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Application News

No. SCA_210_024

LCMS

The alternative for synthetic organic chemists

Farewell to sample preparation …

Fast and direct ionization using DART-LCMS-2020

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Organic synthesis of complex molecular structures often involves several synthesis steps and requires extensive purification of the intermediates and products. Some intermediates can only be detected with conventional analytical methods after time-consuming method development. For such a complex analysis of the intermediates and byproducts there is, however, insufficient time. Therefore simple, universal and fast analytic methods stand for enormous time savings, also in synthetic organic chemistry.

At the Institute of Organic Chemistry of the University of Basel, Switzerland, the synthesis of new structures with unknown characteristics is a daily routine for the scientific assistants. Various mass spectrometric methods are used for synthesis control and structure determination. This is why the selection of a suitable analytical method (GCMS, LCMS with various ionization technologies such as ESI, APCI, APPI, MALDI) for each newly synthesized molecule is a great challenge.

Due to the big amount of different molecular structures, a universal method is not available, so far. Appropriate instrument parameters have to be evaluated for every new synthesized compound. Low molecular weight, non-polar (hydrophobic) compounds are preferably analyzed using GCMS. The LCMS with an ESI and APCI ion source serves either for the detection of more polar, often multiple charged (ESI) or thermally stable, weakly polar compounds (APCI). In addition, many of the structures to be investigated are often thermally unstable. The MALDI technique is especially suitable for the analysis of high molecular weight compounds.

DART: fast analysis of synthesis products directly out of the reaction mixture

The DART (Direct Analysis in Real Time) ionization technology offers a highly promising alternative and allows for direct ionization of gases, liquids and solids under atmospheric pressure. In combination with a Shimadzu LCMS-2020 single-quadrupole mass spectrometer, the rapid analysis of synthesis products directly from the reaction mixture and without any sample preparation becomes possible.

In the DART source, a stream of helium is excited under atmospheric pressure by applying an electric potential to helium (see Figure 1).

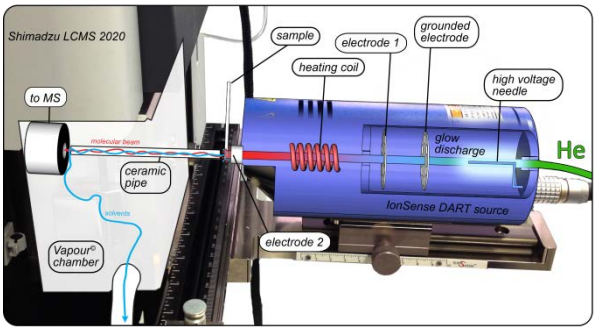

Figure 1: Structure of the DART Ion source with a gas inlet, plasma chamber (for glow discharge), and electrostatic lenses (ions and electrons are removed, leaving only long-lived (metastable) atoms and molecules)

This metastable helium plasma ionizes water molecules, which then transfer protons to the samples molecules.

Various ionization modes

Several ionization mechanisms are possible and summarized in figure 2. When helium is used the dominant positive-ion formation is a proton transfer reaction via ionized water clusters. But also the energy transfer directly from the excited gas to an analyte takes place. Negative-ion formation occurs by formation of O_2^- from atmospheric oxygen. This "indirect" ionization results in simple mass spectra, mainly through the formation of the protonated $[M+H]^+$ molecular ion or the $[M-H]^-$ ion in the negative mode. Depending on the structure of the analytes, M^+ ions (such as for polycyclic aromatic hydrocarbons) or fragments can be formed. Multiple charged ions or adducts with alkali metal cations are not observed.

Proton transfer (mainly with helium):

$He^* + (n+1)\ H_2O \rightarrow (H_2O)nH^+ + OH^- + He$

$(H_2O)_nH^+ + S \rightarrow SH^+ + nH_2O$

O_2^- formation (helium and nitrogen):

$He^* + S \rightarrow S^+\cdot + He + e^-$ or

$He^* + \text{Oberfläche} \rightarrow He + e^-$

$O_2 + e^- \rightarrow O_2^-$

$S + O_2^- \rightarrow S^- + O_2$ or

$S + O_2^- \rightarrow [S - H]^- + HO_2$

Figure 2: Ionization mechanism of excited helium plasma (He^*) with water, the analyte (S) or oxygen (O_2)

The DART source is easily installed and can be used directly "out of the box" without parameter optimization. The He stream transports the ionized sample molecules into the mass spectrometer through a ceramic capillary, which can be easily cleaned, if necessary (see figure 3). Small volatile molecules, such as solvents or water are removed via the vacuum pump.

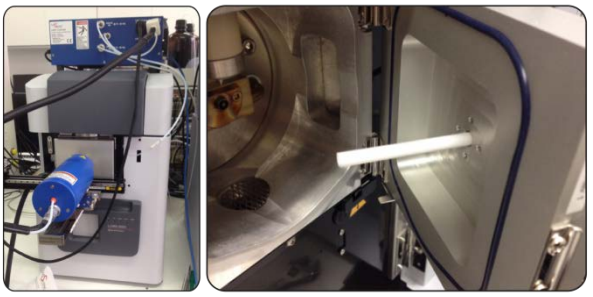

Figure 3: DART ion source from IonSense coupled to Shimadzu's single quadrupole LCMS-2020. The chamber in front of the inlet into the high vacuum stands under slight vacuum in operation.

Figure 4: Sampling out of reaction mixture of unknown concentration using a glass capillary and sample measurement by briefly holding the capillary in the ionization gas stream between the DART ion source and the LCMS-2020.

Figure 4 shows the qualitative analysis of a reaction mixture from a flask without further sample preparation. The sample can be easily removed from the reaction flask using a capillary and be held in the gas stream of the DART source. Three application examples illustrate the capabilities and benefit of the DART source.

Detection of similar polarities in a reaction mixture

A frequent problem are minor modifications of big molecules resulting in insignificant changes of polarity. Figure 5 shows the bromination of a complex methyl ester to 3 different products, which can be clearly characterized without any sample cleaning or another kind of sample preparation, by means of the DART-LCMS (s. figure 6).

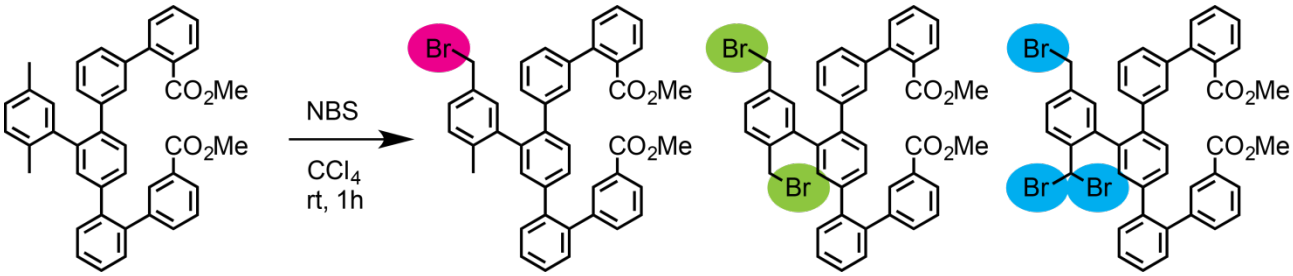

Figure 5: Mixture of a bromination reaction, the target molecule is shown in green colour and the reaction byproducts (pink and blue), which are very similar. The difference of polarity between the molecules is insignificant.

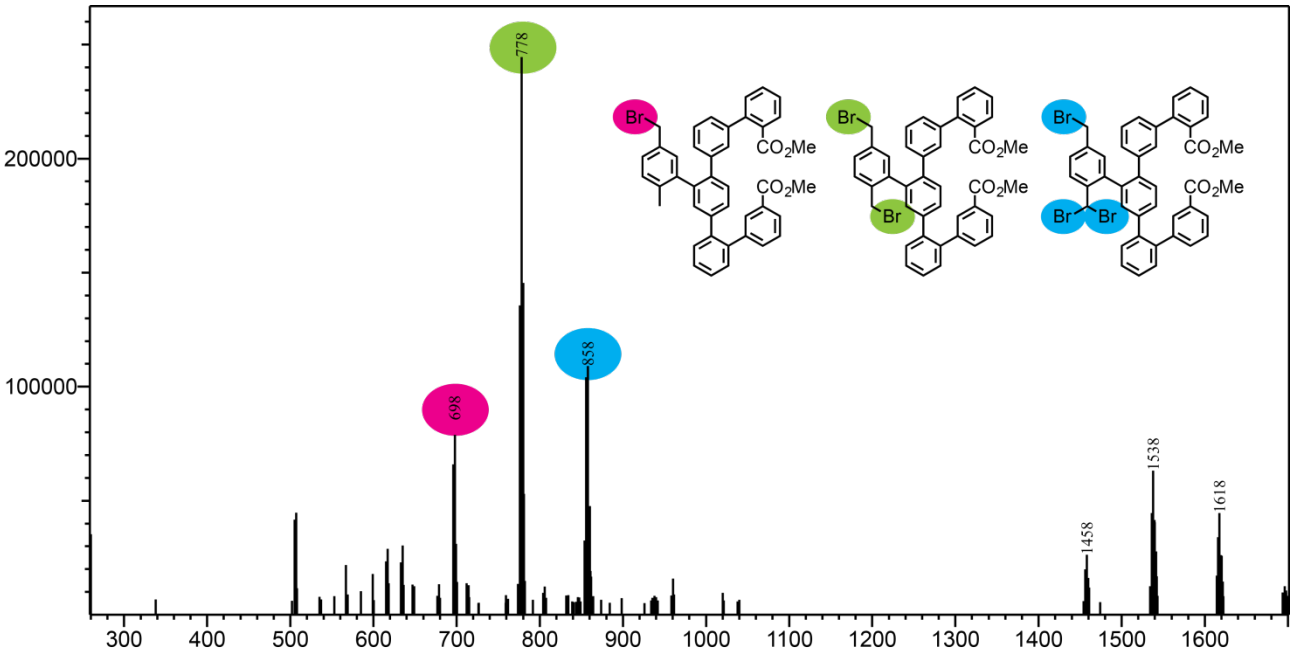

Figure 6: DART-MS-spectrum of the compounds out of the reaction mixture

Although a perfect chromatographic separation of the products can only be achieved by additional efforts, the different level of bromination can be assigned without any difficulties (figure 7). As every chromatographic fraction can be analyzed without sample preparation, a complete rack can be examined very quickly.

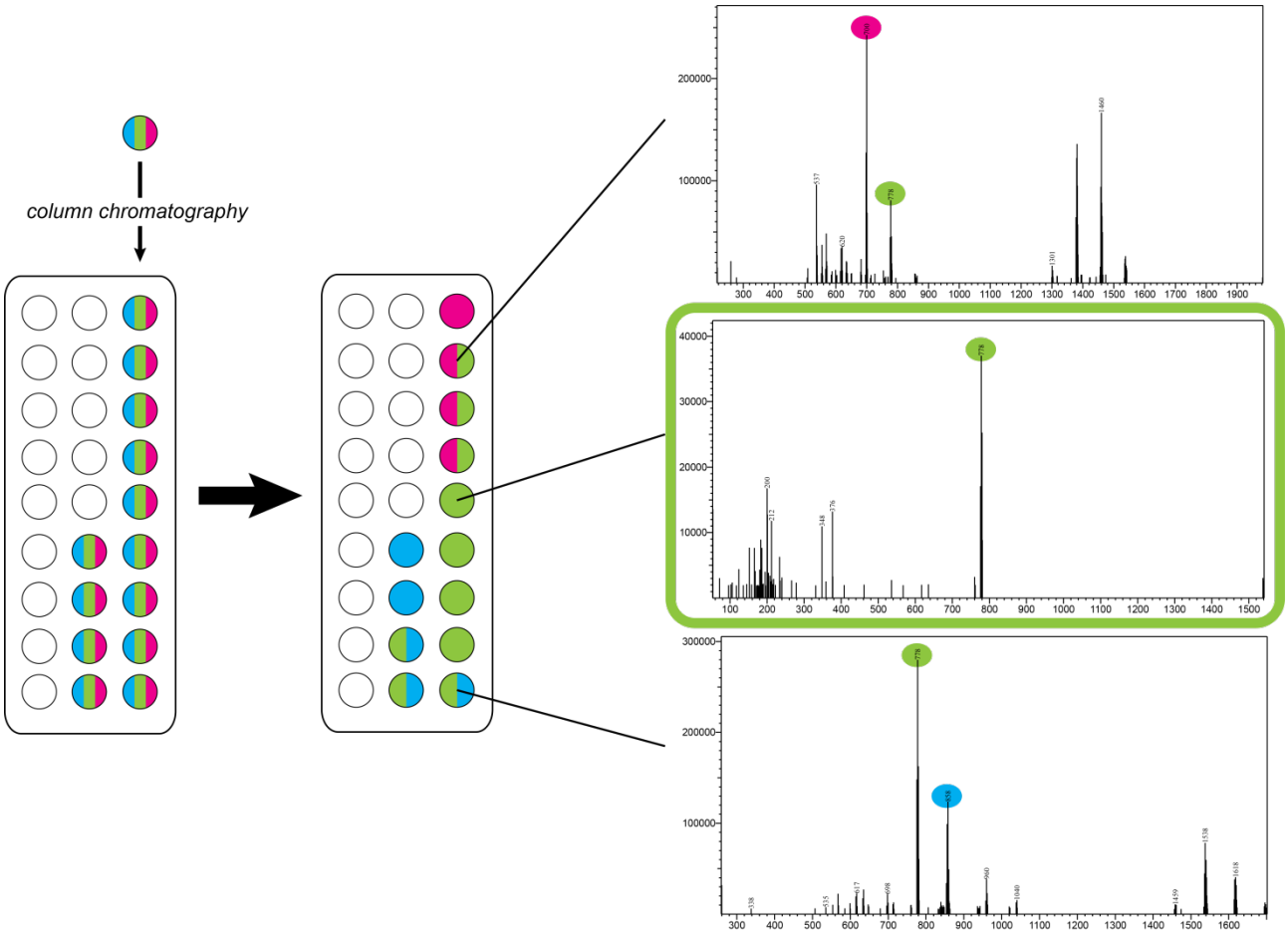

Figure 7: Fractionation of the reaction mixture via column chromatography (left), assignment of the target molecule to the fractions by means of DART-MS (middle) plus control of the purity grade (right).

Characterization of metastable unknown products

Many compounds cannot be detected so easily via mass spectrometry due to their thermal and chemical instability. Often these molecules degrade already during the sample preparation steps. In addition, important parameters like solubility and stability are not known as these compounds are synthesized for the first time.

In figure 8 some of those compounds, which can be characterized directly out of the reaction mixture are presented. Degradation or loss as result of sample preparation could be excluded. The technique of DART-LCMS proved to be the optimal method for the analysis of metastable compounds without any further method development. For instance the detection of the substance with the molecular ion m/z 542.22 succeeded exclusively during the reaction using DART-MS. The characterization via conventional LCMS needed several clean up steps. Just the indication for formation of the targeted product offers the development of dedicated method parameters for the product isolation. Moreover DART-MS is the only possibility to analyze the molecule m/z 1468.12 up to now – again without any further parameter optimization.

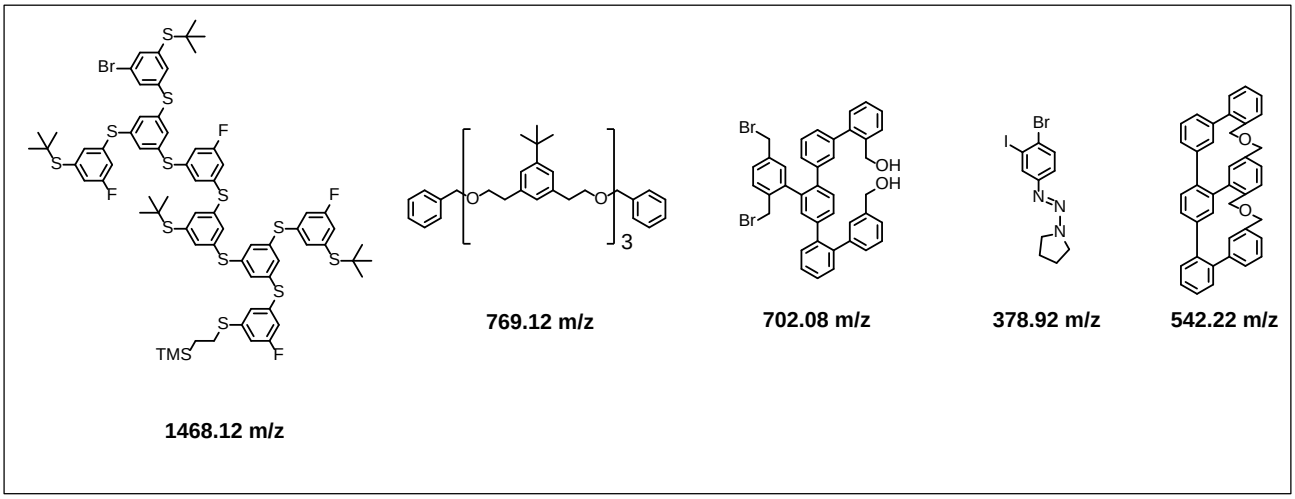

Figure 8: Synthesis products, which could be identified via DART-LCMS-2020

Control of a synthetic route

The analysis of multi-stage synthetic routes is particularly challenging. Because of the continuously changing parent molecules, the mass as well as the polarity and stability of the compounds and, consequently, also the appropriate mass spectrometric methods vary strongly. As an example, Figure 9 shows the development of a cyclic compound with six aromatic rings in 12 synthesis steps.

Each individual stage must be characterized, of which only the first 8 synthesis products could be determined via GCMS. In the figure, the corresponding M^+ molecular ion is shown in blue. The intermediate stages shown in pink were difficult to determine via GCMS due to their thermal instability or their high molecular weight. As of synthesis stage 9, the molecules reach a mass range critical for GCMS.

Analysis via LCMS with an ESI or APCI ion source is also not so easy, due to the low polarity of the compounds. In this case, the DART ion source with which all molecules labeled in green can be analyzed without much effort proves to be the method of choice. The high polarity switching speed (15 ms) of the LCMS-2020 that enables the simultaneous measurement of positively and negatively charged molecules is an additional advantage.

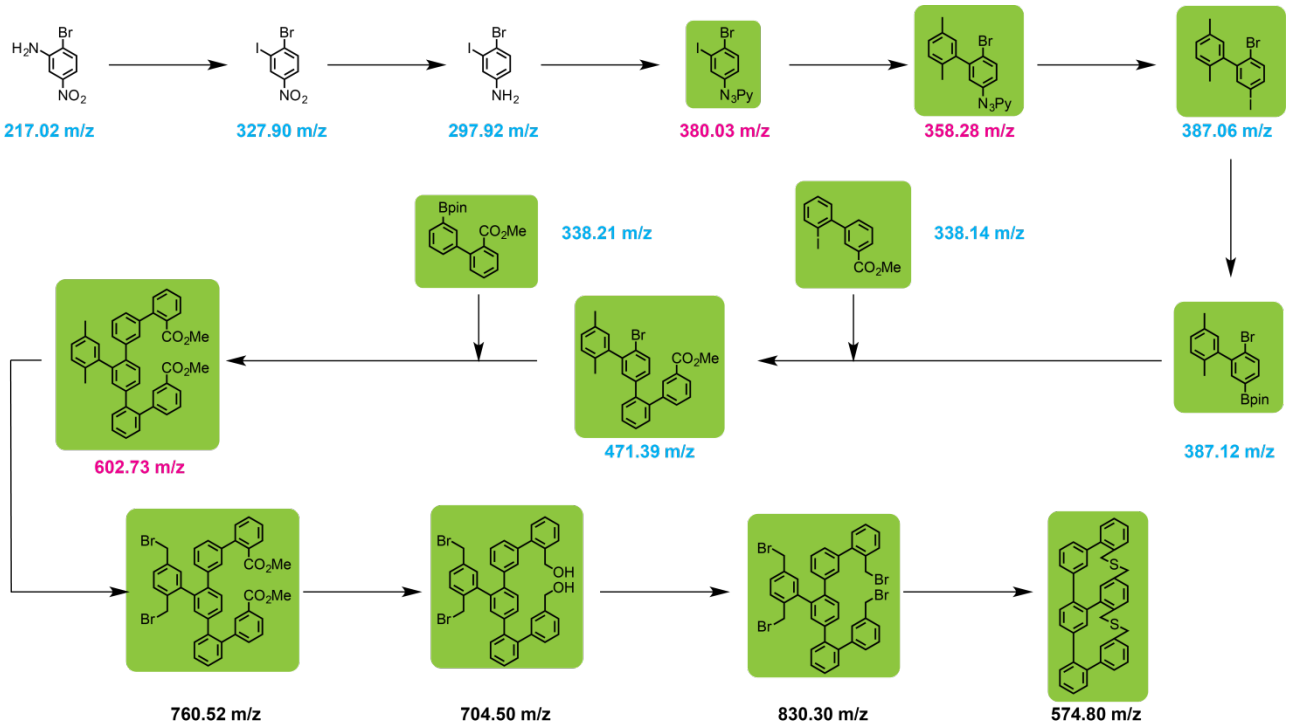

Figure 9: Synthetic pathway of the target molecule m/z 574.8 in 12 synthesis steps. Blue: analysis using GCMS; pink: analysis using GCMS only possible to a limited extent; green: analysis using DART-LCMS.

Summary

Combining the LCMS-2020 with the DART ionization technique enables the analysis of chemically very different polyaromatic compounds directly out of the reaction mixture. For qualitative measurement, a test capillary with a small amount of sample is held in the DART ion source to obtain a mass spectrum. Even large hydrophobic compounds are ionized in the helium stream and be identified reliably. The simple operation and the robustness of this analytical method make the DART-LCMS-2020 essential for daily routine use in organic chemical synthesis at the University of Basel.