JOURNAL OF MAGNETIC RESONANCE **135,** 232–235 (1998)
ARTICLE NO. MN981558

Gradient- and Sensitivity-Enhanced Heteronuclear Multiple-Quantum Correlation Spectroscopy

Guang Zhu,[1] Xiangming Kong, and Konghong Sze

Department of Biochemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

Received May 1, 1998

A gradient- and sensitivity-enhanced HMQC experiment has been developed. The sensitivity of the experiment is increased by factors of $\sqrt{2}$ and 2 over the conventional and gradient-enhanced HMQC experiments, respectively. This improvement is achieved by retaining both the *x* and the *y* magnetization components in the indirectly detected dimension. This experiment will be particularly useful in NMR studies of large biomolecules as the relaxation time of the multiple-quantum coherence is much longer than that of the single-quantum coherence in the slow motion limit.

Key Words: **HMQC; pulsed field gradient; sensitivity enhancement.**

Increasing sensitivity and reducing artifacts are desirable goals in the design of NMR experiments. In multidimensional NMR, quadrature detection in the indirectly detected dimensions is achieved by recording cosine- and sine-modulated signals in two separate experiments to obtain pure absorption spectra, resulting in a loss of sensitivity by a factor of $\sqrt{2}$. This loss can be recovered by retaining both the *x* and the *y* magnetization components in the indirect dimensions, as in the schemes used in the sensitivity-enhanced TOCSY and HSQC experiments (*1–3*). In order to achieve greater suppression of the solvent signal, t_1 noise, and other artifacts in these NMR experiments, pulsed field gradients were introduced and excellent results were obtained (*4–8*). However, in conventional gradient-enhanced experiments, *P*- and *N*-type coherences are not selected simultaneously, which also results in a loss of sensitivity by a factor of $\sqrt{2}$. The way that pulsed field gradients are used in the sensitivity-enhanced HSQC experiment, proposed by Kay *et al.* (*9, 10*), selects both types of coherence and thereby retains the sensitivity. Subsequently, this sensitivity enhancement scheme has been applied to many multidimensional NMR experiments (*11, 12*).

Theoretical calculations (*13–15*) have shown that, for a heteronuclear two-spin system in the slow motion limit, multiple-quantum coherence relaxes much more slowly than single-quantum coherence. Recent reports (*16–19*) describing NMR experiments on biomolecules have also shown that the HMQC experiment is more sensitive than the HSQC experiment in certain circumstances, owing to the slower relaxation rate of the multiple-quantum coherence. This property may have greater application in NMR studies of large biomolecules as the rapid relaxation of magnetization is the major factor responsible for the loss of sensitivity in these experiments. Here, we report that an enhancement in sensitivity can also be obtained for the HMQC experiment by using strategies similar to those given by Palmer *et al.* (*1, 2*) and Kay *et al.* (*9, 10*), in which quadrature detection in the indirectly detected dimension is achieved by postacquisition data processing, and gradients are used to select magnetization pathways without sensitivity loss. The sensitivity of the experiment is enhanced by a factor of $\sqrt{2}$ for a heteronuclear IS spin system when compared with the conventional HMQC method (*21*). To the best of our knowledge, no such kind of experiment has been published. We demonstrate here that a simple modification of the previously published sensitivity-enhanced HMQC experiment (*1, 2*) into the corresponding gradient version does not offer optimal sensitivity enhancement. Optimal enhancement is obtained by modifying the gradient- and sensitivity-enhanced HSQC experiment into the gradient- and sensitivity-enhanced HMQC experiment.

In Fig. 1, we propose three versions of the gradient- and sensitivity-enhanced HMQC pulse sequences based on the method of Palmer *et al.* (*1, 2*) and the gradient method of Kay *et al.* (*9, 10*). In the t_1 evolution period of the first and the second pulse sequences, Figs. 1A and 1B, two G_1 pulsed field gradients serve to dephase the S spin and the final pulsed field gradient, G_2, rephases the transferred magnetization so that the detected I spin magnetization before acquisition becomes

$$\begin{cases} -I_x \cos(\omega_s t_1) - I_y \sin(\omega_s t_1) & \text{if } \phi 2 = x \text{ and } 2G_1 = -G_2 \\ I_x \cos(\omega_s t_1) - I_y \sin(\omega_s t_1) & \text{if } \phi 2 = -x \text{ and } 2G_1 = G_2, \end{cases}$$

where ω_s is the Lamor frequency of the S spin; $G_1 = \gamma_I B^I(z)\tau^I$ and $G_2 = \gamma_S B^S(z)\tau^S$ with γ_I and γ_S being the gyromagnetic ratios of the spins I and S, respectively; $B^I(z)$ and $B^S(z)$ are the *B* fields generated from the pulsed field gradients for spins I and S; and τ^I and τ^S are the durations of these

[1] To whom correspondence should be addressed.

1090-7807/98 $25.00

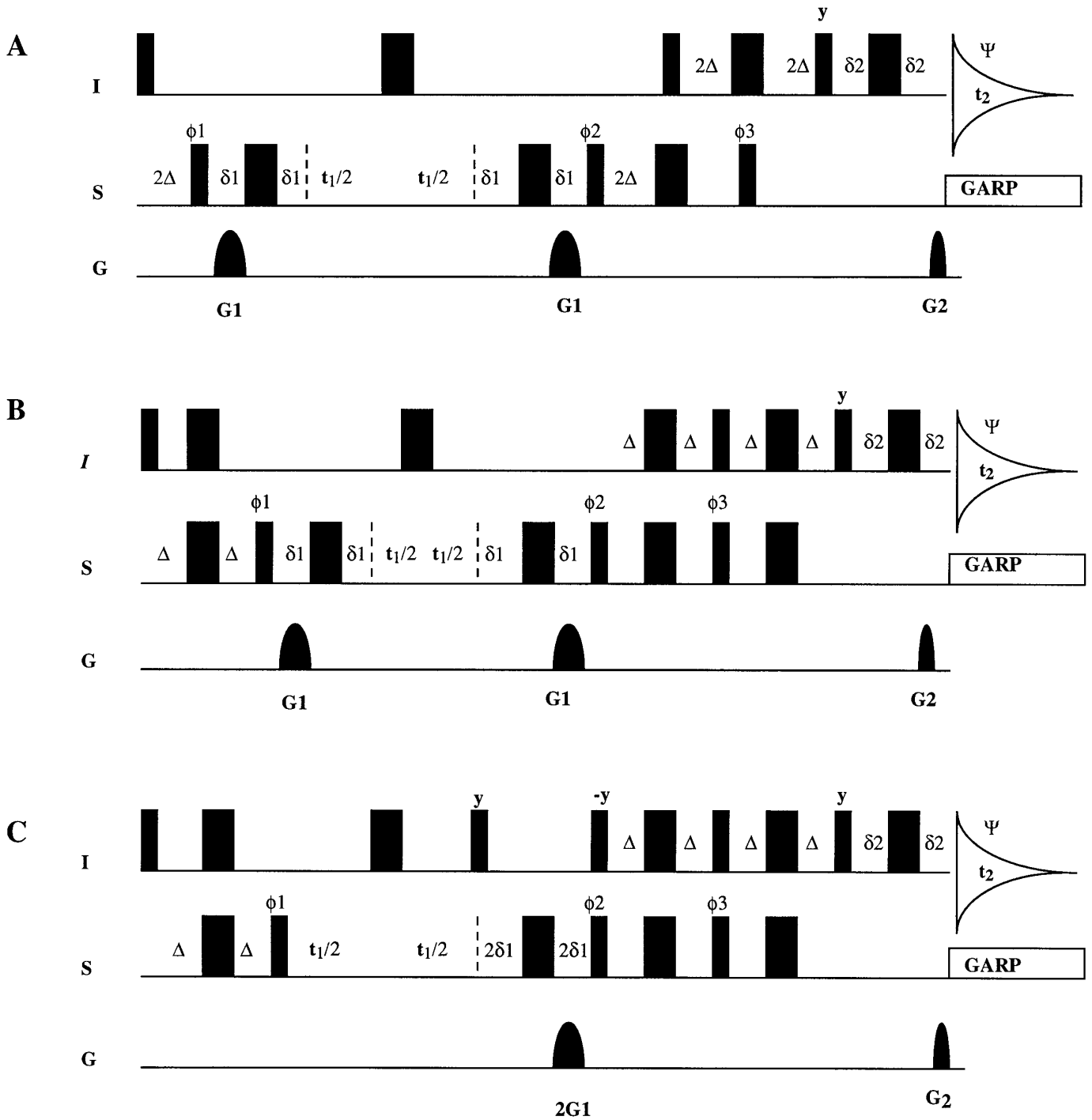

FIG. 1. Pulse sequences of the three versions of the gradient- and sensitivity-enhanced HMQC (g/s-HMQC) experiment. The thin and thick vertical bars represent 90° and 180° pulses, respectively. In these pulse sequences, the delays Δ, δ1, and δ2 are set to $\frac{1}{4J}$ = 2.77, 1.75, and 0.4 ms, respectively. Phase cycling is not necessary; however, we used a four-step phase cycle, which is $\phi 1$ = 0, 0, 2, 2; $\phi 2$ = 0, 2; $\phi 3$ = 1, 3; Rec Ψ = 0, 2, 2, 0. Unlabeled pulses are applied along the *x* axis. For every second t_1 increment, the phase $\phi 2$ and the gradient G_2 are inverted. The strength of gradients G_1 and G_2 is 20 G/cm with durations of 1.5 and 0.3 ms, respectively, and they are applied along the *z* axis. Quadrature detection in the t_1 dimension is achieved by adding and subtracting the two consecutive transients as described in the text.

gradient pulses. For each t_1 value, two transients corresponding to $\phi 2 = x$ and $\phi 2 = -x$ are recorded and stored separately, and are then added and subtracted, with a 90° phase shift being applied to the addition of the transients. The final pure absorption spectrum can be obtained by data processing according to the method of States *et al.* (*20*).

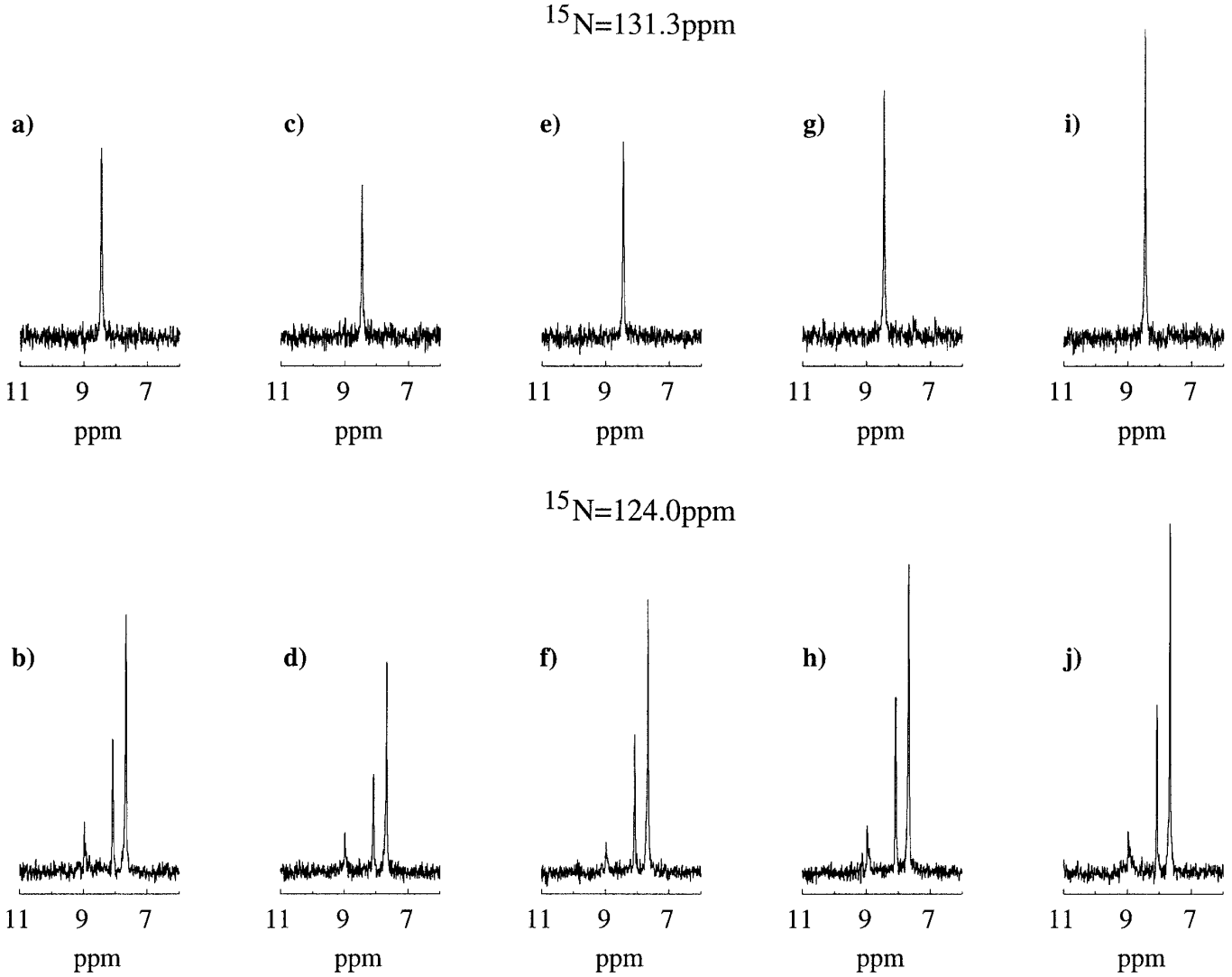

FIG. 2. Cross sections of ^{1}H–^{15}N HMQC spectra of calmodulin recorded with different pulse sequences: conventional HMQC (2a, 2b), gradient-enhanced HMQC as proposed by Davis *et al.* (*8*) (2c, 2d), and g/s-HMQC version 1 (2e, 2f), version 2 (2g, 2h), and version 3 (2i, 2j). In all these experiments, broadband decoupling during the acquisition period was accomplished by using the GARP decoupling sequence with a 0.9-kHz radiofrequency field, and 16 scans per experiment and a relaxation delay of 1 s were used. Spectra were obtained by using the same acquisition and processing parameters for each experiment. The 1D spectra shown here were scaled to the same noise level for comparison.

In the third pulse sequence (Fig. 1C), the second 90° I spin pulse serves to transfer double- and zero-quantum coherences to single-quantum coherences, and the magnetization after the second 90° pulse of spin I becomes

$$2I_z[S_y \cos(\omega_s t_1) - S_x \sin(\omega_s t_1)].$$

This term is exactly the same as that in the sensitivity-enhanced HSQC experiment (*9*) before the application of the first gradient. By then employing the second part of the pulse sequence of the sensitivity-enhanced HSQC and by proper setting of the two gradients, as described above, the detectable magnetization becomes the same as that in the first pulse sequence, except that the sign of I_y is altered. By adopting the same data processing procedures as described for the first experiment, a pure absorption spectrum can be obtained. Although, in theory, the sensitivity gain of these experiments can be as much as $\sqrt{2}$ over the conventional HMQC experiment, this gain may not be achieved in practice because of sensitivity loss due to the larger number of pulses and signal loss through relaxation. It should be noted that this type of experiment, where Δ is used, does not provide sensitivity enhancements for I_2S and I_3S spin systems.

The sensitivity enhancements provided by the proposed gradient- and sensitivity-enhanced HMQC (g/s-HMQC) experiments are demonstrated by ^{1}H–^{15}N correlation spectroscopy of a ^{15}N-labeled calmodulin sample. Recordings were made at

30°C on a Varian Inova 500-MHz NMR spectrometer, and the protein concentration was 1 mM in 90% H_2O, 10% D_2O at pH 6.8. Spectra from the g/s-HMQC experiments are compared with those from the conventional HMQC (*21*) and gradient-enhanced HMQC experiments (*8*). Figure 2 displays cross sections from ^1H–^{15}N HMQC spectra, scaled to the same noise level, at ^{15}N chemical shifts of 131.3 and 124.0 ppm. Figures 2a and 2b show cross sections from the conventional HMQC experiment (*21*) with water suppression achieved by a 70-Hz presaturation field for a duration of 1 s. Figures 2c and 2d present cross sections from the normal gradient HMQC experiment (*8*). Figures 2e and 2f, 2g and 2h, and 2i and 2j show cross sections of the g/s-HMQC experiments, versions 1, 2, and 3 (Figs. 1A–1C), respectively. In all the gradient experiments, suppression of the water signal is achieved solely by the use of pulsed field gradients which avoids sensitivity loss of exchangeable NH signals caused by presaturation.

Based on an analysis of the signal-to-noise ratios (S/N) of 30 well-resolved NH peaks, the g/s-HMQC experiments, versions 2 and 3 (Figs. 1B and 1C), achieve sensitivity enhancements by factors of 1.19 ± 0.25 and 1.42 ± 0.30, respectively, when compared with the conventional HMQC experiment, and sensitivity enhancements by factors of 1.73 ± 0.22 and 2.10 ± 0.42 over the normal gradient-enhanced HMQC experiment while version 1 (Fig. 1A) offers only a little enhancement over the conventional HMQC experiments. The lower sensitivity enhancement in versions 1 and 2 of the proposed g/s-HMQC experiment (Figs. 1A and 1B) compared with version 3 (Fig. 1C) is due to gradient diffusion in the t_1 evolution time and a proton homonuclear coupling effect during $4\delta 1$ and 2Δ in version 1 and $4\delta 1$ in version 2. In addition, version 1 of the experiment has an extra 2Δ delay which will lead to additional relaxation loss.

The experimental results described above indicate that version 3 of the proposed g/s-HMQC experiments, Fig. 1C, offers the best sensitivity enhancement. Moreover, version 3 of the g/s-HMQC experiment offers the most efficient suppression of the solvent signal and other artifacts. This is because the G_1 pulsed field gradient provides an efficient suppression of untransferred I magnetization. In versions 1 and 2 of the proposed g/s-HMQC experiments (Figs. 1A and 1B), the two G_1 pulsed field gradients cannot suppress the untransferred I magnetization. Only G_2 serves to suppress it, and a stronger gradient is required to achieve the same level of suppression of solvent signals and artifacts.

In summary, we have proposed three versions of a gradient- and sensitivity-enhanced HMQC experiment. Version 3 (Fig. 1C) is the best, and it provides significant sensitivity enhancement and superior suppression of the solvent signal and artifacts over the conventional and gradient-enhanced HMQC experiments. This experiment can be used as a basic building block for multidimensional NMR experiments for the study of large biomolecules to take advantage of the slower relaxation rate of the double-quantum coherence. Our preliminary results show that it is possible to further improve the sensitivity of the g/s-HMQC experiment by using a spin lock for ^1H during the t_1 period to remove the homonuclear coupling effect (*17*), and the g/s-HMQC experiment with the use of a spin lock is more sensitive than the g/s-HSQC experiment. These results will be reported later.

ACKNOWLEDGMENTS

The authors thank Mr. Xiangzhong Yan and Dr. David Smith for helpful discussions and Dr. Mingjie Zhang for allowing us to use his ^{15}N-labeled calmodulin sample. This work was supported by grants from the Research Grant Council of Hong Kong (HKUST 563/95M and 6197/97M) and the Hong Kong Biotechnology Research Institute is acknowledged for the purchase of the spectrometer.

REFERENCES

1. A. G. Palmer III, J. Cavanagh, P. E. Wright, and M. Rance, *J. Magn. Reson.* **93,** 151 (1991).
2. A. G. Palmer III, J. Cavanagh, P. E. Wright, and M. Rance, *J. Magn. Reson.* **96,** 416 (1992).
3. J. Cavanagh and M. Rance, *J. Magn. Reson.* **88,** 72 (1990).
4. R. E. Hurd, *J. Magn. Reson.* **87,** 422 (1990).
5. R. E. Hurd and B. K. John, *J. Magn. Reson.* **92,** 658 (1991).
6. G. W. Vuister, R. Boelens, R. Kaptein, R. E. Hurd, B. K. John, and P. C. M. van Zijl, *J. Am. Chem. Soc.* **113,** 9688 (1991).
7. G. W. Vuister, J. Ruiz-Cabello, and P. C. M. van Zijl, *J. Magn. Reson.* **100,** 215 (1992).
8. A. L. Davis, J. Keeler, E. D. Laue, and D. Moskau, *J. Magn. Reson.* **98,** 207 (1992).
9. L. E. Kay, P. Keifer, and T. Saarinen, *J. Am. Chem. Soc.* **114,** 10663 (1992).
10. D. R. Muhandiram and L. E. Kay, *J. Magn. Reson. B* **103,** 203 (1994).
11. M. Sattler, M. G. Schwendinger, J. Schleucher, and C. Griesinger, *J. Biomol. NMR* **5,** 11 (1995).
12. J. Schleucher, M. G. Schwendinger, and C. Griesinger, *Angew. Chem. Int. Ed. Engl.* **32**(10), 1489 (1993).
13. R. H. Griffey and A. G. Redfield, *Q. Rev. Biophys.* **19,** 51 (1987).
14. R. R. Ernst, G. Bodenhausen, and A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Clarendon Press, Oxford (1987).
15. T. J. Norwood, *Prog. NMR Spectrosc.* **24,** 295 (1992).
16. S. Grzesiek, H. Kuboniwa, A. P. Hinck, and A. Bax, *J. Am. Chem. Soc.* **117,** 5312 (1995).
17. S. Grzesiek and A. Bax, *J. Biomol. NMR* **6,** 335 (1995).
18. J. P. Marino, J. L. Diener, P. B. Moore, and C. Griesinger, *J. Am. Chem. Soc.* **119,** 7361 (1997).
19. G. V. T. Swapna, C. B. Rios, Z. Shang, and G. T. Montelione, *J. Biomol. NMR* **9,** 105 (1997).
20. D. I. States, R. A. Haberkorn, and D. J. Buben, *J. Magn. Reson.* **93,** 151 (1982).
21. M. F. Summers, A. Bax, and L. G. Marzilli, *J. Am. Chem. Soc.* **108,** 4285–4294 (1986).