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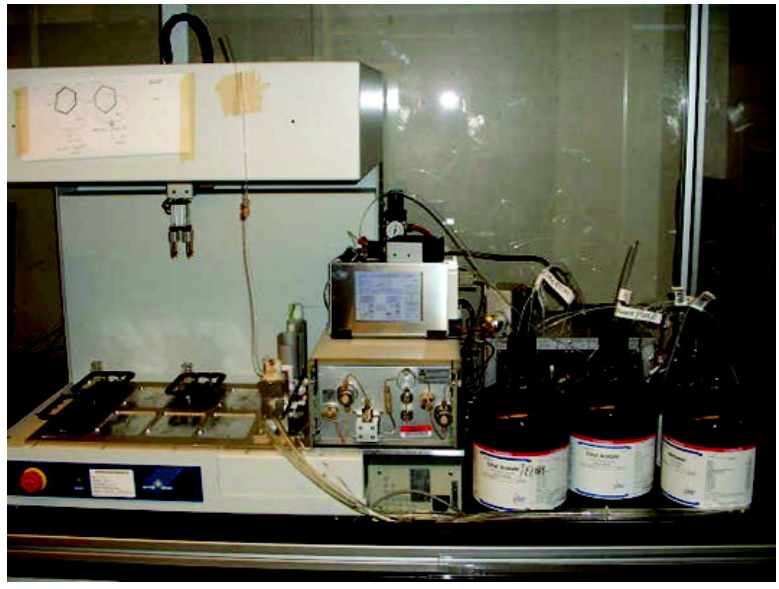

Construction and Validation of an Automated Flow Hydrogenation Instrument for Application in High-Throughput Organic Chemistry

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This manuscript details the construction of a fully automated flow hydrogenation apparatus for use in high-throughput organic synthesis. The instrument comprises of a Bohdan robot platform coupled with a ThalesNano H-cube hydrogenator and a series of solvent valves and pumping mechanisms. Using this instrument, we have been able to fully automate a number of key transformations that could not otherwise be conveniently undertaken in a high-throughput manner.

Introduction

In order to circumvent the rising costs of research and development, the pharmaceutical industry relies upon high-throughput organic synthesis (HTOS) to prepare the vast numbers of chemical analogues required to drive drug discovery programs through library generation, hit-to-lead, and lead development programs.[1] In order for such an approach to be successful, the chemical transformations employed must be clean, high-yielding, and amenable to automation using either parallel or serial reactions and preferably a liquid handling format. In addition, a reaction protocol that utilizes reagents that can be easily removed from the product at the end of the reaction is highly desirable.[2] One type of reaction that would at first appear to be ideal in an HTOS setting is catalytic hydrogenations.[3] These reactions often only require hydrogen gas and a heterogeneous catalyst and, in addition, have workup procedures that require a simple filtration and evaporation to yield essentially pure products. "Workhorse" hydrogenation reactions employed by medicinal chemists include benzyl-protecting group removal, conversion of nitro compounds into amines, conversion oximes to amines, and asymmetric reductions of olefins. However, once the safety hazards of dealing with hydrogen gas and often pyrophoric catalysts are considered, automating such processes becomes very unappealing, especially since an ideal automated chemistry platform will be running unattended. While there are alternative chemical methods such as mercury salts, hydrides, phosphines, tin salts, and transfer-hydrogen reagents that can often be employed to effect the desired reduction,[4] at best they leave additional reagents that need to be removed from the crude reaction medium and, in the worst case, these reagents themselves can be highly toxic or hazardous.

In 2005, ThalesNano developed and marketed the H-cube flow hydrogenation instrument.[5] The instrument utilizes an electrolytic cell that splits water into hydrogen and oxygen. After this occurs, the hydrogen gas is separated, dried, and mixed with a stream of solvent/substrate solution that is supplied by an HPLC pump. The mixture is passed over a cartridge that contains the catalyst of choice, the reaction occurs, and the solution of the product is passed out of the instrument into the collection vial. Since the hydrogen is produced essentially "on demand" from deionized water and the catalyst cartridges are supplied prepackaged, the safety hazards and inconveniences associated with hydrogen cylinders and dispensing catalysts are reduced to essentially zero.[6] In addition, since the H-cube utilizes a catalyst heating block and a backpressure regulator, hydrogen reactions can be performed at elevated temperatures and pressures (20–100 °C, atmospheric pressure−100 bar) without the requirement for specialized reactors. Given these factors, this instrument is being readily accepted by organic chemists to perform routine hydrogenation reactions.[7] We were especially interested in acquiring an automated version of the instrument for application in the HTOS group since it would greatly increase our high-throughput reaction repertoire. Since the instrument and concept is a recently commercialized product, an automated version was not available. Therefore, we designed, constructed, and validated our own automated H-cube platform.

Results and Discussion

The platform for the construction of our instrument was a Bohdan robot. The robot was customized by machining an aluminum sheet to accept eight of our own custom vial racks and attaching it to the deck of the Bohdan. Each rack holds 12 20 mL scintillation type vials, and thus, the instrument accepts a total of 96 vials: 48 20 mL starting material vials and 48 20 or 30 mL[8] product collection vials, Figure 1. The next part of the project was to design and construct a

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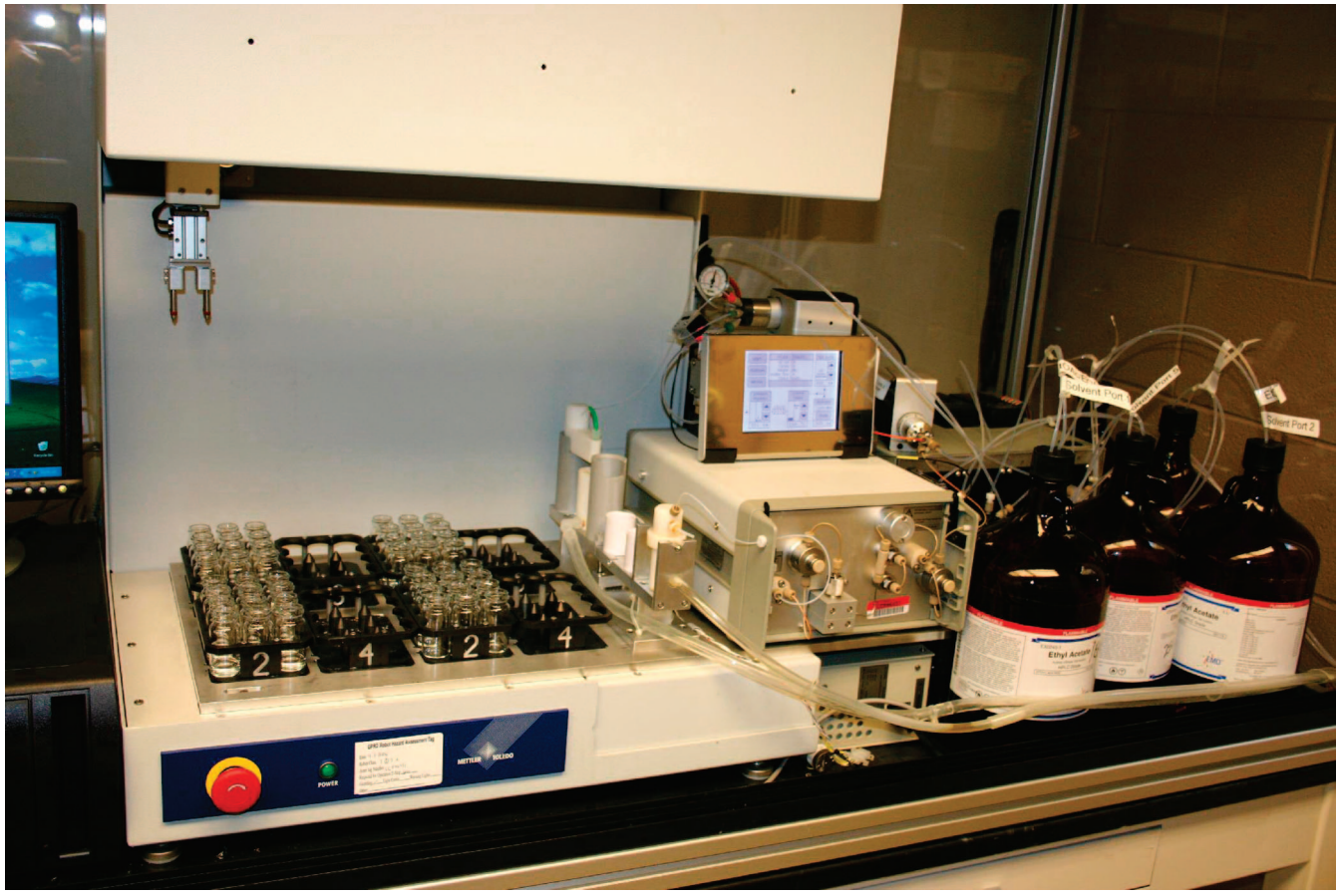


Figure 1. Picture of automated H-cube.

sampling mechanism that would hold the substrate and product collection vials in place while the reaction solution is routed to and from the H-cube. One issue in utilizing flat-bottomed vials is that the removal of all of the solution from the vial using a needle or probe mechanism can lead to significant sample wastage given a dead volume is often required. Although there are 20 mL vials commercially available that possess tapered bottoms so that essentially all of the sample can be withdrawn, prohibitive costs lead us to develop sloped transfer protocols using standard flat-bottomed vials for the majority of our laboratory automation. With this design aspect in mind, an elegant sampling mechanism was designed, Figure 2a−c. The sample vial holder cup was mounted to a spring-loaded cantilever mechanism that holds the cup in a vertical position. The robot gripper then places the vial in the holder, and air pressure is used to tip the lever onto a slant before the sample probe is put in place. After the reaction is complete, the air pressure is withdrawn and the spring returns the sample holder into the vertical position so that the robot gripper can remove the sample vial and move the next vial into position. Since the sample probe was integrated with a bubble detector, essentially the entire sample is withdrawn from the vial before a sample line solvent chase (wash) is signaled; excellent crude yields were observed.

In addition to the sampling mechanism, a 5-port solvent station and a sample switching valve was incorporated, along with an HPLC pump (supplied with the H-cube) that drives the whole solution flow process. The 5-port solvent station was incorporated to enable the broadest range of chemistries to be performed on the instrument in a manner that reaction conditions can be changed "on the fly". Commonly, the solvents employed are ethyl acetate, ethyl acetate/ethanol 1:1, methanol, 2 M NH_3/methanol, and 25% acetic acid/ethyl acetate, and the 5-port station also includes two syringe pumps: The first enables flushing of the system when a new solvent is selected by pulling fresh solvent through the valve. Since this valve is before the HPLC pump, this process also primes this pump to ensure there are no air bubbles present in the system that could cause the HPLC pump to lose prime. The second syringe pump is also connected to the valve, and it serves as a supply of clean solvent to wash the residue of the previous sample from both the main switching valve and the sample probe. Given that the sampling mechanism relies upon sensing a bubble event, this washing step also serves to prime the sample probe sufficiently so that a bubble detection event does not occur prematurely. A schematic of the solvent/sample paths of the automated H-cube system is presented in Figure 3.

A desktop PC and the VisualBasic software program is used to control the entire system, and three operation modes are utilized depending on the project being performed. The first "single sample" protocol enables the user to select any position from the array of 48 substrate positions and process this sample according to a number of parameters including hydrogen method (controlled or full H_2), hydrogen pressure, solvent, catalyst column temperature, sample flow rate, and sample chase (wash volume); this method has been shown

Figure 2. (a) Sampling mechanism.

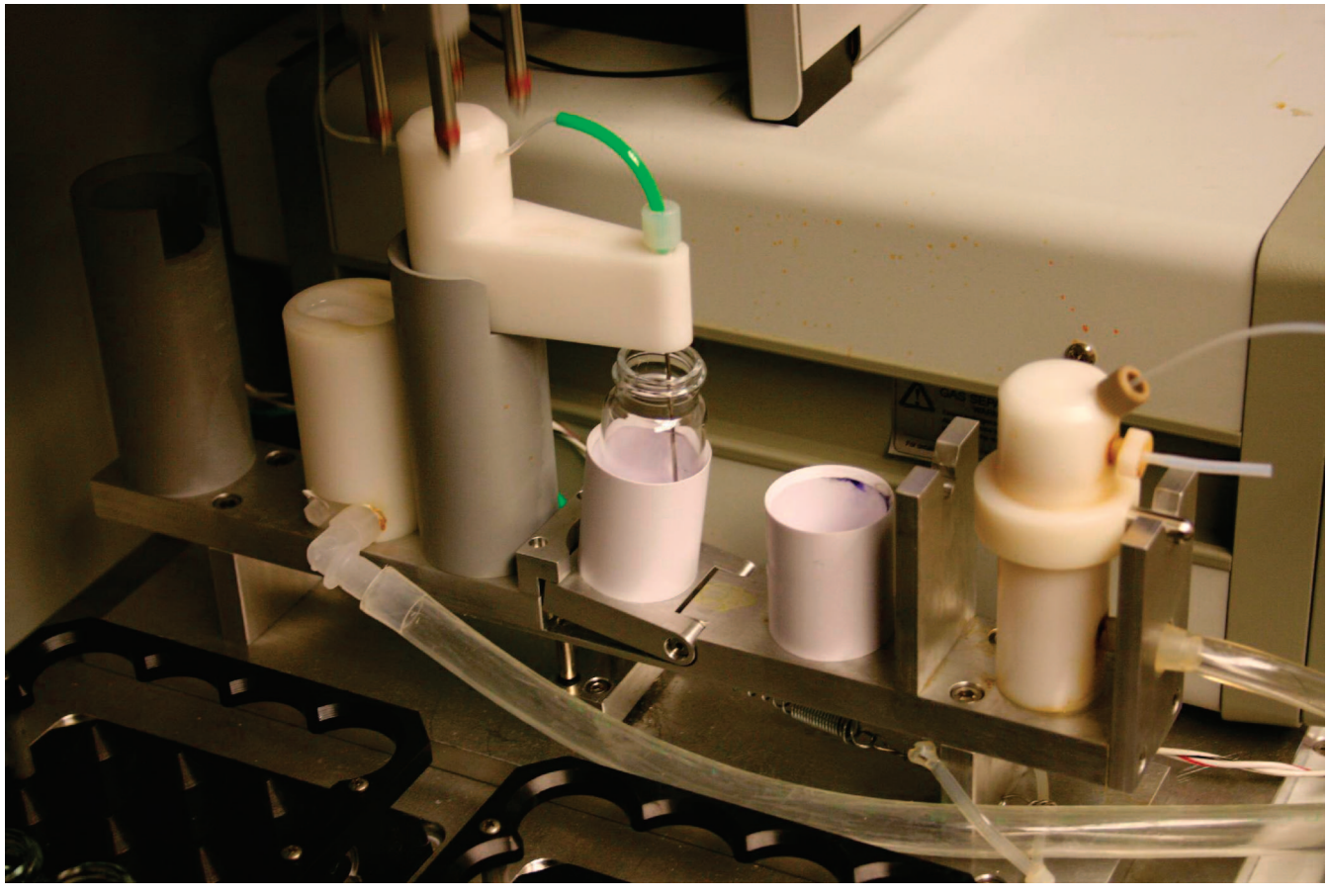


Figure 2. (b) Source vial and sample probe put in place.

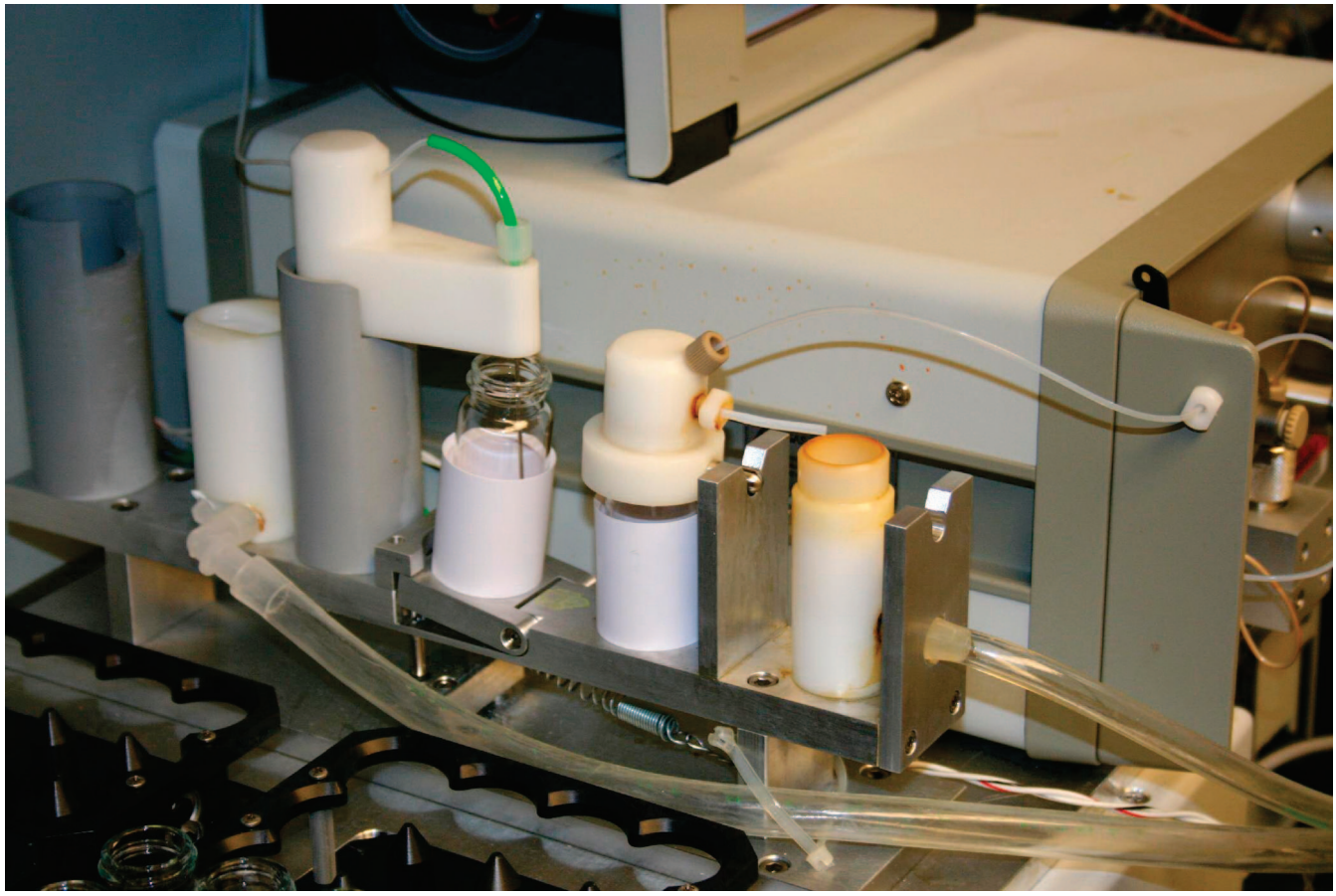


Figure 2. (c) Product collection vial put in place.

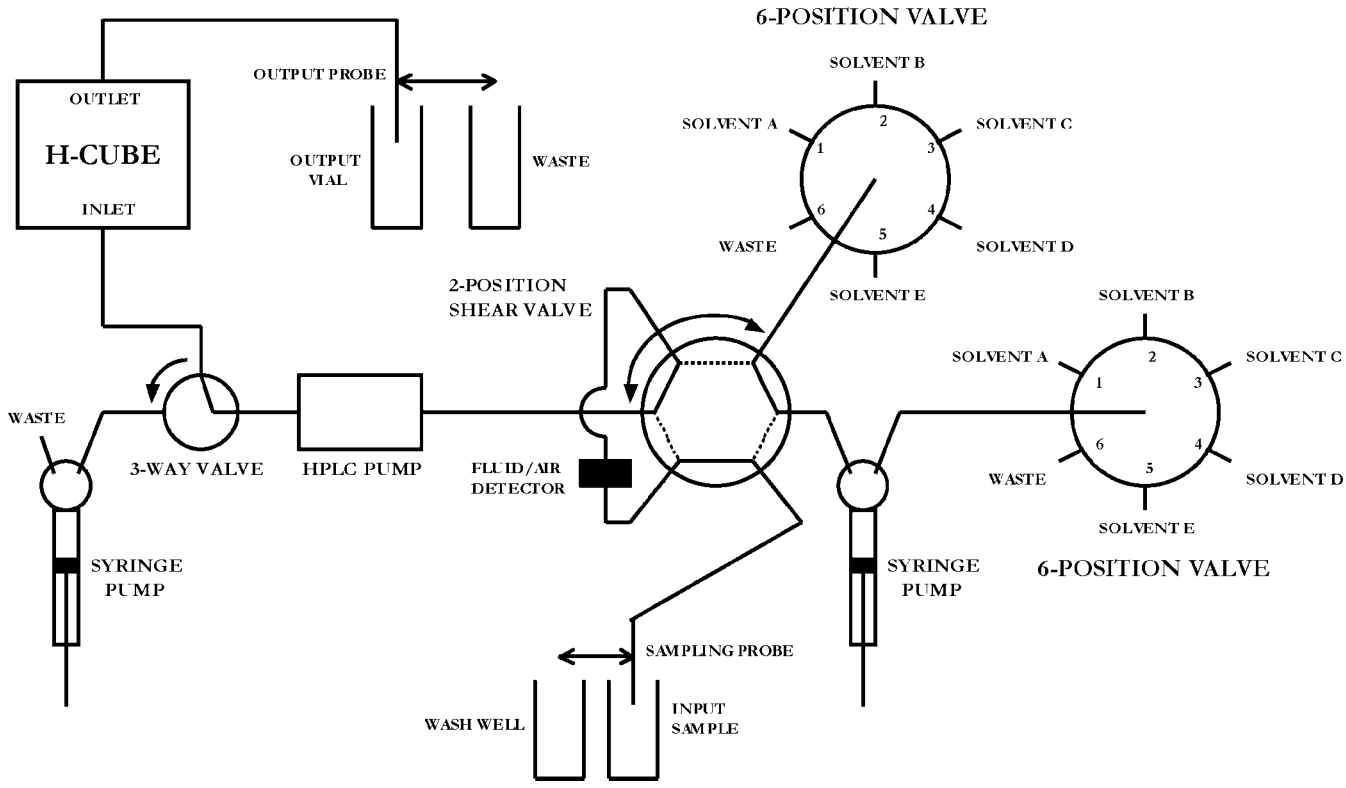


Figure 3. Schematic representation of automated flow hydrogenation instrument.

to be particularly useful for project support and one-off samples where up to 150 mg of substrate with a validated functional group transformation can be performed by simply placing the substrate and product vials on the deck and running the desired conditions. The second "batch method" Figure 4, used predominantly for library synthesis, is

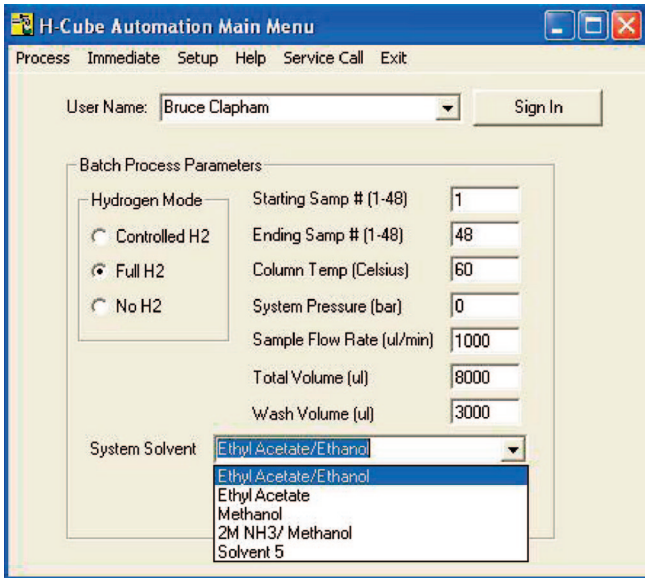
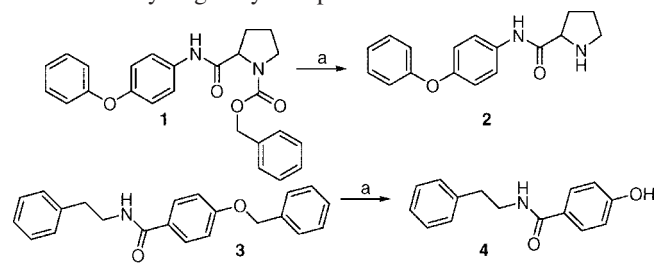


Figure 4. PC interface/automation control window (batch mode).

essentially the same as the single method; however, the instrument runs the desired conditions over a range of sample positions (e.g., 1–48, 1–24, 25–48, etc.). Finally, the third "optimization method" utilizes an Excel spreadsheet to cherry pick any of the 48 substrate positions and process these samples using any independent value of reaction conditions. This method has proved extremely powerful for optimizing new reaction conditions using very small amounts of substrate, and since the product vials can be transferred into a 96 well plate format using a liquid handler, this allows a fast, convenient, and precise method for the analysis of the reaction. Since flow chemistry requires no further scale-up development, the optimal reaction conditions discovered using this mode have been utilized directly in a second stand-alone H-Cube that is used for multigram syntheses.

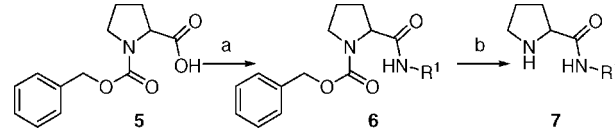
Catalytic hydrogen reactions can be split into two categories, namely hydrogenolysis (cleavage) of protecting groups and hydrogenation (reduction) of unsaturated compounds; both of these transformations figure strongly in medicinal chemistry, and we have studied them accordingly. Benzyl ethers, benzyl esters, and benzyl carbamates or benzylamines are employed as protecting groups in synthesis since they are readily removed under hydrogen reducing conditions to yield the corresponding alcohols or phenols, carboxylic acids, and amines, respectively. They are especially important in multistep library synthesis since they are often more robust than their ^{t}Bu-ether, ester, carbamate (BOC), and silicon based protecting groups which can be sometimes cleaved prematurely by harsh reaction conditions or even during reverse-phase chromatographic purification.[9] The first study using the automated H-cube utilized the optimization mode to establish optimal conditions for the deprotection of both CBZ and benzyl ether protecting groups. Two model compounds **1** and **3** were selected for this study and subjected to hydrogenation, Scheme 1. Both compounds were dissolved in EtOAc/EtOH 1:1 (10 mg/mL) and subjected to hydrogenolysis at 30, 40, 60, and 80 °C in the "full" hydrogenation mode,[10] with a flow rate of 1 mL/min over a Pd/C CatCart.[11] In both cases of conversion of **1** to **2** and **3** to **4**, LC-MS

Scheme 1. Hydrogenolysis Optimization Reactions[a]



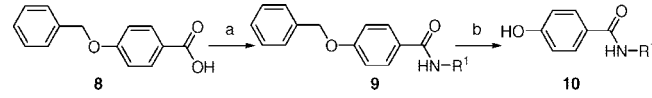
[a] (a) EtOH/EtOAc (10 mg/mL), Pd/C, full H_2, 60 °C, 1 mL/min, 100% conversion.

Scheme 2. Deprotection of the CBZ Protected Library[a]



[a] (a) HATU, Et_3N, R^1-NH_2, DMA, 2 h. (b) EtOH/EtOAc (~10 mg/mL), Pd/C, full H_2, 60 °C, 1 mL/min, 3.0 mL wash.

Scheme 3. Deprotection of a Benzyl Ether Protected Library[a]



[a] (a) HATU, Et_3N, R^1-NH_2, DMA, 2 h. (b) EtOH/EtOAc (~10 mg/mL), Pd/C, full H_2, 60 °C, 1 mL/min, 3.0 mL wash.

analysis indicated optimal conditions of full hydrogen mode, 10 mg/mL in EtOAc/EtOH 1:1, and 1 mL/min with a heater block temperature of 60 °C.

The second experiments performed were to establish the optimal chase (wash) volume that occurs after the end of the sample event occurs (sensed using the bubble detector) and before the next sample is run. In this case, source vials were loaded with either a substrate solution or blank solution in alternating positions and the above conditions were run with 1.0, 2.0, and 4.0 mL wash volumes. LC-MS analysis indicated that 2.0 mL of wash was sufficient to ensure no carryover of sample into the next vial; a standard 3.0 mL wash volume was utilized in all subsequent experiments.

With these results in hand, two pilot libraries that contained these protecting group fragments were synthesized and their deprotection on the H-cube was evaluated. (CBZ)-Proline **5** was coupled with a series of anilines in the presence of HATU to provide 39 amides **6**, Schemes 2 and 3. Each of the amides **6** was isolated and purified by preparative HPLC, and the library was submitted for deprotection using the automated H-cube. Accordingly, each of the amides **6** was redissolved in ethanol/ethyl acetate at a concentration of approximately 10 mg/mL; these compounds were then passed through the H-cube in one run, and the products were collected in a set of preweighed vials. LC-MS and ^{1}H NMR analysis indicated the presence of all 39 of the desired amines **7** with complete conversion having taken place in 34 cases. In addition, LC-MS and ^{1}H NMR analysis showed that the majority of the amine products **7** were present as single homogeneous compounds. In the samples that showed complete conversion to product, crude yields ranged from 68% to quantitative, with an average crude yield of 93%.

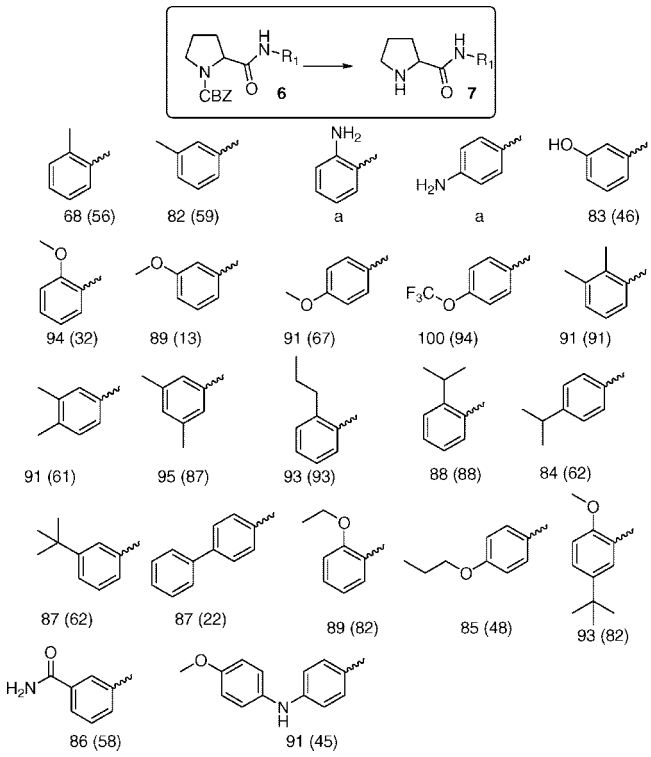


Figure 5. Representative examples of compounds prepared in library 1. Percent crude yield (and yield of purified compounds) is given (in parentheses). The letter a denotes products where deprotection was incomplete, and the product was not isolated.

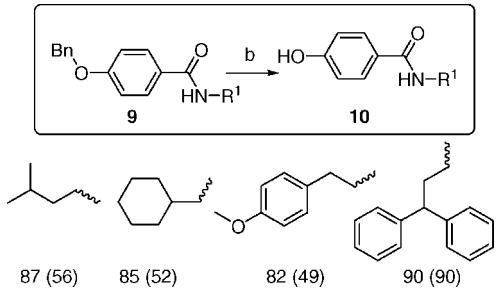


Figure 6. Representative compounds prepared in library 2. Percent crude yield (and yield of purified compounds) is given (in parentheses).

Finally, the crude products were purified using preparative HPLC; purified yields ranged from 13% to quantitative with an average yield of 67%, representative examples are given in Figure 5.

For the second library, 4-benzyloxy benzoic acid **8** was coupled with a series of amines to provide 36 amides **9** that were subsequently purified by preparative HPLC. In a similar fashion to above, each of the amides **9** was redissolved in ethanol/ethyl acetate at a concentration of approximately 10 mg/mL; these compounds were passed through the H-cube in single batch, and the products were collected in a set of preweighed vials. LC-MS and ^{1}H NMR analysis indicated the presence of 36 of the desired phenol products **10**. In addition, LC-MS and ^{1}H NMR analysis showed that the majority of the phenols **10** were present as a single homogeneous compound. In the samples that showed complete conversion to product, crude yields ranged from 83 to 99%, with an average crude yield of 88%. Finally, the crude products **10** were purified using preparative HPLC, and purified yields ranged from 13 to 90% with an average yield of 58% with representative examples given in Figure 6.

In summary, we have designed and constructed a fully automated flow hydrogenation apparatus. The instrument can process up to 48 samples in either a batch or with independent reaction conditions. We are currently investigating more sophisticated methodologies, and these results will be reported in due course.

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Supporting Information Available. Characterization data of selected library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) http://thalesnano.com/.

(6) The catalyst cartridges should be handled and disposed in accordance with the manufacturers recommendations.

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(8) The capability of the Biotage/VapourTec V-10 evaporator (http://www.biotage.com/DynPage.aspx?id=30804) to rapidly dry down 30 mL vials prompted us to add the 30 mL vial capabilities to our instrument. See also: Benali, O.; Davies, G.; Deal, M.; Farrant, E.; Guthrie, D.; Holden, J.; Wheeler, R. *J. Comb. Chem*, published online Nov 3, http://dx.doi.org/10.1021/cc700149w.

(9) Greene, T. W.; Wuts, P. G. M. *Protective groups in organic synthesis*, third ed.; Wiley-Interscience: New York, 1999.

(10) The "full" mode utilizes excess hydrogen at approximately 1 atm; the "controlled" mode uses lower concentrations of hydrogen at higher back pressure.

(11) The catalyst cartridges (CatCarts) are available in 3 lengths with a large variety of catalyst types.

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