Microsaic Systems

Automated reaction monitoring by direct analysis mass spectrometry using the 4000 MiD® and MiDas™

Microsaic Systems plc, GMS House, Boundary Road, Woking, Surrey, GU21 5BX, United Kingdom

OBJECTIVE

Monitor and compare two Boc-deprotection reactions using Microsaic Systems unique mass detection and sampling platform, consisting of the MiDas™, 4000 MiD® and stand-alone on-board Masscape® software.

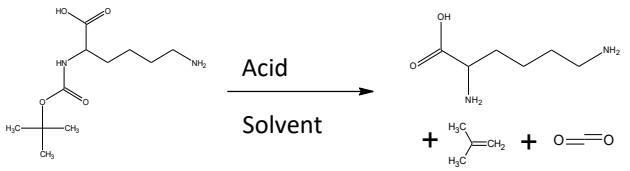

FIGURE 1. *General scheme for the deprotection of N_α-Boc-Lysine*

BACKGROUND

The Boc group can be added to amines to give N-tert-butoxycarbonyl or so-called t-BOC derivatives. These derivatives do not behave as amines, which allows certain subsequent transformations to occur that would have otherwise affected the amine functional group. Removal of the t-BOC amino acid can be accomplished with strong acids such as trifluoroacetic acid neat or in dichloromethane, or with HCl in methanol.

MiDas™ - MiD® FULLY INTEGRATED SAMPLING, DILUTION, ANALYSIS AND DATA MANIPULATION

The MiDas™ - MiD® offers automated monitoring of a chemical process with real-time analysis of data, all possible with the use of the on-board Masscape® software.

With a small footprint and quick pump down time, the MiD® is able to be deployed to the reactor, employing whichever modular arrangement is required for the chemistry and workspace.

Sampling is achieved using a fully customisable dual syringe pump with multi-port valves, and controlled by Masscape®. Integrated into operational methods are user defined values to allow for optimisation where a non-standard tube kit is used.

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Sampling volume, frequency and dilution parameters are customisable by the user to minimise sample consumption or sample transfer time.

REACTION MONITORING BY DIRECT ANALYSIS MS

The 4000 MiD® operates with generic, soft ionisation conditions that result in simple to interpret mass spectra, where pseudomolecular ions of known and unexpected species are easily determinable.

Rapid direct analysis also allows for the profiling of reactions involving transient species that are not observed using conventional off-line techniques. In addition, from point of sampling to ionisation source the system is closed to outside atmosphere, and make-up solvents can be selected to preserve compounds of interest.

Reaction profiles are plotted by Masscape, revealing reaction kinetics, the initiation of unwanted side reactions, stalling, or completion to intended steady state.

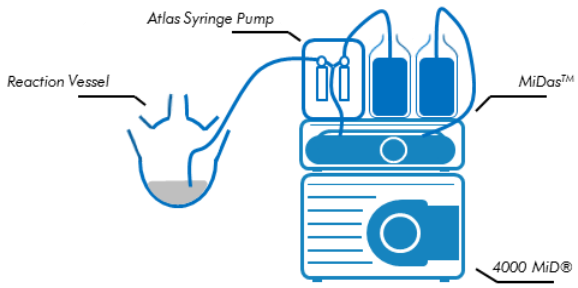

FIGURE 2. *Schematic representation of 4000 MiD® coupled to a reaction vessel via the MiDas™ and dual syringe pump*

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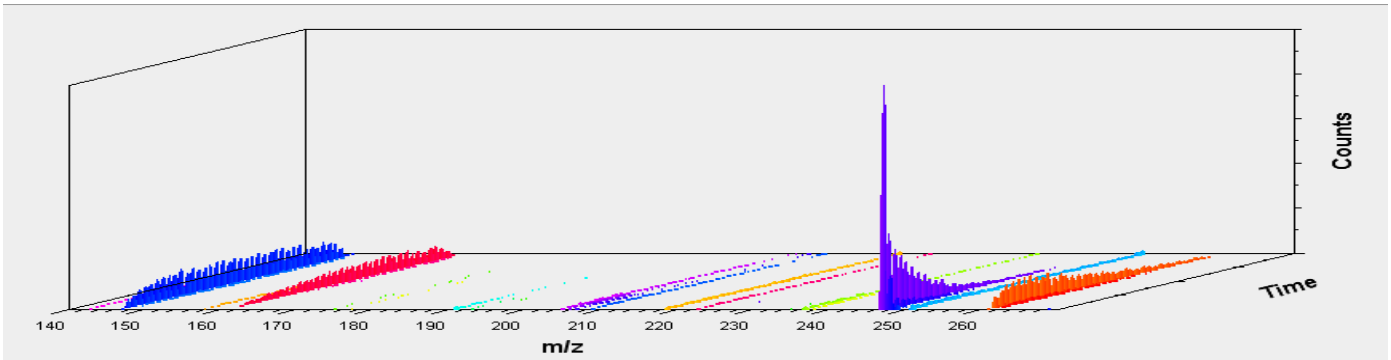


FIGURE 3. *Mass spectra of HCl reaction using the waterfall function to display as 3D stack*

EXPERIMENTAL

A 25mg/mL solution of Nα-Boc-Lysine was prepared in 1:1 Methanol : Acid (neat Trifluoroacetic acid or 25% HCl in water)

RESULTS AND DISCUSSION

Masscape® was used to analyse reaction profiles and changes in mass spectra over time. Additional m/z values can be added to the profile dynamically as they appear (figure 3). The reaction proceeds cleanly using TFA, however several additional species were detected where HCl was used (figure 4). The reaction profile indicates quantitative conversion of Nα-Boc-Lysine to lysine in under 100mins where TFA is used.

Where HCl is used the profile shows rapid consumption of m/z 246 (1) and generation of m/z 146 (2) and m/z 260 (3). This is followed by consumption of (2) and (3) and steady generation of (4), trends which are clearly visible in the 3D graph generated in Masscape® (figure 3). The proposed scheme is shown below.

TABLE 1. *4000 MID® settings*

Scan mode	Full scan
Mass range	m/z 50 - 800
Scan rate	1 scan/sec
Step size	m/z 0.2
Ion polarity	Positive (ESI)
Tip voltage	850 V
Gas flow	2500 mL/min
Vacuum interface	30V

TABLE 2. *MiDAS ™ settings*

Purge & transfer solvent	Methanol
Make-up solvent	50:50 methanol:water (0.1% formic acid)
Make-up flow rate	1mL/min
Sample volume	20µl
Split ratio	1000:1
Sample interval	15 mins (TFA), 8.5 mins (HCl)

FIGURE 4. *Selected EIC reaction profile of TFA reaction (TOP) and HCl reaction (BOTTOM)*

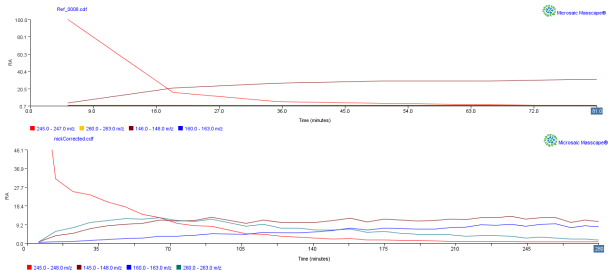


FIGURE 5. *Additional species identified from mass spectra, available at any time during analysis*

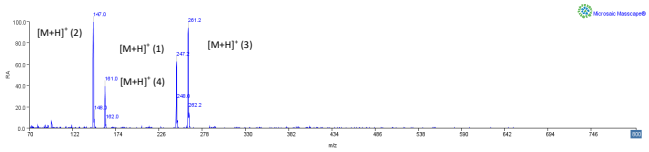
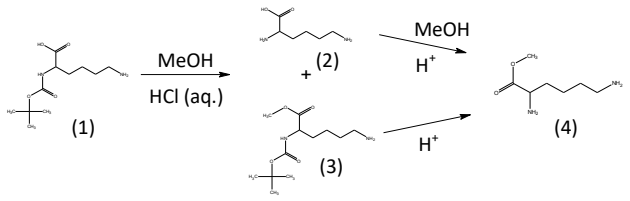


FIGURE 6. *Reaction scheme for de-protection with HCl*



SUMMARY

- Stand-alone automated sampling, dilution and direct analysis using the MiDas™ and 4000 MiD® reliably monitored starting material and several products.
- End of reaction determined for TFA deprotection.
- Profile of products fits with proposed chemistry

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