

A batch-flow hybrid approach for the synthesis of the Schistosomiasis treatment praziquantel

By

Zen Johnston

17062952

Supervisor: Prof. Darren Lyall Riley

Co-supervisor: Dr. Nicole Candice Neyt

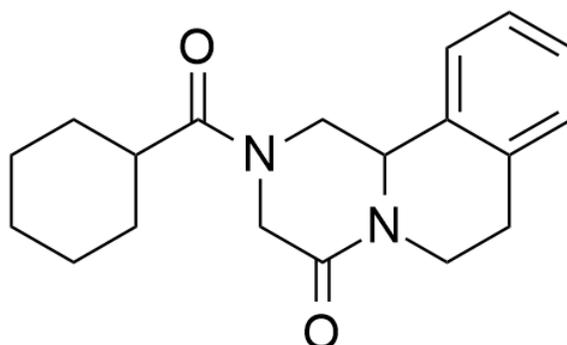
Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae Chemistry

in the Faculty of Natural and Agricultural Sciences

University of Pretoria

2nd December 2022



Declaration

I, Zen Johnston, declare that the dissertation, which I hereby submit for the degree Magister 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.

Signature: 

Date: 02/12/2022

Financial support

This work is based on the research supported wholly by the National Research Foundation (NRF) of South Africa (Application reference number: MND200626537136; Grant number: 130459). I would like to extend my deepest appreciation to the NRF for funding this research project and providing me with this opportunity to better my education and further my career in the field of organic chemistry.



Acknowledgements

I would like to express my deepest gratitude to my excellent supervisor, Prof. Darren Lyall Riley, who is a true expert in the field of research. Without his continual support, understanding, expertise, guidance and motivation, this dissertation would have not been possible.

I would like to extend this appreciation to my co-supervisor, Dr. Nicole Candice Neyt, who is truly brilliant in the field of research. Without her additional guidance, support, understanding and expertise, this dissertation would not have been possible.

I am very grateful for Prof. Riley's research group, Lorinda, Michelle, Lerato, Wessel and Bernice, who have been very supportive friends and excellent laboratory partners.

I would also like to thank Reinard, Clarissa and Simphiwe for being true friends at university. They have shared laughter, love, and support throughout these past two years, which I am extremely grateful for.

I would like to thank the University of Pretoria for the utilisation of the Chemistry Department's facilities and instrumentation which made this research study possible. Furthermore, I'd like to extend this gratitude to the staff who prepared and trained me to make use of the necessary equipment.

I would like to send my deepest appreciation to my loving and supportive parents, Helen and Russell Johnston, who are my role models and have always pushed me to my full potential, ensuring that I achieve what I have set out to. Without their unconditional love, motivation and stress relieving support, this dissertation write-up would have been a much harder and stress filled process.

A special thanks to my brother, Keegan Johnston, who has always supported me in achieving what I set out to. He is an absolute role model and always provides me with emotional strength when it is required. Without his continual support, unconditional love and motivation, this dissertation write-up would have been a much harder process.

Finally, I'd like to express my sincerest gratitude and appreciation to my girlfriend and best friend, Kyra Bawden, who has been there for me throughout this dissertation write-up. Without her unconditional love, support, and motivation, this dissertation process would have been a lot harder, and stress filled.

Abstract

The synthesis and development of active pharmaceutical ingredients (APIs) within the pharmaceutical industry has been achieved using traditional batch production methods for more than a century now. Batch production chemistry on a small scale, within the laboratory, is the use of glassware and manually adding reagents together in order to obtain a final product. However, in the last 20 years, the gradual introduction of continuous flow chemistry has had positive impacts on many fields of synthesis. Continuous flow chemistry uses pressure regulated tubing and pumps to pass reagents at a continuous flow rate through micro-reactors and mixing plates, chips, or coils where the chemical reaction can take place and the resultant product is pumped out of the reactor and obtained for further processing. The use of flow chemistry within the manufacturing of APIs over the last two decades has already shown to be highly productive, at lowered expenses, with reduced waste, all resulting in smaller production footprints. Multiple micro-reactors can be coupled to one another in order to achieve multi-step syntheses. With a rapid increase in publications, the use of flow chemistry is being extensively studied in order to determine where this field of study can be implemented and used to improve the efficiency of medicinal drug synthesis.

Neglected Tropical Diseases (NTDs) are a major concern for many countries throughout the southern hemisphere, and unfortunately are disregarded by the global North as it is not prevalent within majority of these regions. As a result, this means research and development (R&D) into the disease as well as the push to manufacture and supply is not actively pursued and attractive to big pharmaceutical establishments who are more concerned with diseases of the global North like cardiovascular disease and cancer. Schistosomiasis is the focal NTD of this research study with the approved treatment, praziquantel being the API of interest for flow translation.

Here within, we have reported a batch-flow hybrid synthetic procedure for the preparation of praziquantel. This approach consists of three steps whereby the first two steps are flow based and the final step for the conversion to praziquantel is a batch based synthetic step. The first step for the preparation of praziquantel consists of a modified Hofmann procedure for the preparation of the noxious 2-isocynoethylbenzene from the starting material, 2-phenethylamine. Although we initially faced precipitate issues, leading to fouling of the micro-reactors, we were finally successful in translating this step into flow conditions with an optimised yield of 78% with a residence time of 195 min. Critically, when compared with our in-house batch approach for the preparation of this material, we were able to achieve a slight increase in yield of ~ 6% (72% for batch procedure) with a decrease in the reaction time of 45 min (240 min batch procedure).

The second step for the preparation of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide consists of an Ugi four-component reaction whereby 2-isocyanoethylbenzene is condensed with formaldehyde, aminoacetaldehyde dimethyl acetal and cyclohexanecarboxylic acid, all in the polar protic solvent, methanol. Again, we were successful in translating this synthetic step under flow conditions with a good yield of 87% with a residence time of only 60 min. Notably, when compared with our in-house batch procedure, we were able to achieve a comparable yield (89% batch), however, with an appreciable reduction in the reaction time of 47 h (48 h batch). Furthermore, we were able to provide a proof of concept for a telescoped step 1 and 2 for the preparation of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide in an overall yield of 55%. Critically, this was achieved with an off-line separation of the noxious 2-isocyanoethylbenzene from the first step, however, this opens the potential to an integrated in-line separation of 2-isocyanoethylbenzene for the synthesis and consumption of this noxious material on-the-fly.

The final step for the conversion of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide to praziquantel involves an intramolecular Pictet-Spengler type reaction which was performed using traditional batch-based chemistry controls. Unfortunately, we were unsuccessful in translating this step under flow conditions, due to challenges faced with the sodium sulfate additive, however, it exhibits green chemistry techniques being performed solventless with the use of the green, methanesulfonic acid (MSA). We were able to prepare praziquantel in a moderate yield of 61% (81% purity) for this stand-alone step with a reaction time of 6 h. Overall, this entire developed procedure allowed for an overall yield of 41% in 10.25 h, which amounts to a space-time productivity of 0.93 g.L⁻¹.h⁻¹ for the preparation of praziquantel.

List of abbreviations

3D – Three-dimensional

ACN – Acetonitrile

AIBN – Azobisisobutyronitrile

AIDS – Acquired immunodeficiency syndrome

API/s – Active pharmaceutical ingredient/s

BPR/s – Back pressure regulator/s

BTEAC – Benzyltriethylammonium chloride

CDC – Centers for Disease Control and Prevention

COSY – ¹H-¹H Correlation spectroscopy

COX – Cyclooxygenase

CSIR – Council for Scientific and Industrial Research

DAD – Diode array detector

DALYs – Disability adjusted life years

DCC – *N,N'*-Dicyclohexylcarbodiimide

DCE – Dichloroethane

DCM – Dichloromethane

DDAB – Didodecyldimethylammonium bromide

DFT – Density functional theory

DIPEA – *N,N*-Diisopropylethylamine

DMAP – 4-*N,N*-Dimethylaminopyridine

DMF – Dimethylformamide

DMSO – Dimethyl sulfoxide

DNDi – Drugs for Neglected Diseases initiative

DSI – Department of Science and Innovation

DTI – Department of Trade and Industry

EDC/EDCI – 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide / hydrochloride

ESI – Electrospray ionisation

FEP – Fluorinated ethylene-propylene
FOREX – Foreign exchange
FTIR – Fourier-transformed infrared
h – Hour/s
HIV – Human immunodeficiency virus
HMBC – Heteronuclear multiple bond correlation
HOAt – 1-Hydroxy-7-azabenzotriazole
HOBt – Hydroxybenzotriazole
HPLC – High performance liquid chromatography
HRMS – High-resolution mass spectrometry
HSQC – Heteronuclear single quantum coherence
HTTP – Hypertext Transfer Protocol
IDM – Intensified disease management
IR – Infrared
IRMPD – Infrared multiphoton dissociation
JSON – JavaScript Object Notation
LAN – Local area network
LC – Liquid chromatography
LF – Lymphatic filariasis
MDA – Mass drug administration
MDG/s – Millennium Development Goal/s
MFC – Mass flow controller
min – Minute/s
MS – Mass spectrometry
MSA – Methanesulfonic acid
NCE – New chemical entity
NFPA – National Fire Protection Association
NMR – Nuclear magnetic resonance

NMU – Nelson Mandela University

NTD/s – Neglected tropical disease/s

PBT – Persistent, bioaccumulative and toxic

PCT – Preventative chemotherapy treatment

PEEK – Polyether ether ketone

PFA – Perfluoroalkoxy alkanes

PPE – Personal protective equipment

PTC – Phase transfer catalyst

PTFE – Polytetrafluoroethylene

PyBroP – Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate

R&D – Research and development

R_f – Retention factor

SC-XRD – Single crystal X-ray diffraction

S_N2 – Nucleophilic substitution bimolecular reaction

SOBO – Single-Objective Bayesian Optimiser

SQL – Structured Query Language

SSA – Sub-Saharan Africa

STH – Soil-transmitted helminthiasis

TB – Tuberculosis

TEA – Triethylamine

THF – Tetrahydrofuran

TLC – Thin-layer chromatography

TMS – Tetramethylsilane

TMSN₃ – Trimethylsilyl azide

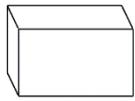
UP – University of Pretoria

UV – Ultraviolet

vPvB – Very persistent and very bioaccumulative

WHO – World Health Organisation

Diagram legend



Sonicator



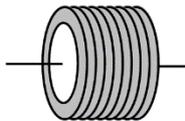
Peristaltic pump



HPLC pump



Back pressure regulator

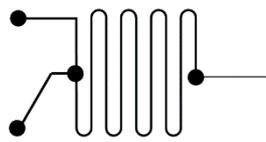


XX mL
XX °C

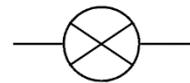
Tubular coil reactor



SAD
selector valve



Micro-chip reactor



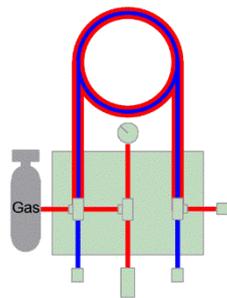
Selector valve



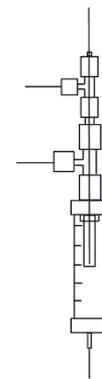
Gas source



Mixing unit



Tube-in-tube reactor



Solvent swapper



Triturator



Packed-bed reactor



Liquid-liquid
separator



Mass flow controller

Table of Contents

Declaration	i
Financial support	ii
Acknowledgements	iii
Abstract	iv
List of abbreviations	vi
Diagram legend	ix
CHAPTER I	1
1.1 Problem Statement	1
1.2 Proposed Solution	2
1.3 Potential impact of research in industry and society	2
1.3.1 Industrial impact	2
1.3.2 Societal and environmental impact	3
1.4 Introduction to Flow Chemistry	4
1.4.1 Principles of flow chemistry	4
1.4.2 Basic set-up of micro-reactor system and components of flow chemistry	5
1.4.2.1 Tubing and connections between flow components	6
1.4.2.2 Solvent bottles and reagent stock solutions	7
1.4.2.3 Reagent introductory devices used within continuous flow systems	8
1.4.2.4 Mixing units	11
1.4.2.5 Flow reactor units	13
1.4.2.6 Back pressure regulators (BPRs)	16
1.4.2.7 Waste/collection selector valve	17
1.4.3 Advantages and Disadvantages of flow chemistry	17
1.4.4 Examples of in-house research with the utilisation of continuous flow conditions	20
1.4.4.1 Batch-Flow hybrid synthesis of the antipsychotic Clozapine	21
1.4.4.2 Flow synthesis of the COX-2 inhibitor celecoxib	23
1.4.4.3 A continuous flow approach for the synthesis of aryldiazonium tetrafluoroborates	25
1.4.4.4 Selective reductions of aldehydes	26
1.4.4.5 Flow-ozonolysis with on-the-fly removal of ozone	27
1.4.4.6 Implementing an automated continuous flow controlling platform for flow chemistry components	28
1.5 Introduction to Neglected Tropical Diseases (NTDs)	30
1.5.1 A brief history and overview of NTDs	30

1.5.2 NTD categories outlined by the WHO with a focus on preventative chemotherapy treatment (PCT) grouped NTDs.....	32
1.5.3 The way forward with regard to NTDs.....	35
1.5.4 Schistosomiasis, the focal NTD of this research project.....	35
1.5.4.1 Introduction and transmission of schistosomiasis.....	35
1.5.4.2 Species of transmission of schistosomiasis.....	37
1.5.4.3 Symptoms and containment of schistosomes within the human body.....	37
1.5.4.4 Occurrences of schistosomiasis and its primary treatment.....	38
1.5.5 Previously reported synthetic approaches to Praziquantel.....	40
1.5.5.1 <i>J. Seubert (E.Merck Company)</i>	40
1.5.5.2 <i>Kim et al. (Shin Poong)</i>	41
1.5.5.3 <i>Berkowitz et al.</i>	41
1.5.5.4 <i>Kim et al.</i>	42
1.5.5.5 <i>Todd et al.</i>	43
1.5.5.6 <i>Ma et al.</i>	45
1.5.5.7 <i>Yixing Xinyu Chemical Co.</i>	47
1.5.5.8 <i>El-Fayyoumy et al.</i>	48
1.5.5.9 <i>Roszkowski et al.</i>	49
1.5.5.10 <i>Cao et al.</i>	49
1.5.5.11 <i>Shou et al.</i>	50
1.6 Initially envisioned flow synthesis of praziquantel 1	52
1.7 Aims	54
1.8 Proposed research objectives and timelines	54
1.8.1 Objective 1: Assessment of praziquantel 1 as a suitable target.....	54
1.8.2 Objective 2: Batch validation of process routes.....	54
1.8.3 Objective 3: Translation and optimization of the process under flow conditions.....	54
1.8.4 Objective 4: Telescoping of flow processes at milligram to multigram gram scale.....	55
CHAPTER II	56
2.1 A flow approach for the synthesis of 2-isocyanoethylbenzene 96 via the modified Hofmann Carbylamine procedure	56
2.1.1 Brief history and chemistry of the isocyanide functional group.....	56
2.1.2 Synthesis of 2-isocyanoethylbenzene 96 using batch-based chemistry methodologies.....	58
2.1.3 Previous synthetic procedures for the preparation of isocyanides under flow conditions.....	59
2.1.4 Batch validation and optimisation of literature method using the Hoffman approach.....	61
2.1.5 Synthesis of isocyanide 96 using the formamide dehydration approach.....	65

2.1.6 Synthesis of 2-isocyanoethylbenzene 96 under flow conditions following the modified Hofmann procedure.....	65
2.1.7 Concluding remarks	73
CHAPTER III	74
3.1 A flow approach for the preparation of <i>N</i>-(2,2-dimethoxyethyl)-<i>N</i>-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide 61	74
3.1.1 Brief history of the Ugi multi-component reaction	74
3.1.2 Ugi four-component reactions under flow conditions	76
3.1.3 “Pre-praziquantel” intermediate 61 synthesis under batch conditions	80
3.1.4 Preparation of “pre-praziquantel” intermediate 61 under continuous flow conditions.....	90
3.1.5 Trial telescoping of stages 1 and 2.....	94
3.1.6 Concluding remarks	95
CHAPTER IV	96
4.1 Stage 3 – Pictet-Spengler cyclisation to afford praziquantel 1	96
4.1.1 Brief history of the Pictet-Spengler reaction	96
4.1.2 Pictet-Spengler reactions previously reported under flow conditions.....	98
4.1.3 Preparation of praziquantel 1 under batch conditions.....	99
4.1.4 Continuation of praziquantel 1 synthesis under batch conditions.....	106
4.1.5 Praziquantel 1 synthesis under flow conditions	107
4.1.6 Concluding remarks	111
CHAPTER V	112
5.1 Techno-economic and safety analysis	112
5.1.1 Cost-of-goods analysis	112
5.1.2 Comparison between in-house developed process and that reported by Cao <i>et al.</i> ⁹⁵	112
5.1.2.1 Step 1	112
5.1.2.2 Step 2	112
5.1.2.3 Step 3	113
5.1.2.4 Comparison between in-house process and two current patents CN111072656A ¹⁶² and CN114195782A ¹⁶³	113
5.1.3 Project specific hazard analysis.....	114
5.1.3.1 Summary of hazards	114
5.1.3.2 Preliminary risk analysis.....	125
5.1.3.3 Chemical interaction data	145
5.1.3.4 Material compatibilities	146
5.1.3.5 Effluent specifications.....	146
CHAPTER VI	147

6.1 Conclusions	147
6.2 Future work	148
6.2.1 Step 1	148
6.2.2 Step 2	149
6.2.3 Step 3	150
CHAPTER VII	152
7.1 Experimental	152
7.1.1 General experimental techniques/details	152
7.1.2 Chromatographic separations.....	152
7.1.3 Spectroscopy and physical data.....	153
7.1.4 SC-XRD Experimental	154
7.1.5 Nomenclature and numbering.....	154
7.2 Chapter II Experimental	154
7.2.1 Preparation of 2-isocyanoethylbenzene 96 , validation of the Cao <i>et al.</i> ⁹⁵ methodology:	154
7.2.2 Optimisation of 2-isocyanoethylbenzene 96 synthesis under batch conditions (Table 2, entries 1 – 8):	155
7.2.3 Formamide dehydration approach for 2-isocyanoethylbenzene 96 adapted from Patil <i>et al.</i> ¹¹⁵	156
7.2.4 Flow synthesis of 2-isocyanoethylbenzene 96 (Table 3, entries 1 – 8):.....	156
7.2.5 Flow synthesis of 2-isocyanoethylbenzene 96 (Table 3, entries 9 – 10):.....	157
7.2.6 Flow synthesis of 2-isocyanoethylbenzene 96 (Table 3, entries 11 – 12):.....	159
7.2.7 Flow synthesis of 2-isocyanoethylbenzene 96 (Table 3, entries 13 – 14):.....	160
7.2.8 Flow synthesis of 2-isocyanoethylbenzene 96 (Table 3, entries 15 – 18):.....	161
7.3 Chapter III Experimental	163
7.3.1 Preparation of <i>N</i> -(2,2-dimethoxyethyl)- <i>N</i> -(2-oxo-2-(2-phenethylamino)ethyl)-cyclohexanecarboxamide (“pre-praziquantel”) 61 , validation of the methodology reported by Cao <i>et al.</i> ⁹⁵	163
7.3.2 Method 1 – Batch approach using paraformaldehyde (Table 5, entry 2):	164
7.3.3 Method 2 – Batch approach using formalin (Table 5, entry 3):.....	164
7.3.4 Method 3 – Batch approach using gaseous formaldehyde (Table 5, entry 4):.....	164
7.3.5 Synthesis of <i>N</i> -(2,2-dimethoxyethyl)- <i>N</i> -(2-oxo-2-(2-phenethylamino)ethyl)-cyclohexanecarboxamide (“pre-praziquantel”) 61 under flow conditions (Table 6, entries 1 – 9):	165
7.3.6 Optimised synthesis of <i>N</i> -(2,2-dimethoxyethyl)- <i>N</i> -(2-oxo-2-(2-phenethylamino)-ethyl)cyclohexanecarboxamide (“pre-praziquantel”) 61 under flow conditions (Table 6, entries 10 – 13):	166
7.3.7 Telescoped synthesis of <i>N</i> -(2,2-dimethoxyethyl)- <i>N</i> -(2-oxo-2-(2-phenethylamino)-ethyl)cyclohexanecarboxamide (“pre-praziquantel”) 61 trial reaction:.....	168

7.4 Chapter IV Experimental	170
7.4.1 Synthesis of 2-(cyclohexylcarbonyl)-2,3,6,7,11b-hexahydro-4 <i>H</i> -pyrazino[2,1- <i>a</i>]isoquinolin-4-one (praziquantel) 1 , validation of the methodology reported by Cao <i>et al.</i> ⁹⁵ (Table 7, entry 2):	170
7.4.2 Attempted synthesis of praziquantel 1 using MSA with DCM (Table 7, entry 4):	171
7.4.3 Attempted synthesis of praziquantel 1 using <i>p</i> -toluenesulfonic acid with MeOH (Table 7, entry 7):.....	171
7.4.4 Attempted synthesis of praziquantel 1 using Amberlyst-15 with MeOH (Table 7, entry 8):	171
7.4.5 Attempted synthesis of praziquantel 1 using <i>p</i> -toluenesulfonic acid with DCM (Table 7, entry 9):	171
7.4.6 Attempted synthesis of praziquantel 1 using Amberlyst-15 with DCM (Table 7, entry 10): .	172
7.4.7 Attempted synthesis of praziquantel 1 under flow conditions (Table 9, entries 1 – 2):	172
7.4.8 Attempted synthesis of praziquantel 1 under flow conditions (Table 9, entries 3 – 5):	173
7.4.9 Batch preparation of praziquantel 1 using sodium sulfate as an additive (Table 10, entry 3):	174
7.4.10: UPLC raw data for the separation of “pre-praziquantel” 61 and praziquantel 1	175
CHAPTER VIII	177
8.1 References	177
Appendix A (Chapter II)	186
A – 1: ¹ H-NMR, ¹³ C-NMR and Dept 135 spectra of 2-isocyanoethylbenzene 96	186
A – 2: ¹ H-NMR and ¹³ C-NMR spectra of 2-phenethylamine 41	188
A – 3: Infrared spectroscopy (400 – 4000 cm ⁻¹) smoothed spectrum and min-max normalised spectrum of 2-isocyanoethylbenzene 96	189
A – 4: Mass spectroscopy spectra of 2-isocyanoethylbenzene 96	190
Appendix B (Chapter III)	191
B – 1: ¹ H-NMR, ¹³ C-NMR, Dept 135, HSQC, HMBC and COSY spectra of <i>N</i> -(2,2-dimethoxyethyl)- <i>N</i> -(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide (“pre-praziquantel”) 61	191
B – 2: ¹ H-NMR and ¹³ C-NMR spectra of cyclohexanecarboxylic acid 51	194
B – 3: ¹ H-NMR and ¹³ C-NMR spectra of aminoacetaldehyde dimethyl acetal 43	195
B – 4: Infrared spectroscopy (400 – 4000 cm ⁻¹) smoothed spectrum and min-max normalised spectrum of “pre-praziquantel” 61	196
B – 5: SC-XRD raw data of “pre-praziquantel” 61	197
B – 6: Mass spectroscopy spectra of “pre-praziquantel” 61	201
Appendix C (Chapter IV)	202
C – 1: ¹ H-NMR, ¹³ C-NMR, Dept 135, HSQC, HMBC and COSY spectra of 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4 <i>H</i> -pyrazino[2,1 <i>a</i>]isoquinolin-4-one (praziquantel) 1	202
C – 2: ¹ H-NMR, ¹³ C-NMR and Dept 135 spectra of the obtained pharmaceutical standard praziquantel 1	205

C – 3: Infrared spectroscopy (400 – 4000 cm ⁻¹) smoothed spectrum and min-max normalised spectrum of praziquantel 1	207
C – 4: Infrared spectroscopy (400 – 4000 cm ⁻¹) smoothed spectrum and min-max normalised spectrum of the pharmaceutical standard praziquantel 1	208
C – 5: Mass spectroscopy spectra of praziquantel 1 synthesised during this study (bottom) juxtaposed with the pharmaceutical standard (top).	209

CHAPTER I

1.1 Problem Statement

Africa is home to many diseases and illnesses that result in millions of infections and deaths annually. Many of these infections are neglected tropical diseases (NTDs), such as human African trypanosomiasis, leishmaniasis, leprosy, schistosomiasis and more.¹ These types of diseases are termed neglected as they largely affect impoverished, deprived communities, whereas highly developed countries remain largely unaffected by NTDs. This means that the urge for research and development (R&D) as well as the manufacturing and supplying of drugs for the treatment of these diseases is scarce. Big pharmaceutical establishments, instead, are more invested in non-communicable diseases prevalent in first world countries, which represent more economically attractive targets.²

The demand for pharmaceutical drugs that are capable of curing and/or preventing many of these neglected diseases is very high. Unfortunately, most prescription drugs in Africa are imported from the global market due to a lack of manufacturing infrastructure capable of handling the required supply and demand.² Resultant of this, we face various problems including extreme price fluctuations due to foreign exchange (FOREX) issues, counterfeiting of pharmaceutical products and constant supply and demand issues.

2-(Cyclohexanecarbonyl)-1,2,3,6,7,11b-tetrahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one (praziquantel) **1** (Figure 1), sold under the trade name Biltricide, is a well-known treatment for schistosomiasis, a parasitic worm infection, which affects a substantial portion of the global population annually. According to the World Health Organisation (WHO), it is estimated that at least “236.6 million people” required preventative treatments (praziquantel being the primary) in 2019 for schistosomiasis,³ however, the amount of people reported to have received treatment amounted to just more than 105.4 million (approximately 44.5%).³ Schistosomiasis is widespread through sub-Saharan Africa (SSA) and is observed to be endemic in at least five of the nine provinces within South Africa. Generic praziquantel **1** (Biltricide by Bayer’s), is available regionally, and although it is reasonably cheap, the current demand remains largely unmet.⁴

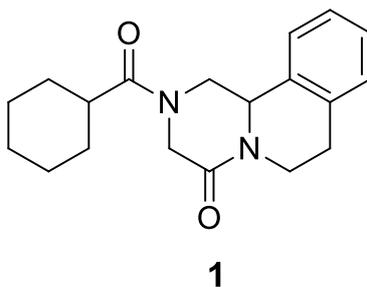


Figure 1: 2-(Cyclohexanecarbonyl)-1,2,3,6,7,11b-tetrahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one. (Praziquantel) **1**

1.2 Proposed Solution

The use of continuous flow manufacturing platforms has, in recent times, received attention as they have small footprints, reduce human error, employ closed system conditions and are convenient to operate. They have been reported to improve product yields, reduce reaction times, are readily up-scaled and often allow synthesis under greener methods.⁵⁻⁸ The cost and demand for imported pharmaceutical drugs in South Africa is high, representing the fifth largest contributor to South Africa's trade deficit. As a result, the development of a pharmaceutical manufacturing sector within South Africa, capable of supplying both local and regional markets, is currently being pursued by both public and private sector players. It is hypothesised that the use of advanced and disruptive manufacturing technologies, such as flow chemistry, could be highly beneficial in realising this goal affording South Africa and Africa the opportunity to leapfrog existing international importers who are currently limited in their abilities to adopt flow technology as a result of capital that is sunk into existing batch manufacturing plants.² With the increased research in flow chemistry, a library of synthetic procedures for the preparation of pharmaceuticals as well as several organic intermediates has been established and will continue to grow over time, promoting its adoption by the pharmaceutical manufacturing sector.

The synthesis of praziquantel **1**, a high-in-demand API, under flow conditions is expected to result in lowered reaction times, greater or comparable overall reaction percentage yields as well as a reduction in waste formation, resulting in safer and greener chemistry controls. In addition, if praziquantel **1** can be mass produced in a more cost and time efficient manner, it will further be improving our confidence in being able to meet the required high demand.

1.3 Potential impact of research in industry and society

1.3.1 Industrial impact

As stated in the bio-economy strategy set out by the Department of Science and Innovation (DSI),⁹ South Africa's national system of health innovation has outlined the development of new drugs, vaccines, diagnostics and medical devices. In 2009, the pharmaceutical industry in South Africa was estimated at a value of R 22.6 billion, however, direct imports of various pharmaceuticals tallied up to R 14.4 billion.⁹ As stipulated within the recent Department of Trade and Industry's (DTI) industrial policy action plan of 2018/19 – 2020/21, the pharmaceutical sector was estimated to have a market value of approximately R 54.1 billion in 2021.¹⁰ The same report outlines the unfortunate heavy reliance on imports of active pharmaceutical ingredients (APIs) at present accounting for R 22 billion in imports alone. The goal of realising a localised pharmaceutical manufacturing sector has been proposed and highlighted within the bio-economy strategy and industrial policy action plan. It has also been

stated that the bio-economy strategy will promote emerging technologies to allow for global competitiveness. The developments and improvements of processes for API production covered within this report will feed into the development of a fledgling pharmaceutical industry in South Africa.

1.3.2 Societal and environmental impact

The research focused on within this report is expected to result in a more economical process for the manufacture of praziquantel **1**, which will in turn have a direct positive impact on the combat of the NTD, schistosomiasis. The implementation and development of a local regional manufacturing sector will result in linked skills development and job creation. Local and/or regional manufacturing will also improve control over supply and demand of pharmaceutical products. Increasing local pharmaceutical production would aid the Pharma sector to meet the ongoing supply demand, in turn reducing the overall disease burden experienced by South Africa and other African countries. A well-developed pharmaceutical manufacturing sector within Africa, will also greatly assist in the fight against NTDs and other illnesses which are largely neglected by the “Global North”.

Unfortunately, there is drug counterfeiting/piracy in South Africa, being driven by the high cost of pharmaceuticals and continued stock-outs. Consequently, many people resort to buying fake or grey pharmaceutical products from unregistered/informal vendors. Local/regional manufacturing is proposed to combat these issues by reducing the price of medicines for the end user and reducing stock-outs. A local manufacturing sector will also require personnel to operate the plants and so another aspect would be development of human capital in the area of advanced manufacturing which is largely missing in South Africa. Currently, there are only two academic continuous flow groups (University of Pretoria (UP) and Nelson Mandela University (NMU)) and one industrial group (Council for Scientific and Industrial Research (CSIR)) that operate and develop syntheses using flow chemistry.

The development of a pharmaceutical manufacturing sector with the use of flow chemistry is expected to result in synthetic procedures with increased yields and purities, and with lowered/comparable reaction times as opposed to batch controls. This increase in yield and purity will result in reduced waste and by-product formation which in turn will reduce the environmental impact. Overall, in terms of the environment, the implementation of flow chemistry is expected to result in synthetic procedures with increased chemical control operating in a greener, environmentally friendly way.

1.4 Introduction to Flow Chemistry

1.4.1 Principles of flow chemistry

For any chemical reaction to occur spontaneously, the Gibbs free energy of the products must be lower than that of the reactants ($\Delta G < 0$). Even though this is necessary, alone it is not a sufficient condition for all reactions, many of which require large activation energies to reach the activated transition complex of a reaction. Furthermore, most reactions occur over a defined amount of time, described by reaction rates, depending on their free energy profiles (Figure 2).¹¹

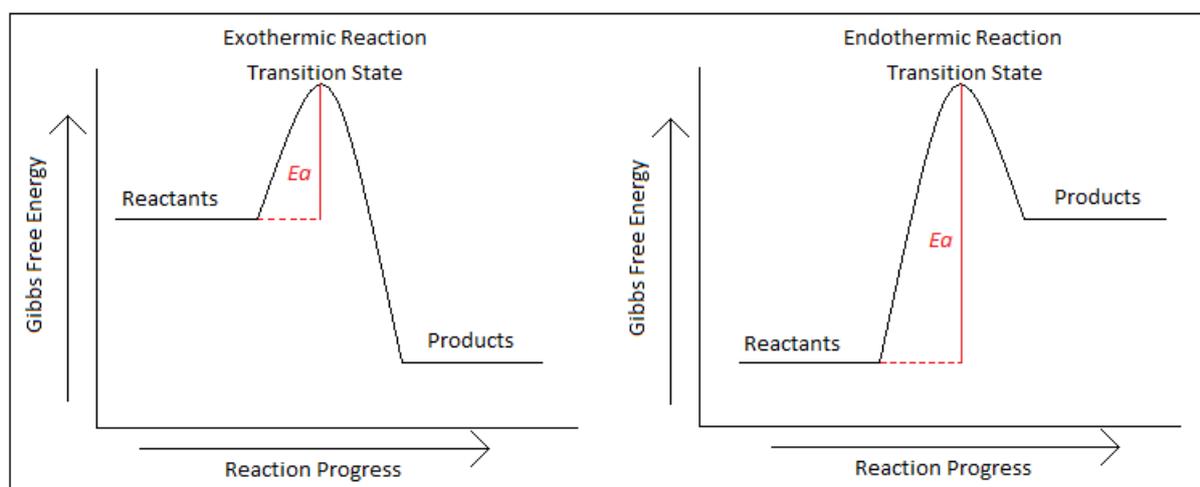


Figure 2: Generic exothermic and endothermic reaction energy profiles going from reactants to products without an additional intermediate formation. E_a is the activation energy of the reaction.

A chemical reaction is either exothermic (releases energy) or endothermic (acquires energy) in nature and is influenced by both enthalpy and entropy. Exothermic reactions are majorly enthalpy driven whereas endothermic reactions (which have products in a higher energy state than their corresponding reactants), rely substantially on the driving forces of entropy. Certain chemical reactions require little to no activation energy at ambient temperature and hence when collisions of reagents occur, they will occur spontaneously. However, many reactions require an extra boost of energy to be able to move from reactants, through a transition state to products. In most cases, this is simply achieved by suitable heat transfer from external energy sources.

The rate of a chemical reaction is highly dependent on the amount of contact reagents have with one another which is generally termed the mass transfer. Consequently, homogeneity or surficial contact (in the case of multiphasic systems) is of a high priority and as such, efficient mixing of a reaction matrix is a critical factor to consider. Simply put, if contact is not achieved, no reaction will occur.

Just like batch chemistry, flow chemistry technologies take advantage of the thermodynamic and kinetic properties of a chemical reaction to afford the best possible product yields. The synthesis and development of APIs has been achieved using traditional batch production methods.¹² Batch chemistry, on a small scale within the laboratory, involves the use of glassware (round bottomed flasks most commonly) into which reagents are manually added in order to obtain a crude product. The required heat transfer is typically facilitated through the use of heating mantles or heater stirrers as heat sources, and the mass transfer is facilitated through the use of magnetic stirrer bars or overhead stirrers. Post-reaction, a large variety of work-up and purification techniques are employed to afford a pure product.

In the past 20 years, the gradual introduction of continuous flow chemistry has had positive impacts on synthetic chemistry in general, showing improvements in heat and mass transfer as well as reaction control. Large-scale manufacturing of APIs under flow conditions show increasing promise, with added benefits such as reducing running costs and waste.²

Flow chemistry involves the use of pumps to continuously move reaction matrixes through reactors comprised of narrow bore tubing or channels, in which a chemical reaction occurs. The relative proportions of reagents used is controlled by their relative flow rates and concentrations. The reagents are typically combined within these micro-reactors at a mixing junction, which take advantage of diffusive mixing properties to allow for much greater homogeneity within the solution mixtures. Alternatively, the use of mixing chips or static mixing elements can also be employed to improve mixing. The resultant crude product can be pumped out of the reactor, recovered and purified off-line,¹² alternatively, downstream work-up and purification techniques are implemented within the continuous flow setup.^{5, 13, 14} Flow systems also typically employ the use of back pressure regulators (BPRs) in order to increase the upstream pressure of the system, in doing so, higher reaction temperatures are accessed (better heat transfer), often beyond the solvent's boiling point, gaseous reagents and products are kept in solution (better mass transfer) and accurate control of reagent flow rates and contact times are achieved.¹⁵

1.4.2 Basic set-up of micro-reactor system and components of flow chemistry

Set-ups under flow conditions are similar to analytical flow systems designed to analyse samples, particularly those using continuous flow modes.¹⁶ The basic components required for such syntheses under continuous flow conditions include, but are not limited to, various pumps for the introduction of gaseous or liquid reagents, a variety of mixers for improving mass transfer within reaction mixtures, flow-through tubular reactors, mixing chips, packed-bed (column) reactors which house solid supported reagents and/or catalysts, and BPRs.^{15, 17} The fabrication of automated continuous flow reactors should be given the most attention as

such reactors are often referred to as the central core of systems for syntheses under flow conditions.^{15, 16, 18-20} A continuous flow setup for a chemical synthesis can be developed using stand-alone devices, tubing and BPRs connected together (Figure 3). Heating and cooling oil/water baths may be employed as a temperature source. Unfortunately, these would need to be operated manually unless an automated system is implemented within the setup.

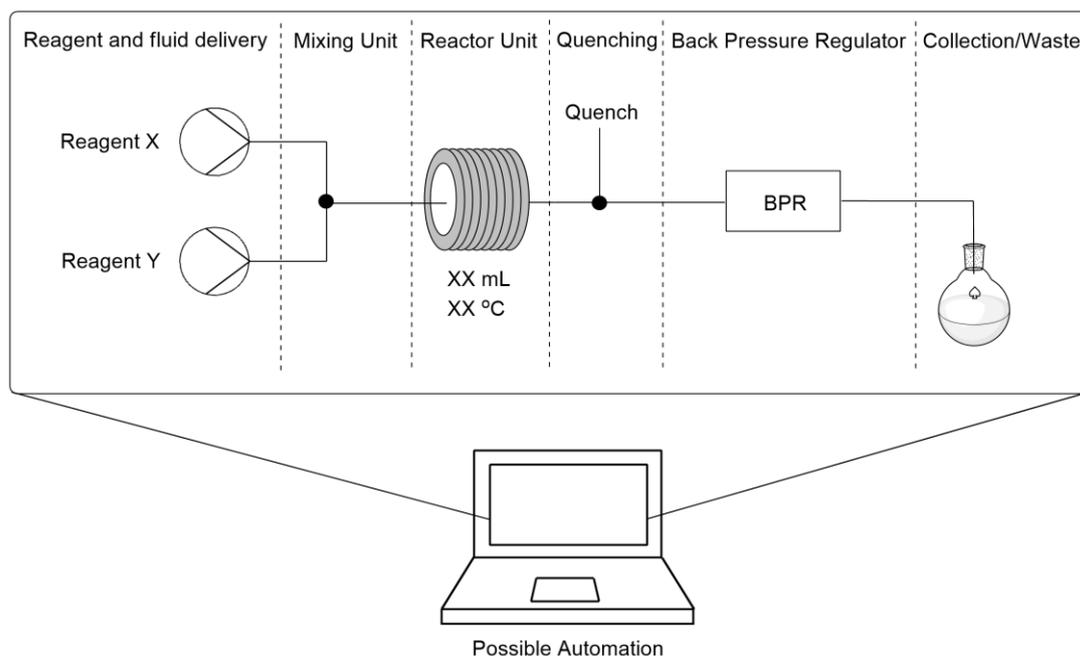


Figure 3: Most basic flow set-up for the introduction and reaction between reagents X and Y.

Commercially available continuous flow units vary substantially in component connections and allow for automation under flow with specifically programmed software. These various components are discussed in detail below.

1.4.2.1 Tubing and connections between flow components

The tubing and fittings used within flow systems are directly in contact with the stream of reagents/solvents and as such needs to be resistant to various chemicals used within synthetic laboratories. The dimensions and composition of tubing differs and must be considered when performing various chemical reactions. Generally, the tubing is composed of fluoropolymers (polytetrafluoroethylene (PTFE), perfluoroalkoxy alkanes (PFA), fluorinated ethylene-propylene (FEP), polyether ether ketone (PEEK) or stainless steel and has a broad range of internal diameters depending on the application.^{15, 17} When performing a reaction aided by flow conditions, the tubing composition must be chemically compatible with the reagents and solvents used within a reaction. The pressures induced onto the system is also an important parameter, ensuring that the pressure limit never exceeds that which the tube can handle. In general, when low and medium pressures are required (< 30 bar), inert perfluorinated polymers are sufficient.²⁰ For high pressured reactions (reactions in which the temperature

induced onto the system is substantially above the boiling point of solvent/reagent and reactions making use of supercritical fluids)²¹, more vigorous and pressure resistant stainless steel, unique alloys or sapphire tube reactors are employed.^{15, 20-22} In a general sense, the connections between multiple components of a flow system consists of different tubing and non-wetted fittings such as nuts and ferrules made of similar materials to that of the tubing. These are used to firmly attach the components and tubing to one another (usually threaded connections (Figure 4)).²³ Generally, the majority of these fittings used for the connection between the respective flow components are the same as those used within standard high performance liquid chromatography (HPLC) devices, hence, they are abundantly available.²⁰



Figure 4: Transparent PTFE tubing with yellow ferrule which is held tightly in place by the threaded brown nut fitting when fitted with another flow component. Image re-used from Bannock et al.²³

1.4.2.2 Solvent bottles and reagent stock solutions

When a chemical reaction is to be performed under flow conditions, the solvents and reagents required in the reaction are to be identified. The reagent stock solutions are made up to a known concentration and volume, which is dependent on the reaction stoichiometry and reagent introduction volumes. These stock solutions are stoppered with a septum during a reaction to minimise volatile compounds from evaporating. Reagent tubes are inserted through the septum into the respective reagent stock solutions which are now ready for introduction into the system (Figure 5).

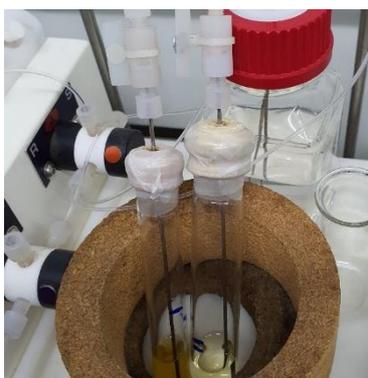


Figure 5: In the foreground, stock solutions prepared and stoppered with septa while pierced with introductory tubes. Red capped solvent bottle in the background. Selector valves seen with orange and purple stickers on the left of photo with labels R and S for reagent and solvent, respectively.

The respective solvents used in the preparation of the stock solutions are to be used as pushing solvents, which are introduced before and after the reagent streams thereby

encapsulating the product stream. These solvents are transferred portion wise to a new secondary solvent bottle to prevent any contamination within the primary solvent source. The solvent introduction tubes are inserted into the solvent bottles which are now ready for introduction into the system. Continuous flow reactors generally have a solvent/reagent selector valve to allow for each introduction when needed.

1.4.2.3 Reagent introductory devices used within continuous flow systems

The introduction of reagents is the first step in initiating a chemical reaction under flow conditions. Reagents are required to meet each other at a single mixing junction or unit at the same time. Careful measurements are required when cutting tube lengths to ensure that reagents travel the same distance (pathlength) to the mixing junction, this then allows one to accurately control when multiple reagent lines will combine and mix. If for some reason different pathlengths are required, the flow rates and fluid concentrations of the stock solutions must be adjusted accordingly to ensure that the reagents combine correctly, and that the reaction stoichiometry is maintained. Reagents and solvents are introduced at known flow rates and volumes made possible by using pumping devices. The most commonly applied fluid pumps within flow chemistry are piston pumps, peristaltic pumps, and syringe pumps.^{14, 20} Pump selection depends on back pressure requirements, flow rate, and fluid phase characteristics (completely homogenous, biphasic or slurry).

Piston pumps, otherwise known as HPLC pumps, are commonly utilised when the flow rate of the system is above $0.1 \text{ mL}\cdot\text{min}^{-1}$ with a variety of low to high system pressures.^{11, 20} Piston pumps operate via a crank mechanism which is connected to a reciprocating piston.²⁴ This crank action results in the piston (moving back and forth) forcefully pushing a fluid through an enclosed cylindrical chamber where the fluid is refilled and emptied as the crank mechanism is in motion (Figure 6).

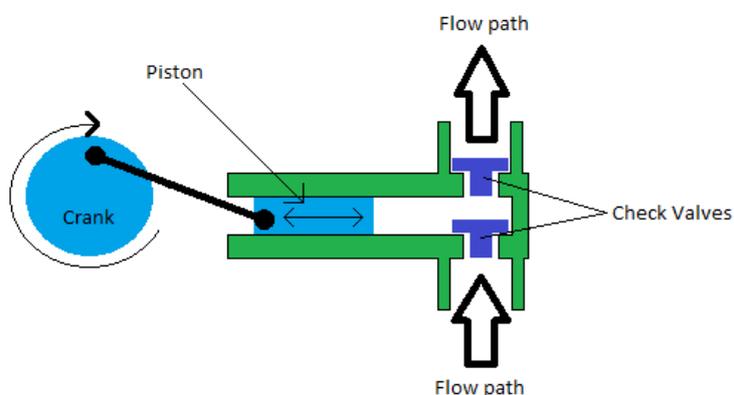


Figure 6: HPLC pump inner working in the most basic sense.

These pumps are often made from different materials including stainless steel, steel, nickel-molybdenum alloys or ceramics, and can operate at pressures as high as 250 bar.²² HPLC

pumps tend to exhibit some complications when pumping volatile solvents like chloroform, diethyl ether and dichloromethane (DCM), however, these complications are easily avoided by pre-pressurising and /or degassing the liquid.²⁰ Piston pumps are often the pump of choice for flow reactions, however, they have associated disadvantages including not being able to process high viscosity fluids and slurries, not being able to smoothly maintain very slow flow rates of a few microliters per min and posing difficulties when processing liquid/gas mixtures.¹¹

Syringe pumps may be utilised for accurate flow rates lower than $0.1 \text{ mL}\cdot\text{min}^{-1}$. At the most basic level, a predefined volume of liquid is dosed into a syringe and introduced into the flow system (Figure 7), which unfortunately is a limiting factor for scale and time of chemical reactions. More advanced syringe pumps make use of dual independent syringes which allows for a continuous operation where one syringe introduces the reagent stream while the other one fills up its dose.^{17, 20} Generally, the syringe pump consists of an external cylinder made from various polymers, glass, stainless steel and alloys with an internal chemically resistant plunger.¹¹ Similarly to the HPLC pumps, syringe pumps are in direct contact with the liquid reagent/solvent stream and consequently, possible precipitate build-up resultant of some reagents may result in fouling or blockages. Swelling and deterioration of seals is often a complication developed over time and as such must be maintained on a regular basis. Syringe pumps, by design, are unable to operate for extended periods of time due to wear and tear,¹⁶ and non-specialised syringe pump units are unable to operate at excessive induced system pressures.¹⁴

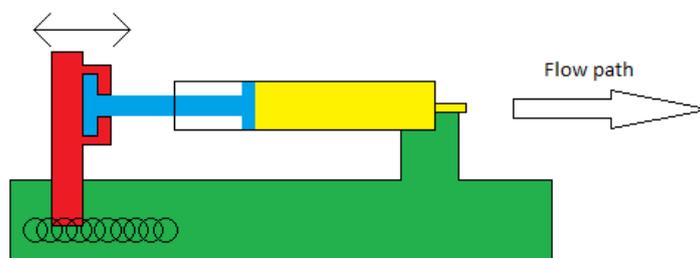


Figure 7: Basic principle of a syringe pump, where a moving arm pushes the syringe plug at a stipulated flow rate.

Critically, piston and syringe pumps are limited to introduction of solutions that are homogenous. When heterogenous mixtures need to be introduced, peristaltic pumps are often employed which are capable of pumping a well-suspended slurry depending on the particle size distribution. This is made possible by the peristaltic pump's mechanism whereby a central rotor squeezes against a flexible tubing fixed between the outer housing and the rotor, through which the fluid stream passes (Figure 8).¹⁴ This avoids contact between the fluids in use and the rotor mechanism reducing fouling of the pump. The tubing is made of various fluoropolymers that exhibit differences in their chemical compatibility and in most instances, are pressure rated at $< 10 \text{ bar}$.¹⁴ The major disadvantage when employing peristaltic pumps

within flow is that they typically have much lower operating pressure limits in contrast to piston or syringe pumps.

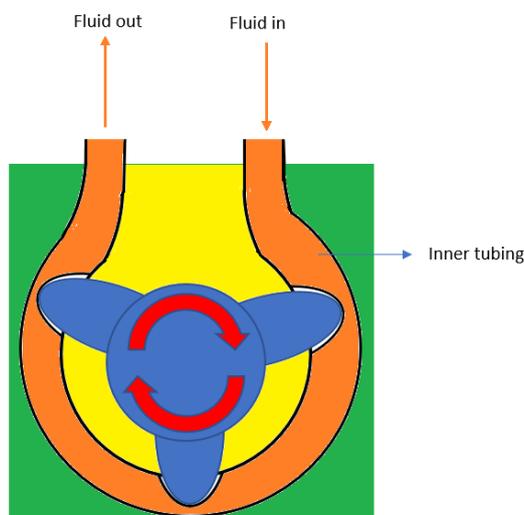


Figure 8: Inner working of a peristaltic pump where the inner rotors squeeze the outer tubing pushing the fluid through the tube.

It is important to note that each of these three pump types (piston, syringe or peristaltic) must be calibrated on a regular basis to ensure accurate volumes and flow rates are maintained. This assists greatly in reproducibility of procedures under flow conditions.

When a chemical reaction requires a reagent that is in the gas phase, flow chemistry is seen to be an ideal tool for such scenarios, especially for gases that are toxic in nature and that are known to present apparent safety issues.^{7, 25} The introduction of gases, in the simplest of cases, into the flow system can be achieved by utilising a pressure regulator. However, most reactions require a stoichiometric amount of the gaseous reagent which is achieved by controlling the gas flow with the implementation of a mass flow controller (MFC), as seen in (Figure 9).²⁶ These devices allow for precise gas flow rates made possible by heat transfer phenomena.²⁷



Figure 9: Mass flow controller connected to a tubular coil reactor commercially available from Vapourtec. Image obtained from Vapourtec products webpage.²⁶

Solid reagents are often required within the synthesis of APIs and therefore still need to be introduced into the flow system. If a solid reagent can be solubilised within a certain solvent, it is in the best interest to attempt this reaction in that respective solvent. Unfortunately, not all solids dissolve in common solvents and as such are either consumed (reagent), form

(product), or remain in the solid phase throughout the reaction. If a solid can be dispensed as a slurry, peristaltic pumps can be employed depending on the particle size distribution and the amount of solid material present. If solids are not consumed within the reaction, it will typically lead to blockages which will ultimately result in pressure build-up, which will trigger an automated reactor shutdown. Consequently, packed-bed reactors through which the reaction mixture can be pumped are often utilised for housing solid reagents or catalysts.^{17, 28, 29} The use of packed-bed reactors can be limiting with regard to the maximum allowable residence time as they have a finite length. Alternatively, recycling of the reaction mixture could allow for longer residence times. Critically, the use of packed-bed reactors is best suited for catalysts that are not consumed stoichiometrically as the latter will require the cumbersome swapping of the packed-bed reactor on a regular basis.

1.4.2.4 Mixing units

As stated previously, in order for a chemical reaction to commence, the reagents in question must have adequate mass transfer. Efficient mixing is a critical factor within a flow process and has a direct impact on the mass transfer and hence the rate of reaction. In single phase reactions, this is achieved by integrating rapid mixing units in the system. Generally, the reagents are pumped through separate pumps and allowed to converge into a single stream where diffusive mixing predominates before passing through the flow reactor. The simplest of these mixing junctions are T-piece, X-piece or Y-piece mixers (Figure 10), which are sufficient for mixing slower reactions which require minutes to hours (min – h) to react and which are not substantially dependent on the speed of mixing.^{20, 30} If rapid mixing is a requirement for a reaction, usually in instances involving highly reactive reagents with reaction times in the range of seconds,³¹ more specialized mixing units are utilised to reduce the reagent mixing time. These specialized units make use of micro-structures or advanced spatial orientation of mixing tubes to enhance turbulence, allowing for increased efficiency in mixing.³² These specialised units include a variety of static mixers, dynamic mixers, and chip micro-reactors (often used as mixers as well as chemical reactors). Mixing components within flow chemistry are mainly composed of fluoropolymers with the more specialized chip reactors being manufactured from glass, stainless steel, ceramics, quartz and more.^{15, 32}



Figure 10: Most basic microfluidic mixers composed of fluoropolymers, where A = T-piece mixer, B = Y – piece mixer and C = X – piece mixer. Image re-used from Britton et al.³⁰

Multi-phase reactions involving biphasic liquids or gas/liquid reagents rely on surficial contact between phases for reactions to occur. Such reactions are highly attractive for applications under flow conditions since a greater interfacial area (alternating biphasic fluid or gas bubbles within small tubes allowing increased contact between reagents of interest) can be created compared to under batch conditions. Similarly to single phase reactions, biphasic systems may utilise Y-pieces, T-pieces, X-pieces, static and dynamic mixers as well as chip micro-reactors (Figure 11).²⁶ The nature of biphasic mixing depends on the flow rates, the chemical properties of the fluids, as well as the characteristics (internal radius, structural obstacles, winding or meandering) of the flow channels.²⁰ Simple Y, T, or X-piece mixers will result in slower mixing resulting in decreased surficial contact between biphasic solutions (thicker slugs of alternating phases) and as such, faster flow rates will promote heightened efficiencies of mixing within these basic units reducing the relative size of slugs. If slower flow rates are required in addition to rapid mixing, the more specialised mixers are utilised.

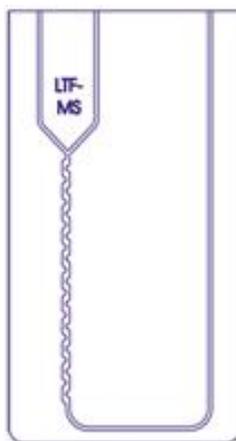


Figure 11: Schematic of a Vapourtec mixing chip primarily used for rapid mixing commercially available from Vapourtec. Image obtained from Vapourtec products webpage.²⁶

Gas/liquid phase reactions can be mixed using gas/liquid static mixers whereby the liquid and gas are passed through a micrometre size frit (PTFE, stainless steel or titanium).¹¹ A widely used approach employed under flow conditions for liquid-gas reactions involves plug-flow/segmented flow regimes. This process allows gas to enter the liquid reagents flow as a gas plug and therefore relies only on the surficial contact between the liquid-gas bubble interface.³³ However, it has been reported that the plug-flow method has limited control over concentration of the gas within the liquid flow and is also not capable of undergoing multistep methods that require heterogeneous catalysis or even solid supported reagents.³³ Additionally, membrane-based tube-in-tube flow reactors, falling-film micro-reactors³⁴ and semi-permeable membrane reactors,^{35,36} which are specialised components combining the mixing and reactor modules of a reactor, are available.³⁷ Permeable membrane reactors have become progressively popular and will be discussed further in the flow reactors sub-section to follow.

It is also important to note that some reactions tend to have slow precipitate formation within the reactor unit and consequently, ultrasonicated devices³⁸ as well as mechanical agitators are often implemented within a flow process to assist in solubilisation, or at least suspension of slow forming precipitates to prevent blockages.

1.4.2.5 Flow reactor units

As mentioned previously, the reactor unit is the heart of a continuous flow system, being the most important component within a reaction under flow conditions. This region is where the reaction is allowed to take place and the amount of time spent within this region is defined by the residence time of the flow process. This must not be confused with the reaction time of a batch reaction, defined by the amount of time the reaction was allowed to proceed in the reacting vessel/flask before being quenched. The type of reactor and material composition utilised within a flow reaction depends on the nature of chemistry that is to be performed (photochemical, multiphase, exothermic, endothermic reactions, etc.).^{6, 22, 39, 40} When a reaction requires addition or removal of heat, the temperature can be maintained using a conventional technique such as submerging the reactor unit into a heating or cooling bath. Unfortunately, this requires attention to regulate the temperature of the bath and is often difficult to maintain sub-zero temperatures. There are, however, more specialised heating/cooling devices and technologies such as inductive heating techniques, microwave irradiation or cryogenic cooling units.⁴¹ If photochemistry is a requirement for a chemical transformation to occur, the reactor needs to be transparent to light and be equipped with a dedicated source of the respective light irradiation.^{41, 42} Flow reactors are commonly categorised into three main types: chip reactors, coil reactors and packed bed reactors.²⁰

Chip micro-reactors (Figure 12), commonly termed “lab-on-chip” devices⁴³ are generally manufactured or machined from silicon, quartz, glass, ceramics, or stainless-steel alloys.^{15, 44} These chip micro-reactors are powerful flow components and can often be implemented into various flow reactions. Chip-based reactors are either used exclusively for rapid mixing where the product stream still passes through another reactor unit, or these reactors can be used as a combined mixing/reactor unit. The fabrication of such reactors involves specialised techniques and are often made with a section devoted to rapid mixing incorporated into one chip unit.²⁰ Chip-based reactors take advantage of very high surface to volume ratio and as such, have excellent heat and mass transfer.¹⁵ Advances in three-dimensional (3D) technology has made designing and printing 3D models of chip-based reactors possible using various polymers.^{45, 46} When deciding on what type of chip reactor to make use of, the nature of chemistry that is to be performed as well as the chemical compatibility with the reactor must be considered.

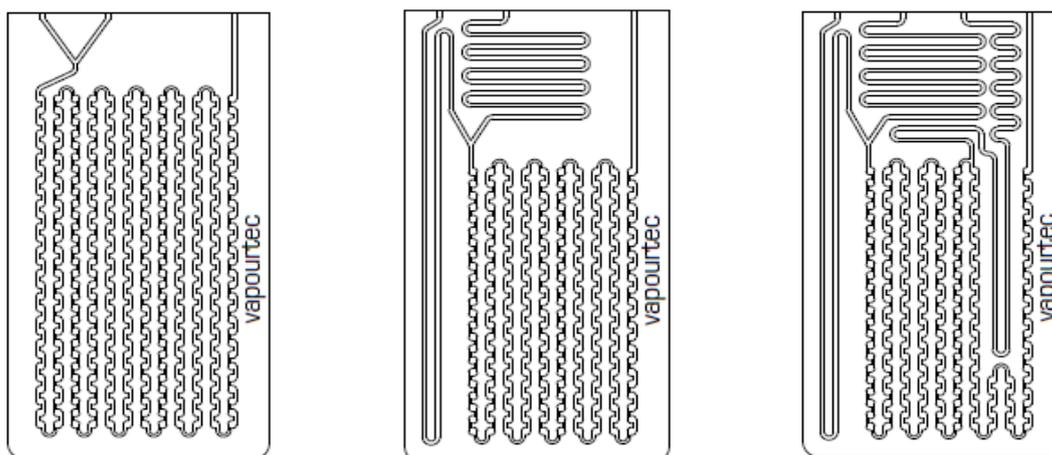


Figure 12: Glass micro-mixing chips commercially available from Vapourtec. Images obtained from Vapourtec products webpage.²⁶

Tubular coil-type reactors are cheaper, more cost effective alternatives to micro-chip reactors.²⁰ Tubular reactors consist of a hollow, coiled tube through which reactants flow, generally being heated or cooled externally for temperature control (Figure 13). The most common materials used for the tubular reactors are fluoropolymers (PTFE, PFA and FEP) or stainless steel, dependent on reaction temperatures and pressure requirements. The volume of a coil reactor is varied by increasing the coil length or increasing the internal diameter of the tubing, with outer diameters of 1/8 " and 1/16 " being most common.²⁰ The internal diameter varies substantially depending on the scale of reaction and application. More specialised coil reactors are available, allowing for full integration with commercially available continuous flow units which presents the coil reactor with integrated temperature control (heating and cooling).



Figure 13: Tubular flow micro-reactors commercially available from Vapourtec. Image obtained from Vapourtec products webpage.²⁶

Some chemical reactions require heterogeneous reagents, solid scavengers or catalysts which should not be introduced through the pumps. These reagents/catalysts are commonly introduced into a flow reaction with the use of a packed-bed reactor.⁴⁷⁻⁴⁹ Packed-bed reactors (Figure 14) consist of an outer column or cartridge composed of glass, polymers or stainless steel which is packed with the heterogeneous catalyst/reagent. Each end of the column

contains a filter to encapsulate the catalyst/reagent and is sealed with resealable caps through which the flow stream passes.²⁰ The reaction takes place within this column and is highly dependent on surficial contact between the phases. The nature of the particle packing plays an important role in the fluid dynamics of the fluid stream and the size of particles within the reactor must neither be too large (decreased surficial contact) nor too small (may result in clogged frits and blocked columns).

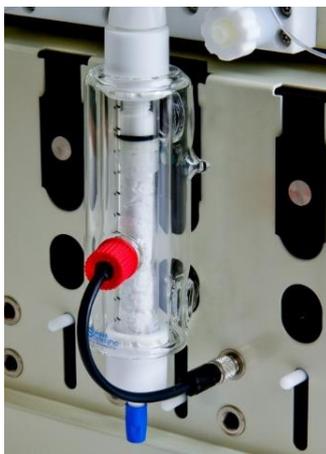


Figure 14: Packed-bed reactor holstered within an outer glass casing allowing for temperature setting. Commercially available from Vapourtec. Image obtained from Vapourtec products webpage.²⁶

As noted previously, flow chemistry is a powerful tool especially when performing liquid/gas reactions, specifically when the reaction requires the introduction of hazardous gases. Permeable membrane reactors (Figure 15), have been widely implemented within continuous flow procedures with the predominant tube-in-tube design already utilised within multiple synthetic procedures.^{33, 37, 50, 51} The basic principle behind a permeable membrane reactor is that a fluid reagent stream will flow in a central gas permeable tube which is encapsulated by a larger diameter impermeable tube through which the gaseous reagent flows or vice versa. As both liquid and gas stream pass through, the gas is allowed to permeate into the adjacent reaction stream. The inner tubing is generally composed of Teflon AF-2400 which is both chemically resistant as well as gas permeable, and the exterior tubing is generally a fluoropolymer such as PTFE.³⁷

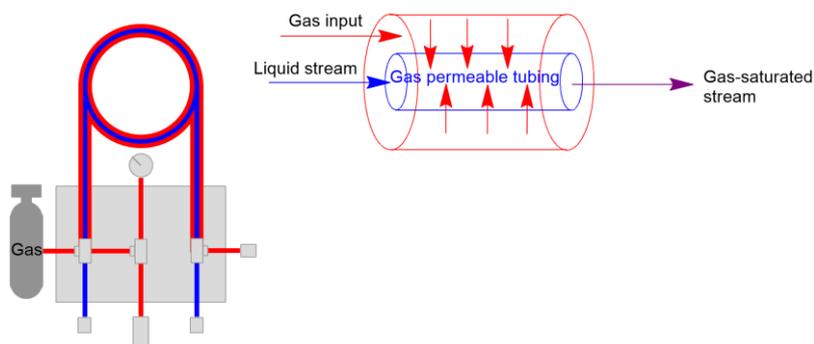


Figure 15: Tube-in-tube permeable membrane gas/liquid flow reactor basic working.

1.4.2.6 Back pressure regulators (BPRs)

As mentioned previously, a key advantage of flow chemistry is the upstream pressure applied onto the reacting medium. This is achieved with the implementation of a pressure regulating unit, usually just before collection, which allows for introduction of a fluid, and limits the output flow resulting in a positive upstream system pressure. The BPR is responsible for superior control and increased reaction rates when gas phase or volatile reagents are introduced into a continuous flow reaction.⁵² Increased system pressures result in higher boiling points (increased required vapour pressure) forcing the reagents to maintain its liquid phase. Most BPRs are not capable of measuring the system pressure, however, this is often determined by pressure sensors which are generally pump integrated or placed in-line anywhere throughout the flow setup.²⁰

BPRs are commonly composed of various chemically resistant polymers and/or stainless steel components. There are two main types of BPRs commonly used within micro fluidics,¹¹ either a pressure pre-determined BPR, or pressure adjusting BPR. Pre-determined BPRs operate at a constant pressure which has been pre-defined (Figure 16). There are multiple options ranging from a few bars to tens of bars in pressure. The basic mechanism of a pre-determined BPR is that the input fluid flow pushes against a spring-loaded plunger which only opens when the required system pressure has been met. The fluid can maintain this flow pass-through provided the system is above the pre-set BPR limit.¹⁷

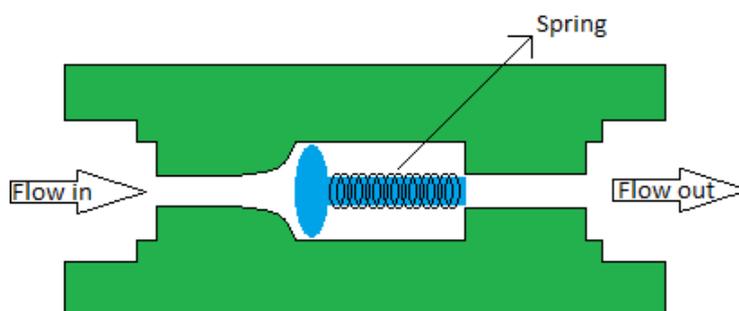


Figure 16: Pre-determined BPR. Where the spring is pre-loaded to achieve a known pre-set BPR.

Pre-set BPRs often need to be interchanged depending on the solvent system used and the temperature required. Consequently, pressure adjusting BPRs are advantageous when various reactions are performed requiring different system pressures. These devices are more versatile, but unfortunately, being more specialised are more costly.²⁰ The most basic adjusting BPR can be adjusted manually by tightening or loosening of a pin that squeezes against an inner tube thereby reducing the internal volume through which the fluid may pass (Figure 17). This decrease or increase in tube volume determines the upstream pressure. More specialised pressure adjusting BPRs make use of a reference pressure determined

against a diaphragm to precisely set the required pressure within the system. This set pressure is made possible with gas pressurisation or mechanical forces.²⁰

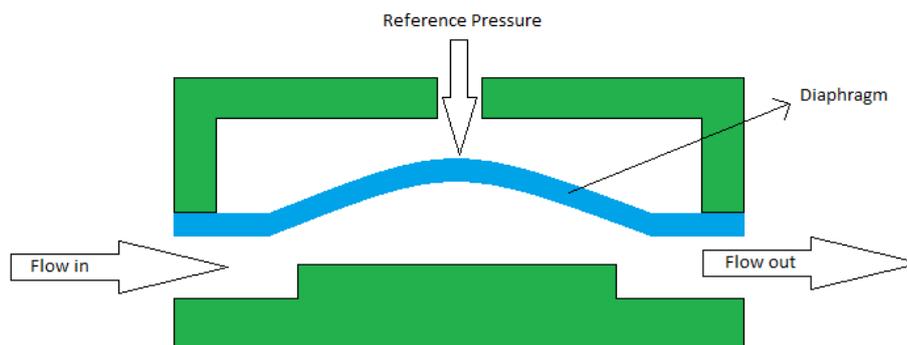


Figure 17: Pressure adjusting BPR.

1.4.2.7 Waste/collection selector valve

When the reaction mixture has passed through the BPR and is allowed to depressurise, the flow stream can be directed into a collection flask for further processing. This can be achieved manually by placing the output tube into a collection flask or automatically with the use of a selector valve. Selector valves have multiple input and output lines which can be opened or closed as needed allowing for preferential flow. A collection/waste selector valve generally has one input stream (reaction mixture) and two output streams, one for the waste and one for the collection of products. The waste is set for the solvents and reagents used within the preconditioning and washing of the flow unit and the collection is only set for when the product stream is passing through.

1.4.3 Advantages and Disadvantages of flow chemistry

Performing a chemical reaction with the utilisation of continuous flow technologies has many advantages which will be discussed below:

- The higher system pressures achieved within a flow reactor allows access to temperatures in excess of a solvents boiling point, generally inaccessible under batch conditions, positively impacting the reaction kinetics.^{6, 18, 53}
- The mixing units mentioned above allow for more efficient mixing of a reagent stream, thereby enhancing the contact reagents have with each other, positively impacting the reaction kinetics and rates. This is responsible for higher mass transfer rates.^{17, 18}
- The heat transfer within a reaction under flow conditions is largely improved as opposed to batch conditions, resultant of very high surface to volume ratios made possible by the small internal diameter tubing, coil reactors, and chip micro-reactors. Heat transfer within flow systems is resultant of promoted conductive and convective effects.^{11, 18}

- The rapid heat transfer achieved within continuous flow reactors allows for more precise temperature control making both exothermic and endothermic reactions easier to handle.^{8, 11, 18, 53}
- This rapid heat and mass transfer mentioned above often leads to lower reaction times when compared to batch conditions.^{6, 17, 53}
- The implementation of a quenching region installed in-line often allows for more precise control over reaction intermediates and by-product formations thereby allowing for a more selective approach to isolating the product of interest with reduced conversion to intermediates or by-products.¹¹ This is achieved by exploiting subtle differences in reaction rates made possible under flow conditions.²⁰
- With the use of continuous flow chemistry, the overall chemical exposure to the chemist is dramatically reduced due to the closed system. This is especially advantageous for reactions that are known to be hazardous under traditional batch conditions.^{18, 54}
- High pressure and heat resistant materials used within flow components reduce hazards when working with explosive and highly exothermic reactions.^{53, 54} These flow components are much easier and safer to handle as opposed to batch-based pressurised systems such as a “bomb” reactor.
- Another positive of flow is that multiple micro-reactors can be coupled to one another in order to achieve multi-step syntheses.^{5, 14} Similarly, work-up and purification techniques are employed in-line thereby allowing for a more continuous approach to isolating the product of interest.⁵
- Another major advantage of flow conditions is the precise control of various parameters achieved due to automation and precise monitoring. This in turn, leads to increased reproducibility⁵³ resultant of reduction in human error.
- Often resultant of higher mass transfer and effective mixing, reagents are often introduced in more precise stoichiometric control, lowering the quantity of excess materials required to achieve successful reactivity.
- Various in-line analytical tools can be integrated within microfluidic systems, allowing enhanced reaction screening and simplification of reaction monitoring and downstream processing.^{53, 55}
- The heightened selective control within a flow reaction⁵³ often leads to lower waste production thereby adhering to greener chemistry controls.
- Flow chemistry often results in higher productivity of various chemical reactions thereby leading to increased space-time yields.^{2, 5}

- Heterogeneous reactions within packed-bed flow reactors have multiple advantages over a batch reaction. Higher effective catalyst/reagent concentrations allow for a potential decrease in reaction times.²⁰ Secondly, since the solid is trapped between two frits on either cap, no subsequent step is required for separation from the mixture unless leaching occurs.⁴⁸
- With the rapid rise in 3D printing technology,^{40, 45, 46, 56} the development of continuous flow platforms with bespoke reactor modules allows the opportunity to design reactors specifically to fit the chemistry at hand.
- When performing scaling up of a flow reaction, dimensional scaling and numbering up approaches are often implemented, allowing for increased product throughput without compromising the physical characteristics of the flow micro-reactors (heat and mass transfer as well as mixing efficiencies). Consequently, reaction results remain similar even when performing larger scale reactions. This is often not the case when performing scaling up in batch conditions, generally using the sizing up approach (larger round bottomed flasks/reactors).⁵³

With all these advantages identified for continuous flow applications, this often leads to increased reaction yields with lowered reaction times. That being said, there are several disadvantages to the technology which are highlighted below:

- Not all chemical reactions show appreciable improvements under flow conditions and certain reactions are performed efficiently using well established batch-based equipment. In such cases, a flow translation may not afford significant improvements in yield and reaction time.
- The major disadvantage of using flow conditions is the handling of solids and precipitates within the system. Solids can easily block the narrow bore tubing used in flow systems resulting in pressure spikes triggering an automated termination of the reactor system. In rare, severe instances, this can also cause damage to the tubing, pumps, fittings, micro-reactor units or BPRs. As a result, method development under flow conditions often requires careful selection of solvent systems to try ensure homogeneity, alternatively solid and solid-supported reagents and catalysts can be housed in packed-bed reactors, and in certain instances slurries can be pumped using peristaltic pumps.^{48, 57} Sonicating baths can also be employed to improve solubility of the reaction mixture. Unfortunately, as more techniques are employed, the overall complexity of the reaction increases. This problem with solid introduction cannot always be overcome and as a result, certain chemical reactions should be performed using a batch-based or a batch-flow hybrid approach.⁵

- The use of immobilized transition metal catalysts in packed-bed flow reactors, commonly used for cross-coupling reactions, have a tendency to leach the catalytic material. This results in column reactor deactivation over time as well as product contamination which then requires a further downstream purification step. The leaching unfortunately cannot be easily overcome as the catalyst continuously moves between the liquid and solid phases during the catalytic cycle and as such, small amounts of the catalyst are carried away in the moving liquid phase.⁵⁷
- Cost is always a factor when comparing batch to flow and unfortunately, the cost of continuous flow technologies required for an initial efficient setup is substantially more than that of standard laboratory equipment.²⁰
- Dispersion of the leading and tailing ends of the reagent stream must be observed with caution, especially on small-scale reactions and when attempting telescoping of multi-step synthetic procedures. Steady-state conditions must be identified to reduce differences in concentrations when linking multiple synthetic steps. This can largely be avoided by implementing in-line analytics such as infrared (IR) or ultraviolet (UV) sensors.^{58, 59}
- Corrosion and wear and tear of check-valves, seals, and springs within components¹⁵ is inevitable, especially if corrosive materials are introduced, and therefore laboratories need to have replacements at hand.

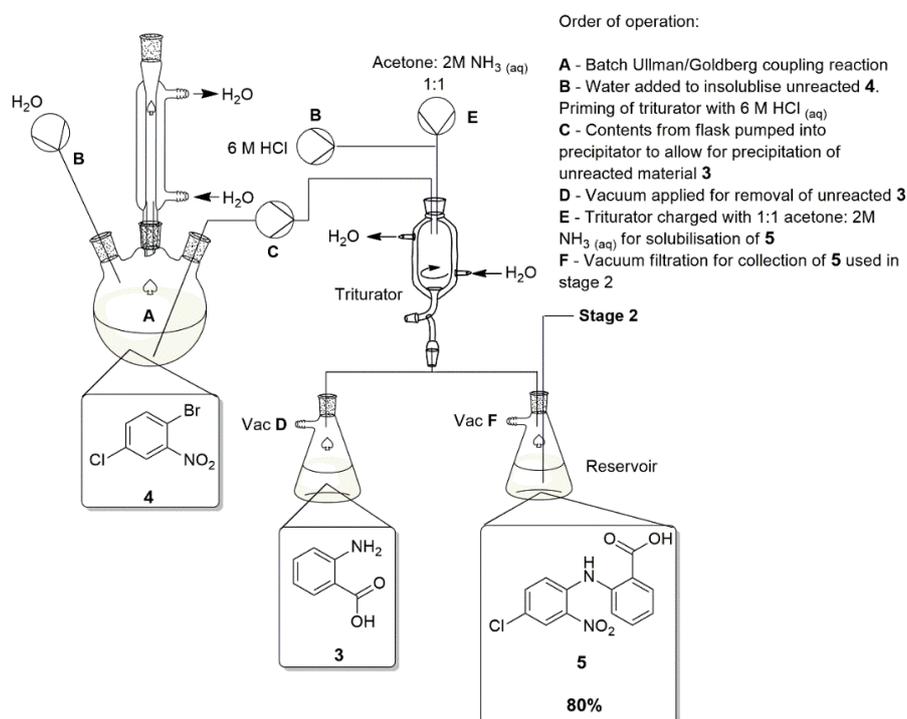
Despite the disadvantages noted above, the continued rapid increase in flow technology innovations may present solutions to many these challenges in the coming years. Ultimately, the advantages have been proved to significantly outweigh the disadvantages and as such flow chemistry is proving to be a prominent tool in the synthetic organic field. That being said, when thinking of translating a synthetic procedure under batch conditions to continuous flow, the advantages, and disadvantages of the reaction of interest should be considered to evaluate whether the flow approach would be beneficial (space-time yield, set-up complexity, cost etc.). It is important to note that batch-flow hybrid synthetic procedures have gained a lot of attention in recent times affording access to the best of both worlds, as such one should always consider how to integrate both flow and batch technologies when designing multi-step syntheses.^{5, 60, 61}

1.4.4 Examples of in-house research with the utilisation of continuous flow conditions

Our research group has a growing interest in the integration of flow chemistry as an efficient synthetic tool. In particular, our research group has looked at the development of flow process routes towards APIs, the design of bespoke flow reactor systems, reaction engineering and automation of flow systems. Highlighted below are several recent examples from our group showcasing the utility of flow chemistry.

1.4.4.1 Batch-Flow hybrid synthesis of the antipsychotic Clozapine

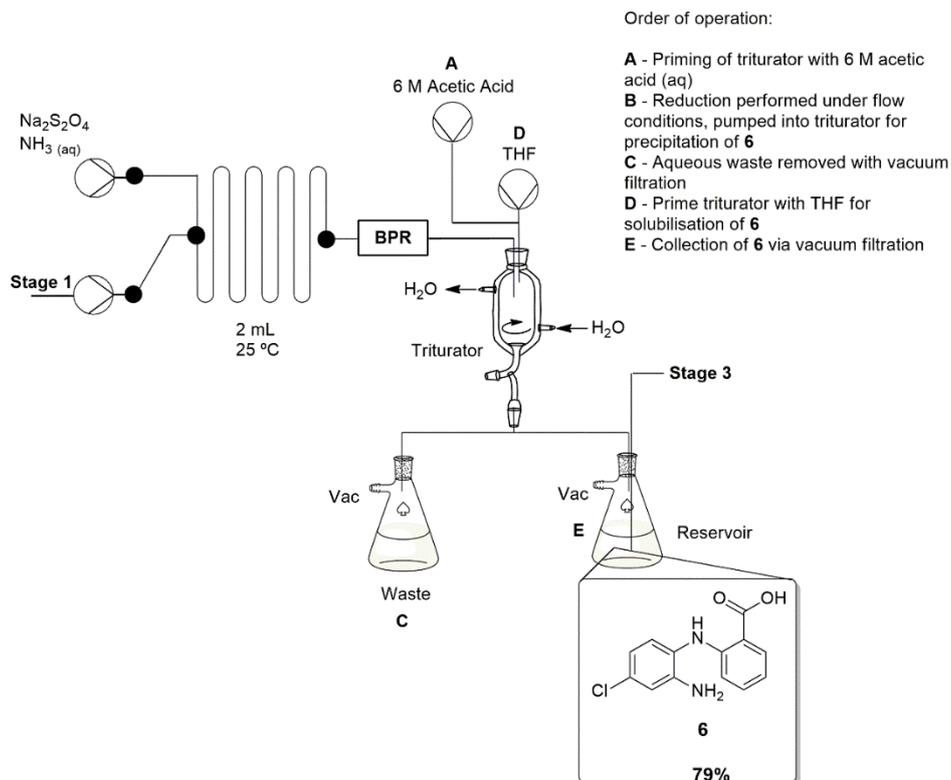
Neyt *et al.* reported a batch-flow hybrid synthesis of the antipsychotic drug, clozapine **2**.⁵ The reported synthetic procedure was divided into four stages (two batch and two flow), which were telescoped together. The first stage involved an Ullmann/Goldberg coupling reaction (copper mediated) between anthranilic acid **3** and 2-bromo-4-chloronitrobenzene **4**. Under flow conditions, precipitation of the product led to reactor fouling with poor yields of 30-38%. Consequently, this stage was run under batch conditions affording 2-(4-chloro-2-nitroanilino)benzoic acid **5** with an isolated yield of 80%. The downstream work-up and purification was performed under flow conditions incorporating the use of a bespoke in-line triturator (Scheme 1).



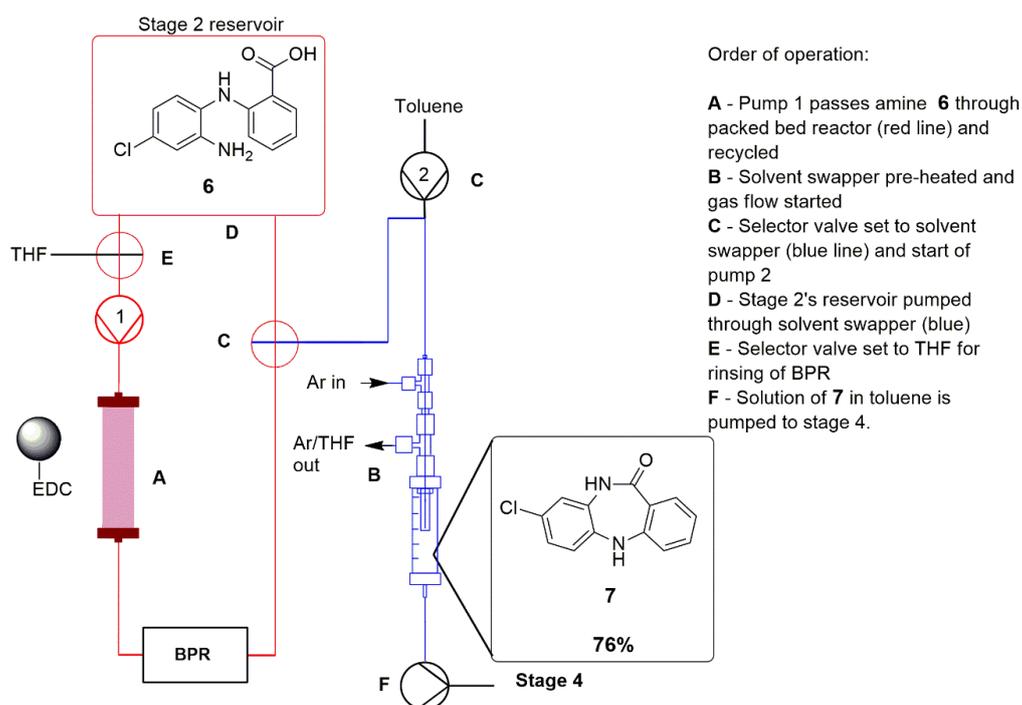
Scheme 1: Stage 1 of the batch-flow approach for the synthesis of clozapine reported by Neyt and Riley, 2018.⁵

The second stage (Scheme 2) was performed under flow conditions in a mixing chip, and involved a sodium dithionite mediated reduction of **5** to 2-((2-amino-4-chlorophenyl)amino)benzoic acid **6** in 79% yield vs. 65% in batch. Notably a residence time of only 92 sec was achieved vs. 15 min reaction time in batch. Stage three was an intramolecular amide coupling achieved using flow conditions. The coupling was mediated through the use of solid-supported EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) which was housed in a PBR, affording 8-chloro-5,10-dihydro-11*H*-dibenzo[*b,e*][1,4]diazepin-11-one **7** in an isolated yield of 76% with a 3.5 h residence time (Scheme 3). The output from stage three was telescoped directly into the final stage, with the aid of an in-line solvent swap. The bespoke aspirating solvent swapper based on a design by the Ley group⁶², was employed allowing the tetrahydrofuran (THF) utilised in stage 3 to be swapped for toluene. The final stage was

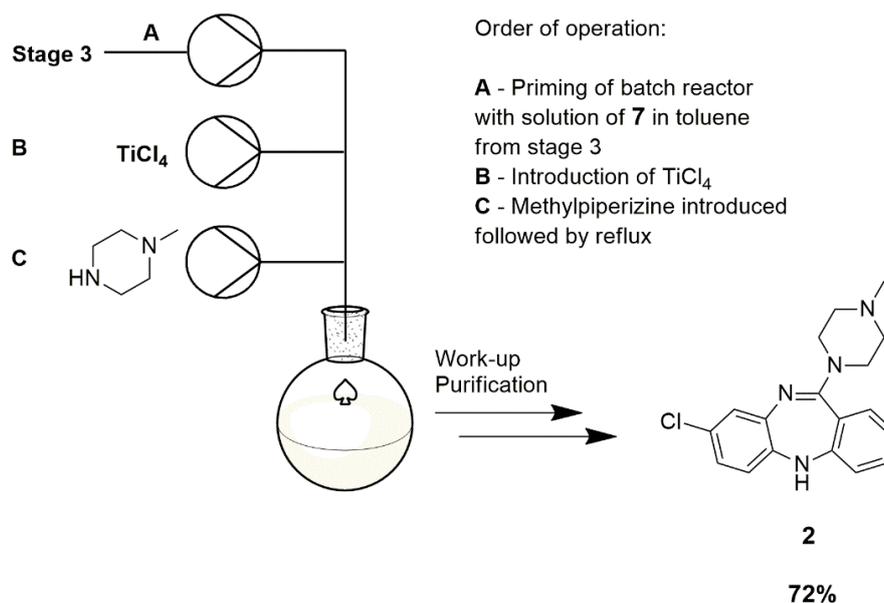
performed utilising batch conditions as the reaction was characterised by severe precipitate formation. This telescoped step allowed for an isolated yield of 72% over the two stages with purification of desired product **2** being performed with the use of column chromatography or recrystallisation (Scheme 4).



Scheme 2: Stage 2 of the batch-flow approach for the synthesis of clozapine reported by Neyt and Riley, 2018.⁵



Scheme 3: Stage 3 of the batch-flow approach for the synthesis of clozapine reported by Neyt and Riley, 2018.⁵

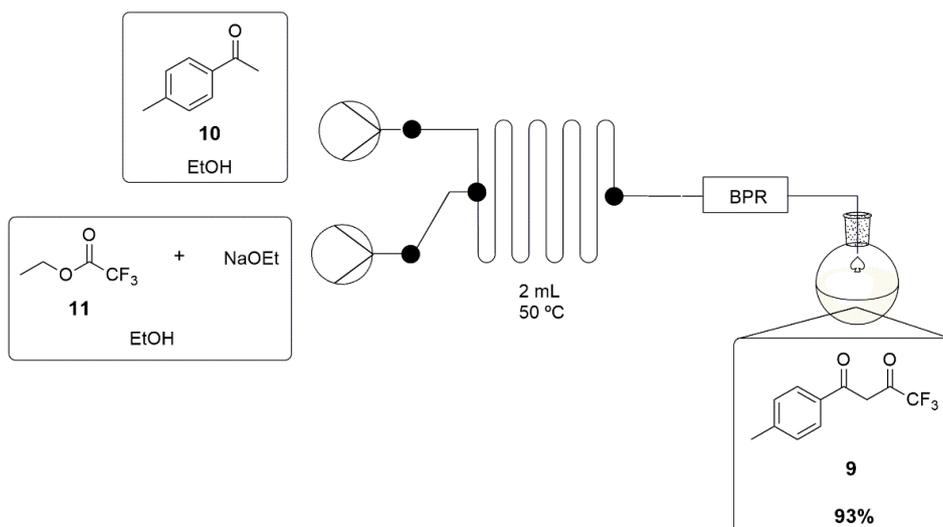


Scheme 4: Stage 4 of the batch-flow approach for the synthesis of clozapine reported by Neyt and Riley, 2018.⁵

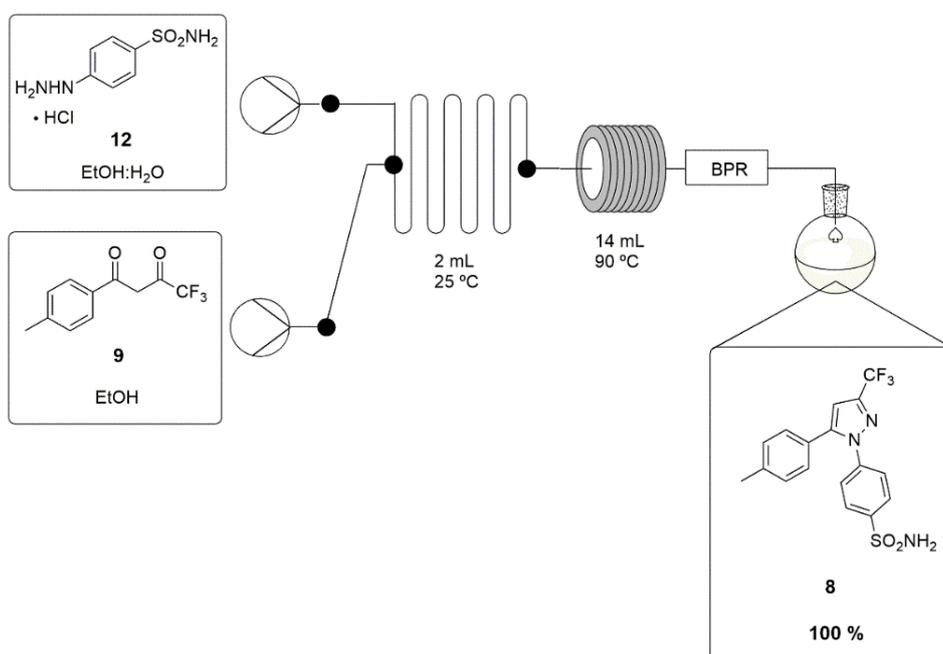
This experimental route with both batch and integrated flow conditions resulted in an overall yield of 45.5% as compared to 27% when obtained using primarily batch conditions.⁵ Furthermore, the overall reaction time taken reduced from 132 h in batch to approximately 44 h in the integrated batch-flow system. It was also noted that the overall safety and chemical exposure hazards posed to the chemists were greatly reduced when using the batch-flow system.⁵

1.4.4.2 Flow synthesis of the COX-2 inhibitor celecoxib

Scholtz *et al.* reported an innovative flow approach for the synthesis of the nonsteroidal anti-inflammatory drug, celecoxib **8**.⁶³ Celecoxib is a cyclooxygenase (COX) enzyme inhibitor with the selective inhibition of COX-2, primarily for the treatment of arthritis and general acute pain relief. Scholtz reported a 2-stage procedure while utilising a micro-flow reactor platform, whereby the first stage involves the preparation of dione **9** by treating 4-methylacetophenone **10** with ethyl trifluoroacetate **11** and sodium ethoxide, dissolved in EtOH. This was achieved with the implementation of a 2 mL mixing chip reactor allowing the reaction to take place with a residence time of 4 min at a temperature of 50 °C. The optimised reaction afforded **9** with a yield of 93% (Scheme 5).⁶³ A subsequent cyclo-condensation reaction with the addition of (4-sulfamoylphenyl)hydrazine hydrochloride **12** afforded celecoxib **8** in quantitative yield. This was achieved with the use of a 2 mL mixing chip (ambient temperature) linked to a 14 mL tubular coil reactor with a residence time of 64 min at 90 °C (Scheme 6).⁶³

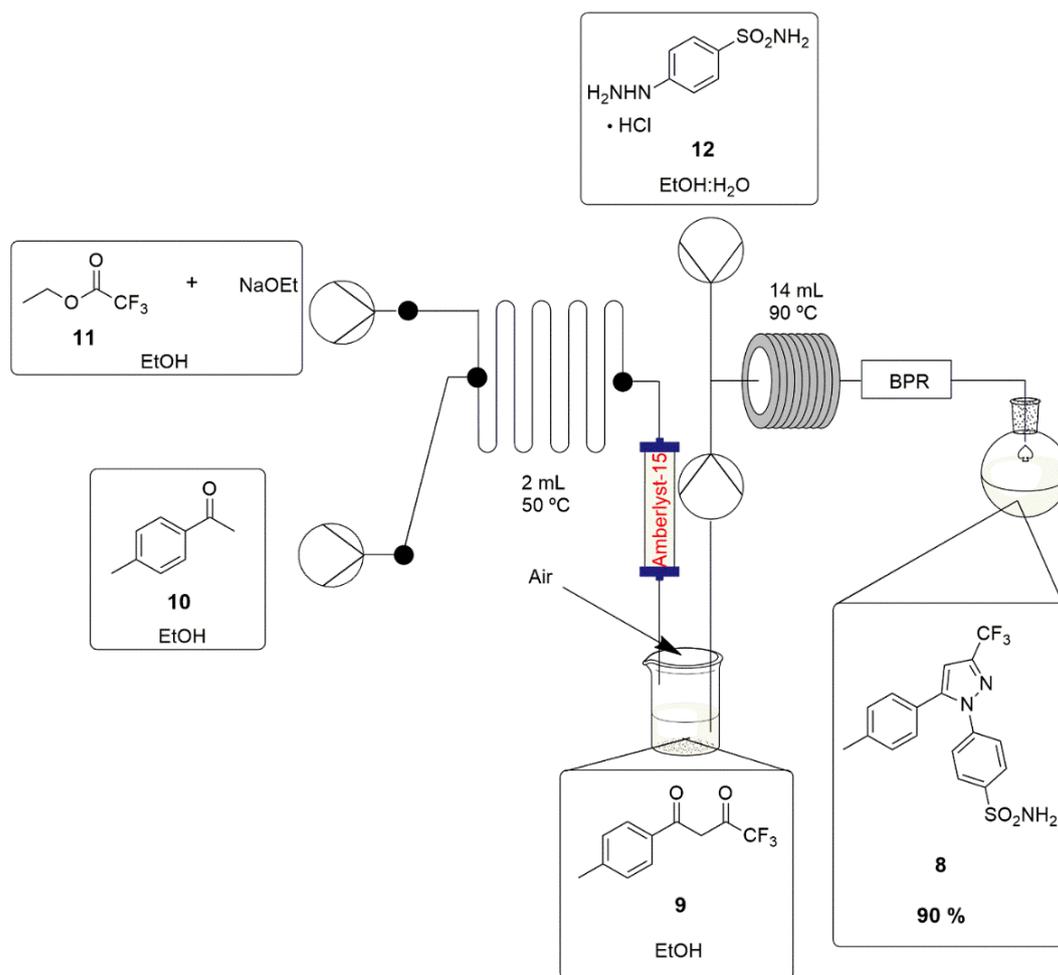


Scheme 5: Stage 1 for the synthesis of celecoxib under flow conditions, reported by C. Scholtz, 2021.⁶³



Scheme 6: Stage 2 for the synthesis of celecoxib reported by C. Scholtz, 2021.⁶³

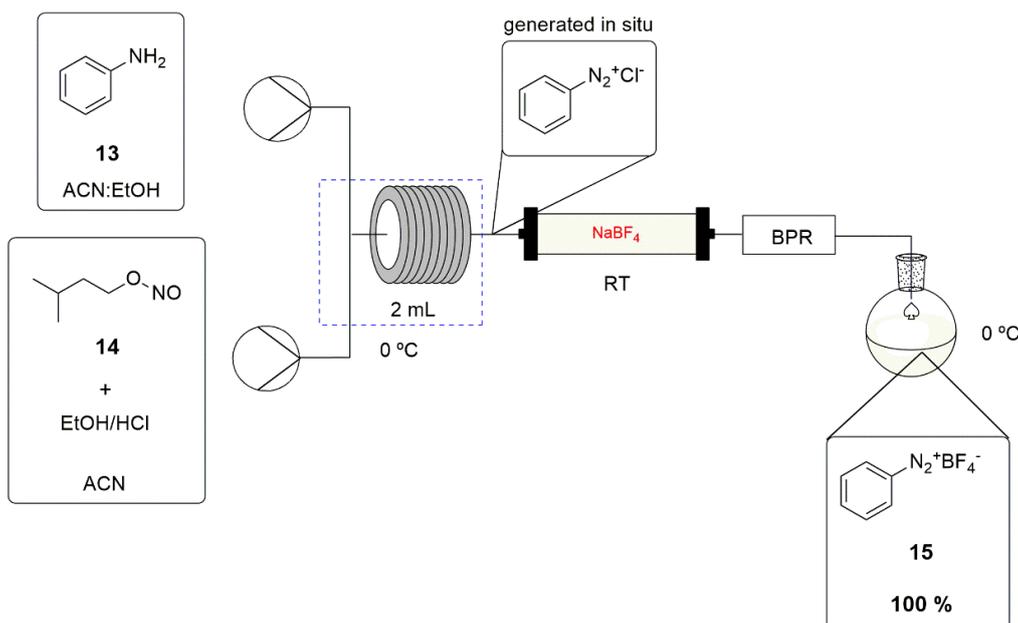
Furthermore, a telescoped process (Scheme 7) was achieved by passing the first stages product stream through a packed-bed reactor filled with Amberlyst-15 in order to neutralise the mixture. The output stream was collected into a pre-calibrated beaker and subsequently made up to a known concentration for introduction into the second stage with the use of an additional pump. A T-piece mixer was put in place of the 2 mL mixing chip, followed by a 14 mL coil reactor and a BPR. This telescoped approach afforded celecoxib **8** with a yield of 90%. The same article reports on an optimised batch approach affording celecoxib **8** in an overall yield of 90% with a total reaction time of 20 h. When comparing the batch and flow approach, the yields were observed to be similar, however the flow approach achieves this with an appreciable reduced 64 min residence time.



Scheme 7: Telescoped process for the synthesis of celecoxib under flow continuous flow conditions reported by C. Scholtz, 2021.⁶³

1.4.4.3 A continuous flow approach for the synthesis of aryldiazonium tetrafluoroborates

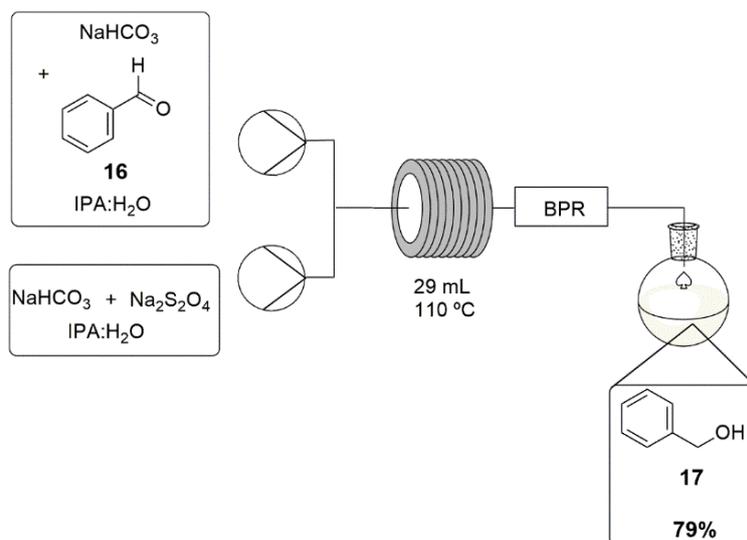
A second article by Scholtz highlights a high-yielding continuous flow approach for the preparation of multiple aryldiazonium tetrafluoroborates.⁶⁴ This was achieved with the use of a T-piece mixer linked to a 2 mL tubular coil reactor, both maintained at 0 °C. The coil reactor was subsequently fitted to a packed-bed reactor, at ambient temperature, filled with sodium tetrafluoroborate and was connected to a BPR. An optimised reaction reported within this article exhibits a diazotisation of aniline **13** with the addition of isopentyl nitrite **14** and ethanolic hydrochloric acid in acetonitrile. The product stream was subsequently passed through packed-bed reactor (packed with sodium tetrafluoroborate) affording the aryl tetrafluoroborate salt **15** in quantitative yields (Scheme 8). The authors reported that when comparing the same chemistry under batch conditions, the yields are comparable or better, however the unstable diazonium chloride salt precursors are generated and consumed *in situ*, thereby avoiding handling, or isolation of these salts.



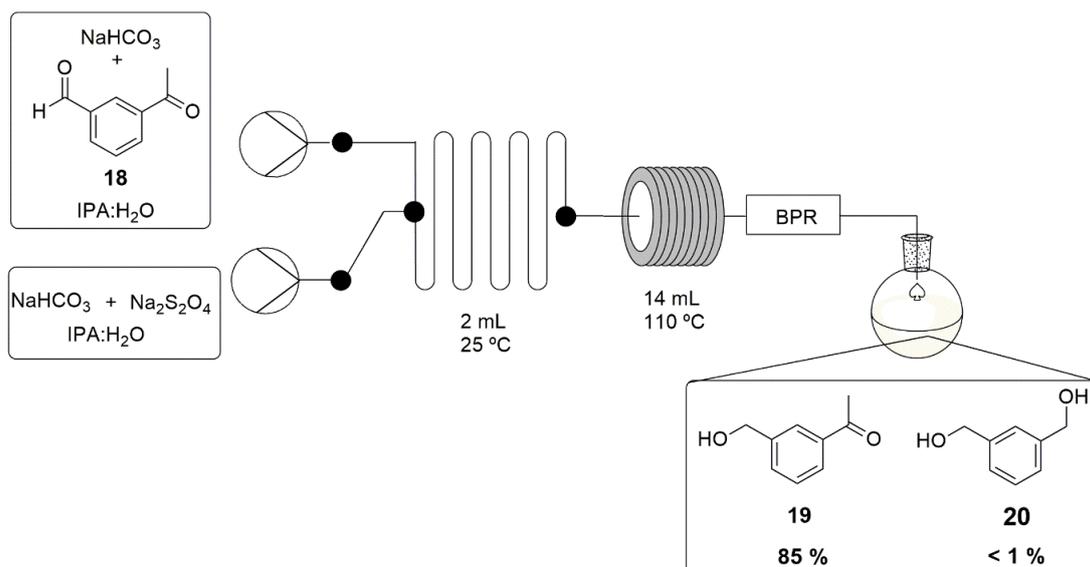
Scheme 8: Synthesis of aryl tetrafluoroborate salt **15**, reported by C. Scholtz, 2020.⁶⁴

1.4.4.4 Selective reductions of aldehydes

Another article by Neyt *et al.* reported the utilisation of flow chemistry technologies for sodium dithionite mediated reductions of aldehydes affording comparable yields to batch with reduced reaction times, from 12 h to 64 min and increasing the space time productivity by $> 3.6\times$.⁶ The reduction of benzaldehyde **16** to afford benzyl alcohol **17** proceeded with a yield of 79% correlating to a procedure productivity of $4.36 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ (Scheme 9). Furthermore, the approach was shown to be selective for the reduction of aldehydes over ketones.⁶ An example of such selectivity was performed in the same research whereby the aldehyde moiety of 3-acetyl benzaldehyde **18** was selectively reduced over the ketone functional group affording 3-acetylbenzyl alcohol **19** with an NMR yield of 85% and $\leq 1\%$ of the unselectively reduced 1,3-dimethanol benzene **20** (Scheme 10).



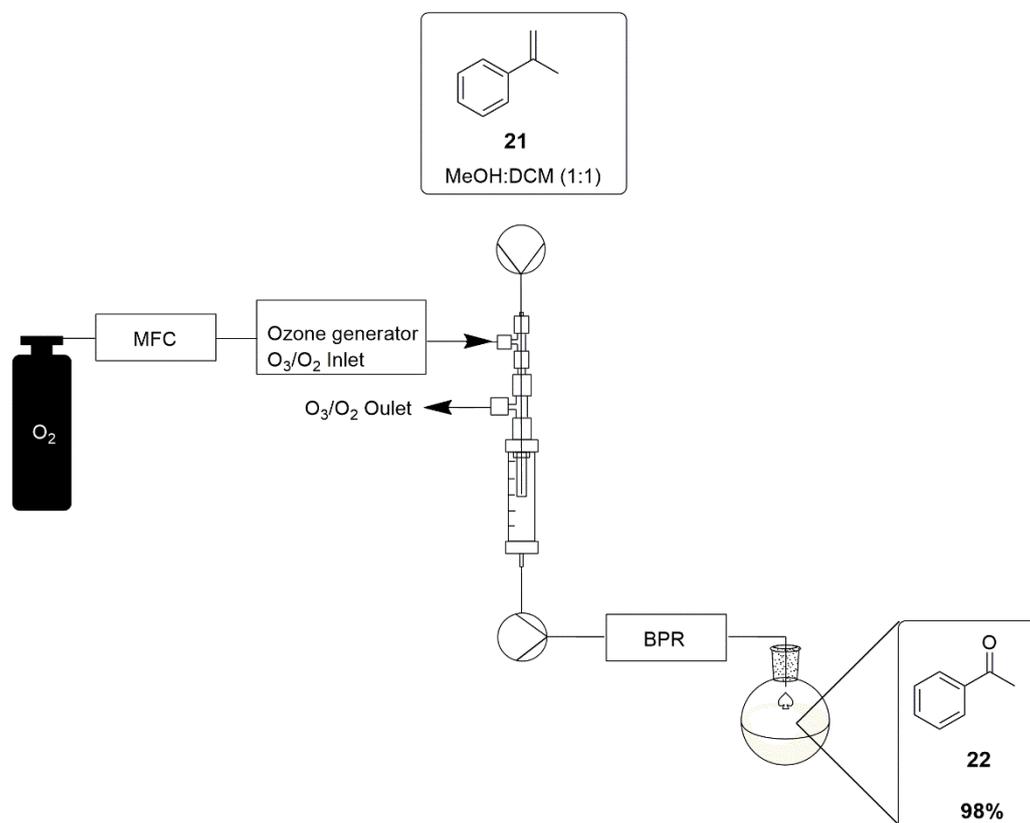
Scheme 9: Flow reduction of benzaldehyde **16** to benzyl alcohol **17**, reported by N. Neyt, 2018.⁶



Scheme 10: Continuous flow procedure for the selective intramolecular reduction of an aldehyde over a ketone.⁶

1.4.4.5 Flow-ozonolysis with on-the-fly removal of ozone

Neyt *et al.* also developed a novel prototype reactor that permits ozonolysis reactions under flow conditions with the aim of addressing safety concerns associated with ozonolysis reactions.⁷ This was achieved by designing an integrated degassing module which facilitated on-the-fly and in-line removal of unreacted oxygen and ozone gas (Scheme 11). The module also minimised contact with the oxygen-rich reaction atmosphere and it allowed the continuous



Scheme 11: Ozonolysis reaction under flow conditions reported by Neyt and Riley.⁷

reduction of ozonide intermediates, thereby preventing the build-up of these potentially explosive agents. The module employed operated by introducing an alkene in a MeOH:DCM solvent mixture to an ozone/oxygen gas stream which was then aspirated into a reaction chamber. The liquid phases were then continuously pumped out of the chamber and the gas phase was vented on-the-fly into the fume hood. The approach was successful in removing 98.5% of all ozone/oxygen and allowed the reaction stream to be telescoped into downstream processes without having to implement an offline ozone degassing step.⁷ The set-up was exemplified by the ozonolysis of methyl styrene **21**, affording acetophenone **22** with a yield of 98% (Scheme 11).

1.4.4.6 Implementing an automated continuous flow controlling platform for flow chemistry components

Van der Westhuizen *et al.*⁶⁵ reported on an automated self-optimising approach utilising several pieces of flow equipment. The system was designed with the use of a low-cost Raspberry Pi which was connected to the laboratory network. While avoiding costly proprietary software packages, an open-source Node-RED software was utilised which allows for flow-based programming and was installed onto the Raspberry Pi. With the aid of Node-RED, a customised dashboard for the monitoring and control of flow chemistry components was designed and altered to fit a specific set of sequences. With the use of the Node-RED platform, complex processes are made possible by aggregating several individual nodes together with each node being responsible for a specified function. Through Hypertext Transfer Protocol (HTTP) requests and sequential JavaScript Object Notation (JSON) messages sent and received between the individual nodes, automation of connected flow components is achieved (Figure 18).

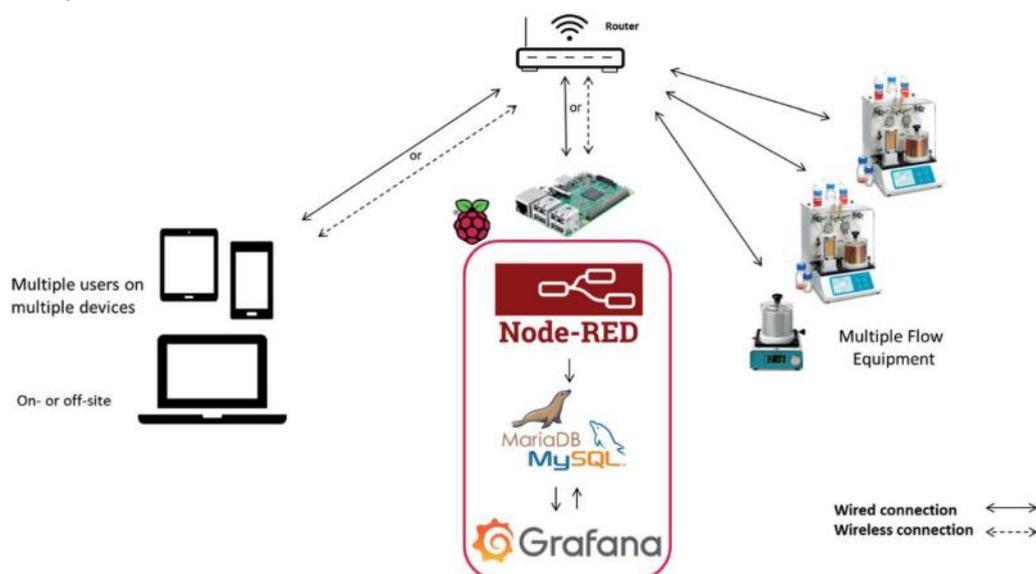
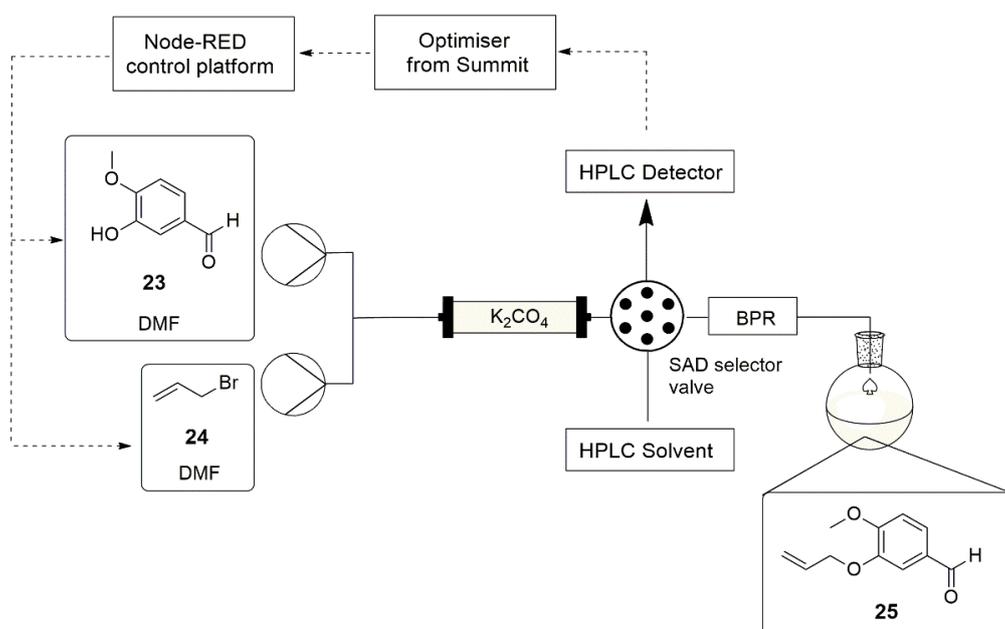


Figure 18: The automated set-up reported and re-used from van der Westhuizen, 2022.⁶⁵ The Raspberry Pi was equipped with Node-RED as well as the installed Grafana. Node-RED can communicate between various flow components allowing for this automated control.

With the use of a database, users are able to record reaction parameters, whereby a Structured Query Language (SQL) database was selected within the article and implemented into the Raspberry Pi. Node-RED is capable of visualising data collected through this dashboard; however, it was noted that graphic representation would result in the dashboard being unresponsive. Consequently, another open-source software, Grafana was utilised for graphic visualisation of the collected data, being capable of connecting to different databases. By using a local area network (LAN) interface, flow chemistry components are connected to the Raspberry Pi, through which they send and receive communication signals when specifying different tasks.⁶⁵

The same report demonstrated the use of this automated system setup for optimisations of an allylation reaction of isovanillin **23** (Scheme 12). This was achieved with the treatment of **23** with allyl bromide **24** while utilising a semi-autonomous closed loop approach to afford **25**. To achieve automated optimisation reactions, a Single-Objective Bayesian Optimiser (SOBO) algorithm was integrated into the system which allowed for automated data collection, analysis and additional automated parameter adjustments in order to achieve the best space-time yield. The optimised reaction conditions were achieved within 33 reaction attempts (approximately 12 h in total) performed automatically and resulted in an optimised space-time yield of 791 g.dm⁻³.h⁻¹ with the reactor unit temperature set at 77.3 °C and a residence time of only 4 min. As exemplified through this article, such closed-loop automated systems exhibit potential in optimisation reactions under flow conditions.



Scheme 12: The closed-loop set-up used for optimisations achieved in an automated approach under continuous flow conditions.⁶⁵

As seen in the research reported above, the use of continuous flow conditions within the pharmaceutical drug industry could be very promising with regards to safer, greener chemistry

methodologies. Continuous flow conditions have assured, in many syntheses, a decrease in reaction times with comparable, if not greater yields than when synthesised under batch conditions. The overall waste is more controlled, with higher product yields and selectivity's, resulting in lower amounts of waste. The use of reagents within a micro-reactor also provides increased safety when working with harmful chemicals and gases. Flow chemistry has also showed to be easily upscaled when requiring larger product yields.

1.5 Introduction to Neglected Tropical Diseases (NTDs)

1.5.1 A brief history and overview of NTDs

South Africa is one of many African countries afflicted by multiple diseases and illnesses, with approximately 75% of the global HIV/AIDS infections, 90% of malaria global deaths, high rates of drug resistant TB,² and in terms of NTDs, we have approximately 40% (400 million) of the global burden.⁶⁶ Despite these high prevalence's, Africa is largely unable to produce medicines for its own people, with almost all pharmaceuticals being imported from overseas vendors. As a result of this reliance on foreign importers we find ourselves facing issues associated with fluctuations in FOREX rates, the influx of counterfeit prescription drugs and constant supply and demand issues.² As a result, the need for local/regional pharmaceutical manufacturing has been identified by the DTI and the DSI as a critical developmental area to relieve some of these burdens.

NTDs are a diverse group of infectious conditions that predominantly affect the poorest people globally, residing primarily in the tropical regions of the globe. The basis of NTDs first arose in the beginning of the 21st century, successive to the agreement of the Millennium Development Goals (MDGs).^{67, 68} Global infectious diseases were outlined as a part of the eight MDGs, namely MDG 6.⁶⁷ HIV/AIDS, malaria and TB were the major targeted illnesses outlined within the MDG 6 framework.⁶⁸ Unfortunately, various other tropical diseases, with high global prevalence and disease burdens, were excluded from MDG 6.⁶⁸ Consequently, the term NTD was introduced, grouping 13 of these "neglected" communicable diseases resultant of bacteria, helminths, and protozoa.⁶⁹ The initial list of 13 NTDs had additions over the years, with the WHO currently having a list of 20 conditions that are categorised under the same NTD banner, constituting more than 1 billion annual global infections. These include "buruli ulcer, Chagas disease, chikungunya and dengue, dracunculiasis, echinococcosis, foodborne trematodiasis, human African trypanosomiasis, leishmaniasis, leprosy, lymphatic filariasis (LF), mycetoma, chromoblastomycosis and other deep mycoses, onchocerciasis, rabies, scabies and other ectoparasitoses, schistosomiasis, soil-transmitted helminthiasis, snakebite envenoming, taeniasis/cysticercosis, trachoma, and yaws".¹

A report by James *et al.*⁷⁰ reveals that the global prevalence of various NTDs, reported in 2017, are significant with ascariasis being responsible for approximately 447 million infections, followed by trichuriasis, hookworm disease, schistosomiasis and LF at 290, 230, 143 and 65 million infections respectively.⁷⁰ Comparably less prevalent are onchocerciasis, leishmaniasis and human African trypanosomiasis with 21, 4.1 and < 0.01 million global infections, respectively.⁷⁰ In the context of SSA, the most prevalent NTDs are helminth (parasitic worms) and protozoan infections.⁷¹ Helminth infections that are seen to be of a major concern and frequent SSA are soil-transmitted helminthiasis (STH) diseases like ascariasis, trichuriasis and hookworm disease. Schistosomiasis and filarial infections like onchocerciasis and LF are also highly prevalent,⁷¹ and the most common and threatening protozoan infections in SSA are leishmaniasis and human African trypanosomiasis.⁷¹ The prevalence of NTD's globally can be seen visually, in figure 19.⁷²

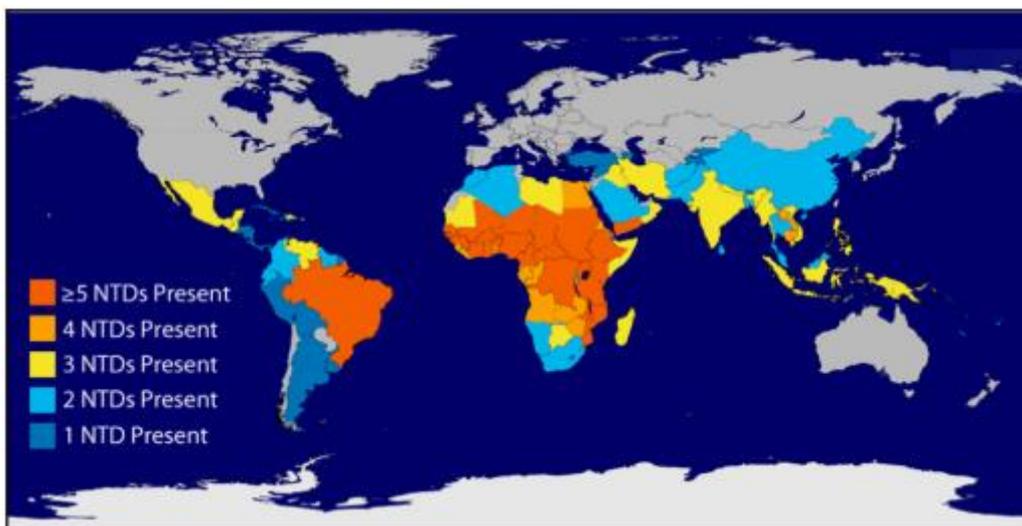


Figure 19: Map illustrating geographical prevalence of NTDs: Image re-used from Bhutta *et al.* 2014.⁷²

Currently, the NTDs that are most common, threatening and outlined by the Drugs for Neglected Diseases initiative (DNDi) are human African trypanosomiasis, leishmaniasis, LF, onchocerciasis and loiasis. Human African trypanosomiasis, commonly termed "sleeping sickness", is a protozoal disease that is resultant of infection by either one of two subspecies of *Typanosoma brucei* (species of parasite), namely *T. b. gambiense* or *T. b. rhodesiense*. The disease is believed to be transmitted to humans via an infected tsetse fly bite, which in final stages of the disease, can result in comas and then death.⁷³ It has been previously reported that throughout different areas of 36 countries in Africa, where human African trypanosomiasis is endemic, approximately 70 million people are at different levels of risk of contracting the human African trypanosomiasis infection.⁷³ Leishmaniasis is an infection which is resultant of the parasite *Leishmania* transmitted by the sandfly and leaves more than 1 billion people at risk of contracting this disease annually. Leishmaniasis is a poverty-associated illness with several forms, where the most common being cutaneous leishmaniasis

and the most fatal, without treatment, visceral leishmaniasis.⁷¹ Filarial diseases, like LF, onchocerciasis and loiasis cause chronic illness and life-long disabilities that lead to suffering and social stigmatisation. According to the DNDi, there is a definite unmet medical need for a pharmaceutical drug that can kill adult worms that cause these types of infections.

NTDs still cause significant morbidity and mortality within the developing world and they are often associated with long-term or chronic illness, impaired development within children, disfigurement, and decreased capacity for productivity.⁷⁴ As a result, they are seen to be large poverty contributors within developing countries which are often burdened by poor health-care systems which cannot efficiently oversee infection rates. Unfortunately, although NTDs are often treatable and/or preventable they are mostly disregarded by the “global North”. As a result, limited interest by big pharma companies in both the development of new drugs for their treatment and the manufacture of existing drugs, are experienced.^{74, 75} In a study undertaken by Pedrique *et al.*,⁷⁵ the authors reported that out of the 850 new approved products for therapeutic usage registered between 2000 and 2011, only 37 (4%) were devoted to neglected conditions. It was also noted that out of the 336 new chemical entities (NCEs) within the same period (2000 – 2011), only 4 (1%) of these NCEs were approved for neglected diseases.⁷⁵ It is also important to mention that the development of drugs, for NTDs, which are subjected to clinical trials is severely lacking. At the end of 2011, approximately 148 000 clinical trials were registered, with trials directed at NTD treatments only accounting for 1% of the total trials.^{73, 75}

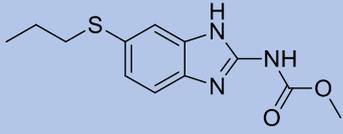
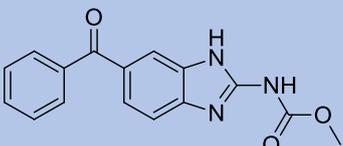
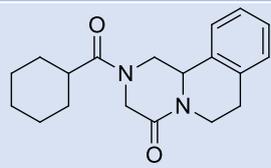
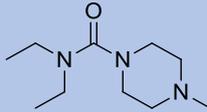
1.5.2 NTD categories outlined by the WHO with a focus on preventative chemotherapy treatment (PCT) grouped NTDs

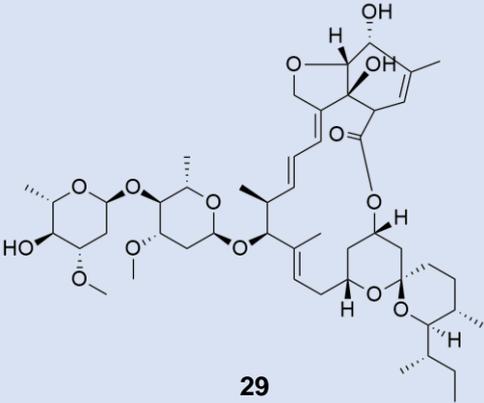
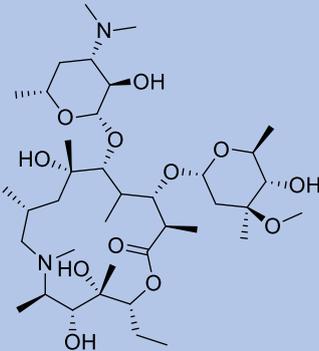
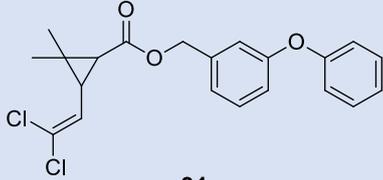
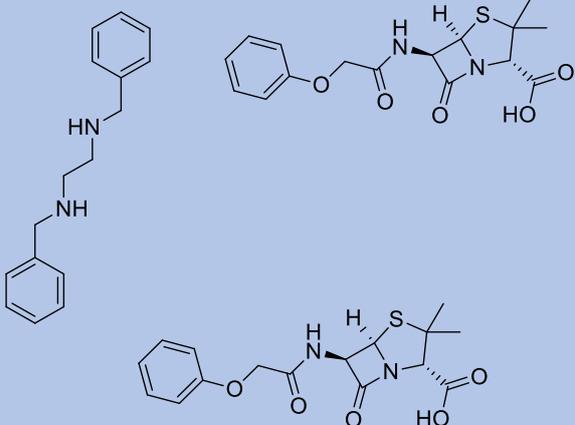
The WHO categorises NTDs into two groups, the one being diseases which are targeted by the use of preventative chemotherapy treatment (PCT) and transmission control; and the other group being innovative and intensified disease management (IDM).⁶⁸ These conditions are resultant of a variety of viruses, bacteria, parasites, fungi and toxins. The PCT-grouped diseases, responsible for the majority of NTD related conditions, were placed within the same group based on the idea that control and prevention of such diseases could be achieved simultaneously through mass drug administration (MDA), otherwise referred to as “preventative chemotherapy”.^{68, 69} Ascariasis, hookworm, LF, onchocerciasis, schistosomiasis, trachoma, trichuriasis, and more recently, scabies and yaws,⁶⁸ are all included within the PCT grouped diseases⁶⁹ and are still dominant amongst poverty stricken populations.⁷⁶

In total, there are currently 9 NTDs that are combated through various MDA initiatives with the implementation of preventative chemotherapy drugs. MDA denotes the distribution of low-

costing or donated pharmaceutical treatments to large populations, prone to various NTDs in countries that have inadequate resources available. MDA is implemented regardless of individual diagnoses, which has proven to be substantially more cost efficient when compared to an individual diagnosis, followed by treatment approach.⁶⁸ MDA is expressed as being a highly sensible approach since the medicines exhibit exceptional safety profiles and show limited documentation of drug resistance.⁶⁸ Table 1 below highlights the MDA treatments for the 9 PCT-classed diseases, including albendazole **26**, mebendazole **27**, praziquantel **1**, diethylcarbamazine **28**, ivermectin **29**, azithromycin **30**, topical scabicides (permethrin **31** for example) and antibiotics such as benzathine penicillin **32**. Often, multiple PCT treatments (rapid impact packages) are delivered to NTD prevalent regions dependent on the risk of multiple NTD infections within the region of interest.⁷⁶

Table 1: PCT categorised NTDs with their respective primary treatments

PCT-grouped NTD	Primary PCT Treatment	Chemical Structure
Ascariasis	Albendazole ^{68, 76}	 26
Hookworm		
Trichuriasis	Mebendazole ^{68, 76}	 27
Schistosomiasis	Praziquantel ^{68, 76}	 1
Lymphatic filariasis (LF)	Albendazole ^{68, 76}	 28
	Diethylcarbamazine ^{68, 76}	
	Ivermectin ^{68, 76}	

<p>Onchocerciasis</p>	<p>Ivermectin ^{68, 76}</p>	 <p>29</p>
<p>Trachoma</p>	<p>Azithromycin ^{68, 76}</p>	 <p>30</p>
<p>Scabies</p>	<p>Topical scabicides ⁷⁷ (Permethrin) Ivermectin ⁷⁷</p>	 <p>31</p>
<p>Yaws</p>	<p>Azithromycin ⁷⁸ Benzathine penicillin ⁷⁸</p>	 <p>32</p>

With the ongoing global warming crisis, it is expected that numerous tropical diseases may become global diseases. It has already been reported that the range of dengue is steadily growing, leishmaniasis is endemic in Italy,⁷⁹ and occurrences of chagas is rising within the Southern United States.⁸⁰

1.5.3 The way forward with regard to NTDs

An updated “2021-2030 roadmap” has been implemented by the WHO, focusing on addressing the disease burden resultant of NTDs. This in turn, is expected to reduce poverty levels within NTD afflicted regions.⁸¹ This road map is intended to allow for opportunities of assessment, evaluation and adjustment of the framework actions when required, throughout this current decade. This is achieved by outlined precise targets and milestones.⁸¹ The overarching 2030 global targets have been defined by the WHO and are identified below:

- It is intended that the amount of people in need of NTD related treatments will experience a reduction of potentially 90%⁸¹
- At least one NTD would be eradicated from a minimum of 100 countries⁸¹
- The extermination of two NTDs (namely dracunculiasis and yaws)⁸¹
- A potential decrease of the disability adjusted life years (DALYs) associated with NTDs by approximately 75%⁸¹

Unfortunately, the COVID-19 pandemic has negatively affected the global economy, the impact of which has been particularly hard felt in the poorer nations and it is a concern that COVID-19 will negatively impact the elimination efforts and preventative control for onchocerciasis (river blindness), LF and schistosomiasis within Africa.^{67, 82} The same is seen to be an issue for India and their control of soil-transmitted helminth infections and lymphatic filariasis.⁶⁷ As such, NTD prevention and elimination needs to be prioritised where possible in order to prevent the derailment of progress against the elimination of NTDs as a result of post COVID-19 impacts.^{67, 82}

1.5.4 Schistosomiasis, the focal NTD of this research project

1.5.4.1 Introduction and transmission of schistosomiasis

Schistosomiasis, commonly termed snail fever or bilharzia, is a chronic circulatory system infection that is resultant of trematode parasites (genus *Schistosoma*, phylum Platyhelminthes, class Trematoda)⁶⁸ which penetrate human skin, subsequently initiating inflammation of the bladder, intestines and liver.⁷⁴ Certain conditions are required for schistosomiasis to be transmitted to humans, including appropriate intermediate snail hosts and tropical climates for a minimum of 4 to 6 months in a year. As such, schistosomiasis is commonly found in the equatorial regions, located majorly between 36°N and 34°S latitude.⁶⁸ The sustainability of schistosome related infections is often promoted by fertilised eggs

contained within infected human faecal and urinary excretions.^{68, 83, 84} This in turn often leads to contamination of surface waters, especially in underdeveloped communities where sanitation supplies and facilities are underfunded, which ultimately enables replication of the ciliated miracidia through hatching of the eggs.⁸³ These ciliated miracidia can infect suitable snail hosts which allows for the parasite to undergo the next stage of their lifecycle, asexual replication, via daughter and mother sporocyst periods.⁸³ This asexual replication stage requires between 4 – 6 weeks, after which, tens of thousands of cercariae are shed from the intermediate snail host into the surrounding waters. These cercariae are infectious towards humans and can enter a human host through penetration of the skin.⁸⁵ Subsequently, these cercariae make their way to the blood stream, allowing migration to the lungs, followed by the liver and lastly, settle in the mesenteric and perivesical venous plexuses.⁷⁴ The now maturing schistosomula require approximately a 5 – 7 week period within the human host before reaching the adult stages of their lifecycle allowing them to produce eggs.⁸³ These adult worms survive within the blood vessels of the human host and the eggs produced die within 1 - 2 weeks and are either excreted from the host or retained within the body. It is important to note that during the 5 – 7 week maturation period within the human host, the infection is ongoing, but the release of eggs is undetectable. Consequently, this cycle repeats itself if the eggs are excreted before dying, accompanied with suitable incubation conditions (freshwater contact) and infection of another appropriate snail host occurs.⁸³ The life cycle of *Schistosoma* spp. is shown in figure 20, the image was obtained from the Centers for Disease Control and Prevention (CDC).⁸⁵

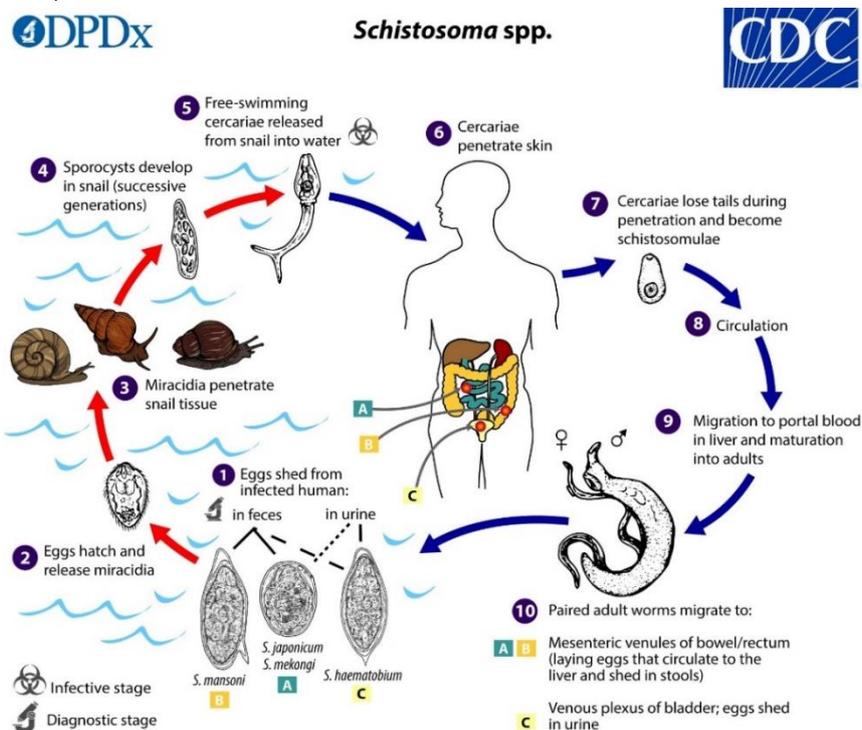


Figure 20: Life cycle of the *Schistosoma* genus through various processes. Image reused from the CDC biology of schistosomiasis webpage.⁸⁵

1.5.4.2 Species of transmission of schistosomiasis

There are many species that affect both mammals and birds, however, only six commonly affect humans. *Schistosoma mekongi* and *Schistosoma malayensis*, which are confined to the central Mekong basin (Laos and Cambodia) and Malaysia, respectively.^{83, 86} *Schistosoma intercalatum* is of a minor concern within some regions in Central and West Africa, however, the three most common species, resulting in the majority of global schistosomiasis cases, are *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*,^{68, 83} with *Schistosoma haematobium* and *Schistosoma mansoni* collectively accounting for approximately 99% of all global schistosomiasis cases.⁸⁷ These species are both widespread in Africa and the Middle East, whereas *Schistosoma japonicum* is endemic in Asia (Philippines and China predominantly). *Schistosoma mansoni* is also occurrent in North and South American regions.⁸³ Specific freshwater snails are capable of transmitting these three species, where the *Biomphalaris* genus allows for transmission of *Schistosoma mansoni*, the *Bulinus* genus is a carrier of *Schistosoma haematobium* and *Oncomelania* genus are intermediate hosts for *Schistosoma japonicum*.^{68, 88} *Schistosoma japonicum* is reported to be zoonoses, being capable of infecting multiple mammalian hosts, consequently, complicating efforts outlined for control and elimination of this genus type. Whereas, *Schistosoma mansoni* and *Schistosoma haematobium* are observed to predominantly target human hosts.⁸³

1.5.4.3 Symptoms and containment of schistosomes within the human body

Schistosoma haematobium is responsible for urogenital schistosomiasis, whereas *Schistosoma mansoni* and *Schistosoma japonicum* lead to intestinal schistosomiasis. Intestinal schistosomiasis causes various symptoms, with the frequency of symptoms often dependent on the intensity of the infection. These symptoms include abdominal pain, diarrhoea as well as rectal bleeding.⁸⁹ It has been reported that in more severe cases, the infected patients are diagnosed with extensive fibrosis as a result of poor immunoregulation of the hosts response towards parasitic egg antigens.⁸³ Urogenital schistosomiasis tends to exhibit haematuria symptoms, associated with an abnormal frequency in urination, suprapubic distress as well as burning micturition. Similarly, to intestinal schistosomiasis, severe cases of urogenital schistosomiasis often lead to chronic fibrosis within the urinary tract. This usually presents itself as obstructive uropathy,⁹⁰ which can ultimately lead to potentially lethal renal dysfunction.⁸³ *Schistosoma haematobium* and *Schistosoma mansoni* have been directly linked to an increase in susceptibility of infection observed amongst HIV-1 positive females, resultant of the urogenital region being more prone to mucosal lesions.^{91, 92}

It is important to note that the symptoms experienced by the infected human is a direct result of the bodies reaction to fertilised eggs that are released and not the adult worm itself.⁸⁵ It has been reported by the CDC that many of the schistosome human related infections are

asymptomatic,⁸⁵ however, the adult worms may be present within the human host. Schistosomes are capable of living an average life cycle of approximately 3 – 10 years within their human hosts, however, various cases have been reported with schistosomes living as long as 40 years in their respective human host.⁸³ When these adult schistosomes live within the human body, they are generally “*in copula*”,⁸³ where the lean female adult worm is mounted within the gynaecophoric duct of the adult male. In this way, as the female delivers her eggs, the male can fertilise them. The adult *Schistosoma haematobium* genus is capable of living within the perivesicular venules, whereas *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi* and *Schistosoma malayensis* are able to live in the mesenteric venules.⁸³ Adult schistosomes survive by the digestion of human erythrocytes with most of their energy being achieved through glucose metabolism,⁹³ however, the egg production is only possible and dependent on oxidation of fatty acids.⁹⁴ Due to the fact that schistosomes are not equipped with a rectum, they are incapable of excreting waste and consequently, regurgitate this back into the bloodstream of the infected host. Fortunately, diagnosis through blood and urine-based bioassays are made possible through some of this expelled waste.⁸³

Schistosomiasis has not only been observed within rural communities, but also reported to have been transmitted within settlements surrounding man-made dams as well as irrigated lands. Urban areas are starting to experience the disease, which is highly problematic in communities without sufficient sanitation.⁶⁸ Intermediate snail hosts are capable of surviving in drainage systems, local canals and irrigated plots within many shantytowns.⁶⁸ Consequently, if the sanitary conditions and water supply are poor, a permanent transmission of schistosomiasis may arise in the city, resultant of people moving between the city and infected rural regions.

1.5.4.4 Occurrences of schistosomiasis and its primary treatment

Schistosomiasis is a disease of important interest as it affects more than 200 million people globally and has over 700 million at risk.^{68, 95} Out of these, more than 80% of occurrences are in SSA (Figure 21).^{95, 96} Praziquantel is a prescription drug accredited as being a de-worming medication to prevent infections related to *Schistosoma* worms. Consequently, it has been approved as being the primary treatment for schistosomiasis, being both safe and greatly effective against all adult *Schistosoma* species as a single dose.⁶⁸ The exact mode of action behind how this drug effects the *Schistosoma* worms is still not certain, however, praziquantel has been suggested to target calcium channels, exhibiting a link between calcium influx and muscular contractions of the schistosomes.⁷⁴ Nucleoside uptake within living worms has also been observed to have been inhibited by praziquantel **1**, which may be directly associated with adenosine uptake or signalling. Notably, said effects have not been observed in mammalian cells, only in schistosomes.⁷⁴

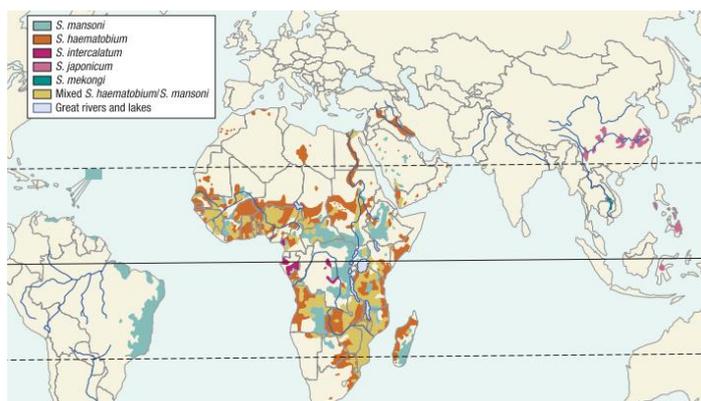


Figure 21: Global distribution of the five major *Schistosoma* genus types with the exemplified dominance of *Schistosoma haematobium* and *Schistosoma mansoni*. Map reused from Ryan et al. 2020.⁶⁸

The implementation of MDA was enabled through international support, pharmaceutical drug donations (i.e. Merck KGaA)⁶⁸ and the synthesis of generic praziquantel treatments. With the increase in MDA, the number of infections and people at different risk levels are declining steadily within areas which have historic schistosomiasis prevention programs, including Brazil, China, and Egypt. This trend is associated with improvements of socio-economic conditions as well as more communities being reached with MDA.^{68, 86}

Although, praziquantel is the primary treatment of choice for schistosomiasis, less frequently used oxamniquine (6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline) **33** (Figure 22) is an available treatment sold under the trade name, Vansil and anti-malarial drug, artemether ((1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,13*R*)-10-methoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecane) **34** (Figure 23) has also exhibited anthelmintic activity against schistosomes.⁹⁷

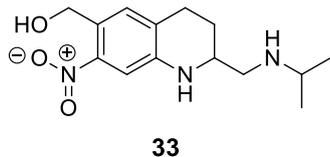


Figure 22: Oxamniquine **33**.

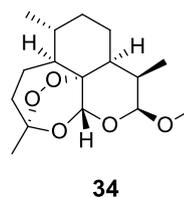


Figure 23: Artemether **34**.

As such, praziquantel **1** is frequently combined with additional anthelmintics, for the handling of schistosomal morbidity.⁶⁸ It has been previously reported that more or less than 100 million adults and 118 million children necessitate frequent praziquantel **1** MDA, however, in 2017 there was only a 28.2% coverage leaving 71.8% without access to this treatment. By 2019, it was estimated that approximately 236.6 million people were in need of preventative treatment, however, the amount of people reported to have received treatment amounted to 105.4 million (approximately 44.5%).³ The WHO reports estimate between 24 000 and 200 000 deaths result from schistosomiasis infections yearly.³ As exhibited from 2017 to 2019, the supply of praziquantel has increased, however the global demand is still not met, requiring more

assistance in praziquantel **1** production and procurement. As such, the development of an economical, safe, and green process for its manufacture is of socio-economic interest.

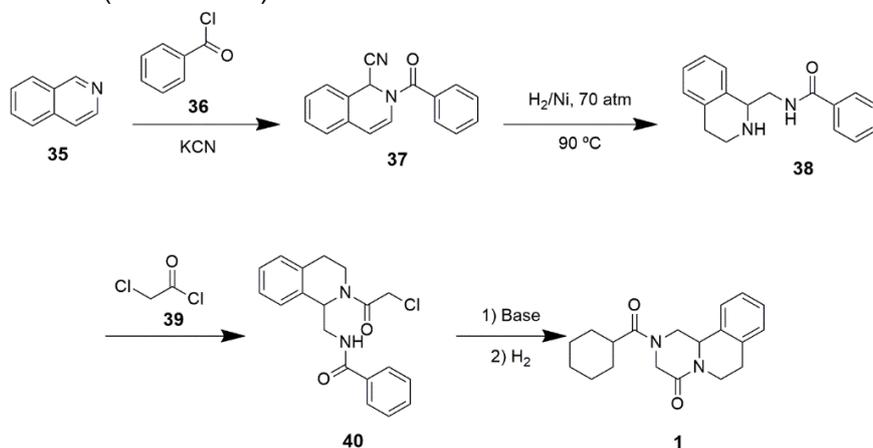
1.5.5 Previously reported synthetic approaches to Praziquantel

Praziquantel **1** was first synthesised in 1977 and has been used extensively for the fight against schistosomiasis ever since. Praziquantel **1** is sold as a racemic mixture, however it has been reported by various researchers that the *R*-configured praziquantel **1** stereoisomer is the most active in killing adult schistosomes.⁷⁴ Fortunately, the racemic form has an excellent safety profile, exhibits appreciable activity against all types of schistosomes and requires less intensive synthetic routes as opposed to the enantiomerically pure (*R*)-praziquantel **1**. As such, the cost-of-goods factor generally favours the production of the racemic praziquantel **1** product.⁷⁴

Several synthetic procedures have been developed for praziquantel **1**; the key approaches are highlighted below.

1.5.5.1 J. Seubert (E. Merck Company)

The first known synthesis of praziquantel **1**, was achieved by J. Seubert *et al.* from the E. Merck Company in the 1970's.^{98, 99} The exceptional anthelmintic activity exhibited by praziquantel **1** was then identified by Bayer AG in collaboration with the E. Merck Company.^{98, 99} Praziquantel **1** represented a breakthrough for the inhibition and treatment of schistosomiasis and ever since, has been the primary treatment. The original procedure was patented in 1975,¹⁰⁰ and later elaborated upon by Dömling and Khoury.⁷⁴ The reaction proceeds via a Reissert type reaction between isoquinoline **35**, benzoyl chloride **36** and potassium cyanide, affording a "Reissert Körper" intermediate **37**. Thereafter, nickel catalysed hydrogenation under high pressure affords *N*-[1,2,3,4-tetrahydro-isoquinolyl-1-methyl]-carboxamide **38** followed by acylation with chloro-acetyl chloride **39** to afford **40**. This is followed by a base-catalysed ring closure and finally, the benzoyl group is hydrogenated to yield praziquantel **1** (Scheme 13).

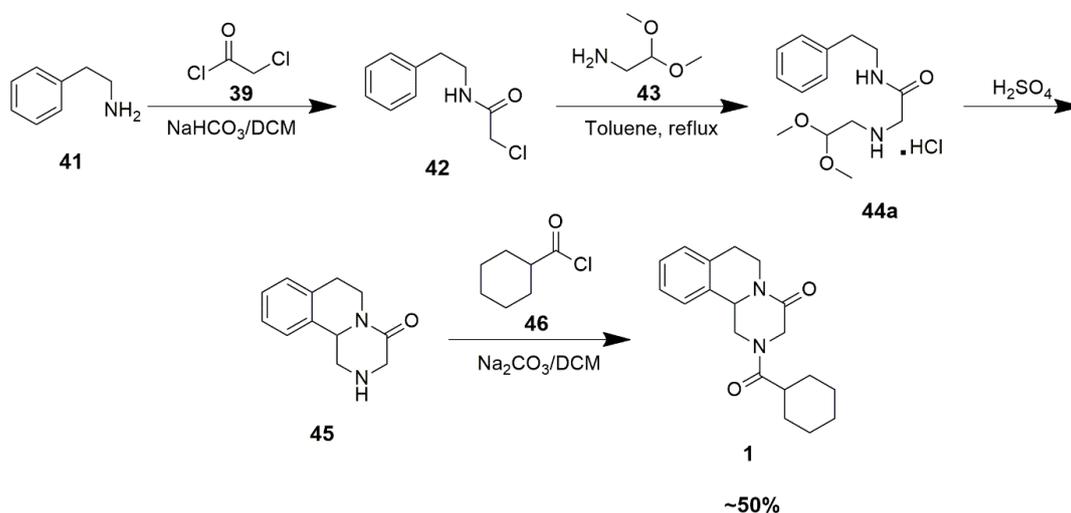


Scheme 13: First known synthesis of praziquantel **1** by the E. Merck Company.⁹⁸

Curiously, reaction yields for this synthetic procedure were not reported in the original works, and as such the overall yield remains a question. Critically speaking, the use of potassium cyanide in step 1 poses a safety and environmental risk and from a process point-of-view the high-pressure hydrogenation (step 2) also has associated safety issues.

1.5.5.2 Kim *et al.* (Shin Poong)

In 1983, the Korean company Shin Poong developed an approach to synthesise praziquantel **1** in bulk using a low-cost strategy and subsequently by 1993 had become the world's largest producer.⁷⁴ The approach employed and reported by Kim *et al.*,¹⁰¹ involved an initial condensation of 2-phenylethylamine **41** and chloro-acetyl chloride **39** with sodium bicarbonate in DCM to afford *N*-(2-phenyl)ethyl chloroacetamide **42**. This precursor was then amino alkylated with amino acetaldehyde dimethyl acetal **43** to yield *N*-2-phenylethyl 2-*N*-(2,2-dimethoxyethylamino)acetamide hydrochloride **44a**. Successive cyclisation was then achieved by the introduction of concentrated sulphuric acid to produce 4-oxo-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*]isoquinoline **45**. Finally, acylation with cyclohexanecarbonyl chloride **46** resulted in praziquantel **1** in an overall reaction yield of approximately 50% (Scheme 14).¹⁰¹ The procedure represented a significant improvement over that developed by the E. Merck Company avoiding the use of cyanides and high pressures, however, from a sustainability point of view half the steps take place in undesirable DCM, which by today's standards is no longer acceptable.

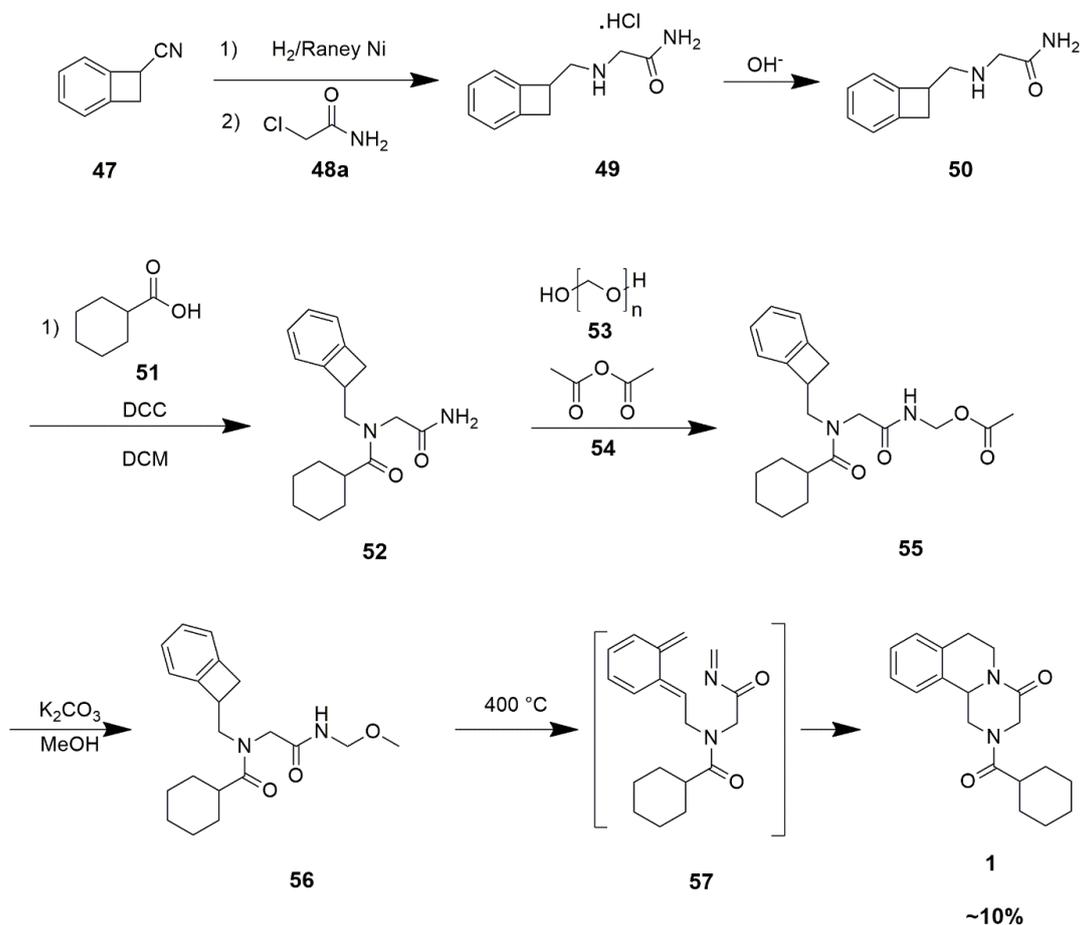


Scheme 14: The Shin Poong process for the synthesis of praziquantel **1**.¹⁰¹

1.5.5.3 Berkowitz *et al.*

Berkowitz *et al.* employed a Diels-Alder approach for the synthesis of praziquantel **1** in 1984.¹⁰² Initially, cyanobenzyl cyclobutene **47** was hydrogenated, followed by the addition of chloroacetamide **48a**. Thereafter, the 2-[(bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethyl)-amino]-acetamide hydrochloride salt **49** formed, was neutralized with sodium bicarbonate, followed by reductive amination to yield 2-[(bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethyl)-amino]-acetamide

50. This newly formed compound **50** was allowed to react with cyclohexanecarboxylic acid **51** and *N,N'*-dicyclohexylcarbodiimide (DCC) in DCM to afford cyclohexanecarboxylic acid bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethyl-carbamoylmethyl-amide **52**. This newly formed compound was subsequently treated with paraformaldehyde (formaldehyde **53** source), acetic acid and acetic anhydride **54** to produce acetic acid [2-(bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethyl-cyclohexanecarbonyl-amino)-acetyl-amino]-methyl ester **55**. Following this, **55** was treated with potassium carbonate in MeOH allowing the formation of cyclohexanecarboxylic acid bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethyl-[(methoxymethyl-carbamoyl)-methyl]-amide **56**. Finally, **56** underwent an internal Diels-Alder reaction at approximately 400 °C yielding praziquantel **1** via reactive intermediate **57**. The entire 7-step procedure afforded praziquantel **1** in an overall yield of approximately 10% (Scheme 15).¹⁰² Overall, this synthetic procedure is very labour and energy intensive with a low yield and notably, uses a cyanide containing reagent which imposes inherent safety risks for the working chemist.

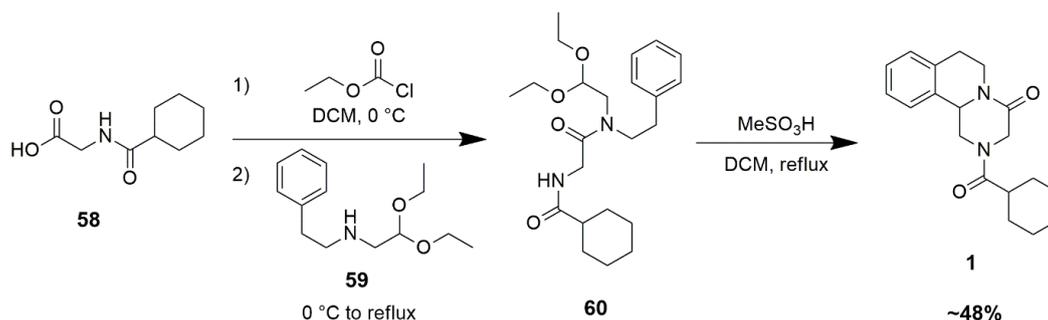


Scheme 15: Diels-Alder approach for the synthesis of praziquantel **1** reported by Berkowitz *et al.*¹⁰²

1.5.5.4 Kim *et al.*

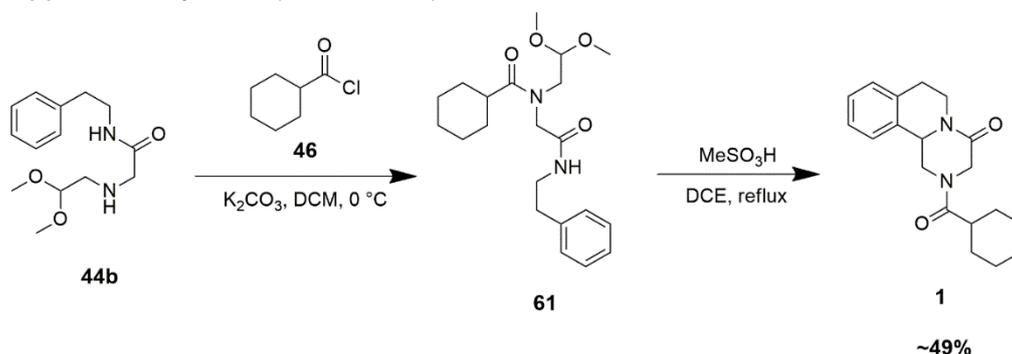
An alternative approach reported in 1998 by Kim *et al.*¹⁰³ starts with the activation of *N*-cyclohexanoyl glycine **58** by the addition of ethyl chloroformate in DCM at 0 °C. Subsequently, an amino acetal, 2,2-dimethoxy-*N*-phenethylethaneamine **59** is introduced into the reaction

mixture, under reflux conditions, to afford the amido acetal *N*-(2,2-diethoxy)ethyl-*N*-2-phenylethyl 2-*N*-cyclohexylcarbonylaminoacetamide **60**. It was noted that the final cyclisation for the conversion to praziquantel **1** was achieved in DCM with the treatment of methanesulfonic acid (MSA) under reflux conditions for 2 days. This synthetic procedure affords the praziquantel product **1** in an overall reaction yield of approximately 48% (Scheme 16).¹⁰³ The procedure is considerably lengthy, unfortunately, limiting the space-time productivity and consists of more than one step using undesirable DCM. Notably, reagents **58** and **59** are complex ingredients and as such would be beneficial to synthesise in-house (avoiding additional costs) adding additional steps and complexity to this route.



Scheme 16: Synthesis of praziquantel **1** reported by Kim *et al.*¹⁰³

The same article¹⁰³ reported a similar last-step cyclisation wherein the dimethyl acetal moiety is bound to the amine functional group instead of the amide (*N*-(2-phenylethyl)-2-(*N*-cyclohexylcarbonyl-2,2-dimethoxyethylamino)acetamide **61**). Intermediate **61** is prepared by the treatment of *N*-2-phenylethyl-2-*N*-(2,2-dimethoxyethylamino)acetamide **44b** with cyclohexanecarbonyl chloride **46** in the presence of sodium carbonate within DCM. It was reported that dichloroethane (DCE) was used instead of DCM for the final conversion to praziquantel **1** promoted by MSA in only 6 h affording praziquantel **1** in an overall reaction yield of approximately 49% (Scheme 17).

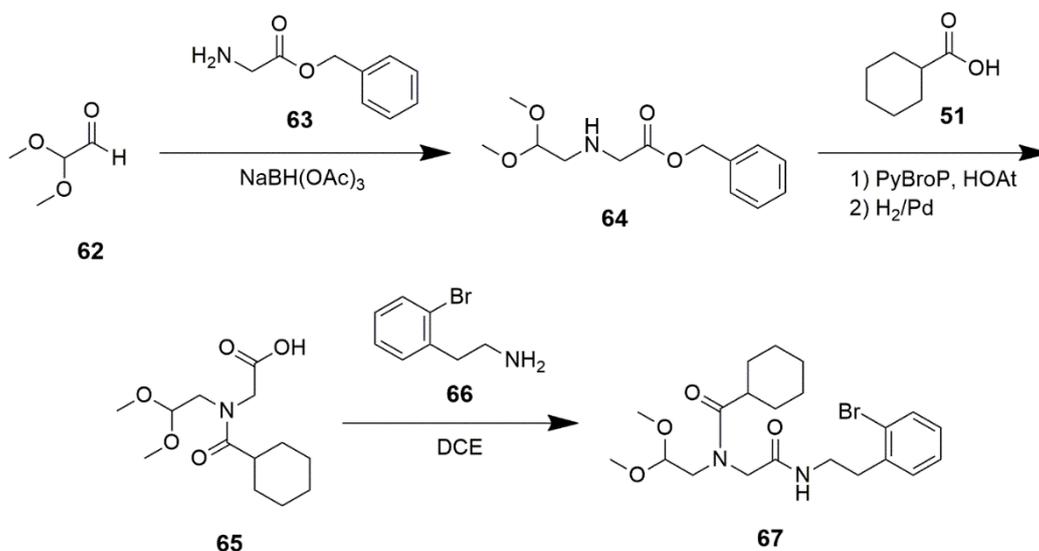


Scheme 17: A different approach for the synthesis of praziquantel **1** reported by Kim *et al.*¹⁰³

1.5.5.5 Todd *et al.*

In 2002, Todd *et al.*¹⁰⁴ reported the synthesis of praziquantel **1** via both a linear and convergent approach. When reviewing the linear approach (Scheme 18), the first step of the reaction is a

reductive amination of 2,2-dimethoxyacetaldehyde **62**, with benzyl glycinate **63** in the presence of sodium triacetoxyborohydride yielding phenylmethyl 2-[(2,2-dimethoxyethyl)amino]acetate **64**. The modest yielding acetal **64** was then treated with cyclohexanecarboxylic acid **51** in the presence of bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP) and 1-hydroxy-7-azabenzotriazole (HOAt), followed by hydrogenation to produce 2-[*N*-(2,2-dimethoxyethyl)cyclohexylcarbonylamino]acetic acid **65**. Finally, 2-bromophenethylamine **66** was allowed to react with **65** in DCE to yield 2-[*N*-(2,2-dimethoxyethyl)cyclohexylcarbonylamino]-*N*-[2-(2-bromophenyl)ethyl] acetamide **67**.

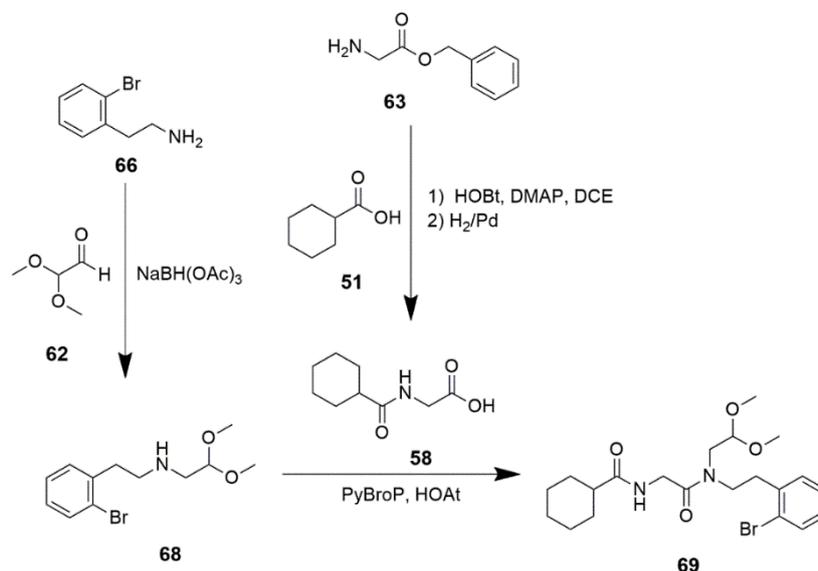


Scheme 18: Linear approach for the synthesis of praziquantel intermediate 2-[*N*-(2,2-dimethoxyethyl)-cyclohexylcarbonylamino]-*N*-[2-(2-bromophenyl)ethyl] acetamide **67** reported by Todd et al.¹⁰⁴

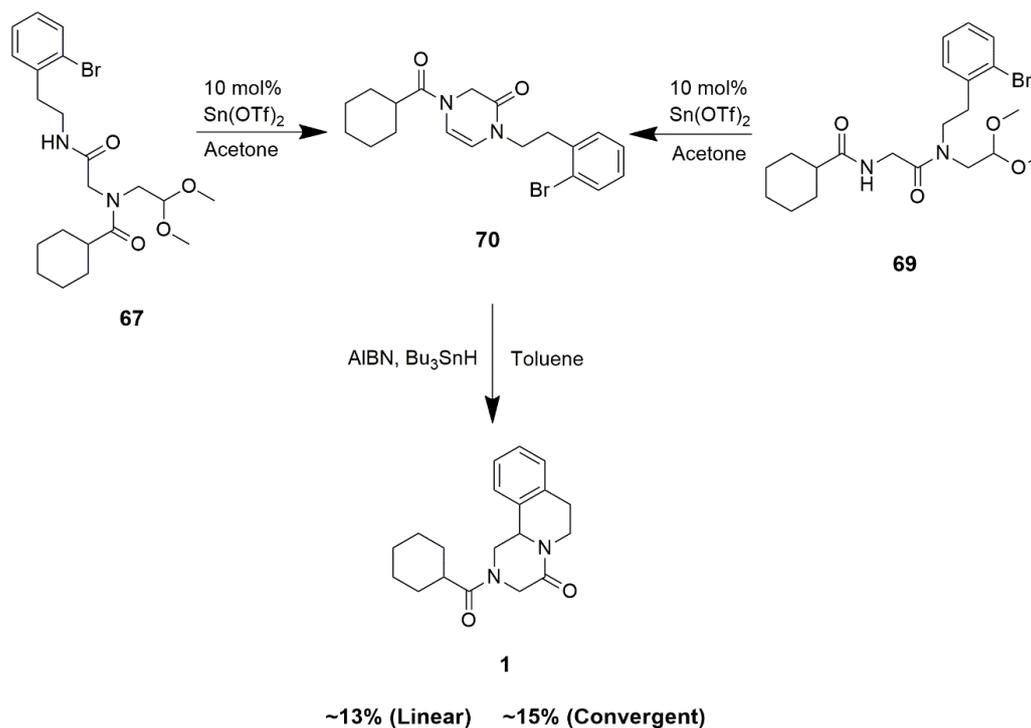
When compared with the convergent approach (Scheme 19), the first step is a similar reductive amination of 2,2-dimethoxyacetaldehyde **62** with ortho-bromophenethylamine **66** affording (2,2-dimethoxyethyl)-[2-(2-bromophenyl)ethyl]amine **68** in the presence of sodium triacetoxyborohydride. Subsequently, benzyl glycinate **63** is allowed to react with cyclohexanecarboxylic acid **51** in the presence of hydroxybenzotriazole (HOBt) and 4-dimethylaminopyridine (DMAP) in DCE. A subsequent hydrogenation is performed to afford *N*-cyclohexanoyl glycine **58**. The modest yielding acetal, **68** was then treated with newly formed glycine **58** in the presence of PyBroP and HOAt yielding *N*-(2,2-dimethoxyethyl)-*N*-[2-(2-bromophenyl)ethyl]-2-(cyclohexylcarbonylamino)acetamide **69**.

In both the linear and convergent approach, acetamides **67** and **69** were subjected to a tin triflate catalysed ring closure in acetone to produce the same pyrazinone 1-[2-(2-bromophenyl)ethyl]-4-(cyclohexylcarbonyl)-1,3,4-trihydropyrazin-2-one **70** in good yields. Subsequently, a key radical cyclisation was exploited in the final step with the use of azobisisobutyronitrile (AIBN) and tributyltin hydride in toluene for the final conversion to

praziquantel **1** (Scheme 20). The approach afforded overall reaction yields of approximately 13% and 15% for the linear and convergent approaches, respectively.¹⁰⁴



Scheme 19: Convergent approach for the synthesis of praziquantel intermediate *N*-(2,2-dimethoxyethyl)-*N*-[2-(2-bromophenyl)ethyl]-2-(cyclohexylcarbonylamino)acetamide **69** reported by Todd et al.¹⁰⁴

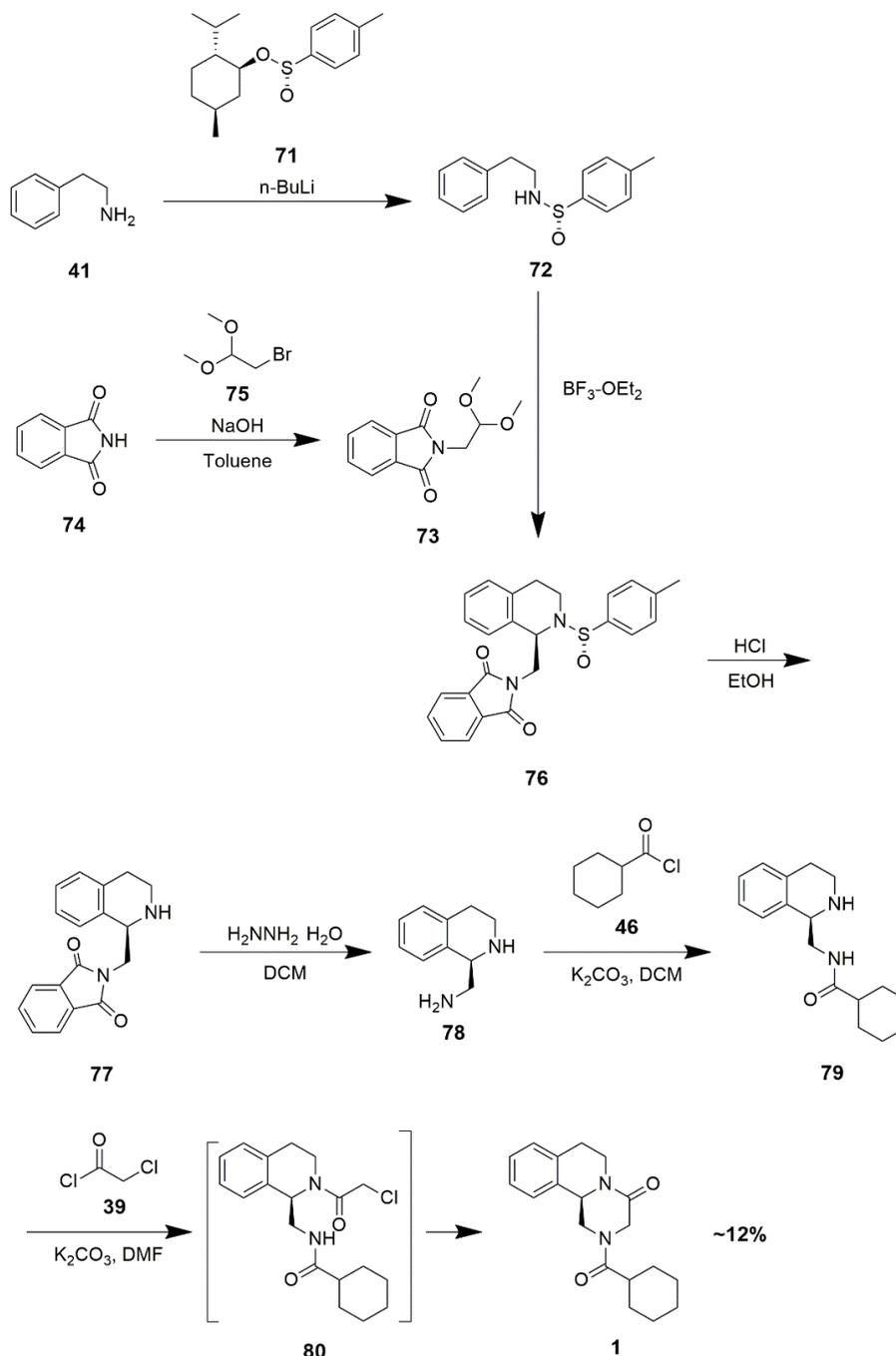


Scheme 20: The last two steps for the synthesis of praziquantel **1** via acetamide intermediates **67** (Linear approach) and **69** (convergent approach) reported by Todd et al.¹⁰⁴

1.5.5.6 Ma et al.

Enantiomerically pure (*R*)-praziquantel **1** has been synthesised by a few research groups and will be discussed briefly.

In 2004 Ma *et al.* reported the first enantioselective synthesis of (*R*)-praziquantel **1** (Scheme 21).¹⁰⁵ The approach started with the introduction of 2-phenylethylamine **41** in THF to an *n*-butyllithium containing hexane solution, followed by the treatment with (1*S*,2*R*,5*S*)-methyl-(*R*)-*p*-toluene-sulfinate **71** in THF affording *N-p*-tolylsulfinyl phenethylamine **72**. Preparation of 2-



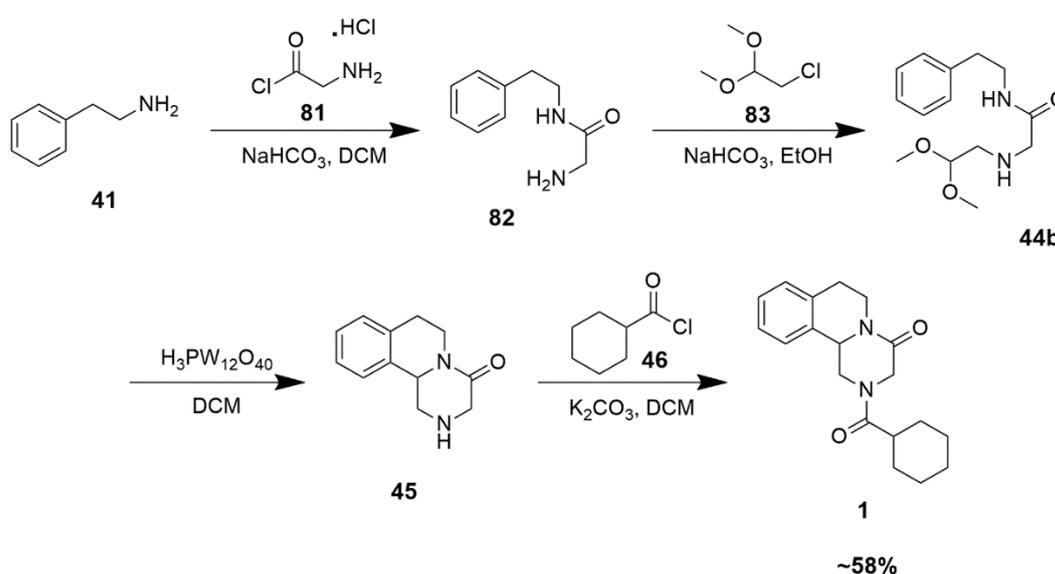
Scheme 21: First synthetic attempt at the enantiomerically pure (*R*)-praziquantel **1** reported by Ma *et al.*¹⁰⁵

(2,2-dimethoxyethyl)isoindoline-1,3-dione **73** was then achieved by reacting phthalimide **74** with 2-bromoacetaldehyde dimethyl acetal **75** under basic conditions in toluene. Subsequently, **72** and **73** were allowed to react with each other in DCM at sub-zero temperatures with the addition of borontrifluoride diethyletherate, producing the

tetrahydroisoquinoline **76**. Subsequent hydrolysis of the tolylsulfinate moiety was performed with concentrated ethanolic HCl affording tetrahydro-isoquinoline enantiomer **77** in an enantiomerically pure form. Thereafter, **77** was treated with hydrazine monohydrate within EtOH to achieve hydrolysis of the phthalimide moiety yielding **78**. Subsequently, cyclohexanecarbonyl chloride **46** and potassium carbonate were introduced to a solution of **78** in DCM to produce 1-[*N*-cyclohexylcarbonylamidomethyl]-1,2,3,4-tetrahydroisoquinolone **79**. Finally, **79** was treated with potassium carbonate and chloroacetyl chloride **39** in dimethylformamide (DMF) to yield (*R*)-praziquantel **1** after ring closure was achieved in intermediate **80**. Unfortunately, this entire 7-step synthetic procedure allows for the synthesis of (*R*)-praziquantel **1** (93% enantioselectivity) with an overall yield of only 12%.¹⁰⁵

1.5.5.7 Yixing Xinyu Chemical Co.

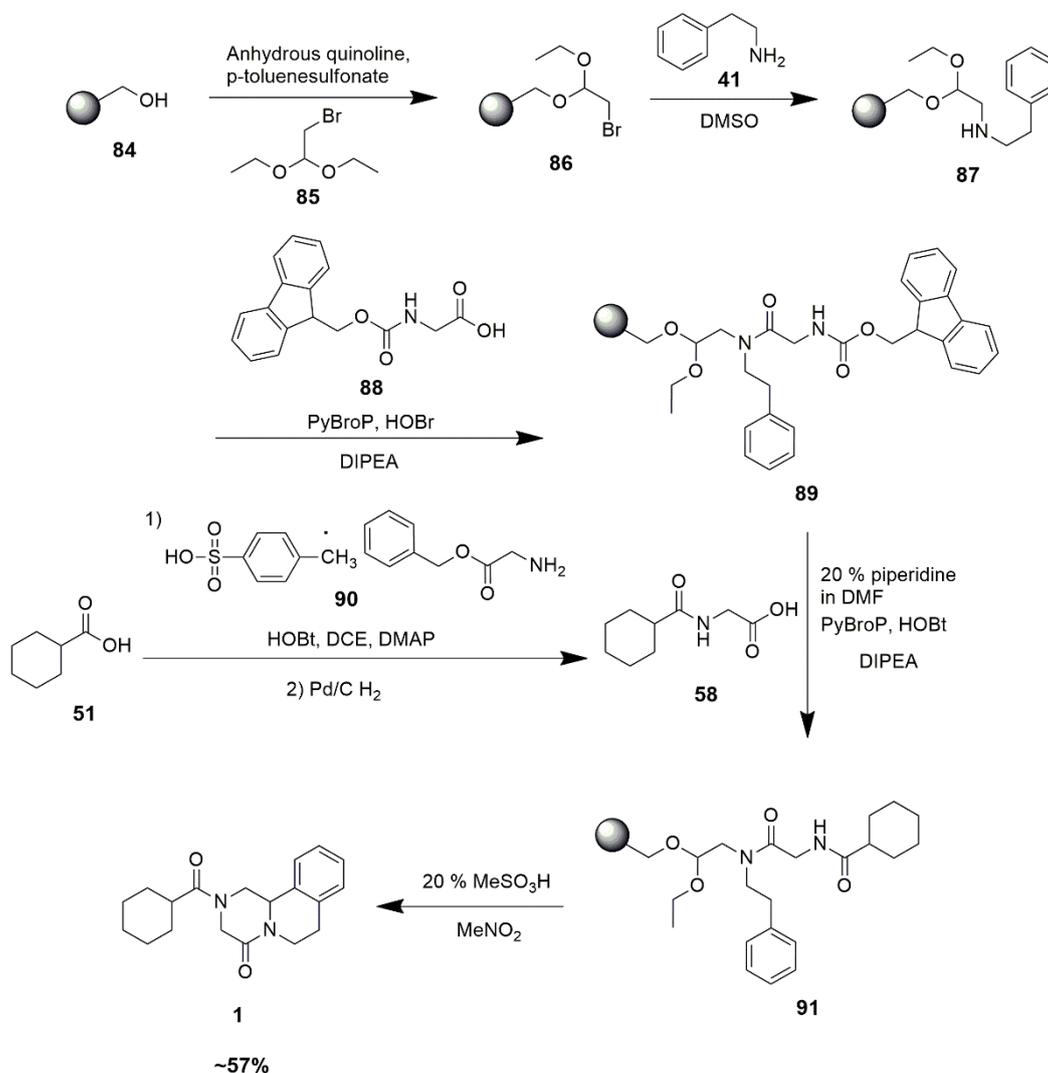
Another approach to the synthesis of praziquantel **1**, patented by the Yixing Xinyu Chemical Co. (Scheme 22)¹⁰⁶ in 2005 was performed, where 2-phenethylamine **41** was allowed to react with glycyl chloride hydrochloride **81** with sodium bicarbonate in DCM affording *N*-phenethylglycinamide **82**. A subsequent amino alkylation was achieved with 2-chloro-1,1-dimethoxyethane **83** to form *N*-2-phenylethyl 2-*N*-(2,2-dimethoxyethylamino)acetamide **44b**. Cyclisation was followed with the utilisation of phosphotungstic acid in DCM, affording 4-oxo-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*]isoquinoline **45**. A final acylation is then performed with the use of cyclohexanecarbonyl chloride **46** to afford praziquantel **1** in yields of 58% over the entire process route. Notably, three of the steps are performed in undesirable DCM and the use of phosphotungstic acid (National Fire Protection Association (NFPA) health rating of 3), imposes inherent safety hazards on the working chemist.



Scheme 22: Synthesis of praziquantel **1** patented by Yixing Xinyu Chemical Co.¹⁰⁶

1.5.5.8 *El-Fayyoumy et al.*

A 2006 report from *El-Fayyoumy et al.* describe the first solid phase synthetic preparation of praziquantel **1** (Scheme 23).¹⁰⁷ Initially, solid-supported hydroxymethyl polystyrene **84** was allowed to react with bromoacetaldehyde diethylacetal **85** in the presence of anhydrous quinoline and *p*-toluenesulfonate, producing solid-supported bromoacetal **86**.



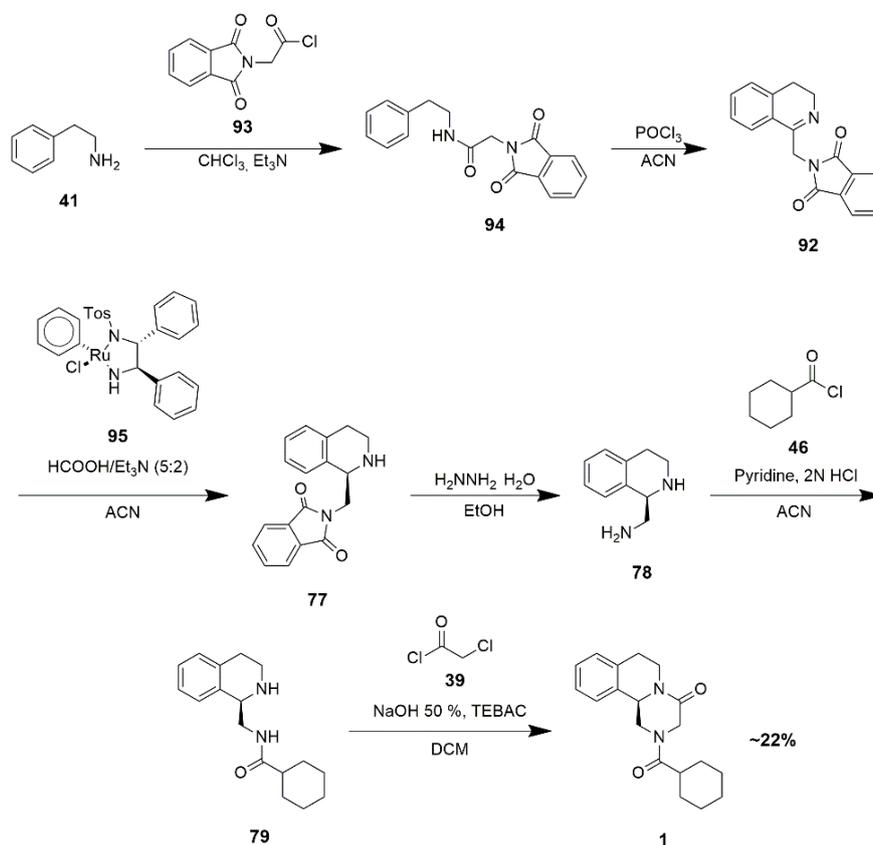
Scheme 23: First solid-supported synthesis of praziquantel **1** reported by *El-Fayyoumy et al.*¹⁰⁷

Subsequently, **86** was treated with 2-phenylethylamine **41** in dimethyl sulfoxide (DMSO), allowing nucleophilic substitution (S_N2) of bromine affording the solid-supported amino acetal **87**. Thereafter, **87** was reacted with Fmoc-glycine-OH **88**, PyBroP and hypobromous acid (HOBr) in *N,N*-diisopropylethylamine (DIPEA) producing the Fmoc protected amine **89**. Subsequently, **89** was washed with 20% piperidine in DMF, followed by the introduction of PyBroP, HOBT, DIPEA and *N*-cyclohexanoyl glycine **58**. Preparation of **58** is achieved by reacting cyclohexanecarboxylic acid **51** with glycine benzyl ester *p*-toluenesulfonate **90** in the presence of HOBT, DCE and DMAP followed by hydrogenation. The afforded compound **91** was then cyclised with the utilisation of 20% MSA in nitromethane yielding the final

praziquantel **1** product. The entire 5 step procedure affords **1** in an overall yield of approximately 57% with the longest linear sequence reported, requiring approximately 36 h reaction time.¹⁰⁷

1.5.5.9 Roszkowski *et al.*

Another 2006 article, reported by Roszkowski *et al.*¹⁰⁸ entails the preparation of prochiral 3,4-dihydro-1-phthalimidomethylisoquinoline **92** by the addition of *N*-phthaloylglycine chloride **93** with 2-phenylethylamine **41**, assisted by a Bischler-Napieralski cyclisation of an intermediate acetamide **94**. Subsequently, compound **92** was subjected to an asymmetric transfer hydrogenation with the use of the key (*R,R*) configured ruthenium catalyst **95** to afford **77**. Similar chemistry is followed for the final stages of the synthetic procedure as reported by Ma *et al.*¹⁰⁵ with a few differences in the reagents employed (Scheme 24). The 6-step process yields the final (*R*)-praziquantel **1** product with an overall yield of approximately 22%.¹⁰⁸

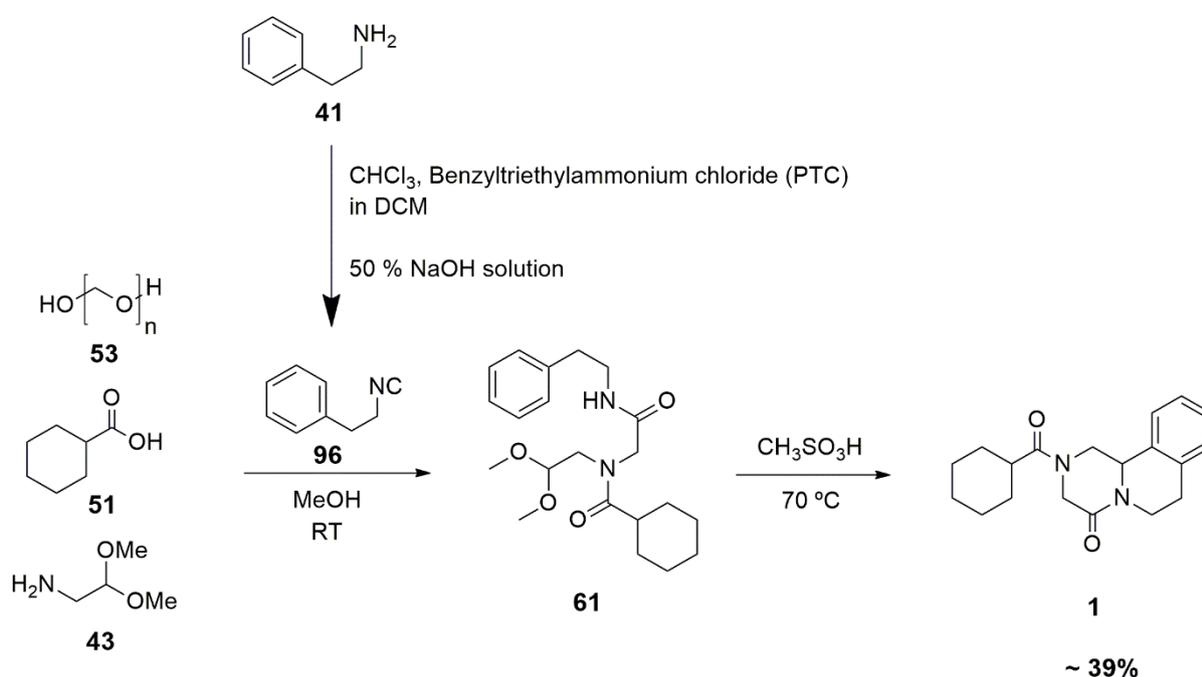


Scheme 24: Synthesis of (*R*)-praziquantel **1** reported by Roszkowski *et al.*¹⁰⁸

1.5.5.10 Cao *et al.*

The shortest known synthetic route (least steps from readily available starting materials) for praziquantel **1** was reported by Cao *et al.* in 2010.⁹⁵ The approach used an Ugi four-component reaction to allow the introduction of acetaldehyde dimethyl acetal **43**, cyclohexanecarboxylic acid **51**, paraformaldehyde (formaldehyde **53** source), and 2-isocynoethylbenzene **96** in order to obtain a “pre-praziquantel” intermediate, *N*-(2,2-

dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide **61** after 48 h stirring at room temp. After intermediate **61** was obtained, the final praziquantel product **1** was afforded by treatment with excess MSA using a Pictet–Spengler type reaction in approximately 6 h (Scheme 25). Notably, the 2-isocyanoethylbenzene **96** employed in the Ugi reaction is not readily available commercially at a reasonable cost and as such was synthesised using a modified Hofmann approach from the commercially available 2-phenylethylamine **41** with the use of carbene chemistry and a phase transfer catalyst (PTC). This synthetic procedure afforded praziquantel **1** in moderately good yields (approximately 39% over 3 steps). Unfortunately, although short in terms of number of steps, the reaction times are long and the use of the 2-isocyanoethylbenzene is undesirable as it has an overpowering noxious odour.⁹⁵

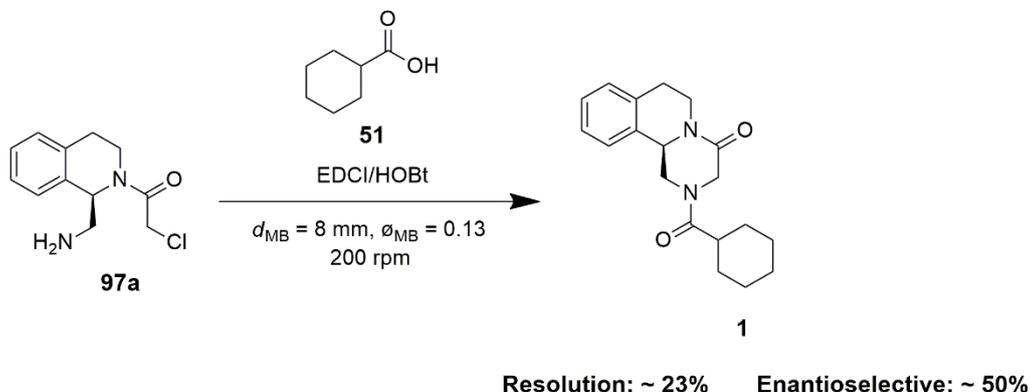


Scheme 25: Three step procedure for synthesis of praziquantel **1** utilising a key Ugi four-component reaction reported by Cao *et al.*⁹⁵

1.5.5.11 Shou *et al.*

More recent, in 2021, two enantioselective approaches for the laboratory scale synthesis of (*R*)-praziquantel **1** were reported by Shou *et al.*¹⁰⁹ whereby the vital (*R*)-1-aminomethyl tetrahydroisoquinoline **97a** is either obtained through an enantioselective synthesis or the resolution of racemic material. The resolution approach (Scheme 26) was reported to have been achieved from the resolution of 1-aminomethyl-2-chloroacetyltetrahydroisoquinoline **97b**, which was produced by performing a mechanochemical Aza-Henry/acylation reaction (utilising a stainless steel ball and vessel) between 3,4-dihydroisoquinoline **98** and nitromethane followed by the addition of chloroacetyl chloride **39** to afford 2-chloro-1-(1-(nitromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethenone **99a**. A subsequent nitro reduction was achieved with the use of Raney nickel catalysis and hydrogen gas to produce **97b**. Resolution of **97b** to

(*R*)-praziquantel **1** product (99.7% ee). The novel mechanochemical procedure for both the 4-step resolution and 3-step enantioselective synthesis approach, afford the final (*R*)-praziquantel **1** product in overall yields of approximately 23% and 50%, respectively (Scheme 28).¹⁰⁹



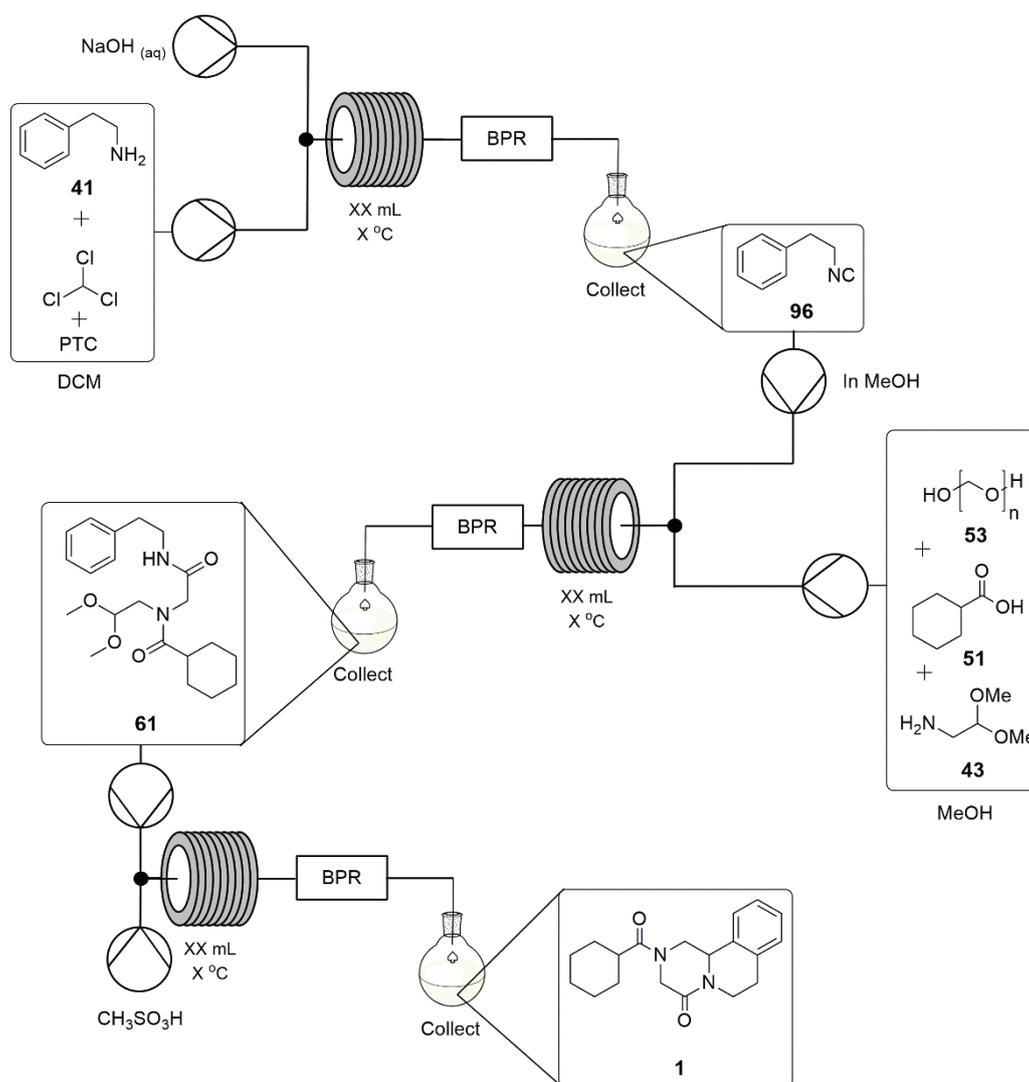
Scheme 28: Conversion of **97a** to (*R*)-praziquantel **1**, using a novel mechanochemical approach reported by Shou *et al.*¹⁰⁹

1.6 Initially envisioned flow synthesis of praziquantel 1

After reviewing the past synthetic procedures for praziquantel **1**, it was decided that the methodology reported by Cao *et al.*⁹⁵ was best suited for potential flow translation due to the fact that the overall yields were moderate and the whole procedure consisted of three stages, all requiring readily available starting materials. From an initial observation, there didn't appear to be any reported precipitation issues, which is highly beneficial for flow conditions. Additionally, Ugi multi-component reactions (second stage) have previously been achieved and reported for different compounds while utilising flow chemistry technologies with appreciable yields and drastically reduced reaction times.¹¹⁰⁻¹¹³ The implementation of flow chemistry within this synthetic procedure is expected to limit the exposure of the foul smelling 2-isocyanoethylbenzene **96** starting material and potentially allow for a continuous telescoped flow approach for the synthesis of praziquantel **1**. In doing so, this procedure was expected to result in comparable or increased overall yields with drastically reduced reaction times (approximately 58 h reaction time in batch), all while being performed under greener chemistry controls.

Upon an initial review of the batch-based synthetic procedure reported by Cao *et al.*⁹⁵, an envisaged flow approach for then synthesis of praziquantel **1** was proposed (Scheme 29). It was envisaged that under flow conditions, step 1 of the procedure would be achieved with the utilisation of a tubular coil reactor wherein a biphasic mixture of aqueous sodium hydroxide, 2-phenethylamine **41**, chloroform and benzyltriethylammonium chloride (BTEAC), a PTC,

would be allowed to react. Upon work-up, the formed, 2-isocyanoethylbenzene **96** would be diluted with MeOH for the successive introduction into step 2. Step 2 was envisioned to be performed using a similar tubular coil reactor wherein acetaldehyde dimethyl acetal **43**, cyclohexanecarboxylic acid **51** and paraformaldehyde (formaldehyde **53** source) in MeOH will be reacted with **96** within MeOH. Upon similar work-up procedures undertaken by Cao *et al.*⁹⁵, the “pre-praziquantel” intermediate **61** will be purified and used in the final step 3. The final step 3 was initially expected to be performed in a similar tubular coil reactor reacting MSA with **61**. Upon neutralisation, extraction and recrystallisation, praziquantel **1** is expected to be yielded.



Scheme 29: Initially envisioned reaction schematic for the synthesis of praziquantel **1** under flow conditions.

To the best of our knowledge, this would be the first reported full synthetic procedure for racemic praziquantel **1** (excluding de-racemisation of racemic praziquantel **1**)¹¹⁴ while utilising a micro-flow reactor platform.

1.7 Aims

The aim of this research project is to develop a synthetic procedure for the Schistosomiasis treatment, praziquantel **1**, under continuous flow conditions and ultimately afford this de-worming medicinal drug with higher yields, lowered reaction times and lowered waste when compared to the pre-existing synthesis of this API under batch conditions. The final optimised synthetic route will then be up-scaled to allow for an efficient synthesis with an appreciable space-time yield. Once synthesised using the best possible synthetic route, the synthetic procedure can be added to a library of synthetic routes of API's performed under flow conditions.

1.8 Proposed research objectives and timelines

1.8.1 Objective 1: Assessment of praziquantel **1** as a suitable target

- **Task 1.1:** Review the current commercial processes, select the most promising routes.
- **Task 1.2:** Rank the top processes in terms of suitability for flow translation and potential as future commercial targets.
- **Task 1.3:** Preliminary cost modelling of batch processes that are identified as being suitable for flow translation, models to act as a benchmark for flow processes.

1.8.2 Objective 2: Batch validation of process routes

- **Task 2.1:** Validation of the best published/patented batch process route to praziquantel **1**.
- **Task 2.2:** Additional optimization of published/patented batch process route.
- **Task 2.3:** Updated cost model based upon optimized batch process

1.8.3 Objective 3: Translation and optimization of the process under flow conditions

- **Task 3.1:** Translation of each stage of the batch process to flow utilising conditions from tasks under objective 2.
- **Task 3.2:** Develop analytical quality control methods. Methods will be developed to analyze the reaction outputs for each step in the batch and flow synthesis of the API targets. These will include nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS) and/or Fourier-transformed infrared spectroscopy (FTIR).
- **Task 3.3:** Optimization of individual process stages in terms of concentration, flowrate/residence time, temperature and reagent stoichiometric excesses. The steps will further be taken to ensure the development of the greenest possible routes employing the twelve principles of green chemistry where possible. Process will further be developed to enhance safety and minimise reagent exposure/handling.

- **Task 3.4:** Initial cost modelling of flow process route

1.8.4 Objective 4: Telescoping of flow processes at milligram to multigram gram scale

- **Task 4.1:** Telescoping of process stages together
 1. Utilising the optimized flow processes from activity 3 and the analytical methods developed in activity 2.3, integrated flow process routes will be developed. Solvent compatibility between stages will be accounted for and where necessary in-line solvent swaps will be conducted. In-line work-up's and purifications using flow-based techniques such as solid phase extractors, membrane extractors, scavenger resins, catch and release techniques will be utilised.
 2. Upscaling to multigram (100 g) scale will be performed under advisement from CSIR Future Pharma
- **Task 4.2:** Perform techno-economic assessment of the developed process route. The data generated from the bench scale process route demonstrations will be integrated into a techno economic assessment of the viability of use for the process route on scale. This will form the basis of a technology demonstrator if the process is successful.

CHAPTER II

2.1 A flow approach for the synthesis of 2-isocyanoethylbenzene 96 via the modified Hofmann Carbylamine procedure

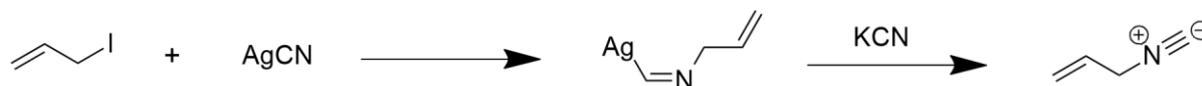
2.1.1 Brief history and chemistry of the isocyanide functional group

An isocyanide, commonly referred to as an isonitrile or carbylamine, is an organic functional group with the general form, $R - NC$, where R represents any alkyl group sigma bonded to the nitrogen atom, which is positively charged, being triply bonded to the terminal negative carbon atom. Isocyanides must not be confused with the isometric cyanide functional group ($R - CN$), which exhibits a terminal nitrogen atom as opposed to a terminal carbon atom. Isocyanides are highly reactive and exhibit reaction plasticity, being able to contribute just a few atoms or play a vital role in large multi-molecular assemblies.¹¹⁵ It is also noted that this functional group generally exhibits a pungent odour, especially if the isocyanide containing compound is a volatile liquid. They are generally considered to not have acute toxicity, but caution should be taken when handling them as toxicity studies are still arguably limited.¹¹⁶

Since the discovery of isocyanides, they have been shown to be of high relevance in multiple disciplines of science including astronomy, biology, chemistry, information technology, and materials science.^{115, 117-119} Within organic chemistry, isocyanides have gained a considerable amount of attention within numerous heterocyclic syntheses as well as multi-component reactions, such as the Ugi four-component condensation and Passerini three component reactions.^{95, 115} Isocyanides have been synthesised for more than a century and a half,¹²⁰ with three general approaches being implemented during this period. Two of the three approaches are largely historic having been mainly utilised within the 19th century.

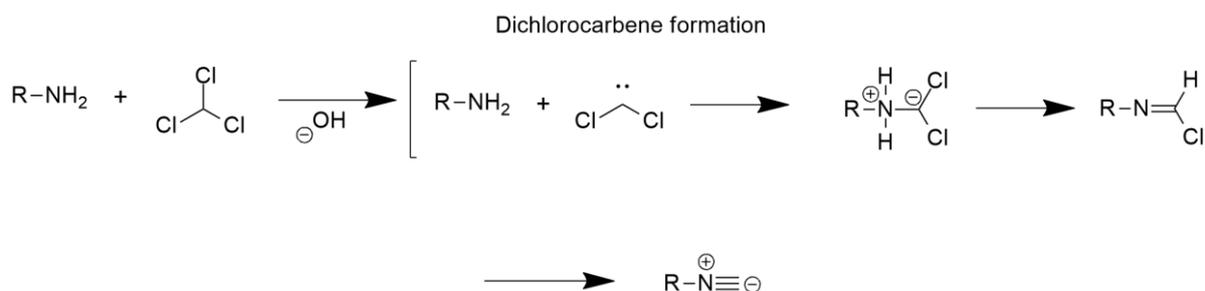
The first known synthesis of an isocyanide containing compound was reported in 1859 by W. Lieke,¹²¹ whereby, silver cyanide was treated with allyl iodide, followed by a substitution reaction promoted by the addition of potassium cyanide, affording an allyl isocyanide product (Scheme 30). This was an unexpected result since alkylation of cyanide, bound to alkali metals, usually yields cyanide containing products. However, the use of the silver transition metal “shields” the carbon atom and promotes the alkylation to occur at the unexpected nitrogen terminal atom instead. Following this discovery, a more general approach for the synthesis of various isocyanides was developed by A. Gautier in 1867,¹²² whereby an isocyanide is prepared by a substitution reaction with the addition of potassium cyanide to isocyanide containing silver complexes, which in turn are prepared by alkylation of silver cyanide with the use of alkyl halides. Besides the use of costly silver complexes, the

approaches have inherent safety hazards associated with the use of cyanide salts and they generate significant toxic waste.



Scheme 30: Synthetic route for allyl isocyanide, the first isocyanide compound synthesised.¹²¹

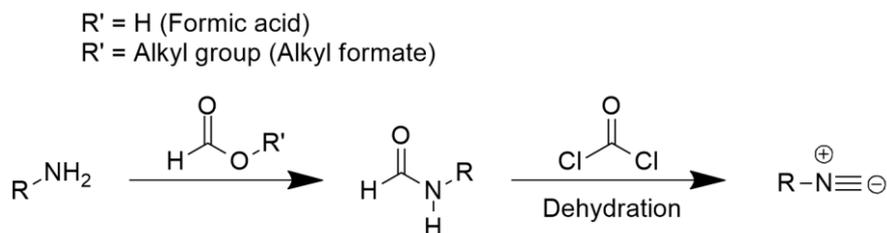
A second approach was developed by A. W. Hofmann in 1867 and was soon to be referred to as the Hofmann carbylamine synthesis (Scheme 31).¹²³ This approach made use of a primary amine which was treated with chloroform in the presence of a strong base (alcoholic solution of potassium hydroxide or sodium hydroxide), generally yielding around 20% of the isocyanide product. When compared with the approach reported by Gautier, the overall safety and cost of reagents of this new approach is clearly superior, however, it only afforded isocyanide products in low yields. Consequently, in 1972, the Hofmann procedure was modified by Weber and Gokel by including a PTC, BTEAC, in addition to a biphasic system comprised of 50% aqueous sodium hydroxide and DCM, the latter of which, hosts the primary amine and chloroform.¹²⁰ This approach promoted the formation of benzyltriethylammonium hydroxide, which is soluble in both the aqueous and organic phases, this encourages partitioning of the basic hydroxide ion into the organic DCM phase initiating dichlorocarbene formation with the accompanying chloroform. The approach allowed the synthesis of various isocyanide products in improved yields averaging 40 to 60%.¹²⁰



Scheme 31: A general reaction scheme for the synthesis of isocyanides following the Hofmann carbylamine procedure.¹²³

The third and most recent approach was formulated in 1956 and was reported by three groups almost simultaneously, namely, I. Hagedorn and H. Tonjes,¹²⁴ W. Hertler and E. Corey,¹²⁵ and I. Ugi and R. Meyr.¹²⁶ This approach involves the dehydration of mono-substituted formamides affording the isocyanide product (Scheme 32). By 1965, Ugi *et al.*¹²⁷ reported the most effective approach with the utilisation of a phosgene-triethylamine system. This general, two-step dehydration procedure involves the initial preparation of a mono-substituted formamide, usually with the treatment of primary amines and formic acid or alkyl formates (commonly methyl and ethyl formate). This newly formed formamide is subsequently treated with a dehydrating reagent (phosgene in Ugi's literature)¹²⁷ affording various isocyanides with overall

yields ranging from 50 to 80%. Unfortunately, toxic dehydrating reagents such as phosgene¹²⁷ or phosphorous oxychloride¹¹⁵ commonly utilised, pose an inherent safety risk. It is also important to note from a flow methodology point of view, the dehydrating reagents utilised are generally characterised by the generation of substantial amounts of precipitate which would complicate any flow translation.

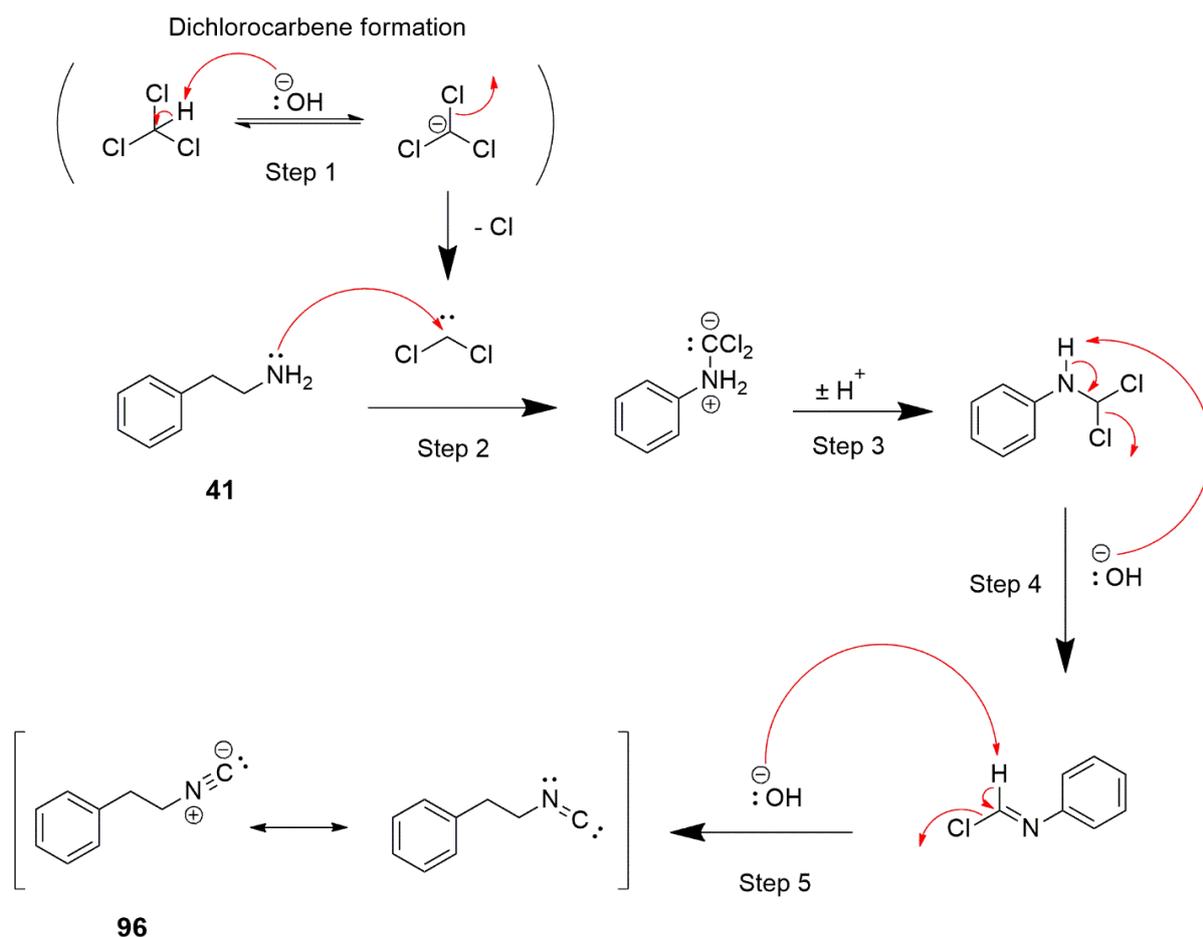


Scheme 32: General approach for the synthesis of isocyanide containing products through the dehydration of a mono-substituted alkyl formamide.

2.1.2 Synthesis of 2-isocyanoethylbenzene **96** using batch-based chemistry methodologies

As mentioned previously, the synthetic route reported by Cao *et al.*⁹⁵ utilises 2-isocyanoethylbenzene **96** in a four-component Ugi reaction. This isocyanide **96** contains an aromatic benzene ring sigma bonded to an ethyl group containing a terminal isocyanide moiety. Unfortunately, a search of fine chemical vendors revealed that **96** is not commonly available commercially, and where available can only be purchased in small quantities with the cheapest found, costing 41 USD per g. That being noted, it can be prepared synthetically from readily available starting materials, 2-phenethylamine **41** and chloroform following a modified Hofmann procedure (carbylamine synthesis)⁹⁵ or via the dehydrating formamide approach (one step if *N*-(phenethyl)formamide is available or two steps including the formamide preparation).¹¹⁵

The general mechanistic approach for the formation of isocyanides via the Hofmann approach starts with a dehydrohalogenation reaction of chloroform (Step 1, Scheme 33) in the presence of an accompanying strong base, usually potassium or sodium hydroxide, producing dichlorocarbene. Dichlorocarbene is a singlet carbene consisting of a vacant p orbital pair and an adjacent sp² hybridized orbital hosting a lone pair of electrons. As such, the carbene is a highly reactive electrophilic reaction intermediate, allowing for a nucleophilic attack from a respective primary amine (Step 2). Thereafter, deprotonation of the positively charged amine nitrogen atom (Step 3), followed by a β-elimination of a single hydrogen chloride molecule affords an intermediate imine (Step 4). Finally, the imine intermediate is subjected to a second dehydrochlorination step (α-elimination) affording the final isocyanide product (Step 5).¹²⁷



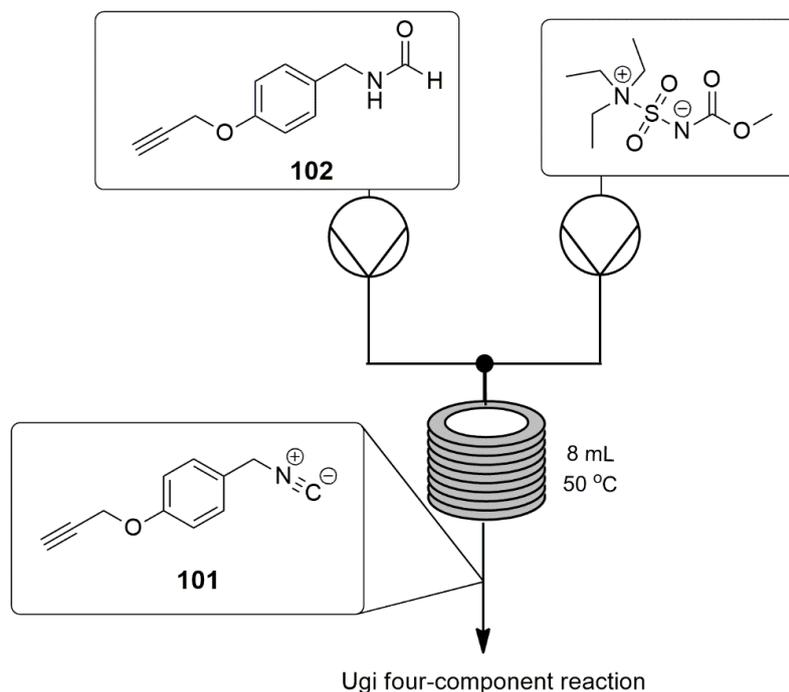
*Scheme 33: Proposed mechanism for the synthesis of 2-isocyanoethylbenzene **96** following a modified Hofmann procedure.*

2.1.3 Previous synthetic procedures for the preparation of isocyanides under flow conditions

To the best of our knowledge, there has been no reported literature utilising the modified Hofmann carbylamine approach under continuous flow conditions, and the preparation of 2-isocyanoethylbenzene **96** has also not been reported under flow conditions. That being noted, there are reported procedures for the synthesis of various other isocyanides under flow conditions, a selection of which are highlighted below.

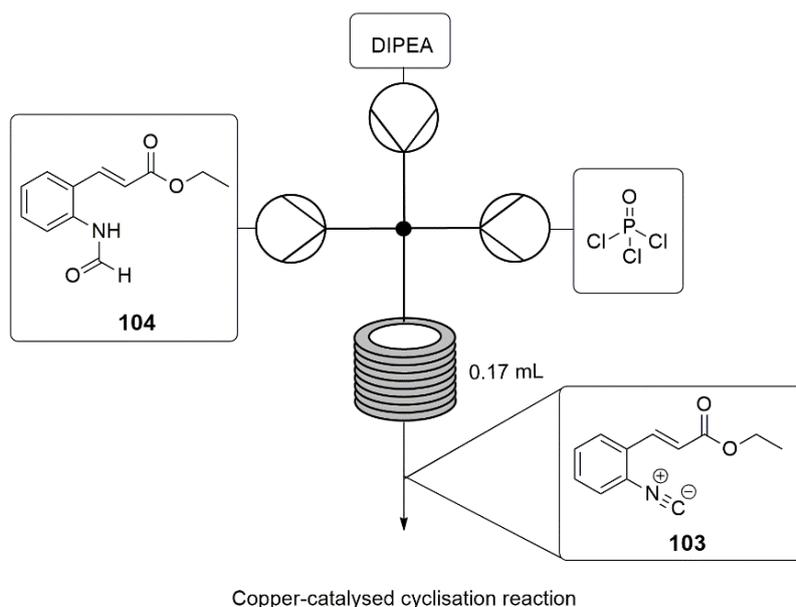
An article reported by Salvador *et al.*¹¹² employed the formamide approach while utilising Burgess reagent (methyl *N*-(triethylammoniumsulfonyl)-carbamate), which surprisingly resulted in a homogenous reaction mixture. The authors were successful in preparing isocyanide **101** from the formamide **102** in quantitative conversion, performed in acetonitrile (ACN). The reaction mixture was allowed to react with Burgess reagent in a coil reactor at 50 °C with a residence time of 20 min. An isolated yield of 90% was reported after subsequent column chromatography was performed off-line. Furthermore, the authors also telescoped the

isocyanide into an Ugi four-component reaction allowing for the in-line generation and consumption of the noxious isocyanide **101** (Scheme 34).



Scheme 34: In-line generation of isocyanide **101** from formamide **102** telescoped to an Ugi four-component reaction.¹¹²

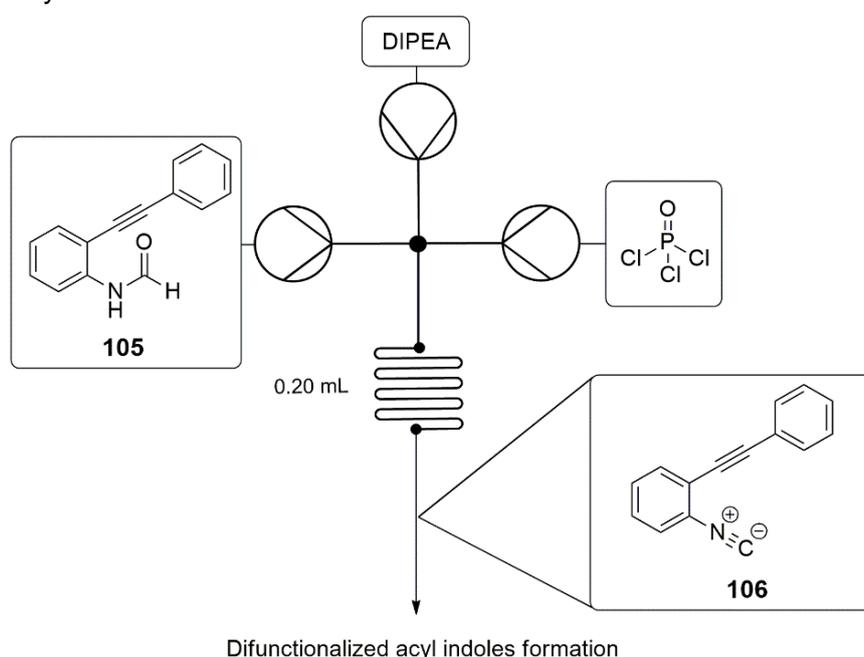
In another report, Heckman *et al.*¹²⁸ developed a continuous flow approach for the preparation of various aryl isocyanides partaking in a subsequent telescoped copper-catalysed cyclisation to generated indole type derivatives (Scheme 35). As an example, the isocyanide **103** was generated from the reaction of aryl formamide **104**, phosphorous oxychloride (dehydrating agent) and DIPEA. Each of these reagents were pumped from separate stock solutions and



Scheme 35: In-line generation of isocyanide **103** from formamide **104** telescoped to a copper-catalysed cyclisation reaction.¹²⁸

allowed to react in a tubular coil reactor with a residence time of 2.5 min (temperature not specified). Following work-up and concentration, isocyanide **103** was afforded in 94% yield as a standalone step. Notably, the authors mentioned nothing about solubility issues faced with the dehydration step, however, it was specified that DCM was efficient at solubilising the starting materials. Furthermore, this procedure was telescoped into a copper-catalysed cyclisation allowing for generation of a variety of indole derivatives.

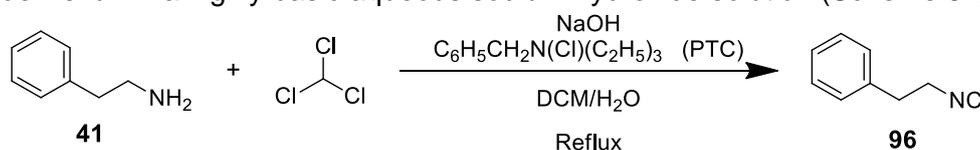
Chen *et al.*¹²⁹ developed a similar procedure to that reported by Heckman *et al.*¹²⁸ whereby formamide **105** was allowed to react with phosphorous oxychloride and DIPEA in DCM in a 0.2 mL glass mixing chip (temperature not specified) with a residence time of 4 min (Scheme 36). The authors also implemented an in-line work-up to telescope the generated isocyanide **106** into a second reaction allowing the formation of a variety of difunctionalized acyl indoles in poor to good yields.



Scheme 36: In-line generation of isocyanide **106** from formamide **105** telescoped to a subsequent step for the formation of difunctionalized acyl indoles.¹²⁹

2.1.4 Batch validation and optimisation of literature method using the Hoffman approach

We initially validated the synthesis of 2-isocyanoethylbenzene **96** following the modified Hoffmann procedure reported by Cao *et al.* using batch-based chemistry methods.⁹⁵ The approach involved the treatment of 2-phenylethylamine **41** with chloroform and BTEAC in DCM under reflux in a highly basic aqueous sodium hydroxide solution (Scheme 37).



Scheme 37: Synthetic step for 2-isocyanoethylbenzene **96**.⁹⁵

The initial validation attempt was performed on a 95 mmol scale utilising BTEAC in catalytic quantities, with 2-phenylethylamine **41** and chloroform in a 1:1 mol equivalence (~ 3.2 M in DCM).⁹⁵ An equal volume of a 50% w/w aqueous sodium hydroxide solution was utilised within this biphasic system as the strong base. The solution started off, light yellow in colour and through the course of a 4 h reflux, got progressively darker ultimately affording a slightly orange, viscous reaction mixture. Over time a sizable amount of off-white precipitate collected on the bottom and sides of the flask (Figure 24) which was dissolved post-reflux on addition of a water quench. It was presumed that the precipitate was either sodium chloride or sodium hydroxide which precipitated out of solution as the aqueous phase was so highly saturated. Work-up of the reaction mixture was achieved with DCM extractions, followed by a cooled 1 M hydrochloric acid and brine wash. After drying, filtration and purification by column chromatography (Figure 25) the noxious 2-isocyanoethylbenzene **96** was isolated as a yellow oil in a 41% yield.



Figure 24: Reaction mixture after 4 h reaction time.



Figure 25: Column chromatography for purification of isocyanide **96**.

When converting 2-phenethylamine **41** to 2-isocyanoethylbenzene **96**, the ¹H-NMR spectrum (Figure 26) showed the disappearance of the signal at 1.20 ppm integrating for two protons which is characteristic of the primary amine in **41** (depicted in Appendix A – 2). In addition, the two CH₂ peaks present in both compounds, shift downfield in the 2-isocyanoethylbenzene **96** spectrum due to the additional de-shielding effect from the newly formed isocyanide functional group (CH₂ adjacent to NH₂ shifts from 2.96 to 3.60 ppm and the CH₂ adjacent to the aromatic benzene ring, shifts from 2.74 to 2.98 ppm). When comparing the ¹³C-NMR spectrum of these two compounds (depicted in Appendix A – 1 and A – 2), the major distinctive change is the appearance of an extra signal due to the isocyanide carbon atom at 156.49 ppm. The isocyanide quaternary carbon atom at 156.49 ppm is also evidenced through its disappearance in the DEPT 135. When comparing the IR spectra of these two compounds,

the major distinction observed is the disappearance of the broad primary amine stretch between 3100 and 3500 cm^{-1} and the appearance of a new signal at 2147 cm^{-1} , (2152 and 2154 in literature)^{130, 131} which corresponds well with the isocyanide functional group (depicted in Appendix A – 3). Lastly, the mass spectrum obtained (depicted in Appendix A – 4), displays an $[\text{M} + \text{H}]^+$ ion located at 132.0781 m/z corresponding well with the calculated 132.0813 m/z .

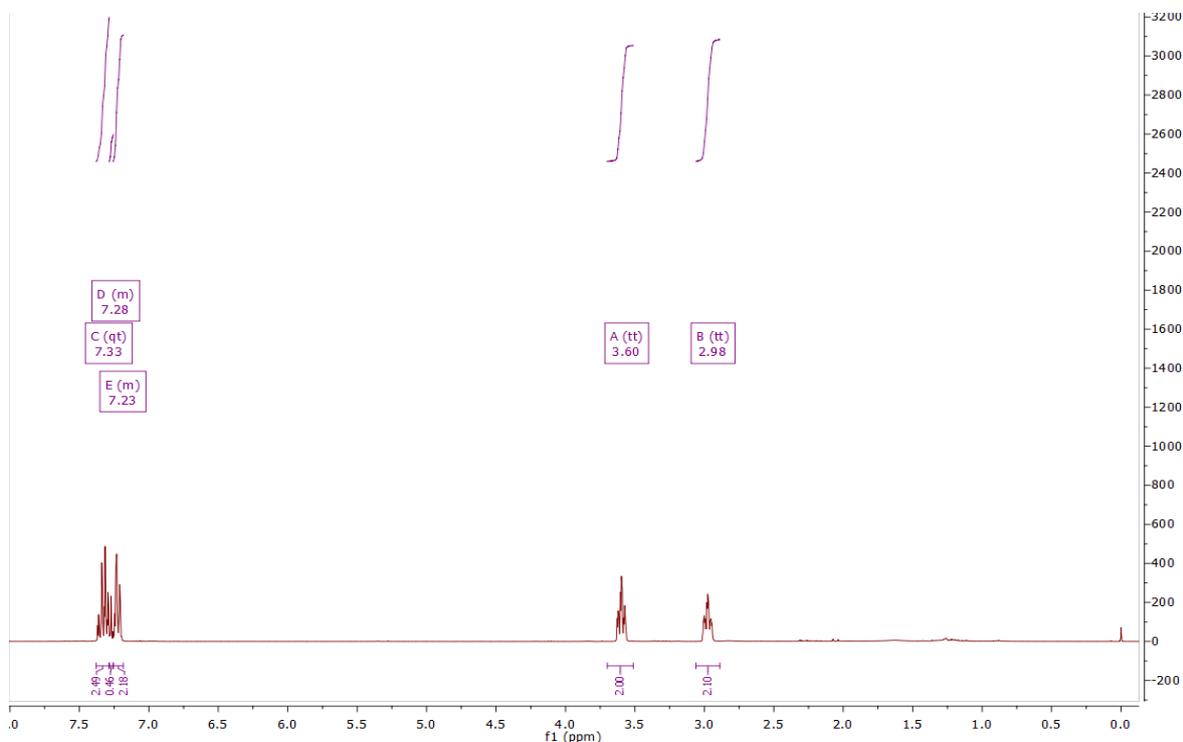


Figure 26: ^1H -NMR obtained for 2-isocyanoethylbenzene **96** during this research.

Unfortunately, the precipitate formation during the reaction was problematic for the envisaged flow translation, and as is, would likely result in rapid reactor fouling. Consequently, we assessed various reaction conditions to assess if the precipitation could either be avoided or minimized. We initially screened a series of reaction conditions by maintaining the concentration of the reagents in the DCM phase (2-phenethylamine **41** and chloroform) while varying the concentration of the basic sodium hydroxide solution.

Mechanistically, the isocyanide synthesis requires 3 hydroxide ions per conversion (Scheme 38), but previous reports suggested the use of sodium hydroxide in at least 7.9 fold excess.⁹⁵ We hypothesized that reducing the amount of sodium hydroxide may reduce the amount of precipitate and opted to screen the reaction using 10 M aqueous sodium hydroxide (6.0 equivalents). Under these conditions, no precipitate was observed throughout the reaction and purification, unfortunately, after 4 h reflux, the approach only afforded a modest yield of 36% (Table 2, entry 1).



Scheme 38: 2-Isocyanoethylbenzene **96** stoichiometric reaction for this modified Hofmann synthesis approach.

Increasing the concentration of sodium hydroxide to 12.5 M (7.6 equivalents), resulted in minor precipitation in a comparable yield (Table 2, entry 2). Increasing the concentration further to 15 M (9.1 equivalents) resulted in a substantial increase in the amount of precipitate but again the yield at 37% remained comparable to previous attempts (Table 2, entry 3). At this stage, it was evident that in order to avoid precipitation, we would need to limit the sodium hydroxide concentration to a maximum of 12.5 M. Subsequently, it was decided to run the reaction with an increased reflux time of 18 h, and in all instances, an increase in yield was observed (Table 2, entries 5-7), with the best yield of 60% achieved when utilising a 25 M aqueous sodium hydroxide solution. A subsequent literature review revealed an article by Patil *et al.*¹¹⁵ which suggested that the use of an aqueous acid wash could lead to losses in yield due to unwanted hydrolysis of the isocyanide. As such the reaction was repeated with a 25 M aqueous sodium hydroxide solution (7.6 equivalents), while concurrently doubling the concentration of the remaining reagents to 0.0165 mol. The reaction was refluxed for 4 h, and during the downstream processing the questionable acid wash was avoided (Table 2, entry 8). Encouragingly a significant increase in yield to 72% was observed, however, the reaction was marred by the formation of a thick precipitate.

Table 2: Results observed for test reactions run in batch.

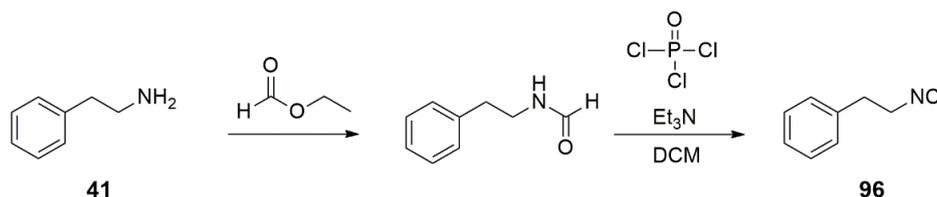
Batch entry:	[NaOH]/ In H ₂ O (mL)	2-Phenethylamine 41 (mol)	Chloroform (mol)	BTEAC (PTC) (mol)	In DCM: (mL)	Reflux (h)	% Yield (pure)
1	[10]/ 5 mL	0.00825	0.00825	0.0001	5	4	36
2	[12.5]/ 5 mL	0.00825	0.00825	0.0001	5	4	35
3	[15]/ 5 mL	0.00825	0.00825	0.0001	5	4	37
4	[25]/ 5 mL	0.00825	0.00825	0.0001	5	4	39
5	[12.5]/ 5 mL	0.00825	0.00825	0.0001	5	18	55
6	[15]/ 5 mL	0.00825	0.00825	0.0001	5	18	56
7	[25]/ 5 mL	0.00825	0.00825	0.0001	5	18	60
8	[25]/ 5 mL	0.0165	0.0165	0.0002	5	4	72

As observed from the batch results reported (Table 2), the reaction tends to favour longer reaction times with a high concentration of base. Notably, the precipitate was abundantly present when using any concentration of base at or above 15 M, but at or below 12.5 M it was

mostly soluble, even though the molar equivalents of the base was greater. It has been established that the precipitate is dependent on the concentration of base (related to the saturation levels of the aqueous phase), therefore the more concentrated the aqueous phase, the more precipitation present in the reaction.

2.1.5 Synthesis of isocyanide **96** using the formamide dehydration approach

As a result of the moderate yielding batch-based results obtained using the modified Hofmann procedure,⁹⁵ it was decided to perform another procedure following the formamide dehydration approach reported in 2020 by Patil *et al.*¹¹⁵ In this instance, 2-phenethylamine **41** was refluxed in ethyl formate, generating the formamide quantitatively which was subsequently concentrated and diluted in DCM (~ 2 M). This mixture was used directly in the following dehydration step with triethylamine (5.0 equivalents) and phosphorous oxychloride (1.0 equivalents). After reaction completion (monitored by TLC at < 10 min), the reaction mixture was passed through a short silica column with the use of diethyl ether and DCM (100% diethyl ether increasing in polarity to end with 100% DCM) affording 2-isocyanoethylbenzene **96** (Scheme 39) with an isolated yield of 83% (16.5 mmol scale). Unfortunately, significant precipitate formation was noted upon the initial introduction of the phosphorous oxychloride, and as a result, although the yields were superior to those achieved using the Hoffman approach, we decided not to pursue this approach any further.



Scheme 39: Preparation of 2-isocyanoethylbenzene **96** following the formamide dehydration approach.

2.1.6 Synthesis of 2-isocyanoethylbenzene **96** under flow conditions following the modified Hofmann procedure

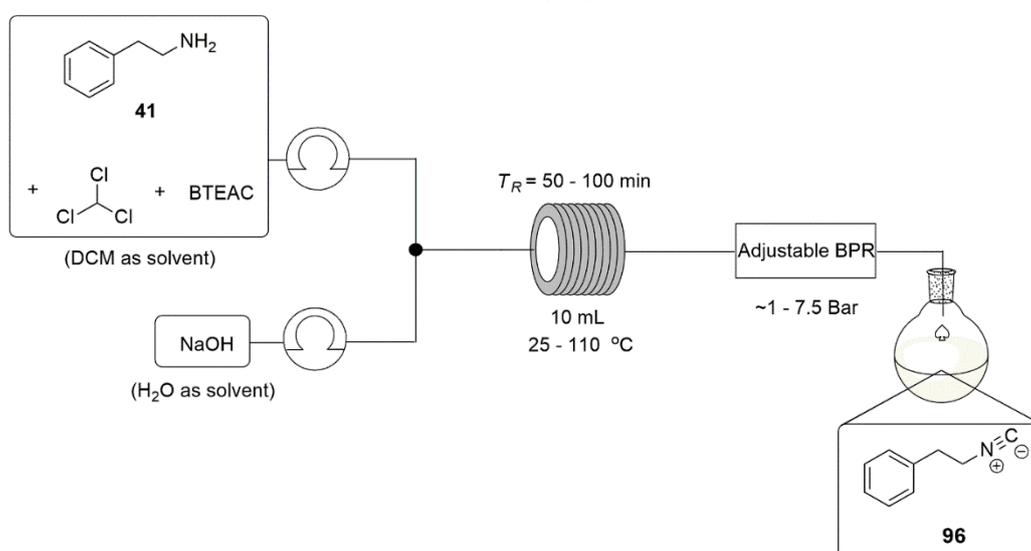
Since the reaction was shown to exhibit a propensity to form precipitates following the modified Hofmann procedure, and as the sodium hydroxide solutions were appreciably viscous, we decided to employ the use of a peristaltic flow platform which can handle slurries to reduce the chances of reactor fouling. A Vapourtec E-series flow reactor unit was utilised for the flow translation. This Vapourtec unit makes use of two Vapourtec V-3 peristaltic pump heads which can be fitted with a variety of easily interchanged chemically resistant colour-coded tubes manufactured from various fluoropolymers. The two main types (colour coded red and blue) cover a broad range of chemicals, and are pressure limited to a maximum of 10 bar. More specialized tubing is available for strong acids and bases, colour coded green, but unfortunately these have reduced pressure limits with a maximum operating pressure of only 5 bar. In all runs reported herein, blue colour coded tubes (Figure 27) were utilised for the

introduction of both the aqueous sodium hydroxide solution and the DCM solution containing 2-phenethylamine **41**, chloroform and BTEAC.



Figure 27: Peristaltic pumps utilised during this research with the blue colour coded inner tubing.

In this flow setup, two peristaltic pumps were connected to two stock solutions respectively (Scheme 40). The pumps were plumbed to a standard Y-piece mixer using 32 cm long, 1.0 mm internal diameter PFA tubing. The Y-piece mixer was then connected, again using a 32 cm, 1.0 mm internal diameter PFA tube to a 10 mL Vapourtec tubular coil reactor (PFA, 1.0 mm internal diameter) unit, and finally, the output of the coil reactor was passed through a BPR prior to collection in a round bottom flask. We planned to screen the reaction at various temperatures above the boiling point of DCM, and for convenience, we employed the use of a pressure adjustable BPR instead of interchanging between pre-set BPRs.



Scheme 40: The flow set-up for flow described in entries 1-8 (Table 3).

The initial screen under flow conditions was performed at ambient temperature with the use of a 12.5 M (7.6 equivalents) sodium hydroxide stock solution (5.0 mL). The DCM stock solution contained **41** and chloroform at a 1.65 M concentration (0.00825 mol / 5.0 mL) and the PTC, (BTEAC) in catalytic quantities (Table 3, entry 1). The selector valves were primed with the stock solutions and the reactor was primed with water and DCM as the pushing solvents in a 1:1 volume ratio. The system was set to introduce 3.00 mL (theoretical yield of 0.65 g) from each stock solution with a residence time of 50 min. While no visible precipitation

was observed during initial mixing at the Y-piece, downstream, off-white crystals were observed prior to entering into the BPR. The precipitates rapidly blocked the BPR, resulting in an automated termination of the reaction triggered by a pressure spike. Increasing the temperature to 80 °C and reducing the concentration of the base to 10 M (also at 80 °C) (Table 3, entries 2-3) also led to reactor blockages, although when performed at 10 M there was a visual decrease in the amount of precipitate formed. We then decided to attempt the reaction with a 7.5 M sodium hydroxide solution, which, encouragingly showed no precipitation (Table 3, entry 4), however subsequent purification off-line, afforded a modest 35% yield.

Table 3: Results obtained from test reactions under flow conditions.

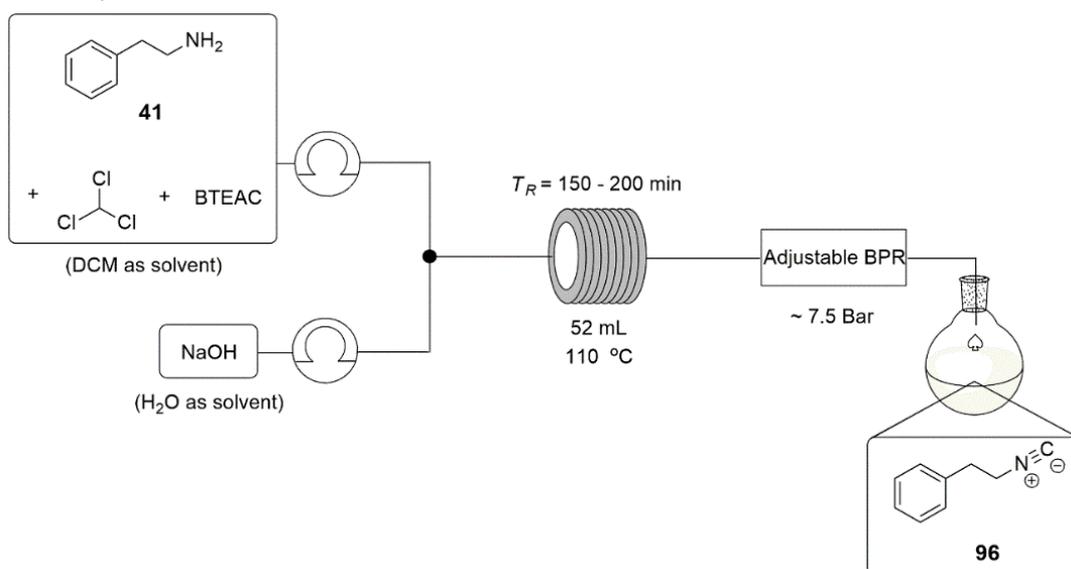
Flow entry	A [NaOH]	B ^a [Reactants]	Volume Ratio A:B	Volume input from each tube	Flow Rate (mL.min ⁻¹)	Residence Time (min)	Reactor Temperature (°C)	% Yield (pure)
1	12.5	1.65	1:1	3.00 mL	0.100	50	25	Blocked
2	12.5	1.65	1:1	3.00 mL	0.100	50	80	Blocked
3	10	1.65	1:1	3.00 mL	0.100	50	80	Blocked
4	7.5	1.65	1:1	3.00 mL	0.100	50	80	35
5	7.5	1.65	1:1	3.00 mL	0.100	50	90	36
6	7.5	1.65	1:1	3.00 mL	0.100	50	100	39
7	7.5	1.65	1:1	3.00 mL	0.100	50	110	44
8	7.5	1.65	1:1	3.00 mL	0.100	100	110	53
9	7.5	1.65	1:1	3.00 mL	0.130	200	110	33
10	7.5	1.65	2:1	6.00 : 3.00 mL	0.232 : 0.116	150	110	49
11	8.75	2.2	2:1	7.00 : 3.50 mL	0.232 : 0.116	150	110	46
12	8.75	2.9	2:1	5.30 : 2.65 mL	0.232 : 0.116	150	110	35
13	10	1.25	1:1:2 (quench)	7.60 mL	0.173: 0.173: 0.356	150 + 45 min delay*	110	67
14	12.5	1.56	1:1:2 (quench)	6.10 mL	0.173: 0.173: 0.356	150 + 45 min delay*	110	78
15	12.5	1.56	1:1:2 (quench)	6.10 mL	0.173: 0.173: 0.356	150 + 45 min delay*	110	76

16	12.5	1.25	1:1:2 (quench)	7.60 mL	0.173: 0.173: 0.356	150 + 45 min delay*	110	60
17	12.5	2.08	1:1:2 (quench)	4.60 mL	0.173: 0.173: 0.356	150 + 45 min delay*	110	69
18	12.5	1.56 in 2-Me-THF	1:1:2 (quench)	6.1 mL	0.173: 0.173: 0.346	150 + 15 min delay*	110	56

^a B = Amine **41**, chloroform and catalytic amounts of BTEAC in DCM unless stated otherwise.

Thereafter, we proceeded to screen the reaction at increased temperatures while maintaining the concentration of the sodium hydroxide stock solution at 7.5 M and the reaction residence time at 50 min (Table 3, entries 5-7). An increase in yield was noted with increasing temperature culminating in a yield of 44% when performed at 110 °C. Unfortunately, we could not screen temperatures above 120 °C as at that point the system pressure kept exceeding the 10-bar pressure limit. To try and increase the yield further we elected to connect a second 10 mL tubular reactor in series. In doing so the residence time was increased to 100 min (Table 3, entry 8), which resulted in an appreciable increase in yield to 53%. Critically, as the yield increased, the reaction visually appeared to form more precipitate. This is expected as an increased conversion to the product would be accompanied by an increase in sodium chloride by-product resulting in oversaturation of the aqueous phase. Fortunately, at this stage, the precipitate was still fine enough to be able to easily pass through the BPR.

We wished to increase the residence time further, however, the Vapourtec E-series reactor used was limited to the use of two 10 mL coil reactors. As such, we elected to employ the use of an external 52 mL (1.5 mm internal diameter) stand-alone coil reactor (Uniqsis Hotcoil) (Scheme 41).



Scheme 41: The flow set-up for flow entries 9 through 10 (Table 3).

Initially, we screened the reaction employing the same conditions used previously (Table 3, entry 8), however, increasing the residence time to 200 min. Unexpectedly, this resulted in a decrease in yield to 33% (Table 3, entry 9). In hindsight, this could be explained by the observation that the biphasic reaction mixture separated into distinct alternating slugs of the organic and aqueous phases (Figure 28) which in the thicker bore tubing of the 52 mL coil reactor at slow flow rates resulted in decreased surficial contact between the phases (i.e. longer slugs).

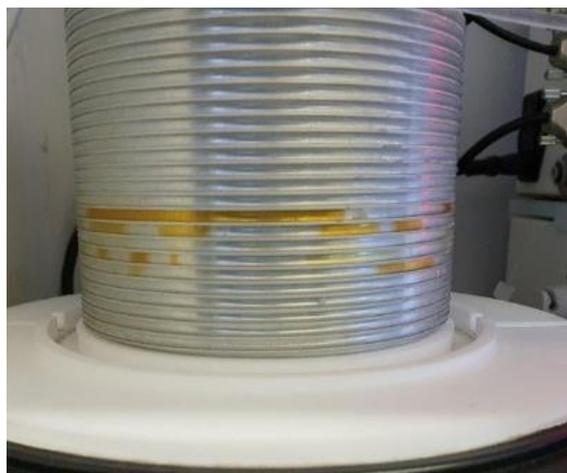
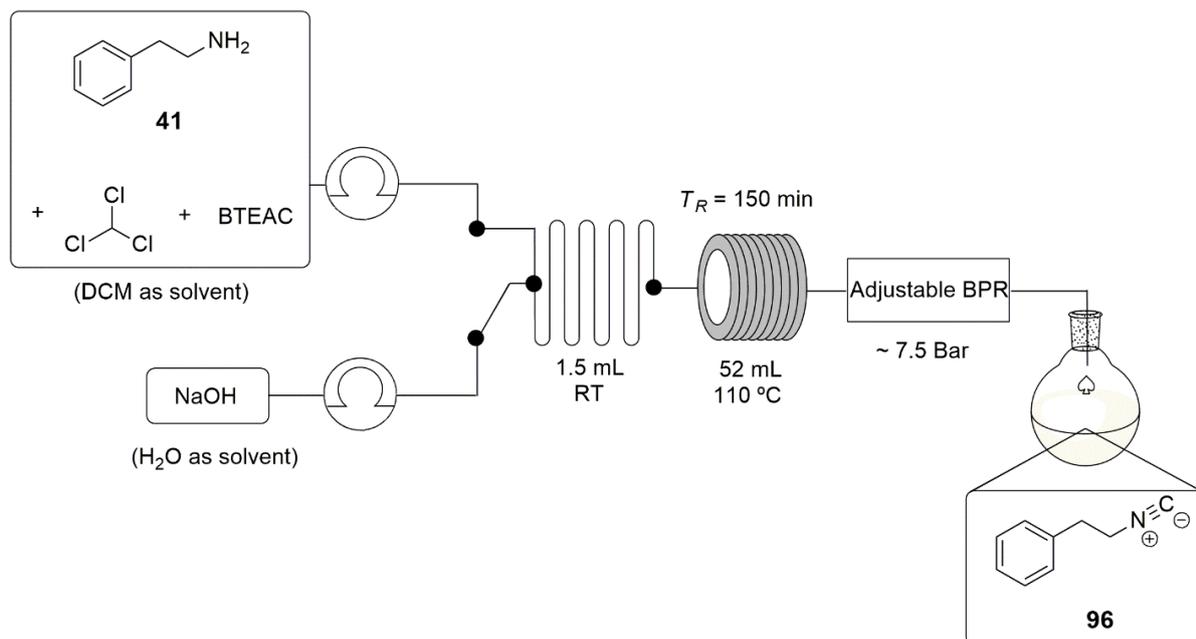


Figure 28: Definitive alternating slugs observed for flow entry 9 (Table 3).

In light of this, we elected to introduce the sodium hydroxide stock solution at double the volume of base in hopes of narrowing the organic phase slugs, and thereby increasing the surficial contact between the biphasic phases (Table 3, entry 10). Under these conditions, a residence time of 150 min was achieved, and as expected, the volume of the individual slugs decreased. The yield increased to 49% but when compared to entry number 8 (Table 3), this still represented a slight decrease in yield (49 vs. 54%). This was likely since the slugs were still larger than those achieved previously in the narrower bore tubing. Interestingly, the amount of precipitate was visually reduced when thicker bore tubing was used. This is possibly occurring as the wider bore tubing has a reduced surface area relative to the thinner tubing. As a result, there is a reduced surface on which nucleation can occur and precipitates can build up.

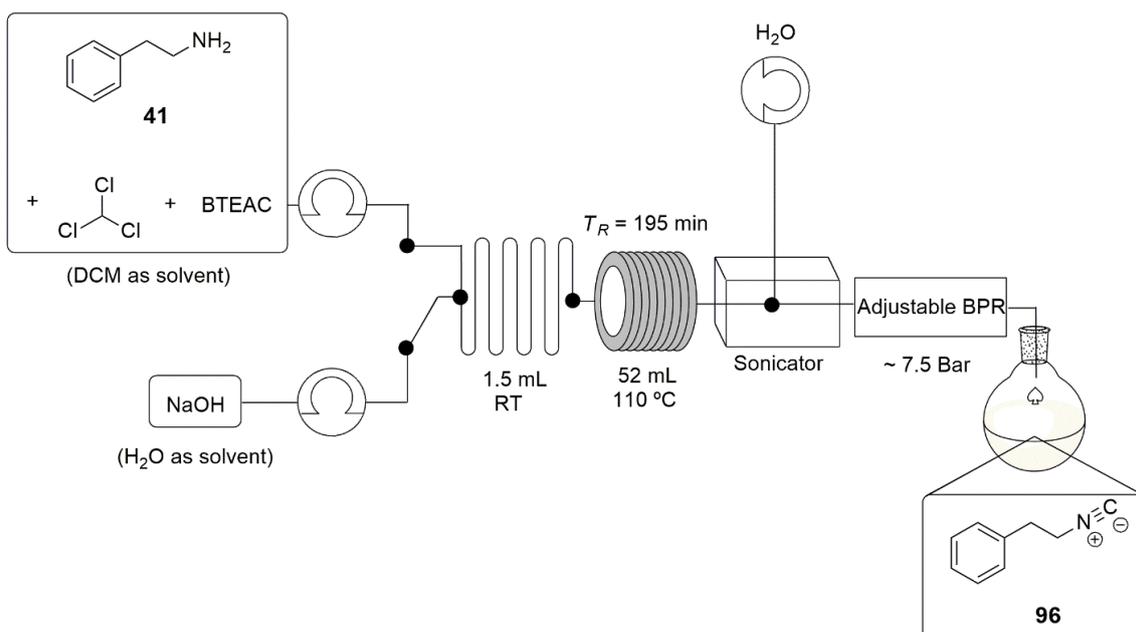
At this stage, it was hypothesized that a faster mixing process was needed to decrease the size of the biphasic slugs, thereby increasing the surficial contact between the phases. It had also become clear that the precipitate only developed in the heated coil reactor and not in the Y-piece mixer where initial mixing occurred at ambient temperature. Consequently, we elected to exchange the Y-piece mixer for a 1.5 mL Vapourtec mixing chip which promotes rapid mixing through structural channels designed to create eddies resulting in a more turbulent flow environment (Scheme 42).



Scheme 42: The flow set-up for flow entries 11 through 12 (Table 3).

Using this new setup, we initially reacted 8.0 equivalents of sodium hydroxide (8.75 M) with the remaining reagents in the DCM stock solution (2.2 M) in a 2:1 ratio to maintain the equivalents (Table 3, entry 11). The use of a mixing chip had a substantial impact on the rate of mixing and visually, the reaction exhibited more consistent bands of the alternating phases. Unexpectedly, a minor decrease in yield to 46% was observed. Nonetheless, we then decided to repeat the reaction with reduced equivalents of sodium hydroxide (6.0 equivalents), and as expected, this resulted in a noticeable decrease in yield to 35% (Table 3, entry 12). At this stage, as the concentration of sodium hydroxide was increased, the yield showed no substantial difference, however, in light of the batch results which exhibited a general trend of increasing yields with increasing sodium hydroxide concentration, we decided to perform further flow reactions while progressively introducing higher concentrations of sodium hydroxide solutions.

As the mixing chip improved the consistency of the alternating biphasic slugs, we were able to revert to mixing the two stock solutions in a 1:1 volume ratio, and even though we anticipated an increase in the amount of precipitation we felt that the use of the wider bore 52 mL coil reactor (1.5 mm internal diameter) would readily handle these solids. That being said, we were still concerned that the BPR would block and to mitigate this issue, we elected to install a quench line delivering distilled water after the coiled reactor. This would ensure the rapid dissolution of the precipitate prior to entering the BPR (Scheme 43).



Scheme 43: The flow set-up for flow entries 13 – 14 (Table 3). Where entry 13 does not contain the sonicator.

The concentration of sodium hydroxide was increased to 10 M, the two stock solutions were mixed in a 1:1 ratio, and the post-coil reactor water quench line was introduced in a 1:1 ratio relative to the exiting flow stream (Table 3, entry 13). This reaction resulted in an appreciable increased yield to 67%. Critically, prior to the quench line, there was a substantial build-up of precipitate within the coil reactor which subsequently impeded the flow of the liquid phase slowing the overall flowrate (an issue associated with the use of peristaltic pumps when pumping slurries). As a result, the residence time was approximately 195 min as opposed to 150 min. Additionally, although the water quench largely dissolved the precipitate (and diluted the sodium hydroxide which also suppresses the precipitation) small solids were still visually present. We were again concerned that over time the BPR would eventually block resulting in the reaction aborting. To mitigate this risk, we again modified the flow setup to have the quench line join the main flow stream at a Y-piece mixer housed inside a sonicating bath (Figure 29).

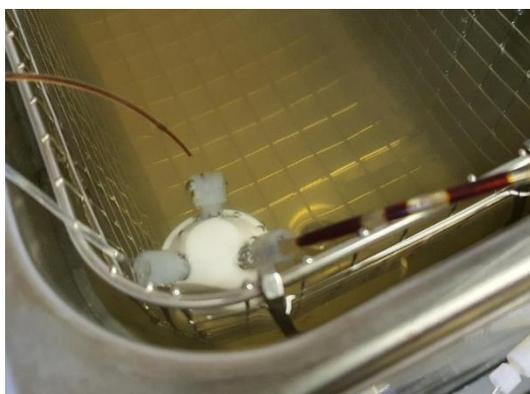


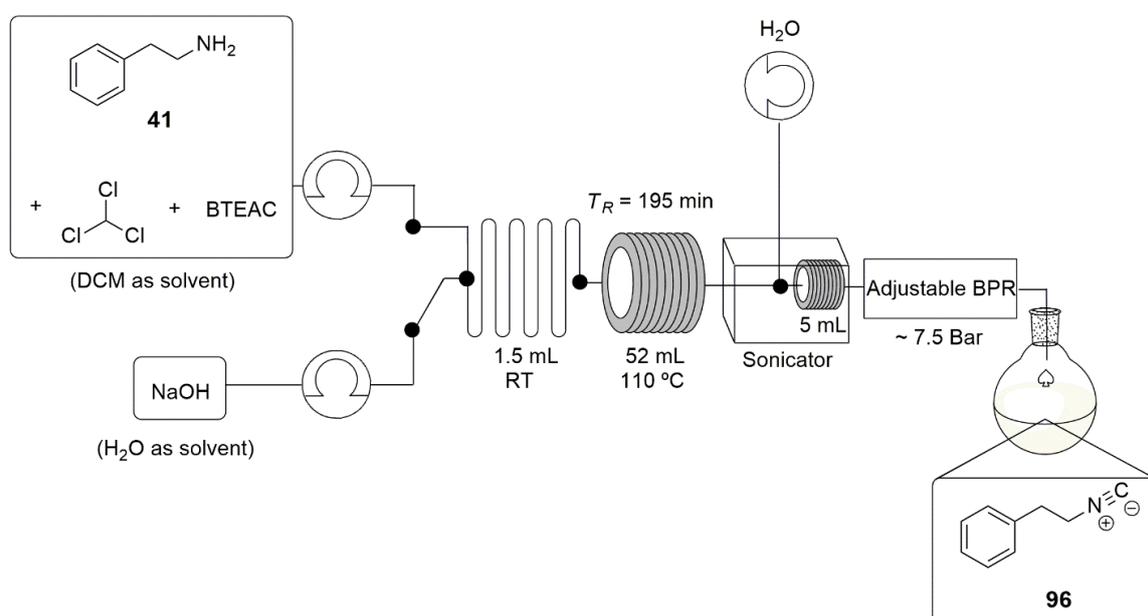
Figure 29: Y-piece mixer for additional quench line submerged in the sonicating bath.



Figure 30: More consistent alternating slugs observed for entry 14 (Table 3) with substantial amounts of precipitate present in the alternating aqueous phase.

The reaction was repeated under comparable conditions to those listed under entry 13 of table 3, but with the introduction of an increased concentration of 12.5 M sodium hydroxide (8.0 equivalents). As expected, visually there was a significant amount of precipitation (Figure 30), however, the precipitate moved through the coil reactor smoothly and thereafter solubilised upon quenching. The use of the sonicating bath greatly improved the dissolution of the precipitate by keeping the solid particles mobilised and preventing solids layering in the tube. Once again, the total residence time amounted to approximately 195 min and a large increase in yield to 78% (1.25 g scale) was achieved. This is higher than both the previously reported performance of 61% as well as the best yield of 72% achieved in-house under batch conditions. Notably, this was achieved in 195 min residence time as opposed to 240 min under batch conditions.

The setup was subsequently modified to include a 5 mL dissolution loop fitted directly after the quench line (Scheme 44), this afforded additional time for complete dissolution and under these conditions, the process performed similarly with a 76% isolated yield (Table 3, entry 15). We again investigated the effect of reducing the equivalents of sodium hydroxide solution (Table 3, entries 16 and 17), but as expected neither of these attempts resulted in an increase in yield.



Scheme 44: The flow set-up for flow entries 15 – 18 (Table 3).

Finally, we also attempted to prepare the 2-isocyanoethylbenzene **96**, employing the use of 2-methyl tetrahydrofuran (2-Me-THF) as an alternative green solvent to DCM (Table 3, entry 18) and in this instance, we elected to use water as the pushing solvent. Unfortunately, although the reaction proceeded smoothly it only afforded a yield of 56%. The decrease in yield was hypothesized to have resulted from the PTC (BTEAC) exhibiting poor solubility in

the 2-Me-THF (BTEAC added first to a volumetric flask, followed by a minimum amount of 2-Me-THF) and only upon addition of the required chloroform into the same flask did the BTEAC solubilise completely. This is problematic as the chloroform is used stoichiometrically in a 1.0 mol equivalence, as a result, the partitioning of the BTEAC between the organic and aqueous phase decreases as the chloroform is consumed, which in turn decreases the rate of dichlorocarbene formation.

2.1.7 Concluding remarks

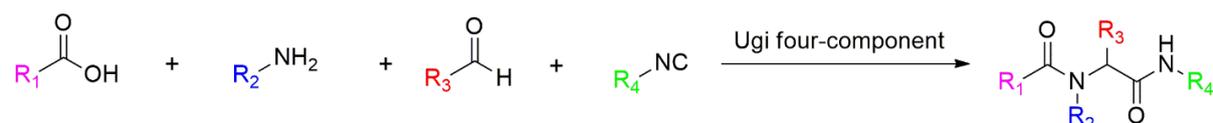
To conclude, we have successfully demonstrated a synthetic procedure for the formation of 2-isocyanoethylbenzene **96** under flow conditions while implementing the modified Hofmann approach. This reported procedure afforded a significant increase in yield (78%) when compared with the previous batch methodology (61%) reported in literature by Cao *et al.*⁹⁵ and, a reduction in residence/reaction time of ~ 20%. This amounts to a space-time yield of 5.7 g.L⁻¹.h⁻¹. Critically, this flow procedure also reduced the concentration of base required from a 50% w/w (~ 19 M) sodium hydroxide solution to 12.5 M which, from a safety aspect, is highly beneficial for the working chemist. When comparing this flow procedure to that of the batch-based formamide dehydration approach performed in-house, the results were slightly inferior (78% vs. 83%), however this flow approach is substantially “greener” as there is no use of the questionable dehydrating reagent, phosphorous oxychloride, excess triethylamine as well as an additional step for the formamide formation (12 h reflux in ethyl formate). This in turn results in an arguably more economical reaction requiring only one step from the same 2-phenethylamine **41** starting material in a drastically reduced overall reaction time (195 min residence time as opposed to 12 h reflux before the dehydration step). Furthermore, this flow procedure shows potential to allow for the in-line work-up and consumption of the noxious isocyanide **96**, which in our case would allow telescoping into the next Ugi four-component reaction for the preparation of the “pre-praziquantel” intermediate **61**.

CHAPTER III

3.1 A flow approach for the preparation of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide **61**

3.1.1 Brief history of the Ugi multi-component reaction

The second step of the synthetic procedure utilises a key four-component Ugi-reaction for the preparation of the “pre-praziquantel” intermediate, *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide **61**.⁹⁵ The first multi-component reaction utilising this type of chemistry was discovered by Ivar Ugi in 1959,¹³² and soon after the eponymous “Ugi four-component reaction” name was coined. This general approach for preparing complex α -acetoamido carboxamide derivatives (Ugi adducts or sometimes referred to as peptoids) requires the introduction of an isocyanide, aldehyde, amine and carboxylic acid (Scheme 45).¹³³ Notably, the introduction of the carboxylic acid surrogate, hydrazoic acid (HN₃) was implemented in 1961 by Ugi and Steinbrückner¹³⁴ for the analogous preparation of tetrazole derivatives and is still utilised today, often involving trimethylsilyl azide (TMSN₃).¹³⁵

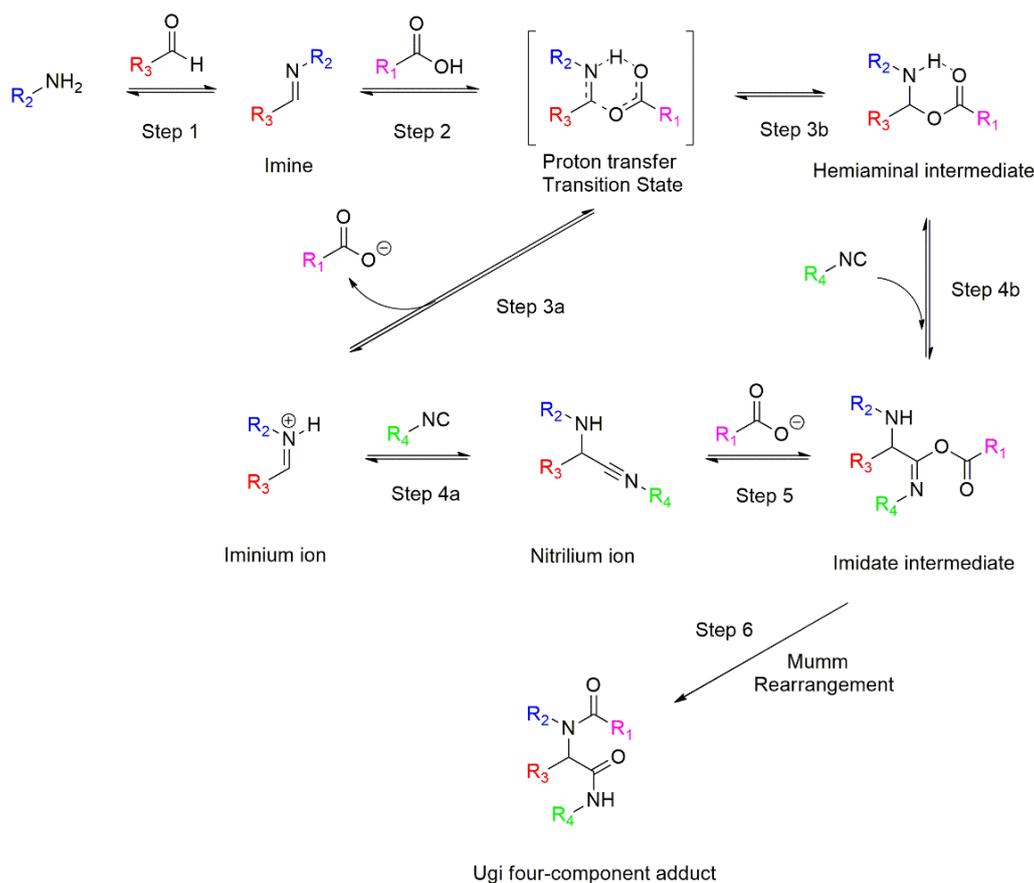


Scheme 45: General schematic for an Ugi four-component reaction with the introduction of an isocyanide, aldehyde, amine and carboxylic acid.¹³³

Subsequently, the use of this type of chemistry which allows access to complex heterocyclic building blocks required for various organic products containing an α -acetoamido carboxamide skeletal structure or as a required precursor, has grown in popularity. The approach has been extensively scrutinised, targeting different reaction applications, conditions, catalysts, diversity of syntheses, effects, and features, and today it can be regarded as a highly developed synthetic tool within organic chemistry.¹³³ The most common choice of solvent when performing an Ugi reaction are polar protic solvents, as they conform to the reaction mechanism, with polar derivative reaction intermediates being stabilised. That being said, it has also been reported by Madej *et al.* that polar aprotic solvents can be employed successfully affording the desired products in good yields for specific reactions.¹³⁶ Furthermore, the use of an aqueous system with the addition of surfactants like didodecyldimethylammonium bromide (DDAB) has also proved efficient, allowing the preparation of the Ugi adducts in appreciable yields.¹³⁶ The Ugi four-component reaction has proven its versatility in organic chemistry, and not limited to, allowing for efficient single step syntheses of various 1,6 enynes in good yields,¹³⁷ dimeric α -aziridine aldehyde intercepted Ugi reactions affording β -(acylaziridiny)- α -aminoamide derivatives,¹³⁸ novel *N*-substituted

alkenylamides,¹³⁹ and constituting an important step in the synthetic preparation of the core fragment hosted in the animal metabolite massadine.¹⁴⁰

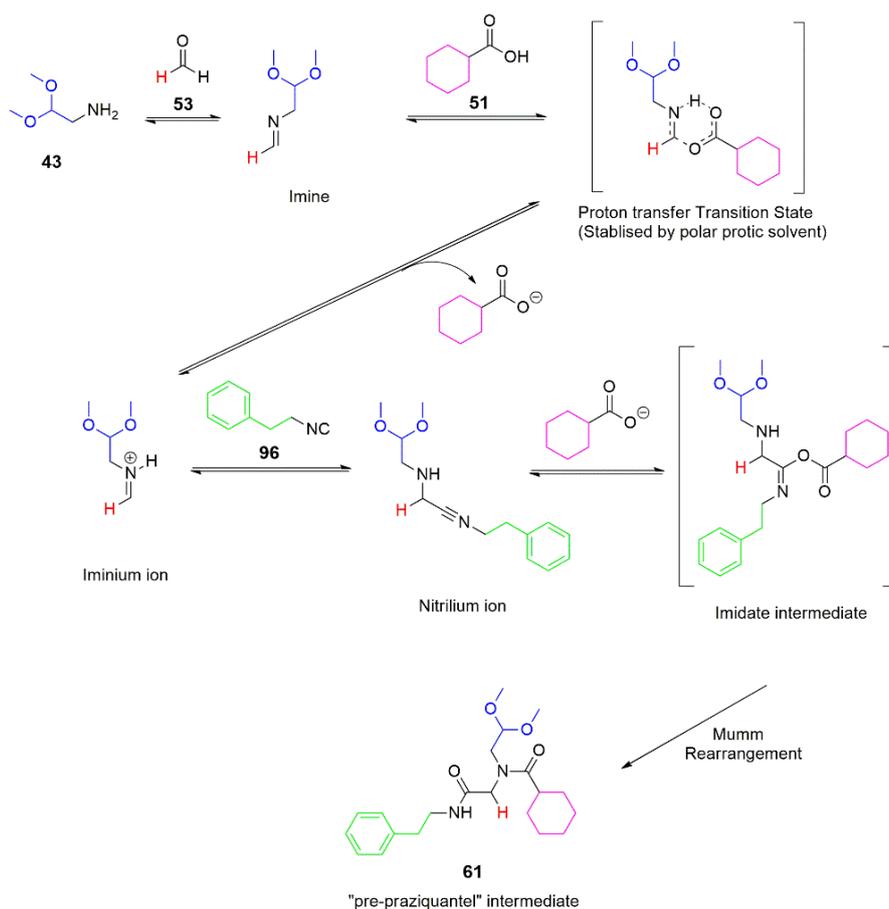
The key mechanistic features of the four-component reaction were originally described by Ugi and in 2012, a second competitive pathway gained widespread acceptance (Scheme 46).¹³³ Both pathways involve the initial formation of the imine intermediate by reaction of the aldehyde and amine functional groups (Scheme 45, Step 1). The added carboxylic acid promotes the proton transfer transition state (Step 2), which allows for either i) the activation of the iminium ion through the expulsion of the carboxylate ion (Step 3a) or ii) the formation of a hemiaminal intermediate (Step 3b) (proposed in 2012 by Chéron *et al.*)¹⁴¹. The iminium ion, which has been proven to be a key intermediate,¹⁴² is then subjected to an isocyanide addition (first widely accepted approach forming a nitrilium ion intermediate) (Step 4a) followed by the carboxylate ion re-insertion (Step 5). Using the alternative approach, the hemiaminal is subjected to an isocyanide insertion (Step 4b). Notably, both of these approaches (from hemiaminal or nitrilium ion) result in the formation of the same imidate intermediate, which then subsequently undergoes a Mumm rearrangement to afford the desired Ugi adduct (Step 6).¹³³



Scheme 46: Current two competitive reaction mechanisms for the Ugi four-component reaction.¹³³

In an attempt to understand which mechanistic approach was correct, Medeiros *et al.*¹⁴² undertook a study in 2014 utilising charge tagged reagents which were detectable by

electrospray ionisation mass spectrometry (ESI-MS(/MS)) techniques. The authors reported that the first approach, involving the nitrilium ion intermediate, was the most viable route with several of the expected intermediates being detected including the iminium and nitrilium ions as well as the final Ugi adduct. Notably, none of the intermediates expected for the hemiaminal mechanistic approach or the convergent imidate intermediate were detected prompting the authors to reject the hemiaminal mechanism.¹⁴² A second mechanistic study reported by Iacobucci *et al.* in 2014,¹⁴³ made use of ESI-MS(/MS) which was coupled with infrared multiphoton dissociation (IRMPD) spectroscopy in order to characterise the nitrilium ion as well as the convergent imidate intermediate. The hemiaminal ion was again not observed, even when attempting to push its formation. The authors also reported density functional theory (DFT) calculations that were in good agreement with their experimental results and consequently, based on their findings they also supported the classical nitrilium ion mechanistic approach.¹⁴³ To aid the reader, the supported mechanism has been redrawn for “pre-praziquantel” intermediate **61** targeted in this study in scheme 47.



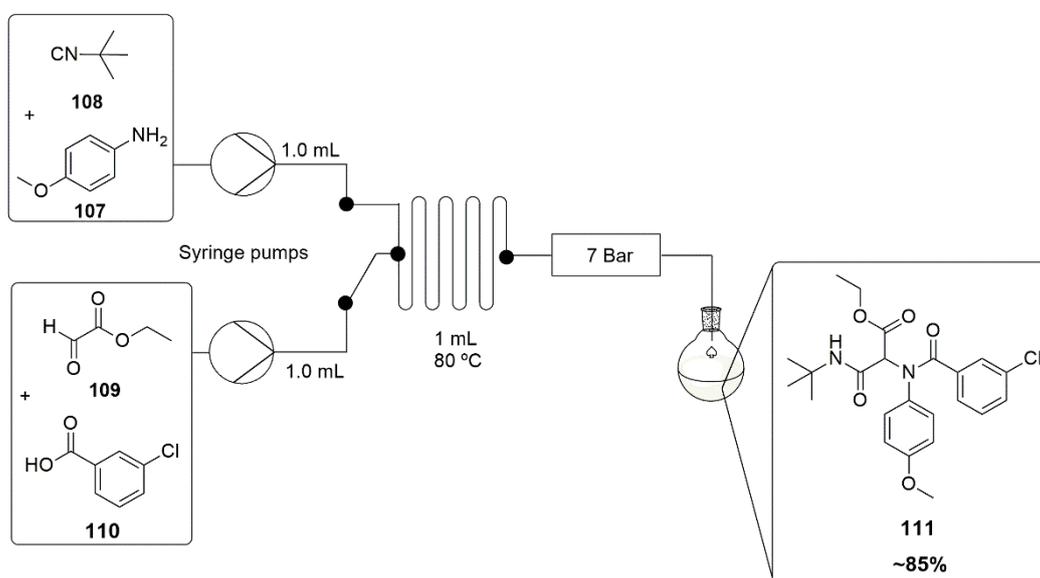
Scheme 47: Proposed mechanism for the preparation of the “pre-praziquantel” intermediate **61** obtained during this study.

3.1.2 Ugi four-component reactions under flow conditions

Ugi multi-component reactions have been implemented under flow conditions by several research groups and have been shown to gain many advantages when compared to

analogous batch syntheses, most notably are appreciable decreases in reaction times with improved or comparable yields. Selected examples for the Ugi four-component reaction are highlighted below.

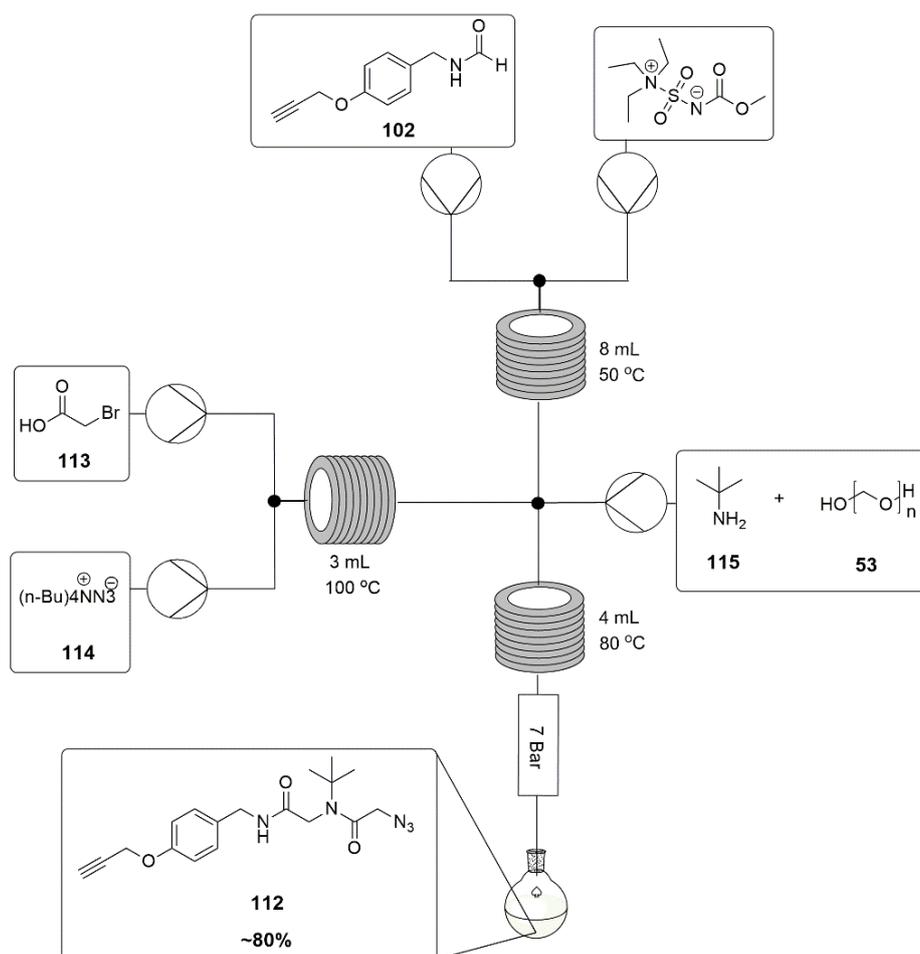
An article by Vasconcelos *et al.*¹¹⁰ reports the synthesis of various α -amino-1,3-dicarbonyl compounds with the utilisation of flow chemistry technologies. The optimised setup employed by the authors involved the introduction of the required isocyanide with the respective amine in MeOH from one stock solution and the respective carboxylic acid and aldehyde in MeOH from another stock solution. In both instances, syringe pumps were employed and the stocks were combined in a 1 mL mixing chip at 80 °C with a residence time of 10 – 20 min, after which time the flow stream passed through a 7 bar BPR. Off—line purification by column chromatography afforded a range of compounds in moderate to good yields.¹¹⁰ To highlight an example from the report, amine **107** (1.0 M) with isocyanide **108** (0.5 M) in MeOH from one stock solution and aldehyde **109** (2.0 M) with carboxylic acid **110** (1.0 M) in MeOH from a second stock solution were reacted in a 1 mL mixing chip (held at 80 °C with a residence time of 20 min) affording the Ugi product **111** in 85% yield after off-line purification by column chromatography (Scheme 48). When compared with conventional batch procedures for the same chemistry, the authors drastically reduced the overall reaction times (with an increase in yield) from 24 h while stirring at room temperature (69% isolated yield) to 20 min residence time under flow conditions at 80 °C (85% isolated yield).¹¹⁰



Scheme 48: Flow set-up for the preparation of **111**. Reported by Vasconcelos.¹¹⁰

Salvador *et al.*¹¹² exemplified another Ugi four-component reaction under continuous flow conditions for the preparation of the linear peptoid **112** (Scheme 49). The authors reported an in-line generation of the respective isocyanide, by reacting formamide **102** in ACN (0.25 M) with Burgess reagent in ACN (0.5 M) in a tubular coil reactor set at 50 °C with a residence

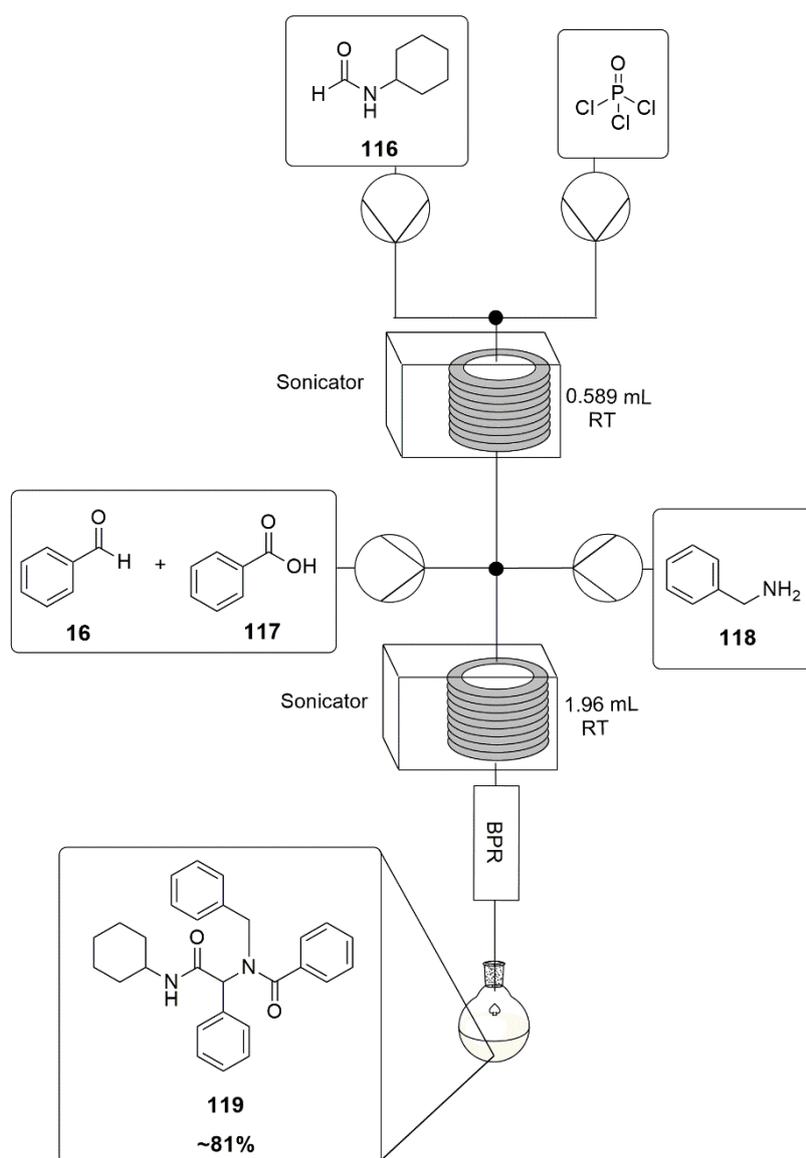
time of 20 min, as well as an in-line formation of the desired azide by reacting 2-bromoacetic acid **113** in ACN (1.0 M) and tetrabutylammonium azide **114** in ACN (1.5 M) in a tubular coil reactor set at 100 °C with a residence time of 15 min. The two output streams were then combined with a stock solution containing amine **115** and paraformaldehyde (formaldehyde **53** source) in MeOH (0.5 M) at an X-type mixer prior to passage through a 4 mL tubular reactor set at 80 °C with a total residence time of 5 min. Following off-line column purification, the linear peptoid **112** was afforded with a good yield of 80%.¹¹² Notably, the authors also reported a batch procedure with comparable yields, making use of a microwave reactor at 80 °C and 4 min, however, this was not a telescoped procedure and required stand-alone preparations of the required starting materials.¹¹²



Scheme 49: Continuous flow reaction for the preparation of peptoid **112**, reported by Salvador *et al.*¹¹²

As a final example, Sharma *et al.*¹⁴⁴ reported a continuous flow approach for the synthesis of various Ugi four-component adducts with yields of 76 – 86%. The authors achieved this by firstly preparing the required isocyanide in-line with quantitative yields, which was further telescoped into an Ugi four-component reaction performed at room temperature while exposed to ultrasonic irradiation. As an example, the authors prepared the cyclohexyl isocyanide by reacting cyclohexyl formamide **116** in DIPEA (1.0 M) with phosphorous

oxychloride in toluene (2.0 M at half the flow rate) in a capillary microreactor submerged in an ultrasonicating bath at room temperature with a residence time of 12 min. This was subsequently worked up in-line and introduced (2.0 M in toluene) into an X-type mixer along with benzoic acid **117** and benzaldehyde **16** in one DMF stock solution (2.0 M) and benzylamine **118** in another DMF stock solution (4.0 M at half the flow rate) (Scheme 50). The resulting outgoing mixture was passed through a PFA tubing submerged in an ultrasonicating bath while at room temperature for a total residence time of 24.5 min. Following purification via column chromatography, the Ugi adduct **119** was afforded in 81% yield.¹⁴⁴



Scheme 50: Continuous flow reaction for the preparation of Ugi adduct **119** reported by Sharma et al.¹⁴⁴

To the best of our knowledge, the Ugi adduct used within this research has, to date, not been synthesised under flow conditions.

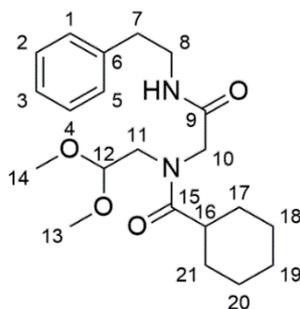
3.1.3 “Pre-praziquantel” intermediate **61** synthesis under batch conditions

Initially the Ugi batch methodology reported by Cao *et al.*⁹⁵ was validated. In the approach, paraformaldehyde (formaldehyde **53** source), aminoacetaldehyde dimethyl acetal **43** and cyclohexanecarboxylic acid **51** were all dissolved/suspended in MeOH at 0 °C, followed by a subsequent portionwise addition of 2-isocynoethylbenzene **96**. All reagents were introduced in an equimolar ratio on a 19.1 mmol scale in 20 mL of MeOH (~ 1 M in reagents). The mixture was left to stir at room temperature for 48 h, followed by an aqueous wash, separation, drying and concentration to afford the “pre-praziquantel” intermediate **61** in near quantitative conversion. Unfortunately, assessment of the material by TLC analysis revealed a baseline spot, and two spots with higher R_f values than the desired **61**, furthermore, careful analysis of the ¹H-NMR spectra displayed minor discrepancies in the integral traces. It was evident that the product required an additional purification step, which was achieved using column chromatography affording the “pre-praziquantel” intermediate **61** in 89% yield. Upon standing, this light yellow/white oil (Figure 31) slowly crystallises.



Figure 31: “Pre-praziquantel” intermediate Ugi adduct **61** obtained from the batch validation reaction.

To correlate the ¹H- and ¹³C-NMR signals with that of the structure of “pre-praziquantel” **61**, we performed additional heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC) and ¹H-¹H correlation spectroscopy (COSY) analyses (depicted in Appendix B – 1) to get an in depth understanding of the intramolecular coupling that is taking place and in turn, assist with the structural analysis. Based on the HSQC, we were able to distinguish between CH/CH₃ and CH₂, where the HSQC would conveniently colour code the signals by red (CH/CH₃) and blue (CH₂) in line with the correlating signals observed on the ¹H-NMR (horizontal axis in our spectrum). The HMBC was only used for a few final confirmations which will be discussed to follow. Lastly, the COSY analysis was used for final confirmation to ensure the correlated protons couple to expected protons from observing the structure. Figure 32 has been added for clarification of peak and structure correlation of the “pre-praziquantel” intermediate **61**.



61

Figure 32: “Pre-praziquantel” intermediate Ugi adduct **61** numbered structure for reference to discussion.

- I. Based on the $^1\text{H-NMR}$ analysis, we could confidently identify the aromatic protons, the cyclohexyl protons as well as the methoxy group protons based on their relative chemical shift regions and integration values. However, correlating each C and H from these groups was achieved with the additional spectral data.
- II. For the aromatic protons, based on the COSY, it was evident that the multiplet, integrating for three protons, located between 7.15 – 7.24 ppm was coupled with the two triplets around 2.78 and 2.82 ppm, which could only be the nearest protons bonded to C-7 with the correlated C atom split at 35.64 and 35.59 ppm. Inversely, this confirms that this is most likely from the closest aromatic protons on C-1 and C-5. Notably, $\sim 1/3$ of this aromatic multiplet didn't display coupling, giving reason to be the proton on C-3 (non-equivalent with respect to C-2 and C-4). Therefore, the multiplet at 7.25 – 7.34 ppm, integrating for the remaining two protons, correlates with the protons on C-2 and C-4. Using the HSQC, we correlated the respective C atoms on the spectrum. C-1 (128.76 ppm), C-5 (128.68 ppm), C-3 (128.60 ppm), C-2 (126.63 ppm), C-4 (126.47 ppm). The remaining quaternary C-6 atom was identified by being the only peak left in the aromatic region of the $^{13}\text{C-NMR}$ as well as no coupled proton from the HSQC. This signal is split at 138.58 and 138.82 ppm.
- III. The methoxy groups can ultimately rotate to match the others orientation so therefore we decided on the signal correlations as if it was in the orientation in figure 32. The protons were identified with additional confidence from the HSQC as they were red and further allowed us to correlate them with the respective C signals whereby the protons on C-13 correlate to the singlet signal at 3.37 ppm with the C atom at 55.49 ppm (closest to carbonyl O atom possibly forming a H-bonding interaction so more deshielded). As for the protons on C-14, correlate to the singlet at 3.33 ppm with the C atom at 55.08 ppm.
- IV. The cyclohexyl protons were classified in a similar fashion where the multiplet, integrating for five protons, located between 1.54 – 1.83 ppm correspond to the protons

on C-17, C-21 and one proton from C-20. The multiplet between 1.38 – 1.51 ppm, integrating for two protons correspond to the remaining proton on C-20 and one proton for C-18. As for the remaining multiplet between 1.15 – 1.35 ppm corresponds with the remaining proton on C-18 and both protons on C-19. Lastly, the C atoms were correlated with the HSQC spectrum to conclude C-17 (29.39 ppm), C-21 (29.32 ppm), C-20 (25.75 ppm), C-18 (25.67 ppm), C-19 (25.56 ppm).

- V. The C-H (C-16) proton on the cyclohexyl moiety, was easily identified since it appeared as two red signals in the HSQC, located in an expected region where a carbonyl group usually deshields a C-H, integrated for half a proton each and presented as a doublet of triplets (expected since adjacent to two CH₂ groups, coupled to its neighbouring proton on C-16 and an adjacent quaternary carbonyl). This was correlated (HSQC) with the C atom split peaks at 40.29 and 41.06 ppm.
- VI. Furthermore, this quaternary carbonyl C-15 atom was concluded to be located and split at 178.03 and 177.83 ppm, due to the coupling observed in the HMBC with the C-H from the cyclohexyl moiety. The other carbonyl C-9 atom was subsequently concluded to be the split peaks at 169.28 and 169.55 ppm.
- VII. The next major distinctive peaks that were identified was the only N-H proton present in the molecule, since it had no singly bonded coupled C atom (broad triplets, each integrating for half a proton, at 7.03 and 6.51 ppm).
- VIII. The proton on C-12 was identified by the remaining two red signals observed on the HSQC, also the peak experienced significant deshielding due to the two adjacent methoxy O atoms. The proton was split across two triplets, each integrating for half a proton, located at 4.39 and 4.58 ppm. Using the HSQC spectrum, C-12 was observed as a split signal at 102.73 and 103.49 ppm.
- IX. The remaining protons on C-8, C-10 and C-11 were the most challenging to confidently identify based on the HSQC analysis. Consequently, the HMBC analysis was observed in order to correlate multiple bond couplings. Since C-10 is situated between a carbonyl (C end of the one amide) and an amide functional group, it is expected to exhibit the highest deshielding effects (for protons and C atom) when compared with C-8 and C-11. As such, it was observed as a doublet (splitting amongst these two protons on C-10), integrating for two protons, at 3.99 ppm. Furthermore, this was confirmed by the HMBC displaying proton coupling to both carbonyl C atoms (C-8 and C-11 are too far away to couple with both carbonyl C atoms). HSQC was used to correlate these protons with the C-10 atom split at 51.52 and 50.33 ppm.

- X. The protons on C-8 were identified from the HMBC since they displayed coupling with the quaternary aromatic C-6 as well as C-1 and C-5 (C-12 is too far for this coupling to occur). Therefore, the protons on C-8 appear as a doublet of triplet of doublets, integrating for two protons, situated at 3.52 ppm. Using HSQC, the C-8 atom split at 40.50 and 40.69 ppm.
- XI. The remaining protons on C-11 correlate to the triplet, integrating for two protons, situated at 3.42 ppm. HSQC was used for correlating with the C-11 atom split at 52.11 and 54.04 ppm. This was further confirmed by the proton coupling with the close carbonyl C-15 and neighbouring C-12 in the HMBC.

When comparing these chemical shifts with that of the starting materials, the proton NMR spectrum (Figure 33) exhibits a disappearance of the broad singlet peak around 11.77 ppm (carboxylic acid proton) confirming that a reaction indeed took place at the carboxylate oxygen position of cyclohexanecarboxylic acid moiety forming the new amide. With regards to the cyclohexyl moiety CH₂ groups (protons on C-17 – C-21; Figure 32), multiplets integrating for a total of ten protons were observed, as expected, in the region between 1 – 2 ppm. The cyclohexyl CH group (proton on C-16) adjacent to the carbonyl carbon which presents as a triplet of triplets at 2.34 ppm, integrating for one proton in the starting material **51** (depicted in Appendix B – 2), splits into two separate triplet of triplets located at 2.25 and 2.58 ppm respectively, each integrating for half a proton, giving evidence of successful linkage forming a part of a larger rotationally bound system.

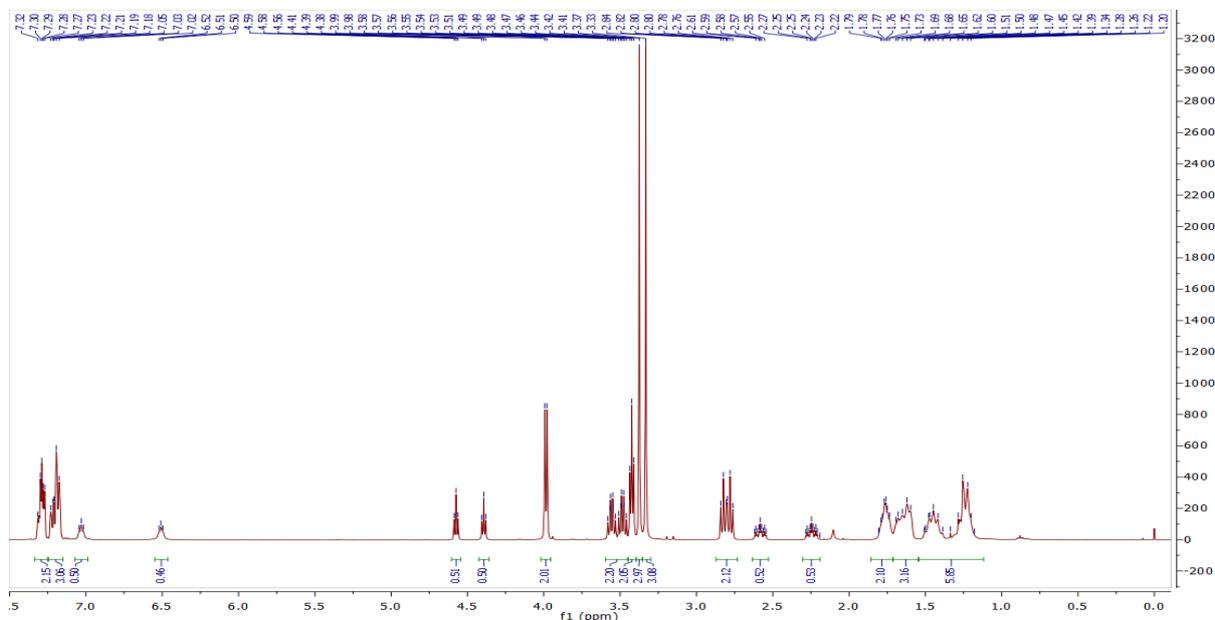


Figure 33: ¹H-NMR obtained for “pre-praziquantel” **61** during this research study.

A further characterising feature for the reaction is that there is no evidence of an aldehyde proton (from formaldehyde **53**) confirming that the C-C bond formation was successful (C-10).

When comparing the aminoacetaldehyde dimethyl acetal **43** spectrum (depicted in Appendix B – 3) with the newly formed “pre-praziquantel” intermediate **61**, a major distinction is the unequal proton environment formation observed for the two methoxy functional groups (protons on C-13 and C-14) which exhibits as a singlet integrating for six protons at 3.39 ppm in the starting material **43** but display as two singlets, each integrating for three protons at 3.33 and 3.37 ppm in the spectrum of **61**. This suggests that the close proximity carbonyl and aromatic groups influence the shielding of these protons, and it forms a part of a rotamer system. The same is true for the CH group (proton on C-12) bonded to these methoxy oxygen atoms (one triplet at 4.29 ppm in **43**) which now exhibits two separate triplets, each integrating for half a proton at 4.39 and 4.58 ppm, respectively.

Finally, the CH₂ group (protons on C-11) adjacent to the amine functional group presents as a doublet at 2.79 ppm in the spectrum of **43** whereas in the spectrum of **61**, this doublet appears as a triplet at 3.42 ppm owing to the new adjacent amide functional group as well as additive effects from the surrounding deshielding electronegative atoms. Lastly, confirmation of the isocyanide **96** reaction gives rise to two broad triplet peaks at 7.03 and 6.51 ppm, each integrating for half a proton responsible of the newly formed amide proton (C-8 – NH – C-9).

When comparing the ¹³C-NMR of the starting materials and the “pre-praziquantel” intermediate **61** (Figure 34), as expected there are two clear carbonyl carbon atoms, each showing two peaks, observed at 178.03/177.83 ppm and 169.55/169.28 ppm, which arise from the cyclohexyl carbonyl (C-15; Figure 32) and the amide carbonyl (C-9), respectively. In the aromatic region, the carbon atoms (C-1 – C-6) are observed in a similar region when compared with the 2-isocyanoethylbenzene **96** spectrum (depicted in Appendix A – 1), between 120 – 140 ppm. Likewise, the CH₂ carbon atoms on the cyclohexyl moiety (C-17 – C-21) also remain in a similar region of 25 – 30 ppm to those in **51** (depicted in Appendix B – 2). The cyclohexyl CH carbon (C-16) exhibits a split in the signal observed at 40.29 and 41.06 ppm in the “pre-praziquantel” **61** spectrum vs. 43.08 ppm in the spectrum of **51**. As for confirmation that the aminoacetaldehyde dimethyl acetal **43** successfully reacted, the methoxy carbon atoms (C-13 and C-14) exhibit a split in their peak observed at 55.08 and 55.49 ppm in the “pre-praziquantel” **61** vs. 53.87 ppm as one signal in the spectrum of **43** (depicted in Appendix B – 3). The CH carbon (C-12) situated between the two methoxy oxygen atoms also exhibited a split in its signal observed at 102.73 and 103.49 ppm vs. 105.68 ppm in the spectrum of **43**. Finally, the carbon atom from the CH₂ group (C-11) adjacent to the amine (43.54 ppm in the aminoacetaldehyde dimethyl acetal **43** spectrum), exhibited a split and deshielding with signals observed at 52.11 and 54.05 ppm owing to the new adjacent amide functional group being observed in the spectrum of **61**.

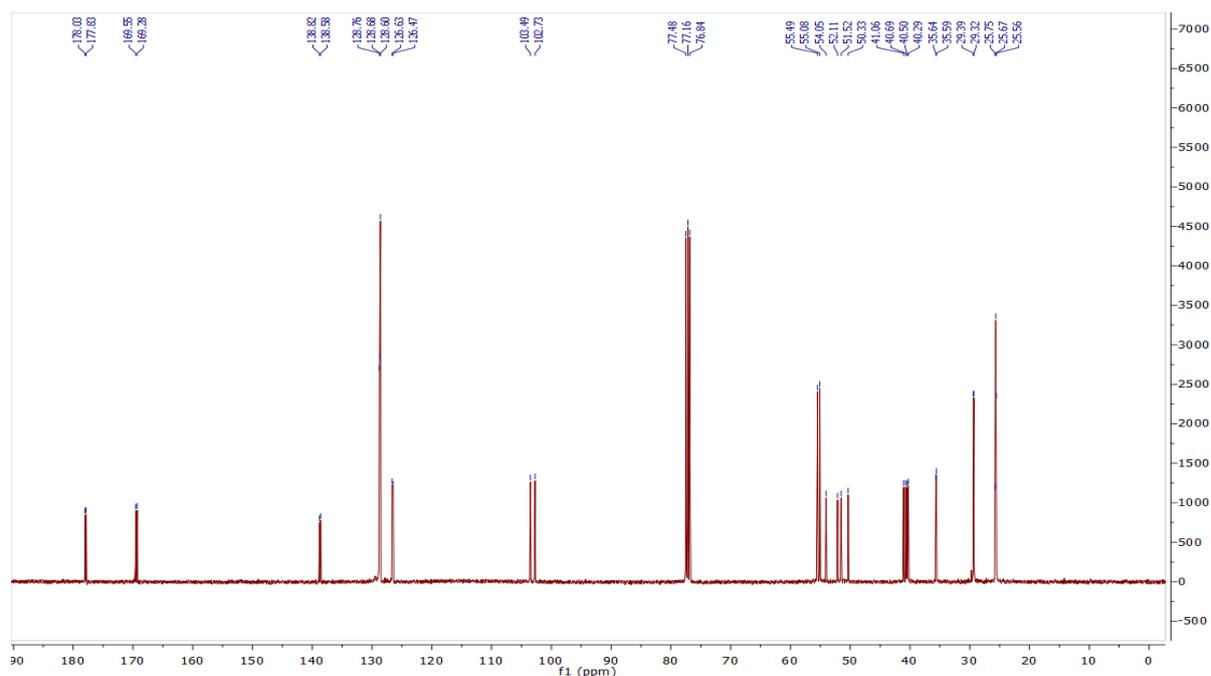


Figure 34: ^{13}C -NMR obtained for “pre-praziquantel” **61** during this research study.

When observing the IR spectrum obtained for **61** (depicted in Appendix B – 4), the disappearance of the strong isocyanide stretch at 2147 cm^{-1} , in **96**, is evidence that the isocyanide functional group has successfully reacted. Additionally, an appearance of a broad medium peak at 3283 cm^{-1} corresponds well with an additional secondary amine functional group N-H stretch due to the amide formation. An additional confirmation of the reaction is that the aldehyde C-H stretch from **53**, which presented as a medium doublet generally observed between $2695 - 2830\text{ cm}^{-1}$, disappeared. As for the C=O stretch, distinctive of this secondary amide formation as well as the secondary amide formation resultant of the reacted primary amine (aminoacetaldehyde dimethyl acetal **43**), this is generally present as a strong peak around 1675 cm^{-1} . In addition, when identifying if the aminoacetaldehyde dimethyl acetal **43** reacted successfully, it is evident that there is no primary amine N-H stretch at around 3500 cm^{-1} giving evidence that the reaction was successful at the nitrogen atom site. As for the aliphatic ether C-O stretch, corresponding to the methoxy moieties (aminoacetaldehyde dimethyl acetal **43**), these are still strong peaks observed around $1150 - 1040\text{ cm}^{-1}$ giving evidence that these groups are still present. Lastly, the reaction of the cyclohexanecarboxylic acid **51** was evidenced as there was no weak broad peak observed between $2700 - 3200\text{ cm}^{-1}$, which is distinctive of a carboxylic acid O-H stretch and no strong carboxylic acid C=O strong stretch at 1760 cm^{-1} .

Further definitive identification for the “pre-praziquantel” **61** structure was obtained through single crystal X-ray diffraction (SC-XRD) (Figure 35; For raw data, please see Appendix B – 5).

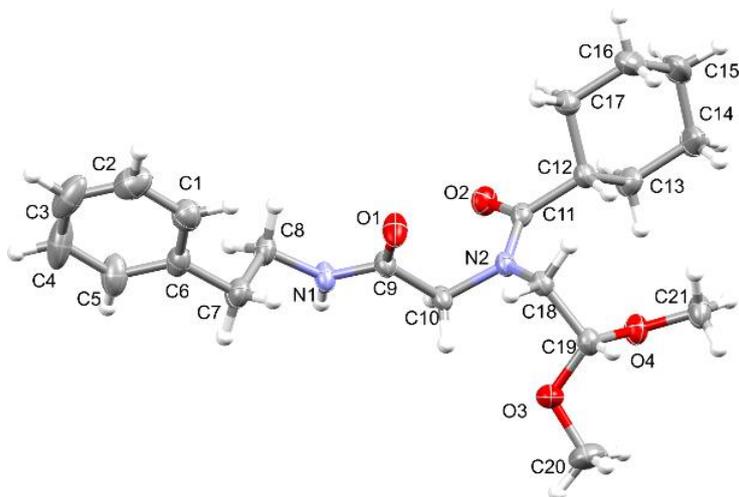


Figure 35: "Pre-praziquantel" **61** crystal structure obtained during this research study.

Figure 36 shows strong hydrogen bonding interactions (as observed down the *b*-axis shown in blue, hydrogen atoms hidden for clarity) between two adjacent molecules resulting in a dimeric crystal structure of **61**. Figure 37 has been added to assist in viewing this interaction (as observed down the *b** axis) between two molecules while including the hydrogen atoms for clarity. This interaction is present between the hydrogen atom on N1 (Figure 35) and the oxygen atom O2 from the respective molecules.

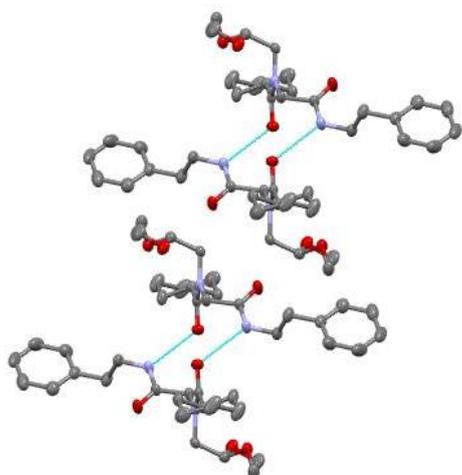


Figure 36: "Pre-praziquantel" **61** crystal structure highlighting the dimer configuration, represented by the blue lines as viewed down the *b*-axis.

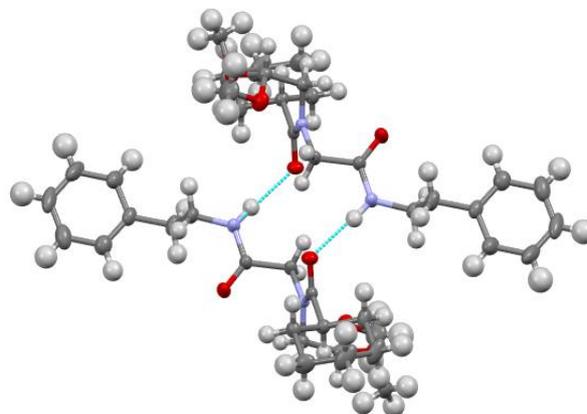


Figure 37: "Pre-praziquantel" **61** crystal structure highlighting the hydrogen bonding as viewed down the *b**-axis with hydrogen atoms included for clarity.

Finally, the mass spectrum obtained (depicted in Appendix B – 6) displays an $[M+H]^+$ ion located at 377.2433 *m/z* which corresponds well with the expected calculated $[M+H]^+$ of 377.2440 *m/z*. In addition, a comparison of the ^{13}C and ^1H -NMR spectral data obtained during this research study and that reported by Cao *et al.*⁹⁵ has been tabulated (Table 4) showing good correlation.

Table 4: Comparison of chemical shifts observed for “pre-praziquantel” **61** during this research with that of Cao *et al.*⁹⁵

¹ H-NMR Shift (ppm)		¹³ C-NMR shift (ppm)	
Cao <i>et al.</i> ⁹⁵	This work	Cao <i>et al.</i> ⁹⁵	This work
7.17 – 7.31 (m, 5H)	7.25 – 7.34 (m, 2 H)	178	178.03
	7.15 – 7.24 (m, 3 H)		
7.01 (br s, 0.5 H)	7.03 (br t, 0.5 H)	177.8	177.83
6.50 (br s, 0.5 H)	6.51 (br t, 0.5 H)	169.5	169.55
4.58 (dd, 0.5 H)	4.58 (t, 0.5 H)	169.2	169.28
4.39 (dd, 0.5 H)	4.39 (t, 0.5 H)	138.7	138.82
3.99 (s, 1 H)	3.99 (d, 2 H)	138.5	138.58
3.98 (s, 1 H)		128.72	128.76
3.55 (dd, 1 H)	3.52 (dtd, 2 H)	128.6	128.68
3.48 (dd, 1 H)		128.5	128.60
3.42 (t, 2 H)	3.42 (t, 2 H)	126.6	126.63
3.37 (s, 3 H)	3.37 (s, 3 H)	126.4	126.47
3.33 (s, 3 H)	3.33 (s, 3 H)	103.4	103.49
2.82 (t, 1 H)	2.80 (dt, 2 H)	102.6	102.73
2.78 (t, 1 H)		55.4	55.49
2.60 (tt, 0.5 H)	2.58 (tt, 0.5 H)	55.0	55.08
2.25 (tt, 0.5 H)	2.25 (tt, 0.5 H)	54.04	54.05
1.59 – 1.78 (m, 5 H)	1.54 – 1.83 (m, 5 H)	52.1	52.11
1.44 (m, 2 H)	1.38 – 1.51 (m, 2 H)	51.4	51.52
1.23 (m, 3 H)	1.15 – 1.35 (m, 3 H)	50.3	50.33
		41.0	41.06
		40.6	40.69
		40.4	40.50
		40.2	40.29
		35.6	35.64
		35.5	35.59
		29.3	29.39
		29.2	29.32
		25.7	25.75
		25.6	25.67
		25.5	25.56

Notably, the use of solid paraformaldehyde as the formaldehyde **53** source was not ideal for flow translation as it was consumed slowly over the 48-h period, and as a result appreciable amounts of insoluble paraformaldehyde were present throughout the duration of the reaction (validated procedure; Table 5, entry 1). As a result, we elected to screen the use of different formaldehyde **53** sources (Table 5, entries 2-4).

Table 5: Batch reactions performed for the preparation of the “pre-praziquantel” intermediate Ugi adduct **61**.

Entry	Mol ratio A:B:C:D ^a (formaldehyde 53 source)	A (mmol)	MeOH (mL)	Reaction time (h)	% Yield
1	1:1:1:1 (Paraformaldehyde)	19.1	20	48	89
2	1:1:1:1 (Paraformaldehyde)	4.76	5.0	48	98% impure (>99% conversion)
3	1:1:1:1 (Formalin 37%)	4.76	5.0	48	89% impure (>99% conversion)
4	1:1:1:1 (Gaseous formaldehyde)	4.76	5.0	48	75% impure (>99% conversion)
5	1:1:1:1 (Paraformaldehyde)	6.27	6.0	48	85
6	1:1.1:1.1:1.1 (Paraformaldehyde)	5.7	5.0	48	84
7	1:1:1:1 (Formalin 37%)	10.7	10.0	48	68
8	1:1.5:1.5:1.5 (Formalin 37%)	4.15	6.0	4	74

^a A - 2-isocyanoethylbenzene **96**, B - formaldehyde source **53**, C - aminoacetaldehyde dimethyl acetal **43** and D - cyclohexanecarboxylic acid **51**.

We employed the use of paraformaldehyde according to the original procedure for reference (Table 5, entry 2), the use of a 37% aqueous formalin solution (formaldehyde **53** source) (Table 5, entry 3; Figure 38), and the use of gaseous formaldehyde (prepared by cracking excess paraformaldehyde and bubbling the formaldehyde gas produced into a flask containing **43** and **51** in MeOH for 1.5 h, followed by the dropwise addition of **96** (Table 5, entry 4; Figure 39). Analysis of the ¹H-NMR and ¹³C-NMR spectra in all three cases displayed the characteristic peaks for the “pre-praziquantel” species **61** with no peaks distinctive of 2-isocyanoethylbenzene **96** being evident. The NMR data suggested a quantitative conversion for all three of these attempts. The isolated crude yield when using paraformaldehyde was

near quantitative, while the crude yields when using formalin and gaseous formaldehyde were 89 and 75%, respectively.



Figure 38: Reaction flasks for the original procedure reported in literature and the other flask labelled 2, for the formalin solution mixture.



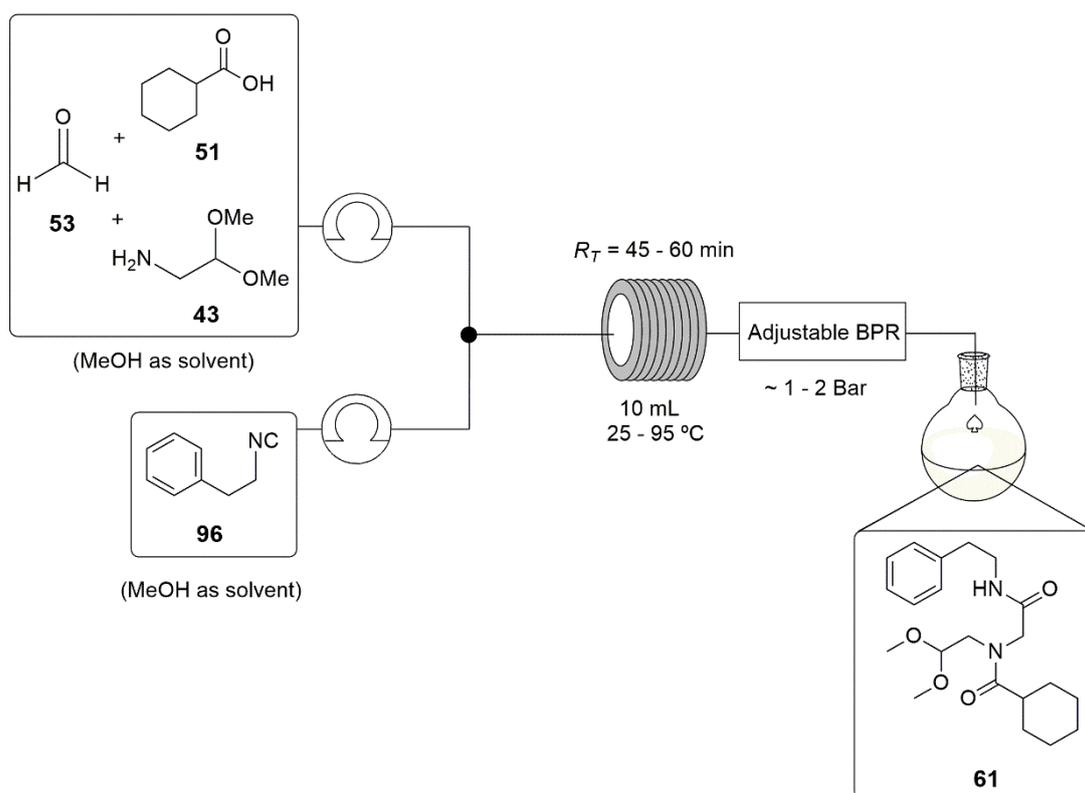
Figure 39: Reaction flask number 3 with the Nitrogen gas inlet top left, paraformaldehyde heated in oil bath and the flask is seen bottom right.

Notably, as was the case with entry 1 (Table 5), the 2-isocyanoethylbenzene **96** could not be observed in the crude $^1\text{H-NMR}$ spectra, however, the TLC again displayed the distinctive baseline spot, and the two spots with higher R_f values than the spot corresponding to **61**, suggesting possible by-product formation or decomposition. The main objective from these experiments was to observe if the reactions would afford the desired product **61** without the presence and/or formation of any precipitates. In the case where formalin or gaseous formaldehyde was used, no precipitates were observed suggesting that the approaches were suitable for flow translation. We next repeated the process using equimolar equivalents of all reagents with paraformaldehyde (Table 5, entry 5) and formalin (Table 5, entry 7) affording pure **61** in 85 and 68% yield, respectively. When using paraformaldehyde as the formaldehyde **53** source and increasing the mol ratio of all the reagents to 1.1 equivalents relative to **96** afforded a comparable purified yield of approximately 84% (Table 5, entry 6). Encouragingly, when using a 37% formalin solution and increasing the mol ratio of all reagents relative to **96** to 1.5 equivalents while decreasing the reaction time from 48 to 4 h (at which time complete consumption of 2-isocyanoethylbenzene **96** was observed) a marginal increase in yield from 68 to 74% was observed, and critically the solution remained homogeneous (Table 5, entry 8).

Despite the positive result when using gaseous formaldehyde (Table 5, entry 4), we did not proceed with its use as we had concerns that it may rapidly repolymerise and precipitate out of solution, in a flow system. We have formulated a possible engineering solution to utilise gaseous formaldehyde under flow conditions which will be discussed as part of the future work chapter.

3.1.4 Preparation of “pre-praziquantel” intermediate **61** under continuous flow conditions

We next decided to screen and optimise the Ugi reaction under flow conditions using 37% aqueous formalin as the source of formaldehyde **53**. The reaction screens were performed on a Vaportec R2 S+ flow system. The setup comprised of two peristaltic pumps connected to two stock solutions respectively. The first stock solution was comprised of 2-isocynoethylbenzene **96** in MeOH (1 or 2 M) and the second stock solution consisted of a mixture of 37% formalin, aminoacetaldehyde dimethyl acetal **43** and cyclohexanecarboxylic acid **51** (1 or 2 M). The pumps were plumbed to two separate selector valves using 30 cm, 1.0 mm internal diameter PFA tubes. An additional 50 cm, 1.0 mm internal diameter PFA tube was plumbed from each of these selector valves to a Y-piece mixer. The Y-piece mixer was then connected, again using a 32 cm, 1.0 mm internal diameter PFA tube to a 10 mL Vapourtec tubular coil reactor (PFA, 1.0 mm internal diameter) unit, and finally, the output of the coil reactor was passed through a pressure adjustable BPR prior to collection in a round bottom flask (Scheme 51).



Scheme 51: Flow set-up (Table 6, entries 1 – 9) for the preparation of “pre-praziquantel” **61**.

We initially performed a series of small-scale optimisations estimating the percentage conversion in relation to 2-isocynoethylbenzene **96** by $^1\text{H-NMR}$ (Table 6, entries 1 – 8). We first attempted the reaction under flow conditions at ambient temperature with a residence time of 60 min. The two 1 M reagent stocks were combined in an equimolar ratio and the input

volume for each was set to 1 mL affording a 47% conversion (Table 6, entry 1) which was encouraging considering the dramatic reduction in reaction time relative to that reported by Cao *et al.*⁹⁵ (48 h vs. 1 h). A further three reactions were then screened at analogous concentrations while increasing the temperature by 15 °C per reaction while retaining the residence time at 60 min (Table 6, entries 2-5) resulting in a best conversion of 79% at 70 °C (Table 6, entry 4). Increasing the temperature further resulted in a minor decrease in conversion to 72%, notably, the conversion was also accompanied by the appearance of a murky solution which gave us reason to believe that some partial decomposition of the product or starting material was occurring (Table 6, entry 5).

Table 6: Results obtained from the various reactions run under flow conditions.

Flow entry	Mol ratio	[A]	Volume Ratio A:B ^a	Volume input from each tube	Flow Rate (mL.min ⁻¹)	Residence Time (min)	Reactor Temperature (°C)	% Yield
1^b	1:1:1:1	1 M	1:1	1.00 mL	0.083	60	25	30 impure (47% conversion)
2^b	1:1:1:1	1 M	1:1	1.00 mL	0.083	60	40	77 impure (68% conversion)
3^b	1:1:1:1	1 M	1:1	1.00 mL	0.083	60	55	77 impure (66% conversion)
4^b	1:1:1:1	1 M	1:1	1.00 mL	0.083	60	70	84 impure (79% conversion)
5^b	1:1:1:1	1 M	1:1	1.00 mL	0.083	60	95	78 impure (72% conversion)
6^b	1:1:1:1	1 M	1:1	1.00 mL	0.111	45	55	32 impure (89% conversion)
7^b	1:1:1:1	1 M	1:1	1.00 mL	0.111	45	70	46 impure (93% conversion)
8^b	1:1:1:1	1 M	1:1	1.00 mL	0.111	45	95	60 impure (86% conversion)
9^c	1:1:1:1	1 M	1:1	3.00 mL	0.083	60	70	85
10^d	1:1:1:1	1 M	1:1	3.30 mL	0.083	60	70	87
11^e	1:1:1:1	1 M	1:1	2.70 mL	0.083	60	70	80
12^f	1:1:1:1	2 M	1:1	2.00 mL	0.083	60	70	70
13^g	1:1:1:1	2 M	1:1	1.50 mL	0.083	60	70	79

^a A - 2-isocyanoethylbenzene **96** in MeOH and B – formaldehyde source **53**, aminoacetaldehyde dimethyl acetal **43** and cyclohexanecarboxylic acid **51** in MeOH. ^b Flow reactions 1 to 8 were performed on approximately 376.5

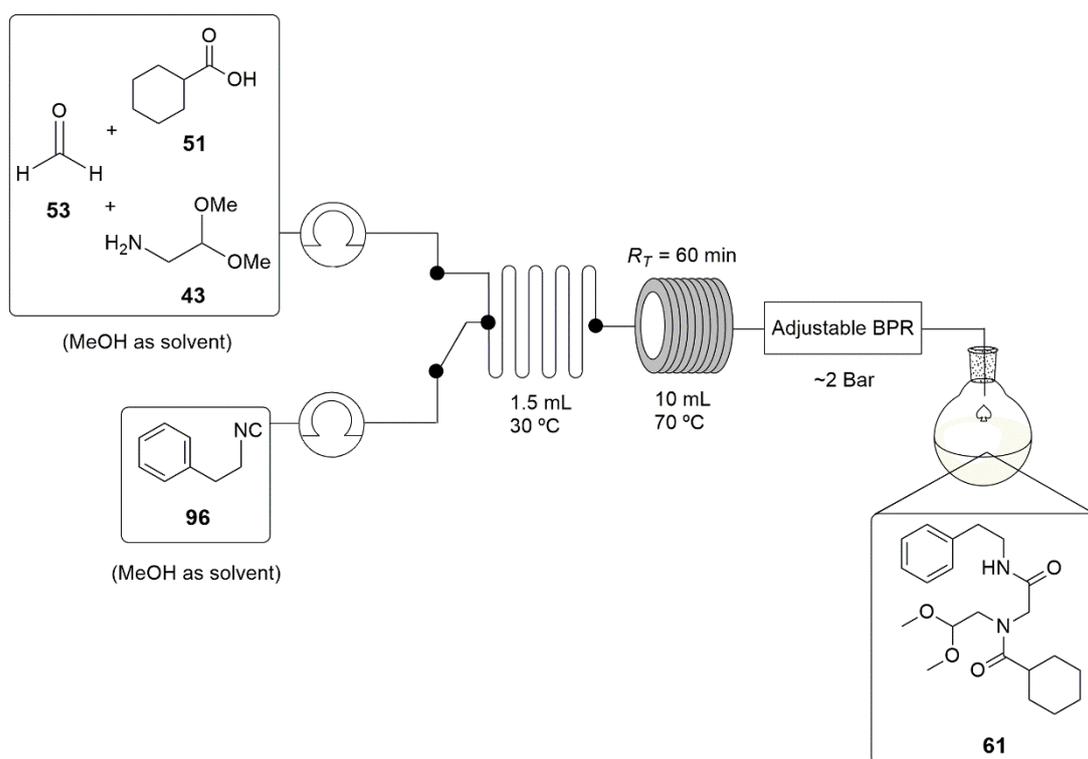
mg scales. ^c Flow reaction number 9 on approximately 1.13 g scale. ^d Flow reaction 10 on 1.25g scale. ^e Flow reaction 11 on 1.02 g scale. ^f Flow reaction 12 on 1.51 g scale. ^g Flow reaction 13 on 1.13 g scale.

Concurrently, we also ran the reaction with a residence time of 45 min at 55, 70 and 95 °C, respectively (Table 6, entry 6 – 8). Interestingly, although the percentage conversions (based on the isocyanide **96** consumption) exhibited an increase, the collected impure material (mass balance) was surprisingly lower when compared with the same conditions performed at 60 min residence time. This observation is not easily explained, however, we hypothesized that it may be attributed to leading or tailing dispersion effects. The reactor through built in software estimates the exit time for the reaction plug based upon the volume of the plug, the flow rate and a dispersion model, however, the model often underestimates the plug size and as a result the tailing ends are often not collected leading to discrepancies in the mass balance of the reaction.

To test this, we next elected to increase the scale of the reaction 3-fold (Table 6, entry 9). In doing so, the relative influence of the dispersion would be reduced with the reaction plug increasing in volume but the leading and tailing dispersion remains comparable to the smaller scale reactions. When run while maintaining a residence time of 60 min, an isolated yield (after off-line purification by column chromatography) of 85% was achieved, suggesting our hypothesis regarding the dispersion was correct. Notable, this is comparable in yield with the batch processes (using paraformaldehyde), however, a drastic decrease in reaction time from 48 h to 1 h was realised. Furthermore, when compared with the best batch result using 37% aqueous formalin solution, an increase in yield of approximately 11% was observed with an accompanied decrease in reaction time of approximately 3 h.

In an attempt to further increase yield, the Y-piece mixer was replaced with a 1.5 mL Vapourtec mixing chip in an effort to improve mixing prior to entry into the heated tubular reactor. The peristaltic pumps were now plumbed to a 1.5 mL mixing chip using 32 cm, 1.0 mm internal diameter PFA tubes. The mixing chip was then connected, again using a 32 cm, 1.0 mm internal diameter PFA tube to the 10 mL Vapourtec tubular coil reactor (PFA, 1.0 mm internal diameter) unit used previously, and finally, the output of the coil reactor was then passed through a pressure adjustable BPR prior to collection in a round bottom flask (Scheme 52).

When using this new setup and performing an analogous reaction to that detailed in Table 6, entry 9, albeit on a slightly larger scale (1.25 g) a comparable yield of 87% was achieved (Table 6, entry 10). When performing this similar reaction, instead with a minor decrease in the scale (1.02 g), the purified product (Figures 40 & 41) was afforded with a slight decrease in yield of approximately 80% (Table 6, entry 11).



Scheme 52: Flow set-up (Table 6, entries 10 – 13) for the preparation of “pre-praziquantel” **61**.



Figure 40: Column used for purification of the “pre-praziquantel” intermediate **61**. Test tubes used during collection of eluate for efficient separation.

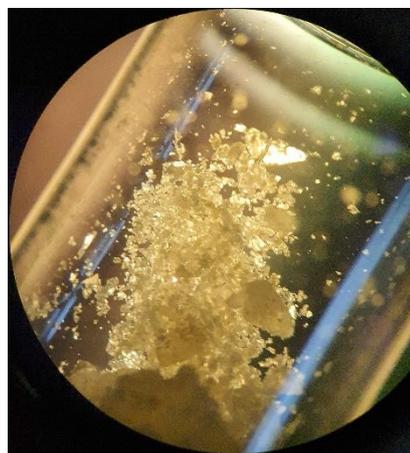


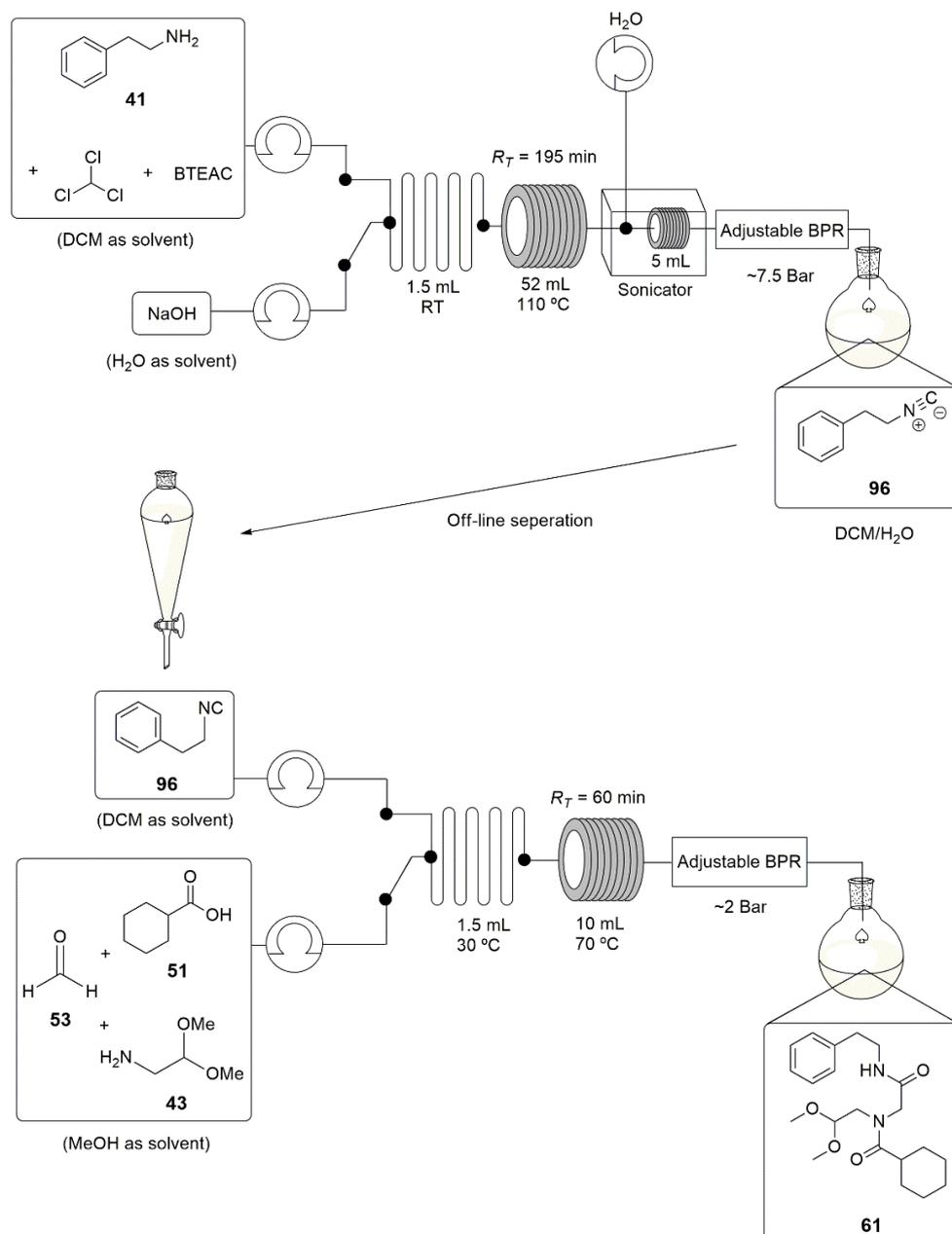
Figure 41: “Pre-praziquantel” intermediate **61** purified crystallised product under flow. As observed through microscope.

Finally, we decided to perform the reaction at a higher concentration (2 M) to reduce solvent usage, with the maximum yield obtained being 79% (Table 6, entry 13). The slight decrease in yield when compared with the best 1 M reaction attempt, was again suggested to be resultant of greater relative dispersion of a more concentrated reagent stream over a smaller volume. Critically, this may be less apparent on a larger scale, and we anticipate being able

to improve the performance at higher concentrations as the reaction is scaled-up. Due to time constraints, up-scaling was not attempted during this research project but will be discussed further in the future work section.

3.1.5 Trial telescoping of stages 1 and 2

In an attempt to avoid handling and isolating the noxious 2-isocyanatoethylbenzene **96**, an initial trial telescoping was attempted in order to assess whether, under flow conditions, it would be possible to link stages 1 and 2 together (Scheme 53).



Scheme 53: Telescoped flow reaction set-up with off-line separation. No purification performed for isocyanide **96** before introduction to the next step.

We employed the optimised set-up and conditions from stage 1 (with the additional 5 mL quench coil, 12.5 M sodium hydroxide and 1.56 M 2-phenethylamine **41**) to prepare ~ 1 g of

the crude isocyanide **96** material. We collected a steady-state fraction from midway through the run and manually separated the organic and aqueous phases, affording approximately 4.5 mL of the DCM/isocyanide **96** mixture which was estimated to have a concentration of ~ 1.25 M (~ 80% conversion). Thereafter, this material was reacted with cyclohexanecarboxylic acid **51**, formalin (formaldehyde **53** source) and aminoacetaldehyde dimethyl acetal **43** in excess (~ 1.7 M) in MeOH using the optimised flow setup for the Ugi reaction (Table 6, entry 10). The “pre-praziquantel” intermediate **61** was obtained with a purified yield of 69% for the second step (~ 55% over both steps with a space-time yield of 2.88 g.L⁻¹.h⁻¹) (Scheme 53). Due to time constraints, no further telescoped reactions were attempted, however based on this initial result, it suggests that telescoping these two first steps with appreciable yields is plausible and will form part of future work for this project. An overview of how the manual separation employed will be substituted with an in-line automated continuous flow separation and will be discussed in the future work chapter.

3.1.6 Concluding remarks

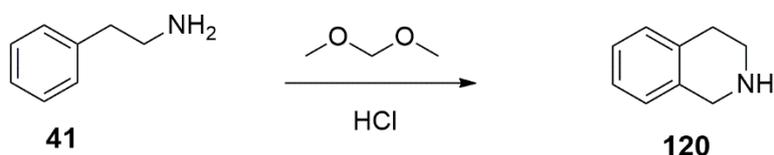
With the implementation of flow chemistry technologies for the preparation of the “pre-praziquantel” intermediate Ugi adduct **61**, we were able to achieve comparable yields (87% vs. 89%) to that achieved in-house under batch conditions when using paraformaldehyde (formaldehyde **53** source), with a dramatic decrease in the reaction time of 47 h. This developed stand-alone process amounts to a space-time yield of 107 g.L⁻¹.h⁻¹. It is important to note that although the ¹H-NMR spectra, obtained prior to purification by column chromatography purification under the optimised flow conditions, exhibited an arguably clean spectrum, the TLC analysis consistently suggested that additional purification was required for both the batch and flow-based approach. Going forward, more experimentation with regards to the purity of this adduct should be performed using more sensitive analytical techniques (such as HPLC) to confirm whether this column purification is absolutely necessary or alternatively, would it be more beneficial to use the crude product directly in the final step thereby limiting material loss that occurs during the purification step.

CHAPTER IV

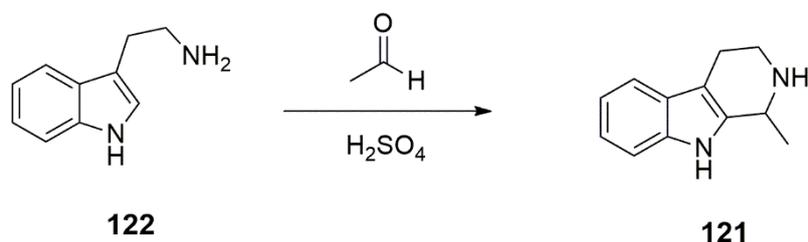
4.1 Stage 3 – Pictet-Spengler cyclisation to afford praziquantel 1

4.1.1 Brief history of the Pictet-Spengler reaction

The final step for the conversion of the “pre-praziquantel” intermediate **61** formed in step 2, to the desired praziquantel **1** makes use of an intramolecular Pictet-Spengler type reaction with the addition of MSA under solvent-free conditions. The chemistry behind the Pictet-Spengler reaction was first disclosed by Amé Pictet and Theodor Spengler in 1911, whereby 2-phenethylamine **41** was treated with dimethoxymethane in the presence of hydrochloric acid affording 1,2,3,4-tetrahydroisoquinoline **120** (Scheme 54).^{145, 146} Soon after, the newly developed “Pictet-Spengler” reaction was implemented for a general preparation of tetrahydroisoquinoline derivatives and by 1928, G. Tatsui¹⁴⁷ implemented this type of chemistry in the preparation of 1-methyl-1,2,3,4-tetrahydro- β -carboline **121** with the indole base, tryptamine **122** and acetaldehyde in the presence of sulphuric acid (Scheme 55). Both the tetrahydroisoquinoline and tetrahydro carboline skeletal structures form part of thousands of indole and isoquinoline alkaloids found in nature.¹⁴⁸ In addition, these classes of alkaloids have been shown to have appreciable physiological and therapeutic effects,^{148, 149} and consequently, the synthesis of these alkaloids by this approach has proven to be a powerful tool for synthetic chemists.



Scheme 54: First Pictet-Spengler reaction reported by Pictet and Spengler.¹⁴⁶



*Scheme 55: Modified Pictet-Spengler reaction utilising tryptamine **122** reported by Tatsui.¹⁴⁷*

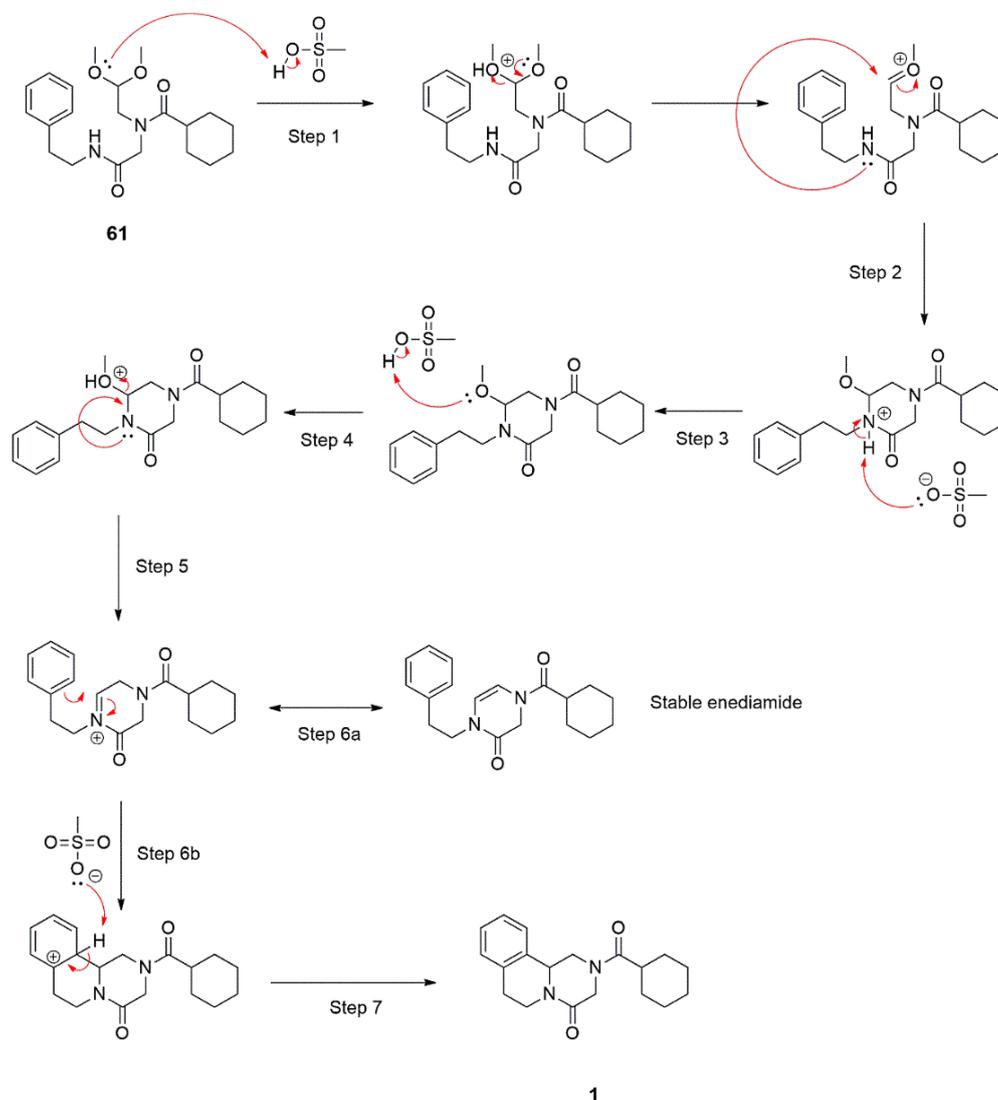
In general, the Pictet-Spengler reaction allows for the condensation of a β -arylethylamine with an aldehyde (including masked aldehydes such as acetals, enol ethers, hemiaminals, aminonitrils and more),¹⁴⁸ or a ketone (including structural variances) followed by a subsequent ring closure. This reaction is usually performed in a protic solvent with the addition

of an acid catalyst,¹⁴⁵ however, a review article by Cox *et al.*¹⁴⁵ reports the use of several tryptophan methyl ester derivatives in place of tryptamine, without the need for an acid catalyst, all within an aprotic solvent. Surprisingly, when comparing this same chemistry performed within an aqueous acidic medium, the aprotic solvent system afforded increased yields.¹⁴⁵

The Pictet-Spengler reaction has been extensively utilised for a wide variety of alkaloids and as such has led to a large number of applications in the field of synthetic organic chemistry, to name a few, the synthesis of pyrimidoquinolines,¹⁵⁰ formation of triazabenzazulene heterocycles,¹⁵¹ syntheses of 3-phosphorylated β -carboline derivatives,¹⁵² and the generation of 4-substituted 1*H*-2,3-benzoxazines.¹⁵³ Enantioselective Pictet-Spengler methods for the synthesis of tetrahydroisoquinoline and tetrahydrocarboline scaffolds has gained appreciable attention over the last 3 – 4 decades which arose from the initial discovery of a stereoselective condensation promoted by enzyme catalysis in 1977. The class of enzymes allowing for this condensation are typically referred to as Pictet-Spenglerases, with the first being detected in nature, known as strictosidine synthase.^{148, 154-156} Furthermore, in 1985 Czarnocki *et al.*¹⁵⁷ reported a highly enantioselective procedure for the preparation of simple isoquinoline alkaloids through the condensation of dopamine hydrochloride with (*R*)-(+)-glyceraldehyde. This was achieved in a refluxing MeOH solution affording a mixture of diastereomers in a 93% yield and a 9:1 ratio. It is important to note that this method allows for elaboration and variance in the synthesis of increasingly complex alkaloids with the same precursor scaffold.

The general mechanistic approach for the formation of tetrahydroisoquinoline derivatives (praziquantel **1** being one) occurring via an acid catalysed Pictet-Spengler type reaction has been proposed to proceed via an initial condensation of the respective aldehyde with a β -arylethylamine, generating an imine which is activated by treatment with an accompanying Bronsted acid.¹⁵¹ This activated imine is then subjected to a 6-endo-trig cyclisation step with an adjacent aryl ring, while compromising the aromaticity. A final deprotonation promotes restoration of the aromatic ring affording the desired tetrahydroisoquinoline derivative.¹⁵¹ The conversion of “pre-praziquantel” **61** to praziquantel **1** involves a modified mechanism starting from an acetal-disguised aldehyde instead of an aldehyde. The added MSA initiates the partial deprotection (exposing the carbonyl type carbon atom) of this acetal (Scheme 56; Step 1), followed by an intramolecular nucleophilic attack from the secondary amine (from the amide closest to aromatic ring) (Step 2). This positively charged amide nitrogen is then deprotonated, promoting neutrality of the newly formed ring (Step 3) followed by a subsequent protonation step of the methoxy group (Step 4). This in turn allows for the expulsion of MeOH as a leaving group through the transfer of the lone pair of electrons on the closest neighbouring nitrogen atom (Step 5) forming a double bond and positively charged nitrogen atom (on the amide).

From this stage, the intermediate is either i) capable of resonating to a stable enediamide (Step 6a) or ii) undergoes a ring closure with the aromatic ring while compromising the integrity of the aromaticity (Step 6b). Lastly, the aromaticity is restored by a subsequent deprotonation step generating praziquantel **1** (Step 7).

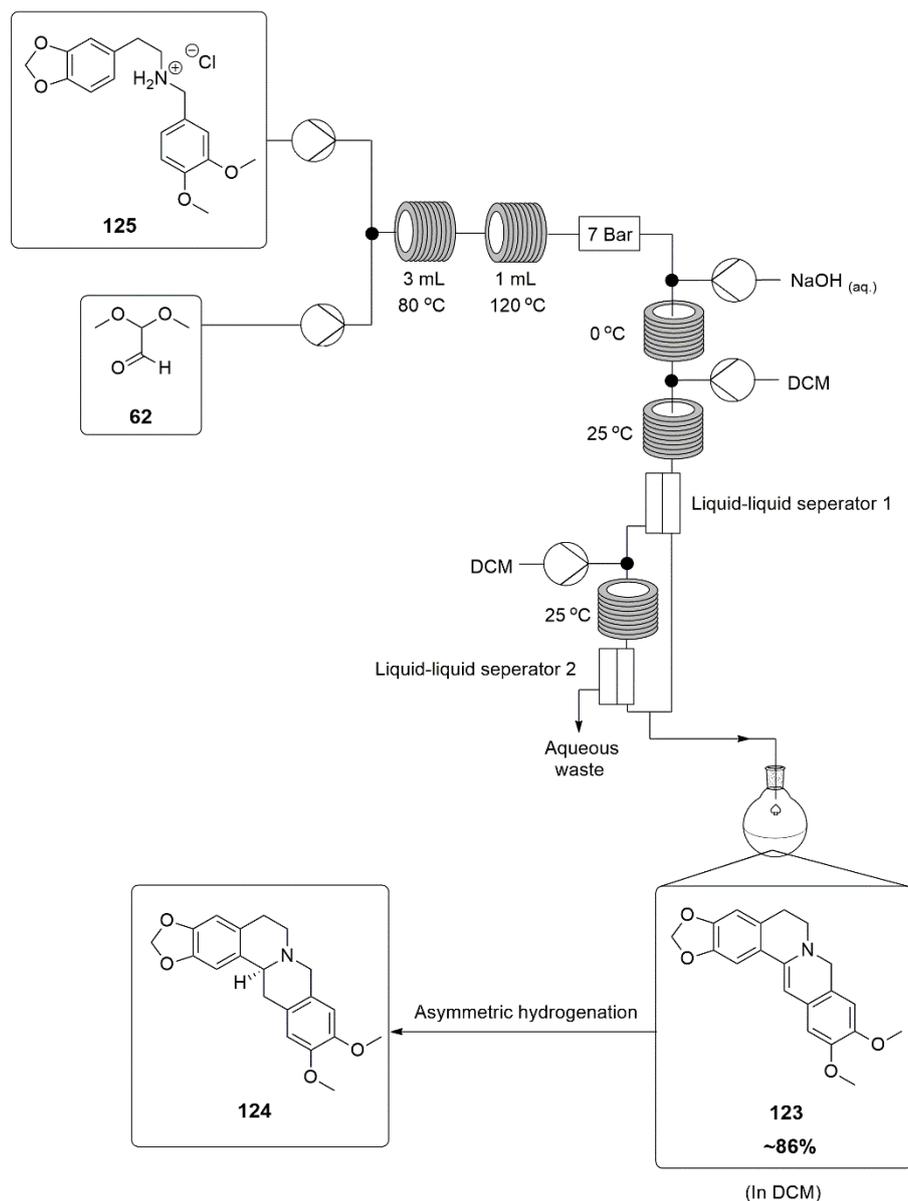


Scheme 56: Mechanistic approach for the conversion of "pre-praziquantel" **61** to praziquantel **1**.

4.1.2 Pictet-Spengler reactions previously reported under flow conditions

The Pictet-Spengler cyclisation, to the best of our knowledge has only been reported once under flow conditions. A recent article reported by Li *et al.*¹⁵⁸ makes use of a continuous Pictet-Spengler/Pomeranz-Fritsch cascade cyclisation in order to prepare dihydroisocanadine **123**, which partakes in a subsequent asymmetric hydrogenation for the generation of (-)-isocanadine **124** (Scheme 57). The optimised conditions for the preparation of **123** were achieved by allowing the amine hydrochloride salt **125** (0.2 M in 98% formic acid) to react with 2,2-dimethoxyacetaldehyde **62** (1.0 M in 98% formic acid) in two tubular coil reactors (first 3

mL coil reactor set at 80 °C with a residence time of 7.5 min and second 1 mL coil reactor set at 120 °C with a residence time of 2.5 min). The mixture was then allowed to undergo an in-line basification, extraction, and a subsequent separation (through two liquid-liquid separation units) affording **123** in a yield of 86%. Without an additional purification step, this mixture was introduced into a final stereoselective iridium-catalysed hydrogenation step for the generation of the desired (-)-isocanadine **124**.¹⁵⁸ The authors utilised this set-up for the preparation of various tetrahydroprotoberberine alkaloids, including (-)-nandinine, (-)-stylopine and (-)-tetrahydropseudocoptisine.



Scheme 57: Preparation of dihydroisocanadine **123** utilising a continuous Pictet-Spengler/Pomeranz-Fritsch cascade cyclisation step reported by Li *et al.*¹⁵⁸

4.1.3 Preparation of praziquantel **1** under batch conditions

The conversion of the “pre-praziquantel” intermediate **61** to the final praziquantel product **1**, as reported by Cao *et al.*⁹⁵, makes use of MSA in excess without any additional solvent. The

approach was employed in an attempt to render this step more environmentally friendly without requiring the commonly employed chlorinated solvents like DCE and DCM, in addition, MSA is also considered a green acid.¹⁵⁹ The initial validation of the Cao *et al.*⁹⁵ procedure was attempted, involving the addition of “pre-praziquantel” **61** (~ 0.4 g product scale) to MSA (~ 20 mol equivalents) in a portionwise manner at 0 °C. Thereafter, the reaction mixtures temperature was increased to 70 °C and matured for 6 h during which time the yellow mixture turned progressively darker in colour. Following the work-up, a thick crude yellow residue was obtained and analysed, from which ¹H-NMR spectroscopy confirmed that trace amounts of praziquantel were formed (Table 7, entry 1). Unfortunately, we were unable to crystallise and isolate the material from this crude mixture leading to us repeating the process on a larger scale, in hopes that it would facilitate in crystallisation. Encouragingly, at the increased scale of ~ 1.7 g, the same approach afforded a purified yield of 23% (Table 7, entry 2). Nonetheless, when compared with the literature (65% recrystallised), this was disappointingly low.

Table 7: Batch reactions initially performed for the final conversion to praziquantel 1.

Batch Entry	Acid source (mol)	Solvent	Pre-Praziquantel 61 (mol)	Reaction time (h)	Temperature (°C)	% Yield
1	MSA (0.0266)	N/A	(0.0013)	6	70	Trace
2	MSA (0.1064)	N/A	(0.0053)	6	70	23 (recrystallised)
3	MSA (0.0266)	DCM	(0.0013)/[0.26]	24	Reflux	No product
4	MSA (0.0266)	DCM	(0.00133)/[0.53]	48	Reflux	Trace
5	MSA (0.0539)	DCM	(0.00318)/[0.64]	72	Reflux	Trace (suspected decomposition)
6	MSA (0.0266)/[5.32]	EtOH	(0.0013)/[0.26]	24	70	No product
7	Para-toluene sulphonic acid (0.00293)	0.4 mL MeOH	(0.000146)	6	Reflux	No product
8	Amberlyst 15 (0.00537)	2 mL MeOH	(0.00132)	6	Reflux	No product
9	Para-toluene sulphonic acid (0.0320)	DCM	(0.00166)/[0.28]	72	Reflux	No product
10	Amberlyst 15 (0.0323)	DCM	(0.00163)/[0.11]	72	Reflux	No product

To correlate the ^1H - and ^{13}C -NMR signals with that of the structure of praziquantel **1** (Figure 42), we performed additional HSQC, HMBC and COSY analyses (depicted in Appendix C – 1) to get an in depth understanding of the intramolecular coupling that is taking place and in turn, assist with the structural analysis.

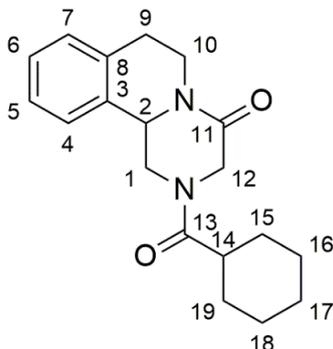


Figure 42: Praziquantel **1** structure with labels included for clarity in discussion.

- I. Based on the ^1H -NMR analysis, we could confidently identify the aromatic protons and the cyclohexyl protons based on their relative chemical shift regions and integration values. However, correlating each C and H from these groups was achieved with the additional spectral data.
- II. For the aromatic protons, the multiplet is unresolved and consequently, all four of the integrated protons, located between 7.14 – 7.35 ppm correlate to the protons on C-4 – C-7 (Figure 42). Using the HSQC spectrum, we correlated the respective C atoms. C-4 (129.22 ppm), C-5 (126.90 ppm), C-6 (125.40 ppm), and C-7 (127.37 ppm). The remaining quaternary C-3 and C-8 atoms were identified by being the only peaks left in the aromatic region of the ^{13}C -NMR as well as no coupled proton from the HSQC. These signals are 132.69 (C-3) and 134.65 ppm (C-8).
- III. The cyclohexyl protons were classified in a similar fashion where the multiplet, integrating for five protons, located between 1.65 – 1.95 ppm correspond to the protons on C-15, C-19 and one proton from C-18. The multiplet between 1.44 – 1.64 ppm, integrating for two protons correspond to the remaining proton on C-18 and one proton for C-16. As for the remaining multiplet between 1.19 – 1.42 ppm corresponds with the remaining proton on C-16 and both protons on C-17. Lastly, the C atoms were correlated with the HSQC spectrum to conclude C-15 & C-19 (28.92 ppm), C-16 & C-18 (28.64 ppm), and C-17 (25.63 ppm).
- IV. The C-H (C-14) proton on the cyclohexyl moiety, was easily identified since it appeared as a red signal in the HSQC, located in an expected region where a carbonyl group usually deshields a C-H, integrated for one proton each and presented as a

triplet of triplets (expected since adjacent to two CH₂ groups and a quaternary carbonyl; 2.47 ppm). This was correlated (HSQC) with the C atom peak at 40.71 ppm.

- V. Furthermore, the quaternary carbonyl C-13 atom was concluded to be located at 174.69 ppm, due to the coupling observed in the HMBC with the C-H from the cyclohexyl moiety (C-14). The remaining carbonyl C-11 atom was subsequently concluded to be at 164.33 ppm.
- VI. The proton on C-2 was identified by the remaining red signal observed in the HSQC spectrum. The proton forms part of the multiplet, located at 4.81 ppm. Using the HSQC spectrum, C-2 was correlated and observed at 54.87 ppm.
- VII. The remaining protons on C-1, C-10 and C-12 were the most challenging to confidently identify based on the HSQC analysis. Consequently, when observing the COSY spectrum, it was evident that the two doublets located at 4.08 and 4.47 ppm, each integrating for one proton, were not displaying any prominent coupling to adjacent protons. This was expected since C-12 is situated between a carbonyl (C end of the one amide) and an amide functional group, which gives further evidence of the large deshielding effects. Furthermore, this was confirmed with additional evidence by the HMBC displaying proton coupling to both carbonyl C atoms (C-1 and C-10 are too far away to couple with both carbonyl C atoms). HSQC was used to correlate these protons with the C-12 atom at 48.94 ppm.
- VIII. The protons on C-1 were identified from the HMBC since they displayed coupling with the quaternary carbonyl C-13 as well as C-12. Additional confirmation was the multiplicity splitting pattern being a doublet of doublets due to the coupled neighbouring proton on C-1 as well as the adjacent C-H proton on C-2. Therefore, the protons on C-1 were confirmed at 5.17 ppm. Using the HSQC spectrum, C-1 was observed at 45.06 ppm.
- IX. As such, the remaining protons on C-10 were observed as a split signal across the two multiplets at 4.81 ppm and 2.75 – 3.04 ppm, respectively. HSQC was used for correlating with the C-10 atom at 39.01 ppm. In addition, the COSY spectrum confirming coupling across these two multiplets as well as the HMBC confirming coupling present with the quaternary aromatic C-3 and C-8 atoms.
- X. The remaining protons on C-9 were identified due to coupling with the quaternary aromatic C atoms C-3 and C-8, evident in the HMBC spectrum. Consequently, these protons form part of the multiplet between 2.75 – 3.04 ppm, making up for the final two protons of the integration of four protons. Using the HSQC spectrum, this C atom was observed at 29.16 ppm.

When observing whether the reaction was successful, there were distinctive chemical shift regions in the ^1H -NMR spectrum when compared with “pre-praziquantel” **61**. The cyclisation was supported by:

- i) The integration of the aromatic protons (7.14 – 7.35 ppm) changing from five to four protons (C-4 – C-7; see Figure 42),
- ii) The disappearance of the two broad triplets at 7.03 ppm and 6.51 ppm, corresponding to the secondary amide of the “pre-praziquantel” **61** (no proton on C-10 – N – C-11),
- iii) The appearance of a doublet of doublets at 5.17 ppm, integrating for one proton, corresponding to one of the non-equivalent protons on C-1 (shifted from 3.42 ppm in “pre-praziquantel” **61**),
- iv) The appearance of a multiplet at 4.81 ppm, integrating for two protons, one corresponding to the proton on C-2 (Shifted from the two triplets at 4.39 and 4.58 ppm in “pre-praziquantel” **61**) and the other corresponding to one of the non-equivalent protons on C-12,
- v) The appearance of two doublets at 4.08 and 4.47 ppm, each integrating for one proton, corresponding to the two non-equivalent protons on C-12.
- vi) The disappearance of the large doublet at 3.99 ppm observed in the “pre-praziquantel” **61** spectra (shifted to 4.08 and 4.47 ppm) owing to the protons on C-12.
- vii) The disappearance of the two major singlets at 3.37 and 3.33 ppm, which correspond to the methoxy groups in the “pre-praziquantel” **61**.
- viii) The shifting of the two protons on C-10 was observed at approximately 2.91 (part of multiplet) and 4.81 ppm (part of multiplet) from 3.52 ppm in the “pre-praziquantel” **61** and the shifting of the two protons on C-1 was observed at 5.17 and 2.82 ppm from a triplet at 3.42 ppm.

When observing the ^{13}C -NMR and Dept-135 spectra of these two compounds (depicted in Appendix B – 1 (**61**) and C – 1 (**1**)), there were distinctive chemical shift regions allowing confirmation of the cyclisation which was supported by:

- i) The two carbonyl quaternary C atoms have shifted slightly upfield to 174.69 (Figure 42; C-15) and 164.33 ppm (C-10), owing to increased shielding effects observed by these C atoms when compared with “pre-praziquantel” **61**,
- ii) The new quaternary C-3 is now suppressed in the Dept-135 spectrum and exhibits a downfield shift when compared with the same C atom present in **61**,
- iii) The disappearance of the two methoxy C atoms, in both the Dept-135 and ^{13}C -NMR at 55.49 and 55.08 ppm observed in the spectra of **61**,

- iv) The extreme upfield shift experienced by C-2 (same C atom from CH group between both methoxy O atoms split at 103.49 and 102.73 ppm in **61**) to 54.87 ppm due to the loss of these deshielding O atoms from the expelled methoxy groups,
- v) There are now only lone peaks observed for each C atom (except for those that are equivalent in the cyclohexyl moiety) in the spectrum of praziquantel **1** when compared with that of **61** which exhibited a split in majority of C atom's signal giving rise to more peaks than C atoms.

The observed peaks in the proton and carbon spectra correspond well with those obtained from a commercial pharmaceutical standard of **1** and those reported by Cao *et al.* (Table 8).⁹⁵ Figures 43 and 44 display the stacked ¹H and ¹³C-NMR spectra, respectively, of the pharmaceutical standard and that of praziquantel **1** synthesized during this research study. It is important to note that the integral traces are slightly off, with some multiplets integrating for more protons than expected in the praziquantel **1** structure. This is due to the apparent S-(+)-isomer with peaks overlapping as well as decided peaks that are not a part of the racemic structure integral trace.¹⁶⁰ Notable, this is observed in the spectra recorded for that of **1** synthesised during this research study, that reported in the supplementary information of Cao *et al.*⁹⁵ as well as the obtained pharmaceutical standard. For clarity, the reported peak ranges in table 8 are those correlating with the reported values by Cao *et al.*⁹⁵

Table 8: A comparison of the ¹H and ¹³C-NMR chemical shifts obtained during this study with that of Cao *et al.*⁹⁵ and the pharmaceutical standard of praziquantel **1**.

¹ H-NMR δ (ppm) (Cao <i>et al.</i> ⁹⁵)	¹ H-NMR δ (ppm) (This study)	¹ H-NMR δ (ppm) (Pharmaceutical standard)	¹³ C-NMR δ (ppm) (Cao <i>et al.</i> ⁹⁵)	¹³ C-NMR δ (ppm) (This study)	¹³ C-NMR δ (ppm) (Pharmaceutical standard)
7.18 – 7.29 (m, 4 H)	7.14 – 7.35 (m, 4 H)	7.15 – 7.35 (m, 4H)	174.7	174.69	174.70
5.17 (dd, 1 H)	5.17 (dd, 1 H)	5.16 (dd, 1 H)	164.3	164.33	164.33
4.81 (m, 2 H)	4.81 (m, 2 H)	4.81 (m, 2 H)	134.7	134.65	134.66
4.47 (d, 1 H)	4.47 (d, 1 H)	4.47 (d, 1 H)	132.7	132.69	132.70
4.08 (d, 1 H)	4.08 (d, 1 H)	4.08 (d, 1 H)	129.2	129.22	129.23
2.78 – 2.99 (m, 4 H)	2.75 – 3.04 (m, 4 H)	2.74 – 3.04 (m, 4 H)	127.4	127.37	127.37

2.47 (tt, 1 H)	2.47 (tt, 1 H)	2.47 (tt, 1 H)	126.9	126.90	126.91
1.72 – 1.83 (m, 5 H)	1.65 – 1.95 (m, 5 H)	1.65 – 1.95 (m, 5 H)	125.4	125.40	125.41
1.53 – 1.57 (m, 2 H)	1.44 – 1.64 (m, 2 H)	1.44 – 1.64 (m, 2 H)	54.9	54.87	54.88
1.27 – 1.28 (m, 3 H)	1.19 – 1.42 (m, 3 H)	1.19 – 1.42 (m, 3 H)	48.9	48.94	48.95
			45.1	45.06	45.08
			40.7	40.71	40.72
			39.0	39.01	39.02
			29.2	29.16	29.16
			28.9	28.92	28.93
			28.6	28.64	28.65
			25.7	25.63	25.64



Figure 43: Praziquantel **1** ¹H-NMR obtained for the pharmaceutical standard (top spectrum) juxtaposed with that synthesised during this study (bottom spectrum).

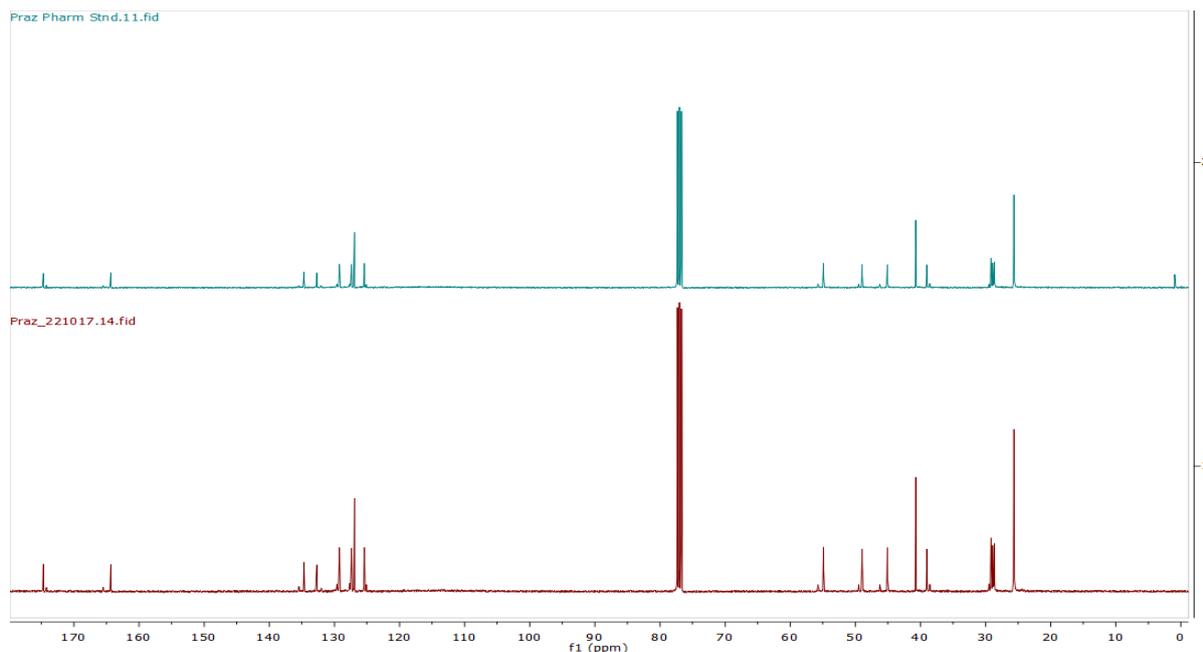


Figure 44: Praziquantel **1** ^{13}C -NMR obtained for the pharmaceutical standard (top spectrum) juxtaposed with that synthesised during this study (bottom spectrum).

In the infra-red spectrum (depicted in Appendix C – 3), the most appreciable distinction is the disappearance of the N-H stretch at 3283 cm^{-1} and the amide II band at 1553 cm^{-1} due to the reaction of this secondary amide, now converted into a tertiary amide functional group in praziquantel **1**. Unfortunately, the disappearance of the C-O ester stretch at 1189 cm^{-1} in the “pre-praziquantel” **61** spectrum cannot be clearly discerned as the same region is “busy” in the praziquantel **1** spectrum. That being said, it is important to note that there is no defined strong peak in this region as there was in the “pre-praziquantel” **61** spectrum.

Lastly, when obtaining a mass spectrum of praziquantel **1**, the $[\text{M}+\text{H}]^+$ value was found at 313.1891 m/z , which corresponds well with the pharmaceutical standard $[\text{M}+\text{H}]^+$ found at 313.1906 m/z as well as the calculated value expected at 313.1916 m/z (depicted in Appendix C – 5).

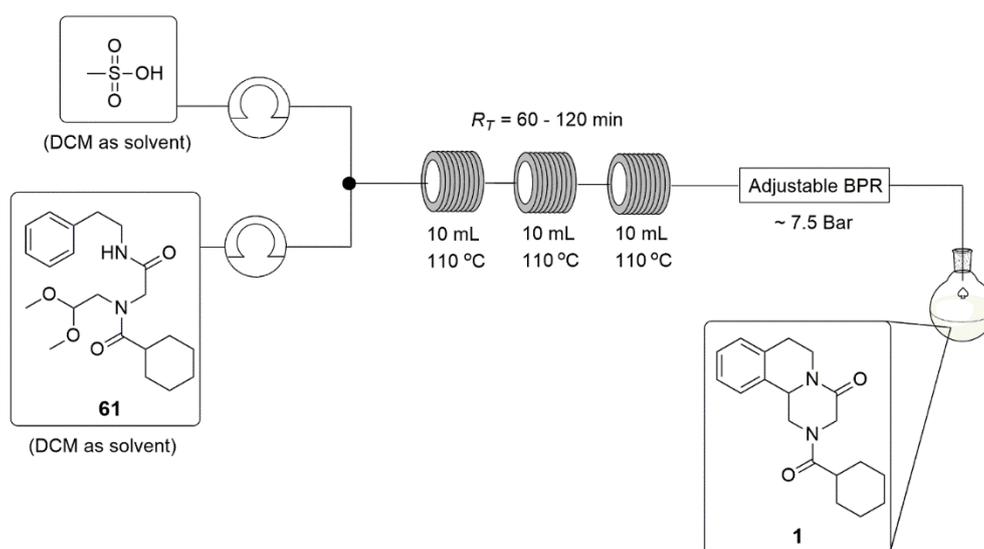
4.1.4 Continuation of praziquantel **1** synthesis under batch conditions

Following these disappointing results, it was decided to attempt this reaction while in a DCM medium for a period of 24, 48 and 72 h, respectively (Table 7, entries 3-5). We elected to screen longer reaction times in DCM, as Kim *et al.* previously reported the use of MSA in DCM under reflux over 48 h.¹⁰³ Unfortunately, no product formation was observed after 24 h (Table 7, entry 3), after 48 h trace amounts of praziquantel **1** were observed (Table 7, entry 4) and after 72 h trace product was again observed, but the NMR spectrum suggested that significant decomposition was occurring (Table 7, entry 5). We next attempted the reaction in EtOH over 24 h, but again, no product formation was noted (Table 7, entry 6). In hindsight, the failure in

this instance is possibly the result of the unwanted formation of the ethyl methanesulfonate ester.¹⁶¹ As a last resort, *p*-toluene sulfonic acid and amberlyst 15 were screened as potential acid sources when performed in MeOH, unfortunately, once again no product formation was noted (Table 7, entries 7 – 8). Switching the solvent to DCM with prolonged heating also afforded no product formation (Table 7, entries 9 – 10).

4.1.5 Praziquantel **1** synthesis under flow conditions

When performed under batch conditions, it was clear that the desired product formation was favoured, albeit in lower than desired yields, when using MSA under solvent-free conditions. Critically, using MSA as a solvent in a flow system is problematic as it has a relatively low melting point of 16 °C, being a solid at 0 °C. This could be overcome by introducing it at ambient temperature, however the “pre-praziquantel” **61** has poor solubility within this acid at ambient temperature and would require additional heat prior to entry into the flow apparatus leading to some reaction occurring prior to being introduced into the flow system. Furthermore, it would be very costly as a pushing solvent and potentially lead to corrosion of the flow components. As such, and despite the negative results under batch conditions, we decided to screen several reactions under flow conditions using DCM as a solvent. The initial flow setup comprised of two peristaltic pumps connected to the two respective stock solutions (MSA in DCM, and “pre-praziquantel” **61** in DCM). The pumps were plumbed to a standard Y-piece mixer using 32 cm long, 1.0 mm internal diameter, PFA tubing. The Y-piece mixer was then connected, again using a 32 cm, 1.0 mm internal diameter, PFA tube to three 10 mL Vapourtec tubular coil reactor (PFA, 1.0 mm internal diameter) units in series, and finally, the output of the coil reactor was passed through a pressure adjustable BPR (~ 7.5 bar) prior to collection in a round bottom flask (Scheme 58).



Scheme 58: Flow set-up for the flow entries 1 – 2 (Table 9).

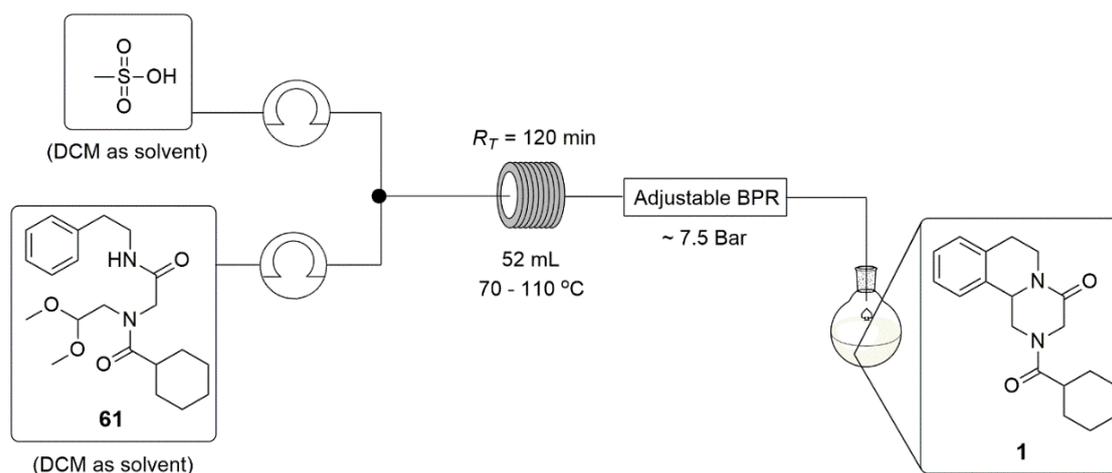
The first screen was attempted using a MSA stock solution (in DCM) accompanied by a “pre-praziquantel” **61** stock solution in DCM. A residence time of 1 h was selected at a temperature of 110 °C (the maximum allowable temperature for DCM while using a peristaltic pump system). Unfortunately, no product formation was noted (Table 9, entry 1). Doubling the residence time to 2 h, again didn’t afford any product formation (Table 9, entry 2).

Table 9: Flow reactions performed during this research for the attempted preparation of praziquantel **1**.

Flow Entry Number	[MSA] Stock A	Solvent	[Pre-praziquantel 61] Stock B	Solvent	Volume intro A:B	Reaction time (h)	Flow rate A:B	% Yield
1	[10.7]	DCM	[0.53]	DCM	3.00 : 3.00	1 (110 °C)	0.250: 0.250	No product
2	[10.0]	DCM	[0.50]	DCM	3.25 : 3.25	2 (110 °C)	0.125: 0.125	No product
3	[10.0]	DCM	[0.50]	DCM	3.25 : 3.25	2 (110 °C)	0.217: 0.217	No product (Possible decomp.)
4	[10.0]	DCM	[0.50]	DCM	3.25 : 3.25	2 (80 °C)	0.217: 0.217	No product
5	[10.6]	Same Stock	[0.53]	DCM	3.05	2 (70°C)	0.217: 0.217	No product

In a further attempt to promote product formation, the set-up was modified, replacing the three 10 mL coil reactors with a 52 mL, 1.5 mm internal diameter coil (Scheme 59). This allowed us to maintain the same residence time (2 h) but with an increased flow rate (combined flow rate of 0.434 vs. 0.250 ml.min⁻¹) which in turn afforded improved mixing efficiency. Unfortunately, once again no product formation was noted, and spectral analysis suggested possible decomposition (Table 9, entry 3). Consequently, we decided to decrease the temperature to 80 °C but without any luck (Table 9, entry 4).

Finally, we decided to introduce the “pre-praziquantel” **61** and MSA dissolved in one stock solution (almost solventless with a minimum amount of DCM of < 0.5 mL in a 5 mL volumetric flask) while using DCM as the pushing solvent to pass the reaction matrix through the system. Yet again, no product formation was noted, however, peaks were identified in the crude ¹H-NMR spectrum suggesting that the acetal deprotection was successful, however, no praziquantel **1** was present (Table 9, entry 5).



Scheme 59: Flow set-up for the flow entries 3 – 5. Where entry number 5 was achieved using a single stock solution.

We thereafter revisited the Cao *et al.*⁹⁵ article and noted that the authors made use of magnesium sulfate as an additive, however, this was not reported in the experimental descriptions and no quantities were reported. As a result, we re-validated the approach under batch conditions using MSA in excess (~ 20 mol equivalents) and magnesium sulfate (2.5 mol equivalents) as an additive under solvent-free conditions. To our delight, praziquantel **1** was isolated in 60% yield after recrystallisation (Table 10, entry 1). We then decided to repeat this experiment while increasing the equivalents of magnesium sulfate (~ 3.6 mol equivalents) in hopes of improving the yield, however, under these conditions a comparable yield of 58% was achieved (Table 10, entry 2).

Table 10: Batch reactions performed with the sulfate additive.

Batch entry	“Pre-praziquantel” 61 (mol)	Solvent	Acid Source (~20 mol eq.)	Na ₂ SO ₄ / MgSO ₄ (mol)	Reaction time (h)	% Yield
1	0.0048	N/A	MSA	MgSO ₄ (0.012)	6 (70 °C)	60
2	0.0033	N/A	MSA	MgSO ₄ (0.012)	6 (70 °C)	58
3	0.0033	N/A	MSA	Na ₂ SO ₄ (0.0082)	6 (70 °C)	61

In a final attempt to observe if a different alkali metal sulfate would result in better yields, sodium sulfate was used in place of magnesium sulfate (~ 2.5 mol equivalents) which again, afforded a comparable yield of 61% with a purity of ~ 81% determined via UPLC analysis (Table 10, entry 3). It is important to note that this final run was recrystallised using diethyl ether as opposed to an ethyl acetate/hexane mixture, affording praziquantel as a clean white

solid after only 1 – 2 additional ice-cold ether washes (Figure 45). Although the choice of diethyl ether is not the most preferable solvent, it resulted in the cleanest, quickest, and most efficient recrystallisation. Notably, when using an ethyl acetate/hexane recrystallisation solvent mixture, the praziquantel solid required 2 – 3 additional washes. Acetone/water, acetone, absolute EtOH or an EtOH/water mixture were investigated as suitable recrystallisation solvents, but in our hands, none of these performed as well as diethyl ether (praziquantel **1** is appreciably soluble in ether when heated and substantially insoluble when ice-cold, with the impurities still dissolved in ether).

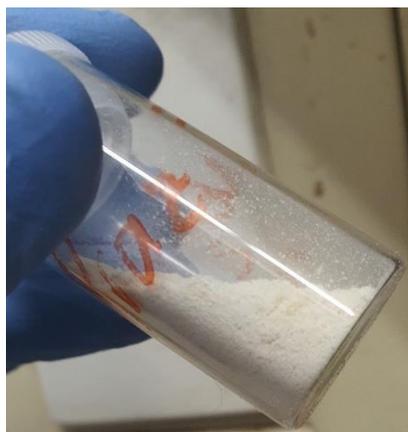
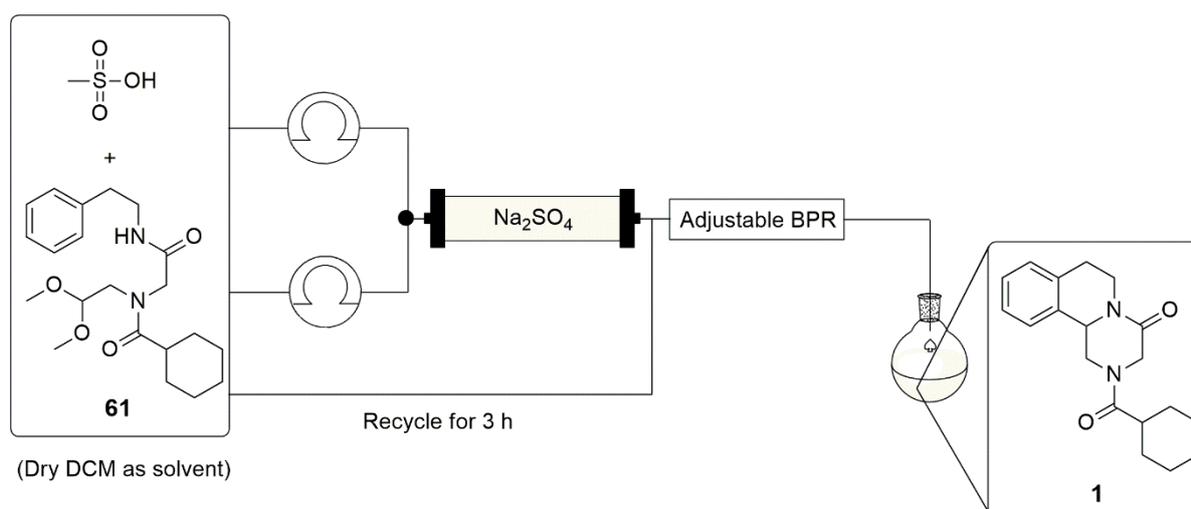


Figure 45: Praziquantel **1** recrystallised during this research study. Appearance of a clean white amorphous solid.

Encouragingly, this led us to attempt one final reaction under flow conditions with the use of a packed-bed reactor filled with sodium sulfate while passing through a single reaction stream of MSA and “pre-praziquantel” **61** with distilled DCM to reduce the amount of water in the system, but unfortunately, the reactor blocked upon contact with the reacting stream and the sodium sulfate (Scheme 60).



Scheme 60: Final flow set-up which unfortunately blocked.

This concluded our trial reactions for this final step due to time constraints and consequently, we decided it was best for this step to proceed under batch-based conditions.

4.1.6 Concluding remarks

We have successfully validated the synthetic procedure reported by Cao *et al.*⁹⁵ The final conversion of “pre-praziquantel” **61** to praziquantel **1** was achieved in a comparable recrystallised yield of 61% (vs. 65% reported by Cao *et al.*⁹⁵). This stand-alone process step results in a space-time yield of 24.4 g.L⁻¹.h⁻¹. Unfortunately, due to time constraints and challenges faced with the sulfate additive, we were unable to successfully translate this final step to flow. Furthermore, due to time constraints, we were also unable to investigate the role of the magnesium/sodium sulfate, however, we hypothesize that it is required to either direct the ring closure while chelating the enediamide intermediate (displayed in the mechanistic approach) or remove any water that may have been present in the reaction mixture, preventing the final ring closure. Looking forward, it would be in the best interest to decrease the sulfate equivalents or alternatively attempt the reaction using a phosphate buffer in order to determine whether the product may be isolated in higher yields.

CHAPTER V

5.1 Techno-economic and safety analysis

5.1.1 Cost-of-goods analysis

We next performed a high-level cost-of-goods analysis to benchmark our in-house process against that reported by Cao *et al.*⁹⁵ as well as current patent process routes to praziquantel **1**. The analysis only includes the costs of the fine chemicals and solvents employed and does not include operational costs like power consumption, water consumption, manpower, etc. It is important to note that costs alter over time, as a result of price and exchange rate fluctuations and furthermore, they will vary significantly depending on vendors. To simplify the analysis, we elected to benchmark the different approaches using the cost of reagents from Sigma Aldrich South Africa (Obtained 15/11/2022) and solvents/salts from Radchem South Africa (14/11/2022) unless otherwise stated. It is important to note that these values cannot be used to benchmark the process relative to the current cost of commercial praziquantel **1**, as existing commercial processes source reagents from large-scale and bulk distributors. They can, however, give one an indication of the relative performance of the different process routes.

5.1.2 Comparison between in-house developed process and that reported by Cao *et al.*⁹⁵

5.1.2.1 Step 1

The preparation of 2-isocyanoethylbenzene **96** accumulates to approximately R 10.25 per gram when following the synthetic procedure reported by Cao *et al.*⁹⁵ vs. R 8.79 per gram following our procedure. This is majorly resultant of the reduced concentration of base required in addition to the hydrochloric acid wash which is avoided in our procedure. Notably, these costs have been calculated excluding the costs of the purification step which would be similar for both procedures with the major costs being that of the silica gel. However, it is important to note that this cannot be calculated per gram of product since the amount of silica and eluent required will vary with respect to a range of expected yields. That being said, the cost of a 25 kg drum through Labfriend South Africa (Obtained 15/11/2022), amounts to R 24'488.75.

5.1.2.2 Step 2

As for the preparation of the “pre-praziquantel” intermediate **61**, the costs of the reagents, excluding the amounts for the generation of **96**, accumulates to approximately R 7.80 per gram when following the procedure by Cao *et al.*⁹⁵ Our process totals to a slightly increased R 8.23 per gram, which is primarily due to the formaldehyde **53** replacement employed (37% formalin vs. paraformaldehyde). Our procedure makes use of an additional column purification step

which would add to the cost, that being noted, this purification step is to be scrutinised as part of the future work and potentially be excluded while telescoping the crude material to the final step 3. When comparing the costs for the preparation of **61**, taking into consideration the costs for **96** required, our procedure accumulates to approximately R 11.80 per gram of **61**, whereas that of Cao *et al.*⁹⁵ costs a comparable R 11.55 per gram.

5.1.2.3 Step 3

This brings us to the final preparation of praziquantel **1**, taking into consideration the costs required for the preparation of **61** and **96**. The procedure reported by Cao *et al.*⁹⁵ accumulates to approximately R 49.16 per gram, whereas our procedure costs a heightened R 72.65 per gram. This is due to the additional sodium sulfate required (~ 2.5 equivalents) whereas for the route reported by Cao *et al.*⁹⁵, we assumed a conservative 1 equivalent ratio of magnesium sulfate since no values were provided in the literature. If compared while using 2.5 equivalents of magnesium sulfate, the cost of the procedure reported by Cao *et al.*⁹⁵ accumulates to a slightly increased R 49.89. We also used a larger volume of diethyl ether for the final extractions which significantly added to the cost of the process. If compared with the same volume of diethyl ether to mol ratio reported by Cao *et al.*⁹⁵ then our approach accumulates to R 58.50 per gram. Notably, once further optimisations of this batch step have been performed as a part of the future work, the overall costs would be expected to be drastically reduced resultant of potentially lowered equivalents of sodium sulfate as well as the minimum amount of diethyl ether required for the extraction steps. Critically, the MSA as well as the diethyl ether are the costliest reagents for this final step. This could be overcome by replacing diethyl ether with a cheaper alternative such as ethyl acetate and potentially decreasing the amount of equivalents required for MSA if possible.

Critically, it is important to note that our approach drastically reduces the overall reaction times by 47.75 h, which, if one was to consider additional costs such as power consumption and manpower would very possibly make our approach comparable or superior to that reported by Cao *et al.*⁹⁵

5.1.2.4 Comparison between in-house process and two current patents CN111072656A¹⁶² and CN114195782A¹⁶³

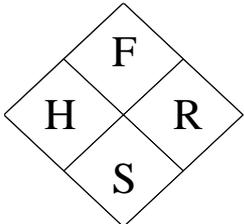
The total costs for the preparation of praziquantel **1** reported in the patents CN111072656A¹⁶² and CN114195782A¹⁶³ accumulates to R37.92 and R 28.91 per gram, respectively. Critically, the first patent CN111072656A¹⁶², makes use of tap water as their reaction solvent, which has not been accounted for during the cost calculations. That being said, the use of water drastically reduces the costs required for common solvents. Furthermore, they specify neutralisation steps from a basic medium but don't specify the volumes or acids utilised, however, this was performed from a pH above 7 to neutral, not specifying what the basic pH

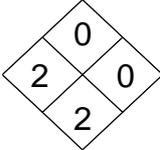
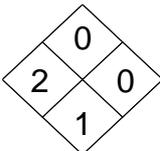
was to begin with. As such, this has not been included as apart of these calculations and would result in additional costs, nonetheless. As for the patent CN114195782A¹⁶³, the patent holders make use of a Ru/Ni catalyst which is not specified in the patent and would add additional costs to their process route. That being said, catalysts are not used in large amounts and therefore, would not add an excessive amount to the overall costs for this process. Furthermore, they specify a hydrogenation step with hydrogen gas but don't specify the exact amount required for this step, except report a required pressure of 3 MPa which they add hydrogen until this is reached. As such, the calculations were assumed as approximately 100 mL of hydrogen gas for this step. Critically, although both of these patents result in lower overall costs when compared with our procedure, they required longer reaction times of 27 h (CN111072656A)¹⁶² and 17 h (CN114195782A)¹⁶³ vs. 10.25 h residence time for our procedure. This again, as with the Cao *et al.*⁹⁵ process suggests that our approach could potentially be competitive when accounting for energy and manpower costs. Unfortunately, from a process point of view, it is not easy to perform direct relationships with the overall costs (including the laboratory running costs) but we feel that if we are able to reduce the cost-of-goods through further alterations and optimisations of our procedure, this may balance out with the appreciable decreased reaction times potentially allowing for a viable process route. Critically, it is important to note that our approach has not yet been subjected to translational scaled-up studies and telescoping options are still being investigated. We believe that this initial high-level cost analysis suggests that the process certainly has promise and deserves further investigation.

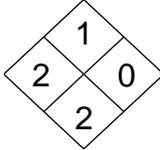
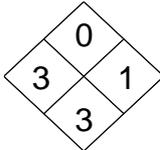
5.1.3 Project specific hazard analysis

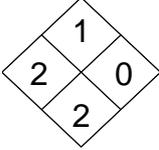
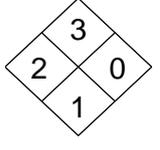
We performed a hazard analysis on the reagents and solvents utilised for our developed process route for the preparation of praziquantel **1** achieved during this research study. This was to ensure that the necessary safety protocols were in place and to ensure that handling of the required chemicals were done so with the necessary precautions.

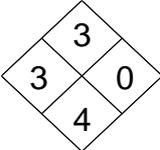
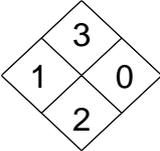
5.1.3.1 Summary of hazards

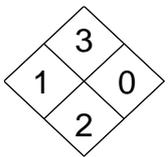
	F: Flammability H: Health R: Reactivity S: Skin contact	0: None 1: Low 2: Medium 3: High 4: Extreme
---	--	---

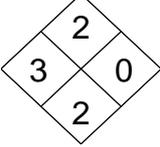
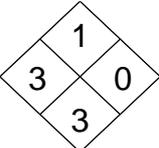
Chemical name	Potential Hazard	Severity	Preventative control measure
2-Phenethylamine 41	<ul style="list-style-type: none"> H290: May be corrosive to metals. H301: Toxic if swallowed. H314: Causes severe skin burns and eye damage. 	  	<ul style="list-style-type: none"> P234: Keep only in original packaging. P270: Do not eat, drink, or smoke when using this product. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: If on skin (or hair): Take off all contaminated clothing immediately. Rinse skin with water. P304 + P340 + P310: If inhaled: Remove person to fresh air and keep comfortable for breathing. Immediately call a poison centre/doctor. P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Chloroform	<ul style="list-style-type: none"> H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H331: Toxic if inhaled. H336: May cause drowsiness or dizziness. H351: Suspected of causing cancer. H361d: Suspected of damaging the unborn child. 	  	<ul style="list-style-type: none"> P261: Avoid breathing vapours. P281: Use personal protective equipment as required. P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P311: Call a poison centre or doctor/ physician.

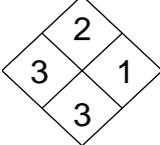
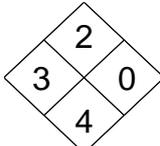
Chemical name	Potential Hazard	Severity	Preventative control measure
Benzyltriethyl-ammonium chloride (BTEAC)	<ul style="list-style-type: none"> H372: Causes damage to organs through prolonged or repeated exposure. 	 	<ul style="list-style-type: none"> P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P264: Wash skin thoroughly after handling. P271: Use only outdoors or in a well-ventilated area. P280: Wear protective gloves/ eye protection/ face protection. P302 + P352: If on skin: Wash with plenty of water. P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Sodium Hydroxide 50% w/w	<ul style="list-style-type: none"> H290: May be corrosive to metals. H314: Causes severe skin burns and eye damage. 	 	<ul style="list-style-type: none"> P234: Keep only in original packaging. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: If on skin (or hair): Take off all contaminated clothing immediately. Rinse skin with water. P304 + P340 + P310: If inhaled: Remove person to fresh air and keep comfortable for breathing. Immediately call a poison centre/ doctor.

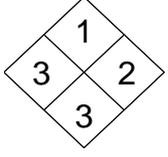
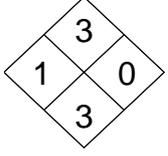
Chemical name	Potential Hazard	Severity	Preventative control measure
Dichloromethane	<ul style="list-style-type: none"> • H315: Causes skin irritation. • H319: Causes serious eye irritation. • H336: May cause drowsiness or dizziness. • H351: Suspected of causing cancer. 	  	<ul style="list-style-type: none"> • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. • P363: Wash contaminated clothing before reuse.
Chemical name	Potential Hazard	Severity	Preventative control measure
Ethanol	<ul style="list-style-type: none"> • H225: Highly flammable liquid and vapour. • H319: Causes serious eye irritation. • UEL: 27.7% v/v • LEL: 3.1% v/v • Flash point 9.7 °C 	 	<ul style="list-style-type: none"> • P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. • P233: Keep container tightly closed. • P240: Ground and bond container and receiving equipment. • P241: Use explosion-proof electrical/ventilating/lighting/equipment.

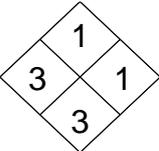
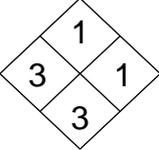
			<ul style="list-style-type: none"> • P242: Use non-sparking tools. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Triethylamine	<ul style="list-style-type: none"> • H225: Highly flammable liquid and vapor. • H302: Harmful if swallowed. • H311 + H331: Toxic in contact with skin or if inhaled. • H314: Causes severe skin burns and eye damage. • H335: May cause respiratory irritation. 	   	<ul style="list-style-type: none"> • P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. • P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. • P301 + P312: If swallowed: Call a poison centre/ doctor if you feel unwell. • P303 + P361 + P353: If on skin (or hair): Take off all contaminated clothing immediately. Rinse skin with water. • P304 + P340 + P310: If inhaled: Remove person to fresh air and keep comfortable for breathing. Immediately call a poison centre/ doctor. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Ethyl Acetate	<ul style="list-style-type: none"> • H225: Highly flammable liquid and vapor. • H319: Causes serious eye irritation. 		<ul style="list-style-type: none"> • P210: Keep away from heat/sparks/open flames/hot surfaces. No smoking. • P233: Keep container tightly closed.

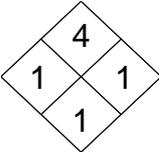
	<ul style="list-style-type: none"> H336: May cause drowsiness or dizziness. 	 	<ul style="list-style-type: none"> P241: Use explosion-proof electrical/ventilating/lighting/equipment. P243: Take precautionary measures against static discharge. P264: Wash skin thoroughly after handling. P271: Use in a well-ventilated area or outdoors. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection P304+340: If inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P303 + P361 + P353: If on skin (or hair): Remove/ Take off all contaminated clothing immediately. Rinse skin with water/ shower. P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P501: Dispose of contents/container.
Chemical name	Potential Hazard	Severity	Preventative control measure
Hexane	<ul style="list-style-type: none"> H225: Highly flammable liquid and vapor. H304: May be fatal if swallowed and enters airways. H315: Causes skin irritation. H336: May cause drowsiness or dizziness. 	 	<ul style="list-style-type: none"> P201: Obtain special instructions before use. P210: Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking. P273: Avoid release to the environment. P301 + P310: If swallowed: Immediately call a poison centre/ doctor.

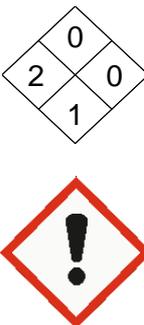
	<ul style="list-style-type: none"> • H361f: Suspected of damaging fertility. • H373: May cause damage to organs (Nervous system) through prolonged or repeated exposure if inhaled. • H411: Toxic to aquatic life with long lasting effects. 	 	<ul style="list-style-type: none"> • P303 + P361 + P353: If on skin (or hair): Take off all contaminated clothing immediately. Rinse skin with water. • P331: Do not induce vomiting.
Chemical name	Potential Hazard	Severity	Preventative control measure
Amino-acetaldehyde dimethyl acetal 43	<ul style="list-style-type: none"> • H226: Flammable liquid and vapour. • H314: Causes severe skin burns and eye damage. 	  	<ul style="list-style-type: none"> • P280: Wear protective gloves/protective clothing/eye protection/face protection. • P310: Immediately call a poison centre or doctor/physician. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Cyclohexane-carboxylic acid 51	<ul style="list-style-type: none"> • H302: Harmful if swallowed. • H314: Causes severe skin burns and eye damage. • H335: May cause respiratory irritation. 	  	<ul style="list-style-type: none"> • P261: Avoid breathing dust. • P280: Wear protective gloves/ eye protection/ face protection. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Chemical name	Potential Hazard	Severity	Preventative control measure
Paraformaldehyde (Formaldehyde 53 source)	<ul style="list-style-type: none"> H228: Flammable solid. H302 + H332: Harmful if swallowed or if inhaled. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. 	    	<ul style="list-style-type: none"> P210: Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P301 + P312: If swallowed: Call a poison centre/ doctor if you feel unwell. P304 + P340 + P312: If inhaled: Remove person to fresh air and keep comfortable for breathing. Call a poison centre/ doctor if you feel unwell. P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308 + P313: If exposed or concerned: Get medical advice/ attention.
Chemical name	Potential Hazard	Severity	Preventative control measure
Formalin 37% (Formaldehyde 53 source)	<ul style="list-style-type: none"> H226: Flammable liquid and vapor. H301 + H311: Toxic if swallowed or in contact with skin. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H330: Fatal if inhaled. 	  	<ul style="list-style-type: none"> P201: Obtain special instructions before use. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: If on skin (or hair): Take off all contaminated

	<ul style="list-style-type: none"> • H335: May cause respiratory irritation. • H341: Suspected of causing genetic defects. • H350: May cause cancer. • H370: Causes damage to organs (Eyes, Central nervous system). 	 	<p>clothing immediately. Rinse skin with water.</p> <ul style="list-style-type: none"> • P304 + P340 + P310: If inhaled: Remove person to fresh air and keep comfortable for breathing. Immediately call a poison centre/ doctor. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
2-Isocynoethylbenzene 96	<ul style="list-style-type: none"> • H302: Harmful if swallowed. • H312: Harmful if in contact with skin. • H315: Causes skin irritation. • H319: Causes serious eye irritation. • H332: Harmful if inhaled. • H335: May cause respiratory irritation. 	 	<ul style="list-style-type: none"> • P264: Wash thoroughly after handling. • P270: Do not eat, drink, or smoke when using this product. • P280: Wear protective gloves/protective clothing/eye protection/face protection. • P261: Avoid breathing dust/fume/gas/mist/vapours/spray. • P271: Use only outdoors or in a well-ventilated area.
Chemical name	Potential Hazard	Severity	Preventative control measure
Methanol	<ul style="list-style-type: none"> • H225: Highly flammable liquid and vapor. • H301 + H311 + H331: Toxic if swallowed, in contact with skin or if inhaled. • H370: Causes damage to organs (Eyes, Central nervous system). 	  	<ul style="list-style-type: none"> • P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. • P233: Keep container tightly closed. • P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. • P301 + P310: If swallowed: Immediately call a poison centre/ doctor.

			<ul style="list-style-type: none"> • P303 + P361 + P353: If on skin (or hair): Take off all contaminated clothing immediately. Rinse skin with water. • P304 + P340 + P311: If inhaled: Remove person to fresh air and keep comfortable for breathing. Call a poison centre/ doctor.
Chemical name	Potential Hazard	Severity	Preventative control measure
Methanesulfonic acid (MSA)	<ul style="list-style-type: none"> • H290: May be corrosive to metals. • H302: Harmful if swallowed. • H314: Causes severe skin burns and eye damage. • H335: May cause respiratory irritation. 	  	<ul style="list-style-type: none"> • P234: Keep only in original packaging. • P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. • P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. • P301 + P312: If swallowed: Call a poison centre/doctor if you feel unwell. • P303 + P361 + P353: If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water. • P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Para-toluenesulfonic Acid	<ul style="list-style-type: none"> • H290: May be corrosive to metals. • H314: Causes severe skin burns and eye damage. • H335: May cause respiratory irritation. 	 	<ul style="list-style-type: none"> • P234: Keep only in original packaging. • P260: Do not breathe dusts or mists. • P273: Avoid release to the environment. • P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

	<ul style="list-style-type: none"> H412: Harmful to aquatic life with long lasting effects. 		<ul style="list-style-type: none"> P303 + P361 + P353: If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P305 + P351 + P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Chemical name	Potential Hazard	Severity	Preventative control measure
Diethyl ether	<ul style="list-style-type: none"> H224: Extremely flammable liquid and vapour. H302: Harmful if swallowed. H336: May cause drowsiness or dizziness. EUH019: May form explosive peroxides. EUH066: Repeated exposure may cause skin dryness or cracking. 	  	<ul style="list-style-type: none"> P210: Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking. P233: Keep container tightly closed. P240: Ground and bond container and receiving equipment. P241: Use explosion-proof electrical/ ventilating/ lighting equipment. P301 + P312: If swallowed: Call a poison centre/doctor if you feel unwell. P403 + P233: Store in a well-ventilated place. Keep container tightly closed.
Chemical name	Potential Hazard	Severity	Preventative control measure
"Pre-praziquantel" 61	<ul style="list-style-type: none"> No MSDS available, to the best of our knowledge. Treat with extra caution. 	No data available. Treated with extra caution.	<ul style="list-style-type: none"> Treat with caution and dispose of as hazardous organic waste.

Chemical name	Potential Hazard	Severity	Preventative control measure
Praziquantel 1	<ul style="list-style-type: none"> H412: Harmful to aquatic life with long lasting effects. 		<ul style="list-style-type: none"> P273: Avoid release to the environment. P501: Dispose of contents/ container to an approved waste disposal plant.

5.1.3.2 Preliminary risk analysis

Chemical: 2 – Phenethylamine 41

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500	
Sensitisation	Extreme	Moderate – Severe	Unknown, Mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating	Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4	A5	X
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous

Carcinogenicity: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible, or confirmed human carcinogen by IARC.

Ecotoxicity / bioaccumulation: Toxicity to fish LC₅₀ - *Leuciscus idus* (Golden orfe) - 32 - 46 mg/l - 96 h (Phenethylamine). Does not bioaccumulate.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Work in fume hood.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents, Copper, Copper alloys, Strong acids, Brass						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)									
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent		Neutralise with		Sweep up	
Comments	Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations. Keep in suitable, closed containers for disposal.								

Chemical: Chloroform

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating		Unknown, moderately irritating	X	Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3	X	A4		A5	
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: NO DATA

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents, Strong bases, Magnesium, Sodium/sodium oxides, Lithium.						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)	Keep in tightly closed container in cool area away from direct sunlight or heat sources.								
Comments	Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Absorb spill with inert material (e.g., vermiculite, sand, or earth), then place in suitable container. Dispose of as hazardous waste. Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation. Approach spill from upwind.								

Chemical: Benzyltriethylammonium chloride

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: Do not flush into surface water or sanitary sewer system. Do not allow material to contaminate ground water system. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. The product contains following substances which are hazardous for the environment. This substance/mixture contains no components considered to be either persistent, bio accumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful		Non-harmful	X	Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Combustibles						

Disposal

Acid		General organic	X	Halogenated	X	Toxic		Solid	X
Other (specify)	Relatively unreactive organic reagents should be collected in container A. If halogenated, they should be collected in container B. For solid residues use container C.								
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company.								

Spill/Leak

Evacuate area		Add sand		Add absorbent		Neutralise with		Sweep up	X
Comments	Clean up immediately by sweeping or vacuum.								

Chemical: Sodium Hydroxide 50% w/w

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500	X	(Non-toxic) >500	
Sensitisation	Extreme	Moderate – Severe	X	Unknown, mild		Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4		A5	X
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible, or confirmed human carcinogen by IARC.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases	X	Oxidant		Reductant	
Incompatibilities	Acids. Organic materials. Metals.						

Disposal

Acid		General organic		Halogenated		Toxic		Solid	
Other (specify)	Neutralize solution with HCl to dispose of in general aqueous waste.								
Comments									

Spill/Leak

Evacuate area		Add sand		Add absorbent	X	Neutralise with	X	Sweep up	
Comments	Cover drains. Collect, bind, and pump off spills. Take up with liquid-absorbent and neutralising material (e.g., Chemizorb® OH ⁻ , Merck Art. No. 101596). Dispose of properly. Clean up affected area.								

Chemical: Triethylamine

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500	X	(Non-toxic) >500	
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4		A5	X
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Acute toxicity: LD50 Oral - Rat - male and female - 730 mg/kg (OECD Test Guideline 401)

LC50 Inhalation - Rat - male and female - 4 h - 3,63 mg/l (OECD Test Guideline 403)

LD50 Dermal - Rabbit - male - 580 mg/kg (OECD Test Guideline 402)

Ecotoxicity: Aquatic vertebrates: LC50 - *Oryzias latipes* (Orange-red killifish) - 24 mg/l - 96 h (OECD Test Guideline 203)

Daphnia and other aquatic invertebrates semi-static test LC50 - *Ceriodaphnia dubia* (water flea) - 17 mg/l - 48 h (US-EPA)

Algae EC50 - *Pseudokirchneriella subcapitata* (green algae) - 8 mg/l - 72 h (OECD Test Guideline 201)

Bacteria static test EC50 - *Pseudomonas putida* - 95 mg/l - 17 h (DIN 38421 TEIL 8)

Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	X
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Avoid contact with skin, eyes, and clothing. Wash hands before breaks and immediately after handling the product. Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands. Flame retardant antistatic protective clothing required.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases	X	Oxidant		Reductant	
Incompatibilities	Oxidising agents. Keep in tightly closed container in cool area away from direct sunlight, heat, flames and sparks..						

Disposal

Acid		General organic		Halogenated		Toxic	X	Solid	X
Other (specify)	Keep in tightly closed container in cool area away from direct sunlight or heat sources.								
Comments	Consult state, local or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Do not discharge into drains or rivers. Discharge into the environment must be avoided. Mix with vermiculite if absorbent is required. Transfer to a closable, labelled salvage container for disposal by an appropriate method.								

Chemical: Dichloromethane

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5		(Highly toxic) 5-50		(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme		Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion			Corrosive, severely irritating		Unknown, moderately irritating	X	Non- or mildly irritating	
Carcinogenicity	A1 or 2	X	A3		A4		A5	
Hazard Classification	Extreme Hazard		High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: Limited evidence of carcinogenicity in animal studies. Suspected human carcinogen.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator	X	Apron	
Other (specify)	Avoid contact with skin, eyes, and clothing. Use personal protective equipment. Avoid breathing vapours, mist, or gas. Ensure adequate ventilation. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Rubber, various plastics, Light metals, Metals, Mild steel						

Disposal

Acid		General organic	X	Halogenated	X	Toxic		Solid	
Other (specify)	Keep in tightly closed container in cool area away from direct sunlight or heat sources.								
Comments									

Spill/Leak

Evacuate area	X	Add sand		Add absorbent	X	Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Take up with liquid-absorbent material (e.g., Chemizorb®). Dispose of properly. Clean up affected area.								

Chemical: Ethanol

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: NO DATA

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Take measures to prevent the build-up of electrostatic charge. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.								

Storage

Toxic		Harmful		Non-harmful	X	Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Oxidising agents, rubber, various plastics. Avoid heat, flames, and sparks. Keep in tightly closed container in cool area away from direct sunlight or heat sources.						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place. Hygroscopic.								
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Do not discharge into drains. Use personal protective equipment. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas. Mix with vermiculite if absorbent is required. Transfer to a closable, labelled salvage container for disposal by an appropriate method.								

Chemical: Ethyl Acetate

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising

Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3	A4	X	A5	
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous	

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Various plastics						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)									
Comments	Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Offer surplus and non-recyclable solutions to a licensed disposal company.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent	X	Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.								

Chemical: Hexane

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising	

Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3	A4	X	A5	
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous	

Carcinogenicity: NO DATA.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities							

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)									
Comments	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Collect spillage. On land, sweep or shovel into suitable containers. Soak up spills with inert solids, such as clay or diatomaceous earth as soon as possible.								

Chemical: Aminoacetaldehyde dimethyl acetal 43

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating

Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents, Strong acids.						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)									
Comments	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Contain spillage, and then collect with non-combustible absorbent material, (e.g., sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations.								

Chemical: Cyclohexanecarboxylic acid 51

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4		A5	X
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible, or confirmed human carcinogen by IARC.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful		Non-harmful	X	Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents, Strong bases						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	X
Other (specify)									
Comments	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.								

Spill/Leak

Evacuate area		Add sand		Add absorbent		Neutralise with		Sweep up	X
Comments	Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.								

Chemical: Paraformaldehyde (Formaldehyde 53 source)

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4	X	A5	
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: NO DATA.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Iron, Copper, Nickel, Zinc, various alloys						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	X
Other (specify)									
Comments	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent		Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions. Take up carefully. Dispose of properly. Clean up affected area. Avoid generation of dusts.								

Chemical: Formalin 37% solution (Formaldehyde 53 source)

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5		(Highly toxic) 5-50		(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme		Moderate – Severe		Unknown, mild		Non-sensitising
Irritation/Corrosion			Corrosive, severely irritating		Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	X	A3		A4		A5
Hazard Classification	Extreme Hazard		High Hazard	X	Medium Hazard		Non-Hazardous

Carcinogenicity: Presumed to have carcinogenic potential for humans.

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use only outdoors or in a well-ventilated area. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands.								

Storage

Toxic	X	Harmful		Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents						

Disposal

Acid		General organic		Halogenated		Toxic	X	Solid	
Other (specify)									
Comments	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent	X	Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions. Take up carefully with liquid-absorbent material (e.g., Chemizorb®). Dispose of properly. Clean up affected area.								

Chemical: Methanol

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity/Bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
----------	---	----------------	---	----------------	---	--------------	---	-------------	--

Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use personal protective equipment. Avoid contact with skin and eyes. Keep away from sources of ignition - No smoking. Take measures to prevent the build-up of electrostatic charge.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Acid chlorides, Acid anhydrides, Oxidizing agents, Alkali metals, Reducing agents, Acids						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.								
Comments	Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. Handle uncleaned containers like the product itself. Offer surplus and non-recyclable solutions to a licensed disposal company.								

Spill/Leak

Evacuate area		Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Contain spillage, and then collect with non-combustible absorbent material, (e.g., sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations.								

Chemical: Diethyl ether

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity/Bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Use personal protective equipment. Avoid contact with skin and eyes. Keep away from sources of ignition - No smoking. Take measures to prevent the build-up of electrostatic charge.								

Storage

Toxic		Harmful		Non-harmful	X	Flammable	X
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Rubber, various plastics						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.								
Comments	Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Offer surplus and non-recyclable solutions to a licensed disposal company.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent	X	Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions. Take up with liquid-absorbent material (e.g., Chemizorb®). Dispose of properly. Clean up affected area.								

Chemical: 2-Isocynoethylbenzene 96

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity/Bioaccumulation: NO DATA

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator	X	Apron	
Other (specify)	Use personal protective equipment. Avoid contact with skin and eyes. Foul smelling chemical, use respirator when inadequate ventilation.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	NO DATA						

Disposal

Acid		General organic		Halogenated		Toxic	X	Solid	
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.								
Comments	Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Offer surplus and non-recyclable solutions to a licensed disposal company.								

Spill/Leak

Evacuate area	X	Add sand	X	Add absorbent	X	Neutralise with		Sweep up	X
Comments	Collect and arrange disposal. Keep the chemical in suitable and closed containers for disposal. Remove all sources of ignition. Use spark-proof tools and explosion-proof equipment. Adhered or collected material should be promptly disposed of, in accordance with appropriate laws and regulations.								

Chemical: Methanesulfonic acid (MSA)

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500		(Non-toxic) >500	X
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3		A4	X	A5	
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous	

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid	X	Bases		Oxidant		Reductant	
Incompatibilities	Various metals, i.e., Iron, Copper, brass, Mild steel						

Disposal

Acid	X	General organic		Halogenated		Toxic		Solid	
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Store in cool place. Hygroscopic.								
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste								

Spill/Leak

Evacuate area		Add sand		Add absorbent	X	Neutralise with	X	Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions. Take up with liquid-absorbent and neutralising material (e.g., Chemizorb® Merck Art. No. 101595). Dispose of properly. Clean up affected area.								

Chemical: *p*-Toluenesulfonic acid

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50		(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe		Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	X	Unknown, moderately irritating		Non- or mildly irritating
Carcinogenicity	A1 or 2	A3		A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard		Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity/Bioaccumulation: NO DATA

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	

Other (specify)	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.
-----------------	---

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid	X	Bases		Oxidant		Reductant	
Incompatibilities	Oxidising agents. Keep in tightly closed container in cool area away from direct sunlight or heat sources. Avoid contact with water or humidity.						

Disposal

Acid	X	General organic		Halogenated		Toxic		Solid	X
Other (specify)	Keep container tightly closed in a dry and well-ventilated place. Store in cool place. Hygroscopic								
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent		Neutralise with		Sweep up	X
Comments	Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Transfer to a closable, labelled salvage container for disposal by an appropriate method. Do not let product enter drains.								

Chemical: Praziquantel 1

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	(Non-toxic) >500	X	
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising	
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating	
Carcinogenicity	A1 or 2	A3	A4	X	A5	
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard		Non-Hazardous	X

Carcinogenicity: NO DATA

Ecotoxicity / bioaccumulation: This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.								

Storage

Toxic		Harmful		Non-harmful	X	Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	Strong oxidizing agents						

Disposal

Acid		General organic		Halogenated		Toxic		Solid	X
Other (specify)									
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste.								

Spill/Leak

Evacuate area		Add sand		Add absorbent		Neutralise with		Sweep up	X
Comments	Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions. Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.								

Chemical: *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)-cyclohexanecarboxamide ("Pre-praziquantel" 61)

Toxicity Oral (LD₅₀, mg/kg)	(Extremely toxic) ≤5	(Highly toxic) 5-50	(Toxic or unknown) 50-500	X	(Non-toxic) >500
Sensitisation	Extreme	Moderate – Severe	Unknown, mild	X	Non-sensitising
Irritation/Corrosion		Corrosive, severely irritating	Unknown, moderately irritating	X	Non- or mildly irritating
Carcinogenicity	A1 or 2	A3	A4	X	A5
Hazard Classification	Extreme Hazard	High Hazard	Medium Hazard	X	Non-Hazardous

Carcinogenicity: NO DATA

Ecotoxicity/Bioaccumulation: NO DATA

Control Measures and PPE

Lab coat	X	Nitrile gloves	X	Safety glasses	X	Safety shoes	X	Face shield	
Overall		4 H gloves		Safety goggles		Respirator		Apron	
Other (specify)	Avoid contact with skin and eyes. Provide appropriate exhaust ventilation when working with compound.								

Storage

Toxic		Harmful	X	Non-harmful		Flammable	
Acid		Bases		Oxidant		Reductant	
Incompatibilities	No data is available. Treat with caution and store separate.						

Disposal

Acid		General organic	X	Halogenated		Toxic		Solid	X
Other (specify)	Oil before standing for a while to yield solid.								
Comments	Consult state, local, or national regulations for proper disposal. Hand over to authorised disposal company as hazardous waste.								

Spill/Leak

Evacuate area	X	Add sand		Add absorbent	X	Neutralise with		Sweep up	X
Comments	Use personal protective equipment. Avoid breathing vapours, mist, or gas. Ensure adequate ventilation. Transfer to a closable, labelled salvage container for disposal by an appropriate method. Do not let product enter drains.								

5.1.3.3 Chemical interaction data

Legend:
 d = desired reaction
 K = known reaction
 - = no hazards
 x = chemicals will not be used in the same reaction

Chemicals	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
A 2-Phenethylamine 41		d	d	d	-	-	-	-	X	X	X	X	d	X	X	-	X	X	X	X
B Chloroform			d	d	-	-	-	-	X	X	X	X	d	X	X	-	X	X	X	X
C Benzyltriethylammonium chloride				d	-	X	X	X	X	X	X	X	d	X	X	X	X	X	X	X
D NaOH (aq.) 50% w/w					-	X	X	X	X	X	X	X	d	X	X	X	X	X	X	X
E Dichloromethane (DCM)						-	-	-	-	-	X	-	-	-	-	-	-	X	X	X
F Ethyl Acetate							-	-	-	X	X	X	-	X	X	X	-	X	X	X
G Triethylamine								-	-	X	X	X	-	X	X	X	-	X	X	X
H Hexane									-	X	X	X	-	X	X	X	-	X	X	X
I Cyclohexanecarboxylic acid 51										-	X	X	d	d	d	X	d	X	X	X
J Methanol											X	-	-	-	-	-	-	X	X	X
K Ethanol												X	X	X	X	X	X	X	X	-
L Aminoacetaldehyde dimethyl acetal 43														d	d	d	-	d	X	X
M 2-Isocyanoethylbenzene 96															d	d	-	d	X	X
N Paraformaldehyde (Formaldehyde 53 source)																X	X	d	X	X
O Formalin 37% (Formaldehyde 53 source)																	X	d	X	X
P Diethyl ether																		-	-	-

CHAPTER VI

6.1 Conclusions

We have successfully validated the full synthetic procedure utilising batch-based chemistry methodologies for the preparation of praziquantel **1** as reported by Cao *et al.*⁹⁵ However, based on our initial results obtained for the validated procedure, our overall yields were observed to exhibit a decrease of ~ 17% (22% vs. 39% reported by Cao *et al.*⁹⁵). It is important to note that our test reactions were performed on a substantially smaller scale to that of Cao *et al.*⁹⁵ which may be responsible for the difference observed. We optimised all stages using batch-based chemistry, and in doing so were able to increase the yields to afford praziquantel **1** in an overall yield of 39% which is comparable to that reported by Cao *et al.*⁹⁵

Furthermore, we have successfully translated the first two of the three steps to flow conditions with a combined yield of ~ 68%. When comparing this with the corresponding yields reported by Cao *et al.*⁹⁵ our flow-based procedure exhibits an increase in the yield ~ 8% for these two steps (~ 60% reported by Cao *et al.*⁹⁵). Notably, our flow-based approach allowed this increase in yield with a drastic decrease in the reaction times of around 47.75 h over both steps (4.25 h combined residence time vs. 52 h reaction time reported by Cao *et al.*⁹⁵). In addition to this, based on a single attempt to telescope steps 1 and 2 together, we were able to prepare “pre-praziquantel” **61** in an overall yield of 55%. This approach served to provide proof of concept, and critically involved an off-line separation of the organic and aqueous phases after step 1. That being noted, an in-line separation could be readily realized (see future work) to allow the synthesis and consumption of the noxious 2-isocyanoethylbenzene **96** on-the-fly. Critically, however, the yield over steps 1 and 2 obtained is slightly lower than that achieved under stand-alone flow conditions (68%) and under batch conditions (60%) as reported by Cao *et al.*⁹⁵ Nonetheless, this was a once-off trial reaction that has not yet been optimised. As such we believe that it opens potential for an odourless reaction for the preparation of **61** with appreciable yields, reduced waste and decreased reaction times.

As for the final conversion of **61** to **1**, we achieved comparable yields with that of Cao *et al.*⁹⁵ (61% vs. 65%). Unfortunately, we were unable to translate this step to flow conditions. However, the step, as reported by Cao *et al.*⁹⁵, is actually considered a green step having been performed under solvent-free conditions and it employs the use of a green organic acid. Consequently, our final procedure was performed as a batch-flow hybrid synthesis. The approach affords **1** in a yield of 41% which is comparable to that reported by Cao *et al.*⁹⁵ (39%) but with a significant decrease in the reaction times of ~ 47.75 h over the entire procedure (10.25 h vs. 58 h). Critically, this in turn allows for a greater overall space-time yield for the

preparation of praziquantel **1** ($0.93 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ vs. $0.84 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ for that of Cao *et al.*)⁹⁵ which is highly beneficial from a process point of view.

When comparing the high-level cost analysis of the process reported by Cao *et al.*⁹⁵ with that of our developed process route, we were able to prepare praziquantel **1** with an overall cost-of-goods of approximately R 58.50 per gram of praziquantel **1** produced when compared with the slightly lowered R 49.89 per gram when calculated using the approach reported by Cao *et al.*⁹⁵ Notably, once further optimisations of this batch step have been performed as a part of the future work, the overall costs would be expected to be lowered by reducing the amount of sodium sulfate and diethyl ether employed. Overall, the batch-flow hybrid approach proved to be advantageous in terms of productivity by drastically reducing the reaction time while affording comparable yields. The new hybrid process afforded safer working conditions, in particular step 1 was conducted under less basic conditions, and the trial telescoping suggests that the noxious isocyanide can be synthesised and consumed on-the-fly leading to a lower odour approach.

When comparing the cost-of-goods of two recent patents (CN111072656A¹⁶² and CN114195782A¹⁶³) with that of our developed process route, the patents result in lower overall costs (R 37.92 and R 28.91 per gram, respectively), however, they required substantially longer reaction times of 27 h and 17 h, respectively (vs. 10.25 h residence time for our procedure). This again, as with the Cao *et al.*⁹⁵ process suggests that our approach could potentially be competitive when accounting for energy and manpower costs. Furthermore, our developed procedure has not been performed on as large scales, due to time constraints, and still requires fine tuning and optimisations to be more beneficial from a process point of view.

6.2 Future work

Due to time constraints, we were unable to scale-up the process under the optimised batch-flow hybrid conditions, however, this is something that would be highly beneficial to afford a direct comparison on the same scale reported by Cao *et al.*⁹⁵ as well as assessing the potential for a viable process route to praziquantel **1** on a multi-gram to kilogram scale. As such, this is proposed to be performed as a part of the future work together with reproducibility studies. We anticipate scaling the reaction to 10 g, 100 g and finally 1 kg (all demonstrated in triplicate). A techno-economic assessment will be performed after the 100 g runs to assess process viability; this assessment will form a stage-gate to the envisaged kilo-scale runs.

6.2.1 Step 1

As for step 1 of this synthetic procedure, it would be beneficial to attempt further optimisations under flow conditions to potentially increase the yield with a decreased residence time. We

propose that this may be achieved by implementing additional 1.5 mm internal diameter coil reactors to allow for increased flow rates while at the same time having sufficient internal volume to handle the precipitate formation. Furthermore, the possible implementation of HPLC pump heads allowing for greater pressure limits would allow one to increase the reaction temperature above 110 °C, even with the use of DCM. On the downside, HPLC pumps have issues with pumping viscous solutions and as such, the aqueous base concentration would need to be decreased, however, from a safety aspect, this would be beneficial. If the yield could be further increased with a decrease in the base concentration, this would be highly advantageous and bring some new light to this modified Hofmann procedure.

In addition, it would be in the best interest to attempt the formamide dehydration method under flow conditions for the preparation of 2-isocynoethylbenzene **96** while using mild reducing agents such as Burgess reagent. In doing so, a direct comparison with the modified Hofmann approach can be performed in order to assess which method would be most beneficial, taking into account the environmental friendliness (type of solvent usage, waste generation and atom economy), safety, reaction set-up complexity, reproducibility, scale-up capabilities and overall costs.

Optimisations of the telescoping reaction of step 1 into 2 to potentially increase the overall yield, with an integrated in-line work-up of **96**. We propose for this to be achieved with the introduction of an acidic stream post-reactor, followed by a Zaiput or Syrris FLEXX liquid-liquid separator with the organic phase stream directed towards the step 2 reactor set-up which would be met by the accompanying reagents.

6.2.2 Step 2

It would be in the best interest to implement more precise analytical techniques for the analysis of **61** pre-purification to assess whether it would be more beneficial to direct this unpurified material into the final conversion to **1**. In doing so, this would further decrease the amount of organic waste generated as well as the solid silica gel waste post-column. Unfortunately, due to time constraints we were unable to utilise the HPLC method developed for the separation of **61** from **1** (used for purity calculation) to achieve separation of **61** from **96**. As such, it would be in the best interest to attempt this similar HPLC method for such purposes. Furthermore, it would be highly advantageous to attempt telescoping step 2 directly into step 3. We propose that this may be achieved with an in-line work-up with the output stream directed into a rotary evaporator to concentrate **61** prior to being pumped into a batch reactor housing the MSA and sodium sulfate.

As an alternative source of formaldehyde **53**, gaseous formaldehyde is to be attempted under flow conditions as the introductory source with a piece of glassware (designed in-house) in

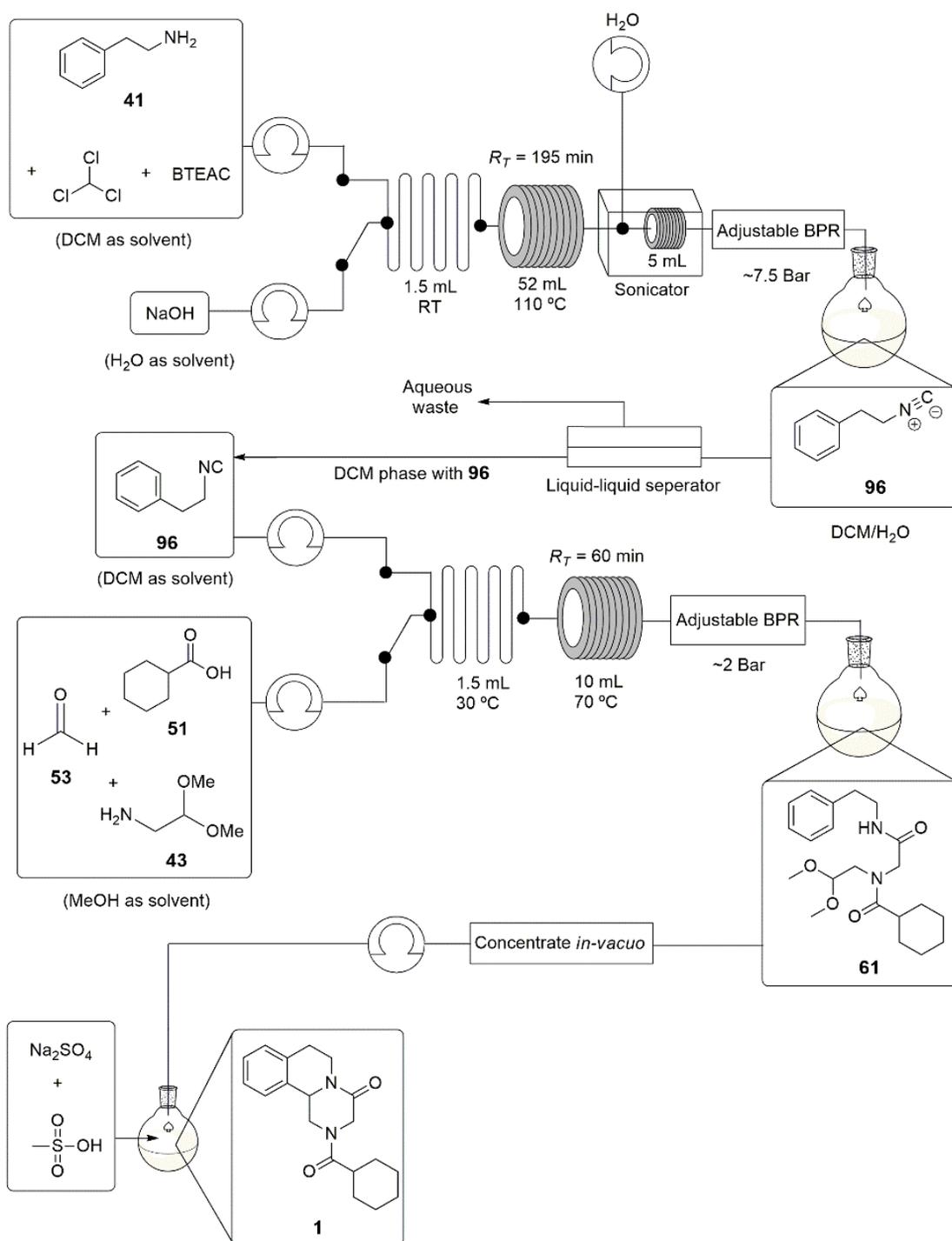
the final steps of being produced. In principle, we envision that paraformaldehyde would be cracked and consumed on-the-fly while limiting the potential of rapid polymerization, which we envisage to be problematic utilising a standard tube-in-tube reactor. This is to be achieved with an attachment to the flow apparatus allowing for communication with the flow apparatus and autonomous operation while maintaining an accurate temperature control.

6.2.3 Step 3

Due to time constraints, we were unable to attempt multiple flow trial reactions with the use of the crucial magnesium/sodium sulfate. However, at this stage, we propose that the sulfate additive may be packed within a packed-bed reactor with an additional packing material (possibly silica gel) to “bulk up the material” which would assist in flow paths without excessive compaction leading to fouling of the reactor unit. It would be in the best interest to identify the mode of action of this sulfate additive in order to potentially replace this with a reagent that is more suitable for flow translation. As such, it would be of interest to attempt a trial reaction while utilising an in-line moisture trap (moisture absorbent beads) without sodium sulfate in order to observe if this was a primary mode of action of the sulfate additive. If flow conditions prove to be futile for this final step, then optimise this reaction using batch-based methodologies to at least allow for a heightened yield. Furthermore, if the MSA equivalents could be reduced to catalytic amounts with the use of a green solvent, this would be highly advantageous for this procedure.

As for the purity of the final product (80.9%), this could be easily increased with consecutive recrystallisation steps or column chromatography. However, this was not achieved during this research due to time constraints and minimal amounts of the synthesised praziquantel **1** product at hand. Possible impurities are still to be investigated and isolated as a part of the future work which would provide more insight into purifying the final praziquantel **1** product.

To conclude, we envisage a continuous telescoped reaction for the preparation of praziquantel **1** whereby the flow set-ups for step 1 and step 2 will be telescoped with the use of an in-line liquid-liquid separator, as discussed previously. The generated “pre-praziquantel” **61** is envisaged to be concentrated *in-vacuo* and pumped directly into the batch reactor for the final conversion to praziquantel **1** (Scheme 61).



Scheme 61: Envisioned telescoped reaction for the preparation of praziquantel **1** to be achieved as a part of the future work.

CHAPTER VII

7.1 Experimental

7.1.1 General experimental techniques/details

Optimisations of previously reported batch processes were performed using traditional organic and synthetic chemistry techniques with the utilisation of typical glassware/equipment as found in standard synthetic laboratories. All starting materials and reagents required, excluding those synthesised during this research study, were purchased from fine chemical vendors and used as received with no further purification. Concentration *in-vacuo* signifies the removal of solvent under reduced pressures (approximately 50 – 200 mmHg) at temperatures between 40 °C and 70 °C depending on the identity of the solvent. This was performed with the aid of a standard Heidolph rotary evaporator. Final drying of products/intermediates were performed with the help of a high-pressure pump, additional applied heat and was connected to a solvent trap submerged in liquid nitrogen. Unless otherwise stated, yields were calculated from the immediate synthetic precursors.

7.1.2 Chromatographic separations

The retention factor (R_f) values reported are for thin-layer chromatography (TLC) on aluminium-backed Macherey-Nagel Alugram Sil G/UV254 plates pre-coated with 0.25 mm silica gel 60. A commonly used potassium permanganate dip was applied onto thin-layer chromatography plates to visualize compounds that were not UV active or to increase the visibility on diluted spots. Macherey-Nagel Silica gel 60 (particle size 0.063 – 0.200 mm) was used as the adsorbent for conventional preparative column chromatography. The silica was packed into a column of suitable size and the indicated eluent mixture was passed through the column with additional pressure to ensure that no air bubbles were trapped in the column. The crude product was adsorbed onto silica, loaded onto the pre-packed silica in the column and covered with a cotton wool plug. The elution process was performed with the indicated eluent mixture under gravitational conditions. When performing dry vacuum column chromatography for the batch-based formamide dehydration procedure for the preparation of 2-isocyanoethylbenzene **96**, the silica particles (same dimensions as specified above) were tightly packed, followed by a portion of the mobile phase eluent. This was dried with the use of the vacuum, after which a filter paper was placed on top of the silica and wet-loaded the reaction slurry. Using applied vacuum, the desired eluent mixture was passed through in a portion-wise manner while collecting each portion separately.

7.1.3 Spectroscopy and physical data

^1H -NMR (300 MHz or 400 MHz) and ^{13}C -NMR (75 MHz or 101 MHz) spectra were recorded on a Bruker AVANCE-III-300 or Bruker AVANCE-III-400 spectrometers at 300.13 and 400.13 MHz respectively using standard pulse sequences. All spectra were recorded in deuterated chloroform with tetramethylsilane (TMS) as the internal standard (CDCl_3 at 7.26 ppm, TMS at 0 ppm) while placed in 5 mm NMR spectroscopy tubes. Chemical shifts, δ , are reported in parts per million (ppm) and splitting multiplicity patterns are given as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of triplet of doublets (dtd), triplet (t), broad triplet (br t) triplet of triplets (tt), quartet (q), and multiplet (m). Coupling constants, J , are expressed in Hertz (Hz). In noted cases, conversions were estimated from ^1H -NMR by comparison of definitive integral areas of starting materials and products. All NMR spectra recorded (^1H and ^{13}C -NMR) were compared to known literature spectra and that of praziquantel **1** to an additional purchased pharmaceutical standard.

High-resolution mass spectra (HRMS) were recorded on a Waters Synapt G2 Mass Spectrometer at 70 eV and 200 mA. For analysis, the instrument was operated under the following conditions: a capillary voltage of 2.8 kV (positive mode) and 2.5 kV (negative mode), a sampling cone (ramped from 20 V – 40 V), an extraction cone of 4 V, a source temperature of 100 °C, a desolvation temperature of 200 °C, cone gas of 100 L.h⁻¹, desolvation gas of 500 L.h⁻¹, inert gas source: nitrogen. Samples were made up in analytical grade methanol to an approximate concentration of 10 $\mu\text{g.mL}^{-1}$.

Infrared spectra were obtained on a Bruker ALPHA Platinum ATR spectrometer. The wavenumber (cm^{-1}) of absorptions is reported in the range of 400 – 4000 cm^{-1} , after normalisation (min-max) and smoothing of points (25 smoothing points) processing of the raw spectra, where “vs” denotes very strong signals (75 – 100% relative intensity), “s” for strong signals (50 – 75% relative intensity), “m” for medium signals (25 – 50% relative intensity) and “w” for weak signals (< 25% relative intensity).

Melting points were determined on a Stuart Melting Point SMP10 determination unit with the use of Marienfeld Superior Microhaematocrit capillary tubes (75 +/- 0.5 mm). All reported melting points are uncorrected.

High-performance liquid chromatography for the purity study (HPLC-DAD) performed on the final praziquantel **1** product was achieved on an Agilent 1260 Infinity II series set-up. This comprised of an Agilent 1260 Infinity II series quaternary pump (G7111 B), an Agilent 1260 series autosampler (G7129 A), an integrated column compartment oven (ICC, G7130 A) and an Agilent Infinity 1260 series diode-array detector (DAD, G7115 A). The injection volume was set to 5 μL with the DAD data recorded at several wavelengths with the peakwidth at 2.5 Hz.

The system was controlled by OpenLAB CDS ChemStation software (Edition Rev. C.01.10 [201]). An Agilent Poroshell 120 EC-C18 column (695975-902, 4.6 × 100 mm, 2.7 μm particles) was utilised for all analyses (See Appendix C – 6 for method preparation).

7.1.4 SC-XRD Experimental

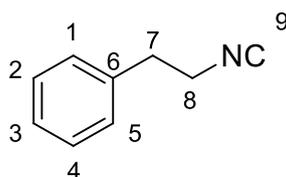
Single crystals of “pre-praziquantel” **61** were analyzed on a Rigaku XtaLAB Synergy R diffractometer, with a rotating-anode Cu X-ray source and a HyPix CCD detector. Data reduction and absorption were carried out using the CrysAlisPro (version 1.171.40.23a) software package.¹⁶⁴ All X-ray diffraction measurements were performed at 150(1) K, using an Oxford Cryogenics Cryostat. All structures were solved by direct methods with SHELXT-2013¹⁶⁵ using the OLEX2¹⁶⁶ interface. All H atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. For data collection and refinement parameters, see Appendix B – 5; Tables B-5.1 – B-5.6.

7.1.5 Nomenclature and numbering

Compounds were named using the standard International Union of Pure and Applied Chemistry (IUPAC) rules and handbooks. However, the numbering system utilised in the illustration of compound diagrams, is one implemented for convenience and is not meant to reflect the systematic numbering of these compounds.

7.2 Chapter II Experimental

7.2.1 Preparation of 2-isocyanoethylbenzene **96**, validation of the Cao *et al.*⁹⁵ methodology:



96

Figure 46: Structure of 2-isocyanoethylbenzene **96** labelled for clarity of NMR peak correlation.

A round-bottomed flask is equipped with a stirrer bar, reflux condenser, and a pressure equalising dropping funnel was charged with 30.00 mL H₂O. NaOH (30.0184 g; 0.7505 mol) was added portion wise to the flask and the mixture was stirred until full dissolution was observed. Subsequently, a mixture of 2-phenylethylamine **41** (11.95 mL; 0.0951 mol), chloroform (7.62 mL; 0.0951 mol) and BTEAC (205.2 mg; 0.901 mmol) in DCM (30.00 mL) was added dropwise over 10 minutes to the reaction flask. The mixture was then allowed to reflux for 4 h after which time it was transferred to an ice and water solution (25 mL). The organic layer was separated and collected, and the aqueous phase was extracted with DCM

(2 × 25 mL). The combined organic phases were washed with a cooled HCl solution (1 M, 3 × 50 mL) and brine (50 mL). The organic phases were collected and dried over anhydrous magnesium sulphate. The drying agent was then removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v with 1% TEA) to afford the desired 2-isocyanoethylbenzene **96** (Figure 46) as a yellow oil (5.14 g, 41 % yield). **¹H NMR** (300 MHz, CDCl₃) δ 7.38 – 7.30 (m, 2H, H-2 & H-4), 7.30 – 7.25 (m, 1H, H-3), 7.25 – 7.18 (m, 2H, H-1 & H-5), 3.60 (tt, *J* = 7.1 & 1.9 Hz, 2H, H-8), 2.98 (tt, *J* = 7.1 & 2.1 Hz, 2H, H-7); **¹³C NMR** (101 MHz, CDCl₃) δ 156.49 (t, *J* = 5.5 Hz, C-9), 136.64 (C-6), 128.71 (C-2 & C-4), 128.65 (C-1 & C-5), 127.18 (C-3), 42.91 (t, *J* = 6.5 Hz, C-8), 35.54 (C-7); **IR** $\nu_{\max}/\text{cm}^{-1}$ (neat) 3064 (C-H stretch aromatic, w), 3030 (C-H stretch aromatic, m), 2941 (m), 2869 (w), 2147 (NC stretch, vs), 1956 (C-H bend aromatic overtone, w), 1877 (C-H bend aromatic overtone, w), 1812 (C-H bend aromatic overtone, w), 1652 (C-C stretch aromatic, m), 1602 (C-C stretch aromatic, w), 1496 (C-C stretch aromatic, s), 1451 (vs), 1353 (m), 1254 (C-H in-plane bend, w), 1082 (C-H in-plane bend, m), 1029 (C-H in-plane bend, m), 982 (m), 946 (m), 907 (w), 820 (w), 745 (vs), 697 (vs), 577 (vs), 498 (vs) and 465 (w); **HRMS** *m/z* (**ES+**) [*M* + *H*]⁺ 132.0781 found, 132.0813 calc; [*M* – NC]⁺ 105.0678 found, 105.0704 calc.

7.2.2 Optimisation of 2-isocyanoethylbenzene **96** synthesis under batch conditions (Table 2, entries 1 – 8):

A representative example (Table 2, entry 8) for the batch optimisations is provided below, please refer to Table 2 for additional details and conditions. A round-bottomed flask was equipped with a stirrer bar, reflux condenser, and a pressure equalising dropping funnel. A 25 M NaOH solution was prepared by weighing NaOH (5.0174 g; 0.125 mol) in a 5.00 mL volumetric flask, following by portion-wise addition of H₂O to the calibration mark and transferred to the round-bottomed flask. Subsequently, a mixture of 2-phenylethylamine **41** (2.08 mL; 0.0165 mol), chloroform (1.32 mL; 0.0165 mol) and BTEAC (75.1 mg; 0.330 mmol) in DCM (5.00 mL) was added dropwise over 10 minutes to the reaction flask. The mixture was allowed to reflux for 4 h after which time it was transferred to an ice and water solution (5 mL). The organic layer was separated and collected, and the aqueous layer was extracted with DCM (2 × 5 mL). The combined organic layers were washed with distilled H₂O (2 × 10 mL) and brine (10 mL). The organic mixture was dried over anhydrous magnesium sulfate, and the drying agent removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford the desired 2-isocyanoethylbenzene **96** as a yellow oil (1.55 g, 72% yield).

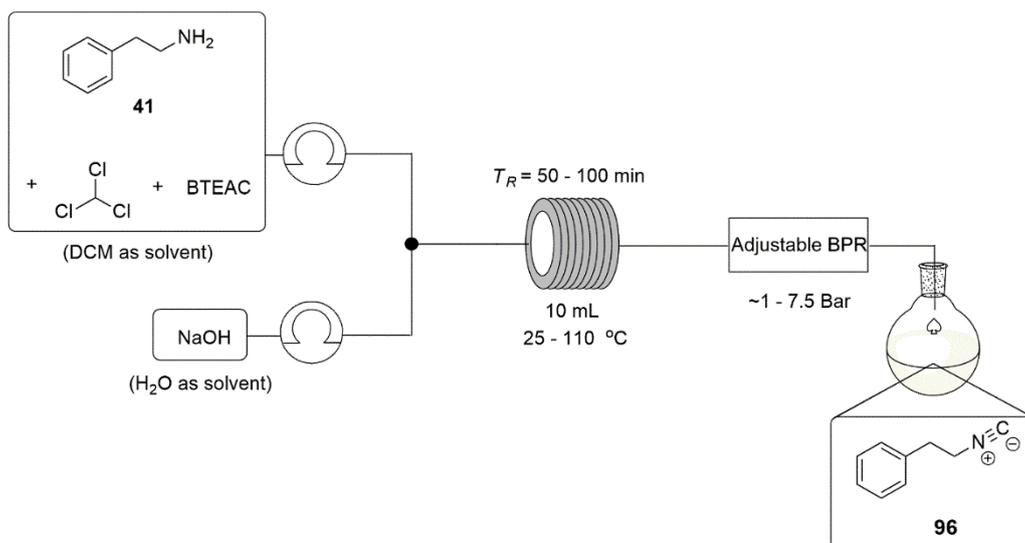
7.2.3 Formamide dehydration approach for 2-isocyanoethylbenzene **96** adapted from Patil *et al.*¹¹⁵

2-Phenethylamine **41** (2.08 mL; 0.0165 mol) was added to a two-neck 50 mL round-bottomed flask followed by the addition of ethyl formate (3.34 mL; 0.0413 mol). This mixture was heated to reflux for 18 h, after which time it was concentrated *in-vacuo* (quantitative conversion with no additional purification). The concentrate was dissolved in DCM (8.25 mL) followed by the addition of NEt₃ (11.50 mL; 0.0825 mol) at ambient temperature. The reaction mixture was allowed to stir for 5 – 10 minutes after which time the flask was lowered into an ice bath at 0 °C and allowed to re-equilibrate. Thereafter POCl₃ (1.54 mL; 0.0165 mol) was added in a dropwise manner and the reaction mixture was allowed to stir for a further 10 – 15 minutes at 0 °C. The resulting slurry was subsequently wet loaded onto a short silica column and eluted with Et₂O (2 × 50 mL) under an applied vacuum and collected. Subsequently, 25 mL portions (90/10 Et₂O/DCM increasing in polarity (10%) until 0/100 Et₂O/DCM) were passed through the column and collected, whereby the product was absent of impurities (excluding the volatile Et₃N which is evaporated off) until the fourth portion of eluent (60/40 Et₂O/DCM). The pure product containing portions (and those with minor Et₃N) were concentrated *in-vacuo* affording 2-isocyanoethylbenzene **96** as a yellow oil (1.79 g, 83%).

7.2.4 Flow synthesis of 2-isocyanoethylbenzene **96** (Table 3, entries 1 – 8):

Entries 1-8, Table 3 were performed on a Vapourtec easy-PhotoChem fitted with two Vapourtec V-3 peristaltic pump heads (Scheme 62).

PFA tubing (32 cm, 1.0 mm internal diameter) was plumbed from each peristaltic pump (threaded fittings hand-tightened) to a standard Y-piece mixer. An additional length of PFA tubing (32 cm, 1.0 mm internal diameter) was plumbed from the Y-piece mixer outlet to a 150 °C rated 10 mL Vapourtec tubular coil reactor (PFA, 1.0 mm internal diameter). A thermocouple was connected from the coil reactor to the Vapourtec unit for direct communication with the interface. A single coil was utilised for entries 1-7 (Table 3), and two 10 mL Vapourtec tubular coil reactors (PFA, 1.0 mm internal diameter) were connected in series via a union for entry 8. A 32 cm, 1.0 mm internal diameter, PFA tubing connected the reactor unit output, via the use of a union, to a pressure adjustable BPR (adjusted manually to prevent DCM gassing out). Finally, another 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 62). Shorter, 1.0 mm internal diameter PFA tubings were then connected to this selector valve, one for collection and one for waste.



Scheme 62: The flow set-up for the preparation of **96** as described in flow entries 1 – 8 (Table 3).

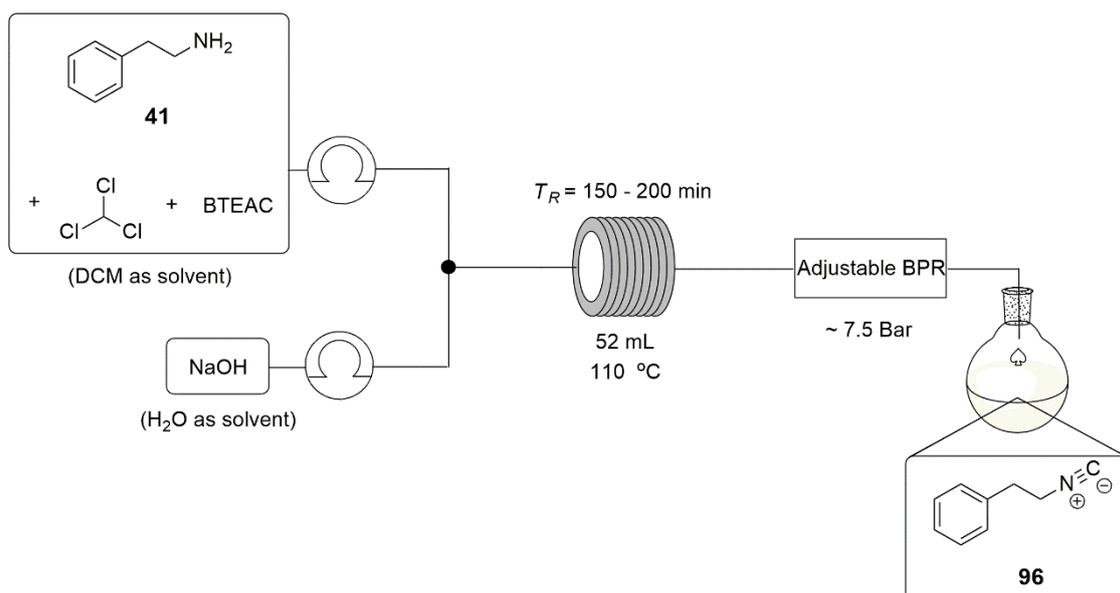
A representative example (Table 3, entry 8) follows. A 7.5 M NaOH stock solution “A” was prepared by dissolving NaOH (1.5031 g; 0.0376 mol) in distilled water in a 5.00 mL volumetric flask. A 1.65 M reactant stock solution “B” was prepared by weighing BTEAC (24.8 mg; 0.109 mmol) in a 5.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.04 mL; 8.27 mmol) was added, followed by chloroform (0.66 mL; 8.24 mmol). Finally, the stock was diluted further to the 5.00 mL calibration mark with DCM.

Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these pushing solvents resulting in a biphasic solvent stream. A volume of 3.00 mL of each stock solution was introduced at a flow rate of 0.100 mL.min⁻¹ ($T_R = 100$ minutes), the two 10 mL Vapourtec tubular coil reactors were heated to 110 °C and the BPR was adjusted to ~ 7.5 bar. Upon collection from the reactor, the organic phase was separated and collected, the aqueous phase was extracted with DCM (2 × 5 mL) and thereafter the organic phases were combined. The combined organic phases were washed with distilled water (2 × 10 mL) and brine (10 mL). The organic phase was dried over anhydrous magnesium sulfate, and then filtered to remove the drying agent. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA). The collected eluent was concentrated *in-vacuo* to afford **96** as a yellow oil (0.34 g, 53 % yield).

7.2.5 Flow synthesis of 2-isocynoethylbenzene **96** (Table 3, entries 9 – 10):

A Vapourtec easy-PhotoChem platform was utilised for entries 9 and 10 (Table 3). A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump to a standard Y-piece mixer. The output stream from the Y-piece mixer was connected to a third 32 cm, 1.0

mm internal diameter, PFA tubing, which in turn was connected to an external coil reactor (Uniqsis HotCoil, 52 mL, 1.5 mm internal diameter, PFA). Thereafter, the output line was connected, with an additional 32 cm, 1.0 mm internal diameter, PFA tubing to a pressure adjustable BPR. Finally, an additional 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 63). Shorter, 1.0 mm internal diameter PFA tubes were connected to this selector valve, one for collection and one for the waste.



Scheme 63: The flow set-up for the preparation of 96 as described in flow entries 9 – 10 (Table 3).

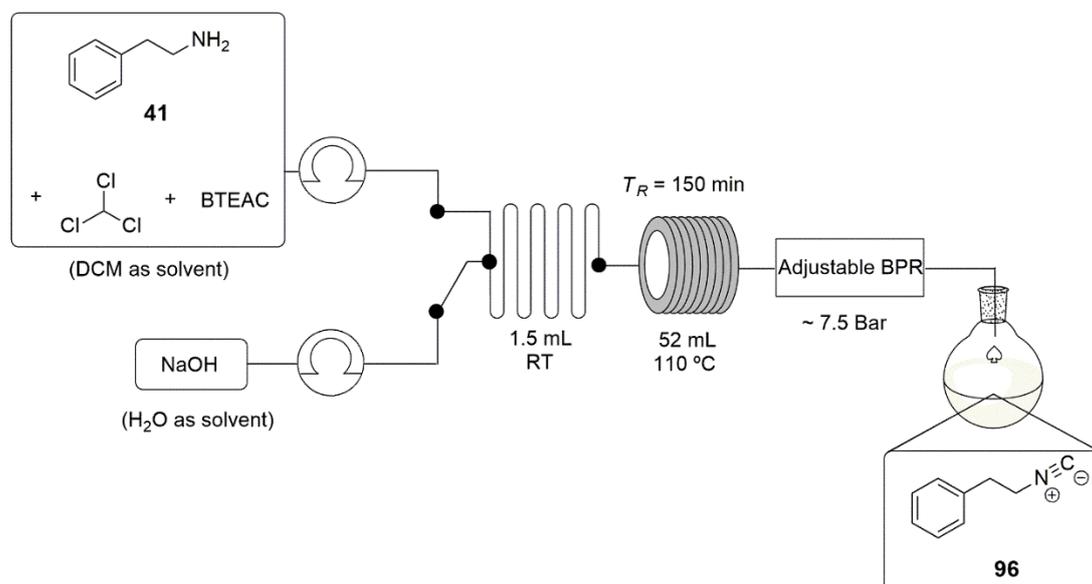
A representative example (Table 3, entry 10) follows. A 7.5 M NaOH stock solution “A” was prepared by dissolving NaOH (3.0027 g; 0.0751 mol) in distilled water in a 10.00 mL volumetric flask. A 1.65 M reactant stock solution “B” was prepared by weighing BTEAC (25.3 mg; 0.111 mmol) in a 5.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.04 mL; 8.27 mmol) was added, followed by chloroform (0.66 mL; 8.24 mmol). Finally, the stock was diluted further to the 5.00 mL calibration mark with DCM.

Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these pushing solvents resulting in a biphasic solvent stream. The flow reactor was set to introduce 6.00 mL from stock solution “A” and 3.00 mL from stock solution “B”. Stock solution “A” was pumped at 0.232 mL.min⁻¹ and “B” at 0.116 mL.min⁻¹ ($T_R = 150$ minutes). The hotcoil coil reactor was heated to 110 °C and the BPR adjusted to ~ 7.5 bar. Upon collection from the reactor, the organic phase was separated and collected, and the aqueous phase was extracted with DCM (2 × 5 mL). The combined organic phases were washed with distilled water (2 × 10 mL) and brine (10 mL). The organic phase was dried over anhydrous

magnesium sulfate, and thereafter, the drying agent was removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford 2-isocyanoethylbenzene **96** as a yellow oil (0.32 g, 49 % yield).

7.2.6 Flow synthesis of 2-isocyanoethylbenzene **96** (Table 3, entries 11 – 12):

A Vapourtec easy-PhotoChem unit was utilised for entries 11 and 12 (Table 3). A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump to a 1.5 mL Vapourtec mixing chip. The output stream was connected to a 50 cm, 1.0 mm internal diameter, PFA tubing, which in turn, was connected to an external coil reactor (Uniqsis HotCoil, 52 mL, 1.5 mm internal diameter, PFA). The output line was connected, with an additional 32 cm, 1.0 mm internal diameter, PFA tubing to a pressure adjustable BPR. A 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 64). Finally, shorter, 1.0 mm internal diameter PFA tubings were connected to the selector valve, one for collection and one for waste.



Scheme 64: The flow set-up for the preparation of **96** as described in flow entries 11 – 12 (Table 3).

A representative example (Table 3, entry 11) follows. An 8.75 M NaOH stock solution “A” was prepared by weighing NaOH (3.5017 g; 0.0875 mol) into a 10.00 mL volumetric flask and dissolving in distilled H₂O to the calibration mark. A 2.2 M reactant stock solution “B” was prepared by weighing BTEAC (31.1 mg; 0.137 mmol) in a 5.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.38 mL; 11.0 mmol) was added, followed by chloroform (0.88 mL; 11.0 mmol). Finally, the stock was diluted to the 5.00 mL calibration mark with DCM.

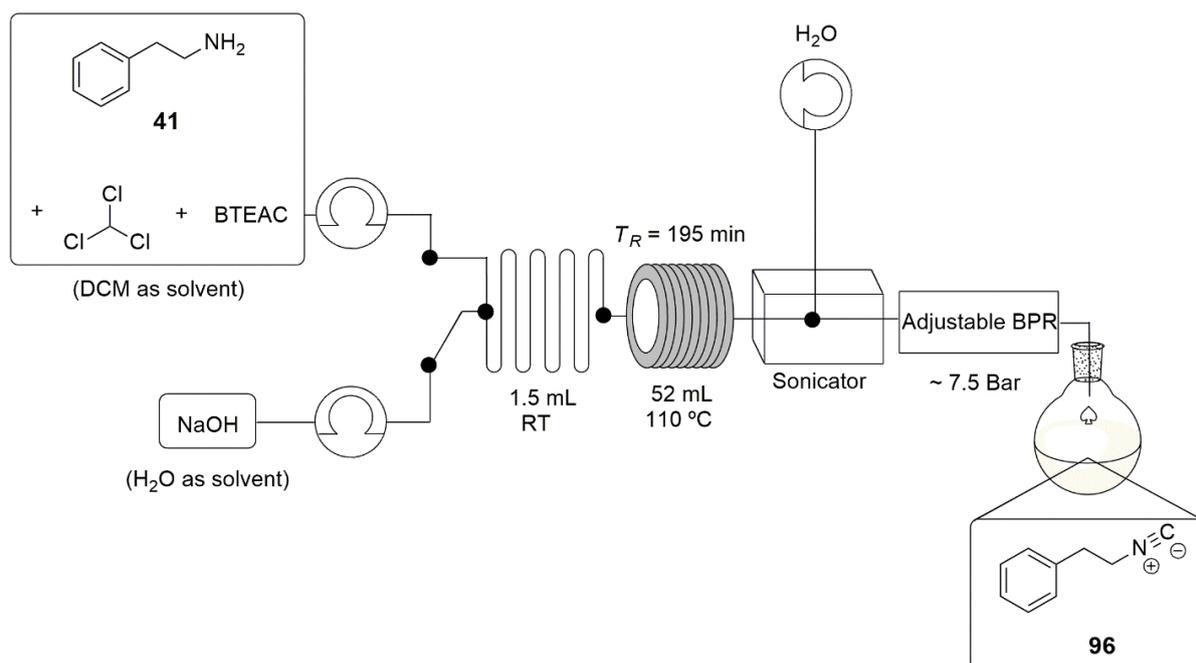
Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these

pushing solvents resulting in a biphasic solvent stream. The flow reactor was set to introduce 7.00 mL from stock solution “A” and 3.50 mL from stock solution “B”. Stock solution “A” was pumped at 0.232 mL.min⁻¹ and “B” at 0.116 mL.min⁻¹ ($T_R = 150$ minutes). The mixing chip was at ambient temperature, hotcoil coil reactor was heated to 110 °C and the BPR adjusted to ~7.5 bar. Upon collection from the reactor, the organic phase was separated and collected, and the aqueous phase was extracted with DCM (2 × 5 mL). The combined organic phases were washed with distilled water (2 × 10 mL) and brine (10 mL). The organic phase was dried over anhydrous magnesium sulfate, and thereafter, the drying agent was removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford 2-isocyanoethylbenzene **96** as a yellow oil (0.46 g, 46 % yield).

7.2.7 Flow synthesis of 2-isocyanoethylbenzene **96** (Table 3, entries 13 – 14):

A Vapourtec easy-PhotoChem unit was utilised for entries 13 and 14 (Table 3). A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump to a 1.5 mL Vapourtec mixing chip. The output stream was connected to a 50 cm, 1.0 mm internal diameter, PFA tubing, which in turn, was connected to an external coil reactor (Uniqsis HotCoil, 52 mL, 1.5 mm internal diameter, PFA). The output line was then directly connected to a second Y-piece mixer, at which point a third peristaltic pump (fitted with red colour-coded tubing) introduced a stream of distilled water. In the case of entry 14 (Table 3), the second Y-piece mixer was submerged in a sonicating bath, at ambient temperature while increasing in temperature (as sonication occurs) as the reaction proceeds. In both instances, the output lines were connected, with an additional 32 cm, 1.0 mm internal diameter, PFA tubing to a pressure adjustable BPR (~ 7.5 bar). Thereafter, a 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 65). Finally, shorter, 1.0 mm internal diameter PFA tubings were connected to the selector valve, one for collection and one for waste.

A representative example (entry 14, Table 3) follows. A 12.5 M NaOH stock solution “A” was prepared by weighing NaOH (4.9974 g; 0.1249 mol) in a 10.00 mL volumetric flask and dissolving to the calibration mark with distilled H₂O. A 1.56 M reactants stock solution “B” was prepared by weighing BTEAC (47.5 mg; 0.209 mmol) in a 10.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.96 mL; 15.6 mmol) was added to this same flask, followed by chloroform (1.25 mL; 15.6 mmol). Finally, the stock was diluted to the 10.00 mL calibration mark with DCM.



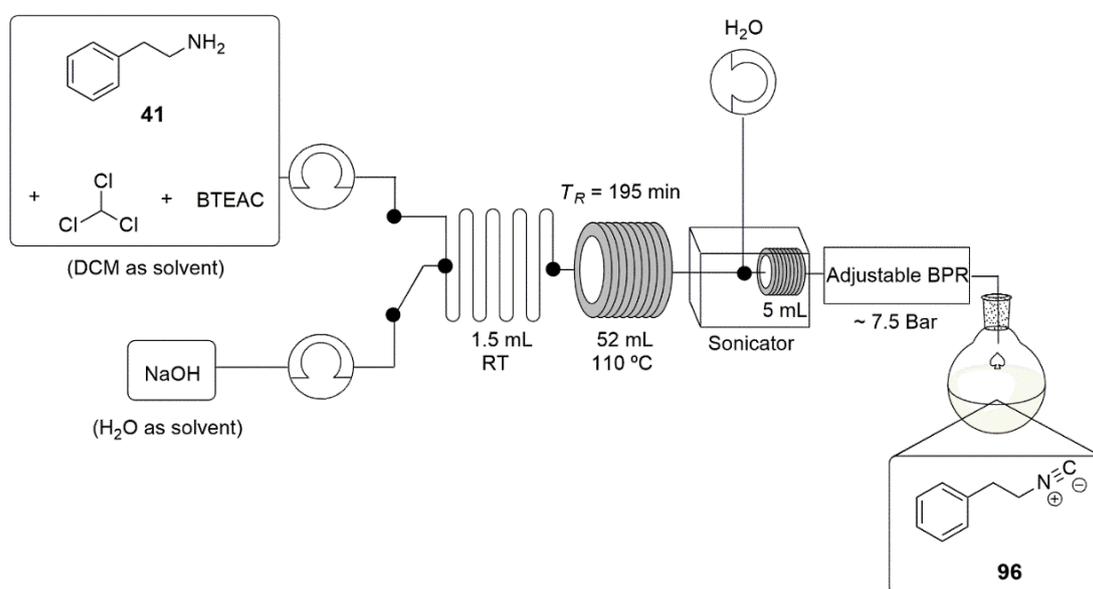
Scheme 65: The flow set-up for the preparation of **96** as described in flow entries 13 – 14 (Table 3). Where entry 13 does not contain the sonicator.

Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these pushing solvents resulting in a biphasic solvent stream. The flow reactor was set to introduce 6.10 mL from both stock solutions “A” and “B”. The stocks were both pumped at 0.173 mL·min⁻¹ (expected $T_R = 150$ minutes). The mixing chip was at ambient temperature, hotcoil coil reactor was heated to 110 °C and the BPR adjusted to ~ 7.5 bar. Due to the additional unplanned residence time, the waste/collection valve was manually operated to only start collection when the reaction stream was visually pumping out of the BPR (visible from yellow DCM phase in reaction stream). Upon collection from the reactor, the organic phase was separated and collected, and the aqueous phase was extracted with DCM (2 × 10 mL). The combined organic phases were washed with distilled water (2 × 15 mL) and brine (15 mL). The organic phase was dried over anhydrous magnesium sulfate, and thereafter, the drying agent was removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford 2-isocyanatoethylbenzene **96** as a yellow oil (0.97 g, 78 % yield).

7.2.8 Flow synthesis of 2-isocyanatoethylbenzene **96** (Table 3, entries 15 – 18):

A Vapourtec R2S+ unit was utilized for entries 15-18 (Table 3). A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump to a 1.5 mL Vapourtec mixing chip. The output stream was connected to a 50 cm, 1.0 mm internal diameter, PFA tubing, which in turn, was connected to an external coil reactor (Uniqsis HotCoil, 52 mL, 1.5 mm

internal diameter, PFA). The output line was then connected to a second Y-piece mixer, with an additional 50 cm, 1.5 mm internal diameter, PTFE tubing. A third stand-alone peristaltic pump (fitted with red colour-coded tubing) was plumbed to the mixer for the introductory stream of distilled water. The Y-piece mixer was fitted directly with a 5 mL, 1.5 mm internal diameter, PTFE tubing and was submerged in a sonicating bath, at ambient temperature while increasing in temperature (as sonication occurs) as the reaction proceeds. This tubing was connected, with an additional 32 cm, 1.0 mm internal diameter, PFA tubing to a pressure adjustable BPR (~ 7.5 bar). Thereafter, a 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 66). Finally, shorter, 1.0 mm internal diameter PFA tubings were connected to the selector valve, one for collection and one for waste.



Scheme 66: The flow set-up for the preparation of 96 as described in flow entries 15 – 18 (Table 3).

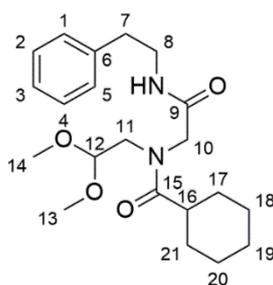
A representative example (entry 15, Table 3) follows. A 12.5 M NaOH stock solution “A” was prepared by weighing NaOH (5.0128 g; 0.125 mol) into a 10.00 mL volumetric flask and dissolved to the 10.00 mL calibration mark with distilled H₂O. A 1.56 M reactants stock solution “B” was prepared by weighing BTEAC (48.3 mg; 0.212 mmol) in a 10.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.96 mL; 15.6 mmol) and chloroform (1.25 mL; 15.6 mmol) was added to this same flask. Finally, the stock was diluted to the 10.00 mL calibration mark with DCM.

Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these pushing solvents resulting in a biphasic solvent stream. The flow reactor was set to introduce 6.10 mL from both stock solutions “A” and “B”. The stocks were both pumped at 0.173 mL.min⁻¹ ($T_R = 150$ minutes). The mixing chip was at ambient temperature, hotcoil coil reactor was

heated to 110 °C and the BPR adjusted to ~ 7.5 bar. Due to the additional unplanned residence time, the waste/collection valve was manually operated to only start collection when the reaction stream was visibly pumping out of the BPR (visible from yellow DCM phase in reaction stream). Upon collection from the reactor, the organic phase was separated and collected, and the aqueous phase was extracted with DCM (2 × 10 mL). The combined organic phases were washed with distilled water (2 × 15 mL) and brine (15 mL). The organic phase was dried over anhydrous magnesium sulfate, and thereafter, the drying agent was removed by filtration. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford 2-isocynoethylbenzene **96** as a yellow oil (0.95 g, 76% yield).

7.3 Chapter III Experimental

7.3.1 Preparation of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)-cyclohexanecarboxamide (“pre-praziquantel”) **61**, validation of the methodology reported by Cao *et al.*⁹⁵



61

Figure 47: Structure of “pre-praziquantel” **61** labelled for clarity of NMR peak correlation.

To a mixture of paraformaldehyde (0.5723 g; 0.0191 mol), aminoacetaldehyde dimethyl acetal **43** (2.08 mL, 0.0191 mol) and cyclohexanecarboxylic acid **51** (2.4426 g; 0.0191 mol) in methanol (20 mL); 2-isocynoethylbenzene **96** (2.5036 g; 0.0191 mol) was added dropwise at 0 °C (Table 5, entry 1). The mixture was then stirred at ambient temperature for 48 h, and thereafter, concentrated *in-vacuo*. The crude residue was dissolved in diethyl ether (25 mL) and washed with distilled H₂O (20 mL), and brine solution (20 mL). Subsequently, the organic phase was dried over anhydrous magnesium sulfate and filtered to remove the drying agent. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA). The collected eluent was concentrated *in-vacuo* to afford “pre-praziquantel” **61** (Figure 47) as a pale-yellow oil which crystallises slowly on standing (6.41 g, 89 % yield). *R_f* = 0.4 (ethyl acetate/hexane 3:2 v/v 1% TEA); *Mp* = 86 – 88 °C ¹H NMR (400 MHz, CDCl₃) δ 7.34 – 7.25 (m, 2H, H-2 & H-4), 7.24 – 7.15 (m, 3H, H-1 & H-3 & H-5), 7.03 (br t, *J* = 5.9 Hz, 0.5H, N-H), 6.51 (br t, *J* = 5.9 Hz, 0.5H, N-H), 4.58 (t, *J* = 5.1 Hz, 0.5H, H-12),

4.39 (t, $J = 5.2$ Hz, 0.5H, H-12), 3.99 (d, $J = 5.9$ Hz, 2H, H-10), 3.52 (dtd, $J = 28.8, 7.1$ & 5.8 Hz, 2H, H-8), 3.42 (t, $J = 5.1$ Hz, 2H, H-11), 3.37 (s, 3H, H-13), 3.33 (s, 3H, H-14), 2.80 (dt, $J = 17.2$ & 7.1 Hz, 2H, H-7), 2.58 (tt, $J = 11.5$ & 3.4 Hz, 0.5H, H-16), 2.25 (tt, $J = 11.5$ & 3.4 Hz, 0.5H, H-16), 1.83 – 1.54 (m, 5H, H-17 & H-21 & H-20), 1.51 – 1.38 (m, 2H, H-20' & H-18), 1.35 – 1.15 (m, 3H, H-18' & H-19); ^{13}C NMR (101 MHz, CDCl_3) δ 178.03 (C-15), 177.83 (C-15), 169.55 (C-9), 169.28 (C-9), 138.82 (C-6), 138.58 (C-6), 128.76 (C-1), 128.68 (C-5), 128.60 (C-3), 126.63 (C-2), 126.47 (C-4), 103.49 (C-12), 102.73 (C-12), 55.49 (C-13), 55.08 (C-14), 54.05 (C-11), 52.11 (C-11), 51.52 (C-10), 50.33 (C-10), 41.06 (C-16), 40.69 (C-8), 40.50 (C-8), 40.29 (C-16), 35.64 (C-7), 35.59 (C-7), 29.39 (C-17), 29.32 (C-21), 25.75 (C-20), 25.67 (C-18), 25.56 (C-19); IR $\nu_{\text{max}}/\text{cm}^{-1}$ (neat) 3283 (N-H stretch secondary amide, m), 3076 (C-H stretch aromatic, w), 2924 (s), 2852 (m), 1676 (C=O stretch secondary amide, s), 1608 (C-C stretch aromatic, vs), 1554 (C-C stretch aromatic, s), 1451 (m), 1415 (m), 1355 (m), 1294 (w), 1249 (m), 1184 (w), 1116 (C-O stretch aliphatic ether, s), 1083 (C-O stretch aliphatic ether, s), 1058 (vs), 1017 (s), 906 (w), 896 (w), 877 (w), 857 (w), 779 (w), 755 (s), 703 (vs), 604 (w), 570 (s), 538 (s), 498 (s) and 438 (w); HRMS m/z (ES+) $[\text{M} + \text{H}]^+$ 377.2433 found, 377.2440 calc; $[\text{M} - \text{OCH}_3]^+$ 345.2166 found, 345.2178 calc.

7.3.2 Method 1 – Batch approach using paraformaldehyde (Table 5, entry 2):

To a mixture of paraformaldehyde (0.1428 g; 4.76 mmol), aminoacetaldehyde dimethyl acetal **43** (0.52 mL, 4.77 mmol) and cyclohexanecarboxylic acid **51** (0.6095 g; 4.76 mmol) in methanol (5 mL); 2-isocyanoethylbenzene **96** (0.6238 g; 4.76 mmol) was added dropwise at 0 °C. This mixture was then stirred at ambient temperature for 48 h and subsequently concentrated *in-vacuo*. An ^1H -NMR was performed on the crude mixture and revealed an approximate conversion of >99%.

7.3.3 Method 2 – Batch approach using formalin (Table 5, entry 3):

To a mixture of formalin (37%) (0.35 mL; 4.70 mmol), aminoacetaldehyde dimethyl acetal **43** (0.52 mL, 4.77 mmol) and cyclohexanecarboxylic acid **51** (0.6103 g; 4.76 mmol) in methanol (5 mL); 2-isocyanoethylbenzene **96** (0.6247 g; 4.76 mmol) was added dropwise at 0 °C. This mixture was then stirred at ambient temperature for 48 h and subsequently concentrated *in-vacuo*. An ^1H -NMR was performed on the crude mixture revealed an approximate conversion of >99%.

7.3.4 Method 3 – Batch approach using gaseous formaldehyde (Table 5, entry 4):

Aminoacetaldehyde dimethyl acetal **43** (0.52 mL, 4.77 mmol) and cyclohexanecarboxylic acid **51** (0.6097 g; 4.76 mmol) in methanol (5 mL) was stirred in a round bottomed flask. In a separate vessel, paraformaldehyde (0.5588 g; 18.61 mmol) was cracked by submerging the vessel into an oil bath set at ~ 120 °C, while in the presence of nitrogen gas, whereby the

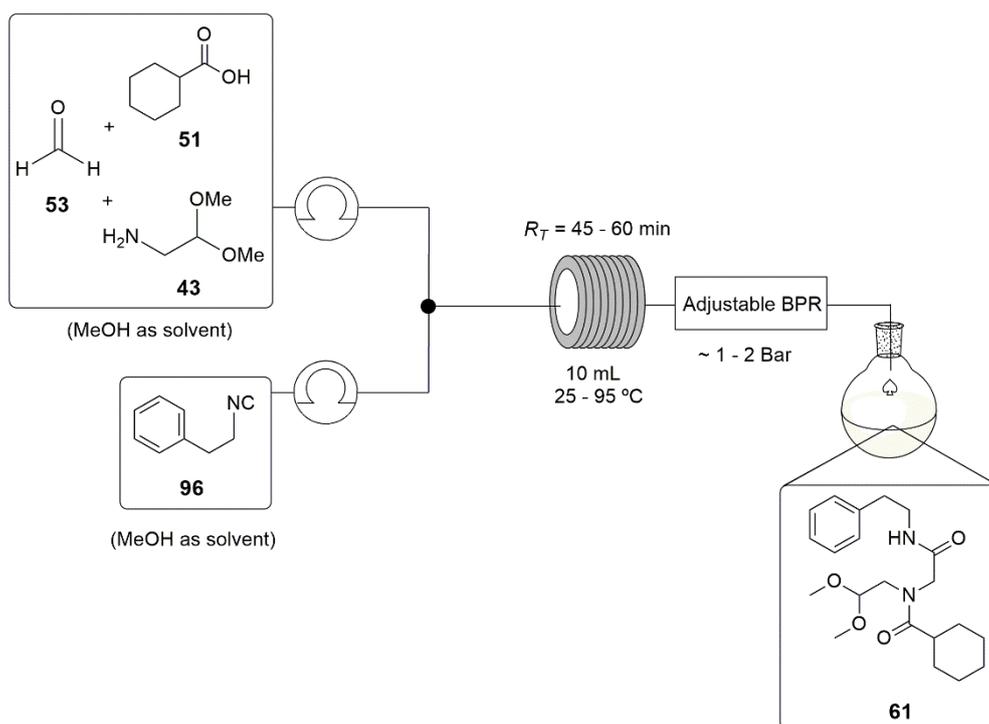
resulting formaldehyde gas was bubbled through the reaction mixture, maintained at ambient temperature, for 1.5 h. Thereafter, 2-isocyanoethylbenzene **96** (0.6238 g; 4.76 mmol) was then added dropwise at 0 °C. This mixture was then stirred at ambient temperature for 48 h and subsequently concentrated *in-vacuo*. An ¹H-NMR was performed on the crude mixture revealed an approximate conversion of >99%.

7.3.5 Synthesis of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)-cyclohexanecarboxamide (“pre-praziquantel”) **61** under flow conditions (Table 6, entries 1 – 9):

A Vapourtec R2S+ flow unit fitted with two Vapourtec V-3 peristaltic pump heads (with blue colour-coded tubes) was employed for entries 1 – 9 (Table 6). A 30 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump (threaded fittings screwed in until hand-tight) to two separate selector valves, which were in turn connected via two 50 cm, 1.0 mm internal diameter, PFA tubes to a standard Y-piece mixer. The mixer outlet was then connected via 32 cm, 1.0 mm internal diameter, PFA tubing to a 150 °C rated 10 mL Vapourtec tubular coil (PFA, 1.0 mm internal diameter) reactor. A thermocouple was connected from the coil reactor to the Vapourtec unit for direct communication with the interface. A 75 cm, 1.0 mm internal diameter, PFA tubing was connected from the reactor unit output, via the use of a union, to a pressure adjustable BPR (adjusted to various pressures required in order to prevent MeOH from gassing out dependent on the temperature). Finally, a 32 cm, 1.0 mm internal diameter, PFA tube was connected from the BPR to the collection/waste selector valve (Scheme 67). Shorter, 1.0 mm internal diameter PFA tubings were connected to this selector valve, one for collection and one for waste.

An equimolar ratio of 2-isocyanoethylbenzene **96** in methanol (Stock solution “A”) and formalin, cyclohexanecarboxylic acid **51** and aminoacetaldehyde dimethyl acetal **43** in methanol (Stock solution “B”) were introduced into the reactor employing methanol as a pushing solvent.

A representative example, entry 9 (Table 6) follows: 2-Isocyanoethylbenzene **96** (0.6559 g, 5.00 mmol) was added to a 5.00 mL volumetric flask, dissolved in MeOH, and diluted to the calibration mark to afford stock solution “A”. An aqueous formalin solution (37%) (0.37 mL, 4.97 mmol), aminoacetaldehyde dimethyl acetal **41** (0.55 mL, 5.05 mmol), and cyclohexanecarboxylic acid **53** (0.6413 g, 5.00 mmol) were added to a 5.00 mL volumetric flask, dissolved, and diluted to the calibration mark to afford stock solution “B”.



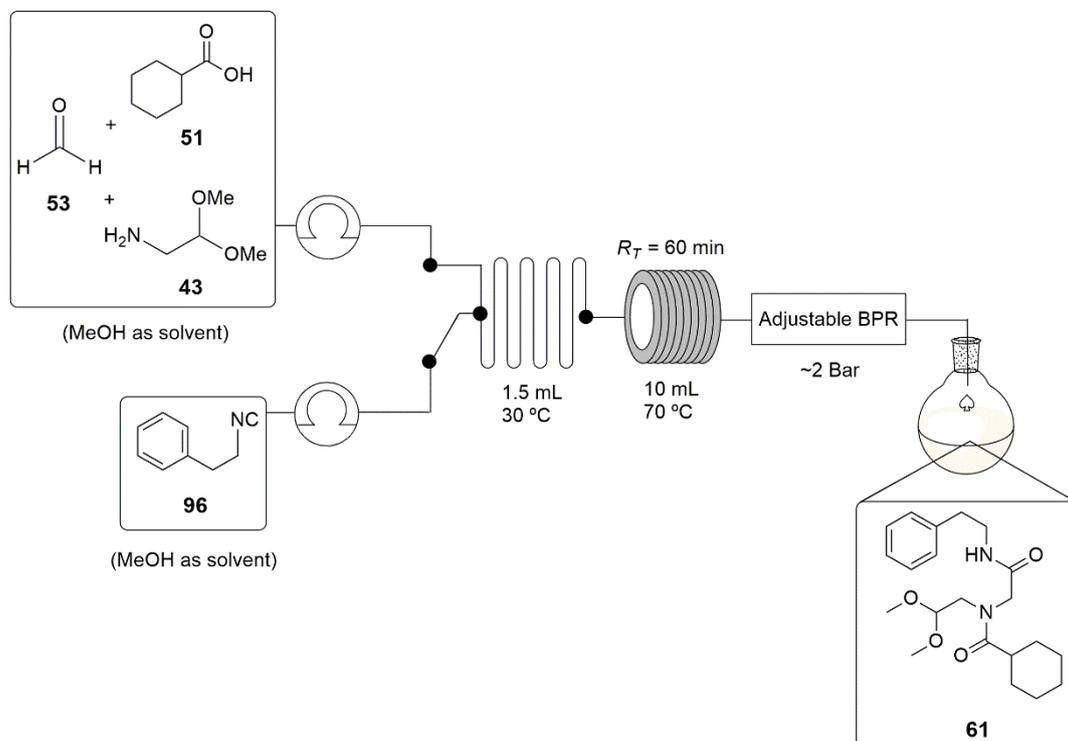
Scheme 67: The flow set-up for the preparation of **61** as described in flow entries 1 – 9 (Table 6).

The reactor was primed with MeOH, which was also used as the pushing solvent. The flow reactor was set to introduce 3.00 mL from both stock solutions “A” and “B”. The stocks were both pumped at $0.083 \text{ mL}\cdot\text{min}^{-1}$ ($T_R = 60$ minutes). The coil reactor was heated to $70 \text{ }^\circ\text{C}$ and the BPR adjusted to ~ 2 bar. Upon collection, the reaction mixture was concentrated *in-vacuo* and the resulting residue was re-dissolved in diethyl ether (15 mL) and washed with distilled H_2O (10 mL) and a brine solution (10 mL). Thereafter, the organic phase was dried over anhydrous magnesium sulfate and filtered to remove the drying agent. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA) to afford “pre-praziquantel” **61** as a pale-yellow oil which crystallised upon standing (0.95 g, 85 % yield).

7.3.6 Optimised synthesis of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)-ethyl)cyclohexanecarboxamide (“pre-praziquantel”) **61** under flow conditions (Table 6, entries 10 – 13):

A Vapourtec R2S+ flow unit fitted with two Vapourtec V-3 peristaltic pump heads (with blue colour-coded tubes) was employed for entries 10 – 13 (Table 6). A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump (threaded fittings screwed in until hand-tight) to a 1.5 mL Vapourtec mixing chip (held at $30 \text{ }^\circ\text{C}$), which was connected in series, with the use of an additional 32 cm, 1.0 mm internal diameter, PFA tubing, to a $150 \text{ }^\circ\text{C}$ rated 10 mL Vapourtec tubular coil (PFA, 1.0 mm internal diameter) reactor. A thermocouple was connected from the coil reactor and mixing chip to the Vapourtec unit for direct

communication with the interface. A 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the reactor unit output, via the use of a union, to a pressure adjustable BPR. Lastly, a 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 68). Shorter, 1.0 mm internal diameter PFA tubings were connected to this selector valve, one for collection and one for waste.



Scheme 68: The flow set-up for the preparation of **61** as described in flow entries 10 – 13 (Table 6).

A representative example, entry 10 (Table 6) follows: 2-Isocyanatoethylbenzene **96** (0.6589 g, 5.02 mmol) was added to a 5.00 mL volumetric flask, dissolved in MeOH, and diluted to the calibration line to afford stock solution “A”. An aqueous formalin solution (37%) (0.37 mL, 4.97 mmol), aminoacetaldehyde dimethyl acetal **41** (0.55 mL, 5.05 mmol), and cyclohexanecarboxylic acid **53** (0.6405 g, 5.00 mmol) was added to a 5.00 mL volumetric flask, dissolved in MeOH, and diluted to the calibration line to afford stock solution “B”.

The reactor was primed with MeOH, which was also used as the pushing solvent. The flow reactor was set to introduce 3.30 mL from both stock solutions “A” and “B”. The stocks were both pumped at $0.083 \text{ mL}\cdot\text{min}^{-1}$ ($T_R = 60$ minutes). The coil reactor was heated to $70 \text{ }^\circ\text{C}$ and the BPR adjusted to ~ 2 bar. Upon collection, the mixture was concentrated *in-vacuo* and the resulting residue was dissolved in diethyl ether (15 mL) and washed with distilled H_2O (10 mL) and a brine solution (10 mL). Thereafter, the organic phase was dried over anhydrous magnesium sulfate and filtered to remove the drying agent. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA). The

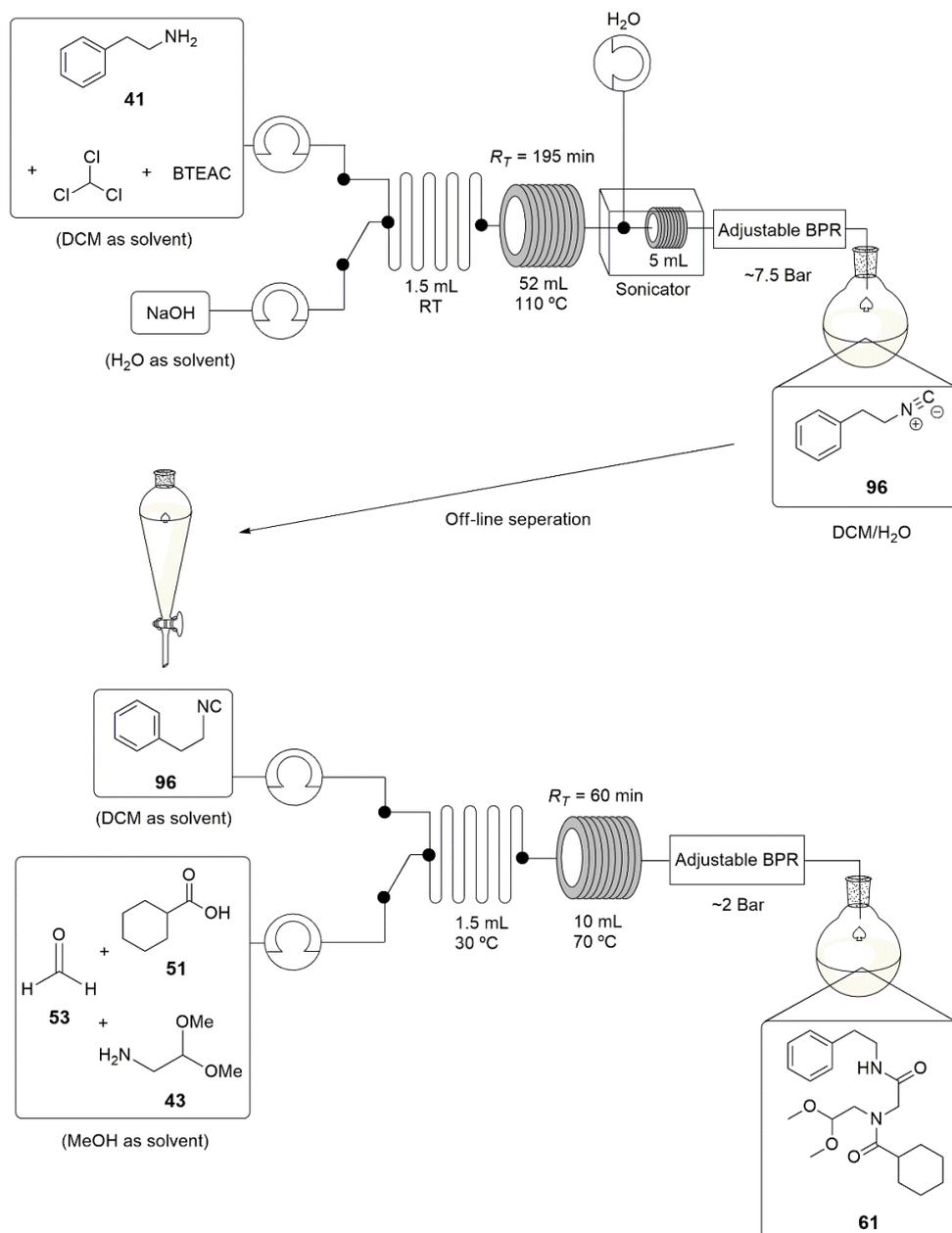
collected eluent was concentrated *in-vacuo*, to afford “pre-praziquantel” **61** as a pale yellowish oil that crystallised on standing (1.07 g, 87 % yield).

7.3.7 Telescoped synthesis of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)-ethyl)cyclohexanecarboxamide (“pre-praziquantel”) **61** trial reaction:

A Vapourtec R2S+ unit was utilized for this telescoped reaction. The flow set-up for step 1 was achieved as described previously for entry 15, Table 3 (Scheme 66). A 12.5 M NaOH stock solution “A” was prepared by weighing NaOH (5.0068 g; 0.1252 mol) into a 10.00 mL volumetric flask and dissolved to the 10.00 mL calibration mark with distilled H₂O. A 1.56 M reactants stock solution “B” was prepared by weighing BTEAC (47.4 mg; 0.208 mmol) in a 10.00 mL volumetric flask followed by a minimum addition of DCM for dissolution. Subsequently, 2-phenethylamine **41** (1.96 mL; 15.6 mmol) and chloroform (1.25 mL; 15.6 mmol) was added to this same flask. Finally, the stock was diluted to the 10.00 mL calibration mark with DCM.

Distilled H₂O was utilised as the pushing solvent for stock solution “A” and DCM for stock solution “B”. Prior to the injection of the stock solutions the reactor was primed with these pushing solvents resulting in a biphasic solvent stream. The flow reactor was set to introduce 6.10 mL from both stock solutions “A” and “B”. The stocks were both pumped at 0.173 mL.min⁻¹ ($T_R = 150$ minutes). The mixing chip was at ambient temperature, hotcoil coil reactor was heated to 110 °C and the BPR adjusted to ~ 7.5 bar. Due to the additional unplanned residence time, the waste/collection valve was manually operated to only start collection after the leading tail (~ 0.75 mL) was pumped to waste to ensure the collected stream was at an approximate steady state. The collection was changed to waste after 4.50 mL of DCM (excluding upper layer aqueous phase) was collected with the use of a measuring cylinder. Upon collection from the reactor, the organic phase (4.50 mL) was separated and collected (73.7% of total DCM product stream). Based on the assumption of ~ 80% conversion, this amounts to a 1.25 M 2-isocynoethylbenzene **96** stock solution which was used directly in the next step as stock solution “A”.

A Vapourtec R2S+ flow unit was employed for step 2 and set-up as described previously for entry 10, Table 6 (Scheme 68). Stock solution “A” in DCM was already prepared as described above from step 1. An aqueous formalin solution (37%) (0.64 mL, 8.59 mmol), aminoacetaldehyde dimethyl acetal **41** (0.93 mL, 8.54 mmol), and cyclohexanecarboxylic acid **53** (1.0954 g, 8.55 mmol) was added to a 5.00 mL volumetric flask, dissolved in MeOH, and diluted to the calibration line to afford a 1.7 M stock solution “B” (Scheme 69).



Scheme 69: The telescoped flow set-up for the preparation of **61** as described for the trial telescoped reaction.

The reactor was primed with DCM and MeOH, which was also used as the pushing solvent for stock solutions “A” and “B”, respectively. The flow reactor was set to introduce 2.35 mL from both stock solutions “A” and “B”. The stocks were both pumped at $0.083 \text{ mL}\cdot\text{min}^{-1}$ ($T_R = 60$ minutes). The coil reactor was heated to 70 °C and the BPR adjusted to ~ 2 bar. Upon collection, the mixture was concentrated *in-vacuo* and the resulting residue was dissolved in diethyl ether (10 mL) and washed with distilled H₂O (7.5 mL) and a brine solution (7.5 mL). Thereafter, the organic phase was dried over anhydrous magnesium sulfate and filtered to remove the drying agent. The filtrate was concentrated *in-vacuo* and purified by column chromatography (ethyl acetate/hexane 3:2 v/v 1% TEA). The collected eluent was

concentrated *in-vacuo*, to afford “pre-praziquantel” **61** as a pale yellowish oil that crystallised on standing (0.76 g, 69% yield). This amounts to ~ 55% over both steps.

7.4 Chapter IV Experimental

7.4.1 Synthesis of 2-(cyclohexylcarbonyl)-2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one (praziquantel) **1**, validation of the methodology reported by Cao *et al.*⁹⁵ (Table 7, entry 2):

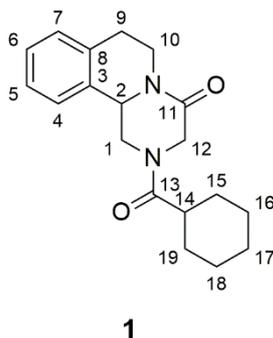


Figure 48: Structure of praziquantel **1** labelled for clarity of NMR peak correlation.

“Pre-praziquantel” **61** (1.9954 g, 5.30 mmol) was added portion-wise to MSA (6.91 mL, 106.4 mmol) at 0 °C. The mixture was heated to 70 °C for 6 h and thereafter, it was poured into an ice-water mixture. The pH was adjusted to 8 with an aqueous 20% NaOH solution. The solution was then extracted with diethyl ether (4 × 10 mL). The combined organic layers were washed with brine (15 mL), dried with anhydrous magnesium sulfate and concentrated *in-vacuo* to afford praziquantel **1** as a yellowish solid. The crude product was recrystallised from an ethyl acetate/ hexane mixture (1:1) to afford praziquantel **1** (Figure 48) as a pale yellow/white solid (0.38 g, 23 % yield). R_f = 0.6 (ethyl acetate/chloroform 1:4 v/v); **Mp** = 134 – 137 °C; **¹H NMR** (400 MHz, CDCl₃) δ 7.35 – 7.14 (m, 4H, H-4 & H-5 & H-6 & H-7), 5.17 (dd, J = 13.4, 2.6 Hz, 1H, H-1), 4.95 – 4.68 (m, 2H, H-2 & H-10), 4.47 (d, J = 17.4 Hz, 1H, H-12), 4.08 (d, J = 17.4 Hz, 1H, H-12'), 3.04 – 2.75 (m, 4H, H-9 & H-9' & H-1' & H-10'), 2.47 (tt, J = 11.4, 3.2 Hz 1H, H-14), 1.95 – 1.65 (m, 5H, H-19 & H-15 & H-18), 1.64 – 1.44 (m, 2H, H-18' & H-16), 1.42 – 1.19 (m, 3H, H-16' & H-17); **¹³C NMR** (101 MHz, CDCl₃) δ 174.69 (C-13), 164.33 (C-11), 134.65 (C-8), 132.69 (C-3), 129.22 (C-4), 127.37 (C-7), 126.90 (C-5), 125.40 (C-6), 54.87 (C-2), 48.94 (C-12), 45.06 (C-1), 40.71 (C-14), 39.01 (C-10), 29.16 (C-9), 28.92 (C-15 & C-19), 28.64 (C-16 & C-18), 25.63 (C-17); **IR** $\nu_{\max}/\text{cm}^{-1}$ (**neat**) 2926 (s), 2854 (s), 1625 (C=O stretch tertiary amide, vs), 1495 (C-C stretch aromatic, m), 1422 (vs), 1357 (s), 1325 (s), 1296 (s), 1252 (C-H in-plane bend, s), 1213 (C-H in-plane bend, s), 1129 (C-H in-plane bend, s), 1087 (C-H in-plane bend, m), 1055 (C-H in-plane bend, w), 1028 (C-H in-plane bend, m), 995 (m), 961 (w), 888 (m), 767 (vs), 727 (m), 692 (m), 623 (m), 569 (w) and 446 (m); **HRMS** m/z (**ES+**) [M + H]⁺ 313.1891 found, 313.1916 calc.

7.4.2 Attempted synthesis of praziquantel **1** using MSA with DCM (Table 7, entry 4):

“Pre-praziquantel” **61** (0.5007 g; 1.33 mmol) was dissolved in DCM (2.51 mL) and added portion-wise to MSA (1.73 mL; 26.6 mmol) at 0 °C. After addition was completed, the mixture was heated to reflux for 48 h after which time it was poured into an ice-water mixture. The pH was adjusted to 8 with aqueous 20% NaOH while stirring rapidly. The solution was then extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a thick yellow residue. ¹H-NMR analysis of the crude material showed trace amounts of product formed, isolation attempts proved futile.

7.4.3 Attempted synthesis of praziquantel **1** using *p*-toluenesulfonic acid with MeOH (Table 7, entry 7):

“Pre-praziquantel” (0.0551 g; 0.146 mmol) is added portion wise to *p*-toluenesulfonic acid (0.5047 g; 2.93 mmol) in MeOH (0.4 mL) at 0 °C. After the addition was complete, the mixture was refluxed for 6 h and poured into an ice-water mixture which was then adjusted to pH 8 with an aqueous 20% NaOH solution. The solution was then extracted with diethyl ether (4 × 10 mL). The combined organic layers are washed with brine (20 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a yellow residue. Unfortunately, based on NMR analysis of the crude residue, no product formation was observed.

7.4.4 Attempted synthesis of praziquantel **1** using Amberlyst-15 with MeOH (Table 7, entry 8):

“Pre-praziquantel” (0.4982 g; 1.32 mmol) is added portion wise to Amberlyst-15 (1.6893 g; 5.37 mmol) in MeOH (2 mL) at 0 °C. After addition complete, mixture was refluxed for 6 h, filtered off amberlyst beads, concentrated *in-vacuo* and re-dissolved in a minimal amount of diethyl ether. The mixture was poured into an ice-water mixture which was then adjusted to pH 8 with an aqueous 20% NaOH solution while rapidly mixing. The solution is then extracted with diethyl ether (4 × 10 mL). The combined organic layers are washed with brine (20 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a yellow residue. Unfortunately, based on NMR analysis of the crude residue, no product formation was observed.

7.4.5 Attempted synthesis of praziquantel **1** using *p*-toluenesulfonic acid with DCM (Table 7, entry 9):

“Pre-praziquantel” **61** (0.6242 g; 1.66 mmol) in DCM (6 mL) was added portion-wise to anhydrous *p*-toluenesulfonic acid (5.5131 g; 32.0 mmol) at 0 °C. After the addition was complete, the mixture was heated to reflux for 72 h and thereafter poured into an ice-water

mixture. The pH was adjusted to 8 with aqueous 20% NaOH solution and the solution was then extracted with DCM (3×10 mL). The combined organic layers are washed with brine (15 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a yellow residue. Unfortunately, based on NMR analysis of the crude residue, no product formation was observed.

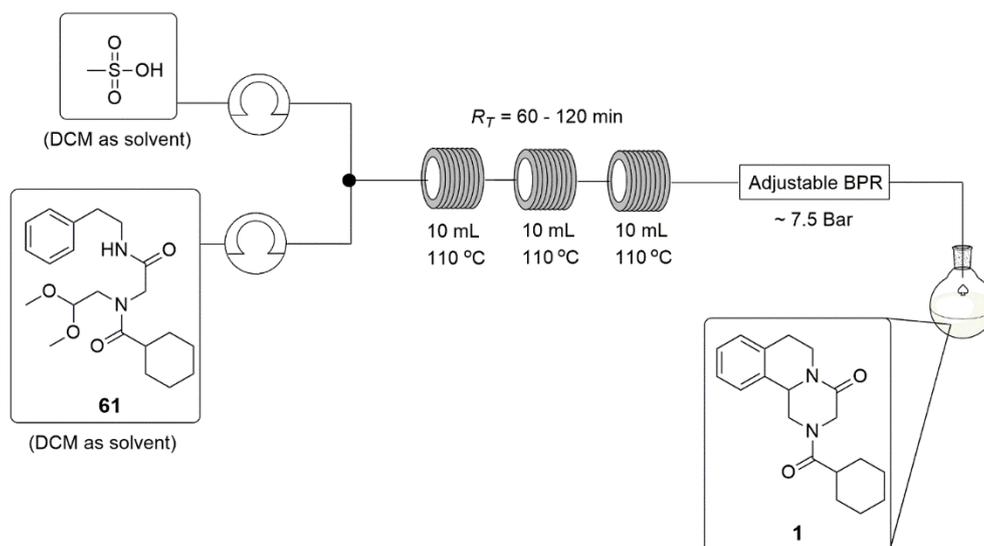
7.4.6 Attempted synthesis of praziquantel **1** using Amberlyst-15 with DCM (Table 7, entry 10):

“Pre-praziquantel” **61** (0.6118 g; 1.63 mmol) in DCM (15 mL) was added portion wise to Amberlyst-15 (10.1637 g; 32.3 mmol) at 0 °C. After the addition was complete, the mixture was heated to reflux for 72 h while stirring slowly. Thereafter the amberlyst beads were removed by filtration and the reaction mixture was poured into an ice-water mixture. The pH was adjusted to 8 with aqueous 20% NaOH solution while stirring rapidly. The solution was then extracted with DCM (3×10 mL). The combined organic layers were washed with brine (15 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a yellowish solid. Unfortunately, based on NMR analysis of the crude residue, no product formation was observed.

7.4.7 Attempted synthesis of praziquantel **1** under flow conditions (Table 9, entries 1 – 2):

A Vapourtec R2S+ flow unit was employed fitted with two Vapourtec V-3 peristaltic pump heads (blue colour-coded tubing). A 32 cm long, 1.0 mm internal diameter, PFA tube was plumbed from each peristaltic pump (threaded fittings screwed in until hand-tight) to a standard Y-piece mixer, which was in turn connected, with the use of an additional 32 cm, 1.0 mm internal diameter, PFA tubing, to three 150 °C rated 10 mL Vapourtec tubular coil (PFA, 1.0 mm internal diameter) reactors, connected in series. A thermocouple was connected from each of the coil reactors to the Vapourtec unit for direct communication with the interface. A 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the third reactor unit output, via the use of a union, to a pressure adjustable BPR. Finally, another 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve

(Scheme 70). Shorter, 1.0 mm internal diameter PFA tubings are connected to this selector valve, one for collection and one for waste.



Scheme 70: The flow set-up for the preparation of **1** as described in flow entries 1 – 2 (Table 9).

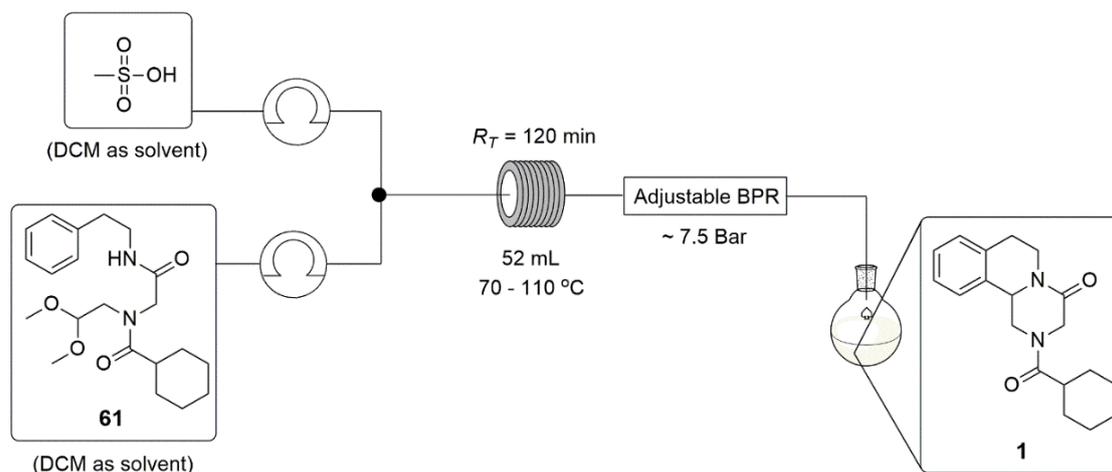
A representative example, entry 1 (Table 9) follows. A 10.7 M MSA stock solution was prepared by adding MSA (3.46 mL; 53.3 mmol) to a 5.00 mL volumetric flask and diluted with DCM until the calibration line to afford stock solution “A”. A 0.53 M “pre-praziquantel” **61** stock solution was prepared by weighing out **61** (1.0041 g; 2.67 mmol) into a 5.00 mL volumetric flask and diluted with DCM until the calibration line to afford stock solution “B”.

The flow reactor was primed with DCM, which was also used as the pushing solvent. The flow reactor was set to introduce 3.00 mL from each stock solution. The stocks were introduced at a flow rate of 0.250 mL·min⁻¹ ($T_R = 60$ min), the coil reactors were heated to 110 °C and the adjustable BPR was set to ~ 7.5 bar. Upon collection, the reaction mixture was poured into an ice-water solution. The pH was adjusted to 8 with aqueous 20% NaOH solution while stirring rapidly. The solution was then extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a thick yellow residue. Unfortunately, based on NMR analysis of the crude residue no product formation was observed.

7.4.8 Attempted synthesis of praziquantel **1** under flow conditions (Table 9, entries 3 – 5):

A Vapourtec easy-PhotoChem flow unit fitted with two V-3 peristaltic pump heads (with blue colour-coded tubing) was employed. A 32 cm, 1.0 mm internal diameter, PFA tubing was plumbed from each peristaltic pump (threaded fittings screwed in until hand-tight) to a Y-piece mixer, which was then connected, with the use of an additional 32 cm, 1.0 mm internal diameter, PFA tubing, to an external PFA coil reactor (Uniqsis HotCoil, 52 mL, 1.5 mm internal

diameter). A 32 cm, 1.0 mm internal diameter, PFA tubing was then connected from the reactor unit output, via the use of a union, to a pressure adjustable BPR. Finally, a 32 cm, 1.0 mm internal diameter, PFA tubing was connected from the BPR to the collection/waste selector valve (Scheme 71). Shorter, 1.0 mm internal diameter PFA tubings are connected to this selector valve, one for collection and one for waste.



Scheme 71: The flow set-up for the preparation of **1** as described in flow entries 3 – 5 (Table 9).

A representative example, entry 3 (Table 9) follows. A 10.0 M MSA stock solution was prepared by adding MSA (3.25 mL; 50.0 mmol) to a 5.00 mL volumetric flask and diluted with DCM until the calibration line to afford stock solution “A”. A 0.50 M “pre-praziquantel” **61** stock solution was prepared by weighing out **61** (0.9465 g; 2.51 mmol) into a 5.00 mL volumetric flask and diluted with DCM until the calibration line to afford stock solution “B”.

The flow reactor was primed with DCM, which was also used as the pushing solvent. The flow reactor was set to introduce 3.25 mL from each stock solution. The stocks were introduced at a flow rate of 0.217 mL·min⁻¹ ($T_R = 120$ min), the coil reactors were heated to 110 °C and the adjustable BPR was set to ~ 7.5 bar. Upon collection, the reaction mixture was poured into an ice-water solution. The pH was adjusted to 8 with aqueous 20% NaOH solution while stirring rapidly. The solution was then extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous magnesium sulfate, filtered, and then concentrated *in-vacuo* to afford a thick yellow residue. Unfortunately, based on NMR analysis of the crude residue, no product formation was observed.

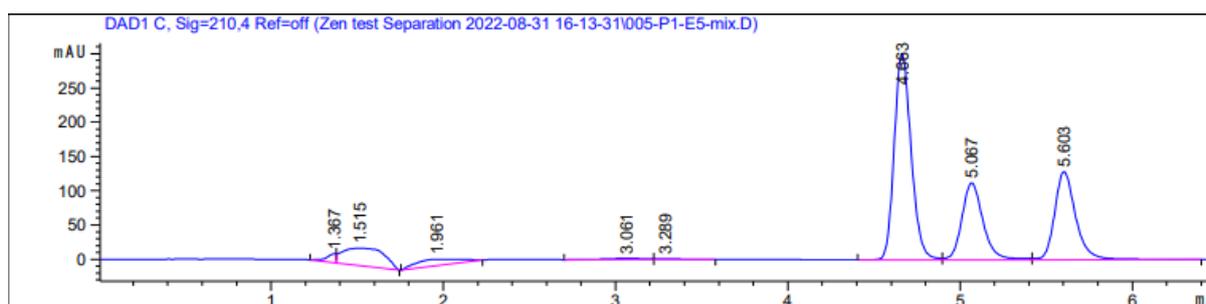
7.4.9 Batch preparation of praziquantel **1** using sodium sulfate as an additive (Table 10, entry 3):

Sodium sulfate (1.1647 g; 8.20 mmol) was added to MSA (4.32 mL; 66.5 mmol) at ambient temperature. “Pre-praziquantel” **61** (1.2518 g; 3.32 mmol) was then added to the mixture in a portion-wise manner. After the addition was complete, the mixture was heated to 70 °C for 6 h and poured into an ice-water mixture. The pH was adjusted to 8 with an aqueous 20% NaOH

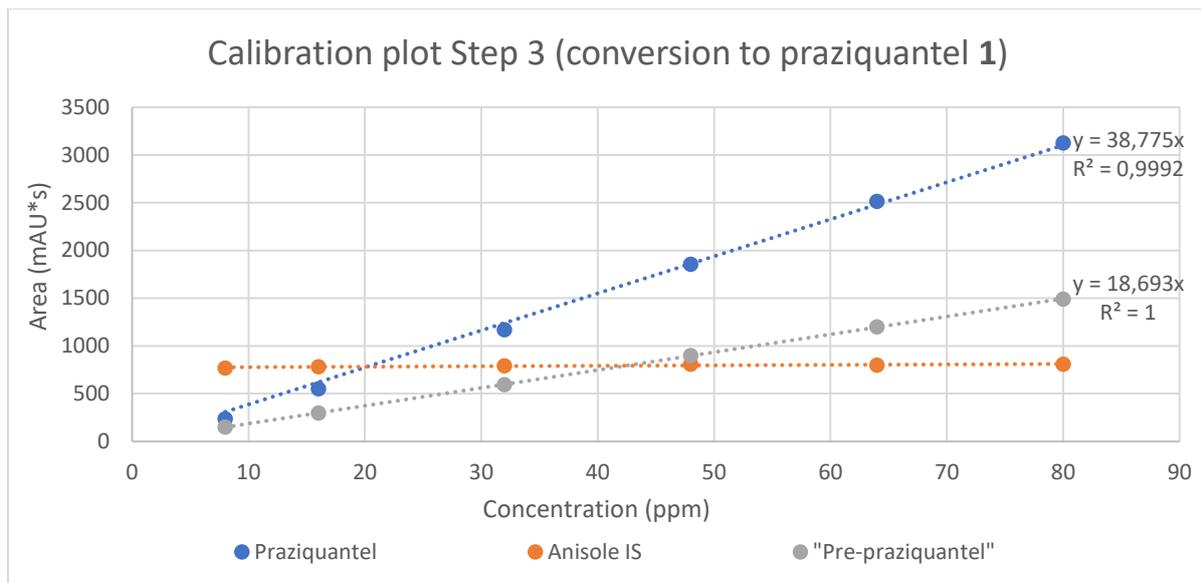
solution. The solution is then extracted with diethyl ether (4 × 15 mL) and the combined organic layers were washed with brine (20 mL), dried with anhydrous sodium sulfate, filtered, and then concentrated *in-vacuo* to afford a yellowish solid. Purification by recrystallisation from diethyl ether to afford pure praziquantel **1** as a white solid (0.63 g, 61% yield, 81% purity).

7.4.10: UPLC raw data for the separation of “pre-praziquantel” **61** and praziquantel **1**

The method utilised consisted of a mobile phase being 40% (10 mM phosphate buffer aqueous solution), 45% (ACN) and 15% (water). The input volume was set at 5 µL with a total runtime of 6.5 min. All samples were prepared using HPLC grade ACN. Praziquantel **1** eluted with a residence time of 4.663 min, anisole (internal standard) eluted in 5.067 min and “pre-praziquantel” **61** eluted in 5.603 min with the best responses observed at 210,4 nm wavelength. The calibration plot was achieved performing triplicate analyses of the respective sample mixtures (varying concentration between 8 – 80 ppm) prepared with “pre-praziquantel” **61** purified during this research study, HPLC grade anisole and a pharmaceutical praziquantel **1** standard.



[PPM]	Praziquantel 1 (210,4 nm DAD1 C)			“Pre-praziquantel” 61 (210,4 nm DAD1 C)			Anisole 32 ppm (IS) (210,4 nm DAD1 C)		
8	237,4869	242,86447	229,82599	149,35857	151,52831	148,15759	781,94629	788,3725	736,92694
16	566,8772	546,69598	542,44562	301,74182	290,69766	296,28305	792,6192	781,96106	773,0603
32	1181,789	1143,10278	1189,86414	590,63293	601,42859	597,68927	788,04425	792,68256	798,04565
48	1867,745	1874,42957	1822,4137	896,72406	902,63892	897,98602	812,56854	808,8642	804,28003
64	2516,45	2529,51318	2500,59741	1208,95801	1204,88489	1191,118	808,55646	775,92261	809,46497
80	3114,15	3164,26123	3103,73657	1485,04846	1493,59192	1494,9863	803,09229	819,09003	802,0672
	Average			Average			Average		
8		236,725793			149,68149			769,08191	
16		552,006267			296,24084			782,546853	
32		1171,58521			596,583597			792,924153	
48		1854,86263			899,116333			808,570923	
64		2515,52018			1201,65365			797,981347	
80		3127,38257			1491,20890			808,083173	



We prepared a theoretical “40 ppm” praziquantel 1 solution starting with a weighed-out amount of 61.2 mg of praziquantel synthesised using sodium sulfate as the additive (Table 10, entry 3). We then ran the solution in duplicates for the purity calculation and recorded the observed areas for each run obtained from the UPLC. The data recorded is tabulated below:

UPLC run	Observed area (mAU*s)
1	1266,26135
2	1244,26392
Average area (mAU*s)	
1255,262635	
Actual concentration (ppm)	
32,37298865	
% Purity	
80,9324716 %	

Using the average area obtained and substituting into the linear equation of best fit for the prepared calibration plot, we were able to determine that the praziquantel 1 synthesised was 80.93% (~ 81%) pure.

CHAPTER VIII

8.1 References

1. WHO Neglected tropical diseases. https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1 (accessed 2022/07/27).
2. Riley, D. L.; Strydom, I.; Chikwamba, R.; Panayides, J.-L., Landscape and opportunities for active pharmaceutical ingredient manufacturing in developing African economies. *Reaction Chemistry & Engineering* **2019**, *4* (3), 457-489.
3. WHO Schistosomiasis. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis> (accessed 2022/07/27).
4. Melissa, D. B.; Vinogrin, D.; Jagidesa, M., Schistosomiasis infections in South African pregnant women: A review. *Southern African Journal of Infectious Diseases* **2020**, *35* (1), 1-7.
5. Neyt, N. C.; Riley, D. L., Batch-flow hybrid synthesis of the antipsychotic clozapine. *Reaction Chemistry & Engineering* **2018**, *3* (1), 17-24.
6. Neyt, N. C.; Riley, D. L., Mild and selective reduction of aldehydes utilising sodium dithionite under flow conditions. *Beilstein Journal of Organic Chemistry* **2018**, *14*, 1529-1536.
7. Neyt, N. C.; van der Westhuizen, C. J.; Panayides, J.-L.; Riley, D. L., Design and testing of an ozonolysis reactor module with on-the-fly ozone degassing under flow conditions. *Reaction Chemistry & Engineering* **2022**, *7* (8), 1718-1727.
8. Riley, D. L.; Neyt, N. C., Approaches for performing reductions under continuous-flow conditions. *Synthesis (Germany)* **2018**, *50* (14), 2707-2720.
9. The bio-economy strategy. Department of Science and Technology (DST), South Africa: **2013**; pp 26 - 30. <https://www.gov.za/documents/bio-economy-strategy>
10. Industrial policy action plan (IPAP). The Department of Trade and Industry (DTI): **2018/19 - 2020/21**; pp 141 - 160. https://www.gov.za/sites/default/files/gcis_document/201805/industrial-policy-action-plan.pdf
11. Darvas, F.; Hamlin, T. A.; Hessel, V.; Dorman, G.; Jensen, K. F.; Becker, R.; Delville, M. M. E.; Fekete, M.; Fülöp, F.; Glasnov, T., *Flow Chemistry - Fundamentals : Fundamentals*. De Gruyter, Inc.: Berlin/Boston, GERMANY, **2014**; Vol. 1.
12. Darvas, F.; Dormán, G.; Hessel, V., *Flow Chemistry, Volume 1 - Fundamentals*. De Gruyter: **2014**; pp 9-57.
13. Jiao, J.; Nie, W.; Yu, T.; Yang, F.; Zhang, Q.; Aihemaiti, F.; Yang, T.; Liu, X.; Wang, J.; Li, P., Multi-step continuous-flow organic synthesis: Opportunities and challenges. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2021**, *27* (15), 4817-4838.
14. Murray, P. R. D.; Browne, D. L.; Pastre, J. C.; Ley, S. V.; Butters, C.; Guthrie, D., Continuous flow-processing of organometallic reagents using an advanced peristaltic pumping system and the telescoped flow synthesis of (E/Z)-tamoxifen. *Organic Process Research and Development* **2013**, *17* (9), 1192-1208.
15. Jensen, K. F.; Reizman, B. J.; Newman, S. G., Tools for chemical synthesis in microsystems. *Lab on a Chip* **2014**, *14* (17), 3206-3212.
16. Trojanowicz, M., Flow chemistry vs. flow analysis. *Talanta* **2016**, *146*, 621-640.
17. Britton, J.; Majumdar, S.; Weiss, G. A., Continuous flow biocatalysis. *Chemical Society Reviews* **2018**, *47* (15), 5891-5918.
18. Wegner, J.; Ceylan, S.; Kirschning, A., Ten key issues in modern flow chemistry. *Chemical Communications (Cambridge, England)* **2011**, *47* (16), 4583-92.
19. Hartman, R. L.; McMullen, J. P.; Jensen, K. F., Deciding whether to go with the flow: Evaluating the merits of flow reactors for synthesis. *Angewandte Chemie - International Edition* **2011**, *50* (33), 7502-7519.
20. Plutschack, M. B.; Pieber, B.; Gilmore, K.; Seeberger, P. H., The hitchhiker's guide to flow chemistry II. *Chemical Reviews* **2017**, *117* (18), 11796-11893.

21. Han, X.; Poliakoff, M., Continuous reactions in supercritical carbon dioxide: problems, solutions and possible ways forward. *Chemical Society Reviews* **2012**, *41* (4), 1428-1436.
22. Razzaq, T.; Kappe, C. O., Continuous flow organic synthesis under high-temperature/pressure conditions. *Chemistry - An Asian Journal* **2010**, *5* (6), 1274-1289.
23. Bannock, J. H.; Krishnadasan, S. H.; Heeney, M.; de Mello, J. C., A gentle introduction to the noble art of flow chemistry. *Materials Horizons* **2014**, *1* (4), 373-378.
24. Skoog, D. A.; West, D. M.; Holler, F. J.; Crouch, S. R., *Fundamentals of analytical chemistry*. Ninth edition. ed.; Brooks/Cole, Cengage Learning: Belmont, CA, **2014**; pp 916-917.
25. Ramezani, M.; Kashfipour, M. A.; Abolhasani, M., Minireview: Flow chemistry studies of high-pressure gas-liquid reactions with carbon monoxide and hydrogen. *Journal of Flow Chemistry* **2020**, *10* (1), 93-101.
26. Vapourtec Flow chemistry products <https://www.vapourtec.com/wp-content/uploads/2015/05/Vapourtec-micromixer-reactor-datasheet.pdf> (accessed 21/09/2022).
27. Couturier, P., Advanced control strategy for a digital mass flow controller. *Mechatronics* **2009**, *19* (4), 443-449.
28. Cravotto, G.; Bonrath, W.; Tagliapietra, S.; Speranza, C.; Gaudino, E. C.; Barge, A., Intensification of organic reactions with hybrid flow reactors. *Chemical Engineering & Processing: Process Intensification* **2010**, *49* (9), 930-935.
29. Lin, W.-Y.; Wang, Y.; Wang, S.; Tseng, H.-R., Integrated microfluidic reactors. *Nano Today* **2009**, *4* (6), 470-481.
30. Britton, J.; Raston, C. L., Multi-step continuous-flow synthesis. *Chemical Society Reviews* **2017**, *46* (5), 1250-1271.
31. Yoshida, J.-i.; Takahashi, Y.; Nagaki, A., Flash chemistry: flow chemistry that cannot be done in batch. *Chemical Communications* **2013**, *49* (85), 9896-9904.
32. Brivio, M.; Verboom, W.; Reinhoudt, D. N., Miniaturized continuous flow reaction vessels: influence on chemical reactions. *Lab on a Chip* **2006**, *6* (3), 329-344.
33. Brzozowski, M.; O'Brien, M.; Ley, S. V.; Polyzos, A., Flow chemistry: intelligent processing of gas-liquid transformations using a tube-in-tube reactor. *Accounts of Chemical Research* **2015**, *48* (2), 349-362.
34. Russo, V.; Milicia, A.; Di Serio, M.; Tesser, R., Falling film reactor modelling for sulfonation reactions. *Chemical Engineering Journal* **2019**, *377*.
35. Hao, Y.; Huang, Y.; Gong, M.; Li, W.; Feng, J.; Yi, Q., A polygeneration from a dual-gas partial catalytic oxidation coupling with an oxygen-permeable membrane reactor. *Energy Conversion and Management* **2015**, *106*, 466-478.
36. Tian, T.; Wang, W.; Zhan, M.; Chen, C., Catalytic partial oxidation of methane over SrTiO₃ with oxygen-permeable membrane reactor. *Catalysis Communications* **2010**, *11* (7), 624-628.
37. Polyzos, A.; O'Brien, M.; Petersen, T. P.; Baxendale, I. R.; Ley, S. V., The continuous-flow synthesis of carboxylic acids using CO₂ in a tube-in-tube gas permeable membrane reactor. *Angewandte Chemie - International Edition* **2011**, *50* (5), 1190-1193.
38. Hessel, V.; Löwe, H.; Schönfeld, F., Micromixers—a review on passive and active mixing principles. *Chemical Engineering Science* **2005**, *60* (8), 2479-2501.
39. Palmieri, A.; Ley, S. V.; Hammond, K.; Polyzos, A.; Baxendale, I. R., A microfluidic flow chemistry platform for organic synthesis: The Hofmann rearrangement. *Tetrahedron Letters* **2009**, *50* (26), 3287-3289.
40. Maier, M. C.; Leitner, M.; Kappe, C. O.; Gruber-Woelfler, H., A modular 3D printed isothermal heat flow calorimeter for reaction calorimetry in continuous flow. *Reaction Chemistry & Engineering* **2020**, *5* (8), 1410-1420.
41. Ley, S. V.; Fitzpatrick, D. E.; Myers, R. M.; Battilocchio, C.; Ingham, R. J., Machine-assisted organic synthesis. *Angewandte Chemie - International Edition* **2015**, *54*, 10122-10137.

42. Cambié, D.; Bottecchia, C.; Straathof, N. J. W.; Hessel, V.; Noël, T., Applications of continuous-flow photochemistry in organic synthesis, material science, and water treatment. *Chemical Reviews* **2016**, *116* (17), 10276-10341.
43. Petrucci, G.; Caputo, D.; Lovecchio, N.; Costantini, F.; Legnini, I.; Bozzoni, I.; Nascetti, A.; de Cesare, G., Multifunctional system-on-glass for lab-on-chip applications. *Biosensors and Bioelectronics* **2017**, *93*, 315-321.
44. Watts, P.; Wiles, C., Micro reactors, flow reactors and continuous flow synthesis. *Journal of Chemical Research* **2012**, *36* (4), 181-193.
45. Maier, M. C.; Lebl, R.; Sulzer, P.; Lechner, J.; Mayr, T.; Zdravec, M.; Slama, E.; Pfanner, S.; Schmölzer, C.; Pöchlauer, P.; Kappe, C. O.; Gruber-Woelfler, H., Development of customized 3D printed stainless steel reactors with inline oxygen sensors for aerobic oxidation of Grignard reagents in continuous flow. *Reaction Chemistry & Engineering* **2019**, *4* (2), 393-401.
46. Maier, M. C.; Valotta, A.; Hiebler, K.; Soritz, S.; Gavric, K.; Grabner, B.; Gruber-Woelfler, H., 3D printed reactors for synthesis of active pharmaceutical ingredients in continuous flow. *Organic Process Research and Development* **2020**, *24* (10), 2197-2207.
47. Frost, C. G.; Mutton, L., Heterogeneous catalytic synthesis using microreactor technology. *Green Chemistry* **2010**, *12* (10), 1687-1703.
48. Munirathinam, R.; Huskens, J.; Verboom, W., Supported catalysis in continuous-flow microreactors. *Advanced Synthesis & Catalysis* **2015**, *357* (6), 1093-1123.
49. Roda, N. M.; Tran, D. N.; Battilocchio, C.; Labes, R.; Ingham, R. J.; Hawkins, J. M.; Ley, S. V., Cyclopropanation using flow-generated diazo compounds. *Organic & Biomolecular Chemistry* **2015**, *13* (9), 2550-2554.
50. Buba, A. E.; Koch, S.; Kunz, H.; Löwe, H., Fluorenylmethoxycarbonyl-N-methylamino acids synthesized in a flow tube-in-tube reactor with a liquid-liquid semipermeable membrane. *European Journal of Organic Chemistry* **2013**, *2013* (21), 4509-4513.
51. Gross, U.; Koos, P.; O'Brien, M.; Polyzos, A.; Ley, S. V., A general continuous flow method for palladium catalysed carbonylation reactions using single and multiple tube-in-tube gas-liquid microreactors. *European Journal of Organic Chemistry* **2014**, *2014* (29), 6418-6430.
52. Bedore, M. W.; Zaborenko, N.; Jensen, K. F.; Jamison, T. F., Aminolysis of epoxides in a microreactor system: A continuous flow approach to β -amino alcohols. *Organic Process Research and Development* **2010**, *14* (2), 432-440.
53. Akwi, F. M.; Watts, P., Continuous flow chemistry: where are we now? Recent applications, challenges and limitations. *Chemical Communications* **2018**, *54* (99), 13894-13928.
54. García-Lacuna, J.; Domínguez, G.; Pérez-Castells, J., Flow chemistry for cycloaddition reactions. *ChemSusChem* **2020**, *13* (19), 5138-5163.
55. Bristow, T. W. T.; Ray, A. D.; O'Kearney-McMullan, A.; Lim, L.; McCullough, B.; Zammataro, A., On-line monitoring of continuous flow chemical synthesis using a portable, small footprint mass spectrometer. *Journal of the American Society for Mass Spectrometry* **2014**, *25* (10), 1794-802.
56. Sagandira, C. R.; Siyawamwaya, M.; Watts, P., 3D printing and continuous flow chemistry technology to advance pharmaceutical manufacturing in developing countries. *Arabian Journal of Chemistry* **2020**, *13* (11), 7886-7908.
57. Cantillo, D.; Kappe, C. O., Immobilized transition metals as catalysts for cross-couplings in continuous flow—A critical assessment of the reaction mechanism and metal leaching. *ChemCatChem* **2014**, *6* (12), 3286-3305.
58. Lange, H.; Carter, C. F.; Hopkin, M. D.; Baxendale, I. R.; Ley, S. V.; Burke, A.; Goode, J. G., A breakthrough method for the accurate addition of reagents in multi-step segmented flow processing. *Chemical Science* **2011**, *2* (4), 765-769.
59. Hopkin, M. D.; Baxendale, I. R.; Ley, S. V., A flow-based synthesis of Imatinib: the API of Gleevec. *Chemical Communications* **2010**, *46* (14), 2450-2452.
60. Fitzpatrick, D. E.; Ley, S. V., Engineering chemistry: integrating batch and flow reactions on a single, automated reactor platform. *Reaction Chemistry & Engineering* **2016**, *1* (6), 629-635.

61. Hergert, T.; Mátravölgyi, B.; Örkényi, R.; Éles, J.; Faigl, F., Multistep batch-flow hybrid synthesis of a terbinafine precursor. *Journal of Flow Chemistry* **2022**, *12* (1), 51-57.
62. Deadman, B. J.; Battilocchio, C.; Sliwinski, E.; Ley, S. V., A prototype device for evaporation in batch and flow chemical processes. *Green Chemistry* **2013**, *15* (8), 2050-2055.
63. Scholtz, C.; Riley, D. L., Improved batch and flow syntheses of the nonsteroidal anti-inflammatory COX-2 inhibitor celecoxib *Reaction Chemistry & Engineering* **2021**, *6* (1), 138-146.
64. Scholtz, C.; Riley, D. L.; Cutler, A., A concise, rapid and high yielding flow synthesis of aryldiazonium tetrafluoroborates. *Arkivoc* **2020**, *2020* (5), 119-128.
65. van der Westhuizen, C. J.; du Toit, J.; Neyt, N.; Riley, D.; Panayides, J.-L., Use of open-source software platform to develop dashboards for control and automation of flow chemistry equipment. *Digital Discovery* **2022**.
66. WHO Promising progress on neglected tropical diseases in Africa. <https://www.afro.who.int/news/promising-progress-neglected-tropical-diseases-africa> (accessed 10/08/2022).
67. Hotez, P. J.; Fenwick, A.; Molyneux, D., The new COVID-19 poor and the neglected tropical diseases resurgence. *Infectious Diseases of Poverty* **2021**, *10* (1).
68. Ryan, E. T.; Hill, D. R.; Solomon, T.; Endy, T. P.; Aronson, N., Hunter's tropical medicine and emerging infectious diseases. Tenth edition / ed.; Elsevier: Edinburgh, **2020**. <https://public.ebookcentral.proquest.com/choice/publicfullrecord.aspx?p=5742492>.
69. Molyneux, D. H.; Hotez, P. J.; Fenwick, A., "Rapid-impact interventions": How a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. *PLoS Medicine* **2005**, *2* (11), e336.
70. James, S. L.; Abate, D.; Abate, K. H.; *et al.*, Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *The Lancet* **2018**, *392* (10159), 1789-1858.
71. Hotez, P. J.; Kamath, A., Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases* **2009**, *3* (8), e412.
72. Lassi, Z.; Bhutta, Z.; Sommerfeld, J.; Salam, R.; Das, J. Global burden, distribution, and interventions for infectious diseases of poverty *Infectious Diseases of Poverty* [Online], **2014**, p. 1-7. WorldCat.org.
73. Njoroge, M.; Njuguna, N. M.; Mutai, P.; Ongarora, D. S.; Smith, P. W.; Chibale, K., Recent approaches to chemical discovery and development against malaria and the neglected tropical diseases human African trypanosomiasis and schistosomiasis. *Chemical Reviews* **2014**, *114* (22), 11138-11163.
74. Dömling, A.; Khoury, K., Praziquantel and Schistosomiasis. *ChemMedChem* **2010**, *5* (9), 1420-1434.
75. Pedrique, B.; Strub-Wourgaft, N.; Some, C.; Olliaro, P.; Trouiller, P.; Ford, N.; Pécou, B.; Bradol, J.-H., The drug and vaccine landscape for neglected diseases (2000–11): A systematic assessment. *The Lancet Global Health* **2013**, *1* (6), e371-e379.
76. Hotez, P. J.; Fenwick, A.; Ray, S. E.; Hay, S. I.; Molyneux, D. H., "Rapid impact" 10 years after: The first "decade" (2006-2016) of integrated neglected tropical disease control. *PLoS Neglected Tropical Diseases* **2018**, *12* (5), e0006137.
77. WHO Scabies. <https://www.who.int/news-room/fact-sheets/detail/scabies> (accessed 12/08/2022).
78. WHO Yaws. <https://www.who.int/news-room/fact-sheets/detail/yaws> (accessed 12/08/2022).
79. Weld, E. D.; Waitt, C.; Barnes, K.; Garcia Bournissen, F., Twice neglected? Neglected diseases in neglected populations. *British Journal of Clinical Pharmacology* **2022**, *88* (2), 367-373.
80. Hotez, P. J., The rise of neglected tropical diseases in the "new Texas". *PLoS Neglected Tropical Diseases* **2018**, *12* (1), e0005581-e0005596.

81. WHO Neglected tropical diseases <https://www.who.int/news-room/questions-and-answers/item/neglected-tropical-diseases> (accessed 10/08/2022).
82. Ehrenberg, J. P.; Zhou, X.-N.; Fontes, G.; Rocha, E. M. M.; Tanner, M.; Utzinger, J., Strategies supporting the prevention and control of neglected tropical diseases during and beyond the COVID-19 pandemic. *Infectious Diseases of Poverty* **2020**, *9* (1), 86.
83. Colley, D. G.; Bustinduy, A. L.; Secor, W. E.; King, C. H., Human schistosomiasis. *Lancet (London, England)* **2014**, *383* (9936), 2253-2264.
84. Steinmann, P.; Keiser, J.; Bos, R.; Tanner, M.; Utzinger, J., Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet. Infectious diseases* **2006**, *6* (7), 411-425.
85. CDC Parasites - Schistosomiasis biology <https://www.cdc.gov/parasites/schistosomiasis/biology.html> (accessed 12/08/2022).
86. Gryseels, B.; Polman, K.; Clerinx, J.; Kestens, L., Human schistosomiasis. *Lancet (London, England)* **2006**, *368* (9541), 1106-1118.
87. Hotez, P. J.; Bottazzi, M. E.; Bethony, J.; Diemert, D. D., Advancing the development of a human Schistosomiasis vaccine. *Trends in Parasitology* **2019**, *35* (2), 104-108.
88. Ross, A. G.; Sleigh, A. C.; Li, Y.; Davis, G. M.; Williams, G. M.; Jiang, Z.; Feng, Z.; McManus, D. P., Schistosomiasis in the People's Republic of China: prospects and challenges for the 21st century. *Clinical microbiology reviews* **2001**, *14* (2), 270-295.
89. Mohamed, A. R.; al Karawi, M.; Yasawy, M. I., Schistosomal colonic disease. *Gut* **1990**, *31* (4), 439-442.
90. Khalaf, I.; Shokeir, A.; Shalaby, M., Urologic complications of genitourinary schistosomiasis. *World Journal of Urology* **2012**, *30* (1), 31-38.
91. Secor, W. E., Interactions between schistosomiasis and infection with HIV-1. *Parasite Immunology* **2006**, *28* (11), 597-603.
92. Downs, J. A.; Dupnik, K. M.; van Dam, G. J.; Urassa, M.; Lutonja, P.; Cornelis, D.; de Dood, C. J.; Hoekstra, P.; Kanjala, C.; Isingo, R.; Peck, R. N.; Lee, M. H.; Corstjens, P. L. A. M.; Todd, J.; Changalucha, J. M.; Johnson, W. D., Jr.; Fitzgerald, D. W., Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. *PLoS Neglected Tropical Diseases* **2017**, *11* (9), e0005968.
93. Barrett, J., Forty years of helminth biochemistry. *Parasitology* **2009**, *136* (12), 1633-1642.
94. Stanley Ching-Cheng, H.; Tori, C. F.; Eyal, A.; Bart, E.; Erika, L. P.; James, B. L.; Edward, J. P. Fatty acid oxidation is essential for egg production by the parasitic flatworm *Schistosoma mansoni* *PLoS Pathogens* [Online], **2012**. WorldCat.org.
95. Cao, H.; Liu, H.; Dömling, A., Efficient multicomponent reaction synthesis of the Schistosomiasis drug praziquantel. *Chemistry - A European Journal* **2010**, *16* (41), 12296-12298.
96. Liu, H.; William, S.; Herdtweck, E.; Botros, S.; Dömling, A., MCR synthesis of praziquantel derivatives. *Chemical biology & drug design* **2012**, *79* (4), 470-477.
97. Ahmed, S. H. Schistosomiasis (Bilharzia) medication: Anthelmintics. <https://emedicine.medscape.com/article/228392-medication#1> (accessed 20/09/2022).
98. Seubert, J.; Pohlke, R.; Loebich, F., Synthesis and properties of praziquantel, a novel broad spectrum anthelmintic with excellent activity against Schistosomes and Cestodes. *Experientia* **1977**, *33* (8), 1036-1037.
99. Brossi, A., 175 years of isoquinoline drugs. *Heterocycles* **1978**, *11* (1), 521-547.
100. Seubert, J. Process for preparing (1-acylaminomethyl)-1,2,3,4-tetrahydroisoquinolines. US4362875A, **1975**.
101. Kim, J. H.; Lee, Y. S.; Park, H.; Kim, C. S., Formation of pyrazinoisoquinoline ring system by the tandem amidoalkylation and N-acyliminium ion cyclization: an efficient synthesis of praziquantel. *Tetrahedron* **1998**, *54* (26), 7395-7400.
102. Berkowitz, W. F.; John, T. V., An internal imino-Diels-Alder route to a tetrahydroisoquinoline. *The Journal of Organic Chemistry* **1984**, *49* (26), 5269-5271.

103. Kim, J. H., Synthesis of praziquantel via N-acyliminium ion cyclization of amido acetals through several synthetic routes. *Heterocycles* **1998**, *48*, 2279-2285.
104. Todd, M. H.; Ndubaku, C.; Bartlett, P. A., Amino acid derived heterocycles: lewis acid catalyzed and radical cyclizations from peptide acetals. *The Journal of Organic Chemistry* **2002**, *67* (12), 3985-3988.
105. Ma, C.; Zhang, Q.-F.; Tan, Y.-B.; Wang, L., Total synthesis of (-)-praziquantel: An anthelmintic drug. *Journal of Chemical Research* **2004**, *2004* (3), 186-187.
106. Yuhua, S. F., L.; Shunfu, Y.; Ping X. Praziquantel synthetic process. CN100503582C, **2005**.
107. El-Fayyoumy, S.; Mansour, W.; Todd, M. H., Solid phase synthesis of praziquantel. *Tetrahedron Letters* **2006**, *47* (8), 1287-1290.
108. Roszkowski, P.; Maurin, J. K.; Czarnocki, Z., Enantioselective synthesis of (R)-(-)-praziquantel (PZQ). *Tetrahedron: Asymmetry* **2006**, *17* (9), 1415-1419.
109. Shou, H.; He, Z.; Peng, G.; Su, W.; Yu, J., Two approaches for the synthesis of levo-praziquantel. *Organic & Biomolecular Chemistry* **2021**, *19* (20), 4507-4514.
110. Vasconcelos, S. N. S.; Fornari, E.; Stefani, H. A.; Caracelli, I., Synthesis of α -amino-1,3-dicarbonyl compounds via Ugi flow chemistry reaction: access to functionalized 1,2,3-triazoles. *Molecular Diversity* **2017**, *21* (4), 893-902.
111. Alfano, A. I.; Buommino, E.; Ferraro, M. G.; Irace, C.; Zampella, A.; Lange, H.; Brindisi, M., Coupling interrupted Fischer and multicomponent Joulie-Ugi to chase chemical diversity: from batch to sustainable flow synthesis of peptidomimetics. *ChemMedChem* **2021**, *16* (24), 3795-3809.
112. Salvador, C. E. M.; Pieber, B.; Neu, P. M.; Torvisco, A.; Kleber Z Andrade, C.; Kappe, C. O., A sequential Ugi multicomponent/Cu-catalyzed azide-alkyne cycloaddition approach for the continuous flow generation of cyclic peptoids. *The Journal of Organic Chemistry* **2015**, *80* (9), 4590-4602.
113. Van Mileghem, S.; Veryser, C.; De Borggraeve, W. M., Flow-assisted synthesis of heterocycles via multicomponent reactions. In *Flow Chemistry for the Synthesis of Heterocycles*, Cham : Springer International Publishing : Springer: **2018**; pp 133-159.
114. Valenti, G.; Tinnemans, P.; Baglai, I.; Noorduyn, W. L.; Kaptein, B.; Leeman, M.; ter Horst, J. H.; Kellogg, R. M., Combining incompatible processes for deracemization of a praziquantel derivative under flow conditions. *Angewandte Chemie - International Edition* **2021**, *60* (10), 5279-5282.
115. Patil, P.; Ahmadian-Moghaddam, M.; Dömling, A., Isocyanide 2.0. *Green Chemistry* **2020**, *22* (20), 6902-6911.
116. Galli, U.; Tron, G. C.; Purghè, B.; Grosa, G.; Aprile, S., Metabolic fate of the isocyanide moiety: Are isocyanides pharmacophore groups neglected by medicinal chemists? *Chemical research in toxicology* **2020**, *33* (4), 955-966.
117. Lim, F. Y.; Won, T. H.; Raffa, N.; Baccile, J. A.; Wisecaver, J.; Rokas, A.; Schroeder, F. C.; Keller, N. P., Fungal isocyanide synthases and xanthocillin biosynthesis in *aspergillus fumigatus*. *mBio* **2018**, *9* (3).
118. Arcadia, C. E.; Kennedy, E.; Geiser, J.; Dombroski, A.; Oakley, K.; Chen, S.-L.; Sprague, L.; Ozmen, M.; Sello, J.; Weber, P. M.; Reda, S.; Rose, C.; Kim, E.; Rubenstein, B. M.; Rosenstein, J. K., Multicomponent molecular memory. *Nature Communications* **2020**, *11* (1).
119. Javanbakht, S.; Shaabani, A., Multicomponent reactions-based modified/functionalized materials in the biomedical platforms. *ACS applied bio materials* **2020**, *3* (1), 156-174.
120. Weber, W. P.; Gokel, G. W., An improved procedure for the Hofmann carbylamine synthesis of isonitriles. *Tetrahedron Letters* **1972**, *13* (17), 1637-1640.
121. Lieke, W., Ueber das cyanallyl. *Justus Liebigs Annalen der Chemie* **1859**, *112* (3), 316-321.
122. Gautier, A., Ueber die einwirkung des chlorwasserstoffs u. a. auf das aethyl- und methylcyanür. *Justus Liebigs Annalen der Chemie* **1867**, *142* (3), 289-294.
123. Hofmann, A. W., Ueber eine neue reihe von homologen der cyanwasserstoffsäure. *Justus Liebigs Annalen der Chemie* **1867**, *144* (1), 114-120.
124. Hagedorn, I.; Tonjes, H., Konstitutions-aufklärung von xanthocillin, einem neuen antibioticum. *Die Pharmazie* **1956**, *11* (6), 409-410.

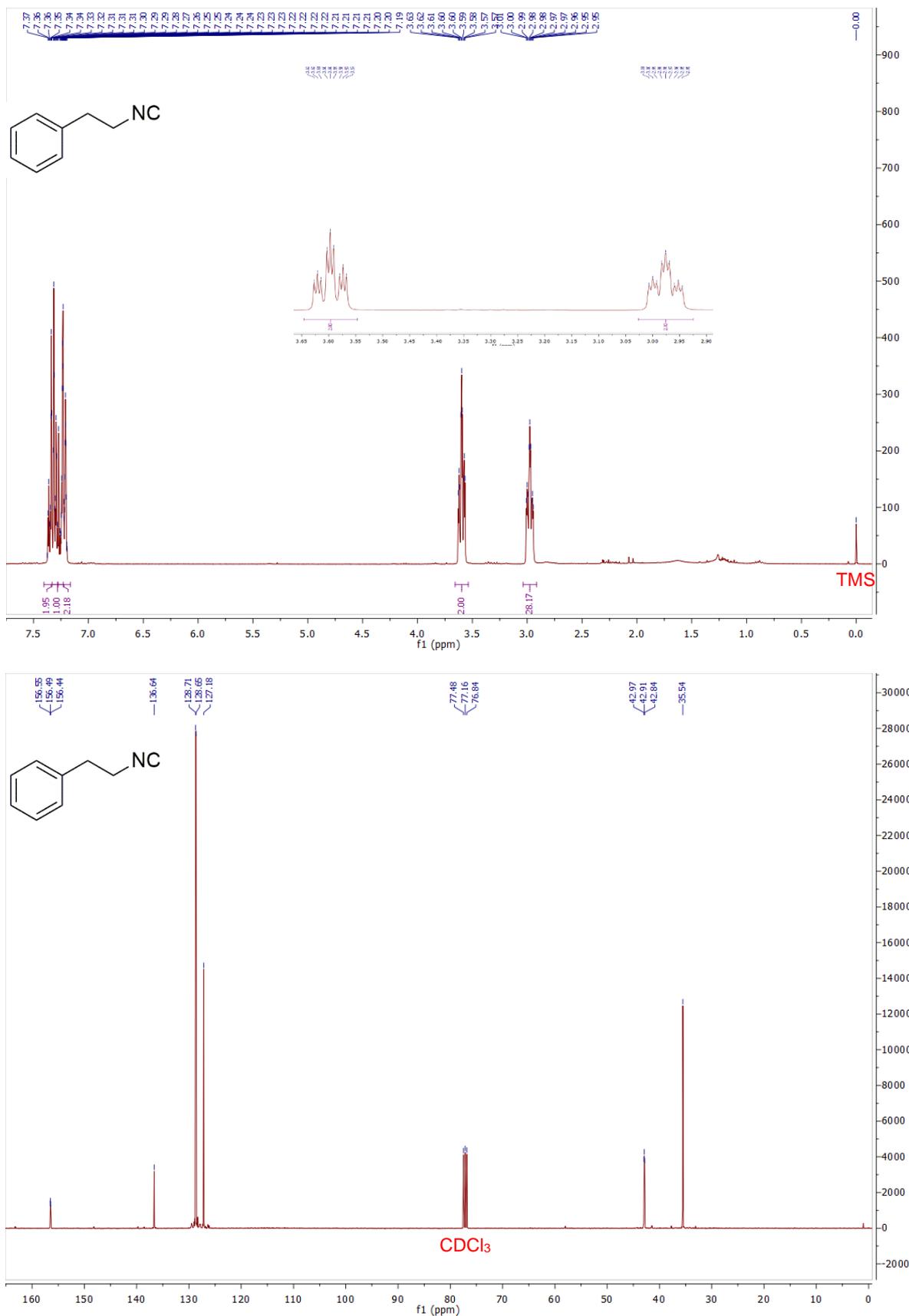
125. Hertler, W.; Corey, E., A novel preparation of isonitriles. *The Journal of Organic Chemistry* **1958**, *23* (8), 1221-1222.
126. Ugi, I.; Meyr, R., Neue darstellungsmethode für isonitrile. *Angewandte Chemie* **1958**, *70* (22-23), 702-703.
127. Ugi, I.; Fetzer, U.; Eholzer, U.; Knupfer, H.; Offermann, K., Isonitrile syntheses. *Angewandte Chemie - International Edition* **1965**, *4* (6), 472-484.
128. Heckman, L. M.; He, Z.; Jamison, T. F., Synthesis of highly substituted 2-arylindoles via copper-catalyzed coupling of isocyanides and arylboronic Acids. *Organic Letters* **2018**, *20* (11), 3263-3267.
129. Chen, S.; Oliva, M.; Van Meervelt, L.; Van der Eycken, E. V.; Sharma, U. K., Palladium-catalyzed domino synthesis of 2, 3-difunctionalized indoles via migratory insertion of isocyanides in batch and continuous flow. *Advanced synthesis & catalysis* **2021**, *363* (13), 3220-3226.
130. Hess, U.; Brosig, H.; Komenda, J., Electrochemical synthesis of isocyanides and 1,n-diisocyanides. *Pharmazie* **1999**, *54* (6), 412-417.
131. Hess, U.; Brosig, H.; Fehlhammer, W. P., Electrochemical synthesis of isocyanides. *Tetrahedron Letters* **1991**, *32* (40), 5539-5542.
132. Ugi, I., Versuche mit isonitrilen. *Angewandte Chemie-International Edition* **1959**, *71* (11), 386-386.
133. Rocha, R. O.; Rodrigues, M. O.; Neto, B. A. D., Review on the Ugi multicomponent reaction mechanism and the use of fluorescent derivatives as functional chromophores. *ACS Omega* **2020**, *5* (2), 972-979.
134. Ugi, I.; Steinbrückner, C., Isonitrile, II. Reaktion von isonitrilen mit carbonylverbindungen, aminen und stickstoffwasserstoffsäure. *Chemische Berichte* **1961**, *94* (3), 734-742.
135. Neochoritis, C. G.; Zhao, T.; Dömling, A., Tetrazoles via multicomponent reactions. *Chemical Reviews* **2019**, *119* (3), 1970-2042.
136. Madej, A.; Paprocki, D.; Koszelewski, D.; Żądło-Dobrowolska, A.; Brzozowska, A.; Walde, P.; Ostaszewski, R., Efficient Ugi reactions in an aqueous vesicle system. *RSC Advances* **2017**, *7* (53), 33344-33354.
137. Welsch, S. J.; Umkehrer, M.; Ross, G.; Kolb, J.; Burdack, C.; Wessjohann, L. A., PdII/IV catalyzed oxidative cyclization of 1,6-enynes derived by Ugi-4-component reaction. *Tetrahedron Letters* **2011**, *52* (47), 6295-6297.
138. Rotstein, B. H.; Yudin, A. K., Aziridine-2-carboxaldehyde dimers undergo homo-Ugi 4-component-5-center reactions. *Synthesis* **2012**, *44* (18), 2851-2858.
139. Balalaie, S.; Motaghedi, H.; Tahmassebi, D.; Bararjanian, M.; Bijanzadeh, H. R., A facile and efficient synthesis of 2,2,2-trifluoroethyl 2-[(E)-N-phenylcinnamamido]-2-phenylacetates in trifluoroethanol via sequential Ugi four-component reaction/esterification. *Tetrahedron Letters* **2012**, *53* (46), 6177-6181.
140. Chinigo, G. M.; Breder, A.; Carreira, E. M., Ugi-4-component reaction enabling rapid access to the core fragment of massadine. *Organic letters* **2011**, *13* (1), 78-81.
141. Chéron, N.; Ramozzi, R.; El Kaïm, L.; Grimaud, L.; Fleurat-Lessard, P., Challenging 50 years of established views on Ugi reaction: a theoretical approach. *The Journal of Organic Chemistry* **2012**, *77* (3), 1361-1366.
142. Medeiros, G. A.; da Silva, W. A.; Bataglion, G. A.; Ferreira, D. A. C.; de Oliveira, H. C. B.; Eberlin, M. N.; Neto, B. A. D., Probing the mechanism of the Ugi four-component reaction with charge-tagged reagents by ESI-MS(/MS). *Chemical Communications (Cambridge, England)* **2014**, *50* (3), 338-340.
143. Iacobucci, C.; Reale, S.; Gal, J.-F. o.; De Angelis, F., Insight into the mechanisms of the multicomponent Ugi and Ugi-Smiles reactions by ESI-MS(/MS). *European Journal of Organic Chemistry* **2014**, *2014* (32), 7087-7090.
144. Sharma, S.; Maurya, R. A.; Min, K.-I.; Jeong, G.-Y.; Kim, D.-P., Odorless isocyanide chemistry: An integrated microfluidic system for a multistep reaction sequence. *Angewandte Chemie* **2013**, *125* (29), 7712-7716.

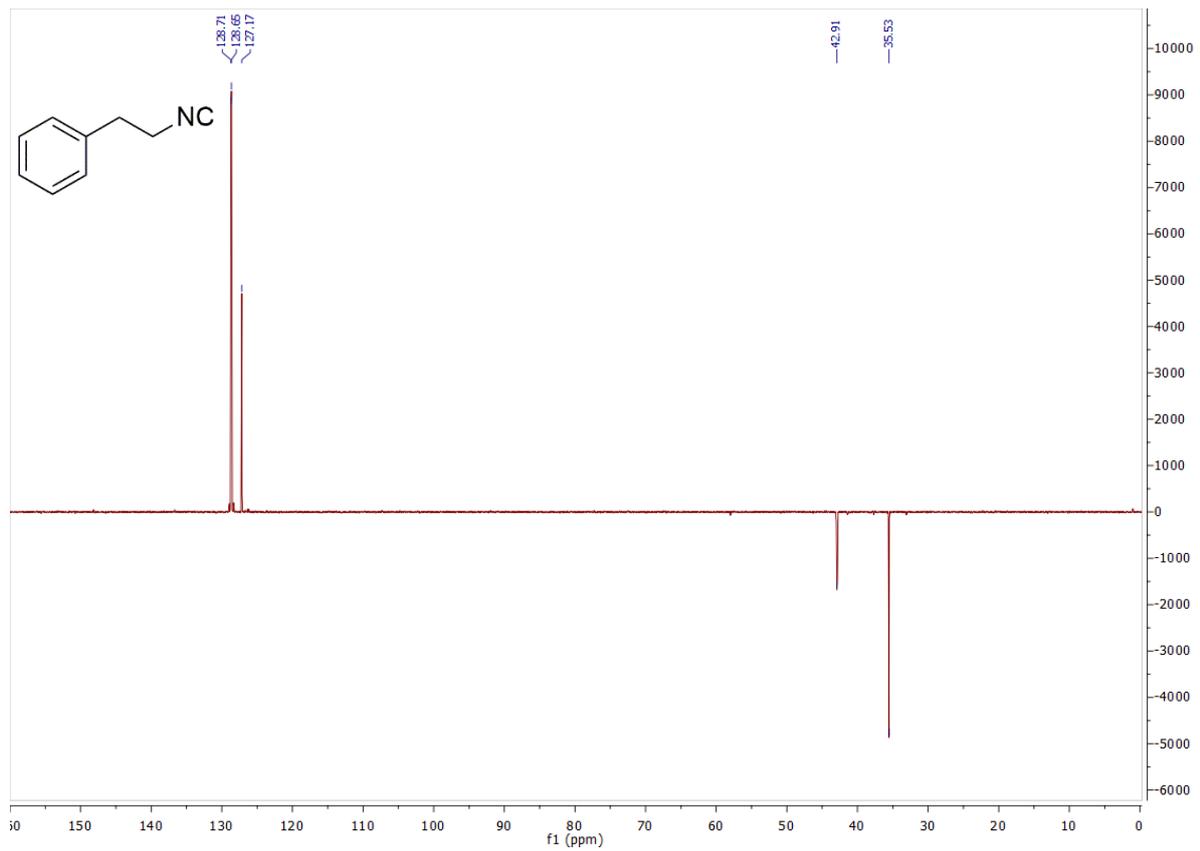
145. Cox, E. D.; Cook, J. M., The Pictet-Spengler condensation: a new direction for an old reaction. *Chemical Reviews* **1995**, *95* (6), 1797-1842.
146. Pictet, A.; Spengler, T., Ueber die bildung von isochinolin-derivaten durch einwirkung von methylal auf phenyl-äthylamin, phenyl-alanin und tyrosin. *Berichte der Deutschen Chemischen Gesellschaft* **1911**.
147. Tatsui, G., Über die synthese von carbolinderivaten. *Yakugaku Zasshi* **1928**, *48* (5), 453-459.
148. Stöckigt, J.; Antonchick, A. P.; Wu, F.; Waldmann, H., The Pictet-Spengler reaction in nature and in organic chemistry. *Angewandte Chemie - International Edition* **2011**, *50* (37), 8538-8564.
149. Manske, R. H. F.; Holmes, H. L.; Rodrigo, R. G. A. The alkaloids. chemistry and physiology **1950**. WorldCat.org. <http://www.sciencedirect.com/science/bookseries/18760813>.
150. Agarwal, P. K.; Sharma, S. K.; Sawant, D.; Kundu, B., Application of the Pictet-Spengler reaction to aryl amine-based substrates having pyrimidine as a π -nucleophile: synthesis of pyrimidoquinolines with structural analogy to benzonaphthyridines present in alkaloids. *Tetrahedron* **2009**, *65* (6), 1153-1161.
151. Kundu, B.; Sawant, D.; Partani, P.; Kesarwani, A. P., New application of Pictet-Spengler reaction leading to the synthesis of an unusual seven-membered heterocyclic ring system. *The Journal of Organic Chemistry* **2005**, *70* (12), 4889-92.
152. Viveros-Ceballos, J. L.; Sayago, F. J.; Cativiela, C.; Ordóñez, M., First practical and efficient synthesis of 3-phosphorylated β -carboline derivatives using the Pictet-Spengler reaction. *European Journal of Organic Chemistry* **2015**, *2015* (5), 1084-1091.
153. Zheng, X. W. X.; Chang, J. Z. K., A new application of the Pictet-Spengler reaction to the preparation of 4-substituted 1H-2,3-benzoxazines. *Synlett*. **2006**, *17* (19), 3277.
154. Stöckigt, J.; Zenk, M. H., Isovincoside (strictosidine), the key intermediate in the enzymatic formation of indole alkaloids. *FEBS Letters* **1977**, *79* (2), 233-237.
155. Stöckigt, J.; Zenk, M. H., Strictosidine (isovincoside): the key intermediate in the biosynthesis of monoterpenoid indole alkaloids. *Journal of Chemical Society* **1977**, (18), 646-648.
156. Stöckigt, J., Enzymatic formation of intermediates in the biosyntheses of ajmalicine: Strictosidine and cathenamine. *Phytochemistry* **1979**, *18* (6), 965-971.
157. Czarnocki, Z.; MacLean, D. B.; Szarek, W. A., Application of the Pictet-Spengler condensation in enantioselective synthesis of isoquinoline alkaloids. *Journal of Chemical Society* **1985**, (19), 1318-1319.
158. Li, W.; Jiang, M.; Liu, M.; Ling, X.; Xia, Y.; Wan, L.; Chen, F., Development of a fully continuous-flow approach towards asymmetric total synthesis of tetrahydroprotoberberine natural alkaloids. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2022**, *28* (33), e202200700.
159. Kulkarni, P., Methane sulphonic acid is green catalyst in organic synthesis. *Oriental Journal of Chemistry* **2015**, *31* (1), 447-451.
160. El-Subbagh, H. I.; Al-Badr, A. A., Praziquantel. In *Analytical Profiles of Drug Substances and Excipients*, Brittain, H. G., Ed. Academic Press: **1998**; Vol. 25, pp 463-500.
161. Teasdale, A.; Delaney, E. J.; Eyley, S. C.; Jacq, K.; Taylor-Worth, K.; Lipczynski, A.; Hoffmann, W.; Reif, V.; Elder, D. P.; Facchine, K. L.; Golec, S.; Schulte Oestrich, R.; Sandra, P.; David, F., A detailed study of sulfonate ester formation and solvolysis reaction rates and application toward establishing sulfonate ester control in pharmaceutical manufacturing processes. *Organic Process Research and Development* **2010**, *14* (4), 999-1007.
162. Bin, L.; Yongcheng, L.; Gen, L.; Chuan, Y.; Shuangxi, L.; Xin, W. Jiangsu Chengxin Pharmaceutical Co LTD; Synthetic method of praziquantel. CN111072656A, **2020**.
163. Feixiang, Z.; Pengqian, H.; Junting, Z.; Xingying, C.; Jibo, J. Hebei Jiayi Pharmacological Co LTD; Preparation method of praziquantel. CN114195782A, **2022**.
164. Rigaku Oxford Diffraction, CrysAlisPro Software system, **2018**.
165. Sheldrick, G. M., SHELXT – Integrated space-group and crystal-structure determination. *Acta Crystallographica. Section A, Foundations and Advances* **2015**, *71* (Pt 1), 3-8.

166. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H., OLEX2: a complete structure solution, refinement and analysis program. *Journal of Applied Crystallography* **2009**, *42* (2), 339-341.

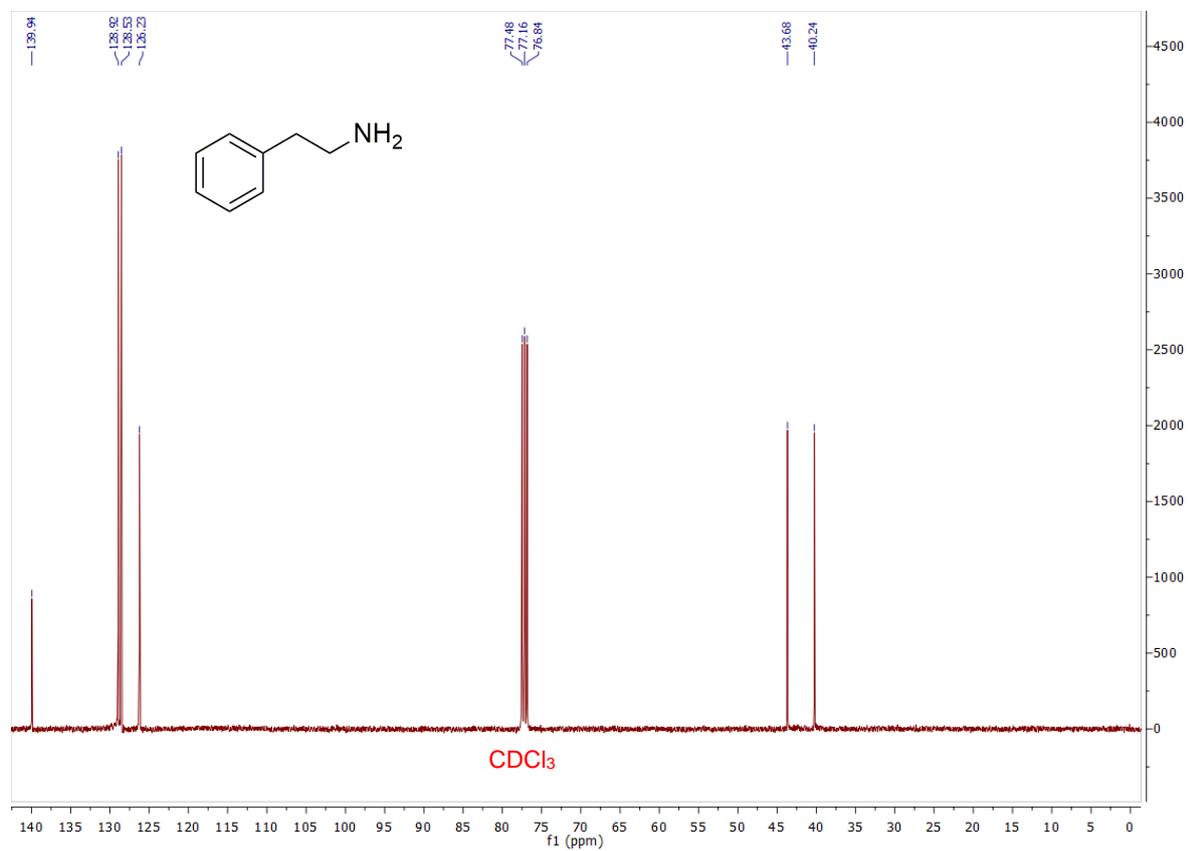
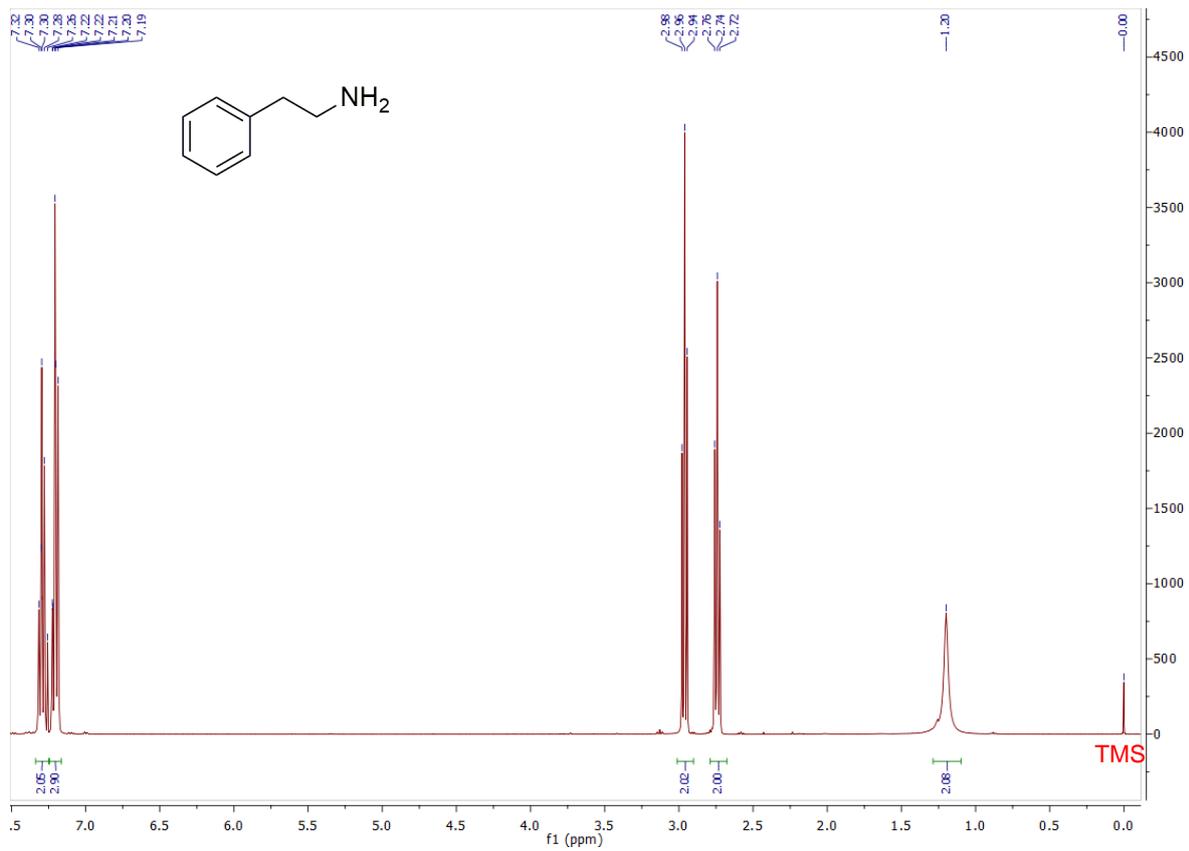
Appendix A (Chapter II)

A – 1: $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and Dept 135 spectra of 2-isocyanoethylbenzene **96**

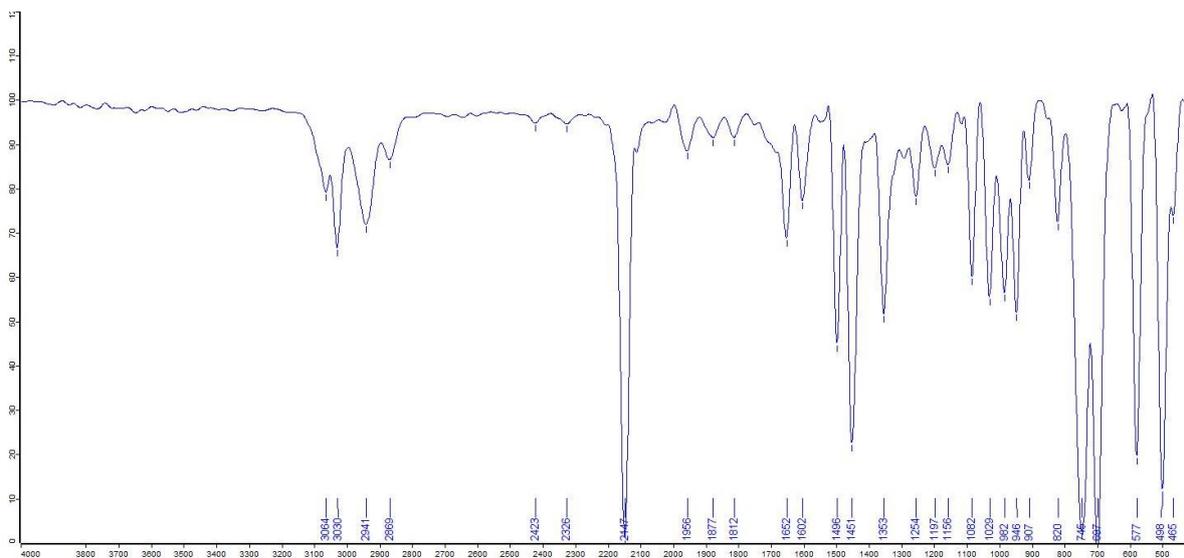
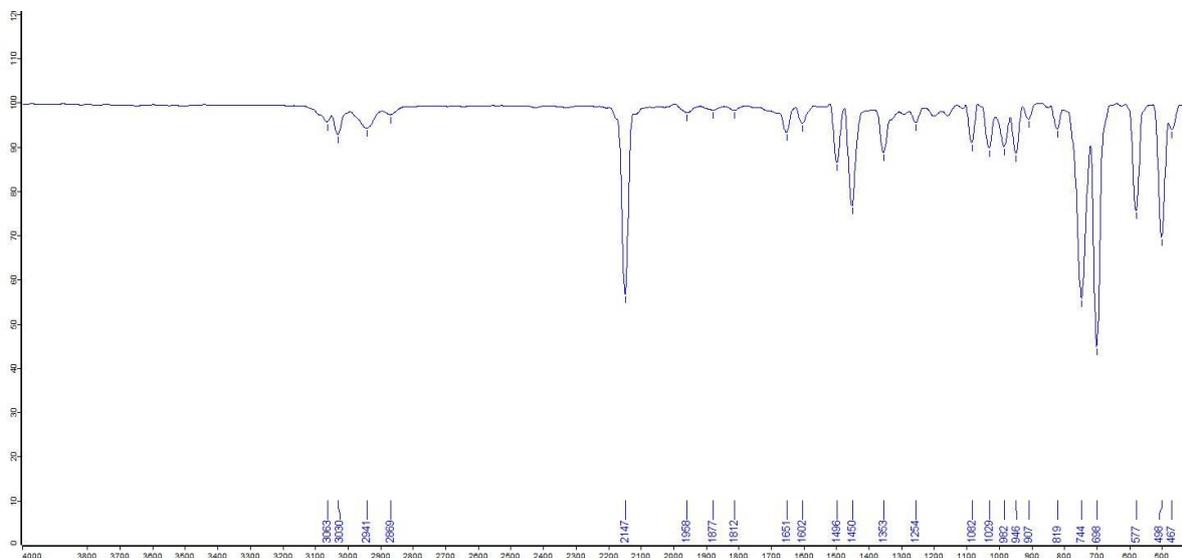




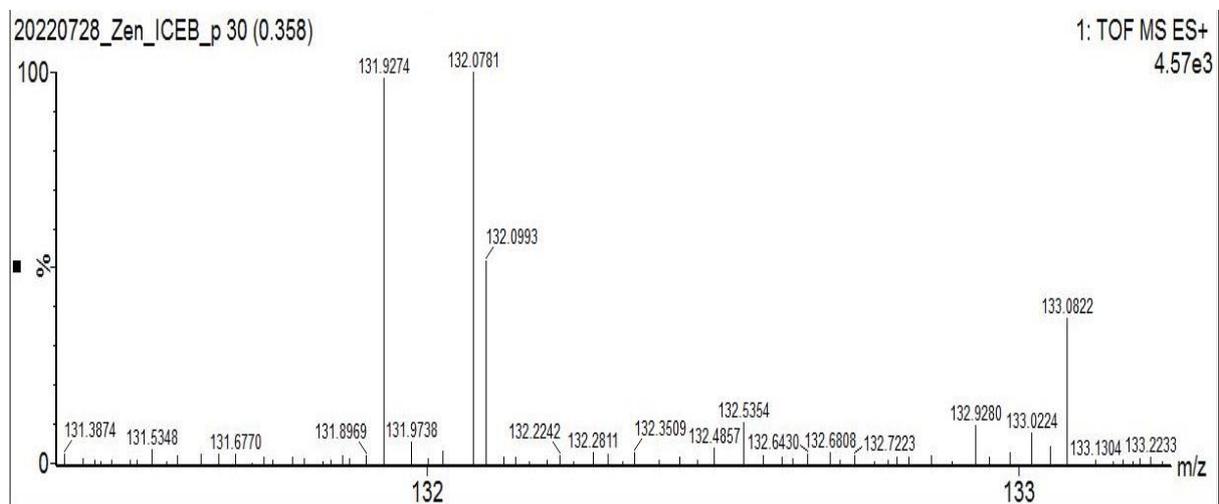
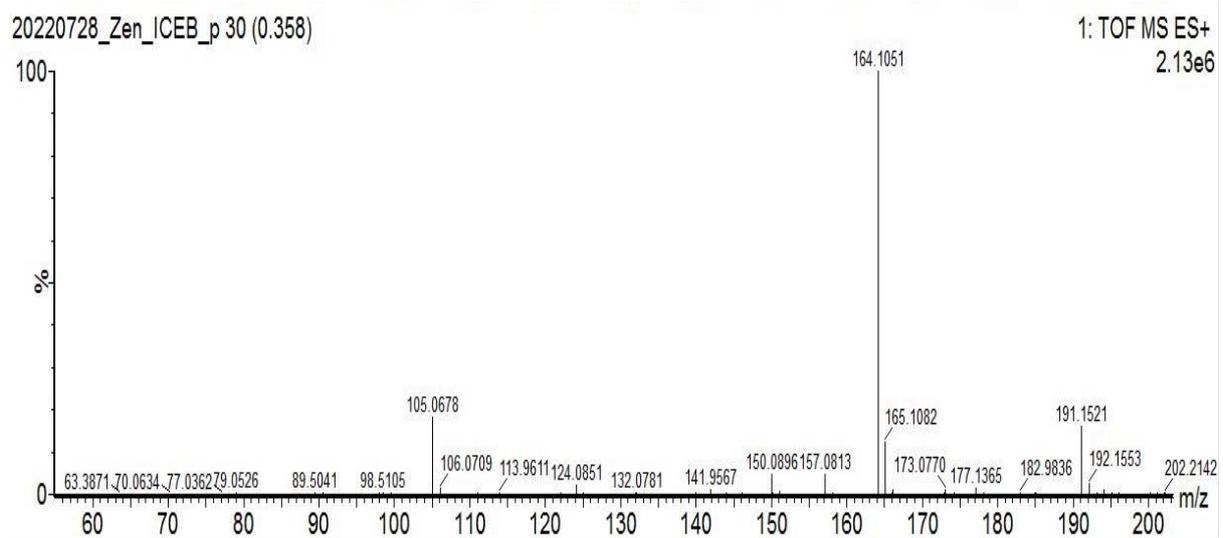
A – 2: $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of 2-phenethylamine 41



A – 3: Infrared spectroscopy (400 – 4000 cm^{-1}) smoothed spectrum and min-max normalised spectrum of 2-isocyanoethylbenzene **96**

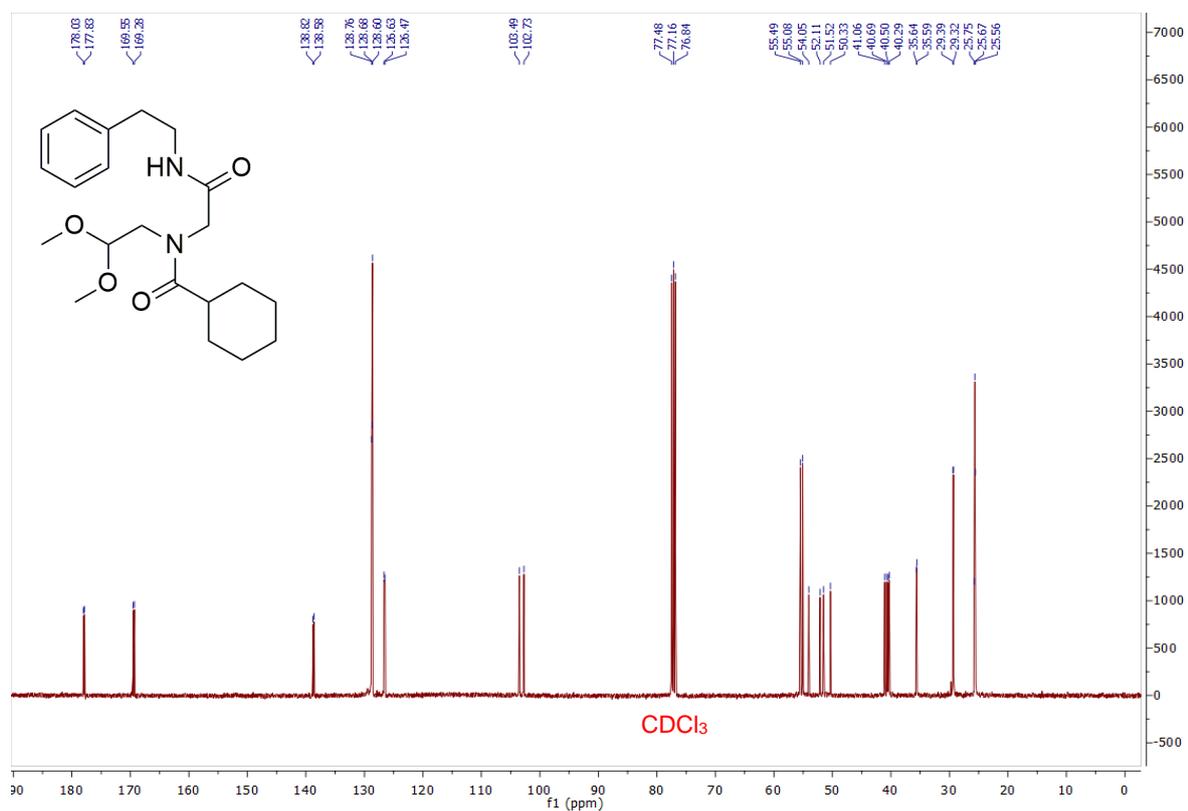
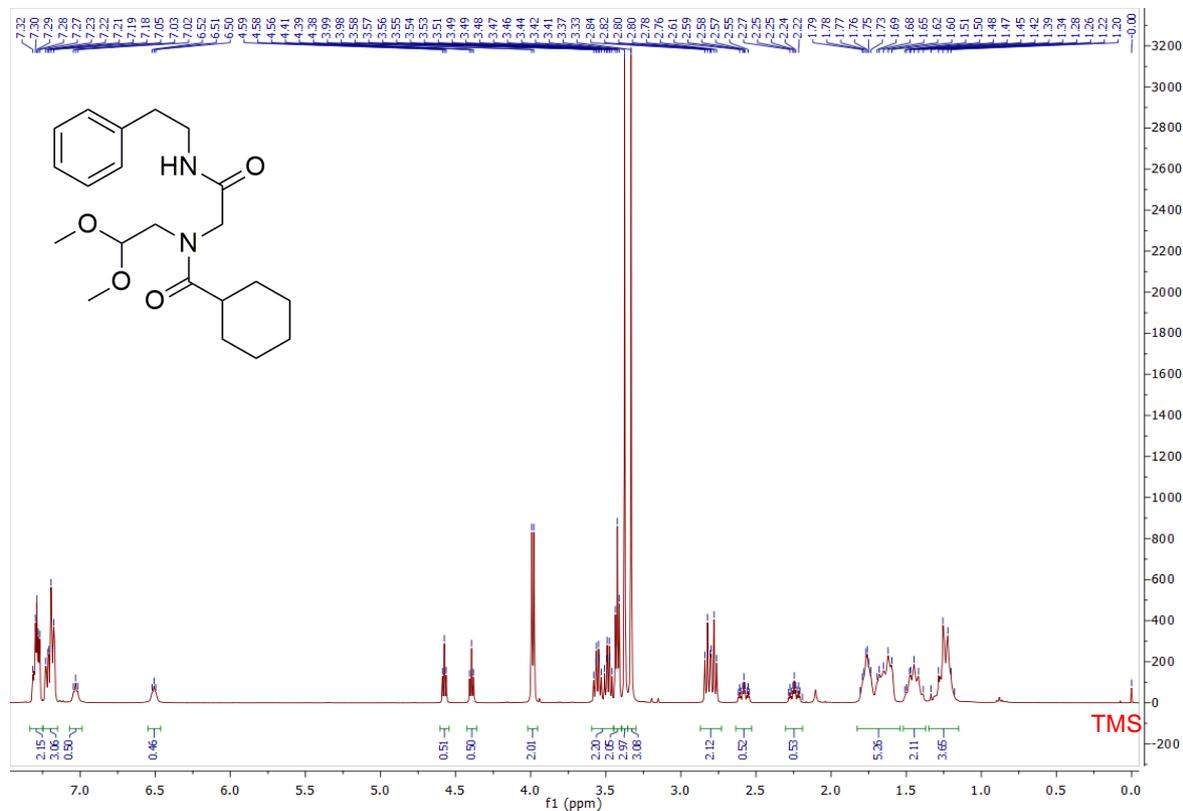


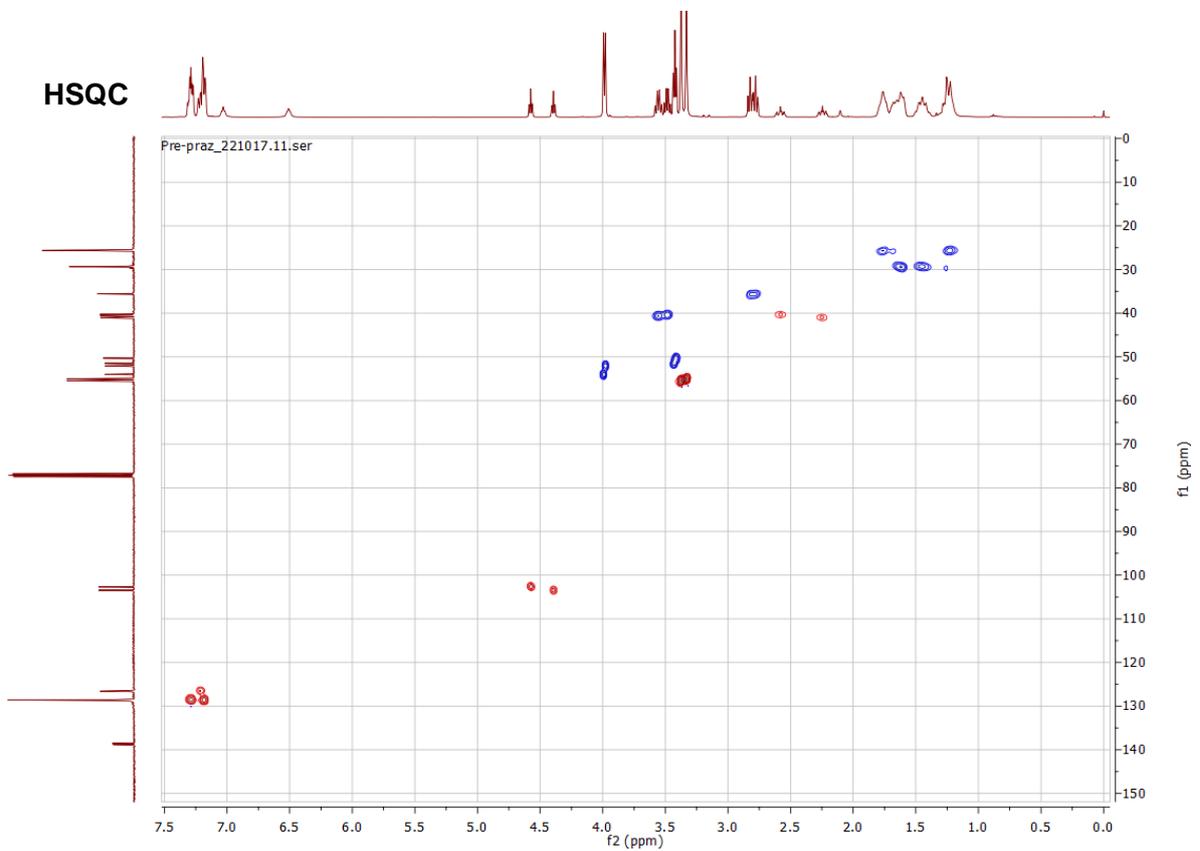
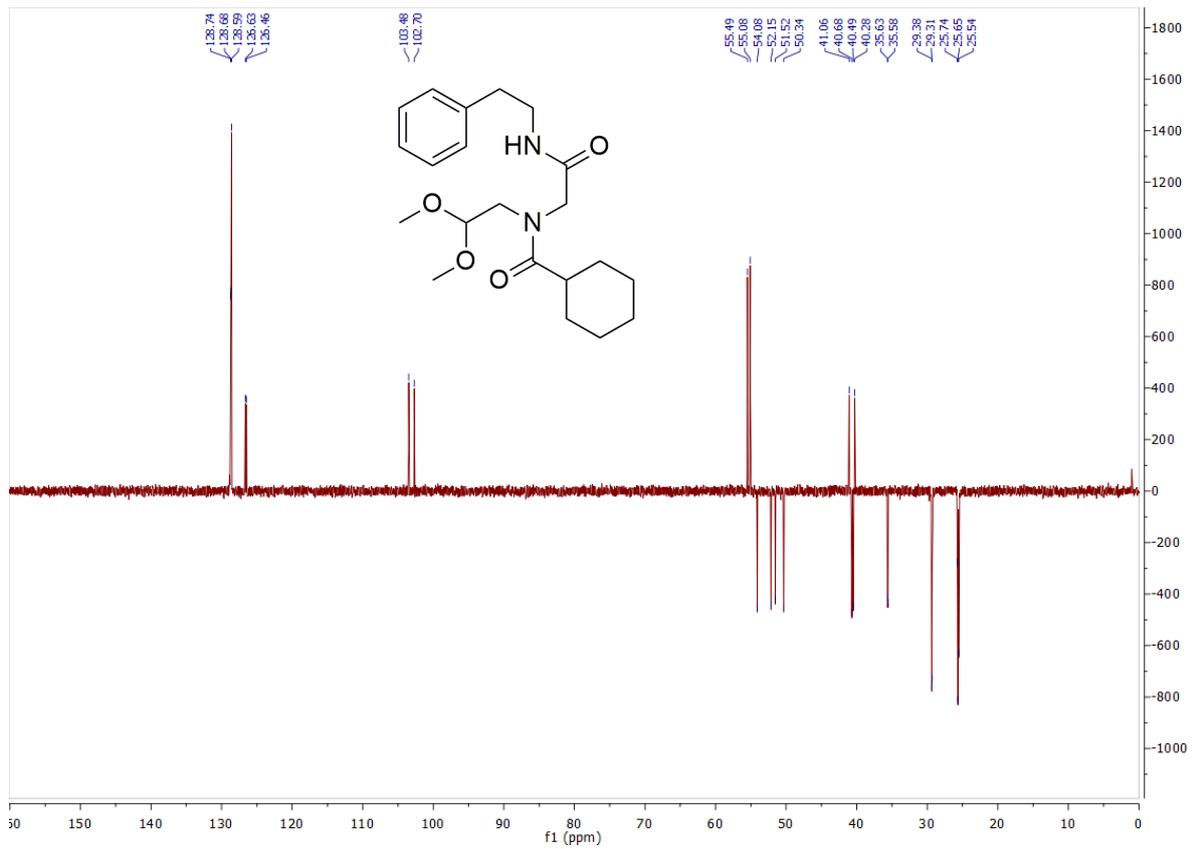
A – 4: Mass spectroscopy spectra of 2-isocyanoethylbenzene 96



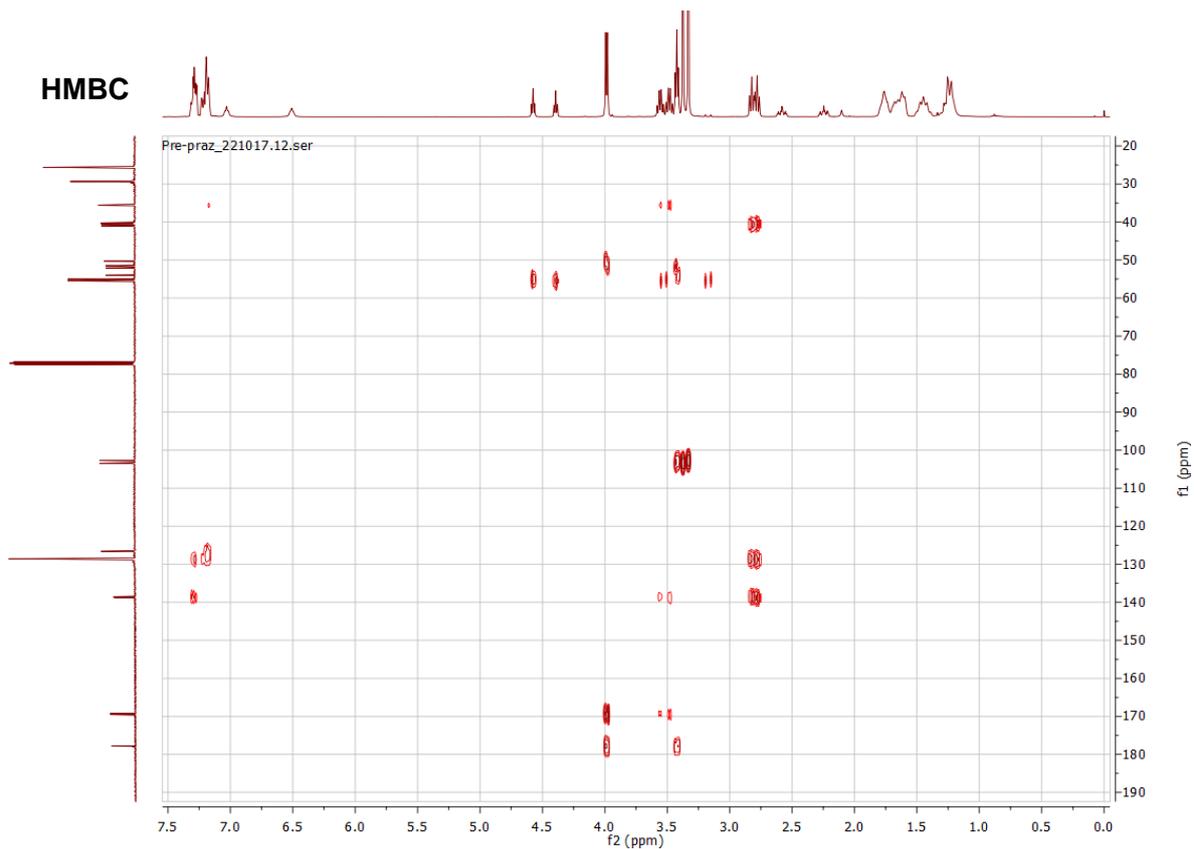
Appendix B (Chapter III)

B – 1: $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, Dept 135, HSQC, HMBC and COSY spectra of *N*-(2,2-dimethoxyethyl)-*N*-(2-oxo-2-(2-phenethylamino)ethyl)cyclohexanecarboxamide (“pre-praziquantel”) **61**

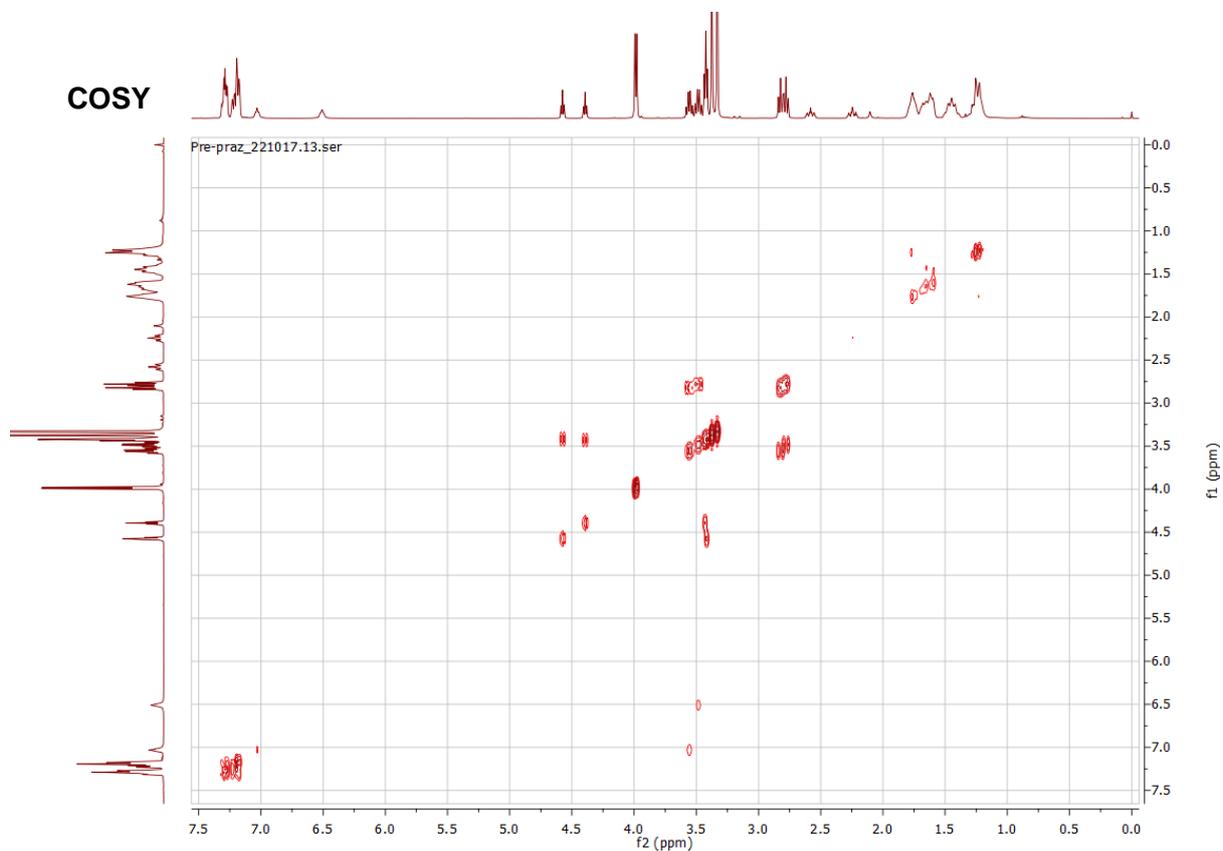




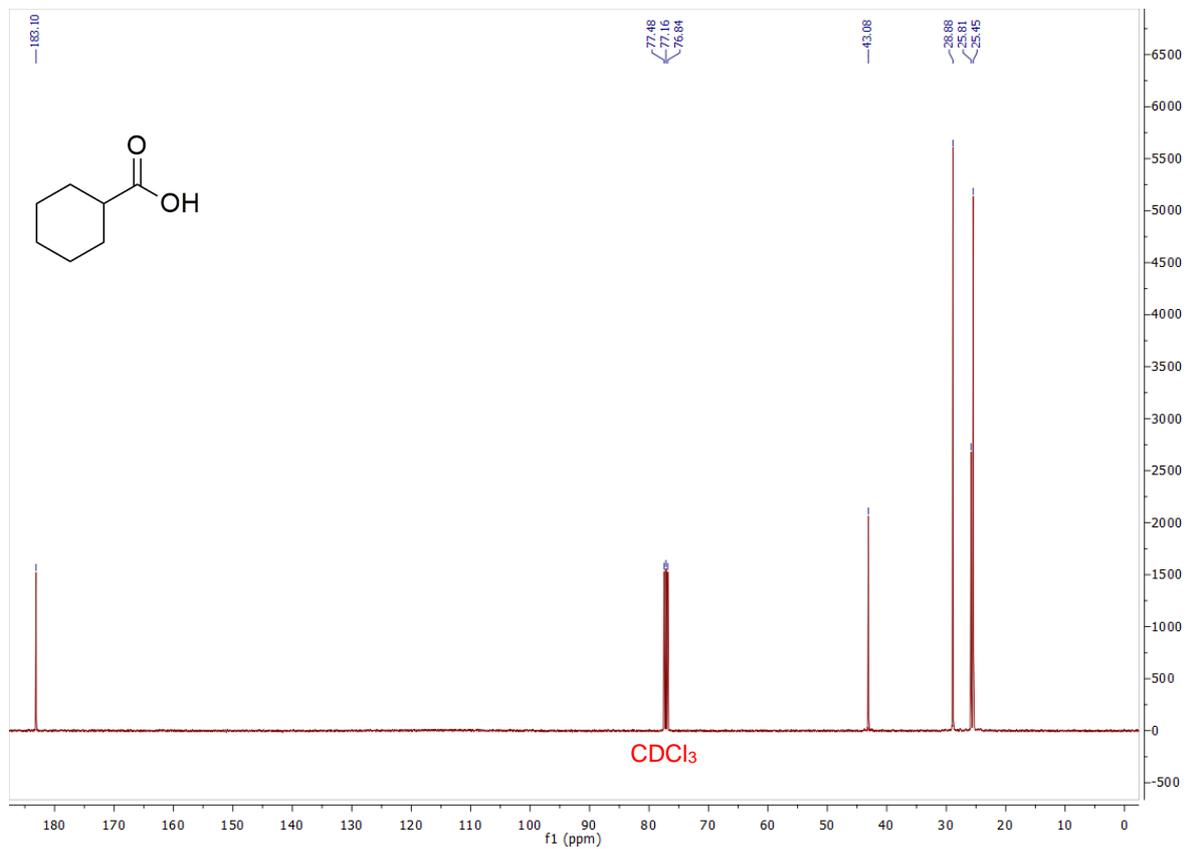
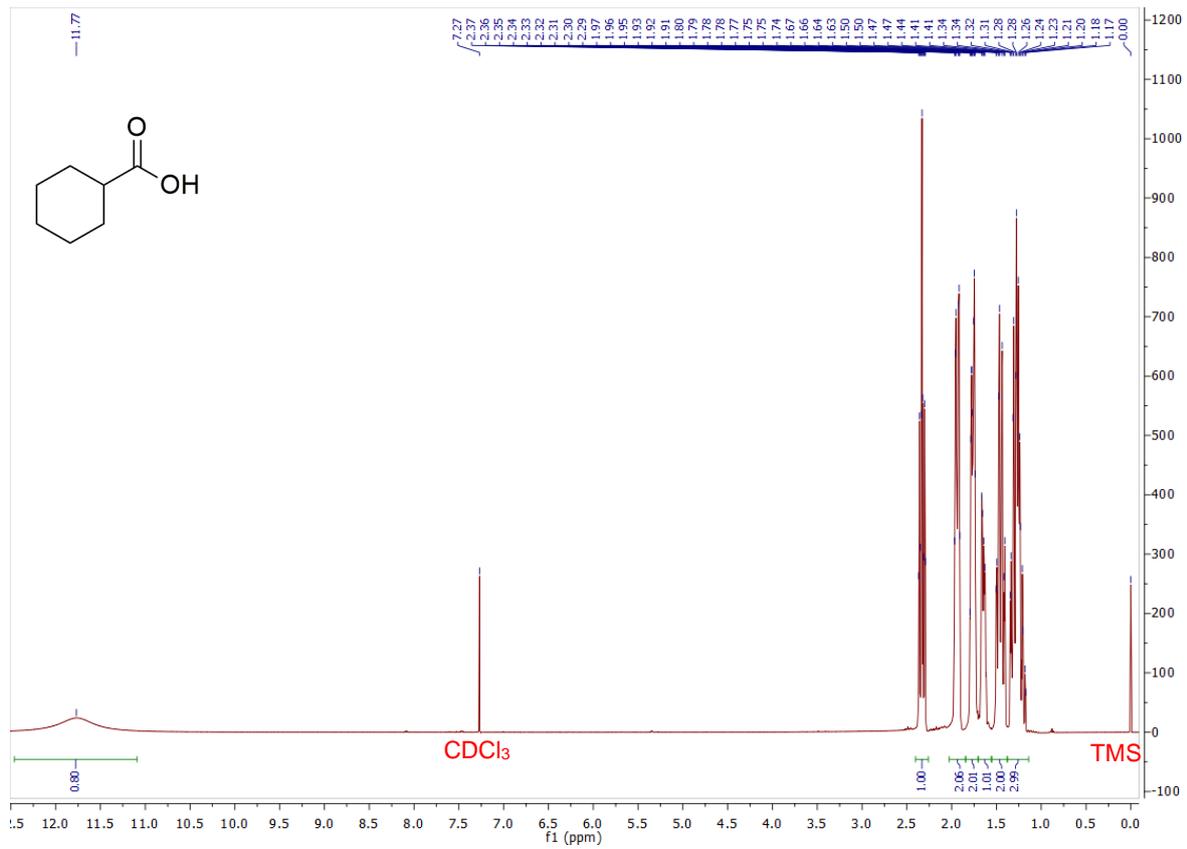
HMBC



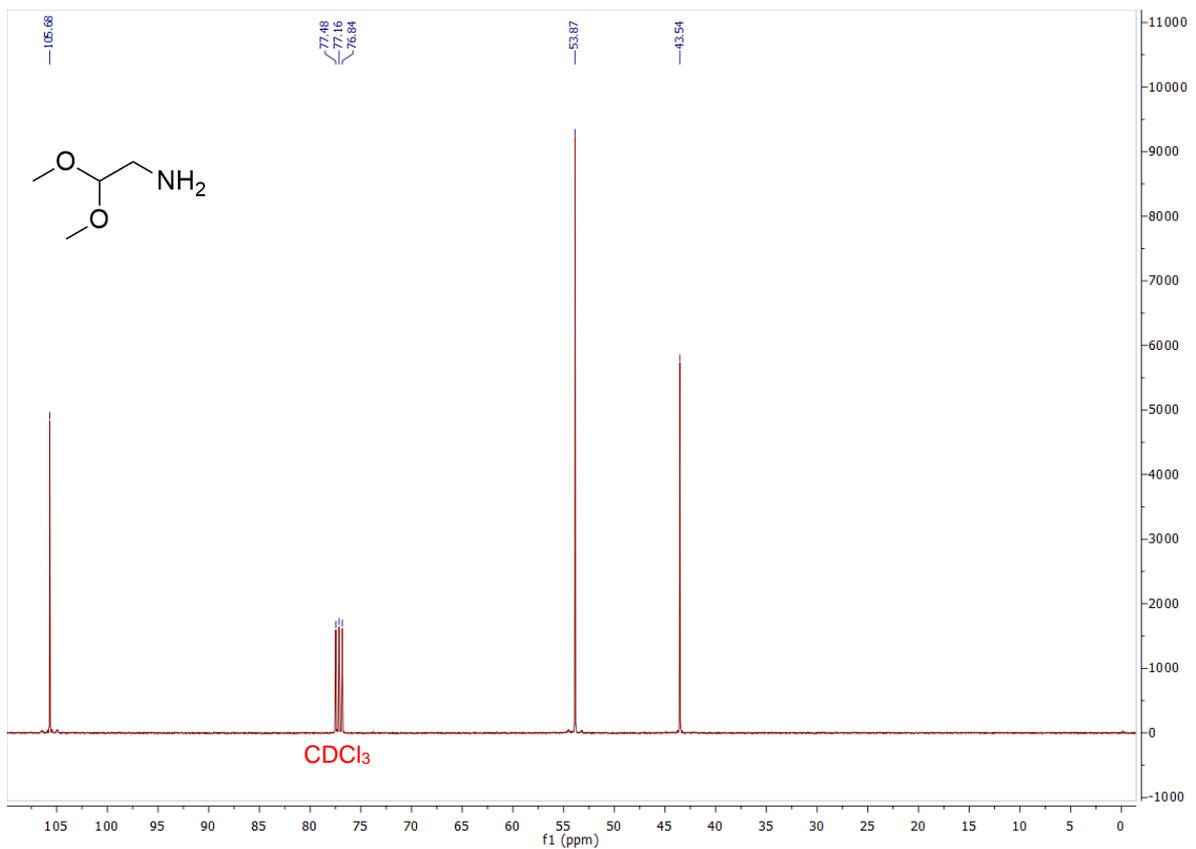
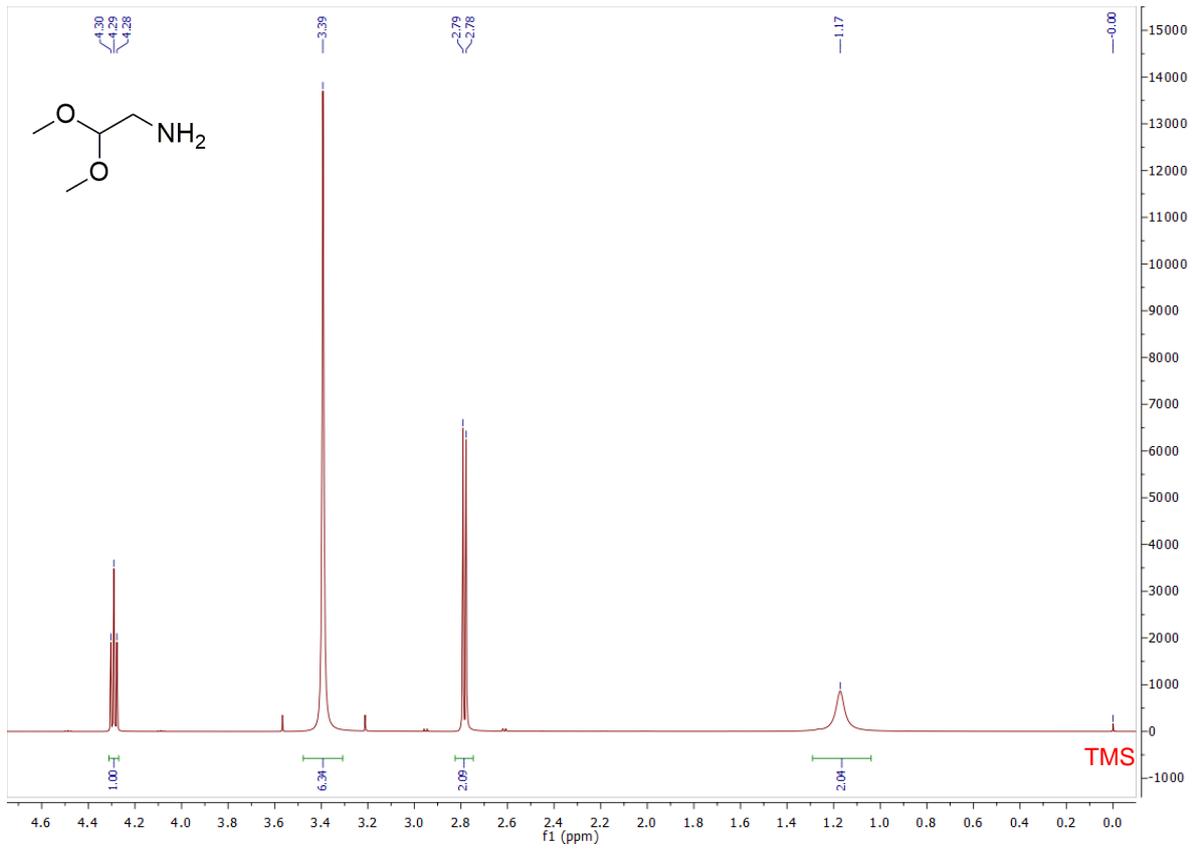
COSY



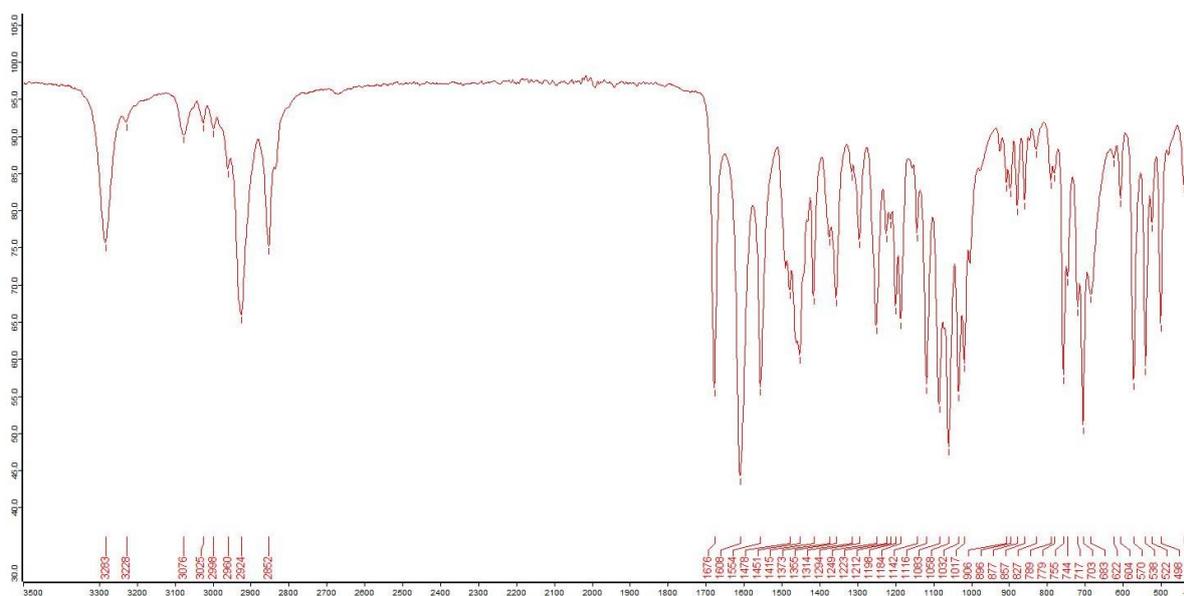
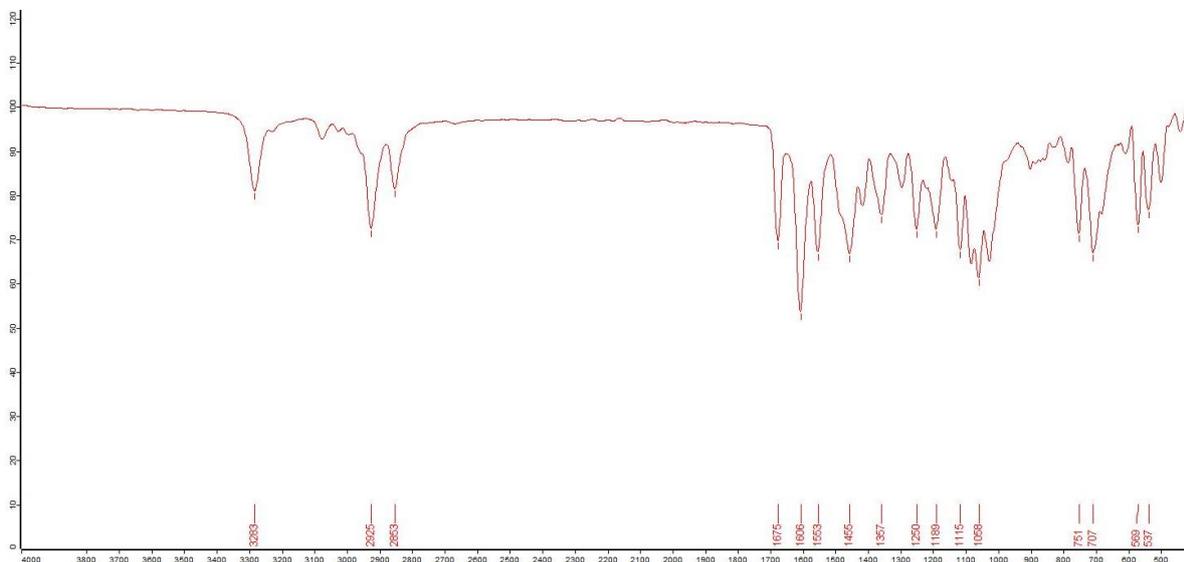
B – 2: ^1H -NMR and ^{13}C -NMR spectra of cyclohexanecarboxylic acid **51**



B – 3: ^1H -NMR and ^{13}C -NMR spectra of aminoacetaldehyde dimethyl acetal **43**



B – 4: Infrared spectroscopy ($400 - 4000 \text{ cm}^{-1}$) smoothed spectrum and min-max normalised spectrum of “pre-praziquantel” **61**



B – 5: SC-XRD raw data of “pre-praziquantel” 61

Table B – 5.1: Crystal data and structure refinement for ZJ01_LT_auto.

Identification code	ZJ01_LT_auto
Empirical formula	C ₂₁ H ₃₂ N ₂ O ₄
Formula weight	376.48
Temperature/K	150.15
Crystal system	triclinic
Space group	P-1
a/Å	10.1553(2)
b/Å	10.2110(2)
c/Å	11.5149(2)
α/°	97.1990(10)
β/°	106.729(2)
γ/°	109.527(2)
Volume/Å ³	1044.78(4)
Z	2
ρ _{calc} /cm ³	1.197
μ/mm ⁻¹	0.664
F(000)	408.0
Crystal size/mm ³	? × ? × ?
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	8.27 to 158.342
Index ranges	-12 ≤ h ≤ 12, -13 ≤ k ≤ 12, -14 ≤ l ≤ 14
Reflections collected	23154
Independent reflections	4346 [R _{int} = 0.0636, R _{sigma} = 0.0360]
Data/restraints/parameters	4346/0/246
Goodness-of-fit on F ²	1.094
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0553, wR ₂ = 0.1646
Final R indexes [all data]	R ₁ = 0.0597, wR ₂ = 0.1672
Largest diff. peak/hole / e Å ⁻³	0.23/-0.25

 Table B – 5.2: Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters (Å²×10³) for ZJ01_LT_auto. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij} tensor.

Atom	x	y	z	U(eq)
O2	860.7(13)	3315.9(14)	5243.0(12)	32.3(3)
O4	5005.5(14)	5726.5(14)	8440.9(12)	32.4(3)
O3	4905.1(16)	7737.4(14)	7800.4(12)	36.9(3)
O1	2928.2(16)	5711.7(17)	3491.8(12)	39.7(4)
N2	3081.2(15)	5162.5(15)	5824.2(13)	23.0(3)
N1	996.4(17)	6381.3(17)	3367.5(14)	30.4(3)
C11	2247.3(18)	3781.0(18)	5703.9(15)	24.0(3)
C18	4711.9(18)	5826.7(18)	6289.3(15)	24.6(3)
C9	2102.6(19)	6014.8(19)	3972.3(16)	26.5(4)
C12	2976.5(19)	2764.8(18)	6119.0(16)	25.9(4)
C10	2308(2)	6068.2(19)	5348.0(15)	26.2(4)
C19	5404(2)	6625.7(19)	7657.5(17)	28.4(4)
C6	1268(2)	7820(2)	513.4(18)	34.2(4)
C8	665(2)	6424(2)	2058.8(17)	32.6(4)
C17	2579(2)	1541(2)	4996.5(17)	30.9(4)
C13	2440(2)	2145(2)	7123.6(18)	37.8(4)

C21	6131(2)	5244(2)	9025.1(19)	40.0(5)
C7	1581(3)	7862(2)	1884.1(18)	39.4(5)
C1	2034(3)	7303(2)	-123(2)	42.9(5)
C5	191(3)	8258(3)	-145(2)	48.7(6)
C16	3337(3)	531(2)	5405(2)	48.1(6)
C2	1712(3)	7196(3)	-1396(2)	53.4(6)
C14	3159(3)	1104(2)	7537(2)	51.6(6)
C3	617(3)	7617(3)	-2045(2)	60.0(8)
C20	5643(3)	8687(3)	9017(2)	53.7(6)
C15	2866(3)	-74(2)	6437(2)	57.8(7)
C4	-137(3)	8153(4)	-1425(2)	63.9(8)

Table B – 5.3: Anisotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for ZJ01_LT_auto. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\dots]$.

Atom	U ₁₁	U ₂₂	U ₃₃	U ₂₃	U ₁₃	U ₁₂
O2	24.3(6)	39.1(7)	34.2(7)	8.8(5)	9.0(5)	14.5(5)
O4	34.4(7)	41.7(7)	28.2(6)	16.0(5)	13.4(5)	18.7(6)
O3	46.9(8)	30.1(7)	30.7(7)	2.3(5)	7.7(6)	18.3(6)
O1	45.0(8)	66.5(10)	29.6(7)	19.4(6)	20.4(6)	39.4(7)
N2	25.4(7)	28.5(7)	22.1(7)	9.8(5)	9.8(5)	16.4(6)
N1	34.3(8)	44.3(9)	26.5(7)	17.4(6)	15.1(6)	25.4(7)
C11	26.0(8)	31.3(9)	19.6(7)	7.7(6)	9.9(6)	15.1(7)
C18	25.7(8)	28.5(8)	26.7(8)	11.7(7)	13.7(7)	13.4(7)
C9	30.4(8)	32.0(9)	25.5(8)	12.4(7)	12.6(7)	18.2(7)
C12	27.2(8)	26.2(8)	25.7(8)	8.6(6)	7.9(7)	12.6(7)
C10	33.4(9)	31.5(9)	23.9(8)	10.9(7)	12.5(7)	21.6(7)
C19	27.8(8)	30.3(9)	29.2(9)	10.8(7)	10.5(7)	12.1(7)
C6	36.1(10)	34.5(10)	28.0(9)	15.0(7)	9.0(7)	8.4(8)
C8	34.7(9)	43.3(10)	24.8(9)	15.3(8)	10.1(7)	19.0(8)
C17	35.6(9)	31.8(9)	28.7(9)	7.4(7)	12.6(7)	16.3(7)
C13	51.3(12)	36.1(10)	26.0(9)	12.2(8)	13.9(8)	15.4(9)
C21	46.2(11)	55.6(13)	31.3(10)	20.2(9)	14.9(8)	31.2(10)
C7	49.4(12)	40.6(11)	26.1(9)	13.4(8)	9.4(8)	16.4(9)
C1	46.6(12)	41.2(11)	40.5(11)	14.6(9)	15.0(9)	15.4(9)
C5	39.6(11)	70.0(15)	45.3(12)	33.8(11)	18.5(10)	22.0(11)
C16	55.7(13)	36.4(11)	50.0(13)	0.8(9)	8.4(10)	27.5(10)
C2	63.3(15)	49.2(13)	41.2(12)	9.8(10)	26.6(11)	7.6(11)
C14	75.7(16)	32.2(10)	34.9(11)	15.3(9)	3.8(10)	17.4(10)
C3	52.7(14)	69.3(16)	30.3(11)	20.9(11)	9.9(10)	-8.6(12)
C20	61.9(15)	41.1(12)	40.9(12)	-8.0(10)	6.9(11)	15.4(11)
C15	79.7(17)	27.8(10)	49.1(13)	9.7(9)	-2.3(12)	21.8(11)
C4	36.9(11)	100(2)	50.2(14)	48.8(15)	8.8(10)	15.5(13)

Table B – 5.4: Bond Lengths for ZJ01_LT_auto.

Atom	Atom	Length/\AA	Atom	Atom	Length/\AA
O2	C11	1.245(2)	C12	C17	1.534(2)
O4	C19	1.411(2)	C12	C13	1.533(3)
O4	C21	1.421(2)	C6	C7	1.510(3)
O3	C19	1.400(2)	C6	C1	1.387(3)
O3	C20	1.426(2)	C6	C5	1.381(3)
O1	C9	1.226(2)	C8	C7	1.526(3)
N2	C11	1.346(2)	C17	C16	1.524(3)

N2	C18	1.462(2)		C13	C14	1.528(3)
N2	C10	1.462(2)		C1	C2	1.388(3)
N1	C9	1.335(2)		C5	C4	1.396(3)
N1	C8	1.456(2)		C16	C15	1.528(4)
C11	C12	1.513(2)		C2	C3	1.379(4)
C18	C19	1.516(2)		C14	C15	1.516(3)
C9	C10	1.529(2)		C3	C4	1.375(4)

Table B – 5.5: Bond Angles for ZJ01_LT_auto.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C19	O4	C21	113.37(14)	O3	C19	O4	109.18(14)
C19	O3	C20	113.05(16)	O3	C19	C18	108.01(14)
C11	N2	C18	126.04(14)	C1	C6	C7	120.53(18)
C11	N2	C10	117.81(14)	C5	C6	C7	121.1(2)
C18	N2	C10	115.91(14)	C5	C6	C1	118.38(19)
C9	N1	C8	121.73(15)	N1	C8	C7	113.00(16)
O2	C11	N2	120.49(15)	C16	C17	C12	110.02(15)
O2	C11	C12	118.84(15)	C14	C13	C12	110.22(18)
N2	C11	C12	120.67(14)	C6	C7	C8	110.90(16)
N2	C18	C19	114.03(13)	C6	C1	C2	121.3(2)
O1	C9	N1	123.59(16)	C6	C5	C4	120.5(2)
O1	C9	C10	122.11(15)	C17	C16	C15	110.46(19)
N1	C9	C10	114.25(14)	C3	C2	C1	119.6(2)
C11	C12	C17	109.84(14)	C15	C14	C13	111.71(18)
C11	C12	C13	109.93(15)	C4	C3	C2	119.8(2)
C13	C12	C17	109.55(15)	C14	C15	C16	111.91(19)
N2	C10	C9	111.56(13)	C3	C4	C5	120.3(2)
O4	C19	C18	112.07(14)				

Table B – 5.6: Hydrogen Atom Coordinates ($\text{\AA} \times 10^4$) and Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for ZJ01_LT_auto.

Atom	x	y	z	U(eq)
H1	454	6603	3770	36
H18A	5029	6503	5784	30
H18B	5102	5072	6168	30
H12	4085	3299	6475	31
H10A	2890	7070	5849	31
H10B	1318	5744	5441	31
H19	6516	7043	7904	34
H8A	-410	6227	1673	39
H8B	864	5657	1615	39
H17A	1483	1005	4639	37
H17B	2909	1940	4341	37
H13A	2707	2933	7853	45
H13B	1340	1638	6785	45
H21A	6393	4768	8385	60
H21B	5758	4566	9500	60
H21C	7017	6065	9593	60
H7A	1334	8625	2273	47
H7B	2657	8092	2309	47
H1A	2792	7016	322	51
H5	-332	8634	276	58
H16A	4434	1054	5716	58
H16B	3060	-266	4680	58

H2	2244	6834	-1817	64
H14A	4249	1637	7959	62
H14B	2756	669	8147	62
H3	385	7538	-2918	72
H20A	5251	9441	9063	81
H20B	6718	9122	9181	81
H20C	5467	8151	9644	81
H15A	1788	-695	6092	69
H15B	3422	-673	6731	69
H4	-884	8452	-1870	77

Crystal structure determination of [ZJ01_LT_auto]

Crystal Data for $C_{21}H_{32}N_2O_4$ ($M = 376.48$ g/mol): triclinic, space group P-1 (no. 2), $a = 10.1553(2)$ Å, $b = 10.2110(2)$ Å, $c = 11.5149(2)$ Å, $\alpha = 97.1990(10)^\circ$, $\beta = 106.729(2)^\circ$, $\gamma = 109.527(2)^\circ$, $V = 1044.78(4)$ Å³, $Z = 2$, $T = 150.15$ K, $\mu(\text{CuK}\alpha) = 0.664$ mm⁻¹, $D_{\text{calc}} = 1.197$ g/cm³, 23154 reflections measured ($8.27^\circ \leq 2\theta \leq 158.342^\circ$), 4346 unique ($R_{\text{int}} = 0.0636$, $R_{\text{sigma}} = 0.0360$) which were used in all calculations. The final R_1 was 0.0553 ($I > 2\sigma(I)$) and wR_2 was 0.1672 (all data).

Refinement model description

Number of restraints - 0, number of constraints - unknown.

Details:

1. Fixed Uiso

At 1.2 times of:

All C(H) groups, All C(H,H) groups, All N(H) groups

At 1.5 times of:

All C(H,H,H) groups

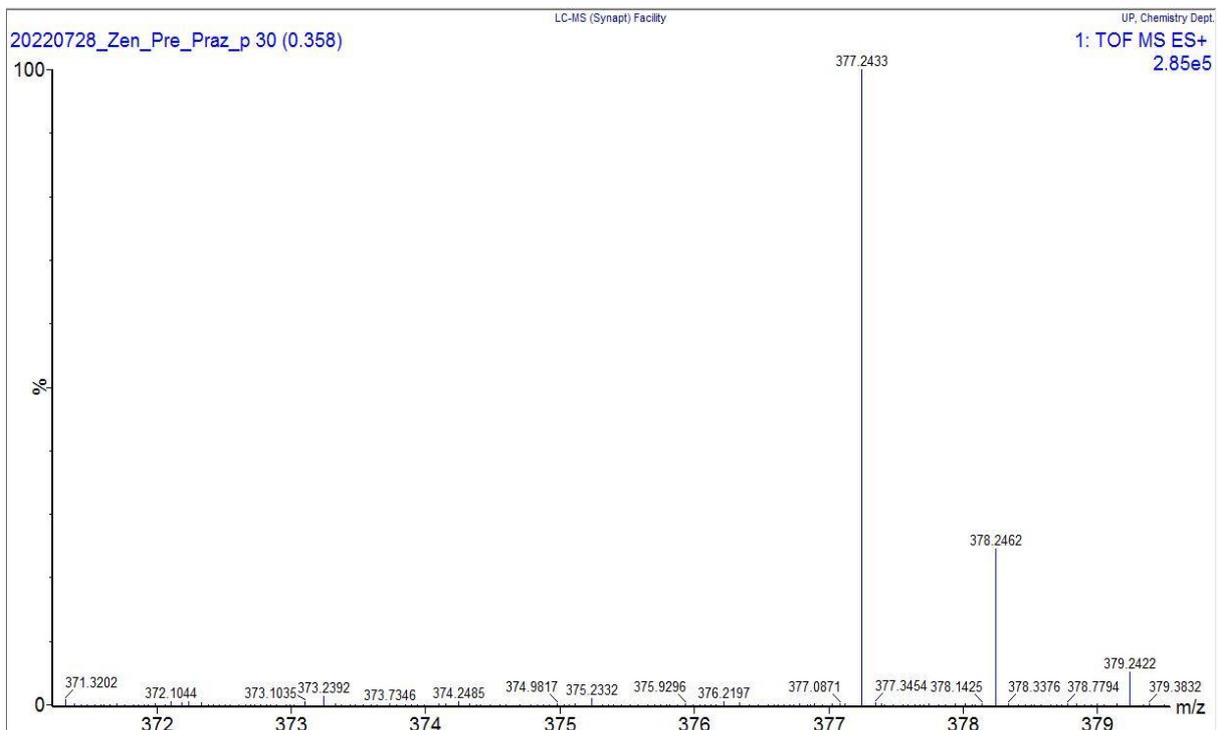
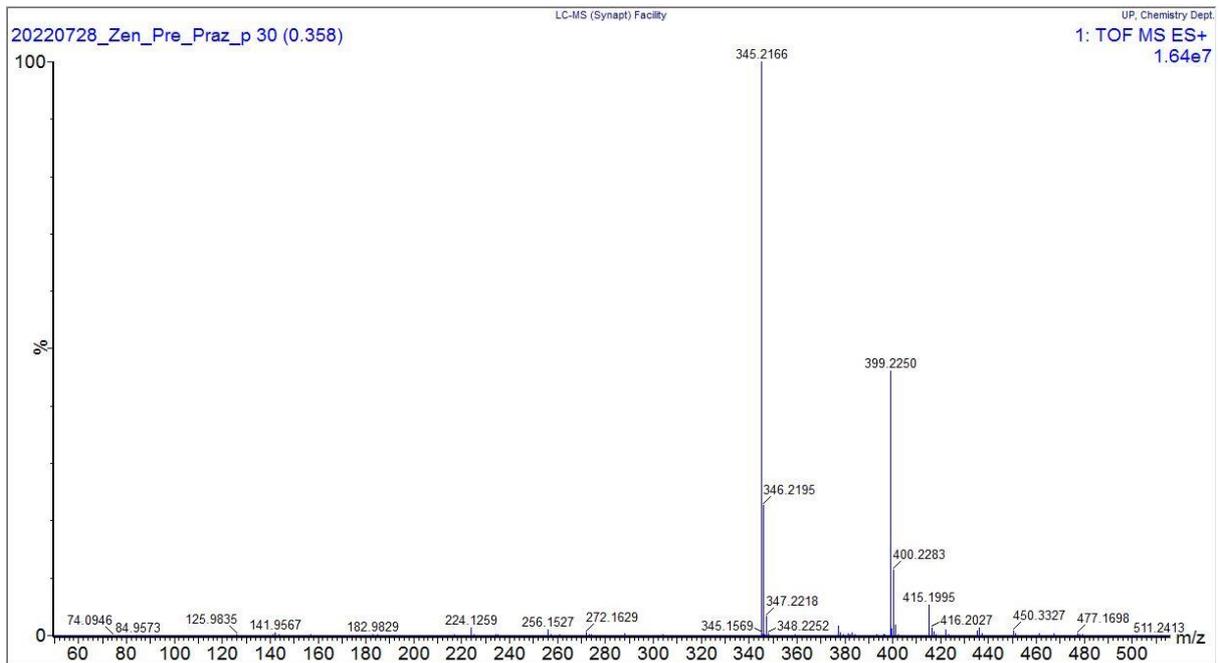
2.a. Ternary CH refined with riding coordinates: C12(H12), C19(H19)

2.b. Secondary CH₂ refined with riding coordinates: C18(H18A,H18B), C10(H10A,H10B), C8(H8A,H8B), C17(H17A,H17B), C13(H13A,H13B), C7(H7A,H7B), C16(H16A,H16B), C14(H14A,H14B), C15(H15A,H15B)

2.c. Aromatic/amide H refined with riding coordinates: N1(H1), C1(H1A), C5(H5), C2(H2), C3(H3), C4(H4)

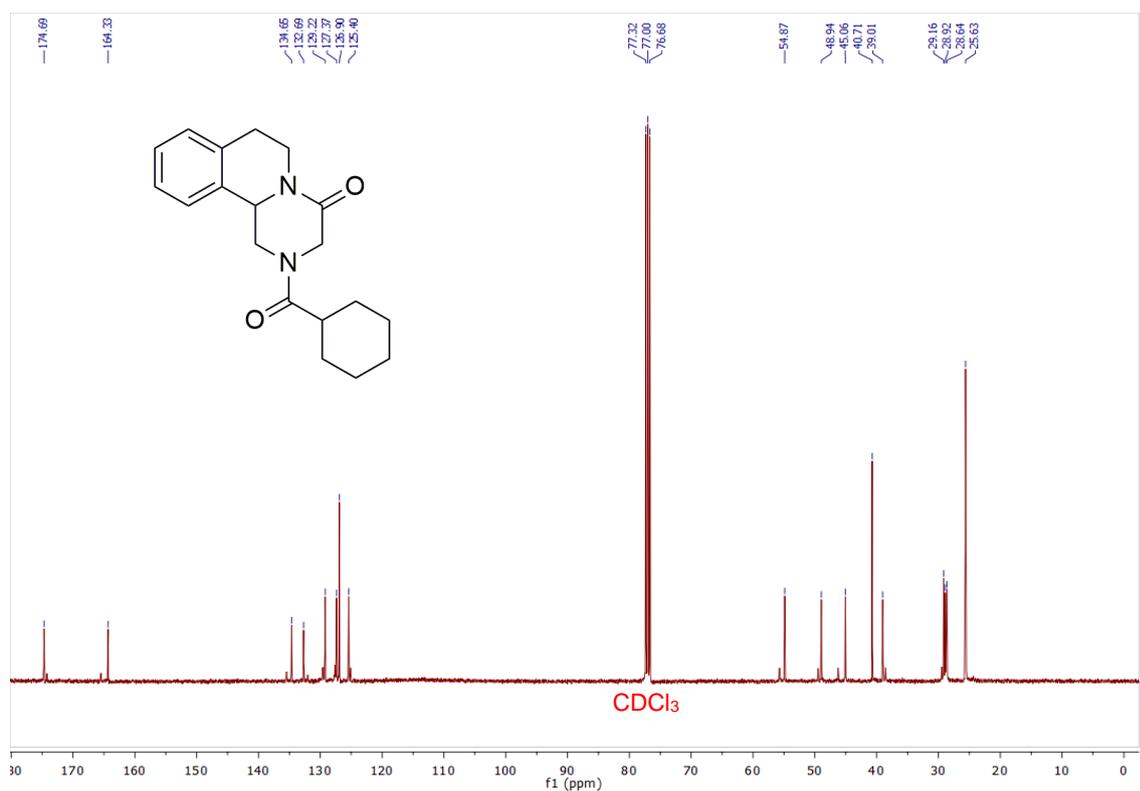
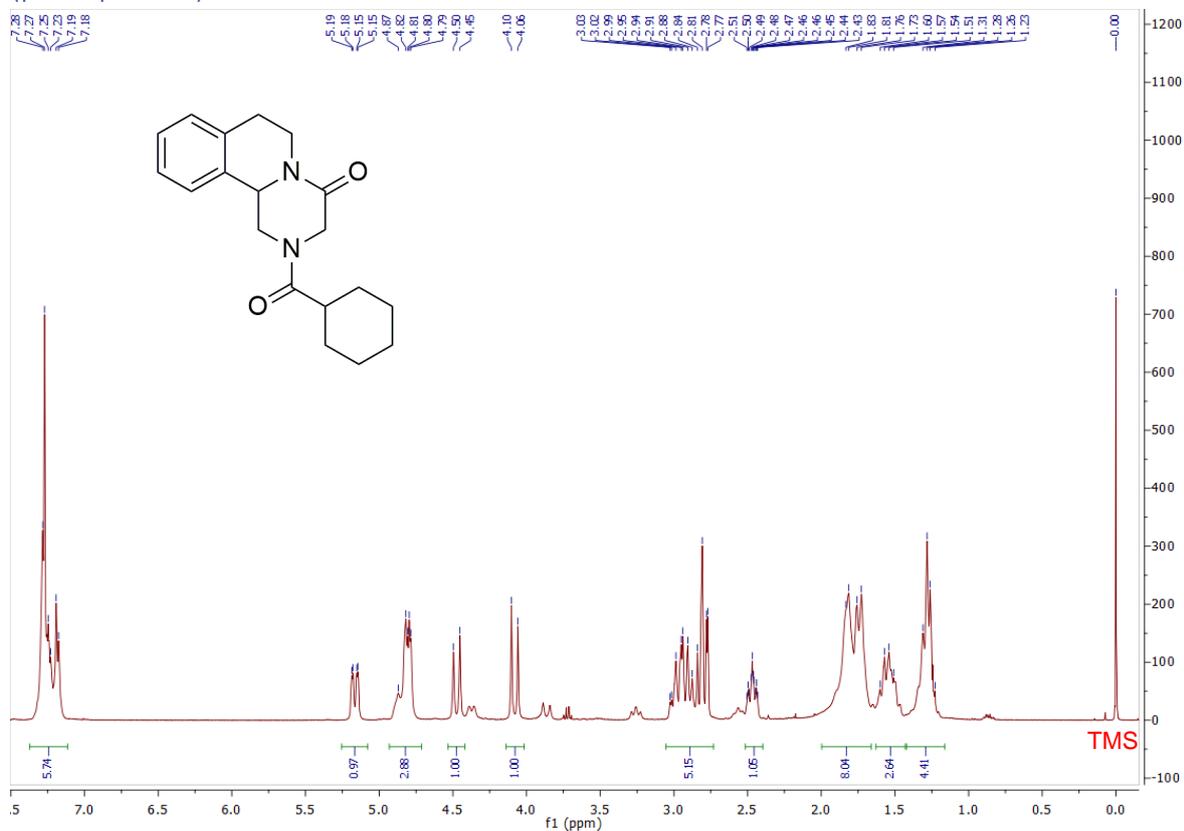
2.d. Idealised Me refined as rotating group: C21(H21A,H21B,H21C), C20(H20A,H20B,H20C)

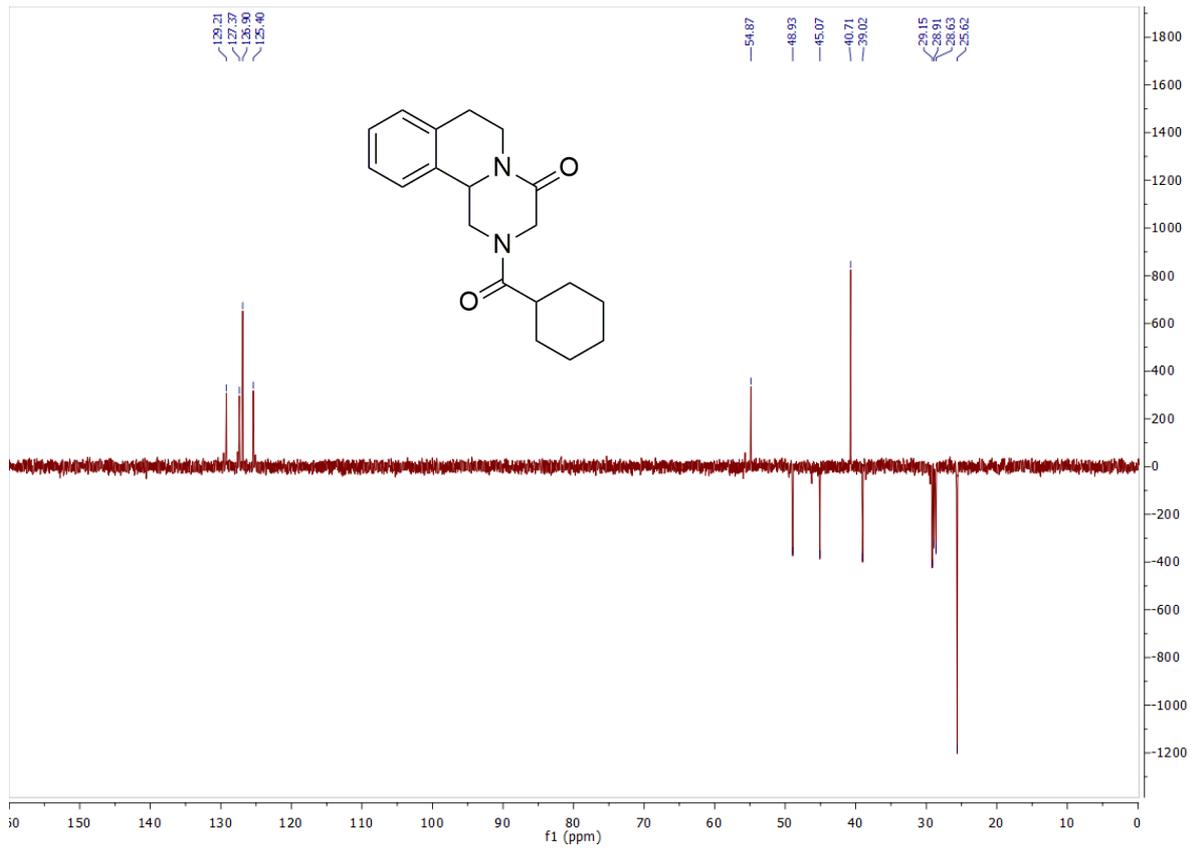
B – 6: Mass spectroscopy spectra of “pre-praziquantel” 61



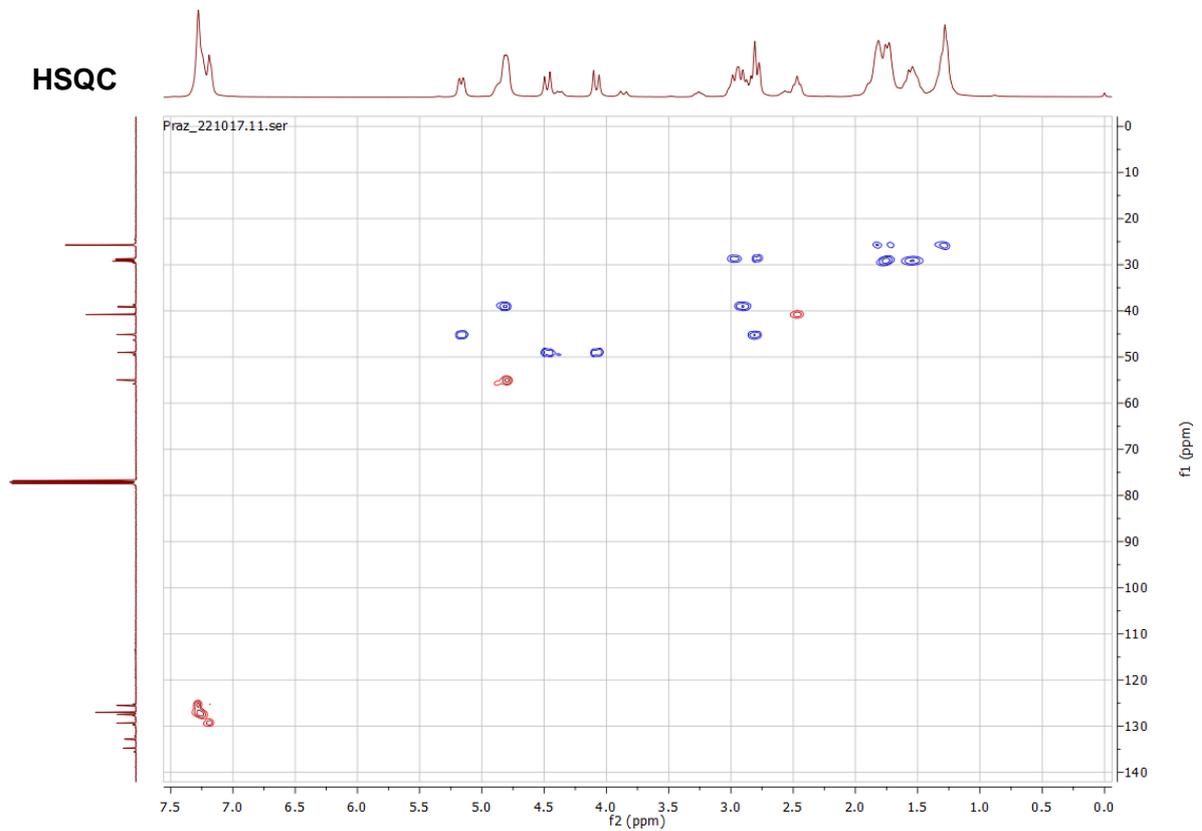
Appendix C (Chapter IV)

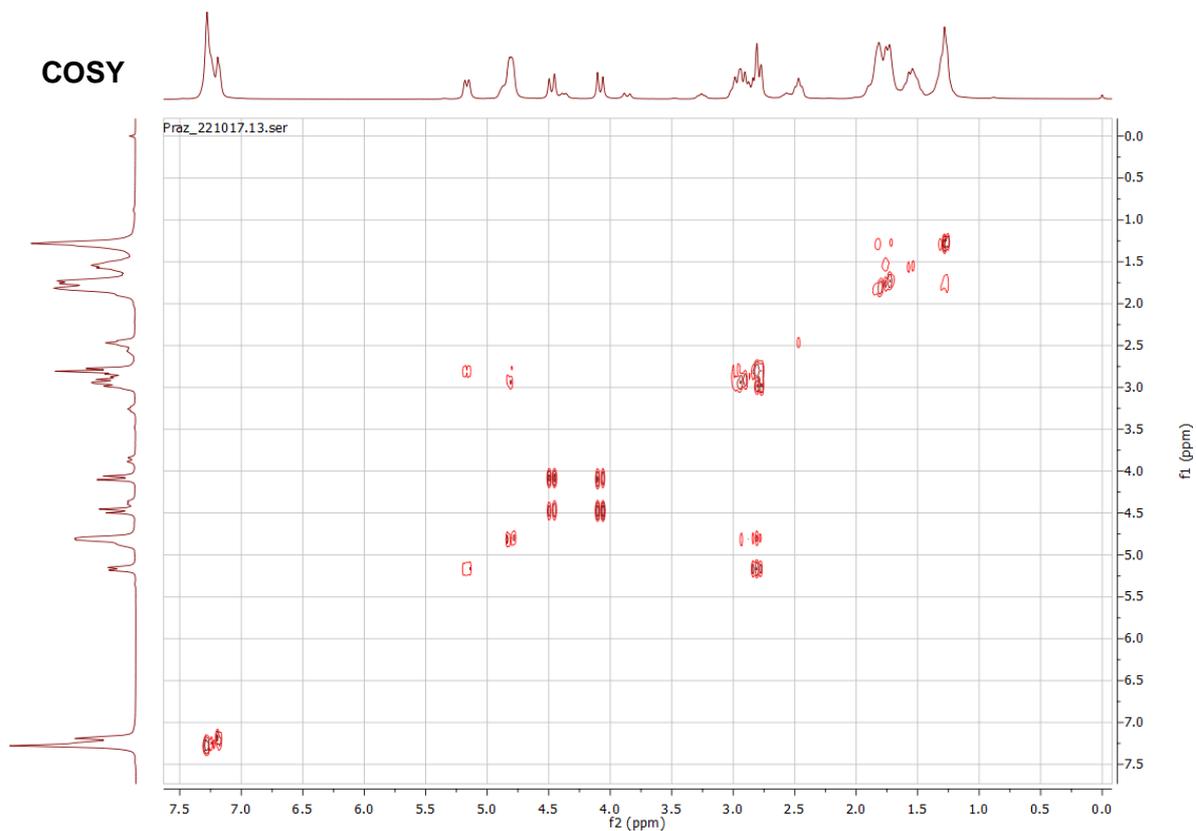
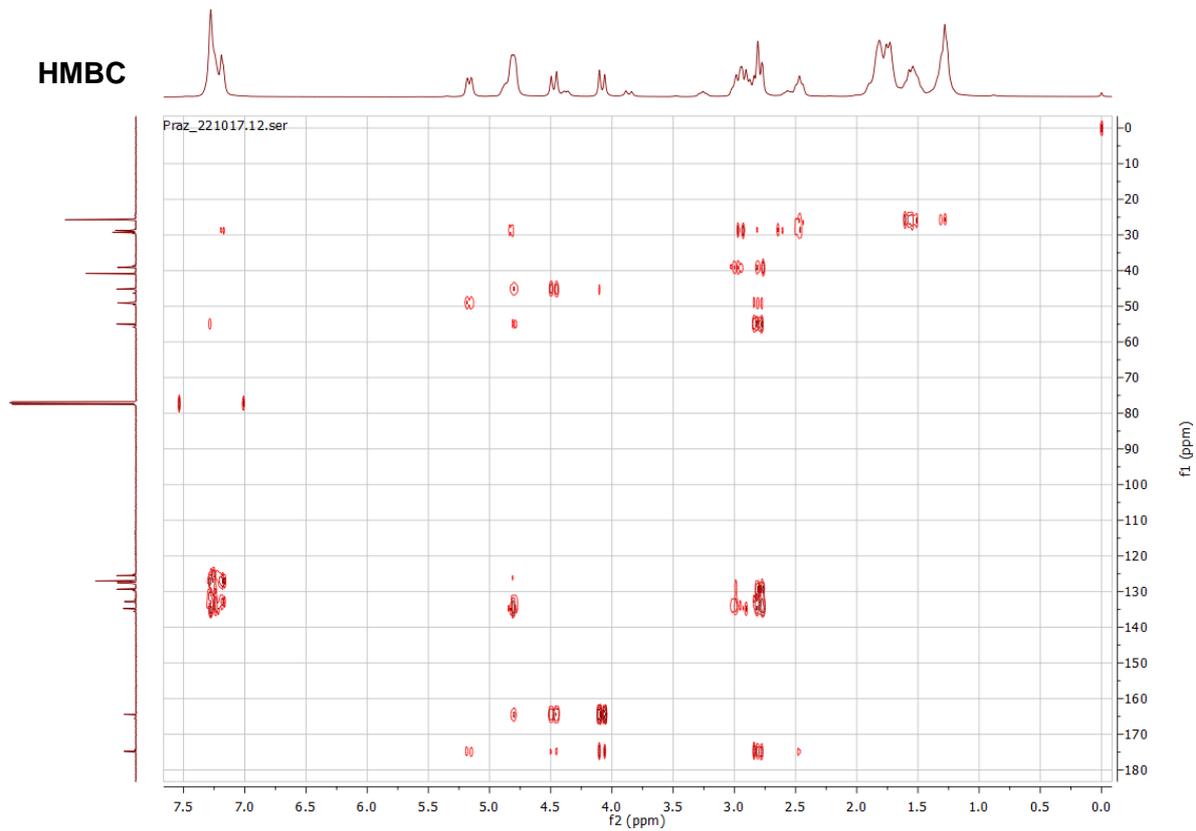
C – 1: $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, Dept 135, HSQC, HMBC and COSY spectra of 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1a]isoquinolin-4-one (praziquantel) **1**



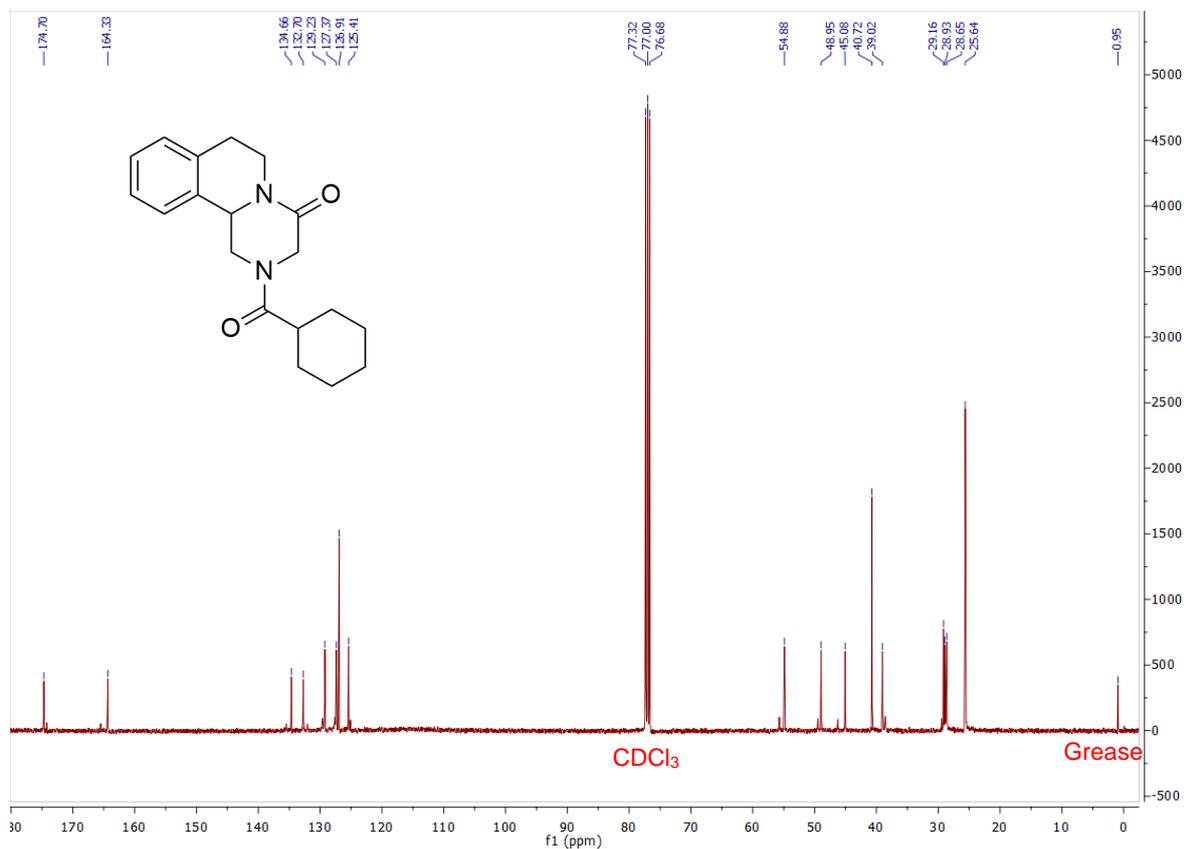
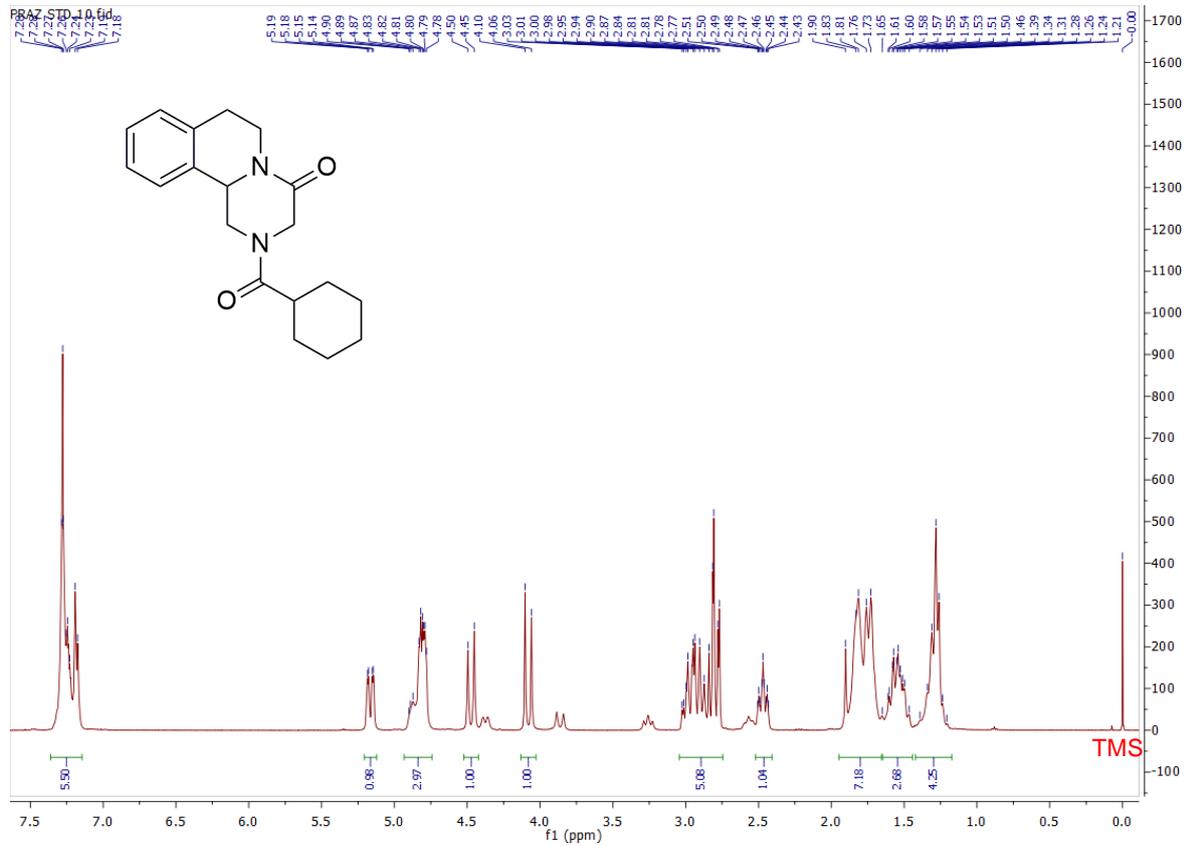


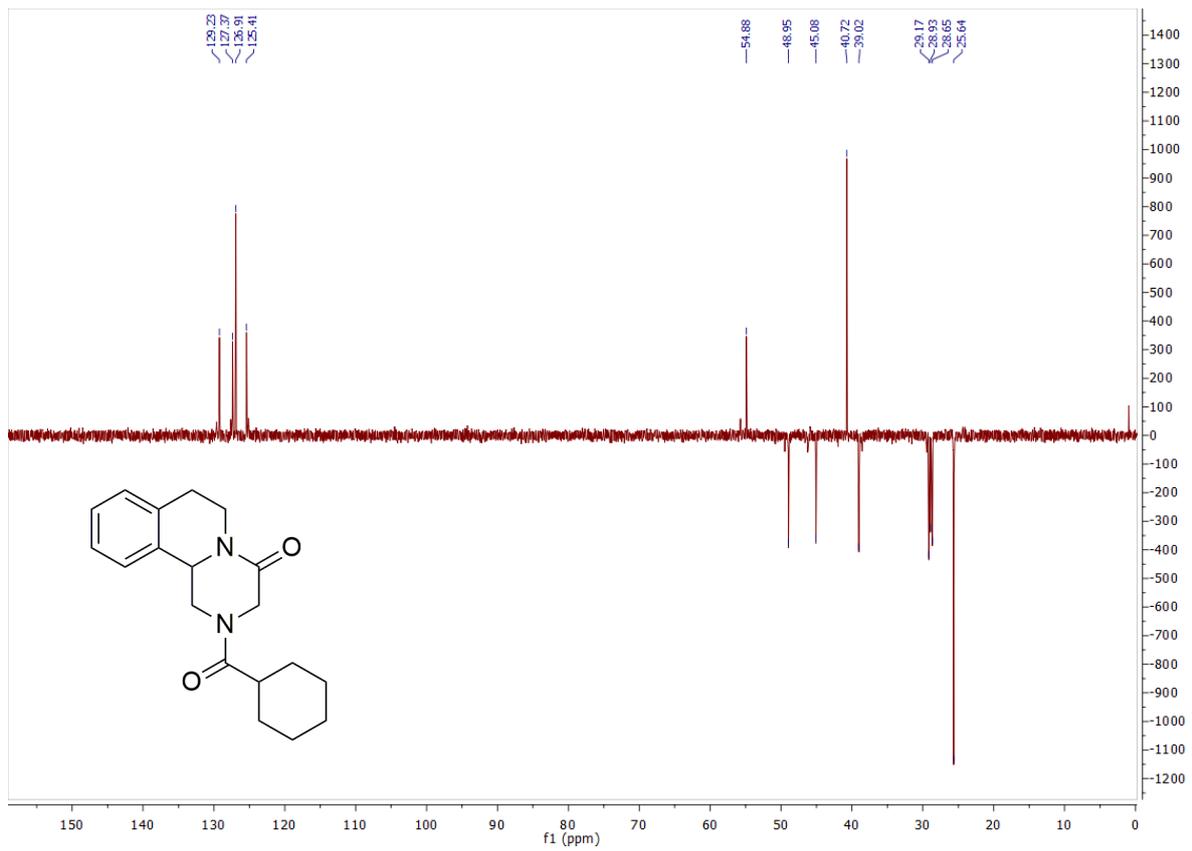
HSQC



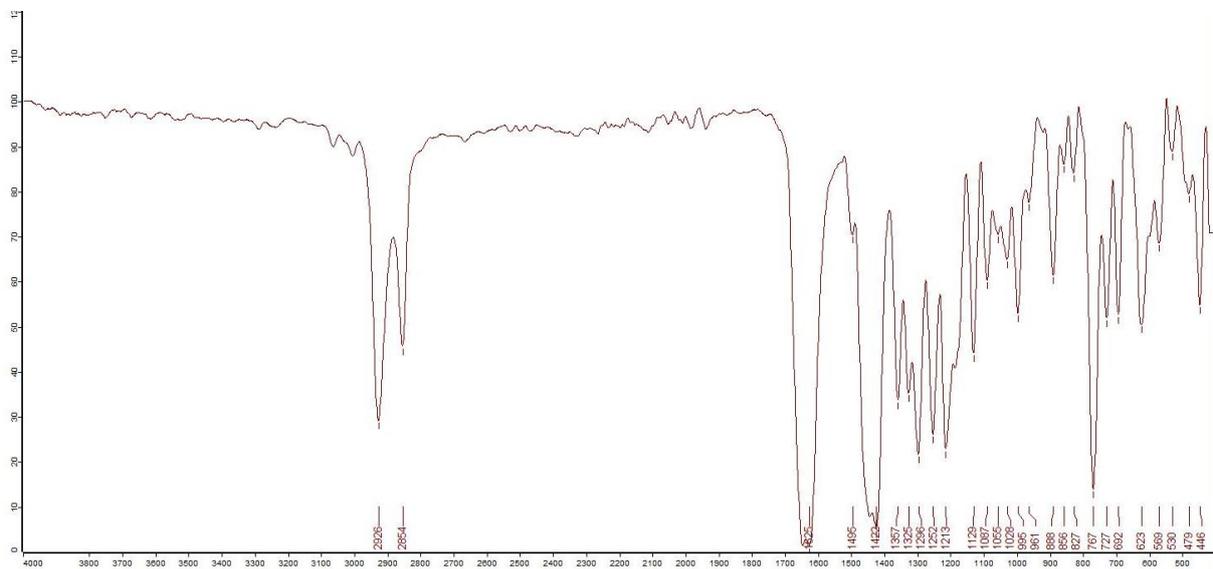
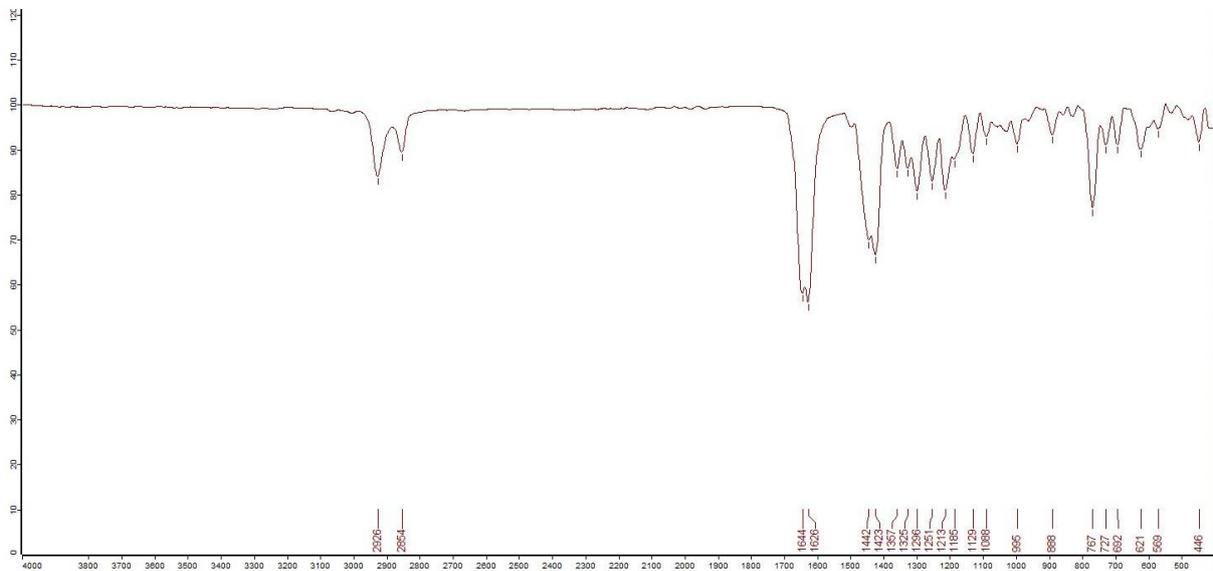


C – 2: $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and Dept 135 spectra of the obtained pharmaceutical standard praziquantel **1**

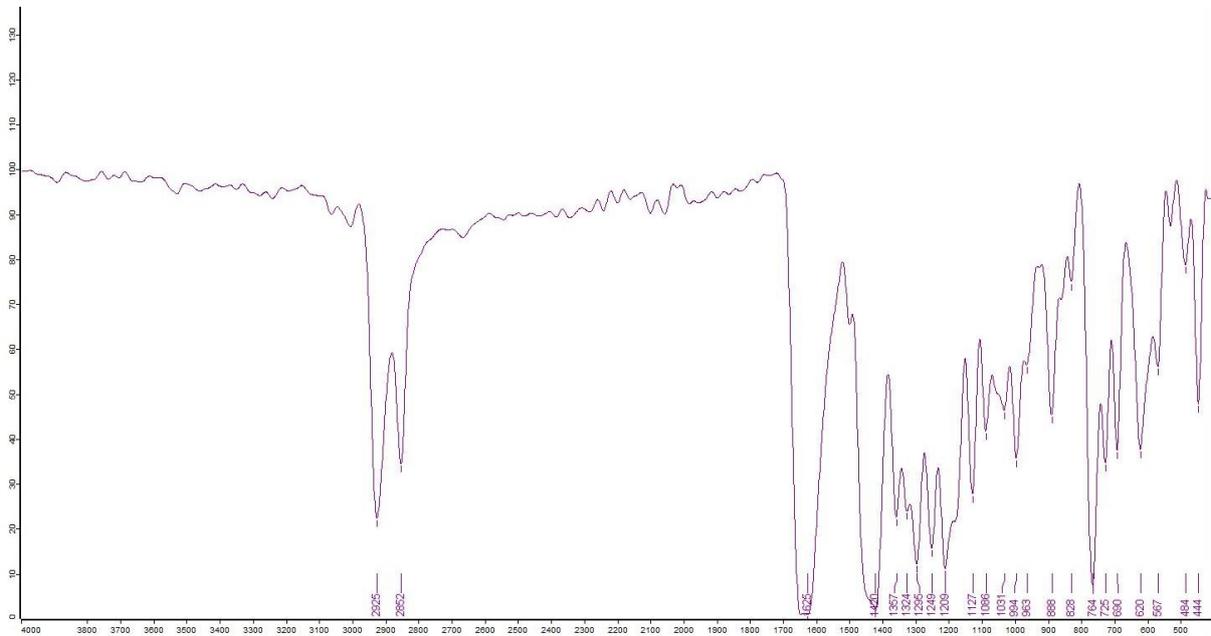
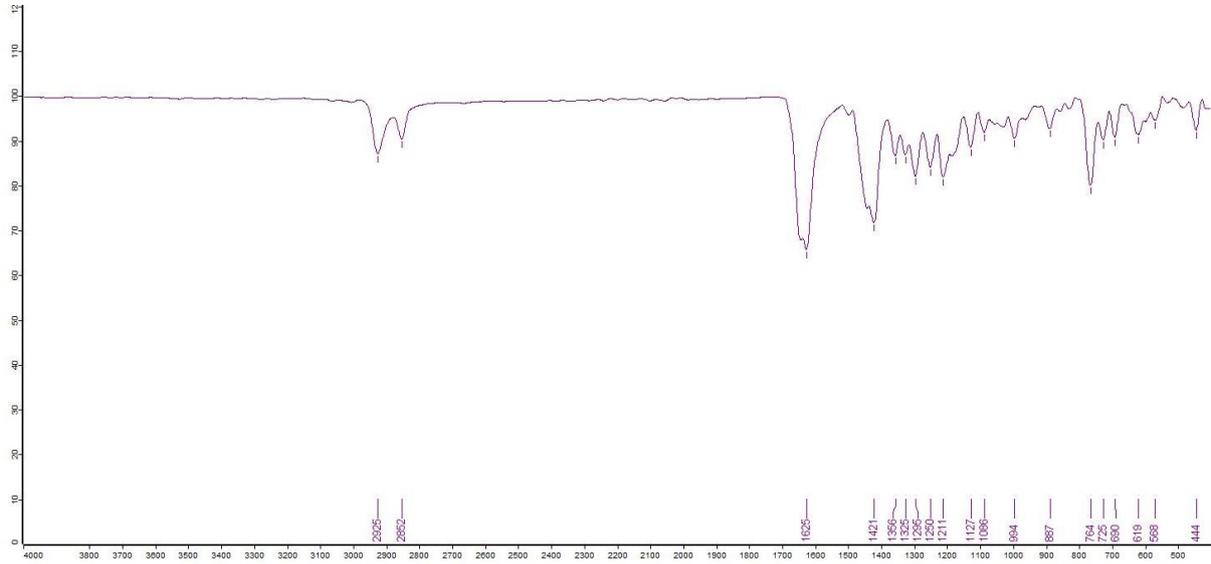




C – 3: Infrared spectroscopy (400 – 4000 cm^{-1}) smoothed spectrum and min-max normalised spectrum of praziquantel **1**



C – 4: Infrared spectroscopy (400 – 4000 cm^{-1}) smoothed spectrum and min-max normalised spectrum of the pharmaceutical standard praziquantel **1**



C – 5: Mass spectroscopy spectra of praziquantel **1** synthesised during this study (bottom) juxtaposed with the pharmaceutical standard (top).

