Host–Guest Chemistry

Multivalency in the Gas Phase: The Study of Dendritic Aggregates by Mass Spectrometry**

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Insights into the strength and dynamics of noncovalent interactions in complex molecular aggregates are essential for the understanding of all forms of soft matter.[1–3] Most, if not all, studies are performed under thermodynamically controlled conditions, in which association and dissociation are in competition.[4] Exciting progress in mass spectrometry has enabled gas-phase studies of protein complexes[5–8] and organic supramolecular aggregates to be carried out.[9–11] Electrospray ionization mass spectrometry (ESI-MS) has proven to be most successful in achieving the delicate transfer of large molecules, including dendrimers,[12] from solution to the gas phase.[13–15] The stability of ions in the gas phase can be probed with collision-induced dissociation (CID), in which an ion is selected, accelerated, and subsequently collided in a cell with neutral gas atoms, for example, argon.[16,17] In the collision process a part of the ion's kinetic energy is transformed into internal energy, which eventually results in fragmentation. The extent of fragmentation depends on the internal energy of the excited ion, which in turn is related to the imposed acceleration. Despite widespread application of CID in the analysis of covalent structure,[18] it appears to be difficult to analyze the dissociation of noncovalent structures in the gas phase. Moreover, the relationship between the strength of the aggregate in solution (thermodynamically controlled) and in the gas phase (kinetically controlled) remains controversial.[19–21] Here we report on dendritic aggregates with one to eight guest molecules, which were designed to assemble at the periphery of the dendrimer. Analysis of the aggregates by collision-induced dissociation mass spectrometry shows that the guest molecules are removed one by one in a highly controlled way. The analysis presented unequivocally proves the existence of multicomponent dynamic libraries and also allows the relative stabilities of the host–guest interactions to be determined when different guests are simultaneously anchored to the same dendrimer. The selectivity in the dissociation of the individual guests is analyzed in the molecular center of mass frame with a simple kinetic model. New insights into the behavior of multivalency in supramolecular aggregates including libraries of oligopeptides are presented here, in which mass spectrometry acts as the tool to analyze these multivalent interactions in great detail.

The compounds used in this study are based on third generation adamantylurea-substituted poly(propylene imine) dendrimers (**D**, Scheme 1), which are excellent hosts for guest molecules based on urea acids (**1**–**4**) in solvents such as chloroform. The multivalency of dendritic macromolecules[22–25] is generally seen as the way to obtain highly efficient bioactive synthetic structures.[26] An attractive methodology for dynamically modifying the periphery of dendrimers was recently disclosed, in which the end groups of the dendrimer act as the scaffold for guest molecules that assemble around the dendrimer.[27,28] If both ionic and hydrogen-bonding interactions are considered, the maximum number of guests that can be bound specifically is set by the number of end groups, thus eight guests can be bound by the third-generation dendrimer (Scheme 1). The assembly process has been studied previously with different generations of dendrimers and other guest molecules by a variety of techniques, including NOESY NMR spectroscopy.[27] Although firm experimental evidence has been presented to confirm the assignment of these supramolecular structures, definite proof of these multivalent supramolecular architectures is inherently difficult, because of the dynamic nature of the multicomponent aggregates.[29]

Electrospray ionization mass spectrometry was used to analyze mixtures containing **D** and a one- to eightfold excess of guest **2** in chloroform (10^{-6} M **D**). Triply and quadruply protonated complexes of the different aggregates were observed. Deconvolution provided the overall spectrum and, surprisingly, all possible assemblies between **D** and **2** are observed as well as a small amount of free **D** (Figure 1a, b). The nine major peaks are separated by the mass of **2** (M_r = 244 Da) and are interpreted as the assembly of x guests **2** with **D** (notation used through the paper for the assemblies is: $\mathbf{D}\cdot\mathbf{2}_x$, with $x = 0$–8). Selecting one of the assemblies in the quadrupole (for example, $[\mathbf{D}\cdot\mathbf{2}_4]^{3+}$; Figure 1a) and subsequently colliding the isolated ion with argon buffer gas in the collision cell resulted in dissociation of the aggregate. The products of this dissociation were analyzed by TOF-MS. Figure 1c shows a stacked plot of mass spectra obtained with increasing acceleration voltages. Dissociation of guest **2** is observed as the voltage is increased, which eventually results in empty **D**. The guests are removed as neutral species and only the tricationic dendritic aggregates are observed. This is a general observation for all aggregates (**D** with **1**–**4**) and all possible values of x. Not only do the spectra prove that the different complexes are indeed formed, ionized, and stable enough to be analyzed, the voltage needed for dissociation is a guide to the stability of the aggregate in the gas phase.

We have also analyzed complexes of **D** with **1**–**4**, and thus we now have a set of molecules that differ in acidity (**1** versus

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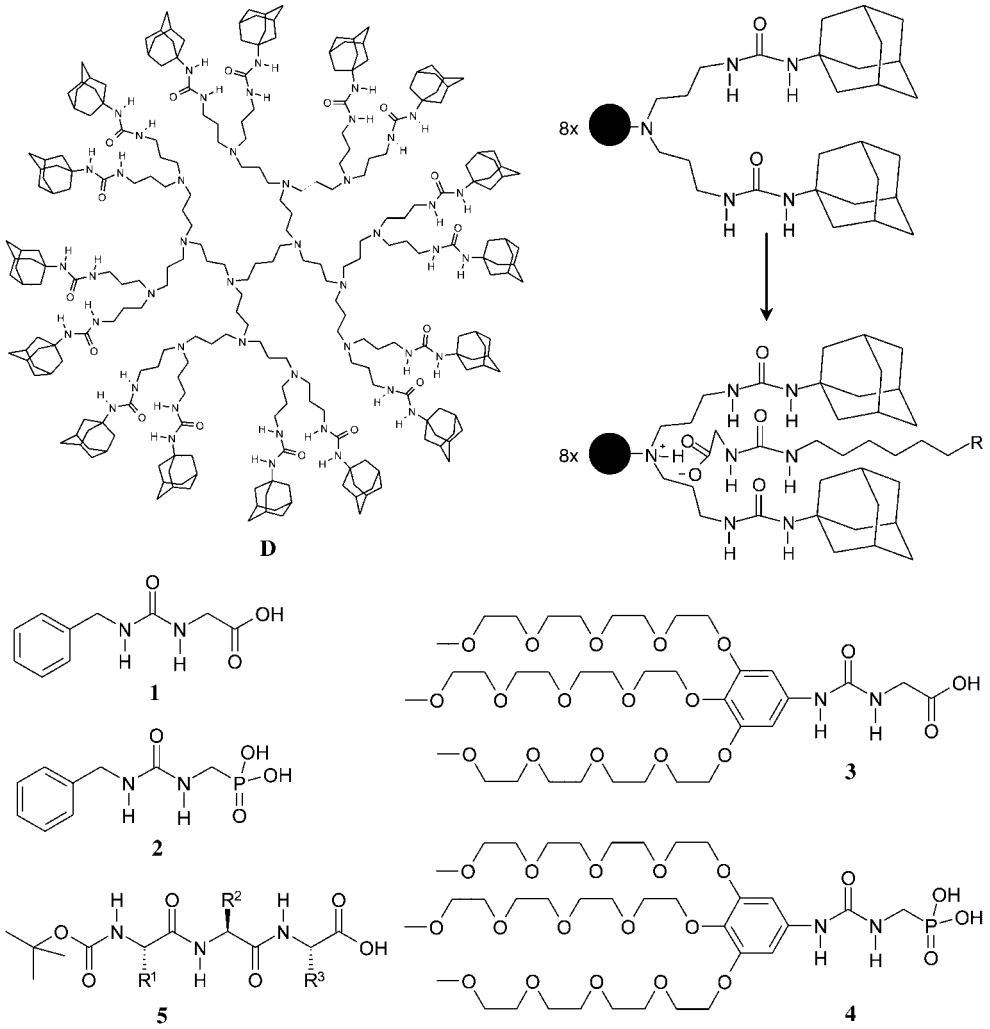

Scheme 1. Representation of the structures used. Third generation adamantylurea-modified poly(propylene imine) dendrimer **D** acts as a host for urea acid guests **1**–**4** which differ in size and acid strength. The Boc-protected tripeptides (**5**) also investigated are: Boc-GGG, Boc-GGA, Boc-AAA, Boc-FGG, Boc-FFF, and Boc-GVV. The symbols for the amino acids are: G = glycine (R = H), A = alanine (R = CH_3), F = phenylalanine (R = $CH_2C_6H_5$), V = valine (R = $CH(CH_3)_2$). Boc = *tert*-butoxycarbonyl.

2 and **3** versus **4**) and molecular weight (**1** versus **3** and **2** versus **4**). Importantly, the multivalency of the system makes it possible to assemble different guest molecules with the same dendrimer and perform selection and analysis of these multicomponent ions. This type of analysis is unique for comparing different guests, since the internal energy of collision is distributed over all the guests in the same aggregate. When an aggregate containing two guests of both **1** and **2** (denoted as $\mathbf{D}\cdot(\mathbf{1}_2+\mathbf{2}_2)$) was subjected to CID, guests **1** dissociated before guests **2** started to dissociate (Figure 2a). This selective dissociation of **1** over **2** is independent of the combination of the guests attached to the dendrimer and is indicative of a difference in the binding strength in the gas phase which arises from differences in acidity. These results are in good agreement with observations of solution experiments, where a higher acidity leads to a higher association constant in chloroform. When both **2** and **4** are assembled on the same dendrimer and $\mathbf{D}\cdot(\mathbf{2}_2+\mathbf{4}_2)$ subjected to CID, the small guests are significantly less strongly anchored than the large guests (Figure 2b). Evidence for fragmentation of the covalent bonds in the dendrimer appears, even before all of **4** is removed. In contrast to the gas-phase data, the difference in the binding strength measured for **2** and **4** with **D** in chloroform is marginal. This discrepancy between the solution and gas phases is proposed to result from additional van der Waals interactions between the ethylene glycol chains of **4** and the dendrimer scaffold in the gas phase. In chloroform the chains of the guests are solvated and the association constant is then primarily determined by the urea acid motif.

To analyze the data in a more quantitative way we compared the voltage required to reach an ion survival yield of 50% of the selected ion. Dendrimers with the smaller guest **2** require significantly less voltage then dendrimers with the larger guest **4** (Figure 3a). The same trend is observed for **1** versus **3** (not depicted). However, it is important to remember when comparing different ions that the fraction of kinetic energy of an ion that can be converted into internal energy

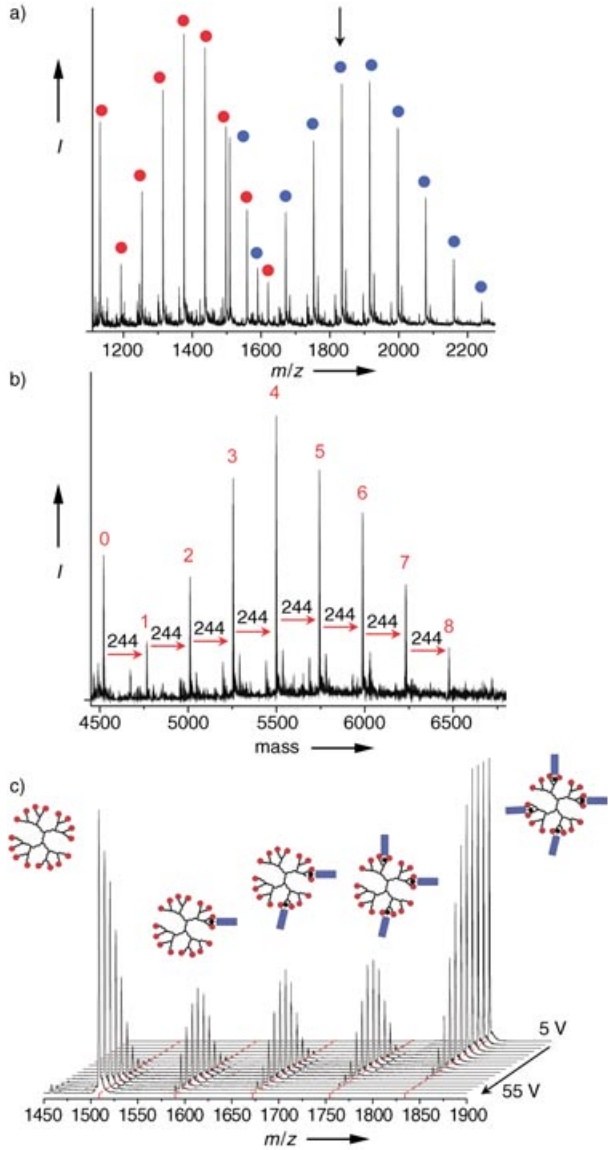

Figure 1. a) Mass spectrum of a sample of **D** with six equivalents of **2**. Two clusters of ions are observed, which consist of one to eight guests bound to the dendrimer with a 4^+ (red) or 3^+ (blue) charge. b) The mass difference between the peaks after deconvolution corresponds to the mass of **2** (244 Da). c) Selection of one ion $[\mathbf{D}\cdot\mathbf{2}_4]^{3+}$ (indicated with the arrow in (a)) followed by collision with increasing acceleration voltage in the collision cell results in sequential dissociation of the guests from the dendrimer. The charge of the complex does not change, which means that the guest dissociates as a neutral species, which is not observable by MS.

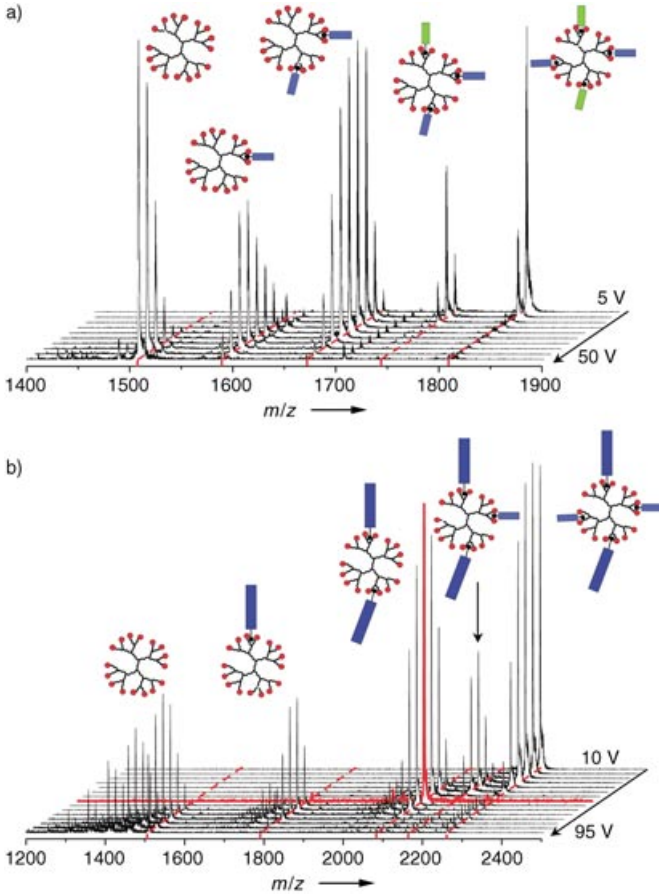

Figure 2. a) Selection of the $[\mathbf{D}\cdot(\mathbf{1}_2+\mathbf{2}_2)]^{3+}$ ion followed by CID results in selective dissociation of **1** over **2**. These differences result from the difference in the acid strengths of the guests. b) Selective dissociation also occurs for smaller guest **2** over **4** in the CID experiments of the $[\mathbf{D}\cdot(\mathbf{2}_2+\mathbf{4}_2)]^{3+}$ ion. In this case the selectivity is almost 100%: at 55 V $[\mathbf{D}\cdot\mathbf{4}_2]^{3+}$ is the only major signal (red trace). Interestingly the dendrimer itself also starts to fragment at high voltages (the adamantly groups dissociate), which indicates that covalent and noncovalent dissociation compete at that stage.

(which causes dissociation) is inversely proportional to the total mass of the complex. Therefore, the acceleration voltage has to be converted into the center of mass energy to compare the assembly of **2** with **4** in separate complexes.[16] A significant difference remains in the center of mass frame of $\mathbf{D}\cdot\mathbf{2}_x$ and $\mathbf{D}\cdot\mathbf{4}_x$, which indicates there is stronger binding of the larger guests (Figure 3b). The data for $\mathbf{D}\cdot\mathbf{2}_x$ and $\mathbf{D}\cdot\mathbf{4}_x$ are similar to the data of the mixed $\mathbf{D}\cdot(\mathbf{2}_2+\mathbf{4}_2)$ system (see above) if this correction is applied. The difference in dissociation energy in going from $\mathbf{D}\cdot\mathbf{2}_3$ to $\mathbf{D}\cdot\mathbf{2}_7$ and $\mathbf{D}\cdot\mathbf{4}_1$ to $\mathbf{D}\cdot\mathbf{4}_5$ (Figure 3b) is small.

We have simulated the distributions of the complexes formed during CID by a simple model to investigate the relationship between the number of guests bound to the dendrimer and the ion stability in more detail. The release of guests from a complex through collisions with argon atoms can be modeled as consecutive reaction steps that are pseudo-first order because of the constant argon concentration. We have used the $\mathbf{D}\cdot\mathbf{2}_4$ complex and defined four reaction rate constants (k_1, k_2, k_3, k_4) as parameters. After integrating the rate equations, we can calculate the fractions of the various species ($\mathbf{D}\cdot\mathbf{2}_4$, $\mathbf{D}\cdot\mathbf{2}_3$, $\mathbf{D}\cdot\mathbf{2}_2$, $\mathbf{D}\cdot\mathbf{2}_1$, and **D**) as a function of time. The effect of increasing the acceleration voltage upon injection of ions into the collision cell was then simulated by taking the concentrations of the different fractions at increasing times.[30] The best fit of the model with the experimental data was achieved when the k values increase slightly in value, thus indicating that the guest molecules

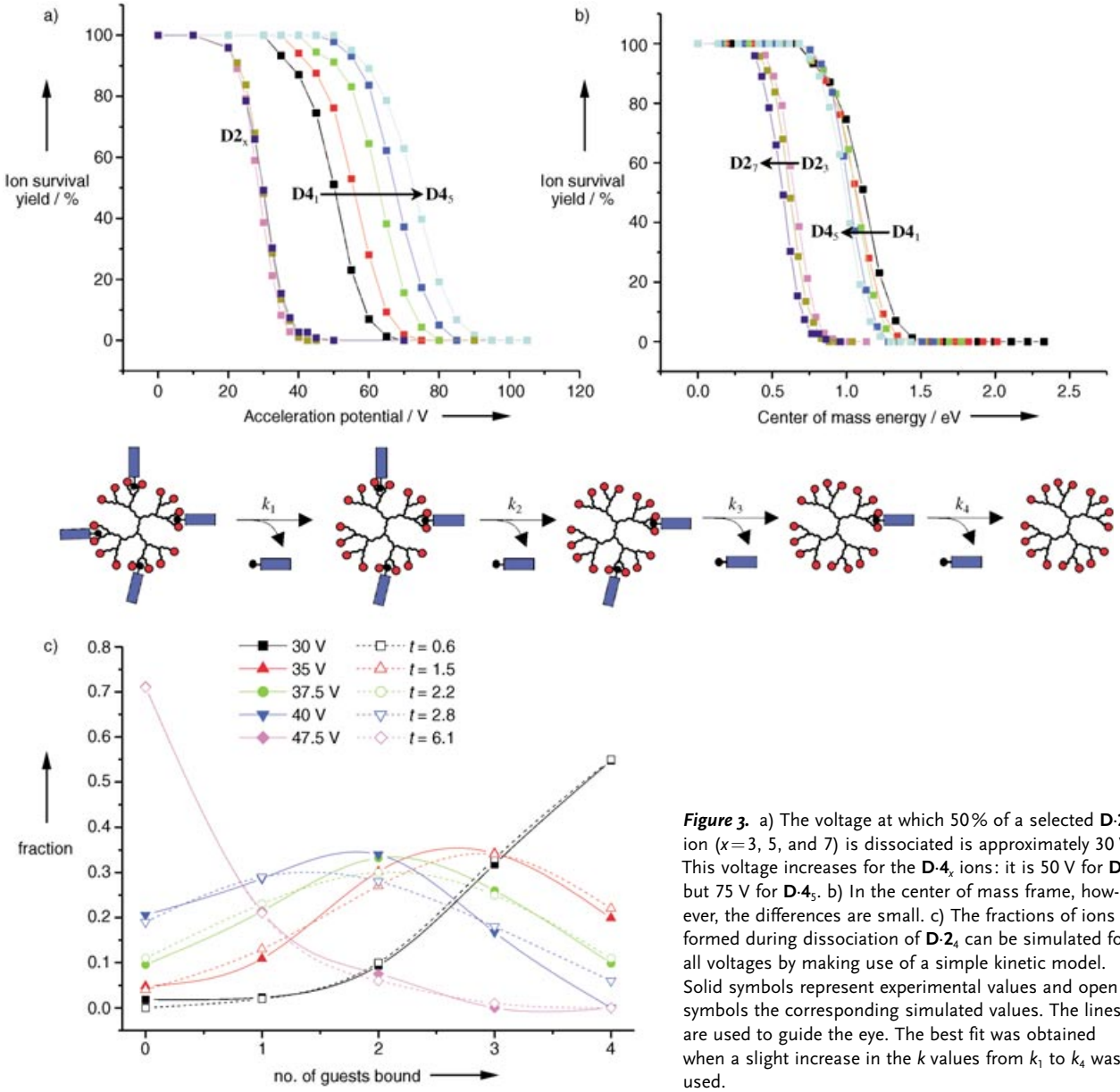

Figure 3. a) The voltage at which 50% of a selected **D·2**$_x$ ion (x = 3, 5, and 7) is dissociated is approximately 30 V. This voltage increases for the **D·4**$_x$ ions: it is 50 V for **D·4**$_1$ but 75 V for **D·4**$_5$. b) In the center of mass frame, however, the differences are small. c) The fractions of ions formed during dissociation of **D·2**$_4$ can be simulated for all voltages by making use of a simple kinetic model. Solid symbols represent experimental values and open symbols the corresponding simulated values. The lines are used to guide the eye. The best fit was obtained when a slight increase in the k values from k_1 to k_4 was used.

dissociate slightly easier when less guest molecules are bound. However, the influence of loading on the stability remains very small and is close to the statistical dissociation of the guests.

Since oligopeptides with free carboxylic acid and Boc-protected amine end groups can also be assembled at the periphery of **D** in chloroform,[28] we tested the versatility of our method by using tripeptide–dendrimer aggregates in CID-MS. A large variety of oligopeptides can be assembled in a dynamic way. Figure 4a and b show the results of two typical mass spectra for a mixture of two and three different peptides. All statistically possible structures are observed and the full dynamic library can be analyzed. By selecting a specific trication with two or three different oligopeptides it is possible to determine the different binding affinities. Remarkably, very small structural differences lead to significant changes in the dissociation. Figure 4c, for example, shows a clear preference for the dissociation of Boc-AAA over Boc-GGG, although the guests differ only by three methyl groups, which can sterically interfere with the binding motif. This effect could also explain the preference for the dissociation of Boc-GVV over Boc-FGG (not depicted), where the two isopropyl groups of Boc-GVV interfere with the binding motif. On the other hand, Boc-FFF binds more strongly than Boc-GGG, a result that is indicative of the presence of additional van der Waals interactions between the guest and host. Clearly, by now studying a large variety of peptide structures it should be possible to gain insight into protein aggregation in the gas phase.

We have shown in an unprecedented clean and simple way that collision-induced dissociation in mass spectrometry allows for detailed investigations of multicomponent supramolecular interactions. The formation of the dendritic associate in combination with the CID-MS method provides

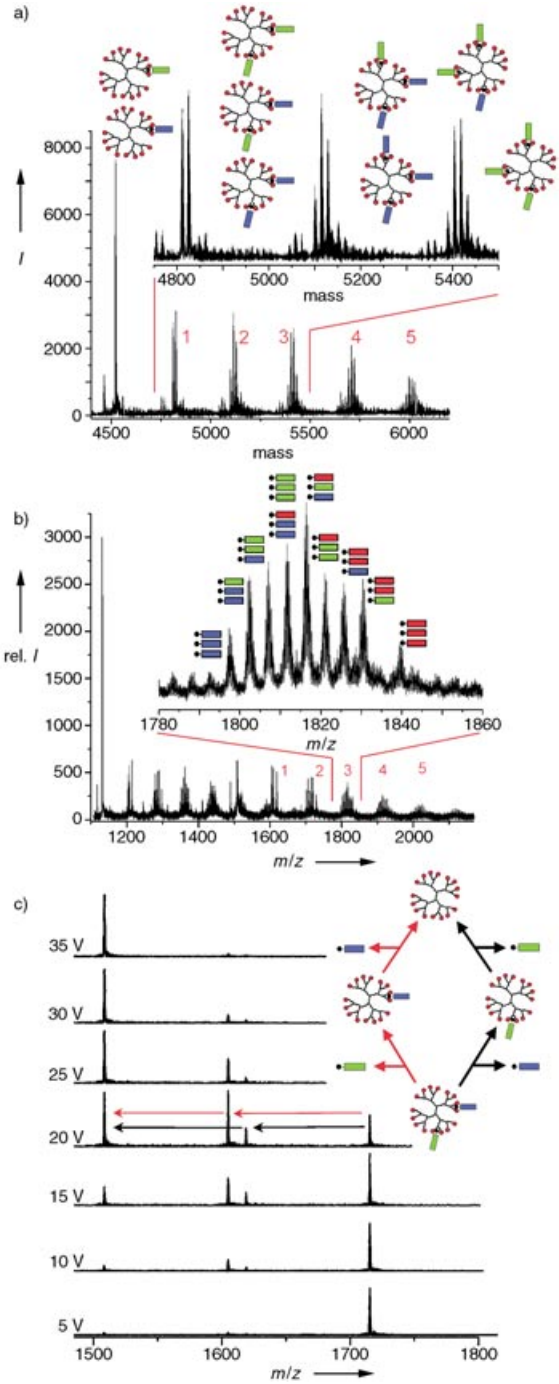

Figure 4. a) Mass spectrum after deconvolution of two tripeptides, Boc-GGG (blue) and Boc-GGA (green), bound to the dendrimer. The small difference in mass between the guests results in clusters of peaks that correspond to one to five guest molecules bound to the dendrimer (shown in red). All peaks in the clusters can be assigned to the different possible combinations of guests. b) Mass spectrum (no deconvolution) of three different tripeptides added to the dendrimer (Boc-GGG (blue), Boc-GGA (green), and Boc-AAA (red)), which results in more complicated clusters. Inspection of the cluster of signals of the dendrimer with three peptides attached to it show that ten possible combinations can be formed, all of which are visible. Two combinations result in an identical mass. c) CID-MS spectra of a dendrimer with one Boc-GGG (blue) and one Boc-AAA guest (green). Two pathways can be envisioned, of which the one indicated with the red arrow predominates.

us with a wealth of information concerning important secondary interactions of supramolecular aggregates in the gas phase.

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[30] A higher acceleration voltage in the experiment yields a higher kinetic energy for the complexes, and this in effect raises the reaction temperature for the release of the guests. As a result, the rate constants for the dissociation of the guests increase, and higher conversions are reached (more fragmentation of the initial complex). We have modeled this in a constant-temperature approach by using longer reaction times to give similarly higher conversions. In other words, we are using a temperature–time transformation, so that the fractions of the various complexes at a certain time simulate the effect of the collision cell. In the model we have assumed that the individual rate constants depend in the same way on the temperature. For a detailed description see the Supporting Information.

