β-Alanine-Based Dendritic β-Peptides: Dendrimers Possessing Unusually Strong Binding Ability Towards Protic Solvents and Their Self-Assembly into Nanoscale Aggregates through Hydrogen-Bond Interactions

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Abstract: A series of poly(β-alanine) dendrimers **1**–**4** with Boc-carbamate as the surface functionality, β-alanine as the dendritic branch, 3,5-diaminobenzoic acid as the branching agent, and 1,2-diaminoethane as the interior core has been synthesized by a solution-phase peptide-coupling method. The structural identities and purities of the products have been fully characterized by spectroscopic and chromatographic methods. ^{1}H NMR studies on the dendrimers indicated that the Boc-carbamate surface groups exist as a mixture of *syn* and *anti* rotamers in solution, and that the dendrimers adopt an open structure in polar solvents; this allows the free interaction of the interior core functionality with solvent molecules. Due to the cooperative effect of a large number of carbamate and amide groups, the dendrimers exhibit an unusually strong binding ability towards protic solvents and behave as H-bond sponges. As a result, the H/D exchange rates of the N–H protons are significantly enhanced in such dendritic structures, as compared to those of nondendritic carbamates and amides. These dendritic peptide dendrimers also exhibit a strong tendency to form nanoscopic aggregates in nonpolar or polar aprotic solvents through intermolecular H-bond interactions.

Keywords: aggregation · amides · dendrimers · peptides

Introduction

There is an increasing interest in the synthesis of peptide-based[1] (i.e. those in which the amino acid units are directly connected to each other) and α-amino acid based (i.e. those in which the amino acid units are not directly linked to each other) dendrimers[2] owing to their structural resemblance to globular proteins. Such biomimetic dendrimers represent potentially novel drug-delivery agents or bio-drugs with enhanced biocompatibilities. They have also been used as biological mimics for the exploration and understanding of the biological functions of proteins.[3] However, the synthesis of such peptide- or polyamide-based compounds also presents a substantial synthetic challenge because of their high polarity. To facilitate their preparations, amino acid based dendrimers with nonpolar linkages such as C–C [2a] or C–O [2c] bonds to connect the amino acid residues together have been reported.

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We have been interested in using synthetic dendritic systems to model the properties of redox proteins[4] and enzyme molecules.[5] Prompted by the impressive work on dendritic peptides by Zimmerman[1d] and the recent surge of interest in the conformational and biological properties of β-peptides,[6] we began to look into the preparation of dendritic macromolecules constructed from β-amino acid residues and to investigate their peptide-like properties. In fact, β-peptide dendrimers, apart from poly(amidoamine) (PAMAM) dendrimers,[7] have been less well studied than α-peptide dendrimers. Herein, we report a facile solution-phase convergent synthesis of a series of poly(β-alanine) dendrimers **1**–**4**, in which the β-alanine units are connected by amide linkages by using aromatic branching units, and show that they form nonspecific yet discrete self-assembling aggregates of different nanoscopic sizes in various solvent systems. Furthermore, due to the presence of a large number of amide functionalities inside the dendritic structure, they exhibit unusually high cooperative binding abilities towards protic solvents as a result of H-bond interactions relative to their nondendritic counterparts.

Although the formation of well-defined aggregates from carefully pre-designed dendritic subunits has been well documented,[8] the preparation and self-assembly mechanism of nonspecific dendritic aggregates from simple dendritic fragments has been less well studied. This approach deserves

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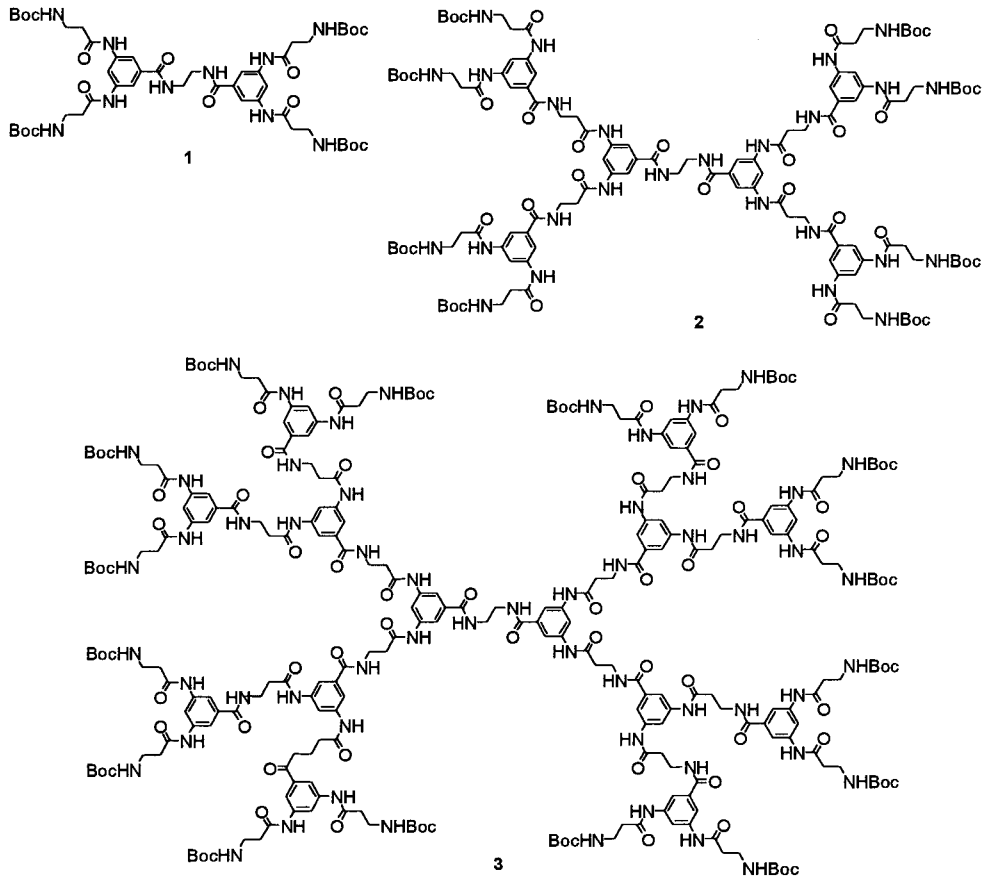


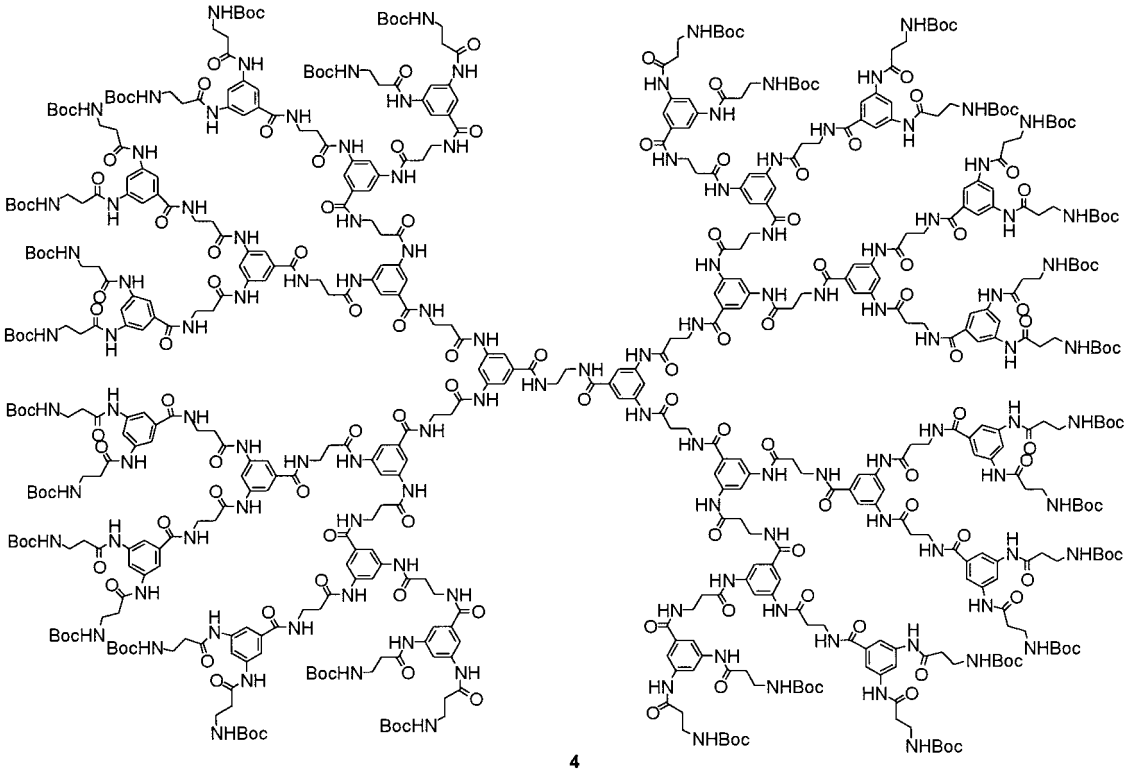
further attention because it allows the construction of nanosystems with novel functions and properties without resorting to elaborate syntheses of highly functionalized subunits. It therefore represents an alternative and efficient strategy for the preparation of large nanoscopic systems. For example, cylindrical and spherical nanostructures have been generated through the self-assembly of flat tapered and conical polyether monodendrons, respectively, presumably as a result of hydrophobic interactions.[9] Highly spherical metallodendrimers of 200 nm in diameter have also been assembled by virtue of the coordination chemistry of AB_2 organopalladium monomers.[10] Aggregates with long fibrous rod-like structures have been prepared from arborols, again through hydrophobic interactions.[11] In this report, we wish to demonstrate that large nanoscopic aggregates may also be generated by hydrogen bonding from this series of poly(β-alanine) dendrimers.

Abstract in Chinese:

本文采用液相多肽合成法制备了一系列以叔丁氧羰基为表面功能基团，β-丙氨酸为连接单元，3,5-二氨基苯甲酸为支化单元，1,2-乙二胺为内核的聚(β-丙氨酸)树状分子 **1**–**4**，其结构和纯度用光谱及色谱方法进行了表征。^{1}HNMR 研究表明分子表面的叔丁氧酰胺基部分在溶液中是以旋转异构体 *syn*- 和 *anti*- 的形式存在；并且，这些树状分子在溶剂中是以开放的结构形式存在，使溶剂分子和树状分子内部的功能团可以自由地相互作用。由于分子中大量氨基甲酸酯基和酰胺基的共同作用，这些树状分子与质子溶剂之间表现出不寻常的氢键作用，其行为如同吸附氢键的海绵体。因此，与非树状结构的氨基甲酸酯基以及酰胺基相比，这些树状分子中的 N-H 中的 H/D 交换速度显著提高。同时，在非极性或极性非质子溶剂中，这些树状分子还通过分子间氢键形成纳米级聚集体。

Results and Discussion

Structural features of the poly(β-alanine) dendrimers 1–4: The target poly(β-alanine) dendrimers consist of β-alanine as the dendritic branch, 3,5-diaminobenzoic acid **5** as the branching agent, and 1,2-diaminoethane **6** as the central core unit. With this design, both the amino and carboxylic acid moieties in the various building blocks could be coupled to β-alanine solely through amide/peptide linkages.[12] Furthermore, we wished to develop a synthetic route that could be extended to allow the incorporation of different amino acid residues into the dendritic architecture if we were to replace the β-alanine residue with different amino acids in the future. The synthetic operation should also be amenable to large-scale preparation of the dendrimers in order to facilitate investigation of their properties. A convergent synthetic scheme[13] was chosen because it provided a better control over the placement of different amino acid residues in different dendritic layers and a better chance of producing structurally perfect dendrimers. To avoid uncontrolled coupling between 3,5-diaminobenzoic acid **5** and β-alanine, selective protection of the amino and carboxylic acid functions was necessary. Our initial goal was to prepare a series of



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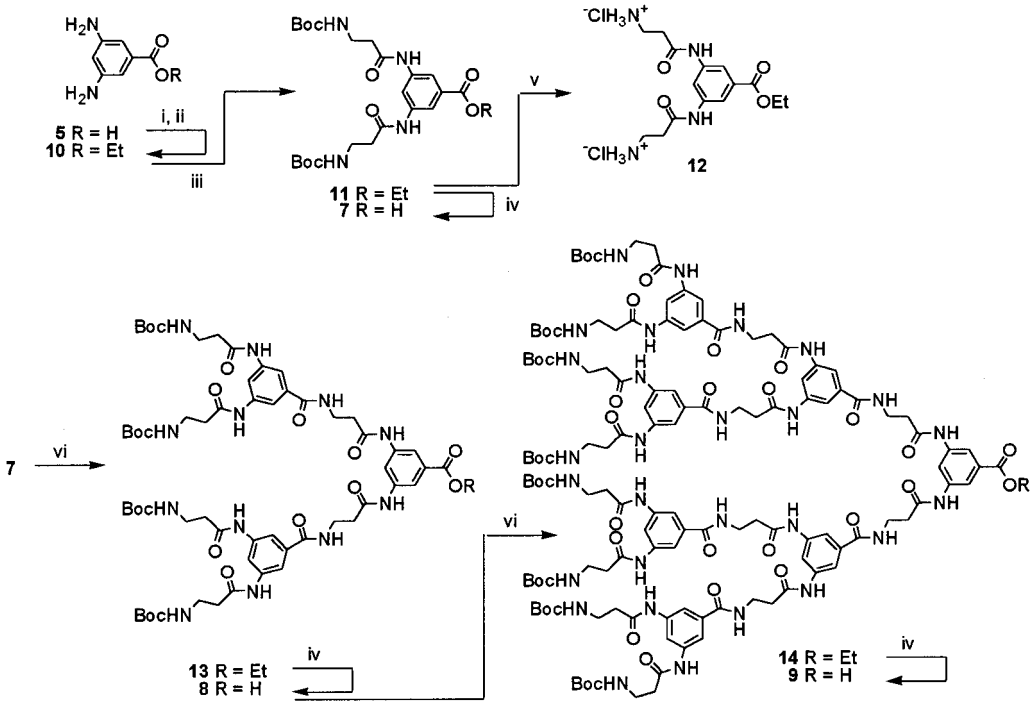
carboxylic acid dendrons **7**–**9**, which were subsequently coupled to the central 1,2-diaminoethane core to furnish the target dendrimers.

Synthesis: Ethyl 3,5-diaminobenzoate **10**, prepared by acid-catalyzed esterification from the acid **5**, was treated with two equivalents of Boc-β-alanine in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[14] to provide the G1-ester **11** as the key AB_2 monomer unit (Scheme 1). Base hydrolysis of the ester group of **11** and subsequent work-up afforded the carboxylic acid **7** as a crystalline solid. Alternatively, the Boc-protecting groups could be removed by treatment of **11** with HCl (1.2 M) to furnish the diammonium salt **12**.



Scheme 1. i) H_2SO_4, EtOH; ii) Na_2CO_3; iii) Boc-β-alanine, EEDQ, THF; iv) NaOH, H_2O, MeOH, THF; v) HCl, EtOH, H_2O; vi) DCC, HOBt, DIPEA, DMF, **12**.

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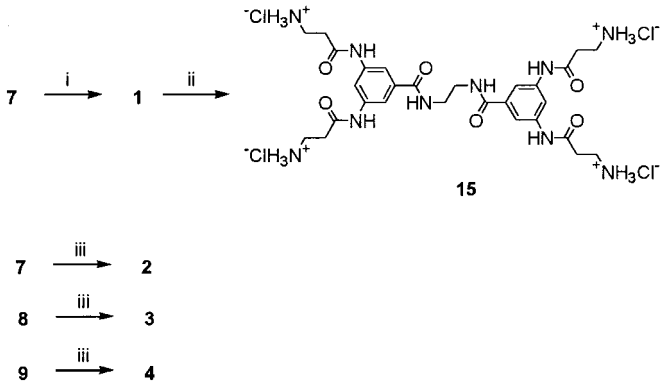
Reaction of **7** and **12** in the presence of EEDQ and triethylamine failed to produce the desired G2-ester dendron **13**. After several attempts, it was found that effective coupling between these two fragments could be realized through the use of dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIPEA) in DMF, providing the G2-ester **13** in 70% yield. Compound **13**, despite having eight amide and four carbamate functionalities, proved to be soluble in a number of organic solvents such as alcohols, THF, and DMF. Ester **13** was then subjected to base hydrolysis to furnish the Boc-protected G2-(carboxylic acid) **8**, which could be obtained in a pure state (74% yield) after flash chromatography on silica gel. By using the same iterative reaction sequence, the Boc-protected G3-ester **14** was synthesized as a white amorphous solid in 70% yield from the carboxylic acid **8** and the diammonium salt **12**. Base hydrolysis of the ester **14** gave the Boc-protected G3-(carboxylic acid) **9** in 70% yield. The overall yield of the Boc-protected G3-ester **14** based on Boc-β-alanine was 23%.

The target dendrimers **1**–**3** could, in principle, be assembled by treating the respective Boc-protected G*n*-(carboxylic acid) dendrons **7**–**9** with 1,2-diaminoethane. In practice, however, a multivalent central core unit **15** was synthesized in order to shorten the synthetic route (Scheme 2). This core unit



Scheme 2. i) DCC, HOBt, DMF, 1,2-diaminoethane **6**; ii) HCl, EtOH, H_2O; iii) DCC, HOBt, DIPEA, DMF, DMSO, **15**.

15 was prepared in two steps from the Boc-protected G1-(carboxylic acid) **7**. First, two equivalents of the dendron **7** were treated with 1,2-diaminoethane **6** in the presence of DCC and HOBt to yield the G1-dendrimer **1** as a solid in 80% yield. Secondly, the four Boc-protecting groups were removed by treatment of **1** with HCl (1.2 M) in aqueous ethanol to produce the tetraammonium salt **15** (80%). The use of this 'hypercore' had the advantage of bypassing the need to synthesize the G4-(carboxylic acid) dendron for use in the preparation of the G4-dendrimer **4**.

The tetraammonium salt **15** was then converted in situ to the corresponding free tetraamine by reaction with four equivalents. of DIPEA, which was then coupled to the respective carboxylic acid dendrons **7**–**9** in the presence of DCC and HOBt to furnish the G2- to G4-dendrimers **2**–**4**. The target compounds were purified by flash chromatography on either neutral alumina or silica gel to remove the by-products. Due to the extremely high polarity of these compounds, substantial loss of material, especially for the higher generation dendrimers, was noted. Hence, the percentage yield of the G2-dendrimer **2** was 70%, while those of the G3- and G4-dendrimers **3** and **4** were both 40%.

Characterization

^{1}H NMR spectroscopy: All of the poly(β-alanine) dendrimers and the relevant intermediates were characterized by ^{1}H and ^{13}C NMR spectroscopy. Because the poly(β-alanine) dendrimers possess a large number of polar amide bonds, they tend to entrap solvent molecules. Generally, the sample was placed under high vacuum at elevated temperature (~80 °C) for two days to remove the residual solvents. This operation was essential in order to obtain a satisfactory NMR spectrum free of solvent interference. Due to the poor solubility of the dendrimers in chloroform, their NMR spectra were recorded in [D_6]DMSO. Because the residual proton signals and the signals of the dissolved water in [D_6]DMSO partially overlapped with the resonance signals of the methylene protons of the β-alanine moiety and those of the central 1,2-diaminoethane core, the relative integrals in this region could not be determined accurately.

The ^{1}H NMR spectra of the various dendrons and dendrimers are shown in Figures 1 and 2, respectively. Despite their large molecular size, the symmetrical architecture of these products simplified the task of peak assignment. Hence, the aromatic proton signals were located in the range $\delta = 7.5$–8.5, while the resonance signals of the methylene protons of the β-alanine were identified in the range $\delta = 2.4$–3.6. Three groups of N–H signals were noted. A set of downfield signals

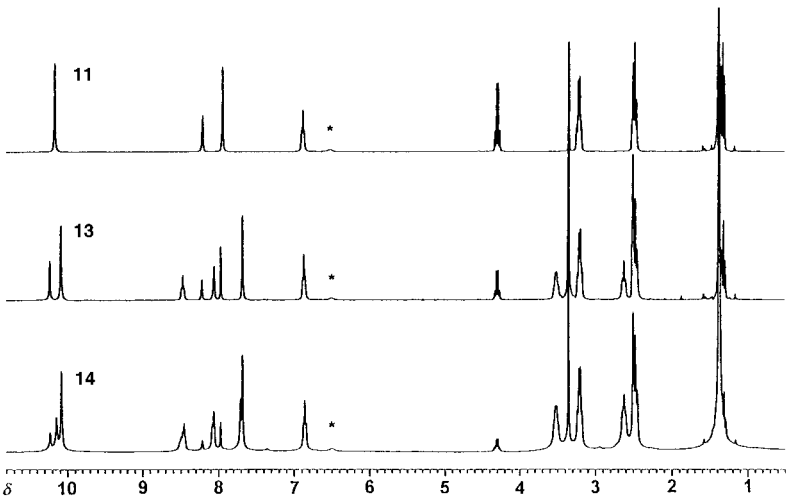


Figure 1. ^{1}H NMR spectra of the various G*n*-ester dendrons **11**, **13**, **14**.

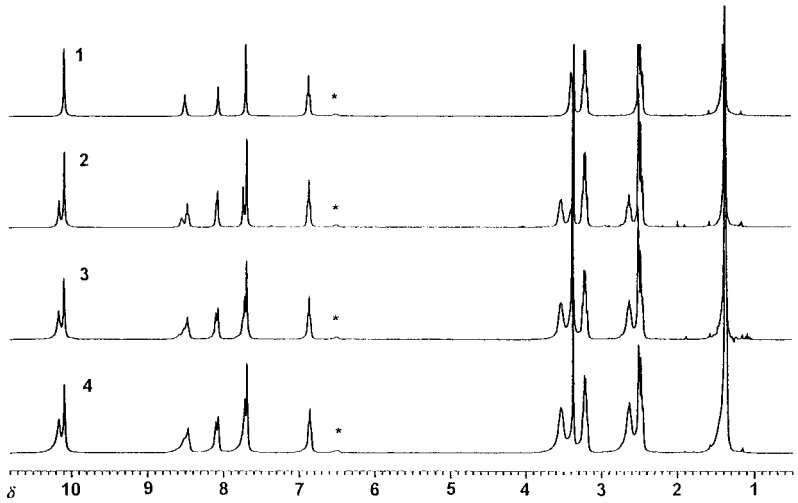


Figure 2. ^{1}H NMR spectra of the various G*n*-dendrimers **1**–**4**.

at $\delta \approx 10$ was attributed to the amide protons adjacent to the aromatic ring, while a second set of signals ($\delta = 8.4-8.6$) could be assigned to the amide protons of the β-alanine unit. On the other hand, the signals of the carbamate N–H appeared at around $\delta = 6.87$. Finally, the *tert*-butyl proton signal of the Boc group appeared as a sharp singlet, invariably at $\delta = 1.37$.

Further examination of the ^{1}H NMR data revealed additional information regarding the gradual change of the dendritic microenvironment with increasing dendrimer generation. First, functional groups of the same nature located on the surface sector of the various generations were found to have almost identical chemical shift values (Table 1). Thus, the resonance signals of the two different aromatic protons located on the surface sector of the various dendrimers and dendrons appear at $\delta \approx 7.68$ and 8.06. Likewise, the surface anilide N–H protons of all the compounds resonate at $\delta =$ 10.08. Secondly, ^{1}H nuclei exhibit a gradual shift of δ value on moving towards the interior core of the dendrimer. In general, ^{1}H nuclei located in the middle layer of the dendrimer are shifted downfield by about 0.03–0.07 ppm when compared to ^{1}H nuclei of the same nature located on the surface sector. This downfield shift is more pronounced for nuclei situated in the inner core (~0.07–0.15 ppm). Similar shifts of NMR signals have also been reported by Meijer et al. and Stoddart et al.[15] in their ^{1}H NMR studies and by Seebach et al. in ^{19}F NMR studies.[16]

Syn and anti rotamers: Upon close scrutiny of Figures 1 and 2, a small signal (labeled *) appears at about 0.35 ppm upfield from the carbamate N–H signal ($\delta = 6.87$), irrespective of the dendrimer generation. The relative integral of this small peak was found to be approximately one-tenth of that of the carbamate signal. A similar finding was also reported by Schlüter et al. in his study on Boc-terminated dendritic building blocks, in which this small signal was proposed as being due to the intermolecular H-bonding dendrimer aggregates.[17] This rationale was supported by the immediate disappearance of the minor signal upon addition of H-bonding solvents such as methanol, which effectively broke-up the intermolecular H-bond interaction.

In the case of the poly(β-alanine) dendritic molecules, the ^{1}H NMR spectra in [D$_6$]DMSO were found to consist of sharp signals. This suggested that there was little or no aggregate formation, at least in DMSO. Although a dynamic laser light scattering (DLLS) study (see later) on the G2-dendrimer **2** did reveal the presence of aggregates in DMSO, they were found to be present at much lower concentration (<2%) than would account for the observed ^{1}H NMR signal intensity (~10%). On the basis of literature data and the results of our own experiments, we conclude that the dominant Boc-carbamate N–H resonance signal at $\delta = 6.87$ is due to the *anti* rotamer, while the smaller upfield signal is caused by the corresponding *syn* rotamer.

The characteristics of *syn*/*anti* rotamers of carbamates in solution has been thoroughly studied by Nudelman et al.[18] Due to the poor solubility of the dendritic poly(β-alanine) molecules in chloroform, we were unable to monitor their ^{1}H NMR characteristics in $CDCl_3$. Hence, an aliphatic carbamate, Boc-heptylamine **16**, was prepared and used as a model compound to examine its NMR spectroscopic behavior in solvent systems (~3% *w*/*v* solution) in which the poly(β-alanine) dendrimers are soluble. The ^{1}H NMR spectral characteristics of compound **16** were found to be similar to the spectra obtained by Nudelman et al. Thus, its ^{1}H NMR spectrum in $CDCl_3$ at room temperature exhibited one N–H proton signal at $\delta = 4.54$. Meanwhile, addition of the donor solvent [D$_6$]DMSO to the $CDCl_3$ solution resulted in a downfield shift and a splitting of the N–H signal. Hence, in $CDCl_3$/[D$_6$]DMSO (3:1, *v*/*v*) solution, two N–H proton signals could be identified at $\delta = 5.54$ and 5.84. In pure [D$_6$]DMSO

Table 1. Selected ^{1}H NMR chemical shifts for the G*n*-dendrimers **2**–**4**, G*n*-(carboxylic acid)s **8**, **9**, and G*n*-esters **13**, **14**.

	G*n*	ArNH			ArCONH			ArH			BocNH	*t*Bu
		surface	middle	inner	surface	middle	inner	surface	middle	inner	surface	surface
2	G2	10.08	–	10.15	8.46	–	8.54	7.68, 8.06	–	7.73, 8.08	6.86	1.37
3	G3	10.09	10.16	10.16	8.47	8.51	8.57	7.69, 8.06	7.71, 8.10	7.74, 8.10	6.86	1.37
4	G4	10.09	10.16	10.16	8.46	8.51	8.57, 8.61	7.69, 8.07	7.72, 8.10	7.72, 8.10	6.86	1.37
8	G2	10.08	–	10.18	8.46	–	–	7.67, 8.07	–	7.94, 8.18	6.86	1.37
9	G3	10.09	10.15	10.18	8.47	–	8.48	7.69, 8.07	7.71, 8.10	7.95, 8.16	6.86	1.37
13	G2	10.09	–	10.23	8.48	–	–	7.68, 8.06	–	7.97, 8.22	6.87	1.37
14	G3	10.08	10.15	10.23	8.46	–	8.50	7.68, 8.06	7.71, 8.08	7.97, 8.21	6.86	1.37

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solution, the two N–H signals were further shifted downfield to $\delta = 6.40$ and 6.75 with a relative integrals of 1:11. The smaller hump was therefore assigned accordingly to the N–H signal of the *syn* rotamer, whilst the larger peak was attributed to the corresponding signal of the *anti* rotamer. Furthermore, addition of acetic acid to the above $CDCl_3$/[D_6]DMSO (3:1, *v*/*v*) solution resulted in a large downfield shift of the *syn*-rotamer N–H protons (Figure 3), which is consistent with the formation of stable intermolecular dimers between the *syn* rotamer of compound **16** and acetic acid.[18]

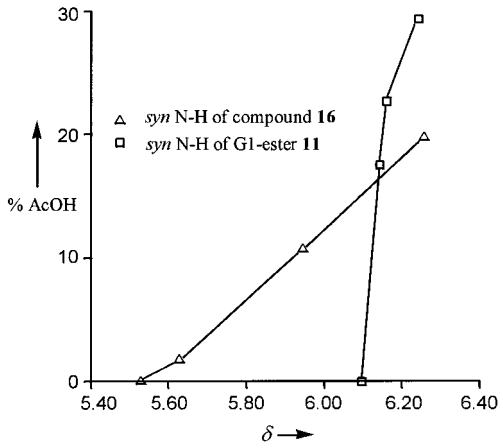

Figure 3. Plot of the chemical shift values (δ) of the *syn*-carbamate N–H protons of compounds **11** and **16** against the % concentration of acetic acid in $CDCl_3$/[D_6]DMSO (3:1, *v*/*v*).

For the series of poly(β-alanine) dendrimers, we chose the Boc-protected G1-ester **11** as a representative in our study due to its lower tendency to form aggregates in solution; the observed NMR behavior could thus be assumed not to be the consequence of intermolecular aggregation. In either $CDCl_3$/[D_6]DMSO (3:1, *v*/*v*) or [D_6]DMSO, two carbamate N–H signals were observed for the G1-ester **11**. In both cases, the predominant N–H signal due to the *anti* rotamer appeared about 0.35 ppm further downfield than that of the *syn* rotamer. Titration of a [D_6]DMSO solution of the G1-ester **11** with acetic acid resulted in a gradual but noticeable downfield shift of the *syn*-carbamate N–H signal. Thus, in all cases the ^{1}H NMR spectroscopic patterns of the G1-ester **11** were found to be similar to those of the model compound **16**. Hence, we conclude that the small peak adjacent to the N–H signal is in fact due to the minor *syn* rotamer, and not due to aggregates.

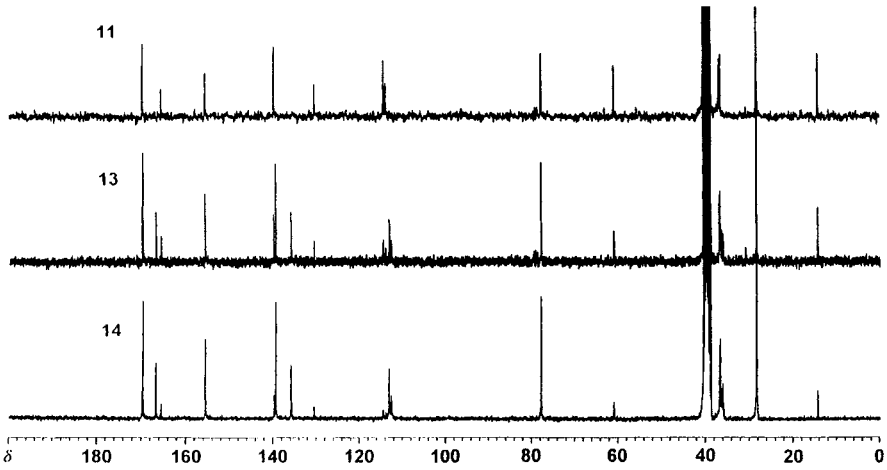

Figure 4. ^{13}C NMR spectra of the various G*n*-ester dendrons **11**, **13**, **14**.

^{13}C NMR spectroscopy: The ^{13}C NMR resonance signals of the various functional entities of the poly(β-alanine) dendrimers were located in distinct spectral regions. Stacked plots of ^{13}C NMR spectra for the poly(β-alanine) dendrons and dendrimers are shown in Figures 4 and 5. Two types of amide carbonyl could be differentiated; one originates from the β-alanine moiety and the other is due to the benzamide brancher. The former gave rise to ^{13}C signals in the range $\delta = 169-171$, the latter at $\delta = 167$. By expanding the spectrum in this region, the β-alanine amide moieties situated at the different locations (i.e., inner, middle, or surface) in the dendrimer could be revealed. On the other hand, the ^{13}C signal of the Boc-carbamate carbonyl was seen at $\delta = 155$. The ^{13}C signals of the aromatic carbons were found in the range $\delta = 110-142$. In the aliphatic region, the tertiary and primary carbons of the prominent *tert*-butyl group resonate at $\delta = 77$ and 28, respectively. The two methylene carbons of the β-alanine branch were observed in the range $\delta = 33-38$. For the G1- to G4-dendrimers **1**–**4**, the ^{13}C signal

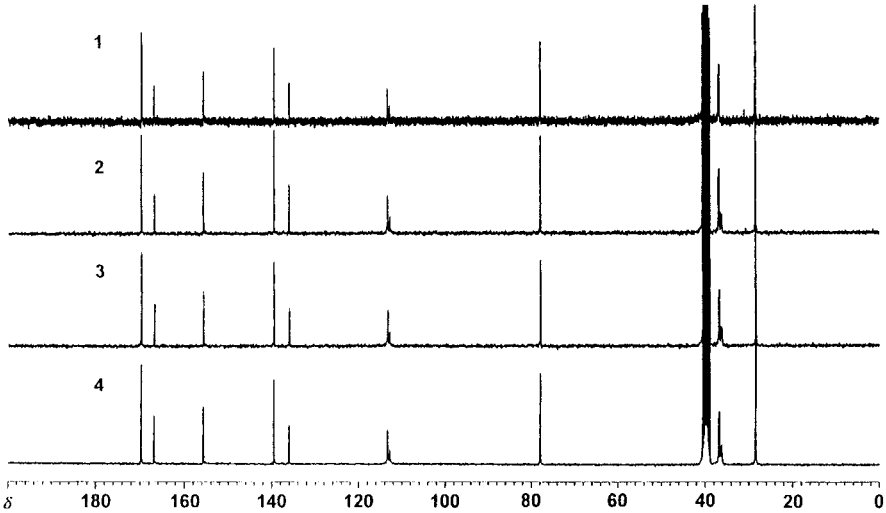

Figure 5. ^{13}C NMR spectra of the various G*n*-dendrimers **1**–**4**.

of the core ethylene unit was found to be obscured by the ^{13}C signals of [D_6]DMSO. However, its presence in the G1- and G2-dendrimers **1** and **2** could be revealed by heteronuclear magnetic correlation resonance (HMQC) spectroscopy.

Mass spectrometry: The development of modern ionization techniques has allowed polymers of high molecular weight (MW) to be conveniently characterized by mass spectrometry. For the poly(β-alanine) dendrimers, the fast-atom bombardment (FAB) technique has proved to be more useful for species with MW <1000 amu, while secondary ion mass spectrometry (L-SIMS) method has been found to provide more satisfactory results for those with MW >3000 amu Unfortunately, both methods failed to detect the molecular ions of poly(β-alanine) dendrimers with MW >4000 amu. For dendrimers for which a mass spectrum could satisfactorily be obtained, molecular ions in the form of $[M]^+$, $[M+H]^+$, $[M+Na]^+$, or $[M+Ag]^+$ could always be detected. Furthermore, a characteristic fragmentation pattern involving the successive loss of Boc groups (100 a.m.u.) from the molecular ion could be noted. This was seen most clearly in the L-SIMS mass spectrum of the G2-dendrimer **2** (Figure 6), in which the abundance of the molecular ion $[M+Na]^+$ was relatively low and was followed by a number of ion fragments with m/z corresponding to $[M+H-(100\times n)]^+$, in which n is the number of Boc groups lost.

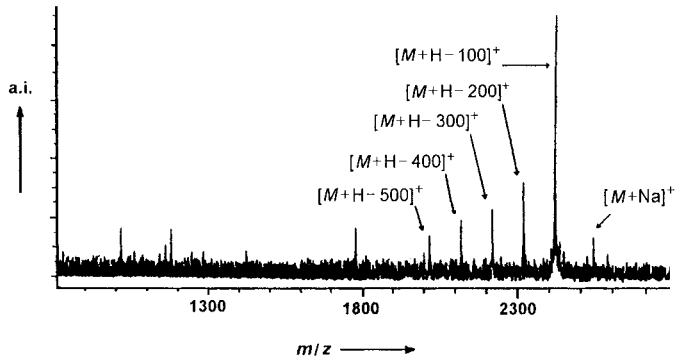

Figure 6. The L-SIMS mass spectrum of the G2-dendrimer **2** showing successive loss of Boc groups.

Gel-permeation chromatography and weight-average molar mass determination: Although all the synthesized dendrimers were isolated and purified by flash chromatography, it was still necessary to assess their purities before studying their physicochemical properties. One of the most convenient methods was to subject the dendrimers to gel-permeation chromatographic analysis (GPC) (solvent: DMF) with laser refractometer (LR)/multi-angle laser light scattering (MALLS) detectors. Both the LR (Figure 7) and MALLS chromatograms of all the dendrimers exhibited one single sigmoidal peak. No defective products or unreacted starting materials could be detected. Furthermore, no peak of lower retention time than the main peak was seen in any of the chromatograms, suggesting the absence of aggregates in DMF. Due to the lack of mass spectral data for the G3- and G4-dendrimers **3** and **4**, the weight-average molar masses (M_w) of the target dendrimers **1**–**4** were determined by MALLS

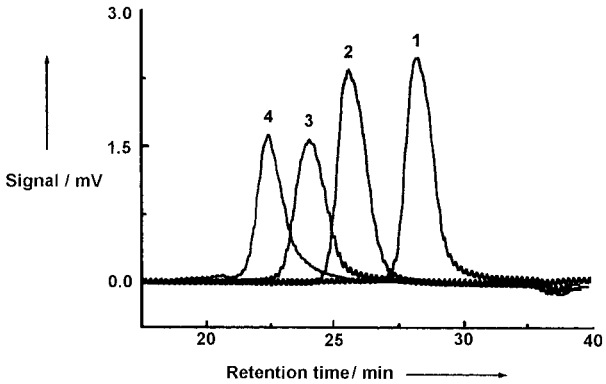

Figure 7. GPC chromatogram (detector: LR) of the G*n* -dendrimers **1**–**4**.

(Table 2). The deviation from the theoretical value was found to be less than 7% for the G2- to G4-dendrimers **2**–**4**, which is within the acceptable error range for the GPC/MALLS technique. The larger deviation in the M_w value for the smallest G1-dendrimer **1** probably stemmed from the relatively weak scattering signal of this small-size molecule.

Table 2. Weight-average molar mass M_w, polydispersity, and hydrodynamic radii of dendrimers **1**–**4**.

	Calcd M_w	Measured M_w[a]	Polydispersity[a]	Hydrodynamic radius [nm][b]
1	1013	1.3×10^3	1.03	–
2	2519	2.8×10^3	1.02	1.6[c]
3	5530	5.6×10^3	1.01	2.1[d]
4	11553	1.1×10^4	1.01	2.8[e]

[a] Determined by GPC with a MALLS detector in DMF as the eluent. [b] Determined by DLLS in DMF solution. [c] Concn = 4.4 mM, a small aggregate peak at around 16 nm was noted. [d] Concn = 1.9 mM, a small, broad aggregate peak at around 60 nm was noted. [e] Concn = 0.9 mM, no higher aggregate was detected.

Properties: Some natural proteins and peptides are able to fold spontaneously from a denatured state to a unique conformation. The driving force behind this folding process involves the participation of various noncovalent interactions. These interactions, in turn, originate from the various structural units residing on the linear amino acid sequence of the native peptide. Among these interactions, hydrogen bonding plays a substantial role in dictating the secondary structure of peptides.

Due to the presence of a large number of N–H and C=O functionalities in our poly(β-alanine) dendrimers, we were intrigued to know whether they could also form intra- and intermolecular H-bonding networks, thereby possibly giving rise to some higher-order secondary structures. In this section, we wish to describe our efforts aimed at probing the presence/absence of inter/intramolecular hydrogen bonding in these dendrimers; we considered this to be of great importance in the elucidation of their solution structures and exploration of their physical and chemical properties.

Solution structure

Proton–deuterium exchange (H/D) experiment: In peptides, hydrogen atoms attached to heteroatoms are sufficiently

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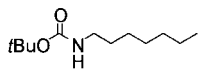
labile to undergo exchange with deuterium provided by deuterated solvents. Lenormant and Blout noted that the rate of exchange was dependent on the local environment of the N–H group.[19] Generally speaking, perseverant or H-bonded N–H protons were found to exchange with protic solvents at a much slower rate than N–H protons exposed to the solvent. To assess the effect of dendrimerization on the N–H H/D exchange behavior, four model compounds were studied—the previously mentioned carbamate **16**, an aliphatic benzamide **17**, an anilide **18**, and a G1-benzamide dendron **19**. The



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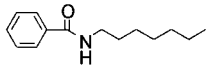

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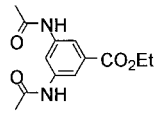

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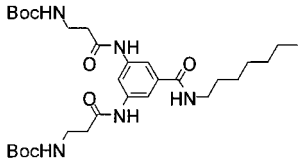

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exchange half-lives ($t_{1/2}$) of the various N–H protons of the model compounds as well as those of the G1- to G4-dendrimers **1**–**4** are given in Table 3. The data were computed by plotting the relative integrals as a function of time, assuming that the H/D exchange process obeyed a pseudo first-order rate law.[20]

For the model compounds, three different types of N–H protons (i.e. the Boc-carbamate, the anilide, and the benzamide N–H) could be identified. Examination of the H/D exchange behavior of the nondendritic models **16**–**18** showed that the $t_{1/2}$ values of the various N–H protons decreased in the following order: Boc-carbamate N–H ($\sim$1000 min) > benzamide N–H ($\sim$500 min) > anilide N–H ($\sim$10 min). The rate of exchange of the anilide N–H was comparatively fast, possibly due to the electronic stabilization effect of the adjacent aromatic ring. The NMR spectra of these compounds consisted of sharp signals, an indication that there was no aggregate formation at the sample concentration range (< 5 %, *w*/*v*) in [D$_6$]DMSO. Therefore, these $t_{1/2}$ values should reflect the "intrinsic" exchange properties of the respective N–H protons in the absence of external factors such as intra- and intermolecular H-bonding effects.

For the G1-benzamide dendron **19**, a hybrid structure that served to bridge the structural features of the simple compounds **16**–**18** and the more complex G1- to G4-dendrimers **1**–**4**, the $t_{1/2}$ values of all the N–H protons, apart from that of the anilide, were found to be slightly lower than the corresponding figures for the model compounds; this indicates that the H/D exchange rates were slightly enhanced in this partially dendrimer-like structure. For the more complex G1- to G4-dendrimers **1**–**4**, the $t_{1/2}$ values of the N–H protons were found to be further reduced, regardless of their location in the dendrimer (i.e. interior or surface). This indicated that the H/D exchange of the N–H protons was further enhanced in a poly(β-alanine) dendritic framework. For example, the $t_{1/2}$ value for the carbamate N–H in the dendrimers decreased to 300 min, while those of the anilide and benzamide N–H protons were significantly reduced to < 20 min. The fast H/D exchange rates of the N–H protons, including those near the interior core of the dendrimer, also suggested that these poly(β-alanine) dendrimers have an open structure with the interior functionalities being fully exposed to the solvent medium.

The enhancement of the H/D exchange rates of the various N–H protons in a dendritic environment, especially those inside the dendrimer core, was unexpected. We attribute this finding to the highly polar internal environment of the dendrimers. Thus, the presence of a large number of carbamate and amide functionalities in a confined space serves as an H-bonding sponge for polar protic molecules such as water. As a result of the strong H-bonding interactions between water molecules and the dendrimers, the local concentration of water in the vicinity and, in particular, in the interior of the dendrimer could be much higher than that in the bulk solvent, thereby accounting for the enhancement in the H/D exchange rates as compared to those of non-dendritic N–H amides and carbamates. This strong water-binding property is also consistent with the elemental analysis data for these poly(β-alanine) dendrimers. It was found that although the analytical data were reproducible for different batches of the same compound, the carbon contents were consistently lower than the expected values due to the inclusion of water. Typically, one to seven water molecules per dendrimer were included, with the higher generation compounds incorporating the largest numbers. The inclusion of water molecules in polar polyamide dendrimers has also been reported by Rosowsky, Voit, and Schlüter.[21]

Temperature coefficients of N–H: The change in the chemical shift value (δ) of N–H protons as a function of temperature (i.e., the temperature coefficient $|\Delta\delta/\Delta T|$) has been employed as a criterion to differentiate intra- from intermolecular hydrogen bonding. Generally, a $|\Delta\delta/\Delta T|$ value of less than 2.0×10^{-3} ppm K^{-1} in [D$_6$]DMSO can be taken to indicate the presence of intramolecular hydrogen bonding. When this coefficient is greater than 4.0×10^{-3} ppm K^{-1}, the existence of intramolecular hydrogen bonds can be excluded.[22] The temperature coefficients of the various N–H protons of the G2-dendrimer **2** in DMSO were therefore determined. Their values were all found to be greater than

Table 3. Exchange half-lives of N–H protons of the G*n*-dendrimers **1**–**4**, aliphatic carbamate **16**, benzamide **17**, anilide **18**, and G1-dendron **19**.

	1	**2**	**3**	**4**	**16**	**17**	**18**	**19**
$t_{1/2}$ of carbamate N–H (min)	300	300	300	200	1000	–	–	400
$t_{1/2}$ of anilide N–H (min)	< 10	< 10	< 10	< 10	–	–	< 10	80
$t_{1/2}$ of benzamide N–H (min)	20	20	20	10	–	500	–	400

6.0×10^{-3} ppm K^{-1} (Figure 8), indicating the absence of intramolecular hydrogen bonding in this solvent and, thus, ruling out the existence of a higher-order, thermodynamically stable secondary structure for these poly(β-alanine) dendrimers in such highly polar solvents.

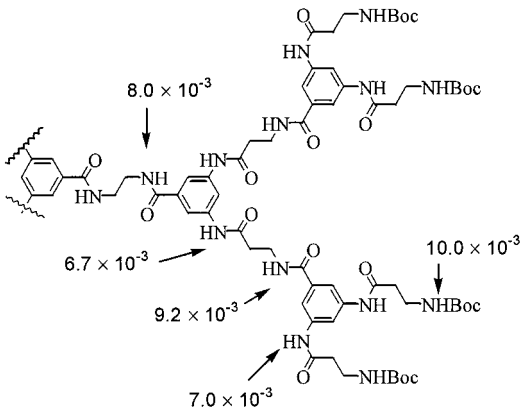

Figure 8. Temperature coefficients (ppm K^{-1}) of G2-dendrimer **2**.

Based on the results of the NMR experiments, the poly(β-alanine) dendrimers are highly solvated in polar solvents and have an open structure that allows the free diffusion of solvent molecules into the interior. The cooperative effect of a large number of highly polar N–H and C=O functionalities within the dendrimer also facilitates the formation of intermolecular hydrogen bonds with polar protic solvents such as water, resulting in an enhancement of the H/D exchange rates.

Aggregation behavior

Dynamic laser light scattering: Recently, several reports dealing with the aggregation and self-association of dendrimers have appeared.[23] The large size and controllable functionalities of dendritic molecules make them ideal building blocks for assembling into large nano- and mesoscopic structures in solutions. On the basis of the aforementioned NMR and GPC studies, both the poly(β-alanine) dendrons and dendrimers appear to be monomeric species in polar aprotic solvents, such as DMSO and DMF. However, such polar dendrimers may be able to form aggregates in less polar solvents. Besides solvent effects, the formation of intermolecular aggregates is also a temperature-dependent process. Here, we wish to report on a study of the aggregation behavior of the poly(β-alanine) dendrimers in various solvent systems and at different temperatures by the more sensitive, dynamic laser light scattering (DLLS) method. This technique has also enabled us to determine the average hydrodynamic radii ($\langle R_h \rangle$) of the dendritic species.

The experimental $\langle R_h \rangle$ values of the G2- to G4-dendrimers **2**–**4** in DMF solution are presented in Table 2. Because of the small size of the G1-dendrimer **1**, its $\langle R_h \rangle$ value could not be determined with accuracy. Each dendrimer exhibited a major peak with an $\langle R_h \rangle$ value slightly larger than that of the PAMAM dendrimer of the same generation.[24] Due to the higher sensitivity of the laser beam used in the DLLS study, in addition to the monomeric peak, an aggregate peak of very low intensity was seen in the hydrodynamic radius distribution diagram for the poly(β-alanine) dendrimers. Thus, the G2-dendrimer **2** in pure DMF solution (4.4 mM) was shown to consist of two narrowly distributed populations at 25 °C (Figure 9). The first peak, located at 1–2 nm, corresponded to

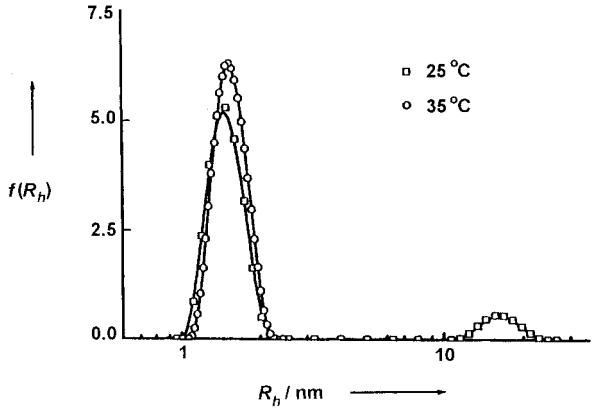

Figure 9. Temperature dependence of the hydrodynamic radius distribution $f(R_h)$ of G2-dendrimer **2** (4.4 mM) in DMF (scattering angle = 15°).

the expected monomeric species. The second peak, located at 10–20 nm, was due to some higher molar mass intermolecular aggregates, which disappeared at slightly elevated temperature (35 °C). Judging from the relative areas of the peaks, the amount of aggregate was very small. The scarcity of the aggregates at 25 °C also accounts for the fact that they were not detectable by the less sensitive GPC on-line MALLS/RI technique. Likewise, an aggregation peak with an even broader distribution centered at around 60 nm was seen in the distribution diagram for the G3-dendrimer **3**. However, no aggregation peak was found for the G4-dendrimer **4**, possibly due to a higher polydispersity and a lower population of the aggregates.

Solvent dependence: The aggregation behavior of the poly(β-alanine) dendrimers was also investigated in different solvent systems. Upon addition of a small amount of protic solvent such as methanol (7% *v*/*v* in DMF), the aggregate peak of the G2-dendrimer **2** gradually disappeared (Figure 10). There

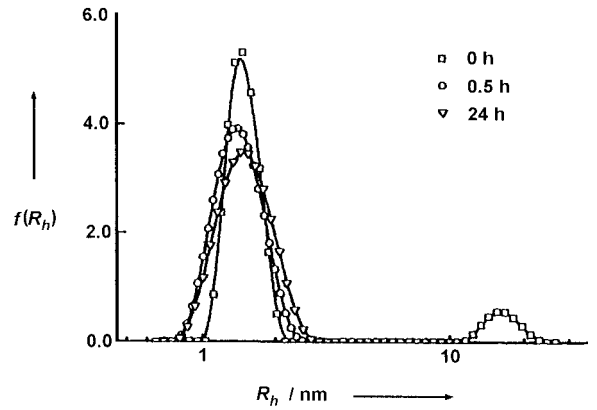

Figure 10. Time dependence of the hydrodynamic radius distribution $f(R_h)$ after addition of methanol to a DMF solution of the G2-dendrimer **2** (4.4 mM) at 25 °C (scattering angle = 15°).

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fore,H-bond donor solvents effectively break-up the intermolecular H-bonding network and stabilize the monomeric state. On the other hand, the DLLS distribution diagram changed dramatically when a nonpolar solvent such as chloroform was added to the DMF solution of the G2-dendrimer **2** (Figure 11). In a 33% (*v*/*v*) $CHCl_3$/DMF

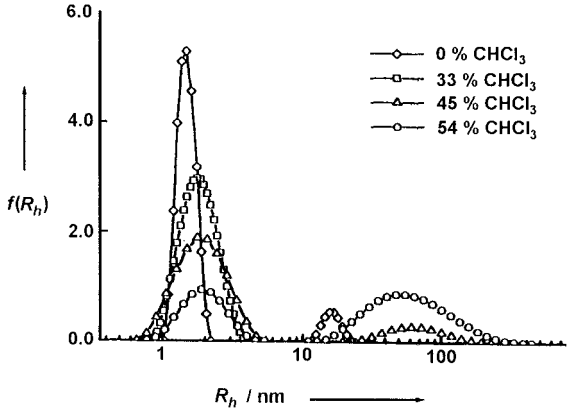

Figure 11. Solvent dependence of the hydrodynamic radius distribution $f(R_h)$ after addition of chloroform to a DMF solution of the G2-dendrimer **2** (2.8 mM) at 25 °C (scattering angle = 15°).

solution, the aggregate peak originally situated at 16 nm disappeared, while another aggregate peak with a larger $\langle R_h \rangle$ value (~56 nm) emerged when the amount of $CHCl_3$ was increased to 45% (*v*/*v*). In other words, the poly(β-alanine) dendrimers self-assembled into larger aggregates in a nonpolar aprotic solvent. This phenomenon could also be confirmed by a ^{1}H NMR study. Solvent titration of solutions of G2- to G4-dendrimers **2**–**4** (~2 to 8 mM range) in [D_6]DMSO or [D_7]DMF with $CDCl_3$ resulted in significant broadening of ^{1}H NMR signals, indicating the formation of intermolecular aggregates in nonpolar solvents.

Conclusion

We have developed a convenient method for the synthesis of a series of highly polar poly(β-alanine) dendrimers from the first to the fourth generation based on a solution-phase coupling procedure. This synthetic operation has proved to be more efficient than the solid-phase method in terms of product yield and homogeneity. Based on ^{1}H NMR studies, these poly(β-alanine)-based dendritic species have been shown to have an open structure in polar aprotic solvents, which allows the free interaction of the interior functionalities with solvent molecules. Due to the presence of a large number of carbamate and amide groups, the dendrimers act as H-bonding sponges, which strongly bind protic solvents such as water. As a consequence, the H/D exchange rates of the N–H protons are significantly enhanced in such dendritic structures as compared with those of nondendritic carbamates and amides. These peptide dendrimers also form intermolecular aggregates of nanoscopic size in both nonpolar and polar aprotic solvents. Furthermore, the size of the aggregates has been shown to be dependent on the nature of the solvent as well as on the dendrimer generation. The aggregates broke up at elevated temperatures or in the presence of H-bond donor solvents. Work is underway aimed at studying the properties of the Boc-deprotected native poly(β-alanine) dendrimers.

Experimental Section

Melting points were measured on an Electrothermal 9100 melting point apparatus and are uncorrected. ^{1}H (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were acquired on a Bruker Advance DPX spectrometer and were recorded in [D_6]DMSO unless otherwise stated. Mass spectrometry was performed either by fast atom bombardment (FAB) on a Hewlett Packard 5989B mass spectrometer or by the liquid secondary ionization mass spectrometry (L-SIMS) method on a Bruker APEX 47E FTMS model spectrometer. The reported molecular mass (*m*/*z*) values refer to monoisotopic masses. Elemental analyses were carried out at MEDAC (UK). Unless otherwise stated, all chemicals were purchased from either Acros or Aldrich and were used without further purification. DMF and DMSO were stirred over calcium hydride overnight and then distilled in vacuo. THF was distilled from sodium and benzophenone. Thin-layer chromatography was performed on Merck silica gel 60 F_{254} plates and spots were visualized by UV illumination. Flash chromatography was carried out on Macherey Nagel silica gel (230–400 mesh) or active Merck aluminium oxide 90, neutral grade (70–200 mesh).

Ethyl 3,5-diaminobenzoate (10): A suspension of 3,5-diaminobenzoic acid **5** (7.0 g, 46 mmol) in ethanol (300 mL) and 98% sulfuric acid (14 mL) was heated to reflux for 18 h. The organic solvent was then removed on a rotary evaporator and the residual syrup was diluted with iced water (200 mL) and made slightly alkaline by the addition of powdered Na_2CO_3. The resulting mixture was extracted with EtOAc (3 × 150 mL) and the combined organic phases were washed with water (2 × 300 mL), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified by flash chromatography on silica gel (eluent: EtOAc/hexane 1:1) to give the ester **10** (7.5 g, 90%) as an amber liquid. ^{1}H NMR: $\delta = 1.27$ (t, ^3J(H,H) = 7 Hz, 3H; CH_3), 4.21 (q, ^3J(H,H) = 7 Hz, 2H; CH_2), 5.00 (s, 4H; NH_2), 6.03 (t, ^4J(H,H) = 2 Hz, 1H; ArH), 6.44 (d, ^4J(H,H) = 2 Hz, 2H; ArH); ^{13}C NMR: $\delta = 14.5$, 60.2, 103.8, 131.1, 149.6, 167.0; MS (FAB): *m*/*z* (%): 181 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_9H_{12}N_2O_2$ (180.2): C 59.99, H 6.71, N 15.55; found C 59.82, H 6.66, N 15.76.

Boc-protected G1-ester dendron (11): A mixture of Boc-β-alanine (10.5 g, 56 mmol) and EEDQ (14.0 g, 56 mmol) was added to a solution of the ester **10** (5.0 g, 28 mmol) in THF (200 mL) at 20 °C. After 24 h, the solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate (300 mL). The product **11** slowly precipitated from the solution and was subsequently collected by filtration and washed with EtOAc/diethyl ether (1:1) to give a white solid (11.7 g, 80%). M.p. 189–190 °C; ^{1}H NMR: $\delta = 1.32$ (t, ^3J(H,H) = 7 Hz, 3H; CH_3), 1.38 (s, 18H; *t*Bu), 2.48 (t, ^3J(H,H) = 7 Hz, 4H; CH_2CO), 3.22 (q, ^3J(H,H) = 7 Hz, 4H; NCH_2), 4.31 (q, ^3J(H,H) = 7 Hz, 2H; OCH_2), 6.89 (t, ^3J(H,H) = 7 Hz, 2H; carbamate-NH), 7.95 (s, 2H; ArH), 8.22 (s, 1H; ArH), 10.17 (s, 2H; NHAr); ^{13}C NMR: $\delta = 14.4$, 28.4, 36.6, 36.9, 61.0, 77.8, 114.1, 114.6, 130.6, 139.9, 155.7, 165.7, 169.9; MS (FAB): *m*/*z* (%): 522 (25) $[M]^+$; elemental analysis calcd (%) for $C_{25}H_{38}N_4O_8$ (522.6): C 57.46, H 7.33, N 10.72; found C 57.30, H 7.26, N 10.73.

General procedure I--hydrolysis of ethyl esters: An aqueous solution of NaOH (1 M, 20 mL, 20 mmol) was added to a solution of the Boc-protected G*n*-CO_2Et (4 mmol) in MeOH/THF (1:1, 100 mL) at 20 °C. The progress of the reaction was monitored by TLC. When the hydrolysis was complete (12–36 h), the excess solvents were removed on a rotary evaporator, and the residue was taken up in a mixture of 10% aqueous citric acid (200 mL) and EtOAc (200 mL) with vigorous stirring. The organic phase was separated and then washed with water (2 × 200 mL). The Boc-protected carboxylic acid dendron G*n*-CO_2H subsequently precipitated from the organic phase and was collected by filtration. Purification by flash chromatography (for details, see below) was required for the G2- and G3-CO_2H dendrons **8** and **9**.

Boc-protected G1-(carboxylic acid) dendron (7): General procedure I: Starting from Boc-protected G1-ester **11** (10.0 g, 20 mmol), after 12 h under hydrolytic conditions the target compound **7** was isolated as a white solid (9.0 g, 90%) after filtration. M.p. 199–200 °C; ^{1}H NMR (COOH not

observed): $\delta = 1.38$ (s, 18H; *t*Bu), 2.48 (t, $^3J(H,H) = 7$ Hz, 4H; CH_2CO), 3.22 (q, $^3J(H,H) = 7$ Hz, 4H; NCH_2), 6.88 (t, $^3J(H,H) = 7$ Hz, 2H; carbamate-NH), 7.93 (s, 2H; ArH), 8.17 (s, 1H; ArH), 10.13 (s, 2H; NHAr); ^{13}C NMR: $\delta = 28.4$, 36.7, 37.0, 77.8, 113.9, 115.0, 131.6, 139.8, 155.7, 167.3, 169.9; MS (FAB): *m*/*z* (%): 517 (17) $[M+Na]^+$; HRMS (L-SIMS): *m*/*z*: 495.2411 ($C_{23}H_{35}N_4O_8$ requires 495.2446).

Boc-protected G2-(carboxylic acid) dendron (8): General procedure I: Starting from Boc-protected G2-ester **13** (5.0 g, 4.0 mmol), after 24 h under hydrolytic conditions the target compound **8** (3.7 g, 74%) was obtained as a white glassy solid after purification by flash chromatography on silica gel (eluent: EtOAc/EtOH, 12:1, gradient to 8:1). ^{1}H NMR (COOH not observed): $\delta = 1.37$ (s, 36H; *t*Bu), 2.45–2.51 (m, 8H; surface CH_2CO), 2.62 (t, $^3J(H,H) = 7$ Hz, 4H; inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 8H; surface NCH_2), 3.52 (q, $^3J(H,H) = 7$ Hz, 4H; inner NCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 4H; carbamate-NH), 7.67 (s, 4H; surface ArH), 7.94 (s, 2H; inner ArH), 8.07 (s, 2H; surface ArH), 8.18 (s, 1H; inner ArH), 8.46 (t, $^3J(H,H) = 7$ Hz, 2H; ArCONH), 10.08 (s, 4H; surface NHAr), 10.18 (s, 2H; inner NHAr); ^{13}C NMR: $\delta = 28.5$, 36.1, 36.4, 36.7, 36.9, 77.9, 112.8, 113.3, 113.8, 115.0, 136.0, 139.5, 139.8, 155.7, 166.9, 167.3, 169.8, 169.9; MS (L-SIMS): *m*/*z* (%): 1269.6 (60) $[M+Na]^+$; elemental analysis calcd (%) for $C_{59}H_{82}N_{12}O_{18}$ (1247.4): C 56.81, H 6.63, N 13.47; found C 56.47, H 6.79, N 13.12.

Boc-protected G3-(carboxylic acid) dendron (9): General procedure I: Starting from Boc-protected G3-ester **14** (8.0 g, 2.9 mmol), after 36 h under hydrolytic conditions the target product **9** (5.5 g, 70%) was obtained as a white amorphous solid after purification by flash chromatography on silica gel (eluent: EtOAc/EtOH, 10:1, gradient to 6:1). ^{1}H NMR (COOH not observed): $\delta = 1.37$ (s, 72H; *t*Bu), 2.45–2.53 (m, 16H, surface CH_2CO), 2.63 (t, $^3J(H,H) = 7$ Hz, 12H; middle and inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 16H; surface NCH_2), 3.50–3.55 (m, 12H; middle and inner NCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 8H; carbamate-NH), 7.69 (s, 8H; surface ArH), 7.71 (s, 4H; middle ArH), 7.95 (s, 2H; inner ArH), 8.07 (s, 4H; surface ArH), 8.10 (s, 2H; middle ArH), 8.16 (s, 1H; inner ArH), 8.47 (t, $^3J(H,H) = 7$ Hz, 4H; surface ArCONH), 8.48 (t, $^3J(H,H) = 7$ Hz, 2H; inner ArCONH), 10.09 (s, 8H; surface NHAr), 10.15 (s, 4H; middle NHAr), 10.18 (s, 2H; inner NHAr); ^{13}C NMR: $\delta = 28.4$, 36.1, 36.4, 36.7, 36.9, 77.8, 112.8, 113.3, 136.0, 139.5, 155.7, 166.8, 169.7, 169.8, 169.9; MS (L-SIMS): *m*/*z* (%): 2774 (60) $[M+Na]^+$; elemental analysis calcd (%) for $C_{131}H_{178}N_{28}O_{38}\cdot 2H_2O$ (2789.1): C 56.41, H 6.58, N 14.06; found C 56.05, H 6.75, N 13.88.

Boc-protected G1-dendrimer (1): DCC (1.13 g, 5.5 mmol) was added to a stirred mixture of the G1-(carboxylic acid) dendron **7** (2.5 g, 5.0 mmol), HOBt (0.74 g, 5.5 mmol), and 1,2-diaminoethane (0.17 mL, 2.5 mmol) in DMF (150 mL) at 0 °C. The mixture was stirred at 0 °C for 12 h and then at room temperature for an additional 24 h. It was then filtered and the solvents were removed in vacuo. The residue was redissolved in EtOAc/EtOH (12:1, 200 mL) and the resulting solution was washed successively with 5% $KHSO_4$ (2 × 200 mL), water (2 × 200 mL), and brine (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated to dryness on a rotary evaporator. The residue was chromatographed on silica gel (eluent: EtOAc/EtOH/THF, 14:1:1) to give the target G1-dendrimer **1** (2.0 g, 80%) as a white amorphous solid. ^{1}H NMR: $\delta = 1.38$ (s, 36H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 8H; CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 8H; NCH_2), 3.35–3.43 (m, 4H; core NCH_2), 6.87 (t, $^3J(H,H) = 7$ Hz, 4H; carbamate-NH), 7.69 (s, 4H; ArH), 8.06 (s, 2H; ArH), 8.50 (t, $^3J(H,H) = 7$ Hz, 2H; ArCONH), 10.09 (s, 4H; NHAr); ^{13}C NMR (core NC overlapped with solvent signals): $\delta = 28.4$, 36.7, 36.9, 77.8, 112.8, 113.3, 136.0, 139.5, 155.7, 167.0, 169.8; MS (L-SIMS): *m*/*z* (%): 1035.5 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{48}H_{72}N_{10}O_{14}$ (1013.2): C 56.90, H 7.16, N 13.82; found C 56.30, H 6.70, N 13.99.

General procedure II—removal of the Boc group: An ethanolic solution of HCl (1.2 M, 20 mL, 24 mmol) was added to a solution of the Boc-protected compound (4 mmol) in EtOH/H_2O (2:1, 100 mL) at 40 °C. The progress of the reaction was monitored by withdrawing aliquots and examining them for complete disappearance of the *tert*-butyl signal by ^{1}H NMR spectroscopy. Upon complete cleavage of the Boc group, the solvent was removed in vacuo and the ammonium salt was precipitated from an EtOH/H_2O mixture (8:1, *v*/*v*).

Ethyl 3,5-di(*β*-aminopropionamido)benzoate dihydrochloride (12): General procedure II: Starting from Boc-protected G1-ester dendron **11** (4.0 g, 8 mmol), after 12 h of reaction the diammonium salt **12** (2.5 g, 80%) was isolated as a white amorphous solid. ^{1}H NMR: $\delta = 1.31$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 2.79 (t, $^3J(H,H) = 7$ Hz, 4H; CH_2CO), 3.07 (t, $^3J(H,H) = 7$ Hz, 4H; NCH_2), 4.31 (q, $^3J(H,H) = 7$ Hz, 2H; CH_2), 8.02 (s, 2H; ArH), 8.05–8.20 (brs, 6H; N^+H_3), 8.20 (s, 1H; ArH), 10.60 (s, 2H; NHAr); ^{13}C NMR: $\delta = 14.4$, 33.5, 35.0, 61.1, 114.2, 115.0, 130.7, 139.7, 165.7, 168.9; MS (FAB): *m*/*z*: 323 (100) $[M-2HCl+H]^+$; HRMS (L-SIMS): *m*/*z*: 323.1722 ($C_{15}H_{23}N_4O_4$ requires 323.1714).

Tetraammonium core (15): General procedure II: Starting from the Boc-protected G1-dendrimer **1** (2.0 g, 2.0 mmol), after 24 h of reaction the product **15** (1.2 g, 80%) was obtained as a white hygroscopic amorphous solid. ^{1}H NMR: $\delta = 2.80$ (t, $^3J(H,H) = 7$ Hz, 8H; CH_2CO), 3.07 (q, $^3J(H,H) = 7$ Hz, 8H; NCH_2), 3.40 (brs, 4H; core NCH_2), 7.74 (s, 4H; ArH), 8.10 (s, 2H; ArH), 8.16 (brs, 12H; N^+H_3), 8.53 (s, 2H; ArCONH), 10.52 (s, 4H; NHAr); ^{13}C NMR: $\delta = 33.5$, 35.1, 113.1, 113.8, 136.1, 139.3, 167.0, 168.7; MS (FAB): *m*/*z* (%): 613.32 (100) $[M-4HCl+H]^+$; HRMS (L-SIMS): *m*/*z*: 613.3207 ($C_{28}H_{41}N_{10}O_6$ requires 613.3202).

General procedure III—synthesis of Boc-protected ester dendrons: A solution of the diammonium salt **12** (1.0 g, 2.5 mmol) and DIPEA (0.87 mL, 5.0 mmol) in DMF (25 mL) was added in one portion to a stirred solution of the appropriate carboxylic acid dendron (5.0 mmol) and HOBt (0.74 g, 5.5 mmol) in DMF (150 mL) at 0 °C. DCC (1.13 g, 5.5 mmol) was then added and the reaction mixture was stirred at 0 °C for 12 h and thereafter at room temperature for a further 36–60 h. The reaction mixture was then filtered and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in EtOAc/EtOH (12:1, 200 mL) and the resulting solution was washed successively with saturated $NaHCO_3$ (2 × 200 mL), 5% $KHSO_4$ (2 × 200 mL), and water (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated on a rotary evaporator. The concentrate was further purified by flash chromatography.

Boc-protected G2-ester dendron (13): General procedure III: Starting from G1-(carboxylic acid) dendron **7** (2.5 g, 5.0 mmol), after 48 h of reaction the crude product was purified by flash chromatography on silica gel (eluent: EtOAc/EtOH/THF, 20:1:1, gradient to 12:1:1) to give the product **13** (2.2 g, 70%) as a white amorphous solid. ^{1}H NMR: $\delta = 1.31$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 1.37 (s, 36H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 8H; surface CH_2CO), 2.62 (t, $^3J(H,H) = 7$ Hz, 4H; inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 8H; surface NCH_2), 3.52 (q, $^3J(H,H) = 7$ Hz, 4H; inner NCH_2), 4.31 (q, $^3J(H,H) = 7$ Hz, 2H; OCH_2), 6.87 (t, $^3J(H,H) = 7$ Hz, 4H; carbamate-NH), 7.68 (s, 4H; surface ArH), 7.97 (s, 2H; inner ArH), 8.06 (s, 2H; surface ArH), 8.22 (s, 1H; inner ArH), 8.48 (t, $^3J(H,H) = 7$ Hz, 2H; ArCONH), 10.09 (s, 4H; surface NHAr), 10.23 (s, 2H; inner NHAr); ^{13}C NMR: $\delta = 14.4$, 28.4, 36.1, 36.4, 36.7, 36.9, 61.0, 77.8, 112.8, 113.3, 114.1, 114.7, 130.7, 136.0, 139.5, 139.9, 155.7, 165.7, 166.9, 169.7, 170.0; MS (L-SIMS): *m*/*z* (%): 1297.6 (50) $[M+Na]^+$; elemental analysis calcd (%) for $C_{61}H_{86}N_{12}O_{18}\cdot H_2O$ (1293.4): C 56.65, H 6.86, N 12.99; found C 56.77, H 6.77, N 13.31.

Boc-protected G3-ester dendron (14): General procedure III: Starting from the G2-(carboxylic acid) dendron **8** (6.2 g, 5.0 mmol), after 72 h of reaction the crude product was purified by flash chromatography on silica gel (eluent: EtOAc/EtOH/THF, 14:1:1, gradient to 6:1:1) to yield the final product **14** (4.8 g, 70%) as a white amorphous solid. ^{1}H NMR: $\delta = 1.31$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 1.37 (s, 72H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 16H; surface CH_2CO), 2.62 (brs, 12H; middle and inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 16H; surface NCH_2), 3.52 (q, $^3J(H,H) = 7$ Hz, 12H; middle and inner NCH_2), 4.30 (q, $^3J(H,H) = 7$ Hz, 2H; OCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 8H; carbamate-NH), 7.68 (s, 8H; surface ArH), 7.71 (s, 4H; middle ArH), 7.97 (s, 2H; inner ArH), 8.06 (s, 4H; surface ArH), 8.08 (s, 2H; middle ArH), 8.21 (s, 1H; inner ArH), 8.46 (t, $^3J(H,H) = 7$ Hz, 4H; surface ArCONH), 8.50 (t, $^3J(H,H) = 7$ Hz, 2H; inner ArCONH), 10.08 (s, 8H; surface NHAr), 10.15 (s, 4H; middle NHAr), 10.23 (s, 2H; inner NHAr); ^{13}C NMR: $\delta = 14.4$, 28.4, 36.1, 36.4, 36.6, 36.7, 36.9, 37.1, 61.0, 77.9, 112.8, 113.3, 114.1, 114.7, 130.7, 136.0, 139.5, 140.0, 155.7, 165.7, 166.9, 169.7, 169.8, 170.0; MS (L-SIMS): *m*/*z* (%): 2802.2 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{133}H_{182}N_{28}O_{38}\cdot H_2O$ (2799.1): C 57.07, H 6.63, N 14.01; found C 56.72, H 6.77, N 13.62.

General procedure IV—syntheses of Boc-protected G2- to G4-dendrimers (2–4): A solution of the tetraammonium salt **15** (1 equiv) and DIPEA (5 equiv) in DMSO (20 mL) was added in one portion to a mixture of the G*n*-(carboxylic acid) dendron (5 equiv) and HOBt (5 equiv) in DMF

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(150 mL). DCC (5 equiv) was then added and the reaction mixture was stirred at room temperature for 48–72 h. The precipitate was filtered off and the solvents were removed in vacuo to leave a syrup, which was redissolved in EtOAc/EtOH (10:1, 200 mL). The organic phase was then washed with 5% aqueous $KHSO_4$ (2 × 200 mL) and water (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated on a rotary evaporator. The concentrate was then purified by flash chromatography.

Boc-protected G2-dendrimer (2): General procedure IV: Starting from the G1-(carboxylic acid) dendron **7** (2.5 g, 5 mmol), after 48 h of reaction the target product **2** (1.8 g, 70%) was obtained as a white amorphous solid after flash chromatography on silica gel (eluent: EtOAc/EtOH/THF, 12:1:1, gradient to 6:1:1). ^{1}H NMR: $\delta = 1.37$ (s, 72H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 16H; surface CH_2CO), 2.63 (t, $^3J(H,H) = 7$ Hz, 8H; inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 16H; surface NCH_2), 3.39 (br s, 4H; core NCH_2), 3.52 (q, $^3J(H,H) = 7$ Hz, 8H; inner NCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 8H; carbamate-NH), 7.68 (s, 8H; surface ArH), 7.73 (s, 4H, inner ArH), 8.06 (s, 4H; surface ArH), 8.08 (s, 2H; inner ArH), 8.46 (t, $^3J(H,H) = 7$ Hz, 4H; surface ArCONH), 8.54 (t, $^3J(H,H) = 7$ Hz, 2H; inner ArCONH), 10.08 (s, 8H; surface NHAr), 10.15 (s, 4H; inner CONHAr); ^{13}C NMR (core NC overlapped with solvent signals): $\delta = 28.4$, 36.1, 36.4, 36.7, 36.9, 77.8, 112.8, 113.2, 136.0, 139.5, 155.7, 166.8, 166.9, 169.7, 169.8; MS (L-SIMS): *m*/*z* (%): 2540.2 (10) $[M + Na]^+$; elemental analysis calcd (%) for $C_{120}H_{168}N_{26}O_{34} \cdot H_2O$ (2536.8): C 56.82, H 6.75, N 14.36; found C 56.51, H 6.66, N 14.79.

Boc-protected G3-dendrimer (3): General procedure IV: Starting from the G2-(carboxylic acid) dendron **8** (6.2 g, 5 mmol), after 72 h of reaction the target compound **3** (2.2 g, 40%) was obtained as a white amorphous solid after flash chromatography on alumina (eluent: EtOAc/EtOH/H_2O, 7:1:0, gradient to 7:1:0.02) followed by precipitation from MeOH/EtOAc. ^{1}H NMR: $\delta = 1.37$ (s, 144H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 32H; surface CH_2CO), 2.63 (br s, 24H; middle and inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 32H; surface NCH_2), 3.40–3.60 (m, 28H; middle, inner, and core NCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 16H; carbamate-NH), 7.69 (s, 16H; surface ArH), 7.71 (s, 8H; middle ArH), 7.74 (s, 4H; inner ArH), 8.06 (s, 8H; surface ArH), 8.10 (s, 6H; middle and inner ArH), 8.47 (t, $^3J(H,H) = 7$ Hz, 8H; surface ArCONH), 8.51 (t, $^3J(H,H) = 7$ Hz, 4H; middle ArCONH), 8.57 (t, $^3J(H,H) = 7$ Hz, 2H; inner ArCONH), 10.09 (s, 16H; surface NHAr), 10.16 (s, 12H; middle and inner NHAr); ^{13}C NMR (core NC overlapped with solvent signals): $\delta = 28.4$, 36.1, 36.4, 36.7, 36.9, 77.9, 112.8, 113.3, 136.0, 139.5, 155.7, 166.9, 169.8, 169.9; elemental analysis calcd (%) for $C_{264}H_{360}N_{58}O_{74} \cdot 3H_2O$ (5584.2): C 56.78, H 6.61, N 14.55; found C 56.54, H 6.56, N 14.50; M_w (MALLS) = 5600 ± 200 g mol^{-1}.

Boc-protected G4-dendrimer (4): General procedure IV: Starting from the G3-(carboxylic acid) dendron **9** (6.9 g, 2.5 mmol), after 72 h of reaction the product **4** (2.3 g, 40%) was obtained as a white amorphous solid after flash chromatography on alumina (eluent: EtOAc/EtOH/H_2O, 6:1:0, gradient to 6:1:0.02) followed by precipitation from MeOH/EtOAc. ^{1}H NMR: $\delta = 1.37$ (s, 288H; *t*Bu), 2.48 (t, $^3J(H,H) = 7$ Hz, 64H; surface CH_2CO), 2.52–2.75 (m, 56H; middle and inner CH_2CO), 3.21 (q, $^3J(H,H) = 7$ Hz, 64H; surface NCH_2), 3.53 (br s, 60H; middle, inner, and core NCH_2), 6.86 (t, $^3J(H,H) = 7$ Hz, 32H; carbamate-NH), 7.69 (s, 32H; surface ArH), 7.72 (s, 28H; middle and inner ArH), 8.07 (s, 16H; surface ArH), 8.10 (s, 14H; middle and inner ArH), 8.46 (t, $^3J(H,H) = 7$ Hz, 16H; surface ArCONH), 8.51 (t, $^3J(H,H) = 7$ Hz, 8H; middle ArCONH), 8.57 (t, $^3J(H,H) = 7$ Hz, 4H; inner ArCONH), 8.61 (t, $^3J(H,H) = 7$ Hz, 2H; inner ArCONH), 10.09 (s, 32H; surface NHAr), 10.16 (s, 28H; middle and inner NHAr); ^{13}C NMR (core NC overlapped with solvent signals): $\delta = 28.4$, 36.1, 36.4, 36.7, 36.9, 77.9, 112.8, 113.3, 136.0, 139.5, 155.7, 166.9, 169.7, 169.8; elemental analysis calcd (%) for $C_{552}H_{744}N_{122}O_{154} \cdot 7H_2O$ (11678.8): C 56.77, H 6.54, N 14.63; found C 56.33, H 6.73, N 14.53; M_w (MALLS) = 11000 ± 600 g mol^{-1}.

(*N*-*tert*-Butyloxycarbonyl)heptylamine (16): A mixture of 1-heptylamine (2.1 g, 10 mmol) and di(*tert*-butyl) carbonate (2.2 g, 10 mmol) in THF (20 mL) was stirred at 20°C for 12 h. The solvent was then removed in vacuo and the residue was extracted with EtOAc (2 × 50 mL). The organic extracts were washed with saturated $NaHCO_3$ (2 × 50 mL), 5% $KHSO_4$ (2 × 50 mL), and water (2 × 50 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated to dryness on a rotary evaporator and the residue was purified by column chromatography on silica gel (eluent: EtOAc/hexane, 1:20) to give the target product **16** (1.5 g, 70%) as a colorless liquid. ^{1}H NMR: $\delta = 0.85$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 1.20–1.24 (m, 10H; CH_2), 1.36 (s, 9H; *t*Bu), 2.88 (q, $^3J(H,H) = 7$ Hz, 2H; NCH_2), 6.76 (t, $^3J(H,H) = 7$ Hz, 1H; NH); ^{13}C NMR (NC overlapped with solvent signals): $\delta = 14.1$, 22.2, 26.4, 28.5, 28.6, 29.7, 31.5, 77.4, 155.7; HRMS (L-SIMS): *m*/*z*: 216.1950 ($C_{12}H_{26}NO_2$ requires 216.1957).

Heptyl benzamide (17): 1-Heptylamine (1.48 mL, 10 mmol) and triethylamine (1.2 mL, 15 mmol) were added to a stirred solution of benzoyl chloride (1.75 mL, 15 mmol) in dry THF (30 mL). The mixture was stirred at 0°C for 6 h and then the excess solvents were removed in vacuo. The residue was redissolved in EtOAc (2 × 50 mL) and washed with saturated $NaHCO_3$ (2 × 50 mL), 5% aqueous $KHSO_4$ (2 × 200 mL), and water (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated to dryness on a rotary evaporator. The residue was chromatographed on silica gel (eluent: EtOAc/hexane, 1:8) to give the product **17** (1.5 g, 70%) as a white solid. M.p. 34–35°C; ^{1}H NMR: $\delta = 0.85$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 1.20–1.35 (m, 8H; CH_2), 1.45–1.60 (m, 2H; CH_2), 3.24 (q, $^3J(H,H) = 7$ Hz, 2H; NCH_2), 7.40–7.52 (m, 3H; ArH), 7.80–7.88 (m, 2H; ArH), 8.44 (t, $^3J(H,H) = 7$ Hz, 1H; NH); ^{13}C NMR (NC overlapped with solvent signals): $\delta = 14.1$, 22.3, 26.7, 28.7, 29.3, 31.5, 127.3, 128.4, 131.1, 134.9, 166,2; HRMS (L-SIMS): *m*/*z*: 220.1699 ($C_{14}H_{22}NO$ requires 220.1696).

Ethyl 3,5-di(acetamido)benzoate (18): A mixture of acetic anhydride (4.0 mL) and the diamine **10** (0.4 g, 2.2 mmol) was stirred at 40°C for 12 h. The volatiles were then evaporated in vacuo and the residue was extracted with EtOAc (2 × 30 mL). The organic extracts were washed with saturated $NaHCO_3$ (2 × 50 mL), 5% aqueous $KHSO_4$ (2 × 200 mL), and water (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated to dryness on a rotary evaporator. The residue was chromatographed on silica gel (eluent: EtOAc/hexane, 3:1) to give the product **18** (0.4 g, 70%) as a white solid: M.p. 142–143°C; ^{1}H NMR: $\delta = 1.31$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 2.04 (s, 6H; CH_3CO), 4.30 (q, $^3J(H,H) = 7$ Hz, 2H; CH_2), 7.90 (s, 2H; ArH), 8.18 (s, 1H; ArH), 10.15 (s, 2H; NH); ^{13}C NMR: $\delta = 14.4$, 24.2, 61.0, 113.8, 114.4, 130.7, 140.1, 165.7, 168.8; MS (FAB): *m*/*z* (%): 264 (90) $[M]^+$; HRMS (L-SIMS): *m*/*z*: 265.1188 ($C_{13}H_{17}N_2O_4$ requires 265.1184).

Heptyl 3,5-di[β-(*N*-*tert*-butyloxylcarbonylamino)propionamido]benzamide (19): A mixture of pentachlorophenol dicyclohexylcarbodiimide[25] (0.8 g, 0.8 mmol) and G1-(carboxylic acid) dendron **7** (0.4 g, 0.8 mmol) in DMF (20 mL) was stirred at 20°C for 12 h. The reaction mixture was then filtered and diluted with THF (20 mL). 1-Heptylamine (0.13 mL, 0.9 mmol) and DIPEA (0.15 mL, 0.9 mmol) were added and the resulting mixture was stirred at 20°C for a further 12 h. The solvents were then evaporated on a rotary evaporator, and the residue was taken up in ethyl acetate (30 mL). The organic phase was washed with saturated $NaHCO_3$ (2 × 50 mL), 5% aqueous $KHSO_4$ (2 × 200 mL), and water (2 × 200 mL), dried (Na_2SO_4), filtered, and concentrated to dryness on a rotary evaporator. The residue was chromatographed on silica gel (eluent: EtOAc/hexane, 3:1) to give the product **19** (0.35 g, 75%) as a white solid. M.p. 92–93°C; ^{1}H NMR: $\delta = 0.84$ (t, $^3J(H,H) = 7$ Hz, 3H; CH_3), 1.20–1.40 (m, 10H; CH_2), 1.38 (s, 18H; *t*Bu), 2.47 (t, $^3J(H,H) = 7$ Hz, 4H; CH_2CO), 3.20–3.35 (m, 6H; NCH_2), 6.85 (t, $^3J(H,H) = 7$ Hz, 2H; carbamate-NH), 7.65 (s, 2H; ArH), 8.05 (s, 1H; ArH), 8.32 (t, $^3J(H,H) = 7$ Hz, 1H; ArCONH), 10.05 (s, 2H; NHAr); ^{13}C NMR (one NC obscured by solvent signals): $\delta = 14.2$, 22.3, 26.6, 28.4, 28.7, 29.3, 31.5, 36.7, 36.9, 77.8, 112.6, 113.2, 136.4, 139.4, 155.7, 166.7, 169.7; elemental analysis calcd (%) for $C_{30}H_{49}N_5O_7$ (591.7): C 60.89, H 8.53, N 11.84; found C 60.73, H 8.46, N 11.69.

Proton–deuterium exchange experiments: The experimental methodology of the proton exchange study was an adaptation of that reported by Krishna.[26] [D_6]DMSO was treated with 3 Å molecular sieves for 2 days and the dendrimer samples were stored at 60°C under high vacuum (0.01 mmHg) for 3 weeks to remove the residual solvents and moisture. In a typical example, a 4% (*w*/*v*) solution of G2-dendrimer **2** in [D_6]DMSO (0.50 mL) was prepared in a pre-dried NMR tube. D_2O (50 μL) was then added by means of a microsyringe and a series of ^{1}H NMR spectra was recorded at regular time intervals (~10 min, acquisition time ~1 min per spectrum) until all N–H signals had vanished. From the relative integrals thus obtained, a pseudo first-order rate constant for the PDX process of a particular proton–deuterium exchangeable functionality was computed, from which an exchange half-life $t_{1/2}$ (time for 50% of the protons in such a functionality to be exchanged) was evaluated.[20]

Variable-temperature NMR experiments: A 3% (*w*/*v*) solution of the G2-dendrimer **2** in [D_6]DMSO was prepared in an NMR tube, tetramethylsilane was added as an internal standard, and the tube was sealed. The VT-NMR experiment was performed on a Varian 400 MHz NMR spectrometer. Spectra were recorded at 298, 303, 313, and 323 K, as set by a

temperature programmer. The temperature coefficient $|\Delta\delta/\Delta T|$ was obtained by measuring the slope of a graph of chemical shift (δ) versus temperature (K).

Determination of dendrimer purity and M_w by gel-permeation chromatography with multi-angle laser light scattering/refractive index detectors (GPC-MALLS/RI)

General: Polystyrene standards were purchased from Aldrich and used for the calibration of the laser refractometer, normalization of the MALLS instrument, and as test samples for the GPC-MALLS/RI system. For calibration of the MALLS detector, HPLC grade toluene was used. Distilled (over CaH_2) DMF was used as the mobile phase for both GPC-MALLS/RI and off-line *dn*/*dc* measurements. For the preparation of standard and sample solutions, the distilled DMF was further clarified by passage through an Anotop 10 membrane filter (purchased from Whatman) of 0.2 μm pore size. The GPC-MALLS/RI system was configured by connecting the various detector modules with polyether ether ketone (PEEK) tubing of 0.01 inch internal diameter to minimize the dead volume. A pressure damper was installed just before the injector port to ensure a smooth mobile-phase delivery. As mentioned above, DMF was employed as the mobile phase and was delivered by means of a Waters 510 HPLC pump. Two styragel HR-1 and HR-3 GPC columns (purchased from Waters) were connected in series for sample separation. The static light scattering and refractive index signals were measured with a multi-angle DAWN® DSP laser photometer (He-Ne laser, output power = 5 mW at $\lambda_0 = 632.8$ nm, purchased from Wyatt Technology) and an LR40 refractometer (purchased from Viscotek), respectively.

dn/*dc measurement*: The *dn*/*dc* measuring system was set up by connecting the HPLC pump directly to the LR40 refractometer and the *dn*/*dc* values of the dendrimers were measured in off-line mode. Stock solutions (20.0 mL, $\sim$3.4 mg mL^{-1}) of known concentrations of the G1- to G4-dendrimers **1**–**4** were prepared in clarified DMF and were allowed to stand overnight to ensure complete dissolution. For each of the stock solutions, a series of dilutions was made so as to obtain five sample solutions of different concentrations (at approximately 0.4, 0.9, 1.4, 1.9, and 2.4 mg mL^{-1}), each with a volume of 5.0 mL. A 1.0 mL sample loop was used for sample loading. At least 2.5 mL of the sample solution was injected for each analysis. The sample in the loop was delivered by a DMF mobile phase at a flow rate of 0.6 mL min^{-1}. The temperature of the refractive index measurement was maintained at 21 °C. The recorded RI signal was converted to the refractive index by multiplying the value by the calibration constant of the laser refractometer. A graph of refractive index values plotted against the corresponding dendrimer concentrations was obtained. The slope of the graph thus gave the *dn*/*dc* of the dendrimer. The measured *dn*/*dc* values of the G1- to G4-dendrimers **1**–**4** were 0.100, 0.121, 0.132, and 0.134 mL g^{-1}, respectively.

M_w *determination*: Six sample solutions were prepared from the G1- to G4-dendrimers **1**–**4** ($\sim$40 mg) and two polystyrene standards ($\sim$7 mg) by individually dissolving the samples in 2.0 mL aliquots of clarified DMF; these solutions were allowed to stand overnight to ensure complete dissolution. A 200 μL sample loop was used for sample loading and the flow rate of the mobile phase was set at 1.0 mL min^{-1}. The temperature of the MALLS and RI detectors was kept at 21 °C. The *dn*/*dc* values of the dendrimers obtained from the off-line measurement were entered manually into an ASTRA program (purchased from Wyatt Technology). Data from the MALLS and RI detectors were processed by the ASTRA software to generate the M_w of the injected dendrimer. The molar masses of the G1- to G4-dendrimers **1**–**4** were determined by injecting 1.5–2.5 % (*w*/*v*) sample solutions in DMF into the GPC-MALLS/RI system. Two polystyrene standards ($M_w = 3.0 \times 10^3$ and 3.0×10^4) were also analyzed under same experimental conditions to check the reliability of the method. The measured M_w values of the dendrimers **1**–**4** are given in Table 2.

Aggregation study by dynamic laser light scattering (DLLS): Details of the DLLS instrumentation and theory have been reported previously.[27] Sample solutions of 10.0 mg mL^{-1} of the G2- to G4-dendrimers **2**–**4** were prepared by dissolving the samples in clarified DMF. The solutions were allowed to stand overnight to ensure complete dissolution and then clarified by passage through a 0.2 μm pore size filter to achieve a dust-free state. The hydrodynamic radii of the dendrimers were measured on a modified commercial LLS spectrometer (ALV/SP-125) equipped with a multi-τ digital time correlation unit (ALV5000) and a solid-state laser (ADLAS DPY425II, output power 400 mW at $\lambda_0 = 532$ nm) as light source. The effects of varying the temperature and solvent on the hydrodynamic radius distribution function were studied for the G2-dendrimer **2** by raising the temperature from 25 °C to 35 °C and by titrating the initial DMF sample solution with the solvent of interest.

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