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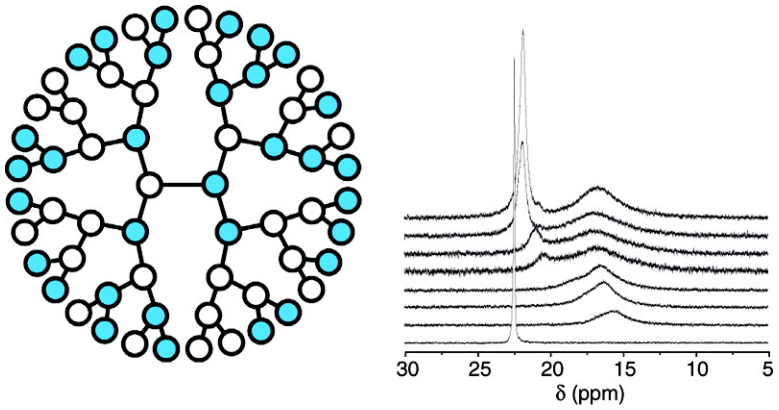
J. Am. Chem. Soc., **2005**, 127 (29), 10334-10343• DOI: 10.1021/ja052074m • Publication Date (Web): 23 June 2005



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Multicomponent Host−Guest Chemistry of Carboxylic Acid and Phosphonic Acid Based Guests with Dendritic Hosts: An NMR Study

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Abstract: A new way to analyze supramolecular dendritic architectures is reported by making use of ^{13}C NMR and ^{31}P NMR. Two ethylene glycol guest molecules have been synthesized containing a ^{13}C labeled carboxylic acid headgroup (**2**) and a phosphonic acid headgroup (**3**). The binding of these guests to urea-adamantyl modified poly(propylene imine) dendrimers has been investigated with ^{13}C NMR and ^{31}P NMR next to 1D and 2D ^{1}H NMR techniques. Different amounts of guest **2** have been added to fifth generation dendrimer **1e**, and the observed chemical shift values in ^{13}C NMR were fitted to a model that assumes 1:1 binding between guest and binding site. An association constant of 400 ± 95 M^{-1} is obtained for guest **2** with 41 binding sites per dendrimer. When different amounts of phosphonic acid guest **3** are added to dendrimer **1e**, two different signals are observed in ^{31}P NMR. Deconvolution gives the fractions of free and bound guest, resulting in an association constant of $(4 \pm 3) \times 10^4$ M^{-1} and 61 ± 1 binding sites. A statistical analysis shows that guest **2** forms a "polydisperse supramolecular aggregate", while guest **3** is able to form a "monodisperse supramolecular aggregate" when the amount of guest is high enough. The NMR results are compared with dynamic light scattering experiments, and a remarkable agreement is found. Phosphonic acid guest **3** is able to exchange with guest **2**, which is in agreement with the obtained association constants, and shows that these techniques can be used to analyze multicomponent dendritic aggregates.

Introduction

Dendrimers are multivalent, well-defined, and highly branched macromolecules that tend to form a globular shape in solution.[1,2] The stepwise synthesis of dendrimers allows for a large degree of control over the molecular architecture and has enabled organic chemists for over 20 years to create dendrimers with different morphologies and properties.[3−8] Next to covalent modification, several noncovalent interactions such as hydrogen bonding,[9,10] electrostatic interactions,[11,12] hydrophobic interactions,[13−15] $\pi-\pi$ interactions,[16,17] or metal coordination[18−20] have been employed to obtain supramolecular dendritic architectures. As the strength of these interactions depends on external factors such as temperature, solvent polarity, or pH, an adaptive system is obtained that can be designed to change under specific stimuli. Moreover, decorating the periphery of dendrimers in a noncovalent fashion is particularly interesting as many biological processes occur via supramolecular interactions at surfaces.

Recently, our group developed a methodology to modify the periphery of dendrimers in a noncovalent manner.[21−29] Poly(propylene imine) dendrimers modified with urea-adamantyl end

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10.1021/ja052074m CCC: $30.25

groups can bind ureido-acetic acid guest molecules via electrostatic interactions and hydrogen bonding. The electrostatic interactions occur through an acid−base interaction between the carboxylic acid functionality of the guest and a tertiary amine of the dendrimer. Hydrogen bonding occurs between the urea-groups of the guest and host and should direct complexation to the periphery of the dendrimer. A major increase in binding strength can be achieved by increasing the acid strength of the guest molecules. This has been observed both in the gas phase[25] and in chloroform,[26,30] when the carboxylic acid group was replaced with a phosphonic or sulfonic acid group.

Although mass spectrometry and NMR spectroscopy have given useful information about the binding of these types of guests to dendrimers, specific information about the number of guests that bind to the host, the binding strength between guest and dendritic host and the exchange kinetics between free and bound guest in solution remains difficult to obtain. Overlap of the signals in ^{1}H NMR of guest and host hampers the analysis of several complexes. Ideally, we would like to have a general analytical methodology to investigate the binding of any guest to the dendrimer in any solvent. In this report, we present the results obtained by making use of ^{13}C NMR and ^{31}P NMR next to ^{1}H NMR to investigate the guest−host interactions of ^{13}C labeled carboxylic and ^{31}P phosphonic acid containing guest molecules in more detail. The principle of the methodology is investigated in detail for the fifth generation dendrimer.

Designing the System. In an apolar aprotic solvent like chloroform, an acid (HA) and a base (B) are usually in equilibrium with a tight ion pair. The formed anion (A^-) and cation (BH^+) are in close proximity of each other due to electrostatic attraction and hydrogen bonding (eq 1), and it is generally believed that no free ions are present in solution.[31−48]

$$A\text{-}H + B \rightleftharpoons A^{\ominus}\cdots H\text{-}B^{\oplus} \qquad (1)$$

It is known that both the ^{13}C chemical shift of carboxylic acids and the ^{31}P chemical shift of phosphonic acids are strongly dependent on the pH. In water, the ^{13}C chemical shift of carboxylic acids can shift over 5 ppm downfield when the acid is deprotonated and the carboxylate salt is formed.[49−53] For phosphonic acids, both upfield and downfield shifts in ^{31}P NMR have been reported upon deprotonation.[54−57] This inspired us to use ^{13}C NMR and ^{31}P NMR as a tool to investigate the binding of ureido-acetic acid and ureido-phosphonic acid type guests to urea-adamantyl dendrimers in chloroform. To make analysis easier and to prevent long measurement times, a ^{13}C label has been incorporated in the carbonyl group of the acid functionality of the ureido-acetic acid guest molecules.

For solubility reasons, guest **2** and **3** were synthesized and used. Complexation experiments of both guests have been performed to several dendritic hosts (**1a**−**1e**) (Scheme 1), and the experimental results in case of host **1e** were fitted to get an idea of the binding stoichiometry and the association constant. Exchange experiments have been performed in which both guest molecules were simultaneously added to the dendrimer host, to investigate the difference in binding strength between guest **2** and **3** in more detail.

Results

Covalent Synthesis. The starting material for the ^{13}C label in guest **2** was the commercially available 1-^{13}C-glycine (Scheme 2). Reaction with benzyl alcohol in toluene in the presence of *p*-toluenesulfonic acid resulted in the benzyl ester of 1-^{13}C-glycine as the *p*-toluenesulfonic acid salt (**4**). A Dean−Stark setup was used to azeotropically remove the water that is formed during the reaction. As a starting material for the oligoethylene glycol part of guest **2**, 3,4,5-tri(tetraethyleneoxy)-benzoic acid was used, which was synthesized according to a described procedure.[58] This acid was reacted with ethylchloroformate in THF with triethylamine to afford the mixed anhydride. This mixture was added to a solution of sodium azide in water, and extractions with dichloromethane resulted in the acyl azide derivative. The acyl azide could subsequently be converted to the isocyanate **7** via the Curtius rearrangement in

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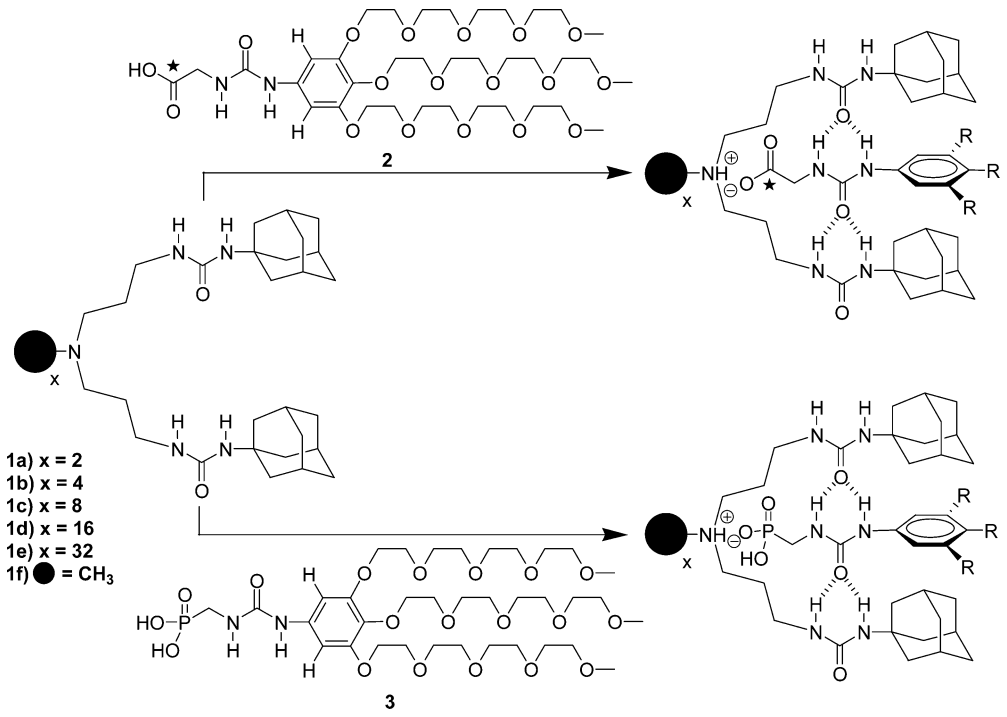
Scheme 1



refluxing toluene. The isocyanate was reacted with **4**, after triethylamine was added to liberate the free amine. Ester **8** could be purified using column chromatography. Finally the benzyl ester was converted to free acid **2** by catalytic hydrogenolysis with H_2 gas and Pd/C as a catalyst. Benzyl ester **8** has to be treated carefully. A first attempt to purify **8** by a basic workup with 1 M NaOH (aq) resulted in hydantoin **9**. When a saturated $NaHCO_3$ (aq) is used, this side reaction can be prevented. Though initially unwanted, hydantoin **9** was purified and analyzed and has been used for several control experiments. Phosphonic acid guest **3** was synthesized from phthalimide derivative **5**, which has been synthesized according to an adapted literature procedure.[59] Treatment with hydrazine resulted in amine **6**, which was immediately used for reaction with isocyanate **7**. Product **10** could be purified using column chromatography, and treatment with TFA to remove the *tert*-butyl groups resulted in pure **3**. All compounds were analyzed with ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, FT-IR spectroscopy and (high resolution) mass spectrometry.

Supramolecular Synthesis of the Complexes. All complexes were prepared in a similar fashion, in which the concentration of the guest was kept constant. For both guest **2** and **3**, 10 mg were weighed and an amount of dendrimer was added to obtain the desired guest/host ratio. The compounds were dissolved in 0.5 mL of $CDCl_3$ and shaken for 5 min. Before we investigated the complexation behavior of guest **2** and **3** with ^{13}C NMR and ^{31}P NMR, we have analyzed the complexation to dendrimer **1e** with several ^{1}H NMR techniques to ascertain that binding occurred. This was done for both guests on a sample with a guest/host ratio of 32. This is denoted as **1e**+$\mathbf{2}_{32}$ and **1e**+$\mathbf{3}_{32}$, and this notation is used for all guests and dendrimers throughout this paper. The observed changes in ^{1}H NMR upon addition of guest **2** and **3** to dendrimer **1e** (S7, S8) are in agreement with previously reported results.

If guest and host are bound, they should be in close proximity to each other. This has been investigated with ^{1}H−^{1}H-NOESY NMR spectroscopy. ^{1}H−^{1}H-NOESY spectra of **1e**+$\mathbf{2}_{32}$ and **1e**+$\mathbf{3}_{32}$ (Figure 1b and 1d) show cross-peaks between the oligoethylene glycol tails of the guests (in all spectra indicated as 2) and the adamantyl groups (signal 3) of the dendrimer, indicating that these protons are close to each other through space. Also a weak cross-peak is observed between the aromatic protons of guest **2** and **3** (signal 1) and the adamantyl signals of **1e**, which is an indication that the headgroup of the guest is in close proximity to the periphery of the host and is responsible for complexation, as has been investigated in earlier work.[27] This is also confirmed by ^{1}H−^{1}H-NOESY spectra that have been recorded for **1e**+$\mathbf{9}_{32}$ and **1e**+$\mathbf{10}_{32}$. In both cases no intermolecular NOE contacts were observed between the dendrimer and the guests, showing that no complexation occurs.

When a guest molecule binds to fifth generation dendrimer **1e**, its diffusion constant should change as the dendrimer has a much higher molecular weight and consequentially diffuses slower. Actually, if binding of a guest to **1e** is strong enough, both guest and host should diffuse with an equal rate as they belong to the same supramolecular aggregate. Therefore, ^{1}H-DOSY-NMR has been performed on several complexes. When samples **1e**+$\mathbf{9}_{32}$ and **1e**+$\mathbf{10}_{32}$ are analyzed with ^{1}H-DOSY-NMR, different apparent diffusion constants were found for guest and host (Figure 1). The apparent diffusion constants of hydantoin **9** and phosphonic ester **10** are higher than that of dendrimer **1e**, as they are smaller molecules that diffuse faster. These differences again support the idea that **9** and **10** do not interact with the dendrimer. However, this is not the case for guest molecules **2** and **3**. When samples **1e**+$\mathbf{2}_{32}$ and **1e**+$\mathbf{3}_{32}$

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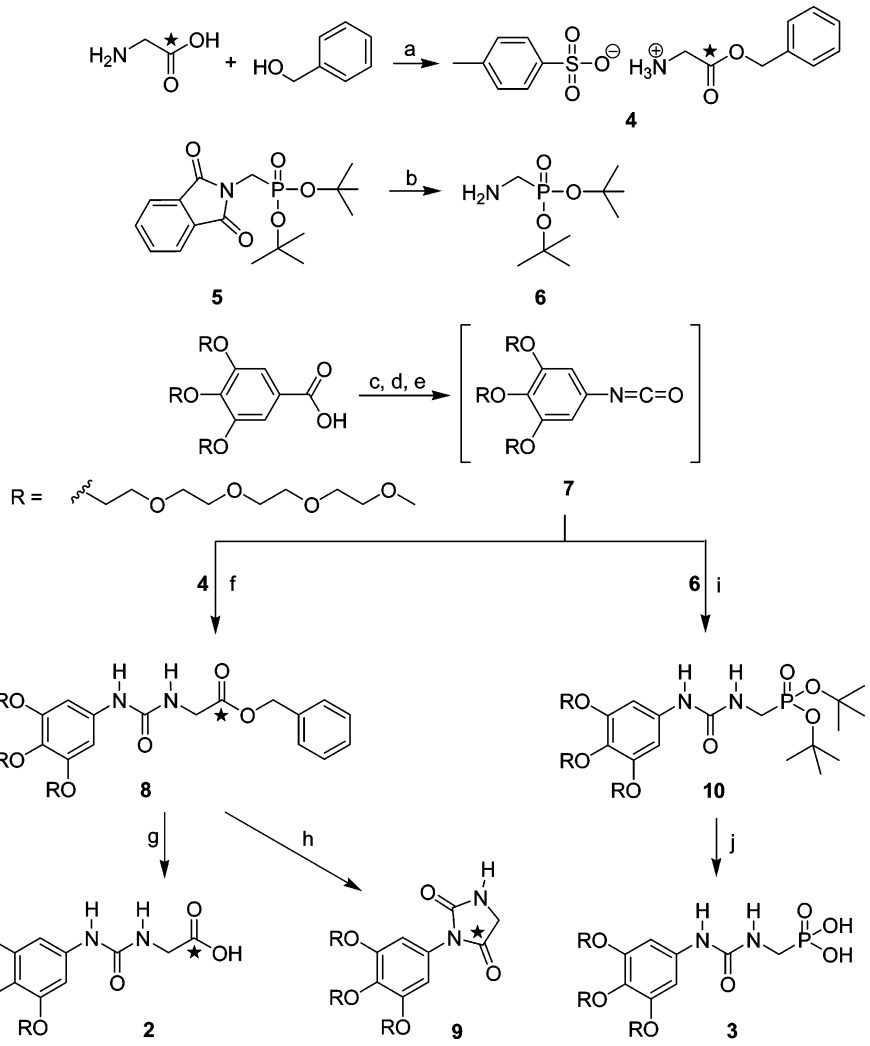
Scheme 2. Synthesis of Guest **2** and **3**[a]



[a] Reagents and Conditions: (a) *p*-toluenesulfonic acid, toluene; (b) H_2NNH_2, EtOH; (c) ethylchloroformate, triethylamine, THF; (d) NaN_3, H_2O; (e) toluene, reflux; (f) CH_2Cl_2, triethylamine; (g) *t*-BuOH, H_2O, H_2(g), Pd/C; (h) 1 M NaOH(aq); (i) CH_2Cl_2; (j) TFA, CH_2Cl_2.

are analyzed with ^{1}H-DOSY-NMR, the apparent diffusion constants of **2** and **3** are similar to host **1e**. This confirms that guest and host are bound to each other.

To exclude the possible formation of dendrimer−dendrimer aggregates next to dendrimer−guest complexes, dynamic light scattering experiments have been performed in nondeuterated chloroform. Measurements performed on dendritic host **1e** alone in chloroform and complexes with guest **2** and **3** in different guest/host ratios show one predominant process of small particles. The hydrodynamic radius (R_H) found for the sample with only dendrimer **1e** is 2.2 nm. This value is in good agreement with the dimensions of a single dendrimer.[3] For the different host/guest complexes R_H increases upon increasing guest/host ratio, with $R_H = 2.8$ nm for **1e**+$\mathbf{2}_{34}$ and $R_H = 3.0$ nm for **1e**+$\mathbf{3}_{32}$ (S11). The results also show that no dendrimer aggregation is taking place.

Complexation of Guest 2 to Dendrimer 1e Observed by ^{13}C NMR. Having obtained evidence that guest **2** binds to dendrimer **1e** in chloroform, we can analyze the complexation behavior with ^{13}C NMR. This has been performed by investigating how the ^{13}C chemical shift of the guest depends on the composition of the sample. The ^{13}C NMR spectrum of pure **2** in $CDCl_3$ shows a signal at 172.5 ppm, which corresponds to the carboxylic acid carbon (Figure 2).

For the free guest the line width of the peak at half-height is approximately 3 Hz. For the sample with a guest/host ratio of 8, the peak shifts downfield to 175.6 ppm and broadens slightly. The downfield shift is also observed when an excess of triethylamine is added to guest **2**, but not when hydantoin **9** is added to **1e** (S10). Clearly the shift is due to deprotonation of the acid. As in chloroform a tight ion pair is formed between an acid and a base, the downfield shift is a direct measure for complexation of the guest to the dendrimer. When we look at the results for increasing guest/host ratio, two trends are observed. First of all, the carbonyl signal shifts upfield. The change in chemical shift is small when **1e**+$\mathbf{2}_{8}$ is compared to **1e**+$\mathbf{2}_{16}$ but is clearly present when the guest/host ratio (from now on referred to as *G*/*H*) is further increased to 32, 48, 64, 96, and 128. Furthermore, the signal broadens when *G*/*H* increases until the value of 64 is reached. A further increase of *G*/*H* results in a sharper signal again. We propose that the results can be interpreted in the following manner. Only one peak is observed, and this indicates that the signal is an average signal for both free and bound guest. This shows that exchange

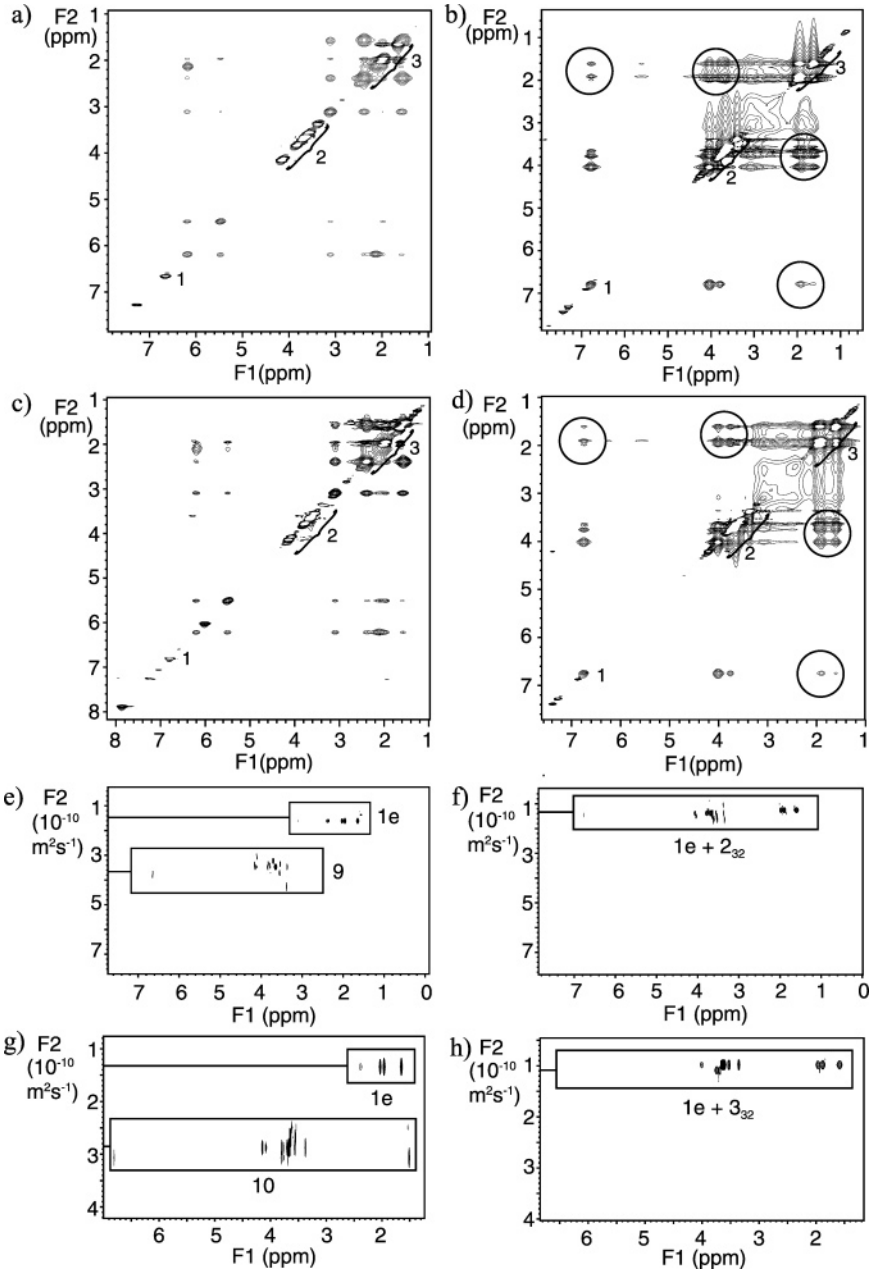


Figure 1. ^{1}H−^{1}H-NOESY spectra (a−d) and ^{1}H-DOSY spectra (e−h) recorded for (a) **1e**+$\mathbf{9}_{32}$, (b) **1e**+$\mathbf{2}_{32}$, (c) **1e**+$\mathbf{10}_{32}$, (d) **1e**+$\mathbf{3}_{32}$, (e) **1e**+$\mathbf{9}_{32}$, (f) **1e**+$\mathbf{2}_{32}$, (g) **1e**+$\mathbf{10}_{32}$, and (h) **1e**+$\mathbf{3}_{32}$ in $CDCl_3$ at 25°C.

between free and bound guest is fast on the spectral time scale.[60] For increasing *G*/*H* the signal shifts back in the direction of the free guest, indicating that the amount of unbound guest increases.

Two effects govern the broadening of the signal. Complexation to a large macromolecule results in a decrease in mobility of the headgroup of the guest. This causes an increase in T_2 relaxation and consequentially in broadening of the signal. However, this is not very plausible as the peak becomes sharper again after 64 equiv of guest. When the exchange rate between free and bound guest comes close to the spectral time scale, broadening can also occur due to coalescence.[61] In this case the relative amount of free and bound guest should influence the peak broadness, and this is exactly what happens when *G*/*H* is increased. When we start at **1e**+$\mathbf{2}_8$, bound guest is the predominant species. When the relative amount of **2** is increased more unbound guest starts to become present, resulting in a broader signal. At **1e**+$\mathbf{2}_{64}$ the line width is highest, which suggests that the amount of free and bound guest is approximately equal in this case. A further increase in *G*/*H* results in an excess of unbound guest and thus in a sharper signal. As spectral line shape perturbations are dependent on the magnetic field, complex **1e**+$\mathbf{2}_{64}$ was analyzed at different field strengths (Figure 3). This resulted in a sharper signal at lower field strength and is an indication that coalescence is indeed taking place. This means that although T_2 relaxation and coalescence

(60) The spectral time scale represents the inverse width of the NMR spectrum, measured in frequency units. The chemical shift difference between free and bound guest corresponds to 390 Hz (3.1 ppm), which results in a spectral time scale of 2.6 ms. Levitt, M. H.; Spin Dynamics, John Wiley & Sons: 2002; p 485.

(61) Sandström, J. *Dynamic NMR Spectroscopy*; Academic Press, 1982.

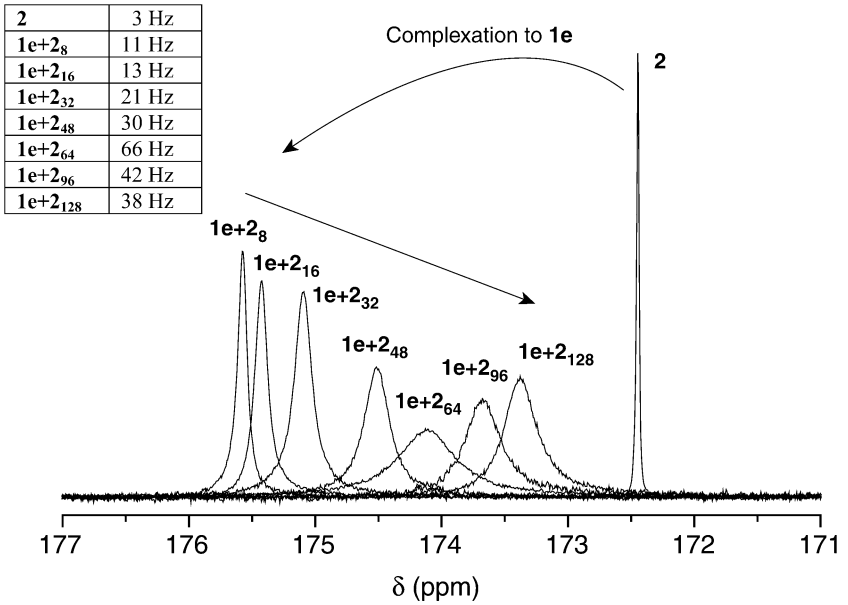


Figure 2. ^{13}C NMR spectra of different guest/host ratios of **2** and **1e**. The concentration of guest is kept constant at 2.46×10^{-2} M. The line widths of the peaks are indicated in the inset. The intensities are not normalized.

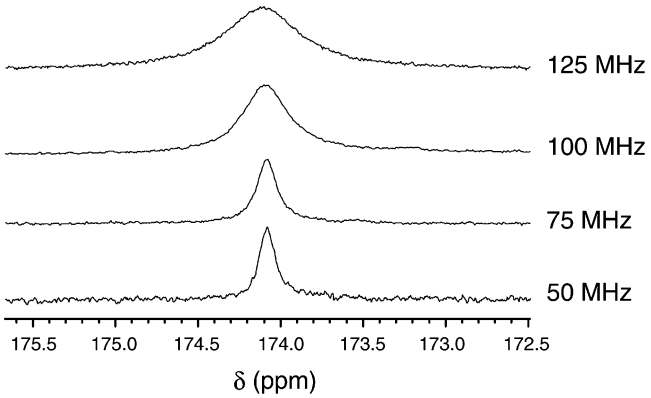


Figure 3. Dependency of the signal of **1e**+2$_{64}$ on the magnetic field. The signal becomes more narrow when the field strength of the spectrometer decreases.

both play a role in line broadening, coalescence is the major contributor of the two.

When guest **2** is added to lower generation dendrimers, the same trend in chemical shift dependency is observed when *G*/*H* is increased, and complexation can be followed in exactly the same way. Apparently, the method is applicable to all dendrimer generations, but this is beyond the scope of this paper. The broadening of the signal upon increasing *G*/*H* has not been observed for lower generation dendrimers, indicating that the exchange kinetics are different for these dendrimers.

Complexation of Guest 3 to 1e Observed by ^{31}P NMR. In a manner similar to guest **2**, we have investigated the complexation of guest **3** to dendrimer **1e** using ^{31}P NMR (Figure 4). Free guest **3** gives a sharp peak at 22.5 ppm in $CDCl_3$. This peak completely disappears when 16 (not depicted) or 32 guests are added to **1e** and is a broad signal at 16 ppm. When *G*/*H* is increased to 56, no big changes occur. At *G*/*H* = 64, two distinct signals start to become present: a narrow peak at 21 ppm and the very broad signal at 17 ppm. A further increase of *G*/*H* shows that the peak at 21 ppm becomes more intense and shifts a little downfield.

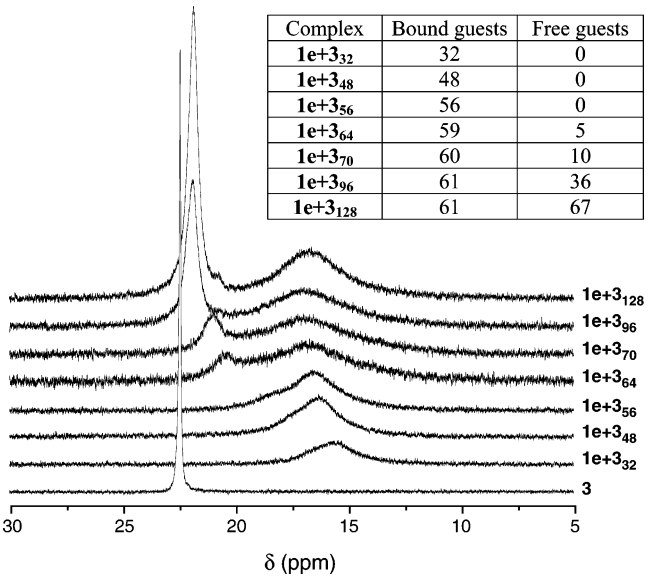


Figure 4. ^{31}P NMR spectra of different *G*/*H* ratios of **3** and **1e**. The concentration of guest is kept constant at 2.36×10^{-2} M. The deconvolution results are depicted in the table.

The broad peak remains present. These results show completely different trends than the observations for complexation of guest **2** to **1e** in ^{13}C NMR. The chemical shift of the phosphonic acid group shifts upfield instead of downfield due to deprotonation. It is known that the chemical shift of phosphonic acid derivatives strongly depends on the pH. Both upfield and downfield shifts have been reported for different compounds. In our case, deprotonation of the phosphonic acid and the formation of a tight ion pair apparently results in an upfield shift, and this has also been observed when an excess of triethylamine was added to guest **3** (S10). We observe two separate signals when *G*/*H* is increased, indicating that we now have slow exchange on the spectral time scale.[62] The signal of the free guest is located around 22 ppm and relatively sharp,

(62) The chemical shift difference of 1315 Hz corresponds to a spectral time scale of 0.76 ms.

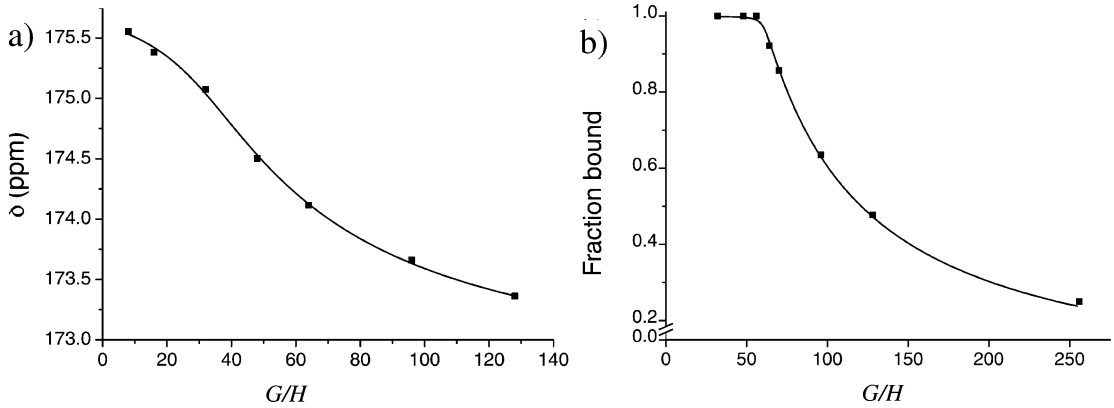


Figure 5. (a) Chemical shift values of guest **2** as a function of *G*/*H* (points). The solid line represents the fit. (b) The fraction of bound guest **3** to host **1e** as a function of the amount of *G*/*H*. The solid line represents the fit.

and the signal of the bound guest is located around 16 ppm and very broad. It is possible to deconvolute the two signals and to directly determine the ratio of free and bound guest. This has been done for *G*/*H* = 64, 70, 96, and 128 (Figure 4, table) and shows that the amount of bound guest remains fairly constant around 60 equiv. The amount of free guest increases from 5 to 67 guests. The numbers suggest that approximately 60 guests can bind to **1e**. This amount comes very close to the 62 tertiary amines that are present inside the dendrimer.

The signal of the bound guest is very broad. Again, both T_2 relaxation and coalescence can cause this broadening. However, as we have slow exchange on the spectal time scale coalescence is less likely to be the major contributor. This was confirmed by the observation that measurements at other magnetic field strengths did not show a change in the spectrum. To test whether T_2 relaxation could be the reason for the severe broadening, we examined the phosphorus NMR spectra of guest **3** added to the lower generation dendrimers **1c**, **1a**, and pincer molecule **1f** (S10). In all cases the stoichiometry of one guest per two endgroups was maintained. The spectrum of **1f**+$\mathbf{3_1}$ shows a downfield shift of the phosphorus signal, but no big change in line width, in contrast to **1a**+$\mathbf{3_2}$, **1c**+$\mathbf{3_8}$, and **1e**+$\mathbf{3_{32}}$. Clearly the line width increases upon increasing size of the dendrimer host. A decrease in mobility of the headgroup of the guest due to complexation to such a large molecule could explain the results.

For lower generation hosts a similar chemical shift dependency is observed for different values of *G*/*H*, meaning that the signal of the guest shifts upfield upon deprotonation. However, the exchange kinetics also depend on the dendrimer generation and are faster for lower generation dendrimers as the two different signals for free and bound guest are not always observed. For example, different amounts of guest added to pincer **1f** always results in one signal (S11). So, exchange is faster for the pincer. In this case, the broadening is most likely due to coalescence again.

Quantitative Analysis of Binding of Guest 2 and 3 to 1e. The obtained chemical shift values can be used to analyze the binding of guest **2** to dendrimer **1e** in a quantitative manner. The observed chemical shift (δ) can be seen as a mixture of free and bound (deprotonated) guest since we are in a fast exchange situation, and can be represented as

$$\delta = p_b\delta_b + p_f\delta_f$$

where p_b = fraction of bound guest, δ_b = chemical shift of the bound guest, p_f = fraction of free guest, and δ_f = chemical shift of the free guest.

The chemical shift of the free guest is known (δ_f = 172.5 ppm), but that of the bound guest is not. However, most likely it is very close to the 175.6 ppm obtained for **1e**+$\mathbf{2_8}$. We assume there is no cooperativity: all binding sites in the host are identical and do not influence each other. When a 1:1 binding of guest and binding site is assumed, an equation can be derived that gives the fraction of bound guest as a function of *G*/*H* (S12). Fitting this equation to the measured chemical shift values with a nonlinear least-squares fit (Figure 5a) gives us values for the number of binding sites (n), the association constant for carboxylic acid guest **2** (K_C), and the chemical shift of the bound guest (δ_b). We find

$$n = 41 \pm 2$$

$$\delta_b = 175.61 \text{ ppm}$$

$$K_C = 400 \pm 95 \text{ M}^{-1}$$

The obtained association constant is in the same order of magnitude as the association constant found from fluorescence measurements on model host compounds.[30]

For guest **3** we followed the same reasoning as that for guest **2**, but now we have the fraction of bound guest (p_b) directly available from the deconvolution of the spectra. We can directly fit p_b to the equivalents of guest added (Figure 5b). We then find

$$n = 61 \pm 1$$

$$K_P = (4 \pm 3) \times 10^4 \text{ M}^{-1}$$

The fit shows a higher association constant for guest **3** than for guest **2**. This is in agreement with earlier results that show a stronger binding for the phosphonic acid guest. The results are different from the traditional model as more than 32 guests can apparently bind to the dendrimer. The discrimination between guests bound at the periphery or the interior of the dendrimer cannot be made based on ^{31}P NMR.

Exchange Experiments. As we are now able to follow complexation of guest **2** and **3** with two orthogonal NMR techniques, we investigated what happens when both guests are simultaneously added to dendrimer **1e**. The amount of carboxylic

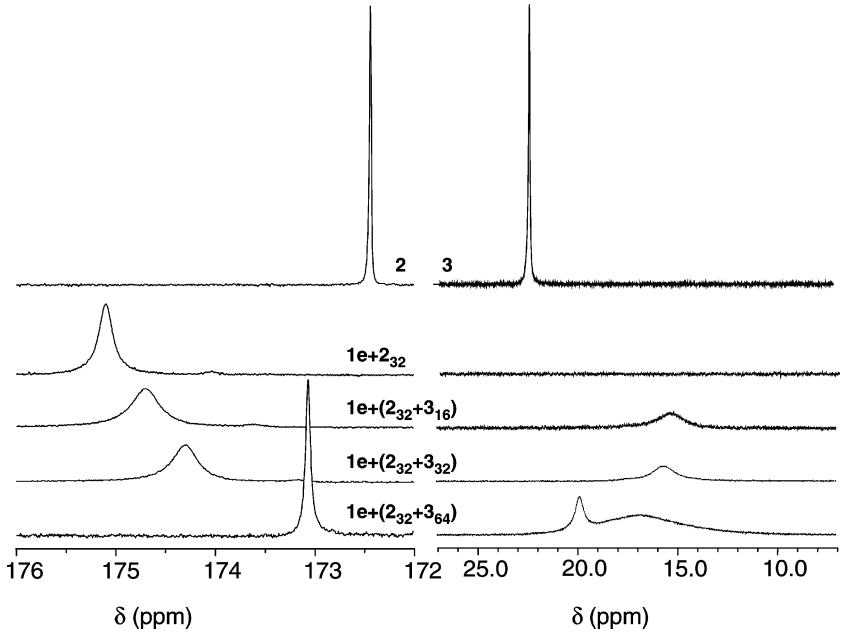


Figure 6. Influence of the addition of **3** to **1e**+**2**$_{32}$ observed in ^{13}C NMR (left) and ^{31}P NMR (right). The concentration of guest **2** was kept constant at 2.46 $\times$ 10^{-2} M.

acid guest **2** was kept constant at $G/H = 32$. To this sample was added 16, 32, and 64 equiv of phosphonic acid guest **3**, and the results were monitored by both ^{13}C NMR and ^{31}P NMR (Figure 6).

When 32 equiv of **2** are added to **1e**, the characteristic downfield shift to 175.1 ppm is observed in ^{13}C NMR. As **3** is not present, the ^{31}P NMR spectrum does not show anything. When 16 equiv of **3** are added to this sample, resulting in **1e**+-**(2**$_{32}$+**3**$_{16}$**)**, an upfield shift in ^{13}C NMR is observed to 174.7 ppm, indicating that **2** dissociates partially from the dendrimer. When **1e**+**(2**$_{32}$+**3**$_{16}$**)** is analyzed with ^{31}P NMR, a broad signal at 16 ppm is observed, indicating that all of phosphonic acid guest **3** is bound to **1e**. An increase of the amount of **3** to **1e**+-**(2**$_{32}$+**3**$_{32}$**)** results in an upfield shift to 174.3 ppm in ^{13}C NMR, so **2** dissociates further from **1e** due to **3**. In ^{31}P NMR we still observe complete complexation of phosphonic acid guest **3** to the dendrimer. When the amount of guest **3** is increased to **1e**+-**(2**$_{32}$+**3**$_{64}$**)** the signal of guest **2** in ^{13}C NMR shifts to 173.1 ppm. This is much further upfield than observed for **1e**+**2**$_{96}$ or **1e**+**2**$_{128}$, which give values of 173.7 and 173.4 ppm, respectively (Figure 2). Also the line width of the peak, 10 Hz, is much lower than that for **1e**+**2**$_{96}$ or **1e**+**2**$_{128}$ and indicates the amount of bound **2** is lower. In ^{31}P NMR, we now start to observe two signals, so some free **3** is present.

Discussion

The results regarding the binding strength and binding stoichiometry of guest **2** and **3** to urea-adamantyl dendrimer **1e** have some major implications for our molecular picture. With the availability of association constant and number of binding sites for both guests, we can calculate the amount of free and bound guest for every guest/host ratio (S13, S14) at a certain concentration. The relatively low association constant of guest **2** has the consequence that when 32 equiv of guest **2** are added to **1e**, only 26 guests are bound *on average* and also free **2** is present in solution. The term on average is deliberately used to indicate that, as expected, we do not have a monodisperse supramolecular aggregate in solution but a distribution in the number of bound guests. When the amount of **2** is further increased, more guests will be bound on average, but the amount of free guest increases significantly too. For guest **3** the same statistical rules apply, also resulting in a polydisperse supramolecular aggregate. However, below $G/H = 60$ virtually all guests are bound to the dendrimer due to the higher association constant. An almost monodisperse supramolecular aggregate can be obtained for guest **3**, but only at high guest/host ratios when a lot of free guest is present (S14). Obviously, the number of bound guests is based on a certain concentration and alters with changes in the concentration.

To visualize the polydispersity and the ratio of bound and free guest more clearly, two distributions have been depicted in Figure 7. Although both graphs are based on simple statistical calculations, we can use them to expatiate the often too simplified view of a multicomponent aggregate, in which it is presented as a monodisperse entity with complete binding.

The binding stoichiometries of guest **2** and **3** obtained with NMR are in agreement with dynamic light scattering experiments. The absolute scattering intensity R_{vv}/c, normalized to the concentration of dendrimer at large probing lengths ($q \approx 0$), has been determined from several guest−host complexes. It is proportional to the molar mass of the probed aggregate in solution. This quantity is plotted against the number of guests that are bound to the dendrimer based on the NMR results. A monotonic, almost linear increase is observed for both guest−host systems (Figure 8). This confirms the obtained relations between the number of added guest and the number of bound and free guest found for guests **2** and **3** to dendrimer **1e** by NMR. According to the fitted NMR data, 55 equiv of guest **2** added to dendrimer **1e** should result in 32 equiv bound. For guest **3**, addition of 32 equiv to dendrimer **1e** should also give 32 equiv bound. These points also overlap in the graph.

The differences between guest **2** and **3** are also reflected in the exchange experiment. If we assume that the binding equilibria are independent for **2** and **3**, the amount of free and

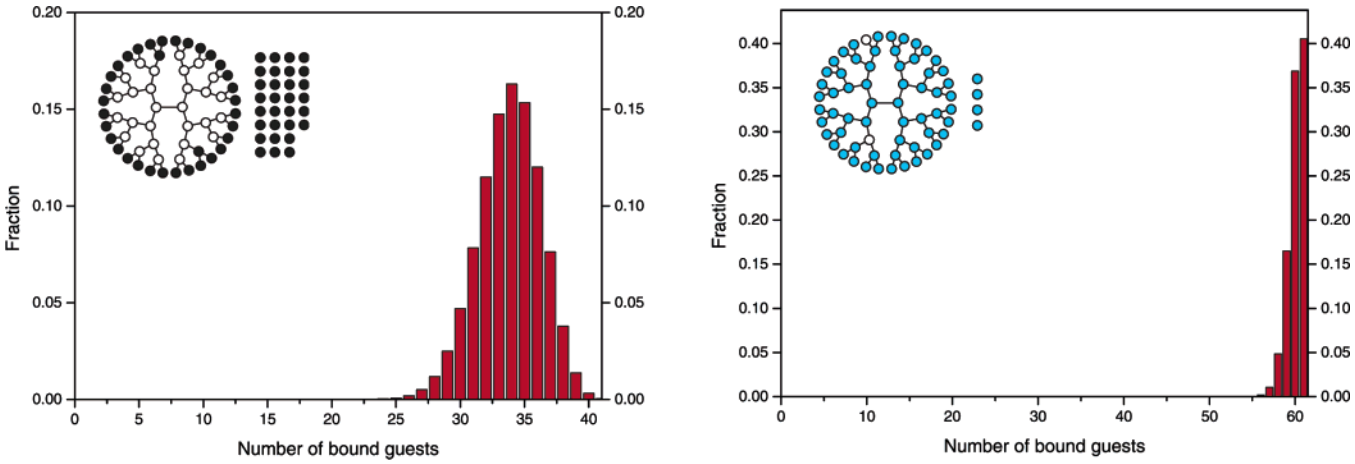


Figure 7. Calculated distributions of the number of bound guests **2** (left) or **3** (right) to dendrimer **1e** in the case of noncooperative binding. In both cases the guest/host ratio corresponds to 64. The concentration of **2** and **3** is 2.46×10^{-2} M and 2.36×10^{-2} M respectively.

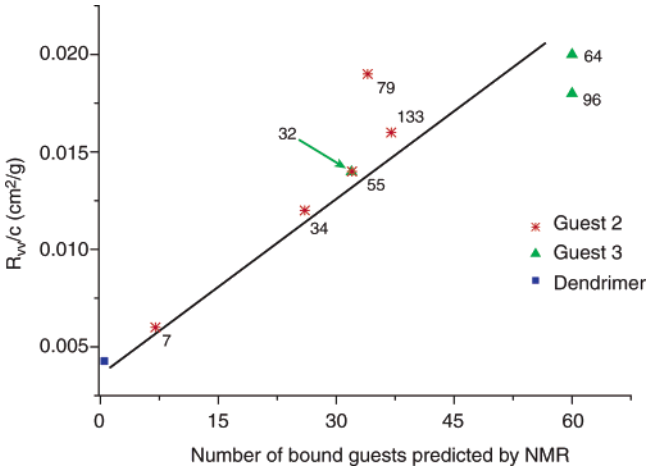


Figure 8. Absolute scattering intensity R_{vv}/c plotted against the number of bound guests **2** and **3** according to the fitted NMR data. The numbers in the graph represent the guest/host ratio of the sample. With the number of binding sites and the binding constant for guest **2** and **3** from NMR, the number of bound guests have been calculated and put on the *x*-axis. The line is to guide the eye.

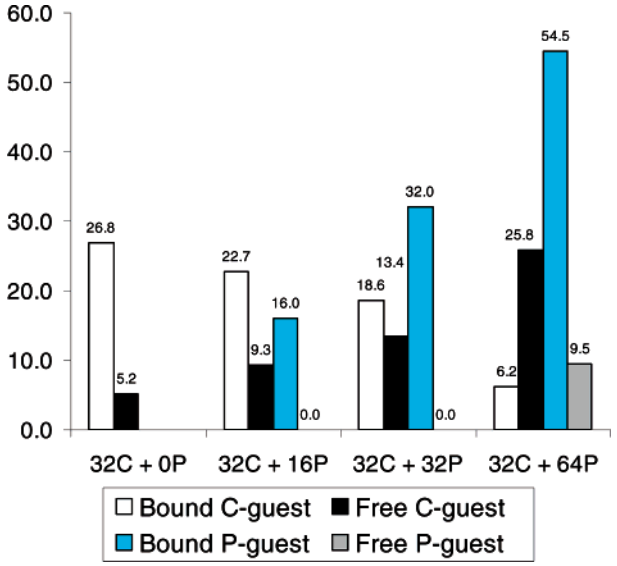


Figure 9. Amounts of free and bound guests **2** and **3** as obtained from the exchange experiment depicted in Figure 6.

bound carboxylic acid guest **2** can be determined from the chemical shift, and the amount of free and bound phosphonic acid guest **3** can be determined from deconvolution of the spectra (Figure 9). The obtained numbers of bound and free guest **2** and **3** show that initially guest **2** and **3** coexist on the dendrimer. Although the association constant of guest **3** is higher, it does not compete severely with carboxylic acid guest **2** as guest **3** has 61 possible sites to bind to. However, when 64 equiv of **3** are added to **1e**+$\mathbf{2}_{32}$, **3** competes with all the binding sites of guest **2**, and this results in almost complete dissociation of **2** from **1e**. The amounts of free and bound **2** and **3** that are found in the mixing experiment are in accordance with the expected values based on the obtained association constants. From these experiments we can conclude that it is possible to make mixed aggregates and that phosphonic acid guest **3** can expel carboxylic acid guest **2** from the dendrimer.

Knowing all of these details, we can say that the number of guests that bind to the dendrimer is mainly governed by the acid−base interaction. When the acid strength is increased, the amount of guests that bind to the dendrimer and the binding strength increases similarly. The hydrogen bonds most likely help to direct and strengthen the binding to the periphery of the dendrimer, as previous ^{1}H−^{1}H-NOESY experiments have shown that carboxylic acid based guest molecules are mainly located at the periphery of the dendrimer. However, we must conclude that as more than 32 equiv of guest **2** can bind to the dendrimer, some guests should be bound to the inner tertiary amines of the dendrimer. This does not exclude the formation of hydrogen bonds with the dendrimer, as its structure is highly flexible and certainly not always completely stretched, but implies a more complex mode of binding than the initial "pincer model" that has been presented (Introduction).

It is important to stress that the association constant 400 M^{-1} obtained for guest **2** is not general for every carboxylic acid guest. For a cyanobiphenyl-containing guest[21] the association constant was estimated to be 10^5 M^{-1} in chloroform. The discrepancy in association constant is caused by a difference in solubility. The cyanobiphenyl guest has a low solubility in chloroform but can be solubilized by complexation to the dendrimer. Therefore the equilibrium (eq 1) is shifted in the direction of the complex, resulting in a higher apparent association constant.[31] Clearly the apparent association constant can be influenced over several orders of magnitude by the

solubility of guest and complex, as has been nicely demonstrated by Gillies and Fréchet.[29]

For guest **3** we have observed that it can bind to virtually all tertiary amines, indicating that the acid−base interaction dominates the binding to the host. The urea groups of guest **3** might still help to direct binding to the peripheral tertiary amines of the dendrimer, but this effect is probably weaker than for guest **2**. Actually, based on our experiments we cannot discriminate between binding to the peripheral tertiary amines and binding to the amines further inside the dendrimer. Especially for guest **3**, Figure 10 gives a more realistic representation for the binding to dendrimer **1e**. Currently we are making use of molecular simulations and crystal structures to get detailed information on the way the guest molecules bind to the dendrimer in three dimensions.

Conclusions

A new methodology has been introduced to get insights in the binding of carboxylic and phosphonic acid type guest molecules to dendrimers by making use of ^{13}C NMR and ^{31}P NMR. The method is generally applicable for any type of guest and host in chloroform and gives information about binding strength, binding stoichiometry, and binding dynamics. In this way it is possible to analyze multicomponent aggregates of different guest molecules simultaneously bound to the dendrimer in great detail. With simple statistical calculations, we have used the results to expatiate the often too simplified view of a multivalent aggregate, in which it is presented as a monodisperse entity with complete binding.

Acknowledgment. The authors would like to thank Mrs. Antje Larsen for technical assistance of the DLS measurements and acknowledge financial support of the EU (HPRN-CT-2000-2003) and grants from the department of Biomedical Engineering of the Eindhoven University of Technology and the Council for Chemical Science of The Netherlands Organization for Science Research (CW-NWO).

Supporting Information Available: Experimental details for the synthesis of compounds **2**, **3**, **4**, **8**, **9**, and **10**. ^{1}H NMR spectra for the samples **1e**+$\mathbf{2}_{32}$, **1e**+$\mathbf{3}_{32}$, **1e**+$\mathbf{9}_{32}$, and **1e**+$\mathbf{10}_{32}$, ^{13}C NMR spectra of samples **1e** + $\mathbf{9}_{32}$ and **2** in $CDCl_3$ with an excess of triethylamine, and ^{31}P NMR spectra of samples **1e** + $\mathbf{10}_{32}$, **1f**+$\mathbf{3}_{1}$, **1f**+$\mathbf{3}_{2}$, **1f**+$\mathbf{3}_{4}$, **1a**+$\mathbf{3}_{2}$, **1c**+$\mathbf{3}_{8}$, and **3** with an excess of triethylamine. Dynamic light scattering experiments of complexes of **1e** with **2** and **3** with different guest/host ratios. Details on the equations derived for the quantitative analysis of the binding of **2** and **3** to **1e**. Calculations of the fractions of bound and free guest for several *G*/*H* values and the corresponding distributions. This material is available free of charge via the Internet at http://pubs.acs.org.

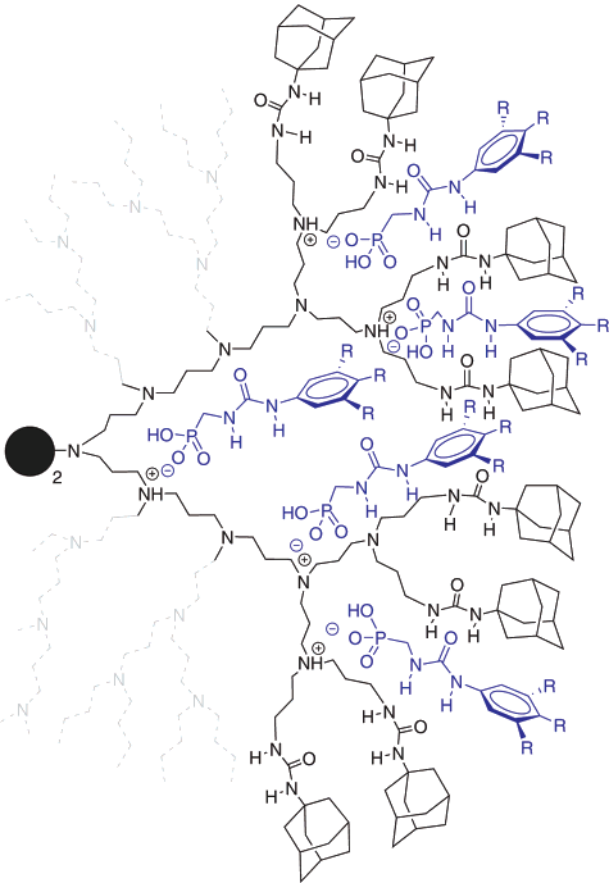


Figure 10. Representation for the binding of guests to urea-adamantyl dendrimers that shows that guest molecules (especially with phosphonic acid headgroups) can also bind to the inner tertiary amines of the dendrimer. Only a part of the dendrimer structure is depicted. The hydrogen bonds between guest and host have been omitted for clarity.

JA052074M