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# Optimisation of Conditions for *O*-Benzyl and *N*-Benzyloxycarbonyl Protecting Group Removal using an Automated Flow Hydrogenator

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This paper is dedicated to Professor Masakatsu Shibasaki on the occasion of his 60th birthday.

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

**Abstract:** A versatile, fully automated flow hydrogenator has been developed that is able to perform sequential flow optimisation experiments, flow library hydrogenation, or iterative scale-up hydrogenation. The behaviour of a palladium catalyst in effecting removal of *O*-benzyl and *N*-benzyloxycarbonyl protecting groups has been investigated. Significant observations relating to maintaining optimal throughput are reported. A small library of peptidic derivatives has been deprotected in high yield and purity.

**Keywords:** automation; debenzylation; deprotection; flow hydrogenation; palladium; peptides

Microfluidic[1] and mesofluidic[2] flow processes are receiving ever-increasing attention in an attempt to identify and develop more efficient synthesis technologies[3] that assist in the rapid, early stage identification of potential small molecule modulators of emerging therapeutic targets.[4] Key advantages associated with flow processes in comparison with more traditional batch techniques include the ability to independently vary and precisely control reaction parameters such as stoichiometry, temperature, pressure and flow rate leading to high reproducibility and facilitated scale-up.[5] We are currently developing protocols that invoke the use of supported reagents in reactor cartridges[6] that may be combined to implement multi-step, flow-through compound synthesis.[7]

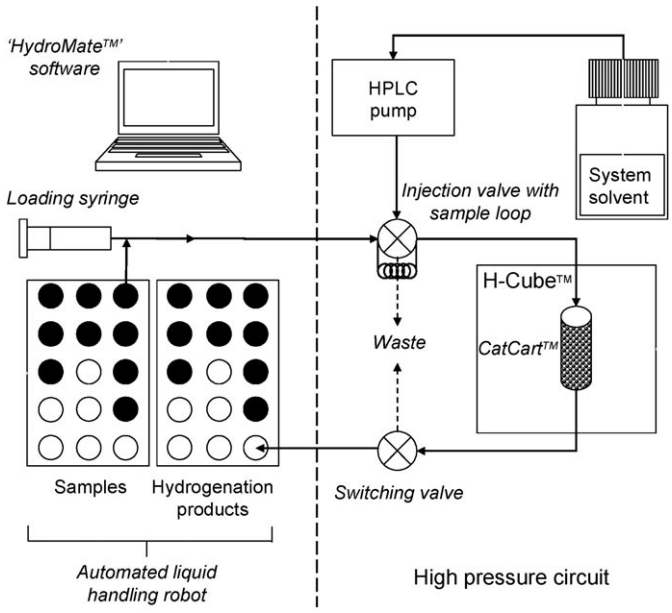
Catalytic batch hydrogenation constitutes an important synthetic method that is typically high-yielding and affords clean products that often require no further chromatographic purification. Continuous flow hydrogenation[8] presents an attractive alternative approach that, following the introduction of the H-Cube™,[9,10] may be safely and conveniently performed without the need for special facilities using a portable device.

Here we report on the development and use of an automated flow hydrogenation platform that facilitates the study and optimisation of catalytic flow processes and present our findings in effecting the catalytic hydrogenolysis of benzyl and Cbz protecting groups with high conversion to afford high purity products under flow-through conditions (Scheme 1). A significant difference between the present platform and a recently reported[11] combination of an H-Cube™ with a robotic liquid handler resides in the implementation of a simple single graphical user interface (HydroMate™) that controls all hardware devices and enables an automated series of experiments to be performed.[12]

In order to develop an optimised procedure for *O*-debenzylation that maximises throughput under continuous flow hydrogenation conditions, we selected *O*-benzyl protected *N*-Boc-tyrosine as a representative substrate. A series of experiments was performed to probe the effects of flow rate, temperature and concentration (Figure 1). The 10% Pd/C catalyst cartridge was changed only for each new set of reaction conditions.

As expected the efficiency of the catalyst progressively deteriorates with increasing throughput; the rate of deterioration being faster with increased concentration and flow rate. However, although increasing pressure (up to 60 bar; results not shown) had little effect, increasing temperature was found to have a significant effect in maintaining catalyst activity.




**Scheme 1.** Schematic representation of the automated flow hydrogenation platform.

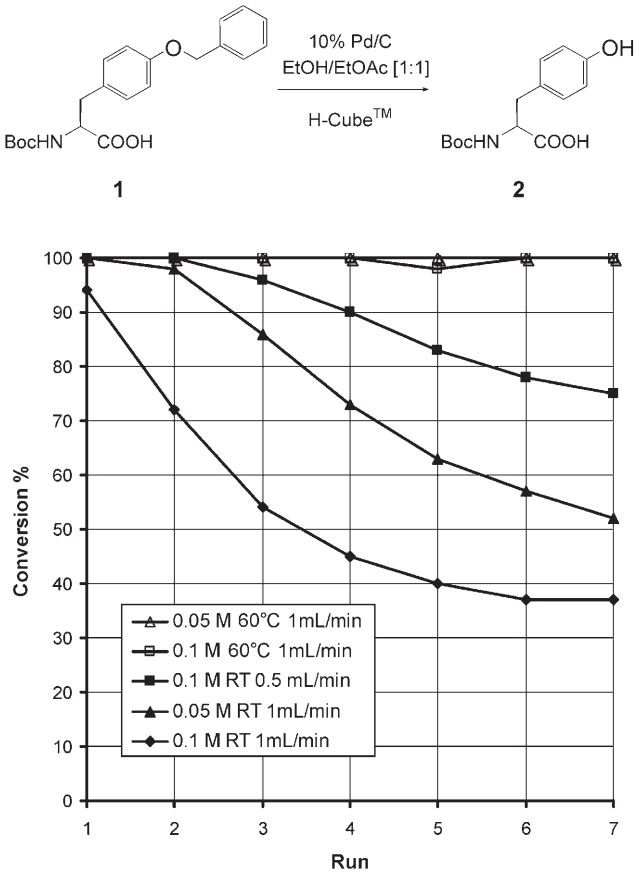



**Figure 1.** Study of the effect of temperature, flow rate and concentration on throughput for repeated catalyst use in an automated flow-though hydrogenator.

To study this phenomenon further, a series of experiments at a concentration of 0.1 M and flow rate of 1.0 mL min$^{-1}$ was performed. For the first six runs the temperature was maintained at 30 °C and a rapid deterioration in catalytic activity was observed. However, increasing the temperature to 60 °C lead to an immediate recovery in catalytic activity giving high conversion for the following eight runs (Figure 2). The

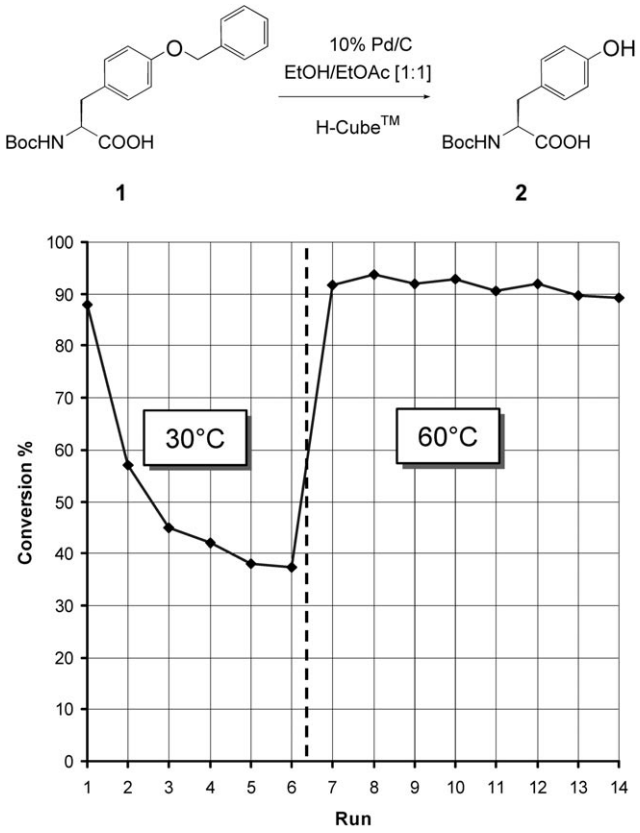



**Figure 2.** Sequential hydrogenation of 5-mL aliquots of a 0.1 M solution of *O*-benzyl *N*-Boc tyrosine in a flow-through catalytic hydrogenator at 30 °C (samples 1–6) and then at 60 °C (samples 6–14).

higher conversions observed under these conditions may be attributable to more effective solvolytic cleaning of the catalyst surface or an increase in catalytic off-rate with increasing temperature. In general, therefore, in order to maximise turnover it is advantageous to perform flow-through catalytic hydrogenation at elevated temperatures whenever possible.

Typically, under continuous flow hydrogenation conditions the catalyst has no opportunity to regenerate in the absence of substrate. A series of automated experiments was performed to contrast throughput and conversion for both continuous and iterative loop injection flow hydrogenation protocols.[13] In each of the experiments shown in Figure 3, the total quantity of substrate **1** processed was identical. However, by

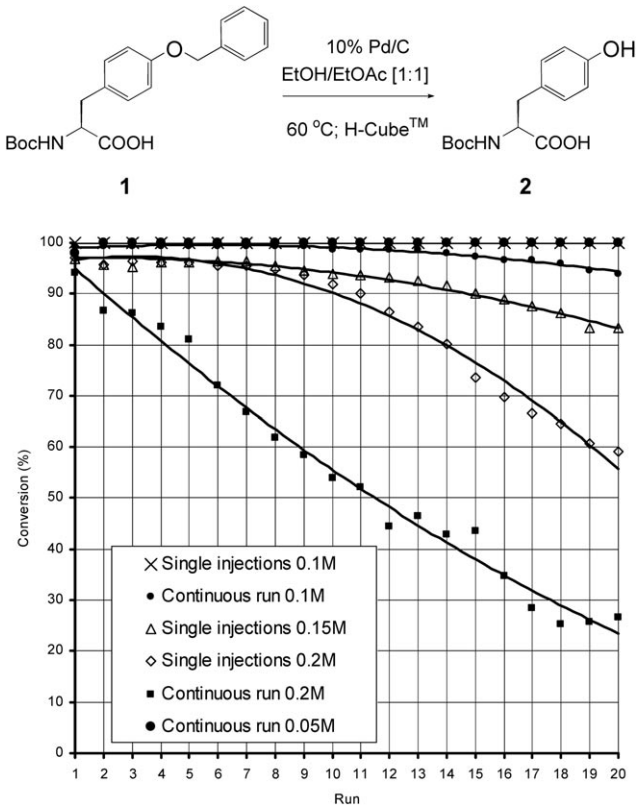



**Figure 3.** Comparison of a large scale reaction run as either a continuous run fractionated by the liquid handler or a series of single injections with recovery period for the catalyst.

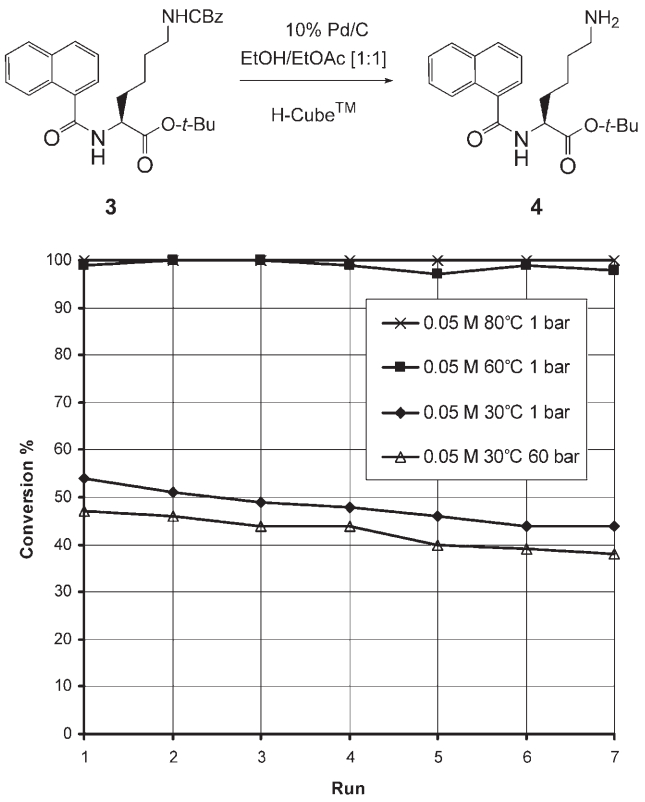



**Figure 4.** Optimisation of *N*-Cbz protecting group removal.

allowing for a period of catalyst recovery in the absence of substrate, it is apparent that more concentrated solutions may be processed with full conversion. Although, ultimately, maximum throughput was obtained under continuous flow conditions, allowing for a period of catalyst regeneration is beneficial in preserving activity of the catalyst over an extended period of time.

Finally, in order to establish general conditions that would be suitable for hydrogenation of a small compound library of *N*-Cbz derivatives, we selected ε-[*N*-Cbz] lysine *tert*-butyl ester **3** as a test substrate. Again, optimal throughput was obtained at elevated temperature, in this case 80 °C at 0.05 M (Figure 4).

A small library of eight *N*-Cbz protected compounds, including the dipeptide Z-Thr-Tyr(O-*t*-Bu)-O-*t*-Bu was selected and the compounds were subjected to sequential automated deprotection under the optimised conditions (Table 1). In all cases the deprotected derivatives were isolated in good yields and high purities. In particular, both Cbz and benzyl protecting groups were simultaneously removed from Z-(OBn)Tyr-OMe under these experimental conditions.

In conclusion, we have shown that the optimisation of flow-through hydrogenation processes may be facilitated using an automated platform. A combination of elevated temperature and period regeneration were found to be beneficial in terms of maximising throughput and extending the lifetime of the catalyst.

We have applied this methodology to effect the *O*- and *N*-deprotection of a range of substrates including amino acids, simple amines and dipeptides demonstrating that this strategy has utility in peptide synthesis. This aspect is currently under further investigation within our group.[15]

**Table 1.** Deprotection of eight *N*-Cbz protected compounds.

| Substrate | Conversion [%][a] | Yield [%] |
|---|---|---|
| Cbz-Pro-OMe | 99 | 99[b] |
| Cbz-Piperazine | 99 | 80[b] |
| Cbz-Asp(OMe)-OMe | 99 | 77 |
| Cbz-(OBn)Tyr-OMe | 97 | 83[c] |
| Cbz-Ser-OMe | 98 | 82 |
| Boc-(N-Cbz)-Lys-O(Naph) | 95 | 86 |
| Cbz-Thr-Tyr(O-*t*-Bu)-O-*t*-Bu | 99 | 96 |
| Cbz-Pro-tetrazole | 99[d] | 99 |

[a] Measured by $^1$H NMR and GC-MS; each reaction was performed by automated processing of 3.5 mL of a 0.05 M solution at 1.0 mL min$^{-1}$ at 80 °C.
[b] Isolated as hydrochloride salt.
[c] Both protecting groups removed.
[d] This reaction was also performed on 3-g scale; 30% AcOH was added to the solvent, see ref.[14]

## Experimental Section

### Typical Experimental Procedure

A 40-mL sample vial containing 35 mL of a 0.1 M *N*-Boc-(OBn)-tyrosine solution in EtOH/EtOAc (1:1) was placed at a designated sample position on the liquid handler robot. A sequence of 7 reactions was programmed, in each case introducing a 5-mL aliquot into the flow hydrogenator using loop injection . The system automatically stabilises at the designated temperature and flow rate (e.g., 60 °C, 1.0 mL $min^{-1}$) before commencing each experiment. For each reaction, the product was collected as an individual fraction for the designated time before the robot progressed to the next experiment. Conversion was measured by HPLC. HPLC: $t_R$ = 4.00 min (product), $t_R$ = 4.52 min (starting material). All other reactions were run in a similar manner except continuous flow experiments where the system solvent was replaced with a stock solution of substrate.

### Supporting Information

More detailed description of the system configuration and general experimental details.

## Acknowledgements

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