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Elucidation of Fragmentation Pathways of Nitrile-Terminated PPI Dendrimer-Metal Complexes

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ELUCIDATION OF FRAGMENTATION PATHWAYS OF NITRILE-TERMINATED PPI DENDRIMER-METAL COMPLEXES

Thesis submitted to
the Graduate College of
Marshall University

In partial fulfillment of the
requirement for the degree of
Master of Science in Chemistry

by

Kristen Nicole Pack

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Marshall University

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Abstract:

The complexity of branching macromolecules around a core unit, such as poly(propylene)imine (PPI) dendrimers, has caught the attention of researchers for several years\textsuperscript{1,2,3,4,5}. The structural framework of the core unit for these dendrimers is simply 1,4-diaminobutane. As the synthesis of higher-order generations of the macromolecule progresses, the molecules become very complex and tightly woven, but have predictable geometries and properties. The PPI dendrimers can be functionalized at the terminal ends of the branches in order to elicit different properties. The PPI dendrimer examined in this project has been terminated with a nitrile group rather than the usual amine group. Using mass spectrometry, the gas-phase fragmentation pathways under low energy collision conditions of the modified PPI dendrimer complexed with transition and alkali metals will be examined. The current project focuses on the fragmentation pathways of complexes consisting of alkali and transition metals with first and second-generation nitrile-terminated PPI dendrimers. The current project also utilizes a \textsuperscript{15}N-labeled nitrile-terminated first generation PPI dendrimer for comparison with the unlabeled first generation dendrimer fragmentation pathways. The dendrimers are synthesized in-house using 1,4-diaminobutane and acrylonitrile. Deuterium-labeled dendrimers are synthesized from d\textsubscript{4}-succinonitrile and d\textsubscript{4}-succinamide and reduced using LAD and LAH. The fragmentation pathways for the metal-dendrimer complexes tend to follow a similar pattern, with acetonitrile and acrylonitrile as the primary neutral losses. The complexes also utilize a radical mechanism for the loss of acetonitrile, with the nickel complex being an exception. The overall ease and efficiency of the elucidation of these fragmentation pathways makes mass spectrometry a very valuable method of analysis for these dendrimer-metal complexes.
DEDICATION

I want to dedicate this thesis to my husband and my family, whose love and support gave me the drive to succeed in my academic pursuits. I am blessed to have such wonderful and caring people to look to for encouragement and strength.
ACKNOWLEDGEMENTS

First and foremost, I want to thank God for giving me an analytical mind that allowed me to finish this project.

I also want to thank Dr. William Price for his guidance in this project. Your patience and encouragement helped me to become a more confident scientist and person. Your dedication to helping me succeed in this project made all the difference in the world. I owe all of this to you.

I want to thank Dr. Leslie Frost for her instruction in the fields of mass spectrometry and modern instrumental methods. Your enthusiasm for the material was contagious, and I have you to thank for my solid interest in analytical methods.

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Introduction:

Dendrimers are a group of highly branched molecules that consist of three main parts: a central core, branches, and functionalized terminal groups. Dendrimers are characterized by their size or generation, their terminal groups, and the central core. The generation number is determined by the amount of branching from the central core. According to Tomalia et al., “The dendrimers possess ‘reactive end groups’ that allow (a) controlled molecular weight building (monodispersity), (b) controlled branching (topology), and (c) versatility in design and modification of the terminal end groups”. The number of terminal functional groups is directly related to the amount of branching of the dendrimer; as the generation number increases, the number of terminal ends increases.

The first dendrimer was synthesized by Buhleier, Wehner, and Vögtle in 1978. They named the dendrimer a “cascadane”. Maciejewski worked on dendrimer syntheses in 1982 and Pierre de Gennes introduced the "de Gennes dense packing" phenomenon in 1983 that explained the fundamental dendrimer surface-congestion properties. The Tomalia group then began their own research based on the work of Buhleier, Wehner, and Vögtle. The Vögtle group used a divergent iterative methodology in the synthesis of their dendrimers using acrylonitrile. Divergent synthesis involves starting at the core and synthesizing outward towards the functional terminal ends. However, their synthesis gave low yields and purification problems. The Tomalia group used a divergent methodology to prepare dendrimers as well; however, they used acrylate monomers instead of acrylonitrile. They did not have the same problems that Vögtle faced, and their methodology is still used today to create dendrimers, including the Starburst®, or PAMAM dendrimers.
Two other synthesis groups, Wörner/Mülhaupt of Freiburg University and de Brabander-van den Berg/Meijer of the DSM (De Nederlandse Staatsmijnen/Dutch State Mines) group modified the divergent synthesis used by Vögtle in order to create true poly(propyleneimine) dendrimers. The synthesis method used by de Brabander-van den Berg/Meijer of the DSM group is used commercially to prepare the poly(propyleneimine) dendrimers. Table 1 shows the evolution of dendrimer synthesis.

<table>
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<tr>
<td>1990</td>
<td>Miller/Neenan</td>
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<tr>
<td>1988</td>
<td>Odian/Tomalia</td>
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<tr>
<td>1990</td>
<td>Kim/Webster</td>
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<tr>
<td>1991</td>
<td>Fréchet/Hawker</td>
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<tr>
<td>1991</td>
<td>Tomalia</td>
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<tr>
<td>1991</td>
<td>Gauthier/Möller</td>
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Table 1: Dendrimer syntheses reported by early synthetic groups.

Dendrimers have a multitude of internal cavities due to the parameters of their synthesis. As such, dendrimers can be used in a variety of ways for transportation. In 1994, the discovery that dendrimers could encapsulate dye molecules in a stable way paved the way for the research into different guest molecules that dendrimers could efficiently encapsulate. One important way dendrimers are being used is for the delivery of drugs. According to Klajnert et al., the drug
molecules can be attached in two places on the dendrimer: the terminal groups or the interior of the dendrimer. Higher generation dendrimers may prove more useful in experiments in which the drugs are attached to the peripheral terminal groups, as higher generations have more functional terminal ends to which the drug can attach. However, if the drug is encapsulated within the dendrimer, the branches of the dendrimer can protect the drug from becoming degraded or can allow the drug to be slowly released into the system. The drug can be linked to the interior of the dendrimer covalently during synthesis with the remainder of the synthesis occurring to encapsulate the drug, or the drug can be associated with the dendrimer non-covalently because of the properties of the dendrimer interior. For example, a nonpolar dendrimer interior provides sites for non-covalent bonding for a nonpolar guest molecule.

The ability for the dendrimer to encapsulate a drug is dependent on the dendrimer generation. Smaller generations cannot encapsulate a drug efficiently as their size is more ellipsoidal than globular. The ability for the dendrimer arms to fold back around is greater in the larger dendrimers which allows for the greater encapsulation efficiency. Larger generations can form a dense shell on their surface that allows ligands to be incorporated.

Dendrimers have been studied using many analysis techniques, including chromatographic methods, potentiometric titrations, and electron paramagnetic resonance. Two types of mass spectrometry ionization methods are used regularly in the characterization of dendrimers: matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI). MALDI is used for large, nonvolatile compounds, as is ESI. Both techniques are considered to be “soft” ionization techniques, meaning that they would ionize the dendrimer without causing excessive fragmentation to the point that assignment of peaks in relation to the structure of the full dendrimer would be impossible. ESI also has allowed synthesis errors
resulting in dendrimer impurities to be observed\textsuperscript{8}, which makes ESI a better choice than MALDI. ESI has been shown to be effective in carefully transferring the large dendrimers from solution phase to gas phase\textsuperscript{10}. Tandem mass spectrometry (MS/MS) can be used to determine whether the molecular mass peaks found at a lower molecular mass than the dendrimer itself are due to fragmentation of the dendrimer or due to defects stemming from the synthesis of the dendrimer. Mass spectrometry can be used in order to determine whether a dendrimer has been protonated or not as well as where the dendrimer has been protonated\textsuperscript{2,6}.

ESI is an ionization method typically used for larger, stable, nonvolatile compounds such as peptides, proteins, polymers and lipids\textsuperscript{13}. The reason ESI is preferred for these compounds is because the method is well-known for producing ions with multiple charges. The typical mass range for ESI is 70,000 Da; with multiply charged species, however, molecules with even higher molecular weights than 70,000 Da can be observed using ESI as long as they can carry more than one charge. ESI has very good sensitivity, as it can detect in the high femtomole to the low picomole range\textsuperscript{13}. One stipulation for the use of ESI is that the solution sent through the capillary must contain analytes that can be charged. The solvents used in ESI must be volatile so that they are able to be evaporated away. Common solvents used for ESI include 50/50 methanol/water and 50/50 acetonitrile/water\textsuperscript{13}.

ESI works by “creating a fine spray of highly charged droplets in the presence of an electric field”\textsuperscript{13}. Figure 1 shows the creation of the droplets. The solution is sent through a fused silica capillary that in inside the needle of the source. The voltage on the needle causes separation of charges in the surface of the solution. This causes the solution to be forced through the needle as a Taylor cone. The Taylor cone extends from the needle until the Rayleigh limit is reached. The Rayleigh limit is the point at which the electrostatic repulsions in the solution equal
the surface tension of the solution. When this limit is hit, highly charged droplets start breaking away from the Taylor cone. These droplets are then accelerated toward the mass analyzer entrance since the mass analyzer entrance carries a high voltage that is opposite of the needle charge; for example, in positive ESI mode, the needle voltage is positive and the mass analyzer voltage is negative. This would cause the cations of the solution to travel toward the mass analyzer while the anions remain near the needle\textsuperscript{13}.

As the highly charged droplets move toward the mass analyzer, the nitrogen gas in the source aids in evaporation of the solvent encasing the ions. As this happens, the Coulombic repulsion on the surface of the droplet exceeds the surface tension, and the ions break away from the droplet. The ions can break away in one of two ways: Coulombic explosion or ion ejection. In Coulombic explosion, the droplet continues to become smaller and smaller until the Coulombic repulsion exceeds the surface tension; the droplet then explodes, releasing the ions from their confinement in solution. In ion ejection, as the solvent is evaporated away, the repulsion between the ions in the droplet increases and overcomes the surface tension. Ions are then ejected from the droplet through a Taylor cone\textsuperscript{13}. Figure 2 shows the two methods by which the ions are released.

Figure 1: Creation of the highly charged droplets using electrospray ionization.
Figure 2: Depiction of the two methods for the creation of ions in electrospray ionization: Coulombic explosion and ion ejection.

The mass analyzer used in the project is the quadrupole ion trap, or QIT. Figure 3 shows the QIT as part of an ESI-QIT mass spectrometer. The QIT was created by Wolfgang Paul, who also created the quadrupole mass analyzer. The QIT is made up of a ring electrode and two endcap electrodes. In a QIT, the ions are trapped within a radio frequency, or rf, field. As the radio frequency is changed, different mass-to-charge ratios are excited and then ejected through the endcap to the detector, while the other ratios remain in the trap. The design of the QIT allows one mass-to-charge ratio to be selected and fragmented at a time; however, this leads to loss of
signal since almost 99% of the ions are ejected. The mass range of a typical QIT is 2,000 Da, although analytes weighing up to 4,000 Da can be observed.

*Figure 3: Set-up of an ESI-QIT mass spectrometer.*

The mass spectra are generated from data obtained through the fragmentation of the analyte of interest. The analytes of interest are fragmented through a process called collision-activated dissociation, or CAD\textsuperscript{13}. In CAD, the ion of interest is hit by a neutral species in the collision cell, inducing fragmentation. The fragmentation occurs through the conversion of some of the translational energy of the ion to internal energy in the ion\textsuperscript{13}. The neutral species in the collision cell is typically an inert gas such as argon or helium.

Many types of dendrimers have been created; the one examined for this project is the nitrile-terminated poly(propyleneimine), or nPPI, dendrimer. Dendrimers have specific fragmentation patterns that can vary when complexed with another molecule such as metals. This project focuses on those specific fragmentation patterns when the nitrile-terminated PPI
dendrimer is complexed with alkali and transition metals. The fragmentation patterns will be compared in order to determine where the metal is located in the complex and how variation in the metal affects the fragmentation of the complex.

**Materials:**

The nickel (II) acetate used for the metal solutions was purchased from EM Science and the copper (II) chloride was bought from Alfa Products. The zinc (II) acetate, acrylonitrile and the lithium acetate were purchased from Matheson, Coleman & Bell. The first generation amine-terminated PPI dendrimer, $^{15}$N-labeled acrylonitrile, methanol and glacial acetic acid were purchased from Sigma Aldrich. The acetonitrile and the 1,4-diaminobutane used were bought from Acros Organics. The materials were used without further purification. The results were viewed using Xcalibur software. Computer-generated spectra were obtained from iMass.

**Methods:**

A scaled-down synthesis$^{12}$ of the $1^{st}$ generation nitrile-terminated PPI dendrimer (nPPI) involved adding 11g distilled H$_2$O to 1.47g 1,4-diaminobutane and 4.43g acrylonitrile. The mixture was heated at 80°C for one hour in a water bath and stirred using a spin bar. After cooling overnight, the mixture was noted to have separated into two layers: a thick, colorless and clear aqueous top layer and a yellow dendrimer bottom layer. Figure 1 shows the reaction of 1,4-diaminobutane and acrylonitrile.

The procedure for synthesis of the $1^{st}$ generation $^{15}$N-labeled PPI dendrimer was the same as for the $1^{st}$ generation dendrimer; however, $^{15}$N-labeled acrylonitrile was used.
Figure 4: Reaction of 1,4-diaminobutane with acrylonitrile in a 1:4 ratio to create the nitrile-terminated first generation poly(propylene) imine dendrimer.

The synthesis of the 2nd generation nitrile-terminated PPI dendrimer involved adding 3g distilled H$_2$O to 1.47g 1st generation amine-terminated PPI dendrimer and 2.215g acrylonitrile. The mixture was heated at 80°C for one hour in a water bath and stirred using a spin bar. The solution was allowed to cool overnight. The PPI dendrimer formed white needle-like crystals after cooling completely. Figure 2 shows the reaction of the PPI dendrimer with acrylonitrile.

Figure 5: Reaction of the first generation amine-terminated PPI dendrimer with acrylonitrile to create the nitrile-terminated second generation poly(propylene) imine dendrimer.

A 0.1M nickel (II) acetate solution was formed by adding 0.2475g Ni(OAc)$_2$·4H$_2$O to 10mL distilled H$_2$O. A 0.1M copper (II) chloride solution was made by adding 0.1705g CuCl$_2$·2H$_2$O to 10mL distilled H$_2$O.
The $^{15}$N-labeled 1st generation nPPI-metal complex solutions were formed by adding 970μL distilled water, 10μL nPPI, 10μL metal solution, 10μL methanol, and 1 drop of glacial acetic acid. The 1st and 2nd generation nPPI-metal complex solutions were formed by adding approximately 0.5mL distilled water, 4-6 drops of the metal solution, 2-3 drops acetonitrile, 2-3 drops nPPI dendrimer (1 part dendrimer to 100 parts water), and one drop of glacial acetic acid.

Approximately 500μL of the nPPI-metal complex solution were injected into a Thermo Finnigan LCQ ion trap mass spectrometer at a spray voltage at 4.1kV and a flow rate of 6μL/min for analysis. Xcalibur software was used for data collection and analysis.

The $d^2$-labeled nPPI dendrimer (the $d_2$ signifying that the dendrimer arms each have two deuteriums) was formed by adding 0.55g D$_2$O, 0.074g 1,4-diaminobutane and 0.22g deuterated acrylonitrile (deuteron on second carbon) to a round bottom flask. The mixture was heated at 80°C in a water bath for one hour. The heat was then removed and stirring was allowed to continue overnight. The solution was a dark purple color after the reaction proceeded to completion. One deuterium on each arm came from the deuterated acrylonitrile while the other came from D$_2$O.

![Reaction of 1,4-diaminobutane with deuterated acrylonitrile to form the deuterated 1st generation nPPI dendrimer.](image)

The deuterium-labeled 1,4-diaminobutane used in the creation of the $d_4$-labeled nPPI dendrimer (the $d_4$ signifying that the α-carbons in the core each have two deuteriums) was
formed by adding 0.1g succinamide to an evacuated round-bottom flask. The flask was hooked to a Schlenk line to prevent contamination from water in the outside air. The flask was placed into an ice bath and 0.2g LiAlD$_4$ in THF was added to the flask. The apparatus was then heated to 50°C and allowed to cool. The solution was allowed to stir overnight. The dendrimer was then synthesized using the same procedure as outlined for the creation of the nPPI dendrimer.

*Figure 7: Reduction of succinamide to deuterated 1,4-diaminobutane using lithium aluminum deuteride.*

The deuterium-labeled 1,4-diaminobutane used for the synthesis of the d$_4$-labeled nPPI dendrimer (with the deuteriums on the β-carbons) was synthesized utilizing the same procedure as the synthesis of the d$_4$-labeled nPPI dendrimer (with the deuteriums on the α-carbons) except for the following change to the procedure: 0.24g LiAlH$_4$ in THF was added to 0.1g d$_4$-succinonitrile. The dendrimer was then synthesized using the same procedure as outlined for the creation of the nPPI dendrimer.

*Figure 8: Reduction of d$_4$-succinonitrile to deuterated 1,4-diaminobutane using lithium aluminum hydride.*
Results and Discussion:

First Generation Cu-nPPI Complex

The first complex to be studied was the labeled Cu-nPPI metal-dendrimer complex. Various studies have been conducted on the amine-terminated PPI dendrimers, but the nitrile-terminated PPI dendrimers provide a unique polar, aprotic nanoenvironment whereas the normal PPI dendrimers provide a polar, protic environment. Figures 9 and 10 shows the isotopic distributions for the Cu-nPPI complex obtained from the computer and obtained experimentally.

Interesting to note was the charge state of the complex. The full spectrum of the $^{15}$N-labeled Cu-nPPI complex showed the parent peak at 367 m/z, which was the complex with a +1 charge. However, no peak was found at 183.5 m/z, which would have indicated the presence of the +2 complex. This indicated that the charge of the copper was being reduced instead of the charge of the entire metal-dendrimer complex, since no labile protons were available for ionization from the metal-dendrimer complex to leave the complex with a +1 charge with the metal remaining divalent. Fragmentation of a Cu(I) metal-dendrimer complex yielded the same fragmentation pattern as the Cu(II) metal-dendrimer complex, which confirmed that the metal was being reduced instead of the complex. No hydride attachment occurs with the Cu complexes. The reduction of the copper may be due to nonthermal collisions with the inert gases in the ion trap post-ionization. The isotopic distribution obtained through scanning verified the presence of a copper ion associated with the nPPI dendrimer, since the isotopic distribution for the nPPI by itself only has two isotope peaks, and also confirmed that the complex carried a +1 charge.
Figure 9: Computer-generated isotopic distribution for the Cu-nPPI complex. The mass shifts to 367 m/z for the $^{15}$N-labeled complex.
Figure 10: Experimentally observed isotopic distribution pattern for the Cu-nPPI complex. The mass shifts to 367 m/z for the $^{15}$N-labeled complex. The difference in one amu between isotope peaks indicates that the complex has an overall charge of +1.

The $^{15}$N-labeled parent compound was isolated and then fragmented to begin the process of neutral loss determination and structure verification. Figure 11 shows the CAD spectrum for the parent complex. The full isotopic distribution of the Cu was not seen in the spectrum obtained since only the monoisotopic mass of Cu was isolated for fragmentation. The neutral losses most commonly observed were a loss of 54, 42, and 96 amu. The loss of 54 amu is consistent with the loss of an acrylonitrile branch from the dendrimer through a retro-Michael reaction; this loss is indicated by a fragment at 313 m/z. Figure 12 shows the structure of the dendrimer after this loss. The loss of 42 amu was observed at 325 m/z. The peak at 271 m/z indicated the neutral loss of 96 amu.
Figure 11: Fragmentation spectrum of the parent dendrimer-metal complex at 367 m/z.

Figure 12: Loss of an acrylonitrile branch from the $^{15}$N-labeled Cu-nPPI complex to show the neutral loss of 54 m/z. The hydrogen attached to the nitrogen from which the branch fragmented comes from the acrylonitrile branch as it leaves through a retro-Michael reaction.

A loss of 42 m/z is proposed as a loss of acetonitrile. Upon further examination of possible structures after a loss of 42 m/z, three structures were proposed that involve a hydrogen
abstraction from a branch of the dendrimer. A radical mechanism is proposed for the loss of acetonitrile in which a homolytic bond cleavage occurs between the terminal carbon of the acetonitrile and the remaining carbon in the acrylonitrile branch. The radical acetonitrile and the radical CH₂ each pull a hydrogen from either the core or the nearest dendrimer acrylonitrile branch. A double bond is created in either the dendrimer acrylonitrile branch or the dendrimer core to compensate for the loss of the two hydrogens. Figure 13 shows the potential final structures for the dendrimer after the loss of acetonitrile.

Figure 13: Scheme showing the potential final products after the loss of acetonitrile and the hydrogen abstraction. Due to the acidity of the carbons in the core that are next to the core nitrogens, only those carbons were thought to be involved in the mechanism.
The loss of 96 amu is consistent with a loss of an acetonitrile as well as a dendrimer acrylonitrile branch. Figure 14 shows the structure of the Cu-nPPI complex after the neutral loss of 96 amu. This loss also involves the hydrogen abstraction process. The acetonitrile then leaves, creating the CH$_2$ radical attached to the secondary amine. The radical then abstracts the hydrogen from the nitrogen, forming the double bond.

Figure 14: Cartoon showing the final structure of the complex after the loss of 96 m/z.

A schematic showing the fragmentation pattern from the original dendrimer is shown in Figure 15. Figure 16 shows the fragmentation spectrum from 271 m/z and Figure 17 shows the proposed fragmentation pathways.
Figure 15: Schematic showing the losses from the parent $^{15}$N-labeled Cu-nPPI complex and the structures resulting from those losses.
Figure 16: Fragmentation spectrum of m/z 271. Primary neutral losses are 42 (229 m/z), 54 (m/z 217) and 96 (m/z 175).
The first generation Cu-nPPI complex was also studied in order to see whether terminal nitrogens from the dendrimer branches or tertiary nitrogens from the core were part of the neutral losses. Figure 18 shows the CAD spectrum of the parent ion. The parent ion was found at 363 m/z, which also indicated that the synthesis of the 1\textsuperscript{st} generation nPPI was successful since the dendrimer would weigh 301 with the proton attached and 363 with the Cu metal attached. The fragmentation pattern of the Cu-nPPI complex was similar to the fragmentation pattern of the $^{15}$N-labeled complex, in which losses of 41 (acetonitrile), 53 (acrylonitrile branch), and 94 (acetonitrile and an acrylonitrile branch) m/z were observed. Figure 19 shows the CAD spectrum for the dendrimer-metal complex after the loss of acetonitrile.
Figure 18: Mass spectrum showing the fragmentation of the Cu-nPPI complex. The parent ion appears at 363 m/z.
Figure 19: MS/MS fragmentation of the ion found at 322 m/z. This ion demonstrates the same fragmentation pattern as the fragmentation pattern for its analog, the 325 m/z fragment for the labeled Cu-nPPI complex.

Second Generation Cu-nPPI Complex

The fragmentation of the 2nd generation Cu-nPPI complex was compared to the 1st generation Cu-nPPI complex. The parent ion was found at 803 m/z. The mass of the parent ion verified the synthesis of the 2nd generation nitrile-terminated PPI dendrimer since the mass of the parent ion minus the Cu metal would be shown at 741 m/z (after protonation since the dendrimer would have to carry a charge to be observed using ESI-MS).
The fragmentation of the parent ion results in fragments at 750 and 697 m/z. The primary loss to result in the fragment at 750 m/z is a loss of 53, which is most likely the loss of an acrylonitrile branch from the dendrimer. The fragment at 697 m/z results from a neutral loss of 106, which is the loss of two acrylonitrile branches from the parent Cu-nPPI complex. Figure 20 shows the spectrum obtained from the isolation and fragmentation of the parent complex.

Figure 20: CAD mass spectrum of the 2nd generation nitrile-terminated Cu-PPI complex.

The parent ion is at 803 m/z.

Isolation of the fragment at 750 m/z results in neutral losses of 53, 106, and 159 m/z. The pattern indicates successive losses of acrylonitrile branches from the parent dendrimer. Figure 21
shows the mass spectrum obtained from the fragmentation of the ion at 750 m/z. The main fragmentation pathways are displayed in Figure 22.

Figure 21: MS/MS spectrum of the fragment ion found at 750 m/z in the original MS spectrum taken of the parent ion. The spectrum shows a primary neutral loss of 53 which indicates the loss of an acrylonitrile branch.
Figure 22: Main fragmentation pathways for the 2\textsuperscript{nd} generation nitrile-terminated Cu-PPI complex. The losses are shown coming from only one side of the dendrimer for simplicity; the losses could be coming from any arm of the dendrimer.
The main difference between the fragmentation pathways for the 1st generation and 2nd generation Cu-nPPI complexes is that the 2nd generation complex does not lose acetonitrile. Instead, it only loses acrylonitrile branches from the dendrimer. The 1st generation complex also loses acrylonitrile branches primarily but it also loses acetonitrile through the hydrogen abstraction. It is unclear at this point why the 2nd generation complex prefers to only lose acrylonitrile rather than to lose the acetonitrile in addition to acrylonitrile.

First Generation Ni-nPPI Complex

The 15N-labeled and the Ni-nPPI metal-dendrimer complexes were also studied. The labeled Ni-nPPI complex was studied first. The parent dendrimer was found at 362 m/z, and the isotopic distribution obtained through scanning verified the presence of a nickel ion associated with the nPPI dendrimer. The computer-generated isotopic distribution is shown in Figure 23. The isotopic distribution shown in Figure 24 as well as the full CAD spectrum obtained for the Ni-nPPI complex indicated that either the nickel was being reduced to a monovalent state or the complex itself was being reduced. It seems unlikely that the metal would be reduced since nickel(I) would be d⁰ and thus unstable. Since the dielectric constant for a vacuum is 1 and the dielectric constant for water is 80, this could explain why the nickel could exist in a monovalent state in the gas-phase while it only exists in the divalent state in the solid and liquid phases. However, since nickel exists in the divalent state in solid form, determining whether or not the complex or the metal is reduced cannot be done.
Figure 23: Isotopic distribution for the Ni-nPPI parent peak at 358 m/z. The mass shifts to 362 m/z for the $^{15}$N-labeled complex.
Figure 24: Experimentally observed isotopic distribution for the Ni-nPPI complex. The mass shifts to 362 m/z for the $^{15}$N-labeled complex.

Figure 25 shows the CAD spectrum for the $^{15}$N-labeled Ni-nPPI parent complex. The most common losses seen for the $^{15}$N-labeled Ni-nPPI complex were neutral losses of 28, 41, 54, and 96 m/z. The loss of 28 m/z is a loss of hydrogen cyanide from one of the ends of the dendrimer. The loss of 41 m/z appears to be a loss of acetonitrile; however, the hydrogen abstraction that occurs with the Cu-nPPI dendrimer complex does not occur here. Instead, the CH$_2$ remains a radical. If the hydrogen abstraction were occurring to leave the double bond either in the acrylonitrile branch or in the core, the peak for the final structure would be seen at 320 m/z. This could be occurring because the lone pair on the nickel or the single electron left in the nickel orbital may be stabilizing the radical. Figure 26 shows the structure of the dendrimer after the loss of acetonitrile.
Figure 25: Spectrum obtained by the fragmentation of the parent metal-dendrimer complex at 362 m/z.

Figure 26: Cartoon showing the loss of acetonitrile to form the CH$_2$ radical.
The loss at 54 m/z is the loss of an acrylonitrile branch from the dendrimer-metal complex as was observed in the fragmentation of the Cu-nPPI complex. The neutral loss at 96 m/z appears to be the same loss observed for the Cu-nPPI complex in which the complex first loses an acrylonitrile branch and then loses an acetonitrile; it appears that the process involves a hydrogen abstraction from the secondary amine to form the double bond with the core nitrogen and the radical CH₂. Figure 27 shows the fragmentation scheme for the ^15N-labeled Ni-nPPI complex.
Figure 27: Schematic showing the losses from the $^{15}$N-labeled Ni-nPPI complex and the structures that result from those losses.

The first generation Ni-nPPI complex follows the same fragmentation pattern as the labeled complex. The CAD spectrum for this complex is shown in Figure 28. The parent ion is found at 358 m/z. When the parent ion is fragmented, neutral losses of 27, 40, 53 and 94 occur. The loss of 27 is indicative of a loss of hydrogen cyanide. The loss of 40 is a loss of acetonitrile.
which leaves the CH$_2$ as a radical just as the fragmentation of the parent ion of the labeled complex does. The loss of 53 is the loss of a branch from the dendrimer and the loss of 94 is a loss of an acrylonitrile branch as well as acetonitrile. This loss is interesting because instead of seeing the loss of acetonitrile with a CH$_2$ radical on the end, the acetonitrile is lost normally as CH$_3$CN.

Figure 28: Spectrum displaying the fragmentation pattern of the parent ion found at 358 m/z. 

The primary loss is 40, which is indicative of acetonitrile with a CH$_2$ radical on the end instead of the usual CH$_3$. 
Isolation of the ion at 318 m/z results in fragment ions appearing at 291 and 278 m/z. The neutral loss of 27 to leave the fragment ion at 291 m/z is the loss of hydrogen cyanide. The neutral loss of 40 is a loss of acetonitrile with a CH$_2$ radical on the end instead of the CH$_3$ normally observed for the structure of acetonitrile. Figure 29 shows the CAD spectrum obtained for the fragmentation of 318 m/z.

Figure 29: Spectrum displaying the fragmentation pattern of the ion at 318 m/z. The primary loss is 27, which is indicative of hydrogen cyanide.
Second Generation Ni-nPPI Complex

The second generation Ni-nPPI complex was also fragmented; its CAD spectrum is displayed in Figure 30. The parent ion appeared at 797 m/z. Upon isolation and fragmentation, the parent ion fragmented into four main ions: 770, 745, 692, and 674 m/z. The neutral loss of 27 from the parent ion to obtain the peak at 770 m/z is a loss of hydrogen cyanide from one of the terminal ends of a branch. The neutral loss of 53 from 798 to 745 m/z is the loss of an acrylonitrile branch. The neutral loss of 106 for creation of the ion at 692 m/z is the loss of two acrylonitrile branches. The peak at 674 m/z indicates a loss of 124. This peak results from the neutral loss of acetonitrile, acrylonitrile and hydrogen cyanide.

Figure 30: Fragmentation of the parent ion for the second generation nitrile-terminated Ni-PPI complex. The parent ion appears at 798 m/z. The ion primarily loses 53 m/z, which is a loss of a branch.
The ion at 745 m/z was isolated and further fragmented. The primary loss was a neutral loss of 53, which is the loss of one of the branches from the dendrimer. The fragment also loses 106, which is the loss of two branches from the dendrimer. Figure 31 shows the fragmentation spectrum for the ion at 745 m/z. Figure 32 shows the main fragmentation pathways for the 2nd generation Ni-nPPI complex.

Figure 31: MS/MS spectrum for the fragment that appears at 745 m/z. The ion primarily loses 53 m/z, which is a loss of a branch.
Figure 32: Main fragmentation pathways for the 2nd generation nitrile-terminated Ni-PPI complex.
First Generation Zn-PPI Complex

Zinc, with a monoisotopic mass of 64 amu, was complexed with the first generation nitrile-terminated PPI dendrimer and fragmented to see if its fragmentation patterns were similar to the other metal-dendrimer complexes. The second generation Zn-dendrimer complex could not be evaluated as the parent peak was unable to be isolated.

The parent ion was isolated and its isotope distribution was observed in order to confirm that the Zn-nPPI was successfully formed. Much like the nickel complex, the zinc complex holds a 1+ charge. Whether this charge results from a reduction of the metal or a reduction of the entire complex remains unable to be tested since zinc does not normally exist in a monovalent state. Figures 33 and 34 show the isotope distributions generated by iMass and Xcalibur.

Figures 35 and 36 show the mass spectrum obtained upon fragmentation of Zn-nPPI and the fragmentation pattern of the metal-dendrimer complex. The Zn-nPPI parent complex appears at 363 m/z, which is one mass unit away from where it should appear at 364 m/z. The complex may exist as a radical through the loss of a hydrogen from the backbone, a zinc hydride anion (ZnH\(^-\)) may be abstracting a proton to form zinc hydride, or a zinc cation may be pulling a neutral hydrogen from the dendrimer.
Figure 33: Computer-generated isotopic distribution of the first generation Zn-nPPI complex centered at 364 m/z.

Figure 34: Experimentally observed isotopic distribution of the first generation Zn-nPPI complex centered at 363 m/z.
The first thing noted with the fragmentation was the array of Zn-nPPI complexes with extra molecules attached. When the parent complex was isolated, it picked up water and acetonitrile in the trap. Thus, the complexes seen at 381, 404, 422, and 463 m/z indicate that the Zn-nPPI complex picked up varying combinations of water and acetonitrile. These additions show that the Zn is not entirely enclosed in the dendrimer. This means that the Zn has ligand sites available for complexation with various molecules in the trap and solvent. Table 2 lists the complexes seen due to the complexation with the available ligand sites.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>362.8</td>
<td>Zn-nPPI complex</td>
</tr>
<tr>
<td>380.5</td>
<td>Zn-nPPI + water</td>
</tr>
<tr>
<td>403.5</td>
<td>Zn-nPPI + MeCN</td>
</tr>
<tr>
<td>421.6</td>
<td>Zn-nPPI + MeCN + water</td>
</tr>
<tr>
<td>461.7</td>
<td>Zn-nPPI + 2MeCN + water</td>
</tr>
</tbody>
</table>

Table 2: The zinc ion in the Zn-nPPI complex was not entirely closed in the dendrimer, thus leaving open ligand sites. The table lists adducts seen in the spectra at their respective mass-to-charge ratios.

The most common losses seen in the fragmentation of the zinc-dendrimer complex are the loss of acetonitrile (corresponding to a neutral loss of 41 amu), the loss of an arm (corresponding to a neutral loss of 53 amu), and the loss of an arm and acetonitrile concurrently (corresponding to a neutral loss of 94 amu). Some dehydrogenation is also seen in further fragmentation, but is not seen often. Table 3 shows the neutral losses associated with the Zn-nPPI fragmentation.
Figure 35: CAD mass spectrum of the parent complex at 363 m/z. The primary losses observed in the spectrum include acetonitrile, acrylonitrile and a combination of both.
Figure 36: The main fragmentation scheme of the first generation Zn-nPPI complex. The parent peak is found at 363 m/z.
Neutral losses (amu) | Structure
---|---
41 | ![Structure](image1)
53 | ![Structure](image2)
94 | ![Structure](image3) and ![Structure](image4)

Table 3: The neutral losses associated with the fragmentation of the Zn-nPPI complex.

First Generation Li-nPPI Complex

Lithium was chosen as the next metal for complexation since alkali metals had not yet been studied with the nPPI dendrimer. Confirmation of the complexation was obtained by searching out the parent ion at 307 m/z and viewing its isotope distribution. Figure 37 shows the experimentally obtained isotope distribution which indicates proper creation of the complex.
Figure 37: Mass spectrum showing the isotopic distribution of the first generation Li-nPPI complex centered at 307 m/z.

The Li-nPPI complex produced a fragmentation pattern very similar to those already seen with other metals. The most common neutral losses observed were 41, 53, 94, and 106 amu. Collision-activated dissociation of the parent ion resulted in fragment ions at 266, 254, 213, 201 and 160 m/z. Upon further fragmentation of these ions, losses of acetonitrile and acrylonitrile dendrimer arms were observed. Isolation and fragmentation of the 266 m/z ion yielded a variety of product ions. Hydrogen cyanide was a product ion not previously seen in the fragmentation of the parent ion. Figure 38 displays the fragmentation scheme for the metal-dendrimer complex. Table 4 lists the commonly observed neutral losses.
Table 4: The neutral losses associated with the fragmentation of the first generation Li-nPPI complex.
Second Generation Li-nPPI Complex

The fragmentation scheme of the second generation Li-nPPI complex is shown in Figure 39. Upon isolation and fragmentation of the parent ion at 747 m/z, product ions at 694, 641 and
588 m/z were obtained. The neutral losses to get these product ions are 53, 106 and 159 amu. These losses correspond to consecutive losses of dendrimer arms (acrylonitrile). This fragmentation pattern continues as the complex undergoes further fragmentation. From MS\(^2\) to MS\(^6\) (the number designates the round of fragmentation that the analyte of interest has undergone), the dendrimer continues to lose only arms. However, at MS\(^7\), the complex begins losing acetonitrile. Table 5 shows the neutral losses observed through fragmentation of the second generation Li-nPPI complex.

<table>
<thead>
<tr>
<th>Neutral loss (amu)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>106</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>159</td>
<td><img src="" alt="Structure" /></td>
</tr>
</tbody>
</table>

*Table 5: The neutral losses associated with the fragmentation of the second generation Li-nPPI complex.*
Figure 39: The main fragmentation scheme of the second generation Li-nPPI complex.
First Generation Na-nPPI Complex

Fragmentation of the first generation Na-nPPI complex followed the same fragmentation pattern as Li-nPPI. The scheme is shown in Figure 40. Losses of acetonitrile, arms, and a combination of the two are prevalent in the fragmentation scheme. The parent ion was found at 323 m/z, with the most abundant product ion seen at 282 m/z (see Appendix for spectrum). This peak corresponds to a neutral loss of 41 amu, which indicates loss of CH₃CN. Table 6 lists the losses seen through the fragmentation of the metal-dendrimer complex.

Figure 40: The main fragmentation scheme of the first generation Na-nPPI complex.
Table 6: The neutral losses associated with the fragmentation of the first generation Na-nPPI complex.

<table>
<thead>
<tr>
<th>Neutral loss (amu)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>53</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>94</td>
<td><img src="image3.png" alt="Structure" /> and <img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>106</td>
<td>2 <img src="image5.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

Second Generation Na-nPPI Complex

Figure 41 shows the fragmentation scheme for the second generation Na-nPPI complex. This complex follows the same pattern as the second generation Li-nPPI complex, although neutral losses of 41 and 94 amu corresponding to losses of acetonitrile and a concurrent loss of acetonitrile and a dendrimer arm are observed. Table 7 displays the structures of the neutral losses observed.
Figure 41: The fragmentation pathway of the second generation Na-nPPI complex.
<table>
<thead>
<tr>
<th>Neutral loss (amu)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>[Image]</td>
</tr>
<tr>
<td>53</td>
<td>[Image]</td>
</tr>
<tr>
<td>94</td>
<td>[Image]   and [Image]</td>
</tr>
<tr>
<td>106</td>
<td>[Image]   2</td>
</tr>
<tr>
<td>159</td>
<td>[Image]   3</td>
</tr>
</tbody>
</table>

Table 7: Common neutral losses seen in the fragmentation of the second generation Na-nPPI complex.

First Generation Ag-nPPI Complex

The parent dendrimer was found at 407 m/z, and the isotopic distribution obtained through scanning verified the presence of a silver ion associated with the nPPI dendrimer. Figure 42 shows the computer-generated isotopic distribution and Figure 43 shows the experimentally-derived isotopic distribution for the first generation Ag-nPPI complex.
Figure 42: Computer-generated mass spectrum showing the isotopic distribution of the first generation Ag-nPPI complex centered at 407 m/z.
Figure 43: Experimentally obtained mass spectrum showing the isotopic distribution of the first generation Ag-nPPI complex centered at 407 m/z.

Figure 44 shows the fragmentation scheme for the first generation Ag-nPPI complex. The most common losses from the complex included the loss of acetonitrile and the loss of arms. The complex also tended to lose both acetonitrile and arms at the same time. The main losses were neutral losses of 41, 53, 94 and 147 m/z. These masses correspond to a loss of acetonitrile, the loss of an arm, the loss of acetonitrile and an arm, and the loss of acetonitrile and two arms. The loss of acetonitrile from the Ag-nPPI complex follows the same radical mechanism as seen in the other metal-dendrimer complexes.

As the complex was further broken down, the loss of H₂ was noticed with the loss of multiple arms. Hydrogen cyanide was also lost as further fragmentation occurred. Table 8 lists the neutral fragments lost during the fragmentation process.
Figure 44: The main fragmentation scheme of the first generation Ag-nPPI complex. Unique to this dendrimer-metal complex is the loss of $H_2$. 
<table>
<thead>
<tr>
<th>Neutral loss (amu)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>$\text{H}_2$</td>
</tr>
<tr>
<td>41</td>
<td><img src="image" alt="" /></td>
</tr>
<tr>
<td>53</td>
<td><img src="image" alt="" /></td>
</tr>
<tr>
<td>94</td>
<td><img src="image" alt="" /> and <img src="image" alt="" /></td>
</tr>
<tr>
<td>147</td>
<td><img src="image" alt="" /> 2 and <img src="image" alt="" /></td>
</tr>
</tbody>
</table>

*Table 8: Common neutral losses seen in the fragmentation of the first generation Ag-nPPI complex.*

**Second Generation Ag-nPPI Complex**

The fragmentation scheme for the second generation Ag-nPPI complex is shown in Figure 45. The metal-dendrimer complex followed the same general fragmentation pathway as the Li-nPPI and Na-nPPI complexes. The complex primarily showed losses of acetonitrile and acrylonitrile arms. The parent mass was found at 847 m/z. Subsequent fragmentation showed successive losses of acrylonitrile branches. Table 9 displays the main fragmentation products.
Figure 45: The main fragmentation pathway of the second generation Ag-nPPI complex. The complex primarily loses arms and acetonitriles.
Deuterium-labeled nPPI Complexes

In order to determine which hydrogens were abstracted during the radical mechanism seen in the fragmentation pathway of the Cu-nPPI dendrimer, the d$_2$-labeled nPPI dendrimer and the d$_4$-labeled nPPI dendrimer were synthesized in-house using deuterium-labeled materials. D$_2$-labeled materials (acrylonitrile) and d$_4$-labeled materials (succinonitrile) were used. The synthesis utilizing the deuterium-labeled acrylonitrile put deuteriums on the second carbon from the nitrogen in the nitrile group in each arm. Lithium aluminum hydride (LAH) and lithium aluminum deuteride (LAD) were used for reduction of the dendrimer in order to put deuteriums on different carbons in the core. LAD allowed for the deuteriums to be on the α-carbons in the core (those closest to the core nitrogens) while using LAH for the reduction put the deuteriums on the β-carbons (second carbons from the core nitrogens). Figure 46 shows the structure of the arm-labeled dendrimer while Figure 47 shows the two nPPI dendrimers with the labeled cores.

<table>
<thead>
<tr>
<th>Neutral loss (amu)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>53</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>106</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
</tbody>
</table>

*Table 9: The neutral losses associated with the fragmentation of the second generation Ag-nPPI complex.*
Figure 46: The \( d_2 \)-designated nitrile-terminated PPI dendrimer with deuterated \( \beta \)-carbons on the arms.

\[
\text{\( d_2 \)-labeled Cu-nPPI Complexes}
\]

The \( d_2 \)-labeled Cu-nPPI complex was studied first. The parent peak appeared at 371 m/z. Fragmentation of the parent peak yielded peaks at 328, 317, 274 and 273. The peak of interest was seen at 328 m/z. This peak indicates a neutral loss of acetonitrile weighing 43 amu. The structure of the neutral loss is shown in Figure 48. The addition of 2 amu from a typical loss of acetonitrile indicates that the acetonitrile includes the deuterium from the arm from which the acetonitrile fragmented as well as another deuterium from another arm. The inclusion of this
deuterium shows that during the radical mechanism, the acetonitrile leaves with a hydrogen from the same arm and a hydrogen that comes from a different arm.

\[ \text{Figure 48: The loss of acetonitrile from the metal-dendrimer complex. The acetonitrile leaves as a neutral loss of 43 amu. This indicates the presence of two deuterons.} \]

d\textsubscript{2}-labeled Ni-nPPI Complexes

The d\textsubscript{2}-labeled Ni-nPPI complex was also studied to see if the results would be the same as for the d\textsubscript{2}-labeled Cu-nPPI complex. The parent peak should have appeared at 366 m/z; however, the main peak in the full spectrum was found at 428 m/z. This peak is indicative of the parent Ni-nPPI complex with NiD\textsubscript{2} attached. Since deuterated water and deuterated acetic acid were used in the creation of the solution, and since this same addition was seen with the Ni-nPPI complex (added as nickel hydride), this addition of nickel deuteride was expected. Fragmentation of the parent peak resulted in product ions at 366, 337, 323, 266, 180 and 139 m/z. The product ion at 366 m/z is the Ni-nPPI complex. The main peak of interest out of the product ions is the peak found at 323 m/z since this peak represents the loss of acetonitrile from the Ni-nPPI parent peak. The neutral loss from the parent peak was 43 amu; this correlates with the loss of a doubly deuterated acetonitrile. This loss of 43 amu indicates again that one of the hydrogens in the radical mechanism comes from the same arm and one comes from an adjacent arm.
The \( d_4 \)-labeled Cu-nPPI complex was studied in order to verify that the other hydrogen comes from an adjacent arm in the loss of acetonitrile. In this study, the core of the dendrimer was labeled with deuteriums. The deuteriums were either on the \( \alpha \)- or \( \beta \)-carbons. Figure 49 shows the mass spectrum of the \( \alpha \)-complex and Figure 50 shows the mass spectrum of the \( \beta \)-complex. When the Cu-nPPI complex with the deuteriums on the \( \alpha \)-carbons was run, the parent peak appeared at 367 m/z. When the parent peak was isolated and fragmented, product ions were found at 326, 314 and 273 m/z. The peak at 326 m/z was of main interest since this peak resulted from a neutral loss of 41, or acetonitrile, from the parent peak. A neutral loss of 41 amu indicated that no deuteriums were lost with the acetonitrile.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mass_spectra.png}
\caption{Mass spectrum of the deuterium-labeled Cu-nPPI complex with the deuteriums on the \( \alpha \)-carbons in the core.}
\end{figure}
The Cu-nPPI complex with the deuteriums on the β-carbons was also fragmented, and the product ions appeared at the same m/z as the complex with the deuteriums on the α-carbons. This indicated that in the β-complex, the deuteriums were not lost with the neutral loss of acetonitrile. As a result, it was realized that the second hydrogen in the radical mechanism also comes from the dendrimer arm. Figure 51 shows the mechanism of the loss of acetonitrile.

Figure 50: Mass spectrum of the deuterium-labeled Cu-nPPI complex with the deuteriums on the β-carbons in the core.
**Figure 51:** The mechanism by which acetonitrile fragments from the metal-dendrimer complex.

In A) the bond between the α- and β-carbons in the dendrimer arm undergoes homolytic cleavage. In B) the CH$_2$CN radical abstracts one of the hydrogens from the adjacent arm of the dendrimer. The CH$_2$ radical also abstracts a hydrogen from that arm. In C) the double bond is formed in the dendrimer arm and the acetonitrile leaves.

**d$_4$-labeled Ni-nPPI Complexes**

The d$_4$-labeled Ni-nPPI complex was also studied to verify that the hydrogen was indeed coming from the arm and not the core. Both the α- and β-complexes were studied. The parent ion was found at 362 m/z for both. When both complexes were fragmented, a peak at 322 m/z indicating a neutral loss of 40 amu was seen. This loss indicated that the acetonitrile was
fragmenting as a radical and staying as a radical, rather than abstracting a hydrogen from the dendrimer arm from which it fragmented. Figure 52 shows the acetonitrile neutral loss and the structure of the remaining metal-dendrimer complex.

\[
\begin{align*}
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N} \\
&\text{N} \quad \text{N}
\end{align*}
\]

\[+ \quad \text{H}_2\text{C} \equiv \text{N}\]

*Figure 52: The products of the acetonitrile loss from the deuterated Ni-nPPI complex. No hydrogen abstraction occurs.*

**Conclusions:**

Fragmentation pathways of nitrile-terminated PPI dendrimers complexed with metals tend to follow the same scheme. Because ESI-MS was used, and neutral losses using this method tend to be stable molecules, this can explain why the main neutral losses for the dendrimer-metal complexes were acetonitrile and acrylonitrile. It was observed that only one metal cation was complexed with the dendrimer instead of multiple cations; if another metal was complexed, it was through the complexation of a metal hydride that was not encapsulated by the dendrimer like the main metal cation. It was also observed through the molecular weights of the dendrimer peaks observed that only one dendrimer, not multiple dendrimers, was complexed with a metal. Variation of the metals complexed from alkali metals to transition metals appears to have little effect on the fragmentation pathways. The Ni-nPPI complex seems to be the stand-out among the other metal-dendrimer complexes in that its neutral losses remain radicals and do not abstract any hydrogens from the dendrimer. The zinc complex also varies slightly from the expected
behavior of the metal-nPPI complex since the metal does not become fully encapsulated in the dendrimer, allowing for the addition of neutral adducts while the complex is isolated in the trap. Mass spectrometry has allowed researchers the ability to accurately and efficiently reveal the mechanisms by which the complexes fragment.
Appendix:

This appendix includes mass spectra for the metal-dendrimer complexes.

**Cu-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 310**

![Mass spectrum of m/z 310](image)

**Cu-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 269**

![Mass spectrum of m/z 269](image)
Cu-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 697

Cu-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 644
Cu-nPPI $2^{\text{nd}}$ Gen: CAD mass spectrum of m/z 591

Ni-nPPI $1^{\text{st}}$ Gen: CAD mass spectrum of m/z 305
Ni-nPPI 1st Gen: CAD mass spectrum of m/z 291

Ni-nPPI 1st Gen: CAD mass spectrum of m/z 263
Ni-nPPI 1st Gen: CAD mass spectrum of m/z 235

Ni-nPPI 2nd Gen: CAD mass spectrum of m/z 770
Ni-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 692

Ni-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 639
Zn-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 322

Zn-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 310
Zn-nPPI 1st Gen: CAD mass spectrum of m/z 269

Li-nPPI 1st Gen: CAD mass spectrum of m/z 307
Li-nPPI 1st Gen: CAD mass spectrum of m/z 266

![Graph 1: CAD mass spectrum of m/z 266](image1)

Li-nPPI 1st Gen: CAD mass spectrum of m/z 254

![Graph 2: CAD mass spectrum of m/z 254](image2)
Li-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 225

Li-nPPI 1\textsuperscript{st} Gen: CAD mass spectrum of m/z 213
Li-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 747

Li-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 694
Li-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 641

Li-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 588
Na-nPPI 1st Gen: CAD mass spectrum of m/z 323

Na-nPPI 1st Gen: CAD mass spectrum of m/z 270
Na-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 763

![Mass Spectrum of m/z 763](image)

Na-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 710

![Mass Spectrum of m/z 710](image)
Na-nPPI 2nd Gen: CAD mass spectrum of m/z 657

Ag-nPPI 1st Gen: CAD mass spectrum of m/z 407
Ag-nPPI 1st Gen: CAD mass spectrum of m/z 366

Ag-nPPI 1st Gen: CAD mass spectrum of m/z 354
Ag-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 847

Ag-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 794
Ag-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 741

Ag-nPPI 2\textsuperscript{nd} Gen: CAD mass spectrum of m/z 688
Cu-nPPI d$_2$: CAD mass spectrum of m/z 371

Cu-nPPI d$_2$: CAD mass spectrum of m/z 328
Cu-nPPI $d_2$: CAD mass spectrum of m/z 317

Ni-nPPI $d_2$: CAD mass spectrum of m/z 428
Ni-nPPI $d_2$: CAD mass spectrum of m/z 366

Cu-nPPI $\alpha$-$d_4$: CAD mass spectrum of m/z 367
Cu-nPPI β-d₄: CAD mass spectrum of m/z 367

Ni-nPPI α-d₄: CAD mass spectrum of m/z 362
Ni-nPPI β-d₄: CAD mass spectrum of m/z 362
References:


