

# The Two-Dimensional Nuclear Magnetic Resonance Spectroscopy of Macromolecules

FRANK A. BOVEY\* and PETER A. MIRAU

AT&T Bell Laboratories, Murray Hill, New Jersey 07974

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The study of polymers by nuclear magnetic resonance has made tremendous advances in the past few years, not only with the development of superconducting magnets with increasing field strength, permitting greater sensitivity and the resolution of increasingly fine structural detail, but also in the development of new techniques. Among these is two-dimensional (2D) NMR. The 2D NMR spectrum, instead of having the conventional single horizontal frequency scale, has two frequency scales at right angles to each other. The spectrum is actually a surface in three-dimensional space, as will be shown. Two-dimensional NMR was proposed by Jeener in 1971,<sup>1</sup> but was not widely appreciated until Ernst and his colleagues performed the first actual experiments in 1976.<sup>2</sup> It has found increasing use in organic chemistry and in the study of the structure and conformation of natural polymers—polypeptides, proteins, and nucleic acids—but until recently, there was little or no application to synthetic polymers, possibly because its application here is less obvious and the problems presented are somewhat different.

There are now many types of 2D NMR experiments, each consisting of a particular sequence of radio-frequency (rf) pulses and delays. The information contained in these experiments depends on the width, phasing, and timing of the pulses. Among the most common forms of 2D NMR are *correlation* experiments, in which the spectrum reveals the chemical shift positions of nuclei that are in some manner interacting with each other. This correlating interaction may be through-bond  $J$  coupling, through-space dipole-dipole interaction, or chemical exchange.

In conventional or 1D NMR one typically applies a 90° pulse (i.e., one that rotates the macroscopic nuclear magnetization into a plane transverse to the applied magnetic field) to the sample and measures the time-dependent voltage induced in an rf coil. This time-domain signal, or free induction decay, is stored in computer memory (perhaps after signal averaging) and then Fourier transformed into the corresponding frequency-domain spectrum. Thus, data are acquired during a single time period, i.e., the free induction decay, and consequently the final spectrum has only a single frequency scale. In 2D NMR a second time

variable is introduced in addition to the free induction decay. Following Fourier transformation of the signal during the data acquisition period, the data are transformed with respect to the second time variable to obtain a spectrum with two frequency coordinates. The additional frequency coordinate thus corresponds to the modulation of the signal intensities or phases by the second time variable.

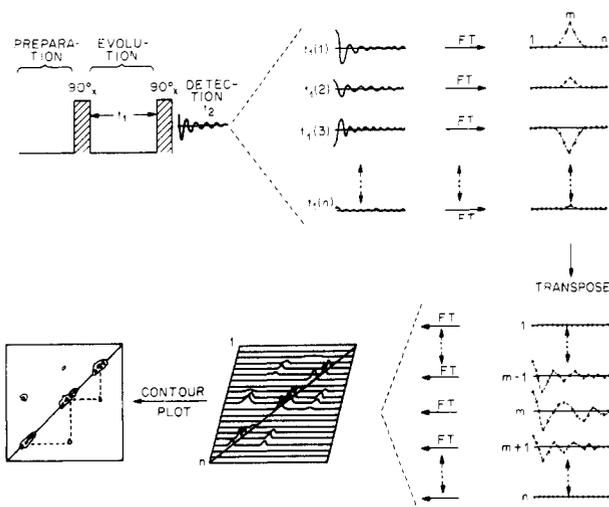
This description is made somewhat more concrete in Figure 1. The pulses and delays in the development of a 2D spectrum are typically divided into three periods, as shown in the upper left portion of the figure. During the *preparation* period, the populations of the Zeeman levels are allowed to equilibrate with their surroundings through spin-lattice relaxation. During the *evolution* period,  $t_1$ , the  $x$ ,  $y$ , and  $z$  components evolve under all the forces acting on the nuclei, including the interactions between them indicated above. We introduce the second time dependence by systematically incrementing the evolution time  $t_1$ , as shown. Following each interval  $t_1$ , a second 90° pulse is applied, after which exchange of magnetization between spins may occur. The FID is then acquired in  $t_2$  and transformed. The pulse sequence shown is actually appropriate for the production of a *chemical shift correlated* or COSY spectrum, in which the correlating influence is the  $J$  coupling between spins. In a typical example, we might employ 1K or 1024 such  $t_1$  increments, increasing from 0.5 to 500 ms. The free induction decay is different for each increment because the interacting spins modulate each other's response. Each FID is detected in  $t_2$  and transformed. We thus generate a series of 1024 matrix *rows*, each consisting of 1024 points (for a square matrix) representing the frequency-domain spectrum for a particular value of  $t_1$ . The *columns* of the matrix contain information about how the free induction decays were modulated as a function of  $t_1$ . We now perform a *transpose* operation, which consists of looking vertically down the columns at 1024 successive points and constructing 1024 new free induction decays from the information thus obtained. (For simplicity, the spectrum is represented as a single resonance at this stage, although in reality it will always be more complex.) We then perform a second Fourier transform on these new FID's, obtaining a square two-dimensional data matrix which is actually a surface in three-dimensional space and may be represented either as a stacked plot or as a contour plot, as shown. The stacked plot conveys relative peak intensities more vividly than the contour plot but is very time-consum-

Frank A. Bovey received a B.S. degree in chemistry in 1940 from Harvard College and Ph.D. in physical chemistry from the University of Minnesota in 1945. He joined AT&T Bell Laboratories in 1962. His research has concerned mainly the application of NMR to the study of the structure and conformation of polymer chains.

Peter A. Mirau obtained his B.A. in chemistry in 1976 from the University of California at Santa Barbara and his Ph.D. in pharmaceutical chemistry from the University of California at San Francisco in 1981. He is currently a Member of Technical Staff at AT&T Bell Laboratories. His research interests include liquid- and solid-state NMR studies of polymer structure and dynamics and the biophysics of DNA.

(1) Jeener, J. Ampère International Summer School, Basko Polje, Yugoslavia, 1971.

(2) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* 1976, 64, 2229.



**Figure 1.** Schematic representation of the generation of a two-dimensional (2D) correlated (COSY) spectrum in which the correlating influence is the  $J$  coupling between chemically shifted nuclei. (Jelinski, L. W. *Chem. Eng. News* 1984, Nov 5, p 26.)

ing to record and does not clearly show complex relationships. The contour plot is much preferred for most purposes.

Those nuclei that did not exchange magnetization have the same frequencies during  $t_1$  and  $t_2$ —designated  $F_1$  and  $F_2$ , respectively—and give rise to the normal spectrum along the diagonal, corresponding at each point to  $F_1 = F_2$ . Those nuclei that exchange magnetization owing to scalar coupling have a final frequency differing from the initial. This gives rise to off-diagonal contours or *cross peaks* connecting the coupled nuclei, as shown in Figure 1. We thus obtain in a single experiment a picture of all the  $J$ -coupled connectivities in the molecule. It may be imagined that this technique is of great importance in assigning the resonances of complex molecules.

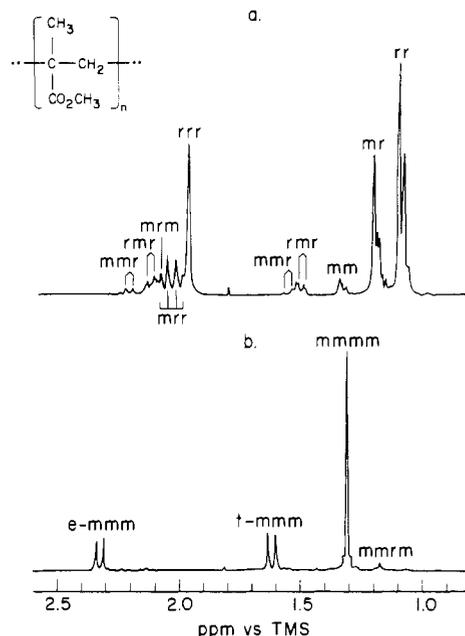
By a closely related technique, we may also obtain the direct through-space connectivities, in effect a map of all the intra- and intermolecular (usually interproton) distances of less than ca. 4 Å. Both techniques are examples of correlated spectroscopy, as we have seen. It is now customary to refer to the first of these, employing  $J$  coupling for the correlating interaction, as COSY, while the second, which uses a 2D version of the nuclear Overhauser effect or NOE, is termed NOESY. We present two examples of the application of each.

The above description of 2D NMR is very brief and incomplete. The reader is referred to the review of Lerner and Bax<sup>3</sup> for a fuller discussion.

### Stereochemical Configuration

Beginning about 35 years ago, polymer science has undergone a major revolution initiated by Ziegler's discovery of metal halide-metal alkyl initiators for ethylene polymerization. This finding has been greatly extended by Natta and his colleagues,<sup>4,5</sup> who showed how such catalysts can be used to produce polymers of *controlled stereochemistry* from a wide variety of vinyl and diene monomers. NMR is particularly informative concerning stereochemical microstructure, revealing details that can be seen by no other technique. We

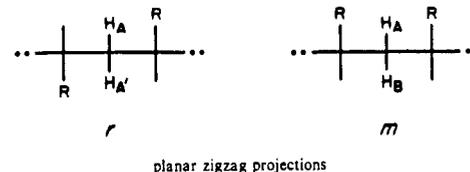
(3) Bax, A.; Lerner, L. *Science (Washington, D.C.)* 1986, 232, 960.  
 (4) Ziegler, K. *Angew. Chem.* 1964, 76 (Nobel Prize Address).  
 (5) Natta, G. *Chem. Ind. (Milan)* 1964, 46, 397 (Nobel Prize Address).



**Figure 2.** 500-MHz proton spectra of (a) free radical (predominantly syndiotactic) and (b) isotactic poly(methyl methacrylate) observed in 10% (w/v) solution in chlorobenzene- $d_5$  at 100 °C. (Schilling, F. C.; Bovey, F. A.; Bruch, M. D.; Kozłowski, S. A. *Macromolecules* 1985, 18, 1418.)

discuss two examples illustrating the use of 2D NMR in making resonance assignments in polymer spectra.

**Poly(methyl methacrylate).** In Figure 2 are shown the 500-MHz proton spectra of poly(methyl methacrylate) prepared (a) with a free radical initiator and (b) with fluorenyllithium in toluene, an anionic initiator. The profound effect of the nature of the initiator is evident in the marked differences between these spectra.<sup>6,7</sup> To interpret these spectra we consider the chain in terms of two monomer units or diads. The syndiotactic or *racemic* diad ( $r$ ) has a twofold symmetry axis and consequently the methylene protons are in equivalent environments on a time average over the chain conformations. The protons therefore have the same chemical shift and appear as a singlet. The isotactic or *meso* diad ( $m$ ) has a plane of symmetry but no twofold axis and so the two protons are nonequivalent and have different chemical shifts.

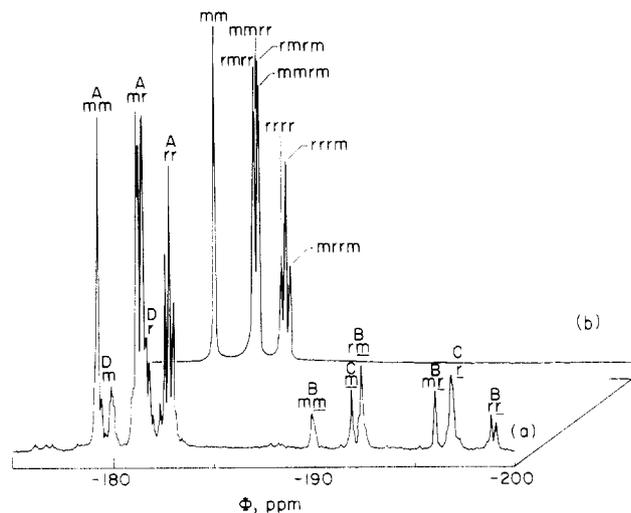


When there is no vicinal coupling to neighboring protons, as is the case in poly(methyl methacrylate), the syndiotactic sequences should exhibit a methylene singlet while the isotactic form should give two doublets, each with a spacing equal to the geminal coupling, ca. 15 Hz. We see in Figure 2 that the methylene spectrum of the anionic polymer (b) is almost exclusively a pair of doublets; quantitative assessment shows that this polymer is 95% isotactic. The methylene spectrum of the free radical polymer (a) is more complex, but the principal resonance—at ca. 1.9 ppm—is a singlet,

(6) Bovey, F. A.; Tiers, G. V. D. *J. Polym. Sci.* 1960, 44, 173.

(7) Schilling, F. C.; Bovey, F. A.; Bruch, M. D.; Kozłowski, S. A. *Macromolecules* 1985, 18, 1418 and references therein.



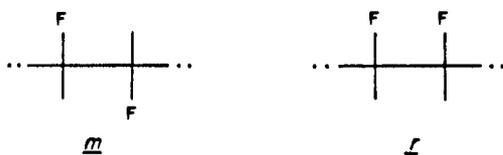


**Figure 4.** 188-MHz  $^{19}\text{F}$  spectrum of (a) commercial poly(vinyl fluoride) and (b) inversion-free poly(vinyl fluoride); both spectra observed as 8% solutions in *N,N*-dimethylformamide- $d_7$  at 130 °C. (Cais, R. E.; Kometani, J. M. In *NMR and Macromolecules*; Randall, J. C., Ed.; ACS Symposium Series 247; American Chemical Society: Washington, DC, 1984; pp 153-166.)

$J$  coupling has been removed by proton irradiation. The resonance assignments are indicated by the capital letters in the spectrum and the formula above. The stereochemical assignments are also indicated in Figure 4. The *m* and *r* designations that are not underlined represent the usual relationships between substituents in 1,3-positions (in planar-zigzag projection):



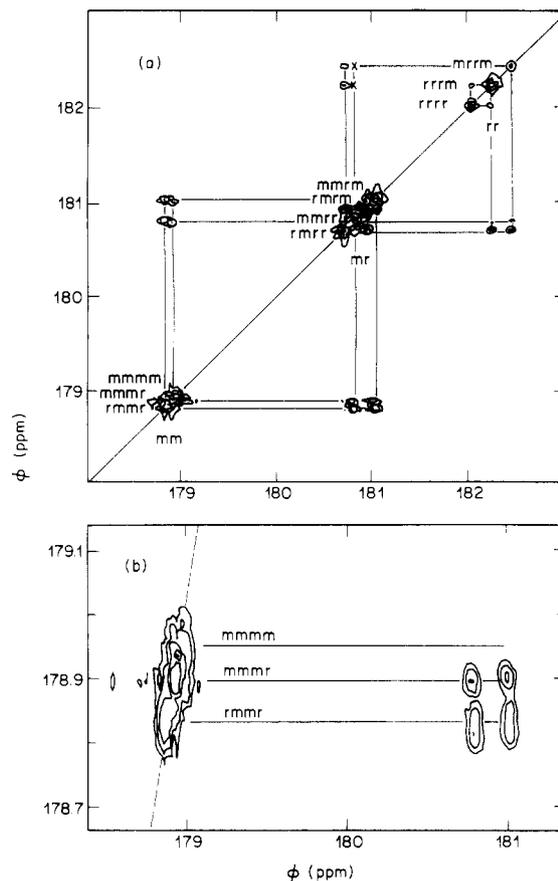
The underlined designations represent the substituents in 1,2-positions (also in planar-zigzag projection) in the head-to-head:tail-to-tail junctions:



One should note in connection with the B resonances that  $\underline{rm}$  and  $\underline{mr}$  are quite different structures and do not differ merely in direction as with *rm* and *mr*.

Spectrum b in Figure 4 is that of a poly(vinyl fluoride) prepared without inversions by a special chemical route. The upfield portion of spectrum a, as well as the D resonances, are absent, thus clearly identifying them as associated with monomer inversions. This material has a melting point of 210 °C, compared to the normal polymer's 190 °C. Both polymers are nearly atactic. The indicated assignments to stereochemical sequences present a difficult problem. The only clue provided by 1D  $^{19}\text{F}$  NMR is that in the spectrum of a polymer prepared by polymerization in a urea matrix—generally assumed to favor syndiotactic propagation—the most shielded of the A resonances

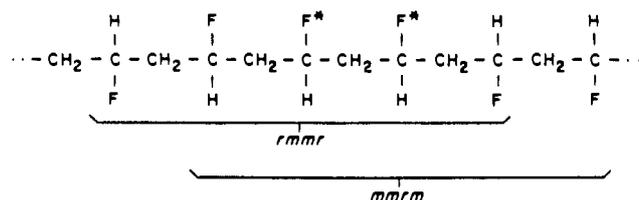
(14) Cais, R. E.; Kometani, J. M. In *NMR and Macromolecules*; Randall, J. C., Ed.; ACS Symposium Series 247; American Chemical Society: Washington, DC, 1984; pp 153-166.



**Figure 5.** (a) 188-MHz  $^{19}\text{F}$  2D  $J$ -correlated ("COSY") spectrum of an 11% solution of inversion-free poly(vinyl fluoride) in *N,N*-dimethylformamide- $d_7$  at 130 °C;  $\times$  denotes peaks that are visible at lower contour levels. (b) Expansion of *mm* region of spectrum a. (Bruch, M. D.; Bovey, F. A.; Cais, R. E. *Macromolecules* 1984, 17, 2547.)

shows increased intensity at the expense of the least shielded.<sup>15</sup> This group of resonances may therefore be assigned to 5-monomer (pentad) steric sequences centered on *rr* triads. But the remaining assignments in Figure 4 require 2D spectroscopy.

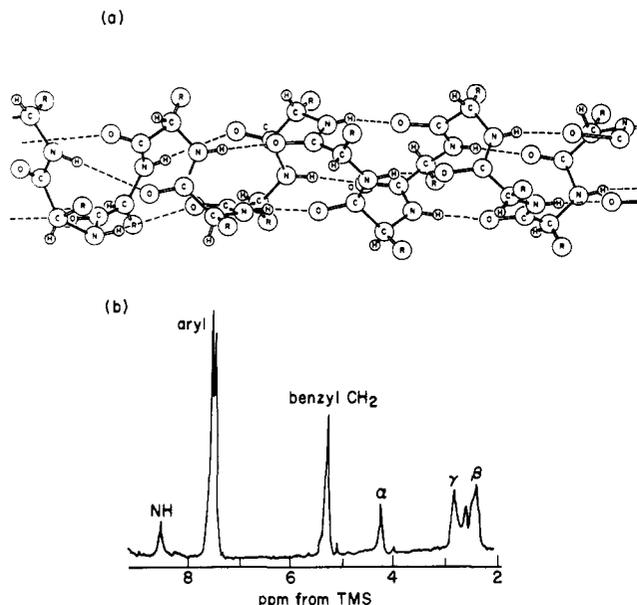
The 188-MHz  $^{19}\text{F}$  COSY spectrum of the inversion-free polymer is shown in Figure 5a.<sup>16</sup> We take advantage of the four-bond  $^{19}\text{F}$ - $^{19}\text{F}$   $J$  coupling between neighboring fluorines. This has a magnitude of about 7 Hz<sup>17</sup> and is too small to give rise to spectral splittings, but nevertheless generates the cross peaks shown in Figure 5a. These off-diagonal peaks can be used to make stereosequence assignments since there will be coupling between the central pair of fluorines in pentad sequences which share a common hexad. Thus, for example, in the hexad sequence



the coupling between the starred fluorines results in

(15) Cais, R. E., unpublished observations.  
(16) Bruch, M. D.; Bovey, F. A.; Cais, R. E. *Macromolecules* 1984, 17, 2547.

(17) Elleman, D. D.; Brown, L. C.; Williams, D. *J. Mol. Spectrosc.* 1961, 7, 307, 322.



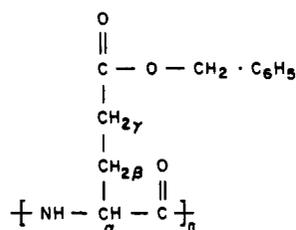
**Figure 6.** (a) Conformation of  $\alpha$ -helix (14-mer). (b) 500-MHz proton spectrum of  $\alpha$ -helical poly( $\gamma$ -benzyl L-glutamate) (PBLG) in 95:5 (v/v) chloroform-trifluoroacetic acid. The trifluoroacetic acid serves to break up aggregates of  $\alpha$ -helices, which themselves remain intact. (Mirau, P. A.; Bovey, F. A. *J. Am. Chem. Soc.* 1986, 108, 5130.)

cross peaks connecting the *mmmr* and *mmrm* resonances in the COSY spectrum. All the remaining pentad assignments can be made unambiguously from the correlations observed in this COSY spectrum. In Figure 5b the *mm*-centered region near  $\phi$  178.9 is expanded to show the 2D resonances more clearly.

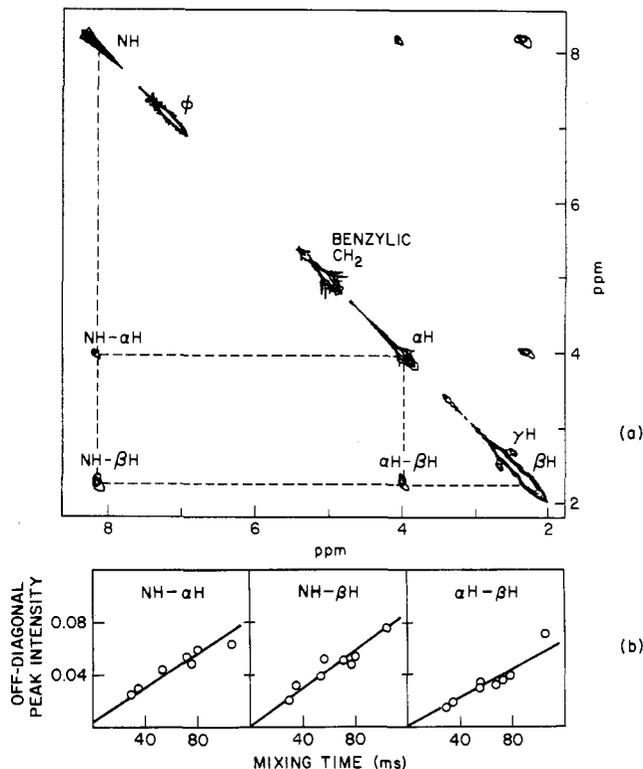
We see that in the 1D  $^{19}\text{F}$  spectrum (Figure 4) the *mm*-centered region shows no observable resolution of the pentad fine structure which is so clearly resolved in the *mr* and *rr* regions. However, the 2D spectrum does show such resolution; this is seen more clearly in the expanded spectrum of Figure 5b. Here, the *mm* diagonal resonance can be seen to consist of three regions, two of which correlate with the *mr* region and one of which does not. The latter may be identified as *mmmm*, which (like *rrrr*) shares no hexad with an *mr*-centered pentad.

The 2D  $^{19}\text{F}$  spectrum of the commercial poly(vinyl fluoride) provides detailed and unambiguous assignments of the inversion resonances in Figure 4a, but we do not have space to describe this in detail here.<sup>16</sup>

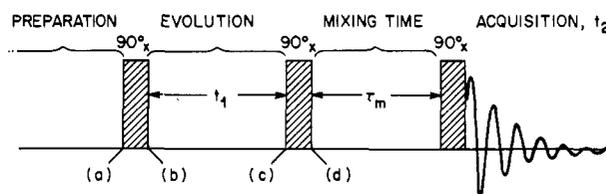
**Poly( $\gamma$ -benzyl L-glutamate).** We next consider the application of the NOESY form of 2D NMR spectroscopy to cases where the question at issue is not the covalent structure of the polymer chain—which is known—but rather the *conformation* of the chain. We first discuss the synthetic polypeptide poly( $\gamma$ -benzyl L-glutamate) or PBLG:



This polypeptide may exist in either  $\alpha$ -helical (Figure 6a) or random coil form in solution, depending on the



**Figure 7.** (a) Absorption-phase 2D NOE spectrum of poly( $\gamma$ -benzyl L-glutamate) (PBLG) in the  $\alpha$ -helical state in 95:5 (v/v) chloroform-trifluoroacetic acid. (b) Initial rise of cross peak intensities in spectrum a as a function of mixing time,  $\tau_m$  (ms); spectrum a corresponds to  $\tau_m = 76$  ms. (Mirau, P. A.; Bovey, F. A. *J. Am. Chem. Soc.* 1986, 108, 5130.)



**Figure 8.** Pulse sequence for generation of NOESY spectrum.

solvent and temperature. We are concerned here with the  $\alpha$ -helical form. The conformation of the  $\alpha$ -helix in the crystalline state is well-known from X-ray diffraction. The existence of the  $\alpha$ -helix in solution in solvents such as chloroform can be demonstrated by its optical properties; it is assumed to have a conformation very similar to that of the solid state. This may be tested through measurement of interproton distances by NOESY.

In Figure 6b is shown the 500-MHz 1D proton spectrum of a PBLG 20-mer in  $\alpha$ -helical form (Figure 6a represents a 14-mer), observed in  $\text{CDCl}_3$  solution. The proton resonances that principally concern us are identified: NH,  $\alpha\text{H}$ , and  $\beta\text{H}$ . The protons of the  $\beta\text{-CH}_2$  and  $\gamma\text{-CH}_2$  groups differ from each other in chemical shift within each group but the difference is not resolved in the helix spectrum. The side chains of PBLG are large enough that the helical 20-mer is nearly as broad as it is long and may be treated as tumbling isotropically in solution. In Figure 7a is shown the 500-MHz NOESY spectrum, observed under the same conditions. In order to make clear the interpretation of this spectrum, we must add a few words of explanation of the NOESY experiment. In Figure 8 is shown

Table I  
Cross-Relaxation Rates, Calculated Interproton Distances,  
and X-ray Distances for  $\alpha$ -Helical PBLG

interaction	$\sigma, \text{s}^{-1}$	$r_{\text{HH}}, \text{\AA}$	
		from NOESY	expected for $\alpha$ -helix (X-ray)
NH- $\alpha$ H	0.54	2.20	2.48
NH- $\beta$ H	0.55	2.20	2.26
$\alpha$ H- $\beta$ H	0.72	2.10	2.20

the NOESY pulse sequence. Between the two  $90^\circ$  pulses of the COSY experiment an additional  $90^\circ$  pulse is inserted. Following this there is a mixing time,  $\tau_m$ , before the final pulse and spectrum acquisition. This mixing time is of the order of the spin-lattice relaxation time ( $T_1$ ) of the observed protons. During  $\tau_m$  some spins exchange magnetization by direct, through-space dipole-dipole interaction. The result of this is that spins labeled by their frequencies during the evolution time  $t_1$  may precess at a different frequency at the end of  $\tau_m$ . This gives rise to cross peaks in the 2D spectrum. When  $\tau_m$  is very short compared to the rate of magnetization exchange, the cross peaks will be very weak compared to the diagonal peaks. If we choose  $\tau_m$  to be long enough to allow the  $z$  magnetizations of the spins in each interacting set to equilibrate, the intensities of the diagonal and cross peaks will be equal. (If  $\tau_m$  greatly exceeds  $T_1$ , the whole system will come to thermal equilibrium and no peaks will be detected, either on or off the diagonal.) By measuring peak intensities as a function of  $\tau_m$ , i.e., by carrying out several NOESY experiments at varied  $\tau_m$  values, one may determine rates of magnetization transfer between spins and from these one may calculate interproton distances.

The 2D spectrum of Figure 7a was obtained with a  $\tau_m$  of 76 ms. In Figure 7b the growth of the cross peaks is shown as a function of  $\tau_m$ . Interproton distances may be obtained from the slopes of these plots by the relationship

$$\text{slope} = \sigma = 5.7 \left[ \frac{6\tau_c}{1 + 4\omega^2\tau_c^2} - \tau_c \right] \times 10^{10} r_{\text{HH}}^{-6}$$

where  $\omega$  is the observing frequency ( $3.14 \times 10^9 \text{ rad s}^{-1}$ ) and  $\tau_c$  the correlation time of the helix, found by other measurements<sup>18</sup> to be  $10^{-9}$  s; this may be taken as the average time for the molecule to turn through one radian. In Table I are shown the three interproton distances concerned as found from the X-ray structure of the crystalline  $\alpha$ -helix and as measured by the 2D experiment. The values are comparable, but the main-chain NH- $\alpha$ H distance differs by more than the experimental error ( $\pm 0.15$ ) and represents a real difference between the crystalline and solution conformations.

**1:1 Alternating Styrene-Methyl Methacrylate Copolymer.** As a final example, we briefly consider the more difficult case of the solution conformation of a polymer chain having a high degree of local segmental freedom in solution rather than a fixed structure like that of PBLG. Here there is no X-ray structure to refer to and we must make comparison instead to the results of theoretical rotational isomeric state calculations. It has in the past been experimentally difficult to study the molecular behavior of such chains at the local level, and indirect methods, such as the measurement of

(18) Mirau, P. A.; Bovey, F. A. *J. Am. Chem. Soc.* **1986**, *108*, 5130.

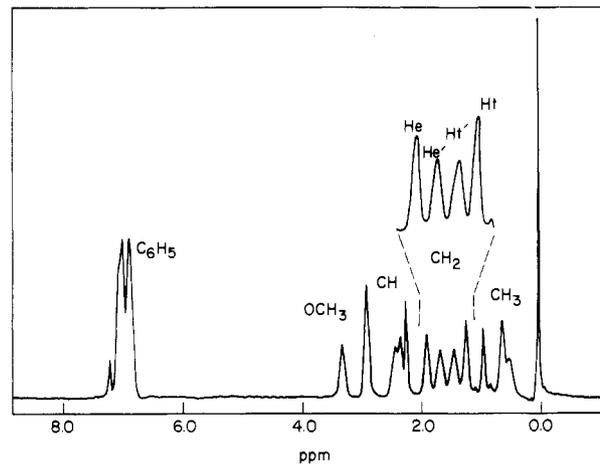
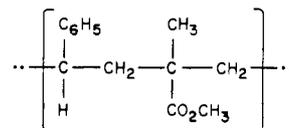
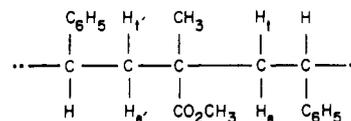


Figure 9. 500-MHz proton spectrum of a 1:1 alternating styrene-methyl methacrylate copolymer in 90:10 (v/v) hexachlorobutadiene-benzene- $d_6$  at  $65^\circ\text{C}$ . (Mirau, P. A.; Bovey, F. A.; Tonelli, A. E.; Heffner, S. A. *Macromolecules* **1987**, *20*, 1701.)

end-to-end distances or dipole moments, have been the only recourse.<sup>19,20</sup> However, observation of the interproton distances by cross-relaxation measurements can yield a direct measure of the local conformational preferences, averaged (in inverse-sixth-power space) over the available conformations.<sup>21,22</sup> A 1:1 alternating copolymer of styrene and methyl methacrylate has been employed for such measurements:<sup>21,22</sup>



This copolymer has been shown to be strictly alternating in comonomer sequence<sup>21</sup> and random in stereochemical configuration.<sup>19,20</sup> Its principal advantage for such a study is that the "coheterotactic" triad (50% of the chain structure)



exhibits neighboring methylene groups with four clearly distinguishable protons, designated by an extension of the erythro-threo nomenclature described above for poly(methyl methacrylate). This is shown in the 500-MHz proton spectrum in Figure 9, in which the methylene region is also shown expanded.

A rotational isomeric state modeling of this copolymer chain,<sup>10,12,22,23</sup> as well as examination of space-filling molecular models, shows that only the conformations in Scheme I need to be considered, other conformers being too highly hindered to contribute appreciably.

In Figure 10 is shown the NOESY spectrum of the methylene proton region (Figure 9, inset). The strongest cross peaks, marked  $\text{H}_e\text{---H}_t$  and  $\text{H}_e\text{---H}_t'$ , rep-

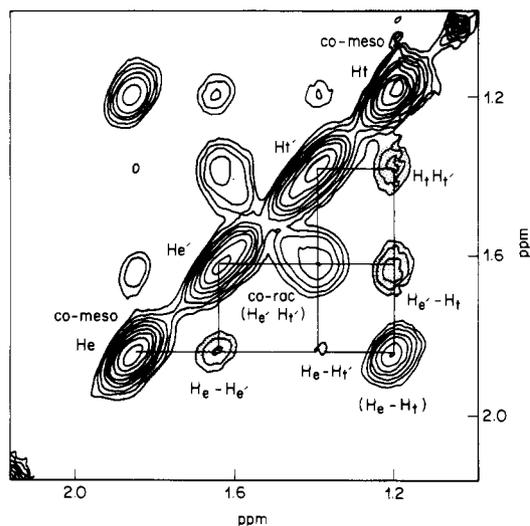
(19) Flory, P. J. *Statistical Mechanics of Chain Molecules*; Wiley-Interscience: New York, 1969.

(20) Bovey, F. A. *Chain Structure and Conformation of Macromolecules*; Academic: New York, 1982.

(21) Heffner, S. A.; Bovey, F. A.; Verge, L. A.; Mirau, P. A.; Tonelli, A. E. *Macromolecules* **1986**, *19*, 1628.

(22) Mirau, P. A.; Bovey, F. A.; Tonelli, A. E.; Heffner, S. A. *Macromolecules* **1987**, *20*, 1701.

(23) Koinuma, H.; Tanabe, T.; Hirai, H. *Macromolecules* **1980**, *13*, 383 and references therein.



**Figure 10.** 500-MHz 2D NOE spectrum of 1:1 alternating styrene-methyl methacrylate copolymer (same conditions as in Figure 9). This is the methylene portion of the spectrum showing geminal cross peaks ( $H_e-H_t$ ;  $H_{e'}-H_{t'}$ ) and vicinal cross peaks ( $H_e-H_{e'}$ ,  $H_e-H_{t'}$ ,  $H_{e'}-H_t$ ,  $H_{e'}-H_{t'}$ ). (Mirau, P. A.; Bovey, F. A.; Tonelli, A. E.; Heffner, S. A. *Macromolecules* 1987, 20, 1701.)

**Table II**

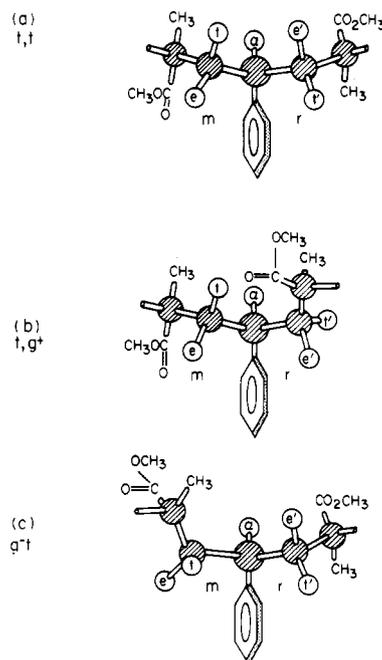
conformer	fraction	
	calcd from RIS	exptl from NMR
tt	0.53	$0.58 \pm 0.05$
$tg^+$	0.20	$0.24 \pm 0.05$
$g^+t$	0.27	$0.18 \pm 0.05$

represent the geminal cross relaxation in each methylene group, co-meso and co-racemic. Here the internuclear distance is fixed at 1.8 Å; this relaxation rate does not provide conformational information but serves as an interproton distance calibration for the conformationally sensitive interactions:  $H_e-H_{e'}$ ,  $H_t-H_{t'}$ ,  $H_e-H_{t'}$ , and  $H_{e'}-H_t$ . It can be seen that these interactions vary in intensity,  $H_e-H_{t'}$  being markedly weaker than the others. These interproton distances are sensitive to the conformer preferences. Sufficient information is available from these rates of cross relaxation—i.e., from the cross peak intensities measured as a function of mixing time—to yield not only the internuclear distances but also to provide an estimate of  $\Delta\phi$  (Scheme II), the deviation of the torsional angles away from the exact-staggered values, expected to occur in order to relieve the phenyl- $C_\alpha$  steric interaction. A value of  $12^\circ$  is consistent with the NMR data; a value of  $20^\circ$  was employed in the RIS calculations.<sup>23</sup> In Table II, the conformer fractions calculated by the RIS model (with  $\Delta\phi = 20^\circ$ ) are compared with the NMR values. The agreement is good for the trans-trans conformer, but the deviations for the others are outside of estimated experimental error.

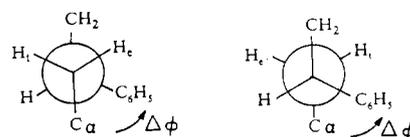
### Concluding Remarks

We have described examples illustrating the usefulness of 2D NMR in the study of polymer structure. In the determination of covalent structure, COSY, or correlated NMR spectroscopy in which  $J$  coupling provides the correlating interaction, enables one to assign resonances of stereochemical sequences with

**Scheme I**



**Scheme II**



certainty—often a difficult problem. In the case of isotactic poly(methyl methacrylate) the COSY spectrum also reveals a weak four-bond coupling, not detectable in the 1D spectrum, which indicates a predominantly trans-trans conformation in solution.

The 2D nuclear Overhauser effect or NOESY spectrum reveals direct, through-space dipole-dipole interactions. From the rates of growth of these cross peaks, proton-proton distances may be obtained both in polymers of fixed conformational structure, such as  $\alpha$ -helical poly( $\gamma$ -benzyl L-gutamate), and in flexible, random coil polymers, illustrated by a 1:1 alternating styrene-methyl methacrylate copolymer. In the latter case, the proton-proton distances obtained represent an inverse-sixth-power average. In both cases, preferred conformations in solution may be deduced.

These studies show that 2D NMR experiments developed for structure elucidation and determination can be easily applied to synthetic and natural macromolecules. In the future we may expect to see the application of more complex 2D NMR experiments capable of determining the properties of polymers at increasingly subtle levels. In addition to the solution studies, 2D has been used to examine the organization and dynamics of polymers in the solid state. As the technology for the measurement of 2D NMR in the solid state becomes more routine, it will play an increasingly important role in our understanding of the relationship between the chemical structure of polymers and their physical and mechanical properties.