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Electrospray Mass Spectrometry of Poly(propylene imine) Dendrimers—The Issue of Dendritic Purity or Polydispersity

Jan C. Hummelen, Joost L. J. van Dongen, and E. W. Meijer*

Abstract: Electrospray mass spectrometry (ESI-MS) is used to analyze in detail the structure of the first five generations of the poly(propylene imine) dendrimers with 4–64 end groups. Although the reaction sequence to prepare these dendrimers is highly optimized, an accumulation of statistical defects is observed at higher generations. A simple statistical simulation allows the assignment of all peaks in

the deconvoluted spectrum and the determination of yields for each side reaction in the formation of each generation. The

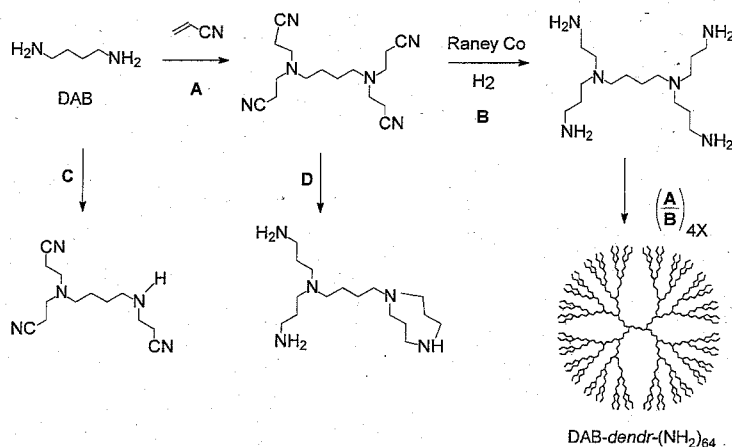
Keywords

defects · dendrimers · electrospray · mass spectrometry · polydispersity · selectivity

fifth generation of the poly(propylene imine) dendrimer containing amine end groups, which is formed after 248 consecutive reaction steps, possesses a dendritic purity of approx. 20%, which corresponds to a polydispersity of approximately 1.002. The defects in the divergent growth patterns of dendrimers are compared to those observed in the Merrifield synthesis of polypeptides.

Introduction

Dendritic macromolecules, prepared by divergent or convergent syntheses, are generally depicted as well-defined organic compounds of single composition.^[1] This representation is correct for most of the convergent dendrimers, which are of high purity due to their method of formation, isolation, and purification.^[2] In contrast, the divergent approach starts with a core molecule, which is transformed into higher-generation dendrimers by a multiple reaction sequence without purification. This strategy has been developed in most detail for the poly(amino amido) dendrimers,^[3] the arborols,^[4] and the poly(propylene imine) dendrimers.^[5] The poly(propylene imine) dendrimers with amine or nitrile end groups have been synthesized on a large scale following the reaction sequence given in Scheme 1.^[5] The alternating sequence of Michael and hydrogenation reactions leads in ten steps from diaminobutane (DAB) to DAB-dendr-(NH₂)₆₄. Only after 248 successful consecutive reactions is the ideal structure formed. In the past we have acknowledged the possibility of incomplete Michael addition (path C) and the formation of cyclic structures during hydrogenation (path D). Therefore, the product should suffer from a small number of statistical imperfections and, hence, should be polydisperse, even when the reactions involved are highly optimized.^[6] This phenomenon of the expression of small imperfections is well known in the area of the Merrifield synthesis of polypeptides, as is illustrated for the synthesis of the 124-amino-acid ribonucle-



Scheme 1. The synthesis of poly(propylene imine) dendrimers and the possible side reactions (C, D).

ase, which is obtained in 17% purity after 369 chemical conversions, while the other 83% of the material possesses one or more defects in the amino acid sequence.^[7]

The divergent synthesis of dendrimers is ideally suited to probe dendritic growth patterns, provided that a powerful analytical technique is available. Recently, mass spectrometry has been applied very successfully to the characterization of dendrimers, namely, through the use of fast atom bombardment (FAB),^[8] liquid secondary ion,^[9] electrospray (ESI),^[10] and matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) spectrometry.^[11] In this paper we will present a detailed analysis, supported by simulations, of ESI-MS data on poly(propylene imine) dendrimers by which all imperfections present at the level of ca. 0.1% are assigned. The statistical

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origin and the low level of imperfections are used to introduce the term "dendritic purity" for dendrimers, and we discuss how this term relates to the term polydispersity.

Results and Discussion

The commercially available series DAB-*dendr*-(CN)_x and DAB-*dendr*-(NH₂)_x are both very polar and polybasic compounds, and are partially protonated when dissolved in methanol–water mixtures.^[12] This property allows the direct analysis of such solutions by positive ion ESI-MS. Samples with a concentration of ca. $25 \times 10^{-3} \text{ mmol L}^{-1}$ in MeOH/H₂O (75/25) are directly injected into the Perkin-Elmer Sciex API 300 triple quadrupole mass spectrometer by means of a syringe pump at a flow rate of $5 \mu\text{L min}^{-1}$. In the positive ion mode a voltage of 5.5 kV is applied to the capillary. The actual and deconvoluted ESI-MS spectra of DAB-*dendr*-(NH₂)₆₄ are given in Figure 1. The spectrum measured shows a repetition of different clusters of peaks, corresponding to dendrimers with 4–12 charges per dendrimer (m/z with $z = 4-12$), while no counterion interactions are observed. Deconvolution by standard methods yields a spectrum in which the largest peak at $M_r = 7168$ corresponds to the perfect DAB-*dendr*-(NH₂)₆₄. A series of peaks of lower abundance are found at regular intervals of $\Delta M = 57.1$ (missing propylamine units via path C) from the perfect dendrimer at $M_r = 7168$. Furthermore, the peak at $M_r = 7151$ ($\Delta M = 17$, missing ammonia via path D) is assigned to the dendrimer with 62 primary amine end groups and one cyclic secondary amine structure; there is also a series of peaks with intervals of 57.1 from $M_r = 7151$ (again via path C in earlier generations).

In order to fully understand the origin of the smaller peaks, ESI-MS spectra of all generations with both end groups (NH₂, CN) were recorded. The deconvoluted spectra of the DAB-*dendr*-(CN)_x series with $x = 8-64$ are given in Figure 2. The lowest generations (up to $x = 8$) are seemingly defect-free, and a molecular ion peak is found at $M_r = 740.6$ for $x = 8$. For the third generation with $x = 16$, the perfect dendrimer is found at $M_r = 1622.0$, while at least one defect in the dendrimer synthesis is recorded at 1569.0 ($\Delta M = 53$; acrylonitrile). For the fourth and fifth generation, a repetition of defects at intervals of $\Delta M = 53$ is found, assigned to missing acrylonitrile units. The

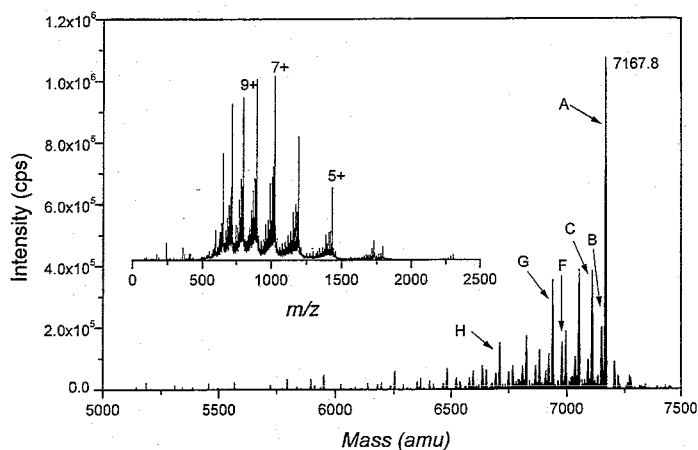


Figure 1. The experimental (inset) and deconvoluted ESI-MS data of DAB-*dendr*-(NH₂)₆₄; the assignments of A–H are given in Table 1 and Figure 4.

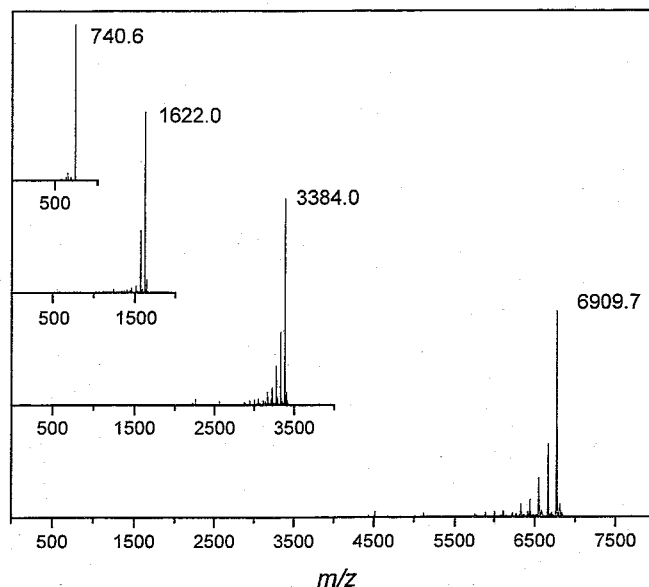


Figure 2. Deconvoluted ESI-MS data of DAB-*dendr*-(CN)_x with $x = 8, 16, 32, 64$.

presence of peaks at $\Delta M = 17$ originates from path D in the synthesis of the earlier generations, while the peaks at $\Delta M = 57$ originates from path C in earlier generations. A number of dendrimers with small defects were further investigated with the MS/MS techniques and fragmentation products assigned, in order to verify that no fragmentation had occurred in the spectra presented here.^[13]

All the defects follow the rules of statistics for both side reactions (paths C and D), and no other defects are found in the ESI-MS spectra of the two series of poly(propylene imine) dendrimers. Once the probability of both side reactions in each step is known from the individual ESI-MS spectra, it is possible to simulate the ESI-MS spectra of all generations, provided that the isotope distributions are accounted for and assuming that the response factors are equivalent for the perfect and imperfect dendrimers. These simulated spectra can then be fitted to the actual reconstructed spectra in an iterative process. The results of the DAB-*dendr*-(NH₂)₆₄ simulation are given in Figure 3, and an almost perfect fit is found with the actual spectrum (Figure 1). Based on this simulation, we are able to assign all peaks

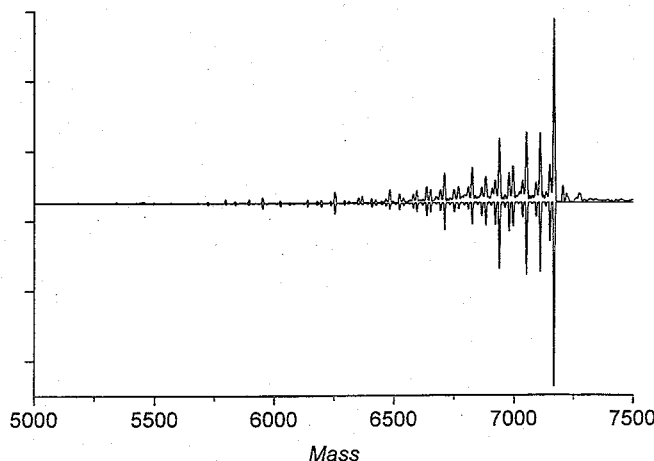


Figure 3. The deconvoluted (upper trace) and the simulated (lower trace) ESI-MS spectra of DAB-*dendr*-(NH₂)₆₄.

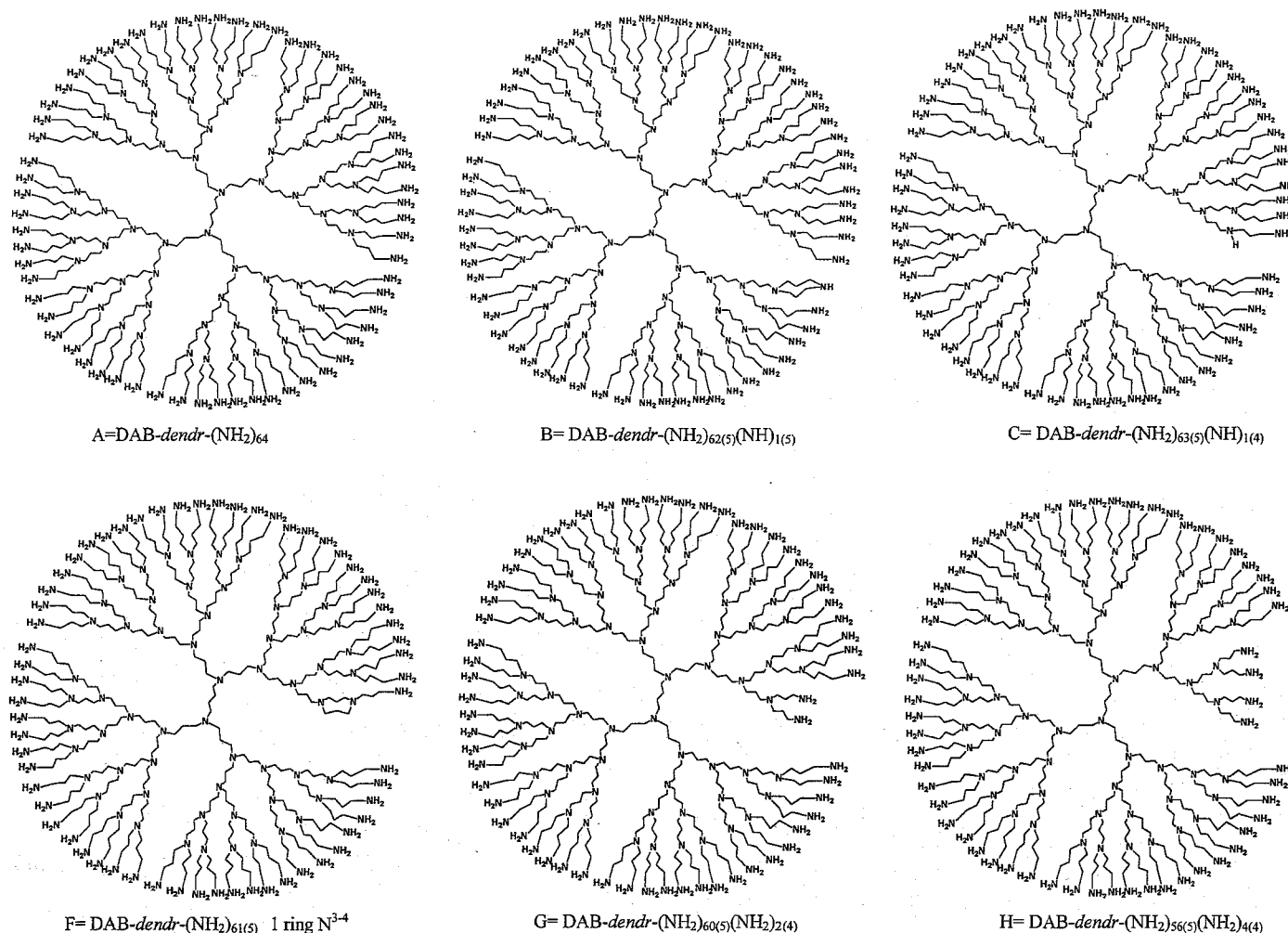


Figure 4. Prominent structures present in DAB-dendr-(NH₂)₆₄; see also Figure 1 and Table 1.

in the spectrum and to determine the yield of each side reaction in the formation of each generation. A number of assignments are illustrated in Table 1 and Figure 4.

Despite the fact that the IR spectra of the DAB-dendr-(NH₂)_x series sometimes indicate the presence of small amounts of residual CN groups, the detailed analysis of the reconstructed and simulated ESI-MS spectra clearly shows that no shoulders at $M_r - 4$ (or $n \times 4$) are present. Remarkably in these cases, however, the ESI-MS spectra contain signals corresponding to traces of starting material DAB-dendr-(CN)_x (see the small cluster around $m/z = 1728.7$ in Figure 1). This observation was investigated further by using material obtained by interrupting the hydrogenation of DAB-dendr-(CN)₃₂.

Table 1. Interpretation of the most prominent structures present in the mass spectrum of DAB-dendr-(NH₂)₆₄.

Cluster [a]	M_r	Structure [b]	Defect history [c]	A/% [d]
A	7167.8	DAB-dendr-(NH ₂) ₆₄₍₅₎	no defects	100
B	7150.6	DAB-dendr-(NH ₂) ₆₂₍₅₎ (NH) ₁₍₅₎	1D at gen. 5	18.7
C	7111.7	DAB-dendr-(NH ₂) ₆₃₍₅₎ (NH) ₁₍₄₎	1C at gen. 5	36.2
D	7053.2	DAB-dendr-(NH ₂) ₆₂₍₅₎ (NH ₂) ₁₍₄₎ DAB-dendr-(NH ₂) ₆₂₍₅₎ (NH) ₂₍₄₎	1C at gen. 4 2C at gen. 5	35.8
E	6995.6	DAB-dendr-(NH ₂) ₆₁₍₅₎ (NH ₂) ₁₍₄₎ (NH) ₁₍₄₎ DAB-dendr-(NH ₂) ₆₁₍₅₎ (NH) ₃₍₄₎	1C at gen. 4 + 1C at gen. 5 3C at gen. 5	17.1
F	6979.1	DAB-dendr-(NH ₂) ₆₁₍₅₎ 1 ring N ³⁻⁴ DAB-dendr-(NH ₂) ₅₉₍₅₎ (NH ₂) ₃₍₄₎ (NH) ₁₍₅₎	1D at gen. 4 3C at gen. 5 + 1D gen. 5	14.1
G	6939.7	DAB-dendr-(NH ₂) ₆₀₍₅₎ (NH ₂) ₂₍₄₎ DAB-dendr-(NH ₂) ₆₀₍₅₎ (NH) ₂₍₃₎	1C at gen. 3 2C at gen. 4	37.8
H	6711.2	DAB-dendr-(NH ₂) ₅₆₍₅₎ (NH ₂) ₄₍₄₎	1C at gen. 2	12.4

[a] Isotope clusters taken from the spectrum of Figure 1 (see also Figure 4). [b] Notation: (NH₂)_{x(y)} means x NH₂ endgroups at generation y . [c] Notation: “ x C at gen. y ” means x randomly missing Michael additions (path C, Scheme 1) in the generation y , followed by normal growth; “1D at gen. y ” means one ring formation (path D, Scheme 1) in the generation y , followed by normal growth. [d] Normalized abundance out of the deconvoluted mass spectrum. [e] Main constituent.

The ESI-MS spectrum, deconvoluted by means of two envelopes, clearly shows the presence of only two products: fully converted dendrimer and completely unreacted starting material (Figure 5). Obviously the kinetics of reaction and diffusion of the dendrimer favor full hydrogenation as soon as the nitrile dendrimer adsorbs on to the Raney-Co catalyst. This all-

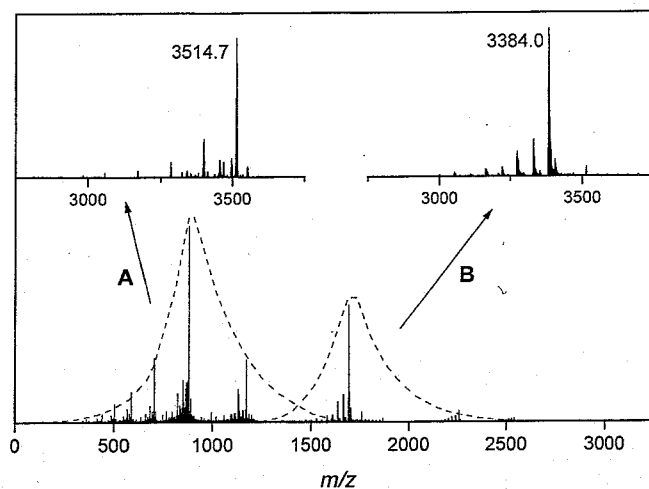


Figure 5. ESI-MS spectrum of the mixture obtained from interrupted hydrogenation of DAB-dendr-(CN)₃₂. Inset A depicts the selective deconvoluted spectrum of the low-mass region (see deconvolution envelope) and shows clearly fully converted material (DAB-dendr-(NH₂)₃₂, $M_r = 3514.7$). Inset B depicts the selective deconvoluted spectrum of the high-mass region and shows unreacted DAB-dendr-(CN)₃₂ ($M_r = 3384.0$).

or-nothing phenomenon has been observed on other occasions^[14] and will be investigated in more detail.

Using the data available from the ESI-MS spectra of the dendrimers and the simulation for the region of 4000–7250 amu, we have calculated the polydispersity (1.0018 and 1.0024 from the deconvoluted and simulated spectra, respectively) as well as the dendritic purity of DAB-dendr-(NH₂)₆₄ (15.3 and 23.2% from the deconvoluted and simulated spectra, respectively). The polydispersity M_w/M_n is calculated by the standard method for macromolecules, while the dendritic purity is defined as the number of error-free dendrimers divided by the total number of dendrimers multiplied by 100%.^[15]

The overall branching efficiency, as typically used for hyper-branched polymers, is calculated to be $\alpha = 0.987$.^[16] Furthermore, the yield of each individual reaction for all ten steps can be estimated from the simulated spectra and the results are given in Table 2. This ESI-MS analysis raises the question whether the statistical defects in dendrimers made by the divergent route should be discussed in terms of polydispersity or in terms of dendritic purity. Since it is highly preferred to use the terminology that is most informative, we would like to propose here that, in the case of dendrimers in which the fully converted and perfect product is the dominant species, the mixture should be discussed in terms of dendritic purity. Polydispersity of dendrimers is only useful when an imperfect dendrimer is the main product or when it is impossible to detect the individual mole-

Table 2. Data on the DAB-dendr-(NH₂)_x series calculated from the simulated spectrum of DAB-dendr-(NH₂)₆₄.

Product	Path C % per end group	Path D % per end group	Dendritic purity % of total
DAB-dendr-(NH ₂) ₄	1.0	0.0	96
DAB-dendr-(NH ₂) ₈	1.0	0.55	86.7
DAB-dendr-(NH ₂) ₁₆	1.65	0.50	63.8
DAB-dendr-(NH ₂) ₃₂	0.97	0.77	41.3
DAB-dendr-(NH ₂) ₆₄	0.58	0.65	23.1

cules. In this case it is also difficult to use the methodology presented here, because too many defects in the different m/z regions will not allow the ESI-MS spectrum to be deconvoluted.

Conclusion

We have shown that electrospray mass spectrometry (ESI-MS) in combination with a simple statistical simulation program allows full characterization of the polar poly(propylene imine) dendrimers, including their statistical errors. These commercially available dendrimers, which are currently used in a variety of modification reactions, are of high dendritic purity. The term dendritic purity is introduced next to polydispersity, because the latter is not very useful for almost defect-free dendrimers. The divergent growth of high-generation dendrimers can be compared to the Merrifield synthesis of polypeptides and polynucleotides; even with reactions that are highly optimized, it is impossible to obtain defect-free products when the synthesis uses a large number of conversions. The ESI-MS techniques now available can be used to analyze these dendrimers in detail and thus open the way to a classification based on dendritic purity for dendrimers synthesized by a divergent approach. However, if the number of defects is large, it is not possible to use the technique presented here; it is then better to classify these dendrimers in terms of polydispersity as determined by more classical techniques for polymer characterization.

Experimental Section

Materials: The poly(propylene imine) dendrimers of all five generations, with primary amine and nitrile end groups, were obtained from DSM Research and made according to the procedure published in refs. [5] and [6].

Electrospray analysis: Electrospray mass spectra were recorded on a API 300 MS/MS mass spectrometer (PE-Sciex, Foster city, USA) with a mass range of 3000. The compounds were dissolved (150 ppm) in a mixture of 75% methanol and 25% water. The sample solutions were delivered directly to the ESI-MS by a Harvard syringe pump (Harvard Apparatus, Massachusetts, USA) at a flow rate of 5 $\mu\text{L min}^{-1}$. The mass spectrometer was used in positive ion mode by applying a voltage of 5.5 kV to the capillary, while the orifice was set at 35 V. Mass spectra were collected in full scan mode, scanning over the range $30 < m/z < 2500$ in 25 s. Dry air was used as nebulizer gas at a flow rate of 1.04 L min^{-1} (ion spray mode). Nitrogen was used as the drying bath gas at a flow rate of 0.63 L min^{-1} . Electrospray data were deconvoluted by the Bio-reconstruct program available on the Apple power-PC of API 300.

Simulation: The simulated ESI-MS spectra were obtained by Monte Carlo calculations. Spectra of single dendrimers that could be present in the mixture were generated, starting at DAB and allowing both amino groups to react with a chance $f\hat{A}(C)$; subsequently, the cyano groups were quantitatively converted into amino groups. For every two amino groups present a ring closure reaction (chance $f\hat{A}(D)$) was then allowed in the conversion of the cyano group to the amino group. During all reactions we kept track of the molecular weight as well as the number of reactive groups. This sequence was repeated to obtain higher generation dendrimers. From the total formula the ESI-MS spectrum of the single dendrimer was calculated from the exact mass and natural abundance of the atoms. In this manner many spectra were generated and added to give the final simulated ESI-MS spectrum of the mixture.

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- [16] $\alpha = \sqrt[5]{\alpha(C)_{1-5}(1 - \alpha(D)_{1-5}/2)}$, where α is the overall branching efficiency, $\alpha(C)_{1-5}$ the efficiency of the Michael reaction (route C in Scheme 1) in generations 1 to 5, and $\alpha(D)_{1-5}$ the efficiency of the ring closure reaction (route D in Scheme 1) in generations 1 to 5.