Guest–Host Chemistry with Dendrimers: Stable Polymer Assemblies by Rational Design

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ABSTRACT: A new type of guest has been designed and synthesized for the exo-type supramolecular functionalization of adamantyl-urea-terminated poly(propylene imine) dendrimers. This new type of guest motif features a uriedo methane sulfonic acid moiety that binds very selectively to the surfaces of dendrimers via a combination of noncovalent interactions forming well-defined complexes. The guest–host properties have been examined for a fifth-generation adamantyl-urea-functionalized poly(propylene imine) dendrimer capable of binding 32 guest molecules and for a model host molecule that can bind only one guest molecule. The guest–host chemistry has been studied with ^{1}H NMR spectroscopy, nuclear Overhauser enhancement spectroscopy NMR spectroscopy, T_1-relaxation NMR experiments, and IR spectroscopy. The 1:32 ratio with the dendrimer has been confirmed unambiguously from ^{1}H NMR spectra of the complex after size exclusion chromatography. Competition experiments with guests bearing a carboxylic acid instead of a sulfonic acid in the binding motif have demonstrated that the sulfonic acid has superior binding strength. Also, the importance of a combination of noncovalent interactions has been shown via competition experiments with a guest lacking the uriedo moiety. © 2004 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 42: 3792–3799, 2004

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INTRODUCTION

Guest–host chemistry with dendrimers is an area of dendrimer chemistry that has attracted the attention of several research teams around the world in recent years.[1–10] For guest–host systems in which the dendrimer acts as the host, two main types of systems have been defined: endosystems, in which the guest is located inside the dendrimer, and exosystems, in which the guest is located at the periphery of the dendrimer.[2,6] Maciejewski[11] and de Gennes and Hervet[12] suggested the existence of empty voids inside the three-dimensional structures of dendrimers, and so far guest–host chemistry with dendrimers has mainly been concerned with endo-type complexation. Typically, the guests have been different types of dye molecules or various organic and inorganic ions.[13–18]

The perspectives in exocomplexation are wide, ranging from model systems for multivalent biological systems to dynamic combinatorial libraries and to the construction of large supramolecu-

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lar nanoscale aggregates.[2,6] Some examples of exocomplexes have been presented in the literature, and they often rely solely on hydrogen bonding, metal–ligand bonding, or electrostatic interactions. Astruc and coworkers[19,20] prepared selective anion binders for ions such as $H_2PO_4^{2-}$ and Cl^- with ferrocene-modified dendrimers. Crooks et al.[21] prepared inversed micelles based on electrostatic interactions on the surfaces of dendrimers and compared them with covalently bound dendrimer-based inverse micelles. Catalytic metal complexes have been prepared on the surfaces of dendrimers with known scaffolds as binding sites; Breinbauer and Jacobsen's[22] salen complexes are representative examples.

Previously, our group introduced a new guest–host motif for the exocomplexation of poly(propylene imine) dendrimers that relies on a combination of supramolecular interactions.[23–26] This design is outlined in Figure 1, where *X* in the original system is a carboxylic acid. The adamantyl-urea-functionalized poly(propylene imine) dendrimer (Fig. 1) serves as a multivalent host for urea acetic acid guest molecules in a very selective manner, enabling the isolation of well-defined complexes with one guest per host motif at the periphery of the dendrimer. The complexation is due to a combination of multivalent hydrogen bonding between the urea parts of the guests and the host and to an electrostatic interaction between the acidic part of the guest and the tertiary amine in the host moiety. It has also been demonstrated that this type of dendrimer can serve as a multivalent host for the C terminus of small peptides; this expands the perspective of the use of dendrimers as drug carriers via exocomplexation.[25] Through changes in the adamantyl-urea binding motif on the dendrimer to a adamantylthiourea moiety, it has been proven possible to increase the binding affinity slightly, as demonstrated by the use of isothermal calorimetry.[24] Still, the association constants are in the range of 10^4 M^{-1} (comparable to the values obtained for the original design) in $CHCl_3$, and the complexes can still be dissociated by the addition of a competing solvent such as methanol. Recently, the acid strength of the guest has been increased, and urea-phosphonic acid molecules bind significantly more strongly than the corresponding molecules of urea acetic acid.[27]

In this contribution, we demonstrate that the binding strength of the guest–host complex can be further increased through changes in the design of the guest molecule. The structures of the guest molecules are shown in Figure 2, and the change in the design is achieved through a substitution of the carboxylic acid part from the guest motif with a sulfonic acid, so that we go from a moiety of a urea acetic acid [(3-benzyl-ureido)-acetic acid (**2**)] to a urea methane sulfonic acid [pyridinium (3-benzyl-ureido)-methane sulfonate (**1**); $X = SO_3^-$ in Fig. 1]. This change in design not only changes the acid strength but also changes the geometry of the acidic part of the guest from planar to tetrahedral. By this change in design, we achieve higher binding affinities than those of the guest containing the carboxylic acid. Also, the binding properties of a simple methane sulfonic acid [pyridinium methane sulfonate (**3**)] have been studied to verify that the binding of the new guest motif is due to a combination of effects (hydrogen bonding and electrostatic effect) and is not just an effect of the acid strength. The sulfonic acid guests have been synthesized as their pyridinium salts to achieve an acidic moiety that has acidity comparable to that of an aliphatic carboxylic acid. The binding properties have been studied for both a fifth-generation dendrimer (**5**, Fig. 3) and a simple model compound that only contains one host motif (**6**, Fig. 3).

EXPERIMENTAL

All starting materials were obtained from commercial suppliers and used as received. Analytical thin-layer chromatography (TLC) was performed on Kieselgel F-254 precoated silica plates. Column chromatography was carried out on Merck 60 silica gel (70–230 mesh). Preparative size exclusion chromatography was performed on Bio-Rad S-X1 Bio-Beads swollen in CH_2Cl_2. The $CDCl_3$ used for the NMR titrations, T_1-relaxation (the spin-lattice relaxation time) experiments, and ^1H–^1H nuclear Overhauser enhancement spectroscopy (NOESY) experiments was filtered through basic Al_2O_3 before it was used. All standard ^1H NMR and ^{13}C NMR spectra were recorded on a 300-MHz NMR instrument (Varian Gemini; 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) or a 400-MHz NMR instrument (Varian Mercury; 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). The NMR relaxation time experiments and the two-dimensional ^1H–^1H NOESY NMR experiments were carried out on a Varian Inova 500 spectrometer operated at 500.618 MHz and equipped with a 500 SW/PFG 5-mm probe from Varian. Proton chemical shifts are reported in

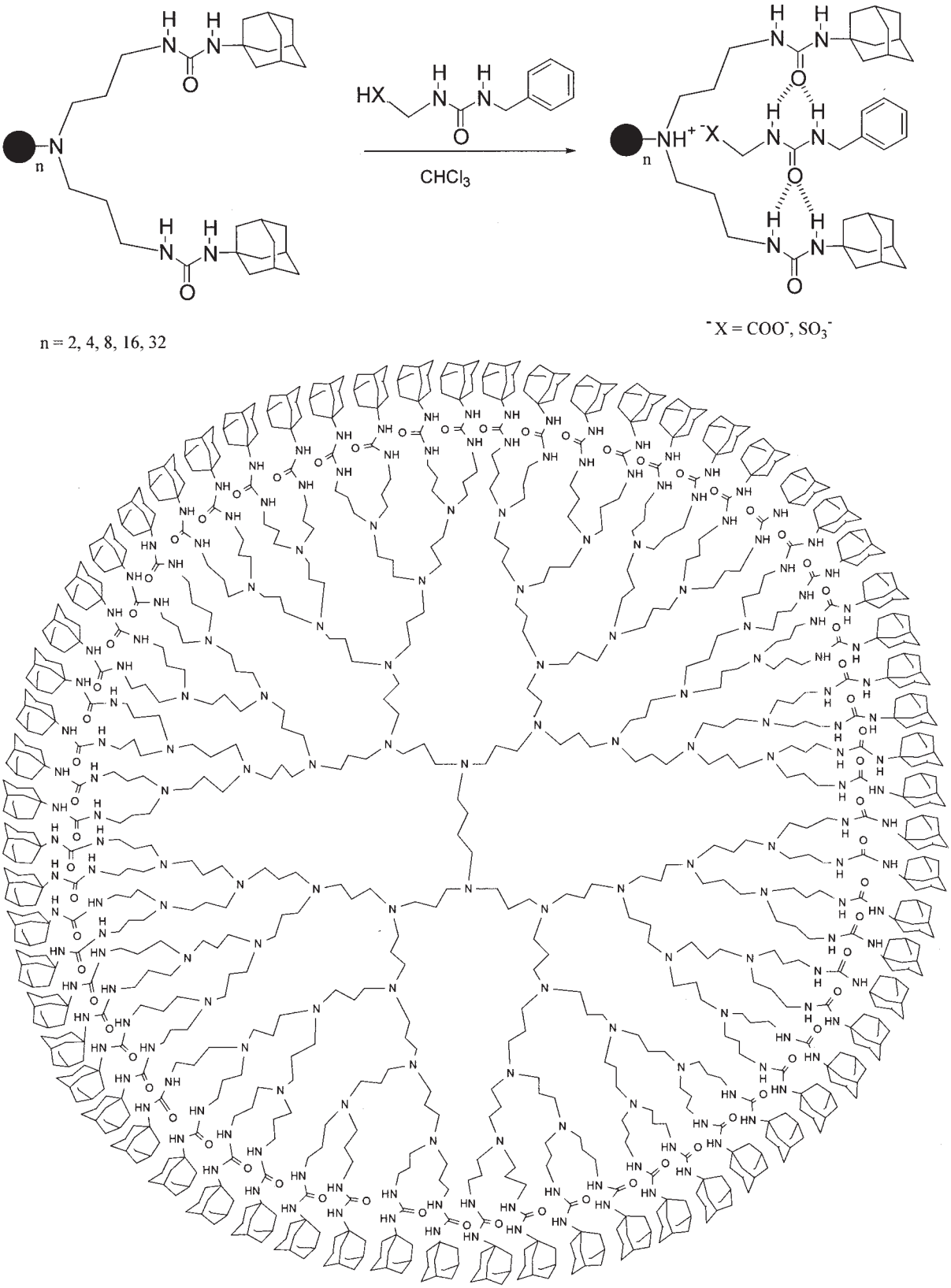
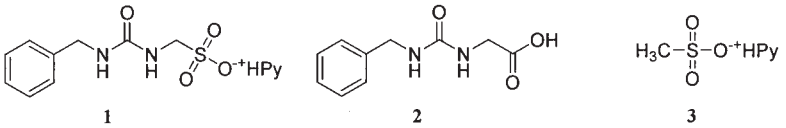


Figure 1. Schematic illustration of the guest–host system and the structure of the full fifth-generation adamantyl-urea-functionalized poly(propylene imine) dendrimer. With black-ball notation, *n* illustrates the generation of the dendrimer. When *n* is 32, it illustrates the fifth-generation dendrimer.

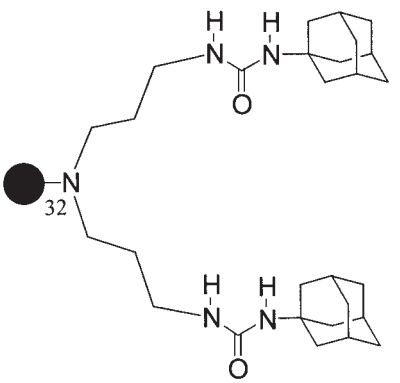


1 2 3

Figure 2. Guest molecules studied in this work.

parts per million downfield from tetramethylsilane (TMS), and carbon chemical shifts are reported in parts per million downfield from TMS, with the resonance of the deuterated solvent as an internal standard. IR spectra were measured on a PerkinElmer Spectrum One attenuated total reflection/Fourier transform infrared machine. The elemental analyses were performed on a PerkinElmer Series II 2400 instrument. Melting points were measured on a Büchi B-140 apparatus and are uncorrected. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-HX 110 A tandem mass spectrometer in either positive or negative ion mode with *m*-nitrobenzyl alcohol (*m*-NBA) as the matrix.

The synthesis of the new sulfonic acid guest (**1**) as its pyridinium salt and the carboxylic acid analogue (**2**) is outlined in Scheme 1. **3**,[28] the modified fifth-generation dendrimer **5**, and the model compound **6** were synthesized according to literature procedures.[23]



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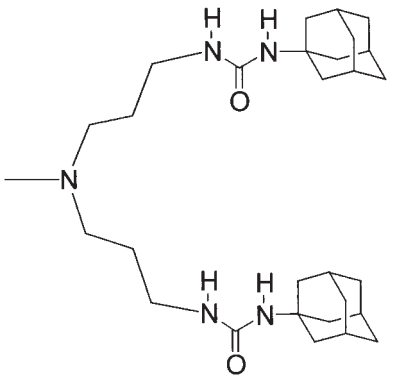


6

Figure 3. Structures of hosts **5** and **6**.

The preparation and purification of the dendrimer–guest complexes were performed with the general procedure introduced previously.[23] This was done through the mixing of the dendrimer and the guest molecule in the appropriate amounts (an excess of the guest) and subsequent flushing through a preparative Bio-Beads (S-X1) column. Both the sample preparation and the column chromatography were carried out in CH_2Cl_2.

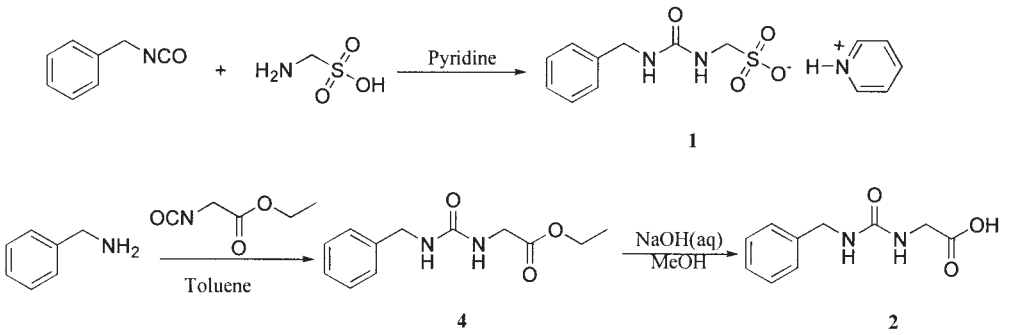
Pyridinium (3-Benzyl-ureido)-methane Sulfonate (1)

Benzyl isocyanate (0.54 g, 4.1 mmol) was added via syringe to a stirring solution of aminomethane sulfonic acid (0.43 g, 3.87 mmol) in pyridine (25 mL) under a nitrogen atmosphere; this resulted in a turbid solution. The reaction mixture was refluxed overnight, and this resulted in a clear solution. Water (50 mL) was added, and after the filtration of a small amount of a white solid (symmetrical urea from an excess of isocyanate), the solvent was evaporated *in vacuo*. The resulting solid material was recrystallized from ethanol; 0.90 g (72%) of a white solid was yielded.

mp: 130–132 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.89–8.94 (m, 2H), 8.53–8.60 (m, 1H), 8.0–8.08 (m, 2H), 7.16–7.32 (m, 5H), 6.53–6.73 (br s, 1H), 6.10–6.30 (br s, 1H), 4.20 (d, J = 8.8 Hz, 2H), 3.84 (d, J = 10.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 158.2, 146.9, 143.0, 141.4, 128.9, 127.9, 127.6, 127.6, 57.4, 43.5. ELEM. ANAL. Calcd. for $C_{14}H_{17}N_3O_4S$: C, 51.99%; H, 5.31%; N, 13.00%. Found: C, 51.86%; H, 4.98%; N, 12.67%. MS (FAB−) *m/z*: 243 [M − pyridinium]$^-$.

(3-Benzyl-ureido)-acetic Acid (2)

(3-Benzyl-ureido)-acetic acid ethyl ester (**4**; 2.50 g, 10.58 mmol) was dissolved in methanol (10 mL) and aqueous NaOH (15 mL, 4 M). After 10 min of stirring at room temperature, the starting material was no longer detectible by TLC (4% metha-



Scheme 1. Synthesis of guests **1** and **2**.

nol in dichloromethane). The product was precipitated through the addition of aqueous HCl. The solids were filtered off and washed with cold water; 2.03 g (92%) of a white solid was yielded.

mp: 168–170 °C (with gas evolution). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.4 (br s, 1H), 7.3 (m, 5H), 6.60 (t, 1H), 6.2 (t, 1H), 4.2 (d, 2H), 3.7 (d, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 173.2, 158.7, 141.4, 128.9, 127.7, 127.2, 43.5, 42.3. ELEM. ANAL. Calcd. for $C_{10}H_{12}N_2O_3$: C, 57.67%; H, 5.82%; N, 13.46%. Found: C, 57.83%; H, 5.54%; N, 13.30%. MS (FAB−) *m/z*: 207 $[M - H]^-$.

(3-Benzyl-ureido)-acetic Acid Ethyl Ester (4)

Benzyl amine (1.42 g, 13.24 mmol) in toluene (10 mL) was added dropwise to an ice-cooled stirring solution of ethyl isocyanatoacetate (1.71 g, 13.24 mmol) in toluene (20 mL). A white precipitate was formed immediately upon the addition, and after 1 h of stirring at room temperature, the precipitate was filtered off and washed with cold toluene. Recrystallization from ethanol yielded the title compound as a white solid.

Yield: 2.84 g (91%). mp: 78–79 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.3 (br s, 5H), 5.0 (m, 2H), 4.40 (m, 2H), 4.18 (q, 2H), 3.98 (m, 2H), 1.24 (t, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172.0, 158.2, 139.5, 128.8, 127.6, 127.4, 61.5, 44.6, 42.4, 14.3. ELEM. ANAL. Calcd. for $C_{12}H_{16}N_2O_3$: C, 60.99%; H, 6.84%; N, 11.86%. Found: C, 60.77%; H, 6.54%; N, 11.89%. MS (FAB+) *m/z*: 237 $[M + H]^+$.

RESULTS AND DISCUSSION

A simple way of detecting binding between the dendrimer and the different guest molecules is to monitor the change in the ^{1}H NMR spectrum of model host compound **6** upon the addition of the guest molecules. The model compound contains only one binding site, and this simplifies the ^{1}H NMR spectrum and provides an inexpensive and

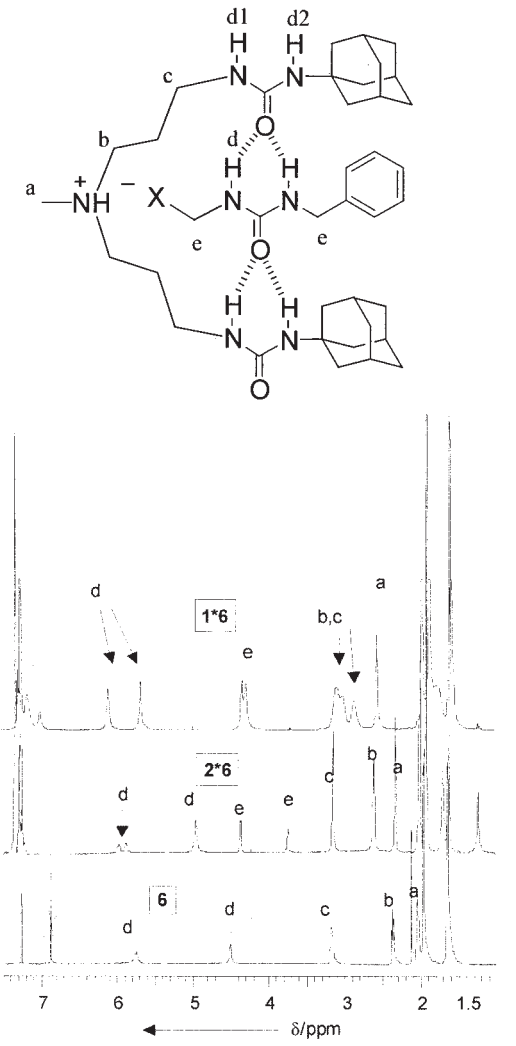


Figure 4. ^{1}H NMR ($CDCl_3$) spectra of model host **6** alone (bottom) and upon the addition of guest **1** (top) and guest **2** (middle).

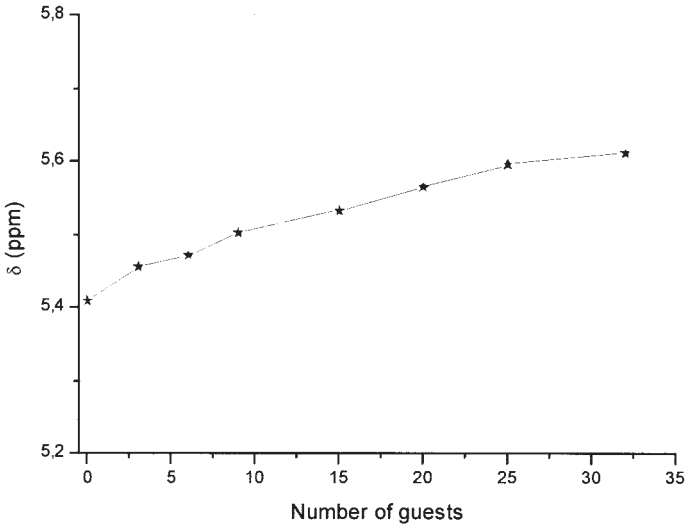


Figure 5. ^{1}H NMR titration of dendrimer **5** with sulfonic acid guest **1**. The chemical shift of the urea protons in the host changes gradually upon the addition of the guest.

reliable method of detection. In Figure 4, the ^{1}H NMR spectrum of the model host compound **6** is shown together with spectra of the complexes between **6** and guests **1** and **2**. From a simple inspection of the spectra, it is clear that a downfield shift for the protons in the binding site takes place, indicating binding. Another indication of binding, though somewhat qualitative, is that the guest molecules are very slightly soluble in $CDCl_3$, but upon the addition of the host, a homogeneous solution rapidly forms.

The fifth-generation dendrimer **5** has 64 adamantyl-urea end groups and can therefore bind 32 guest molecules. This has been demonstrated unambiguously for carboxylic acid guests in a previous work.[23] Sulfonic acid guest **1** also makes a 1:32 complex with the dendrimer, as calculated from the integrals in the ^{1}H NMR spectrum after purification by Bio-Beads column chromatography. The spectra after the simple addition of 32 equiv of the guest and after purification are similar, except that the spectrum of the purified complex has sharper signals. A ^{1}H NMR titration, following either one of the urea protons on the dendrimer or the methylene group closest to the tertiary amine in the host motif of the dendrimer, reveals that 32 guest molecules can indeed be bound. This is true for sulfonic acid guest **1**, whereas **3** does not seem to form any well-defined complexes. It is most likely that the simple sulfonic acid (**3**) binds randomly to the amine functionalities within the dendrimer. The titration curve for **1** is shown in Figure 5.

Measuring the T_1 relaxation of the methylene protons closest to the tertiary amine in the binding motif on the dendrimer (Fig. 6) was performed with different ratios of the dendrimer to guests **1** and **3**. The complexed product, when titrating with **1**, shows much less flexibility in the guest motif upon increased amounts of the guest, in comparison with guest **3**. The surface of the dendrimer becomes less dynamic and, therefore, more solid-state-like. This is a very strong indication of selective complexation near the periphery of the dendrimer when it is titrated with designed guest **1**.[29,30] This type of experiment was previ-

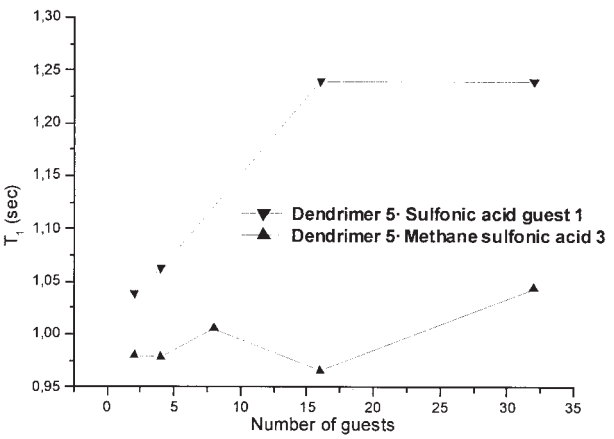


Figure 6. T_1-relaxation values for dendrimer **5** (the proton closest to the amine in the binding motif, i.e., proton b in Fig. 4) plotted as a function of added guests **1** and **3**.

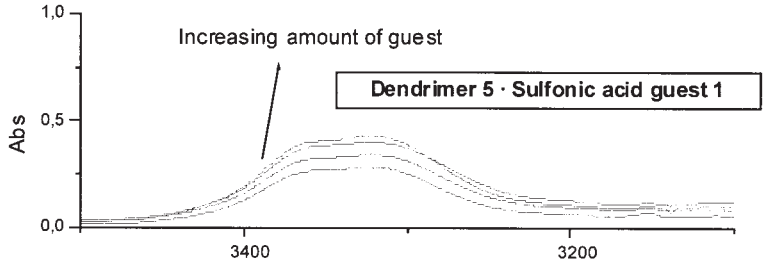


Figure 7. Hydrogen-bonding area of the IR spectra of fifth-generation dendrimer **5** upon the addition of guest **1**.

ously carried out with a guest with a urea acidic acid binding motif, and a similar result was obtained.[23]

IR spectra of the samples from the titration indicate that increased hydrogen bonding occurs when guest **1** is added to the fifth-generation dendrimer. Also, the resonances shift to lower wave numbers, indicating stronger hydrogen-bonding interactions, because of the participation of the guest ureas. This is in agreement with earlier findings for this type of system (Fig. 7).[23–26]

Final proof that guest molecule **1** indeed is bound to the periphery of the dendrimer comes from the NOESY spectra of a 1:32 complex with the fifth-generation dendrimer (Fig. 8). The NOESY spectra show cross peaks between the protons in the binding motif and no apparent cross peaks between the guest and the interior of the dendrimer. A NOESY spectrum of the dendrimer with guest **3** reveals a myriad of cross peaks (not shown). Because the simple sulfonic acid does not form a well-defined complex, it is not surprising that the NOESY spectrum shows cross peaks between both the binding motif and the interior of the dendrimer, indicating that this guest does not bind specifically to the periphery of the dendrimer.

A competition experiment was carried out to evaluate, in a qualitative manner, the binding affinities of sulfonic acid guest **1** and carboxylic acid guest **2**. An excess of each guest (64 equiv) was added to dendrimer **5**, and the mixture was then flushed through a Bio-Beads column. This resulted in a pure 1:32 complex between **5** and **1** (as shown by the ^{1}H NMR spectrum). This clearly proves that the new sulfonic acid design has a stronger affinity for the dendrimer than the carboxylic acid guest. These findings have led us to look into the conditions for the release of the guests from the dendrimer. In a recent study on the binding of peptides to this type of dendrimer,[25] it was shown that the peptides were dissociated quantitatively by the addition of a competing solvent, which broke the hydrogen bonds in the complex. Pure components were obtained by column chromatography on silica gel; the guest was eluted from the column, leaving the dendrimer behind. This procedure was investi-

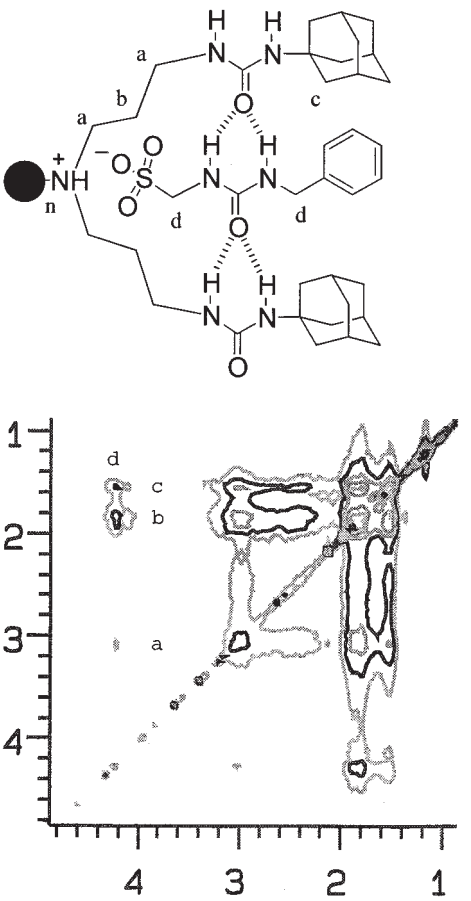


Figure 8. Partial NOESY ($CDCl_3$, 25 °C) spectra of fifth-generation poly(propylene imine) dendrimer **5** with guest **1** complexed.

gated for the guest–host systems with fifth-generation dendrimer **5** and guests **1** and **2**. The complexes were prepared with the aforementioned Bio-Beads protocol. The release experiments were performed by the guest–host complex being placed on a column of silica gel and eluted with an eluent with increasing polarity. In the case of carboxylic acid guest **2**, it was possible to release the guest quantitatively from the dendrimer with methanol as an eluent. With sulfonic acid guest **1**, release was not possible simply through elution with methanol, and even when the polarity of the eluting solvent was increased (1:10 hexafluoro-2-propanol/methanol), release was not accomplished. This result indicates that sulfonic acid guest **1** is bound significantly more tightly to the dendrimer than carboxylic acid guest **2**. Also, it points toward the intriguing possibility of the polarity-mediated selective release of guests from mixed guest–host complexes.

CONCLUSIONS

We have synthesized a new type of guest molecule for the exocomplexation of urea-adamantyl-functionalized poly(propylene imine) dendrimers. This guest motif makes strong and well-defined complexes, thus expanding the supramolecular toolbox for this type of guest–host chemistry with dendrimers. In future work, we will quantify the association constants for this new guest–host system in different solvents. Finding applications for the guest–host system in competing solvents such as water is the long-term goal.

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