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CAGEBIRD: improving the GBIRD filter with a CPMG sequence

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Abstract

An improvement of the GBIRD-filter is presented. The current approach utilizes Carr–Purcell–Meiboom–Gill type pulse train during the BIRD delay. The method enables recording of purely absorptive 1D spectrum using only one isotope editing element. In the current method, the parent signal leakage due to J_{HH} evolution during the BIRD delay is considerably smaller than in the conventional approach. As a consequence, the t_1-noise is smaller also in 2D applications, such as GBIRD-filtered HSQC.

Keywords: Isotope editing; GBIRD; CPMG; HSQC

1. Introduction

Proton-detected multi-dimensional experiments have established their superiority over methods employing X-nuclei detection. The enhanced sensitivity can be readily utilized either to reduce the measurement times, or to study more dilute samples. However, the inverse-detection contains some aspects, which need to be considered. Quite frequently, the natural abundance of the involved X-nuclei is significantly less than 100%. For example, in ^{1}H–^{13}C correlation experiments only 1.1% of the protons contribute to the desired signal, whereas 98.9% of the protons are responsible for the unwanted parent signal. An opposite problem occurs in long-range ^{1}H–^{13}C correlation experiments, were the short-range correlation peaks from ^{13}C-bound protons are not desired. As a result of incomplete suppression of directly ^{13}C-bound protons, the appearance of long-range correlation spectrum will be more complex, and in the worst case the residual unwanted peaks may overlap with the long-range cross peaks. Especially in biomolecular NMR, the analysis of molecular interactions between ^{13}C, ^{15}N-labeled and unlabeled species demands high performance from the isotope editing and/or filtering, in order to produce reliable data. Consequently, a legion of editing and filtering sequences has been designed to overcome these problems [1–11].

In 1995 Emetarom et al. [12] introduced an excitation sculpting-based isotope editing pulse sequence that relies on the combined use of BIRD propagators [13] and gradient pulses. This gradient-BIRD (GBIRD) provides an excellent signal suppression of ^{12}C-bound protons (parent signal). Thus, GBIRD has been applied to e.g., HMQC and HSQC pulse sequences as an isotope-editing element [14,15]. However, the BIRD propagator has some shortcomings, which compromise the performance of GBIRD. These aspects are discussed, and some improvements are presented in the following text.

2. Description of the method

The pulse sequences for 1D isotope editing experiments used in this study are presented in Fig. 1. Due to the J_{HH} evolution during the BIRD delay Δ, the final gradient pulse of GBIRD will refocus a part of the non-^{13}C-bound proton magnetization. This will lead to parent signal leakage for J_{HH} coupled systems even when the dephasing capabilities of the gradients are

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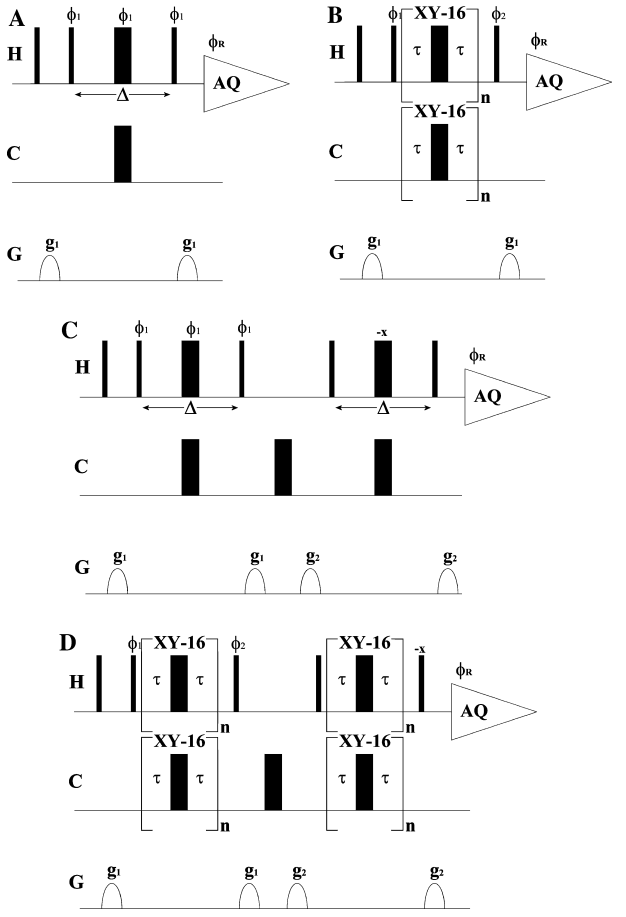

Fig. 1. Pulse sequences for single-GBIRD (A), single-CAGEBIRD (B), double-GBIRD (C), and double-CAGEBIRD (D). Narrow and thick bars represent the 90° and 180° RF-pulses, respectively. The 90° pulse durations were 5.97 μs for ^{1}H and 11.60 μs for ^{13}C. The pulse phases were along x if not indicated otherwise. Phase cycles were: $\phi_1 = \{x, y, -x, -y\}$, $\phi_2 = \{-x, -y, x, y\}$, and $\phi_R = \{x, -x, x, -x\}$. The phases of the pulses in XY-16 sequence were: $\{x, y, x, y, y, x, y, x, -x, -y, -x, -y, -y, -x, -y, -x\}$. The BIRD delay Δ was set to correspond the reciprocal of average $^{1}J_{CH}$ coupling (7.14 ms). The τ was set for approximately 100 μs. The number of cycles (n) in CAGEBIRD was set so that the duration of consecutive XY-16 cycles equals with delay Δ, and τ was further fine-tuned in order to fulfill this condition. The pulsed field gradients are represented by half-ellipses. The PFG duration was 1 ms, with recovery delay of 100 μs. The applied PFG strengths were: $g_1 = 36.6$ G/cm and $g_2 = 42.2$ G/cm.

ideal. If the pulses are assumed to be perfect, the residual parent signal intensity of a single-GBIRD element will follow Eq. (1).

$$I_{\text{Single-GBIRD}} \propto -0.5[1 - \cos(\pi J_{\text{HH}'}\Delta)]H_y + 0.5[\sin(\pi J_{\text{HH}'}\Delta)]H_z H'_x. \quad (1)$$

Apparently, the COSY type transfer to the coupled H' proton is considerable, and it will introduce parent signal leakage to the spectrum. Moreover, the BIRD propagator does not refocus the $^{1}J_{CH}$ coupling. This is because the BIRD inverts both ^{13}C-spins and ^{13}C-bound ^{1}H-spins, thus the $^{1}J_{CH}$ evolution during the gradient delays will twist the ^{13}C-satellite signals. Consequently, a second successive GBIRD element with an extra 180° ^{13}C-pulse in between the two GBIRD elements is needed to produce a pure phase spectrum. For the double-GBIRD filter the leakage intensity of unwanted magnetization is shown in the Eq. (2).

$$I_{\text{Double-GBIRD}} \propto -0.25[1 - 2\cos(\pi J_{\text{HH}'}\Delta) + \cos(2\pi J_{\text{HH}'}\Delta)]H_y + 0.25 \times [2\sin(\pi J_{\text{HH}'}\Delta) - \sin(2\pi J_{\text{HH}'}\Delta)]H_z H'_x. \quad (2)$$

In this case, the leakage is considerably smaller, as shown in the Fig. 2. However, the demand for the second GBIRD element is unfortunate if the experiment is to be used to study molecules with short transverse relaxation times. When considering the above, two improvements can be considered: (i) how to refocus J_{CH} during the single GBIRD element, and (ii) how to minimize the parent signal leakage caused by J_{HH}.

It should be noted that, in theory, the long-range coupled proton magnetization could also contribute to the apparent parent signal intensity. The product operator calculations show that the intensity of the ^{13}C-bound proton signal for single-GBIRD is (neglecting homonuclear proton coupling):

$$I_{^{13}\text{C-H}} \propto 0.5[1 - \cos(\pi J_{\text{CH}}\Delta)]. \quad (3)$$

For a ^{12}C-bound proton with heteronuclear long-range coupling of 5 Hz, the intensity of ^{12}C-bound proton is about 0.3% of the intensity of ^{13}C-bound proton, if BIRD is optimized for one-bond coupling of 145 Hz. Therefore, the long-range heteronuclear couplings will have only marginal effect to the intensity of residual parent signal.

Earlier studies have shown that the CPMG [16,17] sequence can be used to suppress the J evolution [18–20], if the condition $\tau < 1/(2\sqrt{J^2 + \Delta\nu^2})$ is satisfied. The symbols τ and $\Delta\nu$ represent half of the inter-pulse delay, and the chemical shift difference of the coupled spins, respectively. For weakly coupled spin systems the approximated condition $\tau < 1/(2\Delta\nu_{\max})$, where $\Delta\nu_{\max}$ is the largest shift difference of the coupled spins, is sufficient. The lower limit of τ is defined by the duty cycle of the spectrometer. For protons, the J and $\Delta\nu$ are usually small enough to enable the J coupling suppression. Consequently, if CPMG pulse train is applied at the same time on a heteronucleus, polarization can be transferred through J_{XH} as the chemical shift difference between the proton and the heteronucleus is large enough, while the J_{HH} evolution can be suppressed [21,22]. This concept should not be mixed with HEHAHA [23–25], as in the current approach the Hartmann–Hahn condition $\gamma_H B_{1H} = \gamma_C B_{1C}$ should not be fulfilled. However, typically this is not an issue, because to attain the Hartmann–Hahn condition between heteronuclei needs

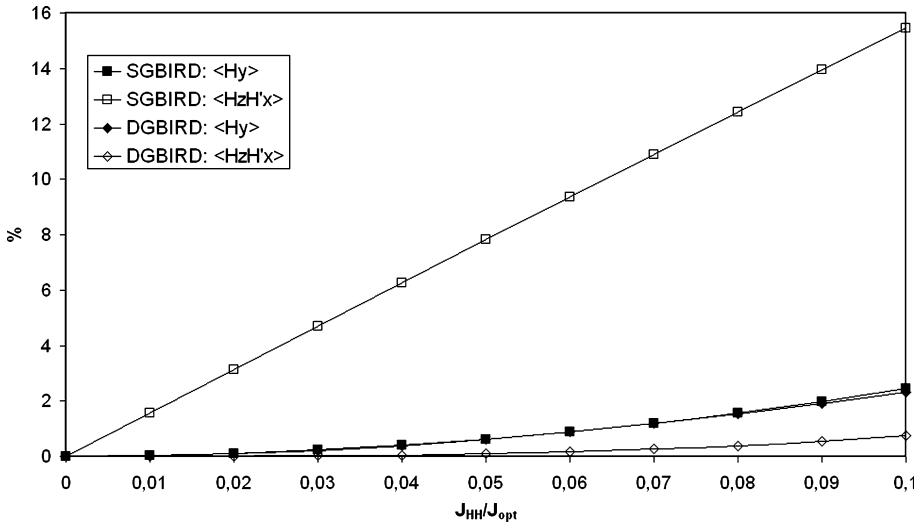

Fig. 2. Theoretical parent signal leakage (terms $<H_y>$ and $<H_zH'_x>$) for homonuclear AX-spin system (HH′) as a function of J_{HH}/J_{opt} ratio with single-GBIRD (SGBIRD) regarding Eq. (1) and double-GBIRD (DGBIRD) regarding Eq. (2). The J_{opt} stands for a coupling to which the delay Δ is optimized for. The intensity is shown in percentages from the total proton magnetization.

precise adjustment as such. For polarization transfer between ^{1}H and heteronucleus a CPMG type sequence called XY-16 [26] is preferred over the original CPMG pulse train, as the XY-16 sequence will preserve the magnetization on all of the Cartesian coordinates, and it has a good tolerance for B_1 inhomogeneity. Consequently, it has been used as a building block of a CPMG-INEPT sandwich in heteronuclear shift correlated experiments due to its benefits with the studies of biomolecules with exchange-broadened protons [27,28].

Therefore, if the $\Delta/2 - 180_{H,C} - \Delta/2$ element of BIRD is replaced by a XY-16 pulse train, one should achieve much less parent signal leakage due to the suppressed J_{HH} evolution. Moreover, as the XY-16 pulse train consists of an even number of 180° ^{13}C-pulses, the J_{CH} evolution is refocused, and a pure phase spectrum can be achieved with resulting single CAGEBIRD (CPMG and gradient-enhanced bilinear rotational decoupling) element (Fig. 1B).

3. Results and discussion

The hypothesis was tested by recording a series of GBIRD and CAGEBIRD spectra from 1,1-dichloroethane. The single-GBIRD spectrum (Fig. 3A) shows

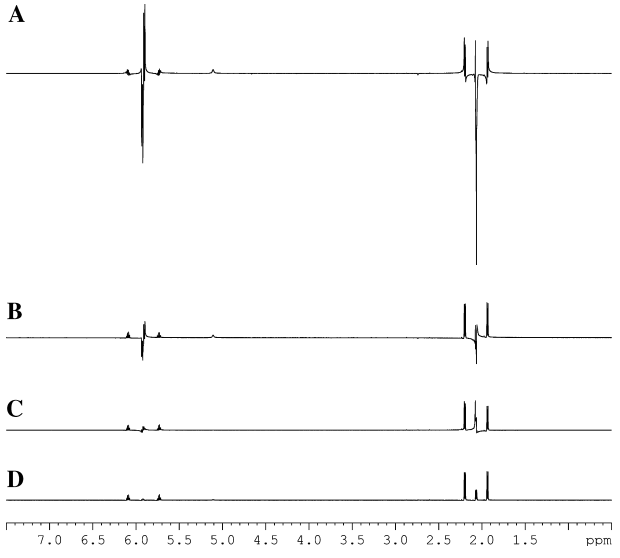

Fig. 3. 1D proton spectra of 1,1-dichloroethane in $CDCl_3$ recorded with single-GBIRD (A), single-CAGEBIRD (B), double-GBIRD (C), and double-CAGEBIRD (D).

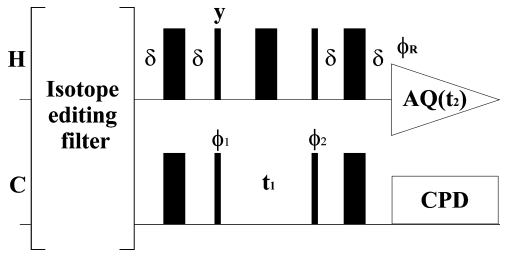

Fig. 4. Pulse sequence for phase-sensitive HSQC sequence with isotope editing filter. The isotope filter was one of the sequences presented in the Fig. 1. For the filters only the first phases presented in the Fig. 1 legends were used. For the HSQC the phase cycles were: $\phi_1 = \{x, -x, x, -x\}$, $\phi_2 = \{x, x, -x, -x\}$, and $\phi_R = \{x, -x, -x, x\}$. The delay δ was 1.72 ms, and the delay Δ used in the isotope editing filter was 6.90 ms. Composite pulse decoupling (CPD) was performed with GARP. Quadrature detection was accomplished by employing TPPI, i.e., by incrementing phase ϕ_1 by 90° in concert with t_1 increment. Spectral widths were 6 and 160 ppm in F2 and F1 dimensions, respectively, with total of 4 k × 400 acquired data points. Squared cosine window function was performed on both dimensions prior to the Fourier transform, and the final spectrum size was 4 k × 512 complex points.

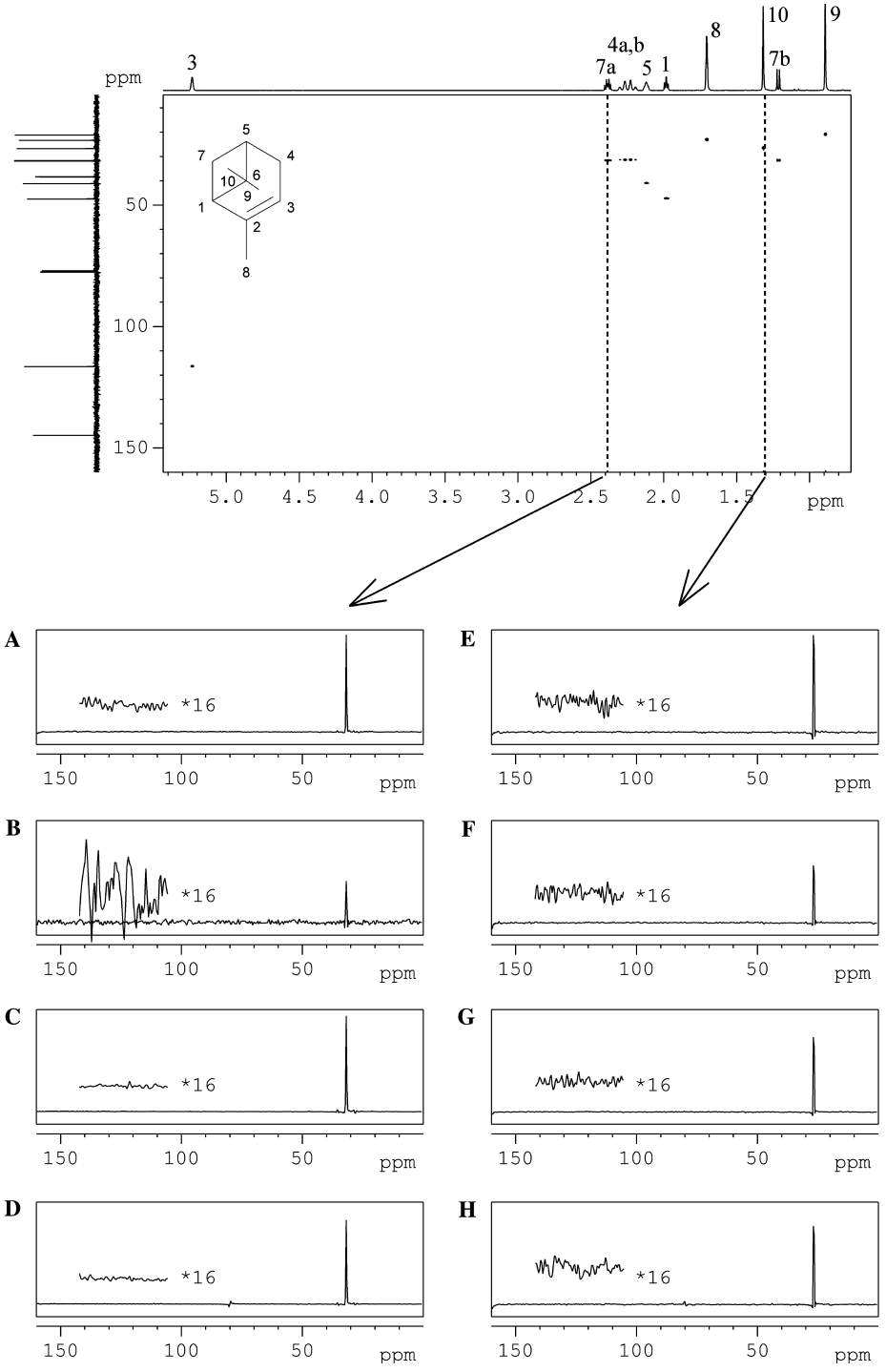

Fig. 5. F1-projections of phase-sensitive HSQC spectra of α-pinene in $CDCl_3$ utilizing single-CAGEBIRD (A, E), single-GBIRD (B, F), double-CAGEBIRD (C, G), and double-GBIRD (D, H) isotope editing. The F1 projections were extracted on the correlation peaks of H-7a (left column) and H-10 (right column) to illustrate intensities of peaks with various filtering schemes. A part of base line is expanded with 16-fold magnitude to illustrate the level of t_1-noise. Spectra intensities in projections are calibrated relative to the intensity of H-7a and H-10 peaks on single-CAGEBIRD-HSQC (A, E).

significant residual parent signals, and ^{13}C-satellite signals are not purely absorptive. In the corresponding single-CAGEBIRD (Fig. 3B) spectrum the parent signals are considerably smaller, and the ^{13}C-satellite signals appear in pure phase. The double-GBIRD improves parent signal suppression as anticipated from Eq. (2), and the J_{CH} evolution is refocused (Fig. 3C). However, the parent signal suppression can be further improved with the double-CAGEBIRD filter (Fig. 3D).

The effect on 2D applications was tested with a phase-sensitive HSQC where the coherence selection was performed by four-step phase cycling (Fig. 4). The method for the parent signal suppression was varied in the beginning of HSQC, but all the other parameters were kept the same. HSQC spectra with four different isotope filters (cf. Fig. 1) were measured from α-pinene. F1 projections on the correlation peaks of H-10 (a proton without homonuclear couplings) and H-7a (a proton with homonuclear couplings) were inspected to compare the effect of different isotope filters to the intensity of correlation peak and t_1-noise (Fig. 5).

First of all, for the proton with homonuclear couplings (H-7a) the t_1-noise is higher in the single-GBIRD filtered spectrum than in the single-CAGEBIRD filtered spectrum (Figs. 5A and B). As was demonstrated in the Fig. 3, the parent signal leakage with the single-GBIRD element can be significant, and the corresponding magnetization can more easily surpass the HSQC, thus contributing to the acquired signal, and causing higher level of t_1-noise compared to the single-CAGEBIRD. Moreover, the single-GBIRD-HSQC gives a little poorer peak intensity than the single-CAGEBIRD-HSQC. This is because the $^1J_{CH}$ evolves during the filter section, and consequently only amount relative to $\cos(\pi J_{CH}\tau_G)$ of the ^{13}C-bound proton magnetization (τ_G is the total length of the gradient delays in GBIRD) will be transferred to carbon during the following INEPT period. For example, if $^1J_{CH}$ is 145 Hz and τ_G is 2.2 ms, only 54% of the magnetization is in-phase with respect to the $^1J_{CH}$ prior to subsequent INEPT period. As expected, for the proton with no homonuclear couplings (H-10) the parent signal leakage is not a major issue, and the t_1-noise levels do not differ significantly between GBIRD and CAGEBIRD (Figs. 5E and F). However, the intensity of peak with the GBIRD is again smaller than with the CAGEBIRD due to the reasons discussed above.

If double filtering scheme is used for the GBIRD, the aforementioned problems can be minimized, but the intensity of correlation peaks will decrease due to the relaxation during the lengthened pulse sequence. The double-CAGEBIRD does not give significant benefit compared to the double-GBIRD filter (Figs. 5C, D, G, and H), which is due to the fact, that although the double-CAGEBIRD will yield better parent signal suppression, the residual parent signals in both methods will be small enough to be effectively suppressed by the four-step phase cycle of HSQC. However, the axial peaks seem to be more pronounced in the double-GBIRD filtered one. The reason can be that the phase cycle employed on the HSQC was not enough to suppress these artifacts caused by imperfect 180° carbon pulses, as has been discussed by Hammarström and Otting [29]. The XY-16 sequence employed in CAGEBIRD is more tolerable to imperfections in B_1-field homogeneity, thus no axial peaks are visible.

At first it may seem that replacing a single 180° pulse with a multi-pulse sequence could cause a strong intensity dependency of resonance offset. This could be unpleasant with an X-nuclei with a large chemical shift region. However, the test have indicated that the XY-16 has a good offset-compensation capabilities (data not shown), thus e.g., 21.55 kHz RF field strength, that was used throughout the experiments for the carbon channel, was sufficient for 200 ppm chemical shift region.

In conclusion, an enhanced version of the GBIRD filter, CAGEBIRD, is presented. The current approach, contrary to the original method, gives improved parent signal suppression, and pure in-phase spectrum can be recorded utilizing only single element. This can be considered beneficial in pulse sequences that have previously employed a GBIRD type editing, for example in many 2D applications. The CPMG approach should also be applicable to BIRD propagator to be used in any BIRD-related experiments.

4. Experimental

The spectra were measured with a Bruker DRX 500 spectrometer equipped with a 5 mm BBI probe with z-gradient at 300 K. The 1D experiments were performed with a sample prepared by dissolving 63 mg of 1,1-dichloroethane in 0.5 ml of $CDCl_3$. The 2D experiments were performed with a sample made of 68 mg of α-pinene dissolved in 0.5 ml of $CDCl_3$.

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