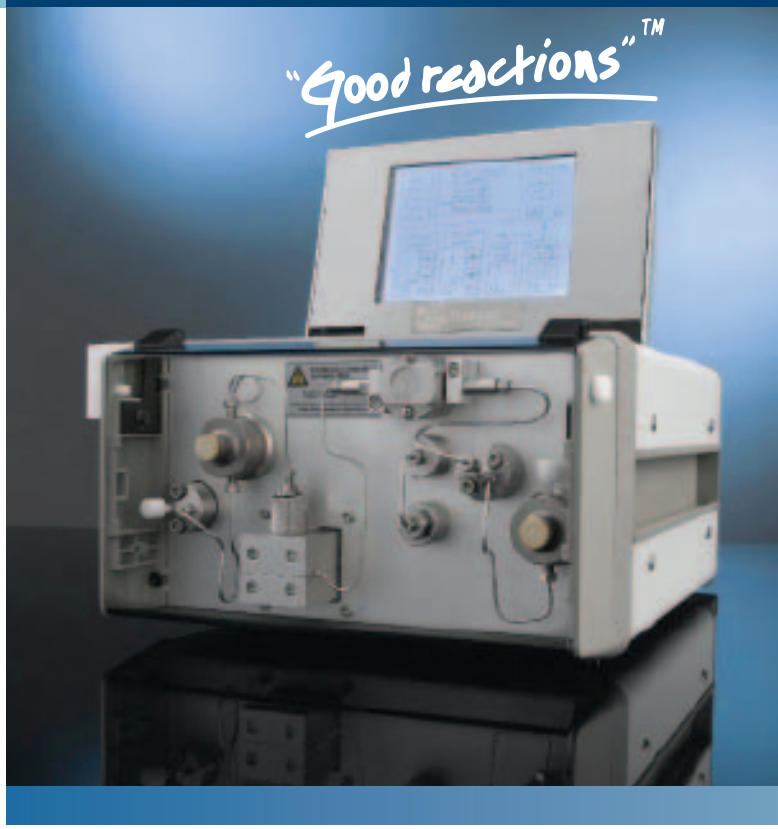
THALESNano



Rapid Library Synthesis of Biological Active Compounds using the H-Cube® Flow Reactor

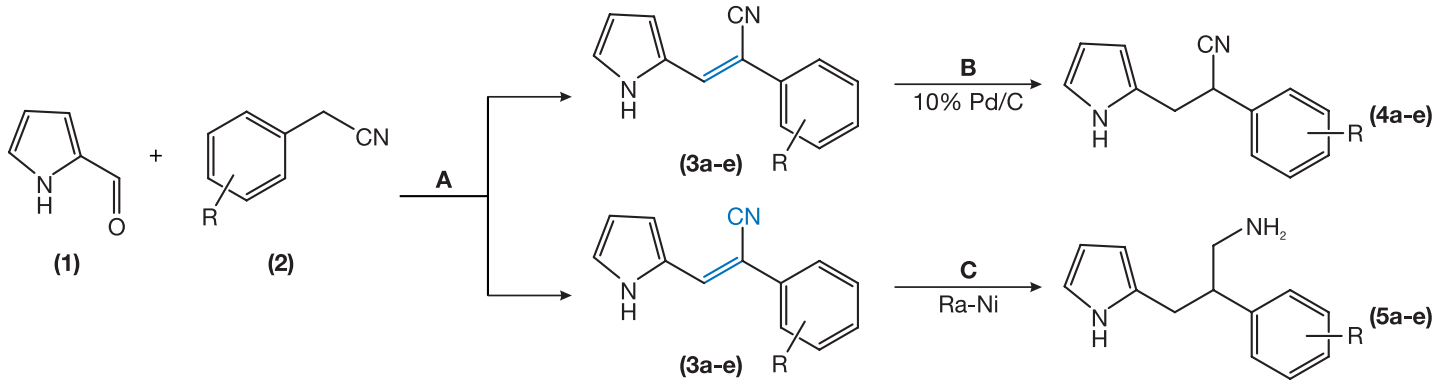
All medicinal chemistry programs require elegant, and rapid synthetic techniques that can deliver novel building blocks from milligrams to several grams. The following application note demonstrates a number of alleviated library synthesis techniques, where the H-Cube® flow reactor afforded several high yield and selective building block syntheses for biological screening.

INTRODUCTION

Certain bis-aromatic acrylonitriles reported by Ali et al. show potent bioactivity.[1] Interestingly, the propylamine derivatives of the corresponding acrylonitriles are known pharmacophores in various novel protein phosphatase inhibitors as well. Intrigued by these facts Tarleton et al. set about developing a series of highly focused libraries to investigate the bioactivity of these nitrile derivatives in focus against a panel of ten human cancer cell lines.[2] The synthesized bis-aromatic acrylonitriles were also reduced to their corresponding bis aromatic amines that were further utilized as pharmacophore building blocks in novel norchantharidine derivatives.[3]

EXPERIMENTAL PROCEDURE

The desired compounds were synthesized in a two step process, in which the (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl) acrylonitrile derivatives (***3a-e***) were obtained firstly via a Knoevenagel condensation from *pyrrole-2-carboxyaldehyde* (**1**) and a range of substituted *benzyl nitriles* (**2a-e**). The resulted analogues were then reduced using an H-Cube® flow hydrogenator, pre-packed catalyst cartridges (CatCart®s) and, in-situ hydrogen generation alleviating safety concerns, which are normally associated with hydrogenation and the handling of catalyst materials. Short reaction times and rapid condition modifications allowed multiple runs in a single day, which led to an agile reaction optimization.



Scheme 1. Synthetic routes to the desired compounds. Conditions: A) H_2O, $PhCH_2NMe_3(OH)$, 50°C, 5h; B) 0.05 M (**3a-e**) in acetone, 50°C, 50 bar, 1.0 mL/min, H-Cube®; C) 0.05 M (**3a-e**) in 1 M NH_3/MeOH, 70°C, 70 bar, 0.5 mL/min, H-Cube®

The first series of optimization reactions involved passing 0.05 M solution of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl) acrylonitrile (**3a**) at 1 mL/min flow rate through three different types of CatCart®s: 5% Pd/C, 10% Pd/C and Ra-Ni respectively. Bearing in mind the highest possible reduction efficiency in each instance, the reactions were conducted at 100°C and 100 bar in *'Controlled Mode'*. No reaction was observed with the 5% Pd/C catalyst under these conditions, but having used the 10% Pd/C one afforded a selective olefinic double bond reduction. In this case both the conversion rate and the isolated yield were 100%. In case of using Ra-Ni, however, reduction occured both on the olefinic double bond and the nitrile moiety. Total conversion of the starting material was obtained by having the feeder solution recirculated through the system.

In comparison to the 10% Pd/C usage, a 5% isolated yield decrease occured due to product stability and workup issues.

Since the 10% Pd/C and Ra-Ni catalyst yielded two different products and no conditions were found under which only the nitrile moiety would have been reduced, the group sought to optimize the reaction in which the Ra-Ni afforded the bisaromatic amines.

Therefore, keeping the flow rate at 1 mL/min they commenced their evaluation with fixing the temperature at a constant 100°C and decreased the H_2 pressure in 5 steps from 90 bar to 40 bar. Even though in each of these cases the conditions resulted in the desired product, they afforded significantly lower conversions (***Table 1, Entries 1-6***). Later on, it was the pressure that they maintained at a constant 100 bar and the temperature was decreased in 10 °C decrements from 90°C to 40°C (***Table 1, Entries 7-12***). Having the temperatures decreased at a constant 100 bar pressure resulted in falling conversion rates too, where below 50°C no conversion was observed. As a result of the rapid reaction condition

Entry	p [bar]	T [°C]	Conversion [%]
1	90	100	30
2	80	100	30
3	70	100	30
4	60	100	30
5	50	100	25
6	40	100	25
7	100	90	25
8	100	80	15
9	100	70	12
10	100	60	5
11	100	50	0
12	100	40	0

Table 1: *Temperature and pressure optimization for the reduction of **3a** to **5a**. Flow rate: 1 mL/min. Catalyst: Ra-Ni*

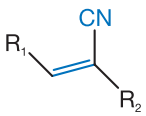
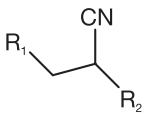
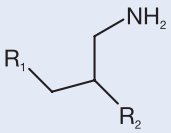
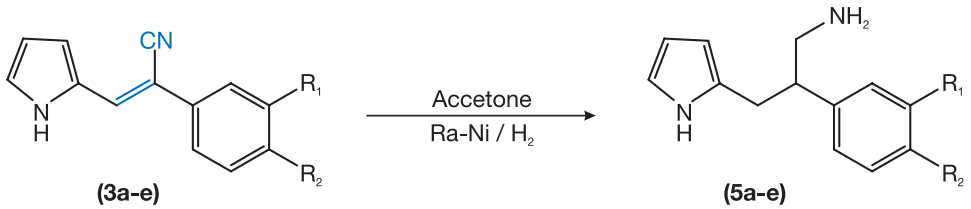
Entry	Substrate	Catalyst	Product	Results
1		10% Pd/C		100% Conversion 100% Isolated Yield
2		Ra-Ni		100% Conversion 95% Isolated Yield
3		5% Pd/C	N/A	NO REACTION WAS OBSERVED

Table 2. Catalyst screening. Conditions: 0.05 M solution of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (3a) at 100 °C, 100 bar pressure and 1 mL/min flow rate, H-Cube® (Controlled Mode)



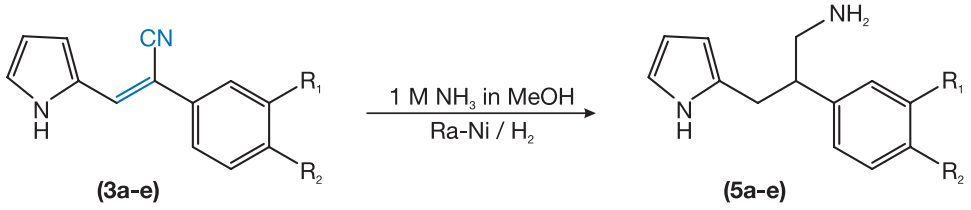
(3a-e) (5a-e)

Entry	Substrate	R_1	R_2	Pressure [bar]	Product	Isolated Yield [%]
1	3a	H	H	50	5a	100
2	3a	H	H	100	5a	100
3	3b	H	$NO_2(NH_2)^{\#}$	50	5b	100
4	3c	H	F	50	5c	100
5	3d	H	Cl	50	5d	100
6	3e	Cl	Cl	50	5e	100

Table 3: Flow hydrogenation of bis-aromatic acrylonitrile analogues (3a-e) to the corresponding amines (5a-e) at 60°C and 50 bar at 1.0 mL/min flow rate. [# - denotes that -NO_2 was reduced to -NH_2]

survey, they applied the mildest full conversion conditions to the series of acrylonitrile analogues (*60°C and 50 bar,* ***Table 3***). 20 minute sample collection afforded adequate product amount for purification, identification and yield calculations. As it can be seen in ***Table 3***, only the unsubstituted acrylonitrile analogue (***Entry 1, 2***) was converted quantitatively to the desired amine (***5a***) under the applied conditions. In the case of substrate **3b**, a simultaneous -NO_2 moiety reduction was also observed. Poor conversions to the desired bis-aromatic amines complicated product isolation and purification.

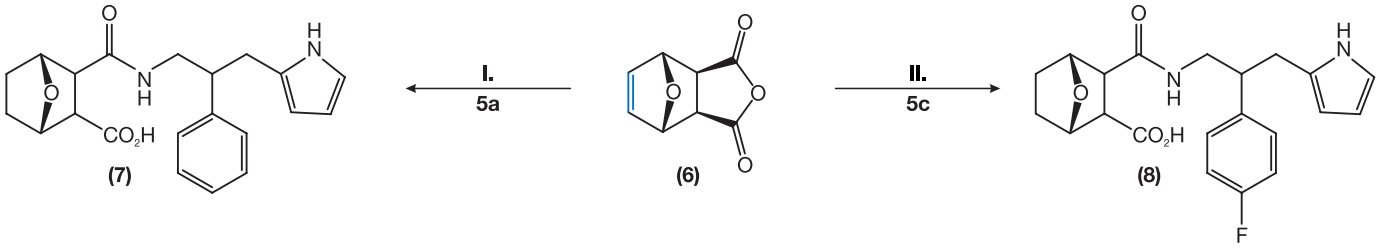
Since the given results reflected poor yields in the series of these reactions, the group initiated another optimization run by having the flow reductions of ***3a-e*** repeated in 1M NH_3/MeOH. System pressure and reaction temperature was kept at 70 bar and 70°C, while the flow rates were fixed at 1.0 and 0.5 mL/min. Each of the α,β- unsaturated nitriles (***3a-e***) were then converted quantitatively to the desired amines under the above mentioned conditions (***Table 4***). Having the H-Cube® further utilized, novel norcantharidine derivatives were synthesized from the previously prepared bis-aromatic amines.



(3a-e) (5a-e)

Entry	Substrate	R_1	R_2	Flow rate [ml/min]	Product	Isolated Yield [%]
1	3a	H	H	0.5	5a	100
2	3a	H	H	1	5a	100
3	3b	H	$NO_2(NH_2)^{\#}$	0.5	5b	100
4	3c	H	F	0.5	5c	100
5	3d	H	Cl	0.5	5d	100
6	3e	Cl	Cl	0.5	5e	100

Table 4: Flow hydrogenation of bis-aromatic acrylonitrile analogues (3a-e) to the corresponding amines (5a-e) in 1M NH_3/MeOH at 70°C, 70 bar pressure, 0.5 and 1.0 mL/min flow rate./# - denotes that -NO_2 was reduced to -NH_2/



Scheme 2. Reagents and conditions: I) 0.05 M of **6** in acetone, 50°C, 50 bar pressure at 1.0 ml/min, 0.05M **5a** in 1M NH_3/MeOH. II) 0.05 M solution of **6** in acetone, 50°C, 50 bar, 1.0 mL/min; 0.055 M solution of **5c** in 1M NH_3/MeOH, 50°C, 50 bar, 1.0 mL/min

5a and ***5c*** were subjected to flow hydrogenation conditions in the presence of 5,6- dehydronorcantharidine (***6***), which resulted in the corresponding protein phosphatase 1 and 2A inhibitor amides (***7*** and ***8***, ***Scheme 2***). IC_{50} values of compound 7 are 18.5 ± 3.5 µM in terms of PP2A inhibition and 14.1 ± 7.9 µM in terms of PP1 inhibition. In case of compound ***8*** the measured half maximal inhibitory concentration against PP2A is 7.25 ± 1.25 µM and 4.25 ± 1.55 µM against enzyme PP1.

CONCLUSION

Judicious choice of catalyst selection allowed the selective reduction of either the olefin double bond to the corresponding propanenitrile derivative (10% Pd/C) or the reduction of unsaturated nitriles to the corresponding propaneamine derivatives (Ra-Ni) in a flow manner. The involvement of the H-Cube® flow reactor in the synthesis steps allowed rapid condition screening and parameter optimization.

The prepared scaffolds were then collected long enough to gather compounds for either subsequent building block syntheses or for biological screening.

REFERENCE

1. A. Ali; M. Bliese; J.-A. M. Rasmussen; R. M. Sargent; S. Saubern; D.G. Sawutz; J.S. Wilkie; D.A. Winkler; K.N. Winzenberg; and R.C. J. Woodgate; *Bioorg. Med. Chem. Lett*.; **2007**; 17; 993-997.
2. M. Tarleton; J. Gilbert; M. J. Robertson; A. McClusky and J A. Sakoff; *Med. Chem. Commun*.; **2011**; 2; 31-37.
3. M. Tarleton, A. McClusky, *Tetrahedron Letters*; **2011**; 52; 1583-1586.

LEGAL

ThalesNano Nanotechnology Inc.
Zahony u. 7.
H-1031 Budapest
Hungary
Tel.: +36 1 880 8500
Fax.: +36 1 880 8501
E-mail: sales@thalesnano.com

US Office
50 S. Penn St. Suite B-2
Hatboro
PA. 19040
USA
Phone: +1 732 274 3388
E-mail: USAsales@thalesnano.com

www.thalesnano.com