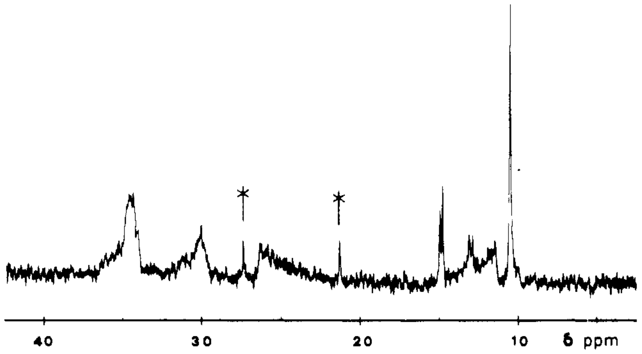

Figure 1. ^{13}C NMR spectrum of the benzene-soluble fraction of isotactic poly[(*RS*)-3-methyl-1-pentene]. The resonances marked by asterisks are due to ^{13}C-enriched $\beta\beta$ (21.5$_5$ ppm) and $\alpha\gamma$ (27.6$_0$) methylene carbons (HMDS scale).

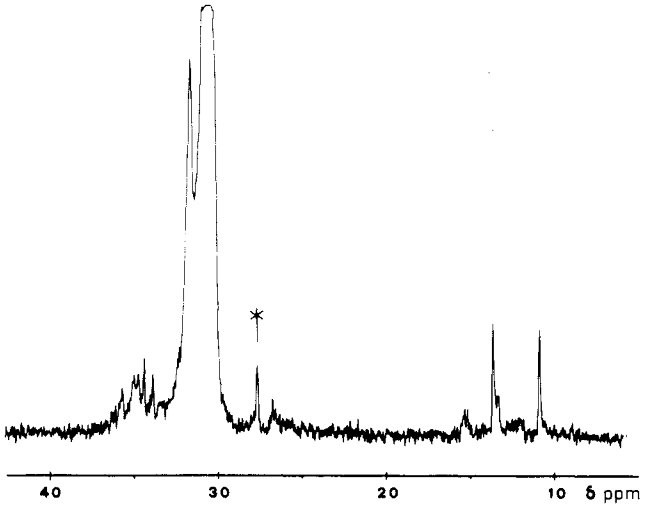

Figure 2. ^{13}C NMR spectrum of the benzene-soluble fraction of poly[(*RS*)-3-methyl-1-pentene-*1*-^{13}C]. The resonance marked by an asterisk is due to ^{13}C-enriched $\alpha\gamma$-methylene carbons (HMDS scale).

from enriched ethylene or from 1-^{13}C-enriched 3-methyl-1-pentene, while $\beta\beta$-enriched carbons can only come from enriched ethylene.

It can be concluded that ethylene incorporation occurs via a decomposition route. The absence of metathesis is some not irrelevant evidence for the mechanism of insertion of α-olefins in Ziegler–Natta polymerization.[6]

Experimental Section. The synthesis of (*RS*)-3-methyl-1-pentene-*1*-^{13}C) and of $Al(^{13}CH_3)_3$, together with the polymerization and fractionation conditions, were described in ref 1.

^{13}C NMR analysis of the polymers dissolved in 1,2,4-trichlorobenzene containing 1% HMDS as an internal standard was carried out at 140 °C in the PFT mode on a Bruker HX-90 spectrometer operating at 22.63 MHz. Pulse width: 3.6 μs.

Acknowledgment. Financial support by the Italian "Programma Finalizzato di Chimica fine e Secondaria" is gratefully acknowledged.

Registry No. $Al(CH_3)_3$, 75-24-1; $Zn(CH_3)_2$, 544-97-8; poly[(*RS*)-3-methyl-1-pentene) (homopolymer), 66906-10-3; ethylene, 74-85-1; isotactic poly[(*RS*-3-methyl-1-pentene] (homopolymer), 88611-18-1.

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(2) The assignment has been confirmed by observing the ^{13}C NMR spectrum of a copolymer obtained by adding ethylene-*1*-^{13}C in the feed. The backbone methylene carbons are labeled according to the distance from the tertiary carbons. Carman, C. J.; Wilkes, C. E. *Rubber Chem. Technol.* **1971**, *44*, 481.

(3) Two resonances should be expected for the $\alpha\gamma$-methylene carbons in view of the stereochemical effect on the chemical shift of the substituent of the neighboring 3-methyl-1-pentene unit. Most probably, one of them is overlapped with the other resonances.

(4) The metallocarbene species could be formed, for example, by hydrogen migration from the α carbon to the metal. Ivin, K. J.; Rooney, J. J.; Stewart, C. D.; Green, M. L. H.; Mathab, R. *J. Chem. Soc., Chem. Commun.* **1978**, 604.

(5) Another possibility could be

$$Mt\text{-}(^{13}CH_3)_2 \rightarrow {}^{13}CH_2{=}^{13}CH_2 + \text{decomposition products}$$

(6) Zambelli, A.; Locatelli, P.; Sacchi, M. C.; Rigamonti, E. *Macromolecules* **1980**, *13*, 798.

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Proton Resonance Assignments in Copolymer Spectra by Two-Dimensional NMR

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for the analysis of the microstructure of copolymers since the comonomer sequences can be directly observed. If the resonance lines can be assigned to specific sequences, the line intensities can be used to characterize the kinetics of the copolymerization process. We report a novel application of two-dimensional nuclear Overhauser effect (2D NOE) spectroscopy to the assignment of tetrad sequence resonances in the proton spectrum of a copolymer.

2D NOE spectroscopy is only one of a number of two-dimensional NMR experiments that are all based on the same general principles. Two-dimensional NMR was first conceived by Jeener in 1971[1] but his ideas were not widely appreciated until Ernst et al. performed the first experiment in 1976.[2] Performed the first experiment in 1976.[2] 2D NMR spectroscopy can be divided into two broad categories: 2D resolved and 2D correlated. Two-dimensional resolved spectroscopy separates two different interactions along two frequency axes. An example is homonuclear 2D *J*-resolved spectroscopy, which displays the chemical shift along one axis and the *J* coupling along the other. In 2D-correlated spectroscopy, chemical shifts are plotted along both axes but are correlated by a particular interaction between them. In 2D NOE spectroscopy, the correlating interaction is the nuclear Overhauser effect. The result of a 2D NOE experiment is a map of the entire network of nuclear Overhauser effects between protons in the molecule.

Two-dimensional NMR techniques have been applied extensively to biological molecules.[3] In particular, 2D NOE experiments have been performed by Wüthrich, Ernst, and others on proteins,[8–14] cyclic peptides,[15,16] micelle-bound glucagon,[17] nucleic acids,[18,19] and synthetic biopolymers.[20] Macura and Brown[21] have applied 2D *J*-resolved spectroscopy to poly(vinyl chloride), and Gerig[22] has applied ^{19}F–^{1}H 2D chemical shift correlated NMR to poly(*p*-fluorostyrene). However, there have been no applications of 2D NOE spectroscopy to synthetic polymers.

2D NOE Spectroscopy. The pulse sequence for 2D

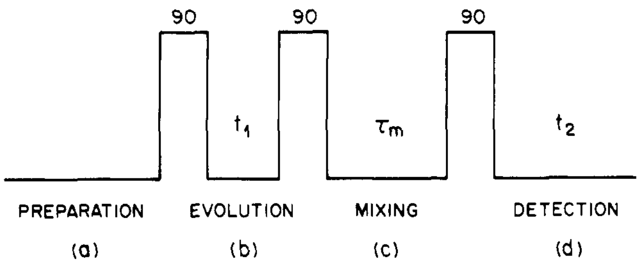
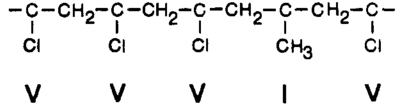


Figure 1. 2D NOE pulse sequence: (a) system relaxes back to equilibrium; (b) protons are labeled by their initial precession frequencies; (c) dipolar-coupled spins exchange magnetization; (d) final precession frequencies are detected.

NOE spectroscopy is shown in Figure 1. The *preparation* period (a) is a relaxation delay to ensure that the spin system is in equilibrium. The first 90° pulse causes each proton to precess at its characteristic frequency during the *evolution* period (b) of length t_1. This in effect labels each proton according to its precession frequency during t_1. During the *mixing* period (c) following the second 90° pulse, dipolar-coupled protons exchange magnetization via the nuclear Overhauser effect. The *mixing time*, τ_m, has a constant value chosen to be approximately equal to the spin-lattice relaxation time, T_1. The final frequency is observed during the *detection* period (d) of length t_2. The evolution time t_1 is systematically incremented and the resultant free induction decay detected in t_2 for a series of t_1 values. A two-dimensional Fourier transform is performed on the resulting data matrix to yield a two-dimensional spectrum as a function of two frequencies, ω_1 and ω_2, the precession frequencies during t_1 and t_2, respectively. Those protons that did not exchange magnetization during the mixing period have the same initial and final frequency. This gives rise to the normal spectrum along the diagonal corresponding to $\omega_1 = \omega_2$. Those protons that exchange magnetization due to dipole–dipole cross relaxation during τ_m have a final frequency differing from the initial. This gives rise to off-diagonal peaks connecting these protons. These off-diagonal peaks generally occur in pairs that are symmetric about the diagonal peaks. By matching all pairs of off-diagonal peaks, one obtaines the complete system of nuclear Overhauser interactions in a single 2D spectrum. However, caution must be exercised in the interpretation of a 2D NOE spectrum. As in the conventional double-resonance experiment to measure the nuclear Overhauser effect, magnetization transfer from spin i to spin j does not necessarily occur to the same extent as from spin j to spin i.[23] Consequently, the off-diagonal peak with coordinates (ω_i, ω_j) is not necessarily the same size as the off-diagonal peak at (ω_j, ω_i). It is even theoretically possible for one of these peaks to be absent. This seldom happens in practice, but the possibility should be recognized in order to avoid erroneous conclusions.

Results and Discussion. The vinylidene chloride–isobutylene copolymer system has been studied previously by proton NMR,[24-27] and line assignments have been made for comonomer tetrad sequences. This copolymer is an ideal case for testing a new method of line assignment as there are no complications from stereochemical configuration or scalar coupling. The structure of two overlapping tetrads is as follows:


$$-\mathrm{CCl_2}-\mathrm{CH_2}-\mathrm{CCl_2}-\mathrm{CH_2}-\mathrm{CCl_2}-\mathrm{CH_2}-\mathrm{C(CH_3)_2}-\mathrm{CH_2}-\mathrm{CCl_2}-$$

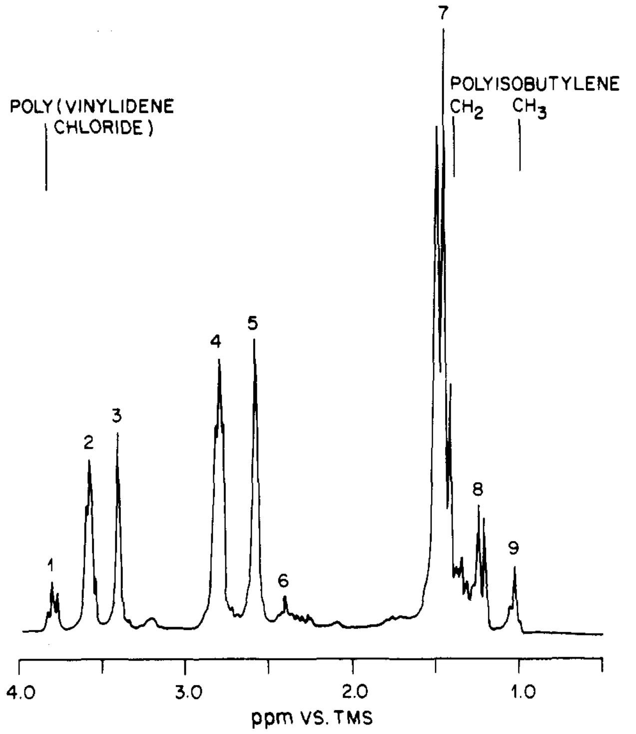


Figure 2. 200-MHz proton spectrum of a vinylidene chloride–isobutylene copolymer (65 mol % vinylidene chloride); observed at 40 °C in a 20% solution in $CDCl_3$. The assignments are listed in Table I.

Table I
Proton Resonance Assignments in Vinylidene Chloride-Isobutylene Copolymer Spectrum

peak no.	sequence assignment	chem shift, ppm vs. Me_4Si
1	VVVV CH_2	3.80
2	VVVI CH_2	3.58
3	IVVI CH_2	3.41
4	VVIV CH_2	2.80
5	VVII + IVIV CH_2	2.59
6	IVII CH_2	2.33
7	VIV CH_3	1.46
8	VII CH_3	1.24
9	III CH_3	1.03

Here, V represents vinylidene chloride and I isobutylene. Nuclear Overhauser effects are observed between neighboring methylene protons and can be used to make assignments. For example, a proton–proton Overhauser effect between the two central methylene groups in this pentad sequence results in a pair of off-diagonal peaks in the 2D NOE spectrum connecting the VVVI and VVIV tetrads. In a similar manner, the central methylene protons in each tetrad sequence have Overhauser interactions with the methylene protons of several other overlapping tetrad sequences.

The one-dimensional proton NMR spectrum of a 65:35 (mol fraction) vinylidene chloride–isobutylene spectrum is shown in Figure 2. The numbers on the peaks correspond to the assignments given in Table I. The resonances are grouped in three chemical shift ranges: VV-centered CH_2 resonances at low field, IV-centered CH_2 resonances at intermediate field, and II-centered CH_2 resonances together with CH_3 peaks at high field.

Spin–lattice relaxation times of VV and VI methylene protons were 345 ± 10 and 275 ± 10 ms, respectively, at 40 °C. The methyl protons all exhibit T_1 values of ca. 600 ms. T_1 values measured at 25 °C were found to be shorter: 300 ± 20 and 220 ± 20 ms, respectively, for VV and VI

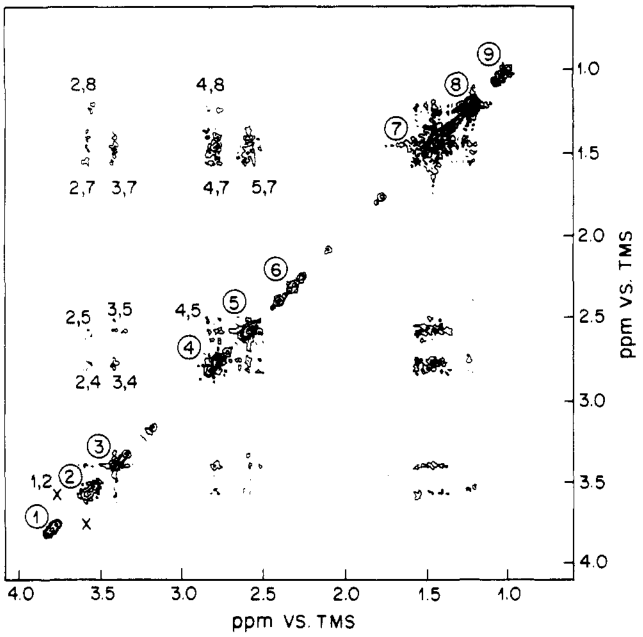


Figure 3. Contour plot of 200-MHz 2D NOE spectrum of a vinylidene chloride–isobutylene copolymer (65 mol % vinylidene chloride) in $CDCl_3$. The temperature was 40 °C and the mixing time 500 ms. The cross peaks indicate nuclear Overhauser effects between the indicated peaks. The carrier frequency is at the left edge of the spectrum to eliminate quadrature image peaks. "X" designates cross peaks which can be seen at lower contour levels.

methylenes (ca. 450 ms for CH_3), indicating that the proton motions are on the fast-motion side of the T_1 minimum, i.e., that

$$\omega_0\tau_0 < 1$$

τ_0 being the proton correlation time and ω_0 the observing frequency of 200 MHz. Under these circumstances, there will be measurable Overhauser effects only between protons that are separated in space by no more than ca. 4 Å; no appreciable transfer further down the chain by spin diffusion is to be expected. It is anticipated that the NOE will be positive (vide infra; 2D contour spectra give absolute intensities only and not relative signs.)

The 2D NOE spectrum of the copolymer is shown in Figure 3. Peak 1 can be assigned to the VVVV tetrad by comparison to the vinylidene chloride homopolymer, as in the 1D spectrum (Figure 2). Since a VVVV overlaps only a VVVI tetrad, the weak cross peaks, designated 1,2, between peaks 1 and 2 identify the latter peak as arising from VVVI. VVVI should have Overhauser effects with VVIV and VVII. The two cross peaks designated 2,4 and 2,5 identify 4 and 5 as VVIV and VVII, but with reservations discussed below. There are also cross peaks between 3 and 4 (designated 3,4) and between 3 and 5 (designated 3,5). Peak 3 must logically correspond to the IVVI tetrad. No Overhauser effect is expected between VVIV and VVII, since these sequences do not overlap appropriately. Nevertheless, apparent cross peaks between these sequences are evident, designated 4,5. To explain this, it may be observed that an interaction is expected between VVIV and VIVI. It follows that the VIVI and VVII resonances must overlap in the proton spectrum, contrary to a previous assignment.[27] This is reasonable on structural grounds. The resonances designated 6 are too weak to have observable Overhauser effects so this region is assigned to IVII on the basis of intensity and chemical shift considerations.

It should be noted that the 2D NOE spectrum cannot be used to make unambiguous assignments of peaks 4 and 5. It is clear that one is VVIV and the other contains both VVII and VIVI, but one cannot readily tell which is which. This could, in principle, be done, since VVII should interact with VIIV (and VIII), but the latter are in the strongly overlapping upfield region around 1.4 ppm and cannot be clearly assigned (vide infra). However, from peak intensities one can assign resonance 4 to VVIV and peak 5 to (VVII + VIVI) since from the overall compositon the former must be the larger.

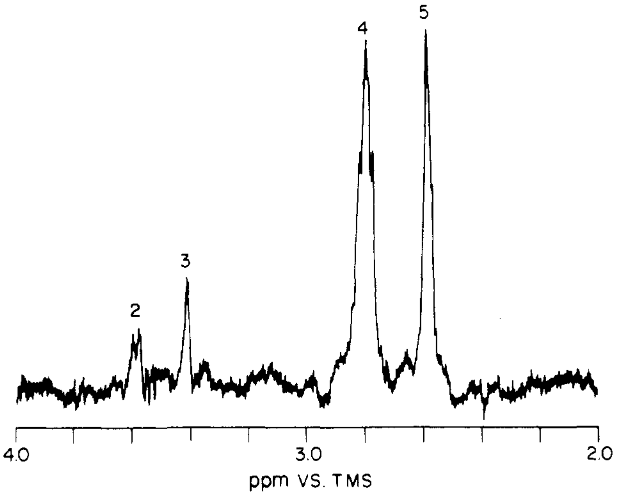


Figure 4. One-dimensional NOE difference spectrum of a vinylidene chloride–isobutylene copolymer at 40 °C in $CDCl_3$. The methyl protons were saturated, and only the Overhauser effects (which are positive) in the upfield region are shown.

The cross peaks observed in the upfield part of the 2D spectrum are principally due to interactions between methyl protons and methylene protons. Methyl protons interact not only with immediate neighboring methylene protons but also with those of the next monomer unit. We thus see strong interactions between the methyl groups of VIV and the methylene groups of VVIV (cross peak 4,7) and IVIV (cross peak 5,7), as expected. But there are also observable interactions between VIV and the central methylenes of VVVI (cross peak 2,7) and of IVVI (cross peak 3,7), as well as apparent VII cross peaks with VVVI and VVIV. Such effects are not useful for peak assignments but clearly point to a very crowded structure with many $CH_3\cdots CH_2$ distances of less than 4 Å. Space-filling models confirm this conclusion, which is consistent with the known conformations of the homopolymers.[28,29]

A one-dimensional NOE difference experiment was performed to verify this result. The methyl protons were saturated and the methylene resonances observed. The difference spectrum, shown in Figure 4, is in complete agreement with the 2D NOE spectrum. There is a *positive* Overhauser effect of ca. 5% between the methyl protons and their nearest methylene proton neighbors (in VVIV and VVII + IVIV) and a weaker effect of ca. 3% with their next-nearest neighbors (VVVI and IVVI). The fact that the NOE is positive is further strong indication that these results are due to spatial proximity and not to spin diffusion.

Conclusions. We have demonstrated specific nuclear Overhauser effects between the methylene protons of neighboring monomer units in a vinylidene chloride–isobutylene copolymer in solution. The entire network of such effects has been observed in a single 2D NOE experiment, and the 2D contour plot has been used to make tetrad line assignments. Methyl–methylene proton interations have also been observed. They are less specific than $CH_2\cdots CH_2$ interactions and are therefore not employed for assignments. However, they clearly reflect the conformation of the copolymer chain. We anticipate that this method will provide useful information concerning the average conformations of synthetic polymers in solution, as it already has for the more rigid conformations of biopolymers.

The conclusions drawn from the 2D NOE observations have been confirmed by 1D experiments, but the former has the advantage that it involves no problems in the selective irradiation of closely spaced peaks. The 2D NOE spectrum also gives a more vivid demonstration of these relatively small effects (2–5%), in part because of the absence of the Bloch–Siegert shifts and subtraction artifacts that accompany the 1D experiments.[30]

Experimental Section. Materials. The 65:35 (mol ratio) vinylidene chloride–isobutylene copolymer was prepared by Dr R. E. Cais from a 50:50 molar feed ratio of comonomers at 60 °C in bulk using 0.1 mol % azobis(isobutyronitrile) initiator. Conversion was carried to ca. 5%. For proton NMR measurements it was dissolved in $CDCl_3$ and transferred to a 5-mm NMR tube which was then degassed with argon for ca. 15 min to remove dissolved oxygen. The final concentration was approximately 20% (w/v). The molecular weight was determined by gel permeation chromatography in tetrahydrofuran using polystyrene calibration; found: $\bar{M}_w = 4100$; $\bar{M}_n = 2400$.

NMR Spectroscopy. All spectra were obtained at a controlled temperature of 40 °C on a Varian XL-200 spectrometer operating at a proton frequency of 200 MHz. T_1 values were measured by using a $180°-\tau-90°$ inversion–recovery pulse sequence and a least-squares analysis of the integral data. Sixteen transients were accumulated for each of 13 τ values using a recycle time of 10 s.

The 2D NOE experiment was performed with a mixing time τ_m of 500 ms and a recycle time of 2.9 s to ensure that the spins have relaxed from the 90° sampling pulse so that the maximum signal is obtained upon repetition of the pulse sequence. A four-cycle phase cycling routine was employed to eliminate spectral artifacts. Quadrature detection was used, the carrier frequency being placed at the left edge of the spectrum to eliminate quadrature image peaks. Hence, twice the normal sweep width was employed in both dimensions.[31] The data matrix consisted of 512 spectra of 838 points each. This matrix was zero-filled to 1024 points covering 1400 Hz in each dimension. The free induction decays were multiplied by a pseudo-echo window function in both dimensions to restore the resolution lost by calculation of the absolute value spectrum, which broadens the bases of the peaks.[7] The transformed data matrix was symmetrized[32] to eliminate the ridges of "t_1 noise" running parallel to the ω_1 axis. A total of 32 transients was recorded for each t_1 value.

To obtain the one-dimensional NOE spectra, 100 transients were accumulated with the second radio-frequency field at the resonance frequency of the proton to be saturated, and another 100 transients were accumulated with the radio-frequency field off-resonance. The transformed spectra were phase corrected and subtracted to obtain the difference spectrum. The decoupler power used was the minimum required to saturate selectively the protons of interest. Quadrature detection was employed and the saturating power was gated off during acquisition so that coupled spectra were obtained. A saturating time of 3 s was used to ensure that a steady state was achieved.

Acknowledgment. We thank Dr. R. E. Cais for the sample of copolymer and Mrs. M. Y. Hellman for the molecular weight determination.

Registry No. (Vinylidene chloride)·(isobutylene) (copolymer), 25231-99-6.

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(31) It should be noted that although this procedure eliminates quadrature image peaks, it results in a loss of digital resolution in both dimensions by a factor of 2 compared to the corresponding experiment with the transmitter frequency located at the center of the spectrum. This loss of resolution could be avoided without introducing quadrature image peaks by placing the transmitter frequency at the edge of the spectrum and recording a pure absorption mode spectrum.[7] However, this would require special data manipulation which is not routinely available on all spectrometers. In the present case, the loss of resolution did not impair our ability to distinguish tetrad sequences. Hence, a pure absorption mode spectrum was not necessary and was not recorded.
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