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Dendrimer Disassembly in the Gas Phase: A Cascade Fragmentation Reaction of Fréchet Dendrons

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Abstract: The mass spectrometric characterization of Fréchet-type dendrons is reported. In order to provide the charges necessary for electrospray ionization, dendrons bearing an OH group at the focal point can be deprotonated and observed in the negative ion mode. Alternatively, the corresponding bromides can be converted to quaternary ammonium ions that can easily be detected in the positive mode. If the latter ions are subjected to collision-induced dissociation experiments, a fragmentation cascade begins with the dissociation of the focal amine. The focal benzyl cation quickly decomposes in a fragmentation cascade from the focal point to the periphery until the peripheral benzyl (or naphthylmethyl) cations are formed. Five different mechanisms are discussed in detail, three of which can be excluded based on experimental evidence. The cascade fragmentation is reminiscent of self-immolative dendrimers.

Keywords: dendrimers · fragmentation mechanisms · gas-phase reactions · mass spectrometry

Introduction

Dendrons and dendrimers[1] are highly branched, ideally monodisperse and regularly shaped macromolecules with a significant impact on biomedical[2] and materials sciences.[3] They are particularly interesting with respect to their nanosized structures and their Aufbau principle in generations.[4] Recently, the idea of self-immolative dendrimers has been developed, which is particularly promising for the application of dendrimers in medicine. Self-immolative dendrimers[5] carry multiple copies of a prodrug at their periphery. At the focal point they bear a disease-specific trigger. Upon arrival at the location of the disease in the body, a specific signal activates the trigger, which leads to complete fragmentation of the dendrimer scaffold into small subunits. From the periphery, the drug is released at high concentrations because of the many branches to which the prodrug is attached. This strategy is thus a way to amplify the trigger signal into the liberation of a multitude of drug molecules.

Dendrimer research has been accelerated significantly by the development of analytical methods that permitted to characterize their structures in detail, analyze their purity, and characterize their properties as a function of increasing generation number. Among these methods[6] are nuclear magnetic resonance (NMR), size-exclusion chromatography (SEC), high-performance liquid chromatography (HPLC) and, more recently[7] scanning probe methods (SPM). Since the development of the so-called soft ionization methods, mass spectrometry has proven to be a very valuable tool,[8] because it provides detailed information on defects and impurities.[9] Dendrimers have thus been ionized by fast atom bombardment (FAB-MS),[10] matrix-assisted laser desorption/ionization (MALDI),[11] and electrospray ionization (ESI).[12] The two latter techniques involve very gentle ionization that minimizes the fragmentation of the analyte molecules, although there are examples for artifacts formed during electrospray ionization[13] and through the destruction of dendrons and dendrimers during the MALDI process.[14]

The utility of mass spectrometry in dendrimer chemistry is not limited to the determination of molecular masses of dendrimers and their purity. Their chemistry in the gas phase is an interesting and novel area of research.[15] Mass spectrometry may provide even more information, for ex-

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ample on the self-assembly of dendrimers,[16] or on weak, non-covalently bound host-guest complexes of dendritic species.[17] These results demonstrate the power of mass spectrometry for a detailed characterization of dendrimers without which the fast pace of development in this field would not have been possible.

In this contribution, we discuss the mass spectrometric characterization of Fréchet dendrons such as those shown in Figure 1, which are synthesized in a convergent way.[9a,18]

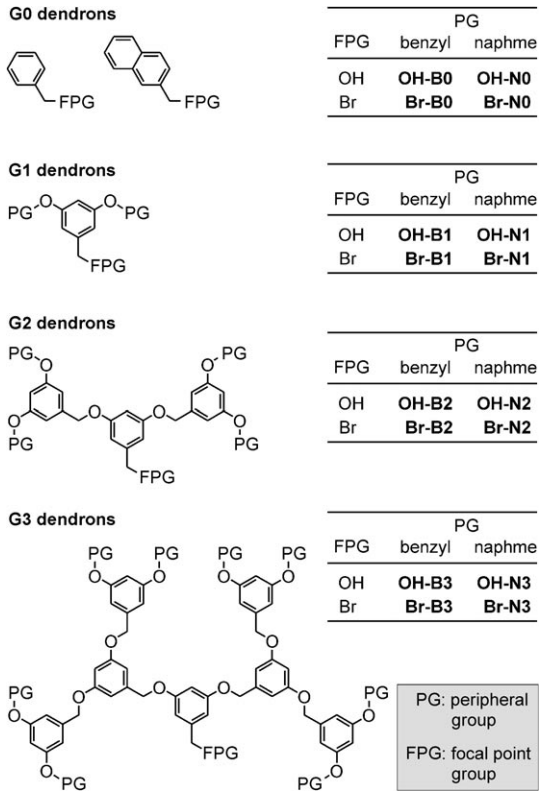


Figure 1. G0–G3 Fréchet dendrons under study. At the focal points (FPG) they bear a benzylic hydroxy group or bromide; the periphery (PG) is decorated either with benzyl or with 2-naphthylmethyl ("naphme") groups.

The final dendron carries a benzylic alcohol or a benzyl bromide at the focal point with which it can be attached to a core molecule. After some brief comments on the analytical characterization and the analysis of defects, we will focus on the fragmentation behavior of Fréchet dendrons with quaternary ammonium ions at their focal points (Figure 2). Some of them have a viologen core and are doubly charged (Figure 2). These dendrons exhibit a fast fragmentation cascade, for which five different mechanisms are discussed. Three can be ruled out based on experimental evidence from tandem mass spectrometry. The remaining two are closely related to each other and also supported by theoretical calculations. Although the initial step is the loss of an amine from the focal point, the fragmentation cascade proceeds so quickly that intermediate fragments are either not observed at all or appear merely with low intensities. As the final fragmentation products, the peripheral benzyl and naphthylmethyl groups are detected with the highest intensities. The suggested mechanism also rationalizes a few side reactions such as a methyl migration to the peripheral benzyl or naphthylmethyl groups. A number of control experiments also supports this fragmentation mechanism.

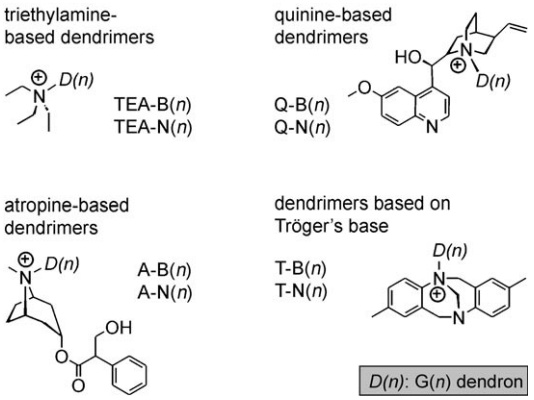


Figure 2. Dendrimers (G0–G3) with quaternary ammonium ions at the focal points. With the exception of the triethyl ammonium derivatives, these dendrimers bear chiral amino moieties (B(*n*): benzyl-terminated dendron of *n*th generation; N(*n*): naphthylmethyl-terminated G(*n*) dendron)

Results and Discussion

Synthesis of Fréchet dendron bromides: All Fréchet dendrons were synthesized by the previously published procedure.[9a,18] Up to generation G2, column chromatography can easily be used to purify the dendrons. For the preparation of the G3 dendrons, two literature procedures exist to convert the benzylic alcohol into the benzyl bromide: One makes use of PBr_3 and creates acid which may rearrange the benzyl ethers within the already existing dendron scaffold. The second one uses the Appel reaction and generates the benzyl bromide under very mild conditions with CBr_4 as the bromide source and Ph_3P as the oxygen scavenger. The final benzyl bromide was attached to the tertiary amine cores by simple nucleophilic displacement reactions.

ESI-MS characterization of Fréchet dendrons: The Fréchet dendrons terminated with a benzylic hydroxy group at the focal point can easily be characterized by negative-ion electrospray ionization Fourier-transform ion-cyclotron resonance (ESI-FTICR) mass spectrometry. Deprotonation of the OH group during the electrospray process occurs, when for example methanol is used as the spray solvent. In addition to the deprotonated dendron, a quite intense signal for the dimeric species bridged by an O···H-O hydrogen bond is observed. The fact that the dimer survives even quite harsh ionization conditions can be rationalized by the substantial strength of this ionic hydrogen bond in the absence of any competing solvent (ca. 130 kJ mol^{-1}).[19]

The corresponding benzyl bromides, however, are difficult to ionize with electrospray ionization, because they neither carry a charge nor bear any functional groups to which a charge could easily be attached. All attempts to characterize them directly by ESI-MS yielded more intense signals of some traces of easy-to-charge impurities. For example, the sodium adduct of triphenyl phosphine oxide generated as a side product in the above-mentioned Appel reaction was observed as one of the major signals, although it was not detected in the NMR spectra and thus can only be present in the sample in trace amounts.

Consequently, 1 equiv of triethyl amine has been added to the sample solutions of the benzyl bromide dendrons in methanol to generate **TEA-B(*n*)** and **TEA-N(*n*)** in situ before conducting the MS experiment.[20] Each of these solutions gave excellent results with the parent ion being the most intense signal. Since this approach turned out to be successful, other, more complex tertiary amines, that is, atropine (A-B(n)), quinine (Q-B(n) and Q-N(n)), and Tröger's base (T-B(n) and T-N(n)) were used instead of triethyl amine (Figure 2). These compounds were isolated before performing the MS experiments. For all dendrons bearing an ammonium cation at their focal point, clean mass spectra with excellent signal-to-noise ratio could be obtained (Figure 3). Not unusual for the electrospray ionization of salts, dimers held together by electrostatic forces through one counter ion (bromide in all cases) can be seen with low intensity. The exact masses and isotope patterns determined by experiment are in excellent agreement with those calculated.

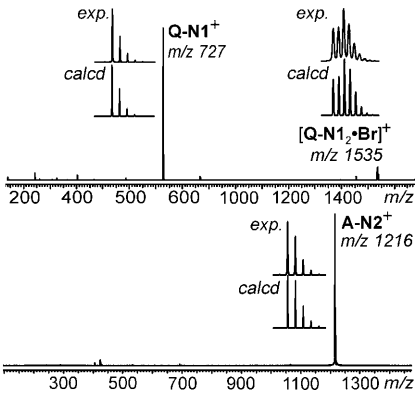


Figure 3. Two representative examples for positive-ion ESI-FTICR mass spectra of dendrimers bearing an ammonium cation at the focal point: **Q-N1** (top) and **A-N2** (bottom). Not unexpectedly, a bromide-bridged dimer is observed for **Q-N1**. Experimental and calculated isotope patterns agree well.

When the PBr_3 reaction was used for the preparation of the G3 Br-dendron, the co-generated acid induced the formation of defects in the dendron scaffold. These defects appear in the mass spectra at mass distances of $\Delta m = 212$ below and to a minor extent also above the benzyl-terminated parent ions (Figure 4) and of $\Delta m = 264$ for the 2-naphthylmethyl terminated ones. These mass differences correspond to the masses of one branching unit plus a peripheral end group. For some cases, the defect structures shown in Figure 4 represent several possible isobaric isomers. Since the corresponding second-generation dendrons do not show any substantial impurities, the defects in the third-generation dendrons must originate from the last steps in the convergent synthesis. During the conversion of the benzyl alcohol into the benzyl bromide with PBr_3 traces of acids induce rearrangements of the benzyl ether linkages. While it is easy to separate the analogous defects from the intact parent ion for the lower generations, the chromatographic separation of the intact dendrons becomes increasingly difficult for higher generations. While the cleavage of benzyl ethers does not occur in the absence of water, group transfer reactions are observed in which whole branching units can change places and even be transferred from one dendron to another. Using CBr_4/PPh_3 for the production of the bromides, which is also advantageous because of the higher yields obtained, no defects were observed in the mass spectrum of these compounds (Figure 4, bottom).[18] Consequently, the defects that prevail in the samples even after chromatography are easily seen by ESI mass spectrometry.

Dendritic viologens—The effect of dendron size on dication stability: Quite different from the ammonium salts discussed so far are the dendritic viologens shown in Figure 5. These compounds have been examined by mass spectrometry before with respect to their host–guest chemistry and they represent excellent guests for Klärner-type molecular tweezers.[21] The dications are quite stable in solution due to the presence of stabilizing counterions. However, they decompose slowly over time, when the solvent is nucleophilic. Due to the short distance between the two charges, significant charge-repulsion effects can be expected to affect their gas-phase behavior. Figure 6a shows the ESI- FTICR mass spectrum of **Viol-G0**. Most remarkably, the dication in its bare form (asterisk in Figure 6a) has never been observed irrespective of the ionization conditions applied. Instead, the sample ions avoid being a dication suffering from charge repulsion by forming singly and doubly charged $(\mathbf{M^{2+}})_n(PF_6^-)_{2n-1}$ (n = 1–3) and $(\mathbf{M^{2+}})_n(PF_6^-)_{2n-2}$ (n = 3–6) clusters. In these clusters, the high positive charge can be compensated by the counter ions and thus the compounds are significantly stabilized.

Other signals also speak of a strong tendency to avoid bare dications: Signals at m/z 203 and m/z 359 are due to fragmentation of the dication by cleavage of one of the benzylic C–N bonds. A benzyl cation is then created together with a mono-substituted, singly charged bipyridinium and charge repulsion is avoided by separating the two charges on two independent ions. Interestingly, a signal at m/z 561 showed an isotope pattern in the broadband mode of the FT-ICR instrument, whose relative intensities changed with the ionization conditions. This pointed to the fact that two overlapping, non-resolved isotope patterns are observed. Changing the ionization conditions also changed the relative amounts of the two species contributing to the overall pat-

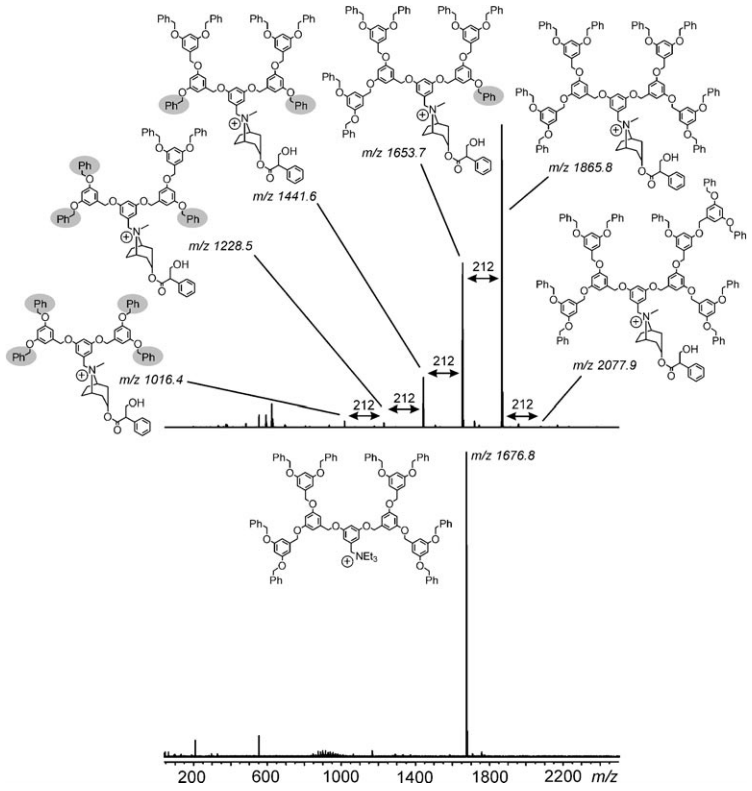


Figure 4. ESI-FTICR mass spectra of the third generation dendrimers **A-B3** (top) and **TEA-B3** (bottom). Top: Defects become visible at regular spacings of 212 Da below and to a minor extent above the parent ion of the intact dendrimer, when the PBr_3 reaction is used for dendron preparation. Bottom: Dendrons prepared with the Appel reaction are essentially defect-free.

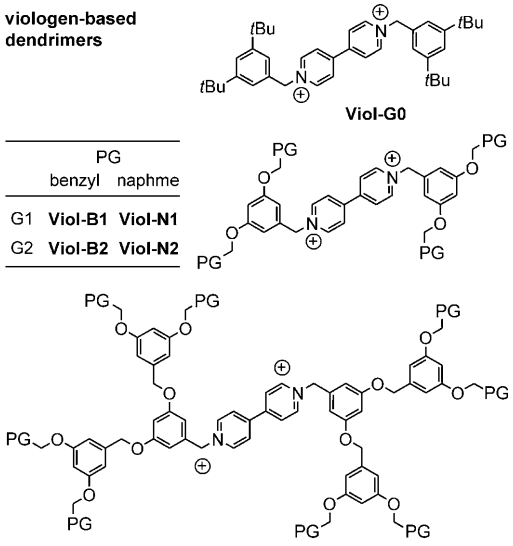


Figure 5. Doubly charged dendrimers (G0–G2) with viologen cores ("naphme" = 2-naphthylmethyl).

tern. A high-resolution mass spectrum confirmed that two ions differing by only 1 Da correspond to i) a one-electron reduction occurring during the ionization process (*m*/*z* 562) and ii) the loss of a proton from the dication (*m*/*z* 561). Since the proton has a mass differing from the exact difference between ^{12}C and ^{13}C, both patterns can be resolved and independently seen at high resolution in Figure 6. Likely, the proton loss occurs at the benzylic position adjacent to one of the nitrogen atoms yielding a zwitterionic structure, which nevertheless is well stabilized by conjugation of the anion with the aromatic ring. Consequently, four different ways exist for a cation to avoid the charge repulsion within the dication: Compensation of positive charges by counter ions, proton loss, one-electron reduction, and fragmentation leading to the separation of two singly charged ions.

With the larger dendrimers **Viol-B1**, **Viol-B2**, **Viol-N1**, and **Viol-N2** the same experiments gave similar results. However, one significant difference was observed: Substitution with dendrons of the Fréchet-type stabilizes the bare dications so that signals for them can be observed in the ESI-FTICR mass spectra (Figure 6b,c). A clear ranking of stabilities with increasing dendron generations was observed depending on the harshness of the ionization conditions. In particular, the capillary exit voltage can be tuned at our instrument. This accelerates the ions to different velocities with which they then undergo collisions with residual gas molecules. At higher settings of this voltage, only **Viol-B2** and **Viol-N2** gave clearly observable signals for the corresponding dications, but no dications of the smaller generations were observed. By softening the conditions, all four dendritic viologens of first and second generation gave signals for bare dications, while **Viol-B0** did not yield any bare dications irrespective of the ionization conditions. This ranking was confirmed by other experiments with the Klärner-type tweezers complexes which showed a pronounced dendritic effect on their gas-phase reactions.[21a]

Collision-induced decomposition of dendrons bearing ammonium ions at their focal points: The fragmentation pattern of the dendrons under study can be examined in tandem MS experiments as shown for **A-N2** in Figure 7. First, the monoisotopic parent ion is mass-selected from all ions present in the ESI mass spectrum (Figure 7a and b). Then, the dendron ions of interest are subjected to collisions with Ar as the collision gas. Chemical intuition would certainly predict that the primary fragment would correspond to the loss of the tertiary amine giving rise to the corre-

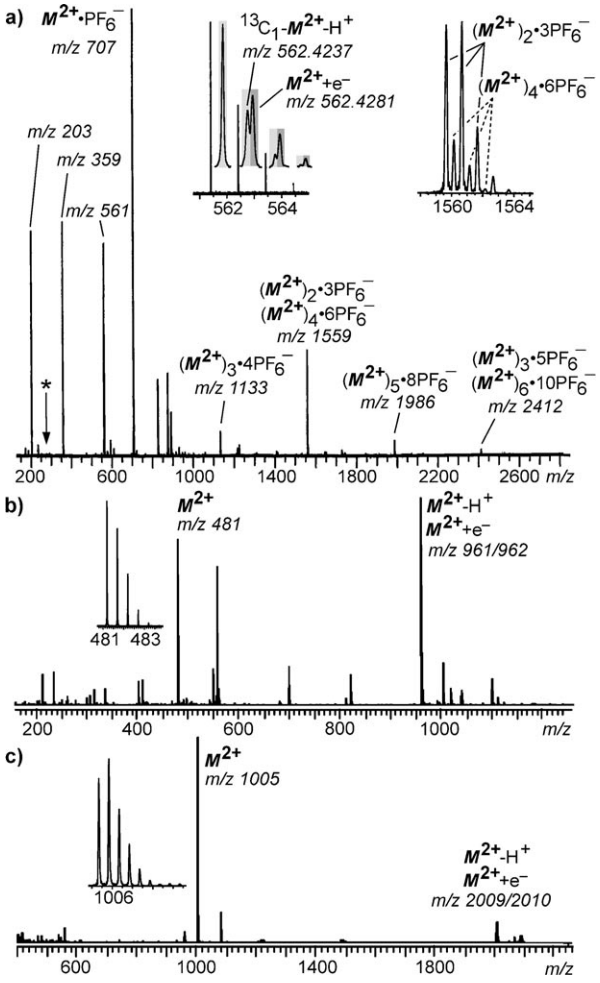


Figure 6. Positive-ion ESI-FTICR mass spectra of MeOH solutions (50 μM) of a) **Viol-G0**, b) **Viol-N1**, and c) **Viol-N2** (the latter two optimized for maximum dication intensities). Insets: High resolution isotope pattern of the signal at m/z 561 revealing that both electron capture and proton loss overlap (top left), isotope pattern of the signal at m/z 1559 providing evidence for a superposition of singly and doubly charged clusters (top right), and experimental isotope patterns of the bare **Viol-N1** and **Viol-N2** dications.

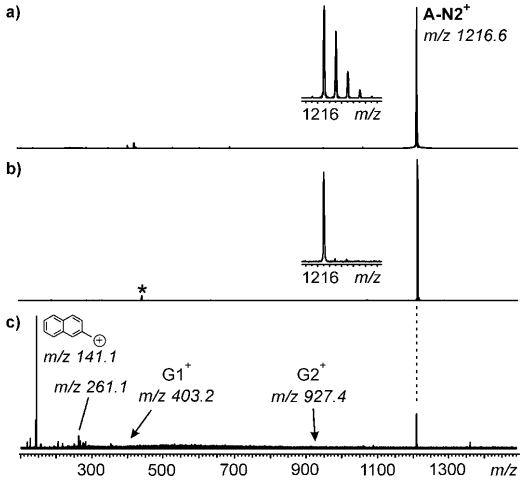
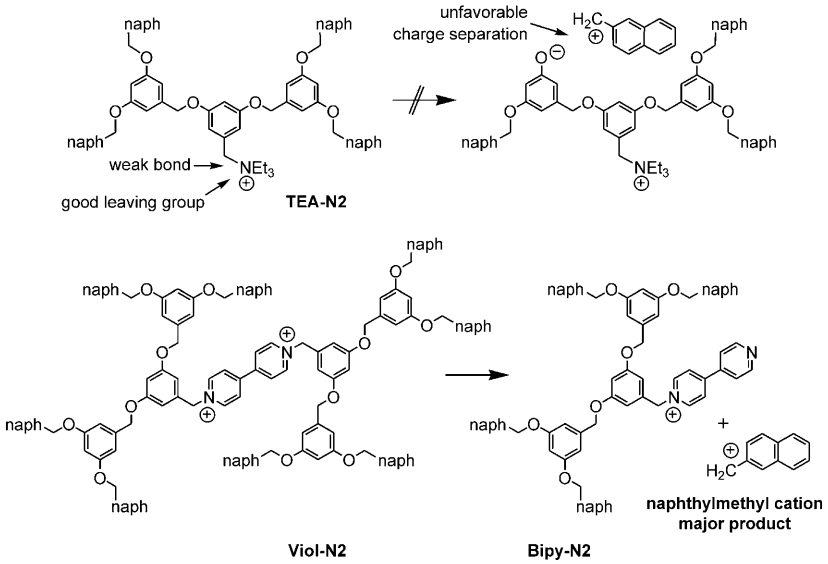


Figure 7. Top: ESI-FTICR mass spectrum of **A-N2**. Center: Isolation of the monoisotopic molecular ion. Bottom: Collision-induced decay (CID) experiment. The most intense fragment ion is the 2-naphthylmethyl cation at *m*/*z* 141. The positions are marked, at which the intermediate 2nd and 1st generation benzyl cations are expected to appear. (*=overtone)

sponding benzyl cation of *n*th generation. In our example **A-N2**, the G2 benzyl cation would thus be expected to appear at *m*/*z* 927. The tertiary amine is a good and stable leaving group and the benzyl cation is stabilized by conjugation of the cation with the aromatic ring next to it. Figure 7c shows the CID mass spectrum of **A-N2** as a representative example. The experimental result differs significantly from expectation for any of the dendrons under study: Regardless of the generation number, the most intense (and often the only) signal in all CID mass spectra corresponds to either benzyl or 2-naphthylmethyl ions depending on the peripheral end group incorporated in the dendrons. Signals for the expected benzyl cations of 1st to 3rd generation are instead either not seen at all (G2 and G3) or hardly exceed the noise (G1). The question arises, which mechanism might account for this unexpected fragmentation behavior. Why do we only observe a cation originating from the periphery, when the first reaction step should be C–N bond cleavage at the focal point?

Direct bond cleavage mechanism: The simplest mechanism that can be imagined involves the direct cleavage of a peripheral benzyl ether bonds (Scheme 1). However, it is not likely to take place because of the following reasons: First of all, it is hard to imagine that it could energetically compete with the expected amine loss, if one considers that it would require a charge separation through formation of the benzyl or 2-naphthylmethyl cations and the corresponding anionic phenolic oxygen, which would compensate for the charge on the ammonium group of the neutral fragment. Such a charge separation in the gas phase requires several hundreds of kJ mol^{-1}.[22] A second argument against such a mechanism is the fact that no other dendritic benzyl cations are observed, although for example the first generation Fréchet dendron is connected to the next branching unit in the G2 dendrons through the same benzyl ether bond. Another evidence against such a mechanism comes from the CID experiments on viologen-based dendrimers **Viol-N1** and **Viol-N2**. In the CID spectra of the dications, a bipyridinium fragment is observed (e.g., **Bipy-N2** in Scheme 1) which indicates the cleavage of the benzyl–N bond. The corresponding benzyl cation dendron is however missing in the CID mass spectrum. Instead, the major fragment is again the peripheral naphthylmethyl cation. Consequently, there must be a mechanism connecting an initial amine loss with the cleavage of the peripheral benzyl ether bond. The direct bond cleavage can thus be ruled out as a viable alternative.

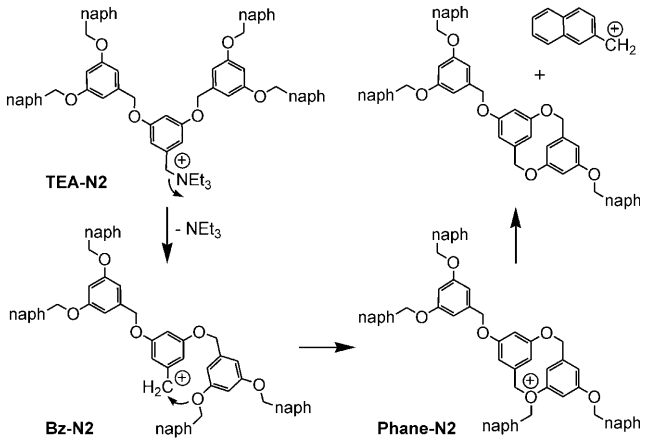
Fragmentation cascade involving cyclophanes: A similar fragmentation behavior is observed for dendrons of all gen-



Scheme 1. A direct bond cleavage mechanism (top) is unlikely because of the weak C–N bond and unfavorable charge separation. Also, it does not provide an explanation for the fragments observed for viologen-based dendrimers such as **Viol-N2** (bottom).

erations under study. Any reaction mechanism connecting the initial amine loss with cleavage of the peripheral benzyl ether bond must thus involve a fragmentation cascade which works itself through the generations: Loss of the tertiary amine forms a G(n) benzyl cation, which undergoes a quick rearrangement to yield the G($n-1$) benzyl cation of the next lower generation and so on until the periphery is reached and no further rearrangements are possible anymore.

One candidate for such a fragmentation cascade is depicted in Scheme 2. Amine loss from second generation **TEA-N2** is followed by an electrophilic attack of the benzylic carbon atom on an oxygen atom from the next shell of branching units. Consequently, dioxa-meta[2.2]cyclophane **Phane-N2** is generated. The neutral cyclophane is an excellent leaving group and cleavage of the oxonium–CH_2 bond

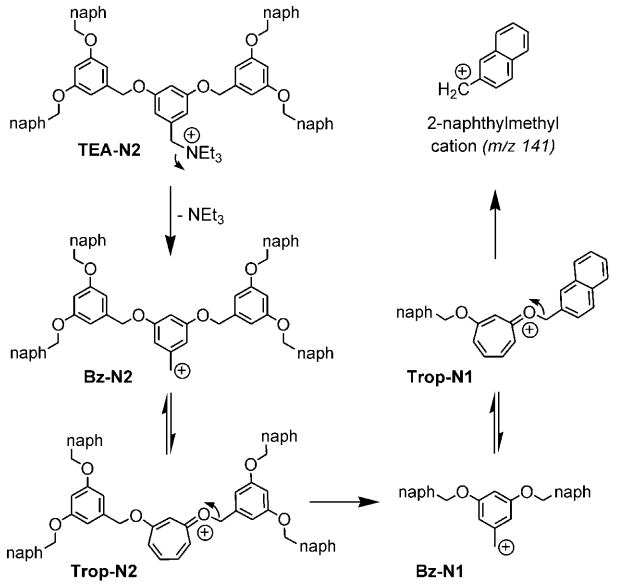


Scheme 2. A fragmentation cascade involving the formation of cyclophanes.

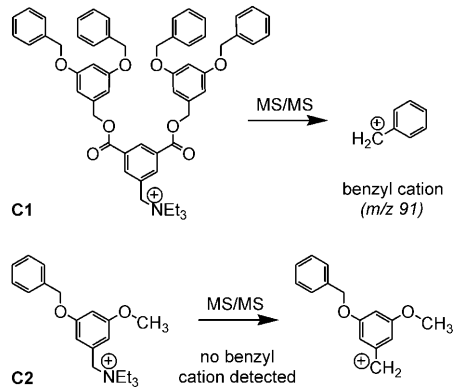
liberates the naphthylmethyl cation. However, the same mechanism applied to G1 and G3 dendrons should generate G1 benzyl cation, which cannot fragment further through this same mechanism. One would therefore expect an alternating fragmentation pattern: The cascade should terminate at G1 benzyl cations for G1 and G3 dendrons, while it forms the peripheral benzyl or naphthylmethyl cations for G2 dendrons. This behavior is not observed. All generations form predominantly benzyl or naphthylmethyl cations. Consequently, this mechanism can also be ruled out.

Benzyl–tropylium rearrangement cascade: A third mechanistic scenario avoids this alternation (Scheme 3). It involves the formation of a G(n) benzyl cation, which rearranges into a G(n) tropylium ion. In the tropylium ion, the positive charge can be delocalized over all seven carbon atoms and the two benzyl ether oxygens attached to the tropylium core. This creates a good leaving group and is followed by the formation of the G($n-1$) benzyl cation for which the same process occurs until the periphery is reached. According to the literature on the benzyl/tropylium rearrangement, the tropylium structure is energetically more favorable than its benzyl analogue.[23] This would provide an explanation, why the intermediate benzyl cations are not observed with higher intensities. The ion would gain internal energy from the reaction energy of each rearrangement step and thus the individual steps in the fragmentation cascade proceed at higher and higher rates from step to step.

However, theoretical calculations indicate the rearrangement barrier to be quite substantial (ca. 270 kJ mol^{-1}).[24] Consequently, doubts arise that the benzyl–tropylium rearrangement scenario holds true. Two control experiments have been performed. On their basis, we can also rule out this mechanism (Scheme 4): In control compound **C1**, the benzyl ethers at the first branching units have been replaced by esters. After amine loss and the benzyl–tropylium rearrangement, one would expect the fragmentation cascade to stop, because the tropylium leaving group cannot be formed. Against expectation, the peripheral benzyl cation is still the predominant fragment ion. If one of the peripheral benzyl groups is replaced by methyl as in **C2**, no benzyl fragment is observed anymore. Instead, the fragmentation cascade is terminated directly after the amine loss. After a benzyl–tropylium rearrangement, both substituents are however activated and one would expect to observe the remaining benzyl group to be cleaved off, because this cation



Scheme 3. A fragmentation cascade involving sequential benzyl–tropylium rearrangements.

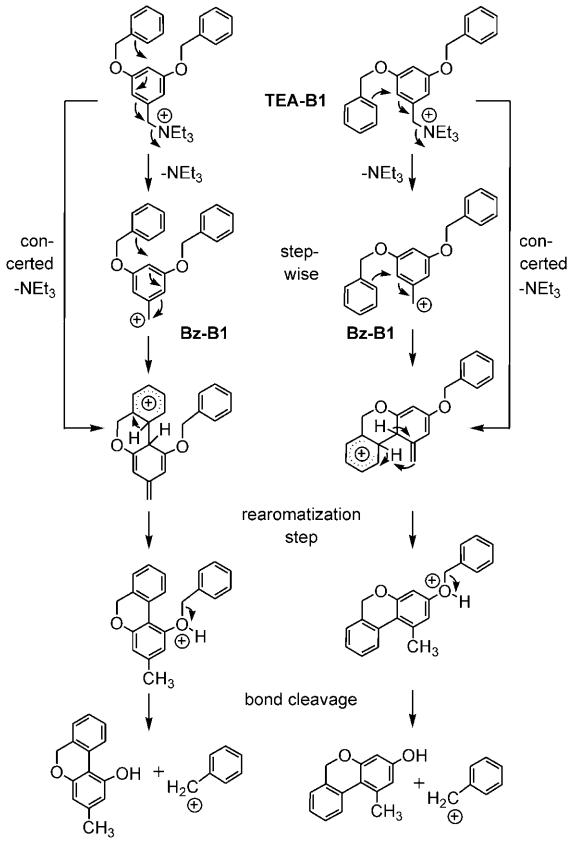


Scheme 4. Control experiments ruling out the benzyl–tropylium fragmentation cascade.

would certainly be more stable than the methyl cation from the second branch. These two control experiments clearly rule out the benzyl–tropylium rearrangement to be involved.

Fragmentation cascades involving electrophilic aromatic substitutions: In particular, the latter control experiment brings us to two additional mechanisms which are quite similar and both involve intramolecular electrophilic aromatic substitution steps (Scheme 5).

The fragmentation starts either with the stepwise loss of the tertiary amine from the focal point and then creates the corresponding G(n) benzyl cation, or it commences with a concerted reaction step, in which the amine is lost simultaneous with the electrophilic attack. In a stepwise sequence, some ions should have sufficient internal energy to accomplish the loss of NEt_3, while not enough internal energy is left to overcome the barrier for the electrophilic substitution



Scheme 5. Two similar fragmentation mechanisms involving electrophilic aromatic substitution reactions. Note that rearomatization of both rings can occur for both intermediates due to the presence of mobile protons.

step. These G(n) benzyl cations should then be seen in the mass spectra. Consequently, the concerted reaction is most likely to happen in all those cases, where the G(n) dendron is *not* observed as an intermediate. In those few cases (e.g., see below), where the G(n) benzyl cation is indeed observed as a minor fragment, both mechanisms may well compete.

Rearomatization of the attacked rings occurs and generates a mobile proton[25] which can easily move across the whole aromatic ring system. Finally, this proton is anchored to one of the oxygen atoms. When attached to the remaining benzyl ether oxygen, again a good leaving group is generated and the benzyl cation (or 2-naphthylmethyl, respectively) is liberated. If this benzyl cation is still dendritic, it can react through the same sequence of steps until the periphery is reached.

The fact that a quick reaction cascade is observed is not in agreement with endothermic reaction steps. In line with this argument, theoretical calculations predict the products to be more favorable than the precursor benzyl cation by more than 50 kJ mol^{-1} (Figure 8). Since reaction energy is gained in each step, they proceed faster and faster so that the intermediates along the fragmentation cascade are not observed in the mass spectra. The dendron thus immolates itself in a chain reaction.

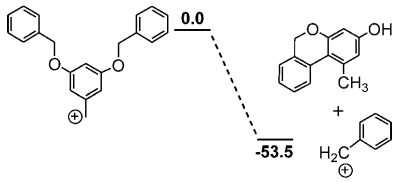
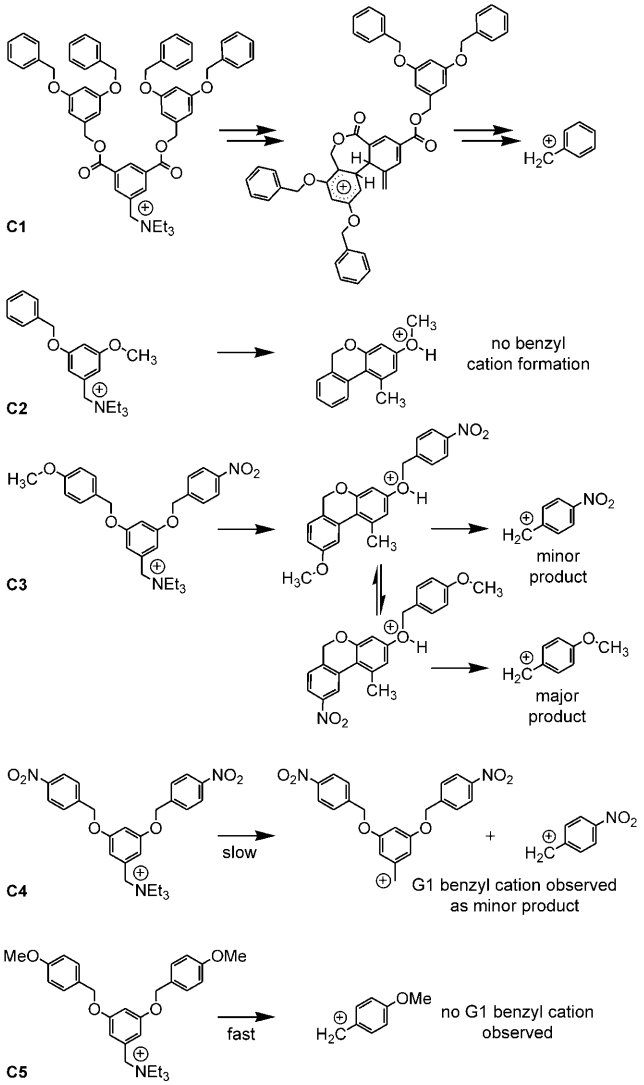


Figure 8. Calculations at the B3LYP/TZVP level of theory predict the products of the mechanisms shown in Scheme 5 to be more stable in energy than the dendron benzyl ion so that each subsequent step in the cascade should be faster than the preceding one (relative energies in kJ mol^{-1}). Note that the dissociation threshold is not shown and the Figure is just a comparison of the relative reactant and product energies.

The electrophilic substitution mechanism is also in line with the fragmentation behavior of the control compounds **C1**–**C5** (Scheme 6). Only the *ortho*-attack (Scheme 5, right branch) is considered here. An analogous reaction can of course occur in the *para*-position (Scheme 5, left branch). The ester analogue **C1** fragments to yield the benzyl cation, because the same mechanism can be applied to this compound. The only difference is the formation of a seven- instead of a six-membered ring in the neutral fragment, but this difference does not interfere with the cascade. It is also clear, why no benzyl cation is formed from **C2**. The electrophilic substitution step attacks the only benzyl group present and connects it tightly to the core. Since the methyl cation is too small for the mass range of our instrument, we do not know whether this cation is formed instead, but it is clear that no benzyl cation can be liberated anymore. **C3** forms the methoxybenzyl cation as the major product, although one would expect the electrophilic attack to be faster at the more electron-rich methoxybenzyl ring. After this reaction, only the nitrobenzyl would be available for further reaction. However, the fact that the methoxybenzyl cation is the major product is in good agreement with the assumption that all rearrangement steps proceed below any of the available exit channels. In such a situation, rearrangements are reversible and the more stable cation is expected to form. From **C3**, the methoxybenzyl cation is better stabilized as compared to the nitrobenzyl cation and thus is the major product. Finally, symmetrically substituted **C4** and **C5** have been synthesized. While no G1 benzyl cation is observed as an intermediate for **C5**, the corresponding nitro-substituted G1 benzyl cation is visible in the CID mass spectrum of **C4** as a minor fragment. This indicates the consecutive reaction steps to be faster for the methoxy derivative as compared to the nitro compound—in line with the expected rates of the electrophilic attacks involved in the fragmentation of these ions.



Scheme 6. Control experiments in support of the intramolecular $S_E(ar)$ reaction pathway.

Collision-induced decomposition of viologen-based dendrimers: Finally, we should take a closer look at the CID mass spectrum of **Viol-N1** as a representative of the doubly charged dendritic viologens (Figure 9). As discussed above, the mass-selected dication forms two singly charged fragments through a cleavage of one of the benzylic C–N bonds. In the CID mass spectrum, the bipyridinium fragment is therefore an intense signal. The corresponding G1 benzyl cation, however, is only observed with low intensity. Nevertheless, this is one of the rare cases, in which the G1 benzyl cation intermediate of the fragmentation cascade is indeed observed. In addition, a few additional fragments appear which are usually either very low in intensity or absent in the CID mass spectra of the other dendrimers. A very similar situation is observed for **Viol-N2**. Because of charge repulsion, the first dissociation step into the bipyridinium fragment and the G(n) benzyl cation occurs at lower internal energies. Consequently, the resulting benzyl cation is less excited, if generated from **Viol-N1** or **Viol-N2** and becomes visible as an intermediate. Also, one expects that this intermediate is longer-lived so that more time is available for rearrangements that finally lead to the additional fragments at higher intensities than observed for the singly charged ammonium dendrimers.

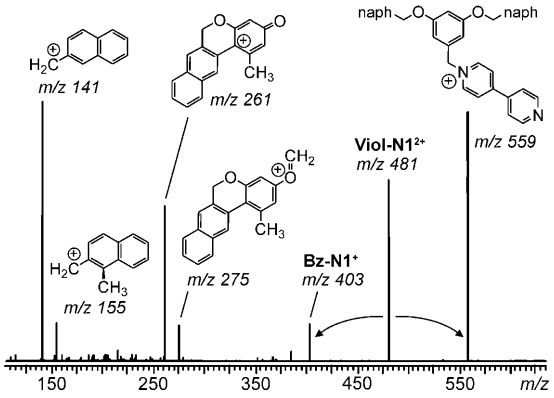
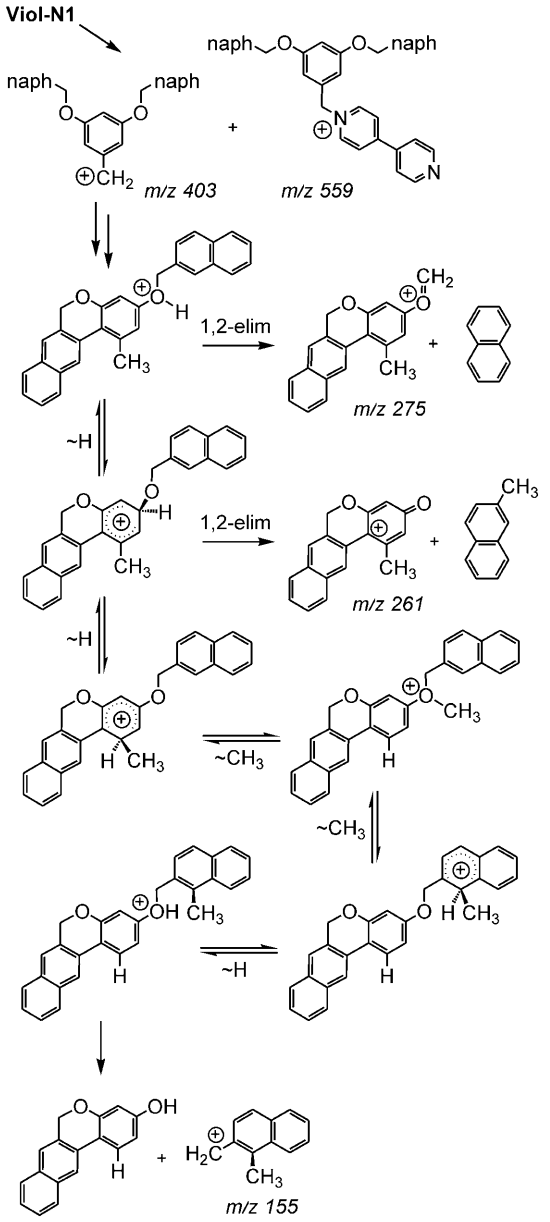


Figure 9. For **Viol-N1**, the G1 benzyl cation is observed as the fragmentation intermediate. Also, some other side products besides the peripheral benzyl loss are observed, which are in agreement with the postulated mechanism.

Scheme 7 provides a plausible mechanistic interpretation of the additional fragments observed in the CID experiments with **Viol-N1**. The mobile proton can shift across different positions of the aromatic rings. Similarly, a methyl cation can be involved. By combining different sequences of such shifts, different intermediates are formed, which form the products in energetically quite favorable 1,2-elimination reactions of simple bond cleavages. Most importantly, the methyl group can be shifted to the naphthyl ring explaining the methyl-naphthylmethyl fragment at *m*/*z* 155. This shift also indicates that an intact methyl group is present in the intermediates and thus lends further support to the formation of the exocyclic methyl group during the rearomatization step in Scheme 5.



Scheme 7. A mechanistic rationalization of the side products observed in the fragmentation of **Viol-N1**. All prominent side products can be explained by invoking a series of simple proton and methyl cation shifts across the aromatic system. The major naphthyl methyl fragment is formed in analogy to the mechanism shown in Scheme 5 (right) and not repeated here.

Conclusion

ESI-FTICR mass spectrometry proved useful for the ionization and characterization of dendrimers and is a sensitive tool for the detection of defects. While the mass spectra are quite simple to interpret for the dendritic alcohols (negative mode) and dendritic ammonium ions (positive mode), the spectra of the doubly charged dendritic viologens are more complex. In particular, they show a pronounced stabilization of the bare dication with increasing dendron size.

Most importantly, however, the Fréchet dendrimers, which are certainly one of the most important classes of dendrimers in the chemical literature, exhibit a fascinating fragmentation behavior. Collision-induced decomposition (CID) experiments provided insight into a surprising fragmentation cascade initiated by the loss of the neutral amine. Through several rapid reaction steps of energetically highly favorable rearrangements of the intermediate benzyl cations, the peripheral benzyl or 2-naphthylmethyl cations are generated as the most prominent signals in the CID mass spectra. This cascade mechanism is reminiscent of the self-immolative dendrimers mentioned in the beginning of this article. As well, the fragmentation cascade reminds of the first name "cascadanes" given to the early dendritic species synthesized by Vögtle et al.[26] Based on control compounds, a number of mechanistic alternatives can be ruled out leaving a mechanism, which involves an electrophilic aromatic substitution of the benzyl cation core at one of the aromatic rings in the next dendron shell. This mechanism also provides a rationalization for minor fragments that are for example observed in the fragmentation of the doubly charged viologen-based dendrimer ions.

Experimental Section

Syntheses: All dendrons under study here were synthesized, purified, and characterized according to well-established procedures published previously.[20,27] Syntheses and analytical data of the control dendrons **C1**–**C5** are provided in the Supporting Information.

ESI-FTICR mass spectrometry: 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, acetonitrile served as the spray solvent and 30–50 μM solutions of the analytes were used. Analyte solutions were introduced into the ion source with a syringe pump (Cole-Parmers Instruments, Series 74900) at flow rates of about 3–4 $\mu L\,min^{-1}$. Ion transfer into the first of three differential pumping stages in the ion source occurred through a glass capillary with 0.5 mm inner diameter and nickel coatings at both ends. Ionization parameters—some with a significant effect on signal intensities—were adjusted as follows: capillary voltage: −4.1 to −4.4 kV; endplate voltage: −2.8 to −3.5 kV; capexit voltage: +200 to +300 V; skimmer voltages: +8 to +12 V; temperature of drying gas: 200 °C. The flows of the drying and nebulizer gases were kept in a medium range (ca. 10 psi). The ions were accumulated in the instruments hexapole for 0.5–1 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 to 512 scans were averaged to improve the signal-to-noise ratio.

Tandem MS experiments: For MS/MS experiments, the whole isotope patterns of the ions of interest were isolated by applying correlated sweeps, followed by shots to remove the higher isotopes. After isolation, argon was introduced into the ICR cell as the collision gas through a pulsed valve at a pressure of about 10^{-8} mbar. The ions were accelerated by a standard excitation protocol and detected after a 2 s pumping delay. A sequence of several different spectra was recorded at different excitation pulse attenuations in order to get at least a rough and qualitative idea of the effects of different collision energies on the fragmentation patterns.

Theoretical calculations: The calculations were performed with the TURBOMOLE program package[28] employing Kohn-Sham density functional theory (DFT) with the B3LYP functional[29] and the TZVP[30] basis set. The SCF convergence criterion for these calculations was chosen to be 10^{-8}.

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