AUTOMATED INJECTION, SAMPLING AND REAL-TIME ANALYSIS IN THE CONTINUOUS FLOW HYDROGENATION OF A NITRO MOIETY ON AN API PRECURSOR

INTRODUCTION

Nitro group hydrogenations are one of the most common transformations in synthetic organic chemistry. This reaction is used mainly in medicinal chemistry and drug discovery[1], since it is the most important technique for the synthesis of primary amines. Continuous flow hydrogenation offers a facile, convenient and inherently safe method to perform this reaction2. ThalesNano offers instruments that generate hydrogen on demand via water electrolysis, without the need to store large amounts of the gas. In this work, we demonstrate how the H-Cube® Pro instrument can be coupled with an automatic liquid handler ("Autosampler") from Brooks, as well as a Spinsolve® in-line NMR spectrometer from Magritek, to perform the parameter optimization, analysis and sampling of the reduction of 2,4-difluorobenzene (**1**) to its corresponding aniline derivative **3**, an intermediate analogous to Linezolid's key precursor.

INSTRUMENTATION AND RISK ASSESSMENT

The H-Cube® Pro instrument uses water electrolysis in its cells to generate hydrogen gas in situ at flow rates up to 60 NmL/min. It can be equipped with pre-filled and sealed catalyst cartridges (CatCarts®), which protect the user from contact with pyrophoric materials, and allow chemists to avoid the catalyst filtration step during work-up. The instrument can achieve temperatures from 10 to 150°C, as well as pressures between 1-100 bar. The touch screen also provides an intuitive, easy-to-handle interface, where all reaction parameters can be controlled conveniently. The extremely low amount of stored hydrogen, combined with the numerous safety features of the instrument, provides an inherently safe way to perform laboratory-scale hydrogenations without the need for gas cylinders.

The Brooks Autosampler is capable of injecting starting material solutions and washing solvents into the reactor system using a robotic arm equipped with a needle and tubing. It can also automatically collect samples from the reaction mixtures into empty vials. The Autosampler can be equipped with racks of different sizes, capable of holding 60 x 8 mL vials, 21 x 30 mL vials, or 5 x 300 mL bottles. It also has a washing station and a separate waste collector, in order to ensure that your materials and samples can never mix with each other or be contaminated.

The Spinsolve® benchtop NMR spectrometer can be equipped with either a regular NMR tube holder or a flow cell, the latter being especially useful for continuous flow chemistry applications. The ability of the instrument to suppress many solvent signals makes it possible to apply in-line analysis of the reaction mixture after it flows out of the reactor dissolved in regular protonated solvents (without the need of any deuterated solvents). Its software contains many pre-programmed settings, allowing for a convenient way to monitor many reaction types. Its probe can detect up to eight different nuclei and it can also measure 2D-NMR spectra such as COSY, ROESY, TOCSY, HSQC and HMBC, many of them also with solvent suppression.

The combined instrument platform allows the user to preset multiple combinations of conditions, and/or prepare multiple different starting material stock solutions. The instruments will automatically set up the pre-defined conditions and then inject each sample, measure its NMR spectrum, and then collect the products into separate vessels. This enables either a rapid

parameter optimization of a single reaction, or an automated way to perform molecule screening and the synthesis of small libraries.

The instrument system was built according to the following schematic representation (Figure 1).

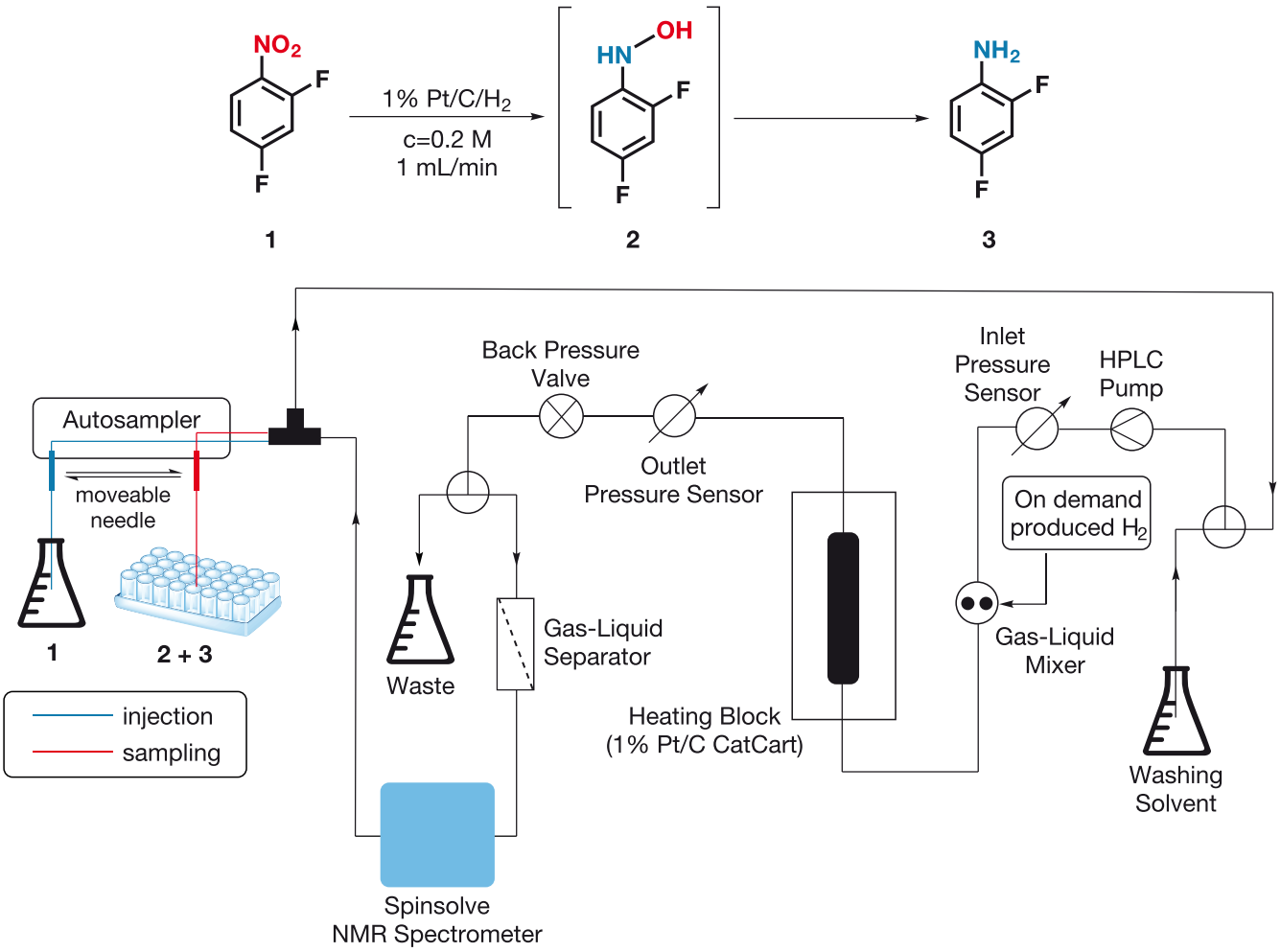

Figure 1: Reaction equation and the schematic representation of the instrument platform

EXPERIMENTAL RESULTS AND DISCUSSION

A 0.2 M solution of 2,4-difluoro-nitrobenzene (**1**) was prepared from 9.55 g (60 mmol) **1** and 300 mL methanol. This solution was filled into the starting material vials of the Autosampler's rack. The H-Cube® Pro was equipped with a 70 mm 1% Pt/C CatCart®. Since the gas-liquid mixtures influences the quality of the NMR-spectrum, a Zaiput SEP-10 phase separator was included between the outlet of the H-Cube® Pro and the flow cell of the Spinsolve Spectrometer, which was responsible for separating the excess hydrogen gas from the liquid flow.

The spectrometer was set up with the following parameters: The Spinsolve software includes the reaction monitoring module, RMX, where monitoring loops can easily be set up. For these experiments we used the continuous flow mode of the software to monitor the output of the flow reactor at regular time intervals. The RMX loop was set up with a Fluorine HDEC WALTZ sequence to acquire ^{19}F in the presence of ^{1}H decoupling.

Each measurement took 78 seconds. This sequence allows to collapse the multiplets, which come from the coupling of the respective fluorine atom's direct neighbour protons in the aromatic ring. The following experimental parameters has been put into the H-Cube® Pro's software:

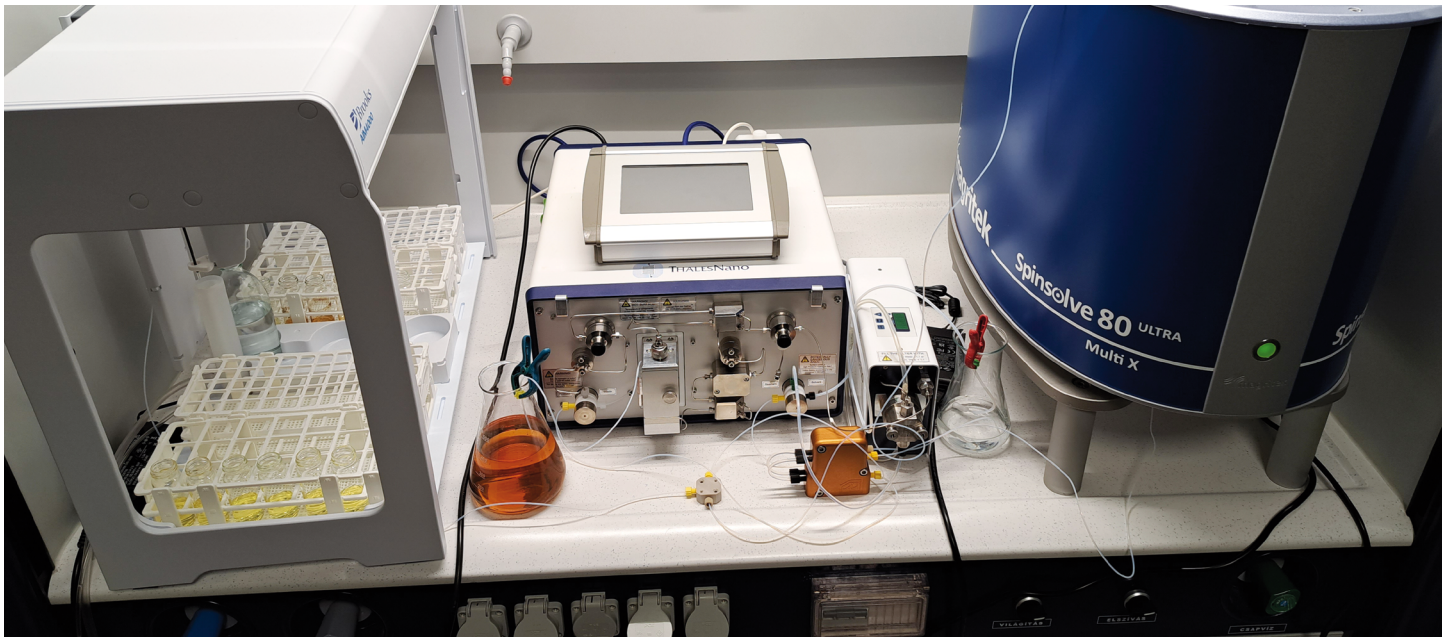

Figure 2: Photograph of the Complete Instrument Platform

# of Exp.	*T [°C]*	*p [bar]*	Flow rate [mL/min]	H_2 production capacity [%]	Conversion of **1** based on NMR [%]	Selectivity (**2:3** ratio) based on NMR [%]
1	20	1	1	0	0*	---
2	50	20	1	100	54	49
3	75	30	1	100	83	79
4	100	40	1	100	99	97
5	125	55	1	100	98	99
6	150	80	1	100	99	99

*The goal was to measure the ^{19}F-NMR spectrum of the starting material (**1**)

In each case, the Autosampler was responsible for injecting 5 mL of the starting material **1** into the H-Cube® Pro, as well as to collect 10 mL sample once the reaction mixture has reached the end of the flow path. The entire system, including the reactor zone and all the tubing were washed with clean methanol in-between each experiment to avoid cross-contamination of the samples across the run.

The reaction mixture from each experiment has been passed through the Spinsolve instrument's flow cell to detect the 19F-NMR spectrum of the materials that are present in the solution. After that, the samples were collected automatically into the vials in the Autosampler's receiver rack.

This technique allows the monitoring of the conversion of **1**, as well as the amount of the intermediate **2**, while also detecting the spectra of the desired product **3**. Complete conversion of **1** was first observed in experiment 4 (100°C, 40 bar), however the intermediate N-hydroxylamine **2** was still present in the solution.

By further increasing the temperature and pressure of the reaction, a full selectivity could be achieved, leading to a quantitative yield of the desired aniline derivative **3** in experiment **5** (125°C, 55 bar). The conversion of 1, as well as the presence of the intermediate **2** and the product 3, are depicted in Figure 3.

The NMR spectra for the six different reactions conditions are shown in the stack plot in Figure 4. After a 10 minute setup of the conditions prior to the start, no further time had to be invested from the user until all 6 runs have been completed. The six experiments were completed in 4 hours and 25 minutes.

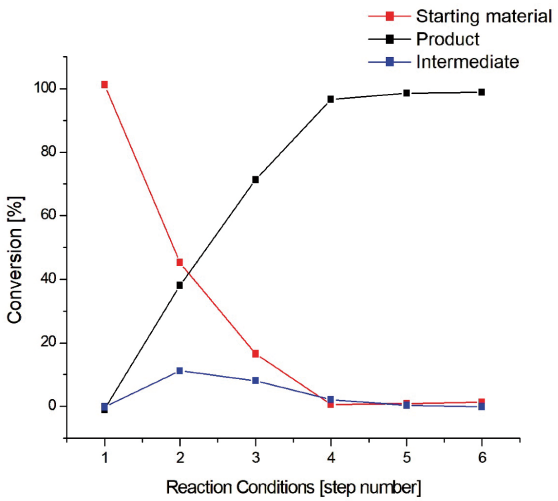

Figure 3: Conversion curve extracted from the NMR spectra shown in Fig. 4.

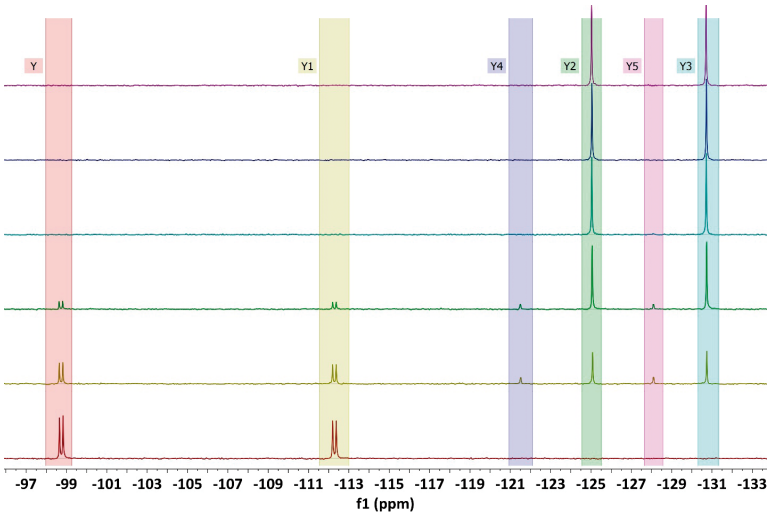

Figure 4: Stacked NMR spectra of all 6 experiments (peak assignations: **1**: Y and Y1, **2**: Y4 and Y5, **3**: Y2 and Y3)

SUMMARY AND CONCLUSIONS

By combining a Brooks Autosampler and a Magritek Spinsolve NMR spectrometer with our H-Cube® Pro continuous flow reactor, we were able to show the automated procedure for a rapid parameter screening and real-time monitoring of a nitro group hydrogenation reaction. The optimum conditions were found among the first attempted run involving 6 cycles of injection, reaction, analysis and sampling, easily fitting into one single working day, with a 10 minute actual human input time.

REFERENCES

1. Downing, R. S.; Kunkeler, P. J.; van Bekkum, H.: Catalytic Syntheses of Aromatic Amines, *Catal. Today*, **1997**, 37, 121.
2. F. Darvas, Gy. Dormán, V. Hessel: Flow Chemistry Textbook Volume 1: Fundamentals, De Gruyter, **2021**, ISBN 978-3-11-028915-2.

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