Fragmentation behavior of DOTA complexes under different

activation conditions

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Abstract

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) is a macrocycle that is able to complexate a large variety of metals. The stability of these metal complexes is generally very high. This has led to the use of these complexes in medical applications, e.g. magnetic resonance imaging. Here, we have investigated the fragmentation behavior of a number of DOTA-metal complexes under collision-induced dissociation (CID), infraredmultiphoton dissociation (IRMPD) and higher-energy collisional dissociation (HCD) activation conditions. Both, positive and negative ion mode electrospray ionization was applied. The covered metals range from sodium, aluminium, iron and indium to yttrium and several lanthanides. Furthermore, the protonated and deprotonated species of DOTA have also been investigated.

The results show that characteristic fragmentations were obtained for the metal-complexes under the investigated conditions. All elemental compositions of fragment ions have been unambiguously identified by high resolution-accurate mass measurements. Certain trends, for instance eliminations of carbon dioxide, alkyl and amine residues, were observed that coincide with the size of the metal and its location within the periodic table. Additionally, lanthanide, aluminium and indium species with even oxidation state or containing a radical

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have been detected. To further investigate the observed water capture during activation, deuterium labeling experiments have also been carried out.

Introduction

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, H₄L, Figure 1) is a chelating agent that complexates a wide variety of metal ions.¹ These range from earth alkali metals like calcium and strontium, via transition metals like cobalt, nickel, copper and zinc to lanthanides and even actinides.²⁻³ Me^{II} and Me^{III} ions form very stable complexes, as stability constants (*log*K) range from 11.2 (Zn^{II}-DOTA) to 30.3 (Bi^{III}-DOTA).^{1,4} For a variety of these complexes even crystallographic data is available.^{3, 5} This extraordinary high stability makes them an ideal tool in order to connect inorganic ions with organic molecules. Hence, these complexes and its derivatives are widely used in medical diagnosis and therapy. The applications range from radiopharmaceuticals in nuclear medicine, optical imaging probes, to magnetic resonance imaging (MRI) contrast agents. Regarding MRI experiments, Gd complexes are widely used as contrast agents.⁶⁻⁷ Moreover, the lanthanide-DOTA complexes (Ln-DOTA) are used in protein and peptide quantification.⁸ All these fields benefit from the high thermodynamic stability and kinetic inertness of these complexes. Based on the frequent use of Gd-DOTA complexes as unspecific MRI contrast agent and the associated increasing anthropogenic presence,⁹⁻¹⁴ analytics of DOTA complexes has mainly focused on environmental speciation, mostly by identification via electrospray (ESI) - mass spectrometry (MS) and quantification with inductively-coupled plasma (ICP) - MS. Based on the so far low natural background, very low limits of detection (LODs) have been achieved by employing the latter method.^{12, 15-24} However, a detailed fragmentation study of these complexes is so far lacking.

The mass spectrometric behavior of metal-ethylenediaminetetraacetic acid (EDTA) complexes has been investigated by Baron and Hering, but this study only focused on the detectability of a number of EDTA complexes.²⁵ A further study investigated Eu^{III}-, Am^{III}- and Pu^{III}-DOTA complexes in aqueous solution.²⁶ Here, the influence of the cone (skimmer) voltage and collision-induced dissociation (CID) collision energy on the intensity of the molecular ions was investigated in positive and negative ion electrospray. Furthermore, CID-MS/MS spectra were acquired and loss of CO₂ and C₂H₄, as well as, the addition of water molecules was reported from ion trap (IT) spectra.²⁶ Nevertheless, this study lacked high resolution data to support further conclusions.

Here, we have investigated the fragmentation behavior of various metal-DOTA complexes in positive and negative ionization by CID, higher-energy collisional dissociation (HCD), and infrared-multiphoton dissociation (IRMPD) in combination with high resolution-accurate mass detection. Additionally, the free (no metal containing) DOTA and the sodium complex of DOTA were also investigated.

Experimental section

Materials

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, $\geq 97\%$) and AlCl₃ (99%) were purchased from Sigma-Aldrich. FeCl₃ ($\geq 98.5\%$), GdCl₃ · 6 H₂O (99.99%), LaCl₃ · H₂O (99.99%), ammonium acetate ($\geq 97\%$), formic acid ($\geq 98\%$), deuterium oxide (99.8atom%D) and methanol-d₄ (99.8atom%D) were supplied by Carl Roth. PrCl₃ · 6 H₂O (99.9%), HoCl₃ · 6 H₂O (99.9%), InCl₃ (98%+), YCl₃ · x H₂O (99.9%) and LuCl₃ · 6 H₂O (99.9%) were purchased from Alfa Aesar and formic acid-d₂ (95wt% D₂O, 98atom% D) from Aldrich. Ultrapure water (18.2 MΩ⁻cm⁻¹) was obtained from a Millipore system (Milli-Q Plus system). Methanol (gradient grade) was purchased from J.T. Baker.

Sample preparation

DOTA-metal complexes were prepared by mixing 249 μ L of a 2.5 mM solution of DOTA in 0.2 M ammonium acetate buffer (pH 8.5) with 62 μ L of the metal chloride solution (50 mM in water) and incubation for 2 h at 37°C to yield a 2 mM solution of the corresponding DOTA-metal complex. For measurements of DOTA and the sodium-DOTA complex, DOTA was dissolved in 0.2 M ammonium acetate buffer (pH 8.5) to a concentration of 5 mM. Prior to measurements these solutions were diluted 1:100 (v/v) in 50% methanol, 0.1% formic acid and directly infused into the MS. Deuterated samples were prepared identical in fully deuterated solvents.

Mass spectrometry

CID and HCD were performed on an Orbitrap XL (Thermo Scientific) and IRMPD activation on a LTQ FT MS ULTRA (Thermo Scientific), both with ESI interface. Samples were introduced at a flow rate of 5 μ L^{min⁻¹}. The ESI voltage was set to 5 kV, the sheath gas flow (nitrogen) to 15 arb, and the temperature of the transfer capillary to 230°C. Ion transfer parameters were tuned automatically for maximum intensity of the [M+H]⁺ in positive and the [M-H]⁻ ions in negative mode. Helium was used as collision gas in CID and nitrogen in HCD, correspondingly. Additionally, HCD experiments with helium as collision gas were performed. Fragmentation spectra were acquired with a resolution of 60,000 in the Orbitrap and 100,000 in the FTICR. Precursor ions were isolated with a $\Delta m/z$ of 8 with the exception of Fe-DOTA (see Results). Normalized collision energies, IRMPD irradiation intensities and times (ms) are given in figures. Activation time in CID experiments was always set to 30 ms.

Results and Discussion

MS spectra

The investigated Me^{III}-DOTA (Me = Al, In, Y, La, Pr, Gd, Ho, Lu) complexes showed the expected [M+H]⁺ ([MH₂L]⁺) species in positive and [M-H]⁻ ([MeL]⁻) species in negative ionization as most abundant ions. Lanthanides harbor nine coordination sites, while DOTA only provides eight, complexes of the form $MeL(H_2O)^{-1}$ are the final solution form and have also been identified from crystal structures.^{1, 26} As ESI is a soft ionization technique, molecular ions of the form $[MeH_2L+H_2O]^+$ and $[MeL+H_2O]^-$ would have been expected. However, we did not observe any water adducts in positive or negative ionization as previously reported.²⁶ Even when lowering the temperature of the transfer (desolvation) capillary to 70°C and using decreased ESI spray voltages (down to 0.2 kV) no such adducts were detected in both ionization modes. Furthermore, also no methanol adducts were observed in any of the performed experiments. In case of iron, $[Fe^{III}H_2L]^+$ was also observed as most abundant signal in positive MS spectra. In negative ESI-MS, the Fe-DOTA complex was mainly detected as $[Me^{II}H_2L]^+$ with a reduced oxidation state. This behavior is readily understandable, as iron is easily reduced under electrospray conditions.²⁷ As a consequence the isolation width had to be limited to a $\Delta m/z$ of 1 for the Fe^{III}-DOTA complex to avoid the generation of mixed fragment spectra that originate from both the Fe^{II}- and the Fe^{III}complexes. To exclude major abundance changes in the resulting fragment spectra that stem from the reduced isolation width, we also performed MS/MS experiments with an isolation width of $\Delta m/z = 8$ and did not observe noticeable differences in intensities of Fe^{III}-DOTA derived fragments.

The non-metal containing DOTA was also easily detected in both ionization modes, yielding the $[H_5L]^+$ and the $[H_3L]^-$ ions as most abundant signals. Additionally, the sodiated species were also detected in both ionization modes from these samples with intensities of the same order of magnitude as the protonated/deprotonated species, although no sodium salts had been added.

Positive CID

In positive CID using helium as collision gas, the protonated DOTA macrocycle eliminated water, carbon dioxide, and C_3H_7N (Figure S1). Interestingly, the isolated loss of a single water molecule was observed in these spectra, while the elimination of CO₂ was mostly accompanied by losses of C_3H_7N or C_3H_7N and water. MS³ of the most abundant fragment in the MS/MS resulted in the most abundant fragment $[C_8H_{15}O_4N_2]^+$. Additional water and C_3H_9N fragmentations were also apparent.

The sodiated form $[H_4L+Na]^+$ exhibited the losses of water and CO₂, while here the C₂H₂O₂ loss was more abundant than elimination of CO₂ and C₃H₇N, as compared to the protonated form $[H_5L]^+$ (Figure S2). In general, cleavage of C₃H₇N (or other nitrogen containing neutral losses) comprised only a very minor extend of the fragmentations. Instead, only small neutral losses of H₂O and CO₂ represented the majority of the signals. Even in the performed MS⁵ spectrum no dramatic breakdown of the cyclen ring was observed. Instead, only eliminations of H₂O, NH₃ and formally $\frac{1}{2}$ O₂ occurred.

In the MS/MS spectra of Ln-DOTA (Ln = Y, La, Pr, Gd, Ho, Lu) complexes in positive CID only the losses of one to three CO₂ groups were observed as major ions (Figure S3). In contrast to the elimination of H₂O of the protonated and sodiated DOTA, Ln-DOTA complexes underwent addition of water, as previously reported.²⁶ This has been unambiguously proven in this study by high resolution accurate mass data. Whether these water additions are caused by remaining water in the ion trap ($p \approx 2 \cdot 10^{-5}$ mbar) or from neutral losses of other ions however remains unknown. Up to the MS³ experiments no major disintegration of the cyclen ring was observed and in the performed MS⁴ experiments the majority of abundant fragment species also harbored all four nitrogen atoms contained. Only in case of La, $[C_8H_{19}N_3La]^+$ was detected as base peak ion, while the Y, Pr, Gd, Ho and Lu complexes mostly formed $[C_{10}H_{24}N_4Ln]^+$ or $[C_{10}H_{22}N_4Ln]^+$ by elimination of ethylene or acetylene in the MS⁴ spectra.

For the investigated non-lanthanide Me^{III} -DOTA complexes (Me = Al, Fe, In) the fragmentation is less homogeneous. Here, the elimination of CO₂ in case of Fe and two CO₂ together with a CH₂ group for Al and with a methane group (CH₄) for In represented the most abundant fragment ions in the MS/MS spectra (Figure S4). Furthermore, eliminations of water, C₂H₄, and H₂CO₂ were observed in these experiments. In plays an exceptional role among the investigated metals, as the MS/MS spectra of the In-DOTA complex already showed a breakdown of the cyclen ring that was not observed for Al and Fe complexes.

Furthermore, the In-DOTA complex is the only investigated complex that showed release of the metal core in CID fragmentation spectra.

Interestingly, in the MS³ spectra of the Fe^{III}-DOTA complex also a species containing Fe^{II} was observed. $[C_{12}H_{23}O_2N_4Fe]^+$ is only possible with a Fe^{II} central atom, thus, the elimination of a methyl radical species ([•]CH₃) had taken place. Even more unexpected was the detection of a number of species were an Al^{II} or In^{II} is enclosed in the MS³, to form $[C_{11}H_{19}O_2N_4Me]^+$ (Me = Al, In), with the elemental composition being unambiguously identified. Here also eliminations of [•]CH₃ radicals had occurred. In the higher order MSⁿ spectra, further Me^{II} species were detected. For iron $[C_{11}H_{21}N_4Fe]^+$ and for aluminium $[C_{13}H_{19}O_3N_4Al]^+$, both in the corresponding MS⁴ spectra. Surprisingly, the addition of water molecules has only been observed for the Al-DOTA complex, as in the MS⁵ spectra the species $[C_7H_{15}O_3N_3Al]^+$ was detected. The addition of water being the only explanation for this elemental composition. The Fe- and In-DOTA complexes did not show any water addition in these fragmentation experiments although iron is known to be very oxophilic.²⁸

The relative amounts of the dominant CO_2 eliminations are not similar among all investigated species. By comparing the relative abundances of the four possible CO_2 eliminations, it is apparent that in case of the protonated, sodiated and iron-containing DOTA the single loss of CO_2 is predominant in comparison to multiple CO_2 losses (Figure S5).

In the fragmentation of Al-DOTA no CO_2 loss was apparent at all and for the different lanthanide, yttrium and indium complexes the threefold CO_2 eliminations were most abundant. The abundance of the threefold CO_2 elimination also increased, as did the abundance of the fourfold CO_2 loss, with increasing atom number within the lanthanides, with the exception of yttrium that behaved like a later rare earth element; while the abundances of the single and twofold CO_2 loss decreased.

Negative CID

The fragmentation behavior of the deprotonated DOTA $[H_3L]^-$ in negative CID was similar to the corresponding positive ion mode fragmentation (Figure S6). The eliminations of water, CO₂ and a combination of the two formed the majority of detected ions. Surprisingly, no cleavages involving the cyclen ring were observed in MS/MS. Also in the performed MS³ of $[C_{15}H_{27}O_6N_4]^-$ no such fragmentation was observed. Fragmentation of the cyclen ring was only apparent in the MS³ of $[C_{13}H_{27}O_2N_4]^-$ with slightly higher activation energy. Further MSⁿ experiments were not possible, albeit the intensity of the $[H_3L]^-$ ion was similar to the metalized species. The distribution of the initial intensity over a higher number of abundant fragment ions made it impossible to acquire further MSⁿ spectra (Figure 2).

- The sodiated DOTA $[NaH_2L]^-$ negative CID was again very similar to the positive counterpart, as the losses of water, CO₂, H₂C₂O₂, H₂CO₂, and a combination of these were apparent (Figure S7). In difference to the positive mode, more fragments having lost nitrogen from the molecule were observed in the MS³ and MS⁴.
- The Ln-DOTA complexes [LnL] showed cleavages of CO₂ in combination with the eliminations of C₅H₉N, C₂H₇N, C₂H₅N and C₂H₄ (Figure S8). The addition of water in combination with the loss of a single CO₂ occurred for all lanthanides, including yttrium. In positive mode CID, the addition of water was observed mostly for the threefold CO₂ lacking ions (Figures S3 & S5). Here, for negative ions, this water addition was only observed for ions that lacked one CO₂ moiety and ions that also missed larger parts of the cyclen ring. While $[C_{15}H_{24}O_6N_4Ln]^{-1}$ was the most abundant fragment ion in MS/MS spectra for all investigated lanthanide and vttrium complexes, in the MS³ this changed. While the La and Pr complexes yielded $[C_{10}H_{14}O_5N_2Ln]^-$ as most abundant ions, Gd-, Ho-, and Lu-DOTA resulted in $[C_{13}H_{19}O_6N_3Ln]^-$ as being the dominant signals (Figure S9). Again, Y-DOTA behaved similarly to the later rare earth elements, as it also formed $[C_{13}H_{19}O_6N_3Y]^{-1}$ as most abundant species in the MS³. In subsequent MSⁿ experiments the intensity of ions originating from the six investigated lanthanide and yttrium complexes further diverged. Generally, the later lanthanide complexes tended to form smaller complexes. Additionally, the addition of up to three water molecules was observed to form $[C_9H_{16}O_7N_2Ln]^{-1}$ in the MS⁵ of $[C_{11}H_{17}O_6N_3Y]^{-1}$ and [C₁₁H₁₇O₆N₃Ho]⁻.

For the investigated non-lanthanide metal complexes, up to a threefold water addition was observed for the Al-DOTA complex in MS/MS, to result in formation of $[C_{14}H_{27}O_9N_3A1]^-$ (Figure S10). For the Fe- and In-DOTA complexes no such signals were observed. All three, Al-, Fe- and In-DOTA complexes have shown $[C_{15}H_{24}O_6N_4Me]^-$, as most abundant signals in MS/MS experiments after elimination of CO₂. While $[C_{15}H_{24}O_6N_4Me]^-$ is an even electron system, iron also formed fragment ions with odd number of electrons. $[C_{14}H_{23}O_6N_4Fe]^-$ and $[C_{14}H_{21}O_6N_4Fe]^-$ were both observed, containing Fe^{II} within DOTA. Here, **°**CH and **°**CH₃ were lost from Fe-DOTA. No reaction products of these radicals to other ions were observed. Further Fe^{II}-containing signals were also observed, namely, $[C_9H_{15}O_6N_3Fe]^-$ and $[C_8H_{11}O_4N_2Fe]^-$ in MS/MS, $[C_{11}H_{18}O_4N_3Fe]^-$ and $[C_{10}H_{18}O_2N_3Fe]^- / [C_8H_{14}O_2N_3Fe]^-$ in MS⁴ and MS⁵, respectively. Surprisingly, also Al-DOTA showed $[C_5H_{13}O_6N_2A1]^-$, a fragment

containing Al^{II} and four acquired water molecules in the MS^5 . No further Al^{II} species have been observed in negative CID experiments. While no In^{II} species was observed in these experiments at all, the presence of an In^{I} species was detected as neutral loss in the MS^4 . Here the transition from $[C_{14}H_{24}O_4N_4In]^-$ to $[C_{14}H_{23}O_3N_4]^-$ clearly proved the elimination of $In^{I}OH$. The tendency to lose one or multiple CO_2 was not similar within the investigated precursor ions. The loss of three CO_2 was again most pronounced among the lanthanide- (including Y), Al- and Fe-DOTA complexes, while the deprotonated and sodiated forms showed a higher relative intensity for the single and two fold CO_2 eliminations (Figure S11).

Positive HCD

As HCD can only be performed as single stage fragmentation in the available instruments, preceding fragmentation steps were performed using CID in higher MSⁿ experiments. HCD is known to be very similar to CID, as both generate fragments by collision with neutral molecules.^{11, 29} Here, HCD was performed with nitrogen as collision gas.

The HCD of the protonated $[H_5L]^+$ and the sodiated DOTA $[NaH_4L]^+$ were very similar to positive CID. No major differences were observed for the protonated DOTA (Figure S12). In case of the sodiated DOTA, the only observed differences, among minor changes in abundances of fragment signals, was the appearance of $[C_{13}H_{24}O_6N_4Na]^+$ and the dramatically lower abundance of $[C_{14}H_{24}O_5N_4Na]^+$ (Figure S13). The lack of a number of detected fragments in the MS⁴ in HCD, was not caused by a different fragmentation behavior in comparison to CID, but due to a much lower general intensity of ions and so only the two most abundant signals were detected in HCD.

The positive ion HCD fragment spectra of the lanthanide- and yttrium-DOTA complexes showed the elimination of three CO₂ and the capture of water as most abundant signals (Figure S14). The strong abundance of $[C_{13}H_{28}O_3N_4Ln]^+$ (- 3 CO₂, + H₂O) ions within the HCD spectra again raised the question whether this water is acquired from 'moisture' within the instrument of from neutral losses of other ions. As the pressure within the HCD cells (\approx 1^{-10⁻²} mbar) is much higher than in CID (\approx 2^{-10⁻⁵} mbar), a water uptake of residual moisture is most likely. In general, smaller fragment ions were observed within HCD fragment spectra. This was caused by the use of 32 eV as collision energy that is higher in comparison to the 30 normalized collision energy (NCE) in the ion trap. As a result, the loss of four CO₂ and the addition of one or two water molecules were also observed in case of all investigated lanthanides and yttrium complexes. Also in the MS³ with HCD the water addition was very prominent (Figure S15). All major signals from all investigated lanthanides and yttrium complexes showed the capture of one or two water molecules, accompanied with the elimination of CO₂, H₂CO₂ and C₂H₄. This behavior was further expanded in the MS⁴ and MS⁵, as also here all major signals bore at least one added water molecule. In the MS⁴ and MS⁵, as also the previously described [LnO]⁺ signals were observed for La-, Pr- and Gd-DOTA complexes.³⁰ Furthermore, also ions of the type [(HO)₂Ln]⁺ were observed in both MS stages. Differences between the five investigated lanthanides and yttrium complexes were observed in the MS³ and higher MSⁿ experiments with HCD. In the MS³, La-, Pr-, and Gd-DOTA showed [C₁₀H₂₄ON₄Ln]⁺ as most abundant fragment in the ON₄-group, while Y-, Ho-, and Lu-DOTA mainly yielded [C₁₂H₂₈ON₄Ln]⁺ in this group. In the MS⁴ also the base peaks were unequal in within the lanthanides and yttrium complexes. While Gd-, Ho-, and Lu-DOTA formed [C₁₂H₃₀O₂N₄Ln]⁺ as base peak, La- and Pr-DOTA mainly fragmented to [C₈H₂₃O₂N₃Ln]⁺. Yttrium did behave differently to the remaining investigated lanthanide complexes, as in the corresponding MS⁴ of the Y-DOTA complex [C₄H₁₀O₂N₂Y]⁺ was formed as the most abundant signal (Figure S14).

The DOTA complexes of aluminium, iron and indium showed a very distinct behavior in HCD (Figure S16). Here also smaller fragments were observed in comparison to CID. Already at the MS/MS stage, a Fe^{II} species ($[C_{12}H_{23}O_2N_4Fe]^+$) was observed. Further Fe^{II} species were detected in subsequent MSⁿ experiments, while only one Al^{II}/In^{II}-species was detected in the performed HCD experiments. $[C_{11}H_{19}O_2N_4Me]^+$ (Me = Al, In) was formed in MS^3 and MS^4 experiments after elimination of three $\mathrm{CO}_2,\,\mathrm{C}_2\mathrm{H}_6$ and a hydrogen radical. In context of the fragmentation here, these losses are not remarkable, thus it remains unclear why aluminium and indium generated this species. Water capture was also observed in positive ion HCD fragmentation of Al-DOTA. As in positive ion CID experiments, very limited water uptake was detected in HCD, as only in the MS⁵ two species with one added water $([C_{11}H_{18}O_3N_4A1]^+$ and $[C_7H_{15}O_3N_3A1]^+)$ each were formed. In case of iron and indium no water uptake was detected in HCD experiments, identical to CID experiments. Nevertheless, very unusual fragmentation occurred during HCD experiments of nonlanthanide DOTA complexes, e.g. the transition from $[C_{11}H_{22}N_4In]^+$ to $[C_{13}H_{12}N_4]^+$. The release of the metal core does not appear to be unusual, as this has been observed before in CID experiments of the In-DOTA complex. However, the increase in number of carbon is only explainable by uptake of neutrals from corresponding losses of other ions within the HCD cell.

As the collision gas plays an important role in collision activation, we also investigated the effect of a different collision gas (helium) in HCD.³¹ In comparison to the standard collision gas nitrogen, helium requires higher energies but yielded the same fragment ions, while the intensity of individual fragments was only slightly changed (Figure S18).

In general, the loss of three CO_2 and the addition of water represented the dominant fragmentation of the yttrium and lanthanide complexes in positive ion HCD, while the iron and indium complexes mainly eliminated one CO_2 group. The sodium, aluminium, and protonated DOTA complexes did not show any of these reactions to a noticeable extend (Figure S17).

Negative HCD

The investigation of the fragmentation behavior of the deprotonated DOTA $[H_4L-H]^-$ in HCD was limited to MS³ by the very low abundance of molecular ions in these experiments. In the MS/MS, DOTA yielded, the from CID expected, fragments like $[C_{16}H_{25}O_7N_4]^-$ (- H₂O), $[C_{15}H_{25}O_5N_4]^-$ (- CO₂, - H₂O), $[C_{14}H_{25}O_3N_4]^-$ (- 2 CO₂, - H₂O), and $[C_{13}H_{27}O_2N_4]^-$ (- 3 H₂O), also accompanied by additional formal eliminations of CH₂ and H₂ (Figure S19). In general, similar fragments were obtained in CID and HCD during negative MS/MS fragmentation of DOTA, while the intensities of individual fragments ions were different. At the MS³ stage only one fragment ion ($[C_7H_{13}O_2N_2]^-$ after loss of two C₃H₇N from $[C_{13}H_{27}O_2N_4]^-$) was observed, due to the already mentioned low intensity of signals.

The sodiated DOTA $[NaH_3L-H]^-$ showed very similar reactions to the fragmentations of $[H_3L]^-$ under HCD conditions, as well as the sodiated DOTA $[NaH_3L-H]^-$ in CID (Figure S20).

The Ln-DOTA complexes [LnHL-H]⁻ likewise exhibited analog fragmentations in negative HCD as in negative CID (Figure S21). The loss of a single CO₂ marked the most abundant signal for all investigated lanthanide (including yttrium) complexes. Also the dominant elimination of CO₂ in combination with C_5H_9N was observed. Nevertheless, there appeared to be a wider variety in amine losses. While in CID MS/MS mostly the cleavage of C_5H_9N and C_2H_5N were detected, in HCD also the eliminations of C_4H_9N , C_3H_7N , and C_2H_4N occurred. This might again be attributable to the higher collision energy employed in HCD. In higher MSⁿ, smaller fragments in comparison to negative CID were observed. In general, the tendency for the capture of water appeared higher in HCD as in CID. In negative CID only about two thirds of the abundant fragment signals among the different MSⁿ stages originated from the acquisition of water inside the MS, in HCD almost all of the detected

abundant signals stemmed from water capture. Interestingly, no fragments similar to $[(HO)_2Ln]^+$ or $[OLn]^+$, observed in positive ion HCD, were detected in negative ion mode.

For the Al-, Fe- and In-DOTA complexes, the most abundant fragments in negative HCD MS/MS were $[C_{15}H_{24}O_6N_4Al]^{-}$ (- CO₂), $[C_{12}H_{20}O_4N_4Fe]^{-}$ (- 2 CO₂, - C₂H₄) and $[C_{15}H_{24}O_6N_4In]^{-}$ (- CO₂) (Figure S22). While the losses of one and two CO₂ were detected for all three metal complexes, the additionally observed loss of CH₂ for Al- and In-DOTA and C₂H₄ for Fe- and In-DOTA were not detected for iron and aluminium, respectively. For Al- and In-DOTA the addition of water was observed to form $[C_6H_{10}O_5N_2Me]^{-}$. Other fragments representing the eliminations of C₂H₉N, C₂H₇N, C₃H₇N, C₄H₇N, C₄H₉N, and C₅H₉N were also apparent. In the HCD MS³ a number of Fe^{II}-complexes were detected. These originated from $[C_{15}H_{24}O_6N_4Fe]^{-}$ by loss of hydrogen, C₂H₃[•], C₃H₆N[•], and C₅H₈N[•] radicals. This formation of Fe^{II} ions further increased in the MS⁴, as here more than half of the detected abundant ions were represented by Fe^{II} species. Why Fe^{II} appears to be more stable under these isolated MS conditions remains unknown, however, no abundant Al^{II} or In^{II} species were detected in these HCD experiments. Furthermore, the uptake of water by the Al- and In-DOTA appeared to be limited to one water molecule in HCD, while in negative CID the capture of up to four water molecules was detected.

Again, the effect of helium as collision gas in HCD was investigated for a number of DOTA complexes, while no major differences were observed (Figure S18).

In summary, for negative ions, HCD mostly resembled the CID. Furthermore, we saw only a small dependency on the metal for the intensity of the CO_2 losses in HCD, as for all ions (apart from the deprotonated and deprotonated sodiated DOTA) the single CO_2 elimination was always more abundant than the loss of two CO_2 (Figure S23). While for the bare DOTA and the sodiated DOTA the elimination of two CO_2 was more abundant; all other investigated metal ion complexes showed a higher tendency to lose only one CO_2 . Aluminium possesses a higher oxophilicity in comparison to iron and so the loss of only one CO_2 was far more abundant than the loss of two CO_2 molecules.³²

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Positive IRMPD

As IRMPD is only a single stage fragmentation technique in the employed instrument, thus, higher order MS^n experiments had to be performed similar to HCD with the aid of CID, were only the last step in fragmentation was IRMPD activated. In positive IRMPD activation the protonated DOTA $[H_5L]^+$ showed combinations of the losses of CO₂, water, C₃H₇N, and C₃H₉N (Figure S24). Further MSⁿ experiments were not successful due to the low intensity of signals.

The sodiated DOTA $[NaH_4L]^+$ exclusively showed elimination of the CO₂ and adjacent CH₂ groups in IRMPD activation (Figure S25). Nevertheless, the dominance of the CO₂ loss seemed reasonable as the employed IRMPD laser was a CO₂-laser with an output wavelength of 10.6 µm that directly stimulates the carbonic acid groups within DOTA. However, this behavior was not observed in the protonated DOTA, which in this case should have also showed a limitation to CO₂ eliminations.

In IRMPD spectra of the Ln- and Y-DOTA complexes the loss of three and four CO_2 groups to form $[C_{13}H_{26}O_2N_4Ln]^+$ and $[C_{12}H_{26}N_4Ln]^+$ marked by far the most abundant signals. All other fragment signals were typically an order of magnitude less abundant. This tendency was most dominant at the later investigated lanthanides (Ho, Lu), as here the losses of one and two CO_2 groups were at least two order of magnitudes less abundant (Figure S26).

Another correlation between fragment intensity and atomic number was also observed for the loss of H_2CO_2 resulting in $[C_{15}H_{24}O_6N_4Ln]^+$ and $[C_{14}H_{24}O_4N_4Ln]^+$. Compared to the intensities of the single and twofold CO_2 lacking fragments $[C_{15}H_{26}O_6N_4Ln]^+$ and $[C_{14}H_{26}O_4N_4Ln]^+$, their relative intensity changed. While the first two were not at all observed for La-, and Pr-DOTA the intensity of the two latter increased throughout the lanthanide series and in case of Lu-DOTA $[C_{14}H_{24}O_4N_4Lu]^+$ became more intense than $[C_{14}H_{26}O_4N_4Lu]^+$ (Figure S27). Capture of water was not observed in IRMPD MS/MS of the lanthanide or yttrium complexes. In the IRMPD MS³ of lanthanide and yttrium complexes the capture of a single water molecule to form $[C_{12}H_{28}ON_4Ln]^+$, $[C_8H_{21}ON_3Ln]^+$ and $[C_7H_{17}ON_3Ln]^+$ was observed. The most abundant signal in the IRMPD MS³ of $[C_{13}H_{26}O_2N_4Ln]^+$ (m/z 435.1360 for Ho-DOTA) in case of the lanthanide and yttrium complexes was the loss of the last remaining CO₂ to yield $[C_{12}H_{26}N_4Ln]^+$ (m/z 391.1452 for Ho-DOTA), while the second most abundant signal in all cases was the additional uptake of water $[C_{12}H_{28}ON_4Ln]^+$ (m/z 409.1561 for Ho-DOTA, Figure 3). All other signals were significantly lower in abundance. In the IRMPD MS³, [C₈H₂₁ON₃Ln]⁺, [C₇H₁₇ON₃Ln]⁺, and $[C_7H_{13}N_3Ln]^+$ were detected in all cases. Within the higher order MSⁿ IRMPD spectra, the

smallest observed fragment in MS^5 was $[C_4H_8N_2Ln]^+$, which has also been the most abundant fragment for La-, Pr-, and Gd-DOTA in the N₂-group.

The Fe- and In-DOTA complexes also showed eliminations of CO₂ groups in positive IRMPD MS/MS (Figure S28). The intensities of the CO₂ losses were in the order fourfold>single>twofold≈threefold for Fe-DOTA and fourfold>>single≈ twofold≈ threefold for In-DOTA (Figure S29). Why the threefold CO₂ loss was comparatively low remains unclear, as it marked the most abundant signal of the CO₂ losses for Y- and the Ln-DOTA complexes. Furthermore, the Al-DOTA complex did not show any single or multiple CO₂ eliminations at all in the IRMPD spectra. For Al-DOTA the loss of one or more carboxylic acid functions only occurred as H₂CO₂ or was always bound to a water or alkyl loss or even a combination of these. Only in the MS⁵ the single loss of CO₂ to $[C_{12}H_{20}N_4A1]^+$ was observed. Again, iron and aluminium formed Me^{II}-species, while indium did not show any In^{II} species.

The addition of water was only observed for one iron species in the MS^3 to result in $[C_{13}H_{25}O_7N_3Fe]^+$. While under CID and HCD conditions aluminium and indium captured water; under IRMPD conditions this was not observed. This points towards an uptake of 'moisture' from inside the instruments for the water additions, as in IRMPD the lowest pressure (< 4.10^{-10} mbar) is used. In case of iron the situation is not so clear, as here also at least one water-added species was detected in IRMPD experiments under low pressure conditions.

In summary, under positive IRMPD conditions the CO_2 moieties were most prone to fragmentation, while there was a strong dependence on the metal core. In the simplest system (added proton) and Al-DOTA, no CO_2 eliminations were observed. With sodium, a single CO_2 loss marked the most dominant fragmentation and for the Fe, Y and the lanthanide complexes ions multiple CO_2 eliminations were predominant (Figure S29).

Negative IRMPD

In negative IRMPD activation of the deprotonated DOTA $[H_3L]^-$, a dramatically reduced laser energy (30% in comparison to 60% or 100% in other cases) and irradiation time (30 msec in comparison to 80 msec and more in other cases) were already sufficient to reduce the precursor ion to about 10% of its original intensity. While the eliminations of water and CO₂ to form $[C_{16}H_{20}O_4N_3]^-$ and $[C_{15}H_{27}O_6N_4]^-$ only comprised minor signals, the elimination of three CO₂ formed the base peak. In contrast to positive ion CID, were this was not observed at all, in negative IRMPD DOTA lost CO₂ (Figure S30). Additional water, alkyl and amino eliminations were also observed. In MS³ only two signals could be identified due to the low abundance of ions. These comprised a CO_2 and water loss as well as losses of CO_2 and H_2CO_2 .

The negatively charged sodiated DOTA $[NaH_2L]^-$ in IRMPD yielded water and one and two CO_2 molecules deprived fragment ions plus a number of alkyl eliminations (Figure S31). The losses of three and four CO_2 were not detected. In comparison to the deprotonated DOTA a higher laser energy and irradiation time (here 60% and 60 msec) were necessary for fragmentation. Interestingly, at least one CO_2 group was retained in the molecule in all fragments of the sodiated and the deprotonated DOTA. This appeared to be essential to stabilize the negative charge of the ions.

For the Y- and Ln-DOTA complexes in negative IRMPD an overwhelmingly large number of different fragments were detected (Figure 4). In the range of m/z 300 - 450 for example in case of Ho-DOTA almost every mass was occupied with a fragment. As base peaks in these spectra Y- and Ln-DOTA showed the elimination of two CO₂ groups in combination with two C₃H₇N units to form $[C_8H_{10}O_4N_2Ln]^-$ (Figure S32). Within the number of CO₂ losses in negative IRMPD, the single CO₂ loss was by far the most abundant for the Y- and Ln-DOTA complexes (Figure S33). The twofold loss of CO_2 was also observed but only to a very minor extend and the three and fourfold losses of CO₂ were not detected. Furthermore, the twofold elimination of CO₂ was often accompanied with not only the loss of two C₃H₇N, but also $C_4H_{12}N_2$, $C_7H_{14}N_2$, and $C_8H_{16}N_2$ that marked a major breakdown of the precursor ion complexes. Additionally, ions (e.g. [C₆H₇O₄NLn]⁻ or [C₇H₉O₄NLn]⁻) were observed that proved extensive reactions. Earlier lanthanides (La, Pr) and yttrium complexes exhibited a strong loss of two C_3H_7N ($C_6H_{14}N_2$). While these additional amine losses remained most abundant throughout the lanthanides, their intensity decreased with increasing atomic number (Figure S34). The combinational loss of C_3H_7N and CH_5N ($C_4H_{12}N_2$) showed the same trend. While the relative intensities of the loss of C₇H₁₄N₂ and C₈H₁₆N₂ increased, the loss of $C_5H_{14}N_2$ seemed rather unaffected. In the MS³ different base peaks for the different lanthanide complexes were observed. Y-, Gd-, Ho- and Lu-DOTA resulted in [C₈H₁₀O₄N₂Ln]⁻ as the most abundant signal, while La- and Pr-DOTA mostly formed $[C_8H_8O_3N_2Ln]^{-}$, the difference being an additional water elimination. Surprisingly, in negative MS^3 IRMPD the lanthanides lost water to form $[C_8H_8O_3N_2Ln]^-$ rather than capture it up as in previous experiments (see above). However, when bearing in mind that only elemental compositions were determined and not reactions, the identical sum formula could also be reached by an addition of water ($[C_{15}H_{24}O_6N_4Ln]^2 - 2 CO_2 - C_5H_{16}N_2 + H_2O$), rather than the loss of water $([C_{15}H_{24}O_6N_4Ln]^2 - CO_2 - C_6H_{14}N_2 - H_2O)$. In the context of the so far

observed fragmentation, both paths would be reasonable. Whether $[C_8H_8O_3N_2Ln]^-$ originated solely by one of the two possibilities or even a combination of both remains unsolved. In the IRMPD MS⁴ however, the capture of water molecules was undoubtedly observed again. Here, $[C_6H_7O_4NLn]^-$, $[C_4H_5O_3Ln]^-$ and $[C_3H_3O_3NLn]^-$ were detected. In case of the other lanthanides complexes the intensity was unfortunately not high enough to observe any fragment ions in the corresponding MS⁴ experiments. In the MS⁴ also for the first time, a number of Ln^{II} species were observed. This could already be deducted from the nitrogen rule, where Ln^{III} (and theoretical Ln^{I} species) comprise sum formulae with nitrogen-hydrogen combinations of even-even or uneven-uneven. For $[C_{11}H_8ON_3Ln]^-$, $[C_{11}H_6ON_3Ln]^-$, $[C_{10}H_8ON_3Ln]^-$, $[C_{10}H_6ON_3Ln]^-$ and $[C_9H_6ON_3Ln]^-$ this combination is uneven-even. Thus, a radical species or a species with even oxidation state had to be contained within the ions.

For Al-, Fe- and In-DOTA only IRMPD experiments up to MS^3 were possible. The Al-DOTA complex did not show any simple CO₂ loss as in positive IRMPD (Figures S33 & S35). The loss of CO₂ was always bound to breakdown of the cyclen ring. However, this was not accompanied with formation of Al^{II} -species. In case of iron-DOTA the majority of fragment ions in negative IRMPD contained Fe^{II} rather than Fe^{III}. For In-DOTA, again the loss of the metal core was observed for this element. In non-lanthanide metal cases no water addition was observed.

Deuterium labeling

In order to investigate the origin of water that is captured by fragment ions, we generated a number of deuterated Me^{III}-DOTA species. However, for Al-, La-, Ho-, Lu-DOTA, no signals of deuterated species were observed in either positive or negative ionization, although the complexation reaction were carried out in completely deuterated solvents. For negative ionization this is not unusual, as here no exchangeable protons are present after ionization. Why several attempts to detect deuterated lanthanide-DOTA complexes in the positive ion mode failed remains unknown. In case of Fe-DOTA however, about a third of the complex could be detected as monodeutero Fe^{III}-DOTA. We could also exclude that the signal observed at m/z 459.1158 originated from Fe^{II}-DOTA ([C₁₆H₂₇O₈N₄Fe]⁺) or ¹³C₁-Fe^{III}-DOTA, as the exact masses clearly proved that [C₁₆H₂₅DO₈N₄Fe]⁺ was formed (Figure S36). A dideutero Fe^{III}-DOTA was not observed. Unfortunately, Fe^{III}-DOTA only showed one water addition in positive IRMPD. In the resulting MS³ spectrum (m/z 459 \rightarrow (CID@30) m/z 415 \rightarrow (IRMPD@60/80) \rightarrow) both, [C₁₃H₂₅O₇N₃Fe]⁺ (m/z 391.1036) and [C₁₃H₂₄DO₇N₃Fe]⁺

(m/z 392.1099) were observed. However, based on the relative abundance of the nondeuterated and the deuterated Fe^{III} -DOTA in MS scans, a clear change was obvious. In the MS³, the relative abundance of the deuterated species was dramatically lower than the corresponding non-deuterated species (Figure S36). While extensive rearrangement reactions, were the extra water contained non-exchangeable protons from the cyclen ring cannot be fully excluded, this clearly suggests that Fe-DOTA acquired additional water from inside the MS rather than from neutral losses of other ions. This is in line with the general observation that the water uptake shows a tendency that follows the pressures (HCD > CID > IRMPD).

Conclusion

Using CID; HCD and IRMPD, we studied the fragmentation behavior of different DOTA complexes. The loss of the carboxylic acid functions as carbon dioxide molecules represent the dominant and omnipresent fragmentation for DOTA and the investigated metal complexes under all activation conditions for positive and negative ions. Depending on the activation condition, a tendency for the elimination or addition of water molecules was observed. The yttrium- and lanthanide-DOTA complexes favored water uptake, as under all collision activation conditions and in both polarities the capture of water molecules was clearly detected. Under non-collision activation (IRMPD), however, the addition of water was less dominant. Furthermore, unusual ions were observed. Al^{III}-, Fe^{III} and In^{III} transformed to Me^{II}-DOTA species. For iron this behavior is not unusual, as the redox potential for the Fe^{III} to Fe^{II} reduction is low. However, for aluminium and indium this was not expected and has so far only been observed in nanocondensates.³³

Additionally, lanthanide species with the lanthanide in an even oxidation state or alternatively containing a radical have been detected. Yttrium is often regarded as a pseudo-lanthanide.³⁴ In the performed fragmentation experiments it clearly behaved like a lanthanide. Moreover, it can be pointed out that in the majority of the experiments yttrium performed similar to the later lanthanides (Ho, Lu) with respect to abundances of formed fragment ions. Table 1 displays the general tendencies in the various fragmentation activations of the different complexes.

In summary, the investigated DOTA complexes at first glance show comparable behavior when activated under different conditions. However, at a close examination the differences are quite vast. References

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Table 1 General tendencies in the various fragmentation activations of the different complexes.

Activation	DOTA	<u>Na-DOTA</u>	<u>Al-DOTA</u>	<u>Fe-DOTA</u>	In-DOTA	Ln-DOTA
technique						
	Loss of water,	Loss of water, CO ₂ ,	Loss of water, alkyl &	Loss of water, CO ₂ /H ₂ CO ₂ ,	Loss of water, multiple	Loss of multiple CO ₂ ,
+ CID	CO_2, C_3H_7N	C ₃ H ₇ N	amine residues	alkyl residues	CO ₂ /H ₂ CO ₂ , alkyl residues,	alkyl & amine residues;
					amines, indium core	water addition
	Loss of water,	Loss of water,	Loss of CO ₂ , alkyl &	Loss of CO ₂ , alkyl & amine	Loss of CO ₂ , alkyl &	Loss of CO ₂ , alkyl
- CID	multiple	CO ₂ /H ₂ CO ₂ , C ₃ H ₇ N	amine residues; water	residues; reduction to Fe^{II}	amine residues, indium	residues; water addition
	CO ₂ /H ₂ CO ₂		addition	species	core; water addition	
	Loss of water,	Loss of water, C ₃ H ₇ N,	Loss of water, alkyl	Loss of water, CO ₂ , alkyl	Loss of water, (multiple)	Loss of multiple CO ₂ ,
+ HCD	C ₃ H ₇ N	alkyl residues	residues	residues	CO ₂ , alkyl residues,	alkyl residues; water
					indium core	addition
	Loss of water,	Loss of water,	Loss of CO ₂ , amine	Loss of water, CO ₂ , amine	Loss of CO ₂ , alkyl &	Loss of CO ₂ , amine
- HCD	multiple CO ₂ ,	(multiple) $CO_2/C_2H_2O_2$,	residues	residues; reduction to Fe^{II}	amine residues, indium	residues; water addition
	C ₃ H ₇ N	amine residues		species	core; water addition	
	Loss of water,	Loss of water,	Loss of water, alkyl	Loss of water, multiple CO ₂ ,	Loss of water, multiple	Loss of multiple CO ₂ ,
+ IRMPD	C_3H_7N	$CO_2/H_2CO_2/C_2H_2O_2$	residues	alkyl & amine residues	CO ₂ , amine residues,	alkyl residues; water
					indium core	addition
	Loss of water,	Loss of water,	Loss of water, alkyl &	Loss of water, multiple CO ₂ ,	Loss of water, (multiple)	Loss of CO ₂ , amine
- IRMPD	multiple CO ₂	(multiple) CO ₂ , alkyl	amine residues	alkyl & amine residues;	CO ₂ , alkyl & amine	residues
		residues		reduction to Fe ^{II} species	residues, indium core	
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					e is protected by copyrigi	it. All fights reserved.



Figure 1 Structure of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).

Accepted



Figure 2. MS/MS spectra of A – deprotonated DOTA and B – deprotonated Ho-DOTA in negative CID activation.



Figure 3 FTICR MS³ fragment spectrum of the Ho-DOTA complex in positive IRMPD fragmentation (m/z 567 (CID@30) \rightarrow m/z 435 (IRMPD@60/80) \rightarrow). Magnification of individual ranges (10-fold, 100-fold) are given on top of the spectrum.

Accepted



Figure 4 FTICR MS/MS fragment spectrum of the Ho-DOTA complex in A – positive and B - negative IRMPD.

Acce