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New 3D NMR Pulse Sequences for Characterization of Polymer Chain End Structures

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A series of three-dimensional (3D) nuclear magnetic resonance (NMR) pulse sequences, utilizing pulsed-field gradients (PFG) techniques, were developed or adapted from biological experiments for applications in the characterization of the structures of polymers and other heteroatom-containing organic materials, in much the same way that the data from multiple 3D NMR experiments have been used in biological structure determination. This initial Communication describes variations of an ^{1}H/X/Y chemical shift correlation (HXY) experiment, and an HCX sequence (Y = ^{13}C) is combined with ^{13}C homonuclear isotropic mixing to generate new pulse sequences which provide additional structural information. Spectra of polystyrene and poly(α,β-$^{13}C_2$-styrene) (PS) prepared by diphenylphosphinyl radical (DPPR) initiated polymerization of α,β-$^{13}C_2$-styrene are used to illustrate the application of these techniques for characterization of polymer chain end structures. While polymers are used to illustrate the applications of these pulse sequences, they can just as easily be used to study other organic structures containing an NMR-active X nucleus. Organometallic chemistry is especially suited for applications of these NMR experiments. © 1998 Academic Press

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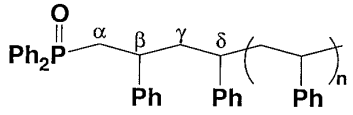
A knowledge of polymer chain end structures is important for the development of a complete understanding of the initiation, transfer, and termination reactions involved in polymerization processes. Previously, we have demonstrated that the ^{1}HXY (HCAP, where $Y = {}^{13}C_{\alpha}$ and $X = {}^{31}P$) correlation 3D NMR experiment can be used to selectively detect signals originating from CH_n groups directly bonded to ^{31}P introduced at the polymer chain end, and filters all other signals from the spectrum of diphenylphosphinyl radical (DPPR) initiated polystyrene (*1*). A complete understanding of the initiation reaction with this radical requires chemical shift information about the backbone CH_n nuclei from at least the first two monomer repeat units (i.e., C_{α}, C_{β}, C_{γ}, and C_{δ}) at the ^{31}P-containing chain end. Detection of signals based on long-range J_{CP} or performing $^{13}C-{}^{13}C$ relay-type experiments when ^{13}C is present at natural abundance are extremely difficult because of the low chain end concentrations and efficient relaxation which broadens the resonances. Therefore, α,β-$^{13}C_2$-styrene was polymerized by initiation with DPPR to aid in the identification of resonances from the structure fragments formed by the addition of the first two monomer units in the polymerization.

One of the most probable structures formed at the poly(α,β-$^{13}C_2$-styrene) (PS) chain ends is



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which results from an initiation step that involves addition of a DPPR to the methylene carbon of styrene. Configurational isomers of this structure are expected because meso and racemic configurations of styrene–styrene enchainments exist. The ^{31}P 1D NMR spectrum of PS shows signals from at least four major components and a number of minor components (*1*).

If ^{13}C-labeling of the polymer backbone is performed, the HCACO–TOCSY experiment, developed by Kay *et al.* (*2*), might be used to characterize the structures present; however, a spectrum obtained with this pulse sequence produces TOCSY type $^{1}H-{}^{1}H$ correlations with cross peaks between the resonances of protons which are *J*-coupled. Most polymers produce ^{1}H spectra with broad overlapping resonances, precluding the extraction of structure information. Here, we demonstrate another type of ^{13}C spin-lock experiment which includes a ^{13}C chemical shift evolution period after the ^{13}C spin-locking period. Thus, in this experiment, we can take advantage of the large ^{13}C chemical shift dispersion to characterize the polymer.

Figure 1 shows the pulse sequences used in this work. The sequence in Fig. 1a is the constant time (CT) HCAP

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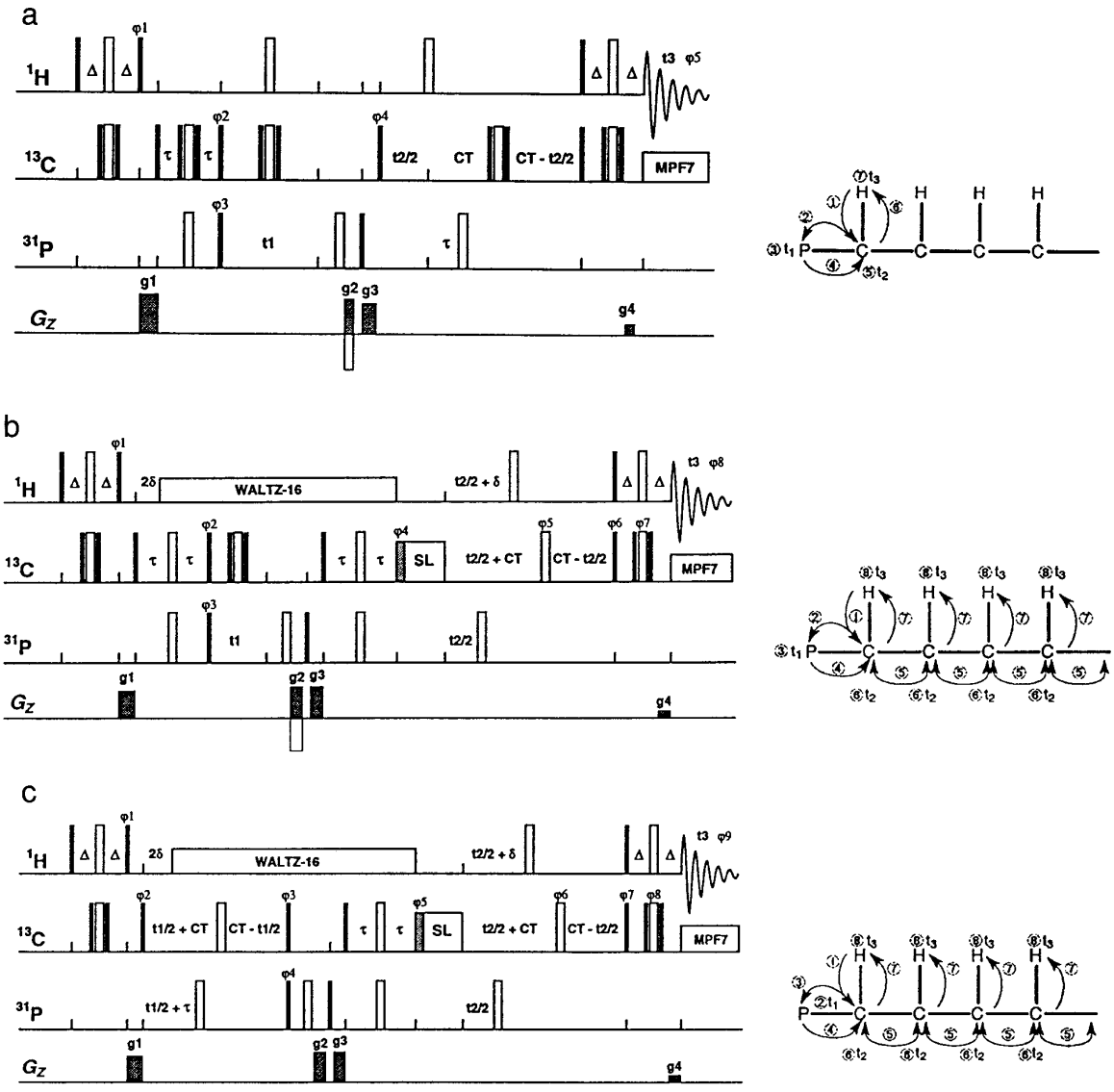

FIG. 1. Pulse sequences for the (a) CT-HCAP, (b) H(CA)P-CC-TOCSY, and (c) HCA(P)-CC-TOCSY experiments. Solid and open pulses are 90° and 180° pulses, respectively. All composite ^{13}C pulses have relative phase of *x,y,x* and all other pulses are applied along the $+x$ axis unless otherwise noted. (a) In the CT-HCAP experiment, delays were set to $\Delta = 1.78$ ms, $\tau = 4.17$ ms, and CT = 14.3 ms, respectively. The phase cycling was $\varphi 1 = y; \varphi 2 = y; \varphi 3 = x, -x$ (incremented in t_1 according to the States method (7)); $\varphi 4 = 2y,2(-y)$ (incremented in t_2 according to the States method); $\varphi 5 = x, -x, -x,x$. (b) In the H(CA)P-CC-TOCSY experiment, the delays were set to $\Delta = 1.78$ ms, $\delta = 1.19$ ms, $\tau = 4.17$ ms, and CT = 14.3 ms, respectively. The phase cycling is as follows: $\varphi 1 = y, -y; \varphi 2 = x, -x; \varphi 3 = 2x,2(-x); \varphi 4 = 4x,4(-x); \varphi 5 = 4x,4y,4(-x),4(-y); \varphi 6 = x$ (incremented in t_2 according to the States method); $\varphi 7 = 4x,4(-x); \varphi 8 = 4x,4(-x)$. Two spectra were collected with different signs of the second PFG and combined to obtain a pure absorption spectrum in f_1 (*8*). (c) In the HCA(P)-CC-TOCSY experiment, the delays were set at $\Delta = 1.78$ ms, $\delta = 1.19$ ms, $\tau = 4.17$ ms, and CT = 14.3 ms, respectively. The phase cycling is as follows: $\varphi 1 = y,-y; \varphi 2 = x$ (incremented in t_1 according to the States method); $\varphi 3 = x,-x; \varphi 4 = 2x,2(-x); \varphi 5 = 4x,4(-x); \varphi 6 = 4x,4y,4(-x),4(-y); \varphi 7 = x$ (incremented in t_2 according to the States method); $\varphi 8 = 4x,4(-x); \varphi 9 = 4x,4(-x)$.

correlation experiment which is a variation of that reported by Marino *et al.* (*3*) for studying oligonucleotides. The coherence transfer path is illustrated by the structure in Fig. 1a. Sequential INEPT-type transfers are performed to move magnetization from ^1H to ^{13}C (using $^1J_{CH}$) then from $^{13}C_\alpha$ to ^{31}P (using $^1J_{CP}$); ^{31}P chemical shift evolution occurs during t_1; then magnetization transfer occurs from ^{31}P back to $^{13}C_\alpha$; a constant evolution period, CT, is used to encode ^{13}C chemical shift during t_2; and magnetization is transferred from ^{13}C to ^1H for detection. The end result is a 3D spectrum with ^1H, ^{13}C, and ^{31}P shifts defined along the three axes and cross peaks which correlate the shifts of these atoms when they form an H−C−P structure fragment. With uniformly ^{13}C-labeled samples, the constant evolution period is neces-

sary to eliminate the effects of $^{13}C-{}^{13}C$ homonuclear coupling during t_2. Since all heteronuclear one-bond J values present in the PS sample studied here are large, the optimum delays are short. Therefore, the CT delay $(1/(2\times{}^1J_{cc}))$ is based on $^{13}C-{}^{13}C$ homonuclear coupling.

Figures 1b and 1c show the new spin-lock pulse sequences developed for this work. Both experiments provide correlations of the chemical shifts of the atoms anchored to a polymer chain end bearing an X nucleus having $I = \frac{1}{2}$ such as ^{31}P, while filtering the main-chain resonances from the spectrum. The coherence transfer pathways in these experiments are similar to that in the CT-HCAP experiment with a few exceptions. These experiments both use the DIPSI-3 (*4*) isotropic mixing scheme for the spin-locking ^{13}C. In both experiments, ^{13}C chemical shift evolution takes place after the spin-lock period so that these experiments can take advantage of wide dispersion of the ^{13}C resonances.

The pulse sequences in Fig. 1b will be referred to as the H(CA)P-CC-TOCSY experiment; it correlates the chemical shifts of ^1H, ^{13}C, and ^{31}P nuclei along the three axes. After $H \rightarrow C_\alpha \rightarrow P(t_1) \rightarrow C_\alpha$ sequential magnetization transfer, ^{13}C spin-locking transfers coherence down the backbone of mutually coupled ^{13}C atoms; magnetization is then transferred from C to directly bound H atoms for detection. A slice from the 3D spectrum at one ^{31}P shift in f_1 will contain C–H correlations for each of the first 2–6 CH_n groups on the chain end attached to that ^{31}P. Alternatively, if the ^{13}C shift of a carbon near the chain end is known, an $f_1 f_3$ slice at its shift in the f_2 dimension will contain cross peaks correlating the shifts of the ^{31}P and α-^1H atoms at that chain end.

In some instances, the resonances of the X nuclei on the various chain ends are not well resolved. In these circumstances, it can be useful to disperse the resonances from the different chain ends based on the ^{13}C chemical shifts of C_α. This is accomplished with the pulse sequence shown in Fig. 1c, which will be referred to as the HCA(P)-CC-TOCSY experiment. This sequence has a coherence transfer pathway identical to that of the sequence in Fig. 1b; it differs only by the fact that a constant evolution time CT is inserted before the spin-lock period to encode ^{13}C chemical shift during t_1, and the ^{31}P evolution period is replaced with a fixed polarization transfer delay so that only coherence originating from the ^{13}C bound to ^{31}P is relayed to the end of the sequence for detection.

Figure 2a shows the $f_2 f_3$ ($\delta^{13}C$ vs δ^1H) projection of the 3D CT-HCAP spectrum from PS. This spectrum displays signals from CH_n groups directly bonded to ^{31}P much like the single quantum (SQ)-HCAP spectrum (*1*). This CT-HCAP spectrum of PS shows only resonances originating from C_α, similar to the resonances observed in the spectrum of the unlabeled sample. The $f_2 f_3$ projection of the H(CA)P-CC-TOCSY spectrum (Fig. 2b) shows cross peaks in four regions. The first group of cross peaks (Fig. 2b, region 1) is also found in the CT-HCAP spectrum. These correlations

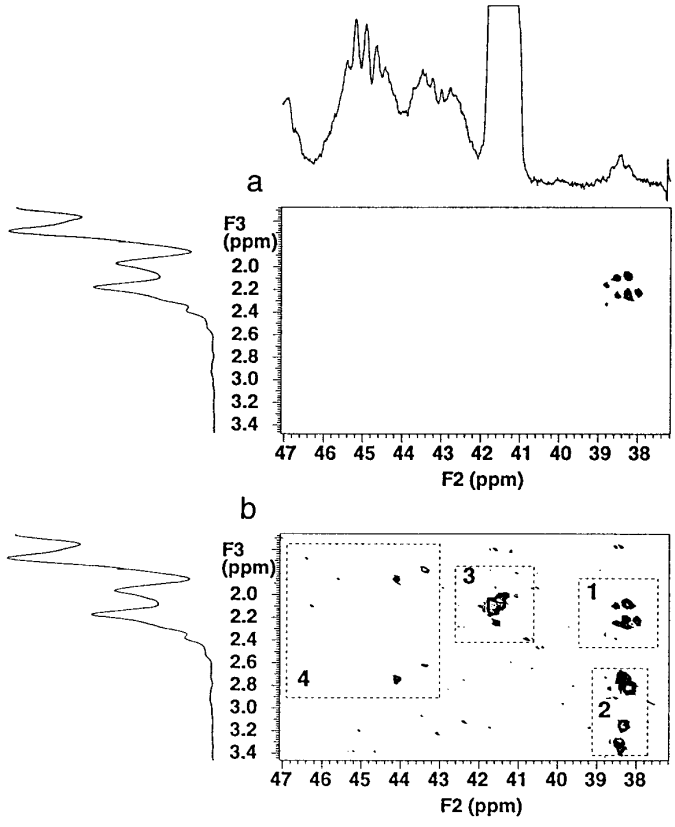

FIG. 2. The $f_2 f_3$ projections of the 3D spectra of DPPR-initiated PS observed with (a) CT-HCAP and (b) H(CA)P-CC-TOCSY pulse sequences. Corresponding regions from the ^1H and ^{13}C spectra are plotted along the side and top axes, respectively. The parameters used to collect these spectra are found in the legend of Fig. 3.

relate the H_α and C_α resonances of CH_n groups directly bonded to ^{31}P atoms at the chain end ($\delta^1H = 2.2$, $\delta^{13}C = 38.5$). The second group of cross peaks (Fig. 2b, region 2) correlate the ^1H resonances at 2.7–3.3 ppm with the ^{13}C resonances near 38.5 ppm; the third group of cross peaks (Fig. 2b, region 3) are correlations to the ^{13}C resonances around 41.5 ppm; and the last group of cross peaks (Fig. 2b, region 4) correlate the ^{13}C resonances at 47–43 ppm with the resonances of two nonequivalent ^1H atoms. The cross peaks in regions 2–4 originate from carbons two to four bonds away from the chain end ^{31}P nucleus. It is important to assign these resonances in order to determine the chain end structures.

Figure 3 illustrates the combined use of these 3D NMR techniques to establish the connectivities of the first four CH_n groups from the chain end structure **1.** This figure contains selected slices from the three experiments shown in Fig. 1. The assignments start with the $f_1 f_3$ ($\delta^{31}P$ vs δ^1H) slice from the CT-HCAP spectrum (Fig. 3a) at the shift of C_α in f_2; this spectrum identifies the resonances of $H_{\alpha1}$, $H_{\alpha2}$, and P from the chain end of **1.** Since there are two nonequivalent

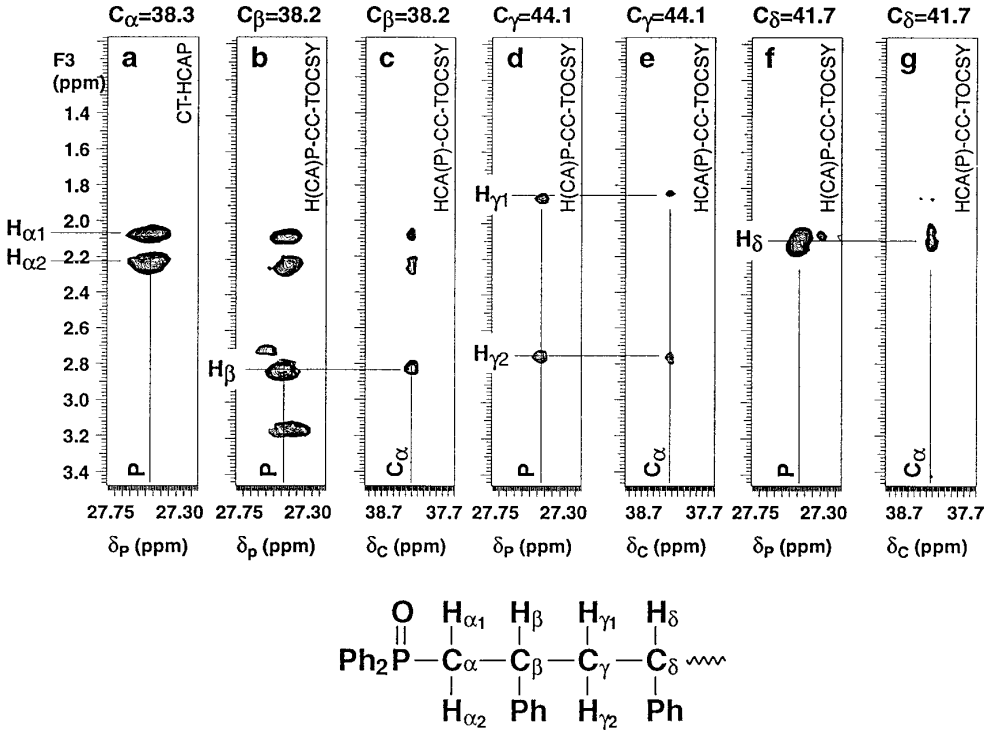

FIG. 3. Slices from a variety of 3D NMR spectra of poly(α,β-$^{13}C_2$-styrene) using (a) the CT-HCAP pulse sequence in Fig. 1a, (b, d, and f) the H(CA)P-CC-TOCSY pulse sequence in Fig. 1b, and (c, e, and g) the HCA(P)-CC-TOCSY pulse sequence in Fig. 1c. Lines indicate the connectivities from C_α through C_δ. The slices are each labeled at the top with the ^{13}C SQ chemical shift, and the carbon number responsible for that slice. All spectra were obtained on a Varian Unity*plus* 600 MHz spectrometer equipped with Ultra·shims, a pulsed field gradients (PFG) accessory, and a Nalorac $^1H/^{13}C/X$ PFG triple-resonance probe, using 34 mg of PS in C_6D_6 contained in a 5-mm NMR tube. The temperature was regulated at 30.0 ± 0.1°C. The following parameters were used to collect the spectra: ^1H, ^{13}C, and ^{31}P 90° pulse widths of 10.5, 20.0, and 17.0 μs, respectively; 606.2-, 2556.9-, and 8000.0-Hz spectral windows in the $^{31}P(f_1)$, ^{13}C (f_2), and ^1H (f_3) dimensions, respectively; 16 complex t_1 (^{31}P) and 64 complex t_2 (^{13}C) increments; a 1.0-s relaxation delay; and a 0.048-s acquisition time with ^{13}C MPF7 (*9, 10*) decoupling using a field strength of 2.49 kHz. Gradients g1 through g4 were applied for 3.0, 1.0, 2.0, and 1.0 ms, respectively, with strengths of 0.247, 0.309, 0.309, and 0.125 T m^{-1}, respectively. The second and fourth PFGs provide coherence selection between ^{31}P and ^1H nuclei, while the first and third PFGs were used for purging undesired signals. All data processing was performed on a Sun SPARCstation 10 using Varian's Vnmr software. Digital signal processing was applied to reduce the spectral window in f_3 from 8000.0 to 2000.0 Hz, and the data were processed with a combination of shifted sinebell and Gaussian weighting in all dimensions. Linear prediction was used to forward extend the data in the t_1 and t_2 time dimensions to twice the original size (12 complex points were used to calculate two coefficients). Three-dimensional Fourier transforms were performed on 256 × 256 × 256 matrices. Spectrum (a) was obtained with the CT-HCAP pulse sequence shown in Fig. 1a by averaging four transients per free induction decay (FID). The spectra which produced the slices in (b–g) were obtained with the following additional parameters: 16 transients per FID, a 10.4-ms isotropic mixing time using the DIPSI-3 (*4*) sequence with a field strength of 5.21 kHz followed by a 2.0-ms trim pulse, and ^1H WALTZ-16 (*11*) decoupling with a field strength of 2.00 kHz.

protons correlated to this ^{31}P resonance, the chain end must contain a methylene group bound to phosphorus. These H_α–P correlations were also found in the f_1 slice at the shift of C_β in the H(CA)P-CC-TOCSY spectrum (Fig. 3b), permitting the assignment of C_β and H_β resonances. Confirmation of these resonance assignments comes from the presence of these same ^1H resonances in the f_1 slice at the shift of C_β in the HCA(P)-CC-TOCSY spectrum (Fig. 3c), and from the CT-HCCH spectrum (*5*) (not shown). The resonances of nonequivalent H_γ atoms can be correlated with the ^{31}P and C_α resonances in the f_2 slices at the shift of C_γ from the H(CA)P-CC-TOCSY and HCA(P)-CC-TOCSY spectra in Figs. 3d and 3e, respectively. Similarly, the H_δ resonances can be correlated with the ^{31}P and C_α resonances in the f_2 slices at the shift of C_δ from the H(CA)P-CC-TOCSY and HCA(P)-CC-TOCSY spectra in Figs. 3f and 3g, respectively. Correlations from atoms further down the chain could

TABLE 1
Chemical Shift Assignments for the Chain End Structure 1 of DPPR Initiated PS

Nucleus	^{31}P	^{13}C	^1H
α	27.4 ~ 27.7	38.3	2.08, 2.24
β	—	38.2	2.82
γ	—	44.1	1.86, 2.72
δ	—	41.7	2.09

not be detected. The spin-locking time was qualitatively set to obtain the best signal strength for the first few carbons on the chain. While longer spin-locking times might promote coherence transfer further down the chain, permitting the identification of ϵ and ζ resonances, longer spin-locking times also significantly attenuate all of the signals. The resonance assignments for the chain end structure of **1** are summarized in Table 1.

The use of HCAP correlation NMR experiments provides spectra which can be used to characterize the minor structures at polymer chain ends. When ^{13}C enrichment is used, performance of ^{13}C homonuclear spin-locking experiment are possible. The spectra of PS not only provide directly attached CH_n–P signals but also CH_n resonances that are few bonds away from the ^{31}P atoms, permitting the identification of the first few monomer units at the chain end. Pulsed-field gradient (PFG) techniques (*6*) were essential for detection of the signals of interest in this work. Without their use for coherence selection, severe dynamic range problems would significantly hamper the ability to detect weak resonances from chain end structures in the presence of the intense signals originating from ^{13}C-enriched repeat units of the polymer backbone. Techniques related to those which that have applied to biological systems can be enormously useful for characterizing the structures of polymeric materials. Although NMR studies of polymers are described here, similar techniques can also be helpful for the study of other organic materials when NMR-active heteroatoms such as ^{31}P, ^{15}N, and ^{19}F are present.

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