A Two-Dimensional NMR Study of Poly(vinyl (dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol)

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ABSTRACT: The microstructure of amine-modified poly(vinyl acetate-*co*-vinyl alcohol) (P(VAM−VOH−VAC)) copolymers was studied by a combination of 2D NMR techniques COSY (correlated spectroscopy), $^{1}H-^{13}C$ HMQC (heteronuclear multiple-quantum correlation), and HMBC (heteronuclear multiple-bond correlation). A complete signal assignment was achieved on a representative sample poly(vinyl 3-(dimethylamino)propylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol). The microstructures of the poly(vinyl alcohol) (PVA) backbone and the amine substitution are thus determined. The correct assignment of methylene peaks in the ^{1}H spectrum of previously reported PVA was verified on the basis of COSY spectra. Tacticities obtained from the methine ^{13}C signals reveal that mainly isotactic triads react with the activated diamine. By using the $^{1}H-^{13}C$ HMBC technique, the covalent bond between PVA backbone and amine was clearly confirmed. Furthermore, the tacticity assignment of pure PVA could be substantiated by the HMBC spectra.

Introduction

It is commonly accepted now that polymer properties depend basically on their microstructure. 2D NMR techniques are very useful in terms of structural analysis.[1] In a recent review, Tonelli illustrated the usefulness of homonuclear 2D NMR techniques in characterizing polymer comformations.[2] The application of heteronuclear 2D NMR techniques in polymer microstructure studies was illustrated by Moore et al.[3] These multidimensional NMR techniques were developed for resonance assignments of complicated small organic molecules[4] and for the structure determination of polypeptides and biological macromolecules.[5] One type of these techniques is based on the covalent bond interactions. Examples are COSY, $^{1}H-^{13}C$ HMQC (heteronuclear multiple-quantum correlation), and $^{1}H-^{13}C$ HMBC (heteronuclear multiple-bond correlation). The heteronuclear correlation experiments HMQC and HMBC use the inverse spectroscopy of proton detection and thus improve the sensitivity of the ^{13}C spectrum to 32-fold theoretically. On the basis of the covalent interactions, these two methods together make all carbons in the molecule detectable at natural abundance and thus deliver structural information along chemical bond network of the molecule. More recently, this type of multidimensional NMR technique has been applied to determine the microstructure of copolymers of aliphatic polyesters and polycarbonates.[6,7] The usefulness of these NMR techniques in polymers has also been highlighted by Rinaldi et al. in their studies of the microstructure of copolymers of ethylene and α-olefins[8,9] and the chain-end structures of poly(allyl alcohol).[10] All these examples show the applicability of these NMR techniques in the structure studies of polymers.

The design of polymers for gene and drug delivery remains a formidable challenge since structure−function relations are still scarce.[11,12] We recently investigated self-assembling nanocomplexes prepared from peptides, proteins, or DNA with positively charged branched polyesters.[13−15] These amphiphilic polycations form inter-polyelectrolyte complexes from aqueous solutions in a spontaneous manner, protecting the drug from degradation.[16] Alternatively, more hydrophobic polyesters of this type can be used to prepare nanoparticles relying on the Marangoni effect.[13] In both cases charge-modified poly(vinyl alcohol) backbones were grafted with biodegradable poly(lactide-*co*-glycolide) side chains, allowing the adjustment of drug loading and release as well as polymer degradation.[13] Since many proteins and also DNA are negatively charged under physiological conditions, these branched polyesters based upon amine-modified poly(vinyl alcohol) (PVA) backbones and poly(lactide-*co*-glycolide) side chains are of particular interest. To allow the precise design of the polymer structure to drug delivery objectives, detailed structural information about the polymer is necessary.

Many NMR studies about poly(vinyl alcohol) (PVA) and its copolymer with vinyl acetate have been performed.[17−28] Nevertheless, only a few studies have used multidimensional techniques.[23,26,27] To our knowledge, no studies on both constitutional and configurational microstructures of modified PVA consisting of three different components have been published until now.

In this paper, we show the power of multidimensional NMR methods in solving constitutional and configurational microstructure of PVA-based copolymers with three components. Thus, results of COSY, $^{1}H-^{13}C$ HMQC, and $^{1}H-^{13}C$ HMBC studies on the microstructure of the amine-modified PVAs (Scheme 1) are presented. A complete assignment of constitutional and configurational microstructures is given for a representative copolymer poly(vinyl 3-(dimethylamino)propylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol) (M(32)).

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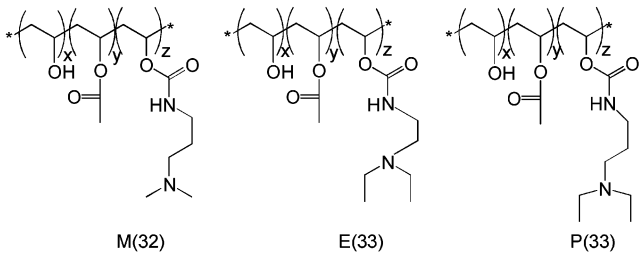
Scheme 1



Reassignment of the reported[21,22] methylene peaks in the ^{1}H spectrum is performed on the basis of COSY spectra. Using ^{1}H−^{13}C HMBC, we demonstrate the covalent bond between PVA backbone and the amine substituents.

Experimental Section

Materials. 2-(Diethylamino)ethylamine (DEAEA) (purum, >98%), 3-(diethylamino)propylamine (DEAPA) (purum, >98%), 3-(dimethylamino)propylamine (DMAPA) (purum, >98%), poly(vinyl alcohol) (M_W 15 000 g mol^{-1}; degree of polymerization 300 (P = 300); degree of hydrolysis 86−89%), carbonyldiimidazole (purum, ~97%), *N*-methylpyrrolidone (NMP) (absolute), dimethylacetamide (DMAc) (for HPLC, 99.8%), and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) (puriss., absolute, over molecular sieve) were purchased from Fluka GmbH (Germany) and used as received. Tetrahydrofuran (THF) (BASF, Germany) was dried over sodium and distilled under nitrogen before use. All other chemicals including lithium bromide (extra pure) (Merck) were used as received without further purification.

Synthesis. The synthesis of poly(vinyl (dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol) was described earlier.[13] Using carbonyldiimidazole chemistry, the diamines were activated in THF solution. After removal of the solvent the calculated amount of the reaction mixture was injected into a solution of PVA in NMP at 80 °C and stirred for 4.5 days. The resulting polymers were purified by ultrafiltration (YM1 membrane, cutoff 1000 g mol^{-1}, Millipore) and then freeze-dried (Edward Freeze Dryer Modulyo, standard conditions).

NMR Experiments. 50−100 mg of polymer was solved in 0.75 mL d_6-DMSO (euriso-top, <0.02% HDO + D_2O). ^{1}H (400.13 MHz), ^{13}C (100.21 MHz), and ^{1}H−^{13}C correlation spectra were recorded on a Bruker DRX-400 spectrometer. COSY experiment was performed on a Bruker DRX-500 spectrometer. ^{1}H and ^{13}C were referenced to the d_6-DMSO solvent signal. A 5 mm multinuclear gradient probe and gs-HMQC and gs-HMBC pulse sequences[29,30] were used for the ^{1}H−^{13}C correlation experiments. While the HMQC experiment was optimized for C−H coupling of 140 Hz, with decoupling applied during acquisition, the HMBC experiment was optimized for coupling of 8 Hz, without decoupling during acquisition. HMBC and HMQC data were acquired with 512 points in t_2. The number of increments for t_1 was 256. 64 scans and 4 dummy scans were used for both HMQC and HMBC. COSY spectra were recorded with 1024 points in t_2 and 256 increments for t_1. A relaxation delay of 1 s was used for all 1D experiments and 2 s for all 2D experiments. The typical experiment time was about 12 h for HMQC and HMBC. All the measurements were performed at 340 K.

Results and Discussion

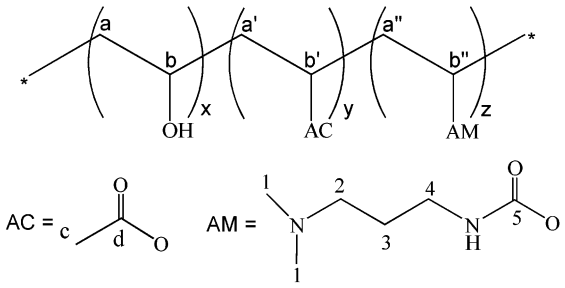
The microstructure of branched polyesters is complicated by the tremendous possibilities in constitutional and configurational variations of the copolymer (containing five different comonomers). Therefore, we initiated the investigation with the starting copolymer, that is, the amine-modified PVA backbone with three comonomers. Characteristic data of polymers used in this study are listed in Table 1. In this paper we selected three polymers M(32), E(33), and P(33) (Scheme 1) as model systems and show a complete structure analysis using 1D and 2D NMR techniques. The polymers were synthesized from the same commercially available PVA (Table 1). The degree of polymerization of this PVA was 300. The amine substitution is 10.8, 10.9, and 10.9% for M(32), E(33), and P(33), while the respective acetate substitution is 4.1, 4.1, and 0.8%. The numbers 32 and 33 correspond to the total number of the amine substitution (degree of polymerization of PVA times degree of substitution).

Table 1. Polymerization Data of Poly(vinyl (dialkylamino)alkylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol)s

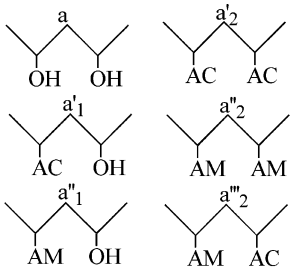
polymer	M_w (g mol^{-1})	M_w/M_n	DS: amine (%)[a]	DS: acetate (%)[a]
M(32)	14 400	1.2	10.8	4.1
E(33)	17 200	1.2	10.9	4.1
P(33)	19 800	1.2	10.9	0.8
PVA	14 000	1.2	0.0	11.8

[a] DS calculated from ^{1}H NMR spectra.

Scheme 2

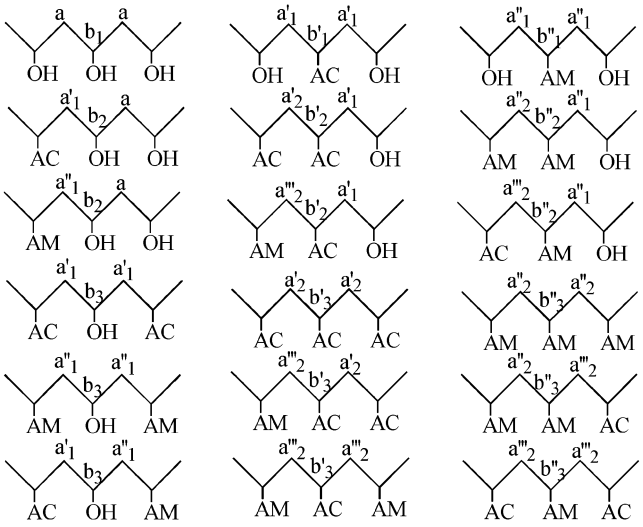


Scheme 3



Even with these model polymers, the ^{1}H and ^{13}C spectra are rather complicated (see Figure 1 and Figure 4). This is due to an overlap of signals from various constitutional and stereo configurations. It will be shown later that only with the help of two-dimensional techniques is it possible to obtain correct assignments. Because of the complexity of the system, it is necessary to analyze the possible microstructures before we go into details of the NMR studies. Thus, Scheme 2 presents a detailed notation of the monomers of M(32), and its possible microstructures up to triads are listed in Schemes 3 and 4. As shown in Scheme 2, without consideration of microstructures, the methylene and methine of the PVA backbone are denoted as a and b, respectively. The acetate-substituted ones are denoted as a′ and b′, while the amine-substituted ones are denoted as a″ and b″, respectively. When the constitutional microstructures are considered, all the positions are further split. According to the rule of Tonelli,[31] six diads are assigned to methylene (Scheme 3), while 18

Scheme 4



triads are assigned to methine (Scheme 4). Thus, the diads are (VOH, VOH), (VOH, VAC), (VOH, VAM), (VAC, VAC), (VAM, VAM), and (VAC, VAM) for methylenes a, a'_1, a''_1, a'_2, a''_2, and a'''_2. (For clearness, the substituted methylenes will be denoted as a^{sub} in figures, and for the help to later assignments (see section COSY Studies), a^{sub} will be further denoted as follows: $a^{sub} = a^{sub}_1 + a^{sub}_2$, $a^{sub}_1 = a'_1 + a''_1$, $a^{sub}_2 = a'_2 + a''_2 + a'''_2$.) In the case of methine where triads are considered, some microstructures may overlap. With a sufficient separation of chemical bonds (in this work five bonds for ^{13}C and six bonds for ^1H), the difference in chemical shift due to the different substituents is negligible. Therefore, the triads are assigned as follows: (VOH, VOH, VOH) for b_1, (VAC, VOH, VOH) and (VAM, VOH, VOH) for b_2, (VAC, VOH, VAC), (VAM, VOH, VAM), and (VAC, VOH, VAM) for b_3; (VOH, VAC, VOH) for b'_1, (VAC, VAC, VOH) and (VAM, VAC, VOH) for b'_2, (VAC, VAC, VAC), (VAM, VAC, VAC), and (VAM, VAC, VAM) for b'_3; (VOH, VAM, VOH) for b''_1; (VAM, VAM, VOH) and (VAC, VAM, VOH) for b''_2; (VAM, VAM, VAM), (VAM, VAM, VAC), and (VAC, VAM, VAC) for b''_3. On the basis of these schemes, NMR analyses can be performed.

^{1}H and COSY Studies. The ^1H spectrum of M(32) and its assignment are shown in Figure 1. The peaks for DMAPA are (Figure 1a) NH, 6.78−6.63 ppm; CH_2(4), 2.99 ppm; CH_2(2), 2.22 ppm (t, 7.10 Hz); CH_3(1), 2.12 ppm; and CH_2(3), 1.53 ppm (quintet, 7.30 Hz). The CH_3 group of acetate shows a peak band of 1.99−1.94 ppm. The correctness of this assignment is confirmed by 2D experiments COSY (Figure 2) and HMBC (Figure 7). The COSY cross-peaks observed for DMAPA show connections between NH and 4, 3 and 4, and 3 and 2. The assignment of 1 is further confirmed by the observation of HMBC cross-peaks between 1 and 2 (2.12−56.4 ppm and 2.22−44.7 ppm, respectively).

The assignment of the PVA backbone is referenced to van der Velden et al.[22] and Budhlall et al.[28] and further confirmed by the COSY experiment. In contrast to our work, both authors investigated systems with only two different structural components. Here the amine modification leads to a three-component system, which results in more complicate signal splitting. Therefore, first we assign the signal bands without considering splittings due to microstructures. Thus, the peaks for the PVA backbone appear as follows (Figure 1a): CH(b′), 5.10−5.00 ppm; CH(b″), 4.92−4.82 ppm; OH, 4.40 ppm; CH(b), 3.91−3.86 ppm and 3.72−3.68 ppm; CH_2(a′+a″), 1.79−1.57 ppm; and CH_2(a), 1.55−1.34 ppm. The assignment of CH(b′) against CH(b″) is based on a ^1H measurement on P(33) (10.9% amine and 0.8% acetate), which shows almost disappearing b′, with the signal b″ remaining unchanged. Further assignment to the fine structures requires the consideration of the polymer microstructures. Since methine protons shown more resolved signals (b, b′, and b″) than methylene protons (a, a′, and a″), we start with the analysis of methine. As stated by van der Velden, three different triads exist for the AC-substituted methine. For M(32) with three comonomers there are 18 different compositional triads (Scheme 4). Thus, three components are observed for both b′ and b″ (Figure 1b). The triad b'_1 is assigned to the signal at the lowest field position and b'_3 to the one at highest field.[22] Similarly, the amine-substituted methine shows three types of components, b''_1, b''_2, and b''_3, with increasing field. For the nonsubstituted methine only two components b_1 and b_2 are observed (Figure 1c). Because of the blocky property of

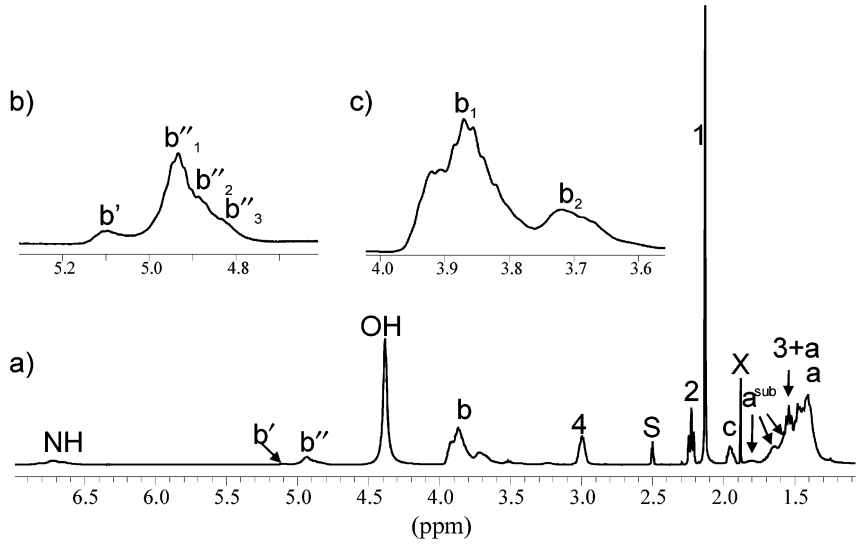


Figure 1. (a) ^1H NMR spectrum of the amine-modified PVA copolymer M(32). (b) and (c) are extended insets. The labels S stands for the solvent DMSO and X for impurity for all the figures.

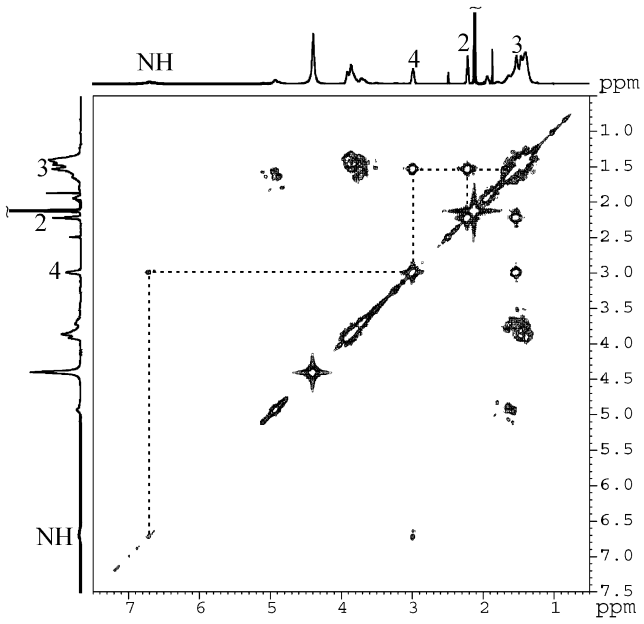
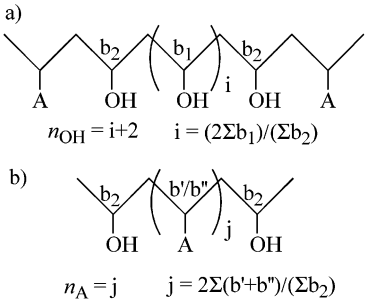


Figure 2. COSY spectrum of M(32).

Scheme 5



the PVA,[22] triads showing b_3 have very little contribution and b_3 is not detectable. This assignment of b_1 and b_2 is verified by a HMQC study (see Figure 6).

On the basis of the fine structure assignment of the methine ^{1}H signals details of the compositional microstructure of the copolymer can be obtained. A deconvolution of the amine-substituted methine (b″) shows 54, 30, and 16% for b''_1, b''_2, and b''_3. This means that the amines exist in the copolymer in 54% as separated substitution (VOH, VAM, VOH), in 30% as (VCM, VAM, VOH) (VCM = VAC or VAM) and only in 16% as pure vinyl alkylcarbamate or mixed vinyl acetate/vinyl alkylcarbamate triads (VCM, VAM, VCM). At this point we would like to calculate the average block length of the substitution. If b''_1 presents a length of 1.0 for the substitution and b''_2 presents for 2.0, then b''_3 should include contribution from all blocks equal and higher than triads (VCM, VAM, VCM), centering on the triads. Because of this overlap, it is not possible just by using assignment of triads to calculate the average length of substitution. The calculation is realized by analyzing the ^{1}H signal integrals of all the different methines. Thus, the integrals obtained are 16% for (b′ + b″), 65% for b_1, and 19% for b_2, with the sum normalized to be 100%. As shown in Scheme 5a, a block index n_{OH} for the PVA polymer sequence can be defined as the average block length of nonsubstitution. The experimental value obtained implies an average length of 9.0 for the PVA block. Similarly, for substitution A (amine or acetate), a block index n_A is defined as the average

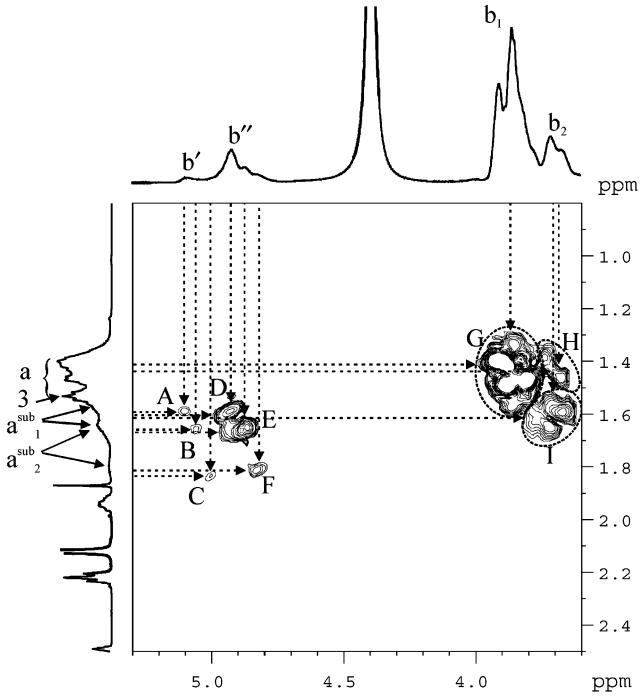


Figure 3. COSY spectrum of M(32) in the regions (F_2) 5.3−3.6 ppm and (F_1) 2.5−0.8 ppm.

length of the overall substitution (Scheme 5b). The experimental value observed implies an average length of 1.72 for the substitution. Next we present the assignment of methylene protons.

As shown in Figure 1, the methylene protons have broad and overlapping signal bands. This is due to an overall contribution of spin−spin coupling and compositional and configurational microstructures. In addition, in contrast to the situation of methine proton, the two protons of the methylene group within the modified copolymer are heterosteric (also called diastereotop): having different chemical environment therefore showing different chemical shifts.[23] Assignments up to compositional diads for poly(vinyl alcohol-*co*-vinyl acetate) have been published.[21,22,28] These assignments are ambiguous because none of them considered the heterosteric property of the methylene protons. Because of chiral center sitting on the substituted methines, protons of the methylene groups (a′ and a″) adjacent to these centers are heterosteric, with a difference of about 0.15 ppm in their chemical shifts (predicted by ACDLabs).[32] Therefore, the assignment of three signal bands to (VAC, VAC), (VOH, VAC), and (VOH, VOH) with increasing field strength is not accurate.[21,22,28] A reassignment to methylene is presented here based on the COSY spectrum.

To have a reasonable assignment of the methylene protons, we start with the following question: which COSY cross-peaks are available and can be used? The methylene protons possess spin−spin coupling with methine proton separated by three bonds ($^3J_{HH}$). Therefore, we examine the cross-peaks between methylene and methine protons. According to the microstructures shown in Scheme 4, the expected cross-peaks can be divided into three groups, corresponding to the three columns of the microstructures on the scheme. The extended COSY spectrum shown in Figure 3 also presents three groups of cross-peaks: (G, H, I), (D, E, F), and (A, B, C). These cross-peaks are thus reasonably

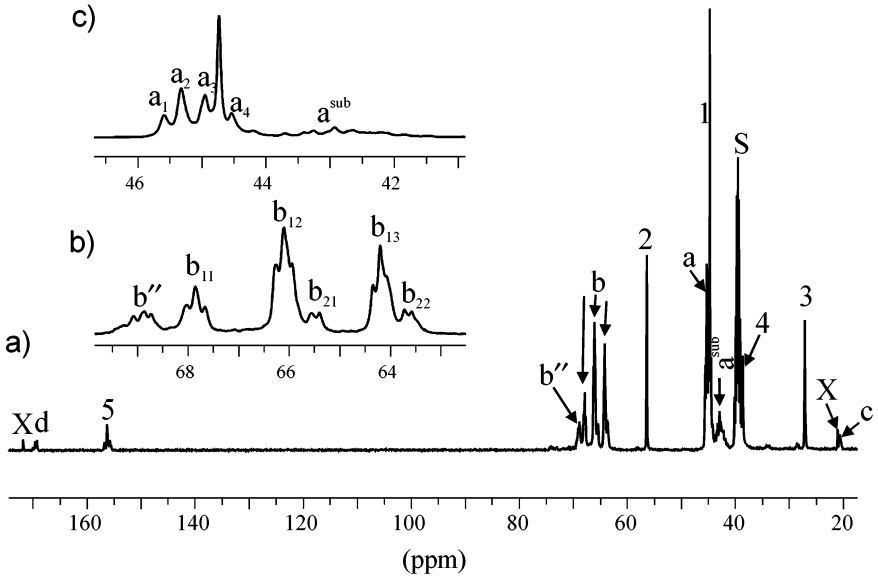


Figure 4. ^{13}C spectrum of M(32).

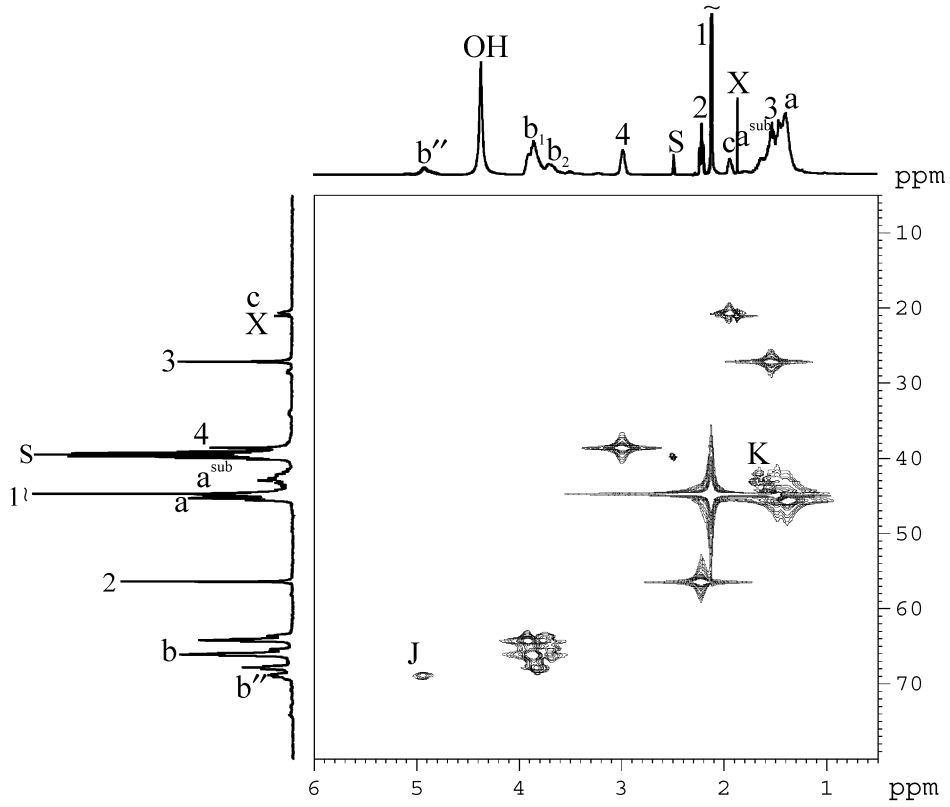


Figure 5. $^{1}H-^{13}C$ HMQC spectrum of M(32).

assigned as follows: G for correlation between b_1 and a(b_1 − a); H for (b_2 − a); I for (b_2 − a'_1) and (b_2 − a''_1); D for (b''_1 − a''_1); E for (b''_2 − a''_1), (b''_2 − a''_2), and (b''_2 − a'''_2); F for (b''_3 − a''_2) and (b''_3 − a'''_2); A for (b'_1 − a'_1); B for (b'_2 − a'_1), (b'_2 − a'_2), and (b'_2 − a'''_2); C for (b'_3 − a'_2) and (b'_3 − a'''_2). On the basis of these cross-peaks, a correct assignment of the methylene groups can be made (with additional subscripts a and b to distinguish the two heterosteric CH_2 protons): a'_{1a} and a''_{1a} appear at 1.57 ppm; a'_{1b} and a''_{1b} at 1.65 ppm; a'_{2a}, a''_{2a} and a'''_{2a} at 1.65 ppm; a'_{2b}, a''_{2b} and a'''_{2b} at range 1.79−1.81 ppm. This assignment is further confirmed by a COSY measurement on P(33) (10.9% amine and 0.8% acetate), which shows no more cross-peaks A, B, and C (all due to a′ and b′). (For the COSY spectrum of P(33) see the Supporting Information.)

^{13}C and HMQC Studies. Up to now we discussed the microstructure of M(32) by using ^{1}H and COSY spectra. By taking advantage of the rather well-resolved methine spectra, compositional microstructure up to triads has been verified. Since ^{13}C spectra have much larger chemical shift range than ^{1}H spectra, they are expected to show more resolved signals and to provide information on configurational microstructure. The ^{13}C spectrum of M(32) is shown in Figure 4. Using $^{1}H-^{13}C$ HMQC techniques (Figure 5) and the proton assignment (Figure 1), the aliphatic ^{13}C signals of the side chains can be completely assigned. The acetate methyl group

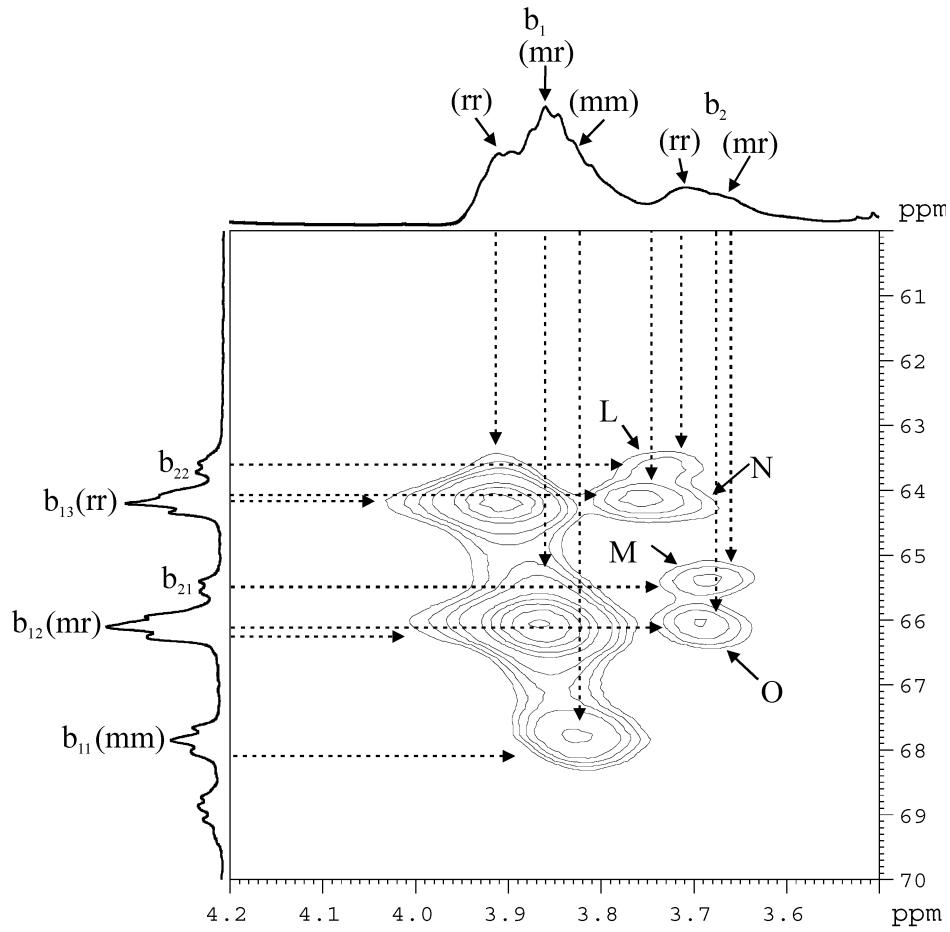


Figure 6. $^{1}H-^{13}C$ HMQC spectrum of M(32) in the regions of (F_2) 4.2−3.5 ppm and (F_1) 70−60 ppm.

appears at 20.7 ppm; the signals at 27.1 and 38.6 ppm are assigned to the amine methylene groups at positions 3 and 4, respectively; the methyl group (1) of amine appears at 44.7 ppm; and the signal at 56.4 ppm is assigned to the amine methylene 2. By further using the HMQC spectrum, signals of the PVA backbone are assigned and shown in Figure 4a. It should be mentioned that cross-peak J (Figure 5) is mainly due to amine-substituted methine (b″), and the signal band 69.1−68.7 ppm is thus assigned to b″. The signal of b′ may be too weak and not detectable. The methylene groups overlap together and cause the cross-peak K. The signal band at 43.7−41.4 ppm is thus assigned to (a′ + a″).

Signals of the methylene and methine groups of the nonsubstituted PVA backbone show resolved peaks due to stereo configurations. Multiples a_1, a_2, a_3, a_4 (Figure 4c) and b_{11}, b_{12}, b_{13}, b_{21}, b_{22} (Figure 4b) are observed for a and b, respectively. A complete tacticity assignment of the methylene and methine ^{13}C signals of pure vinyl alcohol has been performed by chemical shift calculation considering the γ-gauche effect[33] and recently verified by 2D spectroscopy HSQC-TOCSY.[26] The four peaks for the methylene are thus assigned to the following tacticities: a_1 (at 45.6 ppm), rrr; a_2 (45.3 ppm), (rmr + mrr); a_3 (44.9 ppm), (mmr + mrm); and a_4 (44.5 ppm), mmm. Signals of methine show two additional peaks b_{21} and b_{22} (Figure 4b) as compared with the corresponding signals of the pure PVA. To have the ratio between the substituted and the total methine signals correct for the real degree of substitution, the integral of these two peaks has to be taken into consideration as a component to the nonsubstituted methine. This means that peaks b_{21} and b_{22} are due to certain sort of nonsubstituted methine. Furthermore, a ^{13}C measurement on a commercial PVA (Fluka, 12% acetrate) detected no peaks of b_{21} and b_{22}. Therefore, peaks b_{21} and b_{22} are due to nonsubstituted methine related to the amine modification. To have a clear assignment of these peaks, a special HMQC spectrum with 1024 increments in F_1 dimension was recorded. With high resolution in F_1, this HMQC spectrum provides clearly resolved methine cross-peaks (Figure 6). The major peaks b_{11}, b_{12}, and b_{13} show cross-peaks with b_1 of the unmodified triad (VOH, VOH, VOH), while the weak peaks b_{21} and b_{22} are correlated to b_2 of triad (VOH, VOH, VCM). Therefore, signals due to compositional and configurational microstructures can now be assigned separately. The triads (VOH, VOH, VOH) overlap with (VOH, VOH, VAC)[21] and have signals of configurations: mm (b_{11}), mr (b_{12}), and rr (b_{13}). These signals are further split into multiplicities due to pentads.[26,33] Thus, peaks (b_{11}) at 68.0, 67.9, and 67.7 ppm are due to pentads rmmr, mmmr, and mmmm; peaks (b_{12}) at 66.3, 66.1, and 65.9 ppm are due to rmrr, (mmrr + rmrm), and mmrm; and peaks (b_{13}) at 64.3, 64.2, and 64.1 ppm are due to rrrr, mrrr, and mrrm. The weaker signals due to the hetero triads (VOH, VOH, VAM) are assigned as mr at 65.5 and 65.4 ppm (b_{21}) and rr at 63.7 and 63.6 ppm (b_{22}). No mm tacticity is observed for the hetero triad. The cross-peaks related to the ^{1}H signal of methine b_2 for both triads (VOH, VOH, VAC) and (VOH, VOH, VAM) are resolved into four peaks: L, M, N, and O (Figure 6). The cross-peaks L and M are due to triad (VOH,

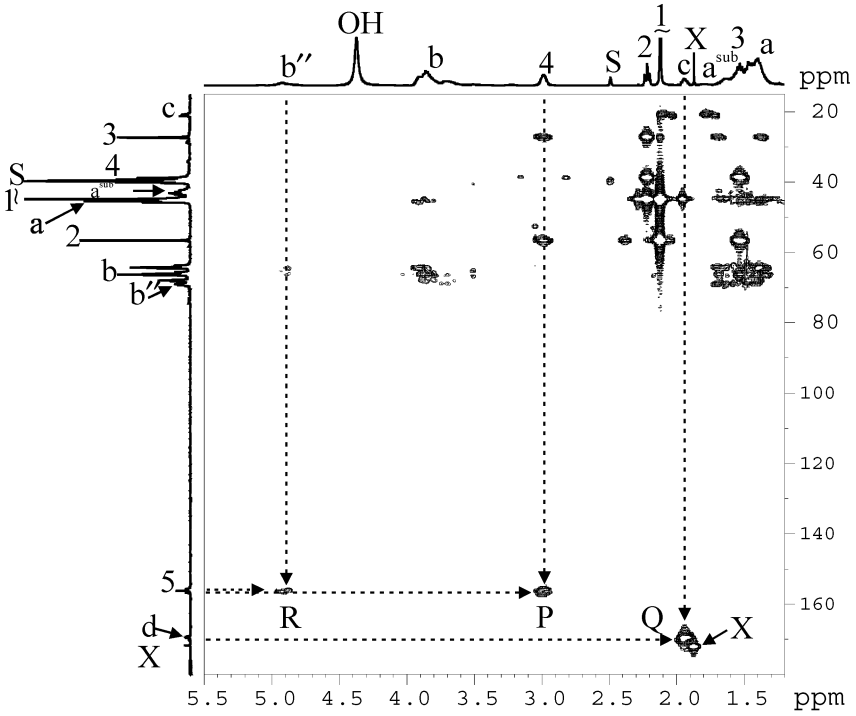


Figure 7. $^{1}H-^{13}C$ HMBC spectrum of M(32).

VOH, VAM), while cross-peaks N and O are due to the triad (VOH, VOH, VAC). The assignment of the triad (VOH, VOH, VAC) is not straightforward because its ^{1}H signal overlaps with that of the triad (VOH, VOH, VAM) (b_2) and ^{13}C signal overlaps with that of the triad (VOH, VOH, VOH). The ^{1}H spectrum of b_1 and b_2 shows signal bands for different tacticities up to triads. By using HMQC cross-peaks and the ^{13}C assignment, the tacticities of b_1 and b_2 are assigned and labeled in Figure 6. It is noteworthy that there is no cross-peak of hetero triads with tacticity of mm. We propose that mostly isotactic triads react with the activated diamine. In the mm configuration the hydroxyl groups can easily form hydrogen bonds with themselves, with the catalyzing DMPU and the amine-CI. The observation of only one cross-peak due to b″ confirms these considerations.

HMBC Studies. In $^{1}H-^{13}C$ HMBC long-range couplings of the protons with carbons in beta, gamma, or delta positions are detected. This technique enables the recognition of couplings across heteroatoms like nitrogen and oxygen. The quaternary ^{13}C signals can thus be assigned, and of even greater importance the covalent bonds between PVA backbone and diamine can be proved.

Figure 7 shows the HMBC spectrum of M(32). Cross-peak P shows a $^{3}J_{CH}$ coupling between methylene protons 4 and the carbonyl carbon 5 of amine. The ^{13}C signals at 156.8, 156.3, and 155.8 ppm are thus assigned to the carbonyl carbon 5. Cross-peak Q shows a $^{2}J_{CH}$ coupling between methyl proton c and the carbonyl carbon d of acetate. The ^{13}C signals at 170.0, 169.6, and 169.3 ppm are thus assigned to carbonyl carbon d.

In the case of a covalent urethane bond between PVA backbone and diamine cross-peak between methine proton b″ and carbonyl carbon 5 shall be detectable in $^{1}H-^{13}C$ HMBC measurements. This is confirmed by the observation of cross-peak R in Figure 7.

Both of the carbonyl carbons 5 and d have three signals. These multiple components of the ^{13}C signals reflect the compositional microstructures of the amine-modified copolymer PVA. This is confirmed by the observation of HMBC cross-peaks between the amine-substituted methine proton b″ and the carbonyl carbon 5. Because of the low acetate concentration, a HMBC cross-peak between acetate carbonyl d and the corresponding acetate-substituted methine b′ is too weak to be detected. Nevertheless, three ^{13}C signals of the carbonyl d are observed and are assumed to be mainly due to the three different compositional microstructures.

Brar et al. used ^{13}C edited $^{1}H-^{1}H$ total correlation (HSQC-TOCSY) to determine for the first time the tacticity of an unmodified pure PVA completely.[26] In the course of our study on the modified PVA, we noticed another possibility of doing this, that is, by observing the $^{3}J_{CH}$ of the HMBC correlation. This is shown in Figure 8. The carbon of a pentad methine can have two $^{3}J_{CH}$ couplings with the methine protons of its first neighboring comonomers. Thus, the ^{13}C of the rr-centered pentads mrrm, mrrr, and rrrr show $^{3}J_{CH}$ cross-peaks with protons of triads rr and mr; the mr-centered ones mmrm, (mmrr + rmrm), and rmrr arise correlation with all the three triads mm, mr, and rr; and the mm-centered pentads mmmm, mmmr, and rmmr give cross-peaks with triads mm and mr. These HMBC cross-peaks highlighted in Figure 8 confirm the tacticity assignment.

Comparison between M(32) and E(33). The structure analysis of the modified copolymer is further confirmed by performing complete NMR measurements on the DEAEA modified copolymer E(33) (amine 10.9%, acetate 4.1%). Results similar to M(32) are obtained. The DEAEA are found to have 46% as separated substitution of triad (VOH, VAM, VOH), 26% as (VCM, VAM, VOH), and 28% as pure vinyl alkylcarbamate or mixed vinyl acetate/vinyl alkylcarbamate triads (VCM, VAM, VA). The block index of substitution n_A observed is 1.90 (for M(32) the experimental value is 1.72). The block index n_{OH} of the nonsubstituted PVA polymer observed implies an average length of 9.8 for the PVA block in copolymer E(33) (9.0 for M(32)). The analysis confirms that similar amine modifications to PVA with

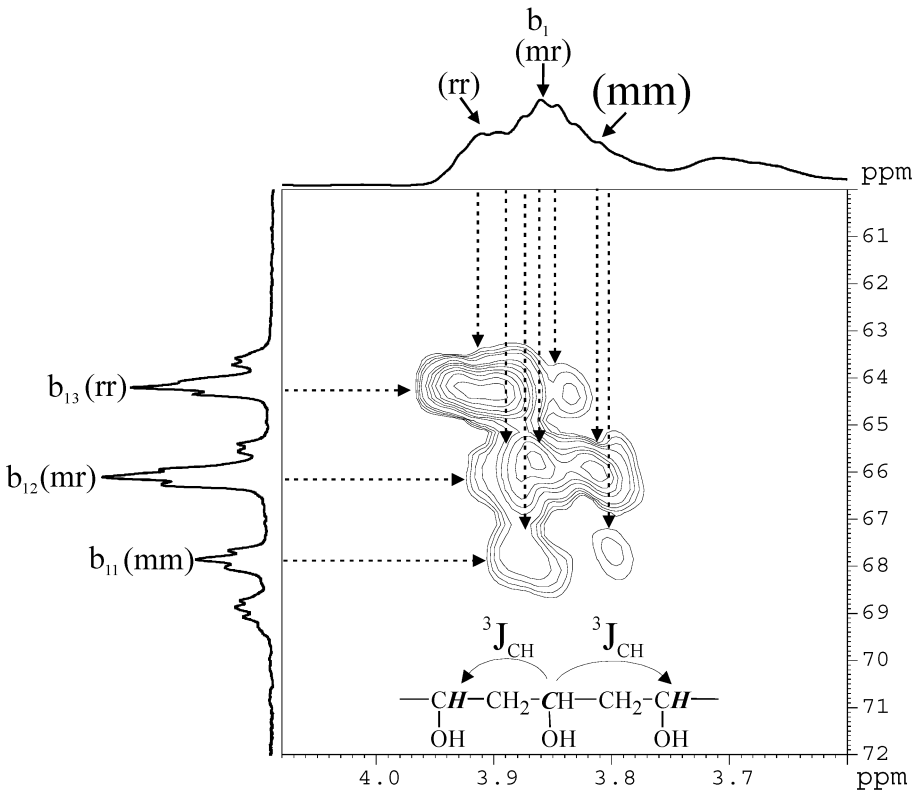


Figure 8. $^{1}H-^{13}C$ HMBC spectrum of M(32) in the regions of (F_2) 4.1−3.6 ppm and (F_1) 72−60 ppm.

the same degree of substitution result in similar microstructure of the modified copolymers.

Conclusions

Using COSY and the $^{1}H-^{13}C$ HMQC and HMBC spectroscopy, a complete assignment of the ^{1}H and ^{13}C spectra of the amine-modified PVA M(32) has been done. The methine ^{1}H spectrum revealed 54% of (VOH, VAM, VOH), 30% of (VCM, VAM, VOH), and 16% of (VCM, VAM, VCM) to be the microstructures of the amine modification. On the other hand, an average block length of 9 VOH is estimated for the PVA backbone. The methylene protons are heterosteric, and their spectra are properly reassigned. The ^{13}C spectrum of methine contains components of both compositional and configurational: tacticities rr, mr, and mm for (VOH, VOH, VOH), whereas rr and mr for triad (VOH, VOH, VAM). We may thus deduce that mainly isotactic triads react with activated diamines. The HMBC cross-peaks clearly confirmed the covalent bond between PVA backbone and amine. The tacticity assignment of pure PVA could be substantiated by the HMBC spectra.

In this work we presented the first NMR study of the complete microstructure of the PVA backbones for tailor-made branched polyesters. Beyond the routine one-dimensional experiments, we used two-dimensional homonuclear and heteronuclear NMR spectroscopic techniques. The constitutional and configurational microstructures are thus clearly determined. Furthermore, the covalent bond between PVA backbone and amine was clearly confirmed. This paper thus provides structural evidence for a targeted manipulation in the design of polymers for gene and drug delivery and serves as a good example for the power of the sophisticated NMR methods to elucidate the structure of complex polymers in biopharmacy.

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Supporting Information Available: A section of COSY spectrum of P(33). This material is available free of charge via the Internet at http://pubs.acs.org.

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