Università degli Studi di Ferrara

DOCTORAL COURSE IN CHEMISTRY

CYCLE XXXIII

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Flow chemistry development in the synthesis of pharmaceutical products, the example of Domperidone and related compounds

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1 Abstract

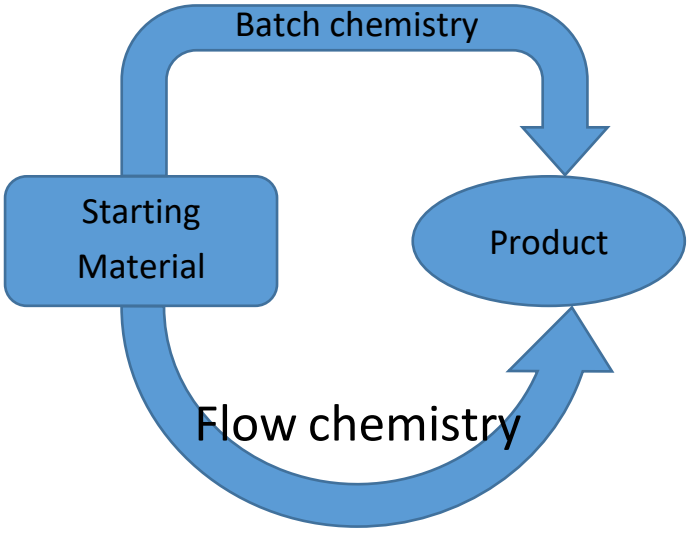
The flow synthesis of Domperidone and some structurally related analogues was approached using a statistical approach (DoE) to maximize the product conversion, minimize the impurities, and if necessary recycle the side product to generate the desired intermediates. Several synthetic approaches were investigated starting from the commercial synthesis, then switching to another three to adapt the route based on the synthetic issues encountered. During this activity a compound initially considered an impurity was reused as starting material to give a key intermediate. Despite some confirmation runs during the development work not performing completely in line with the predicted response, the final run through, with the conditions optimized during the development work, resulted in a 42.8% molar yield. The work could be further expanded investigating whether different parameters affect the overall yield, for example the isolation of domperidone and the solvents used in the reactions.

2 Introduction

2.1 Continuous flow chemistry

Continuous flow chemistry is not a new chemistry, it is a different approach to reach, at the end of the day, the same target, ie. synthesis of the desired product, using different equipment.

The main difference from batch chemistry is that the reagents are pumped continuously into the reactor and the product is continuously removed from the reactor.



During a webinar hosted by Corning the speaker ended the event with this phrase “The purpose of a flow experiment is not to obtain a good yield but to know how to obtain a good yield”; this sentence summarizes the capability of flow chemistry to collect data, and knowledge on the reactions performed with this technology, but not all the reactions performed in flow chemistry will result in high yields.

2.1.1 The Flow chemistry in industry

Flow chemistry is a recent concept in pharmaceutical industries, but it is 100 years old.

One of the current commercial productions of ammonia was patented in 1908 and uses a flow of N_2 and H_2 in contact with a catalyst (Fe) at very high temperature [1]. The petrochemical industries have also used continuous processes at commercial level for about 100 years, for example the Fischer–Tropsch process [2], where carbon monoxide and hydrogen were transformed into liquid hydrocarbons in the presence of a metal catalyst [3].

Currently, the petrochemical industry is based on flow chemistry as the crude oil continuously enters into the distillation equipment and then the product is collected continuously, taking advantage of the large quantity of material processed.

In 1970 Charles Howard Kline introduced the classification of chemical products, related to their production quantity and their value added.

		Value added	
		LOW	**HIGH**
Production quantity	**LOW**	**specialty chemical:** low production and low value added	**fine chemical:** low production and high value added
	HIGH	**pseudo-commodity (or branded commodity):** high production and low value added	**true commodity:** high production and high value added

The classes of product that could take advantage of flow chemistry in their production are the true commodities and the fine chemicals as both could take advantage of the process intensification obtained by the introduction of flow chemistry.

Moving from the economic advantages listed so far to the other advantages always mentioned for flow chemistry [4–10], the latter are:

- Safety
- Faster scale-up
- Smaller footprint

The advantage with regards safety is intrinsic to the small reaction volume, the precise temperature control and the possibility to accommodate high pressure. The improved safety allows access to reactions that are not accessible in large batch reactors due to the risk of more hazardous reactions such as exothermic reactions [4].

The quickest way to scale-up a flow reaction, once optimized, is just increasing the reagents feeding, running the reactor for more time and then collecting more output. A more sophisticated method to increase the output of a process is intrinsic in how the flow reactions are performed: it is very common in flow synthesis to telescope a reaction sequence, in which the output from the first reactor enters a second reactor where a new reagent is added for the second reaction. The longest linear sequence reported in flow conditions was the total synthesis of ciprofloxacin sodium salt; a 5 linear steps flow synthesis of the sodium salt is followed by two off-line acidifications to give first ciprofloxacin and then its hydrochloride; the total time of the synthesis moves from 24h for the patented synthesis to 9 minutes for the reported flow synthesis [11].

More commonly in flow processes a non-flow step is introduced between flow transformations and this is called a batch-flow process [5]. Another way to perform flow reactions is that in which the reaction is optimized on a chip reaction unit and then for the scale up it is necessary just to increase the number of chip units and increase the flow rate to keep the same residence time (reaction time for flow reactions).

To see how the foot print of a flow reactor could impact chemical plants we can consider the Corning G1 Reactor (Figure 1), a multipurpose flow reactor for development and small scale production with a capacity of about 80 t/y, with a reactor size of 88 x 38 x 72 cm; on the other hand, the Corning G4 SiC Reactor (Figure 2), suitable for commercial production with a capacity of about 3500 t/y, has a reactor size of 60 x 74 x 96 cm. The footprint of the second reactor is c.a. 1.5 times that of the development reactor, the main difference being the number of reactors in sequence: 10 x 10 ml on G1 Reactor and 1 x 260 ml on G4 SiC Reactor. In June 2020 Corning introduced to the market the G5 reactor and the press release reported the fantastic achievement of 10000 t/y of throughput “Zhejiang Weihua New Materials Co., Ltd., in collaboration with Corning Advanced-Flow Reactor Technology Co., Ltd. and Shanghai Hybrid-Chem Technology Co., Ltd., recently achieved 10,000 metric tons annual throughput of agrochemicals using Corning’s G5 reactor” [12]. The Chambroad holding group was able to maintain the production reducing the footprint from 4000 m^2 to 400 m^2 moving the production from batch reactor to flow reactor.

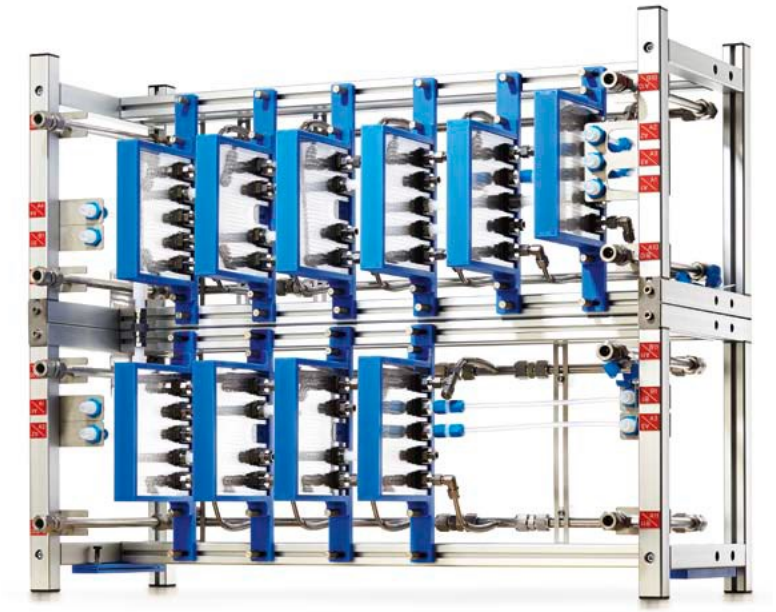


Figure 1 G1 Reactor from Corning S.A.S.

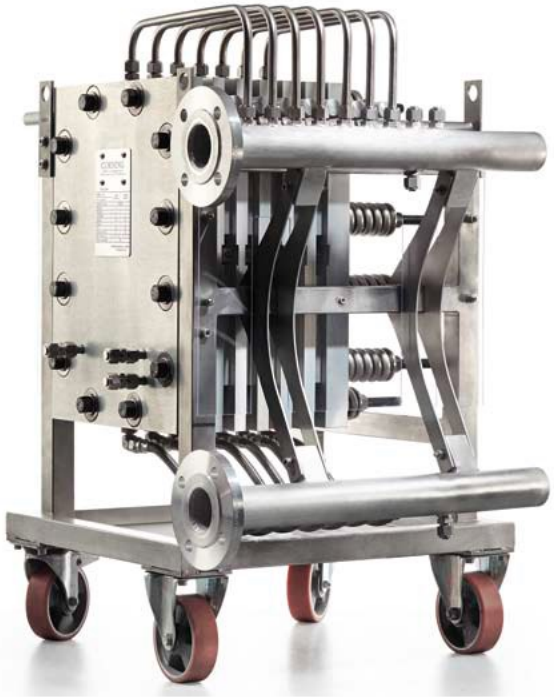


Figure 2 G4 SiC Reactor from Corning S.A.S.

Despite the advantages described above, the application of continuous flow reactors in pharma industries is low.

Baumann et al. [4] identified three main factors that have slowed the penetration of continuous flow reactors in chemical industries:

- **Cultural change**, both Pharma Companies and Contract Research Organizations (CROs) are orientated on achieving ambitious timelines to amaze stakeholders, clients for CROs and investors for Pharma Companies, and often do not have time to redesign a batch process into a flow process.
- **Education**, for the lack of specific university courses for the next generations of chemists, which includes not only fundamental aspects of chemical reactions but also chemical engineering knowledge.
- **Management support**, as management decides the budget and resource allocations; this point is the most important of the three factors for continuous flow reactor penetration in the chemical industry.

For example Syngenta Crop Protection AG introduced flow chemistry in 2014 to solve scale-up issues, building on the know-how it established in collaboration with academic and industrial partners. The most important collaboration was with Prof. Steven Ley that helped accelerate the construction and the development of their own platform and boosted their knowledge of flow chemistry [13].

The switch of a process already in place from batch to flow could help pharmaceutical companies to maintain their profit margins as continuous manufacturing requires less human supervision and the reduced amount of material processed with an accurate control will decrease the loss of material, the labour cost and the loss of revenue, if the product is out-of-specification [14].

The research on switching a process from batch to continuous, in the last couple of decades, was focalized on performing experimental performance comparison of the two processes [15–18]. Due to the expense of experimentally comparing the two procedures, several authors have developed a screening method to determine if there is an advantage to move a process from batch to flow; the most basic is a decision tree as in Figure 3 in which the answer decides if the process is suitable for continuous flow manufacturing [19]; several other papers report qualitative analysis to decide if a batch process could take advantage to move to flow [20].

A first quantitative methodology to investigate the economic convenience of switching to a flow process was first introduced by Goršek and Glavič [21–23] and recently adapted on a modelling simulation [24]. A more precise comparison of batch and continuous process is available if there is a precise knowledge of the batch process [14].

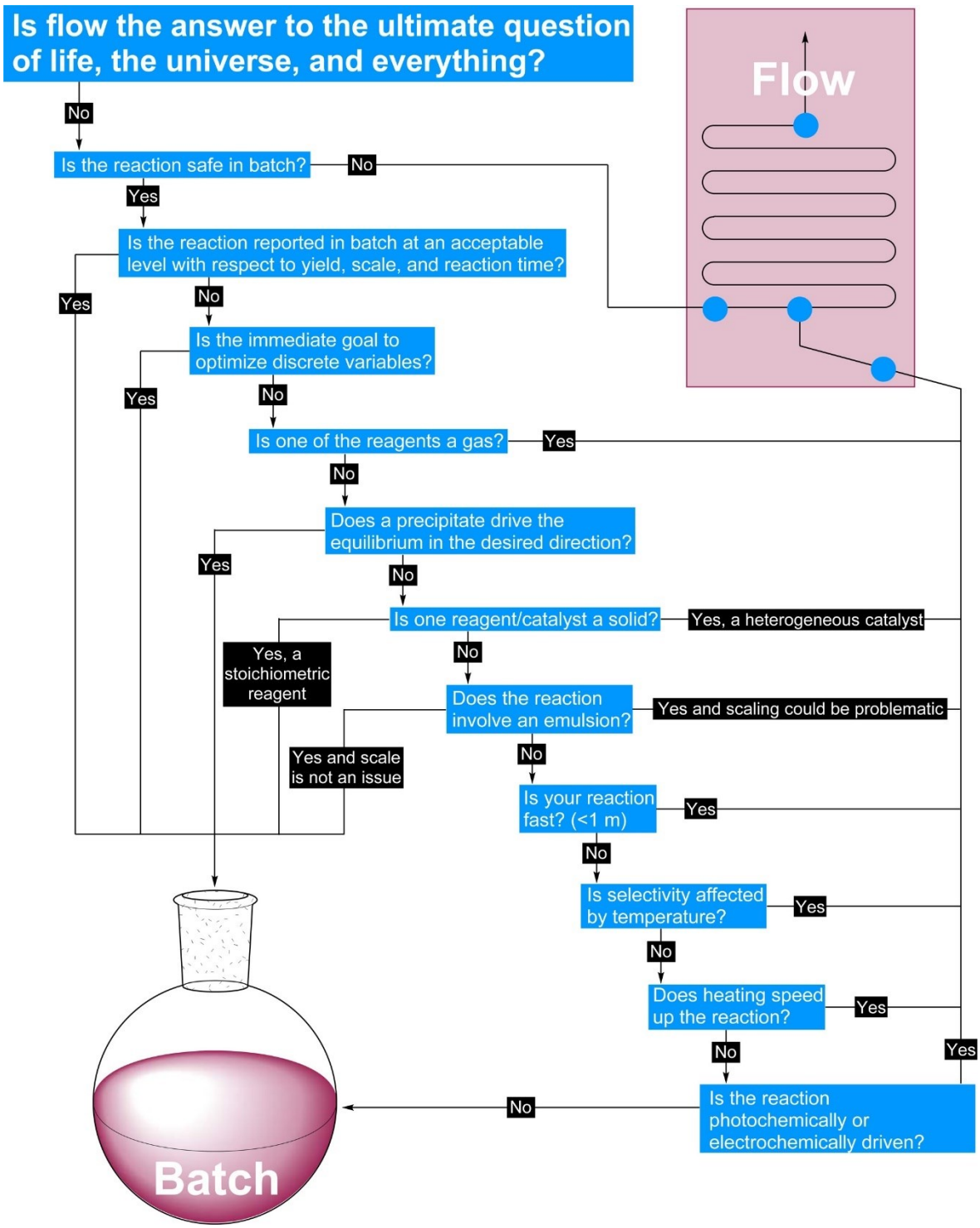


Figure 3 Decision tree to Flow or not to Flow (Reprinted with permission from Chem. Rev. 2017, 117, 18, 11796–11893. Copyright 2020 American Chemical Society.)

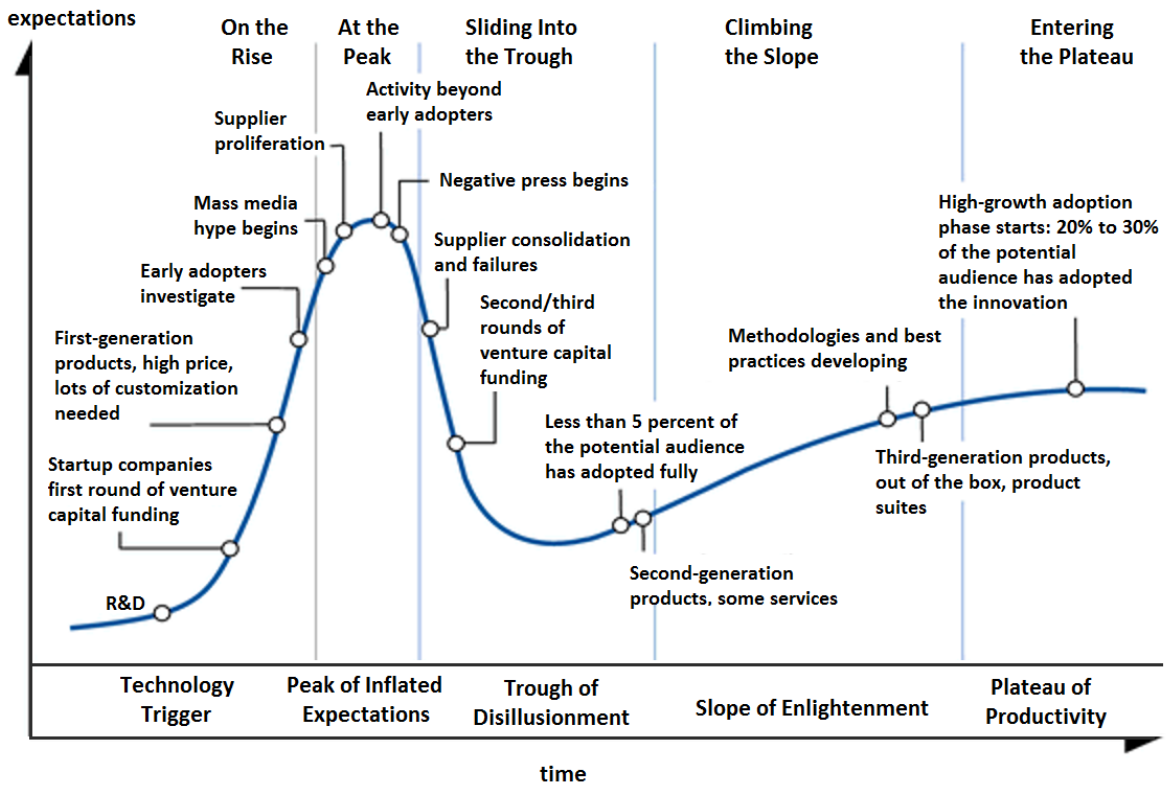


Figure 4 Hype cycle applied to continuous flow chemistry [25]

If we consider the number of publications related to continuous flow chemistry synthesis as a measurement of the interest for that technology [26] and we report it in the Hype cycle (Figure 4), we are now in the area denominated “peak of Inflated Expectations”. As it is possible to see observing the number of references in Scifinder-n reported for the search of “ “continuous flow” chemistry synthesis ” there have been 5591 documents since 1927, as shown in Figure 5 below, and the maximum number of publications were in 2019.

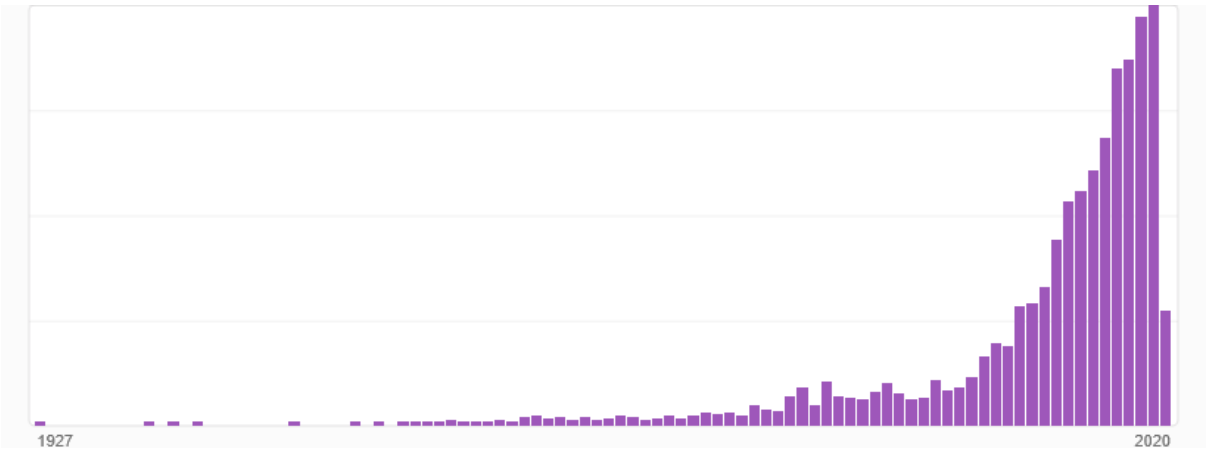


Figure 5 References in Scifinder-n for " ”continuous flow” chemistry synthesis" per year[1]

[1] Data extrapolated in April 2020.

In the graph above, separating the journal articles from the patents published each year (Figure 6), we can observe that the first publications in the period 1999-2007 (555 articles and 37 patents) were primarily from universities or public institutions, which supported the initial growth of continuous flow chemistry.

In the period 2017-2019, there were 59 publications from industries or non-public institutions from a total of 1565 publications, showing that industries are now introducing continuous flow chemistry into their manufacturing sites / research centres.

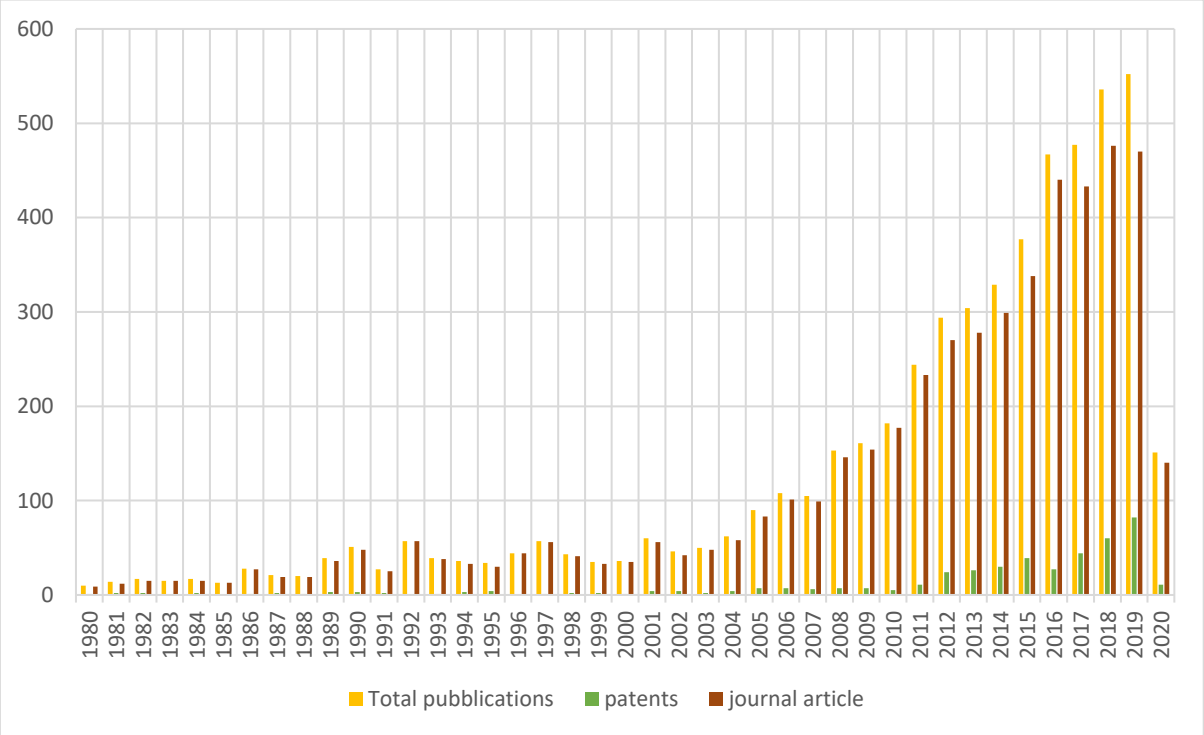


Figure 6 Combined publications in Scifinder-n for " "continuous flow" chemistry synthesis" per year[2]

If we disassemble the hype curve, we can distinguish two phases: the first phase in which the basic research pushed for a new technology (dotted line in Figure 7 below) and the second phase in which the industry introduced that technology and generated patents (dashed line in Figure 7 below).

[2] Data extrapolated in April 2020.

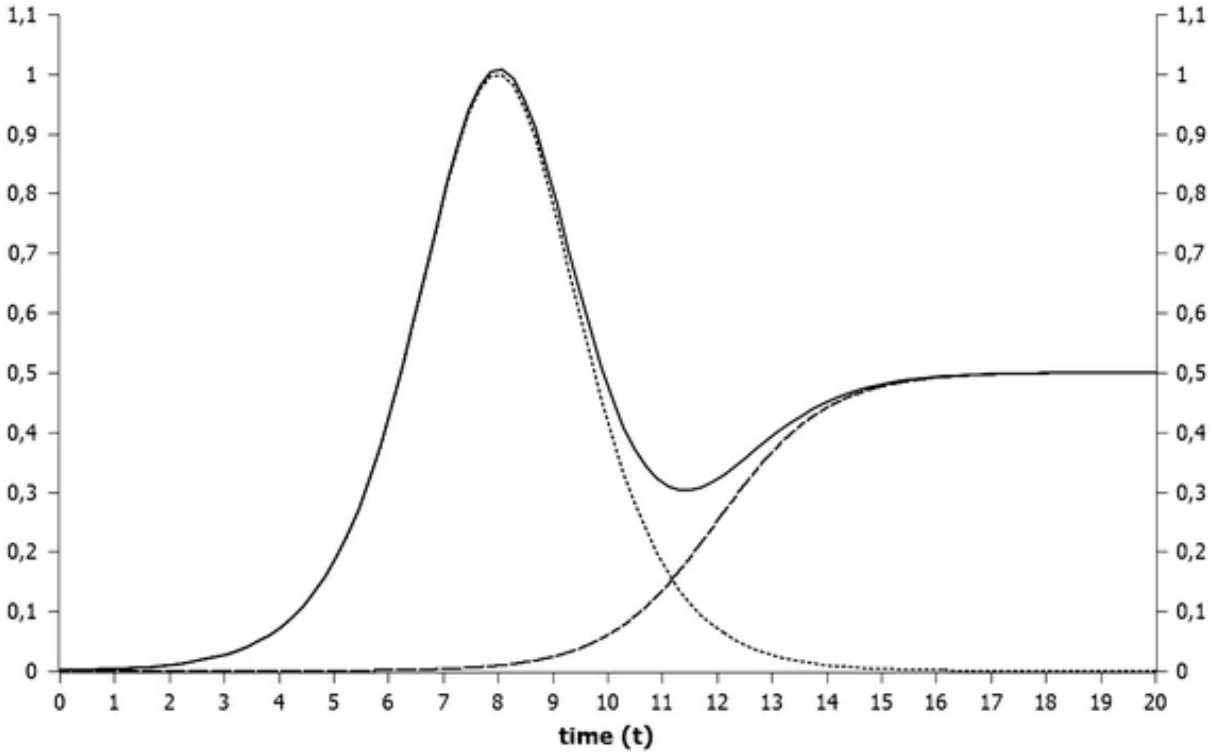


Figure 7 Hype curve disassembled [26]

In the near future if the Hype cycle is confirmed (see Figure 19 to observe the hype cycle applied to domperidone (1)) we could expect a reduction of academic interest in flow chemistry with a reduced number of publications associated to an increase of industrial applications.

On the other hand, the possibility to expand the knowledge and practical applications to reactions currently unsuitable for industrialization, such as photo- and electro-chemistry, could keep high the academic interest for flow chemistry for further industrial applications.

The main advantage of flow chemistry in the photochemistry field is the accurate and reproducible control of the energy applied to the reaction mixture.

Similarly to photochemistry, electrochemistry was also the object of interest from industry for its potentiality [5].

Now photo- and the electro-chemical reactions can run in a scalable condition without the necessity of a large capital expenditure (CAPEX).

The limited footprint of a continuous flow reactor would allow entrepreneurs to enter unregulated chemical markets and gnaw space from companies that have not been able to evolve.

Another point of interest for manufacturing is within drug discovery where integration of design-synthesis-screening-analysis and optimization will reduce the time necessary to identify a potential drug candidate. This end-to-end process was partially achieved by GSK that created a platform in which synthesis and biological tests were integrated [27]; this was a pioneering work followed by others who subsequently reported the integration of flow synthesis and biological testing.

Now the progress in automated synthesis, compound activity prediction, and automated biological testing give a tremendous opportunity to medicinal chemistry to not be the limiting step for new drugs [28].

2.1.2 Regulatory aspects

Moving back to pharma industries, the regulatory agencies have also started to consider continuous flow manufacturing in their guidance to help the API and drug product manufacturers to produce high quality drugs: “guidance describe the Agency’s current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited” [29].

In 2013 the FDA with their “Strategic plan for preventing and mitigating drug shortages” [30] proposed the “Redundancy, capability, and capacity” as a recommendation for manufacturers to avoid drug shortages. Some drug shortages related to exportation restrictions, especially in this pandemic period, could be solved by continuous flow manufacturing due to the limited space necessary for a continuous plant and quick process transfer.

Expanding the concept of continuous flow chemistry to continuous flow manufacturing, we could have a continuous flow of material from the API starting material to the tablet or other drug product (for example see Figure 8 below). However, the FDA considers a continuous process as a process with at least two connected unit operations where the material is continuously fed and from which the material/product is continuously removed.

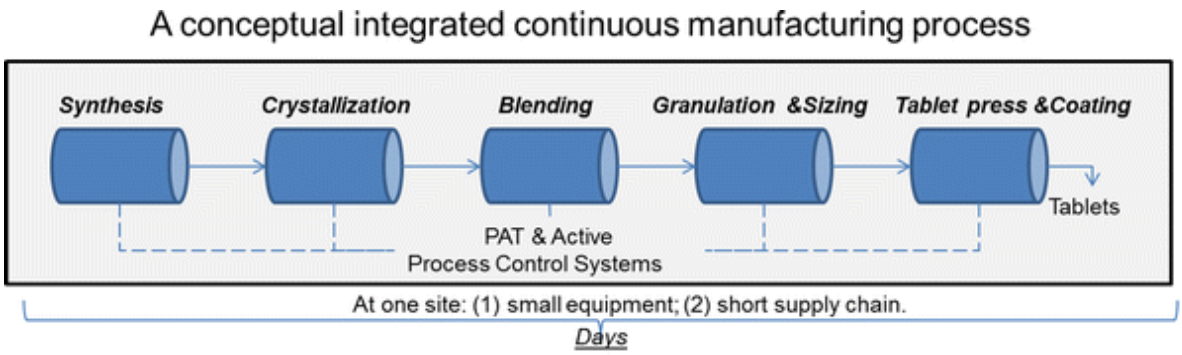


Figure 8 Conceptual fully integrated drug substance manufacturing [31] (with permission from Springer Nature)

From a regulatory point of view, in continuous flow manufacturing the concept of batch is not so clear, as in "batch manufacturing", where the material introduced into the reactor makes the product, the material is continuously introduced into the reactor and continuously the material is released from the reactor.

The FDA clearly identifies a batch at point 2 of 21CFR210.3 [32]:

"Batch means a specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture".

From the point above it was necessary to introduce the concept of residence–time distribution that describes how long the material remains in the process which can be determined by direct measurement of a tracer, by online measurement of product properties and also by mathematical methods.

In the same document the FDA expands the concept of batch using the "lot" at point 10 of 21CFR210.3 [32]:

"Lot means a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits."

In both definitions the necessity to have a uniform character and quality is clear however the product was manufactured.

Continuous flow synthesis is a powerful tool to increase the knowledge of chemical transformations [33] and the effects in the quality attributes of the product.

The knowledge of the process allows the definition of a control strategy to ensure the quality of the product. The first line of control is the in-line monitoring that is fully automated, that has not to be confused with autonomous [34] feedback control to adjust the reaction parameters in case of variation from the acceptance criteria. The second line of control is the knowledge on the impact of starting material quality to the final product. The third line of control is an accurate and tight control of the reaction parameters and the starting material specifications [31].

A thermodynamic and kinetic process model can be developed using specific software that helps to understand the intricate mechanism that connects chemistry and technology; in a conference Flavien Susanne (at that time employed in GSK) reported that the largest part of reactions, for the optimization of a process, were simulated. There are several commercially available programs that perform process modelling such as AspenPlus [35] or gProms [36] that can help the understanding of process dynamics.

In the case that the above controls fail for whatever reason, the non-conforming material needs to be handled by segregation of the material directly impacted and of the material potentially-impacted, for example the material just before and after the deviation, as the diffusion of the “contamination” makes the material out of specification. Also in such a case the knowledge of the reaction helps to decide the segregation point and where it is appropriate to restart with the “collection” of the product [31].

The FDA in the same guidance [29] includes the possibility to move a batch process to a continuous process after the completion of clinical trials. This change requires a discussion with the FDA and a subsequent study on bioequivalence in vitro; in case of “significant changes” an additional in vivo study of bioequivalence will be necessary.

The international council for harmonisation of technical requirements for pharmaceuticals for human use (ICH) is also preparing a new quality guideline, the Q13 for “Continuous Manufacturing of Drug Substance and Drug Products”[37]; this guideline is currently at an initial stage (Step 1) and the most up to date plan foresees the final adoption by November 2022[38].

2.2 Continuous flow chemistry equipment

The parts present in a continuous flow reactor are clearly described in “The Hitchhiker’s Guide to Flow Chemistry” by Plutschack et al [19] for their function. Visual examples of the equipment are reported.

- **The delivery system.**
  The use of HPLC pumps is very common (Figure 9a) as they are cheap and suitable for low and high pressures. Syringe pumps (Figure 9b) are limited by the material contained in the syringes; a recent advance in this delivery system consists of using two syringes which operate independently, the first delivers the product to the reactor while the second is being refilled and once the delivering syringe is empty the roles are switched. Peristaltic pumps are the most flexible (Figure 9c) allowing also the delivery of gases and slurries, for example the Vapourtec V-3 peristaltic pump has been used to perform a continuous flow hydrogenation using ammonium formate, and a heterogeneous palladium on charcoal slurry [39].

a) example of an HPLC pump suitable for continuous flow manufacturing from GL Sciences	
b) example of a syringe pump for continuous flow manufacturing from Syrris	

c) example of a peristaltic pump for continuous flow manufacturing from Vapourtec	

Figure 9 Examples of pumps for continuous flow manufacturing

- **The mixer.**

  In the case of small tubes and relatively slow reactions a simple T o Y mixer (Figure 10a) is enough. For a very fast reaction or in the case of process intensification, a more specific mixer needs to be included in the initial part of the reactor such as the static mixer inserted by InnoSyn in their 3D-printed reactor (Figure 10b) obtained using the Sintering Laser Melting (SLM) technology.

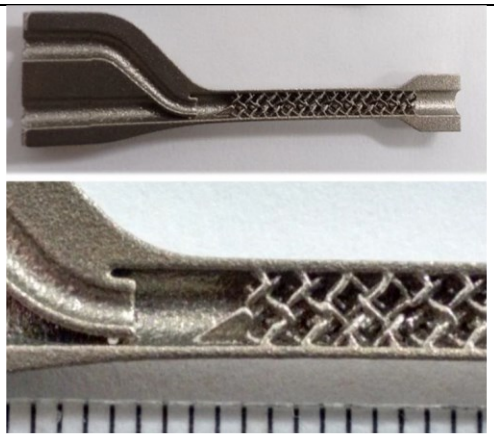
	
a) PEEK Y mixer	b) Section of a “3D printed” static mixers from INNOSYN B.V.

Figure 10 Examples of static mixers

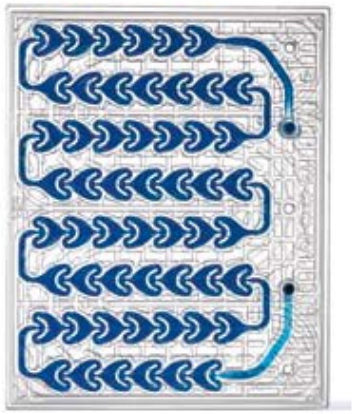
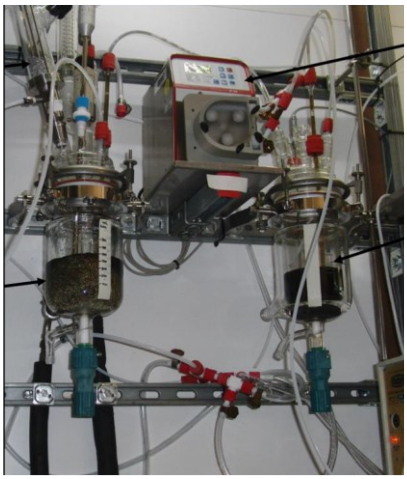
- **The reactor.**

  The reactor is the central part of a continuous flow reactor. The most common types of reactors are:

  - **Chip based reactors** with their high surface-to-volume ratio provide an accurate temperature control. The construction materials include glass,

SiC, stainless steel and all materials that are possible to 3D print (Figure 11a and Figure 11b).

- **Coil reactors** are the most used reactors in flow chemistry due to their limited cost. Temperature control can be achieved by submerging the coil in an oil bath or mounting it on a dedicated thermostatic unit like the Vapourtec R4 module. The construction materials are stainless steel or inert fluoropolymers (Figure 11c).
- **Packed bed reactors** are usually used where a heterogeneous catalyst is required. As the reaction is performed on a catalyst surface, the particle size plays a critical role in the reaction; big particles show a low surface-to-volume ratio and this will affect the conversion, on the other hand small particles show a high surface-to-volume ratio but the small particles could block the column. The construction materials are glass, chemical resistant plastic and stainless steel, all equipped with a filter to keep the catalyst inside the reactor (Figure 11d). One other type of packed bed reactor is the H-CUBE pictured later in Figure 16.
- **The continuous stirred tank reactor** (Figure 11e) is very similar to the classic batch reactor and the main difference is in the continuous feeding of reagents and consequent product removal. This type of reactor could be used to produce Grignard reagents, as the magnesium chips could block the other types of reactor.
- **The continuous loop reactor** (Figure 11f) has its typical application for high exothermic reactions such as the catalytic manufacturing process of polypropylene at commercial scale.
- Other types of reactors are the **electrochemical reactors** (Figure 11g), for electrochemical reaction; the **tube-in-tube reactor** (Figure 11i) for reaction of gasses with highly reactive species; in case of solids formed during the reaction an **agitated cell reactor** could be used to avoid clogging, such as the reactor manufactured by AM Technology, Runcorn, UK (Figure 11h); the design of the **spin reactor** manufactured by Flowid (Figure 11j) is very particular, this type of reactor is suitable also to make emulsions.

	
a) Chip reactor from Corning S.A.S.	b) “3D printed” chip reactor from InnoSyn B.V.
	
c) Coil reactor from Vapourtec ltd	d) Packed bed reactor from Vapourtec ltd
	
e) Continuous Stirred Tank Reactors at Evonik Health Care [8]	f) Loop reactor for polypropylene catalytic manufacturing process

	
g) electrochemistry module from Syrris	h) dynamically-stirred reactor from AM Technology , UK
	
i) Gas-Liquid reactor from Cambridge Reactor Design, UK	j) Spin reactor from Flowid BV, NL

Figure 11 Examples of reactors used in flow.

- **The quenching part**

  All chemical reactions need to be quenched once finished and flow reactions have the same requirement. Typically quenching consists in the addition of an aqueous solution that will be removed using appropriate equipment (see the liquid-liquid separator below). In low temperature reactions the addition of the quenching mixture needs to be performed at low temperature, for this reason the low temperature coil of Vapourtec shows an entrance for the quenching mixture just

before the exit from the cooled area. In the case of photo- or electro-chemical reactions the quenching happens once the reaction leaves the reactor, as well as using a heterogeneous catalyst.

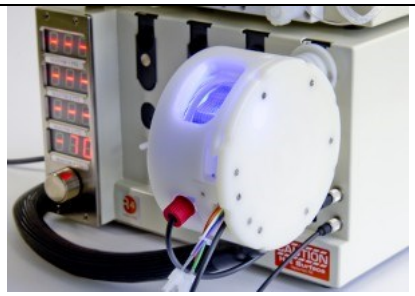
Cooled coil reactor from Vapourtec ltd	

Figure 12 Example of quenching applied to a low temperature coil

- **The back pressure regulator (BPR)**
  At the end of the reaction sequence there is the part of the equipment which allows the flow reactor to work under pressure to avoid solvent evaporation despite being heated to temperatures higher than its boiling point. There are 2 types of BPR, the pre-set type (Figure 13a) and the adjustable type (Figure 13b): in the first, a calibrated spring maintains the liquid inside the tube until the calibration pressure is reached; in the second, a diaphragm releases the liquid once the pressure reaches the reference pressure on the other side of the diaphragm.

	
a) Pre-set BPR	b) adjustable BPR from Zaptuit

Figure 13 Examples of BPRs

- **The collection system.**

  After the BPR the reaction mixture can be collected in a flask or using an automated fraction collector. The fraction collector is usually manufactured by a specialized company and integrated into the flow system (Figure 14a and Figure 14b).

	
a) Asia Product Collector from Syrris	b) FC 204 Fraction Collector from Gilson

Figure 14 Examples of fraction collectors

- **The optional part**
  - **In–line analysis equipment** automatically collect a sample from the reaction mixture and perform the analysis; the in-line analysis can be performed continuously using non-destructive techniques like NMR (Figure 15a), FT-IR (Figure 15b), Raman (Figure 15c) and UV-Vis spectroscopy. HPLC/UPLC and GC have also been integrated into continuous flow reactors but these techniques require addition sample preparation and a subsequent delay, therefore they do not show a real picture of the process output at that time [40].
  - Purification consists mainly in liquid-liquid separation performed using semipermeable membranes such as in the Zaptuit equipment (Figure 15d).

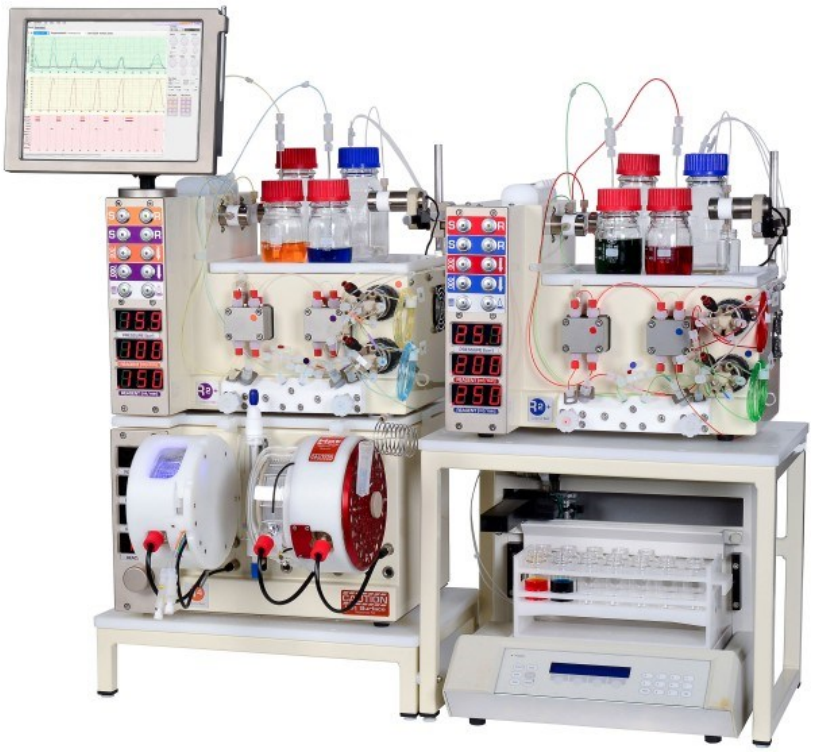
	
a) In-line benchtop NMR from Magritek	b) In-line FTIR system from Mettler Toledo
	
c) Electrochemical flow cell equipped with Raman probe from Redoxme AB	d) Liquid-liquid/gas-liquid continuous separators from Zaptuit

Figure 15 Examples of optional parts: a) in-line NMR, b) in-line FTIR, c) Electrochemical flow cell with Raman probe, d) continuous phase separator.

- The use of scavengers requires their regeneration/replacement once saturated.
- In line crystallization is possible but has not found application at the moment in the laboratory routine.
- Automation of the reaction in continuous flow chemistry can be reached integrating a logic machine into the flow equipment to provide feedback from the in-line analysis equipment to the delivery system in the case of an established process to avoid deviation or to provide data for the optimization performing the reaction under of thousands of conditions [41].

In contrast to custom-built flow systems there are also fully automated systems available

- Fully automated systems are on sale from different manufacturers well known in the field, some of which are displayed in Figure 16 below.

Vapourtec RS-300	
Syrris Asia Flow Chemistry System	

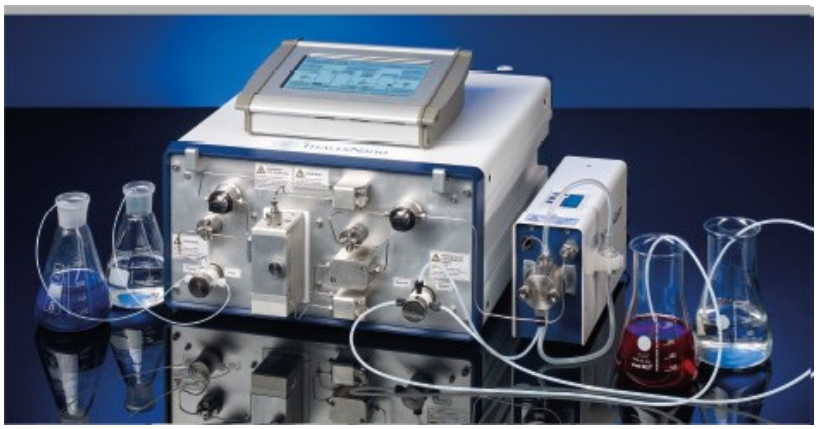
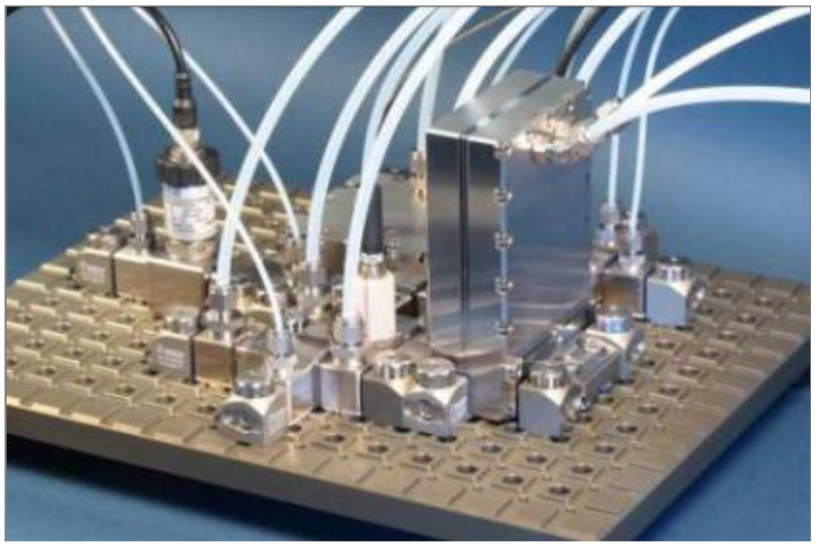
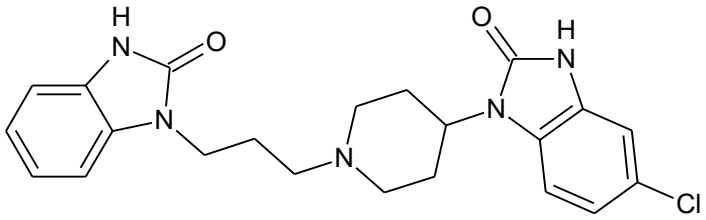
Uniqsis Ltd FlowSyn Auto-LF	
Thalesnano Inc. H-Cube® Pro	
Ehrfeld Mikrotechnik MMRS (Modular MicroReaction System)	

Figure 16 Automated continuous flow chemistry systems

The first four instruments reported show low flexibility with regards to further implementation/integration but they are completely automatic with their own control software. The last instrument is completely flexible as each piece can be placed anywhere in the plate providing a starting platform to integrate third party control systems for in-process monitoring by process analytical technology (PAT) [40].

2.3 Domperidone (1)

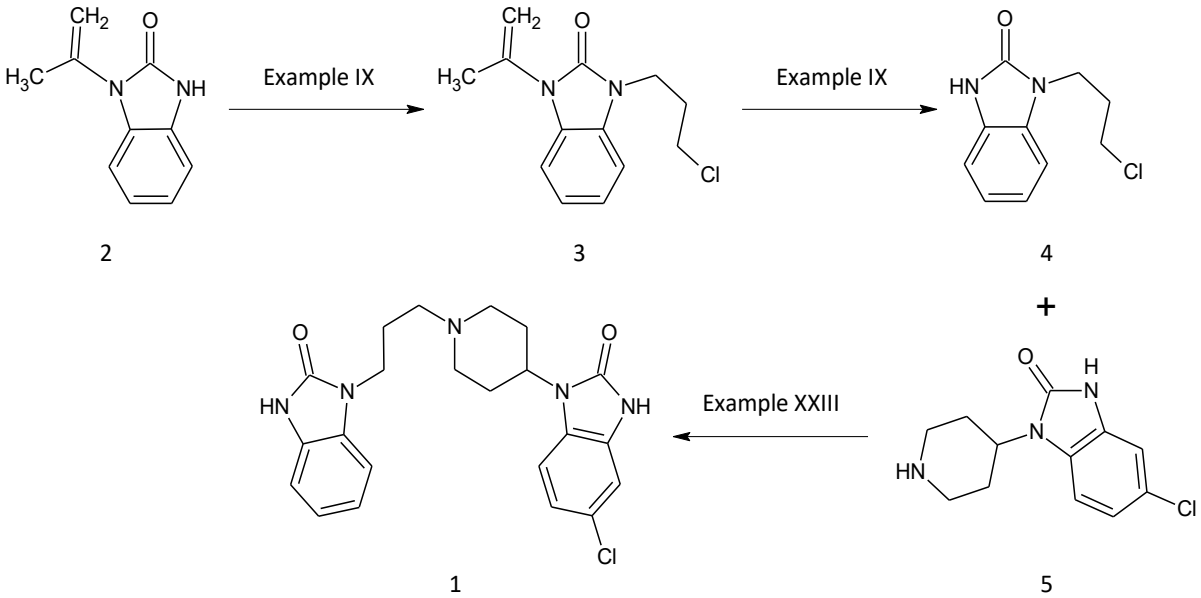
Domperidone (1), pictured in Figure 17, was first synthesized in 1974 [42] and was patented in 1978 (3th January) under patent US4066772 in the USA by Janssen Pharmaceutica N.V., the inventors were Jan Vandenberk, Ludo E. J. Kennis, Marcel J. M. C. Van der Aa and Albert H. M Th. Van Heertum. The title of the patent is "1,3-Dihydro-1-[3-(1-piperidinyl)propyl]-2H-benzimidazol-2-ones and related compounds" and it was the continuation of the application Ser, No 597793 filed in 1975 and at that time abandoned [43].



1

Figure 17 Domperidone (1)

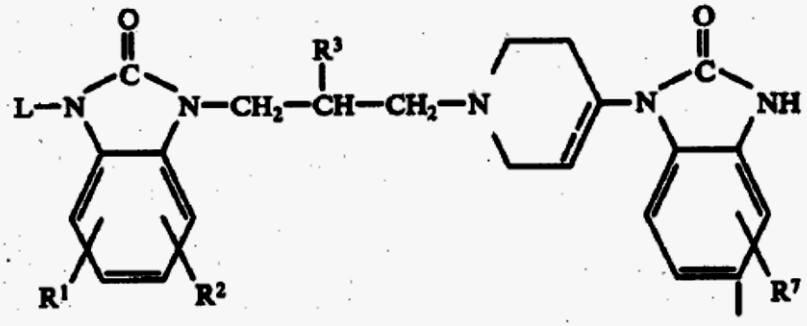
The synthetic route presented in US4066772 is reported in Scheme 1 below. For the synthesis of the starting material the patent [43] refers to "the procedure outlined in J. Chem. Soc., 1960, p. 308 and p. 314" [44].



Scheme 1 Synthetic scheme of Domperidone reported in US4066772

The same patent [43] reports the activity (ED_{50} - the dose that protects 50% of animals from emesis) of Domperidone in blocking apomorphine induced vomiting in dog as show in Figure 18 below.

TABLE II



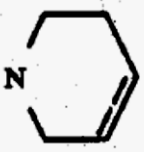
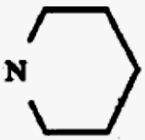
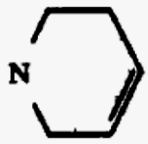
L	R^1	R^2	R^3		R^7	Salt and Solvate form	ED_{50} mg/kg s.c.
H	H	H	H		H	—	0.02
							
H	5-CL	H	H	"	H	—	0.10
H	5-CH_3	6-CH_3	H	"	H	CH_3—CHOH—CH_3	0.45
H	H	H	CH_3	"	5-Cl	—	0.06
H	H	H	H	"	5-Cl	—	0.01
H	5-Cl	H	H	"	5-Cl		0.12
CH_3	H	H	H	"	5-Cl	—	0.16
CH_2=CH—CH_2	H	H	H	"	%-Cl	—	0.12
H	H	H	H		H	—	0.04
							
H	5-F	H	H		5-Cl	HCl H_2O	0.004
							
H	5-CH_3	H	H	"	5-Cl	HCl H_2O	0.12
C(CH_3)=CH_2	H	H	H	"	5-Cl	—	0.20
H	5-CH_3	H	H	"	H	HCl H_2O	0.20

Figure 18 Activity of Domperidone on emesis in dog

Domperidone has a molecular weight of 425.9. In human depending on intramuscular (IM) or oral administration, peak plasma levels of Domperidone occur anywhere from 10 to 30 min after administration. Peak levels after rectal administration of suppositories are

usually achieved after 1–2 h. Bioavailability is high after intra-muscular administration (90%), and much lower with oral administration (13–17%). This is probably a result of both incomplete absorption and a first-pass effect with oral administration. The bioavailability of oral Domperidone is further decreased by increasing the pH of the stomach via antacid medications due to the poor solubility of Domperidone free base. The plasma protein binding is 92% and it is rapidly metabolized by the liver to inactive metabolites. Following oral administration, 32% of the adsorbed drug is excreted in the urine [42].

On PubMed in April 2020, 2669 journal articles that cite “domperidone” as a keyword were reported. The distribution of the articles by publication year is reported in Figure 19 below, the graph follows the hype curve reported in Figure 7.

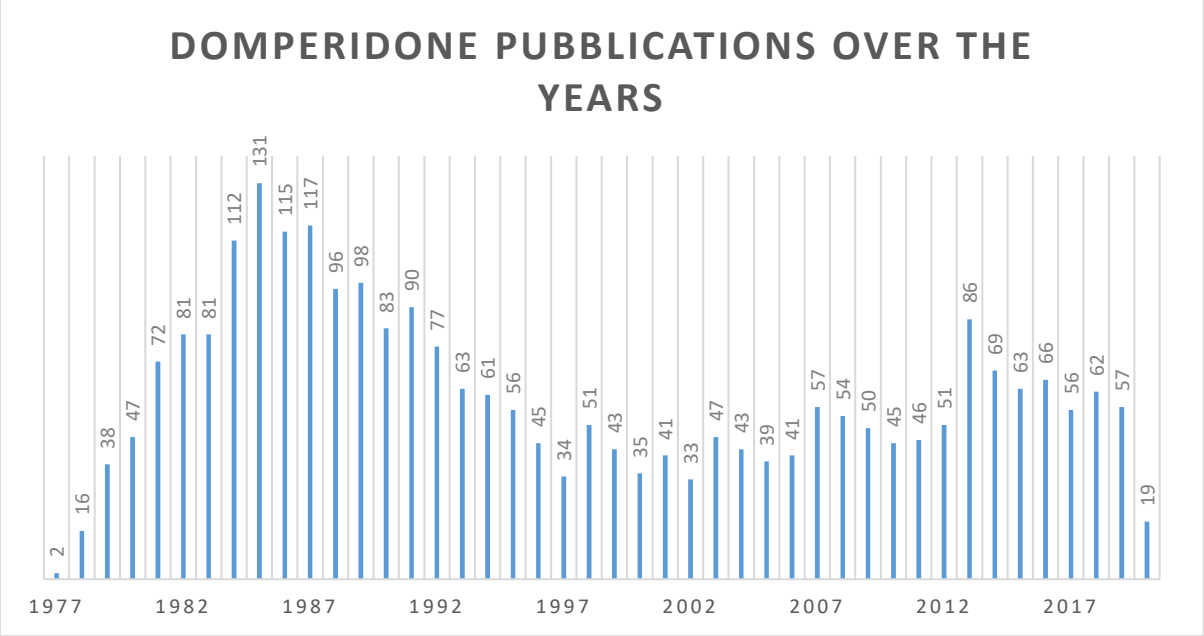


Figure 19 Number of publications for "domperidone" in PubMed[3]

Pharmacology books, for example Goodman & Gilman's, place Domperidone in the gastrointestinal disease chapter. The pharmacological basis of therapy 12th Ed. places Domperidone in the chapter “Treatment of disorders of bowel motility and water flux; antiemetic; agents used in biliary and pancreatic disease” [45] and Katzung’s Basic & Clinical Pharmacology 12th Ed. places Domperidone in the chapter “Drugs used in the treatment of gastrointestinal diseases” [46].

Both books describe Domperidone as a dopamine D_2-receptor antagonist without major involvements of other receptors[45]; the gastrointestinal tract activation of dopamine receptors blocks the cholinergic muscle activation. Domperidone and other compounds showing related activity increase the oesophageal peristaltic amplitude, increase the lower

[3] Data extrapolated in April 2020.

oesophageal sphincter pressure, and enhance gastric emptying without effecting the small intestine or colonic motility. The block of dopamine D_2-receptors in the chemoreceptor trigger zone of the medulla (area postrema) results in potent anti-nausea and antiemetic action [46] as the initial activity described in the Domperidone patent [43].

Domperidone is not approved as a drug in the USA. On June 7, 2004, the FDA issued a public warning that distributing any Domperidone-containing products is illegal. The FDA took this action because of concerns regarding the potential serious health risks associated with the use of Domperidone, highlighted in reports, by lactating women to enhance breast milk production [47].
The serious risks associated with Domperidone include cardiac arrhythmias, cardiac arrest, and sudden death. These risks are related to the blood level of Domperidone, and higher levels in the blood are associated with higher risks of these events. The combined use of certain common drugs, such as erythromycin, could raise blood levels of Domperidone and further increase the risk of serious adverse cardiac outcomes [42,48].
The FDA allows physicians to prescribe Domperidone for gastrointestinal symptoms that are refractory to standard medical management, as compassionate use, by requesting and initiating an investigational new drug application (IND) permitting administration of investigational drugs to humans [48].
Domperidone is sometimes used in the treatment of symptomatic Gastroesophageal Reflux Disease (GERD) but prokinetic agents are used mainly in combination with anti-secretory agents in patients with regurgitation or refractory heartburn.

Domperidone is widely used in the treatment of patients with delayed gastric emptying due to postsurgical disorders (vagotomy, antrectomy) and diabetic gastroparesis, and Domperidone leads to symptomatic improvement in a small number of patients with chronic dyspepsia.

The most common adverse effects of Domperidone are related to elevated prolactin levels and it can cause galactorrhoea, a side effect used by lactating women [42], gynecomastia, impotence, and menstrual disorders; neuropsychiatric and extrapyramidal effects are rare.

In Italy Domperidone is approved for use in abdominal pain, nausea and vomiting, functional dyspepsia and reflux. The reported side effects are rare and are hyperprolactinemia, abdominal cramps, rash and extrapyramidal symptoms[49].

On September 01, 2014 the European Medicines Agency (EMA) took the decision to reduce the maximum daily dose of Domperidone to 30 mg. This was the second restriction on the use of Domperidone after the withdrawal from the market due to adverse effects of the intravenous form in 1986[50].

In Italy the authorized products containing Domperidone in March 2020 were 47 (number of AIC) from 20 different pharmaceutical companies. The majority of the products approved are in the form of 10 mg tablets; there are also approved 5mg tablets, effervescent granules, 1mg oral suspension and suppositories[51].

3 Synthesis of Domperidone.

3.1 From the first patent to the commercial synthesis

As mentioned above, the synthesis of Domperidone was first reported in 1978 [43]. The synthesis reported in documents provided to the Ministry of Environment, Forest and Climate Change, Government of India [52] to authorize plant expansion or build-up [53–55] was the same synthesis reported in the Domperidone patent.

Domperidone can be considered as deriving from two principal building blocks, represented with blue ovals, connected by a linker/spacer (red oval) as reported in Figure 20.

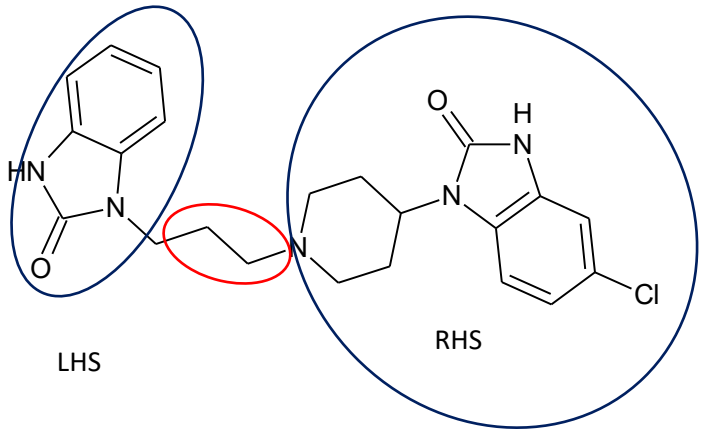
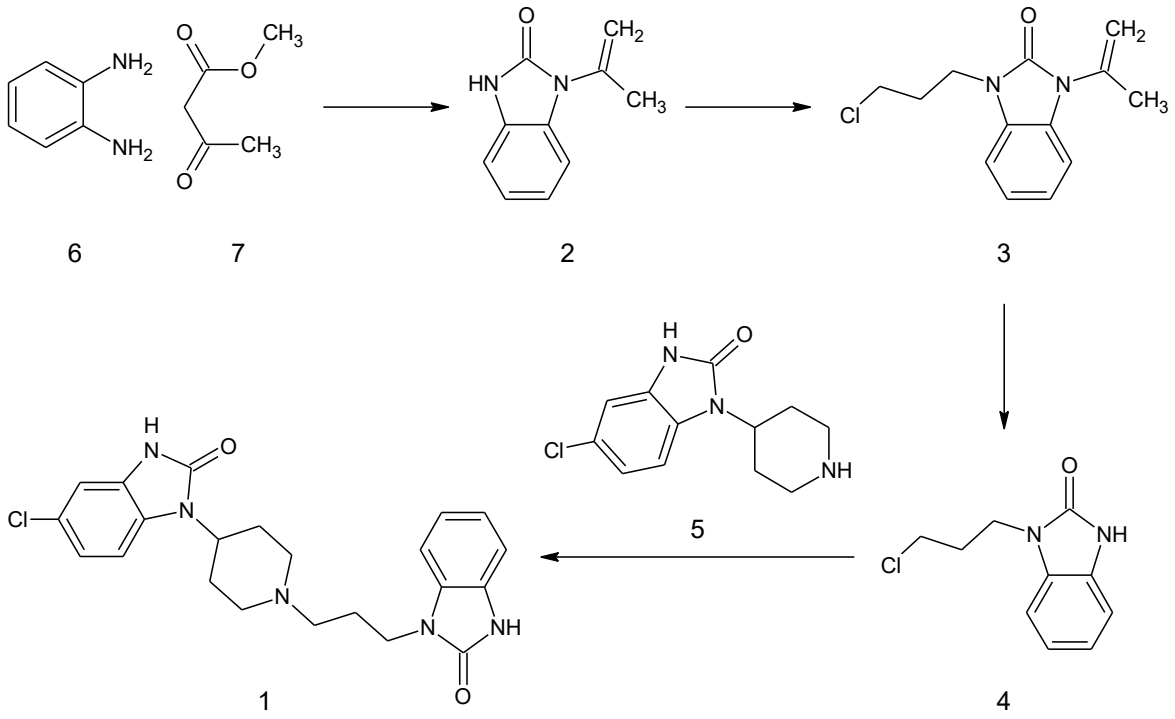


Figure 20 Domperidone and its building blocks

The commercial synthesis, reported in Scheme 2 below, shows the construction of the Left-Hand-Side (LHS) part, then insertion of the linker and at the end connection of the intermediate obtained at that point with the Right-Hand-Side (RHS) part to give Domperidone.

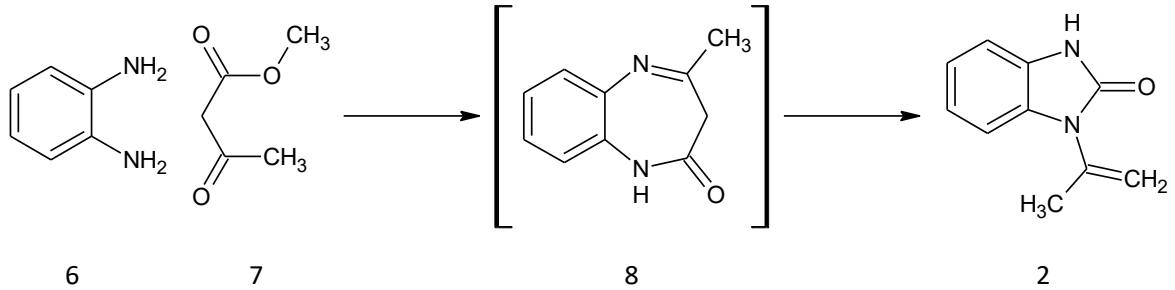


Scheme 2 Commercial synthesis of Domperidone

3.1.1 Synthesis of 1,3-dihydro-2H-benzimidazol-2-one and linker addition.

The LHS 1,3-dihydro-2H-benzimidazol-2-one, in the Domperidone patent [43] was synthesized according to the procedure reported by Sexton [56], who obtained a different compound to that initially assigned as later ascertained by Davoll [44].

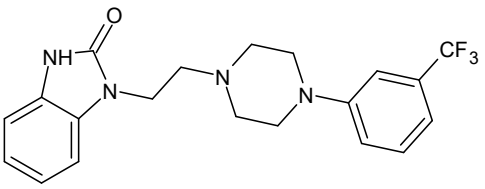
The synthesis of unsubstituted 1,3-dihydro-2H-benzimidazol-2-one could be achieved in several ways[57], but without any differentiation on the two NH positions the selective mono alkylation cannot be achieved. The 1,3-dihydro-2H-benzimidazol-2-one could be obtained by reaction of benzene-1,2-diamine (6) with a carbonyl source such as urea [43], 1,1′-carbonyldiimidazole [58], or phosgene and its safer derivatives.



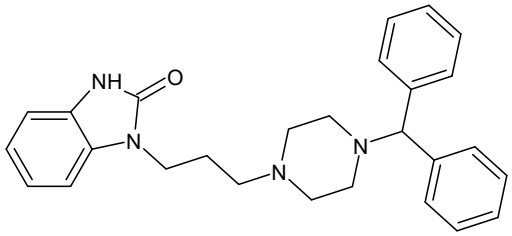
Scheme 3 Synthesis of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2)

1-(Prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (compound 2 in Scheme 3), is a key intermediate for the synthesis of Domperidone and other active compounds such as

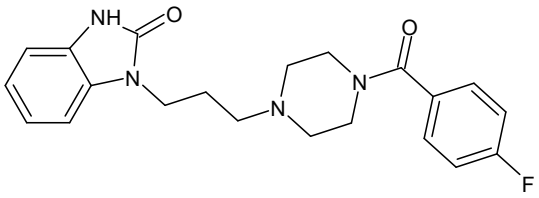
those reported in Figure 21 below. The 1,3-dihydro-2H-benzimidazol-2-one substructure is present in a larger number of approved drugs but in other cases the moiety is related with the 1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one substructure (eg. compound 5 in Scheme 1 and paragraph 3.1.4).



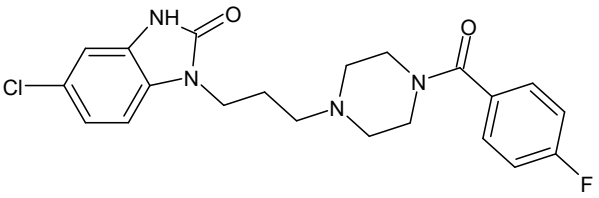
Flibaserin



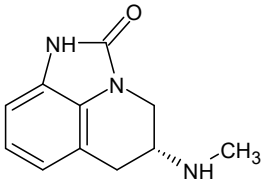
Oxatomide



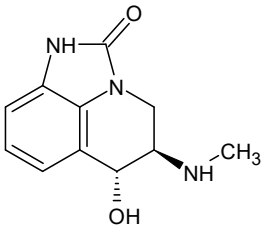
Declenperone



Milenperone



Sumanirole



Zilpaterol

Figure 21 Structures of approved drugs using the mono-protected benzimidazolone in their synthesis

The interest in this building block is not only limited to these approved drugs, but it is also inserted for New Chemical Entities (NCE) under development in “Synthesis and structural study of N-isopropenylbenzimidazolone” (Mondieig et al.) [59].

The first synthetic step starts from an inexpensive material and generates the benzimidazol-2-one; the mono protection on one of the nucleophilic nitrogens allows the subsequent mono-alkylation on the other nucleophilic nitrogen.

Also today the reported synthesis of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2) in the public document provided to the Ministry of Environment, Forest and Climate Change, Government of India [53–55] follows the same route:

“Process: Benzene-1,2-diamine (6) is condensed with Methyl Acetoacetate (7) in Xylene. Xylene is distilled off and the mass is filtered to get crude Stage I which is further purified by dissolving in Lye solution and re-precipitating with hydrochloric acid”. An attempt to perform this reaction with high yield in batch was unsuccessful despite the reported yield of 82 % mol/mol. Attempts to reduce the activation energy for the [1,3] sigmatropic rearrangement with Lewis acid or base were also unsuccessful.

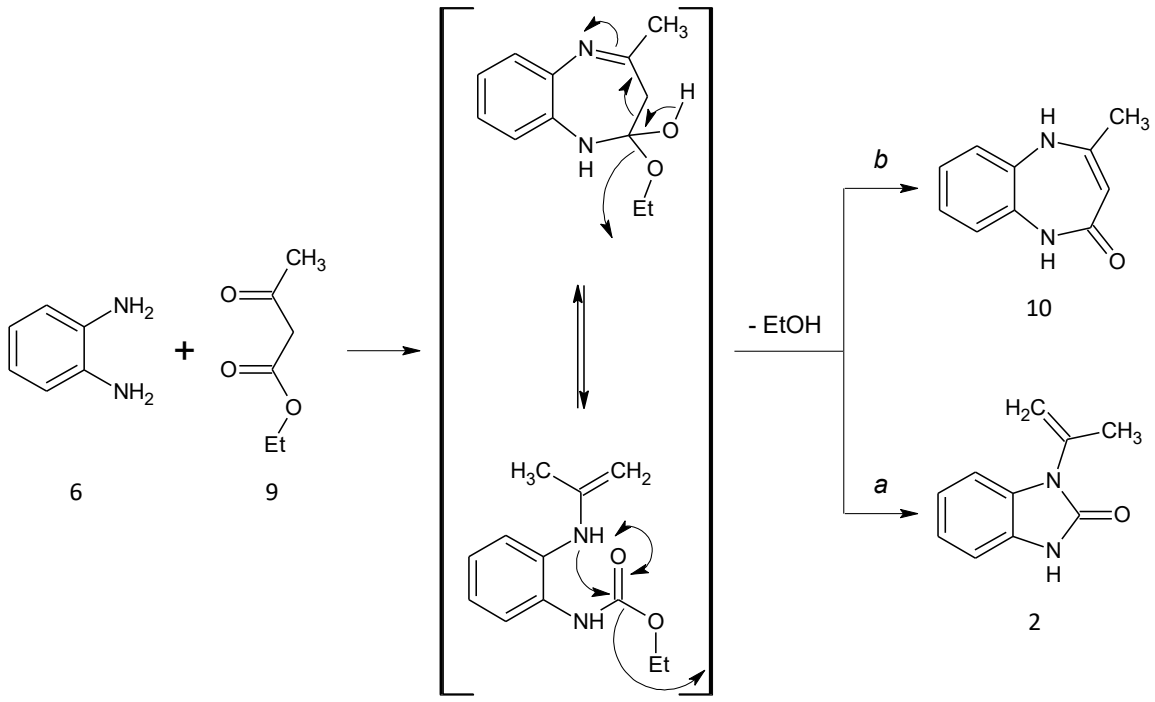
Another problem observed was the poor solubility of benzene-1,2-diamine (6) in xylene, making it incompatible with the continuous flow chemistry approach planned for this work. Also the removal of water [60] and ethanol [61] were unsuitable for the flow equipment available in house.

The reaction time required to perform the cyclization and the subsequent rearrangement was also unsuitable for a continuous flow chemistry system, despite Khajavi et al. [62] having reported the cyclization of benzene-1,2-diamine (6) with ethyl acetoacetate in xylene for 2 minutes with microwave irradiation to give 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one and 1.5 min of irradiation to give the corresponding benzodiazepinones. Burgin et al. [63] first and Koizumi et al. [64] later, performed the cyclization of benzene-1,2-diamine (6) with methyl acetoacetate (7) by irradiation in a microwave and the latter also in an oil bath at 180°C. These experiments resulted in the formation of the 1,5-benzodiazepin-2-one in c.a. 83% yield both under thermal and microwave heating. As the microwave allows to reach, in a solvent free experiment, higher temperatures of up to 250°C, these resulted in a lower yield due to the decomposition of the product at high temperature [64]. So also the increase of the temperature did not force the formation of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2).

A literature research for this transformation reported 32 references (12 patents and 20 journal articles) where this transformation was performed. The transformation could be summarized in the procedure below:

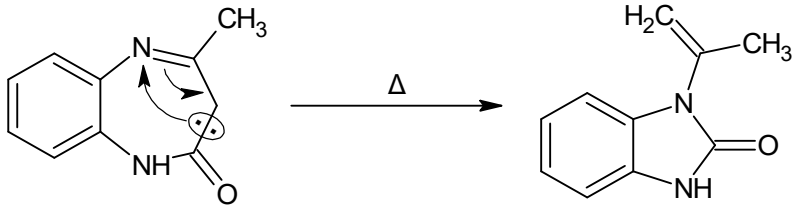
“Ethyl acetoacetate in xylene was added dropwise to a refluxing solution of *o*-phenylendiamine (benzene-1,2-diamine (6)) in xylene under nitrogen, while the water produced during the reflux was removed using a Dean-Stark trap. It was refluxed for several hours to obtain the desired compound”.

The data in the literature confirmed the rapid formation of the 1,5-benzodiazepin-2-one (product 10 in Scheme 4 below), but a slow [1,3] sigmatropic rearrangement [57,65].

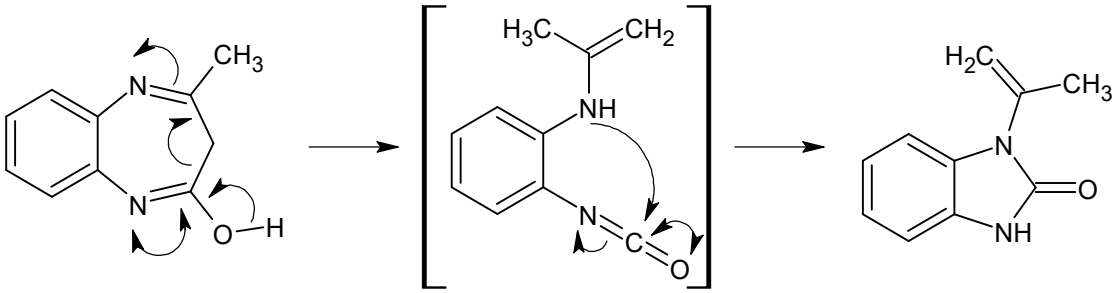


Scheme 4 Possible products from benzene-1,2-diamine (6) and ethyl acetoacetate

However, the literature is not clear on this point[57] as shown in Scheme 5 and Scheme 6 below.



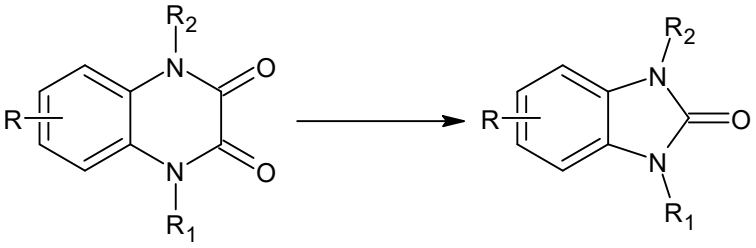
Scheme 5 [1,3]-sigmatropic rearrangement



Scheme 6 Cyclization via isocyanate intermediate

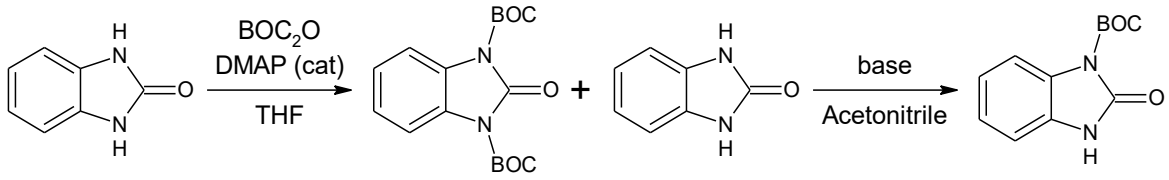
Flibanserin, which contains the same benzimidazolone building block, has been the subject of greater investigation in recent years and several new routes with potential application to Domperidone synthesis were found. The synthesis of O-ethyl benzimidazolone as protected benzimidazolone via reaction of benzene-1,2-diamine (6)

with tetraethyl orthocarbonate [66] showed high yield and the same approach was applied to deuterated Domperidone [67]. This approach was not pursued to avoid patent infringements. More recently, the ring contraction of the benzyl-protected quinoxalinedione was reported (Scheme 7 below) [68].



Scheme 7 Quinoxalinedione ring contraction

A very high yield of mono-protected benzimidazolone was described by Meanwell et al. [58], who reported the migration of the BOC-group from a diprotected-benzimidazolone to an unsubstituted benzimidazolone, in near equimolar ratio, in acetonitrile in the presence of potassium carbonate. The overall yield was 94% over the two steps, Scheme 8 below.

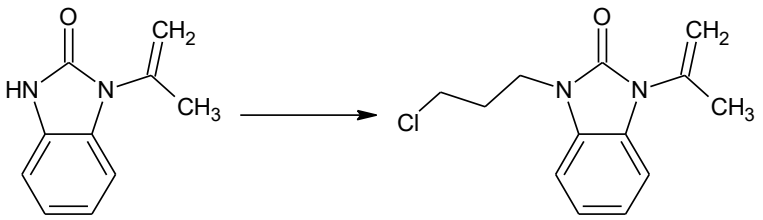


Scheme 8 Meanwell et al. procedure

The most recent application was the article and patent from the same group at Richter Gedeon Nyrt [69,70] that reported the synthetic route for Flibanserin in flow chemistry. This synthetic route uses a completely different approach to insert the linker, performing reductive amination instead of the alkylation; the concern relative to the use of alkylating agents is not mitigated by using aldehyde intermediates as the aldehydes are structures of concern for potential genotoxicity [71].

3.1.2 Alkylation of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2)

The second step in the synthesis of Domperidone is the alkylation of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2) with 1-bromo-3-chloropropane (23) to give 1-(3-chloropropyl)-3-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (3).



2 3

Scheme 9 Synthesis of 1-(3-chloropropyl)-3-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (3)

The alkylation of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2) was reported to be performed in toluene in the presence of anhydrous potassium carbonate, followed by removal of the protecting group [54,55]. The initial procedure in the Domperidone patent [43] reported the alkylation in DMF in the presence of sodium hydride as base. Other alkylation conditions reported in the literature include Schotten-Baumann conditions [72], while a very large part of the references perform the alkylation in a polar solvent in the presence of base; iodide was also used as a catalyst in some examples.

The document provided to the Ministry of Environment, Forest and Climate Change, Government of India [53] reports the procedure below for the second stage.

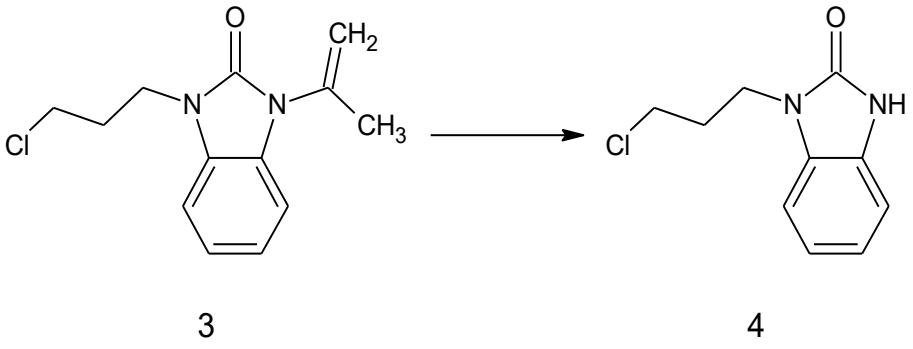
“Process: The Stage–I compound obtained above is reacted with 1-bromo-3-chloro-propane in Toluene in presence of anhydrous potassium carbonate. After the completion of the reaction add water, the toluene layer is separated and washed with dilute alkali to remove unreacted Stage I compound. The toluene layer is hydrolysed in dilute hydrochloric acid and the toluene layer is separated and distilled off to get the title compound.”

This reaction is also unsuitable for continuous flow chemistry due to the use of solid potassium carbonate that, if used in a packed column, will result in an uncontrolled depletion of the number of equivalents of reagent during the reaction. A potential development of this reaction will require a two parallel packed bed reactor with in-line monitoring of the output to allow the switch to the second (freshly) packed bed reactor once the output is out of line with the expectation due to the consumption of the potassium carbonate [73].

3.1.3 Deprotection of 1-(3-chloropropyl)-3-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (3)

The subsequent deprotection with HCl is not supposed to be a problem in continuous flow chemistry if the product remains in solution. A small amount of solid precipitation could be

handled in continuous flow chemistry by reducing the concentration of the reagents. The second approach to handle solids in flow chemistry, while maintaining a small tube bore, is to employ sonication, where the vibrations allow the solid to move along the tubing [19].



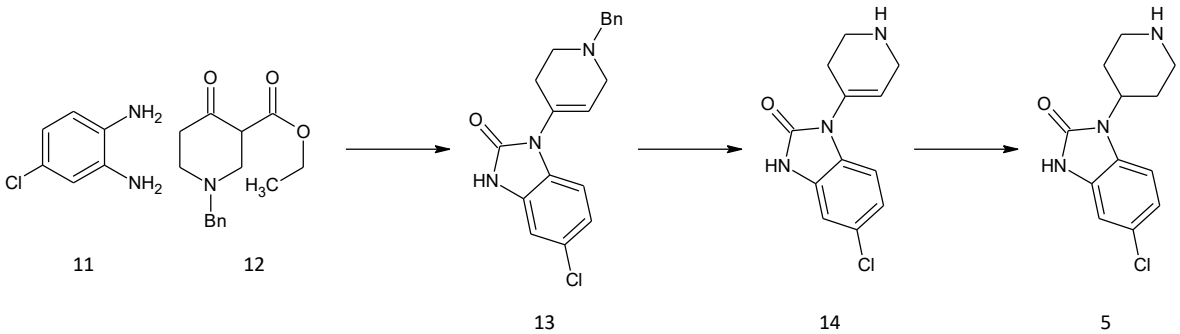
3 4

Scheme 10 Synthesis of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4)

A SciFinder-n search for the removal of the "prop-1-en-2-yl" protecting group showed, as preferred condition, the treatment of the protected compound with hydrogen chloride, but also other acids have been used, such as acetic acid [74] and sulfuric acid [75].

3.1.4 Synthesis of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5)

The Domperidone scaffold is now ready for the alkylation of the second building block on the RHS of Figure 20. The RHS part is now commercially available from various suppliers, but at the time of Domperidone's discovery Janssen had to synthesize it accordingly to Scheme 11 below.



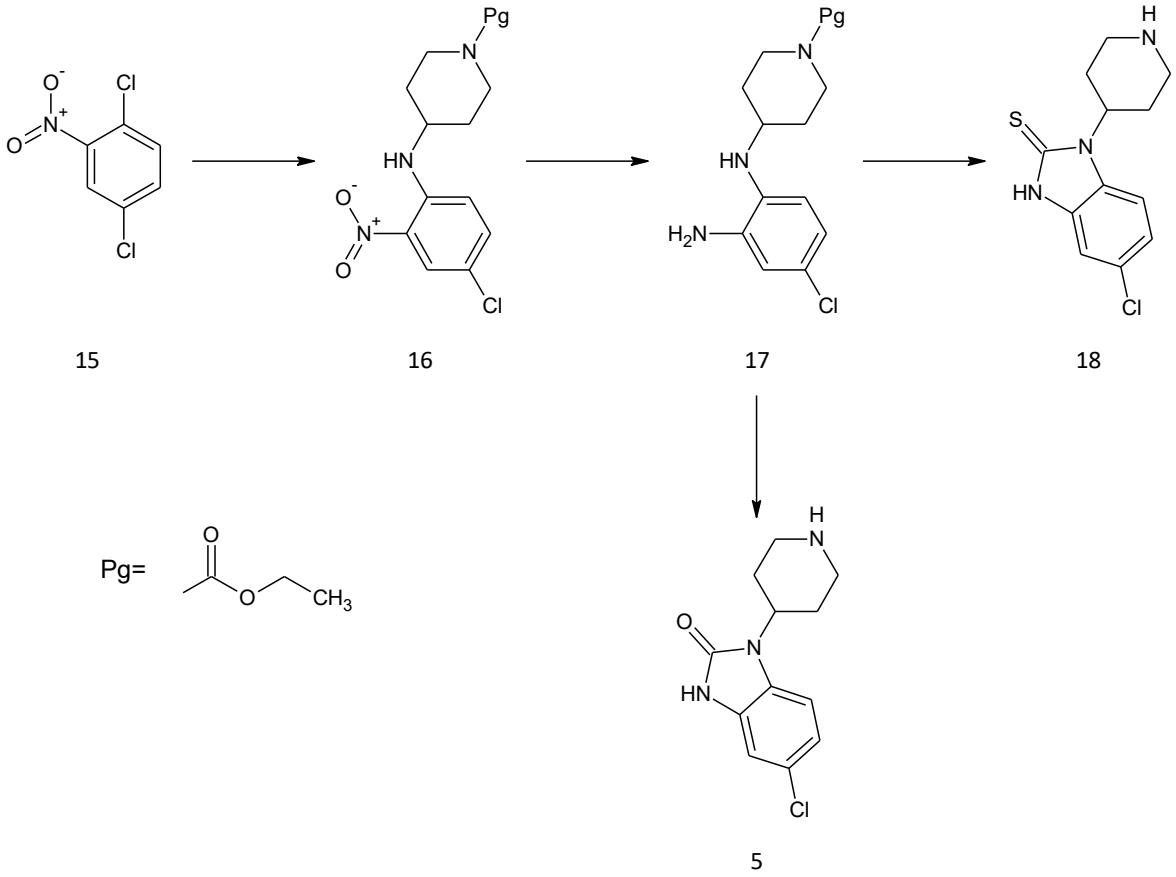
11 12 13 14 5

Scheme 11 Synthesis of the RHS part of Domperidone accordingly to US3161645A

For the synthesis of the RHS part the Domperidone patent [43] refers to US3161645A [76] which describes a similar synthesis in example 1, but a more general description can be found in the claims: "To prepare the 1-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)-5-chloro-1,3-dihydro-2H-benzimidazol-2-one (13) used above as intermediates, 1-benzyl-3-

carbethoxy-2-piperidone (12) is heated with an appropriate 1,2-phenylenediamine, 4-chloro-1,2-phenylenediamine (11) in this case, in an inert solvent such as xylene. This gives the 1-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)-5-chloro-1,3-dihydro-2H-benzimidazol-2-one (13)…" "…Catalytic hydrogenation removes the benzyl group to give the desired tetrahydropyridyl compound (14)" a further hydrogenation in presence of acetic acid and "stopped when two molar equivalents of hydrogen has been taken up" [76] will provide the desired 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5) .

The regiochemistry in this synthesis is not controlled and therefore the Domperidone patent reported the synthesis of a thio-derivative of the RHS building block (18) according to Scheme 12 below.



Scheme 12 Adaptation of the synthesis of the RHS part of Domperidone (compound D (5)), accordingly to US4066772A

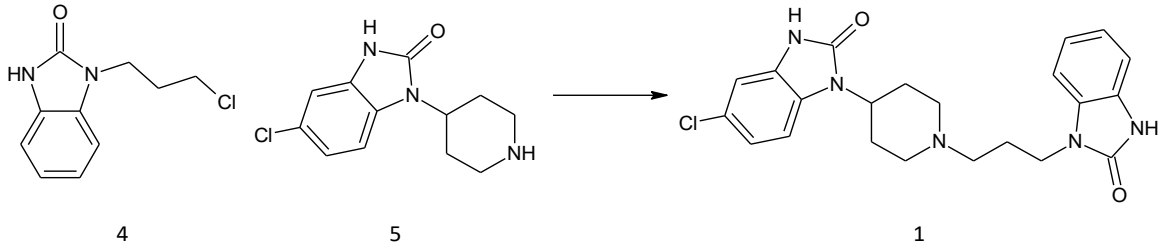
"The starting materials (18) are conveniently prepared by the cyclization of (17) with an appropriate cyclizing agent e.g., carbon disulphide" [43], replacing the cyclizing agent with a carbonyl-group source the cyclization will provide the compound D (5).

The current commercial synthesis of the RHS building block reported in the public document provided to the Ministry of Environment, Forest and Climate Change, Government of India [52] follows the route reported in Scheme 12.

The final cyclization seems to be conducted in solvent free conditions with urea at 180-185°C followed by an aqueous work-up.

From a literature search, the synthesis of compound D (5) follows the same procedure reported in Scheme 12 where the cyclization was performed with 1,1'-Carbonyldiimidazole (CDI) [77] or phosgene [65] (or compounds related to phosgene) as the carbonyl-group source.

3.1.5 Synthesis of Domperidone, final coupling



4 5 1

Scheme 13 Synthesis of Domperidone, final coupling

The last reaction to obtain Domperidone, according to the original patent [43], is the alkylation of compound 5 with compound 4 as reported in Scheme 13 using 4-methyl-2-pentanone as solvent in the presence of sodium carbonate and with potassium iodide catalysis. After an aqueous work-up, the product was purified by silica gel chromatography before recrystallization from DMF/water which give Domperidone in 30% yield.

A literature search on the alkylation reaction shows DMF as the preferred solvent (173 hits out of 303) followed by dichloromethane, acetonitrile and THF. Also different bases were used, including carbonates, hydroxides and organic bases such as triethylamine.

The commercial route to Domperidone reported a further purification, wherein the isolated technical grade or intermediate grade (I.G.) Domperidone was dissolved in methanol and acetic acid and treated with activated carbon. The solution was then basified with gaseous ammonia to give pharma grade Domperidone [54,55].

The manufacturing of Domperidone would be suitable for continuous flow synthesis, both considering it a fine chemical and on practical point of view; but the synthesis of compound 2 seems unsuitable for a continuous flow synthesis for practical reason. Hence, a different intermediate needs to be found. The recrystallization of Domperidone will require specific equipment able to handle solids at the collection point, as the solubility of Domperidone free base is very low as reported in the literature [78].

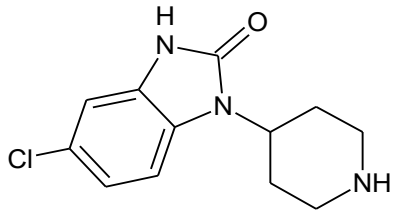
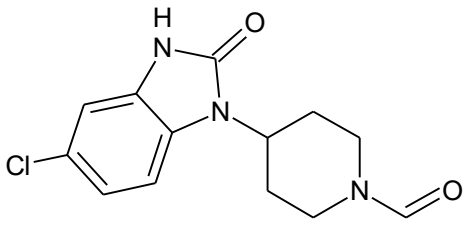
The scale of solubility of Domperidone in different solvents is reported below:

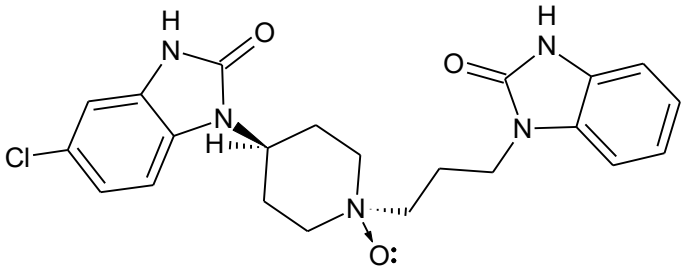
DMA > DMSO > DMF > n-butanol > n-propanol > (isobutanol, ethylene glycol) > ethanol > isopropanol > methanol > acetonitrile > water.

3.2 Domperidone impurities

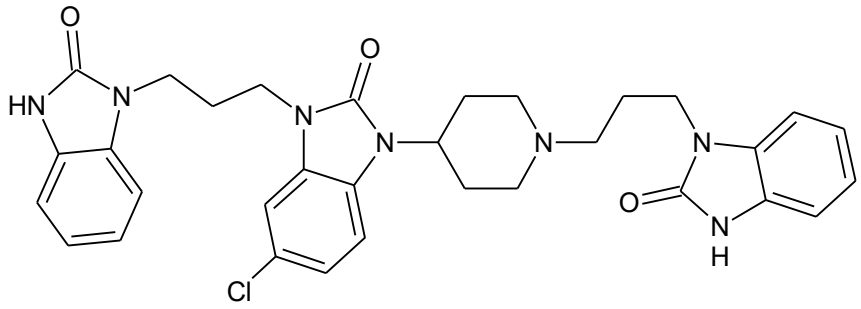
Domperidone is reported in the European Pharmacopoeia in monograph 1009; there are 6 identified impurities, and the relative structures are reported in Table 1 below. There is also the Domperidone maleate monograph 1008 as a more soluble (in water) form of Domperidone; the impurities reported for the maleate are the same as for Domperidone free base.

Three of these impurities, impurity D, impurity E and impurity F, are related to an uncontrolled alkylation at the last step.

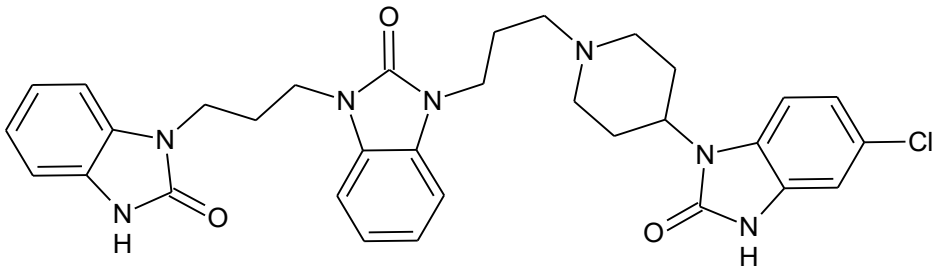
	
Impurity A: 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5)	Impurity B: 4-(5-chloro-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)-1-formylpiperidine



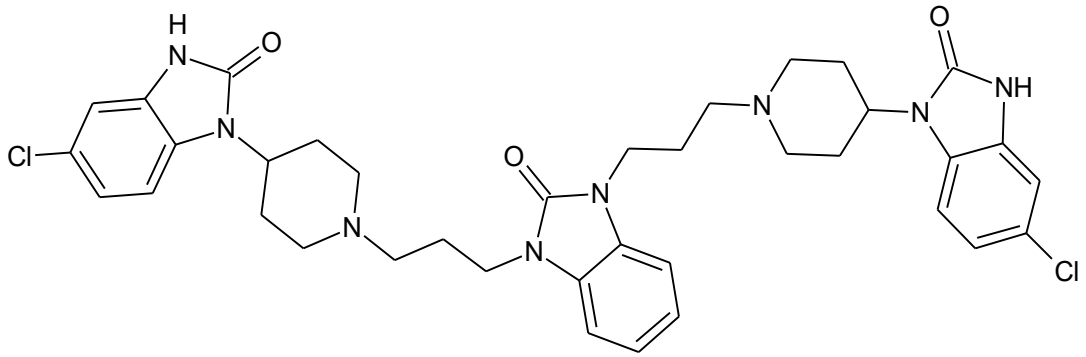
Impurity C: cis-4-(5-chloro-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)-1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]piperidine-1-oxide



Impurity D: 5-chloro-3-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one



Impurity E: 1-[3-[4-(5-chloro-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl]propyl]-3-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]-1,3-dihydro-2H-benzimidazol-2-one



Impurity F: 1,3-bis[3-[4-(5-chloro-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl]propyl]-1,3-dihydro-2H-benzimidazol-2-one

Table 1 Domperidone impurities [79]

4 Design of experiments (DoE) and Quality by Design (QbD)

“Quality by Design (QbD) is an approach that aims to ensure the quality of medicines by employing statistical, analytical and risk-management methodology in the design, development and manufacturing of medicines” [80]. QbD is not a new concept for the pharmaceutical industry as, back in 2004, the QbD principles were described in different ICH guidelines, ICH Q8, ICH Q9, ICH Q10 and ICH Q11.

ICH Q8 defines the term “design space” as the “multidimensional combination and interaction of input variables, process parameters and the assurance of quality for the intended product” [81] and provide a definition of QbD as “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management” [82] and begins with the definition of the objective to be reached on the basis of the knowledge of the process and its control. This approach uses a multivariate analysis to identify and understand the critical attributes for the material and the critical parameters of a process to build the quality of the product. One of the first papers reporting the QbD approach was published in 2007 for the synthesis of Torcetrapib [83]; “the intent of Pfizer was to submit Torcetrapib under the QbD paradigm (route selection, robustness, and reagent/solvent selection during phases I to III are significantly important in establishing a manufacturing process that would have the most flexibility in the final design space)”. One way to reach the knowledge of the process is the Design of experiments (DoE).

“Design of experiments (DoE) is a statistical and mathematical tool to perform the experiments in a systematic way and analyse the data efficiently” [84]. Typically the easiest way to optimize a process is modifying one factor at a time (OFAT) and perform the experiment, check the result and change a bit more the same parameter, and analyse the new result continuing the “optimization” of the parameter until the result is satisfactory. Then the optimization can restart with a new factor in the same manner until the optimization of all the parameters is complete. With the OFAT approach no advanced statistical knowledge is necessary for the data analysis; an example of an OFAT approach is reported below in Figure 22.

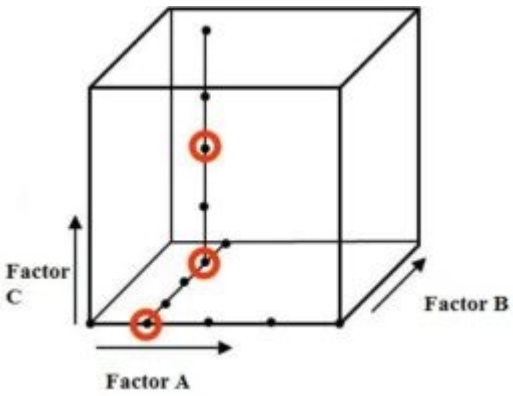


Figure 22 Example of an OFAT design on 3 factors[85]

On the other hand, a DoE approach is a statistical approach based on varying more than one factor at the same time, to also investigate the combination of the individual factors on the outputs inside the space of the design as shown in Figure 23 below.

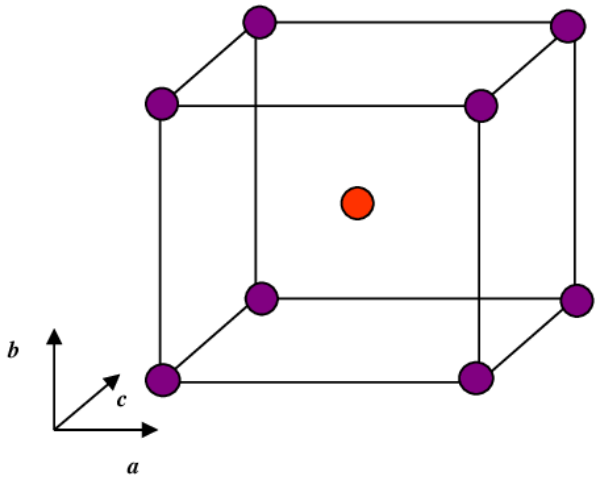


Figure 23 Full factorial design with 3 factor and central point[86]

To investigate 3 factors, A, B and C (n=3), we need to perform 8 experiments (2^n), plus the central point to check the curvature (see Figure 24 to understand the use of the central point). With the 8 experiments we can investigate 7 possible effects: A, B and C, as main effects as in the OFAT approach, but also the interactions between the 3 factors AB, AC, BC and ABC.

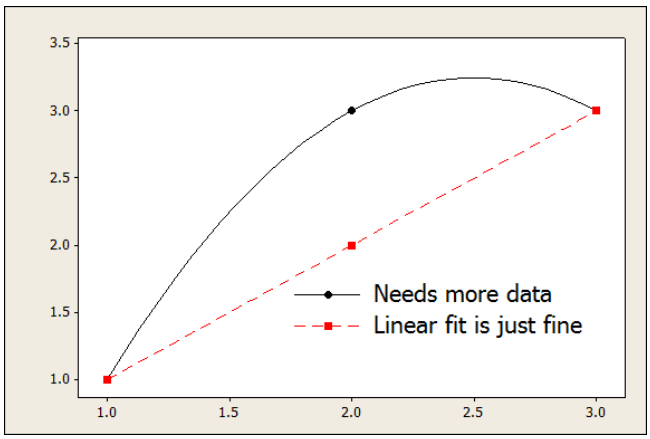


Figure 24 Central point positioning: in line with data (red dashed line) curvature not present, outside the plane (black line) curvature present

The linear model that came out from a factorial design can be expanded in a central composite design in which we need to investigate the quadratic factors A^2, B^2, C^2 and their interactions. This explores part of the space that was not previously investigated, such as a curvature that is very often present in this work. The orange points in Figure 25 below indicate the additional points in the expansion from a full factorial design to a central composite design.

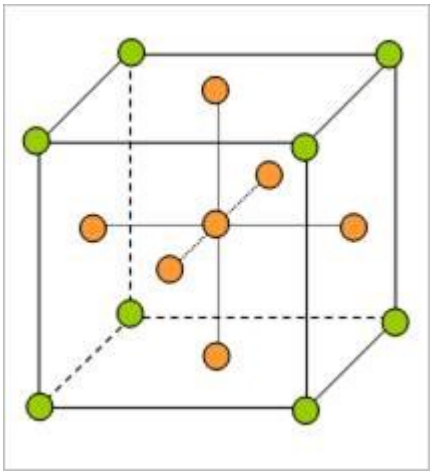


Figure 25 Central composite design with 3 factors and central point

The design to select is crucial and a simplified decision tree can be found in Figure 26 below.

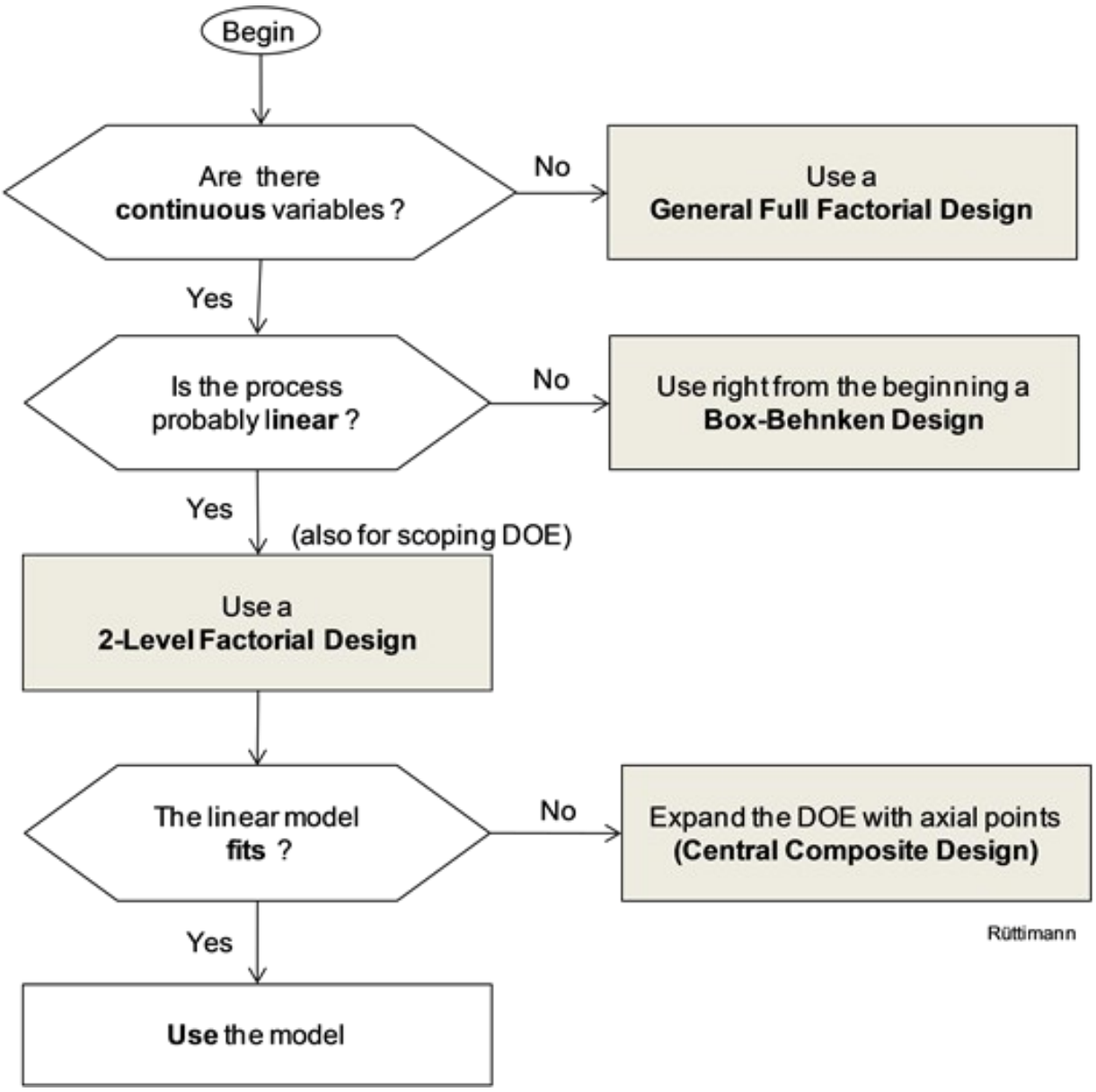


Figure 26 Simplified algorithm to select the appropriate type of DoE[86]

The statistical analysis applied was the ANalysis Of VAriance (ANOVA). ANOVA is used to identify and then quantify the significance of each of the factors and their interaction; this methodology was developed by Sir Ronald Fisher, a British statistician that in 1930s was involved in agricultural experiments with the need to analyse the effects of the experiments in crop yields in different fields. Now we use the same statistical analysis to analyse the effects of a parameter change in the reaction yield.

To check is a parameter is significant for the model statistically elaborated two value were taken into accounts:

F Value: Test for comparing the source's mean square to the residual mean square.

Prob > F: (p-value) Probability of seeing the observed F-value if the null hypothesis is true (there are no factor effects). Small probability values call for rejection of the null hypothesis. The probability equals the integral under the curve of the F-distribution that lies beyond the observed F-value.

Other way to check if a model is statistically significative are the confirmation runs:

Starting from a **predicted mean** generated from the model, for the conditions investigated during the confirmation run, this will create a predicted interval (**PI**) an interval that covers a future outcome from the same population with a pre-determined confidence level, 95% in this case.

Another parameter reported is the standard error of prediction (**SE Pred**), it is the standard deviation associated with the prediction of an individual observation.

During the statistical elaboration if an effect was considered significant o not significant are always on a statistical point of view; if the p-value was less than 0.05 the parameter or the model was considered significant, for example in case a parameter is significant it will impact the response in that case the conversion.

5 Synthetic work

The scope of this thesis is the synthesis of Domperidone in flow chemistry using DoE (Design of experiments) as a statistical method for the development and the optimization of each step of the synthesis.

The study of each synthetic step typically started with a brief investigation to have an idea of the range of reaction parameters for further optimization, as previously only the solubility of the product in each step was tested. Once the parameters for the investigation were decided, the optimization started with a two-level factorial design and, in case it was necessary, the design was expanded in a central composite design to investigate the curvature. At the beginning of my work no one had reported a synthesis in flow of Domperidone or a related compound that follows the same route (Figure 21 above); recently people at Richter Gedeon Nyrt [69,70] reported the synthetic route for Flibanserin in flow chemistry via aldehyde.

All the flow experiments were conducted using the commercially available synthesis platform, Vapourtec R2/R2+/R4 system [87] connected to a Gilson FC203 fraction collector, as shown in Figure 27 below.

Figure 27 Vapourtec flow reactor used for optimization

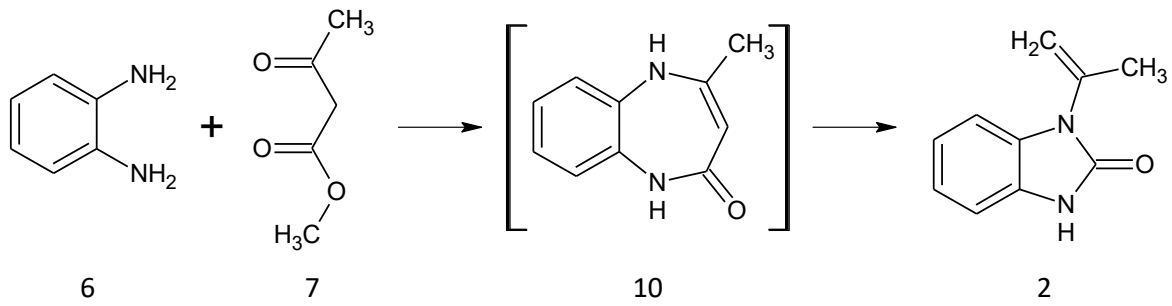
Before starting the flow activities, one batch of all the intermediates of the synthesis were tested to check if they were soluble in the reaction mixture and, as some reactions were planned to be performed at temperatures higher than the boiling point of the solvent, the first batch synthesis was performed only to check the reactivity of the material. The low solubility of Domperidone is known and for this reason was taken into account when performing the last step.

The data reported in the tables present in the experimental section were analysed and used to generate the models discussed in the next sections; the model was further confirmed with the confirmation runs.

5.1 Synthesis of mono-protected 1,3-dihydro-2H-benzimidazol-2-one

The synthesis of the mono protected 1,3-dihydro-2*H*-benzimidazol-2-one is the key point for the synthesis of Domperidone and other drugs that use the same building block. After the initial attempts to replicate the commercial synthesis reported in Scheme 14, attention was focused on the synthesis reported in Scheme 15 were BOC- was used as protecting group.

5.1.1 Synthesis via [1,3] sigmatropic rearrangement of 4-methyl-1,5-dihydro-2H-1,5-benzodiazepin-2-one.



Scheme 14 Synthesis of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one

As the [1,3] sigmatropic rearrangement requires high temperature for numerous hours (up to 34 hours), the possibility to reduce the activation energy for the rearrangement step was investigated.

In order to reduce the activation energy, several catalysts were tested in the original synthetic route which consists in the reaction of benzene-1,2-diamine (6) with methyl acetoacetate (7) (Table 2).

Entry	Solvent	Catalyst (mol%)	Temperature (°C)	Time (h)	Isolated yield (mol%)
1	Xylene	-	120	20	53
2	Xylene	NaOH (25%)	80	20	20
3	Xylene	KOH (25%)	120	5	52
4	Xylene	KOH (2.5%)	120	16	47
5	Xylene	KOH (25%) $CeCl_3$*7 H_2O (2.5%)	120	5	38
	Propionitrile	-	110	20	16[4]
	Toluene	-	110	20	23[4]

Table 2 Attempts to synthesize 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one.

To focus attention on the rearrangement and not on the initial cyclization, a crude material containing both 4-methyl-1,5-dihydro-2H-1,5-benzodiazepin-2-one (10, the transient intermediate) and 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2, the desired product) was synthetized to investigate the possibility to force the rearrangement. As no literature references were found regarding the existence of catalysts which could lower the activation energy, a wide range of catalysts were tested (Table 3), including Lewis-acids, transition metals for π-activation and an organic base.

[4] Conversion monitored by UPLC/MS

Entry	Solvent	Catalyst (mol%)	Temperature (°C)	Time (h)	Conversion (% a/a)
1	Xylene	-	120	20	24 %
2	Xylene	$CeCl_3$*7 H_2O (5 %)	120	20	26 %
3	Xylene	$AlCl_3$ (5 %)	120	20	28 %
4	Acetonitrile	$AlCl_3$ (5 %)	85	14	19 %
5	Acetonitrile	$AlCl_3$ (5 %)	85	14+20	15 %
6	DMF	$AlCl_3$ (5 %)	85	14	25 %
7	DMF	$AlCl_3$ (5 %)	100	14+20	20 %
8	Acetonitrile	$Pd_2(dba)_3$ (5 %)	85	20	30 %
9	Acetonitrile	$Pd(dppf)Cl_2$*CH_2Cl_2 (5 %)	85	20	43 %
10	Acetonitrile	$Pd(dppf)Cl_2$*CH_2Cl_2 (5 %)	85	14	22 %
11	Acetonitrile	$Pd(dppf)Cl_2$*CH_2Cl_2 (5 %)	85	14+20	32 %
12	DMF	$Pd(dppf)Cl_2$*CH_2Cl_2 (5 %)	85	14	22 %
13	DMF	$Pd(dppf)Cl_2$*CH_2Cl_2 (5 %)	100	14+20	26 %
14	Acetonitrile	$PdCl_2[P(cy)_3]_2$	85	20	25 %
15	DMF	DMAP (10 %)	100	20	41 %
16	Acetonitrile	DMAP (10 %)	85	20	59 %

Table 3 Investigation on [1,3] sigmatropic rearrangement

Once suitable conditions to perform the rearrangement were found, the most promising conditions were applied to the reaction starting from benzene-1,2-diamine (6) and methyl acetoacetate (7) to obtain the desired product (Table 4).

Entry	Solvent	Catalyst (mol%)	Temperature (°C)	Time (h)	Conversion (% a/a)
1	Xylene	DMAP (10 %)	120	20	52.5 %
2	Toluene	DMAP (10 %)	110	20	6 %
3	Propionitrile	DMAP (10 %)	110	20	33 %
4	Toluene	DIPEA (10 %)	110	20	13 %
5	Propionitrile	DIPEA (10 %)	110	20	33 %

Table 4 Investigation on the use of different organic bases (as catalyst) and solvents

As in the first investigation (Table 2), where a base (KOH) was present, an equimolar quantity of DIPEA was used to investigate the effect of different quantity of methyl acetoacetate (7) in the reaction mixture (Table 5), the use of toluene as solvent despite the low yield observed in Table 4 was maintained to keep the original idea to reduce the energy to perform the reaction.

Entry	Solvent	methyl acetoacetate (7) (eq)	Base	Temperature (°C)	Time (h)	Conversion (% a/a)
1	Toluene	1.5	DIPEA (1 eq)	110	20	60 %
2	Toluene	3	DIPEA (1 eq)	110	20	64 %
3	Toluene	4.5	DIPEA (1 eq)	110	20	81 %
4	Toluene	6	DIPEA (1 eq)	110	20	68 %

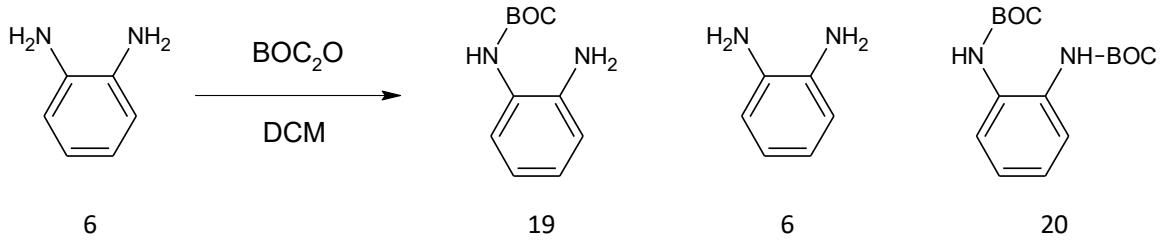
Table 5 Investigation on different equivalents of methyl acetoacetate (7)

Despite the interesting result observed using 4.5 eq. of methyl acetoacetate (7) (Entry 3 Table 5), the poor solubility of benzene-1,2-diamine (6) in toluene discouraged the use of this route as the first step for the synthesis of Domperidone in flow. The commercial route was then abandoned to follow a route that combines routes reported by Janssen [43] and by CinRX [67].

5.1.2 Synthesis of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22) via cyclization of mono-protected benzene-1,2-diamine (6)

As the mono-protected 1,3-dihydro-2H-benzimidazol-2-one (2 and 22) is the key intermediate for the synthesis of Domperidone, a large part of the effort was focused on its synthesis.

The selected route consists of the mono protection of benzene-1,2-diamine (6) with di-tert-butyl dicarbonate, followed by cyclization.



Scheme 15 Possible products obtained reacting benzene-1,2-diamine (6) and di-tert-butyl dicarbonate.

When benzene-1,2-diamine (6) reacts with 1 eq of di-tert-butyl dicarbonate it shows the product distribution reported above with theoretical molar ratio, assuming that the reactivity was not influenced by the insertion of the BOC-group, 50 (19)/ 25 (6) / 25 (20) (the theoretical ratio by weight is 50 / 12.98 / 37.02). The experimental molar distribution observed in batch reaction was 58.2 (19)/ 18.6 (6)/ 23.6 (20) (by weight the ratio was 56.9 / 9.4 / 33.7).

With the view to optimize the process, it was decided to investigate the conditions that could maximize the desired product output, reducing the side products using a statistical approach. The design was Two-Level Fractional Factorial with 4 factors.

One limiting factor was the observed solubility of benzene-1,2-diamine (6) in DCM. Other parameters arose from a risk assessment performed during the route evaluation.

The factors and the ranges are reported below:

- Concentration of benzene-1,2-diamine (6) (from 0.6M to 0.2M)
- Concentration of di-tert-butyl dicarbonate (from 0.6M to 0.2M)
- Equivalents of di-tert-butyl dicarbonate (from 0.95 eq to 1 eq)
- Reaction time (from 5 minutes to 20 minutes)

The resulting graphical version of the effects observed is reported in Figure 28 below:

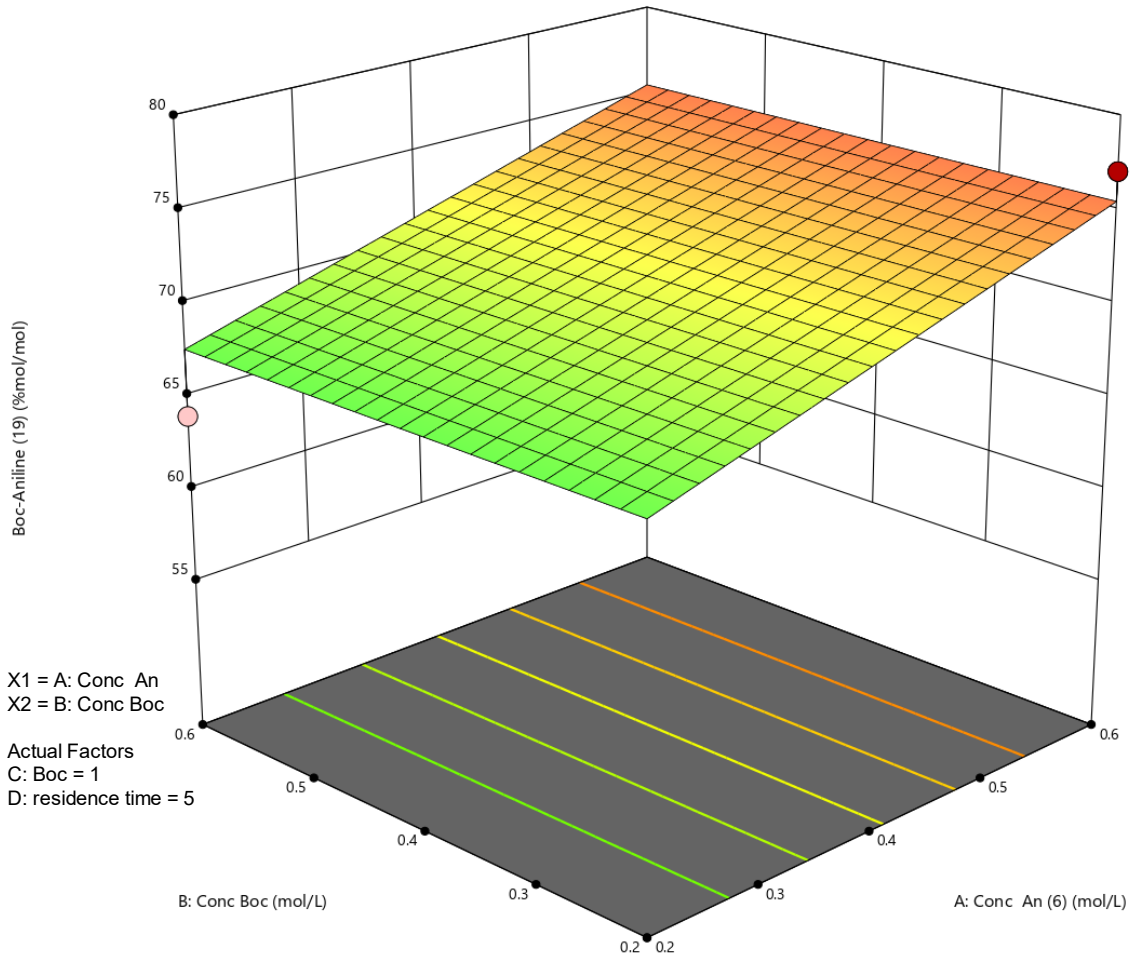
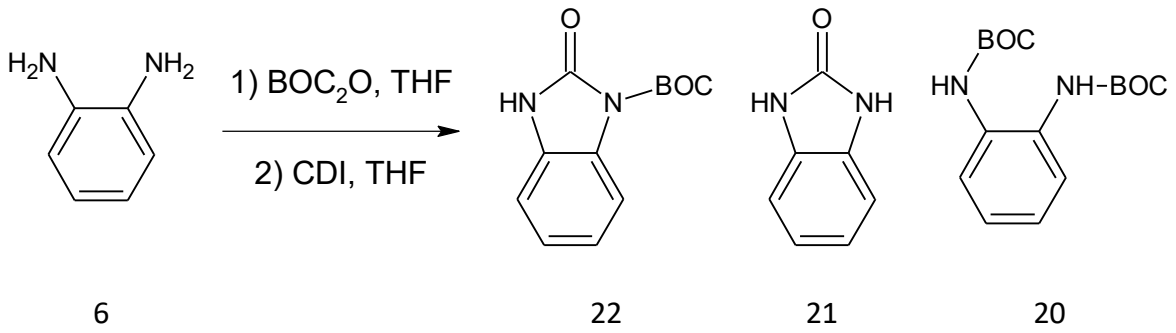


Figure 28 The effects of the reagents concentration on the desired product (19) yield.

The analysis of the data performed with the program “design expert” (DX12) found that the optimal response (minimizing the amounts of unreacted 6 and overreacted 20) was obtained at high concentration of benzene-1,2-diamine (6) (0.6 M) and low concentration of di-tert-butyl dicarbonate (0.2 M). The mean, (74.67%mol/mol) from the confirmation run for the desired response, is within the 95% confidence limit for the selected model. Therefore, despite the modest (0.58) goodness of fit and the low (0.24) predictive capacity estimated for the model, perhaps due to a probable curvature p-value = 0.09, setting the factors (concentration of benzene-1,2-diamine (6) and Equivalents of di-tert-butyl dicarbonate) to optimal values, confirms the predictability of the selected model.

Considering the subsequent cyclization step with a cyclizing agent (CDI), experimentally it was observed that cyclization occurs only on the unreacted benzene-1,2-diamine (6) (giving undesired product 21) and on the mono-protected intermediate, resulting in the formation of desired product (22), as reported in Scheme 16 below.



Scheme 16 Possible products obtained by reacting benzene-1,2-diamine (6) and di-tert-butyl dicarbonate followed by CDI cyclization

In the first set of reactions, the use of DCM as solvent was good for the solubilization of benzene-1,2-diamine (6), but DCM is incompatible with the following steps that include two alkylations, in which the use of a strong base requires a different solvent, so THF was chosen as solvent from this step.

Starting from the result of the first DoE, the possibility to reduce the quantity of unreacted benzene-1,2-diamine (6) as much as possible was investigated by ever increasing the equivalents of di-tert-butyl dicarbonate. It became apparent that undesired side-product (20) in Scheme 16 could be converted into the desired product (22) as will be explained later.

The parameters investigated in this second optimization were:

- Concentration of benzene-1,2-diamine (6) (from 0.4 M to 0.6 M)
- Concentration of di-tert-butyl dicarbonate (from 0.2 M to 0.4 M)
- Equivalents of di-tert-butyl dicarbonate (from 1 to 2 eq)

The design was a 3 parameters, 2 full factorial levels (8 experiments) and 4 central points for a total of 12 experiments.

The quantity of CDI was not investigated, as the only reactions possible were the cyclization of the unreacted benzene-1,2-diamine (6) and the formation of the desired product.

The graphical result of the DoE is reported in Figure 29; this immediately highlights the presence of a curvature, as the central points are outside of the plane.

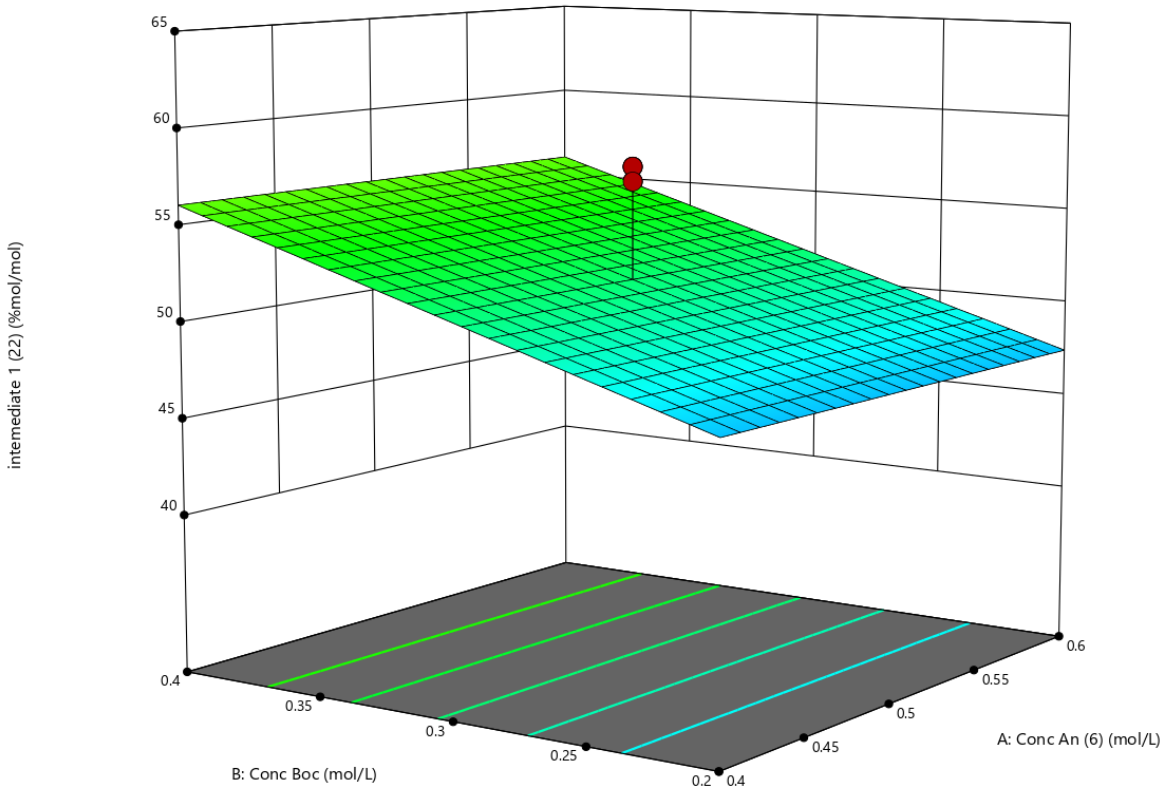


Figure 29 The effects of the reagents concentration on the desired product (22) yield in second DoE

The Model was then expanded to a central composite with an expansion of the initial factorial model, with 8 extra points added to explore the space around the central point as highlighted with the red circle in Figure 30 below, the expansion include further 3 central point.

The new parameter ranges were:

- Concentration of benzene-1,2-diamine (6) (from 0.33 M to 0.67 M)
- Concentration of di-tert-butyl dicarbonate (from 0.2 M to 0.47 M)
- Equivalents of di-tert-butyl dicarbonate (from 0.66 to 2.34 eq)

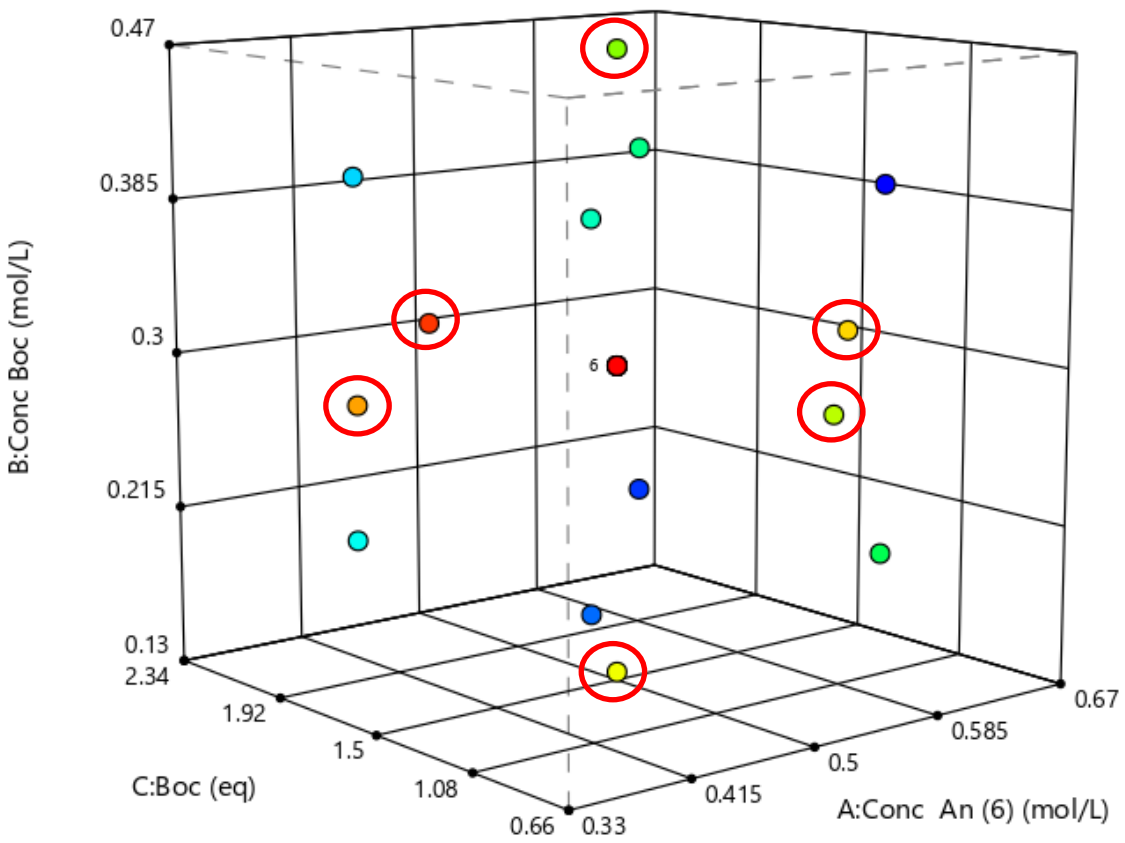


Figure 30 Design expansion

The graphical output of the complete design is reported in Figure 31 below.

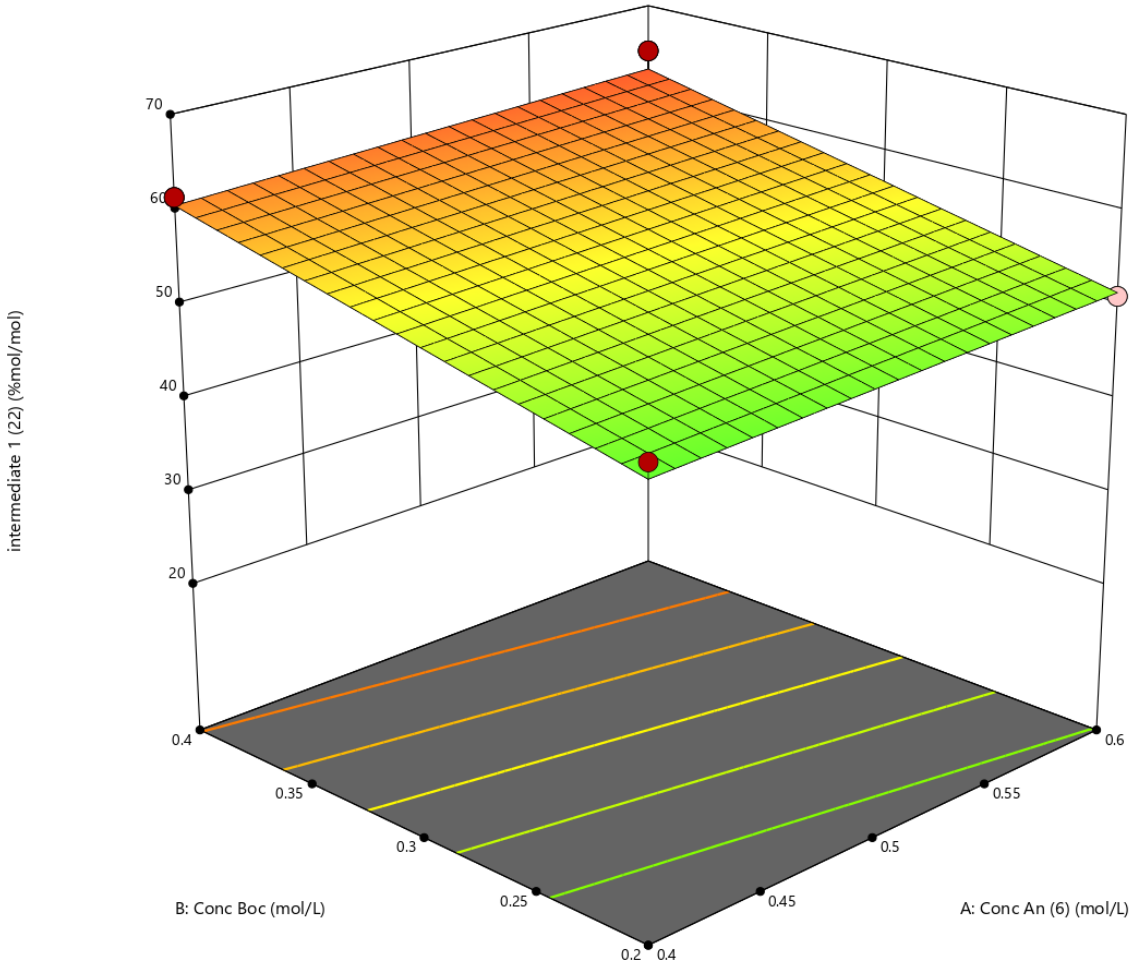


Figure 31 The effects of the reagents concentration on the product (22) yield (equivalents of BOC_2O set at 2)

The ANOVA analysis shows that the Model F-value is 56.73; this implies that the model is significant. There is only a 0.01% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case, the concentration of di-tert-butyl dicarbonate (p-values <0.0001) and equivalents of di-tert-butyl dicarbonate (p-values <0.0001) are significant in the model and also the interaction of these two parameters (p-values 0.0249), as the model was quadratic the equivalents of di-tert-butyl dicarbonate impact as quadratic factor (p-values <0.0001).

The Lack of Fit F-value of 0.95 implies that the Lack of Fit is not significant relative to the pure error. There is a 56.24% chance that a Lack of Fit F-value so large could occur due to noise.

The resulting equation was:

$$\text{Intermediate 1} = -4.88948 + 10.71839\,\mathrm{A} - 11.99762\,\mathrm{B} + 54.90956\,\mathrm{C} + 34.07209\,\mathrm{BC} - 17.75903\,\mathrm{C}^2$$

Where:

A: “Concentration of benzene-1,2-diamine (6)”

B: “concentration of di-tert-butyl dicarbonate”

C: “equivalents of di-tert-butyl dicarbonate”

The Predicted R^2 of 0.8836 is in reasonable agreement with the Adjusted R^2 of 0.9425; i.e. the difference is less than 0.2.

The Adequate Precision, that measures a signal to noise ratio of 29.349, indicates an adequate signal as a ratio greater than 4 is desirable. This model can be used to navigate the design space.

Also the formation of the other side product (21 in Scheme 16) was monitored. From the data analysis it resulted that all the models are significant and that in all cases the “concentration of di-tert-butyl dicarbonate” and the “equivalents of di-tert-butyl dicarbonate” were significant. In all cases the lack of fit was not significant.

Two sets of confirmation runs were performed 3 times each.

In the first set the parameters were:

- Concentration of benzene-1,2-diamine (6) (0.5 M)
- Concentration of di-tert-butyl dicarbonate (0.4 M)
- Equivalents of di-tert-butyl dicarbonate (2 eq)

Response	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	61.7113	1.88052	3	1.54176	58.3521	55.7667	65.0705
not protected (21)	10.2514	2.03076	3	1.53344	6.91031	8.85667	13.5925
Di-Boc (20)	29.208	1.16735	3	0.97095	27.0709	35.3733	31.345

Table 6 First set of confirmation runs for steps 1-2 combined

The first confirmation run did not completely confirm the model as the amount of the desired intermediate 1 (22) was slightly lower than predicted and the amount of di-tert-butyl benzene-1,2-diylbiscarbamate (20) was slightly higher than predicted. There are potentially other parameters that were not investigated and that have an impact on the outcome of the reaction.

The design was forced to obtain the lowest amount of unreacted benzene-1,2-diamine (6) by conversion into 1,3-dihydro-2H-benzimidazol-2-one (21). For this reason the equivalents of di-tert-butyl dicarbonate were set at 3 eq, a value outside of the investigated range; this value was selected as an extremism of the conditions applied until now to form intermediate 1 (22).

In the second set of confirmation runs the parameters were:

- Concentration of benzene-1,2-diamine (6) (0.6 M)
- Concentration of di-tert-butyl dicarbonate (0.4 M)
- Equivalents of di-tert-butyl dicarbonate (3 eq)

Response	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	42.5264	1.88052	3	5.10963	31.3935	40.16	53.6593
not protected (21)	5.23609	2.03076	3	5.16159	-6.01004	1.91667	16.4822
Di-Boc (20)	49.5075	1.16735	3	1.78234	45.5846	57.92	53.4304

Table 7 Second set of confirmation runs for steps 1-2 combined

Also in this case the confirmation run did not completely confirm the model as di-tert-butyl benzene-1,2-diylbiscarbamate (20) was slightly higher than the predicted. In this case the unreacted benzene-1,2-diamine (6), which is then converted into 1,3-dihydro-2H-benzimidazol-2-one (21), was just 1.9 % mol/mol and the di-tert-butyl benzene-1,2-diylbiscarbamate (20, Di-Boc) could be recycled to give more Intermediate 1 (22). As a parameter was outside of the range it was not expected that the model would fit, but the positive result was the minimization of 1,3-dihydro-2H-benzimidazol-2-one (21).

In conclusion, the optimized conditions, in the studied parameter range, to maximize the formation of intermediate 1 were:

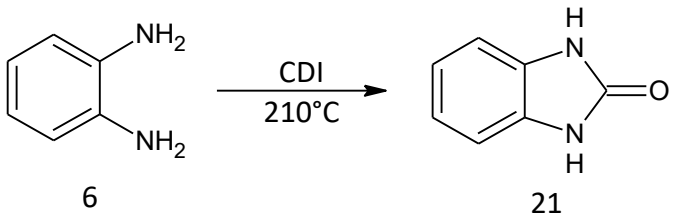
- Concentration of benzene-1,2-diamine (6) (0.5 M).
- Concentration of di-tert-butyl dicarbonate (0.4 M).
- Equivalents of di-tert-butyl dicarbonate (2 eq).

But the optimized conditions to minimize the unreacted benzene-1,2-diamine (6) and its subsequent conversion in 1,3-dihydro-2H-benzimidazol-2-one (21) were:

- Concentration of benzene-1,2-diamine (6) (0.6 M).
- Concentration of di-tert-butyl dicarbonate (0.4 M).
- Equivalents of di-tert-butyl dicarbonate (3 eq).

A recent piece of literature reported the synthesis of 1,3-dihydro-2H-benzimidazol-2-one (21) [88] in flow conditions with the use of CDI as cyclizating agent, as reported in Scheme 17 below; the optimized conditions reported were:

- Residence time: 33.33 min
    - Corresponding to a combined flow rate of 0.3 ml/min in a 10 ml coil reactor
- Temperature 210°C
- Equivalents of CDI: 4.2
    - Corresponding to a 4.2 M solution



6 21

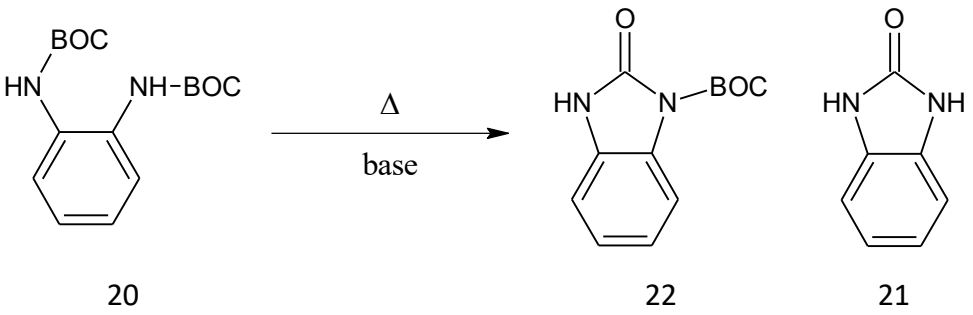
Scheme 17 Reaction performed by Mostarda et al.

Attempts to replicate the experiment, with tert-butyl (2-aminophenyl)carbamate (19) as starting material to obtain tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22), showed an initial difficulty due to solubility issues encountered during the dissolution of CDI (13.6 g in 20 ml) in THF/peg 300 (7/3). After an initial dissolution the reagent precipitated, for this reason it was decided to perform the reaction at the concentration of 2.1 M, reducing also the concentration of tert-butyl (2-aminophenyl)carbamate (19) to 0.5 M to maintain the same flow rate reported in the paper. This procedure resulted in the complete conversion of tert-butyl (2-aminophenyl)carbamate (19) to 1,3-dihydro-2*H*-benzimidazol-2-one (21).

Because di-tert-butyl benzene-1,2-diylbiscarbamate (20) was always present as a side product in this reaction, the possibility to use it as starting material to obtain the desired intermediate 1 (22) as reported by Hegarty[89] was also investigated.

5.1.3 Synthesis via Di-tert-butyl benzene-1,2-diylbiscarbamate (20) direct cyclization.

The synthesis of 1,3-dihydro-2*H*-benzimidazol-2-one (21) via treatment of tert-butyl (2-aminophenyl)carbamate (19) was reported for the first time by Hegarty[89] and recently used by researchers at Richter Gedeon Nyrt [69,70]. In the current development the main objective to reach is to avoid the removal of the protecting group from the 1,3-dihydro-2*H*-benzimidazol-2-one in order to maintain the mono protection, thereby reusing a product that was previously considered an impurity; this improvement could fall under one of the principles of green chemistry: prevention; “It is better to prevent waste than to treat or clean up waste after it has been created” [90].



20 22 21

Scheme 18 Products from di-tert-butyl benzene-1,2-diylbiscarbamate cyclization

5.1.3.1 Optimization using microwave heating

As this reaction is expected to proceed only at high temperature, the reaction was performed using a microwave reactor, more specifically a Biotage initiation, to try and speed up the optimization.

The microwave was chosen as the heating system due to the possibility to reach high temperatures in a very short time and the ability to support high pressures like the flow reactor.

Prof Oliver Kappe [91] suggests the use of a flow reactor to perform the scale-up of reactions performed in microwaves and in the literature there are several examples of translation from batch microwave to continuous flow processing [92–95]. In this case microwave-heating was used to investigate reactions that are planned to be subsequently performed in flow.

The optimization started by investigating the effect of the temperature, the reaction time and the base. For this first set of experiments a response surface D-Optimal design was chosen, based on the use of a categoric factor. As the type of base (DMAP, DIPEA and TEA) for this procedure was not reported a wide range of parameters were selected.

The parameters selected and their ranges were

- Temperature (from 100°C to 150°C)
- Reaction time (from 1 to 10 minutes)
- Type of base (TEA, DIPEA and DMAP)

The fixed condition were:

- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.5 M, 4 ml)
- 1 eq of base
- 5 ml MW vial.

The results of this first set of experiments were discouraging as there was only some formation of product with DMAP. The analysis of the data shows the trend of the reaction increasing the temperature and the reaction time using DMAP in Figure 32 below.

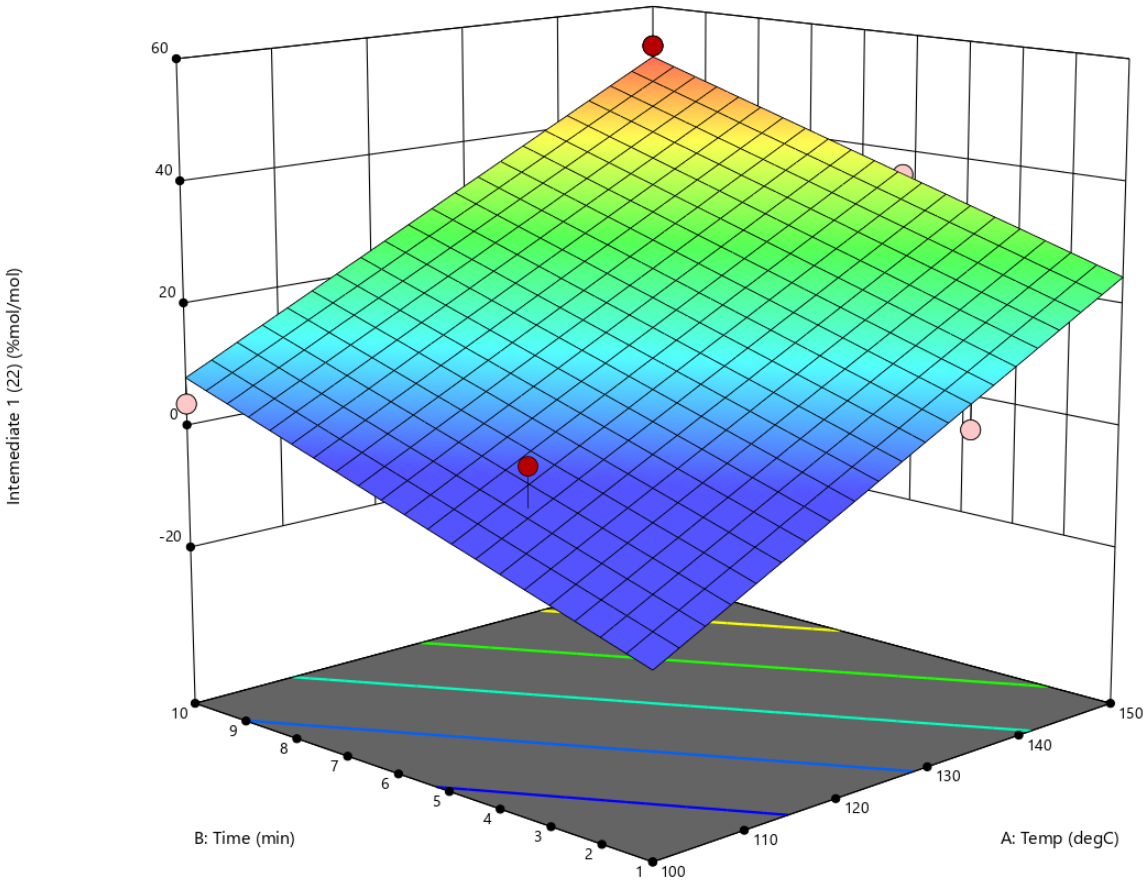


Figure 32 The effects of reaction time and temperature on the product (22) yield (for DMAP as base)

The outputs from the first set of experiments, were used to plan a better investigation of the effect of temperature, reaction time and equivalents of DMAP on the cyclization. The new parameter ranges were:

- Temperature (from 130°C to 180°C)
    - The central point was set at 155°C near the optimum for the temperature in the previous run
- Reaction time (from 1 to 20 minutes)
    - The central point was set at 10.5 minutes near the optimum for the reaction time in the previous run
- Equivalents of DMAP (from 1 to 2)

The design was 3 parameters, 2 full factorial levels (8 experiments) and 2 central points. To increase the robustness of the data, the experiments were performed in duplicate for a total of 20 experiments.

The graphical result of the DoE, reported in Figure 33, Figure 29 immediately highlight the presence of a curvature.

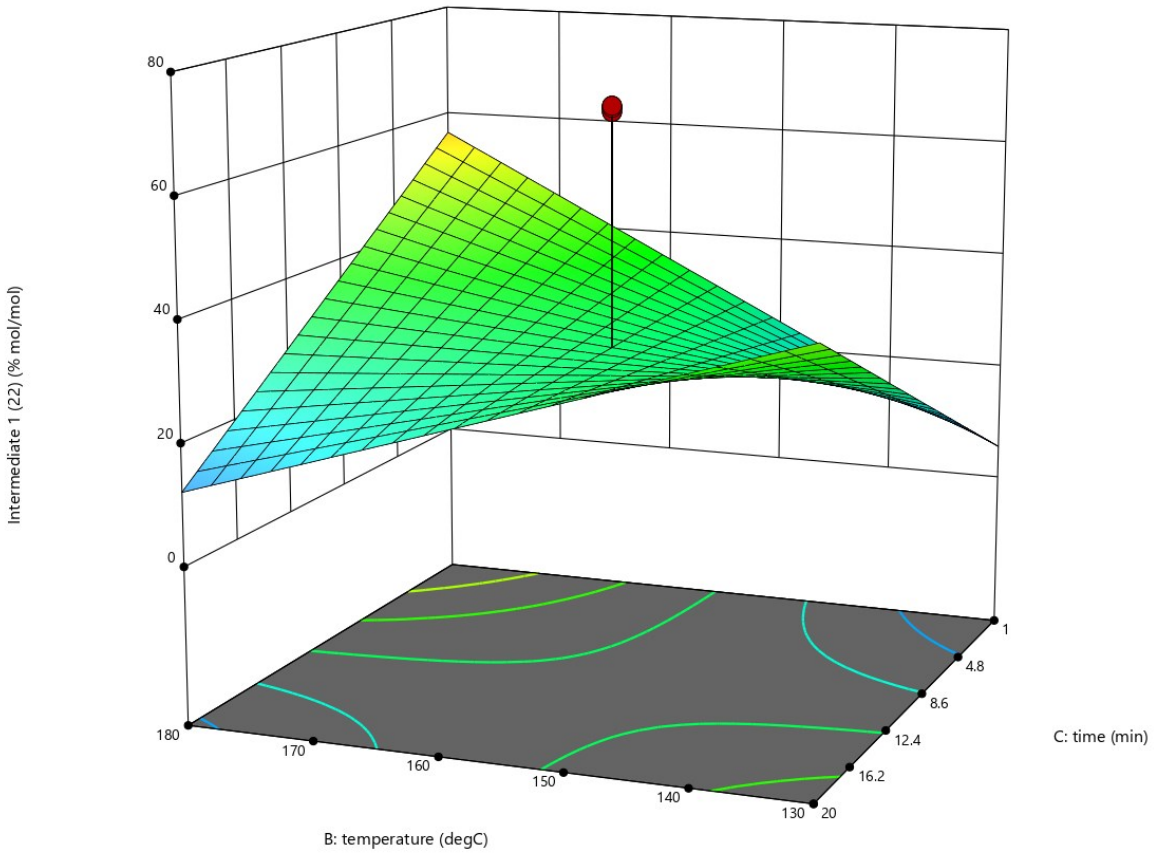


Figure 33 The effects of reaction time and temperature on the product (22) yield (equivalents of DMAP set at 1.5)

Also in this case the other side product and residual starting material were analysed and the curvature was present in the analysis of the unreacted starting material.

Again, the model was expanded in a central composite, the equivalents of DMAP were kept constant at 1.5 as it showed a minimal effect on the reaction, and the reactor range was kept more tightly around the central point.

The new parameter ranges were:

- Temperature (from 145°C to 165°C)
    - Mean at 155°C

- Reaction time (from 4.14 to 16.86 minutes)
    - Mean at 10.5 minutes

The design was 2 parameters, central composite (8 experiments) and 5 central points, for a total of 13 experiments.

The graphical result of the DoE, for the formation of Intermediate 1 (22), reported in Figure 34, immediately highlights the effect of the curvature.

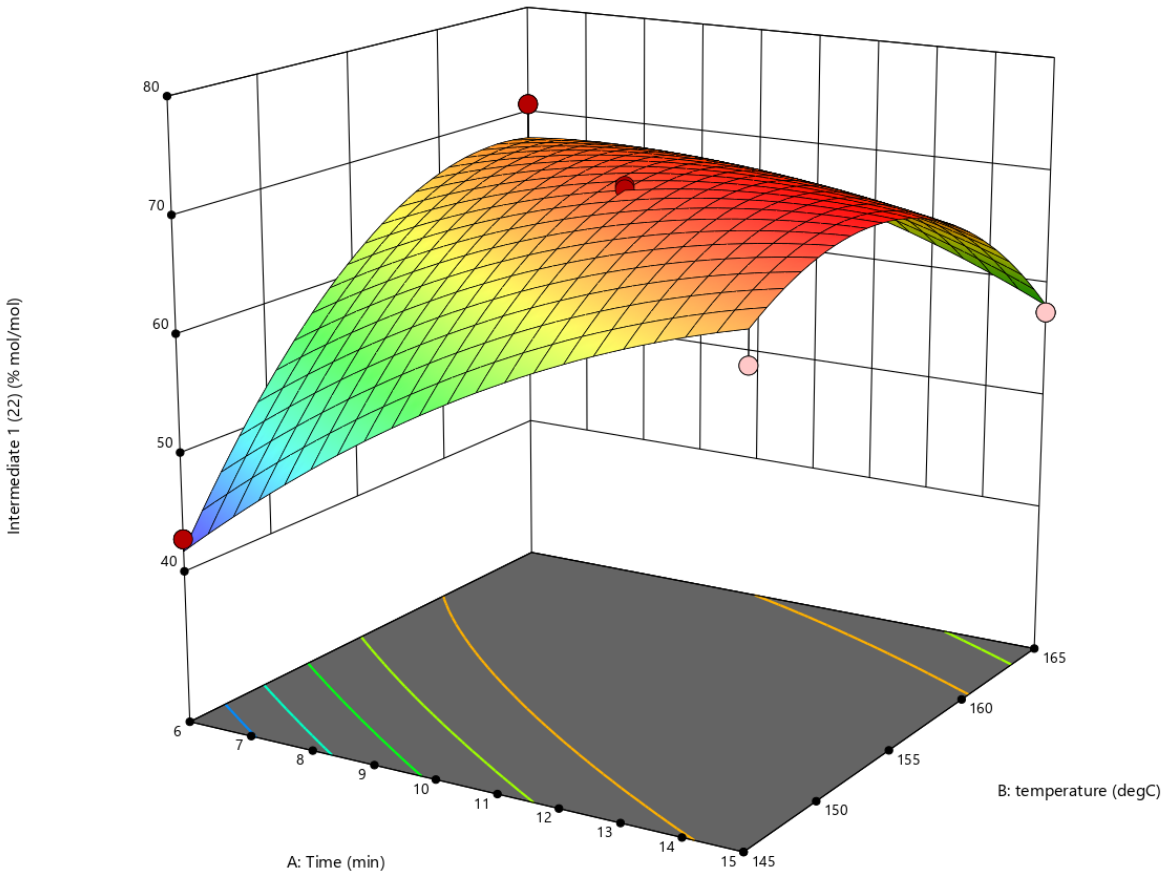


Figure 34 The effects of reaction time and temperature on the product (22) yield (equivalents of DMAP set at 1.5), the curvature was highlighted

The ANOVA analysis for this quadratic model shows an F-value of 38.48 for the model, which implies that the model is significant. There is only a 0.01% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case Time (p-values 0.0016) and Temperature (p-values 0.0018) are significant in the model and also the interaction of these two parameters (p-values 0.0001) as the model was quadratic and both the factors impact as quadratic factor (p-values 0.0058 for the temperature and p-values <0.0001 for the time).

The Lack of Fit F-value of 202.02 implies that the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value so big could occur due to noise.

The resulting equation was:

$$\text{Intermediate 1} = 70.39 + 4.77\,\text{A} + 4.08\,\text{B} - 8.87\,\text{AB} - 3.51\,\text{A}^2 - 8.00\,\text{B}^2$$

Where:

A: “Time”

B: “Temperature”

The Predicted R^2 of 0.7517 is in reasonable agreement with the Adjusted R^2 of 0.9088 as the difference is less than 0.2.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The observed ratio of 17.825 indicates an adequate signal.

Several sets of confirmation runs were performed.

In the first set the parameters were

- Time (15 minutes)
- Temperature (151°C)

Response	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	71.6907	2.36486	3	1.89154	67.2179	71.8155	76.1635
Di-Boc (20)	14.6289	0.496395	3	N/A	13.7009	13.9239	15.5785
not protected (21)	14.5172	0.39612	3	N/A	13.782	14.2176	15.2803

Table 8 Confirmation run in MW

The data above are a mean of 3 runs and the confirmation run was completely in line with the predicted data confirming the model.

Several experiments on the same model were then performed using the flow reactor. The results were very disappointing as none of the runs gave results that fit the model, as shown in the tables below.

In the first set of results in flow the parameters were the same previously tested using the microwave.

- Time (15 minutes)
- Temperature (151°C)
- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	71.6907	2.36486	3	1.89154	67.2179	63.9935	76.1635
Di-Boc (20)	14.6289	0.496395	3	N/A	13.7009	27.9883	15.5785
not protected (21)	14.5172	0.39612	3	N/A	13.782	7.99133	15.2803

Table 9 Confirmation run in flow using MW optimized conditions

In the second set of results in flow the parameters were:

- Time (6 minutes)
- Temperature (145°C)
- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	41.7481	2.36486	1	3.01461	34.6197	24.235	48.8765
Di-Boc (20)	55.8086	0.96961	1	N/A	52.92	73.8997	58.7653
not protected (21)	2.00137	0.05461	1	N/A	1.84268	1.865	2.17209

Table 10 Example run in flow using MW performed conditions

In the third set of results in flow the parameters were:

- Time (15 minutes)
- Temperature (165°C)
- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	58.2604	2.36486	1	3.01461	51.1319	71.695	65.3888
Di-Boc (20)	0.528954	0.09421	1	N/A	0.279599	5.8969	0.84642
not protected (21)	41.7072	1.13803	1	N/A	38.4004	22.4082	45.2651

Table 11 Example run in flow using MW performed conditions

In the fourth set of results in flow the parameters were:

- Time (10.5 minutes)
- Temperature (155°C)

- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	70.3866	2.36486	1	2.59057	64.2609	58.363	76.5123
Di-Boc (20)	16.5094	0.527342	1	N/A	15.1676	35.3648	17.8994
not protected (21)	13.1096	0.357712	1	N/A	12.2106	6.27252	14.0643

Table 12 Example run in flow using MW performed conditions

In the fifth set in flow the parameters were:

- Time (8 minutes)
- Temperature (163°C)
- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	69.0752	2.36486	1	2.65267	62.8026	61.197	75.3477
Di-Boc (20)	10.7846	0.426199	1	N/A	9.67963	30.9961	11.9403
not protected (21)	18.1879	0.496277	1	N/A	16.912	7.80717	19.5454

Table 13 Example run in flow using MW performed conditions

In all the runs performed in flow, where the temperature was also monitored with an external thermocouple, the effect of the temperature was lower than expected. The effect is clear in the third run in flow in which the expected amount of deprotected compound (21), was lower than predicted. The explanation of these results could be found in another article from Prof. Oliver Kappe [96] that focuses attention on the mythical "Microwave Effect". The article reports a series of examples, also using SiC microwave vessels, where the effective internal temperature in microwave vessels was measured; the result of these experiments can be summarized in this phrase: "In other words, the effects reported for most microwave-irradiated chemical transformations can be rationalized by purely thermal/kinetic phenomena, and thus ultimately fall into the category of thermal microwave effects." [96]

In conclusion, it is possible to move a batch microwave reaction to continuous flow conditions, but the optimization needs to be repeated as there is not a correspondence between the microwave temperature and the flow temperature. However, results from the microwave can be used as guidance for the flow optimization, or to explore the feasibility of a reaction; the microwave optimization could be used to decide if a parameter/factor should be included or not in the flow optimization. Indeed, the above findings will be further applied for this intent.

The optimized conditions, in the studied range, to perform the cyclization using microwave heating were

- Time (15 minutes)
- Temperature (151°C)

5.1.3.2 Optimization in continuous flow

Based on the results outlined in the previous section, the optimization restarted in flow taking into consideration what was learnt during the microwave optimization.

The optimization started directly with a central composite design and the parameter ranges were:

- Temperature (from 146°C to 174°C)
    - Mean at 160°C
- Reaction time (from 3 to 17 minutes)
    - Mean at 10 minutes
- di-tert-butyl benzene-1,2-diylbiscarbamate (20) (0.4 M in THF) with 1.5 eq of DMAP

The design was a 2 parameters, central composite (8 experiments) and 5 central points, for a total of 13 experiments.

The graphical result of the DoE, for the formation of the desired product, reported in Figure 35, immediately highlights the effect of the curvature.

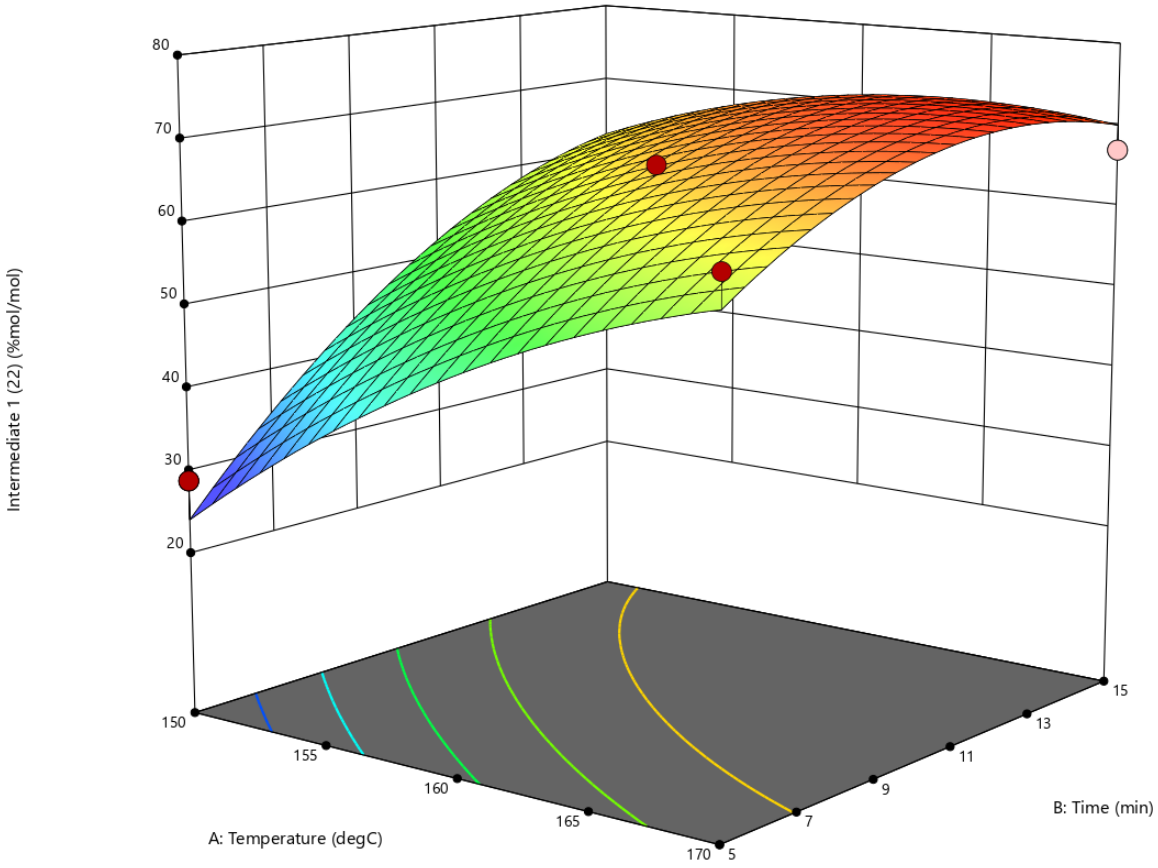


Figure 35 The effects of reaction time and temperature on the product yield (equivalents of DMAP set at 1.5)

The ANOVA analysis for this quadratic model shows an F-value of 33.73 for the model, which implies that the model is significant. There is only a 0.01% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case Time (p-values <0.0001) and Temperature (p-values 0.0001) are significant in the model, also the interaction of these two parameters (p-values 0.0014) is significant, as the model was quadratic, and both the factors impact as quadratic factor (p-values 0.0224 for the temperature and p-values 0.0036 for the time).

The Lack of Fit F-value of 73.31 implies that the Lack of Fit is significant. There is only a 0.06% chance that a Lack of Fit F-value so large could occur due to noise.

The resulting equation was:

$$\text{Intermediate 1} = 64.86 + 10.47\,\text{A} + 12.50\,\text{B} - 6.78\,\text{AB} - 4.47\,\text{A}^2 - 6.56\,\text{B}^2$$

Where

A: “Temperature”

B: “Time”

The Predicted R^2 of 0.7205 is not as close to the Adjusted R^2 of 0.9317 as one might normally expect, due to the fact that the difference is more than 0.2. This may indicate a possible problem with the model and/or data.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The observed ratio of 17.255 indicates an adequate signal.

Several sets of confirmation runs were performed:

In the first set of results the parameters were:

- Time (13.5 minutes)
- Temperature (160°C)

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	70.3966	3.98238	3	2.90394	63.5299	70.7623	77.2633
Di-Boc (20)	15.9836	4.14809	3	2.85646	11.1175	16.5818	24.0411
not protected (21)	12.9542	0.793436	3	0.57558	11.6269	12.6559	14.2815

Table 14 Confirmation run in flow

The data above are a mean of 3 runs and the confirmation run was completely in line with the predicted data confirming the model.

A recently purchased CEM corporation microwave Discover 2.0, that is claimed to have an updated IR sensor, was tested using the optimized flow conditions. Despite the output showing a conversion similar to that observed in flow, as reported in Table 15 below, this equipment did not show the expected temperature accuracy either.

For this run the parameters were:

- Time (13.5 minutes)
- Temperature (160°C)
- intermediate 1 (22) (0.4 M in THF) with 1.5 eq of DMAP

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %mol/mol	95% PI high
intermediate 1 (22)	70.3966	3.98238	3	2.90394	63.5299	71.64	77.2633
Di-Boc (20)	15.9836	4.14809	3	2.85646	11.1175	10.38	24.0411
not protected (21)	12.9542	0.793436	3	0.57558	11.6269	17.98	14.2815

Table 15 Confirmation run in MW using flow optimized conditions

Data were not in the predicted range, however, this microwave equipment could help more than the other equipment tested for the preliminary investigation of a flow experiment.

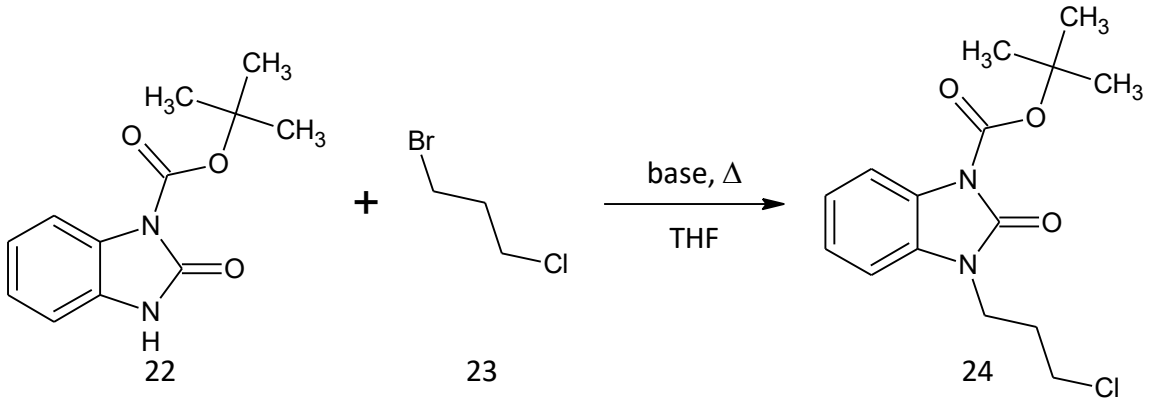
In conclusion, I found a way to reuse di-tert-butyl benzene-1,2-diylbiscarbamate promoting it from waste to alternative starting material for the synthesis of mono-protected 1,3-dihydro-2*H*-benzimidazol-2-one. The optimized conditions, in the studied range, to perform the cyclization in flow were

- Time (13.5 minutes)
- Temperature (160°C)

It was not possible to perform this reaction in batch using standard laboratory equipment as the temperature employed was double that of the solvent's boiling point at atmospheric pressure.

5.2 Synthesis of tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)

The alkylation of mono-protected 1,3-dihydro-2H-benzimidazol-2-one (22) is the step where a change of solvent, from DCM to THF, is indicated in order to have a solvent more suitable for the alkylation step in which a strong base is required[43,54,55].



Scheme 19 Synthesis of tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)

Also in this case the investigation of optimal reaction conditions started using a batch microwave reaction for an initial exploration of the chemical space, and to check the reactivity in such conditions, and also to check whether side products generated in strong conditions can cause clogging of the flow reactor coil.

In the first set of experiments, the use of potassium hydroxide (4 M) as base was investigated.

To increase automation, dispensing of the reagents to the microwave vials was performed with a Hamilton Microstar Lab liquid handler that, once programmed and charged with the stock solutions, dispensed the reagents to 36 vials in 37 minutes.

The 36 experiments were the result of a full factorial design with 5 parameters (32 runs) and 4 central points. The parameter ranges were:

- Temperature (from 100°C to 150°C)
- Reaction time (from 2 to 10 minutes)
- Equivalents of base (from 1.5 to 3)
- Concentration of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22) (from 0.5 M to 1 M)
- Equivalents of 1-bromo-3-chloropropane (23) (from 1 to 2)

To perform the dispensing, a master solution of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22) (1 M) was placed in eight 10 ml vials as well as the solvent (THF), the KOH (4 M) solution and the KI (1M) solution, used as catalyst, were placed in two different 300 ml reservoirs, and the alkylating agent (1-bromo-3-chloropropane (23)) was placed neat in one 10 ml vial.

The aim of this exploration was to include/exclude some of the parameters, but the output of this exploration was completely unexpected as a complete degradation of the starting material to 1,3-dihydro-2*H*-benzimidazol-2-one (21) was observed in the majority of the experiments analysed.

The use of KOH as base was abandoned and two organic bases (DBU and DIPEA) and two inorganic bases (K_2CO_3 and Cs_2CO_3) were tested in a single run of experiments using microwave heating at 100°C with 1.1 equivalents of 1-bromo-3-chloropropane (23) for 10 minutes; the runs were investigated using UPLC/MS for a qualitative analysis of the output:

- DIPEA was the only base that did not show any sign of product formation.
- Cs_2CO_3 showed the formation of the desired product but also a large quantity of a bis-alkylated product, probably due to the formation of 1,3-dihydro-2H-benzimidazol-2-one (21) before the alkylation.
- K_2CO_3 showed the formation of the desired product as well as the formation of 1,3-dihydro-2H-benzimidazol-2-one (21).
- DBU showed the formation of the desired product without degradation, the only side product observed was related to the reaction of the chlorine instead of the bromine with the nitrogen.

Once DBU was chosen as base, the optimization restarted this time directly in flow.

The design is a 2 factorial levels with 5 parameters (16 runs) and 3 central points. The reduced number of experiments should help to identify if a parameter could affect the reaction or not.

The parameter ranges were:

- Temperature (from 70°C to 130°C)
- Reaction time (from 6 to 24 minutes)
- Equivalents of base (from 1 to 2)
- Concentration of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22) (from 0.2 M to 0.5 M)

- Equivalents of 1-bromo-3-chloropropane (23) (from 1 to 3)

The graphical result of the DoE is reported in Figure 36 and immediately highlights the presence of a curvature.

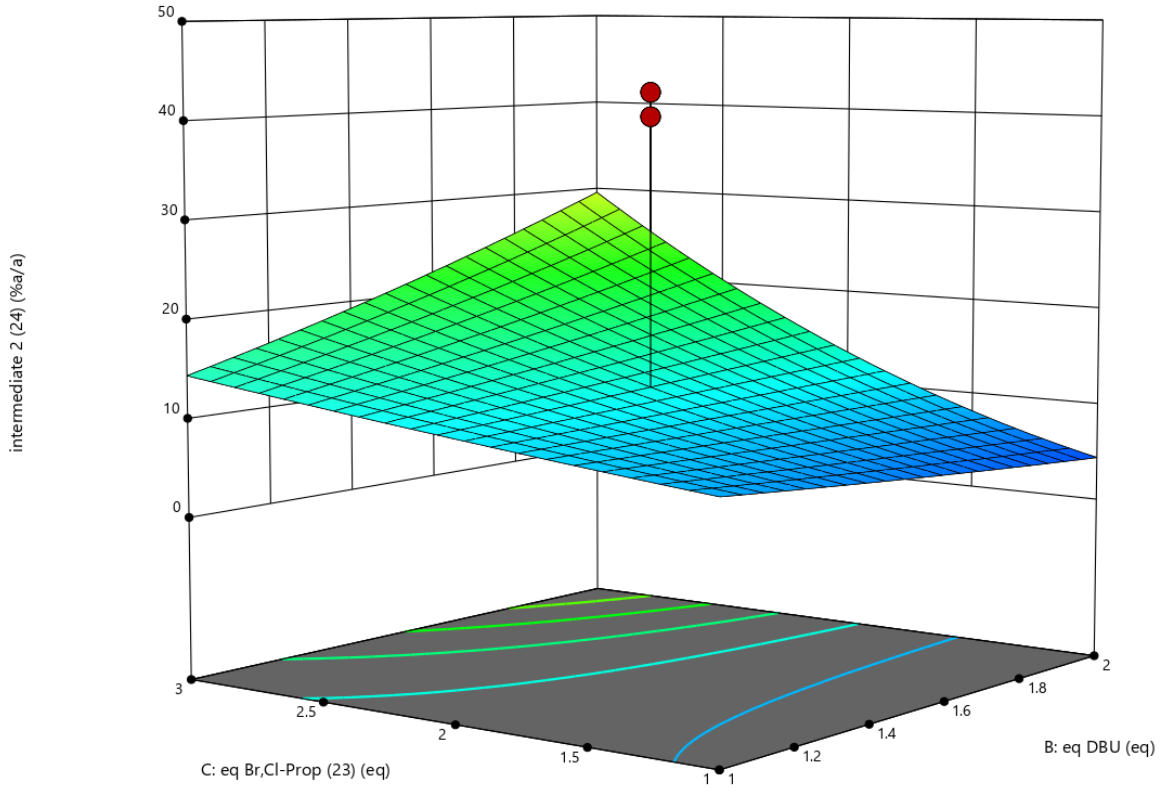


Figure 36 The effects of equivalents of 1-bromo-3-chloropropane (23) and equivalents of base on the product yield (factorial)

Also in this case the other side products were analysed and the curvature was present in the analysis of an impurity formed during the reaction; the other side product observed was the deprotected product (Intermediate 3 (4)) but this is not a problem as the next step will be the removal of the protecting group.

Also in this case the model was expanded in a central composite, the concentration of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22) was kept constant at 0.5 M as it did not show any effect on the reaction output, and the reaction range was adjusted on the basis of the observations from the previous experiments.

The new parameter ranges were:

- Temperature (from 25°C to 100°C)
    - Mean at 62.5°C
- Reaction time (from 0.1 to 20 minutes)

- Mean at 10 minutes
- Equivalents of base (from 1 to 3)
  - Mean at 2
- Equivalents of 1-bromo-3-chloropropane (23) (from 1 to 5)
  - Mean at 3

The design was a 4 parameters, central composite (24 experiments) and 6 central points, for a total of 30 experiments.

The graphical result of the DoE for the alkylation of tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22), reported in Figure 37, did not highlight the effect of the curvature that was observed in the factorial design (Figure 36 above).

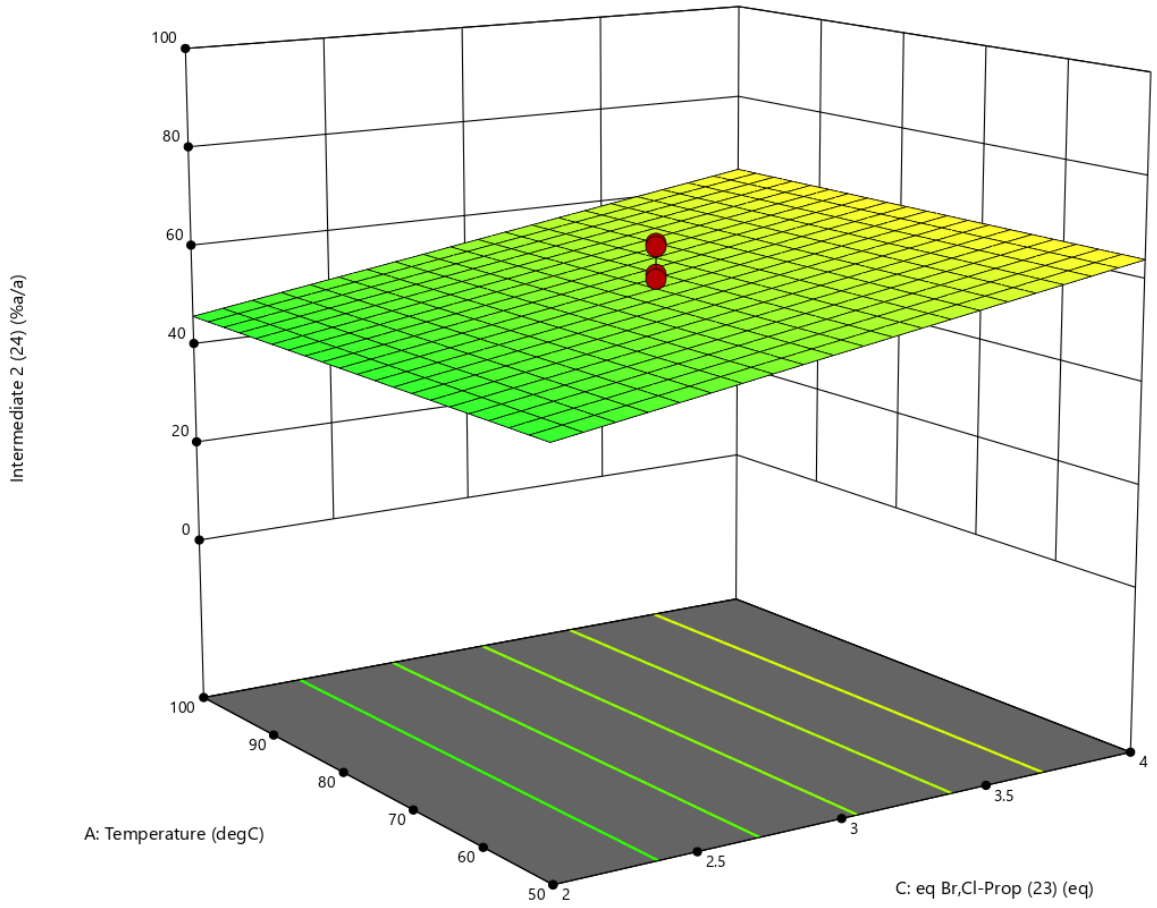


Figure 37 The effects of equivalents of 1-bromo-3-chloropropane (23) and equivalents of base on the product yield (central composite)

The ANOVA analysis for this reduced linear model shows an F-value of 17.63 for the model, which implies that the model is significant. There is only a 0.03% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicates that model terms are significant, in this case only equivalents of 1-bromo-3-chloropropane (23) (p-values 0.0003).

The Lack of Fit F-value of 4.55 implies that the Lack of Fit is significant. There is only a 7.55% chance that a Lack of Fit F-value so large could occur due to noise.

The resulting equation was:

$$\text{Intermediate } 2 = 55.27 + 9.04\ \text{A}$$

Where:

A: “Equivalents of 1-bromo-3-chloropropane (23)”

The Predicted R^2 of 0.2392 is in reasonable agreement with the Adjusted R^2 of 0.3812 as the difference is less than 0.2.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The observed ratio of 12.828 indicates an adequate signal.

One set of confirmation runs was performed in triplicate, the parameters were

- Time (11.6 minutes)
- Temperature (50°C)
- Equivalents of 1-bromo-3-chloropropane (23) (4 equivalents)
- Equivalents of DBU (1,5 equivalents)

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %a/a	95% PI high
intermediate 2 (24)	62.6363	13.821	3	N/A	44.4895	73.49	80.7831
Intermediate 2 related (4, 25, 26)	14.5194	7.18203	3	N/A	5.59764	24.8764	25.1033
Impurity	8.6688	6.11929	3	N/A	1.16839	0	19.365
intermediate 1 (22) (SM)	8.22743	3.72027	3	N/A	3.24044	0.472222	14.3185

Table 16 Confirmation run in flow

The data above are a mean of 3 runs and the confirmation run was not completely in line with the predicted data, probably due to the low R^2 observed (c.a. 0.2) and the lack of fit reported for the product and the product-related species. In any case, the outputs were better than expected, resulting in an almost complete conversion.

The product-related species are the compounds reported in Figure 38 below, and all of them can react and be converted into Domperidone (1).

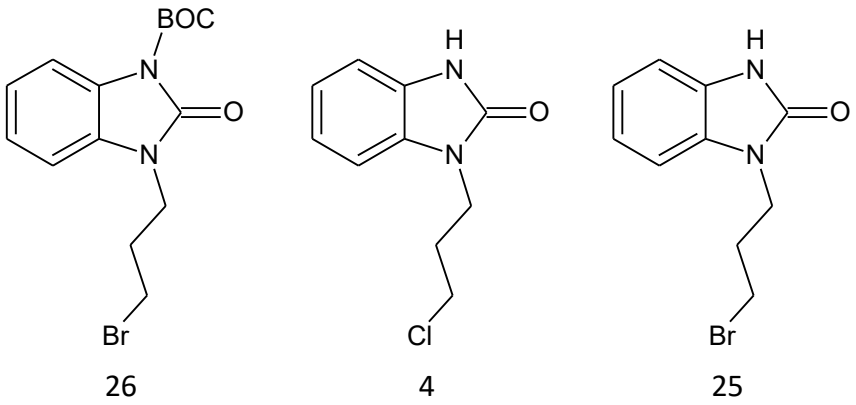


Figure 38 Product-related impurities observed during alkylation

In conclusion, the optimized conditions, in the studied range, are as reported below.

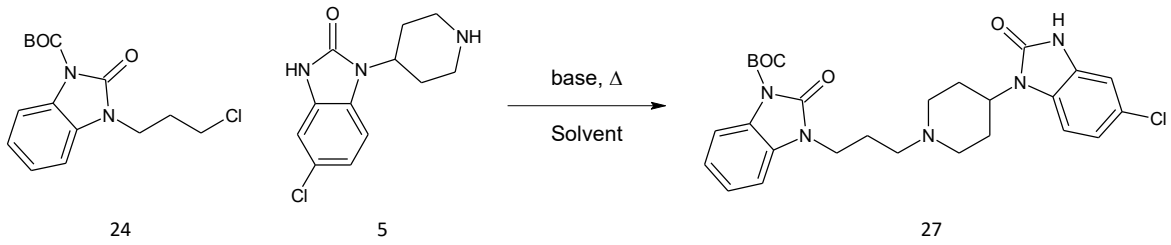
- Time (11.6 minutes)
- Temperature (50°C)

- Equivalents of 1-bromo-3-chloropropane (23) (4 equivalents)
- Equivalents of DBU (1,5 equivalents)

These conditions resulted in an almost compete conversion of the starting material into the desired compound or a useful derivative.

5.3 Synthesis of protected-Domperidone (27)

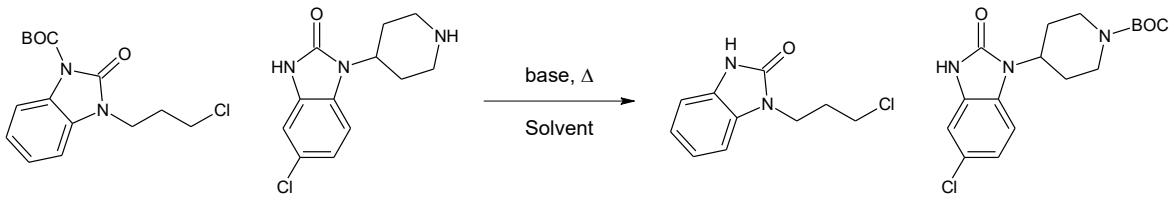
Due to the low solubility of Domperidone free base [78], keeping the structure protected was considered a good idea. In a single MW run, tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24) reacts with 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5) in the presence of a base to give protected-Domperidone (27).



24 5 27

Scheme 20 Planned reaction to obtain protected-Domperidone

The result of the experiment was very disappointing as “migration” of the BOC protecting group from the tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24) to the piperidine’s nitrogen was observed giving tert-butyl 4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (28), as reported in Scheme 21 below.



24 5 4 28

Scheme 21 Reaction of BOC "migration"

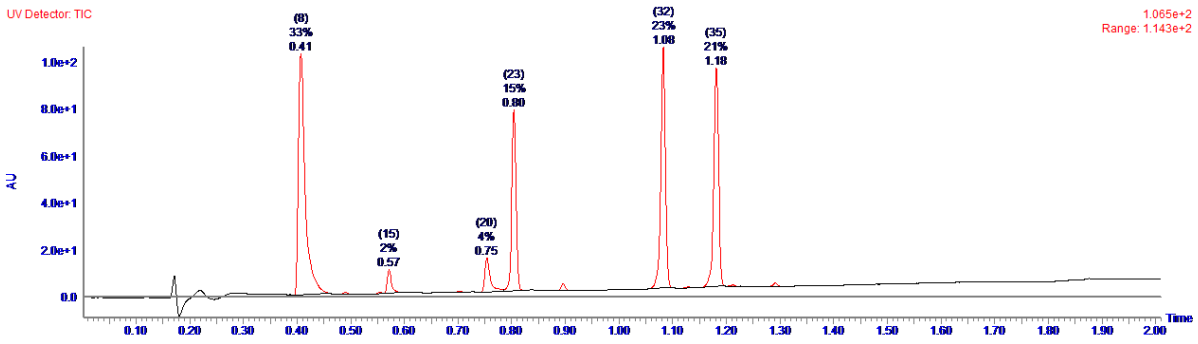


Figure 39 Chromatogram of the alkylation attempt in MW

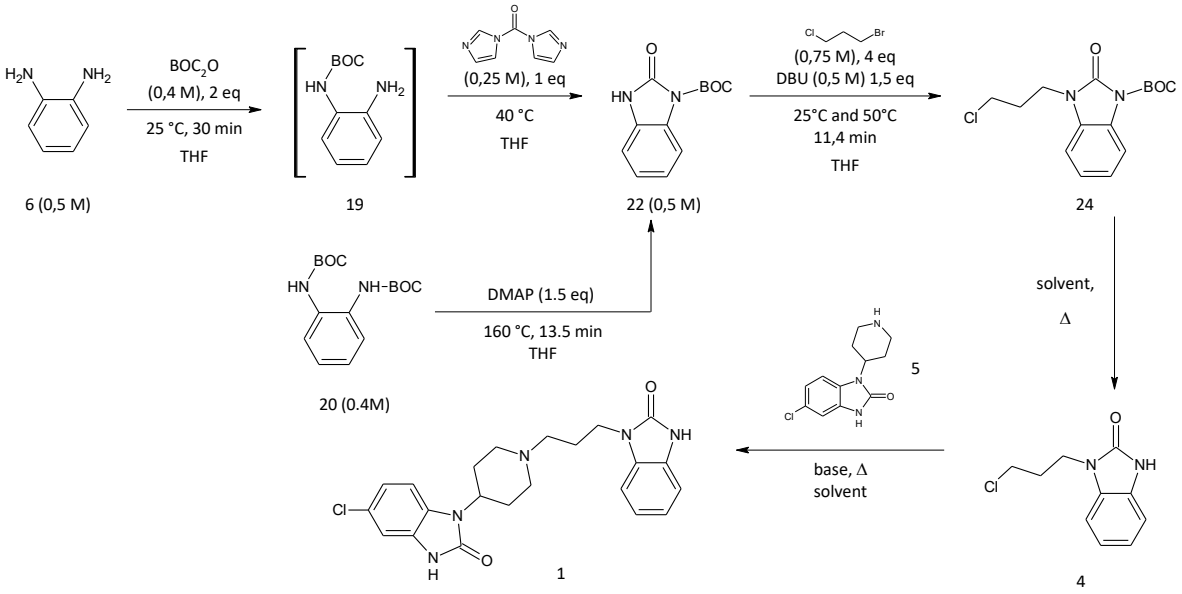
The identified products are reported in Table 17 below.

Retention Time (min)	Observed M/Z	Assignment
0.41	252 (M+1)	5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5)
0.57	426 (M+1)	Domperidone (1)
0.75	526 (M+1)	BOC-Domperidone (27)
0.80	211 (M+1)	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)
1.08	352 (M+1)	tert-butyl 4-(5-chloro-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidine-1-carboxylate (28)
1.18	255 (M+1-56)	tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)

Table 17 Identified products in attempted synthesis of BOC-Domperidone (28)

The data observed forced a change to the synthetic strategy, anticipating the deprotection stage and starting to consider a solvent suitable to keep Domperidone in solution during the synthesis.

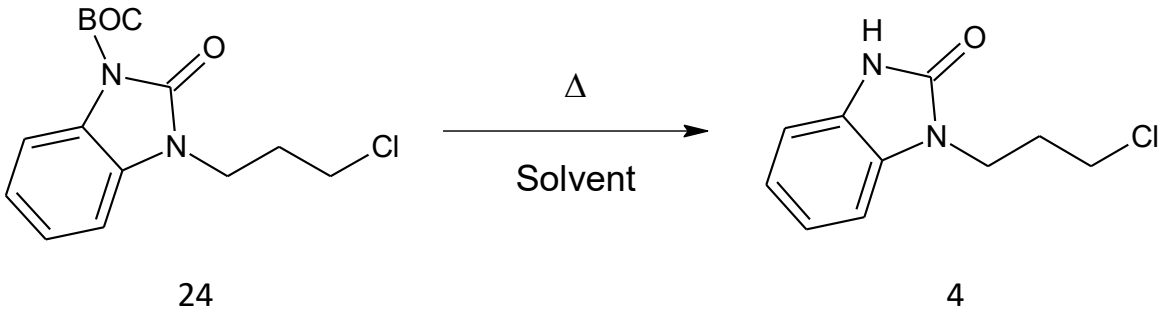
The synthesis that had been planned until now had to be changed following the result just observed. The new synthetic approach can be found in Scheme 22 below.



Scheme 22 Updated synthetic route to Domperidone (1)

5.4 Synthesis of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4)

Due to the lability of the BOC- group at higher temperature, observed in previous experiments, and in order to keep Domperidone in solution once it forms, and to solubilize 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5), DMSO was selected as solvent for the next transformations of deprotection and alkylation.



Scheme 23 Synthesis of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4)

The design is a 2 full factorial levels with 3 parameters (8 runs) and 4 central points.

The parameter ranges were:

- Temperature (from 120°C to 180°C)

- Reaction time (from 5 to 20 minutes)
- Concentration of starting material (24) (from 0.1 M to 0.5 M)

The graphical result of the DoE is reported in Figure 40: it immediately highlights the presence of a curvature as in the other reactions.

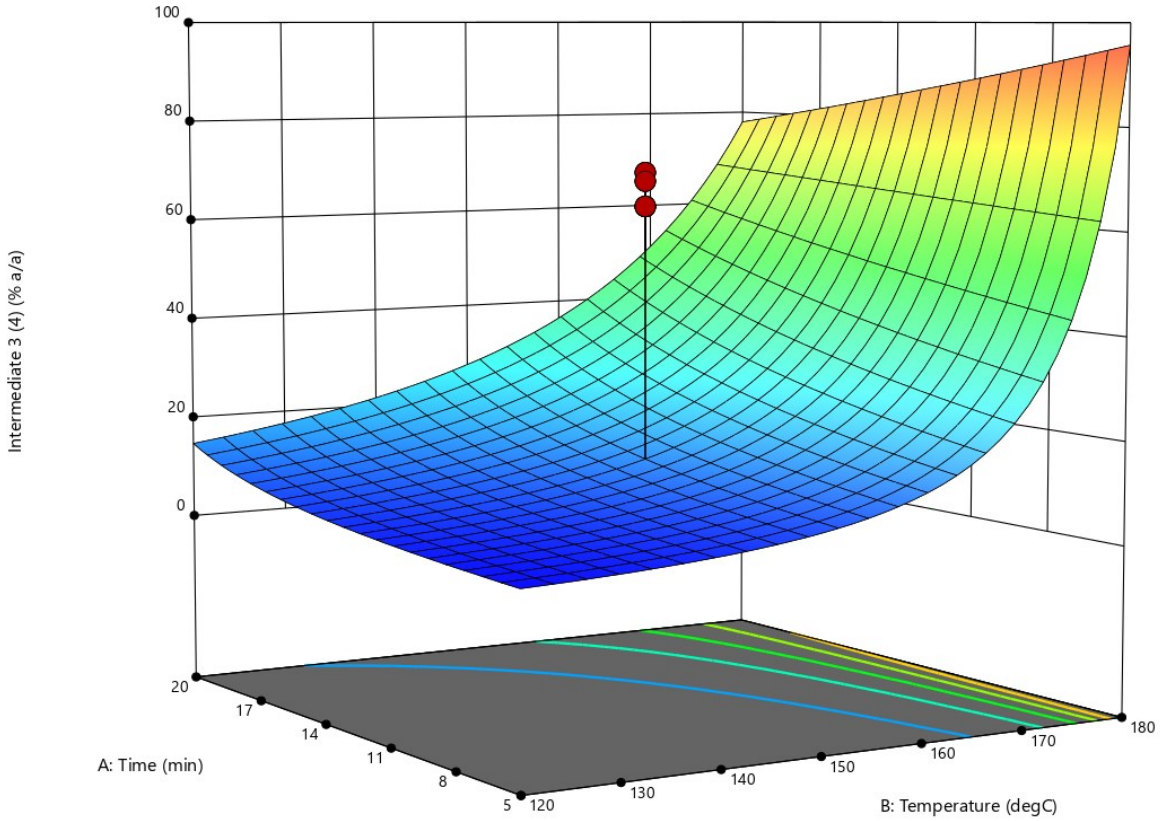


Figure 40 The effects of temperature and time on the product yield at a concentration of 0.3 M.

In this case the complete conversion into the product was observed during the experiments but, as curvature was observed, a central composite design was necessary to better understand the effects of each parameter.

The new parameter ranges were:

- Reaction time (from 2.63 minutes to 22.40 minutes)
- Temperature (from 111°C to 189°C)
- Concentration of starting material (24) (from 0.368 M to 0.56[5] M)

The graphical result for this expansion is reported in Figure 41 below.

[5] For this concentration, the solution was prepared ad hoc.

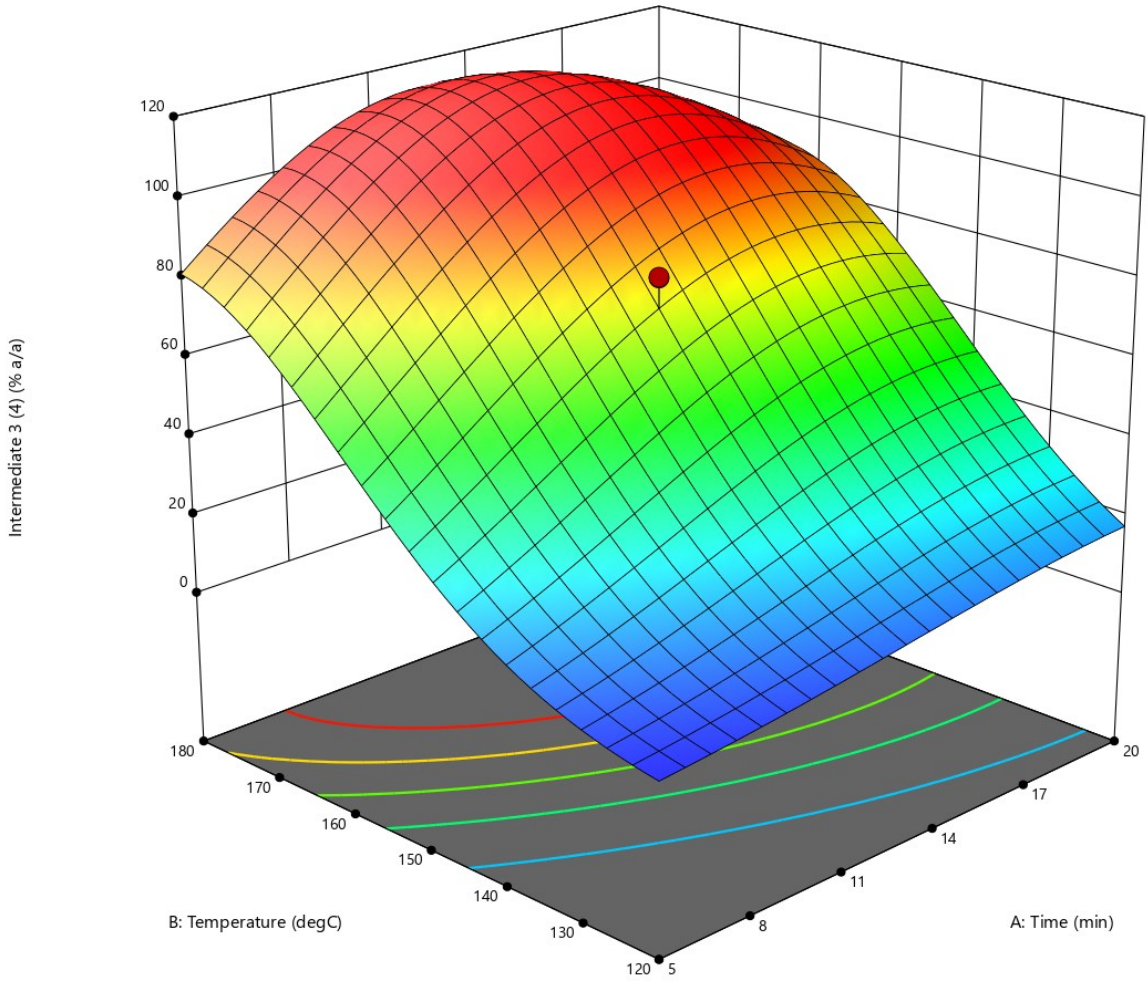


Figure 41 The effects of temperature and time on the product yield after expansion

The ANOVA analysis for this reduced linear model shows an F-value of 141.67 for the model, which implies that the model is significant. There is only less than 0.01% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case, time (A: p-values <0.0001), temperature (B: p-values <0.0001) and the combinations of time and temperature are significant (AB: p-values <0.0001) as well as the square effect of time and temperature (A^2: p-values 0.0002, B^2: p-values <0.0001).

The Lack of Fit F-value of 26.30 implies that the Lack of Fit is significant. There is only a 0.33% chance that a Lack of Fit F-value so large could occur due to noise.

The resulting equation was:

$$\text{Intermediate 3} = -26.05972 + 0.520099\ \text{A} + 0.316608\ \text{B} - 0.002019\ \text{AB} - 0.006455\ \text{A}^2 - 0.000839\ \text{B}^2$$

Where:

A: Time

B: Temperature

The Predicted R^2 of 0.9288 is in reasonable agreement with the Adjusted R^2 of 0.9750; i.e. the difference is less than 0.2.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The observed ratio of 31.152 indicates an adequate signal.

One set of confirmation runs were performed in triplicate, the parameters for the runs were:

- Time (5 minutes)
- Temperature (180°C)
- Concentration (0.1 M)

These confirmation runs coincided with one of the points analysed during the investigation.

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %a/a	95% PI high
intermediate 3 (4)	80.0422	15.5887	3	N/A	56.3397	100	113.717
Impurity	0.319055	15.1814	3	N/A	-0.021672	0[6]	11.8999
intermediate 2 (24) (SM)	4.1305	5.36886	3	N/A	-0.076811	0	15.6646

Table 18 Confirmation run using the experimental point

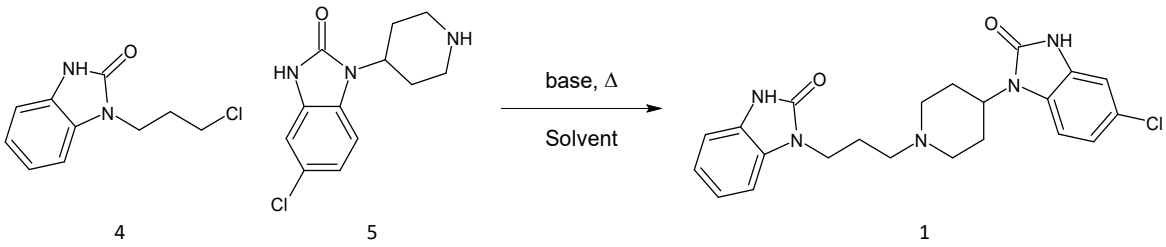
[6] The value reported by the program on the data mean for the impurity was $6.93889 * 10^{-18}$ as the UPLC method was not able to reach such precision the data was rounded to 0

The data above are a mean of 3 runs and the confirmation runs are completely in line with the predicted data and with those observed during the investigation.

In conclusion, this step results in the complete thermal removal of the BOC group without the use of acid in just 5 minutes of residence at 180°C at a concentration of 0.1M.

5.5 Synthesis of 5-chloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one (AKA Domperidone) (1)

The alkylation of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) with 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) was the last chemical step to obtain Domperidone (1).



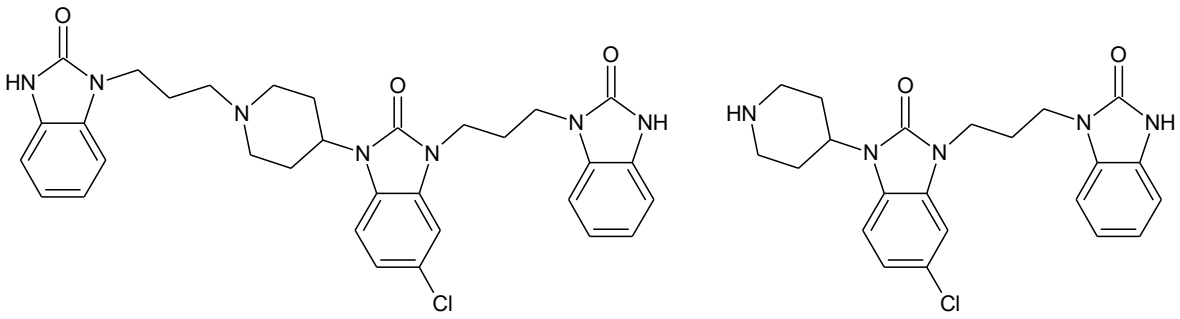
4 5 1

Scheme 24 Last chemical step to obtain Domperidone (1)

The solvent selected was DMSO as Domperidone (1) free base is very soluble in this solvent [78].

Also in this last step the initial test reactions were performed using the microwave to orientate the flow investigation.

The outcome of these experiments was that DIPEA was superior to DBU as base, as the latter was too strong, resulting in the formation of side products as reported in Figure 42 below.



M+1/Z : 600

29

M+1/Z : 426

30

Figure 42 Side products observed in UPLC/MS with DBU as base

The optimization with DIPEA started with a low level full-factorial design for 4 parameters (16 runs) and 4 central points for a total of 20 runs.

The factors investigated were:

- Equivalents of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) (from 1 eq to 2 eq)
- Equivalents of DIPEA (from 1 eq to 2 eq)
- Reaction time (from 10 minutes to 30 minutes)
- Temperature (from 120°C to 180°C)

The graphical result of the DoE, for the formation of Domperidone (1), reported in Figure 43, did not highlight a curvature. A curvature was present in the other responses analysed for the starting materials 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) and 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4).

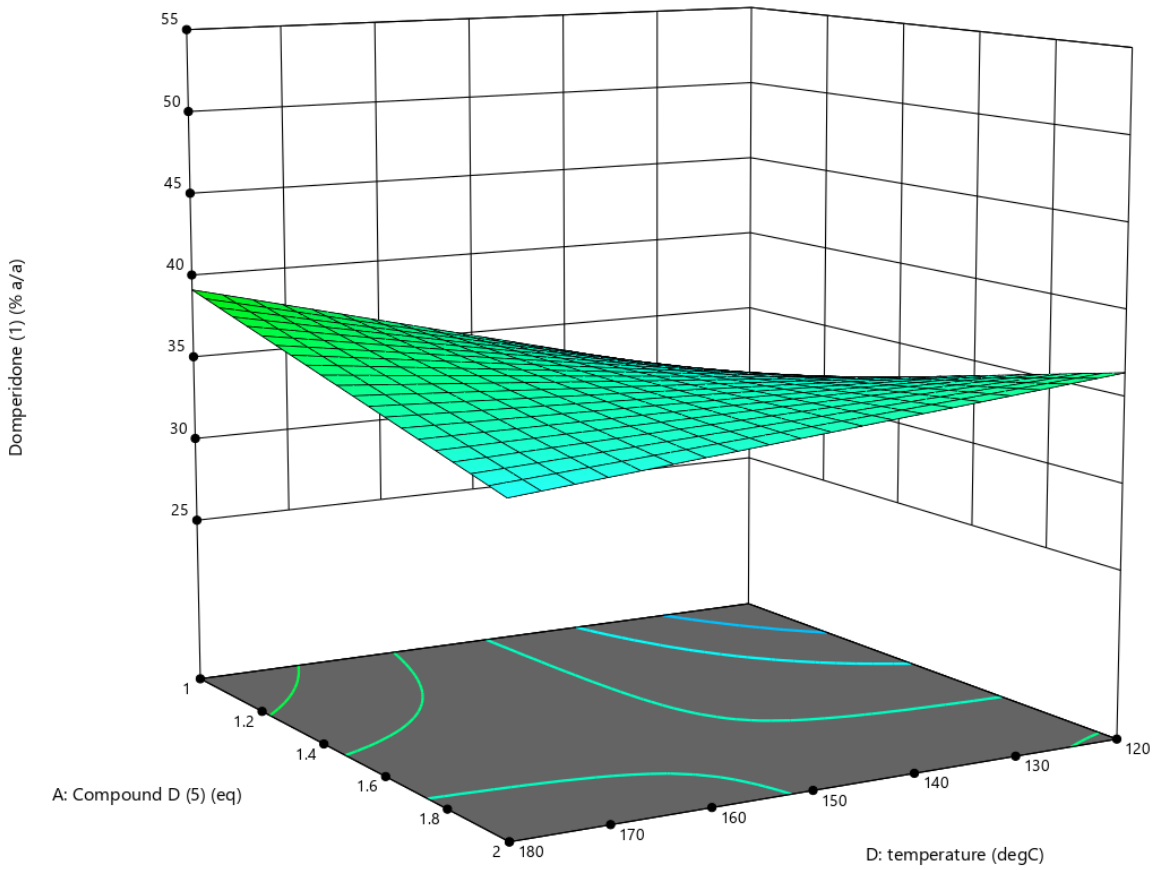


Figure 43 The effects of temperature and 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) on the product yield

To investigate the curvature present in the response analysed for the 2 starting materials, an expansion to a central composite design was performed, resulting in a further 10 experiments including two further central points. The new parameter ranges were:

- Equivalents of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) (0.5 eq – 2.5 eq)
- Equivalents of DIPEA (from 0.5 eq to 2.5 eq)
- Reaction time (from 0.34[7] minutes to 40 minutes)
- Temperature (from 90°C to 210°C)

The graphical result for this expansion is reported in Figure 44 below.

[7] The planned parameter was 0 minute as minimum, but due to pump limits the minimum reaction time was set at 0.34 minutes.

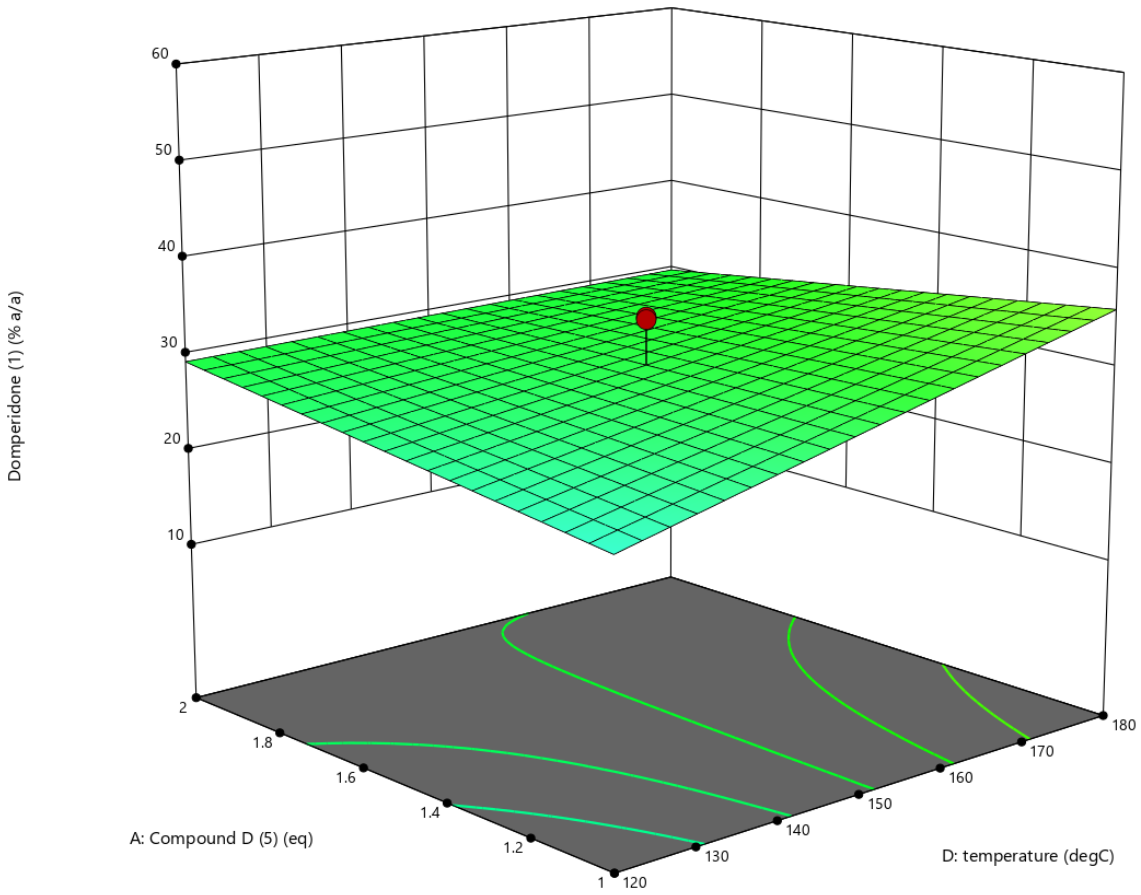


Figure 44 The effects of temperature and 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (compound D (5)) on the product yield after expansion

The ANOVA analysis showed an F-value of 10.37 for this model, which implies that the model is significant. There is only less than 0.01% chance that an F-value so large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case time (p-values 0.0001) and temperature (p-values 0.0011) are significant with the combination of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) (A) and temperature (D) (AD: p-values 0.0101) and the combination of time (C) and temperature (D) (CD: p-values 0.0058).

The Lack of Fit F-value of 3.69 implies that the Lack of Fit is not significant. There is only a 10.70% chance that a Lack of Fit F-value so large could occur due to noise.

The resulting equation was:

$$\text{Domperidone} = -7.348 + 30.255\ \text{A} - 1.242\ \text{C} + 0.194139\ \text{D} - 0.202333\ \text{AD} + 0.010975\ \text{C}D$$

Where:

A: “Equivalents of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one” (5)

C: “Reaction time”

D: “Temperature”

The Predicted R^2 of 0.4343 is in reasonable agreement with the Adjusted R^2 of 0.6258 as the difference is less than 0.2.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The observed ratio of 14.994 indicates an adequate signal.

Two sets of confirmation runs were performed in duplicate, the parameters were:

- Equivalents of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) (1.64 eq)
- Equivalents of DIPEA (1.5 eq)
- Reaction time (30 minutes)
- Temperature (180°C)

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %a/a	95% PI high
Compound D (5)	60.4584	3.84978	2	3.23436	53.7322	56.08	67.1846
Intermediate 3 (4)	0.288667	2.96572	2	2.3878	-4.67703	2.68	5.25436
Domperidone (1)	39.4914	4.33254	2	3.60302	32.038	41.335	46.9449

Table 19 Confirmation run using the central composite optimized conditions

The data above are a mean of 2 runs and the confirmation run was completely in line with the predicted data.

A second set of runs was performed using the “optimized” conditions obtained for the factorial design, the parameters for this run were:

- Equivalents of 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (Compound D (5)) (1.02 eq)
- Equivalents of DIPEA (1.5 eq)
- Reaction time (30 minutes)
- Temperature (180°C)

Response	Predicted Mean	Std Dev	N	SE Pred	95% PI low	Data Mean %a/a	95% PI high
Compound D (5)	49.6415	3.84978	2	3.28004	42.8203	50.025	56.4627
Intermediate 3 (4)	8.33348	2.96572	2	2.51828	3.09644	7.13	13.5705
Domperidone (1)	43.3137	4.33254	2	3.82498	35.4011	42.85	51.2263

Table 20 Confirmation run using the factorial optimized conditions

The data above are a mean of 2 runs and the confirmation run was completely in line with the predicted data. Despite the good conversion, the first set of confirmation runs resulted in a lower amount of unreacted starting material.

In conclusion, the process to obtain Domperidone (1) results in complete consumption of the starting material and the unreacted “compound D” (5) can be removed via the precipitation of Domperidone (1) with water and its subsequent recrystallization.

5.6 Run through to Domperidone

A run through was performed to test all the reactions in a single run using the output of one stage in the following stage, or what in batch chemistry is called telescoped. The main issue in a telescoped process is the unpredicted effect of an impurity generated in one step on the following stage.

The run through started from tert-butyl (2-aminophenyl)carbamate, easily obtained as a solid following the procedure reported in US 2009/0203681 A1[97]. To avoid further divergences from what was carried out during the development work, the first reaction was performed in such a way to have a final concentration equal to that resulting from the collection of all the reagents in one flask. To maximize the output all the available reactors

were placed in series for a total of 35 ml of reaction coil (3 X 10 ml + 5 ml coil) as show in Figure 45 and Figure 46 below and, due to the absence of a continuous work-up equipment, the work-up was performed in batch once all the material had been collected.

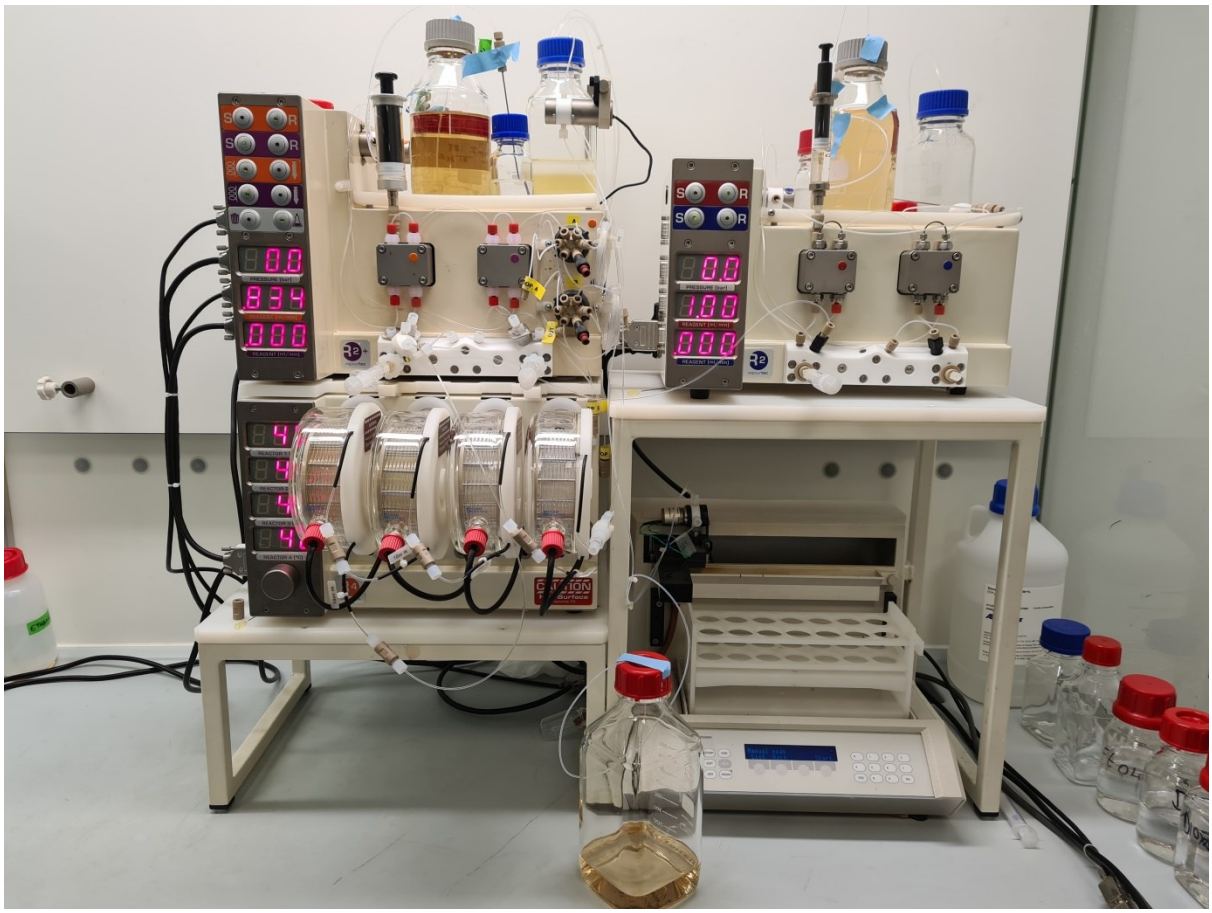


Figure 45 Flow reactor during the run through of step 2

Figure 46 Particular of the 4 reactors in series.

The work-up was performed first with sodium bicarbonate to force the cyclization of the uncyclized intermediate with CDI, followed by aqueous potassium bisulphate (5%) to remove imidazole.

Once obtained, the material was evaporated and dissolved in THF for the next step. The optimization of step 3 was performed with two reactors in sequence, the first 2 ml reactor (at 25°C) to pre-mix the DBU with intermediate 1 (22) and then introduce the 1-bromo-3-chloropropane (23) before the second 10 ml reactor (at 50°C) for 11.6 min in which the reaction takes place. This corresponds to 4.78 min of residence time in the 2 ml reactor at 25°C. During the run through a premixing of the DBU with intermediate 1 (22), in a 5 ml coil reactor, was also included followed by the mixing with the 1-bromo-3-chloropropane (23) in a 30 ml reactor (3 X 10 ml) as show in Figure 47 below. In the attempt to maintain

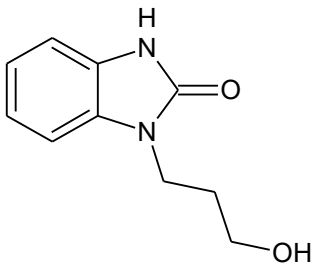
consistency with the development work, the reaction temperature in the 5 ml reactor was increased to 30°C to preserve the same reactivity, accordingly to the Arrhenius equation calculated using a web tool [98].

Figure 47 Flow reactor during step 3

The material from the alkylation step was diluted with ethyl acetate and then the organic phase was washed with water followed by aqueous potassium bisulphate (5%) to remove the excess of DBU. The excess of 1-bromo-3-chloropropane (23) was removed by evaporation under vacuum using heptane to aid the evaporation of the alkylating agent.

Step 4 (BOC-removal) was optimized at high temperature (>150°C) and for this reason required a high temperature stainless steel coil show in Figure 49. As only one support was available for this type of reactor, the overall reaction time was very long. The output of this reaction was used directly in the next step without any further manipulation.

During the run through of step 4, the formation of an unpredicted impurity was observed, corresponding to the insertion of -OH on the chain instead of the chlorine (31) show in Figure 48 below.



31

Figure 48 Impurity observed in UPLC/MS during the deprotection stage in run through.

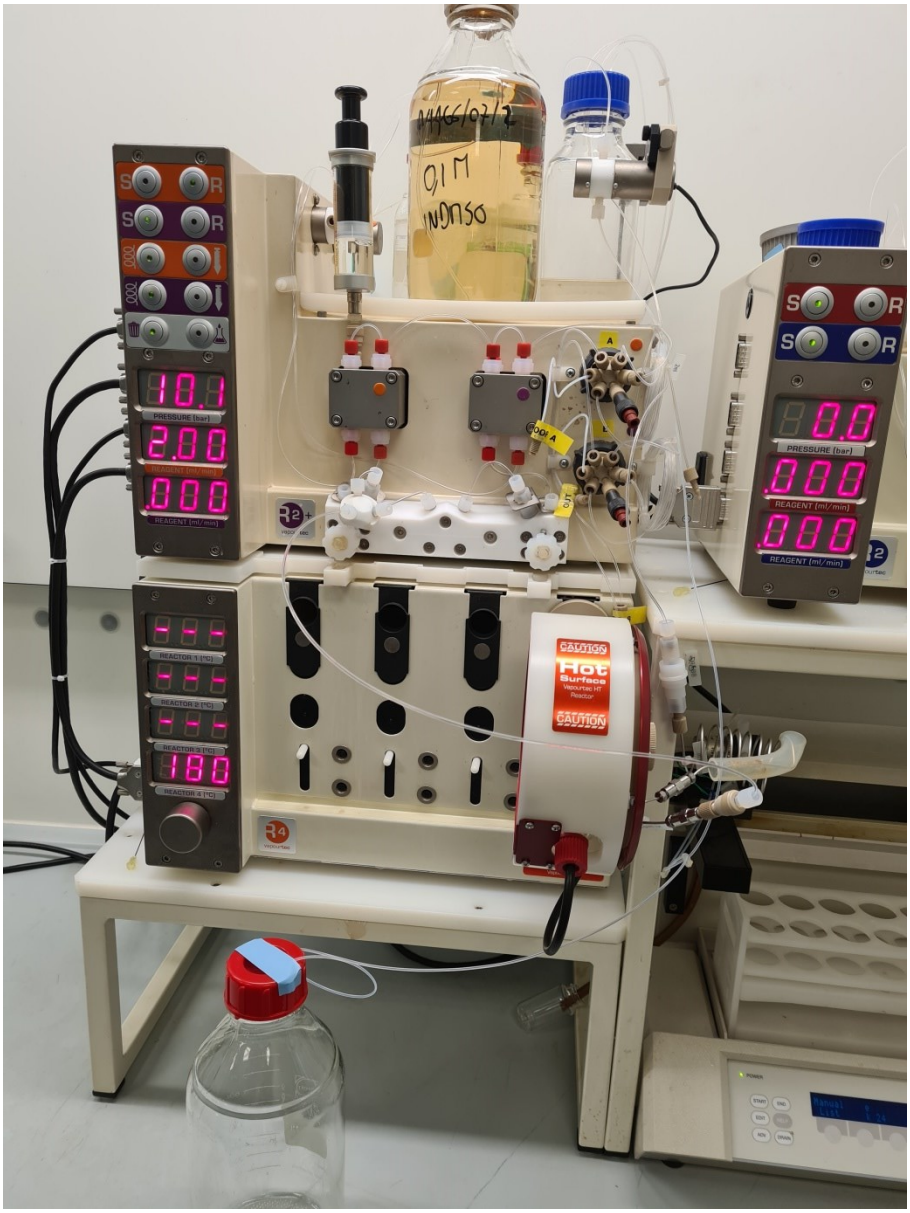


Figure 49 Flow reactor during step 4

The final alkylation needed to be performed at 180°C for 30 min after a pre-mixing stage of the nucleophile (5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5) with the base (DIPEA) the equipment setting was reported in Figure 50 below. As above, the

limited availability of high temperature reactors resulted in a reaction time for step 5 of a total of c.a. 7.5 days, to process all the material manufactured. For this reason only 10% of the original input material was processed in step 5.

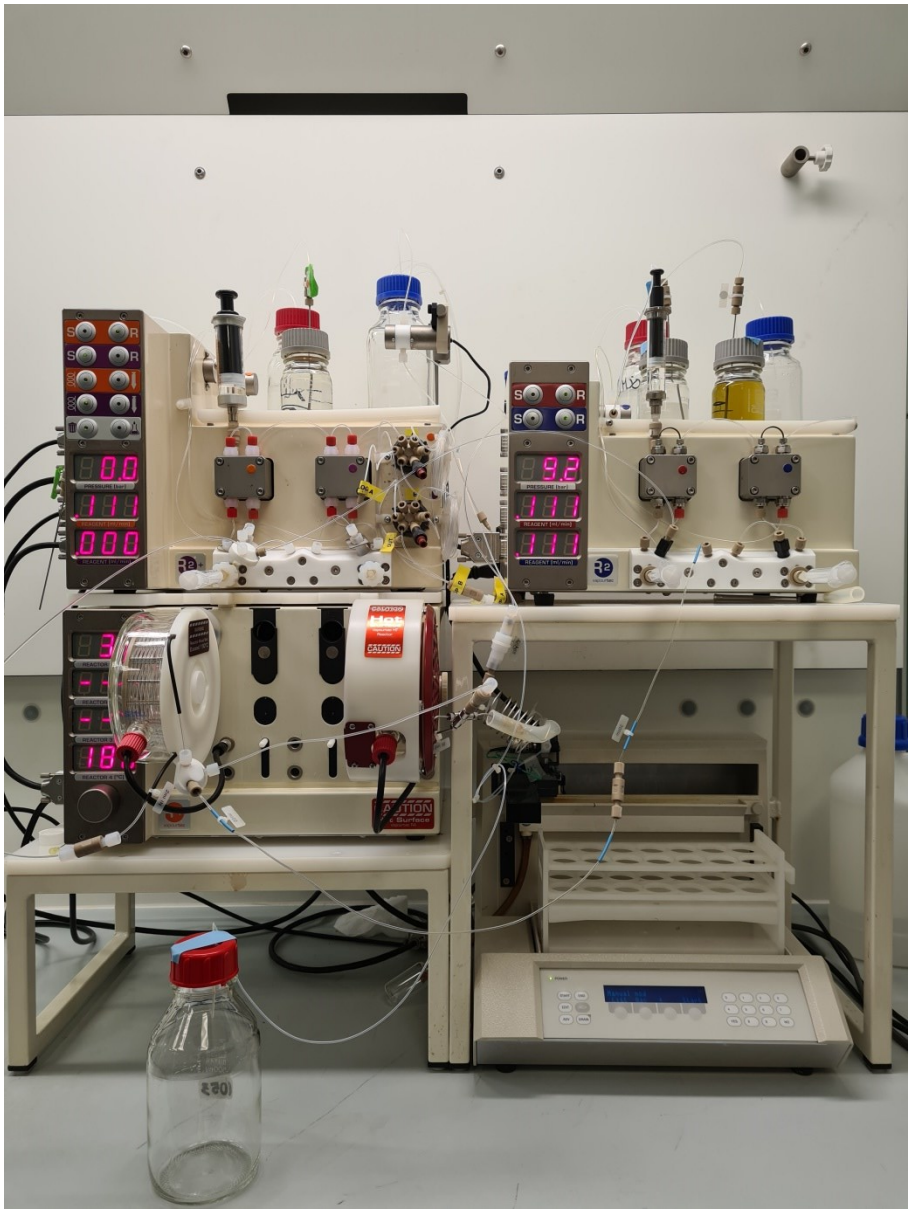
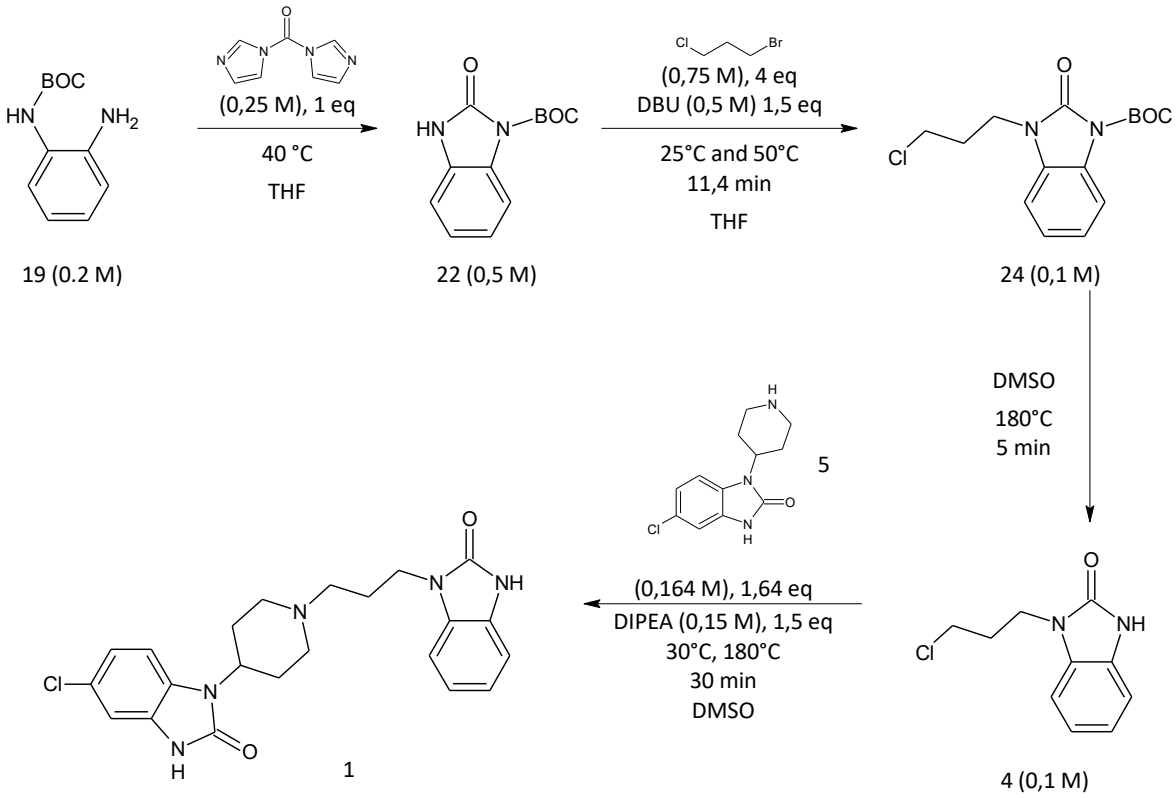


Figure 50 Flow reactor during step 5

The reactions performed during the run through are summarized in Scheme 25 below. The main difference from the optimized process was in the concentration of the reagents in last alkylation step, that during the run through changed for compound D (5), from 0.1 M to 0.164 M, and for DIPEA, from 0.1 M to 0.15 M. These changes were introduced to reduce the reaction time for this step.

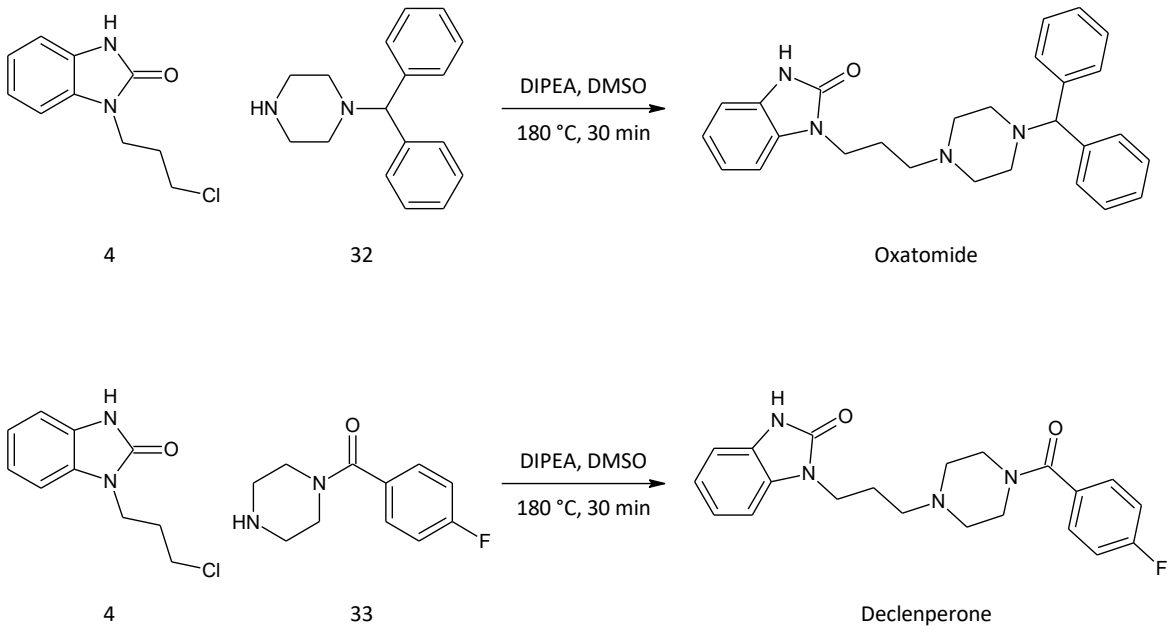


Scheme 25 Domperidone process in run through

This synthesis, summarized in Scheme 25 above, resulted in an overall yield of 42.8 molar %, a value that was significantly impacted by the isolation step that was not optimized and could be part of a further process expansion. The overall reaction time to process all the material was expected to be 9 days, 15 hour, 30 minutes and 41 seconds, if the reactions were performed in sequence. The reaction time was mainly affected by the limited number of reactors available to reach the very high temperatures required to perform the last two steps; the last step accounts for more than 7.5 days in the calculation. For this reason, during the run through only 10% of the material obtained after the deprotection was progressed in the last alkylation step.

5.7 Synthesis of Oxatomide and Declenperone

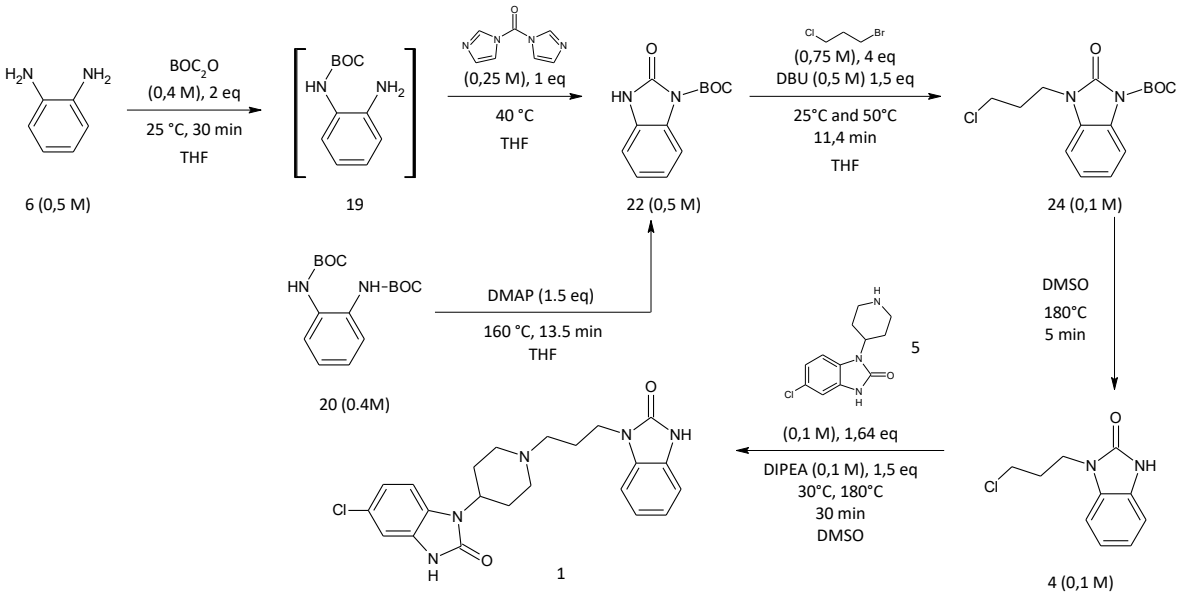
The process described thus far can be easily expanded to other drugs, such as Flibanserin, Oxatomide and Declenperone, that share the same LHS moiety as reported in Figure 21. For Oxatomide and Declenperone we expected that optimization would not be necessary due to the structural similarity with Domperidone with respect to the RHS building block.



Scheme 26 Synthesis of Oxatomide and Declenperone

The two experiments were successfully performed applying the knowledge from the Domperidone process to the synthesis of Oxatomide and Declenperone, once the required RHS parts of both compounds were obtained. For Oxatomide 1-(diphenylmethyl)piperazine (32) was commercially available and was used directly, whereas for Declemperone (4-fluorophenyl)(piperazin-1-yl)methanone (33) was synthetized in batch. The alkylation reactions were performed using the same conditions optimized for Domperidone. In case of a further expansion of the procedure some adjustments may be necessary to re-optimize the process due to differences in the pKa of the reactive nitrogen, 9.33 for 1-(diphenylmethyl)piperazine (32), 7.82 for (4-fluorophenyl)(piperazin-1-yl)methanone (33) and 9.73 for 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5), in which case the current procedure could be used as a starting point.

5.8 Conclusion



Scheme 27 Optimized Domperidone process

The process described up to now, and reported in Scheme 27 above, is suitable for a continuous manufacturing. It will require the implementation of continuous phase separation and continuous evaporation, as a solvent change is necessary to perform the last steps avoiding clogging due to Domperidone precipitation in the reaction coil.

The process was focused on minimizing the unreacted starting materials and recycling the side products (see paragraph 5.1.3).

The overall yield of the run through process was 48.2% mol/mol. Improvements in the isolation part would be necessary for a further industrialization. With an extrapolation considering the unreacted starting materials in each reaction and not considering side reactions, the predicted overall yield was expected to be > 75% mol/mol. Some fine tuning is possible considering this work as a starting point for a further exploration of conditions, as the optimization reported is correct for the parameter range investigated.

The yield data above can be extrapolated from each single step conversion. We expect a 40% molar yield in the synthesis of intermediate 1 (22) and a further 41% molar yield from reprocessing of the di-tert-butyl benzene-1,2-diylbiscarbamate (20) obtained as a side product from the synthesis of intermediate 1 (22). The alkylation of intermediate 1 (22) results in a 99.5% conversion into products that can be converted into Domperidone (1), as reported in Figure 38 above. The deprotection of intermediate 2 (24) did not show the formation of impurities and proceeded to complete conversion, so the yield was considered quantitative. The formation of Domperidone (1) resulted in c.a. 2.5% of

unreacted intermediate 3 (4). Further product loss can be predicted during the precipitation with water.

6 Experimental section

6.1 Flow equipment

As anticipated earlier Vapourtec equipment was utilised for this work including the R2/R2+/R4 system[87] connected with a Gilson FC203 fraction collector. The main R2+ pumping system is composed of two integrated acid resistant HPLC pumps: the flow rates could be regulated and set at any value between 0.01 and 10 ml min^{-1}, working with a system pressure of up to 50 bar (ca. 725 psi); the R2+ system also includes the loop for the use of low amounts of reagents (2 ml). An additional R2 system, composed of two supplementary HPLC pumps, is included to have the possibility of using up to four reagents/solvents. The mix of the reagents' streams was achieved with a simple PTFE Y-piece and the combined output was directed, through perfluoroalkoxy tubing, to the convection-flow coil (CFC), which can be heated up to 250 °C using the stainless steel reactor. The R4 heater guarantees precise temperature control in four independent heating zones with rapid temperature ramping and cooling. The addition of a back pressure regulator (up to 600 psi) is usually applied in-line, allowing access to higher temperature while avoiding solvent boil over. At the top, a large drip tray is located for reagent bottles and waste vessels.

The available reactors are 2 ml, 5 ml and 10 ml; the construction material as well as the reaction temperature influence the pressures allowed for each reactor as shown in Table 21 below.

Tube Reactors (Coil)				
	-70°C to 40°C	**40°C to 99°C**	**100°C to 150°C**	**150°C to 250°C**
PFA tube reactors (and PFA supply tubing)	40 bar (580 psi)	25 bar (362 psi)	15 bar (271 psi)	N/A
Stainless steel Copper Hastelloy®	42 bar (609 psi)			

Omnifit Column Reactors	
	-60°C to 150°C
15mm bore	20 bar (290 psi)
10mm bore	40 bar (580 psi)
6.6mm bore	50 bar (720 psi)

Table 21 Pressure allowed at different temperatures with the available reactors.

The control program associated to the equipment (Flow Commander) automatically calculates the flow rate knowing the type of reactor installed, the residence time, the concentration of each reagent and the molar ratio to perform the reaction.

6.2 Analytical equipment

HPLC

The HPLC used to analyse the samples is an Agilent 1100. The samples were typically prepared by dissolving a small amount of compound in acetonitrile (or in a solvent mixture) to dissolve all the sample.

The HPLC Generic Method (3 min) parameters are reported in table below.

Instrumental Parameter	Value
Column:	Zorbax SB-C18 1.8 µm 3x50 mm; column temperature 60°C
Mobile Phase:	A: 0.05% v/v solution of TFA in water B: 0.05% v/v solution of TFA in acetonitrile
Gradient:	0 min 100% A to 2.50 min 95% B, 2.51 min 95% B
Flow:	1.5 ml/min
Detector:	UV @ 220 nm or at specific wavelength

UPLC/MS

Instrument Name: Acquity UPLC

Method Description: Analytical UPLC/MS (ES+/ES-) 2.0 Minutes Method

LC/MS System: Acquity UPLC coupled with SQD mass spectrometer or ZQ mass spectrometer or coupled with QDA mass spectrometer

LC/MS Conditions:

<table>
<tr><td>Instrumental Parameter</td><td colspan="4">Value</td></tr>
<tr><td>Column:</td><td colspan="4">Acquity UPLC CSH C18 column (50mm x 2.1 mm i.d. 1.7 µm particle size) at 40°C.</td></tr>
<tr><td>Injection mode:</td><td colspan="4">Partial Loop with Needle Overfill</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = 0.1% v/v solution of HCOOH in water
B = 0.1% v/v solution of HCOOH in Acetonitrile</td></tr>
<tr><td rowspan="5">Gradient:</td><td>Time (min)</td><td>Flow Rate (ml/min)</td><td>% A</td><td>% B</td></tr>
<tr><td>0</td><td>1.0</td><td>97.0</td><td>3.0</td></tr>
<tr><td>1.50</td><td>1.0</td><td>0.1</td><td>99.9</td></tr>
<tr><td>1.90</td><td>1.0</td><td>0.1</td><td>99.9</td></tr>
<tr><td>2.0</td><td>1.0</td><td>97.0</td><td>3.0</td></tr>
<tr><td>Acquisition stop time:</td><td colspan="4">2.0 min</td></tr>
<tr><td>UV Conditions:</td><td colspan="4">UV detection range: 210 nm to 350 nm
Acquisition rate: 40 Hz
DAD – MS Rt offset: 0.01 min</td></tr>
<tr><td>MS Conditions:</td><td colspan="4">Ionisation mode: alternate Positive/Negative Electrospray (ES+/ES-)</td></tr>
<tr><td>Scan Range:</td><td colspan="4">100 to 1000 AMU</td></tr>
</table>

NMR

Samples for NMR analysis were prepared by complete dissolution of an appropriate amount of material in approximately 0.75 ml of deuterated solvent (DMSO). ^{1}H-NMR spectra were recorded at 25°C using either a Varian INOVA 400MHz NMR Spectrometer, equipped with a Varian ATB probe or Bruker Avance III 400 MHz NMR Spectrometer, equipped with 5mm BBFO 400 MHz Z-Gradient high-resolution probe. A variable number of scans (16-256) was applied using standard acquisition parameters. The pre-acquisition delay was set to 10 sec whenever NMR quantification was assessed. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, br = broad), integration, and coupling constant (J) in Hertz (Hz).

6.2.1 Sample preparation.

During the optimization the flow reaction were run to collect at least 10 ml of reaction mixture in a 20 ml vial using the fraction collector connected with the instrument, once the reaction was at steady state as indicated by the flow reactor program.

All sample analysed during the optimization are crude reaction mixture, for NMR analysis the reaction mixture was evaporated under nitrogen flow to remove the solvent; for HPLC and UPLC the crude mixture were injected after dilution in acetonitrile/water (9/1).

6.3 Statistical elaboration

The statistical elaboration was performed using the program design expert V12. The designs were chosen starting from a two factorial levels and if necessary increased to a central composite design. The parameters investigated and the range were chosen based on preliminary batch experiments.

6.4 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2)

The experiments performed for the synthesis of 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2) were performed typically in .0.5-5 g scale and the product when isolated was performed following the procedure below.

The organic layer was separated, and acetic acid was added slowly adjusting the pH at 7. The resulting slurry was filtered and washed with cyclohexane obtaining the desired product

The conversion was monitored by UPLC/MS integrating only peaks related to benzene-1,2-diamine (6), 4-Methyl-1,3-dihydro-2H-1,5-benzodiazepin-2-one (10) and 1-(prop-1-en-2-yl)-1,3-dihydro-2H-benzimidazol-2-one (2).

6.5 tert-Butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (22)

6.5.1 Synthesis via cyclization of mono-protected benzene-1,2-diamine (6).

The scheme of the equipment setting is reported in Figure 51 below, and the picture of the equipment during the run in Figure 52 below:

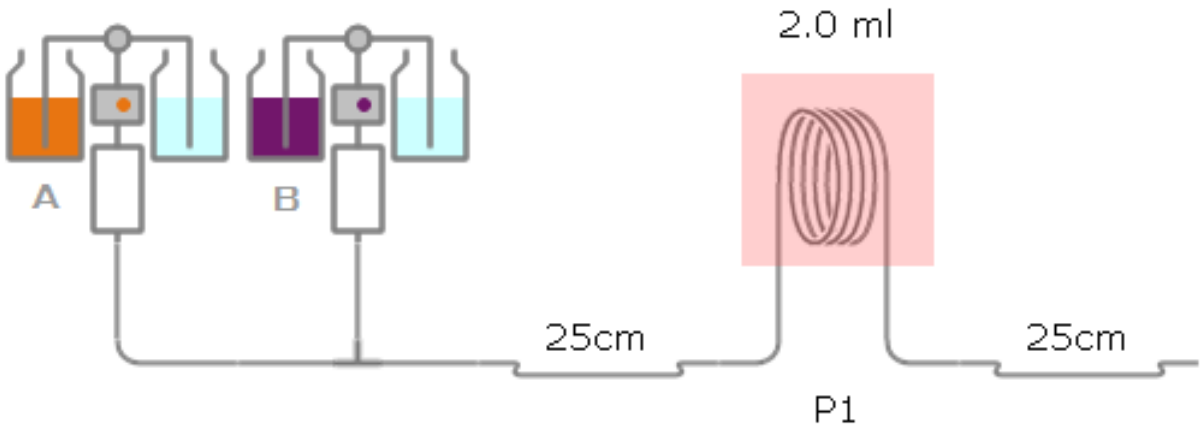


Figure 51 Equipment setting for step 1

In Figure 51 above container A was the container for benzene-1,2-diamine (6), at different concentrations in DCM (0.2 M - 0.6 M), and container B was the container for di-tert-butyl dicarbonate, at different concentrations in DCM (0.2 M - 0.6 M). In Figure 52 below was show the flow reactor during experiment 3 in Table 22 below.

Figure 52 Flow reactor during the optimization

The data from this first DoE are reported in Table 22 below, including the output of the confirmation run, "trial", that shows the optimized conditions.

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
Run	Benzene-1,2-diamine (6)	BOC_2O	Eq BOC_2O	Residence time	tert-butyl (2-aminophenyl) carbamate (19)	Benzene-1,2-diamine (6)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
	mol/L	mol/L	eq	minutes	% mol/mol	% mol/mol	% mol/mol
1	0.2	0.2	0.95	5	63.45	32.5	4.05
2*	0.4	0.4	0.975	12.5	70.93	19.81	9.26
3	0.6	0.6	0.95	5	66.7	26.22	7.08
4	0.2	0.2	1	20	72.6	16.58	10.82
5	0.2	0.6	1	5	64.04	29.34	6.62
6	0.6	0.2	1	5	77.07	7.82	15.11
7	0.2	0.6	0.95	20	64.24	30.35	5.42
~~8*~~	~~0.4~~	~~0.4~~	~~0.975~~	~~12.5~~	~~64.96~~	~~29.25~~	~~5.79~~
9	0.6	0.6	1	20	72.66	17.96	9.38
10	0.6	0.2	0.95	20	73.74	12.92	13.34
11	0.2	0.2	0.95	5	56.17	27.52	16.31
12*	0.4	0.4	0.975	12.5	74.15	16.59	9.26
13*	0.4	0.4	0.975	12.5	76.22	15.67	8.11
	0.6	0.2	1	5	76.73	12.94	10.33
Trial	0.6	0.2	1	5	79.15	6.33	14.52
	0.6	0.2	1	5	68.15	11.10	20.74

Table 22 Experimental data for the first step

A sample NMR for run 1 (in Table 22) is reported below:

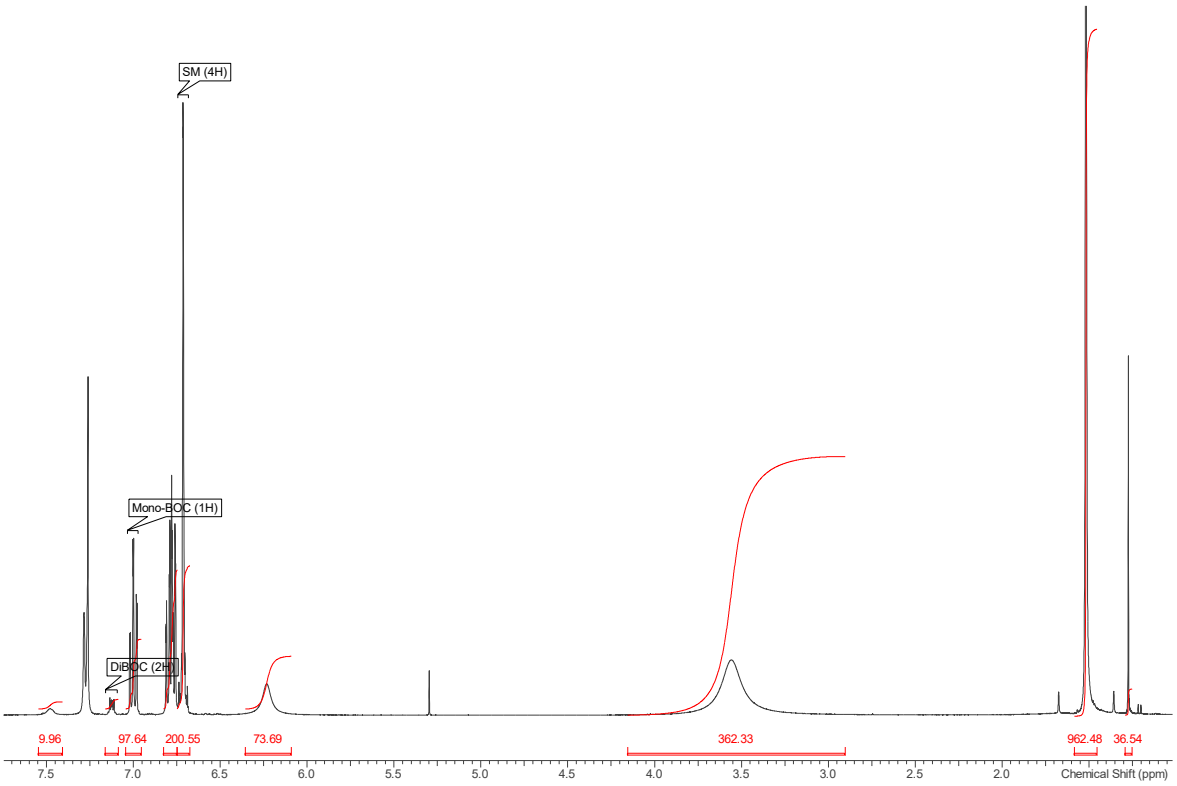


Figure 53 Sample NMR for run 1(in Table 22)

Enlarged aromatic area:

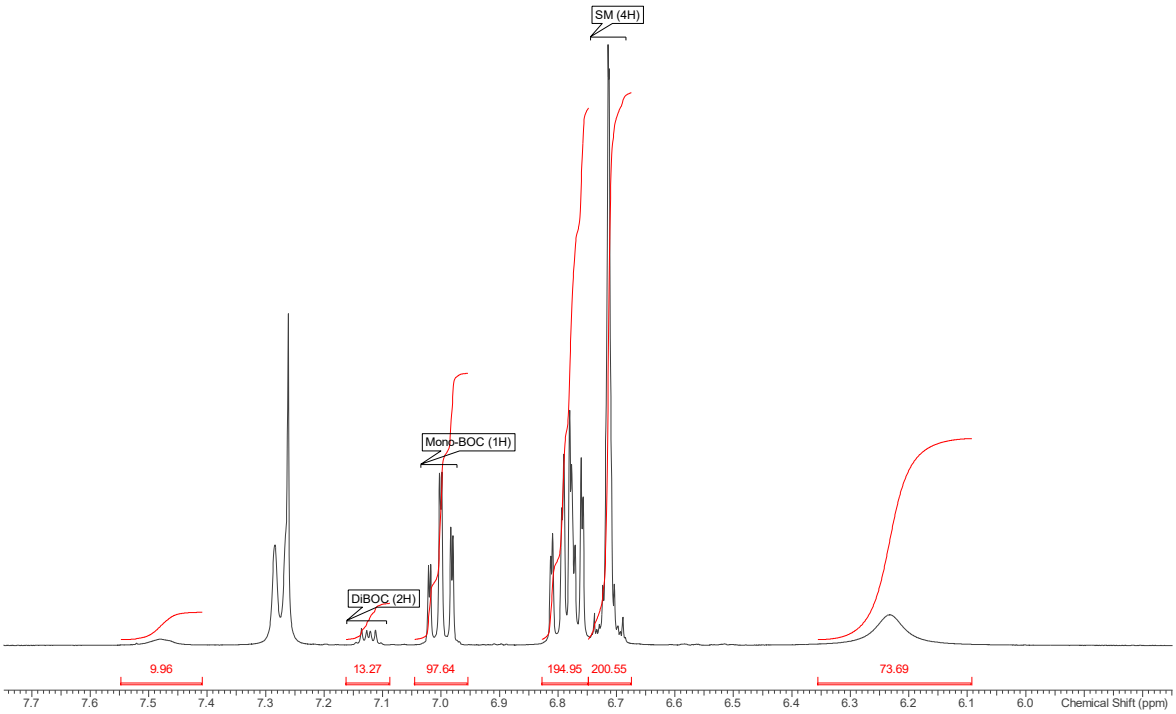


Figure 54 Sample NMR for run 1 (in Table 22) (aromatic part)

The calculated conversion for NMR reported in Figure 53 and Figure 54 was reported in Table 22 above at entry 1 and the equation used was reported below.

$$Intermediate\ 1\ (21) = \frac{\left(\frac{Intermediate\ 1\ (21)\ signal}{integrated\ proton}\right)}{\Sigma\left(\frac{products\ signal}{integated\ proton}\right)} = \%\frac{mol}{mol}$$

To perform the protection and the subsequent cyclization step it was necessary to change the equipment setup, introducing a second reactor in which the reaction mixture reacts with the CDI. The new equipment setting is reported in Figure 55 below:

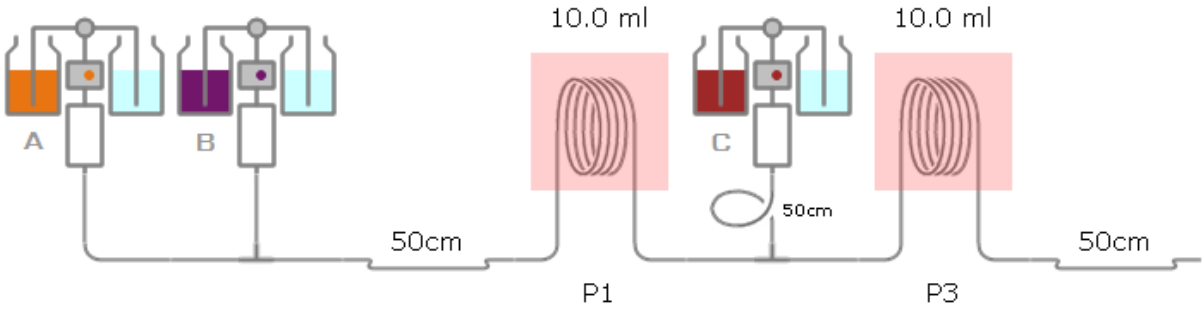


Figure 55 Equipment setting for steps 1-2 in sequence

In the Figure 55 above container A was the container for benzene-1,2-diamine (6), at different concentrations in THF (0.2 M - 0.6 M), container B was the container for di-tert-butyl dicarbonate, at different concentrations in THF (0.2 M - 0.4 M), and container C was the container for CDI at 0.25 M in THF.

The data from this factorial DoE for the protection and cyclization in sequence are reported in Table 23 below. Confirmation runs are not reported as the presence of curvature generated an expansion of the design.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Benzene-1,2-diamine (6)	BOC_2O	Eq BOC_2O	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
	mol/L	mol/L	eq	% mol/mol	% mol/mol	% mol/mol
1	0.4	0.4	2	61.4921	9.73266	28.7752
~~2~~	~~0.5~~	~~0.3~~	~~1.5~~	~~49.8293~~	~~39.7738~~	~~10.3969~~
3	0.6	0.2	2	51.1633	14.7031	34.1336
4	0.4	0.4	1	48.3156	42.7702	8.91423
5	0.6	0.4	1	49.8293	39.7738	10.3969
6	0.4	0.2	1	44.2126	43.1338	12.6536
7	0.6	0.4	2	64.8593	7.15722	27.9835
8	0.5	0.3	1.5	57.7259	23.2592	19.0149
9	0.6	0.2	1	43.3877	44.2511	12.3612
10	0.4	0.2	2	51.0146	13.2753	35.7102
11	0.5	0.3	1.5	56.9509	23.4467	19.6025

Table 23 Experimental data for steps 1-2 in sequence

A sample NMR for run 7 (in Table 23) is reported below:

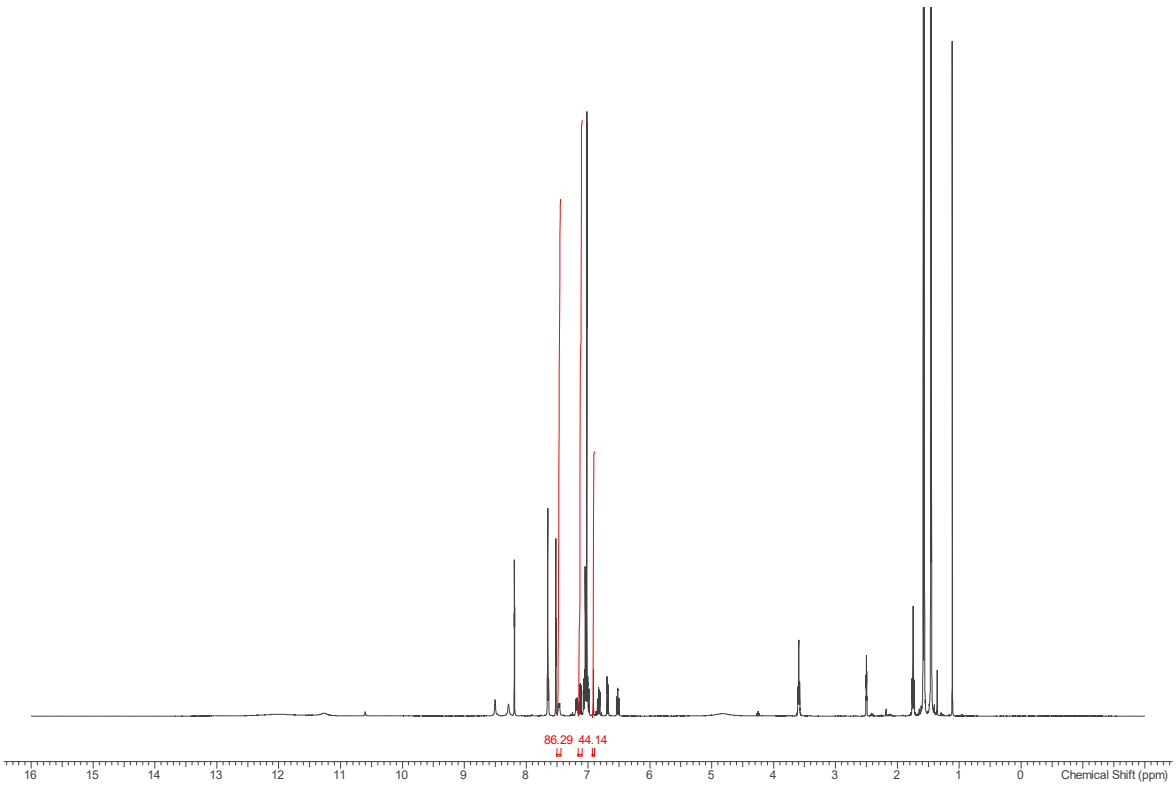


Figure 56 Sample NMR for run 7 (in Table 23)

Enlarged aromatic area:

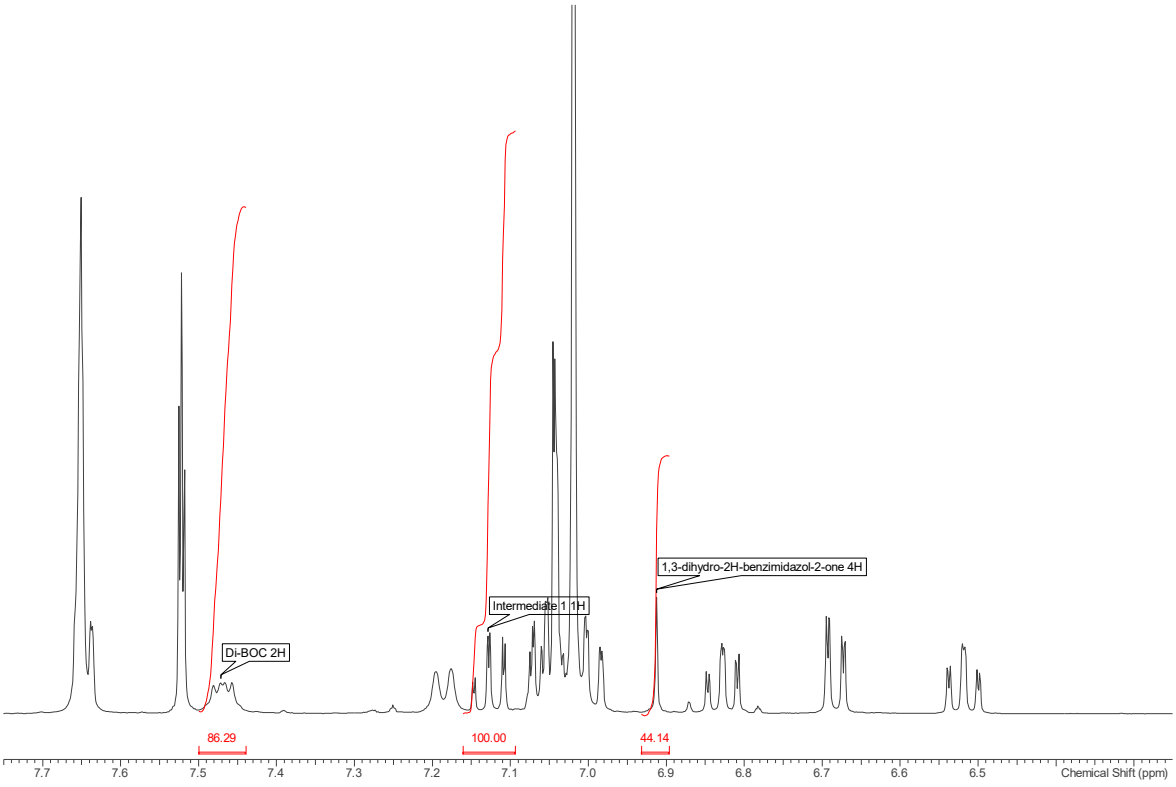


Figure 57 Sample NMR for run 7 (in Table 23) (aromatic part)

The expansion resulted in the creation of blocks to distinguish the two sets of experiments, as reported in Table 24 below, where the first block includes the data already reported in Table 23 above and the second block includes the new experiments that expand the design in a central composite.

Block		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Run	Benzene-1,2-diamine (6)	BOC_2O	Eq BOC_2O	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
		mol/L	mol/L	eq	% mol/mol	% mol/mol	% mol/mol
1	1	0.6	0.4	1	49.8293	39.7738	10.3969
	2	0.6	0.2	2	51.1633	14.7031	34.1336
	3	0.4	0.2	1	44.2126	43.1338	12.6536
	4	0.5	0.3	1.5	57.7259	23.2592	19.0149
	5	0.4	0.4	2	61.4921	9.73266	28.7752
	6	0.4	0.2	2	51.0146	13.2753	35.7102
	7	0.4	0.4	1	48.3156	42.7702	8.91423
	8	0.6	0.4	2	64.8593	7.15722	27.9835
	9	0.6	0.2	1	43.3877	44.2511	12.3612
	10	0.5	0.3	1.5	56.9509	23.4467	19.6025
	11	0.5	0.3	1.5	53.2524	25.5478	21.1998
2	12	0.5	0.3	1.5	54.6172	25.2864	20.0964
	13	0.5	0.47	1.5	57.8219	21.4201	20.758
	14	0.5	0.3	0.66	29.0461	66.9963	3.95753
	~~15~~	~~0.5~~	~~0.13~~	~~1.5~~	~~43.9884~~	~~34.7828~~	~~54.6172~~
	16	0.67	0.3	1.5	57.7134	17.8392	24.4474
	17	0.33	0.3	1.5	51.4986	24.7708	23.7306
	18	0.5	0.3	1.5	52.1037	27.4091	20.4872
	19	0.5	0.3	2.34	51.7183	6.3717	41.91
	20	0.5	0.3	1.5	54.019	25.4024	20.5785
Trial	A	0.5	0.4	2	56.65	9	34.34
		0.5	0.4	2	53.89	9.68	36.43
		0.5	0.4	2	56.76	7.89	35.35
	B	0.6	0.4	3	41.88	1.92	56.19
		0.6	0.4	3	39.77	2.06	58.17
		0.6	0.4	3	38.83	1.77	59.4

Table 24 Experimental data for steps 1-2 in sequence with central composite expansion

The sample NMRs are similar to those reported for the factorial DoE.

6.5.2 Synthesis via Di-tert-butyl benzene-1,2-diylbiscarbamate (20) direct cyclization.

The initial optimization was performed using a Biotage initiator using a solution 0.4 M of Di-tert-butyl benzene-1,2-diylbiscarbamate (20) in THF (4 ml) which was then added with the bases accordingly with Table 25 below; the first set of experiments was focused on the investigation of the effect of the base and the condition range.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Temperature	Time	Base	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
	degC	min	#	% mol/mol	% mol/mol	% mol/mol
1	100	10	DMAP	3.92	96.08	0
2	150	10	TEA	0	100	0
3	150	1	DIPEA	0	100	0
4	124	7.2	TEA	0	100	0
5	150	1	DIPEA	0	100	0
6	100	10	DIPEA	0	100	0
7	133	1	DMAP	5.78	94.22	0
8	100	10	TEA	0	100	0
9	100	1	DIPEA	0	100	0
10	125	4.1	DIPEA	0	100	0
11	106	4.2833	DMAP	3.12	96.88	0
12	150	4.95	DMAP	36.2	60.3847	3.41529
13	115	1	TEA	0	100	0
14	141	3.066	TEA	0	100	0
15	150	10	DIPEA	0	100	0
16	150	10	DIPEA	0	100	0
17	100	10	TEA	0	100	0
18	150	10	DMAP	52.9442	40.4273	6.62845
19	100	1	DIPEA	0	100	0
20	100	10	DIPEA	0	100	0

Table 25 Response surface D-optimal for the initial investigation

The output of this design was that only DMAP was able to perform the cyclization and that the conditions applied were too weak in terms of time and temperature.

The new full factorial design is reported in Table 26 below also in this case a solution 0.4 M of Di-tert-butyl benzene-1,2-diylbiscarbamate (20) in THF (4 ml) added with DMAP was used for the investigation.

Block		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Run	Eq DMAP	Temperature	Time	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
		eq	degC	min	% mol/mol	% mol/mol	% mol/mol
1	1	1	130	1	5.25981	94.6844	0.055754
	2	2	180	1	67.974	21.2419	10.7841
	3	2	130	1	7.08102	92.7738	0.145161
	4	1	130	20	33.4885	64.9359	1.57563
	5	2	180	20	9.3085	0	90.6915
	6	2	130	20	58.2776	37.5395	4.18288
	7	1.5	155	10.5	70.4101	16.1943	13.3955
	8	1	180	20	14.7676	0	85.2324
	9	1.5	155	10.5	69.6682	17.6435	12.6883
	10	1	180	1	46.1979	49.5311	4.271
2	11	1	180	20	14.6891	0	85.3109
	12	1.5	155	10.5	70.3173	17.5723	12.1104
	13	2	130	20	51.7679	44.3521	3.88
	14	2	180	20	10.7651	0	89.2349
	15	1.5	155	10.5	70.6839	16.275	13.0412
	16	2	180	1	65.6362	25.0435	9.32034
	17	2	130	1	6.89898	92.9168	0.184203
	18	1	130	20	34.9092	63.4405	1.65033
	19	1	180	1	46.8033	48.7504	4.44632
	20	1	130	1	3.01225	96.942	0.0457862

Table 26 Full factorial design for the cyclization performed in MW

A sample NMR for run 7 (in Table 26) is reported below:

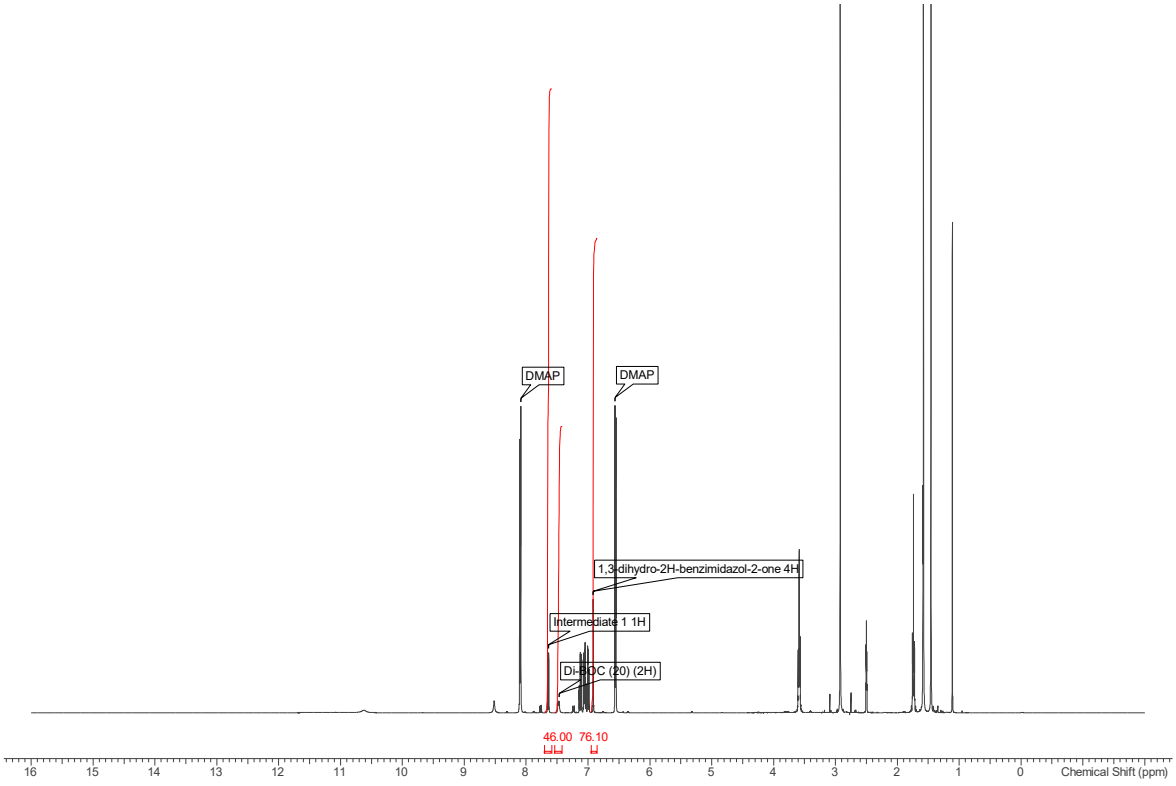


Figure 58 Sample NMR for run 7 (in Table 26)

Enlarged aromatic area:

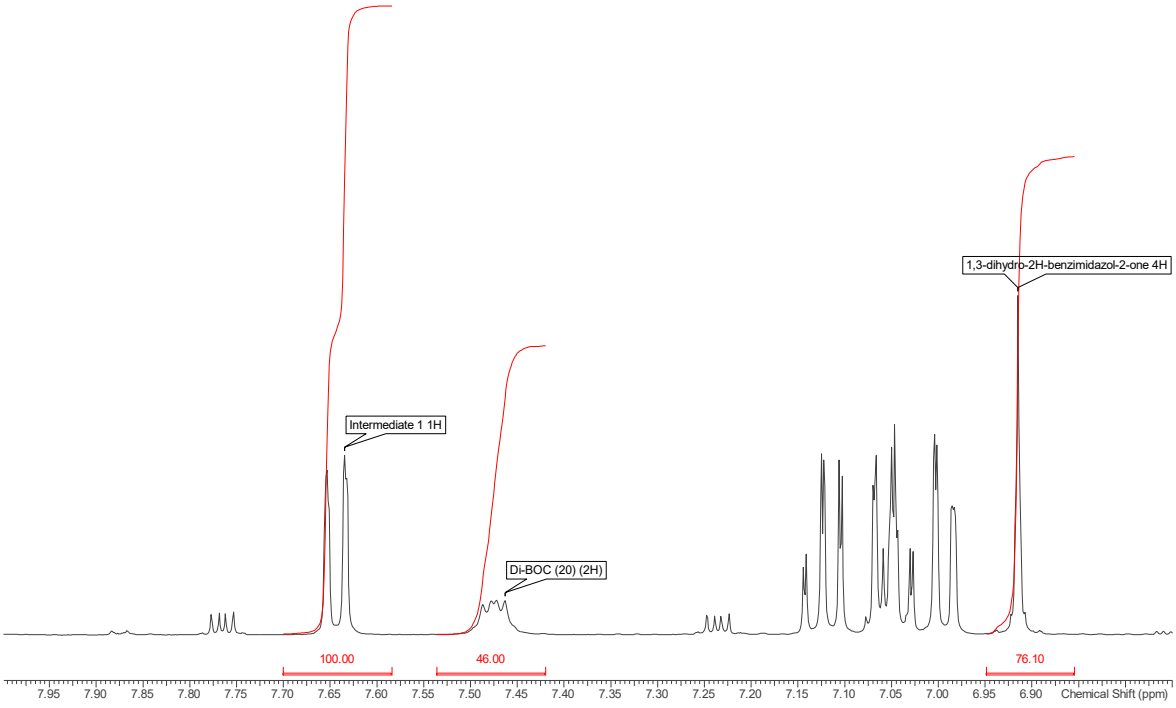


Figure 59 Sample NMR for run 7 (in Table 26) (aromatic part)

The creation of a new design as central composite focused more around the central point of the factorial design is reported in Table 27 below; also in this case a solution 0.4 M of Di-tert-butyl benzene-1,2-diylbiscarbamate (20) in THF (4 ml) added with DMAP was used for the investigation.

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Run	Time	Temperature	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
	min	degC	% mol/mol	% mol/mol	% mol/mol
1	10.5	169	58.4257	1.09548	40.4788
2	10.5	155	70.2186	16.6102	13.1712
3	15	165	57.6319	0.639714	41.7284
4	6	165	70.8604	13.5733	15.5663
5	6	145	42.7931	55.1389	2.06798
6	10.5	155	70.3247	16.5193	13.156
7	10.5	155	70.7764	16.0733	13.1503
8	15	145	65.0629	26.383	8.55414
9	10.5	155	70.1336	17.1266	12.7398
10	16.866	155	71.7746	6.55302	21.6723
11	10.5	141	49.922	46.9317	3.14633
12	10.5	155	70.4796	16.2068	13.3136
13	4.133	155	54.5598	41.8065	3.63369
Trial	15	151	72.6098	12.2819	15.1083
	15	151	71.1959	14.6201	14.184
	15	151	71.6409	14.9479	13.4112

Table 27 Central composite design and confirmation run in MW

A sample NMR is not reported as it is similar to that reported for the factorial DoE.

The flow optimization was performed directly with a central composite design. The scheme of the equipment setting is reported in Figure 60 below; the setting includes also the high temperature reactor due to the temperature that could be above 150°C. Based on what were learned before the container A was filled with solution 0.4 M of Di-tert-butyl benzene-1,2-diylbiscarbamate (20) in THF added with 1.5 eq of DMAP.

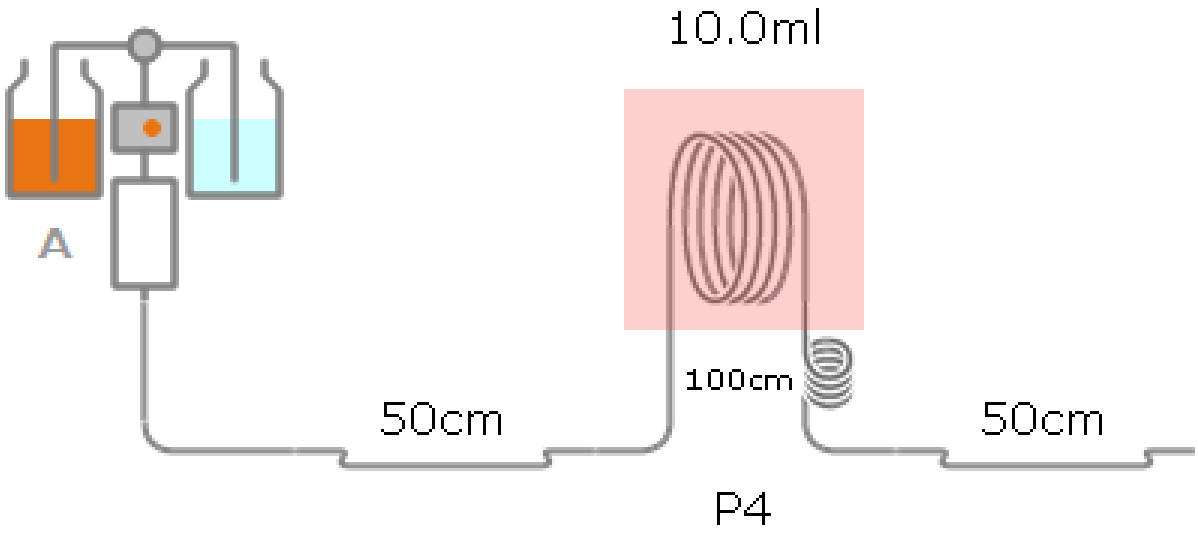


Figure 60 Equipment setting for cyclization

Data for this optimization are reported in Table 28 below and include the confirmation runs:

	Factor 1	Factor 2	Response 1	Response 2	Response 3
Run	Time	Temperature	Intermediate 1 (22)	1,3-dihydro-2H-benzimidazol-2-one (21)	Di-tert-butyl benzene-1,2-diylbiscarbamate (20)
	min	degC	% mol/mol	% mol/mol	% mol/mol
1	10	146	40.0056	55.9718	4.02256
2	10	174	70.4039	4.81915	24.7769
3	5	170	62.6105	26.5124	10.877
4	5	150	28.8659	68.3342	2.79999
5	10	160	66.0982	23.9937	9.90812
6	17	160	73.6838	9.93258	16.3836
7	10	160	64.4444	26.8798	8.67583
8	10	160	64.3677	26.8574	8.77492
9	3	160	28.5351	69.8626	1.60225
10	10	160	64.726	26.8645	8.40952
11	15	170	66.9748	2.76271	30.2625
12	15	150	60.3309	32.82	6.84907
13	10	160	64.727	26.8293	8.44364
Trial	13.5	160	70.7401	16.6133	12.6466
	13.5	160	70.6976	16.7907	12.5117
	13.5	160	70.8491	16.3414	12.8095

Table 28 Central composite design and confirmation run in flow

A sample NMR is not reported as it is similar to that reported for the MW optimization.

6.6 Synthesis of tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)

The initial MW investigation was performed in duplicate and the design is reported in Table 29 below:

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Run	Temperature	Intermediate 1 (22)	Eq KOH	Time	Eq 1-Bromo-3-chloropropane (23)
	degC	mol/L	eq	min	eq
1	100	1	3	2	1
2	125	0.75	2.25	6	1.5
3	150	0.5	1.5	2	2
4	150	1	1.5	2	1
5	150	0.5	3	10	1
6	100	0.5	1.5	10	2
7	125	0.75	2.25	6	1.5
8	150	0.5	1.5	10	1
9	150	1	3	10	1
10	100	0.5	3	2	2
11	150	1	1.5	2	2
12	100	0.5	1.5	2	1
13	100	1	1.5	2	2
14	100	0.5	3	2	1
15	100	0.5	3	10	2
16	150	1	1.5	10	2
17	100	1	1.5	10	2
18	150	0.5	3	10	2
19	100	0.5	3	10	1
20	150	0.5	3	2	1
21	100	1	3	2	2
22	100	1	1.5	2	1
23	150	0.5	1.5	2	1
24	100	1	3	10	2
25	100	1	1.5	10	1
26	150	1	3	10	2
27	150	1	1.5	10	1
28	150	0.5	1.5	10	2
29	125	0.75	2.25	6	1.5
30	100	1	3	10	1
31	100	0.5	1.5	2	2
32	100	0.5	1.5	10	1
33	150	1	3	2	1
34	150	1	3	2	2
35	150	0.5	3	2	2
36	125	0.75	2.25	6	1.5

Table 29 Full factorial design for alkylation with KOH as base

The MW vial was filled using a Hamilton MicroLab Star (Figure 61); the logic scheme used to fill the vial is reported in Figure 62 and Figure 63 below.

Figure 61 Hamilton MicroLab Star used to prepare the MW vials

Variable	Starting materials in solution of THF (1M)
	THF (for dilution)
	KOH in water (4M)
	KI in water
	mL 1-bromo-3-chloropropane (23)
Key point	Volume more than 1000 mL (high limit for pipettors)
	THF (highly volatile and low responsivity to conductive liquid detection)

Main Program

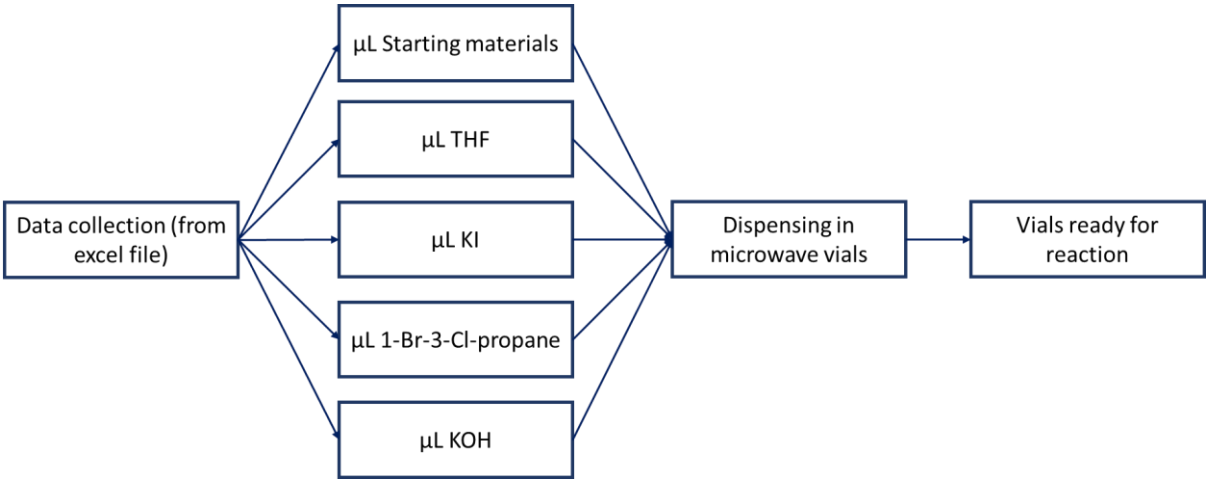


Figure 62 Script for starting material dispensing

The handling of high volumes of liquid was managed via script; the script helped to create a crescent sequence (from 1 to end), with the multiple dispensing after the end of the sequence.

This led to a faster dispensation of liquid.

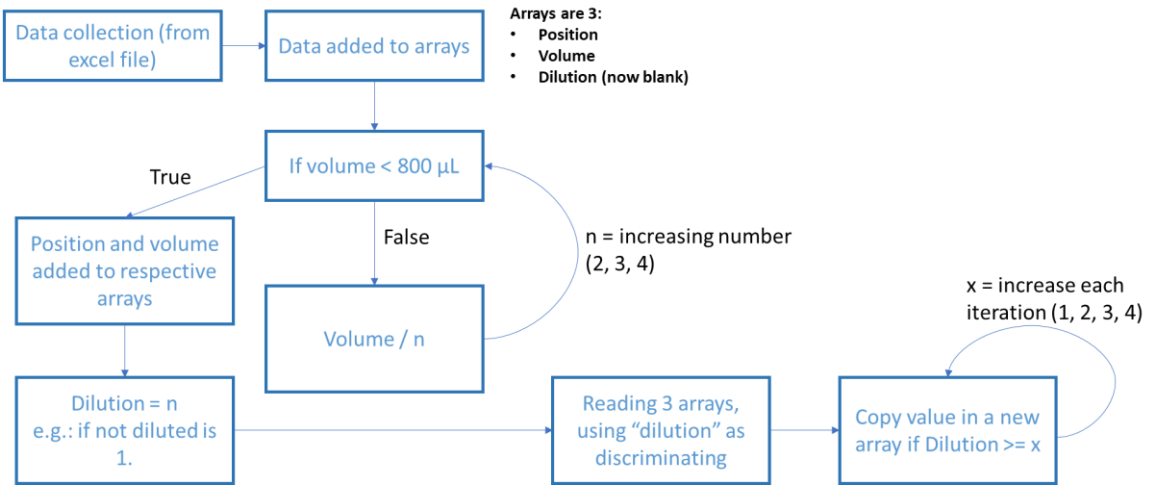


Figure 63 Script for dispensing large amounts of liquid

As THF was not a codified solvent, a liquid class for THF has been prepared.

Liquid class for THF	Liquid class has been prepared ad hoc to reduce the drop from the tips due to gravity.
	The transfer volume has been increased to 30 µL
	A mixing (3 times) before the aspiration helps to reduce the drop
	cLLD has been used (SM in THF is a better conductive respect to pure THF)
	First drop from full tip after 30"

A sample NMR from run 1 is reported below.

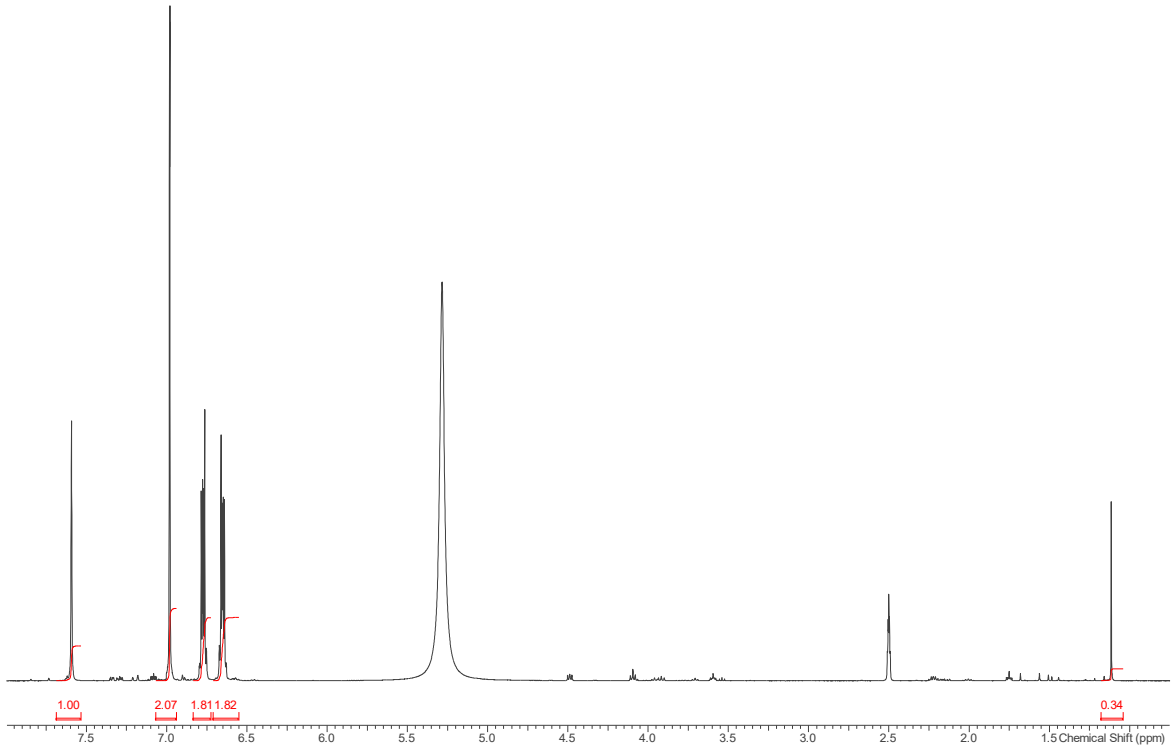


Figure 64 Sample NMR for run 1 (Table 29)

An NMR of the starting material (Intermediate 1 (22)) is reported below.

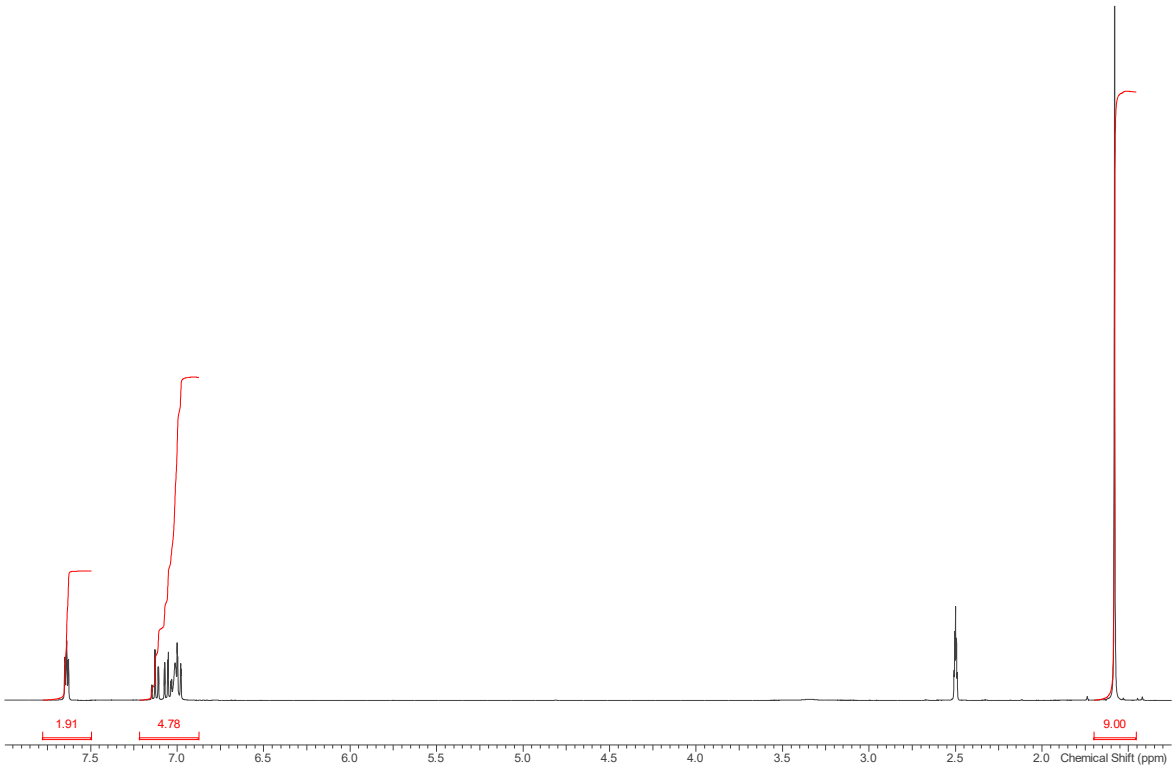


Figure 65 NMR of the starting material (Intermediate 1 (22))

And an NMR sample of the desired product (tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)) prepared in batch is reported below.

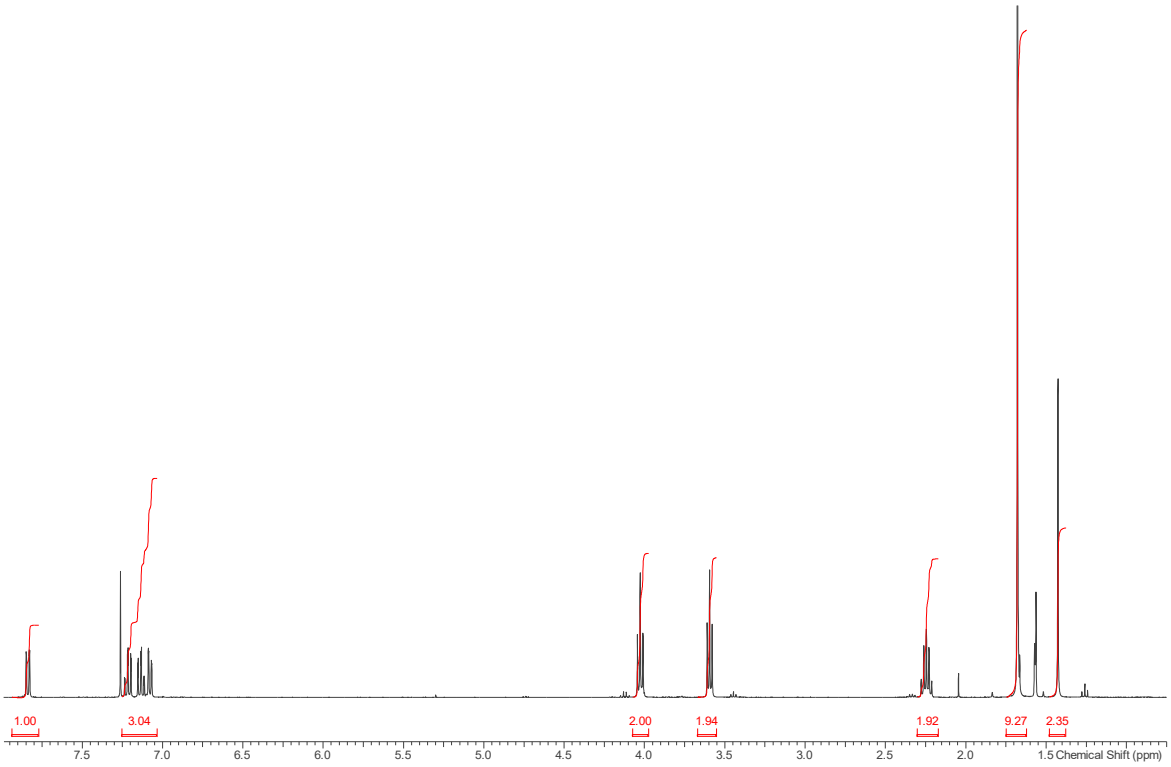


Figure 66 Sample NMR for the desired product (tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (Intermediate 2, 24))

For the selection of the base a quick screening was performed in MW and the UPLC/MS chromatograms are reported below. All the experiments were performed at 100°C for 10 minutes with 1.1 eq of base and 3 eq of 1-bromo-3-chloropropane (23) on a 500 mg of intermediate 1 (22).

Cesium carbonate as base:

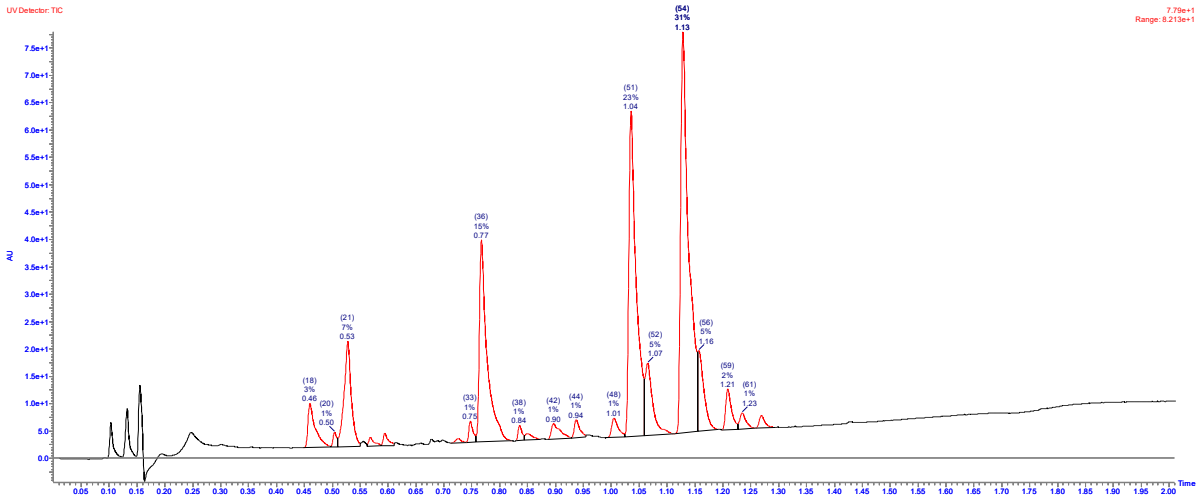


Figure 67 Chromatogram of alkylation reaction using cesium carbonate as base

Potassium carbonate as base:

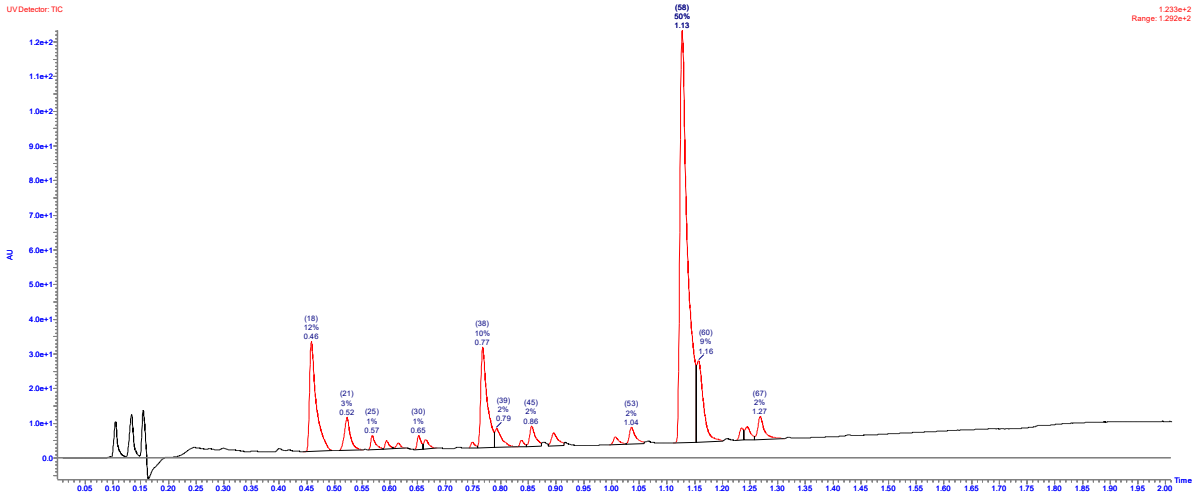


Figure 68 Chromatogram of alkylation reaction using potassium carbonate as base

DIPEA as base:

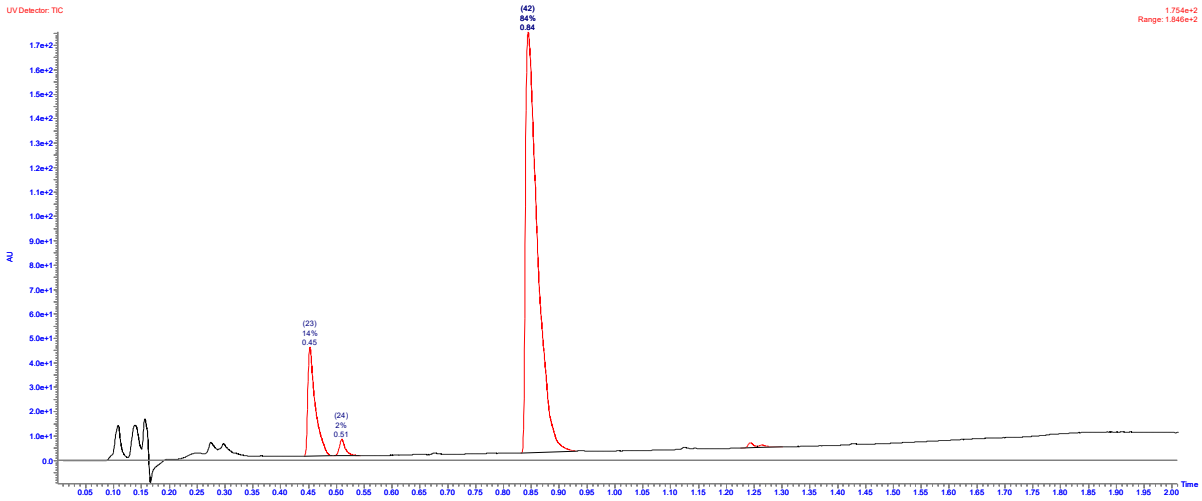


Figure 69 Chromatogram of alkylation reaction using DIPEA as base

DBU as base:

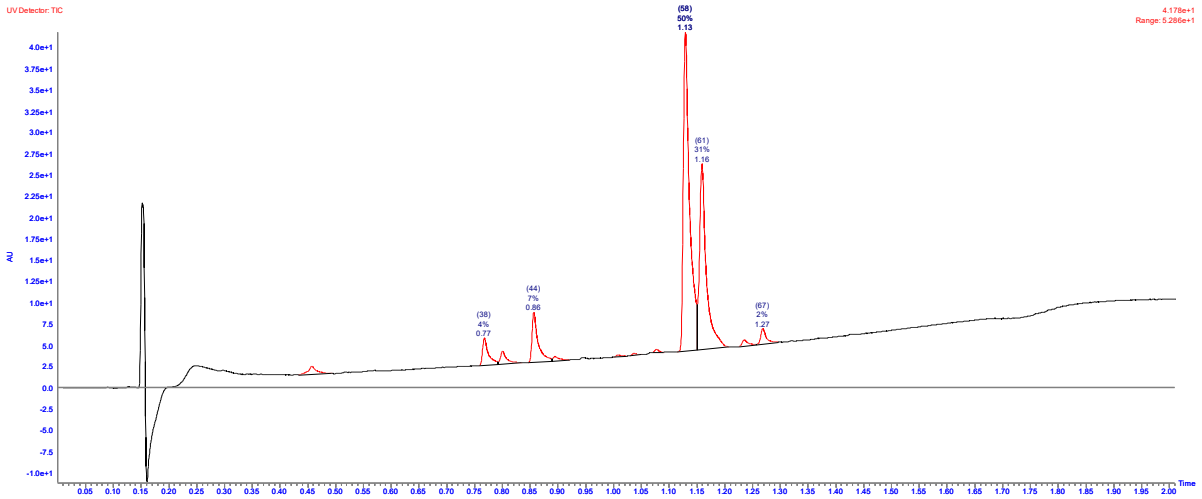
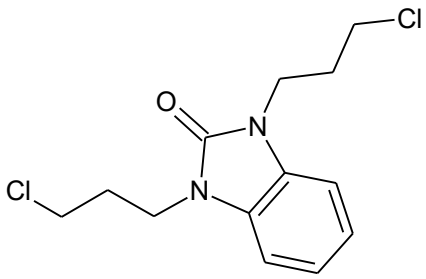


Figure 70 Chromatogram of alkylation reaction using DBU as base

In the chromatograms above the species reported in Table 30 below can be identified.

Retention Time (min)	Observed M/Z	Assignment
0.45	135 (M + H)	1,3-dihydro-2H-benzimidazol-2-one (21)
0.77	211 (M + H)	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)
0.86	257 (M + Na)	*tert*-butyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-1-carboxylate (22)
1.04	287 (M + H)	1,3-bis(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (34)
1.13	255 (M+1-56)	tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)
1.16	377 (M + Na)	tert-butyl 3-(3-bromopropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (26)

Table 30 Mass assignments for the alkylation stage



34

Figure 71 Structure of 1,3-bis(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (34)

Once DBU was chosen as base, the flow optimization was performed starting with a full factorial design. The scheme of the equipment setting is reported in Figure 72 below.

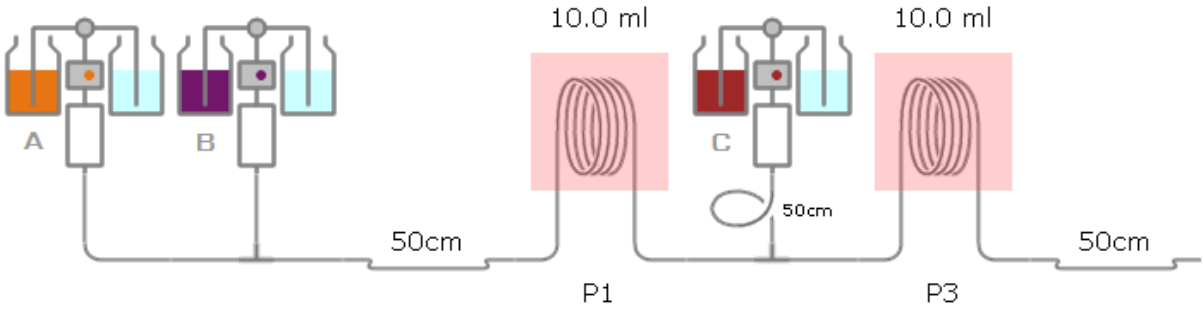


Figure 72 Equipment setting for step 3, full factorial design

In Figure 72 above, container A was the container for Intermediate 1 (22) at 0.5 M in THF, container B was the container for DBU at 0.2 M in THF, and container C was the container for 1-bromo-3-chloropropane (23) at 0.2M in THF.

The data of the full factorial design for the alkylation performed in flow is reported in Table 31 below.

As there are not strong difference on HPLC response between starting material (Intermediate 1 (22)) and product (Intermediate 2 (24)) the conversion analysis will be performed with this technique analysing the percentage area/area (% a/a) of the response.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Response 1	Response 2	Response 3
Run	Intermediate 1 (22)	Eq DBU	Eq 1-Bromo-3-chloropropane (23)	Time	Temperature	Intermediate 2 (24)	Intermediate 2 related compounds (4, 25, 26)	impurity
	mol/L	eq	eq	min	degC	% a/a	% a/a	% a/a
1	0.5	1	1	24	130	7.95	11.25	80.8
~~2~~	~~0.35~~	~~1.5~~	~~2~~	~~15~~	~~100~~	~~28.33~~	~~7.44~~	~~64.23~~
3	0.5	1	1	6	70	12.83	19.57	67.6
4	0.5	2	3	6	70	41.56	16.53	41.91
5	0.5	1	3	6	130	10.26	67.43	22.31
6	0.2	2	3	24	70	39.61	14.73	45.66
7	0.35	1.5	2	15	100	42.75	11.81	45.44
8	0.5	2	1	24	70	6.57	12.43	81
9	0.2	2	3	6	130	26.2	12.48	61.32
10	0.2	2	1	6	70	3.85	11.82	84.33
11	0.5	2	1	6	130	4.55	16.11	79.34
12	0.35	1.5	2	15	100	40.26	12.01	47.73
13	0.5	2	3	24	130	13.94	3.24	82.82
14	0.2	2	1	24	130	1.41	3.13	95.46
15	0.2	1	1	24	70	13.16	21.76	65.08
16	0.2	1	1	6	130	1.07	3.79	95.14
17	0.2	1	3	24	130	6.82	3.2	89.98
18	0.2	1	3	6	70	12.26	20.15	67.59
19	0.5	1	3	24	70	31.26	4.2	64.54

Table 31 Full factorial design for alkylation with DBU as base

The central composite design generated subsequently is reported in Table 32 below. As the design was not an expansion of the previous one, also the reagents concentrations were changed maintaining the scheme of the equipment similar to that reported in Figure 73 below, where container A was the container for Intermediate 1 (22) at 0.5 M in THF, container B was the container for DBU at 0.5 M in THF, and container C was the container for 1-bromo-3-chloropropane (23) at 0.75 M in THF.

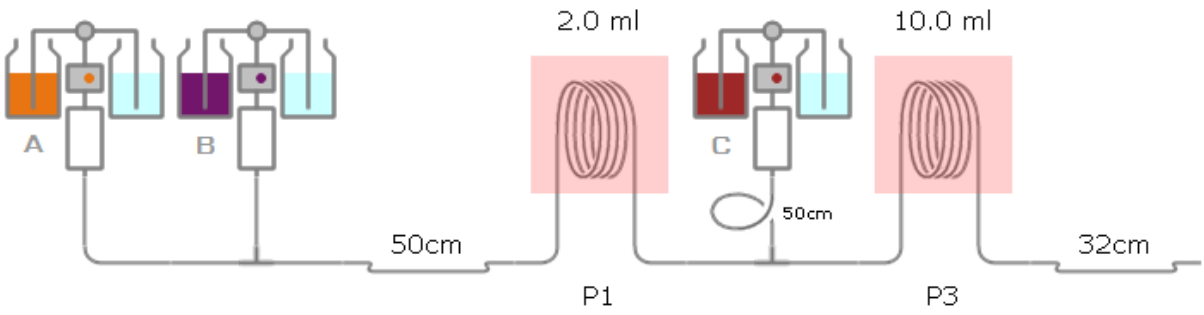


Figure 73 Equipment setting for step 3, central composite design

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3	Response 4
Run	Temperature	Eq DBU	Eq 1-Bromo-3-chloropropane (23)	Time	Intermediate 2 (24)	Intermediate 2 related compounds (4, 25, 26)	impurity	Intermediate 1 (22)
	degC	eq	eq	min	% a/a	% a/a	% a/a	% a/a
1	100	2.5	2	15	49.54	17.66	25.48	7.32
2	75	2	5	10	81.67	7.44	3.24	7.65
3	75	2	3	20	63.19	13.93	15.9	6.98
4	100	2.5	4	15	54.77	6.07	33.04	6.12
5	75	2	3	0.1	56.75	13.64	27.17	2.44
6	50	1.5	4	5	70.65	13.54	9.2	6.61
7	75	3	3	10	58.15	17.1	21.08	3.67
8	50	2.5	2	15	63.54	10.03	22.3	4.13
9	50	2.5	4	15	62.87	10.03	22.85	4.25
10	100	2.5	2	5	30.44	3.13	66.43	0
11	25	2	3	10	54	15.84	25.63	4.53
12	100	1.5	4	5	63.88	8.13	21.01	6.98
13	50	2.5	2	5	42.55	13.43	40.48	3.54
14	75	2	3	10	64.24	18.7	9.52	7.54
15	75	2	1	10	9.95	45.58	33.29	11.18
~~16~~	~~125~~	~~2~~	~~3~~	~~10~~	~~6.83~~	~~0.72~~	~~92.45~~	~~0~~
17	75	2	3	10	63.63	17.93	11.57	6.87
18	50	1.5	4	15	61.3	22.84	10.16	5.7
19	100	1.5	4	15	52.2	0.86	25.01	21.93
20	50	1.5	2	5	44.86	21.46	30.26	3.42
21	100	2.5	4	5	55.64	5.44	34.33	4.59
22	100	1.5	2	15	66.39	11.03	7.75	14.83
23	75	2	3	10	39.63	19.28	40.44	0.65
24	75	1	3	10	59.63	5.24	4.79	30.34
25	75	2	3	10	51.37	20.46	24.24	3.93
26	75	2	3	10	57.18	14.3	23.31	5.21
27	50	2.5	4	5	50.24	18.25	29.62	1.89
28	75	2	3	10	58.12	16.68	19.85	5.35
29	100	1.5	2	5	61.12	19.62	12.51	6.75
30	50	1.5	2	15	39.58	25.91	27.98	6.53

Table 32 Central composite design for alkylation with DBU as base

A sample HPLC for run 24 (in Table 32) is reported in Figure 74 below:

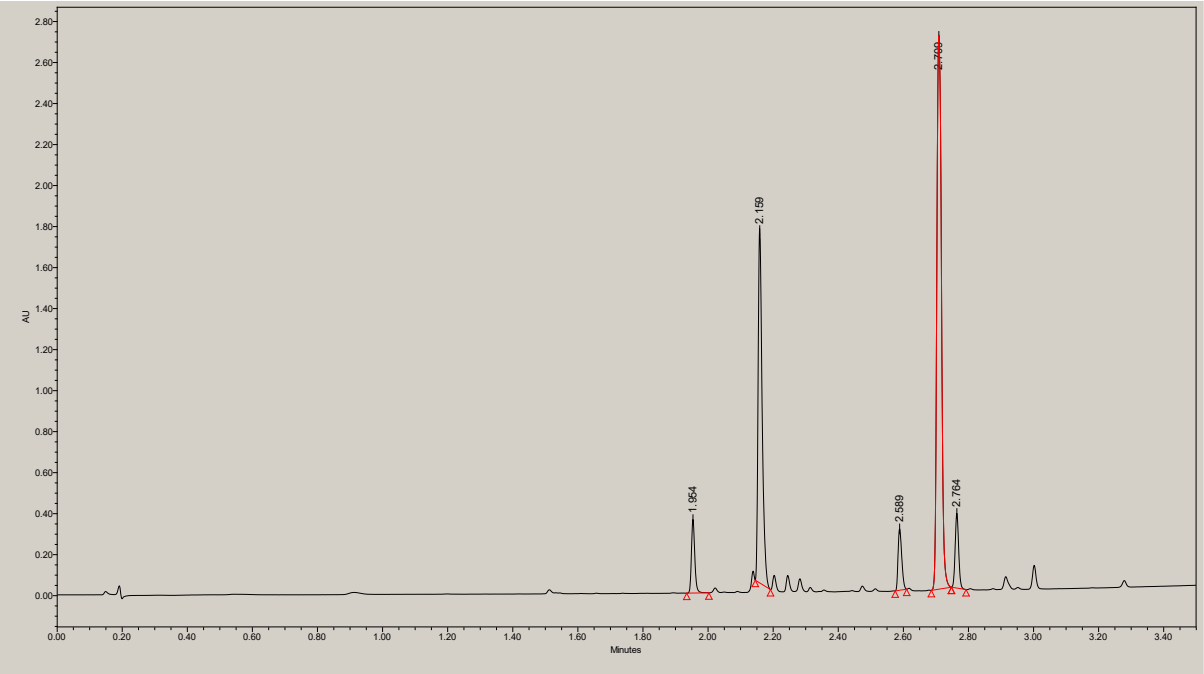


Figure 74 Sample chromatogram for run 24 (in Table 32)

Retention Time (min)	% Area	Assignment
1.954	5.24	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)
2.159	30.34	*tert*-butyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-1-carboxylate (22)
2.589	4.79	Unknown Impurity
2.709	54.12	tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)
2.764	5.51	tert-butyl 3-(3-bromopropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (25)

Table 33 Retention time assignments for the alkylation stage

6.7 Synthesis of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one

The flow optimization was performed starting with a full factorial design. The scheme of the equipment setting is reported in Figure 75 below, where container A was the container for Intermediate 2 (24) at 0.5 M in DMSO and container B was the container for DMSO to change the concentration. The setting also includes the high temperature reactor due to the temperature that could be above 150°C.

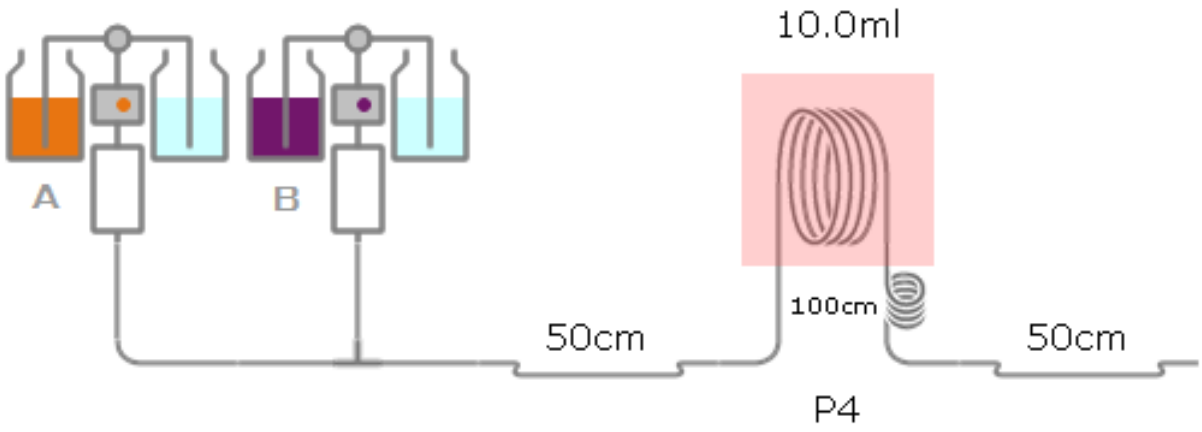


Figure 75 Equipment setting for step 4

The data of the full factorial design for the thermal deprotection performed in flow is reported in Table 34 below.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Time	Temperature	Intermediate 2 (24)	Intermediate 3 (4)	Impurity	Intermediate 2 (24)
	min	degC	mol/L	% a/a	% a/a	% a/a
1	12.5	150	0.3	64.11	0	35.89
2	5	120	0.5	3.02	0	96.98
3	20	180	0.5	88.74	10.71	0.55
4	12.5	150	0.3	70.73	0	29.27
5	20	120	0.1	14.38	0	85.62
6	5	120	0.1	2.82	0	97.18
7	20	120	0.5	15.08	0	84.92
8	12.5	150	0.3	69.04	0	30.96
9	12.5	150	0.3	64.08	0	35.92
10	20	180	0.1	67.5	32.5	0
11	5	180	0.1	100	0	0
12	5	180	0.5	89.04	0	10.96

Table 34 Full factorial design for thermal deprotection

A sample HPLC for run 7 (in Table 34) is reported in Figure 76 below:

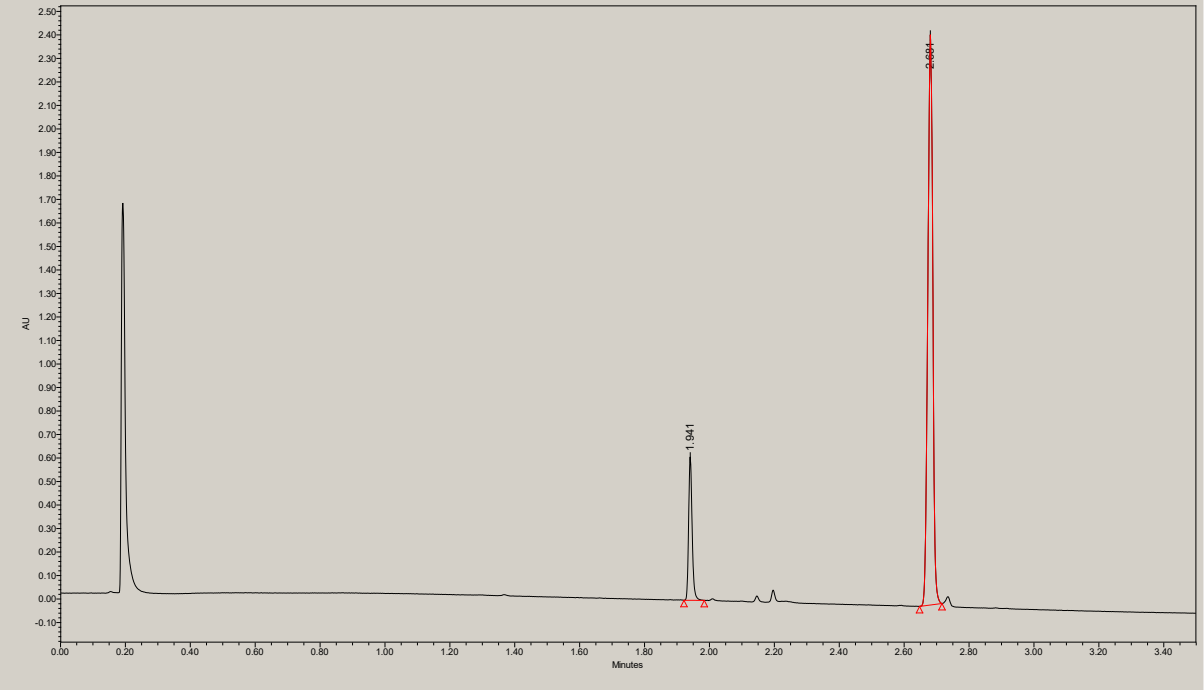


Figure 76 Sample chromatogram for run 7 (in Table 34)

Retention Time (min)	% Area	Assignment
1.941	15.08	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)
2.681	54.12	tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24)

Table 35 Retention time assignments for the deprotection stage

The expansion resulted in the creation of blocks to distinguish the two sets of experiments, as reported in Table 36 below, where the first block includes the data already reported in Table 34 above and the second block the new experiments that expand the design in a central composite.

Block		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Run	Time	Temperature	Intermediate 2 (24)	Intermediate 3 (4)	Impurity	Intermediate 2 (24)
		min	degC	mol/L	% a/a	% a/a	% a/a
DOE	1	12.5	150	0.3	64.11	0	35.89
	2	5	120	0.5	3.02	0	96.98
	3	20	180	0.5	88.74	10.71	0.55
	4	12.5	150	0.3	70.73	0	29.27
	5	20	120	0.1	14.38	0	85.62
	6	5	120	0.1	2.82	0	97.18
	7	20	120	0.5	15.08	0	84.92
	8	12.5	150	0.3	69.04	0	30.96
	9	12.5	150	0.3	64.08	0	35.92
	10	20	180	0.1	67.5	32.5	0
	11	5	180	0.1	100	0	0
	12	5	180	0.5	89.04	0	10.96
Expansion	13	12.5	150	0.56	65.35	1.39	33.25
	14	22.4	150	0.3	76.91	0	23.09
	15	12.5	150	0.0367918	84.22	0	15.78
	16	12.5	189	0.3	68.45	31.55	0
	17	12.5	150	0.3	80.75	7.79	11.31
	18	12.5	150	0.3	80.54	8.36	11.1
	19	12.5	111	0.3	5.33	4.53	90.14
	20	2.63	150	0.3	17.29	3.53	79.12
Trial		5	180	0.1	100	0	0
		5	180	0.1	100	0	0
		5	180	0.1	100	0	0

Table 36 Central composite design for thermal deprotection of BOC

The sample HPLC was similar to that reported in the initial optimization.

6.8 Synthesis of 5-cloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one (AKA Domperidone (1))

For the selection of the base a quick screening was performed in MW and the UPLC/MS chromatograms are reported below. All the experiments were performed at 160°C for 15 minutes with 1.5 eq of base and 1 eq of the two starting materials (210 mg of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) and 252 mg 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5) in 4 ml of THF).

DIPEA as Base:

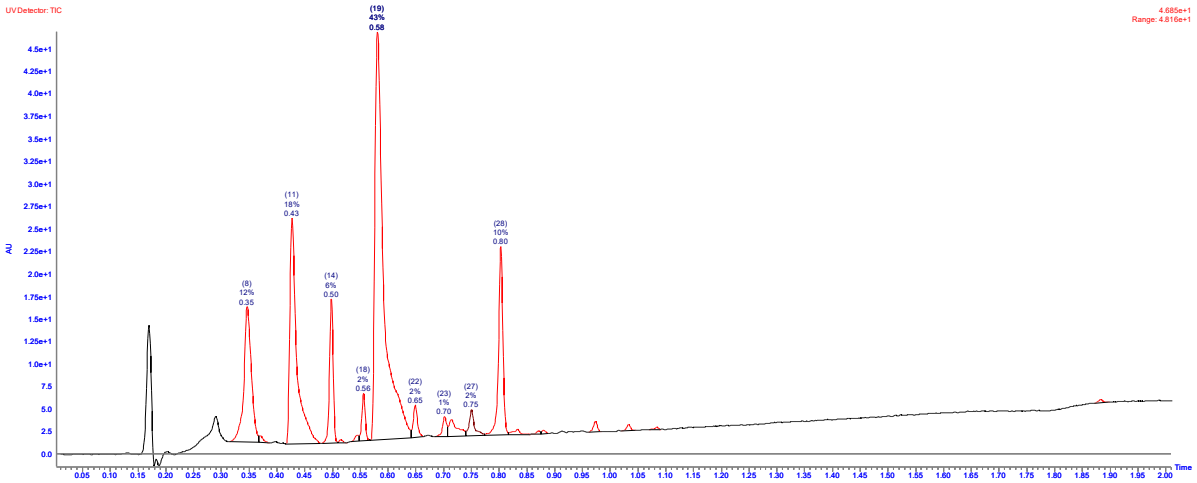


Figure 77 Chromatogram of alkylation using DIPEA as base

DBU as base:

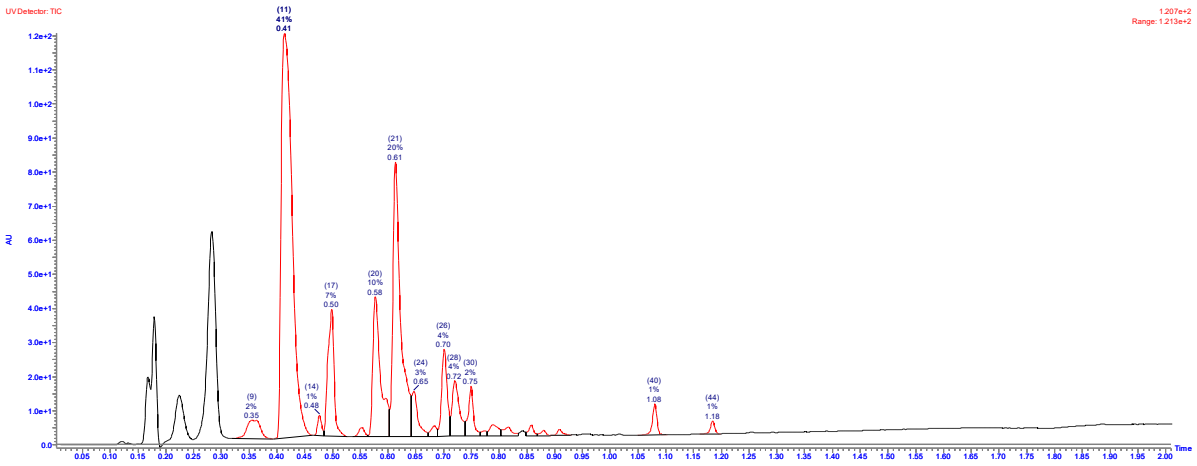


Figure 78 Chromatogram of alkylation using DBU as base

Retention Time (min)	M/Z	Assignment
0.35	126 (M-1)	Iodide
0.41-0.43	252 (M+1)	5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5)
0.50	327 (M+1)	Unknown Impurity
0.58	426 (M+1)	Domperidone (1)
0.61	426 (M+1)	5-chloro-3-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one (27)
0.70-0.72	600 (M+1)	5-chloro-3-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one (26)
0.80	211 (M+1)	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)

Table 37 Mass assignments for the final alkylation

Once DIPEA was chosen as base, the flow optimization was performed starting with a full factorial design. The scheme of the equipment setting is reported in Figure 79 below, where container A was the container for Compound D (5) at 0.1 M in DMSO, container B was the container for DIPEA 0.1 M in DMSO, and container C was the container for Intermediate 3 (4) at 0.1 M in DMSO with 10% mol of KI as catalyst. The setting includes a 2 ml reactor to properly mix compound D (5) with the base, and also the high temperature reactor due to the temperature that could be above 150°C.

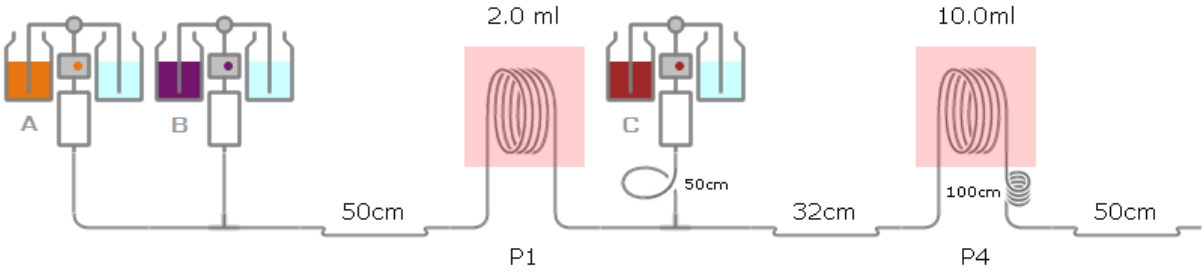


Figure 79 Equipment setting for step 5

The data of the full factorial design for the alkylation performed in flow are reported in Table 38 below.

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
Run	Eq Compound D (5)	Eq DIPEA	Time	Temperature	Compound D (5)	Intermediate 3 (4)	Domeridone (1)
	eq	eq	min	degC	% a/a	% a/a	% a/a
1	1	1	10	180	51.72	18.07	30.21
2	1	2	30	180	49.56	7.83	42.61
3	2	1	30	120	56.78	9.51	33.71
4	2	1	10	120	54.29	13.65	32.05
~~5~~	~~1.5~~	~~1.5~~	~~20~~	~~150~~	~~64.45~~	~~6.51~~	~~29.03~~
6	2	2	30	120	53.02	7.85	39.13
7	1	1	10	120	39.21	29.7	31.1
8	1	2	10	120	40.43	31.91	27.66
9	1	1	30	120	41.45	25.99	32.57
10	2	1	10	180	69.19	3.79	27.05
11	2	2	30	180	62.04	1.9	36.06
12	2	2	10	180	69.51	2.75	27.74
13	2	1	30	180	55.79	1.56	42.65
14	1	2	30	120	43.74	26.58	29.68
15	1.5	1.5	20	150	58.89	6.44	34.71
16	1.5	1.5	20	150	59.3	6.25	34.44
17	1	2	10	180	53.41	14.23	32.36
18	1.5	1.5	20	150	59.23	6.16	34.61
19	2	2	10	120	49.28	9.56	41.16
20	1	1	30	180	40.74	7.42	51.84

Table 38 Full factorial design for the final alkylation

A sample HPLC for run 20 (in Table 38) is reported in Figure 80 below:

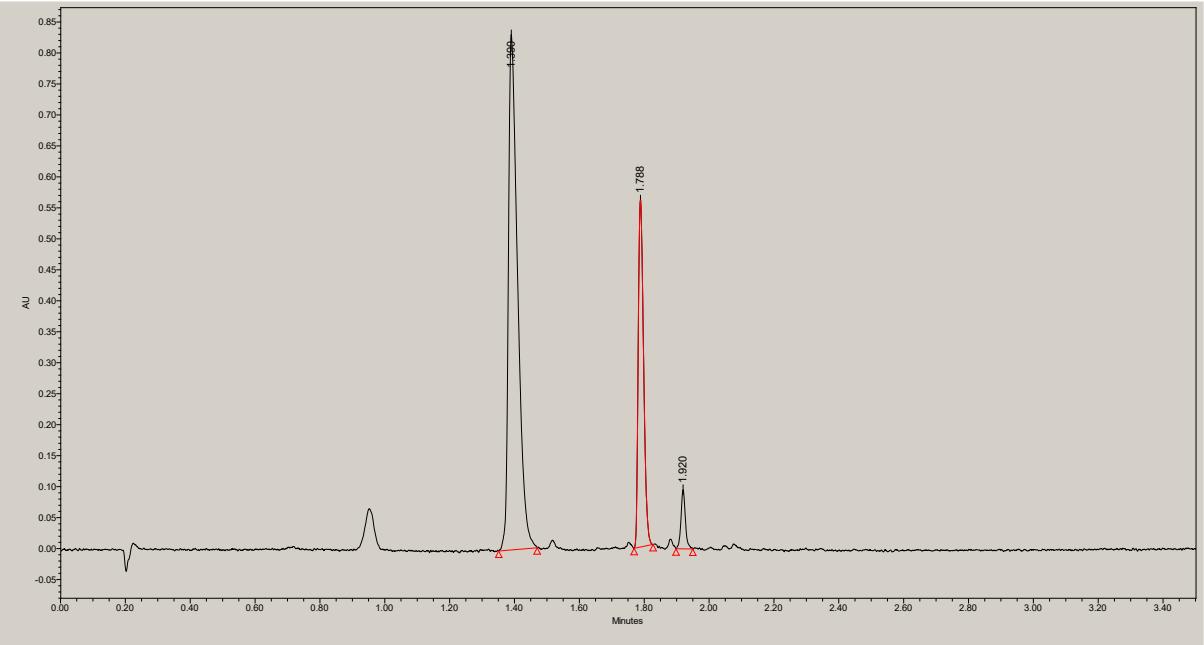


Figure 80 Sample chromatogram for run 20 (in Table 38)

Retention Time (min)	% Area	Assignment
1.390	69.16	5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one tert-butyl 2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (5)
1.788	27.05	Domperidone (1)
1.920	3.70	1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4)

Table 39 Retention time assignments for the run 20

The peak at c.a. 0.95 min is assumed to be the iodide used as catalyst in 10% mol, in the form of potassium iodide, and for this reason it was not integrated.

The expansion resulted in the creation of blocks to distinguish the two sets of experiments, as reported in Table 40 below, where the first block includes the data already reported in Table 38 above and the second block the new experiments that expand the design in a central composite.

Block		Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
	Run	Eq Compound D (5)	Eq DIPEA	Time	Temperature	Compound D (5)	Intermediate 3 (4)	Domeridone (1)
		eq	eq	min	degC	% a/a	% a/a	% a/a
DOE	1	1	1	10	180	51.72	18.07	30.21
	2	1	2	30	180	49.56	7.83	42.61
	3	2	1	30	120	56.78	9.51	33.71
	4	2	1	10	120	54.29	13.65	32.05
	5	1.5	1.5	20	150	64.45	6.51	29.03
	6	2	2	30	120	53.02	7.85	39.13
	7	1	1	10	120	39.21	29.7	31.1
	8	1	2	10	120	40.43	31.91	27.66
	9	1	1	30	120	41.45	25.99	32.57
	10	2	1	10	180	69.19	3.79	27.05
	11	2	2	30	180	62.04	1.9	36.06
	12	2	2	10	180	69.51	2.75	27.74
	13	2	1	30	180	55.79	1.56	42.65
	14	1	2	30	120	43.74	26.58	29.68
	15	1.5	1.5	20	150	58.89	6.44	34.71
	16	1.5	1.5	20	150	59.3	6.25	34.44
	17	1	2	10	180	53.41	14.23	32.36
	18	1.5	1.5	20	150	59.23	6.16	34.61
	19	2	2	10	120	49.28	9.56	41.16
	20	1	1	30	180	40.74	7.42	51.84
Expansion	21	2.5	1.5	20	150	73.33	3.47	23.2
	22	1.5	1.5	20	90	55.86	31.74	12.44
	23	1.5	2.5	20	150	66.52	9.31	24.35
	24	1.5	1.5	40	150	65.44	4.88	29.68
	25	1.5	1.5	20	210	59.64	0	40.36
	26	1.5	1.5	20	150	66.62	8.51	26.87
	27	1.5	1.5	0.34	150	57.45	31.88	10.66
	28	1.5	0.5	20	150	61.72	8.43	29.85
	29	1.5	1.5	20	150	63.98	8.71	27.32
	30	0.5	1.5	20	150	42.41	33.05	24.53
Trial	A	1.64	1.5	30	180	55.06	2.82	42.13
		1.64	1.5	30	180	57.1	2.54	40.54
	B	1.02	1.5	30	180	51.66	7.28	41.07
	C	1.602	1.5	30	180	48.39	6.98	44.63

Table 40 Central composite design for the final alkylation

The sample HPLC was similar to that reported in the initial optimization.

6.9 Run through to Domperidone

The first reaction of this run through was performed with 475 ml of tert-butyl (2-aminophenyl)carbamate (19) 0.2M in THF, which reacted at 40°C with 570 ml of CDI

0.25M in THF, in a sequence of coils (35 ml in total), to obtain 1047 ml of a solution 0.0909M of Intermediate 1 (22) in THF, with unreacted CDI and imidazole, in a total of 13 hours 52 minutes and 10 seconds.

After the aqueous work-up, performed in batch, the material was progressed in the following step. The material recovered after evaporation presented only 221 mg of material loss from the theoretical amount of material; the yield for this step is 99.0 molar %.

The NMR of the product, Intermediate 1 (22), is reported in Figure 81 below

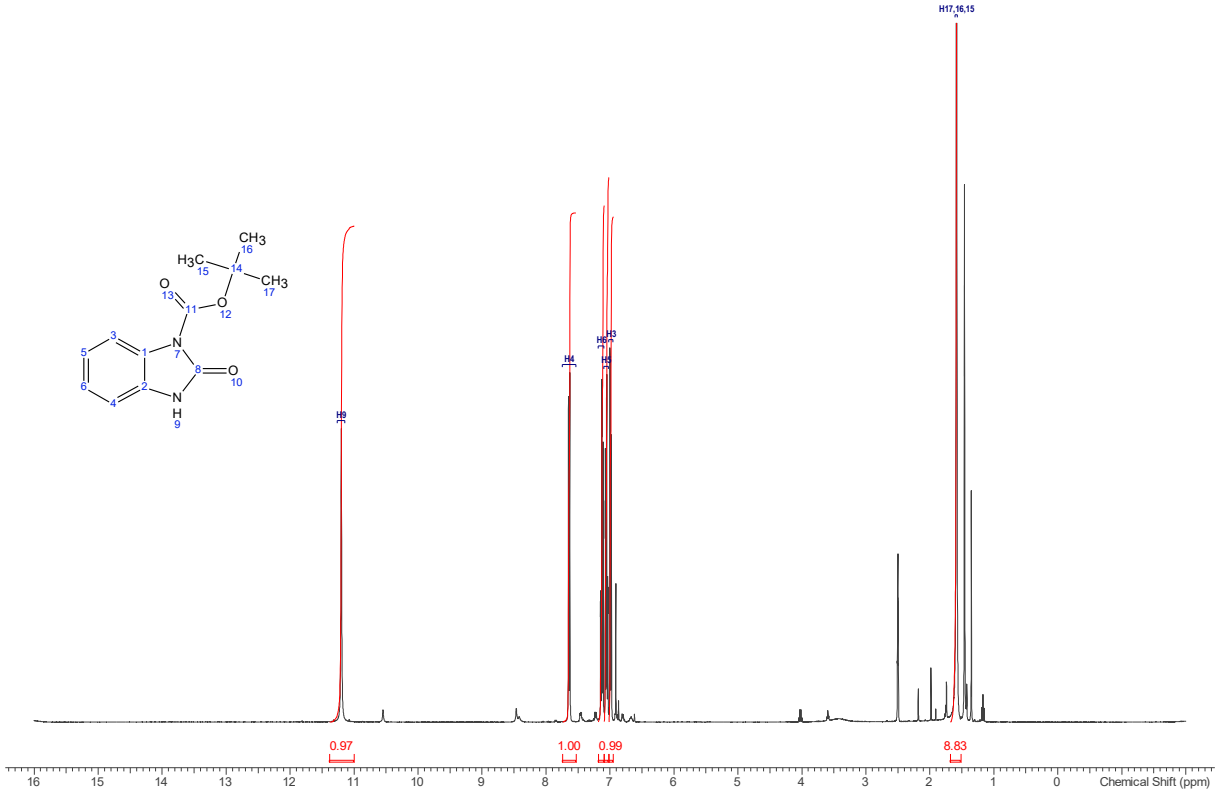


Figure 81 NMR of the product after the first step of the run through

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.20 (s, 1 H) 7.64 (d, *J*=7.92 Hz, 1 H) 7.09 - 7.18 (m, 1 H) 7.02 - 7.09 (m, 1 H) 6.99 (dd, *J*=7.59, 0.77 Hz, 1 H) 1.58 (s, 9 H)

The second step of the reaction was performed using 190 ml of Intermediate 1 (22) 0.5M in THF premixed, in a 5 ml coil reactor at 30°C with 285 ml of DBU 0.5M in THF; then the mixture was reacted at 50°C with 504 ml of 1-bromo-3-chloropropane (23) 0.75M in THF in a sequence of coils (30 ml in total), to obtain 987 ml of a 0.0969M solution of tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24) in THF, in a total of 6 hours 40 minutes and 46 seconds.

After the aqueous work-up, performed in batch, and the evaporation under vacuum performed with the aid of heptane to completely remove the unreacted 1-bromo-3-chloropropane (23), the material was progressed to the following step. The material recovered after evaporation was found to be 27.317 g, 2.206 g below the theoretical amount of material, the overall yield after this step is 92.5 molar %.

The NMR of the product, intermediate 2 (24), is reported in Figure 82 below

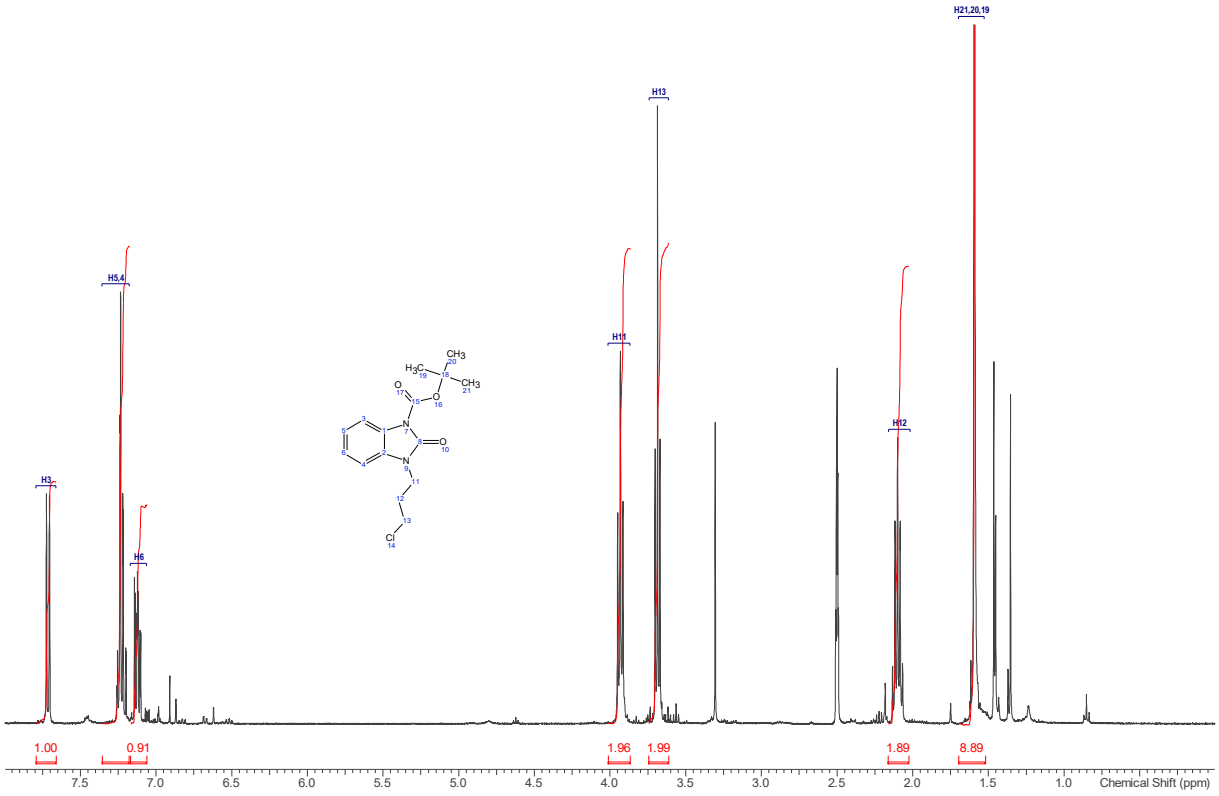


Figure 82 NMR of the product after the second step of the run through

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.71 (d, *J*=7.92 Hz, 1 H) 7.18 - 7.35 (m, 2 H) 7.06 - 7.17 (m, 1 H) 3.93 (t, *J*=6.82 Hz, 2 H) 3.69 (t, *J*=6.38 Hz, 2 H) 2.05 - 2.15 (m, 1 H) 1.59 (s, 9 H)

The third step of the reaction was performed using 879 ml of tert-butyl 3-(3-chloropropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (24) 0.1M in DMSO and the reaction was performed without any additional reagents, just heating the mixture at 180°C for 5 minutes, to obtain 881 ml of a 0.09977M solution of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) in DMSO, in a total of 7 hours 43 minutes and 45 seconds; the material obtained in this step was used directly in the next step. The UPLC of the reaction mixture is reported in Figure 83

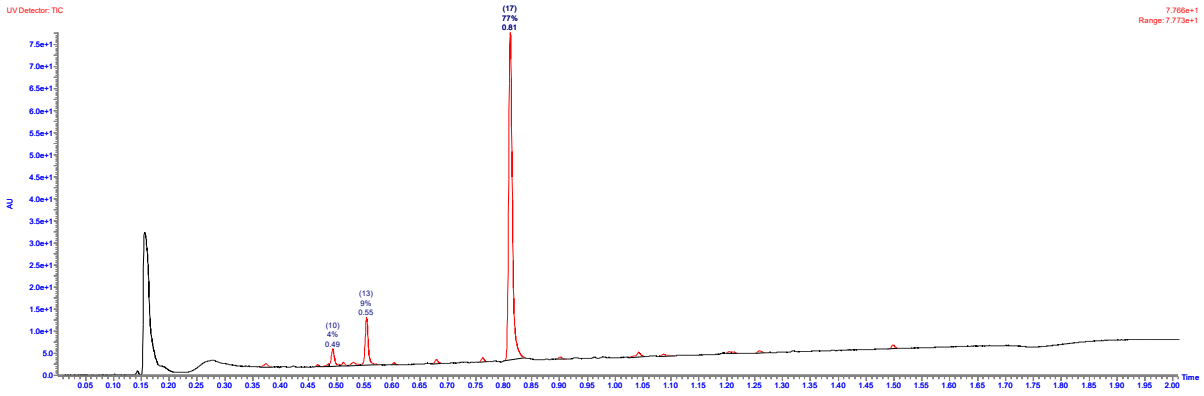
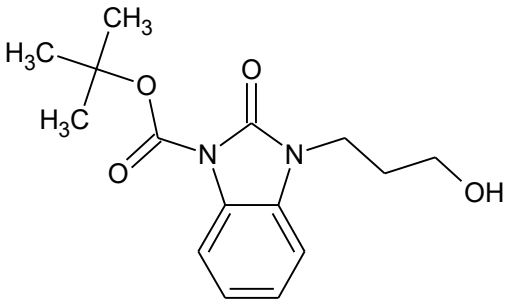


Figure 83 UPLC/MS of the product after the third step of the run through

Retention Time (min)	Observed M/Z	Assignment
0.49	252	1,3-dihydro-2H-benzimidazol-2-one (21)
0.55	193	tert-butyl 3-(3-hydroxypropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (35)
0.81	211	1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (4)

Table 41 Mass assignments for the third step of the run through



35

Figure 84 Structure of tert-butyl 3-(3-hydroxypropyl)-2-oxo-2,3-dihydro-1H-benzimidazole-1-carboxylate (35)

The fourth step of the reaction sequence was initially planned to be performed using 880 ml of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) 0.1M in DMSO, however, the reaction time to react all of this material in the same conditions used during the optimization work would have been c.a. 7.5 days (11005 minutes). Additionally, the idea to

change the concentration of the reagents, to 0.164M for compound D (5) and 1.5M for the DIPEA, would not have significantly reduced the required reaction time, from the previous c.a. 7.5 days to the updated 5.5 days. For this reason it was decided to perform the last step only on 88 ml of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) 0.1M in DMSO to reduce the reaction time. 88 ml of compound D (5) 0.164M in DMSO was premixed, in a 2 ml coil reactor at 30°C with 88 ml of DIPEA 0.1M in DMSO, then the mixture was reacted at 180°C with 88 ml of 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) 0.1M in DMSO to obtain after 14 hours 27 minutes and 3 seconds 266.48 ml of Domperidone (1) in DMSO.

The isolation of Domperidone was attempted by dropwise addition of the Domperidone solution to water (2.6 L) under stirring, the resulting solid was collected and then dissolved in acetic acid (1 M in methanol) followed by precipitation with addition of NH_3 (7 M in methanol) to give 1.821 g of Domperidone (1) with an overall yield of 48.6 molar %.

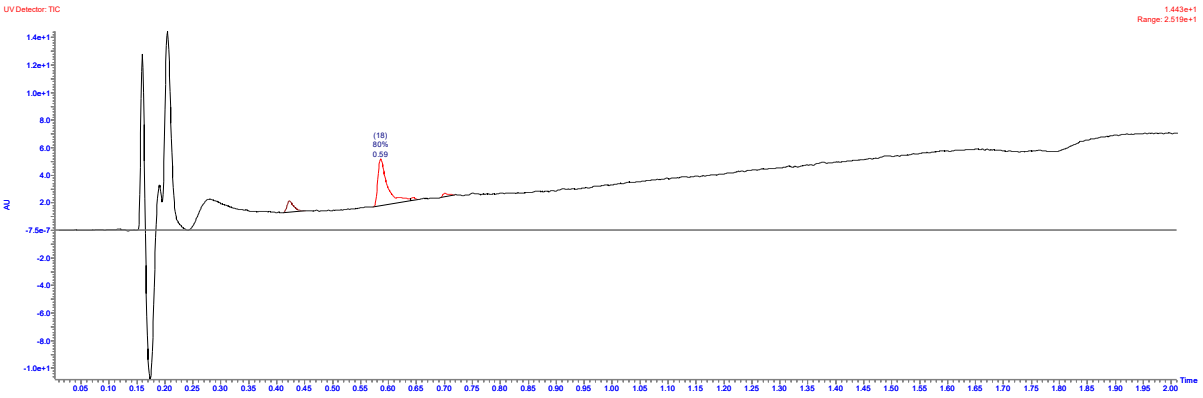


Figure 85 UPLC/MS of Domperidone (1) isolated after the run through

The UPLC/MS of the isolated Domperidone (1) (Figure 85 above) showed the presence of residual 5-chloro-1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one (5), despite a large part of this starting material having been removed during the precipitation in water, as show in the UPLC/MS of the mother liquor reported in Figure 86 below.

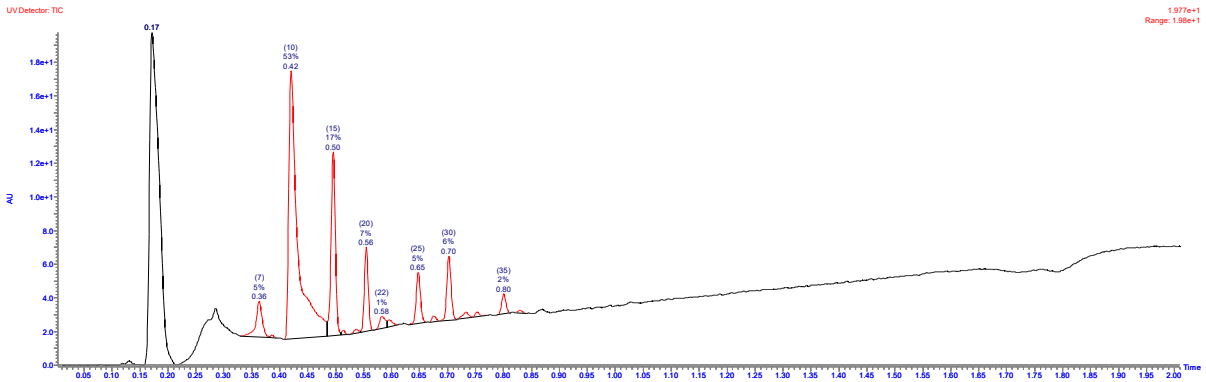
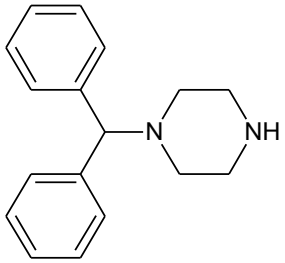


Figure 86 UPLC/MS of the aqueous phase after Domperidone (1) precipitation in run through

6.10 Synthesis of Oxatomide and Declenperone.

For the synthesis of Oxatomide, the 1-(diphenylmethyl)piperazine (32) was commercially available and the product was synthetized using the same conditions optimized for the last step of the synthesis of Domperidone.



32

- 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) 0.1M in DMSO
- 1-(diphenylmethyl)piperazine (32) 0.1M in DMSO
- Equivalents of 1-(diphenylmethyl)piperazine (32) (1.64 eq)
- Equivalents of DIPEA (1.5 eq)
- Residence time (30 minutes)
- Temperature (180°C)
- 14 ml of product were collected

The output of the reaction is displayed in the HPLC in Figure 87 below.

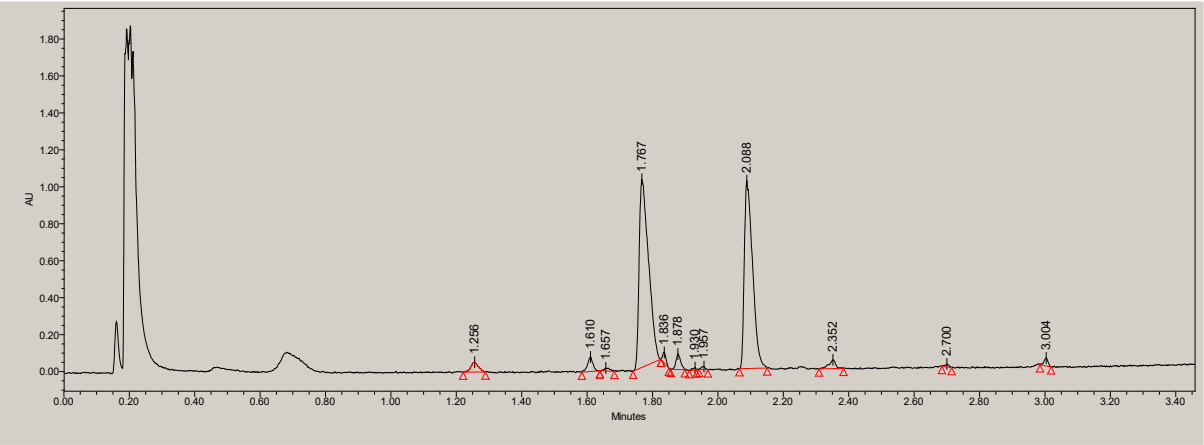
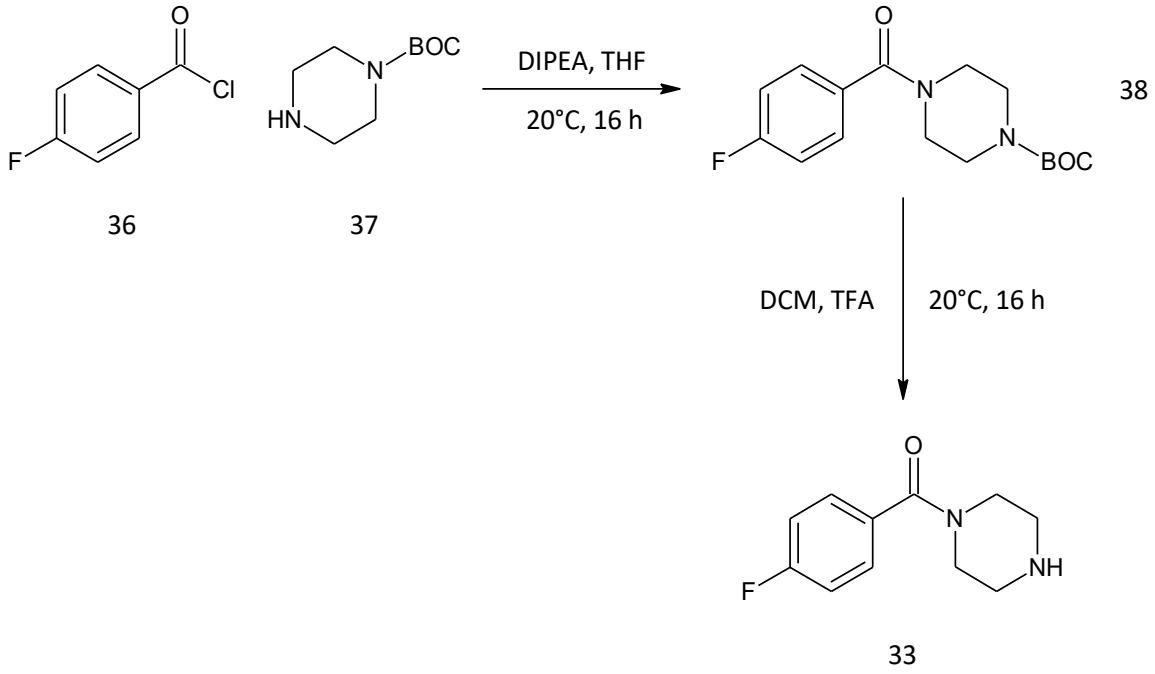


Figure 87 Chromatogram for the synthesis of Oxatomide

Oxatomide signal at 2.088 min, peak at 1.767 min corresponds to 1-(diphenylmethyl)piperazine (32).

For the synthesis of Declenperone, the required building block (4-fluorophenyl)(piperazin-1-yl)methanone (33) was not readily available so it was synthetized using the procedure reported in Scheme 28 below.



Scheme 28 Synthetic scheme for 4-fluorophenyl)(piperazin-1-yl)methanone (29)

For the synthesis of 4-fluorophenyl)(piperazin-1-yl)methanone (33):

In a 100 ml round bottom flask (RBF) tert-butyl piperazine-1-carboxylate (37) (2.05 g, 11 mmol) was dissolved in 50 ml of THF; then DIPEA (2.6 ml, 15 mmol) was added followed by 4-fluorobenzoyl chloride (36) (1.59 g, 10 mmol). The reaction mixture was stirred at

20°C for 16 hours. The reaction mixture was diluted with ethyl acetate (50 ml) and then the organic phase was washed with aqueous $KHSO_4$ 5%. The organic phase was then evaporated under vacuum. The crude material was then purified on silica gel (Biotage Sfar 100g) eluting from cyclohexane (100 %) to ethyl acetate (100 %) in a linear gradient to afford tert-butyl 4-(4-fluorobenzoyl)piperazine-1-carboxylate (38) 2.86 g (92.7 % molar yield).

In a 100 ml RBF tert-butyl 4-(4-fluorobenzoyl)piperazine-1-carboxylate (38) 2.86 g was suspended in DCM (45 ml) then trifluoroacetic acid (2.5 ml) was added to the reaction mixture and it was stirred at 20°C for 16 hours. The reaction mixture was evaporated under vacuum to remove all volatiles. The crude material was diluted with DCM and the organic phase was washed with aqueous $NaHCO_3$ (SAT) to give the desired product 4-fluorophenyl)(piperazin-1-yl)methanone (33) 1.88 g (97.3 % molar yield).

The NMR data for compound 33 is reported below

^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 7.30 – 7.50 (m, 2 H) 6.95 – 7.16 (m, 2 H) 3.19 – 4.21 (br. m, 4 H) 2.44 – 3.09 (br. s, 4 H) 1.87 (br. s, 1 H)

The compound once synthetized was used in the same conditions optimized for the last step of the synthesis of Domperidone.

- 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one (4) 0.1M in DMSO
- (4-fluorophenyl)(piperazin-1-yl)methanone (33) 0.1M in DMSO
- Equivalents of (4-fluorophenyl)(piperazin-1-yl)methanone (33) (1.64 eq)
- Equivalents of DIPEA (1.5 eq)
- Reaction time (30 minutes)
- Temperature (180°C)
- 14 ml of product were collected

The output of the reaction is displayed in the HPLC in Figure 88 below.

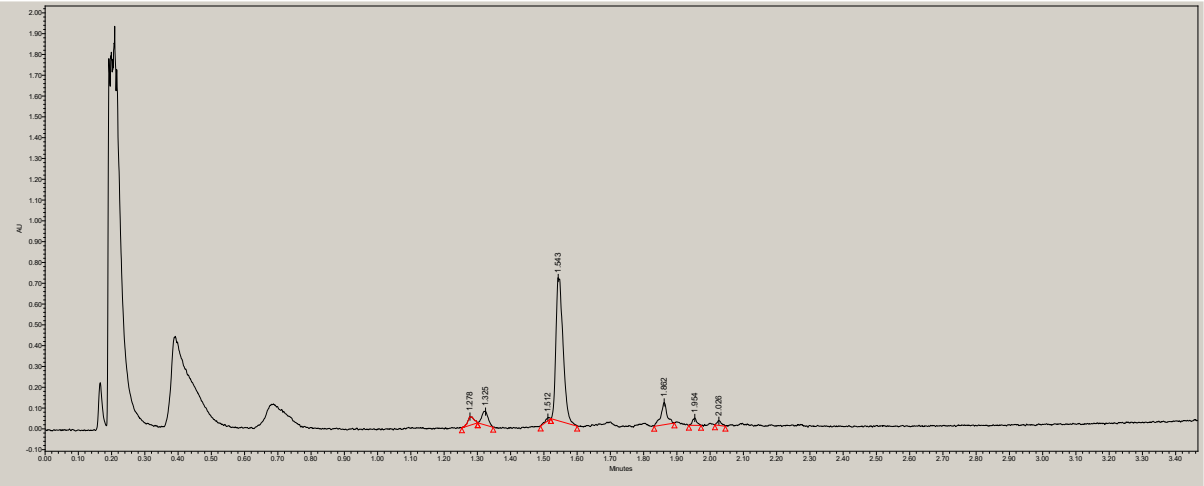


Figure 88 Chromatogram for the synthesis of Declenperone

Declenperone signal at 1.54 min.

7 Table of abbreviations

Abbreviation	Expanded
% mol/mol	Percentage mole-mole
%a/a	Percentage area-area
%v/v	Percentage volume–volume
(CFC)	convection-flow coil
°C	degree Celsius
µm	micrometre
^{1}H NMR	Proton Nuclear magnetic resonance
ANOVA	Analysis Of Variance
API	Active Pharmaceutical Ingredient
BOC_2O	Di-tert-butyl dicarbonate
CAPEX	CAPital EXpenditure
CDI	1,1'-Carbonyldiimidazole
cLLD	capacitive Liquid Level Detection
CRO	Contract Research Organization

Cs_2CO_3	Cesium Carbonate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DoE	Design of Experiment
EMA	European Medicines Agency
eq	Equivalent/Equivalents
FDA	Food and Drug Administration
g/mol	Molar mass or formula weight
GSK	GlaxoSmithKline
h	Hour(s)
HPLC	High Pressure Liquid Chromatography
Hz	hertz

i.d.	Internal Diameter
ICH	The International Council For Harmonisation Of Technical Requirements For Pharmaceuticals For Human Use
J. Chem. Soc	Journal of the Chemical Society
K_2CO_3	Potassium Carbonate
KI	Potassium iodide
KOH	Potassium hydroxide
LHS	Left Hand Side
M	Mol/l, moles per litre
MHz	Megahertz
min	minutes
mm	millimetre
MW	Microwave
NCE	New Chemical Entity
nm	nanometre
NMR	Nuclear Magnetic Resonance
OFAT	One Factor At a Time

PEEK	Polyether ether ketone
PEG 300	Polyethylene glycol with average molecular weight of 300 g/mol
PFA	Perfluoroalkoxy alkane
psi	Pounds per square inch
PTFE	Polytetrafluoroethylene
QbD	Quality by Design
RHS	Right Hand Side
SiC	Silicon carbide
SM	Starting material
ss	Stainless steel
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UPLC/MS	Ultra-high Pressure Liquid Chromatography coupled with mass spectrometer

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9 Bibliography

1. Bosch, C. Process of producing ammonia. (1908).

2. Hellgardt, K. & Mimi Hii, K. K. Continuous flow technologies in the development of “green” organic reactions and processes. in *Advanced Green Chemistry: Part 1: Greener Organic Reactions and Processes* 257–284 (World Scientific Publishing Co., 2017). doi:10.1142/9789813228115_0007.

3. Stranges, A. Germany’s synthetic fuel industry, 1927–1945. in *The German Chemical Industry in the Twentieth Century* 147–216 (2000). doi:10.1007/978-94-015-9377-9_7.

4. Baumann, M., Moody, T. S., Smyth, M. & Wharry, S. A Perspective on Continuous Flow Chemistry in the Pharmaceutical Industry. *Org. Process Res. Dev.* (2020) doi:10.1021/acs.oprd.9b00524.

5. Bogdan, A. R. & Dombrowski, A. W. Emerging Trends in Flow Chemistry and Applications to the Pharmaceutical Industry. *J. Med. Chem.* **62**, 6422–6468 (2019).

6. Akwi, F. M. & Watts, P. Continuous flow chemistry: where are we now? Recent applications, challenges and limitations. *Chem. Commun.* **54**, 13894–13928 (2018).

7. Lummiss, J. A. M., Morse, P. D., Beingessner, R. L. & Jamison, T. F. Towards More Efficient, Greener Syntheses through Flow Chemistry. *Chem. Rec.* **17**, 667–680 (2017).

8. Haefner, B. F., Nonnenmacher, M., Voigtlaender, D. & Ingo, K. Continuous manufacturing of APIs and intermediates under cGMP . A multi-step case study involving a modular continuous plant , and new opportunities for 3D printed reactors. *C&en WHITEPAPERS* (2020).

9. Ley, S. V., Chen, Y., Fitzpatrick, D. E. & May, O. A new world for chemical synthesis? *Chimia (Aarau).* **73**, 792–802 (2019).

10. Brandão, P., Pineiro, M. & Pinho e Melo, T. M. V. D. Flow Chemistry: Towards A More Sustainable Heterocyclic Synthesis. *European J. Org. Chem.* **2019**, 7188–7217 (2019).

11. Lin, H., Dai, C., Jamison, T. F. & Jensen, K. F. A Rapid Total Synthesis of Ciprofloxacin Hydrochloride in Continuous Flow. *Angew. Chemie - Int. Ed.* **56**, 8870–8873 (2017).

12. Corning inc. Corning Advances Flow Reactor Technology For Industrial Chemical Production. *press relase* https://www.corning.com/worldwide/en/about-us/news-events/corning-advances-flow-reactor-technology-for-industrial-chemical-production.html (2020).

13. Godineau, E., Battilocchio, C. & Lal, M. Building up a continuous flow platform as an enabler to the preparation of intermediates on kilogram scale. *Chimia (Aarau).* **73**, 828–831 (2019).

14. Costandy, J. G., Edgar, T. F. & Baldea, M. Switching from Batch to Continuous Reactors Is a Trajectory Optimization Problem. *Ind. Eng. Chem. Res.* **58**, 13718–13736 (2019).

15. Rode, C. V., Ghalwadkar, A. A., Mane, R. B., Hengne, A. M., Jadkar, S. T. & Biradar, N. S.. Selective hydrogenolysis of glycerol to 1,2-propanediol: Comparison of batch and continuous process operations. *Org. Process Res. Dev.* **14**, 1385–1392 (2010).

16. Mane, R. B. & Rode, C. V. Continuous dehydration and hydrogenolysis of glycerol over non-chromium copper catalyst: Laboratory-scale process studies. *Org. Process Res. Dev.* **16**, 1043–1052 (2012).

17. Shen, Y. Maamor, A., Abu-Dharieh, J., Thompson, J. M., Kalirai, B., Stitt, E. H. & David W. Rooney. Moving from batch to continuous operation for the liquid phase dehydrogenation of tetrahydrocarbazole. *Org. Process Res. Dev.* **18**, 392–401 (2014).

18. Mendonça, A. D. M., De Oliveira, A. V. B. & Cajaiba, J. A Comparison between Continuous and Batch Processes to Capture Aldehydes and Ketones by Using a Scavenger Resin. *Org. Process Res. Dev.* **21**, 1794–1800 (2017).

19. Plutschack, M. B., Pieber, B., Gilmore, K. & Seeberger, P. H. The Hitchhiker's Guide to Flow Chemistry. *Chem. Rev.* **117**, 11796–11893 (2017).

20. Teoh, S. K., Rathi, C. & Sharratt, P. Practical Assessment Methodology for Converting Fine Chemicals Processes from Batch to Continuous. *Org. Process Res. Dev.* **20**, 414–431 (2016).

21. Goršek, A. & Glavič, P. Design of batch versus continuous processes - Part I: Single-purpose equipment. *Chem. Eng. Res. Des.* **75**, 709–717 (1997).

22. Goršek, A. & Glavič, P. Design of batch versus continuous processes - Part II:

Multi-purpose equipment. *Chem. Eng. Res. Des.* **75**, 718–723 (1997).

23. Goršek, A. & Glavič, P. Design of batch versus continuous processes. Part III: Extended analysis of cost parameters. *Chem. Eng. Res. Des.* **78**, 231–244 (2000).

24. Jolliffe, H. G. & Gerogiorgis, D. I. Plantwide design and economic evaluation of two Continuous Pharmaceutical Manufacturing (CPM) cases: Ibuprofen and artemisinin. *Comput. Chem. Eng.* **91**, 269–288 (2016).

25. Gartner Hype Cycle - Hype cycle - Wikipedia. https://it.wikipedia.org/wiki/Hype_cycle#/media/File:Hype-Cycle-General.png.

26. Campani, M. & Vaglio, R. A simple interpretation of the growth of scientific/technological research impact leading to hype-type evolution curves. *Scientometrics* **103**, 75–83 (2015).

27. Hawkes, S. Y. F. W., Chapela, M. J. V. & Montembault, M. Leveraging the advantages offered by microfluidics to enhance the drug discovery process. *QSAR Comb. Sci.* **24**, 712–721 (2005).

28. Gioiello, A., Piccinno, A., Lozza, A. M. & Cerra, B. The Medicinal Chemistry in the Era of Machines and Automation: Recent Advances in Continuous Flow Technology. *J. Med. Chem.* **16**, 32 (2020).

29. U.S. Food and Drug Administration Center for Drug Evaluation and Research (CDER). Quality considerations for continuous manufacturing Guidance for industry DRAFT GUIDANCE. 27 (2019).

30. US FDA. Strategic plan for preventing and mitigating drug shortages. in *Persistent Drug Shortages in the United States: Analyses and Strategies* 89–130 (2014).

31. Lee, S. L., O'Connor, T. F., Yang, X., Cruz, C. N., Chatterjee, S., Madurawe, R. D., Moore, C. M. V., Yu, L. X., & Woodcock, J. Modernizing Pharmaceutical Manufacturing: from Batch to Continuous Production. *J. Pharm. Innov.* **10**, 191–199 (2015).

32. FDA. *Code of Federal Regulations Title 21 - 21CRF210.3*. vol. 4 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=210.3 (2019).

33. Waldron, C., Pankajakshan, A., Quaglio, M., Cao, E., Galvanin, F. & Gavriilidis, A. An autonomous microreactor platform for the rapid identification of kinetic models.

React. Chem. Eng. **4**, 1623–1636 (2019).

34. Empel, C. & Koenigs, R. M. Artificial-Intelligence-Driven Organic Synthesis—En Route towards Autonomous Synthesis? *Angewandte Chemie - International Edition* vol. 58 17114–17116 (2019).

35. Aspen Technology Inc. Aspen Plus | Leading Process Simulation Software. *Aspen Technology, Inc.* https://www.aspentech.com/en/products/engineering/aspen-plus (2020).

36. ENTERPRISE PROCESS SYSTEMS. gPROMS | Products | Process Systems Enterprise | Process Systems Enterprise. *PROCESS SYSTEMS ENTERPRISE* https://www.psenterprise.com/products/gproms (2020).

37. ICH. ICH Official web site : ICH. *Ich.Org* (2008).

38. ICH. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Final Business Plan ICH Q13: Continuous Manufacturing for Drug Substances and Drug Products. 1–3 http://www.ich.org (2020).

39. Vapourtec ltd. *Application Note 51 : Palladium on Charcoal Slurries in Continuous Flow for Transfer Hydrogenation*.

40. Sagmeister, P., Williams, J. D., Hone, C. A. & Kappe, C. O. Laboratory of the future: A modular flow platform with multiple integrated PAT tools for multistep reactions. *React. Chem. Eng.* **4**, 1571–1578 (2019).

41. Perera, D., Tucker, J. W., Brahmbhatt, S., Helal, C. J., Chong, A., Farrell, W., Richardson, P. & Sach, N. W. . A platform for automated nanomole-scale reaction screening and micromole-scale synthesis in flow. *Science (80-.).* **359**, 429–434 (2018).

42. Reddymasu, S. C., Soykan, I. & McCallum, R. W. Domperidone: Review of pharmacology and clinical applications in gastroenterology. *Am. J. Gastroenterol.* **102**, 2036–2045 (2007).

43. Vandenberk, J., Kennis L. E. J., Van der Aa M. J. M. C., Van Heertum A. H. M. Th. 1,3-Dihydro-1-[3-(1-piperidinyl)propyl]-2h-benzimidazol-2-ones and related compounds. (1978).

44. Davol, J. 58. The reaction of o-phenylenediamine with αβ-unsaturated acids and

with β-keto-esters. *J. Chem. Soc.* 308–314 (1960) doi:10.1039/JR9600000308.

45. Brunton, L. L., Chabner, B. A. & Knollmann, B. C. *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*. (2011).

46. Katzung, B. G., Masters, S. B. & Trevor, A. J. *Basic & Clinical Pharmacology*. *Annual Reports in Medicinal Chemistry* vol. 12 (2012).

47. FDA. FDA Talk Paper: FDA Warns Against Women Using Unapproved Drug, Domperidone, to Increase Milk Production. *US Food and Drug Administration* https://www.fda.gov/drugs/information-drug-class/fda-talk-paper-fda-warns-against-women-using-unapproved-drug-domperidone-increase-milk-production (2004).

48. FDA Division of Drug Information. How to Request Domperidone for Expanded Access Use. *U.S Food and drug administration* https://www.fda.gov/drugs/investigational-new-drug-ind-application/how-request-domperidone-expanded-access-use (2018).

49. Martini, N., Bozzini, L. & Tosolini, F. Guida all'uso dei farmaci sulla base del British National Formulary. in vol. anno 2002 (Elsevier Masson, 2002).

50. Yüksel, K. & Tuğlular, I. Critical review of European Medicines Agency (EMA) assessment report and related literature on domperidone. *Int. J. Clin. Pharm.* 19–22 (2019) doi:10.1007/s11096-019-00803-9.

51. AIFA. Agenzia Italiana del Farmaco. https://www.aifa.gov.it/trova-farmaco.

52. MOEF. Ministry of Environment, Forest and Climate Change Government of India. 1–5 (2018).

53. Allchem laboratories. *Environmental impact and risk assessment report for proposed expansion of existing advance pharmaceutical intermediate products manufacturing unit*. (2011).

54. *Manufacturing process and mass balance*. http://environmentclearance.nic.in/writereaddata/Online/TOR/0_0_09_Oct_2015_1508009601AdditionalAttachment.pdf.

55. *Pre-Feasibility Report Sri Krishna Pharmaceuticals Limited, Unit I*. http://environmentclearance.nic.in/writereaddata/EIA/150420157B9XC3JIPrefeasibilityreport.pdf.

56. Sexton, W. A. The condensation of o-phenylenediamine with ethyl acetoacetate. *J. Chem. Soc.* 303–304 (1942) doi:10.1039/jr9420000303.

57. Mamedov, V. A. & Zhukova, N. A. *Recent Advances in the Synthesis of Benzimidazol-2-ones via Rearrangements*. *Progress in Heterocyclic Chemistry* vol. 29 (Elsevier, 2017).

58. Meanwell, N. A. Sit, S. Y., Gao, J., Wong, H. S., Gao, Q., St. Laurent, D. R., & Balasubramanian, N. Regiospecific Functionalization of 1, 3-Dihydro-2H-benzimidazol-2-one and Structurally Related Cyclic Urea Derivatives. *J. Org. Chem.* **60**, 1565–1582 (1995).

59. Mondieig, D., Negrier, Ph., Leger, J. M., Lakhrissi, L., El Assyry, A., Lakhrissi, B., Essassi, E. M., Benali B. & Boucetta A. Synthesis and structural study of N-isopropenylbenzimidazolone. *Russ. J. Phys. Chem. A* **89**, 807–811 (2015).

60. Khodarahmi, G. A., Chen, C. S., Hakimelahi, G. H., Tseng, C. T. & Chern, J. W. Design, synthesis, and cytotoxicity of 4-sulfonamide substituted benzamidobenzimidazolones and an acyl benzimidazolone. *J. Iran. Chem. Soc.* **2**, 124–134 (2005).

61. Meth-Cohn, O. & Smith, D. I. N-bridged heterocycles. Part 5. α,ω-bis-(2-oxobenzimidazolinyl) -alkanes and -ethers as selective ligands for group-1 and -2 metals. *J. Chem. Soc. Perkin Trans. 1* 261–270 (1982) doi:10.1039/p19820000261.

62. Khajavi, M. S., Hajihadi, M. & Naderi, R. Synthesis of Heterocyclic Compounds from o-Substituted Anilines under Microwave Irradiation. *J. Chem. Res. - Part S* **27**, 92–93 (1996).

63. Bougrin, K., Bennani, A. K., Tétouani, S. F. & Soufiaoui, M. An easy route to synthesize 1,5-arylodiazepin-2-ones. *Tetrahedron Lett.* **35**, 8373–8376 (1994).

64. Koizumi, H., Itoh, Y. & Ichikawa, T. On the magic of microwave-assisted organic synthesis - 1,5-benzodiazepin-2- one from o-phenylenediamine and ethyl acetoacetate. *Chem. Lett.* **35**, 1350–1351 (2006).

65. Israel, M., Jones, L. C. & Modest, E. J. Thermal rearrangement of condensed dihydrodiazepinones. *Tetrahedron Lett.* **9**, 4811–4814 (1968).

66. Yang, F., Wu, C., Li, Z., Tian, G., Wu, J., Zhu, F., Zhang, J., He, Y., & Shen, J. A Facile Route of Synthesis for Making Flibanserin. *Org. Process Res. Dev.* **20**, 1576–1580 (2016).

67. Soldano, C., Isaacsohn, J. & Patel, P. Deuterated domperidone compositions, methods, and preparation. (2019).

68. Shingare, R. D., Kulkarni, A. S., Sutar, R. L. & Srinivasa Reddy, D. Route to Benzimidazol-2-ones via Decarbonylative Ring Contraction of Quinoxalinediones: Application to the synthesis of flibanserin, a drug for treating hypoactive sexual desire disorder in women and marine natural product hunanamycin analogue. *ACS Omega* **2**, 5137–5141 (2017).

69. Bana, P., Szigetvári, Á., Kóti, J., Éles, J. & Greiner, I. Flow-oriented synthetic design in the continuous preparation of the aryl piperazine drug flibanserin. *React. Chem. Eng.* **4**, 652–657 (2019).

70. Bana, P. & Greiner, I. Process for the multistep continuous-flow preparation of flibanserin. 41 (2020).

71. Hughes, D. L. Applications of Flow Chemistry in the Pharmaceutical Industry—Highlights of the Recent Patent Literature. *Org. Process Res. Dev.* (2020) doi:10.1021/acs.oprd.0c00156.

72. Badarau, E., Suzenet, F., Bojarski, A. J., Fînaru, A. L. & Guillaumet, G. Benzimidazolone-based serotonin 5-HT1A or 5-HT7R ligands: Synthesis and biological evaluation. *Bioorganic Med. Chem. Lett.* **19**, 1600–1603 (2009).

73. Britton, J. & Raston, C. L. Multi-step continuous-flow synthesis. *Chem. Soc. Rev.* **46**, 1250–1271 (2017).

74. Yu, K.-L., Civiello, R. L., Meanwell, N. A. & Combrink, K. D. WO02/26228: Benzimidazolone antiviral agents. (2002).

75. Lakhrissi, B. Benksim, A., Massoui, M., Essassi, E. M., Lequart, V., Joly, N., Beaupère, D., Wadouachi, A. & Martin, P. Towards the synthesis of new benzimidazolone derivatives with surfactant properties. *Carbohydr. Res.* **343**, 421–433 (2008).

76. Jansseni, A. J. P. 1-(1-aroylpropyl-4-piperidyl)-2-benzimidazolinones and related compounds. (1964).

77. Staab, H. A. New Methods of Preparative Organic Chmistry IV. Syntheses Using Heterocyclic Amides (Azolides). *Angew. Chemie Int. Ed. English* **1**, 351–367 (1962).

78. Zheng, M., Chen, J., Chen, G., Farajtabar, A. & Zhao, H. Solubility modelling and solvent effect for domperidone in twelve green solvents. *J. Mol. Liq.* **261**, 50–56 (2018).

79. European Pharmacopoeia. DOMPERIDONE. in 1745–1747.

80. Agency, E. M. Quality by design | European Medicines Agency. https://www.ema.europa.eu/en/human-regulatory/research-development/quality-design#guidance-documents-section.

81. Ahlert, J. ICH Q8 : Pharmaceutical Development . Regulatory Requirements Directed by the New Note for Guidance (EMEA / CHMP / 167068 / 2004) in Comparison to the Previous Guideline (CPMP / QWP / 155 / 96). A Critical View from the Generic Pharmaceutical Industry. *ICH Harmon. Tripart. Guidel.* 53 (2007).

82. Nasr, M. M. Pharmaceutical Development ICH Q8 / Q (8) R. (2008).

83. Ende, D. am, Bronk, K. S., Mustakis, J., O'Connor, G., Santa Maria, C. L., Nosal, R. & Watson, T. J. N. API quality by design example from the torcetrapib manufacturing process. *J. Pharm. Innov.* **2**, 71–86 (2007).

84. Sethuramiah, A. & Kumar, R. Statistics and Experimental Design in Perspective. in *Modeling of Chemical Wear* 129–159 (Elsevier, 2016). doi:10.1016/b978-0-12-804533-6.00006-8.

85. OFAT II | Dr. Muzaffar Bin Zainal Abideen. https://people.utm.my/muzaffar/ofat-ii/.

86. Rüttimann, B. G. & Wegener, K. The Power of DOE: How to Increase Experimental Design Success and Avoid Pitfalls. *J. Serv. Sci. Manag.* **08**, 250–258 (2015).

87. R-Series configurations - Vapourtec. https://www.vapourtec.com/products/r-series-flow-chemistry-standard-configurations/.

88. Mostarda, S., Maz, T. G., Piccinno, A., Cerra, B. & Banoglu, E. Optimisation by design of experiment of benzimidazol-2-one synthesis under flow conditions. *Molecules* **24**, 1–10 (2019).

89. Hegarty, A. F. & Frost, L. N. Elimination-addition mechanism for the hydrolysis of carbamates. Trapping of an isocyanate intermediate by an o-amino-group. *J. Chem. Soc. Perkin Trans. 2* 1719–1728 (1973) doi:10.1039/p29730001719.

90. 12 Principles of Green Chemistry - American Chemical Society.

https://www.acs.org/content/acs/en/greenchemistry/principles/12-principles-of-green-chemistry.html#prevention.

91. Glasnov, T. N. & Kappe, C. O. The microwave-to-flow paradigm: Translating high-temperature batch microwave chemistry to scalable continuous-flow processes. *Chem. - A Eur. J.* **17**, 11956–11968 (2011).

92. Aronow, J., Stanetty, C., Baxendale, I. R. & Mihovilovic, M. D. Methyl glycosides via Fischer glycosylation: translation from batch microwave to continuous flow processing. *Monatshefte fur Chemie* **150**, 11–19 (2019).

93. Nogueira, D. O., De Souza, S. P., Leão, R. A. C., Miranda, L. S. M. & De Souza, R. O. M. A. Process intensification for tertiary amine catalyzed glycerol carbonate production: Translating microwave irradiation to a continuous-flow process. *RSC Adv.* **5**, 20945–20950 (2015).

94. Wiles, C. & Watts, P. Translation of microwave methodology to continuous flow for the efficient synthesis of diaryl ethers via a base-mediated SNAr reaction. *Beilstein J. Org. Chem.* **7**, 1360–1371 (2011).

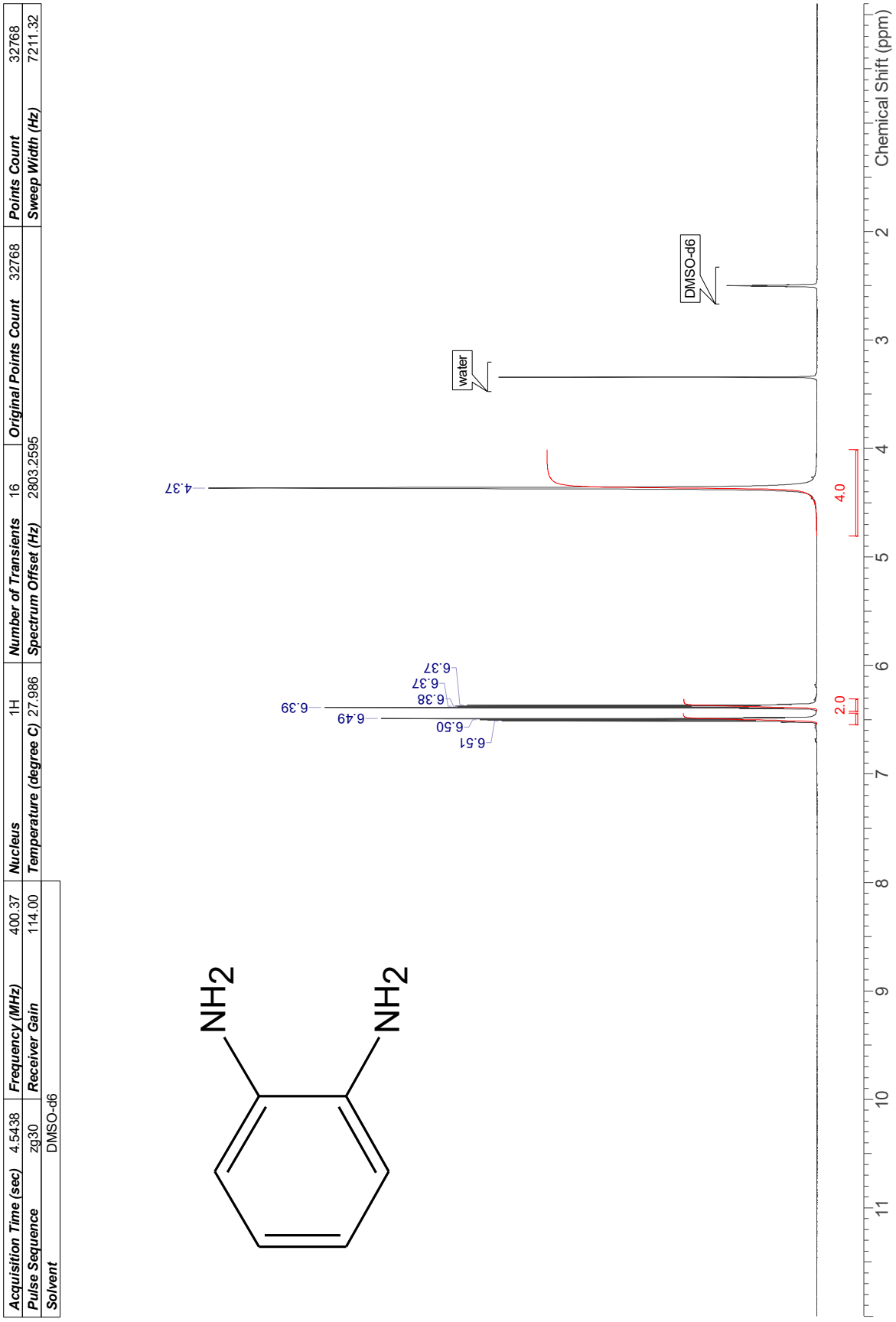
95. Znidar, D., Cantillo, D., Inglesby, P., Boyd, A. & Kappe, C. O. Process Intensification and Integration Studies for the Generation of a Key Aminoimidazole Intermediate in the Synthesis of Lanabecestat. *Org. Process Res. Dev.* **22**, 633–640 (2018).

96. Kappe, C. O., Pieber, B. & Dallinger, D. Microwave effects in organic synthesis: Myth or reality? *Angew. Chemie - Int. Ed.* **52**, 1088–1094 (2013).

97. Chen, L., He, Y. & Wong, J. C. Novel N-(2-amino-phenyl)-amide Derivatives. 91 (2009).

98. Reaction Time Calculator - FlowSyn - Uniqsis. https://www.uniqsis.com/supTime.aspx.

10 Appendix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Sweep Width (Hz) 7211.32
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NH2
4.37
6.39
6.49
6.38
6.37
6.37
6.50
6.51
water
DMSO-d6
4.0
2.0
Chemical Shift (ppm)

Acquisition Time (sec)	4.5438	***Frequency (MHz)***	400.37	***Nucleus***	1H	***Number of Transients***	16	***Original Points Count***	32768	***Points Count***	32768
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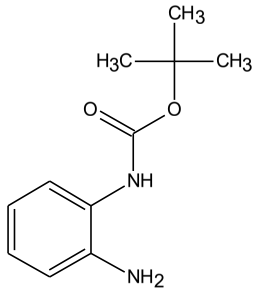

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Pulse Sequence	zg30	***Receiver Gain***	203.00	***Temperature (degree C)***	27.986	***Spectrum Offset (Hz)***	2803.2595			***Sweep Width (Hz)***	7211.32
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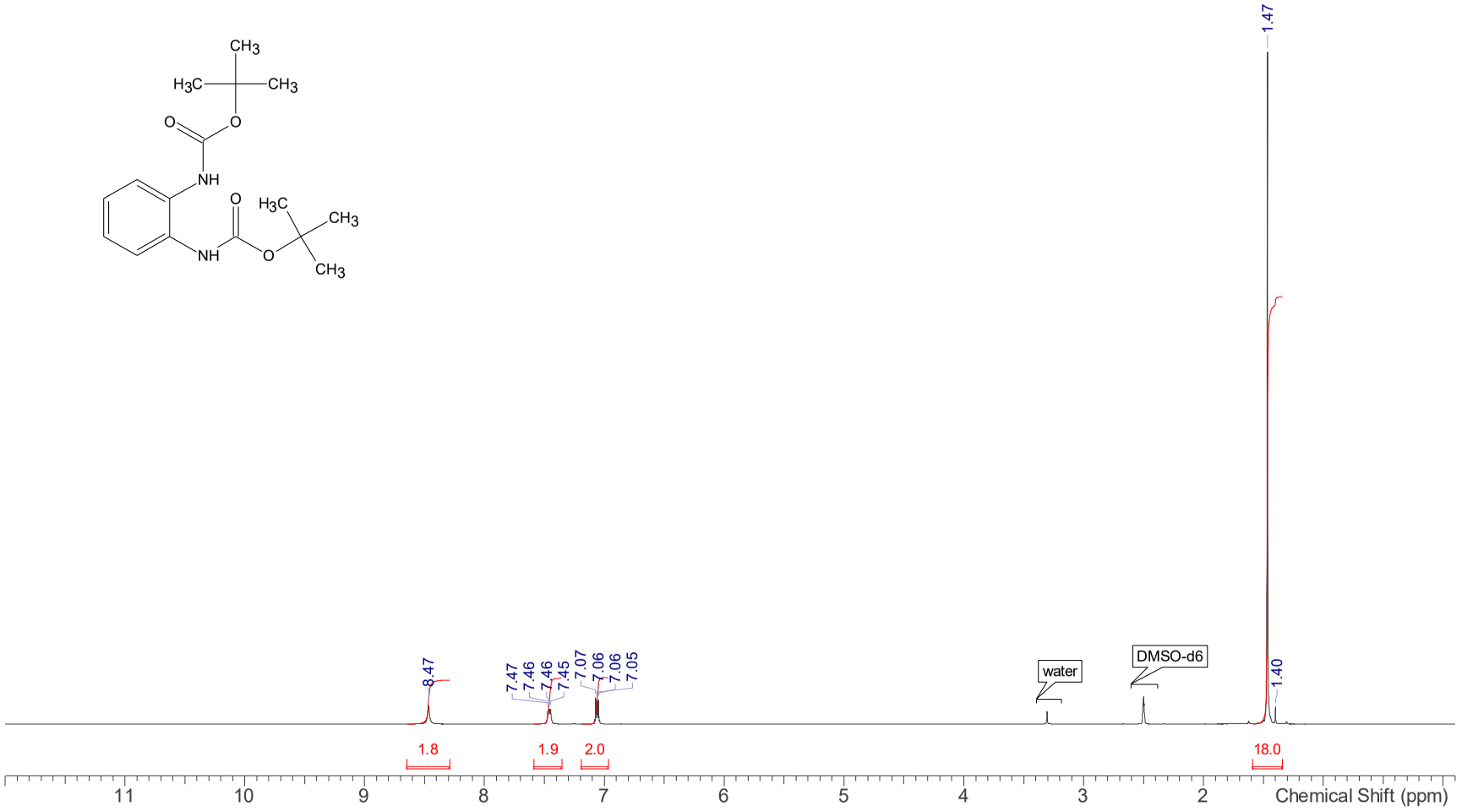

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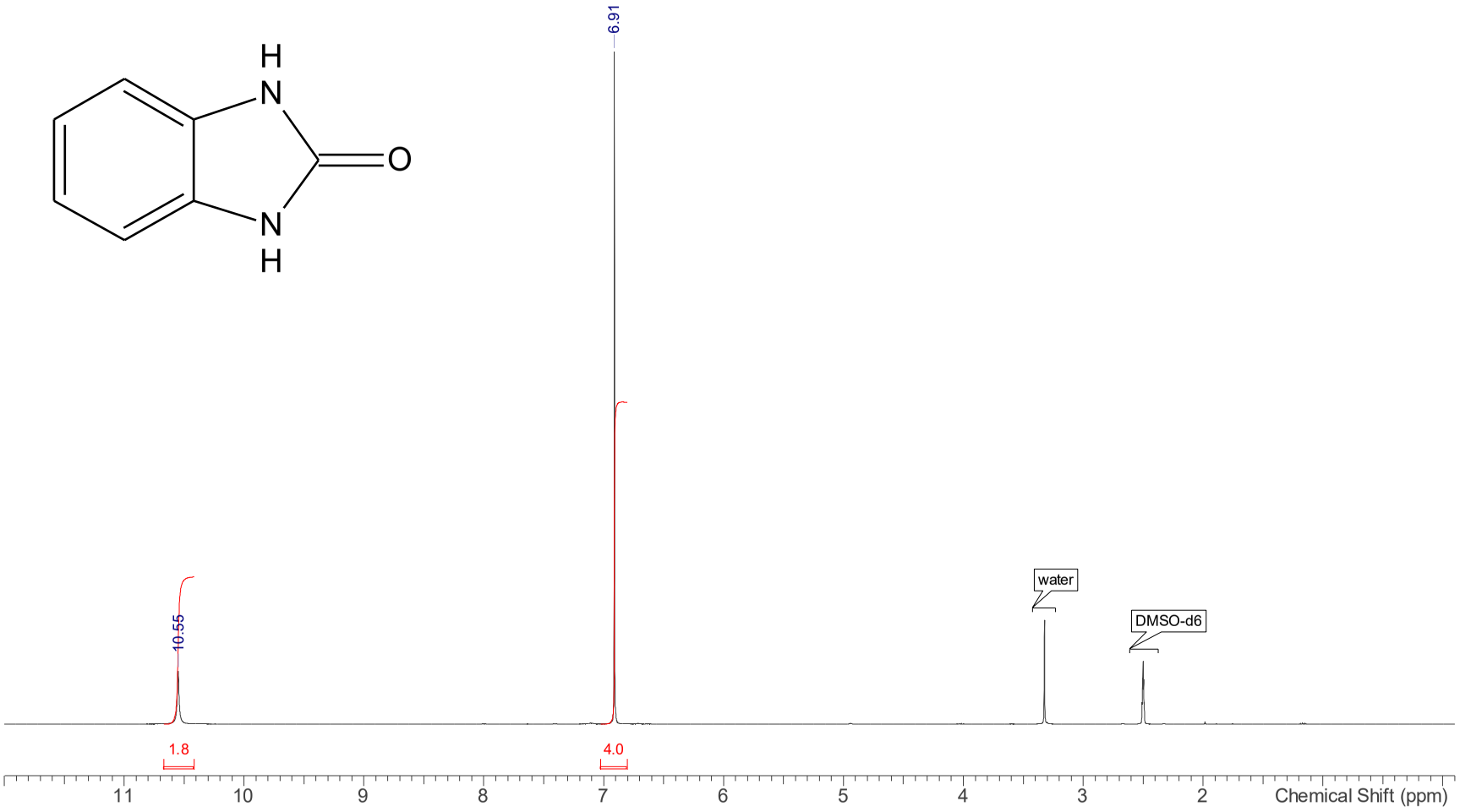

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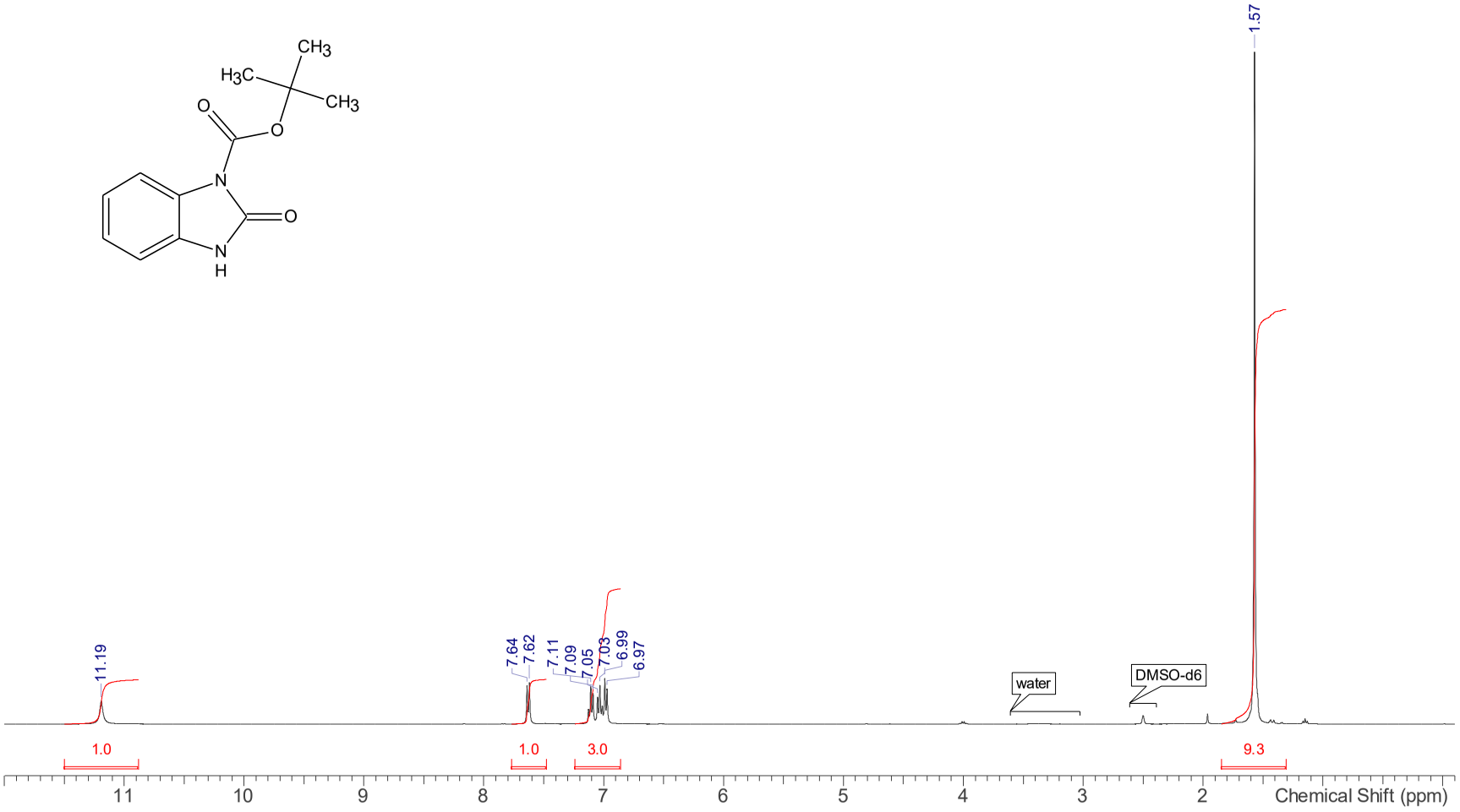

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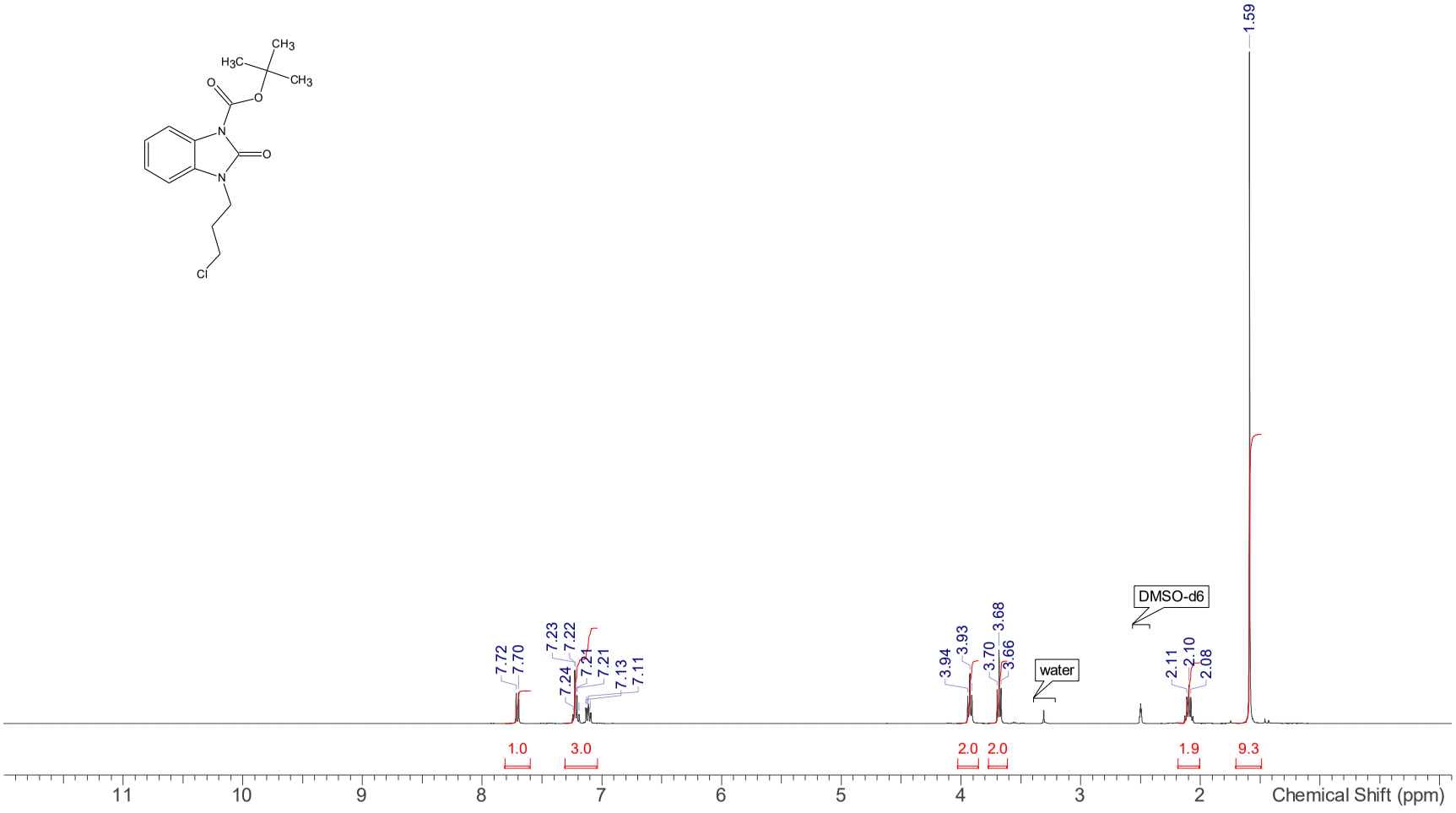

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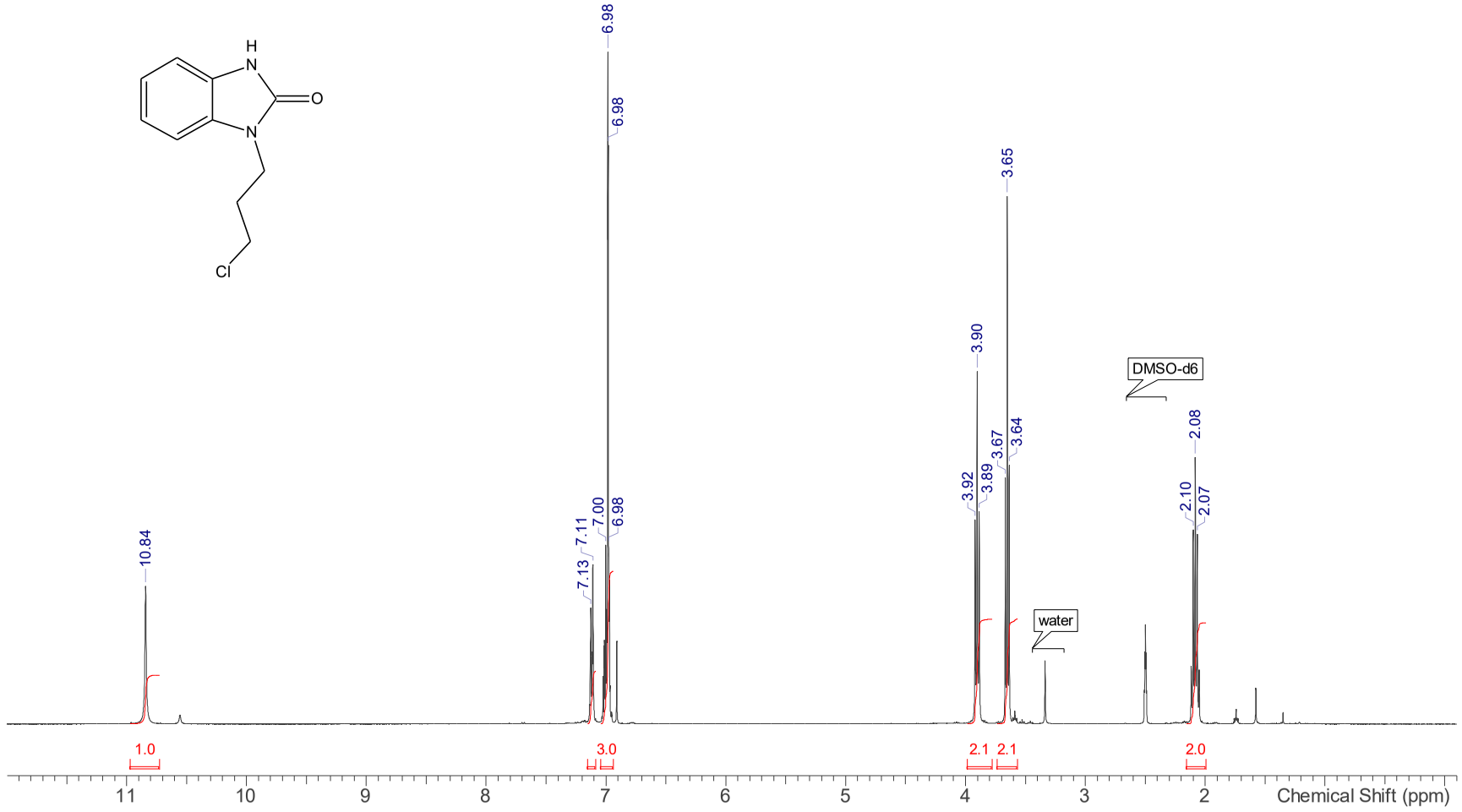

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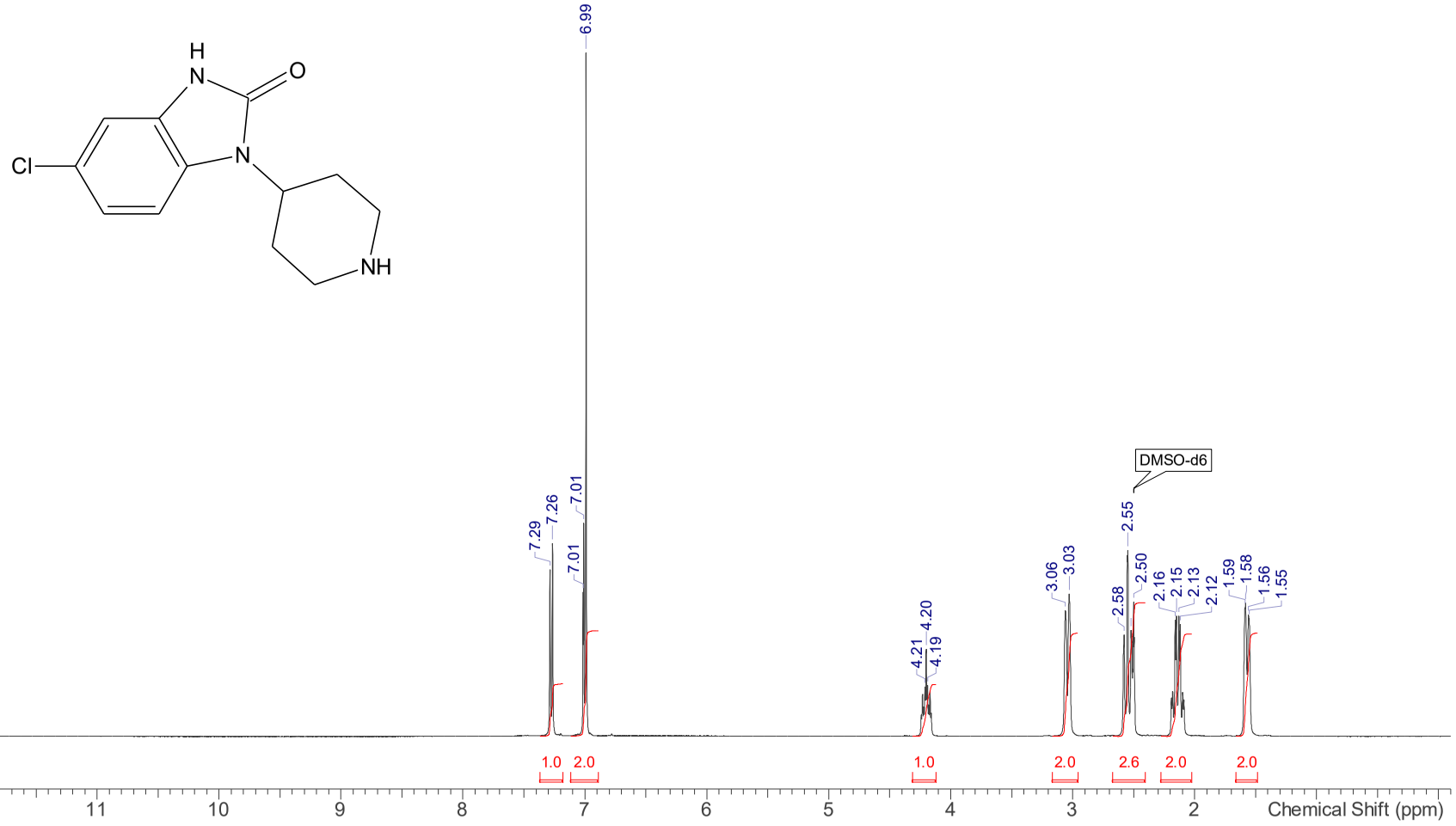

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