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Structural Characterization of Methylenedianiline Regioisomers by Ion Mobility-Mass Spectrometry and Tandem Mass Spectrometry: IV. 3-Ring and 4-Ring Isomers

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Abstract

Matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS) is used to characterize methylenedianiline (MDA) 3-ring and 4-ring species. Building on our previous MALDI-MS 2-ring MDA isomer study, here we compare 3-ring and 4-ring electrospray ionization (ESI) and MALDI results. In ESI, 3-ring and 4-ring MDAs each form one single $[M+H]^+$ parent ion. Whereas in MALDI, each MDA multimer forms three unique precursor ions: $[M+H]^+$, $[M.]^+$, and $[M-H]^+$. In this study, 3-ring and 4-ring MDA precursors are characterized to identify the unique fragment ions formed and their respective fragmentation pathways. In addition to the three possible precursors, the 3-ring and 4-ring species are higher-order oligomer precursors in polyurethane (PUR) production and thus provides additional insight into the polymeric behavior of these PUR hard block precursors. The combination of ion mobility-mass spectrometry (IM-MS) and tandem mass spectrometry (MS/MS) allow the structural characterization of these larger MDA multimers.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: Additional Supporting Information content includes MS/MS, MS3 spectra of 3-ring and 4-ring MDA species, an example of multiple MDA protonation sites, IM-MS curve resolution and raw data, additional fragment ion structures, tables for each precursor's fragment ion formation, and additional CID curves of high energy fragment ions.

Notes

The authors declare no competing financial interest.

Polyurethane (PUR) copolymers are one of the most versatile polymeric materials, commonly manufactured for use in rigid and flexible foams, coatings, adhesives, sealants, elastomers, membrane materials, laminates, fibers, and composites. Methylenedi-aniline (MDA) is a precursor to methylene bisphenyl diisocyanate (MDI), a major hard block component in the manufacturing of PURs. MDA is formed from the reaction between aniline and formaldehyde, generating the 2-ring MDA regioisomers 2,2'-MDA, 2,4'-MDA, and 4,4'-MDA, as well as larger multimeric species including 3-ring and 4-ring MDAs.^{1,2}

In our previous work, 2-ring MDAs (2,2'-MDA, 2,4'-MDA, and 4,4'-MDA) were characterized using electrospray ionization-ion mobility-mass spectrometry (ESI-IM-MS)¹ and matrix assisted laser desorption/ionization-ion mobility-mass spectrometry (MALDI-IM-MS),³ two MS techniques commonly used to analyze PUR polymers.⁴⁻⁸ In those studies, one unique parent ion ($[M+H]^+ = 199$ Da) was observed for each isomer using ESI-MS; however, when studied by MALDI-MS we observed three distinct precursor ions, $[M-H]^+ = 197$ Da and $[M]^+ = 198$ Da, in addition to the $[M+H]^+ = 199$ Da species.

More recently, we used ESI-IM-MS to structurally characterize 3-ring and 4-ring MDAs by studying their preferred sites of protonation, gas phase stability, and fragmentation pathways.² In this present study, 3-ring and 4-ring MDAs were similarly characterized using MALDI-IM-MS and the results were compared to our previous ESI-IM-MS findings. In Figure 1, 3-ring and 4-ring MDA parent species are illustrated with their respective protonation sites denoted with asterisks. Consistent with our 2-ring MDA study, we observed one unique parent ion formed during ESI for both 3-ring ($[M+H]^+ = 304$ Da) and 4-ring MDA ($[M+H]^+ = 409$ Da). During the MALDI process we observe three parent ions; two of which are unique to the MALDI process for each 3-ring and 4-ring MDA: $[M+H]^+ = 304, 409$ Da, $[M]^+ = 303, 408$ Da, and $[M-H]^+ = 302, 407$ Da, respectively. The additional rings in these species provide greater flexibility and thus more complex consequences for the fragmentation patterns observed. The novelty of this work compares ESI and MALDI-MS ionization and the unique precursor species that arise from 3-ring and 4-ring MDA species. This work highlights the complexity when investigating fragmentation pathways of larger multimeric MDA species. The combination of IM-MS and MS/MS allow for unique structural characterization of the three precursor ions formed and their respective fragment ion pathways observed during the MALDI process.

EXPERIMENTAL SECTION

Materials.

Sample preparation and experimental details have been reported previously for 3-ring and 4-ring MDA ESI studies² and 2-ring MDA ESI and MALDI studies^{1,3}, but a few details will be mentioned here. Methylenedianiline (MDA) 3-ring and 4-ring samples were provided by Dr. Stefan Wershofen (Bayer MaterialScience AG, 47812 Uerdingen, Germany). Optima grade methanol and water with 0.1% formic acid were obtained from Fisher Scientific (Waltham, MA, USA). Tetralkylammonium salts, α -cy-ano-4-hydroxycinnamic acid (CHCA) and alkali salts were obtained from Sigma-Aldrich (St. Louis, MO, USA). Samples were dissolved at a concentration of 1 mg/mL in 9:1 methanol/water containing 0.1% formic acid (v/v). Each MDA isomer was combined in a 10:1 CHCA matrix-to-analyte ratio and two or three layers of 0.5 μ L of samples were spotted on a 100-well MALDI plate.

Traveling Wave MALDI-IM-TOF/MS.

A traveling wave IM-MS (TWIM-MS) Synapt G2-S (Waters Corporation, Milford, MA) was used for acquiring ESI and MALDI MS, MS/MS, and IM-MS data.⁹ Collision cross section (CCS) values cannot be obtained directly from TWIM experimental drift time values, therefore quaternary ammonium salts were used as CCS calibration standards to obtain CCS data from TWIM.^{10–12}

The Synapt G2-S mass spectrometer MALDI source has a frequency-tripled Nd:YAG laser which emits 355 nm at 1kHz pulse repetition rate. Laser attenuation ranged from 190–300 (arbitrary units), with 270 selected for all experiments unless noted otherwise. CHCA matrix cluster peaks were used for TOF calibration. Further experimental details can be found from our earlier study², but briefly the TWIM drift cell settings were as follows: TWIM pressure, 3 mbar nitrogen (2.25 Torr); electrodynamic wave height, 35 V; wave velocity, 700 m/s; TOF resolving power, ca. 18,000 m/z at 200 m/z . Collision-induced dissociation (CID) experiments were performed using argon gas. In our previous studies, experiments were conducted to evaluate the influence of the MALDI laser fluence and the resolving power of the MS/MS resolving quadrupole on the resulting precursor ion intensities. From those studies, the m/z resolving quadrupole was set to 18 resolving power for the MS/MS experiments providing the best separation of the three precursor ions, without significant loss of signal.³ To perform MS³ experiments, the ion of interest is selected by the quadrupole and is fragmented in the “Trap” region located prior to the TWIM drift cell. The first generation of product ions are then separated by the TWIM and subjected to a second stage of CID in the “Transfer” region to produce 2nd generation and 1st generation product ions (i.e., MS/IM/MS experiments).

RESULTS AND DISCUSSION

Differences between ESI and MALDI Spectra for [M+H]⁺

The full ESI-MS and MALDI-MS spectra in Figure 2 were acquired for both 3-ring (Figure 2a-b) and 4-ring MDA (Figure 2c-d), respectively. The ESI-MS and MALDI-MS spectra for the 3-ring and 4-ring MDA species are vastly different. Using ESI, we observed one

precursor species $[M+H]^+$ and additional characteristic peaks at 211 and 106 Da for the 3-ring (Figure 2a) and 316, 211, and 106 Da for 4-ring MDA (Figure 2c). In addition, peaks observed at 152.6 m/z and 158.6 m/z correspond to the doubly charged 3-ring and 4-ring MDAs (Figure 2a,c). With MALDI we observe more complex mass spectra for both 3-ring and 4-ring MDA (Figure 2b,d). Each of the three unique precursor ions is observed in the 3-ring and 4-ring MALDI spectra, similar to our previous study on 2-ring MDA regioisomers. In MALDI, we observe a characteristic peak at 211 Da for 3-ring MDA and CHCA matrix cluster peaks at 337, 335, 190, and 172 Da (Figure 2b). In addition, the MALDI spectra for 4-ring MDA show the $[M+Na]^+$ peak at 431 Da, characteristic peaks at 316 and 211 Da, and CHCA matrix cluster peaks at 379, 335, 190, and 172 Da (Figure 2d). Precursor ions unique to 3-ring MDA include: $[M+H]^+ = 304$ Da, $[M.]^+ = 303$ Da, and $[M-H]^+ = 302$ Da (Figure 2b). In addition, precursor ions characteristic to the 4-ring MDA include: $[M+H]^+ = 409$ Da, $[M.]^+ = 408$ Da, and $[M-H]^+ = 407$ Da (Figure 2d). In MALDI, the 3-ring and 4-ring $[M+H]^+$ precursor is the least abundant precursor ion compared to $[M.]^+$ and $[M-H]^+$ species (Figure 2b,d). In ESI, the 3-ring and 4-ring $[M+H]^+$ species and characteristic fragment peaks appear more stable than the $[M+H]^+$ precursor and fragment ions produced by the MALDI process. This difference observed for the $[M+H]^+$ precursor indicates that these MDA multimers behave significantly differently when ionized by MALDI than by ESI.

Characterization of 3-Ring and 4-Ring MDA by MS/MS

Figure 3 details the MALDI MS/MS spectra for each precursor ion formed: 3-ring (Figure 3a-c) and 4-ring (Figure 3d-f) MDAs, $[M+H]^+$, $[M.]^+$, and $[M-H]^+$. In the MS/MS spectra, different collision energies were used to generate fragment ions for each precursor due to differing precursor stabilities. Figures S1 and S2 in Supporting Information compare all precursor species fragmented at 15 eV compared to 20 eV (laboratory frame). Due to an increase in the number of fragment ions observed for the $[M+H]^+$ species for both 3-ring and 4-ring MDA, a 15 eV collision energy was used to characterize specific fragment ions derived from the $[M+H]^+$ precursor species, and 20 eV was used to characterize fragment ions derived from the $[M.]^+$ and $[M-H]^+$ species. The MS/MS spectra in Figure 3 show unique fragment ions for each 3-ring and 4-ring precursor species: 15 eV was used for $[M+H]^+$ (Figure 3a,d) and 20 eV was used for $[M.]^+$ and $[M-H]^+$ species (Figure 3b-c,e-f). Additional MS/MS spectra at various collision energies for both 3-ring and 4-ring MDA precursors can be found in Supporting Information (Figures S3-S4). Due to the numerous fragment ions formed uniquely for each precursor, this manuscript will focus on the interpretation of major low energy fragment ions formed by the MALDI process and will be introduced by schemes proposing fragment ion pathways and collision-induced dissociation ion breakdown curves (CID curves). Additional MS³ data found in Supporting Information (Figures S5 and S6) was also acquired and used to support the interpretation of unique fragment ion pathways.

It should be noted that there is a major difference between interpretation of the MALDI spectra of the 3-ring and 4-ring MDAs relative to the 2-ring MDAs from our previous work. This difference arises from the multiple amine groups in the 3-ring and 4-ring compounds. Not only do the amine groups have somewhat differing basicities, but their steric effects can vary significantly. These effects were observed to some extent in the ESI spectra of these

compounds,² but the complexities in the MALDI spectra are more severe due to the larger MDA species and formation of three unique precursor ions in the MALDI process. The net result indicates that it is generally not possible to identify a unique structure for a given mass loss. Supporting Information Figures S7 and S8 address this problem in greater detail with an illustration provided to highlight the complexity of these specific compounds. Therefore, specific structures described in the manuscript are tentative and may not be the only structures possible for a given fragmentation.

Characterization by Ion Mobility-Mass Spectrometry

The experimental IM spectra provided in Figure 4 are shown for the $[M+H]^+$ (blue), $[M.]^+$ (black), and $[M-H]^+$ (red) ion forms of the 3-ring and 4-ring MDA precursor species. In Figure 4a, the TWIM spectrum obtained for the 3-ring species indicate a single distribution for each precursor, whereas in Figure 4b the 4-ring MDA species each exhibit two partially-resolved structural populations, indicative of multiple gas-phase conformations. In our previous ESI studies, computational modeling was used to study the $[M+H]^+$ 3-ring and 4-ring MDA. Theoretical structures were generated to align with experimental CCS values. In those studies, the $[M+H]^+$ 3-ring MDA was shown to undergo the most favorable protonation on the internal amine, when compared to the external amines. In the ESI $[M+H]^+$ 4-ring MDA studies, two conformations were seen, which is similar to what is experimentally observed in the MALDI studies described here. These two conformational families were attributed to external amine protonation and internal amine protonation, respectively. Computational modeling in the ESI study suggests protonation on the external amines is more favored due to the 4-ring MDA species wrapping around the proton to share between the two external amine groups. As demonstrated in previous literature, IM-MS separations can differentiate between both aniline protomers coexisting in the gas-phase: Nprotonated and ring protonated aniline.¹³⁻¹⁴ Similar to aniline studies, we can confirm that IM-MS techniques can aide in identifying multiple sites of protonation within 3-ring and 4-ring MDA precursor species. In Figure 4a, the IM-MS results show 304 Da $[M+H]^+$ and 303 Da $[M.]^+$ species to have the same CCS ($140.8 \text{ \AA}^2$). However, the 302 Da $[M-H]^+$ species show a smaller CCS ($139.2 \text{ \AA}^2$; this difference in CCS for the 3-ring MDA can be attributed to the structural differences exhibited by the $[M-H]^+$ bridged fluorene-backbone, as depicted in Figure 1. The 4-ring MDA precursors behave much differently than the 3-ring species. First, we notice two conformations present for each precursor, each conformation having different abundances. Next, the 409 Da $[M+H]^+$ and 408 Da $[M.]^+$ species each share the same CCS both for the larger conformation ($158.3 \text{ \AA}^2$) and smaller conformation ($164.1 \text{ \AA}^2$). The 407 Da $[M-H]^+$ species is uniquely different from all of the 3-ring and 4-ring precursors. The 407 Da IM-MS trace shows two isomeric species; the less abundant isomer exhibits the smaller CCS ($156.0 \text{ \AA}^2$), whereas the more abundant isomer has the larger CCS ($164.2 \text{ \AA}^2$). When comparing the $[M-H]^+$ species between 3-ring and 4-ring MDAs, we notice that the 302 Da species has a smaller CCS, whereas the 407 Da species (more abundant isomer) has a larger CCS value. The differences observed between the precursors and 3-ring and 4-ring MDA can be attributed to either structural differences or multiple sites of protonation. Structural differences between 3-ring and 4-ring MDA include the addition of a bridged methyl-aniline and differences between $[M+H]^+$ and $[MH]^+$ for each MDA multimer include the bridged fluorene backbone. Possible protonation sites on the 3-ring and

4-ring MDAs are illustrated in Figure 1, differences include exterior or interior amine protonation. As seen in our previous 3-ring and 4-ring ESI studies, the MDA species must be oriented such that the additional proton is in proximity to the most basic site on the adjacent aniline ring to represent the most energetically favorable position. Curve resolution analysis for the 4-ring MDA precursors (Supporting Information S9) and additional IM-MS data can be found in Supporting Information on Pages S10-S11

3-Ring MDA

In the MS/MS spectrum for each 3-ring MDA precursor, Figure 3a-c shows major fragment peaks observed at low lab frame collision energies: $[M+H]^+ = 15$ eV, $[M.]^+ = 20$ eV, and $[M-H]^+ = 20$ eV. The conversion from precursor ion to major fragment ion was monitored as a function of applied collision energy as shown in Figure 5. Collision energy was ramped from 0 to 50 eV for all unique precursor species in 5 eV increments and performed in triplicate. Figure 5a,c,e monitors CID of major fragment ions having a percent relative ion current (%RIC) of 20% or more, and Figure 5b,d,f illustrates %RIC of low abundance fragment ions formed in the range of 5–20% along the 0–50 eV ramp. All of the CID curves in Figure 5 represent %RIC values of 5% or more along the 0–50 eV ramp. Additional CID curves for minor fragment ions formed at 35 eV or higher are provided as Supporting Information (Figure S12). Major fragment ions observed for each precursor species will be discussed in further detail.

3-Ring MDA $[M+H]^+$ Species (304 Da)

In Figure 3a, the MS/MS spectrum highlights major fragment ions observed for the 304 precursor species at 15 eV. The MS/MS spectrum shows the 304 Da species dissociating into four major fragment ions 303, 211, 209, and 198 Da, as seen in Scheme 1a and in the total percent ion current table found in Supporting Information Figure S13. These fragment ions are derived directly from the 304 Da species and each forms with an intensity greater than 5%. In Figure 5a-b, the $[M+H]^+$ precursor depletes at 15 eV forming fragment ions 303 ($[M-1]^+$), 211 ($[M-93]^+$), 209 ($[M-95]^+$), and 198 Da ($[M-106]^+$); a dashed line is drawn for visual alignment of major and minor fragment ion formation at 15 eV. Major fragment ion 303 Da corresponds to the loss of one hydrogen, and the transfer of charge to the ring (8%, 15 eV). Major fragment ion 211 Da forms from the loss of aniline (7%, 15 eV), from its parent species 304 Da. Fragment ion 209 Da corresponds to the loss of aniline and two hydrogens; this fragment ion forms at higher collision energy (6%, 30 eV). In addition, mass 198 Da forms in high abundance due to the loss of methylene-aniline (30%, 15 eV). Scheme 1a outlines a proposed fragmentation pathway for each fragment ion (>5%) originating from the $[M+H]^+$ 304 Da species. As seen in Scheme 1a, minor fragment ions such as 210 ($[M-93]^+$), 197 ($[M-106]^+$), 181 ($[M-17]^+$), and 180 Da ($[M-17]^+$) form by depletion of the major fragment ions at higher collision energy. Scheme 1a proposes that mass 303 Da can form two possible fragments ions: 210 Da and 197 Da. Mass 210 Da is formed by the loss of aniline (10%, 25 eV) and ion 197 Da forms from the loss of methylene-aniline. In Figure 5a-b we observe the depletion of mass 198 Da leading to the formation of the 181 Da (25%, 40 eV) species, resulting from the loss of $-NH_2$ and one hydrogen forming the fluorene-bridge. Similarly, minor fragment ion 180 Da (16%, 40 eV) results from the loss of $-NH_2$ and one hydrogen forming the fluorene-bridge, from major fragment ion 197 Da. The MS3 data

found in Supporting Information Figure S5 is consistent with the pro-posed Scheme 1a outline. Additional possible structures of the proposed fragment ions derived from the 304 Da $[M+H]^+$ are outlined in Supporting Information (Figure S14). Proposed fragmentation pathways, CID curves, and intensity values for additional ions formed at 35 eV or higher are also described in Supporting Information (Figures S12).

3-Ring MDA $[M.]^+$ Species (303 Da)

When comparing the Figure 3b MS/MS spectrum of the $[M.]^+$ species to Figure 3a $[M+H]^+$ MS/MS spectrum, we observe an increase in ion stability. In Figure 5 precursor $[M.]^+$ requires additional collision energy to generate fragment ions compared to the $[M+H]^+$ species. This increase in stability results from delocalization of charge for the 303 Da species. In Figure 5c-d, fewer fragment ions are observed for the 303 Da $[M.]^+$ species at 20 eV collision energy compared to Figure 5a-b (vertical dashed line is drawn for visual reference). Found in the Supporting Information, Figure S15 shows percent total ion current values of major and minor fragment ions formed from mass 303 Da depletion. In Scheme 1b, the major fragment ions observed to form directly from mass 303 Da are outlined: mass 302 ($[M-1]^+$), mass 210 ($[M-93]^+$), and mass 197 ($[M-106]^+$). At 25 eV, fragment ion 302 Da forms from the loss of one hydrogen (9%, 25 eV) and mass 197 Da results from the loss of methylene-aniline (42%, 25 eV). Fragment ion 210 Da forms at higher collision energy (10%, 30 eV), resulting from the loss of aniline. Several minor fragment ions are also observed from the depletion of these major fragment ions: 209 ($[M-93]^+$), 181 ($[M-29]^+$), 180 ($[M-17]^+$), and 165 Da ($[M-15]^+$). In Scheme 1b, species 209 Da forms directly from mass 302 Da through an M-93 loss of aniline (16%, 35 eV). At higher collision energy, mass 210 Da fragments into 181 Da due to loss of $-NH$ and $-CH_2$ (9%, 45 eV). In Figure 5c, the depletion of 197 Da can be monitored to illustrate the formation of fragment ion 180 Da (30%, 50 eV, loss of $-NH_2$ and one-hydrogen). Increasing fragmentation energy to 50eV causes mass 180 Da to form mass 165 Da (9%, loss of $-NH_2$). Additional structures of the major and minor fragment ions formed from the 303 Da species can be found in Supporting Information Figure S16.

3-Ring MDA $[M-H]^+$ Species (302 Da)

The $[M-H]^+$ species is the most stable precursor compared to $[M+H]^+$ and $[M.]^+$ (see Figure 5). The increase in stability is due to the fluorene-backbone, as seen in the 2-ring $[M-H]^+$ species versus the $[M+H]^+$ species, discussed previously.³ In Figure 3c, the MS/MS spectrum shows three major fragment ions deriving from the 302 Da precursor ion at 20 eV: 285 ($[M-17]^+$), 209 ($[M-93]^+$), and 106 Da ($[M-196]^+$). The dashed line in Figure 5e-f at 20 eV is drawn for visual alignment to illustrate fragment ions formed at %RIC above 5%. Here we observe the increased stability of the $[M-H]^+$ precursor ion, higher collision energy is needed to form fragment ions. Total percent ion current values of major and minor fragment ions formed from the 302 Da species can be found in Supporting Information Figure S17. The formation of mass 285 Da corresponds to M-17, loss of ammonia (4%, 20 eV), this species is not one of the more intense fragment ions for the mass 302 Da precursor, but loss of $-NH_3$ occurs prominently in the MALDI spectra for all species. In Scheme 1c the proposed fragment ion pathways are illustrated for the 302 Da precursor. The major fragment ion formed from the 302 Da precursor is an ion at mass 209 Da (36%, 20 eV),

formally M-93 corresponding to loss of aniline; it can be assumed to come from charge-remote fragmentation (Supporting Information Figures S7-S8). Fragment ion 106 Da has been consistently characterized for the MDA 2-ring, 3-ring, and 4-ring species. For the 3-ring MALDI MS/MS studies, mass 106 Da forms from a loss of 4,4'-MDA and one hydrogen (5%, 30 eV). There are five additional minor fragment ions of interest, all formed at higher collision energies: 208 ($[M-1]^+$), 193 ($[M-16]^+$), 192 ($[M-1]^+$), 180 ($[M-29]^+$), and 165 Da ($[M-28]^+$). Fragment ion 208 Da (16%, 35 eV) forms from 209 Da due to a loss of one hydrogen localizing the charge on the ring. Fragment ion 193 Da (18%, 30 eV) forms from a loss of $-NH_2$ directly from fragment 209 Da. Furthermore, the 193 Da ion depletes into mass 192 Da (8%, 30 eV) from loss of the bridge-hydrogen; in addition, higher collision energy causes mass 165 Da (30%, 50 eV) to form from the 193 Da species due to the loss of $-CHN-$ from the protonated species. The 180 Da (26%, 50 eV) ion also forms at high collision energy and is formed from the 209 Da species from a loss of $CH_2=NH$. Additional structures of the major and minor fragment ions formed from the 302 Da precursor can be found in the Supporting Information Figure S18.

4-Ring MDA

The three 4-ring MDA precursors behave similarly to the 3-ring MDA. Here we observe $[M+H]^+$ as the least stable precursor amongst $[M.]^+$ and $[M-H]^+$ species, forming more fragment ions at lower collision energy (Figure 3d-f); this is consistent with our previous 2-ring studies.³ The MALDI MS/MS spectra for the 4-ring MDAs show major fragment ion peaks at low collision energy for each precursor species: $[M+H]^+$, $[M.]^+$, and $[M-H]^+$. It is interesting to note that an increase in fragment ions is observed in Figure 3d for the $[M+H]^+$ species (15 eV) compared to $[M.]^+$ and $[M-H]^+$ in Figure 3e-f (20 eV). In Figure 6, we monitor the depletion of each precursor species as a function of applied collision energy from 0 to 50 eV in 5 eV increments. Figure 6a,c,e shows CID breakdown curves for the major fragment ions having a %RIC of 20% or more, and Figure 6b,d,f illustrates the minor fragment ions (less than 20% RIC) formed along the 0–50 eV ramp. Major fragment ions to be discussed form in high abundance at collision energies below 35 eV, additional CID curves for fragment ions formed at 35 eV or higher are provided as Supporting Information (Figure S19). Total percent ion current tables are provided in the Supporting Information (Figures S20-S22) for each precursor; these tables highlight fragment ion (%RIC) formation as CID energy is increased. The major fragment ions observed for each precursor species will be discussed in further detail.

4-Ring MDA $[M+H]^+$ Species (409 Da)

The MS/MS spectrum in Figure 3d highlights the 409 Da $[M+H]^+$ precursor forming three major fragment ions at low collision energy (15 eV): 316 Da ($[M-93]^+$), 315 Da ($[M-94]^+$), and 303 Da ($[M-106]^+$). In Figure 6a-b, the depletion of 409 Da species is monitored over a 0–50 eV span at 5 eV increments, a dashed line is drawn for visual alignment. The three major fragment ions are observed to originate directly from the depletion of 409 Da. Proposed structures of these fragment ions are provided in Scheme 2a. The CID curves show that the most abundant fragment ion, mass 316 Da (37%, 15 eV), corresponds to the loss of aniline through charge-remote fragmentation. Based on the CID curves and Supporting Information Table S20, mass 209 Da forms directly from the 316 Da species through M-107

loss of methylene-aniline and one hydrogen (15%, 35 eV). The second major fragment ion at mass 315 Da (15%, 30 eV) also forms from the 409 Da species due to the loss of aniline and one hydrogen. The third major fragment ion, mass 303 Da has the same structure as the 3-ring MDA $[M.]^+$ species discussed above. The charge is delocalized on the ring, and the 303 Da (14%, 35 eV) fragment ion forms from the loss of a methylene-aniline. Additional fragmentation of mass 303 Da leads to the formation of a minor fragment ion, mass 210 Da (M-93, 14%, 35 eV), which represents the loss of aniline through charge remote fragmentation. Higher collision energy fragment ions formed from the $[M+H]^+$ precursor can be found in Supporting Information Figure S19.

4-Ring MDA $[M.]^+$ Species (408 Da)

As observed in Figure 3e, the 408 Da species is more stable than the 409 Da precursor. The collision energy is increased to 20 eV for the $[M.]^+$ species and only a few fragment ions are observed compared to the $[M+H]^+$ spectrum. In Figure 6c-d, 408 Da is monitored across an increasing collision energy gradient, here the $[M.]^+$ species reaches 50% depletion at 25 eV. Scheme 2b illustrates the two major fragment ions derived from the 408 Da species: mass 315 Da ($[M-93]^+$) and 302 Da ($[M-106]^+$). Major fragment ion 315 Da initially forms due to loss of aniline (23%, 25 eV). This major fragment ion can further fragment into two minor fragment ions at higher collision energy: 314 Da ($[M-1]^+$) and 106 ($[M-209]^+$). Fragment 314 Da forms from the loss of one hydrogen at the bridge-carbon (16%, 45 eV), and fragment 106 Da (30%, 50eV) forms due to charge-remote fragmentation. MS³ data support 314 Da and 106 Da species originating from the 315 Da species, see Supporting Information Figure S6. The third major fragment ion formed from the 408 Da precursor is mass 302 Da (11%, 30 eV); this species is formed from the loss of a methylene-aniline and the structure resembles 3-ring MDA with the loss of a hydrogen from the bridged methylene. Further depletion of the mass 302 Da species increases the formation of mass 209 Da (30%, 50 eV) through loss of aniline (M-93). Additional supporting data for 408 Da fragment ions can be found in the Supporting Information (Figures S19 and S21).

4-Ring MDA $[M-H]^+$ Species (407 Da)

The 407 Da $[M-H]^+$ species is also more stable than the 409 Da $[M+H]^+$ ion. In Figure 3f the MS/MS spectrum at 20 eV shows few fragment ions and a strong 407 Da precursor peak at 62% relative intensity. Figure 6e-f illustrates the major and minor fragment ions formed above 5% as the collision energy was monitored from 0–50 eV. As the 407 Da precursor depletes, four major fragment ions are observed to derive directly from the $[M-H]^+$ species. Scheme 2c shows mass 314 Da ($[M-93]^+$), 211 Da ($[M-196]^+$), 209 Da ($[M-198]^+$), and 106 Da ($[M-301]^+$) all forming from the 407 Da $[M-H]^+$ precursor. Fragment ion 314 Da (16%, 25 eV) is formed from the loss of aniline and then further depletes into minor fragment ion 297 Da ($[M-17]^+$, 9%, 25 eV), formed from the loss of an ammonia. Additionally, ions 211 Da (6%, 30 eV) and 209 Da (24%, 35 eV) are both formed by fragmentation at the center CH₂, causing the loss of methylene-dianiline. The difference in these fragment ion masses correspond to alternative structural rearrangement and loss of two hydrogens. The 106 Da fragment forms at high collision energy as seen in our previous studies;² this ion forms in 37% abundance at 50 eV. Further exploration of high energy fragment ions formed due to 407 Da depletion can be found in Supporting Information (Figures S19). A complete

breakdown of fragment ion %RIC over 0–50 eV ramp can also be found in Supporting Information (Figure S22).

CONCLUSIONS

In this study, 3-ring and 4-ring MDA have been extensively characterized using MALDI-MS, MALDI-IM-MS, and multi-stage fragmentation (MS/MS, MS³) techniques. The combination of MS/MS and IM-MS is demonstrated to be a successful tool in the structural characterization of these unique precursor species and their respective fragment ions. In previous work, we concluded that both the 3-ring and 4-ring MDA only form the [M+H]⁺ precursor in ESI, whereas in this current MALDI study we observe three unique precursor species as seen in the 2-ring studies: [M+H]⁺, [M]⁺, and [M-H]⁺. Here we observe both the 3-ring and 4-ring MDA forming these three unique precursor species. Using energy-resolved ion fragmentation studies, the [M+H]⁺ species was found to be the least stable of the three precursor ions. The [M]⁺ and [M-H]⁺ species were both more stable, generating fewer fragment ions at a higher collision energy. The IM spectra exhibited one narrow structural population for the 3-ring precursor species, whereas the 4-ring MDA precursors each had two unique conformational families present. IM-MS separations can identify the presence of multiple protonation sites coexisting in the gas-phase. Future studies will include the characterization of the 4-ring MDA IM-MS spectra. These findings provide insight into the complex nature of PUR precursors and their unique fragmentation behavior.

Supplementary Material

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Figure 1.
MDA 3-ring and 4-ring $[M+H]^+$, $[M]^+$, and $[M-H]^+$ species. Asterisks (*) denote sites of possible protonation

Figure 2.
Comparison of ESI-MS and MALDI-MS of 3-ring (a-b) and 4-ring (c-d) MDA.

Figure 3. MALDI MS/MS spectra for (left) 3-ring MDA and (right) 4-ring MDA. Lab-frame energies are shown; corresponding energies are 15 eV for (a,d) and 20 eV for (b-c,e-f). Results for $[M+H]^+$ are shown in (a,d), for $[M]^+$ in (b,e), and $[M-H]^+$ in (c,f).

Figure 4. MALDI-IM-MS profiles for (a) 3-ring MDA and (b) 4-ring MDA. [M+H]⁺ results are shown in blue, [M]⁺ results are shown in black, and [M-H]⁺ results are in red.

Figure 5.

Collision-induced dissociation curves monitoring the transition of 3-ring MDA precursor to fragment ions for (a-b) $[M+H]^+$ ions, (c-d) $[M]^+$ ions, and (e-f) $[M-H]^+$ ions. Fragment ions formed above 20% relative ion current are represented in (a,c,e); fragment ions formed between 0–20% relative ion current are represented in (b,d,f) for each precursor ion. Dashed lines for visual alignment are added for each precursor species: $[M+H]^+$ dashed line represents 15 eV, precursor $[M]^+$ and $[M-H]^+$ dashed line represents collision energy at 20 eV.

Figure 6.

Collision-induced dissociation curves monitoring the transition of 4-ring MDA precursor to fragment ions for (a-b) $[M+H]^+$ ions, (c-d) $[M]^+$ ions, and (e-f) $[M-H]^+$ ions. Fragment ions formed above 20% relative ion current are represented in (a,c,e); fragment ions formed between 0–20% relative ion current are represented in (b,d,f) for each precursor ion. Dashed lines for visual alignment are added for each precursor species: $[M+H]^+$ dashed line represents 15 eV, precursor $[M]^+$ and $[M-H]^+$ dashed line represents collision energy at 20 eV.

Scheme 1.

MDA 3-ring (a) $[M+H]^+$, (b) $[M]^+$, and (c) $[M-H]^+$ fragmentation pathways of major low energy fragment ions. Fragment ions identified through MS³ spectra are denoted, MS³.

Scheme 2.

MDA 4-ring (a) $[M+H]^+$, (b) $[M]^+$, and (c) $[M-H]^+$ fragmentation pathways of major low energy fragment ions. Fragment ions identified through MS^3 spectra are denoted, MS^3 .