

Development of NMR Instrumentation to Achieve Excitation of Large Bandwidths in High-Resolution Spectra at High Field

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A prototype 2.5-mm ^1H high-resolution probe for an 18.8-T (800 MHz) nuclear magnetic resonance spectrometer has been designed, together with a dedicated amplifier capable of delivering up to 1 kW of power. This probe permits a 90° pulse length of $2\ \mu\text{s}$ to be achieved at 300 W, corresponding to an excitation bandwidth of $\pm 125\ \text{kHz}$. Probe performances were tested on samples commonly used for this purpose as well as on protein and paramagnetic model compound samples. It is shown that this probe is useful for a wide range of applications at high magnetic field, especially in the study of systems characterized by very broad and far-shifted resonances and in experiments that require high-power radiofrequency irradiation. © 2001 Academic Press

Key Words: high-resolution probe; excitation bandwidth; high-power radiofrequency irradiation; paramagnetic molecules.

INTRODUCTION

The increase in NMR demand in the past decades has been flanked by an improvement in instrumental performances and by a continuous increase in magnetic field strength available. Improving resolution and sensitivity of NMR spectrometers opens the way toward the study of larger biological molecules and of macromolecular assemblies (1). In addition, high field NMR spectrometers allow one to address many field-dependent phenomena, including partial orientation of molecules for solution structure determination (2–5). Higher fields, availability of new probes, advances in data acquisition, and processing strategies over the next few years promise to increase NMR sensitivity by about a factor of 5, with a resulting increase in the potential of NMR applications.

At high fields the bandwidth of excitation, which depends on the Larmor frequency and on the duration of the 90° pulse, may become a general problem. Indeed, increasing Larmor frequency results in an increasingly small excitation bandwidth, in terms of parts per million, for a given duration of the 90° pulse. An insufficient excitation bandwidth also results in an inefficient spin-lock field as, for instance, in TOCSY experiments, and to overcome this limitation adiabatic pulses have been introduced in some sequence schemes (6). A constant excitation profile turns out to be a particularly relevant feature when one deals

with paramagnetic molecules, as a large excitation bandwidth is often a requirement for detecting all signals of interest (7, 8).

To this end, we developed a high-resolution capable probe, which differs from the standard probes by a low quality factor (hereafter Q -factor) and a high voltage capability. The Q -factor of a standard probe is so high that it acts as a center-weighted bandpass filter within the spectral area desired. Moreover, it makes the effective rise time of the pulses considerably longer. As a disadvantage, a lower Q reduces the sensitivity and requires longer pulses for the same pulse duration. This can only be achieved in a 3-mm coil.

We report here the realization and the testing of a prototype 2.5-mm ^1H probe developed for a 18.8-T (800 MHz) high-resolution NMR spectrometer. Such a probe (HP probe, hereafter) has been designed, together with a dedicated amplifier capable of delivering up to 1 kW of power, in order to have a 90° pulse length of $2\ \mu\text{s}$, which corresponds to an excitation bandwidth of $\pm 125\ \text{kHz}$.

To test probe performances, besides standard experiments on samples commonly used for this purpose, three different sets of experiments were performed: (i) 1D NMR spectra on a paramagnetic sample characterized by very large spreading of NMR signals; (ii) 2D TOCSY spectra on a diamagnetic protein sample; (iii) 1D NMR spectra of samples characterized by fast relaxing signals with a wide range of linewidths.

The aim is to show that this HP probe has some interesting features, e.g., the possibility of exciting a large spectral bandwidth without the need of a small flip angle; comparable performances for any conventional 2D homonuclear application; the ability to apply continuous strong radiofrequency fields avoiding sample heating and coil mismatch.

RESULTS

Probe Features

The probe has been designed for a Bruker Avance 800 NMR spectrometer. It is equipped with a separate lock channel for deuterium (operating at 122.8 MHz) with a 10-mm-diameter saddle-type geometry tuned to ^2H . Radiofrequency (RF) excitation and

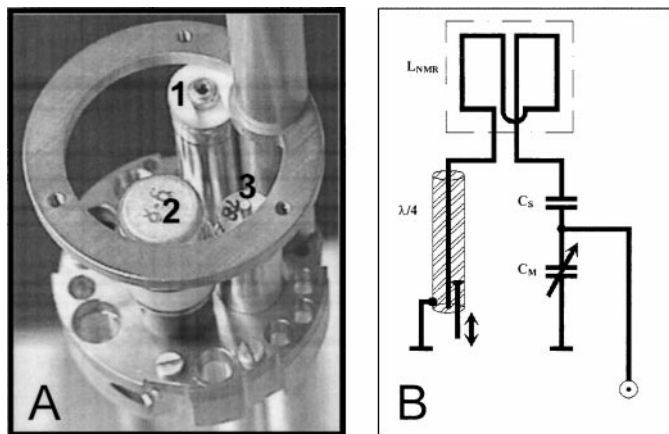


FIG. 1. (A) View of the HP probe upper section showing circuit elements used in the RF circuitry (NMR saddle coil not shown). (1) Upper part of the $\lambda/4$ resonator tunable to 800 MHz; (2) and (3) Additional tuning capacitors. (B) Circuit diagram of the proton channel with a $\lambda/4$ line of variable length (movable shortcut). L_{NMR} represents the saddle coil, C_{M} the matching trimmer capacitor, and C_{s} the symmetry capacitor (balancing the circuit).

detection is achieved by an NMR RF excitation/receiving coil with 3-mm-diameter saddle-type geometry tuned to ^1H . The high-frequency (^1H) channel of the probe comprises a resonant transmission line circuit that is capacitively coupled to an RF matching network (Figs. 1A and 1B). This ^1H circuit has been built as a balanced circuit in order to optimize RF efficiency during pulsed excitation, to minimize electric fields in the sample, and to maximize signal-to-noise ratio of the NMR signal.

All elements used in the circuit (fixed and trimmer capacitors, transmission line) were selected for high-voltage stability in order to ensure the operation of the probe at RF power levels larger than 100 W. An external Q -factor for the ^1H channel of $Q \approx 215$ (at 800 MHz) has been achieved. A 90° pulse length of $3 \mu\text{s}$ at an RF input power of approximately 150 W was measured. We achieved 90° pulses of $1.9 \mu\text{s}$ at approximately 300 W. For the RF coil the same type of wiring material is used as for standard high-resolution probes. Low-susceptibility materials were used to provide high spectral resolution free from B_0 inhomogeneity artifacts. All materials used in the construction of the probe were thoroughly inspected to minimize residual ^1H signals over a wide spectral range, far beyond the usual narrow spectral window of ^1H high-resolution NMR.

The probe unifies the features of standard high-resolution probes (appropriate coil geometry, low-susceptibility materials) with the advantage brought by the high efficiency and high-voltage stability of circuitry used in solid state probes.

Standard Tests

Figure 2A represents the results of the ^1H NMR lineshape test (1% CHCl_3 in acetone- d_6), resulting in <6 and <13 Hz linewidth at 0.55 and 0.11% intensity levels (nonspinning sample). These values are comparable with those obtained using

a triple-resonance inverse detection probe (TXI, hereafter). The HP probe yielded quite high sensitivity, which results in a S/N of 288 : 1 with a 0.1% ethyl benzene sample, as shown in Fig. 2B. This value is again comparable with the typical S/N ratio of 5-mm probes, if the factor 4 reduction in volume is taken into account. Figure 2C shows the water suppression for 2 mM sucrose in 90% $\text{H}_2\text{O}/10\%$ D_2O . Again, the result is comparable with that obtained with a standard TXI probe. The probe itself shows only very small spurious signals originating from residual protons in materials close to the NMR coil, as can be inferred by the clean and flat spectrum shown in Fig. 2D. The origin of ^1H background signal traces back to residual protons in the ceramics material and fluorinated plastics material (Teflon) in the high-voltage capacitors and fluorinated plastics materials (Teflon) in the transmission line used in the circuitry. In order to keep RF retardation effects to a minimum, these elements are located relatively close to the NMR coil and may still contribute a spurious proton NMR signal.

Large Bandwidth Excitation

Efficiency in bandwidth excitation has been tested over the paramagnetic complex $[N, N'-(4\text{-methyl-4-azaheptane-1,7-diyl}) \text{ bis}(5\text{-chlorosalicylideneaminato})\text{nickel(II)(II)}(5\text{-Cl-NiSAL-MeDPT})]$, dissolved in CDCl_3 . This molecule, whose NMR began many years ago (9), shows NMR signals which, due to the hyperfine interaction with the paramagnetic Ni(II) ion, are shifted up to 460 ppm (10). The spectrum of this complex has been successfully investigated at low field (300 MHz), where a typical 90° pulse of $4 \mu\text{s}$ produces an almost uniform excitation over about 500 ppm (11).

A standard 5-mm 800-MHz ^1H probe has a 90° pulse of about $10 \mu\text{s}$ at a probe RF input power of about 30 W. This implies that only a spectral window of ± 25 kHz can be uniformly excited. Under these conditions, the first null of the excitation profile is at ± 100 kHz from the transmitter. This corresponds, at 800 MHz, to a 250-ppm useful spectral region, which is insufficient to record the NMR spectrum of 5-Cl-NiSAL-MeDPT in one shot.

The NMR spectrum of 5-Cl-NiSAL-MeDPT recorded with the HP probe using a 90° pulse of $2 \mu\text{s}$ (Fig. 3) shows the expected large bandwidth excitation. All expected NMR signals can be observed in the recorded spectrum, in which the carrier frequency has been set at 250 ppm. The relative intensities of the observed signals show that the excitation profile is almost flat ($\geq 80\%$) over a ± 250 -ppm spectral region, consistent with expectations. The spectrum shown in Fig. 3 clearly demonstrated that ^1H NMR spectra over a spectral width of at least 500 ppm can be obtained, corresponding to an increase of a factor of 5 of the spectral window with respect to standard probes at 800 MHz. Of course, it would be possible to obtain the same excitation bandwidth also on a standard TXI by using a small flip angle. However, only the proper setting of the 90° pulse will permit an optimal exploitation of most of the commonly used sequences.

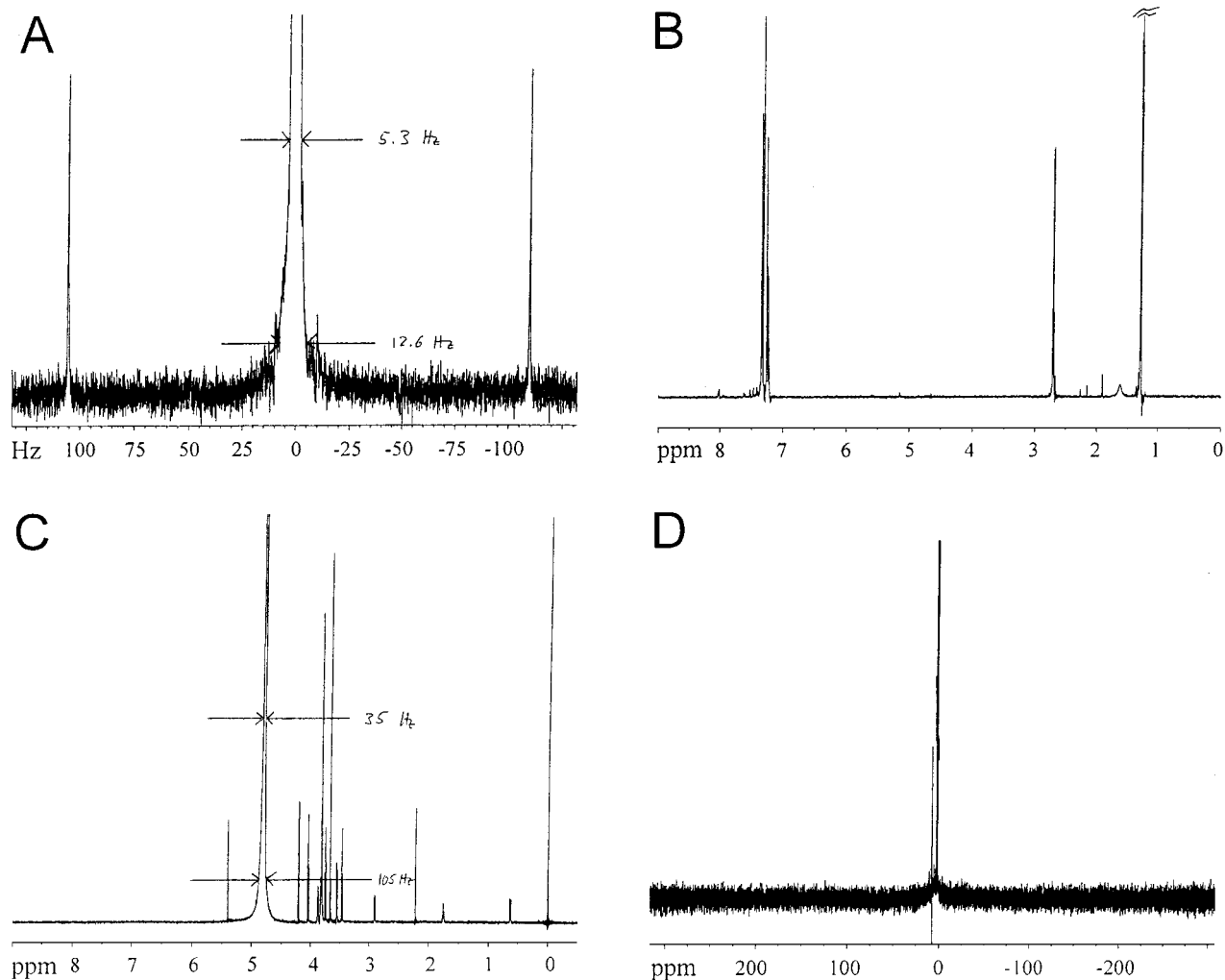


FIG. 2. (A) ^1H NMR lineshape sample spectrum (CHCl_3 in acetone- d_6); (B) ^1H NMR spectrum of ethyl benzene 0.1%; (C) ^1H NMR spectrum of 2 mM sucrose in $\text{H}_2\text{O}/\text{D}_2\text{O}$ with water suppression; (D) ^1H NMR spectrum of CHCl_3 in acetone- d_6 taken over a spectral range of 600 ppm with small spurious signals (approximately $2\times$ noise level), indicating that the materials in the close environment of the NMR coil are proton free.

2D TOCSY Experiments on a Diamagnetic Protein

Manufacturer's specifications for standard 800-MHz TXI probes indicate about 10 kHz as the maximum spin-lock field strength under "safety" conditions, with 30 W being the maximum power bearable by the probe. The much higher power bearable by the HP probe makes it particularly suitable for recording TOCSY spectra, which can be safely performed using a spin-lock field of 20 kHz. This also makes the HP probe a useful tool for studying diamagnetic systems. Indeed, a 10-kHz bandwidth is barely sufficient to homogeneously cover the spectral window required to collect the spectrum of a diamagnetic protein, thus giving rise to TOCSY spectra at 800 MHz which may present artifacts (6).

To test the performances of the HP probe in this respect, we used the 30,000-Da diamagnetic protein zinc(II) bovine carbonic anhydrase isoenzyme II (ZnBCA) as a test protein. Figure 4

shows a TOCSY experiment recorded with the HP probe using an MLEV as spin-locking sequence (12). No phase distortions are observed over the entire spectral window investigated. This permits the observation of TOCSY patterns also for the most downfield shifted amide protons which, in the TOCSY experiment collected using the TXI probe, have a much weaker intensity. Additionally, TOCSY experiments recorded with the HP probe show no evidence of artifacts arising from ROESY-type cross peaks, which are observed in the analogous experiment collected on the TXI using a 6-kHz RF field. Although these artifacts can be minimized by replacing MLEV sequence with its clean version (12, 13), an increase in the effective RF field eliminates such artifacts.

No detectable heating effects are observed when a 20-kHz spin-locking field is applied, as chemical shift values are identical to those measured when a much lower field (6 kHz) is applied on the TXI probe. This is a consequence of the higher conversion

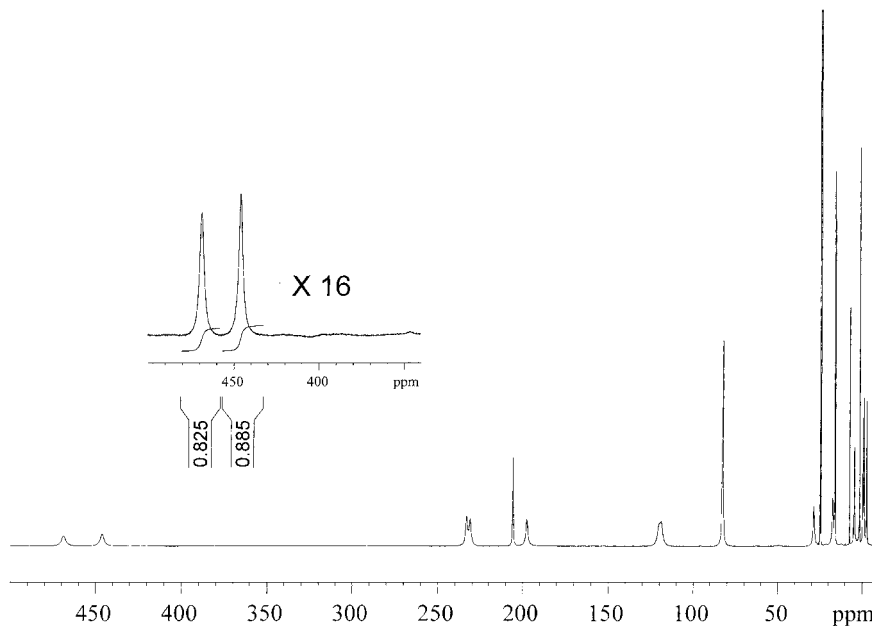


FIG. 3. An 800-MHz ^1H NMR spectrum of 5-Cl-NiSAL-MeDPT (*19*) dissolved in CDCl_3 . In the inset a vertical expansion of the far downfield region of the spectrum is reported. The observed fractional intensity of the far downfield shifted signals is fully consistent with the theoretical excitation profile.

rate of incident RF power into RF magnetic field amplitude for smaller coils. In addition, the sample volume is smaller, such that keeping the sample at constant temperature for a given air-flow is more efficient, thus preventing sample heating even at high RF field amplitudes.

Detection of Barely Detectable Signals Using Broadband Presaturation

One possibility of enhancing the detectability of fast-relaxing signals is based on a broadband presaturation as a relaxation-rate-based filter. This sequence was originally proposed when dealing with NOESY experiments at 600 MHz (*14*) and was later applied in 1D, 2D, and NOE difference experiments (*15*). The idea is to enhance the intensity of fast-relaxing signals with respect to slow-relaxing signals by inserting, prior to the 90° observation pulse, a broadband presaturation period. Under the above conditions, all resonances within the spectral region covered by the broadband presaturation will be partially suppressed. Because the saturation factor of a signal depends on its T_1 and T_2 values, signals sensing a hyperfine interaction will be affected by broadband presaturation to a much lesser extent than diamagnetic ones. The method can be optimized case by case by inserting a recovery delay between the broadband presaturation and the observation pulse. The signal intensities of fast-relaxing signals in the final spectrum will depend on the recovery delay. A very short delay will enable the detection of the fastest relaxing signals only.

The intrinsic limitations of this approach are sample heating and limited bandwidth saturation. These problems could be

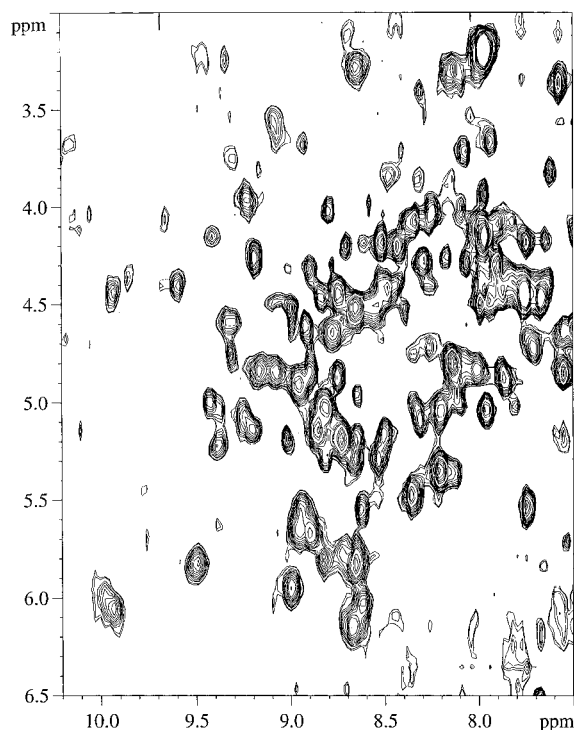


FIG. 4. Results of 800-MHz TOCSY experiments on ZnBCA. Only the fingerprint region is shown. Mixing time and recycle delays were 20 and 500 ms, respectively. The spin-lock field was 20.8 kHz, corresponding to a 90° low power pulse of $12 \mu\text{s}$. The 90° high power pulse was $1.9 \mu\text{s}$. A 2048×466 data point matrix was acquired, using 256 scans for each transient. ZnBCA was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The lyophilized protein (20 mg) was dissolved in unbuffered H_2O and the pH was adjusted at 6.0. A 10% D_2O was added to allow deuterium lock.

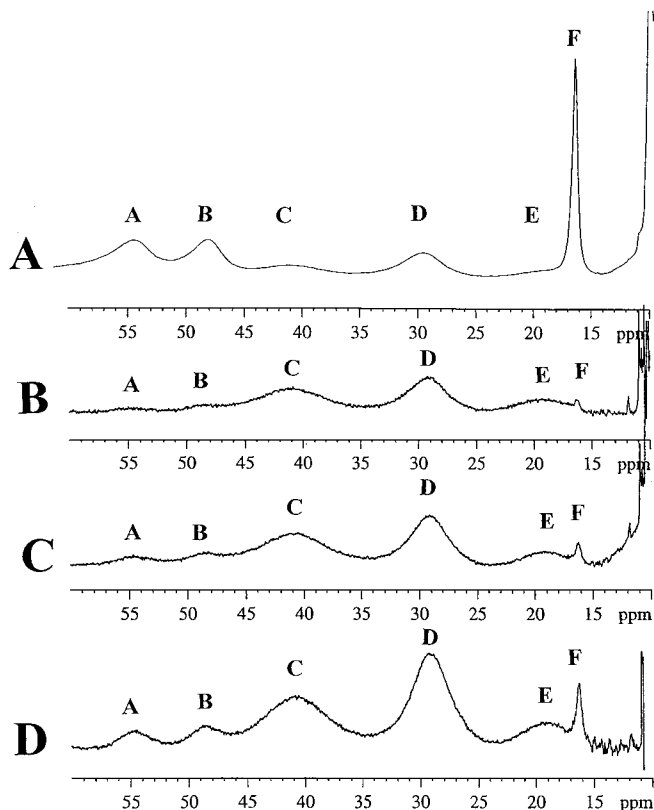


FIG. 5. An 800 MHz, 1D NMR spectrum of CuSt. (A) A 1D spectrum obtained upon a 90° observation pulse preceded by a selective saturation to suppress solvent signal. From B to D: The 90° observation pulse is preceded by a 10-ms broadband saturation (GARP) and by a recovery delay of 50 μ s (B), 100 μ s (C), and 200 μ s (D). *Rhus vernicifera* CuSt was prepared in 50 mM phosphate buffer–water solution at pH 6.0. A 10% D_2O was added to allow deuterium lock.

partly overcome by replacing any conventional broadband decoupling scheme with an adiabatic decoupling (16). This probe, however, provides a straightforward solution, thanks to its capability of providing strong RF fields, as outlined earlier.

We have tested this approach with the blue copper protein stellacyanin from *Rhus vernicifera* in the oxidized paramagnetic state (CuSt) (17). Such a system shows very broad signals, barely detectable from the noise, for which the use of very high fields provides an extremely useful gain in sensitivity.

The spectrum obtained using only water presaturation is shown in Fig. 5A, while spectra obtained with broadband presaturation are shown in Figs. 5B–5D. A 10-ms broadband presaturation was performed using the GARP sequence (18) for composite pulse decoupling with a field strength of about 22,000 Hz. This ensures almost complete saturation of all the observable NMR signals up to 60 ppm. Upon insertion of a relaxation delay between the broadband presaturation and the observation pulse, the recovery of the fast-relaxing signal is observed. As shown in Figs. 5B–5D, the choice of the relaxation delay allows one to modulate the recovery of signals to allow the detection of pre-

viously unobserved signals in a large spectral window. This is, in the present example, the case of signal E (19 ppm), which is almost unobservable in the conventional 1H NMR spectrum but which is clearly observed in the broadband saturated spectrum.

CONCLUSIONS

Paramagnetic systems have challenging properties also in terms of equipment. As a consequence of large hyperfine shifts, the availability of a probe that is specifically designed toward the analysis of large spectral windows is important. Here we have reported the first example of a probe that has been specifically designed to match the needs of researchers working with paramagnetic molecules at very high field.

Nowadays, the available probes for very high field spectrometers have been optimized to work with small or relatively small spectral regions, in which oversampling and digital acquisition can be applied. As these characteristics are inadequate when dealing with paramagnetic molecules, the availability of a probe designed to work using large spectral windows is extremely important. Nevertheless, further improvements in terms of spectrometer/probe dead time (becoming noticeable at large spectral widths of 500 kHz) are desired in terms of pulse sequence design and software development. These developments are currently in progress.

Additionally, we showed that such a probe may have interesting applications also in diamagnetic systems, to overcome the limitations associated with spin-lock-type experiments at very high field. The availability of shorter hard pulses, the high efficiency to convert the incident RF power into RF magnetic field in conjunction with suitable circuit and coil design, and the more effective temperature control are of great help to collect high-quality TOCSY experiments. These features would be also useful when adiabatic mixing schemes are considered.

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