KILOGRAM SCALE SYNTHESIS OF A LINEZOLID ANALOGUE INTERMEDIATE VIA TANDEM *N*-ARYLATION AND NITRO GROUP REDUCTION IN A PHOENIX™ II. PROFESSIONAL CONTINUOUS FLOW REACTOR SYSTEM

INTRODUCTION

The morpholine ring bears utmost importance in synthetic chemistry and drug design[1], since it can be found in numerous bioactive compounds. Analgesics Phenadoxone and Dextromoramide, antihypertensive Timolol, antidepressants Moclobemide and Reboxetine, antibiotics Linezolid and Finafloxacin, as well as the anticancer drug Gefitinib are all FDA approved drugs that contain a morpholine moiety. Using morpholine itself as a nucleophile is one of the most important techniques in the synthesis of such compounds. In this work, we present a large scale, two-step tandem technique for the synthesis of a Linezolid analogue key intermediate via the *N*-arylation of morpholine followed by a nitro group reduction[2], in a flow reactor system that conveniently fits inside a single fume hood.

INSTRUMENTATION AND RISK ASSESSMENT

The reactions were performed in a Phoenix™ II. Flow Reactor system, equipped with a liquid pump, a back pressure regulator, and for the reduction step, a H-Genie® II. as hydrogen source. This setup enables the execution of high temperature and high pressure reactions involving liquids or gas-liquid mixtures at high-throughput rates. The Phoenix™ II. Flow Reactor is capable of reaching temperatures up to 450°C and pressures up to 200 bars for liquids, or 100 bars for gas-liquid mixtures. The reactor module can be equipped with coiled tubes for homogenous reactions, or columns loaded with wide range of catalysts. The Pressure Module™, responsible for building back pressure in the system, allows for safe overheating of most organic solvents without the danger of boiling or instantaneous evaporation. The liquid pump is capable of producing stable liquid flow of up to 50 mL/min at pressures up to 150 bars (200 bars with 10 mL/min pump). The H-Genie® II. hydrogen generator has a maximum production capacity of 1000 NmL/min with a maximum output pressure of 100 bars. Benchtop hydrogen generation allows chemists to evade the use of hydrogen cylinders, since it produces hydrogen on-demand *via* water electrolysis, without the need for storing large amounts of gas, making it inherently safe compared to conventional cylinders.

Fig. 1.: Modules of the Phoenix™ II. Professional Reactor System

Experimental procedures and discussion of results. Preliminary experiment for the N-arylation of morpholine.

Two solutions were made mixing 80 g (0.50 mol) 2,4-difluoronitrobenzene (**1**) in 100 mL IPA, and 96 g (1.1 mol, 2.2 eq.) morpholine (**2**) in 100 mL IPA.

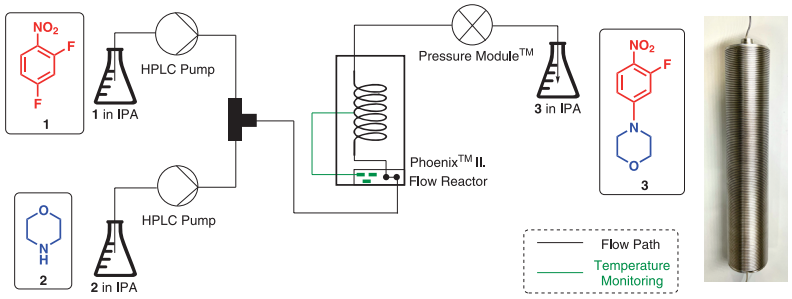


Fig. 2: Flow reactor setup for the *N*-arylation of morpholine (**2**)

The Phoenix™ II. flow reactor was equipped with a 16 mL stainless steel loop and a back pressure regulator. Table 1 summarizes the attempted parameter sets during the preliminary experiments.

Entry	*T* [°C]	*p* [bar]	Combined flow rate [mL/min]	Gas flow rate [NmL/min]	Residence time [min]	Calculated throughput [mmol/min]
1	100	10	1.6	10	2.3	Full conversion
2	100	10	3.2	5	4.6	Full conversion
3	100	10	6.4	2.5	9.2	SM detected
4	150	30	1.6	10	2.3	Full conversion
5	150	30	3.2	5	4.6	Full conversion
6	150	30	6.4	2.5	9.2	SM detected
7	150	30	4.7	3.4	6.76	Full conversion

Table 1: Optimization experiments for the *N*-arylation reaction step

Out of experiments 1-6, the best result was obtained in experiment 2, which had the highest productivity with full conversion. To fine tune the maximum throughput, a new experiment was conducted without the use of solvents, however, in the first few minutes of the experiment, we experienced a blockage in the system due to the precipitation of morpholine hydrofluoride side product. Batch experiments were carried out which showed that the reactant mixture should contain at least 50 V/V% IPA to avoid this adverse event. Since this value is close to the concentration used in the preliminary experiments, we have attempted to increase the flow rate to 4.7 mL/min (residence time: 3.4 min), resulting in full conversion with a throughput of 6.76 mmol/min.

LC-MS analysis of the above 7 experiments revealed that the regioselectivity of the reaction is the same under every attempted set of conditions. The desired product was obtained with 88% selectivity, with 5% of the 2-(*N*-morpholinyl) regioisomer (**3a**) and 7% of the 2,4-bis(*N*-morpholinyl)nitrobenzene (**3b**) also present in the solution. This solution was transferred to the next synthetic step without purification.

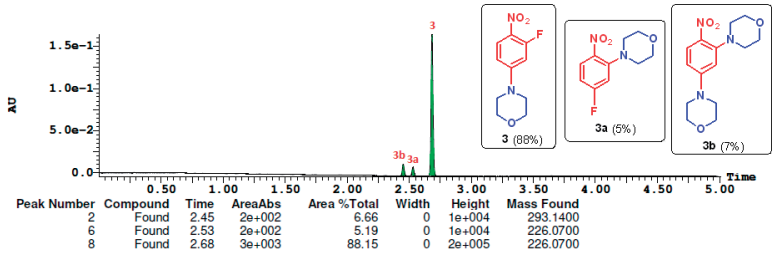


Fig. 3: LC-MS chromatogram of the crude product after the *N*-arylation step

Preliminary experiment for the reduction of the nitro group.

For this set of experiments, a Phoenix™ II. Flow Reactor was connected to a H-Genie® II. and a Pressure Module™ as depicted on Fig. 4. First, we attempted to run the solution from the previous experiment directly through the system, however, the hydrogen gas carried some solvent vapour with itself, resulting in a more concentrated solution. Since the solution was already very close to saturation to begin with, this phenomenon caused the precipitation of morpholine hydrofluoride inside the column, resulting in a blockage and overpressure shutdown. To avoid this issue, we added 1.23 L methanol - a better solvent for organic ammonium salts - to set the concentration of the solution to 0.35 M. To determine the optimum parameters and maximum throughput rate, the following experiments were carried out as described in Table 2. A 1" wide 250 mm long metal-metal sealed column was filled with 5% Pd/C granules. The void volume of this column was determined as 30 mL. Before the experiments, the catalyst bed was activated at room temperature and 15 bars of pressure by pumping methanol and hydrogen gas through the system.

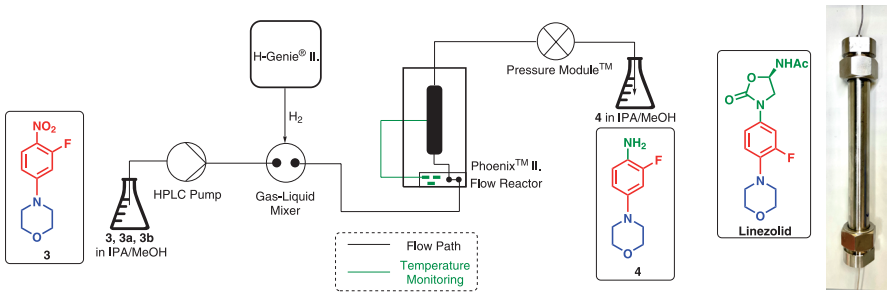


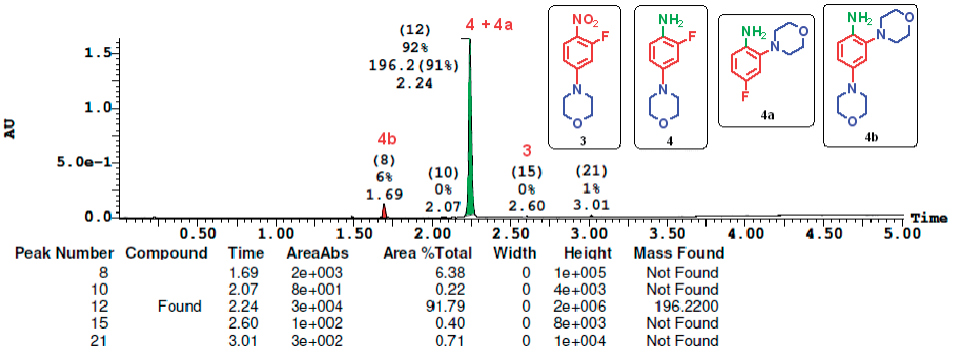
Fig. 4: Flow reactor setup for the nitro group reduction

Entry	*T* [°C]	*p* [bar]	Liquid flow rate [mL/min]	Gas flow rate [NmL/min]	Residence time [min]	Calculated throughput [mmol/min]	Result (TLC)
1	25	10	5	300	~1 min	1.75	Full conversion
2	25	10	10	500	~30 sec	3.5	Full conversion
3	25	10	20	700	~20 sec	7.0	17% SM detected
4	25	30	40	1000	~13 sec	14.0	No significant conversion
5	50	35	20	700	~45 sec	7.0	2% SM detected
6	50	35	40	1000	~28 sec	14.0	86% SM detected

Table 2: Optimization experiments for the nitro group reduction step

In entries 1-2, the full conversion of the starting material **3** was observed, while the experiment at 20 mL/min liquid flow rate started to drop in conversion. Harsher reaction conditions were attempted to improve the conversion of the high-throughput experiments, which resulted in a 98% conversion at 20 mL/min liquid flow rate. Table 2. entry 5. was determined as the optimum set of parameters having the highest throughput with good conversion.

The selectivity of the reduction step was determined by LC-MS measurments (Fig. 5.). In all cases, only the desired amine products (**4, 4a, 4b**) were detected, corresponding to the regioisomers **3, 3a, 3b** observed in the previous synthetic step. Therefore it can be concluded that the nitro reduction was carried out with nearly quantitative conversion and selectivity, without affecting the aromatic ring, cleaving the C-N bonds in the morpholine moiety, or detecting any of the partially reduced intermediates such as the *N*-hydroxyl amine (peaks that don't correspond to any of the 3 regioisomers having less than 1% intensity in the chromatogram).



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
8		1.69	2e+003	6.38	0	1e+005	Not Found
10		2.07	8e+001	0.22	0	4e+003	Not Found
12	Found	2.24	3e+004	91.79	0	2e+006	196.2200
15		2.60	1e+002	0.40	0	8e+003	Not Found
21		3.01	3e+002	0.71	0	1e+004	Not Found

Fig. 5: LC-MS chromatogram of the crude product after the reduction step

Tandem reactor system setup for the multi-step synthesis of 4

Using the results of the previous two experiments, we have combined the two reactor systems into one tandem continuous flow reactor setup to consecutively perform the *N*-arylation and the nitro group reduction (Fig. 6.). In the first step, two HPLC pumps were connected to a T-mixer, followed by the Phoenix™ II. Flow Reactor equipped with a 16 mL stainless steel loop. These two pumps carried the 1:1 mixtures of IPA and the two starting materials. The outlet of the coil was then connected to a T-mixer, which was responsible for diluting the solution to 0.35 M using a third HPLC pump that carried methanol. This solution was then transferred to a similar reactor setup as described in the nitro group reduction step: the diluted mixture was combined with hydrogen from a H-Genie® II. using a static gas-liquid mixer, and transferred further into a second Phoenix™ II. Flow Reactor, fitted with a 1" MMS column and a back pressure regulator. The flow rates of the pumps were adjusted such that the morpholine (**1**) is at a 2.2 equivalent excess compared to the 2,4-difluoronitrobenzene (**2**), followed by the dilution with methanol to 0.35 M.

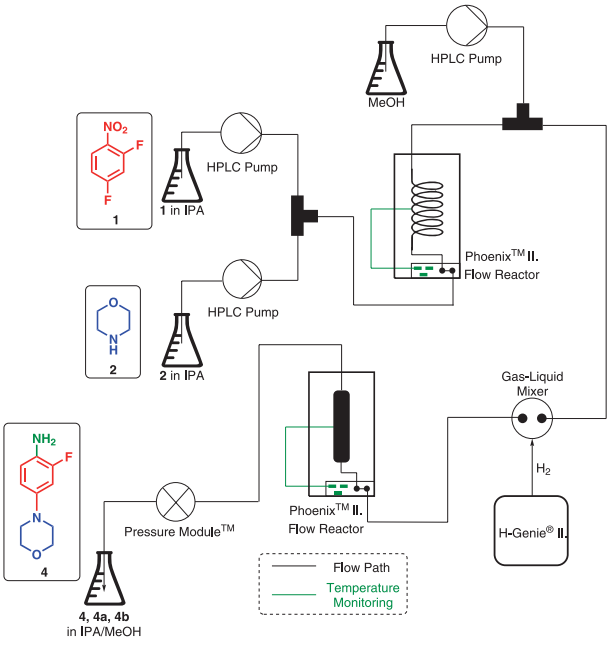


Fig. 6: Flow reactor setup for the tandem synthesis of **4**

The combined flow rates of all liquid paths (4.7 ml/min from the first two pumps combined, followed by a dilution with methanol at 15.3 ml/min with a third pump) gave an overall flow rate of 20 mL/min, equating to a theoretical throughput of 7 mmol/min. The *N*-arylation step was performed under 100°C, while for the nitro reduction, the reactor zone was heated to 50°C. The Pressure Module™ was set to 35 bars which was the optimum value for the second synthetic step. The experiment was left to run continuously for 14 hours (two 7 hour periods over the course of two days), LC-MS samples were prepared after every hour. This analysis revealed that the system maintained full conversion for 12 hours, after which the catalyst started to deactivate and a small amount of the morpholine-substituted nitro compound started to appear in the chromatogram (Fig. 7.). Therefore, we have concluded that the reaction should be stopped after 12 hours in order to replace or reactivate the catalyst column.

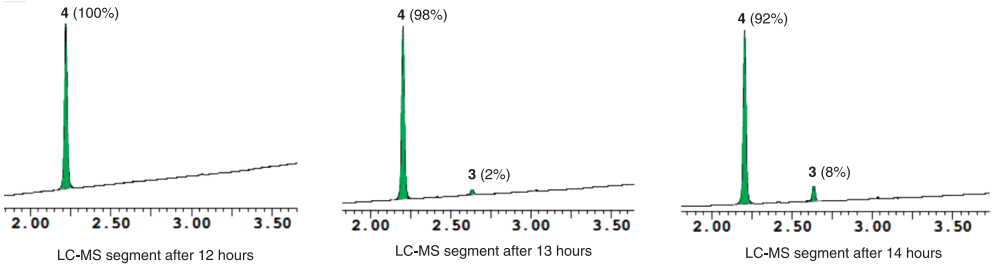


Fig. 7: LC-MS segments taken from the reaction mixture after 12/13/14 hours

A small amount of the product was collected, the solvent was evaporated and the crude mixture was purified by column chromatography (hexane: EtOAc=5:1), resulting in a 81% isolated yield of the desired pure product **4**, combined for the two steps. Using the theoretical throughput of 7 mmol/min, it can be concluded that the 81% isolated yield corresponds to 5.67 mmol/min actual product throughput. Because the catalyst column requires changing or activation after 12 hours, we calculated that a full day of three 8 hour shifts is capable of producing the product for 22 hours, equating to ~7.5 moles, or ~1.47 kg/day production rate of the pure product.

SUMMARY AND CONCLUSIONS

The efficient and safe two-step synthesis of a Linezolid analogue key intermediate **4** was demonstrated using a tandem continuous flow reactor setup that conveniently fits into a standard fume hood. The reactions proceeded with quantitative conversion and great selectivity for both steps, resulting in 81% isolated yield and a 1.47 kg/day throughput capacity for the desired product.

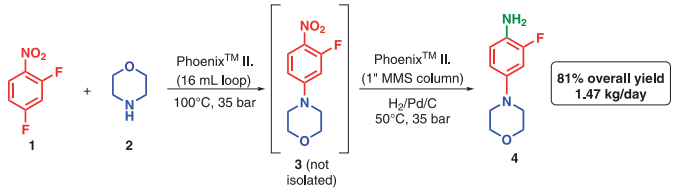


Fig. 8: Summarized reaction scheme and results

REFERENCES

[1]A. P. Kourounakis, D. Xanthopoulos, A. Tzara: **Morpholine as a privileged structure: A review on the medicinal chemistry and pharmacological activity of morpholine containing bioactive molecules**, Med. Res. Rev., 40(2), **2020**, 709-752.

[2]For earlier results on small scale synthesis, see: R. Örkényi, J. Éles, F. Faigl, P. Vince, A. Prechl, Z. Szakács, J. Kóti, I. Greiner: **Continuous Synthesis and Purification by Coupling a Multistep Flow Reaction with Centrifugal Partition Chromatography**, Angew. Chem., 129, **2017**, 8868-88.

For more information, please visit
www.thalesnano.com
linkedin.com/company/thalesnano-inc-/
twitter.com/thales_nano
instagram.com/thalesnano_inc/
facebook.com/ThalesNano/

ThalesNano Inc.
Záhony utca 7. | H-1031 Budapest | Hungary
Phone: +36 1 880 8500
Fax: +36 1 880 8501
Email: sales@thalesnano.com
www.thalesnano.com

US Office
50 S. Penn St. Suite B-2
Hatboro PA. 19040
USA
Phone: 215-534-3365
E-mail: USAsales@thalesnano.com