A flow process for the multi-step synthesis of the alkaloid natural product oxomaritidine: a new paradigm for molecular assembly

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A flow process for the multi-step synthesis of the alkaloid natural product (±)-oxomaritidine is described, mediated through the use of microfluidic pumping systems that progress material through various packed columns containing immobilized reagents, catalysts, scavengers or catch and release agents; our route involves the combination of seven separate synthetic steps linked into one continuous sequence utilizing flow chemistry.

The often low availability of natural products from their sources of origin, combined with the lack of structural analogues, creates a need for laboratory synthesis. This is especially true if these materials are to be used as biological probes for drug discovery programs. Consequently, new techniques are urgently required to overcome many of the bottlenecks associated with conventional organic synthesis. Also more use of automation is needed to facilitate scale-up and to improve the current synthesis standards in terms of reaction optimisation and product clean-up practices. For some years, we have been evolving the concept of using immobilised reagents, scavengers and catch and release techniques to overcome many of these problems.[1] It has always been a part of our vision that these techniques would eventually be incorporated into micro-[2] and meso-flow[3] reactors to greatly enhance current processing capabilities, developing new strategies for how future synthesis programmes may be conducted in a far more effective fashion.

In recent years, the search for new synthetic methods has moved beyond the traditional quest for new reactions and reagents. The scope of the search has now broadened to incorporate new practical preparative methods for increasingly complex organic transformations. One of the most exciting and potentially significant developments is the innovative incorporation of flow chemistry into lab based synthesis platforms. Flow chemistry replaces traditional glassware with columns and cartridges that can be pre-packed with immobilised reagents and catalysts, or through the use of precision manufactured reaction chips, that permit controlled mixing and precise temperature control of reaction sequences. This essentially transforms conventional reaction methods into a flowing, dynamic system by either passing the starting materials through an immobilized reagent or combining the starting materials and reagents on a reaction chip. The product then exits from the reactor into a chemically unreactive environment that is beyond the reach of any further chemical transformation. These methods allow for the immediate collection of a pure product upon elution from the reactor in an expedited cost-conscious manner. An additional benefit to this mode of chemistry is that it allows for the linking of individual reactions into multi-step sequences, allowing for one reaction to flow seamlessly into another, creating a rapid route to the desired more complex product by combining multiple synthetic steps into a continuous operation. In addition, the incorporation of automated and real-time analysis facilitates rapid optimisation and excellent quality control whilst also ensuring reproducibility. By varying flow rates, solvents, temperatures and pressures, these information-rich sequences create opportunities for sensitive modification of chemoselective processes and constitute a paradigm shift for the on-demand delivery of chemical substances. Flow chemistry extends the opportunities available to organic chemists, providing as it does, improved reactivity, better selectivity and simple in-line analysis.

Here we report on the first multi-step flow through preparation of a natural product, utilising flow methods and techniques. What is significant in this work is that no product isolation at intermediate stages was necessary, no use was made of labour intensive techniques such as product distillation or crystallisation, nor was column chromatography required to furnish materials of appropriate quality in order to progress to the next step in the synthesis. The time saved by using these flow through methods compared to conventional procedures is dramatic. For example, even when supplied with an optimised procedure using standard protocols, one might expect a typical synthesis using conventional round-bottomed flasks of the Amaryllidaceae alkaloid natural product oxomaritidine (**1**) (Fig. 1) to require some four days of laboratory manipulation,[4] involving, as it does, setting up of the apparatus, conducting and monitoring of the individual reactions, determination of the completion of the reactions, quenching (usually by water washing, solvent extraction and evaporation), and subsequently column chromatography to purify individual products at all the intermediate stages.

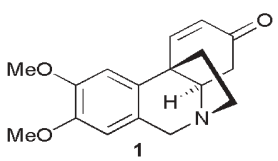

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Fig. 1 The natural product (±)-oxomaritidine (**1**).

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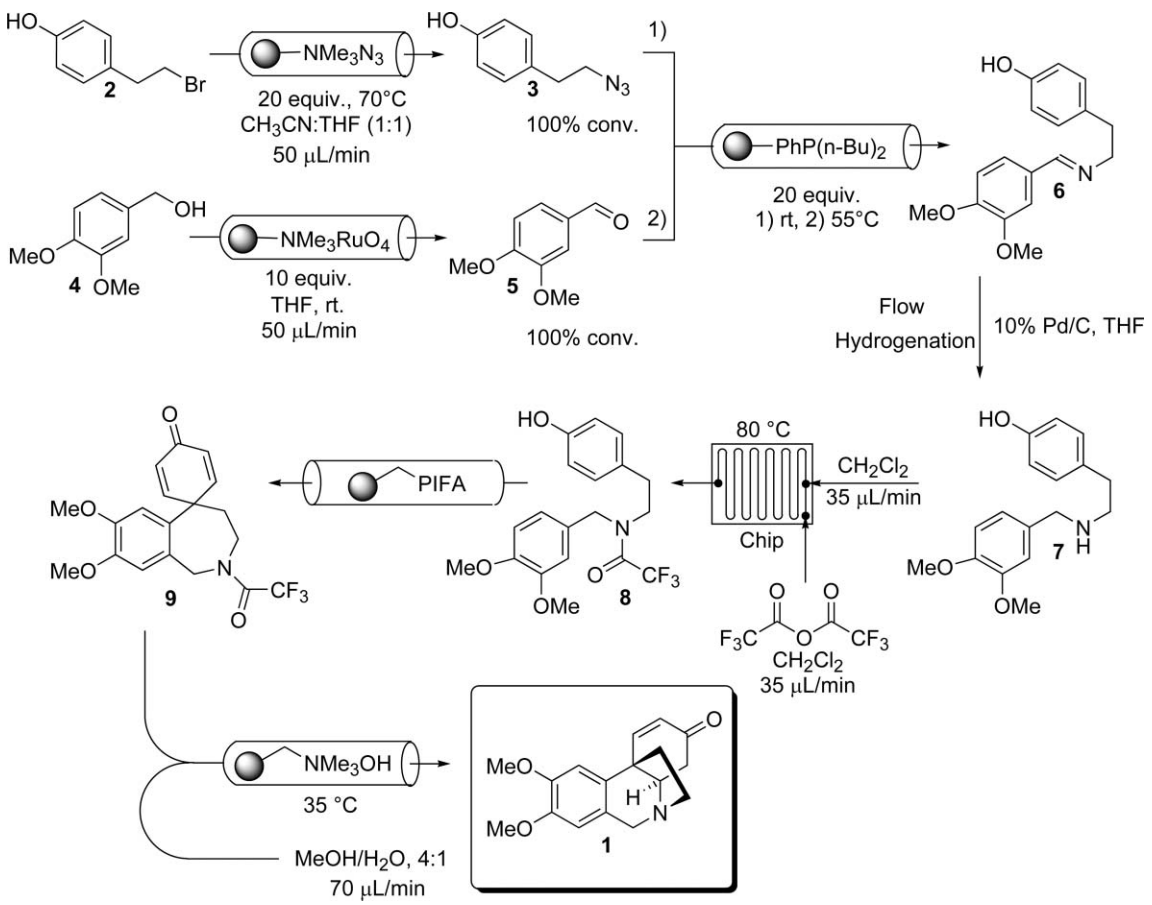
(±)-Oxomaritidine (**1**) is a cytotoxic alkaloid of the Amaryllidaceae family of natural products.[5] We have previously reported a total synthesis of this biologically important compound using only polymer-supported reagents at every individual stage of the synthesis pathway.[4] The significance of this report was the development of a route that was completed without the need for column chromatography or the use of aqueous work-ups at any stage. Here we build upon this prior knowledge to devise a flow through reaction sequence that produces the natural product in an automated sequence from readily available starting materials in less than a day (Scheme 1). This was accomplished by directly coupling glass reaction columns[6] in series, thereby providing multiple synthetic transformations in one flow-through process.

In the first step of the sequence a commercially available bromide, 4-(2-bromoethyl)phenol (**2**), was quantitatively converted to the corresponding azide (**3**). This was accomplished by packing a glass column with an azide exchange resin[7] and flowing a solution of the bromide through the supported reagent using a Syrris AFRICA® system.[8] The output reaction stream from this step containing the newly generated alkyl azide (**3**) was coupled directly into a second column containing a polymer-supported phosphine,[9] furnishing the corresponding aza-Wittig intermediate trapped on the supported material. Simultaneously, within a separate, but convergent channel, was prepared the aldehyde coupling partner (**5**). A pre-packed column of tetra-*N*-alkylammonium perruthenate (PSP)[10] was used to facilitate the oxidation from the commercially available, 3,4-dimethoxybenzyl alcohol (**4**). This product stream was then passed through the column containing the immobilized aza-Wittig intermediate, producing the desired imine (**6**). The tetrahydrofuran (THF) solution of imine (**6**) was then subjected to continuous flow hydrogenation utilizing a Thales H-Cube® flow hydrogenator,[11] which contained a cartridge of 10% palladium on carbon as catalyst. A more detailed report of this work has been given previously by our group.[12] The product of this reaction (**7**) was then collected on-line and the THF solvent was removed using a Vapourtec V-10® solvent evaporator[13] and re-dissolved in dichloromethane (DCM), an operation that can be readily accomplished in less than ten minutes. It should be noted that the simple solvent-switch procedure following the flow hydrogenation step, from THF to DCM, represents the only product handling operation provided by the user and serves as a useful point of concentrating the product for the final transformations.

In the last series of coupled reactions, passage of the secondary amine (**7**) onto a microfluidic reaction chip that combines an additional stream of trifluoroacetic anhydride (TFAA) in DCM resulted in the trifluoroacetylation of the amine to give the amide (**8**). The reaction stream was then passed through a short scavenging column containing a silica-supported primary amine[14] that removed any excess TFAA or residual trifluoroacetic acid (TFA). The optimal conditions for this reaction were found to be 5 equivalents of the TFAA, a temperature of 80 °C, and a residence



Scheme 1 Synthesis in flow of (±)-oxomaritidine.

time of just over 3.5 minutes. It should also be noted that a back-pressure regulator was essential in this procedure and was connected in-line with the exiting reaction stream, allowing heating of DCM to 80 °C, well above its usual boiling point. This reaction stream was then directed into a column containing polymer-supported (ditrifluoroacetoxyiodo)benzene (PS-PIFA)[15] which performed the oxidative phenolic coupling, generating a seven-membered tricyclic intermediate (**9**). The resulting product was then passed directly into a column that contained a polymer-supported base[16] which promoted cleavage of the amide bond, allowing for a 1,4-conjugate addition to spontaneously take place, generating the target compound (±)-oxomaritidine (**1**). The output from the completed synthesis in flow was evaporated *in vacuo* and the reaction product was found to have in excess of 90% purity by ^{1}H NMR spectroscopy. The purity assay is even more impressive when considering that the only impurity was identified as the ortho-coupled product derived from the phenolic oxidation and was easily separated from the desired product using preparative HPLC, producing 20 mg of **1**.

The reproducible yield over the entire sequence was found to be slightly greater than 40%. In an effort to identify the fate of the unrecovered material, the reaction sequence was dissected stepwise. It was found that the phenolic oxidation gave only a moderate 50% yield, with the resulting impurities and unreacted starting material being scavenged out by the polymer-supported base during the last step of the flow synthesis. The other six steps in the sequence occurred with quantitative to near quantitative conversion. This indicates that the only issue in our sequence of synthetic transformations was the inability of current supported reagents to offer a high yielding synthetic alternative, a matter we plan to address and solve in the near future.

While in this work we describe just one multi-step preparation of a natural product using flow methods, we believe that such an approach could be readily applied to the synthesis of other compounds. The application of these techniques constitutes a new paradigm for molecular assembly and will certainly have a very significant impact on how synthesis programmes will be conducted in the future. The benefits attained are in terms of both the cost and efficiency gained through the avoidance of extensive purification and work-up procedures, the rapid optimisation and precise control of reaction conditions, and a reduction in manual handling. The swift adoption of these techniques and principles will provide chemists with methods that allow for the rapid production of chemical targets in a fashion far superior to that permitted by conventional methods. With this ability to rapidly access on demand targets it becomes possible to quickly interrogate and probe biological systems, allowing for the immediate integration of new screening and assay knowledge into the next iteration of molecular design and, in turn, simplifying and expediting the decision-making processes in the lead generation and optimisation stages of drug discovery programs.

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