

Sensitivity Improvements in Selective $^1\text{H}-^{13}\text{C}$ 1D Polarization-Transfer Schemes

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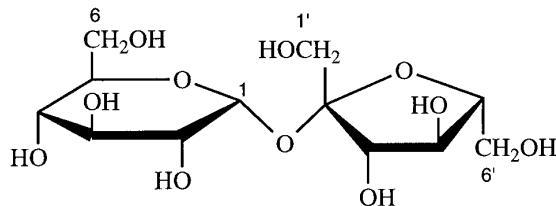
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Recently, many new high-resolution NMR experiments using pulsed field gradients (PFG) for coherence selection have been proposed which offer a much better spectral quality than analogous phase-cycled experiments in generally shorter total experiment time (1). Specifically, the implementation of PFG in selective homo- and heteronuclear 1D experiments (2) affords a powerful tool for obtaining high-quality 1D spectra without the presence of undesirable artifacts arising from the imperfect subtraction inherent in the conventional phase-cycling selection. Special attention has been focused on some heteronuclear gradient-based 1D schemes featuring selective polarization transfer from carbon to proton using the basic HMQC and HSQC pulse trains (3–6), which can also be used as starting building blocks in more sophisticated 1D sequences such as 1D ^{13}C -edited TOCSY, NOESY, or ROESY experiments (4). Interesting advantages of such gradient-based experiments are the lack of need for a complete phase-cycle process, the perfect suppression of the large unwanted $^1\text{H}-^{12}\text{C}$ magnetization without the need of the BIRD cluster, the minimization of dynamic range limitations, and the better tolerance to errors in parameter setup or to spectrometer instabilities. However, these gradient 1D experiments suffer from the theoretical loss of sensitivity by a factor of 2, compared to phase-cycled analogues, due to the selection by the gradients of only one of the two coherence-transfer pathways (CTPs) that can contribute to the detected signal. In practice, the use of a much higher setting of the receiver gain in PFG experiments partially overcomes these sensitivity drawbacks.

In the present work, the effectiveness of heteronuclear polarization transfer for several gradient-based 1D schemes is experimentally examined in terms of sensitivity. In addition, new modified 1D pulse sequences offering improved sensitivity for this heteronuclear polarization transfer are also proposed and discussed. These improved experiments use the known principle of the preservation-of-equivalent-pathways (PEP) approach (7, 8), currently implemented in many multidimensional heteronuclear experiments. This is

based on the simultaneous selection in a single scan of both orthogonal magnetization components present during an evolution period, and when applied to 1D experiments, no extra requirements in the acquisition or processing steps are needed. As a result, and in accordance with the theoretical predictions (9–11), a maximum increase of the signal-to-noise ratio by a factor of 2 and 1.41 can be achieved for IS and I_2S systems, respectively. To the best of our knowledge, this is the first application of the PEP methodology in a one-dimensional NMR experiment.

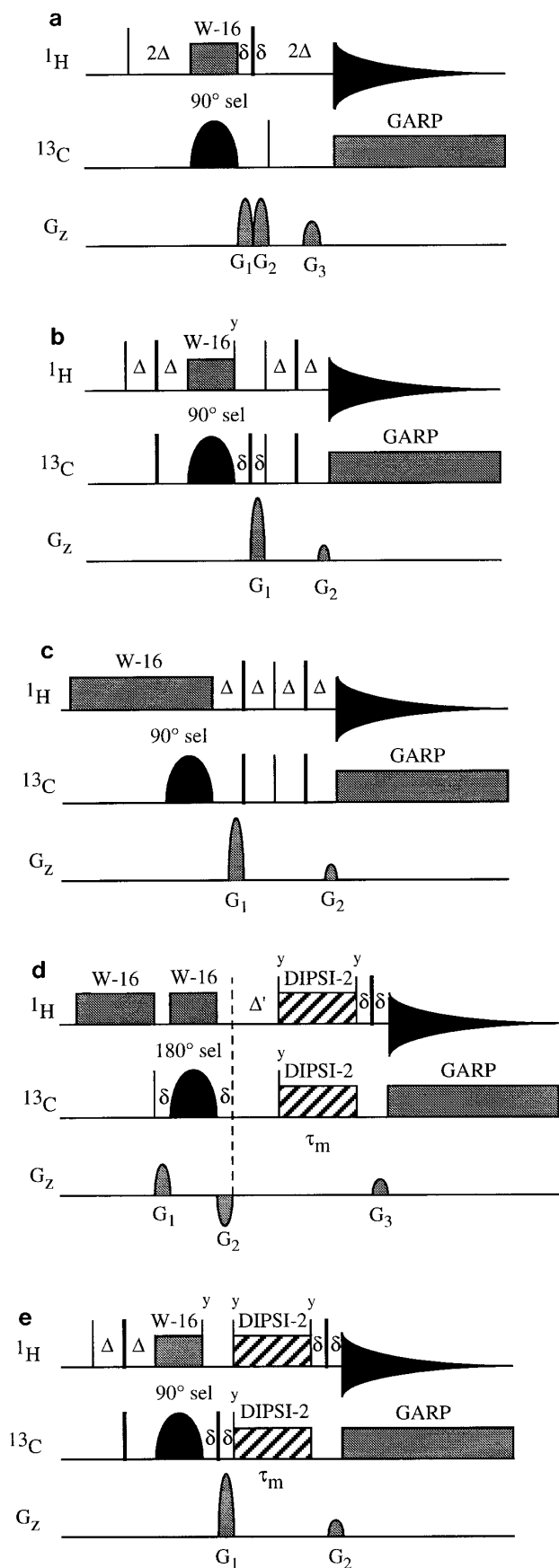
Figure 1 shows several pulse sequences that we have experimentally tested. They are all designed to achieve selective polarization transfer from a specific carbon to its directly attached proton via $^1J_{\text{CH}}$. In order to make an accurate comparative study, all experiments have been recorded using the same experimental conditions in a Bruker ARX400 spectrometer using an inverse broadband probehead incorporating a self-shielded Z-gradient coil. As a model, we have chosen the H-1/C-1 pair ($^1J_{\text{CH}} = 169.8$ Hz) of a 0.2 M sucrose solution in D_2O :



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Four scans preceded by two dummy scans were acquired for each 1D spectrum using a prescan delay of 1 s, an interpulse delay Δ ($=1/4^1J_{\text{CH}}$) of 1.5 ms, and an acquisition recording time of 1.27 s (Fig. 2). The length of each gradient (shaped as a 5% truncated Gaussian) was 1 ms and the maximum strength was set at 20 G/cm. Selective 90° or 180° carbon pulses with a duration of 12 ms also had a Gaussian profile truncated to 5%. A WALTZ-16 pulse train was simultaneously applied to achieve better selectivity during the application of these selective carbon pulses and to

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avoid chemical-shift precession (12). Figure 3 shows the performance of these experiments as a function of both length and field strength of the selective carbon pulse. As shown, these experiments were found to be very tolerant to miscalibration of the selective carbon pulse.

The first sequence (Fig. 1a) is based on the classical four-pulse HMQC experiment, in which one of the two carbon pulses is made selective (2–4). As known for the analogous 2D experiment, several gradient combinations can be used for proper signal rephasing (13). In practice, they all gave similar sensitivity results, and we have used a 3:5:0 ratio which selects DQC (I^+S^+) and ZQC (I^-S^+) during the first two gradients. The second approach is based on the HSQC pulse train (Fig. 1b) (2, 4–6) in which a single gradient G_1 is used as a dephasing gradient during the carbon evolution period. This gradient is inserted into a spin-echo period in order to avoid chemical-shift evolution. Finally, the gradient G_2 applied with a 4:1 ratio just prior to acquisition selects the desired $S^+ \rightarrow I^-$ pathway. Theoretically, the sensitivities of ge-1D HMQC and HSQC should be the same. However, in our hands, the ge-1D HMQC experiment works slightly better than the ge-1D HSQC experiment, probably because it contains fewer pulses (Fig. 2a vs 2b). Poorer results (Fig. 2c) are obtained using the scheme based on a reverse INEPT pulse train (named ge-1D XINEPT experiment) with a 4:1 gradient ratio (Fig. 1c). In this case, the pulse sequence starts with a selective 90° carbon pulse and the enhancement factor is therefore governed by the initial NOE buildup step. An additional drawback of this sequence is that the repetition time between scans (prescan delay) depends on the T_1 relaxation time of the selected carbon. As a comparison, the dependence of the signal intensity vs the prescan delay for the ge-1D HSQC and the ge-1D XINEPT experiments showed that full relaxation is achieved after 0.1 and 1.5 s, respectively (Fig. 4).

Another possible approach is the heteronuclear polarization transfer by cross polarization (CP), termed a ge-1D CP experiment (Fig. 1d), in which the heteronuclear transfer is achieved by applying simultaneous spin-lock fields fulfilling the Hartmann–Hahn condition for both proton and carbon nuclei (14–16). Gradient selection avoids the need for proton presaturation during the relaxation period, and selective carbon excitation is achieved at the start of the

FIG. 1. (a) Selective ge-1D HMQC, (b) selective ge-1D HSQC, (c) selective ge-1D XINEPT, (d) ge-1D CP, and (e) ge-1D HSQC-CP pulse schemes. Hard 90° and 180° pulses are indicated by vertical narrow and wide black bars. Selective 90° or 180° carbon pulses are indicated by shaded shapes. Proton decoupling during the selective carbon pulse is achieved by applying a WALTZ-16 pulse train. Pulsed field gradients of duration δ are also indicated by shaded shapes on the line G_z . The PFG recovery time was $100 \mu\text{s}$ and the maximum strength was 20 G/cm . All pulses are applied from the x axis unless otherwise indicated. A minimum two-step phase cycle was applied in which the initial carbon 90° pulse and the receiver are inverted on alternate scans.

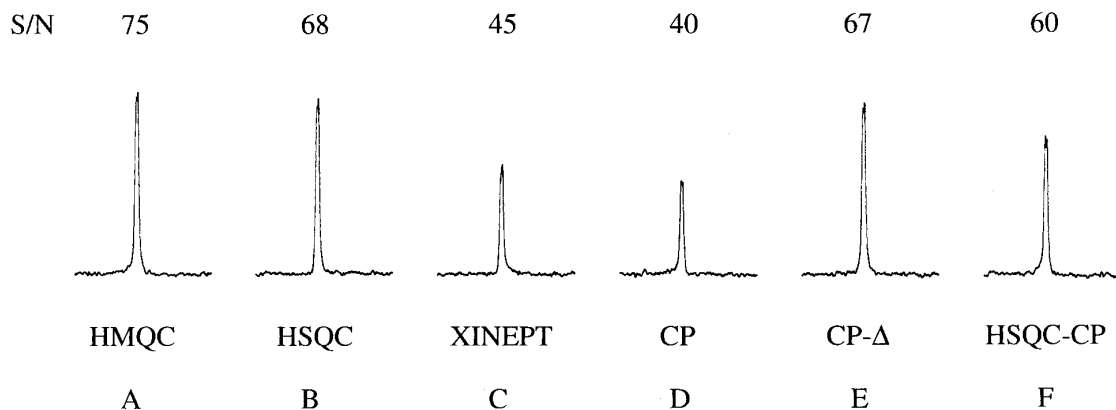


FIG. 2. Relative intensity of the H1 signal after applying the pulse sequences of Fig. 1. All spectra are plotted with the same scale. Figures above each signal give the experimental signal-to-noise ratio obtained.

sequence by using a single pulsed-field-gradient echo (SPFGE) consisting of a 90° (^{13}C) — G_1 — 180° (^{13}C , selective) — G_2 . After this, carbon magnetization is allowed to evolve during a period of $\tau_m = 1/|J_{\text{CH}}|$ (in this case, 6 ms) under the effect of a z -filtered DIPSI-2 scheme applied simultaneously for proton and carbon nuclei. Other mixing sequences give similar sensitivity ratios, and details about how to implement these different transfer schemes in several gradient-based cross-polarization experiments will be published later (17). At the end of the CP mixing period, a third gradient G_3 inserted into a spin-echo period is applied to rephase the desired magnetization (2:–2:1 gradient ratio), and the proton signal is finally detected. This experiment shows lower sensitivity than the other proposed experiments, but the signal-to-noise ratio can be improved by a factor of ≈ 2 if a delay Δ' is inserted between the SPFGE scheme and the mixing period (Fig. 2). In this case, sensitivity ratios similar to those obtained with the HMQC and HSQC sequences are achieved. On the other hand, as described for the ge-1D XINEPT experiment, the ge-1D CP experiment starts with carbon excitation, and therefore, the prescan delay must be set according to the carbon T_1 relaxation. In order to reduce the length of this delay and the total experiment time, the initial SPFGE block can be replaced by a carbon-selective INEPT pulse train (experiment ge-1D HSQC-CP in Fig. 1e), but the overall sensitivity does not improve (Fig. 2f).

As noted above, only one of the two possible CTP contributing to the detected signal is rephased by the gradients in all experiments of Fig. 1, and therefore, the achievable signal-to-noise ratio is reduced by a theoretical factor of two in comparison with analogous experiments based in phase-cycle selection (18). However, improved sensitivity by a factor of two for IS systems can be obtained using the PEP methodology. We, therefore, propose the new experiments ge-1D HMQC-PEP, HSQC-PEP, and XINEPT-PEP (Fig. 5), in which the PEP methodology is introduced in the usual way (7, 8). Experimentally, this can be achieved by inserting additional blocks (pulses inside the

frames in Fig. 5) into the conventional pulse sequences of Figs. 1a–1c, in order to select both orthogonal components in a single scan. In practice, an improvement by a factor

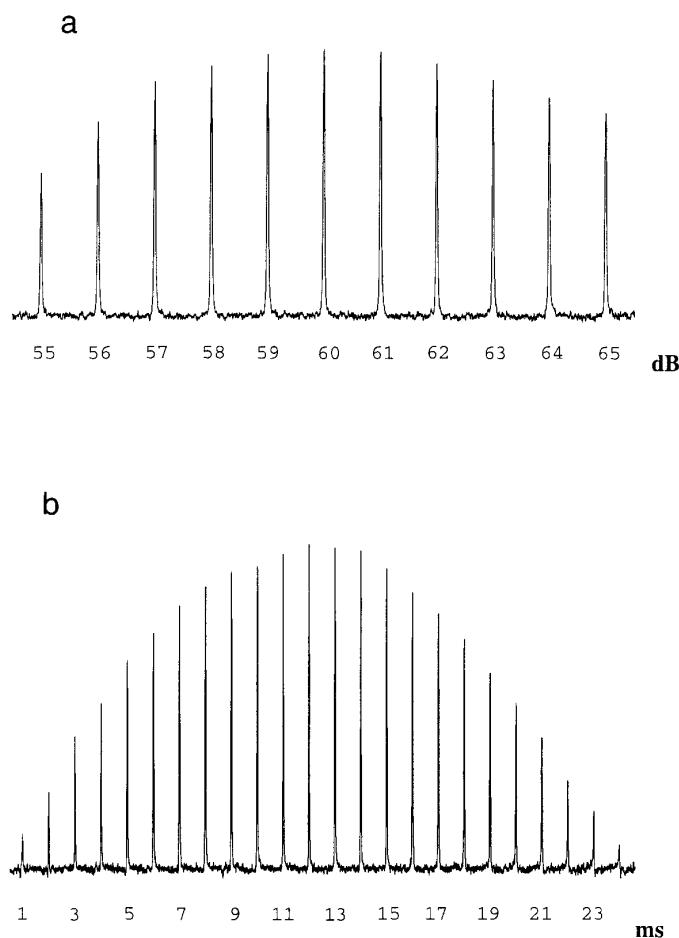


FIG. 3. Experimental dependence of the signal intensity with respect to the (a) field strength and (b) the duration of the selective carbon 90° pulse in the ge-1D HSQC experiment. Eleven and 24 experiments have been performed, respectively, starting from 55 dB with a step of 1 dB in (a) and starting from 1 ms with a step of 1 ms in (b).

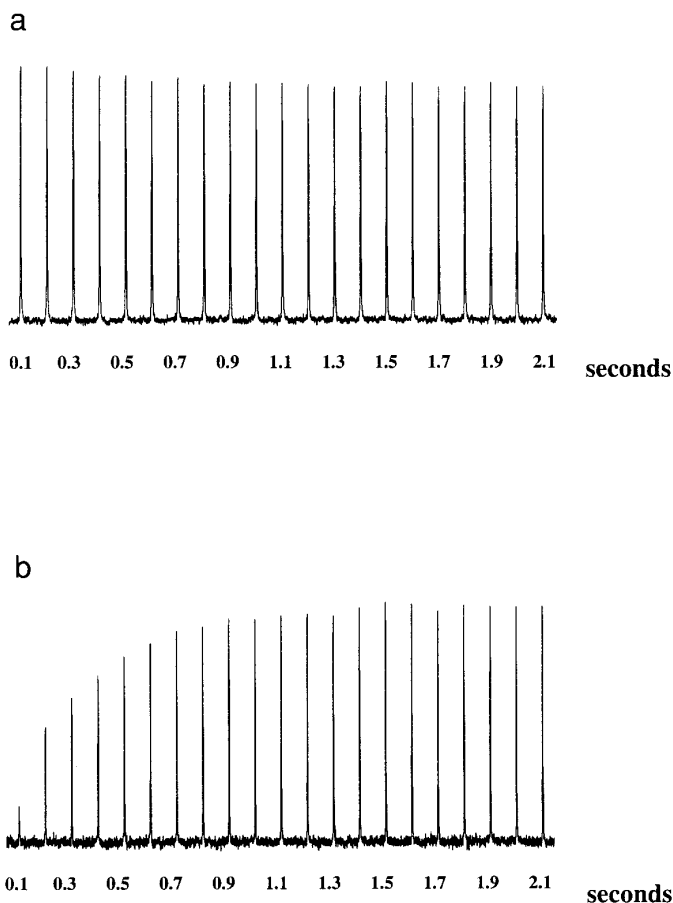


FIG. 4. Experimental dependence of the signal intensity with respect to the prescan delay in the ge-1D HSQC (a) and the ge-1D XINEPT (b) experiments. Twenty-one experiments starting from 0.1 s have been performed with a step of 0.1 s.

of $\approx 1.85\text{--}1.9$ is achieved for all these improved versions (Fig. 6) because more pulses and delays are involved and relaxation effects can become more important. Again, as shown for conventional experiments, the HMQC-based experiment seems to be the more sensitive approach.

A full analysis of these experiments based on the product-operator formalism can be found in Ref. (8). Thus, the classical HMQC, HSQC, and XINEPT blocks allow one to obtain the desired in-phase ^{13}C -labeled proton magnetization. In the PEP-based experiments, a second block (pulses inside the frames in Fig. 5) is placed immediately after the first block in order to convert the usually non-detected coherence into antiphase proton magnetization that after a refocusing period is converted to a detectable in-phase signal. Meanwhile, the magnetization selected by the first block is flipped to the z axis in order to avoid any evolution during the second block. The final 90° proton pulse converts the longitudinal z magnetization into transverse magnetization while the second selected transverse magnetization is unaffected. The final gradient inserted into a spin-echo period serves to rephase the desired signal.

As a crucial point to select both orthogonal components, the second block must be applied with a phase difference of 90° (from the y axis) with respect to the first block (from the x axis).

In summary, we have shown how PEP methodology can be successfully implemented in heteronuclear 1D experiments with gradient selection in order to improve their sensitivity. In addition, no extra acquisition and/or processing conditions are required for these experiments. The experi-

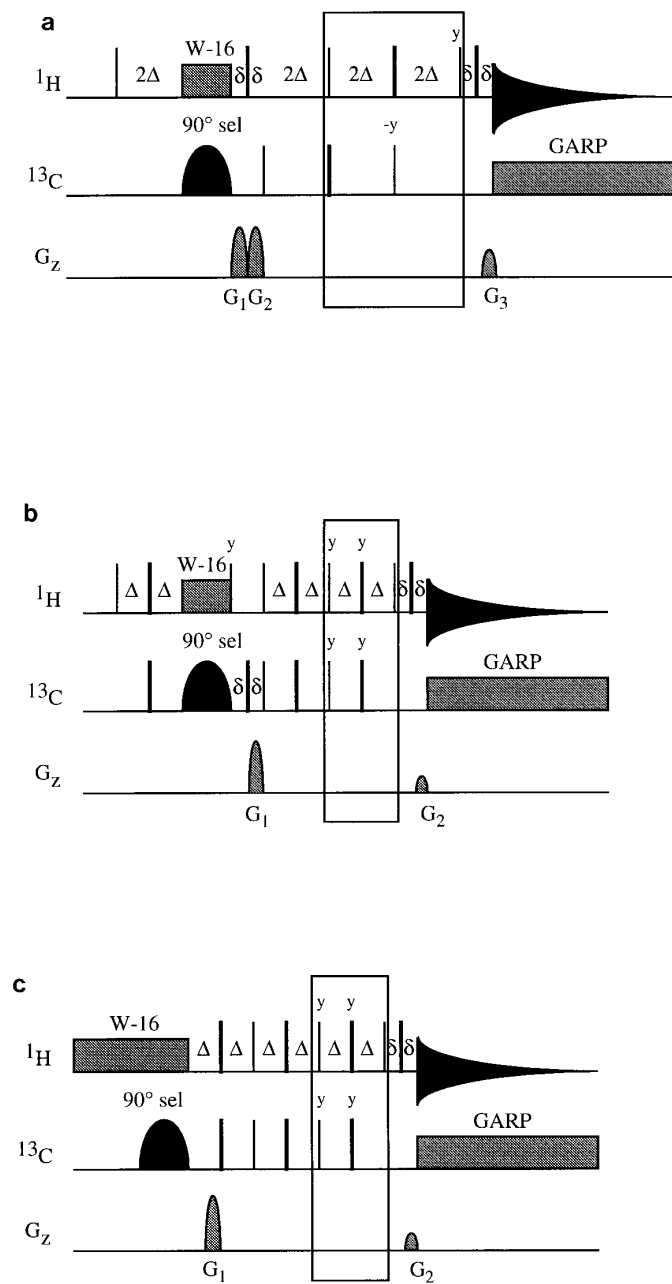


FIG. 5. Pulse sequences of the selective sensitivity-improved (a) ge-1D HMQC-PEP, (b) ge-1D HSQC-PEP, and (c) ge-1D XINEPT-PEP experiments. All details as described in the legend to Fig. 1.

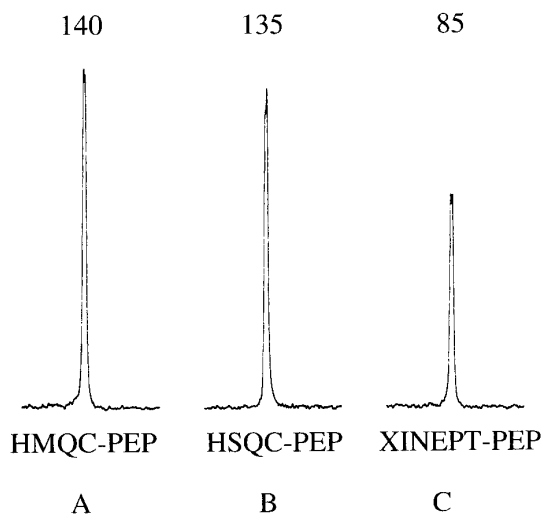


FIG. 6. Relative intensity of the H1 signal after applying the pulse sequences of Fig. 5. All spectra are plotted with the same scale as Fig. 3. Figures above each signal give the experimental signal-to-noise ratio obtained.

mental results obtained confirm the theoretical predictions, and the possible relaxation effects due to additional delays inserted into the new proposed sequences can therefore be neglected. This approach could be also applied to analogous phase-cycled experiments (18).

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