# Convergence of multiple synthetic paradigms in a universally programmable chemical synthesis machine

* The connection of multi-step syntheses in a single machine to run many different protocols and reactions is not possible, as manual intervention is required.
* In this research the researchers show how the chemputer synthesis robot can be programmed to perform many different reactions, including solid-phase peptide synthesis, iterative cross-coupling and accessing reactive, unstable diazirines in a single, unified system with high yields and purity.
* The researchers’ system, performs around 8,500 operations while reusing only 22 distinct steps in 10 unique modules, with the code able to access 17 different reactions.
* The researchers also demonstrate a complex convergent robotic synthesis of a peptide reacted with a diazirine – a process requiring 12 synthetic steps.
* The main problem with organic synthesis in not only labor intensive but also highly specialized, requires years of training.
* The lack of a universal approach means that current technologies are highly specialized and focus on specific niches.
* There is a need of paradigm that not only capture the expertise and numerous hours spent discovering and optimizing batch reactions but also is amenable to the development of an overarching ontology that allows universality.
* Previously the concept of the Chemputer, a programmable batch synthesis robot, was designed and developed to demonstrate the proof of the principle for general approach.
* However, the ability to converge existing disparate automation strategies is a significant challenge.
* A good example highlighting these limitations is the classic iterative formation of peptides, one of the first classes of reaction to be automated.
* No system can perform the final precipitation of the product without manual intervention.
* Once produced in the peptide synthesizer, the product compounds cannot then be used in the same type of robot for any further automatic procedure beyond peptide coupling chemistry except for some limited additional bespoke post-synthetic modifications, and these are not generally programmable across different systems.
* We need a general system that can generalize all the above aspects into one system, that is it can also be programmable and modular.
* In this the researchers develop the Chemputer paradigm such that a single system, is capable of the automatic execution of iterative cross-coupling (ICC) MIDA-boronate-based Suzuki-Miyaura coupling reactions, solid-phase peptide synthesis (SPPS), cleavage from the resin, deprotection and isolation, and the synthesis of succinimidyl4, 4’-azipentanote (NHS-diazirine)- a photoreactive crosslinker with important applications in biology and materials science.
* During this process the researchers also designed a range of new ‘chemical hardware’ modules and showed how these could be versioned and improved to create new iterations of the hardware.
* They also find that this approach is critical to enhancing the reliability and functionality of the hardware whilst ensuring interoperability of the software.
* Result: - the path towards a universal synthesis machine was paved by previous efforts to automate specific reaction classes, such as SPPS and ICC chemistry based on the Suzuki-Miyaura reaction.
* At first step the researchers aimed to merge these efforts and automate them in a uniform way, using standard synthetic chemistry unit operations.
* For doing this we need to do a series of automated base steps. For example, the ‘Evaporation’ unit operation consists of the following base steps: -
  + A move base step to move a liquid to an evaporator
  + A StartEvaporation base step to being the evaporation
  + A wait base step to wait for a given time
  + A StopEvaporation base step to stop the evaporator
  + A final Move base step to move the concentrated liquid to the target destination.
* One key principle of the researchers’ strategy was to mimic the manual organic synthesis workflow closely.
* The advantage of this approach is that existing batch procedures, which have been developed for manual syntheses, can be easily automated using the platform, and this defines a universal, programmable system architecture or ontology – critical for the digitization of chemical synthesis and currently lacking.
* The ideal ‘chemical hardware’ has been designed so that it can be constantly versioned, allowing continuous evolution to maximize the universality of the Chemputer whilst ensuring backward compatibility.
* A key feature of the architecture was to design the hardware modules so that they are logically and physically self-contained units.
* These modules are connected via the liquid-handling backbone, it connects all the modules to each other.
* This connectivity is described as graph. This graph is used by the software to locate all available hardware resources.
* It is highly modular approach in which the architecture is readily extendible, and it is easy to make changes in one module without affecting the rest of the system.
* This modularity of the architecture in hardware and software allows a formal ontological description that is universal for all chemical syntheses, which is a key feature of the Chemputer.
* The first step towards the automation of the three target syntheses – ICC, NHS-diazirine synthesis and SPPS-was to analyses the underlying unit operations.
* After these unit operations were identified, it was possible to develop a universal software ‘wrapper’ to link each unit operation to a corresponding hardware module on the basis of the provided graph.
* Automated reaction: - the first reaction class developed by researchers was used for automation in their system it is based on the ICC sequence, which consists of three high-level steps: deprotection, coupling and purification.
* The deprotection could be routinely performed using the standard hardware, the Suzuki-Miyaura cross-coupling reaction required a new deoxygenation unit operation as strict inert-gas and moisture-free conditions were required.
* The key ‘catch-and-release’ purification step, requiring the product to be dry-loaded onto a silica column, needed to be implemented in the researchers’ architecture.
* Initial research shows that improvements in the handling and storage of sensitive chemicals were needed owing to low yields arising from the decomposition of reagents left for a prolonged period in the laboratory.
* At last, the researchers enabled the Chemputer to perform solid-phase peptide synthesis.
* The peptide synthesis is simple and robust, the challenge is to perform each synthesis is simple and robust, the challenge is to perform each synthetic step with high conversion and purity.
* To achieve this, it is essential to avoid cross-contamination of reagents and solvents during the synthesis.
* The agitation of the heterogenous mixture of the solid-phase resin and the liquid reagent solutions needed to be fine-tuned so that mixing is effective but does not damage the resin.
* Emulation of the highly specialized automation procedure used for the dedicated ICC system.
* The researchers’ general platform required a new suite of unit operations. First, to allow for the strict moisture and oxygen-free conditions required, these unit operations were combined into a programmable gas-vacuum manifold (PM).
* The manifold consists of two rows of solenoid valves allowing switching between vacuum and a high- or low-pressure line of inert gas.
* In addition to the six switchable gas/vacuum ports, the PM provides nine passive inert-gas lines, which can be used to keep reagents under a protective atmosphere.
* The ICC reactions required solutions and solvents to be dried, filtered and loaded onto a solid silica support at various stages in the procedure.
* A cartridge carousel was developed for these requirements.
* The carousel accommodates up to six cartridges that were filled with either silica (for the catch-and-release step), Celite (for filtrations), molecular sieves or magnesium sulphate (for drying solvents), and this forms one of the ten modules built for the system.
* The NHS-diazirine also required a moisture-free environment; moreover, it posed a formidable challenge as temperature- and light-sensitive reagents were required in this synthesis.
* Therefore, the researchers developed a reagent module that allowed chilling, stirring and maintaining an inert atmosphere.
* The solids can be kept in the flask until required, whereupon they could be dissolved by addition of the appropriate solvent to the flask.
* The reagent module ensured that sensitive chemicals could be stored at low temperatures and dissolved directly before they were used in the synthesis.
* This new module was an important factor that substantially enhanced the conversion of the diaziridine formation step, as it allowed for the preparation of the hydroxylamine-*O*-sulfonic acid (HOSA) solution at 5 during the run.
* A crucial part of the hardware library is the liquid-liquid extraction module.
* The critical operation of this module is to detect the phase boundary formed between two immiscible liquid phases using a conductivity sensor.
* A switchable system with two conductivity sensing modes was developed.
* The sensitivity was increased by more than an order of magnitude- the signal-to-noise ration of the sensor was improved from around 5 to 60 in a typical use case.
* For the NHS-diazirine synthesis, a column chromatography module was also required to purify the final product.
* The efflux of the cartridge is attached to a value that acts as a distributor in the fraction collector.
* The valve directs the eluting solvent from the column either to the solvent waste or to one of the five collection flasks on the basis of predefined elution volumes.
* Finally, to achieve high yields in solid-phase peptide synthesis on the platform, extra cleaning routines and priming steps were added to the executable code to prevent cross-contamination of reagents.
* It was found by the researchers that typical three-necked flask used in all previous syntheses on the Chemputer was not suitable for solid-phase peptide synthesis.
* The researchers developed a suitable reactor that was designed with a filter frit to capture the solid-phase resin, and a fast-responding heating element that allowed for rapid heating cycles was required for efficient iterative synthesis steps.
* Gentle mixing of the system to avoid damage to the resin could be achieved in two ways: with an overhead stirrer or via the purging of gas through the reaction mixture.
* The final model of the NHS-diazirine synthesis was coupled with an overhead stirrer or via the purging of gas through the reaction mixture.
* After the required modules were built, the precise configurations of the Chemputer were implemented.
* The first step, the reagent and solvent were added to the separator module to deprotect the MIDA boronate **1**.
* A liquid-liquid extraction was then performed, followed by filtration and evaporation of the organic solvent in the rotary evaporator.
* The free boronic acid **2** was redissolved and deoxygenated by purging with argon. The deoxygenated boronic acid solution was slowly added to the reactor vessel, which precharged with the catalyst, base and bromoaryl coupling partner.
* The final product **3** was obtained after liquid-liquid extraction, filtration, evaporation of the organic solvent and purification via the catch-and-release protocol in a yield comparable to the specialist platform.
* The researchers select the NHS-diazirine **7** because its own the high value of the final product and the operational challenges the synthesis poses.
* The imine was formed in the first step and transformed to the diaziridine 5 in situ by adding the corresponding reagents in the appropriate sequence while maintaining low temperature.
* The diaziridine in **5** was oxidized to the diazirine, followed by liquid-liquid extraction, drying of the organic solvent phase and concentration to yield 62% of the 4-diazirinrprntanoic acid intermediate **6**.
* The carboxylic acid in 6 was transformed into an activated ester, which required temperature control and exclusion of humidity.
* The final product was purified by column chromatography without manual intervention to give NHS-diazirine 7 in 21% yield.
* The solid-phase peptide synthesis involved a repetitive cycle of adding reagents and removing them after the reaction by filtration, followed by a washing step by a drying step.
* The fully assembled peptide 9 must be cleaved from the resin and the side chains must be deprotected.
* In the Fmoc-SPPS, the final deprotection step is usually performed with a cocktail of concentrated trifluoroacetic acid (TFA) and scavenger reagents, which must be freshly prepared each time before use.
* To ensure quantitative transfer of the final product from the filter, the peptide was redissolved in acetonitrile-water solvent mixture and then moved to a tared storage vial, purified by preparative HPLC and lyophilized as required.
* The Chemputer SPPS gave the target materials **10a -10c** in good yield and purity.
* The synthesis of **10a** involved a light-sensitive and high-value NHS-diazirine 7 building block, which was prepared previously by another modular build of the Chemputer as described.
* The system give result that is equivalent to the yield and purity obtained with a highly specialized SPPS system.
* We should also note that this commercial system was not able to perform cleavage, deprotection and precipitation; hence, these steps had to be performed manually.
* The Chemputer has shown the successful demonstration of execution of 17 different synthetic protocols with a total of 8,500 units operations.
* To achieve this breadth of chemistry only, 10 different modules and 22 distinct steps were needed, highlighting how a single function can be reused in many different synthetic contexts in the Chemputer.
* Discussion: - this work illustrated by the successful preparation of three target molecules representing a range of different compound classes.
* If enough space is available, a single hardware instance of the platform that contains all the modules required in the three syntheses can be built.
* It was also demonstrated how two instances of the Chemputer can work in tandem to deliver a high-value final product.
* The first instance produces the activated ester of the diazirine-containing molecule 7, which was then used by the second instance in the peptide synthesis.
* This type of products are key tools in biochemistry and medicinal chemistry, and are usually very expensive.
* Methods: - Chemputer software – the Chempiler software suite consists of three Python modules: Chempiler, SerialLabware and ChemputerAPI.
* SerialLabware provide a Python library that allows us to control typical commercially available laboratory hardware such as hotplates or rotary evaporators.
* The main task of the ChemupterAPI is to communicate with the custom-made pumps and values of the Chemputer.
* Chempiler brings the functionality of the different devices together.
* Chempiler reads in a graph file that describes the topology of the physical setup. A web app called Chemputer Graph has been developed to conveniently generate the required graph files.
* Chemputer architecture and hardware modules: - once all the unit operations required in a given synthesis were implemented by the hardware modules, the process could be executed.
* The unit operations were implemented in a general way in the hardware modules so that they could be reused many times in different contexts – for example, during one multi-step synthesis or between different syntheses.
* The different modules from the Chemputer module library could be combined freely to build a functional Chemputer hardware assembly.
* Material transport between different modules was performed by the liquid-handling backbone - a series of pumps and valves that were required to be manufactured from polyether ether ketone (PEEK) or polytetrafluoroethylene (PTEE).
* Every Chemputer hardware assembly constituted its own network with a PoE-capable switch.
* Two options to control vacuum and gas supply were developed. The first option was based on a Chemputer valve as used in the liquid-handling backbone and provided one switchable vacuum/inert gas line.
* As a more general solution, a pneumatic manifold was designed, which provided six lines that could be switched between vacuum and either of two different pressures of inert gas or two different types of gas.
* The pneumatic manifold consisted of two rows of six 12 V solenoid valves. These were operated by an Arduino board with a custom-made shield.
* Reagents were either charged in the storage flasks as solutions or provided as solids, which were then dissolved on the fly during the synthesis.
* The advanced reagent bottle setup consisted of a standard reagent bottle, a magnetic stirrer and a cooling jacket.
* The standard Chemputer reactor consisted of a multi-neck round bottom flask on a stirrer hotplate and was connected to the liquid-handling backbone as well as to the inert gas supply.
* The SPPS reactor consisted of a filter frit, a 60W heating mat and a temperature sensor.
* A PID feedback loop implemented on Arduino controlled the power output of the heating mat to maintain the target temperature.
* The inlet of the filter frit was connected to the inert gas supply and the liquid-handling backbone.
* The outlet of the filter frit was connected to a Chemputer valve that switched between vacuum, inert gas or the liquid-handling backbone.
* Similar to the SPPS reactor, the inlet of the jacketed filter was connected to (1) the liquid-handling backbone to add reagents and (2) to the inert gas supply to establish an inert atmosphere.
* The content of the jacketed filter was agitated with an overhead stirrer. The flow tube consisted of two isolated steel tubes and consisted one leg of the voltage divider. The other leg of the voltage divider consisted of a capacitor for noise filtering and a circuit that switched between two different reference resistances.
* Two different models of rotary evaporators were integrated into the Chemputer architecture: a BUCHI Rotavapor R-300 and an IKA RV10.
* The rotary evaporator was connected to the liquid-handling backbone at two points. One tube reached the bottom of the evaporation flask to add the solution to be evaporated and to remove the concentrated product.
* A second connection was made to the outlet of the collection flask at the bottom of the condenser to remove the distilled solvent.
* The outlet of these cartridges was combined via a six-way check valve into one line again, which was connected back to the liquid-handling backbone.
* The six-way check valve was replaced with a second Chemputer valve. A third Chemputer valve was connected to the outlet of the chromatography column and switched between different fractions.
* Automated Suzuki-Miyaura ICC: - the separator module was manually charged with 6-methyl-2-(m-tolyl)-1,3,6,2-dioxazaborocane-4,8-dione **1** and the reactor module was charged with 2-(4-bromophenyl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione, second-generation XPhos Buchwald Precatalyst and K3PO4.
* The automatic procedure started with the dissolution of the MIDA building block **1** in THF and aqueous NaOH. After stirring, potassium phosphate buffer was added, followed by diethyl ether. The phases were separated and the organic layer was washed with brine.
* The free boronic acid **2** was dissolved in THF and transferred to a holding flask. THF was added to be reactor and the resulting suspension was deoxygenated and heated to 55. The solution of the free boronic acid **2** was slowly added to the reactor over 4h.
* The crude product **3** was dissolved in THF. The resulting solution was loaded onto a silica column in nine portions of approximately 3 ml each. The column was then washed with diethyl ether. After that the product was eluted with THF. The THF solution was transferred to the rotary evaporator and concentrated to dryness to give the pure product **3** as an off-white solid.
* Automated diazirine synthesis: - the jacketed filter was manually charged with anhydrous MgSO4.
* Hydroxylamine-O-sulfonic acid (HOSA), a solution of ammonia in methanol and 1-ethyl-3-carbodiimide (EDCI) were placed in advanced reagent bottles, capable of stirring and temperature control, and kept under argon at 5 .
* The operations were performed, Step 1: ammonia in methanol was added to a jacketed filter followed by a solution of 4-oxopentanoic acid **4** in methanol. The mixture was cooled to -10 and stirred for 3h.
* After that a fresh solution of HOSA in methanol was prepared and added slowly to the reaction mixture.
* The reaction mixture was filtered and concentrated in vacuo at ambient temperature to give ammonium 3 propanoate 5.
* Step 2, a reaction vessel was charged with aqueous KOH and cooled to 0 . The crude product 5 in the rotary evaporate flask was dissolved in aqueous KOH and transferred to the reaction vessel.
* A solution of iodine in diethyl ether was added, when the reaction was complete, the reaction was stirred at 0 .
* The phase was separated, the aqueous phase was acidified by the addition of aqueous HCI and extracted with diethyl ether. The organic phases were dried by passing them through a cartridge packed with MgSO4 and sand and concentrated in vacuo to give pure propanoic acid **6**.
* Step 3: the product **6** in the rotary evaporator was dissolved in dichloromethane and transferred to a new reaction vessel.
* A solution of EDCI in dichloromethane was freshly prepared and added to the reaction mixture. Then a solution of *N*-hydroxysuccinimide in acetonitrile was added and the reaction was stirred.
* The crude material was redissolved in dichloromethane, loaded onto to the column and eluted with dichloromethane.
* The fraction from 150ml to 330ml was collected, concentrated and dried in vacuo to give the pure product, succinimidyl 4, 4’-azipentanote 7, as a white solid.
* Automated SPPS: - the SPPS reactor was manually charged with Fmoc-Ala-Wang resin **8**.
* The following steps are performed automatically:- the resin was swelled in DMF at 25 for 1h,
* Double Fmoc deprotection was performed with piperidine solution in DMF for 3 min and 12 min, respectively. After five washing cycles with DMF, the amino acid in DMF was coupled with HBTU in DMF and diisopropylethylamine in *N*-methyl-2-pyrrolidone.
* The coupling was done twice, the resin was washed five times with DMF after each coupling step.
* After the peptide sequence was assembled, the resin was washed five times with DCM. Now the diazirine functionalization of the *N*-terminus was performed. The solid succinimidyl 4, 4’-azipentanoate 7 was dissolved in DCM.
* Then, a solution of diisopropylethylamine in DCM was added to the reactor followed by the solution of 7 in DCM.
* Then, the resin was washed five times with DCM and dried under a flow of argon, the cleavage mix was freshly prepared.
* After that the cleavage mix was filtered and the filtrate was added to the precooled diethyl ether.
* The SPPS reactor was rinsed with the cleavage mix and the washings were added to the jacketed filter with diethyl ether.
* Then, the precipitate was filtered and the filter cake was washed with diethyl ether at -25 .
* The jacketed filter was washed once with CAN and water, and this liquid was added to the receiver vial.
* The material was further purified manually by preparative HPLC to give **10a** as a white solid.