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Microchip-based synthesis and total analysis systems (μSYNTAS): chemical microprocessing for generation and analysis of compound libraries

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A miniaturised-SYNthesis and Total Analysis System (μSYNTAS) integrating a silicon-machined chemical microprocessor and time-of-flight mass spectrometry (TOF-MS) is used for the generation of compound libraries based on sub-reactions of an Ugi multicomponent reaction (MCR). The microreactor—based on the concept of an AND logic operator—allowed the coupling of serially-switched solution-phase library generation with on-line compound analysis and identification. In addition, the μSYNTAS allowed real-time parallel-processing of MCR sub-reactions; in contrast to combinatorial techniques employing a solid support for reagent and product isolation, the μSYNTAS protocol required no additional preparation or work-up procedures.

Introduction

Parallel synthetic protocols utilising resin bead,[1] magnetic bead,[2] multipin,[3] disk (or 'wink')[4] and 'tea-bag'[5] technologies have become highly developed in recent years, providing routes for drug discovery *via* the coupling of compound library generation with high-throughput screening.[6,7] However, the widespread dependence of these approaches on solid-support technologies for reagent and product handling has constrained them in terms of their operational flexibility. For example, efficient attachment and detachment to and from the support are crucial for successful library generation, increasing the number, time and financial cost of the required process steps. Issues relating to the possible influence of solid supports on reaction chemistry are well documented,[1,8] and rapid, facile optimisation of solid-supported chemistries is problematic.[9] Correspondingly, the reasons to pursue solution-phase combinatorial chemistries for library generation are numerous: unlimited numbers and types of reactions may be used; the large excesses of solvents and reagents typically used in solid-phase syntheses are not required, and the development and monitoring of such chemistries is more easily performed.[10]

Developments in miniaturised-Total Analysis Systems (μTAS)[11] in recent years have been driven by the benefits of reduced analysis times, increased efficiencies of mixing and separation, and reduced consumption of reagents. The potential gains of increased performance arising from miniaturised *analysis* systems combined with miniaturised *reaction* methods have been the rationale behind the development of miniaturised-SYNthesis and Total Analysis Systems (μSYNTAS). In this paper we describe the integration of continuous-flow synthesis and on-line analysis within a microfabricated structure,[12] to provide a highly effective route for the solution-phase generation of compound libraries. Such a system, based on a distributive micromixing device coupled with time-of-flight mass spectrometry (TOF-MS), has allowed discrete multicomponent reaction (MCR) chemistries to be performed, analysed and optimised in real-time.[13] The potential of this strategy to deliver mechanistic and kinetic information on synthetic processes, and to perform chemistries under unusual reaction environments has thus provided an additional impetus for the development of the μSYNTAS protocol.

In order to exploit fully the potential benefits associated with chip-based solution-phase chemistries, it has been the aim of our research to examine the flexibility of the μSYNTAS approach under a variety of operational modes. The ability to perform sequences of discrete reactions in a serial, switching manner is highly desirable for subsequent integration with screening methods ('target-oriented synthesis'). Alternatively, the ability to perform parallel solution-phase reactions with on-line, real-time identification of reaction components is desirable for high-throughput library generation and diversity-oriented protocols. In this paper we demonstrate that serially-switched *and* parallel chemical processing in a μSYNTAS is, indeed, a viable approach for continuous flow solution-phase generation of compound libraries on the microscale. Such developments should have far-reaching consequences for high-throughput reaction screening technologies and automated product library synthesis.

Experimental

Design and principle of presented micromixer

The microreactor used for all experiments operates on the principle of distributive mixing, *i.e.* two inlet flows are split into a series of multichannel streams which, when combined within the silicon manifold, provide an extremely large diffusional surface area for rapid, efficient mixing. The mixer structure is made up of a glass–silicon–glass sandwich, has an internal volume of ~600 nL and measures 2 × 5 × 10 mm. Fabrication and design methods are discussed in detail elsewhere.[12]

Materials

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

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Experimental conditions and set-up

The micromixer was coupled to a TOF-MS (Mariner, Perseptive Biosystems, Foster City, CA, USA) *via* an electrospray unit. Fused silica capillaries (TSP150375, Composite Metal Services Ltd, Hallow, UK) were coupled to the surface of the micromixer and clamped in place with a poly(tetrafluoroethylene) (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, using a Rheodyne injection valve (50 nL injection loop) for introduction of discrete sample pulses into the μSYNTAS. 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.

Switching reagent injection

A methanol solution of formaldehyde (20 mM) was infused continuously (3 μL min^{-1}) into one inlet of the micromixer. Into the remaining inlet of the micromixer, methanol solutions of piperidine hydrochloride (0.2 mM) and 4,4′-bipiperidine dihydrochloride (0.2 mM) were alternately injected at intervals of 90 s.

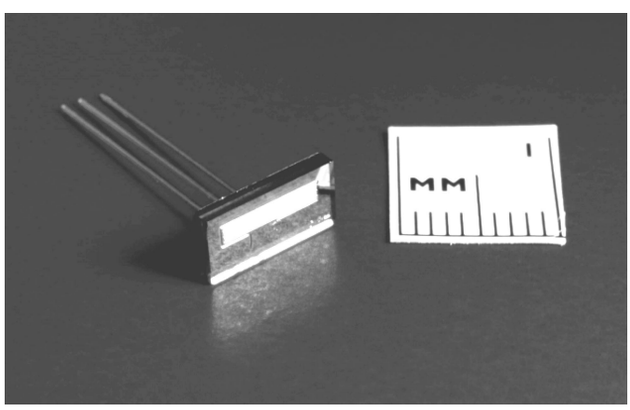

Fig. 1 Two-input, one-output glass–silicon microreactor.[12]

Serial reagent injection

A methanol solution of formaldehyde (20 mM) was infused continuously (3 μL min^{-1}) into one inlet of the micromixer. Into the remaining inlet of the micromixer, methanol solutions of 3-hydroxypiperidine hydrochloride (0.2 mM), 2,2,6,6-tetramethyl-4-piperidone hydrochloride (0.2 mM), piperidine hydrochloride (0.2 mM), 4,4′-bipiperidine dihydrochloride (0.2 mM) and 4-hydroxypiperidine monohydrate hydrochloride (0.2 mM) were alternately injected at intervals of 120 s.

Parallel reagent injection

A methanol solution of formaldehyde (20 mM) was infused continuously (3 μL min^{-1}) into one inlet of the micromixer. Into the remaining inlet of the micromixer, a methanol solution comprising 3-hydroxypiperidine hydrochloride (0.04 mM), 2,2,6,6-tetramethyl-4-piperidone hydrochloride (0.04 mM), piperidine hydrochloride (0.04 mM), 4,4′-bipiperidine dihydrochloride (0.04 mM) and 4-hydroxypiperidine monohydrate hydrochloride was injected.

Results and discussion

The μSYNTAS is composed of two core elements: chemical microprocessing and chemical analysis. The chemical microprocessor is based upon a silicon-machined micromixer (Fig. 1).[12] which utilises distributive mixing in order to achieve extremely rapid rates of diffusional mixing on the microscale. Mixing motifs within microstructures have been examined by a number of groups.[14,15]

The two-input, one-output arrangement of the chemical microprocessor allows us to make a conceptual analogy with an electronic logic gate. If we consider a logic gate which performs the AND operation under the rules of Boolean algebra, we see that an output of '1' is only obtained when both inputs have the value '1' (where '1' denotes an active input/output; '0' inactive) [Fig. 2(a)]. Similarly, we can consider a two-input, one-output reaction device—the microreactor—in much the same way. Here we would only expect to see a desired product, ***C*** (gate output value '1') when both reagents ***A*** and ***B*** are delivered to

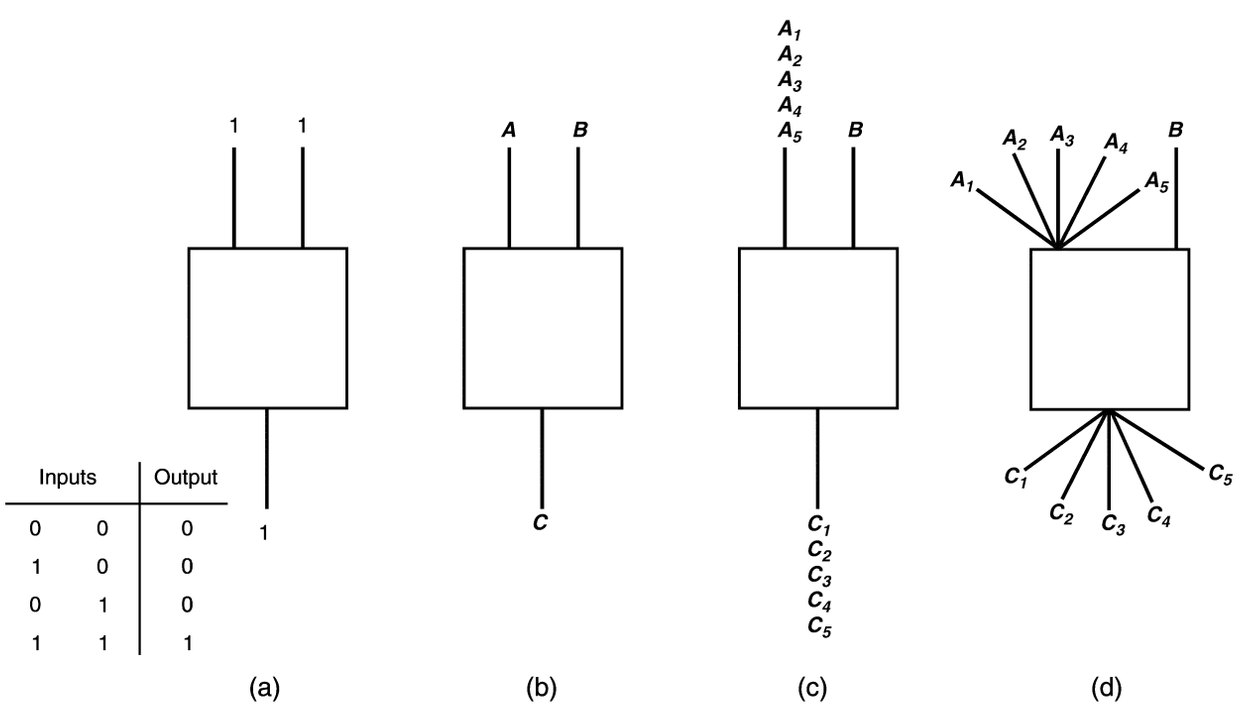

Fig. 2 (a) Logic gate and truth table functioning under the rules of the AND operator; (b) chemical AND microprocessor; (c) chemical microprocessor operating under continuous-flow conditions for the serial synthesis of C_1–C_5 derived from reagents A_1–A_5 and ***B***; (d) chemical microprocessor operating under parallel conditions for continuous-flow compound library synthesis.

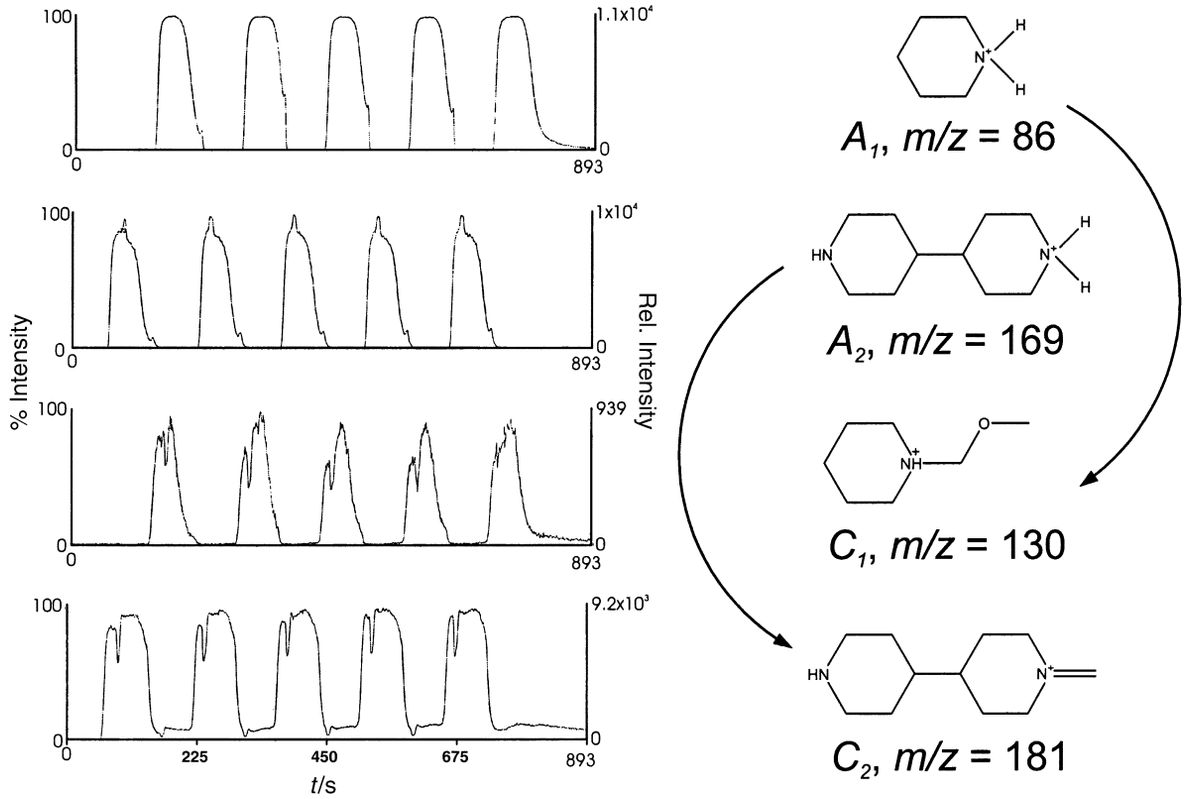

Fig. 3 The alternating injection of methanol solutions of piperidine hydrochloride (A_1) (0.2 mM) and 4,4′-bipiperidine dihydrochloride (A_2) (0.2 mM) into a stream of formaldehyde in methanol (20 mM) under continuous-flow conditions. The peak shape observed for A_1 (with a flattened profile) is most likely due to the signal intensity reaching the maximum for the detector during peak elution. The peak shapes observed for A_2, C_1 and C_2 are explained in terms of the influence of the dead-volume of the valve during injection. The 1-methylenepiperidinium cation (expected from the reaction with piperidine hydrochloride, A_1) is not detected directly under these conditions; instead 1-methoxymethylpiperidine (C_1) the product of further reaction with methanol, is observed.[22]

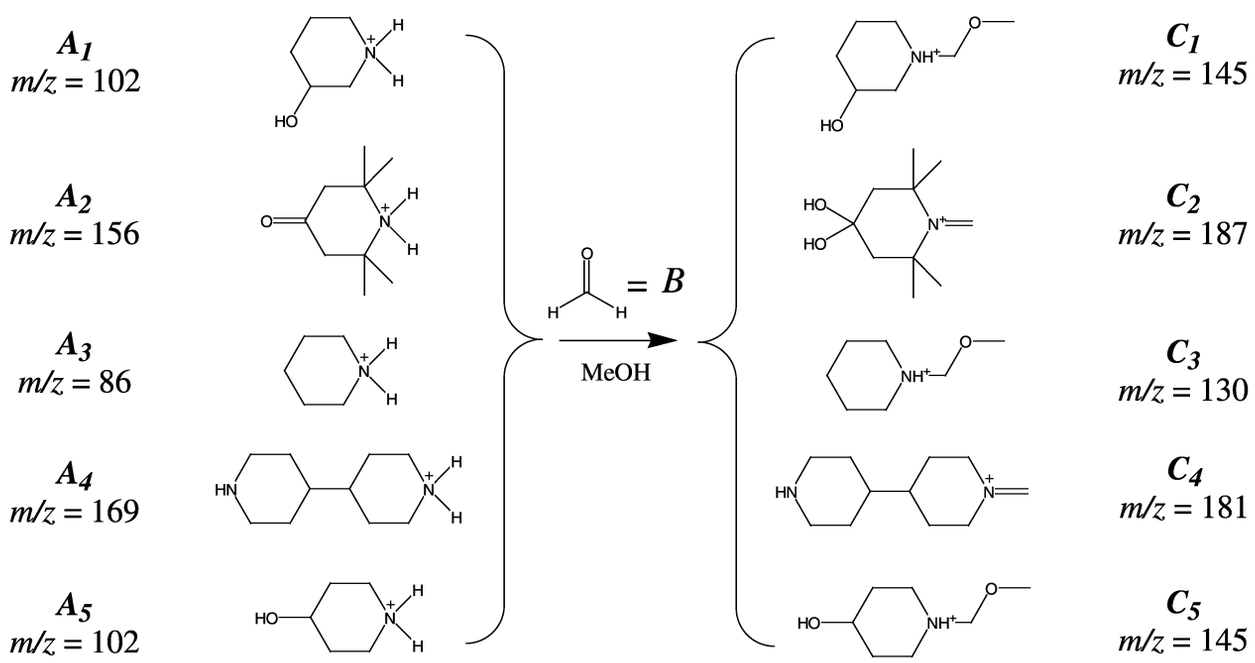

Fig. 4 Compound library synthesis: the transformation of five piperidine-based hydrochloride salts (A_1–A_5) with formaldehyde (B) to give products (C_1–C_5).

the two inputs (gate input values '1') simultaneously [Fig. 2(b)]. Thus, the concept of a switchable μSYNTAS in which reagents are 'pulsed' or 'switched' between '1' and '0' (reagent 'present' and 'absent') could form the basis of a highly automated reaction-screening device. The notion of applying Boolean logic to molecular chemistries has become popular in recent years[16,17] and for good reason; such approaches have yielded fundamental advances in the development of electron- or photon-activated molecular switches and numerous groups are engaged in reproducing the functions of semiconductor logic gates at a molecular level.[18] To our knowledge, however, no-one has yet attempted to use logic-based microsystems for *chemical synthesis*. Of course, the μSYNTAS concept is not constrained to operate purely under binary conditions (the presence or

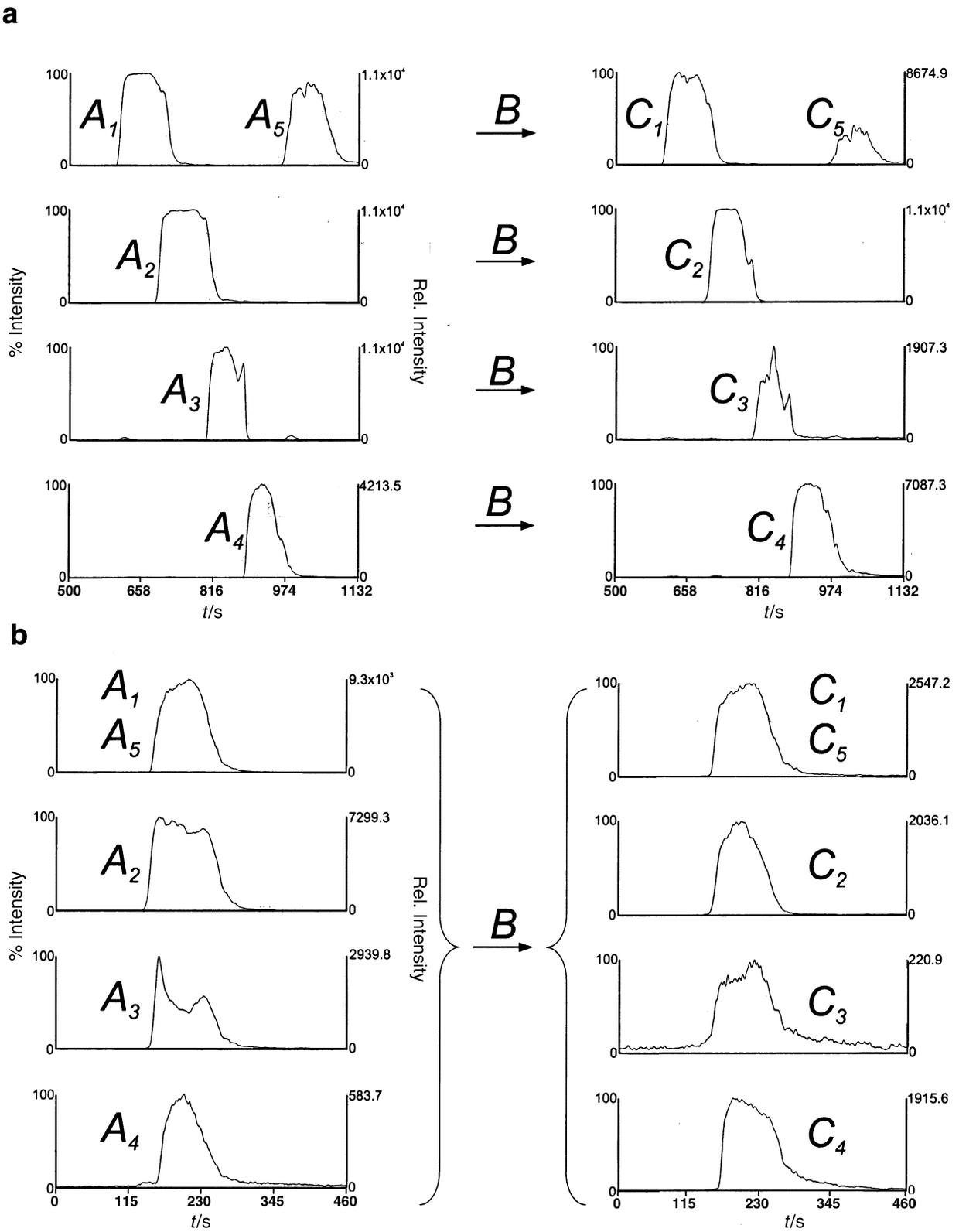

Fig. 5 (a) Mass chromatograms for the sequential injection of A_1–A_5 (0.2 mM) into a stream of formaldehyde in methanol (20 mM) under continuous-flow conditions to give corresponding products (C_1–C_5). Serial injections of the reagents were made at intervals of 120 s. It should be noted that since A_1 and A_5 are structural isomers (differing only in the position of the hydroxy moiety) they are resolved in the mass chromatograms with the same *m*/*z* ratio. (b) Mass chromatograms showing the parallel injection of A_1–A_5 to provide products (C_1–C_5). A single injection of a methanol solution containing equimolar concentrations (each 0.2 mM) of reagents (A_1–A_5) was performed. Discrimination between structural isomers (A_1 and A_5; C_1 and C_5) cannot be inferred directly from these chromatograms since they are resolved in the same mass chromatogram.

absence of a single reagent); in this paper we demonstrate the operation of a μSYNTAS performing reaction chemistries with high orders of system complexity, *i.e.* 'switching' serial [Fig. 2(c)] and parallel [Fig. 2(d)] chemical processing. Such developments should have far-reaching consequences for high-throughput reaction screening technologies and automated product library synthesis, in addition to the possibilities of molecular computation.

In order to examine the behaviour of the μSYNTAS operating under serial- and parallel-mode conditions, it was necessary

to use a set of reaction chemistries typical of those used for solution-phase combinatorial library generation. A number of solution-phase approaches have been developed and are summarised in a recent review.[10] The utility of multicomponent reactions (MCRs) for library generation is well-known [19] and an early Ugi MCR [20] was chosen to examine the behaviour of the μSYNTAS as a microreaction/microanalysis device. One of the sub-reactions of the MCR, *viz.* the production of iminium cations by the reaction of secondary amine hydrochlorides with formaldehyde, was initially chosen as a model reaction. Fig. 3 illustrates a period of 15 minutes during which pulses of piperidine hydrochloride (***A*** $_1$) and 4,4′-bipiperidine dihydrochloride (***A*** $_2$) were alternately injected into one inlet of the micromixer at intervals of 90 s; a continuous flow of formaldehyde (***B***) was infused into the remaining inlet. A flow-rate of 3 μL min^{-1} was maintained at both inlets (total flow-rate, 6 μL min^{-1}). In this system, the zone (or 'reagent plug') corresponding to each injected reagent (***A*** $_1$ or ***A*** $_2$) takes a finite amount of time (*ca.* 120 s) to reach the point of confluence within the micromixer. It is only at that moment that mixing between the two inlet streams is achieved and the reaction between formaldehyde and ***A*** $_1$ or ***A*** $_2$ may begin. Thus, when the reagent plug reaches the outlet of the micromixer, a mixture of reagents and products will be present in the outlet stream. The reagent plug takes *ca.* 15 s to reach the detector of the TOF-MS and reagents and products are observed simultaneously. The alternate injection of ***A*** $_1$ and ***A*** $_2$ results in an alternating pattern of reagents and products (Fig. 3). It can be seen that the peaks of each product and each reagent appear with excellent reproducibility in the peak shape. It is also apparent that cross-contamination between reagent flows through the micromixer channel is negligible. This illustrates the operation of a *truly switching chemical microprocessor operating in 'serial' mode*.

The performance of the microprocessor was investigated in serial mode and parallel mode with reactions between five secondary amine hydrochloride salts (***A*** $_1$–***A*** $_5$) and formaldehyde (***B***) (Fig. 4). In serial mode, the five reagents are injected (3 μL min^{-1}) into a continuous flow of formaldehyde (3 μL min^{-1}) at intervals of 120 s [Fig. 5(a)]. The peaks corresponding to the unreacted amine salts (***A*** $_1$–***A*** $_5$) and the products of reaction (***C*** $_1$–***C*** $_5$) are clearly resolved. It is noted that there is some significant overlap between reagent peaks and between product peaks, with *no* discernible effect on the peak shapes; it may be concluded, therefore, that baseline separation is *not* a requirement for the identification of reagents and products in serial-mode synthesis. This observation has obvious implications for transferring high-throughput screening methods to this μSYNTAS.

Of greater significance is the trace shown in Fig. 5(b). Here, all five reagents (***A*** $_1$–***A*** $_5$) are injected *simultaneously* under the conditions described above. All reagents and all products are *fully resolved* by their corresponding *m*/*z* ratios except, of course, the isomeric reagents (***A*** $_1$ and ***A*** $_5$) and isomeric products (***C*** $_1$ and ***C*** $_5$). Examination of the mass spectrum for the parallel-mode reaction indicates the presence of no additional product peaks in comparison with the serial-mode reaction; cross-reaction between the reagents is therefore minimal. Clearly, the combined use of serial ('time-encoded') and parallel ('mass-encoded') modes of operation would be required for the optimal synthesis and analysis of a wide range of reaction chemistries. These ideas are currently being addressed.

The concepts described in this paper have many potential applications, but the most exciting directions for future work build upon the concept of the chemical microprocessor as but one component within a μSYNTAS. The significance of operating under continuous-flow conditions cannot be overstated as this protocol will allow the integration of library generation, component identification and screening to be performed fully on-line and in real-time. Arrays of microdevices could feasibly be used for the synthesis, derivatisation and subsequent analysis of products with extremely high throughput capacity. As the pharmaceutical industry moves towards the development of drugs 'tailored' to specific population genotypes (pharmacogenomics),[21] the synthesis and screening of large numbers of structurally-related molecules gain ever-greater importance. Arrays of μSYNTAS devices will provide a route towards the automation of such processes.

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