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A Comparison of Inverse-Detected Heteronuclear NMR Performance: Conventional vs Cryogenic Microprobe Performance

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Abstract: We report a comparison of the results obtained at 500 MHz for heteronuclear shift correlation (HSQC) experiments with very small natural product samples using conventional and cryogenically cooled 3 mm NMR probes. The cryo probe affords a 12- to 16-fold reduction in data acquisition time for a comparable signal-to-noise ratio.

Structure elucidation has been undeniably changed by the advent of 2D NMR methods. NMR, despite its inherent insensitivity, is capable of providing a wealth of molecular connectivity information. Fundamentally, there have been three principal avenues utilized in the pursuit of improved NMR sensitivity. First, the development of superconducting NMR solenoids capable of higher magnetic fields, with correspondingly greater sensitivity, has been pursued continually for the last several decades. Second, experimental methods, e.g., inverse-detected heteronuclear shift correlation methods, have been developed to augment sensitivity. Third, there has been a considerable effort expended in the area of NMR probe development. It is in this latter area that the present effort is reported.

The need to characterize small samples by NMR has driven much of the probe development work that has been reported. Early examples include direct ^{13}C observation experiments in capillary (1.7 mm) NMR tubes by Shoolery and co-workers.[1−3] The performance of the first commercial 3 mm micro inverse-detection NMR probe (Nalorac MIDG500-3) was described in 1992 by one of the present authors,[4] and such performance was compared to conventional 5 mm probe performance (Nalorac IDG500-5) later that year.[5] Since these initial reports, numerous structural elucidation studies using 3 mm micro NMR probe technology have been reported. Varian subsequently reported the development of the magic angle liquid Nano-probe.[6−9] The first reports on micro-coil NMR probes began to appear in 1995,[10] and refinements in this area of probe development have continued.[11−14] The first reports of 1.7 mm submicro (SMIDG) NMR probes appeared in 1998[15,16] followed by a comparison of the performance of a 1.7 mm SMIDG (Nalorac SMIDG-500-1.7) vs a conventional 3 mm micro NMR probe (Nalorac MIDG-500-3) in 1999.[17]

In an effort to push NMR sensitivity still higher, a number of preliminary reports have appeared, predominantly as posters presented at various NMR meetings,which have described the development of NMR probes in which the rf coils and associated electronics are cooled to 25 K or lower.[18−21] Efforts in this area have focused initially on the development of 5 mm cryogenic probes. A report appeared in 1999 comparing the results of nuclear Overhauser difference spectra obtained for a small sample of Taxol (paclitaxel) in both conventional 5 mm and 5 mm cryogenic probes.[22] Earlier this year, preliminary results obtained using a 2.5 mm cryogenic probe were reported.[23] We now report the comparison of results obtained with a 3 mm CryoSPEC micro inverse NMR probe (Nalorac CryoM[H]C500-3) with those obtained using a conventional 3 mm micro inverse probe (Nalorac MIDG500-3) for low-level acquisition of heteronuclear shift correlation data.

Data for the present study were acquired using a sample containing 40 μg of strychnine (**1**, 120 nmol) dissolved in 150 μL of benzene-d_6 (Cambridge Isotope Laboratories) in a 3 mm NMR tube (Wilmad) prepared by serial dilution. The sample was prepared under an inert argon atmosphere in a glovebox and then flame sealed. The 3 mm CryoSPEC probe was cooled using an open-loop helium delivery system to a stable temperature of <10 K. The conventional

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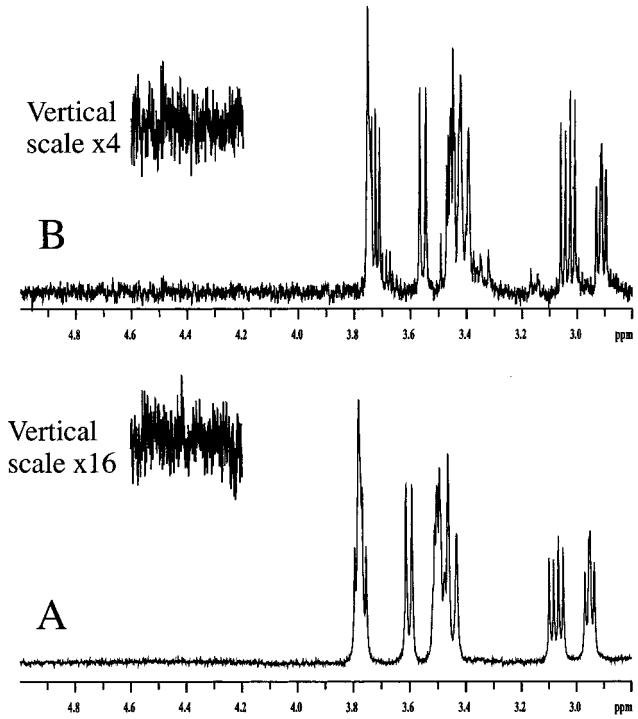
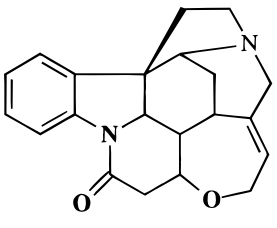


Figure 1. Comparison ^{1}H reference spectra for a sealed 40 μg sample of strychnine (**1**) in 150 μL benzene-d_6 prepared by serial dilution. (A) Segment from a spectrum recorded by accumulating 16 transients using a CryoSPEC micro inverse NMR probe (Nalorac CryoM[H]C500-3) operating at a temperature of ~8 K. The noise region inset is plotted with a vertical scale 16 times greater than that of the spectral segment. (B) Spectral segment from the same sealed sample recorded using a conventional Z-SPEC micro inverse NMR probe (Nalorac MIDTG-500-3) also recorded by accumulating 16 transients. The noise region inset is plotted with a vertical scale 4 times greater than that of the spectral segment shown. The actual S/N difference between the two spectra was a factor of ~3.5 times greater for the cryoprobe. Differences in line width in the spectra recorded with the two probes may be attributed to the fact that the cryoprobe is a developmental prototype on which temperature susceptibility compensation has not been fully optimized. We anticipate commensurate improvements in line shape and performance when this optimization is completed.

3 mm NMR probe was operated at a temperature of 293 K. All experiments were performed on a Varian INOVA 500 MHz NMR spectrometer. Pulse widths were 5.0 μs at a power of 51 dB (63 dB max) for ^{1}H and 13.4 μs for ^{13}C at a power of 60 dB (63 dB max) for the conventional Nalorac MIDG500-3 probe; pulse widths were 9.9 μs at a power of 50 dB (63 dB max) for ^{1}H and 12 μs for ^{13}C at a power of 51 dB (63 dB max) for the Nalorac CryoM [H]C500-3 probe. Cool down for the cryo probe using a stream of helium gas required approximately 2 h, with the probe attaining a stable temperature of ~8 K.



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A segment of the ^{1}H reference spectrum of the 40 μg sample of strychnine (**1**) recorded using the cryoprobe is shown in Figure 1A. An HSQC spectrum of strychnine spectrum was recorded. The data were acquired as 2K × 48 (×2 hypercomplex files), accumulating 32 transients/t_1 increment, giving a total data acquisition time of 90 min. The delay for the one-bond heteronuclear coupling was set for 135 Hz. Completely interpretable data were observed in 45 min; the aliphatic region of the noise-free spectrum recorded in 90 min is shown in Figure 2A. Using the same sample, a ^{1}H reference spectrum was next recorded using a conventional 3 mm probe, a segment of which is shown in Figure 1B. An HSQC spectrum was then recorded using exactly the same parameters in the conventional 3 mm probe at 293 K as were used for the acquisition of the spectrum shown in Figure 2A with the cryoprobe. As would be expected, the 90 min HSQC data were of lower quality. The 90 min conventional probe spectrum is shown in Figure 2B. The spectrum is noisy and contains only some of the direct correlation responses observed in the 90 min spectrum recorded using the cryogenic probe.

On the basis of a comparison of the signal-to-noise ratios in one-dimensional reference spectra acquired in both probes using the data shown in Figure 1, the relative difference in S/N was ~3.5-fold higher for the cryoprobe. Using this S/N difference as a basis for computation, comparable HSQC data would be expected for the conventional 3 mm probe by acquiring ~12.5 times the number of transients/t_1 increment as were acquired with the cryoprobe. The second HSQC spectrum with the conventional 3 mm probe was acquired taking the relative S/N ratio into account. These data were acquired as 2048 × 48 (×2 hypercomplex files), accumulating 352 transients/t_1 increment, giving an acquisition time of 17.5 h. These data are shown in Figure 2C. As expected, the 17.5 h data are comparable to those acquired in 90 min using the 3 mm cryogenic probe.

Advantages inherent to the use of cryogenic NMR probes are self-evident. Time compression afforded by the S/N difference between normal and cryoprobes in the range of 12−16-fold can be expected to open the door for the investigation of chemical structures at unprecedentedly low levels in reasonable periods of time. While the investigation of a 120 nmol sample of strychnine in 17.5 h is not time prohibitive, acquiring the long-range heteronuclear shift correlation spectrum that would be required to fully characterize an unknown at this level would require~80 h (assuming the HMBC,[24] CIGAR−HMBC,[25] or another long-range experiment was employed to be roughly one-fourth the relative sensitivity of the direct correlation experiment). Alternatively, acquiring direct correlation data at half the level used in this study (20 μg of strychnine) would also be expected to consume ~80 h of instrument time. In many laboratories, either of these experiments would be time prohibitive. In contrast, the direct and long-range heteronuclear shift correlation data would be accessible using a 3 mm cryogenic NMR probe in <10 h total.

The high sensitivity offered by cryogenic NMR probes with the corresponding compression of data acquisition times can be expected to be exploited for the characterization of very small samples of natural products[22] and for the identification of metabolites of pharmaceuticals. It is also probable the sensitivity of cryoprobes will be utilized for the rapid acquisition of data to characterize unstable species, as has already been demonstrated for 1.7 mm SMIDG NMR probes.[26,27] Cryoprobes, relative to conventional 1.7 mm submicro NMR probes, can be anticipated to afford roughly a 1.5× performance advantage. Adddditional performance gains should result from the utilization of Shigemi micro NMR cells in conjunction with the cryogenic NMR probes. Further examples might include

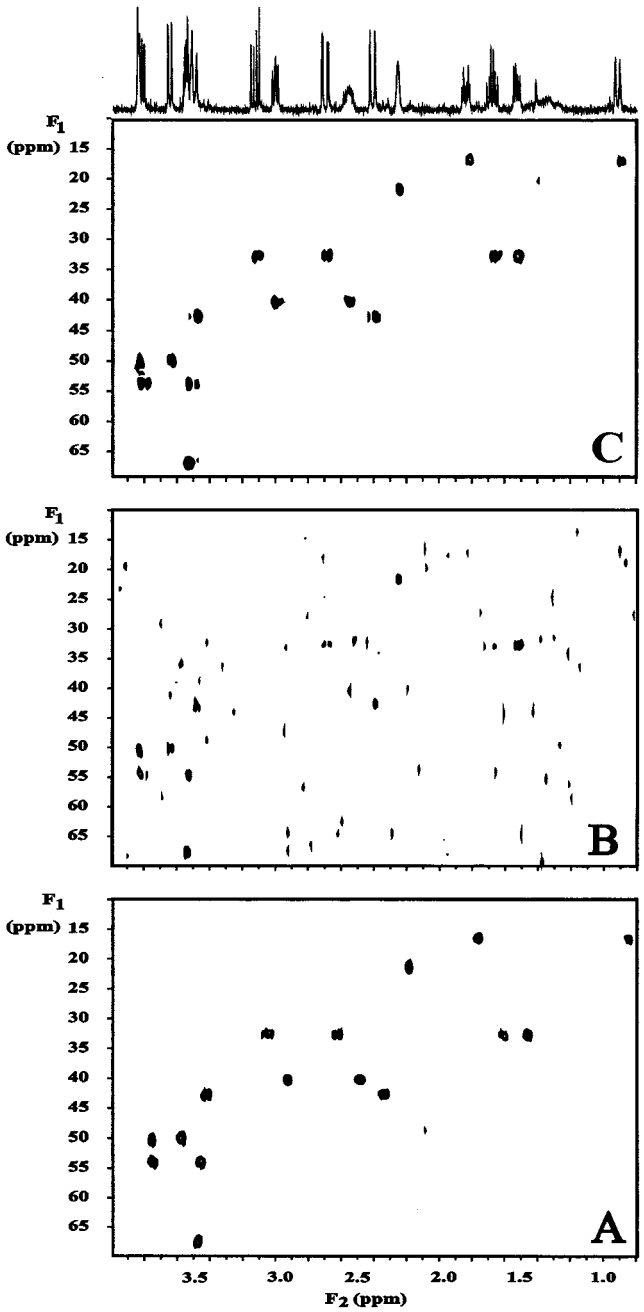

Figure 2. Segments of the aliphatic region of the HSQC spectra of a sealed 40 μg sample of strychnine (**1**) prepared by serial dilution. (A) Spectrum recorded by accumulating 32 transients/t_1 increment as 2048 × 48 (hypercomplex files × 2) points linear predicted in the second time domain to 192 points and zero-filled to 256 points during processing using a CryoSPEC micro inverse NMR probe (Nalorac CryoM[H]C500-3). The data were acquired in a total of 90 min. (B) Spectrum recorded under conditions identical to those used for the spectrum shown in panel A but recorded using a Z-SPEC micro inverse NMR probe (Nalorac MIDTG-500-3). Processing and scaling parameters were identical to those used for the data shown in panel A. The data are obviously of lower quality and contain only a portion of the actual number of responses that should be observed. (C) Spectrum recorded using the conventional 3 mm NMR probe as in panel B taking into account the S/N ratio difference calculated from the spectra shown in Figure 1. The data were recorded by accumulating 352 transients/t_1 increment giving an acquisition time of 17.5 h. The data were again processed identically to those shown in panel A. The responses observed and the relative S/N ratio for these data are also comparable to what is shown in panel A.

the study of kinetic processes and the possible acquisition of data for transient species. Applications of cryoprobes in screening for biologically active molecules have been described by Fesik and co-workers,[28] and the characterization of metabolites has also recently been reported.[23] Applications have also begun to appear describing the structural characterization of proteins,[29,30] including the use of ^{13}C-detection cryoprobes,[31] which facilitates the development of experiments that can be specifically applied to ^{13}C-labeled proteins.

Supporting Information Available: Supplemental information comparing B1 homogeneity of the 3 mm cryo and conventional microprobes is available free of charge via the Internet at http://pubs.acs.org.

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