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Microchip-based synthesis and analysis: Control of multicomponent reaction products and intermediates

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A miniaturised-SYNthesis and Total Analysis System (μSYNTAS) was used for the solution-phase synthesis and on-line analysis (TOF-MS) of Ugi multicomponent reaction (MCR) products. This approach provides an unusually high degree of control of the MCR and delivers detailed, novel information on reaction intermediates in real-time. Specifically, the Ugi 4 component condensation (4CC) involving the reaction of an amine, acid, aldehyde and isocyanide species was performed at room temperature in a controllable fashion. Furthermore, observation of the nitrilium intermediate, cyclohexyl(2-piperidin-1-ylethylidyne)ammonium chloride, is presented for the first time.

Introduction

The field of miniaturised-Total Analysis Systems (μTAS)[1] has been an area of great activity in recent years and the development of microfabrication technologies has been vital to the pursuit of these goals.[2] Some of the most notable applications which have benefited from the integration of analytical systems on the microscale ('lab-on-a-chip') include separation science[3,4] nucleic acid analysis,[5] DNA amplification[6,7] and detection techniques.[8–10] However, the field of on-chip *solution-phase synthesis* and analysis, termed μSYNTAS (miniaturised-SYNthesis and Total Analysis Systems), has seen a more moderate rate of growth, primarily due to the wide range of processes required during a typical synthetic methodology.[11] These may include, but not be limited to, reagent preparation, derivatisation, product separation and isolation steps, *in addition* to the particular reaction of interest. The motivating factors behind the development of μSYNTAS, nevertheless, are the same as those for μTAS, *viz.* miniaturised processing systems yield significant enhancements in efficiencies of mixing and separation. Furthermore, the extremely small amounts of materials used in such systems make them ideal for processing particularly valuable or hazardous reaction components. Importantly, the large surface-to-volume ratios encountered in micro reactor environments allow for rapid interruption of chemical processes, which may lead to higher reaction selectivity and thus 'higher quality' products.

To date, microsystems have been applied to a number of key areas in reaction technology. These include; isothermal processing,[12] chemical processing within explosive regimes,[13] process optimization,[14] enzymatic reactions,[15] DNA amplification,[6] electrochemical reactions,[16] radical polymerizations,[17] and fine-chemical production.[18] In all these systems structure elucidation is the key process which ultimately allows for analyte identification. In recent years, mass spectrometry (MS) has become one of the most powerful and dominant tools for this purpose. In large part this has been due to its facile interfacing with standard separation techniques such as capillary electrophoresis (CE)[19] and high-performance liquid chromatography (HPLC).[20] Furthermore, the high sensitivity and specificity of MS analysis obviates the need for sample preparation, which often complicates the analytical procedure.

More recently, the development of electrospray ionization (ESI) MS has allowed the analysis of extremely small samples at low flow rates (nL–μL min^{-1}).[21] Since these flow rates closely match those encountered in many microfluidic chip systems, the ability to perform structural identification of analytes processed on μ-TAS devices has become a reality. Karger and co-workers demonstrated the first microfabricated glass chip coupled to ESI-MS. Peptide and protein samples were pumped by pressure and aligned by a precision translation stage to the MS inlet.[22] Figeys and co-workers have also used ion trap MS[23] and ESI-MS[24] with electro-osmotic pumping for the analysis of protein samples. Ross *et al.* demonstrated coupling of MALDI-TOF-MS to a miniaturized analytical thermal cycling instrument. This approach allowed for complete polymerase chain reaction (PCR) and product analysis in 50 min.[25] More recently much research has concentrated on clean-up procedures for complex biological samples in ESI-MS.[26]

In this paper we describe a μSYNTAS developed in our laboratory, which is based on the integration of a silicon-machined micro reactor[27] with time-of-flight mass spectrometry (TOF-MS). The coupling of the micro reactor with rapid on-line analysis provides a means for obtaining highly detailed information on reaction processes *in real-time*. Consequently, the integration of synthetic and analytical process elements, and the highly unusual reaction environment within the μSYNTAS (very high mass and thermal transfer coefficients) means that real-time *optimization* of chemical processes may be achieved with very high throughput. Such a methodology is simply not possible with the solid-supported techniques currently favoured in the pharmaceutical industry. The fact that rapid TOF-MS analysis is performed *on-line* also means that reaction *intermediates* may be observed *and identified* with a flexibility unknown using other traditional techniques. Specifically, in this paper, we address the Ugi 4 component condensation (4CC) reaction. Ugi 4CCs involve the reaction of an amine, acid, aldehyde and isocyanide[28–30] (Fig. 1). A number of Ugi-type multicomponent reactions (MCRs) have been developed since publication of the first examples in 1959,[28] and their utility is illustrated by the variety of biologically active compounds, *e.g.* substituted amino acids and β-lactams, which may be generated *via* the Ugi protocol.[29,31] Multicomponent reactions (MCRs) such as this are extremely interesting model processes due to their relevance in compound library generation for the synthesis of pharmaceutically relevant intermediates. MCRs are one-pot reactions in which a particular reaction sequence occurs only when all the components have been introduced into the reaction

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vessel. As a result, great savings are made in terms of time and process complexity, since intermediate work-up steps are not required. Also, a high degree of molecular complexity is derived from several simple starting components, and the reaction products have multiple points for the introduction of molecular diversity. Consequently, MCRs are widely used in compound library generation *via* combinatorial methods.[31] However, the widespread use of solid-support technologies for combinatorial chemistry has the potential for limiting process flexibility, and on-line real-time optimization strategies are only viable with a fully solution-phase approach. The μSYNTAS described here illustrates such an approach and demonstrates the power of the integration of synthesis and analysis performed *on-line* and in *real-time*.

Experimental methods

Design and principle of presented micromixer

The micro reactor used for all experiments operates on the principle of distributive mixing. The microstructure is a two-layer device made up of a glass/silicon/glass sandwich. It has an internal volume of ~600 nL and measures 2 × 5 ×10 mm. Fabrication and design methods have been discussed in detail elsewhere.[27] Briefly, two inlet flows are split into a series of separate multichannel streams (16 partial flows). This is achieved by repeated splitting of the channels in such a way that an array of symmetrical elements results. Wafer-through nozzles connecting the two fluidic layers allow the two liquid streams to converge and mix. Channels are then sequentially combined in a reverse network until all partial flows are united in one broad outlet channel. The extremely large diffusional surface areas created within the device allow for rapid, efficient mixing.[27]

Experimental conditions and set-up

The micromixer was coupled to a TOF-MS (Mariner, Perseptive Biosystems, Foster City, CA, USA) *via* an electrospray unit (Fig. 2). Fused-silica capillaries (TSP150375, Composite Metal Services, Harlow, Essex, UK) were coupled to the surface of the micromixer and clamped in place with a PTFE jig. Electrospray conditions were achieved using an applied voltage of 4 kV and nebulizing gas flow. Solutions were infused under continuous-flow conditions into both inlets of the micromixer. Acquisition of data from the mass spectrometer was initiated manually. Data scans were made at a rate of 1 Hz for *m/z* 90–1000.

Materials

Piperidine hydrochloride, 4-piperidone monohydrate hydrochoride, 3-hydroxypiperidine hydrochloride, 4-hydroxypiperidine hydrochloride and 2,2,6,6-tetramethyl-4-piperidone hydrochloride were purchased from Aldrich (Gillingham, Dorset, UK). 4,4′-Bipiperidine dihydrochloride and formaldehyde (aqueous solution, 37% w/w) were purchased from Lancaster Synthesis (Morecambe, Lancs., UK). All reagents were used as supplied without further purification. Methanol (AnalaR) was purchased from BDH Laboratory Supplies (Poole, Dorset, UK) and de-gassed prior to use.

Results and discussion

The high rates of thermal and mass transfer within the micromixer chip provide a particularly suitable environment for exothermic reactions such as the Ugi 4 component condensation (4CC).

When performed on a typical laboratory-scale, the reaction in Fig. 1 is reported to proceed in a highly exothermic 'violent' manner.[28–30] Under such conditions, active steps are required to cool the reaction vessel. This has obvious process implications when 'scaling-up' the reaction for preparative synthesis. However, it was anticipated that the high thermal transfer characteristics of a microfluidic system should allow the reaction to proceed in a controlled manner with *no additional cooling*. High rates of thermal transfer have been successfully exploited, for example, in microscale devices for continuous-flow PCR chemistries.[6] A micro reactor capable of performing exothermic synthetic chemistries with no requirement for active cooling would be of great importance to the fine chemical industry, as compounds could be produced on a preparative scale by 'scaling out' the number of reactors rather than 'scaling up' their size. On transferring the MCR to the micromixer, a

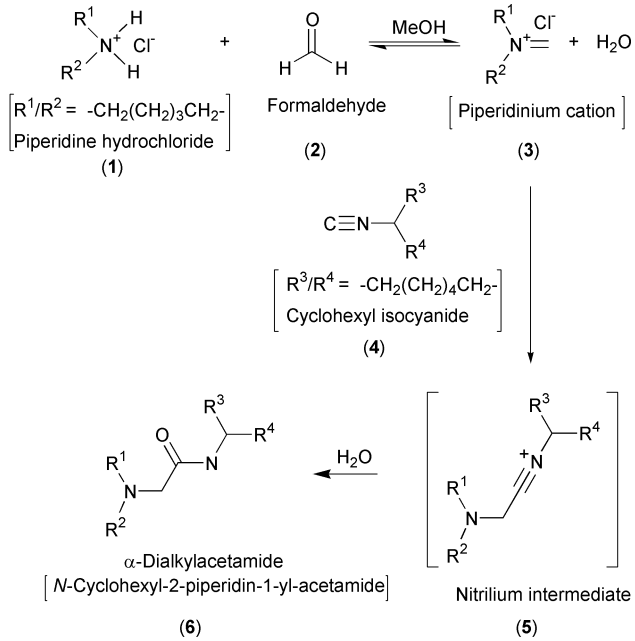

Fig. 1 The Ugi multicomponent reaction (MCR) of piperidine hydrochloride, formaldehyde and cyclohexylisocyanide.

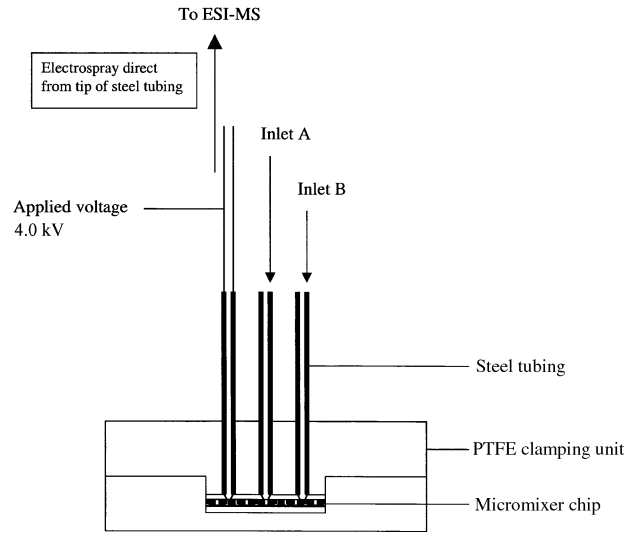

Fig. 2 Schematic diagram of micromixer/ESI-MS interface (not to scale).

methanol solution of formaldehyde (0.20 M) was infused into one of the inlets of the micro reactor as a *continuous* flow (10 μL min^{-1}) at room temperature. Solvent only was admitted to the second inlet at the same rate. The remaining MCR components (piperidine hydrochloride:cyclohexylisocyanide, ratio 0.1 : 1) were then pulsed into the micromixer *via* an injection loop (50 nL). The outlet flow of the micromixer was analyzed on-line using TOF-MS with an ESI source, and *no batch collection or purification steps*.

The detailed information yielded by the mass spectrum [Fig. 3(i)] illustrates the power and sensitivity of the micromixer-TOF-MS protocol for chemical synthesis. The $[M+H]^+$ ion for *N*-cyclohexylpiperidin-1-ylacetamide (*m/z* = 225.2) is clearly observed as the major reaction product. In addition, a series of starting materials and reaction intermediates are also observed [Fig. 3(ii)]. The fact that the desired reaction product is obtained in such excess is extremely interesting as this particular reaction is typically carried out at a reduced temperature (0 °C) for 'bench-top' scale preparation. The high intensity of the α-dialkylacetamide signal may be explained by consideration of the thermal characteristics of micro reactors; the dimensions of the microscale channels result in very high surface area:volume ratios (*ca.* 10^3–10^4 times greater than 'conventional' laboratory glassware). This results in rapid heat dissipation within the reactor environment and even an exothermic reaction such as this does not raise the local temperature significantly. Consequently, by-product formation is limited (observed mainly as two peaks, *m/z* 239 and 417, not shown) and the α-dialkylacetamide is dominant in the product mixture. Such behaviour has great implications for the future *control* of highly exo- or endothermic reactions by carrying them out on a microfluidic platform.

The utility of the system for dynamic control of the progress of a reaction is shown in Fig. 4. A series of reactions were performed in which piperidine hydrochloride [(Fig. 4(a) and (c)] and 4,4′-bipiperidine dihydrochloride [Fig. 4(b) and (d)] were allowed to react with formaldehyde at a variety of flow rates (20–2 μL min^{-1} total flow rate). With both reaction systems, reduced flow rates result in increased intensities of reaction products relative to the starting materials. Indeed, the use of TOF-MS as the analysis component of the μSYNTAS allows the course of the complex reaction of 4,4′-bipiperidine dihydrochloride with formaldehyde [Fig. 4(b)] to be followed very closely [Fig. 4(d)]. A variety of singly and doubly charged products and by-products are observed, and their peak intensities may be manipulated by judicious control of the flow

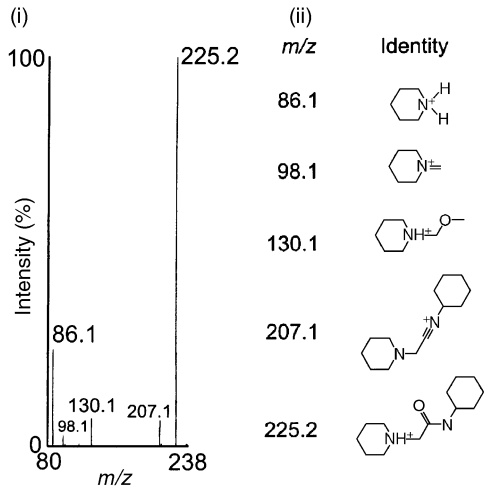

Fig. 3 (i) The mass spectrum obtained from the on-chip Ugi MCR. Electrospray conditions were achieved using an applied voltage of 4 kV and nebulizing gas flow. A methanol solution of formaldehyde (0.20 M) was infused under continuous-flow conditions into one inlet of the micromixer; pure methanol was infused into the remaining inlet of the micromixer. A methanol solution containing piperidine hydrochloride (0.020 M) and cyclohexylisocyanide (0.20 M) was then injected into the pure methanol flow *via* an injection loop (50 nL). (ii) Major ions observed in the mass spectrum of the on-chip Ugi MCR.

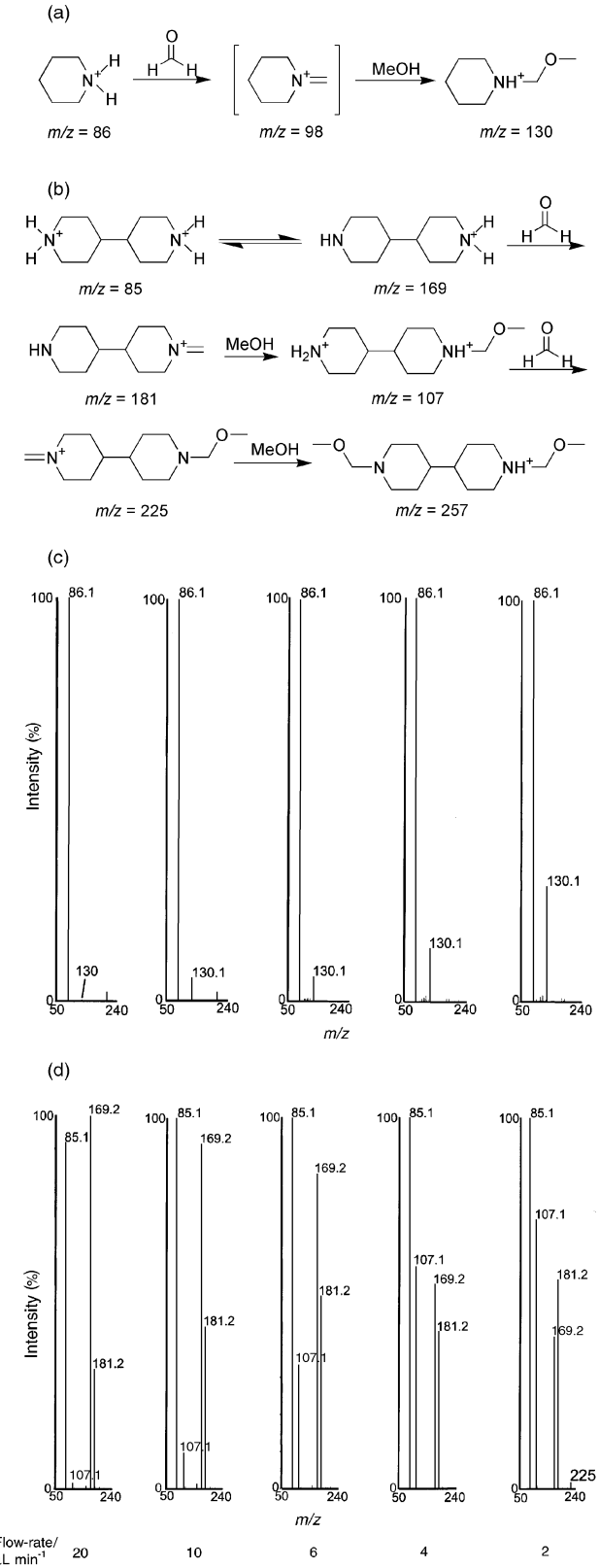

Fig. 4 (a) The reaction of piperidine hydrochloride (0.2 mM) with formaldehyde (20 mM) in methanol. (b) The reaction of 4,4′-bipiperidine dihydrochloride (0.2 mM) with formaldehyde (20 mM) in methanol. (c) Mass spectra (*m/z*, 0–240) at flow rates of 20–2 μL min^{-1} for the reaction of piperidine hydrochloride with formaldehyde in methanol. (d) Mass spectra (*m/z*, 0–240) at flow rates of 20 –2 μL min^{-1} for the reaction between 4,4′-bipiperidine dihydrochloride and formaldehyde in methanol.

rate. It is therefore seen that optimization of the experimental conditions to favour one product over another may be performed with ease, offering a route towards automated, high-yielding chemical syntheses. The on-line nature of the coupling between synthesis and analysis steps is clearly a significant development in the progress towards a μSYNTAS for reaction optimization.

An extremely interesting aspect of this particular reaction relates to the issue of reaction intermediates. It has been suggested[32] that this particular MCR initially involves the production of an iminium species (**3**) (Fig. 1) followed by the attack of cyclohexylisocyanide (**4**) to yield a putative nitrilium cation intermediate (**5**) prior to formation of the final α-dialkylacetamide product (**6**). To our knowledge, this is the first instance of the experimental confirmation of the production of (**5**) (*m/z* 207.1). Furthermore, it is apparent in Fig. 3 that there is evidence of a side-reaction product (*m/z* = 130.1) previously unreported within this reaction scheme. Further evidence for the provenance of this species was obtained *via* a number of studies involving the microscale reaction between methanol solutions of piperidine hydrochloride and formaldehyde. Under the Ugi reaction conditions, it is clear that the iminium cation (**3**) undergoes two competing reactions, one involving (**4**) to yield the final product (**6**), and one involving reaction solvent (methanol) to give 1-methoxymethylpiperidine. The possible role of this by-product in determining the overall pathway of this particular Ugi reaction is particularly interesting and is currently under examination in this laboratory.

As has been demonstrated, the high level of structural information provided by the continuous-flow μSYNTAS provides a means for determining mechanistic characteristics of even highly complex reaction systems. Work is currently underway in this laboratory towards the dynamic optimization of reaction products by manipulation of other system parameters in real-time. In comparison with solid-phase technologies, the automation of the cycle involving parameter manipulation and on-line analysis may be developed to a much higher degree in a solution-phase system. Consequently, the use of μSYNTAS for optimization of compound library syntheses may be a useful addition to the combinatorial chemistry toolkit.

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