Synthesis and Properties of New Thiourea-Functionalized Poly(propylene imine) Dendrimers and Their Role as Hosts for Urea Functionalized Guests

Ulrik Boas, Annika J. Karlsson, B. F. M. de Waal, and E. W. Meijer*

Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, NL-5600 MB Eindhoven, The Netherlands

E.W.Meijer@tue.nl

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Five generations of poly(propylene imine) dendrimers have been modified by palmityl and adamantyl endgroups via a thiourea linkage. The synthesis of the thiourea dendrimers DAB-*dendr*-$(NHCSNHAd)_n$ and DAB-*dendr*-$(NHCSNHC_{16}H_{33})_n$ ($n = 4, 8, 16, 32, 64$) proceeds smoothly via the amino-terminated DAB dendrimer and the adamantyl and palmityl isothiocyanates, respectively. The properties of the thiourea dendrimers have been studied by IR and ^1H NMR, including relaxation (T1, T2) measurements. The thiourea dendrimers are used as multivalent hosts for a number of guest molecules containing a terminal urea−glycine unit in organic solvents. The host−guest interactions have been investigated using 1D- and NOESY-NMR. These investigations show that the guest molecules bind to the dendritic host via thiourea (host)−urea (guest) hydrogen bonding, and ionic bonding between the terminal guest carboxylate moiety and the outer shell tertiary amines of the dendrimer. The ability to bind guest molecules of the adamantyl- and palmitylthiourea dendrimers has been compared with their respective urea containing dendrimer analogues, by NMR-titration, and competition experiments. Upon complexation, the thiourea dendrimer hosts show a larger downfield NH shift than the corresponding urea dendrimer hosts, indicative of stronger hydrogen bonding in the complexed state. Furthermore, microcalorimetry has been used to determine binding constants for formation of the host−guest complexes; the binding constants are typically in the order of 10^4 M^{-1}. Both NMR and microcalorimetric studies show that the thiourea dendrimers bind the urea containing guests with somewhat higher affinity than the corresponding urea dendrimers.

Introduction

Many of the typical physical properties of dendrimers, such as solubility, macroscopic shape, and multivalency, are determined by the nature of the peripheral groups.[1] Most end-group modifications of dendrimers are based on covalent bonding, and the use of secondary interactions to obtain new dendritic structures is limited. However, there is a growing interest in building new supramolecular dendritic structures using noncovalent interactions. There are several contributions in the literature highlighting the use of multiple secondary interactions to create self-assembled nanostructures.[1−6] The role of dendrimers as macromolecular hosts in supramolecular chemistry is also a topic of great current interest.[7] And their role in host−guest chemistry have been well documented.[1,4,6,8]

Recently, we presented results on a new strategy for modifying end groups at the periphery of the poly(propylene imine) dendrimer using a supramolecular approach.[9] In this methodology, end groups are self-assembled at the periphery of the dendrimer by multiple secondary interactions using ionic and hydrogen bonding. The poly(propylene imine) dendrimer was functionalized with adamantyl end groups and urea linkages, which were used as a scaffold to take in the glycine−urea building blocks (Scheme 1).

Our interest in extending the usage of multiple secondary interactions in order to modify the periphery of dendrimers prompted us to search for alternatives to urea-modified dendrimers. Previous reports suggest that thioureas may have improved properties as hydrogen-bond donors (e.g., lower pK_a),[10] form more flexible hydrogen-bonding networks,[11] and show lower tendency to self-associate (i.e., poor H-acceptors) than the corresponding urea analogues.[10] Therefore, replacing the urea moieties in the dendrimer with thiourea is of interest to

* To whom correspondence should be addressed. Tel: 31-40-2473101. Fax: 31-40-2451036.

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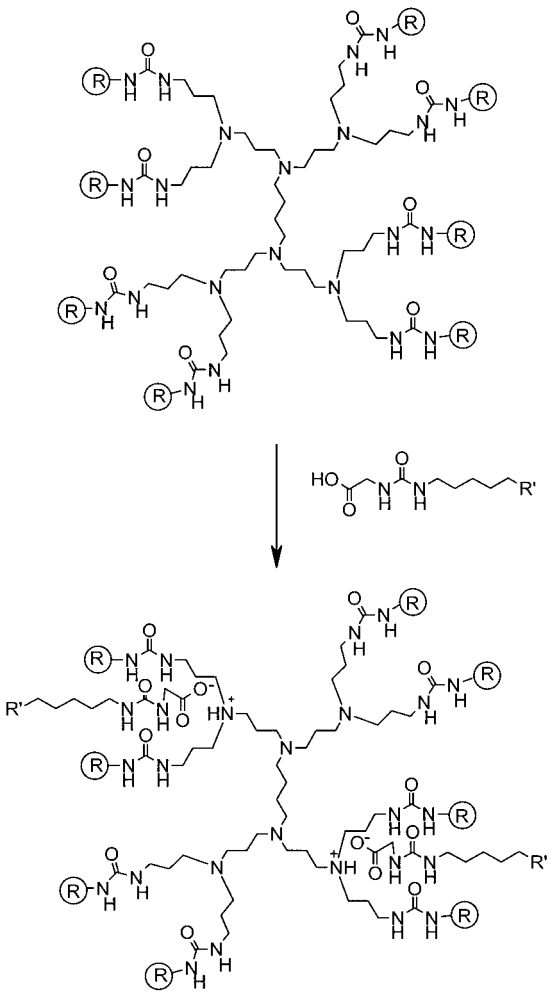
Scheme 1. Host−Guest Interactions: Second-Generation Urea Modified Dendrimer, DAB-*dendr*-(NHCONHR)$_8$[a]



[a] Two of the four adamantyl or palmityl-bis(propylurea) pincers are occupied by the guest. R = adamantyl or palmityl, R′ = e.g., CH_3.

increase the extent of hydrogen bonding with the urea-functionalized guests, compared to the native state.

In this paper, we report the synthesis of adamantyl- and palmitylthiourea end group modified poly(propylene imine) dendrimers and their use as hosts to assemble glycine−urea functionalized guest molecules. Physical properties of the dendrimers and host−guest complexes have been investigated in detail by IR and 1D- and 2D-NMR spectroscopy, including T1- and T2-relaxation measurements. The complexation properties of the guest compounds with the fifth-generation thiourea dendrimer hosts, vs urea analogues, have been revealed from competition experiments. Isothermal titration calorimetry have been used to determine the binding strength for the host−guest complexes.

Results and Discussion

Synthesis. The adamantylthiourea-functionalized dendrimers were prepared by reaction of commercially available 1-adamantyl isothiocyanate with DAB-*dendr*-$(NH_2)_n$ (**1**) (n = 4, 8, 16, 32, 64 for **1a**,**b**,**c**,**d**,**e**, respectively) for 48 h. The DAB-*dendr*-(NHCSNH-Ad)$_n$ (**2**) (n = 4, 8, 16, 32, and 64 for **2a**,**b**,**c**,**d**,**e**, respectively) were obtained in typical yields of 75−98% (Scheme 2).

The palmitylthiourea-functionalized dendrimers were prepared by reaction of **1a**−**e** with hexadecyl isothiocyanate (**3**), obtained from hexadecylamine and carbondisulfide, using DCC as desulfurylating agent.[12] As for the adamantyl-functionalized dendrimers, 48 h of reaction time was nessesary to complete the formation of thiourea bonds. The DAB-*dendr*-(NHCSNH-$C_{16}H_{33}$)$_n$ (**4**) (n = 4, 8, 16, 32, and 64 for **4a**,**b**,**c**,**d**,**e**, respectively) were obtained in yields of 83−90% (Scheme 2). The functionalized dendrimers were characterized by ^{1}H NMR, ^{13}C NMR, MALDI-TOF, elemental analysis, and IR spectroscopy. The synthesis and characterization of the adamantylurea-functionalized dendrimers, i.e., DAB-*dendr*-(NHCONH-Ad)$_n$ (n = 4, 8, 16, 32, and 64), the palmitylurea-functionalized dendrimers, i.e., DAB-*dendr*-(NHCONH-$C_{16}H_{33}$)$_n$ (n = 4, 8, 16, 32, and 64), and [3-(6-(4-cyano)-1,1′-biphenyloxyhexyl)ureido]acetic acid (**10**) have been reported earlier.[9,13] (3-Hexylureido)acetic acid (**6**) was obtained from the reaction of commercially available hexyl isocyanate with glycine methyl ester using triethylamine as base, followed by saponification of the methyl ester (**5**) by lithium hydroxide (Scheme 3).

A chloroform-soluble guest, 3,4,5-tridodecyloxybenzoic acid-6-(3-carboxymethyl-ureido)-hexyl ester (**9**) was synthesized by the esterification of [3-(6-hydroxyhexyl)-ureido]acetic acid *tert*-butyl ester (**7**) with 3,4,5-tridodecyloxy-benzoic acid, using DCC and DMAP in 76% yield. 3,4,5-Tridodecyloxybenzoic acid-6-(3-*tert*-butoxycarbonylmethylureido)hexyl ester (**8**) was deprotected by TFA in dichloromethane in 85% yield. Compound **7** was synthesized in a one pot procedure, via 6-hydroxyhexyl isocyanate, in quantitative yield. The isocyanate was synthesized from the corresponding amine and di-*tert*-butyl tricarbonate[14] (Scheme 3).

IR and ^{1}H NMR Spectroscopy of the Hosts in Solution. Infrared spectroscopy was used to achieve qualitative information on the hydrogen-bonding interactions in solution between the end groups in the dendrimers **2a**−**e** and **4a**−**e** depending on generation. This technique has earlier been used by Gellman et al. to determine the ratio between hydrogen-bonded and non-hydrogen-bonded states in solution.[15] The IR measurements were performed in chloroform at 298 K at constant end group concentration of 23 mM. The resonances at 3400−3450 cm^{-1} correspond to non-hydrogen-bonded states, whereas the resonances at lower wavenumbers 3250−3270 cm^{-1} originate from the intramolecular hydrogen-bonded states, Figure 1a,b.

Upon increasing dendrimer generation in **2a**−**e** and **4a**−**e**, the intensity of the non-hydrogen-bonded states is decreased while the intensity of the hydrogen-bonded states is increased. This observation is in agreement with IR studies on a *N*-*t*-BOC-glycine-functionalized poly(propylene imine) dendrimer reported by Bosman et al.[1,16] Moreover, upon increasing dendrimer generation, small

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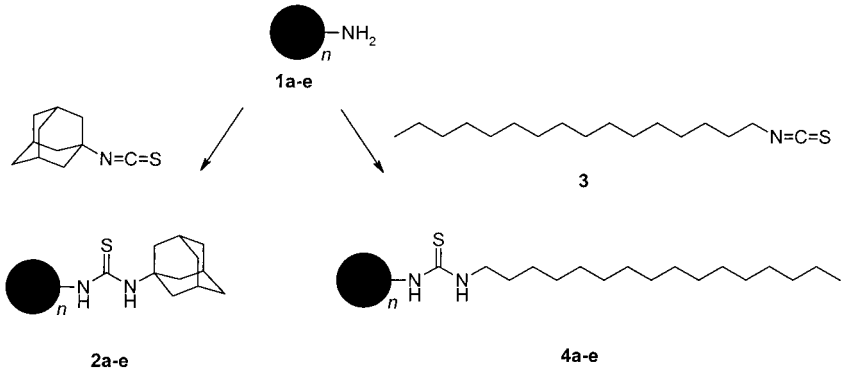
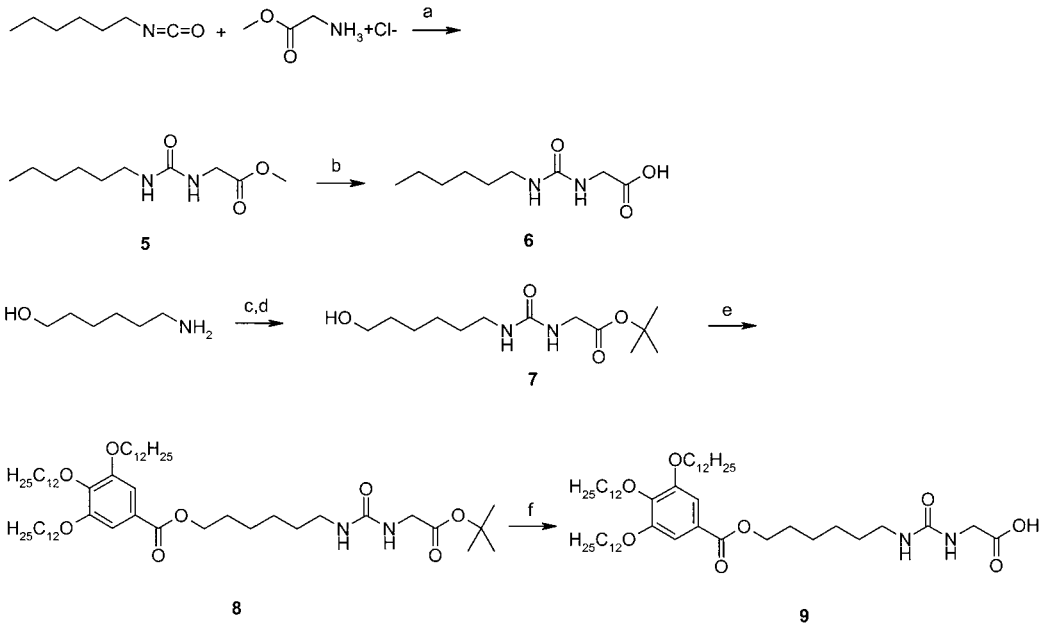
Scheme 2



Scheme 3. Synthesis of Guest Compounds 6 and 9[a]



[a] Key: (a) NEt_3, 2 h, rt; (b) LiOH (aq), 16 h, rt; (c) $Bu^tO(CO)O(CO)O(CO)OBu^t$, 20 min, rt; (d) glycine *tert*-butyl ester, 16 h, rt; (e) 3,4,5-tridodecyloxybenzoic acid, DCC, DMAP, 48 h, ambient temperature; (f) TFA, 3 h, rt.

shifts toward lower wavenumbers are observed for both dendrimers **2a**−**e** and **4a**−**e**, respectively. This result is indicative of an increase in hydrogen-bonding strength with dendrimer generation.[17] Molecular dynamics calculations on amino acid functionalized poly(propylene imine) dendrimers also suggest that hydrogen bonding increases with higher generation.[18] The lower wavenumber for **4a**−**e**, 3250 cm^{-1}, compared to **2a**−**e**, 3270 cm^{-1}, suggests that the hydrogen-bonding interactions in the palmitylthiourea-functionalized dendrimers are slightly stronger than in the adamantylthiourea analogue. A similar behavior has been observed by Baars for the urea-linked adamantyl- and palmityl-functionalized dendrimers, although in these cases the ratio between non-hydrogen-bonded/hydrogen-bonded is much lower.[19] Upon increasing the dendrimer concentration, no shifts are observed, indicative of intermolecular hydrogen bonding. For the higher generations, the IR profile is preserved upon increasing/decreasing the concentration.[20]

The hydrogen-bonding interactions in **2a**−**e** and **4a**−**e** were further investigated by ^1H NMR spectroscopy. All measurements were performed in $CDCl_3$ solutions at constant end group concentration, 23 mM. The changes in chemical shift for the two N−H resonances for both **2a**−**e** and **4a**−**e** were studied. Upon increasing dendrimer generation, **2a**−**e** showed downfield shifts for the two N−H protons from 5.90 and 6.78 ppm to 6.39 and 6.94 ppm, respectively. Similar downfield shifts from 6.28 and 7.33 ppm to 6.73 and 7.45 ppm for the N−H protons in **4a**−**e** were observed. These results indicate an increase in hydrogen-bonding interactions between the thiourea moieties in the higher generation dendrimers. For the urea-linked dendrimer analogues DAB-*dendr*-$(NHCONH\text{-}Ad)_n$ and DAB-*dendr*-$(NHCONH{-}C_{16}H_{33})_n$ a similar tendency has been found.[19] The increased strength of the hydrogen bonds was explained by a closer proximity of the end group units in the higher generation dendrimers.[1,21] For the fifth-generation adamantyl- and

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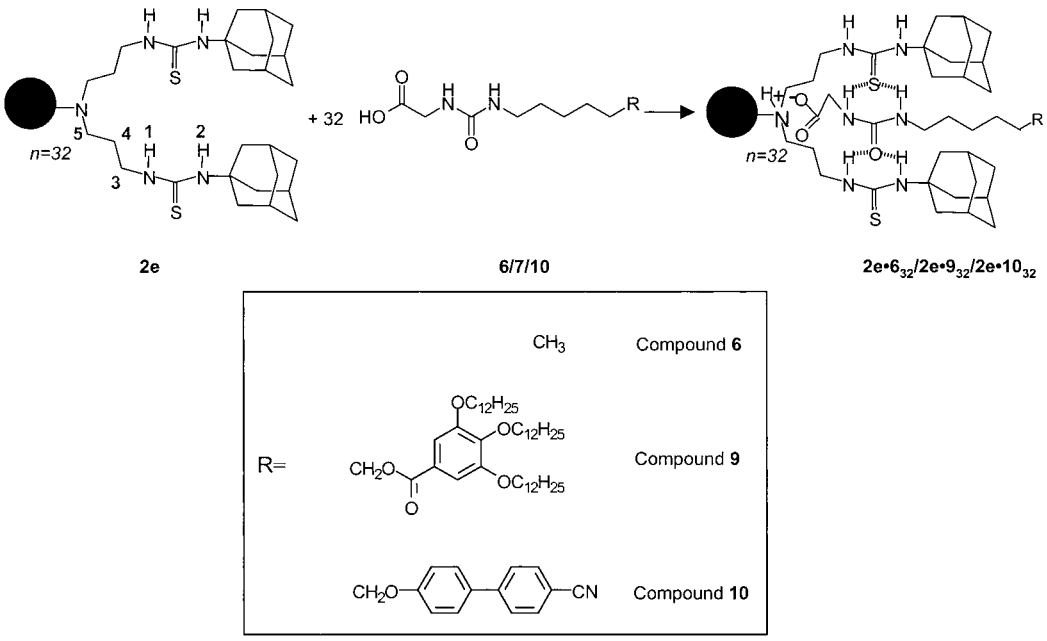
Scheme 4. Host−Guest Interactions between the Thiourea Pincer in the DAB-*dendr*-(NHCSNHAd)$_{64}$ and Guest Molecules 6, 9, and 10[a]



[a] "*n*" referes to the number of pincers in the dendrimer.

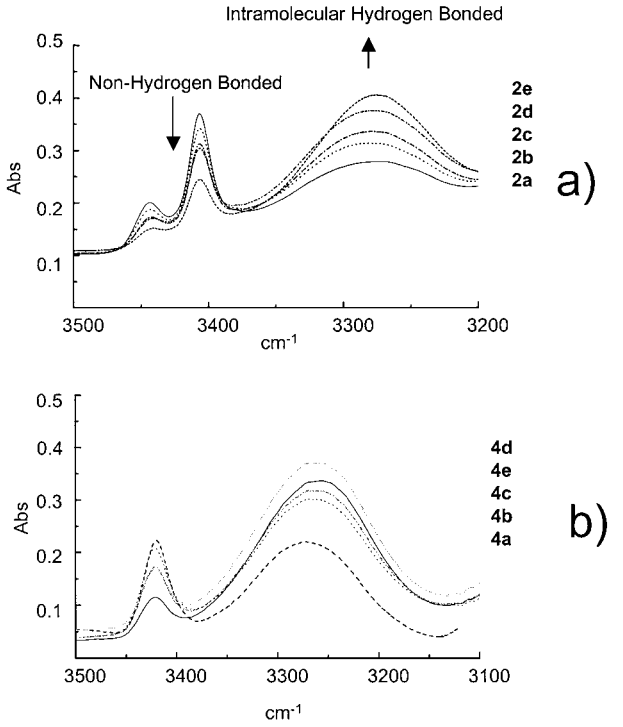


Figure 1. Partial IR spectra (showing the N−H stretch vibrations) obtained at 25 °C of typically 0.5 mM chloroform solutions of (a) **2a**−**e** (bottom to top) and (b) **4a**−**d** (bottom to top).

palmitylthiourea dendrimers, no significant change in NH chemical shifts is observed, upon increasing/decreasing the concentration, indicating a strictly intramolecular nature of the hydrogen bonding.[20] Thiourea gives lower NH-stretch wavenumbers (both in hydrogen-bonded and non-hydrogen-bonded states) and higher NH chemical shifts than the corresponding urea analogues.[19] Furthermore, thiourea shows larger $\Delta\delta$ for NH protons upon increasing dendrimer generation than the respective urea analogues. These observations can be explained by the higher ability of thiourea to act as a hydrogen donor in formation of hydrogen bonds in comparison with the urea analogues. These results are in full agreement with earlier reports.[10,22,23]

Complexation Studies of Hosts and Guests. We investigated the complexation between the adamantyl-bis(propylthiourea)amine-functionalized dendrimer **2e** and the nonaromatic glycine−urea-functionalized guest molecule **6** in chloroform, leading to complexes **2e·6$_n$** (Scheme 4).

Typically, mixtures of **2e** and **6**, as powders were dissolved in $CDCl_3$ in ratios of 1: 0, 2, 4, 8, 16, 24, and 32, respectively. The complexation was followed by ^{1}H NMR spectroscopy. Upon complexation of dendrimer **2e** and compound **6** in a 1:32 ratio a downfield shift of ca. 0.2 ppm was observed for the urea N−H protons of the guest compound. Furthermore, a downfield shift from 2.49 to 2.90 ppm for the methylene protons adjacent to the tertiary amines in the bis(propylthiourea)amine pincer in **2e** was observed upon formation of complex **2e·6$_{32}$**, Figure 2.

This result is indicative of protonation of the tertiary amine nitrogens in the dendritic host **2e**. Downfield shifts from 7.12 to 7.57 ppm and from 6.4 to 6.83 ppm, respectively, were also observed for the two-dendrimer thiourea N−H protons. These changes in chemical shifts indicate that upon complexation there is an increase in the hydrogen bonding interactions. ^{1}H NOESY was used in order to elucidate the solution structure of the complex **2e·6$_{32}$**. The ^{1}H NOESY spectra of complex **2e·6$_{32}$** were obtained in $CDCl_3$ at 25 °C and resulted in the NOE interactions shown in Figure 3.

^{1}H NMR spin−lattice (T1) relaxation experiments were conducted to verify the idea, that the dendritic shell

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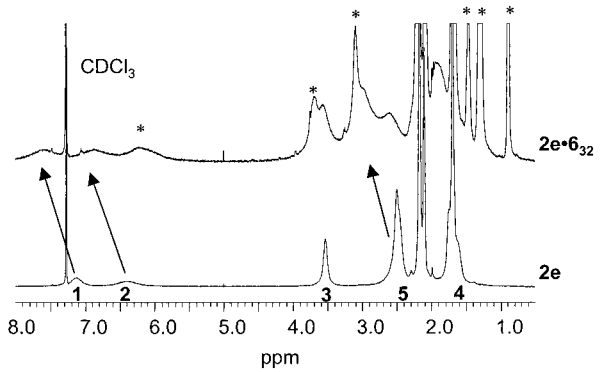

Figure 2. ^{1}H NMR spectra (obtained at 500.618 MHz) of **2e** and **2e**·6_{32} obtained at 25 ± 0.5 °C in chloroform. NMR signals from guest compound **6** are denoted with an asterisk.

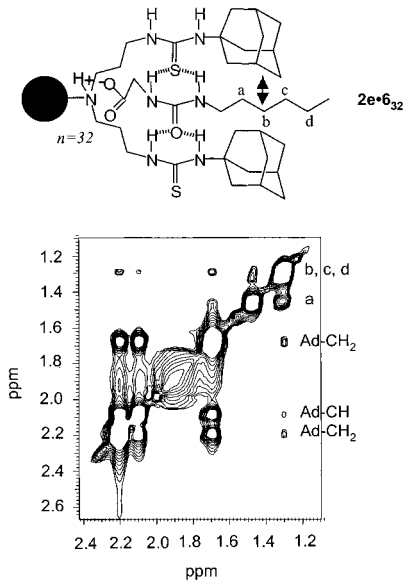

Figure 3. Partial ^{1}H,^{1}H-NOESY spectrum (obtained at 500.618 MHz) of complex **2e**·6_{32} obtained at 25 ± 0.5 °C in chloroform showing NOE interactions between **2e** and **6**.

becomes denser due to complexation. We performed T1 relaxation measurements for complexes between **2e** and **6** in different ratios. In Figure 4a, changes in T1 relaxation times for some selected atoms of the guest upon increasing the guest molecule−dendrimer ratio are shown.

The strong complexation was used to purify the **2e**·$\mathbf{6_{32}}$ complexes. Biobead chromotography does not lead to dissociation; the stoichiometry of the complex was preserved after repeated chromotography. Guest molecules bound by adhersion were removed by this technique.

The T1 relaxation times of **6** "internal" positions (β, ϵ) decrease with increasing guest molecule−dendrimer ratios up to ca. 12 equiv of **6**; thereafter, an increase of relaxation times was observed. These results show that the dendrimer **2e** effectively assembles guest molecule **6**. The increase in T1s after a guest−host ratio of ca. 12 can be explained by a decrease in molecular motions of encapsulated **6** molecules.[9] However, no significant alteration in relaxation times of the "end" position (γ) was observed, showing that only the "internal" part of **6** is involved in complexation with **2e**, Figure 4a. Changes in T1 relaxation times are shown for the dendrimer upon increasing the guest−dendrimer ratio. For dendrimer **2e**, the recorded T1 relaxation times increase upon increasing the guest−dendrimer ratio. These results suggest that upon increasing the guest−dendrimer ratio there is a decrease in conformational motion of the outer bis(bispropylthiourea) amine groups in **2e**. These results agree well with denser shell packing when more guest molecules are complexed. Furthermore, we investigated the changes in packing of the dendritic shell as a function of generation in compounds **2a**−**d** complexed with [3-(6-(4-cyano)-1,1′-biphenyloxyhexyl)ureido]acetic acid (**10**), i.e., **2a**−**d**·$\mathbf{10}_n$ (**a**, $n = 2$; **b**, $n = 4$; **c**, $n = 8$; **d**, $n = 16$), Figure 4b. The T1 relaxation times for the guest molecule atoms in close proximity to the bis(propylthiourea) unit increase for higher dendrimer generations, i.e., the mobility of these atoms decreases, Figure 4b. The T1s for the atoms next to the cyanobiphenyl unit remain constant; i.e., the mobility of these atoms remains unperturbed. Moreover, we found that the T1 relaxation data for the atoms in the dendritic shell increases with dendrimer generation. These results are indicative of an almost solid-phase behavior for higher generation dendrimers, also found for the dendritic box[24] and indicative of dense-shell packing in solution.

For comparison, we investigated interactions between the adamantyl-bis(propylurea)-functionalized dendrimer, i.e., DAB-*dendr*-(NHCONH-Ad)$_n$ and guest molecule **6**. In a similar way, mixtures of DAB-*dendr*-(NHCONH-Ad)$_{64}$ and **6** were prepared in $CDCl_3$ in ratios of 1:0, 2, 4, 8, 16, 24 and 32, respectively. The formation of complex DAB-*dendr*-(NHCONH-Ad)$_{64}$•$\mathbf{6}_n$ was followed by ^{1}H NMR. Upon complexation of compound **6** with the dendrimer DAB-*dendr*-(NHCONH-Ad)$_{64}$, a downfield shift of ca. 0.2 ppm is observed for the guest compound glycine urea N−H protons. Furthermore, a downfield shift from 2.39 to 2.92 ppm for the methylene protons adjacent to the tertiary amines in the bis(propylurea)amine "pincer" in DAB-*dendr*-(NHCONH-Ad)$_{64}$ was observed. Downfield shifts from 6.20 to 6.40 ppm and from 5.47 to 5.75 ppm, respectively, were also observed for the two N−H protons in DAB-*dendr*-(NHCONH-Ad)$_{64}$.[20]

We compared the complexation properties of the adamantylthiourea dendrimer **2e** with the urea analogue, i.e., DAB-*dendr*-(NHCONH-Ad)$_{64}$, to elucidate which of the two dendrimers were most effective in binding **6**. To a 1:1 mixture of **2e** and DAB-*dendr*-(NHCONH-Ad)$_{64}$ in $CDCl_3$ was added 32 molar equiv of compound **6**. The number of guest molecules complexed to each of the two dendrimers was followed by the changes in chemical shifts for the two N−H protons in **2e** and DAB-*dendr*-(NHCONH-Ad)$_{64}$, respectively, Figure 5.

These investigations concluded that approximately 24 ± 2 guest molecules were complexed per thiourea-linked dendrimer **2e** and approximately 8 ± 2 guests were complexed per urea-linked dendrimer, DAB-*dendr*-(NHCONH-Ad)$_{64}$, respectively. Control experiments without any guest molecules present showed only small changes in NH chemical shift ($\Delta\delta = 0.00-0.04$ ppm), excluding the existence of homo- and hetero-complexation between the dendrimers.[20] The result competition experiment shows, that the bis(propylthiourea)amine scaffold is somewhat more selective for complexation of the urea-containing guest molecules, than the bis(propylurea)-amine analogue.

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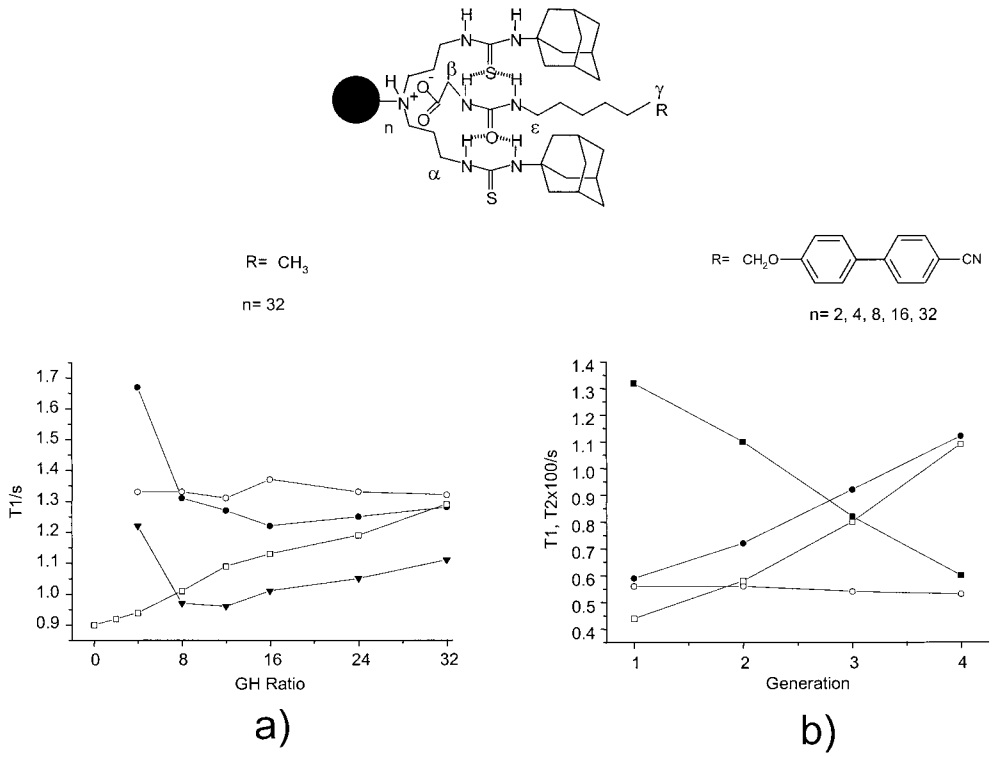

Figure 4. T1 and T2 ^{1}H NMR relaxation data vs (a) guest−host ratio and (b) generation. The selected atoms are α (-□-), *β* (-●-), *ϵ* (-▼-), *γ* (-○-), and T2 × 100 (-■-).

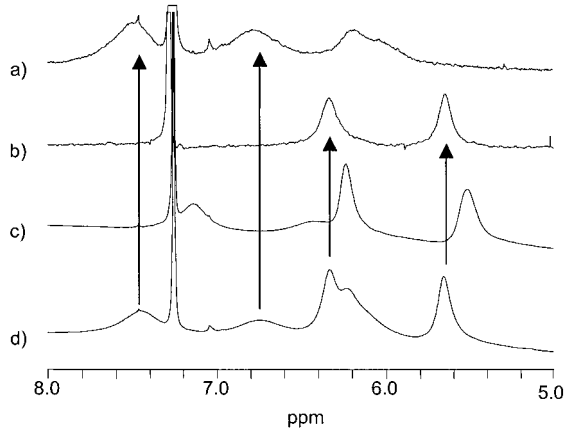

Figure 5. Competition experiment. ^{1}H NMR spectra obtained at 25 ± 0.5 °C in chloroform of a) complex **2e**·6_{24}. b) complex DAB-*dendr*-$(NHCONHAd)_{64}$·6_8 c) **2e** and DAB-*dendr*-$(NHCONHAd)_{64}$ before addition of guest **6** and d) **2e** and DAB-*dendr*-$(NHCONHAd)_{64}$ competing for 32 equivalents of guest **6**.

A similar set of experiments were set up for the palmityl-modified dendrimers to investigate the complexing ability of DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$ and **4e** with guest compound **6**. Upon titration of DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$ with **6**, a downfield shift of ca. 0.15 ppm for the guest compound urea N−H protons was observed. Furthermore, we observed a downfield shift from 2.36 to 2.69 ppm for the methylene protons adjacent to the tertiary amines in the bis(propyl urea) amine pincer. Downfield shifts from 6.47 to 6.49 ppm and from 6.04 to 6.09 ppm, respectively, were observed for the two dendrimer urea N−H protons. The small changes in chemical shifts suggests that there is only a small increase in the hydrogen-bonding interactions upon complexation with the guest molecules, compared to the native state. This indicates that intramolecular interactions (hydrogen bonding, hydrophobic interaction) in the uncomplexed palmityl urea dendrimer are so strong that intake of guests does not result in significant additional hydrogen bonding.

Titration studies between the dendritic host **4e** and the guest compound **6** showed a downfield shift of ca. 0.1−0.4 ppm for the guest urea NH protons and a downfield shift from 2.40 to 2.72 ppm for the methylene protons adjacent to the tertiary amines in the dendrimer bis(propyl thiourea) amine pincer. Interestingly, relatively large downfield shifts from 7.36 to 7.77 ppm and from 6.71 to 7.43 ppm, respectively, were observed for the thiourea N−H protons in **4e**.[20] These rather large changes in NH chemical shifts indicate that upon formation of complex **4e**·$\mathbf{6}_n$, hydrogen bonding between the dendrimer thiourea units and the guest molecule urea units is more pronounced, compared to what was observed for complex DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$·$6_n$.

Competition between palmitylthiourea-modified dendrimer **4e** with DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$ for uptake of guest compound **6** was investigated by ^{1}H NMR. From the competition experiment, it was concluded that approximately 24 ± 2 guest molecules were complexed per thiourea dendrimer **4e** and approximately 8 ± 4 guests were complexed per urea dendrimer, DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$, respectively (Figure 6). As for the adamantyl dendrimers, control experiments, without guest molecules present, showed only small changes in NH chemical shift.[20] The competition experiment shows that the bis(propylthiourea) amine pincer is somewhat more selective for complexation of guest molecules than the bis(propylurea)amine pincer. Hence, both the adamantyl- and the palmitylthiourea dendrimer hosts show

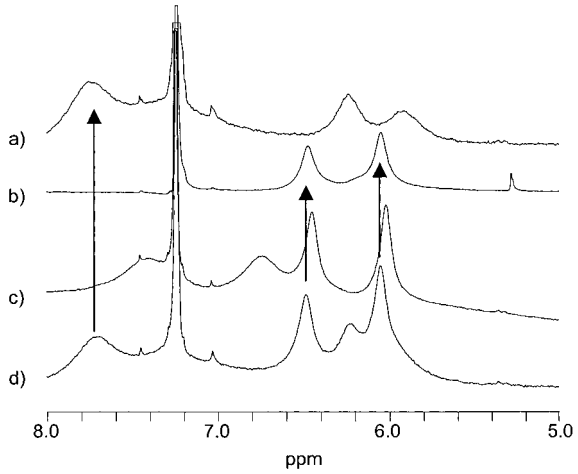

Figure 6. Competition experiment. ^{1}H NMR spectra obtained at 25 ± 0.5 °C in chloroform of (a) complex **4e**·6_{24}, (b) complex DAB-*dendr*-$(NHCONHC_{16}H_{33})_{64}$·$6_8$, (c) **4e** and DAB-*dendr*-$(NHCONHC_{16}H_{33})_{64}$ before addition of guests, and (d) **4e** and DAB-*dendr*-$(NHCONHC_{16}H_{33})_{64}$ competing for 32 equiv of **6**.

a somewhat higher ability to take in the guest molecules, compared to their respective urea analogues.

Binding Studies Using Microcalorimetry. To perform experiments to estimate the binding constant, the guest molecule (as well as the host molecule) needs to be completely soluble in the solvents used; guest **9** was synthesized for this purpose. ^{1}H NMR titration experiments indicated that the binding constant, using a model dendrimer compound, i.e., the bis(propylurea)amine pincer and guest compound **9**, was above the detection limit of the NMR method. Therefore, titrations using microcalorimetry were employed to acquire thermodynamic profiles for binding. The titrations were performed using the dendrimer compound and guest **9** in pure chloroform solution at 25.0 ± 0.5 °C. To provide more information about the host−guest complex stabilities and the factors influencing the binding strength, we performed ITC experiments of the urea linked dendrimer DAB-*dendr*-$(NHCONH\text{-}Ad)_{64}$ and the thiourea analogue **2e** with guest **9**, respectively. The DAB-*dendr*-$(NHCONH\text{-}Ad)_{64}$ (0.2 mM) was titrated with guest **9** (2 mM) in pure chloroform at 25.0 ± 0.5 °C, and an association constant of (1.5 ± 0.5) × 10^4 M^{-1} was obtained. Similarly, the thiourea dendrimer **2e** (0.2 mM) was titrated with guest **9** (2 mM) and the association constant was determined to be (2.1 ± 0.5) × 10^4 M^{-1}. As reference experiments, the guest or dendrimer host molecules were added to pure chloroform determining the heat of dilution. We performed similar binding studies on the urea and thiourea palmityl functionalized dendrimers, i.e., DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$ and **4e**, respectively, with compound **9**. Dendrimer **4e** (0.2 mM) was titrated with guest **9** (2 mM) in pure chloroform at 25.0 ± 0.5 °C, and an association constant of (1.0 ± 0.1) × 10^4 M^{-1} was obtained. However, titration of DAB-*dendr*-$(NHCONH\text{-}C_{16}H_{33})_{64}$ with **9** resulted in a much lower association constant and the stoichiometry number indicated that only a few guests were complexed with the dendrimer. The weaker binding of the palmityl-modified dendrimers can be explained by the strong intramolecular interactions (hydrogen bonding, hydrophobic bonding) in the native dendrimer.[9]

Conclusions

In summary, the synthesis of five generations adamantyl- and palmityl-functionalized poly(propylene imine) dendrimers with thiourea linkages have been reported in this paper. From IR and ^{1}H NMR spectroscopy, it has been shown that the hydrogen-bonding interactions increase for higher generation dendrimers due to closer proximity of the end group units. It was also demonstrated that the adamantane-bis(propylthiourea) and palmityl-bis(propylthiourea) dendrimers can be used as hosts to complex glycine−urea guests by multiple secondary interactions in a way similar to that of their urea-linked analogues. ^{1}H NMR competition experiments showed a somewhat higher ability for the thiourea adamantyl- and palmityl-functionalized dendrimers to take in the urea-functionalized guests. However, estimating the number of guests taken in by the palmitylurea-functionalized dendrimer is accompanied with some error due to the small changes in chemical shifts upon complexing with the guests. Isothermal titration microcalorimetry, using a chloroform-soluble guest, also showed that the bis(propylthiourea) scaffold has a somewhat stronger ability to complex the glycine-urea derivatives than the bis(propylurea) scaffold. Analysis of the binding isoterms in chloroform showed that the binding constant for the complexes was in the range of 2 × 10^4 M^{-1}. It was shown, that the palmityl-bis(propyl thiourea) dendrimer is more effective in uptake of glycine urea guest molecules, compared to the palmityl-bis(propyl urea) analogue. These results can be explained by more flexible, i.e., "soft" hydrogen bonding between thiourea moieties in the native dendrimer, giving a less dense structure, more accessible to take in guests.

Experimental Section

General Remarks. Chloroform (Biosolve), dichloromethane (Biosolve), acetone (Biosolve), diethyl ether (Biosolve), and carbon disulfide (Janssen Chemicals) were of *p.a.* quality and used as received. 1-Adamantyl isothiocyanate (Aldrich, 99%), *n*-hexadecylamine (Lancaster, tech.), and dicyclohexyl carbodiimide (Aldrich, 99%) were used as received. The dendrimers DAB-*dendr*-$(NH_2)_n$ (n = 4, 8, 16, 32, 64) were kindly provided from DSM and were, prior to use, stripped three times with toluene in order to remove water. Biobeads S-XI Beads (200−400 mesh) with a cutoff of 14 kD were obtained from Bio-Rad Laboratories; dichloromethane was used as eluent. Melting points were measured on a Büchi B-140 apparatus and are uncorrected. For adamantylthiourea dendrimers, melting points and glass transition temperatures (T_g) were measured on a Perkin-Elmer Pyris 1 differential scanning calorimeter, with a scanning speed of 40 °C/min and a temperature range of 50−180 °C. GC−MS measurements were performed on a Shimadzu GC-MS QP5000 using a GC-17A gas chromatograph. MALDI-TOF were performed at Voyager System 6020 from PE Biosystems, using α-cyano-4-hydroxycinnamic acid and cobalt as matrix.[25] Elemental analyses were performed on a Perkin-Elmer, Series II, 2400. IR spectra were obtained on a Perkin-Elmer Spectrum One ATR-FT-IR machine, using a semipermeable solution cell (1.0 mm, F-05NT, NaCl windows) or attenuated reflection (ATR) technique. Standard ^{1}H NMR experiments were performed on a Varian Gemini 300 MHz instrument and a Varian Inova 500 MHz. ^{13}C NMR experiments were performed at a Varian Gemini 300 MHz and a Varian Inova 500 MHz instrument, operating at 75 and 125 MHz, respectively. Chemical shifts are reported in ppm

(25) Strong fragmentation is observed for the higher generation dendrimers.

downfield from TMS. NMR solvents, deuteriochloroform $CDCl_3$, and DMSO-d_6 were purchased from Cambridge Isotope Laboratories ^{1}H NMR measurements: The NMR relaxation time experiments and the 2D NMR ^{1}H,^{1}H NOESY experiments were carried out on a Varian Inova 500 spectrometer operating at 500.618 MHz and equipped with a 5 mm 500 SW/PFG probe from Varian. Deuteriochloroform (>99.95 atomic % ^{2}H) was used as solvent, and ^{1}H chemical shifts were referenced to $CDCl_3$ (δ 7.26). The spectra were obtained at 25 °C. One-dimensional proton spectra were recorded with standard parameters. The 2D NMR spectra were acquired using non-spinning 5 mm samples with deuterium field-frequency locking. For the ^{1}H,^{1}H-NOESY spectra, the following parameters were used: spectral width, 5086 Hz (f2) and 5086 Hz (f1); 256 increments and 16 scans per increment in t1; calibrated mixing time 0.1 s. Spin−lattice relaxation time (T1) measurements were conducted using a standard ^{1}H inversion−recovery experiment supplied by Varian. Typical conditions: puls width 12.5 μs (calibrated), pulse delay >4T1, 20 s, 12 points per run, 16 accumulations per point. The concentrations of the host−guest complexes were kept constant at 0.4 mM. The constancy of the equilibrium signal intensity was always investigated over the whole d2-array period.

DAB-*dendr*-(NHCSNH-Ad)$_4$ (2a). DAB-*dendr*-$(NH_2)_4$ (0.12 g, 0.37 mmol) was dissolved in chloroform (3 mL). 1-Adamantyl isothiocyanate (0.37 g, 1.90 mmol) was added and the mixture stirred 48 h at room temperature. Precipitation during the reaction occurred for the first-generation dendrimer. The mixture was added dropwise into stirred diethyl ether (20 mL), and the product was collected by filtration: yield 0.30 g (75%); mp 156 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.46 (s, 4H) 1.68 (br s, 24H), 1.75 (br qn, 8H), 2.10 (br s, 36H), 2.40−2.49 (s + br t, 12H), 3.53 (br s, 8H), 5.90 (br s, 4H), 6.78 (br s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2, 27.1, 29.6, 36.3, 42.2, 44.0, 51.9, 54.2, 180.5; IR (ATR) ν (cm^{-1}) 1227.9 (C═S), 2905.3 (C−H sat.), 3270.7 (N−H stretch); MS (MALDI-TOF) calcd ($C_{60}H_{100}N_{10}S_4$) 1089.94, found 1091.41. Anal. Calcd for $C_{60}H_{100}N_{10}S_4$: C, 66.11; H, 9.27; N, 12.85. Found: C, 65.60; H, 10.07; N, 12.72.

DAB-*dendr*-(NHCSNH-Ad)$_8$ (2b). DAB-*dendr*-$(NH_2)_8$ (0.30 g, 0.40 mmol) dissolved in chloroform (8 mL) was mixed with adamantyl isothiocyanate (0.68 g, 3.50 mmol). Same procedure as for compound **2a**: yield 0.840 g (90%); mp 161 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.41 (s, 4H) 1.58 (s, 8H)1.70 (br s, 48H), 1.78 (qn, 16H), 2.13 (br s, 64H), 2.42−2.51 (m, 36H), 3.55 (br s, 16H), 6.00 (br s, 8H), 6.91 (br s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 25.2, 26.4, 29.8, 36.4, 42.3, 44.0, 52.3, 52.5, 54.3, 180.4; IR (ATR) ν (cm^{-1}) 1215.5 (C═S), 2907.0 (C−H sat.), 3273.6 (N−H stretch); MS (MALDI-TOF) calcd ($C_{128}H_{216}N_{22}S_8$) 2320.14, found 2361.83. Anal. Calcd for ($C_{128}H_{216}N_{22}S_8$): C, 66.26; H, 9.40; N, 13.28. Found: C, 64.82; H, 9.20; N, 12.83.

DAB-*dendr*-(NHCSNH-Ad)$_{16}$ (2c). DAB-*dendr*-$(NH_2)_{16}$ (0.14 g, 0.08 mmol) dissolved in chloroform (3 mL) was mixed with adamantyl isothiocyanate (0.34 g, 1.75 mmol). Same procedure as for compound **2a**: yield 0.390 g (98%); T_g 127 °C; mp 158 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm)1.40 (s, 4H), 1.56 (m, 56H), 1.68−1.75 (m, 96H) 2.10 (br s, 192H), 2.41−2.48 (m, 84H), 3.53 (br s, 32H), 6.08 (br s, 16H), 6.89 (br s, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 26.5, 29.8, 36.5, 42.3, 43.9, 44.0, 52.3, 52.5, 52.8, 54.3, 180.5; IR (ATR) ν (cm^{-1}) 1220.4 (C═S), 2906.0 (C−H sat.), 3271.0 (N−H stretch); MS (MALDI-TOF) calcd ($C_{264}H_{448}N_{36}S_{16}$) 4780.54, found 4799.18. Anal. Calcd for $C_{264}H_{448}N_{36}S_{16}$: C, 66.32; H, 9.47; N, 13.48. Found: C, 64.88; H, 9.55; N, 13.23.

DAB-*dendr*-(NHCSNH-Ad)$_{32}$ (2d). DAB-*dendr*-$(NH_2)_{32}$ (0.14 g, 0.04 mmol) dissolved in chloroform (3 mL) was mixed with adamantyl isothiocyanate (0.33 g, 1.72 mmol). Same procedure as for compound **2a**: yield 0.39 g (98%); T_g 113 °C; mp 158 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.40 (s, 4H) 1.56 (br s, 120H), 1.68−1.75 (m, 192H) 2.10−2.13 (m, 288H), 2.41−2.48 (m, 180H), 3.53 (br s, 64H), 6.15 (br s, 32H), 6.94 (br s, 32H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2, 26.4, 29.7, 36.3, 42.2, 43.7, 43.8, 52.0, 52.2, 180.1; IR (ATR) ν (cm^{-1}) 1228.0 (C═S), 2906.3 (C−H sat.), 3277.7 (N−H stretch); MS (MALDI-TOF) calcd ($C_{536}H_{912}N_{94}S_{32}$) 9701.34, found 9700.80. Anal. Calcd for $C_{536}H_{912}N_{94}S_{32}$: C, 66.36; H, 9.49; N, 13.57 Found: C, 64.81; H, 9.43; N, 13.30.

DAB-*dendr*-(NHCSNH-Ad)$_{64}$ (2e). DAB-*dendr*-$(NH_2)_{64}$ (0.93 g, 0.13 mmol) dissolved in chloroform (15 mL) was mixed with adamantyl isothiocyanate (2.00 g, 10.35 mmol). Same procedure as for compound **2a**: yield 2.50 g (85%); T_g 115 °C; mp 159 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.40−1.80 (br s, 636H) 2.10−2.16 (m, 576H), 2.41−2.49 (br s, 372H), 3.52 (br s, 128H), 6.39 (br s, 64H), 6.94 (br s, 64H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7, 29.9, 36.5, 42.3, 52.3, 54.3, 180.3; IR (ATR) ν (cm^{-1}) 1245.8 (C═S), 2905.6 (C−H sat.), 3274.6 (N−H stretch); MS (MALDI-TOF) calcd ($C_{1080}H_{1840}N_{190}S_{64}$) 19542.94, found 19584.52. Anal. Calcd for $C_{1080}H_{1840}N_{190}S_{64}$: C, 66.37; H, 9.51; N, 13.62. Found: C, 65.16; H, 9.63; N, 13.27.

Hexadecyl Isothiocyanate (3). DCC (35.00 g, 0.17 mol) and carbon disulfide (71.00 mL, 1.19 mol) were dissolved in stirred diethyl ether (400 mL). The mixture was cooled to −5 °C on an ice/ethanol bath, and hexadecylamine (40.00 g, 0.17 mol) was added in the cold. The mixture was stirred overnight, allowing the mixture to warm to room temperature. The precipitated dicyclohexylthiourea was filtered off, and solvent and residual carbon disulfide were removed by evaporation. The residue was distilled at 130−135 °C (0.01 mbar) yielding 31.00 g (65%) of the isothiocyanate as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.90 (t, J 6.8 Hz, 3H), 1.25 (br s, 24H), 1.40 (qn, J 6.8 Hz, 2H), 1.69 (qn, J 6.8 Hz, 2H), 3.5 (t, J 6.8 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.3, 22.9, 26.8, 29.1, 29.6−30.2, 32.2, 45.3, 129.9; IR (ATR) ν (cm^{-1}) 2084.4 (N═C═S), 2921.3 (C−H sat.); LRMS calcd ($C_{17}H_{33}NS$) 283.6, found 282; GC 100% purity.

DAB-*dendr*-(NHCSNH-C$_{16}$H$_{33}$)$_4$ (4a). Compound **3** (1.63 g, 5.77 mmol) was dissolved in chloroform (5 mL) and added to DAB-*dendr*-$(NH_2)_4$ (0.33 g, 1.03 mmol) in a round-bottomed flask. The mixture was stirred for 48 h at room temperature and added dropwise to stirred acetone (80 mL). The product was collected by filtration and air-dried: yield 1.25 g (83%); mp 91−93 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.88 (br s, 12H), 1.25 (m, 104H), 1.52−1.58 (m, 12H), 1.75 (br s., 8H), 2.40−2.49 (m, 12 H), 3.48 (br s,16H), 6.28 (br s, 4H), 7.33 (br s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 22.8, 27.1, 29.4, 29.5, 29.7, 29.8, 32.0, 43.6, 45.0, 52.8, 54.0, 181.1; IR (ATR) ν (cm^{-1}) 1214.8 (C═S), 2917.7 (C−H sat.), 3245.8 (N−H stretch); MS (MALDI-TOF) calcd ($C_{84}H_{172}N_{10}S_4$) 1450.9, found 1453.04. Anal. Calcd for $C_{84}H_{172}N_{10}S_4$: C, 69.53; H, 11.97; N, 9.66. Found: C, 69.37; H, 12.18; N, 9.55.

DAB-*dendr*-(NHCSNH-C$_{16}$H$_{33}$)$_8$ (4b). Compound **3** (1.57 g, 5.52 mmol) was dissolved in chloroform (5 mL) and added to DAB-*dendr*-$(NH_2)_8$ (0.379 g, 0.49 mmol), same procedure as for compound **4a**: yield 1.25 g (83%); mp 70−73 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.88 (br t, 24H), 1.25 (m, 208H), 1.57−1.74 (m, 44H), 2.39−2.49 (m, 36H), 3.47 (br s, 32H), 6.32 (br s, 8H), 7.35 (br s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 22.9, 26.8, 27.3, 29.6, 29.7, 29.9, 30.0, 32.2, 43.6, 45.3, 52.0, 52.3, 180.6; IR (ATR) ν (cm^{-1}) 1214.8 (C═S), 2918.0 (C−H sat.), 3247.5 (N−H stretch); MS (MALDI-TOF) calcd ($C_{176}H_{360}N_{22}S_8$) 3042.06, found 3040.36. Anal. Calcd for ($C_{176}H_{360}N_{22}S_8$): C, 69.48; H, 11.95; N, 10.13. Found: C, 69.15; H, 11.95; N, 9.97.

DAB-*dendr*-(NHCSNH-C$_{16}$H$_{33}$)$_{16}$ (4c). Compound **3** (1.53 g, 5.38 mmol) was dissolved in chloroform (5 mL) and added to DAB-*dendr*-$(NH_2)_{16}$ (0.41 g, 0.24 mmol), same procedure as for compound **4a**: yield 1.30 g (87%); mp 67−69 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.88 (br t, 48H), 1.25 (m, 416H), 1.57−1.74 (m, 92H), 2.39−2.49 (m, 84H), 3.49 (br s, 64H), 6.52 (br s, 16H), 7.34 (br s, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 22.9, 26.8, 27.3, 29.0, 29.6, 29.7, 29.8, 29.9, 30.0, 30.2, 43.6, 45.3, 52.1, 52.3, 52.6, 52.8, 181.2; IR (ATR) ν (cm^{-1}) 1214.7 (C═S), 2917.6 (C−H sat.), 3246.3 (N−H stretch); MS (MALDI-TOF) calcd ($C_{360}H_{736}N_{46}S_{16}$) 6224.38, found 6243.00. Anal. Calcd for $C_{360}H_{736}N_{46}S_{16}$: C, 69.46; H, 11.94; N, 10.35. Found: C, 69.12; H, 12.13; N, 10.02.

DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{32}$ (4d). Compound **3** (1.53 g, 5.38 mmol) was dissolved in chloroform (5 mL) and added to DAB-*dendr*-$(NH_2)_{32}$ (0.42 g, 0.12 mmol), same procedure as for compound **4a**: yield 1.35 g (90%); mp 60−63 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.88 (br t, 96H), 1.25 (m, 832H),

1.57−1.73 (m, 188H), 2.40−2.47 (m, 180H), 3.46 (br s, 128H), 6.56 (br s, 32H), 7.40 (br s, 32H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 14.4, 22.9, 26.8, 27.4, 29.6, 29.8, 29.9, 30.0, 32.2, 45.3, 52.3, 52.8, 181.75; IR (ATR) ν (cm^{-1}) 1248.5 (C═S), 2916.8 (C−H sat.), 3246.8 (N−H stretch); MS (MALDI-TOF) calcd ($C_{728}H_{1488}N_{94}S_{32}$) 12589.02, found 12576.90. Anal. Calcd for $C_{728}H_{1488}N_{94}S_{32}$: C, 69.45; H, 11.94; N, 10.46. Found: C, 69.19; H, 12.01; N, 10.36.

DAB-*dendr*-(NHCSNH-$C_{16}H_{33}$)$_{64}$ (4e). Compound **3** (7.50 g, 26.46 mmol) was dissolved in chloroform (100 mL) and added to DAB-*dendr*-$(NH_2)_{64}$ (2.47 g, 0.35 mmol). The mixture was stirred for 48 h at room temperature. The solvent was removed in vacuo. The residue was dissolved in chloroform (25 mL), and the mixture was added dropwise to vigorously stirred acetone (300 mL). The product was collected by filtration and air-dried: yield 7.36 g (84%); mp 82−83 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.88 (br t, 192H), 1.25 (m, 1664H),1.57−1.72 (m, 380H), 2.41−2.50 (m, 372H), 3.46 (br s, 256H), 6.73 (br s, 64H), 7.45 (br s, 64H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 22.9, 27.4, 29.6, 29.8, 29.9, 30.0, 31.1, 32.2, 43.53, 45.3, 52.4, 181.2; IR (ATR) ν (cm^{-1}) 1214.8 (C═S), 2921.0 (C−H sat.), 3247.3 (N−H stretch); MS (MALDI-TOF) calcd ($C_{1464}H_{2992}N_{190}S_{64}$) 25318.30, found 25328.41. Anal. Calcd for $C_{1464}H_{2992}N_{190}S_{64}$: C, 69.45; H, 11.94; N, 10.51. Found: C, 69.08; H, 11.98; N, 10.44.

(3-Hexylureido)acetic Acid Methyl Ester (5). Hexyl isocyanate (2.05 g, 16.12 mmol) and glycine methyl ester hydrochloride (2.12 g, 16.88 mmol) were suspended in dry chloroform (30 mL). The mixture was cooled on an ice bath, and triethylamine (3 mL) was added, creating a clear solution. The mixture was stirred overnight at room temperature. Washing with 1 M HCl (aq, 2 × 30 mL), brine (30 mL), drying (Na_2SO_4), and evaporation of solvent gave a white glasslike residue which was recrystallized from diethyl ether to give 2.40 g (69%) fo the title compound as a white crystalline: mp 50−52 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.89 (t, *J* 7.0 Hz, 3H), 1.25−1.35 (m, 6H), 1.50 (m, 2H), 3.18 (m, 2H), 3.76 (s, 3H), 4.01 (d, *J* 5.5 Hz, 2H), 4.85 (br t, 1H), 5.16 (br t, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.4, 26.5, 30.1, 31.4, 40.3, 41.8, 51.8, 158.4, 171.9; IR (ATR) ν (cm^{-1}) 1618.8 (C═O, urea), 1723.8 (C═O, ester), 3328.2 (N−H stretch); LRMS calcd ($C_{10}H_{20}N_2O_3$) 216.3, found 216.1.

(3-Hexylureido)acetic Acid (6). Compound **5** (1.4 g, 6.47 mmol) was dissolved in a mixture of THF (20 mL) and water (5 mL). Lithium hydroxide monohydrate (0.31 g, 7.38 mmol) was added and the mixture stirred overnight at room temperature. Dilute hydrochloric acid (20 mL, 1.0 M) was added, and the resulting precipitate was filtered off and washed with water. Drying in vacuo afforded 1.25 g (95%) of the title compound as a white powder: mp 153−154 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 0.86 (t, *J* 7.0 Hz, 3H) 1.45−1.20 (m, 8H), 2.95 (m, 2H), 3.70 (d, *J* 5.6 Hz, 2H), 6.00 (br t, 1H), 6.10 (br t, 1H); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 14.4, 22.6, 26.5, 30.4, 31.5, 39.0, 41.9, 158.4, 173.0; IR (ATR) ν (cm^{-1}) 1620.3 (C═O, urea), 3312.6 (N−H stretch); MS (MALDI-TOF) calcd ($C_9H_{18}N_2O_3$) 202.29, found 241.57 (M + K). Anal. Calcd for $C_9H_{18}N_2O_3$: C, 53.43; H, 8.99; N, 13.85. Found: C, 54.12; H, 9.16; N, 13.56.

[3-(6-Hydroxyhexyl)ureido]acetic Acid *tert*-Butyl Ester (7). Di-*tert*-butyl tricarbonate[14] (4.00 g, 15.20 mmol) was dissolved in dry chloroform (90 mL). 6-Aminohexanol (1.80 g, 15.2 mmol) dissolved in dry chloroform (60 mL) was injected into the mixture with rapid stirring (evolution of CO_2). The mixture was stirred for 20 min at room temperature, TLC (ethyl acetate) showed conversion to isocyanate (ninhydrine test shows weak positive). Triethylamine (4 drops) was added to quench excess tricarbonate, glycine *tert*-butyl ester[26] (2.00 g, 15.2 mmol) dissolved in dry chloroform (30 mL) was added, and the mixture was stirred overnight at room temperature. Solvent was removed in vacuo, and polar impurities were removed on a short silica gel coloumn (ethyl acetate/methanol 8:2 mixture). Solvent and residual *tert*-butyl alcohol were removed by evaporation on an oil pump, obtaining the product as a bright yellow viscous oil: yield 4.20 g (quantitative); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.30−1.40 (m, 4H), 1.42−1.56 (m, 13H), 2.21 (br s, 1H), 3.15 (q, *J* = 6.4 Hz, 2H), 3.60 (t, *J* = 6.4 Hz, 2H), 3.86 (d, *J* = 5.4 Hz, 2H), 5.19 (t, *J* = 5.4 Hz, 1H), 5.36 (t, *J* = 5.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 26.5, 28.1, 30.1, 32.5, 40.1, 42.8, 62.2, 81.8, 158.8, 170.9; IR (ATR) ν (cm^{-1}) 1639.0 (C═O, urea), 1736.9 (C═O, ester), 3332.0 (N−H stretch); MS (MALDI-TOF) calcd ($C_{13}H_{26}N_2O_4$) 274.41, found 297.96 (M + Na).

3,4,5-Tridodecyloxybenzoic Acid 6-(3-*tert*-Butoxycarbonylmethylureido)hexyl Ester (8). 3,4,5-Tridodecyloxybenzoic acid (4.00 g, 5.92 mmol), compound **7** (1.80 g, 6.52 mmol), and DMAP (0.072 g, 0.59 mmol) were dissolved in dry chloroform (50 mL). The mixture was cooled on an ice bath, DCC (1.35 g, 6.52 mmol) was added in the cold, and the mixture was stirred for 48 h with warming to room temperature. TLC (pentane/ethyl acetate 8:2) showed conversion to the new adduct. Dicyclohexylurea (DCU) was removed by filtration, and solvent was removed by evaporation. The residue was taken up in diethyl ether (150 mL) and residual DCU filtered off. Traces of DCU were removed on a short silica gel column (diethyl ether): yield 4.2 g (76%) as a white foam; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.89 (t, *J* 6.9 Hz, 9H), 1.20−1.56 (m, 63H), 1.70−1.86 (m, 8H), 3.19 (q, *J* 6.0 Hz, 2H), 3.90 (d, *J* 5.0 Hz, 2H), 4.02 (t, *J* 6.6 Hz, 6H), 4.30 (t, *J* 6.9 Hz, 2H), 4.64 (br t, 1H), 4.92 (br t, 1H), 7.28 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 22.7, 25.7−26.5, 28.8, 29.4−29.8, 30.1−32.0, 39.1, 43.0, 65.0, 69.3, 73.6, 81.9, 108.2, 125.0, 142.5, 152.9, 158.0, 166.6, 170.6; IR (ATR) ν (cm^{-1}) 1581.7 (Ar−H, vibr), 1625.2 (C═O, urea), 1739.2 (C═O, ester), 3342.8 (N−H stretch); MS (MALDI-TOF) calcd ($C_{56}H_{102}N_2O_8$) 931.60, found 954.87 (M + Na).

3,4,5-Tridodecyloxybenzoic Acid 6-(3-Carboxymethylureido)hexyl Ester (9). Compound **8** (0.80 g, 0.86 mmol) was dissolved in dry dichloromethane (10 mL), and the mixture was flushed with nitrogen gas. TFA (10 mL) was added and the mixture stirred 3 h at room temperature. Solvents were removed by evaporation, and the residue was stripped with toluene (4 × 30 mL), obtaining a light gray solid. The product was dissolved in hot ethanol and hot filtered, followed by cooling overnight at 4 °C, and the solid was washed with cold ethanol and air-dried: yield 0.600 g (80%); mp 87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.88 (t, *J* = 7.3 Hz, 9H), 1.20−1.56 (m, 63H), 1.70−1.85 (m, 8H), 3.17 (br t, 2H), 3.95 (br s, 2H), 4.00 (m, 6H,), 4.28 (t, *J* = 6.4 Hz, 2H), 5.30 (br s, 1H), 5.47 (br s, 1H), 7.26 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 22.8, 25.5−28.6, 29.4−29.7, 30.4−32.0, 40.5, 42.7, 65.1, 69.3, 73.6, 108.2, 124.9, 142.6, 152.9, 161.8, 166.9, 180.0; IR (ATR) ν (cm^{-1}) 1577.9 (Ar−H, vibr), 1617.6 (C═O, urea), 3319.9 (N−H stretch); MS (MALDI-TOF) calcd ($C_{52}H_{94}N_2O_8$) 875.48.30, found 898.64 (M + Na). Anal. Calcd for ($C_{52}H_{94}N_2O_8$): C, 71.33; H, 10.84; N, 3.20. Found: C, 71.90; H, 11.04; N, 3.32.

IR Determination of Hydrogen Bonding Depending on Dendrimer Generation. DAB-*dendr*-$(NHCSNH\text{-}Ad)_n$, where *n* = 4, 8, 16, 32, 64 (7 mg), or DAB-*dendr*-$(NHCSNHC_{16}H_{33})_n$ (9.3 mg) was dissolved in $CDCl_3$ (1.00 mL) and put into an IR solution cell. The mixtures were measured, using air as blank scan. In both the adamantyl and the palmityl case, the first-generation functionalized dendrimer needs gentle heating in order to dissolve. The changes in the NH stretch region (3200−3500 cm^{-1}) were investigated.

Formation of Host−Guest Complexes. Crystalline fifth-generation dendrimer (1 equiv) and guest molecule (2−32 equiv), were suspended in dry chloroform (dendrimer concentration approximately 7 mg/mL). The mixture was sonicated for 30 min at rt (gentle heating was sometimes required). Guest compounds **6** and **10**, insoluble in chloroform, gradually dissolved upon complexation with the dendrimer, and a clear solution was formed. The complex was purified using a biobead column, eluting with dichloromethane, or used directly for further investigation.

Competition Experiments. Titration of DAB-*dendr*-

(26) Glycine-*tert*-butyl ester was synthesised analogous to procedures published by: (a) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962−1965. (b) Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411−416.

(NHCONHAd)$_{64}$/DAB-*dendr*-(NHCSNHAd)$_{64}$ with Compound 6. DAB-*dendr*-(NHCONHAd)$_{64}$ (19.8 mg, 0.0011 mmol) or DAB-*dendr*-(NHCSNHAd)$_{64}$ (21.0 mg, 0.0011 mmol) was mixed with, respectively, 0, 2, 4, 8, 16, 24, and 32 equiv of compound **6** and dissolved in $CDCl_3$ (3 mL). One milliliter of each of these solutions was transferred to a NMR tube, and the changes in chemical shifts of protons in the NH region and in the methylene group next to the protonated amino group in the outer shell of the dendrimer (host) were investigated by NMR.[20]

Competition between DAB-*dendr*-(NHCONHAd)$_{64}$ and DAB-*dendr*-(NHCSNHAd)$_{64}$ for Uptake of 32 Guests. DAB-*dendr*-(NHCONHAd)$_{64}$ (6.6 mg, 4 × 10−4 mmol) and DAB-*dendr*-(NHCSNHAd)$_{64}$ (7.0 mg, 4 × 10^{-4} mmol) were dissolved together with 32 equiv of compound **6** (2.3 mg, 0.012 mmol) in $CDCl_3$ (1 mL), and the changes in chemical shifts of protons in the NH region of the host were investigated. To see whether the dendrimers interacted with each other, DAB-*dendr*-(NHCONHAd)$_{64}$ (6.6 mg, 4 × 10−4 mmol) and DAB-*dendr*-(NHCSNHAd)$_{64}$ (7.0 mg, 4 × 10^{-4} mmol) were dissolved in $CDCl_3$ (1 mL) and the change in chemical shifts of protons in the NH region of the dendrimer (host) was investigated by NMR.

Titration of DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{64}$/DAB-*dendr*-(NHCONHC$_{16}$H$_{33}$)$_{64}$ with Compound 6. DAB-*dendr*-(NHCONHC$_{16}$H$_{33}$)$_{64}$ (26 mg, 0.0011 mmol) or DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{64}$ (28 mg, 0.0011 mmol) was mixed with, respectivel,y 0, 2, 4, 8, 16, 24, and 32 equiv of hexylurea guest and dissolved in $CDCl_3$ (3 mL). One milliliter of each of these solutions was transferred to an NMR tube, and the changes in chemical shifts of protons in the NH region, and in the methylene group next to the protonated amino group, in the outer shell of the dendrimer (host) were investigated by NMR.[20]

Competition between DAB-*dendr*-(NHCONHC$_{16}$H$_{33}$)$_{64}$ and DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{64}$ for Uptake of 32 Hexylurea Guests. DAB-*dendr*-(NHCONHC$_{16}$H$_{33}$)$_{64}$ (8.7 mg, 4 × 10−4 mmol) and DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{64}$ (9.3 mg, 4 × 10^{-4} mmol) were dissolved together with 32 equiv of hexylurea guest (2.3 mg, 0.012 mmol) in $CDCl_3$ (1 mL), and the change in chemical shifts in the NH region of the host was investigated. To see whether the dendrimers interacted with each other, DAB-*dendr*-(NHCONHC$_{16}$H$_{33}$)$_{64}$ (6.6 mg, 4 × 10−4 mmol) and DAB-*dendr*-(NHCSNHC$_{16}$H$_{33}$)$_{64}$ (7.0 mg, 4 × 10^{-4} mmol) were dissolved in $CDCl_3$ (1 mL), and the change in chemical shifts of protons in the NH region of the dendrimer (host) was investigated by NMR.

^{1}H NMR T1 Relaxation Experiments. DAB-*dendr*-(NHCSNHAd)$_{64}$ (21.0 mg, 0.0011 mmol) was mixed with, respectively, 0, 2, 4, 8, 16, 24, and 32 equiv of hexylurea guest and dissolved in $CDCl_3$ (3 mL). One milliliter of each of these solutions was transferred to a NMR tube, and the changes in the spin−lattice relaxation time (T1) for protons in the "end" methyl group (δ approx 0.87 ppm), the methylene group next to the urea nitrogen of the guest (δ approx 3.09 ppm), and T1 for protons in the methylene group next to the thiourea nitrogen (δ approx 3.54 ppm) of the dendrimer (host) were investigated.

ITC Microcalorimetry. Microcalorimetric measurements were performed using an Omega titration microcalorimeter from Microcal, Inc. The data analysis were performed using the Origin 5.0 software supplied with the titration calorimeter. Typically, the calorimetric experiment was performed by addition of a chloroform (dry and free of acid) solution of guest **9** (1.99 mM) to the dendrimer (end group concentration 0.40 mM) in chloroform. The dendrimer solution (1.7 mL) was placed in the sample cell, and the temperature was adjusted to 25.0 ± 0.5 °C. The dendrimer solution was titrated with the guest solution in 50 injections of 5 μL each and with 4 min of equilibrium time between each injection. The stirring speed of the syringe was 300 rpm. The compensating power required to maintain thermal equilibrium between the sample cell and reference cell was recorded as a function of time and integrated to yield a plot of enthalpy per injection as a function of molar ratio. The heat evolved during each injection is a function of enthalpy of binding, ΔH and the amount of complex formed during that specific injection. The enthalpy of binding is a function of the concentration of free guest, free host, and guest−host complex and the association constant K_a. A nonlinear least-squares fit (using one set of binding sites) of the equation was performed to estimate the number of binding sites (n) on the dendrimer host, the association constant Ka and the enthalpy of binding ΔH. In our case, the titration curve is too gradual to allow unambiguous estimation of n therefore we have performed curve fitting both with the stoichiometry number n fixed to 1 and when n was allowed to deviate from 1. And the resulting difference in association constant is taken into account in the error presented. Control experiments were performed under identical conditions by injections of the guest compound **9** to chloroform and injections of chloroform to dendrimer host solutions. The integrated heat effects were corrected relative to the control experiments.

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Supporting Information Available: Interpreted ^{1}H and ^{13}C NMR spectra for all new compounds; ^{1}H NMR and IR data on the titration and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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