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Optimizing the Deprotection of the Amine Protecting *p*-Methoxyphenyl Group in an Automated Microreactor Platform

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Abstract:

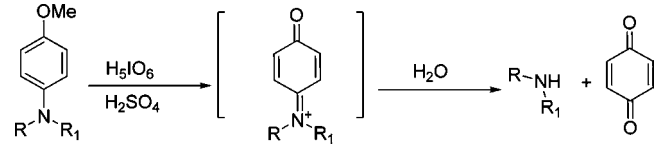
Three factors (temperature, stoichiometry and reaction temperature) were investigated in continuous flow microreactors in an automated fashion for optimization of the removal of the *p*-methoxyphenyl (PMP) protecting group, thereby consuming only minute amounts of substrate (0.2 mg/sample). The optimal reaction conditions were also applied to a larger microreactor system, in which the corresponding free amine was obtained at a preparative scale.

Introduction

In the recent past, the interest in using microreactors for synthetic purposes has increased enormously.[1−7] Traditionally, the emphasis has been either on the production of chemicals in microstructured flow reactors providing several benefits over conventional batch reactors or on rather specialized and novel reaction processes on a very small scale.[8,9] Scaling up, or scaling out using microreactor setup multiplication, has been a particular subject of investigation.[10] Surprisingly, only a few examples exist on the application of microliter or nanoliter volume reactors for screening purposes, in particular for reactions that are commonly used in organic synthesis laboratories.[11,12]

With the advent of new catalytic strategies to produce enantiopure products, asymmetric one-pot direct crossed-Mannich reactions constitute an elegant entry into β-aminoketones.[13,14] In many of these reactions, the *p*-methoxyphenyl (PMP) group stood out as a crucial protecting group for the amine function, giving rise to optimal enantio- and diastereoselectivity. The inevitable removal of the PMP group, however, appeared an important drawback for scale-up of this methodology, since common deprotection methods require the use of toxic and expensive reagents (e.g., ceric ammonium nitrate (CAN), or $PhI(OAc)_2$) and the use of column chromatography. Recently, Verkade et al.[15] developed a mild and efficient method to remove the PMP-group, leading smoothly to the corresponding amines in a one-pot procedure (see Scheme 1).[16,17]

Scheme 1. **General scheme for the deprotection of *p*-methoxyphenyl-protected amines**



The latter method involved the use of either periodic acid or trichloroisocyanuric acid (TCCA) in the presence of one equivalent of sulfuric acid and water. This causes oxidation of the aromatic ring to give the corresponding quinone-derived iminium ion, which is then hydrolyzed, resulting in overall removal of the PMP group. Due to the cheap reagents and favorable atom economy, these conditions are well-suited for large-scale application in an industrial setting. These particular conditions, however, have not been fully optimized yet.

We have previously shown that small microreactors with internal volumes in the range of a few microliters can be successfully applied to screen and optimize chemical reactions using tiny amounts of reaction fluids.[18,19] Although these microreactors in combination with a robot and efficient analytical means are capable of screening many reaction parameters in a short time frame, we chose to apply the principles of Design of Experiment (DOE), more specifically D-optimal design,[20]

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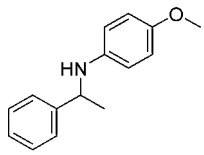
Scheme 2. **p-Methoxyphenyl (PMP)-protected model substrate**



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in order to come up with a rationalized set of reaction conditions. Traditional experimental designs (Full Factorial Designs, Fractional Factorial Designs, and Response Surface Designs) are appropriate for calibrating linear models in experimental settings where factors are relatively unconstrained in the region of interest. In some cases, however, models are necessarily nonlinear. In other cases, certain treatments (combinations of factor levels) may be expensive or impossible to measure. D-optimal designs are model-specific designs that address these limitations of traditional designs. With D-optimal design the number of experiments can be reduced compared to those for standard factorial or fraction factorial designs so that less time and resources are needed.

The first goal was to optimize the removal of a PMP protecting group in a continuous flow microreactor platform using D-optimal design. A second goal was to apply the optimized reaction conditions to a larger microreactor to scale up the process to preparative amounts. We selected the protected amine **1** (Scheme 2) as a representative example for PMP group removal.

Results and Discussion

Initially, the deprotection was studied in small batch reactors at various temperatures (60−90 °C) and monitored by taking small samples from the reaction medium for subsequent HPLC analysis, resulting in the graph depicted in Figure 1. This clearly shows that elevating the reaction temperature leads to an enhancement of the reaction rate. Thus, both factors (reaction time and temperature) have a significant effect and have to be invoked as factors in the reaction model. Additionally, the stoichiometry (ratio PMP-substrate:periodic acid) was taken as a factor in the DOE.

In order to be able to optimize the reaction in the microreactor platform, it also had to be translated into a continuous flow process. We envisioned that the design shown in Scheme

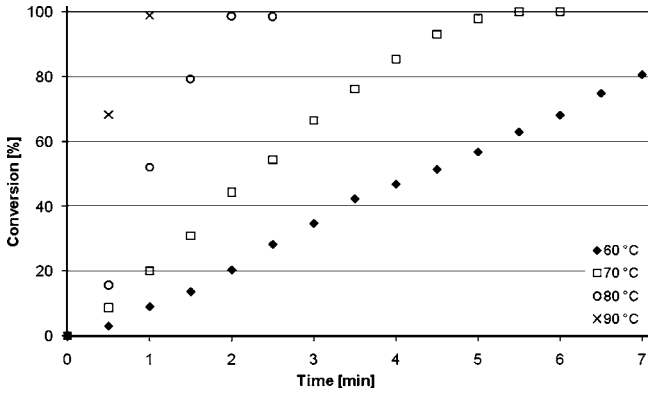
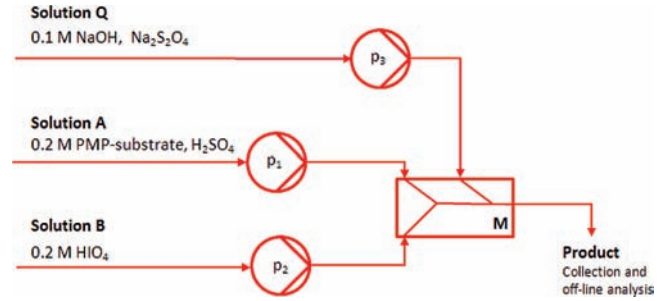


Figure 1. **Conversion of the PMP-substrate into its corresponding free amine (in batch reactors).**

Scheme 3. **Schematic of the microreactor setup (internal microreactor (M) reaction volume 7 μL)**



3 would be well-suited to study the flow process. Due to the fact that sulfuric acid can deactivate the periodic acid over time, we reasoned that sulfuric acid had to be combined with the PMP-substrate instead of periodic acid. Since the deprotection is efficiently stopped at higher pH values, we used aqueous sodium hydroxide and sodium dithionite for quenching the process.

During the continuous flow optimization experiment, the reaction temperature was varied between 60 and 90 °C, the reaction time between 0.5 and 4 min, and the stoichiometry between 1 and 4. The design for screening the domain contained 51 samples and was transferred to the automated microreactor platform,[18] in which the individual 51 reactions (approximately 0.2 mg per sample) were automatically conducted in a total sampling time of 5.6 h. The samples were analyzed by HPLC resulting after polynomial fitting depicted in the contour plots in Figure 2 (raw data available in Table S1, Supporting Information). Note that the response is shown in a logarithmic scale to better visualize the modeled response at high conversions.

The contour plots indeed show that the factors, reaction time and temperature, have a considerable effect on the yield. On the other hand, the effect of an increasing stoichiometry is less pronounced. The reaction conditions for a conversion of >99% combined with the shortest reaction time (highest flow rate) were observed at a reaction time of 1.3 min, a stoichiometry of 3.2, and a reaction temperature of 90 °C.

The next step was to transfer the optimal conditions from the 7 μL to a 950 μL internal volume microreactor in order to conduct the same deprotection at a preparative scale. For this purpose, a standard commercially available stainless steel continuous flow reactor was selected and the optimal settings from the screening experiments were applied. At a reaction temperature of 90 °C, however, the solvents (water and acetonitrile) started to boil inside the continuous flow reactor, giving rise to an unstable outflow of the product. In order to circumvent this issue, the temperature was lowered to 80 °C, while the reaction time was increased to 4 min in order to achieve 100% conversion (according to the screening experiments displayed in Figure 2). The reaction fluids were continuously pumped through the reactor for approximately 4 h. The flow rates of the individual pumps were set to 176 μL/min (approximately 10 mL/h), leading to a continuous deprotection of PMP-protected amine **1** at a rate of 213 mg/h. The conversion of the outflow was monitored at intervals and always appeared >99%, confirming that the initially identified optimal deprotection conditions can also be successfully used in the larger continuous flow reactor.

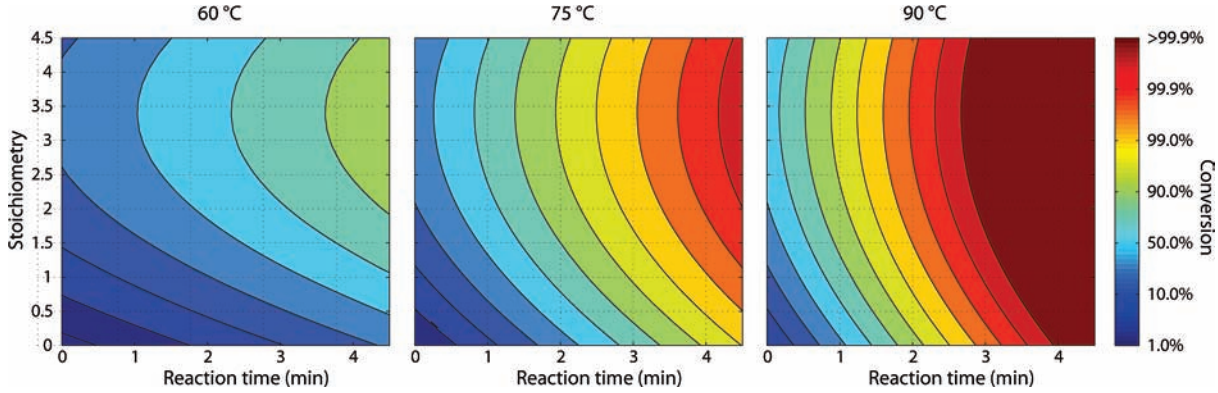


Figure 2. **Contour plots of the optimized model of the PMP-group removal in the automated microreactor platform (polynomial fit).**

Conclusion

Three factors (temperature, stoichiometry and reaction time) were investigated in continuous flow microreactors in an automated fashion for optimization of the removal of the *p*-methoxyphenyl (PMP) amine protecting group. As a result of the small dimensions of the microreactor, only tiny amounts of reagents and solvents were required in order to identify optimal reaction conditions. The latter conditions were also applied to a larger microreactor system to synthesize the free amine product at a preparative scale. In conclusion, the PMP group could be conveniently removed in 100% conversion within two minutes in a continuous flow microreactor, both at small and at preparative scale, which clearly underlines the potential of flow chemistry in organic synthesis. Although we efficiently used HPLC as the analysis method for determining reaction conversion, online detection such as IR and UV will be considered to decrease the time required for optimization even further.

Experimental Section

HPLC Analysis. HPLC analysis was performed on a Shimadzu VP LC10 equipped with a 250 mm × 4 mm × 4.6 mm Inertsil ODS-3 column, using a gradient program with acetonitrile and 20 mM phosphate buffer (pH 3) with a flow rate of 2 mL min^{-1}. Detection took place at a wavelength of 254 nm.

Synthesis of PMP-Protected Amine 1. See procedure described by Verkade et al.[15]

Microreactor Setup. All syringes (Harvard apparatus; high pressure syringe, 2 mL) mounted on a syringe pump (New Era; type NE-1000 or NE 500) were connected to FEP tubing (1.59 mm OD, 254 μm ID). At the end of each piece of tubing, a special 'flat bottom headless nut' (Upchurch Scientific; type: M 660) was mounted which pressed down onto a flat bottom ferrule (Upchurch Scientific; type: M 650) to achieve a leak-free fluid connection to the microreactor. The microreactor was placed in a custom-designed chip holder[18] with threaded holes on the top side in which the nuts were screwed. For temperature control, a custom-designed heater (peltier element) was used, which could easily slide into the microreactor chipholder. A stainless steel needle (UpChurch Scientific; type U 106 1/100 in. ID 1/16 in. OD, custom-filed needle tip) was used as outlet. A sample robot (Gilson Aspec XL) with dedicated software (Gilson 735 Sample software, Version 1.00) was used to dispense all samples during reaction screening. The pumps, robot, and temperature controller were automatically controlled with a custom-designed software program (developed by Fraunhofer IMS, Duisburg, Germany).

Microreactor. The microchannel structure was designed using the software program CleWIN. The actual microreactor was fabricated from borosilicate glass by Micronit Microfluidics BV, Enschede, The Netherlands (HF etched). Chip dimension: length 45 mm, width 15 mm, height 2.2 mm. Channel dimension: width 120 μm, depth 60 μm, total length 132 cm. Reactor reaction volume 7.02 μL.

Microreactor Experiment for Deprotection Using D-Optimal Design. The first syringe was loaded with solution A containing substrate **1** (200 mg, 0.880 mmol), 1 M sulfuric acid (0.88 mL, 1 equiv) and *p*-nitrophenol (100 mg, 0.718 mmol, internal standard) dissolved in MeCN/H_2O (10 mL, 1:1). The second syringe was loaded with solution B containing periodic acid (201 mg, 0.880 mmol, 1 equiv) and benzoic acid (100 mg, 0.819 mmol, internal standard) dissolved in MeCN/H_2O (10 mL, 1:1). The third syringe was loaded with the quenching solution (Q) containing sodium dithionite (391 mg, 2.24 mmol, 2.55 equiv) dissolved in MeCN/0.1 M NaOH (25 mL, 1:1). Solution C was prepared by dissolving biphenol (30 mg, 0.161 mmol, internal standard) in MeCN/0.1 M NaOH (25 mL, 1:1). Solution D was made by diluting solution C (3 mL) to 25 mL with MeCN/0.1 M NaOH (1:1). Solutions A, B, and Q were then connected to the microreactor system. Of each reaction mixture, 20 μL was collected in 500 μL of solution D. Due to the varying flow rates, sampling times differ for every experiment. All reaction conditions were randomized except for the temperature. The samples were analyzed with HPLC. The retention times are 6.23, 8.20, 8.51, 9.59, and 10.97 min, respectively, for the deprotected amine, benzoic acid, PNP, 2,2-biphenol, and PMP substrate **1**.

Deprotection in the Larger-Scale Continuous Flow System. A stainless steel reactor (internal volume 0.95 mL) was used in combination with a commercial available T-junction (Upchurch) acting as a mixer. The reactor was submerged in an oil bath, and the two inlets of the T-junction were connected to the syringes. Both syringes

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(50 mL) were mounted on a syringe pump (New Era; type NE-1000) and were connected with FEP tubing (1.59 mm OD, 254 μm ID) to the T-junction. The following solutions were prepared: Solution A: compound **1** (1.01 g, 4.46 mmol), *p*-nitrophenol (internal standard, 49.3 mg), and 1 M H_2SO_4 (4.4 mL) were diluted to 50 mL with acetonitrile/H_2O (1:1). Solution B: periodic acid (1.00 g, 4.41 mmol) was diluted to 50 mL with acetonitrile/H_2O (1:1). Both solutions were degassed for 50 min with ultrasound. The flow rates of the pumps were set to a total of 167 $\mu L \cdot min^{-1}$ corresponding to a total reaction time of 4 min. After stabilizing the system for 15 min, the outflow was collected for 255 min. During this time frame, the conversion was regularly measured and shown to be >99% according to HPLC analysis. Subsequently, the collection vial was worked up according to the procedure by Verkade et al.[15] resulting in an isolated yield of 60% (329 mg) of the corresponding free amine.

Acknowledgment

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Supporting Information Available

Raw data from the optimization experiment is shown in Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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