

# Fragmentation of $B_{12}H_{11}S-R(2-)$ in electrospray mass spectrometry

Irina Slepukhina <sup>a,\*</sup>, Thomas Duelcks <sup>a</sup>, Hans-Martin Schiebel <sup>b</sup>, Detlef Gabel <sup>a</sup>

<sup>a</sup> Department of Chemistry, University of Bremen, P.O. Box 330440, D-28334 Bremen, Germany

<sup>b</sup> Institute of Organic Chemistry, TU Braunschweig, Germany

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## Abstract

The electrospray ionization (ESI) mass spectrometry (MS) of (3-carboxyethyl)-thio-undecahydro-*closo*-dodecaborate(2-) shows the appearance of additional peaks corresponding to  $B_{12}H_{11}SH(2-)$  and  $B_{12}H_{11}(1-)$ . Their origin can be traced to fragmentation reactions in the skimmer-CID region and the ion trap of the spectrometer.

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## 1. Introduction

The synthesis of boron-containing compounds that show an affinity for a wide variety of tumors has been attempted by many investigators [1,2]. For this purpose, the boron-carbon bond must be sufficiently stable in the final target molecule. Here, we report the new synthesis of a polyhedral borane carboxylic acid (Scheme 1) which can form stable bonds with amino groups in the target molecules. In the course of the characterization of this compound, we found in electrospray ionization mass spectrometry (ESI-MS) signals which did not correspond to the signals expected from the presumed product, but which could be interpreted as signs of an incomplete reaction, or a degradation of the compound upon storage. We investigated this phenomenon and found that ESI-MS induces fragmentation of the compound. The fragmentation pathways and the place in the ESI-MS could be clarified.

## 2. Experimental

### 2.1. General

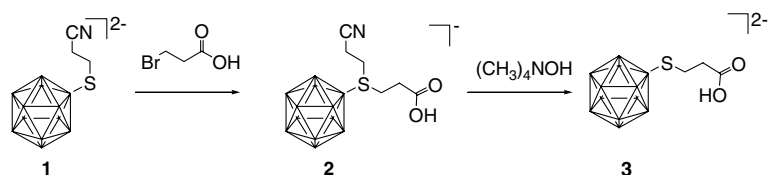
The NMR measurements ( $^{11}B$ ,  $^1H$ ) were carried out on a Bruker DPX 200 spectrometer. IR ( $cm^{-1}$ ) spectra were determined as KBr disc on a Biorad FTS-7 spectrometer. HPLC was carried out on a RP-18 LiChroCart  $125 \times 4$  mm column [3] with 57% MeOH/43%  $H_2O$  10 mM tetrabutylammonium hydrogen sulfate pH 7.0 as mobile phase. Detection was at 220 nm. Cyanoethyl- $B_{12}H_{11}S$  was prepared as described in the literature [4].

ESI-MS measurements were performed on a Bruker Esquire-LC ion trap mass spectrometer. Samples were dissolved in acetonitrile at concentrations of about  $10^{-5}$ – $10^{-6}$  mol/L and injected into the mass spectrometer via a syringe pump at a flow rate of 2  $\mu$ L/min. Spectra were recorded in the positive and negative ion mode for 1 min and averaged to correct for signal fluctuations.

The  $m/z$  values given correspond to the maxima of the isotopic distributions. In the analytical description of the compounds, peaks containing boron are indicated by “\*”. As the boron-containing compounds a broad isotopic distribution and therefore allow the assignment of the charge of the compound,  $z$  is given in parentheses after each  $m/z$  value.

\* Corresponding author. Tel.: +49 421 2182381; fax: +49 421 2183720.

E-mail address: irina.slepukhina@chemie.uni-bremen.de (I. Slepukhina).



Scheme 1.

## 2.2. Tetramethylammonium-(2-cyanoethyl)-(3-carboxyethyl)-sulfonium-undecahydro-closo-dodecaborate(-) 2

Bis-(tetramethylammonium)-(2-cyanoethyl)-thio-undecahydro-closo-dodecaborate (0.4 g, 1.07 mmol) was suspended in 15 ml of acetonitrile at room temperature. A solution of 0.25 g (1.61 mmol) 3-bromopropionic acid in 3 ml of acetonitrile was added slowly. The reaction mixture was refluxed during 3 h and stirring at room temperature continued overnight. Acetonitrile was evaporated and the resulting green oil was crystallized from diethylether. Subsequent recrystallization from water:acetone 1:1 gave 0.265 g (66%) of **2**. m.p. >250 °C. HPLC 3.62 min;  $^1\text{H}$  NMR (DMSO- $d_6$ ): 12.65 (s, -COOH), 3.13 (t, -CH<sub>2</sub>COOH), 3.1–3.03 (m, -CH<sub>2</sub>-CH<sub>2</sub>COOH, -N(CH<sub>3</sub>)<sub>4</sub>), 2.8–2.71 (m, -CH<sub>2</sub>CH<sub>2</sub>CN), 2.3–0.2 (broad, B<sub>12</sub>H<sub>11</sub>). IR (KBr): 3424, 2961, 2928, 2502, 1714, 1484, 1414, 1269, 1044, 949, 822, 712 cm<sup>-1</sup>. MS: (pos. ESI) 74.0 (cat<sup>+</sup>), 448.5\* (A<sup>-</sup> + 2cat<sup>+</sup>); (neg. ESI) 141.2\* (see text), 300.2\* (A<sup>-</sup>).

## 2.3. Bis-(tetramethylammonium)-(3-carboxyethyl)-thio-undecahydro-closo-dodecaborate(2-) 3

A 0.265 g (0.7 mmol) sample of **2** was dissolved in a minimal amount of hot acetone. Tetramethylammonium hydroxide as a 25% solution in methanol was added in equimolar amounts. Compound **3** precipitated,

was filtered off, and recrystallized from hot water:methanol 1:1. Yield: 0.20 g (71%). m.p. >250 °C. HPLC 4.68 min;  $^1\text{H}$  NMR (DMSO- $d_6$ ): 3.1 (s, -N(CH<sub>3</sub>)<sub>4</sub>), 2.36 (t, -CH<sub>2</sub>COOH), 2.07 (t, -CH<sub>2</sub>CH<sub>2</sub>COOH), 1.8–(-0.3) (broad, B<sub>12</sub>H<sub>11</sub>).  $^{11}\text{B}$  NMR (DMSO- $d_6$ ): -6.21 (B1), -14.22 (B2–6), -15.72 (B7–11), -18.25 (B12). IR (KBr): 3432, 3027, 2484, 1729, 1486, 1211, 1049, 950, 843, 721 cm<sup>-1</sup>. MS: (pos. ESI) 74.0 (cat<sup>+</sup>); (neg. ESI) 87.1\* (see text), 123.1\* (A<sup>-</sup>), 141.2\* (see text).

## 2.4. Electrospray MS

To induce fragmentation of the sample ions, collision-induced dissociation (CID) in the nozzle-skimmer region (“skimmer-CID” or sCID) as well as tandem-MS was used (see Fig. 1).

In sCID, fragmentation is induced by colliding the sample ions with the background gas in the intermediate-pressure region (the so-called “nozzle-skimmer region”, gas pressure about 10<sup>-1</sup> mbar) of the ESI interface. Increasing the voltage difference between the nozzle and the skimmer ( $V_{\text{sCID}}$ ) raises the kinetic energy of the ions passing through this region, hereby increasing the collisional energy of the ions with the background gas (consisting mainly of nitrogen and of some residual solvent vapor) and eventually causing fragmentation. The  $V_{\text{sCID}}$  applied in this investigation were between 75 and 100 V and are indicated in the mass spectra shown in Section 3 (see Fig. 2).

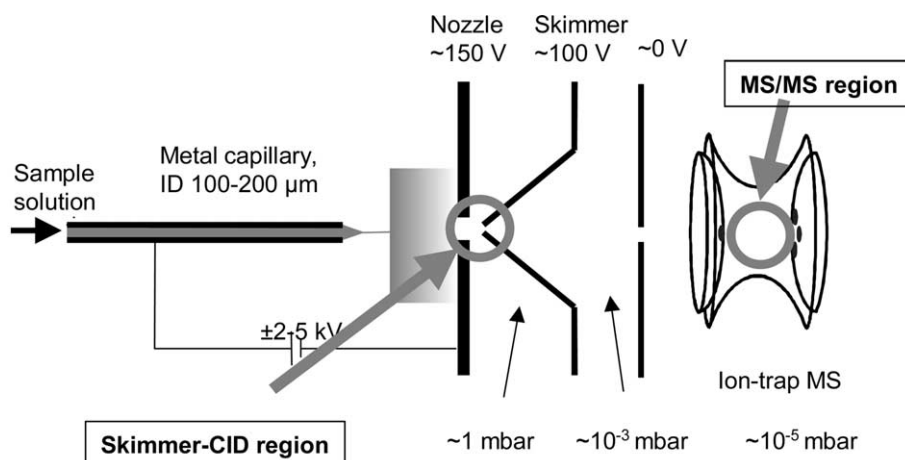


Fig. 1. Schematic diagram of an ESI mass spectrometer. Fragmentation can occur in the skimmer-CID region, or in the MS/MS region.

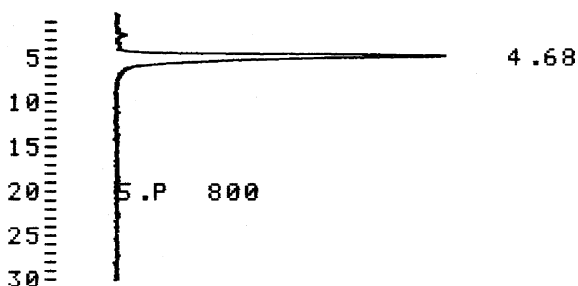


Fig. 2. HPLC chromatogram of **3**. For eluent and column used, see text. Detection was at 220 nm.

In contrast, tandem-MS (or MS/MS) takes place in the mass analyzer, i.e., in the high-vacuum region of the MS (pressure about  $10^{-5}$  mbar). In MS/MS, the ion of interest is isolated within the ion trap by ejecting all other ions out of the trap. Then this ion is accelerated (by applying a suitable high-frequency AC voltage) and collides with a collision gas (usually He) present at a pressure of  $5 \times 10^{-6}$  mbar. The fragment ions thus generated are then detected by a normal mass scan. The degree of fragmentation depends on the AC voltage amplitude; in this investigation, this amplitude was adjusted empirically so that a low intensity signal of the parent ion remained visible in the fragment ion spectrum. Alternatively, the residence time of the ion was increased.

### 3. Results and discussion

The synthesis of **3** proceeded as expected for similar compounds prepared in the past [4]. HPLC separation on RP-18 [3] showed one peak at 4.68 min.

Generally, ESI-MS is considered to be the softest of all known MS ionization methods [1]; consequently, fragmentation of sample ions is usually not observed unless it is deliberately induced by either sCID or MS/MS.

Hence, the negative-ion ESI mass spectrum of **3** was expected to show only the signal of the doubly charged molecular anion at  $m/z$  123 (2<sup>-</sup>). However, the spectrum (Fig. 3) shows two additional peaks: a doubly charged ion at  $m/z$  87 (2<sup>-</sup>), which was attributed to  $[\text{B}_{12}\text{H}_{11}\text{SH}]^{2-}$ , and a singly charged ion at  $m/z$  141 (1<sup>-</sup>) whose mass and isotopic pattern suggest the molecular formula  $[\text{B}_{12}\text{H}_{11}]^{-}$ .

Thus, the mass spectrum seemed to indicate a mixture of compounds rather than a pure compound, which, however, was not in accordance with results from HPLC, IR, and NMR. In order to investigate whether the additional ion signals originated from the MS measurement, a series of sCID experiments were carried out. The results are shown in Fig. 4.

It is seen that with increasing  $V_{\text{sCID}}$  the relative intensity of the ion signal at  $m/z$  123 (2<sup>-</sup>) decreases while the other two ion signals increase (since all spectra are normalized to the highest intensity peak, which is  $m/z$  87 (2<sup>-</sup>) in all cases, the relative intensity increase of this ion is not directly visible). Since generally upon increasing the fragmentation energy the intensity of a parent ion is expected to decrease while that of the fragments should increase, this finding clearly shows that the ion signals at  $m/z$  87 (2<sup>-</sup>) and 141 (1<sup>-</sup>) must be fragments of the molecular ion at  $m/z$  123 (2<sup>-</sup>).

We have observed the signal at  $m/z$  141 (1<sup>-</sup>) rather frequently in ESI mass spectra of substituted  $\text{B}_{12}$ -clusters; its origin has not yet been investigated. In the case of the molecule considered here, the mass spectrum shows two fragment peaks, which raises the question of the fragmentation pathway. With sCID this cannot be clarified because all ions present in the nozzle-skimmer region at a given time might be fragmented. Hence it cannot be distinguished whether the primary fragmentation product of the molecular ion is  $m/z$  87 (2<sup>-</sup>) or whether  $m/z$  141 (1<sup>-</sup>) arises independently. We therefore performed an MS/MS experiment to obtain further information on this issue. In contrast

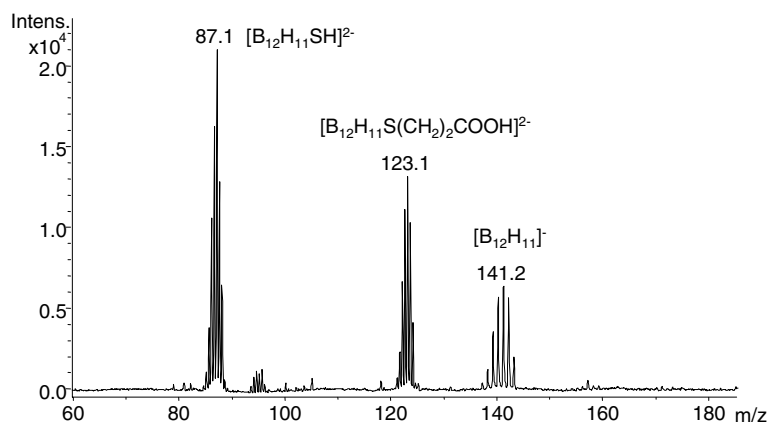


Fig. 3. ESI-MS of **3**.

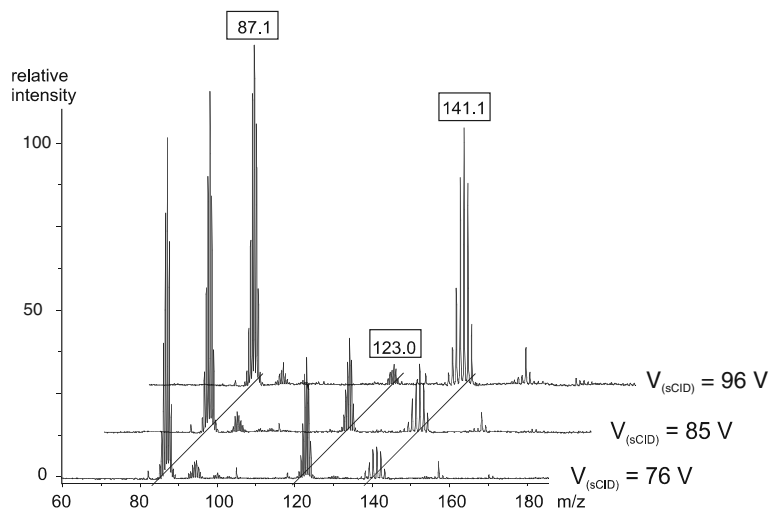


Fig. 4. ESI-MS of **3**, using increasing sCID voltage. The peaks are normalized to the peak at  $m/z$  87.

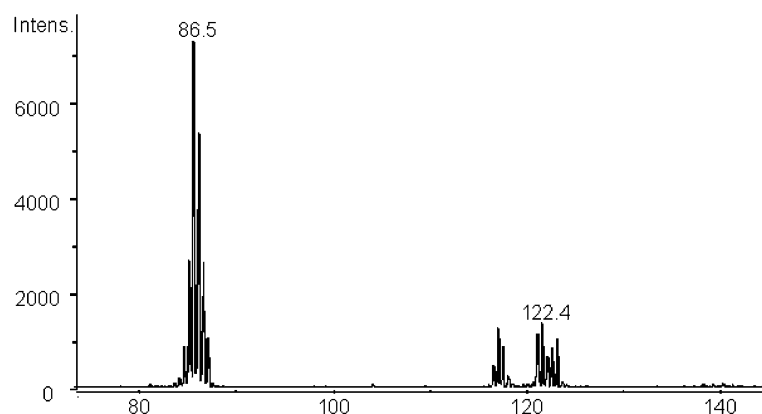


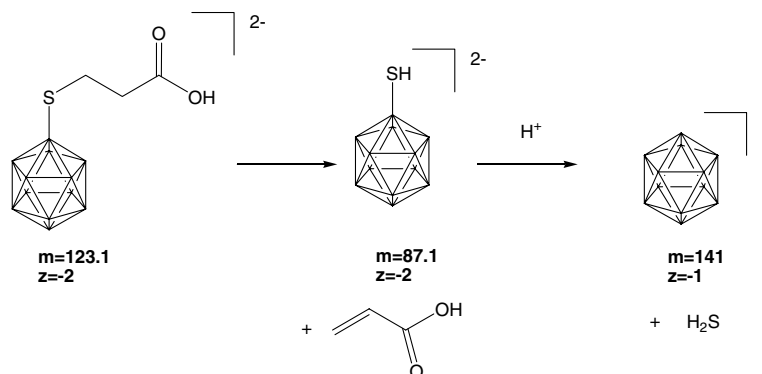
Fig. 5. MS/MS of peak 123.

to sCID, MS/MS is selective because the ion to be investigated is isolated before fragmentation. The negative-ion ESI-MS/MS mass spectrum of  $m/z$  123 ( $2-$ ) is shown in Fig. 5.

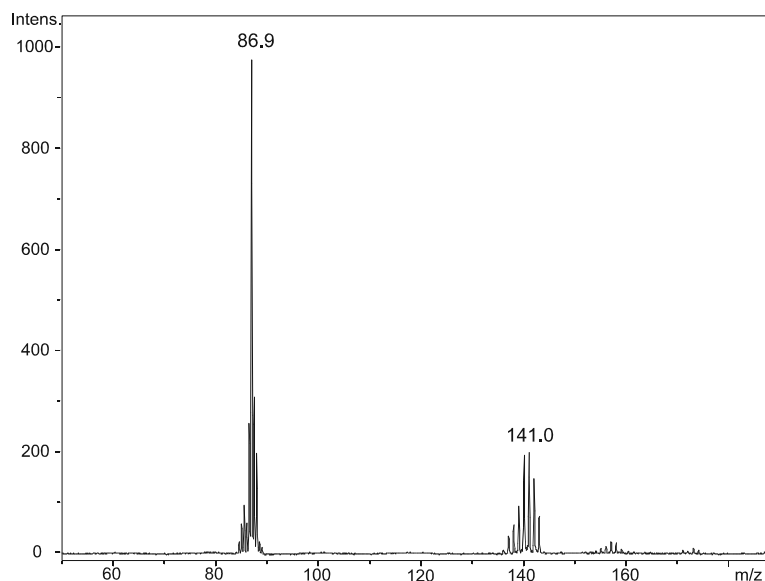
The spectrum shows that the main fragmentation product is  $m/z$  87 ( $2-$ ), i.e., the  $[B_{12}H_{11}SH]^{2-}$  ion. This

fragmentation reaction can be easily explained by abstraction of acrylic acid (see Scheme 2). Upon protonation, the resulting  $[B_{12}H_{11}SH]^{2-}$  ion can lose  $H_2S$ , to give  $m/z$  141 ( $1-$ ).

This is also in accordance with the empirical rule that mass spectrometric fragmentation of even-electron ions



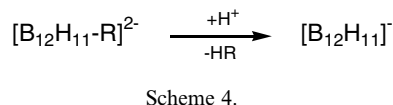
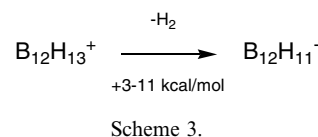
Scheme 2. Generation of fragment ions of **3** in ESI-MS.

Fig. 6. MS/MS of  $B_{12}H_{11}SH^{2-}$ .

occurs preferentially by abstraction of small neutral molecules; frequently, the driving force for the reaction is the stability of the neutral molecule. In this case, both the resulting fragment ion and the neutral acrylic acid are stable particles. For  $[B_{12}H_{11}SH]^{2-}$ ,  $H_2S$  as the stable neutral fragment is generated by protonation of the sulfur.

The obvious conclusion from this result is that the ion at  $m/z$  141 ( $1-$ ) must be a secondary fragmentation product of  $m/z$  123 ( $2-$ ), i.e., it must originate from the  $[B_{12}H_{11}SH]^{2-}$  ion ( $m/z$  87 ( $2-$ )). To test this, we subjected the ion at  $m/z$  87 ( $2-$ ) from the MS/MS spectrum shown in Fig. 5 again to MS/MS conditions, i.e., we performed an  $MS^3$  experiment. The resulting spectrum showed a weak signal at  $m/z$  141 ( $1-$ ), which was found to increase with increasing residence time of the  $m/z$  87 ( $2-$ ) ion in the ion trap. Thus, the  $m/z$  141 ( $1-$ ) signal does not only stem from the sCID region, but can also be formed under MS/MS conditions. The same signal was observed with an MS/MS spectrum of  $B_{12}H_{11}SH^{2-}$  (Fig. 6).

Obviously, the formation of  $m/z$  141 ( $1-$ ) requires the presence of the background gas which is present in the nozzle-skimmer region, but whose concentration is low in the high vacuum of the ion trap. In the skimmer region, this background gas, although it consists mainly of nitrogen, also contains residual solvent vapor as source for protons; we can tentatively explain this fragmentation reaction to occur via a gas-phase proton attachment to the doubly charged BSH anion, followed by abstraction of  $H_2S$  (see Scheme 2). The same explanation might hold for the high vacuum conditions in the ion trap; here, due to the low background (and hence also solvent)



gas pressure, longer residence times of the precursor ions are needed to form detectable amounts of the product ion.

From the protonated form of  $B_{12}H_{12}$  ( $2-$ ) ion,  $H_2$  abstraction has been found to be energetically possible and leads to a rather stable  $B_{12}H_{11}(1-)$  anion. The energy required has been calculated to only 3 kcal/mol [5] to 7.5–11 kcal/mol [6] (Scheme 3).

#### 4. Conclusion and outlook

The mass spectrometric experiments described here have shown that the  $[B_{12}H_{11}]^-$  ion is easily formed from the investigated substituted  $B_{12}$ -clusters under suitable conditions, such as ESI-MS with sCID. Further investigations will have to show whether this is a fragmentation reaction common to substituted  $B_{12}$ -clusters, according to Scheme 4.

In addition, the proposed reaction mechanism via proton transfer must be verified, which could possibly be done by isotope labeling experiments.

### **Acknowledgment**

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