

Progress towards the flow synthesis of antibiotics vital to the treatment of tuberculosis in South Africa

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Declaration

I, Wessel Johannes Bonnet, declare that the thesis/dissertation, which I hereby submit for the Degree Magisterii Scientiae Chemistry at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed:  _____

Date: 29 July 2022

I dedicate this dissertation to my grandmother, parents, sisters, and friends for all their support and words of encouragement.

I would also like to thank my supervisor Professor Darren Riley for his patience, input, and countless pep talks.

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Abstract

The burden of tuberculosis (TB) on the world has been explored in recent literature and described as a major source of human suffering. More specifically, the socio-economic implications of TB on South Africa were described and key problems identified, chief of which was the financial complexities of providing treatment to those suffering from this disease, especially in remote and impoverished communities. Uncertainty in regard to the availability of active pharmaceutical ingredients (APIs) to countries such as South Africa were in part attributed to the reliance on foreign imports for obtaining crucial medicines needed for the treatment of TB, MDR-TB, and XDR-TB. The local production of APIs for the treatment of TB is worth pursuing to ensure a constant supply of drugs and timely delivery of lifesaving medicines in a financially sound manner, especially in scenarios where global supply chains are disrupted to such a degree that reliance on foreign suppliers is no longer an option (as seen with the SARS-CoV-2 pandemic).

The core drug regimen for treating TB usually includes isoniazid and ethambutol (together with rifampicin and pyrazinamide), while resistant TB is treated with second-line TB drugs like the fluoroquinolones and thioamides. Thus, three of the most common TB medications were chosen to demonstrate the potential for translation to flow syntheses, namely ethionamide, ethambutol, and isoniazid.

For the attempted total synthesis of ethionamide, only the first step could be successfully completed, albeit delivering the enolated form of the expected product. It was shown that this step still offers a unique way to access a ring system featuring a nitrogen adjacent to a carbonyl group.

Steps 1 and 2 of the three-step ethambutol synthesis were successfully reproduced in batch and translated into flow. The use of methanol-stabilised formalin as a substitute for gaseous formaldehyde in the first step proved to be successful, eliminating the need for more complicated methods relying on specialised flow equipment when handling gases in flow. The highest yield obtained for 1-nitrobutanol was 59 %, 30 % higher than its batch equivalent. Furthermore, it was

demonstrated that hydrotalcite, a reusable solid catalyst, was an effective substitute for the liquid base triethylamine.

Step 2 of the ethambutol synthesis in flow using Raney-Nickel delivered high yields, plateauing at 85 %, 53 % higher than its batch equivalent. The dangers of using hydrazine, however, were clear after a column reactor burst during a reduction reaction. Initial attempts at using gaseous hydrogen instead showed some promise, but only trace yields were recovered by the end of the experimental phase of this project. The final step of the ethambutol synthesis was unsuccessful, and requires further study. Special attention will be given to fully elucidating the reaction mechanism, as this might provide alternative strategies to obtaining the final product.

For the planned synthesis of isoniazid, only the first of the two steps could be replicated in flow and batch. The flow synthesis reaction in step 1 delivered a superior yield at 73 %, 26 % above that of the batch method. The hydrazinolysis of isonicotinamide, however, proved unsuccessful and requires further study.

In summary, the goal of translating each of the three target APIs onto a flow platform was partially accomplished. Those steps that proved viable in flow serve to demonstrate that the local production of valuable intermediates is indeed feasible using steady-state chemistry. South Africa currently has almost no batch manufacturing plants for pharmaceuticals, and thus adopting flow production with the safer, cleaner, and more reliable processes associated with this burgeoning field can solve the many difficulties associated with the importation of APIs.

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List of abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AMK	Amikacin
APIs	Active Pharmaceutical Ingredients
BCG	Bacillus Calmette-Guerin
BPR	Back Pressure Regulator
CAP	Capreomycin
CLOF	Clofazimine
CYS	Cycloserine
DALYs	Disability-Adjusted Life-Years
DR-TB	Drug-resistant TB (resistance to any TB drug)
ELISA-based	Enzyme-Linked Immunosorbent Assay-based
ELISPOT	Enzyme-Linked Immunosorbent Spot
EMB	Ethambutol
ETA	Ethionamide
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
Hr-TB	Isoniazid-Resistant TB
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
KM	Kanamycin
LEV	Levofloxacin
LIN	Linezolid
LTBI	Latent Tuberculosis Infection
MDR-TB	Multidrug-Resistant Tuberculosis
MNPCs	Multi-National Pharmaceutical Companies
MXF	Moxifloxacin
NAD+	Nicotinamide Adenine Dinucleotide
National Department of Health	NDoH
National Strategic Plan	NSP
NTM	Non-Tuberculosis Mycobacterium
PAS	Para-Amino Salicylic acid
PBMCs	Peripheral Blood Mononuclear Cells
PLHIV	People Living with HIV
PPD	Purified Protein Derivate
ppm	Parts Per Million
preXDR-TB	MDR-TB with resistance to either a fluoroquinolone OR a second-line injectable drug
PZA	Pyrazinamide

QFT	QuantiFERON-TB Gold In-Tube
RMP	Rifampicin
RMR-TB	Rifampicin Mono-Resistant TB (resistance to rifampicin and susceptibility to isoniazid)
RR-TB	Rifampicin-Resistant TB (TB resistant to rifampicin, regardless of resistance to other drugs)
SADC	Southern African Development Community
SM	Streptomycin
TB	Tuberculosis
T-cells	T-lymphocytes
TNF	Tumour Necrosis Factor
TST	Tuberculin Skin Test
WHO	World Health Organization
XDR-TB	Extensively Drug Resistant Tuberculosis

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Chapter 1 - Introduction and Problem Statement

1.1 Introduction Tuberculosis (TB) is a disease that is a major cause of illness across the globe. As of 2020, tuberculosis remains one of the top ten causes of death, and is the leading cause of death from a single infectious agent.¹ The deadliness of tuberculosis is exacerbated by new forms of the disease that show increasing resistance to the selection of antibiotics available to medical professionals.²

The bacillus *Mycobacterium tuberculosis* is responsible for TB. As an airborne pathogen, TB is spread by exhalation of aerosol particles from infected persons, and is also present in saliva.² The infection affects mainly the lungs (pulmonary tuberculosis), but can also spread to other parts of the body (extrapulmonary tuberculosis). The World Health Organization (WHO) reports that up to 25% of people worldwide have the infection, although some show only minor symptoms or no symptoms at all (classified as 'latent' TB).¹ Latent TB is predominantly diagnosed through blood tests or epidermal-swab methods.

Even though TB is present across the globe, it is often labelled as a disease affecting only developing countries. TB is indeed more prevalent in third-world countries, and countries with limited public health care facilities. The severity of outbreaks is fanned by the close living quarters conditions present in vulnerable communities, and patients are often stigmatized. More developed societies often view TB as an antiquated disease, seeing as the prevalence and deadliness of TB infections in these countries have dwindled in comparison to that of a hundred years ago.

A successful approach to controlling the spread of TB therefore requires an all-inclusive strategy that considers all factors contributing to the spread and mortality rate of the disease.³ TB is curable and even preventable. Treatment usually consists of a course of antibiotics, and often more than one drug is employed. Treatment can also significantly reduce one's risk of contracting the disease again.⁴

Even though most cases of TB are easily curable, a disproportionate number of deaths occur in countries where healthcare is scant due to shortages in medicines. The bulk of TB cases in the

year 2019 were in the regions of South-East Asia (~44%), Africa (~25%), Western Pacific (~8.2%), Americas (~2.9%), and Europe (~2.5%). Eight countries account for two thirds of the global total: India (~26%), Indonesia (~8.5%), China (~8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (~3.6%), and South Africa (~3.6%). The other countries on the WHO's list of TB-burdened nations make up the remaining 21% of the global total (Figure 1).

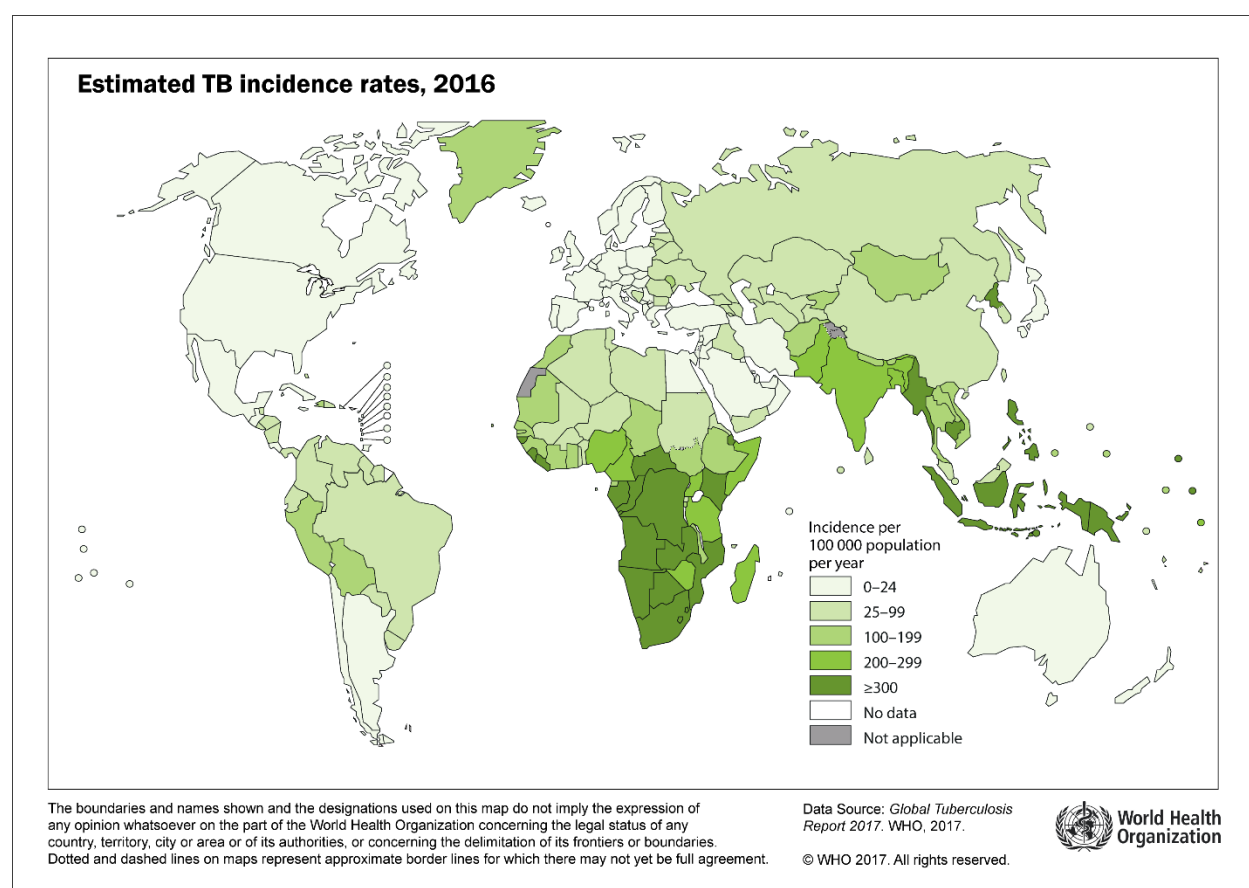


Figure 1 - Worldwide distribution of TB⁵

1.2 - Problem Statement

As of 2020, South Africa has one of the largest populations of TB sufferers (3.6%) in the world.¹ Pillay *et al.* recently published the first survey conducted on the prevalence of TB in South Africa.⁶ Previously, the WHO used modelling exercises to estimate the burden of TB in South Africa, but these results were not always reliable. Thus, the South African study was executed via a TB survey of persons older than 15 years of age in ten selected clusters in the nine provinces, yielding a total of 54 873 respondents (Figure 2).

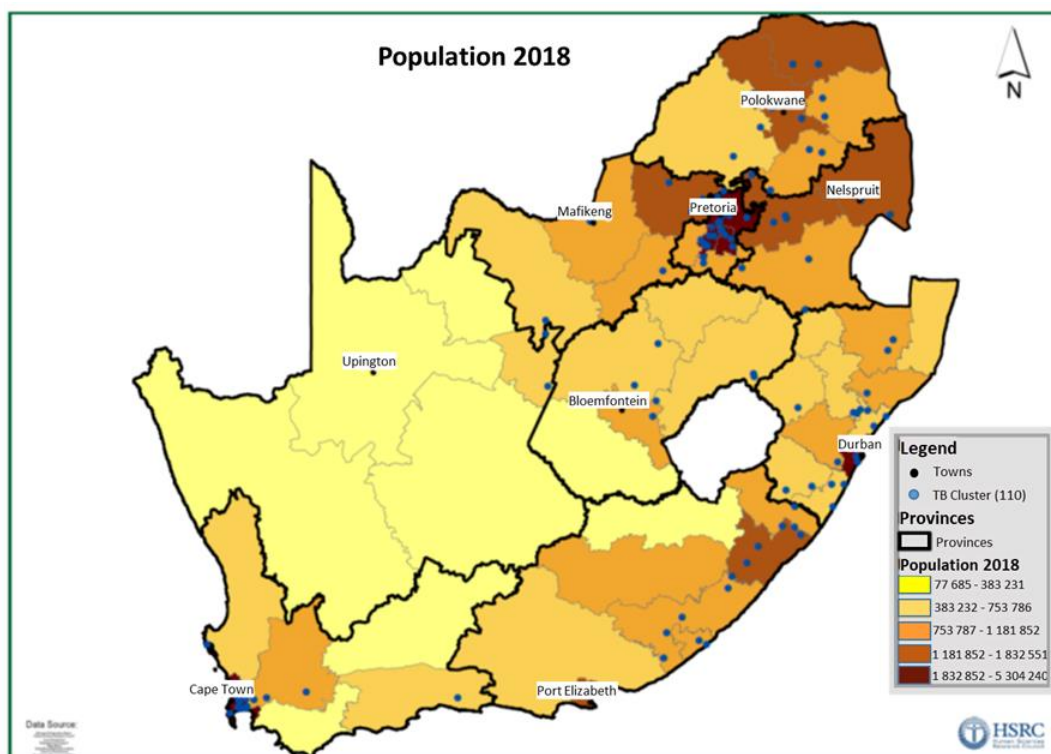


Figure 2 - Clusters identified for the South African TB survey⁶

The participants underwent chest X-ray screening and symptoms were listed in each case. Participants with positive screening outcomes were then tested using the Xpert Ultra (Cepheid) test and TB cultures. The overall participation rate was 66.1%, translating to 35 191 participants, with the majority being female. The results are depicted in Table 1 below.

Table 1 - Survey Screening Results⁶

Total population enumerated at household level	68,771
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Individuals eligible to participate at household level	53,250 (77.4% of enumerated)
Eligible individuals who participated	35,191 (66.1% all eligible = participation rate)
Participants screened positive (symptoms and/or abnormal CXR)	9,066 (25.8% of participants) eligible for sputum collection
Valid Xpert Ultra result	7,521 (82.9% of screened positive)
Valid culture result	7,305 (80.6% of screened positive)

The prevalence of TB in SA was estimated to be 737 per 100 000 population with the number of positive males significantly higher than females. A big cause for concern was the finding that many participants with at least one symptom do not seek health care, causing an under estimation of the prevalence of TB. Of further concern is the finding that the biggest prevalence to notification gap occurred in the 15-24 years age group and in those 65 years and older. 65% of participants indicated that they would indeed seek care if they develop symptoms.

A high number of symptomatic participants (86%) seek care from community clinics, followed by private clinics/hospitals (8.1%), and district hospitals (4.5%). The average cost for the patient and his family during the first 3 - 5 months of TB treatment came to about R1 000 per month, including loss of earnings.⁷ Thus, poor patients are reluctant to seek and complete treatment, and often suffer malnutrition that leads to poor treatment outcomes. The emergence of drug resistant TB escalates the cost tremendously, as does comorbid diseases like human immunodeficiency virus (HIV) and diabetes. Various reasons were given for not seeking care, as shown below (Figure 3).

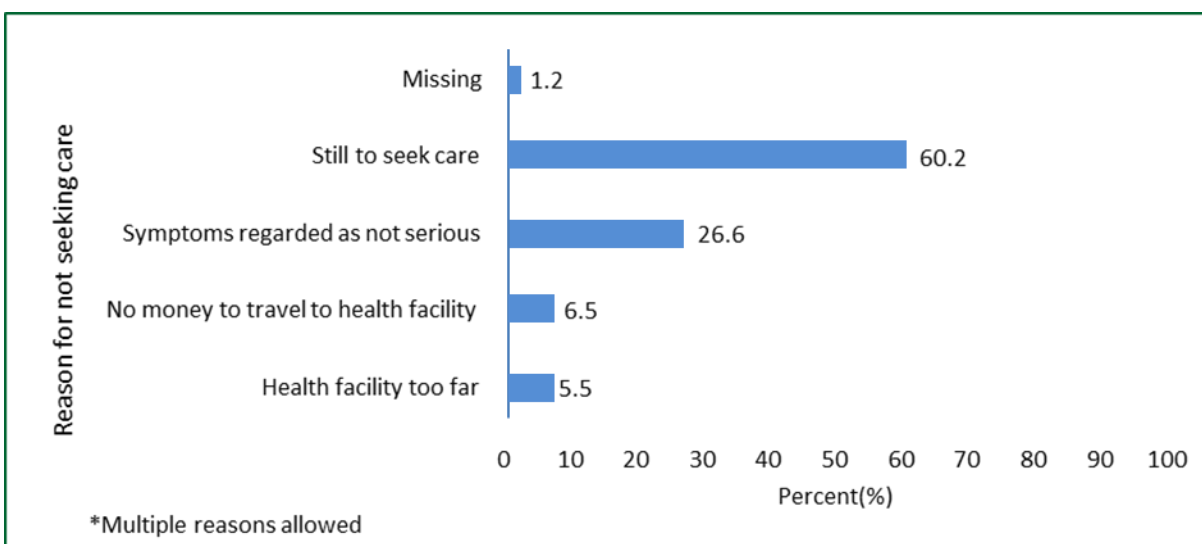


Figure 3 – Symptomatic participants’ reasons for not seeking care (N = 3442)⁷

This survey highlighted the severity of the burden of TB in South Africa, and many people with TB are never diagnosed. Considering that a TB patient can infect 4 - 12 people in their lifetime, urgent action is needed to reach undiagnosed citizens.

In 2014 the WHO announced its post-2015 “End TB Strategy”, which aims to reduce global TB incidence by 50% and mortality by 75% by 2025.³ To reach these targets, high-burden countries like South Africa will have to make major advances in TB prevention, treatment, and care, which will translate into astronomical costs. In South Africa, the government funds about 80% of the treatment cost, supplemented by funding from the USA (20%) and global funds. The government has increased allocations to the National Department of Health (NDoH) for HIV and TB by 7% annually, from R11.0 billion in 2013/14 to an expected R25 billion in 2020/21.⁵ Menzies *et al.* investigated the cost-effectiveness and resource implications of aggressive anti-TB strategies in China, India, and South Africa via multiple independently developed *M. tuberculosis* transmission models. They concluded that in South Africa, the first five years of the strategy will need almost double the TB health care budget of 2015, and the cost will remain high for the next 15 years. However, the decrease in disability-adjusted life-years (DALYs) and substantial decrease in patient costs may render these programmes cost-effective, even if it is just in a societal perspective.⁸

1.3 - Pharmaceutical Industry of South Africa

By the late 1990s, the South African pharmaceutical manufacturing industry was well developed, and had become the largest manufacturer of pharmaceuticals in Africa.⁹ Many Multi-National Pharmaceutical Companies (MNPCs) ran strategically located plants inside South Africa, with the bulk of the produced drugs going directly to local markets. Such an approach is especially inviting for niche Active Pharmaceutical Ingredients (APIs), which are in low demand in the developed world but are essential to the nation hosting the plant. Drugs used to treat TB and acquired immunodeficiency syndrome (AIDS), for example, are crucial to the South African healthcare system, whereas these diseases are of little concern in developed countries, making local production for the purpose of local distribution very inviting.

At the turn of the century, however, various local and global shifts in the pharmaceutical industry had caused a decline in both the number of manufacturers and employees inside of South Africa. One such factor was the global boom in the manufacturing of generic medicines by emerging pharmaceutical giants such as India and China, leading to more competitive pricing and a dip in profits for smaller-scale operations in South Africa. A glut of cheaply produced medicines had made it more economical for pharmaceutical companies to either import the medicine directly or import the APIs for final packaging in South Africa. In order to strengthen the local API manufacturing sector, the Southern African Development Community (SADC), comprised of sixteen member states, singled out flow chemistry as a key development area, advancing high-tech continuous processing in South Africa.¹⁰

Thus, the aim of this project was to translate the synthesis of several commonly used TB antibiotics onto a flow chemistry platform, both as a proof of concept and to demonstrate the practical applications of manufacturing important medicines that are relevant to solving local shortages of vital treatment drugs. The drugs selected for process development on flow are ethionamide (**1**), isoniazid (**2**), and ethambutol (**3**) (Figure 4).

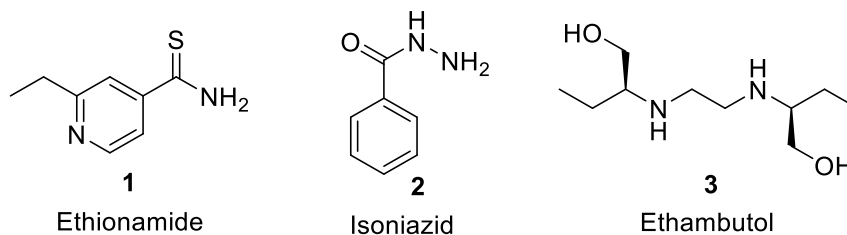


Figure 4 - Chemical structures of targeted drugs

Projects such as these go a long way to proving that the local, large-scale production of APIs is not only possible, but vital to building a self-sustaining pharmaceutical industry that will lead to reduced treatment costs and the retention of skilled workers that may look to other countries for better job offers. We believe that flow chemistry and flow chemists have a place in the fledgling pharmaceutical industry in South Africa and can yield valuable research and industrial outputs that would benefit the country as a whole.

Chapter 2 – Introduction to Tuberculosis

2.1 - The Disease of Tuberculosis

Tuberculosis is a highly infectious disease caused by the *Mycobacterium tuberculosis* pathogen. It is a member of the tuberculosis complex, a group of closely related mycobacterium pathogens which includes *M. bovis* (which infects cattle and may also infect humans), *M. microti*, *M. africanum* (which causes TB in West Africa), *M. avium*, *M. intracellulare*, *M. leprae* (the causal agent for leprosy), *M. lepraemurium* (infection in rats and cats), and *M. scrofulaceum* (causing opportunistic infections in patients with AIDS).¹¹ Today, TB remains a serious health problem in many regions of the world, especially in developing countries. It is a contagious disease and is becoming epidemic in some parts of the world. The emergence of multidrug-resistant tuberculosis (MDR) and extensively drug resistant tuberculosis (XDR) strains have complicated the treatment of TB. Compounding the problem is diseases like HIV and diabetes, which are common risk factors for the development of active TB from latent TB. Thus, treatment and prevention of TB remains a challenge throughout the world.

2.1.1 - History of Tuberculosis

M. tuberculosis has been active for millennia and was the cause of millions of deaths due to its high mortality rate. The first documented descriptions of TB were found in India (3000 years ago) and China (2300 years ago), respectively.¹² During the same period, mummies found in Egypt and Peru showed signs of deformities of the spine due to Pott's disease (tuberculosis of the spine), often caused by spreading of the disease from the lungs to other parts of the body (Figure 5).



Figure 5 - Pott's Disease, Tuberculosis of the Spine¹³

Early Greek scientists like Hippocrates, Isocrates and Aristotle accurately described TB's symptoms, the formation of lung lesions, and that the disease is contagious. Another Greek physician, Galen, in 174 BC described the symptoms of TB as fever, coughing, blood-stained sputum, and sweating.

Advances during the Middle Ages were minimal, and the few medical writings referred to TB as phthisis, the "white plague," or consumption. Treatments included bloodletting, purgatives, a healthy diet, and exercise.¹⁴ In 1679 Francis Sylvius described how tubercles could progress to abscesses and cavitory lesions in the lungs and in other sites of TB patients in his work *Opera Medica*.¹⁵ A British surgeon named Percivall Pott, first identified spinal tuberculosis, which leads to the collapse of vertebrae and spinal cord paralysis, in 1779.¹⁶ During the 18th and 19th centuries TB spread rapidly over Europe and the USA, with death rates reaching 800 – 1000 per 100,000 of the population. Doing autopsies on TB casualties in Paris at the time, René' Laennec gained vast experience on the disease and in 1819 published his findings. This comprehensive work described not only the pathology of TB, but also coined definitions for observed symptoms that are still used today.¹⁷ Hermann Koch made a breakthrough in 1882 when he isolated the tubercle bacillus, a small, rod-shaped bacterium, and succeeded in reproducing the disease by inoculating the bacillus into laboratory animals. He further developed a staining technique to visualize the *M. tuberculosis* bacillus and noted that tuberculosis is transmitted through droplets released while sneezing or coughing.¹⁸

The discovery of X-rays in 1895 facilitated the diagnosis of TB and the ability to follow up on patients. However, at that stage the treatment of choice was still at sanatoriums where healthy living, good nutrition, and rest were prescribed to cure sufferers. Progress was made in 1906, when Calmette and Guerin developed the *Bacillus Calmette-Guerin* (BCG) vaccine against TB.¹⁹ However, data from mass immunizations in the 1920's suggested that BCG vaccination only prevents serious forms of childhood TB and has no effect on adults. Streptomycin was the first effective antibiotic released in 1943, but it was hampered due to the fact that it had to be injected and toxicity prevented long term treatment. In 1949, *para*-amino salicylic acid was discovered, followed rapidly by isoniazid, pyrazinamide, ethambutol, cycloserine, and rifampin.

Despite the development of many anti-TB drugs in the last century and early 21st century, the disease is still prevalent in many countries across the world, and thus a comprehensive strategy is necessary to combat the problem. This includes the improvement of treatment options, improved prevention strategies, improvement in the availability of diagnostic instruments, and improvement in the provision of information to the public.

2.1.2 - *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is the slow-growing, rod-shaped aerobic bacterium responsible for tuberculosis.²⁰ It is non-motile, non-spore producing, and colonies often manifest as entwined bundles resembling cords. It is highly aerobic, and as such it thrives in the highly oxygenated environment of the lungs (Figure 6).²¹



Figure 6 - Chest X-ray of Serious TB Case²¹

Under optimal breeding conditions, *M. tuberculosis* can be expected to divide about once a day, which is significantly slower than most other bacteria, and can take weeks to produce a visible colony on a microbial growth medium.

M. tuberculosis has a complicated lifecycle and exploits the host's own immune system as part of its reproductive sequence (Figure 7).

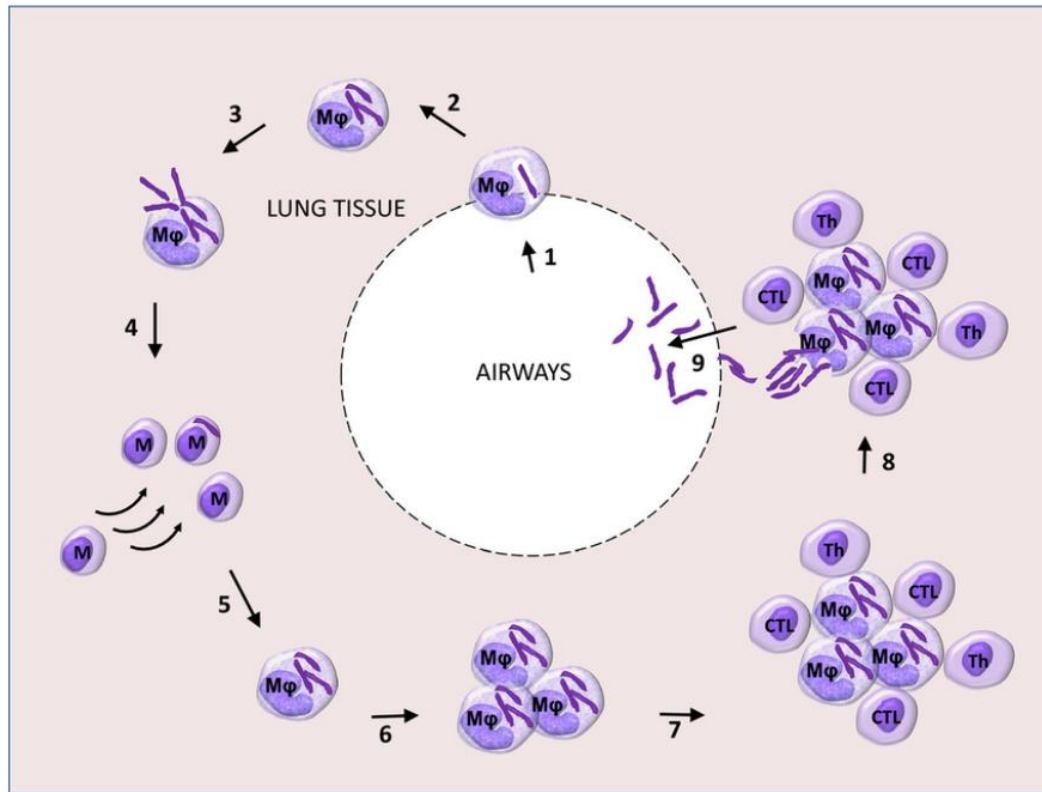


Figure 7 - Replication Cycle of *M. tuberculosis*²²

Upon being taken up via inhalation, *M. tuberculosis* is engulfed by alveolar macrophages inside of the lungs (Step 1). *M. tuberculosis* is able to survive inside the host macrophage, and commences to replicate (Step 2) until the macrophage dies (Step 3). The macrophage disintegrates, releasing the *M. tuberculosis* colony (Step 4). This leads to further recruitment of macrophages to the site of infection, leading to further dissemination of the bacteria (Step 5). A localised area of inflammation (a granuloma) eventually develops at the infection site (Step 6). Cytolytic cells are recruited to the site of infection, forming a layer around the granuloma (Step 7). After a period of time, the granuloma becomes necrotic (Step 8), bursts, and releases viable

M. tuberculosis into the alveolar space (Step 9), enabling it to spread via exhalation. The cycle then repeats.

2.1.3 - Mycobacterial Cell Wall

The cellular wall acts as the interface between the highly regulated environment of the bacterial cytoplasm and the external environment in which the cell finds itself. The cell wall has a number of very important functions that are essential to the cell's survival, making it one of the most attractive targets for bactericides (Figure 8).

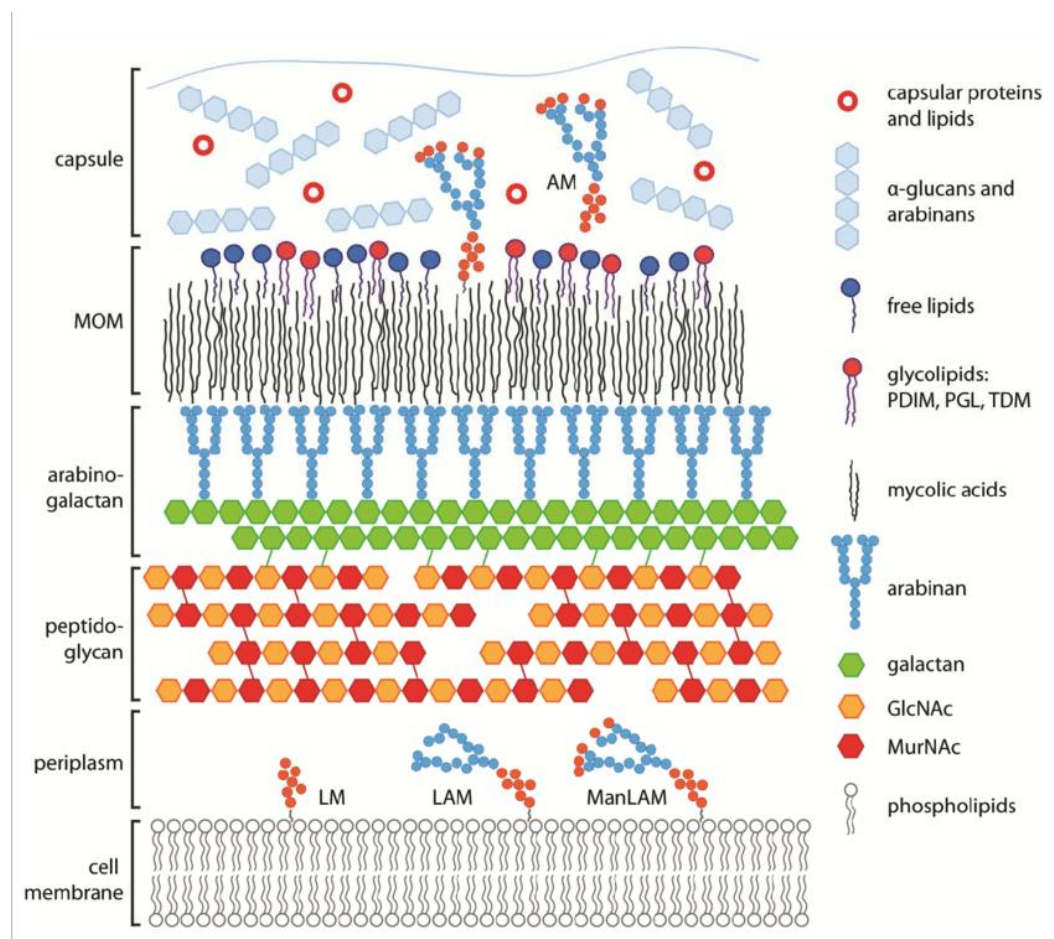


Figure 8 - The cell wall of *M. tuberculosis*. MOM = mycobacterial outer membrane, LM = lipomannan, AM = arabinomannan, LAM = lipoarabinomannan, ManLAM = mannose-capped LAM, GlcNAc = N-acetyl-glucosamine, MurNAc = N-acetyl-muramic acid.²³

Some of the cell wall's functions include the transportation of nutrients into the cell, the balancing of osmotic pressures between the cytoplasm and the external environment, and

creating favourable conditions outside of the cell necessary for its survival (for example, changing the chemical environment of the host medium to aid the survival of the entire bacterial colony and maintaining the cell's overall shape).²⁴ Most species of bacteria are classified as either Gram-positive or Gram-negative. Gram-staining (named for its inventor Hans Christian Gram) is a method of distinguishing between these two main branches of bacteria, based on the nature of their cellular walls.

The resilience of *M. tuberculosis* to antibiotics can in part be attributed to its unusual cell wall, which features a waxy coating containing unique long-chain fatty acids, that have an alkane sidechain and an hydroxyl group at positions α and β to the carboxyl group, respectively (mycolic acids).²¹ This waxy layer presents a barrier to most commonly used antibiotics, especially those with a more hydrophilic nature.²⁵ Three main types of mycolic acids form part of the cell wall of *M. tuberculosis*, namely, α -mycolic acids (**4**) (about 70% of the total mycolic acids), methoxy-mycolic acids (**5**) (10-15%), and keto-mycolic acids (**6**) (10-15%) (Figure 9).²⁶ Mycolic acids conceal the developing bacteria in macrophages, thus screening it from the host's immune system. The survival and pathogenesis of *M. tuberculosis* is dependent on the biosynthesis of these mycolic acids.²⁷

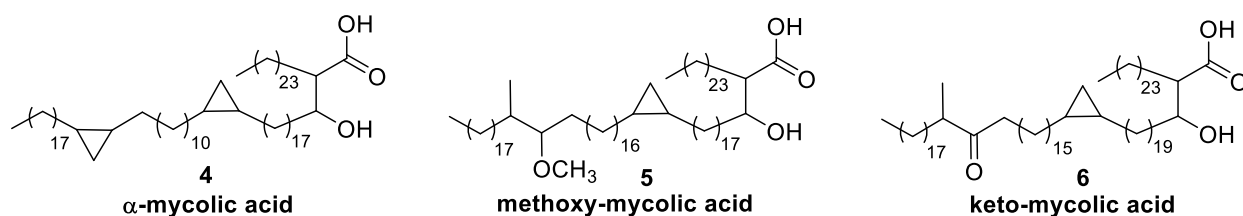


Figure 9 - The three major mycolic acids that play a role in drug resistance²⁶

2.1.4 - Latent and Active TB

Latent tuberculosis infection (LTBI) is defined as a state of persistent cellular immune response against *M. tuberculosis* antigens. LTBI shows no clinical symptoms or radiological abnormality.²⁸ In 2016, Houden and Dodd adjusted the then accepted rate of LTBI of 33% to 25% via mathematical modelling, using data accumulated from 1950-2011. They reported that the South-East Asia, Western-Pacific, and Africa WHO regions contributed almost 80% to the global LTBI burden, while LTBI infection in the South African population is estimated to be as high as 75-

85%.²⁹ According to The WHO global tuberculosis report of 2018, only about 5 – 10% of TB infected individuals develop active TB from LTBI in the first 2 – 5 years after exposure, and that the duration of latency is unpredictable. Some individuals may have LTBI though their entire lifetime.¹ There are several risk factors for progression from LTBI to active TB infection. These are divided into (i) high-risk factors of which HIV is the most prominent, as well as patients with renal failure and transplant patients on immune suppressants; (ii) moderate-risk factors like diabetes and patients treated with tumour necrosis factor alpha (TNF- α) inhibitors for autoimmune and inflammatory conditions, and (iii) low-risk factors like smoking, alcohol abuse, and malnutrition.²⁹

Two tests are currently used to identify LTBI: the tuberculin skin test (TST) and the interferon gamma (IFN- γ) release assay (IGRA).³⁰ During the TST, intradermal purified protein derivate (PPD) is injected into the forearm of the patient. PPD is a mixture of derivatized proteins, precipitated from mycobacterial culture filtrates. The injection site is observed for 48-72 hours and formation of a skin induration (thickening of the skin) of 0.5-2.0 cm indicates a positive test (Figure 10). A negative response is indicated by redness without swelling, or skin thickening of less than 0.5 cm. Owing to the low cost of the TST it is the preferred testing method in low-income countries with a high TB burden.



*Figure 10 - Skin Induration (Localized Thickening of the Skin)*³¹

Unfortunately, false positives can be observed in individuals who had other recent mycobacterium infections, those who received the BCG vaccination, and people with long-term LTBI.

The IGRA is a blood test that measure T-cell release of IFN- γ after stimulation by *M. tuberculosis* complex antigens, but without the BCG vaccine substrains that can give false positives. Most species of non-tuberculosis mycobacterium (NTM) are also not encoded except for *M. marinum* (infects fish and can spread to humans via skin lesions), *M. kansasii* (non-contagious tuberculosis-like infection that mostly infects the lungs), *M. szulgai* (rare pathogen that is the cause of less than 1% of all cases of NTM infections), and *M. flavescens* (atypical aquatic organism).³² The QuantiFERON-TB Gold In-Tube (QFT) assay is a commercial enzyme-linked immunosorbent assay-based (ELISA-based) whole blood test that uses the TB antigens ESAT-6 and CFP-10. The secretion of early secretory antigenic 6 kDa protein (ESAT-6) and filtrate protein 10 (CFP-10) is essential for the virulence of *M. tuberculosis*. A positive *M. tuberculosis* infection test is indicated by an IFN- γ response higher than the test cut-off (0.35 IU/mL for latent tuberculosis).

A second IGRA is the T-SPOT.TB assay - an ELISPOT- (enzyme-linked immunosorbent spot) assay performed on ESAT-6 and CFP-10 peptides incubated with separated and counted peripheral blood mononuclear cells (PBMCs). The number of IFN- γ -producing T-cells (spot-forming cells) indicates a positive test if the number of spots in the TB-antigen wells are higher than the negative control wells (Figure 11).

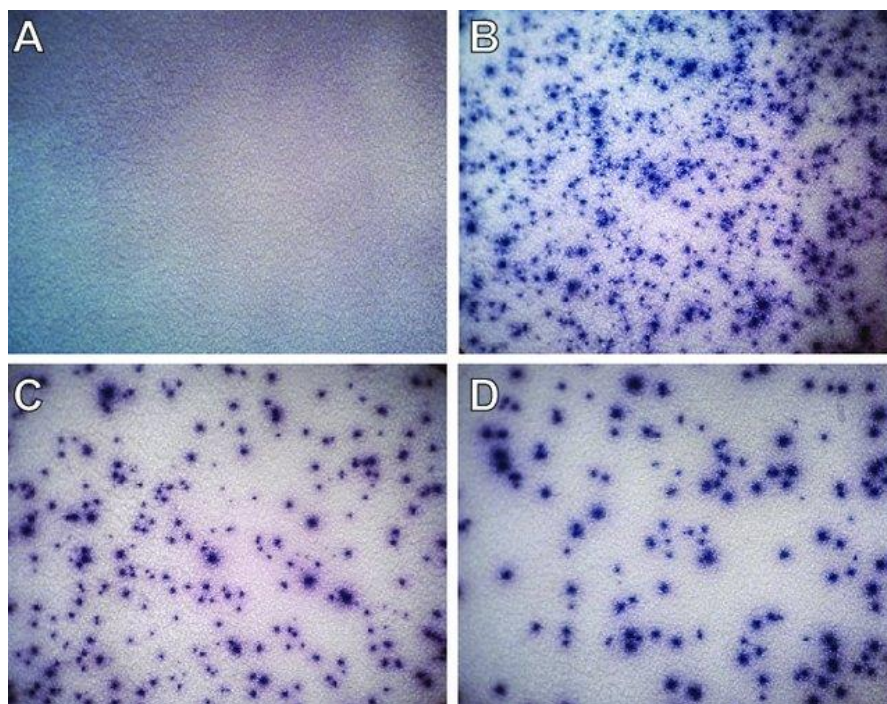


Figure 11 - T-SPOT. TB test performance results on pleural effusions. (A) Nil control. (B) Positive control. (C) Positive result for ESAT-6 antigen. (D) Positive result for CFP-10 antigen.³³

A drawback with both tests is that they do not discriminate between active and latent tuberculosis. Thus, a positive test in the TST or IGRA needs to be followed up by chest X-rays or a sputum test to rule out active TB. Treatment of LTBI will decrease the active TB caseload, but it is only feasible in low-burden countries, because the cost of such treatment in high-burden countries is prohibitive, since up to 60-80% of populations in these countries may have LTBI.

2.1.5 - MDR and XDR *M. tuberculosis*

The global treatment of *M. tuberculosis* has relied on the same drugs for decades, resulting in the formation of drug resistant *M. tuberculosis* strains. The WHO reported that 3.5% of new TB cases and 18% of previously treated cases were infected with multi-drug resistant (MDR) and Rifampicin resistant (RR)-TB in 2017.³ Extremely drug resistant (XDR) TB was diagnosed in 8.5% of the MDR-TB patients. The different TB-strains and the drugs involved are summarized in Figure 12 and Table 2.

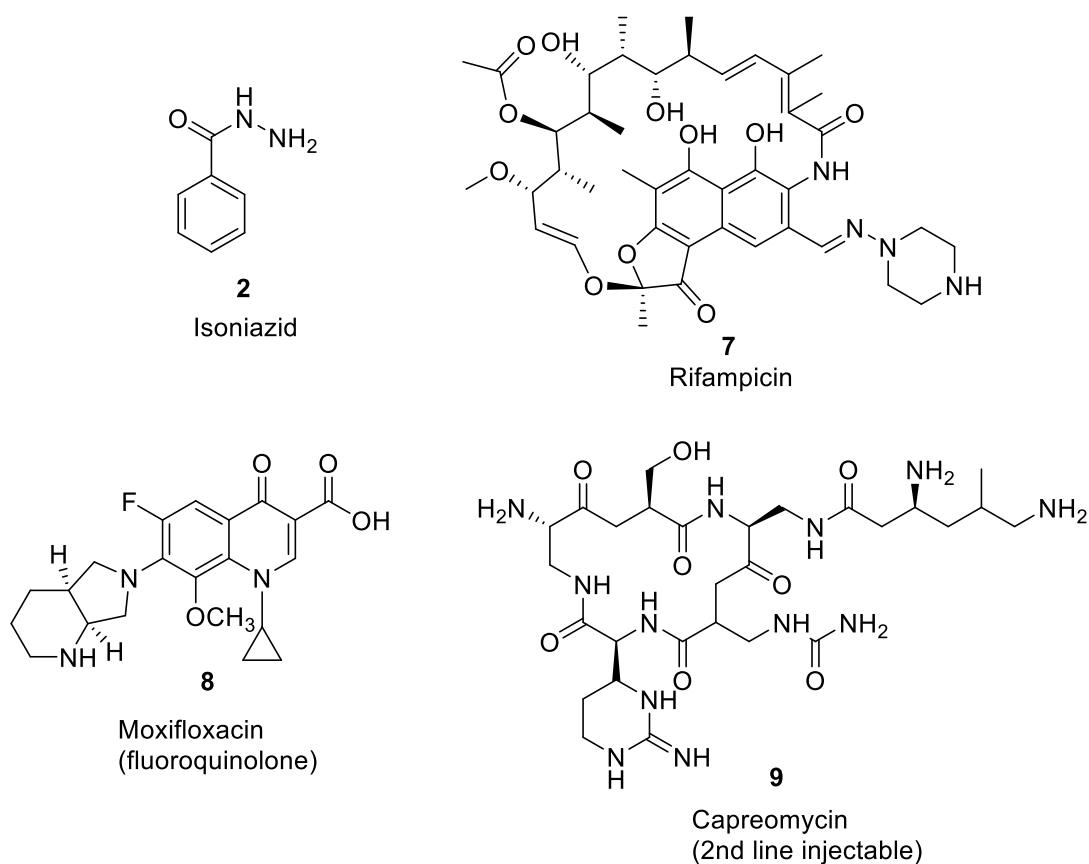


Figure 12 - Drugs that have become ineffective against MDR and XDR TB strains

Table 2 - Different Strains of TB

Disease	Definition
TB	Tuberculosis
DR-TB	Drug-resistant TB (resistant to any one TB drug)
RMR-TB	Rifampicin (4) mono-resistant TB (resistant to rifampicin and susceptible to isoniazid (2))
MDR-TB	Multi-drug resistant TB (resistant to isoniazid (2) and rifampicin (4))
RR-TB	Rifampicin-resistant TB (resistant to rifampicin (4), regardless of resistance to other drugs)
preXDR-TB	MDR-TB with resistance to either a fluoroquinolone (5) OR a second-line injectable drug (6)
XDR-TB	Extensively drug-resistant TB (MDR-TB with resistance to both a fluoroquinolone, e.g. (5) and a second-line injectable drug, e.g. (6))

2.1.6 TB-HIV co-infection

HIV is one of the main factors for progression from LTBI to active TB infection, and conversely, *M. tuberculosis* remains the highest cause of death in people living with HIV (PLHIV) and AIDS patients. Globally, TB/HIV co-infection resulted in about 9% of new TB cases and about 20% of all TB-deaths.³⁴ Aaron *et al.* reported that the risk of TB activation in PLHIV is 5-10% annually, while HIV negative individuals have a risk of 5-10% during their lifetime.³⁵ Figure 13 highlights the global HIV burden in new and relapse TB cases in 2017.

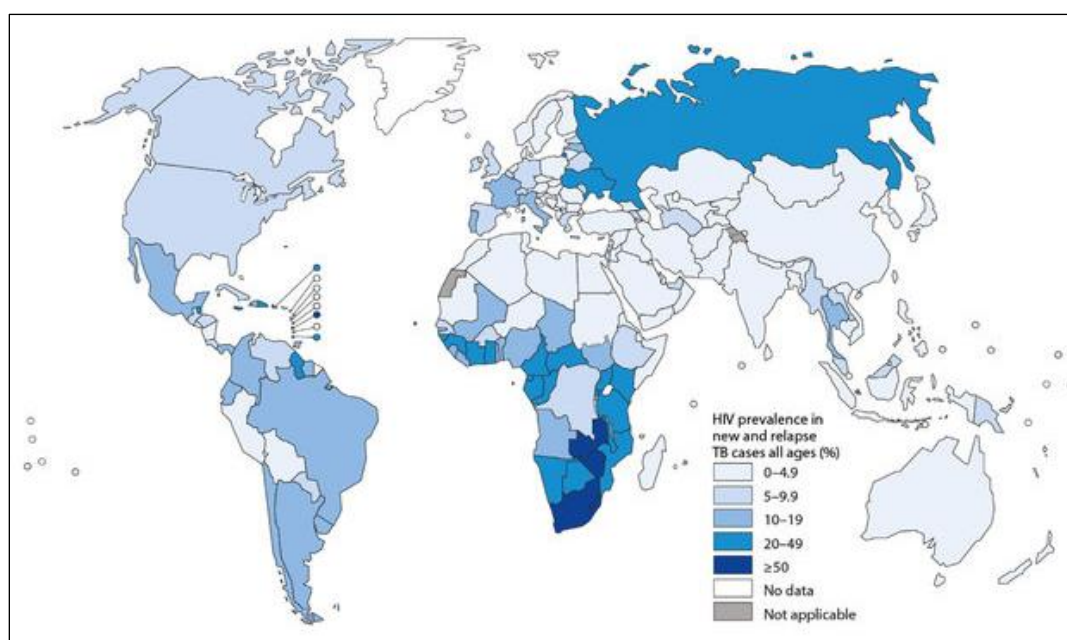


Figure 13 - Global HIV burden in new and relapse TB cases, 2017³⁶

As can be seen in Figure 13, South Africa has one of the highest burdens of TB/HIV coinfection in the world. The current crisis has its roots in a poorly managed pre-HIV tuberculosis epidemic, which was then exacerbated by the explosion of the HIV epidemic. In 2004, there were South African communities where more than 30% of pregnant women were HIV positive, and annual TB rates peaked at 1,500/100,000.³⁷ Currently, 7.1 million South Africans are living with HIV, and more than 50% of new TB cases are diagnosed from PLHIV.³⁸

In the period 2009-2016, both HIV and TB infection rates decreased due to better governance of all aspects of these diseases. The adoption of the fourth National Strategic Plan (NSP) for HIV, TB,

and sexually transmitted diseases 2017- 2022 further advanced the curtailment of these burdens. The NSP objectives were stipulated as follows:³⁹

- Focus on social and behaviour change communication to ensure social mobilisation and increasing awareness,
- Build strong social systems, including strengthening families and communities, to decrease risks of transmission and to mitigate the impact of the epidemics,
- Effectively integrate HIV, TB and sexually transmitted infections (STI) interventions and services,
- Strengthen procurement and supply chain systems, and
- Ensure that the human resources required are sufficient in number and mix, trained and located where they are needed.

Unfortunately, the emergence of the SARS-COV-2 pandemic in 2019 has hampered these goals due to the disruption of TB diagnosis and treatment, and disruption in the prevention of HIV.⁴⁰ As a consequence of the high burden on the health services due to the outbreak of the SARS-COV-2 pandemic, early diagnosis and treatment of new TB cases have reduced significantly, while the interruption of antiretroviral therapy played a significant role in the case of HIV prevention and therapy.

A review of the published data on TB/HIV coinfection reveals that the two diseases work in synergy and potentiate one another. T-lymphocytes (T-cells) are white blood cells that play an integral role in the body's immune response. In PLHIV the reduction in CD4⁺ and CD8⁺ T-cells is characteristic of an antigen immune response, and constant antigenic stimulation leads to an accelerated T-cell turnover and immune imbalance. Nunes-Alves *et al.* reported that CD8⁺ T-cells assist in the control of LTBI.⁴¹ Several mechanisms of TB activation due to HIV have been postulated, including HIV manipulation of macrophage bactericidal pathways, deregulated chemotaxis, up-regulated *M. tuberculosis* entry receptors on macrophages, and decreased tumour necrosis factor (TNF)-mediated macrophage apoptosis.⁴² Conversely, Whalen *et al.* in 1995 reported that TB accelerate the progression of HIV to AIDS, although the exact mechanism has not been established.⁴³ Probable causes include the fact that active TB leads to accelerated

loss of CD4⁺ T-cells and exposure to opportunistic infections; an increase in the replication of HIV-1 in the blood due to chronic T-cell antigen activation for TB; and TB infection sites in the lungs provide a micro-environment suitable for HIV-1 replication.⁴⁴ Thus, TB and HIV co-infection remains a challenge in South Africa, and diagnostic and therapeutic improvements are of critical importance.

2.2 - Treatment of the Different Forms of Tuberculosis

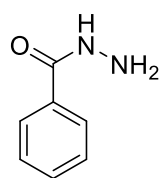
TB medications are grouped according to compatibility and similar effectiveness to aid in the design of treatment regimens.⁴⁵ The groups are as follows, with commonly used abbreviations for some (Table 3, Figures 14 – 19):

Table 3 - TB Medication Groupings

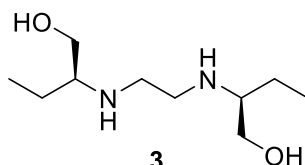
First-line		Abbreviation
<i>Group 1</i>	Isoniazid (2)	INH
	Ethambutol (3)	EMB
	Rifampicin (7)	RMP
	Pyrazinamide (10)	PZA
	Streptomycin (11)	SM
Second-line		
<i>Group 2</i>	Moxifloxacin (8)	MXF
	Gatifloxacin (12)	
	High-dose levofloxacin (13)	LEV
<i>Group 3</i>	Linezolid (14)	LIN
	Bedaquiline (15)	-
	Delamanid (16)	-
<i>Group 4</i>	Amikacin (17)	AMK
	Capreomycin (9)	CAP
	Kanamycin (18)	KM

Group 5	Clofazimine (19)	CLOF
	Ethionamide (1)	ETA
	Carbapenems (20-22)	-
Group 6	Cycloserine (23)	CYS
	Para-amino salicylic acid (24)	PAS
	Amoxicillin (25)	-

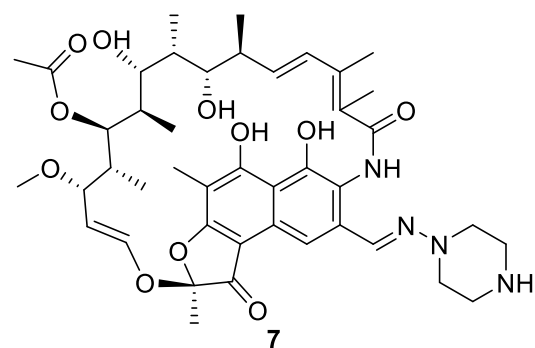
GROUP 1



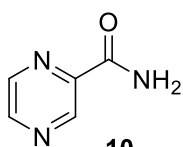
2
Isoniazid



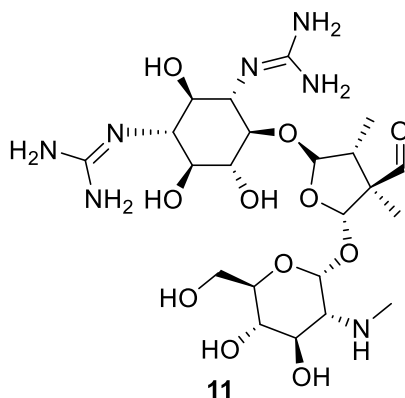
3
Ethambutol



7
Rifampicin



10
Pyrazinamide



11
Streptomycin

Figure 14 - First-line TB medications in Group 1

GROUP 2

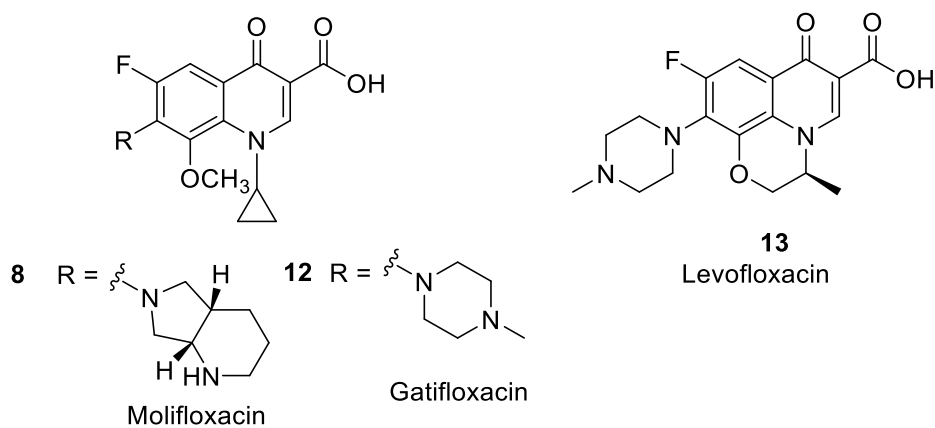


Figure 15 - Second-line TB medications in Group 2

GROUP 3

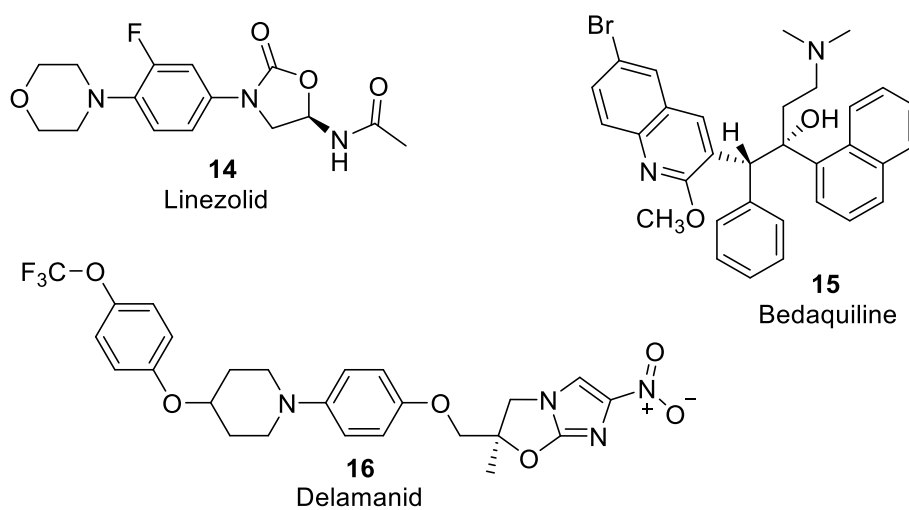


Figure 16 - Second-line TB medications in Group 3

GROUP 4

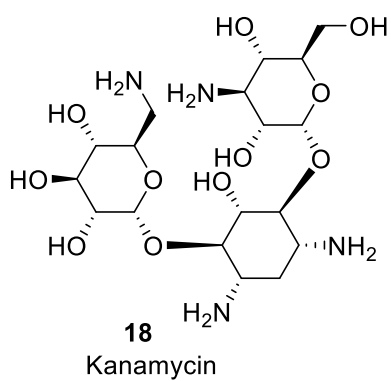
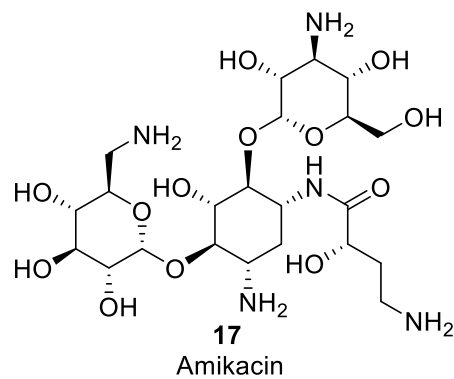
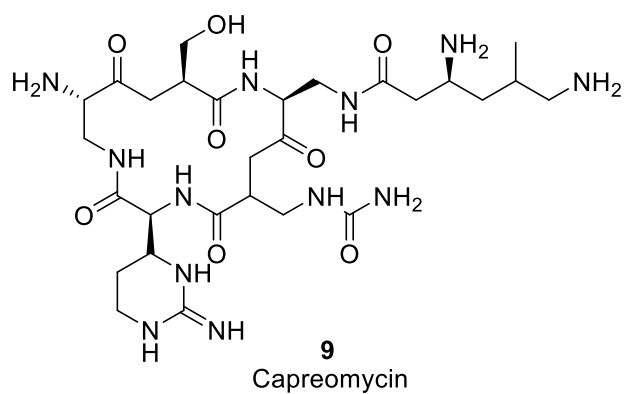


Figure 17 - Second-line TB medications in Group 4

GROUP 5

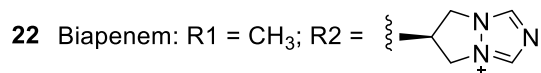
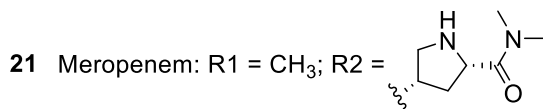
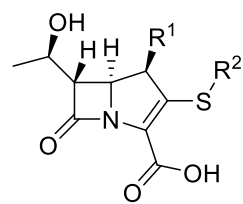
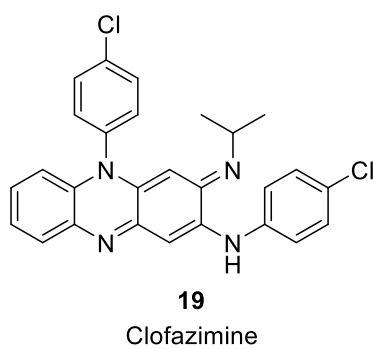
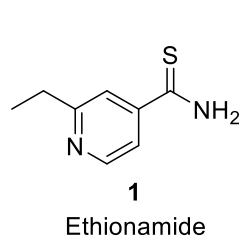


Figure 18 - Second-line TB medications in Group 5

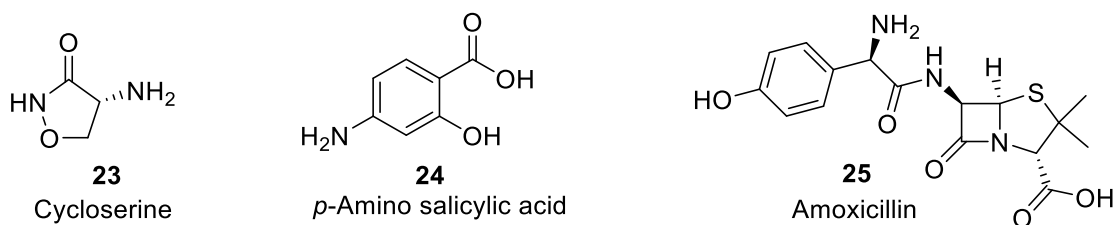
GROUP 6

Figure 19 - Second-line TB medications in Group 6

2.2.1 - Treatment of Non-Resistant TB

The standard regimen for treating new active TB infections includes all five first-line medications from Group 1, namely ethambutol (**1**), isoniazid (**2**), rifampicin (**7**), pyrazinamide (**10**), and streptomycin (**11**). Streptomycin (**11**) is sometimes omitted if it is deemed unnecessary or is unavailable. Several modifications can be made to the standard regimen if one or more of these medications is judged to be hazardous to the patient, or has been proven to be ineffective against the infection. These include:

i) *Regimens omitting ethambutol (1)*⁴⁶

Even though resistance to ethambutol (**1**) is considered rare, a regimen comprised of **2**, **7**, and **10** may still prove successful, at the cost of longer treatment duration.

ii) *Regimens omitting isoniazid (2)*⁴⁷

Isoniazid (**2**)-resistant TB infections are common. If the patient has undergone at least two months of the standard treatment and does not show signs of complete remission, the treatment may be extended beyond the usual treatment duration with high-dose rifampicin (**7**) and ethambutol (**1**). Adding a quinolone such as moxifloxacin (**8**) or levofloxacin (**13**) may also help.

iii) *Regimens omitting pyrazinamide (7)*⁴⁸

Although pyrazinamide (**10**) resistance is rare, its adverse effect profile is a common complaint among patients. Pyrazinamide (**10**) may be excluded from the treatment regime at the cost of longer treatment times. Higher dosages of the remaining drugs may once again shorten treatment duration, provided the patient finds the increased side-effects tolerable.

iv) *Regimens omitting rifampicin (7)*⁴⁹

Resistance to **7** usually goes hand-in-hand with resistance to isoniazid (**2**), but this is not always the case. Many patients find **7** intolerable, and it is especially associated with hepatitis and thrombocytopaenia. Rifampicin (**7**) is also the most expensive of the first-line drugs, which may limit its use in poorer countries. Removal of **7** from the standard regimen can significantly increase treatment times. Increasing the dosage of the remaining drugs and adding a quinolone may once again shorten TB therapy.

2.2.2 - Multidrug-resistant TB (MDR-TB) Treatment

MDR-TB is a form of TB that is resistant to treatment with most effective first-line medications, namely isoniazid (**2**) and rifampicin (**4**).¹ This form of *M. tuberculosis* is mostly attributed to one strain of the disease, often referred to as the Beijing lineage. After sweeping across most of Eurasia through the course of the 20th century, the strain has now been detected in every country on the planet in some form or another.⁵⁰ MDR-TB is treated with various combinations of second-line TB medications (Table 4, Figure 20). Treatment times for MDR-TB tend to be longer compared to TB and is more expensive. In addition, more severe side effects are often present.

Table 4 - WHO-recommended TB classifications

Group	Class of Drugs	Drugs
Group A	Fluoroquinolones	Moxifloxacin (8)
		Gatifloxacin (12)
		Levofloxacin (13)
Group B	Second-line injectables	Capreomycin (9)
		Amikacin (17)
		Kanamycin (18)
Group C	Other second-line agents	Ethionamide (1)
		Linezolid (14)
		Clofazimine (19)
		Cycloserine (23)
		Prothionamide (26)
		Terizidone (27)

Group D1	(Add-on agents)	Ethambutol (3)
		High-dose isoniazid (2)
		Pyrazinamide (10)
Group D2	(Add-on agents)	Bedaquiline (15)
		Delamanid (18)
Group D3	(Add-on agents)	<i>p</i> -Aminosalicylic acid (24)
		Imipenem (20)-cilastatin (28)
		Meropenem (21)
		Amoxicillin (25)-clavulanate (29)
		Thioacetazone (30)

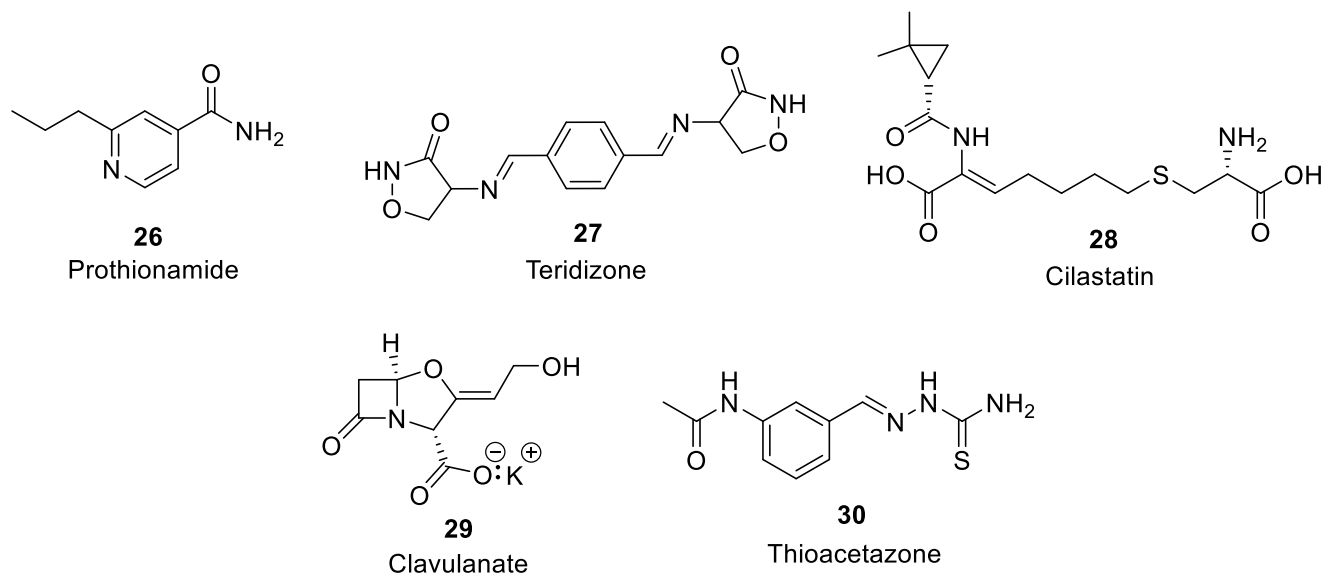


Figure 20 - Additional 2nd line drugs used to combat TB

The WHO recommends the following guidelines for the treatment of MDR-TB, which has its own revised categorisation of medicines (Table 4):

i) *Isoniazid (2)-resistant TB (Hr-TB)*⁵¹

Patients with rifampicin (**7**)-susceptible and isoniazid (**2**)-resistant TB should be treated with ethambutol (**3**), rifampicin (**7**), pyrazinamide (**10**), and levofloxacin (**13**) for a duration of 6 months. It is not recommended to add streptomycin (**11**) or other injectable agents to the regimen as this may result in a more complex adverse effect profile.

ii) *Longer MDR-TB regimens*⁵¹

In MDR/RR-TB patients on lengthy treatment regimens, all Group A agents and at least one agent from Group B must be included, such that at least four effective TB agents are administered at the start of medication. If less than three Group A agents are used, all Group B agents must be employed. If the regimen cannot be composed with agents solely from Groups A and B, the necessary number of Group C agents are added to complete the regimen.

- Longer treatments for MDR/RR-TB must not contain capreomycin **(9)** or kanamycin **(18)**.
- Either moxifloxacin **(8)** or levofloxacin **(13)** must be included in longer MDR/RR-TB regimens.
- Bedaquiline **(15)** must be part of longer MDR-TB regimens in patients above 17 years of age. Bedaquiline **(15)** can also be included in lengthier MDR-TB regimens for patients between 6-17 years of age.
- Linezolid **(14)** should be included in longer MDR/RR-TB regimens.
- Clofazimine **(19)** and cycloserine **(23)**, or terizidone **(27)**, can be included in longer MDR/RR-TB regimens.
- Ethambutol **(3)** can be included in longer MDR/RR-TB regimens.
- Delamanid **(16)** may be included in patients aged 3 years or more on longer MDR/RR-TB regimens.
- Pyrazinamide **(10)** may be included in longer MDR/RR-TB regimens.
- Imipenem **(20)**-cilastatin **(28)**, or meropenem **(21)** can be included in longer regimens.
- Amikacin **(14)** can be included in MDR/RR-TB regimens for patients aged 18 years or more when susceptibility has been demonstrated and adequate measures are in place to monitor for excessive adverse effects. Streptomycin **(11)** can replace amikacin if similar precautions are taken.
- Either ethionamide **(1)** or prothionamide **(26)** can be included in the treatment of MDR/RR-TB patients on lengthy regimens only if linezolid **(14)**, bedaquiline **(15)**, delamanid **(16)**, or clofazimine **(19)** are not used or if better options to compose a regimen are not possible.

- *p*-Aminosalicylic acid (**24**) may be included in the treatment of MDR/RR-TB patients on longer regimens only if **14**, **15**, **16**, and **19** do not form part of the regimen, or if more effective options are not available.
- Clavulanate (**29**) should not be included in longer regimens.

2.2.3 - Length of Treatment Regimens for MDR/RR-TB

- MDR/RR-TB patients on longer regimens have a recommended treatment time of 18-20 months. The duration may be modified according to the patient's response to treatment.
- MDR/RR-TB patients on longer regimens that include streptomycin (**11**) or amikacin (**17**) should have an intensive treatment phase of 6-7 months. Duration may be modified according to the patient's response to treatment.
- Patients without fluoroquinolone-resistant MDR/RR-TB may undergo a shorter treatment time of between 9-12 months.

2.2.4 Extreme drug resistant TB (XDR-TB) Treatment⁵²

XDR-TB treatment is often unpleasant and dangerous in terms of side-effects. These regimens are not as rigid as the aforementioned ones and are better described as a loose set of rules built upon clinical observations. This set of rules is a last resort in battling XDR-TB, and treatment will last until either the infection is eliminated, or the patient dies. The guidelines are as follows:

- Pyrazinamide (**10**) and any other group 1 agent must be included in the regimen.
- An injectable agent to which the strain is susceptible can be added to the regimen for the entire duration of treatment.
- If an injectable agent is judged to be effective but shows unacceptable levels of toxicity, consider administration of the drug by inhalation via a nebuliser.
- Higher-generation fluoroquinolones such as moxifloxacin (**8**) or gatifloxacin (**12**) can be included in the regimen.
- All group 4 agents considered to be effective may be used if they have not formed part of a previously attempted regimen.

- Consider adding at least two group 5 drugs, including bedaquiline (15).
- If sanctioned by the WHO compassionate use scheme, consider adding drugs still undergoing trials.
- High-dose isoniazid (2) treatment may be helpful if low-level resistance or absence of the katG gene has been confirmed by culture analysis.
- Consider adjuvant surgical resection of infected tissues in addition to the treatment regimen.

Careful management of comorbid HIV is necessary if present.

2.3 - Adverse Effects of Commonly Used Anti-TB Drugs

All antibiotics available for the treatment of TB have potentially serious side effects, worsened by the fact that treatment can last many months. Benign adverse effects may become intolerable over a long period of time, leading many to prematurely terminate their course of antibiotics. Often it is not clear whether the symptoms result from the disease itself or the medication administered to treat it. Also, side effects may vary when a medication is used in conjunction with other drugs as part of a treatment regimen, further complicating the adverse effect profile.⁵³ For some treatments, the severity of side-effects is strongly dosage dependent. Given the option, lower dosages can lead to safer treatment at the cost of treatment brevity.

Despite the seriousness of these potential complications, many patients experience only mild discomfort from the treatment itself.⁵⁴

2.3.1 - Hepatotoxicity

One of the more serious side effects associated with TB drugs is hepatitis, with the risk usually increasing as the length of treatment does. Patients with either pre-existing liver conditions or persons with liver damage acquired through lifestyle choices are especially vulnerable, often to the point where treating the TB infection is riskier than simply doing nothing, as has been noted with isoniazid (2).⁵⁵ Hepatotoxicity is one of the main causes of drug cessation, accounting for about 11% of treatment cessations. Most TB drugs can lead to hepatic damage. Those that do not (for

example, ethambutol) are usually nevertheless used as part of a treatment regimen that includes a drug capable of causing liver damage (such as **2**).⁵⁶

2.3.2 - Peripheral Neuropathy

Peripheral neuropathy refers to damage or illness pertaining to the peripheral nerves of the body, that is, nerves located outside of the brain and spinal cord.⁵⁷ This condition affects motor, sensory, and autonomic functions, resulting in impaired motor skills, loss of sensation, and disruption of autonomous bodily processes. Neuropathy stemming from TB treatment is almost always sensory, usually leading to loss of the tactile senses, intermittent paraesthesia, impaired kinaesthesia, and allodynia. Many of these sensory side-effects disappear during treatment but may become chronic as the length of treatment increases. Sensory neuropathy is most commonly associated with isoniazid (**2**) and quinolones like moxifloxacin (**5**). If neuropathic symptoms appear during treatment with a regimen that includes **2**, the drug must be stopped and substituted with another. High-dose pyridoxine (vitamin B6) is often administered to treat the neuropathy, and permanent damage can often be avoided.

2.3.3 – Thrombocytopenia

Thrombocytopenia is a condition associated with a deficiency in thrombocytes (platelets) in the blood.⁵³ As these platelets are responsible for clotting of damaged blood vessels, individuals with this disorder may experience increased and longer bleeding in case of injury. Patients undergoing TB treatment may develop spontaneous bleeding of the nose and gums, and may also experience fatigue, malaise, and frequent bruising. Thrombocytopenia is most commonly associated with rifampicin (**4**) and is thus often omitted from regimens without replacement.

2.3.4 - Ototoxicity

Ototoxic substances cause damage to the inner ear and can lead to hearing loss and impaired balance.⁵³ Inner ear damage stemming from TB medication is often irreversible, and is especially prominent in infants and persons over the age of 40. Streptomycin (**8**) is most commonly associated with ototoxicity, along with ethionamide (**1**), and the fluoroquinolones to a lesser degree.

2.3.5 - Tendinitis

Tendinitis is the inflammation of tendons due to infection or physical injury.⁵⁸ In severe cases of tendinitis, even minor physical activity can lead to a tendons bursting or detaching from the muscle. Tendinitis stemming from TB treatment is quite rare and mainly associated with the fluoroquinolone family of antibiotics. Non-steroidal anti-inflammatory agents can lessen the degree of damage.

2.4 - Conclusion

The primary focus of the research reported in this dissertation was the development of improved process routes to the anti-TB agents ethionamide (**1**), isoniazid (**2**), and ethambutol (**3**), and using advanced manufacturing technologies in the form of flow chemistry. An overview of these four treatments together with the synthetic approaches used to access them is described in Chapter 3.

Chapter 3 – Introduction to Flow Chemistry

3.1 - Introduction to Flow Chemistry

Flow chemistry involves the synthesis of compounds using continuous streams of reagents pumped through a series of tubes and mixing interfaces. Although large-scale flow processes are common in process engineering, smaller, gram-scale flow chemistry instruments are less common.⁵⁹

3.1.1 - Advantages of Flow Chemistry over Traditional Batch Synthesis

Flow technology offers some advantages over traditional batch synthesis in terms of yield, reaction speed, and safety.⁶⁰ For example, a reaction solvent can be heated to above its boiling point due to the fact that flow reactors are closed systems and can be pressurized as needed, whereas a pressurized batch reactor poses additional challenges in terms of safety. In flow chemistry, mixing of reagents can be achieved within seconds due to the small scale of these reactions, conversely large batch reactors tend to suffer from poor mixing requiring careful interventions to ensure uniformity in the reaction mixture. The large interfacial area and short molecular diffusion paths in narrowly spaced flow reactors can drastically improve the efficiency of a reaction above that of a batch reaction. The reaction mixture carries a significantly lower thermal mass than the flow system itself, allowing faster and more precise control of reaction temperature throughout. An additional benefit of the smaller scale flow reactions is the fact that at any given time in a run, the amount of reagents in contact with one another is minimal compared to a batch process. This greatly reduces the danger of a runaway reaction, even though the same molar throughput is achieved by the end of the flow process. The sealed environment of a flow reactor also lends itself well to reactions involving gases, which would otherwise require a pressurized 'bomb-type' reaction vessel.

The modular nature of flow reactors allows a chemist to link up multiple reactors in sequence, which allows one to perform complex multistep reactions that would otherwise be difficult in terms of post-reaction workups. A linked system can also be beneficial in reactions where isolating intermediate products is difficult or impossible⁶¹. Some reactions may even lend

themselves to in-line purification, where a product is continuously isolated in the flow system as it is produced.

Perhaps one of flow chemistry's greatest advantages, compared to traditional batch methods of synthesis, is the tendency for flow reactions to consume less solvent⁶². Due to the smaller reaction volumes, and the more efficient heating and mixing of reactions, the solvent required is reduced. Reactions that see a significant rise in yield when switched to flow might also decrease the amount of solvent necessary to purify a reaction crude product. Cleaner reaction crude products also allow for easier solvent recycling.

Flow chemistry is not limited to gram-scale reactions. In fact, the concepts of a flow setup are akin to larger industrial processes that involve continuous flow production.⁶³ Once a reaction has been fine-tuned on a benchtop flow reactor, an attempt can be made to scale it up to multi-gram or kilogram-scale, considering the various challenges this would present (for example, the heating speed and efficiency of a microreactor may be lost as reaction volumes increase in size).⁶⁴ Kilogram-scale flow reactors also offer a smaller laboratory footprint without the mass and heat transport limitations of a reactor scale-up in batch.

3.1.2 - Disadvantages of Flow Chemistry

Flow chemistry does, however, have disadvantages that might make a traditional batch synthesis the preferred option. One of the larger downsides to flow chemistry is the large initial capital investment needed to procure and set up a flow reactor when compared to the lower cost of most batch reactors.

Also, the fact that flow chemistry tends to work best with homogenous liquid reactions is another factor limiting the scope of reactions.⁶⁵ Undissolved reagents and precipitates can easily block the narrow tubes and mixing channels of various components in the flow line, which can be partially mitigated through the inclusion of in-line sonication or alternatively the use of peristaltic or similar pumping devices that are designed to pump slurries. Workarounds for the inclusion of heterogeneous reagents or catalysts include the use of a dedicated 'packed-bed' reactor module, containing a solid reagent packed into a column that forms part of the flow line.⁶⁰

Furthermore, designing a reaction methodology for a flow process can be cumbersome as a number of variables must be optimised separately (flow speed, temperature, pressure etc). However, once this has been accomplished, the same methodology may be applicable to similar reactions.

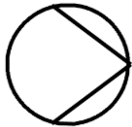
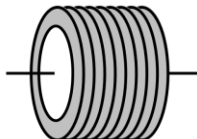
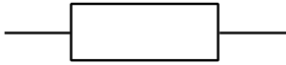
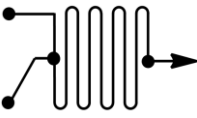
Dispersion of reagents and products throughout the flow line can become a significant problem as the length of the reaction path increases, along with a possible increase in reaction time. The difference in concentration between the reaction plug and the pushing solvent will lead to tailing and fronting dispersion. This can make it more difficult to accurately gauge when to start collecting product and when to stop, or in the case of multi-step syntheses when to time the addition of reagents in subsequent steps, all of which can lead to a loss of yield. That being said, these dispersion issues are more problematic in smaller scale reactions. Once scaled-up, this problem becomes less prominent with the leading and tailing fractions typically being discarded to focus on processing reactions under steady state conditions.

3.2 - Basics of Flow Reactors

Flow reactors can range from a simple single-pump setup to an array of many pumps, specialised reactor modules, and in-line reaction monitoring via various analytical systems. A variety of components can be used to configure a flow system for a specific reaction. Different reactor configurations can also be linked to form a continuous system capable of performing a multi-step synthesis.

Some of the commonly utilised components together with the symbols used to represent them are shown in Table 5.

Table 5 - Common Flow Instrument Components

Component	Flow Scheme Symbol	Example
Pump		
Coil Reactor		
Column Reactor (Packed-bed reactor)		
Mixing Chip		
Back Pressure Regulator (BPR)		

A particular flow setup can be drawn as a simple diagram using the various component symbols. For example, a reaction between two reagent solutions A and B, using two pumps and a catalyst-packed column, can be illustrated as shown in Figure 21:

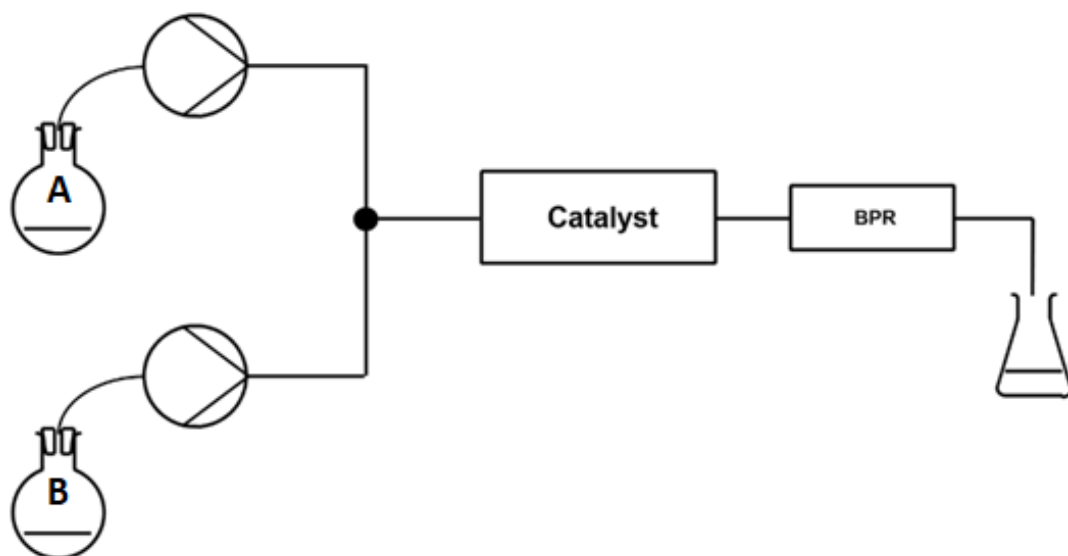


Figure 21 - Example Flow-setup Diagram

3.2.1 - Pumps

A variety of different pumps are used in flow chemistry, depending on the application. Some of the types of pumps available are described below.

3.2.1.1 - Peristaltic Pumps

A peristaltic pump, or roller pump, is a displacement pump that uses continuous circular or linear motion to force a fluid or slurry forwards through a flexible tube (Figure 22). By pinching the tube and forcing the pinched section forwards through mechanical action, the fluid ahead of the pinched section can be made to move forwards.

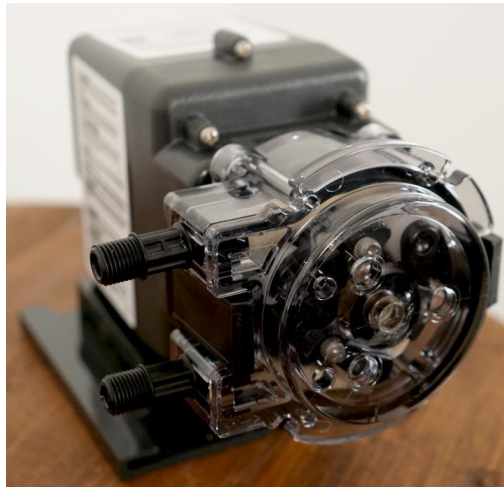


Figure 22 - Example of a peristaltic pump

This is analogous to emptying a tube of toothpaste by squeezing the back of the tube between one's fingers and sliding them towards the opening. Many biological processes function through linear peristaltic motion to displace material, such as the swallowing of food or movement of chyme through the digestive tract. For a continuous pumping motion, a circular portion of tube is placed around two or more rollers inside a housing. By rotating the rollers such that they pinch a portion of tube against the outer housing, a continuous peristaltic motion can be achieved. Figure 23 demonstrates a pumping cycle, with the blue portion representing a volume of liquid being moved through the tube in a clockwise fashion.

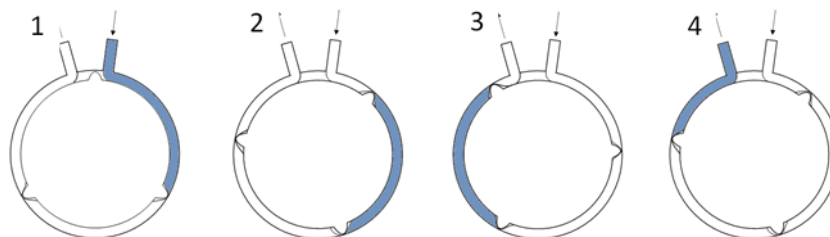


Figure 23 - Rotary peristaltic pumping cycle

In step 1, the forward movement of the pinched section of tube creates a vacuum aft of the roller, pulling in liquid from the feedstock. In step 2, a second roller creates another pinched section of tube, effectively trapping a volume of fluid between two rollers. The roller housing continues to rotate clockwise in step 3, pushing and pulling the volume of solvent as the pinched section of tubing moves forwards. In step 4, the leading roller clears the tube, allowing it to open. The rear roller is still pinching the tube, pushing the fluid out of the loop and into the forward flow line. This cycle runs continuously and allows for uninterrupted pumping. A major perk of a peristaltic pumps is their ability to pump both viscous fluids and even slurry-like mixtures. For example, this type of pump is used in dialysis to pump blood, which is very viscous and sticky.

3.2.1.2 - Tandem Syringe Pumps

Two syringe-like pumps operating in tandem can provide a steady and accurate rate of fluid flow. An example of such a system is pictured in Figure 24.⁶⁶



Figure 24 - A series of syringe pump pairs

As shown in Figure 25, a tandem syringe pump consists of a pair of plungers connected in parallel, emptying into the same fluid line.

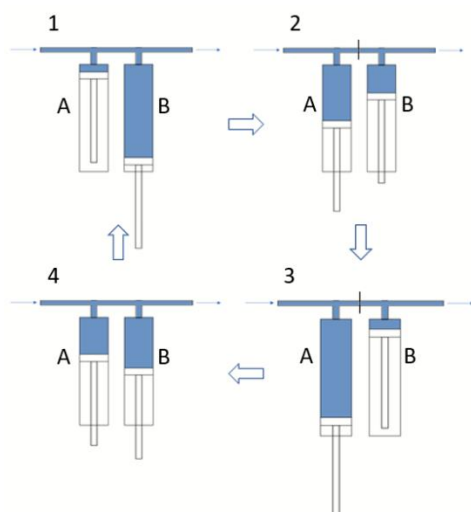


Figure 25 - Tandem syringe pump

Syringe A is twice the volume of syringe B. Starting with the configuration in step 1, syringe B is filled with fluid and syringe A is empty. In step 2, the line between pump A and B is closed off, pump A fills, and pump B injects into the flow line. In configuration 3, syringe A has reached its end of travel, and syringe B has been emptied. In step 4, the flow line between pumps A and B is opened, and pump A begins to inject its contents into the flow line. Pump A (which is twice the volume of pump B) both fills pump B and injects fluid into the downstream flow line. The cycle then repeats when the valve is closed, allowing pump A to fill and pump B to inject into the flow line. This allows for an uninterrupted pumping action.

3.2.1.3 - HPLC Pumps

High performance liquid chromatography (HPLC) pumps are used in liquid chromatography, where very high pressures are required to move fluid through tightly packed analytical columns. They are therefore ideal for other high-pressure applications such as flow chemistry (Figure 26).⁶⁷

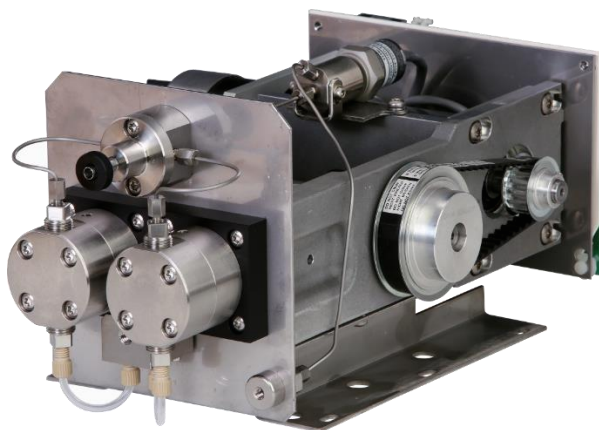


Figure 26 - Dual-piston HPLC module

Figure 27 demonstrates a typical HPLC pumping cycle, which relies on a pair of check-valves to orchestrate the forward movement of fluid through the flow line (the valves are indicated by A and B).

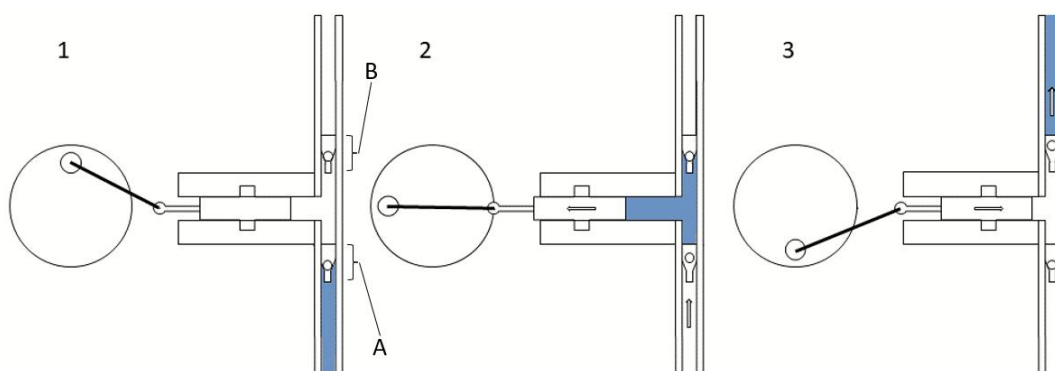


Figure 27 - Cutaway diagram of single-piston HPLC Pump

These valves allow for fluid movement in one direction only, ensuring that there is only forward flow. As shown in step 1, both check valves are closed. As the cam rotates anticlockwise in step 2, the piston retracts and check-valve A opens, allowing fluid to move into the pumping chamber. When the piston reaches its end of travel in step 3 and reverses its direction of movement, check-valve A closes and prevents the liquid in the pumping chamber from moving backwards. The piston compressing the pumping chamber forces check-valve B open and the chamber empties into the forward flow line. When the piston once again reaches end of travel, check-valve B closes, and the system returns to the configuration in step 1. The cycle then repeats.

The high-pressure capabilities of HPLC pumps are counterbalanced by the check-valves' sensitivity to precipitates. Also, a single HPLC pump doesn't allow for continuous flow. Two HPLC pumps can be set up in parallel such that continuous flow is achieved; while one pump refills, the other injects into the flow line, and vice versa.

3.2.2 - Coils and Tubular Reactors

Coil/tubular reactors are lengths of flow tubing typically wound around a cylindrical housing or mandrel. These function as an elongated reaction pathway which can be uniformly heated or cooled. An example of a transparent 10 mL reactor coil is pictured in Figure 28.⁶⁸



Figure 28 - Vapourtec 10 mL reactor coil

These coils may be manufactured from the same flow tubing that connects the different components of a flow setup or can be made of other materials depending on the application. For example, stainless steel or Hastelloy may be desirable should reaction temperatures exceed 250 °C, whereas polymers like PTFE (Polytetrafluoroethylene) or PEEK (Polyether ether ketone) are more desirable if high chemical resistance is required. Many commercially available Teflon coils can be manually rewound with new tubing if damaged or blocked and are usually built such that they can be slotted onto a module for cooling or for heating.

3.2.3 - Mixing Chips

A mixing chip is a flow component that is designed to achieve rapid mixing of incoming flow reagent lines (Figure 29).⁶⁸



Figure 29 - Glass mixing chip

Through careful design of the chip, fully turbulent flow is created along the flow pathway of the mixing unit such that maximum homogeneity is achieved. These components double as a reaction chamber, and as such come in commercial designs that can be both heated and cooled depending on the reaction requirements.

3.2.4 - Back Pressure Regulators

A back pressure regulator acts to exert backwards resistance on the pressure created by the flow instrument pumps. This is essential to keep the reactor flow path under the desired system pressure. The fluid path moves through the BPR for further downstream processing. Several types of BPR exist, some of which include:

2.3.4.1 - Passive Spring-loaded BPR

Passive BPRs are manufactured to exert a fixed amount of backwards pressure (Figure 30).⁶⁹



Figure 30 - Example of passive BPR

As shown in Figure 31-a, a spring made to exert a constant forward pushing force on the valve head serves to close the inlet when the system pressure is below the BPR's rated pressure. In Figure 31-b, the system pressure is high enough to overcome the resistance of the spring and can move the valve head backwards to allow fluid to flow through the BPR.

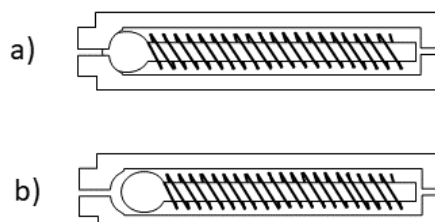


Figure 31 - Passive BPR Modes

Fluid will move through the BPR at any pressure higher than its calibrated rating. The downside of such a BPR is of course the fact that its backwards resistance cannot be adjusted to allow for higher or lower pressures, and as such must be swapped with a unit rated for the desired back pressure. This can be easily accomplished but necessitates the purchasing of multiple BPRs.

3.2.4.2 - Adjustable Membrane BPR

An adjustable BPR allows the chemist to dial in a specific backpressure to suit their needs. Most adjustable BPRs operate with the use of a non-permeable membrane. Such a BPR is pictured in Figure 32.⁷⁰



Figure 32 - Compact gas-regulated membrane BPR

Figure 33 shows a generalized schematic of an adjustable membrane BPR. In Figure 33-1, the applied force on the membrane (indicated by the arrows) pushes down on the membrane, sealing the fluidic pathway and blocking the passage of liquid. In Figure 33-2, the system pressure is able to overcome the force pushing down on the membrane, lifting it up sufficiently to allow fluid to pass through. The force applied to the membrane can be tweaked to increase or decrease the system pressure needed to allow the passage of fluid. Membrane resistance can be adjusted either mechanically (with, for example, the use of a variable force spring), or with adjustable gas pressure.

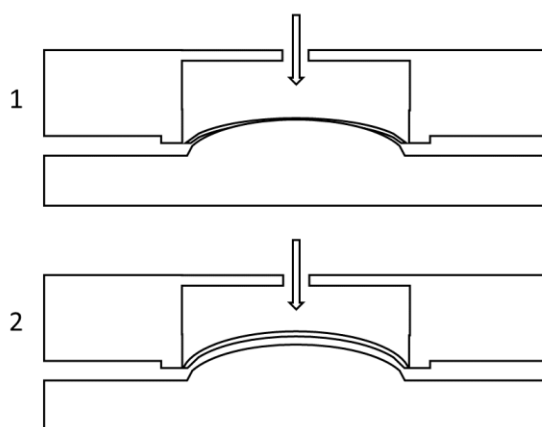


Figure 33 - Schematic of membrane-operated BPR

3.2.5 - Column/Packed-Bed Reactors

A column/packed-bed reactor is a tubular flow component into which is packed a solid reagent (example pictured in Figure 34).⁶⁹



Figure 34 - Column reactor

The column forms part of the flow line and fluid moves through it, coming into contact with the solid reagent. This component is ideal for integrating solid reagents into a flow system that would otherwise block the narrow fittings and tubes. Insoluble catalysts are often used inside these

columns. As with coils and mixing chips, columns can be mounted on special devices that allow it to be heated or cooled during a reaction.

3.2.6 - Photochemical Reactors

Photochemical reactors can be integrated into a continuous flow system to effect electromagnetic-radiation catalysed reactions (Figure 35).⁶⁸

These reactors usually operate in the UV-spectrum of wavelengths. The component consists of a glass or quartz reaction chamber that is exposed to UV-light. Photons can be supplied with either bulbs or lasers. Photospectrometers can also be integrated into the reaction line, which can provide continuous spectral analysis of the product as it forms.



Figure 35 - Vapourtec UV reactor

3.3 - Basics of Fluid Dynamics

A fluid is defined as a material that will continually deform when placed under a shearing force.⁷¹ This definition applies to both liquids and gases. Figure 36 demonstrates the different reactions of a solid (a) and a fluid (b) in response to an applied shear stress τ .

Figure 36-a represents a metal plate fixed at the base, assumed to be perfectly elastic. When a shearing force τ is applied, the plate will deform laterally a certain distance before stopping, and

will remain deformed for as long as the force is exerted. As soon as the force is removed, the plate will return to its initial configuration.

Figure 36-b represents a volume of fluid. When a shearing stress τ is applied, the portion of fluid acted upon by the shear force will continue moving laterally for as long as the force is exerted, while the remainder of the fluid will remain stationary. When the shearing force is removed, the system will not return to its initial configuration.

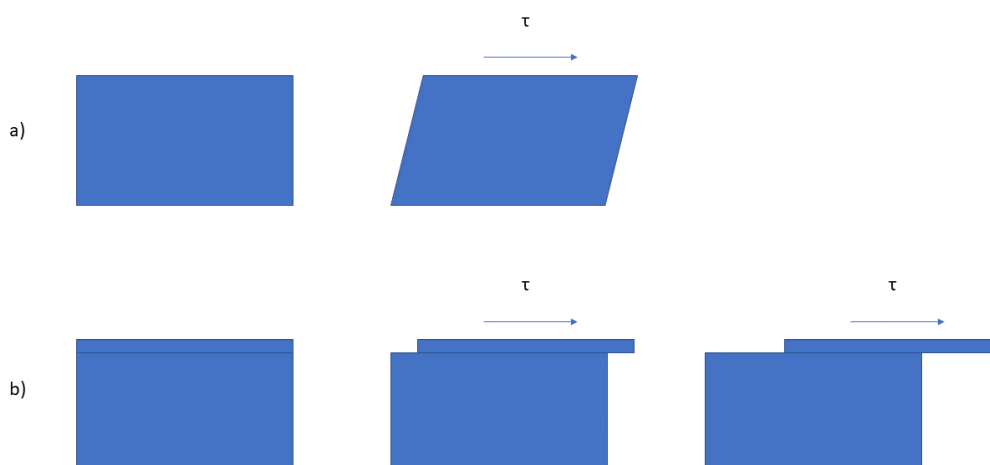


Figure 36 - Different response of a solid (a) and a liquid (b) to a shearing force

Several theoretical models exist to describe the behaviour of fluids under various velocities and in a variety of environments. Knowledge of the type of fluid flow is important in determining parameters related to mass and heat transfer, which are essential when designing continuous flow reactors. Two of the most commonly described types of fluid movement are laminar flow and turbulent flow.

3.3.1 - Laminar Flow

In this flow model, fluid is viewed as moving in parallel sheets, similar to a pack of playing cards sliding past one another.⁷² This is illustrated in Figure 37, where the parallel arrows represent a stationary unit of fluid.

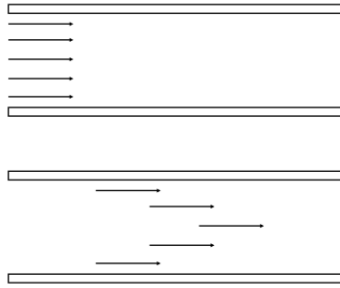


Figure 37 - Body of fluid transitioning into laminar flow.

When the fluid starts moving, the layers travel forwards uniformly. There is no movement of fluid perpendicular to the direction of flow. What little mixing does occur is mainly attributed to kinetic diffusion caused by the collision between molecules in motion. It must be noted that the layers in contact with the sides of the pipe tend to move slower than those located at the centre due to friction between the fluid layer and the pipe surface. Layers away from the inside surface of the pipe only experience friction from adjacent layers of fluid.

3.3.2 - Turbulent Flow

As shown in Figure 38, when laminar flow increases in speed, it will eventually transition into turbulent flow. Molecules in the fluid line will start to travel in random directions as they move down the pipe.

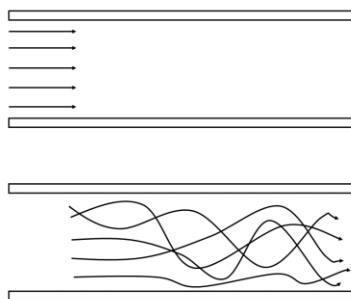


Figure 38 - Body of fluid transitioning into turbulent motion.

The transition from laminar to turbulent flow can be clearly seen in a waterfall. Upon clearing the ledge, water starts travelling downwards in laminar flow. As it accelerates under the force of gravity, the water moves over into turbulent flow, creating a foamier appearance before it hits the water below.

Both of these types of flow can be described and distinguished mathematically by what is known as the Reynold's number.

3.3.3 - Reynold's Number

A Reynolds number is a dimensionless value that can theoretically distinguish a mass of fluid in motion as either laminar or turbulent. As a single fluidic system is often comprised of both laminar and turbulent flow, the Reynold's number is used to predict where each type of flow is most prominent in the system. It can also be used to determine the point at which laminar flow transitions in turbulent flow, or vice versa. This in-between state is known as transitional flow, and consists of segments where the flow will switch randomly between laminar and turbulent movement.

The Reynolds equation for a circular pipe is as follows:

$$Re = \frac{\rho Q D_H}{\mu A}$$

Where:

ρ is the density of the fluid.

Q is the volumetric flowrate of the fluid.

D_H is the inside diameter of the pipe.

μ is the dynamic viscosity of the fluid (the fluid's resistance to flow under an applied external force due to its own intermolecular friction).

A is the cross-sectional area of the pipe.

For Reynolds numbers below 2300, laminar flow is predicted.⁷³ For Reynolds numbers above 2900, turbulent flow is predicted. For Reynolds numbers between these two values, transitional flow will dominate, where the flow type will flip spontaneously between laminar and turbulent motion, with laminar flow dominating mostly in the centre, and turbulent flow dominating nearer the inner surface of the pipe.

This equation adequately explains fluid behaviour as observed experimentally. For example, to increase mixing in the flow line, one can:

- i. Use a denser solvent system. As ρ increases, so does Re .
- ii. Increase the flowrate Q . As Q increases, so does Re .
- iii. Use a less viscous solvent system. As μ decreases, Re increases.
- iv. Decrease the pipe diameter D_H . Despite A being dependent on D_H according to the equation $A = \frac{\pi D_H^2}{4}$, the value of A decreases faster than D_H , leading to a larger Re .

Even though a stock solution will consist of both solvent and reagents, the equation parameters may be defined only in terms of the pushing solvent as it is assumed the bulk of the stock is made up of solvent. If desired, ρ and μ for the prepared stock solution can be obtained experimentally as a whole for increased accuracy.

3.3.4 - Secondary Flow

When a fluid in motion encounters a curved section of flow path, an interesting phenomenon occurs. As a volume of fluid enters the curved section, centripetal forces will act on the flowing particles to change their main direction of travel by creating a transverse pressure gradient. However, the pressure gradient needed for the faster moving fluid in the centre of the pipe to follow the curve is greater than that of the slower moving fluid nearer the outside. This results in the fluid in the centre of the pipe being swept towards the outer side of the bend and the fluid near the pipe surface being swept towards the centre. This creates a pair of counter-rotating cells known as Dean vortices (Figure 39).

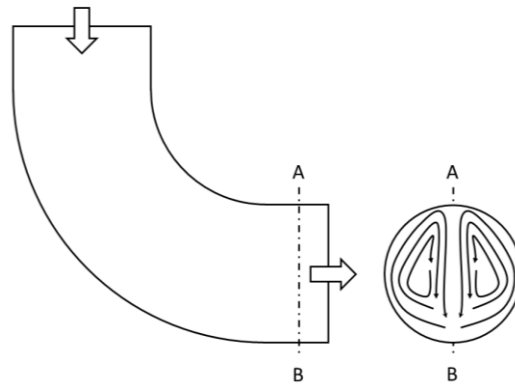


Figure 39 - Formation of Secondary Flow Vortices (Dean Vortices)

3.3.5 - Stagnation Zones

A stagnation zone, or “dead-zone”, is a localised portion of fluid in a pipe where highly turbulent swirls form, trapping fluid in a spinning vortex. This can be problematic as it leads to higher levels of friction, increasing backpressure. Figure 40 displays a selection of three 90° bends, namely, (a) a smooth bend, (b) a bend featuring a single sharp edge, and (c) a bend featuring two sharp corners. Secondary flow effects are not considered in these scenarios.

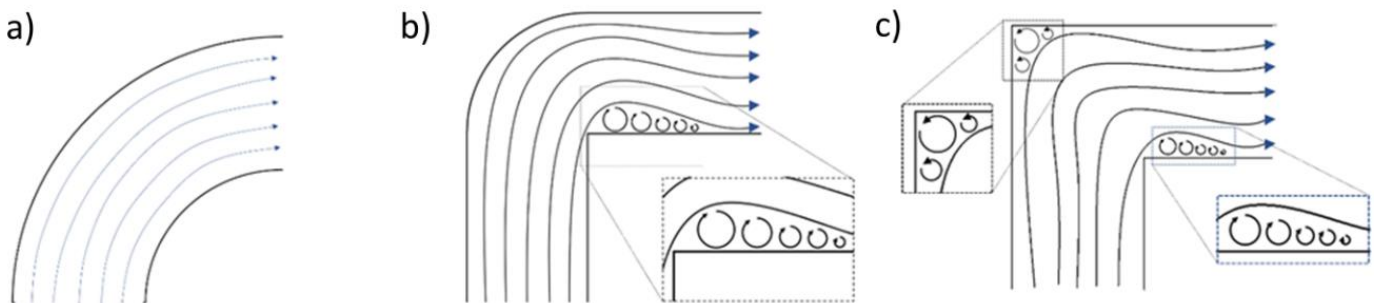


Figure 40 - Fluid flow responses to encountering various 90-degree bends.

Figure 40-a displays the highly ordered, smooth laminar fluid movement around the gradual 90 ° change of flow direction. Formation of stagnant swirls are kept to a minimum, and the creation of unwanted friction is diminished.

When a single sharp corner is introduced on the innermost edge of the bend (Figure 40-b), a localised pocket of stationary fluid forms on the other side of the turn. These stagnant swirls are the result of a low-pressure zone created by complex fluidic effects. Intuitively, the effect may be

attributed to the stream of fluid close to the inner wall of the pipe in effect “overshooting” the sharp corner due to its momentum, creating a low-pressure pocket on the other side of the bend. The vortices form due to a difference in friction experienced by this stationary pocket; the pocket experiences more friction from the pipe wall than from the layer of fluid above it, causing it to ‘roll’ in a clockwise fashion.

Introducing a second sharp corner (Figure 40-c) creates a second vortex area. Unlike the swirls formed on the inside bend of the pipe, the vortex area on the outside of the bend is an area of high pressure. Once again this can be visualised as being caused by the moving fluid in effect ‘slamming’ into the wall of the 90° bend as a result of its momentum. This creates a pocket of stationary fluid in the corner of the bend. Once again, the friction experienced by the pocket causes it to spiral; in this case, the vortices rotate counterclockwise.

These stagnation effects are seen not only with full 90° angles. Any angle upwards of 0° is likely to create this effect.

3.3.6 - Relevance of Fluid Analysis to Flow

Describing and predicting the behaviour of fluids is especially important in the design of mixing chips. By incorporating, for example, a series of zig-zags, bends, and friction-heavy surfaces in the flow line of the mixing chips, rapid maximal mixing of reagents can be achieved. Figure 41 illustrates a top-down hypothetical design for a mixing chip of a large volume. Several perks and flaws of the design are explained in terms of previously discussed fluid dynamics.

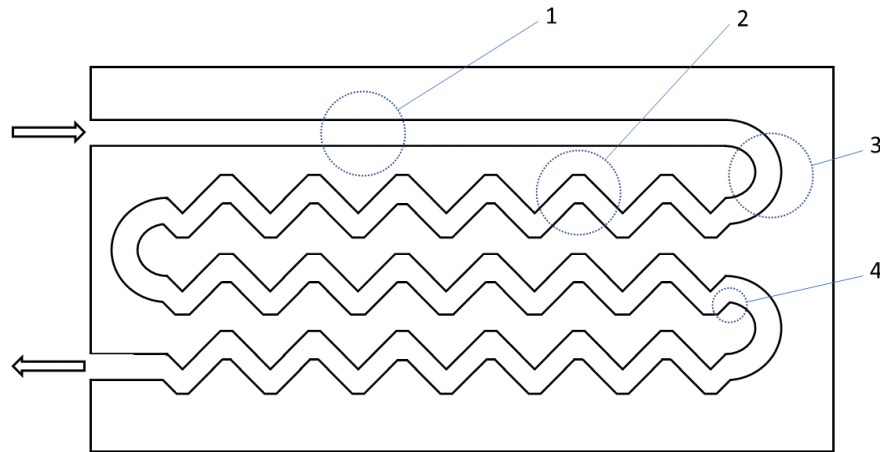


Figure 41 - Hypothetical Mixing Chip Design

Area 1 denotes a section of straight flow path. Depending on flowrate, the liquid in this flow path displays either laminar flow (minimal mixing) or turbulent flow (maximal mixing). Additional passive mixing can be achieved by having a roughened surface on the inside of the channel (at the cost of greater friction and therefore a higher pressure-drop across the chip).

Area 2 features a series of zig-zag bends, superimposing secondary fluid folding on top of the prevailing main flow regime (either laminar or turbulent). Notice that the bends are not fully rounded; this can reduce manufacturing cost at the expense of a higher backpressure. Stagnation effects produced by these low-angle edges may or may not lead to reduced mixing, but will likely increase dispersion and result in a larger pressure drop penalty.

Area 3 indicates a smooth 180° bend reversing the direction of flow towards the left side of the chip. Secondary flow effects dominate in this region, folding and mixing the fluid as it passes through. Depending on the flowrate, additional mixing may be provided by primary turbulent flow. This type of bend introduces a large amount of mixing with minimal friction, minimising the pressure drop between the start of the bend and the bend's exit.

Area 4 shows a sharp corner leading into another rounded 180° bend. This sharp edge may result in reduced mixing inside the bend, as it will likely reduce the overall speed of the fluid entering the turn, limiting the effects of secondary mixing. Also, the formation of stagnation zones post-edge may trap fluid in a vortex, reducing mixing and leading to increased dispersion. However,

due to the complexities of fluidic interaction with the surrounding chip structure, the combination of these effects might in fact lead to increased mixing.

3.3.7 - Computational Fluid Dynamics

This intuitive description of this hypothetical mixing chip is based on hydrodynamic first principles but, in reality, falls far short of an accurate analysis. Specific sections of the chip and their global contribution to the mixing effects of the device can be sufficiently described using basic fluid dynamics. However, due to the complex interplay and randomness of fluids in various scenarios, simple mathematic analysis falls short in describing the behaviour of liquids and gases in varying environments to a greater degree of accuracy. Fluidic systems are more commonly described using computational methods, making use of Computational Fluid Dynamics Software, in conjunction with experimental confirmation of the results. This process usually proceeds as follows:

- i. Pre-processing, where a theoretical fluidic system is transformed into a discreet, ideal computer model. Analysts make several assumptions based on their desired computational approach, such as the flow type, compressibility of the fluid, and the fluid's viscosity (among others).
- ii. Solving, where powerful algorithms are employed to model the flow system over time. Depending on the number of flow factors in the simulation, calculations may take days or even weeks.
- iii. Post-processing, where analysts screen and visualise the results.
- iv. Confirmation of findings in a physical, experimental environment.

A host of commercial fluid analysis software packages exist, some tailored to specific fluidic problems (for example, some may focus on liquid analysis as opposed to gaseous analysis). Some of the software available include Autodesk CFD, SimScale, and Ansys. Open-source software is also available, such as OpenFoam.⁷⁴

The various aspects of flow chemistry discussed in this chapter allow the flow chemist to design, tweak, and commercialize a wide variety of syntheses should they prove viable for a flow-based

synthesis. Knowledge of the inner workings of mobile fluids provides insight into the various complexities of a continuous chemical synthesis, and paves the way to manufacturing essential medicines in a more reliable, more affordable, and more environmentally friendly manner.

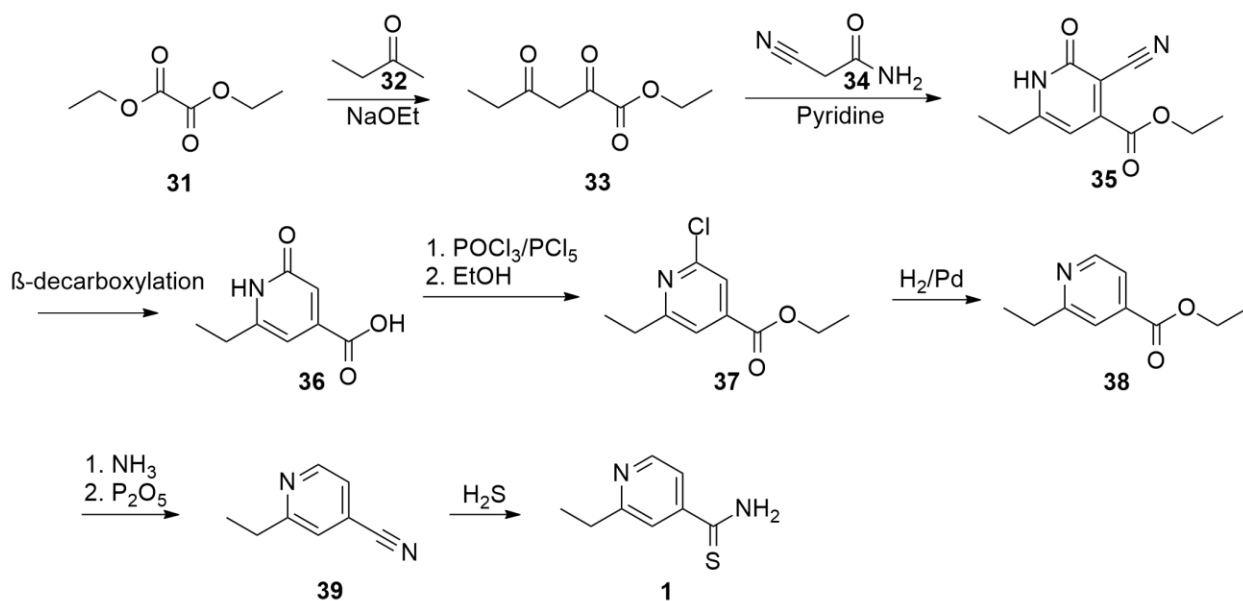
Chapter 4 - Results and Discussion

As stated before, the aim of this project was to translate the synthesis of several commonly used TB antibiotics onto a flow chemistry platform, both as proof of concept and to demonstrate the practical applications of manufacturing important medicines that are relevant to solving local shortages of vital treatment drugs. The synthesis of these drugs was performed in batch as well as in flow to compare the results obtained. Optimization of the flow processes was of utmost importance. The drugs that were synthesized were Ethionamide, Ethambutol, and Isoniazid.

4.1 – *Ethionamide*

A TB drug from the fifth group (Table 3), ethionamide (ETH) was first introduced in 1965 as a treatment for TB and leprosy (although it is not often used for the latter).⁷⁵ Ethionamide functions as a prodrug, and is converted to prothionamide by a mono-oxidase as soon as it enters the cell. Prothionamide then binds to nicotinamide adenine dinucleotide (NAD⁺) before inhibiting a 2-trans-enoyl-acyl carrier protein reductase called InhA. Ethionamide produces similar side-effects compared to other TB drugs, but is associated with a higher risk of hepatotoxic complications.⁵³

Ethionamide has a lengthy synthesis consisting of seven steps, as outlined in Scheme 1.⁷⁶



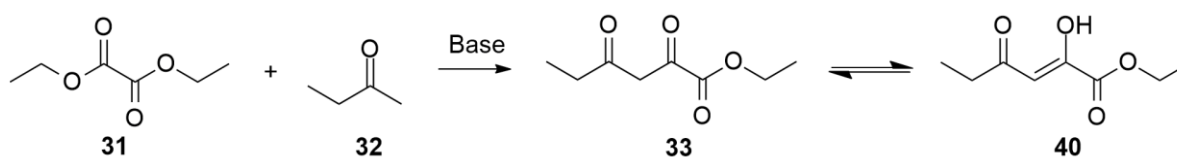
Scheme 1 - Common Synthesis of Ethionamide (**1**) (all steps employ ethanol as solvent).

The first step of the synthesis is an aldol condensation between diethyl oxalate (**31**) and 2-butanone (**32**) with sodium ethoxide as base to deliver ethyl 2,4-dioxohexanoate (**33**). Cyclization is achieved via reaction between 2-cyanoacetamide (**34**) and (**33**) in the presence of pyridine to form the necessary ring structure (**35**). Hydrolysis of **35** with hydrochloric acid, is followed by chlorination of **36** with either phosphoryl chloride or phosphorous pentachloride, and then re-esterification with ethanol to afford ethyl 2-chloro-6-ethylisonicotinate (**37**). The material is then passed over palladium in the presence of hydrogen, producing 2-ethylisonicotinic acid (**38**). Aminolysis of this compound using ammonia, followed by a dehydration reaction with phosphorous pentoxide to form the necessary cyano group, yields 2-ethylisonicotinonitrile (**39**). Thionation with hydrogen sulfide or Lawesson's reagent afforded the target molecule, ethionamide (**1**).

The synthesis of ethionamide in batch and flow is described in the next section.

4.1.1 - Condensation of diethyl oxalate (**31**) and 2-butanone (**32**) to produce (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate (**40**)

Scheme 2 illustrates the proposed synthesis of (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate. Starting with the batch aldol condensation between diethyl oxalate (**31**) and 2-butanone (**32**), an ethanol-sodium ethoxide solvent-base system was considered for reasons of base-reagent compatibility. Alkoxide bases are strong nucleophiles and would inevitably lead to the base attacking the carbonyl centre of **31** instead of abstraction of the α -proton of **32**, leading to the formation of side-products and the depletion of base in the reaction mixture. The combination of sodium ethoxide as base, ethanol as solvent, and diethyl oxalate as electrophile ensures that any nucleophilic addition of either sodium ethoxide or deprotonated solvent to diethyl oxalate would simply regenerate the starting material.



Scheme 2 - Synthesis of (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate (**40**)

The reactions were conducted under various conditions as outlined in Section 4.1.1.1 (a) – (c).

4.1.1.1 - Results

a) 2-Butanone (**32**) added to solution of diethyl oxalate (**31**) and sodium ethoxide

The rationale behind this method was to introduce **32** into a solution where there is an abundance of base to deprotonate the α -carbon, after which it can immediately encounter a molecule of the excess oxalate **31**. This ensures that a molecule of deprotonated **31** is less likely to react with another molecule of **32** in a side reaction, due to the low concentration of the unreacted ketone being present in the reaction mixture at any given time. The reaction was quenched with HCl, and after distillation, product **33** was obtained in a maximum yield of 18%.

b) Diethyl oxalate (**31**) added to a solution of 2-butanone (**32**) and sodium ethoxide

The rationale behind this method was to introduce small amounts of **31** to a stirred solution of already deprotonated **32**, allowing for immediate contact between **31** and a molecule of ketone. It was hoped that the deprotonated excess ketone would not self-condense in solution before the reaction was driven to completion. The reaction was quenched with HCl, and after distillation, a maximum yield of only 6% was obtained.

c) Diethyl oxalate (**31**) and 2-butanone (**32**) added to sodium ethoxide

This approach was intended to be a compromise between the perceived benefits of the aforementioned two methods. As soon as a drop of 1:1 oxalate:butanone enters the sodium ethoxide solution, the excess base can deprotonate **32**, after which it is in immediate contact with a molecule of diethyl oxalate (**31**). It was theorized that the drop disperses into the reaction solution slowly enough for the bulk of it to react. A yield of 39% was obtained after distillation.

Step one of the synthesis of ethionamide was attempted using flow chemistry. Two stock solutions were prepared: one containing both butanone (**32**) and diethyl oxalate (**31**), and a second containing an *in-situ* prepared solution of sodium ethoxide. The two stock solutions are simply combined at a T-piece before entering a reaction coil. The setup is displayed in Figure 42.

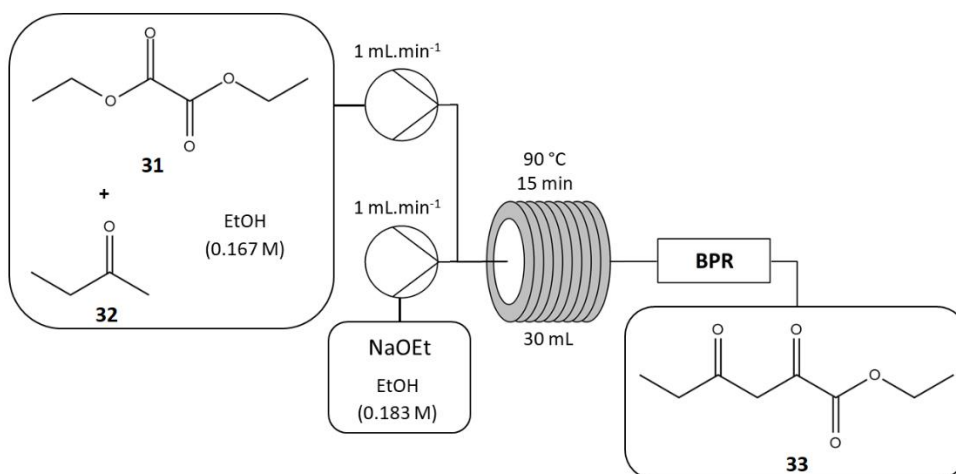


Figure 42 - Ethionamide Flow Step 1 Setup

As the two reagents and the base meet, however, the short length of tubing between the T-piece and the entrance to the reaction column invariably blocks before the reaction can be completed. A white precipitate forms slowly over time, likely traces of the reaction salt. Once the reaction solution enters the heated reaction coil, no precipitation occurs at any point downstream. Two modifications were therefore attempted to eliminate this blockage, both involving heating the T-piece. Firstly, the T-piece was simply suspended in a bath of boiling water in an effort to create a similar heated environment to that of the coil. This offered only marginal improvement, allowing the reaction to proceed a bit further before once again blocking the T-piece. The water bath was replaced by a sonication bath, to both heat the T-piece and attempt to break up any precipitate with mechanical force. This allowed the reaction to proceed yet further still, but inevitably resulted in blockage. Also, sonicating the T-piece for prolonged periods of time can damage the delicate threads of the tube heads such that they start to leak.

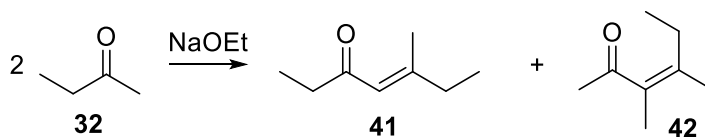
4.1.1.2 - Discussion

The method where 2-butanone (**32**) is added to a solution of diethyl oxalate (**31**) and sodium ethoxide delivered the second highest yield of the three reaction attempts. Significant amounts of unreacted **31** are recovered during workup, suggesting that not enough of **32** is deprotonated to drive the reaction to completion. It may be possible that sodium ethoxide is somehow lost during the time of addition. Theoretically, a reaction between sodium ethoxide and **31** should simply regenerate both the base and the oxalate. It is not known for sure, however, if another molecule of ethoxide is produced after reacting with diethyl oxalate (**31**).

Adding **31** to a stirred solution of **32** and sodium ethoxide delivered the lowest yield of the three methods. The failure of this approach is most likely due to self-condensation between molecules of 2-butanone (Scheme 3). The reaction crude mixture features a complex assortment of products, resulting in a difficult workup and messy distillation. Usually, the crude reaction mixture is a thick tar with elements that fail to dissolve during funnel separation.

The most successful approach was to slowly add the two starting materials simultaneously in one solution to the prepared solution of base. This suggests that both **31** and **32** interact with sodium ethoxide in an undesirable manner that leads to a host of side-reactions and loss of product. 2-

Butanone (**32**) in isolation with sodium ethoxide likely results in self-condensation to afford the self-condensation products **41** and **42**, leading to loss of yield mainly through side-product formation. Diethyl oxalate (**31**) in isolation with sodium ethoxide seems to lead to a loss of available ethoxide in solution, through a mechanism that does not destroy **31** (as most of it is recovered upon workup).



Scheme 3 - Self-condensation of 2-butanone

It is not clear what kind of effect the distillation process has on the amount and types of products isolated. There seems to be an indication that excessive distillation times result in a more complex product profile, and indeed, one particular product that stands out is the carboxylate of the diketone **33**.

The ester group is removed in the third step of the planned total synthesis but hinders the preceding ring formation. This might suggest that the ester group plays a role in the ring-formation mechanism, or possibly serves as a protective group to block nucleophiles. Also, isolation of this by-product is difficult, as it distils at a high temperature and readily cools and solidifies before reaching the collection bulb.

It became apparent early on that the reaction was indeed taking place, but through NMR analysis it became clear that the desired product **33** was being obtained only in its enolized form **40** (Figure 43).

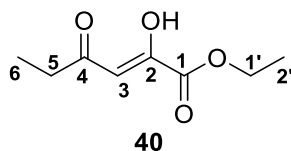


Figure 43 - Enolized product NMR designations

The obtained product **40** was analysed by both ¹H and ¹³C spectroscopy (spectrum A1 and A2, respectively). The molecule integrated for eleven protons and the expected eight carbon

resonances were observed in the ^{13}C spectrum. Close inspection of the proton spectrum revealed the presence of two endmost ethyl groups represented by two triplets integrating for three protons each at 1.19 ppm (H-6) and 1.39 ppm (H-2'), respectively, and two quartets at δ_{H} 2.55 (H-5) and 4.37 (H-1'), respectively. These two sets of resonances were distinguished based on the fact that the signal of H-1' (the ester group) shifted downfield as it is located on an oxygenated carbon. The corresponding triplet (H-2') was also deshielded relative to its non-oxygenated ethyl group counterpart H-6. Finally, a singlet denoting vinylic proton H-3 is observed at δ_{H} 6.39, and a second singlet corresponding to the hydroxyl group at δ_{H} 7.29, confirming that the product is predominantly obtained in the enolate form. Notable is the presence of a very weak singlet resonance at 3.94 ppm, attributed to the diketone form where H-3 would otherwise integrate for two protons. Thus, according to NMR spectroscopy, both forms are present in any given sample. The two peaks always exist in a fixed ratio of about 1:35 (keto:enol) on ^1H NMR spectra, suggesting that the enolate and the diketone settle into an equilibrium state, with the enolized form present in an overwhelming excess. Intuitively, one could theorize that the enolized form is the preferred product due to its capacity to form a potential hydrogen bond as shown in Figure 44. Attempts to manipulate the product ratio by adjusting the pH of the product solution were unsuccessful. These observations are echoed in literature.⁷⁷

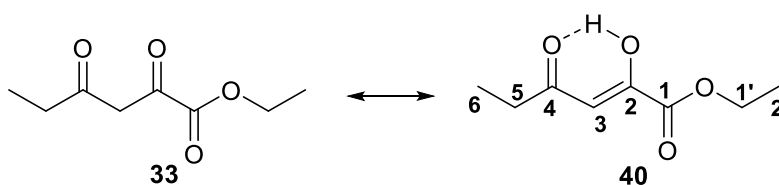


Figure 44 - Keto-enol tautomers of **33** and **40** with possible hydrogen bond formation in the enol form

Salient in the ^{13}C NMR spectrum was the presence of two carbonyl resonances at δ_{C} 204.2 and 165.9 corresponding to the ketone (C-4) and ester (C-1), respectively. The enolate carbon C-2 resonates at 162.2 ppm and the vinylic carbon C-3 at 101.3. The two methylene carbons are observed at δ_{C} 62.5 (oxygenated C-1') and 34.2 (C-5), respectively, and the two methyl groups at δ_{C} 13.8 and 8.6, respectively.

4.1.1.3 - Conclusion

NMR analysis indicated that the target molecule **33** was indeed obtained, but predominantly in its enol form **40**. Literature sources suggest that this is the rule for molecules of this general structure.⁷⁷ It was shown that the flow synthesis of **40** was unsuccessful due to the formation of precipitates in the reaction line.

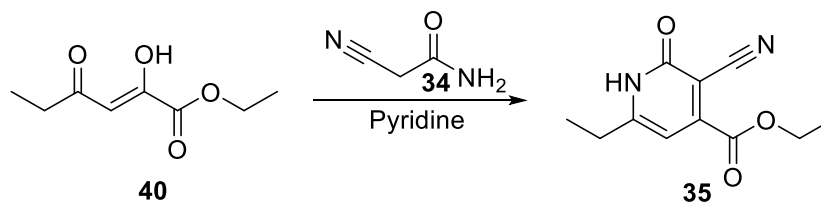
4.1.1.4 - Future Work

Further refinement of the batch procedure will be undertaken to increase the yield, focusing on the method whereby the two starting materials are added as one to the prepared solution of sodium ethoxide. Experiments will be performed with the goal of decreasing the time of reaction, as longer reaction times lead to an increase in undesired by-products. An optimised balance of temperature, rate of reagent addition, and equivalents of base in solution will deliver a cleaner reaction crude mixture and increased yield.

The flow synthesis of **33** and **40** will be revisited, focusing on eliminating blockages upon reagent combination. Larger diameter tubes might be more forgiving of precipitation formed in this way. Additionally, identification of the substance responsible for the blockages may shed light on how and why the precipitate forms, and why this precipitate does not appear once the combined reaction solution enters the heated coil reactor.

4.1.2 – Synthesis of ethyl 3-cyano-6-ethyl-2-oxo-1,2-dihydropyridine-4-carboxylate (**35**) via cyclization through condensation of (**40**) and cyanoacetamide (**34**)

The ring formation step was attempted despite not having the correct tautomer of the starting material (Scheme 4).



Scheme 4 - ethyl 3-cyano-6-ethyl-2-oxo-1,2-dihydropyridine-4-carboxylate (**35**)

4.1.2.1 – Batch Synthesis Results

The ring closure reaction between cyano acetamide (**34**) and **40** was first attempted using the acidic catalyst *p*-TsOH according to the method of Chen and co-workers⁷⁸ in toluene as solvent. A Dean-Stark trap was used to remove water released during the reaction and served to indicate that the reaction was indeed taking place. Unfortunately, this method did not deliver any product, and returned only starting materials and catalyst. Water was then considered as solvent, allowable since it is released during the reaction in any case and a basic catalyst was selected.

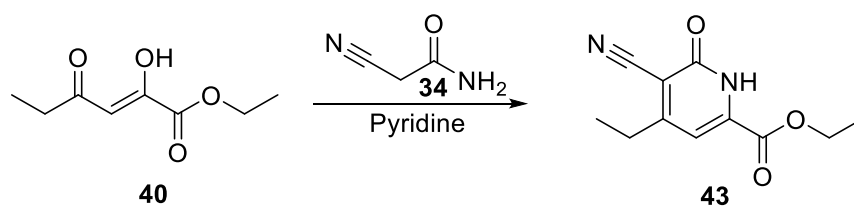
For the first base catalysed ring closure attempts, pyridine was used, serving as both a base and solvent. The benefits of pyridine were deemed to outweigh its hazards (which include its carcinogenic properties and its ability to damage fertility in mammals).⁷⁹ However, all reactions proved unsuccessful, as the reaction crude products obtained were tar-like in nature and could not be purified for analysis. Reasons for the failure are unclear, but are likely related to overreaction of reagents due to the abundance of base in the system. If product **35** is indeed formed during the reaction, it is likely consumed in side-reactions or degraded.

Consequently, triethylamine was used solely as a base, with ethanol as solvent. The reaction sets were performed under reflux and quenched using hydrochloric acid. The results are tabulated in Table 6.

Table 6 - Batch results for triethylamine-catalysed ring-formation

Condition Set	Cyanoacetamide equiv.	Base equiv.	Time	Yield % (35)
1	1	0.5	3 h	6
2	1	1	3 h	14
3	1	1.5	3 h	15
4	1	0.5	6 h	5
5	1	1	6 h	17
6	1	1.5	6 h	19
7	2	0.5	3 h	9
8	2	1	3 h	24
9	2	1.5	3 h	25
10	2	0.5	6 h	11
11	2	1	6 h	23
12	2	1.5	6 h	22
13	3	0.5	3 h	12
14	3	1	3 h	36
15	3	1.5	3 h	33
16	3	0.5	6 h	10
17	3	1	6 h	31
18	3	1.5	6 h	28

However, due to the use of the enolate **40** instead of the diketone **33** the product was identified as the geometric isomer of **35**, compound **43** (Scheme 5).

Scheme 5 - Geometric isomer of **35** formed during ring formation

The batch reaction condition set 14, using 1 equivalent of **40**, 3 equivalents **34**, and 1 equivalent Et₃N refluxed for three hours delivered the highest yield of compound **43** at 36%.

4.1.2.2 – Flow Synthesis Results

After optimising the batch reaction using 1 equivalent of base and 3 equivalents **34**, it was decided to translate this reaction onto a flow chemistry platform using the same equivalents. The final flow setup consisted of one stock solution (containing both **34** and **40**) first being fed through a 1 mL mixing chip heated to 60 °C before mixing with the triethylamine stock at a T-piece. The flow line then runs into a heated 20 mL coil, after which the crude product is quenched as it exits. However, solubility issues limited the amount of **34** that could be dissolved in the dual **34/40** stock solution, and therefore its mol contribution had to be reduced to 1.5 equivalents, and necessitated the addition of a small amount of water to fully dissolve it. The flow setup is pictured in Figure 45.

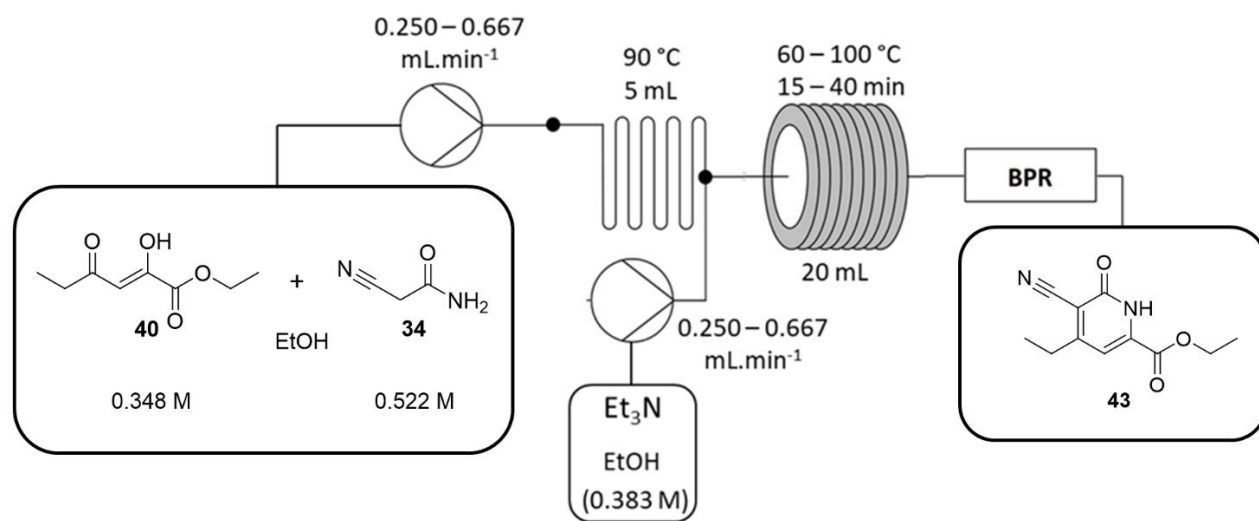


Figure 45 - Flow setup for the synthesis of **43**

Only the reaction time and temperature were varied to optimise the flow reaction. The results of these runs are tabulated in Table 7.

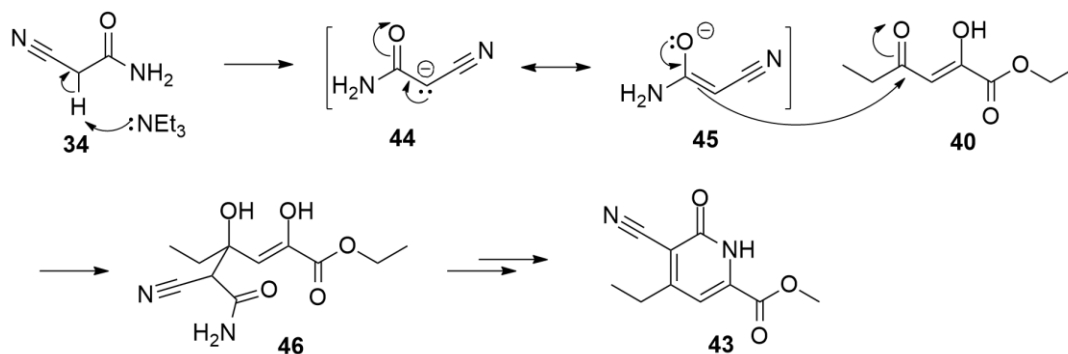
Table 7 - Flow results for triethylamine-catalysed ring-formation

Condition Set	Temperature (°C)	Residence Time (min)	Yield % (43)
1	60	15	13
2	60	20	15
3	60	25	14
4	60	30	20
5	60	35	22
6	60	40	23
7	70	15	15
8	70	20	13
9	70	25	17
10	70	30	19
11	70	35	23
12	70	40	26
13	80	15	25
14	80	20	24
15	80	25	37
16	80	30	43
17	80	35	56
18	80	40	55
19	90	20	35
20	90	25	37
21	90	30	39
22	90	35	46
23	100	30	31
24	100	35	35
25	100	40	38

The yield of **43** plateaued at 56%, using a residence time of 35 minutes at 80 °C. As the temperature was further increased, yields dropped off rapidly at similar residence times.

4.1.2.3 - Discussion

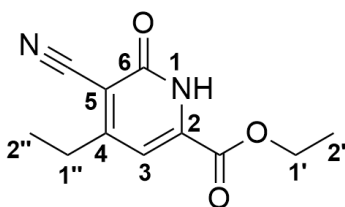
The formation of product **43** from reagents **34** and **40** probably forms via attack of deprotonated C-2 of transition state **44/45** at the electrophilic centre C-4 of enolate **40**, giving rise to intermediate **46** (Scheme 6). The subsequent ring closure afforded product **43**.



Scheme 6 - Proposed mechanism of the formation of product (43)

For the batch portion of this reaction, condition set 14 delivered the highest yield at 36%, using 3 equivalents of cyanoacetamide (**34**) and 1 equivalent of triethylamine. The results suggest that the reaction proceeds non-catalytically, as base equivalents below those matching the limiting reagent **40** deliver noticeably lower yields. As the equivalents of compound **34** are raised, the reaction time starts to show a noticeable effect on the yields obtained. Reaction times above 3 h often resulted in lower yields and more complex reaction crude mixtures, presumably as a result of product degradation and side reactions. Excess triethylamine tends to result in reduced yields, especially at longer reaction times in conjunction with greater equivalents of **34**. One might conclude that one source of reduced yields is due to an interaction between the base and cyanoacetamide (**34**) that reduces the available triethylamine in solution. In other words, the main reaction suffers from a lack of base, leading to reduced yields. However, raising the equivalents of triethylamine above 1 does not lead to major changes in the obtained yields, and when it does, the yields tend to decrease rather than increase. This suggests that a higher amount of available base actually has a deleterious effect on the main reaction, likely as a result of the product decomposing through interactions with triethylamine. The fact that this trend holds true at longer reaction times featuring higher equivalents of base, supports this. Furthermore, the purification process of the more viscous, darker crude products afforded by these reactions

consistently yielded crystals of lower quality, even after several recrystallization attempts. The crystals obtained were also smaller. NMR analysis of these crystals show small traces of various impurities. The inconsistency of the peak ratios, for example, the peak intensity ratio between products **43** and **34** vary randomly, suggesting that it does not integrate into the crystal structure itself, but simply appears as a surface impurity.



ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate

Figure 46 - NMR designations for **43**

Structure **43** was elucidated via ^1H and ^{13}C NMR (spectra A3 and A4, respectively), and IR spectrometry (A5).

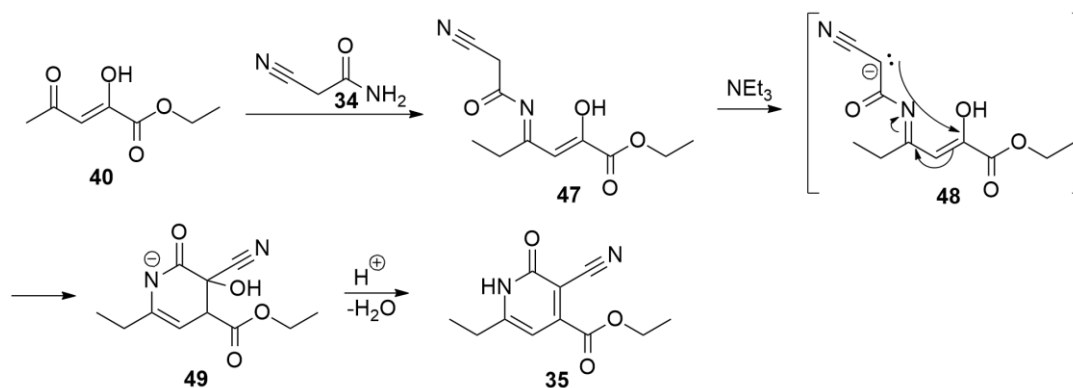
Close inspection of the ^1H NMR spectrum of **43** revealed the presence of the expected 11 protons. The signal splitting is quite similar to that of **33**, due to the fact that the backbone of **33** remains the only observable hydrogen-bearing moiety in product **43**. Salient is the appearance of characteristic resonances for two ethyl groups, as seen in the spectrum of precursor **33**. Thus, two triplets integrating for three protons each were observed at δ_{H} 1.37 and 1.45 ppm and were assigned to H-2'' and H-2', respectively. The triplet located more downfield is attributed to H-2' based on the fact that it forms part of an ester group. Two observed quartets integrating for two protons each at 2.81 ppm and 4.47 ppm were identified as H-1'' and H-1', respectively, with H-1' connected to the oxygenated C-1 and thus falling in the 2.5 – 5.0 ppm range. It is assumed the N-H proton is exchanged with deuterium. Lastly, a vinylic singlet is present at 6.68 ppm, integrating for a single proton and attributed to H-3.

The ^{13}C spectrum showed 11 resonances, including two methyl signals at 12.3 and 13.9 ppm, corresponding to C-2'' and C-2', two methylene resonances at δ_{C} 27.1 ppm (C-1'') and 63.3 ppm (C-1'), distinguished based on the fact that C-1' is oxygenated, and the vinylic C-3 resonance at 101.0 ppm. Of importance is the two carbonyl resonances at 162.8 and 163.9 ppm, corresponding

to the amide and ester carbonyl groups, respectively. The resonance at δ_c 158.1 ppm is assigned to the vinylic carbon C-2 as it is connected to the amide nitrogen atom and thus deshielded.

Regarding the IR-spectrum, the presence of the major functional groups was confirmed; the nitrile group is present at 2225 cm^{-1} , the ester moiety at 1715 cm^{-1} , the amidic carbonyl group at 1645 cm^{-1} , the aromatic C-H bonds at around 1371 cm^{-1} , and the methylene ester moiety at 1011 cm^{-1} .

A modification of the reaction procedure yielded an interesting result. Instead of concurrently introducing the three reagents (**34**, **40**, and triethylamine) into the reaction vessel, the weighed portions of **34** and **40** were instead vigorously refluxed for an hour before the base was slowly dropped in. It was hoped that **34** would first form an imine bridge at C-4 of **40** to yield **47**. Only then would C-2 on the bound molecule of cyanoacetamide (**34**) be deprotonated to yield intermediate **48**, and would be forced to react at the carbon centre C-2 of **48**, necessary for the formation of the correct cyclic geometric isomer **35**, as the other is protected through the imine bridge (Scheme 7).



*Scheme 7 - Modified reaction procedure to yield the correct product (**35**)*

After recrystallization, the obtained crystals were coloured a dark green instead of the usual light yellow, and crystallised as a radial snowflake-like solid instead of the longer, splintery crystals afforded by the unmodified reaction. However, NMR data for the two different products were identical. It is possible that they share the exact same spectra, but this is unlikely, thus the product once more is compound **43**. It is also possible that minute quantities of different contaminants are responsible for the green colouration. Further analysis through other analytical

methods can provide a more definitive answer. Regardless of the change of crystal shape and colour, this method delivered a similar maximum yield to the original method using the same reaction conditions, at 33%.

The flow translation of this reaction delivered only the green crystals. This is likely due to the heated mixing chip preceding the T-piece where the two stocks meet (Figure 47).

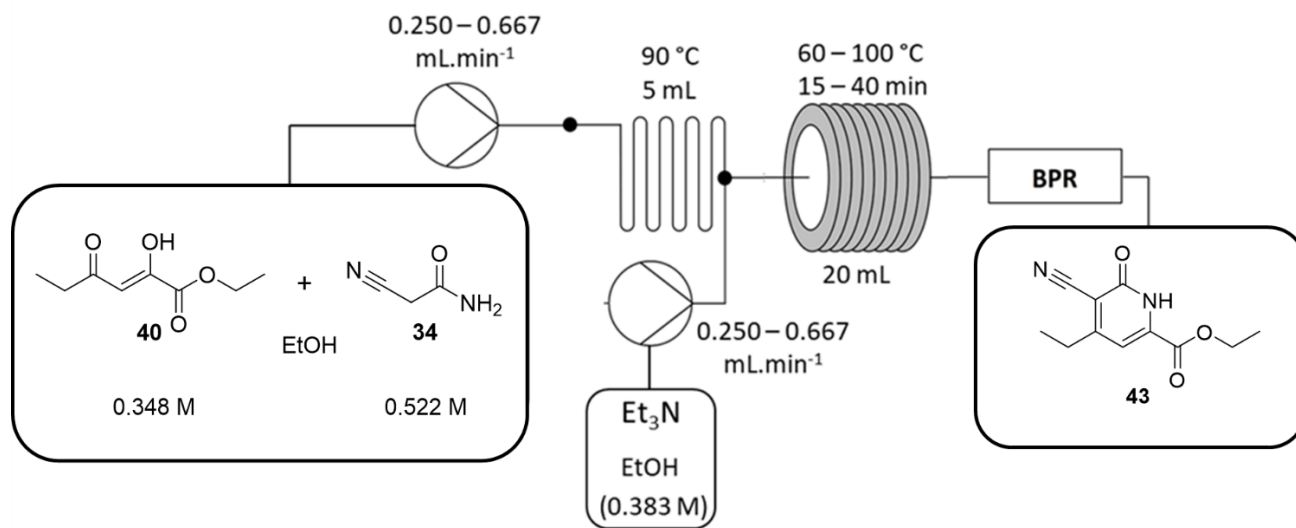


Figure 47– Mixing chip inserted to fully homogenise stock solution A containing **34** and **40**

The chip was introduced mainly for mixing purposes, with the heat aiding in keeping the cyanoacetamide fraction fully dissolved. This further supports the assumption that there is some form of interaction between **34** and **40** in the absence of the base that results in the green colouration.

The flow method delivered a far superior yield compared to the batch route, surpassing it by 20%. In addition, crude mixtures were noticeably cleaner and less tar-like compared to those of the batch method. This was especially true for samples obtained from residence times of 25 min or lower, at temperatures between 80–90 °C (for example, runs 15 and 19 as listed in Table 7). If so desired, cleaner, more manageable reaction crude mixtures can be prioritised at the cost of a higher yield. In certain scenarios, a cleaner crude product may be preferred if a flow step is to be linked directly into a second reaction step, where fewer interfering side-products may be a crucial factor in a successful telescoping.

4.1.2.4 - Conclusion

According to NMR analysis the target compound **35** was likely not synthesized. Due to the close similarity of their NMR spectra, further analysis using other analytical techniques (for example, mass spectrometry) may give a definitive answer. Further study of the reaction mechanism may also elucidate which mass isomer is obtained from this reaction, as well as methods of increasing the reaction yield.

4.1.2.5 - Future Work

Additional analytical techniques in the form of mass-spectrometry and crystallography will be employed to differentiate the two possible products. Further investigation of the imine-formation reaction route will also be done to elucidate the overall mechanism, as this will lead to a better understanding of the reaction process and where procedures can be tweaked to obtain the correct target molecule.

Further work on the total synthesis of ethionamide was halted due to time constraints, as it was judged that the sum of difficulties encountered would require considerably more work to overcome than initially thought. One major obstacle to be solved includes the precipitation issue in the first step of the planned flow synthesis in step 1. Additionally, and despite the higher yield achieved in flow for step 2 of the total synthesis, the amount of unreacted starting material present after this step is likely to negatively impact subsequent steps should a telescoped synthesis be attempted using flow chemistry.

4.2 – Ethambutol

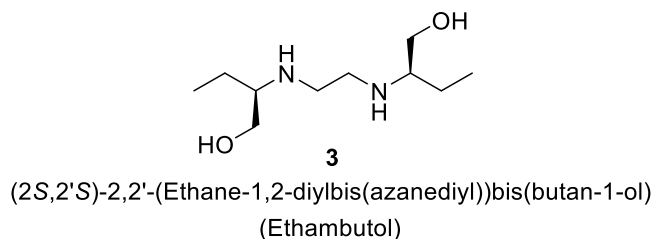
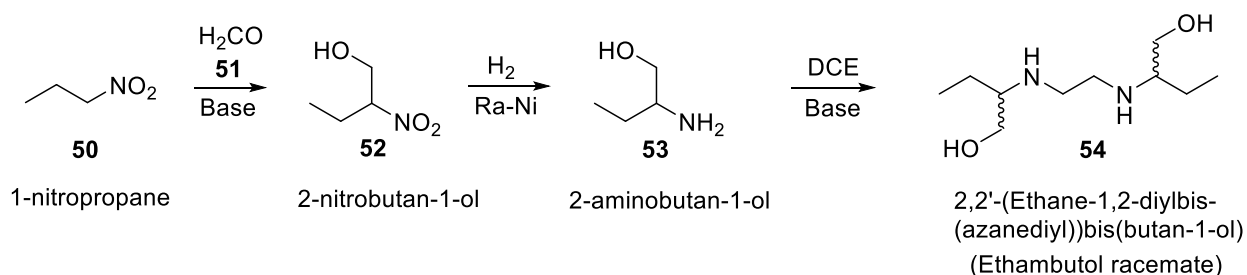


Figure 48 - Therapeutically active isomer of ethambutol (**3**)

Ethambutol (**3**) was first described in 1961, and demonstrated good bacteriostatic action against *M. tuberculosis* when it entered widespread use about five years later.^{76,80} Its complete mechanism of action is still disputed due to the complex pleiotropic cascade the drug sets in motion, making it unclear which intercellular mechanisms it targets to get the desired outcome. However, it is known that it inhibits the diffusion of mycolic acid into the cellular membrane of *M. tuberculosis*, once again interfering with the proper formation of the bacterial cell wall. More specifically, a recent study suggested that ethambutol inhibits three arabinosyltransferases linked to the production of both arabinogalactan and lipoarabinomannan, both vital to the formation of the cell wall.⁸⁰ By manipulating the level of expression of the genes coding for these enzymes, researchers were able to determine that a decrease in cellular concentration of these enzymes inevitably leads to stunted bacterial growth and colony dispersion. The reverse also held true for two of the enzymes under investigation, with overexpression of these molecules resulting in acquired immunity against ethambutol.

Ethambutol is commonly synthesized as a racemate (**54**) in a three-step procedure according to Scheme 8.



Scheme 8 - Common Synthesis of Ethambutol **54**

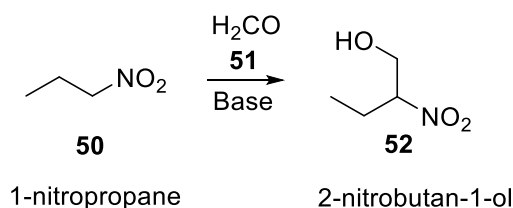
Firstly, in a Henry reaction, 1-nitropropane (**50**) is reacted with formaldehyde (**51**) under basic conditions to yield 2-nitrobutanol (**52**), followed by reduction over Raney-Nickel in the presence of hydrogen to deliver 2-aminobutanol (**53**). In the final step, two molecules of **53** are added to 1,2-DCE in a double nucleophilic displacement reaction under basic conditions to produce three stereoisomers of ethambutol (**54**).

Ethambutol was initially prescribed as a racemic mixture of the L-and D forms. Since only the dextro isomer provides a therapeutic benefit, every dose of racemic ethambutol carried with it

an amount of inactive levo isomer that does not treat the infection but still contributes to the medication's side-effect profile. It is therefore now prescribed only in its D-form. The synthesis envisioned in this project would thus need to incorporate a purification step of some form to separate the two isomers.

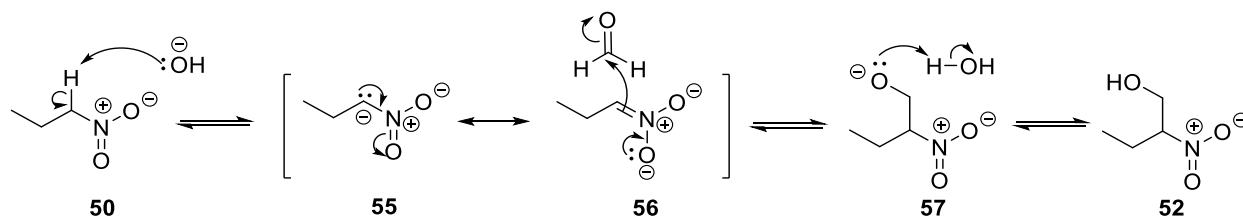
4.2.1 - Synthesis of 2-nitrobutan-1-ol (**52**) from 1-nitropropane (**50**) and formaldehyde (**51**)

In order to form the necessary C-C bond in the first step, the Louis Henry Carbon-Carbon bond formation reaction was proposed. The reaction is essentially a nitro aldol condensation reaction, employing an aliphatic nitro compound and an aldehyde or ketone (Scheme 9).⁸¹



Scheme 9 - Synthesis of 2-nitrobutanol-1-ol **52** via the Henry reaction

Due to formaldehyde (**51**) being both a gas and susceptible to spontaneous polymerisation (to form paraformaldehyde), its use in a batch synthesis poses several challenges. As a result, we decided to use a 37% (*m/m*) aqueous formalin solution to provide the carbonyl centre necessary for the reaction. Fortunately, water was always intended to be part of the solvent system for this reaction. Unfortunately, the unmodified Henry reaction suffers from poor enantioselectivity, leading to a mixture of enantiomers. Also, due to the absence of a committed step in the reaction, an equilibrium between the various precursors and the final product exists (Scheme 10).⁸²



Scheme 10 - Equilibrium Steps in the Henry Reaction

4.2.1.1 – Batch Results

The reaction was initially attempted using NaOH as base and a solvent system of 1:1 H₂O:Methanol was employed. The reactions were carried out using 1.2 equivalents of **51** and quenching with acetic acid. The results are tabulated below (Table 8).

Table 8 - Batch synthesis results of (**52**)

Condition Set	Base	Base equiv.	Temperature (°C)	Time	Yield % 52
1	NaOH	0.5	RT	5h	Trace
2	NaOH	1	RT	5 h	Trace
3	NaOH	1.5	RT	5 h	Trace
4	NaOH	2	RT	5 h	1
5	NaOH	0.5	RT	10 h	Trace
6	NaOH	1	RT	10 h	2
7	NaOH	1.5	RT	10 h	5
8	NaOH	2	RT	10 h	6
9	NaOH	0.5	Reflux (~70)	5 h	1
10	NaOH	1	Reflux (~70)	5 h	2
11	NaOH	1.5	Reflux (~70)	5 h	5
12	NaOH	2	Reflux (~70)	5 h	8
13	NaOH	0.5	Reflux (~70)	10 h	1
14	NaOH	1	Reflux (~70)	10 h	2
15	NaOH	1.5	Reflux (~70)	10 h	11
16	NaOH	2	Reflux (~70)	10 h	10

It is shown that increases in temperature leads to increases in yield. In terms of reaction time, increases in yield are modest with increased reaction time. This is due to the possibility that the reaction system reaches an equilibrium state where the final product and its precursors exist in a fixed ratio. A fundamentally different approach to the reaction might be to remove the product as it forms in order to drive the reaction forwards.

In lieu of this, a different base was considered, namely triethylamine. The results are tabulated below in Table 9. All experiments used formaldehyde in a 1.2 equivalent and reactions were quenched with aqueous HCl.

Table 9 - Batch synthesis results of **52** using triethylamine

Condition Set	Base equiv.	Temperature (°C)	Time	Yield % (52)
1	0.1	RT	2 h	29
2	0.5	RT	2 h	27
3	1	RT	2 h	5
4	0.1	Reflux	2 h	32
5	0.5	Reflux	2 h	26
6	1	Reflux	2 h	4
7	0.1	RT	5 h	23
8	0.5	RT	5 h	21
9	1	RT	5 h	10
10	0.1	Reflux	5 h	25
11	0.5	Reflux	5 h	19
12	1	Reflux	5 h	5

Condition set 4 delivered the highest yield at 32 % using 0.1 equivalents of base in a 2-hour reaction at reflux temperatures. It is clear the reaction favours more modest equivalents of base and shorter reaction times.

4.2.1.2 – Flow Results

Translation of these reactions to flow had no significant obstacles, as none of the batch reactions attempted showed signs of precipitation.

A 3-way combination flow setup was employed for the synthesis using NaOH (Figure 49).

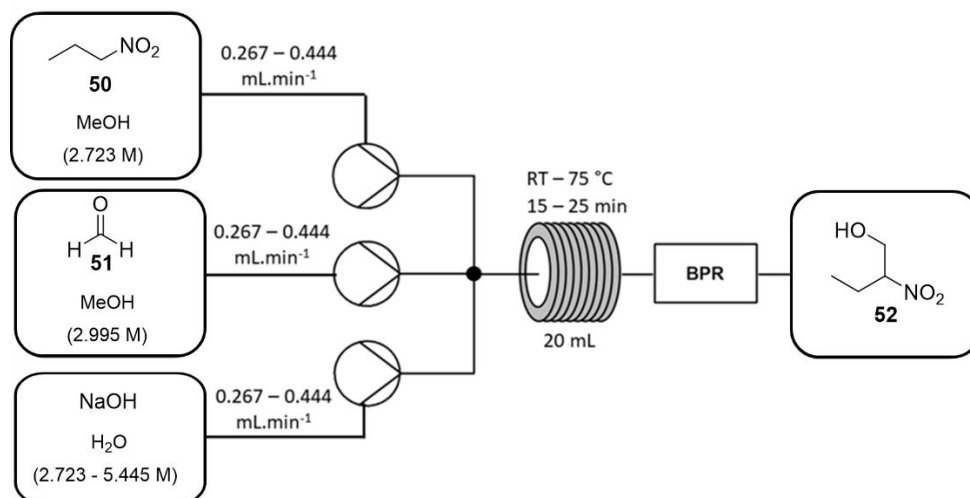


Figure 49 -3-Way flow setup for the synthesis of **52** using NaOH as base

Unfortunately, the yields obtained for the NaOH system were low and inconclusive. The results are tabulated below in Table 10. 1-Nitropropane and formaldehyde were used in fixed equivalents of 1 and 1.2, respectively. All reactions were quenched with HCl.

Table 10 - Flow synthesis results of **52** using NaOH

Condition Set	Base Concentration equiv.	Temperature (°C)	Residence Time (min)	Yield %
1	1	RT	15	5
2	2	RT	15	6
3	1	50	15	7
4	2	50	15	5
5	1	60	15	9
6	2	60	15	7
7	1	75	15	8
8	2	75	15	6
9	1	RT	25	5
10	2	RT	25	6
11	1	50	25	6
12	2	50	25	9
13	1	60	25	9
14	2	60	25	5
15	1	75	25	6

16	2	75	25	9
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As shown, no clear trend can be established from the obtained yields. NaOH as a base was therefore abandoned due to its lacklustre performance in both batch and flow, and Et₃N was employed for the remainder of the flow syntheses. The setup used is pictured in Figure 50.

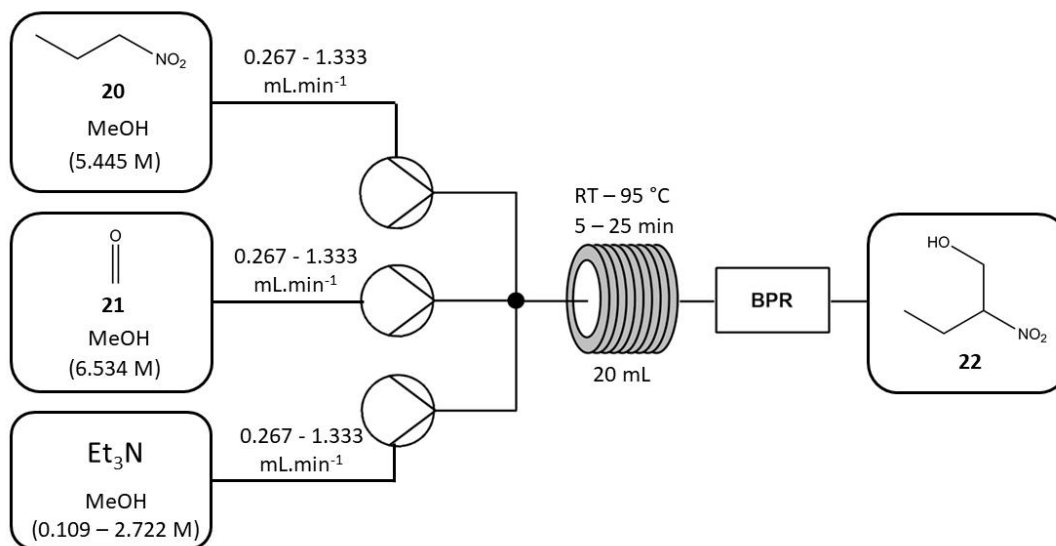


Figure 50 - 3-Way flow setup for the synthesis of **52** using Et₃N

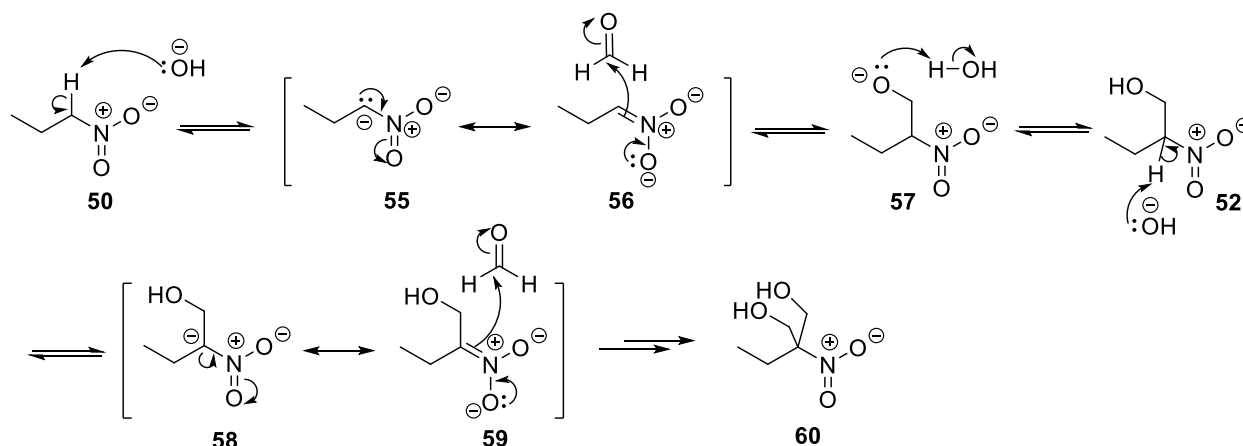
The results are tabulated in Table 11.

Table 11 - Flow synthesis results of **52** using Et₃N

Conditions	Base Concentration equiv.	Temperature (°C)	Residence Time (min)	Yield %
1	0.1	RT	15	5
2	0.5	RT	15	6
3	0.1	50	15	10
4	0.5	50	15	9
5	0.1	60	15	14
6	0.5	60	15	13
7	0.1	70	25	46
8	0.5	70	25	35
7	0.1	85	25	51
8	0.5	85	25	36

9	0.1	95	25	48
10	0.02	50	5	26
11	0.02	90	5	48
12	0.05	50	5	23
13	0.05	90	5	47
14	0.02	50	10	27
15	0.02	90	10	59
16	0.05	50	10	24
17	0.05	90	10	48

A flow synthesis at 90 °C with a residence time of 10 min and a 2% catalytic base solution delivered product at 59%, the highest yield of **52** of all the methods attempted. Furthermore, this method delivers the cleanest crude product and the fewest side-products. In fact, as the residence time and temperature of the reaction increases past this point, NMR data suggests that the Henry condensation happens twice on the same molecule. This is within the scope of the reaction mechanism, as nothing stops the catalytic cycle from occurring twice (Scheme 11).



Scheme 11 - Double Addition of Formaldehyde to Nitrobutane to yield 60

Once again, however, it is possible that the yield cannot increase past 60% due to the limitations imposed by the equilibrium of the reaction system. If an equilibrium does indeed exist, methods of shifting it towards increased product formation could be beneficial. Several modifications of

the standard Henry reaction were therefore explored, centred on the use of an alternative catalyst.

4.2.1.3 - Flow Experiments using Al-Mg Hydrotalcite^{83 84}

Hydrotalcite is a layered double-hydroxide mineral, generally of the formula $\text{Mg}_6\text{Al}_2\text{CO}_3(\text{OH})_{16}\cdot 4\text{H}_2\text{O}$. Hydrotalcites have found new applications in anion exchange, catalysis, and catalyst supports. These compounds consist of brucite-like octahedral layers, with part of the M^{2+} cations isomorphously substituted with M^{3+} cations. The excess positive charge resulting from this substitution is counteracted by interstitial layers of anionic CO_3^{2-} and crystalline H_2O . As a solid catalyst, hydrotalcite can be packed into a reaction column for use in flow synthesis. Once the solution leaves the column, the reaction is effectively quenched as the reagents are no longer in contact with the catalyst. If the reaction can be driven towards maximum yield while in contact with the catalyst, product reversal cannot take place once it has cleared the column, which should result in a higher final yield.

Initial attempts to use a packed hydrotalcite column were unsuccessful, as the system pressure would rise to levels where the pumps are automatically turned off. It was clear that the hydrotalcite was not allowing sufficient liquid to pass through the column at the desired flowrates. In fact, when wetted with either water or IPA, the hydrotalcite takes on a thick, clay-like form that is quite resistant to fluid flow. It was therefore decided to add another solid to the hydrotalcite as a dispersing agent to create microscopic channels through which the solvent could pass more easily. Three solids were considered, namely acid-washed sand, NaCl, and celite. Each bulking solid was used in the minimum amount necessary to facilitate fluid flow at the fastest flowrate used. Catalytic columns were repacked between each experiment.

a) Hydrotalcite-Sand

Hydrotalcite and sand were used in a ratio of 1:1.2 by mass. The addition of the sand indeed allowed for faster fluid flow, and no blockages were experienced. Sand-bulked hydrotalcite columns had internal volumes averaging around 6 mL, the smallest of the three methods. This is likely due to the large size of the sand particles which take up more space than, for example, diatomaceous earth particles. The flow setup used is pictured in Figure 51.

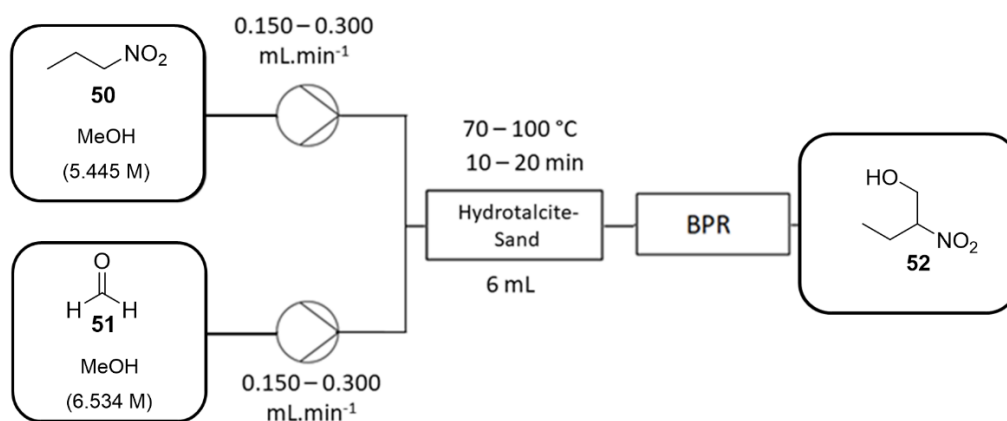


Figure 51 - Sand-bulked hydrotalcite column

1-Nitropropane (**50**) and formaldehyde (**51**) were used in fixed equivalents of 1 and 1.2, respectively. The results for the Hydrotalcite-Sand catalytic column system are shown in Table 12.

Table 12 - Flow synthesis results of **52** using hydrotalcite-sand catalyst

Condition Set	Temperature (°C)	Residence Time (min)	Yield %
1	70	10	3
2	80	10	2
3	90	10	4
4	100	10	6
5	70	15	3
6	80	15	8
7	90	15	8
8	100	15	11
9	70	20	13
10	80	20	14
11	90	20	20
12	100	20	19

b) NaCl used to create *in-situ* flow channels

By packing a column with a 1:2 mixture by mass of hydrotalcite and NaCl, hot water can be pumped through at very low flowrates to dissolve and remove the salt particles such that small catalytic channels form throughout, facilitating fluid flow through the column. Depending on the size and distribution of the NaCl particles, different sized channels can be formed. The concept is illustrated in Figure 52 (salt particle sizes exaggerated for visibility purposes).

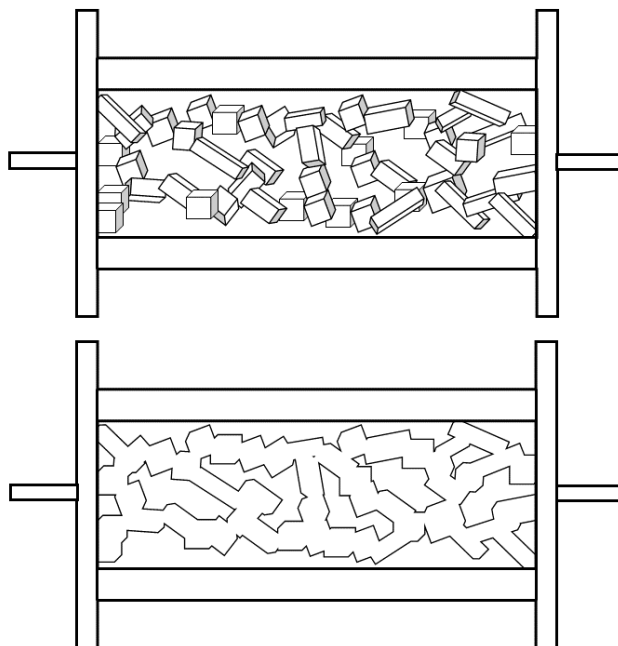


Figure 52 - Diagrammatic illustration of salt particles before H₂O wash (top) and channels formed after H₂O wash (bottom)

Channels must be as narrow as possible to maximise the available catalytic exposure, and so finely ground salt was used. Warm water is pumped through the prepared column at low flowrates to slowly remove the NaCl, presumably leaving empty flow channels in their place. The pumping is continued until salt is no longer eluted from the flow line. However, the prepared channels are fragile, as higher flowrates seem to collapse the tunnels. This is evident from the shortening of the packed solid inside of the column as time passes, also worsened by higher temperatures. The effect of temperature on the stability of the channels is not clear. This method of catalyst preparation delivered columns with the largest void volume of the three methods, averaging at 10.5 mL. The flow setup is pictured in Figure 53.

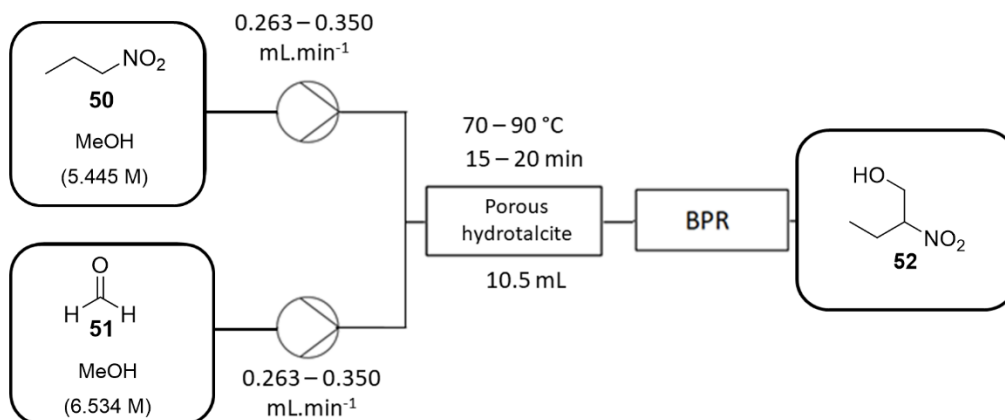


Figure 53 - Porous hydrotalcite column

Due to these limitations, only a handful of experiments could be completed before the prepared column collapsed, compressed the catalyst, and halted the flow of liquid through the solid hydrotalcite, despite the fact that the reactions for this method were done at half the scale of the other two methods. Only experiments with residence times of 15 min or higher, and reaction temperatures lower than 90 °C, were able to run to completion. 1-Nitropropane and formaldehyde were used in fixed equivalents of 1 and 1.2, respectively. The results are tabulated in Table 13.

Table 13 - Flow synthesis results of **52** using NaCl-formed channels

Condition Set	Temperature (°C)	Residence Time (min)	Yield %
1	70	15	25
2	80	15	39
3	70	20	20
4	80	20	23
5	90	20	28

c) Hydrotalcite-Celite

Hydrotalcite and Celite were used in a 1:1.5 mass ratio. No blockages at the necessary flowrates were experienced. Packed columns using hydrotalcite and celite had internal void volumes averaging 9.4 mL in size, larger than that determined for the hydrotalcite-sand column. The experimental setup is pictured in Figure 54.

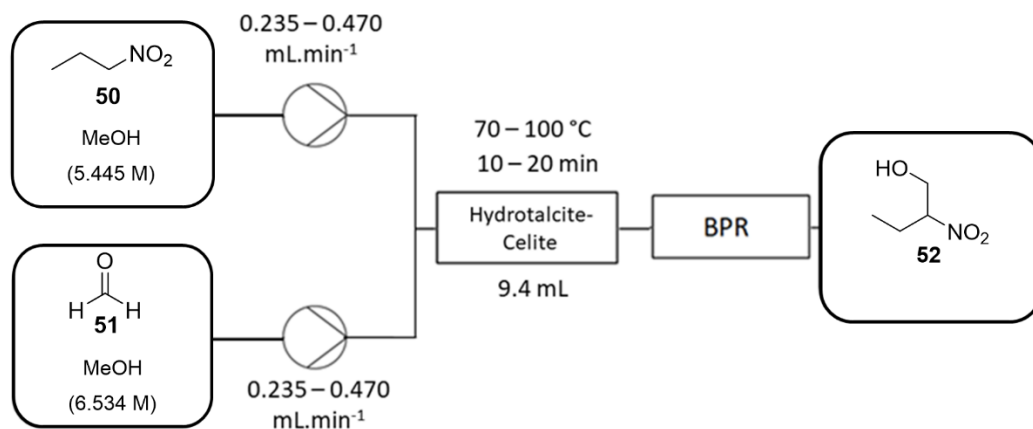


Figure 54 - Celite-bulked hydrotalcite column flow setup

The hydrotalcite-celite system contains a larger mass of bulking agent compared to the hydrotalcite-sand column, but delivers a column with a larger void volume (likely due to the smaller size of diatomaceous earth particles compared to grains of sand).

1-Nitropropane and formaldehyde were used in fixed equivalents of 1 and 1.2, respectively. The results are tabulated in Table 14.

Table 14 - Flow synthesis results of **52** using hydrotalcite-celite mixture

Condition Set	Temperature (°C)	Residence Time (min)	Yield %
1	70	10	13
2	80	10	14
3	90	10	16
4	100	10	19
5	70	15	42
6	80	15	45
7	90	15	44
8	100	15	47
9	70	20	41
10	80	20	43
11	90	20	47
12	100	20	51

4.2.1.4 - Discussion

The formation of product **52** was confirmed via NMR experiments (spectra A5 and A6) and IR spectrometry (spectrum A7).

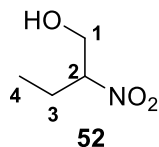


Figure 55 - 2-nitrobutanol NMR designations

A total of eight integrated protons are expected (assuming the -OH group interchanges for deuterium). In the ^1H NMR spectrum, a multiplet observed at δ_{H} 4.55 ppm corresponds to H-2, deshielded due to the resonance of the nitro group, leading to a partial positive charge on the nitrogen atom that shifts the H-2 signal downfield. The two doublet of doublets (dd) at δ_{H} 4.02 and 3.87 ppm respectively, correspond to the oxygenated H-1_a and b. The two multiplets at 1.57 - 2.04 ppm correspond to H-3_a and b, while the three-proton triplet observed at 1.00 ppm was assigned to H-4.

The presence of the alcohol and nitro moieties was confirmed in the IR spectrum, with one absorbance peak at 3389 cm^{-1} corresponding to the C-OH group, and two more at 1545 and 1367 cm^{-1} corresponding to the C-NO₂ group.

The expected four resonances were observed in the ^{13}C NMR spectrum. The downfield resonance observed at δ_{C} 91.0 ppm correlates to the deshielded nitro-coupled carbon. The oxygenated C-1 resonates at 62.9 ppm, and the remaining methylene and methyl carbons at δ_{C} 23.3 and 10.1 ppm, respectively.

It is clear that Et₃N acts as a better catalytic base than NaOH. The obtained data suggests that the NaOH reaction actually proceeds non-catalytically, suggesting that the base is either not being regenerated at the end of the catalytic cycle or that the base is being consumed by a side reaction. One possible route of by-product formation is by a Cannizzaro-type reaction, which would sacrifice two molecules of formaldehyde to produce an alcohol and carboxyl salt.⁸⁵ In this

particular case, methanol and sodium formate would be formed. As the yield tends to increase with increasing equivalents of NaOH, this might well be the case.

With regards to Et₃N, equivalents of base nearing or matching those of the limiting reagents results in tar-like crude products, which are difficult to distil and show increased by-product formation compared to those experiments using a 10% or less concentration of base. Temperature-wise, it was noted that prolonged reaction delivers both lower yields and unmanageable crude products. Still, it is clear that more modest reaction times with Et₃N deliver the highest yields of **52** in both batch and flow. However, it is still likely that the overall reaction eventually settles in an equilibrium as previously discussed. If the final product could be selectively complexed to remove it from the equilibrium, increased yields will likely be obtained.

For the hydrotalcite catalyst, the lowest, least consistent yields of **52** were obtained using the hydrotalcite-sand system. The highest yield obtained was 20% at a temperature of 90 °C with a residence time of 20 minutes. Despite the lacklustre yield, this method did, however, suffer the fewest blockages and experiments could be run as long as necessary without the pumps failing due to pressure spikes. It is not clear why this method, which had the largest ratio of hydrotalcite to bulking agent, delivered the least satisfactory yields. Despite having the largest theoretical total catalytic surface area, hydrodynamic factors might be to blame for the lacklustre performance. Figure 56 illustrates a hypothetical situation occurring in a hydrotalcite-sand column, with a line tracing the path of a microscopic unit of fluid through hydrotalcite and sand particles (sand particles exaggerated in size).

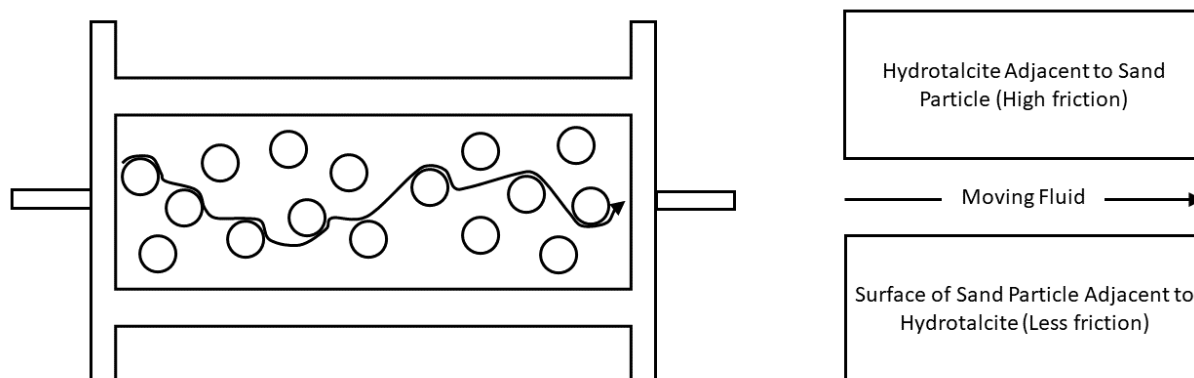


Figure 56 – Fluid moving through mixed catalyst, following the path of least resistance.

As shown, the fluid molecules tend to hug the large sand particles as they move through the column, which allows the moving phase to follow a path of lesser resistance as opposed to having to push through the dense, clay-like hydrotalcite. If the bulk of the reagents flow through the column in this manner, they are in effect being exposed to a reduced amount of catalyst. Additionally, if a large amount of fluid prefers to follow paths around the sand particles, a higher flowrate is in effect created in these areas compared to the fluid portions having to move through only hydrotalcite, reducing the residence times for these streams of reagents. This should result in greater dispersion compared to other methods.

Additionally, the acid-washed sand has a more neutral pH versus that of celite (which has a pH of about 10). It might be possible that the sand reduces the basicity of the catalyst in some way, leading to decreased catalytic activity.

As for the hydrotalcite-NaCl system, only a few experiments could be completed (as well as requiring smaller scale reaction). Despite this, the method showed promising results, affording a yield of 39% at a temperature of 80 °C with a residence time of 15 minutes.

It was, however, not proven if these channels were in fact being formed, and other analytical methods could shed light on this. It is likely that no channels spanning the entire column are formed. Many channels may be dead ends, but the method still seems to work to some degree. Even though a series of unconnected tunnels still offers a path of lower resistance to a volume of fluid moving through the column, the pressure exerted likely aids in collapsing and compressing the hydrotalcite matrix. Figure 57 proposes a hypothetical before-and-after illustration of columns collapsing.

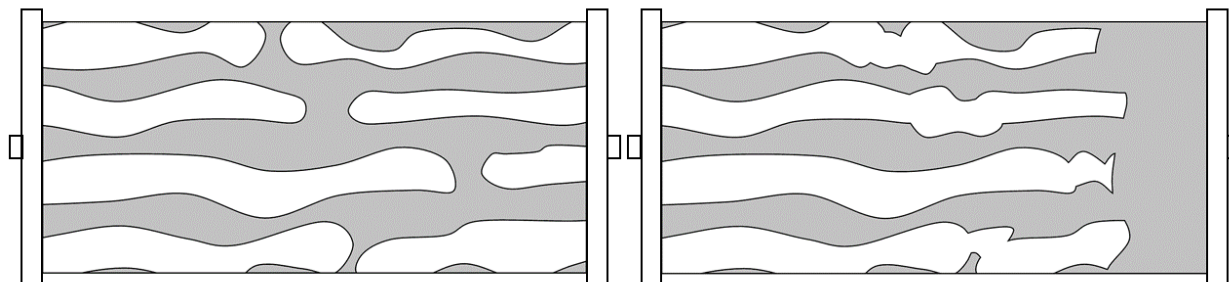


Figure 57 - Collapsing hydrotalcite channels

Pressure is exerted on the dead-end of a given channel as fluid flows from left to right through the column. If the flowrate is sufficiently high, a pressure difference forms between the hydrotalcite wall separating two channels, eventually causing it to break through. This, in conjunction with erosion of the channel walls caused by passing fluid, leads to the channels disintegrating and the hydrotalcite accumulating towards the column's end, leading to a compacted mass of catalyst. This eventually leads to a pressure build-up and termination of the pumps. Another downside of this type of packed column is the high level of dispersion, requiring that several column volumes of pushing solvent be sent through to collect all the tailing product.

The hydrotalcite-celite system proved to be the most stable, consistent, and highest yielding column of the three attempted. It achieved a peak yield at 51% with a residence time of 20 min at a temperature of 100 °C. Further increases in yield might be achieved with temperatures beyond this. System blockages did occur, but once a column had been flushed with pushing solvent and proven to be stable, a reaction was almost guaranteed to run to completion. Compared to the sand-bulked column, the catalyst mixture featuring hydrotalcite and celite was likely more homogenous in terms of material dispersion, as both feature particles of similar macroscopic size.

4.2.1.5 - Conclusion

Ethambutol intermediate **52** was synthesised successfully in both batch and flow processes. The flow synthesis using a Et_3N catalyst yielded the largest amount of product, at a yield of 59%, versus its batch counterpart, yielding only 32% of **52**. The alternative hydrotalcite system (specifically, that which uses diatomaceous earth as bulking agent) gave a maximum flow yield of 51%, quite similar to that of the Et_3N catalyst. The larger channels formed by the NaCl-method may be beneficial to higher throughput applications in scaled-up reactions, provided that the catalyst can be made sturdier for longer, hotter reactions. Introducing the porosity to the packed catalyst without the need of first flushing out a bulking agent would be preferable.

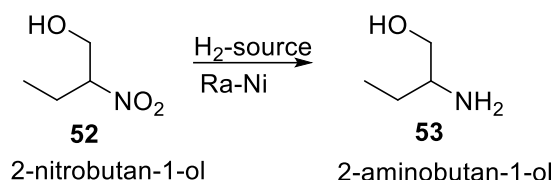
4.2.1.6 - Future Work

There is great room for improvement on the flow portion of this synthesis, particularly with regards to the hydrotalcite-catalyzed reaction. The solid catalyst is especially attractive due in

part to its ease of reusability. Even though the Et₃N system delivered the highest yield, the use of hydrotalcite presents additional perks in the form of easier catalyst reclamation. Residual Et₃N must be reclaimed by distillation after reactions, whereas hydrotalcite can simply be filtered out, reactivated under heating, and repacked into the catalytic column for further reactions. This ease of reusability makes it more attractive compared to Et₃N. Alternatively, solid-supported Et₃N can offer the same benefit with the possibility of higher yields.

4.2.2 - Synthesis of 2-aminobutan-1-ol (**53**) from 2-nitrobutan-1-ol (**52**) using Raney-Nickel

The second step in the synthesis of ethambutol involves a simple hydrogenation reaction to reduce 2-nitrobutanol to 2-aminobutanol, using a heterogeneous catalyst. The reaction proceeds as follows (Scheme 12):



*Scheme 12 - Synthesis of 2-aminobutan-1-ol **53***

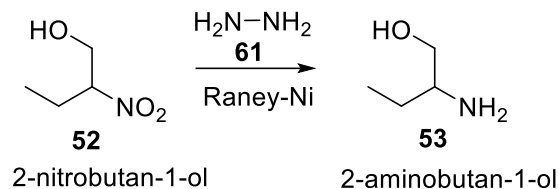
The catalyst chosen for this reaction was Raney-Nickel. Activated Raney catalyst consists largely of spongy nickel metal, featuring a large surface area for reactions to take place. Raney catalyst is prepared from a 1:1 mass ratio alloy of nickel and aluminium. During catalyst activation using heat and NaOH, most of the aluminium metal is leached from the solid to deliver a large surface area of nickel reagent capable of effectively catalysing hydrogenation reactions.

4.2.2.1 – Batch Synthesis Results

The batch attempt at this reaction used the solid Raney-nickel catalyst in a hydrogen-saturated reaction flask. The reaction is straightforward and delivers a maximum yield of 32% after distillation, with an optimum reaction time of 2.5 hours at 60 °C. Reaction times beyond this do not deliver additional product (despite additional hydrogen-loading mid-reaction), and catalytic effectiveness is limited to only a single reaction. Further batch optimisation is therefore needed.

4.2.2.2 – Flow Synthesis Results

For the flow translation of the reaction, the hydrogen source chosen was hydrazine monohydrate (**61**) (Scheme 13).



Scheme 13 - Flow synthesis of 2-aminobutan-1-ol **53**

Having a liquid source of hydrogen was deemed beneficial for continuous synthesis as introducing molecular hydrogen into a flow line requires specialised equipment. To minimize the toxic effects of clean hydrazine (which according to the MSDS include dermatitis, irritation to the eyes, nose, and throat, vomiting, dyspnea, pulmonary edema, headache, dizziness, central nervous system depression, lethargy, temporary blindness, seizures and coma), a solution of hydrazine monohydrate was used instead.⁸⁶ Figure 58 depicts the final flow setup used for the reaction, featuring a stock solution of amino alcohol **52** and a stock solution of hydrazine monohydrate **61** meeting at a T-piece before entering a packed catalytic column of Raney-Nickel.

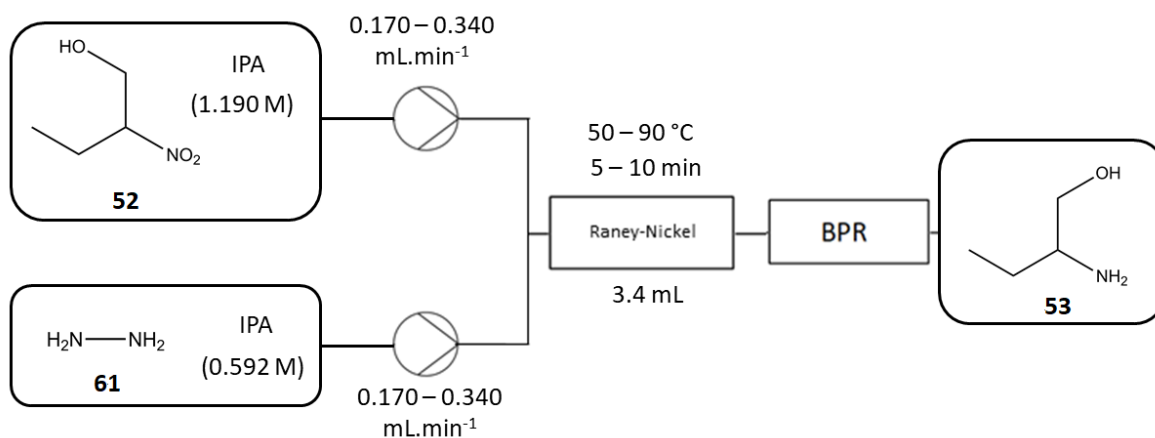


Figure 58 - Flow setup for the synthesis of 2-aminobutan-1-ol

All runs were completed without blockages. The results are tabulated in Table 15.

Table 15 - Flow results for syntheses of 2-aminobutan-1-ol (**53**)

Condition Set	61 Equiv.	Temperature (°C)	Residence Time (min)	Yield %
1	0.5	50	5	69
2	1	50	5	70
3	0.5	60	5	71
4	1	60	5	73
5	0.5	70	5	72
6	1	70	5	70
7	0.5	80	5	81
8	1	80	5	72
9	0.5	90	5	85
10	1	90	5	79
11	0.5	50	10	74
12	1	50	10	72
13	0.5	60	10	75
14	1	60	10	66
15	0.5	70	10	78
16	1	70	10	71
17	0.5	80	10	80
18	1	80	10	65
19	0.5	90	10	78
20	1	90	10	57

A residence time of 5 minutes at a temperature of 90 °C with a hydrazine (**61**) molar equivalent of 0.5 delivered the highest yield at 85%.

An additional experiment was attempted using a modified approach to what was used before. Instead of introducing **52** and **61** concurrently, the catalyst would instead be first 'primed' with hydrogen by passing undiluted 64% hydrazine monohydrate (**61**) through the column, after which **52** would be sent through in the neat state. The motivation behind this was to eliminate the blackish side-products of hydrazine decomposition (especially seen at higher temperatures and

longer residence times) by first loading the catalyst with hydrogen and then pumping solvent through the column until the exiting solution became clear. After pumping the hydrazine monohydrate solution through the column and further flushing of the column with IPA until the exiting solution was clear, 5 mL of 2-nitrobutan-1-ol (**52**) was loaded into a 5 mL loop and introduced into the system at the flowrate necessary to achieve the desired residence time inside the column. About a minute before the experiment's end, a large volume of gas was seen escaping the collection line of the flow system. A few seconds later, an explosion occurred, destroying the column, and damaging the column housing.

Several inherent safety features of flow systems greatly reduced the extent of the damage. Firstly, the pumps immediately shut down as soon as the system pressure spiked above the maximum allowed pressure. The column housing greatly reduced the amount of glass shrapnel released when the column burst. Also, due to the absence of large volumes of solvent in the flow system, the danger of explosive solvent combustion was minimal. All that was required to get the system operational again was to wipe away cosmetic stains on the column heater and replace two flow line sockets.

The explosion suggests that this reaction step is significantly exothermic when done in the neat state. As the column was stable and no longer eluting gas at the time the neat 2-nitrobutan-1-ol (**52**) was introduced, one might conclude that the sudden spike in pressure was caused by the rapid heating and expansion of the column contents due to the condensation reaction, and not from explosive hydrazine decomposition (although it may be a combination of both).

4.2.2.3 - Discussion

The identity of the product was confirmed via NMR spectroscopy (spectrum A7).

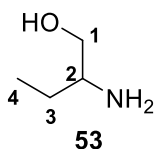
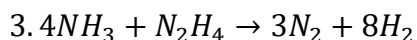
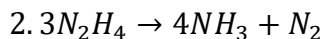
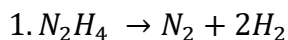


Figure 59 - 2-Aminobutan-1-ol (**53**) NMR designations

The ^1H NMR spectrum of **53** displayed the same number of hydrogens and resonance multiplicities as those present in compound **52**, indicating the same skeleton. However, the large upfield shift of H-2 from δ_{H} 4.55 ppm in **52** to 3.19 ppm in **53** indicates that the nitro group in **52** has been reduced to the amino group in **53**.

The highest yield of **53** was 85% at a residence time of 5 minutes at a temperature of 90 °C with a hydrazine equivalent of 0.5 delivered. The results suggest that shorter reaction times deliver the highest yields at higher temperatures. It is noticeable that higher temperatures result in more complex crude mixtures, which are more difficult to handle during distillation. As per the hydrazine decomposition reaction, two equivalents of molecular hydrogen are produced per one molecule of hydrazine, making 0.5 equivalents of hydrazine the theoretical minimum amount needed to match the limiting reagent (in this case, nitrobutanol **52**).⁸⁷



Reactions 1 and 2 are highly exothermic, generating large amounts of heat and voluminous gas in a small amount of time. This explosive decomposition reaction has led to **61** being a commonly used propellant in RCS (Reaction Control System) thrusters on spacecraft, where various forms of hydrazine in conjunction with a heated catalyst are used to achieve fine positional adjustments in space-faring vehicles.⁸⁸ In our flow-chemistry application, hydrazine decomposition in conjunction with the heat supplied by the reactor module likely leads to a localised hotspot inside the catalytic nickel column, facilitating unwanted side-reactions. As the only other decomposition products besides molecular hydrogen are gaseous ammonia and nitrogen, it is likely that the two latter compounds are responsible for the various side-products seen in this reduction reaction. As molecular nitrogen is especially unreactive in most chemical environments, the produced ammonia is likely responsible for any further interfering reactions. It is unclear whether the reduced yields seen at higher temperatures and longer reactions times are due to product degradation or hindrance of the hydrogenation reaction.

4.2.2.4 - Conclusion

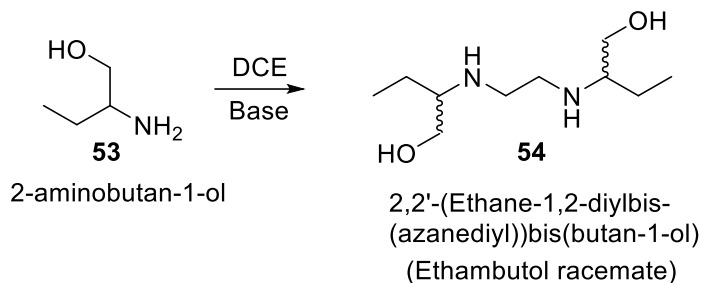
Molar equivalents of hydrazine above 0.5 lead to a more complex side-product profile and reduced yields after purification. It is evident that shorter reaction times in conjunction with lower temperatures lead to higher yields, and it was observed that cleaner, more workable crude products are obtained from experiments using these parameters.

4.2.2.5 - Future Work

The explosive incident using hydrazine (**61**) prompted a search for a safer hydrogen source. One method of hydrogen supply is readily available with the appropriate equipment. Tube-in-tube hydrogen introduction uses a special segment of flow tubing, where the usual flow tube is in turn covered in a second section of tubing, through which the hydrogen gas is pumped. The semi-permeable inner tube allows the pressurized hydrogen gas to diffuse into the reagent stream in the inner tube, saturating the flow line with elemental hydrogen. The reagent stream is then passed through the catalytic column in the normal fashion to effect the reduction reaction. This removes the need to introduce hydrogen into the catalyst column by using an intermediate molecule such as hydrazine, thereby eliminating the formation of various breakdown products. Despite the increased intricacy and cost of using such a specialised flow system, the benefits obtained overshadow these drawbacks. As the decomposition products of **61** appear to be a major factor in reduced yields as it pertains to this reaction step, usage of a cleaner source of hydrogen may well result in more favourable results.

4.2.3 - Attempted synthesis of Ethambutol (**54**) from 2-aminobutan-1-ol (**53**) and DCE by double nucleophilic substitution

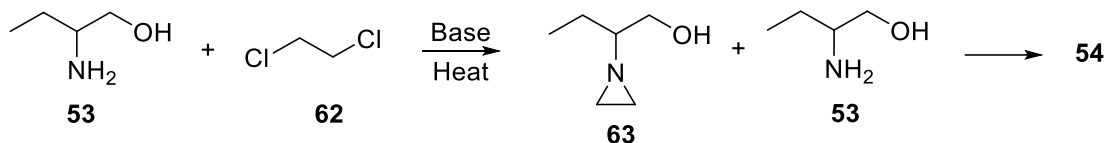
The final step to produce ethambutol (**54**) proceeds via two nucleophilic substitution reactions as shown in Scheme 14.



Scheme 14 - Final step in the synthesis of ethambutol

This particular reaction, however, has the added difficulty of requiring the substitution reaction to occur twice on the same molecule of 1,2-DCE. A common procedure for nucleophilic substitution with an amine was followed, and the reaction attempted with either NaOH or KOH, in water, ethanol, and IPA (for solvent compatibility in flow).⁸⁹ Unfortunately, no product was obtained using this method.

A second method uses one of a series of metal iodides (such as MgI_2 , NaI, KI, CaI_2) as catalyst to form a diamine from 1,2-DCE.⁹⁰ The catalyst is used in a 1 mol% or less concentration. According to similar examples from literature, it is likely that the reaction proceeds via an aziridine intermediate, in other words, the nitrogen analogue of an epoxide.⁸¹ Scheme 15 illustrates how an aziridine can be opened in a manner similar to opening an epoxide to deliver ethambutol.



Scheme 15 - Synthesis of 54 proposing the presence of an aziridine intermediate.

Knowing this, it might suggest that a different approach may afford the final product. If 1,2-DCE is instead used as the initial reagent in excess, every molecule of 2-aminobutan-1-ol (**53**) might be consumed to form 2-(aziridin-1-yl)butan-1-ol (**63**) *in-situ*. Only then would a second equivalent of **53** be added to react with the formed aziridine **63**, delivering the maximum amount of **54**. Spectral evidence in the form of NMR and MS may confirm this mechanistic route if **63** is found to be present after the initial reaction, assuming it can be isolated.

The reason for the failure of the final step in the synthesis of ethambutol is unclear, as nucleophilic substitution reactions of this nature are well-understood and ostensibly simple. Interference from the HCl produced from any substitution of a chlorine moiety on 1,2-dichloroethane may be partially to blame. The ability of this HCl to protonate certain functional groups in the reaction solution may be deactivating nucleophilic centres. For example, protonation of the amine moiety would drastically reduce its nucleophilicity. In conjunction with this, it is possible that HCl neutralises the base in solution (a reaction between HCl and either NaOH or KOH would produce NaCl and KCl, respectively). Using a greater equivalent of base might mitigate this.

3.3 – Isoniazid (Pyridine-4-carbohydrazide)

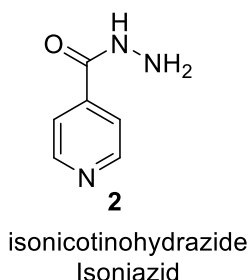
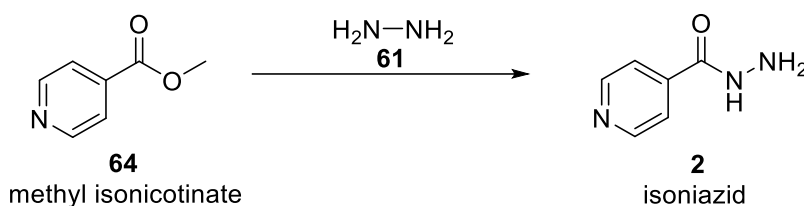


Figure 60 – Isoniazid (**2**)

Isoniazid (**2**) is a synthetic drug used to combat tuberculosis. Introduced in 1952, it remains a frontline treatment for active TB thanks to its potency and high selectivity against *M. tuberculosis*.⁵⁴ Isoniazid (**2**) is a prodrug that functions either as bactericide or as a bacteriostatic agent, depending on the rate of Mycobacterium division. It inhibits the synthesis of the mycolic acid component of the bacterial cell wall, leaving the bacterium vulnerable to various forms of degradation it would otherwise have survived.⁹¹ Isoniazid (**2**) shows a broad spectrum of activity against several Mycobacterium species, including *M. tuberculosis*, *M. kansasii*, and *M. bovis*.⁹² Another factor contributing to its longevity as an outstanding frontline treatment is its very low minimum inhibitory concentration, averaging about 0.4 μM against *M. tuberculosis*.⁹³ As most TB medicines can show significant side-effects over long-term use, drugs capable of successful TB

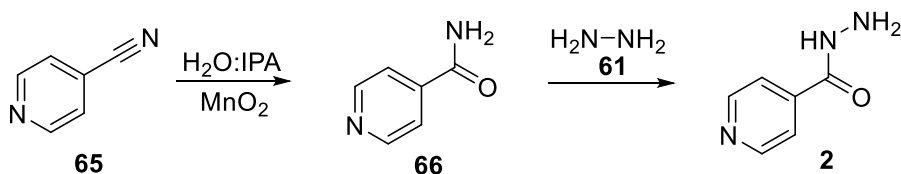
treatment at low concentrations become more important in terms of alleviating patient discomfort over the long period of treatment necessary, ensuring stronger adherence to their prescribed drug regimes. Completion of treatment and the outright elimination of the TB infection is critical as later attempts at thwarting the resurgent infection may prove unsuccessful using the same treatment a second time, and contributes to the growing threat of untreatable, drug-resistant TB strains.

Isoniazid (**2**) is commonly synthesized in one step by reacting methyl isonicotinate (**64**) with hydrazine (**61**) (Scheme 16). Hydrazine monohydrate is often used instead of neat hydrazine to limit the risk of explosion or poisoning.



Scheme 16 -Common Synthesis of Isoniazid

In this investigation, a different approach was used by starting from reduction of isonicotinonitrile (**65**) to yield isonicotinamide (**66**), followed by the addition of hydrazine (**61**) to yield **2** (Scheme 17).

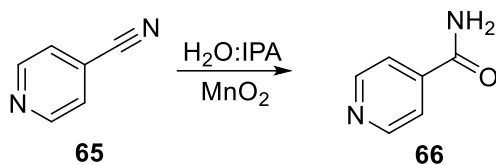


Scheme 17 - Method for synthesis of isoniazid used in this project

This alternative approach uses the more affordable isonicotinonitrile (**65**) instead of methyl isonicotinate (**64**) at the cost of an additional reaction step.

4.3.1 - Synthesis of isonicotinamide (**66**) from isonicotinonitrile (**65**) using MnO_2 catalyst

The first step of the synthesis of isoniazid involves a hydration of the nitrile group on isonicotinonitrile, as follows (Scheme 18):

Scheme 18 - Nitrile reduction of **65**

The hydration of isonicotinamide to isonicotinamide was attempted in both batch and flow, with the flow method taken directly from previous experiments by Ley *et al.*⁹⁴ Both methods make use of MnO_2 as reducing agent acting as a heterogenous catalyst.

4.3.1.1 – Batch Synthesis Results

The batch procedure for the preparation of **66** was analogous to the flow method in terms of solvent and catalyst used, both employing MnO_2 as reducing agent and a 1:1 mixture of H_2O :IPA as reaction medium. An overnight batch reaction at reflux delivered a yield of 47% isonicotinamide (**66**) after removal of the solvent under reduced pressure and recrystallization from hot ethanol.

4.3.1.2 – Flow Synthesis Results

For the flow syntheses, a simple single-pump setup was used, pumping the sole stock solution through a packed column containing MnO_2 . The setup is outlined in Figure 61.

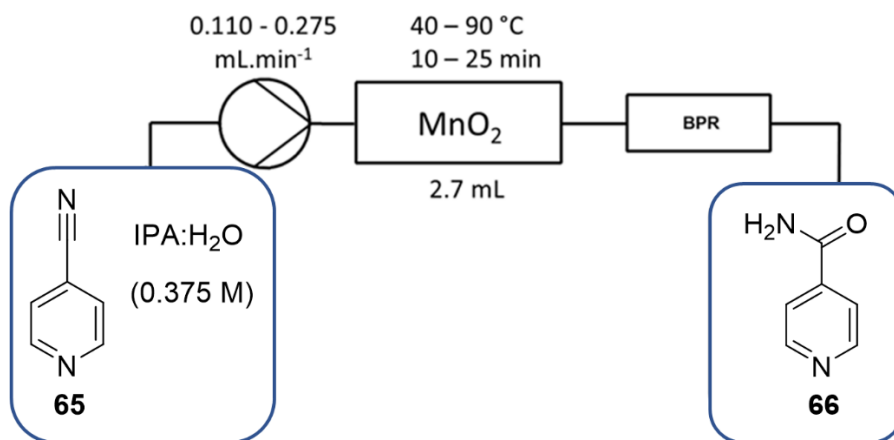


Figure 61 - Flow setup for the synthesis of isonicotinamide

The flow experiments proceeded smoothly with no blockages due to precipitates. The results are tabulated in Table 16.

Table 16 - Flow results for isonicotinamide synthesis

Condition Set	Temperature (°C)	Residence Time (min)	Yield (%)
1	40	10	34
2	50	10	39
3	60	10	55
4	70	10	57
5	80	10	53
6	60	16	73
7	70	16	70
8	80	16	72
9	90	16	69
10	60	25	68
11	70	25	66
12	80	25	62
13	90	25	58

A flow synthesis at 60 °C with a residence time of 16 min delivered the highest yield of **66** at 73%. At temperatures and residence times beyond these two values, yields either plateau or drop-off to varying degrees.

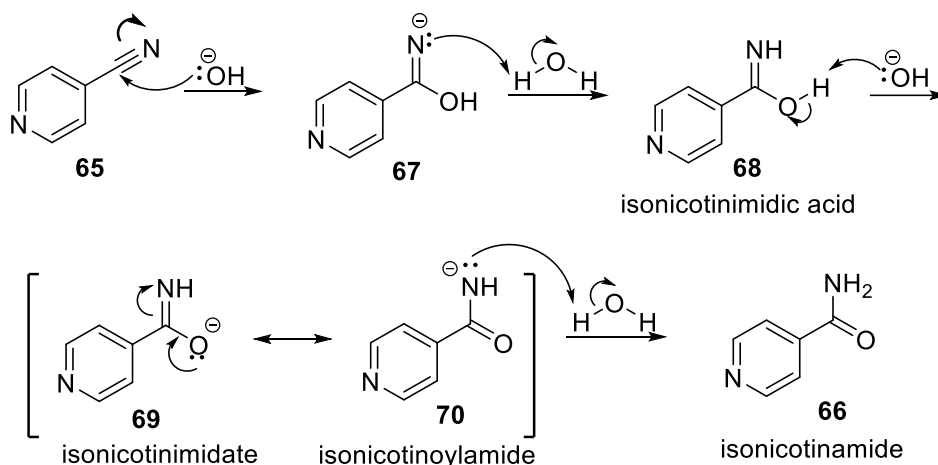
4.3.1.3 - Discussion

The flow synthesis of isonicotinamide delivered yields up to 26% higher than that of the batch procedure. Furthermore, use of MnO₂ in batch proved to be messy and cumbersome. The solid is a fine powder, and adheres to glassware. It is difficult to remove even by filtration, and the batch reactions required thorough cleaning of glassware afterwards. The flow reaction, however, proceeded smoothly and as described by Ley et al.⁹⁴ The catalyst is simply packed into a column, and attached to the heating rack. The method as reported in literature claimed a quantitative conversion at 60 °C. In practice, however, purification of the crude product is problematic as isonicotinamide readily sublimes, especially at the temperatures required to remove the reaction solvent (1:1 IPA:H₂O). Furthermore, due to the limited solubility of isonicotinonitrile (**65**) in the solvent system, stock solutions need to be quite dilute (generally less than 0.4 M). This limits

throughput significantly when columns of sizes 2-4 mL are used, and delivers a reaction crude mixture with a large solvent volume to molar product ratio, meaning that longer vacuum evaporation times are necessary to isolate the product **66**. In fact, when concentrated on a rotary evaporator, up to half of **66** travels upwards and collects in the condenser, where it has to be rinsed out and once again concentrated. The condenser itself can be effectively cleaned by distilling deionised water through it. It is, however, quite likely that a near quantitative yield is being obtained. As the starting material does not sublime or evaporate at the temperatures required to remove the reaction solvent *in vacuo*, it would be unable to escape the evaporation flask as the crude product is concentrated. NMR analysis of the dried reaction crude mixture shows only traces of **65**. As mentioned, however, simply removing the crude mixture's solvent post-reaction leads to an unacceptable loss of product **66** (a single pass on the rotary evaporator only yields about 50%), making repeated washings of the condenser and a final recrystallisation from ethanol the preferred method of collecting the isonicotinamide.

4.3.1.4 – Mechanism of Nitrile Hydration

Metal oxides in an aqueous environment tend to form hydroxyl groups on their catalytically active surface. The degree of basicity of the OH^- ions is determined by the polarization of the metal-oxygen bond. Factors influencing the degree of polarization include the charge and radius of the metal ion involved, the metal-oxygen bond length, the coordination geometry of the metal complex, and the nature of any other surrounding ligands. The mechanism of nitrile hydration using MnO_2 is proposed as follows (Scheme 19):⁹⁵



Scheme 19 - Mechanism of nitrile hydration

The reaction begins with a nucleophilic attack on the cyano carbon of **65**, delivering a negatively charged imine **67**. The nitrogen then abstracts a hydrogen atom from a molecule of water to form isonicotinimidic acid (**68**), whose hydroxyl group is then deprotonated. The molecule then tautomerizes between isonicotinimide (**69**) and isonicotinoylamide (**70**), until isonicotinoylamide (**70**) abstracts a proton from a second molecule of water to form compound **66**. Notice that the hydroxyl ion has been regenerated by the end of the catalytic cycle.

The obtained flow results show that higher temperatures at longer reaction times lead to decreased yields. NMR scans of these crude products show no molecules present besides either the product or starting material. This suggests that the loss in yield is likely due to a dip in catalytic activity brought about by excessive heat, and not from product degradation. For the experimental sets featuring residence times of either 10 min or 16 min, yields tend to plateau from 60 °C and onwards, whereas residence times beyond this show marked reductions in the amount of product obtained, with yields decreasing markedly with each 10 °C increment in temperature. The product identity was confirmed via NMR spectroscopy.

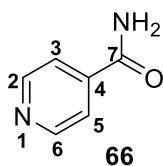


Figure 62 - Isonicotinamide NMR designations

The only non-exchangeable protons are all located on the aromatic moiety of both the starting material **65** and product **66**. From the reaction crude mixture itself, one can clearly see the disappearance of the two AA'BB' aromatic doublets of **65** located at 7.56 and 8.41 ppm, respectively, and the appearance of the downfield-shifted doublets of **66** located at 7.84 ppm (H-2/6), due to the proximity to the heterocyclic nitrogen, and 8.72 ppm (H-3/5), respectively. The downfield shift can be explained by the deshielding of the aromatic ring as a result of the introduction of a benzylic carbonyl group. From the ^{13}C spectrum, the four carbon signals one would expect were observed (this does not equal the total number of carbon atoms in the molecule due to symmetry across the aromatic moiety). These are located at 121.7 (C-3/5) and 149.6 ppm (C-2/6), respectively. Each account for two carbons, as the pair of signals are stronger than the other two likely as a result of symmetry. The remaining carbons are located at 142.1 ppm (C-4), as it falls in the benzylic range, and at 168.4 ppm (C-7), as it falls within the range of amidic carbonyl shifts. From this data we can conclude that isonicotinamide (**66**) was successfully synthesized from 4-pyridinecarbonitrile (**65**).

4.3.1.5 - Conclusion

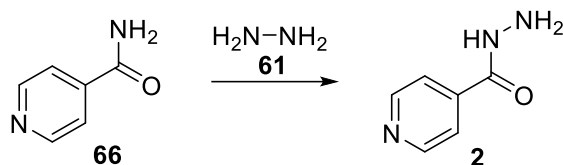
The isoniazid intermediate isonicotinamide (**66**) was successfully synthesized in a flow process in good yields at mild temperatures and short reaction times, delivering a maximum yield of 73%, surpassing that of the batch reaction by 26%. However, the quantitative conversion of the starting material claimed by Ley *et al.* could not be achieved, likely due to the difficulty in product purification experienced in this project. NMR scans of crude products not recrystallised from ethanol show only traces of residual starting material, suggesting that a near-quantitative conversion is indeed taking place. It was also demonstrated that longer reaction times at temperatures above 70 °C reduce catalytic activity, resulting in rapid space-time yield drop-off.

4.3.1.6 - Future Work

Alternative analytical techniques such as liquid chromatography and mass spectrometry can confirm if **66** is obtained quantitatively during the flow reaction through direct analysis of the eluted reaction crude product. This process can be automated and executed continuously so as to monitor the flow reaction from start to finish. Monitoring a flow reaction in this manner can also shed light on the rate of catalytic drop-off.

4.3.2 - Attempted Synthesis of isoniazid (**2**) from isonicotinamide (**66**)

The planned final step in the synthesis of isoniazid proceeds by hydrazinolysis of isonicotinamide (Scheme 20).



Scheme 20 - Hydrazinolysis of isonicotinamide to yield isoniazid.

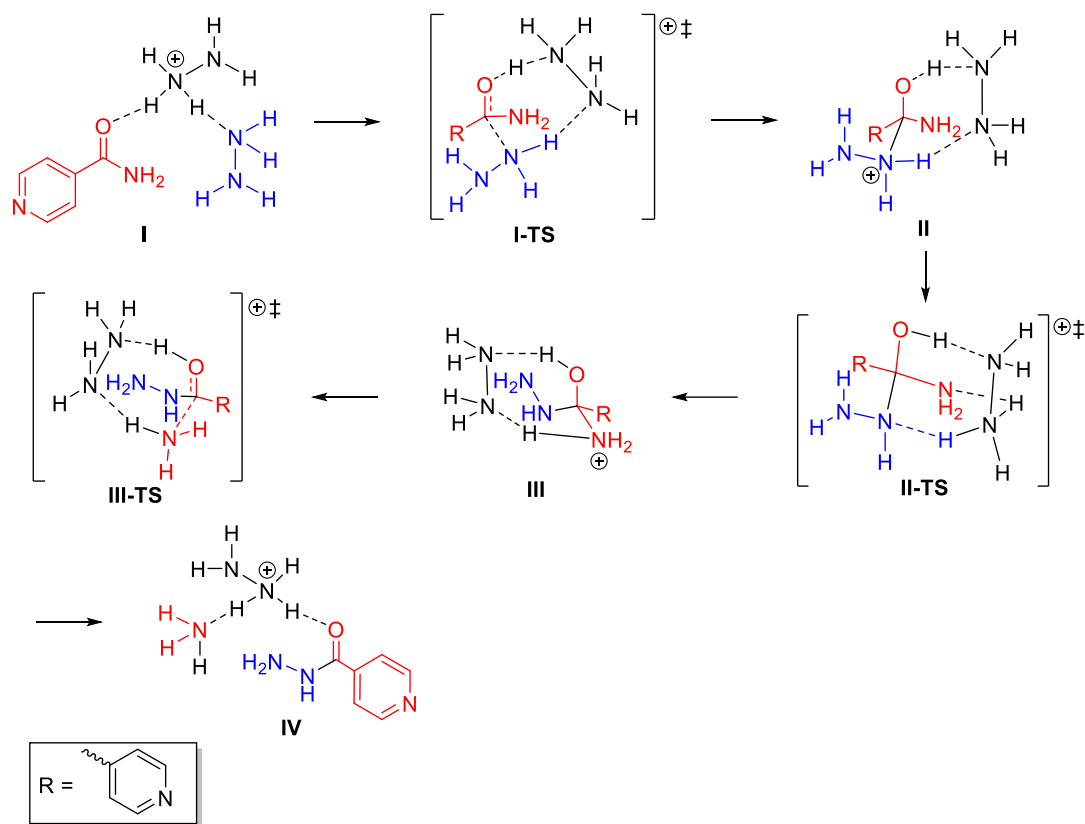
The final step of the 2-part synthesis of **2** could not be completed in either batch or flow. Literature sources suggested the reaction could proceed uncatalyzed and at mild temperatures, which was not the case despite having similar starting materials.⁹⁶ In this section, an alternative strategy to effect the final reaction is detailed, focusing on increasing the reactivity of the amide moiety (generally considered to be quite stable) through activation of the carbonyl centre.⁹⁷

4.3.2.1 - Hydrazinolysis Overview

Hydrazinolysis involves the cleaving of the carbonyl-nitrogen bond in an amide to deliver a hydrazide (for example, isoniazid), and either an amine or ammonia.⁹⁸ Hydrazides have received special interest due in part to their application as building blocks for various uses in molecular synthesis in both organic and inorganic chemistry, including Schiff bases, coordination compounds, and hydrazones. As mentioned, use of only the starting amide and hydrazine monohydrate was unsuccessful. Several modifications to this procedure, however, have delivered various target hydrazides of a similar nature.

4.3.2.2 - Ammonium Salt Accelerated Hydrazinolysis

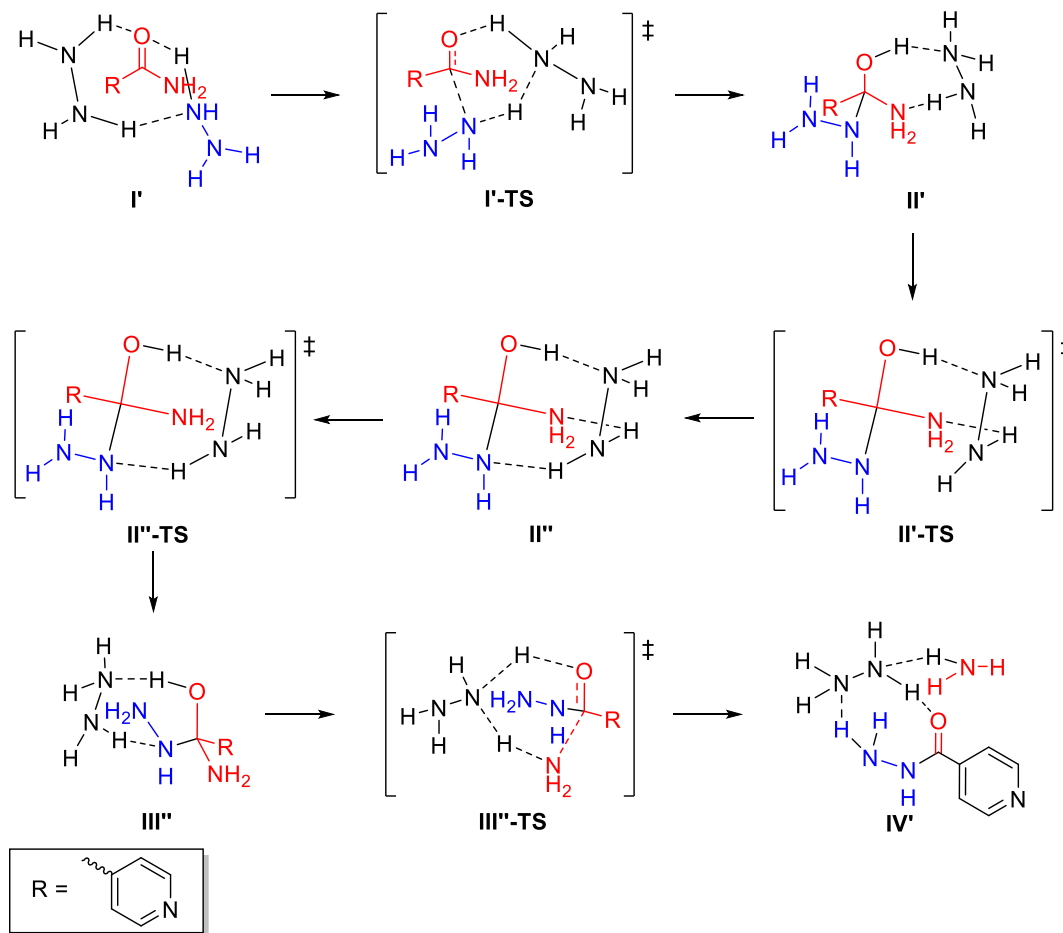
Noshita and co-workers employed a method of cleaving the amide bond through the cooperation of a hydrazinium salt and hydrazine.⁹⁸ Their reactions did not proceed in the absence of an ammonium salt. They postulate that hydrazinium salts generated from the ammonium salts and hydrazine hydrate are the active species in the reaction process. Scheme 21 illustrates a DFT-calculated reaction process that delivers the cleaved amine and its associated hydrazide.



Scheme 21 - DFT-calculated reaction sequence in the presence of a hydrazinium salt

The results showed that both the addition of hydrazine to the amide (indicated in I-TS) and the elimination of the amine from the tetrahedral intermediate (indicated in III-TS) require high activation energies. The presence of the hydrazinium salt provides additional energy to surmount this. These calculations proved to be consistent with obtained experimental observations. In short, the hydrazinium salt activates the amine carbonyl group, stimulating the simultaneous nucleophilic addition of the hydrazine molecule and the elimination of the amine moiety from the tetrahedral transition state. It is postulated that the elimination of the amine from the tetrahedral transition state was the rate-limiting step in the overall reaction pathway. This is ascribed to the higher nucleophilicity of the hydrazine compared to the amine, decreasing the activation energy for the addition step vis-à-vis the activation energy needed for the elimination step. In both cases, however, the proton transfer steps were faster than both of these. This was especially true when a larger ratio of ethanol:water solvent was employed. To elucidate the role of the activated hydrazinium salt in the hydrazinolysis of amides, the reaction pathway in the

absence of the salt was also evaluated. Scheme 22 demonstrates this process, in this case featuring two molecules of hydrazine.



Scheme 22 - DFT-calculated reaction sequence in the absence of a hydrazinium salt

The additional hydrazine molecule facilitates the proton-transfer steps during the reaction. These steps require extremely high activation energies compared to the salt-aided process, introducing yet more rate-limiting steps when compared to the salt-assisted reaction pathway.

4.3.2.3 - Hydrazinolysis on a Flow Platform

The salt-assisted method of hydrazinolysis was previously successfully translated onto a flow platform, utilising an inline microwave flow reactor.⁹⁸ The flow reactor setup is pictured in Figure 63.

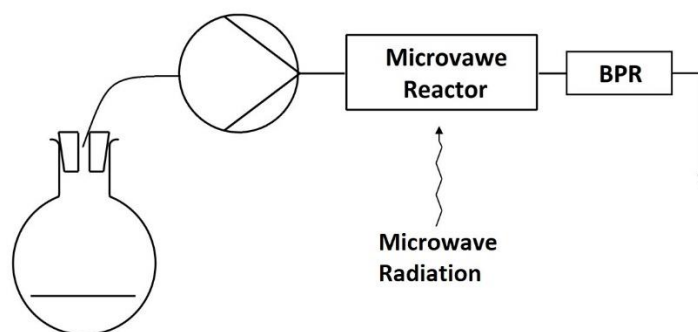


Figure 63 - Flow setup for salt-assisted microwave-catalysed reaction

Using the flow system, the yields of the model reactions were increased by factors of 3 to 4 versus that of the batch processes. This is another example of the increased efficiency of flow chemistry in both yield and mass-throughput.

Chapter 5 – Conclusion and Future Work

Although none of the planned syntheses could be completed in their entirety, it was demonstrated that every API in the project has good viability for either partial or total translation onto a flow platform. All steps successfully translated into flow delivered higher yields, cleaner crude products, and had greatly reduced reaction times compared to their batch counterparts. Furthermore, every reaction step of each of the three syntheses required less solvent when performed in flow as opposed to batch.

Corollaries of these findings offer many valuable insights into the benefits of flow chemistry. Firstly, it was shown that flow processes offer greatly increased space-time yields compared to batch on laboratory scale as a larger amount of product is generated per unit reactor volume per unit time. Alternatively, this can also be seen as a larger yield delivered per unit man-hour; if these syntheses are performed in a commercial setting, fewer chemists and less time is needed to produce an amount of product comparable to that of a batch reactor. Secondly, the increased simplicity of the operation of an optimised flow setup compared to batch laboratory methods allows the use of minimally trained personnel in the synthesis of complex molecules on a kilogram scale. With the supervision of, for example, a career chemist and a process engineer, multiple technicians can be employed to run flow instruments on a commercial scale at a greatly reduced cost in terms of training and wages. Thirdly, the intrinsic similarity between benchtop-scale flow systems and multiple-ton scale flow-process production plants in terms of operation, fluid physics, and safety allows for the expedited scale-up of laboratory-proven syntheses into a commercially viable endeavour. The versatility and modular design of flow chemistry instruments greatly simplifies this difficult process.

Despite the initial cost of obtaining flow chemistry equipment, the reduced production costs stemming from the aforementioned perks can make it an attractive alternative to batch synthesis.

Future work will be centred around completing those steps that could not be completed or fully optimised in this project, with the goal of scaling these reactions from gram level to kilogram

level in order to perform techno-economic evaluation on pilot plant production scale. The aim of this is to demonstrate that research and development at the laboratory level can be telescoped to commercial production capacities in an expedited and reliable manner through discreet, methodical steps.

The scaling up of the various steps explored in this thesis will be accomplished on a FlowSyn Maxi instrument (Figure 64).⁹⁹



Figure 64 - FloSyn Maxi Reactor

This commercially available instrument is focused on high throughput processes, and can achieve flow rates of up to $100 \text{ mL} \cdot \text{min}^{-1}$ to deliver multiple kilograms of product per day. The first API of focus for scaling up will be ethambutol, as it offers the fewest issues in terms of precipitates and features no solid starting materials in any of its three synthesis steps (although the third step remains to be accomplished, and newly added reagents may change this). Of particular interest is the second step featuring the hydrotalcite catalyst. If the yield of ethambutol intermediate **52** cannot be brought up to quantitative levels, the accumulation of residual starting materials such as formalin (**51**) and 1-nitropropane (**50**), and the presence of various side products stemming from these, may greatly complicate the work-up process of the delivered crude product. Even though large-scale purification of **51** can be accomplished by the use of a distillation column of appropriate size, the ultimate goal of coupling each synthesis step in an automated sequence will

likely not allow for this at present (an integrated distillation step, however, is another attractive goal in the pursuit of automated flow setups. Figure 65 suggests how this might be accomplished).

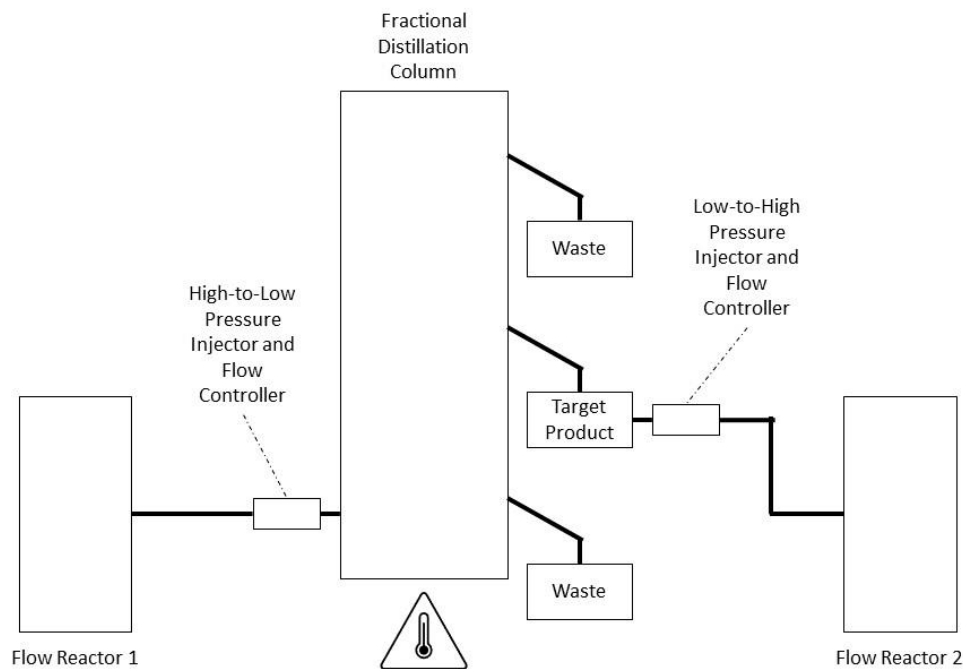


Figure 65 - Proposed integration of a distillation step to a scaled-up flow process

As the crude product exits flow reactor 1, a high-to-low pressure injector capable of controlling flowrates injects the reactor eluent into the fractional distillation column. The distillation column features a temperature gradient, being at its hottest at the base and decreasing upwards. Depending on the temperature at which the desired product is collected, unwanted waste components will have to be removed at temperatures above and below that of the target compound. If this can be achieved concurrently, the process can be run in steady state conditions. However, non-ideal mixtures will likely have a complex azeotropic profile in addition to their varying boiling points. For this approach to operate in true steady state conditions, the composition of the vapour leaving the heated base must remain constant. Also, due to the fact that the reactor eluent is usually comprised mostly of solvent, removing the product selectively becomes increasingly difficult. If the solvent and product can be collected at a similar temperature junction, however, the mixture can be injected back into the flow line and the

concentration adjusted as needed. If the sum of these challenges proves too great, a flow-batch-flow hybrid approach would be needed, where a volume of collected reactor eluent is first separated by normal distillation before being re-diluted and introduced into the next flow step.

In conclusion, this project has demonstrated that flow chemistry can become a valuable contributing technology towards the elimination of South Africa's reliance on imported TB medications.

Chapter 6 - Synthetic Methods

6.1 General Synthetic Details

NMR analysis was performed using either a Bruker AVANCE-III-300 and Bruker AVANCE-III-400 spectrometer, at 300 MHz and 400 MHz respectively. Chemical shifts (δ) are described in parts per million (ppm). Peak multiplicity is described as singlets (s), doublets (d), triplets (t), quartets, and multiplets (m). Coupling constants (J) are reported in Hertz (Hz). Trimethylsilane was selected as internal standard, set to 0 ppm.

When reporting packed column volumes, the volume was obtained by first loading the column with dry catalyst and weighing it. After wetting the column with solvent, it is once again weighed and the mass difference is recorded. The difference is then divided by the solvent density to obtain the true void volume of the packed column.

Reference to *in-vacuo* solvent removal entails the use of a heated water bath rotary evaporator. High-vacuum distillation refers to the use of very low-pressure heated distillation accomplished through the use of a Edwards RV5 capable of providing a vacuum pressure of 2.0×10^{-3} mbar.

All ethanol used was of ≥ 99.9 % purity.

Flow-chemistry instruments used originate from Syrris Ltd. (UK) and Vapourtec Ltd. (UK).

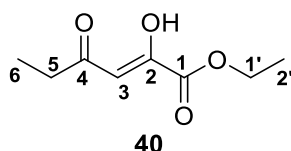
Main Syrris components used consist of an Asia Pressurized Input Store, Asia Syringe Pump modules, Asia Reagent Injector, Asia Chip Climate Controller, Asia Heater, Asia Pressure Controller, as well as various sundries including mixing chips, tubing and connectors, column reactors, and coil reactors.

Main Vapourtec systems used include an E-series easy-Medchem 3-pump peristaltic flow reactor, glass microchip reactor and housing, and 10 mL Vapourtec reactor column, as well as various sundries including tubing and connectors.

6.2 Synthetic Methods

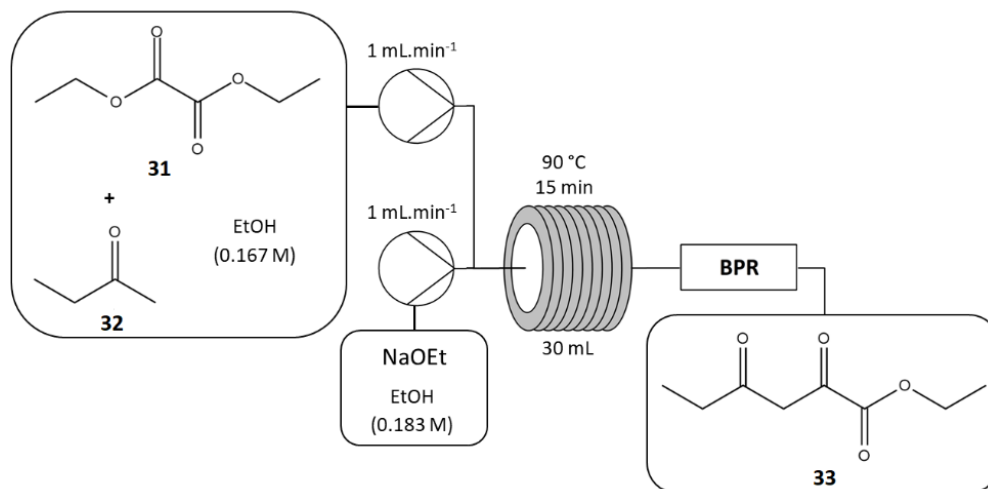
Synthesis of (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate (**40**) in Batch

A solution of sodium ethoxide was prepared by slowly feeding slivers of sodium metal (1.264 g, 55 mmol, 1.1 equiv.) into an ice-cooled flask of ethanol (74 mL) under counter-current argon flow. Once all the sodium had dissolved, a solution of 2-butanone (**32**) (3.606 g, 50 mmol, 1 equiv.) and diethyl oxalate **31** (7.301 g, 55 mmol, 1.1 equiv.) in ethanol (75 mL) was added dropwise into the sodium ethoxide solution over the course of an hour, using a capped dropping funnel. After this time the reaction mixture was allowed to warm up to room temperature, and was further stirred for 8 hours. The reaction mixture changed from a clear solution to a brownish-red colour by the end of the reaction. The ethanol solvent was removed *in vacuo* to yield a splintery light-red solid. The solid was redissolved in H₂O (150 mL) and ethyl acetate (100 mL), and the solution was partitioned between an organic phase and an aqueous phase. Any remaining diethyl oxalate was removed in the organic phase and can be easily reclaimed. The aqueous fraction was then quenched at 0°C with aqueous HCl (1 M, 5 equiv.). An organic fraction was then extracted 5 times with aliquots of ethyl acetate (15 mL), and washed twice with H₂O (15 mL). The organic fraction was dried over Na₂SO₄ and concentrated *in vacuo*. The product was then purified by distillation under reduced pressure at 60 °C to yield a light-yellow oil (3.354 g, 39%).



¹H NMR (300 MHz, CDCl₃)¹⁰⁰: δ 1.19 ppm (t, *J* = 6 Hz, 3H, H-6), δ 1.39 ppm (t, *J* = 6 Hz, 3H, H-2'), δ 2.55 ppm (q, *J* = 9 Hz, 2H, H-5), δ 4.37 ppm (q, *J* = 9 Hz, 2H, H-1'), δ 6.39 ppm (s, 1H, H-3); **¹³C NMR (300 MHz, CDCl₃)**¹⁰⁰: δ 8.67 ppm (C-2' or C-6), δ 13.83 ppm (C-2' or C-6), δ 34.21 ppm (C-5), δ 62.55 ppm (C-1'), δ 101.34 ppm (C-3), δ 162.29 ppm (C-2), δ 165.96 ppm (C-1), δ 204.26 ppm (C-4).

Synthesis of (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate (**40**) in Flow



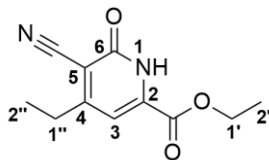
A stock solution of 2-butanone **32** (0.166 M) and diethyl oxalate **31** (0.166 M) was prepared with ethanol as solvent. A third stock solution consisted of *in-situ* prepared sodium ethoxide in ethanol (0.183 M). The pushing solvent was pure ethanol. The flow setup consisted of a T-piece feeding into a 30 mL heated coil reactor. A reactor temperature of 90 °C at a residence time of 15 min was employed. The reaction crude mixture was added dropwise to a stirred, ice-cold solution of HCl (0.915 M). All experiments attempted inevitably resulted in blockages at the T-piece placed at the entrance to the column reactor due to the gradual accumulation of a white precipitate. Trace product **40** was recovered from each experiment.

¹H NMR (300 MHz, CDCl₃)¹⁰⁰: δ 1.19 ppm (t, *J* = 6 Hz, 3H, H-6), δ 1.39 ppm (t, *J* = 6 Hz, 3H, H-2'), δ 2.55 ppm (q, *J* = 9 Hz, 2H, H-5), δ 4.37 ppm (q, *J* = 9 Hz, 2H, H-1'), δ 6.39 ppm (s, 1H, H-3); **¹³C NMR (300 MHz, CDCl₃)**¹⁰⁰: δ 8.67 ppm (C-2' or C-6), δ 13.83 ppm (C-2' or C-6), δ 34.21 ppm (C-5), δ 62.55 ppm (C-1'), δ 101.34 ppm (C-3), δ 162.29 ppm (C-2), δ 165.96 ppm (C-1), δ 204.26 ppm (C-4).

Synthesis of ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate **43** in Batch

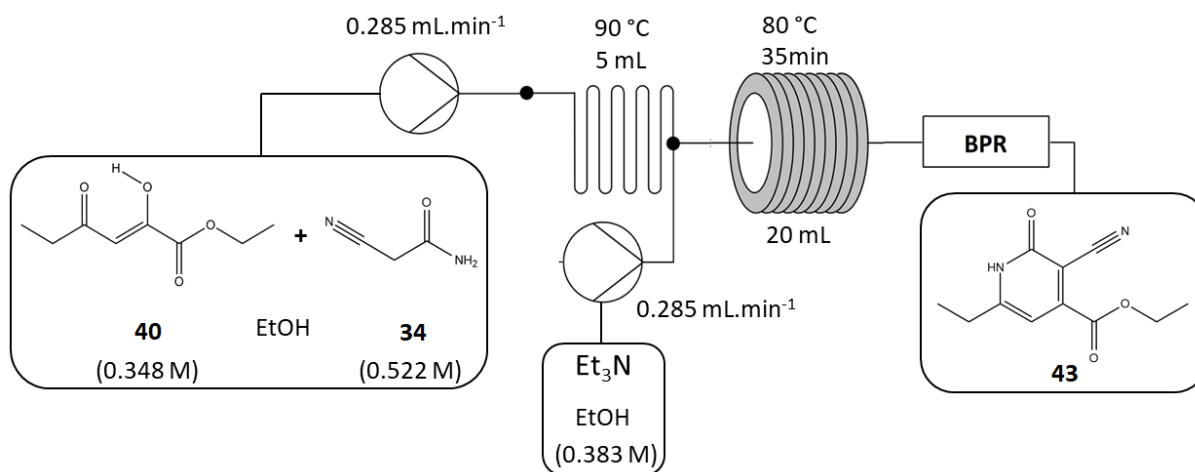
(Z)-Ethyl 2-hydroxy-4-oxohex-2-enoate **40** (1.000 g, 5.8 mmol, 1 equiv.), 2-cyanoacetamide **34** (0.537 g, 6.4 mmol, 3 equiv.), and triethylamine (0.647 g, 6.4 mmol, 1 equiv.) were combined in ethanol (50 mL) and refluxed for 5 hours. The reaction was then quenched with aqueous HCl (6 M, 1.45 mL). An organic fraction was then extracted 3 times with aliquots of ethyl acetate (15

mL), washed once with H₂O (15 mL), and dried over Na₂SO₄. The solvent was removed *in vacuo* and the product recrystallized from hot ethanol to yield **43** as green crystals (0.460 g, 36%).



¹H NMR (300 MHz, CDCl₃): δ 1.37 ppm (t, J = 9 Hz, 3H, H-2''), δ 1.45 ppm (t, J = 9 Hz, 3H, H-2'), δ 2.81 ppm (q, J = 9 Hz, 2H, H-1''), δ 4.47 ppm (q, J = 6 Hz, 2H, H-1'), δ 6.68 ppm (s, 1H, H-3); **¹³C NMR (300 MHz, CDCl₃):** δ 12.29 ppm (C-2''), δ 13.91 ppm (C-2'), δ 27.10 ppm (C-1''), δ 63.33 ppm (C-1'), δ 101.03 ppm (C-3), δ 104.96 ppm (C-), δ 113.80 ppm, δ 148.36 ppm, δ 158.08 ppm, δ 162.79 ppm, δ 163.87 ppm. **IR ν_{max} /cm⁻¹:** 2763 (alkyl C-H), 2225 (-CN), 1715 (ester C=O), 1645 (amide C=O), 1613, 1538, 1488, 1371 (aromatic C-H), 1344, 1310, 1239, 1194, 1117, 1011 (ester COCH₂), 962, 930, 865, 798, 756, 681, 593, 545, 465.

Synthesis of ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate (**43**) in Flow



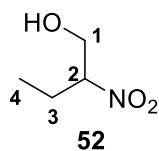
Two stock solutions were prepared, namely a combined stock consisting of (*Z*)-ethyl 2-hydroxy-4-oxohex-2-enoate **40** (0.348 M) and cyanoacetamide **34** (0.522 M), as well as a single stock of triethylamine (0.383 M) in ethanol. A 10:1 solution of ethanol:H₂O was used as pushing solvent. The reactor setup consisted of a pair of feeding pumps, with the combined stock of **40** and **34** first feeding into a 5 mL mixing chip at 90 °C before encountering the base solution at a T-junction. The reagent stream then enters a 20 mL coil reactor ($FR = 0.571 \text{ mL}\cdot\text{min}^{-1}$, $R_T = 35 \text{ min}$, $T = 80 \text{ }^\circ\text{C}$).

40 mL of each stock solution was pumped through the system. Ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate **43** was recovered as green crystals after removing the solvent from the reaction crude mixture *in vacuo* and recrystallizing from ethanol (3.064 g, 56%).

¹H NMR (300 MHz, CDCl₃): δ 1.37 ppm (t, J = 9 Hz, 3H, H-2''), δ 1.45 ppm (t, J = 9 Hz, 3H, H-2'), δ 2.81 ppm (q, J = 9 Hz, 2H, H-1''), δ 4.47 ppm (q, J = 6 Hz, 2H, H-1'), δ 6.68 ppm (s, 1H, H-3); **¹³C NMR (300 MHz, CDCl₃):** δ 12.29 ppm (C-2''), δ 13.91 ppm (C-2'), δ 27.10 ppm (C-1''), δ 63.33 ppm (C-1'), δ 101.03 ppm (C-3), δ 104.96 ppm (C-), δ 113.80 ppm, δ 148.36 ppm, δ 158.08 ppm, δ 162.79 ppm, δ 163.87 ppm. **IR ν_{max} /cm⁻¹:** 2763 (alkyl C-H), 2225 (-CN), 1715 (ester C=O), 1645 (amide C=O), 1613, 1538, 1488, 1371 (aromatic C-H), 1344, 1310, 1239, 1194, 1117, 1011 (ester COCH₂), 962, 930, 865, 798, 756, 681, 593, 545, 465.

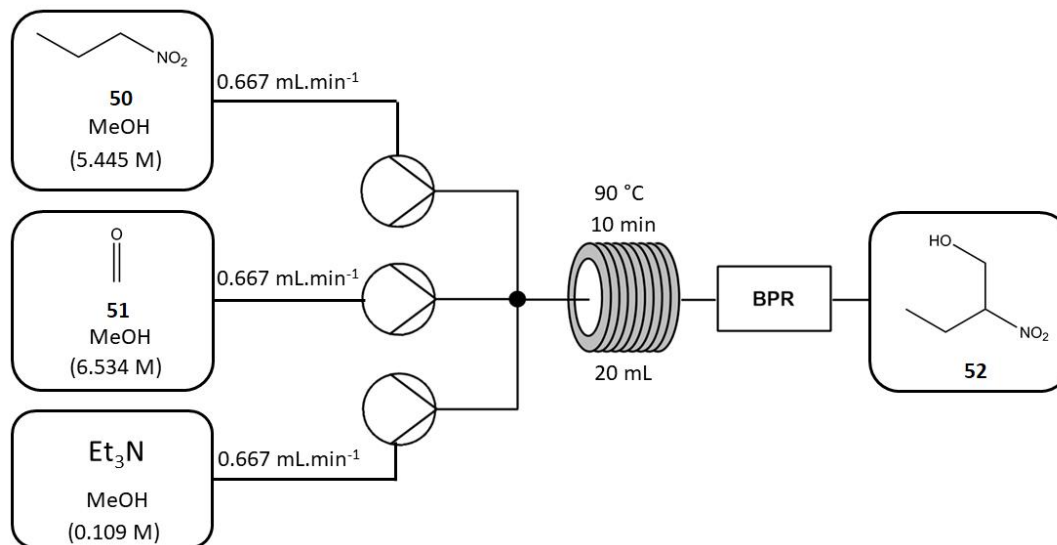
Synthesis of 2-nitrobutan-1-ol (**52**) in batch

1-Nitropropane **50** (5.956 g, 0.05 mol, 1 equiv.), 37% aqueous formalin solution **51** (3.78 mL, 1.5 equiv.), and catalytic triethylamine (0.506 g, 5 mmol, 10 mol%) were added to 20 mL methanol. The reaction was refluxed for 1 h and then quenched with aqueous HCl (6 M, 1.25 mL, 0.15 equiv.). The solvent was removed *in vacuo* and the product distilled off under reduced pressure at 95 °C to yield a clear oil (1.718 g, 29%).

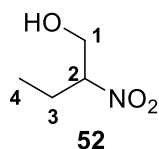


¹H NMR (300 MHz, CDCl₃): δ 1.00 ppm (t, J = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, J = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, J = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃):** δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2). **IR ν_{max} /cm⁻¹:** 3389 (C-OH), 2977, 2942, 2885, 1545 (C-NO₂), 1460, 1367 (C-NO₂), 1346, 1330, 1256, 1221, 1052, 1002, 970, 921, 863, 798, 620, 515, 458.

Synthesis of 2-nitrobutan-1-ol (**52**) in flow using Et₃N

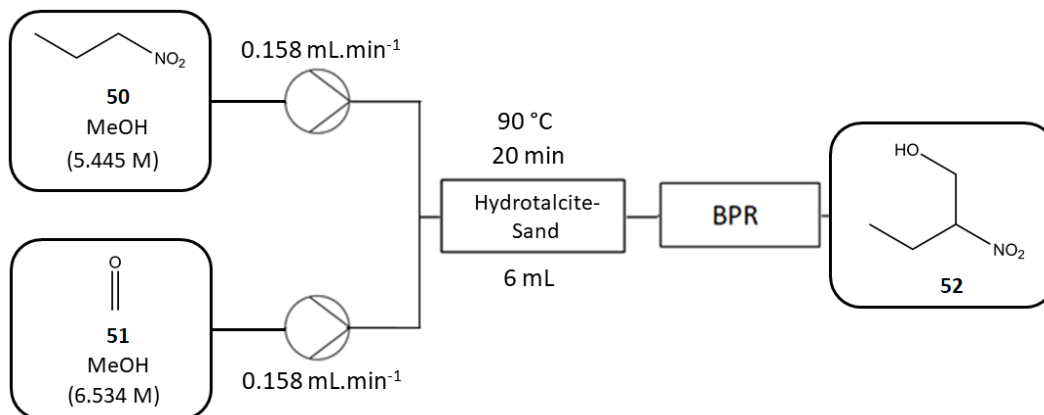


Three stock solutions, namely 1-nitropropane **50** (5.445 M, MeOH, 1 equiv.), formalin **51** (6.534 M, MeOH, 1 equiv.), and catalytic triethylamine (0.109 M, MeOH, 2 mol%), were prepared. The pushing solvent used was MeOH. 40 mL of each of the three stock solutions were combined at a 3-way junction fitting before entering a 20 mL (FR = 2.00 mL.min⁻¹, R_T = 10 min, T = 90 °C). An additional 30 mL of pushing solvent was collected after the theoretical end of the collection time to collect any tailing product. The solvent and remaining starting material was removed *in vacuo* and the product was purified by distillation under reduced pressure at 95 °C to obtain a clear oil (15.307 g, 59%).

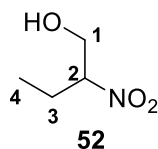


¹H NMR (300 MHz, CDCl₃): δ 1.00 ppm (t, *J* = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃):** δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2). **IR ν_{max}/cm⁻¹:** 3389 (C-OH), 2977, 2942, 2885, 1545 (C-NO₂), 1460, 1367 (C-NO₂), 1346, 1330, 1256, 1221, 1052, 1002, 970, 921, 863, 798, 620, 515, 458.

Synthesis of 2-nitrobutan-1-ol (**52**) in flow using hydrotalcite:sand catalyst

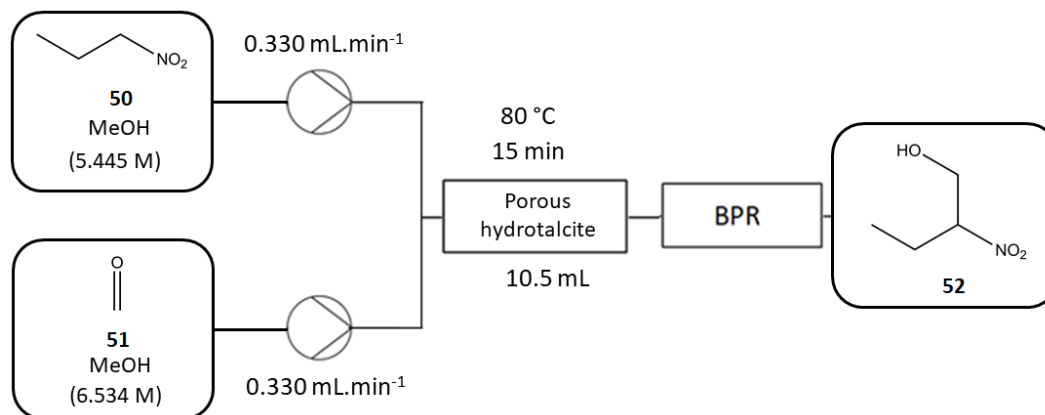


Two stock solutions, namely 1-nitropropane **50** (5.445 M, IPA, 1 equiv) and formalin **51** (6.534 M, IPA, 1 equiv) were prepared. The pushing solvent used was IPA. The stock solutions were combined at a T-junction fitting before entering a column dry-packed with a 1:1.2 hydrotalcite:sand by mass mixture (column void volume 6.3 mL). 40 mL of each stock was sent through the system (FR = 0.315 mL.min⁻¹, R_T = 20 min, T = 90 °C). An additional 15 mL of pushing solvent was collected after the theoretical end of the collection time to collect any tailing product. The solvent and remaining starting material was removed *in vacuo* and the product purified by distillation under reduced pressure at 95 °C to obtain a clear oil (5.19 g, 20%).

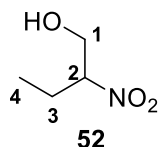


¹H NMR (300 MHz, CDCl₃): δ 1.00 ppm (t, *J* = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃):** δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2). **IR ν_{max}/cm⁻¹:** 3389 (C-OH), 2977, 2942, 2885, 1545 (C-NO₂), 1460, 1367 (C-NO₂), 1346, 1330, 1256, 1221, 1052, 1002, 970, 921, 863, 798, 620, 515, 458.

Synthesis of 2-nitrobutan-1-ol (**52**) in flow using NaCl-formed channels

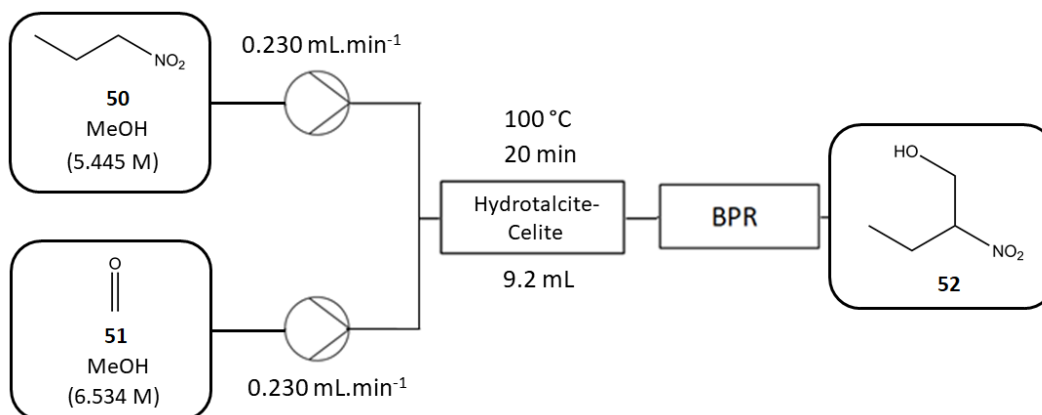


Two stock solutions, namely 1-nitropropane **50** (5.445 M, IPA, 1 equiv) and formalin **51** (6.534 M, IPA, 1 equiv) were prepared. The pushing solvent used was IPA. A catalytic column was prepared by first mixing dried activated hydrotalcite with powdered NaCl in a 1:2 mass ratio. The mixture is then packed into a column before warm water is sent through until salt is no longer eluted. The final column void volume was 9.9 mL. 15 mL of each stock was then sent through the system (FR = 0.660 mL.min⁻¹, R_T = 15 min, T = 80 °C). An additional 5 mL of pushing solvent was collected after the theoretical end of the collection time to collect any tailing product. The solvent and remaining starting material was removed *in vacuo* and the product purified by distillation under reduced pressure at 95 °C to obtain a clear oil (3.79 g, 39%).

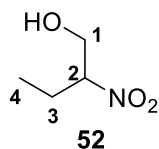


¹H NMR (300 MHz, CDCl₃): δ 1.00 ppm (t, J = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, J = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, J = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃):** δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2). **IR ν_{max} /cm⁻¹:** 3389 (C-OH), 2977, 2942, 2885, 1545 (C-NO₂), 1460, 1367 (C-NO₂), 1346, 1330, 1256, 1221, 1052, 1002, 970, 921, 863, 798, 620, 515, 458.

Synthesis of 2-nitrobutan-1-ol (**52**) in flow using hydrotalcite:celite catalyst



Two stock solutions, namely 1-nitropropane **50** (5.445 M, IPA, 1 equiv) and formalin (6.534 M, IPA, 1 equiv) were prepared. The pushing solvent used was IPA. 40 mL of each stock solution was combined at a T-junction fitting before entering a column dry-packed with a 1:1.5 hydrotalcite:celite (column void volume 9.2 mL). 40 mL of each stock was sent through the system (FR = 0.46 mL.min⁻¹, R_T = 20 min, T = 100 °C). An additional 15 mL of pushing solvent was collected after the theoretical end of the collection time to collect any tailing product. The solvent and remaining starting material was removed *in vacuo* and the product was purified by distillation under reduced pressure at 95 °C to obtain a clear oil (13.23 g, 51%).



¹H NMR (300 MHz, CDCl₃): δ 1.00 ppm (t, *J* = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃):** δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2). **IR ν_{max}/cm⁻¹:** 3389 (C-OH), 2977, 2942, 2885, 1545 (C-NO₂), 1460, 1367 (C-NO₂), 1346, 1330, 1256, 1221, 1052, 1002, 970, 921, 863, 798, 620, 515, 458.

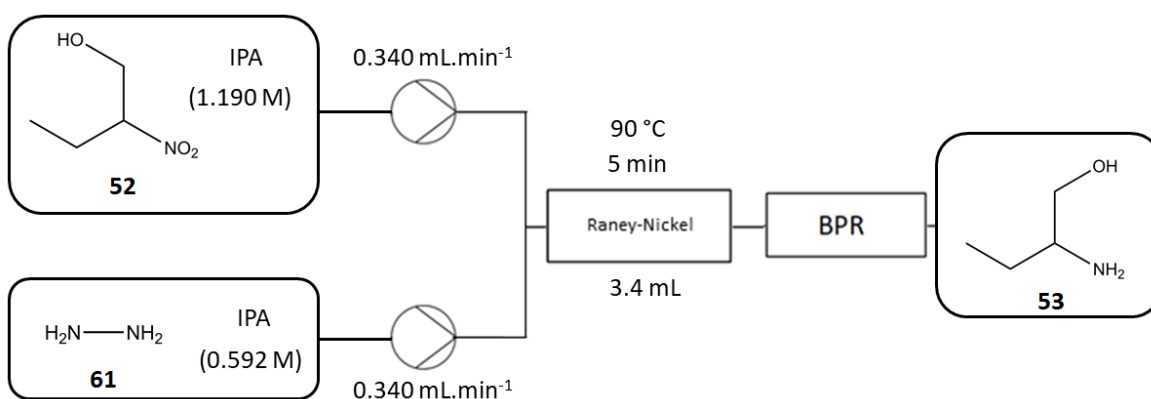
Synthesis of 2-aminobutan-1-ol **53** in batch

2-nitrobutanol **52** (5.003 g, 42 mmol, 1 equiv.) was added to a hydrogen-saturated solution of IPA (100 mL) containing Raney-Nickel (1 g weighed as aqueous slurry). The reaction mixture was

stirred at 60 °C for 4 h, after which time the catalyst was filtered out and the solvent removed *in vacuo*. The product was distilled off at 115 °C to yield **52** as a light-yellow oil (1.197 g, 32%).

¹H NMR (300 MHz, CDCl₃)¹⁰¹: δ 1.00 ppm (t, *J* = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃)**¹⁰¹: δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2).

Synthesis of 2-aminobutan-1-ol (**53**) in Flow

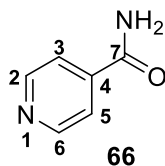


Two stock solutions were prepared, namely 2-nitrobutanol **52** (1.190 M) and hydrazine monohydrate **61** (0.592 M), both using IPA as solvent. IPA was used as pushing solvent. The reactor setup consisted of a T-piece feeding into a packed column of Raney-Nickel (3.4 mL packed void volume). 40 mL of each stock was pumped through the system (FR = 0.680 mL.min⁻¹, *R_T* = 5 min, *T* = 90 °C). The solvent was removed from the collected crude product *in-vacuo*, and subsequently distilled off at 67 °C under high-vacuum to deliver **53** as a light yellow oil (3.107 g, 85%).

¹H NMR (300 MHz, CDCl₃)¹⁰¹: δ 1.00 ppm (t, *J* = 9 Hz, 3H, H-4), δ 2.04 ppm (m, 2H, H-3), δ 3.87 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.02 ppm (dd, *J* = 12 Hz, 1H, H-1), δ 4.55 ppm (m, 1H, H-2); **¹³C NMR (300 MHz, CDCl₃)**¹⁰¹: δ 10.06 ppm (C-4), δ 23.25 ppm (C-3), δ 62.94 ppm (C-1), δ 90.99 ppm (C-2).

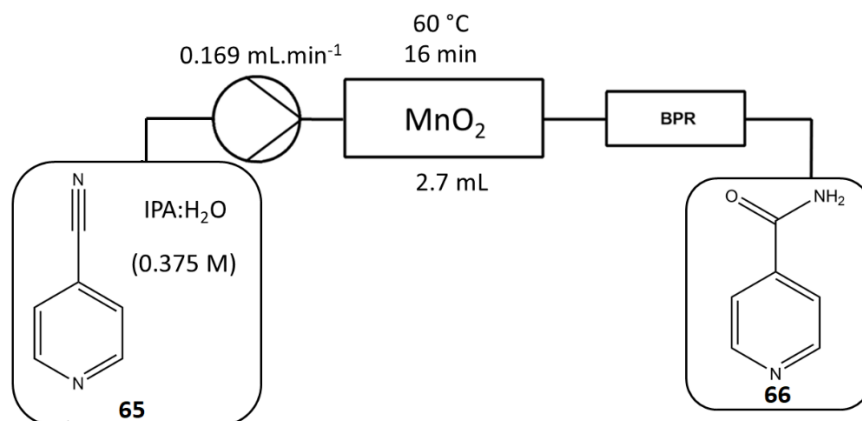
Synthesis of Isonicotinamide in Batch

Isonicotinonitrile **65** (1.562 g, 15 mmol, 1.0 equiv.) was added to a stirring solution of MnO₂ catalyst (0.163 g, 1.9 mmol, 13 mol%) in IPA:H₂O (1:1, 100 mL), and allowed to reflux overnight. The reaction mixture was paper filtered twice while hot and once through Celite to remove all traces of catalyst. The solvent was removed *in vacuo*, requiring multiple recoveries of sublimed product from the condenser. Once all the crude product was collected, the obtained solid was recrystallized from hot ethanol to deliver isonicotinamide **66** as a white solid (0.861 g, 47%).



¹H NMR (300 MHz, MeOD)⁹⁴: δ 7.84 ppm (d, *J* = 6 Hz, 2H, H-2/6), δ 8.72 ppm (d, *J* = 6 Hz, 2H, H-3/5); ¹³C NMR (300 MHz, MeOD)⁹⁴: δ 121.74 ppm (C-3/5), δ 142.05 ppm (C-4), δ 149.57 ppm (C-3/5), δ 168.35 ppm (C-7).

Synthesis of Isonicotinamide in Flow



A solution of pushing solvent was prepared (IPA:H₂O 1:1). The reactor setup consisted of a single reagent feed line connected to a packed-bed reactor (PBR) housing MnO₂ (2.7 mL) and the PBR was mounted in a column heater. The PBR was then directly connected to a BPR prior to collection. A total of 40 mL of isonicotinonitrile stock solution **65** (0.375 M, IPA:H₂O 1:1) was pumped (FR = 0.169 mL.min⁻¹, T_R = 16 min, T = 60 °C) through a packed column of MnO₂ (4.7 mL

with catalyst packed). An additional 5 mL of pushing solvent was pumped through after the theoretical collection time had elapsed in order to collect any tailing product. The solvent was removed *in vacuo*, requiring multiple recoveries from the condenser to collect all crude product. The crude mixture was recrystallized from hot ethanol to obtain the pure product **66** (1.342 g, 73%).

¹H NMR (300 MHz, MeOD)⁹⁴: δ 7.84 ppm (d, J = 6 Hz, 2H, H-2/6), δ 8.72 ppm (d, J = 6 Hz, 2H, H-3/5); **¹³C NMR (300 MHz, MeOD)⁹⁴:** δ 121.74 ppm (C-3/5), δ 142.05 ppm (C-4), δ 149.57 ppm (C-3/5), δ 168.35 ppm (C-7).

Attempted synthesis of Isoniazid in batch

Isonicotinamide (1.391 g, 11.4 mmol, 1 equiv.) was added to a stirring solution of Fe(NO₃)₃·9H₂O catalyst (83 mg, 342 μ mol, 3 mol%) in EtOH (120 mL) and H₂O (2 mL). After stirring for 5 min, aqueous hydrazine monohydrate solution (1.0 mL, 64%, 1.2 equiv.) was added dropwise to the reaction mixture. The reaction was refluxed overnight. TLC analysis seemed to indicate the formation of a new compound. The RM was hot-filtered through celite and the solvent removed *in vacuo*. Only starting material was recovered.

Attempted synthesis of Isoniazid in Flow

A 2:1 ethanol:H₂O (v/v) solution was prepared as pushing solvent. Two stock solutions were prepared, namely isonicotinamide (0.5 M) and hydrazine monohydrate (1.5 M). The reactor setup consisted of two reagent lines meeting at a T-piece, which fed first into a 1 mL mixing chip, followed by a heated 20 mL coil and finally a BPR. Residence times of 10, 15, 20, and 30 min were attempted, at temperatures of 50, 60, 70, 80, and 90 °C. None of the experiments delivered any isolatable product and only starting material was recovered.

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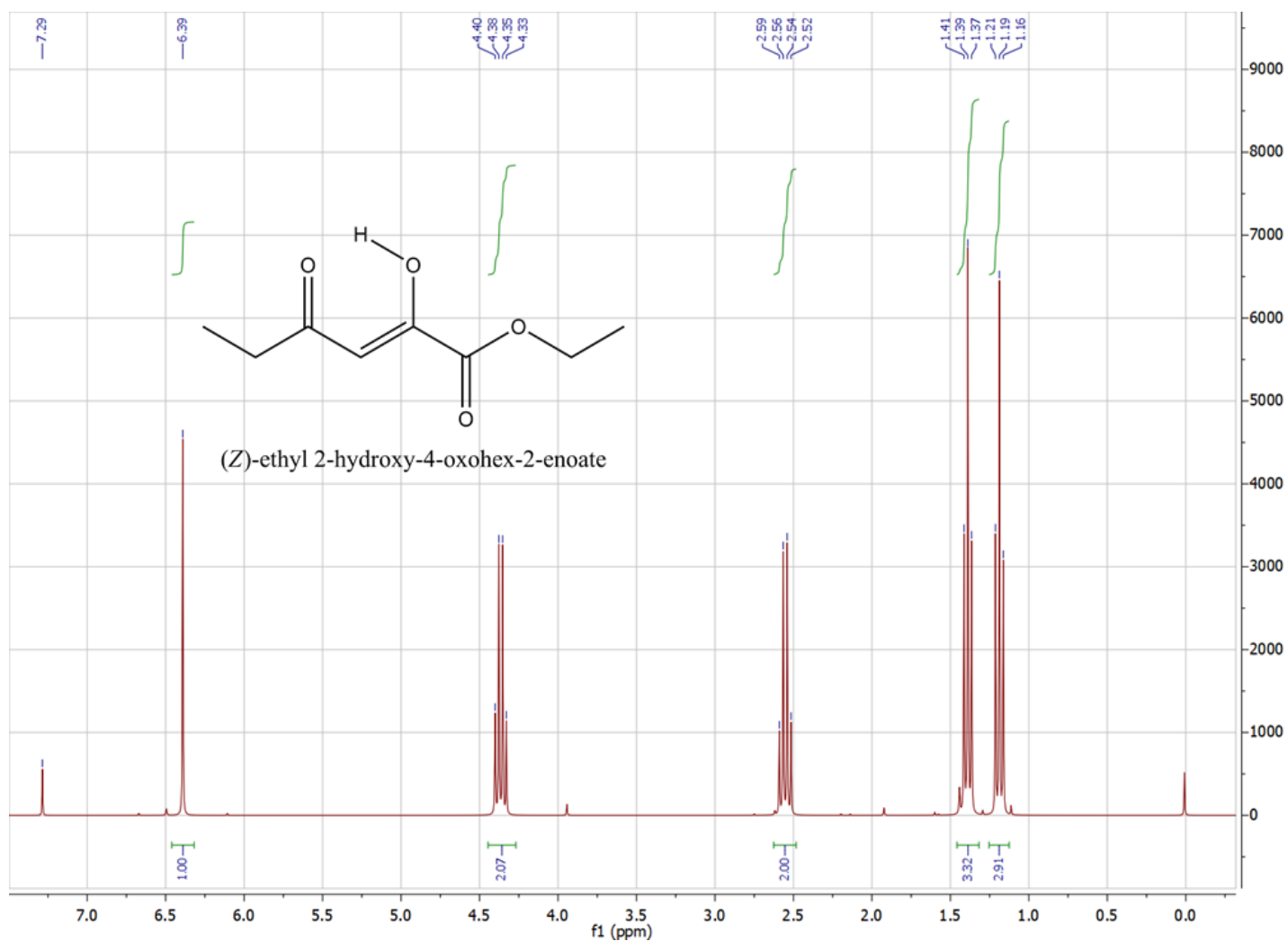
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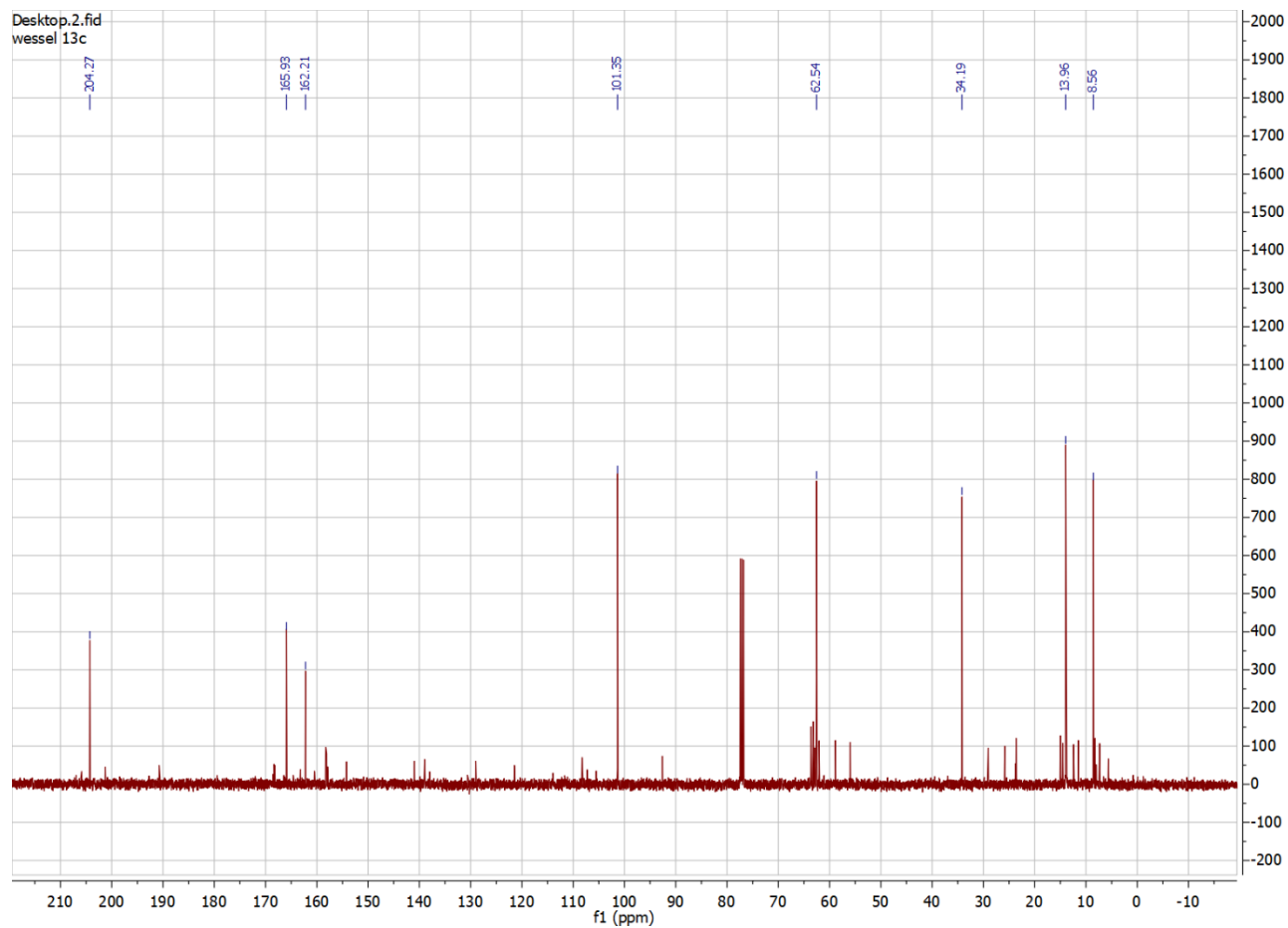
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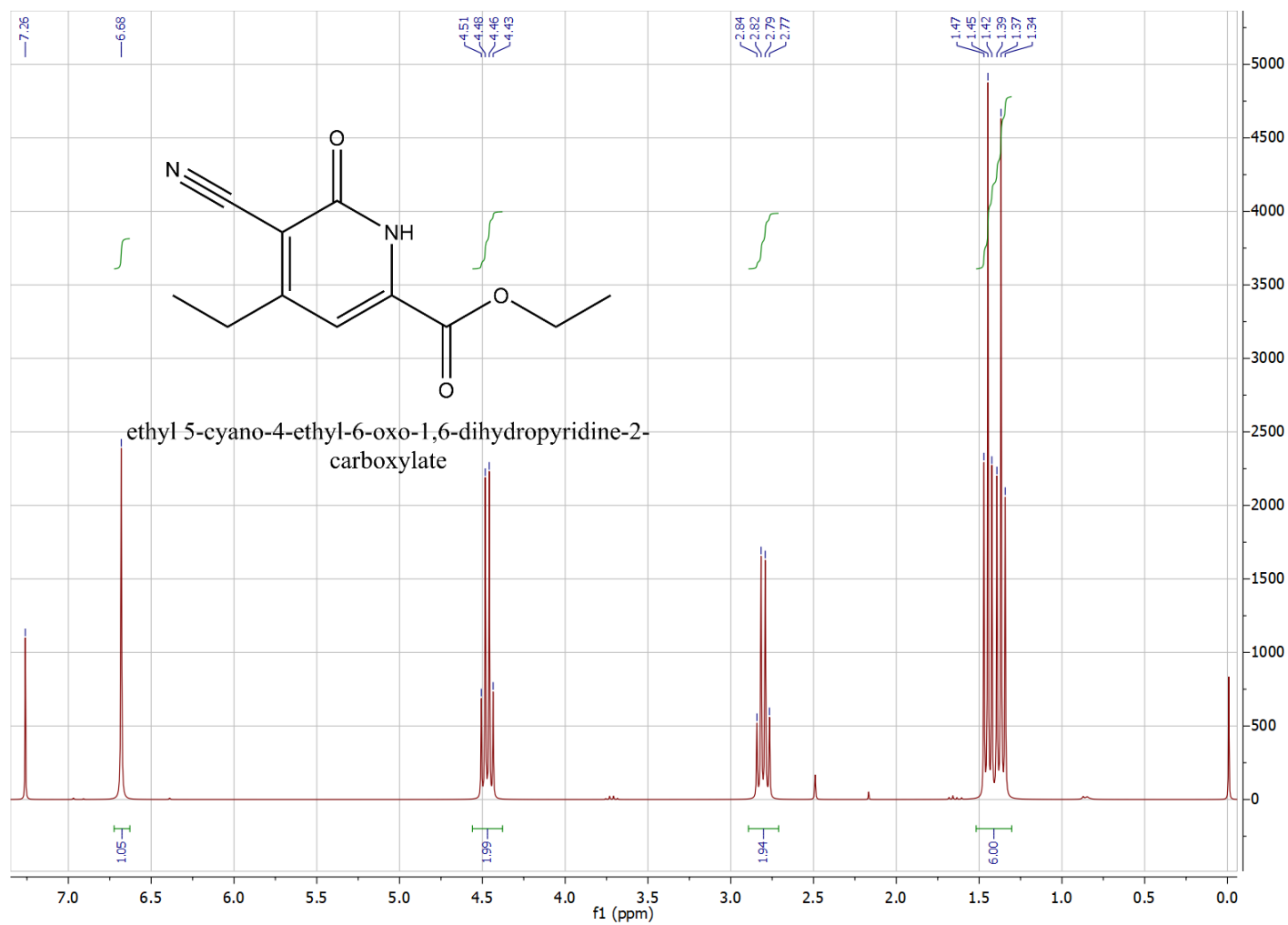
Appendixes

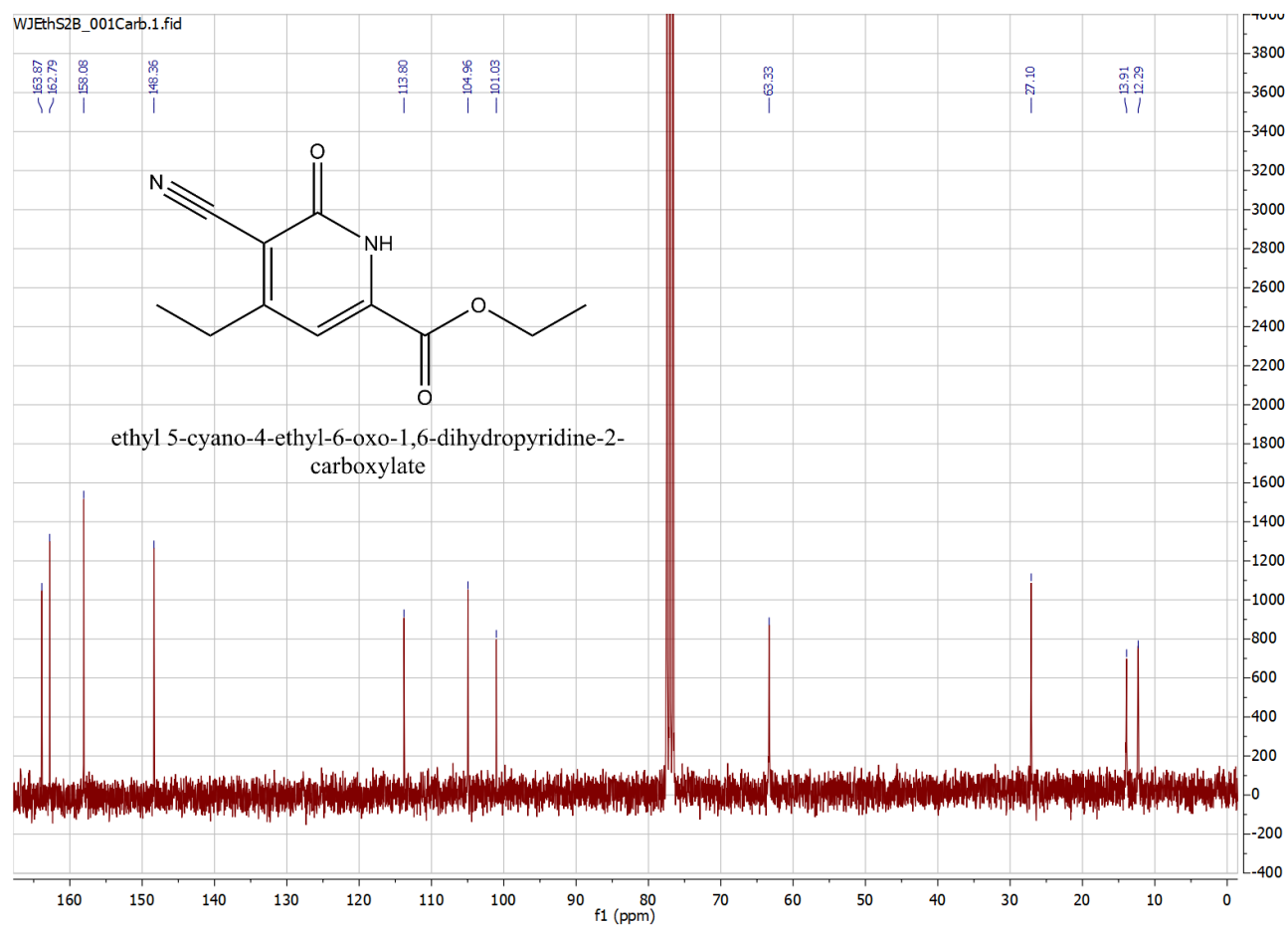
Appendix A – NMR Spectra

A1 - (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate ^1H Spectrum

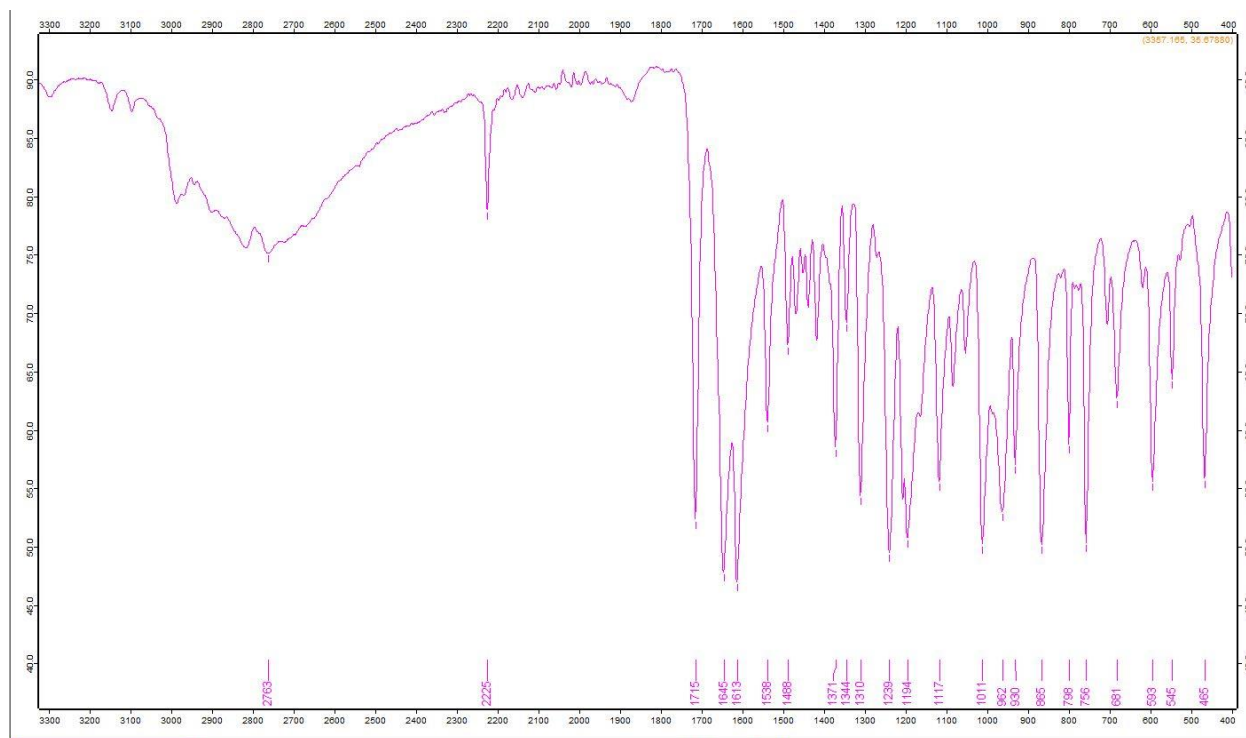


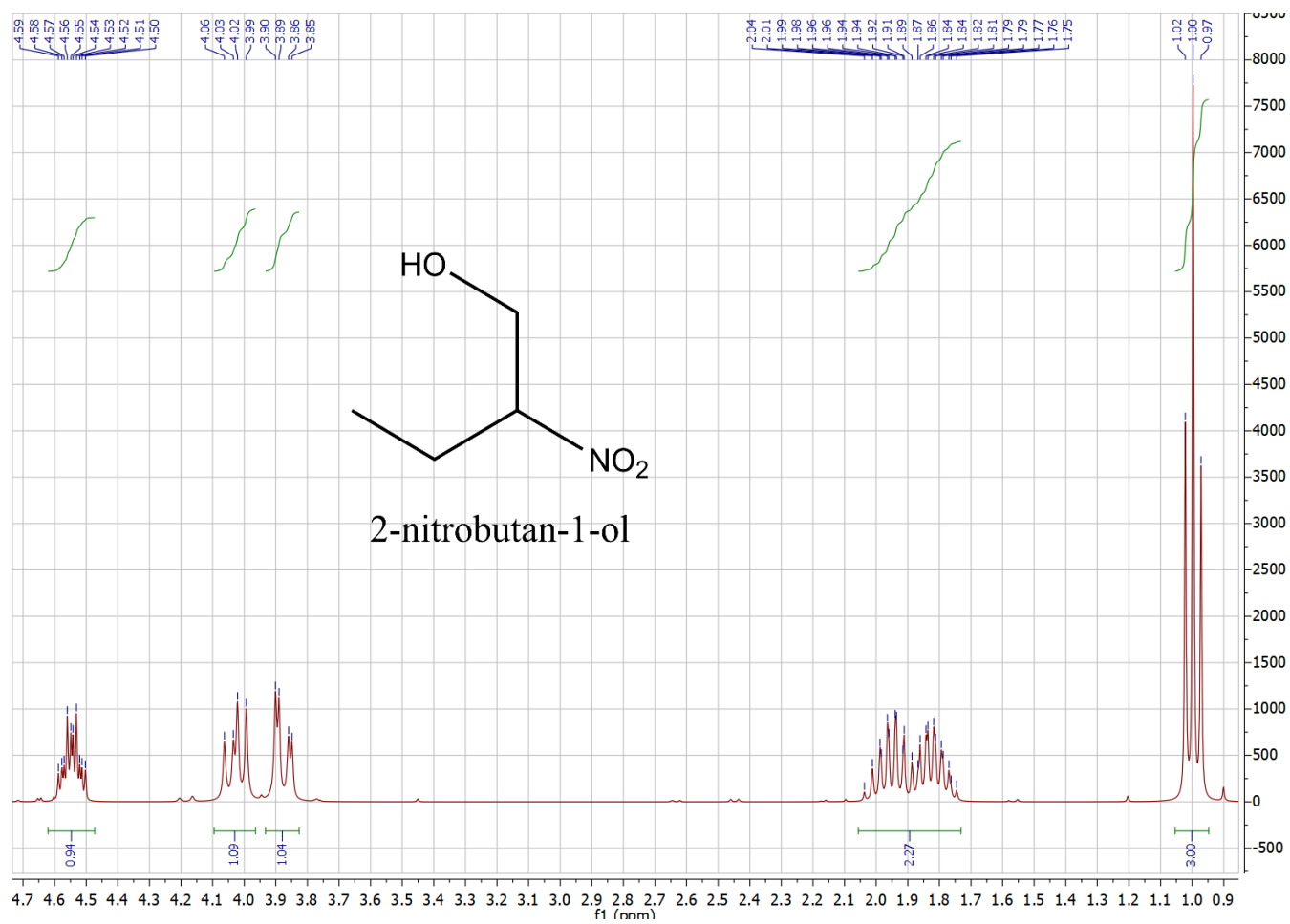
A2 - (Z)-ethyl 2-hydroxy-4-oxohex-2-enoate ^{13}C Spectrum

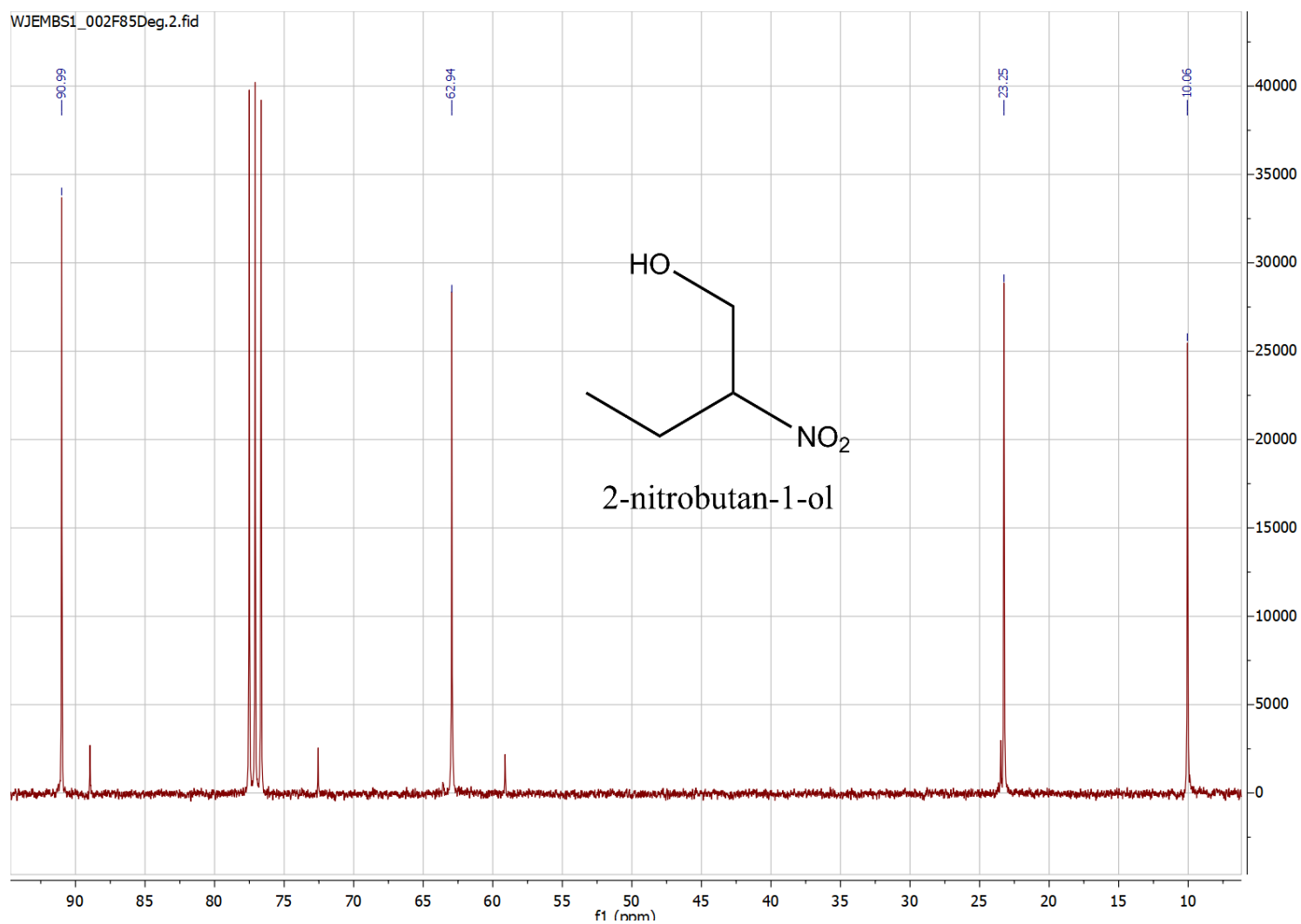
A3 - Ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate ^1H Spectrum

A4 - Ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate ^{13}C Spectrum

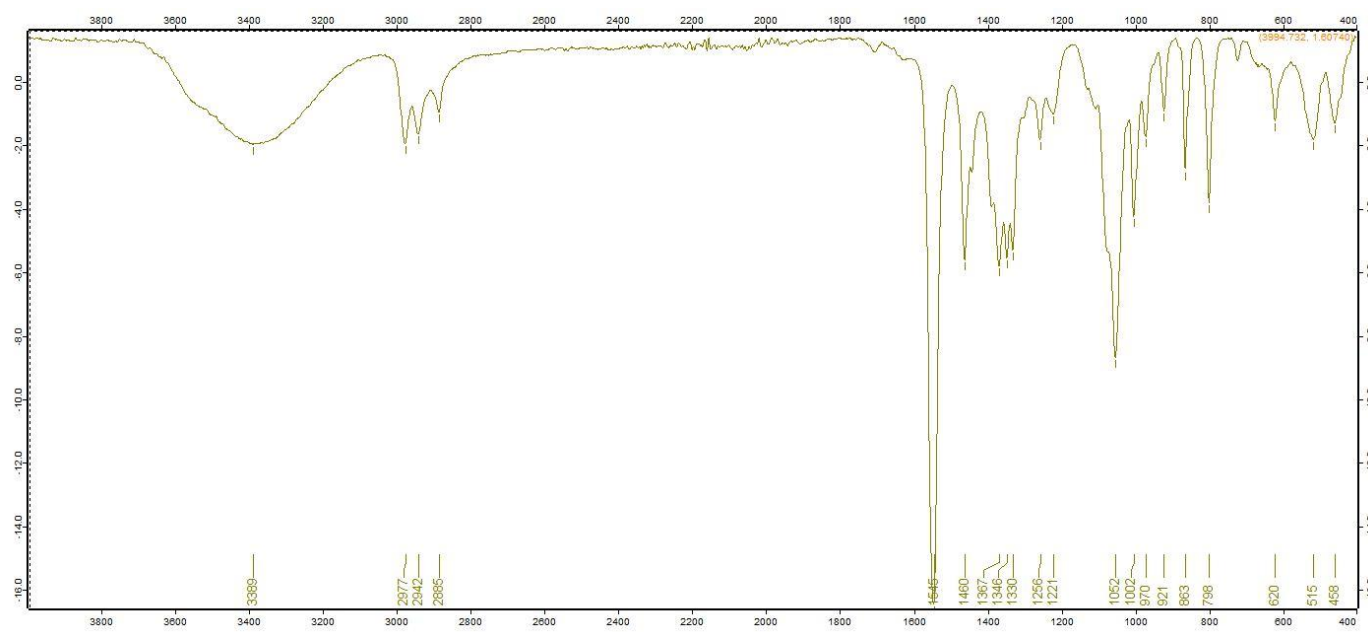
A5 - Ethyl 5-cyano-4-ethyl-6-oxo-1,6-dihydropyridine-2-carboxylate IR spectrum

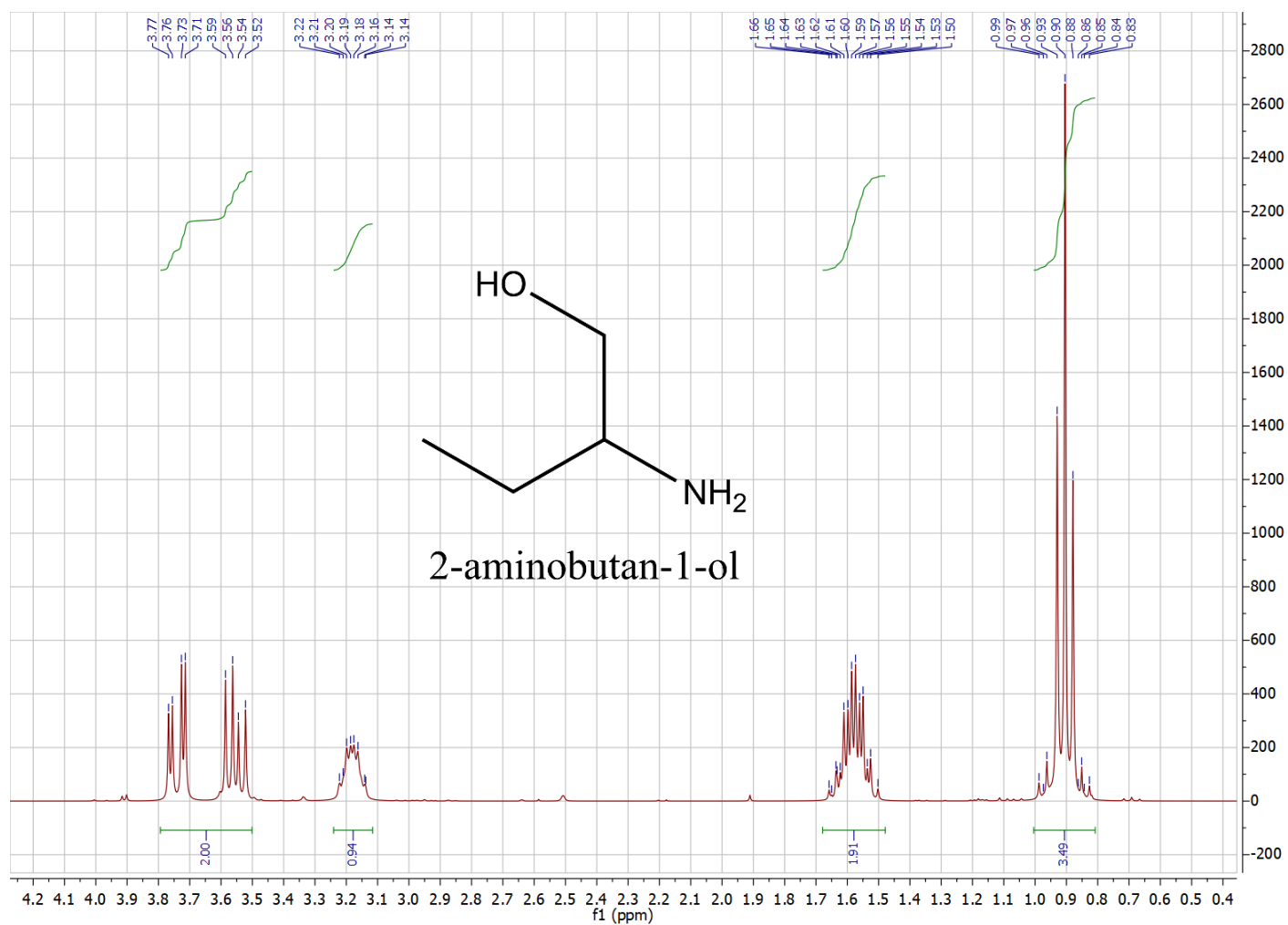


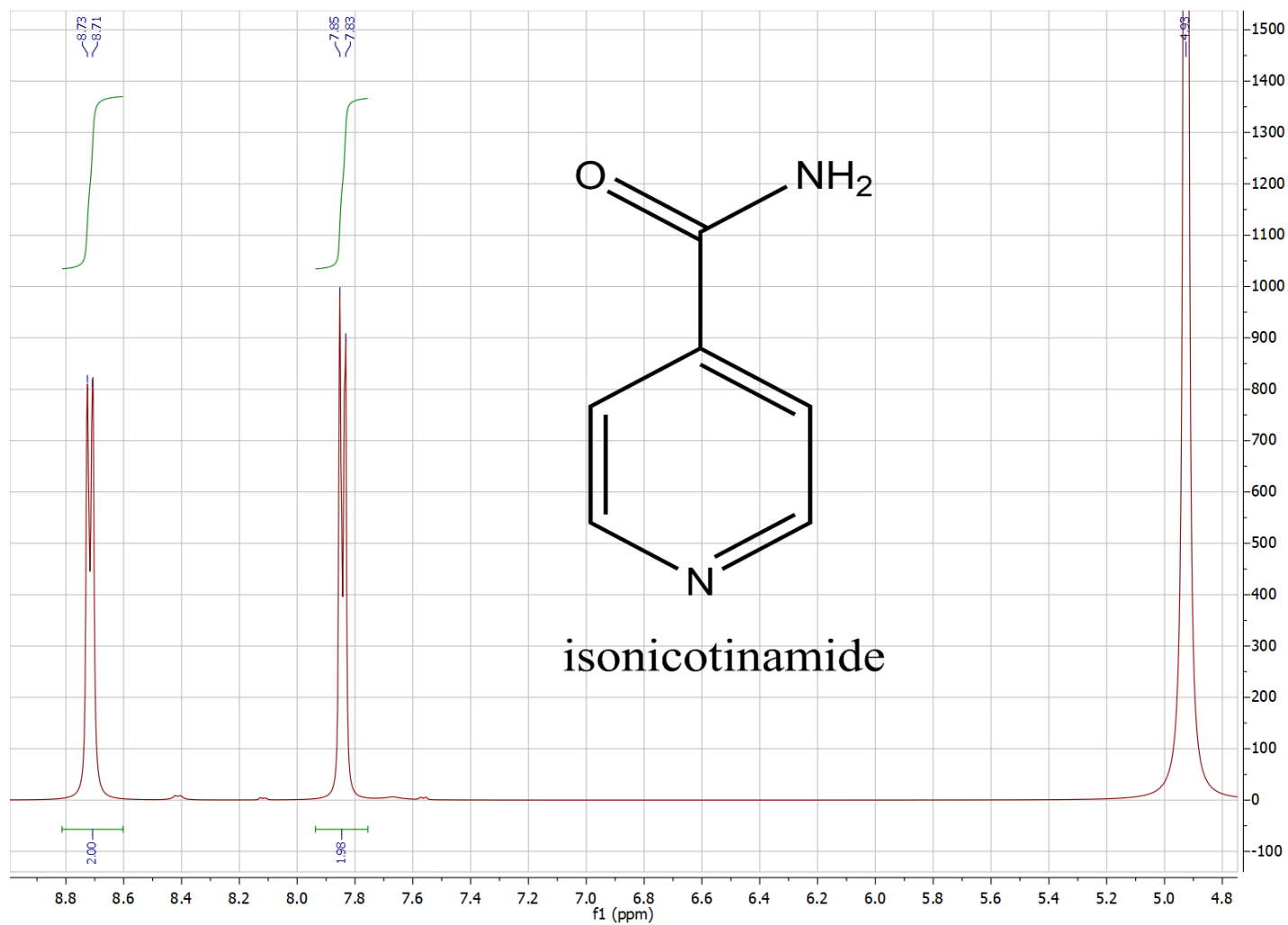
A6 - 2-Nitrobutan-1-ol ^1H Spectrum

A7 - 2-Nitrobutan-1-ol ^{13}C Spectrum

A8 – 2-Nitrobutanol IR spectrum



A9 - 2-Aminobutan-1-ol ^1H Spectrum

A10 - Isonicotinamide ^1H Spectrum

A11 - Isonicotinamide ^{13}C Spectrum