

Application News

No. C159

Direct Probe Ionization Mass Spectrometer

Real-Time Monitoring of Chemical Reactions

In the field of chemistry, monitoring the reaction process and rate of chemical reactions is very important in order to accurately understand the type of reaction that is occurring.

Probe electrospray ionization (PESI) is a direct ionization technique that uses a probe to ionize sampled target components by sampling a sample at a constant frequency with high voltage applied to the probe tip. This technique enables rapid monitoring of sample changes without the need for a chromatograph.

The DPiMS-2020 (Fig. 1), which combines PESI with a mass spectrometer, enables accurate understanding of the progression of chemical reactions through real-time monitoring of changes in the molecular weight information of components targeted for analysis.

This article introduces the result of using the DPiMS-2020 to perform real-time analysis of the deprotection reaction of a peptide protecting group that was produced on a sample plate.

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■ Peptide Deprotection Reaction

When a solution of 23.0 mg of Fmoc-Tyr(tBu)-OH(N-(9-Fluorenylmethoxycarbonyl)-O-tert-butyl-L-tyrosine) dissolved in 5 mL of DMF (solution ①) is mixed together with a solution of piperidine and DMF mixed at a 1:4 ratio (solution ②), a deprotection reaction of the Fmoc group will occur at room temperature (Fig. 2). 4.5 μ L of both solution ① and solution ② were injected onto a small (10 μ L) sample plate used in PESI solution analysis to induce a deprotection reaction and the reaction process was analyzed in real time.

Table 1 lists the drive conditions of the PESI probe and Table 2 lists the analysis conditions of the mass spectrometer.

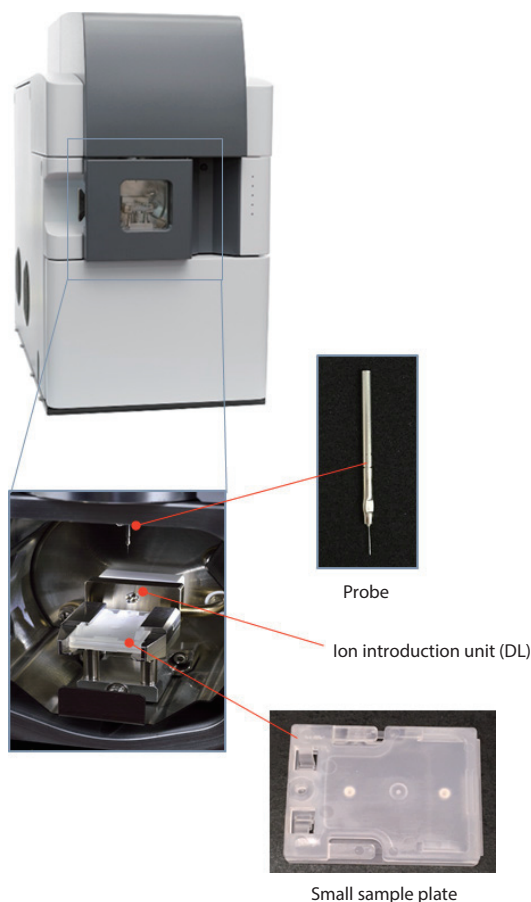


Fig. 1 DPiMS-2020

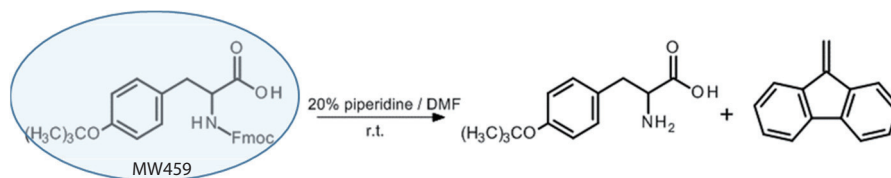


Fig. 2 Deprotection Reaction of Fmoc-Tyr(tBu)-OH

Table 1 PESI Drive Conditions

Ionization position	: -37 mm
Ionization stop time	: 100 msec
Sampling position	: -46 mm
Sampling stop time	: 50 msec
Probe speed	: 250 mm/s
Probe acceleration	: 0.63 G

Table 2 Mass Spectrometer Analysis Conditions

DL temperature	: 250 °C
Heater block temperature	: 50 °C
Interface voltage	: -2.45 kV (ESI - Negative mode)
Scan speed	: 5,000 u/sec

Real-Time Monitoring of the Reaction Process

By monitoring the attenuation in negative ion intensity of Fmoc-Tyr(tBu)-OH from the mass spectrum obtained through scanning, it was possible to monitor the deprotection reaction in real time at 0.1-minute intervals. Fig. 3 shows decreasing of the negative ion intensity of Fmoc-Tyr(tBu)-OH from the 3-minute mark to the 4.5-minute mark following solution mixture.

Influence of Sampling Volume Reduction Due to Volatilization

Although our intent is to monitor the reaction that occurs by mixing the two solution types on the sample plate, there is a possibility that the volume of the solution mixture decreases over the course of analysis due to volatilization since the sample plate is placed directly below the DL, which is set to a high temperature. This may cause a reduction in the volume of solution sampled by the probe and decreasing of the ion intensity. In order to negate this effect and only monitor the decreasing of ion intensity caused by the progression of the deprotection reaction, the speed at which the reaction progresses following solution mixture was determined by setting ions at m/z 474 as the internal standard peak and plotting the m/z 458 to m/z 474 intensity ratio against time after solution mixture (Fig. 4). As a result, we were able to monitor the progression of the deprotection reaction in real time.

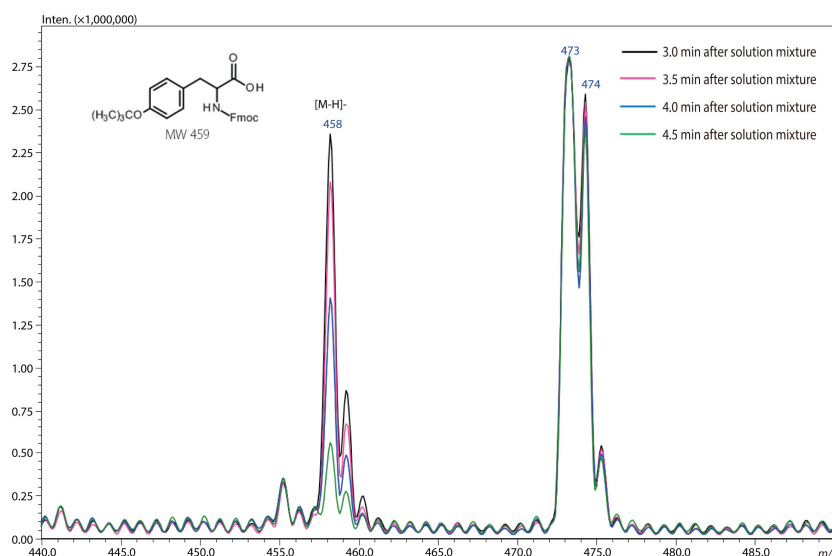


Fig. 3 Time Series Mass Spectra of Deprotection Reaction

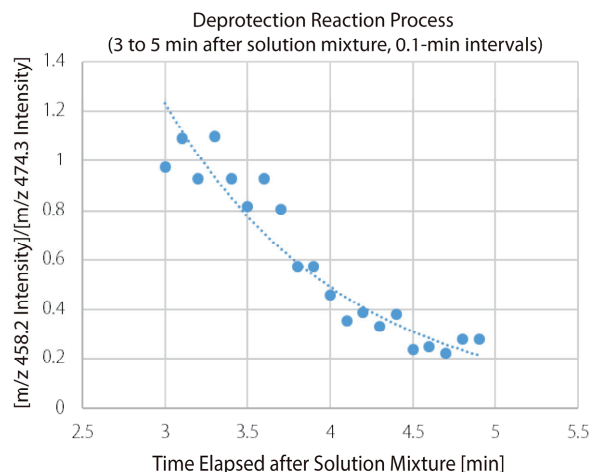


Fig. 4 Time Series Variation of Deprotection Reaction

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