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An Auxiliary RF Channel with Convenient Phase Control for NMR Spectrometers

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When $^{13}C/^{15}N$ double-labeled proteins are available, coherence transfer through backbone atoms can be efficiently accomplished using triple-resonance experiments first introduced by Kay *et al.* (*1*). A judicious combination of efficient triple-resonance experiments allows assignment of backbone resonances. Most of these experiments are actually quadruple-resonance experiments, exciting ^{1}H, ^{15}N, $^{13}C\alpha$, and $^{13}C{=}O$ nuclei. Irradiating four nuclei puts heavy demands on spectrometer hardware. Shifting from $^{13}C\alpha$ to $^{13}C{=}O$ resonance frequencies may be performed for some experiments but is problematic if it disrupts phase coherence or if simultaneous pulses must be delivered at these two frequencies. Thus, adding one or two auxiliary channels to a total of four is desirable to perform these experiments. In many experiments, phase shifting of the external channel is necessary. However, the approach in which the phase shifting is accomplished by using an external phase shifter operated with TTL lines can make programming of quadrature and phase cycles indirect and cumbersome. Herein, we describe straightforward assembly and use of an auxiliary channel with simple and fast switching of phases under phase table control and, optionally, with switching of power levels. We demonstrate the performance of this channel with a slightly modified CT-HNCA experiment (*2*).

The original CT-HNCA (*2*) experiment has the difficulty that the long, rectangular 180° pulse to decouple the $^{13}C{=}O$ resonances during the $^{13}C\alpha$ chemical-shift evolution period is not selective enough to avoid exciting $^{13}C\alpha$ resonances and therefore reduces the sensitivity. The alternative choice of using a shaped 180° pulse has as a drawback that $^{13}C\alpha$ chemical shift evolves significantly during its course. Since $^{13}C\alpha$ signals decay rapidly, this reduces the sensitivity of the first FID and thus the sensitivity of the entire experiment. Very-low-power composite-pulse decoupling, on the other hand, permits a 60% gain in S/N, in our hands, as it avoids both undesired excitation and initial chemical-shift evolution. Composite-pulse decoupling (preferably WALTZ-16) with an RF field strength of only 300 or 400 Hz decouples the $^{13}C{=}O$ spectral region and introduces only negligible Bloch–Siegert shifts of the $^{13}C\alpha$ resonances as was verified by recording HSQC spectra (not shown). This means of carbonyl decoupling was also employed in the original

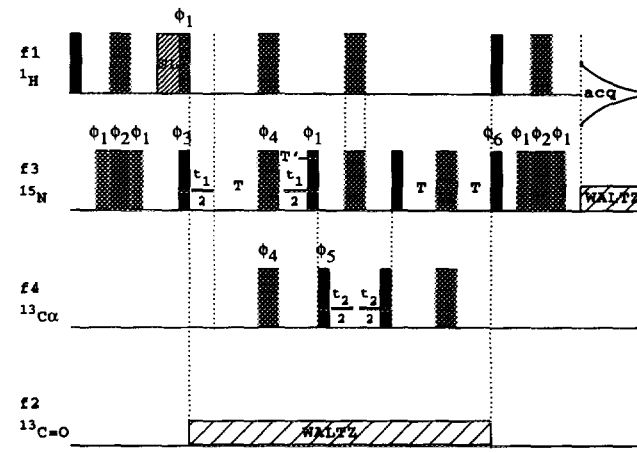

FIG. 1. CT-HNCA (*2*) modified for implementation of an auxiliary fourth channel (f4). Pulses at the $^{13}C\alpha$ frequency are delivered on the auxiliary fourth channel (Fig. 2). This make a native channel available for composite-pulse decoupling of the carbonyl region [WALTZ-16 (*5*) with an RF field strength of 330 Hz]. The phase of the fourth channel is set by the phase of the native, third channel, tuned to ^{15}N. Pulses on these two channels can be cycled independently provided that the pulses do not occur simultaneously. In order to perform the INEPT-like transfer from ^{15}N to $^{13}C\alpha$, the respective 90° pulses are offset (by 3 μs) as shown in order to permit independent phase cycling. A spin-lock pulse of 1 ms is used for water suppression (*6*). The 180° pulses of ^{15}N frequency during INEPT and reverse INEPT transfers between amide proton and nitrogen are of the composite type. Phase cycling is modified from the original sequence (*2*) in order to (a) accommodate the $^{13}C\alpha$ RF being generated from the ^{15}N RF and (b) reduce the phase cycle to 16 steps: $\phi_1 = y, -y$; $\phi_2 = x, -x$; $\phi_3 = x$; $\phi_4 = 4(x), 4(y), 4(-x), 4(-y)$; $\phi_5 = 2(x), 2(-x)$; $\phi_6 = y$; and receiver = $2(x), 4(-x), 2(x)$. Delays, pulse widths, and quadrature detection are performed as described by Grzesiek and Bax (*2*). An example of the syntax for issuing a pulse on the fourth channel, using a Bruker AMX console is

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(3u ph5):f3 ;set phase; "u" represents μsec
1u setf2^5 ;set TTL line high (NMR ctrl f2(5)) to turn on RF
(p7):c4 ; unblank linear amplifier
4u setf2|5 ;set TTL line low to turn off RF
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The power-driven NMR control f2(5) line is accessible from the Bruker AMX console port BP1 S; the RCP4 line is accessible from port BP1 Z.

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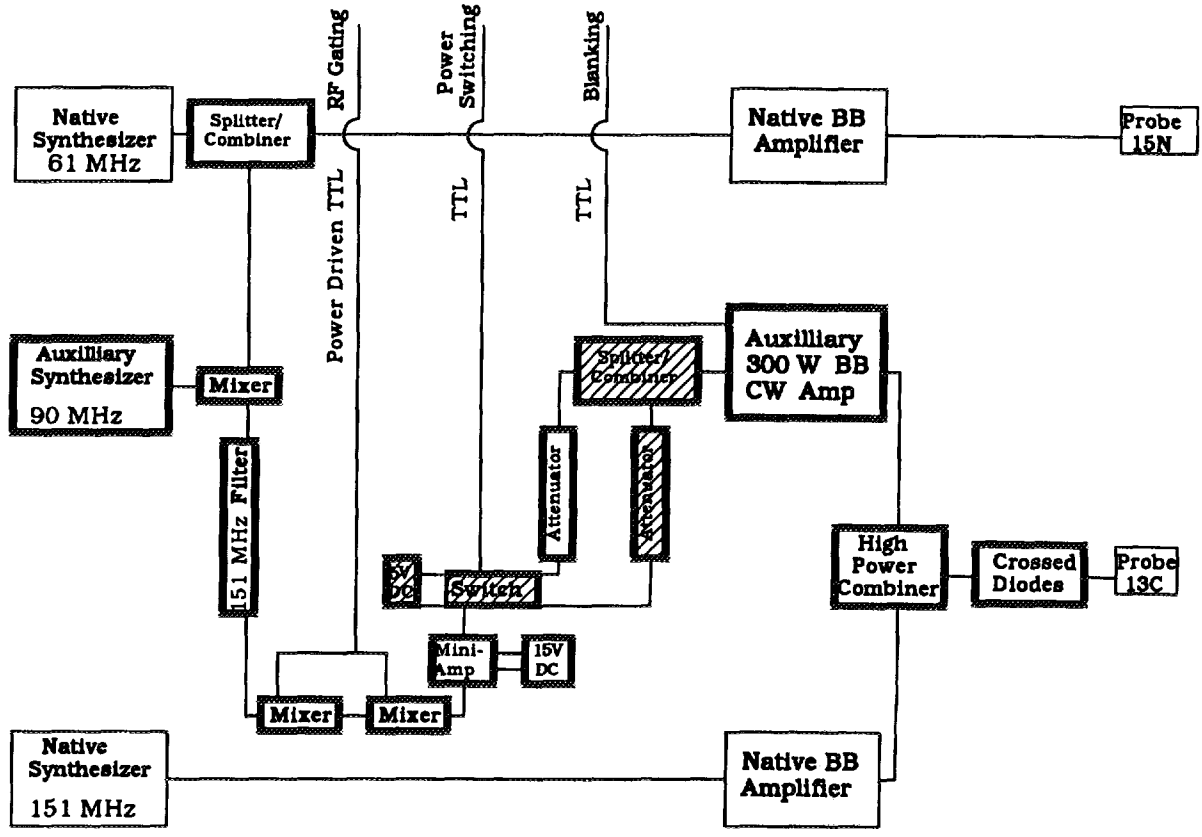

FIG. 2. Configuration of hardware for an auxiliary channel with fast (4 μs) switching of phases and power levels. Off-the-shelf components used to add the fourth channel to the commercial spectrometer are boxed with bold lines. The first mixer, crossed diodes, and Class A linear, broadband, CW amplifier (B-LAX-300) were obtained from Bruker Instruments, Inc. (Billerica, Massachusetts). The auxiliary synthesizer is a Programmed Test Sources, Inc., [PTS]-250 (Littleton, Massachusetts). The power combiner/splitters (ZSC-2-1W), second and third mixers (ZLW-1H or ZAS-1), and low-power amplifier (ZFL-500) were obtained from Mini-Circuits (Brooklyn, New York). The SPDT switch can also be obtained from Mini-Circuits (ZSDR-230). The narrow-bandpass 151 MHz filter (8BE151/10-3-AB) and toggled attenuators (SA-5B) were obtained from Trilithic (Indianapolis, Indiana). The high-power combiner (D1635) was obtained from Werlatone (Brewster, New York). The optional components that can be added to enable power switching are represented by cross-hatched boxes. The auxiliary channel was added to a Bruker AMX-600 equipped with a triple-resonance probe.

HNCA experiment (*1*). More recently, Mueller and coworkers have used shaped pulses in composite-pulse trains to make carbonyl decoupling selective (*3, 4*), but spectrometer limitations may prevent the use of this method. Selective carbonyl decoupling that performs well can be obtained with conventional, low-power WALTZ-16. Figure 1 shows the implementation of the slightly modified CT-HNCA on a three-channel spectrometer (tuned to ^{1}H, ^{13}C, and ^{15}N) with the hardware described below. The carbonyl decoupling with WALTZ is performed on one of the standard (native) channels. ^{15}N frequency pulses are issued on the other native broadband channel. The low-power RF of this channel is mixed with that of an auxiliary synthesizer to generate RF pulses of $^{13}C\alpha$ frequency, to be used for the auxiliary fourth channel.

A diagram of the auxiliary channel as assembled from commercially available components is shown in Fig. 2. The arrangement chosen produces, in addition to native ^{1}H, ^{15}N, and ^{13}C frequencies, an additional ^{13}C frequency. However, other arrangements along the same principles could yield any combination of three heteronuclear frequencies such as those of ^{15}N, ^{13}C, and ^{31}P or $^{13}C{=}O$, $^{13}C\alpha$, and $^{13}C\beta$. The native and auxiliary RF synthesizers are phase-locked to the 10 MHz reference of the spectrometer. The phase of the RF of the native synthesizer is under phase-table control. RF from the native synthesizer is mixed with RF from an external synthesizer and the desired sum or difference frequency is selected by a narrow-bandpass filter. The phase control from the native synthesizer then accurately determines the phase of the mixed RF since no frequency multiplication occurs. Phase selection on the auxiliary channel comes simply by setting the phase of the RF of the native channel with which the external RF is mixed. For example, the phase of the $^{13}C\alpha$ frequency RF is set by changing the phase of the ^{15}N channel using normal phase tables or expressions. This provides the advantage of simple, routine programming of phase cycles and of quadrature. The ^{15}N and $^{13}C\alpha$ channels, although derived from the same synthesizer, can have in-

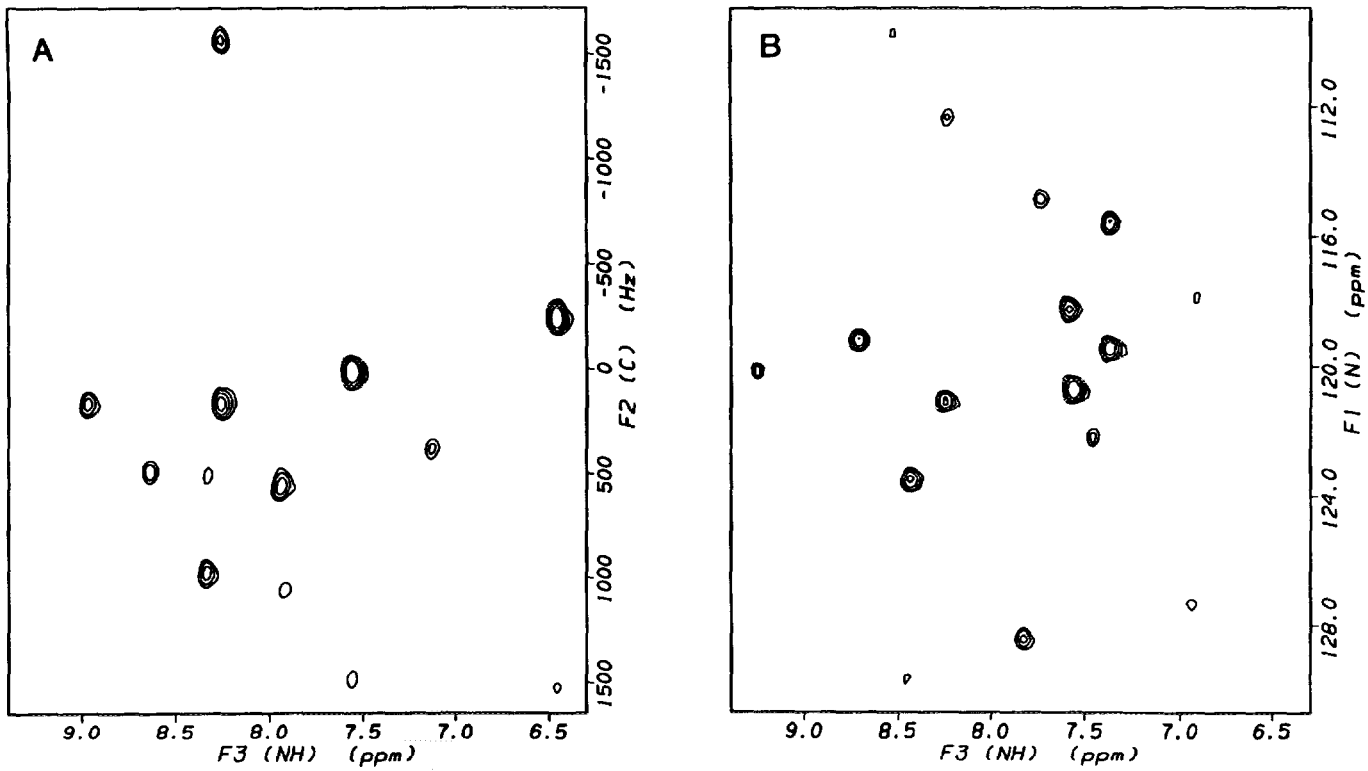

FIG. 3. (A) Amide ^{1}H-^{13}C (F_3, F_2) cross section at 120.7 ppm in F_1 (^{15}N) and (B) amide ^{1}H-^{15}N (F_3, F_1) cross section, at the ^{13}C carrier frequency of the auxiliary channel, from a CT-HNCA experiment performed with the modified pulse sequence on the hardware described. In (B), the ^{13}C carrier is assigned a relative frequency of 0 Hz. Negative features are absent at this contour level. The sample was a 19.5 kDa catalytic fragment of human stromelysin, 1.4 m*M*, pH 7.0 at 32°C. The experiment was collected as a matrix of 36 complex points in t_1, 48 in t_2, and 512 in t_3. The respective spectral widths were ±877 Hz (^{15}N), ±2404 Hz (^{13}C), and ±5435 Hz (^{1}H). A total of 64 acquisitions were collected per t_3 experiment. Quadrature detection in both F_1 and F_2 was obtained by the States–TPPI method (7). Complex "mirror-image" linear prediction to 64 points in t_1 was performed according to Zhu and Bax (8). The data were processed with Felix 2.0 (Hare Research) with modifications written in-house. Note the absence of quadrature images in (A) and the presence of cross peaks in this plane at the ^{13}C carrier frequency (B).

dependent phase programs except when simultaneous pulses are required on the two channels.

Because the blanking of the linear CW amplifier is not adequate to completely turn off amplification of incoming RF, the RF source must be gated. This is accomplished by mixing the RF with a TTL pulse (IF port of mixer) from the console under software control. The TTL line must have a line driver to allow sufficient RF to pass through the mixer. Because of limited isolation between the RF input and output of the mixer employed, a cascade of two mixers using the same power-driven TTL line was used. A small amplifier compensates for RF power losses incurred. Suitable RF power is obtained by a manually switched attenuator. To obtain software-controlled power-level switching, a fast switch with logic can be introduced to route RF to a choice of attenuators. In the application described here, a high-power RF combiner follows the two high-power amplifiers (native and auxiliary) to route two ^{13}C frequencies to the same coil. A pair of crossed diodes blocks any very-low-power background.

The operations included in a pulse program to issue a pulse on the auxiliary channel are listed in pseudocode; e.g., for a $^{13}C\alpha$ pulse,

set TTL line to select attenuator (optional power switching)
set phase of RF of native synthesizer (set to ^{15}N) mixed with auxiliary synthesizer
set TTL line high to turn on RF to auxiliary amplifier
issue TTL pulse to unblank linear amplifier
set TTL line low to turn off RF to auxiliary amplifier

With the equipment described, rise and fall times of pulses on the auxiliary channel of 1 to 2 μs are obtained. An RF field strength of 10 kHz is achieved with the attenuator set to 0 dB, using a Bruker triple-resonance probe capable of delivering 15 μs 90° pulses when driven by 300 W. The RF field strength is limited by the relatively low output of the small amplifier used as input for the linear, auxiliary amplifier.

Shown in Fig. 3A is an F_3–F_2 (^{1}H, ^{13}C) cross section and in Fig. 3B an F_3–F_1 (^{1}H, ^{15}N) cross section at the $^{13}C\alpha$ carrier frequency of a CT-HNCA experiment, with phase-cycled $^{13}C\alpha$ pulses performed on the fourth channel as described above. The absence of quadrature images in Fig. 3A shows that the phase cycling (States–TPPI in both F_1 and F_2 dimensions) performs adequately. If RF were leaking through the fourth channel when pulses are not being issued (i.e., in the absence of gating of RF input to the linear amplifier), cross peaks on a plane corresponding to the $^{13}C\alpha$ carrier would be bleached out. The presence of cross peaks in Fig. 3B demonstrates the efficacy of gating off undesired RF on the fourth channel.

An advantage of the arrangement described is that the spectrometer can be used normally; the auxiliary channel can be ignored when not used. A limitation of the approach is that simultaneous pulses on the auxiliary channel and its "parent," native channel use the same phase table. For example, INEPT-like transfers with simultaneous pulses of independent phase on these two channels need to be displaced, with the pulse on the first nucleus creating zz magnetization and the second pulse completing the transfer to the other nucleus. The necessary components for assembling an auxiliary channel are readily available. Its operation requires only two or three software-addressable TTL lines. The auxiliary channel is a particularly useful addition for older two-channel instruments that have limited RAM for pulse programs and a limited number of free TTL lines.

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REFERENCES

1. L. E. Kay, M. Ikura, R. Tschudin, and A. Bax, *J. Magn. Reson.* **89,** 496 (1990).
2. S. Grzesiek and A. Bax, *J. Magn. Reson.* **96,** 432 (1992).
3. M. A. McCoy and L. Mueller, *J. Am. Chem. Soc.* **114,** 2108 (1992).
4. L. E. Kay, M. Wittekind, M. A. McCoy, M. S. Friedrichs, and L. Mueller, *J. Magn. Reson.* **98,** 443 (1992).
5. A. J. Shaka, J. Keeler, T. Frenkiel, and R. Freeman, *J. Magn. Reson.* **52,** 335 (1983).
6. B. A. Messerle, G. Wider, G. Otting, C. Weber, and K. Wüthrich, *J. Magn. Reson.* **85,** 608 (1989).
7. D. Marion, M. Ikura, R. Tschudin, and A. Bax, *J. Magn. Reson.* **85,** 393 (1989).
8. G. Zhu and A. Bax, *J. Magn. Reson.* **90,** 405 (1990).