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## An Organic Chemistry Odyssey

### Imaging Mass Spectrometry in Neurotherapeutics, Asymmetric Methodology Development and Natural Product Synthesis

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# An Organic Chemistry Odyssey

Imaging Mass Spectrometry in Neurotherapeutics, Asymmetric  
Methodology Development and Natural Product Synthesis

MARVIN VILLACREZ | CENTRE FOR ANALYSIS AND SYNTHESIS | LUND UNIVERSITY





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# An Organic Chemistry Odyssey

Imaging Mass Spectrometry in Neurotherapeutics,  
Asymmetric Methodology Development  
and Natural Product Synthesis

Marvin Villacrez



**LUND**  
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DOCTORAL DISSERTATION

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To be defended at Kemicentrum.

Lecture Hall K:C, 14.00. Friday, May 17, 2024

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<b>Title and subtitle:</b> An Organic Chemistry Odyssey – Imaging Mass Spectrometry in Neurotherapeutics, Asymmetric Methodology Development and Natural Product Synthesis		
<p><b>Abstract:</b> The development of imaging technologies and brain models is crucial for advancements in neurotherapeutic drug discovery and development. They assume a critical role in evaluating essential parameters such as drug blood-brain barrier (BBB) permeation, distribution, and metabolism, all of which are vital for the development of safe and effective medications. In the first part of this thesis, we explore how the antipsychotic drug clozapine (CLO) and its two primary metabolites, <i>N</i>-desmethylclozapine (NDMC) and clozapine-<i>N</i>-oxide (CNO), permeate the blood-brain barrier and how they are distributed as well as metabolised within the brain. This investigation is conducted primarily in a desert locusts (<i>Schistocerca gregaria</i>) <i>ex vivo</i> brain model by employing Imaging Mass Spectrometry (IMS), specifically Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI-IMS) as imaging tool. IMS leverages the capabilities of traditional Mass Spectrometry (MS) but in a two-dimensional format, enabling the creation of ion intensity maps that disclose the precise spatial distribution of molecules within analysed samples. Notably, IMS achieves mapping without the need for molecular imaging probes.</p> <p>Our study was carried out by subjecting isolated desert locust brains to incubation in solutions containing the studied drugs for durations of 15 and 45 minutes. This unique <i>ex vivo</i> approach allowed us to investigate the isolated behaviour of the drugs within the brain, free from the influence of systemic contamination.</p> <p>Obtained ion intensity maps revealed that CLO exhibits a notable capacity to traverse the BBB. After a 15-minute incubation period, the drug was primarily located at the brain tissue's periphery. However, following a 45-minute incubation, it was distributed throughout the entire brain. The maps also reveal that CLO is readily metabolised to NDMC within the brain, with its distribution mirroring that of CLO. Interestingly, these findings diverge significantly from the NDMC administration studies. In the maps derived from these experiments, it becomes apparent that NDMC exhibits a constrained ability to penetrate the BBB, as its distribution remains predominantly limited to the tissue edges even after 45 minutes of incubation. Regarding CNO incubation experiments, the resulting maps clearly illustrate its highly restricted ability to penetrate the BBB, demonstrating an even lower BBB permeability than that observed for NDMC. Furthermore, the maps show the presence of both CLO and NDMC, indicating that CNO is susceptible to brain metabolism. This discovery holds significant implications in the context of CNO's use in the Designer Receptors Activated only by Designer Drugs (DREADD) technique, where it is employed with the assumption of its pharmacological inactivity. The presence of CLO and NDMC, both of which exhibit pharmacological activity on dopamine, serotonin, and muscarine receptors, could potentially have influenced the interpretation of results in previously conducted DREADD studies. This suggests the necessity for a reevaluation of the outcomes of these experiments.</p> <p>The ion intensity maps generated in this study are groundbreaking and showcase the potential of our IMS desert locust brain model platform for application in both pharmaceutical research and neuroscience.</p> <p>The second part of this thesis targets the development of small-molecule compounds. It presents an efficient enantioselective approach for the synthesis of vicinal amino alcohols and our attempts towards the total synthesis of Aspidophylline A. By using asymmetric transfer hydrogenation (ATH) on <math>\beta</math>-amido-<math>\alpha</math>-keto esters undergoing dynamic kinetic resolution (DKR); we managed to synthesise various <i>anti</i>-<math>\beta</math>-amido-<math>\alpha</math>-hydroxy esters in high yields and excellent diastereo- and enantioselectivities. The protocol developed for the hydrogenations uses <math>\text{HCO}_2\text{H}:\text{NEt}_3</math> (2:5) as a hydrogen source and <math>\text{RuCl}[(R,R)\text{-FsDPEN}](p\text{-cymene})</math> as a catalyst, both commercially available. Our work on Aspidophylline A has brought us to the synthesis of an advanced tricyclic intermediate.</p>		
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Marvin Villacrez



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*To my Parents and Life Companion Gabby,*

*without whose never-ending support, love, and encouragement  
this thesis would never have been completed.*

## List of Publications

- I. Evaluation of Drug Exposure and Metabolism in Locust and Zebrafish Brains Using Mass Spectrometry Imaging.**  
M. Villacrez, K. Hellman, T. Ono, Y. Sugihara, M. Rezeli, F. Ek, G. Marko-Varga and R. Olsson  
ACS Chemical Neuroscience. 2018, (9)8, 1994-2000
- II. Enantioselective synthesis of *anti*- $\beta$ -amido- $\alpha$ -hydroxy esters via asymmetric transfer hydrogenation coupled with dynamic kinetic resolution.**  
M. Villacrez and P. Somfai  
Tetrahedron Lett. 2013, (54)38, 5266-5268
- III. A Rapid Construction of an Advanced Tricyclic Intermediate for the Total Synthesis of Aspidophylline A.**  
M. Villacrez and P. Somfai  
*Manuscript in preparation.*

## Contribution to the Publications

- I.** I contributed to the design and planning of drug administration experiments and executed the main portion of the MALDI-IMS experiments. Furthermore, I conducted the majority of the data analysis and drafted the manuscript.
- II.** I participated in both the design and planning phases of the experiments, and subsequently conducted all of them. Furthermore, I conducted all compound analyses, specifically setting up and operating an HPLC apparatus for chiral analysis.
- III.** I conducted all experiments as well as analyses and was actively involved in planning and designing alternative synthetic routes. I also composed the initial draft of the manuscript.

## Abbreviations

<b>ATH</b>	Asymmetric Transfer Hydrogenation
<b>ATH/DKR</b>	Asymmetric Transfer Hydrogenation Coupled with Dynamic Kinetic Resolution
<b>BMEC</b>	Brain microvascular endothelial cell
<b>CHCA</b>	$\alpha$ -cyano-4-hydroxy cinnamic acid
<b>CLO</b>	Clozapine
<b>CNO</b>	Clozapine- <i>N</i> -oxide
<b>CNS</b>	Central Nervous System
<b>CYP</b>	Cytochrome p450
<b>DKR</b>	Dynamic Kinetic Resolution
<b>DMP</b>	Dess-Martin Periodane
<b>DPAE</b>	2-amino-1,2-diphenylethanol
<b>DPEN</b>	1,2-diphenylethane-1,2-diamine
<i>ee</i>	Enantiomeric excess
<b>GABA</b>	Gamma-Aminobutyric Acid Receptor
<b>H&amp;E</b>	Haematoxylin and Eosin Stain
<b>IMS</b>	Imaging Mass Spectrometry
<b>LC-MS</b>	Liquid Chromatography Mass Spectrometry
<b>MALDI</b>	Matrix Assisted Laser Desorption Ionisation
<b>MALDI-IMS</b>	Matrix Assisted Laser Desorption Ionisation Imaging Mass Spectrometry
<b>NDMC</b>	<i>N</i> -desmethylclozapine
<b>TH</b>	Transfer hydrogenation
<b>TsDPEN</b>	<i>N</i> -p-Tosyl-1,2-diphenylethylenediamine
<b>Ts<sub>2</sub>O</b>	<i>p</i> -Toluenesulfonic Anhydride

# Preface

In the dynamic field of drug discovery and development, the journey from initial concept to medication is fundamentally a collaborative endeavour. Central to this challenging pursuit, organic chemistry emerges as a foundational pillar. It offers the universal language of arrows and bonds that unite the diverse array of sciences essential in this quest, encompassing medicine, biology, pharmacology, physics, bioinformatics, and material sciences, among many others. Organic chemists, as masters of the discipline, not only bridge the fields through their mastery of the language but also bring the knowledge and craftsmanship essential to the design and manufacturing of medications. Now, in an era of unprecedented technological advancements, the significance of organic chemists in pharmaceutical research has never been greater.

This thesis stands as a testament to the enduring significance of organic chemistry in pharmaceutical research. It consists of two parts. In the first part, we engage in the collaborative spirit of science to explore the fate of the gold standard antipsychotic, CLO, in an isolated brain. Our objective was to gain insights into its brain dynamics, with the broader goal of developing an IMS-brain platform for advancing research in neuropharmacology and neuroscience (*Paper 1*). The second part delves into the core principles of organic chemistry, focusing on developing small molecule compounds, the backbone of modern pharmacology (*Paper 2, manuscript 1*). For a more comprehensive account of the second part, I kindly refer you to ref. 1.<sup>1</sup>

# Table of Contents

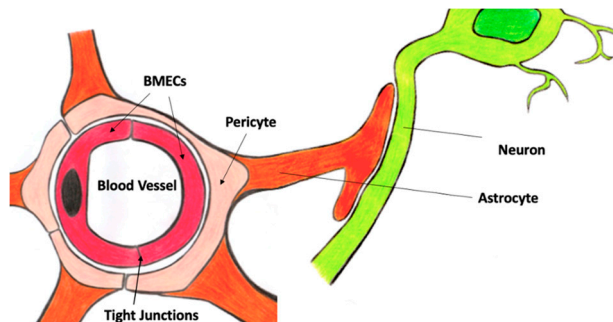
<b>1. Imaging Mass Spectrometry in Neurotherapeutics.....</b>	<b>12</b>
1.2 Background .....	14
1.3 Aim and Motivation .....	18
1.4 Experimental Procedure and Sample Preparation .....	19
1.5 Results .....	20
1.6 Discussion .....	24
1.7 Conclusions .....	26
1.8 Future Perspectives .....	27
<b>2. Asymmetric Methodology Development and Towards the Total Synthesis of Aspidophylline A .....</b>	<b>28</b>
2.1 ATH/DKR for high Enantioselectivity in the Synthesis of Vicinal Amino Alcohols ( <i>Paper II</i> ) .....	28
Enantioselective synthesis of <i>anti</i> - $\alpha$ -amido- $\beta$ -hydroxy esters .....	30
Conclusions .....	34
2.2 Towards the Enantioselective Synthesis of Aspidophylline A ( <i>Manuscript I</i> ) .....	34
Retrosynthetic Analysis.....	34
Progress of the Synthesis.....	35
Conclusions .....	39
<b>3. Concluding Remarks.....</b>	<b>40</b>
<b>Acknowledgements .....</b>	<b>41</b>
<b>Populärvetenskaplig Sammanfattning .....</b>	<b>42</b>
<b>Popular Scientific Summary.....</b>	<b>43</b>
<b>Resumen Divulgativo.....</b>	<b>44</b>

# 1. Imaging Mass Spectrometry in Neurotherapeutics

The 20th century was marked by remarkable scientific and technological progress, leading to the development of Central Nervous System (CNS) medications that completely reshaped the treatment landscape for neurological disorders. However, a major limitation of the majority of these medications lies in their focus on providing symptomatic relief or slowing disease progression, rather than offering definitive cures. In addition, their wide-ranging side effects significantly impact patient quality of life. As a result, many patients on CNS drug treatments gain only limited benefits, creating an urgent need for more effective neuropharmaceuticals. Unfortunately, this demand is expected to surge exponentially in the near future, driven mainly by the global rise in life expectancy. Despite the critical situation, the CNS drug discovery and development sector has experienced a notable slowdown in innovation in recent years.<sup>2-5</sup>

For a neurotherapeutic agent to elicit its effects, it must reach the intended target within the brain at adequate concentrations. Should it fail to meet these essential requirements, its efficacy is compromised. However, even if the drug meets these basic criteria, it can still produce secondary pharmacological or toxic effects, potentially compromising its safety profile. Challenges in developing drugs that strike an optimal balance between efficacy and safety contribute significantly to the limited innovation in neurotherapeutics.<sup>6,7</sup>

The BBB (figure 1.1) plays a central role in issues related to drug efficacy. It acts as the brain's chemical gatekeeper, preventing undesirable solutes (*e.g.*, toxins and pathogens) carried in the blood from entering the brain whilst allowing passage to life's essentials (*e.g.*, amino acids, glucose, and oxygen)<sup>8</sup>.

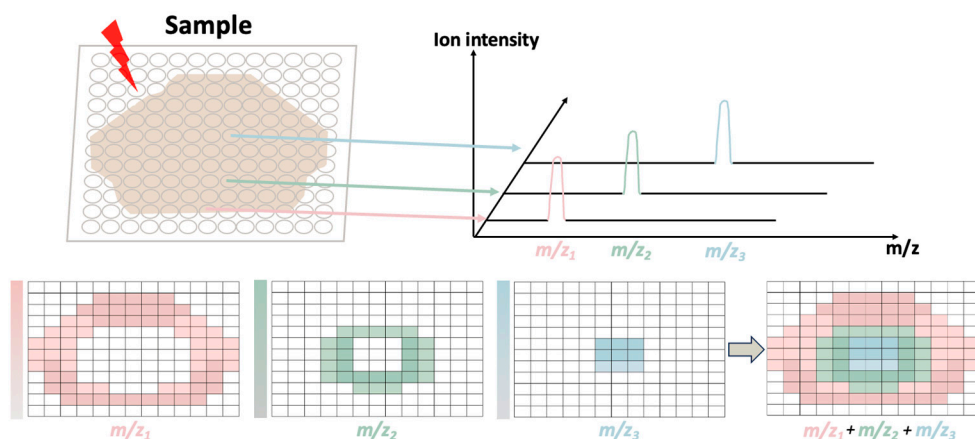


**Figure 1.1:** The BBB is primarily constituted by brain microvascular endothelial cells (BMECs), pericytes, astrocytes, neurons, and tight junction protein complexes. The tight junctions effectively restrict the passage of drugs to the brain by sealing the intercellular spaces between BMECs.

Non-endogenous compounds, such as drugs, typically encounter restricted passage through the BBB, limiting their ability to reach therapeutic concentrations in the brain, thereby impacting their efficacy<sup>9,10</sup>. Challenges in efficacy can also stem from a drug's neuropharmacokinetic (neuroPK) profile, encompassing its absorption, distribution, metabolism, and elimination. Of particular concern are distribution and metabolism, as these processes also can introduce safety concerns through drug distribution to unintended brain regions or the generation of toxic metabolites<sup>11,12</sup>.

Assessing a drug's ability to permeate the BBB and its neuroPK profile is critically important in modern CNS drug discovery and development. To this end, imaging techniques such as immunohistochemistry, Positron Emission Tomography (PET), and autoradiography play a key role<sup>13-15</sup>. They provide spatiotemporal information in a visual manner on how drugs traverse the BBB, distribute within the brain, and undergo metabolism<sup>16</sup>. Despite these techniques' undeniable contribution to neurotherapeutics, their use is to a great extent limited by their reliance on molecular imaging probes, introducing limitations such as restricted image resolution, dynamic range, and sensitivity<sup>17</sup>. The use of imaging probes also requires prior analyte knowledge and precludes the simultaneous assessment of multiple analytes.<sup>18</sup> This presents a significant challenge in drug metabolism studies, given that a single drug can be metabolised into a wide array of compounds, many of which typically are unknown<sup>19</sup>. Moreover, discerning between the parent drug and metabolites that retain the imaging probe introduces yet another layer of complexity.

Addressing these limitations, IMS emerges as a powerful technique used to visualise the spatial distribution of molecules within a sample (figure 1.2). Owing to its foundations in MS, the technique offers the unique ability to target molecules (*e.g.*, drugs, drug metabolites, glycans, lipids, peptides and biomacromolecules) by using their molecular masses as fingerprints. IMS can perform this directly within tissues or cells, without the requirement for molecular imaging probes.



**Figure 1.2:** In IMS, MS data is integrated with spatial coordinates from analysed tissue samples, enabling the creation of ion intensity maps that visually depict the distribution of analytes.



Bypassing the need for molecular imaging probes enables the detection of previously unknown analytes and the simultaneous mapping of multiple compounds. IMS also offers high sensitivity and an extensive dynamic range, features essential for detecting both trace and abundant drugs. Additionally, it is a non-destructive technique, preserving sample integrity for further analysis.<sup>20-22</sup>

With its remarkable mapping capabilities, IMS has rapidly found a central role in neurotherapeutics. For instance, Liu and co-workers used the technique to uncover novel insights into ketamine's potential mechanism of action as an antidepressant<sup>23</sup>. Furthermore, in a landmark study conducted in 2013, ion intensity maps were able to illuminate the diverse effects of tumour vasculature on drug BBB permeation, providing novel perspectives on brain drug delivery<sup>24</sup>. IMS particularly excels in drug metabolism studies for its capability to simultaneously analyse the wide range of metabolites generated upon drug administration. This is highlighted in a study by Castellino's team on the antiretroviral drug candidate Fosdevirine, responsible for causing seizures in trial subjects. IMS was instrumental in identifying early unknown metabolites, one of which accumulated in myelinated axons, thus yielding novel insights into the possible mechanisms underlying epilepsy-related seizures<sup>25, 26</sup>. Moreover, within the novel field of personalised medicine, IMS is increasingly considered a valuable tool. It has been used for mapping the distribution of chemotherapeutics and corresponding metabolites in brain tumours. The generated ion intensity maps have disclosed patient-exclusive drug penetration and metabolic patterns, allowing for a safer and more efficient treatment approach<sup>27, 28</sup>.

This research project is dedicated to exploiting the benefits of IMS to investigate the distribution, metabolism, and permeation across the BBB of CLO, NDMC, and CNO in an isolated neural system.

## 1.2 Background

IMS is built upon the principles of MS, an analytical technique able to separate ionised atoms and molecules by exploiting differences in the ratios of their charges to their respective masses ( $m/z$ )<sup>29</sup>. In an IMS experiment, the sample is ionised at a discrete point, known as a pixel, where a mass spectrum is recorded. This procedure resembles a conventional MS experiment. However, following the acquisition of the first pixel, the tissue sample is repositioned to ionise another part of the sample, where data collection resumes to generate a new pixel. This process iterates until the desired number of pixels has been collected. The recorded MS data is finally compiled to produce an ion intensity map that depicts the distribution of analytes in the scanned tissue sample (figure 1.2).<sup>30, 31</sup>

IMS incorporates a range of platforms, each characterised by unique ionisation sources that offer similar but slightly different properties in terms of speed,

sensitivity, chemical scope and image resolution<sup>32</sup>. The most common ionisation sources are MALDI, secondary ion mass spectrometry (SIMS), and desorption electrospray ionisation (DESI). SIMS-IMS is renowned for its exceptional resolution, allowing for subcellular studies, yet this advantage is counterbalanced by high costs and tedious sample preparations. Of all three platforms, DESI-IMS requires the least sample preparation, but this asset typically comes with limited image resolution. When the identification of a broad spectrum of compounds is required, with a proper balance of resolution and cost-effectiveness, MALDI-IMS is often regarded superior.<sup>32-34</sup>

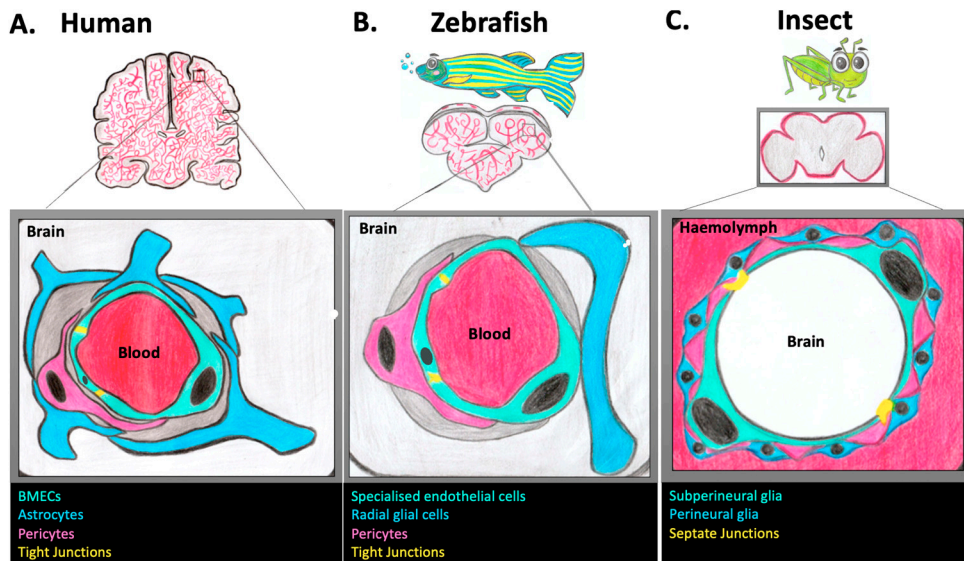
MALDI-IMS was introduced in the late 1990s. The technique relies on irradiation energy from a UV or IR laser and a chemical matrix that absorbs photons at the laser's wavelength. The matrix plays a crucial role in the desorption/ionisation of analytes and their subsequent transportation to the mass analyser.<sup>35,36</sup>

MALDI-IMS has primarily gained popularity for being a soft ionisation technique, meaning that analyte ionisation occurs with minimal fragmentation. This attribute is particularly appealing in drug metabolism studies, where the detection and identification of a broad range of metabolites typically presents a formidable challenge. Furthermore, MALDI's soft ionisation enables the examination of biomacromolecules, a critical aspect in neurotherapeutics given these compounds' role in physiological and pathological conditions.<sup>37,38</sup>

In the early stages of MALDI-IMS development, image resolution was primarily hindered by the size of the laser irradiation area. However, recent technological advancements have enabled laser areas as small as 1-10  $\mu\text{m}$ . Given the average diameter of mammalian brain cell soma is around 20  $\mu\text{m}$ , these technical improvements have significantly advanced MALDI-IMS in neurotherapeutics.<sup>39-42</sup>

IMS has been successfully employed for studying drug metabolism<sup>43</sup>, drug-drug interactions<sup>44</sup>, mapping the distribution of neurotransmitters<sup>45</sup>, and the impact of drugs on biochemical pathways within mammalian brains<sup>46</sup>. Unfortunately, the use of mammals in research is resource-intensive, time-consuming and raises ethical concerns<sup>47,48</sup>. In response, brain organoids have emerged as a recent alternative<sup>49</sup>. These miniature, simplified versions of the human brain offer a valuable platform for testing potential neurotherapeutic interventions in a more efficient and ethically acceptable manner. Yet, the lack of a BBB in these brain models limits their usefulness in CNS drug research.

In recent years, insects and zebrafish have become viable options<sup>50,51</sup>. Insects offer several advantages as experimental animals compared to mammals, including short life cycles, low cost for rearing and few ethical concerns, facilitating high throughput screening of lead compounds<sup>52-54</sup>. Zebrafish, also possess unique features such as external development, optical transparency during embryogenesis, high fecundity, short generation times and limited ethical implications<sup>55,56</sup>. Most importantly, both insects<sup>57-59</sup> and zebrafish<sup>60-62</sup> own a well-developed BBB, structurally and functionally homologous to their human counterpart (figure 1.3).



**Figure 1.3:** The human BBB (A) compared to its counterparts in zebrafish and insects. In zebrafish (B), the major BBB constituents are specialised endothelial cells (green), tight junctions (yellow), pericyte cells (pink), and radial glial cells (blue). The insect BBB (C) main components are perineurial cells (blue) and subperineurial glial cells (green). The latter are tightly connected in between by septate junctions (yellow), which share close similarities with tight junctions.

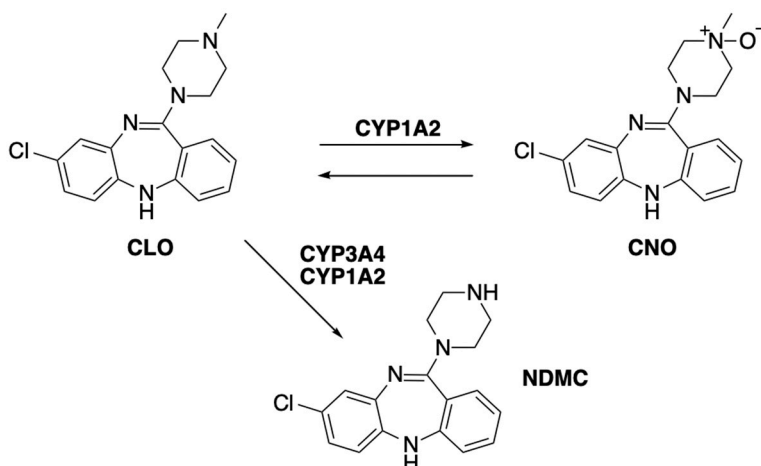
Although the use of insects initially might seem of limited use in neurotherapeutics due to the vast differences between insects and mammals, numerous neurotransmitters are shared between species. These include acetylcholine, GABA, glutamate, serotonin, dopamine, and tyramine<sup>63,64</sup>. Insects also possess octopamine, which exhibits functional similarities to norepinephrine in mammals<sup>65</sup>. In addition, the interaction between P-glycoprotein and CYP3A4, renowned for its role in eliminating xenobiotics from the brain, has been corroborated as conserved across species<sup>66</sup>. These neurochemical resemblances suggest the presence of shared endogenous synthetic pathways and protective mechanisms against foreign substances between species, underscoring the potential of using insect brains in neurotherapeutics<sup>67</sup>.

Zebrafish also hold notable neurochemical commonalities with mammals, including shared neurotransmitters, and enzymes for synthesis and metabolism<sup>68-70</sup>.

The similarities between vertebrate and invertebrate brains make both insects and zebrafish attractive options for studying drug dynamics in neurotherapeutics. However, insects boast an open circulatory system, endowing them with a unique advantage over vertebrates. The open circulatory system allows insects to withstand severe physical trauma. A decapitated insect body can live up to several days after losing its head, dying only because, without a head, it cannot eat or drink. Not only can the body survive decapitation, but the isolated head can also thrive. Studies on

decapitated insects have reported that the isolated heads can continue to exhibit basic reflexive behaviours and sensory responses for several hours to a few days after decapitation. If the head is fed, it can survive up to several weeks.<sup>71-74</sup> With precise surgical techniques, the insect brain can be detached and removed without impairing its function for at least one hour<sup>75, 76</sup>. In stark contrast, removing the brains of vertebrates results in an immediate cessation of neural activity due to their closed circulatory system. Insects thus offer a direct avenue for exposing the brain to particular drugs, presenting an exclusive opportunity to investigate drug BBB permeation, distribution, and metabolism in the brain without the concern of peripheral contamination.

In this project, IMS is employed to investigate the spatiotemporal dynamics of CLO and its two main metabolites, NDMC and CNO (scheme 1.1). Our work centres around the desert locust *ex vivo* BBB/brain model developed by Nielsen and colleagues in 2013<sup>75, 76</sup>. Using this model, BBB permeation, distribution and Cytochrome p450 (CYP) for these compounds were assessed in a previously conducted in-house study<sup>66</sup>. However, this study, involving brain homogenates analysed with Liquid Chromatography-Mass Spectrometry (LC-MS), did not provide any spatiotemporal information on how CLO, NDMC and CNO behave within the brain. To address this gap, this project substitutes LC-MS for MALDI-IMS intending to obtain visual information on the permeation of these compounds through the BBB as well as their distribution and metabolic patterns within the brain. Additionally, our investigation was enriched with a proof-of-concept study on zebrafish (*Danio rerio*), aimed at studying where the drug accumulates within the fish brain.



**Scheme 1.1:** Structures of CLO, NDMC and CNO. CLO is primarily converted to NDMC through an *N*-demethylation reaction, catalysed mainly by CYP3A4 and CYP1A2. CYP1A2 is also the key enzyme in the oxygen insertion process that forms CNO from CLO.<sup>77</sup>

## 1.3 Aim and Motivation

Developing drugs that have both an optimal neuroPK profile and a good ability to cross the BBB is a significant challenge. One of the main reasons for this is the lack of advanced analytical tools to thoroughly study the interaction between drugs and the BBB, as well as their fate within the brain<sup>78-80</sup>. Furthermore, the limitations of existing brain models present an additional obstacle in this process<sup>81-83</sup>. This thesis aims to leverage the advantages of MALDI-IMS as well as the desert locust *ex vivo* model and create an IMS-insect brain tool to drive advancements in neurotherapeutics. To evaluate this tool, our focus was directed towards the study of antipsychotics. Remarkably, since the introduction of chlorpromazine (the first antipsychotic developed) over sixty years ago, no drug that does not block dopamine receptors has been approved for the treatment of psychotic disorders. Given the global impact of psychotic disorders, which rank among the most burdensome and costly mental health illnesses, the scarcity of innovation within this field is concerning.<sup>84-86</sup>

Among several interesting drugs, CLO was selected because, although it ranks as the top antipsychotic of choice, its mechanism of action remains elusive. It is widely accepted that its mild dopamine-2 receptor affinity and a high affinity for serotonin  $5_{HT2A}$  receptors are responsible for part of its pharmacological effects<sup>87</sup>. However, recent studies have begun to challenge this perspective. Two research teams, independently of each other, have proposed a mechanism where NDMC is responsible for the effects of CLO-based therapies<sup>88</sup>. NDMC stands out for its unique ability to bind to dopamine-2 and dopamine-3 receptors, exerting a weak partial agonist activity at these sites similar to that of the novel third-generation antipsychotics aripiprazole and bifeprunox<sup>89</sup>. NDMC's remarkable pharmacological profile suggests the possibility that CLO may function as a prodrug, with NDMC serving as the true active compound. To evaluate NDMC's potential role as an active substance, it underwent a phase IIb trial but demonstrated no efficacy compared to placebo. Peripheral dose-dependent side effects ruled out the option of assessing higher NDMC doses, leaving its precise role in therapies based on CLO unclear.

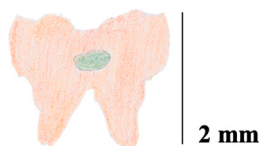
Beyond pharmacological applications, CLO is of interest for its second major metabolite, CNO, a widely employed ligand in DREADD technology<sup>90</sup>. DREADDs are specially engineered receptors that can be selectively activated by ligands, enabling precise manipulation of neural activity. This technique was notably demonstrated in 2007 by Roth and colleagues<sup>91</sup>, who implanted DREADDs into mouse brains, which, when activated by CNO, facilitated precise exploration of diverse neurological and psychiatric conditions. The study established the foundation for the widespread use of CNO in DREADD studies. However, ever since, CNO has been the subject of debate and scrutiny due to concerns about its ability to cross the BBB and its integrity as an inert ligand.<sup>92, 93</sup>

## 1.4 Experimental Procedure and Sample Preparation

After dissecting the insect brains, we exposed them to the desired drugs for 15 and 45 minutes. Test solutions were prepared at 3  $\mu\text{M}$ , a common practice in similar *ex vivo* studies<sup>66, 75, 76</sup>. However, for CNO experiments, a concentration of 10  $\mu\text{M}$  was required. Through extensive literature research, we found that the most commonly utilised matrices in MALDI-IMS studies of small molecule compounds are 2,5-dihydroxybenzoic acid, sinapic acid, and  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA). After rigorous evaluation of all three matrices, we ultimately selected CHCA for allowing simultaneous ionisation of the three analytes. Following numerous trial-and-error experiments and significant time investment, we successfully developed an instrumental protocol for assessing CLO, NDMC, and CNO simultaneously in brain tissues (See Appendix).

Regarding sample preparation, it was imperative to optimise procedures to preserve the native spatial distribution and molecular identity of the analytes in the brain samples. While protocols involving heat stabilisation of tissue samples were initially contemplated, flash-freezing emerged as our preferred method, as this technique swiftly halts all post-mortem processes and prepares the brain for sectioning.<sup>94-96</sup>

At the beginning of our investigation, sample preparation steps were evaluated on zebrafish brains. Despite the small size of the zebrafish brain, obtaining brain tissue samples in a reproducible manner was successfully achieved with hands-on experience. The desert locust brain, in contrast, provided significant handling challenges, owing to its limited size (averaging a volume of approximately 2 x 1.5 x 1 mm<sup>3</sup>) and its gelatinous texture. In addition, its tooth-like shape (figure 1.4) predisposed it to deformation. While we expected challenges during sectioning, difficulties arose as early as flash-freezing. Achieving consistent brain morphology after freezing proved to be a challenging task, primarily because the insect brain was susceptible to significant deformation upon contact with surfaces and dissection instruments.



**Figure 1.4:** Desert locust brain shape from a coronal plane perspective.

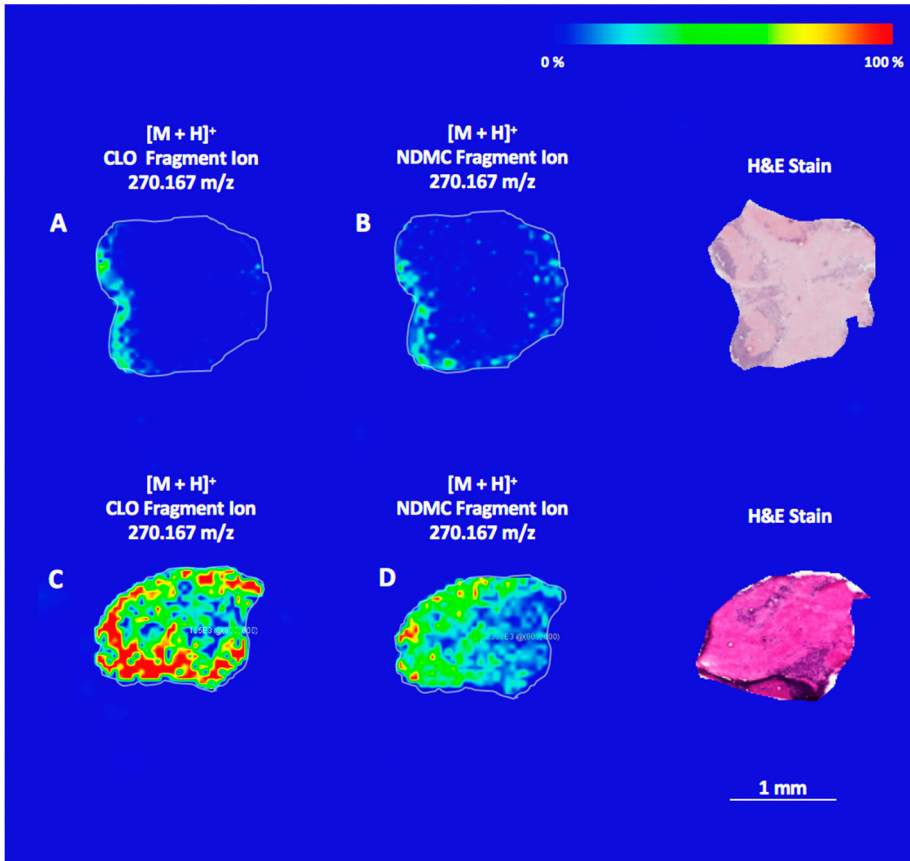
In MALDI-IMS experiments, tissue sections are typically obtained through a frozen sectioning procedure. However, before sectioning can proceed, the sample must be securely attached to the cutting equipment. To this end, adhesives are commonly used. Unfortunately, these adhesives are polymer-based, giving rise to ion suppression effects in our experiments. To overcome this issue, we opted to use

Millipore water/ice as the adherent medium, but attachment difficulties remained. Finally, due to the cryostat's temperature hovering around  $-20^{\circ}\text{C}$ , a feasible adherent was found in saturated NaCl Millipore solution. The transformation of brine into ice occurred at a measured pace, allowing for controlled and reasonably reproducible brain attachment. It must be stressed, however, that the use of brine was not free from issues and only by gaining handling experience was its use as an adherent satisfactory.

The thickness of the tissue sections was yet another critical parameter to evaluate. While thin sections ( $< 12\ \mu\text{m}$ ) were too fragile to handle, thicker sections ( $> 20\ \mu\text{m}$ ), adversely affected both the quantity of tissues that could be collected and the intensity of ion signals. We opted to collect tissue sections with a thickness of 14-18  $\mu\text{m}$ . Without any additional treatment beyond tissue drying, tissue sections were mounted onto microscope slides for the application of the matrix. We evaluated the most inexpensive matrix application method first, consisting of using a painter's airbrush. This time-consuming approach required high demands on individual savoir-faire to obtain acceptable and reproducible results. Satisfactory matrix coating was obtained first after acquiring considerable experience, however, while the method was reproducible between applications by the same operator, person-to-person variability was high. Because of this, we decided to introduce the use of an automated sprayer to obtain reproducible results. While the sprayer indeed is capable of generating excellent coatings and highly reproducible results, the optimisation of the many instrumental parameters was very time-consuming.

## 1.5 Results

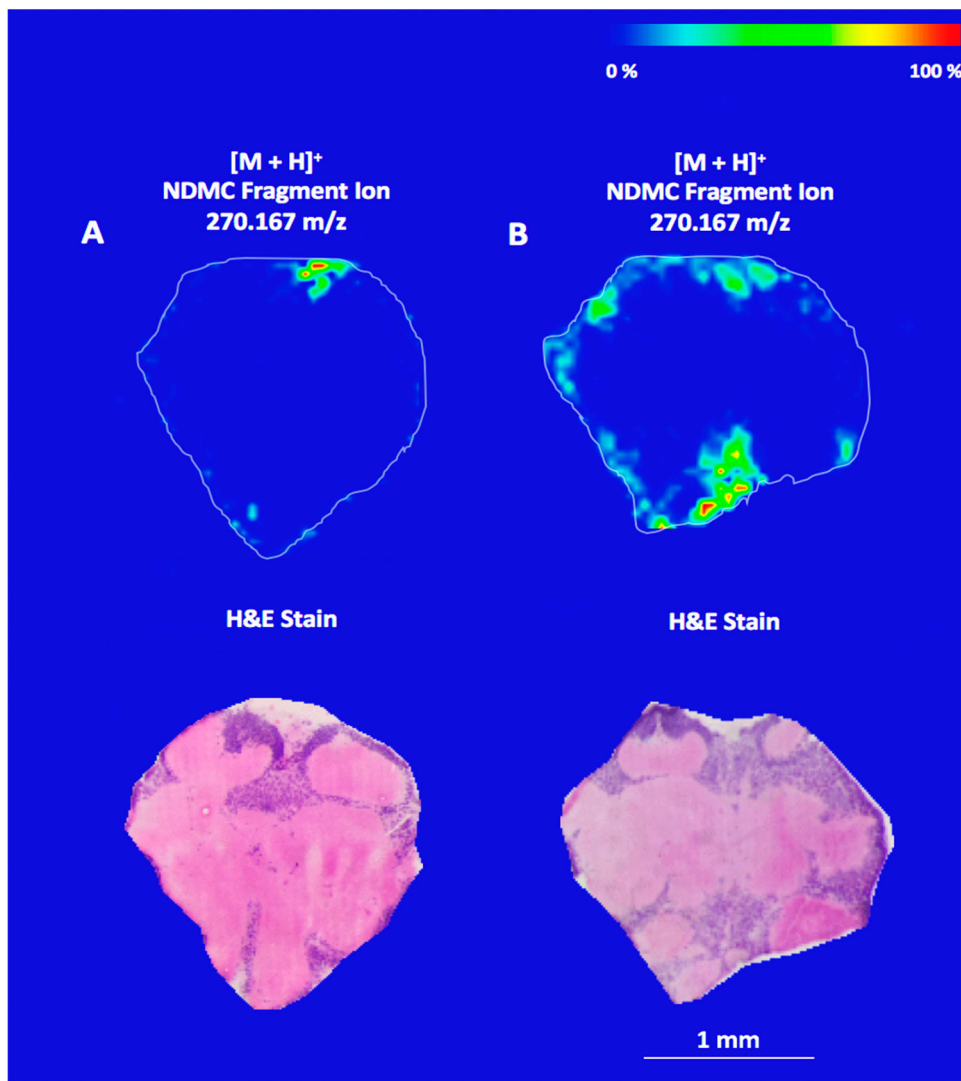
The ion intensity maps obtained from brains incubated with  $3\ \mu\text{M}$  CLO solution for 15 minutes demonstrate permeation of the drug through the BBB (figure 1.5A.) The presence of NDMC can also be observed (figure 1.5B), with its distribution pattern resembling that of CLO. Upon extending the incubation to 45 minutes, CLO is distributed throughout the brain tissue sample, with the highest relative concentration located along the edges (figure 1.5C). The presence of *in situ* generated NDMC after 45 minutes can be observed in figure 1.5D. CNO could not be detected in either of the CLO experiments.



**Figure 1.5:** Ion intensity maps for incubation of brains with CLO. The drug permeates the BBB after 15 minutes of incubation (A) and is predominantly found along one edge of the brain. Metabolism to NDMC (B) is evident. Incubation for 45 minutes (C) reveals the distributions for CLO throughout the entire brain section with a similar distribution for NDMC (D). The colour-coded gradient bar shows the relative concentrations of the analysed compound. Scale bar = 1 mm. H&E staining was performed on analysed tissue samples.

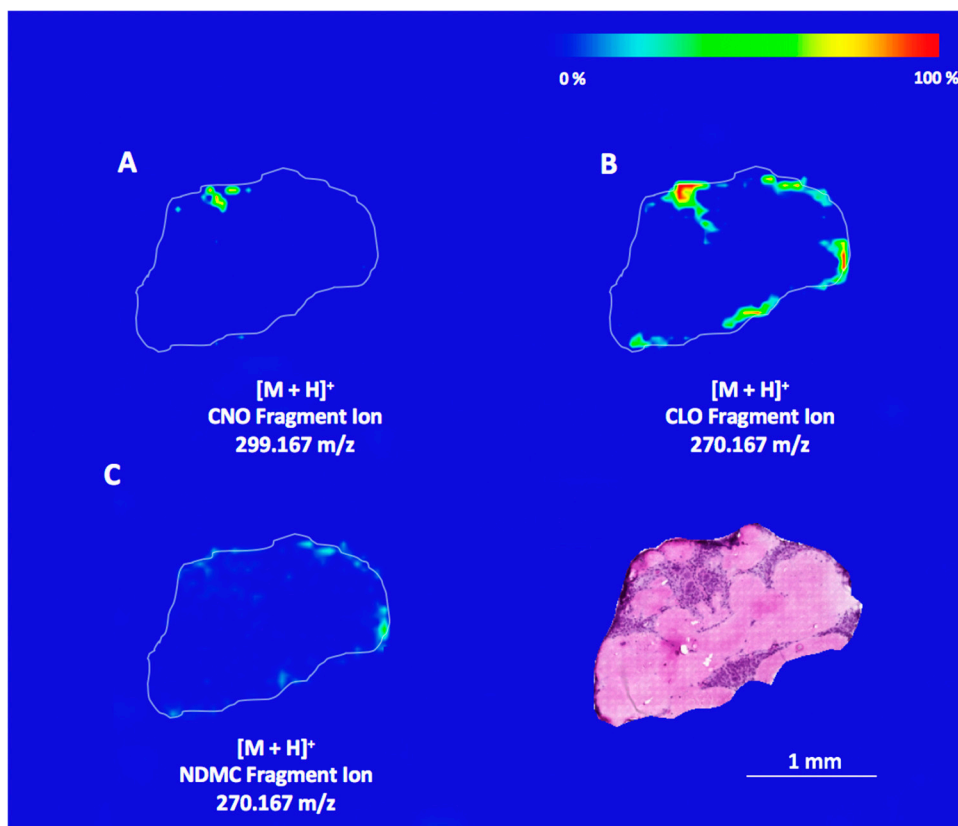


Figure 1.6 illustrates brain incubation in a 3  $\mu\text{M}$  NDMC solution. The 15-minute incubation is shown in figure 1.6A, and the 45-minute experiment in figure 1.6B. Neither CLO nor CNO were detected in these experiments.



**Figure 1.6:** Ion intensity maps for brains incubated with NDMC for 15 minutes (**A**) reveal a limited drug penetration through the BBB. Extending the incubation time to 45 minutes (**B**) shows that NDMC permeates the BBB to a greater extent. The colour-coded gradient bar shows the relative concentrations of the analysed compound. Scale bar = 1 mm. H&E staining was performed on analysed tissue samples.

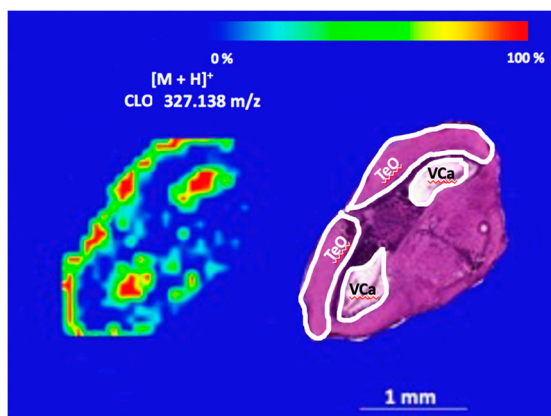
In the CNO experiments, the drug concentration was set to 10  $\mu\text{M}$ , as none of the analytes were identifiable at lower concentrations. No compounds were detected in the 15-minute experiment. However, when incubation was set to 45 minutes, it became evident that CNO exhibits limited BBB permeability (figure 1.7A). Additionally, both CLO and NDMC were detected, as shown in figure 1.7B and 1.7C, respectively. All three compounds were primarily confined to the edges of the brain.



**Figure 1.7:** Ion intensity maps for brains incubated with CNO for 45 minutes. Only a scarce amount of CNO was observed after 45 minutes of incubation at 10  $\mu\text{M}$  (A). CLO was detected (B) as well as NDMC (C). The colour-coded gradient bar shows the relative concentrations of the analysed compounds. Scale bar = 1 mm. H&E staining was performed on analysed tissue samples.

Our study was amplified with a proof-of-concept study where adult zebrafishes were exposed to a 10  $\mu\text{M}$  CLO aquatic environment. After 10 minutes of drug exposure, the zebrafish demonstrated behavioural and movement impairment. Noteworthy was how the shoal was dispelled, and individual fish were constantly at the top of the tank and repeatedly breaching the surface, while the fish in the control tank

showed no such activity. The disturbed *in vivo* activity indicated that a minimum effective concentration of CLO had been achieved. Unfortunately, we ran out of time at this stage, and we could only analyse the dissected brains after 10 minutes of CLO exposure. From figure 1.8, we can deduce high concentrations of CLO in the ventricular cavity (VCa) and optic tectum (TeO).



**Figure 1.8:** Brain tissue for zebrafish exposed to 10  $\mu\text{M}$  of CLO for 10 minutes. Correlating the MALDI ion intensity maps with H&E histology stains reveals that CLO is abundant in the VCa and the TeO. The colour-coded gradient bar shows the relative concentrations of the analysed compound. Scale bar = 1 mm

## 1.6 Discussion

A distinct contrast in BBB permeability and distribution patterns was observed between CLO and NDMC. In the 15-minute NDMC experiment, figure 1.6A, the drug exhibited only minimal presence within the tissue section, with its distribution predominantly confined to the periphery even after 45 minutes, figure 1.6B. In contrast, CLO was localised at the tissue edge after 15 minutes, figure 1.5A, and over 45 minutes, figure 1.5C, the drug was spread throughout the entire tissue sample, implying a higher BBB permeability for CLO. Regarding drug metabolism, the maps reveal a significant difference in NDMC's distribution when derived from CLO metabolism versus its direct administration. Especially, in the 45-minute CLO experiment, NDMC is found distributed throughout the brain tissue, contrasting sharply with its edge-concentrated presence when directly administered. These observations align with the outcomes of the phase IIb studies on NDMC, where its limited BBB permeability was posited as a potential factor in the inability to administer higher doses.

Unfortunately, we were unable to confirm the presence of CNO in the CLO experiments, making it essential to consider and address technical issues that might

have influenced this outcome. During the optimisation of the experimental protocol, CNO was the most difficult compound to detect. Adjusting the laser energy level allowed us to detect CNO, but regrettably, this compromised the detection of CLO and NDMC. Consequently, we settled on laser energy of 14  $\mu$ J for all experiments, as this level represented a compromise strategy to attain acceptable simultaneous ionisation of all three compounds. Additionally, it is crucial to recognise that the nature of MALDI-IMS renders it susceptible to localised ion suppression effects. This means that when CNO is co-localised with CLO and NDMC, it can lead to its reduced ionisation. Since our earlier LC-MS investigations revealed CNO to be present at significantly lower concentrations than both CLO and NDMC under identical experimental conditions, ion suppression is a likely explanation for the ostensible absence of CNO.

Having addressed the technical challenges in detecting CNO, we can now delve deeper into the CLO experiments in figure 1.5. Notably, the presence of NDMC in regions where CLO is absent can be observed. These observations could stem from the technical issues recently mentioned. Alternatively, we posit that these observations may be rationalised by three potential factors: 1) fast metabolism of CLO in certain brain compartments due to high or specific CYP expression, 2) different diffusion rates and 3) different efflux rates between CLO and NDMC.

Focusing now on the CNO experiment, the ion intensity map displayed in figure 1.7A confirms its penetration through the BBB. However, its limited presence and the requirement of a 10  $\mu$ M administration concentration implies that CNO is not an efficient BBB permeant. What also stands out is the appearance of CLO and NDMC, as shown in figure 1.7B and figure 1.7C, respectively. This indicates that CNO is susceptible to brain metabolism, questioning its previously assumed inertness in DREADD experiments. If CLO is produced in concentrations high enough to influence neural receptors, its pharmacological properties could potentially lead to complex and unintended effects beyond those mediated by CNO acting at DREADDs. The complexity deepens when considering NDMC, which exhibits distinct neuropharmacological activity compared to CLO. Given its potentially unique actions, NDMC might have had a more significant impact on DREADD studies than CLO. Obviously, this takes us into the realm of speculation, and more research is needed before jumping to conclusions.

In the proof-of-concept experiments conducted on zebrafish, the ion intensity map in figure 1.8 discloses a noticeable accumulation of CLO in both the VCa and the TeO. The TeO, analogous to the superior colliculus in mammals, serves as a crucial processing centre for sensory information. We hypothesise that the accumulation of CLO within the TeO may be linked to the observed impairments in behaviour and movements, although further research is essential to investigate this potential correlation. Additionally, we observed the accumulation of CLO within the VCa. The observed accumulation potentially corroborates the hypothesis of a less developed BBB at the VCa in zebrafish.

This study underscores our platform's potential for studying the spatiotemporal dynamics of drugs. However, a key limitation was the MALDI-IMS sensitivity. In the previous in-house study using LC-MS, experiments at concentrations as low as 1  $\mu\text{M}$  were able to be conducted, and CNO was reliably detected in both CLO and CNO experiments, highlighting the superior sensitivity of LC-MS. Besides instrumental limitations, considerable challenges with sample preparation need to be tackled for our approach to gain relevance in neurotherapeutics. From the moment the desert locust brain is detached from the skull, it poses handling difficulties due to its minute size and peculiar shape. First, it is difficult to avoid affecting its morphology when held with a pincer, and the deformation will carry over to the sectioning stage because of snap-freezing. Moreover, preparing the small brain for subsequent sectioning poses challenges, and the method of using brine to attach the brain is suboptimal due to the adverse effects of salts in MS. Moreover, the insect brains are fragile and proved difficult to section without embedding support. Consequently, we assessed the use of the embedding material Optimal Cutting Temperature (OCT) compound, documented in various MALDI-IMS studies. However, its use ended up introducing contaminants into our experiments due to OCT's polymeric nature, thus complicating analyte detection. The lack of suitable embedding support made it extremely challenging to achieve consistent sectioning of the desert locust brain, representing the most significant limitation of our study.

## 1.7 Conclusions

Before concluding our work, it is crucial to stress that our findings were derived from studies on animal brains. This requires a cautious interpretation of the results. That being said, our insect experiments have yielded intriguing results. The stark differences observed in BBB permeation and brain distribution between NDMC and CLO administration experiments are remarkable. Moreover, administering CLO leads to widespread distribution of NDMC throughout the brain, unlike direct NDMC administration. These findings suggest that CLO can potentially act as a prodrug for NDMC, but further investigation is required to validate these findings. In addition, our research challenges the notion of CNO as a suitable ligand in DREADD studies, suggesting that observed effects might have resulted from *in situ* generation of CLO or NDMC.

Broadly, our study confirms the potential of using IMS in tandem with the *ex vivo* brain model to expedite preclinical BBB permeation and neuroPK lead screenings. However, our approach is still in its infancy and requires further development, in particular regarding sample preparation.

## 1.8 Future Perspectives

Our findings highlight the potential of our IMS/ insect brain tool. However, a pivotal factor in extending and refining our study lies in the identification of an appropriate embedding material for brain sectioning. Such a material would not only facilitate the replication of our initial experiments, potentially yielding more precise results, but also expand the scope of our research to include the examination of additional compounds. Moreover, a suitable embedding material would enable us to section the brain from edge to edge, thereby allowing us to create comprehensive 3D ion intensity maps of the desert locust brain. These 3D maps would not only allow us to study BBB permeation and neuroPK more comprehensively but also delve into the neurochemistry of the brain by targeting neurotransmitters, lipids, glycans, proteins, and peptides. The creation of such 3D brain maps would undoubtedly make a significant impact in the field of neurotherapeutics and insect neuroscience. Finding the right embedding support is therefore a top priority for broadening the scope of our future research.

## 2. Asymmetric Methodology Development and Towards the Total Synthesis of Aspidophylline A

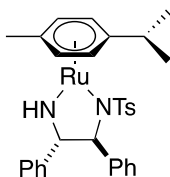
The discovery and development of new pharmaceuticals is a tremendously complex undertaking that requires general and specific knowledge of a wide range of scientific areas such as pharmacy, medicine, and chemistry. The role of organic chemistry in the pharmaceutical industry continues to be one of the main drivers in the drug discovery process, and developments in modern drug discovery go hand in hand with developments in organic synthesis methodology.

### 2.1 ATH/DKR for high Enantioselectivity in the Synthesis of Vicinal Amino Alcohols (*Paper II*)

Hydrogenation reactions are among the most fundamental transformations in organic synthesis<sup>97</sup> and often used in the reduction of many functionalities including alkenes,<sup>98</sup> ketones,<sup>99-101</sup> and imines<sup>102-104</sup>. Direct hydrogenation with a pressure of H<sub>2</sub> gas and transfer hydrogenation (TH) are the two employed strategies for hydrogenation, and both utilise transition-metal catalysts involving first-, second-, and third-row transition metals of groups 8, 9, 10, and 11. However, while direct hydrogenation uses molecular hydrogen to achieve reduction, TH uses hydrogen sources other than H<sub>2</sub> (g), such as secondary alcohols and formate. TH becomes an attractive alternative to direct hydrogenation for a couple of reasons, 1) the method does not require hazardous pressurised H<sub>2</sub> gas nor elaborate experimental setups, 2) the hydrogen sources are normally readily available, low-cost, and easy to handle, 3) the metal catalysts involved are normally readily accessible and robust.<sup>105, 106</sup>

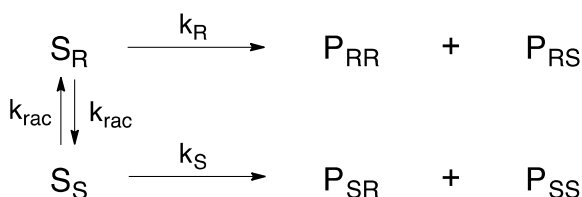
A remarkable milestone in the field was the introduction of chiral ligands to achieve enantioselective hydrogenations. In particular, the catalyst derived from the chiral monotosylated diamine 1,2-diphenylethane-1,2-diamine (TsDPEN) and the resultant Ru complex (Ru-TsDPEN) has received considerable attention due to its versatile application in a wide range of enantioselective reactions.<sup>107-110</sup> The Ru-TsDPEN catalyst, developed by Noyori and co-workers (scheme 2.1), can catalyse

reactions with high enantioselectivity, up to 99% ee, and is one of the most popular catalyst for asymmetric transfer hydrogenation (ATH) reactions.<sup>111</sup>



**Scheme 2.1:** Noyori's Ru-TsDPEN catalyst.

Recently, the field of metal-catalysed ATH has been boosted by applying dynamic kinetic resolution (DKR).<sup>112</sup> In DKR a racemic substrate possesses a configurationally labile stereogenic centre that undergoes racemization during the reaction (scheme 2.2). In this case,  $S_R$  is converted with a rate constant of  $k_R$  to diastereomers  $P_{RR}$  and  $P_{RS}$  while  $S_S$  is converted to  $P_{SR}$  and  $P_{SS}$  with a different rate constant,  $k_S$ . If  $k_{rac} > k_r \gg k_s$  the enantioselective synthesis of one diastereomer is possible and can be obtained in 100 % theoretical yield from racemic starting material. Hence, the asymmetric transfer hydrogenation assisted by dynamic kinetic resolution (ATH/DKR) can be used as an efficient technique for establishing the stereochemistry of two adjacent stereogenic centres.<sup>113-115</sup>



**Scheme 2.2:** Dynamic kinetic resolution.

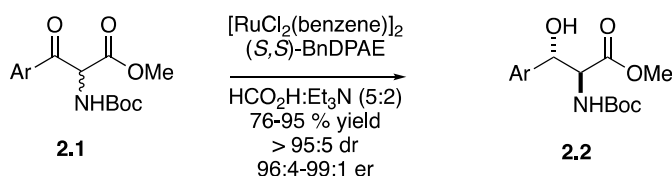
Modern applications of ATH/DKR include, transformations  $\beta$ -ketoamides,<sup>116</sup>  $\alpha$ -substituted- $\beta$ -ketoesters,<sup>116-118</sup> cyclic  $\alpha$ -oxy- $\beta$ -ketoamides,<sup>119</sup> 1,3-diketones,<sup>120-122</sup> 1,2-diketones<sup>123, 124</sup>,  $\alpha$ -ketophosphonates,<sup>125</sup> 2-substituted-cycloalkanones,<sup>126-128</sup>  $\beta$ -ketosulfones<sup>129</sup>. The approach has emerged as a powerful and practical tool for reduction reactions in both academia and industry, due to its operational simplicity, high reaction rate and enantioselectivity and broad substrate scope.<sup>130, 131</sup>



## Enantioselective synthesis of *anti*- $\alpha$ -amido- $\beta$ -hydroxy esters

The  $\beta$ -amino- $\alpha$ -hydroxy ester functionality, and the related vicinal amino alcohol moiety, are found in various biologically active natural products. The importance of 1,2-amino alcohols is also well recognised in asymmetric synthesis, where the need for chiral auxiliaries and ligands is continuously increasing. Not surprisingly, the asymmetric synthesis of these structures has received considerable attention.

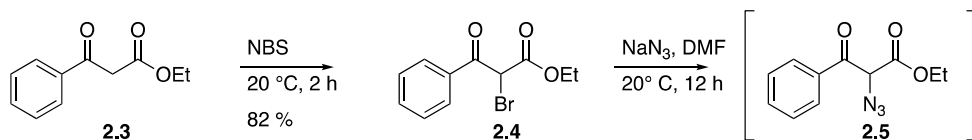
It has been shown that asymmetric transfer hydrogenation (ATH) coupled with dynamic kinetic resolution DKR can be applied to the formation of  $\alpha$ -substituted- $\beta$ -hydroxy esters. Previous reports on ATH/DKR on  $\alpha$ -substituted compounds include  $\alpha$ -methoxy- $\beta$ -keto esters<sup>132</sup>,  $\alpha$ -alkyl- $\beta$ -keto amides<sup>116</sup>, and *N*-Cbz protected  $\alpha$ -amido- $\beta$ -keto ester<sup>118</sup>. The enantioselective synthesis of *anti*- $\beta$ -hydroxy- $\alpha$ -amido esters using ATH/DKR has earlier been developed in our research group. The approach relies on the stereochemical lability of the  $\alpha$ -stereocenter in **2.1** scheme 2.3, allowing for two stereocentres to be introduced in the asymmetric reduction.<sup>113-115</sup>



**Scheme 2.3:** ATH/DKR reactions for the reduction of  $\alpha$ -amido- $\beta$ -keto esters.

Excellent results in terms of yield, diastereo- and enantioselectivities were achieved for a wide range of  $\alpha$ -amido- $\beta$ -keto esters. We rationalised that the high diastereoselectivity resulted from an intramolecular hydrogen bonding in the substrate and became interested if the same methodology could achieve a *syn* diastereoselectivity if applied to a substrate unable to form an intramolecular hydrogen bond. In theory, enantioenriched *syn*  $\alpha$ -amido- $\beta$ -hydroxy esters could be obtained from  $\alpha$ -azido- $\beta$ -keto esters using a similar ATH/DKR approach.<sup>133-137</sup>

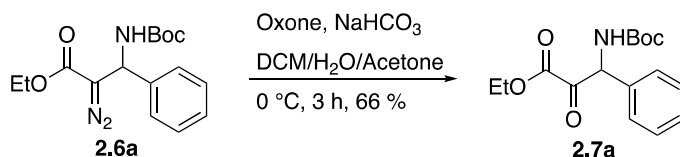
To test our hypothesis, we initiated our studies by preparing  $\alpha$ -bromo- $\beta$ -keto ester **2.4** under solvent-free conditions from commercially available keto ester **2.3** and *N*-bromosuccinimide (NBS) (scheme 2.4).<sup>138</sup> Our intension was to transform intermediate **2.4** to the corresponding  $\alpha$ -azido- $\beta$ -keto ester **2.5** through an  $\text{S}_{\text{N}}2$  substitution with  $\text{NaN}_3$  in DMF.



**Scheme 2.4:** Attempt to prepare azide **2.5** Synthesis of  $\alpha$ -bromo- $\beta$ -keto ester **2.4**.

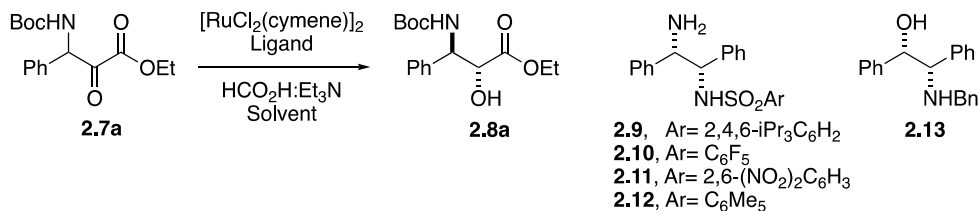
However, despite the successful bromination, the rapid and spontaneous decomposition of compound **2.5** during the work-up phase hindered further investigation. We suspect that the formation of bubbles in the reaction mixture indicates the evolution of nitrogen gas. If this is the case, there is a possibility that an  $\alpha$ -imino ketone intermediate was generated, although its precise formation within the mixture was not confirmed. Recently, an iron-catalysed alkylazidation of dehydroamino acids using peroxides as alkyl radical precursors has been presented by Waser and co-workers<sup>139</sup>.

We redirect our attention to the synthesis of  $\beta$ -amido- $\alpha$ -keto esters, a regioisomer of the previously investigated  $\alpha$ -amido- $\beta$ -keto esters. In theory, enantioenriched  $\beta$ -amido- $\alpha$ -hydroxy esters could be obtained by using a similar ATH/DKR approach to that used for obtaining *anti*- $\beta$ -hydroxy- $\alpha$ -amido esters. Encouraged by the asymmetric synthesis of diverse glycolic acid scaffolds via ATH/DKR of  $\alpha$ -keto esters<sup>140</sup>, we began our studies by generating a suitable  $\beta$ -amido- $\alpha$ -hydroxy ester to be evaluated under ATH/DKR conditions. The  $\alpha$ -diazo ester **2.6a** was oxidized to the desired keto ester **2.7a** using oxone (scheme 2.5).<sup>130</sup>



**Scheme 2.5:** Synthesis of  $\beta$ -amido- $\alpha$ -hydroxy ester **2.7a**.

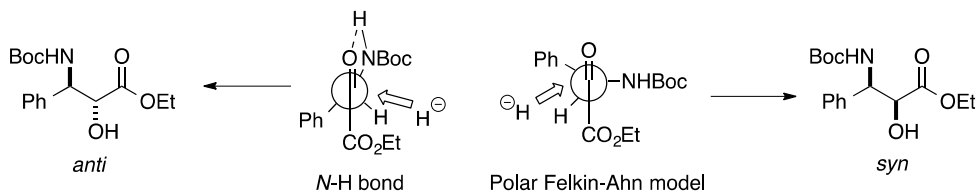
With substrate **2.7a** in hand, we initiated the ATH/DKR investigations by screening reaction conditions and catalysts (table 2.1).

**Table 2.1:** Optimization of ATH/DKR reaction conditions.<sup>a</sup>

Entry	Ligand	Solvent	Yield <sup>b</sup>	Time	dr <sup>b</sup>	er <sup>c</sup>
1	<b>2.9</b>	DMF	95 %	1h	>20:1	94:6
2	<b>2.10<sup>d</sup></b>	DMF	95 %	1h	>20:1	95:5
3	<b>2.11</b>	DMF	73 %	5h	>20:1	78:22
4	<b>2.12</b>	DMF	97 %	1h	>20:1	81:19
5	<b>2.13</b>	DMF	88 %	0.5 h	>20:1	83:17
6	<b>2.10<sup>d</sup></b>	Toluene	90 %	1 h	>20:1	90:10
7	<b>2.10<sup>d</sup></b>	DMSO	94 %	1 h	>20:1	94:6
8	<b>2.10<sup>d</sup></b>	Et <sub>3</sub> N:HCO <sub>2</sub> H(2:5)	93 %	1 h	>20:1	92:8
9 <sup>e</sup>	<b>2.10<sup>d</sup></b>	H <sub>2</sub> O:CH <sub>2</sub> Cl <sub>2</sub>	95 %	1 h	3:1	n.d. <sup>f</sup>

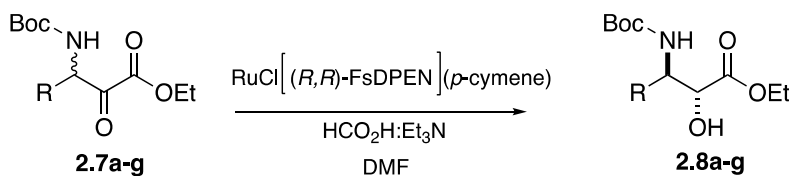
<sup>a</sup>Reactions performed by heating [Ru(*p*-cymene)Cl<sub>2</sub>]<sub>2</sub> (0.05 eq.) and the ligand (0.15 eq.) in 2-propanol (0.1M) at 80 °C for 1 h. After cooling to rt the solvent was removed and the catalyst was added to a solution of **2.7a** (1 eq., 0.1 M) and HCOOH/Et<sub>3</sub>N (5:2, 5 eq.). <sup>b</sup>Yield and *dr* determined by <sup>1</sup>H NMR spectroscopy of the crude reaction mixture. <sup>c</sup>Determined by chiral HPLC analysis of the crude reaction mixture. <sup>d</sup>Commercially available catalyst RuCl[(*R,R*)-FsDPEN](*p*-cymene) (0.05 eq.), purchased from Sigma-Aldrich, was added to a solution of **2.7a** (1 eq., 0.1 M) and HCO<sub>2</sub>H/Et<sub>3</sub>N (5:2, 5 eq.). <sup>e</sup>Reaction performed using emulsion conditions, see ref 53. <sup>f</sup>Not determined.

Substrate **2.7a** was screened using a series of catalyst complexes based on the (*p*-cymene)RuCl(ligand) framework. Chiral ligands **2.9–2.12** were manufactured based on the (*S,S*)-1,2-diphenylethane-1,2-diamine (DPEN) framework and differed by the aryl group attached to the sulfonamide. Chiral ligand **2.13** was prepared by a reductive amination between (*S,S*)-(-)-2-amino-1,2-diphenylethanol and benzaldehyde. Ester **2.8a** was obtained in good yields and excellent diastereoselectivity, favouring *anti* stereochemistry in all observed reactions. We rationalised that the diastereofacial selectivity was the result of the formation of an intramolecular hydrogen bond between the *N*-H and carbonyl moiety (figure 2.1). This conformation is believed to facilitate the hydride addition from the sterically least hindered side of the carbonyl group, thus leading to the observed *anti* diastereomer while the polar Felkin–Ahn model predicts the wrong diastereoselectivity.

**Figure 2.1:** Models offering different relative stereochemistry.

The enantioselectivity was determined by chiral HPLC analysis and revealed a wide range of variations in enantiomeric ratios (*er*) for the experiments performed in DMF (Entries 1-5). The optimal reaction conditions used the pentafluorinated DPEN derivative **2.10** as ligand affording **2.8a** in excellent 95:5 *er* (Entry 2). The influence of the solvent system on the ATH/DKR reaction was also investigated. Unfortunately, solvent systems other than DMF resulted in inferior results, (Entries 6-9), with the emulsion showing exceptionally poor diastereomeric ratios (*dr*) (Entry 9). Another observation was the decrease in *dr* from 20:1 (*anti:syn*) to 3:1 (*anti:syn*) when the reaction was left overnight. This is probably due to an epimerisation process with the low *dr*'s representing the equilibrium ratio. Finally, we were surprised by the large difference in the observed reaction rates (1 h) compared to the ATH/DKR of the regioisomeric  $\alpha$ -amido- $\beta$ -keto esters (5-7 days). We hypothesise that the difference in reactivity can be attributed to an increased electrophilicity of the  $\alpha$ -carbonyl carbon due to the induction effect exerted by the vicinal electron-withdrawing ester functionality.<sup>141</sup> With the optimised conditions in hand we set out to investigate the scope of the reaction (table 2.2).

**Table 2.2:** Substrate scope of the ATH/DKR reaction.<sup>a</sup>



Entry	R	Yield <sup>b</sup>	<b>2.7/2.8</b>	<i>dr</i> <sup>b</sup>	<i>er</i> <sup>c</sup>
1	Ph	95 %	<b>a</b>	>20:1	95:5
2	<i>p</i> -MeO-C <sub>6</sub> H <sub>5</sub>	96 %	<b>b</b>	>20:1	98:2
3	<i>m</i> -Br-C <sub>6</sub> H <sub>5</sub>	68 %	<b>c</b>	>20:1	62:38
4	<i>p</i> -F-C <sub>6</sub> H <sub>5</sub>	89 %	<b>d</b>	>20:1	99:1
5	<i>p</i> -Me-C <sub>6</sub> H <sub>5</sub>	96 %	<b>e</b>	>20:1	94:6
6	3-thiophene	92 %	<b>f</b>	>20:1	94:6
7	<i>o</i> -Br-C <sub>6</sub> H <sub>5</sub>	78 %	<b>g</b>	>20:1	94:6

<sup>a</sup>Catalyst RuCl[(*R,R*)-FsDPEN](*p*-cymene) (0.05 eq.), was added to a solution of **2.7a-g** (1 eq., 0.1 M) and HCOOH/Et<sub>3</sub>N (5:2, 5 eq.). <sup>b</sup>Yield and *dr* determined by <sup>1</sup>H NMR spectroscopy of the crude reaction mixture. <sup>c</sup>Determined by chiral HPLC analysis of the crude reaction mixture.

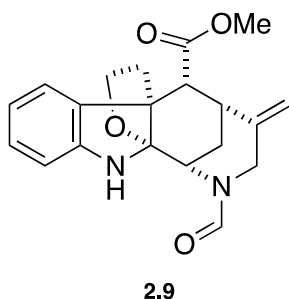
The results presented in table 2.2 show a reliable performance of the ATH/DKR methodology for  $\alpha$ -amido- $\beta$ -keto esters. The only exception is found in compound **2.7c**, unfortunately offering **2.8c** in moderate yield and poor *er* (Entry 3).

## Conclusions

We have developed a method for the ATH/DKR transformation of  $\beta$ -amido- $\alpha$ -keto esters into  $\beta$ -amido- $\alpha$ -hydroxy esters. Our method provides the corresponding *anti*- $\beta$ -amido- $\alpha$ -hydroxy esters in high yields, *dr*'s and *er*'s by using commercially available ligand–catalyst complex, thus making it easy and operationally straightforward to perform.

## 2.2 Towards the Enantioselective Synthesis of Aspidophylline A (*Manuscript I*)

Our recent advancements in the carbopalladation-carbonylation domino sequence inspired our core strategy for the synthesis of Aspidophylline A (figure 2.2).<sup>142, 143</sup> Aspidophylline A was isolated in 2007 by Kam and co-workers from the Malayan stem-bark *Kopsia Singaporensis*. The remarkable structural features of Aspidophylline A, coupled with its notable bioactivities, have rendered it an attractive target molecule for the synthetic community.<sup>144</sup>

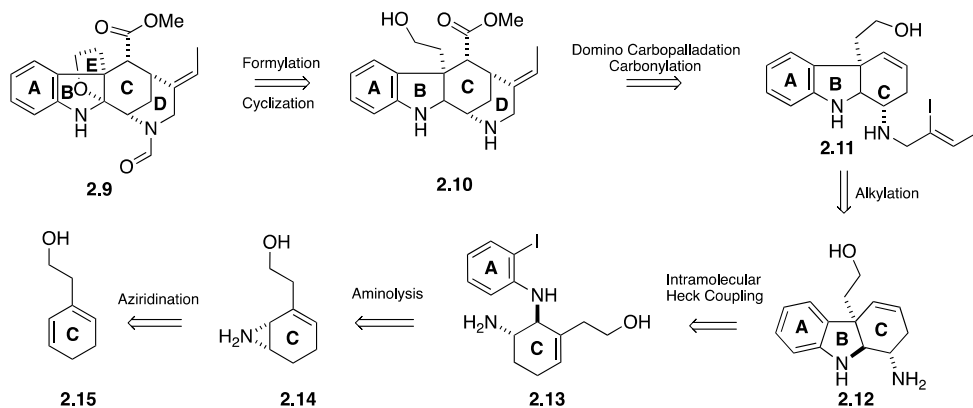


**Figure 2.2:** Aspidophylline A.

### Retrosynthetic Analysis

A noteworthy feature of Aspidophylline A is its intricate pentacyclic framework, entailing a bridged [3.3.1] bicycle and five stereogenic centres, including an all-carbon quaternary one. The target compound was planned to be derived from tetracyclic intermediate **2.10** (scheme 2.6) after formylation of the piperidine nitrogen and oxygen-carbon bond formation yielding tetrahydrofuran ring **E**. The key transformation in the total synthesis was envisioned to be a domino carbopalladation–carbonylation sequence, converting compound **2.11** to **2.10** by installing **D** ring followed by *syn* insertion of carbon monoxide. We envisioned that intermediate **2.11** could be synthesized from species **2.12** after *N*-alkylation.

Formation of the tricyclic framework **2.12** was projected to occur by implementation of a regioselective  $S_N2$  aminolysis ring-opening reaction<sup>145</sup> of aziridine **2.14** to afford diamine **2.13**, followed by an intramolecular Heck reaction<sup>146</sup>. We aimed to introduce the precise stereochemistry in the final target compound by executing an asymmetric regioselective aziridination on diene **2.15** to yield bicyclic compound **2.14**.

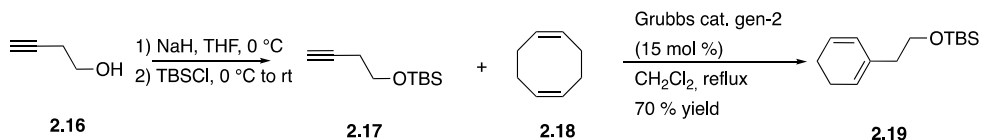


**Scheme 2.6:** Retrosynthetic scheme for the total synthesis of Aspidophylline A.

With a retrosynthetic approach in hand, we launched our studies towards the synthesis of Aspidophylline A by identifying a feasible synthetic route to conjugated diene **2.15**.

## Progress of the Synthesis

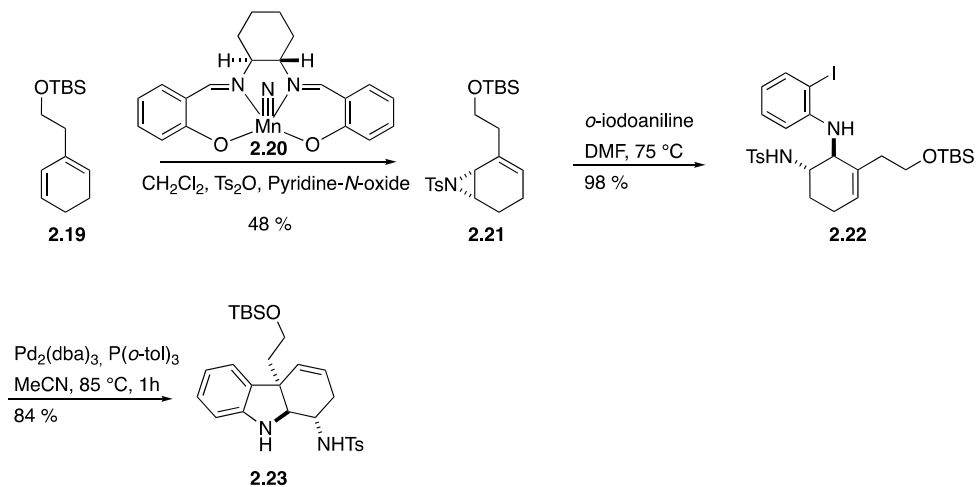
The protected analogue **2.19** of 1,3-diene **2.15** was synthesized by tandem enyne metathesis<sup>147-150</sup> between 1,5-cyclooctadiene **2.18** and alkyne **2.17** (scheme 2.7).<sup>151</sup> Alkyne **2.17** was obtained after protection of commercially available alcohol **2.16**.



**Scheme 2.7:** Synthesis of protected diene **2.19**.

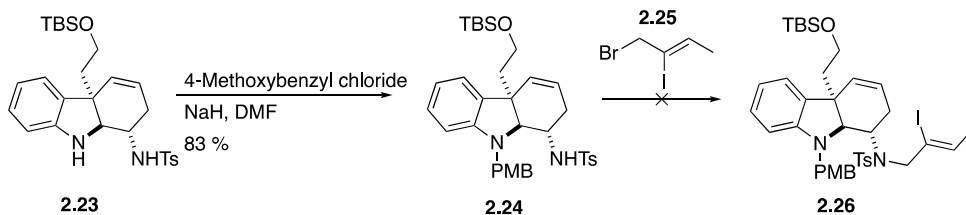
With species **2.19** in hand, we continued the synthesis towards Aspidophylline A (scheme 2.8). Our synthetic route offered rapid access to the tricyclic scaffold **2.23**. Compound **2.19** was transformed into the bicyclic aziridine **2.21** by using

aziridination chemistry developed by Komatsu<sup>152</sup>. The olefin aziridination reaction offered a non-typical insertion of the nitrogen into the least substituted double bond, *i.e.*, the bond expected to possess the lowest reactivity towards metal nitrene complexes like **2.20**. No regioisomer of **2.21** was found in the reaction mixture despite modest yields of 48 %. Without further optimisation, we continued with the synthesis via ring opening<sup>145</sup> of aziridine **2.21** with 2-iodoaniline in DMF, a reaction offering near quantitative yield of the vicinal diamine **2.22**. The tricyclic compound **2.23** was prepared through Heck coupling in high yields.



**Scheme 2.8:** A three-step approach to compound **2.23** starting from diene **2.19**.

Our aim to promptly evaluate the carbopalladation–carbonylation key step was hindered by the unexpected chemoselectivity in the projected *N*-alkylation step. Unfortunately, the sulphonamide nitrogen proved to be the least reactive nitrogen upon treatment with allylic bromide **2.25**<sup>153</sup>. After protection of intermediate **2.23** with 4-methoxybenzyl chloride (scheme 2.9) to yield compound **2.24**, several known procedures for *N*-alkylation of sulphonamides were evaluated for the synthesis of **2.26**. (Table 2.3).<sup>154-157</sup>

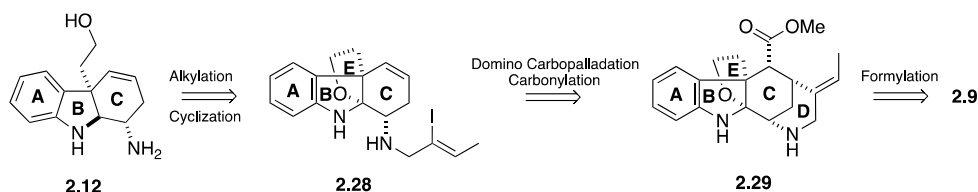


**Scheme 2.9:** Attempts to prepare compound **2.26**.

**Table 2.3:** Screening of reaction conditions for the alkylation of compound 2.24.

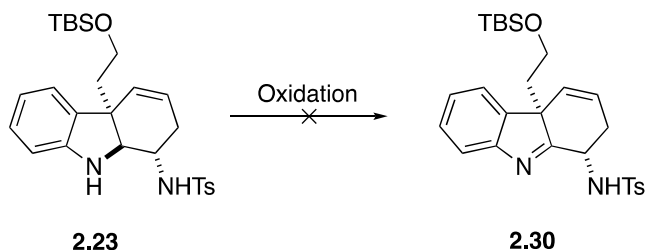
Entry	Base	Solvent	Temperature	Result
1	K <sub>2</sub> CO <sub>3</sub>	DMF	rt	no rxn
2	Cs <sub>2</sub> CO <sub>3</sub>	MeCN	rt	no rxn
3	Cs <sub>2</sub> CO <sub>3</sub>	MeCN	50 °C	decomp.
4	<i>t</i> -BuOK	THF	0 °C	decomp.
5	NaH	DMF	0 °C	decomp.
6	LDA	THF	-78 °C to rt	decomp.

Alkali metal carbonates were not basic enough to perform the desired *N*-deprotonation at room temperature (Entries 1-2). Both the addition of allylic bromide **2.25** or methyl iodide caused no alkylation of **2.24**. The addition of 18-crown-6-ether (0.1 eq.) to the reaction mixture proved also to be unsuccessful. Before turning our attention towards stronger bases, the influence of the temperature was studied using caesium carbonate as base (Entry 3). However, the colour of the reaction mixture went from yellow to black even before alkene **2.25** was added and TLC as well as NMR analysis indicated decomposition of the starting material. Due to the lack of deprotonation, we decided to use stronger deprotonating agents. Unfortunately, the use of *t*-BuOK, NaH and lithium diisopropylamide (LDA) prompted decomposition of compound **2.24**. Consequently, an alternative strategy for the introduction of the *N*-alkyl moiety had to be developed (scheme 2.10). It was envisioned that tetracycle **2.28** could be obtained after the introduction of furan ring **E** and alkylation with **2.25**, thus, allowing us to pursue the critical carbopalladation–carbonylation sequence. If successful, this approach can potentially provide the pentacyclic framework **2.29** only in need of formylation to conclude the total synthesis.

**Scheme 2.10:** Diversification of initial route towards Aspidophylline A.

We therefore continued our total synthesis by pursuing imine **2.30** which we intended to obtain by oxidation of sulphonamide **2.23** (scheme 2.11). We envisioned that access to the imine would give us the freedom to try out the essential alkylation before a cyclization or vice versa, but unfortunately, no imine was ever recovered (Table 2.4).





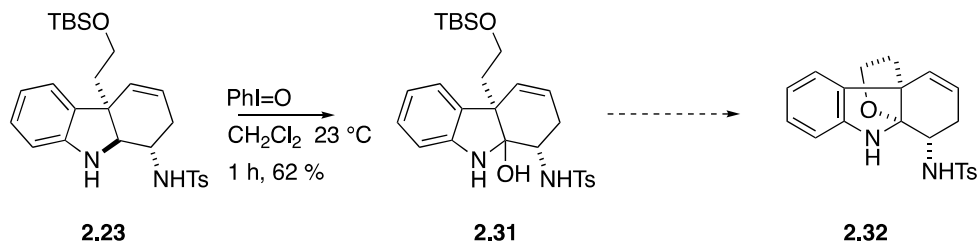
**Scheme 2.11:** Attempted oxidation of sulphonamide **2.23** to furnish imine **2.30**.

**Table 2.4:** Oxidation of compound **2.23**.

Entry	Oxidation Source	Solvent	Temperature	Yield
1	DMP	CH <sub>2</sub> Cl <sub>2</sub>	0 °C	decomp.
2	MnO <sub>2</sub>	CH <sub>2</sub> Cl <sub>2</sub>	0 °C	decomp.
3	KMnO <sub>4</sub>	Benzene	rt	decomp.
4	KMnO <sub>4</sub>	Benzene	0 °C	decomp.
5	Swern Oxidation	CH <sub>2</sub> Cl <sub>2</sub>	-78 °C to rt	undesired
6	PhIO	CH <sub>2</sub> Cl <sub>2</sub>	rt	62 % <sup>a</sup>

<sup>a</sup> Aminal **2.31** was obtained.

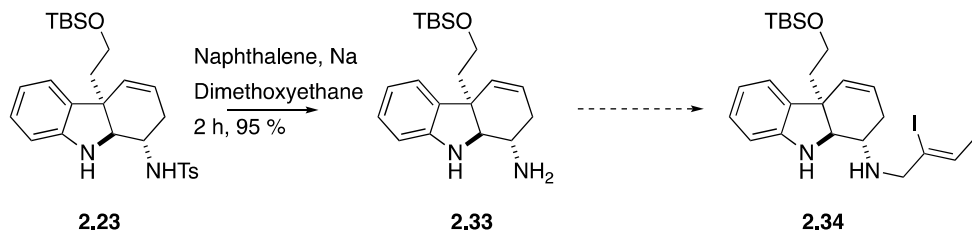
The first attempt was executed with commercially available Dess–Martin periodinane (DMP) and resulted in slow decomposition of starting material (Entry 1). Subjecting to manganese-based oxidants resulted in immediate decomposition of the starting material (Entries 2-4). Swern oxidation delivered a clean reaction to a yet unknown compound (Entry 5). However, treatment with iodosobenzene at room temperature for one hour generated hemiaminal **2.31** instead of the anticipated imine (entry 6) and scheme 2.12.<sup>158</sup> Next, compound **2.31** will be exposed to acidic conditions in an attempt to access tetracycle **2.32**. We hypothesize that acidic reaction conditions will result in desilylation and *in-situ* imine formation followed by cyclization to give intermediate **2.32**.



**Scheme 2.12:** Obtained hemiaminal **2.31** will be used to furnish intermediate **2.32**.

Compound **2.23** was also transformed into amine **2.33**, upon treatment with naphthalene and sodium (scheme 2.13). This deprotection increases our synthetic

options for alkyl installation by enabling the potential use of reductive amination to render diene **2.34**.



**Scheme 2.13:** Amine **2.33** can potentially be transformed into diene **2.34** by alkylation or reductive amination.

## Conclusions

To date, we have developed a 5-step route to the A/B/C tricyclic scaffold **2.31** present in Aspidophylline A **2.9**. A key feature of our approach is the straightforward installation of the correct relative stereochemistry of Aspidophylline A through the regioselective opening of aziridine **2.21**. Our synthetic route also provides rapid access to several advanced intermediates where the critical carbopalladation-carbonylation can be evaluated.

### 3. Concluding Remarks

This thesis presents a comprehensive exploration of two distinct yet interconnected domains, IMS in neurotherapeutics and organic synthesis, each offering unique advancements in their respective fields.

In the first part of this thesis, we delved into the dynamics of the antipsychotic drug CLO and its two main metabolites, NDMC and CNO in the brain. Through the innovative use of IMS and a desert locust *ex vivo* BBB/brain model, we were able to visualise and understand the neural distribution of these compounds without the need for molecular imaging probes. Our study sheds light on the limited penetration of CNO through the BBB and its neural metabolism to CLO and NDMC, observations that may carry significant implications for CNO's use in DREADD technology. Furthermore, while directly administered NDMC exhibited limited distribution, NDMC generated from *in situ* CYP-metabolism on CLO, was distributed throughout the brain. Our findings emphasise the need for a thorough understanding of drug dynamics in the brain for optimising drug delivery strategies, ensuring effective targeting, and minimising potential adverse effects. With this understanding, the effectiveness and safety of neurotherapeutic interventions can be improved.

Transitioning to the second part of this thesis, with our developed ATH/DKR strategy, we achieved remarkable yields, diastereo-, and enantioselectivities in the synthesis of aromatic *anti*- $\beta$ -amido- $\alpha$ -hydroxy esters. Our methodology holds promise for the efficient synthesis of biologically active compounds in drug discovery and development. Our work on Aspidophylline A led to the synthesis of an advanced tricyclic intermediate, laying the groundwork for the total synthesis of this biologically intriguing natural compound.

The collaborative efforts highlighted in this thesis exemplify the importance of interdisciplinary collaboration in advancing pharmaceutical research. Our research is poised not only to expedite the pace of innovation but also to enable the exploration of uncharted territories in neurotherapeutic research, potentially enhancing the well-being and quality of life of individuals.

# Acknowledgements

I would like to express my warmest and most sincere gratitude to my supervisor, Prof. Peter Somfai, for his invaluable guidance and support throughout my doctoral journey. Your steady commitment to excellence, profound expertise in chemistry, and dedication to fostering my intellectual growth have been instrumental throughout my Ph.D. journey. Thank you for believing in me and for embodying not only exceptional chemistry prowess but also genuine kindness.

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I am also immensely grateful to my elementary school teachers, Bo Petersson and Gunilla Jönsson, who instilled in me the belief that I could one day become a scientist. Additionally, I owe a debt of gratitude to my first chemistry teacher, Kjell Magnusson, for introducing me to the world of chemistry in such a captivating way.

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I would also like to express my sincere gratitude towards the funders, who made these research projects possible, Lund University, the Estonian Ministry of Education and the Royal Physiographic Society.

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Sist men inte minst vill jag tacka min älskade sambo Gabriella för att alltid ha stöttat mig. Ditt oerhörda tålmod, din omänskliga förmåga att aldrig sluta tro på mig och din ovillkorliga kärlek betyder allt för mig.

## Populärvetenskaplig Sammanfattning

Första delen i denna avhandling fokuserar på '*Imaging Mass Spectrometry*' (IMS), en analytisk teknik med kartografiska egenskaper. Denna teknik används dock inte för att kartlägga geografiska terränger, utan för att skapa kartor över det kemiska landskapet i biologiska prover, från vävnadssnitt av organ eller tumörer till enskilda celler. Dessa kartor visar med hög precision var olika molekyler, såsom kroppsegna ämnen och läkemedel, finns inom de analyserade proverna, och bidrar på så sätt till en djupare förståelse av såväl biologiska som farmakologiska processer på molekylär nivå.

I denna studie använde vi IMS och en insekthjärna för att studera det antipsykotiska läkemedlet klopazin (CLO) och dess två viktigaste metaboliter, *N*-desmetylklopazin (NDMC) och klopazin-*N*-oxid (CNO). Vårt mål var att förstå benägenheten för dessa ämnen att ta sig igenom blod-hjärnbarriären (BBB) samt deras beteende i hjärnan. Vi valde dessa föreningar av följande skäl. Trots att CLO är det vanligaste antipsykotiska läkemedlet utskrivet, är dess verkningsmekanism inte helt kartlagt. NDMC är intressant eftersom den misstänks vara en aktiv substans, delaktig i CLOs effekter. NDMC har utvärderats i kliniska tester men påvisade ingen farmakologisk effekt, något som tros bero på en begränsad förmåga att penetrera BBB. Vad gäller CNO, har den använts flitigt inom neurovetenskap som ett slags fjärrkontroll för att aktivera manipulerade hjärnceller. Dess användning bygger dock på antagandet att den enkelt kan ta sig in i hjärnan och inte genomgår förändringar. Våra IMS-kartor över insekthjärnan visar att CLO effektivt kan passera BBB och fördelar sig jämnt över hela hjärnan. Väl där omvandlas den till NDMC, vars distribution i stort sett matchar CLOs. Däremot, när NDMC administreras direkt, uppvisar den både en inskränkt förmåga att passera BBB och fördela sig i hjärnan. Gällande CNO, indikerar våra kartor att den stöter på svårigheter att passera BBB och att den dessutom omvandlas till både CLO och NDMC inne i hjärnan. Våra resultat ifrågasätter användandet av CNO inom neurovetenskap och tyder på att NDMCs begränsade förmåga att penetrera BBB förmodligen är orsaken till dess bristande effekt som läkemedel. För övrigt framhäver denna studie också användbarheten av vår IMS/insekthjärnplattform för farmakologisk och neurovetenskaplig forskning.

I den andra delen i denna avhandling har en metod för att producera vicinala aminohydroxider, ett vanligt förekommande arrangemang i såväl läkemedel som naturliga föreningar, framgångsrikt utvecklats. Detta lyckades vi med genom att tillämpa asymmetrisk överförings hydrogenering på *anti*- $\alpha$ -amido- $\beta$ -hydroxyl estrar som under våra utvecklade betingelser genomgick dynamisk kinetisk resolution. I denna andra del presenteras också våra framsteg i tillverkningen av Aspidophylline A, en förening som återfinns i en oleanderväxtart och som har potentialen att göra resistent cancerceller mottagliga för behandling. Även om vi inte lyckades fullborda vår syntes har våra ansträngningar satt oss på en lovande väg mot att uppnå detta mål.

## Popular Scientific Summary

The first part of this thesis focuses on 'Imaging Mass Spectrometry' (IMS), an analytical technique with cartographic capabilities. However, this technique is not used to map geographical terrains; instead, it is employed to create maps of the chemical landscape within biological samples, ranging from tissue sections from organs or tumours to individual cells. These maps show with high precision where molecules (*e.g.*, endogenous substances and drugs) are located within the analysed samples, contributing to a deeper understanding of both biological and pharmacological processes at the molecular level.

In this study, we used IMS and an insect brain to investigate the antipsychotic drug clozapine (CLO) and its two main metabolites, *N*-desmethylclozapine (NDMC) and clozapine-*N*-oxide (CNO). Our goal was to understand the propensity of these drugs to cross the blood-brain barrier (BBB) and their behaviour in the brain. We selected these drugs for the following reasons. Although CLO ranks as the top antipsychotic of choice, its mechanism of action is not fully understood. NDMC is of interest because it is suspected to be an active substance involved in CLO's effects. NDMC has been evaluated in a clinical trial but showed no effect compared to placebo. It was hypothesised that this ineffectiveness was due to its limited ability to cross the BBB. As for CNO, it has been widely used in neuroscience as a kind of remote control to activate manipulated brain cells. However, its use relies on the assumption that it can easily enter the brain without undergoing changes. Our IMS maps reveal that CLO has a good ability to cross the BBB and is distributed evenly throughout the brain. Once inside, it transforms into NDMC, whose distribution matches that of CLO. However, when NDMC is administered directly, it struggles to cross the BBB and displays also a limited neural distribution. Regarding CNO, our maps show that it has difficulties crossing the BBB and also transforms into both CLO and NDMC inside the brain. Our results question the use of CNO in neuroscience and suggest that NDMC's limited ability to penetrate the BBB is likely the reason for its ineffectiveness as a drug. Furthermore, this study highlights the applicability of our IMS/insect brain platform for pharmacological and neuroscience research.

In the second part of this thesis, a method for producing vicinal amino alcohols, a common arrangement in both pharmaceuticals and natural compounds, has been successfully developed. We achieved this by applying a process known as asymmetric transfer hydrogenation on compounds undergoing dynamic kinetic resolution. In this second part, we also present our progress in the synthesis of Aspidophylline A, a compound found in an oleander species with the potential to make resistant cancer cells responsive to treatment. Although we did not complete our synthesis, our efforts have placed us on a promising path toward achieving this goal.

## Resumen Divulgativo

La primera parte de esta tesis se centra en '*Imaging Mass Spectrometry*' (IMS), una herramienta analítica con capacidades cartográficas. Sin embargo, esta técnica no se utiliza para mapear terrenos geográficos, está orientada a crear mapas del paisaje químico en muestras biológicas, desde secciones de tejido de órganos o tumores hasta células individuales. Estos mapas muestran con alta precisión la ubicación de moléculas, así como sustancias endógenas y medicamentos, dentro de las muestras analizadas y contribuye a una comprensión más profunda de los procesos biológicos y farmacológicos a nivel molecular.

En este estudio, utilizamos IMS y un cerebro de insecto para investigar el antipsicótico clozapina (CLO) y sus dos principales metabolitos, *N*-desmetilclozapina (NDMC) y clozapina-*N*-óxido (CNO). Nuestro objetivo era entender cómo estos compuestos cruzan la barrera hematoencefálica (BBB) y cómo se comportan en el cerebro. Elegimos estos medicamentos por las siguientes razones. A pesar de que CLO es el antipsicótico más recetado, todavía no se comprende completamente su mecanismo de acción. NDMC es de interés porque se sospecha que es una sustancia activa involucrada en los efectos de CLO. NDMC ha sido evaluado como medicamento, pero no mostró efecto. Se cree que esto se debe a su limitada capacidad para cruzar la BBB. En cuanto a CNO, se ha utilizado ampliamente en neurociencia como un tipo de control remoto para activar neuronas manipuladas. Sin embargo, su uso se basa en la suposición de que puede ingresar fácilmente al cerebro y no sufre cambios. Nuestros mapas revelan que CLO tiene una buena capacidad para cruzar la BBB y se distribuye de manera uniforme en todo el cerebro. Una vez dentro, se transforma en NDMC, cuya distribución se asemeja a la de CLO. Sin embargo, cuando se administra NDMC directamente, tiene dificultades para cruzar la BBB y tiene una distribución limitada. En cuanto a CNO, nuestros mapas muestran que tiene dificultades para cruzar la BBB y también se transforma en CLO y NDMC dentro del cerebro. Nuestros resultados cuestionan el uso de CNO en neurociencia y sugieren que la limitada capacidad de NDMC para penetrar la BBB es probablemente la razón de su falta de eficacia como medicamento. Además, este estudio destaca la aplicabilidad de nuestra plataforma 'IMS/cerebro de insecto' en la investigación farmacológica y neurocientífica.

En la segunda parte de esta tesis, se ha desarrollado con éxito un método para producir aminoalcoholes vicinales, una estructura común tanto en fármacos como en compuestos naturales. Logramos esto aplicando un proceso conocido como hidrogenación asimétrica de transferencia en sinergia con resolución cinética dinámica. En esta segunda parte, también presentamos nuestro progreso en la síntesis de Aspidofilina A, un compuesto natural con el potencial de hacer que células cancerosas resistentes sean receptivas a tratamiento nuevamente. Aunque no completamos nuestra síntesis, nuestros esfuerzos nos han colocado en un camino prometedor hacia la consecución de este objetivo.

# Appendix

## Material and Methods (*Paper I*)

### Chemicals

All chemical used were of analytical reagent grade and purchased from Sigma-Aldrich (Stockholm, Sweden). Ethanol was purchased from Solveco (Rosersberg, Sweden), and acetonitrile was acquired from Fisher Scientific. Chemicals for histology purposes were purchased from Histolab (Gothenburg, Sweden).

### Animal Ethics

The studies in this thesis were conducted in accordance with Swedish national legislation and European Community guidelines for animal studies. Experiments were meticulously planned to minimise the number of animals used

### Zebrafish

Zebrafish sacrificed for the study were from intercrosses of the wild-type AB strain. A 2 L aquarium containing 1 L water was prepared to host 12 fish. The desired drugs were dissolved in 100% dimethyl sulfoxide to generate stock solutions (10 mM). Properly diluted stock solutions were added to the 2 L aquaria in the amount required to obtain the desired concentration and stirred for 2 minutes for homogeneity purposes. Experiments were initiated when fish were transferred to the drug-prepared aquaria, where they were left to swim freely. After behaviour onset, the fish were removed from the tank and anaesthetised by incubation in ice water for 15 minutes before beginning dissection. The dissection began by lightly patting the fish dry on a paper towel before transfer to the dissection dish. The fish was pinned through the fleshy part of the tail and posterior part of the dorsal fin in order to remove the head with a razor blade. The head was cleaned from as much soft tissue as possible, and the skull was gently broken open. Bones were removed, and the eyes were detached by cutting of the optic nerve. The brain was cleaned before submitted to snap-freezing.

### Desert Locust

Insects were obtained from a commercial animal supplier (Petra Aqua, Prague, Czech Republic). Upon arrival, the animals were housed in insect cages supplied by Small-Life Supplies (Peterborough, Great Britain) and adapted to 10:14 h Night/Day cycle with a habitat temperature ranging 25–34 °C depending on the animals' distance to the light bulb. Chinese cabbage and wheat bran were used as alimentary source ad libitum and all experiments were conducted 2–3 weeks after adult emergence. Brains were dissected by cutting off the frontal part of the head which later was detached from the cuticle and cleaned from fat and tissue by using fine



forceps. The isolated brains were placed in microwell plate (96U Microwell; Nunc), all containing 250  $\mu$ L of the drug with the respective concentration of interest dissolved in an insect buffer. The brains were removed from the compound solutions after fixed exposure periods and washed twice in ice-cold insect buffer prior to freezing. The harvested drug treated brains were immediately snap frozen, placed in an Eppendorf tube and kept in a -80 ° freezer until collected for MALDI-IMS experiments.

### Snap Freezing

Brain samples from desert locust and zebrafish were collected in an Eppendorf tube to facilitate for freezing procedure. Immediately after collection, prepared tubes are submerged into liquid nitrogen. Snap-freezing is immediate and the tubes were kept submerged for 1 minute to obtain a homogenous frozen specimen. After 1 minute, the tubes are transferred quickly to a Styrofoam box containing dry ice so to safely transfer the tubes to a -80 °C freezer for long-term storage.

### MALDI-IMS

To avoid interferences from compounds alien to experiments, all brains were mounted on to the cryostat cutting block using only Millipore water/ice. Brain tissue samples ranging from 8-30  $\mu$ m thickness were obtained from both treated and untreated specimens at temperature ranging between -12 °C and -20 °C depending on daily humidity conditions. Brain tissues were thaw-mounted onto a MALDI glass slide (Thermo Fischer, Superfrost Ultra Plus, 25 x 75 x 1,0 mm, Germany) for matrix application. Brains not exposed to drugs were also collected and used as controls to obtain zero value tissues used to compare the signal intensities from the drug-treated brains. Thaw-mounted samples were placed in a vacuum chamber for 30-45 minutes to remove condensate formed from atmospheric water. A CHCA matrix solution was prepared with a concentration of 5.0 mg/mL in (1:1) acetonitrile: water and 0.2 % of TFA. An automated pneumatic sprayer (TM-Sprayer, HTX Technologies) was used to uniformly coat brain samples with matrix solution. The nozzle distance between sprayer and sample was fixed to 46 mm, and the spraying temperature was stabilized at 95 °C. The matrix was sprayed (18 passes) over the tissue sections at a nozzle linear velocity of 700 mm/min and a flow rate set to 0.1 ml/min with a nitrogen pressure permanent at 10 psi. An intermission time of 10 s was pre-set in between each pass in order to give time for the sample to dry completely (*i.e.* let the matrix solvent evaporate) before the next pass. The matrix-treated glass slide had a yellow/whitish frosted appearance when all passes were completed. MALDI-IMS data analyses were performed on a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Ten laser shots at 14  $\mu$ J were used to ionize each pixel examined and entire brain tissue sample were scanned with a spatial resolution of 50  $\mu$ m. Full scans in profile mode, mass acquisition ranging from m/z 200 to 400, were obtained in Orbitrap positive polarity. The following scan event were set to produce collision-induced

dissociation (CID) fragmentation MS/MS spectra, mass ranging from  $m/z$  250 to 320 and analysed using a linear ion trap in positive polarity.

### Imaging Data Analysis

Molecular images of drug distribution were generated using ImageQuest™ software (Thermo Fisher Scientific, San José, CA) from the MS raw files. CLO, NDMC, CNO parent ions and their corresponding fragments ions ( $m/z$ ; CLO 270.167, NDMC 270.167, CNO 299.167) were extracted to determine their localization within tissue sections. Screen shots of the distribution for CLO ( $m/z$  327.138), NDMC ( $m/z$  313.123) and CNO ( $m/z$  343.134) parent ions and of their respective fragment ions were obtained. The scale of the colour pattern in the generated images was used to estimate the relative abundance of a drug within a tissue sample.

### Histological staining

After performed IMS experiment, matrix coated sample slides were submerged in 70 % ethanol until most of the matrix was dissolved. The slides were then submerged in 90 % ethanol for 5 minutes before being immersed in 4 % phosphate-buffered formaldehyde for 30 min and then washed under running distilled water for 5 s. The fixed samples were immersed in Mayer's for 5 minutes, followed by water bluing for 10 minutes. Further staining was achieved by submerging the slides in an eosin solution followed by a wash with deionized water. The slides were then dipped in 70 % ethanol for 30 seconds, in 90 % ethanol for 1 minute, and later in 99 % dehydrated ethanol for 1 min. Finally, the slides were submerged in four different xylene baths for 1 minute in each bath. The stained slides were protracted by applying Pertex® to a cover slip and gluing together slide and cover slip. <sup>159</sup>

### Histological Analysis

The cover-slipped H&E-stained slides were optically examined (Mirax Midi Slide Scanner, Zeiss, Germany) to take non-detailed image for the selection of regions of interest to be scanned at high resolution. The files were opened in Aperio ImageScope Viewer v12.1 (Leica Biosystems Imaging Inc., Vista, CA) where detailed images were taken and saved as \*.tiff files.

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# An Organic Chemistry Odyssey

**MARVIN VILLACREZ**, originally from Huacho, Peru, found a sanctuary amidst the picturesque landscapes of Söderåsen in Billesholm during his early childhood. It was the legacy of Carl von Linné and the abundant natural wonders surrounding him, that ignited his passion for natural sciences. After earning a master's degree in organic chemistry under the guidance of Professor K. Wärnmark at Lund University, he had the privilege to pursue doctoral studies under the esteemed Professor P. Somfai. Later, he had the fortune of conducting multidisciplinary research in Professor R. Olsson's laboratory, delving into the realms of organic chemistry, neuroscience, pharmacology, and analytical techniques.



This thesis condenses my doctoral studies and comprises two parts. In the initial segment, the gold standard antipsychotic, clozapine, is studied in an isolated brain. Our research revolves around the revolutionary mapping technique of Imaging Mass Spectrometry, which is capable of generating detailed maps of the chemical landscape within biological samples.

The second part of this thesis presents an efficient approach for the asymmetric synthesis of vicinal amino alcohols and our attempts towards the total synthesis of the natural product Aspidophylline A.