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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8020

Chemical Reaction Tracking and Reaction Product Confirmation in Organic Synthesis Processes Using a MALDI-TOF Mass Spectrometer

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User Benefits

- Rapid and easy analysis of reaction products with low risk in sample preparation and operation.
- The presence of Br and Cl can be easily identified with class-leading MS resolution.

■ Introduction

In organic synthesis, it is extremely important to identify the reaction products and understand the progress of the reaction. Specifically, it is necessary to know what kinds of compounds exist in the reaction solution and in what proportions. The most simple and general method used for chemical reaction tracking is thin-layer chromatography (TLC). This technique utilizes the adsorptivity of analytes to silica gel as the stationary phase, which is different for each compound contained in the sample. However, because this method separates compounds based on polarity, it is often impossible to separate compounds with similar chemical structures.

As a solution for that problem, this report describes the usefulness of semi-quantitative reaction tracking and product confirmation using a MALDI-TOF MS, which enables rapid measurement of chemical compounds.

■ Method for Replacing Benzyl Halides

Benzyl halides are common electrophiles used to chemically modify proteins.(1) They react selectively with highly nucleophilic thiol groups in cysteine residues. Therefore, fluorochromes and drugs can be introduced into proteins in a site-specific manner by binding them to benzyl halides. Benzyl chloride is less reactive than benzyl bromide and benzyl iodide.

The reaction with proteins requires the use of the appropriate one of these reactive groups. In order to use it universally, it is necessary to synthesize these modifiers with different reactivity.

In this example, a compound with two benzyl chlorides was used as a raw material. The substitution reaction of benzyl chlorides with benzyl bromides was carried out using lithium bromide (LiBr).

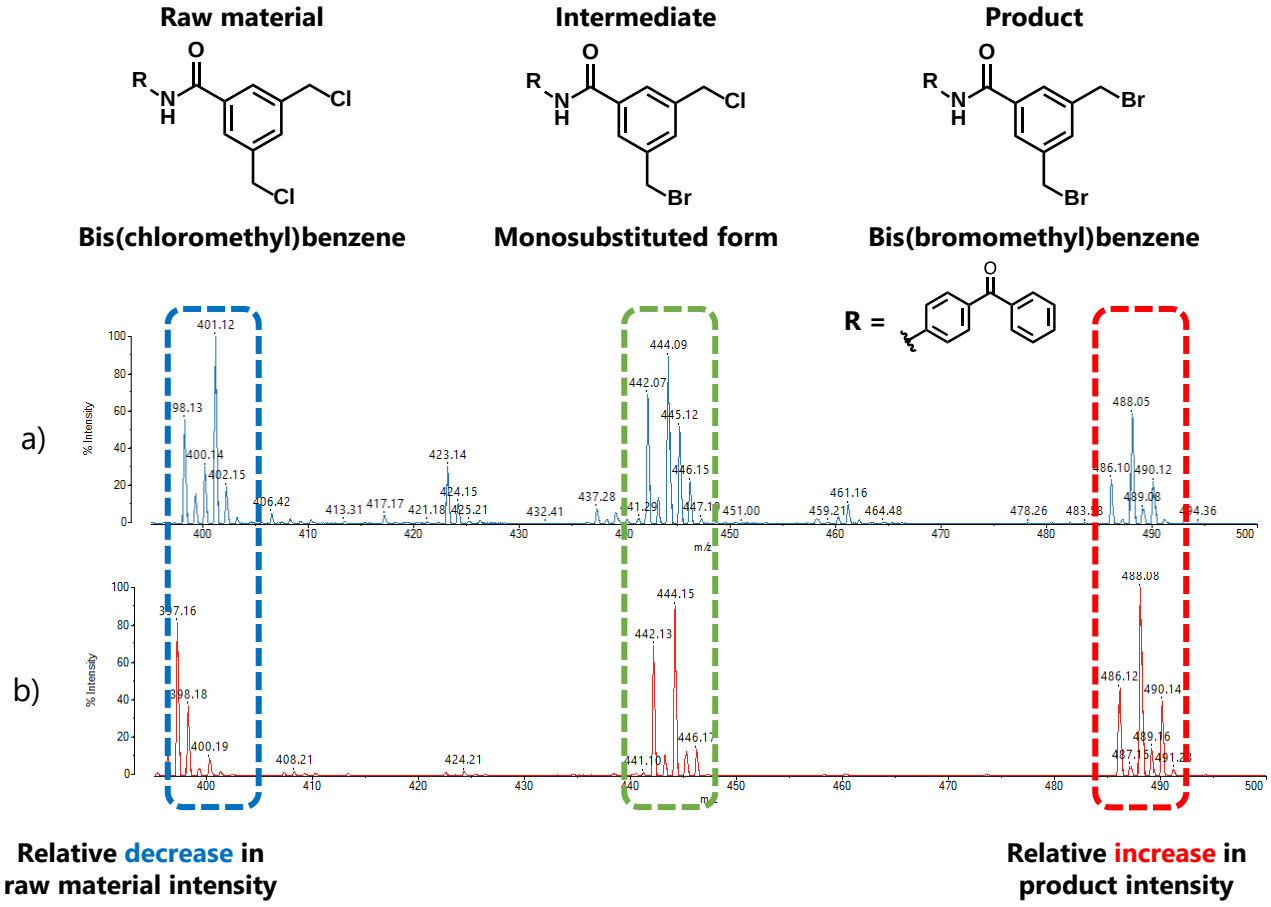

Fig. 1 Comparison of Mass Spectra for Reaction Products Obtained at Two Different Reaction Times
a) Immediately after Starting Reaction, b) After 18 Hours

■ Sample Preparation and Measurement

1 µL of the reaction solution was collected and placed in a glass tube. After drying, the residue in the tube was dissolved in an α-cyano-4-hydroxycinnamic acid (CHCA) solution (water/acetonitrile solution, 0.1% trifluoroacetic acid). The solution of the residue was applied onto a stainless steel MALDI plate and then completely dried.

It is also possible to prepare the sample after separation by TLC. In that case, the reaction solution is separated by TLC with a solution of chloroform/methanol. Then, the UV absorption spots are scraped. The supernatant extracted from the scraped silica with methanol is mixed with the CHCA solution in the same method and the solution is placed on the plate.

■ Results of Chemical Reaction Tracking

An intensity of the raw material (bis(chloromethyl)benzene) observed immediately after the start of the reaction (Fig. 1a) disappeared after 18 hours (Fig. 1b). At the same time, the intensity of the product, bis(bromomethyl)benzene, increased. Fig. 3 shows the mass spectrum of the reaction product obtained after additional reaction time.

There are two major isotopes of Br (^{79}Br and ^{81}Br), with a ratio of approximately 1:1. Therefore, considering an isotopic distribution of three peaks, each compound involving two Br isotopes appears with a peak intensity ratio of 1:2:1 and each peak 2 *m/z* units apart (Table 1 and Fig. 2).

Table 1 Theoretical and Measured Mass Values for the Isotopic Distribution of the Product

Theoretical mass (*m/z*)	Measured value (*m/z*)
485.97 (51.3 %)	485.97
487.97 (100.0 %)	488.03
489.97 (49.0 %)	490.04

The isotopic distribution shown in Fig. 3 is almost the same to the theoretical one in Fig. 2. This indicates that the reaction product contains two benzyl bromides.

■ Conclusion

Using the MALDI-8020, we succeeded in tracking the raw material, monosubstituted intermediate, as well as the target compound (bis(bromomethyl)benzene) levels, and succeeded in identifying the product. The presence or absence of Br and Cl can be easily concluded using the MALDI-8020 even for mixtures that are difficult to separate by TLC.

In addition, unlike LCMS and GCMS that require an interface function to connect chromatography to vacuum, analyte in a dry solid state is directly subjected to measurement in MALDI-TOFMS. Therefore, MALDI-8020 is suitable to use in common facility because the maintenance risk of the vacuum system is low.

Therefore, the MALDI-8020 is useful for easily measuring mass without risk of accidental problems caused by samples.

■ References

(1) Heinis, C., Rutherford, T., Freund, S. & Winter, G. Phage-encoded combinatorial chemical libraries based on bicyclic peptides. *Nat. Chem. Biol.* **5**, 502-507(2009).

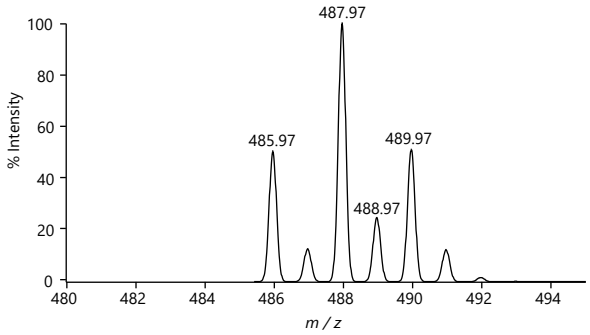

Fig. 2 Theoretical Isotopic Distribution of the Product

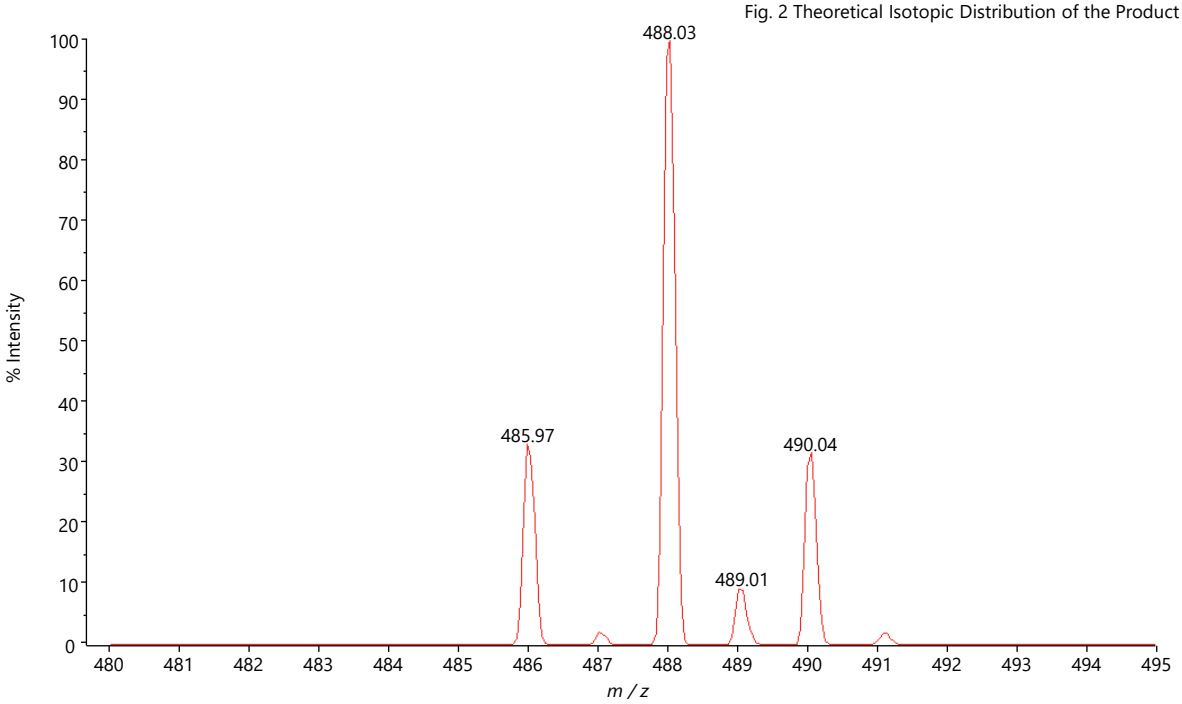

Fig. 3 Mass Spectrum Results of the Recovered Compound
An isotopic distribution with contribution from two bromides was obtained.

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