An autonomous portable platform for universal chemical synthesis

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Robotic systems for synthetic chemistry are becoming common but they are expensive, fixed to a narrow set of reactions, and must be used within a complex laboratory environment. A portable system that could synthesize known molecules anywhere, on demand, in a fully automated way could revolutionize access to important molecules. Herein, we present a portable suitcase-sized chemical synthesis platform containing all the modules required for synthesis and purification. The system uses a chemical programming language coupled to a digital reactor generator to produce reactors and executable protocols based on text-based literature syntheses. Simultaneously, the platform generates a reaction pressure fingerprint, used to monitor processes within the reactors and remotely perform a protocol quality control. We demonstrate the system by synthesizing five small organic molecules, four oligopeptides, and four oligonucleotides, in good yields and purities with a total of 24,936 base steps executed over 329 h of platform runtime.

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.[1-3] Integrating automation into chemical laboratories can increase chemical accessibility, and replaces procedural ambiguities (i.e. add dropwise, fast stirring, room temperature) with defined parameters, increasing the reliability of complex syntheses.[4] 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[4-8] or solid phase synthesis (SPS) (e.g. oligopeptides[9], oligonucleotides[10], oligosaccharides[11,12]). While SPS systems can be applied to many small molecule transformations, such systems require extensive method development[13,14] and remain based on only a small subset of practical chemistries. This means most of the 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 which can perform a greater range of chemical processes are usually modular in nature and can require extensive reconfiguration to switch from one manufacturing process to another.[15-20] Coupled to this, these synthetic platforms are often infrastructure intensive and are commonly lab-based. Compact universal platforms can be challenging to develop due to the laboratory infrastructure needed for different syntheses. A platform that could be compact and prepare any molecule on-demand, autonomously, and on-site could increase accessibility of important molecules across different fields.

Continuous multistep synthesis processes can be complex and technically challenging (using different equipment for each step), requiring reconfigurable systems to complete even a relatively short protocols. One way to remove platform reconfiguration and miniaturize laboratory hardware is the use of bespoke, self-contained, modular reactors for multi-step synthetic procedures such as 3D printed reactors.[21-23] Even though the full 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.

Herein, we present the design, construction and validation of a compact, universal, automated platform to execute multi-step synthesis employing reusable '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, χDL, see Figure 1. This open standard has been designed to allow any chemical transformation to be precisely expressed and reliably ran on any compatible robotic platform. The reaction procedures are automatically translated into the physical modules by using the unit synthetic operations described in the chemical code file (χDL). 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 fabricated and connected to the platform where all the synthetic operations take place. To ensure portability and autonomous operation, the platform was designed around a programmable manifold to control the vacuum/gas flow through the monolith, a liquid handling system and pressure sensors to control the unit operations needed to perform the synthetic sequences. To demonstrate wide applicability, the system was used for the multi-step syntheses of Nardil, an antidepressant drug;[24] Isoniazid, an antibiotic drug for tuberculosis;[25-27] Dihydralazine, an antihypertensive drug;[28] Lomustine, an alkylating agent used in chemotherapeutic cancer treatments;[29] and Arbidol, an antiviral medication for the treatment of influenza.[30] The versatility of reactionware allowed us to use the same platform to perform iterative solid-phase syntheses (oligopeptides: VGSA, GFSVA, FVSGKA, SKVFGA; oligonucleotides: 5'-TACGAT, 5'-CTACGT, 5'-GCTACGAT, and 5'-ATGCTACGGCTACGAT). These syntheses not only included the iterative process of coupling and deprotection of the respective monomers, but also the cleavage from the resin step and purification (typically performed manually in traditional synthesisers). This platform allows the miniaturisation of a chemical manufacture plant into a small footprint (250 mm x 660 mm x 390 mm) synthesizer that only requires an external supply of electricity, gas, and coolant for operation anywhere. However, to operate at its minimal capacity (e.g., in remote locations) without hindering

the platform capabilities, an electricity supply is the sole requirement, which, in principle, can be supplied as a portable source as well.

Results and discussion

Reactors design

The synthesis of any molecule consists of following a series of fixed consecutive steps (e.g., add, filter, evaporate, heat) containing synthesis-specific parameters (e.g., 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).[4] Each χDL step, expands into hardware specific sub-steps, which define unit operations which can be directly executed. 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, Supplementary Tables S1-S3).

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 (Figure 1a). Reactionware systems are comprised of a series of discrete physical reactor modules which are designed to perform linear operations (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[22,23] or by use of specially created reactionware design software.[21]To fully automate the production of reactionware we have developed a cartridge generator software to produce prototypical reactionware systems based on the χDL description of the synthesis. The parameters of the physical modules can be extracted from the information embedded in the χDL file (Figure 1b). Following the structural elements from ChemSCAD[21], the vessels names in the χDL file are based on four basic designs (i.e. reactor, filter reactor, floating filter, double filter reactor). The program iterates through the χDL steps, and based on their physical operation, it will assign one of the basic designs to each operation. For example, a simplified χDL 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 to 50ºC, not generating a new module. Then, water (10 mL) is added to “reactor”, which will increase the volume of the already made module to 35 mL. For 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 transferred. Finally, the product is precipitated, filtered, and washed (see Supporting Information Section 6.3 for the complete Nardil synthesis). This last step adds a “reactor” 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.

From this process, the individual modules can be automatically assembled into a target-specific monolithic cartridge. The entire automated process produces five different files needed for the synthesis execution, see Figure 1c. 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 manufactured. Finally, this monolith can then be fabricated and plugged into the platform for execution of the automated synthesis. To rapidly prototype the reactor designs, we 3D printed polypropylene reactors, nonetheless, the final reactor design can be manufactured using different materials (e.g., PEEK, glass) and methods (e.g., injection moulding, glassblowing).

Platform specifications

Since all the reaction processes are part of the morphology of the reactionware monolith, the automated platform can be simplified to perform minimal operations to the monolith (i.e., heat, cool, evaporate), see Figure 2. For liquid handling, a fluidic backbone consisting of 8 Tricontinent C3000MP syringe pumps equipped with 6-way distribution valves were used, giving the system 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 has 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, while for cooling, a thermal fluid was circulated through a copper coil (cooled in a dry ice/ethylene glycol mixture), allowing working temperatures of between ca. -13ºC and 120ºC (Supplementary Figure S11).

To control the reaction operations within the reactionware vessels, we implemented a programmable manifold. The manifold consists of five solenoids dedicated for supplying nitrogen, and five solenoids for controlling the vacuum input/output (generated with a micro-pump). For monitoring and controlling the pressure within the system, a pressure sensor was added to one reactionware module (Supplementary Figure S14). All the previous components are controlled with a custom-designed Arduino shield (Supplementary Figure S13). This shield allows the precise liquid manipulation within the monolith by operating the solenoids and micro-pump in the correct sequential order (Supplementary Figure S15).

All the components were put together to maximize the capabilities of the platform, while minimising the footprint, see Figure 3. The final portable synthesis platform consists of acrylic plates fixed to a metal framework (250 mm x 600 mm x 330 mm). The back acrylic plate contains all the power supply unit (PSU), two DC-DC convertors (24V -> 3.5V, and 24V -> 12.0V), a 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 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.

Multistep organic synthesis

To demonstrate the capabilities of the platform, we performed the automated synthesis of five different Active Pharmaceutical Ingredients (APIs): Dihydralazine (compound 2), Isoniazid (compound 3), Nardil (compound 5), Lomustine (compound 7), and Arbidol (compound 13). The digitization process starts with extracting the chemical operations from literature procedures into a χDL file. This file, containing the sequential synthesis operations is then automatically converted into functional interconnected modules to form a molecule specific monolith, see Figure 4. For the two-step reaction of Dihydralazine (Figure 4a), the cartridge consists of three different modules: module-1, a filter-reactor for the synthesis and purification of compound 1; module-2, a filter reactor for the precipitation of compound 2; and module-3, a reactor with a round bottom designed for the collection and extraction of solvent waste. This two-step synthesis has 13 χDL steps compacted from 224 base steps, and a total runtime of ca. 24 hours For Isoniazid (Figure 4b), the cartridge consists of two different modules: module-1, a filter-reactor for the synthesis and purification of compound 3; and module-2, a reactor for the collection and extraction of solvent waste. This one-step synthesis has 15 χDL steps compacted from 121 base steps, and a total runtime of ca. 20 hours. The monolith for Nardil (Figure 4c) comprises 4 different modules: module-1, a reactor for the synthesis compound 4; module-2, a floating filter reactor for the 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 χDL 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 χDL steps containing 159 base steps with a total runtime of ca. 30 hours. These four small organic molecules (Isoniazid, Dyhydralazine, Nardil and Lomustine) can be synthesized using the same platform setup, with all the 15 reagents/solvents initially loaded. The only difference in between syntheses would be the bespoke reactor, which is trivial to change. All the steps required 783 χDL *base steps*, with a total of 130 h of runtime. The four APIs were prepared in good purity along with similar yields compared to manual operation of the cartridges (Supplementary Table S15).

To demonstrate the robustness of the platform, Arbidol, an antiviral medication for the treatment of influenza (compound 13) with a six-step synthesis was included as a target. The nature of the synthesis resulted in a three-module monolith: 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 χDL steps enclosing a total of 952 base steps executed over 64 h of continuous platform operation.

One of the main features implemented in the platform is the dynamic use of a pressure sensor to control and monitor all the operations within the monolith. This allows not only to determine the start and end points of automated operations, but also profiling the reaction process itself. This 'fingerprint' can be used as a quality control to validate the reaction process progress, making sure the processes can go to completion. Figure 5 shows the pressure reaction profile for the synthesis of Nardil, composed of 22 different synthesis steps (Figure 5b), associated with a unique pressure profile (a portion of the overall 'fingerprint'). For example, during purging, considering the length of the cartridge, the pressure drops to ca. 0.9 atm. In the first step, for the synthesis of compound **4**, 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 2-bromoethylbenzene. Then the reaction solution is kept at 75ºC to evaporate the solvent using vacuum pulses (~ 4 s every 6 s). During the separation, diethyl ether is added to

module-1 and transferred to module-2, where the separation happens. The diethyl ether solution, containing the product, is transferred to module-3, before continuing with evaporation.

To benchmark and validate the reaction protocols executed in the platform, Nardil was chosen as the test reaction. The synthesis procedure is composed of 22 different χDL steps, each of them correlated with a unique pressure profile (Figure 5b). To define a standardized synthesis profile, and to account for batch differences, the pressure profiles of three different successful reactions were averaged to obtain a single pressure profile. For a new synthesis execution, a similarity score (Wasserstein distance) can be obtained by comparing it with the reaction standard. Performing this analysis over all the χDL steps results in a quality control vector. Figure 5c shows the vectors of successful and failed (# 1, 2, 5, and 7) reactions. At the end of each run, a quick analysis of the quality control vectors can identify failed steps. For example, the vector for the synthesis of Nardil in reaction # 1 suggests that step 16 (red square) differed from the standard significantly. This step corresponds to the acid addition to precipitate phenelzine sulphate, which was corrected by manual addition of H_2SO_4/iPOH. However, because this addition happened in a different time in the reaction, step 17 was also flagged as failed. At the end of the reaction, and upon inspecting the platform, we realized the tubing connecting to the H_2SO_4/iPOH vessel was clogged. For reaction # 2, the tubing was unclogged, however step 16 still showed a significant deviation, probably due to poor backbone cleaning, which was corrected for the next syntheses. For reaction # 5, even though the hardware operations were completed successfully, no product was obtained. However, the pressure profile differed from the standard enough to be flagged as failed. Finally, for reaction # 7 the steps were scoring high (~ 160) overall, but the transfer from module-3 to module-4 during filtration showed a significant deviation. This was due to a leak that developed during the reaction in the sensor case, resulting in an overall different signal profiling. This demonstrates that the generated pressure profile can be used, not only to control the operations within the monoliths, but most importantly, to validate each executed step. Using this analysis, we found that a threshold of 140 (Wasserstein metric) can be used to perform a quality control remotely.

Solid-phase synthesis

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,[9] oligonucleotides,[10] oligosaccharides[11]), and polyolefins.[5]. Considering is an iterative process, the inherent abstraction of χDL can be used to implement a step-reaction (SPPS, Solid Phase Peptide Synthesis) class containing all the necessary sub-steps to complete the sequence of the specified solid-phase synthesis based only on minimum parameters (i.e., sequence, scale, and resin loading) to complete the desired sequence (Supplementary Figure 5).

Based on all the steps needed for the oligopeptide synthesis, 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, a 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 trifluoracetic acid (TFA) and scavenger reagents (TIPS) was added to module-1. The solution was transferred to module-2, where diethyl ether 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 reactor without any detectable cross-contamination. The versatility offered 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), which generated between 1700-2500 χDL base steps depending on the synthesized oligopeptide.

Similarly, oligonucleotides are commonly synthesized using solid-phase synthesis, for which new step-reaction was added (OSPS, Oligonucleotide Solid Phase Synthesis). To ensure the solid support is completely submerged in the reagent solutions during the iterative process a smaller cartridge (2 mL, inner diameter = 28 mm) with a cone-shaped interior (base = 8 mm, top = 25 mm) was designed. The final monolithic cartridge consisted of two modules: module-1, a filter reactor where all the chemical operations will take place; and module-2, a reactor module used for collecting and discarding filtrate waste. To cleave the synthesized oligonucleotide from the solid support, an ammonia solution is added to module-1, filtered to module-2, and the solution is heated to 55ºC for 12 hours for the final heterocyclic base and phosphate deprotections. Finally, the solution is transferred to the receiving flask, ready for further purification methods. This protocol was used for the synthesis of 5'-TACGAT, 5'-CTACGT, 5'-GCTACGAT, and 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, one χDL step is needed (input the oligonucleotide sequence), resulting in outputs containing between 2300-6500 χDL base steps, see Figure 6.

In summary, we have shown a portable automated platform that can execute a wide variety of synthetic procedures that are mapped into a reactionware system. This platform, despite its small footprint, it is capable of executing the synthesis of 13 different targets including the 6-step synthesis of Arbidol, and the solid phase synthesis of oligopeptides and oligonucleotides (along with cleavage from support). The synthetic steps are coded into the blueprint of the reactors, hence switching between chemistries does not require any hardware reconfiguration of the platform (manual or automated), but just switching the reactor. Using pressure sensors to control and monitor the reaction progress, a reaction standard pressure profile can be obtained. Benchmarking the Nardil synthesis in the portable platform suggested that a threshold of 140 (Wasserstein metric) can be used to remotely diagnosed successful procedures without using expensive analytical techniques.

This method is based on the key components: graph, which describes the location and connectivity of all the platform's physical components, and the reactionware monolith, a set of reactor modules connected sequentially containing all the necessary hardware for the chemical operations to obtain the targeted molecule. The bespoke reactors were reused multiple times (> 20) during the development of the platform and validation syntheses, with no sign of degradation, and no significant effect in the synthesis yield (Supplementary Figure 44). As a result of a full digitized process, the system runs using a versionable executable code which is capable of execute all the abstract explicit operations in the chemical programming language. Finally, the platform is designed to be used with minimal requirements such as electric and inert gas supply, and coolant; however, at its minimal capacity, it only requires an electricity supply, which, in principle, can be supplied as a portable source.

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Author Contributions

LC invented the concept and devised the project and the digitization approach, with help from JSM, SSZ and PJK. SSZ developed the initial system design and built the first prototype together with JSM. WH carried out reactionware synthetic routes for the small organic molecules, while PF and HW helped with method development for the synthesis of oligopeptides and oligonucleotides. JSM carried

out all the automated synthesis and developed the necessary code for the platform. JSM, and PJK wrote the paper with help from LC.

Competing Interests

L.C. is the founder and shareholder in Chemify Ltd. The remaining authors declare no competing interests.

Figure Captions

Figure 1. Schematic representation of any synthesis carried out in the compact/portable platform. a) 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 reactionware cartridges unique to the target molecule. The miniaturized laboratory hardware is manufactured and plugged into the platform for an automated execution of all synthetic steps. 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 reactor that contains all the hardware required for the synthesis. c) All the necessary files for the automated synthesis of any molecule: .xdl: a universal chemical code for the synthesis of any molecule extracted from literature procedures; .json a graph representation of the location, connectivity, and capabilities of all the devices needed for the synthesis; .xdlexe: the portable platform executable code for the synthesis; .ccad: editable CAD designs of the reactors needed; and stl: a ready to print monolithic reactor containing all the chemical operations for the synthesis.

Figure 2. Summary of the implemented reaction and platform operations. a) Reaction operations: The synthesis operations are contained within the customized modules. To control the operations within the modules, a series of sequential steps involving the solenoid valves, pressure sensor, and the micropump are executed. For example, to transfer a solution from one module to another one: i) the solenoids connected to the receiving module and following module are closed to cut off the inert gas supply, ii) the vacuum is turned on in the receiving module, iii) the pressure sensor is used to dynamically detect the transfer is completed, iv) the solenoids in the receiving flask and any other following module are turned on to refill the system with inert gas, v) finally, the vacuum is turned off to refill the antire system with inter atmosphere. All the components are controlled from the custom-made Sensorhub shield. b) Platform operations: generic operations needed for any chemical synthesis, which includes liquid handling of solvents and reagents, heating and cooling the reactor.

Figure 3. Physical implementation of the portable platform. a) Back view of the portable platform containing a micropump for liquid transfers within the cartridges, and evaporations; an ethernet switch for communication; DC-DC converters for distributing power to the Sensorhub and the serial-to-ethernet converters; and a valve to control the input of inert gas into the platform. b) Isometric view of the portable platform showing the location of hotplate for heating, stirring, and cooling the reactors; reagents and solvents are located behind the pumps. c) Top view of the platform showing the location of the PumpHub, to control the liquid handling robot, SensorHub along with the manifold, for liquid handling within the monolith, and S2E (serial-to-ethernet) convertors for ethernet communication. Top row: design and location of the different hardware components, bottom row: physical instances of the compact automated platform.

Figure 4. Synthetic schemes of five different APIs prepared using the platform. Synthetic routes for the synthesis of a) Dihydralazine, b) Isoniazid, c) Nardil, d) Lomustine, and e) Arbidol with the respective monolithic cartridges used in the synthesis, yield (purity determined from HPLC), number of base steps executed and runtime. All the monolithic cartridges are composed of three different modules, arranged in different sequence depending on the target molecule: a filter reactor (blue) and a reactor (green) used for stirring, heating, filtering, and evaporating; and a floating filter module (red) used for liquid-liquid extractions.

Figure 5. Fingerprinting and validation of Nardil synthesis using the pressure profile. The reaction pressure profile elucidates all the different processes within the cartridge during the synthesis execution. a) The full pressure profile for the synthesis of Nardil. The plot shows how the pressure changes depending on the physical operation (insets) that occur within the monolith. b) The full pressure profile can be subsequently divided into χDL step specific profiles, showing different features depending on the on-going process. c) Fingerprinting every step in the reaction process can be used to monitor and validate its execution. Comparing any ongoing procedure to a standard profile (an average of three successful reactions) and obtaining a similarity score (Wasserstein metric) for each executed step results in a quality control vector. This metric allows the determination of failed executions, and upon inspection, a specific failed step can be identified (red squares in fingerprint). This analysis can be extended to the reaction profile to obtain an overall similarity metric, and a quality control analysis can be performed to determine successful syntheses (any protocol above 140 metric will be marked as failed) without the use of expensive or complex equipment.

Figure 6. 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 oligopeptide (SPPS) or oligonucleotide (OSPS) sequences are obtained. The reactors can be reused without any cross contamination. a) Oligopeptides prepared in the portable platform using a three-module cartridge system consisting of two filter reactors (blue) followed by a reactor module (green). 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 low-volume filter reactor (blue), where the iterative coupling and cleavage of the final oligonucleotide from the solid support takes place, and module-2, a reactor (green) module, used to remove filtrates from the system.

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Methods

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

Characterization. NMR measurements were performed with Bruker Advance III HD 600 spectrometer operating at 600.1 and 150.9 MHz for 1H and 13C, respectively. Spectra were collected at 298 K, chemical shifts are reported in ppm, calibrated for the (residual) NMR solvent signal.
HPLC analysis was performed on a Thermo Dionex Ultimate 3000 equipped with a 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 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 50-2000 m/z for small molecules and oligopeptides, while 500-5000 m/z for oligonucleotides. Data was analysed using the Bruker DataAnalysis v4.1 software suite.

3D printing. 3D printing reactionware vessels was done using a Ultimaker 2+ FDM. 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. 0ºC), Nozzle temperature: 260ºC, Speed: 15 mm/s. Sensor cases were 3D-Printed 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.

Platform liquid handling and pneumatic system. For solvents/reagents handling, 8 C3000MP Syringe pumps equipped with 12.5 ml syringes 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 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.

Automated Isoniazid synthesis. 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 75ºC and was kept at this temperature for 4 h. Then, the solution was cooled down to 40ºC and stirred for 30min 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 60ºC for 20min Then, the solution was cooled down to 8ºC and stirred for 30min Finally, the solution was filtered and dried for 10 h under vacuum. Isoniazid was obtained as a white solid (2.0 g, 73% yield)

Automated Dihydrilazine synthesis. The monolith was preloaded with 1,4-dicyanobenzene (1.28g, 10 mmol) and urea (3.65g, 61 mmol). The system was purged with nitrogen for three times. Hydrazine hydrate (3.65 mL, 75 mmol) was added to module-1 while stirring. The monolith was heated to 100ºC and kept at this temperature for 3 h. The reactionware was cooled to 30ºC and water (10 mL) was added to module-1. The solution was filtered and washed with water (10 mL, x2). 2M H_2SO_4 (12.5 mL) was added to module-1 over 10min while stirring at 500 rpm. The monolith was heated to 100ºC

for 1 hr. The solution was hot filtered, and then cooled down to 30ºC. The precipitate formed was filtered off and washed with 5 mL of water twice. Dihydralazine sulfate (2.1g, 72% yield) was obtained as a yellow solid after drying under vacuum for 10 h.

Automated Nardil synthesis. The reactor was purged with nitrogen three times. Ethanol (10 mL) and hydrazine hydrate (2.9 mL, 60 mmol) were added to module-1. The solution was heated to 75ºC, and 2-bromoethylbenzene (1.84 mL, 10 mmol) was added to module-1 over 2min Ethanol (5 mL) was added to module-1, and the system was kept at 75ºC for 2 h while applying vacuum pulses of 1 s every 60 s. The solution was cooled down to 40ºC and vacuum was applied to module-1 for 2.5 h 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 35ºC. Isopropanol (20 mL) was added to module-3. Then, a H_2SO_4-isopropanol mixture (1:5, 6 mL) was added to module-3 over 5 mins, and the solution was stirred for 2 h. Hexane (5 mL) was added to module-3 and stirred for 30 min. 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 h.

Automated synthesis of Lomustine. The reactor was purged with nitrogen three times. Diethyl ether (20 mL), 2-chloroethyl isocyanate (1.05 mL, 10 mmol) were added to module-1. The sp;solution was cooled down to 5ºC, and cyclohexylamine (0.5M, 20 mL, 10 mmol) was added over 5 min. The solution was stirred for 3 h, filtered, and washed with diethyl ether twice (5 mL). The obtained solid was dried for 1 h under vacuum. Formic acid (18 mL) was added to module-1, and the solution was cooled down to 5ºC, followed by adding t-butyl nitrate (1.8 mL, 13.5 mmol). The solution was stirred for 2 h at 0→25ºC. Water (36 mL) was added to module-1, and the solution was stirred for an extra 1 h. 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 h to yield compound **5** (1.6g, 69 % yield)

Automated synthesis of Arbidol. $ZnCl_2$ (160 mg) was pre-loaded to module-1. 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 6ºC for 30min and then, the enamine (4.9 mL) was added to module-2 within 10 min. The monolith was heated to 75ºC and kept at this temperature for 2 h and stirred at 200 rpm. Then, the monolith was cooled down to 30ºC. 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. 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 60ºC for 2 h under vacuum pulses (4 s vacuum, 6 s waiting). Methanol (8 mL) was added to module-1, and it was evaporated first at 60ºC for 1 h under vacuum pulses (4 s vacuum, 6 s waiting), and then at 60ºC under vacuum for 2 h. After evaporation, methanol (4 mL) was added to module-1, and the solution was cooled down to 25ºC, 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 grey solid was dried under vacuum for 3 h. 1,2-dichloroethane (10 mL) was added to module-2, and the solution was stirred at 200 rpm for 30min to dissolve Arbidol-B. Then, 48% HBr (2.8 mL) was added to module-1, and the monolith was heated to 70ºC. 10% H_2O_2 (9 mL) was added within 30 mins, and the reaction was stirred at 70ºC for 2 h. DCE was evaporated at 60ºC using vacuum pulses (3 s vacuum, 6 s waiting). Then, the monolith was cooled down to 30ºC, and it was stirred at this temperature for 1h. The solution was filtered, and methanol (15 mL) was added to the residual solid in module-1, and the reaction mixture was stirred for 30min This process was repeated twice. Finally, the white-red solid was dried under vacuum for 1 h. 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-1. The reaction mixture was stirred at room temperature for 2 h. 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. 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 65ºC and stirred at this temperature for 3 h. Water (5 mL) was added to module-1. 15% NaOH (35 mL) was added to module-2, and the monolith was cooled down to 6ºC for 30min Once cooled, the solution in module-2 was transferred to module-3 and it was stirred for 30min Finally, the solution was filtered, washed with water (15 mL), and dried under vacuum at for 2 h to obtain a pale yellow solid. Isopropanol (5 mL) was added to module-2 containing Arbidol-E, and the monolith was heated to 70ºC. Once at this temperature, conc. HCl (1 mL) was added to module-2 within 5min The reaction mixture was stirred at this temperature for 30 mins, before it was cooled down to 30º C, turning off the heating. Then, the solution was stirred for 30min and then filtered and washed with isopropanol (3 mL). Finally, the white-yellow solid was dried under vacuum for 3 h (0.75 g, 5.6 % overall yield)

Automated SPPS synthesis. This general procedure was used for the synthesis of all oligopeptides, by using the amino acids required for the desired sequence. The 3D printed reactor 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. 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 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. 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. 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). The deprotection, resin wash, coupling and resin wash were repeated for each amino acid. 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). 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 0ºC for 3 h 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). The obtained solution was freeze dried to obtain a white solid, that was furthere analyzed by HPLC. The full details can be found in Supplementary Section 7.

Automated oligonucleotides synthesis. This general procedure was used for the synthesis of all oligopeptides, by using the amino acids required for the desired sequence. 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. 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 min. Solution was drained and removed from the system. Anhydrous acetonitrile (ACN, 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 5min 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. 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 2min After that, the solution was added to module-1, and was bubbled with argon for 8min The

solution was drained, and it was removed from the system, and the resin was washed (anhydrous ACN, 3 times). To module-1, 0.1 M Iodine solution (2.0 mL) was added, and the solution was bubbled with argon for 5min The solution was drained, and it was removed from the system, and the resin was washed (anhydrous ACN, 3 times). 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 5min The solution was drained and removed from the system, and the resin was washed (anhydrous ACN, 3 times). The deprotection, resin wash, coupling, resin wash, oxidation, resin wash, capping and resin wash were repeated for each nucleotide. A final deprotection step is performed. The resin was washed two more times with ACN (2.5 mL). 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 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. To purify further the oligonucleotide, Acetonitrile (5 mL) was carefully passed through an Oligonucleotide Purification Cartridge* (OPC), followed by 5 mL of 2M 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 passed through the cartridge another three times. Following the final collection, the eluate was discarded. 15 mL of 0.1M 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.

Data Availability. The supporting volume includes full details to reproduce this work. This consists of full details to reproduce the electronic and mechanical construction of the platform. The XDL files (.xdl), along with the respective graph (.json) and 3D reactor design (.stl), and full analytical data is given including our pneumatic fingerprint for the reactions here: doi:10.5281/zenodo.6542750.

Code availability. Code is available from https://github.com/croningp/PortablePlatform