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3D NMR Studies of DAB-16 Dendrimer

Minghui Chai, Yanhui Niu, Wiley J. Youngs, and Peter L. Rinaldi*

Department of Chemistry, Knight Chemical Laboratory, The University of Akron,
Akron, Ohio 44325-3601

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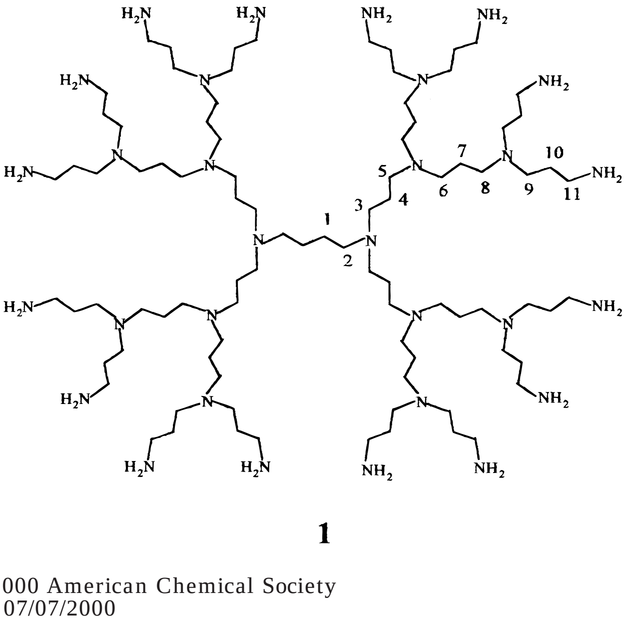
ABSTRACT: The HMQC-TOCSY 3D NMR technique has been used to study the DAB-16 [poly(propyleneimine)] dendrimer (the third generation) on a 750 MHz spectrometer. In the HMQC-TOCSY 3D NMR spectrum, it is possible to disperse ^{1}H signals based on the well-resolved ^{13}C chemical shifts and to obtain both one-bond ^{1}H−^{13}C connectivities and multibond ^{1}H−^{13}C connectivities by tracing the correlations within the same proton spin system from the HMQC-TOCSY slices. The overlapping ^{1}H resonances for core, interior, and exterior methylenes can be clearly resolved and assigned from the 3D NMR.

Dendrimers, which are highly symmetrical branched polymers, have been intensively studied since the first successful synthesis was reported in 1985.[1] The coexisting features, extensive branching and high surface functionality, have distinguished dendrimers from the classic linear and cross-linked polymers.[2] DAB [poly(propyleneimine)] dendrimers were first synthesized by de Brabander-van den Berg and Meijer in 1993.[3] Since then, many studies[2,4] have been done on DAB dendrimers and their derivatives, such as molecular recognition, physical encapsulation, and self-assembly. Meijer's dendritic box[5] has been used to demonstrate the feasibility of temporary selective encapsulation of small molecules within the dendritic voids. This illustrates the tremendous potential for using DAB dendrimers for biomedical applications such as drug and gene delivery systems. Until now, NMR has not been the preferred method for studying dendrimers, despite the wealth of information available from measurable NMR parameters. This is largely a result of the limited ability to resolve the resonances from the many unique, but very similar, groups in these molecules. Most of the thorough NMR studies have been performed on poly(amido amine) (PAMAM) type dendrimers. Because of different structural components, DAB dendrimers are expected to have properties different from those of PAMAM.[6] Therefore, more data describing DAB dendrimers are needed to fully understand their structures and to guide the design and synthesis of DAB type dendrimers for specific applications.

In dendrimer chemistry, NMR can play an important role to help understand the structures and properties of these important compounds. Mainly, 1D NMR techniques have been used for the characterization of dendrimers. Lambert and co-workers have applied ^{29}Si−^{29}Si INADEQUATE 2D NMR to confirm the structure of the first generation dendritic polysilane.[7] It demonstrates the clear connectivity for each of the three types of Si−Si bonds in the molecules. However, this experiment can only be used for silane dendrimers, and the INADEQUATE experiment is a very insensitive method. Because of their high cost and the difficult preparation of isotopically labeled synthetic polymers including dendrimers, the NMR technique used must be very sensitive and give efficient suppression of undesired signal components. In a recent paper,[8] we have shown 3D ^{1}H/^{13}C/^{29}Si triple-resonance 3D NMR combined with pulse field gradient techniques[9] can be used to fully characterize carbosilane dendrimers without resorting to isotopic labeling. In this paper, we demonstrate the application of the double-resonance 3D HMQC-TOCSY NMR experiment[10] for the complete characterization of DAB-16 dendrimer (**1**). This experiment provides better sensitivity (^{1}H detection) and high resolution (dispersion of resonances into 3 dimensions) for structural studies of dendrimers. In the DAB system, there is a unique set of $-CH_2-CH_2-CH_2-$ segments for each generation, isolated from each other by trivalent nitrogen atoms. The 2D HMQC experiment provides ^{1}H−^{13}C correlations for each $-CH_2-$, and the 2D TOCSY produces correlations among the protons within the same spin system. Thus, the 3D HMQC-TOCSY spectrum will contain resonances dispersed based on the well-separated ^{13}C resonances. A plane at the shift of each ^{13}C resonance will contain a diagonal peak at the shift of the ^{1}H bound to that ^{13}C and off-diagonal peaks at the shifts of all the ^{1}H atoms which are a part of the same spin system. It provides a powerful way to resolve and observe the resonances from atoms within each generation of the dendritic structure.



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* Corresponding author: telephone 330-972-5990; Fax 330-972-5256; E-mail PeterRinaldi@uakron.edu.

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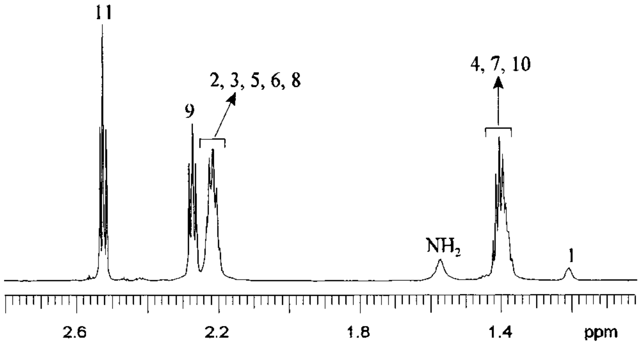

Figure 1. 1D ^{1}H NMR spectrum of DAB-16 dendrimer in $CDCl_3$ obtained at 750 MHz.

In the structure of DAB-16 (**1**), there are 11 unique methylene groups, which are numbered from the core to the exterior of the dendrimer. The exponential growth of dendritic chains provides more methylenes (9−11) for the exterior, which contribute to very strong signals in the NMR spectra. The resonances from the interior methylenes (3−8) of DAB-16 produce strongly overlapping multiplets due to their very similar surroundings. The resonances from core methylenes (1−2) are dramatically weaker because of their very low occurrences in the structure. The 1D NMR spectra of DAB-16 are shown in Figures 1 and 2. The 750 MHz ^{1}H NMR spectrum (Figure 1) exhibits four intense multiplets at ~2.53, ~2.28, ~2.22, and ~1.39 ppm, as well as two small broad peaks at ~1.57 and ~1.21 ppm. Assignments appear above groups of peaks in the spectrum. Further detailed assignments for proton resonances cannot be obtained by simple inspection of the 1D ^{1}H NMR spectrum. Fortunately, the 188.6 MHz ^{13}C NMR spectrum on top of Figure 2 exhibits 11 resolved resonances for DAB-16. On the basis of their relative intensities and expected chemical shifts, peak assignments can be made; these are labeled above each peak.

On the basis of the well-resolved carbon resonances, the 3D ^{13}C edited HMQ-CTOCSY experiment has been used to obtain the proton resonance assignments. To enhance the resolution of the 3D spectrum, to reduce the data size, and to reduce the experiment time, a very narrow ^{13}C spectral window (~3 kHz) was used. Consequently, some peaks are aliased into regions where they do not interfere with other resonances in the spectrum. Additionally, linear prediction was used to extend the data set size to 1024 × 512 × 512 so as to further enhance the digital resolution in the spectrum.

The bottom part of Figure 2 shows *f*2*f*3 slices which have been cut from the 3D HMQC-TOCSY spectrum at the shift of each ^{13}C resonance in the *f*1 dimension. Each 2D slice contains two or three peaks: one corresponding to the diagonal peak (D) and one or two cross-peaks (C). Figure 2a,i displays the TOCSY correlations among the core methylene protons of DAB-16. Only two types of

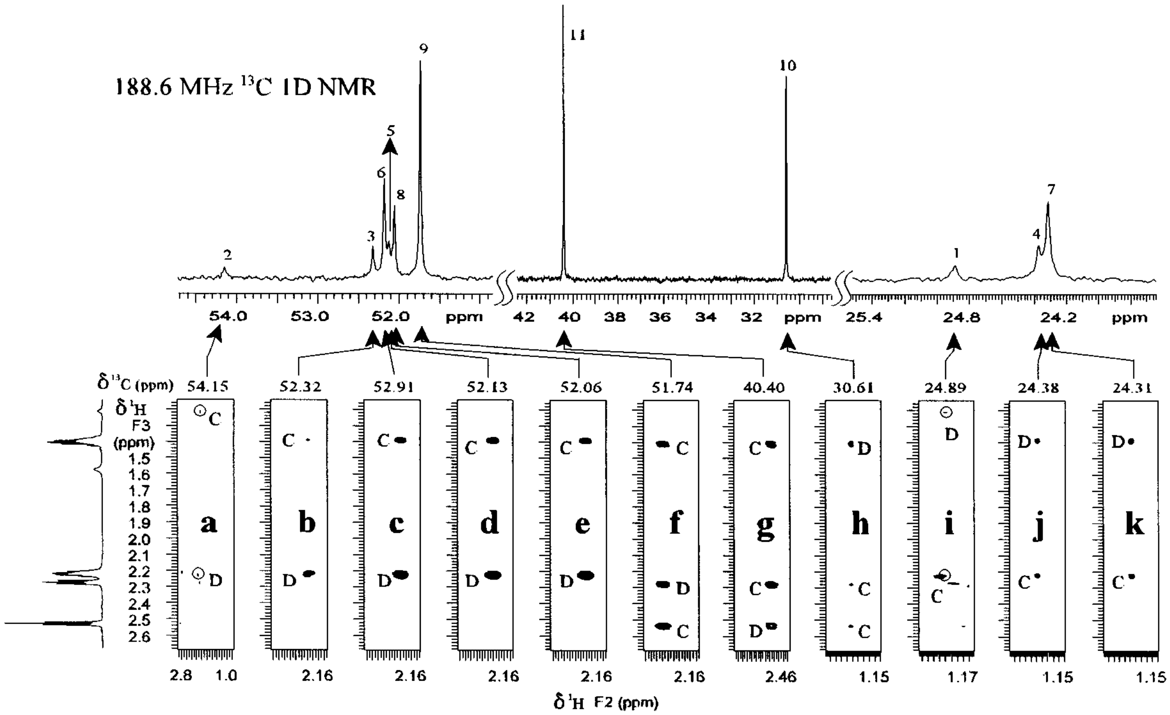

Figure 2. The ^{13}C NMR spectrum of DAB-16 at 188.6 MHz is plotted across the top along with the ^{13}C resonance assignments. Eleven 2D *f*2*f*3 slices from the 3D HMQC-TOCSY spectrum of DAB-16 are displayed in a−k along with the ^{13}C shift of that slice. In each slice, the peak marked D is the diagonal peak and the peak marked C is the cross-peak. The HMQC-TOCSY 3D NMR spectrum of DAB-16 was collected with ^{1}H and ^{13}C 90° pulses of 8.8 and 18.0 μs, respectively, a relaxation delay of 1 s, and $\Delta = (2 \times {}^1J_{HC})^{-1} = 3.57$ ms (optimized for one bond ^{1}H−^{13}C correlations); 3405.1, 1799.9, and 1799.9 Hz spectral windows in the ^{13}C(f_1), ^{1}H(f_2), and ^{1}H(f_3) chemical shift dimensions, respectively; a 0.05 s acquisition time with ^{13}C GARP decoupling with a field strength of 19.7 kHz; and a 50 ms isotropic mixing time using MLEV-17 sequence with a field strength of 11.25 kHz after a 2.0 ms trim pulse; four transients were averaged for each of 2 × 56 complex t_1 increments and 2 × 56 complex t_2 increments. Homospoil gradient pulses were used to purge undesired transverse magnetization. The data were processed with a combination of shifted sinebell and Guassian weighting in all three dimensions; linear prediction was used to forward extend the data in the f_1, f_2, and f_3 dimensions to increase the digital resolution (64 complex points were used to calculate the f_3 coefficient, 48 complex points were used to calculate f_1 *and* f_2 coefficients); and zero filling was used so that 3D FT was performed on a 1024 × 512 × 512 3D matrix. To enhance the resolution of the 3D spectrum, to reduce the data size, and to reduce the experiment time, a very narrow ^{13}C spectral window (~3 kHz) was used. Consequently, some peaks are aliased into regions where they do not interfere with other resonances in the spectrum. The ^{13}C chemical shifts of the 3D spectrum were traced back to those of the 1D spectrum.

Table 1. ^{13}C and ^{1}H Chemical Shift Assignments of DAB-16

carbon no.	chemical shifts [a] $\delta_{^{13}C}$ (ppm)	chemical shifts [a] $\delta_{^{1}H}$ (ppm)	carbon no.	chemical shifts [a] $\delta_{^{13}C}$ (ppm)	chemical shifts [a] $\delta_{^{1}H}$ (ppm)
1	24.89	1.21	7	24.31	1.39
2	54.15	2.24	8	52.06	2.22
3	52.32	2.22	9	51.47	2.28
4	24.38	1.39	10	30.61	1.41
5	52.13	2.22	11	40.40	2.53
6	52.19	2.22	NH_2		1.57

[a] Digital resolution is 0.035 and 0.01 ppm/point in the ^{1}H and ^{13}C dimensions, respectively.

methylene protons are present in the core of the dendrimer, producing unique slices with only two TOCSY correlations. It is straightforward to assign the proton resonance at 2.24 ppm to H_2 and the proton resonance at 1.21 ppm to H_1 on the basis of their relative shifts. Similarly, resonances shown in the remaining slices can be attributed to the interior and exterior methylene protons of the dendrimer. Each of the plots in Figure 2b−e and Figure 2j,k contains two strong peaks at 2.22 and 1.39 ppm. Considering that all methylene protons of the interior are located in the nearly identical environments, all interior $-NCH_2-CH_2-CH_2N-$ units are chemically very similar. Thus, only two types of proton resonances are resolved in each of these slices: at 2.22 ppm from the $-NCH_2-$ groups and at 1.39 ppm from the $-CH_2\mathit{CH_2}CH_2-$. On the basis of their relative intensities, the resonances from C_3/C_5 can be distinguished from the resonances of C_6/C_8. Even though C_5 and C_6 are in different layers of the dendrimer, they are in almost identical environments; both are attached to the same tertiary nitrogen atom. Therefore, the signals at $\delta_c = 52.13$ and 52.19 are assigned to C_5 and C_6, respectively. This makes it possible to distinguish between C_3 and C_5 and between C_6 and C_8. The exterior propylene units of DAB-16 are bound to primary and tertiary amine groups which differentiate them. Therefore, one diagonal peak and two cross-peaks are expected in the TOCSY slices at the shifts of C_9, C_{10}, and C_{11}. Parts f−h of Figure 2 are the TOCSY slices cut at the ^{13}C resonances of each methylene from the 3D HMQC-TOCSY spectrum. In these slices, the signals at 2.53, 2.28, and 1.41 ppm are attributed to H_{11}, H_9, and H_{10}, respectively. In this way all proton resonances in the 3D spectrum can be resolved and assigned. These ^{13}C and ^{1}H chemical shift assignments are summarized in Table 1.

Many computational simulations have been done on dendrimers. However, because of the lack of sufficient experimental data, it has been not been possible to determine the validity of different simulation models, especially with regard to chain conformation and density. Both the conformations in solution and in solid state are still in debate.[11] On the basis of unequivocal ^{1}H NMR resonance assignments and ^{1}H NOE interactions between the dendrimer protons, it should be possible to obtain the needed experimental data. Multidimensional NMR experiments such as 2D NOESY,[12] 3D NOESY-HSQC,[13] and 3D HMQC-TOCSY[14] at very high magnetic field should provide the sufficient information. Multidimensional NMR experiments such as these will also be useful for studying structure changes as a function of solvent environment and generation number.

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(15) Chemicals and sample preparation: The DAB-16 dendrimer was obtained from Aldrich Chemical Co. $CDCl_3$ was purchased from Cambridge Isotope Laboratory (Andover, MA). Chloroform and deuterated chloroform were dried over phosphorus pentoxide and distilled before use. The solvent was freeze−pump−thawed, and the DAB-16 dendrimer was

pumped before use. The preparation of samples was performed in glovebox under argon. DAB-16 (250 mg) was dissolved in $CDCl_3$ solvent and sealed in a 5 mm NMR tube.

(16) Hardware for NMR measurement and data processing: NMR spectra were obtained on a Varian Unityplus 750 MHz NMR spectrometer equipped with four rf channels, a Performa II *z*-axial pulse field gradient (PFG) accessory, and a Varian $^{1}H/^{13}C/^{31}P$ triple-resonance probe with a PFG coil. The solvent signals served as internal references for both ^{1}H (at 7.27 ppm) and ^{13}C (at 77.23 ppm) chemical shifts. All data were processed with Varian's VNMR software on a SUN Ultra-10 workstation.

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