Chapter 15
Relayed Coherence Transfer Experiments

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15.1 INTRODUCTION

In the late 1970s and early 1980s the basic principles of two-dimensional NMR were beginning to become clear. It was also clear that the experiments being used at that time would not be sufficient to solve many interesting problems, especially those concerning nucleic acids and proteins. The experiments that were then in use included INEPT, INADEQUATE, NOESY, homonuclear and heteronuclear J spectroscopy, as well as variants of what has come to be known as COSY type experiments (see Chapters 12–14) and heteronuclear correlation experiments which involved detection of the heteronucleus (see Chapter 22).

The author's interest in two-dimensional NMR began with the use of heteronuclear $^{31}P-^{1}H$ experiments to utilize the ^{31}P nucleus to 'spy' on the proton nuclei.[1] These experiments were originally aimed at obtaining proton–proton and proton–phosphorus scalar couplings and proton chemical shifts via use of experiments such as the one schematically illustrated in Figure 15.1. In this basic type of heteronuclear two-dimensional NMR experiment the free precession information of the protons is transferred to the heteronuclear 'spy' nucleus, in this case ^{31}P, by the simultaneous proton and heteronuclear pulses.

The proton spectrum which is detected in this type of experiment can be thought of as a difference spectrum.[1] The normal proton spectrum can be thought of as arising from the sum of the two subspectra which arise from the two possible polarizations of the heteronucleus. The proton spectrum detected in the heteronuclear experiment is the difference between these two subspectra. This principle is illustrated in Figure 15.2. The spectrum in (a) is the sum of the two subspectra corresponding to the two polarizations of the phosphorus nucleus and is the normal one-dimensional proton spectrum. The spectra in (b) and (c) are the two proton subspectra corresponding to the two different polarizations of the phosphorus nucleus. The spectrum in (d) is the difference spectrum between the two subspectra.

The comparison of the predicted and observed results is shown in the spectra on the right-hand side of Figure 15.2 along with the structure of 2′-guanosine monophosphate (2′-GMP) which was the molecule used for these experiments. There is good agreement between the predicted and observed spectra.

This single quantum experiment and various multiple quantum variations were applied to a number

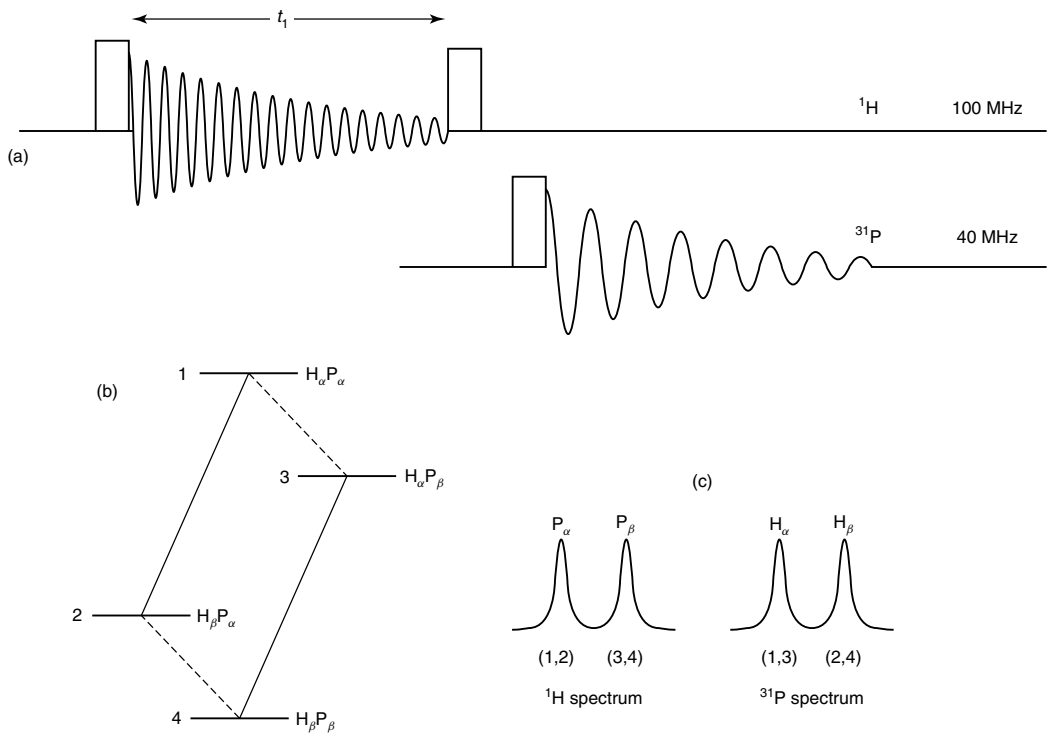


Figure 15.1. (a) A depiction of the basic pulse sequence used for heteronuclear chemical shift correlation spectroscopy. (b) The energy levels of a two-spin heteronuclear spin system and (c) the proton and phosphorus spectra together with labelings of the subspectra. (Adapted from Bolton and Bodenhausen.[1])

of nucleotides including ones which exhibit strong proton–proton scalar coupling and scalar coupling between the protons and more than one phosphate.[2–5] In addition, the flip angle dependence of the intensities of the individual lines in the heteronuclear spectra was extensively investigated.[3,4,6] These studies allowed us to analyze essentially any spectrum obtained in a heteronuclear two-dimensional chemical shift correlation experiment. These methods were even applied to nucleotides bound to an enzyme.[7]

In these heteronuclear two-dimensional experiments, the heteronucleus could report on the protons which had scalar couplings to the heteronucleus but did not report directly on any other protons. For example, in both the predicted and experimental results on 2′-GMP, no information about the chemical shift of the H-3′ proton is obtained. Thus, the results of a heteronuclear experiment on 2′-GMP do not offer complete information about the protons which are not directly coupled to the heteronucleus. As in many cases, the information which is of most interest is not necessarily the most accessible information. The nomenclature which was introduced was to call the protons directly coupled to the heteronuclear 'spy' as 'neighbor' nuclei and the protons coupled to the neighbors as 'remote' nuclei.

One way to obtain information about the remote nuclei was to apply a weak continuous wave decoupling field at the chemical shift of the remote nucleus during the evolution time.[8] When the decoupling field was on resonance then the coupling of the remote nucleus with the neighbor nucleus would be suppressed. Figure 15.3 illustrates the expected change in a heteronuclear two-dimensional experiment. At the time that this double resonance, two-dimensional experiment was first conceived it was not trivial to carry it out. The Varian XL-200 spectrometer that was in use at the time required *milliseconds* to carry out a change in the power level of the proton channel, since the power change was carried out by means of *mechanical* relays which could be monitored by simply listening to the spectrometer. However, application

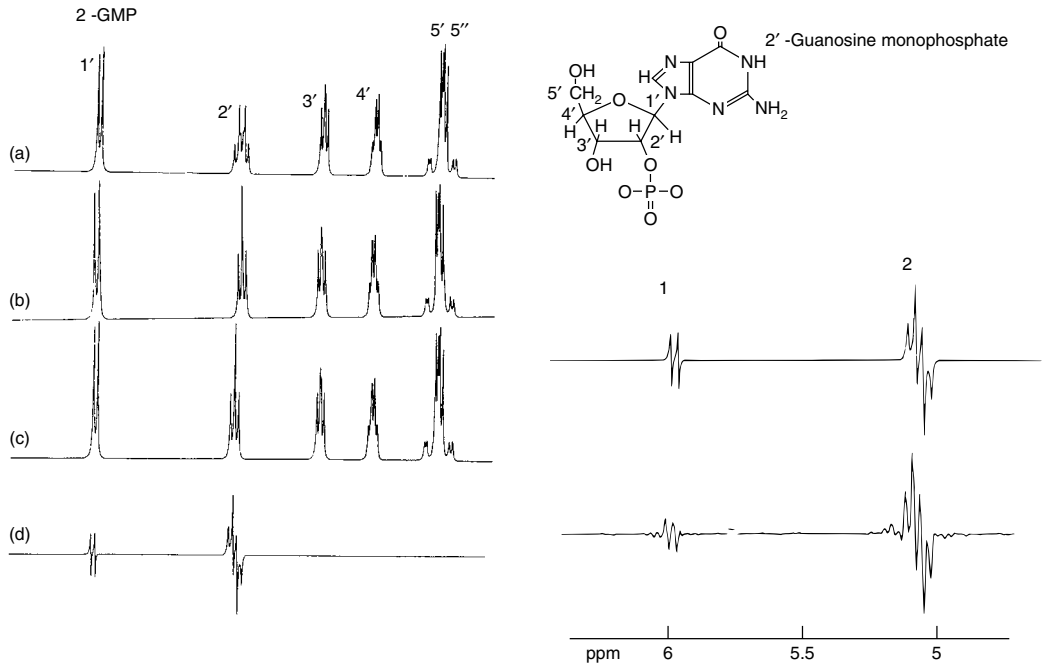


Figure 15.2. The left side of the figure contains the normal proton spectrum of 2′-GMP (a); the two subspectra which arise from the two polarizations of the phosphorus heteronucleus (b,c); and the predicted heteronuclear spectrum of this sample (d). The right side contains a comparison between the predicted heteronuclear spectrum (top) and the observed spectrum (bottom). (Adapted from Bolton and Bodenhausen.[1])

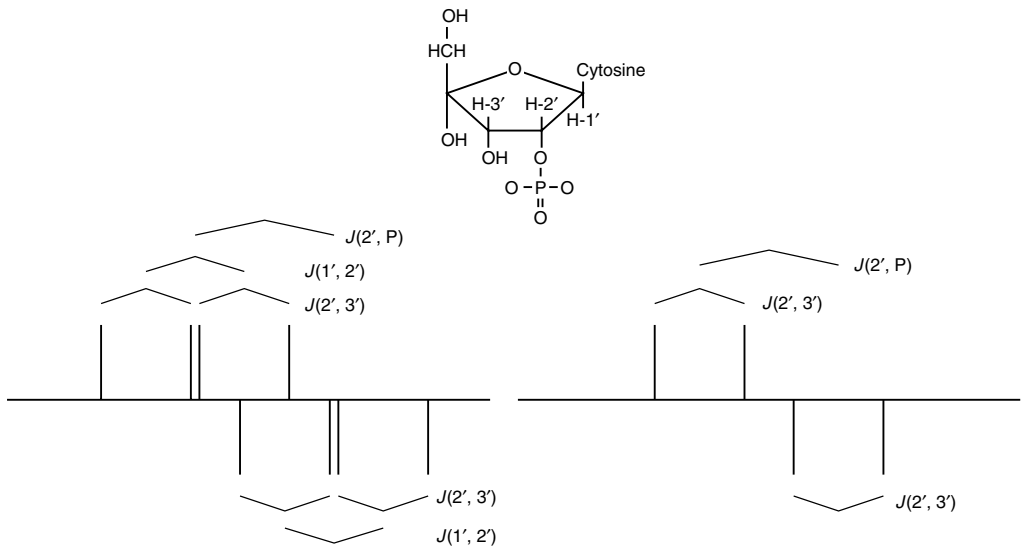


Figure 15.3. The predicted changes in the heteronuclear two-dimensional spectrum of cytosine 2′-monophosphate upon decoupling of the H-1′ proton are shown. The decoupling removes the H-1′–H-2′ coupling from the heteronuclear spectrum. (Adapted from Bodenhausen and Bolton.[6])

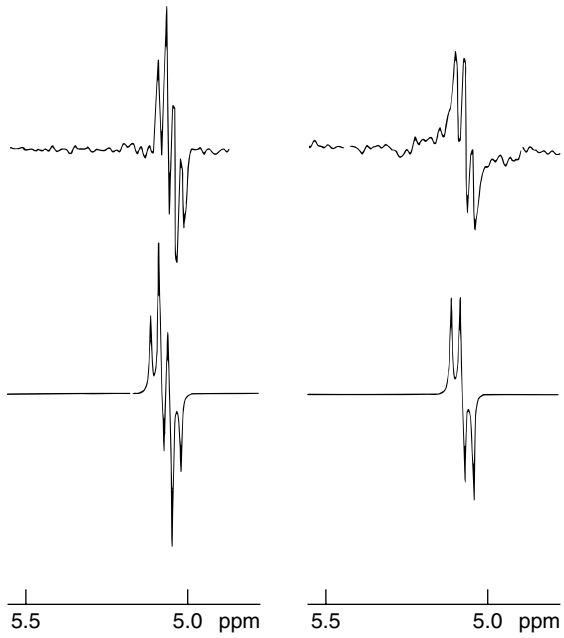


Figure 15.4. The spectrum on the top left is the experimental heteronuclear chemical shift correlation spectrum of 2′-CMP and on the bottom left the predicted spectrum. The spectrum on the top right is the experimental heteronuclear chemical shift correlation spectrum of 2′-CMP obtained with decoupling of the H-1′ proton and on the bottom right the predicted spectrum. (Adapted from Bodenhausen and Bolton.[6])

of a proton decoupling field during the evolution time of a heteronuclear two-dimensional experiment could be implemented and typical results are shown in Figure 15.4. As expected the double resonance, two-dimensional experiment did allow the determination of the couplings between the neighbor and remote protons. The chemical shift of the remote protons could be determined by observing the neighbor protons as a function of the frequency of the decoupling field and this was actually carried out on a nucleotide–protein complex.[7]

15.2 THE ORIGINAL RELAY TRANSFER EXPERIMENTS

About the time that the galley proofs on the above work were being corrected it became apparent that this was not a very clever way to obtain information about the remote protons. The basic heteronuclear two-dimensional experiment can be considered as the successor of the process of obtaining a series of one-dimensional spectra of the heteronucleus as a function of the frequency of a proton decoupling field. A better way to obtain information about the remote protons is to substitute a frequency independent procedure for the decoupling field applied during the evolution time.

This substitution could be based on the same basic idea as the heteronuclear two-dimensional NMR experiment, that is, the transfer of magnetization between nuclei which are scalar coupled by means of pulses rather than a set of experiments varying the frequency of the decoupling field. The basic procedure was to have two, successive magnetization transfers. The first magnetization transfer would be from remote to neighbor protons and the second transfer from the neighbor protons to the heteronuclear spy nucleus whose free precession would be detected.

Thus, the basic idea was simple and so obvious it was surprising no one had implemented it much earlier. The experiment was a straightforward combination of two two-dimensional experiments: a proton–proton COSY experiment and a heteronuclear two-dimensional chemical shift correlation experiment. The pulse sequence for a proton–proton COSY experiment is 90°, t_1, 90°, t_2. The pulse sequence for a heteronuclear two-dimensional experiment is that shown in Figure 15.1. The pulse sequence for the combined heteronuclear experiment is

$$^1\mathrm{H}: 90^\circ,\ t_1,\ 90^\circ,\ t_m,\ 90^\circ\ \mathrm{X}:\ 90^\circ,\ t_2$$

The mixing time, t_m, is chosen to be long enough for the protons to become antiphase with respect to both the heteronuclear and homonuclear couplings. A typical value for a proton–phosphorus experiment is 30 ms. This experiment was named the *relayed coherence transfer* experiment as the magnetization is relayed from the remote to the neighbor to the spy.[9,10]

The first experiments were carried out on phosphothreonine[9] with typical data shown in Figure 15.5. The experiment succeeded in correlating both the remote and neighbor protons with the heteronuclear spy, which in this case includes all of the nonexchangeable protons of the molecule. This demonstrated that two two-dimensional experiments could be combined into a single experiment. In addition, the relay transfer experiment contains

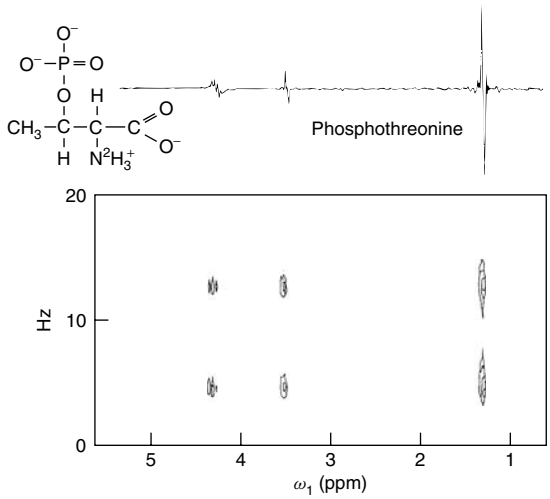


Figure 15.5. The two-dimensional spectrum is a relay transfer data set obtained on a sample of phosphothreonine. Along the vertical axis is the ^{31}P frequency and along the horizontal the proton frequencies. The heteronuclear coupling is present in each dimension. The trace shown at the top is in the phase sensitive mode. (Adapted from Bolton and Bodenhausen.[9])

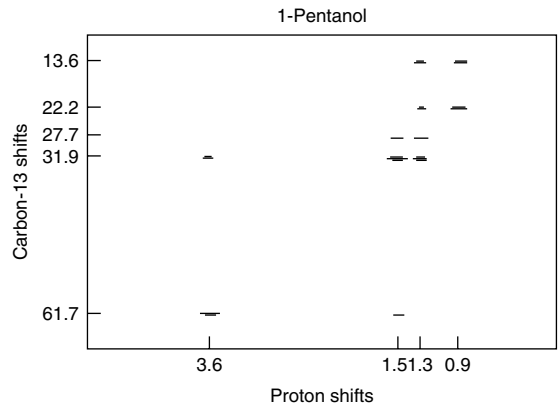
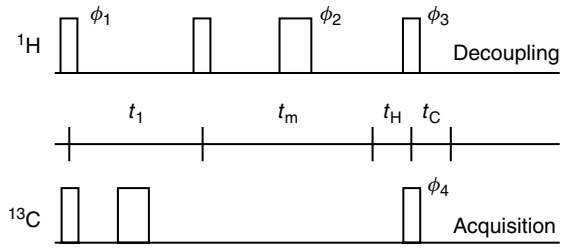


Figure 15.6. A two-dimensional spectrum relay transfer data set obtained on 1-pentanol. (Adapted from Eich *et al.*[11])

essentially all of the information present in the separate two-dimensional experiments.

While the application of the heteronuclear relay transfer experiment to molecules containing phosphorus was a success, the much more interesting application was to use ^{13}C as the spy nucleus.[10] The original pulse sequence is



with t_H the delay time to allow the magnetization to become antiphase with respect to the heteronuclear couplings so that transfer can occur, and t_C the delay time to allow the carbon magnetization to become in-phase to allow acquisition of the data with proton decoupling.

Consider the case of 1-pentanol, which is $C_{(5)}H_3$–$C_{(4)}H_2$–$C_{(3)}H_2$–$C_{(2)}H_2$–$C_{(1)}H_2$–OH. Carbon-5 will correlate with its neighbor protons as well the remote protons attached to carbon-4. Carbon-4 will correlate with the remote protons of carbon-5 and carbon-3 as well as its own neighbor protons. Thus, a single relay experiment on a molecule like pentanol can allow complete sequential assignment of both the proton and ^{13}C spectra, since each carbon will correlate not only with its neighbor protons but the remote protons on adjacent carbons. Thus, the relay data can allow the sequential assignments of the carbon and proton resonances.[10] The spectrum in Figure 15.6 shows that the relay transfer information does indeed allow the sequential connectivities to be made. The only additional information which is needed is to be able to assign cross peaks to the neighbor and remote categories. This can be done either by comparison with a heteronuclear two-dimensional experiment or by elimination of the neighbor signals.

Almost simultaneously with the introduction of the heteronuclear relay transfer experiments, Eich, Bodenhausen and Ernst demonstrated homonuclear relay transfer experiments.[11] These experiments were based on the same principles as the heteronuclear relay transfer experiments.

The original versions of the relay transfer experiments were modified to include improved phase cycling and methods to discriminate between neighbor and remote protons; multiple quantum versions were introduced as were constant time variations. These procedures allowed a number of systems to be investigated. The product operator description of relay transfer experiments can be found elsewhere.[9,12] The

main impact of relay transfer experiments was the demonstration that two, or more, two-dimensional experiments could be combined to yield a new experiment more powerful than any combination of the results of the individual experiments.

15.3 THE DEVELOPMENT OF RELAY TRANSFER EXPERIMENTS

Subsequent to the original introduction of heteronuclear relay transfer experiments there have been three types of significant improvements in the experimental approach. The original experiments relied on free precession of the protons during the mixing time to obtain magnetization which contained at least some antiphase character with respect to both the proton and heteronucleus.[9,10,13] More uniform proton–proton transfer can be obtained by the use of a homonuclear spin lock during the magnetization transfer followed by an INEPT, or analogous, heteronuclear transfer.[14,15] This procedure allows heteronuclear relay transfer experiments to be successfully carried out on a wide range of spin systems. The use of homonuclear spin locks is discussed in Chapter 16.

A major improvement in the sensitivity of the heteronuclear relay transfer experiment was the conversion to proton detection.[16] In the proton detection heteronuclear relay transfer experiments, the proton magnetization is transferred to the heteronucleus which is then allowed to precess. The magnetization is then transferred back to the neighbor protons. The magnetization can then be transferred to the remote protons and, with a spin lock throughout the proton spin system, the magnetizations of both the remote and neighbor protons detected. The original proton detection relay transfer experiments were carried out without the use of spin locks[16] and the first application to a labeled protein occured in 1986.[17] The proton detection versions of heteronuclear relay transfer currently in use are heteronuclear single quantum spectroscopy (HSQC)–TOCSY, heteronuclear multiple quantum spectroscopy (HMQC)–NOESY, and so on.[18–21] Proton–phosphorus relay correlations are now being performed via three-dimensional experiments which transfer magnetization via spin locks from phosphorus to neighbor protons to remote protons on DNA at millimolar concentrations.[22]

The conversion of heteronuclear relay transfer experiments into three-dimensional experiments is accomplished by the use of an evolution time rather than a constant mixing time. This allows experiments to be performed in which the neighbor-remote proton connectivities are in two-dimensional planes resolved by the frequencies of the spy nucleus along the third frequency axis. Three-dimensional HSQC–TOCSY experiments, for example, can be thought of as the combination of two, two-dimensional experiments into a three-dimensional experiment. Extensions to four- and five-dimensional experiments have also been made. The availability of isotopically labeled biological materials has made the development and application of these types of experiments powerful tools for studying the structure and dynamics of proteins and nucleic acids.

The original version of the heteronuclear relay transfer experiment has been vastly superseded by its offspring, both in terms of sensitivity and resolution. Improvements in the sensitivity and resolution of the two-dimensional heteronuclear relay experiment continue to be developed.[23] The fundamental idea of the relay transfer experiments, which is to combine two or more magnetization transfers to obtain a new experiment which contains more information than is present in the results of both of the parent experiments, has remained a very fruitful idea.

RELATED ARTICLES IN THE ENCYCLOPEDIA OF MAGNETIC RESONANCE

Double Resonance

Heteronuclear Assignment Techniques

REFERENCES

1. P. H. Bolton and G. Bodenhausen, *J. Am. Chem. Soc.*, 1979, **101**, 1080.
2. P. H. Bolton, *J. Magn. Reson.*, 1983, **52**, 326.
3. P. H. Bolton, *J. Magn. Reson.*, 1981, **45**, 239.
4. P. H. Bolton, *J. Magn. Reson.*, 1984, **60**, 342.
5. P. H. Bolton, *J. Magn. Reson.*, 1984, **57**, 427.
6. G. Bodenhausen and P. H. Bolton, *J. Magn. Reson.*, 1980, **39**, 399.
7. P. H. Bolton, *J. Magn. Reson.*, 1982, **46**, 91.
8. P. H. Bolton and G. Bodenhausen, *J. Magn. Reson.*, 1981, **43**, 339.

9. P. H. Bolton and G. Bodenhausen, *Chem. Phys. Lett.*, 1982, **89**, 139.
10. P. H. Bolton, *J. Magn. Reson.*, 1982, **48**, 336.
11. G. W. Eich, G. Bodenhausen, and R. R. Ernst, *J. Am. Chem. Soc.*, 1982, **104**, 3731.
12. R. R. Ernst, G. Bodenhausen, and A. Wokaun, *Principles of Magnetic Resonance in One and Two Dimensions*, Oxford University Press, Oxford, 1987.
13. P. H. Bolton, *J. Magn. Reson.*, 1983, **54**, 333.
14. A. Bax, D. G. Davis, and S. K. Sarkar, *J. Magn. Reson.*, 1985, **63**, 230.
15. L. Lerner and A. Bax, *J. Magn. Reson.*, 1986, **69**, 375.
16. P. H. Bolton, *J. Magn. Reson.*, 1985, **62**, 143.
17. J. A. Wilde, P. H. Bolton, N. J. Stolowich, and J. A. Gerlt, *J. Magn. Reson.*, 1986, **68**, 168.
18. G. Otting and K. Wüthrich, *J. Magn. Reson.*, 1988, **76**, 569.
19. L. Müller, *J. Am. Chem. Soc.*, 1979, **101**, 4481.
20. T. J. Norwood, J. Boyd, J. E. Heritage, N. Soffe, and I. D. Campbell, *J. Magn. Reson.*, 1990, **87**, 488.
21. G. Bodenhausen and D. J. Ruben, *Chem. Phys. Lett.*, 1980, **69**, 185.
22. K. Y. Wang, I. Goljer, and P. H. Bolton, *J. Magn. Reson., Ser. B*, 1994, **103**, 192.
23. J. Cavanagh, A. G. Palmer, P. E. Wright, and M. Rance, *J. Magn. Reson.*, 1991, **91**, 429.