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How useful is mass spectrometry for the characterization of dendrimers? "Fake defects" in the ESI and MALDI mass spectra of dendritic compounds

Bilge Baytekin [a], Nicole Werner [b], Friedhelm Luppertz [b], Marianne Engeser [b], Jens Brüggemann [b], Stephan Bitter [b], Roman Henkel [b], Thorsten Felder [b], Christoph A. Schalley [a,*]

[a] *Institut für Chemie und Biochemie, Organische Chemie, Freie Universität, Takustr. 3, D-14195 Berlin, Germany*
[b] *Kekulé-Institut für Organische Chemie und Biochemie der Universität, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany*

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In memory of Chava Lifshitz.

Abstract

Mass spectrometry is usually considered to be one of the very few methods, if not the only reliable one, to investigate dendrimers with respect to the presence and the nature of structural defects. In particular, MALDI mass spectrometry is used routinely for dendrimer characterization. The results reported here emerge from a comparison of ESI and MALDI mass spectra of different dendritic molecules. Two examples are presented for the fact that both methods have their limitations. In the first example, ESI mass spectra of POPAM dendrimers indicate the presence of a high abundance of a new type of defects which are not found in neither the MALDI mass spectra nor ^{1}H or ^{13}C NMR spectra. The second example deals with dendrimers bearing sulfonamide groups in their periphery. Here, the ESI mass spectra provide evidence for sample purity, while MALDI produces signals for defects, which seem to be generated during the synthesis. However, thermal reactions occurring during the ionization within the matrix are responsible for these defects, while synthesis proceeds cleanly to the desired products. When substituted in their periphery with dansyl groups, which absorb light at the wavelength of the MALDI laser, additional fragments are generated through light-induced cleavages of dansyl groups. Consequently, mass spectral data on dendrimer purity needs to be interpreted with care and may be misleading in the sense that falsely negative results are obtained.

Keywords: Dendrimers; Sulfonamides; Mass spectrometry; Electrospray ionization; Matrix-assisted laser desorption ionization; Defect structures

1. Introduction

Dendrimers are onion-type polymers, which bear branching units in each shell [1]. Consequently, the number of branches and their molecular masses increase exponentially from the core outwards. Since the synthesis of dendrimers most often involves the repetition of two distinct steps for the formation of each generation, the building blocks of the *n*th shell are similar to those in the $(n+1)$st shell, but are located in different microenvironments. This usually causes the NMR spectra of higher-generation dendrimers to exhibit broad signals, which cannot precisely be assigned to individual shells. The precise characterization of dendrimers thus becomes increasingly difficult with higher generation numbers. In particular, it is hardly possible to provide evidence for their structural integrity or for the presence of defects such as missing branches. Even if the presence of defects might be detected by NMR methods, e.g., through signal integrals, their exact nature will hardly become clear on the basis of NMR experiments only.

Mass spectrometry is a powerful tool [2] to distinguish structurally perfect dendrimers from those with defects, since they have different elemental compositions and thus different molecular masses [3]. As a first approximation, one should even be able to observe structure-perfect and defect variants with similar ionization efficiencies as long as the differences are not too large, because both still contain similar numbers of the same functional groups, even if some of them are involved in the formation of defects. This is one of the reasons, why soft ionization methods such as electrospray ionization (ESI) [4] and matrix-assisted laser desorption/ionization (MALDI) [5] coupled to the appro-

* Corresponding author. Tel.: +49 30 838 52639; fax: +49 30 838 55817.
E-mail address: schalley@chemie.fu-berlin.de (C.A. Schalley).

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priate mass analyzers have been considered as reliable methods for the detection of structural defects in dendrimers. Recently, we have reported [6] examples of dendrimers persulfonylated at their periphery, where MALDI turns out to induce reactions within the matrix during the ionization process. These reactions lead to the same products, which would form in an incomplete synthesis. Evidence for the absence of such defects and for the monodispersity of the dendrimers under study came instead from ESI mass spectra, in which only signals for the structure-perfect dendrimers were observed. The information that MALDI mass spectrometry may lead to false-negative results is of course not only interesting for the mass spectrometrist, but also of great importance for the synthetic chemist involved in dendrimer synthesis.

Here, we extend the earlier study to two different types of dendrimers. We discuss evidence for reliability problems with both ionization methods. In the first example, electrospray ionization gives rise to signals for protonated POPAM dendrimers which are modified at their periphery. These signals are not at all observed in the corresponding MALDI mass spectra. Here, ESI provides a wrong picture, which is further evidenced by NMR experiments. In the second example, MALDI yields false-negative results by generating defects during the ionization. Again thermal reactions with the matrix are observed which however are superimposed by photocleavages within the dendrimer structure when the dendrimers themselves absorb light at the wavelength of the N_2 laser of the MALDI mass spectrometer (337 nm).

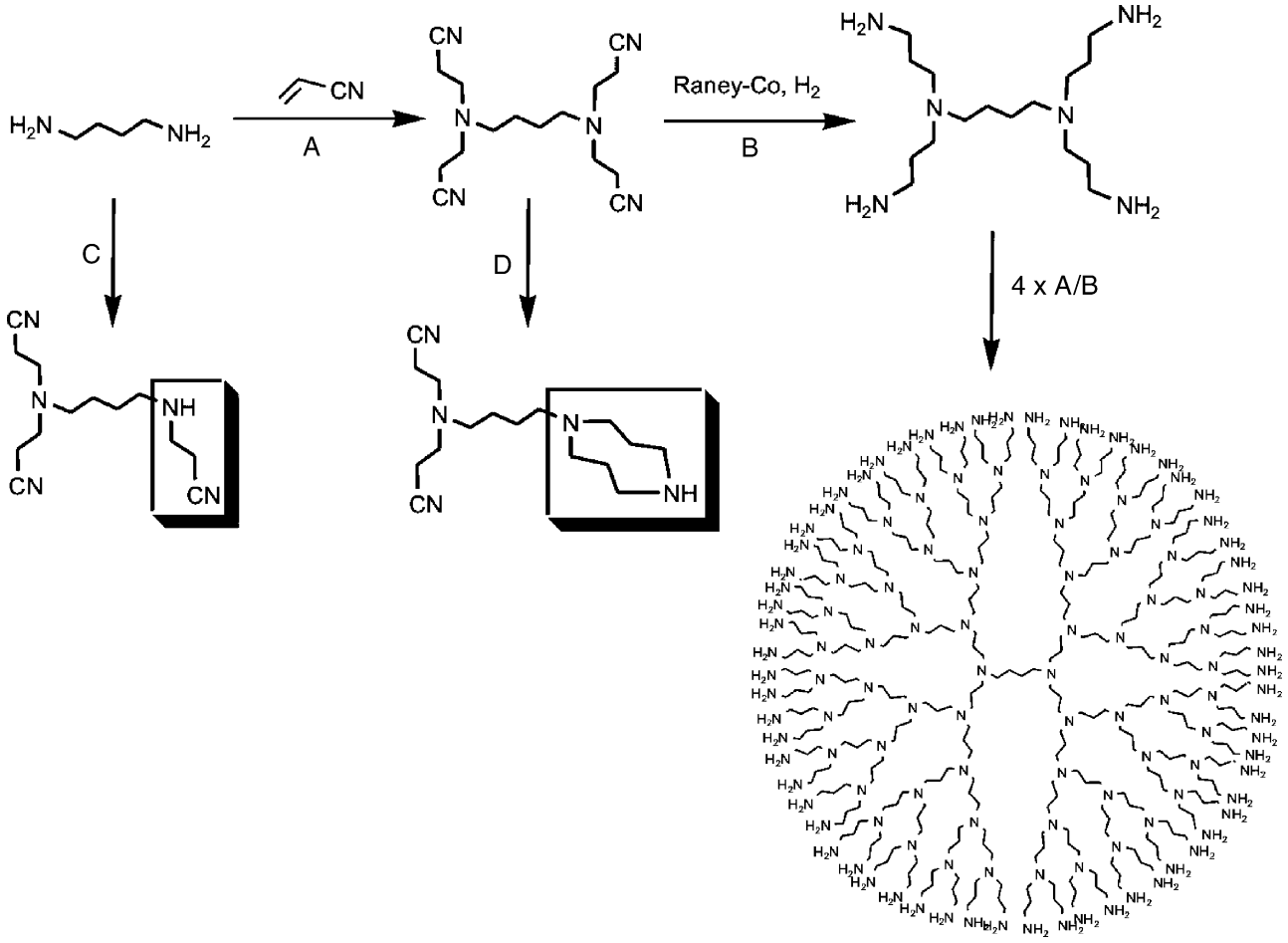
2. Experimental

2.1. Syntheses

POPAM dendrimers were purchased from Aldrich and used without further purification. All sulfonylated dendrimers have been synthesized by standard literature procedures [7].

2.2. Mass spectrometric experiments

ESI mass spectra were recorded on a Bruker APEX IV Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer with an Apollo electrospray ion source equipped with an off-axis 70° spray needle. Typically, methanol with 1% acetic acid served as the solvent and 50 μM solutions of the analytes were used. If solubility of the dendrimers was too low, a small amount of dichloromethane was added to the sample solutions (e.g., MeOH:CH_2Cl_2 = 9:1). Analyte solutions were introduced into the ion source with a syringe pump (Cole-Parmers Instru-



Scheme 1. Synthesis of POPAM dendrimers and potential defect structures.

ments, Series 74900) at flow rates of ca. 3–4 μL/min. Ion transfer into the first of three differential pump stages in the ion source occurred through a glass capillary with 0.5 mm inner diameter and nickel coatings at both ends. Ionization parameters were adjusted as follows: capillary voltage: −4.5 to −5.2 kV; endplate voltage: −4.0 to −4.3 kV; capexit voltage: +200 to +350 V; skimmer voltages: +10 to +16 V; temperature of drying gas: 40–200 °C. The flows of the drying and nebulizer gases were kept in a lower range. The ions were accumulated in the instruments hexapole for 0.5–3 s, introduced into the FT-ICR cell which was operated at pressures below 10^{-10} mbar and detected by a standard excitation and detection sequence. For each measurement 16–64 scans were averaged to improve the signal-to-noise ratio.

MALDI mass spectra were recorded on a Micromass MALDI-TofSpec E mass spectrometer equipped with an N_2 laser (337 nm). All matrices were used as purchased and applied in an 800-fold molar excess relative to the analyte. Matrices and samples (0.5 mg) were dissolved in 400 μl of $CHCl_3$:MeOH = 3:1. Then, 1 μl of the mixture was pipetted onto the stainless steel MALDI target. The matrix crystallized in a spot of ca. 2 mm diameter upon evaporation of the solvent in a stream of air.

3. Results and discussion

3.1. POPAM dendrimers

Polypropyleneamine (POPAM) dendrimers are synthesized in a divergent manner [8] starting from a diaminoalkane core (Scheme 1). In a sequence of a double Michael addition of acrylonitrile at each amino group followed by a heterogeneous Raney-Cobalt-catalyzed hydrogenation of the terminal cyano groups, the next shell of branching units can be added at the periphery of the growing dendrimer. Both reactions proceed with high yields [9] and no purification is necessary between the reaction steps. At higher generation numbers, some defects accumulate, among them missing branches (Scheme 1, side reaction C) and cyclizations connecting two branches at their ends through the formation of a secondary amine (side reaction D) [10].

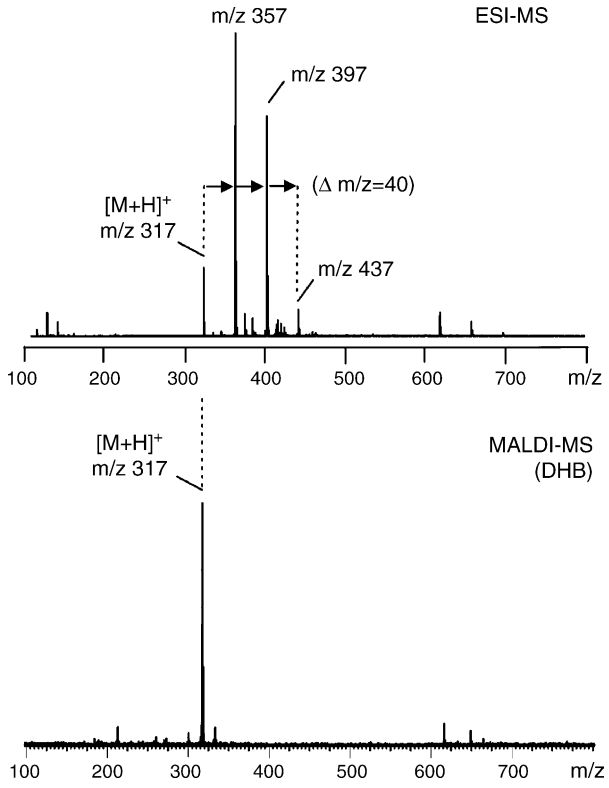
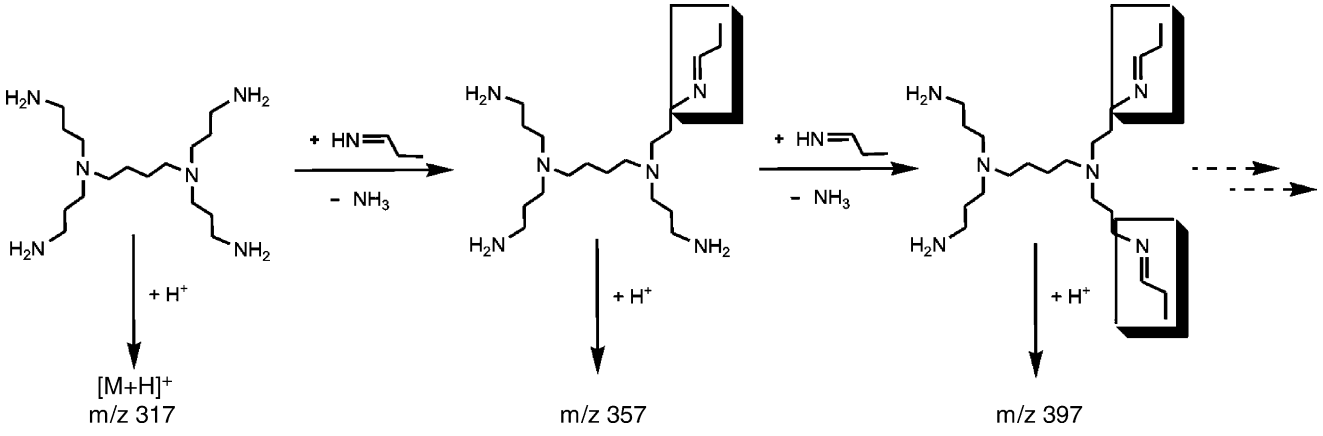


Fig. 1. Top: positive-mode ESI mass spectrum of G1 POPAM dendrimers exhibiting a series of equidistant signals ($\Delta m = 40.031$ Da). Bottom: MALDI mass spectrum (matrix: 2,5-dihydroxy benzoic acid) of the same compound. Only the expected signal for $[M + H]^+$ is observed.

Fig. 1 compares the ESI and MALDI mass spectra of the first generation POPAM dendrimer. When electrosprayed from methanol with 1% of acetic acid added to facilitate protonation, the expected quasimolecular ion $[M + H]^+$ (m/z 317) appears only with rather low intensity. More prominent are signals for a series of ions which differ from each other by a repetitive mass difference of $\Delta m = 40.031$ Da. This mass difference points to additional C_3H_4 units incorporated in the dendrimer structure. In marked contrast, no such additional signals are observed in the MALDI mass spectrum, in which the $[M + H]^+$ ion corresponds to the base peak and is accompanied only by very minor signals.



Scheme 2. Formation of imine defects during the synthesis and the molecular masses of the corresponding ions.

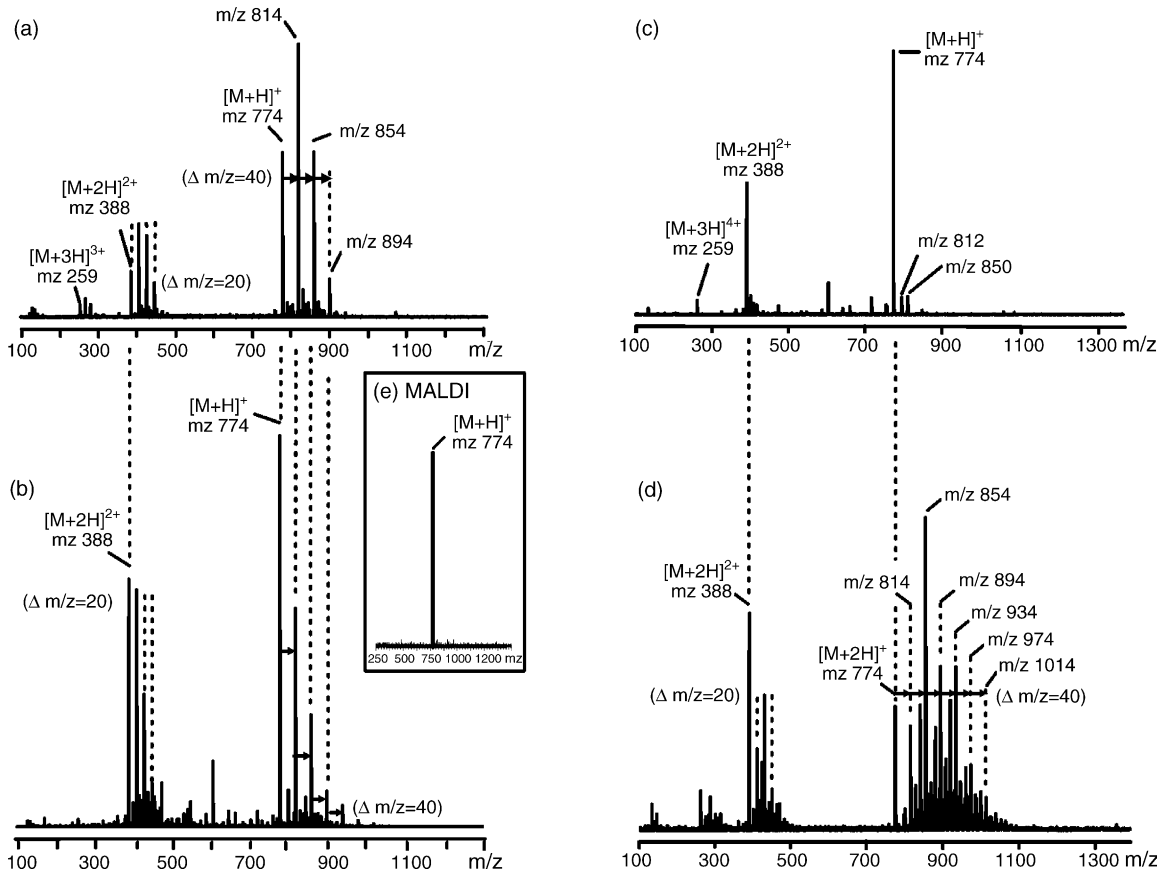


Fig. 2. ESI mass spectra (a) and (b) of two different batches of G2 POPAM dendrimers showing different amounts of defects depending on the batch, (c) of a sample stirred in water for several minutes, and (d) of a sample after addition of propionic aldehyde. The inset (e) depicts the corresponding MALDI mass spectrum, which again does not show any signal for the defects observed in the ESI mass spectra.

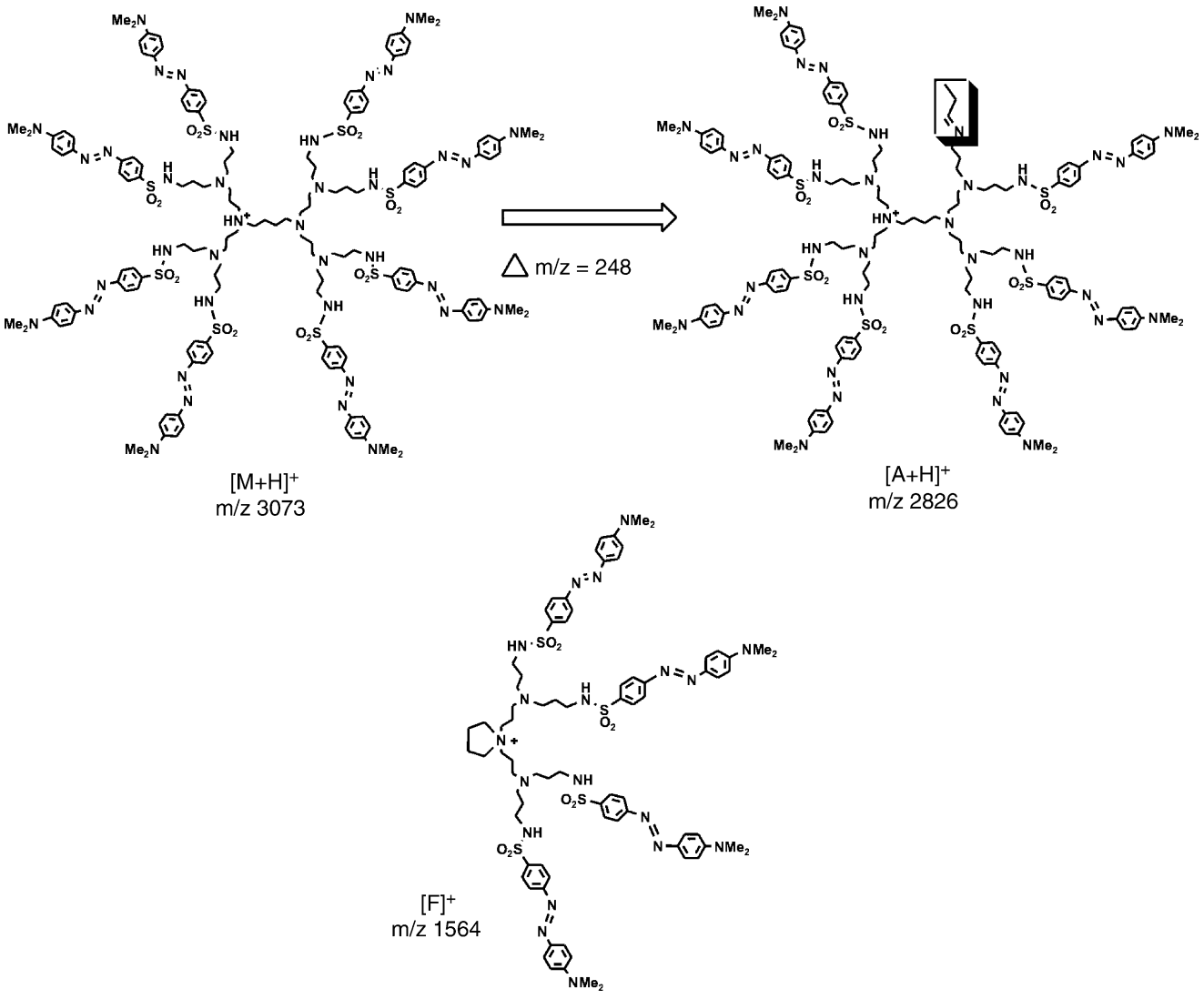
Considering the synthetic procedures by which the (commercial) sample was produced, we attribute the additional signals in the ESI mass spectrum to the defects shown in Scheme 2. When traces of acrylonitrile are left in the product of the Michael addition, they may be reduced in the next step to form an acrylimine intermediate. The exchange of the imine NH group against one of the dendrimer's amino termini then would lead to the observed defects and to the net addition of C_3H_4 units to the dendrimers (Scheme 2). Consequently, a new type of defect was identified which seems reasonable in view of the synthesis.

In order to gather additional evidence for the imine nature of the defects, the following experiments were performed with the second generation dendrimer:

1. Two samples of G2 POPAM from different batches were ionized by ESI and their mass spectra were recorded (Fig. 2a,b). Both spectra show different defect distributions which indicate that the defects are generated in different amounts from batch to batch. In the ESI mass spectra, several signals for multiply protonated dendrimers up to the trication are observed. This is also true for the whole distribution of defects.
2. If the defect is indeed an imine, it should be easily hydrolyzed. Fig. 2c shows the mass spectrum of a sample which has been stirred for several minutes in distilled water. Indeed, the defects are almost completely absent.
3. One might provoke imine formation by addition of propionic aldehyde to the dendrimer sample (Fig. 2d). Indeed, the imine defects are pronounced in their intensities.

From these experiments, we conclude that the assignment of an imine structure to the observed defects is correct.

The next question concerns the fact that no such defects are observed in the MALDI mass spectra. As found for the G1 POPAM dendrimer, the MALDI mass spectrum of the second generation analogue is almost perfectly clean (Fig. 2e). Two alternatives exist to explain this difference: imines are easily hydrolyzed under acidic conditions. Since the matrix used in the MALDI experiments is 2,5-dihydroxy benzoic acid, all imines may have been cleaved either before or during ionization. This would mean that the ESI mass spectra provide a more realistic picture of the sample composition, while the MALDI mass spectra would falsely indicate a clean sample. Alternatively, MALDI might provide the more accurate picture, while ESI exaggerates the amounts of the defects, if they are charged or desolvated more easily as compared to the structure-perfect dendrimers and thus have a higher ionization efficiency.



Scheme 3. Substitution of G2 POPAM dendrimers with methyl orange at their periphery. Imine defects protect branches against substitution. Bottom: one of the gas-phase fragments, which appear in the mass spectra.

Here, NMR experiments may be helpful, since the C–H proton and the carbon atom of the imine double bond as well as the peripheral methyl group should appear in a chemical shift range not superimposed by any other signal from the dendrimers. However, no such signals were observed above a ca. 2% level for any of the POPAM dendrimers irrespective of their generation number. Consequently, we must conclude that the samples at least do not contain significant amounts of the imine-terminated dendrimers. Thus, the intensities observed in the ESI mass spectra are likely exaggerated. Finally, can we exclude that the observed signals are merely an ESI artefact without any imines present in the sample? The following experiment reveals that these defects are real even in solution before ionization. If one reacts a sample of G2 POPAM dendrimers with the sulfonyl chloride derivative of methyl orange [11] as shown in Scheme 3 and records an ESI mass spectrum of the crude product (Fig. 3), a series of equidistant signals with $\Delta m/z = 124$ are observed. Since the corresponding cations are doubly charged, the apparent distance translates into a mass difference of 248 Da which is in agreement with the value expected for a methyl orange branch ($\Delta m = 288$) that is replaced by one of the imines ($\Delta m = 40$). The conclusion from this experiment is that the imine groups protect the branches to which they are attached against substitution with the sulfonyl group. Consequently, they are present before the ESI-MS experiment.

In addition to the imine defects, the ESI mass spectrum (Fig. 4) of 3rd generation POPAM dendrimers exhibits low-intensity signals for the defects A and B shown in Scheme 4 which are also observed in the MALDI mass spectrum. Again, the imines are absent in the MALDI spectrum. Since defects of type A and B have been previously analyzed in detail by Meijer et al. [10] we refrain from an in-depth discussion here. The conclusion from these results is that MALDI provides a more realistic picture of the purity of POPAM dendrimers, while ESI leads to a clear overestimation of the amount of imine defects in the sample.

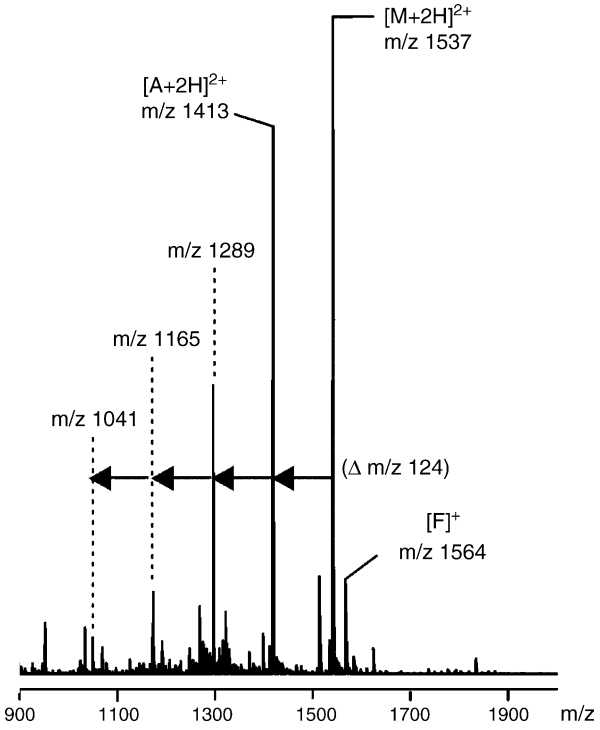


Fig. 3. ESI mass spectrum of the product of the reaction of G2 POPAM dendrimers with the sulfonyl chloride of methyl orange. Note that the ions observed are dications according to the peak spacing of their isotope patterns. The equidistant peaks relate to ions with an increasing number of branches at which no methyl orange substitution was possible due to the presence of a protective imine.

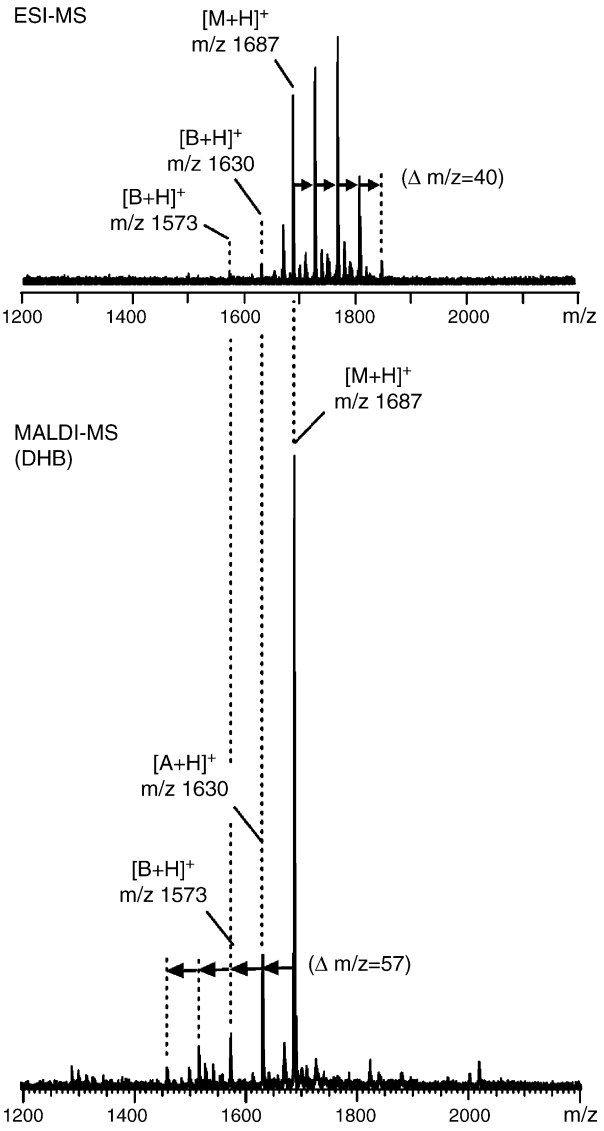


Fig. 4. Comparison of the ESI and MALDI mass spectra of G3 POPAM dendrimers. In addition to the imine defects found in the ESI, but not the MALDI spectrum, signals for ions with missing branches are observed (Scheme 4).

3.2. Sulfonamide-terminated POPAM dendrimers

POPAM dendrimers have earlier been decorated with photoactive sulfonamide groups such as methyl orange [11], azobenzene [12], or dansyl [7b,13] at their periphery, in order to study their photochemical behavior. In the following, we focus on the dansylated dendrimers as representatives for these three different groups. Fig. 5 compares the ESI and MALDI mass spectra of dansylated G1 POPAM dendrimers after purification by column chromatography (Scheme 5).

Clearly, the ESI mass spectrum (Fig. 5a) provides evidence for the perfect structure of the dendrimer. The only signals in the mass spectrum correspond to the expected protonated ion at m/z 1250 and proton- or sodium-bridged dimers at m/z 2499 and 2521. Instead, new signals appear in the MALDI spectrum (Fig. 5b) below the mass of the protonated dendrimer. Peak spacings are $\Delta m = 233$ corresponding to missing dansyl units which are replaced by a proton. Such signals would have been expected for defect structures obtained from an incomplete synthesis in which not all amino termini were reacted with dansyl chloride. However, as evidenced by the ESI mass spectrum, the origin of these signals cannot be due to synthetic failure. Instead, we interpret this result in terms of the mechanism depicted in Scheme 5a. An acidic matrix such as 2,5-dihydroxy benzoic acid may activate the sulfonyl group for an attack of any nucleophile present, e.g., a deprotonated matrix molecule. Such a reaction has been described before for dendrimers bearing sulfonimide groups at their periphery [6]. A photochemical cleavage of the sulfonamides [14] cannot be ruled out, since the absorption maxima of the dansylated POPAM dendrimers ($\lambda_{1,2\ max}$ = 253 nm, 339 nm; acetonitrile/dichloromethane 5:1 (v/v)) [13a,b] correspond quite well to the wavelength of the N_2 laser (337 nm). However, photocleavage is certainly not the only reason for losses of the dansyl groups. Analogous fragmentation is observed for tosylated dendritic derivatives which have their absorption maxima around 254 nm far below the wavelength of the laser, where they do not show any absorption in the UV spectra [6b].

If the mechanism in Scheme 5a is correct, the amount of fragments should depend on the acidity of the matrix [15], while no such pronounced matrix effect is expected, if direct photocleavages occur. Consequently, different matrices were tested with the dansylated G2 POPAM dendrimer (Fig. 6a–c) drawn in Scheme 6, which in the ESI mass spectrum again does not show significant signals for defects with missing dansyl units

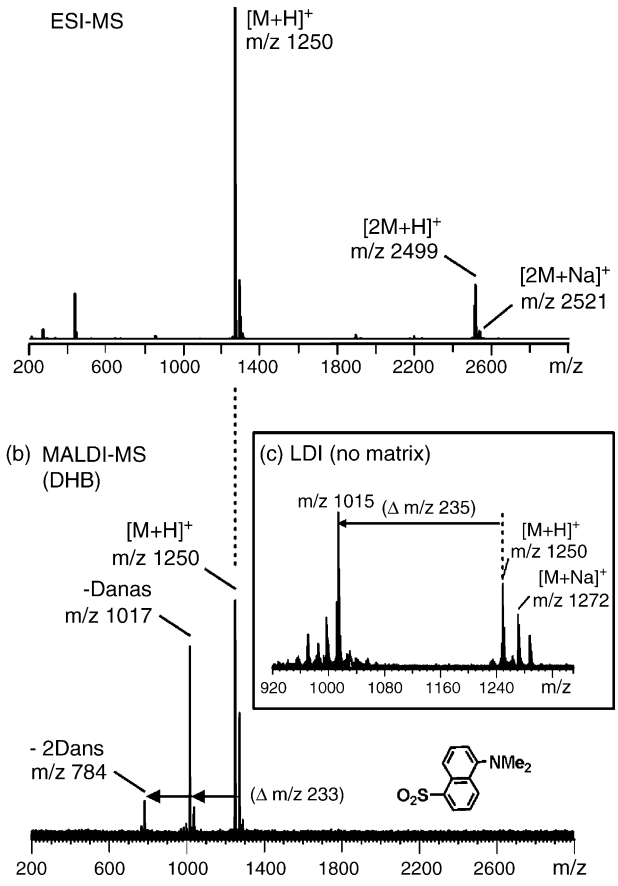


Fig. 5. Comparison of the (a) ESI and (b) MALDI mass spectra of dansylated G1 POPAM dendrimers. While the MALDI spectrum indicates missing dansyl units, the ESI spectrum provides evidence for a clean, fully substituted dendrimer sample. In addition to the expected $[M+H]^+$, only the proton- or sodium-bridged dimers are observed. (c) The inset shows part of the LDI (no matrix) mass spectrum.

(Fig. 6d). When DHB is chosen as the matrix (Fig. 6a), the signals for dansyl-deficient defects are intense and up to three missing dansyl units are detected. 2,4,6-Trihydroxy acetophenone (THAP, Fig. 6b) does not bear any carboxylic acid moiety, although it is still protic with its phenolic OH groups. This matrix reduces the signals for the defect structures significantly and only a low-intensity signal for a structure lacking one dansyl unit is observed. In both spectra signals appear for the fragment shown in Scheme 6 and the corresponding fragments originating from the corresponding dansyl-deficient ions. This fragmentation reaction was described earlier by Meijer et al. [16] for non-dansylated POPAM dendrimers. Finally, 9-nitroanthracene (9-NA) as the matrix yields only a signal for this fragment, while the parent ion has completely been destroyed (Fig. 6c). In conclusion, the expected matrix effect is indeed observed confirming our interpretation of the differences between the ESI and MALDI mass spectra.

In order to address the question in greater detail whether photochemical cleavages occur upon laser irradiation in addition to the reactions discussed above, laser desorption/ionization (LDI) experiments were performed in the absence of any matrix. While the tosylated dendrimers, which do not absorb at the

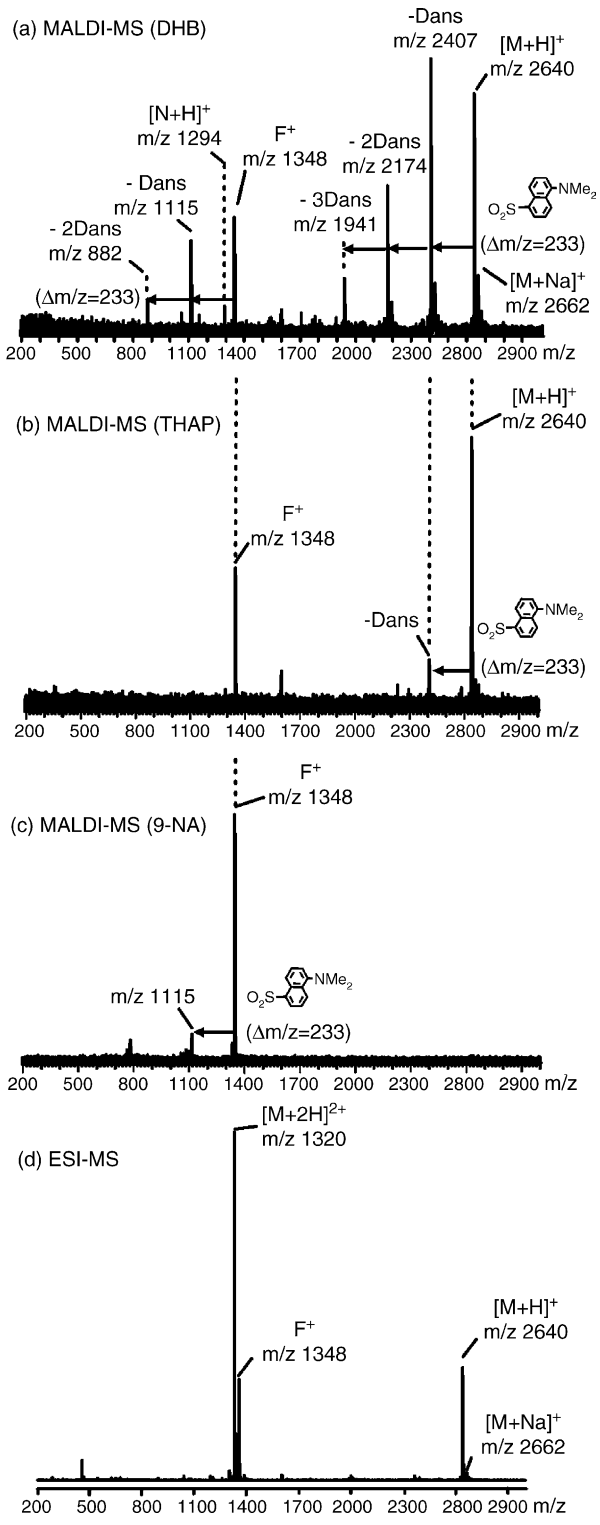
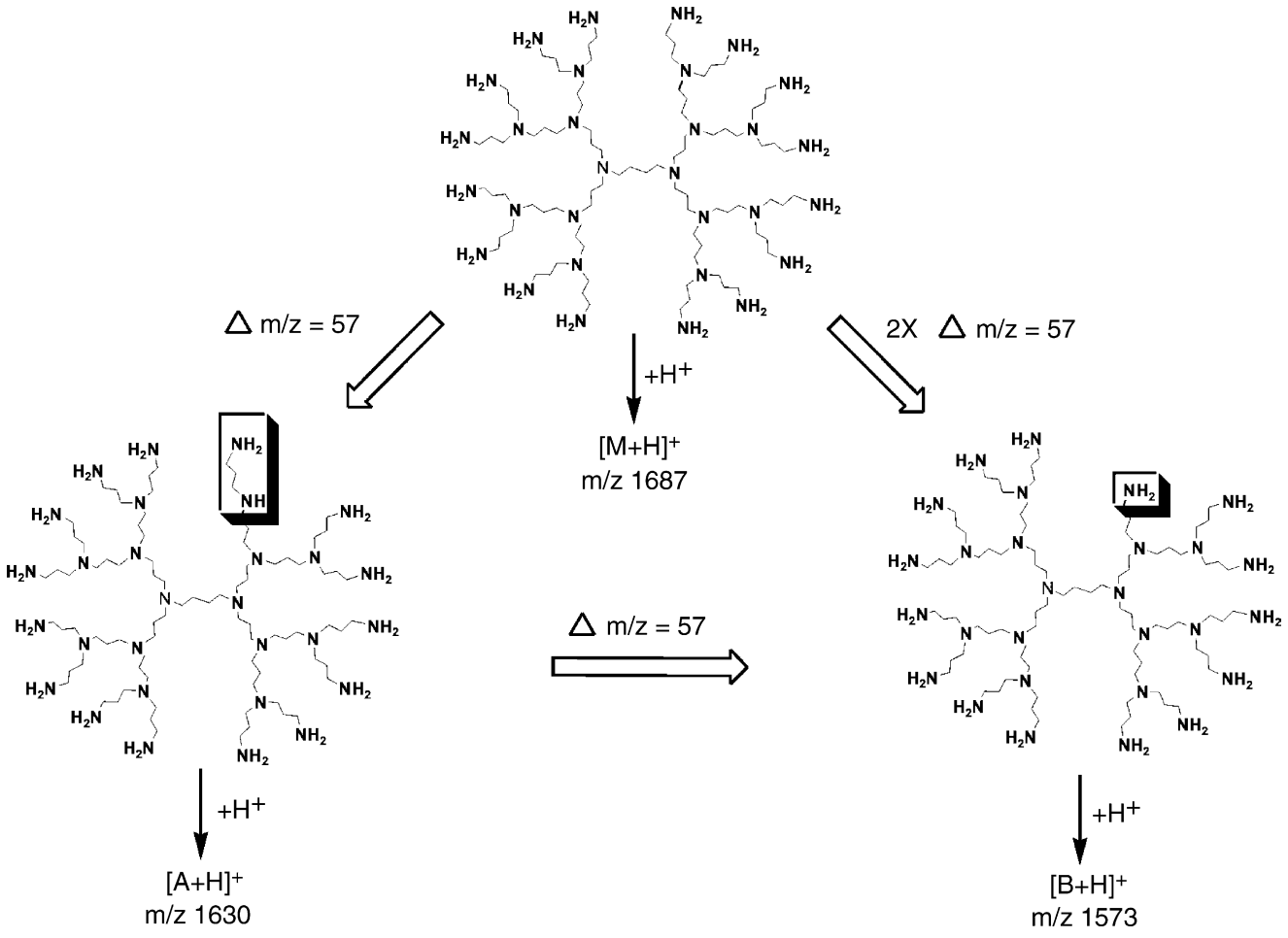


Fig. 6. The influence of matrices of different acidity on the MALDI mass spectrum of perdansylated G2 POPAM dendrimers. (a) 2,5-Dihydroxy benzoic acid (DHB) provokes dansyl losses, which are less pronounced, (b) when 2,4,6-trihydroxy acetophenone (THAP) is used. In addition, a fragment F^+ formed in the gas phase after ionization and the corresponding dansyl loss defects are visible. (c) This fragment corresponds to the major signal when 9-nitroanthracene (9-NA) is the matrix. The parent ion completely vanishes here. (d) Corresponding ESI mass spectrum providing evidence for the purity of the dendrimer. Besides the singly and doubly charged intact dendrimer, only the gas-phase fragment F^+ is formed.



Scheme 4. Molecular ions of G3 POPAM dendrimers and two defects observed in the ESI and MALDI mass spectra in addition to the imine defects appearing only in the ESI spectrum. Note that there exist several isomeric structures for defect B, which lacks two terminal branches. They all have the same elemental composition and thus cannot be distinguished by mass spectrometry alone.

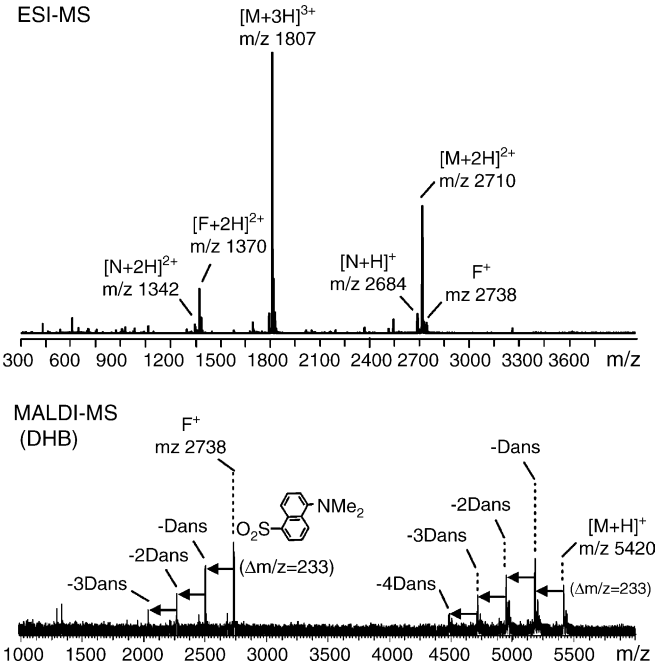
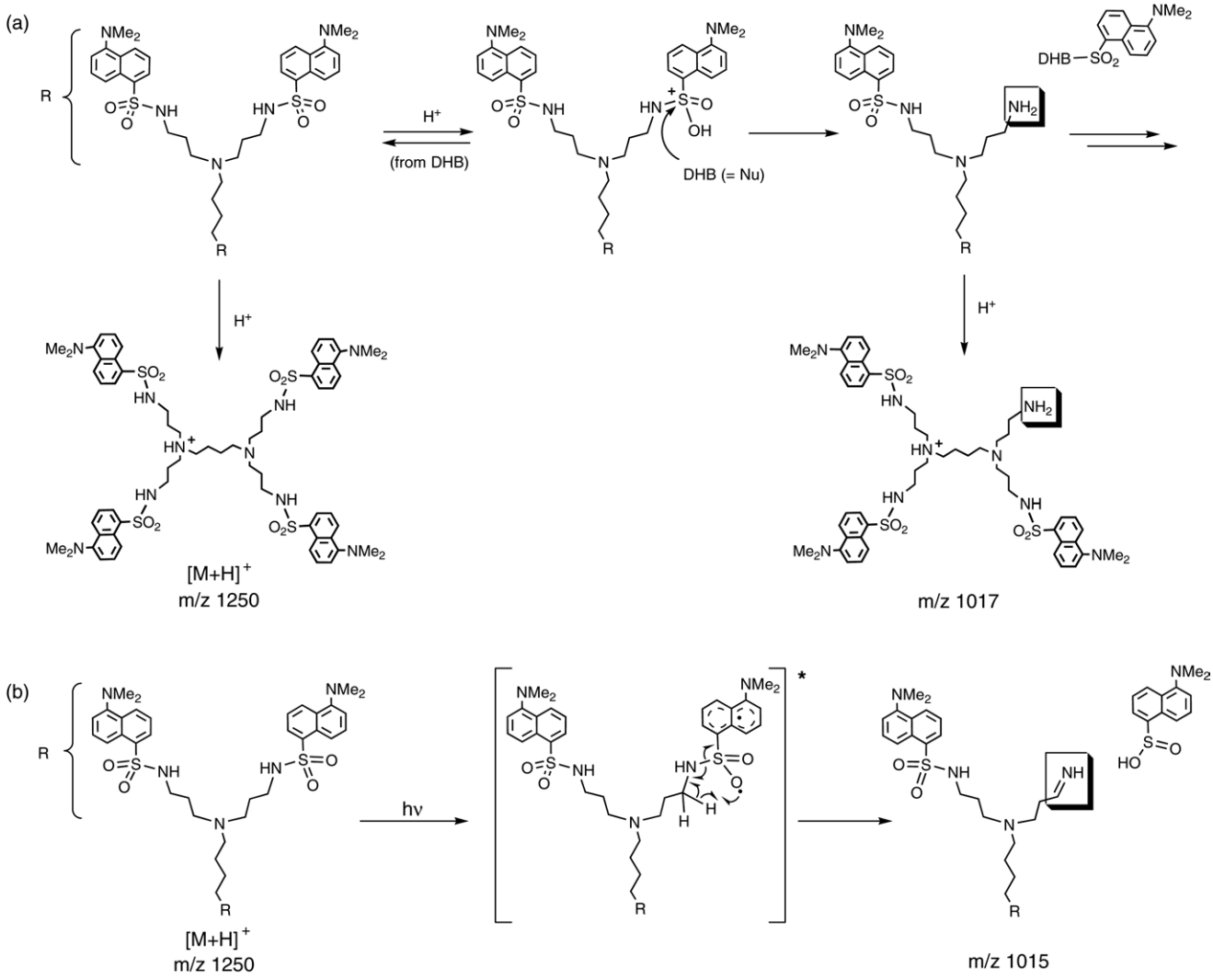


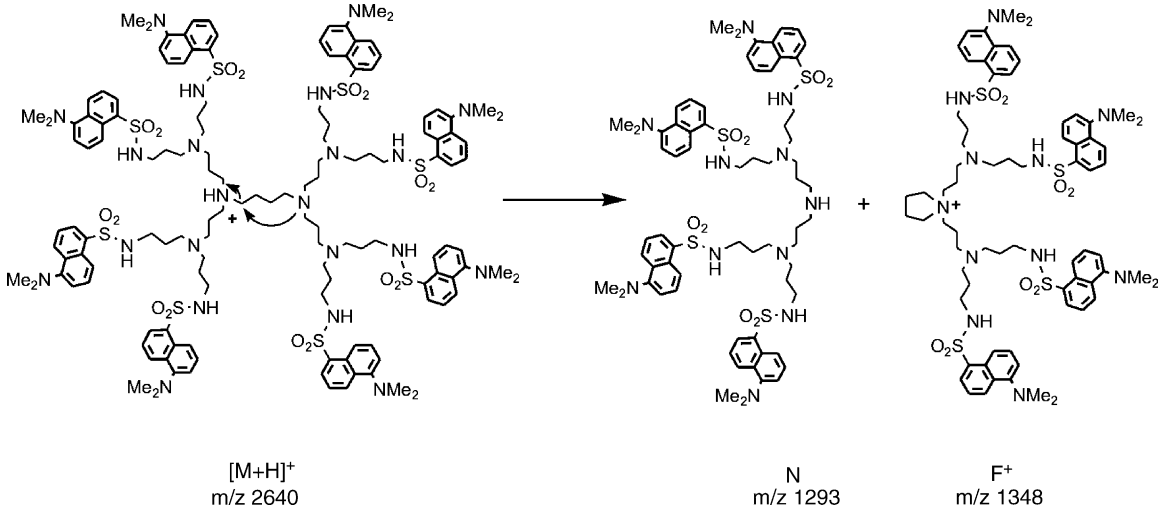
Fig. 7. The dansylated G3 POPAM dendrimers exhibit the same behavior as their lower generation analogues (see Figs. 5 and 6).

laser wavelength do not give useful spectra, the dansylated dendrimers yield signals for the protonated parent ion. Presumably, the proton originates from one of the sulfonamide groups of a second dendrimer, which are slightly acidic and may be transferred under the conditions of the LDI experiment. Gas-phase fragmentation according to the Meijer mechanism [16] is more prominent due to the absence of matrix molecules which remove part of the energy imposed by the laser. This is particularly true for the higher generation dendrimers which have a larger number of chromophores and thus may gain a larger amount of internal energy than the smaller ones. Interestingly, the replacement of a dansyl group by a proton is not observed ($\Delta m = 233$ Da) in the LDI mass spectra. Instead, a mass difference of $\Delta m = 235$ Da is observed (Fig. 5c) indicating a photochemical reaction [17] to occur rather than the decomposition reaction found in the presence of DHB as the matrix. A tentative mechanism rationalizing the observed reaction is shown in Scheme 5b. These reactions are hardly observed in the MALDI mass spectra obtained from DHB matrix.

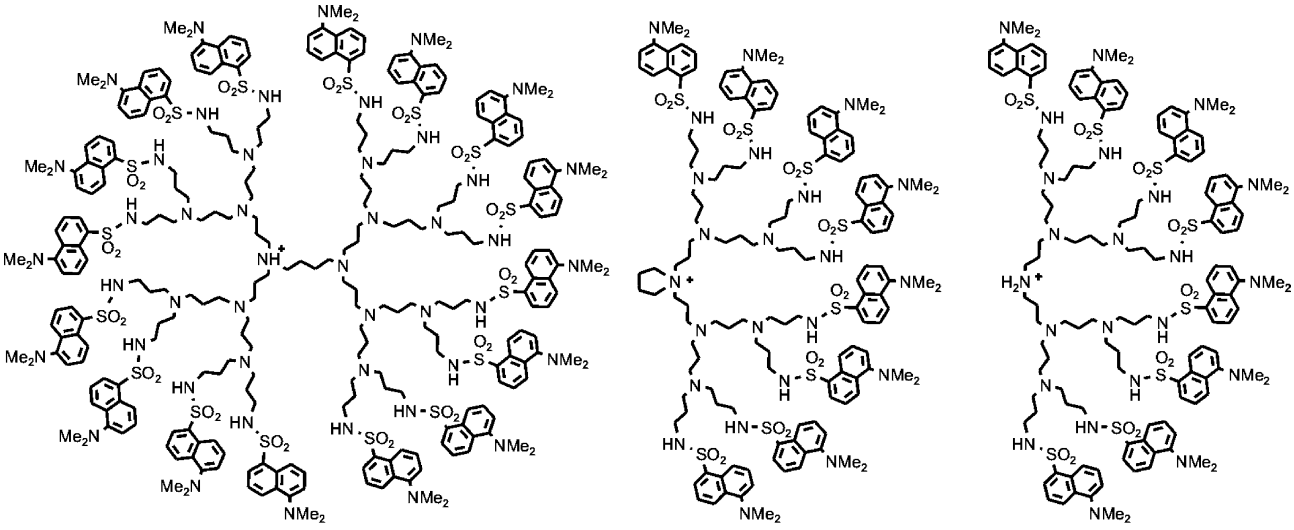
Finally, it should be noted that the dansylated G3 POPAM dendrimers (Scheme 7) behave closely analogous. The only major difference is that they appear in the ESI spectrum only as doubly and triply charged species. Due to the presence of more



Scheme 5. Dansylated G1 POPAM dendrimer and the mechanisms, which lead to (a) thermal replacement of a dansyl group by a proton and (b) to a light-induced loss of a dansyl group.



Scheme 6. Fragmentation mechanism of protonated G2 POPAM dendrimers in the gas phase as described earlier by Meijer et al. [16].



Scheme 7. Dansylated G3 POPAM dendrimer and the two fragmentation products formed in the gas phase after ionization.

than one charge, both fragments F^+ and $[N + H]^+$ are visible. In contrast, the MALDI mass spectrum shows singly charged ions accompanied by analogous series as described above for the G2 analogue (Fig. 7).

4. Conclusions

The two examples discussed in this paper make clear that mass spectrometry is indeed a useful tool for the characterization of dendrimers, although the results need careful evaluation and interpretation. Both ionization methods, ESI and MALDI, may yield a picture which does not precisely reflect the real sample composition. They may drastically overestimate the concentration of defects in the sample as in the case of the ESI mass spectra of POPAM dendrimers. Or they may falsely indicate the presence of defects which are actually completely absent in the sample and are generated upon ionization. This happens, when MALDI is used for the ionization of sulfonamide-terminated POPAM dendrimers. If suitable chromophores are incorporated in the dendrimers, even photochemical reactions may occur that complicate the situation even more.

For the synthetic chemist involved in dendrimer synthesis, it is important to cross-check seemingly unsatisfying results either by applying a second ionization method or by testing the samples with different MALDI matrices in order to judge what the real sample composition is. Since dendrimers with structural defects still bear the same types of functional groups (although their numbers might change) as compared to their structure-perfect analogues, one expects them to give rise to signals under ionization conditions which also permit the analysis of the perfect ones. It is thus unlikely to obtain clean spectra from samples which contain a significant amount of defects, i.e., falsely positive results. Our study demonstrates that negative results may however come from ionization rather than synthesis. It might thus be rewarding for the synthetic chemist to analyze the origin of the signals for defect dendrimers through additional experiments.

Acknowledgments

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