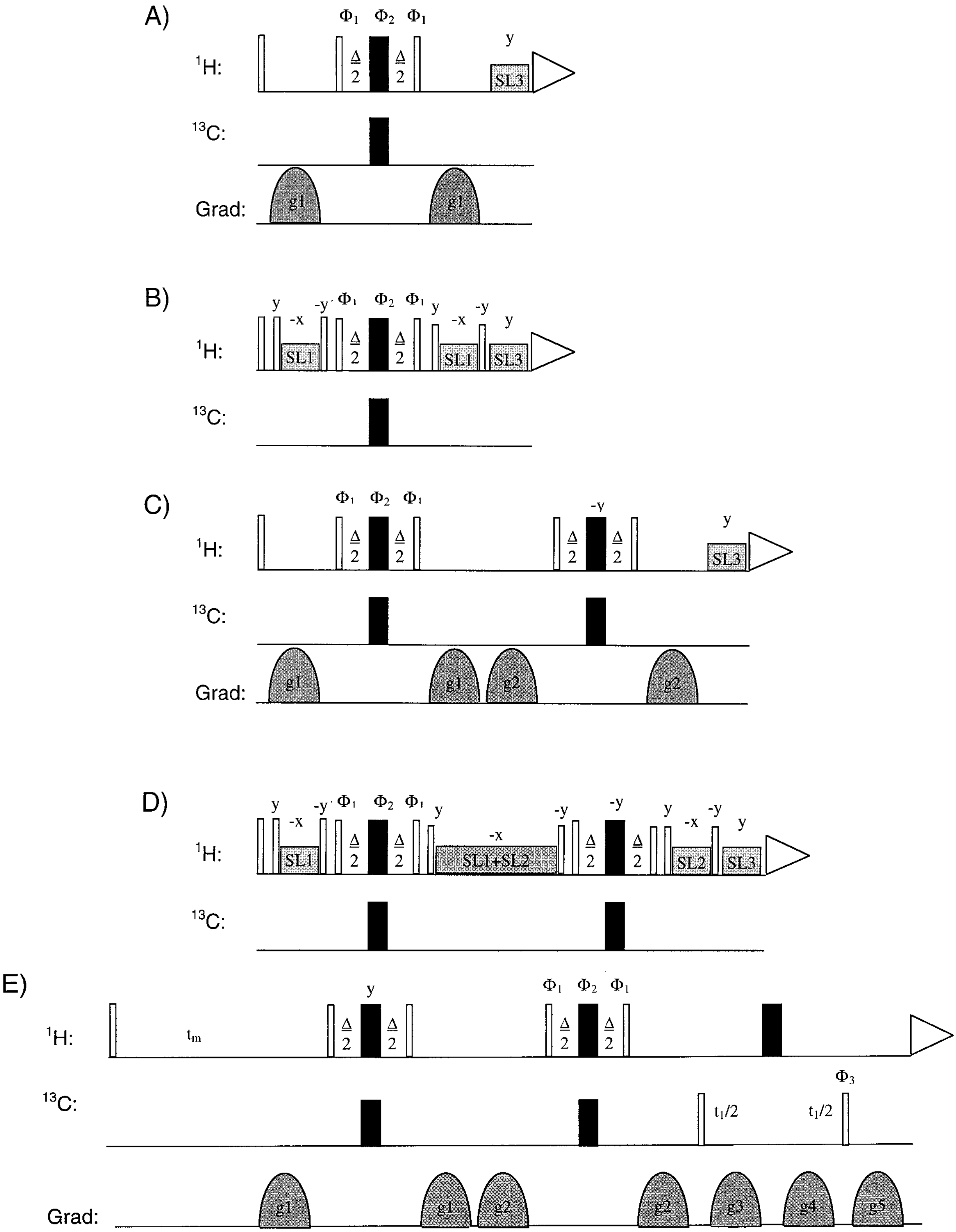

FIG. 1. Pulse sequences for single B_0 $BIRD_R$ (A), single RF $BIRD_R$ (B), double B_0 $BIRD_R$ (C), and double RF $BIRD_R$ filters (D) to select the uncoupled magnetization. Narrow white bars and wide black bars indicate 90° and 180° hard rectangular pulses. Spin-lock pulses are presented with wide gray bars denoted by SL. B_0-gradient pulses are presented as gray half-ellipses with denotation *g.* All pulses have *x*-phase unless otherwise indicated. EXORCYCLE (*13*) is applied on proton pulses of one $BIRD_R$ propagator $\Phi_1 = x, y, -x, -y$; $\Phi_2 = y, -x, -y, x$; receiver $= x, -x$. Purging spin-lock pulse prior to acquisition is optional. (E) Pulse sequence for $GBIRD_R$ filtered HMBC. All pulses have *x*-phase unless otherwise indicated. EXORCYCLE (*13*) is applied on proton pulses of one $BIRD_R$ propagator. Phases for the pulses are $\Phi_1 = x, y, -x, -y$; $\Phi_2 = -y, x, y, -x$; $\Phi_3 = 4(x), 4(-x)$; receiver $= x, -x, x, -x, -x, x, -x, x$.

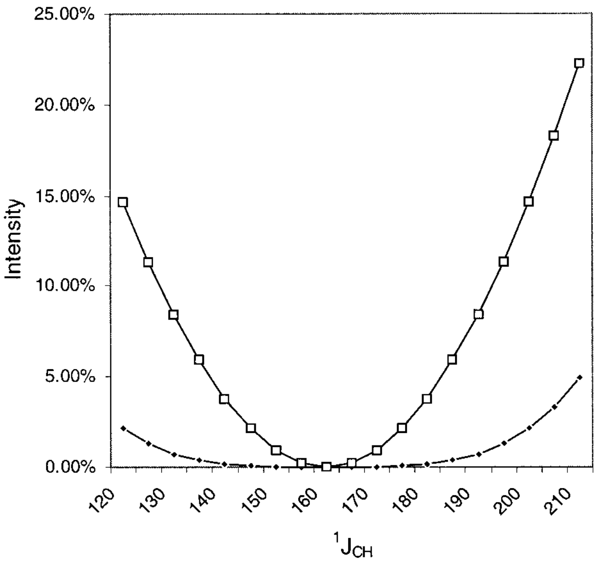

FIG. 2. Leaking intensity of ^{13}C-coupled proton signals for single and double GBIRD$_R$ filters as a function of $^1J_{CH}$. Equation [1] was used to calculate leaking intensities with $\Delta = 1/160$ s and various $^1J_{CH}$ values. Lines corresponding to the single and double GBIRD$_R$ filters are marked with open squares and filled diamonds, respectively.

dephases the leaking magnetization of type H_XC_Z that is formed due to the heteronuclear $^1J_{CH}$-coupling evolution during B_0 gradients when the delay Δ is not correctly matched, i.e., part of the coupled proton magnetization experiences 180° net rotation during the BIRD$_R$ cluster and therefore $^1J_{CH}$ evolves during the gradient pulses. When the length of the gradient period $2t_g$ (t_g is the length of the gradient pulse) is set to $1/2^1J_{CH}$, antiphase magnetization of type H_XC_Z is formed and will be suppressed by the proton spin-lock pulse along the y-axis.

According to Fig. 2, the filter properties of double-BIRD$_R$ are excellent (leaking <5%) for $^1J_{CH}$:s ± 50 Hz from the value used for tuning. For range ±60 Hz from the optimum value, the leaking is still <10%.

Homonuclear J_{HH} will also result in some additional leakage. Product operator calculations were performed for the AMX system (H_1H_2C, where only H_2 is bound to ^{13}C) with the two couplings J_{H1H2} and J_{H2C}. The leaking intensity of double-BIRD$_R$ for this system is presented in Eq. [2]. The terms representing the intensity of the dispersive antiphase term resulting from COSY-type transfer are not shown. For example, leaking intensity is only 0.5% for a system where $J_{HH} = 10$ Hz, $^1J_{CH} = 175$ Hz, and $\Delta = 1/145$ s. Normally, however, the leaking due to homonuclear coupling will be greater as there usually is more than one homonuclear coupling present, and thus complete suppression of the coupled magnetization is not possible.

$$I(\text{double}) \propto (-0.25 - 0.5 \cos \pi\Delta J_{HH} \cos \pi\Delta\, {}^1J_{CH} - 0.25 \cos 2\pi\Delta J_{HH} \cos^2 \pi\Delta\, {}^1J_{CH}) H_Y \quad [2]$$

The long-range ^{1}H–^{13}C couplings will reduce intensities of the desired signals when BIRD filters are used to select the uncoupled magnetization, whereas they will result in leaking when the coupled magnetization is selected.

Interestingly, the intensity of the leaking magnetization of the single GBIRD$_R$ filter presented in Eq. [1] is equivalent to the leaking intensity of the double tuned low-pass filter (double tuned x-half filter) (*10*) when both components of the low-pass filter are tuned for the same coupling constant. Therefore, one can state that the filtering properties are better for the double tuned low-pass filters than for single GBIRD$_R$ filters. When comparing to the double tuned low-pass filter, the double-GBIRD$_R$ method has better filtering properties, as is apparent in Eq. [1]. As a drawback, some decay in signal intensity can be expected with increased filter length.

As the GBIRD$_R$ cluster results in selective rotation for the uncoupled protons and destroys the magnetization of the coupled ones at the same time (and vice versa if the phase of the proton 180° pulse is changed from y to x), the range of applications would be rather wide for BIRD-clusters.

RESULTS AND DISCUSSION

One-Dimensional Experiments

Single or double gradient BIRD$_R$ filters for ^{1}H–^{12}C selection have a considerable filtering bandwidth. In our approach we used both conventional B_0 gradients and radiofrequency gradients (RF gradients). RF gradients were generated with a standard coil using long spin-lock pulses implemented into z-rotation sandwiches to mimic the effects of conventional B_0-gradients (*4, 11–13*). In addition, the effect of the purge spin-lock pulse (y-direction) prior to acquisition was tested.

All filters were tested using 0.5 M D-[1-^{13}C]glucose (mixture of α- and β-forms) in D_2O by monitoring the leaking intensity of the anomeric proton signals as a function of delay $\Delta = 1/^1J_{CH}$. The $^1J_{CH}$:s for anomeric protons are about 170 Hz. The four-step EXORCYCLE (*14*) was applied on the proton pulses of the first BIRD$_R$-cluster to reinforce the echo. Figure 3 presents four series of spectra of the low-field part of the proton doublet at 5.1 ppm recorded using single GBIRD$_R$ and double GBIRD$_R$ filters with B_0 and RF gradients. The first spectrum in each row was recorded with delay Δ optimized for 120 Hz and following with 10-Hz increments. The spectra recorded with B_0 GBIRD$_R$ methods contain phase distortions due to the aforementioned heteronuclear J-coupling evolution during B_0-gradient pulses. No purging spin-lock pulses were applied in these sequences to get the real intensity of leaking magnetization for the BIRD$_R$ filter. Especially in the case of single GBIRD$_R$ with B_0 gradients, a preacquisition purge spin-lock along the y-axis can almost completely destroy the coupled magnetization despite the Δ-value if the lengths of gradient pulses are such that heteronuclear J-evolution forms antiphase

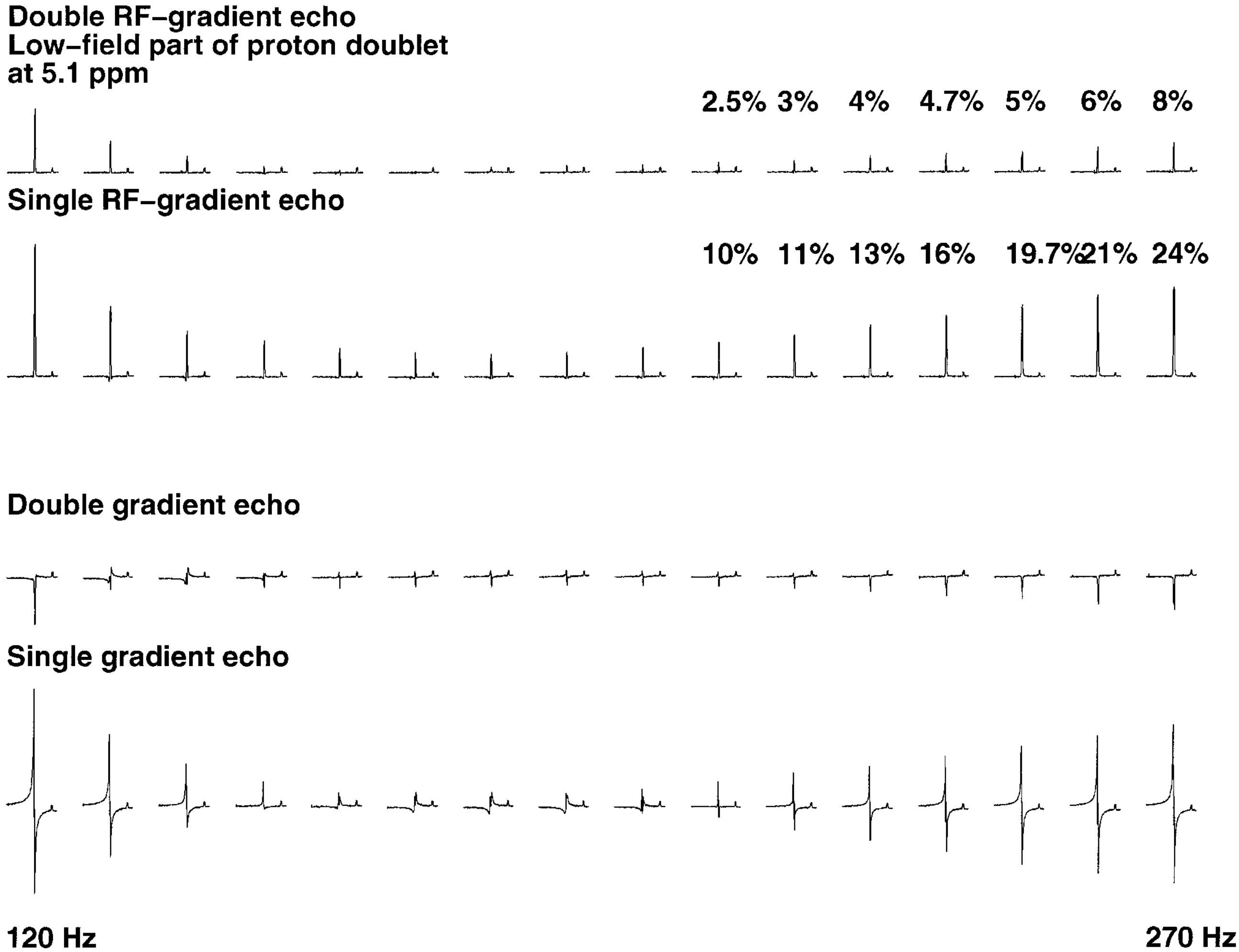

FIG. 3. Low-field part of the proton doublet ($^1J_{CH} \approx 170$ Hz) at 5.1 ppm. Spectra were recorded using sequences A–D in Fig. 1. Intensity of the leaking magnetization was monitored as a function of $^1J_{CH}$ used to tune the delay Δ. No purging spin-lock SL3 was used for the B_0 GBIRD$_R$ sequences. All spectra are plotted with the same relative scale and are thus comparable. Percentage values show the amount of the leaking coupled magnetization relative to the intensity in the normal 1D–^{1}H-spectrum. The spectra were recorded with a Bruker DRX-500 spectrometer equipped with a triple-resonance probehead incorporating a single shielded gradient coil. Number of scans = 4, relaxation delay = 2.0 s, acquisition time = 1.02 s, 90° (^{1}H) pulse = 7.20 μs, 180° (^{13}C) pulse = 14.00 μs, FID was zero-filled, and an exponential weighting function (0.3 Hz) was applied prior to Fourier transform. B_0 gradient methods: gradient shape = sinusoid, gradient pulse length = 1 ms, recovery delay = 500 μs, gradient amplitudes = 7.2 G/cm (single-echo); 7.2 G/cm and 3.0 G/cm (double-echo). RF gradient methods: SL1 = 1.4 ms and SL3 = 1.0 ms (single-echo); SL1 = 1.4 ms, SL2 = 1.8 ms, and SL3 = 1.0 ms (double-echo).

magnetization of type H_XC_Z. This is the case for leaking magnetization, i.e., ^{13}C-bound proton magnetization that is inverted by the BIRD$_R$ propagator. The suppression by purge-pulse is not so significant for double-GBIRD$_R$ as long as delays Δ are "reasonable" (not, for instance, 3 μs).

All four series of spectra are plotted using the same scale, and are thus comparable. Percentage values for double and single RF GBIRD$_R$ spectra show the intensity of the leaking signal compared to the intensity of the corresponding signal in a normal ^{1}H-spectrum.

If H_2O solutions are used, excitation sculpting based suppression sequences (*1*) can be added after the BIRD$_R$ propagator. The double GBIRD$_R$ filter followed by gradient double-echo with $90(x)$–τ–$90(-x)$ as inversion element (*1*) (τ was set to 1 ms) resulted in very good water suppression and filtering performance. Half-EXORCYCLE (pulse: x, y; receiver: x, $-x$) was applied on both the first BIRD$_R$ propagator and on the first $90(x)$–τ–$90(-x)$; thus, four scans were needed to complete the phase cycle. This sequence resulted in a water suppression ratio of about 80,000–100,000 and normal suppression for coupled magnetization (data not shown).

The effect of the proton π-pulse phase is demonstrated in Fig. 4. The selection of the uncoupled magnetization is performed using the pulse sequence described in Fig. 1C. The selection of the coupled magnetization is achieved by changing the proton π-pulse phase by 90°. Heteronuclear coupling is refocused by applying a carbon π-pulse in between the two GBIRD clusters.

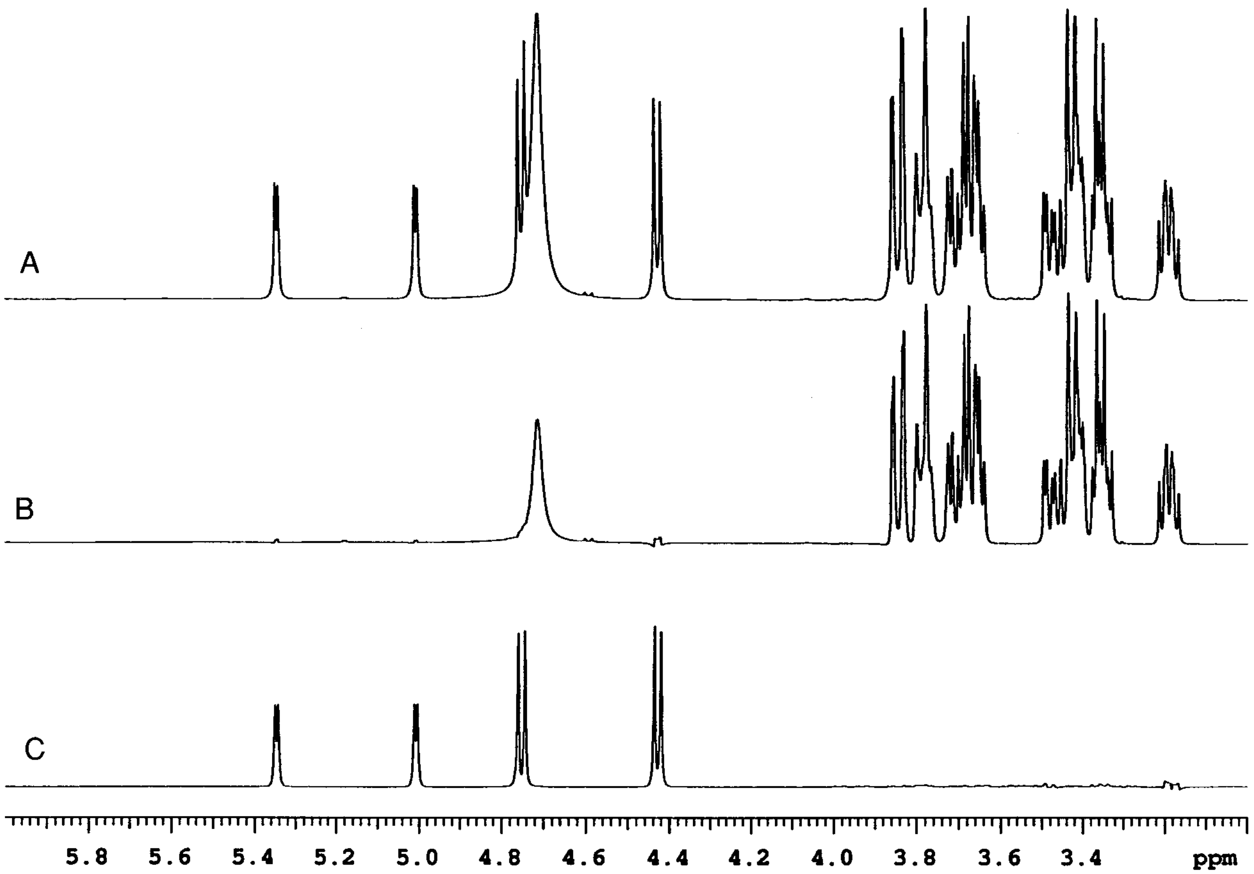

FIG. 4. One dimensional spectra of 0.5 M glucose. (A) Normal ^{1}H spectrum. (B) Double GBIRD$_R$ spectrum to select the uncoupled magnetization (*3, 4*). (C) Double-GBIRD spectrum to select coupled magnetization. For the spectrum in (C) the pulse sequence in Fig. 1C was modified by changing the phase of the BIRD's proton π-pulse by 90°. In addition, a carbon π-pulse was applied in between the two BIRD clusters to refocus the heteronuclear coupling. The spectra were recorded with a Varian Unity 500 spectrometer equipped with a triple-resonance probehead incorporating a single shielded gradient coil. Number of scans = 4, relaxation delay = 2.0 s, acquisition time = 1.02 s, 90° (^{1}H) pulse = 7.40 μs, 180° (^{13}C) pulse = 13.00 μs, FID was zero-filled, and an exponential weighting function (0.3 Hz) was applied prior to Fourier transform. Gradient shape = rectangular, gradient pulse length = 1 ms, recovery delay = 100 μs, gradient amplitudes = 7.2 G/cm and 3.0 G/cm. Purging spin-lock SL3 = 2 ms. Delay Δ was tuned for $^1J_{CH}$ = 145 Hz.

Two-Dimensional Application

The good suppression of the coupled magnetization obtained with GBIRD$_R$ was utilized in an HMBC experiment to filter out correlations arising from directly ^{13}C-bonded protons. In our hands best results were obtained when the double GBIRD$_R$ cluster was placed after the preparation delay rather than after the excitation pulse. The double GBIRD$_R$ HMBC (Fig. 1E) was tested using a 0.5 M sucrose sample in D_2O at 298 K. The filtration of the coupled magnetization was not as perfect as the 1D results with labeled glucose predicted. This was expected because of the numerous proton–proton couplings, whereas the anomeric proton has coupling with only one proton. Still, the filtration efficiency is good, and much better than can be achieved using the conventional low-pass filter (*14, 15*). Figure 5 contains slices taken from the 2D HMBC spectra at the anomeric carbon frequency recorded using double-GBIRD$_R$- and conventional low-pass filters tuned for $^1J_{CH}$ values of 125, 145, 165, and 185 Hz. The signals from the directly ^{13}C-bonded proton are marked with arrows. Double-GBIRD$_R$ tolerates tuning mismatches rather well, whereas in case of the conventional low-pass filter, even a mismatch of 5 Hz results in significant leakage ($^1J_{CH}$ = 170 Hz for anomeric proton). Figure 5 shows clearly the fine performance of the BIRD-based filter, as the worst results with this filter were better than the best obtained using the conventional filter. It should be noted that when the GBIRD$_R$ filter is used to replace the conventional low-pass filter typically used in HMBC, some loss of sensitivity can be expected as the length of the preparation delay increases by a few milliseconds. This may be a problem if the T_2-relaxation time is short.

CONCLUSIONS

J-leakage is a common problem of isotope filters. We have shown here that GBIRD$_R$ filters have very good to excellent filtering performance and they can be easily implemented into

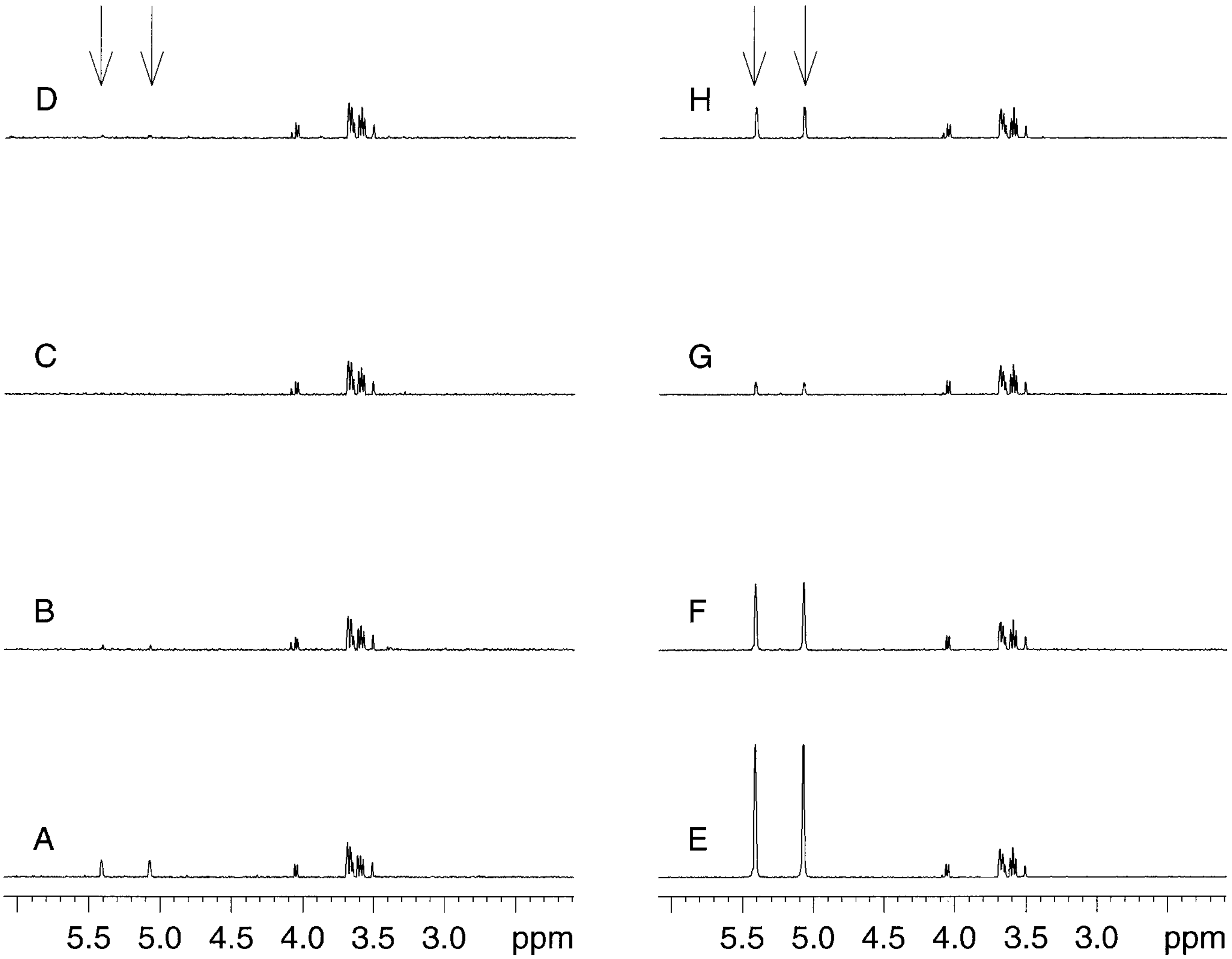

FIG. 5. The anomeric carbon slices of HMBC-spectra of sucrose in D_2O. Spectra A–D were recorded with the sequence presented in Fig. 1E. Spectra E–H were recorded using a normal low-pass filtered HMBC-sequence (*15, 16*). The filters were tuned for 125, 145, 165, and 185 Hz in spectra A and E, B and F, C and G, D and H, respectively. Experimental parameters: Bruker DRX-500 spectrometer equipped with two-channel multiprobe incorporating a single shielded gradient coil, 500 MHz ^{1}H frequency, relaxation delay = 2.0 s, delay for long-range coupling evolution = 50 ms, number of transients = 8, number of time increments = 64, number of f_2 points = 2K, 90° (^{1}H) pulse = 5.90 μs, 180° (^{13}C) pulse = 13.00 μs, gradient pulse length = 1 ms, gradient recovery delay = 100 μs, gradient shape = sinusoid, (A–D) gradient amplitudes *g1, g2, g3, g4, g5* = 10.2, 4.2, 30.0, 18.0, and 24.0 G/cm, (E–H) gradient amplitudes *g3, g4, g5* = 30.0, 18.0, and 24.0 G/cm. The t_1 and t_2 domains were zero-filled and multiplied by sine function prior to Fourier transformation. Signals arising from directly ^{13}C-bonded proton are marked with arrows. The signals at 3.5–4.1 ppm are true HMBC correlations to the anomeric carbon.

a variety of pulse sequences. The implementation of a $GBIRD_R$ filter into HMBC drastically suppresses artifacts arising from one-bond ^{1}H–^{13}C couplings. In HMBC, the increase in the length of the filter period will lead to some decrease in signal intensity due to relaxation. This is a major problem only when T_2 is short, i.e., for macromolecules. When studying small molecules, the relaxation times are much longer and no significant signal loss will occur. If the relaxation is not an issue, the double $BIRD_R$ filtered HMBC is recommended. When macromolecule–ligand interactions are studied, $GBIRD_R$ filters can safely be used to filter out ^{13}C-bound protons of the isotope-labeled macromolecule. For those spectrometers lacking gradient capabilities, the RF gradient method can be useful.

EXPERIMENTAL

The one-dimensional spectra in Fig. 3 were recorded on a Bruker DRX-500 NMR spectrometer (500 MHz ^{1}H frequency) equipped with Bruker triple-resonance probe and *z*-axis gradient system at 298 K. The length of a 90° proton pulse on high power level was 7.2 μs, corresponding to a B_1-field strength of 34.7 kHz. The length of a 90° carbon pulse on high power level was 14.0 μs. The 0.5 M glucose sample was prepared by dissolving D-[1-^{13}C]glucose (mixture of α and β isomers) into 0.7 ml of 99.5% D_2O. The water suppression combined with a double GBIRD$_R$ filter was tested using 5 mM D-[1-^{13}C]glucose (mixture of α and β isomers) in H_2O/D_2O solution (ratio 9:1). Gradient amplitudes used in the water suppression sequence were 7.2, 3.0, 9.0, and 24 G/cm for two BIRD$_R$ and two jump-and-return propagators, respectively.

The one-dimensional spectra of 0.5 M D-[1-^{13}C]glucose (mixture of α and β isomers) in Fig. 4 were recorded on a Varian Unity 500 spectrometer (500 MHz ^{1}H frequency) equipped with Varian triple-resonance probe and z-axis gradient system at 298 K. The pulse lengths: 90° (^{1}H) pulse = 7.4 μs, 90° (^{13}C) pulse = 13.0 μs, trim pulse = 2 ms.

Two-dimensional HMBC spectra were recorded on a Bruker DRX-500 NMR spectrometer (500 MHz ^{1}H frequency) equipped with a Bruker multiprobe and a z-axis gradient system at 298 K. The length of a 90° proton pulse on high power level was 5.9 μs, corresponding to a B_1-field strength of 42.4 kHz. The length of a 90° carbon pulse on high power level was 13.0 μs. The 0.5 M sucrose sample was prepared by dissolving sucrose in 0.7 ml of 99.5% D_2O.

ACKNOWLEDGMENT

This work was supported by the Academy of Finland.

REFERENCES

1. T. L. Hwang and A. J. Shaka, *J. Magn. Reson. A* **112,** 275 (1995).
2. C. Emetarom, T. L. Hwang, G. Mackin, and A. J. Shaka, *J. Magn. Reson. A* **115,** 137 (1995).
3. G. Mackin and A. J. Shaka, *J. Magn. Reson. A* **118,** 247 (1996).
4. S. Heikkinen, E. Rahkamaa, and I. Kilpeläinen, *J. Magn. Reson.* **127,** 80 (1997).
5. A. Sodickson and D. G. Cory, *J. Magn. Reson.* **125,** 340 (1997).
6. J. Garbow, D. P. Weitekamp, and A. Pines, *Chem. Phys. Lett.* **93,** 504 (1982).
7. O. W. Sørensen, G. W. Eich, M. H. Levitt, G. Bodenhausen, and R. R. Ernst, *Prog. NMR Spectrosc.* **16,** 163 (1983).
8. S. Wolfram, "Mathematica. A System for Doing Mathematics by Computer," Addison-Wesley, Redwood City, California (1988).
9. P. Güntert, N. Schaefer, G. Otting, and K. Wüthrich, *J. Magn. Reson. A* **101,** 470 (1985).
10. G. Gemmecker, E. T. Olejniczak, and S. W. Fesik, *J. Magn. Reson.* **96,** (1992).
11. W. E. Maas, F. Laukien, and D. G. Cory, *J. Magn. Reson. A* **103,** 115 (1993).
12. R. Freeman, T. A. Frenkiel, and M. H. Levitt, *J. Magn. Reson.* **44,** 409 (1981).
13. S. Heikkinen, E. Rahkamaa, and I. Kilpeläinen, *J. Magn. Reson.* **133,** 183 (1998).
14. G. Bodenhausen, R. Freeman, and D. L. Turner, *J. Magn. Reson.* **27,** 511 (1977).
15. H. Kogler, O. W. Sørensen, G. Bodenhausen, and R. R. Ernst, *J. Magn. Reson.* **55,** 157 (1983).
16. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **108,** 2093 (1986).