Application Note

Microsaic Systems

Bromination of a pyrrolo-pyrimidine by real-time continuous reaction monitoring mass spectrometry analysis

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

INTRODUCTION

The Microsaic 4000 MiD® is a miniaturised single quadrupole mass detector. By utilising micro-electro-mechanical systems (MEMS) technology, the key components of a traditional mass spectrometer have been miniaturised and designed as "plug and play" components. The system has the vacuum system, electronics and computer within the same enclosure. This means the instrument can be installed in places where no other mass spectrometer can be normally deployed. For instance, the product can be placed into a fume hood next to a flow reactor.

Flow synthesis offer many advantages particularly when applied to processes which are difficult or have dangerous chemical transformations. For continuous chemical synthesis, mass spectrometry can be used to analyse in real-time the reaction stream and optimize transformations. This work-flow improves yield, purity and reaction selectivity by monitoring the presence of transient, reactive intermediates and also determining the steady state conditions of the reaction.

In this application note we report the bromination of ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate by on-line reaction monitoring mass spectrometry. This work was carried out at Durham University in collaboration with Prof. Ian Baxendale.

EXPERIMENTAL

The continuous flow optimization of ethyl pyrrolo[1,2-c] pyrimidine-3-carboxylate was been carried out by coupling the Microsaic 4000 MiD® mass detector to the Vapourtec R-Series flow chemistry system through the MiDas™ interface module (Figure 1).

The time taken to install the Microsaic 4000 MiD® and connect to the Vapourtec flow reactor to acquire meaningful data was less than 1 hour. Using a similar set up with a conventional MS system would take anything from 2 to 5 days.

The bromination of ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate was monitored by the MS using the settings reported in Table 1.

The reaction of ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate with N-bromosuccinimide (Scheme 1) was carried out under different flow reaction temperatures (from 25 to 60°C) using the instrument set up reported in Table 2.

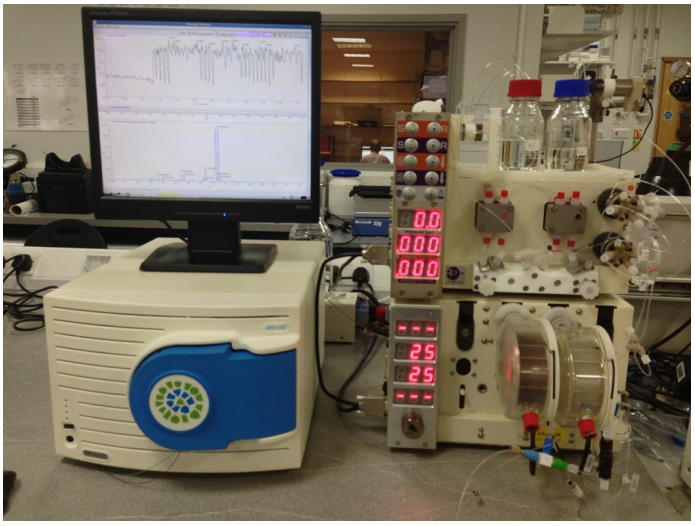
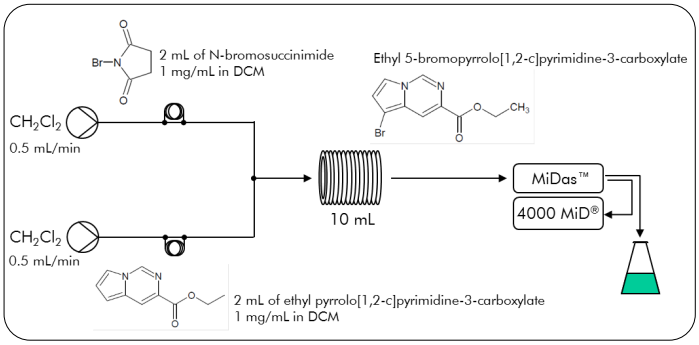


Figure 1. Microsaic 4000 MiD® and *Vapourtec R-Series* flow chemistry system.

Scan mode	Full scan
Mass Range	*m/z* 100 – 800
Scan Rate	1 Hz
Step size	*m/z* 0.2
Ion polarity	Positive (ESI)
Tip voltage	850 V
Gas flow	2500 mL/min
Vacuum interface	50 V
Tube lens	10 V
Plate lens	5 V
Ion guide	1 V

Table 1. Microsaic 4000 MiD® settings for on-line flow reaction monitoring.



Scheme 1. Flow route for the synthesis of ethyl 5-bromopyrrolo[1,2-c]pyrimidine-3-carboxylate.

Reactor	PFA coiled tube
Reactor size	10 mL
Mobile phase A and B	Dichloromethane (DMC)
Flow rate A and B	0.5 mL/min
Reagent A	Pyrrolo[1,2-c]pyrimidine-3-carboxylate, 1 mg/mL in DMC
Reagent B	N-bromosuccinimide, 1 mg/mL in DMC
Injection volume	2 mL (per each reagent)
Make-up pump solvent	Methanol, 0.1% formic acid
Make-up pump flow rate	1 mL/min
Split ratio	3000:1

Table 2. Flow reactor-MiDas™ set up for the chemical transformation of ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate with N-bromosuccinimide.

RESULTS AND DISCUSSION

The generation of the product ethyl 5-bromopyrrolo[1,2-c] pyrimidine-3-carboxylate and consumption of the reagent ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate was monitored in real-time by the Microsaic 4000 MiD®. Figure 2 shows the mass spectrum containing both reagent and product ions at a reaction temperature of 25 °C. Typical isotopic patter for the present of one and two bromine (product and its dimer) is also shown.

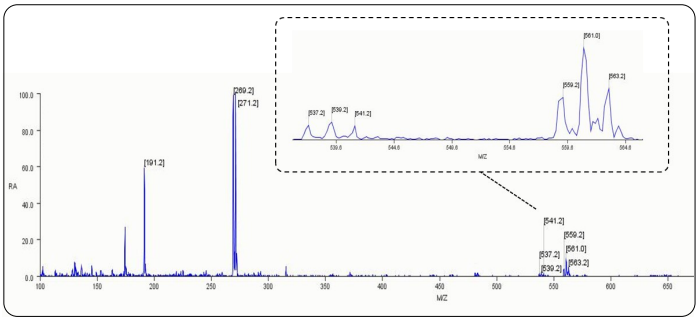


Figure 2. Mass spectrum of protonated ethyl 5-bromopyrrolo[1,2-c]pyrimidine-3-carboxylate at m/z 269.2 and ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate at m/z 191.2 at a reaction temperature of 25°C. Bromine isotopic patter for product at m/z 269.2, product dimer at m/z 537.2 and product dimer adduct with sodium at m/z 559.2) is shown.

Figure 3 shows the formation of the product (m/z 269.2) and consumption of the starting material (m/z 191.2) as a function of the reaction temperature. The temperature profile was obtained in less than two hours from initial experimental set up, reducing significantly the associated costs from off-line analysis usually more time consuming.

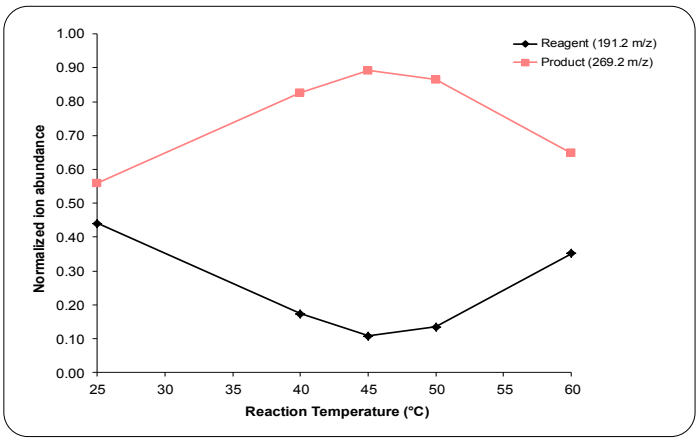


Figure 3. Normalised ion abundance of the ethyl 5-bromopyrrolo[1,2-c]pyrimidine -3-carboxylate (m/z 269.2) and the ethyl pyrrolo[1,2-c]pyrimidine-3-carboxylate (m/z 191.2) as a function of the reaction temperature.

On-line reaction monitoring data was used to identify the optimum reaction temperature, monitor impurity generated in real-time and calculate conversion and yield at each reaction temperature (Table 3).

Reaction Temperature (°C)	Conversion (%)	Yield (%)
25	72.5	50.5
40	90.7	86.8
45	91.2	92.4
50	90.8	90.1
60	81.0	65.9

Table 3. Estimated conversion and yield for the bromination of ethyl pyrrolo[1,2-c] pyrimidine-3-carboxylate.

The effect of ion suppression was evaluated by comparing the intensities of reagent and product individually injected into the system with the intensities generated when components were injected as a mixture. The results are displayed in Table 4. From these results it is apparent the effect of ion suppression is not significant. If ion suppression was present then a quantitative approach would be achieved by using deuterated standards spiked into the make-up solvent as a quantitative comparison.

Compound	m/z	Conc. (mg/mL)	Intensity
Reagent individually injected	191.2	0.5	17742
Reagent injected as mixture	191.2	0.5	18656
Product individually injected	269.2	0.5	7909
Product injected as mixture	269.2	0.5	7794

Table 4. Comparison of the intensities of the reagent and product parent ions when singly and both injected into the flow-MS system.

CONCLUSIONS

The experiments carried out represent a cursory demonstration of the Microsaic 4000 MiD® for on-line flow reaction monitoring with minimal experimental set up and no optimisation of solvents or source conditions. The user was able quickly acquire meaningful data unlike the alternative off-line analysis methods associated with an open-access mass spectrometry service.

Ion suppression was also investigated but not found to be present.

The data was used to optimize chemical transformations, monitor impurities, intermediates and determine reactions steady state in real time. This information could also enable improvements to product yield, purity, and reaction selectivity.

The experiment carried out yielded invaluable information not previously available in real-time.

Finally, the benefit of using the Microsaic 4000 MiD® to decrease the time to reaction optimisation is clearly apparent.