

METHODS AND PLATFORM FOR CHEMICAL SYNTHESIS

Related Applications

- 5 This application claims priority from, and the benefit of, GB 2213747.5 filed 20 September 2022 (20.09.2022), the contents of which are incorporated by reference in their entirety.

Field of the Invention

- 10 The invention provides a method and apparatus for performing and characterising a chemical synthesis, such as those performed with an automated chemical synthesis platform, including such that are portable platforms.

Background

- 15 The synthesis of complex organic molecules requires a very high degree of manual labour from highly trained experts who work in well controlled laboratory environments that provide sufficient infrastructure for synthesis (Nicolaou 1996; Nicolaou 2003; Nicolaou 2011). Integrating automation into chemical laboratories can increase chemical accessibility, and
20 replaces procedural ambiguities (i.e. add dropwise, fast stirring, room temperature) with defined parameters, increasing the reliability of complex syntheses (Mehr). Current automated technologies typically focus on strictly circumscribed subsets of synthetic chemistry for discrete target molecule classes, resulting in different platforms for small molecule synthesis (Li; Coley; Steiner; Angelone; Mehr) or solid phase synthesis (SPS) e.g.
25 oligopeptides (Merrifield), oligonucleotides (Alvarado-Urbina), oligosaccharides (Plante; Joseph).

- While SPS systems can be applied to many small molecule transformations, such systems require extensive method development and remain based on only a small subset of practical
30 chemistries (Jiang; Ley). This means the majority of synthetic approaches already employed by chemists are neglected, and new synthetic routes must be designed and tested for even well-known compounds with established syntheses. Examples of small molecule synthesis platforms (either in batch or in flow) which can perform a greater range of chemical processes are usually modular in nature and can require extensive reconfiguration to switch
35 from one manufacturing process to another (Schotten; Chatterjee; Britton; Bédard; Ghislieri; Zhang). Coupled to this, these synthetic platforms are often infrastructure intensive, commonly lab-based and occupy large spaces in research facilities. In addition, compact universal platforms can be challenging to develop due to the laboratory infrastructure needed for syntheses. A platform that could be compact and prepare any molecule on-
40 demand, autonomously, and locally could increase accessibility of important molecules.

Continuous multistep linear synthesis processes can be complex and technically challenging (using different equipment for each step), requiring reconfigurable systems to complete even a relatively short synthetic protocol. One way to remove the necessity for extensive platform reconfiguration and miniaturize laboratory hardware is the use of bespoke, self-contained,
5 modular reactors for multi-step synthetic procedures such as 3D printed reactors can be used (Kitson *et al.*; Zalesskiy *et al.*; Hou *et al.*). Nonetheless, even though the entire synthetic route is enclosed within the reactors, manual execution is still needed, and the system must ideally be situated in a highly controlled and well serviced laboratory.

10 Recent developments have exploited the use of suitably programmed robotic systems together with modular reactors for the development of standardised reaction platforms for chemical synthesis. Such systems are intended to reduce the experimental burden on the operator by making available suitable coding for the production of all the components of the reactionware, which is itself developed responsive to the encoding of the reaction protocols
15 for the synthesis of the desired product.

For example, the present inventor has shown how literature methods in natural language may be converted into a machine-readable instruction set: see WO 2021/219999. This instruction set may then be used to generate a hardware index, which is the required
20 reactionware for the performance of a synthetic procedure.

Similarly, the present inventor has also shown how a method of synthesis for a target product can be translated into digital model of that method: see WO 2019/137954. This digital model comprises a digital description of the chemical and/or physical steps within the
25 method of synthesis. From this, a digital reactionware may be developed, which provides a digital reaction module for each step in the process sequence, and the digital modules are digitally interconnected for the digital production of a target product. From this digital reactionware the physical reactionware is generated, with the physical reactionware has a module for each step in the process sequence.

30 Using such machine-readable instruction sets and physical reactionware developed from digital model that are responsive to the machine-readable instructions, it is possible to develop and utilise standardised models for chemical synthesis that can be executed by automated synthesiser. Whilst different synthesisers may operate under the control of
35 similar instructions, and may utilise similar reactionware developed from a common digital model, there still exists uncertainty in the conduct of reactions between these different synthesisers, and the quality of the products that are produced. Consequently, there is a need for an adaptable chemical synthesis platform for use in conducting chemical syntheses under reproducible conditions, and with certainty as to the progression of the synthesis and
40 with validation of the final product.

Summary of the Invention

In a general aspect the present invention provides methods and apparatus for performing a chemical synthesis, and for characterising the chemical synthesis in the form of a profile, or fingerprint, for comparison against a reference profile for the synthesis. A profile is generated from analytical data recorded over the time course of the chemical synthesis and it may be regarded as a characterisation of the synthesis.

The creation of the profile, and its comparison against a reference profile, permits an operator to assess the success of any chemical synthesis performed, and also allows the operator to recognise failures in expected performance, for example where there is a divergence of the profile from the reference profile for the synthesis.

The profile may itself provide validation for the chemical synthesis and may provide a guarantee that the synthesis has been conducted in an appropriate manner, and consistent with the approved synthesis from which a reference profile is obtained. In this way, a third party can assume the identity and the quality of a product based on the profile that is generated during its production in the chemical synthesis. Here, the profile is the evidence that the chemical synthesis has been followed in accordance with the approved synthesis, with the appropriate reactions and purifications steps undertaken, as needed, and at the appropriate times, and therefore the desired product must then inevitably result.

Similarly, where there is a difference between the profile and the reference profile, then it can be understood that the chemical synthesis was not performed according to the approved synthesis, whether unintentionally or by design. Where the recorded analytical data for a chemical synthesis departs from the data for the reference profile, the chemical synthesis may be managed to bring it into line with the profile, or the chemical synthesis may be abandoned, as the product of the process will be deemed not to have the necessary reaction profile matching that of the approved process.

The present invention is particularly concerned with methods that are intended to replicate approved, chemical syntheses based on common reaction platforms, such as common chemical synthesiser platforms. Such platforms typically share similar, if not identical, components, and may be based on programmable robotic synthesisers using standardised modules.

Accordingly, in a first aspect of the invention there is provided a method for validating a chemical synthesis, the method comprising the steps of:

- performing a chemical synthesis in a chemical synthesiser;
- recording analytical data during the chemical synthesis, and developing a profile for the analytical data recorded over time,

comparing the profile for the chemical synthesis against a reference profile, which reference profile is the analytical data recorded over time for a reference chemical synthesis, wherein the chemical synthesis and the reference chemical synthesis share at least the same reagents and method steps for the same intended product.

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A chemical synthesis may comprise one or more consecutive chemical reaction steps. A reactionware may be provided for the chemical reactions, and this may include multiple reaction vessels which are fluidically connected.

10 In a second aspect of the invention there is provided an apparatus for use in the method of validating a chemical synthesis, the apparatus comprising a chemical synthesiser, an analytical unit and a control unit, wherein:

the chemical synthesiser comprises a reactionware for the performance of chemical reactions, which reactionware is optionally also provided with one or modules for work-up and purification, and further comprising reagents, optionally together with solvents and catalysts, for use in the chemical synthesis;

15 the analytical unit is adapted for measuring analytical data from the reactionware over time, and for reporting analytical data to the control unit; and

the control unit is for receiving analytical data from the analytical unit over time, and is adapted to construct a profile from the analytical data, and the control unit further holding a reference profile for comparison against the profile constructed from the analytical data.

The present invention also provides for the chemical synthesiser itself to operate autonomously. Thus, the apparatus may include a suitably programmed robotic system that is capable of comparative assessment of a synthesis profile against an appropriate reference profiles, and is suitably programmed to react as needed once such comparisons have been made. The apparatus may also be suitably programmed to collect the analytical data, and to create a profile against which comparisons may be made to the reference profile.

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30 The apparatus may also include an autonomous synthesiser which undertakes the chemical synthesis

The analytical data for the profile may be generated throughout the chemical synthesis. Thus, analytical data may be generated for each chemical reaction in the chemical synthesis. Additionally, the analytical data may be generated during the reaction itself, but may also be generated during the initial setting of the reaction mixture, and also the work-up of a reaction, including purification. In this way, a comparison of the profile against that of the reference profile is truly indicative of the similarities in the chemical syntheses.

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40 The analytical data may be recorded at high frequency or continuously. Modern analytical techniques all data generation in this way, and provide a greater number of data points for

comparison, which provides a greater degree of reassurance in the comparison between the profile and the reference profile. Further, where there is a departure between the profile and that of the reference, it is more likely to be identified where there is high frequency or continues recording of analytical data.

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Similarly, the comparison between the generated reaction profile and the reference profile may be continuous during the reaction course, and may be performed in real time. Thus, the operator is provided with rapid notification of any divergence from a reference profile, and corrective measures may then be rapidly employed to bring the chemical synthesis back to its intended course.

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Similarly, the real time development of data during the synthesis showing that there is a compliment between the profile and the reference profile provides contemporary reassurance to the operator that a chemical synthesis, such as a chemical reaction that is part of the synthesis, is proceeding as intended, without the operator needing to wait for some later data point to provide that assurance.

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Where a reaction is performed and its profile is matched to that of a reference profile, such that there is substantial similarity in the profiles, the profile may be used as a confirmation for the identity and quality, such as purity, of the reaction product. Thus, the recording of a profile for a chemical synthesis and its comparison against a reference profile may provide a validation of the process and its product.

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Where there is a close match between the reference profile and that of the reaction, the operator may reasonably assume that the methods and the product produced in the reaction is substantially identical to the method and product associated with the reference profile. As such, with a reaction profile matching that of the reference, the operator can advantageously not undertake further, often time consuming and costly, analytical analysis of the reaction product.

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Where the profile for a reaction differs from that of a reaction profile, a reaction product may be rejected or it may be further analysed to determine its worthiness. During the performance of a chemical synthesis, where there is a departure of the reaction profile from the reference, the system may act to counter such a divergence.

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The action taken by the system may include the step of halting the reaction. The operator may then subsequently undertake a further performance of the chemical synthesis, for example in order to obtain a reaction product with an associated profile matched to that of the reference profile.

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The action taken may include the step of acting upon the reaction system in order to alter its performance. The act here may include physical steps, such as changes in heating, cooling

and mixing, and may include chemical steps, such as the addition or removal of reagents, solvents and catalysts from the reaction mixture. Such steps may be intended to bring the course of a synthesis into line an approved synthesis, for example such that the reaction profile is brought into accordance with the reference profile for the subsequent parts of the chemical synthesis.

The method of the invention may include the comparison of a generated reaction profile against multiple reference profiles. Here, one reference profile may represent the profile for the intended performance of the chemical synthesis, and the remaining profiles may represent the profiles for reactions having common differences to the reference profile. Here, there remaining profiles may represent common experimental mistakes or other known problems in the synthesis. Thus, where there is a departure from an approved reference profile, the operator may easily determine why that departure from the profile has arisen, and the operator may be able to provide a suitable remedy, based on suitable corrections that are reported together with those remaining profiles.

The reaction profile may be the characterisation of a single reaction, or it may be the characterisation of series of reaction steps. The reaction profile typically includes the characterisation of the preparatory steps for the performance of the reaction, as well as the subsequent steps that work up the reaction mixture, including those steps for the purification of a reaction product.

The methods and apparatus of the present case are particularly suited for use in portable synthesis platforms.

The methods and apparatus of the present invention are particularly well-suited to the performance of chemical syntheses where an operator of the apparatus is a lay or inexperienced chemist. The combination of autonomous synthesis, analysis and comparison, optionally coupled with the autonomous intervention into the chemical synthesis, removes the need for substantive intervention by the operator themselves. A suitable control system may therefore take the place of the operator for the performance of the chemical synthesis, the comparison of data, and also for intervention into the synthesis where needed, for example where there is a departure of the profile from that of the reference profile.

The invention also provides a method for establishing a profile, which profile may be used as a reference profile against which profiles recorded for subsequent reactions may be compared against.

The profile is typically a physical or chemical property of the reaction environments that is recorded over time, and may be recorded continuously over the course of the reaction.

The reaction environment may refer to the reaction mixture as held in a reaction vessel, and may include the headspace over the reaction mixture, where such is present.

- 5 The profile may simultaneously record more than one, such as two or three, different physical or chemical properties of the reaction to give a multifaceted reaction profile.

In one embodiment, the profile is the change in reaction vessel pressure over time. Such an embodiment is exemplified in the worked examples of the present case.

- 10 The methods of the invention are advantageously performed on a portable automated platform that can execute a wide variety of synthetic procedures that are mapped into a reactionware system.

- 15 The reaction profile - or fingerprint - that is generated over the course of a chemical synthesis performed on the automated platform allow the monitoring of the process, confirmation of completion, and the remote diagnosis of a problem with the execution of a synthesis.

- 20 Accordingly, in a first aspect of the invention there is provided a method for generating a profile for a chemical synthesis, the method comprising the steps of autonomously performing a chemical reaction in a reaction vessel and recording an analytical property of the reaction over time thereby to generate a reaction profile, and comparing the reaction profile against a reference profile for the chemical reaction.

- 25 The reaction vessel may be a component of an apparatus that is a reaction system for the performance of a chemical synthesis.

- 30 In a second aspect of the invention there is provided an apparatus that is a reaction system for the performance of a chemical synthesis. Typically the apparatus comprises one or more reaction vessels, an analytical device for analytical measurement of the chemical synthesis in the reaction vessels, and a control unit for collecting analytical data from the analytical device over time, and for preparing a reaction profile from the analytical data for comparison against a reference profile stored in the control unit.

- 35 Accordingly, the apparatus for use in the method of validating a chemical synthesis comprises a chemical synthesiser, an analytical unit and a control unit, wherein:

- 40 the chemical synthesiser comprises a reactionware for the performance of chemical reactions, which reactionware is optionally also provided with one or modules for work-up and purification, and further comprising reagents, optionally together with solvents and catalysts, for use in the chemical synthesis;
- the analytical unit is adapted for measuring analytical data from the reactionware over time, and for reporting analytical data to the control unit; and

the control unit is for receiving analytical data from the analytical unit over time, and is adapted to construct a profile from the analytical data, and the control unit further holding a reference profile for comparison against the profile constructed from the analytical data.

- 5 The apparatus may be a robotic chemical synthesiser for autonomous performance of the chemical synthesis, and for autonomous control of the analytical device.

In a further aspect there is provided a method for generating a reference profile, the method comprising the step of autonomously performing a chemical reaction in a reaction vessel and
10 recording an analytical property of the reaction over time thereby to generate a reaction profile.

The method may comprise the steps of:
performing a chemical synthesis in a chemical synthesiser;
15 recording analytical data during the chemical synthesis, and developing a reference profile for the analytical data recorded over time; and
providing the reference profile together with the instruction set for the chemical synthesis, wherein the instruction set is for the performance of the chemical synthesis using a reactionware, the instruction set optionally also for the preliminary generation of the
20 reactionware for the chemical synthesis.

In a further aspect of the invention there is provided a method for preparing an apparatus that is a reaction system for performance of a reaction, including the steps of generating a code for the reaction, generating a virtual reaction platform to that code, and generating the
25 physical reactionware for the reaction from the virtual platform.

The apparatus of the invention, and for use in the methods of the invention may be provided as a portable platform. Thus, the scale of the components of the apparatus are such as to allow movement of the apparatus between locations. In this way, the apparatus may be
30 moved to locations where it is needed, or where it is desirable to have ready and immediate access to the product of the chemical synthesis.

The apparatus of the invention is suitable for use outside of the traditional laboratory environment, and allows for the production of target products in locations that would
35 otherwise not be suitable for the preparation of chemical products. It is in such environments that it is particularly desirable to have validation of the chemical synthesis and the target product through the use of a profile compared with an approved reference profile. Alternative methods of analysis, and analytical equipment generally, may not be available to the operator, and therefore the profile from the chemical synthesis serves as the only
40 characterisation of that synthesis, and the only means by which to guarantee the process and its products.

These and other aspects and the embodiments of the invention are described in further detail herein.

Summary of the Figures

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The present invention is described with reference to the figures listed below, which are related and encompass various aspects and embodiments of the invention.

Figure 1 is a schematic representation of a general synthesis carried out on a compact/portable platform, where a) shows the synthetic operations and variables are extracted from the literature procedure and converted into an executable chemical code (χDL). The operations and variables are used to generate single monolithic reactionware cartridge specific to the molecule. The miniaturised laboratory hardware is 3D printed and plugged into the platform for an automated execution of all synthetic steps; and b) From χDL steps, the reaction parameters are encoded into reactionware modules. Using the linearity of chemical processes, these modules can be assembled into a monolithic cartridge that contains all the infrastructure to prepare the targeted molecule. c) All the necessary files for the automated synthesis of any molecule: i) *.xdl*: a universal chemical code for the synthesis of any molecule extracted from literature procedures; ii) *.json* a graph representation of the location, connectivity, and capabilities of all the devices needed for the synthesis; iii) *.xdl.exe*: the portable platform executable code for the synthesis; iv) *.ccad*: editable CAD designs of the reactors needed; and v) *.stl*: a ready to print monolithic reactor containing all the chemical operations for the synthesis.

Figure 2 is a summary of the implemented reaction and platform operations, where a) *Reaction operations*: The synthesis operations are contained within the 3D printed modules. To control the liquid movement within the monolith, a combination of operations between the solenoid valves (pneumatic supply), pressure sensors, and the micropump is applied. All the components are controlled from the custom-made Sensorhub shield; and b) *Platform operations*: generic operations needed for any chemical synthesis, which include liquid handling of solvents and reagents, heating and cooling the reactor.

Figure 3 shows the synthetic schemes of four different APIs prepared using a synthesis platform. Synthetic routes for the synthesis of a) Dihydralazine, b) Isoniazid, c) Lomustine, d) Nardil, and e) Arbidol with the respective monolithic cartridges used in the synthesis, yield (purity determined from HPLC), number of base steps executed and the runtime.

Figure 4 shows the reaction profile for dihydralazine synthesis. The reaction profile elucidates all the different chemical process happening within the cartridge based on a pressure sensor attached to the reactor, where a) shows the full pressure profile for the synthesis of dihydralazine; b) shows the sequential steps executed in a portable platform

according to one embodiment of the invention; and c) shows the pressure profiles for the different processes executed in the platform as part of the chemical synthesis.

Figure 5 shows a schematic representation of the oligopeptides and oligonucleotides synthesized in the platform. The syntheses are based on solid-phase approach, where the iterative steps are executed until the desired peptide or oligonucleotide are obtained. The monolithic cartridges for both syntheses are composed of two filter reactors and a collector. The cartridges can be recycled or reused without any cross contamination. a) Oligopeptides prepared in the portable platform using a three-module cartridge system consisting of two filter reactors followed by a collector cartridge. The iterative coupling and cleavage of the oligopeptide from the solid support happens in *module-1*, precipitation of the final oligopeptide takes place in *module-2*, and *module-3* is used to extract waste solvents from the system. b) Oligonucleotides sequences prepared in the portable platform. All the oligonucleotide sequences were prepared in the same monolithic cartridge consisting of two modules: *module-1* a filter reactor, where the iterative coupling and cleavage of the final oligonucleotide from the solid support takes place, and *module-2* is used to extract waste solvents.

Figure 6 (a) shows the pressure profile for a liquid transfer from a module-A to a module-B (blue, top), and pressure change (green, bottom) to determine when the transfer/filtration is finished depending on the established threshold (red, dashed centre line); and Figure 6 (b) shows the pressure profile for a slow (pulse transfer, mid line) liquid transfer from module-A to module-B (blue, top line), and pressure change (green) to determine when the transfer/filtration is finished depending on the established threshold (red, dashed line).

Figure 7 shows the pressure profile for the synthesis of Isoniazid.

Figure 8 shows the pressure profile for the synthesis of Dihydralizine.

Figure 9 shows the pressure profile for the synthesis of Nardil.

Figure 10 shows the pressure profile for the synthesis of Lomustine.

Figure 11 shows the pressure profile for the synthesis of Arbidol.

Figure 12 shows the pressure profile for the synthesis of VGSA.

Figure 13 shows the pressure profile for the synthesis of GFSVA.

Figure 14 shows the pressure profile for the synthesis of FVSGKA.

Figure 15 shows the pressure profile for the synthesis of SKVFGA.

Figure 16 shows the pressure profile for the synthesis of 5'-TACGAT.

Figure 17 shows the pressure profile for the synthesis of 5'-CTACGT.

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Figure 18 shows the pressure profile for the synthesis of 5'-GCTACGT.

Figure 19 shows the pressure profile for the synthesis of 5'-ATGCTACGGCTACGAT.

10 Figure 20 shows a photograph of an example reactionware of glass construction and a triangular orientation, including two filter vessels (1 and 2) and a collector vessel connected in series by transfer tubing. Figure 20a is a view of filter 1, transfer tubing, and filter 2 with a support holder above the transfer tubing. Figure 20b shows a view of filter 2 in the front and two transfer tubes and support holder. Figure 20c shows a view of filter 2, transfer tubing and collector with a support holder above the transfer tubing. Figure 20d shows a view of
15 Filter 1 and collector with filter 2 in the back.

Figure 21 shows a schematic representation of an example reactionware including two filter vessels and a collector vessel connected in series by transfer tubing, such as that in
20 Figure 20, and its use in the two steps of the example Lomustine synthesis.

Figure 22 shows a schematic representation of an example reactionware including two filter vessels and a collector vessel connected in series by transfer tubing, such as that in Figure 20. Figure 22 shows a transfer scheme illustrating liquid transferring from filter 1
25 through filter 2 to the collector. Figure 22a shows nitrogen being flowed in from filter 2 and collector to hold the solution above the filter. Figure 22b shows a flow of nitrogen in filter 1 while pulling vacuum from filter 2 and collector to initiate the transfer from filter 1 to filter 2. Figure 22c shows stabilization of the pressure and holding of the solution in filter 2 by flowing nitrogen into filter 1 and the collector. Figure 22d shows a flow of nitrogen into both filter 1
30 and filter 2 and pulling a vacuum in the collector to transfer the solution from filter 2 to the collector. Figure 22e shows stabilization of the pressure by flowing nitrogen into both filter 1 and filter 2. Figure 22f shows the use of a syringe pump to transfer the solution from the collector to the desired location.

35 Figure 23 shows a schematic representation of an example reactionware, such as that in Figure 20, and shows how it may be used for filtration and evaporation to collect solid. Figure 23a shows a flow of nitrogen in filter 1 while pulling vacuum from filter 2 and collector to initiate the transfer of liquid from filter 1 to filter 2 and then onto the collector, leaving the solid behind in filter 1. The system continues to pull vacuum in filter 2 to dry the solid while
40 using the syringe pump to remove the solution in collector. Figure 23b shows pulling a vacuum by pulses from filter 1 and supplying N₂ to filter 2 and collector, to slowly evaporate the solution in filter 1, and when the solution level is low pull full vacuum to dry the solid.

Figure 24 shows the pressure profile for the synthesis of Lomustine.

5 Figure 25 shows a monolithic cartridge composed of a filter reactor and a reactor connected with a siphon.

Figure 26 shows a monolithic cartridge composed of two filter reactors (blue) and a reactor (green) connected with siphons.

10 Figure 27 shows a monolithic cartridge composed of one reactor (yellow), a floating filter reactor (red), a filter reactor (blue) and a reactor (green) connected with siphons.

15 Figure 28 shows a monolithic cartridge composed of one filter reactor (blue) containing all synthetic steps of Lomustine, and a reactor (green) that is used to collect, and extract waste from the system.

Figure 29 shows a monolithic cartridge composed of two filter reactors (blue) and a reactor (green) that is used to collect, and extract waste from the system.

20 Figure 30 shows a monolith composed of a *filter reactor* (blue), followed by another *filter reactor* (blue), and a *reactor* (green).

Figure 31 shows a monolith composed of a *filter reactor* (blue, *module-1*), and a *reactor* (green, *module-2*).

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Detailed Description of the Invention

30 The present invention provides methods and apparatus for performing a chemical synthesis, and for characterising the chemical synthesis in the form of a profile, or fingerprint, for comparison against a reference profile for the synthesis. A profile is generated from analytical data recorded over the time course of the chemical synthesis and it may be regarded as a characterisation of the synthesis.

35 The generation of the profile, and its comparison against a reference profile is described in further detail below.

40 The work in the present case also exemplifies the design, construction and validation of a compact, universal, automated platform to execute multi-step synthesis using reusable 3D printed 'module-monolith' reactionware cartridges which are automatically generated from literature procedures using an intelligent software system based on the open-source universal chemical programming language standard and format, χ DL (see Figure 1). This open standard has been designed to allow any chemical transformation to be precisely

expressed and run on any compatible robotic platform. Thus, described herein is a system which can generate all the components necessary to execute an automated synthesis directly from a literature procedure using a universal chemical description language.

- 5 The reaction procedures are automatically translated into the physical modules using the unit synthetic operations described in the chemical code file (χ DL). The physical modules are then automatically assembled into a single monolithic unit that contains all the infrastructure needed for the synthesis of the targeted molecule. The resulting monolith is 3D printed and then connected to the compact platform where all the essential operations
- 10 take place. To ensure portable and autonomous operation, the platform is designed around a programmable manifold to control the vacuum/gas flow through the monolith, a liquid handling system and pressure sensors to control and monitor the unit operations for the synthetic sequences.
- 15 The system may include a *graph*, which describes the location and connectivity of all the platform's physical components and a *reactionware monolith*, a set of 3D printed reactor modules connected sequentially containing all the necessary hardware for the chemical operations to obtain the targeted molecule. The system may be run using a versionable *executable code* which is capable of execute all the abstract explicit operations in the
- 20 chemical programming language.

WO 2007/017738 describes an automated manufacturing system for the industrial preparation of food products and pharmaceuticals, which may include cross batch analysis and review. WO 2007/017738 does not relate to universal, automated platform capable of

25 executing multi-step chemical synthesis.

CN 115389703 describes the production of ethanol in a biological fermentation process, and a predictive model for determining the ethanol concentration in the fermentation based on historical data. The data for ethanol concentration is not recorded over the course of the

30 fermentation, but is instead recorded at four discrete stages of the fermentation. This differs from the methods of the invention where the data is measured frequently, and preferably continuously, throughout a synthesis to allow comparison to a reference profile which is the change in the data over time. CN 115389703 also does not relate to the operation of a universal, automated platform capable of executing multi-step chemical synthesis.

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Chemical Synthesis

The methods of the invention are for analysing and characterising a chemical synthesis as performed in a chemical synthesiser, and typically as performed autonomously by a

40 chemical robot.

Typically, the chemical synthesis is a multistep synthesis. Here, the chemical synthesis may include the step of preparing an intermediate compound from one or more reagents, and the subsequent transformation of that intermediate compound into a target product. The one or more reagents may themselves be upstream intermediate compounds, prepared in their turn from suitable reagents. Accordingly, a chemical synthesis may comprise one or more chemical reactions, and preferably two or more chemical reactions.

The chemical synthesis is not particularly limited and may encompass methods for the production of small organic compounds, metal complexes, supramolecular structures and polymers, amongst others.

The chemical synthesis may also encompass biological reactions, or reactions where biomolecules participate as reagents or catalysts. Such biomolecules may include polypeptides, such as proteins, including enzymes and antibodies, polynucleotides, polysaccharides, metabolites and cofactors, amongst many others.

The worked examples provided in the present case exemplify the preparation, in multi-step syntheses using a chemical synthesiser under autonomous control, of Nardil, an antidepressant drug (Agnew); Isoniazid, an antibiotic drug for tuberculosis (Youatt; Timmins; Zhang); Dihydralazine, an antihypertensive drug (Heilmann); Lomustine, an alkylating agent used in chemotherapeutic cancer treatments (Chakkath); and Arbidol, an antiviral medication for the treatment of influenza (Blaising).

The chemical synthesis may also include, and typically includes, methods of preparing a reaction mixture for a chemical reaction, as well as methods for the work-up of a reaction mixture following a chemical reaction, and methods for purifying a product of a chemical reaction. As discussed in further detail below, the profile for the chemical synthesis may include the recording of analytical data for the profile during any and all of these steps. Thus, the profile may be used to validate each chemical reaction, but also the preparative steps before the reaction, and the follow up steps after the reaction is completed. In this way, the profile can provide a complete overview of the steps undertaken in the chemical synthesis and as such the profile can be a comprehensive characterisation of the chemical synthesis for comparison against a reference profile.

In a preferred embodiment, the chemical synthesis comprises two or more chemical reactions, such as three or more chemical reactions. The chemical synthesis may comprise a series of chemical reactions, as well as parallel chemical reactions, that are later brought to convergence for the production of a target product.

Where there are multiple chemical reactions, these reactions are typically convergent. Where there are multiple chemical reactions, these may be in series.

Reactions may be performed within a chemical synthesis in parallel, but ultimately they are brought to convergence for at least final chemical reaction step.

5 A downstream chemical reaction may be performed in a reaction vessel different to that of an upstream chemical reaction. On one embodiment, each chemical reaction may be performed in a different reaction vessel. Here, the chemical synthesis will require the transfer of material between reaction vessels as the synthesis progresses. Such may be achieved fluidically or by other suitable material transfer methods, and such may be undertaken by a chemical synthesiser and may be autonomously controlled.

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A chemical synthesis may comprise one or more work-up, purification or preparation steps.

For each chemical reaction in a chemical synthesis, the chemical reaction may include one or more work-up, purification or preparation steps.

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Profile, Analysis and Analytical Unit

In the methods of the invention, a profile is generated from a chemical synthesis. The profile is analytical data recorded over time, and this profile may characterise the chemical synthesis.

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The analytical data is a physical or chemical property that is repeatedly or continuously measured during the chemical synthesis. In the worked examples of the present case a profile is recorded across a chemical synthesis as a change in pressure in a reactionware over time. The pressure is recorded as the pressure in the one or more reactions vessels holding a reaction mixture over the course of the chemical synthesis.

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Thus, in one embodiment, the analytical data is a measure of pressure over time. This pressure may be the pressure within a reaction vessel used within the chemical synthesis.

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Other physical or chemical properties of a chemical synthesis may be recorded over time to generate alternative profiles for the characterisation of the method performed within a chemical synthesiser. The properties include those of a reaction mixture or more broadly the reaction space that holds a reaction mixture for performance in the synthesis.

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Example physical or chemical properties of the reaction mixture that may be analysed include impedance, conductivity, colour, such as visible colour, opacity, acoustic, gas evolution. Video imagery of the chemical synthesis may also be recorded, with the images analysis for, amongst others, colour, evolution events and movement.

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For example, the spectroscopic properties of the chemical synthesis may be recorded over time, including the spectroscopic properties of reaction mixtures. Changes in the

spectroscopic properties may be observed as reagents are added to a reaction mixture and are consumed, and as new products and by-products are formed. Changes in spectroscopic properties may be observed as products are worked-up and purified, and when the product is prepared for subsequent reaction. Here, common spectroscopic methods may be used,
5 such as UV-vis, IR, NMR and mass spectroscopy.

Analytical data may be recorded continuously, for example, where the property is a physical or chemical property that can be measured in this way, such as temperature, pressure and absorption. Analytical data may also be recorded at high frequency, under circumstances
10 where it is not practicable to recorded continuously, or where it is not useful to do so.

The analytical data may be recorded without sampling from a reaction mixture. In certain circumstances, and for certain analytical techniques, particularly those associated with separation and destruction, a sample may be taken for analysis. Sampling may be used for
15 mass spectroscopy measurements and NMR, for example, and this may also be associated with chromatographic purification of the sample prior to analysis, as undertaken for liquid chromatography mass spectroscopy (LC-MS) analysis.

Changes in spectroscopic properties may also be associated with inputs into a chemical reaction, for example, where photosynthetic methods are used, and the analysis of the spectroscopic properties may provide confirmation that the correct photosynthesis conditions have been applied.
20

Alternatively, the temperature of the reaction mixtures prepared over the synthesis may be measured over time. Changes in temperature may be observed as reagents are dissolved into a reaction solution, and are permitted to react. Changes in temperatures may also be associated with inputs into a reaction mixture, for example, where heating and cooling steps are used for the reaction, or for a work-up or purification, and the analysis of the spectroscopic properties may provide confirmation that the correct heating or cooling steps have been undertaken.
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Changes in mass may also be recorded over time, and may be linked to the addition and loss of volatile or gaseous components into and out of a reaction mixture. Changes in mass may also be associated with purging steps, as might be used in product work-up and purification.
35

Changes in pressure may be recorded over, and may also be linked to the addition and loss of components into and out of a reaction mixture.

40 Other parameters that may be measured include viscosity and pH amongst others.

The physical or chemical parameters that are measured are generally those that allow for rapid and continuous measurement, and those that are also reasonably expected to change over the course of the chemical synthesis, such that the recorded profile has sufficient variation as to provide a characterisation of the chemical synthesis.

5

The physical or chemical parameters for measurement are also selected on the basis of ease of measurement, and also the relative compactness of the analytical unit. The present invention also provides for a portable platform for performing chemical syntheses, and accordingly the components of the platform should be sufficiently compact (both in size and weight) so as to allow for its portability. It is for this reason that the methods of the invention may measure pressure, temperature, mass, absorption, molecular mass, magnetic resonance and pH, amongst others, as analytical units for the measurement of these parameters are readily available in compact form.

15 The physical or chemical properties are those that may be recorded continuously, or with high frequency, with real time reporting of the analytical data, with preferably real time generation of the profile, and preferably real time comparison of the profiles against the reference profile. The analysis for those properties is typically a non-destructive analytical method.

20

The physical or chemical property may be recorded throughout the chemical synthesis. Thus, the analytical data may be generated across two or more chemical reactions. The generation of analytical data is also not limited to one chemical or physical parameter, and multiple parameters may be analysed to generate a multi-layered profile, where the analytical shows the change in multiple parameters over time. A profile having a multiparameter data set may provide greater validation of a chemical synthesis or a product, as the validation requires matching across a greater number of data points. However, such a multiparameter profile may not be needed, as a judiciously chosen single parameter may provide sufficient information over the course of the chemical synthesis to characterise the method and its product.

30

The analysis is also conducted autonomously without any action required by the operator. The gathering of analytical data and the transfer of that data to a control unit is also performed autonomously. Similarly, the construction of the profile from the recorded data is also handled autonomously.

35

The profile is developed over the course of the chemical synthesis and the profile may be completed once all steps in the synthesis are performed and any work up and analysis as might be needed or desired are completed. However, any comparison with a reference profile need not be delayed until the completion of the synthesis.

40

A visual representation of the profile may be made available to the operator for reference. This reaction profile may also be displayed together with one or more reference profiles, to allow the operator easy understanding of the reaction performance against the reference reaction from which the reference profile has been developed.

5

Although the reaction profile may be displayed in this way, presented against a reference profile, it is not necessary for the operator themselves to conduct a comparison of the profile to the reference profile. This may be undertaken by a control unit provided as a component of the apparatus. This control unit may be suitably programmed to recognise similarities and differences between profiles. The control unit will also be suitably programmed with threshold limits within which it can decide that a profile matches that of the reference profile.

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15

The profile may be regarded as a fingerprint for the chemical synthesis, in that it is characteristic of the reaction undertaken.

Where that profile closely matches that of a reference profile then the product of the chemical synthesis may be regarded as the same as a product of a reference chemical synthesis. Thus, the profile, when matched to its reference, provides a guarantee of product identity and purity. Having such a profile matched to its reference, the operator can use the product as intended, and without the need for further characterisation of the product. Thus, the recording of the profile through the chemical synthesis can avoid the need for costly or burdensome product analysis at the end of the chemical synthesis.

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The profile is for comparison against a reference profile. The reference profile is a profile generated from a chemical synthesis that has been approved, or in some way validated, and may be an optimised synthesis of a product. The reference profile may therefore be derived from a preferred chemical synthesis that is associated with the production of a product under desirable conditions or with a desirable result, such as a desirable yield and/or purity. The reference profile may also be the analytical profile that is associated with the performance of the synthesis by an approved or otherwise known chemist.

The apparatus and chemical synthesiser for use in the present invention provide opportunities for standardisation of reactionware and standardisation of reaction preparation, reaction, work-up and purification. However, even with high levels of standardisation there remains still the possibility of differences in reactions between systems, and these may result from minor differences in set-up, variation owing to operator modifications, variation owing to differences in reagent, catalyst and solvent qualities, or simply with variation within the design tolerances of the system. Thus, although there may be many similarities between systems, it may not be assured that the execution of a chemical synthesis on different platforms will lead to the same product or the same purity of product. For this reason, it is sensible to analyse the chemical synthesis performed on a platform, and compare the analytical data from the synthesis against a standard. Where the profile and the reference

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profile match, under the tolerances set by the operator or the client for the product of the synthesis, there can be validation of the chemical synthesis.

5 The methods of the presents case also provide for the generation of a reference profile for a chemical synthesis performed by a chemical synthesiser. Here, a chemical synthesis may be performed, as described herein, and analytical data for the chemical synthesis may be recorded over time, as described herein, and a profile may be generated.

10 Subsequently, an intended repeat of the chemical synthesis may be performed, using the same chemical synthesiser, or a different chemical synthesiser and a profile may be generated for comparison against the reference, as described above.

Typically, the reference profile is generated from a chemical synthesis for a desirable product, having a desired level of purity.

15 A reference profile may be made available to any operator having a suitable apparatus. The reference profile may be provided on an accessible database for local download to the apparatus, for example to the control unit, where it may made available for comparison.

20 The reference profile may be made available together with an instruction set for generating a reactionware and for the performance of the chemical synthesis using that reactionware. Here, then, the reference profile is the intended analytical result, developed over time, for a chemical synthesis repeated on physical hardware that corresponds to that used for the chemical synthesis giving rise to that reference profile.

25 As described herein, a suitable instruction set may be developed from literature protocols, and these instruction sets may be used to develop a virtual platform from virtual modules, includes reactionware and purification modules. Physical hardware may be developed from such virtual platforms, and the chemical syntheses may be performed in this hardware
30 according to the instruction set, and advantageously autonomously.

Apparatus

35 The apparatus of the invention and for use in the methods of the invention comprises a chemical synthesiser together with an analytical unit for recording analytical data from the reactions performed by the synthesiser. The apparatus includes a control unit, which is in communication with, and receives analytical data from, the analytical unit.

40 An apparatus for use in the method of validating a chemical synthesis, as described herein, comprises a chemical synthesiser, an analytical unit and a control unit, wherein:
the chemical synthesiser comprises a reactionware for the performance of chemical reactions, which reactionware is optionally also provided with one or modules for work-up

and purification, and further comprising reagents, optionally together with solvents and catalysts, for use in the chemical synthesis;

the analytical unit is adapted for measuring analytical data from the reactionware over time, and for reporting analytical data to the control unit; and

- 5 the control unit is for receiving analytical data from the analytical unit over time, and is adapted to construct a profile from the analytical data, and the control unit further holding a reference profile for comparison against the profile constructed from the analytical data.

- 10 The control unit is also in communication with the chemical synthesiser, and is capable of coordinating the operation of the chemical synthesiser with the analytical unit. The control unit may be integrated with the chemical synthesiser.

The analytical unit is integrated with the chemical synthesiser to allow for recording of analytical data from the reactionware of the chemical synthesiser.

- 15 The control unit collects the analytical data from the analytical unit over time and assembles the profile from the data. The control unit is provided with, or has ready access to, suitable data storage, sufficient to hold all the analytical data collected from the analytical unit. The control unit is provided with, or has access to, a reference profile for comparison with the
20 profile generated from the performance of a chemical synthesis by the chemical synthesiser.

- The apparatus may also be used to generate a reference profile. Here, a chemical synthesis is performed by the chemical synthesiser and analytical data is recorded by the analytical unit over time, and reporting to the control unit. Here, the control unit assembles a profile, to
25 be used as a future reference profile. The control unit may provide a reference profile together with a record of the experimental set-up of the chemical synthesiser, and a record of the instruction set for the performance of the chemical synthesis using the chemical synthesiser. This may be made available as package to operators to set up their own apparatus, with the intention of repeating the chemical synthesis.

- 30 In the preferred embodiments of the invention, the apparatus for use in the methods of the invention is a portable platform. Thus, the apparatus can be readily and easily moveable between locations, which may be between laboratories within the same building, but also more usefully between different buildings, where such are not limited to research facilities
35 and chemical laboratories.

It follows that the analytical unit, together with the chemical synthesiser, must be of sufficient size and weight as to be portable.

- 40 The synthesiser typically comprises one or more reaction vessels, and where there are multiple reaction vessels these may be in connection, such as fluid connect, directly or indirectly.

The synthesiser may also be provided with modules interspersed between the reaction vessels for the purification and work up of reaction intermediates and products. These modules are in-line with the reaction vessels, such as fluidically in-line.

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The chemical synthesiser may be a fluidic device, where material is transfer between reaction vessel and other module under fluid control. Thus, the chemical synthesiser may be provided with suitable fluidic lines and pressure systems, including manifolds, to allow for the fluidic transfer.

10

The chemical synthesiser is provided with reagents, optionally together with solvents and catalysts, for use in the chemical synthesis. These may be held in reservoirs within the chemical synthesis and may be deliverable to a reaction vessel or a module, where such is provided, as and when required.

15

The arrangement for the reaction vessels, optionally together with other modules such as for the purification and work up of reaction intermediates and products, may be such as to allow for a continuous flow path through the reactionware from the initial inputs at the upstream end of the chemical synthesis to the downstream output for the product.

20

The chemical synthesiser may be provided with reactionware that is obtained or obtained by 3D-printing. The reactionware may also be glassware, and the chemical synthesiser may be provided with standard glass reactionware for ease of use. The reactionware may be custom glassware for use in the chemical synthesiser. A glassware may include two filter vessels and a collector vessel fluidically connected in series. The vessels may be fluidically connected by fluid passages, such as transfer tubing (for example, as shown in Figures 20-23).

25

In one embodiment, the chemical synthesiser may itself print the reactionware for use in the chemical synthesis, whilst also having the capability of delivering materials, such as reagents, catalysts and solvents, to the reactionware, and for controlling the transfer of material through the reactionware as part of the chemical synthesis.

30

The present inventor has previously described in WO 2013/121230 a system where a 3D-printer can be used to generate reactionware, and also to delivers materials, such as reagents, catalysts and solvents, to that reactionware.

35

The reactionware for use in the chemical synthesiser may be monolithic. Thus, the reactionware for the chemical synthesis may be unified. Such a monolithic reactionware may be obtained by 3D-printing methods, as exemplified in the worked examples of the present case. The preparation of such reactionware is beneficial as it can reduce the footprint of the reactionware within the chemical synthesiser compared with individual pieces

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of reactionware that are required to be linked. In this way, unitary reactionware contributes to the miniaturisation of the apparatus, and the portable nature of the entire platform.

5 The reactionware for use in the chemical synthesiser may be glassware. The glassware may be monolithic. Thus, the glass reactionware for the chemical synthesis may be unified. In other words, the glass reactionware may be formed of a single piece. Such a glass monolithic reactionware may be obtained by standard glass-blowing processes.

10 The glassware may comprise additional components. In some embodiments, the additional components are unitary with the glassware. For example, the unitary additional components may include a glass filter frit formed with the glassware. In some embodiments, the additional components may not be monolithic (e.g., unitary) with the glassware, such as a gasket, transfer tube or bung.

15 The glassware may comprise two filter vessels and a collector vessel fluidically connected in series by transfer tubing (for example, as shown in Figures 20-23). The reactionware may comprise a first filter reactor, a second filter reactor and a collection vessel, fluidically connected in series. This configuration allows for the execution of a large range of reactions, and reduces the complexity of the system by allowing for the use of a single
20 universal glass reactionware. The glass reactionware is also reusable, as it is easier to clean and reduces the chance of contamination compared to 3D printed reactionware. The glass reactionware provides for good pressure responsiveness – the glass construction is typically less flexible than a plastic reactionware and so resists changes in internal volume with pressure. As a result, the pressure profile obtainable from a glass reactionware is more
25 accurate and higher resolution.

A filter reactor is typically a vessel with a filter disposed between the top and the bottom of the vessel. The filter reactor may have a fluidic connection at the top (above the filter) and the bottom (below the filter). The filter may be a glass frit.
30

Gas flow may be used to control the transfer of reagents around the glass reactionware, Positive gas pressure or negative gas pressure (e.g., vacuum) may be applied to the filters and collector independently to control the transfer of reagents. For example, a positive pressure on a first side of the filter or a negative pressure on a second side of the filter may
35 be used to force fluid from the first side to the second side of the filter. A syringe may be used to transfer fluid out of the glass reactionware. This is illustrated in Figures 22 and 23.

Figure 22 shows a transfer scheme illustrating liquid transferring from filter 1 through filter 2 to the collector, of the example glassware shown in Figures 20. Figure 22a shows nitrogen
40 being flowed in from filter 2 and collector to hold the solution above the filter. Figure 22b shows a flow of nitrogen in filter 1 while pulling vacuum from filter 2 and collector to initiate the transfer from filter 1 to filter 2. Figure 22c shows stabilization of the pressure and holding

of the solution in filter 2 by flowing nitrogen into filter 1 and the collector. Figure 22d shows a flow of nitrogen into both filter 1 and filter 2 and pulling a vacuum in the collector to transfer the solution from filter 2 to the collector. Figure 22e shows stabilization of the pressure by flowing nitrogen into both filter 1 and filter 2. Figure 22f shows the use of a syringe pump to transfer the solution from the collector to the desired location.

Figure 23a shows a flow of nitrogen in filter 1 while pulling vacuum from filter 2 and collector to initiate the transfer of liquid from filter 1 to filter 2 and then onto the collector, leaving the solid behind in filter 1. The system continues to pull vacuum in filter 2 to dry the solid while using the syringe pump to remove the solution in collector. Figure 23b shows pulling a vacuum by pulses from filter 1 and supplying N₂ to filter 2 and collector, to slowly evaporate the solution in filter 1, and when the solution level is low pull full vacuum to dry the solid.

A programable manifold may be used to control positive inert gas pressure as well as the vacuum supply.

The chemical synthesiser may be a robotic synthesiser, as described below. Such is a chemical synthesiser that is suitably programmed to perform a chemical synthesis autonomously following an appropriate instruction set. Here, the control unit may be provided with suitable instructions for operation of the synthesiser. The control unit may also control the analytical unit can coordinate the operation of the analytical unit together with the operation of the synthesiser.

Robotic Synthesiser

The methods of the invention and the apparatus for use in the methods of the invention typically make use of a robotic synthesiser. The robotic synthesiser is suitably programmed to perform a chemical synthesis autonomously.

An operator of the robotic synthesiser may need only provide the synthesiser with the instruction set for a chemical synthesis, together with the requisite reagents and optionally together with reactionware, where this is not already present or provided by the chemical synthesiser. The robotic synthesiser may then be permitted to undertake the chemical synthesis autonomously without the need for the operator to make any further contribution. Here, the control unit provides the necessary instructions to the robot and it is suitably programmed to do so. The control unit also coordinates the operation of the analytical unit with the robotic synthesiser, such that the operator is also not required for the analytical data collection.

The control unit, working under autonomous control, and working in coordination with the chemical robot, can also make the necessary comparisons of the profile from the chemical

synthesis against the reference profile and may make suitable reaction decisions following this comparison, and these decisions may be displayed to the operator as might be helpful.

5 The robotic synthesiser may be provided with a selection of instructions for the performance of chemical reactions, and the operator may select the instructions as needed for the intended synthesis and product.

10 Alternatively, the operator can access an appropriate instruction set, including from an online database, and including from a trusted and verified source, and that may be provided to the robotic synthesiser for execution.

15 The instruction set for the performance of a chemical synthesis may also be provided together with one or more reference profiles against which the profile for the chemical synthesis undertaken by the robotic synthesiser may be compared.

The robotic synthesiser may include the analytical unit for analysis a reaction and for generating the profile. The robotic synthesiser may also include the control unit for collecting data from the analytical unit and for generating the profile from the collected data. As components of the synthesiser, the analytical unit and controlled may operate autonomously.

20

Methods for Reference Profile Generation

In one aspect of the invention, there is provided a method for generating a reference profile for a chemical synthesis.

25

Thus, a method for generating a reference profile for a chemical synthesis may comprise the steps of:

performing a chemical synthesis in a chemical synthesiser;
recording analytical data during the chemical synthesis, and developing a reference
30 profile for the analytical data recorded over time; and
providing the reference profile together with the instruction set for the chemical synthesis, wherein the instruction set is for the performance of the chemical synthesis using a reactionware, the instruction set optionally also for the preliminary generation of the reactionware for the chemical synthesis.

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The reference profile may be characteristic of a reaction that yields a desired product, optionally also having a desirable level of purity. The reference profile may also be characteristic of a chemical synthesis for a desired product that is performed under desirable conditions. Thus, whilst there may be many ways to access a desired product at a desired
40 level of purity, the reference profile may reflect the process that is regarded as most appropriate for the operator or the capabilities of the chemical synthesiser, for example

owing to the simplicity of the reaction conditions, the available reactionware and the number of steps, for example.

5 The reference profile may be generated from a chemical synthesis that has been optimised, for example by a skilled chemist.

10 The reference profile may be prepared in combination with multiple other profiles, where these other profiles characterise chemical syntheses and products that do not correspond to the optimal reaction conditions and intended product. For example, these other profiles may represent the profiles for reactions that have commonplace errors in their performance.

15 Such additional profiles may be helpful for understanding the chemical synthesis where it departs from the reference profile. These additional profiles may represent the reaction profiles for reaction methods and products that are not desired, and for which there is an understanding of what reaction conditions led to the generation of those products. Once a particular divergence from the reference profile is noted, and that divergence is mapped to another profile, there is the potential for the system to correct a chemical synthesis to bring that synthesis back on to the desired pathway, and therefore back to the reference pathway.

20 Thus, where additional profiles are provided, these profiles may also be provided together with an instruction set for modifying a chemical synthesis for the purpose of bring about a desirable result.

25 Preferably, where remedial action is required, the system may operate autonomously to bring about a change in the reaction conditions.

Additionally, or alternatively, the system may call for the intervention of the operator to effect a change in the chemical synthesis.

30 Where a reaction deviates from the reference profile it may do to such an extent that it is not practicable to resolve, or such deviations may be associated with a problem in the reaction conditions that is irresolvable, and for such reasons the system or the operator may decide to entirely abandon a chemical synthesis in favour of restarting the synthesis, and optionally together with a reconstruction of the reactionware for the chemical synthesiser, if such was
35 deemed necessary.

Here, then a chemical synthesis may be discarded, and the system may be reset for a further attempt at performing the chemical synthesis, and for the attempted production of a
40 target product.

Other Embodiments

Each and every compatible combination of the embodiments described above is explicitly disclosed herein, as if each and every combination was individually and explicitly recited.

5

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

“and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described above.

20

Experimental Section*Comments*

The synthesis of any molecule consists of following a series of fixed and consecutive steps (e.g. add, filter, evaporate, heat) containing synthesis-specific parameters (time, temperature, volume, mass, etc). Extracting these parameters from any literature protocol and combining them in the correct context results in a chemical code file (χDL file) that can be executed in any robotic platform (as long as the abstractions in the language are universal) (see Mehr). Each χDL step, expands into hardware specific sub-steps, which define unit operations which can be directly executed on the specified platform. To carry out the synthetic protocols, a χDL implementation containing all the executable sub-steps was created. The software is composed of *synthesis steps* (common synthetic steps), *utility steps* (common low-level processes), and *base steps* (directly executable steps) (see Tables 1-3 in the *Software Architecture* section). This demonstrates that the abstraction can be universal as the chemical-code, along with a graph describing the hardware modules can be also compiled into a portable platform.

Given that χDL inherently contains all synthetic steps (including parameters) for the preparation of any molecule, it can be used to define the required hardware that aligns with the sequential synthetic steps (see Figure 1a)). Reactionware systems are comprised of a series of discrete physical reactor modules which are designed to perform linear operations

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(i.e., filtration, evaporation, reaction, separation) to prepare a targeted molecule. The design of these reactionware systems has previously been achieved by either manual CAD design (Kitson; Zalesskiy) or by use of specially created reactionware design software (Hou).

However, in order to fully automate the production of reactionware for the compact synthesis

5 platform we have developed a new automated *cartridge generator* software which can generate prototypical reactionware systems based solely on the χ DL description of the procedure to be automated. The parameters of the physical modules necessary for a synthesis can therefore be extracted directly from the parameters embedded in a χ DL file (Figure 1b)). Following the structural elements from ChemSCAD (a Python based software
10 to generate reactionware cartridges; see Hou), the vessels names in the χ DL file are based on four basic designs (i.e. reactor, filter reactor, floating filter, double filter reactor). The program iterates through all the χ DL steps, and based on their physical operation, it will assign one of the basic designs to each operation.

15 For example, a simplified χ DL procedure for the synthesis of Nardil is shown in Figure 1b). In the first step, ethanol (25 mL) is added to "reactor", which results in a reactor module with a volume of 25 mL. Next, the vessel is heated, which does not generate a new module, since it is a platform operation. Once the reaction is completed, water (10 mL) is added to the same "reactor" vessel, which will increase the volume of the already made module to
20 35 mL. To perform a liquid-liquid extraction, the separate step specifies the solution from "reactor" is going to be extracted twice with diethyl ether (15 mL) through "floating_filter" into a "filter" reactor. This single operation will produce two new individual modules: a "floating filter" reactor with a top volume of 30 mL (organic layer) and a bottom volume of 10 mL (aqueous layer), and a filter reactor with a volume of 30 mL, where the organic phase will be
25 transferred. Finally, the product is precipitated, filtered, and washed (see the *Detailed Materials and Methods* for the full Nardil synthetic procedure). This last step adds a "waste" cartridge, from where all filtrates are disposed to the proper waste through the liquid backbone. This single module is a standard reactor with a round bottom and a volume of 30 mL.

30 From this process, individual 3D printed modules representing each synthesis step can be automatically assembled to produce a target-specific monolithic cartridge. This final cartridge system is a blueprint for the preparation of a targeted molecule, miniaturizing laboratory hardware. Considering 3D printing is used to fabricate the prototypes, size restrictions are
35 employed for the monoliths. In this sense, if the 3D printed reactor exceeds the dimensions of the available 3D printer, the software will scale down the synthesis procedure by a user specified number and produce a new reactor with the respective χ DL file (scaled). Finally, this monolith can then be 3D printed and plugged into the platform to automatically execute all the unit operations. The entire automated process produces five different files (see
40 Figure 1c)) needed for the synthesis execution. The software related files include: a *.xdl* file, a universal chemical code, platform independent file extracted from literature procedures; a *.json* file containing a graph representation of the location, connectivity, and capabilities of all

the platform devices; and a *.xdlexe* file with all the executable unit operations to carry out the synthesis. Additionally to the software set-up, the automated synthesis protocol generates a *.ccad* file, an editable CAD design of the reactor modules; and a *.stl* file of the first monolithic prototype ready to be 3D printed.

5

Since all the reaction processes are part of the morphology of the reactionware monolith (e.g., filtration, liquid-liquid extraction, evaporation), the automated platform can be simplified to perform minimal operations (i.e., heat, cool, evaporate) to the monolith, see Figure 2. For liquid handling (i.e., adding and mixing solutions), a fluidic backbone consisting of

10 8 Tricontinent C3000MP syringe pumps equipped with the 6-way distribution valves was designed.

Thus, this system has a total of 32 inputs/outputs (two ports for each pair of pumps are used for inter-pump connections) for reagents, solvents, cartridges, and waste disposal. This backbone was designed with the ability to move solution from any storage receptacle to any module input. Heating and stirring were accomplished by using a computer controllable hot plate, along with a standard silicone oil bath. For cooling, a coolant (dry ice in ethylene glycol) was circulated through a copper 'cooling element' submerged in the oil bath, allowing working temperatures of between ca. -13°C and 120°C.

20

A programmable manifold was added to the platform to control the reaction operations within the reactionware vessels. The manifold consists of five solenoids dedicated for supplying nitrogen, and five solenoids for controlling the vacuum input/output (generated with a micro-pump). Lastly, to monitor and control the pressure within the system, pressure sensors were added to one of the top ports of each reactionware module. All the previous components are controlled with a custom-designed Arduino shield. This shield allows the precise manifold control to achieve liquid manipulation within the 3D printed vessels by operating the solenoids and micro-pump in the correct sequential order. For example, in a four-module monolithic system, each of the modules is connected to an individual gas and vacuum solenoid. To transfer a solution from *module-1* to *module-2*: i) the solenoids connected to *module-2*, *module-3* and *module-4* are closed, cutting off the inert gas supply for those modules, ii) the micro-pump is turned-on along with opening the vacuum solenoid connected to *module-2*, iii) the pressure sensors are dynamically used to detect when the transfer is completed, iv) solenoids connected *module-3* and *module-4* are opened, allowing the flow of inert gas into the system and preventing any undesired transfer from *module-2* to *module-3*, v) finally the vacuum solenoid connected to *module-2* is closed and the gas solenoid is open to allow the refill of inert gas. Importantly the pressure vs time profile for the entire reaction sequence is diagnostic allowing both successful and unsuccessful executions to be identified and recorded.

40

To demonstrate the capabilities of the platform, we performed the automated synthesis of five different small molecule Active Pharmaceutical Ingredients (APIs) using reactionware

vessels: Dihydralazine (compound **2**), Isoniazid (compound **3**), Nardil (compound **5**), Lomustine (compound **7**), and Arbidol (compound **13**). The digitisation process starts with extracting the chemical operations from literature procedures into a χ DL file. This file, containing the sequential operations that result in a physical molecule is then automatically converted into functional interconnected 3D printed modules to form a molecule specific monolith, see Figure 3. For the two-step reaction of Dihydralazine (Figure 3a), the cartridge consists of three different modules: *module-1*, a filter-reactor designed for the synthesis and purification of compound **1**; *module-2*, a filter reactor designed for the precipitation of compound **2**; and *module-3*, a reactor with a round bottom designed for collection and extraction of solvent waste.

One of the main features implemented in the platform is the dynamic use of a pressure sensor attached to the reactionware vessels. These sensors are used to control and monitor all the operations that occur within the monolith, allowing not only the definition of specific start and end points of automated operations, but also the identification of a 'fingerprint' of the reaction process itself. The 'fingerprint' can be used to monitor the progress of the reaction to validate if the process is progressing as expected and can go to completion. Figure 4 shows the pressure reaction profile for the synthesis of Dihydralazine. The process consists of nine different *synthesis steps* (Purge, Heat, Cool, Transfer, Heat, Transfer, Cool, Transfer, Dry). Each of these processes is associated with a unique pressure profile (a portion of the overall 'fingerprint'). For example, during purging, considering the length of the cartridge and the preloaded reagents added, the pressure drops to 0.8 atm. In the first step, for the synthesis of compound **1**, vacuum pulses are applied to prevent over pressurizing the reactor vessel and an undesired/early transfer to *module-2*. These vacuum pulses are short (~ 1 s every 30 s), and last for the entire reaction of hydrazine hydrate and phthalonitrile. The reaction is then cooled down to 30°C before it is filtered and washed with water.

During the filtration/washing process, the solution is transferred from *module-1* (filter reactor) → *module-2* (filter reactor) → *module-3* (reactor/collector) before it gets extracted to the waste. During these transfers, the sensors can detect subtle changes in the pressure when the transferred is completed (due to the increase in N₂ flow after the transfer of the liquid phase is complete (Figure 4 insets). Once the change is detected, the vacuum is stopped, all the inlets are open to N₂, and the system waits for pressure equilibration before continuing to the next step. For the last synthetic step, 2M H₂SO₄ is added slowly (over 10 mins) to *module-2*, before the reactor is heated to 100 °C for 1h. A hot filtration is performed (transfer-2: *module-2* → *module-3*) to remove impurities. Finally, the reaction is cooled down to 30 °C, before the final filtration/wash sequence is implemented (transfer-3: *module-2* → *module-3*) and the yellow solid is dried for 10 h under vacuum. For Isoniazid (Figure 3b), the cartridge consists of two different modules: *module-1*, a filter-reactor designed for the synthesis and purification of compound **3**; and *module-2*, a reactor designed for collection and extraction of solvent waste. The one step synthesis has 15 χ DL steps compacted from 121 *base steps*, and a total runtime of ca. 20 hours.

The monolith for Nardil (Figure 3c) comprises 4 different modules: *module-1*, a reactor designed for the synthesis compound 4; *module-2*, a floating filter reactor utilised for a liquid-liquid extraction in the purification of compound 4; *module-3* a filter reactor for the synthesis and purification of compound 5; and *module-4*, a standard reactor for the collection and extraction of waste. The two-step protocol consists of 28 χ DL steps compacting a total of 279 *base steps*, and a total runtime of ca. 29 hours. For Lomustine, the monolith was composed of two different modules: *module-1*, a high-volume filter reactor, where the synthesis and purification of compound 6 and 7 takes place; and *module-2*, a reactor for waste collection and extraction. The two-step procedure is composed of 23 χ DL steps containing 159 *base steps* with a total runtime of ca. 30 hours. To demonstrate the robustness of the platform, Arbidol (an antiviral medication for the treatment of influenza, compound 13), a six-step synthesis was included as a target. The nature of the synthesis resulted in a monolith composed of four different modules: *module-1*, a filter reactor for the synthesis of compounds 8-11; *module-2*, a filter reactor for the synthesis of compounds 12 and 13; and *module-3*, a standard reactor used for collection and extraction of solvent waste. Overall, the six-step protocol requires 96 χ DL steps enclosing a total of 952 *base steps* executed over 64 h of continuous platform operation.

Solid-phase synthesis is a process that involves reacting a molecule chemically bound to a solid support using selective protection/deprotection protocols. These methods are commonly used for the synthesis of biological molecules e.g. oligopeptides (Merrifield), oligonucleotides (Alvarado-Urbina), oligosaccharides (Plante), and polyolefins (Li). Considering that during this process all chemical operations occur on the surface of the solid support within a reactor, it can be easily mapped into a reactionware module compatible with the portable platform. To demonstrate this versatility, oligopeptides and oligonucleotides were included as targets. The synthesis of these biological targets includes an iterative process of deprotection, coupling, capping of the respective building blocks until the desired target is acquired, which can be laborious to code. In that sense, the inherent abstraction of χ DL can be used to implement a *step-reaction* class, containing all the necessary sub-steps to complete the sequence of the specified solid-phase synthesis. Thus, the χ DL step will require only the minimum parameters to complete the desired sequence. For example, for synthesizing oligopeptides, a *SPPS* (Solid Phase Peptide Synthesis) χ DL step that contains all the sub-steps (deprotection, coupling, resin washing, and cleavage) was added to the program. Using *step-reaction* χ DL steps, the parameters required to generate a full χ DL script are minimized to synthesis scale and the amino acid sequence, reducing time and coding experience needed to prepare oligopeptides.

Based on all the steps needed for the iterative process, the monolithic cartridge for the *SPPS* synthesis consists of three modules: *module-1*, a filter reactor where the solid support is loaded and all the chemical operations (i.e., deprotection, coupling, cleavage) take place; *module-2*, another filter reactor used for peptide precipitation; and *module-3*, a reactor

cartridge to collect and remove solvent waste. The *SPPS* cycle finishes with a washing and drying step. To cleave the peptide from the solid support, with a -Fmoc protecting group, a freshly prepared solution of trifluoroacetic acid (TFA) and scavenger reagents (TIPS) was added to *module-1*. The solution was transferred (filtered) to *module-2*, where diethyl ether

5 was added to induce the precipitation of the peptide. Since the synthetic protocol is the same, independent of the amino acid sequence, the same monolith can be used for the synthesis of multiple oligopeptides. This protocol was used for the synthesis of VGSA, GFSVA, FVSGKA, and SKVFGA. All the synthetic procedures were carried out using the same 3D printed reactor without any detectable cross-contamination. The versatility offered

10 by the software bound to the platform allowed us to execute the protocols with minimal change in between synthesis (only the oligopeptide sequence was different, with a one letter notation for the amino acids), which generated between 1700-2500 μ DL base steps depending on the synthesized oligopeptide.

15 Similarly, oligonucleotides are commonly synthesised using solid-phase synthesis with different sub-steps compared to *SPPS*. A new *step-reaction* was added called *OSPS* (Oligonucleotide Solid Phase Synthesis), which contained all the necessary steps to perform iteratively a deprotection, capping, coupling, oxidation, resin washes, and cleavage needed for each nucleobase until the desired oligonucleotide sequence is completed. For example,

20 for the synthesis of 5'-ATGCTACGGCTACGT-3', the input contains only the reaction scale and sequence as initial parameters, while the output is composed of 6510 μ DL base steps needed for the synthesis. To design the cartridge for *OSPS*, we must consider the lower volume (< 2 mL) that is generally used for these syntheses to ensure the solid support is completely submerged in the reagent solutions during the iterative process. To do this, a

25 smaller cartridge (inner diameter = 28 mm) with a cone-shaped interior (base = 8 mm, top = 25 mm) was designed, demonstrating the feasibility of adapting new chemical processes to the platform based on the digitization using reactionware. All the reaction operations are still embedded in the reactor, while the platform operates the generic processes (e.g. stir, heat, etc).

30 The final monolithic cartridge is composed of two modules: *module-1*, a filter reactor designed to contain the solid support and where all the chemical operations will take place; and *module-2*, a reactor module used for collecting and discarding waste and the final oligonucleotide solution. To cleave the synthesized oligonucleotide from the solid support

35 (controlled pore glass, CPG), an ammonia solution is added to *module-1*, then the solution is filtered to *module-2*, and the solution is heated to 55 °C for 12 hours to perform the final heterocyclic base and phosphate deprotections. Finally, the solution is transferred to the receiving flask, ready for further purification methods. This protocol, with this bespoke 3D printed reactor was used for the synthesis of 5'-TACGAT, 5'-CTACGT, 5'-GCTACGAT, and

40 5'-ATGCTACGGCTACGAT. All the oligonucleotides were synthesized using the same cartridge, without any detectable cross-contamination, demonstrating the recyclability of these systems. Similarly to *SPPS*, only one μ DL step is needed (input the oligonucleotide

sequence), resulting in outputs containing between 2300-6500 χ DL *base steps* necessary, depending on the entered sequence, see **Figure 5**.

5 Confining all the reaction processes within the reactionware vessels allows the fabrication of a portable and compact platform capable of performing the necessary generic operations. Since the synthetic steps are controlled/coded into the blueprint of the 3D printed vessels, switching between chemistries does not require any reconfiguration of the platform, but just a different monolithic design. This new method was demonstrated to be easily adaptable for the synthesis of 13 different targets including a 6-step small molecule synthesis (Arbidol),
10 solid phase synthesis of peptides (including cleavage from support), solid phase synthesis of oligonucleotides (along with cleavage from support).

All the components were put together to maximize the capabilities of the platform, while minimising the footprint. The final portable synthesis platform consists of acrylic plates fixed
15 to a metal framework (250 mm x 600 mm x 355 mm). The back acrylic plate contains all the power supply unit (PSU), two DC-DC convertors (24V -> 3.5V, and 24V -> 12.0V), the micropump, a main gas inlet and an Ethernet switch for communications. The top-plate contains the gas/vacuum programmable manifold, the PumpHub (PCB for syringe pump communication), SensorHub (custom-designed shield to control the programmable manifold
20 and the sensor framework), and two Serial-to-Ethernet convertors (for the communication with the hotplate and the PumpHub). Finally, the pumps were allocated in the front side of the portable platform in two tiers, while behind the syringe pumps there is space for the reagent, solvent and waste bottles with tailored acrylic shelves.

25 *General Materials and Experimental Details*

Reagents and solvents were used as received from commercial suppliers unless otherwise stated.

30 NMR measurements were performed with Bruker Advance III HD 600 spectrometer operating at 600.1 and 150.9 MHz for ^1H and ^{13}C , respectively. Spectra were collected at 298 K, chemical shifts are reported in ppm, calibrated for the (residual) NMR solvent signal. 3D printing reactionware vessels was done using a Ultimaker 2+ FDM.

35 Polypropylene (PP) filament was purchased from Barnes Plastic Welding Equipment Ltd., Blackburn, UK. All prints were performed on a 12 mm PP sheet as a replacement of the standard glass bed provided by Ultimaker. This is a necessary requirement to achieve a good adhesion of the first PP layer. The main 3D printer settings were: *Bed Temperature*: off (i.e. 0°C), *Nozzle temperature*: 260°C, *Speed*: 15 mm/s. Sensor cases were 3D-Printed
40 using a Connex 500 printer from Stratasys using the Fullcure 720 translucent resin for the major body of the printed parts. Once the print was finished, the supports were scraped manually before washing it thoroughly using a water jet cleaning station (Quill Vogue

Polyjet). Then, the parts were placed in a 0.1 M NaOH_(aq) bath for 30 min. Finally, the parts were again washed thoroughly in the cleaning station.

HPLC analysis was performed on a Thermo Dionex Ultimate 3000 equipped with a
5 LPG-3400 RS pump, a WPS-3000TRS autosampler, a TCC-3000SD column compartment and a DAD-3000 diode array detector. The HPLC was connected to a Bruker MaXis Impact quadrupole time-of-flight mass spectrometer with an electrospray source, operating in negative mode for small molecules and oligopeptides, and positive mode for
10 oligonucleotides. The voltage of the capillary tip set at 4500 V, end plate offset at – 500 V, nebuliser at 1.6 bar, dry gas at 8.0 L/min, funnel 1 RF at 400 Vpp, funnel 2 RF at 400 Vpp, isCID energy at 0 eV, hexapole RF at 100 Vpp, ion energy 5.0 eV, low mass at 50 m/z, collision energy at 5 eV, collision cell RF at 200 Vpp, transfer time at 63.5 µs, and the pre-pulse storage time at 1.0 µs. The mass range was set to 50-2,000 m/z for small molecules and oligopeptides, while 500-5,000 m/z for oligonucleotides. Data was analysed using the
15 Bruker DataAnalysis v4.1 software suite.

Small Organic Molecules

10 µL of each sample was injected on to an Agilent Porodhell 120 EC-C18 2.7 µm, 4.6 x
20 150 mm column, eluting at 1mL/min with mobile phase A being water + 0.1% formic acid and mobile phase B acetonitrile + 0.1% formic acid, detecting using UV (λ = 190, 214, 220 and 254 nm). The total run time was 26 minutes, with the LC method as follows - 0 min- 0% B, 4min – 10% B, 16 min – 70% B, 19 min – 100% B, 23 min – 0% B, 26 min – 0% B. Column compartment was set at 30°C.

25

Oligonucleotides

10 µL of each sample was injected on to a Clarity 2.6 µm Oligo MS 100 Å (100 mm x 2.1 mm) column, eluting at 0.3 mL/min with *mobile phase A* being 100 mM hexafluoro
30 isopropanol (HFIP)/ 4 mM triethylamine (TEA)/ 2% MeOH, and *mobile phase B* 100 mM hexafluoro isopropanol (HFIP)/ 4 mM triethylamine (TEA)/ 98% MeOH, detecting using UV (λ = 260 nm). The LC method was 95% A to 80% B over 30 mins. Column compartment was set at 50°C.

35 3D Printing

Reactionware

3D printing was done using a Ultimaker 2+ FDM. Polypropylene (PP) filament was
40 purchased from Barnes Plastic Welding Equipment Ltd., Blackburn, UK. All prints were performed on a 12 mm PP sheet as a replacement of the standard glass bed. This is a necessary requirement to achieve a good adhesion of the first PP layer. The main 3D printer

settings were: *Bed Temperature*: off (room temperature), *Nozzle temperature*: 260°C, *Speed*: 15 mm/s.

Sensor cases

5

3D Printing of accessories was performed on a Connex Objet 500 printer from Stratasys using the Fullcure 720 translucent resin for the major body of the printed parts. Once the print was finished, the supports were scraped manually before washing it thoroughly using a water jet cleaning station (Quill Vogue Polyjet). Then, the parts were placed in a 0.1 M NaOH_(aq) bath for 30 min. Finally, the parts were again washed thoroughly in the cleaning station.

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Laser Cutting

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Laser-Cutting: was performed on a Monster1060 CO2 Laser system (ML1060 130 W) from Radeval with the RDWorksV8 software. The applied parameters to cut the 6 mm acrylic sheets were:

20

Laser power: 50-65%

Speed: 17 mm/s

Air: On

Laser through mode: Enabled

Air pressure: 0.3 MPa

Flow rate: 33 L/min

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Software Architecture

The portable platform was controlled adapting the previously published Chemical Description Language (χDL) (see <http://xdl-standard.com/> and Mehr).

30

Chemical Description Language (χDL)

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The programming language contains three different modules of steps: base steps, utility steps and synthesis steps. Base steps execute a *Chempiler* object. The *Chempiler* library maps the platform graph to device drivers, providing a platform controller for χDL to execute steps with. This library is programmed to use *ChempilerAPI* (interface for custom-made devices, including the sensorhub shield) or *SerialLabware* (interface for commercial devices, handling different communication protocols) to communicate with the platform. Having this programming hierarchy, the user interacts solely with synthesis steps that will automatically execute operations on the specified platform. Utility steps are composed from base steps and other utility steps, while synthesis steps are constructed from utility steps and other

40

synthesis steps. Currently χ DL is composed of 82 different steps divided in 38 base steps (Table 1), 34 utility steps (Table 2), and 10 synthesis steps (Table 3).

Table 1 - χ DL base steps implemented in the portable platform

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Step	Description	Step	Description
CChillerWaitforTemp	Waits for stirrer to reach the setpoint temperature	CStirrerSetTemp	Sets temperature on stirrer
CStartChiller	Starts recirculating pump	CStirrerWaitForTemp	Waits until the stirrer temperature reaches the setpoint
CStopChiller	Stops recirculation pump	CStopHeat	Stops heating operation of the
CBreakPoint	Execution is halted until operator resumes it	CStopStir	Stops stirring operation of the stirrer
CConnect	Connects two nodes together	CStartVacuum	Switches on vacuum pump
CMove	Moves a specified volume from one node to another	CStopVacuum	Switches off pump and vacuum pump to allow inert gas flow in
Confirm	Waits for user's confirmation	CValveMoveToPosition	Moves valve to given position
CSetRecordingSpeed	Set the time-lapse speed of the camera module	CStirrerHeat	Starts heating operation of the stirrer
CWait	Delays execution for the specified time	CStir	Starts stirring operation
CWaitUntil	Waits until a specified start time is reached	CGetTemperature	Gets the current temperature of the hotplate
CEvaporate	Detects evaporation based on pressure	CIntervalReading	Changes pressure readings frequency
CMonitorPressure	Starts monitoring the pressure in the given channels	CPulse	Vacuum pulses to the specify module
CSwitchArgon	Switches off pump and vacuum pump	CPulseTransfer	Vacuum pulses are used to transfer from module to module

Step	Description	Step	Description
CSwitchVacuum	Switches on vacuum pump	CRestartMonitorPressur	Restarts monitoring pressure
CTransferTo	Reads pressure changes to determine transfers completeness	CWaitForStabilization	Waits until pressure stabilizes after transfers
CStopMonitorPressure	Stops monitoring pressure in given channel	CSwitchEmpty	Closes gas inlet for the specified module
CSeStirRate	Sets stirring speed	CSetStirRate	Sets stir rate for hotplate
CSwitchPump	Switches on/off vacuum pump	CStartRecording	Starts recording
CSwitchStirrer	Switches on/off reagent/solvent	CStopRecording	Stops recording

Table 2 - χ DL utility steps implemented in portable platform

Step	Description	Step	Description
CleanBackbone	Moves 3 mL of a cleaning solvent to every available waste node	StopPurge	Switches pump off and de-energizes valves on the last cartridge
Wait	Waits for given time, changing the recording speed	ShutDown	Iterates through all the devices and switches them off
WaitUntil	Waits until a specified start time is reached, changing the recording speed	Standby	Moves solvent around backbone every specified time to prevent clogging.
Stir	Stirs the specified vessel	SetStirRate	Sets rate of stirring for a reactor, filter or rotary evaporator
HeatChillSetTemp	Sets stirrer temperature	StartStir	Starts stirring reactor, filter or rotary evaporator at given stir speed.

Step	Description	Step	Description
HeatChillToTemp	Heat/Chills vessel to given temp and leave heater/chiller on.	StopStir	Stops stirring reactor, filter or rotary evaporator.
StartHeatChill	Starts heating/Chilling the vessel to given temperature and gives the heater/chiller on	ApplyVacuum	Applies vacuum to a given vessel for a specified time
StirrerReturnToRT	Stops heating the given vessel and let it cool down to RT (30°C)	StartVacuum	Starts vacuum pump attached to given vessel.
StopHeatChill	Stop heater/chiller on given vessel.	StopVacuum	Stops vacuum pump attached to given vessel.
PrimePumpForAdd	Prime pump attached to given reagent flask in anticipation of reagent addition	StartPurge	Switches vacuum pump on the last cartridge
MonitorPressure	Start reading pressures on given vessels	BubbleSolution	Applies vacuum to a specified module to bubble inert gas through
StartCool	Start recirculation pump on given vessel	FlushTubing	Moves gas through backbone to remove residual liquid
StopCool	Stop recirculation pump	RestartMonitorPressure	Restarts monitoring pressure
SwitchArgon	Switches off vacuum pump, turns on gas inlet	HeatChillReturnToRT	Allows heater/chiller return to room temperature and then stop heating/chilling.
SwitchVacuum	Switches on vacuum pump, turns off gas inlet	StopMonitorPressure	Stops monitoring pressure
Transfer	Transfers contents from one vessel to another using the vacuum pump	SwitchArgon	Turns on gas inlet to the specified module
Purge	Switch vacuum pump on the last cartridge for a given time	WaitWhilePulse	Waits for a given time while applying vacuum pulses to a module

Table 3 - χ DL synthesis steps implemented in the portable platform

Step	Description
Add	Adds given volume of given reagent/solvent to given vessel
Dissolve	Dissolves contents of vessel in given solvent, at given temperature.
Dry	Dries given vessel by applying vacuum for a given time
Evaporate	Evaporates contents of give cartridge at given temperature
Filter	Filters contents of given cartridge and transfer them to the collector cartridge
HeatChill	Heat/Chills given cartridge to given temperature for given time
Precipitate	Induces precipitation by changing temperature in given cartridge
Recrystallize	Recrystallizes contents in given cartridge
Separate	Extracts/washes contents of cartridge with given solvent
WashSolid	Washes solid in given cartridge by adding solvent and filtering n times

5 Software Workflow

The *portable platform* χ DL that directly executes the abstract steps from the universal chemical language χ DL includes a *cartridge generator* and *graph generator* packages to guarantee an autonomous execution of the chemical synthesis. In this sense, the χ DL file, obtained from literature protocols (see Mehr), contains all the necessary chemical steps to be executed using the *portable platform* χ DL. Simultaneously, this file automatically generates: a graph, containing all the positions of the monoliths, reagents and solvents to execute the reaction; and a first prototype of a reactionware monolith to execute the synthesis in, generated based on the back-end code of **ChemSCAD** (see Mehr).

This workflow generates five different files that are used for an automated synthesis:

1. **.xdl file:** A universal synthesis language file containing all the necessary steps for the synthesis, independent from the platform used.
2. **.json:** A platform specific graph with all the physical locations, connectivity, properties, and capabilities for each device that will be used in the synthesis.

3. **.xdlexe**: A platform specific executable file containing all the base steps that can be directly executed in the portable platform.
4. **.ccad**: An editable ChemSCAD file of a reactor prototype including all the chemical steps needed for the synthesis.
5. **.stl**: A 3D printable file of the reactors

Cartridge Generator

To automatically produce monolithic reactionware cartridges, a generator was programmed using Python. A χ DL file contains a *Procedure* section with a list of all the synthesis steps needed to produce the targeted molecule. Considering reactionware systems are based on a workflow (i.e. linear synthesis) to create digital blueprints, this list of steps can be used to produce a first draft of monolithic designs containing all the necessary cartridges to execute the respective operations.

The *cartridge generator* iterates through all the steps present in the procedure section of the χ DL file. For each step, it will assign what type of reactor is needed (i.e. reactor, filter or floating filter) based on the vessel's name and the executed function. All the parameters that are defined within this step are used to fine tune the geometric properties of the different modules. For example, if 25 mL of *solvent A* is added to *reactor*, then a reactor-type module with a volume of 25 mL is created. This process continues over all the χ DL steps and finally all the modules are appended in the correct order to produce a single STL file for 3D printing using the backend software of ChemSCAD (Hou). Once the modules are created, all the volumes are increased $\times 1.5$ times. This is to prevent running synthetic procedures with 100% filled modules.

The software contains sanity checks at the end of the process to check the size of the reactors. Considering the modules are 3D printed, they are limited by the printing volume of the 3D printer used. In the case of Ultimaker 2+ the maximum printing height is ~ 200 mm, so this was used as a maximum height for the cartridges. If the cartridge is bigger than this size, the user can input a scale factor to reduce the volume/size of the monolith. When a scale factor is used, a new χ DL file is created containing all the changed volumes to assure compatibility between the 3D printed reactors and the executable script.

Graph Generator

The portable platform is based on a graph containing all the topology information. In the directed multigraph each node represents a physical device location, including all the device properties and capabilities. This also includes reagents/solvents locations, as well as the connections between the pumps and the different modules. The physical operations carried out by the portable platform are fixed, considering all the chemical operations are extracted

into the monolithic designs. This leads to having fixed elements in the graph, such as syringe pumps and the pneumatic manifold and the hotplate.

5 Once having a basic template, the information in the xDL file can be used to identify all the reagents, solvents and cartridges needed in the platform. The reactionware modules are obtained (in the correct sequential order) from the *CartridgeGenerator*. Finally, all the necessary connections (i.e. pump valves ports, manifold ports, and connections in between reactionware modules) are added to obtain a graph ready to be used.

10 Step Reaction Classes

Step reaction can be added as a new type of steps within the xDL software suite. The class is composed of synthetic, utility or base steps and it is designed to contain all the minimum parameters necessary for the execution of a specific type of reaction. For example, the
15 synthesis of oligopeptides is composed of four main processes: deprotection, coupling, washing, and cleavage. These procedures are repeated in an iterative fashion for a specified sequence until the final oligopeptide is completed and cleaved from the solid support. To write an executable file containing all the steps can be tedious and time consuming. However, since these are repetitive processes, they can be compressed into a single step
20 reaction class (SPPS, Solid Phase Peptide Synthesis) that contains only the reaction scale and the sequence as minimum parameters. Having the oligopeptide sequence, xDL can generate all the necessary steps for completing the synthesis that include all xDL steps and base steps. Similarly for the synthesis of oligonucleotides, a different step reaction class can be programmed, to generate a complete executable script based solely on the input
25 sequence.

General Platform Liquid Handling and Pneumatic System

For solvents/reagents handling, 8 C3000MP Syringe pumps equipped with 12.5 mL syringes
30 were used. PTFE plastic tubing with an outer diameter of 1/16 inch was used and connected using standard HPLC low-pressure PTFE connectors and PEEK manifolds (supplied by Kinesis). The pneumatic system is formed by five 3/2 V114A-6LU SMC solenoids dedicated for supplying nitrogen, and five 2/2 LVM11-6C solenoids for controlling the vacuum input/output. For vacuum, a compact diaphragm pump (TopsFlo TF30A-B) was
35 used. The pump was protected with two inline vacuum filters (SMC ZF series), one equipped with a standard filtering cartridge and another one – with basic alumina to neutralize acid vapours coming from the reaction mixtures.

Framework

40 The frame was built from OpenBuilds® parts. The individual V-Slot rails were connected via L brackets and drop in tee nuts. To make a connection, the tee nut is simply placed in the

V-Slot. When tightening the screw, the tee nut will turn inside the bottom slot and make a no-slip connection. More details on construction with OpenBuilds® parts can be found under <https://openbuilds.com/>. Then, the customized acrylic sheets were added to the frame and locked in place.

5

Liquid Handling Backbone

Assembly

- 10 8x C3000MP Syringe Pumps were used in the platform equipped with 6-way distribution valves and 12.5 mL syringes. The pumps were organized into two stacks of 4 pumps each and fixed to the acrylic backplates using M3 screws.

Tubing connections

15

To correctly assemble the backbone, all tubing was cut with an appropriate tube cutter to provide perpendicular cut edge with no burrs.

- 20 The number of possible reagents and solvents that can be used in the system, will vary depending on the number of cartridges present in the monolithic reactionware. This gives a minimum of 24 (monolith of 8 cartridges) and a maximum of 31 (monolith of 1 cartridge) reagents/solvents

Electronics

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To power and communicate with the 8 C300MP Tricontinent syringe pumps, a custom-designed board (PumpHub) was used along with a Serial-to-Ethernet interface converter module. Both components were fixed on the top acrylic plate.

- 30 Cooling Unit

- The cooling unit consists of two copper heat exchangers connected through a peristaltic pump. One of the heat exchangers is immersed in the oil bath where the 3D printed reactors are located, while the remaining one is immersed in a cooling bath of ethylene glycol and dry ice. JULABO Thermal H10 bath fluid is recirculated between the cold bath and the oil batch using a peristaltic pump. Using this set-up, the oil bath can be cooled down to -13°C.

35

SensorHub

- 40 In order to facilitate the control of the pneumatics from the high-level Python-based code, a hardware interface controller was needed. We decided to implement it based on the widely used and easily available Arduino platform. Besides numerous obvious benefits, such as low

price, easy availability and a huge ecosystem, an extra important reason for us was the ability to re-use the previously developed Commanduino framework (see Grizou) which made the integration of new sensors and actuators really fast and easy. We have chosen Arduino Mega2560 as the hardware platform due to a good combination of price,

5 functionality, and robustness.

We developed a custom-made Arduino shield considering that: *i)* contains a relatively large number of on/off outputs to control the pneumatic valves, vacuum pump and peristaltic pump, considering that most common Arduino shields (e.g. RAMPS) usually have 2-4 on/off

10 outputs; *ii)* integrates as many as possible I²C-connected pressure sensors into the system since the BMP280 sensors contain only two choices for address configuration, extra I²C connections are needed; and *iii)* includes an Ethernet port for communication with the portable platform since the Arduino Mega2560 has only serial-over-USB connectivity.

15 The board had a built-in power supply based on TI TPS5430 buck regulator. The power supply accepts 6-30 V DC and outputs 5V DC at up to 3A max current. This 5V power supply is used to power the Arduino board itself as well as to provide power for the external sensors. Next, 5V are fed into the MCP1826 3.3 V LDO which powers the Ethernet to serial converter, I²C multiplexer chip and external sensor connectors. Si3900Dv double N-channel

20 MOSFETs from Vishay with 1A peak current were used to control the pneumatic valves. MOSFETs' gates are controlled from Arduino digital outputs D0-D13. Each MOSFET channel is equipped with an LED that shows the output state as well as the diode-based back-EMF protection, which makes the use of inductive loads (solenoids, motors) safe. Each MOSFET output channel is terminated by a two-pin quick-connect terminal block having V_{in}

25 connected to the other pin. This way full voltage swing from V_{in} to zero can be provided on any channel by applying PWM to the respective Arduino digital output.

The multiplication of the channels for the devices with similar addresses on the I²C bus is provided by a standard PCA9548 I²C multiplexer chip. PCA9548 has 8 fully independent

30 outputs that can be connected to/disconnected from the bus in any possible combination. The chip itself is controlled over I²C as well. Each of eight I²C output channels is routed to a standard 4-way 2.54 mm pitch pin header accompanied with a 3-position pin header with a jumper to select between 3.3 and 5V power supply for each connected sensor. Using the jumpers, every channel can be configured independently to use either 3.3 or 5V power and

35 logic levels.

To provide Ethernet connectivity, a readily available XPort module from *Lantronics* was used. The initial configuration of the modules is done via the *DeviceInstaller* software provided by *Lantronics*. To fully utilize the board space, some of the unused Arduino digital

40 and analog I/Os were routed to a separate set of standard pin headers along with power lines to allow easy connection of extra sensors/actuators. This feature was utilized later for

the control of the conductivity sensor along with the pneumatic manifold for the system (see Angelone).

Pneumatics

5

For the inert gas supply, the compressed argon supply after a secondary pressure regulator (1.2 bar) was connected to the system through a shut-off valve with the low side pressure relief. The output of the valve was connected to a multi-position manifold providing positive argon pressure for the reagent bottles. The outlet of the manifold was connected to the flow regulator that was used to adjust the flow rate of the argon gas coming into reaction chambers. The output of the flow regulator was connected to individual supply valves through another distribution manifold. The outlet of the manifold was vented to the atmosphere through a check valve.

10

15 For the vacuum line, a compact diaphragm pump (TopsFlo TF30A-B) was used as a vacuum source. The pump was protected with two inline vacuum filters (SMC ZF series), one equipped with a standard filtering cartridge and another one – with basic alumina to neutralize acid vapours coming from the reaction mixtures. The outlet of the second filter was connected to a (dry ice/ethylene glycol cooled) trap which was also acting as a vacuum receiver. The outlet of the receiver was connected to the individual distribution valves via standard pneumatic manifold.

20

The programable manifold was designed to control positive inert gas pressure as well as the vacuum supply. To provide this, the unit was designed to include ten 3/2 electromagnetic valves, five used as normally-closed with a common manifold (for the vacuum line) and five normally-open with individual baseplates (for the inert gas supply line). In the default state, when all the valves are de-energized, all the outputs are connected to the positive pressure inert gas supply via the normally-open valves. When a vacuum connection is needed, the normally-open inert gas supply valve for the required output is shut off, while the normally-closed vacuum supply line is opened, connecting the required output to the vacuum line. In the case where a particular output has to be shut off, it can be achieved by energizing only the inert gas supply valve.

25

30

Electronics

35

To power and communicate with the pneumatic manifold, an Arduino fitted with a custom-designed shield was used (SensorHub). The shield consists of:

40

- 12 MOSFET outputs (12V, 2.5A), with back EMF protection diodes
- 8 I²C channels (GND, +5V/3.3V)
- 4 connectors (GND, +5V, digital in, analog in)

Bosch BMP280 sensors were used to monitor pressure changes in the monolithic cartridges. The sensors were connected via the I²C channels in the shield. The sensors were enclosed in a sealed (with epoxy resin) 3D printed case to minimize air leaking. Finally, a micropump was used as a vacuum supply. The micropump was controlled using one of the MOSFET channels.

Other Components

To complete the functions provided in the platform the following components were added:
Power supply unit (PSU); RCT Digital Hot plate; 12 V Peristaltic pump 5 Port Gigabit Ethernet Plus Switch; and a Serial-to-Ethernet plug.

Switch

An Ethernet switch (Entry 4) was included to communicate between the PC running the software and the Portable platform hardware. The switch provides 5 RJ-45 ports and has to be configured by browsing the configuration page and setting up the switch to the gateway address 192.168.1.1 and subnet mask 255.255.0.0 (see the suppliers manual for details).

Complete Assembly

After all components have been prepared, they were attached to respective 6 mm acrylic sheets. The back BB-acrylic sheet is composed of the micropump, ethernet switch, Power Supply Unit (PSU), DC-DC Convertors (BC) and a shut-off valve for the inert gas inlet.

The top acrylic sheet was composed of the PumpHub, SensorHub, Pneumatic supply and the 2 × Serial-to-Ethernet plugs. To control the inert gas flow, a flow regulator was also added.

Detailed Materials and Methods

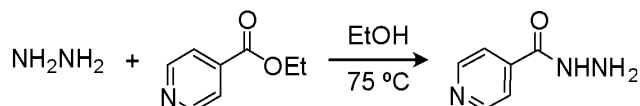
Pressure Profiles

Using the BMP280 sensor, pressure can be used to track important changes within the reactionware monolith. The pressure within the reactor is monitored in the background of the chemical process while the change (Δ) in pressure is calculated. Using this pressure difference single events can be identified. For example, **Figure 6 (a)** shows how a standard transfer module-A → module-B works, the threshold is set at $\Delta P \geq 65$, where the vacuum is stopped, and the reactor is refilled with inert gas.

Similarly, pulse transfers (for slow additions from module-A to module-B without using the platform backbone, see **Figure 6 (b)**) can be detected based on the pressure change in the

system. This kind of transfer is based on applying vacuum pulses to the receiving module and the threshold is set at $\Delta P \leq -400$, where, if detected, the transfer is complete, and the vacuum pulses are stopped.

5 Automated Synthesis - Isoniazid



Synthesis cartridge

10

The monolithic cartridge is composed of a filter reactor and a reactor connected with a siphon, see **Figure 25**. *Module-1* (filter-reactor) is designed to contain all the synthetic procedures, while *module-2* is used to collect, and extract waste from the system.

15 Synthesis Description

Ethyl isonicotinate (3 mL, 20 mmol), hydrazine hydrate (1.5 mL, 31 mmol) and ethanol (12 mL) were added to *module-1*. The monolith was heated to 75°C and was kept at this temperature for 4 hrs. After the reaction was completed, the reaction mixture was cooled down to 40°C and stirred for 30 mins at that temperature. The solution was filtered and washed with ethanol (5 mL). To recrystallize the product, methanol (25 mL) was added to *module-1* to dissolve the product and the reactionware was heated to 60°C for 20 mins. Then, the cartridge was cooled down to 8°C and stirred at that temperature for 30 mins. Finally, the solution was filtered and dried for 10 hrs under vacuum. Isoniazid was obtained as a white solid (2.0 g, 73% yield).

¹H NMR: (600 MHz, D₂O) δ 8.59 (d, J = 5.7 Hz, 2H), 7.60 (d, J = 5.6 Hz, 2H).

¹³C NMR: (151 MHz, D₂O) δ = 121.0, 140.0, 149.0, 166.6.

HPLC-UV/Vis retention time: 0.6 min, purity: > 99 % (at 254 nm).

ESI(M+H)⁺: expected: 138.066 Da, observed: 138.0705 Da.

30 XDL file

<Synthesis>

<Procedure>

35

<Add

reagent="ethyl_isonicotinate"

vessel="filter"

volume="3 mL"

priming_volume="1 mL"

40

/>

```
5      <Add
      reagent="ethanol"
      vessel="filter"
      volume="12 mL"
      />
      <Add
      reagent="hydrazine_hydrate"
      vessel="filter"
      volume="1.5 mL"
10     stir="True"
      priming_volume="1 mL"
      />
      <HeatChill
      vessel="filter"
15     temp="75°C"
      time="4 hrs"
      stir="true"
      />
      <HeatChill
20     vessel="filter"
      temp="40°C"
      time="0.5 hrs"
      stir="true"
      />
25     <Filter
      filter_vessel="filter"
      />
      <WashSolid
      vessel="filter"
30     solvent="ethanol"
      volume="5 mL"
      />
      <Recrystallize
      vessel="filter"
35     dissolve_temp="60°C"
      crystallize_temp="8°C"
      solvent="methanol"
      solvent_volume="25 mL"
      />
40     <Filter
      filter_vessel="filter"
      />
```

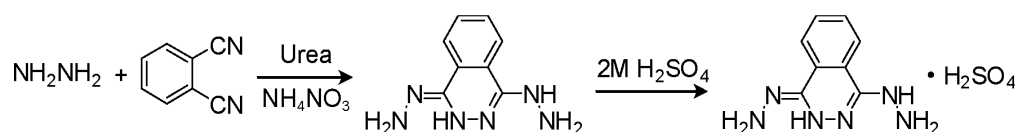
```

    <Dry
      vessel="filter"
      time="10 hrs"
    />
5    </Procedure>
    </Synthesis>

```

The pressure profile for the synthesis of Isoniazid is shown in **Figure 7**.

10 Automated Synthesis - Dihydralazine



Synthesis Cartridge

15

The monolithic cartridge is composed of two filter reactors (blue) and a reactor (green) connected with siphons, see **Figure 26**. Module-1 and module-2 contain all the synthesis steps, while module-3 is used to collect, and extract waste from the system.

20 Synthesis Description

The monolith was preloaded with 1,4-dicyanobenzene (1.28g, 10 mmol) and urea (3.65g, 61 mmol). The system was purged with nitrogen by applying vacuum to it for 60 seconds and refilling back with nitrogen until pressure is stabilized for three times. Hydrazine hydrate (3.65 mL, 75 mmol) was added to *module-1* while stirring. The monolith was heated to 100°C and kept at this temperature for 3 hrs. The reactionware is cooled to 30°C and water (10 mL) was added to *module-1*. The solution was filtered (to *module-3*) and washed with water (10 mL, × 2). All the solvent waste was removed from the system. 2 M H₂SO₄ (12.5 mL) was added to *module-1* over 10 mins while stirring at 500 rpm. The monolith was heated to 100°C for 1 hr. The solution was filtered, while it is hot (transferred to *module-2*), where it has been cooled down to 30°C. Once the solution was cooled down, the precipitate formed was filtered off and washed with 5 mL of water twice. Dihydralazine sulfate (2.1 g, 72% yield) was obtained as a yellow solid after drying under vacuum for 10 hrs.

35 ¹H NMR: (600 MHz, D₂O) δ 8.22 (m, 2H), 8.16 (m, 2H).
 ¹³C NMR: (151 MHz, D₂O) δ = 119.82, 123.46, 134.76.
 HPLC-UV/Vis retention time: 0.45 min, purity: > 99 % (at 254 nm).
 ESI(M+H)⁺: expected: 191.1045 Da, observed: 191.1093 Da.

40 XDL file

```
<Synthesis>
  <Procedure>
    <Purge
      vessel="filter"
5    />
    <Add
      reagent="hydrazine_hydrate"
      vessel="filter"
      volume="3.65 mL"
10    stir="True"
      priming_volume="1 mL"
    />
    <HeatChill
      vessel="filter"
15    temp="100°C"
      time="3 hrs"
      stir="true"
      track="True"
    />
20    <HeatChill
      vessel="filter"
      temp="30°C"
      time="5 mins"
      stir="true"
25    />
    <Add
      reagent="water"
      vessel="filter"
      volume="10 mL"
30    stir="True"
      stir_speed="500 RPM"
    />
    <Filter
      filter_vessel="filter"
35    />
    <WashSolid
      vessel="filter"
      solvent="water"
      volume="10 mL"
40    washes="2"
    />
    <Add
```

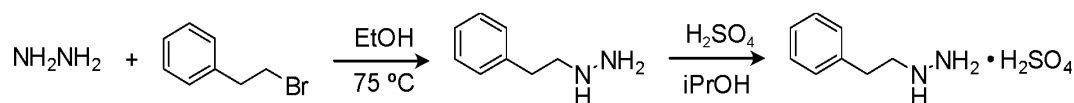
```

    reagent="2M_H2SO4"
    vessel="filter"
    volume="12.5 mL"
    time="10 mins"
5    stir="True"
    stir_speed="500 RPM"
  />
  <HeatChill
    vessel="filter"
10    temp="100°C"
    time="1 hrs"
    stir="true"
  />
  <Transfer
15    to_vessel="filter_2"
  />
  <HeatChill
    vessel="filter"
    temp="30°C"
20    time="1 hrs"
    stir="true"
  />
  <Filter
    filter_vessel="filter_2"
25  />
  <WashSolid
    vessel="filter_2"
    solvent="water"
    volume="5 mL"
30    washes="2"
  />
  <Dry
    vessel="filter_2"
    time="10 hrs"
35  />
</Procedure>
</Synthesis>
```

The pressure profile for the synthesis of Dihydralizine is shown in **Figure 8**.

40

Automated Synthesis - Nardil



Synthesis Cartridge

- 5 The monolithic cartridge is composed of one reactor (yellow), a floating filter reactor (red), a filter reactor (blue) and a reactor (green) connected with siphons, see **Figure 27**. Module-1 and module-2 were designed for the synthesis of Phenelzine, module-3 for the synthesis of Nardil, and module-3 was used to collect and extract waste from the system.

10 Synthesis Description

- The monolith was purged with nitrogen three times (vacuum for 60 seconds each cycle). Ethanol (10 mL) and hydrazine hydrate (2.9 mL, 60 mmol) were added to *module-1*. The reactionware was heated to 75°C, and 2-bromoethylbenzene (1.84 mL, 10 mmol) was added to *module-1* over 2 mins. Ethanol (5 mL) was added to *module-1*, and the system was kept at 75°C for 2 hrs while applying vacuum pulses of 1 seconds every 60 seconds in *module-1*. The reactor was cooled down to 40°C and vacuum was applied to *module-1* for 2.5 hrs to evaporate the solvent. Diethyl ether (15 mL) was added to module-1 to extract Phenelzine and transferred to module-2. The extraction was repeated twice. Water (10 mL) was added to *module-1* and transferred to *module-3*, to push residual diethyl ether from *module-2*. Vacuum was applied to *module-3*, to evaporate diethyl ether, for 2.5 hours, while heating to 35°C. Isopropanol (20 mL) was added to *module-3*. Then, a H₂SO₄-isopropanol mixture (1:5, 6 mL) was added to module-3 over 5 mins, and the solution was stirred for 2 hrs. Hexane (5 mL) was added to module-3 and stirred for 30 mins. The solution was filtered (transferred to *module-4* for collection) and washed with hexanes (10 mL) twice. Nardil was obtained as a pale yellow solid (1.2g, 51% yield) after drying for 10 hrs.

¹H NMR: (600 MHz, D₂O) δ 7.42 (m, 2H), 7.36 (m, 3H), 3.43 (t, *J* = 7.5 Hz, 2H), 3.03 (t, *J* = 7.5 Hz, 2H).

30 ¹³C NMR: (151 MHz, D₂O) δ = 30.6, 52.1, 127.3, 128.8, 136.6.

HPLC-UV/Vis retention time: 8.3 min, purity: > 99 % (at 254 nm).

ESI(M+H)⁺: expected: 137.1034 Da, observed: 137.1077 Da.

XDL file

```
35 <Synthesis>
    <Procedure>
        <Purge
            vessel="reactor"
        />
40    <Add
        reagent="ethanol"
```

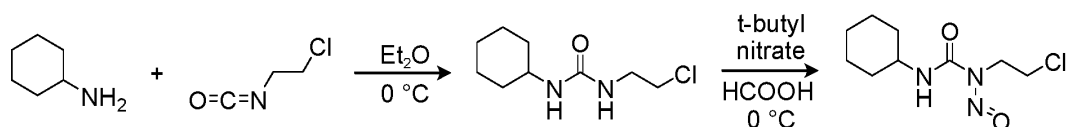
```
        vessel="reactor"
        volume="10 mL"
    />
    <Add
5      reagent="hydrazine_hydrate"
        vessel="reactor"
        volume="2.9 mL"
        stir="True"
        priming_volume="1 mL"
10    />
    <HeatChill
        vessel="reactor"
        temp="75°C"
        time="1 min"
15    stir="true"
    />
    <Add
        reagent="2-bromoethylbenzene"
        vessel="reactor"
20    volume="1.84 mL"
        stir="True"
        time="2 mins"
        stir_speed="400 RPM"
        priming_volume="1 mL"
25    />
    <Add
        reagent="ethanol"
        vessel="reactor"
        volume="5 mL"
30    stir="True"
        stir_speed="400 RPM"
    />
    <HeatChill
        vessel="reactor"
35    temp="75°C"
        time="2 hrs"
        stir="true"
        track="true"
    />
40    <Evaporate
        rotavap_name="reactor"
        solvent="ethanol"
```

```
temp="40°C"
time="2.5 h"
mode="manual"
/>
5 <Separate
  from_vessel="reactor"
  separation_vessel="floating_filter"
  to_vessel="filter"
  product_bottom="False"
10 solvent="diethyl ether"
  solvent_volume="15 mL"
  n_separations="2"
/>
  <Add
15 reagent="water"
  vessel="reactor"
  volume="10 mL"
  stir="True"
  stir_speed="400 RPM"
20 />
  <Transfer
  from_vessel="reactor"
  to_vessel="floating_filter"
  time="10 seconds"
25 />
  <Transfer
  from_vessel="floating_filter"
  to_vessel="filter"
  time="10 seconds"
30 />
  <Evaporate
  rotavap_name="filter"
  solvent="diethyl ether"
  temp="35°C"
35 time="2.5 h"
  mode="manual"
/>
  <Add
40 reagent="isopropanol"
  vessel="filter"
  volume="20 mL"
  stir="True"
```

```
        stir_speed="400 RPM"
    />
    <Add
5      reagent="h2so4_isopropanol"
      vessel="filter"
      volume="6 mL"
      time="5 mins"
      stir="True"
      stir_speed="400 RPM"
10    />
    <Wait
      time="2 hrs"
    />
    <Add
15    reagent="hexane"
      vessel="filter"
      volume="5 mL"
      stir="True"
    />
20    <Stir
      vessel="filter"
      time="30 mins"
      stir_speed="400 RPM"
    />
25    <Filter
      filter_vessel="filter"
    />
    <WashSolid
      vessel="filter"
30    solvent="hexane"
      volume="10 mL"
      washes="1"
    />
    <Dry
35    vessel="filter"
      time="10 hrs"
    />
    </Procedure>
  </Synthesis>
40
```

The pressure profile for the synthesis of Nardil is shown in **Figure 9**.

Automated Synthesis - Lomustine



5 Synthesis Cartridge

The monolithic cartridge is composed of one filter reactor (blue) containing all synthetic steps of Lomustine, and a reactor (green) that is used to collect, and extract waste from the system, see **Figure 28**.

10

Synthesis Description

The monolith was purged with nitrogen three times (vacuum for 60 seconds followed by refilling with nitrogen until the pressure is stabilized). Diethyl ether (20 mL), 2-chloroethyl isocyanate (1.05 mL, 10 mmol) were added to *module-1*. The reactionware was cooled down to 5 °C, and cyclohexylamine (0.5M, 20 mL, 10 mmol) was added over 5 mins. The solution was stirred for 3 hrs, filtered, and washed with diethyl ether twice (5 mL). The obtained solid was dried for 1 hr under vacuum. Formic acid (18 mL) was added to *module-1*, and the solution was cooled down to 5 °C, followed by adding t-butyl nitrate (1.8 mL, 13.5 mmol). The solution was stirred for 2 hrs at 0→25°C. Water (36 mL) was added to *module-1*, and the solution was stirred for an extra 1 hr. The solution was filtered, and the obtained precipitate was washed with water (three times, 5 mL). The obtained pale yellow solid was dried under vacuum for 10 hrs to yield compound **5** (1.6g, 69 % yield).

¹H NMR: (600 MHz, CDCl₃) δ 6.80 (b, 1H), 4.21 (t, J = 6.6 Hz, 2H), 3.94 – 3.87 (m, 1H), 3.52 (t, J = 6.7 Hz, 2H), 2.10 – 2.04 (m, 2H), 1.82 – 1.76 (m, 2H), 1.71 – 1.63 (m, 1H), 1.50 – 1.40 (m, 2H), 1.37 – 1.21 (m, 3H).
¹³C NMR: (151 MHz, CDCl₃) δ = 151.3, 49.5, 39.6, 38.4, 32.6, 24.9, 24.3.
 HPLC-UV/Vis retention time: 1.8 min, purity: > 99 % (at 254 nm).
 ESI(M+H)⁺: expected: 234.1009 Da, observed: 234.1056 Da.

XDL file
 <Synthesis>
 <Procedure>
 <Purge
 vessel="filter"
 />
 <Add
 reagent="diethyl ether"
 vessel="filter"

```
        volume="20 mL"
    />
    <Add
5      reagent="2-chloroethyl isocyanate"
      vessel="filter"
      volume="1.05 mL"
      stir="true"
      priming_volume="0.5 mL"
    />
10    <HeatChill
      vessel="filter"
      temp="5°C"
      time="5 min"
      stir="true"
15    />
    <Add
      reagent="0.5M cyclohexylamine"
      vessel="filter"
      volume="20 mL"
20    time="5 min"
      stir="True"
    />
    <Wait
      time="3 hrs"
25    />
    <Filter
      filter_vessel="filter"
    />
    <WashSolid
30    vessel="filter"
      solvent="diethyl ether"
      volume="5 mL"
    />
    <Dry
35    vessel="filter"
      temp="25 °C"
      time="1 hrs"
    />
    <Add
40    reagent="formic acid"
      vessel="filter"
      volume="18 mL"
```

```
        stir="True"
    />
    <HeatChill
        vessel="filter"
5        temp="5°C"
        time="5 min"
        stir="true"
    />
    <Add
10        reagent="t-butyl nitrite"
        vessel="filter"
        volume="1.8 mL"
        time="1 min"
        stir="True"
15        priming_volume="1 mL"
    />
    <Stir
        vessel="filter"
        time="2 hrs"
20        continue_stirring="true"
    />
    <Add
        reagent="water"
        vessel="filter"
25        volume="36 mL"
        stir="True"
    />
    <Stir
        vessel="filter"
30        time="1 hrs"
    />
    <Filter
        filter_vessel="filter"
    />
35    <WashSolid
        vessel="filter"
        solvent="water"
        volume="5 mL"
    />
40    <Dry
        vessel="filter"
        temp="25 °C"
```

```

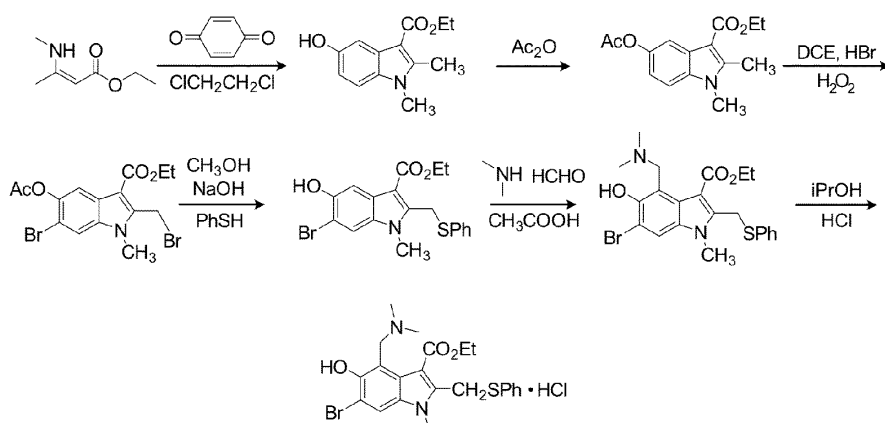
time="10 hrs"
/>
</Procedure>
</Synthesis>

```

5

The pressure profile for the synthesis of Lomustine is shown in **Figure 10**.

Automated Synthesis - Arbidol



10

Synthesis Cartridge

The monolithic cartridge is composed of two filter reactors (blue) and a reactor (green) that is used to collect, and extract waste from the system, see **Figure 29**.

15 Synthesis Description

Ethyl 5-acetoxy-1,2-dimethyl-1H-indole-3-carboxylate (Arbidol-A)

ZnCl₂ (160 mg) was pre-loaded to module-2. A solution of p-benzoquinone (7 mL, 3.32 g in 13 mL of 1,2-dichloroethane) was added to module-2. The monolith was cooled down to 6°C for 30 mins and then, the enamine (4.9 mL) was added to module-2 within 10 mins. The monolith was heated to 75 °C and kept at this temperature for 2 h and stirred at 200 rpm. Then, the monolith was cooled down to 30 °C and heating was stopped. The solution was stirred at 200 rpm for 1 h. Finally, the solution was filtered and dry under vacuum for 1 h to obtain a grey-yellow solid.

25

Ethyl 5-hydroxy-1,2-dimethyl-1H-indole-3-carboxylate (Arbidol-B)

1,2-Dichloroethane (15 mL), acetic anhydride (3 mL) and triethylamine (4.5 mL) were added to module-2. The reaction mixture was stirred at room temperature for 2 h. Then, DCE was evaporated at 60 °C for 2 h under vacuum pulses (4 s vacuum, 6 s waiting). Methanol (8 mL) was added to module-2, and it was evaporated first at 60 °C for 1 h under vacuum pulses

30

(4 s vacuum, 6 s waiting), and then at 60 °C under vacuum for 2 h. After evaporation, methanol (4 mL) was added to module-2, and the solution was cooled down to 25 °C, heating was turned off and the solution was stirred for 1 h. The reaction mixture was filtered and washed twice with methanol (2 mL) and one time with 50% methanol (4 mL). Finally, the
5 grey solid was dried under vacuum for 3 h.

Ethyl 5-acetoxy-6-bromo-2-(bromomethyl)-1-methyl-1H-indole-3-carboxylate
(Arbidol-C)

10 1,2-dichloroethane (10 mL) was added to module-2, and the solution was stirred at 200 rpm for 30 mins to dissolve Arbidol-B. Then, 48% HBr (2.8 mL) was added to module-2, and the monolith was heated to 70 °C. 10% H₂O₂ (9 mL) was added within 30 mins, and the reaction was stirred at 70 °C for 2 h. DCE was evaporated at 60 °C using vacuum pulses (3 s vacuum, 6 s waiting). Then, the monolith was cooled down to 30 °C, and it was stirred at this
15 temperature for 1h. The solution was filtered, and methanol (15 mL) was added to the residual solid in module-2, and the reaction mixture was stirred for 30 mins. This process was repeated twice. Finally, the white-red solid was dried under vacuum for 1 h.

20 Ethyl 5-acetoxy-6-bromo-1-methyl-2-((phenylthio)methyl)-1H-indole-3-carboxylate
(Arbidol-D)

A mixture solution of PhSNa / NaOH in methanol was prepared by mixing NaOH (1.49 g), thiophenol (1.6 mL) and methanol (40 mL). PhSNa/ NaOH solution (22 mL) was added to module-2 containing Arbidol-C. The reaction mixture was stirred at room temperature for 2 h.
25 Then, acetic acid (3 mL) was added to module-2 slowly, and the reaction was stirred for 1 h. Finally, the solution was filtered, the yellow solid was washed with water (3 mL), and it was dried under vacuum at for 1 h.

30 Ethyl 6-bromo-4-((dimethylamino)methyl)-5-hydroxy-1-methyl-2-((phenylthio)methyl)-
1H-indole-3-carboxylate (Arbidol-E)

A solution containing 40% dimethylamine (2.4 mL), acetic acid (10 mL) and 37% formaldehyde (1.4 mL) was pre-prepared. 7 mL of this solutions were added to module-2 containing Arbidol-D. The reaction mixture was heated to 65 °C and stirred at this
35 temperature for 3 h. Water (5 mL) was added to module-2. 15% NaOH (35 mL) was added to module-3, and the monolith was cooled down to 6 °C for 30 mins. Once cooled, the solution in module-2 was transferred to module-3 and it was stirred for 30 mins. Finally, the solution was filtered, washed with water (15 mL), and dried under vacuum at for 2 h to obtain a pale yellow solid.

40 Arbidol

Isopropanol (5 mL) was added to module-3 containing Arbidol-E, and the monolith was heated to 70 °C. Once at this temperature, conc. HCl (1 mL) was added to module-3 within 5 mins. The reaction mixture was stirred at this temperature for 30 mins, before it was cooled down to 30° C, turning off the heating. Then, the solution was stirred for 30 mins and then
5 filtered and washed with isopropanol (3 mL). Finally, the yellow-white (0.75 g, 5.6 % overall yield) solid was dried under vacuum for 3 h.

¹H NMR (600 MHz, DMSO) δ 9.42 (s, 1H), 9.13 (s, 1H), 8.03 (s, 1H), 7.43 – 7.22 (m, 5H), 4.91 (s, 2H), 4.74 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 3.70 (s, 3H), 2.73 (s, 6H),
10 1.25 (t, J = 7.1 Hz, 3H).
¹³C NMR (151 MHz, DMSO): 165.1, 148.9, 144.2, 134.3, 132.7, 131.3, 129.2, 127.6, 125.3, 60.4, 53.1, 42.2, 29.8, 13.9.
HPLC-UV/Vis retention time: 12.8 min, purity (> 95%)
ESI(M+H)⁺: expected: 477.0848, observed: 477.1102

15 XDL file
<Synthesis>
<Procedure>
<Add
20 reagent="p-benzoquinone"
vessel="filter_2"
volume="10 mL"
priming_volume="1.0 mL"
stir="True"
25 stir_speed="150 RPM"
</>
<CleanBackbone
solvent="methanol"
near_waste='waste_3'
30 far_waste="waste_3"
n_cleans="2"/>
<CleanBackbone
solvent="DCE"
near_waste='waste_3'
35 far_waste="waste_3"
n_cleans="3"/>
<HeatChill
vessel="filter_2"
temp="6 °C"
40 time="0.5 hrs"
stir="true"
stir_speed="150 RPM"

```
5      />
      <Add
        reagent="DCE"
        vessel="filter_2"
        volume="1 mL"
        priming_volume="1.0 mL"
        stir="True"
        stir_speed="150 RPM"
      />
10     <Add
        reagent="enamine"
        vessel="filter_2"
        volume="4.9 mL"
        prime="False"
15     time="10 mins"
        stir="True"
        stir_speed="150 RPM"
      />
20     <CleanBackbone
        solvent="methanol"
        near_waste='waste_3'
        far_waste="waste_3"
        n_cleans="2"/>
25     <HeatChill
        vessel="filter_2"
        temp="75°C"
        time="2 hrs"
        stir="true"
        stir_speed="150 RPM"
30     />
        <HeatChillToTemp
        vessel="filter_2"
        temp="30°C"
        stir="true"
35     continue_heatchill="False"
        stir_speed="150 RPM"
      />
      <Stir
        vessel="filter_2"
40     time="1 hrs"
        stir_speed="150 RPM"
      />
```

```
5      <Filter
        filter_vessel="filter_2"
        stir="true"
        removes="None"
        />
      <WashSolid
        vessel="filter_3"
        solvent="methanol"
        volume="5 mL"
10     washes="3"
        />
      <Dry
        vessel="filter_2"
        time="1 hrs"
15     />
      <Confirm
        msg="STEP-2 COMPLETED, Grey white solid?"
        />
      <Add
20     reagent="DCE"
        vessel="filter_2"
        volume="15 mL"
        priming_volume="1.0 mL"
        stir="True"
25     />
      <Stir
        vessel="filter_2"
        time="0.5 hrs"
        stir_speed="200 RPM"
30     />
      <Add
        reagent="acetic anhydride"
        vessel="filter_2"
        volume="3 mL"
35     priming_volume="1.0 mL"
        stir="True"
        stir_speed="200 RPM"
        />
      <Add
40     reagent="TEA"
        vessel="filter_2"
        volume="4.5 mL"
```

```
        priming_volume="1.0 mL"
        stir="True"
        stir_speed="200 RPM"
    />
5    <CleanBackbone
        solvent="DCE"
        near_waste="waste_2"
        far_waste="waste_2"/>
    <Stir
10    vessel="filter_2"
        time="2 hrs"
        stir_speed="200 RPM"
    />
    <Evaporate
15    rotavap_name="filter_2"
        solvent="DCE"
        temp="60°C"
        time="2 hrs"
        mode="pulse"
20    />
    <Add
        reagent="methanol"
        vessel="filter_2"
        volume="8 mL"
25    priming_volume="1.0 mL"
        stir="True"
    />
    <Evaporate
30    rotavap_name="filter_2"
        solvent="methanol"
        temp="60°C"
        time="1 hrs"
        mode="pulse"
    />
35    <Evaporate
        rotavap_name="filter_2"
        solvent="methanol"
        temp="60°C"
        time="2 hrs"
40    mode="manual"
    />
    <Add
```

```

    reagent="methanol"
    vessel="filter_2"
    volume="4 mL"
    priming_volume="1.0 mL"
5    stir="True"
    />
    <HeatChillToTemp
    vessel="filter_2"
    temp="25°C"
10    stir="true"
    continue_heatchill="False"
    />
    <Stir
    vessel="filter_2"
15    time="1 hrs"
    stir_speed="200 RPM"
    />
    <Filter
    filter_vessel="filter_2"
20    removes="None"
    />
    <WashSolid
    vessel="filter_3"
    solvent="methanol"
25    volume="5 mL"
    washes="3"
    />
    <WashSolid
    vessel="filter_2"
30    solvent="methanol"
    volume="4 mL"
    washes="2"
    />
    <WashSolid
35    vessel="filter_2"
    solvent="50% methanol"
    volume="5 mL"
    washes="1"
    />
40    <Dry
    vessel="filter_2"
    time="3 hrs"
```

```
    />
    <Confirm
      msg="STEP-3 COMPLETED, Grey white solid?"
    />
5    <Add
      reagent="DCE"
      vessel="filter_2"
      volume="10 mL"
      priming_volume="1.0 mL"
10    stir="True"
    />
    <Stir
      vessel="filter_2"
      time="0.5 hrs"
15    />
    <Add
      reagent="48% HBr"
      vessel="filter_2"
      volume="2.8 mL"
20    priming_volume="1.0 mL"
      stir="True"
    />
    <CleanBackbone
      solvent="water"
25    near_waste="waste_2"
      far_waste="waste_2"/>
    <HeatChillToTemp
      vessel="filter_2"
      temp="70°C"
30    stir="true"
    />
    <Add
      reagent="10% H2O2"
      vessel="filter_2"
35    volume="9 mL"
      time="30 mins"
      priming_volume="1.0 mL"
      stir="True"
    />
40    <CleanBackbone
      solvent="water"
      near_waste="waste_2"
```

```
        far_waste="waste_2"/>
    <CleanBackbone
        solvent="methanol"
        near_waste="waste_3"
5        far_waste="waste_3"/>
    <HeatChill
        vessel="filter_2"
        temp="70°C"
        stir="true"
10        stir_speed="600 RPM"
        time="2 hrs"
    />
    <Evaporate
        rotavap_name="filter_2"
15        solvent="DCE"
        temp="60°C"
        time="2 hrs"
        mode="pulse"
        pulse_time="3 seconds"
20    />
    <HeatChillToTemp
        vessel="filter_2"
        temp="30°C"
        stir="true"
25        continue_heatchill="False"
    />
    <Stir
        vessel="filter_2"
        time="1 hrs"
30    />
    <Filter
        filter_vessel="filter_2"
        stir="true"
    />
35    <WashSolid
        vessel="filter_3"
        solvent="DCE"
        volume="3 mL"
        washes="2"
40    />
    <Add
        reagent="methanol"
```

```

    vessel="filter_2"
    volume="15 mL"
    priming_volume="1.0 mL"
    stir="True"
5  />
    <Stir
    vessel="filter_2"
    time="0.5 hrs"
10 />
    <Filter
    filter_vessel="filter_2"
    stir="true"
15 />
    <Add
    reagent="methanol"
    vessel="filter_2"
    volume="15 mL"
    priming_volume="1.0 mL"
    stir="True"
20 />
    <Stir
    vessel="filter_2"
    time="0.5 hrs"
25 />
    <Filter
    filter_vessel="filter_2"
    stir="true"
30 />
    <Dry
    vessel="filter_2"
    time="1 hrs"
35 />
    <Confirm
    msg="STEP-4 COMPLETED, Pale red solid?"
40 />
    <Add
    reagent="NaOH/PhSNa/MeOH"
    vessel="filter_2"
    volume="22 mL"
    priming_volume="1.0 mL"
    stir="True"
    />
```

```
5      <CleanBackbone
        solvent="methanol"
        near_waste="waste_2"
        far_waste="waste_2"/>
      <Stir
        vessel="filter_2"
        time="2 hrs"
      />
      <Add
10     reagent="acetic acid"
        vessel="filter_2"
        volume="3 mL"
        time="10 mins"
        priming_volume="1.0 mL"
15     stir="True"
      />
      <CleanBackbone
        solvent="methanol"
        near_waste="waste_2"
20     far_waste="waste_2"/>
      <Stir
        vessel="filter_2"
        time="2 hrs"
      />
25     <Filter
        filter_vessel="filter_2"
        stir="true"
      />
      <WashSolid
30     vessel="filter_2"
        solvent="water"
        volume="3 mL"
        washes="2"
      />
35     <Dry
        vessel="filter_2"
        time="1 hrs"
      />
40     <Confirm
        msg="STEP-5 COMPLETED, yellow solid?"
      />
      <Add
```

```

    reagent="Formaldehyde solution"
    vessel="filter_2"
    volume="7 mL"
    priming_volume="1.0 mL"
5    stir="True"
  />
  <HeatChill
    vessel="filter_2"
    temp="65°C"
10    time="3 hrs"
    stir="true"
  />
  <HeatChillToTemp
    vessel="filter_2"
15    temp="30°C"
    stir="true"
    continue_heatchill="False"
  />
  <Add
20    reagent="water"
    vessel="filter_2"
    volume="5 mL"
    priming_volume="1.0 mL"
    stir="True"
25  />
  <Add
    reagent="15% NaOH"
    vessel="filter_3"
    volume="35 mL"
30    priming_volume="1.0 mL"
    stir="True"
  />
  <CleanBackbone
    solvent="water"
35    near_waste="waste"
    far_waste="waste"/>
  <CleanBackbone
    solvent="isopropanol"
    near_waste="waste"
40    far_waste="waste"/>
  <HeatChill
    vessel="filter_2"
```

```
        temp="6°C"
        time="0.5 hrs"
        stir="true"
    />
5    <Transfer
        from_vessel="filter_2"
        to_vessel="filter_3"
        stir="True"
    />
10   <Stir
        vessel="filter_3"
        time="1 hrs"
        stir_speed="500 RPM"
    />
15   <Filter
        filter_vessel="filter_3"
        stir="true"
    />
    <WashSolid
20   vessel="filter_3"
        solvent="water"
        volume="15 mL"
    />
    <Dry
25   vessel="filter_3"
        time="2 hrs"
    />
    <Confirm
        msg="STEP-6 COMPLETED, pale yellow solid?"
30 />
    <Add
        reagent="isopropanol"
        vessel="filter_3"
        volume="5 mL"
35   stir="true"
    />
    <HeatChillToTemp
        vessel="filter_3"
        temp="70°C"
40   stir="true"
    />
    <Add
```

```

    reagent="HCl"
    vessel="filter_3"
    time="5 mins"
    volume="1 mL"
5    stir="true"
    />
    <CleanBackbone
    solvent="water"
    near_waste="waste"
10    far_waste="waste"/>
    <HeatChill
    vessel="filter_3"
    temp="70°C"
    time="1 hrs"
15    stir="true"
    />
    <HeatChillToTemp
    vessel="filter_3"
    temp="30°C"
20    stir="true"
    continue_heatchill="False"
    />
    <Stir
    vessel="filter_3"
25    time="1 hrs"
    />
    <Filter
    filter_vessel="filter_3"
    stir="true"
30    />
    <WashSolid
    vessel="filter_3"
    solvent="isopropanol"
    volume="3 mL"
35    />
    <Dry
    vessel="filter_3"
    time="3 hrs"
    />
40    </Procedure>
</Synthesis>
```

The pressure profile for the synthesis of Arbidol is shown in **Figure 11**.

Automated Synthesis - Oligopeptides

5 Synthesis Cartridge

All oligopeptides were performed using the same reactionware system. The monolith is composed of a *filter reactor* (blue), followed by another *filter reactor* (blue), and a *reactor* (green), see **Figure 30**.

10

Synthesis Description

All the different oligopeptides were synthesised using the same synthetic procedure. The following stock solutions were prepared manually before the synthesis.

15

Fmoc-O-tert-butyl-L-serine (1.55 g, 4.0 mmol) in DMF (6.85 mL).

Fmoc-L-glycine (1.24 g, 4.0 mmol) in DMF (7.15 mL).

Fmoc-L-valine (1.38 g, 50.0 mmol) in DMF (7.00 mL).

O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) (4.57 g, 12.05 mmol) in DMF (24.0 mL).

20

Piperidine in DMF (100 mL, 20 % v/v, commercially available as a solution).

N,N-Diisopropylethylamine (DIPEA) (4.13 mL, 32 mmol) in N-methyl-2-pyrrolidone (7.89 mL).

The synthesis procedure was divided into the different reaction steps:

25

Swelling

Module-1 was manually charged with Fmoc-Ala-Wang resin (0.82 g, 0.50 mmol, 0.61 mmol/g). DMF (9 mL) was added to *module-1* and stirred for 1 h at room temperature to swell the Fmoc-Ala-Wang resin.

30

Deprotection

Then, a two stage deprotection was performed. Piperidine (9 mL, 20 % v/v in DMF) was added to *module-1* and the solution was stirred at room temperature for 3 min. The resin was filtered and the filtrate was transferred to the waste. Then, fresh piperidine (9 mL, 20 % v/v in DMF) was added to *module-1* and the solution was stirred at room temperature for 12 min. The solution was drained and removed from the system into the waste.

35

Resin wash

DMF (9 mL) was added to *module-1*, and the reaction was stirred for 45 s before the solvent was drained and removed from the system. This washing cycle was repeated five times.

5

Coupling

To *module-1*, the appropriate amino acid solution (4 mL), HBTU (4 mL), and DIPEA (2 mL) were added sequentially. The reaction was stirred at room temperature for 1 h. Then the reagents were drained, and the resin was washed (DMF, 5 times, as *Resin wash*).

10

Repeating cycle

Then, *deprotection*, *resin wash*, *coupling* and *resin wash* were repeated for each amino acid.

15

Then a final *deprotection* step is performed to remove the Fmoc group from the last amino acid coupled. The resin was washed with DCM (9 mL), following the same conditions as in the *resin wash* steps.

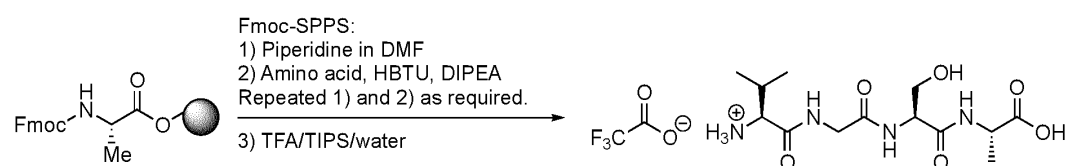
Cleavage

20

To cleave the peptide from the solid support, a cleavage mix was prepared by adding TFA (19 mL) to a mixing flask followed by the addition of TIPS (0.6 mL) and water (0.6 mL), while stirring. The cleavage solution was mixed using the syringe pump, by pumping and delivering the solution to the same flask 4 times. Cleavage solution (10 mL) was then added to *module-1*, and the reaction has been stirred at room temperature for 3 h. Diethyl ether (25 mL) was added to *module-2*, and then the solution (containing the cleaved peptide) was transferred from *module-1* to *module-2*). The reactionware was then cooled to 0 °C for 3 hrs to precipitate the product. The solution was filtered and washed 3 times with diethyl ether (5 mL). The filtrate solution was collected as a precaution if the precipitation method was not successful. The white solid was dissolved in acetonitrile (2 mL) and water (8 mL).

30

VGSA



35

XDL file

<Synthesis>

<Procedure>

<SPPS

```

mmol="0.5"
sequence="VGSA"
resin_loading="0.61"

```

```

/>

```

```

5    </Procedure>

```

```

    </Synthesis>

```

Yield = 42 %

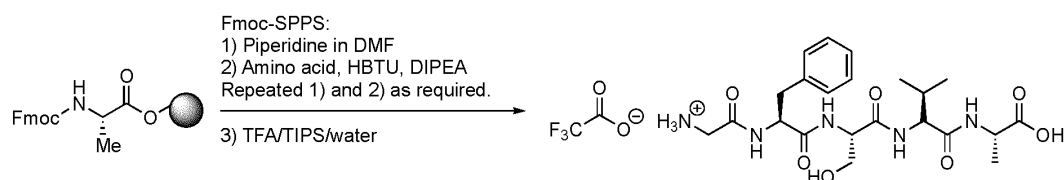
Purity = > 99%

10 HPLC-UV/Vis retention time: 0.8 min, purity: > 99 % (at 214 nm).

ESI(M+H)⁺: expected: 333.1769 Da, observed: 333.1751 Da

The pressure profile for the synthesis of VGSA is shown in **Figure 12**.

15 GFSVA



XDL file

```

20    <Synthesis>

```

```

    <Procedure>

```

```

    <SPPS

```

```

      mmol="0.5"

```

```

      sequence="GFSVA"

```

```

25      resin_loading="0.40"

```

```

    />

```

```

    </Procedure>

```

```

  </Synthesis>

```

30 Yield = 89 %

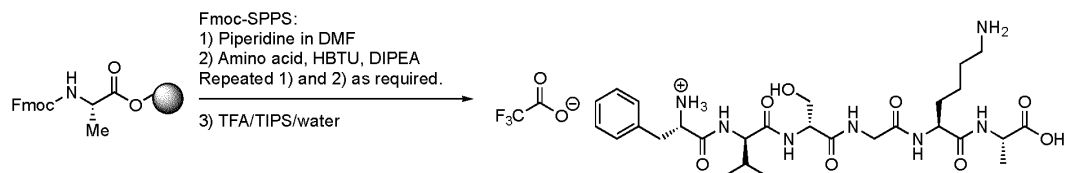
Purity = > 99%

HPLC-UV/Vis retention time: 1.8 min, purity: > 99 % (at 214 nm).

ESI(M+H)⁺: expected: 480.2453 Da, observed: 480.2875 Da

35 The pressure profile for the synthesis of GFSVA is shown in **Figure 13**.

FVSGKA



XDL file

<Synthesis>

<Procedure>

<SPPS

mmol="0.5"

sequence=" FVSGKA"

resin_loading="0.40"

/>

</Procedure>

</Synthesis>

Yield = 31 %

Purity = > 99%

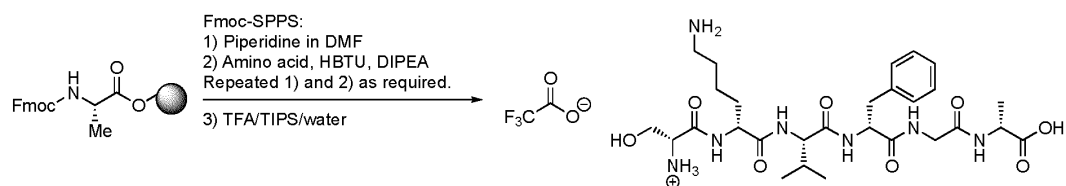
HPLC-UV/Vis retention time: 10.6 min, purity: > 99 % (at 214 nm).

ESI(M+H)⁺: expected: 608.3403 Da, observed: 608.3903 Da

The pressure profile for the synthesis of FVSGKA is shown in **Figure 14**. The flat line at ca. 8.2 h was due to a solvent bottle that finished, so no solvent was added during the washing.

Since no solvent was transferred to *module-2*, no transfer was detected and the system halted until it was manually resumed.

SKVFGA



XDL file

<Synthesis>

<Procedure>

<SPPS

mmol="0.5"

sequence=" SKVFGA"

resin_loading="0.40"

/>

</Procedure>

</Synthesis>

Yield = 42 %

Purity = > 99%

5 HPLC-UV/Vis retention time: 1.8 min, purity: > 99 % (at 254 nm).

ESI(M+H)⁺: expected: 608.3402 Da, observed: 608.3819 Da.

The pressure profile for the synthesis of SKVFGA is shown in **Figure 15**.

10 Automated Synthesis - Oligonucleotides

Synthesis Cartridge

15 All oligonucleotides were performed using the same reactionware system. The monolith is composed of a *filter rector* (blue, *module-1*), and a *reactor* (green, *module-2*), see **Figure 31**. All the synthetic operations were carried out in *module-1*, while *module-2* was used for solvent/waste extraction.

Synthesis Description

20 All the phosphoramidites, 0.5 M 5-Ethylthio-1H-tetrazole in anhydrous acetonitrile (ETT), THF/pyridine/acetic anhydride (CapA), 10% Methylimidazole in THF (CapB), and 0.1 M Iodine solution were purchased from Linktech and used without any further purification. Anhydrous acetonitrile (ACN, extra dry) was purchased from Fisher and used without any
25 further purification. Ammonium hydroxide was purchased from Fisher and used without any further purification.

All the different oligopeptides were synthesised using the same synthetic procedure. The following stock solutions were prepared manually before the synthesis.

30 iBu-dG-CE Phosphoramidite (1.0 g, 1.2 mmol) in anhydrous acetonitrile (11.90 mL)
Bz-dA-CE Phosphoramidite (1.0 g, 1.2 mmol) in anhydrous acetonitrile (11.6 mL)
Bz-dC-CE Phosphoramidite (1.0 g, 1.2 mmol) in anhydrous acetonitrile (12.0 mL)
dT-CE Phosphoramidite (1.0 g, 1.34 mmol) in anhydrous acetonitrile (13.4 mL)

35 *Module-1* was manually charged with CPG (Controlled Pore Glass resin, 10 µmol). The cartridge was purged with Argon (three cycles), and the resin was washed with dry acetonitrile (2.5 mL) twice. The synthesis procedure was divided into the different reaction steps:

40

Deprotection

A three stage deprotection was performed. 3% Trichloroacetic acid in dichloromethane (2 mL) was added to *module-1* and the solution was bubbled with argon (by applying vacuum pulses of 0.1 seconds every 10 seconds to *module-1*) at room temperature for 5 mins. Solution was drained and removed from the system.

Resin Wash

Anhydrous acetonitrile (2.5 mL) was added to *module-1*, and the reaction was bubbled with argon (by applying vacuum pulses of 0.1 seconds every 10 seconds to *module-1*) at room temperature for 5 mins. The solution was drained and removed from the system. This washing cycle was repeated twice. The deprotection and resin wash cycle was repeated three times. At the end of the last washing cycle the solid was dried under vacuum for 1 min.

Coupling

To *module-1*, ETT (1.5 mL) was added to wet the resin with activator. The solution was drained and removed from the system. ETT (1 mL) and the appropriate nucleobase solution (1 mL) were added to the same syringe, and let it mix for 2 mins. After that, the solution was added to *module-1*, and was bubbled with argon for 8 mins. The solution was drained, and it was removed from the system, and the resin was washed (anhydrous ACN, 3 times, as *Resin wash*).

Oxidation

To *module-1*, 0.1 M Iodine solution (2.0 mL) was added, and the solution was bubbled with argon for 5 mins. The solution was drained, and it was removed from the system, and the resin was washed (anhydrous ACN, 3 times, as *Resin wash*)

Capping

CapA (1.5 mL) and CapB (1.5 mL) were mixed in the syringe for 30 seconds before the addition to *module-1*. The solution was bubbled for 5 mins. The solution was drained and removed from the system, and the resin was washed (anhydrous ACN, 3 times, as *Resin wash*).

Repeat!

Then, *deprotection*, *resin wash*, *coupling*, *resin wash*, *oxidation*, *resin wash*, *capping* and *resin wash* were repeated for each nucleotide. Then a final *deprotection* step is performed. The resin was washed two more times with ACN (2.5 mL).

Cleavage and deprotection

To cleave the oligonucleotide from the solid support ammonium hydroxide solution (5 mL) was added to *module-1*, and the reaction was stirred at 55 C for 12 h. The solution
5 containing the targeted oligonucleotide was filtered and the resin was washed with ammonium hydroxide (2.5 mL). The solution was transferred from *module-2* to a collection vial for further purifications.

OPC purification

10 Acetonitrile (5 mL) of was carefully passed through an Oligonucleotide Purification Cartridge* (OPC), followed by 5 mL of 2 M triethylammonium acetate (TEAA) buffer. The eluate was discarded. Aqueous solution of the crude oligonucleotide (1.5 mL) was then passed through the cartridge at a rate of approximately 1 drop per second. The eluate was collected and
15 passed through the cartridge another three times. Following the final collection, the eluate was discarded. 15 mL of 0.1 M TEAA buffer was then carefully passed through the cartridge and the eluate discarded. 1.2 mL of a mixture of water and acetonitrile at 1:1 ratio (by volume), was then passed dropwise through the cartridge, to elute the purified oligonucleotide.

20

5'-TACGAT

XDL file

```
<Synthesis>  
25 <Procedure>  
    <SPPS  
        mmol="0.01"  
        sequence="TACGAT"  
        resin_loading="0.35"  
30    />  
    </Procedure>  
    </Synthesis>
```

Results

35 OD yield: 4.5

ESI:

Species	Expected	Observed
(M+H) ⁺	1789.3426	1789.1365
(M+H) ²⁺	894.6713	894.0672

Species	Expected	Observed
(M+H) ³⁺	596.4475	595.7106

The pressure profile for the synthesis of 5'-TACGAT is shown in **Figure 16**. The flat line around 6.3 h was due to a programmed pause to sample the solid support. After it, the system restarted with a purge.

5'-CTACGT

```

XDL file
10  <Synthesis>
    <Procedure>
      <SPPS
        mmol="0.01"
        sequence="CTACGT"
15    resin_loading="0.35"
      />
    </Procedure>
  </Synthesis>

```

20 Results
OD yield: 3.3
ESI:

Species	Expected	Observed
(M+H) ⁺	1765.3314	1765.1159
(M+H) ²⁺	882.6657	882.0574
(M+H) ³⁺	588.4438	587.7046

The pressure profile for the synthesis of 5'-CTACGT is shown in **Figure 17**.

25 5'-GCTACGT

```

XDL file
    <Synthesis>
30  <Procedure>
      <SPPS
        mmol="0.01"

```

5

```
sequence="GCTACGT"
resin_loading="0.35"
/>
</Procedure>
</Synthesis>
```

Results
OD yield: 2.13
ESI:

Species	Expected	Observed
(M+H) ⁺	2094.3839	-
(M+H) ²⁺	1047.1919	1046.6069
(M+H) ³⁺	698.1280	697.4006
(M+H) ⁴⁺	523.5960	

10

The pressure profile for the synthesis of 5'-GCTACGT is shown in **Figure 18**.

5'-ATGCTACGGCTACGAT

15

```
XDL file
<Synthesis>
  <Procedure>
    <SPPS
      mmol="0.01"
      sequence="ATGCTACGGCTACGAT"
      resin_loading="0.47"
    />
  </Procedure>
</Synthesis>
```

20

25

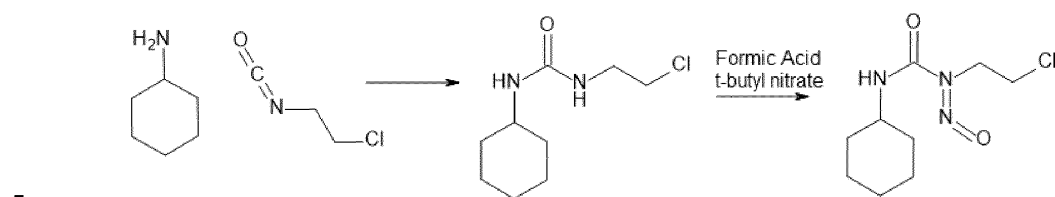
Results
OD yield: 5.37
ESI:

Species	Expected	Observed
(M+H) ⁺	4877.8466	-
(M+H) ²⁺	2438.9233	-

Species	Expected	Observed
(M+H) ³⁺	1625.9489	1625.7477
(M+H) ⁴⁺	1219.4617	1219.0606
(M+H) ⁵⁺	975.56932	975.0482
(M+H) ⁶⁺	812.9744	812.3735
(M+H) ⁷⁺	696.8352	696.1772
(M+H) ⁸⁺	609.7308	609.0304
(M+H) ⁹⁺	541.9830	541.2490

The pressure profile for the synthesis of 5'-ATGCTACGGCTACGAT is shown in **Figure 19**.

Additional Synthesis - Lomustine



Synthesis Cartridge

10 The glass cartridge is composed of a first filter reactor, a second filter reactor and a collection vessel connected in series with transfer tubing. *Module-1* (first filter-reactor) is used to contain all the synthetic procedures.

Synthesis Description

15 The monolith was purged with nitrogen three times (vacuum for 60 seconds followed by refilling with nitrogen until the pressure is stabilized). Diethyl ether (20 mL), 2-chloroethyl isocyanate (1.05 mL, 10 mmol) were added to *module-1*. The reactionware was cooled down to 5 °C, and cyclohexylamine (0.5M, 20 mL, 10 mmol) was added over 5 mins. The solution was stirred for 10 min, filtered, and washed with diethyl ether twice (5 mL). The obtained solid was dried for 5 min under vacuum.

20

Formic acid (18 mL) was added to *module-1*, and the solution was cooled down to 5 °C, followed by adding t-butyl nitrate (1.8 mL, 13.5 mmol). The solution was stirred for 3 min at 0 to 25°C. Water (36 mL) was added to *module-1*, and the solution was stirred for an extra

5 min. The solution was filtered, and the obtained precipitate was washed with water (three times, 5 mL). The obtained pale yellow solid was dried under vacuum for 10 min.

XDL file

```
5
<Synthesis>
  <Hardware>
    <Component
      id="filter"
10    type="cartridge"/>
    <Component
      id="filter_2"
      type="cartridge"/>
    <Component
15    id="collector"
      type="cartridge"/>
    <Component
      id="separator"
      type="separator"
20    />
  </Hardware>

  <Reagents>
    <Reagent
25    name="t-butyl nitrite"
      role="reagent"
      solid="False" />
    <Reagent
      name="cylaclohexmine"
30    role="reagent"
      solid="False" />
    <Reagent
      name="2-chloroethyl isocyanate"
      role="reagent"
35    solid="False" />
    <Reagent
      name="water"
      role="solvent"
      solid="False" />
40    <Reagent
      name="methanol"
      role="solvent"
```

```
    solid="False" />
  <Reagent
    name="diethyl ether"
    role="solvent"
5    solid="False" />
  <Reagent
    name="formic acid"
    role="reagent"
    solid="False" />
10 </Reagents>

  <Procedure>
    <Add
      reagent="diethyl ether"
15    vessel="filter"
      volume="20 mL"
    />
    <Add
      reagent="2-chloroethyl isocyanate"
20    vessel="filter"
      volume="1.05 mL"
      stir="True"
    />
    <Async pid="backbone-cleaning">
25    <CleanBackbone
      solvent="methanol"
      near_waste="waste"
      far_waste="waste"
      n_cleans="1"/>
30    <CleanBackbone
      solvent ="diethyl ether"
      near_waste ="waste"
      far_waste ="waste"
      n_cleans ="2"/>
35    </Async>
    <Await
      pid ="backbone-cleaning"/>
    <Add
      reagent ="cyclohexylamine"
40    vessel ="filter"
      volume ="20 mL"
      stir ="true"
```

```
    stir_speed="500 RPM"
    time="5 min"
    priming_volume="2 mL"
  />
5  <Async pid ="backbone-cleaning">
    <CleanBackbone
      solvent ="methanol"
      near_waste ="waste"
      far_waste ="waste"
10   n_cleans ="1"/>
    <CleanBackbone
      solvent ="diethyl ether"
      near_waste ="waste"
      far_waste ="waste"
15   n_cleans ="1"/>
  </Async>
  <Stir
    vessel ="filter"
    time ="10 min"
20   stir_speed ="600 RPM"
  />
  <Await
    pid ="backbone-cleaning"/>
  <Add
25   reagent ="methanol"
    vessel ="collector"
    volume ="3 mL"
    stir ="true"
  />
30  <Filter
    stir ="False"
    vessel ="filter"
  />
  <Dry
35   vessel ="filter"
    time ="5 min"
  />
  <Add
40   reagent ="formic acid"
    vessel ="filter"
    volume ="18 mL"
    stir ="true"
```

```

/>
<Async pid ="backbone-cleaning">
  <CleanBackbone
    solvent ="methanol"
5    near_waste ="waste"
    far_waste ="waste"
    n_cleans ="1"/>
  </Async>
  <Stir
10    vessel ="filter"
    time ="2 min"
    stir_speed ="600 RPM"
  />
  <Await
15    pid ="backbone-cleaning"/>
  <Add
    reagent ="t-butyl nitrite"
    vessel ="filter"
    volume ="1.8 mL"
20    stir ="true"
    stir_speed ="600 RPM"
    time ="0.5 min"
  />
  <Async pid ="backbone-cleaning">
25    <CleanBackbone
      solvent ="methanol"
      near_waste ="waste"
      far_waste ="waste"
      n_cleans ="1"/>
30    </Async>
    <Stir
      vessel ="filter"
      time ="3 min"
      stir_speed ="600 RPM"
35    />
    <Await
      pid ="backbone-cleaning"/>
    <Add
      reagent ="water"
40    vessel ="filter"
      volume ="20 mL"
      time ="0.5 min"
```

```
    stir ="true"  
    priming_volume ="5 mL"  
    stir_speed ="600 RPM"  
  />  
5    <Stir  
    vessel ="filter"  
    time="5 min"  
    stir_speed ="600 RPM"  
  />  
10   <Filter  
    stir ="False"  
    vessel ="filter"  
  />  
    <WashSolid  
15   vessel ="filter"  
    solvent ="water"  
    volume ="5 mL"  
    repeats="1"  
  />  
20   <Dry  
    vessel ="filter"  
    time ="10 min"  
  />  
    </Procedure>  
25 </Synthesis>
```

The recorded pressure profile for the synthesis of Lomustine is shown in **Figure 24**.

References

- 30 A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.
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CN 115389703

Claims:

1. A method for validating a chemical synthesis, the method comprising the steps of:
performing a chemical synthesis in a chemical synthesiser;
5 recording analytical data during the chemical synthesis, and developing a profile for the analytical data recorded over time; and
comparing the profile for the chemical synthesis against a reference profile, which reference profile is the analytical data recorded over time for a reference chemical synthesis,
wherein the chemical synthesis and the reference chemical synthesis share at least
10 the same reagents and method steps for the same intended product, such as the chemical synthesis and the reference chemical synthesis sharing the same instruction set.
2. The method of claim 1, wherein the analytical data is recorded throughout the chemical synthesis.
- 15 3. The method of claim 1 or claim 2, wherein the analytical data is recorded continuously.
4. The method of any one of the previous claims, wherein the analytical data is
20 pressure data, such as the pressure within a reaction vessel for use in the chemical synthesis.
5. The method of any one of the previous claims, wherein the chemical synthesis comprises two or more, such as three or more, chemical reactions, which chemical reactions
25 are optionally in series.
6. The method of claim 5, wherein each chemical reaction includes one or more associated work-up, purification and/or preparation steps.
- 30 7. The method of any one of the previous claims, wherein the chemical synthesiser is an autonomous chemical synthesiser, such that the chemical synthesis is performed autonomously and the recording of analytical data is performed autonomously, and optionally the comparison of profiles is performed autonomously.
- 35 8. The method of any one of the previous claims, wherein the method comprises the step of generating a reactionware, such as a reaction vessel, for the performance of a chemical reaction within the chemical synthesis.
9. An apparatus for use in the method of validating a chemical synthesis, the apparatus
40 comprising a chemical synthesiser, an analytical unit and a control unit, wherein:
the chemical synthesiser comprises a reactionware for the performance of chemical reactions, which reactionware is optionally also provided with one or modules for work-up

and purification, and further comprising reagents, optionally together with solvents and catalysts, for use in the chemical synthesis;

the analytical unit is adapted for measuring analytical data from the reactionware over time, and for reporting analytical data to the control unit; and

5 the control unit is for receiving analytical data from the analytical unit over time, and is adapted to construct a profile from the analytical data, and the control unit further holding a reference profile for comparison against the profile constructed from the analytical data.

10 10. The apparatus of claim 9, wherein the analytical unit comprises a pressure sensor.

11. The apparatus of claim 9 or claim 10, wherein the apparatus is a portable platform.

12. The apparatus of any one of claims 9 to 11, wherein the chemical synthesiser is an autonomous chemical synthesiser.

15 13. The apparatus of any one of claims 9 to 12, wherein the reactionware is monolithic.

14. The apparatus of any one of claims 9 to 13, wherein the reactionware is glass.

20 15. The apparatus of claim 13 or 14, wherein the reactionware comprises one or more filter reactors and a collection vessel, wherein the filter reactors and collection vessel are fluidically connected in series.

25 16. The apparatus of claim 15 wherein the reactionware comprises a first filter reactor, a second filter reactor and a collection vessel, wherein the first filter reactor, second filter reactor and collection vessel are fluidically connected in series.

30 17. The apparatus of claim 15 or 16, further comprising a manifold for controlling positive gas pressure and negative gas pressure in the reactionware, for transferring fluid between the first filter reactor, a second filter reactor and a collection vessel.

18. A method for generating a reference profile for a chemical synthesis, the method comprising the steps of:
performing a chemical synthesis in a chemical synthesiser;
35 recording analytical data during the chemical synthesis, and developing a reference profile for the analytical data recorded over time; and
providing the reference profile together with the instruction set for the chemical synthesis, wherein the instruction set is for the performance of the chemical synthesis using a reactionware, the instruction set optionally also for the preliminary generation of the
40 reactionware for the chemical synthesis.

19. The method of claim 18, wherein the analytical data is recorded throughout the chemical synthesis.
20. The method of claim 18 or claim 19, wherein the analytical data is recorded
5 continuously.
21. The method of any one of claims 18 to 20, wherein the analytical data is pressure data, such as the pressure within a reaction vessel for use in the chemical synthesis.