NELSON MANDELA UNIVERSITY

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FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

Master of Science: Chemistry

Continuous flow synthesis of imatinib intermediates

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Abstract

In this thesis, an alternative approach using continuous flow chemistry towards imatinib intermediates is described; an important drug in the treatment of acute myeloid leukemia. Various protocols that describe the multistep batch organic synthesis of imatinib are outlined. Many of the batch synthetic protocols require long reaction times in the multistep synthesis towards the various imatinib intermediates.

A broad description into the cancer epidemic such as myeloid leukemia, the cost of drug manufacture and the effect that the high cost of manufacture has on the accessibility to such treatment in Africa is outlined. Use of continuous flow reactors, the exploitation of various technologies and their advantages on organic synthesis compared to batch synthesis are also described.

The batch reaction conditions needed for the multistep transformation towards imatinib were adapted to a continuous flow set up. The optimization investigation shows an improvement in the conversion in the various steps. The flow synthesis of the enaminone provided a conversion of 99% when in *o*-xylene and the ability to use backpressure regulators assisted the investigation at high temperatures. Solution-phase flow synthesis of the guanidinium nitrate, which gave low yields in batch, also showed an improvement in conversion, where in 30 minutes a conversion of 99% was confirmed by altering the co-solvent mixture. The cycloaddition reaction of the enaminone and the guanidinium nitrate salt, achieved 90% conversion to the 2-aminopyridine core at 180^{o}C. The nitro group reduction was achieved in the presence of a greener catalyst, namely iron pentanedionate, in the presence of hydrazine hydrate. The effect of temperature, molar equivalence and solvent on reaction conversions could be observed in these steps.

The thesis is concluded in chapter 4, with the conclusion and recommendations for future work towards a scalable continuous flow synthesis of the imatinib intermediates.

Research output

- Harold Rupapa, Prof. Paul Watts-An alternative synthesis technique towards imatinib intermediates using continuous flow systems (*in preparation*)

Preface

This research was undertaken at North Campus Flow Chemistry research laboratory at Nelson Mandela University in the period February 2018 to December 2019 under the supervision of Prof. Paul Watts. The National Research Foundation and Nelson Mandela University provided the funding that allowed this research to be undertaken.

Acknowledgements

Firstly, I would like to thank Prof. Paul Watts for his unwavering support in my research, patience, honesty when needed and understanding. His support and advice has allowed me to hold strong and overcome the challenges faced in this research. I am also very grateful to the National Research Foundation (NRF) and the Nelson Mandela Research Scholarship, for the financial assistance in this carrying out this research.

The Nelson Mandela Flow Chemistry research group has provided immense support by sharing of ideas, laughter and stimulating discussions. This research would have not been possible without the environment created by my fellow researchers, and for this, I am truly grateful. My heartfelt gratitude, specifically to Dr. Faith Akwi for her friendship and willingness to help always.

Lastly, I am grateful for the support of my family and my mother who raised to this point as single mother and I never went to bed hungry. It has not been an easy journey but all glory, power and honor belong to our Father God.

Declaration

I **Harold Takunda Rupapa** do hereby declare that the work that is described is my own and was carried out under the supervision of Prof. P. Watts between February 2018 and November 2019 at the NMU Flow Chemistry Research Laboratories.

Harold Rupapa

December 2019

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List of Abbreviation

APIs	Active pharmaceutical ingredients
Arom.	Aromatic
BPR	Backpressure regulator
br	Broad signal
$CDCl_3$	deuterated chloroform
D_2O	Deuterium oxide
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
DMSO	Dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
EtOAc	Ethyl acetate
ID	Internal diameter
FT-IR	Fourier transform infrared spectroscopy
HPLC	High Pressure Liquid Chromatography
Hr	Hours
J	Coupling constant
LTF	Little Things Factory
M	multiplet
mins	Minutes
NMP	*N*-Methyl-2-pyrrolidone
Olef	olefin
PTFE	Polytetrafluoroethylene
s	Singlet
TLC	Thin Layer Chromatography
δ	Chemical shift

CHAPTER 1: INTRODUCTION

1.1 The cancer epidemic

Cancer brings a major burden of disease worldwide and is a leading cause of mortality globally. The burden of disease caused by cancer is expected to increase as the world population increases. This greatly applies to low- to middle-income countries, which are projected for a mortality rate of 70% for all cancer incidences.[1] Although the incidence of cancer is higher in high-income countries, the mortality rate in low-income countries is higher. This is owing to the lack of access to essential medication, latest methods of treatment, cost to access essential treatments and early detection that are otherwise available in high-income countries. Such high mortality rates have an enormous impact on the counties that are affected, especially because of premature mortality and lost productive life years of those younger than 65 years old.[1] The risk factors of developing cancer, besides genetic, are further increased by a bad diet, lack of physical exercise, obesity and smoking. Thus, the chances of cancer becoming the number one killer in the low to middle-income counties will continue to increase, unless systems are put in place for improved cancer prevention and management.[1]

The multistep process involved in the development of cancer involves mutation and selection for cells with progressively increasing capacity for proliferation, survival, invasion and metastasis.[2] Genetic and environmental factors have the capability of playing a crucial role in the occurrence and transformation of normal cells to neoplastic cells, whose cell division is abnormal and uncontrolled.[2]

Cancer treatment in our world today has seen improvements in patient management and oncological care because of advances in the knowledge and understanding of the disease. Ever since the declaration of the 'war on cancer' by the former American president Richard Nixon in 1971,[3] major developments in systemic cancer therapy, drug research and trials has been done. The 50 years that would ensue, would see an improvement in precision in laboratory-based diagnostics and treatment availability across the globe. Better staging and detection of cancer has also been made possible due to improvement in imaging services *e.g.* mammograms in the detections of breast cancer.[3]

Most Africans are impacted by their socio-economic status, which presents them with great barriers when any cancer diagnosis is made.[4] The dilemma of providing affordable health care, especially for patients with cancer, has continued to haunt most developing countries across the

globe. This is mainly owing to the high expense of the drugs developed by pharmaceutical companies and lack of insurance and/or medical aid on the part of the diagnosed patients. This directly means the greater population living in poorly performing economies would not have access to these medicines, thus leading to high mortality rates. Since 75% of the world population comprises of developing countries, it is concerning that access to medicines, which are normally patent protected continues to be restricted.[5] The high cost of living and reduced access to disposable income among these populations leads to major problems concerning access to medicine as the drugs are generally high-priced.[5] Standard care for patients who are either partially insured or uninsured remains unaffordable and this produces a huge burden on the states who have to procure treatment for about 85% percent of the population who are dependent on the public health sector.[6]

1.1.1 Cancer treatment in Africa

The population in Africa is expected to rise to 2.5 billion by 2050; the cancer incidence rate is also predicted to grow with the growing population. This will therefore lead to a predicted increase in the need for affordable healthcare and cancer medication for affected patients. This predicted number of people are expected to mainly be those that dwell in low and middle income countries.[7] A rise in cancer incidents is mainly due to lifestyle choices that is lack of exercise, smoking, a higher life expectancy and the improved treatment of diseases that may have been otherwise life threatening.[6,8] The healthcare systems that are currently functioning in most sub-Saharan countries are besieged by the increase in demand for treatment by the ever increasing number of patients.[6]

Although measures have been put in place; whereby developing countries in Africa are able to purchase cancer drugs, access chemotherapy and hormone therapy at a cheaper price as opposed to the higher prices in developed countries such as USA. Sub-Saharan Africa is still affected by the unaffordability of the new generation cancer drugs, even with the discounted prices. One way to counteract this is for South Africa (and Africa as a whole) is find ways to improve the cost of producing the drugs by developing new methods of drug synthesis.[6,9]

South Africa, in comparison to its African counterparts, has made considerable strides into assuring better and affordable healthcare for the greater part of the society, with free access to some medicines in most public hospitals. However, there still remains a need for better financial investment into drug research that seeks to improve and optimize the manufacture of active

pharmaceutical ingredients, which will improve access to medication in Africa as whole.[10] This is owing to the fact that Africa remains a huge importer of APIs and in 2017 active pharmaceutical ingredients importation in Africa accounted for about 85% of the overall pharmaceutical industry trade.[10] This importing of APIs brings great challenges, which include little to no creation of jobs, and thus no overall improvement in the societal standard of living or and the country's economy.

1.1.2 Myeloid leukemia

Leukemia is a form of cancer that is characterized by the over production of immature white blood cells, called myeloblasts. These immature white blood cells are incapable of performing their normal function within the body, such as fighting infections and healing wounds and the normal apoptosis. Leukemia mainly affects the bone and blood marrow, where the myeloblasts fill up the bone marrow and impede the normal production of white blood cells.[11] This eventually leads to an increased risk of infection.[11,12]

Myeloid leukemia is differentiated from other forms of cancer by the presence of the unique abnormality present at chromosome 22 in the human genome.[12] The disease has different stages that it undergoes, which are referred to as chronic/stable, accelerated and blast stage. Characterization of the different stages of myeloid leukemia is dependent on which stage the cancerous myelogenous cells are. The chronic stage is identified by the presence of the myeloid cells with normal differentiation. The blast and accelerated stage almost occur simultaneous as the disease progresses; a transformation occurs by the accumulation of molecular abnormalities that lead to a progressive loss of the capacity for terminal differentiation of the leukemic clone.[13,14]

Targeted cancer therapy serves as a crucial area in the treatment of different cancers, including acute myeloid leukemia (AML), that is, the treatment given works to disrupt certain molecules important in the further proliferation of cancerous cells.[15] These, in the early stages, include but are not limited to stem cell transplantation, hydroxyurea or interferon-α-based regimens. The median age for the incidence of acute myeloid leukemia are 50-60 years,[13] this presents a limitation in being able to identify a suitable bone marrow donor to the majority of the patients and thus eliminates this as an option. With the above explained treatment option, less than 20% of diagnosed patients are able to be cured of the disease.[14] Thus, the next line of drug treatment is of utmost importance to the affected patients, since there is an improvement in survival rates per particular drug dosed to the patients affected by leukemia.

1.2 UTILIZATION OF FLOW CHEMISTRY IN ORGANIC SYNTHESIS

Traditionally, drug synthesis has long relied on batch chemistry in order to synthesize the desired products. Batch operations at a large scale in production facilities are expensive to set up and maintain, which makes continuous flow techniques increasingly utilized because of the advantages that it brings, which include time efficiency, safety and cost saving, which would otherwise be impossible in batch chemistry.[16] Complex products in organic synthesis are synthesized using batch protocols by iterative systematic transformation from simpler precursor. In batch synthesis, as shown in Figure 1(a), most commonly each synthetic step will be followed by purification to remove any undesired byproducts that may interfere in the subsequent synthetic transformations.

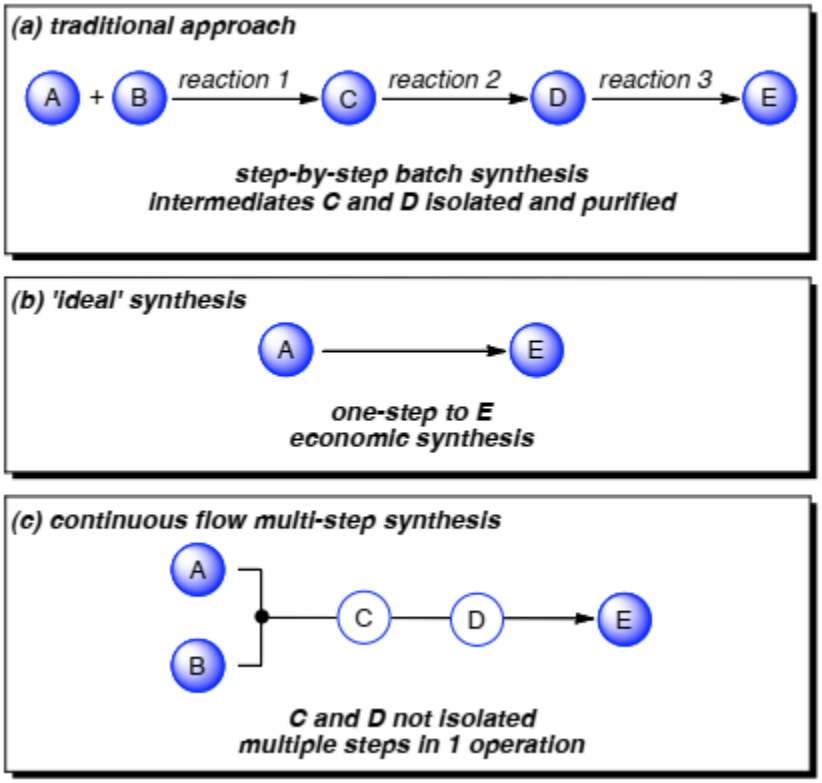


Figure 1: Comparison organic synthetic strategies[17]

This method of organic synthesis is time-consuming, resource-intensive and has a large labour demand, which in turn transfers high costs to the consumer.[17,18] Ideally, in organic synthesis it would be economically ideal to synthesize products directly from simple precursors (Figure 1(b)). This is referred to as “one pot synthesis”, which is an effective strategy, which aims to synthesize

a target compound by way of successive chemical reactions in a single vessel. However, this is unlikely to be achieved in practice due to the different complexities in many organic reactions.[17,19] Figure 1(c) represents a recent advance in organic synthesis to merge multiple synthetic steps[20] in a single continuous reactor network, with the aim of streamlining and avoiding isolating intermediates within the synthetic route.[17,19]

Cost savings can be observed through continuous flow synthesis' ability to simplify reaction processes, and reduce the capital expenditure by up to 50% and can be even more in certain instances.[21,22] It is also important to note that, batch synthesis procedures can be adapted to a condensed and designated continuous flow process.[23] The overall flow system usually shows a reduced footprint to achieve similar yields compared to its batch counterparts. An ideal example is the system shown in Figure 2, which comprised of five reactors coupled with multiple recirculating temperature controllers, weighing scales and pumps for the reactions involving a Grignard reaction, a formylation coupling, a reduction, quench and finally the addition of a cosolvent. The footprint (50 square feet) is greatly reduced with a walk-through double-sided hood.[21]

Figure 2: Flow process design consisting of a series of five reactors coupled with multiple recirculating temperature controllers, scales and pumps[21]

Inputs such as energy consumption and solvent use when using continuous flow synthesis can be reduced, resulting in favorable cost benefits. In certain reactions, because of enhanced process control when using continuous flow synthesis, the use of solvents can be greatly reduced or eliminated.[24,25] Thus, the cost of manufacture, which is also associated with disposal of solvent and chemical waste, are minimized with a resulting process intensification.

Multiple studies that have been carried out have illustrated that continuous flow processes have reduced total operational costs by up to 50% compared to traditional batch protocols, partly due to the lower labor requirements and fewer analytical procedures.[21] In using batch procedures for synthesis of target compounds, there exist some constraints as a result of infrastructure, such as achievable reaction pressures, temperature and equipment cost and availability.[26,27] This results in process engineers and chemists, in some instances, utilizing flawed synthetic routes, which produce impurities problematic in organic synthesis. Continuous flow synthesis can drastically reduce the formation of such impurities by process designs that aim to reduce impurities, such as adapting and manipulating various batch reactions to a single continuous flow design.[21,26,27] Utilization of continuous flow processes in organic synthesis can lead to reduction in extensive work ups, lower footprint and reduction of lengthy reaction times. The benefits of continuous flow synthesis are further discussed in detail in comparison to the batch synthetic approach.

1.2.1 Implications of flow chemistry in organic synthesis

1.2.1.1. Increased Yields and Reaction Selectivity

1.2.1.1.1. Mixing in Batch versus Microreactors

Organic synthesis has long relied on the millennia old technique of synthesis in batch reactions with careful weighing of the starting reagents added and careful control of temperature to produce the desired product. Round bottom flasks and/or batch reactors, which are used in large-scale production, bring a disadvantage of creating a non-uniform flow field when stirring a reaction. Mixing only occurs once the fluid reaches the stirrer's field of stirring, and only then is convection induced and assisting the mixing of the reaction mixture.[28–30] The reactants that occur any further away from the stirrer's field of convection do not experience the same efficient mixing occurring close to the stirrer, which has been studied in depth by visualizations of mixing dyes, which

indicated that a change in the geometry and/or the stirring conditions has a great impact on the mixing efficiency.[31] These regions, which largely remain idle due to their distance from the convection region of the stirrer, experience poor heat transfer and concentration gradients which may lead to decreased product yields, selectivity and need for extremely long reaction times.[29,32]

However, the small dimensions associated with continuously flowing microreactors greatly improves mixing of reagents, even in some instance complete mixing occurring in microseconds. Various strategies have been employed to improve mixing of reagents in flow reaction chemistry. Figure 3 illustrates such strategies such as a Y-junction mixer (A), T-junction mixing (B) and multilamellar layering (C) of fluids like a "sandwich" which permits rapid diffusional mixing.[27] The Y-junction and T-junction mixer (Figure 3(A and B)) allows two fluids to come in contact with each other at a junction in a stable manner, which results in improved and rapid mixing.[33]

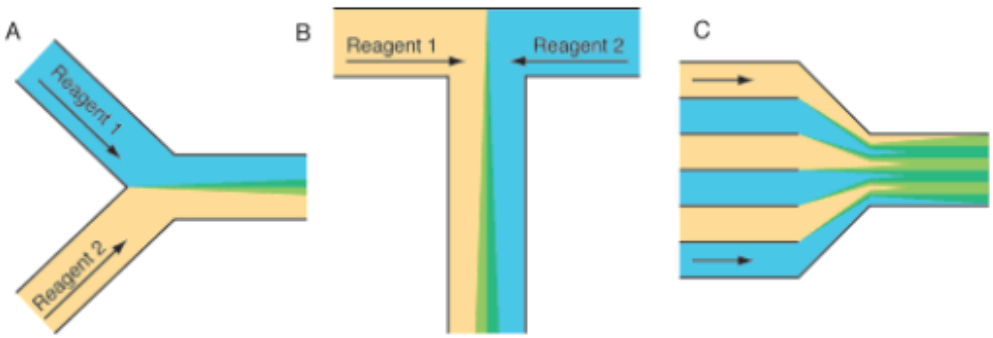


Figure 3: Various channel geometries: (A) Y-junction, (B) T-junction, and (C) interdigitated multilamellar mixer[27]

Figure 4 illustrates micro-scale reactors which often possess special microstructures, or external turbulences in their micro-channels in order to achieve efficient heat transfer and fluid mixing.[34] Special microstructures are usually designed to improve mixing by affecting the flow of the fluid, using different techniques to promote efficient, improved mixing which leads to an overall reduction in mixing time. Such micromixers are mainly used because of the reduced amount of fluids needed for the reaction. Micromixers become particularly useful when the mixing efficiency decreases as the size of the microreactors being using increases.[35] Thus, incorporating a micromixer at the inlet of the reactor can circumvent inefficient mixing and improve flow reactions.[34,35]

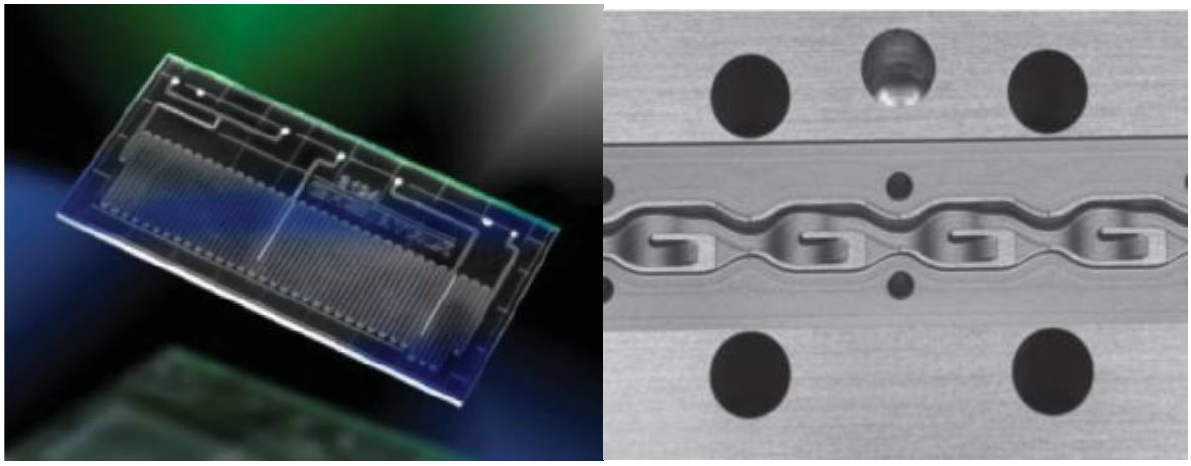


Figure 4: Photographs of glass microreactor (Chemtrix BV) with micro channels and split-recombine micromixer with dedicated microchannel structures for overall mixing efficiency

1.2.1.1.2 Thermal Management

Temperature plays a crucial role in chemical reactions, that is, if it is poorly regulated it can lead to extended reaction times, or if excessive heat is generated, it can lead to explosions that introduces danger. Improper control of temperature in reactions sensitive to temperature changes, can lead to undesirable by-products, and because of varied temperature distribution often associated in batch chemistry.[27,36]

Microreactors provide immense restriction in the temperature distribution, compared to batch reactors. The need for controllable and narrow distribution of temperature is associated with the possibility of side reactions that may occur during a typical reaction. The large surface area to volume ratio available when using microreactors, allows for the input and removal of heat, thus permitting almost equal temperature distribution during the reaction.[29,36] The microreactors used in microfluidics such as those made of borosilicate glass and metal also bring the advantage of being able to withstand high temperatures and due to low thermal expansions, rapid temperature changes may be carried out in flow reactions.[29,37] Figure 5 and 6 illustrates the temperature distribution associated with batch reactors and microreactors respectively. As Figure 5 shows, the uneven temperature distribution that can lead to side reaction occurring, resulting in lower yield of the target compound in some instances. The almost even temperature destitution introduces an efficiency that cannot be easily attained in batch reactors, thereby allowing efficient organic synthesis towards desired products and preventing side reaction from occurring.[27,37,38]

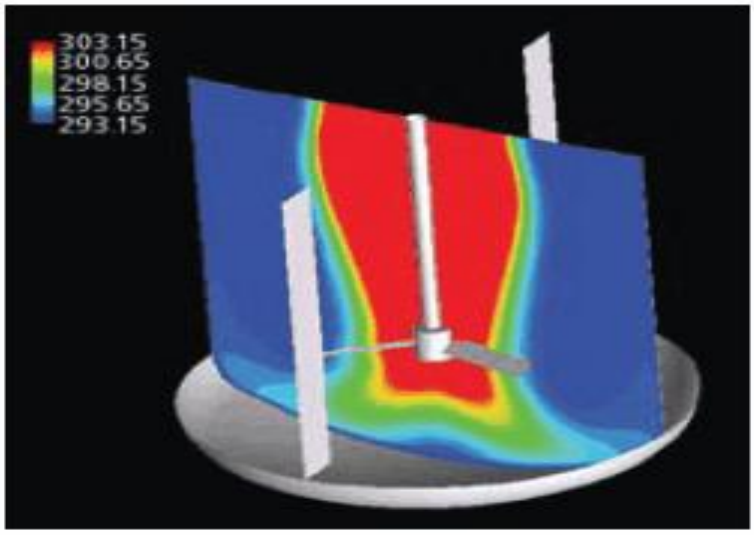


Figure 5: Batch reactor temperature distribution during a typical reflux reaction[38]

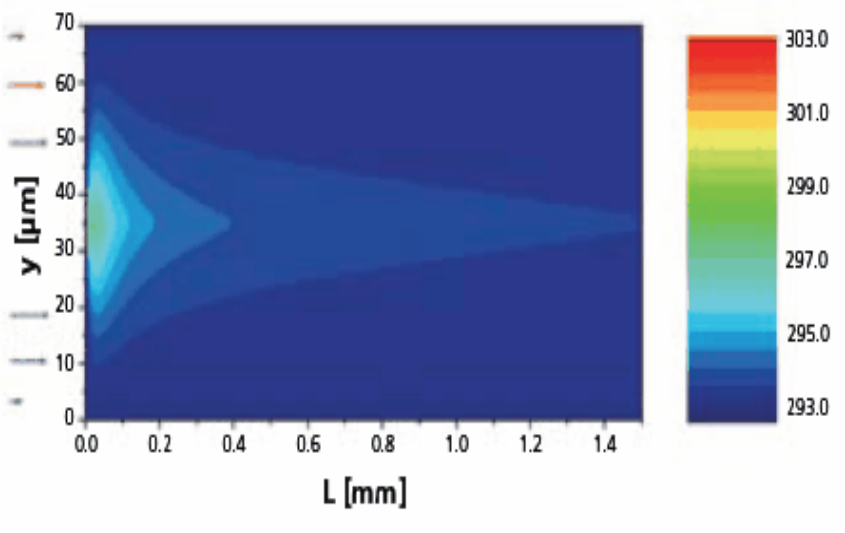
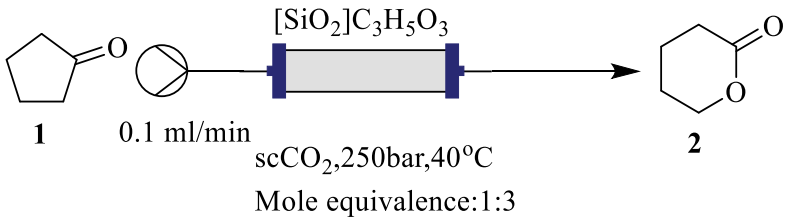


Figure 6: Microreactor temperature distribution shows efficient and equal temperature distubution[38]

1.2.1.1.3. Increased Rates of Reaction, Yields and Selectivity

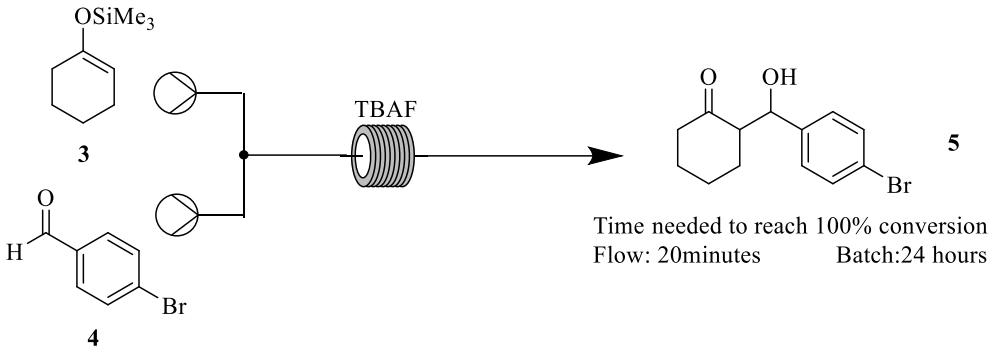
The selectivity in certain reactions, as well as the yield, can be significantly improved when batch reactions are transferred to continuous flow synthesis, mainly because of the rapid heat transfer and improved mixing.[27,39] Improvement in selectivity were illustrated by Mello *et al.*[40] whereby the oxidation of sulfides to sulfoxides in supercritical carbon dioxide ($scCO_2$) was able to be optimized by controlling the pressure of the continuous flow system and the hydration of the solid supported catalytic oxidant (hydrated [2-percarboxyethyl]-functionalized silica). This oxidation reaction required reflux for 48 hours in order to achieve a mixture of the lactone and carboxylic

acid with a yield of 29% of the desired lactone and the carboxylic acid as the by-product. A molar ratio of ketone:peracid (1:3) and $scCO_2$ was pumped through a column packed with the substrate ($[SiO_2]C_3H_5O_3$). The conditions shown in Scheme 1 illustrate a highly pressurized system at 250 bar and a temperature of 40°C, allowed for a 99% yield of the corresponding lactone exclusively, with the system depressurized with a micrometric flow control valve.[40] The batch reaction previously required 48 hours to achieve low yield; the reaction was significantly improved using flow techniques, which saw the time needed to afford a 99% yield reduced to 20 minutes. This illustrated the ability to eliminate the corresponding carboxylic acid by-product and improve selectivity towards the desired lactone **2**, which would have needed to be separated from the final product.[40]



Scheme 1: Oxidation of ketones with packed bed reactor containing [SiO₂]-CH₂CH₂COOOH in scCO₂[40]

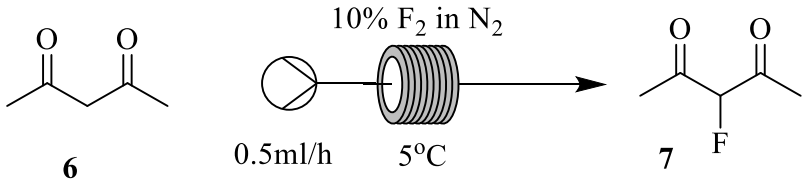
Wiles *et al.* (Scheme 2) carried out an aldol reaction of an aldehyde **3** and a silyl enol ether **4** in the presence of tetrabutylammonium fluoride (TBAF). This aldol reaction, which in batch conditions required 24 hours to reach completion, only needed 20 min to afford a 100% conversion.[41] This reaction is just one of many reactions, which demonstrated dramatic improvements when transferred to microreactors used in flow chemistry. The time needed to achieve a 100% conversion was reduced by 72-fold, from 24 hours to 20 minutes, due to the advantages of rapid heat transfer and improved mixing in microreactors compared to batch reactors.



Scheme 2: Aldol synthesis in both batch and continuous flow synthesis conditions and yield[34,35]

1.2.1.1.4. Increased Safety

Use of flow chemistry in the synthesis of organic compounds brings added advantages over batch synthesis. Continuous flow synthesis provides the ability to safely add small amount of reactants at an instant, which allows highly exothermic reactions to be carried out.[42] The safety concerns surrounding fluorination reactions is one good example because they are generally exothermic, problematic to control the temperature during large-scale synthesis and toxic hydrogen fluoride as a byproduct. Direct fluorination using microreactors is ideal because only a small amount of the fluorine within the microreactor at a given time, thus improving safety concerns normally associated by such reactions. [26,43] Chambers and Spink (Scheme 3), were able to illustrate an ideal example where the safety concerns with the fluorination of ethyl acetoacetate **6** at 5^{o}C to provide a 99% yield to ethyl 2-fluoroacetoacetate **7**, were greatly reduced.[26,43]



Scheme 3: Reagents and condition for the direct fluorination of ethyl acetoacetate[43]

1.2.1.1.4. Compartmentalization

In laboratory reactions and manufacturing industry, use of organic-aqueous biphasic catalysis reactions has been widely adopted because of the advantages these reactions present. These advantages include water-soluble catalysts can be effortlessly recycled, while being concurrently tolerating hydrophobic and hydrophilic reactants.[44,45] The draw back when dealing with such biphasic reactions is that there is complexities involved in separating the catalyst from the product

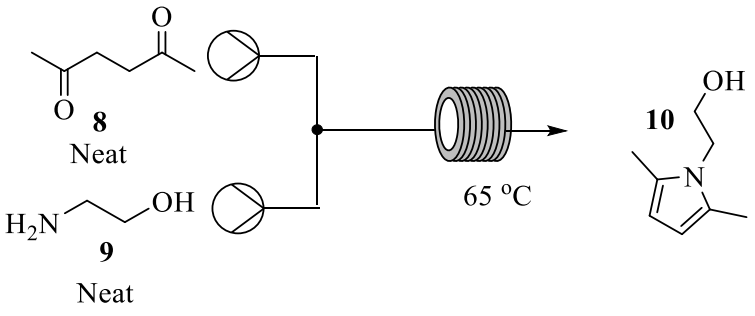
and solvent which would need a batch reactor and there is need to input a mechanical agitation to mix the two phases.[44,46] This type of segmented flow that features alternating "segments" of immiscible liquids or gases presents a problem of phase separation that may occur as the during flow.[47]

In order to address issues that may inadvertently arise where mixing between the reaction and carrier slugs may occur compartmentalized flow was discovered. In compartmentalized flow, a small of volume of immiscible solvent is introduced to bracket each reaction segment.[44,48] The poor solubility of the organic substrates in the presence of immiscible solvent presents a barrier to diffusion, thus eliminating organic phase separation. This approach is unique and eliminates the use the issues of segmented flow by using the immiscible solvent as a spacer that isolates the reactions segments as they move through the reactor.[48]

1.2.1.2 Enhanced reaction efficiency

1.2.1.2.1. Decreased Inputs and Waste

Reactions ran using microreactors, as mentioned earlier, assist in improving reaction yields and selectivity. The rapid transfer heat capability associated with microreactors allow reactions in continuous flow reactions to run at higher concentrations. The continuous developments and introduction of *in situ* purification procedures in continuous flow synthesis create greatly reduced waste.[49] Microreactors can function to reducing waste, by allowing the use of smaller volumes of solvents, starting material and thus byproducts produced per unit product, compared to reactions that are carried out in batch reactors and especially during optimization reactions.[24,49] Flow techniques using microreactors can even more so allow the use of neat reagents, which would otherwise, be exothermic in a batch reactor system.[49]

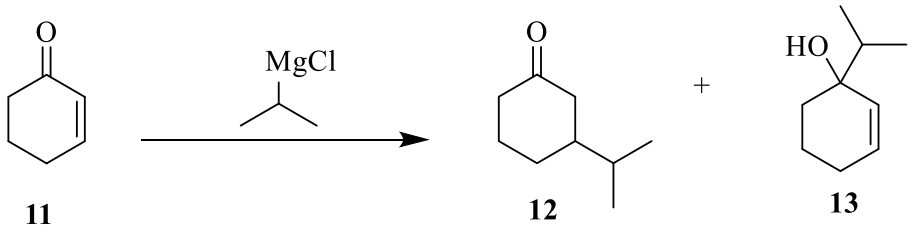


Scheme 4:Flow synthesis 2-(2,5-dimethyl-1H-pyrrol-1-yl)ethan-1-ol in a solvent free system of solvent-free Paal-Knorr[24]

Taghavi-Moghadam *et al.* established a flow synthesis route (Scheme 4) for the reaction towards the synthesis of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl) ethan-1-ol **10**, in a microreactor system and were able to eliminate use of solvent in the reaction producing an isolated yield of 98%. This reduces the waste and costs associated with doing this particular reaction in the presence of a solvent, and this Paal-Knorr reaction is exothermic when done under batch conditions in the absence of a solvent.[24,27]

1.2.1.2.2. Low-Volume Optimization Experiments

Researchers undertaking the cumbersome process of establishing the optimum reaction conditions as those shown in Scheme 5, had to make various adjustments preceding the results. In the process of creating the ideal conditions to obtain the best yield and selectivity towards the desired product in flow synthesis, researchers make various adjustments, such as equivalence, temperature, solvent screening and residence time. Thus, during optimization reactions, using microreactors permit usage of very low volumes and short reaction times.[24,27] Reduction of the material added and used during optimization reactions will ultimately lead to lower costs, faster optimization reactions and overall waste reduction. Taghavi-Moghadam *et al.* demonstrated a selective Grignard reaction using flow chemistry starting with cyclohex-2-en-1-one **11** to yield 78 % of **12**/**13** at a 95:5 ratio compared to the transformation occurring in batch with a yield of 49% with a regioisomer ratio of 65:35 (**12:13**). In order to optimize this reaction, Taghavi-Moghadam *et al.* had to obtain the optimum reaction conditions by investigating 14 different reaction conditions in 6 hours, which during optimization miniscule amounts of the reagents per reaction condition being investigated.[24]



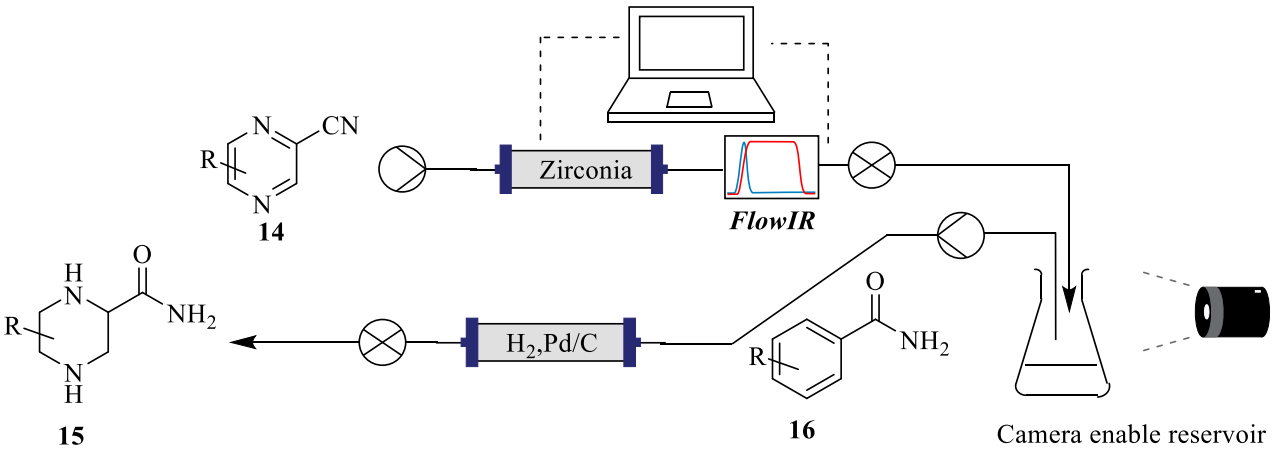
Scheme 5: Optimization of a Grignard reaction toward regioselectivity[24]

1.2.1.2.3 In-Line Reaction Monitoring

Microreactors in continuous flow synthesis brings the ability to analyze the product formed as the reaction progresses. This is particularly advantageous during optimization reactions, thus giving the ability to monitor product formation. One such in-line monitoring technology is inline infrared (IR) monitoring, which use a flow cell that monitors reagents consumption and desired product

formation as the reaction progresses, which assists in the optimization reactions.[50] Batch and flow reaction can use multiple systems to observe the progress of the reaction such as TLC, FTIR and HPLC.[46]

In-line reaction monitoring becomes particularly useful, because it provides the capability to track the formation of the desired product and when an undesired product is generated the system can be stopped to prevent a huge waste of reagents.[27,49,51] Ley *et al*. illustrated the practicality of in-line reaction monitoring in the preparation of piperazine-2-carboxamide (Scheme 6). In this flow route, they were able to illustrate the use of the open pen-source software package and a Raspberry Pi® computer and simultaneously utilize in-line IR monitoring. This allowed for the easy optimization of the initial step as shown in Scheme 6 where the initial nitrile hydration, to synthesize the corresponding benzamide was observed using IR to observe for the benzamide **15** formation.[52]

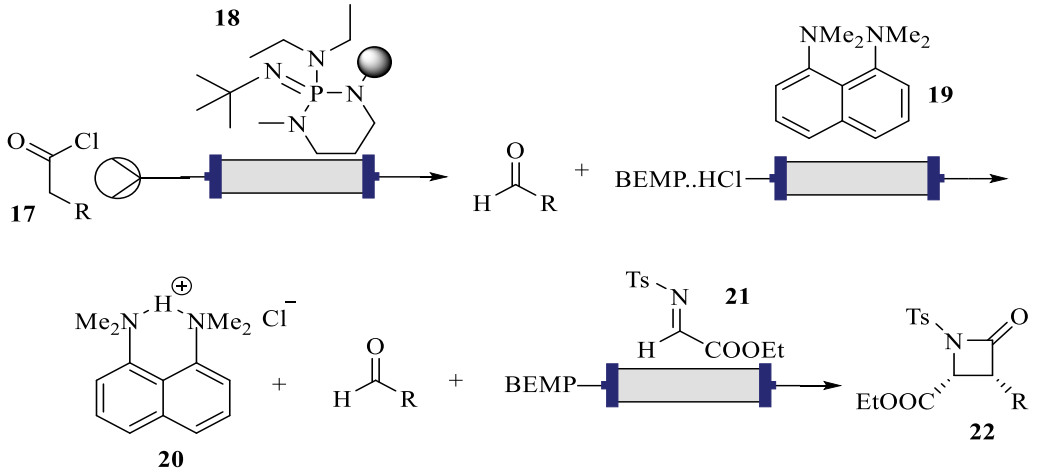


Scheme 6: Flow set up for the automated machine assisted synthesis of (R,S)-piperidine-2-carboxamide[52]

1.2.1.2.4 Introduction of Multiple Transformations with Continuous Flow

Multistep reactions towards a desired product can be carried out using continuous flow techniques; that is microreactors with a potential for upscaling can be connected in series. This can also allow addition of various reagents to the reaction as the reaction progresses, until the final product is isolated. Lectka *et al.*[53] reported, as shown in Scheme 7, a reaction process which utilized a packed-bed reactor 2-tert-Butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine on polystyrene (BEMP) **18** that can be connected in series when using solid-phase reagents. The packed bed reactor columns connected in series to synthesize *β*-lactams, and exhibited excellent enantio- and diastereoselectivity. The use of packed columns in most chemical reactions allows

for ease of reagent recovery, in-line purification and the ability of using various catalysts that would have otherwise been impossible to flow.[53,54]



Scheme 7: Catalytic, shuttle-base route to optically active β-lactams[53,54]

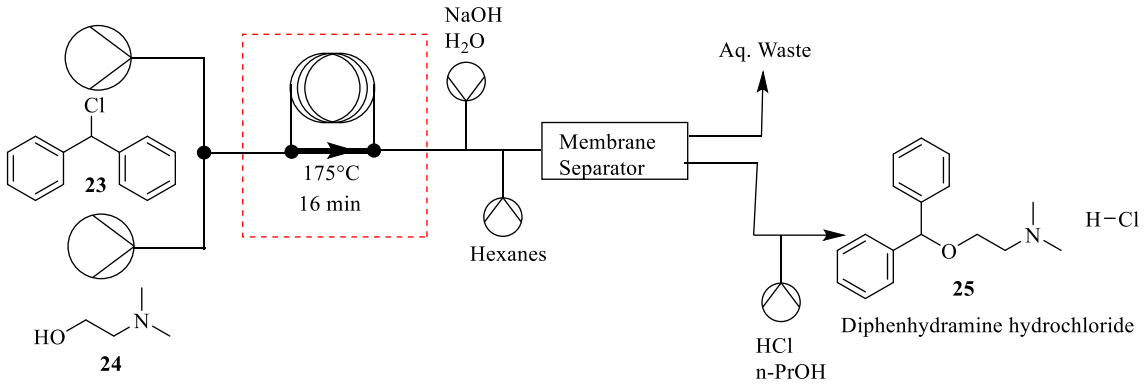
1.3. DRUG MANUFACTURE IN SOUTH AFRICA AND OTHER DEVELOPING COUNTRIES

Establishments such as the LaGray in Ghana and Fine Chemical Corporation (FCC) in South Africa (now part of Aspen) are some of the very modest API manufacturers on the African continent.[55] Countries within the Asia-pacific region *e.g.* India and China are the largest exporters of the APIs as the local drug manufacturing within the continent still remain inadequate to supply the demand to the growing population.[56] The biggest hurdle faced by most developing African countries is the ability to heavily invest in development activities such as research and development to further improve the capability of the African continent to produce its own APIs.[55] Active pharmaceutical ingredients are alone undesirable for human use, but are the active part of the final drug, that release a desirable effect in the treatment process.[55] Active Pharmaceutical Ingredients (APIs) are in the formulation of the final drug that is consumed by patients for treatment. The formulation ingredients are often cheap to obtain, thus the largest percentage of the cost of production is associated with synthesis of the APIs (typically around 70%).[56]

The past decade has seen strides made by developing African countries, to improve on the pharmaceutical manufacturing within Africa. It is important to note that although this is a great stride, the manufacturing plants still formulate towards the final drugs, with the APIs imported.[10]

However, the capacity of these manufacturers can only account for about 30% of the demand in Africa and there still exist a large amount of APIs being imported.[10]

Drug synthesis is advancing as a result of flow chemistry, as seen in the synthesis of organic molecules that include highly functionalized and chiral compounds to mention but a few.[57] This presents a unique solution to problems of sustainability and efficiency in organic synthesis that would have plagued the industry that are faced mainly in batch synthesis.[29,57] The risk associated with the synthesis of organic intermediates and APIs is minimized when utilizing flow chemistry technology, which would have been otherwise harmful and not permissible due to the dangers associated.[57]



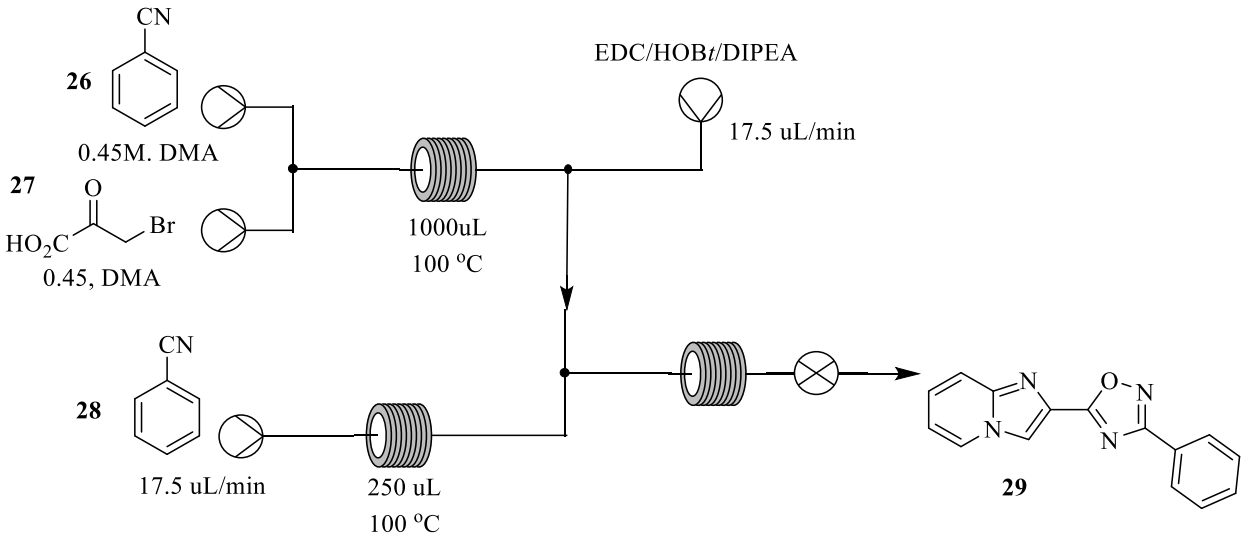
Scheme 8: Continuous flow synthesis of diphenhydramine hydrochloride[57,58]

A good example of the importance of flow chemistry in drug and/or API synthesis is the continuous flow synthesis route described in Scheme 8. Yearly, about a 100 tons of diphenhydramine hydrochloride **25** is in constant demand as an active pharmaceutical ingredient, because it is widely used in medications such as Benadryl, Zzzquil and Tylenol PM.[57,58] In their work, Jamison and Snead endeavored to reduce the production time, minimize waste and need for purification steps by using existing batch synthesis routes.

The optimum condition for the synthesis of diphenhydramine hydrochloride **25** were shown to be at a temperature 175 °C with a residence time of 16 minutes, pumped through a 720 µL PFA tube reactor (internal diameter = 0.5 mm) with the starting precursors chlorodiphenylmethane **23** and dimethylethanolamine **24** all being neat. In their investigations, the reaction rate greatly increased when the reaction was carried out above the boiling point of dimethylethanolamine **24**. Utilization of continuous flow system in the manufacture of product **25**, adds a unique advantage that would

otherwise been impossible to carry out in batch. This adds the ability to run the reaction above the melting point of the salt, which would give a molten form of product and can be easily transported through the flow system. The ammonium salts that formed are quenched with preheated NaOH (3 M solution). An inline membrane separator is used to extract the neutralized tertiary amines. An overall yield of 90% was achieved after addition of 5M HCl in order to precipitate diphenhydramine hydrochloride **25**.[57,58]

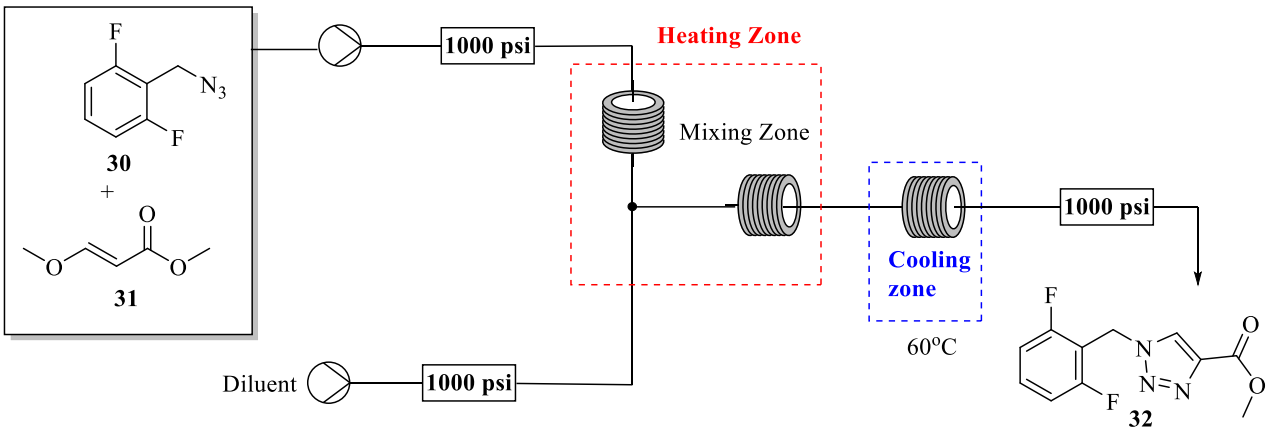
Herath and Cosford (Scheme 9) presented great strides in chemical synthesis utilizing flow techniques to synthesize imidazo[1,2-a]pyridin-2-yl-1,2,4-oxadiazoles **29**.[59] This was achieved by initially investigating the reaction of *N*-hydroxynicotinimidamide **26** with 3-bromobenzoic acid **27** using a single microreactor. The optimum reaction conditions for synthesis of the corresponding 1,2,4-oxadiazole derivative were 150 °C at 10 min residence time with a combination of EDC/HOB*t*/DIPEA (1:1:1).



Scheme 9: Overall flow synthetic route towards highly functionalized imidazo-oxadiazoles[59]

Scheme 9 further illustrates the flow synthesis of the imadazocarboxylic acid was incorporated within the reaction, towards the diverse synthesis of imidazo [1,2-a]pyridin-2-yl-1,2,4-oxadiazoles **29** which are of biological importance. A Syrris AFRICA® flow system was utilized allowing the synthesis of the amidoxime, which was combined with the previously optimized imidazo [1,2-a] pyridine-2-carboxylic acid synthesis by way of using a T-mixer which introduced EDC/HOB*t*/DIPEA 1:1:1. This was ultimately ensued by the addition of another reactor placed in a silicon oil bath at 100°C, and the reagents where pumped through the reactor. Addition of the

liquid-liquid separator assisted in removal of the excess DMA that had been utilized in the reaction. The overall synthesis of the target 5-(6-bromoimidazo [1,2-a] pyridin-2-yl)-3-(4-chlorophenyl)-1,2,4-oxadiazole **29** produced a 70% yield. This further reduced the time taken for the synthesis of highly functionalized imidazo-oxadiazoles, and the safety in the handling of chemicals and application of flow techniques such as fluid extraction, which allows for the removal of excess solvent.[59]

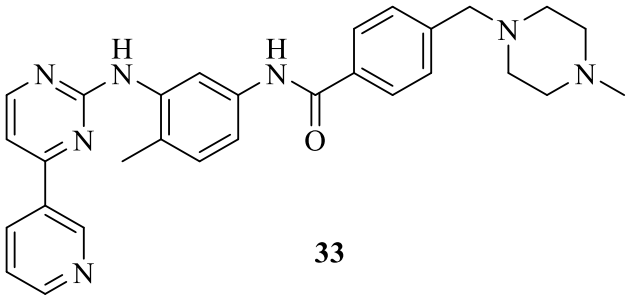


Scheme 10: Flow synthesis of rufinamide precursor via the 1,3-dipolar cycloaddition reaction[16,60]

In 2004, Novartis developed rufinamide, an antiepileptic drug containing a 1,2,3-triazole moiety and is one of the bestselling heterocyclic pharmaceuticals.[16] Under batch conditions, there was a rapid reduction in the yield of the rufinamide intermediate **32**, when a dilute homogenous reaction were used. However, Hessel *et al*[60]. were able to mitigate this problem by using neat reagents in flow synthesis of the rufinamide **32** and this improved the yield of the product. A yield of 82% was achieved in continuous flow at a temperature of 200°C in 1h where previously in batch conditions a yield of 13% was achieved.[60] This serves to show how flow chemistry can be successfully applied in drug synthesis to improve the conditions and yield of the drug.

1.4. INTRODUCTION TO IMATINIB AND ITS SYNTHESIS

Imatinib, namely *N*-(4-methyl-3-(4-(pyridin-3-yl)-pyrimidin-2-ylamino) phenyl)-4-((4-methyl piperazin-1-yl) methyl) benzamide **33**, is a 2-phenylaminopyrimidine derivative which was developed by Novartis Pharma AG, Basel, Switzerland, and licensed for treatment of patients with chronic myeloid leukemia by the U.S. Food and Drug Administration (FDA) on 7 November 2001. Imatinib functions by inhibiting the function of the tyrosine kinase, by competitively binding with the ATP synthetic mechanism of the Bcr-abl protein, which has conclusively established as a leukemic oncogene.[61–63]

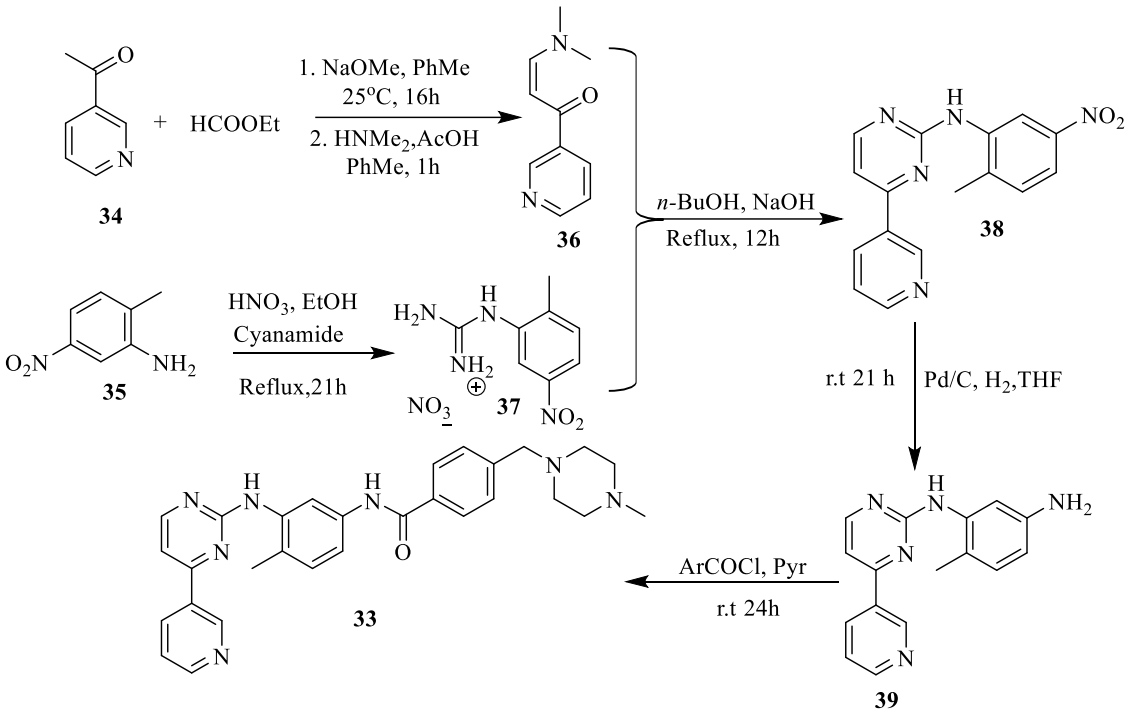


33

Figure 7: Molecular structure of the imatinib

1.4.1 Synthetic Routes for Imatinib

There are many batch synthetic routes towards the synthesis of imatinib. In 1993, Zimmerman *et al.* described the first successful route towards the synthesis of imatinib.[64] In the construction of the imatinib scaffold, Zimmerman *et al*. elucidated this synthetic route (Scheme 11) by using a key target compound phenylaminopyrimidine **38**. This key intermediate is synthesized from the enaminone **36** with a guanidinium salt **37**, obtained from the reaction between 2-methyl-5-nitroaniline **35** and cyanamide, thereby condensing the enaminone **36**. The nitro group in the side chain is reduced in the presence of suitable catalyst, namely Pd/C, to the corresponding aryl amine **39**. *N*-Methyl piperazine is added after reacting the aryl amine **39** with the amine a suitable benzoyl chloride, thus a S_N2 displacement occurs at the chloromethyl moiety of the acid chloride.[65]

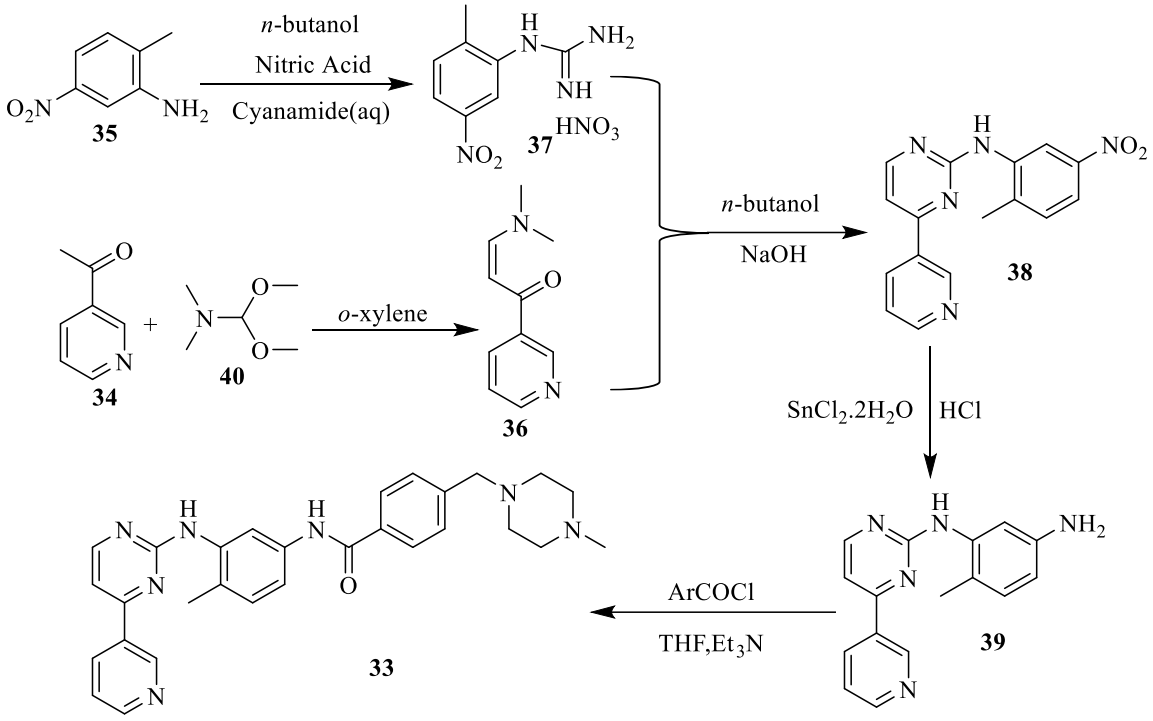


Scheme 11: Original Zimmerman approach to imatinib[65,66]

This particular method (Scheme 11) involved the use poisonous cyanamide, and three steps were needed for the complete synthesis, which involved the metal sodium, low temperature conditions and long reaction times, which complicated the synthesis of enaminone **36**. The yields of all steps were not disclosed in Zimmermann's paper and patent document; overall this reaction takes 74 hours with unknown yields.[66]

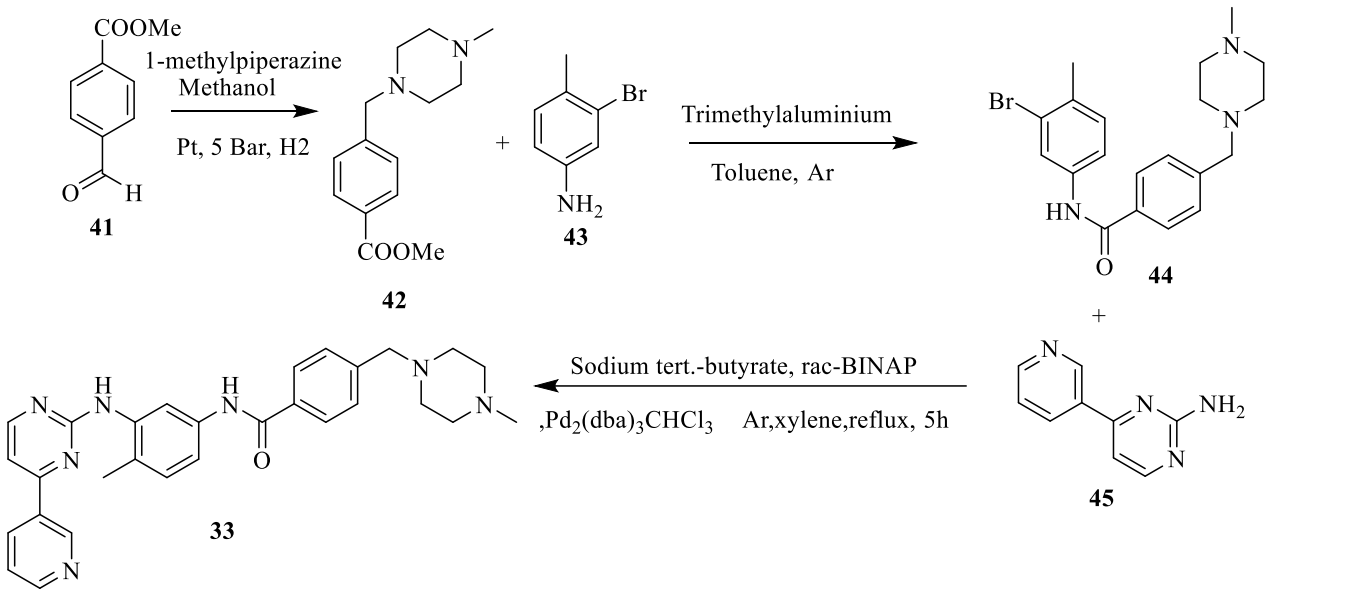
Chang *et al.*[67] elucidated a synthesis approach to imatinib and a new approach to the synthesis of 2-phenylaminopyridine derivatives. These derivatives were shown to possess the capability to inhibit cancer cell proliferation, mainly myeloid leukemia, in human cells. In their synthetic approach (Scheme 12) towards imatinib, 3-acetylpyridine **34** was reacted with *N*,*N*-dimethylformamide dimethylacetal **40** to produce the suitable enaminone namely (*Z*)-3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one **36**. The phenylaminopyrimidine **38** derivative ring system was constructed by reacting enaminone **36** with guanidinium nitrate salt **37**, which was prepared in 66% yield *via* the reaction of *o*-toluidine with 50% aqueous cyanamide in refluxing *n*-butanol. Reduction of the 2-aminopyridine **38** occurs by using $SnCl_2 \cdot 2H_2O$ as catalyst which afforded a 75% isolated yield of the corresponding 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-

2-yl)benzene-1,3-diamine **39**, which when reacted with 4-(chloromethyl)benzoyl chloride, leads to the target imatinib compound.

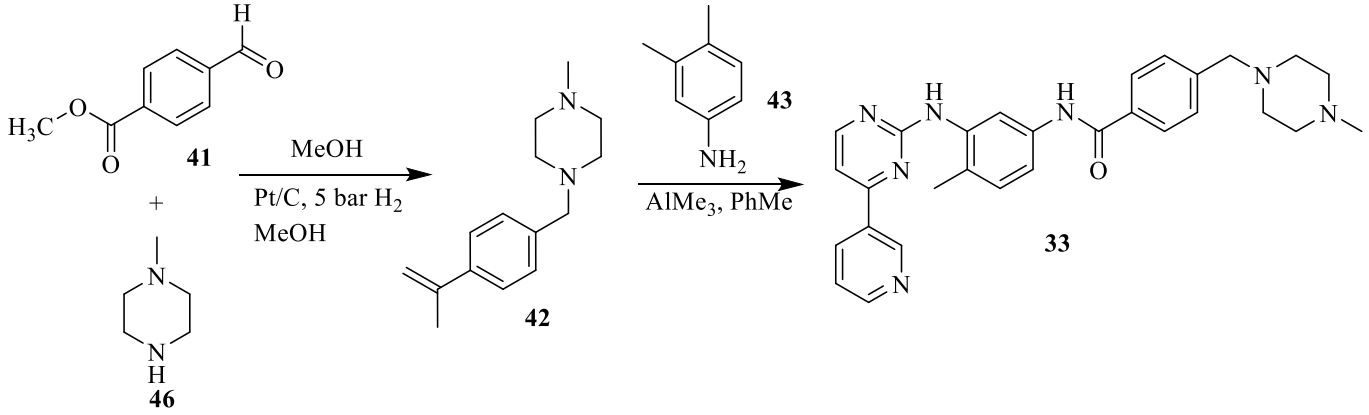


Scheme 12:Chang's approach to Imatinib synthesis starting with 2-methyl-5-nitroanile[67]

A process was described for the preparation of imatinib by Loiseleur *et al.* as shown in Scheme 13, where methyl 4-formylbenzoate **41** was reacted with 1-methylpiperazine in methanol and reduced *via* Pt/C hydrogenation to give 4-((4-methylpiperazin-1-yl) methyl) benzaldehyde **42**. The ligand, *rac*-BINAP, was utilized as an organophosphorus reagent in the $Pd_2(dba)_3$ $CHCl_3$-catalyzed Buchwald Hartwig cross coupling reaction under reflux with xylene as solvent to form the imatinib **33**.[68]

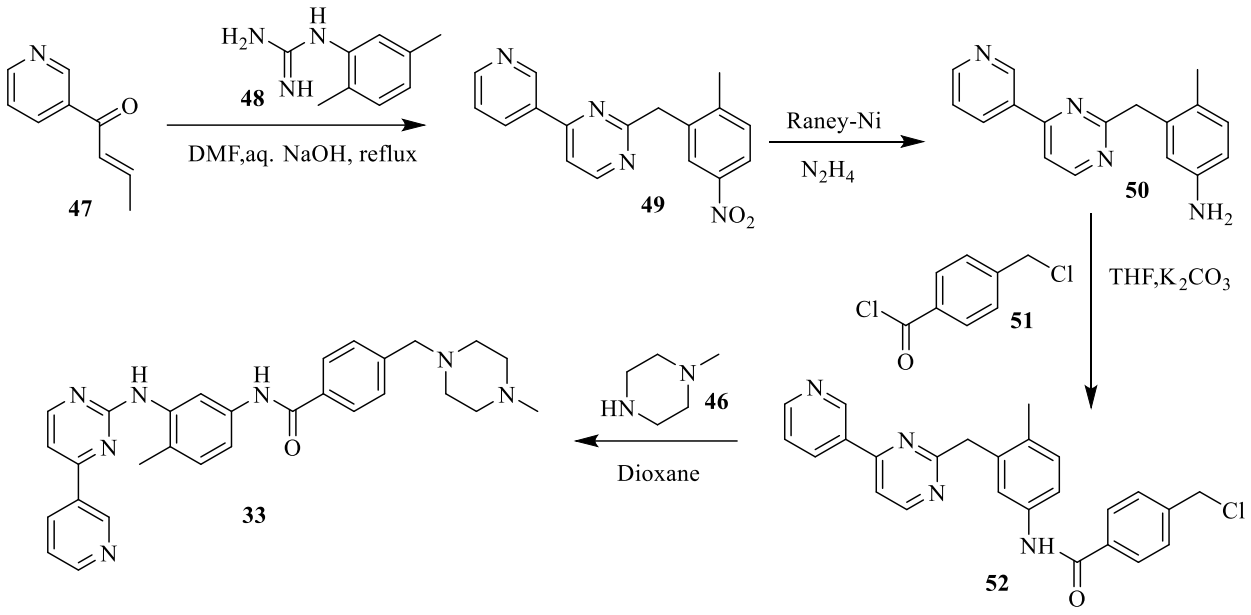


Scheme 13: Loiseleur's route for the preparation of imatinib[64]



Scheme 14: Novartis' route to imatinib utilizing a Buchwald–Hartwig coupling of aminopyrimidine[67]

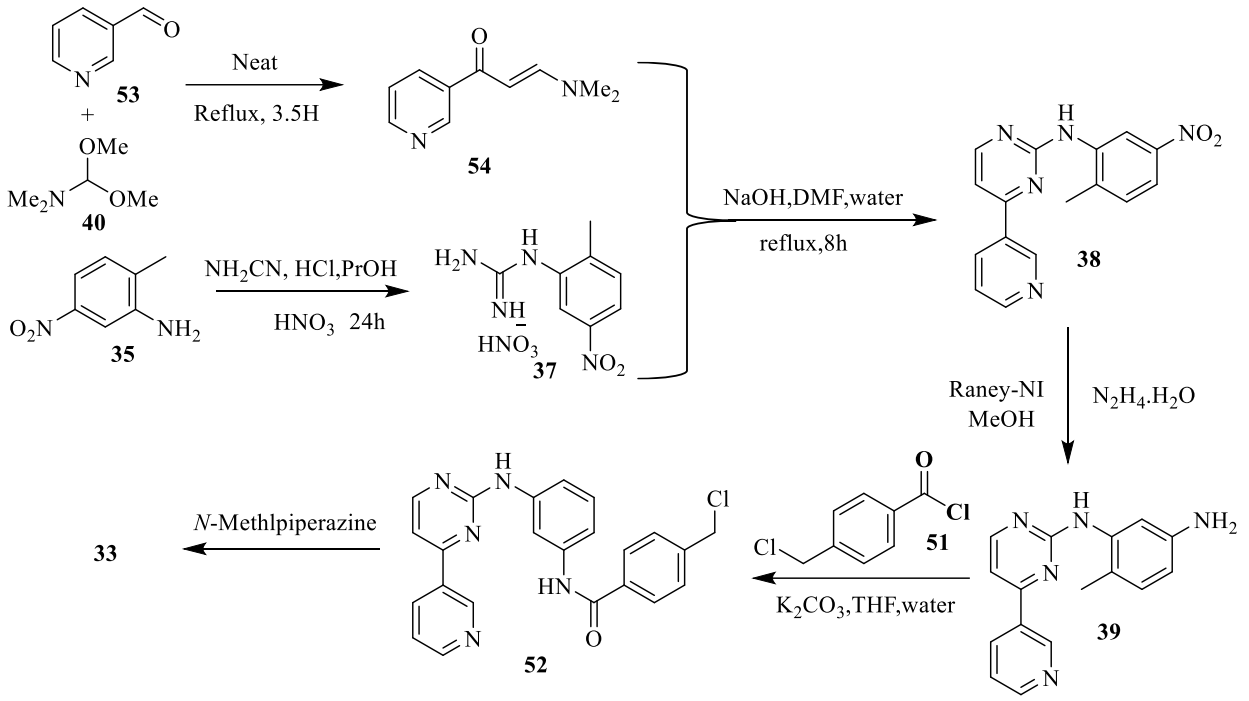
Novartis took a different approach in the synthesis of imatinib (Scheme 14) by starting with *N*-methylpiperazine **46** which when reacted with the formyl benzoate **41** under pressure in methanol over Pt/C. Imatinib **35** is then formed *via* Buchwald–Hartwig coupling procedure with an aminopyridine in the presence trimethylaluminium and toluene as a solvent. However, it was not clear the reason why a 72% yield was given, which did not correspond to the given final mass of the product.[69] Reverse-preparative HPLC was also needed since the product contained other unwanted (not specified) isomers. Even after the purification of the product, there was no final yield provided and this absence of essential details within the Novartis route eliminates this route as a feasible route to imatinib.[69]



Scheme 15: Baumann's alternative route towards the imatinib[70]

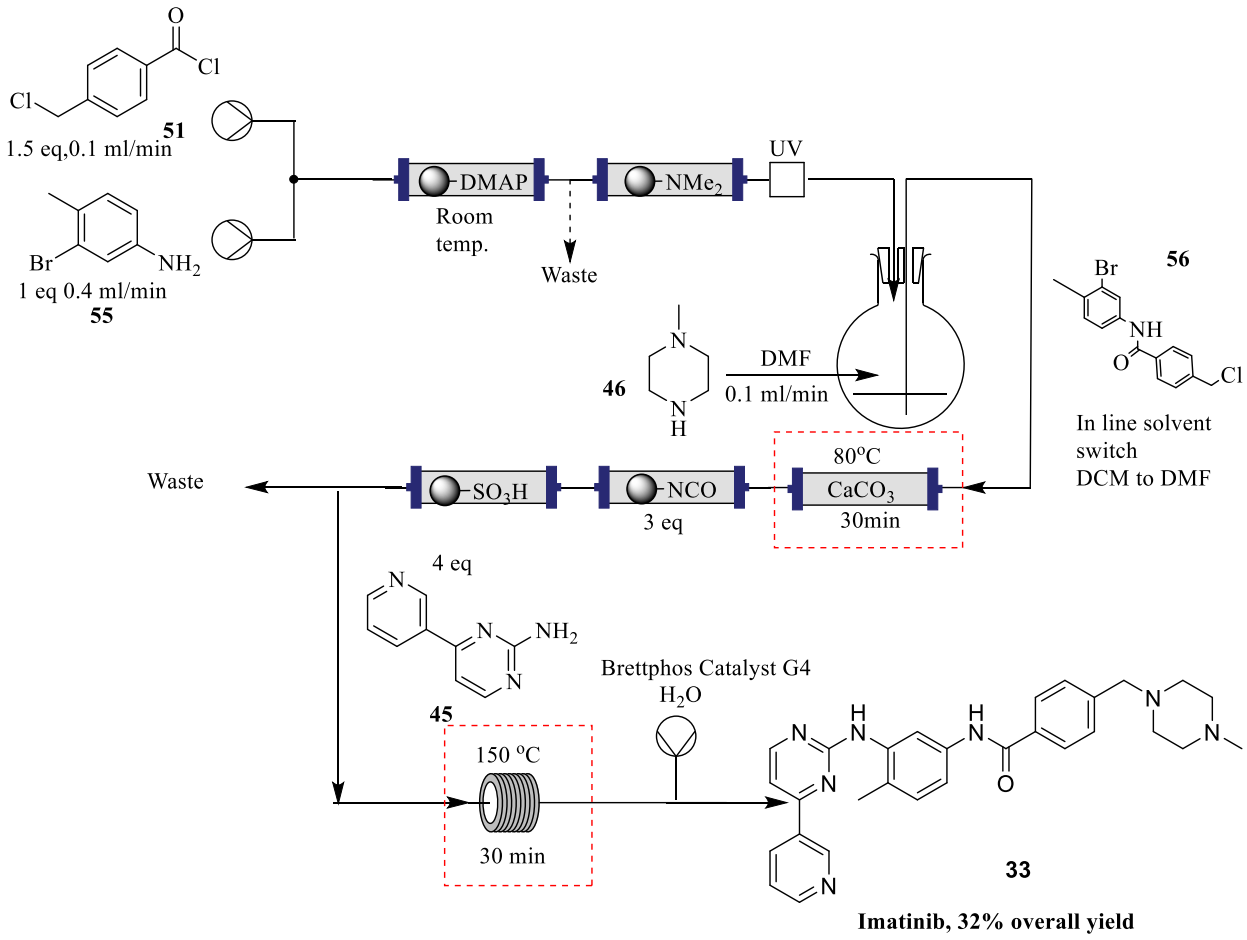
The essential 2-phenylaminopyridine **46** was synthesized in a patented route, where the aldol product **47** was refluxed with guanidine **48** in basic media.[69,70] The Raney-Nickel catalyst in the presence of hydrazine was utilized in the reduction of the nitro group after the generation of the pyrimidine core **49**.

This synthetic route was completed by formation of the amide **50** by use of 4-chloromethyl benzoyl chloride **51** which is later displaced by *N*-methylpiperazine **52**.[71] This imatinib synthetic route is unique because it counters the limited solubility of the intermediates in polar solvents by way of facile isolation of intermediates.[71] However, these advances in the isolation of pure material per step, the synthetic route still limits the telescoping of steps, which would lead to overall efficiency of the process and expenses associated with solvents reduces the ability of upscaling due the expense associated.



Scheme 16: Szczepek's route to imatinib[72,73]

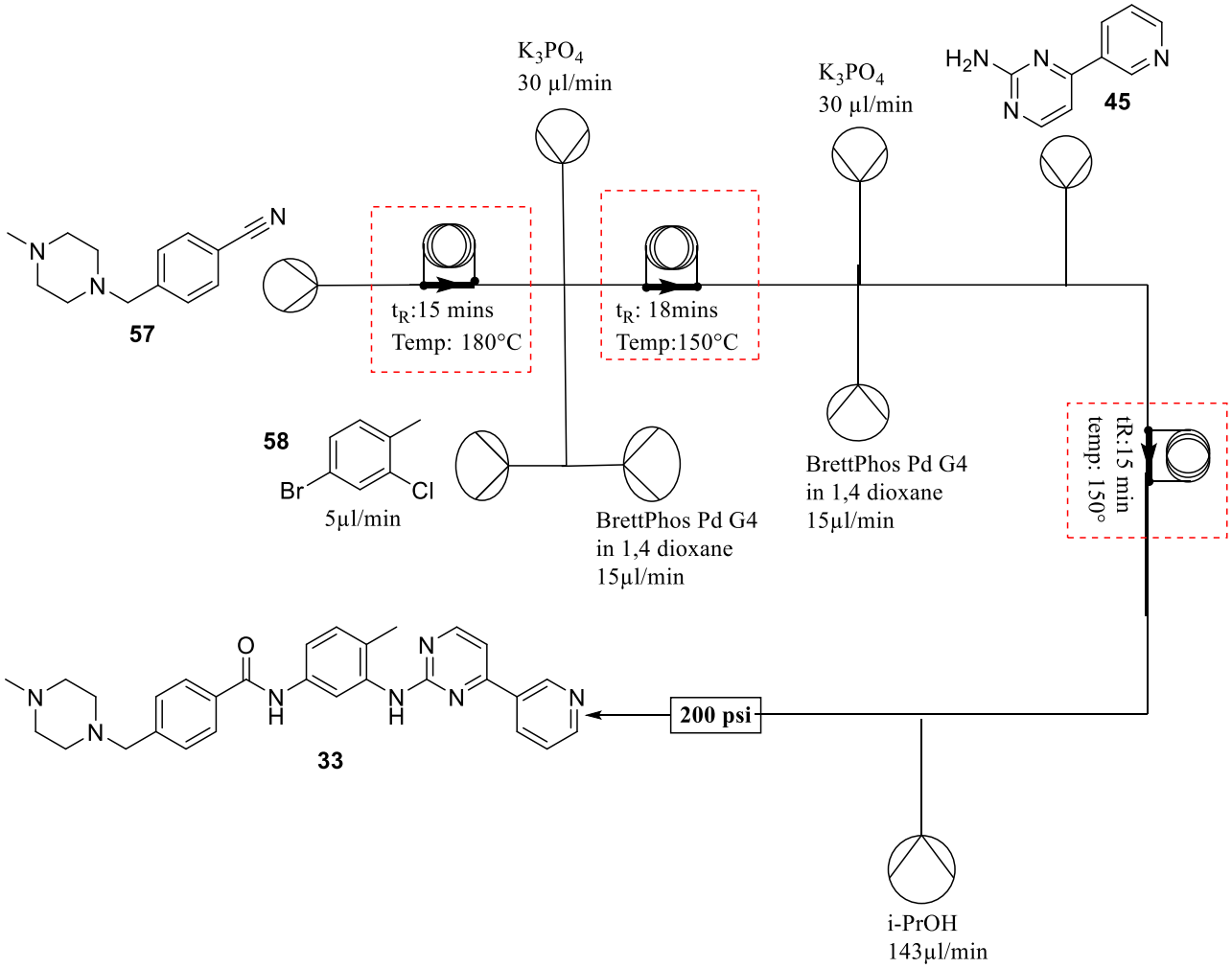
According to the findings of Szczepek *et al.*, the ion exchange of the guanidinium nitrate salt **37** was shown to be more efficient than that of the hydrochloride salt of the same compound. *N*,*N*-Dimethylformamide dimethylacetal **40** was utilized in the coupling reaction with the enone **53** and no solvent was required for this reaction to produce the corresponding enaminone **54**.[72,73] In the reduction and formation to the corresponding amide intermediate **39**, hydrazine hydrate was used over a RANEY® Ni catalyst. The normal route towards imatinib *via* the acid chloride displacement was carried out, to assist in acquiring a greater yield.[73] This synthetic route still possesses lengthy reaction conditions and although substitution for hydrazine hydrate as a hydrogen source in the reduction step towards the amine **39**, the reaction still utilizes a very expensive catalyst, which does not serve to reduce the cost of manufacture.



Scheme 17: Flow Synthetic route towards imatinib as described by Ley et al.[69,73]

The Innovative Technology Centre at Cambridge University described a flow-based synthesis towards imatinib and a small library of imatinib analogues. This route involved the initial loading of the acid chloride **51** in dichloromethane, using an HPLC pump to flow the solution through a glass column containing QP-DMAP. Once the acid chloride solution was trapped and activated, aniline **55** was introduced in dichloromethane, thus releasing the amide intermediate **56**. In order to prepare for the next step, the steam containing the amide was directed to a vial containing *N*-methylpiperazine **46** and DMF. Nitrogen gas was utilized to remove any excess DCM and effect an in-line solvent switch, which eventually led to the final step to imatinib **33** by way of Buchwald Hartwig coupling reaction, giving a 32% overall yield.[71,73] Although the flow synthetic route (Scheme 17) provided access to previously inaccessible imatinib derivatives, there are still great obstacles for upscaling. Usage of monolithic cartridges carries great disadvantages such as those used in Scheme 17; namely the high cost associated with purchase of each cartridge especially

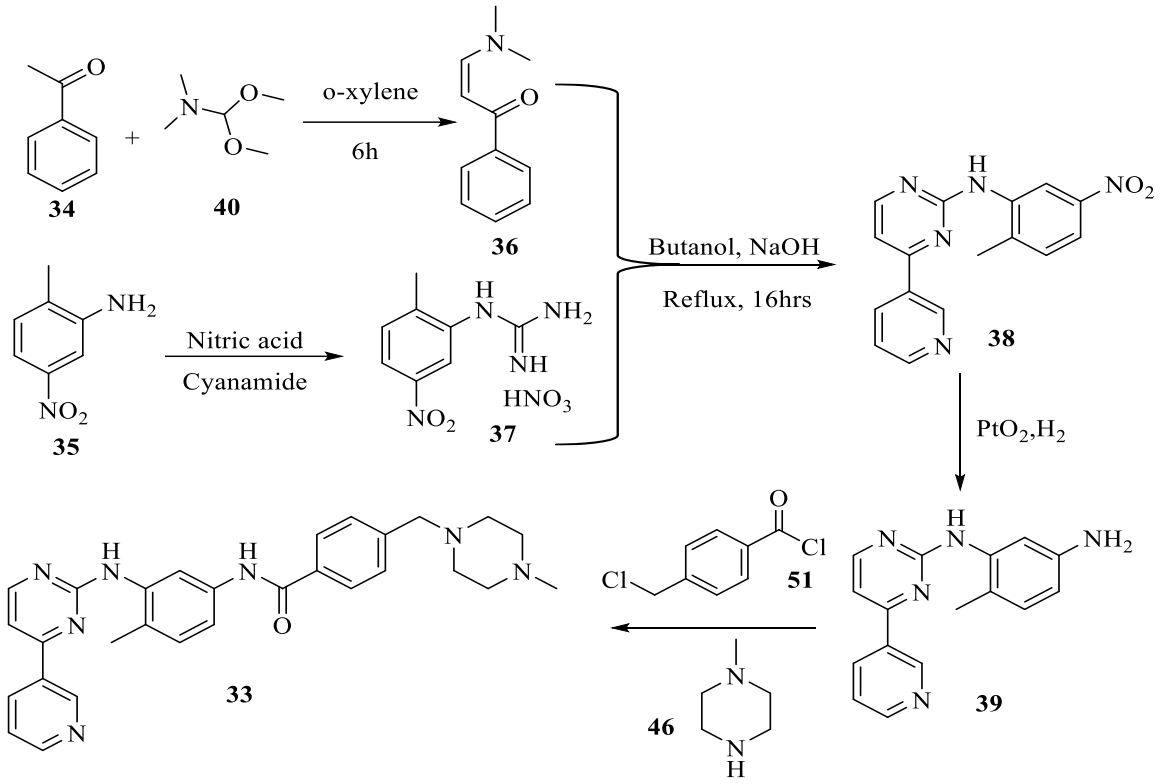
when scaling up is involved, sorbent batch differences lead to low reproducibility in results, which is important in the pharmaceutical industry and the cartridges are prone to blockages due to particles of sample suspended matter.[74]



Scheme 18: Continuous flow synthesis of imatinib and analogues described by Jamison and Chung Fu[75]

Jamison and Fu[75] recently investigated another flow-based approach towards imatinib, where they began with the chemoselective amidation of 2,4-dichlorotoluene with benzamide. In this step cesium carbonate was used to form the chemoselective target compound at high yields in the amidation reaction, and the reactor coil was heated to 150 °C. The utilization of dioxane dominated this reaction series with the 4-bromo-2-chloro-1-methylbenzene **58** and Brettphos catalyst (4th generation) being dissolved in this solvent. The preceding syringes connected and mounted on Harvard syringe pumps were the inorganic salt (tripotassium phosphate) which was dissolved in deionized water for the C–N cross-coupling of 2-aminopyrimidine and 2-chlorotoluene. The

addition of the aryl amine dissolved in isopropanol, and pumped on a Harvard pump at 143µl/min, afforded the overall yield of 55% for imatinib. While this method for flow synthetic route shown on Scheme 18 shows improvements in yield, there is still use for the very expensive Brettphos catalyst.[76] Furthermore, these particular catalysts produce good results only when dealing with primary amines but not with secondary amines, thus reducing the overall adaptive ability of this route towards synthesis of some analogies.



Scheme 19: Improved Zimmerman synthesis to imatinib[65]

Scheme 19 describes an improved approach to synthesis of imatinib originally described by Zimmerman.[65,66] The original synthetic approach to imatinib, which seeks to improve on the original Zimmerman approach to imatinib, only afforded a very low yield of 15% of the guanidinium intermediate. The cyanamide in the presence of hydrochloric acid was utilized to formulate the guanidinium fragment with considerable increase in yield to 85%. Kiningopolou *et al*.[65] show that the imatinib fragment yield was considerably higher (98%), by the initial use of molten cyanamide and the hydrochloride salt of 2-methyl-5-nitroaniline **35**. This, also significantly reduced the original 20 hours reaction time, to 1.5 hours while still affording a higher yield.

In the next step, in order to synthesize the 2-aminopyridine core, use of potassium carbonate (K_2CO_3) instead of sodium hydroxide (NaOH) as the base, and *n*-propanol as solvent gave a pure product. A yield of 81–86% without the formation of byproducts and without the need for purification by column chromatography, which further optimized the condensation reaction between the guanidinium intermediate **37** and the enaminone **36**.[65] The use of Adam's catalyst, PtO_2, showed a noticeable reduction in time, 1.5 hours, needed to for complete nitro-group reduction to the corresponding amide fragment **39**. This improved the method previously use for this reduction, and with no further purification needed. This was followed by coupling the acid chloride **51** to form the imatinib **33**. This synthetic route greatly reduced the reaction time as well as the yield of the original Zimmerman approach towards imatinib **33**.[77]

1.5 PROBLEM STATEMENT

In 2012, there were 8.2 million cancer-related deaths worldwide, making it the second leading cause of death globally and this global mortality rate is predicted to increase to 21.6 million by 2030.[78] The countries that possess the highest percentage of deaths *i.e.* 75%, are the middle to low-income countries and the number of cancer cases in these regions continues to rise every year. The economic impact of this large number of deaths has not gone unnoticed. To put this into perspective, the worldwide economic cost in 2010 was US$ 1.16 trillion (R17 trillion rand). This will ultimately threaten the economies at all income levels, in addition to instigating financial catastrophes for individuals and families.[79]

Many of the cancers that pose the greatest burden in developing countries are amenable to treatment with drugs of proven effectiveness that are off-patent and can be manufactured generically at 'affordable' prices.[8] Some African countries, such as Ghana, Cameroon and Malawi, have seen strides to lower the cost of generic first-line chemotherapy drugs with a cure rate of 50%. A good example is the generic chemotherapy drug used to treat Burkitt's lymphoma, which costs less than US$50 per patient.[8,80] Although this is a significant improvement, the major challenge still remains that for most African countries, the cost of the cancer treatment drugs still remain high leading to reduced accessibility.[81] Use of generic drugs has been widely adopted because of the prohibitive cost of on-patent drugs. These generic drugs, although helpful, are problematic since the newer drugs possess better efficacy and bioequivalence.[82] In Sub-Saharan Africa the influx of generics and APIs from Asia, which are sometimes substandard, remains

poorly regulated in some countries. The issue of access to cancer treatment worsens when it comes to targeted cancer treatment, which are often expensive, and the greater population cannot afford them such as imatinib.[9,82]

Imatinib is reported to have turned around the lives of patients suffering from chronic myelogenous leukemia (CML) in such a way that the cancer is now viewed as a manageable disease and not a death sentence. However, the cost of imatinib remains a major issue, which is largely attributed to the high industrial manufacturing cost attributed to the batch method of synthesis, which has led to its reduced use as a front line treatment for myeloid leukemia.[79] This is despite the fact that this method of synthesis of this drug has been highly optimized over the years to reduce reaction times, energy, cost and overall improved manufacturing processes. Continuous flow synthesis seeks to reduce the impact of manufacturing costs on the cost of final product bought by the consumer. Due to the effectiveness of this drug and with the increase of people affected by this disease, there is a need for an affordable method of synthesis to increase accessibility to low income households.

1.6. RESEARCH AIMS AND OBJECTIVES

The overall aims of this research are to:

1. Investigate the continuous flow synthesis of the intermediates towards imatinib utilizing batch synthetic methods to create a basis of comparison and for preparation of synthetic standards.

2. Explore and develop a flow processes that improves the synthesis of imatinib intermediates in a continuous flow system. We acknowledge that some research has been carried out in this area, but the vision is to develop a scalable route that does not use supported cartridges, as this would not enable local manufacture.

3. To optimize the conversion of the intermediates in the synthetic route towards imatinib by investigating various reaction parameters and greener approaches where possible.

1.7 PROPOSED SYNTHETIC ROUTE

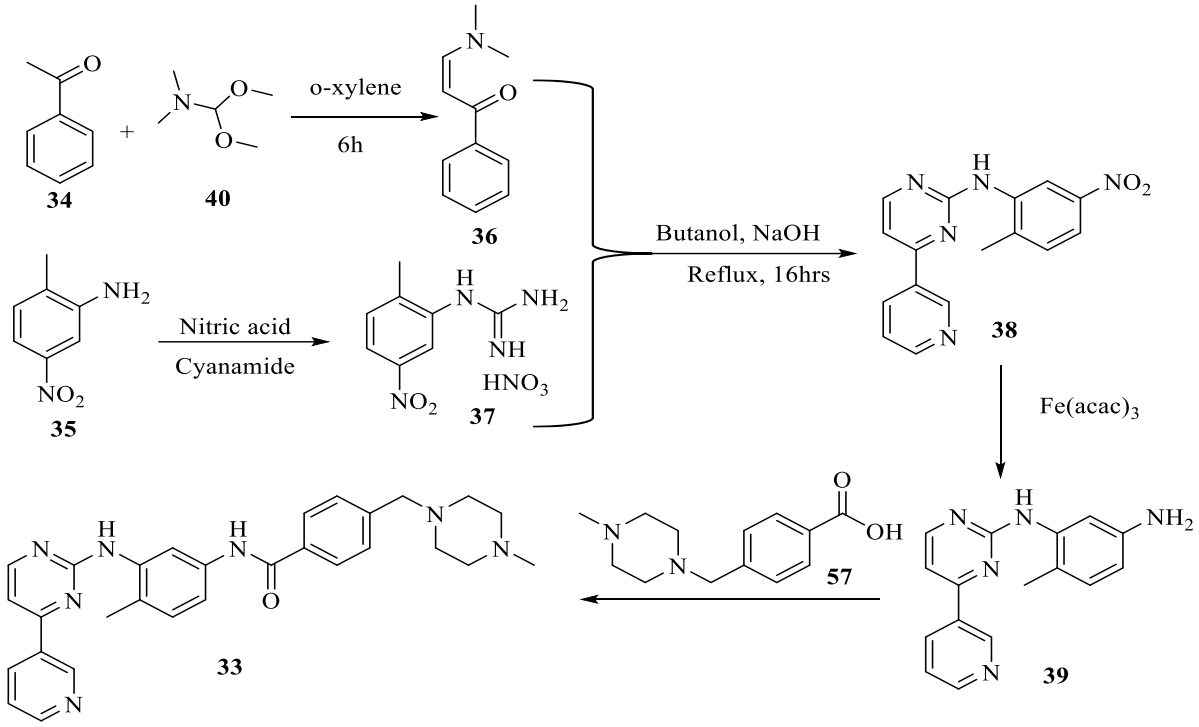


Figure 8: Proposed synthetic route towards imatinib mesylate and its intermediates

CHAPTER 2: EXPERIMENTAL DETAILS

2.1 REAGENTS AND CHEMICALS

All chemical reagents that were used in the research are tabulated below. The tabulated information includes the suppliers, purity and grades for each reagent.

Table 1: Reagents, grades and suppliers used in this research

Chemical Name	Formula	Source	Grade
3-Acetylpyridine	C_7H_7NO	Sigma Aldrich/Merck	98%
N,*N*-Dimethylformamide dimethyl acetyl	$C_5H_{13}NO_2$	Sigma Aldrich	94%
2-Methyl 5-nitroaniline	$C_7H_8N_2O_2$	Alfa Aesar	98%
Cyanamide	CH_2N_2	Alfa Aesar	98%
o-Xylene	C_8H_{10}	Merck	98%
N,*N*-Dimethylformamide	C_3H_7NO	Merck	94%
Toluene	C_7H_8	Minema Chemicals	94%
N-Methyl-2-pyrrolidone	C_5H_9NO	Carbosynth	98%
Methanol	CH_3OH	Merck	98%
Iron pentanedionate	$Fe(C_5H_7O_2)_3$	Merck	99%
Hydrazine hydrate	$N_2H_4.H_2O$	Sigma Aldrich	98%
Hexane	C_6H_{14}	Merck	98%
Dichloromethane	CH_2Cl_2	Merck	99%

2.2 INSTRUMENTAL METHODOLOGY

2.2.1 Nuclear Magnetic Resonance Spectroscopy

Solutions where prepared using deuterated solvent for the respective compound *i.e.* $CDCl_3$, DMSO-d_6 or D_2O. The spectra for the compounds were measured on a Bruker Avance III 400 Nuclear Magnetic Resonance (NMR) Spectrometer operated at 400 MHz and the chemical shifts were recorded in parts per million (ppm) and couplings constants in Hz. Preparation of a sample for analysis was done by dissolving approximately 20mg of the compound in a glass vial and dissolved in 0.6 ml of deuterated NMR solvent; this solution is then transferred to a clean NMR tube.

2.2.2 High Performance Liquid Chromatography

The analysis of flow chemistry conversion from starting reagents to product for the different synthetic steps was carried out using an Agilent 1220 infinity HPLC. A PerkinElmer Brownlee analytical C18 column (100 x 4.6mm) was used in the analysis of all the products in the synthetic routes. The ratio of the mobile phase was determined per compound being investigated, by running a series of HPLC experiments to obtain the optimum solvent ratio, flow rate and run time that show clear separation between the starting materials and the desired product.

Table 2: HPLC conditions for the various compounds investigated

Compound	**Solvent system ratio**	**Flow rate[a]**
3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one	Sodium octanoate buffer: Methanol (70:30)	1 ml/min
1-(2-Methyl-5-nitrophenyl) guanidine nitrate	Methanol with 1% glacial acetic acid: water (70:30)	1 ml/min
N-(2-Methyl-5-nitrophenyl)-4-(pyridin-3-yl)pyrimidin-2-amine	Methanol: water (70:30)	1 ml/min
6-Methyl-*N*1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine	Methanol: water (70:30)	1 ml/min
[a]all run times were 5 minutes		

The sodium octanoate buffer mobile phase was prepared by dissolving sodium octanoate (8.32g) in isopropyl alcohol (100ml) and diluting the solution to 1L with a solution of disodium hydrogen phosphate solution (100mM, pH 7.5).

2.2.3 Melting Point Determination

The Stuart SMP10 digital apparatus was utilized in the determination of melting points. The compounds are placed in a capillary tube made of soda glass at a depth of *ca*. 4 mm. Gentle tapping of the capillary tube allows for the solid powder to settle at the bottom of the tube. The capillary tube containing the sample when placed in the heating chamber of the Stuart SMP10 can be observed through a magnifying glass for viewing. The machine instrument was programmed to ramp up to and plateau at 240^{o}C in 5-degree/min increments and using the view glass on the Stuart SMP10. The temperature at which the compound melts was the noted.

2.2.4 Fourier Transform Infrared Spectroscopy

In order to identify functional groups present, a Bruker Platinum Tensor 27 spectrophotometer was used. The wavenumbers were observed after each run in the range of 4000-600 cm^{-1}. The analysis for each compound was observed with no modifications of the samples.

2.2.5 Thin layer chromatography

Thin layer chromatography (TLC) is a useful technique for the separation and identification of compounds in mixtures. The principle of that allows compounds to separate between the two phases is based on the solubility of the compounds between the two phases. TLC was used routinely to track the progress of the reactions towards the desired product in this research. This was done by checking the disappearance of the reactants and the appearance of the product. The TLC was carried out using solvents of varying polarity. Mecherey-Nagel Alugram® aluminium backed TLC sheets were utilized as the stationary phase. The visualization of the developed TLC plates was carried out using a Spectroline UV (λ: 254nm) analysis cabinet.

Product	**Solvent mixture**	**R_f**
3-Dimethylamino-1-(3-pyridyl)-2-propen-1-one **36**	DCM:hexane (95:5)	0.5
N-(2-Methyl-5-nitrophenyl) guanidinium nitrate **37**	DCM:Methanol:25% Aqueous ammonia (150:10:1)	0.12
N-(2-Methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**	EtOAc: hexane (1:1)	0.1
6-methyl-N^1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1,3-diamine **39**	EtOAc: Hexane (70:30)	0.3

.

2.3 GENERAL BATCH EXPERIMENTAL PROCEDURES

2.3.1 Batch synthesis procedure for 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one

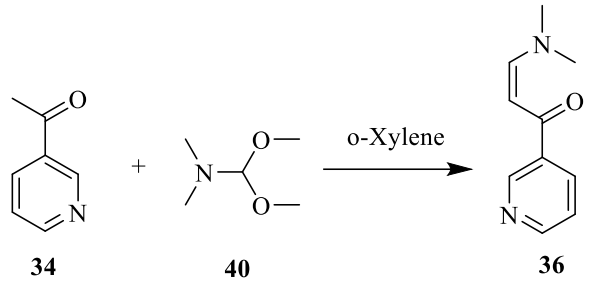


Figure 9: Enaminone synthesis starting with 3-acetylpridine and DMF-DMA[65]

A solution of *N*,*N*-dimethylformamide-dimethylacetal **34** (28.2 ml, 0.14mol) in *o*-xylene (30ml) was added to 3-acetylpyridine **40** (15 ml, 0.14mol) in a 100ml round bottom flask while stirring. The reaction mixture was heated to reflux for 6 hours at 140ºC and monitored using TLC (using 5% hexane in dichloromethane). The reaction mixture was concentrated by use of a rotor vapor and the residue dispersed in diethyl ether. The dispersed solution was then cooled in ice and a precipitate was obtained, which was isolated *via* filtration and further washed with ice-cold diethyl ether to give the product 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one **36** (13.2g, 80%); melting point 83-84ºC (lit value: 84–85ºC).[65] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.95 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.59 (d, *J* =12 Hz, 1 olef. H), 7.26 (dd, *J* =8.00, 4.00 Hz, 1 arom. H), 7.62 (d, *J* =12.24 Hz, 1 olef. H) 8.11 (dt, *J*=8.00, 2.00 Hz, 1 arom. H), 8.45 (dd, *J*=4.00, 1.75 Hz, 1 arom. H), 9.01 (d, *J*=2.01 Hz, 1 arom. H). ^{13}C-NMR (100 Hz, $CDCl_3$): δ:37.38, 45.21, 92.00, 123.31, 135.13, 135.67, 148.84, 151.39, 154.75 and 186.38. FT-IR (neat): 3172, 1641, 1578, 1523, 1449, 1413, 1364, 1247, 1125, 1067, 1024, 903, 761.

2.3.2 Batch synthesis of *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate

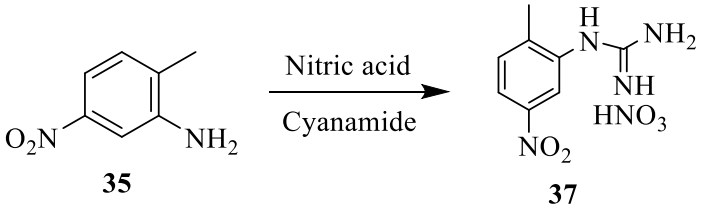


Figure 10: Preparation of N-(2-methyl-5-nitrophenyl) guanidinium nitrate

2-Methyl-5-nitroaniline **35** (22.46g, 135mmol) was added to *n*-butanol (200ml) in a 500ml 3-neck round-bottom flask and 65% nitric acid (10.5ml) was added dropwise. This was followed by the

addition of a 50% cyanamide aqueous solution (22.7g). The reaction mixture was then refluxed at 115ºC for a period of 12 hours and cooled in ice to 0 ºC. The precipitate that resulted was obtained *via* filtration using a Buchner funnel, which preceded washing with ice-cooled solution of methanol and diethyl ether (1:1, 20 mL) and allowed to dry overnight, which afforded *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** (14.99g, 43%). Melting point 217-218ºC (lit.218ºC).[67] ^{1}H-NMR (DMSO-d_6) 2.43(s, 3H, CH_3), 7.54 (3H, m), 7.90 (1H, dd, *J*=8.2 & 2.2 Hz, Ph-H), 8.48 (1H, d, *J*=8.00 Hz), 8.59 (1H, d, *J*=5.41 Hz), 8.75 (dd, 1H), 8.73 (1H, d, *J*=2.19 Hz), 9.31 (1H, d, *J*=1.6 Hz), 9.22 (1H, s, NH). ^{13}C NMR (100 Hz, DMSO-d_6): δ 18.08, 122.98, 123.25, 132.98, 135.00, 144.17, 146.85, 156.63. FT-IR (neat): 3472, 3145, 1694, 1620, 1578, 1514, 1396, 1375, 1345, 1260, 1090, 910, 846, 743, 521.

2.3.4 Batch synthesis of *N*-(2-Methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

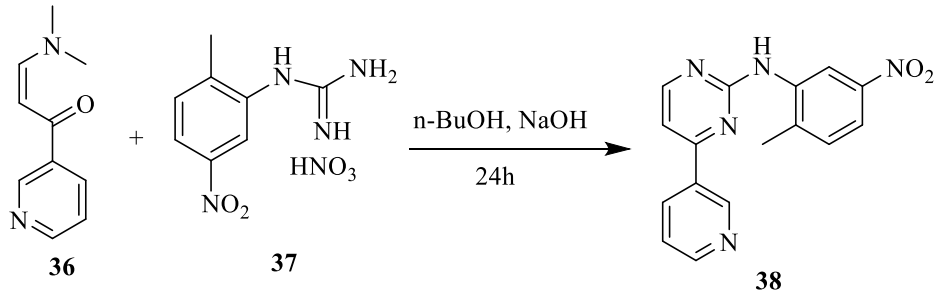


Figure 11: Preparation N-(2-Methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine where the enaminone(36) reacts with the guanidinium nitrate (37)

Into a 100ml round bottom flask 3-dimethylamino-1-(pyridin-3-yl)propenone **36** (2.69g, 15.3mmol) was added to a mixture of *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** (5.14g, 20mmol) in *n*-butanol (20ml). Solid sodium hydroxide (0.863g) was added to the reaction mixture while stirring. The reaction mixture was refluxed for 16 hours at set temperature of 115ºC and monitored by TLC. After 16 hours the reaction was cooled to 0 ºC and filtered using air suction. The precipitate was washed with methanol and diethyl ether (to remove excess unreacted starting material) and air-dried to obtain a pale-yellow solid product (2.9g, 62%). Melting point: 195ºC – 196 ºC (lit. 197ºC). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.48 (s, 3H, CH_3), 6.77 (br s, 1H, NH), 7.50 (d, *J*=5.25 Hz, 1 arom. H), 7.89 (d, *J* = 8.50 Hz, 1 arom. H), 8.32 (dd, *J* = 8.50, 5.00 Hz, 1 arom. H), 8.50 (dd, *J*=8.50, 2.25 Hz, 1 arom. H), 8.63 (d, *J*= 8.00 Hz, 1 arom H), 8.73 (d, *J*=5.25 Hz, 1 arom. H), 8.81 (d, *J*=5.1Hz, 1 arom. H), 9.26 (d, *J* =1.50 Hz, 1 arom. H), 9.31 (d, *J*=2.25 Hz, 1 arom. H); ^{13}C NMR (100 Hz, DMSO-d_6): δ 18.84, 109.36, 117.98, 118.43, 124.36, 131.72,

134.44, 134.78, 139.24, 139.38, 146.31, 148.64, 152.11, 160.18, 160.80, 162.09. FT-IR (neat): 3248, 1580, 1556, 1530, 1405, 1344, 1304, 1084, 1026, 990, 881, 829, 790, 735, 698, 662, 608, 441.

2.3.5 Batch synthesis of 6-methyl-N^1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1, 3-diamine

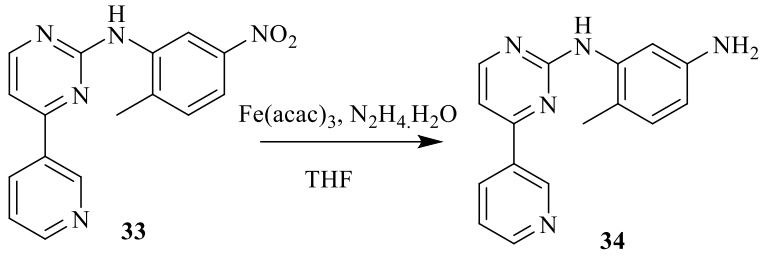


Figure 12: synthesis of 6-methyl-N1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1, 3-diamine

To a solution of *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine) **33** (1g, 0.00325mol) in THF (4ml) 3mol% of iron pentanedionate (0.0344g) and hydrazine hydrate (0.32ml, 0.0065mol) were added while stirring. The reaction was stirred vigorously at 65^{o}C while being monitored by TLC to observe the reaction progression and after 3 hours, only the pure amino-compound was detected with no by-products. The solvent was removed *in vacuo* and the Fe_3O_4 particles formed were removed using a neodymium magnet. The residue obtained was purified with treatment with hexane and diethyl ether (1:1, *v/v*) and dried *in vacuo*, affording the pure aniline derivative **34** (0.98g, 98%). Melting point: 189^{o}C (lit. 182^{o}C-193^{o}C). ^{1}H-NMR (400 MHz) δ: 9.19 (d, *J* =2.2,1H), 8.64 (dd, 1H, *J*= 4.8), 8.55 (s, 1H), 8.42 (d, *J* = 5.1, 1H), 7.97 (dt, *J* = 8.1, 2.0,1H), 7.52 (dd, 1H, *J* = 7.9, *J* = 5.1), 7.35 (d, 1H, *J* = 5.1), 7.26 (d, 1H, *J* = 8.2), 6.84 (d, *J* = 2.3, 1H), 6.58 (dd, *J* = 8.0;2.3), 2.13 (s, 3H, CH_3). ^{13}C NMR (100 Hz): 161.9, 161.77, 159.80, 151.77, 148.57, 147.22, 138.44, 134.73, 132.27, 130.82, 124.27, 119.81, 111.42, 107.55, 103.67, 17.69. FT-IR (neat): 3327, 3210, 2964, 2924, 1622, 1574, 1554, 1445, 1046, 1085, 1026, 872, 802, 800, 735, 652, 559, 441.

2.4 FLOW EQUIPMENT

2.4.1 Residence Time Microreactors

The MR Lab series borosilicate glass reactors (Figure 13) are specially designed for lab scale synthesis by Little Things Factory (Germany). The glass reactors are versatile, allowing investigations at different temperatures, connections are all located at the front for simplicity and reactors can be optionally mounted onto framing when the need arises such as submerging in an oil batch. Figure 13 illustrates the different type of reactors, which possess unique features such as improved and assisted mixing present in the LTF-MX, LTF-MS and LTF-VS.[83]

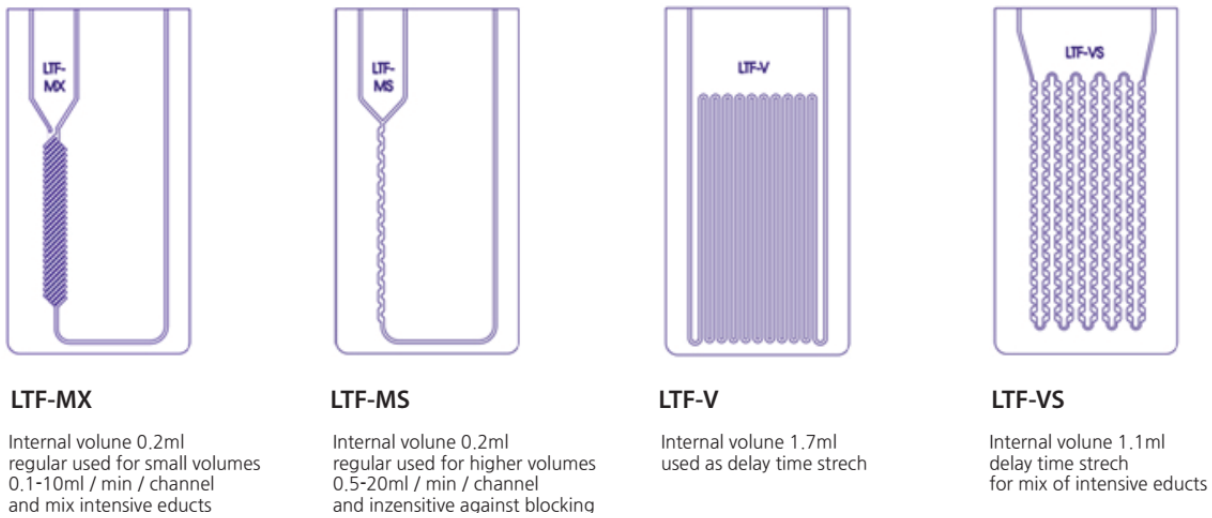


Figure 13: Examples of Little Things Factory reactors starting from left: LTF-M), LTF-MS, LTF-V, LTF-VS[83]

2.4.2 Back Pressure regulator

Figure 14: Zaiput back pressure regulator

These highly versatile backpressure regulators are specially designed for use in flow chemistry lab scale synthesis (Zaiput, USA). These backpressure regulators allow the user to set a reference point for the "back pressure" up to a maximum of 290 psi, which functions by comparing the pressure

of the fluid flowing through the system to the reference point and flow is only allowed when the main stream meets the reference pressure. This permits the flow chemist to work and investigate reactions at temperature above the boiling point of the fluids and the ability to set a reference pressure as selected by the user.

2.4.3 Chemyx Fusion pumps

Figure 15: Chemyx© fusion pump 100

The dual channel syringe pump is designed with the capability to handle a variety of dosing application with an ease of use. In flow chemistry, this particular syringe pump can deliver easily reproducible flow rates *via* a state-of-the-art microstepper motor. The pump is also fitted with an easy to use touch screen to adjust and customize the researchers flow profile. The metal components design of such pumps, means there are resistant to wear and tear damage that is caused by chemicals used in research and development.[84]

2.5 PARAMETER INVESTIGATION IN FLOW SYNTHESIS

2.5.1 Optimum temperature investigation

In order to observe the effect that temperature has on the formation of the various target intermediates towards the synthesis of 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine **39**, the microreactors were submerged in an oil bath. The temperature was able to be controlled accurately using a Eins-Sci E-H550-D hot plate fitted with a temperature probe. The high boiling point of the Malotherm oil (385 - 395 °C), used as oil bath provides the capability of investigating effect of high temperature on reactions.

2.5.2 Mole ratios investigations

In order to investigate the effect of adding reagents in excess relative to the limiting reagent, the mole ratios were initially investigated using the conditions from the batch reactions. The mole ratios were varied at a particular temperature to observe if there is an effect in the conversion towards the desired product. The mole ratios that provided the highest conversion were identified as the optimum condition for the flow synthesis of the target compound.

2.5.3 Solvent screening

The effect of solvent in a reaction was also investigated using various solvents with different properties *i.e.* non-polar and polar solvents. The solvents were utilized in making up the stock solutions of the reagents that were used in the various flow reactions. The previously determined optimum conditions, were utilized in this investigation in order to establish if a solvent of different properties could be improve the residence time needed to achieve high conversion.

2.6 FLOW SYNTHESIS AND OPTIMIZATION OF 3-DIMETHYLAMINO-1-(3-PYRIDYL)-2-PROPEN-1-ONE

Synthesis, screening and optimization of the enaminone **36** was carried out using Little Things Factory glass reactors. The flow setup used in this optimization study was a LTF-V S (1.1ml) glass reactor, a Zaiput back pressure regulator, 2x2.5ml SGE glass syringes and 16.5 cm length of PTFE tubing 1 mm I.D. This system was connected as shown in Figure 16.

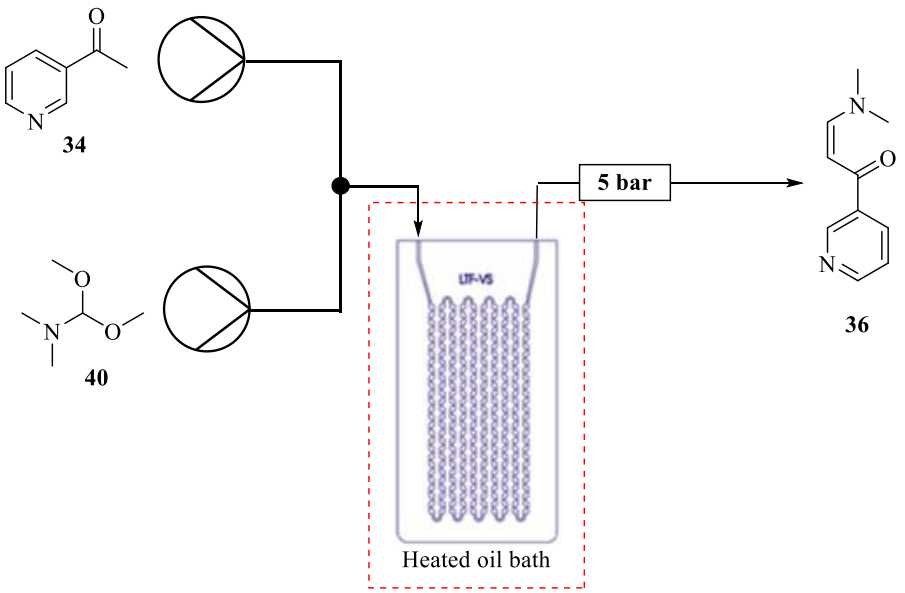
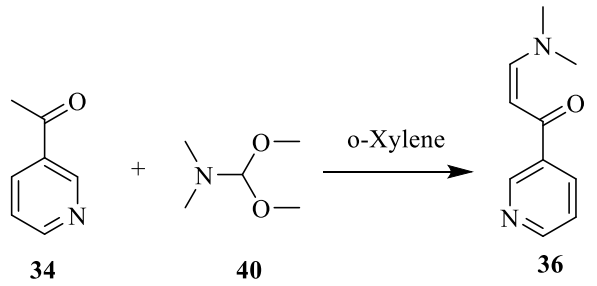


Figure 16: Flow set up for optimization of 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one

Table 3: Flow set up components and specifications of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

COMPONENT	SPECIFICATIONS
Temperature range	120-200ºC
Microreactor	LTF-V S 1.1 ml
PEEK tubing	1mm I.D. (26.5cm, 0.23ml)
Back pressure regulator	Zaiput Back pressure regulator (5 bar)



Scheme 20: Reaction scheme for the synthesis of the enaminone 31, using 3-acetypyridne and DMF-DMA

3-Acetylpyridine **34** and DMF-DMA **40** were dissolved in the appropriate solvents that were being screened such as *o*-xylene, toluene, *N*,*N*–dimethylformamide or *N*-methyl-2-pyrrolidone. The two solution were pumped through the LTF reactor (Figure 16) at varying flow rates depending on the residence time being investigated using a Chemyx© fusion pump 100. The solution obtained containing the product **36** was collected in a vial containing methanol for HPLC analysis, the 3-acetylpyridine **34** and the enaminone **36** eluted at 1.2 min and at 1.3 min respectively. The previously synthesized enaminone permitted for the characterization and identification of the enaminone peak during HPLC analysis using a mobile phase 70:30 of the sodium octanoate buffer: methanol.

2.7 FLOW SYNTHESIS AND OPTIMIZATION OF 1-(2-METHYL-5-NITROPHENYL) GUANIDINIUM NITRATE

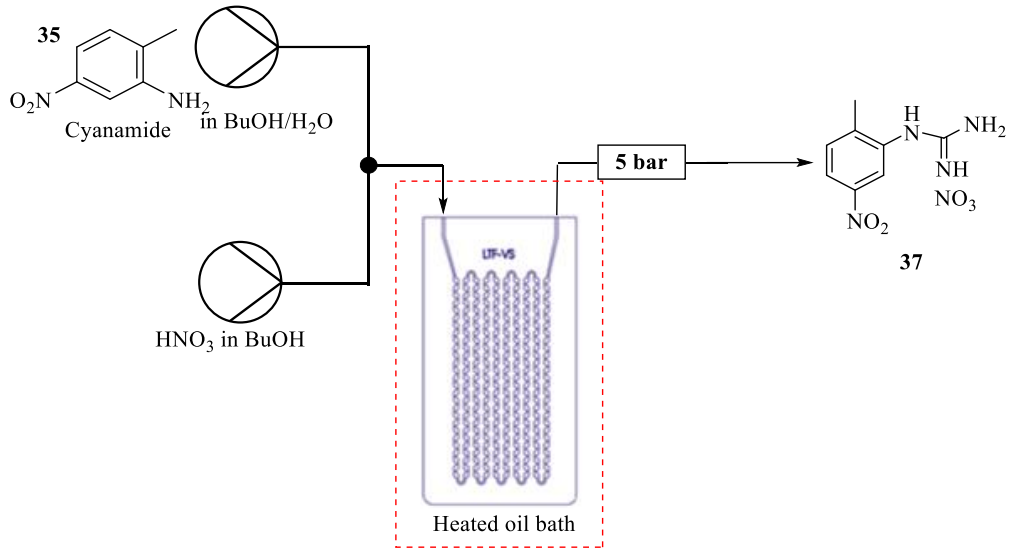


Figure 17: Flow synthesis set up for the 1-(2-methyl-5-nitrophenyl) guanidinium nitrate

Table 4: Components and their specifications utilized in the continuous flow synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate

COMPONENT	SPECIFICATIONS
Temperature range	115-145^{o}C
Microreactor	LTF-Vs 1.1 ml
PTFE tubing	1 mm I.D PTFE 26.5cm, 0.21ml
Back press regulator	Zaiput back pressure regulator (5 bar)

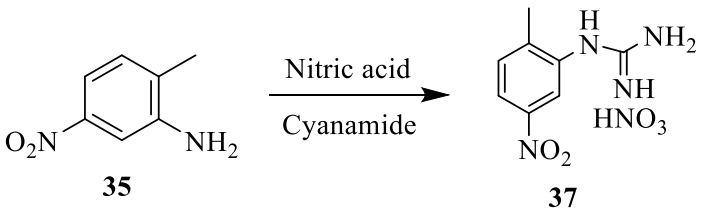


Figure 18: Reaction scheme for the batch synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate

In order to synthesize the guanidinium nitrate salt **37** in flow, the initial reaction was carried out using the batch conditions. A solution of 2-methyl-5-nitraniline **35** (0.01M) was prepared in a 50ml conical flask mixed with cyanamide (0.02M) and in another 50ml conical flask nitric acid (0.95 ml, 0.02M) in *n*-butanol. The solutions were pumped through the 1.1ml LTF-Vs microreactor using a Chemyx© Fusion 100 syringe pump fitted with a Zaiput backpressure regulator calibrated

to a 5 bar. The flow rate was controlled on the Chemyx© Fusion pump in order to investigate the effect of residence time and temperature. The sample was collected into a vial and analyzed *via* HPLC to observe the conversion towards the starting reagents to the desired 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**. The 2-methyl-5-nitraniline **35** eluted at 4.1 min and the guanidinium nitrate salt **37** eluted at 2.4 min. Parameters such as the effect of temperature, residence time and reagents mole equivalence on the conversion towards the guanidinium salt were investigated.

2.8 FLOW SYNTHESIS AND OPTIMIZATION OF *N*-(2-METHYL-5-NITROPHENYL)-4-PYRIDIN-3-YL-PYRIMIDIN-2-YLAMINE

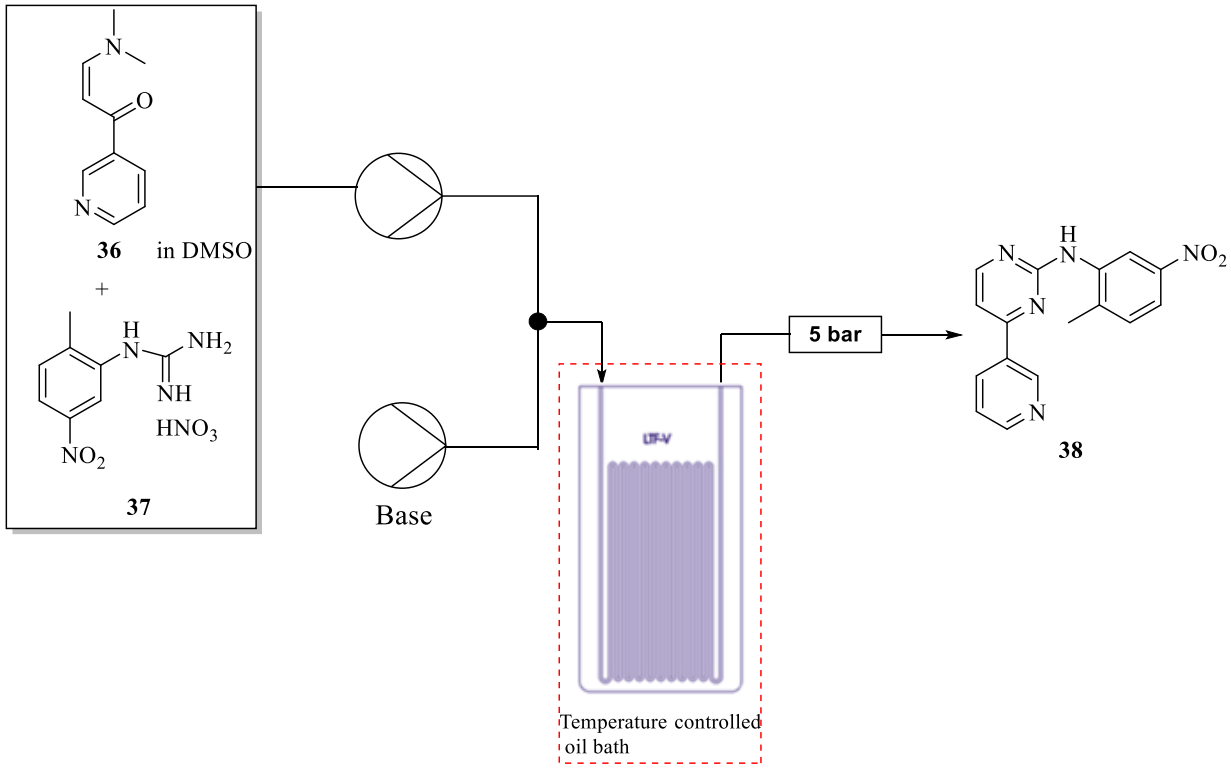
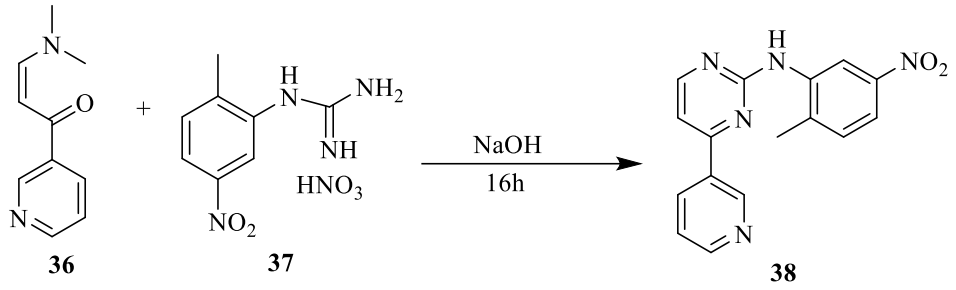


Figure 19: Flow set up for the synthesis of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

Table 5: Components and their specifications utilized in the continuous flow synthesis of the 2-aminopyridine

COMPONENT	SPECIFICATIONS
Temperature range	120-180^{o}C
Microreactor	LTF-V 1.7 ml
PTFE tubing	1 mm I.D PTFE 26.5cm, 0.23ml
Back press regulator	Zaiput back pressure regulator (5 bar)



36 37 38

Figure 20: Batch reaction scheme for the condensation reaction between the enaminone (36) and guanidinium nitrate salt (37) towards the 2-aminopyridine intermediate (38)

In the flow synthesis of the 2-aminopyridine core **38**, a solution of 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one **36** (0.26g, 0.153M) in *o*-xylene with 1-(2-methyl-5-nitrophenyl) guanidinium nitrate (0.514g, 0.2M) **37** in the DMSO (10ml). The base *e.g.* NaOH (0.031M) in 10ml water for the effective condensation reaction to synthesize *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** was dissolved in water. These mixture of the enaminone and the guanidinium salt where pumped at the same flow rate using Chemyx© Fusion 100 syringe pumps through a 1.7ml LTF-V reactor submerged in a heated oil bath. In order to obtain a sample for HPLC analysis, the product obtained after the set residence time was collected in a vial. The collected sample when analyzed *via* the HPLC, showed the 2-aminopyridine **38** eluting at 2.9 min and enaminone **36** at 1.3 min. The effect of other bases was investigated such as potassium carbonate, TEA, cesium carbonate and DBU.

2.9 FLOW SYNTHESIS AND OPTIMIZATION 6-METHYL-N^1-(4-(PYRIDIN-3-YL) PYRIMIDIN-2-YL) BENZENE-1, 3-DIAMINE

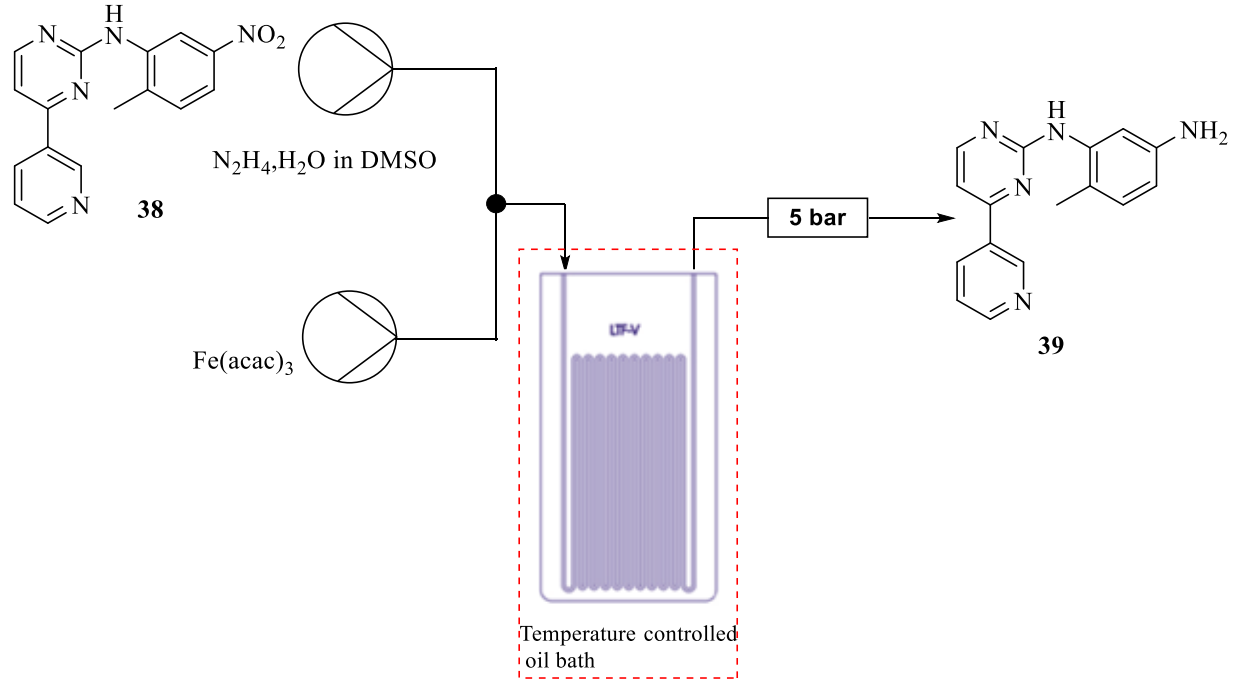


Figure 21: Flow synthesis set up for the nitro group reduction of the 33 towards optimization 6-methyl-N1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1, 3-diamine using iron pentanedionate as catalyst and hydrazine hydrate

Table 6: Components and their specifications utilized in the continuous the flow reduction towards optimization 6-methyl-N^1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1, 3-diamine

COMPONENT	SPECIFICATIONS
TEMPERATURE RANGE	130-190^{o}C
MICROREACTOR	LTF-Vs 1.7 ml
PTFE TUBING	1 mm I.D, 26.5cm (0.23ml)
BACK PRESS REGULATOR	Zaiput back pressure regulator (5 bar)

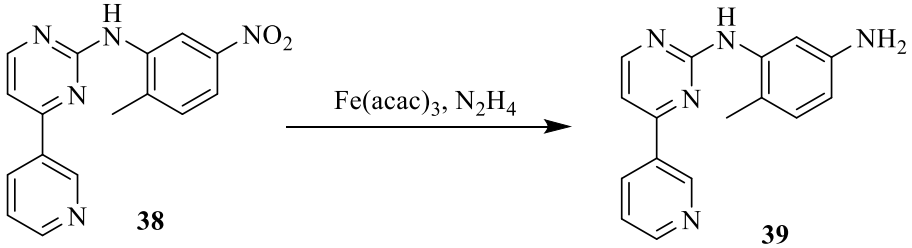


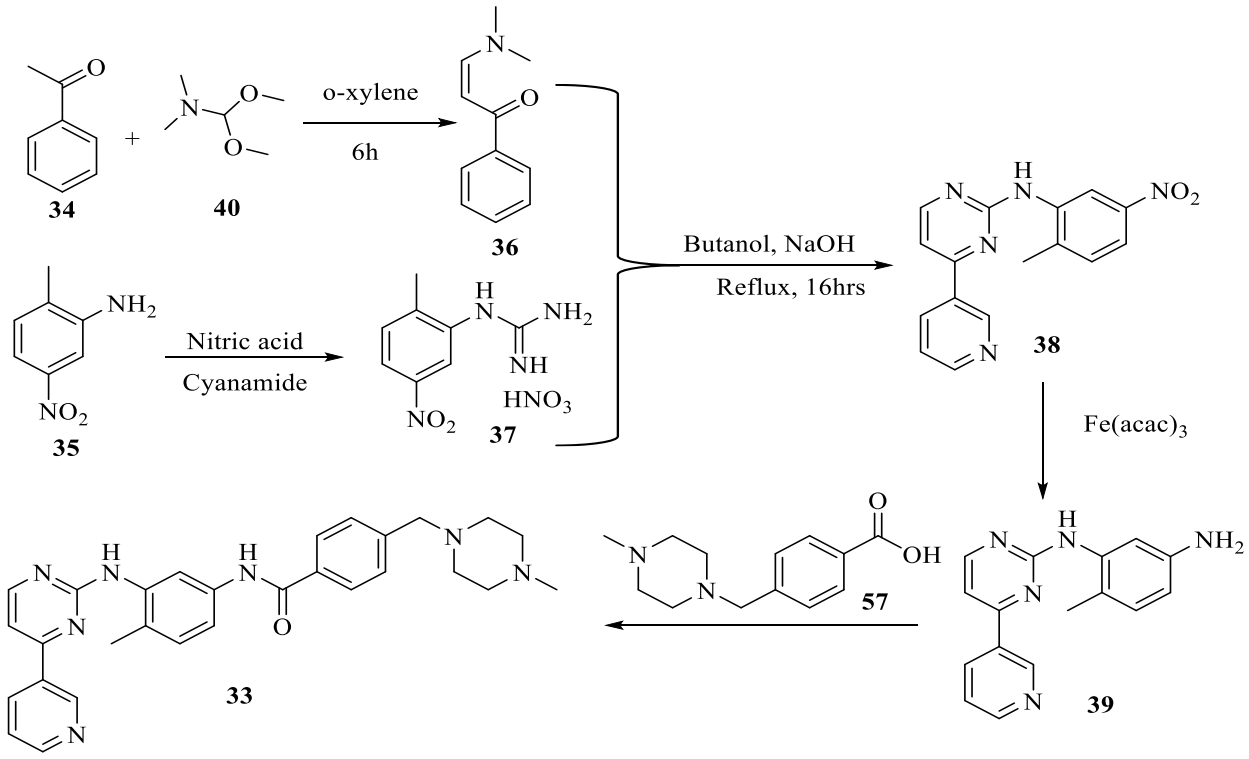
Figure 22: Catalytic reduction to the corresponding amine

In order to investigate the optimum condition for the reduction towards 6-methyl-1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1,3-diamine **38**, preliminary reactions were carried out by using a solution of using *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **33** (0.01M) and hydrazine hydrate (0.012M) prepared in DMSO (10ml) and iron pentanedionate (3mol%) using DMSO (10ml) as solvent. Both solutions were pumped from a single Chemyx Fusion 100 pump into a 1.7ml LTF-V microreactor, which was submerged in an oil bath set at the desired temperature being investigated. The flow rate was varied based on the various residence times, that were investigated and after the time had elapsed, the sample was collected in a vial, filtered to remove the iron particles formed using a syringe filter and analyzed using an HPLC Agilent 1220, the 70:30 methanol to water solvent system. The reduced product **39** eluted at 1.4 min and the *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** at 2.9 min.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 INTRODUCION

Various synthetic routes towards the synthesis of imatinib and the corresponding intermediates leading to the complete synthesis of this drug have been described.[64,65,69,73,75,76] These routes show high associated total cost of synthesis and low overall yield. In our effort to investigate possible ways to reduce the overall cost of synthesis, with high conversion using continuous flow techniques, we sought to adapt the synthetic route shown in Scheme 21, which begins with the synthesis of the enaminone **36,** which when reacted with the guanidinium nitrate **37** yields the 2-aminopyridine core **38**. This aminopyridine core is then reduced, in the presence of a catalyst and hydrazine hydrate. The 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine **39** is then reacted with 4-[(4-methylpiperazin-1-yl)methyl]benzoic acid **57** to yield the imatinib **33**.



Scheme 21: Batch synthetic route towards imatinib to be investigated

3.2 SYNTHESIS AND OPTIMIZATION OF 3-(DIMETHYLAMINO)-1-(PYRIDIN-3-YL)-PROP-2-EN-1-ONE

Batch synthesis of the enaminone **36** using 3-acetylpyridine **34** and *N*,*N*-dimethylformamide dimethylacetal **40** with *o*-xylene as solvent required 6 hours to provide a yield of 80%. The solvent used, *i.e. o*-xylene, has a high boiling point of 144^{o}C, which brings difficulty in separation from the product after the reaction. The advantages that continuous flow synthesis brings is the ability to work above the boiling point of reagents and solvents because of the use to the backpressure regulators. Thus, we can investigate using cheaper alternative solvents with lower boiling points that can potentially replace the difficult to remove *o*-xylene. In addition to this, the starting reagents, 3-acetypyridine **34** and *N*,*N*-dimethylformamide dimethylacetal **40**, are both liquid at room temperature, thus we investigated the possibility of eliminating the use of solvent completely. The reaction time is also particularly long, where 6 hours afforded a yield of 80% in batch; hence, by investigating reaction condition such as increasing the temperature, the mole equivalence of the starting materials would hopefully result in faster reaction in the continuous flow system.

Firstly, the adaptation of the enaminone **36** synthesis in a continuous flow system will be discussed. The enaminone derivative that is synthesized from the 3-acetylpyridine **34** and *N*,*N*-dimethylformamide dimethylacetal **40** is an important intermediate in the synthesis of the imatinib **33.** The reaction shown in Figure 23 between 3-acetylpyridine **34** and *N*,*N*-dimethylformamide dimethylacetal **40** was employed to investigate the optimum reaction conditions.

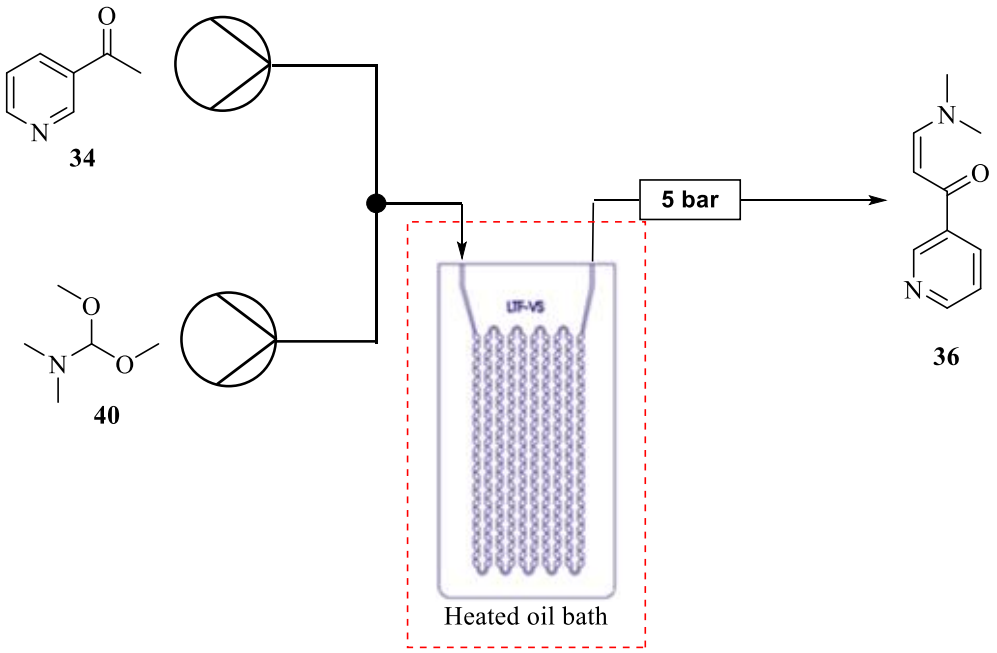


Figure 23: Flow set up for the flow synthesis of the 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one using 3-acetyl

In the initial screening experiments, a solution of 3-acetylpyridine **34** (0.1M in *o*-xylene) was reacted with a solution of DMF-DMA **40** (0.1M in *o*-xylene). The two solutions were pumped, at identical flow rates (0.041 ml/min), through a total reactor volume of 1.31 ml (reactor plus tubing) at the 140^{o}C, which equated to a residence time of 30 minutes. Using HPLC analysis, a conversion of 44% towards the enaminone **36** was observed.

Thus, an investigation into the optimum conditions which are ideal for the complete synthesis of (*Z*)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **36** was undertaken. This comprehensive study involved the study on the effect of residence time (controlled by the flow rate of the starting materials), molar ratios of the starting materials, effect of different solvents and temperature. The initial reaction with a 30-minute residence time investigation at 140^{o}C encouraged a study on the residence time with change in temperature using *o*-xylene as a solvent.

In the initial attempt to increase conversion at a temperature of 140°C (blue line), it can clearly be seen that there was a minimal increase in the conversion between the 15 minutes and 30 minutes. Thus, temperature was increased gradually to 200^{o}C. The highest conversion was obtained at a residence time of 30 minutes at 200^{o}C. Figure 24 illustrates a change in the conversion towards

the desired enaminone **36** as the temperature is increased, with varying residence times while the mole equivalence was kept constant in this study.

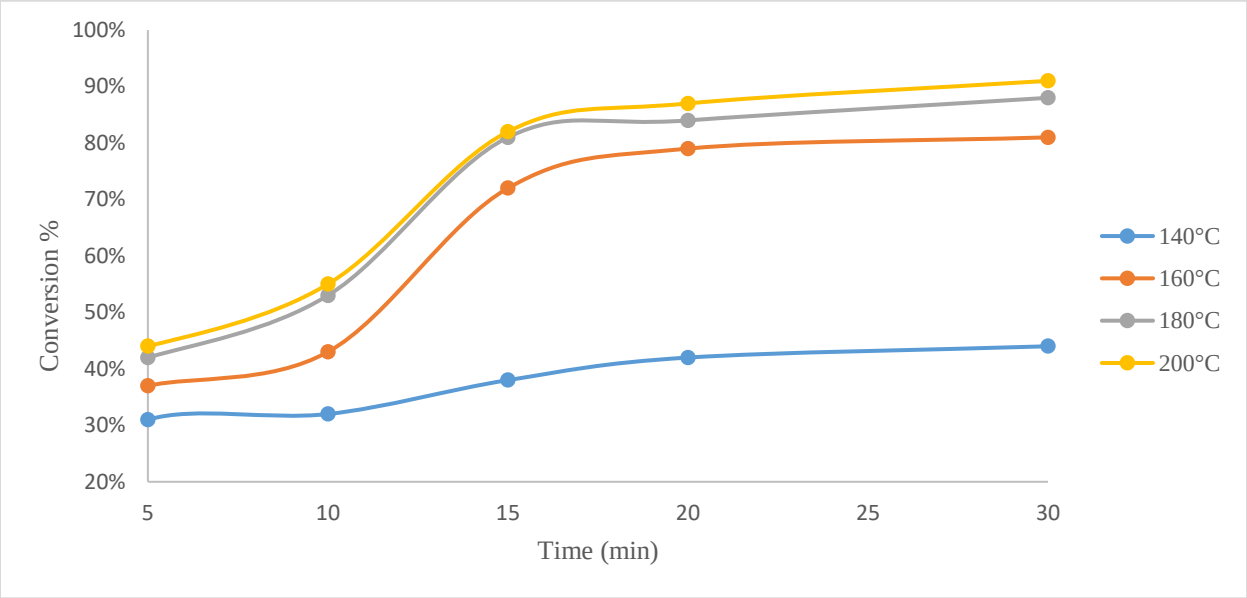
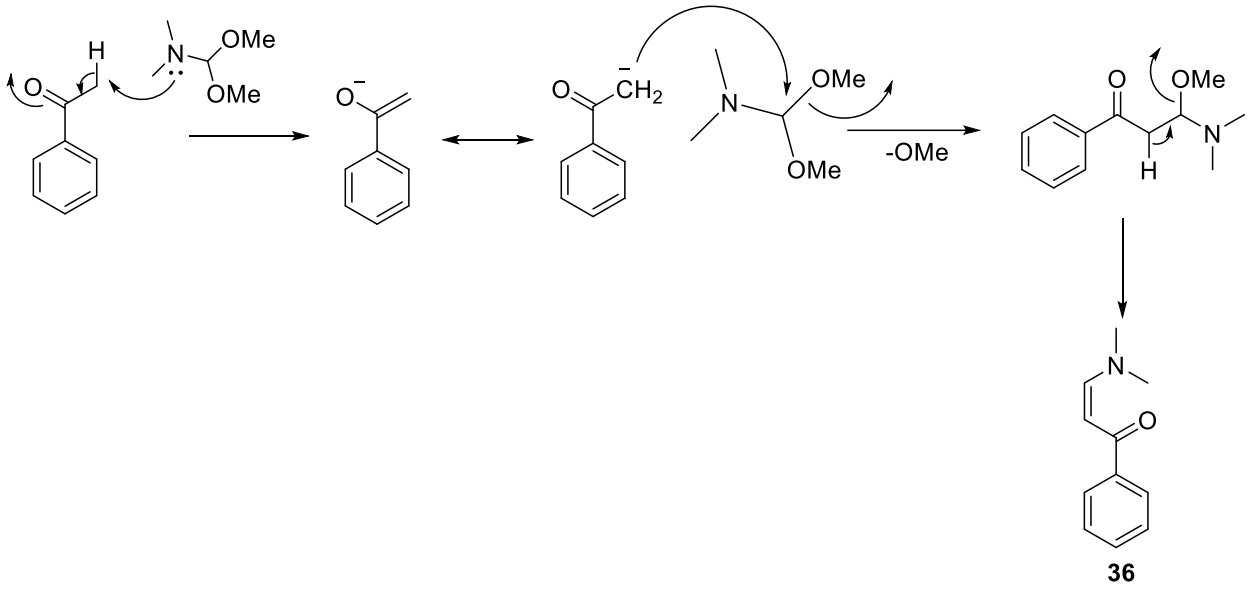


Figure 24: Temperature effect on the conversion % of the enaminone from the reaction with 3-acetypyridine and DMF-DMA in o-xylene

Figure 24 shows the effect of changing temperature on the condensation reaction of 3-acetylridine **34** with DMF-DMA towards the enaminone **36.** The molar ratio of the both starting reagents, 3-acetylpyridine **34** and DMF-DMA **40** was kept constant at 1:1 (0.1M) and temperature was gradually increased. The general trend shows a general increase in the conversion with an increase in temperature and residence time. The batch reaction temperature, 140^{o}C, in the synthesis of 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36**, using *o*-xylene as solvent and transferring these condition to the flow system provided a conversion of 44% in 30 minutes. This conversion towards the desired product is an ideal illustration of the advantages associated using microfluidic systems. The ability of the microreactors to withstand high temperature and use of calibrated backpressure regulators allows investigation of optimum reaction temperatures that are above the boiling point of the solvent and/or the starting materials.



Scheme 22: Plausible reaction mechanism for the synthesis of 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one from 3-acetylpyridine and DMF-DMA

Scheme 22 illustrates the plausible reaction mechanism; which suggests the enolate of 3-acetylpyridine initially forms, which is an excellent nucleophile.[85] This excellent nucleophilicity allows the enolate to substitute the methoxy group present on the *N*,*N*-dimethylformamide dimethylacetal, which is a good leaving group. This forms the resulting carbon-carbon bond, and the preceding step would be the elimination of methanol; the high temperatures used in the flow reactor would facilitate this elimination process. The synthesis of the enaminone **36** follows an initial S_N2 substitution of the methoxy group and E2 elimination, which allows for formation the carbon-carbon double bond.[86]

Figure 24 shows this increase in temperature, a measure of kinetic energy, resulting in an increase in conversion as the temperature is increased. This high temperature, coupled with the improved mixing due to the microreactors' small flow channels, improved heat transfer and the design of the microreactor also assist in creating turbulence to improve mixing of the reagents.[87] This amplified molecular interaction and kinetics between the reagents results in an increase in conversion towards the 3-(dimethylamino)-1-(pyridin-3-yl)- prop-2-en-1-one **36**.

Figure 24 illustrates the various temperature ranges that were investigated, which show a rapid increase with an increase in residence time from 5 mins to 15 mins. However, the difference in conversion between the residence times of 15 min to 30 min is minimal. Shorter residence times

require faster flow rates through the microreactors, thus a more turbulent flow is observed at faster flow rates, leading to improved mixing. A homogenous condition can be achieved by the rapid mixing, hence leading to a rapid increase in conversion towards the enaminone **36**.[88] These results obtained show that the optimum temperature in the flow synthesis of the enaminone is 200°C with a conversion of 91% in 30 mins.

3-Acetylpyridine **34** is the limiting reagent in this reaction, thus we investigated the effect of an increase in the mole equivalence of the DMF-DMA **40** on the conversion towards the synthesis of the desired enaminone **36**. The molar equivalence of the 3-acetylpyridine **34** (0.1M) was kept constant and the DMF-DMA **40** was varied and added in excess and the previously determined optimum temperature of 200°C was used. The molar equivalence of the 3-acetylpyridine **34** and DMF-DMA **40** was varied 1:1, 1:1.5 and 1:2, and the results obtained are shown on Figure 25.

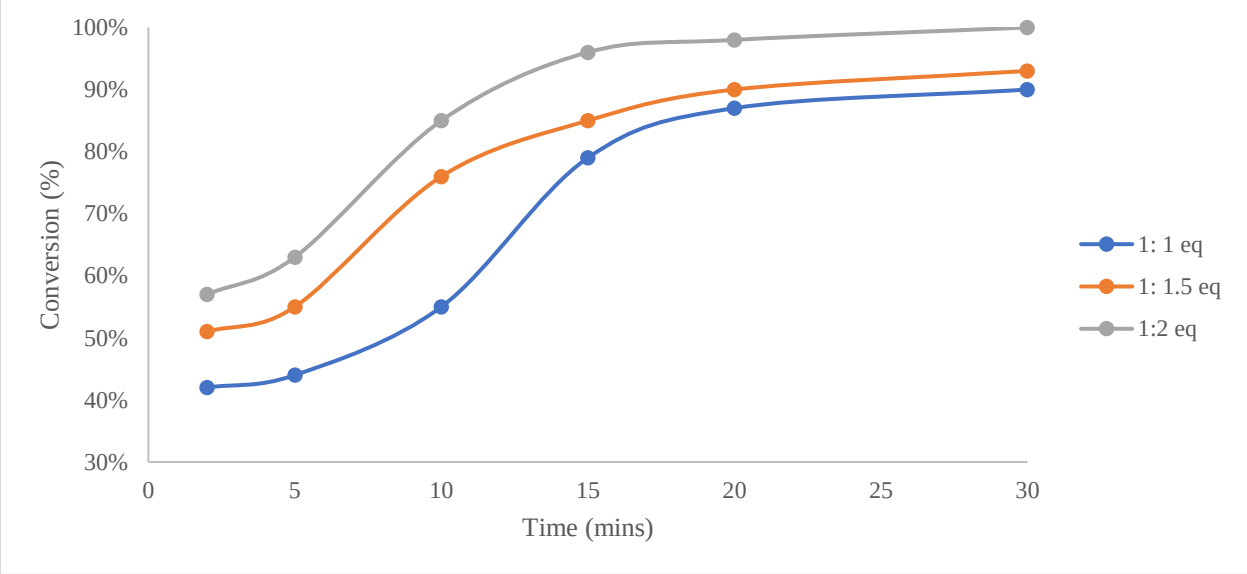


Figure 25: Effect of increase in the molar equivalence of the* N.N-*dimethylformamide dimethylacetal at 200°C, the 3-acetypyridine was kept constant at 0.1M

The graph illustrates an increase in the conversion towards the enaminone as the equivalence an increase in the equivalence of DMF-DMA. This suggest that an excess of DMF-DMA is required in order to improve the synthesis of the desired enaminone. By adding an excess of the non-limiting reagents, the results show an increase in conversion, at the previously determined optimum temperature of 200°C. Increasing the mole equivalence DMF-DMA by two-fold against the limiting reagent, provided a 100% conversion in 30 minutes and 96% in 15 minutes.

Solvents are crucial in the facilitation of chemical reactions, because they facilitate heat transfer, the dissolution of reagents and preventing hot spots and run-away.[89] Thus, the percentage reaction yields can either be decreased or increased depending on the solvent selection.[90]

We further explored the effect of concentration on the conversion of the starting material, 3-acetylpyridne **34** and DMF-DMA **40**. The reaction conditions that had been obtained, that is, reaction temperature of 200°C, 1:2 molar equivalence of the 3-acetylpyridne **34** and DMF-DMA **40**, where both solutions were prepared in *o*-xylene.

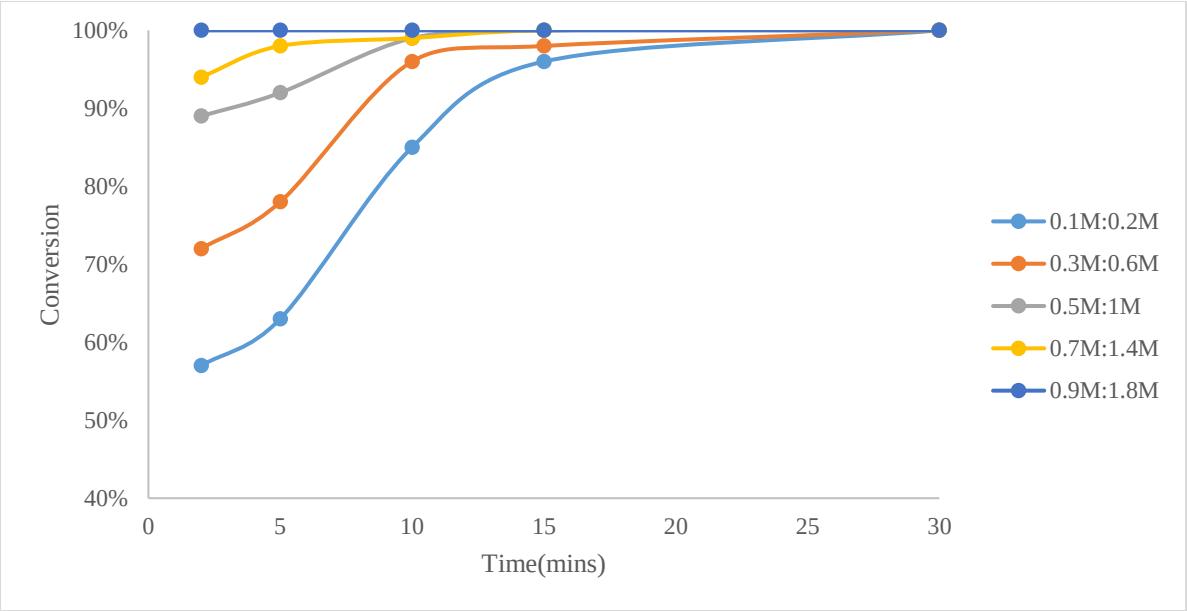


Figure 26: Effect of increase in concentration of the reagents 3-acetylpyridine and DMF-DMA on the conversion towards the desired enaminone

The results that were obtained showed the effect of the concentration on the conversion towards 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36**. This is expected since S_N2 reactions are concentration dependent reaction, thus increase the concentration of the 3-acetylpyridine **34** and the DMF-DMA **40** resulted in the decrease in the time needed to achieve high conversion at 200°C.[91] This is because referring to the reaction mechanism shown in Scheme 22 increasing concentration of the DMF-DMA correlates to a deprotonation of the 3-acetylpyridine **34** to form the enolate nucleophile. The high concentration of the nucleophile in S_N2 reaction and coupled with an increase in temperature means a resulting immense increase in the collision between the nucleophile and electrophilic center.[91] The highest conversion was observed were the

concentration of 3-acetylpyridne **34** was 0.9M concentration and the DMF-DMA **40** was 1.8M, with a 100% conversion with a residence time of 2 minutes.

We were also keen to understand the effect of solvent on the reaction. In the first instance in the solvent study, we arbitrarily used 3-acetylpyridine **34** (0.1M) in order to make comparisons. We investigated the effects that different solvents would have in the conversion towards the desired enaminone **36**. This implied use of 3-acetylpyridine **34** (0.1M) and DMF-DMA **40** (0.2M) with a mole equivalence of 1:2 respectively at 200°C made up in solution using the solvent being investigated.

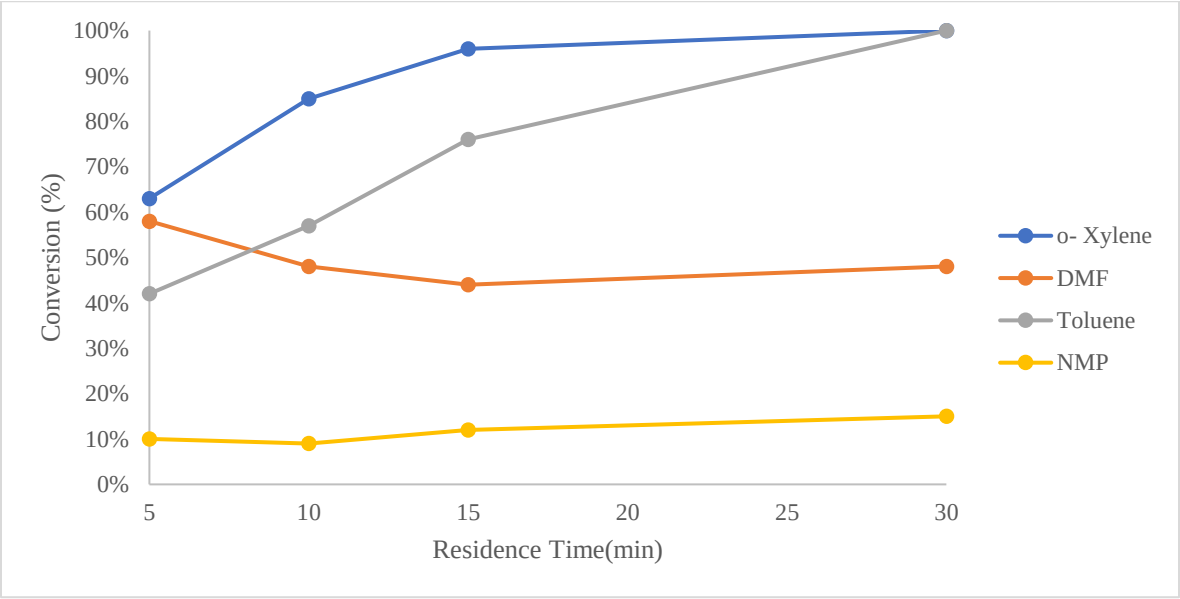


Figure 27: Effect of solvents with different properties on the conversion towards the 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one

Figure 27 illustrates the conversion towards 3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **36** when polar and non-polar solvents were used in the reaction. The solvents, *o*-xylene and toluene, are both non-polar solvents and as shown in Figure 27, there is a gradual increase in the conversion towards 3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **36** with reaction time when these solvents were used. These solvents were particularly desirable to investigate owing to the high boiling points, which would results in an overall reduction in pressure in the flow system and importance of high temperature in this reaction.

Use of the polar solvent, DMF and NMP, resulted in significantly lower conversion compared to the non-polar solvents, with a very slight increase with increasing residence time. DMF, when

used as a solvent, showed a decrease in conversion at longer residence times, but high conversion at low residence times. DMF has a lower solvent polarity compared to NMP among other aprotic solvents[92], thus the reaction is not hindered compared to NMP resulting in higher conversion, although a the conversion decreases with increase in time. The low polarity of the DMF provides some room reaction for flow synthesis of 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one **36** to occur at fast flow rates. Increase in residence time results in the decrease in conversion, this could be attributed to reactants' longer interaction with the solvent, thus the solvent property effects are more apparent.

The formation of the enolate nucleophile is highly reliant in the presence of the DMF-DMA **40** as shown in Scheme 22. Such bimolecular reactions are more favored as the polarity of the solvent decreases owing to the improved charge dispersal in the transition state of elimination reaction, when a nonpolar solvent is used for the reaction.[93] The higher conversion observed using *o*-xylene compared to toluene as a solvent in identical conditions could be attributed to lower relative polarity that *o*-xylene possesses compared to the toluene.[93]

Non-polar solvents *i.e. o*-xylene and toluene did not exhibit any detrimental effect in the flow synthesis of 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one **36.** This is indicated by a steady increase in conversion when residence time was increased. NMP due to its high polarity affected the reaction and achieved very low conversions. However, DMF also showed the ability to achieve high conversions, with 95% conversion achieved in 5 minutes.

Greener reactions that reduce the environmental impact, cost and increasing safety in organic reactions are of great interest. Flow techniques using microreactors can even more so allow the use of neat reagents, which would otherwise, be exothermic in a batch reactor system.[26,49] Organic solvents are costly, volatile, sometimes carcinogenic and toxic, thus the ability to eliminate in the flow synthesis of the enaminone **36** could be beneficial. Both reagents, 3-acetylpyridine **34** and DMF-DMA **40**, are liquid at room temperature and would easily flow through the microreactors. Thus, an investigation was carried out into the effect of temperature on the conversion towards the desired enaminone **36**. In order to achieve a 1:1 mole equivalence of the 3-acetylpyridine **34** and DMF-DMA **40**, the neat reagents were pumped from two different flow pumps with varying flow rates. Achieving accurate mole ratios was done calculating the amount of moles needed per residence time *e.g.* for 15 minutes' residence time with a total reactor volume of 1.31 ml, and using

3-acetylpyridine as the limiting reagent. An example calculation to achieve a 1:1 mole equivalence is shown below:

Flow rate for 15-minute residence with reactor total volume:

1.31 ml = 0.087 ml/min for 3-acetylpyridine **34**

ρ = mass/volume; thus mass= 1.102 g/ml x 0.087 ml = 0.0962 g

Hence, number of moles (n)= 0.0962 g/121.14g/mol = 7.94 x10^{-4} mol

Therefore, I mol eq. of DMF-DMA= (7.94 x10^{-4}) x 119.16 ml = 0.0946g;

Volume = 0.0889g/0.897g/ml= 0.105 ml, Hence the flow rate of the DMF-DMA **40** pump was 0.105 ml/min. This calculation was carried out for all residence times to achieve a 1:1 mole equivalence ratio investigation of the reagents.

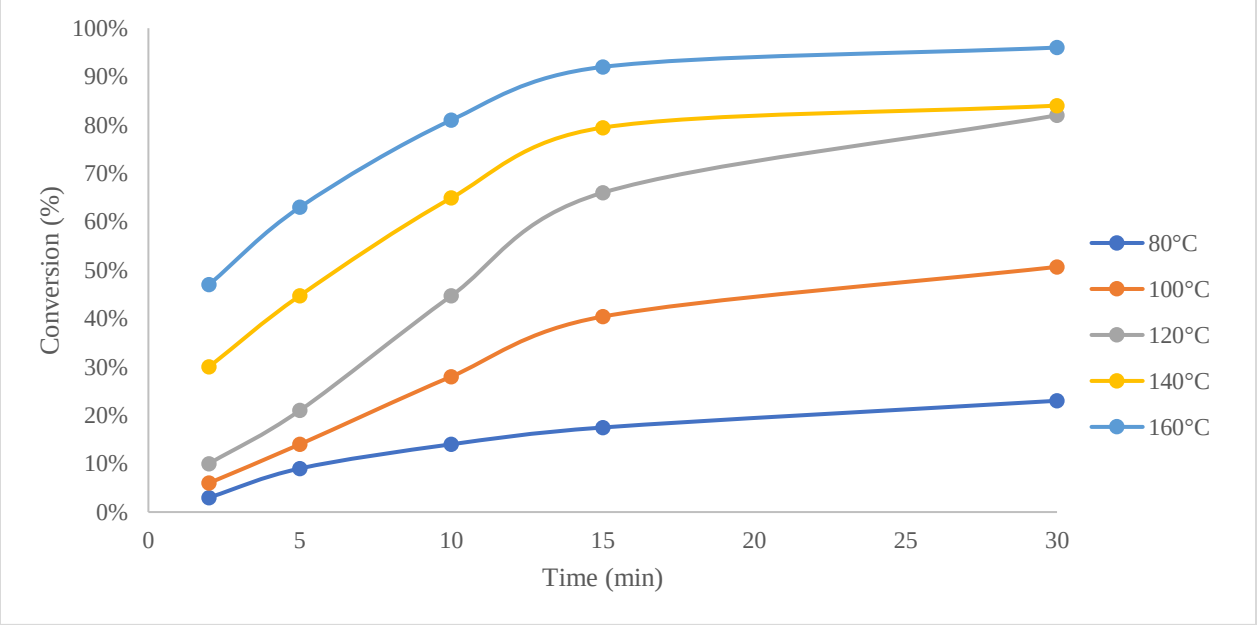


Figure 28: Conversion observed at varied temperatures with neat starting reagents, 3-acetylpyridine and N,N-dimethylformamide dimethylacetal using 1:1 mole equivalence

As shown in Figure 24, the optimum reaction temperature was found to be 200^{o}C in *o*-xylene which is above the boiling point of the solvents and the liquid starting materials. However, this reaction temperature could not be investigated in the optimization of the neat reaction due to Back-pressure regulator problems therefore, the maximum temperature that was as shown on Figure 28 investigated for the neat reaction was 160^{o}C.

Figure 28 shows the results obtained from the neat reaction between 3-acetylpyridine **34** and DMF-DMA in the flow synthesis of 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one **36**. The Zaiput backpressure regulator set to 5 bar provided the ability to work well above the boiling point of the reagents. Figure 28 further illustrates the effectiveness of temperature in this reactions with an increase in conversion per residence time observed as temperature was increased with 160^{o}C giving a total conversion of 96% in 30min. We investigated lower temperature, which had previously provided very low conversions when using a solvent. S_N2 reactions are concentration dependent reactions, thus neat reagents usually result in an increase in conversion at lower temperature and shorter residence times. The dependence on concentration can also be observed on Figure 26, where an increase in concentration resulted in an increase in conversion at shorter residence time. Conversion towards the enaminone **36** is observed at low temperatures *i.e.* at 80^{o}C, when using neat reagents because of higher availability of the reactants in neat reactions.[89] Thus, it is possible to eliminate the solvent in the continuous flow synthesis of 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36**, and still afford high conversions which would be ideal when upscaling. Such optimization at high temperatures would be difficult in a batch reaction because the boiling point for the DMF-DMA is 102^{o}C.

Table 7: Summary of batch and optimum flow reaction conditions in the synthesis of the enaminone

Reaction conditions	**Batch**	**Flow in *o*-xylene**[a]	**Flow neat**
Temperature (^{o}C)	140	200	160
Reaction time	6 hrs	2mins	15mins
Conversion	80%	99%	92%
Throughput	2.2g/h	2.89 g/h	6.54g/h
Solvent	*o*-xylene	*o*-xylene	-
[a]concentration: 3-acetypyridine:DMF-DMA 0.9M:1.8M			

The throughput of the expected mass of product obtained per unit time can be observed in the table above (Table 7). The overall mass per unit time improves in flow processes compared to batch synthesis reaction towards 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36**. The neat reaction showed the highest throughput, suggesting that the system can produce 6.54g.h in the LTF reactor. Thus, using the neat reagents illustrates a greener approach to the synthesis of the enaminone by elimination of the use of any solvent.

3.3 SYNTHESIS AND OPTIMIZATION OF 1-(2-METHYL-5-NITROPHENYL) GUANIDINIUM NITRATE

The batch synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** involved refluxing a mixture of 2-methyl-5-nitroaniline **35** with 50% aqueous solution of cyanamide and 65% nitric acid. The reaction is followed by a work up to remove unreacted starting reagents, to afford a 43% yield; compared to the yield reported by Chang *et al.*[67] of 53% is lower and could be loss of product during work up procedures.

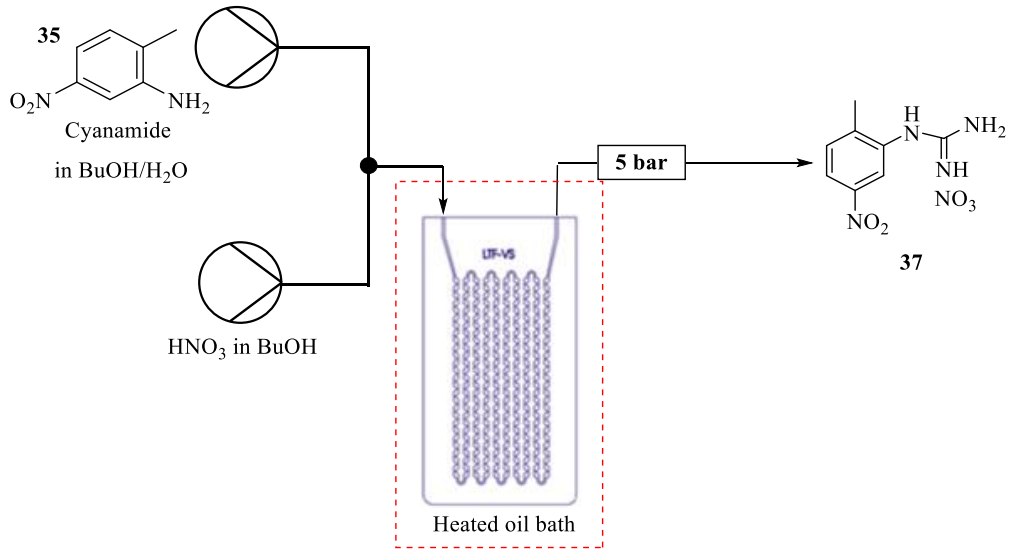
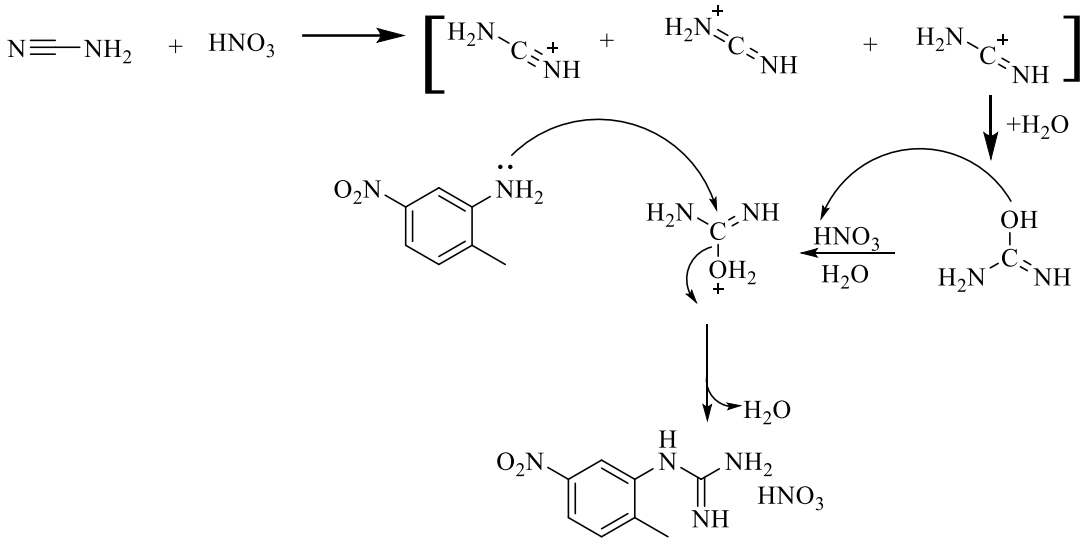


Figure 29: Continuous flow set for synthesis of the guanidinium nitrate salt to the enaminone in the presence of a base

Scheme 23 illustrates the plausible reaction mechanism for the synthesis of the guanidinium nitrate salt **37** salt *via* the acid hydrolysis of cyanamide.



Scheme 23: Plausible mechanism of reaction in the synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate

The importance of adapting this reaction to a continuous flow system prompted the investigation into the parameters that would allow for high conversion towards the guanidinium nitrate. The initial screening flow reaction was carried out using a prepared solution of 2-methyl-5-nitroaniline **35** (0.01M) and cyanamide (0.02M) using a 50:50 butanol:H_2O solvent mixture and nitric acid (0.02M) in butanol. As shown in Figure 29, the reagents where pumped through the a total reactor volume of 1.31 ml at 115ºC using a 30 minute residence time to provide a conversion measured using HPLC of 34%. Thus, using these conditions, we undertook to investigate the effect temperature has on the conversion of towards the *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**.

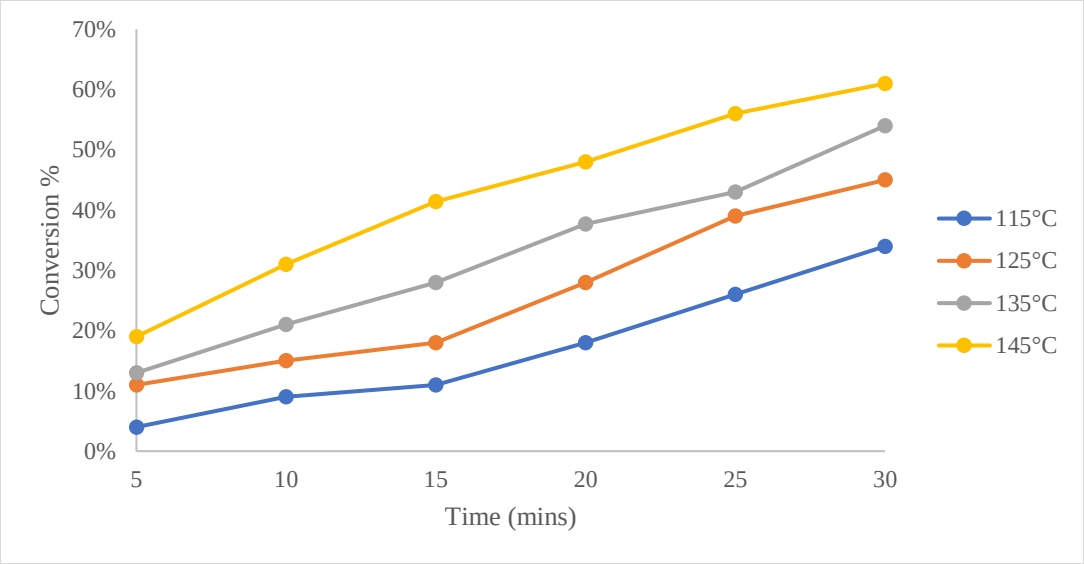


Figure 30: effect of temperature on the conversion towards 1-(2-methyl-5-nitrophenyl) guanidinium nitrate using cyanamide in the presence of nitric acid

The results obtained show an increase in conversion towards the desired product, with an increase in temperature. E1 reactions, as shown in the reaction mechanism shown on Scheme 23, are more favored with an increase in temperature.[94] Thus, an increase in temperature resulted in an increase in conversion towards the target guanidinium nitrate salt **37**. The breaking of bonds of the C-H is also favorable at higher temperature, thus the increase in temperature, allows the bond breakage to occur faster resulting in higher conversion.[95]

In order for the reaction to occur, acid catalyzed hydrolysis of the cyanamide is crucial, which then reacts with amino group resulting in the guanidinium salt **37**. A solution of the 2-methyl-5-nitroaniline **35** (0.01M) and cyanamide (0.02M) were kept constant while the concentration of nitric acid was varied at 145^{o}C.

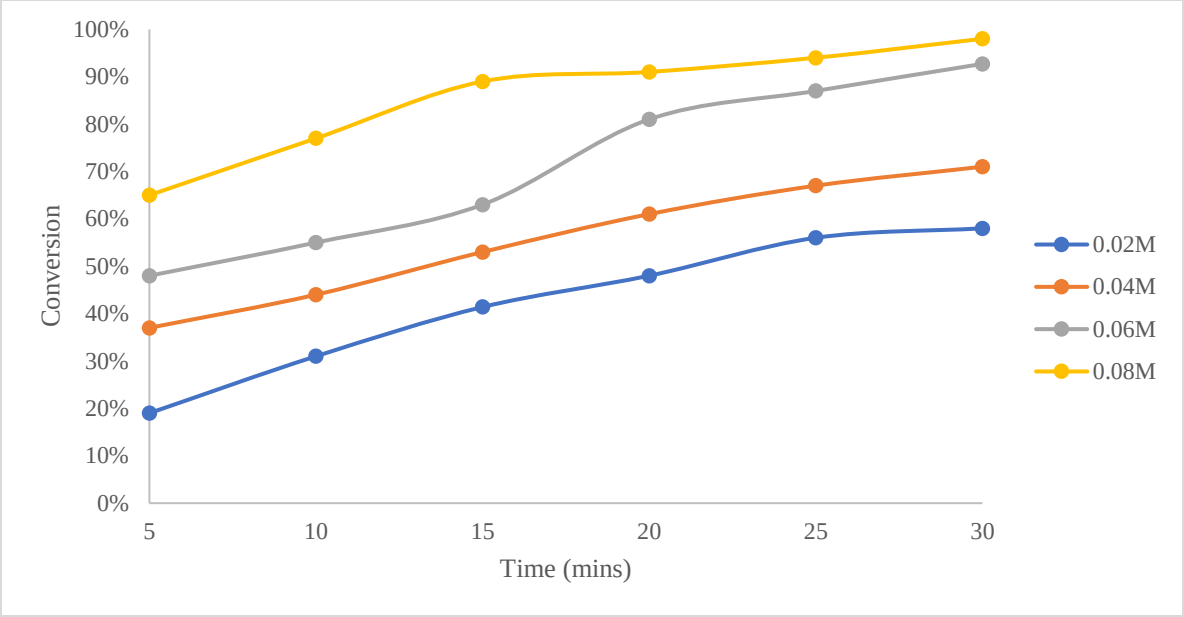


Figure 31: Effect of nitric acid concentration towards the guanidinium salt at 145°C

Increasing the concentration of the nitric acid at 145°C as shown in Figure 31, resulted in an increase in conversion towards the *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**. Nitric acid functions as a nitrating agent and as a hydrolyzing agent towards the complete synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**. Thus, an increase in concentration of the nitric acid with the concentration of the cyanamide kept constant means the faster hydrolysis of the cyanamide to form the intermediate. Thus, this reaction is highly depended on how quickly the cyanamide can be hydrolyzed in to form the intermediates that can react with the 2-methyl-5-nitroanile **34** to form the stable guanidinium salt. Therefore, the data shown on Figure 31 illustrated that increasing the nitric acid concentration resulted in a higher conversion towards the desired product. The high temperature increase the collision between the necessary nitric acid and cyanamide molecules, and the small width the flow reactor channels assist in improved mixing of the reactants.

Water plays a crucial role as a nucleophile in the synthesis of the 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** as shown on Scheme 23. Thus, tabulated below are the results obtained from the effect of the co-solvent mixture, which is butanol and water.

Table 8: Conversion % towards the guanidinium salt with varied co-solvent ratios

Solvent ratio (Water:BuOH) w/w%	Conversion
80:20	99%
70:30	96%
60:40	94%
50:50	89%
40:60	73%
30:70	66%
20:80	57%

*Temperature: 145^{o}C Residence time: 15mins *Nitric acid: 0.08M

Table 8 shows above represents the effect the co-solvent mixture used in the preparation of the 2-methyl-5-nitroaniline **34** and cyanamide stock solution in the continuous flow synthesis of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**. The results obtained showed an increase in conversion towards the desired product, with an increase in water quantity. The highest conversion was observed at an 80:20 water to butanol ratio, used in the preparation of the stock solution. It is important to note that 2-methyl-5-nitroaniline **35** showed partial solubility with at a 90:10 water:butanol mixture, which prevented investigation in this solvent mixture. Solvents such as water that possess a high dielectric constants a considered to be ideal ionizing solvents that organic solvents such ethanol and butanol. Thus, the improved ionization when there is a higher concentration of water, results in an improve ionization of the cyanamide, at low nitric acid concentrations. The solvation of cyanamide improves because of the increased amount of water, which refers to the ability of the solvent to stabilize ions. Increase the amount of water in the reaction correlate to an increase in high conversions, which previously needed high acid concentrations to achieve.[96] Thus, these conditions a comprehensive residence time study was carried out to check the effect of 0.8M concentration of nitric acid and 80:20 water: butanol solvent mixture.

Table 9: Batch and Flow conditions in the synthesis of N-(2-methyl-5-nitrophenyl) guanidinium nitrate

Reaction conditions	Batch Reaction	Flow Reaction
Temperature	115^{o}C	145^{o}C
Reaction time	12 hrs	15 mins
Conversion	40%	99%
Nitric acid eq.	2	8
Throughput	0.012g/h	0.208g/h

Thus, as shown above the conversion towards *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37**, was greatly improved increasing the nitric acid concentration and also indicated that an increase in water improved conversion to the product. The amount of product per hour, that can be obtained using the continuous flow, is significantly higher compared to the batch reaction, by producing at least 0.208 g/h compared to 0.012 g/h.

3.4 SYNTHESIS AND OPTIMIZATION OF *N*-(2-METHYL-5-NITROPHENYL)-4-PYRIDIN-3-YL-PYRIMIDIN-2-YLAMINE

Batch synthesis of *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**, involved reflux in butanol for 16 hours of the 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** and 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36** in the presence of sodium hydroxide (Scheme 21). A yield of 62% was obtained after work up and the time needed to achieve this yield is long (16 hours) and the work up procedure leads to loss of product. Thus, in order to exploit the advantage that flow chemistry possesses, in this reaction we sought to investigate to reduce the time needed to achieve high conversion. This includes investigating the effect of temperature, equivalence of the base used in batch and investigating use of other bases in order to simply the work-up procedure.

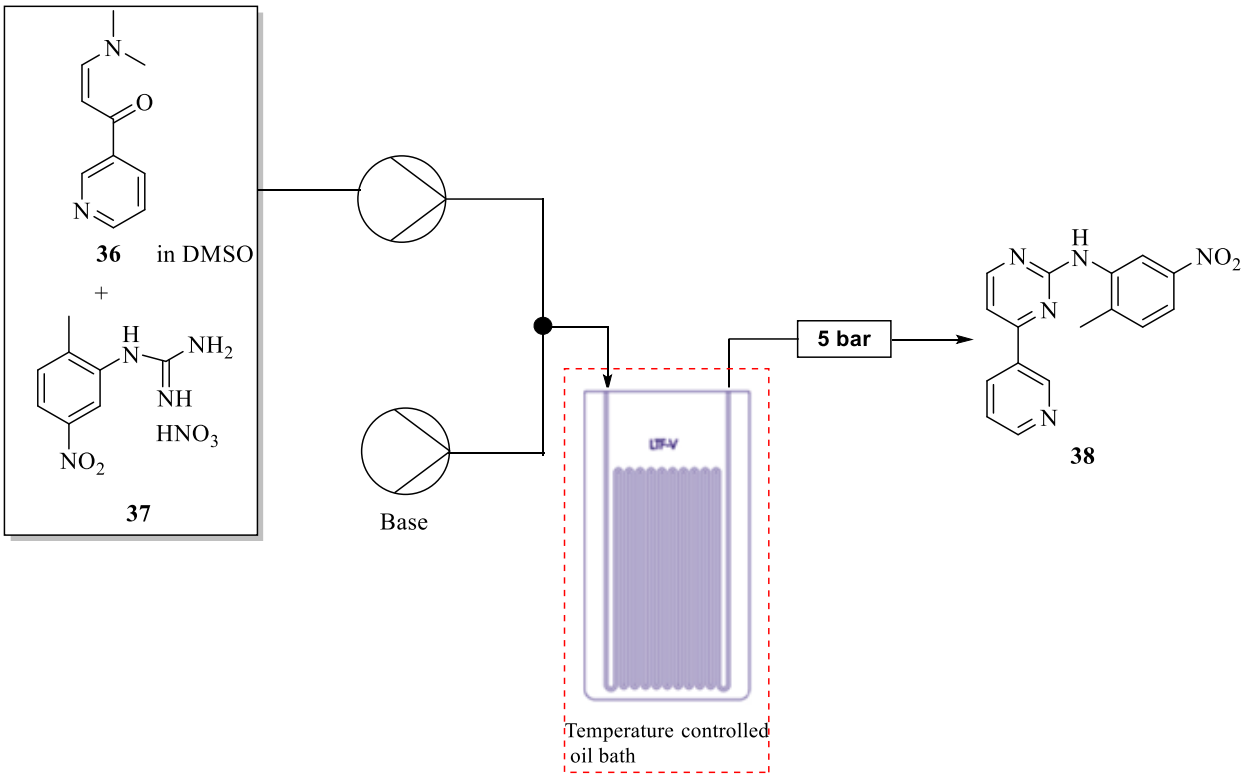


Figure 32: Continuous flow set for the cycloaddition of the guanidinium nitrate salt to the enaminone in the presence of a base

Thus, to investigate the ideal parameters in the condensation reaction between the enaminone **36** and 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** in flow synthesis of the 2-aminopyridine core **38**, conditions such as the mole equivalence of the base to the enaminone **36** and the guanidinium salt **37**, the temperature and different bases were investigated. The plausible reaction mechanism of this reaction is also shown below:

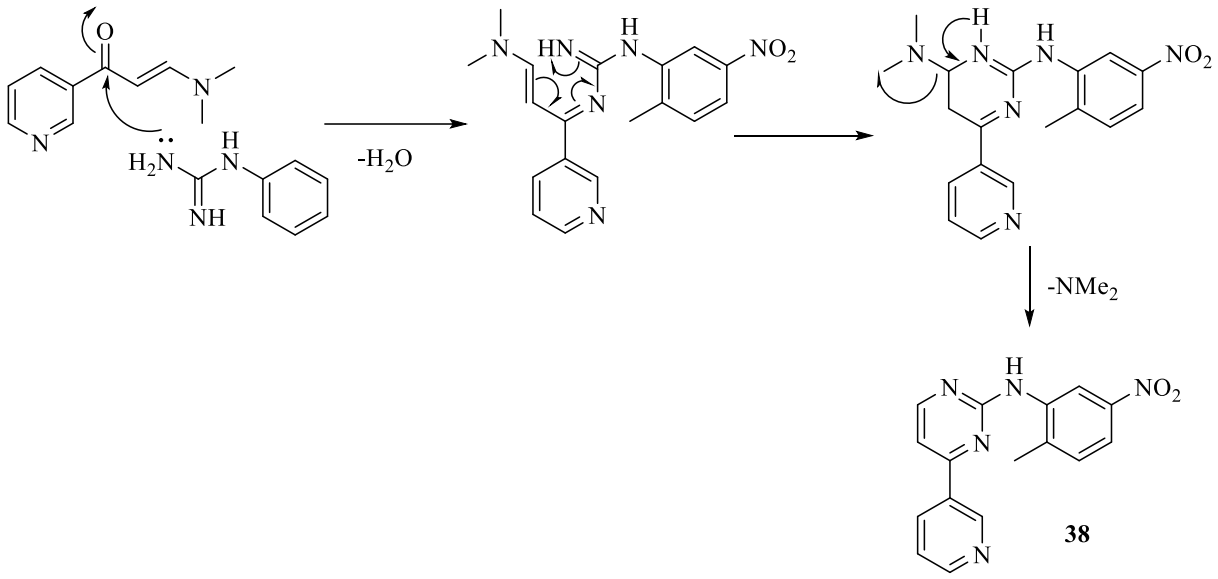


Figure 33: Plausible reaction mechanism on the cycloaddition reaction between the enaminone and 2-methylguanidine nitrate

The preliminary reaction was carried out using 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one **36** (0.0153M) prepared in a stock solution with of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** (0.02M) in DMSO and sodium hydroxide (0.031M, 2eq) in water. The investigations were carried out at low concentration due low quantities of starting materials. However, using 2eq of the NaOH at temperatures from 120^{o}C-200^{o}C for the preliminary reaction provided little to no conversion towards the desired 2-aminopyridine, within a maximum residence time of 30 minutes. Increasing the molar of NaOH (0.186M, 6eq) did not show any improvement in the flow synthesis in the conversion as shown on Table 10. The starting materials could still be observed using HPLC analysis using the batch samples as a standard, which had been confirmed via NMR, thus showing that there was little to no reaction between the reactants.

Thus, we investigated other bases and increasing the molar equivalence of the bases that could be used in order to find an optimum condition that could provide high conversion towards the *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**. Table 10 shows the various bases that were used to investigate the base catalyzed cycloaddition reaction of 2-methyl-5-nitroaniline guanidinium nitrate **37** with the enaminone **36**. The base in this reaction is responsible, once solvated, to precipitate the NO_3^- thus leaving the reactive guanidinium ion. The reactive guanidinium ion can then react with the enaminone **36** *via* a cycloaddition to form the desired

intermediate *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**. Thus, we investigated the effect of the different organic bases and inorganic bases at 120°C.

Table 10: Effect of various bases on the synthesis of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

Base	*Molar Equivalence*	*Solvent*	*Conversion*
Sodium Hydroxide	2	Water/	-
Sodium Hydroxide	4	water	2%
Sodium Hydroxide	6	Water	6%
Potassium carbonate	2	water	3%
Triethylamine	2	water	-
DBU	2	methanol	-
Cesium Carbonate	1.5	Water	6%
Cesium Carbonate	2	water	9%
Cesium Carbonate	2.5	water	13%

*Temperature: 120°C *All residence time is 30minutes

Other bases, shown in Table 10, that were investigated in this cycloaddition reaction, provided minimal conversion towards the desired product **38**. The preliminary reaction that gave promising results was when cesium carbonate (0.023M, 1.5eq) in water and a solution of 1-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** (0.02M) mixed with 3-(dimethylamino)-1-(pyridin-3-yl)-prop-2-en-1-one 36 (0.0153M) at 120°C.

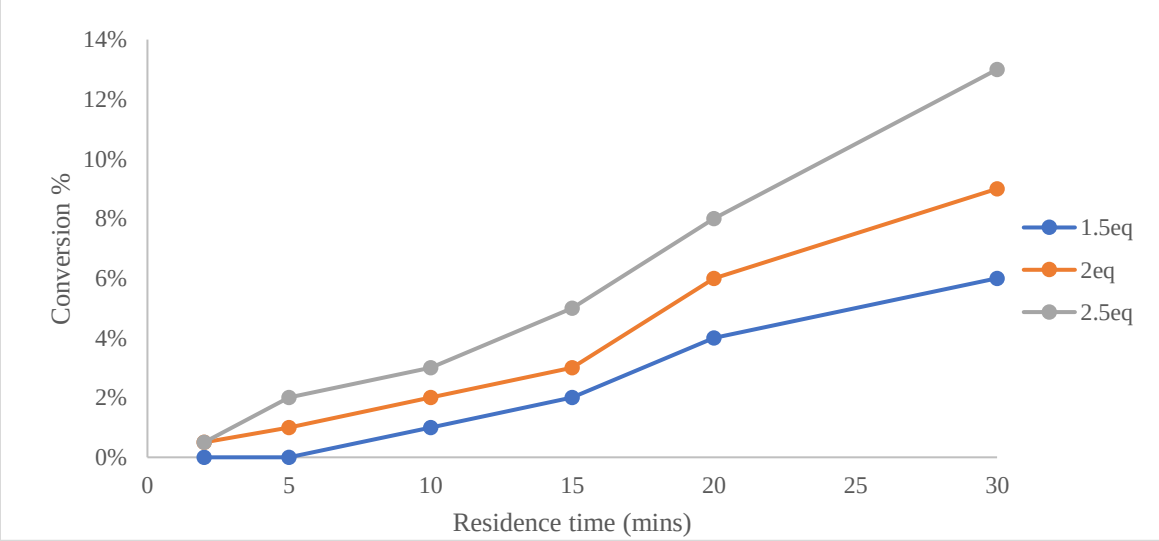


Figure 34: Effect of the increase in mole equivalence of cesium carbonate in the reaction towards the desired 2-aminopyridine core

Cesium carbonate is an ideal choice to utilize as a base catalyst because of its ease of handling and low hygroscopicity.[97] Figure 34 illustrates the investigation into the effect of increase of the cesium carbonate molar equivalence in the flow synthesis of the 2-aminopyridine core **38**. The highest conversion was obtained using 2.5-mole equivalence of the cesium carbonate (0.038M), that is, 13% at a residence time of 30 minutes. This conversion, which was not achieved using other bases could be attributed to the size of a cesium cation, which contributes to the strength of the covalent bond between the cesium and carbonate. The large size of the cesium cation means that a weaker covalent bond exist in the cesium carbonate, meaning the dissociation of the Cs^{+} and CO_3^{2-} is much easier.[98,99] Other bases, shown in Table 10, that were investigated in this cycloaddition reaction, provided minimal conversion towards the desired product **38,** can be attributed to the solvation of the bases in water. The size of a cation, contributes to the strength of the covalent bond, that is, the smaller the cation the stronger the covalent bond and vice versa. The size of the cesium cation result in better solvation, because of the weak covalent bond, which is responsible for the faster reaction rate.[98,99] The cesium cation is needed in order to precipitate the nitrate present plays a crucial role in this reaction with the cesium cation binding to the nitrate anion, to allow the guanidinium to participate in the reaction.[100] Increase in the conversion is a result to the increase in the concentration of the cation present within the reaction, thus the product formed although at low conversion.

The results obtained in Figure 34 prompted a further investigation into the effect of temperature on the conversion towards the *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **37** using a cesium carbonate (0.038M, 2.5eq). Thus, using the base mole equivalence that afforded the highest conversion in 30 minutes at 120^{o}C, there was further investigation on the effect of temperature on the conversion towards the desired product. The base investigation within the 30 minutes residence time allowed us to investigate the effect of temperature on the rate of conversion towards *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**. Using a cesium carbonate as a base, we further investigated the effect of an increase in the mole equivalence of the base using a temperature range of 120^{o}C-180^{o}C.

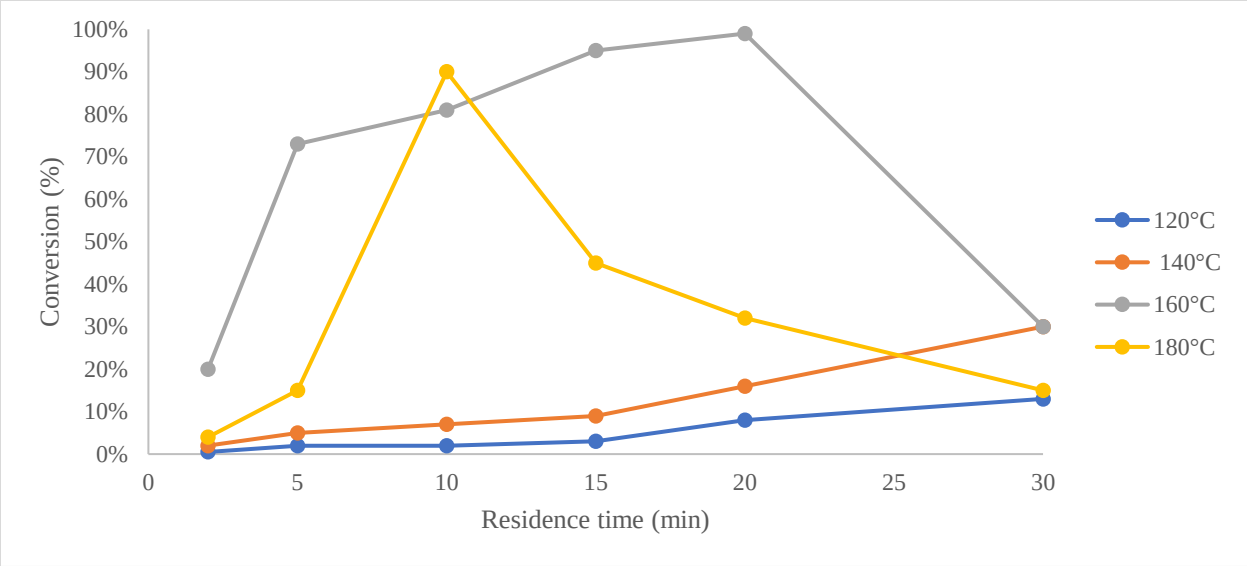


Figure 35: Temperature optimization study for the cycloaddition reaction of the guanidinium nitrate and enaminone in the presence of 2.5 equivalence of cesium carbonate

Figure 35 graphically represents the results obtained in the investigation of the effect of temperature on the condensation of the enaminone **36** and guanidinium salt **37** using cesium carbonate as a base. The conversion towards *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**, at lower temperatures *i.e.* 120ºC-140ºC, is low but a gradual increase in conversion with an increase in residence time. Increase in temperature gave saw a rapid increase in conversion, as the residence time increases, this is owing to improved solvation of the reagents and the bases with an increase in temperature. The solubility of the *N*-(2-methyl-5-nitrophenyl) guanidinium nitrate **37** proved to be difficult and DMSO was capable solubilize the salt at room temperature. Solubility increases as temperature increases, and Diels-Alder reaction show a reliance on temperature based on the size of the molecules that are involved.[101] Thus, the larger the molecules that are involved the higher the temperature is needed to activate the reaction. The shorted residence time of 10minutes needed to achieve the high conversion of 90% towards the *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**. The high temperature investigation, at 160ºC and 180ºC, show a decrease in the conversion with an increase in the residence times. The decrease in conversion at longer residence times could be attributed to the decomposition of the newly synthesized adduct which at elevated temperature and pressure could lead to decomposition, when exposed to heat for long.[101] We suspect that there was decomposition

since new peaks were observed when analyzing the product formed using HPLC. However, we could not isolate to the compounds in order to investigative what had otherwise been formed.. Although, the reaction at 160^{o}C at a residence time of 20 mins, provided a higher conversion compared to the 90% obtained in 10 mins at 180^{o}C. This, is because a high conversion can still be obtained from the flow system at half the time.

Table 11: Batch and flow reaction conditions and conversion comparison in N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine synthesis

Reaction conditions	Batch	Flow
Temperature	115^{o}C	180^{o}C
Reaction time	12 hrs	10 mins
Conversion	60 %	90%
Throughput	0.018g/h	0.04g/h

The ability to increase the temperature, thus working above the boiling point of reagents allowed for improved in the conversion in the reaction to produce the imatinib intermediate, *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **37**. The throughput of the flow reaction, that is, the mass of compound per unit time, is significantly improves when utilizing the flow reaction compared to the batch reaction. Thus, this illustrated a simple method of the flow synthesis and the efficacy of the system with the possibility to be upscaled.

3.4 SYNTHESIS AND OPTIMIZATION 6-METHYL-*N*^{1}-(4-(PYRIDIN-3-YL)PYRIMIDIN-2-YL)BENZENE-1,3-DIAMINE

In the batch reaction, the reduction reaction was carried out by mixing *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** with the catalyst $Fe(acac)_3$ (3mol%) in the presence of hydrazine hydrate. This reaction was refluxed at 140^{o}C at for 3 hours, which resulted in 100% reduction of the NO_2 to the corresponding aniline, which was confirmed *via* TLC and FTIR.

Catalytic hydrogenation by transition metals is usually the method of choice, because the non-catalytic methods such the Bechamp reaction that involves treating the nitroarene with stoichiometric amounts of Fe in acidic conditions.[102,103] A major concern for most metal catalyst such as Pd, Pt or Ru in the reduction of nitroarenes is the lack of selectivity and some are still particularly expensive. Thus we chose, the iron pentanedionate, as our catalyst which has been shown to possess improved selectivity, is inexpensive, and has the capability to be recoverable to be used multiple times as a catalyst. Hydrazine hydrate compared to its anhydrous form is

particularly stable and safe to handle, and as shown in Figure 38 forms only N_2 as byproduct.[103] Although, Pd/H_2 has been widely used in reduction reactions, palladium is still a very expensive and insoluble metal catalyst and compared to the inexpensive $Fe(acac)_3$, which can be easily used in flow due to its solubility in a great number of solvents.[103]

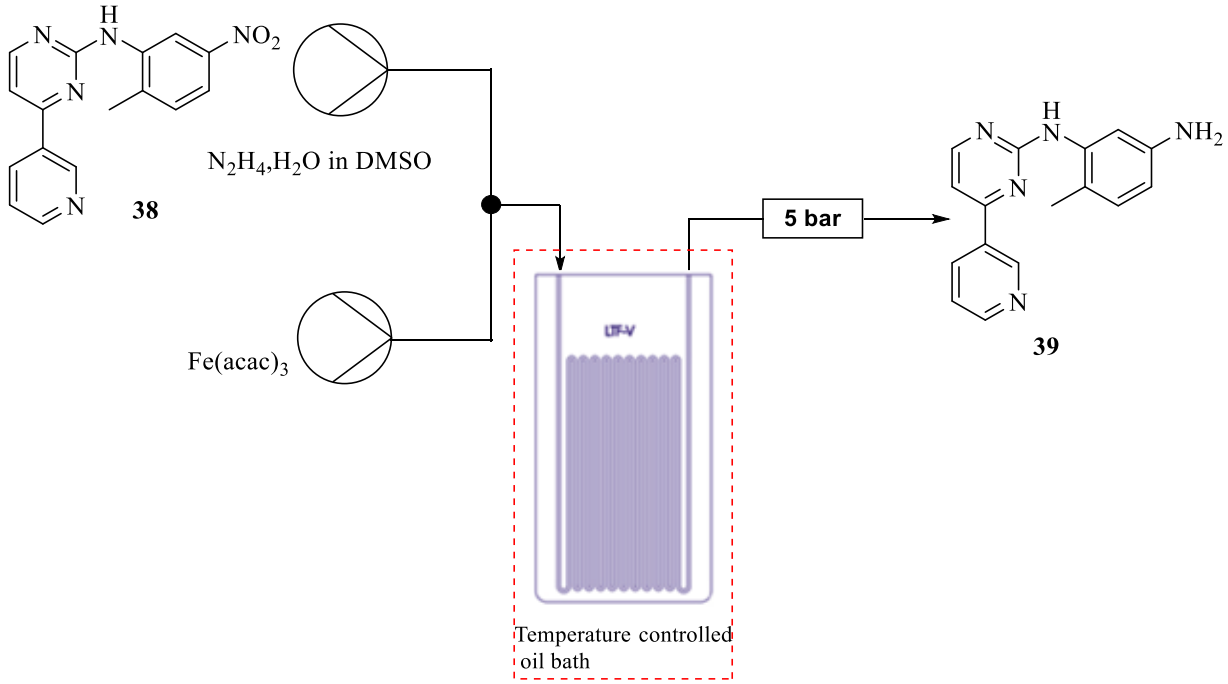


Figure 36: Schematic representation of the flow set up for the catalytic reduction towards 6-methyl-N^1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1, 3-diamine (34)

The reduction of the 2-aminopyridine **38** is the next important step towards the synthesis of imatinib. Figure 36 shows the flow set up that was utilized in the reduction, given the advantage of the easy solubility of iron pentanedionate in various solvents. Preliminary reactions were carried out by using a solution of using *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** (0.01M) and hydrazine hydrate (0.012M) prepared in DMSO and iron pentanedionate (3mol%) using DMSO as solvent at 130°C. The sample was collected after a residence time of 30 minutes and a conversion of 58% was confirmed using an HPLC Agilent 1220.

This success in the nitro group reduction prompted an investigation into the optimum conditions that could provide a high conversion at a shorter residence times. The conditions investigated were the effect of temperature, equivalence of the hydrazine hydrate, mole equivalence of the catalyst and residence time. Iron pentanedionate cystals

Figure 37 illustrates the results obtained during the study on the effect of temperature on the reaction with a residence time maximum of 30 minutes. The reaction parameters: *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** (0.01M), hydrazine hydrate (0.012M) and the iron pentanedionate (3mol %) were kept constant, while temperature was gradually increased.

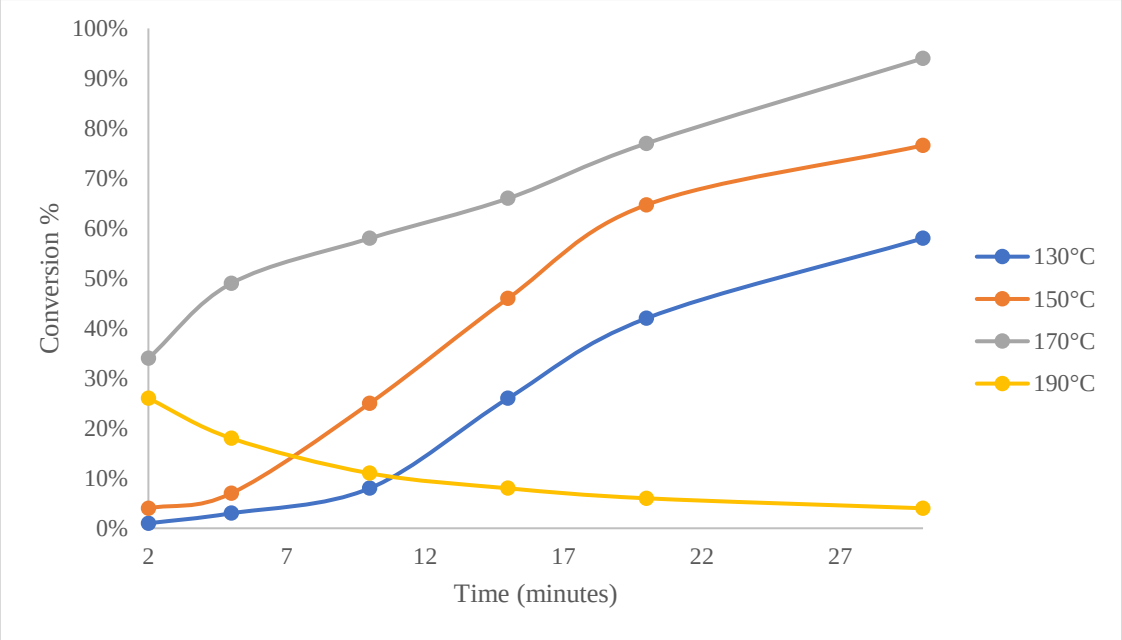


Figure 37: Effect of temperature on the reduction of N-(2-Methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine using iron pentanedionate in the presence of hydrazine hydrate

The reduction step using the catalyst illustrates that it is dependent on temperature, with a dramatic increase in the conversion towards the desired product when the temperature is increased from 150ºC to 170ºC. The mechanism in which this reaction occurs is reliant on the oxidation of the hydrazine hydrate by the $Fe(acac)_3$,[9] in which the hydrazine undergoes the oxidation reaction showing in Figure 38. This in turn provides the electrons from the hydrazine hydrate necessary for reduction needed in NO_2 to NH_2.

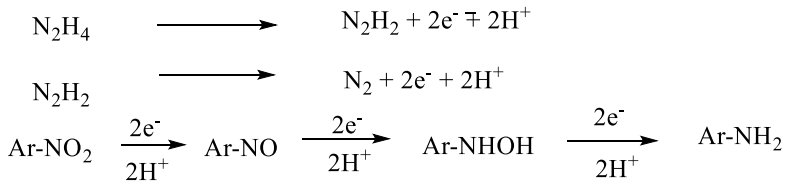


Figure 38: Mechanism for the reduction of nitroarenes[8,103]

The complete oxidation of the hydrazine hydrate yields 4 electrons and N_2 gas, which takes place *via* the diimide intermediate. Increasing in temperature and the small flow channels present within a LTF-V microreactors, improved the interactions between the hydrazine hydrate and the Fe^{3+} ions, thus leading to an increase in the oxidation of the hydrazine hydrate needed to release the 6 electrons for the reduction of the NO_2 group.[104] Thus, the increase in the conversion towards the aryl aniline **34**, is shown to increase with an increase in temperature. The residence time shows a steady increase in conversion, since longer residence times correlate to longer exposure to temperature and the time for reagent interaction is elongated, thus leading to improved conversion.

The temperature was increased to 190^{o}C, in order to investigate whether the residence time needed to afford high conversion towards reduced product could be observed. However, the conversion towards the desired reduced product **39** decreases with an increase in residence time (Figure 37 and Figure 39). This is because the decomposition temperature of hydrazine hydrate is reported to be 183^{o}C. Figure 39 illustrates the trend observed at 190^{o}C, where the conversion is seen to decrease with time. The shorter residence time, which directly translates to faster flow rates, provided better conversion owing to less contact time of the hydrazine hydrate to the temperature compared to longer residence times. The mole equivalence of the hydrazine hydrate was increase at 190^{o}C, to observe the effect of temperature has on the hydrazine hydrate and its role in the reduction of the NO_2. Increase in the equivalence afforded a higher conversion at a shorter residence time, with 1.8 equivalence of the hydrazine hydrate giving 68% in 2 minutes. Further increase in the equivalence of the hydrazine hydrate was not carried out as it became apparent that large amount would be needed for high conversions at short residence times.

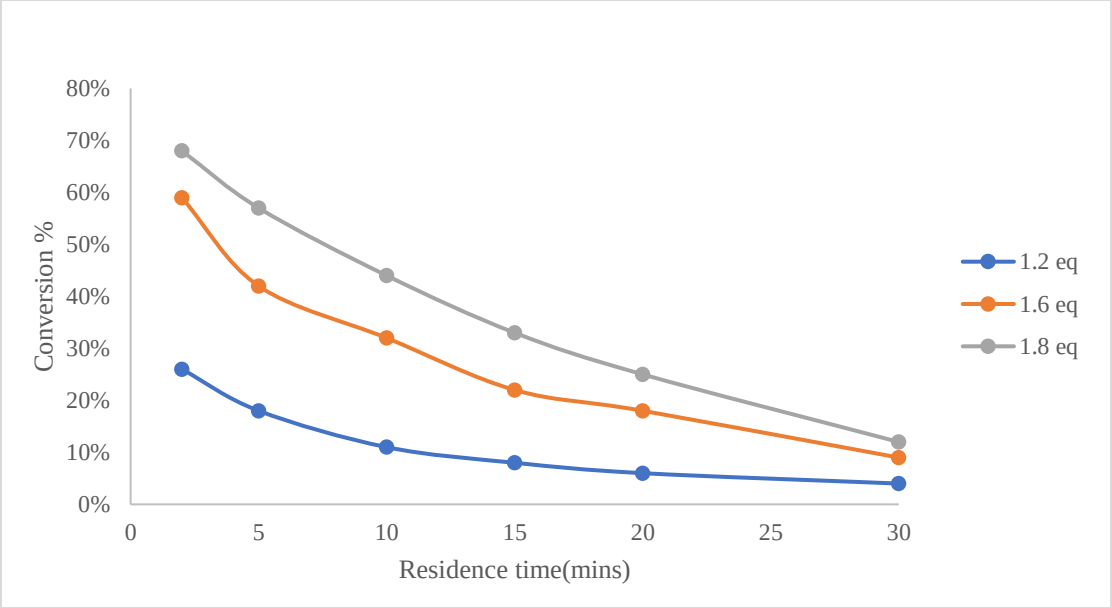


Figure 39: Effect of mole equivalence of hydrazine hydrate on conversion in the reduction of NO_2 above the decomposition temperature of hydrazine hydrate at 190°C

Since, hydrazine hydrate plays a crucial role in the reduction of the NO_2 in the compound *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38**, we investigated the effect an increase in the mole equivalence of the hydrazine hydrate, on the time needed to achieve high conversion. The reaction parameters were as follows: *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** (0.01M) at 170°C, with a solution of $Fe(acac)_3$ (3mol%) in DMSO at the hydrazine hydrate concentration was varied.

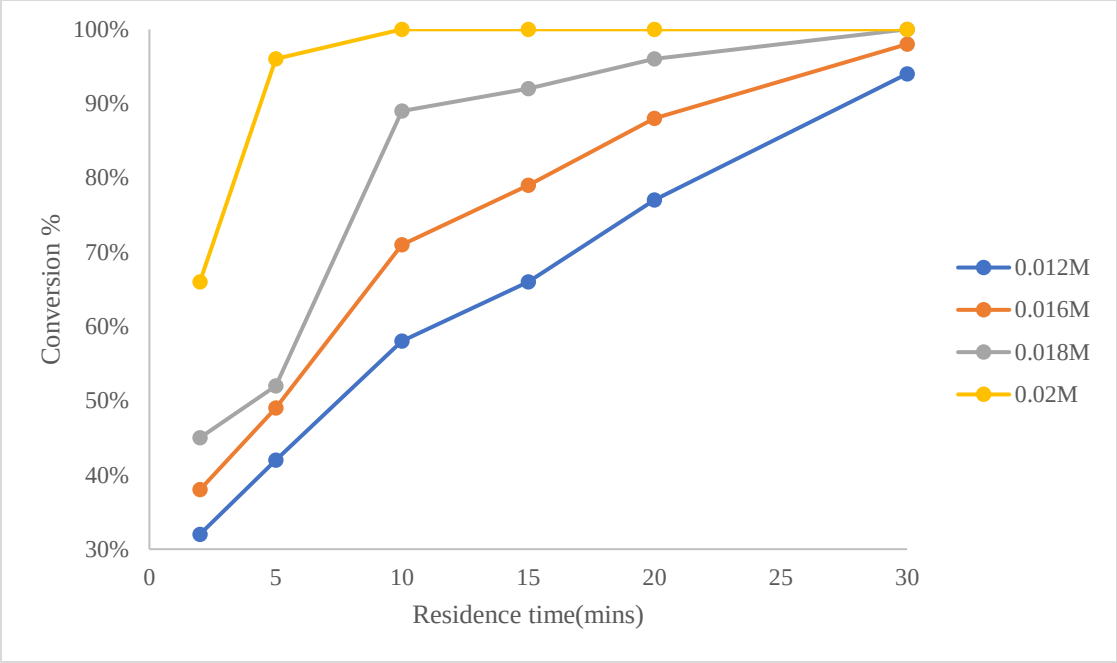


Figure 40: Effect of equivalence of hydrazine hydrate ($N_2H_4.H_2O$) on the rate of the reduction of NO_2 to NH_2 at 170°C

Iron species catalyze the reduction reaction by interaction with the hydrazine and generation of the Fe^{2+} from Fe^{3+}.This reduction reaction is catalyzed by the iron species, which have been indicated to be responsible for the electron transfer needed when in the presence of hydrazine hydrate. The reduction also requires 6 electrons as shown in Figure 38, but only a total of 4 electrons are released from the hydrazine hydrate.[105] This suggest that a higher equivalence is needed to provide the electrons needed for the complete reduction of the NO_2 group. The data obtained agrees with this theory, hence, we observe an increase in conversion with an increase in the equivalence of the source of the electrons needed per residence time. Using 0.02M of the hydrazine hydrate resulted in a maximum conversion of 96 % conversion in 5 minutes towards the reduced product.

In order to observed the effect of an increasing in the concentration of the catalyst, *i.e.* $Fe(acac)_3$, using hydrazine hydrate (0.016M) and *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** (0.01M) at 170°C. There was no noticeable increase or decrease in the conversion towards the 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine **39**. This phenomenon could be attributed to the surface area to volume ratio that the iron pentanedionate nanocrystals possess. There is greater interaction between the hydrazine hydrate and the

nanocrystals, which makes use of these nanocrystals even more attractive since low quantities can be used and recoverability for reuse has also been reported.[103]

Table 12: Reaction conditions in the batch and flow reduction reaction from 2-aminopyridine to 6-methyl-N^1-(4-(pyridin-3-yl) pyrimidin-2-yl)benzene-1,3-diamine

Reaction conditions	Batch	Flow
Temperature (^oC)	140	170
Reaction time	3 hrs	10 mins
Conversion	98%	99%
$N_2H_4.H_2O$ eq.	1.2	2

Thus the increase of the molar equivalence hydrazine hydrate, greatly improve the reduction reaction, since it functions as a hydrogen source. Thus, adding the hydrazine hydrate in the presence of the iron pentanedionate, assist in reducing the time needed to achieve high conversions.

CHAPTER 4: CONCLUSION

4. CONCLUSION

In this research, we were able to adapt the intermediate batch synthetic protocols to flow synthesis towards imatinib. Increasing the temperature in the flow synthesis of the enaminone saw great increase in the conversion, at shorter residence times with the highest conversion obtained at 200^{o}C. The *o*-xylene which has been the solvent of choice due its non-polarity in the synthesis of 3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **36**, showed to be the ideal solvent and by increasing the concentration of the reagents, higher conversions and throughput per unit time was observed compared to the batch reaction. An investigation into eliminating the solvent, which can overall increase the cost of manufacture, by using the neat reagents, was performed and a conversion of 96% at 160^{o}C was obtained in 30mins. This efficacy utilizing neat reagents in the flow synthesis of the enaminone **36**, is further indicated by the high throughput per unit time, i.e. 6.54g/h compared to reactions were solvents was used.

Flow synthesis of the guanidinium nitrate **37**, showed great improvement when the water content in the cosolvent mixture was increased. This is owing to the function of water in the reaction as a nucleophile. The nitric acid concentration also affected the conversion, where increasing the acid concentration resulted in an increase in conversion. The preceding cycloaddition reaction between the enaminone **36** and the guanidinium nitrate **37**, in the presence of the base was successful. Although, preliminary reaction using NaOH, had not been successful we investigated other bases and Cs_2CO_3 was able to provide a high conversion towards the 2-aminopyridine **38**. Thus, a conversion of 90% in 10 mins in the flow synthesis of *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **38** was achieved.

The reduction step using the iron pentanedionate, as catalyst, was achieved in the presence of excess hydrazine hydrate. This saw a 100 % conversion in 10 minutes.

Critically all steps were solution based so could easily be scaled for local production.

4.1 Future Work

The reactions could be scaled up to illustrate ability to transfer these continuous flow set up to an industrial level. The work to connect these steps in a single continuous flow synthesis is also possible, using in-line separation techniques to remove impurities. The final step to synthesize the imatinib **33** can be combined into a single step by reaction with a synthesized 4-[(4-methylpiperazin-1-yl)methyl]benzoic acid.

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APPENDIX

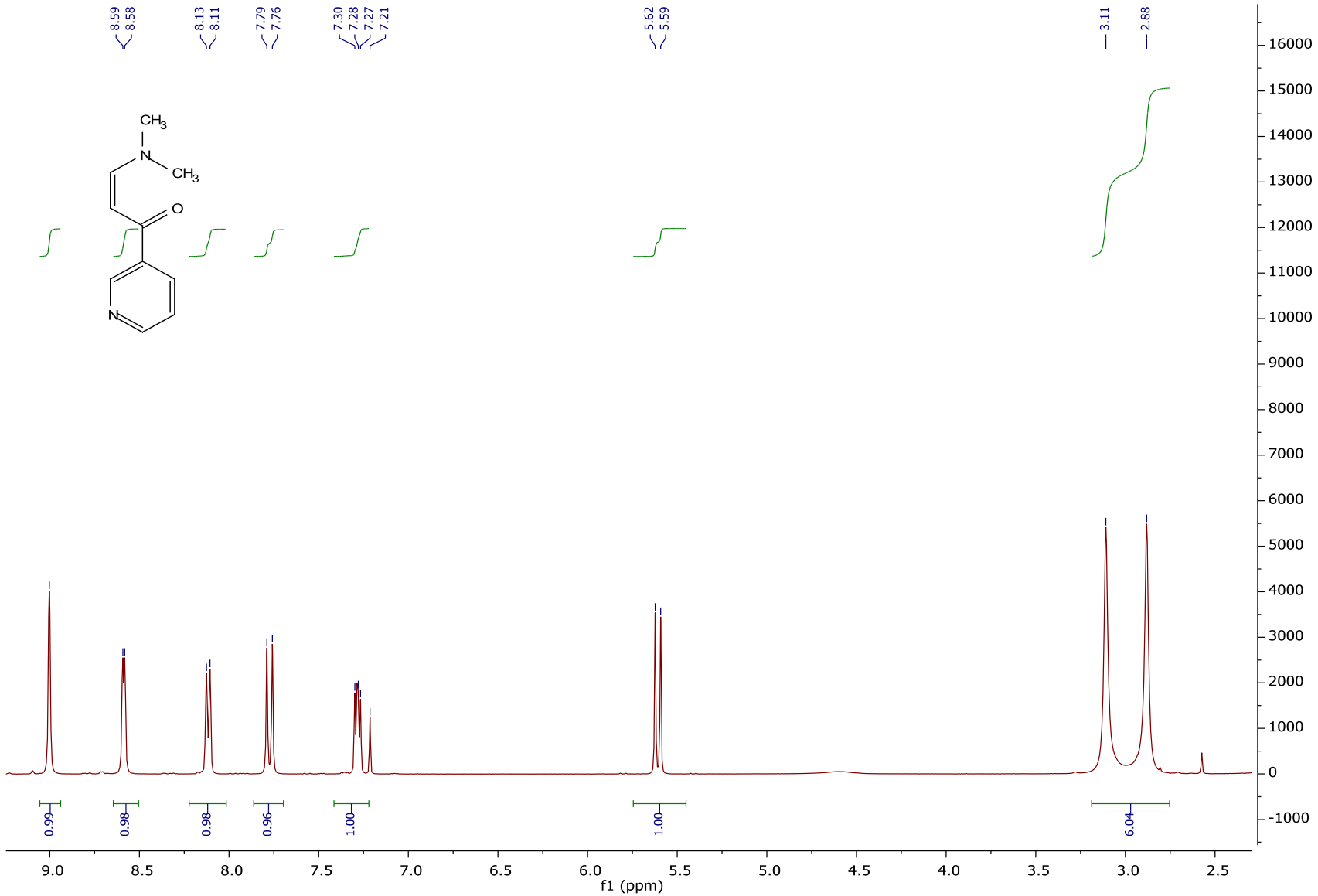

Figure 41:[1]H-NMR obtained from the batch synthesis of (Z)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one

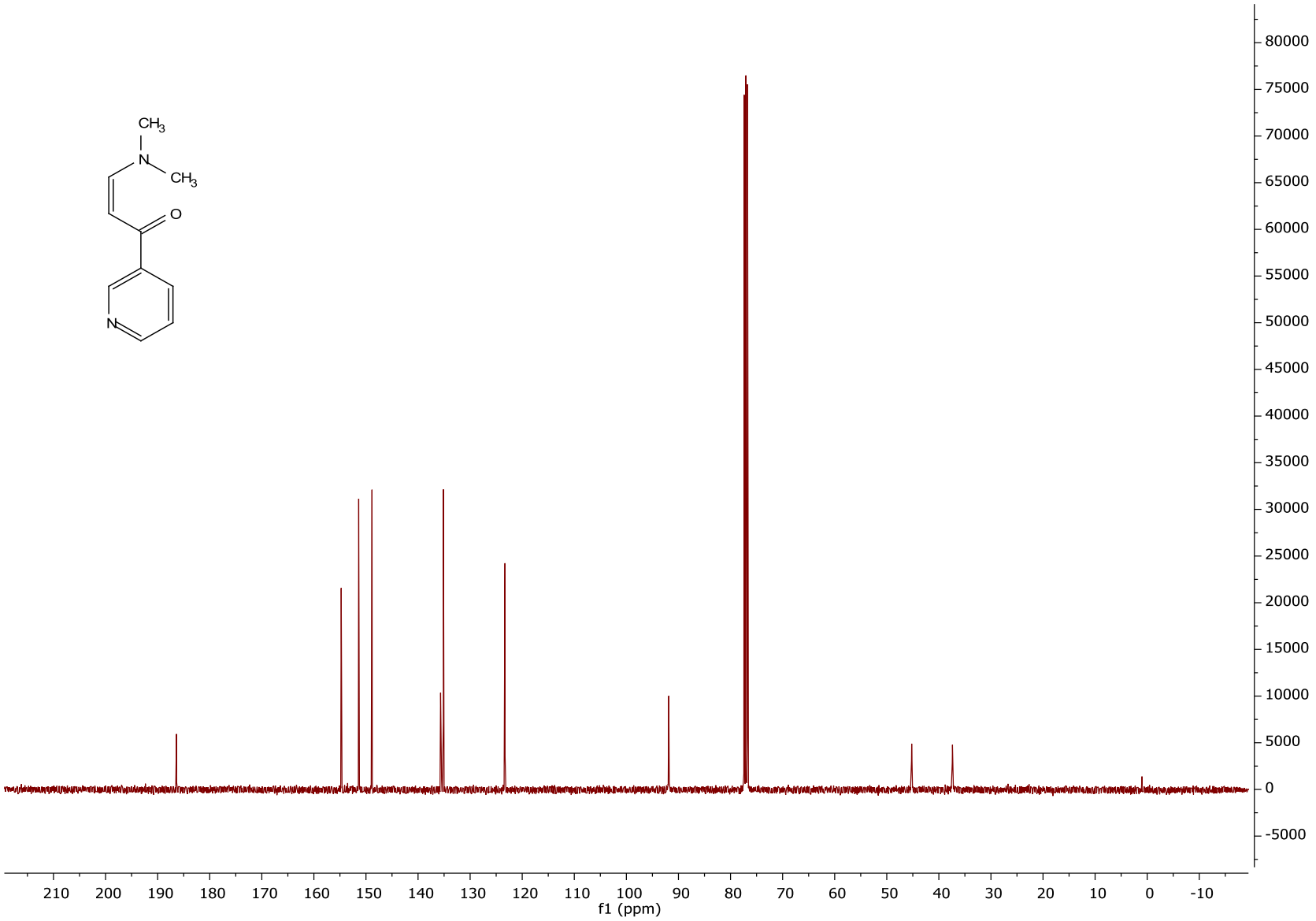

Figure 42: ^{13}C-NMR obtained from the batch synthesis of 3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one

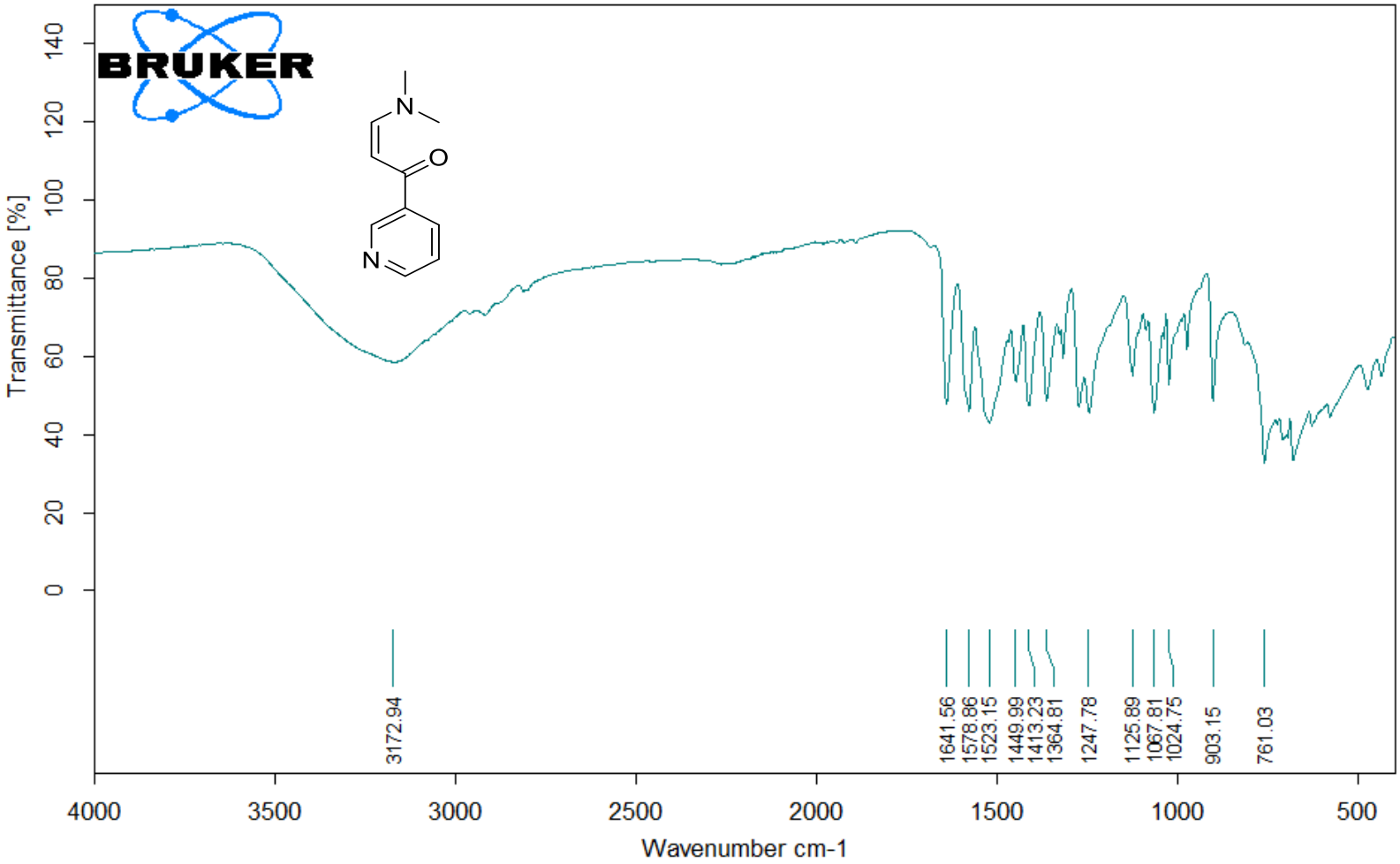

Figure 43: FT-IR of the batch synthesis of 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one

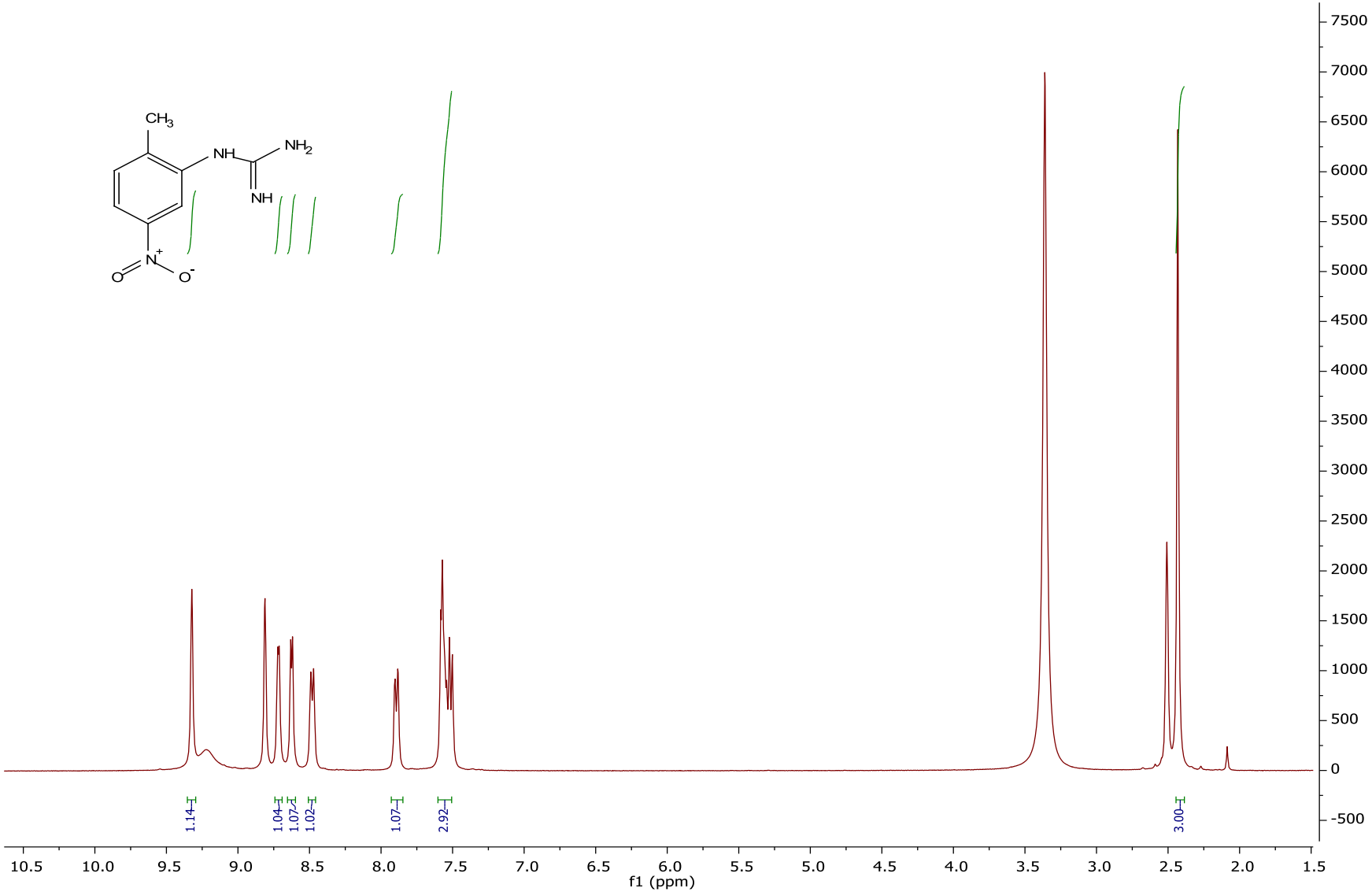

Figure 44: ^{1}H-NMR of N-(2-methyl-5-nitrophenyl) guanidinium nitrate from batch synthesis

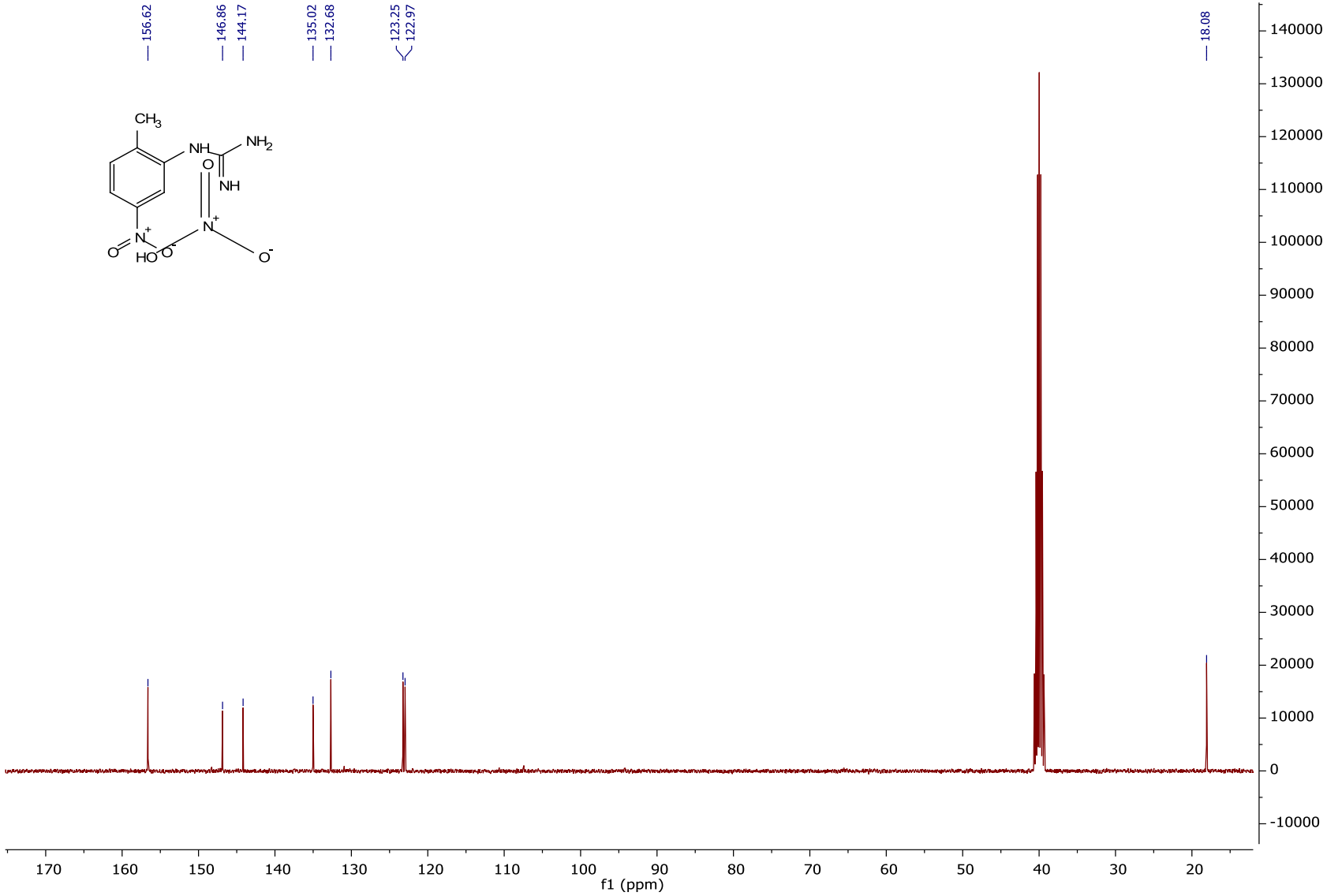

Figure 45:[13]C-NMR from the batch synthesis of N-(2-methyl-5-nitrophenyl)guanidinium nitrate

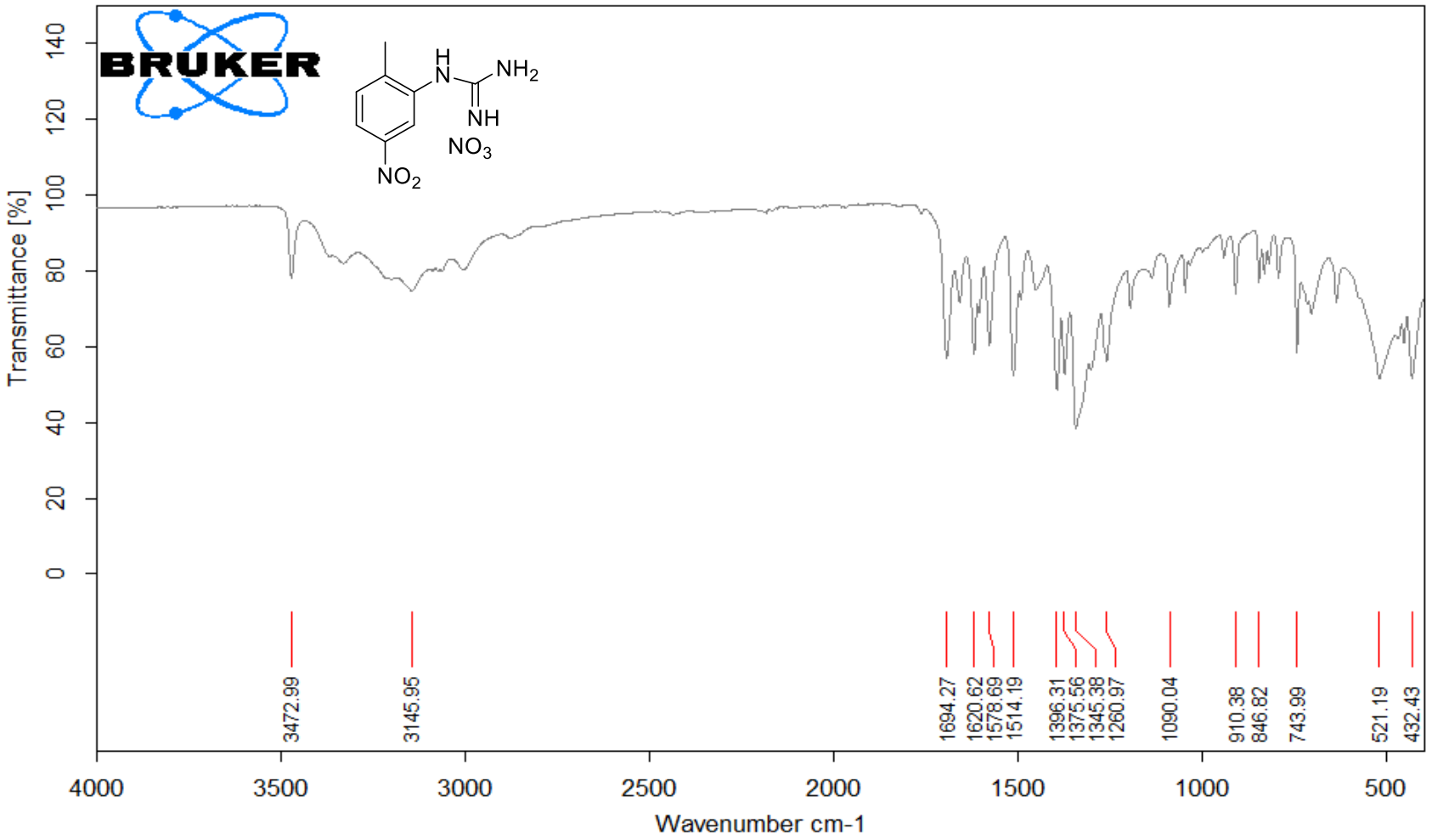

Figure 46: FTIR of batch synthetic standard of N-(2-methyl-5-nitrophenyl) guanidinium nitrate

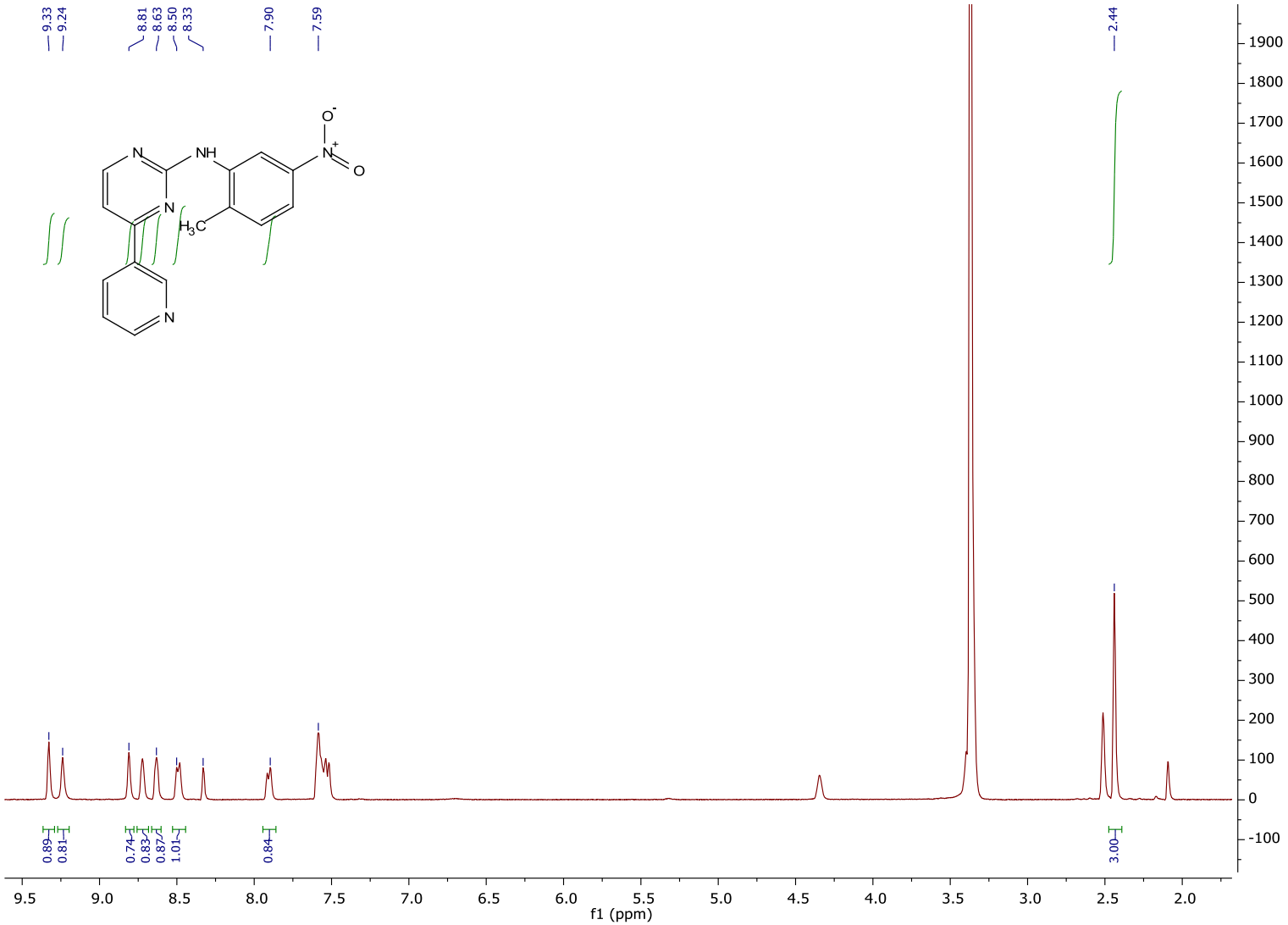

Figure 47: ^{1}H-NMR obtained from the batch synthesis of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

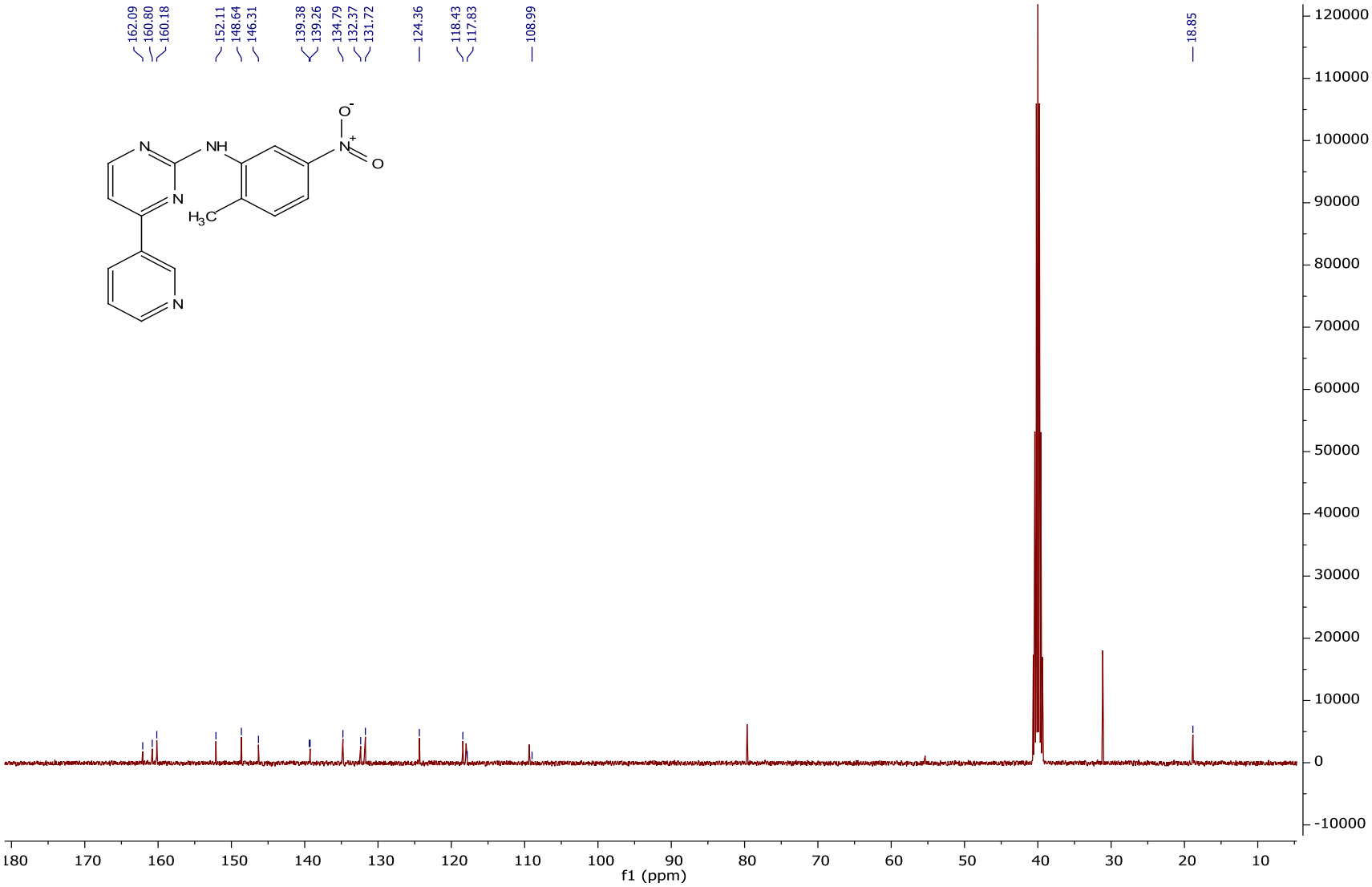

Figure 48:^{13}C-NMR obtained from the batch synthesis of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

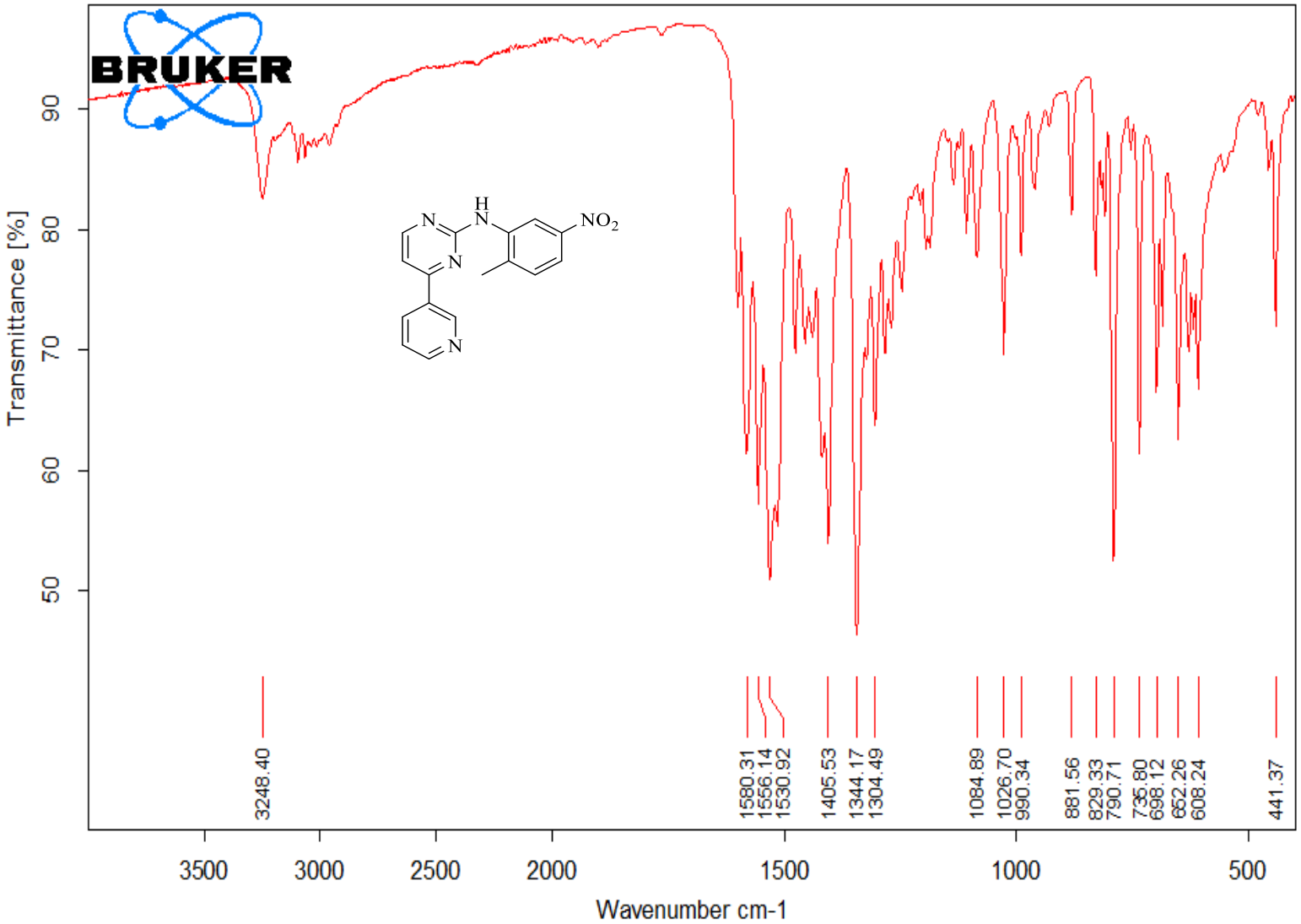

Figure 49: FTIR of batch synthetic standard of N-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine

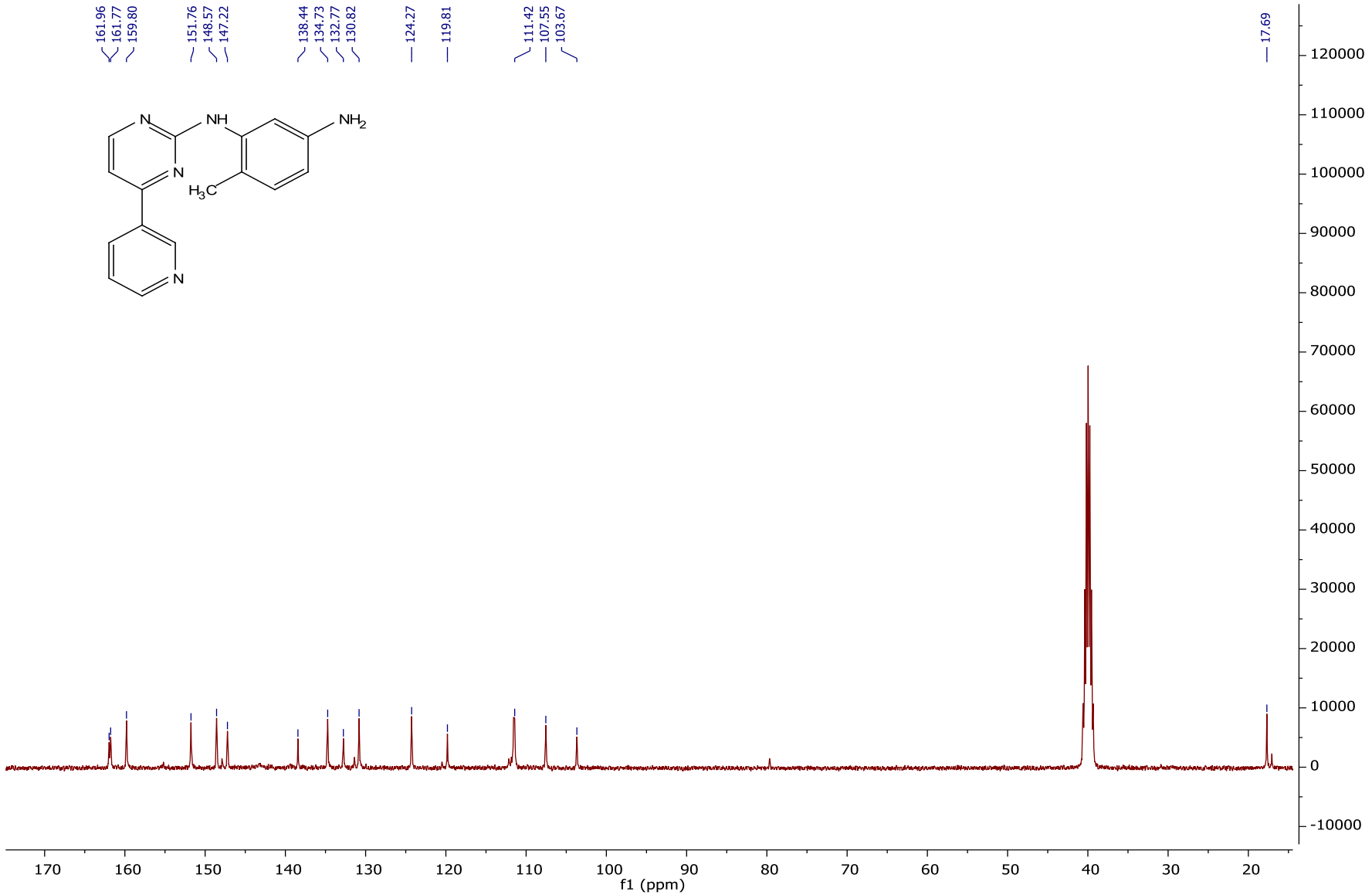

Figure 50: ^{13}C-NMR of obtained from the batch synthesis of 6-methyl-N^{1}-(4-pyridin-3-yl-pyrimidin-2-yl) benzene-1,3-diamine

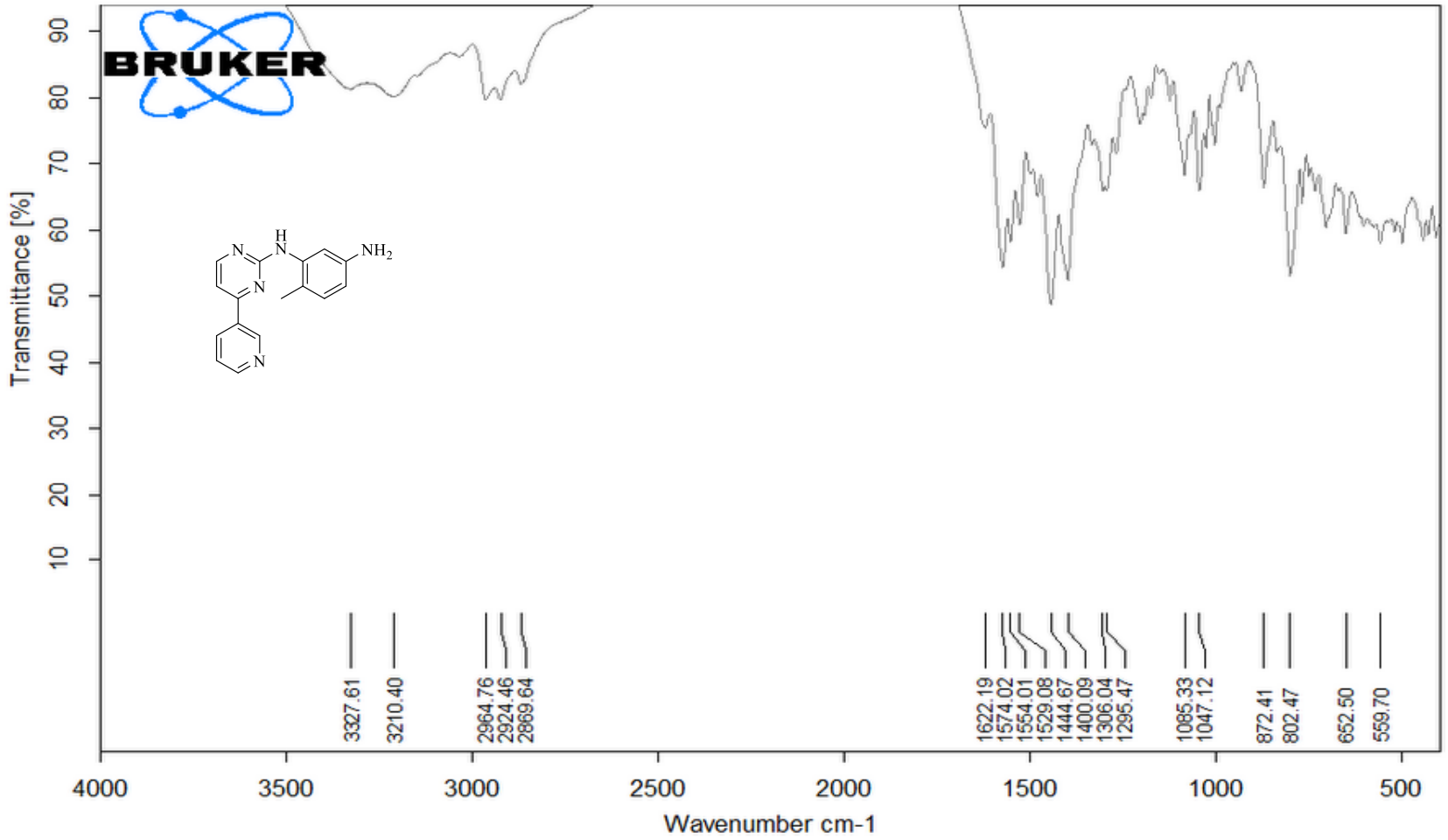

Figure 51: FTIR of obtained from the batch synthesis of 6-methyl-N^1-(4-pyridin-3-yl-pyrimidin-2-yl) benzene-1,3-diamine