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Flow chemistry applied to the preparation of small molecules potentially useful as therapeutic agents

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Chapter 1.
Introduction

1.1. Drug discovery process and enabling techniques

The process of drug discovery involves the identification of clinical candidates, their synthesis and characterization for therapeutic efficacy. During the last 50 years this process significantly changed starting from an approach mostly based around chemistry through a more biological one and finally moving to one more focused on the diseases. Today the advent of molecular biology, coupled with the advances in screening and synthetic chemistry techniques allowed a combination of both knowledge around the biological target and random screening. This transformation was driven by a strategic imperative but was enabled by the impressive technological advances in chemistry and biology.

The process of finding a new drug for a particular disease through the interaction with a chosen target (Figure 1) usually starts with the "Hit finding" step that is accomplished by high-throughput screening (HTS) and/or computational drug design. HTS is performed on libraries sufficiently large and diverse to find novel chemical entities with a relative high probability. Another important function of HTS is to show how selective the compounds are for the chosen target with the goal of finding a molecule which will interact only with the desired target, but not with other related targets (**cross-screening**).
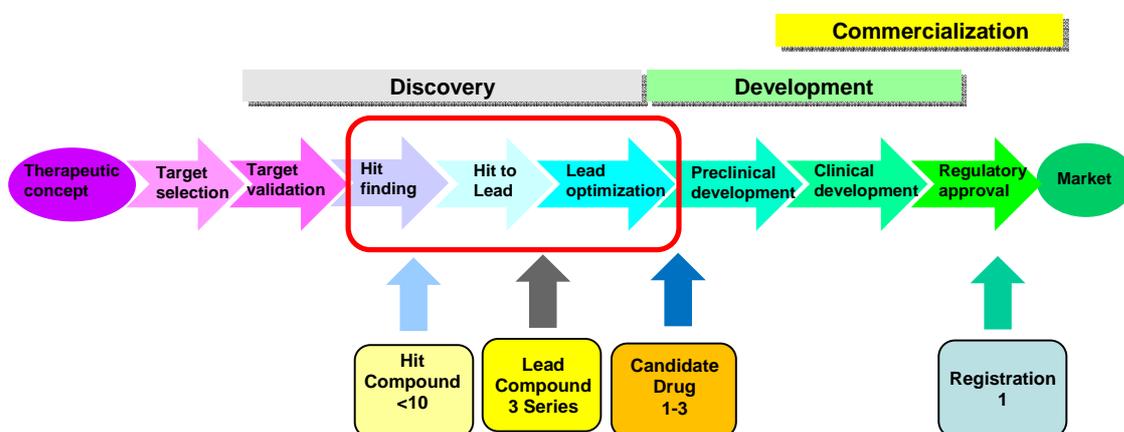
Figure 1. Drug discovery process in the third millennium

In the hit validation stage, hits are assessed and experiments are performed to rule out non-specific hits. The screening of related compounds is important to determine SAR (Structure-Activity Relationships) and to establish the developability profile for interesting hits. Once they have been validated, the "hit to lead" campaign can start, with a detailed set of criteria to be met in order to

initiate a lead optimization project. This includes not only activity criteria but also a range of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties to be optimized. After hit validation and during the lead optimization process medicinal chemists will attempt to use SAR to improve certain features of the lead compound:

● increase potency against the chosen target;

● reduce activity against unrelated targets;

● improve the "drug-likeness" or ADMET properties of the entire molecule.

This process will require several iterative screening runs, during which the properties of the new molecular entities will be improved and the best balanced compounds will go forward to *in vitro* and *in vivo* testing in the disease model of choice.

It is estimated that for every 100.000 compounds screened, about 100 hits are identified. Of these 100 hits only 1 proceeds to the lead compound stage. Between 40% and 60% of these lead compounds fail ADMET testing. In recent years, despite the number of novel and clinically validated targets identified from the human genome project, the number of new drug launches is decreasing and the overall costs for the development of a drug are rising significantly. Pharmaceutical and biotechnology companies are under a strong pressure to produce a steady stream of innovative, well-differentiated drugs and with a reduced cost both for discovery and development. Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy, expensive, difficult, and inefficient process with low rate of new therapeutic discovery. Currently it takes an estimated 10-14 years to develop and market a drug and development cost of each new molecular entity (NME) is approximately US$1.8 billion.

Different and novel technologies (related to synthesis, work-up and isolation) are now available to produce compounds at a higher rate. The so called "*Enabling Techniques*"[1] have emerged in the past decade and were studied in a large extent in academia. They can be applied now both in the hit validation and lead optimization processes. These techniques summarize various traditional as well as new methods which have been developed to speed up synthetic transformations and importantly to make the workup as well as the isolation of products easier. Various successful examples recently appeared in the literature in which different enabling techniques are combined in order to achieve faster synthesis and/or improved work-up.
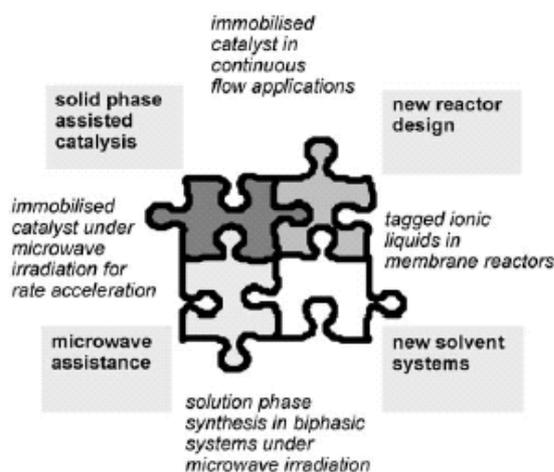
Figure 2. Enabling techniques

Among the most significant technical improvements, the greatest impact has been obtained by polymer-assisted solution-phase synthesis (PASPS), microwave assisted organic synthesis (MAOS) and, more recently, also by continuous-flow processes. These technologies have received great attention in the literature and have demonstrated their potential for improving productivity in organic synthesis and medicinal chemistry.

1.2. Polymer-assisted solution-phase synthesis (PASPS)

In research chemistry, during recent years (from the second part of 1980s), solid-supported reagents and scavengers have been widely employed in organic chemistry, since they allow the simplification of both synthetic procedures and isolation or purification steps, avoiding at the same time the limitations of solid-phase synthesis.[2] The most significant improvement, when PASPS is compared to classical synthesis, is that work-up operations are considerably simplified and reduced to simple filtration. The use of a large excess of reagents (often necessary to drive reactions to completion) is then possible without requiring additional purification steps. Toxic, noxious or hazardous reagents and their by-products can be immobilized and, therefore, not released into the solution thereby improving their general acceptability and safety profile. Owing to site isolation of reagents on the resin bead, species that are incompatible in solution may be used together to achieve one-pot transformations that are not possible under classical homogenous conditions.

1.3. Microwave-assisted organic synthesis (MAOS)

Since the late 1990s, MAOS has become a forefront support for rapid optimization of reactions, for the efficient synthesis of new chemical entities, for discovering and probing new chemical reactivity.

MAOS is mainly based on the efficient heating of materials by the microwave dielectric heating effect (through dipolar polarization and ionic conduction).[3]

The use of microwave irradiation offers significant advantages:

● higher reaction temperatures by combination of microwave heating with sealed vessels;

● reduced reaction times, higher yields and cleaner reaction profiles;

● the use of lower boiling point solvents under pressure in sealed vessels;

● specific heating of strongly microwave-absorbing metal catalysts;

● more reproducible experimental conditions by accurate control of temperature and pressure profile.

MAOS was shown to significantly improve productivity[4] because it dramatically accelerates the rate of many organic reactions (from days to hours and from hours to min), generally improving the yields of the final products. Starting from early reports of microwave-promoted Suzuki coupling,[5] a wide variety of reactions has benefited from MAOS and organic reactivity with microwaves has been extensively explored.[6] In fact high speed microwave-assisted chemistry has been successfully applied to many kinds of organic reactions including cycloaddition reactions, heterocyclic synthesis, transition metal catalysed processes, solvent free reactions and almost all chemical transformations where heating is required.

In literature there are also examples where transformations that did not work using conventional heating were successfully achieved under microwave irradiation.[7]

The increase of the reaction rate often did not improve the productivity because classical methods for work-up and purification of reaction products slow down the entire process.

Anyway MAOS may be advantageously coupled to inorganic-supported solvent-free conditions, thus simplifying work-up procedures (in many cases the pure expected products can be obtained directly by simple extraction, distillation or sublimation) and waste disposal.[8] Similarly, the combination of MAOS and solid-supported organic synthesis or PASPS[9] can be performed. Usually, the synthetic steps involving polymeric supports require repeated runs and longer reaction times than the corresponding solution-phase protocols to reach high conversions. Microwave heating again allows reduction of reaction times and improvement of the loading of

the functionalized solid support, employing not only traditional polystyrene supports but also soluble polymers and fluorous phase synthesis. The main issue associated with MAOS is the scalability of the process. Large-batch reactors,[10] as well as continuous-flow mode[11] have been described. However, the scalability of microwave reactions still requires more development, especially in the technology and engineering field.

1.4. Flow chemistry

Among "Enabling techniques", continuous flow organic synthesis is gaining attention and is moving from a strictly academic level to the wider research and development exploitation. The result of this evolution is the increasing number of reactions successfully performed with this technique and reported in the literature. More recently, thanks to the advent of commercially available micro/meso flow reactors, pharmaceutical companies are embracing flow methodology in drug discovery programs attracted by its potential advantages over the existing batch techniques. Theoretical and practical benefits associated with performing reaction under micro/meso continuous flow have been demonstrated for a number of common organic transformations, ranging from liquid-liquid to solid-liquid-gas systems. In particular for pharma companies, a very attractive feature of continuous-flow processes is the elimination of the risks associated with failing to scale up a process because the reaction conditions set-up on microreactor can be directly transferred to production scale without the need of re-optimisation, either by running the flow-reactor for an extended time or by employing multi-channel parallel reactors (numbering-up process)

In the framework of my PhD thesis exploring the application of the so called "*Enabling Techniques*" in an organic and medicinal chemistry laboratory, my efforts were devoted to the evaluation of the benefits that continuous flow chemistry could provide in Drug Discovery programs and more in general to the preparation of challenging molecules potentially useful as therapeutic agents and to the synthesis of natural products in comparison with traditional synthetic techniques.

Chapter 2.
Flow chemistry

2.1. Flow reactors: structure and design

In flow chemistry, a chemical reaction is performed in a continuously flowing stream in a network of interconnecting channels: where they join one another, the fluids come into contact and the reaction takes place.

Flow reactors are generally composed of the following basic components: one or more fluid control devices which load the solutions of different reactants to the reactor section, the reactor, that usually can be heated or cooled, in which reactions can occur under a precise control of temperature and pressure and suitable reservoirs to collect the resulting mixture (Figure 3).
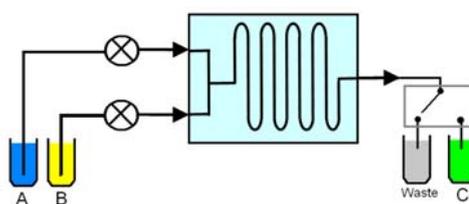
Figure 3. General scheme for a Flow Reactor

Laboratory scale flow reactors can generally be divided into two broad classes on the basis of channels size and volume: micro- and meso-flow reactors. In general, but the distinction is not so sharp and well defined, micro-flow reactors present channel having diameter from 10 to 1000 μm, whereas meso-flow reactors are characterized by larger channels with diameter up to 1000 μm.[12]

The main difference between these two kinds of equipment is related to the shape of the reactors and to the fabrication techniques. In particular micro-flow reactors are designed and produced with methods coming from the field of semiconductor microelectronics, such as photolithography and micro-patterning, and they are usually planar object with the size of a small plate, the "chip". In recent years, a variety of microreactors have been developed and several of them are now commercially available. The applicability of a microreactor is defined by its size, the chemical and physical properties of the material used for its construction, and the mode of reagent and solvent introduction to the system. To illustrate the diversity in miniaturized reaction devices reported to date, a small selection of microreactors is presented in Figure 4. A range of materials, including glass, silicon, stainless steel, metals, and polymers have been used.
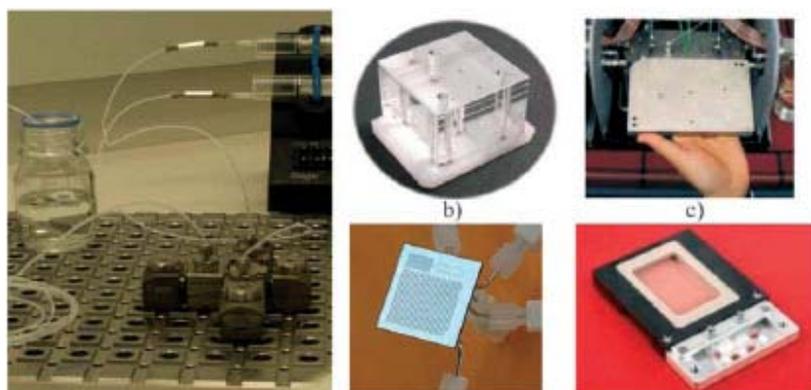
Figure 4. a-c) Stainless steel microreactors; b-e) Glass microreactors; d) Silicon-based microreactor

Meso-flow reactors are instead constructed of plastic tubing (generally the same material used for HPLC equipments) with T or Y shaped junctions (Figure 5).
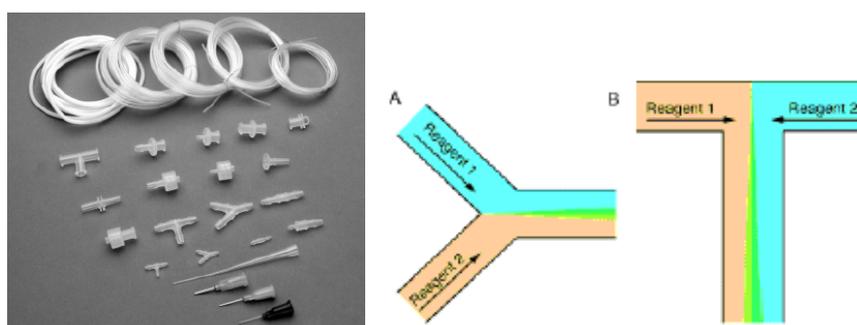
Figure 5. Meso-flow reactors (PTFE); T or Y shaped junctions

In this case the fabrication is a much more simple process that can be easily performed in a common laboratory.[13]

In microreactors (the term microreactor (MR) will be used to indicate both micro and meso-flow reactors as all the listed characteristics are applicable to both categories) the fluid behaviour is mainly non-convective, with "laminar flow"[14] and mixing determined only by diffusion. All flow reactors need a precise control of fluids, achieved by two main techniques: hydrodynamic flow and electrokinetic flow.[15] The former, also called pressure driven flow, is usually associated with syringe or peristaltic pumps that apply a positive pressure to the inlet of the system. The main advantage of these devices is the broad compatibility with any solvent and any construction material. However capillary resistance increases exponentially with decreasing channel dimensions, making pumping almost impossible for too narrow tubing. Moreover the velocity

profile in hydrodynamic flow is parabolic, with faster flow at the centre of the channel and slower flow near the walls; this can lead to non-homogeneous residence times (Figure 6).

Pressure driven flow   *Electroosmotic flow*
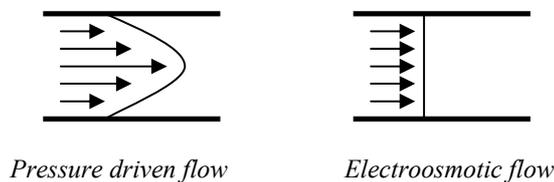
Figure 6. Velocity profiles for pressure driven and electroosmotic flow

The alternative electrokinetic flow is associated with the application of a potential difference at the ends of the system. The first consequence of this is the direct movement of ions in solution toward the electrode of opposite charge. The second component of electrokinetic flow, electroosmotic flow, arises from the electrical double layer that is formed on channels with charged surfaces. At neutral to basic pH, glass and silica surfaces bear a negative charge due to partial ionization of surface hydroxyl groups. In response to the negative surface charge, positive species in the solution form a double layer near the surface of the channel. When an electric potential is applied between the channel ends, the mobile positive ions migrate toward the negative electrode and viscous drag between the moving ions and the rest of the solution causes net flow of the fluid toward the negative electrode (Figure 7).

The velocity of electroosmotic flow is linearly proportional to the applied voltage, allowing precise fluid handling. In this case the velocity profile is nearly flat across the channel, leading to greatly reduced dispersion of reagents if compared to hydrodynamic flow. Unfortunately, the use of electroosmotic flow is restricted to polar solvents such as water, methanol, acetonitrile, dimethylformamide and tetrahydrofuran and to device materials that develop surface charges such as glass, silicon and treated PDMS (Polydimethylsiloxane).
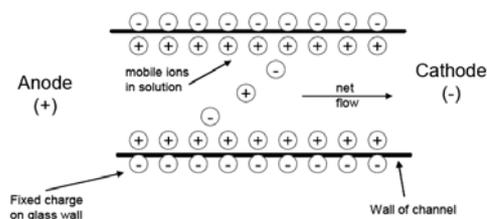
Figure 7. Electroosmotic flow

During the last years a good number of laboratory scale devices were commercialized. Although most of the basic features described in this paragraph are present in all cases, different other characteristics were introduced by producers to give the broadest set of applications to the final user: the possibility to use solid catalyst or polymer-supported reagents, gaseous reagents (*e.g.* for hydrogenations and carbonylations) and more reactors in parallel or in series. Some examples in the literature reported the use of more complex devices, where two or more different techniques were joined to exploit their peculiar advantages; in particular examples of microwave flow reactors and of photochemical flow reactors.[16]

2.2. Principles and key parameters

In batch processes the reaction stoichiometry is defined as the ratio among the moles of reactants while, in flow process, it depends both from the ratio of the reactant concentration and from their flow rate. For this reason the flow system is quite more flexible as concentrations and flow rates can vary in an independent way to find the optimal conditions.

Reaction time in a flow process is defined as the **Residence Time** (**RT**) and is determined by the ratio between the reactor volume and the total flow rate.

RT (min) = Reactor Volume (mL) / Total Flow Rate (mL/min)

The amount of synthesized product per hour, called **Output**, is not related to the classical concept of batch scale, but it is instead defined by a relation among the flow rate, concentration, molecular weight of the product and the reaction yield.

OUTPUT (g/h) = Flow Rate (mL/min) x Conc (mmol/mL) x MW (g/mol) x Yield (%) x 0.0006

2.3. Advantages of the flow technique

The main advantages associated with the flow processes performed in microreactors can be comprised in two broad classes.

The first one is associated with the *small dimensions of the channels* and includes the precise control of the reaction conditions, the efficient mass and heat transfer, the possibility of working under superheating conditions. The second aspect is related to the *continuous nature of the process* and includes the simplicity in reaction scale-up, the possibility of performing sequential synthetic steps with independent control of reaction conditions, the possibility of introducing in-line

purification by means of supported scavengers or sorbents and the possibility of interfacing the reactor with in-line analysis devices for real time monitoring.[17]

The next paragraphs will be dedicated to illustrate these different aspects through the discussion of literature examples.

2.3.1. Advantages related to the small dimensions of the channels

In the classical batch reactors, such as round-bottom flasks, the control of heat and mass distribution is generally achieved by mechanical stirring. In most cases, however, not homogeneous temperatures and mixing are obtained with the formation, in connection with reactor geometry, of concentration gradients and hot spots that can lead to poor yields and low reaction selectivity.

On the other hand microreactors assure a rapid and efficient mixing of reagents because of the continuous and controlled addition of small volumes of reagents, reducing up to milliseconds the time required to obtain a homogeneous solution, avoiding the formation of hot spots.[18]

An example of this advantage is shown in the simulation of the neutralization reaction (HCl/NaOH) performed in batch and in a flow reactor (Figure 8).

The figure shows the concentration distribution in a MR and illustrates its efficient mixing properties. The compound distribution in batch system is less homogeneous than in a MR and provides the formation of hot spots.

Batch synthesis:
Concentration equivalent: 0.8 (blue)-
1.0 (green)-1.2 (red). Formation of
hot spots

Flow synthesis:
Reagent A injected in a flow stream
of B (red); green color means mixture
1:1 of the two reagents.
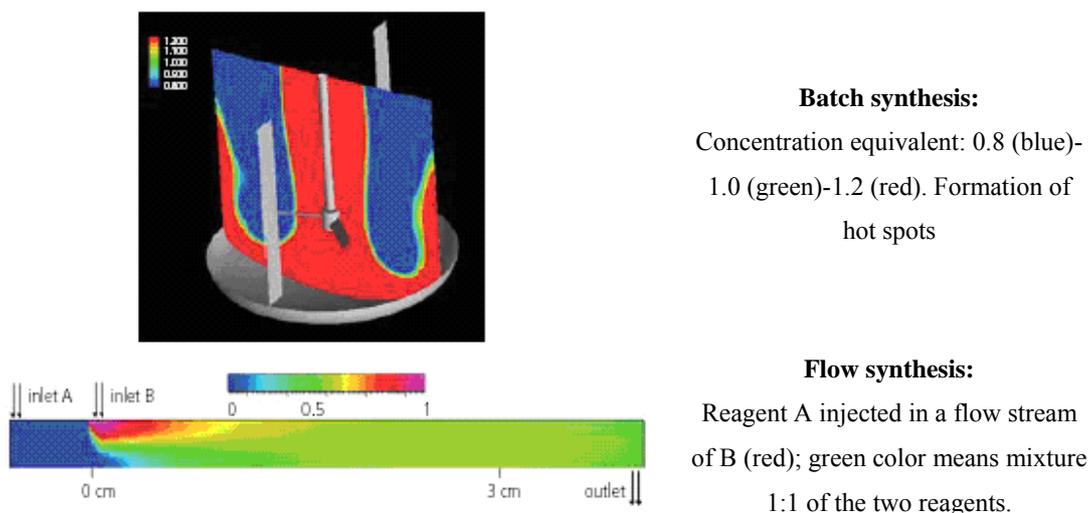
Figure 8. Neutralization reaction of HCl with NaOH

The temperature is another important parameter for obtaining good result in a reaction. Also in this case, the small dimension of the channels permits an efficient heat transfer.

Batch synthesis

Flow synthesis: Temperature: 293 K (blu) 298 K (green) 303 K (red)
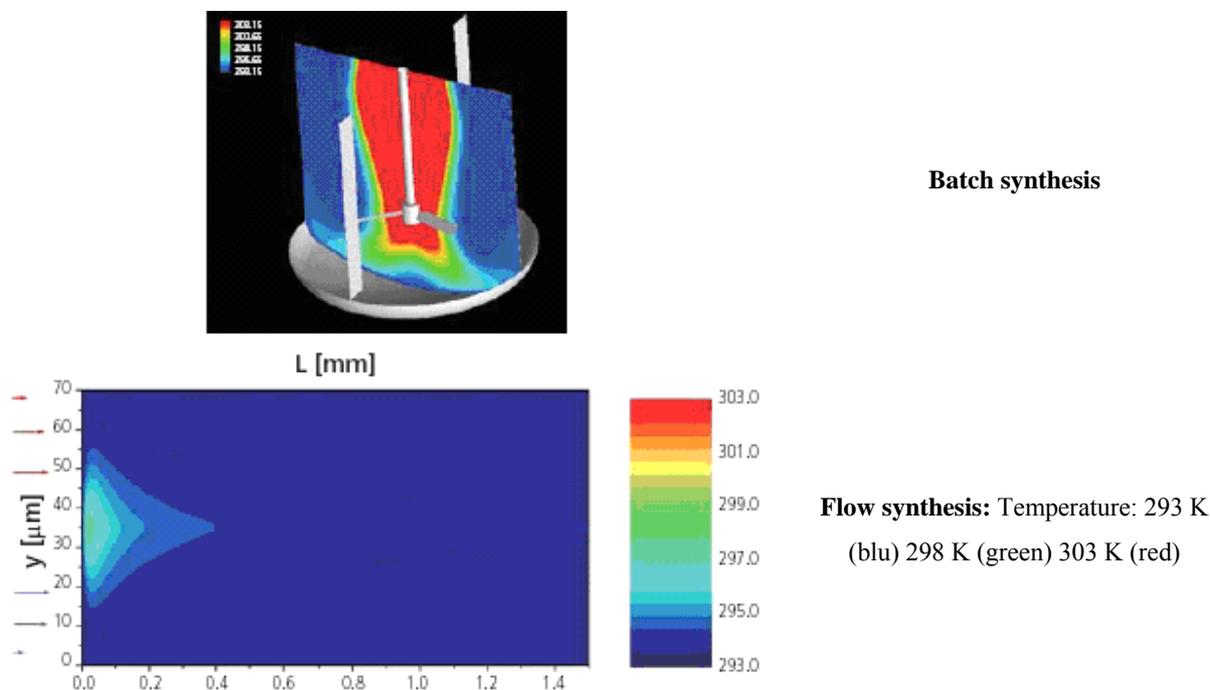
Figure 9. Neutralization reaction of HCl with NaOH

This aspect is underlined in Figure 9 where the temperature distributions for a neutralisation reaction (HCl and NaOH) in a batch vessel and in a channel are simulated.

In MR the average distances from the reagents flow to the heat exchanging walls of microreactors are small. Heat exchange is driven similarly by a steep temperature gradient. At every point along the flow channel concentration and temperature gradients are stable. These stable gradients provide better control of reaction conditions compared to conventional synthesis. In fact the temperature transfer in a multi-m^3 batch vessel is more difficult than in a MR, hence, temperatures are widely fluctuating within the vessel.

The second characteristic of microreactors is the high surface-to-volume ratio, also called specific surface area. This high specific surface area is directly correlated with a high heat-exchanging efficiency, allowing for fast heating and cooling. A qualitative distribution of the inner temperature of a generic reaction, both in a microreactor and in a classical batch reactor is shown in Figure 10a.[19]
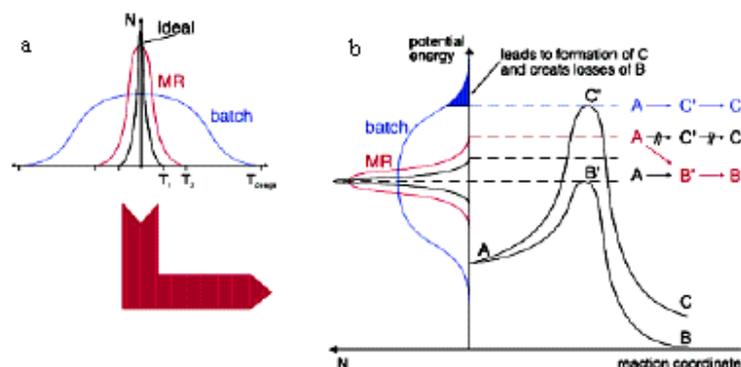
Figure 10. Temperature distribution (a) and its correlation with synthetic pathway (b)

The higher heat transfer capacity of the microreactor is able to avoid that the process temperature moves away from the set one. On the contrary, in a classical reactor, the range of temperature around the set parameter is broader, mainly because of the lower specific surface area and mixing issue. This different distribution may influence the route of the synthetic process.

Figure 10b shows a schematic correlation between reaction temperature and synthetic pathway; potential energy profile is related to reaction temperature along the reaction coordinate and in passing through transition states.

Reaction is shown to proceed from the conversion of starting material A to product B; the formation of B is favoured if the activation energy provided by the reaction is limited, presenting a maximum in correspondence with the set temperature (transition state B'). A side reaction profile is also possible; it requires higher activation energy (transition state C') and affords the by-product C.

Whereas the batch reactor's broad temperature distribution allows the production of the undesired by-product C, the narrow temperature distribution in the MR restricts the reaction to the target product B.

Different examples from the literature can help to better understand these aspects.

2.3.1.1. Higher selectivity, yield and reaction rate

Pennemann[20] benchmarked some classical organic reactions performed both in micro and batch reactors. Gathering simple literature examples, the authors highlighted the advantages of the flow reactors, mainly in terms of reduction of reaction times from hour-long processes to procedure displaying very short residence times. However the success of these processes was often reached

by a significant changing of the experimental protocol to adapt reactions to the needs of the flow reactor engineering. Representative examples are summarized in Scheme 1. The first example is a coupling reaction of the appropriately protected β-alanine reactants, using both DCC (dicyclohexyl carbodiimide) as coupling agent and direct substitution on preactivated carboxylic acid.[21] The second one is a Suzuki coupling catalyzed by immobilized palladium on silica.[22] The last reaction is a 1,4-addition of enolates of 1,3-diketones to Michael acceptors using organic base.[23]

1a. X = H, coupling agent: DCC
1b. X = PFP
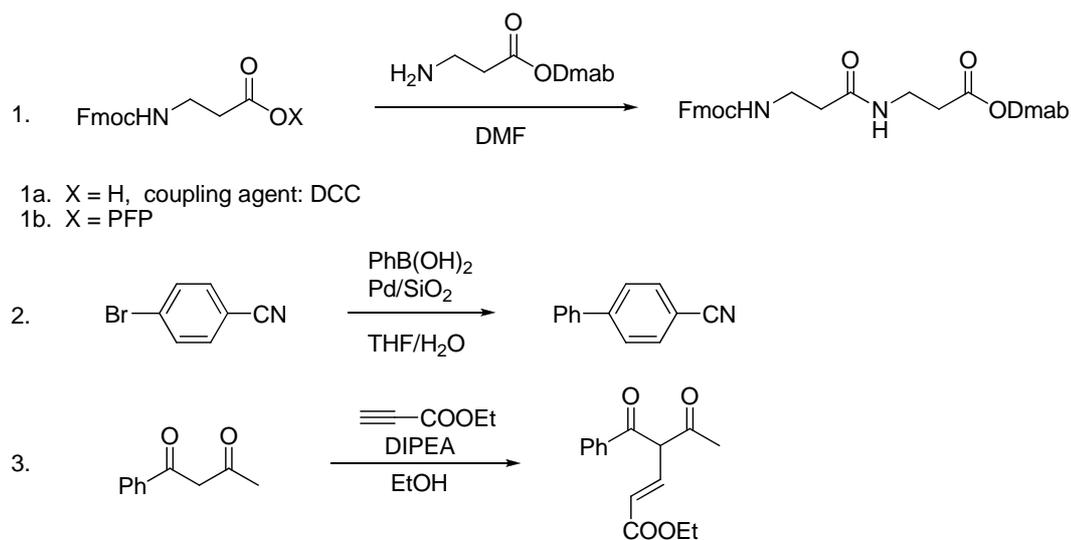
Scheme 1. Organic reactions performed in micro (MR) and batch reactors

The comparison of the results is reported in Table 1. These data demonstrate the potentiality of the microreactors of maintaining high conversions drastically reducing the reaction times.

	MR	Batch reactor
Reaction 1a		
Reaction time	20 min	24 h
Conversion	up to 93%	92%
Reaction 1b		
Reaction time	20 min	120 h
Conversion	100%	60%
Reaction 2		
Reaction time	6 s	8 h
Conversion	68%	60%

	Reaction 3	
Reaction time	20 min	24 h
Conversion	100%	89%

Table 1. Comparison of results for the reactions listed in Scheme 1

2.3.1.2. Pressure control: superheating effects

In batch chemistry the highest reaction temperature depends by the boiling point of the used solvent.

In flow reactors, as in the case of microwave assisted synthesis, working under pressure control permits to perform reactions at temperature higher than the solvent boiling point.

Through opportune back-pressure regulators, lower boiling point solvents may be used in a wider range of temperatures, avoiding, where not strictly necessary, high boiling solvents and thus simplifying the reaction work-up. One example of the application of "superheating" condition was described by Ley. [24]
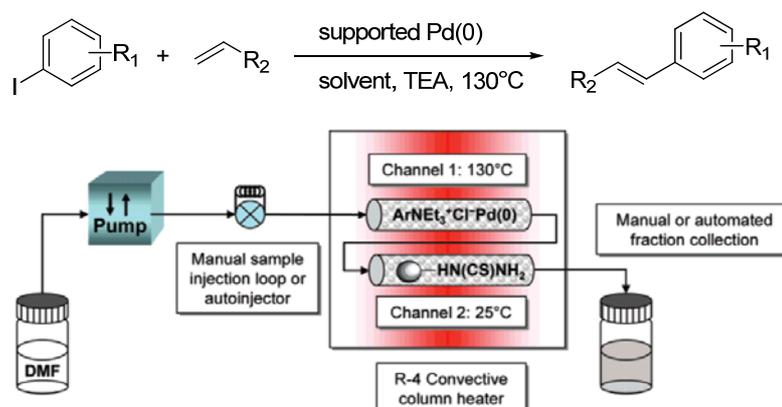
Scheme 2. Heck coupling in continuous flow

A series of Heck coupling were performed in continuous flow condition using ligand-free monolithic palladium(0) nanoparticles in DMF as solvent by an automatic reactor (Scheme 2). The couplings proceeded rapidly at 130°C and DMF, chosen for its high boiling point, was then easily replaced by more benign ethanol. This solvent in superheating conditions under pressure, maintained high yields as reported in Table 2.

The problem of the leaching of the supported metal into solution was solved using thiourea-based metal scavenger resin that permits to limit Pd levels < 5 ppm in the isolated products.

R₁	R₂	Solvent	Yield
4-C(O)CH₃	2-Pyridinyl	DMF	87%
4-C(O)CH₃	2-Pyridinyl	EtOH	86%
4-COOEt	-COOtBu	EtOH	87%
4-COOEt	Phenyl	EtOH	88%
3-CN	2-Pyridinyl	EtOH	85%

Table 2. Heck coupling of various substrates in different solvents

2.3.1.3. Accessibility of exothermic reactions

Microreactors are broadly used for exothermic and potentially explosive reactions as, for example, nitration of aromatic compounds. In fact this reaction is a classical dangerous exothermic process with a high industrial impact that has benefited from continuous reactors. Ducry and Roberge[25] reported a study on autocatalytic nitration of phenol in presence of HNO_3 in acetic acid and water as solvents (Scheme 3).
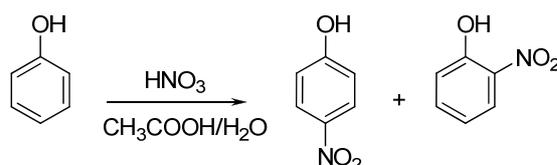
Scheme 3. Nitration of phenol

This reaction leads to two products (4-nitrophenol and 2-nitrophenol) but also to many by-products, such as dinitro compounds, hydroquinone and polymeric products. Exothermic autocatalytic reaction not only yields a complex mixture of products, but also leads to hazardous runaway scenarios especially when performed in large batches. The obtained results are compared in Table 3. Batch conditions led to poor yields, large amount of by-products, mainly polymers, and high inner temperatures. The best results were obtained in a glass microreactor demonstrating how the accurate temperature and mixing control avoids side- and sometimes dangerous reactions.

	T (°C)	HNO₃ (eq)	Yield	Ratio 4-/2- nitrophenol	By-products amount
Batch	0	2.0	30%	1.2	65%
	20	2.0	21%	0.6	77%
MR	5	1.7	74%	0.9	10%
	20	1.4	77%	1.0	17%

| 55 | 1.7 | 65% | 0.9 | 23% |

Table 3. Nitration of phenol in MR and batch conditions

Another similar example is the synthesis in microreactors of 2-methyl-4-nitro-5-propyl-2H-pyrazole-3-carboxylic acid (Scheme 3), a key intermediate of Sildenafil™ (Viagra).

This highly exothermic process is a classical example of transformation characterized by runaway side-reactions. At about 100°C, decarboxylation was found to start, affording the by-product with a dangerous increase of internal pressure (Scheme 4). When the reaction was performed in batch especially if in large scale, the nitrating mixture was added very slowly to avoid reaching high temperature. The reaction takes about 10 h at 50°C to have complete conversion with 75% overall yield. In microreactor system, thanks to the small amount of reactants used at time and to the rapid and efficient heat dispersion, the reaction was conducted at 90°C, increasing the rate but avoiding the undesired decarboxylation. In these conditions the authors obtained a comparable yield (73%) drastically reducing the residence time to 35 min with an output of 5.5 g/h.
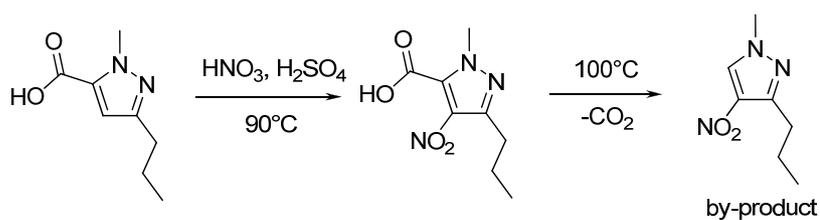
Scheme 4. Synthesis of the carboxylate pyrazole

2.3.1.4. Easy management of poorly stable intermediates

The use of flow chemistry techniques can also allow the conversion of very unstable intermediates into the desired products without loss of yields and side-reaction runaways, thanks to the exact control of temperature and mixing.

The investigation of Moffat-Swern oxidation of alcohols was described by Kemperman[26] in a continuous flow microreactor. The whole synthetic pathway is shown in Scheme 5.
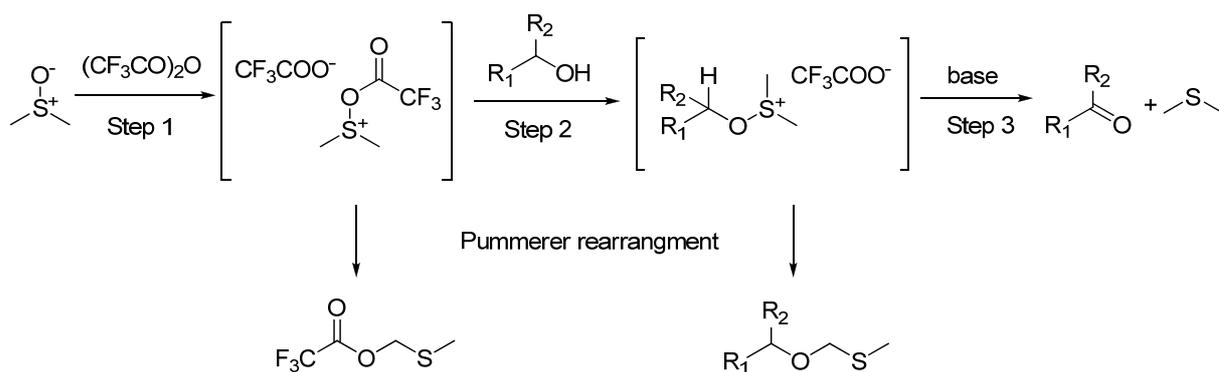
Scheme 5. Moffat-Swern oxidation of alcohols

Mainly for the shortness of the residence times, able to reduce side-reactions such as the exothermic Pummerer rearrangement, the process can be operated at remarkably higher temperatures (0-20°C) in comparison with batch reaction, usually requiring cryogenic temperatures (-70°C) (Table 4).

	MR			Batch		
alcohol	-20°C	0°C	20°C	-70°C	-30°C	-10°C
1	81%	75%	70%	88%	28%	2%
2	83%	82%	76%	88%	20%	0
3	96%	94%	89%	95%	32%	4%
4	97%	96%	86%	98%	45%	3%
5	99%	98%	18%	99%	60%	-

Table 4. Conversion to aldehyde or ketone in MR and batch conditions

2.3.1.5. Increased safety

The microreactor technology is an efficient tool for kilogram scale syntheses in continuous mode and is particularly effective for hazardous reactions that do not allow scale-up in conventional reactors.

In fact in process research, there are many reactions involving explosive or toxic reagents such diazo compounds, azides, etc. Often time and resources have to be devoted to find suitable experimental and engineering designs to safely scale-up these reactions or to design safer alternative syntheses. Since the actual reaction volumes in a microreactor are very small, the safety concerns are minimized (Figure 11).

Batch   **Microeactor**
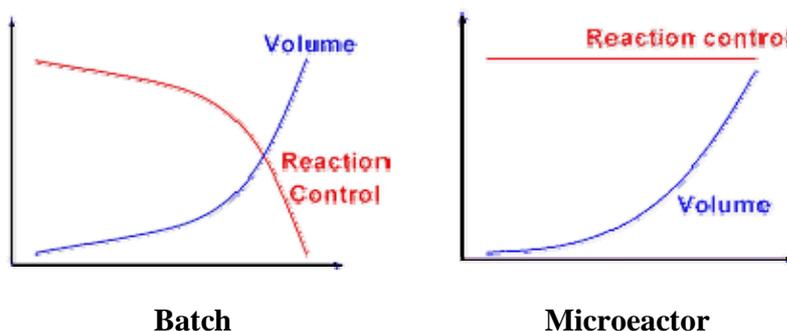
Figure 11. Dependence of the safety by the reaction volume

This has been proven in this example in which a ring-expansion reaction was studied into a microreactor system (Scheme 6).[27]
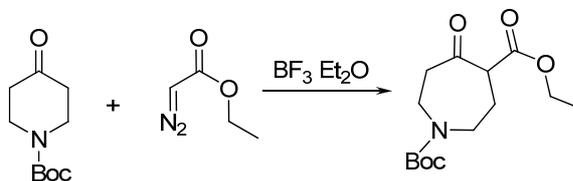
Scheme 6. Ring-expansion reaction

In batch the reaction occurred with a good yield (90%) at -25°C but it was limited to small scale because it is highly exothermic. In fact, after the addition of the diazo reagent the temperature raised at 45°C and the evolution of N_2 caused overpressure in the reactor. Because of these reasons, scaling up this reaction to kilogram scales safely in a conventional reactor is not recommended. When the reaction was performed under flow conditions, the ring expansion occurred with 89% yield, with a residence time of 1.8 min and with an output of 91 g/h.

2.3.2. Advantages related to the continuous nature of the process

To the continuous nature of the flow chemistry technique are associated some benefits that will be described below and in the next paragraph:

- Easy scale-up

- Possible use solid supported reagents or scavenger in-line
- Continuous sequential steps
- Reaction conditions independently varied during the experiment facilitating the optimisation process

2.3.2.1. Easy scale-up

Continuous process offers a quick and easy scale-up procedure. The synthetic conditions set up for few milligrams are easily used for grams or even kilograms by simply using the flow reactor for an extended time or by employing multichannel parallel reactors (numbering-up process), without variations in yields, purities and safety (Figure 12).
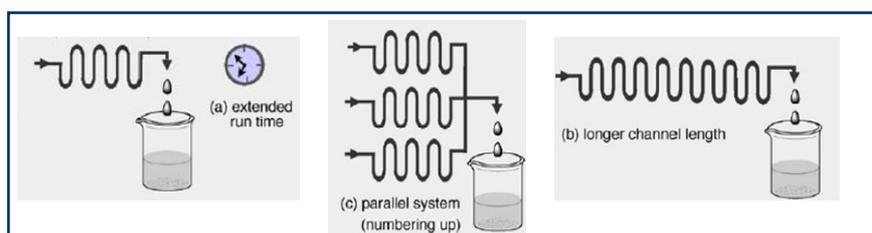
Figure 12. Scale-up in flow chemistry

On the contrary, traditional batch scale-up often requires modifications of laboratory synthetic protocols, optimization of reactor parameters, such as mixing and heat control.

Unsafe synthetic steps and reaction necessitating accurate temperature control are typical scaled-up processes improved by microreactor approach. Some interesting synthetic examples of successful scaling-up are summarized in Scheme 7.[28]
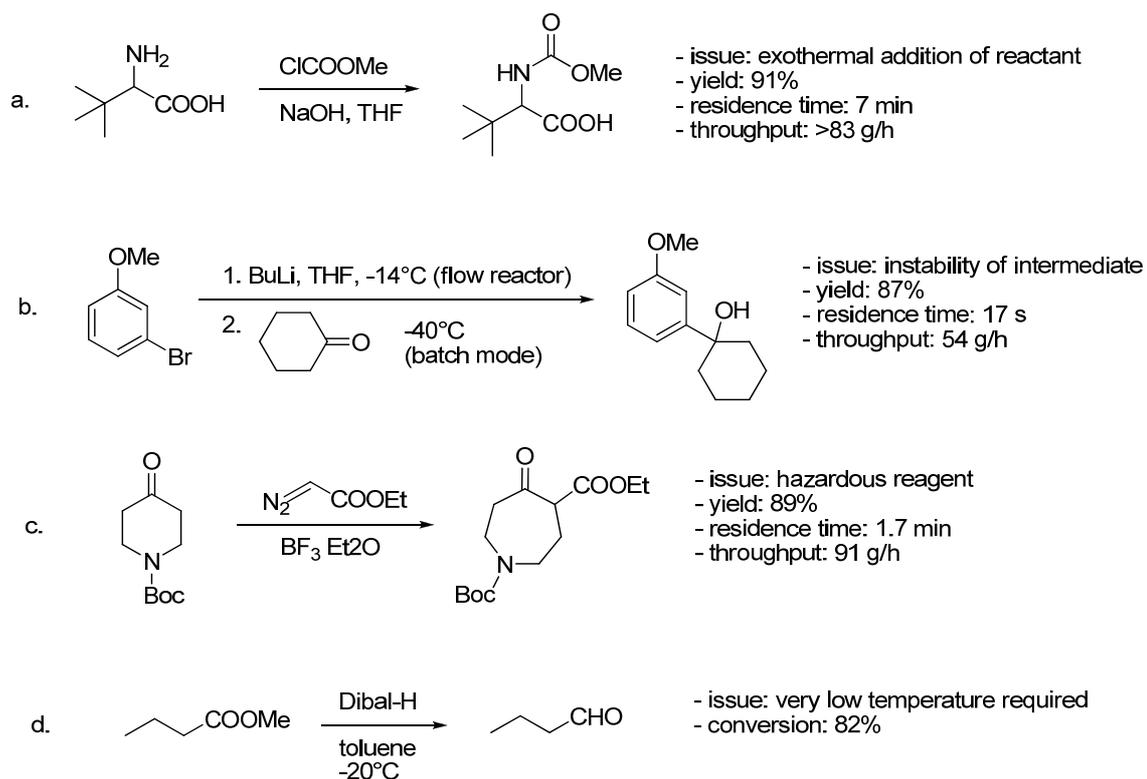
Scheme 7. Examples of successful scale-up

Continuous processing has been shown to be useful in the laboratory and pilot-plant scale-up of pharmaceuticals and fine chemicals. Many processes that cannot be scaled up using batch operations can be readily scaled up in the laboratory and pilot plant through continuous operations.[29] The benefits of continuous processing include greater process control, enhanced margins of safety, increased productivity, and improved yields.

An exemplificative comparison between pilot plant based on 50-litre batch vessel versus microreactor system is illustrated in Table 5.[19] Against higher initial investment, microreactor pilot plant saves scaling efforts, requires fewer operative personnel, increases moderately yields and reduces moderately the consumption of solvent. On the whole, economic benefits demonstrate the profitable applicability of flow chemistry to large industrial production.

	Batch vessel (50 L)	MR
Investment	96.6 k€	460.8 k€
Scale-up effort	10 man days	0 man days
Mean yield	90%	93%

Specific solvent consumption	10 l/kg	8.3 l/kg
Required personnel per facility	2 man	1 man
Production rate	427 kg/a	536 kg/a
Specific production cost	7227 €/a	2917 €/a

Table 5. Comparison between pilot plant versus microreactor system

2.3.2.2. Continuous sequential steps

Another possibility that flow chemistry could offer to organic synthesis is performing sequence of two or more reactions using flow reactors, without breaking the sequence with workup and purification. The sequence of reactions can occur directly through a series of connected reactors, which parameters are accurately set for the specific synthetic step. Flow technique permits the linking of individual reactions into multi-step sequences, allowing for one reaction to flow seamlessly into another and creating a rapid route to the desired more complex product by combining multiple synthetic steps into a continuous operation. Between each step may be present, when necessary, a system for trapping and eliminating possible by-products, without interrupting the flow.

Different examples of multi-step synthesis performed under flow conditions are discussed below.

The first example is the three-step continuous synthesis of a collection of oxadiazole derivatives (Scheme 8).[30] In batch mode the sequence requires long reaction times, mainly for the final cyclization step. The authors, thanks to application of superheating conditions and an accurate mixing of reactants, performed the entire process sequentially through the joint of three microreactors, shortening the reaction time to about 30 min and obtaining a sufficient amount of product for a full characterization and library supply.
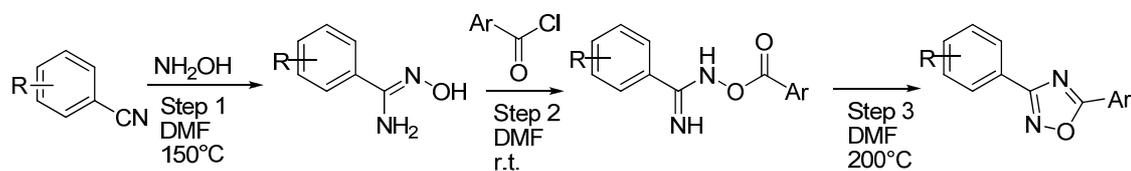
Scheme 8. 1,2,4-Oxadiazole synthesis

In the next recent example of Baxendale and co-workers the synthetic power of performing multistep reaction sequences under flow conditions is impressively demonstrated.[31] The palladium-catalysed acylation of terminal alkynes for the synthesis of yne-ones and their further transformation into various heterocycles is described. Particularly advantageous is the purification

of the final product performed in-line using a suite of packed glass tubes containing appropriate scavenger materials (Scheme 9).
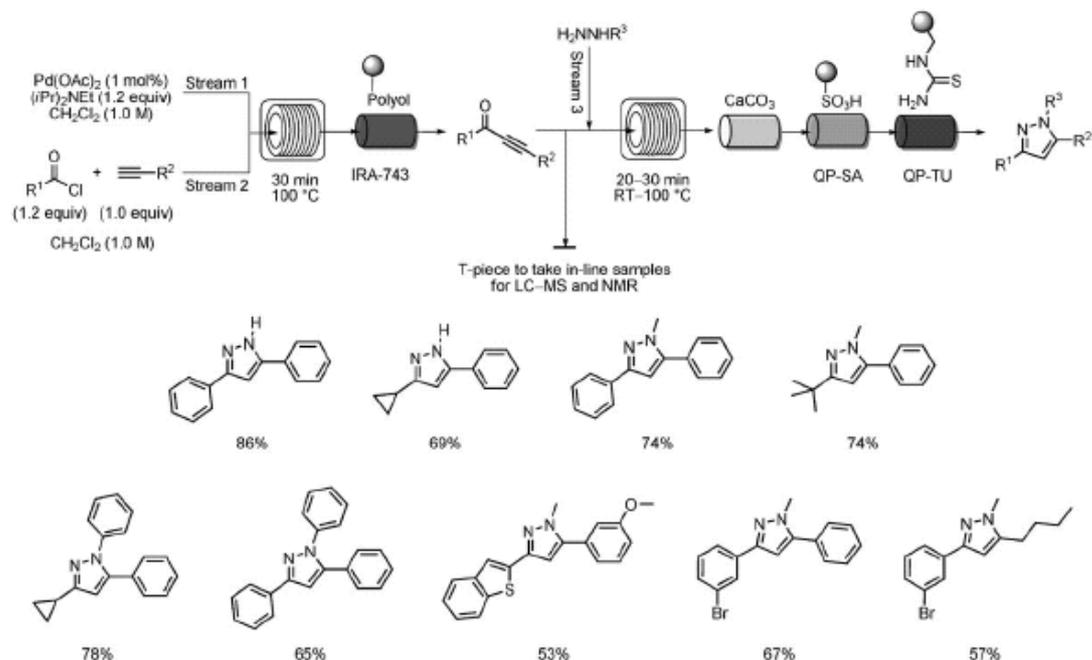
Scheme 9. Two-step formation of pyrazoles from yne-ones

Another example of the combination of multi-step synthesis and solid-supported scavengers and reactants in a flow system was reported by Ley for the preparation of the alkaloid natural product (±)-oxomaritidine (Scheme 10).[32] The whole seven-step synthesis was carried out through a microfluidic pumping system that pushed the solution of reactants into a series of pre-packed columns containing immobilized reagents, scavengers and catalysts. The expected product was obtained in less than a day with 40% yield and 90% purity, through the simple evaporation of the solvent without any isolation and purification of the intermediates. The synthesis of this product and other interesting examples of multi-steps pathway will be describe with more details in Chapter 3 and 4 of this thesis.
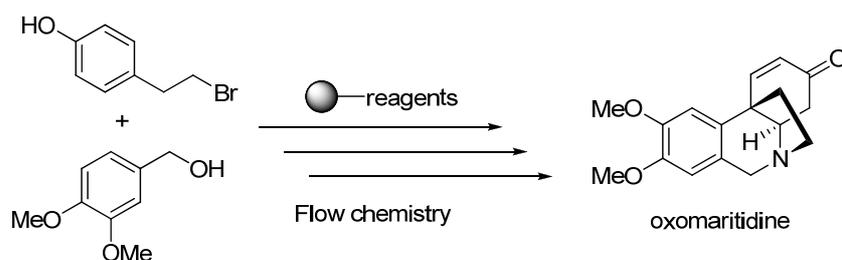
Scheme 10. Synthesis of (±)-Oxomaritidine

The possibility of using separator devices in continuous processes without interrupting the pumped flow opens the way to multi-step syntheses that require intermediate workup stages. An interesting application of this principle and tools was reported by Jensen.[33] This three-step synthesis of carbamate derivatives was performed by Curtius rearrangement starting from commercially available acyl chlorides (Scheme 11). Each step was carried out in a microreactor. A microfluidic separation system was integrated after the first and the second reactor, allowing the ease removal of inorganic salts through liquid/liquid extraction (Step 1) and of produced gas through gas/liquid separator (Step 2). Isocyanate intermediate was then pumped into three microreactors with three different alcohols affording simultaneously three carbamate products. The work demonstrated the simultaneous use of reactors and separator devices in flow mode, allowing the *in situ* generation and consumption of hazardous intermediates such as azides and isocyanates.

Scheme 11. Synthesis of carbamate derivatives by Curtius rearrangement

2.3.3. Multi-phase systems

2.3.3.1. Solid-liquid reactions

The structure of the flow devices, based on the small dimension of channels that improves mixing efficiency, fitted thoroughly to an extensive use in heterogeneous conditions. Solid-liquid, immiscible liquid-liquid, liquid-gas and solid-liquid-gas phase conditions are applications widely studied and exemplified through the flow chemistry.

The use of supported, anchored and encapsulated reagents, scavengers and catalysts perfectly suits with flow conditions, due to the intimate contact between the reactants present in solution and the pad of the stationary reactive phase. In this small space the reaction occurs and then the product is flowed away. In every time the relative amount of bound reactant is large, especially in the case of supported catalysts.

The main limitation is the total amount of supported reagents and scavengers that the system can tolerate, relating on its scale-up threshold. One example of two-phase solid-liquid system, reported

by Watts,[34] illustrates the protection of aldehydes and ketones with dithiol species. In batch-mode, Lewis or Brønsted acid catalyst and, usually, an excess of thiolating agent are necessary to promote the reaction, making the purification step essential. Also the use of supported catalyst does not avoid the need for excess of reactant because of the mechanical degradation of the support, which leads to reduced reagent lifetimes. In a flow system with Amberlyst-15 as acidic supported catalyst, the protection reaction may be performed in very short times (few minutes compared to at least one day in batch mode) using a stoichiometric amount of dithiol (Scheme 12).

R = H, Me
n = 1, 2
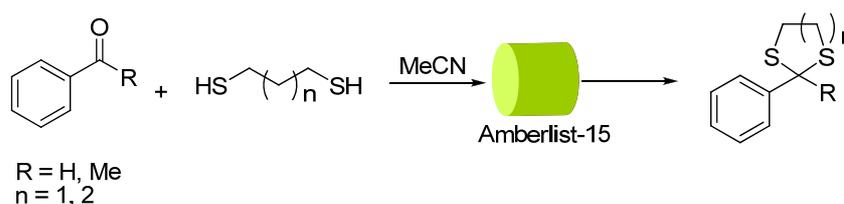
Scheme 12. Protection of aldehydes and ketones with dithiol species

The subsequent simple evaporation of solvent affords the expected thioacetals and thioketals in very high yields (> 99%) and purities.

Another impressive example is reported by Ley and co-workers in the synthesis of 1,4-disubstituted 1,2,3-triazoles in a modular flow reactor (Scheme 13).[35]
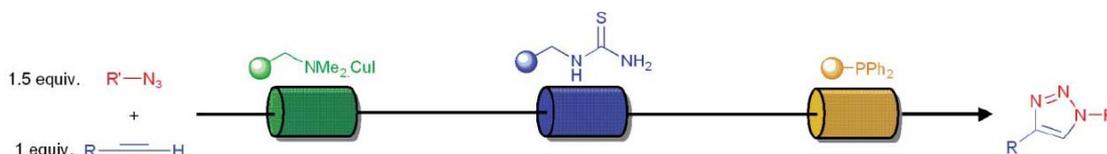
Scheme 13. Synthesis of 1,4-disubstituted 1,2,3-triazoles

The scavengers and the benefits associated to this protocol are described below:

- CuI immobilized on Amberlyst (PS-NMe$_2$: high loading and retention of basic functionality)
- PS-Thiourea used as metal-scavenging resin.
- PS-PPh$_2$ captured the azide excess onto the solid phase as an iminophosphorane.
- No handling work-up required.
- Exclusion of the oxygen from the system preventing Glaser homocoupling.
- Reduces exposure to potentially explosive and highly toxic chemicals.
- Yields 70-93%; purity after solvent evaporation >95%

Another recent application of this type of solid/liquid reaction approach is the use of nanoparticles as supports for catalyst which are rapidly gaining attention due to their promise to show activities as found for homogeneous catalysts but being readily recoverable due to their heterogeneous nature.[36] Among them, magnetic nanoparticles as supports for catalysts are especially attractive for flow processes, since the catalyst can be confined and at the same time agitated in a reactor by a rotating magnetic field, thus avoiding potential problems of clogging membranes or filters that are commonly employed as barriers for immobilized catalysts. This principle was successfully demonstrated in the asymmetric benzoylation of 1,2-diols using copper(II)-azabis(oxazoline) catalysts that had been covalently attached to carbon coated cobalt nanoparticles (Figure 13).[37] In five consecutive runs with a flow rate of 0.2 ml/min excellent yields and enantioselectivities had been achieved for the title reaction with minimal leaching (<1%) of the nanoparticle supported catalyst.
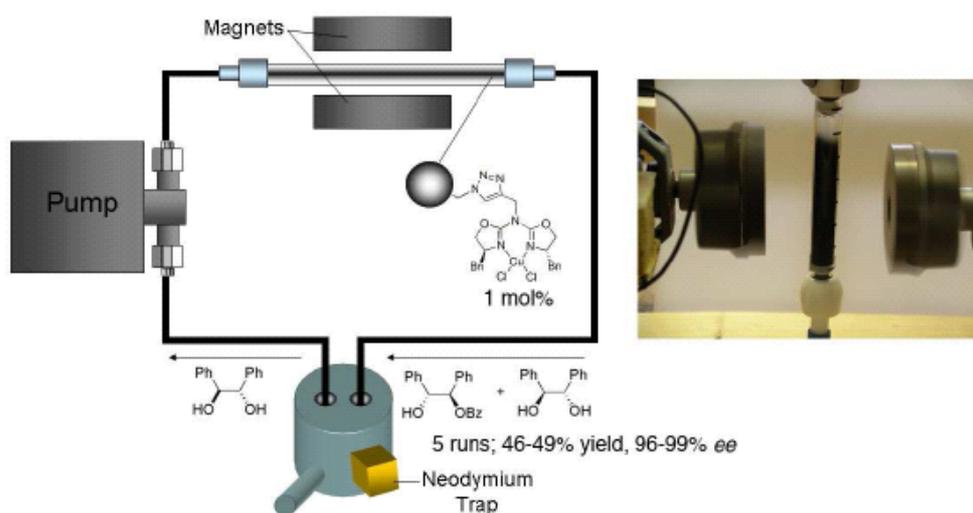
Figure 13.Kinetic resolution of (±)-1,2-diphenylethane-1,2-diol catalyzed by a Cu(II)-azabis(oxazoline) complex immobilized on magnetic nanoparticles

2.3.3.2. Liquid-liquid reactions

Biphasic system obtained by two immiscible liquids is another kind of multi-phase reactions that can be improved by flow apparatus. In the small channels of the flow system, thanks to a T-shaped geometry of the inlet junction, a segmented flow of the two liquids is formed (Figure 14).

Inside each segment, because of the interaction of the liquid with the channel wall, a fluid vortex is generated, causing rapid mixing and a continuous refreshing of the interface. The contact surface between the immiscible liquids is increased, promoting the reactions that occur at the interface.
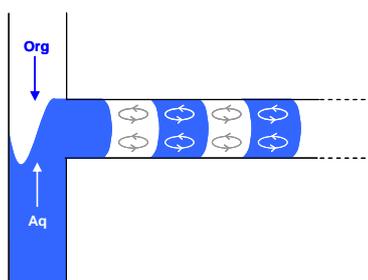
Figure 14. Segmented flow

One example of this use of the flow reactor was reported for the simple biphasic hydrolysis of 4-nitrophenyl acetate, dissolved in toluene, with an aqueous solution of NaOH.[38] Comparison of the results obtained in batch and in a microreactor is summarized in Table 6.

	Reaction time (min)	Yield	Note
Batch	10	53%	
Batch	2	8%	
MR	2	51%	
MR	2	67%	0.1 eq Bu_4NHSO_4
MR	2	82%	sonication
MR	2	88%	0.1 eq Bu_4NHSO_4 and sonication

Table 6. Biphasic hydrolysis of 4-Nitrophenylacetate

Segmented flow was able to afford the same yield (50%) as batch but in shorter time. Moreover, with the same residence time, the yield was further increased by the combination of segmented flow, phase-transfer catalysis (Bu_4NHSO_4) and sonication.

The immersion of the reactor in an ultrasonic bath led to the formation of irregular sized segments that increased the interfacial area thus improving the reaction rate (Figure 15).
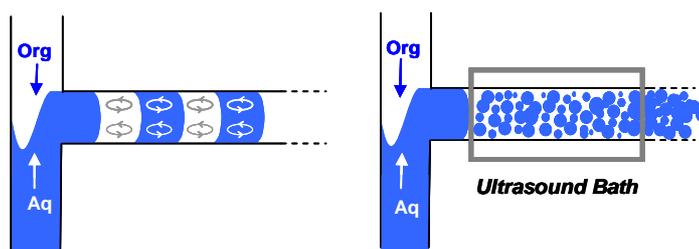
Figure 15. Segmented flow and flow under sonication

Liquid/liquid biphasic segmented flow, which is an excellent solution for the phase-transfer reactions, was used for setting-up Wittig reactions[39] and alkylations of phenols.

Aromatic and heteroaromatic aldehydes were combined with (ethoxycarbonylmethyl) triphenyl phosphonium bromide, in presence of bases, affording the desired substituted cinnamic esters in high yields (Scheme 13). Compared to the classical batch phase-transfer conditions, the reactions required shorter reaction times and were performed without any phase-transfer catalyst. The beneficial effect of the catalyst was replaced by the immersion of the reactor into an ultrasonic bath with consequent increase of the interfacial area between the immiscible solvents. In these conditions, few minutes residence times are enough to reach the complete conversion.
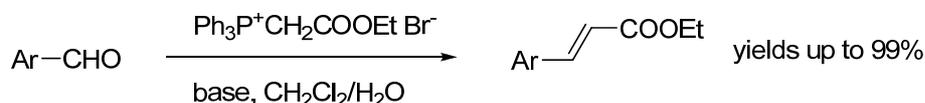
Scheme 14. Wittig reaction

The alkylation of aromatic phenols with benzyl bromide and iodomethane is another successful combination between phase-transfer catalysis and flow systems (Scheme 15). With a catalytic amount of tetrabutylammonium bromide and using sodium hydroxide as base, complete conversions and high yields were obtained just in few minutes, compared to hours required when traditional batch conditions were applied.
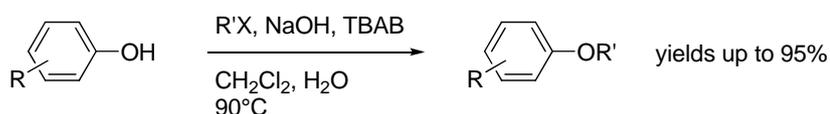
Scheme 15. Phenol alkylation

2.3.3.3. Gas-liquid reactions

Flow chemistry has also showed its potentiality in gas-liquid biphasic reactions. By an accurate regulation of gas pressure and liquid flow, an annular flow regime could be obtained: the liquid is forced to form a thin film against the surface walls, while the gas flows through the centre, generating a very high interfacial area.

The high contact surface, the punctual control of temperature and the small scale typical of the flow reactors allow a safer use of toxic and dangerous gases, such as carbon monoxide, hydrogen, fluorine and chlorine, which often require a sophisticated apparatuses in batch mode.

Carbon monoxide, for example, was used for the first time in a flow device for the formation of amides through carbonylative coupling (Scheme 16). The liquid solution, consisting of a mixture of arylhalide, palladium-phosphine catalyst and benzylamine, was flow into the microreactor while the gas pressure produced an annular flow pattern. With very short residence times (less than 2 min) the obtained yields were comparable and often better than in the classical batch reaction.[40]
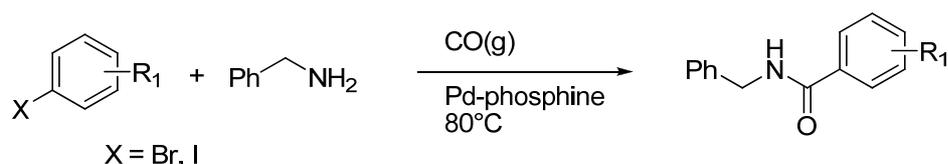
Scheme 16. Formation of amides through carbonylative coupling

Elemental fluorine gas was employed in flow devices for the formation of carbon-fluorine bond. The process is highly exothermic, but thanks to the effective dissipation of the heat and the efficient gas-liquid mixing of flow reactors, direct fluorination may be performed in good yields and high selectivity. Chambers,[41] for example, reported selective mono-fluorination of dicarbonyl compounds and aromatic rings (Scheme 17).
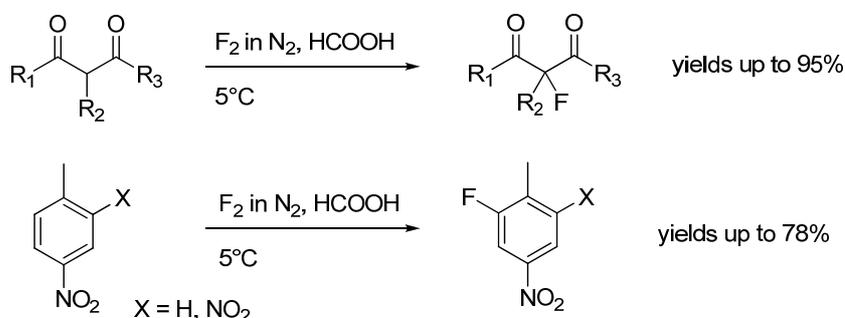
Scheme 17. Selective mono-fluorinations of dicarbonyl compounds and aromatic rings

Fluorine gas was introduced into the system as a 10% mixture with nitrogen, while the reactant was dissolved in formic acid. The process was set up for laboratory scale, but, by simple increasing the number of the channels, the system may be appropriate for synthesis on large scale.

2.3.3.4. Gas-liquid-solid reactions

Further step of multi-phase flow reactions is the combination of solid, liquid and gas phases. An example of instrument dedicated to hydrogenation reactions that reaches this goal is H-Cube^{TM} (ThalesNano) in which a solution of substrate is mixed with gaseous hydrogen, through a pre-packed cartridge containing the heterogeneous catalyst. In the short cartridge path, also thanks to the wide range of pressure and temperature (up to 100 bar and 100°C), the reduction of the substrate is quickly performed and then the product is flowed away and collected.

The instrument is equipped with an electrolytic cell that produces hydrogen directly from water. In this way, gas cylinders or other sources of hydrogen are not necessary, making the system safer than classical batch apparatus. In addition, no catalyst filtration or direct catalyst handling are further advantageous points in term of safety. Many benefits are associated to this tool and are briefly summarized below:

- More efficient and safer process (reduced exposure to the catalyst and the risks involved with pyrophoric catalyst, no filtration required at the end of the experiment).
- The catalyst can be reused for different substrates.
- High catalyst to substrate-hydrogen ratio at each time.
- Good mixing of the three phases.
- Continuous flow process and short residence time allow rapidly reaction optimization on novel substrates.
- T, P and flow may be modified 'on fly'.
- Catalyst cartridges may be quickly exchanged to determine the effect of different catalyst.

Some published papers report the use of H-Cube^{TM} as good and reliable replacement of traditional hydrogenation methods.[42]

An example is the catalytic reduction of imines to amines described by Ladlow and co-workers.[43]

The reduction of imines was assessed using a 10% Pd/C cartridge under 20 bar H_2 pressure (Scheme 18).
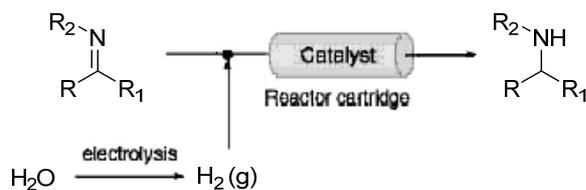
Scheme 18. Reduction of imines to amines

To study the selective reduction of imine in presence of reducible functions as i.e. CN, Bn and pyridine, 8 different substrates were introduced sequentially followed by a short solvent wash to purge the catalyst cartridge. In every case the chemoselective reduction to the desired amine was obtained (yields >93%, purity 84-98%).

2.3.4. Combined technologies

Very good results can often be obtained with the combination of the advantages of two or more of the enabling techniques, thus further improving and enhancing the efficacy of each approach. For example, synthesis of an array of biphenyl derivatives by Suzuki coupling with supported catalyst under microwave irradiation in flow mode has been recently described (Figure 17).[44] The supported catalyst showed enhanced reactivity when used with microwave heating, while poorer results were obtained with conventional heating. Moreover, the use of a flow reactor ensures higher purities than the traditional batch mode, thanks to the very short reaction times in which the local effective catalyst concentration is very high. The proposed protocol and system proved to be very versatile: reactions can be performed in automated sequences for the synthesis of compound arrays or, alternatively, large-scale production can be achieved by running the system for many hours.
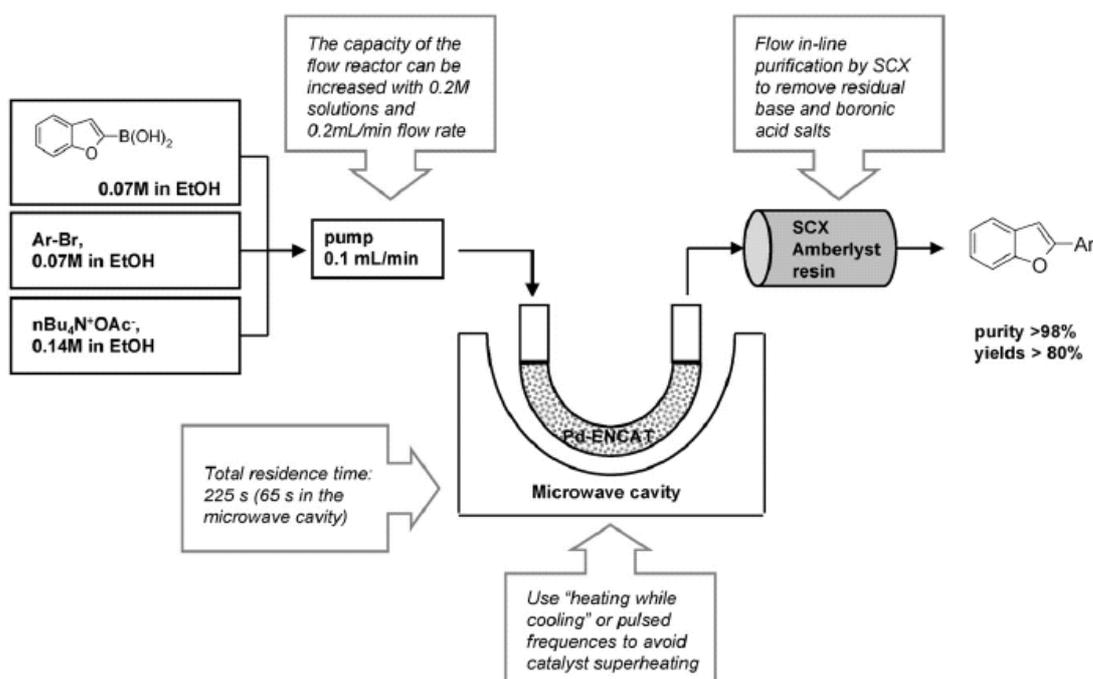
Figure 17. Example of combined technology: PASPS, MAOS and flow chemistry

2.3.5. Conclusion

In the light of all these examples, we can assess that flow chemistry system is not only a mere laboratory or academic new fashion.

The advantages and the drawbacks associated to this technique are summarized and below:

Advantages:

1) Small dimension of the channels

- Precise control of reaction conditions
- Efficient mass and heat transfer
- Superheating
- Safer and more selective processes
- Toxic and explosive intermediates are prepared *in situ* and directly reacted
- Easy management of poorly stable intermediates
- Decreased inputs and waste

2) Continuous nature of the process

- Possible use solid supported reagents or scavengers in-line
- The reaction conditions can be independently varied during the experiment facilitating the reaction optimisation process

36

- Easy scale-up
- Continuous sequential steps

Drawbacks:

- Mainly useful for relatively fast reaction
- Incompatible with solid reagents
- Very sensitive to precipitating product

Chapter 3.
Flow chemistry in Medicinal Chemistry: state of the art

3.1. Introduction

Modern drug discovery tends to be an iterative process which usually begins with a target orientated design phase and then proceeds to the synthesis of a small focused compound collection followed by biological evaluation.[45] The results and observations of these initial assays generate primary information concerning potency, selectivity, pharmacokinetic properties and other parameters which are reincorporated into the design loop to further continue the optimisation process (Figure 18).
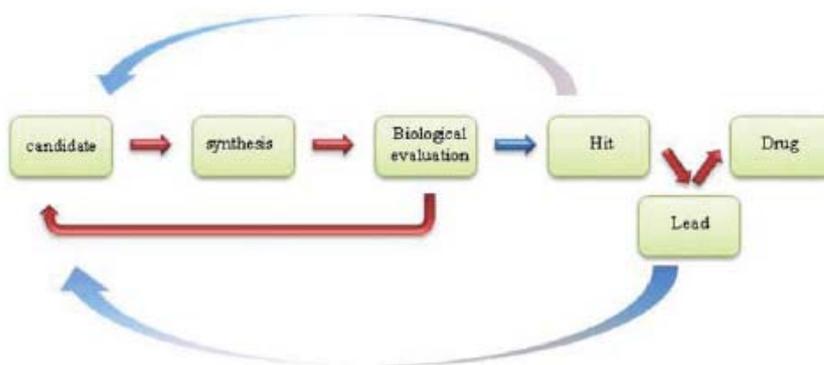
Figure 18. The iterative cyclic drug design

As a result improving any step of the sequence immediately reduces the overall development time in this closed loop approach to drug discovery.[46] While many high-throughput screening methods, computational modeling techniques and predictive software packages exist and have greatly aided medicinal discovery, the synthesis component can still sometimes constitute a significant bottleneck. Consequently, new thinking and alternative approaches for molecular assembly are required.

Of these new enabling technologies continuous processing using chemical microreactors represents an attractive potential solution for medicinal chemists because of the rapid early-stage reaction optimization and scale-up.[47]

In literature a large number of different types of reaction have been already studied showing how flow chemistry can be successfully applied to the organic synthesis (Figure 19).

Carbon-Carbon Bond-Forming	Fluorinations
Aldol condensation	
Claisen Rearr	**Reactions with Diazo Reagents**
Suzuki coupling	
Cyclopropanation	**Radical**
Michael addition of nitromethane	*Hydrosilylation*
Oxidations and Reductions	**Polymerizations**
Moffat-Swern	
	Photochemical
Heterocycle Formations	
	Precipitate-Forming
Carbon-Nitrogen Bond-Forming	
Carbamates	**Aromatic Electrophile Substitution**
Amines	
Peptides	**Aminocarbonylation**
Curtius	
Amide by AlMe$_3$	**Reactions Using Organocatalysts**
Carbon-Oxygen Bond-Forming	**Enzymatic**
Ring opening of terminal epoxides	*Cyanohydrins*
Sulfur-Nitrogen Bond-Forming	

Figure 19. State of the art of single-step continuous-flow reactions.

However the potentiality of this technique is not yet broadly exploited in Medicinal Chemistry;[48] just in recent years flow processes started to be applied in this field.[49]

3.1.1. Application of flow chemistry towards the synthesis of drugs

Flow processes are rapidly finding applications towards the synthesis of biologically active compounds and drugs, taking advantage of shorter reaction times, higher yields, and facile scale-up of a single target compound, but also of more efficient generation of compound libraries by introducing families of building blocks sequentially during a given reaction sequence.

Rimonabant and Efaproxiral (Figure 20), two pharmaceutically active substances, were prepared through a safe, functional-group-tolerant and high-throughput version of the trimethylaluminium mediated amide bond formation developed by Seeberger in a microreactor system.[50]
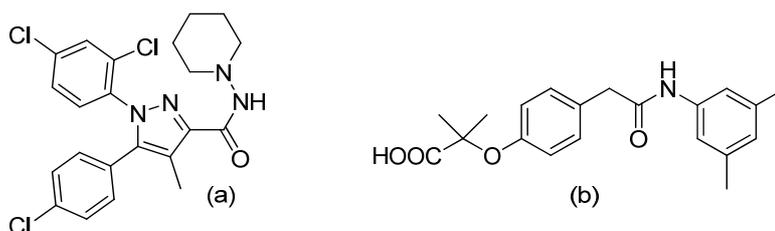
Figure 20. Rimonabant (a) and Efaproxiral (b)

The use of a continuous flow microreactor for β-amino alcohol formation by epoxide aminolysis was developed in the work of Jamison.[51] In particular the aminolysis of epoxides under flow conditions was applied to the synthesis of Metoprolol and Indacaterol™ (selective β$_1$-adrenoreceptor blocking agents). The later compound was produced in a microreactor at a residence time that was 1/60th of that reported in the literature with similar yield (Figure 21).
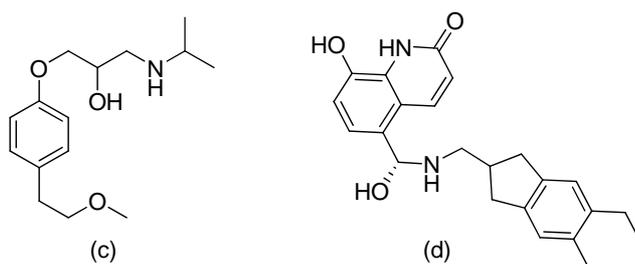
Figure 21. Metoprolol (c) and of Indacaterol™ (d)

Diazoketone is a versatile pharmaceutical intermediate of *N*-Boc-(1*R*,2*S*)-benzylhydroxy-3-chloropropylamine, a precursor to pharmaceutically active ingredients that act as a human immunodeficiency virus (HIV) protease inhibitors (Figure 22).
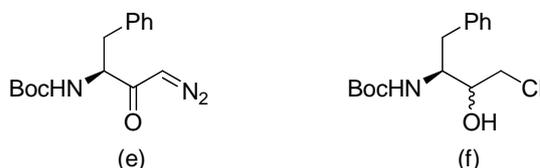
Figure 22. Diazoketone (e) and N-Boc-(1R,2S)-benzylhydroxy-3-chloropropylamine (f)

A continuous flow system, giving the compound in higher yields at more readily attainable temperatures than the analogous batch process was successfully design, build, and implemented. Specifically, quantitative in situ yields were obtained for the transformation of *N*-Boc-(S)-phenylalanine to the correspondent mixed anhydride and its subsequent reaction with hazardous trimethylsilyldiazomethane to the 1-benzyl-3-diazo-2-oxopropylcarbamic acid *tert*-butyl ester (e).[52] The continuous flow process that was designed takes advantage of the superior heat transfer capabilities of packed tubular flow reactors and their inherent increased safety. It allowed to efficiently and safely carrying out a two step synthetic sequence that involves temperature sensitive intermediates and hazardous chemical reactant.

The first continuous flow synthesis of imidazo[1,2-a]pyridine-2-carboxylic acids directly from 2-aminopyridines and bromopyruvic acid was developed, representing a significant advance over the corresponding in-flask method.[53] The process was applied to the multistep synthesis of a Mur ligase inhibitor with potential antibacterial activity, using a two microreactor, multistep continuous flow process without isolation of intermediates (Scheme 19). The in-flask synthesis of this compound was reported to proceed in 16.4% overall yield over two steps. Using the continuous flow method, the Mur ligase inhibitor was prepared in a single process with 46% yield.
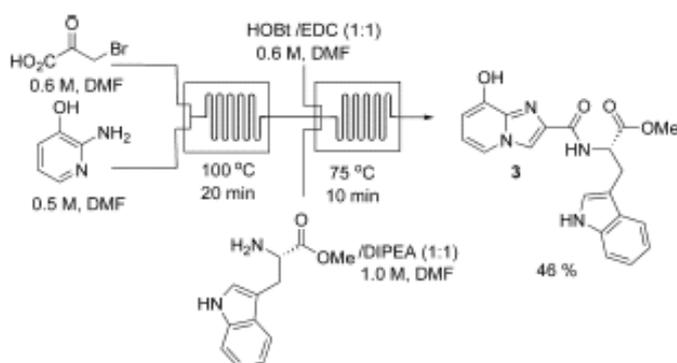
Scheme 19. Continuous flow preparation of Mur ligase inhibitor

Recently a concise, flow-based synthesis of Imatinib (Gleevec; Figure 23), a compound used for the treatment of chronic myeloid leukemia, was described.[54]
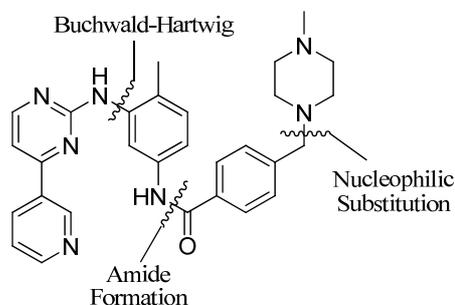
Figure 23. Imatinib and planned disconnections for its flow synthesis

All the steps are conducted in tubular flow coils or cartridges packed with reagents or scavengers to effect clean product formation (Scheme 20). An in-line solvent switching procedure was developed enabling the procedure to be performed with limited manual handling of the intermediates.
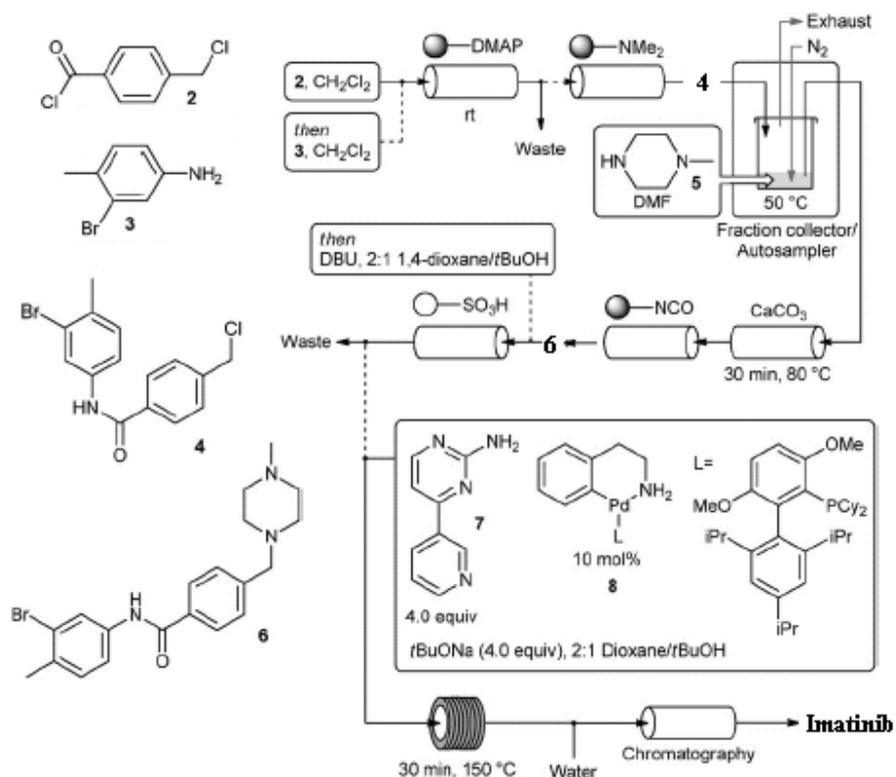
Scheme 20. Flow synthesis of Imatinib

This represents a significant improvement over the existing protocols that require manual intervention. The synthetic route has also the potential to be used for the synthesis of analogue compounds and clearly demonstrates the role of flow chemistry techniques in the assembly of challenging molecules.

3.1.2. Flow chemistry applied to the synthesis of Med-Chem libraries

Flow chemistry may be also applied to the preparation of collections of compounds, using the basic concepts of the parallel/combinatorial chemistry.

In fact multistep continuous-flow systems appear to be particularly suitable for this branch of the medicinal chemistry, where a large number of analogues can be synthesized by the same reaction sequence consisting of a few relatively simple steps, like amide couplings, reductive amination, catalytic hydrogenations, and metal-catalyzed coupling reactions. In particular this approach can be very helpful during the Hit Validation and Lead Identification phases.

The most logical and simple strategy for the preparation of a library of derivatives is the parallel approach, where the number of reaction channels is the same as the number of final products and

starting materials are distributed to all the reaction channels in different combinations.[55] The main limitation of this synthetic strategy is the high number of reactors and the complicated piping system that are necessary for the combination of a large number of starting materials.

An alternative and more favorable approach is the sequential plug flow synthesis. In this case the solutions of reactants are introduced in sequential way into the same reactor. The system offers high flexibility because the reaction conditions (temperature, reaction time, scale, reagent ratio) can be controlled independently and individually for each reaction sequence. All starting materials enter into the system in pulse sequence and their elution profile from the reactor is analyzed by measuring UV absorption, allowing the correct and independent collection of the products. In any case each reaction plug is separated from another plug by a spacer solvent that serves also as washing step, avoiding cross-contaminations.

One of the first examples of this type, even if it was not part of a discovery program, was a fully automated flow-through process for the production of secondary sulfonamides presented by Ladlow and co-workers.[56] Primary sulfonamides were monoalkylated using a two-step "catch and release" protocol to generate library products of high purity. The automated flow synthesis platform incorporates four independent reactor columns and is able to perform automated column regeneration. A 48-member sulfonamide library was prepared as two 24-member sub-libraries, affording library compounds in good yields and high purities without the need for further column chromatographic (Scheme 21).
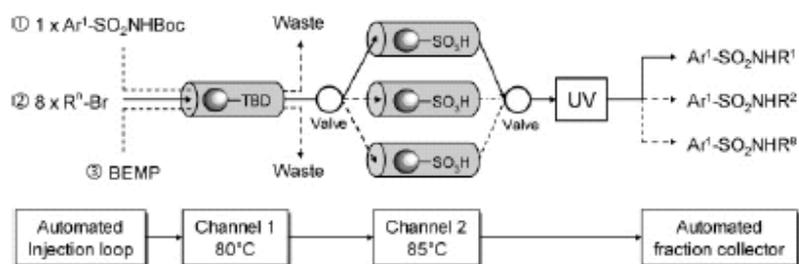
Scheme 21. Automated flow-through synthesizer configuration for sulfonamide library preparation

A general protocol for the preparation of inhibitors of casein kinase I using flow-assisted chemical processing techniques was developed and a small collection of imidazopyridazine (Scheme 22) was synthesized using a four step reaction sequence.
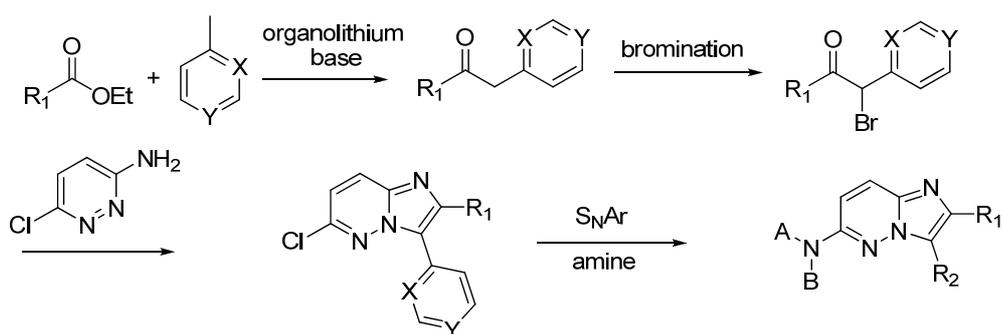
Scheme 22. General route to the imidazo[1,2]pyridazine core

The multi-step synthesis involved a safely scaling up reaction utilizing organometallic bases at low temperature, a monobromination facilitated by application of a solid supported reagent, a cyclocondensation reaction to generate the imidazopyridazine core (Scheme 23). Finally an autosampler was used to perform automated S_NAr reactions to give a collection of diversified imidazopyridazine derivatives.
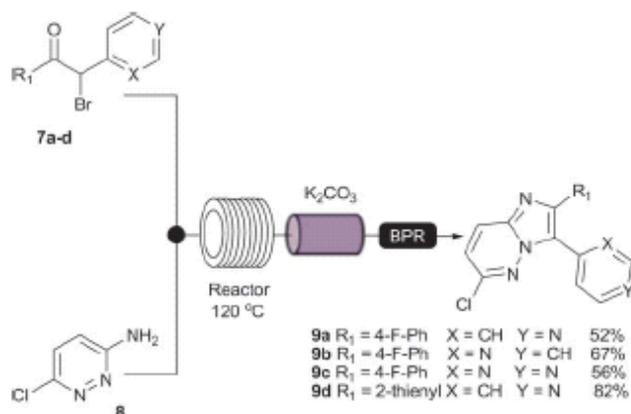
Scheme 23. Flow synthesis of the imidazopyridazine cores

The application of the above strategy was also described by Schwalbe[57] for the sequential synthesis of a library of Ciprofloxacin analogues. The five-step synthetic pathway is reported in Scheme 24. Step 2 (addition/elimination) and Step 4 (aromatic nucleophilic substitution) are the points used by the authors to introduce diversity in the compound collection. The amount of each member of the library was at least 100 mg and purity was satisfactory for drug discovery process. The yields were in 59-99% range and the potential cross-contamination was assessed and excluded, demonstrating the reliability of the sequential synthetic method.
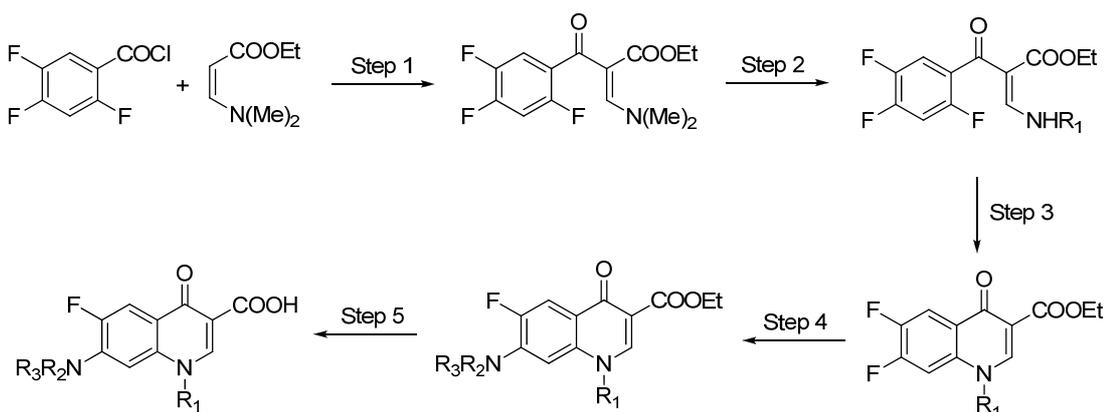
Scheme 24. Sequential synthesis of a library of Ciprofloxacin analogues

An interesting work in this field is the preparation of a library of 1,4-disubstituted 1,2,3-triazoles synthesized using a copper flow reactor.[58] Organic azides, generated *in situ* from alkyl halides and sodium azide, were reacted with acetylenes using the copper-catalyzed Huisgen 1,3-dipolar cycloaddition (Scheme 25). This process eliminates both the handling of organic azides and the need for additional copper catalyst and permits the facile preparation of numerous triazoles in a continuous flow process. Importantly, reaction segments were separated by an immiscible fluorous spacer, perfluoromethyldecalin, that prevented segment diffusion and reaction trailing. Using this approach, multiple segments could flow through the reactor at any one time, maximizing the system's efficiency and permitting rapid library development (medicinal chemistry) or reaction optimization (process research).
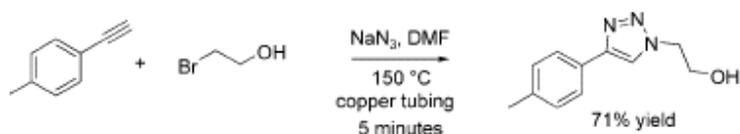
Scheme 25.One-pot click reaction using 4-ethynyltoluene and 2-bromoethanol

In the last example the construction of a continuous-flow sequence designed for rapid synthesis of new ligands in medicinal chemistry by combination of three building blocks was described.[59] The potentiality of the technique was demonstrated by the synthesis of a library of diverse drug-like test compounds. Targeting the chemokine receptor CCR8, which is of interest for treatment of various inflammatory and allergic conditions, a system for on-demand production of compounds with pharmacophores corresponding to known CCR8 ligands was constructed. The sequence consisted of three reaction sequence (two scavengers in between), where a mono Cbz-protected diamine is reacted with an isocyanate, deprotected (H-cube), and reacted further with an alkylating agent (Scheme 26).
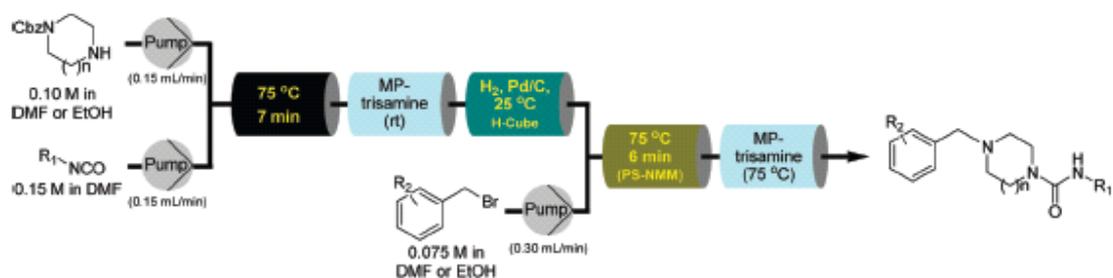
Scheme 26. Three-step continuous-flow for synthesis of receptor ligands

Once the three-step flow sequence was assembled and optimized, it proved to be a powerful and robust tool for rapid production of diverse compounds. Sequential production with a minimum of effort compared to batch or parallel synthesis contributes to minimizing the turnaround time of the critical design-synthesis-screening cycle.

In the next two chapters our works toward the application of flow chemistry to Medicinal Chemistry field will be described. An important aim of these projects was to demonstrate that flow synthesis can be a very effective and flexible tool for rapid synthesis of compounds and that the advantages associated to this technique can be useful in the drug discovery and optimization process.

Chapter 4.
Efficient continuous flow synthesis of hydroxamic acids and SAHA

4.1. Flow synthesis of a collection of hydroxamic acids[60]

In the framework of my PhD thesis exploring the application of the so called "*Enabling Techniques*" in a medicinal chemistry laboratory, my efforts were devoted to the evaluation of the benefits that continuous flow chemistry could provide in Drug Discovery programs and in the synthesis of natural products in comparison with traditional synthetic techniques.

Here we report a general and efficient procedure for the conversion of esters into the corresponding hydroxamic acids with good yields and purities using a commercially available continuous flow reactor. Hydroxamic acids occur in several molecules with a wide spectrum of biological activities[61] such as antibacterial, antifungal, antiinflammatory, antiasthmatic, and anticancer properties. In particular this moiety is present in potent matrix metalloproteinase[62] and hystone deacetylase[63] inhibitors because hydroxamic acids are strong bidentate metal-ion-chelating agents that interact with zinc(II)-containing proteins. Given the importance of this functionality, the development of new methodologies for a general and efficient synthesis are still of great interest, although several methods have been developed and published so far.[64] As a part of a medicinal chemistry project, a simple conversion of ester into hydroxamic acid was envisaged as a suitable and convenient synthetic method for the preparation of a collection of compounds featuring this particular moiety (Figure 24).
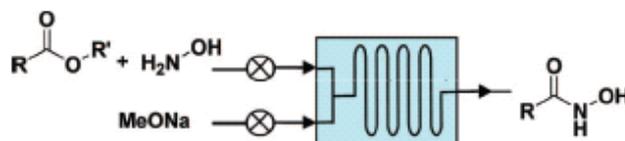
Figure 24. Flow synthesis of hydroxamic acids

Firstly the use of methylbenzoate (**1a**) to optimize the parameters (flow rate, residence time, and temperature) was investigated. A mixture of **1a** (0.5 M in MeOH) and hydroxylamine (1:10 ratio) was simultaneously pumped into the flow reactor with a solution of MeONa (0.5 M in MeOH) of MeONa, allowing efficient mixing in the PTFE tubing at the selected temperature (Scheme 27). The optimization of the experimental parameters was systematically investigated by varying flow rate, temperature, and reactor volume. The reaction set-up was greatly facilitated by continuous flow conditions and a significant number of runs were rapidly conducted in a sequential manner (Table 7).

Entry	Flow Rate (ml/min)	Residence time (min)	T (°C)	Conversion (%)[a]
1	1	5	50	52
2	1	5	70	65
3	1	5	80	58[b]
4	0.5	20	60	76
5	0.5	20	70	74
6	0.5	30	70	80

[a] Conversion was determined by LC/MS at 215 nM
[b] Presence of the corresponding carboxylic acid as by-product
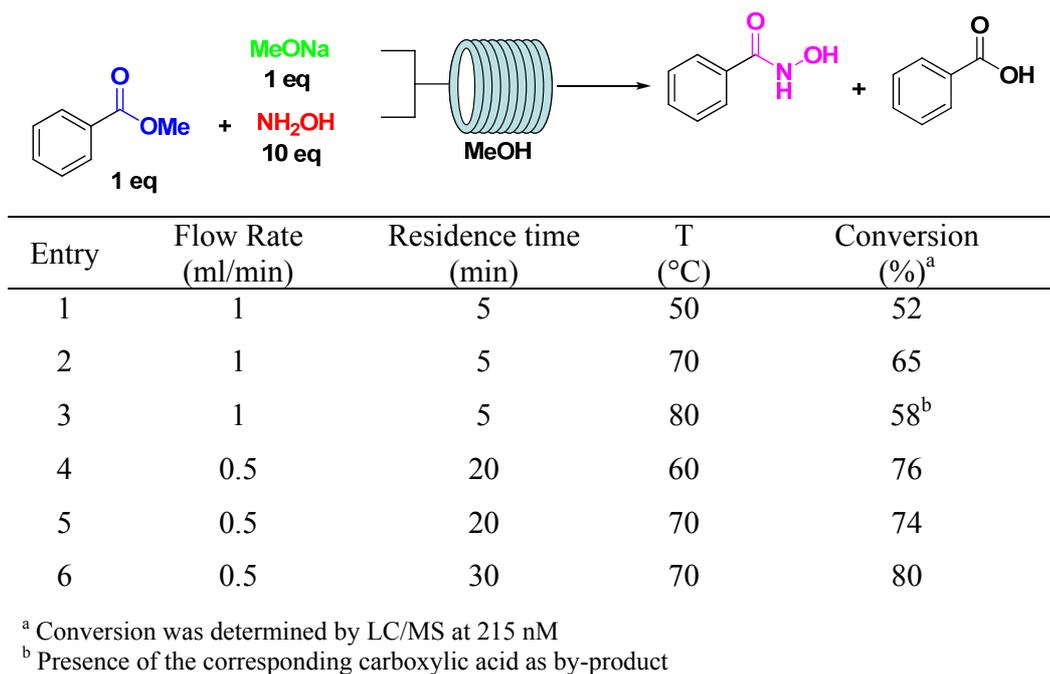
Scheme 27.; Table 7. Synthesis of hydroxamic acid and pptimization of experimental parameters

The temperature of 70°C resulted in the best compromise to have good conversion without the formation of the corresponding carboxylic acid. This by-product was significantly present when the reaction was performed at 80°C. The conversion into the hydroxamic acid was improved by prolonging the residence time by lowering the flow rate and increasing the volume of the reactor. When the reaction was performed at 70°C for 30 min, 80% conversion was achieved by LC/MS, resulting in 82% yield of isolated **2a** (entry 6). For comparative purposes the reaction was run both as a standard batch process and in a sealed tube under microwave irradiation, applying the same conditions of temperature, concentration of reagents, and reaction time as optimized in the flow protocol (Table 8).

Entry	Experimental Condition	Conversion (%)[a]
1	Batch reaction	58
2	Sealed tube with MW irradiation	72
3	Flow system	80

[a] Conversion was determined by LC/MS at 215 nM

Table 8. Comparison of reaction performed under different experimental condition

We found that such conditions gave moderate conversion using conventional equipment (58%, entry 1) and comparable result under microwave irradiation (72%, entry 2). The precise control of temperature distribution and the efficient heating and mixing associated with flow technique minimized the formation of the corresponding carboxylic acid as a by-product and ensured that

this simple chemical transformation proceeded at faster rates compared to the batch system. To verify the effectiveness and reproducibility of the optimized reaction conditions, the synthesis of a small collection of hydroxamic acids was undertaken. The employed flow protocol was based upon the optimized conditions described for the synthesis of **2a** without further optimization (i.e., 70°C, flow rate 0.5 mL/min, residence time 30 min) in a 250 mg scale. All of the desired hydroxamic products were obtained in good to excellent yields (Table 9) with no detection of the corresponding carboxylic acid byproduct.

Entry	Ester	Hydroxamic acid	Yield (%)[a]
1	**1a**	**2a**	82
2	**1b**	**2b**	96
3	**1c**	**2c**	96
4	**1d**	**2d**	95
5	**1e**	**2e**	97
6	**1f**	**2f**	52
7	**1g**	**2g**	100
8	**1h**	**2h**	81
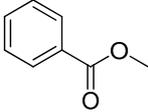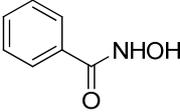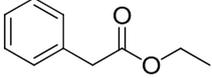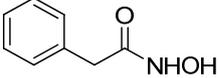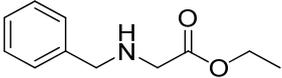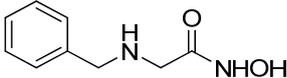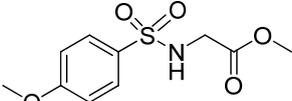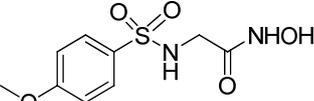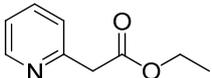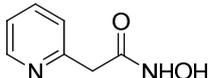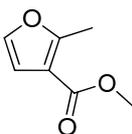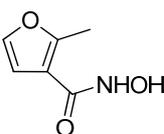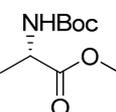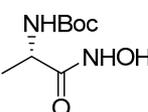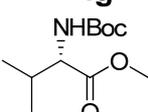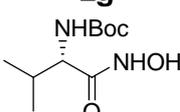
Entry	Ester	Hydroxamic acid	Yield (%)[a]
9	**1j**	**2j**	83

[a] Isolated yields; purity > 95% (LC/MS)

Table 9. Conversion of esters into hydroxamic acids under flow conditions

The data reported in Table 9 clearly show that the formation of hydroxamic acids from esters is quite general and occurs smoothly in the continuous flow system. In fact simple aryl or alkylaryl esters (entries 1 and 2), simple amino esters (entry 3) and Boc-protected amino esters (entries 7-9), sulfonamido-ester (entry 4), and heteroaryl esters (entries 5 and 6) were suitable substrates for the reaction. Moreover the optimized reaction conditions were successfully applied to enantiomerically pure esters without loss of stereochemical integrity. The specific optical rotation for the hydroxamic acid of *N*-Boc-alanine methyl ester **2g** was found to be identical to that reported in literature for the enantiopure compound $\{[\alpha]^{20}_D$ -28; Lit $[\alpha]^{20}_D$ -29 (c 1, MeOH)$\}$.[62a]

With compounds **1b**, **1d**, and **1j** the obtained results show an improvement if compared to the microwave-assisted process described in the literature.[62a] Noticeably for compound **1d** the isolated yield was increased from 33% to 95%; in the other cases an average improvement of about 20% was observed. Simple scale-up makes the continuous flow technique very advantageous when compared to MW systems, as up to now MW heating has proved to be unsuitable for large scale processes. Moreover the risks associated with failing to scaleup a process are limited using continuous flow. The reaction condition setup on the microreactor can be directly transferred to production on larger scale without the need for reoptimization by simply using the flow reactor for an extended time or by employing multichannel parallel reactors (numbering-up process).[65]

A demonstration of this preparative capability was readily obtained. Applying the optimized conditions and doubling the concentration of the starting materials, 4.3 g of *N*-hydroxy-2-phenylacetamide **2b** was straightforwardly produced after 1.5 h (output 2.9 g/h). Yield and purity were similar to that of the smaller scale, proving that the reaction conditions identified for the production of a few milligrams can be transferred to a larger scale without any further optimization. In addition to the easy scalability of the protocol, the careful control of temperature exotherms and the small volumes employed by flow reactor allow the safer use of the highly toxic and potentially explosive hydroxylamine.[66]

4.2. Flow synthesis of SAHA[60]

On the basis of the good results obtained in the development of the continuous flow synthesis of hydroxamic acids, this new methodology was applied to the synthesis of suberoylanilide hydroxamic acid (SAHA, Scheme 27).

This compound, one of the early histone deacetylase (HDAC) inhibitors discovered by Breslow and colleagues,[67] was approved by the U.S. FDA in October, 2006 for the treatment of patients with cutaneous T-cell lymphoma (CTCL).[68] Despite the several synthetic procedures for the preparation of SAHA that have been reported,[69] as far as we know, none of them described the synthesis under flow conditions. Our two-step sequence entails the conversion of the commercially available methyl suberoyl chloride **3** into methyl suberanilate **4** under Schotten-Baumann conditions, followed by the transformation of ester by aqueous hydroxylamine in presence of sodium methoxide (Scheme 28).
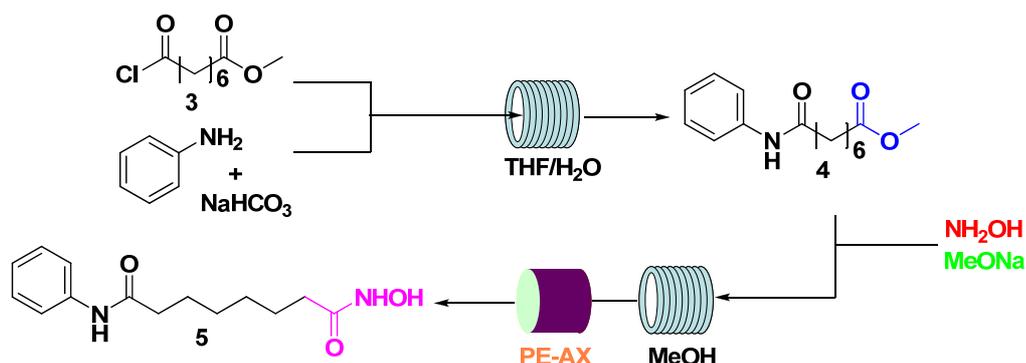
Scheme 28. Two steps synthesis of SAHA (5) (suberoylanilide hydroxamic acid)

In the first step a solution of suberoyl chloride in THF and a mixture of aniline and sodium carbonate in THF/H_2O were simultaneously pumped at room temperature into the reactor. In the optimized protocol the conversion of the acyl chloride into the corresponding amide resulted in clean and quantitative yield in less than 2 min (Table 10).

Entry	[A] (M)	[B] (M)	Flow rate (ml/min)	Residence time (min)	T (°C)	**4**[a] (%)
1	0.05	0.05	2	2.5	r.t.	>95
2	1	1	3.5	1.4	r.t.	>95

[a] Conversion was determined by LC/MS at 215 nM

Table 10. First step (Scheme 1) optimization results

No product isolation at this intermediate stage was necessary: the output of the reaction was evaporated, and the obtained amido ester was dissolved in methanol and used directly in the second step without further purification. Unfortunately the optimized reaction conditions to obtain

hydroxamic acid described above gave only low conversion to **5** (SAHA). A new optimization process was necessary (Table 11), and the best conditions were achieved by flowing a solution of the crude methyl suberanilate and aqueous hydroxylamine in methanol together with a stream of 2 equiv of sodium methoxide in MeOH at 90°C for 50 min.

Entry	MeONa (eq)	Flow rate (ml/min)	Residence time (min)	T (°C)	4[a] (%)	5[a] (%)	6[a] (%)
1	1	0.33	30	70	73	19	8
2	1	0.33	30	90	39	49	12
3	2	0.33	30	90	17	70	13
4	2	0.4	50	90	2	83	15

[a] Conversion was determined by LC/MS at 215 nM

Table 11.Second step (Scheme 1) optimization results

With the aim of avoiding a time-consuming workup procedure and extensive manual purification of the final compound, an integrated sequential flow synthetic pathway was set up, employing an immobilized scavenger. The reaction stream was then directly passed through a short packed column[70] containing silica-supported quaternary amine (ISOLUTE PE-AX)[71] for the selective removal of the carboxylic acid by-product **6** (8-oxo- 8-(phenylamino)octanoic acid). The solution containing the product and traces of the starting material was collected, and after solvent evaporation, crystallization from MeOH afforded SAHA in 84% yield and 99% purity (80% yield over two steps). In conclusion, we have developed a simple and high-yielding method for the synthesis of hydroxamic acids directly from the corresponding carboxylic esters using a flow reactor. The synthetic protocol is amenable both for preparation of compound collections and for the scale-up of single derivatives. Various reaction conditions can be screened very easily and rapidly to set up a more efficient protocol by optimizing temperature, stoichiometry, and residence time. Continuous operations provide an attractive approach for handling hazardous processes and reagents in a safer manner. Remarkably, the method was successfully employed with enolizable esters, such as chiral amino acids, with no trace of racemization at the alpha carbon.

Furthermore once the optimum reaction conditions are established the reaction can be scaled up to provide virtually any amount of product by running the apparatus for longer periods of time. In particular we have demonstrated the possibility of preparing 2.9 g/h of **2b**. Moreover the two-step synthesis of SAHA was developed and the purification of final product was achieved using immobilized scavengers.

Chapter 5.
Reaction of Grignard reagents with carbonyl compounds and synthesis of Tramadol

5.1. Addition of Grignard reagents to carbonyl compound under flow conditions[72]

Organomagnesium reagents were firstly prepared over a hundred years ago by Grignard and still occupy a central place in organic chemistry.[73] They show excellent reactivity towards a wide range of electrophiles and, for this reason, the reactions involving Grignard reagents often need to be controlled by operating at low temperature.

The flow technique represents an innovative way to control such reactivity since its homogeneous mixing and heat transfer narrows the temperature distribution and restricts the reaction output to the target product.[74]

The addition of Grignard reagents to aldehydes and ketones under flow conditions is up-to-date poorly exemplified in the literature. In fact, this reaction was mentioned as an application of CPC-CYTOS microreactor system and its evolution SEQUOA with limited experimental details.[75] While the reaction of a Grignard reagent with thiolactones,[76] diethyl oxalate,[77] and acyl chlorides[78] were reported using specifically developed microreactors often operating at low temperatures. Some examples dealing with the generation and the addition of aryllithium compounds to electrophyles under flow conditions are present in the literature[72b,79] pointing out, once more, the advantages of the flow approach when using highly reactive species.

In the light of the broad application of Grignard reagents in organic synthesis we decided to set up and optimise the addition of Grignard reagents to aldehydes and ketones using a commercially available flow reactor, and to explore the reaction selectivity in the presence of reactive moieties such as nitriles.

In a typical experiment two solutions of 4-isopropylbenzalehyde **7** and (2-methylallyl)magnesium chloride in THF, stored under N_2, were simultaneously pumped at room temperature into the flow apparatus equipped with a 10 ml tubing reactor (Scheme 29). The reaction stream was then directly passed through a short column containing polymer supported benzaldehyde[80] for the scavenging of the excess of Grignard reagent. The optimization of the experimental parameters was investigated by varying the temperature of the stored solution, the residence time and the number of Grignard equivalents (Table 12).
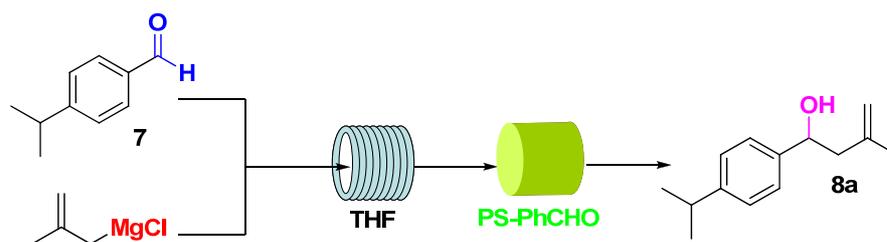
Scheme 29. Flow synthesis of secondary alcohols

Entry	Grignard (eq)	$T\,(°C)^a$	Residence time (min)	Flow rate (ml/min)	Conversion $(\%)^b$
1	2	-78	66 ^{c}	0.30	95
2	2	-78	33	0.30	96
3	2	0	33	0.30	94
4	2	r.t.	33	0.30	97
5	1.2	r.t.	33	0.30	98
6 ^{d}	1.2	r.t.	120	-	29
7 ^{d}	2	-20°C	50	-	95

^{a} Stock solution temperature. ^{b}The conversion was determined by peak integration at 215 nm (UPLC/MS). ^{c}Reaction performed using 20 ml tubing reactor. ^{d} Reaction performed in batch.

Table12.Optimization of the experimental parameters

We started our investigation by pre-cooling both the stock solutions at -78°C and by varying the residence time (entries 1 and 2). Then, the temperature of the reagent solutions was raised up to 0°C and finally to room temperature (entries 3 and 4) without observing any variation in terms of conversion and purity in the reaction profile. This result is a direct consequence of the efficient mixing and heat dispersion due to the high surface area-to-volume ratio in the PTFE tubing that keeps the temperature constant minimizing the occurrence of side reactions.

The Grignard equivalents were then reduced from 2 to 1.2 (entry 5), without affecting the conversion. For comparative purposes the same reaction was performed in traditional batch conditions (entry 6) by adding (2-methylallyl)magnesium chloride to 4-isopropylbenzalehyde 1 at room temperature.

The reaction mixture was thermostated and the temperature was kept constant in the course of the reaction. After 2 hours, the desired product was detected with a conversion of 29% (UPLC-MS 215 nm) showing that, in the specified reaction conditions, the flow system is really advantageous in term of efficacy if compared to the batch one. In order to achieve complete conversion, the number of Grignard equivalents was risen (2 eq) and the temperature was kept at -20°C during the

addition (entry 7), suggesting that the poor conversion in the previous experiment could have been determined by Grignard degradation in the reaction mixture.

In continuous flow synthesis, scale up is generally accomplished by running the microreactor for an extended time or by employing multi-channel parallel reactors (numbering-up process). A demonstration of this preparative capability was readily obtained. Applying the optimized conditions (i.e. stored solution at room temperature; flow rate: 0.30 ml/min; residence time: 33 min) 2 grams of **8a** were straightforwardly produced with an output of 0.9 g/h. Yield and purity were similar to the smaller scale proving that the reaction conditions identified for the production of few milligrams can be directly transferred to a larger scale.

To asses the limit and the scope of this procedure, the same protocol was tested reacting different Grignard reagents (aryl, alkyl, allyl) with 4-isopropylbenzaldehyde and acetophenone (Table 13) on a 200 mg scale.

Entry	Carbonyl compound	Grignard reagent	Alcohol	Yield (%)[a]
1	**7**		**8a**	92
2	**7**		**8b**	93
3	**7**		**8c**	87
4	**9**		**10a**	94
5	**9**		**10b**	95
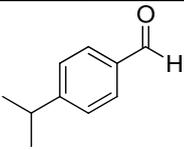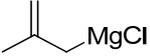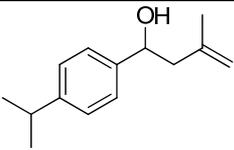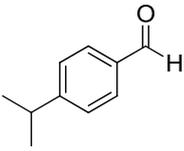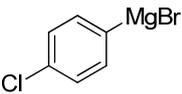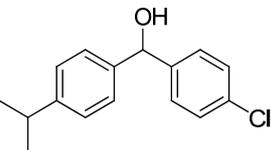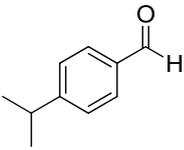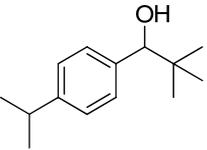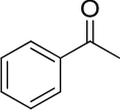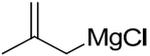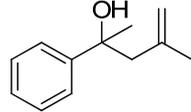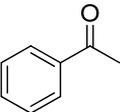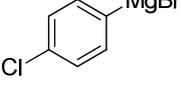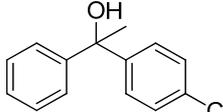
Entry	Carbonyl compound	Grignard reagent	Alcohol	Yield (%)[a]
6	acetophenone **9**	*t*-Bu-MgBr	**10c**	90
7	acetophenone **9**	*i*-Pr-MgBr	**10d**	95
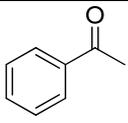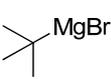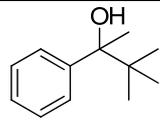
[a] Isolated yields; purity > 95% (UPLC/MS)

Table 13. Conversion of 4-isopropylbenzaldehyde into secondary alcohols and acetophenone into tertiary alcohols

The desired alcohols were efficiently obtained using starting materials such as allyl (entries 1, 4), aryl (entries 2, 5) and alkyl (entries 3, 6, 7) magnesium derivatives. In all examples the isolated yields were excellent: of special interest are the results obtained with the hindered *tert*-butyl magnesium bromide as a reagent (entries 3, 6).

To verify the effectiveness and reproducibility of the optimized protocol, the synthesis of a small collection of secondary and tertiary alcohols was also undertaken. (4-chlorophenyl)magnesium bromide was chosen as reference Grignard reagent and the optimized conditions were applied to different substrates on a 200 mg scale (Table 14).

Entry	Carbonyl compound	Alcohol	Yield (%)[a]
1	benzaldehyde **11**	**12**	90
2	pyridine-2-carbaldehyde **13**	**14**	94
3	1-(pyridin-2-yl)ethanone **15**	**16**	92

59
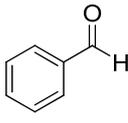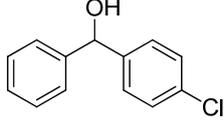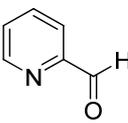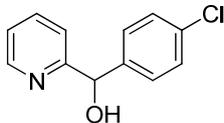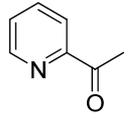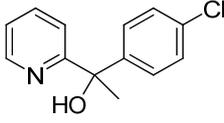
Entry	Carbonyl compound	Alcohol	Yield (%)[a]
4	**17**	**18**	96
5	**19**	**20**	96
6	**21**	**22**	95
7	**23**	**24**	98
8	**25**	**26**	93

[a]Isolated yields; purity > 95% (UPLC/MS)

Table 14. Reaction of different aldehydes and ketones with (4-chlorophenyl)magnesium bromide

The formation of alcohols from aldehydes and ketones was general and occurred smoothly in the continuous flow system. Simple aryl carbonyl compounds, besides reference substrates, (entries 1 and 6), heteroaryl aldehydes and ketones (entries 2, 3, 4 and 5) and alkyl compounds (entries 7 and 8) were suitable substrates for the reaction. All of the desired products were obtained in yields higher than 90%, with no detection of by-products.

5.2. Flow synthesis of Tramadol[72]

The protocol was finally applied to the synthesis of Tramadol ((1R*,2R*)-2-((dimethylamino)methyl)-1-(3-methoxyphenyl)cyclohexanol) **29**, a well known centrally active

analgesic used for treating moderate to severe pain (Scheme 29).[81] This drug is commercialized as racemic mixture and the final step of the published synthesis consists in the addition of (3-methoxyphenyl)magnesium bromide **28** to racemic 2-((dimethylamino)methyl)-cyclohexanone **27**. The reaction affords a diastereomeric mixture with a 8/2 ratio between **29** and **30**.[82] The desired product **30** is obtained after crystallization of the corresponding hydrochloride salt.[83] The flow synthesis of Tramadol is unprecedented. In our study the suitable intermediates were reacted in the flow system using the described optimized protocol to obtain, after chromatographic purification, the diastereomeric mixture in 96% yield, with a significant improvement respect to the data reported in the literature (Scheme 30).[81b] The 8/2 ratio of the two diastereoisomers was confirmed by UPLC-MS and by NMR analyses. Tramadol was converted into its hydrochloride salt and purified by crystallization as reported.[81]
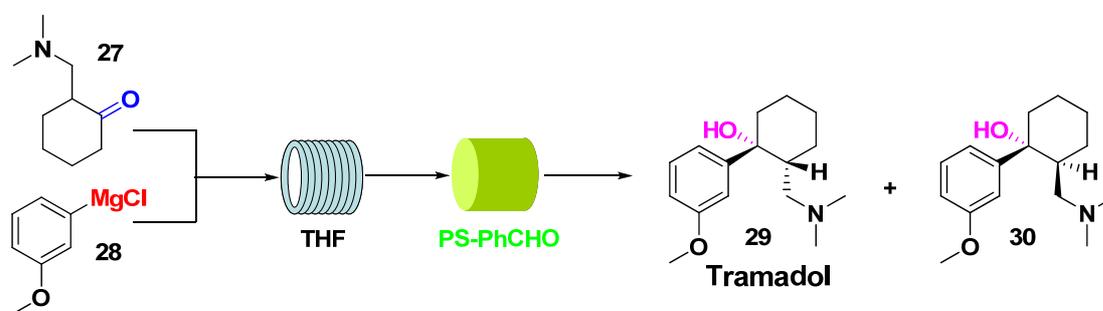
Scheme 30. Flow synthesis of Tramadol

5.3. Addition of Grignard reagents to cyano and bifunctional compounds[72]

The possibility to perform selective addition of Grignard reagent to bifunctional compound was then taken into account. In particular the discrimination of aldehydes and ketones respect to nitriles was investigated. At first, the reactivity of nitriles under the flow conditions was explored (Scheme 31). The addition of phenyl magnesium bromide to benzonitrile was performed, and the obtained product was quenched in 1M HCl in order to afford benzophenone (Table 15). Using the previously optimized conditions (1.2 eq, room temperature, 33 min) the conversion was not complete and around 40% of the starting material remained unreacted (entry 1). In order to drive the reaction to completion, the temperature was risen to 50°C (entry 2), alternatively the residence time had to be doubled (entry 3).
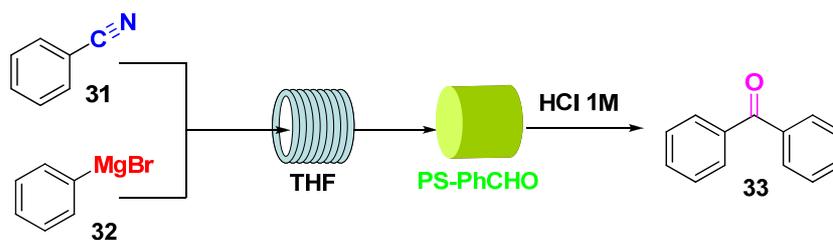
Scheme 31. Addition of Grignard reagent to cyano compound

Entry	Grignard (eq)	T (°C)	Residence time (min)	Flow rate (ml/min)	Yield (%)
1	1.2	r.t	33	0.30	59 [a]
2	1.2	50°C	33	0.30	90
3	1.2	r.t	66[b]	0.30	91

[a]Conversion determined by peak integration at 215 nm (UPLC/MS) [b]Reaction performed using 20 ml tubing reactor.

Table 15. Conversion of benzonitrile into phenylacetophenone

This interesting result let us to envisage the possibility to react aldehydes or ketones in the presence of nitriles (Scheme 32; Table 16).
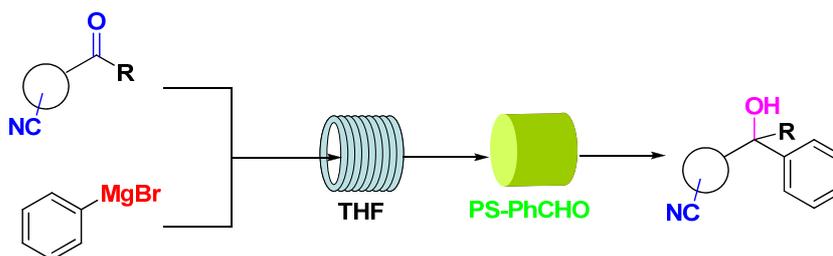
Scheme 32. Addition of Grignard reagent to bifunctional compounds

Entry	Carbonyl compound	Alcohol	Yield (%)
1	**34**	**35**	94
2	**36**	**37**	85

3   89

          38   **39**
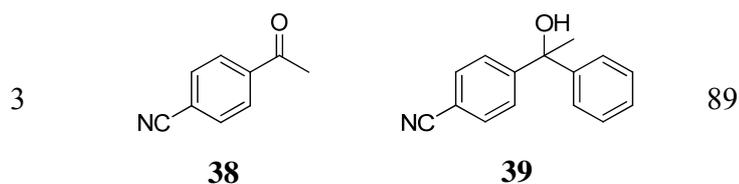
Table 16. Flow addition of benzyl magnesium bromide to aldehydes and ketone in the presence of nitriles

The reaction was performed on aryl and alkyl aldehydes (entries 1 and 2) and on one aryl ketone (entry 3) flowing the starting materials for 10 min at room temperature in presence of 1 eq of Grignard reagent. We observed the selective addition of Grignard reagent to the carbonyl moiety and no product of double addition or deriving from the Grignard reaction on the nitrile group was detected. After purification all of the products were recovered in very good yields with a significant simplification in their synthesis respect to the known methods.[84]

The reaction of phenyl magnesium bromide with 3-oxopropanenitrile (entry 2) is particularly interesting. In fact this example offers an alternative approach to the synthesis of β-hydroxy nitriles, which are commonly prepared by ring opening of epoxides with sodium cyanide,[85] by reaction of cyano-methyllithium with the suitable aldehyde,[86] or by cyanomethylation of carbonyl compounds with trimethylsilyil acetonitrile.[87]

Summarizing a protocol for performing the addition of Grignard reagents to carbonyl compounds in a continuous flow apparatus was set-up. The reaction required very mild conditions avoiding cryogenic temperatures. Other major advantages were the high yields, the excellent reproducibility and the easy scale up. Our results confirm the general applicability of the flow protocol and gain a specific interest if we consider the possibility of synthesizing large amounts of addition products reacting small amounts of potentially dangerous Grignard reagents at a time. The methodology was successfully applied for the preparation of a small collection of secondary and tertiary alcohols using different carbonyl compounds and for the synthesis of the analgesic Tramadol. The possibility of discriminating the carbonyl moiety respect to cyano group was demonstrated offering a new methodology for the preparation of β-hydroxy nitriles.

Working on these two projects, we have demonstrated that flow chemistry methods can facilitate the preparation of medicinally relevant compounds.

In fact, we showed how the application of these techniques constitutes a new paradigm for molecular assembly and will certainly have a very significant impact on how synthetic programs will be conducted in the future. The benefits attained are in terms of both the cost and efficiency

gained through the avoidance of extensive purification and work-up procedures, the rapid optimization and precise control of reaction conditions, and a reduction in manual handling.

Chapter 6.
Flow chemistry applied to the multistep-synthesis of natural products

6.1. Introduction

The innovative incorporation of flow chemistry into laboratory based synthesis platforms for increasingly complex organic transformations could represent one of the most exciting and potentially significant developments.

Although the use of this technique could constitute a new paradigm for complex molecular assembly and an attractive potential solution for multi-step synthesis for the possibility of performing sequence of two or more reactions without breaking the sequence with workup and purification, there are few examples in literature toward this type of application.

Up to now, a number of diverse organic reactions have been performed in single-step continuous-flow systems[88] but the potentiality of multi-step synthesis in a single continuous flow sequence is not yet fully exploited as few examples are available in literature.

In fact only a small handful of multistep systems have been described which can assemble several building blocks to more complex molecules,[89] as for example the Imatinib synthesis or the preparation of a collection of chemokine receptors that was discussed in the previous chapter.

The scarcity of reports of multistep continuous-flow syntheses is probably related to the practical challenges that come out when such systems are assembled, which include the frequent need for different solvents in different reactions, build-up of high back-pressure over several packed columns in series, precipitation and clogging of tubes and columns. Another reason may be the high-tech flavor that the field has, with discussions often focusing on lab chips and microstructured devices, giving the impression that considerable investment is necessary to enter into the field.

In particular the application of flow chemistry to the total synthesis of natural products has to be still explored.

The often low availability of natural products from their sources of origin, combined with the lack of structural analogues, creates a need for their laboratory synthesis. This is especially true if these materials must be used as biological probes for drug discovery programs. Consequently, new techniques are urgently required to overcome many of the bottlenecks associated with conventional organic synthesis. Also more use of automation is needed to facilitate scale-up and to improve the current synthesis standards in terms of reaction optimisation and product clean-up practices.

In this light, flow chemistry could really be an answer for the solution of these problems and the adoption of this technique could provide chemists with methods that allow for the rapid

production of changeling molecules in a fashion far superior to that permitted by conventional methods.

The benefits could be in terms of both the efficiency and cost gained through the avoidance of extensive purification and work-up procedures, the rapid optimisation and precise control of reaction conditions, a reduction in manual handling and easy scale-up process.

6.2. State of the art

As explained before, there are just few applications of flow chemistry to the synthesis of the natural products.

One outstanding example in this field is the preparation of the alkaloid (±)-oxomaritidine by Ley group (Scheme 32).[90]

The whole seven-step synthesis was carried out through a microfluidic pumping system that pushed the solution of reactants into a series of pre-packed columns containing immobilized reagents, scavengers and catalysts. The first steps of the process involved the parallel synthesis of an azide and an aldehyde, which, when combined, reacted in the presence of a polymer supported phosphine to afford the desired imine. Reduction of the imine was followed by trifluoroacetylation and the resulting intermediate subsequently underwent oxidative phenolic coupling, in the presence of polymer-supported (ditrifluoroacetoxyiodo)benzene (PS-PIFA) to afford a seven-membered tricyclic derivative. The resulting product was then passed directly into a column that contained a polymer supported base which promoted cleavage of the amide bond, allowing for 1,4-conjugate addition to spontaneously take place, generating the target compound (±)-oxomaritidine. The natural product was obtained with an overall yield of 40% with 90% purity through the simple evaporation of the solvent.
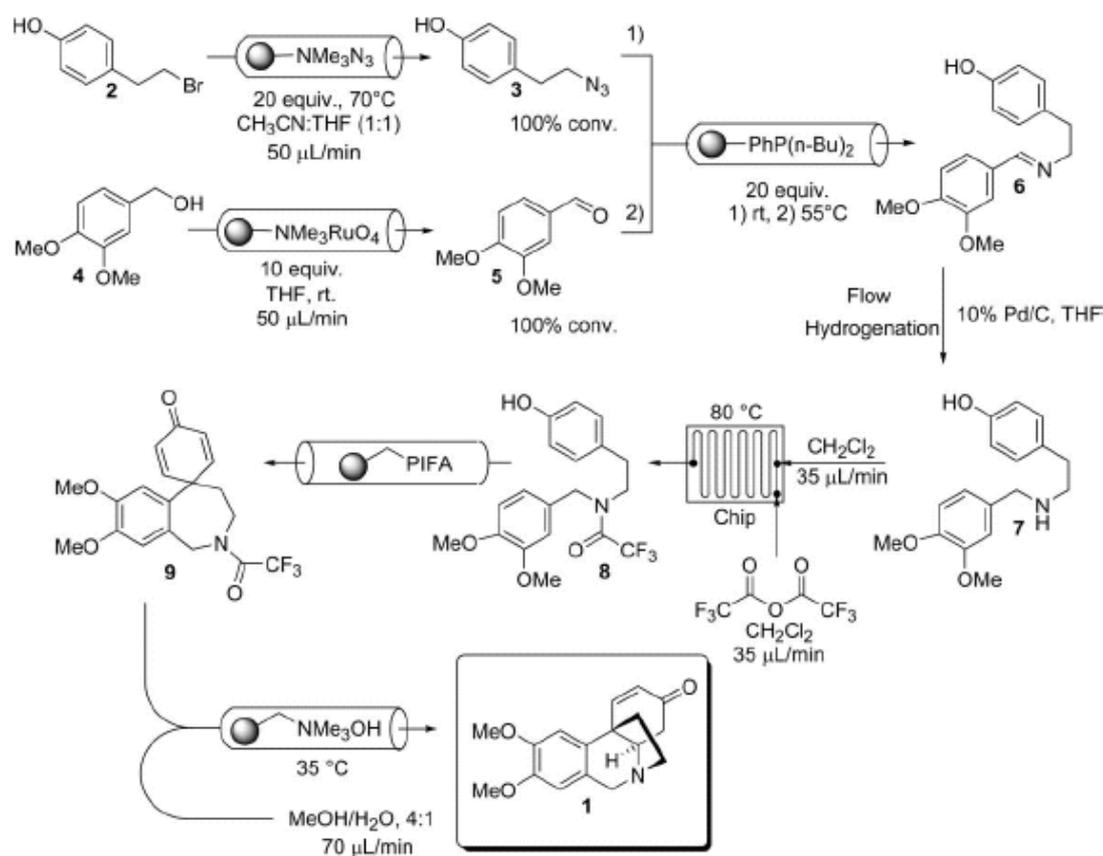
Scheme 32. Flow multistep synthesis of (±)-oxomaritidine

The significance of this work was the development of a route that led to the desired final compound without the need for column chromatography or the use of aqueous work-ups at any stage. Ley group devised a flow through reaction sequence that produces the natural product in an automated sequence from readily available starting materials in less than a day. The time saved by using these flow through methods compared to conventional procedures (minimum four days required for the total synthesis) is dramatic.

The same group developed also the enantioselective synthesis in flow of the neolignan natural product grossamide.[91] Lignanamides, a structurally diverse class of compounds, possess both cyclic and acyclic structures many of which have been shown to regulate biological functions in both plants and microorganisms making them interesting lead discovery candidates for both pharmaceutical and agrochemical investigation.

In particular the design and validation of a flow reactor capable of synthesizing gram quantities of compound was reported. The fully automated continuous flow reactor system was constructed using a simple pumping arrangement with immobilized reagents packed in columns.

As Scheme 33 illustrates, the first step for the preparation of the natural product involved the synthesis of amide, which was achieved via the coupling of ferulic acid and tyramine, in the presence of polymer-supported HOBt in a first column, with the reaction progress monitored by LC-MS. Using this approach, the authors reported an optimal conversion of amide (90% yield). Consequently it was imperative to remove the residual tyramine (10%) before the second step. To achieve this, the reaction mixture was passed through a second column, whereby the amine was sequestered (providing the possibility of recycling the unreacted amine). The pure amide was then premixed with a hydrogen peroxide-urea complex and sodium dihydrogen phosphate buffer, before passing through a third column that contained silica-supported peroxidase (horseradish peroxidase on silica). Collection of the reactor effluent afforded grossamide as the desired product in excellent purity.
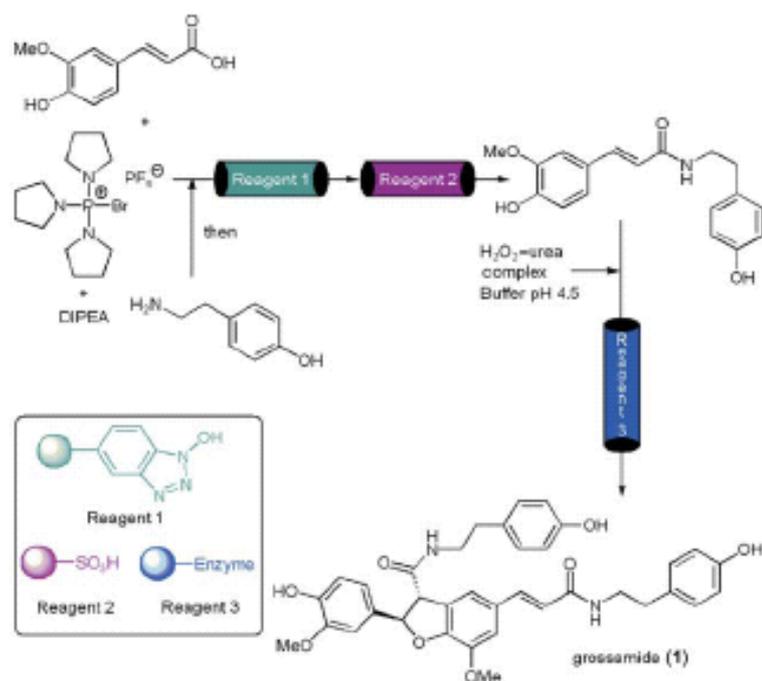
Scheme 33. Enantioselective flow synthesis of grossamide

Along with the ability to generate a natural product in a scalable way, this example is of particular importance as it combines the use of supported chemicals and enzymes to induce synthetic transformations. Due to their cost, limited stability and questionable longevity, enzymes are not readily employed in chemical synthesis. However, through their incorporation into flow reactors,[92] small quantities can be used especially for classically difficult synthetic transformations.

Fukase and co-workers[93] employed the continuous flow dehydration of alkanols as a key reaction step in the multikilogram synthesis of the natural product pristane (2,6,10,14-tetramethylpentadecane), which is now widely used as an adjuvant for monoclonal antibody production (Scheme 34). Since 2002, the availability of pristane has been limited due to the listing of the basking shark *Cetorhinus Maximus* as an endangered species. With this in mind, the authors evaluated a microfluidic approach to the large-scale synthesis of the hydrocarbon. The first two steps of the total synthesis were performed in batch: the treatment of farnesol with MnO_2 to afford the respective aldehyde and subsequently the reaction of it with isobutylmagnesium chloride to afford the allylic alcohol derivative.
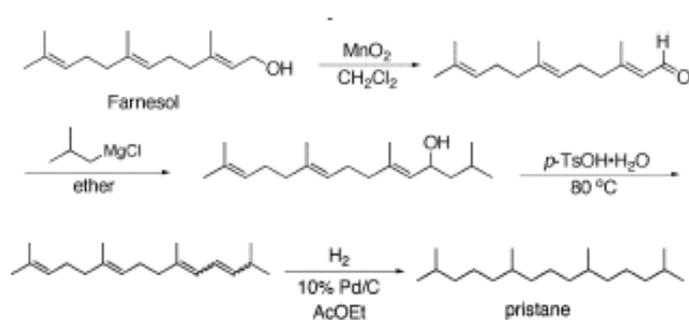
Scheme 34. Synthesis of pristane

The alcohol (1.0 M in THF) was subsequently dehydrated under flow conditions within a micro mixer (Comet X-01) using pTsOH·H_2O in THF/toluene (0.2 to 1.0 M), a total flow rate of 600 μl/min and a reaction temperature of 90°C (Scheme 35).

The final desired product was obtained after the catalytic hydrogenation of the alkene, performed in traditional conditions.

With the help of this flow approach, the authors were pleased to report the synthesis of pristane in 80% yield from farnesol.
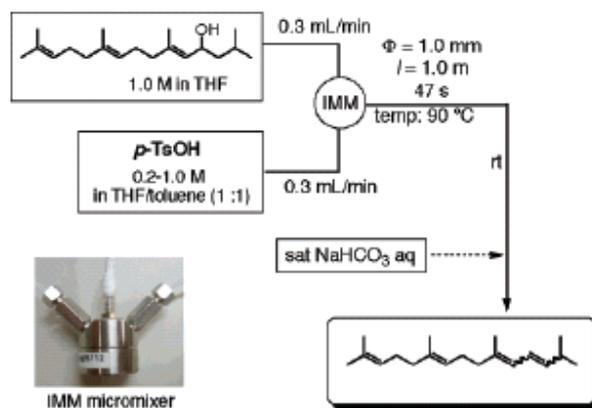
Scheme 35. Optimization of microfluidic dehydration

Compared to conventional batch approach, this synthetic route proved advantageous as it only requires a simple purification to be conducted unlike the multiple distillations previously employed. In fact when the batch acid-catalyzed dehydration of allylic alcohol was performed on a gram scale, various cation-mediated byproducts, such as cyclized products or alkyl group-migrated compounds, were produced. These hydrocarbons were very difficult to separate from the desired diene (yield estimated to be less than 20%), even by repeated distillation or by silica gel chromatography, although the latter is not realistic for kilogramscale purification. Instead working under the established microfluidic conditions, the formation of other byproducts was not detected and the product was obtained in high yield and purity just after a simple work-up.

The route presents significant advancements over traditional techniques in terms of yield and handling purification required, enabling an efficient route to the multi-kilogram synthesis of pristane, which is in-line with the current demand of ca. 5 kg per week.

Chapter 7.
Flow synthesis of
(+)-dumetorine and natural congeners

7.1. Multistep synthesis of (+)-dumetorine

2-Piperidinyl alkaloids are an interesting family of natural products whose convenient synthesis was previously described by our group.[94] The proposed synthesis was based on the use of the common electrophilic synthon **44** that, due to a stereogenic center at position 2 of the piperidine ring and the versatile aldehyde group, is prone to a diversity-functionalization (Scheme 36). The strategic value of this synthon consists in the gram scale availability of both its pure enantiomers by enzyme-catalyzed preparation[94a] of the corresponding hydroxyl derivative 2-(2-hydroxyethyl)piperidine.
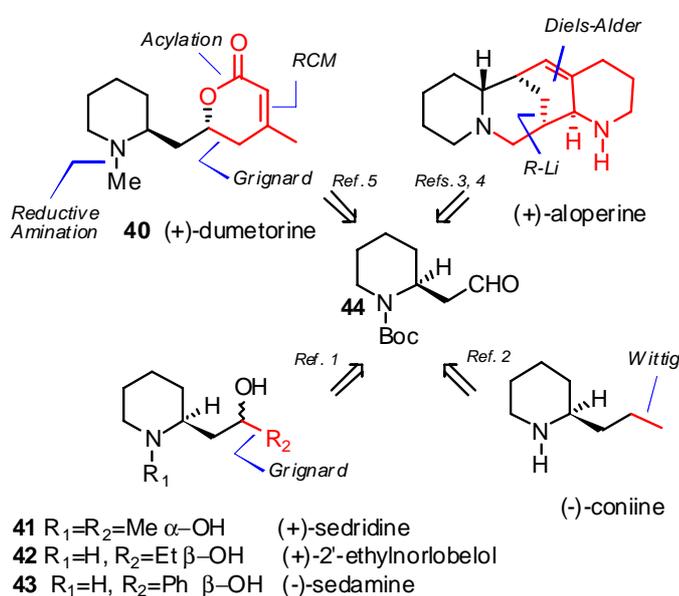
Scheme 36. Structures and synthetic plan of dumetorine and its congeners

(+)-dumetorine (**40**) was isolated in 1985 from the tubers of *Dioscorea dumetorum* Pax, a West Africa yam whose extracts have found a notable use in folk medicine and arrow poisons.[95]

Its total batch-synthesis, recently published by our group, was reported in Scheme 37.[94e]

This synthetic route did not fulfil our expectations because it was affected by a very low overall yield (<1%) showing problems at different steps of the synthetic pathway.
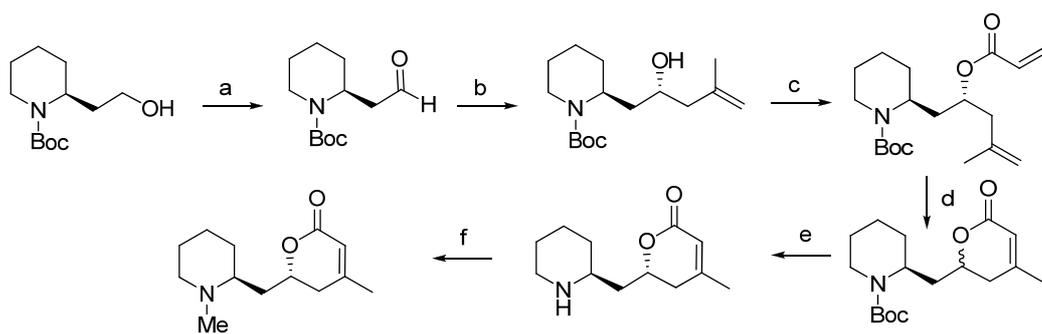
Reagents and conditions: (a) Dess–Martin, DCM, r.t. 6h, 85%; (b) THF, -78°C, CH$_2$=CHCH$_3$CH$_2$MgBr 4h,.58%; (c) i-Pr$_2$Net, CH$_2$Cl$_2$, CH$_2$=CHCOCl, 4 h, 81%; (d) 2nd Grubbs cat., CH$_2$Cl$_2$, 2h, 40°C, 75%; (e) TFA, CH$_2$Cl$_2$,10%; (f) CH$_2$O, NaBH$_3$CN, 39%

Scheme 37. Batch synthesis of (+)-dumetorine

The general interest toward the application of flow-chemistry technology to multistep synthesis focused our attention to devise a more concise and efficient continuous synthetic route to **40** and to its simplified natural congeners (+)-sedridine (**41**) and (-)-sedamine (**43**).

Moreover, we thought that this synthesis represents a remarkable challenge for the evaluation of the potential benefits that continuous flow chemistry could provide to the preparation of natural products in comparison with traditional batch techniques, also in the light of the scarce number of studies published in this area (see Chapter 6).

Basically, we were trusting to make the most of the power of flow chemistry in terms of more selective chemical transformation, safer handling of material and purification efficiency to improve the entire global synthetic pathway.

We decided to assemble the target molecule according to the synthetic plan previously described from *N*-Boc-2-hydroxyethylpiperidine: oxidation to aldehyde, Grignard addition, acylation and RCM reaction, deprotection and methylation. In particular the yields of the two final steps must be improved to obtain a satisfactory overall outcome.

In the first step of our flow sequence towards (+)-dumetorine, the pure *S* enantiomer *N*-Boc-2-(2-hydroxyethyl)piperidine **45**, obtained in gram scale by enzyme-catalyzed preparation, was converted into the corresponding aldehyde **44** (Scheme 38).

74
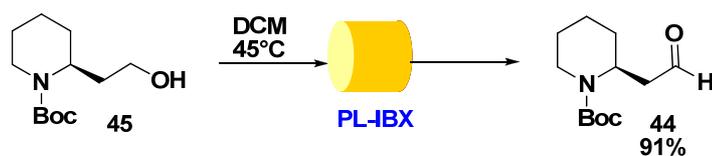
Scheme 38. First step: Oxidation

The reaction was performed continuously cycling the stream of the reagent dissolved in dichloromethane (DCM) through a glass column (Omnifit®) filled with PL-IBX Amide,[96] till complete conversion of the starting material into the desired product was obtained as detected at UPLC/MS (Figure 25).

Figure 25. "Oxidation loop"

In order to speed up the reaction rate, the column containing the resin was heated at 45°C. Temperature higher than 50°C, described as possible in literature,[93a] promoted, in our case, the degradation of PL-IBX Amide: the presence of organic residues of the supported oxidant were detected in the UPLC/MS chromatogram.

Starting from 800 mg of (hydroxyethyl)piperidine, after 8 hours, the pure desired aldehyde was recovered in high yield (91%) by simple solvent evaporation (batch conditions: 6 hours, r.t., 85% yield).

The long reaction time was intrinsically related to the use of the supported reagent and moreover to the relatively limited residence time of the reaction mixture in contact with the resin in the column (about 10 min for each passage). On the contrary no extensive manual purification of the oxidation product was necessary as the only handling procedure required was the evaporation of the reaction solvent.

In the second step, the obtained aldehyde **44** underwent Grignard addition.

The optimal conditions for this reaction were found to be 1.2 equivalents of 2-methylallylmagnesium chloride, room temperature with a residence time of 33 min, as in the general protocol for the addition of Grignard reagent to carbonyl compounds previously described (Chapter 5).[72]

The THF stock solutions of 2-methylallylmagnesium chloride and **45** stored under N_2 were simultaneously pumped at room temperature into the flow apparatus (Scheme 39).

The reaction stream was then directly passed through a short column containing polymer-supported benzaldehyde[80] (2 equivalents) for the scavenging of the excess of the Grignard reagent.
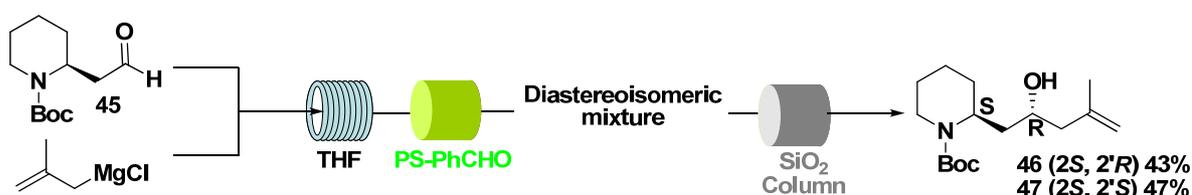
Scheme 39. Second step: Grignard Addition

The reaction required very mild conditions avoiding cryogenic temperatures, necessary in the batch protocol.[94e] This result is a direct consequence of the efficient mixing and heat dissipation due to the high surface area-to-volume ratio in the PTFE tubing that keeps the temperature constant minimizing the occurrence of side reactions.

The output of the reactor was then concentrated *in vacuo* and, after a aqueous work-up, was purified by silica gel chromatography to give the final product **46** in 43% yield with a remarkable improvement compared with the batch process (-78°C, 4h, 58% yield). It is important to underline that this chromatography purification represented the optimal solution for the easily and clean isolation of both the diastereoisomers (**46**; **47**) and was the unique purification in all the process.

The effectiveness and the reproducibility of the previously optimized protocol for the Grignard reaction was confirmed by the very high overall yield (90% for both the isolated diastereoisomers; diastereoisomer (**47**) isolated in 47% yield).

The next step in the synthesis was the acylation of alcohol **46** using acryloyl chloride in presence of a base.

Preliminary flow studies were conducted pumping the reagents dissolved in dry DCM through a pre-packed column containing different type of polymer-supported base to promote the reaction and trap the hydrochloric acid generated in the reaction.

All the attempts performed using either PS-DIPEA or PS-DMAP[97] (6 equivalents; flow rate 0.12 ml/min; residence time ca.10 min) did not give satisfactory results.

In both cases UPLC/MS analysis showed a conversion of the starting material to the desired ester lower than 10%.

In the light of these poor results, probably associated to the scarce reactivity of the supported bases, we planned the use of a classical soluble organic base as pyridine to assess the complete conversion of the alcohol into the desired product.

Two DCM solutions, the first containing **46** and pyridine (3 equivalents) and the second one the acyl chloride (2 equivalents) were simultaneously pumped into the PTFE flow reactor. The reaction occurred in 90 min at a temperature of 90°C exploiting DCM superheating for speeding up the reaction time. A backpressure regulator was essential in this procedure: it was connected in-line and inserted at the end of the reactor configuration, allowing solvent heating well above the usual boiling point.

The output stream was then passed through two glass columns filled with supported scavengers. In fact the in-line purification of product **48** was achieved using PS-Trisamine[98] (3 equivalents) to trap the excess of acyl chloride, and MP-Bicarbonate[99] (4 equivalents) for the neutralization of the pyridine hydrochloride formed during the reaction (Scheme 40).
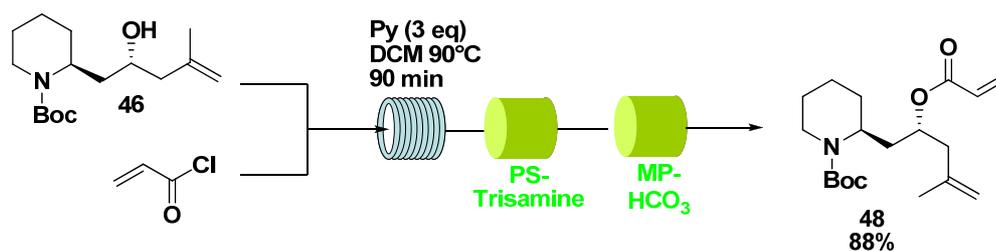
Scheme 40. Third step: Acylation

In this way, compound **48** was obtained pure after simply concentration of the solvents to dryness. Respect to the batch strategy, we switched DIPEA with pyridine because of the lower boiling point of the aromatic base that could be removed easily under *vacuum*..

The obtained ester **48** was directly used without further purification for Ring-Closing Metathesis.

There are generally two ways to introduce a catalyst into microreactors. One method is to dissolve a homogeneous catalyst directly into the reaction mixture; the other is to immobilize the catalyst on a solid supports through which the reagents can flow.

Flowing a soluble catalyst in the reaction mixture offers the well know and already described advantages typical of the flow chemistry. The only drawback of this approach is the removal of the catalyst at the end of the reaction. Since the catalyst is soluble, it cannot be simply filtered off, and, as consequence, the catalyst recycles are quite difficult. On the other hand, when the catalyst is immobilized to an insoluble support, it can be recovered by filtration and potentially recycled.

Packing a column with a supported catalyst and flowing the reaction mixture through it takes this idea a step further. In fact, such systems have been in use industrially for decades in the form of packed-bed reactors (PBRs), and much of the work with immobilized catalysts in microreactors is based on this techniques.

The metathesis reaction on micro scale was previously studied by two different groups. Kirschining and co-workers[100] performed cross and ring-closing metathesis building PASSflow reactors in which Hoveyda-catalyst was immobilized on monolithic polymeric structure.

RCM was also investigated by Trapp employing a column reactor consisted of a capillary coated with Grubbs 2nd generation catalyst and employed an inert carrier gas (He) to transport substrates through the reactor.[101]

We wanted to develop a simple protocol, which could be not only effective but also "friendly", easily assembled and effortless for an organic chemist.

Firstly, we performed RCM flowing the starting material in presence of dissolved 1st or 2nd generation Grubb's catalyst through the PTFE loop, for short time (less than 20 min) at room temperature. These results were comparable with what previously obtained under batch conditions (2 h, 40°C, 75% yield)[94e] but obviously in this way the problem of the final purification by flash chromatography was still present.

On the basis of the PBR concept and for minimizing purification, supported Grubbs catalysts of 1st (a) and 2nd (b) generation (Figure 26) were prepared to perform the RCM reaction flowing the starting material through pre-packed columns., Details of the preparation and use of the supported catalysts were described in the next Chapter.
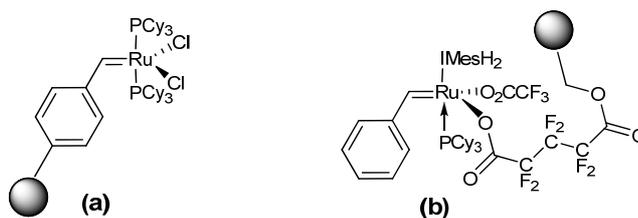
Figure 26. Supported Grubbs Catalysts prepared

In both cases the activity of the freshly prepared catalysts was successfully verified performing RCM reactions using the model molecule (*N*,*N*-diallyl-4-methylbenzenesulfonamide; compound **54**, see Chapter 9) as RCM substrate under both standard solid-phase and flow conditions.

On the other hands, when the reaction was performed on the more complex and hindered compound **48**, no satisfactory results were obtained.

In fact, with catalyst (a), the RCM reaction didn't occur and the use of the supported Grubbs 2nd generation catalyst (b) did not give a acceptable result as well, obtaining a poor conversion (lower than 20% after 48 hours) into the desired product.

In the light of the reported results, the preparation of PEG supported Grubbs catalyst was planned to increase the performance in RCM maintaining the possibility of a simple catalyst recovery. Using this strategy, differently from the use of an insoluble cross-linked polymer, the catalytic reaction can be carried out under homogeneous conditions and then an easy separation of the catalyst is allowed after the reaction end.[102] In particular the polyether supports are most commonly recovered by solvent promoted precipitation and filtration. PEG is usefully soluble in a wide range of solvents including DMF, dichloromethane, toluene, acetonitrile and water while the solvents usually used for the precipitation process are hexane, diethyl ether, *tert*-butylmethyl ether (TBME), isopropyl alcohol, and cold ethanol. This type of solubility facilitates the use of PEG-supported species for carrying out metathesis reactions that usually are performed in DCM.

A newly synthesized PEG-Supported Hoveyda catalyst (Figure 27) was prepared in collaboration with Prof. M. Benaglia and Dr. A. Caselli (Università degli Studi di Milano) and its synthesis is described in details in the next Chapter. This PEG-Supported catalyst is an analogue of PEG-immobilized Ru catalysts described in literature.[103]
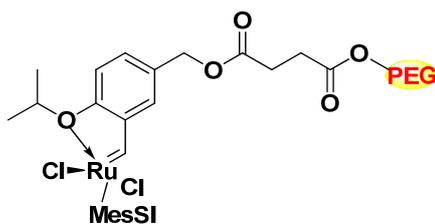
Figure 27. PEG-supported Hoveyda catalyst

This PEG-supported catalyst, led to the total conversion of compound **48** into lactone **49** (Scheme 41).
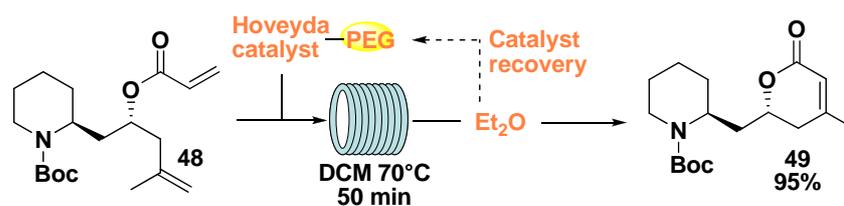
Scheme 41. Fourth step: Ring Closing Metathesis

The streams containing **48** and the PEG-Hoveyda catalyst (6%mol Ru) dissolved in dry DCM were pumped into a PTFE loop reactor and the reaction occurred at 70°C with a residence time of 50 min. As usual the reaction conditions were optimized varying the residence time, the temperature and the catalyst amount. The final high temperature was necessary for assess the total conversion of the starting material in less than one hour. However the presence of DCM in the mixture did not preclude the use of relatively high reaction temperatures which could be achieved by the addition of a 100 psi back-pressure regulator (BPR) inserted at the end of the reactor configuration.

The reaction output was collected into a vial containing a proper volume of diethyl ether that allows the quantitative precipitation of the PEG-bound catalyst. The Hoveyda-catalyst was filtered off on a solid/liquid phase separator cartridge and the desired product **49** was easily obtained by solvent evaporation in high purity and 95% yield. Also the presence of any residue of the Hoveyda catalyst wasn't detected by ^{1}H-NMR spectroscopy.

The PEG-supported Hoveyda catalyst showed high performance in our RCM reaction and guaranteed a very simple catalyst recovery avoiding chromatographic purification. Moreover, the recycle of the catalyst could be possible and was verified in a model reaction (the use of this catalyst on other substrates and its recycle is fully described in the next Chapter).

A new and efficient protocol for Ring Closing Metathesis under continuous flow conditions was developed. In fact the reaction occurred on a complex compound with high yields. Moreover the procedure requires limited and easy manual handling operations, avoiding the use of chromatography column.

The (+)-dumetorine batch synthesis was affected by a low yielding acid catalyzed cleavage of Boc protecting group due to a Michael side reaction of the secondary amine on the α-β unsaturated lactone ring. In particular, the *N*-Boc deprotection resulted in the isolation of small amount of desired product **50** (10% yield) accompanied by the tricyclic by-product **51** (60% yield). Also, the

subsequently reductive amination resulted to be not an easy task to be managed showing a poor yield (35%) (Scheme 42).
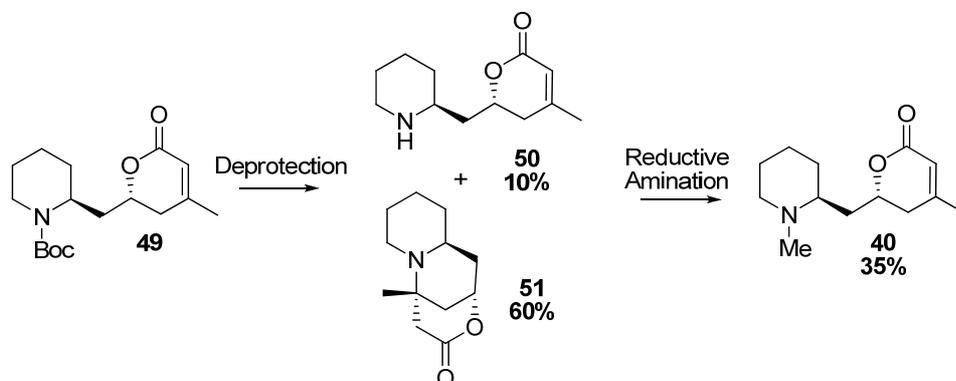
Scheme 42. Deprotection and reductive amination in batch.

We thought that suitable reaction conditions that permit the concomitant Boc deprotection and *in situ N*-methylation could block the formation of the undesired 1,4 addition by-product.

The methylation of secondary amines via treatment with formaldehyde in the presence of formic acid at high temperature - (Eschweiler-Clarke reaction)[104] is a method of proved synthetic utility.[105] In our opinion the acidic conditions and the high reaction temperature could allow to overcome the synthetic problem by permitting the simultaneous N-deprotection and methylation..

We then performed an unprecedented flow Eschweiler-Clarke reaction on the *N*-Boc protected compound (Scheme 43).
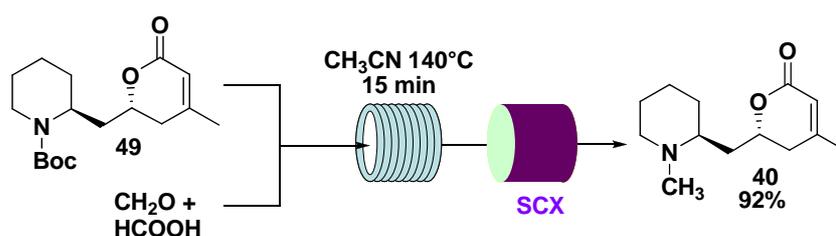
Scheme 43. Fifth step: Concomitant Boc removal and Eschweiler-Clarke reductive amination

In particular, the solution containing **49** dissolved in CH_3CN was combined with the stream of formaldehyde and formic acid in acetonitrile at a static T-junction and entered in a pre-heated PTFE reactor. Here the Boc removal and the reductive amination occurred at 140°C in 15 min assessing the total conversion of the starting material in (+)-dumetorine **40**.

As usual a backpressure regulator was essential in this procedure and was connected in-line with the exiting reaction stream, allowing heating acetonitrile to higher temperature than its boiling point. Usually to assess very high temperatures Eschweiler-Clarke reaction is performed in DMSO, especially under microwave conditions.[104c] This solvent is not suitable for an easy evaporation, so we decided to use CH_3CN obtaining similar results and guarantying its easy removal by *vacuum*.

The optimal reaction conditions were rapidly found by quickly screening various reaction parameters such as residence times and temperature. In particular the influence of the latter on the outcome of the reaction was evaluated and the flash heating at 140°C showed to improve the overall yield and purity profile of this transformation. Although higher temperature resulted in better conversions in a lower reaction time, the increasing of the temperature induced the formation of byproducts probably due to partial degradation of the starting-material.

The final compound was finally purified by a in-line 'catch and release' purification. In fact the flow stream of the newly formed **40** was directly passed through a column containing silica-supported sulfonic acid (SCX) with any unreacted reagents simply passing through to waste. A brief washing sequence (MeOH, 0.4 ml/min) was used to elute any residues prior to release the product by passage of 3% NH_3 in MeOH (3% NH_3 solution, 0.1 ml/min). The product stream was finally eluted and then evaporated *in vacuo* affording (+)-dumetorine (227 mg) with 92% yield and purity >95% assessed by ^{1}H NMR and UPLC-MS. Optical rotation was consistent with those reported in literature $[\alpha]^{25}_D$ + 38 (c 1, CHCl$_3$), [lit. $[\alpha]^{25}_D$ + 37 (c 1, CHCl$_3$)].

This new flow protocol clearly shows remarkable advantages if compared to the existing procedure consisting in two separate steps of Boc removal and reductive amination:

- Total conversion of starting material in (+)-dumetorine
- Simultaneous deprotection and methylation (2 reactions in 1 step)
- No formation of 1,4 addition by-product

Summarizing, the entire process for the multistep flow-based synthesis of (+)-dumetorine was accomplished according to Scheme 44.
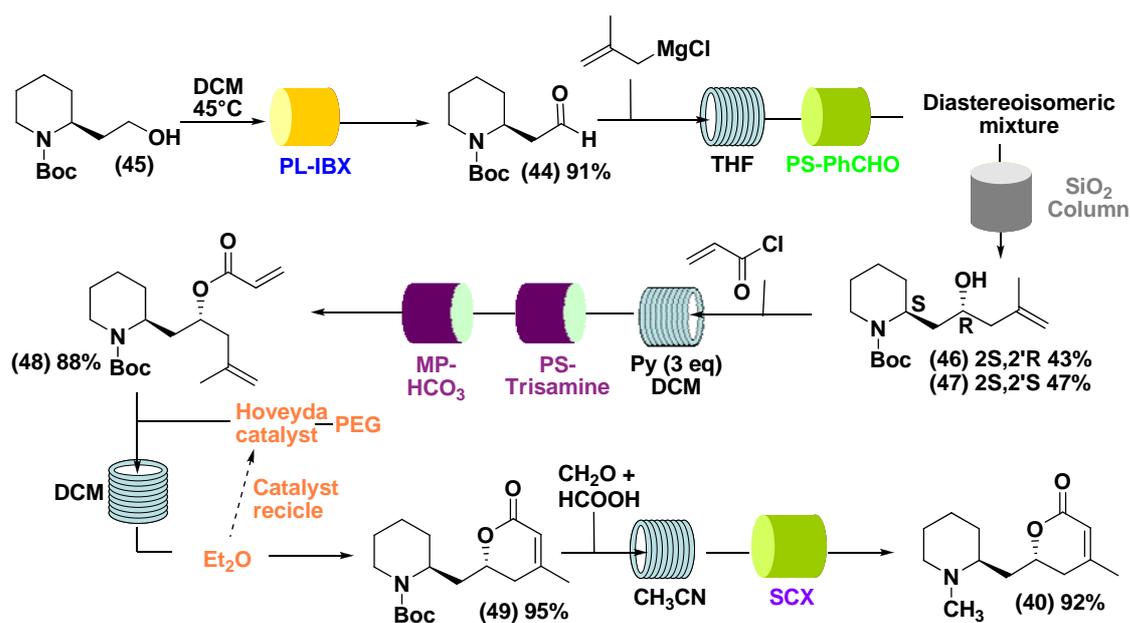
Scheme 44. Total synthesis of (+)-dumetorine

Our flow strategy comprised the combination of 5 separated synthetic steps linked into one continuous sequence to devise a more concise and efficient synthetic pathway.

Remarkable results were obtained in the Grignard reaction, performed at room temperature in an efficient and safe manner avoiding cryogenic temperature; in the RCM reaction, carried out with a PEG-supported Hoveyda catalyst and finally in the unprecedented flow Eschweiler-Clarke *N*-methylation with concomitant Boc deprotection.

Packed columns containing appropriate immobilized reagents, scavenger materials or catch and release agents were used to minimize handling, work-up and purification and to ensure the quality of the final product. Most of the reactions were carried out exploiting solvent superheating. Moreover for synthesizing (+)-dumetorine, new protocols were developed for performing classical reactions under continuous flow conditions.

What is also significant in this work is that just one product purification by flash chromatography was necessary and no use of labour intensive techniques was required to furnish materials of appropriate quality in order to progress to the next step in the synthesis.

This flow total synthesis also represents a significant improvement over the existing protocol. The main advantages and improvements are listed below:

- High yields in all the performed reactions (>85%).
- Purity > 95% (LC/MS) for all intermediates and the final compound.

- Overall yield: 29% (65% for the diastereoisomeric mixture): 227 mg of (+)-dumetorine were obtained starting from 800 mg of **44**.
- Higher yield and lower number of steps compared to batch synthesis.
- Only one chromatographic column in all the process necessary for the separation of the two diastereoisomers.
- New protocols for flow reaction developed for the first time:
  -RCM reaction carried out with a newly synthesized PEG-supported Hoveyda catalyst.
  -Unprecedented flow Eschweiler-Clarke reaction with concomitant Boc deprotection.

This synthesis clearly demonstrates how flow chemistry technique is of great advantage in the assembly of challenging molecules as (+)-dumetorine, in terms of overall yield, reaction time and limitation of handling and purification.

7.2. Multistep synthesis of (+)-dumetorine natural congeners

Planned to exploit the flow procedure for the synthesis of other natural congeners and we selected (+)-sedridine **41** and (-)-sedamine **43** as model targets (Figure 28). The synthesis of the two natural alkaloids was assessed with good results using the same aldehyde **44** as starting material. In the case of (+)-sedridine the synthesis was accomplished by Grignard addition and removal of the Boc protective group. The synthesis of (-)-sedamine is based on a Grignard addition and Eschweiler Clarke reaction as reported for (+)-dumetorine.
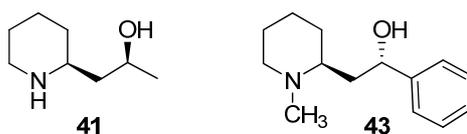
Figure 28. (+)-sedridine (41) and (-)-sedamine (43)

7.2.1. Flow synthesis of (+)-sedridine

(+)-sedridine is a natural piperidine alkaloid isolated from *Sedum acre*.

The addition of the methylmagnesium bromide to **44** was performed using the optimized mild conditions previously described (see Chapter 5).

The THF stock solutions of the Grignard reagent and of **44** were simultaneously pumped at room temperature into the flow apparatus with a residence time of 33 min (Scheme 45).

The reaction stream was then directly passed through a short column containing polymer-supported benzaldehyde[80] for the scavenging of the excess of Grignard reagent.

Scheme 45. First step: Grignard Addition

The output of the reactor was then evaporated and, after an aqueous work-up, it was purified by silica gel chromatography to give the distereoisomer **50** in 49% yield. Also in this case the silica gel chromatography represented the only purification in the process and was required for the easy isolation of both the diastereoisomers (94% for both the isolated diastereoisomers; diastereoisomer **51** isolated in 45% yield)

Such results represented an additional demonstration of the good reproducibility of the Grignard reaction under flow reaction conditions.

Differently from (+)-dumetorine, this natural product does not presents an alkylated piperidine ring.

The Boc removal was achieved by thermal decomposition at high temperature in acidic conditions; two acetonitrile solutions, the first containing **50** and the second HCl (dioxane 4.0 M solution, 1 equivalent) were simultaneously pumped into the PTFE flow reactor. The reaction occurred in 15 min at a temperature of 70°C (Scheme 46).
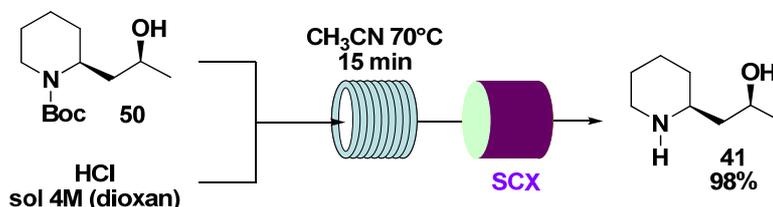
Scheme 46. Second step: Deprotection

(+)-sedridine was purified by the in-line 'catch and release' purification. The reaction mixture was directly passed through a column containing SCX and after a brief washing sequence (MeOH, 0.4 ml/min) the product was released by passage of 3% NH_3 in MeOH (3% NH_3 solution, 0.1 ml/min).

The deprotection occurred in 98% yield and (+)-sedridine was obtained as free base in high purity just removing the solvent under *vacuum*.

In the Scheme below the complete process for the flow synthesis of (+)-sedridine (overall yield 48%) is summarized:
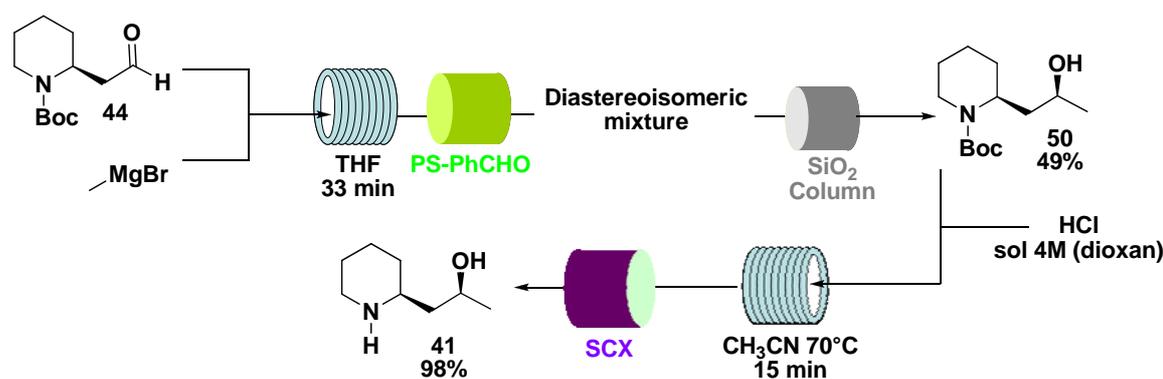
Scheme 47. Flow synthesis of (+)-sedridine

7.2.1. Flow synthesis of (-)-sedamine

(-)-sedamine, an interesting 2-piperidinyl alkaloid, was isolated in *Sedum* species as *Lobelia Inflata* that furnished a crude extract useful for the treatment of respiratory illnesses such as asthma, bronchitis, and pneumonia.

The formation of the alcohol from aldehyde **44** was performed as usual and occurred smoothly in the continuous flow system. As in a typical flow experiment, the Grignard reaction was performed using phenylmagnesium bromide in half an hour at room temperature (Scheme 48) and also in this case the desired alcohol **52** was efficiently obtained as diastereoisomeric mixture.
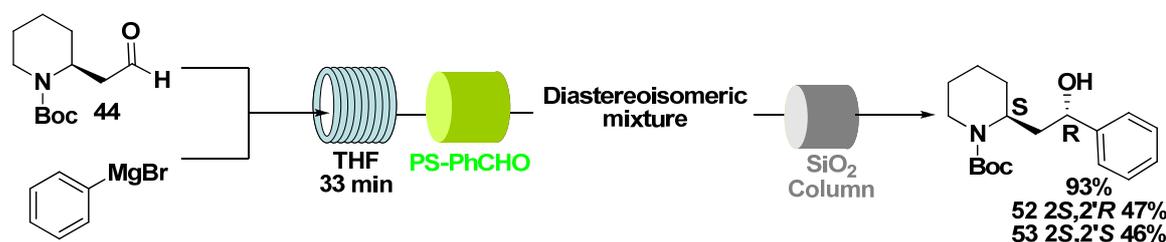
Scheme 48. First step: Grignard addition

After the in-line purification with PS-benzaldehyde and aqueous work-up, the desired diastereoisomer was isolated by chromatographic column (47% yield; diastereoisomer 46% yield).

As mentioned above, the methylation of the nitrogen nucleus was obtained with deprotection and concomitant Eschweiler Clarke reaction. As for the (+)-dumetorine synthesis, the solution containing **52** dissolved in CH$_3$CN was combined with the stream of formaldehyde and formic acid in acetonitrile and flowed into the PTFE reactor. Here the Boc removal and the reductive amination occurred at 180°C in 10 min with the total conversion of the starting material into (-)-sedamine **43** (Scheme 49).
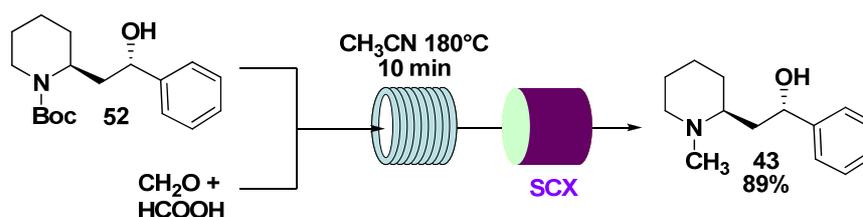
Scheme 49. Second step: Deprotection and concomitant Eschweiler Clarke reaction

As usual a backpressure regulator was connected in-line at the end of the reactor configuration, allowing the solvent superheating. The reaction was performed at 180°C and not at 140°C (as in the (+)-dumetorine protocol) because a higher temperature showed to improve the overall yield and purity of this transformation in a shorter reaction time.

(-)-sedamine was purified by the usual in-line 'catch and release' purification through a SCX containing Omnifit glass column. After solvent evaporation, (-)-sedamine was obtained with 89% yield.

Scheme 50 illustrates the total flow synthesis of (-)-sedamine:
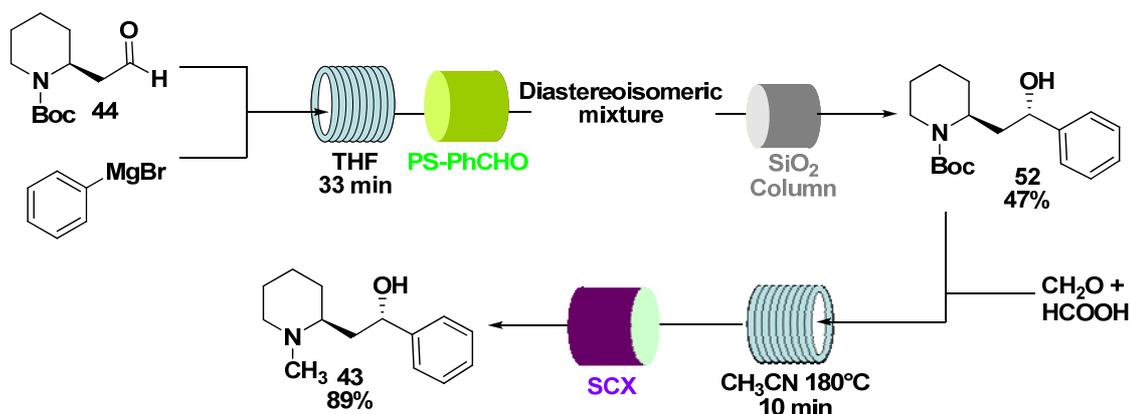
Scheme 50. Flow synthesis of (-)-sedamine

The synthesis of these natural products assessed the effectiveness and reproducibility of the protocols developed during the previous synthesis of (+)-dumetorine.

Chapter 8.
Synthesis of supported catalysts for Metathesis Reactions

8.1. Introduction

Facile catalyst separation and recycle of the catalyst are of prime importance both in industrial and academic field. From the viewpoint of atom economy, a catalyst should ideally be completely recoverable and reusable. In this respect, immobilized or heterogeneous catalyst offer inherent operational and economical advantages over their homogeneous counterparts.

Key issues associated with supported catalysts are:

- preservation of high activity, (enantio-) selectivity and reaction rates observed with homogeneous catalysts
- ease of catalyst separation
- multiple catalyst recycling
- metal- and contaminant-free final products, particularly important in pharmaceutical chemistry

8.2. Synthesis of PS-supported Grubbs catalysts

Metathesis belongs nowadays to the most potent C–C double bond forming reactions,[106] allowing the synthesis of both well-defined, functional polymers and complex architectures including medium-sized and large ring structures. The findings that these reactions can be carried out in an enantioselective way[107] widened the range of applications from commodity chemicals to the synthesis of chiral compounds relevant to pharmaceutical chemistry.

As explained before, in our opinion supported Ru catalyst could offer the access to the development of Ring Closing Metathesis under continuous flow conditions characterized by high reaction performance, easy purification and the possibility of catalyst reusing.

On the basis of our experience in the use of polymer supported reagents and scavengers, we thought as a first approach to prepare polymer supported Grubbs catalyst of 1st and 2nd generation.[108]

Our idea was performing Ring Closing Metathesis simply passing the reaction mixture through a column filled with a polymer supported catalyst (Scheme 51).
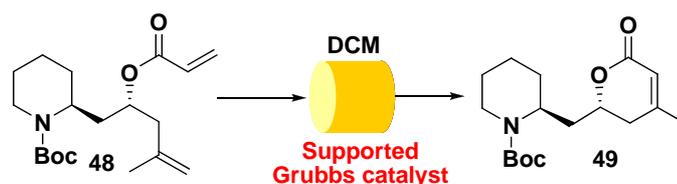
Scheme 51. Flow RCM with Solid Supported Grubbs catalyst

Furthermore, such polymer-supported catalysts seem ideally suited for recycling purposes without the need for chromatography or aqueous work-up regime, thereby avoiding the need for removal of heavy metals from aqueous waste. A drawback could be a lower catalytic activity than its homogeneous counterpart.

The immobilization of metathesis catalysts on standard supports such as silica or polystyrene-divinylbenzene (PS-DVB) has already been addressed by many groups.[109]

Firstly we prepared a vinyl polystyrene supported Grubbs of 1st generation described by Barrett and co-workers.[110]

This polymer-bound catalyst is a 'boomerang' supported catalyst. The "boomerang" mechanism involvs firstly the catalyst release into the solution where the RCM occurs as in the homogeneous catalyst scenario, with concomitant reaction rate advantages, and finally the ruthenium recapture by the styrene vinyl groups of the polymer.

Polymer-supported ruthenium catalyst was obtained by simply shaking a mixture of the Grubbs carbene (approx. 10 mol% based on calculated vinyl resin sites) and vinyl polystyrene for 1-2 h in CH_2Cl_2 and it was isolated as orange-brown beads by filtration. The washings from the filtration were essentially colourless indicating complete incorporation of the Grubbs catalyst onto the support (Scheme 52).
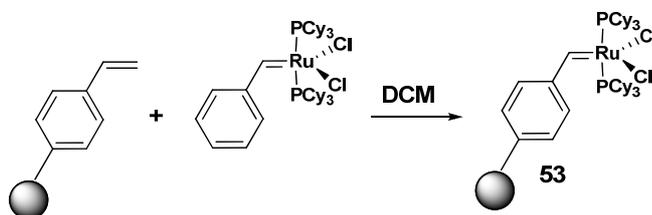
Scheme 52. Synthesis of vinyl polystyrene supported Grubbs of 1st generation

Polymer-supported catalyst was employed for RCM of representative diene substrate **54** (*N,N*-diallyl-*p*-toluenesulfonamide) firstly under traditional solid phase reaction conditions (Scheme 53). Polymer-supported catalyst **53** was suspended in dichloromethane, the diene added and the

mixture shaken for 40 min followed by filtration. The washings were concentrated to provide directly the desired RCM product (Scheme 53).
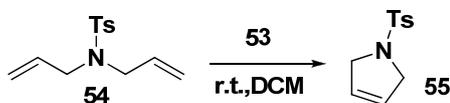
Scheme 53. RCM on model substrate

N,N-diallyl-*p*-toluenesulfonamide was quantitatively converted to the ring closed product in 40 min (r.t.).

The same experiment was conducted on dumetorine intermediate **48** but without obtaining any conversion of the starting material into the desired product (48h, DCM reflux,). Probably this supported Grubbs 1st generation catalyst performs well with simple dienes but is not suitable for more hindered substrate.

Moreover, the 'boomerang systems' could be not ideal because they are released into the solvent during reaction and therefore are prone to elution. At the beginning we thought to solve this problem putting after the column containing the supported catalyst another one filled with vinyl polystyrene resin for the in-line re-catching of the catalyst, but the not satisfactory results obtained on our substrate in batch conditions led us to change our approach.

We then decided to prepare a PS-DVB permanently bound Grubbs 2nd generation catalyst to increase the intrinsic activity of the catalyst and have a system more suitable for continuous flow conditions.

Buchmeiser et al. recently reported on the synthesis of various ruthenium-based metathesis catalysts prepared by the replacement of both chlorine atoms by carboxylates and other ligands containing electron-withdrawing groups. In particular, when fluorinated carboxylates were used, a dramatic increase in reactivity was observed.

A PS-DVB Grubbs 2nd generation catalyst **58** was prepared via replacement of both chlorines by trifluoroacetate groups according to the procedure described by Buchmeiser (Scheme 54).[111]

1,3-Hydroxymethylpolystyrene (PS-DVB-CH_2-OH, 1.7 mmol of CH_2OH/g, cross-linked with 1% DVB) was reacted with perfluoroglutaric anhydride giving **56**. The prepared silver salt of **56** was shaken in the presence of the Grubbs catalyst of 2nd generation obtaining the first replacement of one chlorine substituent; the second was assessed by stirring the supported catalyst and CF_3COOAg in THF. The supported catalyst **58**, filtered and dried in *vacuo,* was obtained as a red-lilac powder.
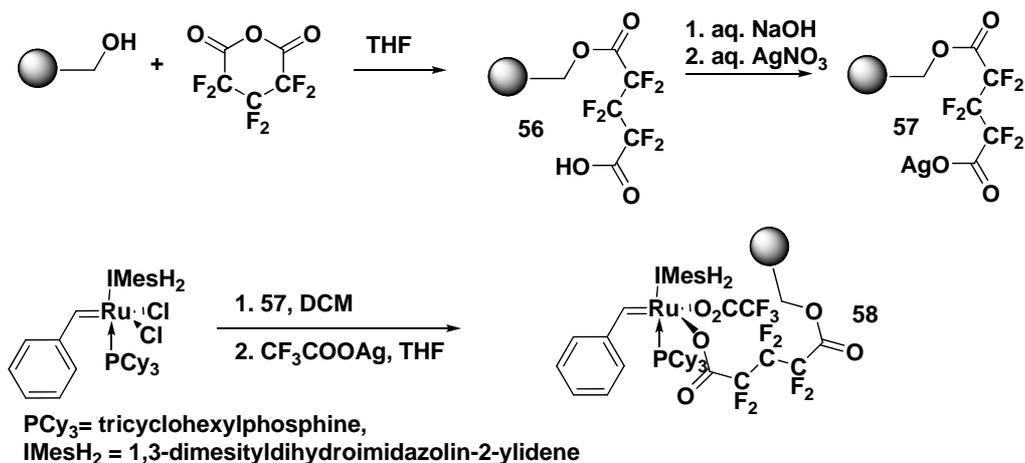
Scheme 54. Synthesis of PS-DVB Grubbs 2nd generation catalyst

Catalyst **58** was tested on the model substrate **54** and on our intermediate **48** under traditional solid phase reaction conditions.

In both cases we observed just a partial conversion (about 25%) of the starting material into the close product (from 6 to 48 h, DCM reflux).

This poor result led us to abandon the idea of testing the PS-DVB supported Grubbs 2nd generation catalyst in flow RCM.

8.3. Synthesis of PEG-supported Hoveyda catalyst

In order to overcome the above mentioned problems encountered with PS-Grubbs catalyst, we evaluated the application of a homogeneous PEG supported Grubbs type catalyst in order to increase the performance in RCM maintaining the possibility of a simple catalyst recovery. PEG has been used to immobilize metathesis catalysts in several ways.[112]

Taking inspiration by two works of Yao,[103] a newly synthesized PEG-Supported Hoveyda catalyst was prepared in collaboration with Prof. M. Benaglia and Dr. A. Caselli (Università degli Studi di Milano).

Catalyst **64** was readily assembled as shown in Scheme 55.
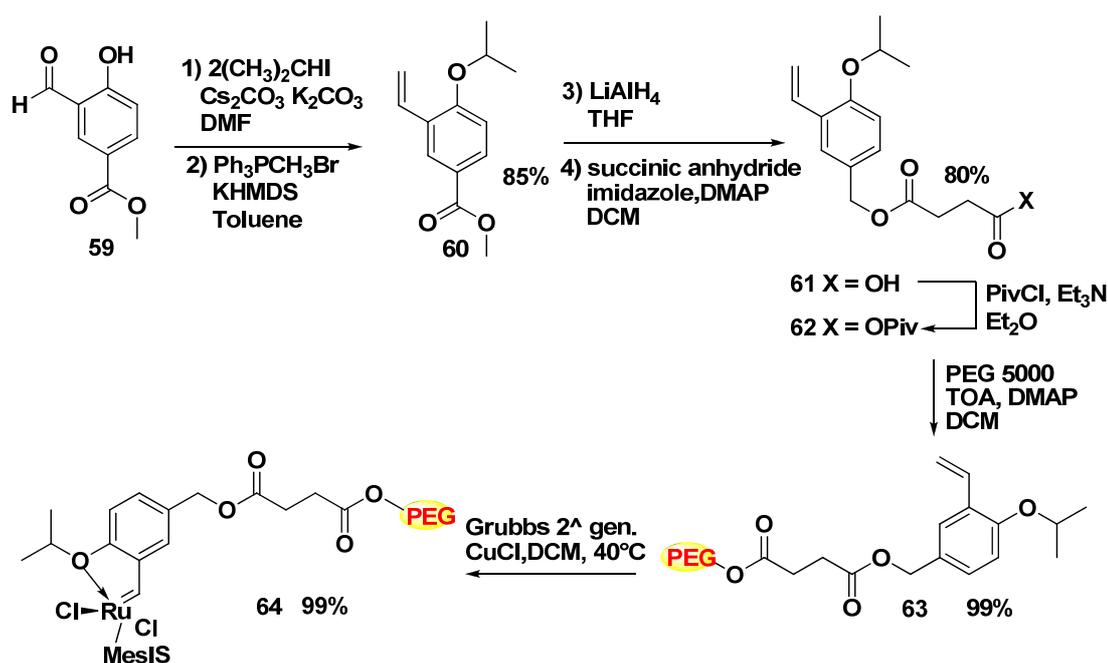
Scheme 55. Synthesis of PEG-Supported Hoveyda catalyst

The isopropoxystyrene obtained from the reduction of **60**, was firstly treated with succinic anhydride in the presence of imidazole and DMAP to give the acid **61**. This compound was then coupled to PEG 5000, through the mixed acid anhydride intermediate **62** affording the PEG-bound ligand **63** in quantitative yield based on 300 MHz ^{1}H NMR spectroscopic analysis as well as on the mass increase of the polymer.

Treatment of **63** with the Grubs 2nd generation catalyst in the presence of CuCl[113] resulted in a clean and quantitative exchange of the styrene ligand as determined by ^{1}H NMR spectroscopy to give **64** as an air stable deep green powder in 99% yield (loading 0.2 mmol/g, stable for 6 months at r.t.).

The activity of **64** was then evaluated with the model diene **54**. *N,N*-diallyl-*p*-toluenesulfonamide underwent RCM in the presence of 3 mol% Ru of **64** in DCM at 40°C in 1 hour to give the cyclic olefin in excellent conversion. Under flow condition the reaction took place in 20 minutes at 70°C in DCM. The catalyst was easily recovered after precipitation with Et$_2$O and importantly the recycled material was reused for up to 6 cycles with only a very slight decrease in activity after each recycling and reuse (Scheme 56; Table 17). The conversion (determined by UPLC-MS analysis) was still exceeding 90% even after 6 consecutive cycles.
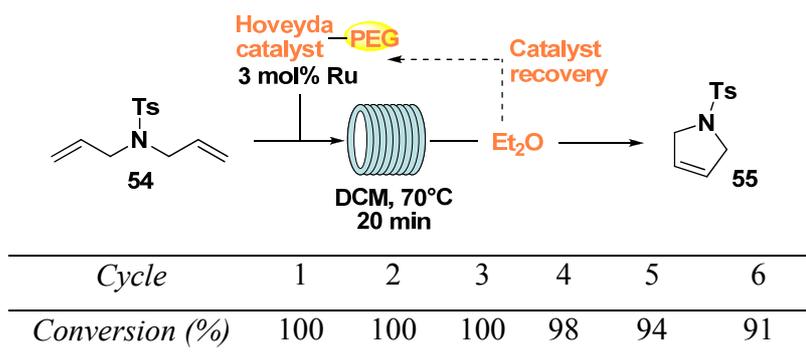
Scheme 56; Table 17. Recycling and reuse of PEG-bound Ru catalyst 64 in the RCM of test diene under flow reaction conditions

Cycle	1	2	3	4	5	6
Conversion (%)	100	100	100	98	94	91

An equivalent performance was obtained with dumetorine intermediate **48**. Its total conversion into lactone **49** was assessed in 3 hours and in the presence of 6 mol% Ru of **64** in refluxing DCM. Again the RCM reaction using this PEG-supported catalyst RCM was successfully performed under flow condition and we observed the total conversion of our starting material in the desired product in 50 minutes at 70°C (DCM superheating). The pure compound was simply obtained by evaporation of the solvent after the precipitation of the catalyst in presence of Et$_2$O.

With the aim at setting up a protocol for the RCM step of the total synthesis of (+)-Dumetorine, we developed a stable, readily recyclable and reusable, PEG-supported Hoveyda catalyst. To broaden the use of catalyst **64** and the reproducibility of our flow RCM protocol, in the future we will investigate the performance of **64** in flow RCM on differently substituted dienes and on its use in flow Cross Metathesis.

Chapter 9.
Conclusions

Despite the rapid development of synthetic methodologies during last decades, chemists have been using essentially the same equipment to run reactions for the last several hundred years.

Round-bottom flasks and complementary large-scale batch reactors remain the cores of modern fine chemical and pharmaceutical synthesis. Using these traditional synthetic methods, organic chemists can have access to almost any organic molecule successfully, however, it is becoming clear that this *modus operandi* is not sustainable and new chemical technologies leading to more efficient processes should be developed. Microreactors offer a realistic solution to this issue because, as described in this thesis, they are inherently less wasteful than traditional techniques and because they provide an unprecedented reaction control.

Continuous processing is common in bulk chemical production but is somewhat uncommon in fine chemical and pharmaceutical synthesis.

Organic chemists have not fully embraced this technology, and just in recent years bench chemists started the development of flow devices for designing new synthetic methodologies. Along with the birth of new laboratory flow reactors different research groups both from academia and from industry started the exploration of possible applications of this technique for laboratory scale organic synthesis.

In the first part of this thesis the unique characteristics of microreactors that make them efficient tools for organic chemistry were investigated using as models two classical, single-step, organic transformation as the Grignard reaction and the preparation of hydroxamic acids.

These examples clearly demonstrated as reactions performed under flow conditions can become faster, cleaner and safer. It was shown how microreactor offers a precise control of reaction parameters including temperature, time, mixing, and permits to use small amounts of precious compounds to rapidly screen a variety of conditions and find the optimal settings. In our experience the use of flow technique gives access to experimental conditions that are often inaccessible with conventional laboratory equipment, exploiting for instance the solvent superheating.

The established protocols were successfully applied to the synthesis of SAHA and Tramadol, two well know commercialized drugs, improving the known procedures for their synthesis

In the second part of this PhD thesis, the synthesis of more complex and synthetically challenging natural compounds was accomplished using this technique. Up to now, the potentiality of multi-

step synthesis in a single continuous flow sequence is not yet fully exploited as very few examples are available in literature.

Our synthesis clearly demonstrates how flow chemistry is of great advantage in the assembly of molecules such as (+)-dumetorine, in terms of overall yield, reaction time and limitation of handling, work-up and purification.

As conclusion, flow chemistry is an attractive new chemical technology for the design of innovative protocol and processes and organic chemistry, in general, can take advantages of this new approach.

Whether or not micro reactors will replace round bottomed flasks remains to be seen; however, the current evolution of this technology appears promising and flow chemistry is emerging as a novel method to build new processes in which the desired product can be obtained in higher yield, purity, and in shorter periods of time if compared with traditional batch reactions.

Chapter 10.
Synthesis of fluorescent compounds for the study of microtubules

10.1. Introduction

During the second and third year of my PhD, I spent few months in the laboratory of Prof. J. S. Snaith in the University of Birmingham (UK) working for a common project, in a different field respect to usual one of my thesis. This research was developed under the umbrella of the COST Actions CM 0602 *("Inhibitors of Angiogenesis: design, synthesis and biological exploitation")*.

10.2. Synthesis of fluorescent derivatives of tiocolchicine

During my second year, in the framework of a wide project for the structural modification of compounds with antitumor activity we conjugated some near infrared fluorophores to a thiocolchicine derivative.

In literature there are few examples about the synthesis of fluorescent or radioactive derivative of different antitubulinic compounds.[114]

Thiocolchicine[115] differs from colchicine owing to the presence of a methylthio group instead of a methoxy at C10 in the tropolonic ring and is readily available from colchicine (Figure 29).
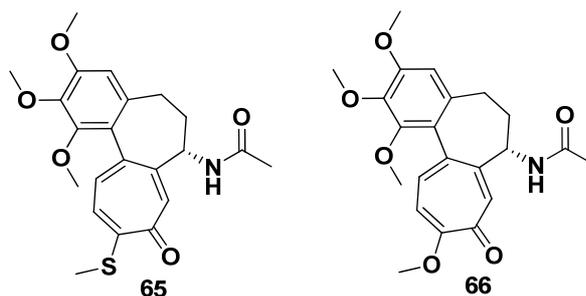
Figure 29. thiocolchicine (65) and colchicine (66)

Colchicine is a plant alkaloid derived from autumn crocus (Colchicum autumnale) and is still one of the most effective treatments to relieve the intense pain associated with an acute gout attack. Thiocolchicine is able to inhibit microtubule dynamics, by hampering microtubule polymerisation. The consequent microtubule paralysis blocks the release of pro-inflammatory mediators in leukocytes and other inflammatory cells. In cancer cells microtubule-interacting drugs act as spindle poisons, thereby blocking the cell cycle at the M phase and inducing apoptosis. This latter feature underlies the antitumour properties belonging to such agents. However, colchicines, thiocolchicines or analogues haven't been extensively used as anticancer drugs, due to the extreme toxicities and the consequent unfavorable therapeutic index noticed in preclinical experimental models and in clinical trials.

The study of anticancer drugs such as thiocolchicine, taxol, epothilone, podophyllotoxin, cephalomannine and the Vinca alkaloids is an important field of interest for our lab.

Bio-active fluorescent derivatives of those products could be a useful tool in the study of these cellular mechanisms. In particular, in the light of the heightened interest in the use of near-infrared (NIR) fluorescent dyes (max 700–900 nm) for in-cellular and in vivo imaging studies, we proposed to conjugate our thiocolchicine derivative with different types of infrared fluorophores. So, availing ourselves of the experience in this field of Prof. Snaith's group, we have synthesized and characterized fluorescently labeled derivatives of thiocolchicine that could be able to bind to microtubules and have cytotoxicities similar to that of thiocolchicine.

The thiocolchicine derivative **67** was prepared in the lab of Prof. Passarella without the loss of the original properties.

After the deprotection using TFA of this compound (95% yield; Scheme 1), the amine was then conjugated with different fluorophores to give the fluorescently labeled derivatives (Scheme 57).
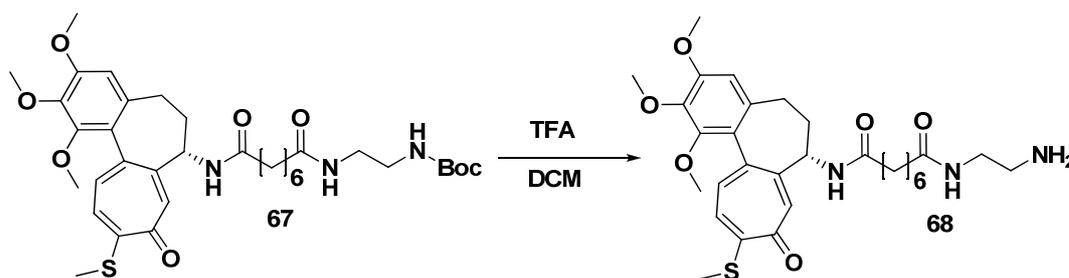
Scheme 57: Deprotection thiocolchicine derivative

Firstly we conjugated the thiocolchicine derivative with Fluorescein isothiocyanate (FITC λ_{ex} 492 nm; λ_{em} 518 nm in 0.1 M phosphate pH 8.0). We became interested in the examination of fluorescein isothiocyanate (FITC) because (a) it is a very commonly used dye in biology; (b) its excitation wave length (488 nm) does not interfere with that of cellular proteins; (c) it is easily accessible and (d) it does not require sophisticated microscopic instruments.
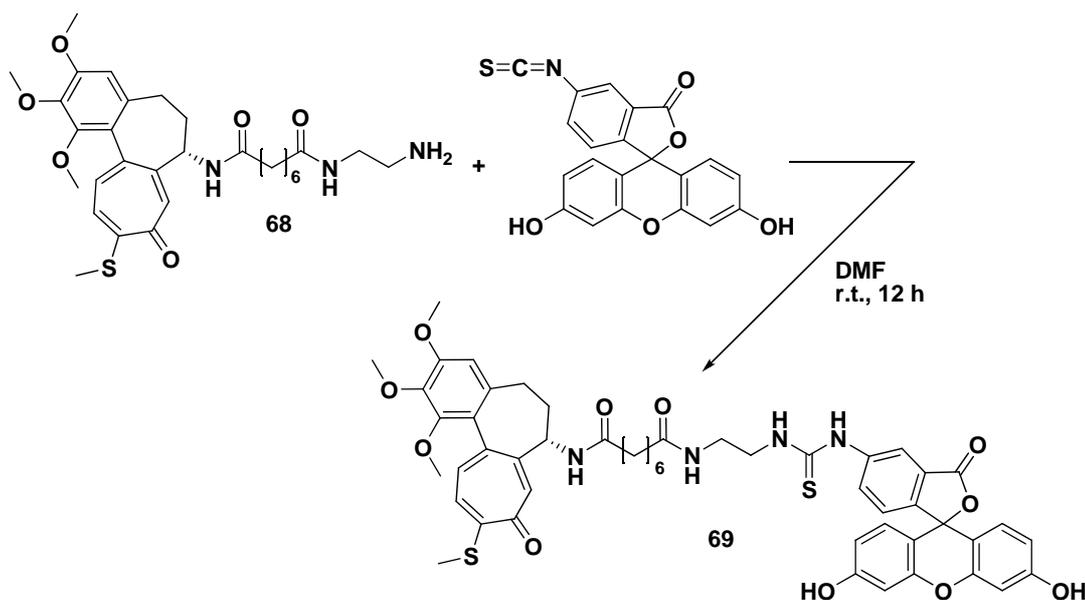
Scheme 58: Synthesis of fluorescein-tagged thiocolchicine derivative

The reaction between the amino group and isothiocyanate group of fluorescein occurred in the dark using DMF as solvent affording the desired fluorescent product **69** (5 mg scale; 80% yield; Scheme 58).

The fluorescent-tagged product was purified by HPLC and characterized by MALDI TOF MS (m/z 960) and ^{1}H NMR. The purity was assessed by HPLC (purity 100%).

The second compound that we synthesized contained, as the fluorescent component, an osmium complex.

The Os complexes were selected for the long wavelength absorption, which allows excitation with wavelengths up to 700 nm. At these wavelengths tissue absorbance and autofluorescence are minimal, and excitation can be accomplished with LEDs or laser diodes.

In particular we conjugated the amino compound with an osmium compound prepared by Prof. Snaith's group. The reaction (5 mg scale; 82% yield; Scheme 59) for the formation of the amide **70** occurred in the dark using DMF as solvent in 48 hours (24 hours at room temperature and 24 hours 60°C).
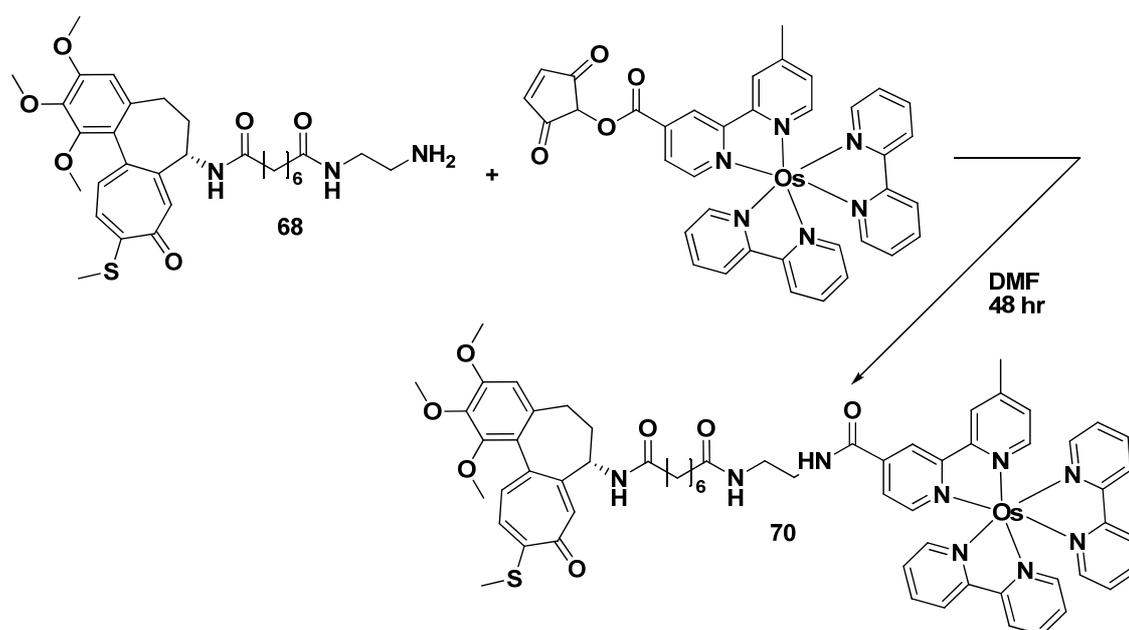
Scheme 59. Synthesis of osmium-tagged thiocolchicine derivative

As for the previous product, the purification of the thiocolchicine derivative labelled with the Os fluorophore was obtained by preparative HPLC and the desired compound was characterized by MALDI TOF (m/z 1271.4) and its purity by HPLC.

We chose NIR 797, a near-infrared cyanine dye for labeling of proteins as third fluorophore that belongs to the class of fluorescent polymethine dyes. This class of dyes is particularly used in a variety of biological applications because of their excellent spectral properties and biocompatibility. The reaction (1 mg scale) was performed in the dark, in buffer pH 9.2 at room temperature for 48 hours (Scheme 60). The formation of the desired product was detected by analytical HPLC and MALDI-TOF (m/z 1428). In the light of the future biological tests on our fluorescein and osmium derivatives, we'll decide to repeat this reaction on a bigger quantity of starting materials in order to obtain the NIR 797 labelled product **71**.
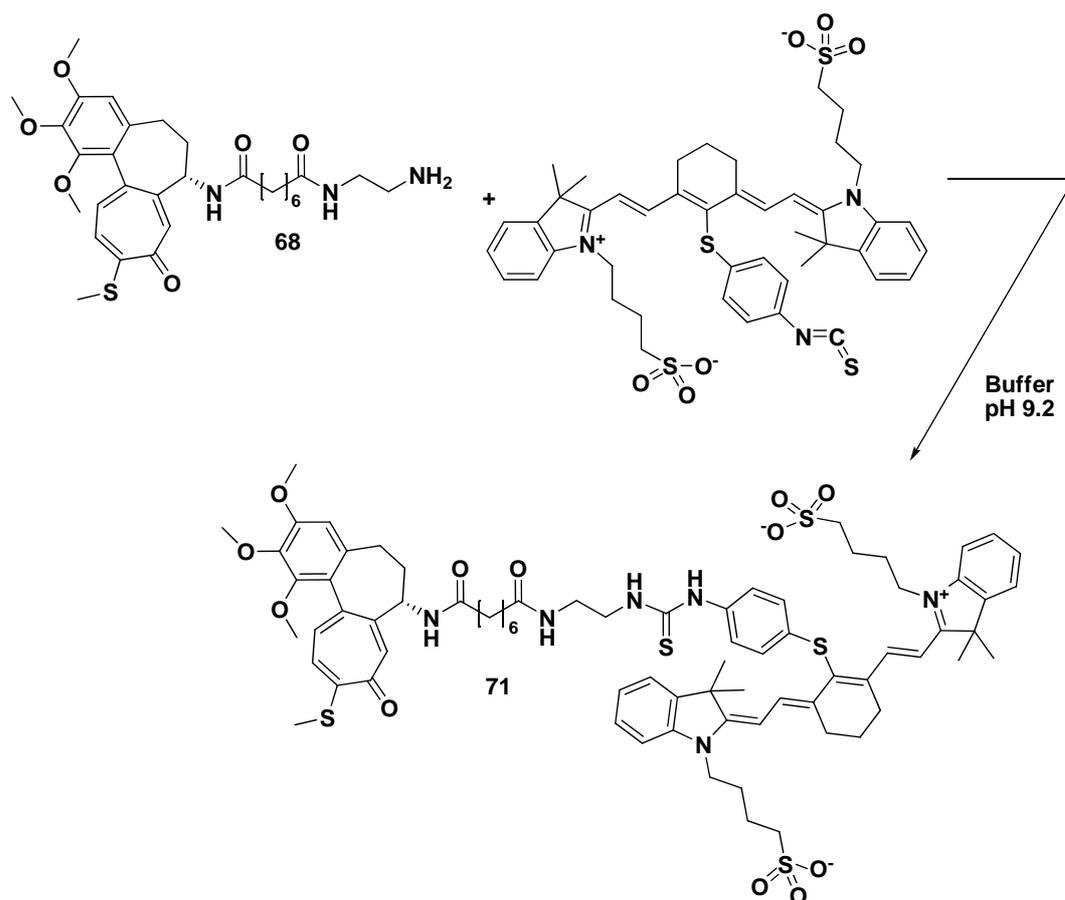
Scheme 60. Synthesis of NIR 797-tagged thiocolchicine derivative

With this work we assessed the possibility to obtain different types of fluorescent thiocolchicine derivatives and the new compounds are now under biological studies that will give us the possibility to evidence their biological behavior.

10.3. Synthesis of fluorescent labelled MPP+ derivative

On the basis of the good results of that we obtained, we then evaluated the possibility of applying ourselves in the preparation of other different luminescent labelled compounds using this effective route.

So during my third year, in collaboration with prof. G. Cappelletti (Università degli Studi di Milano, Biological Department) and again Prof. J. Snaith, we developed the synthesis of fluorescent derivative of MPP+ **72** (Figure 30) for the study of its biological action on microtubules.
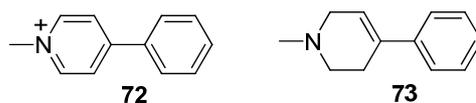
Figure 30. MPP+ (72) and MPTP (73)

MPP+ is the metabolite of MPTP **73** (Figure 30), a neurotoxin responsible of Parkinson's symptoms as tremors, slowness of movements, rigidity etc.[116] In the cell MPP+ has two targets: Complex I of the mitochondrial respiration and microtubules. MPP+ is able to inhibit microtubule dynamics by hampering microtubule polymerisation. The mechanism with which MPP+ destabilizes microtubules is unknown. In particular is not clear if MPP+ interferes directly on microtubules or if the microtubules damage is a consequence of the interaction of MPP+ with Complex I. In fact MPP+ stops the synthesis of ATP and so of GTP in the cells interfering with the normal activity of Complex I.

On the basis of the good results obtained previously in this field, we decided to synthesize a fluorescently labeled derivative of MPP+ in order to prepare a useful tool for the biological study of MPP+ action on microtubules. Availing ourselves of the experience in this field of Prof. Snaith's group, we projected and started to synthesize a derivative of MPP+ that could be able to bind to microtubules and have cytotoxicities similar to MPP+ itself.

The structure of the MPP+ derivative is reported below:

We decided to put a long chain spacer between the anti-tubulinic compound and the fluorophore in order to minimize the influence of this one on the activity MPP+ and to reduce the interaction between the fluorescent label and microtubules.
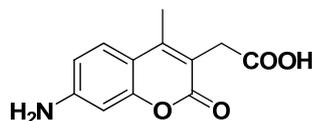
Figure 31. 7-amino-4-methyl-3-coumarinylacetic acid

As fluorophore we chose 7-amino-4-methyl-3-coumarinylacetic acid (Figure 31) that is largely used in the study of proteases and is characterized by excitation and emission wave lengths (UV) that do not interfere with the markers usually employed for mitochondria and microtubules.

Amide links were chosen to join the three different parts of the target derivative in order to avoid the hydrolysis phenomena that occurred into the cell environment.

We also prepared a simply modified derivative of MPP+ that will be tested on cells to analyze the effects that could be generated by a structural modification of MPP+.

We planned the multistep synthetic route reported below (Scheme 61):
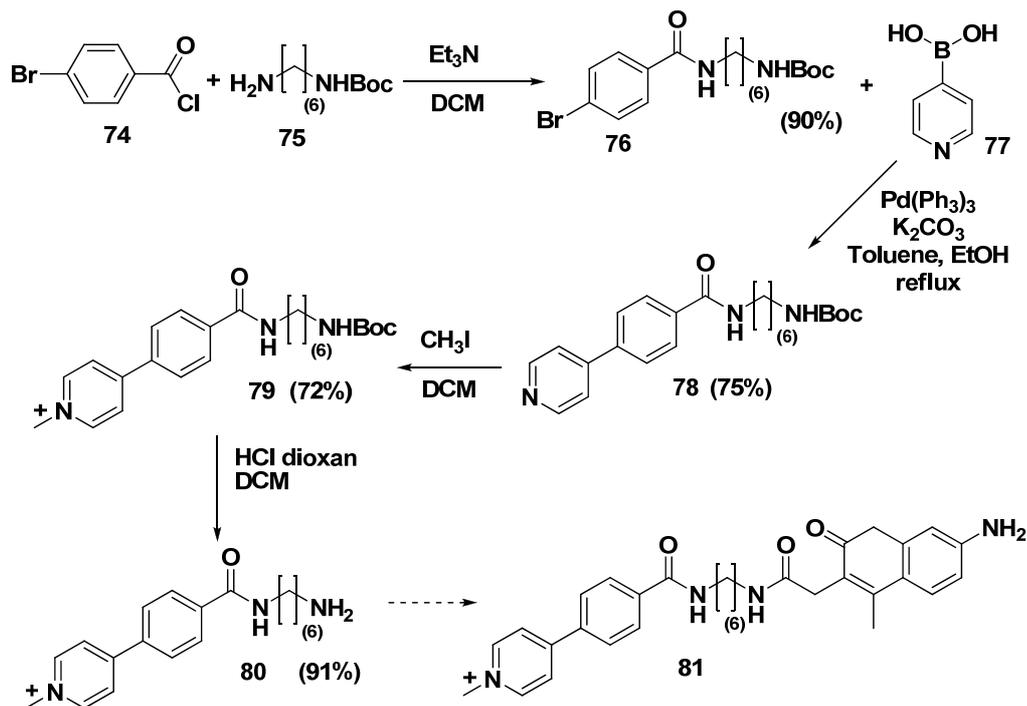
Scheme 61. Synthesis of MPP+ fluorescent derivative

The first step was the preparation of the linker with the mono-Boc protection of hexane-1,6-diamine **76** (Scheme 62):
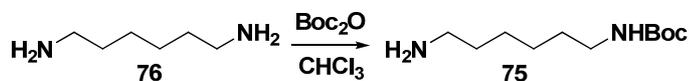
Scheme 62. Deprotection thiocolchicine derivative

So we performed the second step between **74** and the prepared spacer affording the desired compound **76**.

The preparation of the MPP+ nucleus was assessed by a Suzuki coupling between **76** and **77**. On this intermediate compound **78** several proofs of methylation using different solvents were tested and the product **79** was obtained performing the reaction in presence of methyl iodide in DCM.

The deprotection occurred using a dioxan solution 4M of HCl in DCM affording the target compound **80**.

All the reactions were performed before on small scale (20 mg) and then repeated starting the multistep synthesis from a bigger amount of material (800 mg).

In this way we obtained the derivative MPP+ compound that will be reacted with 7-amino-4-methyl-3-coumarinylacetic in order to obtain the tool required for the biological studies.

Using the assessed protocol (Suzuki and consequent methylation), we also obtained the simply modified MPP+ **81** in order to study the modification on the biological activity of the anti-tubulinic compound that a variation in position 1 could cause.
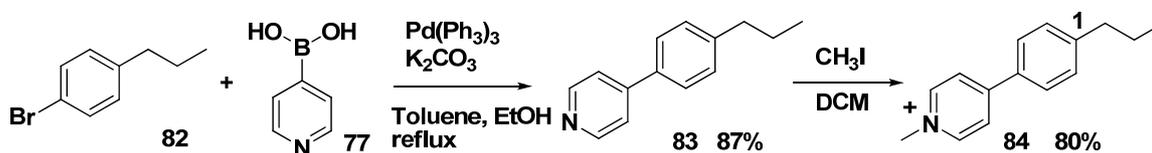
Scheme 63. Synthesis of MPP+ simply modified derivative

With this work we projected a UV fluorescent MPP+ derivative and we started to synthesize it (only one step to the final compound) in order to have a useful tool for biological study that could give us more indications about its mechanism in the depolimerization of microtubules.

Chapter 11.
Experimental section

11.1. Experimental protocol

[1H] spectra were recorded at temperature of 303 K, on a Bruker Avance2 300 Spectrometer at 300.13 MHz (1H) using DMSO-*d6* as solvent. The instrument is equipped with multinuclear inverse probe and temperature controller. Chemical shifts are expressed in ppm, utilizing the solvent peaks as the reference and the coupling constants (J) in Hz. ^{13}C NMR spectra were recorded on the same instrument at 75.47 MHz using DMSO-d_6 as solvent.

LC/MS analyses were performed using an UPLC/MS AquityTM combined with a Micromass ZQTM instrument (column: Acquity BEH C18 2.1X50mm 1.7 um 35°C flow 0.6 mL/min). High resolution electrospray mass spectra (HRESI-MS) were acquired with an FT-ICR (Fourier Transfer Ion Cyclotron Resonance) instrument equipped with a 4.7 Tesla cryo-magnet. Samples were dissolved in CH_3CN and injected into the instrument equipped with its own ESI source. Spectra were recorded in the HR mode with resolutions ranging from 20,000 to 30,000.

Reactions were monitored by TLC using 0.25 mm Merck silica gel plates (60 F254). Flash chromatography was conducted using a Biotage Flash + system and prepacked silica gel columns (KP-SIL, particle size 32-60 μm) or a Biotage Sample Processing Manifold with Isolute Flash cartridges KP-SIL or SCX-2. Anhydrous solvents were purified by MB SPS Solvent Purification System (MBraun).. Unless otherwise specified, solutions of common inorganic salts used in workups are aqueous solutions. Common solvents are abbreviated as follows: DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran. Poly(tetrafluoroethylene) is abbreviated in PTFE.

MW reactions were performed using a Personal Chemistry EmrysTM Optimizer apparatus.

Flow reactions were performed using a combination of R-2 Pump Module and R-4 Reactor Module (Vapourtec[®]).

11.2. Experimental Section of Chapter 4

11.2.1 General Procedure for the synthesis of hydroxamic acid
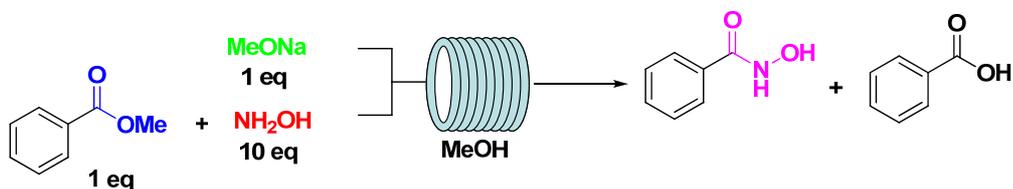
Two 0.5M solutions of starting materials in MeOH were prepared:

Reagent stock bottle A: suitable ester (1 eq); 50% aq. hydroxylamine (10 eq).

Reagent stock bottle B: MeONa, 1 eq.

Using the automated injection system both solutions were transferred at a constant flow rate (0.166 mL/min) onto a preheated PTFE tubing reactor (Reactor Volume 10 mL) maintained at 70°C. The reaction took place into the reactor and the product was collected in an apposite product stock bottle. The solvent was evaporated and the product was purified by the suitable reported method. The reactions were performed on 250 mg scale.

For known products analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.

11.2.2. Experimental for Hydroxamic Acids (compounds 2a-2j)
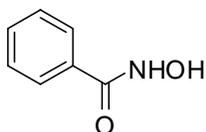
***N*-Hydroxybenzamide (2a):**[117] Starting material: Methyl benzoate; 82% Yield; Off-white solid; Purification: Silica Cartridge (Eluent: CH$_2$Cl$_2$/MeOH from 95/5 to 80/20).
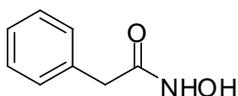
***N*-Hydroxy-2-phenylacetamide (2b):**[117] Starting material: Ethyl 2-phenylacetate; 96% Yield; Off-white solid; Purification: Silica Cartridge (Eluent: CH$_2$Cl$_2$/MeOH from 95/5 to 80/20).
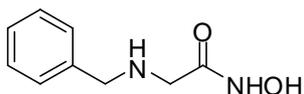
2-(Benzylamino)-*N*-hydroxyacetamide (2c): Starting material: Ethyl 2-(benzylamino)acetate; 96% Yield; Off-white solid; Purification: triturated with 1/1 DCM/MeOH.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.18 (br. s., 2 H), 7.16- 7.36 (m, 6 H), 3.66 (s, 2 H) 3.01 (s, 2 H)

^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 167.60 (s, 1 C), 140.26 (s, 1 C), 128.07 (s, 2 C), 127.86 (s, 2 C), 126.57 (s, 1 C), 52.36 (s, 1 C), 49.13 (s, 1 C)

LC/MS (BPI): Purity 95%

HRMS (ESI): Calcd for $C_9H_{13}N_2O_2$ (+1): 181.09715; Found: 181.09732 error (ppm): 0.9; Calcd for $C_9H_{13}N_2O_2$ (M+Na): 203.07910; Found: 203.07917 error (ppm): 0.7
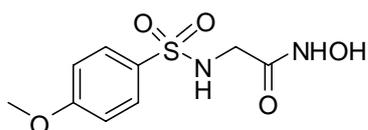
***N*-Hydroxy-2-(4-methoxyphenylsulfonamido)acetamide (2d):**[118] Starting material: Methyl 2-(4-methoxyphenylsulfonamido)acetate; 96% Yield; Off-white solid; Purification: Silica Cartridge (Eluent: CH_2Cl_2/MeOH from 95/5 to 80/20).
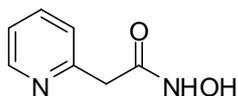
***N*-Hydroxy-2-(pyridin-2-yl)acetamide (2e):** Starting material: Ethyl 2-(pyridin-2-yl)acetate; 97% Yield; Pale rose solid; Purification: SCX Cartridge (Eluent: MeOH/NH$_4$OH from 100/0 to 80/20).

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.75 (br. s., 1 H), 8.90 (br. s., 1 H), 8.37-8.53 (m, 1 H), 7.72 (td, 1 H), 7.28-7.39 (m, 1 H), 7.17-7.28 (m, 1 H), 3.51 (s, 2 H)

^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 166.08 (s, 1 C), 155.93 (s, 1 C), 148.77 (s, 1 C), 136.50 (s, 1 C), 123.69 (s, 1 C), 121.83 (s, 1 C), 42.00 (s, 1 C)

LC/MS (BPI): Purity 98%

HRMS (ESI): Calcd for $C_7H_9N_2O_2$ (M+1): 153.06585; Found: 153.06592 error (ppm): 0.5; Calcd for $C_7H_9N_2O_2$ (M+Na): 175.04780; Found: 175.04792 error (ppm): 0.7
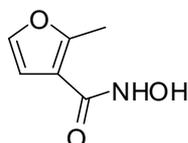
***N*-Hydroxy-2-methylfuran-3-carboxamide (2f):** Starting material: Methyl 2-methylfuran-3-carboxylate; 52% Yield; Yellow solid; Purification: Flash Chromatography (Eluent: CH_2Cl_2/MeOH 95/5).

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 10.69 (br. s., 1 H), 8.83 (br. s., 1 H), 7.49 (d, 1 H), 2.49 (s, 3 H)

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^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 161.06 (s, 1 C), 155.31 (s, 1 C), 140.65 (s, 1 C), 113.31 (s, 1 C), 108.60 (s, 1 C), 12.98 (s, 1 C)

LC/MS (BPI): Purity 98%

HRMS (ESI): Calcd for $C_6H_7N_1O_3$ (M+1): 142.04987; Found: 142.05021 error (ppm): 2.4; Calcd for $C_6H_7N_1O_3$ (M+Na): 164.03181; Found: 164.03211 error (ppm): 1.8
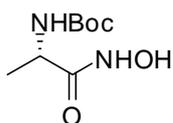
(S)-N-(tert-Butoxycarbonyl)alanine Hydroxamate (2g):[118] Starting material: (S)-N-(tert-Butoxycarbonyl)alanine methyl ester; Quantitative Yield; Off-white solid; Purification: Silica Cartridge (Eluent: CH$_2$Cl$_2$/MeOH from 95/5 to 80/20) $[\alpha]_{20}^{D} = -28.14$ (c 1, MeOH).
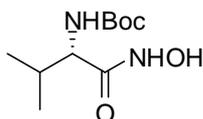
(S)-N-(tert-Butoxycarbonyl)valine Hydroxamate (2h):[119] Starting material: (S)-N-(tert-Butoxycarbonyl)valine methyl ester; 81% Yield; Off-white solid; Purification: Silica Cartridge (Eluent: CH$_2$Cl$_2$/MeOH from 95/5 to 80/20) $[\alpha]_{20}^{D} = -28.78$ (c 1, MeOH). MeOH).
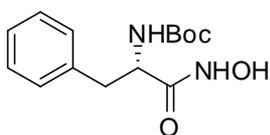
(S)-N-(tert-Butoxycarbonyl)phenylalanine Hydroxamate (2j):[118] Starting material: (S)-N-(tert-Butoxycarbonyl)phenylalanine methyl ester; 84% Yield; Pale rose solid; Purification: Silica Cartridge (Eluent: CH$_2$Cl$_2$/MeOH from 95/5 to 80/20).

11.2.3. Procedure for the scale-up of the synthesis of N-hydroxy-2-phenylacetamide (2e)
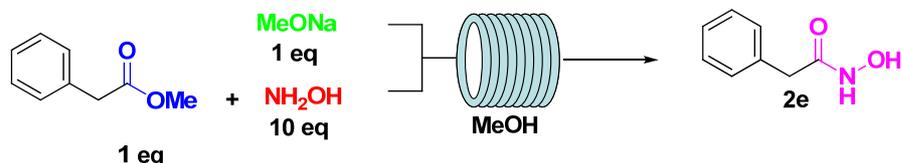
General Reaction Procedure: The flow reactor was configured using a combination of R-2 Pump Module and R-4 Reactor Module (Vapourtec®). Two 0.5M solutions of starting materials in MeOH were prepared:

Reagent stock bottle A: ethyl 2-phenylacetate (4.7 g, 28 mmol, 1 eq); 50% aq. hydroxylamine (17 mL, 280 mmol, 10 eq).

Reagent stock bottle B: MeONa (1.5 g, 28 mmol, 1 eq).

Using the automated injection system, both solutions were transferred at a constant flow rate (0.166 mL/min) to a preheated PTFE tubing reactor (Reactor Volume 10 mL) maintained at 70°C. The flow reactor worked for 1.5 hours. The product was collected in a proper product stock bottle. The solvent was evaporated and the product was purified using a silica cartridge (Eluent: CH_2Cl_2/MeOH from 95/5 to 80/20) to obtain 4.3 g of *N*-hydroxy-2-phenylacetamide. Quantitative yield. OUTPUT = 2.9 g/h.

11.2.4. Procedure for the synthesis of suberoylalanide hydroxamic acid 5 (SAHA)
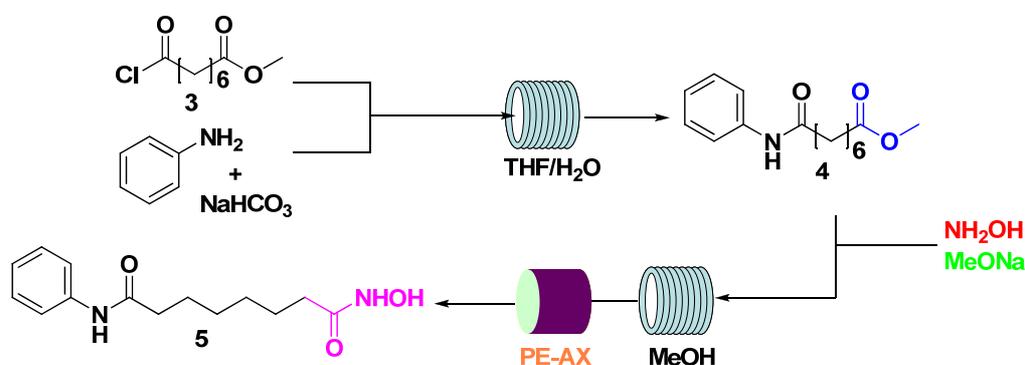
General Reaction Procedure Post Optimization:

First Step. Preparation of Suberanilic Acid Methyl Ester **4**:

The flow reactor was configured using a combination of R-2 Pump Module and R-4 Reactor Module (Vapourtec®). Two 1M solutions of starting materials were prepared:

Reagent stock bottle A: methyl suberoyl chloride (200 mg, 0.97 mmol, 1 eq) in THF.

Reagent stock bottle B: aniline (62.4 mg, 0.97 mmol, 1 eq.); $NaHCO_3$ (81.2 mg, 0.97 mmol, 1 eq); 1/1 THF/H_2O.

Using the automated injection system both solutions were transferred at a constant flow rate (3.5 mL/min) onto a PTFE tubing reactor (Reactor Volume 10 mL) at room temperature (Residence Time 1.4 min). The reaction takes place into the reactor and the product is collected in a suitable product stock bottle. The solvent was evaporated and the crude of methyl suberanilate used for the next step without further purification.

Second Step. Preparation of SAHA (**5**):

The flow reactor was configured using a combination of R-2 Pump Module and R-4 Reactor Module (Vapourtec®). Two tubing reactors (10 mL each) were installed and connected together in

series; the second reactor was connected to an Omnifit® glass column packed with solid supported scavenger. Two solutions of starting materials in MeOH were prepared:

Reagent stock bottle C: methyl suberanilate **4** (255 mg, 0.97 mmol, 1 eq); 50% aq. hydroxylamine (0.58 ml, 9.7 mmol, 10 eq), solution 0.5M.

Reagent stock bottle D: MeONa (105 mg, 1.95 mmol, 2 eq); solution 1M.

Using the automated injection system, both solutions were transferred at a constant flow rate (0.2 mL/min) onto two preheated PTFE tubing reactors maintained at 90°C (Residence Time 50 min). The second reactor was connected to a column (6.6mm id) packed with 1.5 g of silica-supported quaternary amine (ISOLUTE PE-AX®) to trap carboxylic acid, the only by-product of the reaction. After this purification step, the product is collected in an apposite product stock bottle. The solvent was evaporated, 10 ml of water were added and pH was adjusted to 7 with acetic acid. The precipitated solid was filtered, dissolved in hot MeOH and then the solution was cooled to 0°C. The formed solid was filtered to give 219 mg of suberoylalanide hydroxamic acid. 80% Overall Yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm)10.30 (br. s., 1 H) 9.81 (s, 1 H) 8.62 (br. s., 1 H) 7.52 - 7.63 (m, 2 H) 7.19 - 7.33 (m, 2 H) 6.92 - 7.07 (m, 1 H) 2.28 (t, J=7.6 Hz, 2 H) 1.94 (t, J=7.6 Hz, 2 H) 1.41 - 1.66 (m, 4 H) 1.15 - 1.36 (m, 4 H)

Column Preparation: 1.5 g of bulk ISOLUTE PE-AX, acidic excess reagent scavenger (Base material: Silica; Functional Group: Quaternary amine; Capacity: 0.6 meq/g; Counter Ion: Acetate; Biotage®), was transferred to a 6.6mm Omnifit® glass column. The column was eluted with MeOH to obtain a good silica packing before a variable-length end piece was fitted and adjusted to remove solvent gaps and retain the silica supported material under a slightly positive pressure.

11.3. Experimental section of chapter 5

11.3.1. General Procedure for the synthesis of secondary and tertiary alcohols
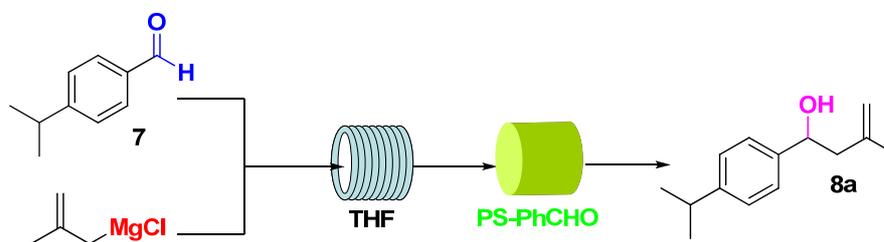
General Optimized Reaction Procedure: Two solutions of starting materials were prepared and stored at room temperature under N$_2$ atmosphere:

Reagent stock bottle A: suitable aldehyde or ketone (1 eq); 200 mg dissolved in dry THF in order to obtain a 0.25 M solution.

Reagent stock bottle B: suitable Grignard reagent (THF or diethyl ether solution; 1.2 eq); dissolved in dry THF in order to obtain a 0.25 M solution.

Using the automated injection system, both solutions were transferred at a constant flow rate (0.3 mL/min) onto a PTFE tubing reactor (reactor volume 10 mL) maintained at room temperature. The reactor was connected to a column (6.6mm id) packed with PS-Benzaldehyde (1 eq) to trap Grignard reagent in excess. After this purification step, the product is collected in an apposite product stock bottle. The solvent was evaporated under vacuum and the crude was partitioned between saturated solution of NH_4Cl and DCM. The organic phase was dried and evaporated and the product was purified by the suitable reported method.

11.3.2. Experimental for secondary and tertiary alcohols

For known products analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.
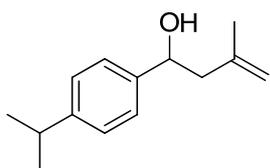
1-(4-isopropylphenyl)-3-methylbut-3-en-1-ol (8a): Starting material: 4-isopropylbenzaldehyde **7**; 92% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9/1).

^{1}H-NMR (300 MHz, CDCl$_3$) δ ppm 7.33 (m, 2 H) 7.23 (m, 2 H) 4.86 - 4.98 (m, 2 H) 4.76 - 4.85 (m, 1 H) 2.93 (spt, J=7.0 Hz, 1 H) 2.39 - 2.53 (m, 2 H) 1.83 (s, 3 H) 1.27 (d, J=7.0 Hz, 6 H)

^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 148.17 (s, 1 C) 142.59 (s, 1 C) 141.52 (s, 1 C) 126.45 (s, 2 C) 125.79 (s, 2 C) 113.81 (s, 1 C) 71.39 (s, 1 C) 48.18 (s, 1 C) 33.81 (s, 1 C) 23.99 (s, 2 C) 22.37 (s, 1 C)

HRMS (ESI): Calcd for $C_{14}H_{20}O_1Na_1$ (+1): 227.14064; Found: 227.14070; error (ppm): 0.4.
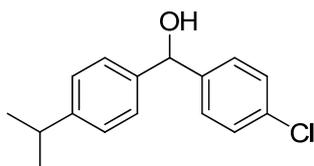
(4-chlorophenyl)(4-isopropylphenyl) (8b): Starting material: 4-isopropylbenzaldehyde **7**; 93% Yield; Off-white solid; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.5/0.5).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.13 - 7.44 (m, 8 H) 5.80 (s, 1 H) 2.92 (spt, 1 H) 2.29 (br. s., 1 H) 1.27 (d, 6 H)

^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 148.62 (s, 1 C) 142.37 (s, 1 C) 140.94 (s, 1 C) 133.15 (s, 1 C) 128.53 (s, 2 C) 127.83 (s, 2 C) 126.71 (s, 2 C) 126.59 (s, 2 C) 75.50 (s, 1 C) 33.81 (s, 1 C) 23.94 (s, 2 C)

HRMS (ESI): Calcd for C$_{16}$H$_{17}$ClO$_1$Na$_1$(+1): 283.08601; Found: 283.08603; error (ppm): 0.2.
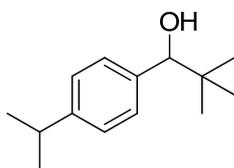
1-(4-isopropylphenyl)-2,2-dimethylpropan-1-ol **(8c):** Starting material: 4-isopropylbenzaldehyde **7**; 87% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.25 (m, 2 H) 7.19 (m, 2 H) 4.40 (s, 1 H) 2.92 (spt, 1 H) 1.61 (br. s., 1 H) 1.27 (d, 6 H) 0.95 (s, 9 H)

^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 139.42 (s, 1 C) 136.28 (s, 1 C) 127.53 (s, 2 C) 125.59 (s, 2 C) 82.33 (s, 1 C) 35.64 (s, 1 C) 33.74 (s, 1 C) 25.94 (s, 3 C) 23.99 (s, 2 C)

HRMS (ESI): Calcd for C$_{14}$H$_{22}$O$_1$Na$_1$(+1): 229.15629; Found: 229.15631; error (ppm): 0.2.
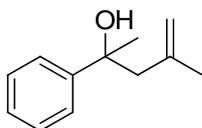
4-methyl-2-phenylpent-4-en-2-ol (10a):[120] Starting material: acetophenone **9**; 94% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).
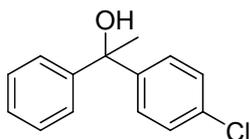
1-(4-chlorophenyl)-1-phenylethanol (10b):[121] Starting material: acetophenone **9**; 95% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).
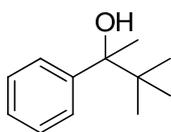
3,3-dimethyl-2-phenylbutan-2-ol (10c):[122] Starting material: acetophenone **9**; 90% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).
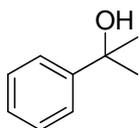
2-phenylpropan-2-ol (10d):[123] Starting material: acetophenone **9**; 95% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.9/0.1).
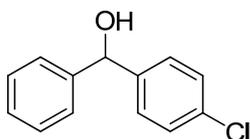
(4-chlorophenyl)(phenyl)methanol (12):[124] Starting material: benzaldehyde **11**; 97% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.7/0.3).
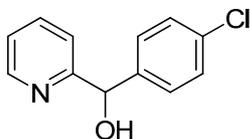
(4-chlorophenyl)(pyridin-2-yl)methanol (14):[125] Starting material: picolinaldehyde **13**; 94% Yield; Colourless oil; Purification: Silica Cartridge (Eluent: from 100% CH_2Cl_2 to CH_2Cl_2/MeOH 95/5).
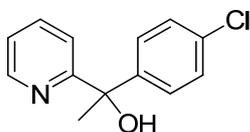
1-(4-chlorophenyl)-1-(pyridin-2-yl)ethanol (16):[126] Starting material: 1-(pyridin-2-yl)ethanone **15**; 92% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.5/0.5).
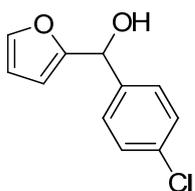
(4-chlorophenyl)(furan-2-yl)methanol (18): Starting material: furan-2-carbaldehyde **17**; 96% Yield; Brown oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.56 (dd, 1 H) 7.34 - 7.50 (m, 4 H) 6.37 (dd, 1 H) 6.17 (dt, 1 H) 6.08 (d, 1 H) 5.73 (d, 1 H)

^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 157.33 (s, 1 C) 142.76 (s, 1 C) 142.11 (s, 1 C) 132.24 (s, 1 C) 128.77 (s, 2 C) 128.47 (s, 2 C) 110.66 (s, 1 C) 106.97 (s, 1 C) 68.14 (s, 1 C)

HRMS (ESI): Calcd for $C_{11}H_9ClO_2Na_1$(+1): 231.01833; Found: 231.01837; error (ppm): 0.4.
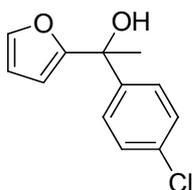
1-(4-chlorophenyl)-1-(furan-2-yl)ethanol (20):[127] Starting material: 1-(furan-2-yl)ethanone **19**; 90% Yield; Yellow oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.7/0.3).
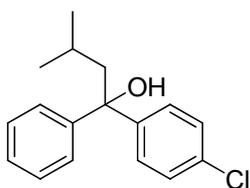
1-(4-chlorophenyl)-3-methyl-1-phenylbutan-1-ol   (22):   Starting   material: 3-methyl-1-phenylbutan-1-one **21**; 95% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.39 - 7.50 (m, 4 H), 7.28 - 7.35 (m, 2 H), 7.23 - 7.28 (m, 2 H), 7.09 - 7.20 (m, 1 H), 5.50 (s, 1 H), 2.17 (d, 2 H), 1.63 (spt, 1 H), 0.77 (d, 3 H), 0.76 (d, 3 H)

^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 149.01 (s, 1 C), 148.45 (s, 1 C), 131.01 (s, 1 C), 128.26 (s, 2 C), 128.21 (s, 2 C), 128.02 (s, 2 C), 126.54 (s, 1 C), 126.20 (s, 2 C), 76.94 (s, 1 C), 49.55 (s, 1 C), 24.91 (s, 2 C), 24.20 (s, 1 C)

HRMS (ESI): Calcd for $C_{17}H_{19}ClO_1Na_1$(+1): 297.10166; Found: 297.10168; error (ppm): 0.2.
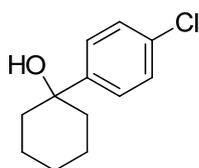
1-(4-chlorophenyl)cyclohexanol (24): Starting material: cyclohexanone **23**; 98% Yield; Off-white solid; Purification: Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.5/0.5).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.45 (m, 2 H) 7.31 (m, 2 H) 1.53 - 1.90 (m, 10 H) 1.18 - 1.43 (m, 1 H)

^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 147.98 (s, 1 C) 132.41 (s, 1 C) 128.24 (s, 2 C) 126.17 (s, 2 C) 72.91 (s, 1 C) 38.81 (s, 2 C) 25.39 (s, 1 C) 22.08 (s, 2 C)

HRMS (ESI): Calcd for C$_{12}$H$_{15}$ClO$_1$Na$_1$(+1): 233.07036; Found: 233.07040 error (ppm): 0.4.
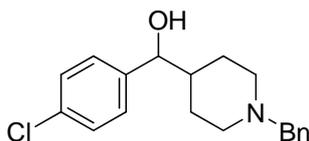
(1-benzylpiperidin-4-yl)(4-chlorophenyl)methanol (26): Starting material: 1-benzylpiperidine-4-carbaldehyde **25**; 90% Yield; Colourless oil; Purification: Flash Chromatography (Eluent: Petroleum ether/AcOEt 9.7/0.3).

^{1}H NMR (300 MHz, DMSO- d_6) ppm δ 7.17 - 7.42 (m, 7 H), 5.19 (d, 1 H), 4.28 (dd, 1 H), 3.39 (s, 2 H), 2.66 - 2.90 (m, 2 H), 1.61 - 1.95 (m, 3 H), 1.33 - 1.52 (m, 1 H), 1.07 - 1.33 (m, 5 H)

^{13}C NMR (75 MHz, DMSO-d_6) ppm δ 144.18 (s, 1 C), 139.24 (s, 1 C), 131.51 (s, 1 C), 129.07 (s, 2 C), 128.84 (s, 2 C), 128.53 (s, 2 C), 128.13 (s, 2 C), 127.17 (s, 1 C), 76.15 (s, 1 C), 62.86 (s, 1 C), 53.69 (s, 1 C), 53.62 (s, 1 C), 43.58 (s, 1 C), 28.63 (s, 1 C), 28.21 (s, 1 C)

HRMS (ESI): Calcd for C$_{19}$H$_{22}$ClONa(+1): 338.12821; Found: 338.12824 error (ppm): 0.3.

11.3.3. General procedure for the synthesis of Tramadol 29
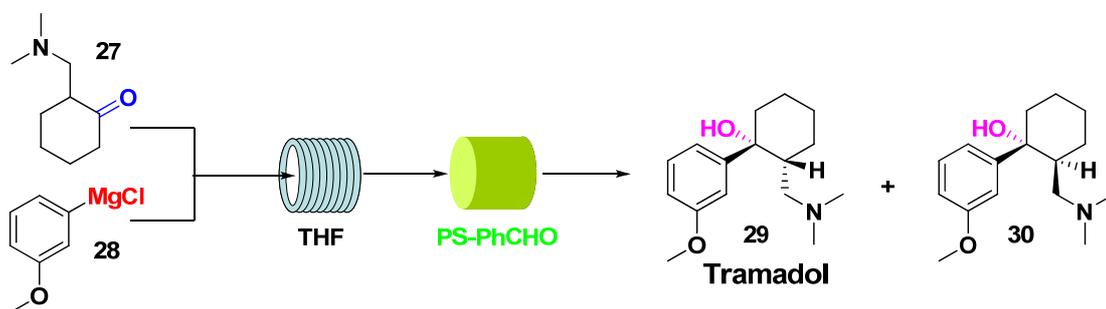
Reaction Procedure: Two solutions of starting materials were prepared and stored at room temperature under N_2 atmosphere:

Reagent stock bottle A: 2–Dimethylaminomethylcyclohexanone[128] (**27**; 1 eq); 200 mg dissolved in dry THF in order to obtain a 0.25 M solution.

Reagent stock bottle B: 3-Methoxyphenylmagnesium bromide (THF) (**28**; 1.2 eq) dissolved in dry THF in order to obtain a 0.25 M solution.

Using the automated injection system, both solutions were transferred at a constant flow rate (0.15 mL/min) onto a PTFE tubing reactor (reactor volume 10 mL) maintained at room temperature. The reactor was connected to a column (6.6mm id) packed with PS-Benzaldehyde (1 eq) to trap Grignard reagent in excess. After this purification step, the product is collected in an apposite product stock bottle. The solvent was evaporated under vacuum and the crude was partitioned between saturated solution of NH_4Cl and DCM. The organic phase was dried and evaporated and the product was purified by Flash Chromatography in order to isolate the product as a diastereoisomeric mixture (96% yield; 8/2 ratio between **29** and **30**).

Water was added and the product was extracted with ethyl ether. The extracts were dried over sodium sulfate, filtered and evaporated in vacuum. The residue was treated ethyl ether saturated with hydrogen chloride; the ethyl ether was evaporated in vacuo and the resulting solid was purified by crystallization from acetone. Tramadol (**29**) hydrochloride was obtained as white crystals.

For known products analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.

11.3.4. Procedure for the synthesis of 4-benzoylbenzonitrile 33
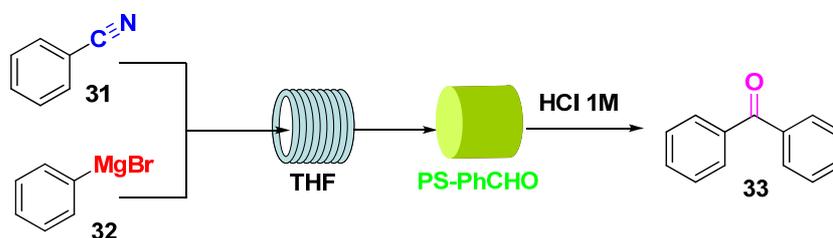
Reaction Procedure 1: Two solutions of starting materials were prepared and stored at room temperature under N_2 atmosphere: Reagent stock bottle A: benzonitrile **(31)** (200 mg; 1.94 mmol) dissolved in 7.8 ml of dry THF. Reagent stock bottle B: phenylmagnesium bromide **32** (diethyl ether solution 1M; 2.33 mmol, 2.33 ml) dissolved in 5.5 ml of dry THF. The system was primed flowing dry THF for 30 minutes. Using the automated injection system, both solutions were transferred at a constant flow rate (0.30 ml/min) onto a preheated PTFE tubing reactor (reactor volume 10 ml) maintained at 50°C (residence time 33 min). The reactor was connected to an Omnifit® glass column (6.6mm id) packed with PS-Benzaldehyde (loading 1.09 mmol/g; 1.78 g) to trap Grignard reagent in excess. After this purification step, the product was directly collected in an apposite product stock bottle containing 1M aqueous HCl. The aqueous phase was extracted by AcOEt and the organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by flash chromatography (eluent: Petroleum ether/AcOEt 9.5/0.5) to obtain 378.3 mg of desired compound as off-white powder. 90% Yield.

Reaction Procedure 2: Two solutions of starting materials were prepared and stored at room temperature under N_2 atmosphere: Reagent stock bottle A: benzonitrile **(31)** (200 mg; 1.94 mmol) dissolved in 7.8 ml of dry THF. Reagent stock bottle B: phenylmagnesium bromide **32** (diethyl ether solution 1M; 2.33 mmol, 2.33 ml) dissolved in 5.5 ml of dry THF. The system was primed flowing dry THF for 30 minutes. Using the automated injection system, the solutions were transferred at a constant flow rate (0.30 ml/min) onto a PTFE tubing reactor (reactor volume 20 ml) maintained at room temperature (residence time 66 min). The reactor was connected to an Omnifit® glass column (6.6mm id) packed with PS-Benzaldehyde (loading 1.09 mmol/g; 1.78 g) to trap Grignard reagent in excess. After this purification step, the product was directly collected in an apposite product stock bottle containing 1M aqueous HCl. The aqueous phase was extracted by AcOEt and the organic layer was dried over Na_2SO_4 and evaporated. The crude was purified by flash chromatography (eluent: Petroleum ether/AcOEt 9.5/0.5) to obtain 382.1 mg of desired

compound as off-white powder. 91% Yield. Analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.

11.3.5. General procedure for the flow addition of benzyl magnesium bromide to aldehydes and ketone in the presence of nitrile
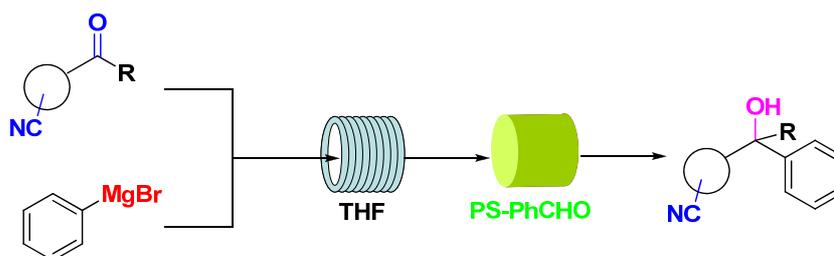
Two solutions of starting materials were prepared and stored at room temperature under N_2 atmosphere: Reagent stock bottle A: suitable aldehyde or ketone (1 eq); 200 mg dissolved in dry THF in order to obtain a 0.25 M solution. Reagent stock bottle B: phenylmagnesium bromide (THF solution; 1 eq); dissolved in dry THF in order to obtain a 0.25 M solution. The system was primed flowing dry THF for 30 minutes. Using the automated injection system, both solutions were transferred at a constant flow rate (0.8 ml/min) onto a PTFE tubing reactor (reactor volume 10 ml) maintained at room temperature (residence time 12.5 min). The reactor was connected to a Omnifit® glass column (6.6mm id) packed with PS-Benzaldehyde (loading 1.09 mmol/g; 1 eq) to trap unreacted Grignard reagent. After this purification step, the product was collected in an apposite product stock bottle. The solvent was evaporated under vacuum and the crude was partitioned between saturated solution of NH_4Cl and DCM. The organic phase was dried and evaporated and the product was purified by the product was purified by the suitable reported method. For known products analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.
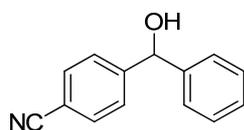
4-(Hydroxy(phenyl)methyl)benzonitrile (35)[129]:

Starting material: 4-formylbenzonitrile **(34)**; 94% Yield; Off-white solid; Purification: flash chromatography (Eluent: Petroleum ether/AcOEt 9.8/0.2).
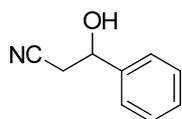
1.1.1. 3-Hydroxy-3-phenylpropanenitrile[130] (37):

Starting material: 3-oxopropanenitrile (36); 85% Yield; Colourless oil; Purification: flash chromatography (Eluent: Petroleum ether/AcOEt 9.9/0.1).
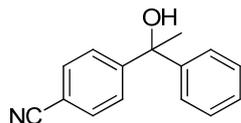
1.1.2. 4-(1-Hydroxy-1-phenylethyl)benzonitrile[131] (39):

Starting material: 4-acetylbenzonitrile (38); 89% Yield; Off-white solid; Purification: flash chromatography (Eluent: Petroleum ether/AcOEt 9.5/0.5).

11.4. Experimental section of chapter 7

11.4.1. Procedure for the synthesis of (+)-dumetorine
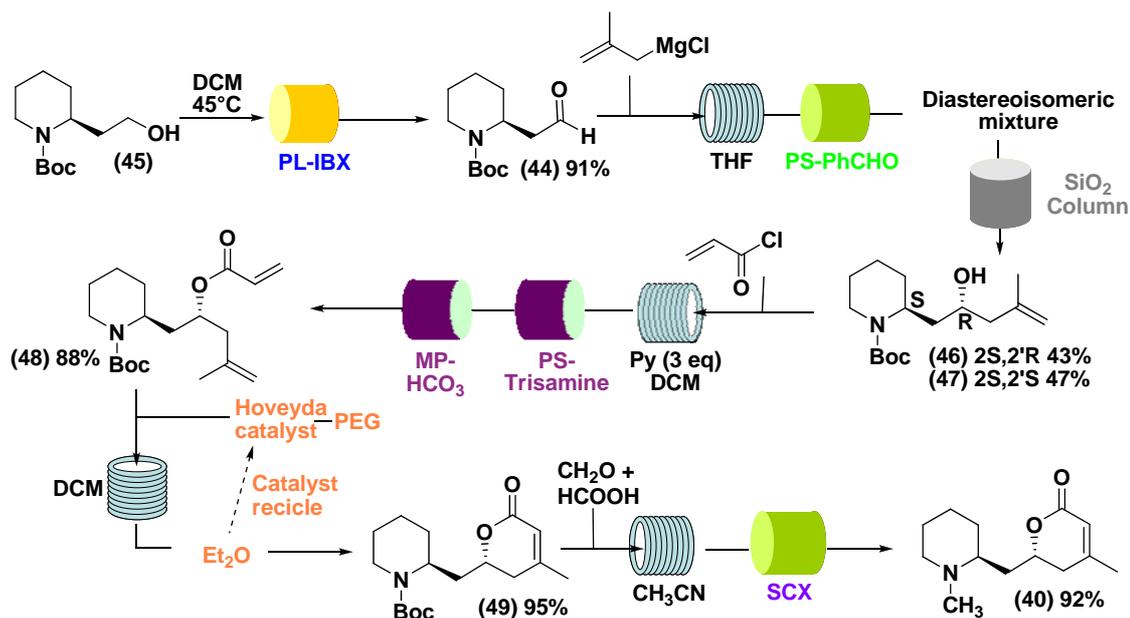
Oxidation Reaction Procedure:
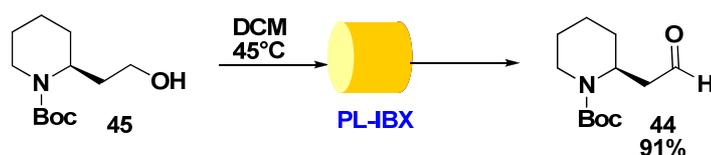
One flow stream driven by the Vapourtec R4/R2+ containing a solution of the alcohol **45** (800 mg, 1 equiv, 3.49 mmol) in DCM was directed through a reagent column containing PL-IBX (loading 1.09 mmol/g, 5 g) heated to 45°C. The reaction was performed continuously cycling the stream through the column until the complete conversion of the alcohol (1) into (2). A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Finally, the solvent was reduced in vacuo provided the desired **44** (725 mg, 3.19 mmol). Yield 91%.

^{1}H NMR (300 MHz, CHLOROFORM-d) δ ppm 9.68 - 9.84 (m, 1 H), 4.78 - 4.97 (m, 1 H), 3.85 - 4.14 (m, 1 H), 2.78 - 2.88 (m, 1 H), 2.75 (ddd, J=15.26, 8.51, 3.23 Hz, 1 H), 2.55 (ddd, J=15.26, 6.46, 2.35 Hz, 1 H), 1.48 - 1.81 (m, 6 H), 1.47 (s, 9 H)

Grignard Addition Reaction Procedure:

Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **44** (725 mg, 1 equiv, 3.2 mmol) in THF (10 ml) and stream 2 containing (2-methylallyl)magnesium chloride (0.5 M THF solution) dissolved in total 10 ml of THF (7.6 ml; 1.2 eq). These were mixed at a T-piece before entering the CFC (volume 10 ml; 33 min residence time) maintained at RT. The stream was then directed through a reagent column filled with PS-Benzaldehyde (loading 1.09 mmol/g; 3.5 g). A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Finally, the solvent was reduced in vacuo and the crude was partitioned between water and DCM. The organic phase was collected, dried by Na$_2$SO$_3$ and the solvent evaporated. The crude was purified by cromatography column (eluent: petroleum ether/AcOEt 10/) providing 380 mg (1.34 mmol, 42% yield) of **46** (S)-tert-butyl 2-((R)-2-hydroxy-4-methylpent-4-enyl)piperidine-1-carboxylate and 432 mg (1.52 mmol, 48% yield) of **47** (S)-tert-butyl 2-((S)-2-hydroxy-4-methylpent-4-enyl)piperidine-1-carboxylate. Total yield 90%.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.60 - 4.80 (m, 2 H) 4.24 - 4.36 (m, 1 H) 4.29 (d, 1 H) 3.73 - 3.93 (m, 1 H) 3.45 - 3.63 (m, 1 H) 2.68 - 2.84 (m, 1 H) 1.98 - 2.25 (m, 2 H) 1.69 (s, 3 H) 1.43 - 1.65 (m, 6 H) 1.39 (s, 9 H) 1.13 - 1.35 (m, 2 H)

Acylation Reaction Procedure:
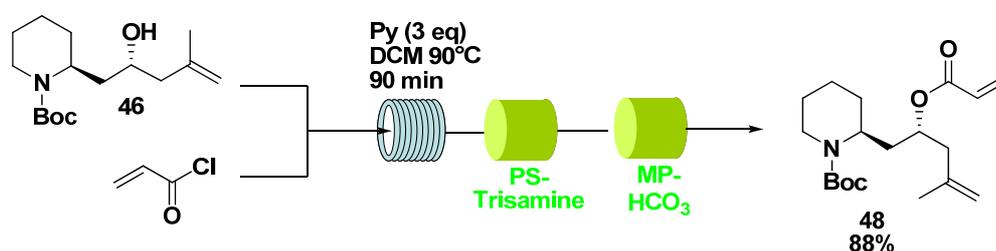
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **46** (380 mg, 1 equiv, 1.34 mmol), pyridine (0.325 ml, 4.02 mmol, 3 eq) in DCM (total solution 10 ml) and stream 2 containing acryloyl chloride (0.217 ml, 2.68 mmol, 2 eq) in DCM (total solution 10 ml). These were mixed at a T-piece before entering the CFC (12 mL in total) for 90 min at 90°C. The stream was then directed through a series of reagent columns beginning with PS-Trisamine (loading 4.11 mmol/g, 1 g) and then MP-HCO$_3$ (loading 1.8 mmol/g, 3 g). A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Finally, the solvent was reduced in vacuo providing **48** (398 mg, 1.179 mmol). Yield 88%.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 6.30 (dd, 1 H) 6.11 (dd, 1 H) 5.92 (dd, 1 H) 4.89 - 5.13 (m, 1 H) 4.76 (s, 1 H) 4.70 (s, 1 H) 4.04 - 4.35 (m, 1 H) 3.72 - 3.97 (m, 1 H) 2.68 - 2.91 (m, 1 H) 2.39 (dd, 1 H) 2.28 (dd, 1 H) 1.85 - 2.00 (m, 1 H) 1.71 - 1.82 (m, 1 H) 1.69 (s, 3 H) 1.44 - 1.61 (m, 5 H) 1.38 (s, 9 H) 1.16 - 1.33 (m, 1 H)

Ring Closing Metathesis Procedure:
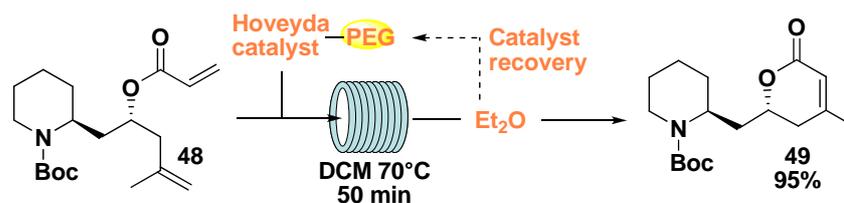
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of the ester **48** (398 mg, 1.179 mmol, 1 eq) in DCM (total solution 5 ml) and stream 2 containing PEG-Hoveyda supported catalyst (loading 0.2 mmol/ g; 353 mg; 0.070 mol; 6%mol Ru) in DCM (total solution 5 ml). These were mixed at a T-piece before entering the CFC (10mL in total) for 50 min at 70°C. A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Diethyl ether was added; PEG-supported catalyst was precipitated and was filtered. Finally, the solvent was reduced in vacuo providing lactone **49** (347 mg, 1.1 mmol). Yield 95%.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 5.81 (s, 1 H) 4.39 - 4.58 (m, 1 H) 4.19 - 4.39 (m, 1 H) 3.86 - 4.09 (m, 1 H) 2.73 - 2.97 (m, 1 H) 2.39 - 2.68 (m, 2 H) 2.23 - 2.39 (m, 1 H) 1.99 (s, 3 H) 1.58 - 1.77 (m, 7 H) 1.47 (s, 9 H)

Eschweiler Clarke Reaction Procedure:
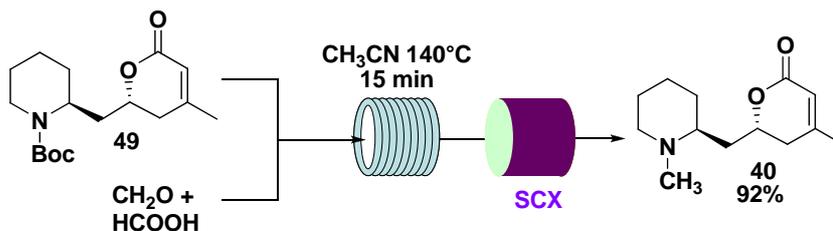
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of the lactone **49** (347 mg, 1.1 mmol, 1 eq) in acetonitrile (total solution 5 ml) and stream 2 containing formaldehyde (aq. solution 36%) (343 µl, 4.49 mmol, 4 eq) and formic acid (172 µl, 4.49 mmol, 4 eq) in acetonitrile (total solution 5 ml). These were mixed at a T-piece before entering the CFC (10 mL in total) for 15 min at 140°C. The flow stream was then directed into a column containing silica-supported sulfonic acid to perform a catch and release purification with any unreacted reagents simply passing through to waste. A brief washing sequence (MeOH, 0.4 mL min^{-1}) was used to elute any residues prior to release the product by passage of NH$_3$ in MeOH (3% NH$_3$ solution, 0.1 mL min^{-1}). The product stream was so eluted into a reaction flask.

Finally, the solvent was reduced in vacuo providing (+)-dumetorine **40** (227 mg, 1.01 mmol). 91%Yield.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 5.82 (s, 1 H) 4.24 - 4.79 (m, 1 H) 2.87 - 3.06 (m, 1 H) 2.42 - 2.56 (m, 1 H) 2.40 (s, 3 H) 2.15 - 2.39 (m, 5 H) 2.00 (s, 3 H) 1.57 - 1.94 (m, 4 H) 1.29 - 1.55 (m, 2 H)

^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 164.89 (s, 1 C) 157.12 (s, 1 C) 116.47 (s, 1 C) 73.91 (s, 1 C) 59.89 (s, 1 C) 56.87 (s, 1 C) 42.46 (s, 1 C) 37.62 (s, 1 C) 35.48 (s, 1 C) 29.93 (s, 1 C) 24.95 (s, 1 C) 23.56 (s, 1 C) 22.92 (s, 1 C)

$[\alpha]^{25}_D$ + 38 (c 1, CHCl$_3$), [lit. $[\alpha]^{25}_D$ + 37 (c 1, CHCl$_3$)]

11.4.2. Procedure for the synthesis of (+)-sedridine
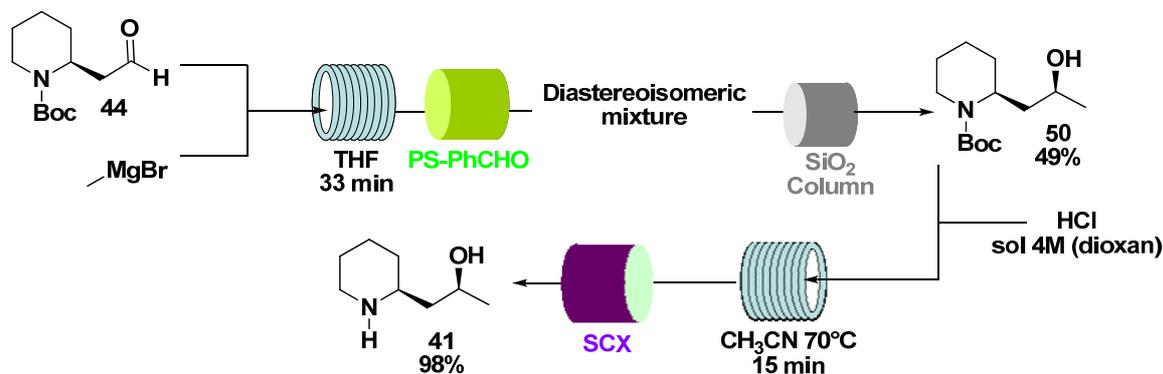
Grignard Addition Reaction Procedure:
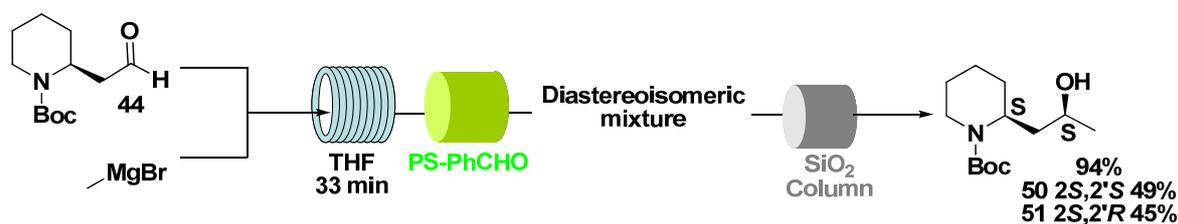
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **44** (100 mg, 1 equiv, 0.44 mmol) in THF (5 ml) and stream 2 containing methylmagnesium bromide (1 M THF solution) (0.52 ml; 1.2 eq) dissolved in total 5 ml of THF. These were mixed at a T-piece before entering the CFC (volume 10 ml; 33 min residence time) maintained at RT. The stream was then directed through a reagent column filled with PS-Benzaldehyde (loading 1.09 mmol/g; 400 mg). A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Finally, the solvent was reduced in vacuo and the crude was partitioned between water and DCM. The organic phase was collected, dried by Na_2SO_3 and the solvent evaporated. The crude was purified by cromatography column (eluent: petroleum ether/AcOEt 10/) providing 52.1 mg (0.21 mmol, 49% yield) of **50** and 47.9 mg (0.2 mmol, 45% yield) of **51**. Total yield 94%.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.22 (3H, d, J=6.7 Hz) 1.42 (9H, s), 1.48– 1.80 (7H, m), 2.00 (1H, dt, J_1=12 Hz, J_2=2Hz), 2.70 (1H, dt, J_1=12 Hz, J_2=2Hz), 3.55 (1H, s,) 3.90 – 4.05 (1H, m), 4.42-4.54 (1H, m)

^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 19.1, 22.5, 25.5, 29.3, 39.4, 46.5, 63.3, 80.1, 110.6

Deprotection Reaction Procedure:
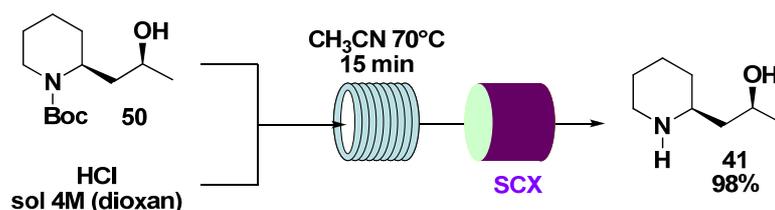
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of the lactone **50** (52.1 mg, 0.21 mmol, 1 eq) in acetonitrile (total solution 2.5 ml) and stream 2 containing dioxane 4 M solution of HCl (52.5 µl, 0.21 mmol, 1 eq) in acetonitrile (total solution 2.5 ml). These were mixed at a T-piece before entering the CFC (10 mL in total) for 15 min at 70°C. The flow stream was then directed into a column containing silica-supported sulfonic acid to perform a catch and release purification with any unreacted reagents simply passing through to waste. A brief washing sequence (MeOH, 0.4 mL min⁻1) was used to elute any residues prior to release the product by passage of NH_3 in MeOH (3% NH_3 solution, 0.1 mL min⁻1). The product stream was so eluted into a reaction flask.

Finally, the solvent was reduced in vacuo providing (+)-Sedridine **41** (29.2 mg, 0.20 mmol). 98%Yield.

¹H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.16 (3H, d, J=5.6Hz), 1.38 – 1.56 (4H, m), 1.58 – 1.68 (3H, m), 1.76 – 1.86 (1H, m), 2.62 (1H, dt, J_1 = 11.2 Hz, J_2=3.7 Hz), 2.90 – 3.00 (1H, m), 3.12 (1H, d, J_1 = 11, 2 Hz), 3.74 (2H, s), 4.05-4.16 (1H, m)

¹³C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 23.4 24.1 25.0 30.8 43.2 46.3 54.5 64.4 $[\alpha]^{25}_D$ +26.2 (c 0,85, EtOH)

11.4.3. Procedure for the synthesis of (-)-sedamine
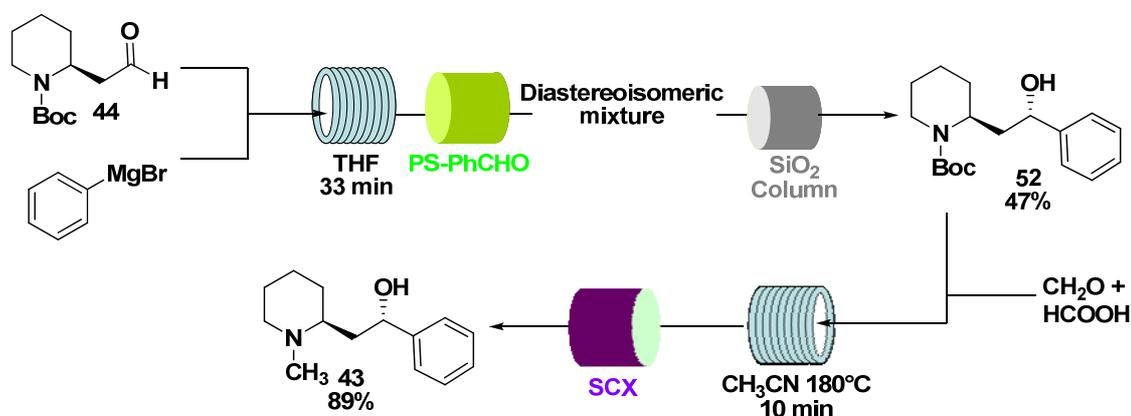
Grignard Addition Reaction Procedure:
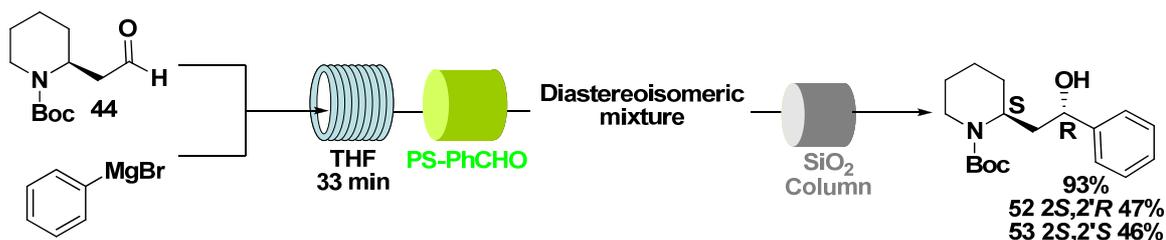
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **44** (100 mg, 1 equiv, 0.44 mmol) in THF (5 ml) and stream 2 containing phenylmagnesium bromide (1 M THF solution) (0.5 ml; 1.2 eq)dissolved in total 5 ml of THF. These were mixed at a T-piece before entering the CFC (volume 10 ml; 33 min residence time) maintained at RT. The stream was then directed through a reagent column filled with PS-Benzaldehyde (loading 1.09 mmol/g; 480 mg). A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Finally, the solvent was reduced in vacuo and the crude was partitioned between water and DCM. The organic phase was collected, dried by Na_2SO_3 and the solvent evaporated. The crude was purified by cromatography column (eluent: petroleum ether/AcOEt 10/) providing 60.5 mg (0.20 mmol, 45% yield) of (S)-tert-butyl 2-((R)-2-hydroxy-2-phenylethyl)piperidine-1-carboxylate **52** and 64.5 mg (0.21 mmol, 48% yield) of (S)-tert-butyl 2-((S)-2-hydroxy-2-phenylethyl)piperidine-1-carboxylate **54**. Total yield 93%.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.45 (9H, s), 1.50 – 1.65 (6H, m), 1.80 – 1.90 (1H, m), 2.02 –2.15 (1H, m), 2.75 (1H, t, J = 14 Hz), 3.80 – 3.95 (2H, m), 4.41 (1H, bs), 4.70 – 4.78 (1H, m), 7.21-7.40 (5H, m)

^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 19.1, 25.4 (2C), 28.5, 29.3, 39.5, 40.5, 48.6, 72.7, 79.8, 125.7 (2C), 127.3, 128.3 (2C), 144.7, 155.4

Eschweiler Clarke Reaction Procedure:
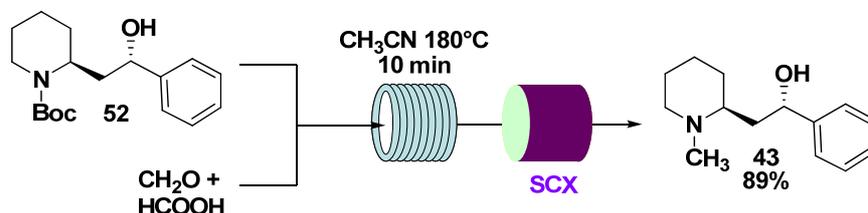
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **52** (60 mg, 0.2 mmol, 1 eq) in acetonitrile (total solution 2.5 ml) and stream 2 containing formaldehyde (aq. solution 36%) (60.1 μl, 0.78 mmol, 4 eq) and formic acid (30.1 μl, 0.78 mmol, 4 eq) in acetonitrile (total solution 2.5 ml). These were mixed at a T-piece before entering the CFC (10 mL in total) for

10 min at 180°C. The flow stream was then directed into a column containing silica-supported sulfonic acid to perform a catch and release purification with any unreacted reagents simply passing through to waste. A brief washing sequence (MeOH, 0.4 mL min^{-1}) was used to elute any residues prior to release the product by passage of NH$_3$ in MeOH (3% NH$_3$ solution, 0.1 mL min^{-1}). The product stream was so eluted into a reaction flask.

Finally, the solvent was reduced in vacuo providing (-)-sedamine **43** (38.3 mg, 0.175 mmol). 89%Yield.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.32 – 1.40 (1H, m), 1.45 – 1.54 (4H, m), 1.55 – 1.70 (3H, m), 1.72 – 1.82 (1H, m), 2.15 (1H, dt, J$_1$ = 14, 10 Hz), 2.58 (3H, s), 2.55 – 2.65 (1H, m), 2.92 (1H, m), 3.08 – 3.15 (1H, m), 4.90 (1H, dd, J$_1$ = 10, 2 Hz), 6.25 (1H, bs), 7.25-7.41 (5H, m)

^{13}C NMR (75 MHz, CHLOROFORM-*d*) δ ppm 20.8, 22.2, 26.3, 39.5, 39.9, 52.6, 61.3, 73.3, 125.5 (2C), 127.1, 128.3 (2C), 145.2.

[α]$^{25}_D$ -38.6 (c 0,80, EtOH)

11.5. Experimental section of chapter 8

11.5.1. Vinyl polystyrene supported Grubbs of 1st generation 53

11.5.1.1. Procedure for the synthesis of vinyl polystyrene supported Grubbs of 1st generation
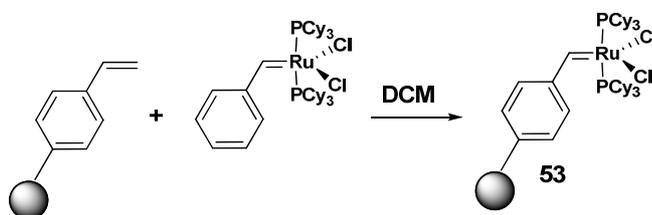
Prepared following the procedure by: Ahmed, M.; Barrett, A. G. M.; Braddock, D. C.; Cramp, S. M.; Procopiou, P. A. *Tetrahedron Letters* **1999**, *40*, 8657.

A mixture of Vinyl-polystyrene (1g, loading 1.5mmol/g) and Grubbs catalyst 1st generation (123 mg, 0.1mmol) was shake in DCM at r.t. for 1.5 hour and then was filtered. The catalyst-resin was washed with DCM (3 times) and then was dried under vacuum for 48 hours to afford 1.11 g of violet catalyst-resin.

11.5.1.2. Ring Closing Metathesis on model substrate 54
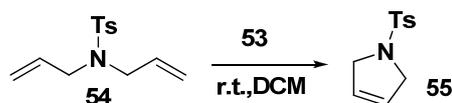
N,N-diallyl-4-methylbenzenesulfonamide (50mg, 0.199 mmol) was added to a suspension of PS-Grubbs 1st generation (50 mg; 25mg of PS-Supported Grubbs 1st gen. catalyst for 0.1mmol substrate according with the literature) in DCM. After shaking for 40 min, the reaction was controlled by UPLC/MS and the total conversion of the starting material was observed. The resin was filtered and the solvent removed under vacuum affording 48 mg of **55**.

11.5.2. PS-DVB Grubbs 2nd generation catalyst 58

11.5.2.1. Synthesis of PS-DVB Grubbs 2nd generation catalyst
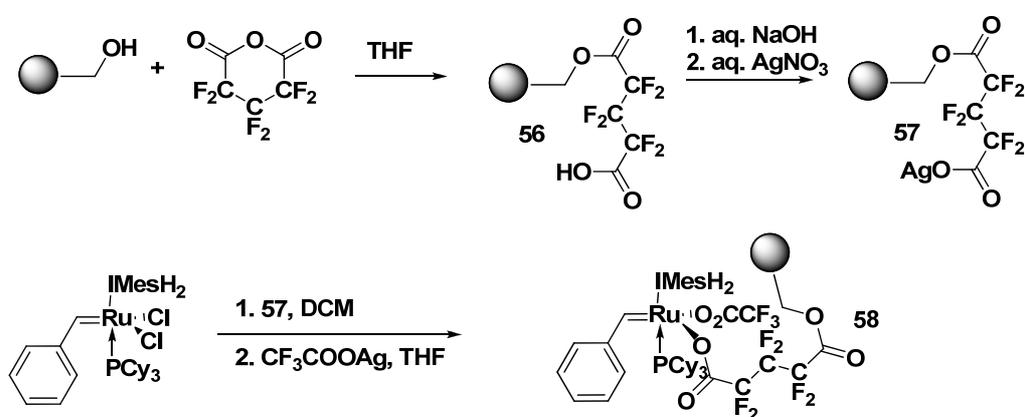
Prepared following the procedure by: (a) Yang, L.; Mayr, M.; Wurst, K.; Buchmeiser, M. R. *Chem. Eur. J.* **2004**, *10*, 5761. (b) Halbach, T. S.; Mix, S.; Fischer, D.; Maechling, S.; Krause, J. O.; Sievers, C.; Blechert, S.; Nuyken, O.; Buchmeiser, M. R. *J. Org. Chem.* **2005**, *70*, 4687.

Synthesis of **56**

PS-CH$_2$OH (1.7 mmol of CH$_2$OH /g, cross-linked with 1% DVB) was suspended in dry THF and perfluoroglutaric anhydride was added. Stirring was continued for 3h then the product was collected by filtration and washed 3 times with THF. It was dried in *vacuo* to give a slightly yellow solid **56** (2.11 g).

Synthesis of **57**

Resin carboxylic acid **56** (2.11 g) was suspended in THF (18 ml) and NaOH (dissolved in water 35 ml) was added. The mixture was stirred for 3 h. The precipitated was filtered, washed 3 times with water and re-suspended in water (25 ml). AgNO$_3$ dissolved in water (15 ml) was added. Stirring was continued for 2 h and the product was filtered off and washed 3 times each with water, Et$_2$O and pentane. Drying in *vacuo* gave of the product as a white solid (1.63 g).

Synthesis of **58**

Resin silver salt **57** (500 mg) was suspended in DCM (15 ml) and Grubbs catalyst 2nd generation (80 mg), suspended in DCM (2 ml), was added slowly. The mixture was stirred for 90 min in the absence of light, and CF$_3$COOAg dissolved in THF (2 ml) was added. The reaction mixture was stirred for further 90 min. The product was filtered and washed three times with THF (10 ml) until the filtrate was colourless. Drying in *vacuo* provided a red-brown powder (420 mg).

(Loading literature: 2.4mg/1g)

11.5.2.2. Ring Closing Metathesis on model substrate 54 and on 48
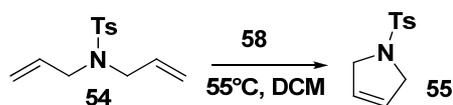
To a solution of N,N-diallyl-4-methylbenzenesulfonamide **54** (20 mg, 0.080 mmol) dissolved in 1 ml of dry DCM, PS-Grubbs 2nd gen. catalyst was added (20mg, following the ratio reported in literature). The mixture was stirred at 55°C under N$_2$ by SynCor thermo shaker apparatus for 6 hr. UPLC/MS showed a conversion of 20%.
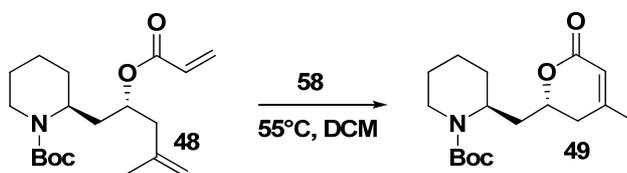
To a solution of tert-butyl 2-(2-(acryloyloxy)-4-methylpent-4-enyl)piperidine-1-carboxylate **48** (20mg, 0.059 mmol) dissolved in 1 ml of dry DCM, resin catalyst Grubbs 2nd gen. (50 mg, following the ratio reported in literature) was added. The mixture was stirred at 55°C under N$_2$ by SynCor thermo shaker apparatus for 48 hours. The conversion was about 30% (UPLC/MS analysis).

11.5.3. PEG-supported Hoveyda catalyst

All PEG samples were melted at 80°C in vacuum for 30 min before use to remove traces of moisture. After reaction PEG-supported product purification involved evaporation of the reaction solvent in vacuum and addition of the residue dissolved in a few ml of DCM to diethylether (50 ml g−1 of polymer), the obtained suspension was filtered through a sintered glass filter, and the solid repeatedly washed on the filter with diethylether (up to 100 mL per gram of polymer, overall).

The yields of the PEG-supported compounds were determined by weight with the assumption that *Mw* is 5000 Da for the PEG fragment. The *Mw* actually ranged from 4500 to 5500. The indicated yields were for pure compounds. The purity of these compounds was determined by ^{1}H NMR analysis in CDCl$_3$ at 300 MHz with pre-saturation of the methylene signals of the polymer at δ=3.63. In recording the NMR spectra, a relaxation time of 6 s and an acquisition time of 4 s were used to ensure complete relaxation and accuracy of the integration. The relaxation delay was selected after T1 measurements. The integration of the signals of the PEG CH$_2$OCH$_3$ fragment at δ=3.30 and 3.36 were used as internal standard. The estimated integration error was +5%. For intermediates analytical characterization (LC-MS, ^{1}H- and ^{13}C-NMR) was in agreement with what reported in the cited literature.

11.5.3.1. Procedure for the synthesis of PEG-supported Hoveyda catalyst
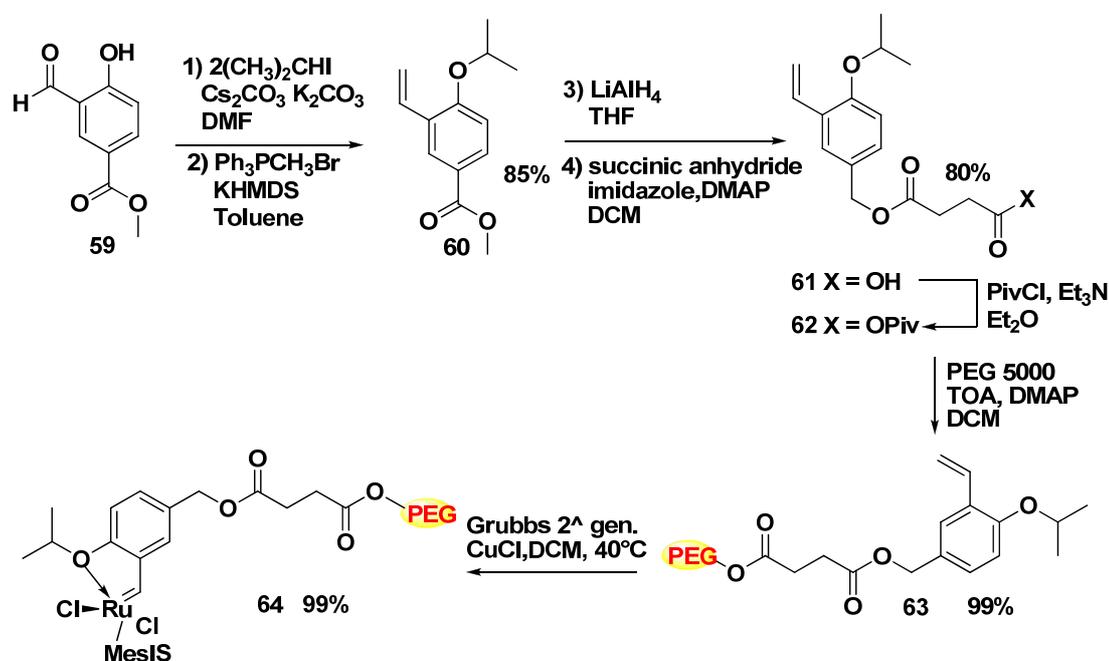
Synthesis of **60**[103]:

Step 1:
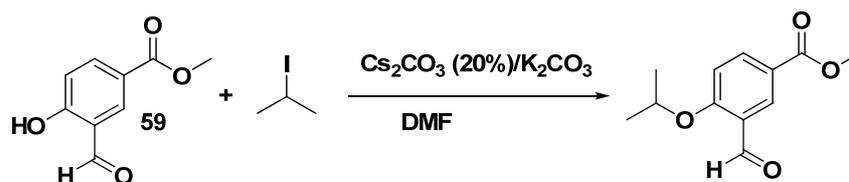
A mixture of methyl 3-formyl-4-hydroxybenzoate **59** (900 mg, 4.99 mmol), 2-iodopropane (0.995 ml, 9.98 mmol), Cs$_2$CO$_3$ (650.0 mg, 1.99 mmol) (1.379 g, 9.98 mmol) and K$_2$CO$_3$ in 20 ml of

DMF was stirred at room temperature under nitrogen atmosphere for 7 hours. The solvent was removed under vacuum and the crude was partitioned between water and DCM. The organic phase was dried and the solvent was removed under vacuum. The crude was purified on a pad of silica gel (eluent hexane/AcOEt 9/1) affording 1100 mg of desired compound. Quantitative yield.

Step 2:
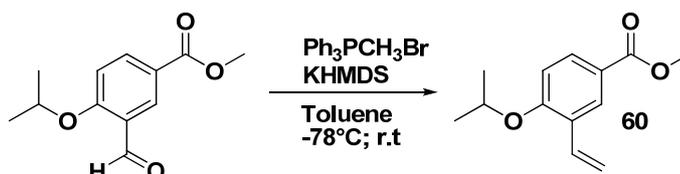
The phosphonium salt (3.53 g, 9.90 mmol) was dissolved in anhydrous toluene (25 ml) and the resulting suspension was stirred and treated with KHMDS (1.97g, 9.90 mmol; dissolved in 25 ml of toluene) affording a brilliant yellow suspension.

After 90 minutes, the reaction mixture was cooled to -78°C and a solution of methyl 3-formyl-4-isopropoxybenzoate (1.1 g, 4.95 mmol) dissolved in toluene (20 ml) was added dropwise. The temperature was slowly warmed to room temperature in 1 hour and stirred at r.t. for 30 minutes. The reaction was quenched with 1N HCl at 0°C and then the solvent was removed under vacuum. The crude was partitioned between NH_4Cl saturated solution and AcOEt. The organic phase was dried over Na2SO4 and then evaporated. The crude was purified by Flash Chromatography (eluent: Hexane/AcOEt 9.5/0.5) affording 926 mg of desired compound. Yield 85%.

Synthesis of **61**[103]:

Step 1:
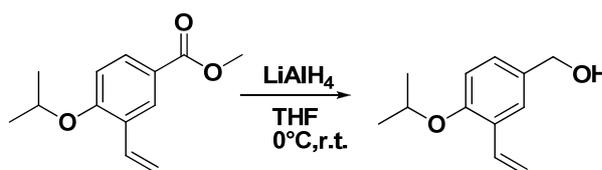
Methyl 4-isopropoxy-3-vinylbenzoate **60** (926 mg, 4.20 mmol) was dissolved in anhydrous THF (10 ml). The reaction mixture was cooled to -0°C and $LiAlH_4$ (160 mg, 4.20 mmol) was added portion wise. The temperature was slowly warmed to room temperature and stirred at r.t. for 1 hour. The reaction was quenched with water at 0°C and then the solvent was removed under vacuum. The crude was partitioned between water and DCM. The organic phase was dried over Na_2SO_4 and then evaporated. The crude was purified by Flash Chromatography (eluent: Hexane/AcOEt 8/2) affording 750 mg of desired compound. Yield 93%.

Step 2:
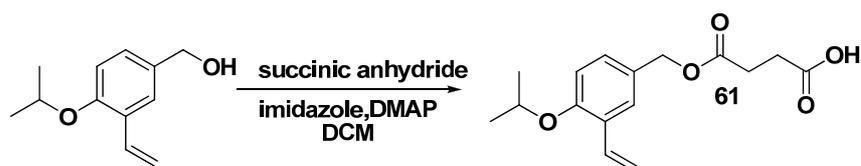
(4-isopropoxy-3-vinylphenyl)methanol **60** (750 mg, 3.9 mmol) was dissolved in anhydrous DCM (20 ml). DMAP (180 mg, 1.56 mmol), imidazole 315 mg, 4.68 mmol) and succinic anhydride (780 mg, 7.8 mmol) were added portion wise. The reaction mixture was stirred at r.t. for 1 night. The solvent was removed under vacuum. The crude was purified by Flash Chromatography (eluent: Hexane/AcOEt 1/1) affording 683 mg of the desired compound **61** as white powder . Yield 60%.

Synthesis of **62**[103]:
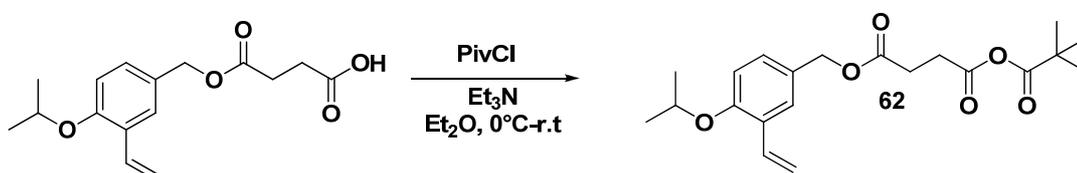
5-(4-isopropoxy-3-vinylbenzyloxy)-5-oxopentanoic acid **61** (150 mg, 0.51 mmol) was dissolved in dry diethyl ether (10 mL) under nitrogen and trimethylacetyl choride (63.2 µl, 0.51 mmol) and triethylamine (52.5 µl, 0.51mmol) were added consecutively to the stirred solution cooled to -78°C. The cold bath was removed and the solution warmed to RT was filtered and solvent removed under reduced pressure. The mixed anhydride **62** as a viscous colourless oil (191 mg) was subsequently used without further purification. Quantitative yield.

Synthesis of **63**:
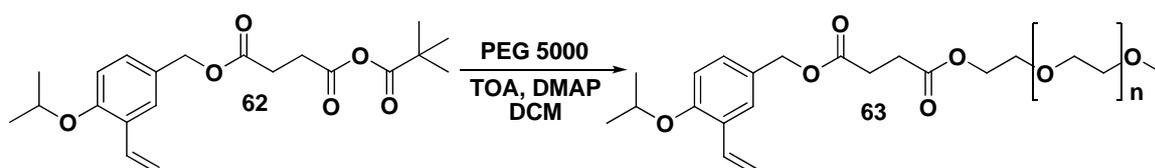
PEG 5000 (1.25 g, 0.25 mmol) was dried in oven for 2 hours and then dissolved in dry DCM (2 ml) under nitrogen atmosphere. TOA (180 mg, 0.51 mmol) was added. The reaction mixture was cooled at 0°C and then 4-(4-isopropoxy-3-vinylbenzyloxy)-4-oxobutanoic pivalic anhydride **62** (191 mg, 0.51 mmol) dissolved in dry DCM (2 ml) and DMAP (15 mg, 0.12 mmol) were added. The cold bath was removed and the solution allowed to warm to room temperature and stirred for 21 hours at r.t. The solution was concentrated under vacuum and Et$_2$O was added. PEG-bound ligand was precipitated from the organic solution and filtered under vacuum obtaining 1.2 g of

PEG-bound ligand. PEG-bound ligand was dried in oven for 1 hour. ^{1}H-NMR confirmed that the desired product **63** was obtained with a loading of 0.2mmol/g. Quantitative yield.

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.35 (6 H, d, J= 6.8 Hz), 2.63 (4H, d, J= 7.1 Hz), 3.30 (2H, m, Me*OCH₂*PEG), 3.36 (3H, s, *Me*OPEG), 4.70 (1H, sept, J = 6.8 Hz), 5.20 (2H, s), 5.34 (1H, dd, J_{trans}=10.0 Hz, J_{gem}=2.1Hz), 5.44 (1H, dd, J_{cis}=16.8 Hz, J_{gem}=2.1Hz), 6.87 (2H, m), 6.90 (1H, dd, J_{trans}=10.0 Hz, J_{cis}=16.8 Hz), 7.11 (1H, s)

Synthesis of **64** :

Grubbs 2^ Generation Catalyst (204 mg, 0.24mmol) and CuCl (24.9 mg, 0.24mmol) were weighed into a 20 ml round bottom flask under nitrogen atmosphere and dissolved in 5 ml of CH₂Cl₂. PEG-bound ligand (1.2 g, loading 0.2mmol/g) was cannulated into the resulting deep red solution in 5 ml of CH₂Cl₂ at r.t. The flask was equipped with a condenser, and the solution was stirred at 40 °C for 1 h. From this point forth, all manipulations were carried out in air with reagent-grade solvents. The reaction mixture was concentrated in *vacuo* to a dark brown solid residue. The unpurified material was dissolved in a minimal volume of 1:1 pentane/ CH₂Cl₂ and then Et₂O was added. PEG-Hoveyda catalyst was precipitated from the organic solution and filtered under vacuum. PEG-Hoveyda catalyst afforded as an air stable deep green powder (1.2 g). Clean and quantitative exchange of the styrene ligand was judged by ^{1}H NMR spectroscopy. Quantitative yield (loading 0.2 mmol/g).

^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 1.35 (6 H, d, J= 6.8 Hz), 2.58 (4H, d, J= 7.1 Hz), 3.30 (2H, m, Me*OCH₂*PEG), 3.36 (3H, s, *Me*OPEG), 4.70 (1H, sept, J = 6.8 Hz), 5.20 (2H, s), 6.80 (2H, m), 6.88 (1H, s), 7.11 (1H, s)

11.5.3.2. Flow RCM on model substrate 54 using PEG-supported Hoveyda catalyst, its recycle and reuse
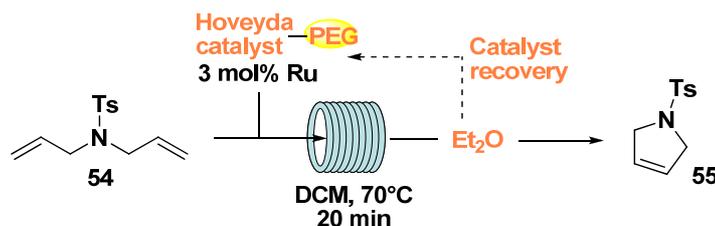
Two flow streams driven by the Vapourtec R4/R2+; stream 1 containing a solution of **54** (50 mg, 0.2 mmol, 1 eq) in DCM (total solution 2 ml) and stream 2 containing PEG-Hoveyda supported catalyst (loading 0.2 mmol/ g; 29.9 mg; 0.0055 mol; 3%mol Ru) in DCM (total solution 2 ml). These were mixed at a T-piece before entering the CFC (10 ml in total) for 20 min at 70°C. A 100 psi BPR ensured the system was pressurized, before eluting into a reaction flask. Diethyl ether was added; PEG-supported catalyst was quantitatively precipitated and was filtered. Finally, the solvent was reduced in *vacuo* providing **55** (49 mg; 0.2 mmol). Yield 100%. The same procedure was repeated 6 times obtaining the following results:

Cycle	1	2	3	4	5	6
Conversion (%)	100	100	100	98	94	91

Chapter 12.
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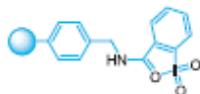
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