NMR Study of Dendrimer Structures Using Paramagnetic Cobalt(II) as a Probe

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Cobalt(II) has been utilized as an external paramagnetic ^{1}H NMR probe for the study of the structure of dendrimers that possess specifically located metal recognition sites. The isotropically shifted ^{1}H NMR signals of the Co(II) complexes of two 2,6-diamidopyridine-containing dendrimers have been fully assigned by means of 1D and 2D NMR techniques, including NOE difference, EXSY, COSY, and TOCSY. T_1 values of the isotropically shifted signals were used to calculate metal−proton distances to build a molecular model of the internal structure of the dendrimers. The presence of sizable cavities within the dendrimers was observed, including a loosely packed conformation for the 2,6-diamidopyridine moiety to bind to potential guest molecules.

Introduction

The unique supramolecular chemistry associated with dendritic macromolecules has raised great interest in the structural and chemical characteristics of these polymers.[1] For example, the incorporation of specific recognition sites located within the dendritic structure has permitted chemical processes such as H-bonding and metal ion complexation to occur at these loci. Indeed, many dendrimers have been suggested to loosely mimic the structure and function of globular proteins owing to their large size, spherical shape, and specific host−guest chemistry.[1b] Unlike proteins, however, dendrimers have not been successfully analyzed by X-ray crystallography due to their fractal nature that interrupts the long-range molecular ordering necessary for crystal packing and X-ray diffraction.[2] Dendrimers also exhibit broad overlapping ^{1}H NMR features, which further hinders the analysis of their conformations in solution.

Paramagnetic metal ions with short electron relaxation times ($<10^{-11}$ s), especially Co(II), have been extensively used as intrinsic and external NMR probes for the analysis of the active sites of metalloproteins.[3] Protons close to these paramagnetic metal centers can exhibit isotropically shifted (or hyperfine-shifted) ^{1}H NMR signals with short relaxation times T_1 and T_2.[3] These signals can be significantly shifted outside the crowded diamagnetic region at 0−10 ppm, which facilitates their detection and assignment. Presumably, isotropically shifted ^{1}H NMR signals for paramagnetic metal-bound dendrimers, if detected, can be especially useful for the characterization of these macromolecules that exhibit poorly resolved overlapping ^{1}H NMR features. For example, a few dendrimers which contain intrinsic paramagnetic Fe−S cores have been shown to display some well-resolved hyperfine-shifted ^{1}H NMR signals with small chemical shifts.[4] For dendrimers lacking any intrinsic paramagnetic centers, simple paramagnetic metal ions such as Co(II) can be utilized as external probes for detailed investigation of the environment about the metal recognition site(s), if present. The availability of dendrimers possessing four potential metal-binding 2,6-diamidopyridine sites[5] (Figure 1) affords a simple system to further evaluate this principle for the first time. The diamidopyridine functional groups in these dendrimers have previously been demonstrated to be involved in H-bonding with guest molecules.[5] Thus, these dendrimers may serve as prototypes for functional dendrimers. We herein describe the use of the paramagnetic Co(II) metal ion as an external NMR probe for detailed investigation of dendrimer structures.

Experimental Section

The 12-ester 2,6-diamidopyridine-containing dendrimer (**1**), the 36-acid 2,6-diamidopyridine-containing dendrimer (**2**) (see structure in Figure 1), and the simple compound that mimics the "dendritic arm" of **1**, *N*-(6-(propionylamino)pyridin-2-yl)propionamide, **3**, were synthesized and characterized according to the literature procedures.[5] The Co(II) complexes of the dendrimers were prepared by the addition of stoichiometric amounts of the metal to the dendrimers in solutions. The ^{1}H NMR spectra of the complexes were obtained by the use of a single 90° pulse with presaturation for solvent suppression on a Bruker

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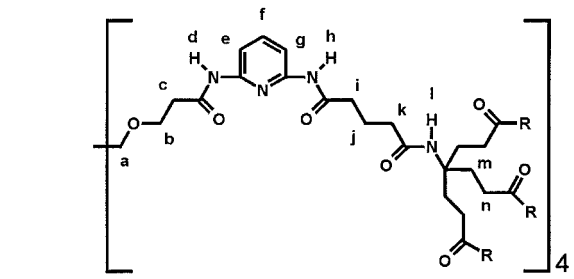

1 R = O-*t*-Bu
2 R = NHC($CH_2CH_2CO_2H$)$_3$

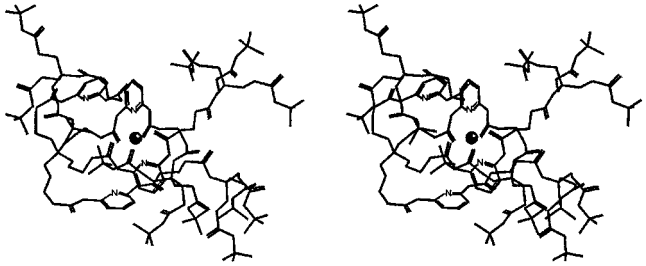

Figure 1. Schematic structure of the 12-ester diamidopyridine dendrimer (**1**) and the 36-acid diamidopyridine dendrimer (**2**) and the 3D model of the Co(II)−**1** complex constructed with distance constraints from NMR relaxation times by the use of the Cerius[2] molecular modeling program. Hydrogen atoms are omitted, and other atoms are not labeled in the structures for clarity.

ADX250 spectrometer at 250 MHz. The T_1 relaxation time was obtained by the use of the standard inversion-recovery method with a three-parameter fitting program on the spectrometer. The EXSY[6] spectra (using the standard phase-sensitive NOESY sequence), which reveals chemical exchange correlations, and the COSY and TOCSY spectra were obtained with 1024 × 256 data points (zero-filled to 1024 × 1024) by the use of the standard pulse programs on the spectrometer with the acquisition parameters properly adjusted, i.e., shorter acquisition, delay, mixing, and spin-lock times.[3] A 0°-shifted sine-bell squared window function was applied to both dimensions of the COSY spectra prior to Fourier transformation and processed in the magnitude mode, whereas a 45° to 60° shifted sine-bell squared window function was used in phase-sensitive EXSY and TOCSY experiments. The NOE difference spectra on dendrimer **2** in CH_3OH (to avoid deuterium exchange) were obtained with the saturation pulse on (addition) and off (subtraction) the signal of interest using the WEFT sequence[7] for solvent suppression.

In paramagnetic molecules, the paramagnetic contribution to the nuclear relaxation rates is proportional to the negative sixth power of the distance between the resonating nucleus and the paramagnetic center, i.e., $T_{1,2M}^{-1} \propto r_{M-H}^{-6}$.[3] The diamagnetic contribution to nuclear relaxation is usually minimal when compared with the paramagnetic contribution, particularly for isotropically shifted signals with observed T_1 less than 100 ms in which the diamagnetic contribution is expected to be less than 10%. Thus, the Co(II)−proton distances can be estimated by the equation $r_{Co-H} = r_{ref}(T_1/T_{1ref})^{1/6}$, in which r_{Co-H} is the distance between a proton with relaxation time T_1 and the Co(II) ion and r_{ref} is the distance between a rigid reference proton and the Co(II) center. Ring proton e (see structure in Figure 1) with a relaxation time T_{1ref} = 39.1 ms is used as the reference which is fixed to be 5.08 Å away from Co(II) according to the structures of several similar pyridine-containing complexes.[8] The structural model for the metal-binding site in the complex Co(II)−**1** was built by the use of Cerius[2] software (Molecular Simulations, San Diego, CA) with distance constraints obtained from the nuclear relaxation times described above.

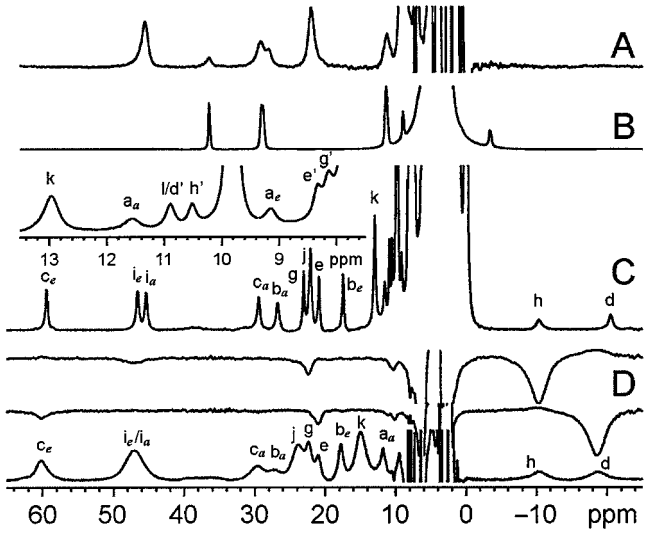

Figure 2. ^{1}H NMR spectra (250 MHz, 298 K, CD_3OH) of complexes Co(II)−(**3**)$_2$ (A), Co(II)−**3** (B), Co(II)−**1** (C), and Co(II)−**2** (D). The 1D NOE difference spectra in part D are obtained with the signals at −11 ppm (top) and −19 ppm (bottom) saturated for 50 ms. Labels of the signals correspond to the structure shown in Figure 1. Signals d, h, and l are solvent exchangeable and disappear when the complexes are dissolved in methanol-d_4.

Results and Discussion

The binding of Co(II) to dendrimer **1** can be clearly monitored through the isotropically shifted ^{1}H NMR features which can be studied in detail by means of 1D and 2D NMR technqiues. Upon addition of less than 1 equiv of Co(II) to the dendrimer, not all the metal-binding diamidopyridine sites of the four arms are occupied by the Co(II) ion. The metal-binding pattern of the dendrimer can be clearly observed under this condition, in which a quasi-selective metal binding pattern was established. As a result, protons from the Co(II)-bound dendritic arms display hyperfine-shifted ^{1}H NMR signals with large chemical shifts in a spectral window of ca. 80 ppm (Figure 2C). A detailed NMR analysis (discussed below) reveals other arms that are not directly bound to the metal ion yet still exhibit noticeable isotropic shifts, which is possibly attributable to the dipolar shift mechanism.[3] Moreover, a third set of signals are also present in the diamagnetic region of ∼0−10 ppm that are due to the metal-free dendrimer molecules. The much larger dendrimer **2** with one more generation also exhibits very similar isotropically shifted ^{1}H NMR features upon addition of <1 equiv of Co(II) (Figure 2D), similar to dendrimer **1**. The similar spectral features indicate that the metal-binding pattern and the ligand configuration around the metal-binding site in this larger dendrimer **2** are similar to those in the smaller dendrimer **1**. However, the broadness of the spectral features of dendrimer **2** due to its larger size hinders fine analysis of the metal-binding environment of this dendrimer in solution. We thus first chose to investigate in detail and discuss here the NMR study of the smaller dendrimer **1**, which exhibits well-resolved hyperfine-shifted ^{1}H NMR features, to shed light on the structure of the larger dendrimer **2** and other similar dendrimers.

The metal-binding properties and the overall structure of dendrimer **1** can be partially revealed upon the binding of 1

(6) Abbreviations: COSY, (through bond) correlation spectroscopy; EXSY, (chemical) exchange spectroscopy; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, (through bond) total correlation spectroscopy; WEFT, water elimination Fourier transformation.

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equiv of Co(II). This dendrimer, with a quaternary sp^3 carbon center, affords a tetrahedral T_d symmetry with four equivalent arms.[5] The detection of three sets of NMR features, far shifted, much less shifted, and diamagnetic, reflects two possibilities for metal binding: (i) metal binding to the four individual arms sequentially and (ii) a cooperative binding of two arms to the metal ion. In the case of (i), the binding of Co(II) to one of the four arms would presumably afford a pseudo-C_{3v} symmetry, leaving three equivalent free arms with respect to a fast rotating Co(II)-bound arm. In the case of (ii), the binding of two arms to Co(II) affords a C_{2v} symmetry and two equivalent free arms. The three sets of NMR signals are observed to correlate with each other in the EXSY spectra (see later), which indicates the presence of fast exchange between the Co(II)-bound and free forms of the dendrimer as shown in the equilibrium

$$\text{Co(II)} + \mathbf{1} \rightleftharpoons \text{Co(II)}-\mathbf{1} \qquad (1)$$

According to this equilibrium, one set of the NMR signals is due to the metal-bound arm(s) in the complex Co(II)−**1**, the second set due to the "proximal" metal-unbound arms in the complex Co(II)−**1**, and the third set due to the metal-free form **1** in eq 1. The NMR results described above, although consistent with equilibrium 1, do not allow the distinction of the two different metal-binding modes (i) and (ii). We thus investigated this problem from a different approach.

Metal ions are known to bind simple ligands in a sequential manner due to the different stepwise constants K_i's (or the overall formation constants β_i's),[9] which afford different complexes with different metal:ligand ratios that presumably should exhibit characteristic NMR spectra. In case of paramagnetic metal ions, the isotropically shifted ^{1}H NMR features of these complexes should be significantly different due to the very different magnetic environments of the ligands in the different complexes, as shown in the metal−daunomycin complexes with different metal-to-ligand ratios.[10] The metal-binding property of the simple molecule **3**, which represents the metal-binding moiety of the dendrimer **1**, was thus studied by means of NMR. The cobalt(II) ion can presumably bind either one or two molecules of the tridentate ligand **3** (via one pyridine and two carbonyl groups) to afford a 1:1 Co(II)−**3** or 1:2 Co(II)−$\mathbf{3}_2$ complex. The 1:1 complex is more favorably formed with excess amount of metal, whereas the 1:2 complex can be formed with an excess amount of ligand. Since the hyperfine-shifted features are detected only for the Co(II)-bound ligands, the 1:1 and 1:2 complexes can be studied in situ despite the presence of an excess amount of the metal and the ligand, respectively. The ^{1}H NMR spectra of these two complexes have been acquired which are quite distinguished from each other (spectra A and B in Figure 2). The isotropically shifted signals of these two complexes can be easily assigned on the basis of their relaxation times and exchanges in EXSY with their diamagnetic metal-free counterparts. The farther shifted signal is due to protons equivalent to those labeled c and i and the less shifted one is an overlap and is equivalent to protons e, g, b, and j. The chemical shifts of the ring 3,5-protons (protons e and g) in the dendrimer complexes Co(II)−**1** and Co(II)−**2** are very close to those in the 1:2 Co(II)−$\mathbf{3}_2$ complex (spectrum A). Moreover, the average of the chemical shifts of the axial- and equatorial-like CH_2 protons (signals $c_{a/e}$, $i_{a/e}$, $b_{a/e}$, and j) in Co(II)−**1** and Co(II)−**2** are also very similar to the chemical shifts of the CH_2 and the methyl signals of the free-rotating propylamide group in Co(II)−$\mathbf{3}_2$ but quite different from those in the 1:1 Co(II)−**3** complex. This observation indicates that Co(II) is coordinated to two dendritic arms of **1** and **2** in a cooperative manner as described in case ii above. This cooperativity of the dendritic arms has to be taken into consideration in the future design of "functional dendrimers".

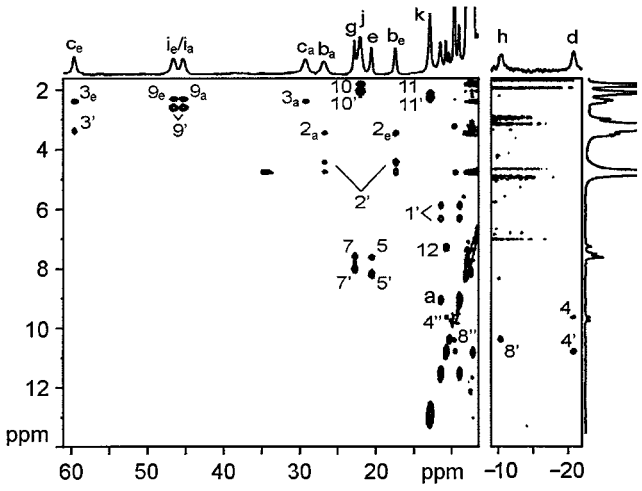

Figure 3. ^{1}H EXSY spectrum (250 MHz, 298 K, 20 ms mixing time, 133 ms recycle time) of Co(II)−**1** in 2:3 CH_3OH/CD_3OD. Labels for the peaks correspond to the structure shown in Figure 1.

The relatively sharp hyperfine-shifted signals of Co(II)−**1** allow all the signals to be assigned by means of 1D and 2D NMR techniques. There are five hyperfine-shifted solvent-exchangeable ^{1}H NMR signals detected in the spectrum of Co(II)−**1** (Figure 2C), including the signals at 10.9 (11.9 and 11.4 ppm at 273 K), 10.5, −10.3, and −20.5 ppm with T_1 values (273 K) of 39.2, 63.7, 60.7, 13.4, and 14.6 ms, respectively. The signals at −10.3 and −20.5 ppm with the shortest relaxation times can be unambiguously assigned to the 2,6-diamidopyridine NH protons (h and d) on the Co(II)-bound arm. These NH protons are likely to be shifted upfield by a through-bond spin polarization mechanism induced by the Co(II) ion coordinated to the amide carbonyl oxygens.[8] A significant dipolar shift contribution can be excluded since there is no indication of such a mechanism to a great extent on any other protons. If the metal ion were coordinated through the amide nitrogens, the NH signals would be shifted farther upfield (in the range −150 to −200 ppm)[11] and their T_1 values would be much shorter (<5 ms) or the NH proton would be deprotonated and not detected; however, this is not the case. A computed structure (see later) also shows that the h and d NH protons are pointed away from the Co(II) ion at 4.25 and 4.31 Å, respectively, with respect to the pyridyl meta protons (39.1 ms) at 5.08 Å. These distances are consistent with the carbonyl oxygen coordination.

The assignment of the five solvent-exchangeable signals can be fully established using the EXSY technique, as they displayed cross peaks to their appropriate diamagnetic counterparts at 7.26, 9.65, 9.76, 9.76, and 9.65 ppm (cross signals 12, 4′, 8″, 8, and 4, respectively, in Figure 3). The signal at 10.9 ppm (11.9 ppm at 273 K) is assigned to the amide NH l proton on the Co(II)-coordinated arm 5.08 Å away from the metal on the basis of its relaxation time. The downfield chemical shift of this signal, as opposed to the upfield-shifted h and d signals described above, indicates an absence of through-bond spin polarization. The h′ and d′ signals at 10.9 (11.4 ppm at 273 K) and 10.5 ppm display

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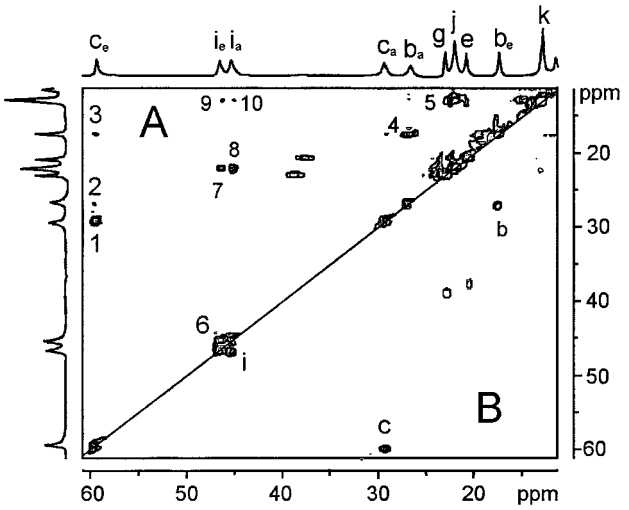

Figure 4. ^{1}H TOCSY (A) and EXSY (B) spectra (250 MHz, ambient temperature, 30 and 80 ms mixing time, and 190 and 240 ms recycle time, respectively) of Co(II)−**1** in methanol-d_4. Labels correspond to the structure shown in Figure 1. The cross signals 1, 4, 5, and 7 are also detected in the COSY spectrum of the complex.

relatively longer T_1 values and are attributed to the 2,6-diamidopyridine NH protons of the "proximal" noncoordinated dendritic arms ~5.5 Å away from the Co(II). However, due to the presence of the fast equilibrium, which may contribute to the measured relaxation, this distance derived from the relaxation times for the noncoordinated arms serves as a minimum distance. Only a small downfield hyperfine shift is observed for these signals, indicating this dendritic arm is not bound to Co(II).

The signals at 59.4 and 29.5 ppm display cross peaks to each other in EXSY, TOCSY, and COSY spectra (cross peaks 1 and c in Figure 4) along with an exchange peak to their diamagnetic counterparts overlapped at 2.4 ppm (cross peaks 3_e and 3_a in Figure 3). These two signals can thus be assigned to the inner methylene CH_2CONH geminal protons (c_e and c_a for the equatorial-like and axial-like protons, respectively; see later). The same protons on the "proximal" noncoordinated dendritic arm at 3.36 ppm (c′) also display an exchange peak with the 2.4 ppm signal. A through-bond correlation in the TOCSY spectrum between the two signals $c_{e/a}$ and the two signals of the adjacent methylene protons ($b_{e/a}$) at 26.7 and 17.5 ppm is also noted (cross peaks 2 and 3 in Figure 4). The latter protons in turn display cross peaks to each other in the EXSY, TOCSY, and COSY spectra (cross peaks 4 and b in Figure 4), along with exchange peaks to their diamagnetic counterpart at 3.4 ppm (cross peaks 2_e and 2_a in Figure 3) and the counterpart on the "proximal" arm (cross peaks 2′ in Figure 3). The 2D NMR studies thus conclude the assignments of these signals. The geminal pair $a_{e/a}$ can also be assigned in a similar way.

The dramatic difference in chemical shift between the geminal protons in the pairs $c_{e/a}$ and $b_{e/a}$ must be attributed to their very different configurations, such as one in an equatorial-like position and the other in an axial-like position with respect to the Co(II)−carbonyl plane. This dramatic difference between geminal pairs has been previously observed and rationalized in several metal complexes of amino acids and amines.[10,12] The equatorial-like protons at 59.4 (c_e) and 17.5 ppm (b_e) are found farther away from the metal ion as indicated by their larger T_1 values (31.6 and 39.1 ms, respectively) compared to the T_1 values of their axial-like geminal partners c_a and b_a (16.4 and 14.8 ms, respectively). The detection of exchange cross peaks among the geminal partners $a_{e/a}$, $b_{e/a}$, $c_{e/a}$, and $i_{e/a}$ (cross peaks a, b, c, and i, respectively, in Figures 3 and 4) suggests the presence of a certain flexibility of the geminal pairs, such as that due to exchange with the free-rotating metal-free form in which the geminal pairs are equivalent.

The TOCSY spectrum of Co(II)−**1** shows cross peaks between the three pairs of adjacent methylene protons distal to the Co(II)-coordinated pyridine, i.e., $i_{e/a}$, j, and k at 46.6/45.4, 22.1, and 12.9 ppm, respectively (cross peaks 5 and 7−10 in Figure 4). This observation allows us to assign these signals to the spin system $CH_2-CH_2-CH_2$. Exchange cross peaks with their diamagnetic counterpart at 2.33/2.33, 1.81, and 2.12 ppm, respectively, are also observed for these hyperfine-shifted signals in the EXSY spectrum (cross peaks 9−11 in Figure 3). The geminal $i_{e/a}$ protons display similar T_1 values of 20.7 and 20.5 ms, respectively, and are thus both pointed away from the Co(II) ion with about the same distance to the metal. The same or much smaller difference in chemical shifts between the geminal pairs in the i−k methylenes near the surface of the dendrimer compared to the significantly larger difference in the a−c methylenes indicates greater rotational freedom for the region closer to the surface that "averages" the two different positions of the methylene protons.

Despite the good resolution of the hyperfine-shifted ^{1}H NMR features of **1** that allows several COSY and TOCSY cross peaks to be detected, the fast nuclear relaxation rates and the small molecular size preclude obtaining more detailed structural information by means of NOE techniques. This is because the NOE intensity in paramagnetic macromolecules is proportional to the rotational correlation time and inversely proportional to the nuclear relaxation rates.[3] The availability of the larger second-generation dendrimer **2** (R = 2 in Figure 1) with 36 terminal carboxylic acids allowed a more detailed investigation of the configuration of the metal-binding environment by the use of NOE techniques due to the significant increase of the molecular size (thus the rotational correlation time); i.e., MW = 4.9 kDa for **2** vs 2.8 kDa for **1**. The complete assignment of Co(II)−**1** greatly assists the assignment of the much broader and less resolved signals for Co(II)−**2** by means of similar techniques described above for Co(II)−**1**. The similar spectral features are indicative of similar metal-binding environments between these two Co(II)−dendrimer complexes. As observed for Co(II)−**1**, the 2,6-bis(amido)pyridine NH protons of Co(II)−**2** are also found in the upfield region at −11 and −19 ppm (h and d), which display strong negative NOE's to their adjacent methylene protons (c and i) and pyridine 3,5-protons (e and g) (difference spectra in Figure 2D). Furthermore, the −19 ppm signal (d) is clearly shown to exhibit an NOE to only the equatorial methylene proton (c_e) at 61 ppm, confirming the configurations for the NH protons and the methylene protons in Co(II)−**1** described above. The computed structure, with distance constraints obtained from relaxation times, indicates a staggered conformation for the b and c methylenes (Figure 1), also consistent with the configuration described here.

The terminal *tert*-butyl methyl groups and the adjacent m and n methylenes of the Co(II)−dendrimer complexes are not isotropically shifted, suggesting that the dendritic termini are not directed inwardly toward the Co(II) ion. This observation supports the presence of mobile structures capable of forming internal cavities where host−guest interactions are possible.[13] If the density of a dendrimer interior were to increase with the

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increase in molecular size as previously proposed,[14] some of the arms of **2** should be folded back in toward the core. This dense packing would significantly change the interior structure of the molecule and, thus, would alter chemical shift patterns associated with the internal functionalities near the metal-binding site. The model that is built on the basis of the relaxation time constraints seems to have a loosely packed structure in which hydrophobic interactions between hydrocarbon chains and/or H-bonding between amido −C═O and amido −NH may take place in solution (Figure 1). The spectral features for dendrimers **1** and **2**, however, appear to be quite similar (spectra C and D in Figure 2), suggesting that dense packing is not evident at these two generations for this dendrimer family.

Concluding Remarks

The size, structure, and accessibility of the internal cavities of a dendrimer can determine, in part, the optimal host−guest interactions of dendrimers practically designed for catalysis, self-assembly, and molecular recognition and encapsulation.[1] Since broad overlapping ^{1}H NMR signals have hindered detailed analysis of the interior structure of dendrimers, examination of the hyperfine-shifted ^{1}H NMR signals resulting from an external paramagnetic probe offers a different route to probe this interesting interior molecular environment. In this case, the use of an external Co(II) probe reveals a relatively accessible conformation for bonding to potential guest molecules for dendrimers **1** and **2**. Moreover, cooperative binding of two dendritic arms to one metal ion has been determined, which should be taken into consideration in the future design of guest−host functional dendrimers. We have demonstrated in this report that a paramagnetic metal ion such as Co(II) can serve as a valuable NMR probe for detailed investigations of the dynamics and structures of synthetic macromolecules.

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