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Communication

Extending the limits of the selective 1D NOESY experiment with an improved selective TOCSY edited preparation function

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Abstract

Compared to its 2D counterpart, the selective 1D NOESY experiment offers greatly simplified spectral interpretation and is invaluable to the structure elucidation of small-to-medium sized molecules, although its application is limited to well-resolved resonances only. The doubly selective 1D TOCSY–NOESY experiment allows the 1D NOESY experiment to be extended to resonances within overlapped spectral regions. However, existing methods do not address the critical issue of zero-quantum interference, which leads to severe anti-phase distortions to the line shape of scalar coupled spins and often complicates the identification of weak NOE enhancements. In this communication, we describe an improved selective TOCSY edited preparation (STEP) function and its application to the selective 1D NOESY experiment. The STEP function incorporates a novel zero-quantum filter introduced by Thrippleton and Keeler [Angew. Chem. Int. Ed. 42 (2003) 3938], which permits essentially complete suppression of zero-quantum coherence in a single scan. Residual anti-phase distortions due to spin-state mixing are removed using the double difference methodology reported by Shaka et al. [45th Experimental NMR Conference, Pacific Grove, USA, 2004]. The combined use of these techniques ensures that the final spectra are free of distortions, which is crucial to the reliable detection of weak NOE enhancements. Although employed as an additional preparation period in the example demonstrated here, the STEP function affords a general editing tool for spectral simplification and can be applied to a range of experiments.

Keywords: Selective TOCSY edited preparation; Zero-quantum coherence; Zero-quantum filter; STEP-NOESY; TOCSY–NOESY; Double difference NOE; Spectral editing

1. Introduction

The two-dimensional (2D) NOESY experiment [1] provides spatial information for all nuclei in a single experiment and has proven to be one of the most valuable NMR experiments in structure elucidation. For small-to-medium sized molecules, however, selective one-dimensional (1D) methods [2–8] for the detection of small NOE enhancements remain an attractive alternative, especially when only a restricted set of NOEs suffices to answer the questions at hand. In such cases, the 1D methods often greatly simplify the spectral analysis, since all irrelevant resonances are absent in the final spectra, and provide the answers significantly faster than their 2D counterpart.

The selective 1D NOE experiments in part owe their popularity to the advent of pulsed field gradients (PFGs) [9,10], which greatly facilitate the suppression of unwanted signals and coherence transfer pathways. Among these, the transient selective NOE experiment utilizing the double pulsed field gradient spin echo (DPFGSE) or so-called "excitation sculpting" technique introduced by Shaka et al. [11] has achieved considerable success. The DPFGSE–NOE sequence makes it possible to detect NOE enhancements as low as 0.02%

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[6,7], thus substantially extending the distance range over which the NOE can be qualitatively interpreted.

However, the usefulness of the selective 1D methods is limited by their reliance on the presence of well-resolved peaks. To extend the applicability of these selective 1D experiments, an additional preparation period that *selectively* transfers magnetization from a targeted, well-resolved resonance to those within overlapped spectral regions can be prepended to the current preparation period. The resultant pulse sequence in this case is a doubly selective NOESY experiment. An appropriate candidate for such an additional preparation period is a selective TOCSY-type transfer step [12], since highly efficient *in-phase* coherence transfer can be achieved using available isotropic mixing sequences.

Applications of the doubly selective 1D TOCSY–NOESY experiment have been previously explored by others [13–16]. However, these proposed pulse sequences do not address the critical issue of homonuclear zero-quantum (ZQ) interference, which is particularly problematic in TOCSY and NOESY experiments. In TOCSY-type magnetization transfer, zero-quantum coherence (ZQC) is explicitly generated by the isotropic mixing sequence; in a selective NOESY experiment, uneven excitation within a given multiplet can also result in ZQC [7]. Zero-quantum coherence usually leads to antiphase dispersive distortions to the line shapes of scalar coupled spins, which can be particularly deleterious in transient NOE difference spectra as they complicate the identification of weak NOEs.

Although substantial efforts have been devoted to the development of effective zero-quantum filters (ZQFs) for the suppression of ZQ interference [17–21], the real breakthrough came only very recently. Thrippleton and Keeler [22] reported a highly efficient ZQF that combines the use of frequency-swept inversion pulses and simultaneously applied strong PFGs. The most prominent feature of this proposed ZQF is that, unlike with the commonly used z-filter [17] where several transients with systematically selected delays must be coadded, suppression of ZQC is achieved in a *single* scan. In this communication, we demonstrate the application of this novel ZQF to the doubly selective 1D TOCSY–NOESY experiment to achieve the highest spectral quality.

2. Results and discussion

The pulse sequences considered in this work are shown in Fig. 1. The ZQF proposed by Thrippleton and Keeler [22] is illustrated in Fig. 1A. At the heart of this sequence is a frequency-swept inversion pulse applied simultaneously with a PFG along the z-axis. Much like in magnetic resonance imaging, the application of the gradient maintains a spatially dependent distribution of the Larmor frequency within the sample volume throughout the course of the inversion pulse. Consequently, spins at different locations within the sample will be inverted at different times by the frequency-swept

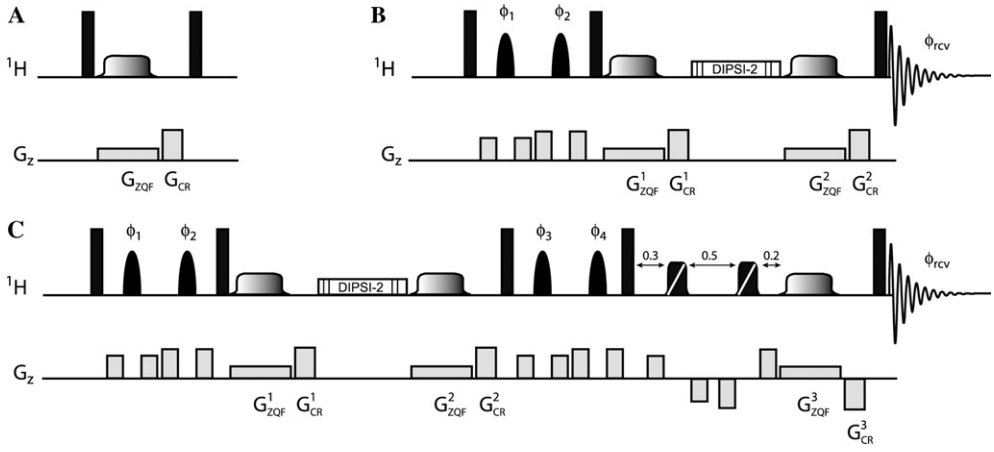

Fig. 1. Timing diagrams of the pulse sequences used in this study. Hard 90° pulses are represented by solid bars, and selective 180° pulses by solid shaped icons. Frequency-swept inversion pulses used in the ZQFs are indicated by the shaded icons. All pulses have phase x unless noted otherwise. All gradient pulses were applied along the z-axis with no shaping. All shaped pulses were generated using the program Pandora's Box [28] available as part of the Varian NMR software. (A) The ZQF proposed by Thrippleton and Keeler [22]. The shaded pulse can be any frequency-swept pulse with a linear inversion profile in frequency. (B) The selective 1D TOCSY pulse sequence incorporating the ZQF. This becomes the selective TOCSY edited preparation (STEP) function if acquisition is omitted. Selective excitation of the target spin for the isotropic mixing period is achieved using the DPFGSE sequence. The ZQFs both before and after the isotropic mixing period need to be carefully adjusted to avoid accidental refocusing of the ZQC. The basic phase cycling is $\phi_1 = x, y, -x, -y$; $\phi_2 = x$; $\phi_{rcv} = x, -x$. If desired, EXORCYCLE phase cycling [29] can be also applied to ϕ_2 to yield a 16-step phase cycle. (C) The doubly selective 1D STEP–NOESY pulse sequence. Selective excitation of the target spins for both the TOCSY and the NOE mixing periods utilizes the DPFGSE sequence. "Nulling" 180° pulses denoted by the scored icons serve to minimize subtraction errors due to relaxation [6,7]. The basic phase cycling is $\phi_1 = x, y, -x, -y$; $\phi_2 = x$; $\phi_3 = 4(x), 4(y), 4(-x), 4(-y)$; $\phi_4 = x$; $\phi_{rcv} = 2(x, -x), 2(-x, x)$. If desired, EXORCYCLE phase cycling can be independently applied to ϕ_1 through ϕ_4 to yield a 256-step phase cycle.

180° pulse. While this has no net effect on the z-magnetization other than the inversion, it causes the ZQC, which evolves at the chemical shift difference between the two scalar coupled spins involved, to acquire a spatially dependent phase at the end of the ZQF, thus leading to effective cancellation of the ZQC in a *single* scan. The ensuing crusher gradient designated G_{CR} serves to dephase any nonzero-order coherence.

Several practical considerations of the ZQF are in order. For the frequency-swept inversion pulse, any shape possessing a linear inversion profile in frequency can be used. The sweep width of the inversion pulse is usually much larger than the spectral window, and the duration of the sweep must be sufficiently long so as to give the ZQC time to dephase. The gradient strength G_{ZQF} needs to be carefully adjusted to match the inversion bandwidth of the frequency sweep. If equipped with triple axis gradients, two shorter ZQFs, one with a z-gradient and the other with a transverse gradient, can improve the suppression still further, as suggested by Cano et al. [23]. In our hand, a ~30–50 ms constant-adiabaticity WURST sweep [24] in conjunction with a properly optimized z-gradient generated satisfactory results.

Fig. 1B shows the timing diagram of the 1D ZQF–TOCSY pulse sequence [14,25]. Selective excitation is achieved using the DPFGSE sequence [11]. The longitudinal TOCSY as shown allows convenient incorporation of the ZQF. Since the strong coupling Hamiltonian governing the isotropic mixing sequence causes an interchange between z-magnetization and ZQC, it is necessary to apply the ZQF both before and after the isotropic mixing period. To avoid accidental refocusing of the ZQC, different durations should be used for the two ZQFs.

To distinguish our method from existing methods [13–16], as well as for the sake of simplicity, we will refer to the selective 1D ZQF–TOCSY sequence of Fig. 1B as the STEP (*s*elective *T*OCSY *e*dited *p*reparation) function hereinafter. Its incorporation into the 1D NOESY experiment is straightforward, as shown in Fig. 1C. This results in a doubly selective experiment, with selective excitation of the target spin for each module achieved using the DPFGSE sequence. The STEP function transfers magnetization from the target spin to its scalar coupled partners, while eliminating all other resonances. The second half of the pulse sequence is simply the DPFGSE–NOESY sequence [6,7] with an additional ZQF at the end of the NOE mixing period. The target spin for the NOE mixing period can now be any resonance within the subspectrum of the scalar coupled spin

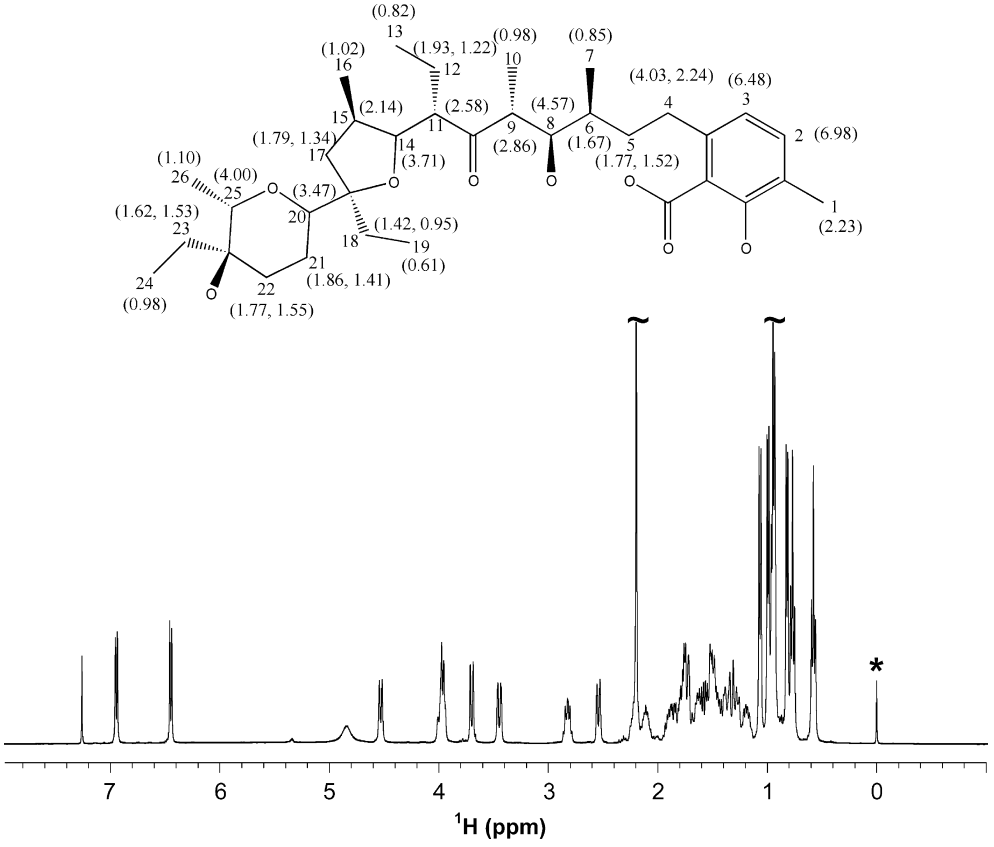

Fig. 2. D ^{1}H spectrum of lasalocid (in $CDCl_3$) and its structure. The proton chemical shift assignments are designated in parentheses. The asterisk in the 1D ^{1}H spectrum indicates TMS used as an internal chemical shift reference. All NMR spectra were recorded at 25 °C on a 400 MHz Varian UnityInova spectrometer equipped with a 5 mm ^{1}H{X} indirect detection probe and z-gradient accessory.

system, provided that it is sufficiently well-resolved from the rest of the peaks. In practice, the isotropic mixing period is optimized so as to maximize coherence transfer to the target spin for the NOESY mixing period.

The 1D ^{1}H spectrum of lasalocid used in this study is shown in Fig. 2 along with its molecular structure and ^{1}H chemical shift assignments. The 1D ^{1}H spectrum shows substantial overlap, with only a small number of well-resolved peaks in the region $\delta > 2.4$ ppm. To demonstrate the efficiency of the new ZQF, Fig. 3 compares the selective 1D TOCSY spectra of proton H_{14} obtained using the pulse sequence of Fig. 1B, with and without the ZQFs. In the case where no ZQ filtration is applied (upper panel), the anti-phase contributions by ZQC to the multiplet patterns are evident. These anti-phase distortions are largely absent when two ZQFs with appropriately adjusted durations and gradient strengths are applied (lower panel). The apparently higher resolution exhibited by H_{15} in the absence of ZQ filtration (upper panel) is only a deception attributable to anti-phase components introduced by ZQ interference, as can be confirmed by comparison with the 1D ^{1}H spectrum.

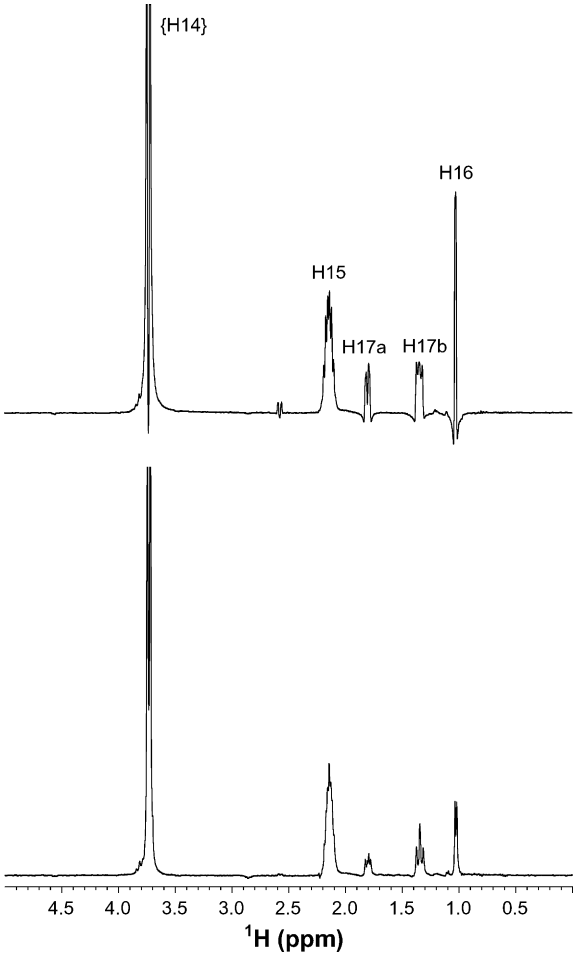

Fig. 3. Selective 1D TOCSY spectra of lasalocid-{H_{14}} obtained with the pulse sequence of Fig. 1B, with (lower panel) and without (lower panel) the ZQFs in place. A 20 ms Gaussian 180° pulse was used in the DPFGSE for the selective excitation of H_{14}. The isotropic mixing period was 25 ms using the DIPSI-2 sequence [30] and a 6.25 kHz B_1 field, which generated optimal magnetization transfer to H_{15}. When the ZQFs are omitted (upper panel), the spectrum shows significant anti-phase distortions due to ZQ interference. These distortions are essentially absent when the ZQFs are included (lower panel). A 30 or 50 ms constant-adiabaticity WURST inversion pulse [24] with a bandwidth of 20 or 30 kHz was used in each of the ZQFs, respectively. The gradients were optimized accordingly as described by Thrippleton and Keeler [22].

The spin system consisting of protons H_{14}, H_{15}, H_{16}, H_{17a}, and H_{17b} provides an excellent example for the STEP–NOESY experiment shown in Fig. 1C. In the 1D ^{1}H spectrum (Fig. 2), with the only exception of H_{14}, all of these peaks show at least some degree of overlap, rendering them unsuitable for direct selective excitation. With the additional spectral editing step provided by the STEP function, it is now straightforward to selectively excite each one of the resonances and observe its NOE correlations.

The doubly selective 1D STEP–NOESY spectrum of {H_{15}{H_{14}}} obtained with the pulse sequence of Fig. 1C is shown in Fig. 4A, where the shorthand notation {H_b{H_a}} denotes H_a as the target spin for the STEP function, and H_b for the NOE mixing period. All of the NOE correlations can be assigned in a straightforward manner. However, there still exist significant distortions to the multiplets of those spins scalar coupled to H_{15}. This is better exemplified in the NOE buildup of H_{17a}, shown in Fig. 4B. The anti-phase distortions are evident for shorter mixing times, while for intermediate to longer NOE mixing times, such distortions are seemingly absent. However, the H_{17a} peak appears to be a doublet, while all other available evidences (e.g., its coupling to both H_{17b} and H_{15}) suggest that it should be a doublet of a doublet.

As pointed out by Shaka and co-workers [26,27], such distortions are not the result of ZQC leakage through the ZQFs. Instead, their origin is a phenomenon known as spin-state mixing, which is encountered in strongly scalar coupled spin systems. Even though these distortions are generally rather small, they pose a significant problem in the NOE experiment, since the signals of interests are usually very weak. These authors further demonstrated that, by subtracting a reference spectrum with ''zero'' mixing time, these distortions can be largely removed. This results in a *double difference* spectrum. The same principle can be applied to the STEP–NOESY experiment. Fig. 4C shows the double difference STEP–NOESY spectrum of {H_{15}{H_{14}}}. Note that there is a slight reduction in the signal-to-noise compared to the single difference spectrum of Fig. 4A. However, the correct multiplet patterns are now restored for all of the spins coupled to H_{15}. For comparison, the distortion-free NOE buildup of H_{17a}

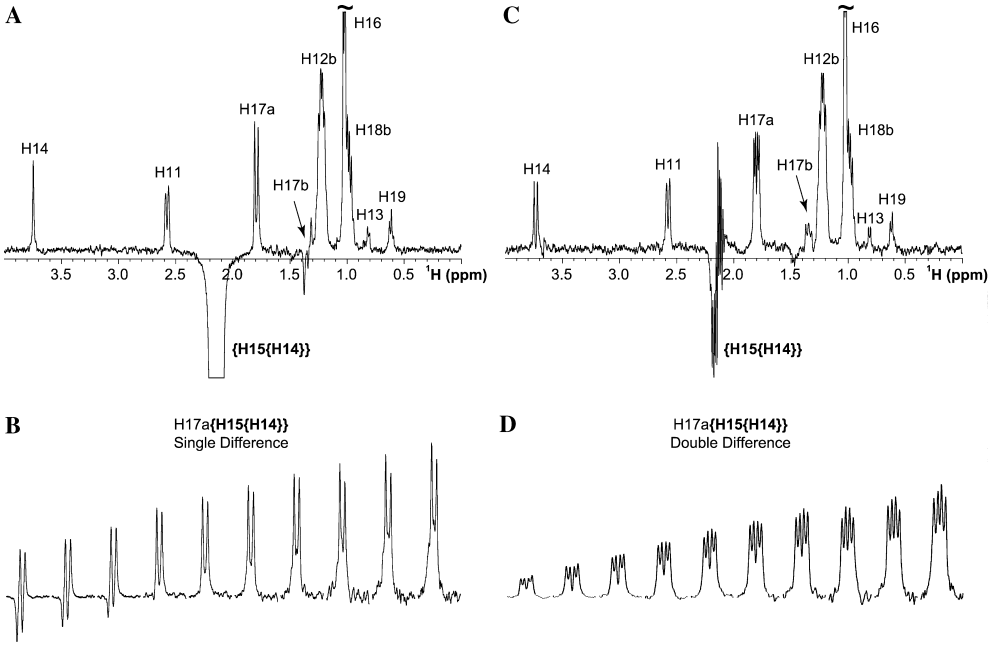

Fig. 4. Comparison of the single (A and B) and double difference (C and D) 1D STEP–NOESY spectra of lasalocid-{H_{15}{H_{14}}} recorded with the pulse sequence of Fig. 1C. Each FID was acquired with 1024 transients. Selective excitation of both H_{14} and H_{15} was achieved with the DPFGSE sequence using a 20 ms Gaussian 180° pulse. The isotropic mixing period for the STEP function was 25 ms, which was optimized using the pulse sequence of Fig. 1B to yield maximum magnetization transfer to H_{15}. The two ZQFs in the STEP function were identical to those described in Fig. 3. The ZQF at the end of the NOE mixing time used a 50 ms constant-adiabaticity WURST sweep with a 30 kHz bandwidth. Two 1.5 ms hyperbolic secant 180° pulses were used for the "nulling" pulses. (A) The single difference 1D STEP–NOESY spectrum of {H_{15}{H_{14}}} using a NOE mixing time of 500 ms. Note the anti-phase distortions for the multiplets of H_{14}, H_{16}, H_{17a}, and H_{17b}. (B) The NOE buildup of H_{17a} {H_{15}{H_{14}}} obtained from the single difference spectra. The NOE mixing time was increased systematically from 100 to 1000 ms in 100 ms steps. The target intensity in each spectrum was normalized, therefore the differences in the signal-to-noise. (C) The double difference 1D STEP–NOESY spectrum of {H_{15}{H_{14}}} using a NOE mixing time of 500 ms. It was obtained by taking the spectral difference between the single difference spectrum of (A) and the nominally "zero" mixing time spectrum, which is recorded by setting the NOE mixing delays to zero, but leaving the ZQF, the two nulling 180° pulses, and the associated gradient pulses intact. (D) The distortion-free NOE buildup of H_{17a}{H_{15}{H_{14}}} obtained from the double difference spectra. The NOE mixing times were the same as in (B).

obtained using the double difference methodology is shown in Fig. 4D.

3. Conclusions

We have demonstrated the application of an improved selective TOCSY edited preparation function to the doubly selective 1D TOCSY–NOESY experiment. The incorporation of a novel ZQF into the STEP function as well as the NOE mixing period permits highly efficient ZQ suppression within a single scan. Additional anti-phase distortions due to spin state mixing can be conveniently removed using the double difference methodology introduced by Shaka et al. [27]. The combined use of these methods allows one to obtain very high quality NOE spectra for the reliable identification of weak NOE enhancements, which would otherwise be hindered by the presence of anti-phase components due to ZQ interference. Moreover, the STEP function described here affords a general strategy for spectral simplification and can be applied to a wide range of experiments. One such example is to incorporate the STEP function into the 2D DOSY experiment [31] as a spectral editing period, which would greatly simplify the analysis of complex mixtures of drug metabolites. A detailed description of applications along this line will be presented elsewhere.

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