Isolation, identification and chemical modification of *Narcissus* alkaloids

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Engineering and Physical Sciences.

2015

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School of Chemistry
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Abstract

Isolation, identification and chemical modification of Narcissus alkaloids

A submission for the degree of Doctor of Philosophy at the University of Manchester

Edmund William Dwerryhouse Burke, 2015

Galanthamine is one of only a few drugs that is licenced for the treatment of Alzheimer’s disease. It is manufactured through an inefficient synthetic process or extracted from members of the Amaryllidaceae plant family.

In this work a range of structurally related alkaloids was semisynthesised from galanthamine and used as standards to assist with the search for alkaloids that may make a good starting point in the semisynthesis of galanthamine. The search was targeted on the effluent from the production line of Alzeim Ltd., a company that extracted galanthamine from members of the Narcissus genus.

In addition to the above there was an examination of specific areas of galanthamine chemistry; focusing on the application of synthetic methodologies to open new chemical space. By far the most successful of these was the use of galanthamine in a urea-mediated intramolecular aryl migration. A rare example of lithiation chemistry being applied to a natural product and also the most complicated structure to date on which the intramolecular aryl migration has been performed.

\[
\begin{align*}
\text{Galanthamine} & \quad \xrightarrow{a} \quad \text{Norgalanthamine} \\
\text{Urea} & \quad \xrightarrow{c} \quad \text{Rearranged product}
\end{align*}
\]

a) Dichloromethane and propan-2-ol (3:1), -10 °C, 3-Chloroperbenzoic acid, then concentrated hydrochloric acid, water, iron(0) powder and Iron(III) chloride hexahydrate. b) Dichloromethane, N-Methyl-N-phenylcarbamoyl chloride, triethylamine. c) Tetrahydrofuran, -42 °C, LDA.
**Declaration**

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I would like to thank my supervisors Jonathan Clayden and Kevin Wall not only for the opportunity to carry out a PhD but also for their extensive and tireless patience and support throughout the course of the work.

Thanks and appreciation are also due to the many technical staff at the University of Manchester for their support, assistance and encouragement: Rehana Sung, Gareth Smith, Carole Webb, Roger Speak, and James Raftery and Madeleine Helliwell along with the other NMR staff and the ever helpful staff from the stores.

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My parents William and Diana and my siblings Helen and Alexander deserve a very special thank you for their support and encouragement over the many years it has taken me to get this far.
Preface

The author graduated from Kingston University with an undergraduate Masters in Chemistry (MChem) in 2010. Later that year the author started at the University of Manchester in the research group of Jonathan Clayden working on “Isolation, identification and chemical modification of Narcissus alkaloids”.
## Abbreviations

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<td>Amyloid-β</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>CHLOROFORM-d</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DMPU</td>
<td>1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DMSO-d6</td>
<td>Deuterated dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>e.e.</td>
<td>Enantiomeric excess</td>
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<tr>
<td>e.r.</td>
<td>Enantiomeric ratio</td>
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<tr>
<td>eq.</td>
<td>equivalents</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GC-MS</td>
<td>Gas chromatography mass spectrometry</td>
</tr>
<tr>
<td>HPCCC</td>
<td>High performance counter current chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatograph</td>
</tr>
<tr>
<td>HMPT</td>
<td>Tris(dimethylamino)phosphine also called hexamethylphosphorous triamide</td>
</tr>
<tr>
<td>IBX</td>
<td>O-iodoxybenzoic acid</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>LC-HRMS</td>
<td>Liquid chromatography high resolution mass spectrometry</td>
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<tr>
<td>LC-MS</td>
<td>Liquid chromatography mass spectrometry</td>
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<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>---------------</td>
<td>-------------------------------------------------</td>
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<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>METHANOL-\textit{d4}</td>
<td>Deuterated methanol</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl ether</td>
</tr>
<tr>
<td>Mpt.</td>
<td>Melting point</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
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<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
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<td>NIH</td>
<td>National Institutes of Health (part of the US Department of Health &amp; Human Services)</td>
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<tr>
<td>OGL</td>
<td>Open Government Licence</td>
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<tr>
<td>PAS</td>
<td>Peripheral anionic site</td>
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<tr>
<td>PIFA</td>
<td>Phenylidinium(III)bis(trifluoracetate)</td>
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<td>Poly(tetrafluoroethylene)</td>
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<td>TMEDA</td>
<td>\textit{N,N,N',N'}-Tetramethylethylenediamine</td>
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<td>UV</td>
<td>Ultra violet</td>
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<td>VT NMR</td>
<td>Variable temperature NMR</td>
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<tr>
<td>wt.%</td>
<td>mass fraction</td>
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1 A brief introduction to Alzheimer’s disease and galanthamine

1.1 The origins of this project

Alzeim Ltd. was a small company, started in the Brecon Beacons by a farmer with an interest in increasing the availability of galanthamine*, also called galantamine and sold under the trade name Reminyl, for the treatment of Alzheimer’s disease. His efforts to extract galanthamine, from daffodils, soon attracted interest from numerous other sources, including those with expertise in the biological sciences and chemical engineering and much work was done in these areas to improve the yield of galanthamine from each crop. The new company had an awareness of the use of semisynthesis in increasing the availability of other naturally sourced medicaments and was keen to see if similar strategies could be used in this instance and as an extension to this they were interested in the transformation of galanthamine to sanguinine, either for the treatment of Alzheimer’s or other undisclosed applications. The other principal areas of interest were in identifying any other potentially useful alkaloids present in the plant extract and the structural modification of galanthamine with a view to improving its efficacy. Thus was this work sponsored, consisting of two distinct parts; the first being the identification of the alkaloidal components of the plant extract and subsequent application of semisynthetic methods and the second being a more general look at methods for the structural modification of galanthamine, potentially, for the investigation of new chemical space.

Unfortunately, Alzeim Ltd. ceased trading before the end of the work resulting in some change in the emphasis and direction of the work.

1.2 Alzheimer’s disease

1.2.a Alzheimer’s disease; history, societal impact, and diagnosis

Alzheimer’s disease is a form of dementia that was first identified and described by the German physician Alois Alzheimer at the beginning of the 20th century. He identified extensive neurofibrillary tangles within neurons in the brain; neurofibrils themselves only

* In the scientific literature for the chemical sciences the compound is generally called galanthamine, however, in the healthcare industry the same compound is called galantamine or sometimes by the trade name Reminyl.
having been reported four years previously. He also reported the presence of plaques, which had first been reported several years before. In the time since, the plaques have been identified as amyloid-β; amino acid chains of up to 42 residues that are derived from the membrane bound amyloid precursor protein. The neurofibrillary tangles have been identified as hyperphosphorylated tangles of tau protein; tau protein’s usual role being the stabilization of microtubules, which are important in cell transport.

Alzheimer’s disease is characterised by cognitive impairment, initially displayed as confusion, forgetfulness, the inability to recall recent events, and mood swings, as the disease progresses these symptoms worsen and the patient may suffer from hallucinations, apraxia, communication problems and a loss of speech, eventually this degenerative disorder will result in the death of the patient. However, the nature of the disease and the fact that it is most evident in the elderly means that assigning an exact cause of death can be difficult and often the cause of death in Alzheimer’s disease patients is related to Alzheimer’s disease if not implicitly caused by it. For example; the patient may die of dehydration due to forgetting to drink, or an inability to consume sufficient fluids, or death may be as a result of an infection, such as pneumonia, which would ordinarily be treated, but the Alzheimer’s disease patient maybe unaware that they are ill, or maybe unable to seek the necessary medical treatment.

The Alzheimer’s Society estimates that about three quarters of a million people suffer from Alzheimer’s disease in the United Kingdom. Due to the fact that the likelihood of developing Alzheimer’s disease grows dramatically with age the continuing trend of increasing longevity in western populations will lead to an escalation in the instances of Alzheimer’s disease; estimated to reach about one million instances by the end of this decade. Current estimates put the financial cost of Alzheimer’s disease in the UK in excess of £20bn a year, the main costs being the increase in the amount of care an Alzheimer’s disease patient needs and the high cost of the medicines available. It is thought that in some areas of the United Kingdom less than one in four cases of Alzheimer’s disease are diagnosed, however, increasing public awareness of Alzheimer’s disease will probably lead to an increase in correct diagnosis, which will of course be accompanied by an increase in expenditure on appropriate medicaments and care.

Alzheimer’s disease is assessed by observations of behaviour and changes in behaviour that may occur over several weeks if not months, cognitive tests, of which the most common is the mini mental state examination (MMSE), scans to identity possible brain damage, and sometimes even a brain biopsy. Consequently it is difficult to give a conclusive diagnosis of Alzheimer’s disease, especially in the early stages of the disease. Hence the qualification and quantification as to the existence and extent of the Alzheimer’s
diseased state in an individual is often determined in a highly subjective manner, such as interviews with the patient, the patient’s carers and family members. This method of prognosis means that Alzheimer’s disease can only be given as a diagnosis following careful consideration and the ruling out of other conditions that may bring about similar symptoms to Alzheimer’s disease, such as depression and dehydration which can both affect memory and other cognitive functions. In some cases Alzheimer’s disease is only confirmed by an autopsy after death.

1.2.b Alzheimer’s disease; causes and pathogenesis

Since Alois Alzheimer’s original description of a new form of dementia there has been considerable investigation of Alzheimer’s disease, but there is still no conclusive evidence as to a specific cause and debate continues as to the exact route of disease pathogenesis. The state of the current aetiology was recently summarised by a National Institutes of Health (NIH) conference, which concluded that while there has been a considerable effort to identify factors that either cause, increase or reduce the risk of developing Alzheimer’s disease, the studies undertaken have provided no conclusive evidence and that the evidence collected cannot be reliably amalgamated or compared due to the extent of variance in the methods used. Not surprisingly the conference recommended that future epidemiological studies should be run for far greater duration (decades, not months or a couple of years) and collect a broader range of peripheral data to allow the effects of the factor in question to be assessed in context and minimise the possibility of hidden associations. Finally the conference recommended that there needed to be technical advancement and standardization in the measurement of the Alzheimer’s disease state, both through analytical equipment (MRI and PETS) and cognitive tests, coupled with a rigorous definition of Alzheimer’s disease and mild cognitive impairment (MCI).

Despite the dour view expressed in the NIH conference report, there has been some evidence published about the genetic propensity of individuals to develop Alzheimer’s disease, it is now well established that early onset (disease progression evident <60 years of age) Alzheimer’s disease is due to specific alleles of three genes, such is the correlation that these genes are considered to have complete penetrance. There is less certainty about the role of genetics in the development of Alzheimer’s disease that starts after 65 years of age, but a statistical correlation between the prevalence of three apo-lipoprotein alleles and the onset of Alzheimer’s disease has arisen. These genes code for proteins that are responsible for the movement of cholesterol, and the brain is the second highest region of expression after the liver. Of the three alleles the apo-lipoprotein ε4 allele is now generally considered to give the greatest risk of developing Alzheimer’s disease. But as with most
genetic investigations considerably more work is required to better illuminate the full relationship between genes and the development of Alzheimer’s disease.

Clearly any studies undertaken, in accordance with the aforementioned NIH conference report recommendations, will, by necessity, require years before they yield reliable information and direction for the prevention of Alzheimer’s disease. Thus, excepting early onset Alzheimer’s disease, for the interim Alzheimer’s disease can only be tackled via a remedial approach. In the absence of a known trigger for the Alzheimer’s disease cascade, such treatments can only target established physiological aspects of Alzheimer’s disease progression; principally amyloid-β plaques and tau neurofibrillary tangles.

The current trends group the theories on Alzheimer’s disease pathogenesis into three main groups known as; the cholinergic hypothesis, the amyloid cascade hypothesis, and the tau and tangle hypothesis.²,¹¹

The cholinergic hypothesis is based on the observation that there is under expression of choline acetyltransferase, the enzyme responsible for the synthesis of the neurotransmitter acetylcholine, in certain regions of the Alzheimer’s diseased brain, namely the basal forbrain, nucleus basalis magnocellularis of Meynert, the cortex and the hippocampus, leading to a reduced availability of acetylcholine, which in turn is thought to impair the transfer of nerve impulses across the synaptic cleft.¹¹

\[
\begin{align*}
\text{O} & \quad + \quad \text{O} \\
\text{CoA} & \quad \text{HO-} \quad \text{N} \\
1 & \quad 2 \\
\text{Choline acetyltransferase} & \quad \text{O} \\
& \quad \text{N} \\
& \quad 3
\end{align*}
\]

Scheme 1.1: Representation of the biosynthesis of acetylcholine, 3.¹²

The extensive research surrounding the cholinergic hypothesis has failed to explain the initial steps in the development of Alzheimer’s disease. However, it has presented potential targets for the treatment of Alzheimer’s disease and is currently the only theory to have yielded licensed medications for the treatment of Alzheimer’s disease.

The amyloid cascade hypothesis, and the tau and tangle hypothesis stem from Alois Alzheimer’s initial observations of amyloid-β plaques and the neurofibrillary tangles in the brain of an Alzheimer’s disease patient. The first argues that the formation of the amyloid-β plaques gives rise to Alzheimer’s disease and the neurofibrillary tangles, while the second argues that the tangles give rise to Alzheimer’s disease and the plaques.²

Resolution of the issues is complicated by the difference in test results from tests on wild
type and mutant animals and the differences between the results observed in animal models and the human brain.\(^2\) The debate is ongoing and increasingly it would seem that neither is responsible for triggering the disease, but it seems clear that both plaques and tangles have a detrimental role in the brain and are neurotoxic.\(^2\),\(^13\) This last point makes them potential targets for drug therapies aimed at attenuation of Alzheimer’s disease.

In addition to the theories outlined above there is persuasive evidence to show that the accumulation of the plaques increases the oxidative stress on the neurons, and that this can lead to cell damage and finally cell death.\(^14\) This has led to the investigation of the therapeutic effects of anti-oxidants and cyclooxengase inhibitors on the amelioration of Alzheimer’s disease; such studies gave mixed results.\(^14\)-\(^16\)

1.2.c Alzheimer’s disease; current therapeutic approaches

The aforementioned cholinergic hypothesis and observed attenuated acetylcholine levels found in the Alzheimer’s diseased brain have led to the principal method for the treatment of Alzheimer’s disease. This method relies on the reversible inhibition of acetylcholinesterase, the enzyme responsible for the metabolism of acetylcholine in the synaptic cleft. It should be noted that this is reversible inhibition as irreversible inhibition is fatal, as shown by the strength of various poisons; e.g. physostigmine and sarin.

In the UK, the National Institute for Health and Clinical Excellence (NICE) recommends the use of three drugs for the treatment of mild to moderate Alzheimer’s disease.\(^17\)-\(^18\) These are donepezil, galantamine (also called galanthamine), and rivastigmine, all of which are acetylcholinesterase inhibitors. NICE also recommends the use of the drug memantine for the treatment of Alzheimer’s disease in patients with moderate to severe Alzheimer’s disease, or patients that cannot be given AChE inhibitors; it is currently the only non-AChE inhibitor that is licensed for the treatment of Alzheimer’s disease in the UK.\(^17\) Notably no new drugs for the treatment of Alzheimer’s have been licenced in the last ten years.

![Figure 1.1: Drugs licensed for the treatment of Alzheimer’s disease in the UK; donepezil (4), rivastigmine (5), galanthamine (6), memantine (7).](image-url)
Of the three AChE inhibitors galanthamine and donepezil are selective for AChE, while rivastigmine is non-selective and will also inhibit butyrylcholinesterase, found in plasma. The costing template issued by NICE estimates the cost of prescribing galanthamine to be between £676 - £1040 per patient per year.\textsuperscript{18} The total cost of galanthamine treatment in 2009 was a little over £19.5 million, making up just over 20% of the total expenditure on the three AChE inhibitors, expenditure on memantine was in the region of £3.7 million.\textsuperscript{18} These costs are set to rise dramatically following amendments to NICE guidelines that will make the AChE inhibitors more widely available to those diagnosed with Alzheimer’s disease.

Currently galanthamine hydrobromide (the commonly supplied formulation of galanthamine) is under investigation by the United States Food and Drug Administration (FDA) for increasing mortality compared to a placebo.\textsuperscript{19} However, this alert has not been updated since the end of September 2009 and galanthamine hydrobromide is still being prescribed.

1.3 Natural products; Amaryllidaceae and the alkaloids they yield - methods of identification, and ethnopharmacology

Natural products, and more recently derivatives thereof, have been used to treat malady for the entirety of human history. Compared to synthetic drugs the molecular structures of natural products used in medicine often contain a more complex rigid structure, including a higher degree of stereochemistry, and often contiguous stereocentres.\textsuperscript{20} Natural products also contain a greater proportion of oxygen and lower proportion of nitrogen compared to synthetic drugs.\textsuperscript{20} Additionally some natural products are actively transported through the body, hence they do not adhere to Lipinski’s rules.\textsuperscript{20-21}

Currently there are a vast array of medicines on the market that are either natural products or natural product derivatives. Some examples are taxol, extracted from \textit{Taxus brevifolia} (pacific yew tree) and other species of yew, used to treat selected refractory cancers, vincristine, from the \textit{Catharanthus roseus} (rosy periwinkle), used to treat childhood leukaemia, and Qinhao (artemisinin) from Qinghaosu (\textit{Artemesia annua}), used as an antimalarial. Availability of all three of these drugs is increased via semisynthesis.

The Amaryllidaceae family of plants is part of the Asparagales order, and consists of plants that grow from a bulb. It is further divided into three subfamilies: agapanthoideae, allioideae, and amaryllidoideae, and thence into genera, and species.\textsuperscript{22} Of greatest relevance here are those of the amaryllidoideae, which includes the \textit{Narcissus} (daffodil)
and *Galanthus* (snowdrop) genera, of which, due to the nature of this work, the genus *Narcissus* is of prime interest. Despite the vast number of plants in the Amaryllidaceae family many of the alkaloids they yield are common across a range of species of the order. There is abundant literature on the identification of the alkaloid content of members of this family, much of it aimed at the search for medicinally useful compounds and some aimed at clarification of evolutionally divergence.\textsuperscript{23}

![Alkaloids](image)

Figure 1.2: Alkaloids representative of the principle structural types found in *Narcissus*. The atoms are numbered according to Ghosal’s numbering system.\textsuperscript{23-24}

The Amaryllidaceae family yield a range of alkaloids with structures based on one of nine principal backbones (see Figure 1.2), although there are other alkaloids present as well. In Ghosal’s numbering system the carbon atoms of the structural skeleton are numbered in accordance to their location in the biological precursor, nonbelladine, heteroatoms and side chains are not numbered; this numbering system is used throughout this report, it is also the numbering system used in many scientific papers.\textsuperscript{23-24}
Of these alkaloids galanthamine is currently the most important; first isolated, from *Galanthus woronowii*, a variety of snowdrop native to eastern Europe, almost sixty years ago. A year before this isolation the Russian scientists Maskovsky and Kruglikova-Lvova demonstrated the acetylcholinesterase (AChE) inhibiting abilities of galanthamine, this was followed by *in vivo* studies a decade later, and twenty years after that galanthamine began to interest those looking for Alzheimer’s disease medications. However, not until 2000 was galanthamine finally licensed as a drug for the treatment of Alzheimer’s disease in the UK, this time lag was no doubt due to the reduced prevalence of Alzheimer’s disease in the slightly shorter lived population of that time and also to restrictions on the free movement of information between the West and the Soviet Union. There are two older reports of the use of snowdrops in the treatment of maladies, the oldest is a suggestion that the ‘molly’ referenced by Homer in the Odyssey might be a variety of snowdrop, and the other is a report made by a pharmacist who claims that locals of the Ural mountains used a concoction made from snowdrops as a remedy for poliomyelitis. However, there is very little evidence to support either of these claims. There is a general lack of evidence as to the early uses of any of the Amaryllidaceae family; this makes a full elucidation of the ethnopharmacology of galanthamine nigh impossible.

Since its first isolation and identification, galanthamine has been isolated from many members of the Amaryllidaceae family; one such member is *Narcissus pseudonarcissus* cv Carlton. Over 100 alkaloids have been reported to be present in extracts of *Narcissus*, a significant proportion of which have been found to be present in *pseudonarcissus*. Of these
other alkaloids, narwedine, 24, and the other alkaloids structurally related to galanthamine have raised the most interest, as they offer a starting point for the semisynthesis of galanthamine.

Table 1.1: Alkaloids based on the galanthamine skeleton

<table>
<thead>
<tr>
<th>Number</th>
<th>Chemical Structure</th>
<th>Alkaloid Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>R¹=OH, R²=H, R³=Me, R⁴=Me</td>
<td>galanthamine</td>
</tr>
<tr>
<td>17</td>
<td>R¹=H, R²=OH, R³=Me, R⁴=Me</td>
<td>epigalanthamine</td>
</tr>
<tr>
<td>18</td>
<td>R¹=OAc, R²=H, R³=Me, R⁴=Me</td>
<td>O-acetylgalanthamine</td>
</tr>
<tr>
<td>19</td>
<td>R¹=OH, R²=H, R³=H, R⁴=Me</td>
<td>norgalanthamine</td>
</tr>
<tr>
<td>20</td>
<td>R¹=H, R²=OH, R³=H, R⁴=Me</td>
<td>epinorgalanthamine</td>
</tr>
<tr>
<td>21</td>
<td>R¹=OH, R²=H, R³=CHO, R⁴=Me</td>
<td>N-formylnorgalanthamine</td>
</tr>
<tr>
<td>22</td>
<td>R¹=OH, R²=H, R³=Me R⁴=H</td>
<td>sanguinine</td>
</tr>
<tr>
<td>23</td>
<td>R¹=OH, R²=H, R³=Ac, R⁴=Me</td>
<td>narcisine</td>
</tr>
<tr>
<td>24</td>
<td>R¹=R, R²=O, R³=Me, R⁴=Me</td>
<td>narwedine</td>
</tr>
<tr>
<td>25</td>
<td>R¹=OH, R²=H, R³=Me, R⁴=Me, ΔΔ'= single bond</td>
<td>lycoramine</td>
</tr>
<tr>
<td>26</td>
<td>R¹=OH, R²=H, R³=H, R⁴=Me, ΔΔ'= single bond</td>
<td>norlycoramine</td>
</tr>
<tr>
<td>27</td>
<td>R¹=H, R²=OH, R³=H, R⁴=Me, ΔΔ'= single bond</td>
<td>epinorlycoramine</td>
</tr>
</tbody>
</table>

Adapted from Bastida et al.23

The most common methods for the identification of alkaloids in whole plant extract are GC-MS, LC-MS, and preparative and semi-preparative HPLC to yield enough for NMR and X-ray studies, although electrophoresis and other techniques have been reported to afford acceptable separation.26,31 However, the structural similarity between many of the alkaloids and the range of structural isomers makes any sure-fire identification without crystal structures and NMR quite difficult. Some of these problems can be overcome by using techniques such as MS-MS which yields a greater level of accuracy in the mass spectrum and increases the ability to identify fragments, thereby making it possible to
distinguish structural isomers. Another way around this is by successive column chromatography to isolate larger samples of the alkaloids.

### 1.4 Galanthamine; total synthesis vs. semisynthesis

As an effective competitive acetylcholinesterase inhibitor, with few side effects, galanthamine has attracted considerable attention in both industry and in academia; a fact clearly shown by the abundance of publications relating to it. The interest focuses on two areas; increasing the availability and production of galanthamine by total or semisynthesis, and using galanthamine as the starting point for derivatization in the search for superior AChE inhibitors.

![Scheme 1.2: The biosynthesis of galanthamine.](image)

The first total synthesis of galanthamine was reported by Barton et al. in 1962, the approach taken was biomimetic, with some steps giving yields of less than 2%. Since that time there have been numerous other methods reported. Many of the total synthesis reported take a biomimetic approach, which involves an ortho-para coupling of a belladine analogue. There is some dispute in the literature as to whether this is $O$-methylnorbelladine or $O,N$-dimethylnorbelladine. After the para-ortho’ coupling the remaining hydroxyl group attacks the dieneone to give narwedine or nornarwedine, which can then be reduced to give either galanthamine or norgalanthamine.
Some other groups avoided the biomimetic pathway due to the low yielding ortho-para phenolic coupling. These methods first link the two six membered rings via an ether linkage and then use palladium and silver catalysts to close the five membered ring. Such methods have been fairly successful with one reporting an overall yield of 63%. However, they have the disadvantage of using expensive palladium and silver catalysts. Sanochemia Pharmazeutika AG, filed their first patent for a total synthesis in 1995, and since that time have produced galanthamine for a subsidiary of Johnson and Johnson. They report a process, outlined in Scheme 1.3, of nine linear steps, with a variable overall yield in the region of 12%.

Briefly, 4,5-dimethoxybenzaldehyde, 30, is brominated, the resulting product, 31, is then regioselectively O-demethylated to give 32. This is then reacted with tyramine to give an imine, which is reduced to the norbelladine analogue, 33, with sodium borohydride. The norbelladine analogue is then N-formylated using ethyl formate to give 34. The ortho-para phenolic coupling of the N-formylated norbelladine analogue, 34, is accomplished using potassium hexacyanoferrate(III) and potassium carbonate. The resulting carbonyl is protected as a propylene glycol ketal, 36, allowing the N-formyl moiety to be reduced to N-methyl. This is followed by deprotection of the ketal to yield racemic narwedine, which is resolved via seeding the solution with crystals of the desired (-)-narwedine to give exclusively (-)-narwedine, 24. The narwedine is then reduced to (-)-galanthamine, 12, with L-Selectride, and the galanthamine is purified via crystallisation as the hydrobromide salt.

An alternative approach to the synthesis of galanthamine involves the use of phenyliodine(III)bis(trifluoroacetate) (PIFA) to afford the key ortho-para phenolic coupling, however, while giving good yields this approach requires trifluoroethanol to be used as the solvent.
1.4.a Methods for the resolution of narwedine

Due to the problems associated with the total synthesis of galanthamine there has also been some effort towards the semisynthesis of galanthamine, usually starting from the biological precursor narwedine, although most of this work has been carried out to facilitate the enantiomeric resolution of narwedine produced as part of biomimetic total syntheses. In solution narwedine readily undergoes a base catalysed isomerisation leading to a racemic
mixture; the resolution of this mixture has been achieved by several groups using a range of methods, aided by the conglomerate nature of narwedine.

The method reported by Shieh\textsuperscript{40} builds on the initial observation of Barton\textsuperscript{32} who noted that the recrystallization of narwedine in the presence of galanthamine resulted in an enantiomeric excess of the opposite enantiomeric form of narwedine. Barton reported that the enantiomeric excess was dependent on the amount of galanthamine present, requiring up to half an equivalent of galanthamine to give complete resolution, however, Shieh \textit{et al.} reported that total conversion could be achieved using only 1\% by mass of galanthamine and a suitable base, such as triethylamine to catalyse the interconversion.\textsuperscript{40}

\begin{center}
\begin{tikzpicture}
\draw[->,blue,thick] (0,0) -- (1.5,1.5) node[midway,above] {(+)-galanthamine};
\draw[->,green,thick] (1.5,0) -- (0,1.5) node[midway,above] {(-)-galanthamine};
\draw[->,red,thick] (0,0) -- (-1.5,-1.5) node[midway,below] {(-)-narwedine (24)};
\draw[->,red,thick] (0,0) -- (1.5,-1.5) node[midway,below] {(+)-narwedine (24)};
\end{tikzpicture}
\end{center}

\textbf{Scheme 1.4:} The galanthamine “catalysed” dynamic resolution of narwedine. An amount (0.5 – 0.01 equivalents) of optically pure galanthamine is added to a solution of racemic narwedine in ethanol and triethylamine.\textsuperscript{32,40} The addition of one enantiomer of galanthamine will result in the isolation of the opposite enantiomer of narwedine. Note that in both instances the phenolic oxygen attacks the same face of the achiral dieneone. a) L-selectride in tetrahydrofuran at -78 °C.

Barton also reported that transformation of the galanthamine O-methyl, and hydroxyl groups on the galanthamine used in the dynamic resolution process had little effect and that epigalanthamine, 21, and lycoramine, 25, could be used in place of galanthamine.\textsuperscript{32} Barton \textit{et al.} suggested that deposition of galanthamine on the surface of narwedine crystals of the corresponding enantiomer retarded further crystal growth, leading to a resolution towards
the opposite enantiomer. However, Shieh et al. were able to recycle the 0.01 equivalents of galanthamine in consecutive resolutions and stated this to be evidence incompatible with the theory of galanthamine adsorption instead suggesting the process was actually “a seeded (galanthamine-controlled), total, spontaneous resolution of enantiomers”. Shieh also dismissed the possibility of the one enantiomer of galanthamine preferentially increasing the solubility of one enantiomer of narwedine in solution by carrying out a series of NMR experiments with various mixtures of galanthamine and narwedine enantiomers, no matter the combination of enantiomers there was no change to the chemical shifts recorded.

Given the discrepancies in the accounts of the narwedine resolution it is not surprising that Chaplin et al. suggested that the kinetic transformation reported by Shieh et al. required the environment to be rigidly controlled to give good results and went on to suggest that a thermodynamically controlled process would be superior. The method they suggested took advantage of the narwedine amine to form a salt with a chiral acid, they reported that di-p-toluoyl-D-tartaric acid, gave the best results. However, rather than the 50% yield of salt they expected, they achieved much higher yields, taken as evidence of a “dynamic diastereomeric salt resolution”. The same method could also be used with only half an equivalent of acid. The narwedine was reduced directly from the salt with two equivalents of L-Selectride, where only half an equivalent of acid had been used the acid could be reclaimed after the reduction step. Interestingly they reported that during the screening for suitable acids they found that some acids did not form salts with the narwedine but instead prompted an enantioenriched crystallization of the narwedine.

![Figure 1.4: di-p-toluoyl-D-tartaric acid, 37.](image)

Shieh et al. also reported a more traditional entrainment method that affected enantiomeric resolution, from a supersaturated ethanol solution, by seeding with enantiomerically pure narwedine crystals, to give resolution to the same enantiomer as the seed. A method in line with that which seems to be used industrially. Using deuteriated water they were also able to confirm the mechanism of interconversion suggested by Barton; that being via a retro-Michael addition to give a dieneone, and then Michael addition to give narwedine again. Interestingly Barton et al. reported that seeding gave negligible resolution.
So far there is little published on the transformation of the other alkaloids to galanthamine.

1.5 Medicinal chemistry of galanthamine and galanthamine derivatives

1.5.a Acetylcholinesterase

The first crystal structure of AChE was for that isolated from Torpedo californica (an electric ray) and this was followed by others over the following decade until the crystal structure of human AChE was determined. AChE is considered a serine hydrolase enzyme, it is a quaternary protein, the subunits of which are held together by disulfide bridges, the protein is then attached to a collagen molecule, which itself is attached to two other AChE proteins and a region of cell membrane adjacent to a cholinergic receptor. As each subunit has an active site, this places twelve active sites close to each cholinergic receptor, reducing the likelihood of an acetylcholine molecule reactivating the same receptor or another receptor. AChE is considered to be one of the fastest and most efficient enzymes with a rate of hydrolysis close to being diffusion limited. The active site contains a catalytic triad, consisting of a nucleophilic serine, which is activated by the proximity of a glutamate residue, and a histidine residue assists with the movement of protons. Other serine hydrolase enzymes contain an aspartate residue instead of the glutamate. The mechanism of hydrolysis is outlined in Scheme 1.5.

Given that acetylcholinesterase is reported to hydrolyse acetylcholine at a rate of greater than $10^4 \text{ s}^{-1}$, it is therefore surprising that the active site is at the bottom of a channel, normally referred to as a gorge, approximately 20 Å deep. How substrate and products can enter and leave the active site so quickly is still not fully elucidated. The gorge is lined by a phenylalanine and two tryptophan residues. These typically hydrophobic residues form a cation-π charge transfer complex with the quaternary amine of acetyl choline, helping to stabilise the substrate in the active site.
Scheme 1.5: A representation of the active site of AChE, the side chains of the catalytic triad residues are shown. Stage 1; serine is activated and attacks the acetylcholine carbonyl. Stage 2; protonation and collapse of the anion back to the carbonyl cleaves the acetyl choline, eliminating choline. Stage 3; the carbonyl undergoes nucleophilic attack by the histidine activated water. Stage 4; reprotonation of the serine oxygen. Stage 5; the carbonyl collapses to form acetic acid, the enzyme acts as the leaving group, and is regenerated in this last step. Throughout the reaction the glutamate acts to stabilize the histidine.\textsuperscript{12}

Near the top of the gorge is an anionic site, actually a tryptophan residue, often called the peripheral anionic site (PAS) or the peripheral binding site.\textsuperscript{42,44} Another pertinent feature of the enzyme is the Ω loop, which in crystal structures of the enzyme can be seen to occlude access to, or egress from, the active site.\textsuperscript{45}
Figure 1.5: *Torpedo californica* AChE with the active site occupied by galanthamine, and a close up of the gorge. Green = galanthamine, yellow = PAS, red = Ω-loop, purple = catalytic triad, grey = rest of enzyme, images prepared with PyMol v 1.4.1, PDB ID 1DX6, X-Ray structure downloaded from the RCB PDB, 1DX6.

Figure 1.6: An alternative view of the active site of AChE, occupied by galanthamine, (hydrogens omitted for clarity). Green = galanthamine, purple = catalytic triad, grey = rest of the enzyme, images prepared with PyMol v 1.4.1; PDB ID 1DX6, X-Ray structure downloaded from the RCB PDB, 1DX6.

In an attempt to elucidate how substrates can enter, and the products then leave, the active site so quickly, Shi et al. used enzymes with cystine mutations to which the fluorophore acrylodan was attached. The quantum yield and the emission spectrum from acrylodan are highly influenced by the environment, with exposure to a more polar environment giving a bathachromic shift. From a comparison of the rate of the enzymes with non-mutants they were able to show that the method had little impact on the rate of enzymatic action. While from the observed changes in emission spectrum and quantum yield they were able to show that certain portions of the enzyme, namely the Ω loop, underwent considerable conformational change during the enzyme cycle.
1.5.b Acetylcholinesterase inhibitors; galanthamine derivatization and SAR

In addition to the work aimed at an effective mass production of galanthamine (vide supra), there have been numerous attempts to enhance the efficacy of galanthamine by means of derivatization. The aim of this work can be divided into two main areas; the first being simple attempts to improve its bioavailability and IC50 values through simple modification. While the second is to increase its effectiveness as an inhibitor and to give it a second action of inhibiting AChE catalysed amyloid-β formation. Although there is considerable overlap in both of these areas.

In vitro studies have shown that the O-demethylgalanthamine, more commonly called sanguinine, is a far more potent inhibitor of AChE than galanthamine; about 3 – 10 times that of galanthamine (the discrepancy is likely due to differing origins of the AChE used for the assays). However, sanguinine has several serious drawbacks when used in vivo, the increase polarity of the molecule leads to an unfavourable change in logP. Additionally, in vivo studies by Bachus et al. have shown that sanguinine is glucuronidated almost instantly and then almost immediately excreted preventing it from having any biological activity. This leads to the observation that the best way of increasing the inhibiting power of galanthamine is to increase its polarity, while the best way of increasing its ability to enter the CNS is to reduce its polarity. The directed modification of galanthamine is complicated by several observations in the literature. Firstly there are a multitude of variants of AChE though-out nature, from invertebrates to vertebrates, with even AChE from closely related sources showing differences. The differences between the exact natures of the AChE can lead to significantly different interactions with a single AChE inhibitor. This is well demonstrated in the study by Greenblatt et al. in which a range of galanthamine inhibitors were screened against both the Electrophorus electricus AChE (EeAChE) and Torpedo californica AChE (TcAChE). The imine equivalent of galanthamine, gave values varying from 0.4 for EeAChE to 1.7 for TcAChE, other imine based derivatives also gave large differences in performance, while another did not give such a large difference.

Such differences in the various forms of AChE show the importance of screening a potential inhibitor against carefully selected variants of AChE. In the same study Greenblatt et al. found that some galanthamine derivatives that should not fit into the active site of AChE due to the presence of large groups on the O-phenyl position were accommodated owing to unpredictable flexibility in one specific section of the enzymes amino acid back bone. Findings such as this clearly illuminate the limitations of in silico studies in this field.
Other groups have screened a range of other structural analogues of galanthamine. Treu et al. synthesised a sulphur analogue, 39, of galanthamine which failed to show any inhibition of AChE, this was taken as an indication that the amine is vital for activity.\textsuperscript{51}

![Figure 1.7: Examples of modifications made to galanthamine for SAR studies.\textsuperscript{50-52}}](image)

In order to determine the pharmacophore of galanthamine, Han et al. systematically altered the structure of galanthamine by modifying the methoxy, hydroxyl, amine, and alkene moieties in turn. In this way they were able to show that the overall structure of galanthamine is required for the desired biological activity.\textsuperscript{52} Hydrogenation of the alkene was shown to have a very detrimental effect on the biological activity.\textsuperscript{52}

In another study on the modification of the N-methyl group, Jia et al. attached, amongst others, benzyl piperidine groups.\textsuperscript{53} The use of the six carbon linker and the benzyl piperidine gave a galanthamine derivative, 40, shown to be more than two hundred times as effective as galanthamine at inhibiting AChE.\textsuperscript{53} Conversely a two or three carbon linker resulted in attenuated activity relative to galanthamine.\textsuperscript{53} They reported that \textit{in silico} docking studies indicated that the phenyl ring interacted with the tryptophan moiety of the PAS, the amine nitrogen and the alkyl ring were also both important for activity, other alkyl groups were either too bulky or too small to give the necessary hydrophobic interaction.\textsuperscript{53}

While not easy to see in Figure 1.5 and Figure 1.6, more careful inspection of the crystal structure, of galanthamine docked in the active site, seems to suggest that the positions at C6, C7 or C8 offer a site better orientated for pendent groups to be directed up the gorge, towards the PAS, than the site offered by the amine nitrogen, a view confirmed by others.\textsuperscript{54}

Another potential advantage posed by the bivalent ligand approach is that it should allow the use of larger hydrophobic groups which should increase the lipophilicity of the galanthamine derivative and thereby increase its penetration of the central nervous system.
It has been shown by Bartolini et al. that the PAS is responsible for catalysing the transformation of soluble amyloid protein to insoluble amyloid-β protein, which then precipitates to form plaques.\textsuperscript{55} Therefore forming a second interaction at the PAS via bivalent ligands and taking advantage of the chelate effect, offers another potential advantage that it may also inhibit the action of this site on amyloid protein.

Surprisingly, few of the bivalent AChE inhibitors synthesised are examined for their ability to inhibit this non-classical action of AChE.

More recently potential drug molecules have been designed to do both of the aforementioned tasks as well as inhibit fibril formation in solution, independent of the presence of the AChE enzyme, and act as anti-oxidants.\textsuperscript{56} This type of design strategy is call “multitarget-direct ligand design strategy” and it has been suggested that in the treatment of diseases for which there is no clear disease pathogenesis such drugs offer a far better potential for successful remediation, some such drugs are reportedly in clinical trials.\textsuperscript{56}
2 A brief introduction to selected topics of lithiation chemistry

2.1 Commercially available organolithiums and general properties of organolithiums in solution

2.1.a The commercial availability of organolithiums

There are a couple of dozen organolithium reagents commercially available today, of which arguably the most common are methyllithium, phenyllithium, \textit{n}-butyllithium, \textit{sec}-butyllithium, and \textit{tert}-butyllithium.\textsuperscript{57} Many organolithiums have reasonable solubility in alkane solvents, indeed \textit{n}-butyllithium, \textit{sec}-butyllithium, and \textit{tert}-butyllithium are all soluble and have reasonable levels of stability in alkane solvents, while the other two aforementioned organolithiums are less soluble and require either diethyl ether or tetrahydrofuran to be added to solubilize them.\textsuperscript{58} The reactivity of organolithiums varies considerably, with the less reactive \textit{n}-butyl simply fizzing upon contact with air while the more reactive \textit{tert}-butyllithium is highly pyrophoric, busting instantly into flame upon contact with air.

![Figure 2.1: The more common commercially available organolithiums. Methyllithium, phenyllithium, \textit{n}-butyllithium, \textit{sec}-butyllithium, \textit{tert}-butyllithium.]

2.1.b The stability, aggregation and interaction with solvents of standard commercially available organolithiums

A carbanion by itself cannot stabilise the lithium ion, therefore, in the absence of any other stabilizing groups, such as in the alkane solvents in which the butyllithium series of organolithiums is commercially supplied, the organolithiums form aggregates.\textsuperscript{58} \textit{n}-Butyllithium forms hexamers, while both \textit{sec}-butyllithium and \textit{tert}-butyllithium form tetramers.\textsuperscript{59} The hexamer aggregation state corresponds to an arrangement of lithium ions at the points of an octahedral with the carbanions taking bridging positions, while the tetramer aggregation state corresponds to an analogous arrangement but with an overall tetrahedral geometry.\textsuperscript{59} The bonding in the aggregate is best thought of as a combination
of delocalised molecular orbitals encompassing the polyhedral structure, however, conceptually it is better understood using a model in which each carbanion interacts with three lithium ions, with each interaction containing only two electrons. Dimers are an additional common aggregate. NMR experiments on organolithiums have shown that the exact extent of aggregation and the rate of dissociation in any given situation is determined by a number of factors including; steric constraints, the presence of any other groups that can stabilize the lithium ions, and the temperature. Even under low pressure and at temperatures in excess of 100 °C mass spectra show that organolithiums remain in an aggregated arrangement.

Using $^7$Li NMR to study a mixture of the tetrameric species tert-butyllithium and trimethylsilylmethylithium, Brown was able to demonstrate that changing the solvent from cyclopentane to toluene resulted in an increase in the rate of dissociation of about 20 times. Hartwell and Brown were also able to show that the intramolecular exchange was dependent on temperature and that the rate of exchange could go from fast to slow, relative to the NMR time scale, over a 20 °C range and that once again solvents could effect this, with solvents such as toluene better able to stabilize intermediates compared to simple alkanes, resulting in a depression of the temperatures required to notably slow the intramolecular exchange. They note that the rate at which organolithiums initiate reactions is far higher than the rate of disassociation and suggest that the reagents interact with the organolithium aggregate forming an intermediate that may bear resemblance to the intermediate state during disassociation.
McGarry and Ogle were able to use NMR to show that in tetrahydrofuran $n$-butyllithium exists as a mixture of dimer and tetramer aggregates. Others used NMR to show that at sufficiently low temperatures, ca. -100 °C, in tetrahydrofuran $sec$-butyllithium and $tert$-butyllithium, severally, exist as a mixture of dimers and monomers. As well as the NMR evidence for the aggregated state of organolithiums there is also crystallographic evidence for the aggregation of some organolithium species in the solid state. The aggregation is an important factor in considering the reaction conditions, as the addition of solvents that lower the degree of aggregation leave the lithium more available to take part in reactions.

Solvents suitable for organolithium reactions are those that contain either ether or tertiary amine moieties. In such groups the lone pairs, of the oxygen or nitrogen, can aid with the stabilization of the lithium ion thereby facilitating a reduction in the aggregation state. It is thought that the attenuation of the aggregation is achieved by the gradual solvation of the polyhedra by solvent molecules approaching the aggregates at the apexes of the polyhedral structures (see Figure 2.2). Clearly this solvated dissociation of the aggregates requires the organization of solvent molecules and is therefore entropically unfavoured. The increase in the reactivity of organolithiums, arising from lower aggregation states as a result of changes in solvent compositions is strikingly exemplified by two reactions. In the first reaction dibenzofuran is lithiated and then subjected to carbonation and hydrolysis to give 4-dibenzofuran carboxylic acid, if diethyl ether is used as the solvent then only very low yields of the acid are formed even at slightly elevated temperatures, while if tetrahydrofuran is used as the solvent then the yield is more than doubled even at much lower sub-zero temperatures. In the second reaction, the treatment of benzene with $n$-butyllithium followed by carbonation and then hydrolysis, using benzene as the solvent results in little if any product, the addition of $N,N,N',N''$-tetramethylethylenediamine (TMEDA), to the lithiation step of the same reaction, results in consistently high yields of benzoic acid.

Suitable solvents for use with organolithiums include $N,N'$-dimethylpropyldeneurea (DMPU), 1,2-dimethoxyethane, TMEDA, tetrahydrofuran, $tert$-butyl methyl ether, and diethyl ether and others. Other more unusual and toxic solvents such as $N,N,N',N'',N'''$-hexamethyphosphoramid, $N,N,N',N''$, $N'''$-pentamethyldiethylenetriamine and sparteine can also be used. It is also possible to combine suitable solvents either for reasons of reagent solubility, economic or environmental reasons. For example the use of tetrahydrofuran with TMEDA or with the highly toxic DMPU allows for the use of the relatively inexpensive tetrahydrofuran as the main solvent while taking advantage of the greater activating ability of the far more expensive amine or urea.
The highly basic nature of the organolithium reagents means they can readily attack and decompose solvents, therefore it is necessary to perform many of the reactions at lower temperatures. The organolithiums are generally more stable in alkane solvents compared to ethereal solvents. For example in tetrahydrofuran the half-life of tert-butyllithium at -20 °C is only 45 minutes and in diethyl ether it is 8 hours, while for n-butyllithium the half-life, in tetrahydrofuran, but at 35 °C is 10 minutes; tert-butyllithium is only ideally used in tetrahydrofuran at temperatures below -30 °C. These half-lives are in contrast to those for the same organolithiums in alkane solvents (such as pentane, cyclohexane, and hexane) in which the reagents are sold and can be stored, at room temperature or in a fridge, for extended periods of time. Jung and Blum found that acetylaldehyde could be synthesised from tetrahydrofuran simply by adding n-butyllithium at room temperature, this gives an indication as to the mechanism for the loss of organolithium in said solvent; that being via hydrogen abstraction, followed by a reverse \([\pi_2 + \pi_4]\) cycloaddition. Clearly for reactions involving organolithiums the choice of solvent is an important consideration, both for the solvent’s impact on aggregation and for the compatibility of the solvent with the organolithium to be used at a temperature that will allow for sufficient solubility of the other reactants to be used.

### 2.2 Lithiation by proton abstraction

While many metals will take part in metation reactions at the site of acidic hydrogens, lithium can give different selectivity even to other group 1 metals and therefore deserves further consideration. An example of the differences in selectivity is outlined in Scheme 2.1.

![Scheme 2.1](image)

Scheme 2.1: The contrast in the reactivity of two group one metals. a) Trimethylsilylmethylpotassium in THF at -78 °C (the site of metation can be determined by trapping with methyl iodide). b) n-Butyllithium with TMEDA in hexane at 25 °C (the site of metation can be determined by trapping with benzophenone).
2.2.a Considerations for the successful lithiation of a compound

The successful lithiation of a compound, by way of deprotonation, is broadly determined by a number of key factors, these include; the proximity of the site to be lithiated to an electron withdrawing group or other functionality that can stabilise the high electron density on the carbon in a carbon-lithium bond, the presence of any heteroatoms in a position that allows them to coordinate with and stabilise the lithium ion, and the acidity of the hydrogen at the position to be lithiated. While it may first appear that the acidity is the most important factor the stabilisation of the lithium ion by the other two considerations can outweigh the significance of the acidity.

That the most acidic, that is the most labile proton, is the first place to be lithiated when treating a compound with an organolithium is clear. However, the impact of coordination and stabilization by local groups is less straightforward.

2.2.b Stabilization by intramolecular coordination

The role of coordination in lithiation reactions has been well studied, with such work demonstrating the clear and important implications not only with regards to the site to be lithiated but also with regards to the final stereochemistry of the products.

Schleyer et al. undertook in silico studies and were able to present collaborative experimental data that showed that in the case of lithiation adjacent to amides the lithium ion forms a complex with the carbonyl oxygen lone pair leading to a highly regiospecific reaction. They demonstrated this using piperidin-1-yl(2,4,6-triisopropylphenyl)methanone and then 2,4,6-triisopropylbenzamides, in the first instance (Scheme 2.2) the piperidine ring is lithiated and then functionalised in an equatorial position, which is attributed to regioselective lithiation arising from coordination between the lithium ion and the carbonyl oxygen. This syn introduction would be energetically less favoured compared to the alternative anti configuration, as the anti-arrangement would give access to the σ* orbital of the C=O bond allowing for a bonding interaction to lower the electron density on the carbanion, however, the coordination makes up for the energetic disparity. Due to the limited rotation of the amides, arising because of the severe steric hindrance of the isopropyl groups, it is possible to isolate single enantiomers of the functionalized products.
They also pointed to the work of others that indicated the same process was occurring and was therefore more generally applicable to the $\alpha$-lithiation of amides and was not a one off occurrence. Schleyer et al. suggested that there were two possible paths by which this regioselectivity could arise, the first involving a rate determining precomplexation of the $\text{sec}$-butyllithium with the carbonyl, the alternative route being the deprotonation and subsequent stabilisation of the syn lithiated product. More recent work by Meyers and Dickman has shown that in the $\alpha$-lithiation of amides lithiation does not display a kinetic isotope effect, which supports the theory that the rate determining step is precomplexation of the butyllithium with the amide carbonyl followed by the deprotonation and lithiation. This precomplexation prior to reaction has been called the “complex induced proximity effect” and there have been extensive attempts to fully elucidate the mechanism surrounding this perceived effect. Beak investigated this by comparing the intramolecular and intermolecular kinetic isotope effects on the lithiation of benzyl-$N,N'$-dimethyl urea (Scheme 2.3). They measured an intermolecular kinetic isotope effect that was only about a quarter of that determined for the intramolecular kinetic isotope effect. This difference in the kinetic isotope effects measured for the two experiments indicates two things; firstly that the reaction proceeds via at least two steps, otherwise both reactions would give the same kinetic isotope effect, and secondly that the complexation and deprotonation steps have competitive rates, otherwise the intermolecular kinetic isotope effect would have been measured as 1, indicating that the complexation step was rate determining.
Further studies of the kinetic isotope effect have been undertaken by others and have shown slightly mixed results depending on the exact substrate to be lithiated. Indeed in the same paper described above Beak et al. present the results of kinetic isotope experiments that show that in the case of ortholithiation there is no clear result leading them to suggest that in the absence of data to the contrary, but where there is precedent of precomplexation, it is reasonable to assume that there is a precomplexation step in the reaction mechanism.

2.2.c Influences of local functional groups and heteroatoms

Local functional groups may influence the success and location of lithiation, either by influencing the acidity of protons at a given site or by means of inductive and mesomeric effects and interference from lone pairs of electrons. This sort of interference is typified by lithiation adjacent to oxygen and nitrogen. In both cases the favourable influence of the electronegativity of the heteroatom is outweighed by the unfavourable interaction of the heteroatom lone pairs with the carbon-lithium bond, resulting in a destabilization of the organolithium. The impact of the lone pairs can be greatly attenuated if they can be lowered in energy by being delocalised into a neighbouring group, such as a carbonyl. Good examples of this are as in the case of lithiation adjacent to amides (see Scheme 2.2), esters and carbamates. As discussed in the previous section carbonyls can also coordinate with the lithium ion making them doubly helpful in lithiation chemistry. Other groups that present interesting positions for lithiation are imines, allyl carbamates, benzylic positions and ureas (see Scheme 2.3). There are many other positions and functional groups that can also have an impact on the lithiation, but they lie outside the scope of this report.
Hoppe reports that allyl carbamates are readily lithiated using $n$-butyllithium at low temperatures in alkane solvents and that the resulting organolithium can be quenched with aldehydes or ketones to give a range of interesting products.\textsuperscript{81} Whether the product is functionalised at the $\alpha$ or $\gamma$ position is largely dependent on the initial substitution of the alkene moiety of the allyl carbamate, with those already more substituted at the $\gamma$ position liable to undergo further substitution at this position.\textsuperscript{81} Hoppe also reports that it is important to use only carbamates with a hindered carbonyl, such as the $N$-diisopropyl carbamate, or there is a possibility that the lithiated allyl carbamates will attack the carbonyl of other unlithiated allyl carbamates to give the nucleophilic addition product, 63.\textsuperscript{81} Hoppe also points out that when lithium diisopropylamide (LDA) is used as the base the secondary amine can reprotonate the lithiated allyl carbonate resulting in only a rearrangement of the position of the double bond, 59.\textsuperscript{81} These reactions are summarised in Scheme 2.4. Hoppe’s work also shows that the chemistry is viable on systems in which the alkene moiety is part of a five or six membered cyclic system.\textsuperscript{81}

![Scheme 2.4](image)

Scheme 2.4: A summary of some lithiation reactions for allyl carbamates. E = electrophile, R = isopropyl. a) LDA, in THF/hexane at -78 °C. b) $n$-butyllithium, TMEDA, in ether/hexane. c) Quench with a suitable electrophile. d) If R = methyl, addition to unlithiated allyl carbamate can occur, which can be trapped by the addition of a suitable electrophile.\textsuperscript{81}

Other carbamates can be lithiated $\alpha$ to the nitrogen. One such pertinent example is the use of the Boc protecting group to direct $N$ $\alpha$ lithiations, as initially reported by Beak and Koo Lee, this method is especially useful as the destabilising effect of amines towards $\alpha$ lithiation usually prevents lithiation in such positions, while the easily installed and removed Boc group effectively reverses this thereby giving access to functionalised amines
that might not otherwise be easily synthesised.\textsuperscript{82} This methodology has good general applicability, being useful on a range of amines from dimethyl amine through to cyclic structures, including $N$-Boc-perhydroazepine systems.\textsuperscript{82,84} Their work strongly indicates that once again the reaction mechanism proceeds via a carbonyl-lithium complex, with 2,4-substituted piperidines assuming a twisted-boat conformation to allow the equatorial lithiation at the 6 position and carbonyl-lithium complex stabilisation.\textsuperscript{84}

As well as the influence of adjacent functionality, more remote groups can also influence the likelihood of successful lithiation; for example favourable arrangement of acidifying groups on an aromatic ring can help to stabilise an organolithium (\textit{vide infra}).

\textbf{2.2.d The conflict between different factors that determine the regioselectivity of lithiation reactions}

It is not always possible to determine the exact position at which lithiation will occur in a molecule without some degree of practical experimentation. While, due to the favourable combination of the influencing factors discussed above or because of structural simplicity, many molecules will clearly exhibit a single position that is the favoured site of lithiation, in more complex molecules this may not be the case. For example; an aromatic structure may have functional groups at different positions about the ring, with groups offering different degrees of stabilization by different mechanisms. In such situations it may not be possible to determine the site at which lithiation will occur without experimentation. Further to this it is worth remembering that in some instances the nature of the lithiating agent or the temperature of the reaction can be sufficient to change the position at which lithiation by deprotonation occurs; there are several examples of this in the literature.\textsuperscript{85} In one such example Beak and Brown demonstrate that lithiation of $N,N$-diisopropyl-$p$-toluamide can yield either the ortholithiated product or the methyl lithiated product depending on the lithiating conditions.\textsuperscript{86} At lower temperatures using the \textit{sec}-butyllithium
and TMEDA ortholithiation is observed, however, at higher temperatures and using the weaker base LDA the methyl group is lithiated.\textsuperscript{86} Beak and Brown suggested that this difference in the product arises because at the higher temperature the energetic advantage of complexation is lost leading to the formation of the thermodynamic product rather than the kinetic product.\textsuperscript{86}

![Scheme 2.6](image)

**Scheme 2.6:** Examples of the changing site of lithiation, by proton abstraction, arising from changes in lithiating agent and other reaction conditions. a) sec-butyllithium and TMEDA in tetrahydrofuran at -78 °C, then quenched with deuterated methanol. b) LDA in THF at 0 °C, then quenched with deuterated methanol.\textsuperscript{86}

### 2.3 Lithiation chemistry and galanthamine

In terms of the current work there are a number of sites on galanthamine that offer potential sites for modification via various lithiation methodologies; all of which start with lithiation via deprotonation. Given the structure of galanthamine there are two sites that could undergo ortholithiation, the C7 and C8 positions on the aromatic ring. The position of the methoxy group at the C9 position makes the C8 position the most obvious target. Successful introduction at this position could potentially facilitate the functionalization of the C7 position. Given the ring already has a methoxy group present and there are already established methods for the demethylation of this group to reveal the hydroxyl, a suitable starting point is with the use of oxygen based directing groups for the ortholithiation reaction.
E. W. D. Burke

Figure 2.3: Galanthamine (12); the carbon positions that could be most easily functionalized by means of lithiation chemistry are indicated.

The C6 position, which is both benzylic and adjacent to an heteroatom, offers an additional site that could be functionalised by deprotonating lithiation. Two methods that could be used are either modification of the amine to a carbamate or urea, followed by lithiation and then quenching the galanthamine organolithium with a suitable electrophile or alternatively using an intramolecular aryl rearrangement. Likewise the allylic hydroxyl could be modified by means of an intramolecular aryl rearrangement or carbamate directed lithiation. These methods are briefly discussed in the following sections.

2.4 Ortholithiation reactions

Ortholithiation is a form of directed lithiation in which an hydrogen ortho to a functional group is replaced by a lithium ion. The resulting organolithiums can then either rearrange, as in the case of O-carbamates undergoing the ortho-Fries rearrangement, or can be quenched with a suitable electrophile to afford a functionalized product.  

![Scheme 2.7](image_url)

Scheme 2.7: A general scheme outlining the ortholithiation reaction. In the ideal situation R is a group that is capable of complexing with the lithium ion both before and after lithiation and also increases the acidity of the ortho hydrogen.

As expected from the outline of deprotonating lithiation reactions given above, ortholithiation is influenced by several factors; the acidity of the hydrogen to be replaced and the ability of the ortho functional group to stabilize the lithium ion by being both electron withdrawing and by its ability to coordinate with the lithium ion, both before and
after deprotonation/lithiation. However, there is not yet a consensus as to the overriding importance of one or other of these two points. The influence of other factors such as the choice of solvent and the aggregation of organolithiums has already been discussed and such considerations remain salient for ortholithiation reactions.

2.4.a The role of directing groups in ortholithiation reaction mechanisms

In contrast to the role of neighbouring groups in other lithiation reactions (cf. section 2.2.c) simple groups containing nitrogen or oxygen heteroatoms can be used as directing groups. However, just as in other lithiation reactions the inclusion of a carbonyl increases the directing ability of the group, so carboxamide groups, such as secondary and tertiary amides and carbamates, are stronger directing groups than simple ether or amine directing groups. The increased directing capability most likely arises from a combination of the strong complexing ability of the basic carbonyl oxygen and the increased acidifying effect of the group as a whole, which arises from a reduction of any mesomeric electron donation and an increase in inductive electron withdrawing capabilities.

The literature contains some discrepancy between the importance of inductive electron withdrawing effects and precomplexation stabilization on the mechanism of ortholithiation reactions. Discrepancies in observed regioselectivities and rates of lithiation have led some to question the role of complexation in at least some ortholithiation reactions. Collum et al. have looked at the role of methoxy groups in ortholithiation reactions and compared this with the work of others to suggest that in such cases the mechanism does not require the formation of a complex between the ortho methoxy group and the lithiating agent. Instead, they suggest that the lithiation is directed solely by the acidifying effect of the inductively electron withdrawing methoxy group and go on to support their theory with in silico studies used to determine activation enthalpies, the results showing that the precomplexation is not required for the directed lithiation. In place of a mechanism that is dependent on precomplexation, Collum et al. propose a mechanism that proceeds via a triple ion and that is dependent on the strength of the inductive effects of the substituents as published by others. In this way the rate of reaction remains dependent on the substituents, while the mechanism is independent of the substituents.
It should be pointed out that others have undertaken NMR analysis of mixtures of n-butyllithium and aryl ethers (namely 1-methoxynaphthalene, 1-methoxy-2-phenoxymethane, and anisole) in hexane and were able to show a change in the chemical shift of the protons $\alpha$ to the n-butyllithium lithium upon changing concentrations of ether. However, the initial studies in this area were shown to be only measuring solvent effects and the studies on 1-methoxy-2-phenoxymethane leave space for further investigation.

Collum et al. do not provide an alternative explanation for the NMR data collected by others.

Experiments by Schlosser et al. on the impact of various degrees and arrangements of fluorine and methoxy substitution on benzene show that the situation is in fact yet more complicated, as the simple addition of acidifying substituents does not result in a simple accumulative effect on the rate of lithiation. Both the number of substituents and their arrangement can have either a positive or a negative influence on the ease with which an aryl compound can be lithiated. For example; although anisole undergoes lithiation almost a thousand times faster than benzene, the addition of a second or third methoxy will have only a minor or even negative impact on the rate of lithiation. A methoxy group positioned para to the site to be metalated (e.g. 1,2,3-trimethoxybenzene, 83) will result in an attenuated rate of lithiation relative to anisole (once the rates are corrected for the number of available lithiation sites the rate is fractionally better, but not as would be expected for a simple additive effect arising from the presence of more methoxy groups).
The reason for this is most likely due to the mesomeric donation of electron density into the aryl \( \pi \)-system resulting in accumulation of electron density at the para position, while the inductively electron withdrawing effect acts over a far shorter distance and so cannot counteract this accumulation of electron density.\(^9^2\) 1,2-Dimethoxybenzene, 82, may have a lower rate of lithiation due to steric interactions between the two methoxy groups requiring that they are orientated in such a way as to reduce the availability of their lone pairs for complexation with the lithium ion, the same is most likely the case with 1,2,3-trimethoxybenzene, 83, with the added disadvantage of a para positioned methoxy group.\(^9^2\) Collum \textit{et al.} also state that in the case of other types of directing groups, most notably carboxamides, they cannot so readily dismiss the importance of complex-induced proximity effects between the directing group and lithiating agent.\(^8^7\)

In general terms the alternative mechanism utilizing complex-induced proximity effects was discussed in section 2.2 and more specifically section 2.2.b. Where directing groups have more obvious means for coordinating interactions with the lithium ion the points made in said sections remain salient in the discussion about the mechanism of ortholithiation reactions.

The literature contains many examples where the regioselectivity of an ortholithiation reaction is dependent on the reagents and conditions of the reaction rather than the substrate and the phrase “optional site selectivity” is used to describe such situations.\(^7^4\) Such examples are, in analogy to the cases reported by Beak \textit{et al. (vide supra)}, best described in terms of kinetic and thermodynamic reaction pathways. One of the oldest know examples of optional site selectivity is in the ortholithiation of (4-methoxybenzyl)dimethylamine, 86, lithiation using unactivated \( n \)-butyllithium in diethyl ether – hexane solvent results in lithiation ortho to the amine containing substituent, however, when the reaction is performed using the same conditions but using \( n \)-butyllithium activated with TMEDA the position ortho to the methoxy group is lithiated in
preference to the alternative position with a regioselectivity of 7.8:1. In both instances the lithiated product was trapped with benzophenone.

Scheme 2.9: Optional site selectivities for the ortholithiation of (4-methoxybenzyl)dimethylamine. a) $n$-Butyllithium in diethyl ether and hexane at 27 °C. b) $n$-Butyllithium with TMEDA in in diethyl ether and hexane at 27 °C.

In this reaction it is thought that the deaggregated and therefore more reactive, $n$-butyllithium attacks the more acidic and kinetically labile aryl hydrogen position first. While in the instance of the unactivated and more aggregated $n$-butyllithium there is more time and indeed necessity for precomplexation prior to hydrogen abstraction; precomplexation not only directing the lithiation but also most likely activating and deaggregating the $n$-butyllithium and thereby assisting the progress of the reaction. Such chance discoveries support the theory that ortholithiation proceeds by more than a single mechanism and also serve to demonstrate the less straightforward nature of deprotonating lithiation reactions in general.

The previous example contrasts favourably with the reaction of anisole with $n$-butyllithium in which the addition of even only a substoichiometric amount of TMEDA results in a massive increase in the rate of reaction. Slocum et al. explain this as a change in the mechanism of reaction; from one in which precomplexation is required to attenuate the mesomeric electron donation from the oxygen into the $\pi$-cloud of the aryl ring thereby increasing the acidity of the ortho hydrogens, to a reaction mechanism in which the more active $n$-butyllithium is able to lithiate the ortho position by means of an “overriding base” mechanism.
Scheme 2.10: Electron donation from the oxygen into the aryl π-cloud is attenuated by a coordinative interaction between the oxygen and the lithium of the n-butyllithium, this leads to acidification of the ortho hydrogens, which in turn allows for metalation of the ortho position.\textsuperscript{94}

With other substituents, capable of more aggressive coordination than simple ethereal groups, such as those that include carbonyl groups, there is still a lack of definitive evidence as to the mechanism of reaction. Kinetic isotope effect experiments having failed to produce clear cut evidence (see section 2.2.b). However, given the existence of optional site selectivities in ortholithiation reactions and the evidence (\textit{vide supra}) and importance of precomplexation in other ortholithiation reactions, it is accepted that in cases where there is precedence for precomplexation and an absence of evidence to the contrary that the role of precomplexation within the reaction mechanism should be accepted.\textsuperscript{58, 80}

2.4.b Directing groups for ortholithiation

Although a definitive reaction mechanism has not been elucidated for ortholithiation reactions, the relative strength of various directing groups has been vigorously investigated by various groups and tables of comparative directing strength have been compiled.\textsuperscript{58} The reliability of such tables is at a level that is synthetically useful, but remains limited by the existence of optional site selectivity and the vast array of reaction conditions that could be employed.

Although anisole undergoes lithiation far more readily than benzene, the methoxy group is considered to be, at best, a mediocre directing group.\textsuperscript{95} The principle redeeming feature of this small group is that it can direct lithiation to a crowded position on the ring, thereby giving access to 1,2,3,-functionalized ring patterns; this can be especially useful to bias the position that is lithiated if the group meta to the methoxy is a powerful directing group.\textsuperscript{58, 96}

As noted above the precise mechanism is still debated and could simply arise from increased acidity at the ortho position between two meta arranged directing substituents or could arise from an increase in the complex induced proximity effect arising as a result of some degree of chelation.\textsuperscript{87, 97} However, it should be noted that the 1,2,3-arrangement of substituents is by far the favoured product, showing that there is at least some accumulative action no matter the mechanism.
Scheme 2.11: Regioselective lithiation of a 1,3-disubstituted aryl to yield a crowded 1,2,3-trisubstituted aryl. Possible complexation and chelation interactions are shown.\textsuperscript{97} a) sec-Butyllithium in THF at -78 °C. b) Quench with an electrophile, e.g. carbon dioxide.\textsuperscript{92}

As a directing group methoxymethyl ether represents a significant improvement over the methoxy group, this is normally attributed to the ability of the additional oxygen atom to better coordinate the lithium ion thereby stabilizing the resulting intermediate structure.\textsuperscript{95}

Scheme 2.12: Stabilization of a lithium ion by the methoxymethyl ether directing group. a) tert-Butyllithium in pentane at 0 °C. b) Quench with 1-chloro-2-iodoethane (or other suitable electrophile).

Sometimes this is even shown as a chelation involving coordination of both oxygens\textsuperscript{95, 98}, however, this is perhaps less probable due to the angles involved in such an arrangement. Although it should be noted that this complexation mechanism has been contested by some who proponent that the increased directing ability arises solely from an enhanced acidifying effect on the ortho hydrogen.\textsuperscript{97} Earlier publications appeared to show that the ortho selectivity of the methoxymethyl directing group was significantly less than ideal, however, in later work Winkle and Ronald were able to demonstrate some exciting tuneable regioselectivity by means of careful solvent and lithiating agent selection.\textsuperscript{95, 99}
In fact, in the example reactions (Scheme 2.13), both reactions gave one or two percent of the other regiosiomer and the reaction done in hexane solvent gave only half the yield of the other reaction. This is not unexpected given the inability of the hexane solvent to deagregate the n-butyllithium (*vide infra*). This regioselectivity is explained in terms of the coordinating ability of the solvent; when a stronger coordinating solvent is used the weaker directing group (in this example the dimethylamine group) acts mainly as a bulky group providing steric hindrance for the bulkier tert-butylithium. However, in the non-coordinative hexane solvent the weaker group also interacts with the n-butyllithium giving rise to substitution at the 2 position.

Overall the methoxymethyl directing groups is of intermediate directing capability, being sufficiently strong that it can direct lithiation to ortho ring positions and away from other sites in the molecules that may otherwise be subject to nucleophilic attack if a less powerful directing group were used. The methoxymethyl ether has the added advantage that it can be removed by simple acid hydrolysis.

**O-Carbamates** and both secondary and tertiary amides, often described as O+N type directing groups, are amongst the very strongest directing groups and generally only require relatively mild conditions to ensure successful lithiation. For carbamates the strength of the directing ability arise from the combination of an oxygen adjacent to the ring which can inductively extract electron density form the ring leading to an increased acidity of the ortho hydrogens, in addition to this the oxygen lone pair will be less available to donate electron density into the π-cloud of the ring due to the presence of the adjacent carbonyl moiety. The carbonyl is a strong Lewis base and can successfully coordinate with the lithium to direct the lithation. The amide acts in an analogous manner, minus the benefit of the oxygen adjacent to the ring system.

In addition to typical ortholithiation/electrophile quench reactions the carbamate can undergo an anionic ortho-Fries rearrangement to give salicylamides. This 1,3-migration
of the amide fraction of the carbamate has some valuable uses as it allows for restoration of a masked phenol group and the introduction of an amide, which itself can be used in further ortholithiation reactions.\textsuperscript{102} The \(N, N\)-dimethylcarbamate is unstable and will migrate upon lithiation of the ring, while the \(N, N\)-diethylcarbamate is more stable and will allow for standard ortholithiation at lower temperatures but upon warming to room temperature will rearrange.\textsuperscript{101} There are examples where the carbamate has been used to successively functionalise an aromatic group; such as in the synthesis of the fungal metabolites ochratoxin A and B, which starting from \(p\)-chlorophenol, relies on three successive ortholithiation reactions to give a 1,2,3,4,5-substituted ring pattern.\textsuperscript{103} While this, combined with the milder reaction conditions needed, is a clear demonstration of the potential use of carbamates as directing groups in ortholithiation reactions Sibi and Snieckus noted that the carbamate showed less discrimination between nonequivalent ortho positions.\textsuperscript{102}

\begin{equation}
\begin{array}{c}
\text{N} \quad \text{O} \\
\text{O} \quad \text{C} \\
\text{N} \quad \text{O} \\
\text{O} \\
\text{N} \quad \text{O} \\
\text{O} \\
\end{array}
\end{equation}

\[ \text{99} \quad \text{a) sec-Butyllithium, TMEDA in THF at -78 °C. b) Quench with a suitable electrophile. c) The reaction is allowed to warm to room temperature and is then quenched with water or methanol.} \]

\begin{equation}
\begin{array}{c}
\text{N} \quad \text{O} \\
\text{O} \quad \text{C} \\
\text{N} \quad \text{O} \\
\text{O} \\
\text{N} \quad \text{O} \\
\text{O} \\
\end{array}
\end{equation}

\[ \text{100} \quad \text{101} \quad \text{102} \]

Scheme 2.14: The ortholithiation of phenyl diethylcarbamate. a) sec-Butyllithium, TMEDA in THF at -78 °C. b) Quench with a suitable electrophile. c) The reaction is allowed to warm to room temperature and is then quenched with water or methanol.\textsuperscript{101-102}

In other respects the reactivity of carbamates and amides is similar.\textsuperscript{101-102}

Ortho lithiated arylamides will attack un lithated molecules, resulting in the formation of the benzylation product; this is best avoided by using sufficiently hindered carboxamides (di-\(i\)-propyl amides, or diethyl carbamates) to prevent such undesirable reactions.\textsuperscript{86, 104} Additionally activating the lithating agent with TMEDA results in an increase in the rate of lithiation leading to insufficient time for the undesired coupling to occur.\textsuperscript{86}

Amides and \(O\)-carbamate groups can be removed by hydrolysis in basic conditions.\textsuperscript{100}
In addition to nitrogen and oxygen containing groups there are a host of other groups that can direct ortholithiation reactions including simple halogens and sulphur containing groups, these are not discussed in this work.

2.5 Intramolecular aryl migration reactions

2.5.a Intramolecular N to C aryl migration in urea based substrates

In 2007 Clayden et al. reported an interesting intramolecular aryl migration of an aromatic ring from a urea nitrogen to a benzylic carbon.\textsuperscript{105}

They reported that a slight excess of \textit{sec}-butyllithium was required as the rearranged product underwent a second lithiation at the benzylic position following the rearrangement.\textsuperscript{105} This second lithiation prevents this method being used to produce a chiral product, however, when an \(\alpha\)-methylbenzylurea is treated in a similar fashion the resulting product displays a high enantiomeric excess showing that the reaction is almost completely stereospecific.\textsuperscript{105} In the original report the scope of the rearrangement included aromatic rings with both electron withdrawing and electron donating substituents.\textsuperscript{105} The scope of the reaction was later extended to include the transfer of hetroaromatic rings, but
to prevent alkylation of the hetroaromatic rings the reaction had to be performed with the less nucleophilic base LDA.\textsuperscript{105-107}

![Scheme 2.16: An example of the intramolecular heteroaryl migration reaction.\textsuperscript{106} a) LDA, THF and DMPU (10:1), at -78 °C. b) Quench with water. R\textsuperscript{1} and R\textsuperscript{2} = benzo, Cl, F, H, Me, or OMe.]

There was only one heteroaryl ring that failed to migrate, that being the 1-methoxy-4-urea pyridyl group, which instead gave a cyclization.\textsuperscript{106}

![Scheme 2.17: The failed migration of the of the 2-methoxy-4-urea pyridyl.\textsuperscript{106} a) LDA, THF and DMPU (10:1), at -78 °C. b) Quench with ammonium chloride in water. c) Oxygen.]

It should be noted that while the reaction has extensive breadth of applicability various specific reaction conditions were needed for the different substrates.\textsuperscript{105-107} For example; they reported that for the rearrangement of tertiary benzylic ureas an additive, \(N,N'\)-dimethylpropylene urea, was needed to ensure that the reaction proceeded in a reasonable amount of time and at a sufficiently low enough temperature to prevent undesirable side
reactions. In reactions in which LDA was used as the base \(N,N'\)-dimethylpropylene urea was needed, as an additive, to ensure the achievement of a useful level of stereospecificity.

That the migration occurs with the preservation of substituent position numbers is indicative of ipso selectivity which is in turn indicative of a formal SNAr reaction. Greater elucidation of the reaction mechanism was achieved by using the naphthyl group as the migrating group and exposing the lithiated urea to oxygen. This allowed the trapping of the intermediate as an enone, the crystal structure of which shows that the reaction is stereochemically retentive and supportive of the proposed intermediate in which the aromaticity of the migrating ring is lost; if the reaction is quenched with water instead of oxygen the aromaticity will be restored.

![Scheme 2.18](image)

Scheme 2.18: Intermolecular aryl rearrangement with the naphthyl group and trapping the rearrangement intermediate as an enone. a) sec-Butyllithium in THF at -78 °C. b) Dry oxygen. \(R^1 = \text{Cl or OMe}\).

While the rearrangement itself is largely stereospecific, reprotonation of the urea proved not to be so, in fact Clayden et al. reported that quenching the reactions with various deuterated solvents revealed that quenching with methanol gave a minor preference for inversion of configuration and quenching with dimethylsulphoxide gave a more significant preference for retention of configuration.

Further work in this area showed that the rearrangement was also applicable to cyclic systems, initially starting with ureas derived from 2-phenylpyrrolidine and moving onto isoindoline, tetrahydroisoquinoline, aminotetralin and aminoindane derived ureas. The reaction conditions required to best promote the rearrangement of each family of ureas varied somewhat, and there was also some variance within each family. The breadth of the conditions needed ranged from those required for \(\alpha\)-methylated tetrahydroisoquinoline, which rearranged under the standard conditions (sec-butyllithium and DMPU in THF at -78 °C) to the conditions for the rearrangement of the unmethylated tetrahydroisoquinoline.
which did not require the additive, but had to be allowed to warm to room temperature instead. In some instances it was also necessary to change the base to LDA.

Clayden et al. propose that the rearrangement reactions occur due to the presence of a carbonyl that can strongly coordinate with the lithium ion and an electron deficient nitrogen bonded to a reactive π-system.

![Scheme 2.19: Intramolecular aryl rearrangement on heterocyclic and carbocyclic structures, derived from the following (listed top to bottom); 2-phenylpyrrolidine, isoindoline, tetrahydroisoquinoline, aminotetralin and aminindane. See references for specific reaction conditions.](image-url)
Following rearrangement the unwanted fragment of the urea could be cleaved by simple solvolysis, achieved by simply heating the products in \( n \)-butanol until methyl isocyanate was eliminated.\(^{106-107} \) The rearrangement, followed by cleavage of the urea to restore a methyl amine, opened a new and synthetically useful route to substituted \( \alpha,\alpha \)-dibenzyldimethylamines and \( \alpha \)-methyl-\( \alpha,\alpha \)-dibenzylmethylamines.

\[
\begin{align*}
\text{Refluxing } n \text{-butanol.}
\end{align*}
\]

### 2.5.b Intramolecular N to C aryl migration in carbamate based substrates

Shortly after reporting the intramolecular aryl migration exhibited by ureas Clayden et al. also reported an analogous N to C aryl migration on carbamate based substrates.\(^{108} \)

\[
\begin{align*}
\text{Scheme 2.21: Intramolecular nitrogen to carbon aryl migration on a carbamate substrate. a) sec-Butyllithium (2.5 equivalents) in THF with DMPU (4:1) at -78 °C for 4 hours or LDA (2.5 equivalents) in THF with DMPU (4:1) at -78 °C for 4 hours. b) Methanol. c) Sodium ethoxide in ethanol at reflux for 2 hours.}\end{align*}
\]

\( R^1 = \text{benzo, Cl, F, H, Me, OMe, or iso-Propyl. } R^2 = \text{H or } p-\text{Cl.}\)\(^{108} \)
The reaction proved effective on a reasonable array of substrates, with only two main groups of reaction conditions. However, attempts to perform the rearrangement on a number of enantiopure O-α-tertiary carbamates yielded racemic products. The problem was ameliorated by changing from the highly coordinating THF and DMPU solvents to the less coordinating diethyl ether solvent, while this greatly increased the enantiomeric ratio of the products the reaction did not repeat the almost perfect enantiospecificity of the same reaction on the urea based substrates, moreover the rate of reaction decreased markedly.

Scheme 2.22: Intramolecular aryl rearrangement of an O-α-tertiary carbamate. a) LDA (2.5 equivalents) in diethyl ether at -78 °C to -35 °C for 24 hours. b) Methanol.

In silico experiments indicated that the energy barrier for the attack on the aryl ring was considerably lower than that for attack on the carbamate carbonyl, therefore the reaction yielded the products of the 1,4-aryl migration rather than the 1,2-acyl migration. The in silico experiments also indicated that during the course of the reaction the substrate becomes associated with two lithium ions; one closely associated to the carbonyl oxygen and a second lithium ion migrating to the vicinity of the other oxygen position. This is thought to make the benzylic carbanion position more reactive. The role of the DMPU and the solvent is considered to be stabilizing the lithium ions.

The migratory reactions present a synthetically useful path to α-diaryl alcohols and α-tertiarydiaryl alcohols.

2.5.c Further development of the intramolecular aryl migration

There has been substantial additional work on the aryl migration with the scope of the reaction being greatly extended to other reagents and to include the use of chiral solvents for selective synthesis of one enantiomer or another, however, this lies beyond the scope of this work.
3 The aims of this work

As outlined at the beginning of the introduction the work consists of two general areas of interest. The first part is the identification of the alkaloidal components of the *Narcissus pseudonarcissus* extract and the subsequent application of synthetic methods to any component that could feasibly be converted to galanthamine and the closely related sanguinine.

The second part is the application of synthetic methodologies to galanthamine in order to identify new ways in which it can be functionalized. From the literature it is evident that the medicinal chemistry undertaken on the galanthamine core focuses on modification and functionalization at the heteroatom positions, however, a visual examination of the crystal structures published by Greenblatt *et al.* seems to indicate that functionalization of the galanthamine core at the C6, C7, and C8 positions could prove more valuable as these positions are better aligned to allow the newly inserted groups to be directed up the gorge of the acetylcholine esterase allowing for more favourable interactions with the wall of the gorge and less unfavourable steric interaction with the wall of the active site. It is hoped that lithiation chemistry could allow functionalization of the galanthamine at some of these alternative positions.

![Figure 3.1: Galanthamine (12); the carbon positions that could be most easily functionalized by means of lithiation chemistry are indicated. Repeated from Figure 2.3 for clarity.](image-url)
4 The semisynthesis of alkaloids and some alkaloid analogues

4.1 The semisynthesis of sanguinine

Given the initial objective of this project and the ready availability of galanthamine hydrobromide, as supplied by Alzheim Ltd., the first task undertaken was the semisynthesis of sanguinine starting from galanthamine. This is widely reported in the literature, with all the procedures using essentially the same basic process: L-Selectride is added to galanthamine in tetrahydrofuran and refluxed overnight. There were minor differences in the work up procedures, however, it was found that the method of Mary et al.\textsuperscript{109} was by far the best, this method required the reaction mixture to be diluted with ethyl acetate and then quenched with water, before working up using more ethyl acetate and water. During workup the sanguinine went into the aqueous layer, the separation of the layers took progressively longer after each extraction; if additional solvent was not added the separation for the third or fourth extraction could take many hours. Without using ethyl acetate for the initial dilution it was found that the product often contained a mixture of inseparable organic borates. The sanguinine could be purified by column chromatography using a solvent system of dichloromethane, methanol and aqueous ammonia solution 35\% in a ratio of 80:19:10, the high proportion of methanol again demonstrating the hydrophilic nature of this alkaloid. The yield was consistently greater than 95\%, both on sub and multi gram scales. Additionally, the method was found to be robust enough that 1 M L-Selectride could be added directly to dry galanthamine at room temperature and then heated and that the exclusion of the additional tetrahydrofuran had no detrimental effect on the outcome of the reaction.

![Scheme 4.1: the semisynthesis of sanguinine, 22, from galanthamine, 12. a) L-Selectride in tetrahydrofuran and refluxed for 16 h.\textsuperscript{109}](image-url)
For comparison and because in natural product chemistry it is often wise to have more than one way to achieve your ends a few other methods for the $O$-demethylation of galanthamine were briefly investigated. The literature reports that sodium ethanethiolate in HMPT gives a slightly lower yield than the use of L-Selectride in tetrahydrofuran.$^{110}$ However, as the solvent is industrially unfavourable the reaction was attempted in other solvents; including tetrahydrofuran, dimethyl sulfoxide, and tetrahydrofuran with 15-crown-5 ether. The crown ether was added as it was hoped that it would complex the cation making the anion more available for reaction with the $O$-methyl group. The reactions were monitored by thin layer chromatography and initially showed negligible reaction progress followed by decomposition, as indicated by the appearance of a multitude of spots and sludgy dark brown colours; no sanguinine was isolated from any of these alternative reactions. The use of boron tribromide is reported as giving yields in the region of 30% and so this was not investigated.$^{52}$ Fortunately throughout the project the lack of an alternative $O$-demethylation reaction did not present any insurmountable obstacles.

### 4.2 The semisynthesis of alkaloids for use as standards in the analysis of the daffodil juice

In total the literature contains details of about 100 alkaloids that can be extracted from the Narcissus family of plants, with over 20 of these already known to be present in Narcissus pseudonarcissus cv Carlton, the primary variety of daffodil farmed and harvested for Alzeim Ltd.$^{23}$ However, from this vast variety of alkaloids only a reasonably small number have a structure that would offer a suitable springboard for the semisynthesis of galanthamine and by extension sanguinine. Due to the fact that the daffodil juice contained a very high number of components it was decided that the best way to analyse it was to synthesise samples of alkaloids known to have a structure similar to galanthamine and use these as standards in chromatographic analysis. Therefore the semisynthetic work, started with the synthesis of sanguinine, was expanded to cover other likely alkaloids (see Table 1.1). The following sections detail the semisynthesis of said alkaloids starting from galanthamine.
4.2.a The semisynthesis of narwedine

Scheme 4.2: The oxidation of galanthamine, 12, to narwedine, 24. a) Oxalyl chloride in chloroform with dimethyl sulfoxide at -60 °C for 5 min, then -50 for 40 min, then triethylamine for 20 min.\textsuperscript{111}

The semisynthesis of narwedine, from galanthamine, is reported using both manganese dioxide and by Swern oxidation.\textsuperscript{32,111} The use of manganese dioxide in dichloromethane gave only a 16% yield, attempts to use tetrapropylammonium perruthenate with 4-methylmorpholine N-oxide were equally unsuccessful. When monitored by thin layer chromatography, 2-iodoxybenzoic acid in acetonitrile at room temperature seemed to oxidise galanthamine slowly, but the initial formation of narwedine was quickly followed by decomposition. The use of Swern oxidation, as reported by Lee \textit{et al.},\textsuperscript{111} gave narwedine in up to 99% yield on multi gram scales with the crude product showing a high degree of purity, if remaining a little smelly. Smaller scale reactions routinely gave slightly lower yields of a dirtier product, in the region of 60%. Although they do not give an explanation as to why, Lee \textit{et al.} do not use the standard -78 °C temperature for the Swern reaction, instead using first -60 °C and then -50 °C.\textsuperscript{111} When the reaction was initially carried out in this investigation it was done entirely at -78 °C and gave a very poor result, given the observations made throughout this report it is highly likely that at -78 °C the galanthamine is not soluble enough to enable the reaction to proceed effectively, unfortunately at such low temperatures ice on the flask made it difficult to see the state of the reagents. When the temperature ranges reported by Lee \textit{et al.}\textsuperscript{111} were used the reaction worked well. That the reaction worked far better at larger scales than at smaller scales is most likely due to the fact that when the reaction was performed on a larger scale the dropwise addition of the galanthamine solution resulted in far smaller, or even negligible, local temperature fluctuations, which in turn would have led to less decomposition of the temperature sensitive dimethylchlorosulphonium or indeed less of any other undesirable side reaction.
4.2.b The semisynthesis of norgalanthamine

The N-demethylation of galanthamine, by way of a non-classical Polonovski reaction, reported by Mary et al.,\textsuperscript{113} in which 3-chloroperbenzoic acid is used to form an N-oxide, by oxidation of the tertiary amine, followed by in situ hydrolysis with iron(II)sulphate heptahydrate to give norgalanthamine, is widely referenced in the literature.\textsuperscript{113-116} The consensus in the literature is that the active species in the second stage of the reaction is iron(II) and that molecular oxygen plays a part in the reaction. There are several variations to this method, used on various other alkaloids, which include varying the temperature of the reaction and the use of various additives including iron(III) chloride, and hydrochloric acid or even replacing the iron(II)sulphate heptahydrate with ferrocene.\textsuperscript{117}

![Scheme 4.3: The N-demethylation of galanthamine. 12, to form norgalanthamine, 19, performed as a one pot reaction. a) 3-Chloroperbenzoic acid in chloroform/isopropanol (3:1) at -10 °C, then hydrochloric acid, b) Water, then iron(0) powder 13 mol% and iron(III) chloride hexahydrate 2 mol%, at room temperature. See text for a full discussion of the experimental details and the yields.](image)

In this investigation both thin layer chromatography and electrospray mass spectrometry indicated the formation of the N-oxide and then the reduction of this to the secondary amine. In fact, it was found that the hydrochloride salt of the N-oxide could be isolated in
good yield (77% after column chromatography) and then stored (the NMRs of the N-Oxide were always very poor showing broad peaks with little coupling visible). However, the second stage of the reaction, the reduction to the amine, was found to be highly capricious.

Initial attempts using iron(II)sulphate heptahydrate gave very low yields, with one of the biggest problems being the removal of the iron. NMR indicated that the N-methyl peak had also been eliminated. However, even the best NMR spectrum achieved was noisy due to the presence of iron in the sample. Attempts to remove the iron by filtering through Celite also resulted in loss of the alkaloid and column chromatography did not notably improve matters. Attempts to extract the norgalanthamine via liquid-liquid extraction by adjusting the pH of the aqueous layer and using various organic solvents were also unsuccessful. Although small quantities of norgalanthamine were isolated they were always contaminated with iron and did not seem to be a fair representation of the actual yield of norgalanthamine formed by the reaction; although the assumed yield was only indicated by the noisy NMRs and therefore the actual degree of success remains unknown.

As was also noted by others the reaction was usable on a small scale where the removal of the iron proved less of a problem, but proved almost unworkable on larger scale reactions. Janssen et al., who also pointed out the difficulty presented by the purification, gave greater detail as to the purification process they developed and this was used to isolate a relatively pure sample but in poor yield. This purification process went via the formation and subsequent extraction of a gel, a lengthy and messy process to give sub 50% yields, although Jannsen et al. did report higher yields. The problem with removing the iron was not alleviated by the addition of additives to the reaction, especially as those additives often contained iron, such as when iron(III)chloride hexahydrate was used as an additive.

The use of two equivalents of ferrocene, as the iron source, returned galanthamine and other organic waste, but no norgalanthamine was found.
Table 4.1: A summary of the reaction conditions used to affect the N-demethylation of galanthamine

<table>
<thead>
<tr>
<th>Entry</th>
<th>Fe(0)</th>
<th>Fe(II) (eq.)</th>
<th>Fe(III) (eq.)</th>
<th>Addition of iron sources</th>
<th>Agitation method</th>
<th>Yield from 50 mg scale norgal/gal (%)</th>
<th>Yield from 100 mg scale norgal/gal (%)</th>
<th>Yield from 2.0 g scale norgal/gal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>Magnetic stirrer</td>
<td>-</td>
<td>43*</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Simultaneous</td>
<td>Magnetic stirrer</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Fe(III), 5 min later Fe(II)</td>
<td>Magnetic stirrer</td>
<td>67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>Fe(III), 5 min later Fe(II)</td>
<td>Magnetic stirrer</td>
<td>&lt;15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>cat.</td>
<td>-</td>
<td>cat.</td>
<td>Simultaneous</td>
<td>Magnetic stirrer</td>
<td>59 / 12</td>
<td>39 / 7</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>13 mol%</td>
<td>2.5 mol%</td>
<td>Simultaneous</td>
<td>Hand Shaken</td>
<td></td>
<td>-</td>
<td>-</td>
<td>63 / 8</td>
</tr>
</tbody>
</table>

* Actually the results from a 289.6 mg scale reaction, purified by the method of Janssen *et al.*

Entries 5 and 6 are for comparison and detail the two best sets of results using the Kok Scammells method.
Eventually the method reported by Kok and Scammells, for the N-demethylation of opiates, which uses sub stoichiometric amounts of iron and an even smaller proportion of iron(III)chloride hexahydrate, was found in the literature. They stated that increasing the amount of iron(III)chloride hexahydrate in the reaction resulted in a reduction in both the amount of product and starting material recovered, this is backed up by the results of this investigation (Table 4.1, entries 3 and 4). In the initial attempts, on small scale reactions, the Kok Scammells method was found to work well (Table 4.1, entry 5). However, when the reaction was scaled up, even by only a small amount, that is from 50 mg to 100 mg scale, the yield was found to fall very dramatically and the reaction became very capricious (Table 4.1, entry 5).

The Kok Scammells method produced much cleaner results than the more iron heavy methods and the purification was far easier. Following simple workup, column chromatography could be used to purify the norgalanthamine, the only slight complication being a compound that eluted after the norgalanthamine despite having a higher $R_f$ by thin layer chromatography. This variance between $R_f$ and actual elution could have arisen due to the use of a gradient solvent system for the column chromatography. Under ultraviolet light this troublesome spot fluoresced powerfully blue, but the compound was only isolated cleanly in one instance. A less than perfect proton NMR was acquired, but before further analysis could be run the sample had turned brown and crystals had started to form. X-ray crystallography showed the crystals to be galanthaminium chloride, 138.

![Chemical structure of galanthaminium chloride](138)

Figure 4.1: The X-Ray structure and chemical diagram of galanthaminium chloride.

Whatever the identity of the blue spot, its elution from the column so close to the already close galanthamine and norgalanthamine fractions, coupled with the close proximity of
some other miscellaneous spots made it hard to isolate both the remaining galanthamine and the norgalanthamine cleanly from a single column. Hence columns were run to favour the norgalanthamine at the expense of the remaining galanthamine that was often only present in lower yields.

In their paper Kok and Scammells state that the reactions were shaken rather than stirred and that the reaction vessel must be left open to the air as molecular oxygen plays a part in the reaction. They demonstrated that exclusion of oxygen resulted in lower total recovery of product and starting material and that the reaction times were greatly extended. As no shaking apparatus were available the reactions were stirred with magnetic stirrers. While this did not seem to have been a problem on the smaller scale reactions it could now be seen that some if not all of the iron was clumping on the tips of the magnetic stirrer bars and it was thought that this may be causing the iron to have too small a surface area to effectively catalyse the reaction. In an attempt to get round this problem a mechanical swirler was used, however, it was quickly observed that the swirling motion was not vigorous enough; thus the iron particles quickly fell out of solution and clumped together in the centre of the flask. Therefore, the reactions were hand shaken. It was observed that if the reactions were shaken carefully, in just the right way, they could be placed on the side for several minutes between shakings without an observable amount of iron falling out of solution. The key in achieving this seemed to be the avoidance of any swirling motion in the shaking, as when the shaking stopped any slight swirling motion quickly translated into rotatory motion of the solution and the heavier iron particles would quickly fall out of solution and congregate together at the bottom of the flask. The initial attempts at hand shaking the reaction worked well, but later attempts proved this too to be a capricious method, especially when the method was scaled up. However, during this investigation the best yield for the N-demethylation of galanthamine was achieved using the hand shaking method.

As a small volume of solvent would have a proportionally much larger surface area compared to the larger volumes of solvent used when the reaction was scaled up it was thought that the reduction in surface area could be leading to a lack of air mixing with the solvent. In an attempt to get round this problem compressed air was bubbled through the reaction mixture, this had the added advantage of stirring the reaction mixture at the same time as aerating it. However, this too failed to produce an increase in the yield, no doubt at least in part because it lead to the evaporation of most of the solvent. The implementation of vigorous stirring, as supplied by an overhead stirrer (using a PTFE coated stirrer to limit interference in the reaction by any other metal sources), in a considerably larger flask to decrease the reaction mixture depth and thus increase its surface area, seemed to produce
better results. The use of an overhead stirrer alone gave a visibly better aeration of the solvent too. However, the next time the reaction was performed the yield was once again poor.

The only change made to the way in which the reaction was carried out was that, due to the increase in scale of the reaction, glass apparatus had been used to measure and add the concentrated hydrochloric acid solution, rather than a stainless steel needles and plastic syringe as had been used previously. In a slightly enraged attempt to kick-start the reaction additional hydrochloric acid was added in haste, this time using a plastic syringe and stainless steel needle for convenience. It was observed that there was possibly a slight reaction, as evidenced by the formation of bubbles, between the hydrochloric acid and the stainless steel needle. The addition of this extra acid did start the reaction working. The slight reaction between the needle and the acid had not been noticed before as the volumes of acid used were so small, but in this instance due to the scale of the reaction the volume of acid was larger. Given that the reaction is thought to be catalysed by iron(II) from the disproportionation reaction of iron(0) and iron(III) it was thought possible that the slight reaction between the needle and the acid may be leading to the introduction of a trace amount of metal ions that were initiating the reaction. Thus, the reaction was repeated again and this time the acid was added using a plastic syringe and stainless steel needle, but once again the reaction failed to proceed. The reaction scale was reduced and attempted several more times, with varying results. It was at this point that it was noticed that the reaction seemed to work better on warm humid days, an observation made from memory rather than records. As the solvent system used is a mixture of chloroform and isopropanol it was thought that possibly the elevated temperature and humidity lead to the solvent having a greater water content, which may in turn increased the solubility of oxygen in the solvent. This would also explain why the second addition of acid had previously proved successful in kick starting the reaction. In an attempt to prove this theory a small volume of the solvent (10.0 cm$^3$) was titrated using a needle and syringe to see how much water it could dissolve until the solution took on a slightly opaque appearance when held up to window light; the cloudiness was taken to be the formation of tiny water droplets, usually in the region of 0.17 cm$^3$ of water could be added. The reaction was then run again, but immediately prior to the iron sources being added the reaction was divided into several identical flasks and to each flask was added a different proportion of water, taking into account the volume of hydrochloric acid that had already been added. Repeating this a couple of times seemed to show that the addition of water to the reaction did have an influence on the outcome of the reaction, however, the effect was less cut and dried then might be desired.
Table 4.2: The yields of N-demethylation reactions, performed using the Kok Scammells method, when various amounts of water were added to the reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Water (as % of saturation)*</th>
<th>Yield of norgalanthamine (%)</th>
<th>Yield of galanthamine reclaimed (%)</th>
<th>Blue spot (%)§</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>36</td>
<td>11 (dirty)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>&gt;54†</td>
<td>10</td>
<td>6.0</td>
<td>Required two columns to purify</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>46</td>
<td>10</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>50</td>
<td>1.4</td>
<td>4.5</td>
<td>Still a little dirty after two columns</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>57</td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>62</td>
<td>3.3</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>57</td>
<td>-</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>44</td>
<td>8.5</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>44</td>
<td>9.6</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Still dirty after column</td>
</tr>
</tbody>
</table>

NB. Entries 1 and 2 were run as individual experiments. Entries 3, 4 and 5, entries 6, 7 and 8, and entries 9, 10 and 11 were run as three groups of reactions.

* These values also include the contribution from the concentrated hydrochloric acid, treated as water for the calculations.

† Some product was lost, due to mishandling of the primary product containing fraction from the column.

§ For the calculation of yields the blue spot was treated as galanthaminium hydrochloride, all blue spots were impure.

The reaction to which no water was added proceeded the most slowly. The reactions with the most water added proceeded the most quickly, but unfortunately also returned a far dirtier product, more decomposition material and generally required more purification.

This apparent rate of reaction was judged by the change in the colour of the reaction and the change in the reaction components as monitored by thin layer chromatography. It was found that the balance point was at about 25% of aqueous saturation of the reaction mixture. With this information in hand it was thought that a secure method had now been
outlined. The reaction was repeated and yet still the reaction was found to be highly capricious sometimes returning relatively good yields and sometimes very poor yields.

By this time the reaction had been repeated many times and it had been observed that when the reaction worked best the mixture changed colour in a very specific manner; from very pale yellow it would turn to a bright golden yellow and then amber, when the reaction was going to return mainly starting material it remained a very pale yellow colour and when it was going to return a poor yield and mainly decomposed material it changed firstly to a bright yellow with a brownish tinge and then a slightly different amber (more rust like) colour. The colour change occurred quickly after the addition of the iron sources and the higher the proportion of water added to the reaction the more quickly the colour change occurred. The experiments into the effect of water on the reaction gave a good opportunity to record the effect of the water on the colour change. In only one instance throughout the investigation did the reaction fail to take on the hoped for final amber colour, instead remaining yellow, but proceed to give a good yield, this was also the cleanest reaction. Of course the combination of water and iron(0) would be expected to lead to rust and no doubt this is one of the causes of the brownish colouration, but even when the iron was removed the brownish colour remained until removed by column chromatography.

A further observation was that the addition of the iron sources had to be accompanied by at least some agitation or tiny spots could form on the surface of the glassware, these were best avoided by adding the iron(0) and then the iron(III)chloride hexahydrate, separated by some light agitation to ensure the iron was suspended in solution. However, no correlation was drawn between the level of success of the reaction and these spots.
As the investigation into the effect of water on the $N$-demethylation of galanthamine had been sparked by the chance observation that the reaction seemed to work best on humid
days it was thought that maybe other environmental factors may be influencing the reaction. Given that the labs are situated in a large urban area it was thought that possibly the presence of tropospheric pollutants may be impacting the reaction. Sunny humid days can result in elevated levels of various gaseous pollutants, therefore the yields of 13 consecutive attempts at \(N\)-demethylation were plotted against tropospheric pollutants using data supplied by DEFRA and collected in Manchester City Centre. The resultant plot clearly shows the total lack of any correlation; at least of the pollutants for which data was available.

![A graph to show the yield of norgalanthamine against tropospheric concentrations of various pollutants](image)

**Figure 4.3:** This graph shows the apparently random distribution of atmospheric pollutants and the yield of norgalanthamine from the \(N\)-demethylation reaction carried out on the corresponding day. Atmospheric data © Crown 2014 copyright Defra via uk-air.defra.gov.uk, licenced under the Open Government Licence (OGL).

Scammells suggested that the presence of trace amounts of unreacted peracid could be interacting with the iron and inhibiting the reaction and suggested that the way round this was to isolate the \(N\)-oxide as the hydrochloride salt and then pursue the second part of the reaction.\(^{118}\) This was done without success; purification of the \(N\)-oxide by column chromatography followed by treatment with iron(0) and iron(III) gave only a 32% yield for the last step. Moreover, in several instances, when the \(N\)-demethylation reaction was run as a single pot experiment, an aliquot of the reaction mixture was taken and analysed by NMR at several points throughout the course of the reaction. These NMR spectra all
clearly show the peaks for 3-chlorobenzoic acid, but not 3-chloroperbenzoic acid. To confirm this, as the fact that the sample contained a mixture of compounds (N-oxide and 3-chlorobenzoic acid) meant that there was some peak shifting, after the initial NMR some of the samples were spiked, either with 3-chlorobenzoic acid or 3-chloroperbenzoic acid or both. A selection of the pertinent NMR spectra are presented in Figure 4.4 and clearly show that if there was any unreacted 3-chloroperbenzoic acid it was present in very low quantities; undetected by NMR and certainly not enough that it could be expected to reliably inhibit 13 mol% of iron(0) catalyst or even 2 mol% of iron(III). The NMRs presented pertain to a reaction that gave a yield greater than 54% (Table 4.2, entry 3) but the NMRs run for less successful reactions (e.g. Table 4.2, entry 2) are essentially indistinguishable.

Figure 4.4: Comparison of ¹H NMR spectra (400 MHz); a) 3-chlorobenzoic acid. b) 3-chloroperbenzoic acid. c and d) aliquots taken from the N-demethylation of galanthamine reaction 30 min and 80 min, respectively, after the addition of 3-chloroperbenzoic acid. e) Rerun of d following the addition of extra 3-chloroperbenzoic acid. X-axis; chemical shift in ppm. The solvent was chloroform-d.

For the sake of curiosity, the formation of the N-oxide, normally run at -10 °C using a salted ice bath, was run at room temperature and all the 3-chloroperbenzoic acid was added in a single portion without any impact on the formation of the N-oxide.
As mentioned previously, Kok and Scammells\textsuperscript{112} have already demonstrated the reaction’s dependency on oxygen, therefore the last two suitable manipulations of the reaction conditions were to use an oxygen environment and the use of different temperatures for the second stage of the reaction.

Hence, the reaction was repeated using an oxygen atmosphere, but this too produced poor results, returning 11\% of the starting material, but only 10\% of very dirty norgalanthamine, additional purification resulted in a yield of less than 5\% compared to a yield of 35\% for the control reaction run in parallel.

Repeating the reaction and then dividing the mixture in two prior to the addition of the iron and gently warming one fraction of the mixture, so as to mimic the warmer more humid days on which the reaction seemed to work best, also failed to produce the terrific improvement hope for. The control experiment, being the fraction run at room temperature, gave a yield of 40\% while the gently warmed reaction (warmed to ca. 30 °C) returned a yield of 45\%, although an improvement on the control experiment it is generally a lower yield and does not explain the better yields that are sometimes achieved.

It should be noted that for all the reactions a single source of iron(0) powder and iron(III)chloride hexahydrate was used, thus eliminating these as uncontrolled variables. Prior to use, the 3-chloroperbenzoic acid was purified (see experimental section for details) and stored in a plastic container in the freezer. Several batches were required throughout the course of the investigation, however, the purity of each batch was assessed by NMR after purification and at other times, especially after reactions failed to proceed. This, combined with the studies into the formation of the N-oxide, reasonably eliminates the 3-chloroperbenzoic acid as the source of the capriciousness of the N-demethylation reaction. Moreover, each batch of purified 3-chloroperbenzoic acid was used for several reactions, which typically gave a range of results.

The Kok Scammells method was by far the best method used for the N-demethylation of galanthamine, primarily for the far easier purification sequence required, which itself arose from the fact the reaction required smaller quantities of iron essentially making it more economical and environmentally friendly, albeit only marginally due to the low cost and benign nature of iron. The only apparent improvement that was made to this reaction was the addition of a little water to the reaction just prior to the addition of the iron, which, while not guaranteeing a high yield, did seem to slightly improve the reliability of the reaction.

Another method reported for the N-demethylation of similar structures was the von Braun reaction.\textsuperscript{119-120} Thin layer chromatography and electrospray mass spectrometry showed the
reaction of galanthamine and cyanogen bromide to proceed cleanly, however, alkaline hydrolysis of the nitrile group, to give norgalanthamine, was inefficient and resulted in considerable degradation, leading to a very low yield of a dirty product. Several attempts were made to tweak the procedure without success. As discussed elsewhere in this work the galanthamine core structure shows some vulnerability to strongly basic environments and so it is most likely that the hydrolysis step is leading to decomposition.

Attempts at \( N \)-demethylation using 1-chloroethyl chloroformate, as reported by others for the dealkylation of tertiary amines, were equally unsuccessful.\textsuperscript{121-122}

Both of these methods are less desirable as the first requires the use of cyanogen bromide and the second requires a protection-deprotection sequence to protect the allylic alcohol.

![Diagram](image1)

**Scheme 4.5:** The \( N \)-demethylation of galanthamine via the von Braun reaction. a) Cyanogen bromide, in tetrahydrofuran and reflux. b) Potassium hydroxide in water/ethylene glycol and reflux.

### 4.2.c The semisynthesis of epigalanthamine

![Diagram](image2)

**Scheme 4.6:** The semisynthesis of epigalanthamine, 17. a) Dilute hydrochloric acid and reflux.\textsuperscript{123}

The semisynthesis of epigalanthamine from galanthamine, using the method of Bhandarkar and Kirby, was moderately successful, but did not proceed cleanly. The minor by-product was 3,4-didehydro-3-deoxygalanthamine, accompanied by considerable amounts of
galanthamine. The method relied on the simple epimerization of the hydroxyl group by heating galanthamine in dilute hydrochloric acid. However, only small amounts of this compound were needed and before much work was done on refining this method other work yielded the necessary amounts as a by-product! See section 10.1 for more details.

4.2.d The semisynthesis of N-formynorgalanthamine

![Scheme 4.7: The semisynthesis of N-formynorgalanthamine, 21. a) Ethyl formate and reflux for 48 hours.](image)

N-Formynorgalanthamine was synthesised, from norgalanthamine, using the method reported by Kodama et al. This was simply the reflux of norgalanthamine with ethyl formate and was found to work in a straightforward manner.

4.2.e The semisynthesis of narcisine and O-acetyl-N-acetyl galanthamine

![Scheme 4.8: The semisynthesis of narcisine, 23. a) Acetic anhydride in dichloromethane for 24 hours. b) Gentle heating in dilute aqueous sodium hydroxide.](image)

O-Acetyl-N-acetyl galanthamine was formed well by reaction of norgalanthamine with acetic anhydride over a period of 24 hours. However, the synthesis of narcisine proved problematic because the attempts to differentiate between the cleavage of the groups
proved tiresome, requiring just the right amount of gentle heating in hydroxide base. No narcisine was produced. However, the fact that the groups were found to cleave so easily meant that it was highly unlikely that either of these compounds would be found in the daffodil extract, as the extraction process used relied on quite harsh acid-base conditions and so any amount of these compounds present would be more likely found as norgalanthamine. Moreover, problems with the synthesis of the starting material, norgalanthamine, meant that a degree of triaging was required as to the exact uses said alkaloid was put to, therefore no further time was spent on this synthesis.

4.2.f The semisynthesis of O-acetyl galanthamine

![Scheme 4.9: The semisynthesis of O-acetyl galanthamine](image_url)

Initial attempts at the semisynthesis of O-acetyl galanthamine were made using the method of Han et al.; this method used 1.8 equivalents of 4-(dimethylamino)pyridine and 1.3 equivalents of acetic anhydride, with a reaction time of just over one hour. However, it was found this method gave a slightly dirty product and initial attempts at purification were problematic. The method was adapted to use only 1.3 equivalents of acetic anhydride and a catalytic amount (spatula tip) of 4-(dimethylamino)pyridine. While these conditions used less reagents the reaction had to be run overnight and the yield was not as good as that reported by Han et al., but the product was cleaner and far easier to purify. Given the double acylation of norgalanthamine in the synthesis of O-acetyl-N-acetyl galanthamine, it is possible the 4-(dimethylamino)pyridine was having only a very small impact on this reaction, possibly because of steric hindrance at the galanthamine hydroxyl position. This may explain why Han et al. used such large amounts of 4-(dimethylamino)pyridine.

It was also found elsewhere in this work that 4-(dimethylamino)pyridine was not wholly compatible with galanthamine (or compounds with a galanthamine core structure) and that its use often resulted in notable levels of decomposition.
4.2.g  The semisynthesis of lycoramine

![Chemical Structures](image)

Scheme 4.10: The synthesis of lycoramine, 25. a) Hydrogen gas, 10% palladium on carbon in dry methanol under hydrogen for 6 days.

Using the simple method of palladium catalysed hydrogenation, adapted from the method used by Han et al. for the hydrogenation of similar structures, the formation of lycoramine proceeded cleanly and the product was easily purified. Thin layer chromatography indicated that the reaction was almost complete after only one night but the reaction was left longer in the hope that the yield would improve. However, after several days there appeared to be no further change in the thin layer chromatography, therefore the reaction was stopped. While there had been some concern that the reaction had stalled the recovered yield of lycoramine was 93%.
5 The isolation and identification of alkaloids

In this work, alkaloids that offered a suitable starting point for the semisynthesis of sanguinine (and thereby also the closely related structure of galanthamine) were of greatest interest. The search for such alkaloids centred on the analysis of solid samples and the liquid extract of Narcissus pseudonarcissus, primarily of the Carlton cultivar, provided by Alzeim Ltd, the industrial sponsor of this work. The liquid extract used was obtained directly from the factory. The secondary objective was the development of an analytical method that Alzeim Ltd. could use to monitor the levels of various alkaloids at different stages in the extraction process, this would obviously require the identification of the different components. Given the limitations, in terms of both technology and expertise, at Alzeim Ltd. the method developed would need to be focused on HPLC.

5.1 Galanthamine and impurities in galanthamine

Galanthamine was supplied as the hydrobromide salt by Alzeim Ltd. Prior to use the free base of galanthamine was extracted using an aqueous solution of saturated sodium hydrogen carbonate and dichloromethane. The organic phase was collected, combined, dried over magnesium sulphate and then evaporated to dryness under reduced pressure. However, the samples of galanthamine supplied often came from different stages in the purification process and additionally Alzeim Ltd. were still in the process of finalising their purification process, consequently there was a significant degree of fluctuation in the purity of the various batches of galanthamine hydrobromide they supplied. Therefore, in most instances further purification was required.

It was found fairly early in this work that trace impurities from the extraction in the galanthamine could have a serious impact on the reactions undertaken.

5.1.a The purification of galanthamine and compounds with a galanthamine core by column chromatography

The two most obvious methods for the purification of large quantities of galanthamine are column chromatography and recrystallization. Unsurprisingly the success of column chromatography was found to fluctuate depending on the purity of the material to be columned and no doubt the nature of the impurities. Later in the work it was found that galanthamine and other compounds with a galanthamine core were better separated using a far slower flow rate, achievable under gravity without any additional pressure. Initially, such gravity columns were done using long stationary phases, however, it was found that
shorter columns and a slower, gravity fed, flow rate resulted in better separations. The biggest problem with the separation of these alkaloids is their surfactant like nature, meaning that they were largely separated in fairly polar solvents systems, typically involving methanol and aqueous ammonia, so if the flow rate were too high they showed a significant degree of tailing and very little separation. Band broadening under reduced flow rates was found to be far less of an issue, what is more the band broadening could be limited further by reducing the length of the stationary phase. However, even using short columns there was an increase in the time required for the successful purification of these compounds.

5.1.b The purification of galanthamine by recrystallization

The investigation, commissioned by Alzeim Ltd. and carried out by Albany Molecular Research Inc., into the purification of galanthamine gave a range of recrystallization methods. However, the report also stated that the success of any of the methods was highly dependent on the purity of the starting material and that they found their results were inconsistent, which they attributed to the differences in the purity of the various batches of galanthamine they were supplied with by Alzeim Ltd. In this work several attempts were made to purify the galanthamine using various recrystallization methods. It was found that it was not too difficult to grow galanthamine crystals in chlorinated organic solvents, however, the crystals often formed growing out of a viscous sticky residue and unfortunately the residue and the crystals proved nigh on impossible to separate, the residue often having to be washed off with solvents that were equally as effective at dissolving the crystals. The most successful method attempted was one detailed in the Albany Molecular Research Inc. report. This involved dissolving galanthamine hydrobromide in deionised water and warming to 30 °C followed by increasing the basicity to a constant pH 10, after half an hour under these conditions the solid was isolated by filtration and then dried under vacuum at 50 °C. The solid was then dissolved in acetone and heated to reflux, upon reaching reflux the solution was filtered hot to remove insoluble material. The filtrate was then allowed to cool slowly and then chilled to less than 5 °C overnight. In the first attempt this method resulted in a loose network of rod like crystals, which appeared tapered at both ends and were of exceptional purity, as measured by NMR and HPLC. Following this initial success, albeit with a lower yield than those in the report, circa <50% (this lower yield could well have arisen from starting from a less pure product), it was decided to scale up this method and use it to purify a larger quantity of galanthamine. Unfortunately, while the initial stages of the process worked as before, the crystal growth stage did not proceed as expected. Instead of the rod like crystals, crystals
with an appearance more like sugar grains formed on the surface of the glass accompanied with a layer of goo. As had been found in the other attempts to separate the crystals from the goo the isolated product was of exceptionally tiny yield and not especially clean. Therefore, the solution was reheated and the crystals dissolved and a second attempt at recrystallization was made. At this point it was observed that the acetone solution seemed to have a slightly darker colour than on the first attempt, again the crystals failed to grow as expected, if anything the result was slightly worse with smaller crystals, so the recrystallization step was attempted a third time. Again it was noted that the acetone solution was now an amber colour, having started as a very pale yellow in the first attempt. The crystals failed to grow again and to make matters worse it was found that thin layer chromatography showed the galanthamine to be less pure now than it had been before the attempts at recrystallization. At this point it was decided that no further attempts would be made at recrystallization and the galanthamine was purified using column chromatography. The galanthamine from these failed recrystallization attempts proved especially difficult and troublesome to purify, while samples of galanthamine from the same batch, but that had not been subjected to recrystallization attempts, could be columned with less trouble than those that had failed this recrystallization process. Both the initial and then the failed larger attempt using this recrystallization method were made on samples of galanthamine from the same batch supplied by Alzeim Ltd, thus eliminating the possibility that the recrystallization failed due to a difference in the nature of the impurities. Apart from the general uncertainties surrounding crystal growth, there was only one factor that was considered to have contributed to the failed recrystallization. That being that while the smaller attempt quickly reached reflux and then after filtering was allowed to cool, the larger sample took longer to reach reflux. It was observed at the time that the larger sample seemed to be a slightly darker colour than the smaller sample had been, this was initially put down to the larger scale of the process. However, given that on subsequent attempts at recrystallization the solution took on a progressively darker colour during each warming stage and displayed a notable decrease in purity, it is possible that these degradation products were what was inhibiting the recrystallization process.

### 5.1.c Impurities isolated from galanthamine hydrobromide

Column chromatography of the galanthamine, both of the sample that failed recrystallization and of the samples from the same batch but that had not undergone recrystallization, yielded the galanthamine-based diene; 3,4-didehydro-3-deoxygalanthamine, 141, used in the Diels Alder experiments detailed in Chapter 10 of this report. 3,4-Didehydro-3-deoxygalanthamine, 141, also known as anhydro galantamine, is
known to be a stubborn impurity in synthetically produced galanthamine and is available as a standard for the analysis of galanthamine.\textsuperscript{126-128}

![Figure 5.1: 3,4-Didehydro-3-deoxygalanthamine, 141.](image)

Given that the extraction of galanthamine from natural sources goes via extended exposure to aqueous acid and basic condition the presence of this diene is probably an indication that the extraction conditions are somewhat harsh and could possibly benefit from a further refinement. The diene most likely forms as a result of acid promoted dehydration.

The numerous other impurities, spots on the thin layer chromatography, were not refined to discrete compounds, instead giving NMRs indicating degradation residues.

### 5.2 The hunt for other alkaloids

#### 5.2.a A brief overview of the extraction method employed by Alzeim Ltd.

Briefly, the process used by Alzeim Ltd., for the extraction of galanthamine, is as follows: daffodils are harvested and then ensilaged for several months, after which time the juice is collected and concentrated. This concentrated daffodil juice is then transferred to the factory where it undergoes various acid-base extractions using different organic solvents. The organic solvents are then reclaimed by way of further acid-base extractions. The solvent reclaiming process results in three distinct aqueous waste phases. These waste phases, the names of which are derived from the relevant step in the extraction process, are called; “acid water”, “toluene recovery”, and “octanol recovery”, of the three the “acid water” fraction was found to hold the most useful organic content. The final step in the purification sequence is the precipitation of galanthamine, as the hydrobromide salt, from methanol. This methanol mother liquor is the last waste phase and the only organic waste solvent, although this too can be reclaimed by distillation. As all the organic solvents are reused in the extraction process the three aqueous waste phases, the methanol, and the galanthamine hydrobromide itself should contain all the remaining alkaloids and indeed any sugars and terpenoids and other non-volatile compounds that were present in the
daffodil juice (or that formed during the extraction process), the methanol was found to contain a significant amount of organic and inorganic matter.

5.2.b Starting the identification and analysis of the daffodil extract

The first attempt at identifying other alkaloids was made on the concentrated juice obtained from ensilaging of the daffodils, as this juice contained so many components attempts were made to extract different alkaloids, or groups thereof, by adjusting the pH. This tack was of limited effectiveness due to the formation of emulsions that did not resolve into separate layers even after a week; heating and the addition of salt both failed to break the emulsions, and changing the organic solvent also had little effect, dilution of the daffodil juice was avoided as this would have negated the advantage of using the concentrated daffodil juice. Consequently, focus was moved to the effluent from the industrial process. This had been avoided in the first instance to try and limit the number of artefacts from the extraction process, which may interfere with the results of the analysis.

It was found that basification of the fractions, followed by filtering to remove inorganic residues (assumed to be inorganic due to a lack of $^1$H or $^{13}$C NMR spectra), gave solutions of many components that showed some more intense spots by thin layer chromatography.
Although, as was obvious from later comparisons with HPLC, the spots were mixtures of compounds. It was found that basification with aqueous ammonia gave the best results, as the excess base could be removed with the water and did not lead to additional inorganic content. From this point the analysis moved in two general directions; the use of analytical equipment and the use of more standard preparative column chromatography for the isolation of those components that were more plentiful in the various effluent fractions.

5.2.c The development of HPLC methods

There are numerous HPLC methods reported for the identification and isolation of small amounts of various alkaloids from various members of the Amaryllidaceae family. However, many of these methods are either designed to identify or isolate only a couple or even a single alkaloid, therefore many of the reported methods give poor overall peak shape or undesirably wide peaks at some points along the chromatogram. The other major issue associated with HPLC methods reported in the literature is the lack of specific detail. An excellent example of the problems that the lack of specific detail presents is demonstrated by the accredited method for the analysis of galanthamine hydrobromide, as detailed in the United States Pharmacopeia. The United States Pharmacopeia states that the monographs do not name specific columns so as not to appear to endorse a specific product. The Official Monographs do detail all the conditions for the analysis including, flowrate, column dimensions, stationary phase, detector wavelength and mobile phase composition and mobile phase gradient. Elsewhere in the United States Pharmacopeia there is more information about the types of stationary phase and each group is given a specific code. The compendial monograph for the analysis of galanthamine hydrobromide states that the stationary phase, or packing as it is called in the United States Pharmacopeia, should be “L1”.

The website of the United States Pharmacopeia has a Column Equivalency Application that includes many columns and is designed to enable the easy selection of similar columns. When attempts were made to run the method as detailed in the United States Pharmacopeia using a Betasil C18 column using all the conditions as published the result was exceptionally poor, giving no clear or well defined peaks. The Betasil C18 column by Thermo Scientific is listed as having an L1 designation on the Column Equivalency Application and certainly the technical documentation for the column shows that it meets the L1 criteria. However, even the warning that accompanies the tables of data, returned by the Column Equivalency Application, stating that the comparison is theoretical, is wistfully worded.

The problem is compounded further by the fact that much of the isolation work reported in the scientific literature was performed some time ago and since that time there have been
numerous changes in the columns that are available and the technology that goes into the stationary phases used in the columns. Fortunately batch to batch variations have attenuated with time due to improvements made by the manufacturers. \(^{131-135}\)

The initial attempt to devise an HPLC method started from the method already used at Alzeim Ltd. (see Table 5.1 for details) for the analysis of the galanthamine isolated in the production line.

**Table 5.1: The gradient HPLC method used by Alzeim Ltd.**

<table>
<thead>
<tr>
<th>Gradient details</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>Water (with additive)</td>
<td>Acetonitrile (no additive)</td>
</tr>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>18</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>19</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>20*</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

**Other details of the method**

Column make: Betasil C18, Thermo Fisher

Column Specifications:

- length: 250 mm, internal \(\varnothing\): 4.6 mm, particle size: 5 \(\mu\)m, pore: 100 Å
- Flowrate (ml min\(^{-1}\)): 1.0
- Additives: Trifluoroacetic acid 0.1%
- Column Temp. (°C): 30.0 ± 0.5
- UV/vis detector wavelength (nm): 240 - 440

*The last, isocratic interval is to reequilibrate the column for the next analysis.

The initial problem was the lack of a column of the same make. The method calls for the addition of trifluoroacetic acid, however, when using the Hypersil ODS-2 C18 column that was available the addition of trifluoroacetic acid gave very poor results, it was found that changing the additive to triethylamine sharpened up the peaks, but still gave far from ideal results. At this point it was thought likely that a C18 column would have to be purchased and therefore it was decided to take this opportunity to examine the performance of a range of C18 columns for the analysis of galanthamine and extracts from *Narcissus pseudonarcissus* cv. Carlton. The other major issue was associated with the HPLC machine, which often gave very different results between consecutive runs of the same sample under the same conditions, as such each sample was run until two consecutive runs gave chromatographs of reasonable similarity (later in the work this issue was negated by the installation of new equipment). Table 5.2 lists the columns and their relative...
performance; the original Alzeim Ltd. method was used for all the analyses, except the trifluoroacetic acid was added to both the acetonitrile and the water as this was found to be a sound improvement to the original method, no doubt due to a more stable and continuous total ionic strength. A galanthamine standard solution was used for all the tests and the peak shapes compared. All the columns used in the tests were listed as having a L1 United States Pharmacopeia designation.

### Table 5.2: Relative performance of some L1 type columns for the HPLC of galanthamine

<table>
<thead>
<tr>
<th>Column name and manufacturer</th>
<th>Column Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betasil C18, Thermo Fisher</td>
<td>Length (mm) 250, Internal ø (mm) 4.6, Particle size (µm) 5, Pore size (Å) 100</td>
<td>Fair</td>
</tr>
<tr>
<td>Hypersil ODS-2, Thermo Fisher</td>
<td>Length (mm) 250, Internal ø (mm) 4.6, Particle size (µm) 5, Pore size (Å) 80</td>
<td>Poor</td>
</tr>
<tr>
<td>Supelco Analytical Column no. 116293-03</td>
<td>Length (mm) 250, Internal ø (mm) 4.6, Particle size (µm) 5, Pore size (Å) n/a</td>
<td>Fair</td>
</tr>
<tr>
<td>Luna 3, Phenomenex</td>
<td>Length (mm) 250, Internal ø (mm) 4.6, Particle size (µm) 10, Pore size (Å) 100</td>
<td>Poor</td>
</tr>
<tr>
<td>ACE 5 C18-AR, ACT</td>
<td>Length (mm) 250, Internal ø (mm) 4.6, Particle size (µm) 5, Pore size (Å) 100</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

All the tests were run using the Alzeim Ltd. method, as detailed in Table 5.1.

By far the best performance was given by the ACE 5 C18-AR column, a column specifically designed to aid in the separation of those compounds that contain aromatic groups, the alkyl chains on the stationery phase contain some aromatic groups to enable better interaction with aromatic groups in the analyte. The galanthamine peaks were symmetrical and sharp. With a suitable column in hand numerous attempts were made at modifying the timings and the gradient of the method, first running a galanthamine standard and then a sample of daffodil juice to better see the effectiveness of the new methods at separating the manifold components of the plant extract. In fact the best method found was only slightly different to the original method already in use by Alzeim Ltd. However, the slight change in method and the change in column type resulted in far sharper peaks and better separation than had been achieved using the original method. Table 5.3 details the parameters for the finalised HPLC method.
Table 5.3: The improved gradient method for the analysis of galanthamine and factory effluent fractions.

| Gradient details |  
|------------------|------------------|
| Time (min)       | Water (with additive) | Acetonitrile (with additive) |
| 0                | 95               | 5                      |
| 25               | 70               | 30                     |
| 30               | 95               | 5                      |
| 45*              | 95               | 5                      |

Other details of the method

Column make: ACE 5 C18-AR, Advanced Chromatography Technologies Ltd.

Column Specifications:
- length: 250 mm
- internal Ø: 4.6 mm
- particle size: 5 µm
- pore: 100 Å

Flowrate (ml min⁻¹): 1.0

Additives: Trifluoroacetic acid 0.1%

Column Temp. (°C): 30.0 ± 0.5

UV/vis detector wavelength (nm): 240 - 440

*The last, isocratic interval is to reequilibrate the column for the next analysis.

At the time the method was developed there was some criticism of the fact that the elution of the peaks did not occur during isocratic flow of the mobile phase, however, given the large number of peaks, for all the peaks to elute during isocratic flow would require an extended period of steady flow and it is doubtful that such conditions could yield successful separation. It should be noted that even after a gradient flow has been changed to isocratic flow the solvent system in the column would take some time to equilibrate to the point at which the eluent passing through the detector would be in an isocratic state. The method reported here is not perfect and greater peak separation would be a great benefit, however, it does represent a method with good peak symmetry and reasonable peak separation. Additionally the use of a column type that shows relatively low bleed, high temperature and pH stability, gives consistently reproducible results even with highly aqueous mobile phases, and with high interbatch fidelity should result in both a method and data that are useful and readily reproducible. Additionally the column is available in a range of sizes and the manufactures guarantee scalability.

5.2.d LC-MS and LC-HRMS for the identification of peaks in the daffodil juice

LC-MS requires a markedly lower flow rate and so the HPLC method was scaled to a lower flowrate and a smaller column using the online Gradient Method Transfer by Thermo Scientific and then further refined by experimentation. Unfortunately due to a lack of column heating there was a slight degree of peak broadening, but not so much as to
prevent collection of mass spectra associated with each peak. A smaller version of the column used for the HPLC was used for LC-MS. Table 5.4 lists the details of the modified method.

Many of the eluted peaks gave mass spectra base peaks in the range $300 \pm 50 \text{ m/z}$, as expected for the molecular ions of alkaloids from *Narcissus pseudonarcissus*. The main problem was the similarity of structure and masses between the alkaloids results in similar fragmentation patterns and effectively the same molecular ion and base peak mass between many of the eluted peaks. Additionally, the soft ES ionization method used by the LC-MS system resulted in minimal fragmentation.

The same LC method was then used for LC-HRMS; while the running of several semisynthesised alkaloids as standards enabled the collection of accurate mass data for those compounds the unknown peaks produced data that were very similar and a lack of fragmentation reduced the usefulness of the information therefore preventing the identification of the unknown peaks by this method. It was thought that a stronger ion source or the availability of HRMS-MS would have yielded more useful data.

### Table 5.4: Details of the new HPLC method scaled for use with LC-MS.

<table>
<thead>
<tr>
<th>Gradient details</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td></td>
</tr>
<tr>
<td>Water (with additive)</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile (with additive)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>15.88</td>
<td>70</td>
</tr>
<tr>
<td>19.14</td>
<td>95</td>
</tr>
<tr>
<td>30</td>
<td>95</td>
</tr>
</tbody>
</table>

Other details of the method

- **Column make:** ACE 3 C18-AR, Advanced Chromatography Technologies Ltd.
- **Column Specifications:** length: 150 mm, internal ø: 3.0 mm, particle size: 3 µm, pore: 100 Å
- **Flowrate (ml min$^{-1}$):** 0.5
- **Additives:** Trifluoroacetic acid 0.1%
- **Column Temp. (°C):** Room temp.
- **UV/vis detector wavelength (nm):** 240 - 440

### 5.2.e Preparative HPLC of daffodil juice and daffodil juice extract

It was thought that preparative HPLC would allow the isolation of numerous components of the daffodil juice, followed by their identification. The HPLC method was scaled for a
preparative HPLC column using the online Gradient Method Transfer by Thermo Scientific. Two versions of the method were developed; a quicker one (detailed in Table 5.5) and a slower one, the slower one being more ideal. Table 5.6 shows a comparison of the analytical and preparative methods.

Table 5.5: The new HPLC method scaled up for preparative HPLC

<table>
<thead>
<tr>
<th>Gradient details</th>
<th>Water</th>
<th>Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>34.89</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>41.68</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>62.52</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

Other details of the method

| Column make: | ACE 10 C18-AR, Advanced Chromatography Technologies Ltd. |
| Column Specifications: | length: 250 mm, internal Ø: 21.0 mm, particle size: 10 µm, pore: 100 Å |
| Flowrate (ml min⁻¹): | 15.0 |
| Additives: | Trifluoroacetic acid 0.1% |
| Column Temp. (°C): | Room temp. |
| UV/vis detector wavelength (nm): | 289 |

This is actually the shorter of two scaled methods, the ideal method has a run time greater than 90 minutes.

Although a successful HPLC method had been developed its transfer to preparative HPLC scale was problematic for a number of reasons. The larger column size and greater solvent flow rate meant that it was not possible to maintaining a stable temperature and the ambient laboratory temperature was only about 26 °C compared to the 30 °C used in the analytical method. This is especially significant given the large number of closely spaced peaks and the fact that a 1 °C change in column temperature can lead to change in retention times of up to about 3%, while lower column temperatures are sometimes a contributing factor to peak fronting. Certainly in this instance there was a marked loss of peak shape, leading to a drop in resolution. The direct scaling of the HPLC method resulted in a method with a run time of over 90 minutes, so some method modification was done to reduce the run time to a more manageable 63 minutes. This had an impact on the resolution, but given the large distortion caused by the lower temperature the shorter run time gave an only slightly less favourable outcome.
Table 5.6: A comparison of the analytical and preparative HPLC methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Analytical</th>
<th>Preparative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>30</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Injection volume (μl)</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Concentration (mg cm$^{-3}$)</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Mass injected</td>
<td>5 μg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Run time (min)</td>
<td>30</td>
<td>63*</td>
</tr>
</tbody>
</table>

*The ideal time is greater than 90 minutes.

A cursory glance at the information in Table 5.6 suggests additional possible pitfalls of preparative HPLC for this sort of work. The chromatogram of one of the factory effluent fractions can have in excess of 40 peaks visible at a single wavelength. Therefore the 5 mg injected contains many different compounds and so a single peak on the chromatogram represents a tiny fraction of a milligram of compound, which would eluted in tens of millilitres of what is largely aqueous solvent, this is a problem that is compounded by the afore mentioned loss of peak shape. It would therefore be necessary to perform many runs and hope that only correctly separated fractions were combined to yield enough compound to make characterization feasible. Had the objective been to only isolate a specific peak it would have been possible to develop a method that better separated the desired peak at the expense of other separations.

The further complexity is added by the fact that peak strength is not an ideal indication of concentration and is dependent on the nature of the chromophores responsible for each peak, a consequence of this is that species will be collected based on how strongly they absorb the wavelength that is being used for analysis rather than on how plentiful they are within the sample that is being examined. This is demonstrated in Figure 5.3. The use of an isoplot can help with the identification of other peaks that may be missed when only one wavelength is examined (see Figure 5.4), but even this cannot distinguish between good chromophores and plentiful components.
Figure 5.3: A chromatogram of “acid water” residue after flash column chromatography, the upper trace was recorded at 289 nm and lower trace at 274 nm. The shoulder on the leading edge of the peak at 16.881 is most likely due to the signal of additional components. Note the substantial inversion of peak intensity for the peaks at 16.881 and 19.366, solely due to a change in wavelength.
5.2.f  The use of external standards in HPLC

Given the lack of success at determining the nature of the constituent components of the daffodil juice and the factory effluent it was decided that standards would be used to hasten the identification process. As the most interesting components are those that could be used as a starting point for the semisynthesis of sanguinine and galanthamine, those alkaloids that were known to be found in various members of the Amaryllidaceae family of plants and had a suitable structure were semisynthesised from galanthamine (see Chapter 4 for details). The semisynthesised alkaloids were then used as external standards in HPLC and in the LC-HRMS. Using these external standards also allowed the ultraviolet spectrum to be collected, this could then be compared to the spectrum corresponding to the peaks on the chromatograms. In this way the presence of a small amount of narwedine was identified in the daffodil juice, however, for the peak to be detectable the sample had to be weak and the peak was only a fraction of the size of the peak for galanthamine, not much more than a bump on the base line.

Given the daffodil growing process had been optimized to give the highest yield of galanthamine possible it is perhaps not surprising that there was not much narwedine, the biological precursor of galanthamine, present in the plant extract. The other alkaloids used as standards were not found in the various fractions of factory effluent or the daffodil juice, here again, given the close structural relationship to galanthamine and the optimization of
the growing process this is perhaps not so unexpected. Moreover, several of the structurally similar alkaloids are essentially galanthamine with various hydrolysable groups at heteroatom positions. Given that the factory process exposes the alkaloids to relatively strong acidic and basic conditions, often for prolonged periods of time, it is possible that acetyl groups at these positions would be removed during the extraction process of the galanthamine. However, this would be expected to lead to the presence of a norgalanthamine peak, which was not observed. Further to this Alzeim Ltd. purchased a sample of narciclasine and this too was used as an external standard.

![Narciclasine](image_url)

Figure 5.5: Narciclasine, 142, purchased for use as an HPLC standard.

Apart from narwedine (and those standards isolated from the plant extract in the first instance) the other standards indicated that they were not naturally present in the various fractions analysed.
Figure 5.6: Alkaloids, from semisynthesis or isolated from the factory output, used as standards in HPLC analysis of daffodil juice and factory effluent. The alkaloids are ordered in increasing retention time: Sanguinine, 22, norgalanthamine, 19, galanthamine, 12, narwedine, 24, haemanthamine, 10, homolycorine, 11, N-formylnorgalanthamine, 21, O-acetylgalanthamine, 18. Haemanthamine and homolycorine were both isolated from the plant extracts and were not semisynthesised.

Table 5.7: A list of standards used in the search for semisynthetically useful alkaloids.

<table>
<thead>
<tr>
<th>Standard</th>
<th>R&lt;sub&gt;t&lt;/sub&gt; (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanguinine, 22</td>
<td>7.239</td>
</tr>
<tr>
<td>Norgalanthamine, 19</td>
<td>8.348</td>
</tr>
<tr>
<td>Galanthamine, 12</td>
<td>9.156</td>
</tr>
<tr>
<td>Narwedine, 24</td>
<td>11.510</td>
</tr>
<tr>
<td>Haemanthamine, 10</td>
<td>11.953</td>
</tr>
<tr>
<td>Homolycorine, 11</td>
<td>12.500</td>
</tr>
<tr>
<td>N-formylnorgalanthamine, 21</td>
<td>12.878</td>
</tr>
<tr>
<td>O-acetylgalanthamine, 18</td>
<td>13.284</td>
</tr>
</tbody>
</table>

The method used was the standard HPLC method as detailed in Table 5.3, the retention times are those from the upper chromatogram in Figure 5.7.
Figure 5.7: Two chromatograms. Upper; a series of standards; synthesised starting from galanthamine or isolated from various fractions of plant extract. Lower; a sample of factory effluent. Although some of the peaks coincide analysis of the UV spectra indicates that the peaks did not arise due to the presence of the same alkaloids in both fractions. The exceptions to this being where the standards were originally isolated from the daffodil juice.
5.2.g Column Chromatography for the isolation of components of daffodil juice and factory effluent.

Given the limited success at identifying the components of the daffodil juice and factory effluents, further attempts were made at isolating the main constituents of the various factory effluents. As has been already stated attempts at further separation of the mixtures by acid base extraction were unsuccessful. This is not so unexpected, as the initial separations carried out in the factory would have been the most general, separating first the acidic and basic components and then those that favoured either organic or aqueous phases. Each successive separation would require finer and more subtle differences to yield a successful separation, not easily accomplished on a useful preparative lab scale. Here high performance counter current chromatography (HPCCC) could offer a significant advantage to more traditional methods. However, given the lack of readily available HPCCC, column chromatography was used. As stated in section 5.1, column chromatography was used with a slow flowrate and a gradient solvent system. This enabled the various fractions of factory effluent to be slowly divided into mixtures of steadily fewer components. Haemanthamine and homolycorine were both successfully isolated in this way (several times each). The unusual structure apohaemanthamine, 145, previously reported by others, was also isolated. This most likely arose from an acid catalysed elimination and cyclisation of haemanthamine and is more likely thought to be an artefact of the extraction process than a natural product.

Scheme 5.1: A proposed mechanism for the formation of an unusual alkaloidal structure.
This theory is supported by the fact that those that previously reported this compound did so after synthesising it from haemanthamine, crinamine or 11-hydroxyvittatine (also called hamayne), simply by gently heating the starting alkaloid in 6 molar hydrochloric acid for a short period of time.\textsuperscript{139-141}

![Alkaloids](image1.png)

Figure 5.8: Alkaloids used as the starting point for the synthesis of apohaemanthamine, 145; haemanthamine, 10, crinamine, 146, and 11-hydroxyvittatine (or hamayne), 147.

One problem with this method is that on occasion spots that appeared strong by thin layer chromatography correlated to negligible levels of product. An additional problem was the fact that the sample contained spots with a wide range of retention factors and therefore finding a suitable solvent gradient was not straightforward with some spots not eluting in the order predicted by thin layer chromatography.

![TLC](image2.png)

Figure 5.9: A typical TLC of the fractions following column chromatography of the small sample of the residue collected from the “acid water” effluent; tailing and co-elution are clearly evident. (TLC conditions; dichloromethane: methanol: concentrated aqueous ammonia 9:0.9:0.1).

In addition to those compounds isolated outright, column chromatography samples were eventually prepared that contained only a few alkaloids, in some instances perhaps as few
as two as shown by the relative simplicity of the $^1$H NMR, although analytical HPLC often indicated a far higher number of components.

![Chromatogram arising from analytical HPLC](image)

Figure 5.10: Chromatogram arising from analytical HPLC; an example of an alkaloid mixture that was successfully reduced to relatively few components (assuming comparable levels of absorption at the monitored wavelength of 289 nm).

### 5.3 The identification of natural products from spectroscopic data

The identification of haemanthamine, 10, is given as an example of the methods used to identify the alkaloids present in the crude daffodil juice.

$^1$H, $^{13}$C, 2D NMR, electrospray mass spectrometry, and infrared spectrometry were used to gather spectroscopic information about the compounds isolated. It had been found that the alkaloids generally gave the best results when the mass spectrum was run in positive mode, however, a few of the galanthamine based compounds were found to give better results in negative electrospray mass spectrometry. Therefore to increase the chance of obtaining the molecular ion both positive and negative electrospray mass spectrometry were run for each unknown compound. The more likely molecular ion was then picked based on the intensity of the signals reported.
Figure 5.11: The electrospray mass spectrometry of haemanthamine, 10. The greater intensity of the positive signal was taken as an indication that this was the molecular ion. The peak at 302 m/z was taken to equate to a mass of 301, the odd mass was taken as an indication that the compound contained a single nitrogen.

Figure 5.12: The infrared spectrum of haemanthamine, 10.
After a mass has been determined the infrared is used to check for the presence of carbonyl, carboxylic acid, hydroxyl or primary or secondary amines. The $^{13}\text{C}$ and 135DEPT NMR are then used to determine the number of carbon environments. Due to the origins of the compounds it was thought unlikely that the compounds would be symmetrical and therefore as a starting point the number of carbon environments was taken to be equivalent to the number of carbons in the compound.

![Figure 5.13: $^{13}\text{C}$ and 135DEPT NMR of haemanthamine, 10.](image)

Together this information indicates the presence of 17 carbon environments; 5 quaternary, 4 CH$_2$, 8 CH or CH$_3$, and a methanol signal (at 50.5 ppm).

A similar strategy is than applied to the $^1\text{H}$ NMR of the compound, in this instance the integrations suggest 19 hydrogens in the compound. The use of the comprehensive review of *Narcissus* alkaloids by Bastida et al.$^{23}$ allowed potential assignments to be made and solvent impurity peaks to be identified. More general knowledge about natural products and more specifically alkaloids from *Narcissus pseudonarcissus* is applied; that is that they contain a number of stereocentres, contain more oxygen than nitrogen, normally contain at least one aromatic ring, often have bridged carbon skeletons and have polycyclic skeletons. Following this an approximate molecular formula can be calculated. Due to the relatively low molecular mass, 301 and the assumption that the compound contains more oxygen than nitrogen and has a 17 carbons and 19 hydrogens, it is assumed that the compound contains only a single nitrogen. This is because the mass of 301 requires an odd number of
nitrogens; more than a single nitrogen would therefore require too many nitrogens and leave too little remaining for a suitable number of oxygens. So far this gives a mass of:

\[ 14 + 19 + (12 \times 17) = 237 \]

![Figure 5.14: $^1$H NMR of haemantamine, 10. The assignments are detailed on the spectrum.](image)

The remaining mass is most likely due solely to the presence of oxygen, which indicates that there are 4 oxygens:

\[ (301 - 237) \div 16 = 4 \]

This gives an overall chemical formula of $\text{C}_{17}\text{H}_{19}\text{NO}_4$. This slightly unorthodox method for determining the chemical formula has the advantage that it does not rely on ultra-pure samples (which can be hard to obtain in sufficient quantities from natural sources). From this point on the 1D and 2D NMR data is interpreted in the usual manner. The well
documented families of alkaloids present in the plant species in question can be used to guide the more tricky assignments, thereby accelerating the elucidation of the final structure.

5.3.a GC-MS for the identification of peaks in the daffodil juice

Following the failed attempts to identify the components of the daffodil juice using LC-HRMS, the potential of GC-MS was briefly examined. The slightly harsher chemical ionization technique used by the GC-MS led to more fragmentation and therefore to a little more useful data. The GC-MS gave good separation using a method published by Witte et al. However, comparing the fragmentation patterns with those published did not yield any new information, only confirming the presence of alkaloids the presence of which was already known. Table 5.8 compares the results for galanthamine and the published results for galanthamine.

Table 5.8: The results of the GC-MS analysis of daffodil juice and factory effluent and comparative literature data.

<table>
<thead>
<tr>
<th>Suggested Alkaloid</th>
<th>Observed peaks (m/z) (intensity in %)</th>
<th>Literature peaks (m/z) (intensity in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galanthamine</td>
<td>287 (93), 286 (100), 244 (40), 216 (66), 174 (64)</td>
<td>287 (97), 286 (100), 244 (30), 216 (45), 174 (43)</td>
</tr>
</tbody>
</table>

The literature values are taken from Witte et al.

Some other fragmentation patterns were matched with patterns for unnamed alkaloids, while many other peaks that eluted from the GC also gave patterns that were similar to those reported in the literature, but slight differences in fragmentation and/or peak intensity coupled with the overall similarity between the spectra prevented identification of the corresponding chromatographic peak. While the GC-MS achieved the identification of some of the components of the plant extract it was of little use to Alzeim Ltd. due to the company’s lack of necessary equipment, so not much time was spent on this aspect of the work.

5.3.b Further work in this area

Although there is still a considerable amount of work to be done in this area after the liquidation of the industrial sponsor, Alzeim Ltd, there was no access to fresh factory effluent, thus work on this area slowed dramatically and focused solely on the column chromatography of the dry residue of the various factory effluents. The reason for this is
that the presence of both 3,4-didehydro-3-deoxygalanthamine and the haemanthamine derivative indicate that at least some of the alkaloids are undergoing chemical reactions either during the extraction process or while waiting for further analysis. Using old samples of effluent would only increase the chances that any new alkaloids identified were simply artefacts of extended storage especially when stored in wet solvents.

Moreover, the winding up of Alzeim Ltd lowered the imperative for the expansion of knowledge in this area, especially given the most obvious starting points for the intended semisynthesis had already been found to be absent in any appreciable quantity.

Furthermore, as this area of work was focused on the factory effluent and daffodil juice from the ensilaging process, any change to the extraction process, growing conditions or the use of a different species of *Narcissus* would require this work to undergo some significant revision. A change in the extraction procedures could alter any reactions that took place during the extraction and therefore change the proportions and possibly the type of any artefacts present. While the other changes could all lead to changes in the proportions and even the varieties of alkaloids found.

In summary four components of the plant extract were isolated and characterized, the presence of narwedine was determined by HPLC. None of the alkaloids found in the daffodil juice or the factory effluent were considered to be a useful starting point for the semisynthesis of galanthamine or sanguinine, the only exception being narwedine, the biological precursor of galanthamine.

Figure 5.15: A summary of the compounds isolated from samples taken from the factory production line.
6  The resolution of narwedine

6.1  The semisynthesis of galanthamine from narwedine

As discussed in the introduction the literature already contains some well-established methods for the synthesis or semisynthesis of galanthamine from narwedine, including methods proposed for used on an industrial scale. In this work we were especially interested in the methods by which racemic narwedine can be resolved. The commercially used entrainment method is well developed. Moreover, entrainment as a general method is well developed and so is not examined here. The dynamic diastereomeric salt resolution method is only briefly examined as it is of lesser interest due to the fact it requires the use of more reagents. The final and more unusual method by means of a galanthamine-controlled spontaneous resolution is examined in more detail.

6.2  The resolution of narwedine by the dynamic diastereomeric salt resolution method

The cocrystallization of narwedine with di-p-toluoyl-D-tartaric acid, as initially reported by Chaplin et al., was only briefly examined for comparative purposes. Attempts to form the 2:1 salt were unsuccessful instead leading to the formation of an oil. The 1:1 salt was formed and it was found that it could be cracked with L-Selectride to yield galanthamine, attempts to purify this by precipitation as the hydrobromide salt were not promising and resulted in minuscule yields, possibly due to the relatively small scale of the attempts. However, while the reduction and cracking were monitored by thin layer chromatography the reaction time reported by others was far longer and this could mean that the reduction did not go to completion, in turn contributing to the low yield. The literature contains mixed reviews of this method with some but not all reporting difficulty in reproducing the results. Although this method looks promising, from an industrial stand point it has several drawbacks. Firstly it requires the addition of stereometric amount of an additional reagent and more rigorous purification, both of which make it a less economical alternative. Additionally, because the method ultimately uses the same reducing agent and solvents, L-Selectride and tetrahydrofuran respectively, there are no financial savings from using cheaper or more benign reagents elsewhere in the process. Consequently, little consideration was given to this area of investigation.
6.3 Galanthamine controlled resolution of narwedine

This unusual method for the resolution of narwedine, 24, is best demonstrated diagrammatically and for convenience the scheme from the introduction is reproduced here (see Scheme 6.1 or Scheme 1.4)

Scheme 6.1: The galanthamine, 12, “catalysed” dynamic resolution of narwedine, 24. An amount (0.5 – 0.01 equivalents) of optically pure galanthamine is added to a solution of racemic solution of narwedine in ethanol and triethylamine. The addition of one enantiomer of galanthamine will result in the isolation of the opposite enantiomer of narwedine. Note that in both instances the phenolic oxygen attacks the same face of the achiral dieneone, 29. a) L-Selectride in tetrahydrofuran at -78 °C. Reproduced from Scheme 1.4 for ease of reference.

It has been reported that as little as 1% by mass of the opposite enantiomer of galanthamine can be used to affect the resolution. The first attempt at reproducing this proceeded to give an enantiomeric excess of 98%, however, due to a miscalculation a 10% level of galanthamine was used and additional solvent was needed to completely dissolve the narwedine. Repeating the experiment with only 1% galanthamine proceeded excellently (97% ee, +411.2, Lit. +412.9) without the addition of any base (triethylamine); moreover there was no discoloration and no foul smell from the recovered narwedine, both of which were a constant feature when resolution was base catalysed.
The crystals of (+)-narwedine were reduced to (+)-galanthamine (100% ee, +96.8) giving no detectable (-)-galanthamine or epigalanthamine by HPLC, although it should be noted that using the chiral columns available there is a slight overlap of the galanthamine peaks. However, the smooth peak shape and the optical rotations indicate that there was no (-)-galanthamine present. Natural (-)-galanthamine has a specific rotation of -97.2° corresponding favourably with that found for the (+)-galanthamine. However, to achieve selective reduction the reaction was run at -78 °C and the cold L-Selectride needed to be added to the crystals and stirred as a suspension. This meant that a lot of the crystals did not dissolve, resulting in a low yield. Identification of the (+)-galanthamine was by NMR, and ES-MS; the spectra were in good agreement with the literature.

After the initial success there were several failed attempts to repeat this degree of resolution. Initially it was thought that the difference in resolution was due to the formation of crystals at the shore of the solvent, where it was observed that crystallization occurred very quickly, even before complete dissolution had occurred. To overcome this the solvent shore was minimised by using vessels of greater aspect ratio to reduce the volume to solvent shore ratio and give over all less shore. The more upright nature of these vessels also meant that there was less of a shore effect. The resolution to the desired (-)-narwedine, precursor to the desired natural (-)-galanthamine also proved to be problematic and it was found that 10% by mass of (+)-galanthamine was not sufficient to give full resolution to (-)-narwedine. The crystalline form of narwedine was decidedly less soluble in ethanol than the more amorphous form, to the extent that even after several hours refluxing in ethanol the crystals did not dissolve, moreover the pure white crystals used in these experiments became discoloured and took on an unpleasant odour. This solubility issue was bypassed by the reamorphousization of the narwedine by dissolving clean crystals in dichloromethane and then removing the solvent under reduced pressure to give a more disordered product that would dissolve in ethanol. However, the amorphous narwedine still resisted attempts to resolve it. The reason for this was initially unclear as the narwedine appeared free of galanthamine as determined by both HPLC and in a 0.156 mol dm⁻³ NMR sample (400 MHz 16 and 32 scans). However, given that 1% addition of galanthamine is sufficient to induce complete resolution it is possible that a very tiny dose would be sufficient to prevent resolution.

The narwedine that had been used for these attempts had already been resolved to (+)-narwedine so it was thought possible that this bias in the starting material was what was causing the problems. However, following dissolution in ethanol the narwedine gave no optical rotation indicating that it had racemised; the problem persisted.
A new and larger batch of narwedine was prepared, yet again the resolution failed to proceed. As the narwedine was semisynthesised from galanthamine and still had a slight smell of Swern reaction to it even after column chromatography it was thought that possibly there were trace impurities that were inhibiting the resolution, hence the narwedine was recrystallized prior to further resolution attempts. It is important to note that this synthesis of narwedine was done on a larger scale and the reaction appeared to have been far cleaner and yielded a less smelly product than the initial attempt. However, attempts at the resolution of the purer narwedine did not yield a better enantiomeric resolution. Following purification by recrystallization the narwedine did not show a bias for either enantiomer, which would be expected if there were a contaminant present that influenced the enantiomeric ratio in a dynamic manner. Neither NMR nor thin layer chromatography of the crude narwedine showed any galanthamine to be present.

By repeating the experiment several times it was elucidated that the reliability of the reaction was determined by impurities, however, rather than impurities limiting the extent of the resolution they aided the resolution and ensured a higher enantiomeric purity. As the natures of the impurities, which were enough to slightly colour and scent the narwedine without giving identifiable structures by NMR, were never discovered it is hard to say in what way they were impacting the resolution. If the published theory, in which the galanthamine is thought to adhere to the surface of the unfavoured crystals of narwedine thereby inhibiting their growth, is correct then there are two more likely roles for the impurities. Either the impurities have a general inhibiting effect on the rate of crystal growth, slowing the whole process allowing more time for the bias in crystal growth to build up, or alternatively by interacting with the galanthamine directly and possibly increasing its effect; say by interaction with the hydroxyl group of the galanthamine making the galanthamine appear larger and therefore more inhibiting to crystal growth. Alternatively, the impurities maybe accelerating the rate of interconversion, say if they had a slightly basic nature. Either way, the nature of impurities, to be uncontrolled and unknown, makes a full analysis of this extremely difficult. Moreover, from a semisynthetic stand-point this information is of limited use as any narwedine extracted from the daffodils would have different impurities that would most likely have different interactions, leading to very different consequences. Therefore, for industrial use the method would need to be developed as required based on the narwedine extracted from the production line. Clearly the development of a method that worked on pristine narwedine could be used industrially, however, this may involve additional purification which may in practice not be necessary, especially if the impurities present were found to have an
advantageous effect on the resolution process. Clearly there is room for substantial speculation here but without industrially extracted samples it is not possible to say.

The observed, if not quantified, impact of the impurities on this resolution process goes some distance to explaining the differences in the amounts of galanthamine that different groups stated was required for the successful resolution.

Industrially this method has the potential benefit that after an initial batch of (+)-galanthamine is prepared it could be used to resolve proportionally vary large amounts of narwedine and as has been shown here if the conditions are correct each batch could be fully resolved in a single attempt. This should lead to economic benefits of saving both time and energy over the entrainment methods that would require several recrystallization attempts to bring about greater than 90% resolution.

It is also worth considering that as part of their work in trying to perform a thermodynamic classical resolution with a chiral acid Chaplin et al. found that some of the acids they screened did not form salts with the narwedine but instead induced the free base of narwedine to crystalize in an enantioenriched form. This poses an interesting question as to whether there is a general possibility to tip the enantiomeric ratio, of a racemic mixture of conglomerate enantiomers, in which the interconversion between enantiomers is facile, by adding a third, possibly entirely unrelated, compound. Certainly, when considered with the work of others, in this case it would seem that a number of “catalysts” can be used; including both those with and without a similar core structure to the conglomerate to be resolved. This is typified by the work of Barton, and Shieh and Carlson who used “catalysts” with a core structure similar to that of galanthamine for the resolution of narwedine, while Chaplin et al. used chiral acids, most notably pyrrolidone-5-carboxylic acid; the use of the (R)-acid gave 99% e.e. of (+)-narwedine, while the use of the (S)-acid gave an equally successful resolution of (-)-narwedine.

A final attempt to resolve clean narwedine, using 50% by mass of galanthamine, gave a resolution comparable with the initial trial made on less pristine narwedine.

## 6.4 The synthesis of narwedine analogues

Barton et al. have already shown that structurally altered galanthamine could be used to resolve the narwedine and Chaplin et al. were able to use chiral acids to facilitate the resolutions in a similar fashion, albeit to a lesser extent. In this investigation, it was decided to strip away the structure of narwedine to see how much of the structure was required for this resolution method to work. Galanthamine would still be used to steer the
resolution. The three obvious analogues of narwedine are those that have the methyl groups removed; leading to a slight reduction in the size of the narwedine but not touching the core structure.

![Chemical structures](image)

Figure 6.1: Three analogues of narwedine; O-desmethylnarwedine, 148, normarwedine, 149, and O-desmethylnormarwedine, 150.

### 6.4.a The synthesis O-desmethylnarwedine

Using the same methods used for the synthesis of alkaloids as outlined in Chapter 4 it was thought that the analogues could be synthesised quickly and efficiently. However, in the first instance after the sanguinine, 22, had been synthesised in the usual fashion it was found that the sanguinine would not oxidise in a satisfactory manner. Swern oxidation, as used for the oxidation of galanthamine, did not work, instead resulting in a mass of dirty products. The use of Dess-Martin periodinane\(^ {142} \) resulted in almost instant degradation. 2-Iodoxybenzoic acid\(^ {143} \) also gave degradation, albeit more slowly than Dess-Martin periodinane. When iodoxybenzoic acid was used the initial thin layer chromatographies of the reaction mixture suggested the product may have been forming, but the rate at which the possible product formed was outstripped by the rate at which the multitude of decomposition products formed. Several solvents were screened to see if they gave more favourable results, all without success. The use of manganese dioxide resulted in a messy mixture of compounds and possibly a sub 1% yield of product. The condition of the manganese dioxide was assessed by oxidising galanthamine to narwedine, the reaction proceeded as reported by Barton et al.\(^ {32} \) The use of catalytic tetrapropylammonium perruthenate with the co-oxidant N-methylmorpholine N-oxide was also found to be an unsuccessful method of oxidation in this instance.

Following these failures, a synthetic route was developed that avoided the direct oxidation of sanguinine. The most obvious route is a simple and typical three step protection deprotection sequence, interspersed with the desired transformation. Taking narwedine, 24, as the starting compound this translates into; the protection of the narwedine carbonyl
as the ketal, 151, followed by cleavage of the \( O \)-methyl group to give 152 and then deprotection of the carbonyl to yield \( O \)-desmethylnarwedine, 148.

![Diagram](image)

**Scheme 6.2:** A proposed, yet unsuccessful, route for the synthesis of \( O \)-desmethylnarwedine, 149.  

- **a)** Either catalytic \( p \)-toluenesulfonic acid, 3 Å mole sieve and ethylene diol refluxed in toluene or catalytic trimethylsilyl trifluoromethanesulfonate, 1,2-bis(trimethylsilyloxy)ethane and pyridine in dichloromethane.  
- **b)** \( L \)-Selectride in tetrahydrofuran and refluxed overnight.  
- **c)** Catalytic \( p \)-toluenesulfonic acid in acetone and reflux.

However, attempts to protect the carbonyl as the ethylene glycol ketal, 151, using both standard and Noyori\(^{144}\) conditions, were unsuccessful, on one occasion proton NMR of the crude reaction mixture seemed to show that some of the protected ketal had formed but none of the desired product was isolated. The failure of this route was unanticipated as the protected narwedine analogue, 36, is known, albeit as a product from a step in a total synthesis of galanthamine rather than from the direct protection of narwedine.\(^{124, 145}\)

The next, and less direct route, was developed from a similar route used for some of the lithiation chemistry and starts from galanthamine. The galanthamine hydroxyl group was protected with a silyl group; using triisopropylsilyl trifluoromethanesulfonate and 2,6-lutidine in dry dichloromethane, which reliably proceeded in 95 – 100% yield. Initially there had been attempts to use the tert-butylidemethylsilyl group as the triisopropylsilyl group had proved difficult to remove in the lithiation chemistry (see chapter 7), however the tert-butylidemethylsilyl was found to be less resilient to the \( O \)-demethylation reaction conditions. Moreover, the tert-butylidemethylsilyl protection only proceeded in 57% yields and that was with heating over two days compared to the mere hours at room temperature required to install the triisopropylsilyl group.
Scheme 6.3: The initial steps of the TBDMS-protected synthetic strategy for the synthesis of O-desmethylnarwedine, 148. a) tert-butylimethylsilyl trifluoromethanesulfonate and 2,6-lutidine in dichloromethane, 0 °C then reflux for 2 days. b) L-Selectride in tetrahydrofuran and reflux overnight.

Scheme 6.4: The TIPS-protected, seven step, synthetic strategy, for the synthesis of O-desmethylnarwedine, 148. a) Triisopropylsilyl trifluoromethanesulfonate and 2,6-lutidine in dry dichloromethane. b) L-Selectride in tetrahydrofuran and reflux overnight. c) Trimethylacetyl chloride and sodium hydride in tetrahydrofuran. d) Tetrabutylammonium fluoride in tetrahydrofuran. e) Swern oxidation. f) 2 M Sodium hydroxide solution in ethanol.

Once the hydroxyl group was TIPS protected to give, 155, the O-methyl group was cleaved using L-Selectride in tetrahydrofuran, analogous to the way in which sanguinine is formed from galanthamine, to give 156. The phenolic hydroxyl group was then protected with a pivaloyl group using trimethylacetyl chloride and sodium hydride to give 157. The silyl protecting group was then cleaved with tetrabutylammonium fluoride to give O-pivaloyl galanthamine, 158, in 63% yield. Pleasingly this deprotection proceeded far more smoothly than the deprotection of the similar compounds in the lithiation chemistry.
This deprotected O-pivaloyl sanguinine, 158, was oxidised to the narwedine analogue, 159, using the usual Swern method (discussed in 4.2.a). The O-pivaloyl narwedine, 159, was then deprotected using aqueous sodium hydroxide in ethanol to give O-desmethylnarwedine, 148.

It was found that this seven step sequence could be shortened by ejecting the silyl protection deprotection steps from the sequence. This was achieved by first synthesising sanguinine, 22, and then taking advantage of the fact that the phenolic hydroxyl group is more acidic than the allylic hydroxyl group and therefore by using a stoichiometric amount of sodium hydroxide it is possible to preferentially protect only one of the two hydroxyl groups to give 158. Moreover, as noticed throughout this report the allylic hydroxyl group stubbornly refuses to react in this sort of reaction. Due to a shortage of trimethylacetyl chloride and time boc anhydride was used to protect the phenolic hydroxyl group resulting in the O-boc narwedine compound 159.

![Scheme 6.5: The refined five step synthesis of O-desmethylnarwedine.](attachment:Scheme_6.5.png)

The O-Boc narwedine, 159, was deprotected by treating with aqueous sodium hydroxide, a little alcohol had to be added to dissolve the narwedine analogue, to give 148.
6.4.b The synthesis of nornarwedine and O-desmethylnornarwedine and N-Boc narwedine

The N-demethylated narwedine analogue, nornarwedine, 149, was synthesised from narwedine using the same one pot method used to synthesis norgalanthamine from galanthamine. Briefly, the narwedine was treated with 3-chloroperbenzoic acid to form the narwedine N-oxide, this was then acidified and catalytic amounts of iron powder and iron(III)chloride hexahydrate were added (for a more complete discussion of this method see 4.2.b). While there was a general variation in the success of this reaction it generally gave at least some product, without the complete failures that were sometimes found for the N-demethylation of galanthamine. However, given the capricious nature of this reaction, without further study no reliable conclusions can be drawn from this observation.

Despite the apparent success of the N-demethylation reaction no nornarwedine, 149, was isolated. Instead the enantiomeric structures, 161, were isolated. NMR analysis showed it to be the product of nucleophilic attack of the secondary amine on the dienone resulting in a new pair of enantiomeric structures, the remainder of the material in the reaction mixture seemed to be a range of decomposition products.

Scheme 6.6: The N-demethylation of narwedine to form nornarwedine, performed as a one pot reaction. a) 3-Chloroperbenzoic acid in chloroform/isopropanol (3:1) at -10 °C, then hydrochloric acid, b) Water, then iron(0) powder 13 mol% and iron(III) chloride hexahydrate 2 mol%, at room temperature. Followed by the formation of new enantiomeric structures, 161, from racemic nornarwedine, 149. The numbering allows for easy comparison against crinine and haemanthamine type alkaloids. The yield of 161 was 19%.
Needless to say this analogue was not resolved. The two enantiomers isolated have a strong resemblance to crinine and haemanthamine type alkaloids, however, the aryl moiety of such structures carry substituents at positions 8 and 9 and not 9 and 10 as see here. However, alkaloids with the same substitution pattern have been isolated from Crinum L., a lily in the Amaryllidaceae family, by Popov et al. who described it as a crinane type alkaloid. In fact this cyclization of nornarwedine, has been observed by others; Mereiter et al. describe it as a Seco-isopowellaminone, a Crinan type alkaloid. The fact that nornarwedine undergoes such rapid cyclization puts doubt on the idea, postulated by some, that nornarwedine is the actual biological precursor to galanthamine.

Left over from some other work (see section 8.2.a) some N-Boc galanthamine, was available and this was oxidised, using the same method as for the oxidation of galanthamine, to narwedine, with a view to deprotecting it and adding it to the nornarwedine, that was being synthesised. However, given the observations above about nornarwedine, it was decided not to deprotect it, instead examining it directly for the ability to be resolved.

Scheme 6.7: The formation of N-Boc nornarwedine, a) Di-tert-butyl dicarbonate and triethylamine in dichloromethane at room temperature. b) Oxalyl chloride in chloroform with dimethyl sulphoxide at -60 °C for 5 min, then -50 for 40 min, then triethylamine for 20 min.

A concomitant attempt to synthesise O-desmethylnornarwedine, from O-desmethylnarwedine, only returned starting material and given the reactivity displayed by nornarwedine, no further attempts were made at the synthesis of O-desmethylnornarwedine.

6.5 The resolution of narwedine analogues

To limit the effects of any impurities on the resolution process the narwedine analogues were used in as pure form as possible, either from recrystallization or column chromatography.
6.5.a Resolution of N-Boc narwedine and O-Boc narwedine

It was found that N-Boc-narwedine, 163, could not be resolved via the unusual recrystallization process. An X-ray structure was obtained from one of the crystals grown and this clearly showed that the compound was not a conglomerate. It is assumed that this is the case for the very similar O-Boc narwedine, 159. Neither were resolved.

Figure 6.2: X-Ray structure of N-Boc galanthamine, 163, showing the racemic nature of the crystal.

Figure 6.3: X-Ray structure of N-Boc galanthamine, 163, (an alternative view), showing the racemic nature of the crystal.

6.5.b The resolution of O-desmethylnarwedine

When O-desmethylnarwedine, 148, was added to ethanol it first dissolved and then immediately began to crystalize giving the mixture a metallic like pearlescent appearance. Changing the solvent for the recrystallization from ethanol to methanol did not notably improve the situation and copious amounts of methanol were not enough to dissolve the
crystals. The crystals were eventually found to be soluble in dimethyl sulfoxide, however, if left for an extended time in this solvent the compound began to decompose, as evidenced by the development of a rich brown colour and deteriorating NMR spectrum. This unfavourable and excessive tendency to crystallise meant that the attempts to resolve \( O \)-desmethylnarwedine, 148, were not successful.

6.5.c The resolution of narwedine analogues; a summary

Clearly issues regarding solubility and the stability of the compounds prevented a better analysis of the resolution process of narwedine and its analogues. The greater propensity of both narwedine, 24, and sanguine, 22, to crystallize compared to galanthamine, 12, indicated that \( O \)-desmethylnarwedine, 148, would display unfavourable solubility, but the extent of such resilience to dissolution could not have been anticipated. Given the low proportion of enantiomers that crystalize as conglomerates it had been expected that at least some of the analogues would not be conglomerate.

The work undertaken here strongly indicates that impurities can play an important role in the resolution process, any future work in this area should perhaps concentrate on searching for other additives that can be used to encourage the resolution process in the same way as galanthamine does.
7 Ortholithiation chemistry for galanthamine C8 functionalization

A search of the literature indicated that while many derivatives of galanthamine are described few include derivatization ortho to the methoxy group; most of those that are reported as derivatized at the C8 position are only intermediates in the total synthesis of galanthamine. Therefore, attempts were made to derivatize galanthamine at this, hitherto largely neglected, position via ortholithiation.

Given the complexity of the galanthamine structure it was thought that a directing group of reasonable ability would be required to ensure that only the desired position was lithiated and so avoid undesirable side reactions. The presence of the methoxy group at the C9 position meant an oxygen containing directing group would be more easily installed; thus the easily removable methoxymethyl ether was selected for ortholithiation trials.

Sanguinine was used as the starting material and both the hydroxyl and phenol groups were protected with the methoxymethyl ether using the method reported by Guedin-Vuong. Proton NMR indicated that the product had formed, but it displayed appalling solubility, only proving to be soluble in alcohols and chlorinated solvents.

In an attempt to overcome the problems with solubility the route was amended to start from galanthamine, the allylic alcohol of which was protected with the TIPS group by way of the method reported by Han et al., to give 155. It was hoped that the greasy nature of the TIPS group would increase the solubility. The TIPS group installed excellently, with near quantitative yields, and the installation was accompanied by loss of the H-bonded hydroxyl group band at 3589 cm\(^{-1}\) from the IR spectrum. The TIPS protected galanthamine was a loamy smelling green tinted oil. Using the method for the preparation of sanguinine the phenol was unmasked to give a solid, 156.† The TIPS protected sanguinine was then protected with the methoxymethyl ether group. Once again the product lacked solubility in solvents suitable for ortholithiation.

Given the solubility problem encountered with the use of the methoxymethyl ether directing group a new directing group was selected; an O-carbamate. This too could be synthesized from the TIPS protected sanguinine, 156, by way of a simple reaction with diethylcarbamoyl chloride, meaning no additional changes to the synthetic strategy. The

† On one occasion the solid product was agitated in a flask and underwent a colour change from yellow to a deeper yellow orange colour, accompanied by the evolvement of a considerable amount of heat. NMR showed that the product was undamaged.
carbamoylation method was adapted from a similar reaction reported by Quasdorf et al.\textsuperscript{150} A further advantage to the use of this group was that because the $O$-carbamate is often considered the most powerful directing group if it did not give a good result with ortholithiation it would suggest that any further work in this area was unlikely to yield a positive result. It was accepted that the carbamate group could not be removed as easily as the methoxymethyl ether, however, carbamates are known to be good inhibitors of AChE due to the way in which they are hydrolysed in the active site.\textsuperscript{12} Therefore the enduring presence of such a group was not thought to be overly damaging and could even prove beneficial.

As it turned out the carbamoylation did not proceed smoothly, while ES-MS and TLC indicated the absence of starting material, after purification there was a significant proportion of starting material present. Pushing the yield to near 40% took an extended period of time. The purification was tricky and that product was an extremely sticky golden orange oil. However, this product, \textbf{164}, did show favourable solubility in tetrahydrofuran.

![Scheme 7.1: The five step ortholithiation strategy.](image)

a) Triisopropylsilyl trifluoromethanesulfonate and 2,6-lutidine in dry dichloromethane. b) L-Selectride in tetrahydrofuran and reflux overnight. c) Sodium hydride and diethylcarbamoyl chloride in THF, stirred overnight. d) sec-Butyllithium and TMEDA in THF at -78 °C, then quench with electrophile. e) TBAF in THF. For \textbf{165} R groups see Table 7.1.
With a suitably soluble substrate, with a powerful directing group, in hand the ortholithiation trials started. The initial trials in this area used sec-butyllithium and TMEDA to promote metalation at the ortho position. The reactions were then quenched with dimethyl disulphide and 1,2-dibromoethane. The reactions appeared to work very successfully, to the point that following workup the crude NMR indicated that the product was already of high purity. However, later attempts to replicate this were less successful, with a greater degree of starting material still present in the reaction. As this was found to be the case using the same electrophile sources as used in the original trials it is thought that the most likely cause of this is the condition of the sec-butyllithium used. The initial samples were prepared using an older, and notably cloudy, sec-butyllithium, while latterly a newer and clearer sec-butyllithium was used. It is possible that the older sec-butyllithium contained a higher proportion of lithium salts and that these assisted with the deaggregation of the butyllithium resulting in greater reactivity and a cleaner reaction. The purification of the products proved difficult and many could not be separated effectively from the starting materials, as a consequence of this they were taken on to the silyl deprotection step in an impure form.

Table 7.1: A summary of the electrophiles used in the synthesis of 165 and the resulting R groups.

<table>
<thead>
<tr>
<th>Electrophile source</th>
<th>R group</th>
<th>Mass Spectr um (m/z) (intensity %, ion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me₂S₂</td>
<td>SMe</td>
<td>575 (100, MH⁺)</td>
</tr>
<tr>
<td>1,2-dibromoethane</td>
<td>Br</td>
<td>609 (100, MH⁺ (⁷⁹Br)), 607 (100, MH⁺ (⁸¹Br))</td>
</tr>
<tr>
<td>N-fluorobenzenesulfonimide</td>
<td>F</td>
<td>547 (100, MH⁺)</td>
</tr>
</tbody>
</table>

The first two entries also gave HRMS data to support that they were successfully synthesised. All mass spectra were taken using positive electrospray ionisation.

Removal of the TIPS group also proved problematic. Attempts to use trifluoroacetic acid to remove the TIPS group were unsuccessful and resulted in the formation of many side products, TBAF gave better results, with a marginally cleaner reaction but did not proceed far.

Following the low yielding carbamoylation step and the subsequent problems with the removal of the TIPS group and the tricky purification steps and inspired by the success of the regioselective Boc protection of sanguinine a final synthetic rout was devised (Scheme 7.2). It was hoped that by avoiding the use of the TIPS group and using a carbamate directing group a simpler cleaner reaction path could be found.
It was hoped that the difference of in the acidities of the two sanguinine hydroxyl groups could be exploited to selectively carbamoylate only the phenol. It was hoped that the apparent inertness of the alternative hydroxyl position would also favour a regioselective reaction. It had been thought that one possible reason for the low yields of the carbamoylation in the previous sequence may have been in part due to steric crowding due to the presence of the large TIPS protecting group. However, the initial attempts at the carbamoylation of sanguinine were less than ideal. The use of elevated temperatures resulted in decomposition, however, later work (See Table 9.1) found that galanthamine was resilient to higher temperatures if 1,4-dioxane was used as the solvent instead of THF, it is probable that the same is also true for sanguinine. Several methods were attempted and finally it was found that the use of the strongly basic sodium hydride in THF at room temperature using potassium iodide as an additive resulted in the highest yield of a selectively carbamoylated product, 167. The product was a white solid, but displayed unfavourable solubility characteristics. To increase the solubility of the compound it was decided that the hydroxyl group should be protected with a hydrophobic group.

Rather than using the TIPS protection it was decided to use the TBDMS protecting group. This group had been used previously with galanthamine and while did not go on in such good yields it was found to be fairly easy to remove. The same was to prove true in this instance too. The TBDMS protected substrate, 168, was suitably soluble and was lithiated and functionalised with a reasonable degree of success. However, the less sticky nature of the compounds and the relative ease with which the TBDMS group could be removed resulted in the successful synthesis of a few galanthamine core structures carrying an O-carbamate and functionalised at the C8 position. The compounds were fairly clean after the lithiation stage and following characterization by mass spectrometry and 1H NMR they were taken directly to the deprotection stage without further purification.

The deprotection step work reasonably well, however, the purification was problematic and all the compounds had to be purified by preparative thin layer chromatography.
Scheme 7.2: The four step strategy to C8 functionalized galanthamine core structures via ortholithiation. a) Sodium hydride and diethylcarbamoyl chloride in THF, stirred overnight. b) tert-Butyldimethylsilyl trifluoromethanesulfonate and 2,6-lutidine in dichloromethane, 0 °C then reflux for 2 days. c) sec-Butyllithium and TMEDA in THF at -78 °C, then quench with electrophile. e) 1% concentrated hydrochloric acid in ethanol for 1 hour.

Table 7.2: A summary of the results of the ortholithiation and deprotection reactions.

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Electrophile source</th>
<th>R group installed</th>
<th>Yield* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>173</td>
<td>Me₂S₂</td>
<td>SMe₂</td>
<td>47</td>
</tr>
<tr>
<td>174</td>
<td>1,2-Dibromoethane</td>
<td>Br</td>
<td>47</td>
</tr>
<tr>
<td>175</td>
<td>Triethyl borate</td>
<td>B(OMe)₂</td>
<td>37</td>
</tr>
<tr>
<td>176</td>
<td>I₂</td>
<td>I</td>
<td>11</td>
</tr>
<tr>
<td>178</td>
<td>N- Fluorobenzenesulfonimide</td>
<td>F</td>
<td>-</td>
</tr>
<tr>
<td>179</td>
<td>N,N-Dimethylformamide</td>
<td>CHO</td>
<td>-</td>
</tr>
<tr>
<td>180</td>
<td>ortho-Fries</td>
<td>C(O)NEt₂</td>
<td>-</td>
</tr>
</tbody>
</table>

*This is the yield over two steps.
Compound 174, 175, and 176 are especially interesting as they offer the potential for use as handles in coupling chemistry, such as Kumada or Suzuki reactions.

Future work in this area could further refine the lithiation conditions and possibly look at the use of alternative protecting groups.
8 Urea-mediated lithiation chemistry for galanthamine C6 functionalization

8.1 Intramolecular aryl migration

The ready availability of galanthamine, presented by this project, offered the opportunity to investigate the effectiveness of the intramolecular aryl migration, first reported by Clayden et al., as a method for the derivatization of natural products and the exploration of new chemical space. It is generally accepted that when medicinal chemists are seeking new drugs by means of the structural modification of natural products they limit themselves to a well-defined chemical toolbox; the expansion of this toolbox is a worthwhile undertaking. The derivatization of galanthamine, by means of the intramolecular aryl migration, requires five separate reactions.

The first reaction is the previously discussed and highly capricious N-demethylation of galanthamine, 12, to afford norgalanthamine, 19, (see section 4.2.b). The norgalanthamine
could then be reacted with an aryl carbamoyl chloride to form a urea, 177. The urea could then be subjected to rearrangement conditions to promote aryl transfer, 178, following which the remainder of the urea moiety could be cleaved to return norgalanthamine functionalized at C6, 179. Then the derivatized norgalanthamine could be methylated to return a galanthamine functionalized at the C6 position, 180.

8.1.a Preparation of carbamoyl chlorides for the urea synthesis

The carbamoyl chlorides, 182, used for the synthesis of the ureas were synthesised according to the method of Lasne et al. (Scheme 8.2).151 This is simple the reaction of triphosgene, 181, with an aniline analogue. No attempt was made to purify the carbamoyl chlorides due to concerns that attempts to column them could lead to degradation moreover NMR showed that they were useably clean already. The carbamoyl chlorides were formed in good yields, however, the volatility of the compounds meant that if extreme care was not taken when removing the solvent there could be significant loss of product. The cleanest synthesis was of the N-methyl-N-2-pyridylcarbamoyl chloride, which was also the only one found to be liquid even after placing in the freezer.

![Scheme 8.2: The synthesis of carbamoyl chlorides: a) Triphosgene in dichloromethane at -78 °C, pyridine, then N-methylaniline analogue and allowed to warm to room temperature.](image-url)
Table 8.1: A summary of the compound numbers for the carbamoyl chlorides synthesised.

<table>
<thead>
<tr>
<th>Position</th>
<th>ortho</th>
<th>meta</th>
<th>para</th>
</tr>
</thead>
<tbody>
<tr>
<td>R=</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl</td>
<td>183</td>
<td>184</td>
<td>185</td>
</tr>
<tr>
<td>Methoxy</td>
<td>186</td>
<td>187</td>
<td>188</td>
</tr>
<tr>
<td>Fluoro</td>
<td>-</td>
<td>189</td>
<td>190</td>
</tr>
<tr>
<td>Chloro</td>
<td>191</td>
<td>192</td>
<td>193</td>
</tr>
<tr>
<td>Pyridyl</td>
<td>194*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Some additional carbamoyl chlorides were also used for the synthesis of the ureas, but these were synthesised by others. * Aryl group is 2-pyridyl.

8.1.b Synthesis of ureas for intramolecular aryl migration reactions

The urea formation proceeded quickly and cleanly, in good to near quantitative yields, typically about 80%, using only stoichiometric ratios of norgalanthamine, 19, and carbamoyl chloride and a slight excess of triethylamine stirred together in dichloromethane at room temperature. The reaction was robust enough that the solids could be combined in a flask and then the solvent and triethylamine added, it was observed that the addition of the triethylamine had the added benefit of fully dissolving the norgalanthamine, which did not otherwise fully dissolve.

The presence of impurities in the carbamoyl chlorides resulted in a slightly less than a stoichiometric amount of the carbamoyl chloride being used in the reactions. This compromise was accepted because there was concern that any excess carbamoyl chloride could react with the hydroxyl position on the norgalanthamine giving rise to a mixture of products that could prove difficult to separate. As it turned out this fear was soundless due to the relative inertness of the galanthamine hydroxyl position towards carbamoyl chlorides, especially under such mild reaction conditions (cf. 9.1.a). Therefore, the yields for the urea syntheseses could be improved simply by increasing the amount of carbamoyl chloride used in the reactions.

Isocyanates could have been used instead of carbamoyl chlorides to form the ureas, however, this would have necessitated the addition of a further reaction step to methylate
the secondary nitrogen that would have been formed; so this path was avoided. Additionally, the carbamoyl chlorides are easier to store, synthesise and handle.

Table 8.2: A summary of the synthesised galanthamine based aryl ureas

<table>
<thead>
<tr>
<th>Ar substituent</th>
<th>ortho (yield, %)</th>
<th>meta (yield, %)</th>
<th>para (yield, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>195 (99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl</td>
<td>196 (84)</td>
<td>197 (86)</td>
<td>198 (84)</td>
</tr>
<tr>
<td>Methoxy</td>
<td>199 (72)</td>
<td>200 (80)</td>
<td>201 (86)</td>
</tr>
<tr>
<td>Chloro</td>
<td>202 (84)</td>
<td>203 (70)</td>
<td>204 (50)</td>
</tr>
<tr>
<td>Fluoro</td>
<td>-</td>
<td>205 (93)</td>
<td>206 (95)</td>
</tr>
<tr>
<td>Naphthyl</td>
<td>207* (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pyridyl</td>
<td>208† (89)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Ar is 1-naphthyl, and the urea is N-ethyl
† Ar is 2-pyridyl

8.1.c Rearrangement of the galanthamine aryl ureas

A search for the ideal conditions to promote aryl migration was started based on the results of other studies into this sort of rearrangement chemistry. Due to the presence of the unprotected hydroxyl group at least one equivalent of base would be needed to deprotonate the hydroxyl group, a second equivalent of base would be needed to deprotonate the benzylic position to which the aryl group would migrate. Following rearrangement, the remaining benzylic proton would be more acidic and therefore preferentially deprotonated compared to the first benzylic deprotonation on a separate molecule, therefore an additional equivalent of base would be needed to ensure that all the urea underwent at least one deprotonation at the benzylic position. However, due to the complex structure of the ureas, there was some concern that any excess strong base would attack the structure leading to significant amount of decomposition. Therefore, the investigation started with the use of three equivalents of base.
The search for suitable conditions to promote rearrangement were carried out on the simplest galanthamine phenyl urea 195. Initially it was decided that a colder temperature should be used to help inhibit any undesirable side reactions and so the trials were started at -78 °C. However, at this low temperature the reactions returned starting material with only trace amounts of the rearranged products 209 and 210 or a small amount of degradation products.

In an attempt to determine if the ureas were being lithiated, but failing to rearrange, some urea was treated with sec-butyllithium and then quenched with methyl iodide (Scheme 8.4). However, this too returned only starting material and no detectable methylated product.

It was finally observed that the urea was precipitating out of solution at lower temperatures and that the surface of the solid was undergoing a colour change, due to lithiation, to give the appearance of the colour change in the solution, this was a little tricky to observe due to the presence of frosting on the glass at lower temperatures. In an attempt to get round this
problem the temperature was increased to -40 °C. However, at this higher temperature there was a marked increase in the amount of degradation. Another attempt to get round the solubility of the urea at low temperatures was to begin the reaction at low temperature (-78 °C) and allow the reaction to warm to room temperature slowly, it was hoped that in this way the reaction would proceed slowly as the material was gradually released into solution. However, as can be seen from entry 7 in Table 8.3, an aliquot of the solution removed after only two hours yielded approximately 60% isolated yield of the rearranged product, while the remainder of the solution yielded only a 40% isolated yield and displayed significantly more degradation. This shows that while higher temperatures resolved the solubility problem they also allowed for more decomposition. Further support came from the result of conducting the experiment at -60 °C which resulted in a lower yield but less decomposition than when the reaction was conducted at higher temperatures. It was also found that increasing to 4 equivalents of base resulted in increased degradation and lower overall yield.

Unlike in other work on aryl migration lithium chloride and DMPU were found to be unsuccessful in promoting the rearrangement; the reactions to which DMPU was added returned very clean starting material. TMEDA was found to promote rearrangement; in other work with galanthamine based compounds it has been observed that the addition of amines such as triethylamine or TMEDA have promoted the solubility of galanthamine based compounds, in this instance it is possible that this is one of the roles that TMEDA was performing the other being the activation of the sec-butyllithium.

Given the problems with balancing solubility against yield and degradation by altering the temperature it was decided to change the base to the alternative and milder base; LDA, synthesised from n-butyllithium and diisopropylamine. Using this milder base the reactions were then run at a higher temperature (-42 °C) and then allowed to warm to room temperature overnight.

Initially 3 equivalents of LDA were used to induce the aryl rearrangement, however, this gave incomplete rearrangement. Increasing to 4 equivalents of base improve the yield. The reactions done using LDA were much cleaner than those done with sec-butyllithium, therefore the amount of product produced by the two reactions could be compared from the crude NMR; from this it was determined that 4.5 equivalents should give complete rearrangement. This was found to be the case; resulting in a repeatable yield of 94%.
Table 8.3: A summary of the reaction conditions screened for the intramolecular aryl rearrangement

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Eq. of base</th>
<th>Additives</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>dr</th>
<th>Notes</th>
<th>Yield of product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sec-BuLi</td>
<td>3</td>
<td>-</td>
<td>-78</td>
<td>3h</td>
<td>1:3.6</td>
<td>Dirty starting material reclaimed</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>sec-BuLi</td>
<td>3</td>
<td>LiCl 13 eq.</td>
<td>-78</td>
<td>3h</td>
<td>-</td>
<td>Starting material recovered</td>
<td>None detected</td>
</tr>
<tr>
<td>3</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-78</td>
<td>3h</td>
<td>1:3.6</td>
<td>Dirty starting material recovered</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>sec-BuLi</td>
<td>3</td>
<td>DMPU 10 eq.</td>
<td>-78</td>
<td>3h</td>
<td>-</td>
<td>Starting material recovered</td>
<td>None detected</td>
</tr>
<tr>
<td>5</td>
<td>sec-BuLi</td>
<td>3</td>
<td>-</td>
<td>-78 to RT</td>
<td>2h</td>
<td>1:1.8</td>
<td>Degradation and starting material</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-78 to RT</td>
<td>2h, overnight</td>
<td>1:2.6</td>
<td>Dirty starting material recovered</td>
<td>54</td>
</tr>
<tr>
<td>7†</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-78 to RT</td>
<td>2h, overnight</td>
<td>1:3.8</td>
<td>Degradation when left overnight</td>
<td>60 and 40</td>
</tr>
<tr>
<td>8</td>
<td>sec-BuLi</td>
<td>4</td>
<td>TMEDA 10 eq.</td>
<td>-40</td>
<td>3h</td>
<td>-</td>
<td>Degradation and starting material</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>sec-BuLi</td>
<td>4</td>
<td>DMPU 10 eq.</td>
<td>-40</td>
<td>3h</td>
<td>-</td>
<td>Starting material recovered</td>
<td>None detected</td>
</tr>
<tr>
<td>10</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-60</td>
<td>3h</td>
<td>1:4.4</td>
<td>Degradation and starting material</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-42</td>
<td>3h</td>
<td>1:4.5</td>
<td>Degradation and starting material</td>
<td>22</td>
</tr>
<tr>
<td>12</td>
<td>sec-BuLi</td>
<td>4</td>
<td>TMEDA 10 eq.</td>
<td>-42</td>
<td>2h</td>
<td>1:2.6</td>
<td>Degradation and starting material</td>
<td>17</td>
</tr>
<tr>
<td>13</td>
<td>LDA</td>
<td>3</td>
<td>TMEDA 10 eq.</td>
<td>-42 to RT</td>
<td>Overnight</td>
<td>1.1:1</td>
<td>Product and starting material</td>
<td>46*</td>
</tr>
<tr>
<td>14</td>
<td>LDA</td>
<td>3</td>
<td>-</td>
<td>-42 to RT</td>
<td>Overnight</td>
<td>1.2:1</td>
<td>Product and starting material</td>
<td>46*</td>
</tr>
<tr>
<td>15</td>
<td>LDA</td>
<td>4</td>
<td>-</td>
<td>-42 to RT</td>
<td>Overnight</td>
<td>1:1.8</td>
<td>Product and Starting material</td>
<td>86*</td>
</tr>
<tr>
<td>16</td>
<td>LDA</td>
<td>4.5</td>
<td>-</td>
<td>-42 to RT</td>
<td>Overnight</td>
<td>1:2</td>
<td>Clean no starting material</td>
<td>91</td>
</tr>
<tr>
<td>17</td>
<td>LDA</td>
<td>4.5</td>
<td>-</td>
<td>0 to RT</td>
<td>Overnight</td>
<td>1:1.7</td>
<td>A little dirty no starting material</td>
<td>96</td>
</tr>
</tbody>
</table>

The reaction conditions in the table relate to the reaction in Scheme 8.3.
* Yield determined from NMR for the crude reaction mixture, after workup, relative to the remaining starting material, ergo the actual yield will be less.
† The first values relate to an aliquot that was removed and quenched, the second values relate to the remainder to the reaction mixture that was left overnight.
While the use of LDA allowed for an elevated starting temperature and warming to room temperature overnight, the initial low temperature was still cold enough to form lumps of solid. On some occasions these lumps did not dissolve during the warming stage; mainly because they either trapped the stirring bar or formed at the lip of the solvent and were therefore insufficiently agitated to redissolve. So, as had been tried when the solubility problem was first observed, the largest stirring bar possible was used for all the rearrangement reactions; this seemed to overcome this particular problem.

In response to this solubility issues and informed by work carried out by Leonard, it was also decided to attempt the rearrangement reaction starting at an initial temperature of 0°C and allowing the reaction to warm to room temperature overnight. The isolated yield increased by 2%, however, the crude product was a little dirtier, for this reason it was decided that the colder conditions would be used to examine the scope of this reaction.

One other interesting observation that can be made from the results listed in Table 8.3 is that, while all the reactions yielded a mixture of diastereoisomers, they all yielded the same minor and the same major isomer, with the exception of the reactions in which LDA was used as the base. When too few equivalents of LDA were used there was an inversion as to which were the major and minor isomers. However, when 4.5 equivalents of LDA were used, as required to take the reaction to completion, the reactions yielded the same major and minor isomers as those reactions in which sec-butyllithium was used as the base.

There is insufficient data to determine why this inversion of stereoselectivity occurs. Given the limited number of reagents, the most likely cause is that there is some degree of aggregation of the lithiated ureas. When two few equivalents of base are used it is possible that the lithiated ureas aggregate in such a way that the more crowded rearrangement product is favoured. Conversely, when sufficient base is used aggregation occurs between the lithiated ureas and the excess base (that base not needed for lithiation), resulting in a less crowded species so the less crowded rearrangement product becomes the favoured product.

From an examination of 3D drawings of the rearranged ureas it could be seen that in one of the diastereoisomers the hydrogens on the 2 and 5 positions of the aryl ring (that had migrated) are in close proximity to the 4a hydrogens of the galanthamine core, as close as only a few Angstroms (Figure 8.1). NOESY NMR experiments showed an interaction between the protons on the 2 and 5 positions of the aryl ring and 4a protons on the galanthamine core, but only for one diastereoisomer from each pair of diastereoisomers. This indicates that the stereochemistry at the bezylic position on the rearranged products can be accurately assigned based on the NOESY data.
Consequently, this information was used to assign the stereochemistry at the benzylic (C6) position of the rearranged products. This assignmet was then later confirmed by X-ray crystallography of some of the rearranged ureas (see Figure 8.2 and Figure 8.3).

Additionally, in some instances the NOESY NMR showed an interaction between the hydrogens at the 2 position on the migrated aryl ring and one of the hydrogens at the 12 position on the galanthamine core; where this was observed this information was used to assign the individual H12 protons. In a few instances this data could be used in conjunction with HMBC NMR data to confirm the specific ring position of some of the protons on the aryl rings.
Figure 8.2: The X-Ray structure and chemical diagram of the major product from the intramolecular aryl migration reaction on the galanthamine based phenyl urea, \textbf{210}. NOESY NMR spectra did not contain any cross peaks for protons on the alkene and protons on the aryl ring that had migrated.

Figure 8.3: The X-Ray structure and chemical diagram of the minor product from the intramolecular aryl migration reaction on the galanthamine based \textit{o}-methoxyphenyl urea \textbf{212}. The distance from the hydrogen at the 5 position on the migrated aryl ring to the hydrogen at the 4a position is 2.75 Å; NOESY NMR spectra showed a cross peak for an interaction between these two protons.
Table 8.4: A summary of the scope of the intramolecular aryl migration reaction on a urea with a galanthamine core structure.

<table>
<thead>
<tr>
<th>Aryl Urea</th>
<th>Remaining starting material (%)</th>
<th>Yield (%)</th>
<th>dr&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Comments</th>
<th>Minor diastereoisomer</th>
<th>Major diastereoisomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>-</td>
<td>91</td>
<td>1:1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Clean reaction</td>
<td><img src="image" alt="Minor diastereoisomer" /></td>
<td><img src="image" alt="Major diastereoisomer" /></td>
</tr>
<tr>
<td>o-Me</td>
<td>-</td>
<td>-</td>
<td>Undetermined</td>
<td>4 products produced</td>
<td><img src="image" alt="Minor diastereoisomer" /></td>
<td><img src="image" alt="Major diastereoisomer" /></td>
</tr>
<tr>
<td>m-Me</td>
<td>-</td>
<td>93</td>
<td>1:2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fairly clean reaction</td>
<td><img src="image" alt="Minor diastereoisomer" /></td>
<td><img src="image" alt="Major diastereoisomer" /></td>
</tr>
<tr>
<td>Substitution</td>
<td>Method</td>
<td>Yield</td>
<td>Product Ratio</td>
<td>Reaction Quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>-------</td>
<td>---------------</td>
<td>-----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-Me</td>
<td></td>
<td>100</td>
<td>1:1.6$^c$</td>
<td>Fairly clean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$o$-Cl</td>
<td></td>
<td></td>
<td>Undetermined</td>
<td>4 products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$m$-Cl</td>
<td>47</td>
<td>26</td>
<td>1:1$^c$</td>
<td>Diastereoisomers unseparated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-Cl</td>
<td>37</td>
<td></td>
<td>1:7.5 (1:2.6)$^{c,e}$</td>
<td>Dirty reaction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^c$ Indicates a specific comment or note relevant to the reaction conditions or product analysis.
<table>
<thead>
<tr>
<th>Aryl Urea</th>
<th>Remaining starting material (%)</th>
<th>Yield (%)</th>
<th>dr&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Comments</th>
<th>Minor diastereoisomer</th>
<th>Major diastereoisomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-OMe</td>
<td>Trace</td>
<td>99</td>
<td>1:1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fairly clean reaction</td>
<td><img src="image" alt="212d" /></td>
<td><img src="image" alt="225" /></td>
</tr>
<tr>
<td>m-OMe</td>
<td>-</td>
<td>73</td>
<td>1:3.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Dirty reaction</td>
<td><img src="image" alt="226" /></td>
<td><img src="image" alt="227" /></td>
</tr>
<tr>
<td>p-OMe</td>
<td>-</td>
<td>99</td>
<td>1:1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Clean reaction</td>
<td><img src="image" alt="228" /></td>
<td><img src="image" alt="229" /></td>
</tr>
</tbody>
</table>

<sup>a</sup> diastereoisomer ratio

<sup>c</sup> reaction conditions
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Yield</th>
<th>Dr</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-F</td>
<td>Trace</td>
<td>73</td>
<td>1:1.2</td>
</tr>
<tr>
<td>1-naphthyl</td>
<td>Trace</td>
<td>80</td>
<td>1:1.9</td>
</tr>
<tr>
<td>2-pyridyl</td>
<td>-</td>
<td>73</td>
<td>1:1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 230" /> <img src="image" alt="Structure 231" /></td>
</tr>
<tr>
<td><img src="image" alt="Structure 232" /> <img src="image" alt="Structure 233" /></td>
</tr>
<tr>
<td><img src="image" alt="Structure 234" /> <img src="image" alt="Structure 235" /></td>
</tr>
</tbody>
</table>

\(^a\) The dr was determined by \(^1\)H NMR spectra of the crude reaction mixture after workup, using the integrations of the H4 or H4a peaks, or an average of both H4 and H4a peaks as these peaks were in a cleaner area of the spectrum.

\(^b\) The assignment of the major and minor diastereoisomers was made from cross peaks on NOESY NMR spectra and confirmed by X-ray crystallography of one of the diastereoisomer.

\(^c\) The assignment of the major and minor diastereoisomers was made from cross peaks on NOESY NMR spectra and by comparison of the NOESY NMR data acquired for those compounds for which the structure was confirmed by X-ray crystallography.

\(^d\) X-ray crystal structures was determined

\(^e\) The second ratio is that of the columned mixture of diastereoisomers, given the dirty nature of the crude and a visual inspection of the two NMRs this second value looks more representative than the first.
The scope of the reaction is good with several aryl migrating groups giving yields higher than those reported for simpler substrates. However, the trifluoromethyl and chloro substituted rings gave far less satisfactory results. The best yields represent over all yields, for the two steps of carbamoylation and rearrangement, of 83%, a result that could be pushed higher by using more carbamoyl chloride in the carbamoylation step.

Generally, the reaction was tolerant of both electron withdrawing and electron donating substituents on the migratory aryl rings. A clear cut reasoning of the reliability of the rearrangements is not completely forthcoming from the data in hand. However, it would seem that the positioning of substituents that can stabilize a negative charge was of most benefit. In the case of both chloro and methoxy substituted rings the meta-substituent resulted in the lowest yield; Wheeland intermediates would distribute the extra electron density around the ring to the ortho and para positions where the electronegative oxygen or chlorine could help to stabilise this additional negative charge.

The most confusing results were yielded by the migration of aryl rings that were either methyl or chloro substituted at the ortho position. NMR analysis seems to show that the rings with ortho methyl or ortho chloro substituents gave 4 distinct products; attempts to separate these have not been entirely successful with only tiny amounts of some of the compounds isolated and others remaining as a mixture. Accurate mass spectra confirm that there is at least one species present with the correct mass for the rearranged compound (or equally the starting material) for both substituent classes. Crucially, in the case of the ortho-chloro substituted ring, close examination of the NMR data seems to indicate that there is no starting material present and that all four products show the presence of the doublet for the N-methyl group; this indicates that the aryl ring has at least left the urea nitrogen. The NMR spectra also shows the presence of the new peak for what could be the remaining benzylic (H6) proton following rearrangement and at the same time shows no peaks for the benzylic CH$_2$ (H6) protons from the starting material. The total mass for the four products from the attempted aryl migration of the ortho-chloro substituted ring equates to a yield of about 58%, based on mass alone. The first consideration was that the ortho substituted compounds were giving diastereoisomeric pairs of atropisomers; with the ortho substituent restricting rotation. However, there seems to be insufficient restriction of movement (see Figure 8.3) to make this the case and more importantly the migration of the ortho-methoxy substituted aryl ring proceeded excellently, giving only two products, likewise the migration of the 1-naphthyl substituted proceeded with good yields and giving clean products. It is unlikely that both the 1-naphthyl group and the ortho-methoxy group both took part in additional coordination during the migration reaction resulting in only
two of the possible four atropisomers of each product being formed. The possibility of rotamers arising from the migration of the ortho-chloro or ortho-methyl groups could have been investigated using VT NMR, however, it is unlikely that they are rotomers as the ortho-methoxy and 1-naphtyl migration products do not show any indication of rotomeric character. Had time permitted more attempts could have been made to separate the products of these more complicated reactions, this would undoubtedly have required the synthesis of larger quantities of the aryl ureas, a synthesis made problematic by the capricious N-demethylation step prior to carbamoylation.

Both the naphthyl and heteroaromatic groups gave good results.

For analytical purposes, once purified the pairs of enantiomers were separated with reverse phase preparative HPLC.

8.1.d Cleavage of the Urea

Attempts to cleave the urea, by heating in n-butanol, to release the C6 aryl functionalised norgalanthamine were unsuccessful.\textsuperscript{106,107} Unfortunately there was insufficient time to attempt other methods.

8.2 Further attempts at lithiation and functionalisation of the C6 position

Following the results of the ortholithiation chemistry and the success of the intramolecular aryl migrations it was decided to look at the possibility of using lithiation chemistry to functionalize the C6 position.

8.2.a Carbamate directed lithiation

A simple strategy was developed starting from norgalanthamine. Norgalanthamine was treated with di-\textit{tert}-butyl dicarbonate, the resulting product, \textit{N}-boc-norgalanthamine, \textbf{162}, was isolated and attempts were made to lithiate it. Unfortunately, lithiation using either \textit{sec}-butyllithium and TMEDA or LDA, followed by quenching with a range of electrophiles, all returned starting material.
8.2.b Urea directed lithiation

A new strategy was developed; the carbamate was changed to a urea, this had originally been avoided due to the potential difficulty in removing the urea. To minimise any difficulty with the removal of the urea after the functionalisation it was decided to use the N-tert-butylmethyl urea. The initial attempts to synthesis this started from the formation of 4-nitrophenyl tert-butyl(methyl)carbamate, 237, from 4-nitrophenyl chloroformate and N-tert-butylmethylamine, which could then be reacted with norgalanthamine to form the desired urea.

![Chemical structure](image)

Figure 8.4: 4-nitrophenyl tert-butyl(methyl)carbamate, 237

Unfortunately, no conditions could be found to drive the reaction between the carbamate and norgalanthamine. It was therefore decided to use a diethyl urea, 238, which could be formed by the reaction between diethylcarbamoyl chloride and norgalanthamine, 19: this reaction was found to work cleanly.
Scheme 8.6: Urea directed lithiation of the galanthamine C6 position. a) Diethylcarbamoyl chloride and triethylamine in dichloromethane, 16 hours. b) sec-Butyllithium, TMEDA in THF at -78 °C, then quenched with electrophile (R).

The screening of conditions for the lithiation of the N'-diethylnorgalanthamine urea, 238, was started from the conditions found to best promote the aryl migration discussed in section 8.1. However, treatment with LDA and quenching with suitable electrophiles (dimethyl disulphide) returned starting material. The harsher conditions of sec-butyllithium and TMEDA in THF at -78 °C were found to lithiate and quenching with dimethyl disulphide installed the methylthio group at the C6 position. The 1H NMR clearly showed the presence of the newly installed methyl group and the loss of the peaks associated with the 6H protons accompanied by the appearance of the singlet for the lone benzylic proton. However, the yield was poor, with the reaction also returning a significant amount of starting material. Therefore, attempts were made to improve on this by firstly increasing the number of equivalents of base and then by elevating the temperature of the reaction but reducing the number of equivalents of base. Increasing the temperature was most unproductive returning a dirty product. Increasing the proportion of base resulted in a side reaction that gave a distinct side product, attempts to elucidate the structure of this product have so far been unsuccessful. To see if the side reaction was influenced by the electrophile the reactions were repeated using 1,2-dibromoethane; changing the electrophile had no positive impact on the reactions. The results are summarised in Table 8.5, page 144. When the newly installed group was relatively small (Br or SCH$_3$) no method could be found to separate the diastereoisomers, even just separating the unknown impurity required the use of preparative HPLC.

While progress in this area was not forthcoming it did show that such lithiation reactions were possible if not perfect; this presented the opportunity to further investigate the configurational stability of the lithiated ureas.
8.2.c Conformational stability of the lithiated galanthamine urea

The tributyltin chloride quench (Table 8.5, entry 7) was included with the express intention of examining the configurational stability of the lithiated galanthamine urea.\textsuperscript{153} The initial lithiation of the galanthamine analogue could occur at either of the two diastereotopic H6 (benzylic) positions, however, stannylation would effectively trap the position of metalation in a more stable compound allowing the metalated compounds to be isolated and separated into the two diastereoisomers. The assignment of the stereochemistry at the benzylic position of the separated stannylated compounds was done using NOESY NMR and by analogy to the products of the aryl rearrangement reactions. For the minor product NOESY NMR spectra show cross peaks between the H4a proton and the protons at H19 and H22 (positions 19 and 22 are the positions at either end of the butyl groups). While for the major product the NOESY NMR spectra does not show these cross peaks. This assignment of the stereochemistry is in agreement with what would be expected for the outcome of this reaction based on the outcome of the aryl migration reactions; that being that the major product is the least sterically hindered.

Scheme 8.7: The determination of the configurational stability of lithiated galanthamine based ureas. a) sec-Butyllithium, TMEDA in THF at -78 °C, then quenched with tributyltin chloride. b) The separated diastereoisomers are treated with sec-Butyllithium, TMEDA in THF at -78 °C, then quenched with iodomethane. The stereochemistry in the product 245 is not known.
Once a single diastereoisomer of the stannylated galanthamine was isolated it was subjected to a repeat of the lithiation conditions and then quenched with iodomethane. Following this second reaction only one of the two possible C6 methylated galanthamine analogues could be found in the reaction mixture.

For comparative purposes a sample of urea 240 was lithiated and the lithiated compound was quenched with iodomethane, this produced a mixture of the two possible diastereoisomers; 243 and 244 (see Scheme 8.8). No HPLC method could be developed to give complete base line separation between these two diastereoisomers, but the HPLC run for the product of the tin lithium exchange reaction (Scheme 8.7) did only seem to show the presence of a single diastereoisomer.

![Scheme 8.8: The lithiation and methylation of a galanthamine based urea. a) sec-Butyllithium, TMEDA in THF at -78 °C, then quenched with iodomethane. The yields are exceptionally low as the preparative HPLC method did not give complete base line separation, therefore only the initial and finial parts of each peak were isolated to give single diastereoisomers.](image)

However, the yields were exceptionally low and needed extensive purification. But even the $^1$H NMR of the crude reaction mixture appeared to only show the presence of one of the diastereoisomers when compared to the NMRs of the individual diastereoisomers produced by, the equally low yielding, direct lithiation-iodomethane quench reaction (Scheme 8.8). This would seem to indicate that the lithiated product is configurationally stable. Unfortunately attempts to determine the stereochemistry of the methylated C6 product, 242, have not been successful.
Table 8.5: A summary of the reaction conditions for the lithiation and functionalization of the C6 position of a galanthamine derived urea.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Eq. of base</th>
<th>Additive</th>
<th>Temp. (°C)</th>
<th>Electrophile for quench</th>
<th>Yield (%)</th>
<th>dr of products</th>
<th>Notes/ R group installed at C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LDA</td>
<td>3</td>
<td>-</td>
<td>-42</td>
<td>Me₂S₂</td>
<td>-</td>
<td>-</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>LDA</td>
<td>3</td>
<td>-</td>
<td>-42</td>
<td>1,2-dibromoethane</td>
<td>-</td>
<td>-</td>
<td>No reaction</td>
</tr>
<tr>
<td>3</td>
<td>sec-BuLi</td>
<td>3</td>
<td>TMEDA</td>
<td>-78</td>
<td>Me₂S₂</td>
<td>48</td>
<td>1:1*</td>
<td>SMe</td>
</tr>
<tr>
<td>4</td>
<td>sec-BuLi</td>
<td>3.5</td>
<td>TMEDA</td>
<td>-42</td>
<td>Me₂S₂</td>
<td>35</td>
<td>-</td>
<td>Dirty reaction / SMe</td>
</tr>
<tr>
<td>5</td>
<td>sec-BuLi</td>
<td>4</td>
<td>TMEDA</td>
<td>-78</td>
<td>Me₂S₂</td>
<td>55</td>
<td>1:1*</td>
<td>SMe</td>
</tr>
<tr>
<td>6</td>
<td>sec-BuLi</td>
<td>4.5</td>
<td>TMEDA</td>
<td>-78</td>
<td>Me₂S₂</td>
<td>54</td>
<td>1:1*</td>
<td>SMe</td>
</tr>
<tr>
<td>7</td>
<td>sec-BuLi</td>
<td>4.5</td>
<td>TMEDA</td>
<td>-78</td>
<td>1,2-dibromoethane</td>
<td>-</td>
<td>-</td>
<td>No product detected</td>
</tr>
<tr>
<td>8</td>
<td>sec-BuLi</td>
<td>4.5</td>
<td>TMEDA</td>
<td>-78</td>
<td>Tributyltin chloride</td>
<td>39</td>
<td>1:4.6†</td>
<td>SnBu₃</td>
</tr>
</tbody>
</table>

*In each case one diastereoisomer seemed to be in a very slight excess (<0.1).

† This is the ratio of the purified products, the crude product was too messy for the dr to be determined.
8.3 A further approach to the functionalization of the C6 positon

Following from the work of Hametner et al.\textsuperscript{154} and Jai et al.\textsuperscript{53} it was decided to try and form a range of similar analogues. Some teething problems with this chemistry and the liquidation of Alzeim Ltd. meant that this work was not pursued to completion. However, the C6 methylation of galanthamine was performed in two steps; first by iminium formation to give 245 and then by reacting this iminium ion with methylmagnesium bromide to give the 6-methyl galanthamine 246.

The formation of galanthaminium bromide by the reaction of galanthamine with \textit{N}-bromosuccinimide (Scheme 8.9) proved to be exceptionally prone to disruption by the presence of trace impurities; it was this observation that lead to the rigorous purification regime being applied to all the galanthamine that did not show itself to be pure by NMR. Whether this susceptibility was due to the nature of the reaction or the impurities is not known and would be hard to determine without knowing the exact nature of the impurities in the galanthamine.

The reaction with the Grignard reagent worked well giving a product that was a mixture of diastereoisomers, 246, but was otherwise clean after workup; this was as far as this work progressed, the diastereoisomers remain unseparated. However, it is an interesting starting point for a further development of the intramolecular aryl migration. The C6 methylated galanthamine, 246, could be \textit{N}-demethylated to give the C6 methylated norgalanthamine analogue 247. This inturn could be converted to the aryl urea, 248, which could be treated with suitable lithiating agents, such as LDA, to promote aryl migration. The usefulness of such a strategy would be somewhat reliant on the ability to separate the diastereoisoemers at
a point before the rearrangement reaction, more specifically at compounds 246, 247, or 248. This would then allow further confirmation of the assessment of the configurational stability of the lithiated galanthamine analogue made from the stannylation reaction detailed in section 8.2.c.

Scheme 8.10: A possible expansion of the intramolecular aryl rearrangement chemistry. Proposed conditions a) 3-Chloroperbenzoic acid in chloroform/isopropanol (3:1) at -10 °C, then hydrochloric acid. Then water, then iron(0) powder 13 mol% and iron(III) chloride hexahydrate 2 mol%, at room temperature.  b) Dichloromethane, N-Methyl-N-phenylcarbamoyl chloride, triethylamine.  c) Lithation conditions, typically; LDA in Tetrahydrofuran at -42 °C then allowed to warm to room temperature.

Given that varying the proportion of LDA in the aryl rearrangements changes the diastereotopic ratio of the products (Table 8.3, entries 13-17, and section 8.1.c) it would be interesting to see the effects of varying the amount of LDA used in the rearrangement of the C6 methylated urea analogues.
9 Functionalisation at the C3 hydroxyl site of galanthamine

9.1.a Carbamate rearrangement

Following the successful rearrangement of galanthamine-based ureas to functionalise the C6 position it was decided to investigate the possibility of carbamate rearrangements analogous to the work of Clayden et al.\textsuperscript{108, 155}

![Scheme 9.1: Suggested route for the structural modification of galanthamine at the C4 position.](image)

However, attempts to synthesis the carbamates at the galanthamine hydroxyl position were unsuccessful; using the carbamoyl chlorides already synthesized no conditions could be found that yielded more than a trace amount of carbamate. Various conditions were tried and are summarised in Table 9.1 (p. 149). Most of the reactions returned either a majority of degradation products or unreacted galanthamine. The reactions using potassium iodide were carried out after its successful implementation in similar work by Leonard\textsuperscript{152}, however, in this instance it did not produce the same good results. As was expected the use of DMAP only served to increase the degree of degradation.

Interestingly, when refluxing the reaction, the use of tetrahydrofuran as solvent resulted in a high degree of degradation while the use of 1,4-dioxane as the solvent resulted in the formation of some product with no appreciable degradation. However, the reaction still returned a significant amount of starting material despite the higher reaction temperature (bp. 100 °C compared with bp. 65 °C for tetrahydrofuran) and refluxing for several days. To ensure that the problem was not the reactivity of the carbamoyl chloride several other carbamoyl chlorides were also used. Only the pyridyl carbamoyl chloride gave any amount of product, still less than a 10% yield, this relative success was most likely due to
the vast excess used simply because the pyridyl carbamoyl chloride was an oil and it was harder to weigh out the desired amount accurately.

The most probable reason for the failure of the reactions is the fact that the hydroxyl group is in a relatively hindered position; being both a secondary alcohol and on the internal face of a ring in a relatively bulky molecule. The crystal structure of galanthamine shows the hydroxyl group to be bent over into the middle of the molecule making it relatively inaccessible, a situation that would only heighten the inertness of this position. Had time allowed, reactions could have been attempted with the more reactive isocyanates in place of the carbamoyl chlorides. Although this would have required a further reaction step to methylate the resulting secondary nitrogen in the carbamate, further there was already great store of various carbamoyl chlorides available from the urea rearrangements.

### 9.1.b Thiocarbonylimidazole rearrangement

Concurrently with the attempts to investigate the intramolecular aryl migration from the carbamate to the C4 position attempts were made to investigate another rearrangement in the same region of the molecule. This was based on the work of Clayden et al. on the [3,3]-sigmatropic rearrangement of O-allyl thiocarbamates. Unfortunately, once again it was found that the galanthamine hydroxyl group could not be made to react readily, due to the failed attempts to form the carbonate this line of investigation was quickly abandoned.

![Scheme 9.2: Proposed method for the functionalization of the galanthamine C4a position. a) Thiocarbonylimidazole and other reagents/conditions (see Table 9.2).](image)

This one pot reaction would have formed a thiocarbamate, **253**, that could have been derivatized further and opening a route to further natural product derivatization. Table 9.2 (p. 149) summarises the conditions that were screened for the synthesis of the thiocarbamates. Here again the use of DMAP resulted in accelerated decomposition, this effect is in common with observations made elsewhere in this report.
Table 9.1: A summary of the reaction conditions screened for the formation of galanthamine carbamates at the C3 position for intramolecular aryl migration investigations.

<table>
<thead>
<tr>
<th>Carbamoyl chloride</th>
<th>Eq. of carbamoyl chloride</th>
<th>Base/Catalyst</th>
<th>Additives</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time</th>
<th>Result/Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>1.0</td>
<td>NEt₃</td>
<td>-</td>
<td>CH₂Cl₂</td>
<td>RT</td>
<td>48 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>Ph</td>
<td>1.5</td>
<td>-</td>
<td>Catalytic DMAP</td>
<td>CH₂Cl₂</td>
<td>RT</td>
<td>20 h</td>
<td>Some degradation</td>
</tr>
<tr>
<td>Ph</td>
<td>1.5</td>
<td>NaH</td>
<td>-</td>
<td>DMF</td>
<td>RT</td>
<td>20 h</td>
<td>Some degradation</td>
</tr>
<tr>
<td>Ph</td>
<td>1.5</td>
<td>NaH</td>
<td>KI</td>
<td>THF</td>
<td>RT</td>
<td>16 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>Ph</td>
<td>1.5</td>
<td>NaH</td>
<td>KI</td>
<td>THF</td>
<td>Reflux</td>
<td>16 h</td>
<td>Some degradation</td>
</tr>
<tr>
<td>Ph</td>
<td>1.5</td>
<td>NaH</td>
<td>-</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>5 days</td>
<td>Trace product</td>
</tr>
<tr>
<td>2-pyridyl</td>
<td>2.2</td>
<td>NaH</td>
<td>-</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>5 days</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>o-OMe, o-Cl, m-Me, and p-F</td>
<td>1.5</td>
<td>NaH</td>
<td>-</td>
<td>1,4-dioxane</td>
<td>reflux</td>
<td>72 h</td>
<td>No reaction/Trace product</td>
</tr>
</tbody>
</table>

Table 9.2: A summary of the reaction conditions tried for the synthesis of galanthamine based thiocarbonylimidazole for rearrangement reactions.

<table>
<thead>
<tr>
<th>Equivalents of Thiocarbonylimidazole</th>
<th>Base/Catalyst</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Times</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DMAP (catalytic)</td>
<td>CH₂Cl₂</td>
<td>Room temp.</td>
<td>8h</td>
<td>Incomplete degradation</td>
</tr>
<tr>
<td>2</td>
<td>NaH (1 eq.)</td>
<td>THF</td>
<td>Reflux</td>
<td>4h</td>
<td>Incomplete degradation</td>
</tr>
</tbody>
</table>
10 The structural modification of the galanthamine core via Diels Alder reactions

10.1 The synthesis of 3,4-didehydro-3-deoxygalanthamine

The literature contains many examples of attempts to improve the efficacy of galanthamine by modifications at the hydroxyl group, which largely demonstrated that the allylic hydroxyl moiety is an essential pharmacophore of the galanthamine structure. However, there has been no serious investigation as to more extensive modifications of the galanthamine core in this region of the molecule. It was thought that the presence of an allylic hydroxyl group presented the opportunity to form a diene, 141, that would allow for further modification using the Diels Alder reaction. We were also curious as to whether the shape of the galanthamine-based diene would result in the formation of only a single diastereoisomer when reacted with a dienophile in a Diels Alder reaction.

The first stage is the dehydration of galanthamine to give the second double bond, of the diene, between the C2 and C3 positions (see Scheme 10.1). The resultant structure is 3,4-didehydro-3-deoxygalanthamine; known as an impurity in galanthamine.

![Scheme 10.1: The dehydration of galanthamine to 3,4-didehydro-3-deoxygalanthamine, 141, see text for a discussion of the conditions needed.](image)

The literature on galanthamine contains several examples where this diene, 141, is the unintended by-product of other reactions. Several methods were examined in the attempt to produce the diene as the main product. The first method was via tosylation, however, attempts to install the tosyl group were unsuccessful and returned only dirty starting material. Briefly, the conditions tried were tosyl chloride, with DMAP, triethylamine and galanthamine in dichloromethane and then the harsher conditions in which sodium hydride was used as the base and the more polar solvents N,N-dimethylformamide or
tetrahydrofuran were used. As was found elsewhere in this work the hydroxyl group proved relatively inert, with NMR showing the absence of any of the desired tosylated product. This perceived inertness is thought to arise from the steric bulk of the rest of the galanthamine structure and so it was thought that a smaller group could be introduced more successfully. With this in mind attempts were made at mesylation. In situ mesylation and elimination using methanesulfonyl chloride in dichloromethane with triethylamine and then a second addition of triethylamine to promote the elimination gave a spot to spot reaction, as monitored by thin layer chromatography, but NMR showed the crude reaction mixture to be very messy and not containing any of the desired product. Due to the spot-to-spot nature of the reaction it was decided to attempt it again at a lower temperature (-78 °C instead of 0 °C) and without the second addition of triethylamine. Doing just this, followed by extraction and purification, gave 3-deoxy-3-chlorogalanthamine, 255, galanthamine, 12, and epigalanthamine, 17, in a 1:3.3:1 ratio and near enough quantitative overall yield. Presumably the chlorinated galanthamine arose as a result of the nucleophilic attack of the chloride ions, themselves liberated by the successful mesylation of the hydroxyl group, on the C3 position. While the epigalanthamine would most likely have formed from a similar nucleophilic attack on the C3 position, only this time by water from the reaction quench and work up. Unfortunately, due to the fact that only a few milligrams of 3-deoxy-3-chlorogalanthamine, 255, was isolated and that this was not clean and because some galanthamine was found amongst the products it is not possible to say whether the substitution reaction went via an SN1 or SN2 type mechanism. The epigalanthamine could have arisen either from steric control or an SN1 type reaction. Notably this method was producing better yields of epigalanthamine than the deliberate attempts at its semisynthesis detailed in section 4.2.c.

![Scheme 10.2](image)

Scheme 10.2: a) Methanesulfonyl chloride 1.5 eq. in dichloromethane with triethylamine 1.5 eq.

In an attempt to drive the elimination reaction to completion the 3-deoxy-3-chlorogalanthamine, 255, was treated with potassium tert-butoxide, a non-nucleophilic
base, in tetrahydrofuran and refluxed. The reaction gave a 43\% yield which equates to an overall yield, for the two steps, for the synthesis of 3,4-didehydro-3-deoxygalanthamine, 141, of 8.5\%. A yield most notable for being 4.5\% lower than the accidental yield of this same product reported by Han et al.\textsuperscript{52} in their attempts to synthesize 3-deoxy-3-iodogalanthamine.

The mesylation method was attempted a final time, but in this instance exposure to water was kept to a minimum, after the initial mesylation step the reaction was filtered through silica and then the solvent was removed. The crude was then subjected to the same potassium tert-butoxide and tetrahydrofuran conditions as before, however, no more than a trace of the desired product was obtained. It had been hoped that by not exposing the reaction to so much water the epigalanthamine would not form and that the mesylated product would survive to undergo elimination. At this point it was decided that the use of mesylation was clearly problematic and alternative methods were examined.

Hoffman et al. reported that anhydrous copper(II) sulphate could be used to dehydrate allylic alcohols and yield the olefinic products.\textsuperscript{157} However, attempts to do this using \(N,N\)-dimethylformamide as a solvent and refluxing for several days returned only a reduced yield of the starting material, notably in their paper Hoffman et al.\textsuperscript{157} did not use a solvent and demonstrated this method using the alcohol, to be dehydrated, as the solvent.

It was noticed that the concomitant attempts to synthesis epigalanthamine (for use in the analysis of the daffodil juice, (see section 4.2.c) were in fact producing small samples of 3,4-didehydro-3-deoxygalanthamine, 141. Therefore, it seemed that simply intensifying the reaction conditions could potentially yield useful amounts of the desired diene. The epimerization of galanthamine to epigalanthamine was done simply by heating galanthamine in dilute hydrochloric acid (see section 4.2.c), so for the dehydration galanthamine was heated in more concentrated acids; including hydrochloric acid, sulphuric acid, and phosphoric acid. The actual product of this acid catalysed reaction was not the intended diene, 141, but the aromatized product 259 (see Scheme 10.3). The reaction was found to be very effective at forming this product, which was often clean after work up. The conditions and the results of the attempted dehydrations using acid catalysis are summarised in Table 10.1. Clearly this shows that it is possible to use acid catalysis to form the elimination product, but given that it may be reasonably assumed that the second elimination proceeds more quickly than the first it may prove difficult to find the precise conditions needed to affect only the first elimination giving the diene.
Scheme 10.3: A proposed mechanism for the aromatization of galanthamine under strongly acidic conditions. Details of the first step, a standard acid catalysed dehydration are omitted for brevity.

Table 10.1: A summary of the conditions and results of the acid catalysed dehydration and aromatization of galanthamine.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Temperature (°C)</th>
<th>Time (hours:mins)</th>
<th>Yield (%)</th>
<th>Condition of product after workup</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃PO₄</td>
<td>Reflux</td>
<td>1:33</td>
<td>85</td>
<td>Slightly dirty</td>
</tr>
<tr>
<td>HCl</td>
<td>Reflux</td>
<td>0:25</td>
<td>71</td>
<td>Clean</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>Reflux</td>
<td>0:25</td>
<td>-</td>
<td>Dirty/degradation</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>Reflux</td>
<td>0:25</td>
<td>95</td>
<td>Clean</td>
</tr>
</tbody>
</table>

It was thought that the aromatized product may display atropisomerism, however, attempts to investigate this with variable temperature NMR did not prove successful as there was only very minor movement of a few of the peaks over a 92 K temperature range; one of the H6 peaks shifted 0.07 ppm down field, while the higher field resonances for the H11 and H12 peaks shifted down field by 0.07 and 0.04 ppm respectively. The higher field H11 peak also showed a slight degree of sharpening, larger than that shown by the H12 peak, however, it overlapped with the singlet arising from the presence of the methyl group on the benzazocine nitrogen, a problem compounded further by the close proximity of the dimethyl sulfoxide residual solvent peak. Chiral HPLC gave no decisive evidence, yielding two peaks in a 50:50 ratio.
At this point in the attempt to synthesise 3,4-didehydro-3-deoxygalanthamine, 141, the compound was found to be an impurity in one on the batches of galanthamine supplied from Alzeim Ltd. The separation from galanthamine was time consuming but this source gave enough material for an initial investigation into the feasibility of using it as the diene in Diels Alder reactions and so no further attempts were made to synthesise 3,4-didehydro-3-deoxygalanthamine, 141.

While 3,4-didehydro-3-deoxygalanthamine, 141, is known to form as an impurity in the industrial synthesis of galanthamine it was more surprising to find it present in naturally sourced galanthamine. Possible sources are discussed previously (see 5.1.c) it is interesting in the context of this work as it was unintentionally forming while attempts to form it by submitting galanthamine to conditions similar to those that most likely lead to the formation of the impurity were not working out favourably.

While the attempts to synthesise 3,4-didehydro-3-deoxygalanthamine, 141, detailed above gave exceptionally underwhelming success the small quantity of product obtained hints that the most reliable route may be to go via a halogenated product and then elimination in an E2 mechanism. The E2 elimination step gave a 43% yield, but this was without optimisation so it is not unreasonable to expect that it could be coax ed to a higher yield. Additionally opting for a more suitable halide leaving group such as bromine or iodine may also encourage the elimination step, a prospect supported by the work of Han et al.\textsuperscript{52}

Thus presenting a possible route to useful quantities of 3,4-didehydro-3-deoxygalanthamine, 141. Suitable methods for the halogenation, to give 260, may be the Appel reaction or treatment with triphosgene and pyridine in dichloromethane or even simply mesyl chloride and then treatment with an ionic iodine or bromine source. The elimination step, to give the diene 141, could best be performed with a non-nucleophilic base at elevated temperature, much as was already done and possibly enhanced by screening for suitable solvents and alternative bases.

Scheme 10.4: A possible route to 3,4-didehydro-3-deoxygalanthamine, a) Halogenation conditions, b) Elimination conditions (see text for discussion of possible conditions).
10.2 The use of 3,4-didehydro-3-deoxygalanthamine as a diene in Diels-Alder reactions

The Diels Alder reaction of 3,4-didehydro-3-deoxygalanthamine, 141, was attempted with two dienophiles, chosen for their reputation at readily taking part in this sort of pericyclic reaction; 4-phenyl-1,2,4-triazoline-3,5-dione, 261, and dimethyl acetylenedicarboxylate, 262.

![Figure 10.1: 4-phenyl-1,2,4-triazoline-3,5-dione, 261, and dimethyl acetylenedicarboxylate, 262, the dienophiles used in the Diels Alder reactions with the diene 3,4-didehydro-3-deoxygalanthamine, 141.](image)

The addition of the dienophile 4-phenyl-1,2,4-triazoline-3,5-dione, 261, dissolved in toluene, to the diene, 141, resulted in an almost instant disappearance of the characteristic purplish-red colour associated with the dienophile. The reaction was stirred a little longer and then worked up. The crude NMR was a little messy and chromatography did not separate the remaining unreacted diene and the Diels Alder product. The first fractions of product off the column contained a few milligrams of a dirty and unidentified compound, but the NMR did contain some clear indications of organised structure derived from the galanthamine core, for example the singlets for the OCH$_3$ and NCH$_3$, and the doublet of doublets for the H6 protons were intact if a little shifted, but other features were less clear and the impure nature of the sample meant the integrations were inconsistent. The middle fractions contained a mixture of the unreacted diene and a single diastereoisomer, 263, of the Diels Alder product in a 1:1.94 ratio, but the mixture was otherwise clean. Unfortunately, some of the peaks on the NMR overlapped and so a full characterisation was not performed. However, the presence of some clear peaks in the NMR were highly indicative of the formation of the expected product. Specifically, the appearance of the peaks at 6.44 and 6.40 ppm corresponding to the protons (H3 and H4) on the newly formed double bond from C3 to C4 and the movement of the signals for the protons H1 (4.86 ppm) and the two bridge head position protons H2 and H4a (5.37 and 5.62 respectively). The expected coupling for this region of the molecule was displayed in the COSY.
Unfortunately, the peaks for the H11 and H12 protons overlapped starting material peaks for the corresponding positions, with the exception of one of the H11 peaks which collided with the singlet arising from the protons on the NCH$_3$ group (see Scheme 10.5 for an explanation of the numbering in the altered part of the structure).

Scheme 10.5: a) 4-phenyl-1,2,4-triazoline-3,5-dione in toluene, at room temperature. The numbers in blue delineate the atom numbering in the altered part of the structure as used in the text and for NMR assignment.

The reaction probably only gave a single diastereoisomer due to the fairly bulky nature of both the diene and the dienophile resulting the in the outcome of the reaction being under a significant degree of steric control. An approximate yield for the Diels Alder product, 263, of 42%, was calculated from the combined mass of starting material and product and the relevant ratios determined from the NMR. 22% of the starting material was also present in the mixture. The final fractions contained only a trace of another galanthamine based structure, but not enough for characterisation. It should be noted that the trace products isolated were not especially clean and this combined with the tiny quantities available made the NMRs less than ideal. However, neither trace sample is thought to relate to the alternative diastereoisomer as the NMRs contained no peaks that could be readily assigned to the bridge head hydrogens. Later attempts to improve upon this by increasing the reaction time from 5 minutes to 1 hour pushed the yield to 50% but also gave a dirtier reaction.

Attempts to react 3,4-didehydro-3-deoxygalanthamine, 141, with dimethyl acetylenedicarboxylate, 261, using the same method as outlined above, resulted in only a messy reaction mixture from which none of the desired product, 264, was isolated.
10.3 Summary of the attempts to use 3,4-didehydro-3-deoxygalanthamine as a diene in Diels Alder reactions

The failed attempts to synthesise the diene, 3,4-didehydro-3-deoxygalanthamine, 141, delayed progress on this area of work and while a source of the diene was found it was of limited supply and proved difficult to purify. However, the brief enquiry into the usefulness of 3,4-didehydro-3-deoxygalanthamine as a diene in Diels Alder reactions seemed to suggest that it could be used and with the added advantage that it apparently only gave a single diastereoisomer, a feat that could no doubt be repeated by using other large dienophiles. Clearly more work is needed in this area and a ready supply of the diene, 141, possibly via the route outlined above, would make a proper screening of both the conditions and suitable dienophiles a feasible undertaking.
\section*{11 Conclusion}

The project clearly had two related components; the first associated with the isolation and identification of natural products and the second with the application of synthetic methods to the same natural products. Progress throughout the work was hampered primarily by the problems associated with the purification of the natural product starting material and to a lesser degree the purification of the synthetically modified natural products. These problems no doubt arose due to the specific solubility characteristics of the compounds, meaning they required highly polar solvent systems for purification. A further problem arose as a result of the solubility of the compounds, with more than one synthetic strategy needing to be reworked due to the product of a reaction being insoluble in solvents suitable for the next step – this was especially a problem in several areas of the organolithium chemistry.

Haemanthamine, 10, homolycorine, 11, 3,4-didehydro-3-deoxygalanthamine, 142, and apohaemanthamine, 146, were isolated in factory effluent or as impurities in the galanthamine supplied from the factory. Two of these, namely 3,4-didehydro-3-deoxygalanthamine, 141, and apohaemanthamine, 145, are most likely thought to form from other alkaloids present in the daffodil extract and suggest that the extraction process is rather harsh, a fact backed up by the isolation of several other components that turned out to be waste material.

![Structural formulas of haemanthamine, homolycorine, 3,4-didehydro-3-deoxygalanthamine, and apohaemanthamine](image)

Figure 11.1: A summary of the compounds isolated from samples taken from the factory production line. (Reproduced from Figure 5.15).
The semisynthesis of some standards for analytical work allowed the daffodil juice and factory effluent to be examined for the presence of the most likely and most useful alkaloids for the semisynthesis of galanthamine and sanguinine, none, save narwedine, were present.

Some progress was made in eliminating the possible causes for the capricious nature of the iron-mediated N-demethylation reaction, a potentially highly useful reaction. The most useful improvement to the reaction was the addition of a small volume of water, although further work is still needed to perfect this reaction and allow it to be run reliably.

Scheme 11.1: Scheme 11.2: The N-demethylation of galanthamine, 12, to form norgalanthamine, 19, performed as a one pot reaction. a) 3-Chloroperbenzoic acid in chloroform/isopropanol (3:1) at -10 °C, then hydrochloric acid, b) Water, then iron(0) powder 13 mol% and iron(III) chloride hexahydrate 2 mol%, at room temperature. See text for a full discussion of the experimental details and the yields.

The structural modification of the galanthamine core was undertaken via various routes although the aforementioned solubility problems hampered the rate of some of this work. The capricious nature of the N-demethylation reaction also greatly slowed this work. But nevertheless some valuable transformations were undertaken; most notably the intramolecular aryl migration, which often worked in excellent and repeatable yields. This is the first demonstration of this chemistry being applied to such complex natural products and is a rare application of organolithium chemistry to a natural product derived starting material. The reaction was tolerant of a range of substituents on the migrating aryl ring and the use of a 2-pyridyl group as the migrating ring.
Scheme 11.3: The intramolecular aryl migration reaction on a galanthamine based phenyl urea, 195. a) LDA (4.5 eq.) in THF at -42 °C and then allowed to warm to room temperature overnight. Quenched with methanol.

It is this intramolecular aryl migration that allows for the greatest potential in further work and the potential for this was discussed at the end of chapter 8.
12 Experimental – Part 1: Natural products; isolated and semisynthesised

Proton nuclear magnetic resonance spectra were taken using either a Bruker Avance 300 (300 MHz), a Bruker Avance III 400 UltraShield (400 MHz), or a Bruker Avance II+ UltraShield 500 (500 MHz). Carbon 13 spectra were taken using the same machines, with resonance frequencies at 75, 100, and 125 MHz respectively. The NMR data are presented as chemical shift δ (ppm relative to tetramethylsilane), integration, multiplicity, coupling constant J (Hz), and assignment. Where necessary DEPT135, COSY, and HMQC experiments were performed to aid spectral assignment. Deuteriated chloroform, dimethyl sulfoxide (DMSO), or methanol was used as the solvent; the residual solvent peak was used as an internal reference.

Mass spectra were determined on either a Micromass Platform II or a Walters QTOF, both using ES ionisation.

Infrared spectra were taken using an ATi Perkin Elmer Spectrum RX1 FTIR or a Thermo Scientific ATR. All samples were run as an evaporated film, as a Nujol mull or a solid. Only significant characteristic absorptions are reported.

Melting points were measured using a Melting Point SMP10 apparatus, resolution to 1 °C, manufactured by Stuart, Bibby Scientific.

Optical rotations were determined using an AA-100 Polarimeter, Optical Activity Ltd., using a cell with a path length 2.50 cm³, concentrations (c) are quoted as mmol dm⁻³.

Tetrahydrofuran (THF) was distilled over sodium wire under nitrogen, using benzophenone indicator. Other dry solvents were obtained from the solvent purification system (SPS), and other reagents were used as received unless otherwise stated.

Reactions were cooled using ice-water baths, or a thermostatically controlled cryo-cooler.

Thin layer chromatography (TLC) was done using Polygram SIL/UV254 TLC plates, Macherey-Nagel, stationary phase thickness 0.20 mm.

Standard techniques were used to handle air and moisture sensitive compounds.
12.1 The compounds of Chapter 4: The semisynthesis of alkaloids and some alkaloid analogues

12.1.a The preparation of galanthamine, 12

Saturated aqueous sodium hydrogen carbonate (100 cm³) and distilled water (100 ml) were added to Galanthamine hydrobromide (5.00 g, 13.6 mmol) and stirred. The pH of the mixture was adjusted to pH 10 by the addition of sodium hydroxide pellets. The mixture was then extracted with dichloromethane (3 × 200 cm³). The organic fractions were combined and dried over magnesium sulphate and then the solvent was removed under reduced pressure to return galanthamine as the free base. Where needed the galanthamine free base was further purified by column chromatography using a gradient solvent system eluting with dichloromethane and 9:1 methanol/35% aqueous ammonia (98:2, 96:4, 94:6, 92:8, 90:10), depending on the impurities present the gradient was adjusted, for example the gradient may have been started at 95:5 trending towards the 90:10.

It should be noted that on many occasions the extraction was done directly from saturated aqueous sodium hydrogen carbonate, the exact conditions used were adapted for each batch of galanthamine hydrobromide supplied by Alzeim Ltd. The method given in full detail was the one used for the batch of galanthamine hydrobromide that yield the cleanest product.

Rf (9.0:0.9:0.1 DCM:methanol:35% aqueous ammonia) 0.300; [α]D²² -97.2 ° (c. 34.8 in ethanol) [Lit.¹⁵⁸, -97 in ethanol]; MS m/z (ES) 288 (100%, MH⁺); ¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.66 (1 H, d, J=8.0 Hz, H8), 6.62 (1 H, d, J=8.0 Hz, H7), 6.06 (1 H, dd, J=10.5, 1.5 Hz, H4a), 5.99 (1 H, ddd, J=10.0, 5.0, 1.0 Hz, H4), 4.59 - 4.62 (1 H, m, H1), 4.11 - 4.16 (1 H, m, H3), 4.08 (1 H, d, J=15.0 Hz, H6), 3.83 (3 H, s, OCH₃), 3.67 (1 H, dd, J=15.0, 1.0 Hz, H6), 2.39 (3 H, s, NCH₃), 3.26 (1 H, ddd, J=14.5, 13.0, 1.5 Hz, H12), 3.05 (1 H, br dt, J=14.5, 3.5 Hz, H12), 2.68 (1 H, ddt, J=15.5, 3.5, 1.5, 1.5 Hz, H2), 2.08 (1 H, td, J=13.5, 3.5 Hz, H11), 2.00 (1 H, ddd, J=15.5, 5.0, 2.5 Hz, H2), 1.57 (1 H, ddd, J=14.0, 4, 2.0 Hz, H11); ¹³C NMR (101 MHz, CHLOROFORM-d) δ ppm 145.7
(C10), 144.0 (C9), 133.0 (C10a), 129.3 (C6a), 127.5 (C4), 126.8 (C4a), 122.0 (C7), 111.4 (C8), 88.7 (C1), 62.0 (C3), 60.6 (C6), 55.8 (OCH$_3$), 53.8 (C12), 48.2(C10b), 42.1 (NCH$_3$), 33.7 (C11), 29.9(C2). Spectroscopic data is in reasonable agreement with that reported in the literature (see Table 11.1 and Table 11.2), the discrepancies most likely arise due to minor impurities or differences in the concentration of the NMR samples.$^{159, 160}$ The crystal structure was found to be in agreement with that reported in the literature.

Table 12.1: A comparison of experimental and literature $^1$H NMR chemical shifts for galanthamine.

<table>
<thead>
<tr>
<th>Proton assignment</th>
<th>Experimental δ (ppm) (400 MHz)</th>
<th>Literature δ (ppm)$^{159}$ (200 MHz)</th>
<th>Literature δ (ppm)$^{160}$ (600 MHz)</th>
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<tr>
<td>1</td>
<td>4.59-4.62</td>
<td>4.73</td>
<td>4.64</td>
</tr>
<tr>
<td>2</td>
<td>2.00 and 2.68</td>
<td>2.12 and 2.80</td>
<td>2.03 and 2.70</td>
</tr>
<tr>
<td>3</td>
<td>4.11-4.16</td>
<td>4.25</td>
<td>4.15</td>
</tr>
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<td>4</td>
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<td>6.10</td>
<td>6.03</td>
</tr>
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<td>6.06</td>
<td>6.21</td>
<td>6.08</td>
</tr>
<tr>
<td>6</td>
<td>4.08 and 3.67</td>
<td>3.79 and 4.21</td>
<td>3.78 and 4.21</td>
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<tr>
<td>7</td>
<td>6.62</td>
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<td>1.57 and 2.08</td>
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<td>12</td>
<td>3.26 and 3.05</td>
<td>3.16 and 3.39</td>
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<td>OH</td>
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All NMR spectra were taken for samples in CDCl$_3$. 

163
Table 12.2 A comparison of experimental and literature $^{13}$C NMR chemical shifts for galanthamine.

<table>
<thead>
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<th>$^{13}$C assignment</th>
<th>Experimental δ (ppm) (101 MHz)</th>
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<td>OCH$_3$</td>
<td>55.8</td>
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<td>55.8</td>
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</table>

All NMR spectra were taken for samples in CDCl$_3$.

Figure 12.1: Left; X-ray structure of galanthamine. Right; X-ray structure of galanthamine hydrobromide

The crystal structure for galanthamine hydrobromide came from some large crystals (up to about 5 mm diameter) that formed during the extraction process in the factory. The staff at
Alzeim Ltd had been unable to identify them, they were found to be insoluble in a range of solvents including aqueous sodium hydroxide. They were eventually found to dissolve in concentrated hydrochloric acid.

12.1.b The semisynthesis of known alkaloids

**Sanguinine, 22**

The method for the synthesis of sanguinine adapted from the method reported by Mary et al.\textsuperscript{109} Galanthamine (1.00 g, 3.48 mmol) was placed in a dry flask, under a nitrogen atmosphere, 1 mol dm\textsuperscript{-3} L-Selectride (18.0 cm\textsuperscript{3}, 18.0 mmol, 5.17 eq.) was added and the mixture was then refluxed for 20 hours. The reaction mixture was allowed to cool to room temperature before being diluted with ethyl acetate (25 cm\textsuperscript{3}) and then quenched by the addition of distilled water (25 cm\textsuperscript{3}), initially drop wise and then as a single portion. Then the reaction mixture was extracted with distilled water (3 × 25 cm\textsuperscript{3}), for each successive extraction the layers took progressively longer to separate (in some instances the last separation was left overnight, this could be mitigated by the addition of more solvent). The combined aqueous fractions were evaporated and the residue purified by column chromatography (80:19:1 dichloromethane / methanol / 35% ammonia in water) to give the title product as a pale yellow solid. Yield 98%, 0.9374 g, 3.4300 mmol.

\textbf{Rf} (9.0:0.9:0.1 DCM:methanol:35% aqueous ammonia) 0.10; \textbf{MS} m/z (ES) 274 (100%, \textit{MH}\textsuperscript{+}); \textbf{\textit{1H NMR}} (500 MHz, DMSO-\textit{d6}) δ ppm 9.02 (1 H, br s, \textit{OH}), 6.49 (1 H, d, \textit{J}=8.0 Hz, H8), 6.42 (1 H, d, \textit{J}=8.0 Hz, H7), 6.03 (1 H, d, \textit{J}=10.5 Hz, H4), 5.80 (1 H, dd, \textit{J}=10.5, 4.0 Hz, H4a), 4.43 - 4.46 (1 H, m, H1), 4.04 - 4.10 (1 H, br s, H3), 4.03 (1 H, br d, \textit{J}=15.0 Hz, H6), 3.50 (1 H, d, \textit{J}=15.0 Hz, H6), 3.15 (1 H, br t, \textit{J}=13.0 Hz, H12), 2.88 (1 H, m, \textit{J}=14.5 Hz, H12), 2.26 (1 H, br dd, \textit{J}=16.5, 3.0 Hz, H2), 2.23 (3 H, s, NCH\textsubscript{3}), 2.03 (1 H, dt, \textit{J}=15.5, 4.5 Hz, H2), 1.97 (1 H, td, \textit{J}=13.0, 3.0 Hz, H11), 1.44 (1 H, br dd, \textit{J}=13.5, 2.0 Hz, H11); \textbf{\textit{13C NMR}} (126 MHz, DMSO-\textit{d6}) δ ppm 145.2 (C10), 140.5 (C9), 132.9 (C10a), 128.1 (C4), 127.9 (C4a), 127.1 (C6a), 121.0 (C7), 115.1 (C8), 86.5 (C1), 60.1 (C3), 59.6 (C6), 53.2 (C12), 47.8 (C10b), 41.3 (NCH\textsubscript{3}), 33.6 (C11), 30.9 (C2).
E. W. D. Burke

$^1$H NMR (400 MHz, METHANOL-d$_4$) δ ppm 6.58 (1 H, d, $J$=8.0 Hz, H7), 6.54 (1 H, d, $J$=8.0 Hz, H8), 6.12 (1 H, d, $J$=10.5 Hz, H4a), 5.93 (1 H, ddd, $J$=10.5, 5.0, 1.0 Hz, H4), 4.52 - 4.55 (1 H, m, H1), 4.17 (1 H, br t, $J$=5.0 Hz, H3), 4.11 (1 H, d, $J$=15.0 Hz, H6), 3.69 (1 H, ddd, $J$=15.0, 1.0 Hz, H6), 3.25 (1 H, dm, $J$=14.0 Hz, H12), 3.06 (1 H, br d, $J$=14.0 Hz, H12), 2.50 (1 H, ddt, $J$=16.0, 3.5, 1.5 Hz, H2), 2.43 (3 H, s, NCH$_3$), 2.05 - 2.15 (2 H, m, H2 and H11), 1.69 (1 H, ddd, $J$=14.0, 4.0, 1.5 Hz, H11); $^{13}$C NMR (75 MHz, METHANOL-d$_4$) δ ppm 147.0 (C10), 143.0 (C9), 134.4 (C10a), 128.8 (C4), 128.3 (C4a), 125.8 (C6a), 123.6 (C7), 117.1 (C8), 88.9 (C1), 62.6 (C3), 61.4 (C6), 55.5 (C12), 49.2 (C10b), 43.4 (NCH$_3$), 35.3 (C11), 31.5 (C2). Spectroscopic data is in reasonable agreement with that reported in the literature, the discrepancies most likely arise due to minor impurities or differences in the concentration of the NMR samples.$^{109}$

Table 12.3: A comparison of experimental and literature $^{13}$C NMR chemical shifts for sanguinine.

<table>
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<th>Literature δ (ppm)$^{109}$ (250 MHz)</th>
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<td>1.65 and 2.05</td>
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All NMR spectra were taken for samples in CD$_3$OD.
Table 12.4: A comparison of experimental and literature $^{13}$C NMR chemical shifts for sanguinine.

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All NMR spectra were taken for samples in CD$_3$OD.

Narwedine, 24

Narwedine was synthesised by the method reported by Lee et al.$^{111}$ Oxalyl chloride (3.50 cm$^3$, 41.4 mmol) in dichloromethane (87.0 cm$^3$) was cooled to -60 °C, to this dry dimethyl sulphoxide (6.00 cm$^3$, 84.5 mmol) in dichloromethane (16.5 cm$^3$) was added over 5 minutes. The mixture was allowed to warm to -50 °C and then Galanthamine (5.00 g, 17.4 mmol) in dichloromethane (8.0 cm$^3$) was added over 5 minutes and the reaction left to stir for 50 minutes, after which triethylamine (25.0 cm$^3$, 179 mmol) was added over 30 minutes. The mixture was stirred for a further 5 minutes and then allowed to warm to room temperature before being quenched with ice cold water (50 cm$^3$). The layers were
separated and the aqueous fraction extracted with dichloromethane (3 × 50 cm³), the combined organic fractions were then washed with water (50 cm³) and brine (50 cm³). The residue was purified by flash column chromatography eluting with dichloromethane, Methanol and 35% aqueous ammonia (9:0.9:0.1). The product was an off white solid, Yield 4.90 g, 17.2 mmol, 99%.

Rf (9.0:0.9:0.1 dichloromethane:methanol:35% aqueous ammonia) 0.403; IR
υmax(film)/cm⁻¹ 1680 (C=O); Mpt. 190-191 °C (recrystallized from ethanol) [Lit.¹⁶, 188-190 °C, recrystalized from ethanol]; MS m/z (ES) 286 (100%, MH⁺); ¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.96 (1 H, dd, J=10.5, 2.0 Hz, H4a), 6.70 (1 H, d, J=8.5 Hz, H8), 6.66 (1 H, d, J=8.5 Hz, H7), 6.05 (1 H, d, J=10.5 Hz, H4), 4.74 (1 H, dt, J=4.0, 2.0 Hz, H1), 4.09 (1 H, d, J=15.5 Hz, H6), 3.85 (3 H, s, OCH₃), 3.75 (1 H, d, J=15.5 Hz, H6), 3.10 - 3.29 (3 H, m, H2, H12 and H12), 2.76 (1 H, dd, J=17.5, 4.0 Hz, H2), 2.45 (3 H, s, NCH₃), 2.28 (1 H, td, J=13.0, 3.5 Hz, H11), 1.86 (1 H, ddd, J=14.0, 3.5, 2.0 Hz, H11); ¹³C NMR (101 MHz, CHLOROFORM-d) δ ppm 194.4 (C3), 146.9 (C10), 144.3 (C4a), 143.9 (C9), 130.5 (C10a), 129.3 (C6a), 127.1 (C4), 122.0 (H7), 111.7 (H8), 87.9 (C1), 60.6 (C6) 55.9 (OCH₃), 54.1 (C12), 48.9 (C10b), 42.4 (NCH₃), 37.3 (C2), 33.2 (C11). Spectroscopic data is in reasonable agreement with that reported in the literature, the discrepancies most likely arise due to minor impurities or differences in the concentration of the NMR samples.²³,¹¹¹ The crystal structure was found to be in agreement with that reported in the literature.

Figure 12.2: X-Ray structure of narwedine, taken from a crystal of (-)-narwedine.
Table 12.5: A comparison of experimental and literature $^1$H NMR chemical shifts for narwedine.

<table>
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<th>Literature δ (ppm) (400 MHz)</th>
<th>Literature δ (ppm) (600 MHz)</th>
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<td>1.85 and 2.27</td>
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<td>3.10-3.29</td>
<td>3.14 and 3.27</td>
<td>3.15-3.32</td>
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<td>OCH$_3$</td>
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All NMR spectra were taken for samples in CDCl$_3$.

Table 12.6: A comparison of experimental and literature $^{13}$C NMR chemical shifts for narwedine.

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All NMR spectra were taken for samples in CDCl$_3$. 

Galanthamine \( N \)-oxide or \((4aS,6R,8aS,11R)-6,11\text{-dihydroxy-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-11-iium chloride, 137\)

Galanthamine (2.00 g, 6.99 mmol) was dissolved in propan-2-ol – chloroform (1:3) (207 cm\(^3\)) and cooled to -10 °C. 3-Chloroperbenzoic acid (1.21 g, 7.00 mmol) was added slowly over 10 minutes and the reaction was stirred for a further 10 minutes. After which time the reaction mixture was extracted with 1 mol dm\(^{-3}\) hydrochloric acid (3 × 20 cm\(^3\)). The aqueous phase was evaporated to dryness to give a white solid. Yield 83\%, 1.97 g, 5.81 mmol.

**MS** m/z (ES+) 304 (100\%, M-Cl); **MS** m/z (ES-) 338 (100\%, M\(^{35}\text{Cl})-\text{H}) , 340 (30\%, M\(^{35}\text{Cl})-\text{H}); **\(^1\text{H} NMR** (300 MHz, CHLOROFORM-\(d\)) \( \delta \) ppm 6.69 (2 H, s, H7 and H8), 5.84 - 6.12 (2 H, m, H4 and H4a), 4.78 (1 H, m, H6), 4.55 - 4.61 (1 H, m, H1), 4.31 (1 H, m, H6), 4.10 - 4.19 (1 H, m, H3), 3.87 - 4.17 (1 H, m, H12), 3.79 (3 H, s, OCH\(_3\)), 3.59 - 3.81 (1 H, m, H12), 2.98 (4 H, s, NCH\(_3\) and OH), 2.64 (1 H, dm, \( J = 16.0 \) Hz, H2), 2.00 (1 H, ddd, \( J = 16.0, 5.0, 3.0 \) Hz, H2), 1.77 - 2.09 (2 H, m, H11); **\(^{13}\text{C} NMR** (75 MHz, CHLOROFORM-\(d\)) \( \delta \) ppm 146.3 (C10), 145.9 (C9), 131.8 (C10a), 130.2 (C4a), 124.1 (C4), 123.6 (C7), 119.6 (C6a), 112.0 (C8), 88.5 (C1), 75.4 (C6), 69.7 (C12), 61.2 (C3), 55.8 (OCH\(_3\)), 49.8 (NCH\(_3\)), 46.2 (C10b), 34.7 (C11), 30.1 (C2). The **\(^{13}\text{C} NMR** assignments were made by comparison with other galanthamine based compounds.

**Norgalanthamine, 19**

Norgalanthamine was synthesized using an adaption of the method of Kok and Scammells.\(^{112}\) Briefly galanthamine (0.4957 g, 1.725 mmol) was dissolved in propan-2-ol
– chloroform (1:3) (56.0 cm$^3$) and cooled to -10 °C. To this was added 3-chloroperbenzoic acid (pre-purified by extraction into ether from aqueous pH 7.5 solution) (0.3246 g, 1.881 mmol) in portions over ten minutes, the mixture was stirred for a further 30 minutes after which concentrated aqueous hydrochloric acid (0.153 cm$^3$, 1.85 mmol) was added dropwise. The magnetic stirrer was removed and the reaction vessel removed from the proximity of any magnetic objects. Iron powder (12.6 mg, 0.0226 mmol, 13 mol-%) and iron(III) chloride hexahydrate (9.3 mg, 0.034 mmol, 2.5 mol-%) were added in a single portion and the mixture shaken vigorously for approximately 5 minutes, after which time it was shaken briefly, but vigorously, every few minutes until TLC showed the reaction was complete. Chloroform (84.0 cm$^3$) was added and the mixture washed with 5% aqueous sodium hydroxide (2 × 2.8 cm$^3$). The organic phase was then dried over magnesium sulphate and the solvent removed under vacuum. The rust-red residue was purified by gravity chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient 98:2, 96:4, 94:6, 92:8, 90:10, to give norgalanthamine. Yield 0.2916 g, 1.067 mmol, 62%) as a white solid.

**MS** m/z (ES+) 274 (100%, MH$^+$); **$^1$H NMR** (500 MHz, CHLOROFORM-d) δ ppm 6.65 (1 H, d, $J$=8.0 Hz, H8), 6.63 (1 H, d, $J$=8.0 Hz, H7), 6.06 (1 H, dd, $J$=10.5, 1.5 Hz, H4a), 6.01 (1 H, ddd, $J$=10.5, 5.0, 1.0 Hz, H4), 4.61 - 4.64 (1 H, m, H1), 4.13 - 4.17 (1 H, m, H3), 4.03 (1 H, d, $J$=15.5 Hz, H6), 3.96 (1 H, d, $J$=15.5 Hz H6), 3.84 (3 H, s, OCH$_3$), 3.36 (1 H, dt, $J$=14.5, 3.5 Hz, H12), 3.23 (1 H, ddd, $J$=14.5, 12.5, 2.0 Hz, H12), 2.70 (1 H, ddt, $J$=15.5, 3.0, 1.5 Hz, H2), 2.02 (1 H, ddd, $J$=15.5, 5.0, 2.0 Hz, H2), 1.86 (1 H, ddd, $J$=13.5, 4.0, 2.0 Hz, H11), 1.75 (1 H, ddd, $J$=13.5, 12.5, 3.5 Hz, H11); **$^{13}$C NMR** (101 MHz, CHLOROFORM-d) δ ppm 146.1 (C10), 144.0 (C9), 133.1 (C10a), 127.6 (C6a), 127.0 (C4), 122.0 (C4a), 120.6 (C7), 110.9 (C8), 88.5 (C1), 62.0 (C3), 55.9 (OCH$_3$), 53.8 (C6), 48.7 (C10b), 47.0 (C12), 40.3 (C11), 29.9 (C2).

The NMR data for this compound did not match exactly with that reported in the literature. However, the chemical shifts published in the literature are not in agreement with each other either.$^{23,148}$ It was noticed that the chemical shifts of this compound were strongly effected by impurities and that one less pure sample gave chemical shifts in better agreement with one of the literature sources. The chemical shifts reported here are for a sample that gave a clean NMR, in which more detailed coupling was visible than was reported in either of the literature sources referenced above.
Table 12.7: A comparison of experimental and literature $^1$H NMR chemical shifts for norgalanthamine.

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All NMR spectra were taken for samples in CDCl$_3$.

Table 12.8: A comparison of experimental and literature $^{13}$C NMR chemical shifts for norgalanthamine

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<th>Experimental δ (ppm) (101 MHz)</th>
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<td>OCH$_3$</td>
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All NMR spectra were taken for samples in CDCl$_3$. 
Norgalanthamine (10.8 mg, 0.395 mmol) was dissolved in ethyl formate and refluxed for 48 hours. The reaction was cooled and then evaporated under vacuum to give a residue which was subjected to flash column chromatography, eluting with dichloromethane, methanol and 35% aqueous ammonia; 10:0.9:0.1 to give the product as an off white residue. Yield 84%, 10.0 mg, 0.0332 mmol. The NMR data shows a mixture of rotamers in a 1:1.5 ratio.

**MS** m/z (ES+) 324 (100%, MNa\(^+\)); \(^1^H\) NMR (500 MHz, CHLOROFORM-d) δ ppm 8.17 (0.58 H, s, CHO), 8.12 (0.42 H, s, CHO), 6.86 (0.42 H, d, J=8.0 Hz, H7), 6.68 - 6.72 (1.58 H, m, H7 and H8), 6.02 - 6.11 (1.42 H, m, H4 and H4a), 5.99 (0.58 H, d, J=10.5 Hz, H4a), 5.22 (0.42 H, d, J=15.0 Hz, H6), 4.60 (1.58 H, m, H1 and H12), 4.52 (1 H, s, H6), 4.14 – 4.21 (1 H, m, H3), 4.04 (0.42 H, d, J=15.0 Hz, H6), 3.88 - 3.94 (0.42 H, m, H12), 3.86 (1.74 H, s, OCH\(_3\)), 3.84 (1.26 H, s, OCH\(_3\)), 3.67 - 3.76 (0.48 H, m, H12), 3.21 - 3.31 (0.52 H, m, H12), 2.68 - 2.76 (1 H, m, H2), 2.36 - 2.43 (1 H, m OH), 2.01 - 2.08 (1 H, m, H2), 1.94 - 1.99 (0.48 H, m, H11), 1.78 - 1.92 (1.52 H, m, H11); \(^1^C\) NMR (CHLOROFORM-d, 125 MHz) δ 162.4 (C=O\(_{major}\)), 161.8 (C=O\(_{minor}\)), 144.9 (Aryl), 144.7 (Aryl), 128.7 (Aryl), 128.5 (C4), 128.5 (C4), 127.8 (Aryl), 126.2 (C4\(_{major}\)), 125.8 (C4\(_{minor}\)), 121.8 (C7), 120.1 (C7), 111.6 (C8), 111.6 (C8), 88.3 (C1), 88.1 (C1), 61.8 (C3), 56.0 (OCH\(_3\)), 56.0 (OCH\(_3\)), 52.9 (C6\(_{major}\)), 48.3 (C10b), 48.3 (C10b), 46.8 (C6\(_{minor}\)), 46.6 (C12\(_{minor}\)), 41.0 (C12\(_{major}\)), 39.4 (C11\(_{minor}\)), 36.1 (C11\(_{major}\)), 29.9 (C2). NMR were in good agreement with those reported in the literature.\(^{124}\)
O-Acetyl-N-acetyl galanthamine, 140

Acetic anhydride (2.00 cm³, 21.2 mmol) was added to norgalanthamine (99.7 mg, 0.365 mmol) in dichloromethane (2.0 cm³) and left to stir for 24 hours. After this time the reaction was not complete and so triethylamine (1.00 cm³, 7.17 mmol) was added and the reaction stirred for a further 1 hour. Saturated sodium hydrogen carbonate aqueous solution (15 cm³) was added and the mixture was extracted with dichloromethane (3 × 15 cm³), the organic fractions were combined and dried over magnesium sulphate and evaporated under reduced pressure to give O,N-diacetylnorgalanthamine as an off white solid. Yield 0.1054 g, 0.295 mmol, 81%.

¹H-NMR (CHLOROFORM-d, 300 MHz) δ ppm 6.70 (1H, d, J=8.0 Hz, ArH), 6.64 (1H, d, J=8.0 Hz, ArH), 6.21 (1H, d, J=10.0 Hz, H4a), 5.93 (1H, ddd, J=10.0, 5.0, 1.5 Hz, H4), 5.31 - 5.38 (1H, m, H3), 4.70 (1H, dt, J=13.5, 3.5 Hz, H12), 4.51 - 4.59 (2H, m, H1, H6), 4.47 (1H, d, J=16.5 Hz, H6), 3.86 (3H, s, OCH₃), 3.13 - 3.26 (1H, m, H12), 2.65 - 2.75 (1H, m, H2), 2.10 (3H, s, OC(O)CH₃), 2.05 (3H, s, OC(O)CH₃), 1.96 - 2.03 (1H, m, H2), 1.87 - 1.95 (1H, m, H11), 1.73 - 1.81 (1H, m, H11). This compound was not fully characterized as it was only an intermediate in the attempted synthesis of narcisine (N-acetylnorgalanthamine).

O-Acetylgalanthamine, 18

Acetic anhydride (0.050 cm³, 0.455 mmol) was added slowly to a solution of galanthamine (0.1006 g, 0.3501 mmol) in dichloromethane (3.50 cm³) at 0 °C. The reaction was stirred and then a catalytic amount (spatula tip) of 4-(dimethylamino)pyridine was added and the reaction stirred overnight at room temperature. The reaction was quenched with water (1.0
cm$^3$) and then washed with saturated sodium hydrogen carbonate aqueous solution (3.0 cm$^3$), and then brine (3.0 cm$^3$). The organic phase was evaporated under vacuum and the residue purified by gradient flash column chromatography, eluting with acetone:methanol (10:0 to 10:1) to yield *O*-acetylgalanthamine as an off white solid, yield 50%, 58.1 mg, 0.176 mmol.

**MS** m/z (ES$^+$) 330 (100%, MH$^+$); **$^1$H NMR** (500 MHz, CHLOROFORM-$d$) δ ppm 6.67 (1 H, d, $J=8.0$ Hz, H8), 6.60 (1 H, d, $J=8.0$ Hz, H7), 6.27 (1 H, d, $J=10.5$ Hz, H4a), 5.91 (1 H, dd, $J=10.5$, 5.0 Hz, H4), 5.33 (1 H, br t, $J=5.0$ Hz, H3), 4.55 - 4.58 (1 H, m, H1), 4.19 (1 H, d, $J=15.0$ Hz, H6), 3.84 (3 H, s, OCH$_3$), 3.73 (1 H, d, $J=15.0$ Hz, H6), 3.33 - 3.41 (1 H, m, H12), 3.07 - 3.15 (1 H, m, H12), 2.68 (1 H, ddd, $J=16.5$, 3.0, 1.5 Hz, H2), 2.41 (3 H, s, NCH$_3$), 2.15 (1 H, br td, $J=13.5$, 2.0 Hz, H11), 2.08 (1 H, ddd, $J=16.5$, 5.0, 3.0 Hz, H2), 2.03 (3 H, s, OC(O)CH$_3$), 1.59 - 1.66 (1 H, m, H11); **$^{13}$C NMR** (126 MHz, CHLOROFORM-$d$) δ ppm 170.9 (C=O), 146.6 (C10), 144.2 (C9), 131.9 (C10a), 130.3 (C4a), 127.9 (C6a), 123.0 (C4), 121.6 (C7), 111.5 (C8), 86.2 (C1), 63.1 (C3), 59.9 (C6), 55.9 (OCH$_3$), 53.3 (C12), 47.8 (C10b), 40.9 (NCH$_3$), 33.8 (C11), 27.6 (C2), 21.4 (OC(O)CH$_3$). The spectroscopic data was in agreement with the literature.$^{28}$ However, based on the position of cross peaks on the HSQC NMR the assignment of the $^{13}$C peaks for C4a and C7 were swapped compared to those reported in the literature.$^{28}$

**Lycoramine, 25**

This method was adapted from a method reported by Han *et al.* for the hydrogenation of similar structures.$^{52}$ Briefly galanthamine (0.7000 g, 2.436 mmol) was dissolved in dry methanol (12.0 cm$^3$) and to this was added palladium on carbon (0.1403 g). The nitrogen atmosphere was evacuated and the mixture placed under hydrogen and stirred overnight. The reaction mixture was filtered over silica, eluting with dichloromethane:methanol:35% aqueous ammonia (9:0.9:0.1). The solvent was removed under vacuum to give lycoramine as a white solid. Yield 93%, 0.6535 g, 2.258 mmol.

**MS** m/z (ES$^+$) 290 (100%, MH$^+$); **$^1$H NMR** (500 MHz, CHLOROFORM-$d$) δ ppm 6.65 (1 H, d, $J=8.0$ Hz, H8), 6.60 (1 H, d, $J=8.0$ Hz, H7), 4.36 - 4.39 (1 H, m, H1), 4.06 - 4.12 (1 H, m, H3), 4.00 (1 H, d, $J=15.0$ Hz, H6), 3.86 (3 H, s, OCH$_3$), 3.62 (1 H, d, $J=15.0$ Hz, 175
H6), 3.20 (1 H, ddd, J=14.5, 12.5, 2.0 Hz, H12), 3.01 - 3.07 (1 H, m, H12), 2.55 (1 H, d, J=9.0 Hz, OH), 2.50 (1 H, ddd, J=16.0, 4.0, 2.5 Hz, H2), 1.96 (1 H, ddd, J=13.5, 12.5, 3.0 Hz, H11), 1.90 (2 H, ddd, J=16.0, 4.5, 3.5 Hz, H2), 1.67 - 1.86 (3 H, m, H4, H4a, and H4a), 1.65 (1 H, ddd, J=13.5, 3.8, 1.9 Hz, H11), 1.53 - 1.61 (1 H, m, H4); $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ ppm 145.9 (C10), 143.9 (C9), 136.2 (C10a), 128.9 (C6), 121.7 (C7), 110.6 (C8), 89.9 (C1), 65.3 (C3), 60.4 (C6), 55.8 (OCH$_3$), 54.0 (C12), 46.7 (C10b), 41.8 (NCH$_3$), 31.5 (C2), 31.1 (C11), 27.6 (C4), 23.6 (C4a). Spectroscopic data were in agreement with the literature.

12.2 The compounds of Chapter 5: The isolation and identification of alkaloids

Homolycorine, 11

Homolycorine crystallized out of the factory production line at various points, usually in the form of the hydrobromide salt, which could be purified by recrystallization from a mixture of hot toluene and methanol. A specimen of the crystals was basified using ammonia solution, 35% (30 cm$^3$) and extracted with toluene (3 × 30 cm$^3$), the organic fractions were combined, dried over magnesium sulphate and then the solvent was removed under reduced pressure. The residue was analysed.

At a further stage in the investigation more homolycorine was also isolated, from the residue of factory effluent, by column chromatography, eluting with dichloromethane: methanol-35% aqueous ammonia (9:1) in a gradient 98:2, 96:4, 94:6, 92:8, 90:10.

MS m/z (ES) 316 (100%, MH$^+$), IR $\nu_{\text{max}}$ 1712 cm$^{-1}$ (\textit{\nuC=O}); $^1$H NMR (300 MHz, CHLOROFORM-d) δ ppm 7.55 (1 H, s, H7), 6.94 (1 H, s, H10), 5.45 - 5.52 (1 H, m, H3), 4.79 (1 H, ddd, J=4.0, 2.0, 2.0 Hz, H1), 3.94 (3 H, s, OCH$_3$), 3.93 (3 H, s, OCH$_3$), 3.12 (1 H, ddd, J=9.5, 6.0, 4.0 Hz, H12), 2.66 - 2.74 (1 H, m, H4a), 2.57 - 2.65 (3 H, m, H10b, H11 and H11), 2.44 - 2.54 (2 H, m, H2), 2.21 (1 H, ddd, J=9.5, 9.5, 9.5 Hz, H12), 1.98 (3 H, s, NCH$_3$); $^{13}$C NMR (75 MHz, CHLOROFORM-d) δ ppm 165.8 (C6), 153.0 (C9),
E. W. D. Burke

148.8 (C8), 141.0 (C4), 137.7 (C10a), 116.9 (C6a), 115.1 (C3), 111.9 (C7), 110.7 (C10), 77.6 (C1), 66.5 (C4a), 56.6 (C12), 56.3 (OCH₃), 56.1 (OCH₃), 44.3 (NCH₃), 43.7 (C10b), 31.2 (C2), 28.0 (C11). NMR data is in agreement with the literature.¹⁶¹ ¹⁶² X-ray crystallography of homolycorine hydrobromide confirmed the identity of the compound.

Figure 12.3: X-Ray structure of homolycorine hydrobromide.

Haemanthamine, 10

A specimen of “Acid water” (200 cm³) factory effluent was basified by the addition of ammonia solution, 35%, and then evaporated, using toluene to aid with the removal of the water. The residue was then dissolved in a minimal amount of distilled water and methanol was added, the addition of methanol caused the formation of a precipitate. The solid (1.30 g) was collected by filtration and the filtrate was evaporated to dryness to give a residue (1.78 g). The filtrate residue was then subjected by column chromatography, eluting with dichloromethane:methanol:35% ammonia solution (9.0:0.9:0.1). Many fractions were collected. Fractions 24 - 26 were combined and analysed. Initial NMR showed that the fractions were impure, however, upon standing colourless crystal formed. The crystals were collected and analysis revealed the compound to be haemanthamine, yield 74.1 mg, 0.246 mmol. The appendix contains a brief description of the slightly
unorthodox process used to elucidate the structure. The residue was bright yellow and could not be successfully identified.

**MS** m/z (ES) 302 (100%, MH+); **IR** ν_{max} 3030 cm⁻¹ (ν_{Ar-H}), 2927 cm⁻¹ (ν_{C-H}); **¹H NMR**

(400 MHz, CHLOROFORM-d) δ ppm 6.84 (1 H, s, H10), 6.48 (1 H, s, H7), 6.45 (1 H, d, J=10.0 Hz, H1), 6.39 (1 H, ddd, J=10.0, 5.0, 1.0 Hz, H2), 5.91 (1 H, d, J=1.5 Hz, OCH₂), 5.89 (1 H, d, J=1.5 Hz, OCH₂), 4.34 (1 H, d, J=17.0 Hz, H6), 3.97 - 4.02 (1 H, m, H11), 3.88 (1 H, td, J=4.4, 2.0 Hz, H3), 3.71 (1 H, d, J=17.0 Hz, H6), 3.33 - 3.44 (2 H, m, H4α and H12), 3.38 (3 H, m, OCH₃), 3.27 (1 H, dd, J=14.0, 3.0 Hz, H12), 2.13 (1 H, td, J=13.5, 4.5 Hz, H4), 2.03 (1 H, ddd, J=13.5.0, 5.0, 2.0, 1.0 Hz, H4), 1.85 (1 H, d, J=2.5 Hz, OH);

**¹³C NMR** (126 MHz, CHLOROFORM-d) δ ppm 146.4 (C9), 146.0 (C8), 135.3 (C10α), 131.7 (C1), 127.4 (C2), 126.7 (C6α), 106.8 (C7), 103.2 (C10), 100.7 (OCH₂O), 80.0 (C11), 72.7 (C3), 63.6 (C6), 62.6 (C4α), 61.3 (C12), 56.5 (OCH₃), 50.0 (C10b), 28.2 (C4).

Spectroscopic data are in agreement with the literature.²³

### 3.4-Didehydro-3-deoxygalanthamine or anhydro galanthamine, 142

3,4-Didehydro-3-deoxygalanthamine was isolated from samples of galanthamine (released from the hydrobromide salt by extraction from saturated sodium hydrogen carbonate) and the residue from failed attempts to purify galanthamine by recrystallization. In both cases the isolation was by column chromatography, eluting with a gradient of dichloromethane and methanol-35% aqueous ammonia (9:1) (98:2, 96:4, 94:6, 92:8, 90:10). In the best instance the yield, by mass, was about 7%. (See 0, p. 242 for spectroscopic details).

### Apoahaemanthamine, 145

![Apoahaemanthamine](145)

A specimen of “Acid water” (200 cm³) factory effluent was evaporated under reduced pressure and the residue (3.7 g) was filtered through a plug of silica gel, eluting with a gradient from dichloromethane:methanol:35% ammonia solution (9:0:9:0.1). The filtrate was evaporated to dryness under reduced pressure and subjected to column chromatography eluting with dichloromethane:methanol-ammonia solution 35% (9:1) (98:2, 96:4, 94:6, 92:8, 90:10, 300 cm³ of each mixture). Numerous fractions were collected; fractions 44-47 contained the product in an identifiable, but impure form. Yield 0.1684 g, 0.6253 mmol.
MS m/z (ES) 270 (90%, MH⁺); IR νmax (film)/cm⁻¹ 2936 and 2900 (C-H); ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 6.84 (1 H, s, H10), 6.77 (1 H, dd, J=8.5, 5.5 Hz, H2), 6.64 (1 H, dd, J=8.5, 1.5 Hz, H1), 6.49 (1 H, s, H7), 5.92 (1 H, d, J=1.5 Hz, OCH₂O), 5.91 (1 H, d, J=1.5 Hz, OCH₂O), 4.41 (1 H, ddt, J=5.5, 4.0, 1.5 Hz, H3), 4.31 (1 H, d, J=1.5 Hz, OCH₂O), 4.31 (1 H, d, J=17.0 Hz, H6), 3.72 (1 H, dd, J=4.5, 1.5 Hz, H11), 3.72 (1 H, d, J=17.0 Hz, H6), 3.30 (1 H, d, J=13.5 Hz, H12), 3.11 (1 H, ddd, J=13.5, 4.5, 1.5 Hz, H12), 3.06 - 3.10 (1 H, m, H4a), 1.90 (1 H, ddd, J=14.0, 4.0, 2.0 Hz, H4), 1.82 (1 H, ddd, J=14.0, 10.0, 1.5 Hz, H4); ¹³C NMR (126 MHz, CHLOROFORM-d) δ ppm 146.8 (C₉), 146.6 (C₈), 137.5 (C₂), 131.2 (C₁₀a), 129.6 (C₁), 126.5 (C₆a), 106.5 (C₇), 106.3 (C₁₀), 100.9 (OCH₂O), 80.9 (C₁₁), 65.1 (C₃), 61.8 (C₁₂), 60.9 (C₄a), 59.9 (C₆), 47.2 (C₁₀b), 35.4 (C₄).

Spectroscopic data are in reasonable agreement with the literature, some minor differences most likely arising due to the slightly impure nature of the sample isolated.¹⁴¹

12.3 The compounds of Chapter 6: The resolution of narvedine

Some of the compounds from chapter 6 were also synthesised as part of the work in other chapters; the synthesis and spectroscopic details of such compounds are detailed under their alternative sections within the experimental.

Narwedine, 24, see section 12.1.b.

O-Triisopropylsilylgalanthamine, 155, and O-Triisopropylsilylsanguinine, 156, see section 13.1, starting at p. 189.

(4aS,6R,8aS)-6-((tert-Butyldimethylsilyl)oxy)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine, 153

tert-Butyldimethylsilyl protected galanthamine was synthesised using a variation of a method reported by Han et al. for the installation of a triisopropylsilyl protecting group.⁵² Galanthamine (0.2866 g, 0.9974 mmol, 1 eq.) was dissolved in dichloromethane (2.0 cm³), and cooled to 0 °C, tert-butylidemethylsilyl trifluoromethanesulfonate (0.690 cm³, 3.00 mmol, 3 eq.) and 2,6-lutidine (0.350 cm³, 3.00 mol, 3 eq.) were added, and the mixture...
stirred for 15 minutes and then heated to reflux for 2 days. The mixture was allowed to cool to room temperature and then dichloromethane (20 cm³) was added and the reaction mixture washed with saturated sodium hydrogen carbonate (10 cm³), and then brine (10 cm³). The solvents were removed and the residue purified by column chromatography, eluting with a sharp gradient of dichloromethane:methanol (95:5, 90:10) to give an oil. Yield 57%, 0.2301 g, 0.5729 mmol.

**IR** \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2926 and 2854 (C-H); **MS** m/z (ES+) 402 (100%, MH\(^+\)); **\(^1\)H NMR** (500 MHz, CHLOROFORM-\(d\)) \(\delta\) ppm 6.61 (1 H, d, \(J=8.0\) Hz, H8), 6.52 (1 H, d, \(J=8.0\) Hz, H7), 6.07 (1 H, dd, \(J=10.5, 1.0\) Hz, H4a), 5.83 (1 H, dd, \(J=10.5, 4.0\) Hz, H4), 4.56 (1 H, td, \(J=4.0, 1.0\) Hz, H1), 4.23 - 4.28 (1 H, m, H3), 4.13 (1 H, d, \(J=15.0\) Hz, H6), 3.79 (3 H, s, OCH\(_3\)), 3.62 (1 H, d, \(J=15.0\) Hz, H6), 3.28 (1 H, ddd, \(J=14.5, 13.0, 1.5\) Hz, H12), 3.00 (1 H, m, H12), 2.38 (1 H, dt, \(J=15.0, 4.0\) Hz, H2), 2.34 (3 H, s, NCH\(_3\)), 2.10 (1 H, ddd, \(J=13.5, 13.0, 3.0\) Hz, H11), 2.07 (1 H, ddd, \(J=15.0, 5.0, 4.0\) Hz, H2), 1.49 (1 H, ddd, \(J=13.5, 4.0, 1.5\) Hz, H11), 0.86 (9 H, s, SiC(CH\(_3\)_3)), 0.07 (3 H, s, SiCH\(_3\)), 0.02 (3 H, s, Si(CH\(_3\)_3)); **\(^{13}\)C NMR** (126 MHz, CHLOROFORM-\(d\)) \(\delta\) ppm 146.9 (C10), 143.8 (C9), 132.3 (C10a), 129.0 (C4), 128.8 (C6a), 127.2 (C4a), 120.9 (C7), 111.6 (C8), 86.9 (C1), 62.0 (C3), 60.1 (C6), 56.1 (OCH\(_3\)), 53.8 (C12), 48.1 (C10b), 41.6 (NCH\(_3\)), 34.9 (C11), 32.8 (C2), 25.7 (SiC(CH\(_3\)_3)), 18.0 (SiC(CH\(_3\)_3)), -4.6 (SiCH\(_3\)), -4.7 (SiCH\(_3\)).

(4aS,6R,8aS)-6-((tert-Butyldimethylsilyl)oxy)-11-methyl-4a,5,9,10,11,12-hexahydro-6\(H\)-benzo[2,3]benzofuro[4,3-\(cd\)]azepin-3-ol, 154

O-demethylation of (4aS,6R,8aS)-6-((tert-butyldimethylsilyl)oxy)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6\(H\)-benzo[2,3]benzofuro[4,3-\(cd\)]azepine (0.1420 g, 0.3536 mmol) was performed using the same method as for the synthesis of sanguinine (see section 12.1.b). However, the yield was less than 8% and still very impure after column chromatography. Presence of the desired product was indicated by accurate mass (HRMS: found 388.2304, MH\(^+\) requires 3882303) and the presence of the H8, H7, H4a, H4, H1, H3, and H6 protons (visible in a cleaner part of the \(^1\)H NMR), the peak for the methoxy
group was clearly absent. The remaining peaks were also clearly visible although accompanied by a significant degree of impurity.

\[(4aS,6R,8aS)-11\text{-Methyl-6-}((\text{triisopropylsilyl})\text{oxy})-4a,5,9,10,11,12\text{-hexahydro-6H-benzo[2,3]benzofuro[4,3-}cd\text{]}azepin-3-yl \text{pivalate, 157}\]

\[(4aS,6R,8aS)-11\text{-methyl-6-}((\text{triisopropylsilyl})\text{oxy})-4a,5,9,10,11,12\text{-hexahydro-6H-benzo[2,3]benzofuro[4,3-}cd\text{]}azepin-3-ol (99.8 mg, 0.232 mmol, 1 eq.) was dissolved in tetrahydrofuran (1.25 cm\(^3\)) and added to sodium hydride, 60% dispersion in mineral oil, (29.0 mg, 0.725 mmol, 3.1 eq.) in tetrahydrofuran (0.25 cm\(^3\)) at 0 °C. The reaction was stirred for 30 minutes and then trimethylacetyl chloride (0.115 cm\(^3\), 0.934 mmol, 4 eq.) was added, the reaction was stirred for a further hour and then allowed to warm to room temperature and stirred overnight. The reaction was quenched with water (1.0 cm\(^3\)) and then the organic solvent was removed under reduced pressure and the residue worked up with saturated sodium hydrogen carbonate (20 cm\(^3\)) and dichloromethane (4 × 20 cm\(^3\)). The organic fractions were combined, dried over magnesium sulphate and the solvent removed under reduced pressure to give the title compound with only trace impurities which could be used without further purification. Yield 94%, 0.1128 mg, 0.2195 mmol.

**MS** m/z (ES+) 514 (100%, MH\(^+\)); **HRMS**: found 514.3356, MH\(^+\) requires 514.3348; \(^1\)H NMR (300 MHz, CHLOROFORM-d) δ ppm 6.74 (1 H, d, \(J=8.0 \text{ Hz, H8}\)), 6.56 (1 H, d, \(J=8.0 \text{ Hz, H7}\)), 6.07 (1 H, br d, \(J=10.5 \text{ Hz, H4a}\)), 5.89 (1 H, dd, \(J=10.5, 4.0 \text{ Hz, H4}\)), 4.57 (1 H, td, \(J=3.5, 1.0 \text{ Hz, H1}\)), 4.31 - 4.38 (1 H, m, H3), 4.18 (1 H, d, \(J=15.5 \text{ Hz, H6}\)), 3.68 (1 H, d, \(J=15.5 \text{ Hz, H6}\)), 3.33 (1 H, ddd, \(J=14.5, 13.0, 1.5 \text{ Hz, H12}\)), 2.98 - 3.09 (1 H, m, H11), 2.40 - 2.49 (1 H, m, H2), 2.39 (3 H, s, OCH\(_3\)), 2.13 (1 H, ddd, \(J=13.5, 13.0, 3.0 \text{ Hz, H11}\)), 1.98 - 2.08 (1 H, m, H2), 1.43 - 1.53 (1 H, m, H11), 1.33 (9 H, s, C(CH\(_3\))\(_3\)), 1.02 - 1.07 (21 H, brs, Si(CH(CH\(_3\))\(_2\))\(_3\)); \(^13\)C NMR (75 MHz, CHLOROFORM-d) δ ppm 176.3 (C=O), 149.7 (C10), 134.1 (C9), 133.9 (C6), 133.6 (C10a), 128.8 (C4), 126.7 (C4a), 121.6 (C8), 120.7 (C7), 87.2 (C1), 61.8 (C3), 60.2 (C6), 53.7 (C12), 48.0 (C10b), 41.4 (NCH\(_3\)), 39.0 (C(CH\(_3\))\(_3\)), 34.4 (C11), 32.4 (C2), 29.6 (Si(C(CH\(_3\))\(_2\))\(_3\)), 27.1 (C(CH\(_3\))\(_3\)), 18.1 (Si(CH(CH\(_3\))\(_2\))\(_3\)), 12.3 (Si(CH\(_3\))\(_3\)).
TBAF 1 mol dm\(^{-3}\) 5% water in THF (2.40 cm\(^3\), 2.40 mmol, 2 eq.) was added dropwise to a solution of (4aS,6R,8aS)-11-methyl-6-((triisopropylsilyl)oxy)-4a,5,9,10,11,12-hexahydro-6\(H\)-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl pivalate (0.6175, 1.202 mmol, 1 eq.) in THF (5.50 cm\(^3\)) at 0 °C and stirred for 20 minutes, after which the reaction was allowed to warm to room temperature and stirred for 3 hours 20 minutes after which hydrochloric acid 0.6 M (1.00 cm\(^3\), 0.600 mmol, 0.5 eq.) was added and the reaction stirred overnight. The THF was removed under vacuum and saturated sodium hydrogen carbonate (20.0 cm\(^3\)) was added, the pH was increased to pH 9 by the addition of concentrated aqueous ammonia and the solution was extracted with dichloromethane (3 × 20.0 cm\(^3\)). The organic phase was dried over magnesium sulphate and the solvent removed under vacuum, the residue (0.7569 g) was purified by column chromatography eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10), to give the product as an oil (0.2505 g, 0.7008 mmol, 58%).

**MS** m/z (ES+) 358 (100%, MH\(^+\)); **HRMS**: found 358.2020, MH\(^+\) requires 358.2013; **\(^1\)H NMR** (400 MHz, CHLOROFORM-d) \(\delta\) ppm 6.78 (1 H, d, \(J=8.0\) Hz, H8), 6.62 (1 H, d, \(J=8.0\) Hz, H7), 6.02 - 6.05 (2 H, m, H4 and H4a), 4.59 - 4.64 (1 H, m, H1), 4.08 - 4.14 (1 H, m, H3), 4.11 (1 H, d, \(J=15.0\) Hz, H6), 3.72 (1 H, d, \(J=15.0\) Hz, H6), 3.29 (1 H, ddd, \(J=14.5, 12.5, 2.0\) Hz, H12), 3.00 - 3.10 (1 H, m, H12), 2.56 - 2.63 (1 H, m, H2), 2.43 (3 H, s, NCH\(_3\)), 2.12 (1 H, ddd, \(J=14.0, 13.5, 3.5\) Hz, H11), 1.95 (1 H, ddd, \(J=15.5, 5.0, 2.5\) Hz, H2), 1.59 (1 H, ddd, \(J=14.0, 4.0, 2.0\) Hz, H11), 1.33 (9 H, s, C(CH\(_3\))\(_3\)); **\(^{13}\)C NMR** (101 MHz, CHLOROFORM-d) \(\delta\) ppm 175.9 (C=O), 148.5 (C10), 134.7 (C9), 134.1 (C6), 133.5 (C10b), 127.8 (C4), 126.4 (C4a), 121.6 (C7), 120.8 (C8), 88.3 (C1), 61.7 (C3), 60.7 (C6), 53.7 (C12), 48.5 (C10b), 42.1 (NCH\(_3\)), 39.0 (C(CH\(_3\))\(_3\)), 33.1 (C11), 30.0 (C2), 27.1 (C(CH\(_3\))\(_3\)).
11-Methyl-6-oxo-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3cd]azepin-3-yl pivalate, 159

Dimethyl sulphoxide (0.225 cm³, 2.61 mmol) in dichloromethane (0.67 cm³) was added, dropwise, to oxalyl chloride (0.140 cm³, 1.61 mmol) in dichloromethane (3.5 cm³) at -60 °C over 10 minutes. The mixture was allowed to warm to -50 °C and then pivaloyl protected sanguinine (0.2505 g, 0.7008 mmol) in dichloromethane (1.00 cm³) was added dropwise over 10 minutes. The reaction was stirred at this temperature for 35 minutes, and then triethylamine (1.00 cm³, 13.6 mmol) was added dropwise over 18 minutes. The reaction was stirred for a further 10 minutes and then allowed to warm to room temperature, at which point it was quenched by the addition of water (2.00 cm³). Dichloromethane (25.0 cm³) was added and the mixture was washed with water (25.0 cm³), brine (25.0 cm³) and then the organic phase was dried over magnesium sulphate, following which the solvent was removed under vacuum to give a residue (0.2852 g) which was purified by flash column chromatography eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) (9:1) to give the title product as an off white solid (0.1071 g, 0.3013 mmol, 43%).

MS m/z (ES+) 378 (100%, MNa⁺); HRMS: found 356.1840, MH⁺ requires 356.1857; IR νmax (film)/cm⁻¹ 2912 (Alkyl CH), 1746 (C=O piv), 1679 (C=O ab-unsaturated); ¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.98 (3 H, br d, J=10.5 Hz, H4a), 6.83 (3 H, d, J=8.0 Hz, H8), 6.67 (3 H, d, J=8.0 Hz, H7), 6.05 (1 H, d, J=10.5 Hz, H4), 4.73 (1 H, ddd, J=4.0, 2.5, 2.0 Hz, H1), 4.12 (3 H, br d, J=15.5 Hz, H6), 3.78 (1 H, d, J=15.5 Hz, H6), 3.22 - 3.32 (1 H, m, H12), 3.10 - 3.20 (3 H, m, H12), 3.06 (4 H, ddd, J=18.0, 2.5, 1.0 Hz, H2), 2.73 (3 H, ddd, J=18.0, 4.0 Hz, H2), 2.46 (3 H, s, NCH₃), 2.29 (4 H, ddd, J=14.0, 13.5, 3.5 Hz, H11), 1.85 (3 H, ddd, J=13.5, 3.5, 2.5 Hz, H11), 1.33 (9 H, s, C(CH₃)₃); ¹³C NMR (101 MHz, CHLOROFORM-d) δ ppm 194.5 (C3), 176.1 (C=O), 149.7 (C10), 144.4 (C4a), 134.8 (C9), 134.2 (C6), 131.4 (C10b), 127.1 (C4), 122.6 (C8), 121.7 (C7), 88.0 (C1), 60.5 (C6), 53.9 (C12), 48.8 (C10b), 42.2 (NCH₃), 39.0 (C(CH₃)₃), 37.2 (C2), 32.7 (C11), 27.1 (C(CH₃)₃).
tert-Butyl ((4aS,6R,8aS)-6-hydroxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl) carbonate, 158

Sanguinine (0.2003 g, 0.7328 mmol, 1 eq.), sodium hydride, 60% dispersion in mineral oil (0.0301 g, 0.752 mmol, 1 eq.) and di-tert-butyl dicarbonate (0.1596 g, 0.7313 mmol, 1 eq.) were combined in a flask and tetrahydrofuran was added. The reaction was stirred for 24 hours and then quenched by the addition of saturated aqueous sodium hydrogen carbonate (10 cm³). The organic solvent was removed under reduced pressure and then the mixture was extracted with dichloromethane (4 × 10 cm³). The organic phases were combined and dried over magnesium sulfate and then the solvent was removed under reduced pressure to give the product in a usably clean form. Yield 87%, 0.2379 g, 0.6370 mmol.

¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 6.85 (1 H, d, J=8.0 Hz, ArH), 6.61 (1 H, d, J=8.0 Hz, ArH), 6.01 - 6.07 (2 H, m, H4 and H4a), 4.62 - 4.66 (1 H, m, H1), 4.09 - 4.15 (1 H, m, H3), 4.10 (1 H, br d, J=15.5 Hz, H6), 3.72 (1 H, d, J=15.5 Hz, H6), 3.29 (1 H, ddd, J=14.5, 13.0, 1.5 Hz, H12), 3.04 - 3.11 (1 H, m, H12), 2.86 (1 H, br d, J=12.0 Hz, OH), 2.62 (1 H, ddd, J=15.5, 3.0, 1.5 Hz, H2), 2.43 (3 H, s, NCH₃), 2.12 (1 H, ddd, J=14.0, 13.0, 3.0 Hz, H11), 1.97 (1 H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.60 (1 H, ddd, J=14.0, 4.0, 1.5 Hz, H11), 1.54 (9 H, s, C(CH₃)₃); ¹³C NMR (126 MHz, CHLOROFORM-d) δ ppm 150.8 (C=O), 148.4 (Aryl), 135.1 (Aryl), 134.2 (Aryl), 133.7 (Aryl), 127.8 (C4a), 126.4 (C4), 121.6 (Aryl CH), 120.6 (Aryl CH), 121.8 (Aryl CH), 134.2 (Aryl), 133.7 (Aryl), 127.8 (C4a), 126.4 (C4), 121.6 (Aryl CH), 120.6 (Aryl CH), 88.5 (C1), 84.0 (C(CH₃)₃), 61.7 (C3), 60.8 (C6), 53.8 (C12), 48.6 (C10b), 42.3 (NCH₃), 33.2 (C11), 30.0 (C2), 29.7 (s), 28.4 (s), 27.5 (C(CH₃)₃).
tert-Butyl ((4aS,8aS)-11-methyl-6-oxo-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl) carbonate, 159

Oxidation of the allylic alcohol was carried out using the same method as for the synthesis of narwedine or pivaloyl protected sanguinine. The column chromatography, eluting with dichloromethane – methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10) returned a slightly dirty product. Yield 75%, 2.26 g, 6.08 mmol.

MS m/z (ES+) 372 (100%, MH+); ¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.95 (1 H, d, J=8.5 Hz, H8), 6.92 - 6.98 (1 H, m, H4a), 6.66 (1 H, d, J=8.5 Hz, H7), 6.04 (1 H, d, J=10.5 Hz, H4), 4.73 - 4.77 (1 H, m, H1), 4.11 (1 H, br d, J=15.5 Hz, H6), 3.77 (1 H, d, J=15.5 Hz, H3), 3.21 - 3.31 (1 H, m, H12), 3.10 - 3.18 (1 H, m, H12), 3.09 (1 H, dd, J=18.0, 2.5 Hz, H2), 2.74 (1 H, dd, J=18.0, 3.5 Hz, H2), 2.44 (3 H, s, NCH₃), 2.23 - 2.33 (1 H, m, H11), 1.85 (1 H, br d, J=13.9 Hz, H11), 1.51 (9 H, s, C(CH₃)₃).

O-Desmethylnarwedine or 3-hydroxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-6-one, 148

The method used for the cleavage of the protecting group on the O-desmethylnarwedine analogue was achieved using the method reported by Brancour et al. Briefly pivaloyl or Boc protected O-desmethylnarwedine (0.1071 g, 0.3013 mmol) was stirred in 2 M aqueous sodium hydroxide solution (9.30 cm³), ethanol (4.00 cm³) was added to increase the solubility of the alkaloidal analogue, finally methanol was added slowly until complete dissolution was achieved. The reaction was stirred at room temperature until TLC showed the reaction to be complete (approximately 1-3 hours), the ethanol and methanol were removed under vacuum and the remaining aqueous residue was extracted with
dichloromethane (3 × 10.0 cm$^3$). The solvent was removed under vacuum and the residue purified by column chromatography, eluting with dichloromethane – methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10), to give a white solid, tinged with yellow (59.5 mg, 0.219 mmol, 73%).

When the reaction was performed on a larger scale the extraction procedure had to be modified. The pH of the aqueous reaction mixture was adjusted to pH 7-8 and then extracted with chloroform – propan-2-ol (3:1) the result was a solution of golden metallic appearance. This solution was evaporated to dryness under reduced pressure and the residue was washed with methanol and then copious amounts of hot ethanol. The residue was dried in the vacuum oven at 35 °C for 6 hours to return the product as a silvery solid. Yield 52%, 1.15 g, 4.23 mmol. **Warning!** Once crystalized this compound resists dissolving in almost anything, only DMSO was found to dissolve it!

**MS** m/z (ES+) 272 (100%, MH$^+$), (ES-) 270 (100%, M-H$^+$); **HRMS**: found 272.1286, MH$^+$ requires 272.1282; **$^1$H NMR** (500 MHz, DMSO-d$_6$) δ ppm 9.24 (1 H, br s, ArOH), 7.15 (1 H, dd, $J$=10.5, 2.0 Hz, H4a), 6.56 (1 H, d, $J$=8.0 Hz, H8), 6.50 (1 H, d, $J$=8.0 Hz, H7), 5.92 (1 H, d, $J$=10.5 Hz, H4), 4.66 - 4.70 (1 H, dd, $J$=3.5, 2.0 Hz, H1), 4.11 (1 H, br d, $J$=15.5 Hz, H6), 3.57 (1 H, d, $J$=15.5 Hz, H6), 3.15 - 3.24 (1 H, m, H12), 3.04 (1 H, dd, $J$=17.5, 3.5 Hz, H2), 2.92 - 3.01 (1 H, m, H12), 2.79 (1 H, dd, $J$=17.5, 2.0 Hz, H2), 2.27 (3 H, s, NCH$_3$), 2.12 - 2.19 (1 H, m, H11), 1.79 - 1.86 (1 H, m, H11); **$^{13}$C NMR** (126 MHz, DMSO-d$_6$) δ ppm 195.2 (C3), 145.7 (C4a), 145.6 (C10), 140.6 (C9), 131.0 (C10a), 128.4 (C6a), 126.0 (C4), 121.6 (C7), 115.8 (C8), 87.0 (C1), 59.4 (C6), 53.0 (C12), 48.8 (C10b), 41.1 (NCH$_3$), 37.6 (C2), 31.4 (C1).

**tert-Butyl 3-methoxy-6-oxo-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxylate, 163**

\[
\text{N-Boc-galanthamine was oxidised to the narwedine analogue using the same procedure used for the synthesis of narwedine. The product was purified by recrystallization from ethanol. Yield 82%, 1.2414 g, 3.342 mmol.}
\]
\[ ^1H\ NMR\ (500\ MHz,\ CHLOROFORM-d)\ \delta\ ppm\ 6.80 - 6.90\ (1.4\ H,\ m,\ H4a\ and\ H7),\ 6.68 - 6.74\ (1.6\ H,\ m,\ H7\ and\ H8),\ 6.05\ (1\ H,\ d,\ J=10.5\ Hz,\ H4),\ 4.93\ (0.4\ H,\ br\ d,\ J=16.0\ Hz,\ H6),\ 4.75\ (0.6\ H,\ d,\ J=16.0\ Hz,\ H6),\ 4.69 - 4.73\ (1\ H,\ m,\ H1),\ 4.34 - 4.41\ (0.6\ H,\ m,\ H12),\ 4.21 - 4.28\ (0.4\ H,\ m,\ H12),\ 4.17 - 4.21\ (1\ H,\ br\ d,\ J=16.0\ Hz,\ H6),\ 4.12\ (1\ H,\ br\ d,\ J=16.0\ Hz,\ H6),\ 3.85\ (1.8\ H,\ s,\ OCH_3),\ 3.84\ (1.2\ H,\ s,\ OCH_3),\ 3.66 - 3.44\ (0.4\ H,\ m,\ H12),\ 3.25 - 3.34\ (1\ H,\ m,\ H12),\ 3.14 - 3.22\ (1\ H,\ m,\ H2),\ 2.72 - 2.80\ (1\ H,\ m,\ H2),\ 2.11 - 2.20\ (0.6\ H,\ m,\ H11),\ 1.97 - 2.09\ (1.4\ H,\ m,\ H11\ and\ H11),\ 1.43\ (3.6\ H,\ s,\ C(CH_3)_3),\ 1.38\ (5.4\ H,\ s,\ C(CH_3)_3);\ \(^{13}C\ NMR\ (126\ MHz,\ CHLOROFORM-d)\ \delta\ ppm\ 194.2\ (C_3^{\text{major}}),\ 194.1\ (C_3^{\text{minor}}),\ 154.9\ (C=O^{\text{major}}),\ 154.5\ (C=O^{\text{minor}}),\ 147.6\ (C_{10}^{\text{major}}),\ 147.5\ (C_{10}^{\text{minor}}),\ 144.2\ (C_9^{\text{major}}),\ 144.1\ (C_9^{\text{minor}}),\ 143.9\ (C_{4a}^{\text{major}}),\ 143.6\ (C_{4a}^{\text{minor}}),\ 129.9\ (C_{10a}^{\text{minor}}),\ 129.8\ (C_{10a}^{\text{major}}),\ 129.6\ (C_6),\ 129.6\ (C_6),\ 127.4\ (C_4^{\text{major}}),\ 127.3\ (C_4^{\text{minor}}),\ 121.6\ (C_7^{\text{minor}}),\ 121.0\ (C_7^{\text{major}}),\ 111.9\ (C_8^{\text{minor}}),\ 111.5\ (C_8^{\text{major}}),\ 87.9\ (C_{11}^{\text{major}}),\ 87.7\ (C_{11}^{\text{minor}}),\ 80.1\ (C(CH_3)_3^{\text{major}}),\ 56.0\ (OCH_3\ \text{major}\ and\ \text{minor}),\ 52.0\ (C_6^{\text{major}}),\ 51.4\ (C_6^{\text{minor}}),\ 49.1\ (C_{10b}),\ 45.9\ (C_{12}^{\text{minor}}),\ 45.7\ (C_{12}^{\text{major}}),\ 37.3\ (C_{2}^{\text{major}}),\ 37.2\ (C_{2}^{\text{minor}}),\ 36.7\ (C_{11}^{\text{minor}}),\ 35.6\ (C_{11}^{\text{major}}),\ 28.5\ (C(CH_3)_3^{\text{minor}}),\ 28.3\ (C(CH_3)_3^{\text{major}}).\]

\((4aS,5R,10bR)-10\text{-Hydroxy-9-methoxy-4,4a-dihydro-3H,6H-5,10b-ethanophenanthridin-3-one, 161}\)

\(N\text{-boc-nornarwedine} (0.7003\ g, 1.885\ mmol)\) was dissolved in dichloromethane (14.0 cm\(^3\)) and trifluoroacetic acid (14.0 cm\(^3\)) was added, the mixture was stirred for 1 hour 45 minutes. The solvents were then removed under reduced pressure. Saturated aqueous sodium hydrogen carbonate (30 cm\(^3\)) was added to the residue and the mixture was extracted with dichloromethane (4 \times 30\ cm\(^3\)). The organic fractions were combined, dried over magnesium sulphate and the solvent was removed under reduced pressure. The product was purified by columned chromatography, eluting with dichloromethane – methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10, 90:10, 88:12, 86:14). Yield 19%, 0.0995 g, 0.367 mmol.
An alternative method is the N-demethylation of narwedine by means of the same method used to synthesise norgalanthamine from galanthamine. On a 0.1000 g, 0.3504 mmol, scale this alternative method gave a yield of 65%, 0.0616 g, 0.227 mmol.

**MS** m/z (ES+) 272 (60%, MH⁺), (ES-) 270 (65%, M-H⁺); **HRMS**: found 272.1296, MH⁺ requires 272.1287; **IR** νmax (film)/cm⁻¹ 3087 (Aryl C-H), 2912 (Alkyl CH), 1670 (C=O);

**¹H NMR** (400 MHz, METHANOL-d₄) δ ppm 8.95 (d, J=11.0 Hz, H1), 6.81 (d, J=8.5 Hz, H8), 6.51 (d, J=8.5 Hz, H7), 5.96 (d, J=11.0 Hz, H2), 4.37 (d, J=16.5 Hz, H6), 3.84 (3 H, S, OCH₃), 3.75 (d, J=16.5 Hz, H6), 3.61 - 3.68 (1 H, m, H4a), 3.52 (1 H, ddd, J=13.0, 10.5, 3.3 Hz, H12), 2.94 (ddd, J=13.0, 9.0, 7.0 Hz, H12), 2.52 - 2.59 (2 H, m, H4 and H4a), 2.45 (ddd, J=12.5, 9.0, 3.5 Hz, H11), 2.26 (ddd, J=12.5, 10.5, 7.0 Hz, H11); **¹³C NMR** (101 MHz, METHANOL-d₄) δ ppm 200.1 (C3), 155.0 (C1), 147.8 (C9), 144.7 (C10), 129.2 (C10A), 128.7 (C2), 127.0 (C6), 118.8 (C7), 111.3 (C8), 70.5 (C1), 62.0 (C6), 56.8 (NCH₃), 54.5 (C12), 44.0 (C2), 41.0 (C2).
13 Experimental – Part 2: Synthetic methods for the structural modification and functionalization of galanthamine

13.1 The compounds of Chapter 7: Ortholithiation chemistry for galanthamine C8 functionalization

*O*-Triisopropylsilylgalanthamine or \((4aS,6R,8aS)-3\)-methoxy-11-methyl-6-((triisopropylsilyl)oxy)-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine, 155

*O*-Triisopropylsilylgalanthamine was synthesised by the method of Han *et al.*

Galanthamine (0.5740 g, 1.998 mmol, 1 eq.) was dissolved in dichloromethane (29.0 cm\(^3\)), and cooled to 0 °C, triisopropylsilyl trifluoromethanesulfonate (0.645 cm\(^3\), 3.00 mmol, 1.5 eq.) and 2,6-lutidine (0.560 cm\(^3\), 4.82 mmol, 2.4 eq.) were added, and the mixture stirred for 20 minutes. The mixture was allowed to warm to room temperature and then stirred for 3 hours. Ether (100 cm\(^3\)) was added and the mixture was washed with aqueous sodium carbonate (5%, 50 cm\(^3\)), and then brine (50 cm\(^3\)). The solvents were removed and the residue purified by flash column chromatography, eluting with dichloromethane:methanol:35% aqueous ammonia (95:4:1) to give a pale very green oil. Yield 93%, 0.8262 g, 1.862 mmol. A few drops of the oil were dissolved in acetone and left to crystallize into fine needle like, very pale green crystals.

R\(_f\) (9.0:0.9:0.1 dichloromethane:methanol:35% aqueous ammonia) 0.481; Mpt. 117-119 °C (recrystallized from acetone); MS m/z (ES+) 444 (100%, MH\(^+\)); \(^1^H\) NMR (400 MHz, CHLOROFORM-\(d\)) \(\delta\) ppm 6.62 (1 H, d, \(J=8.0\), ArH), 6.53 (1 H, d, \(J=8.0\) ArH), 6.08 (1 H, d, \(J=10.5\), H4a), 5.91 (1 H, dd, \(J=10.5\) and 4.0, H4), 4.59 (1 H, m, \(J=4.5\), H1), 4.37 (1 H, m, \(J=4.0\) and 1.5, H3), 4.14 (1 H, d, \(J=15.0\), H6), 3.80 (3 H, s, OCH\(_3\)), 3.62 (1 H, d, \(J=15.0\), H6), 3.28 (1 H, tt, \(J=13.0\) and 1.5, H12), 3.00 (1 H, m, \(J=14.5\) and 3.5, H12), 2.44 (1 H, m, \(J=15.0\) and 4.0, H2), 2.35 (3 H, s, NCH\(_3\)), 2.17 - 2.08 (2 H, m, \(J=5.0\) and 3.5, H11 and H2), 1.48 (1 H, m, \(J=13.5\) and 2.0, H11), 1.01 - 1.08 (25 H, m, TIPS group); \(^1^C\) NMR
O-Triisopropylsilylsanguinine or (4aS,6R,8aS)-11-methyl-6-((triisopropylsilyl)oxy)-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-ol, 156

O-Triisopropylsilylgalanthamine, 155, (2.19 g, 4.94 mmol) and L-Selectride (1 mol dm\(^{-3}\), 24.6 cm\(^3\)) were refluxed for 24 hours and then allowed to cool, once at room temperature ethyl acetate (50 cm\(^3\)) was added followed by water (50 cm\(^3\)). The mixture was extracted with dichloromethane (3 \times 60 cm\(^3\)). The solvent was removed and the residue purified by flash column chromatography (9:0:9:0.1 dichloromethane:methanol:35% aqueous ammonia) to give a pale bright yellow solid (2.11 g, 4.91 mmol, 99%).

**R\(_f\)** (9:0:9:0.1 dichloromethane:methanol:35% aqueous ammonia) 0.284; **IR** \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3580 (O-H); **Mpt.** 173-175 °C (recrystallized from dichloromethane); **MS** m/z (ES\(^{+}\)) 430 (100%, MH\(^{+}\)); **\(^1\)H NMR** (500 MHz, CHLOROFORM-d) \(\delta\) ppm 6.56 (1 H, d, \(J=8.0\), ArH), 6.47 (1 H, d, \(J=8.0\), ArH), 6.07 (1 H, d, \(J=10.5\), H4a), 5.91 (1 H, dd, \(J=10.5\) and 4.5, H4), 4.58 (1 H, m, \(J=4.0\), H1), 4.38 (1 H, m, \(J=4.50\), H3), 4.17 (1 H, d, \(J=15.0\), H6), 3.66 (1 H, d, \(J=15.0\), H6), 3.33 (1 H, m, \(J=13.0\), H12), 3.05 (1 H, m, \(J=14.50\) and 4.0, H12), 2.43 (1 H, m, \(J=15.0\) and 3.70, H2), 2.37 (3 H, s, NCH3), 2.17-2.09 (2 H, m, \(J=13.20\), 4.70, and 2.50, H11 and H2), 1.49 (1 H, m, \(J=14.0\) and 2.0, H11), 1.01 - 1.10 (22 H, m, TIPS group); **\(^13\)C NMR** (125 MHz, CHLOROFORM-d) 145.6 (Arly), 140.4 (Aryl), 132.0 (Aryl), 129.0 (C4), 127.4 (Aryl), 127.1 (C4a), 121.4 (Aryl), 115.0 (Aryl), 87.2 (C1), 62.1 (C3), 59.9 (C6), 53.6 (C12), 48.5 (C10b), 41.0 (NCH3), 34.5 (C11), 33.2 (C2), 18.1 and 18.0 (SiCHCH3), 12.3 (SiCH).
(4aS,6R,8aR)-11-Methyl-6-((triisopropylsilyl)oxy)-5,6,9,10,11,12-hexahydro-4aH-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 164

**O-Triisopropylsilylsanguinine, 155**, (1.50 g, 3.49 mmol) was dried by consecutive evaporation from dry THF (3 × 3 cm³). The residue was dissolved in dry THF (1.5 cm³) and added dropwise, over 2 minutes, to a suspension of sodium hydride (60% suspension in mineral oil, 0.418 mg, 10.5 mmol) in dry THF (3.5 cm³) at 0 °C. The reaction mixture was warmed to room temperature for 10 minutes and then cooled to 0 °C. Diethyl carbamoyl chloride (0.885 cm³, 7.00 mmol) was added dropwise, after 10 minutes the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with a few drops of water, and the solvent removed. The residue was dissolved in dichloromethane (100 cm³) and washed with saturated aqueous sodium hydrogen carbonate (2 × 100 cm³). The dichloromethane was removed under vacuum and the residue applied to the top of a silica plug and washed with hexane, and then eluted with dichloromethane and methanol (9:1). The solvent was removed and the residue purified by flash column chromatography (9:0.9:0.1 dichloromethane:methanol:35% aqueous ammonia) to give a very pale yellow oil (0.485 g, 0.917 mmol, 18%). Other fractions contained the starting material, O-Triisopropylsilylsanguinine (0.470 g, 1.09 mmol, 31%), and a mixture of starting material and product (0.596 g).

**Rf** (9:0.9:0.1 dichloromethane-methanol-35% aqueous ammonia) 0.29; **MS** m/z (ES) 529 (100%, MH⁺), **IR** ν max 1722 cm⁻¹ (υC=O); **¹H NMR** (500 MHz, CHLOROFORM-d) δ ppm 6.86 (1 H, d, J=8.0, ArH), 6.54 (1 H, d, J=8.0, ArH), 6.0 (1 H, d, J=10.0, H4a), 6.0 (1 H, dd, J=10.5 and 4.5, H4), 4.58 (1 H, m, J=4.0, H1), 4.36 (1 H, m, J=4.0 and 3.0, H3), 4.19 (1 H, d, J=15.0, H6), 3.67 (1 H, d, J=15.5 H6), 3.48-3.31 (5 H, m, J=7.5 and 6.5, H12 and N-ethyl CH₂), 3.03 (1 H, m, J=14.5, H12), 2.44 (1 H, m, J=15.0 and 3.0, H2), 2.37 (3 H, s, NCH₃), 2.13 (1 H, m, J=13.0 and 3.0, H11), 2.0 (1 H, m, J=15.0 and 4.5, H2), 1.45 (1 H, m, J=14.0 and 2.0, H11), 1.20 (6 H, m, J=7.5, CH₃), 1.00 - 1.09 (22 H, m, TIPS group); **¹³C NMR** (125 MHz, CHLOROFORM-d) δ ppm 153.7 (EtN(C)(O)O), 149.8 (Aryl), 134.5 (Aryl), 133.7 (Aryl), 133.4 (Aryl), 128.7 (C4), 127.0 (C4a), 122.2 (Aryl), 120.6 (Aryl),
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87.1 (C1), 61.9 (C3), 60.1 (C6), 53.7 (C12), 48.1 (C10b), 42.1 and 41.8 (ethyl CH2), 41.1 (NCH3), 34.3 (C11), 32.6 (C2), 18.0 (SiCHCH3), 14.1 and 13.3 (N-ethyl CH3), 12.3 (SiCH).

(4aS,6R,8aS)-6-Hydroxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 167

Tetrahydrofuran (13 cm3) was added to sanguinine (1.28 g, 4.67 mmol, 1 eq.), sodium hydride (60% dispersion in mineral oil) (0.1867 g, 4.67 mmol, 1 eq.) and potassium iodide (0.8540 g, 5.14 mmol, 1.1 eq.) and stirred at room temperature for 24 hours. The reaction was then quenched by the slow addition of saturated aqueous sodium hydrogen carbonate (1 cm3). The organic solvent was removed under reduced pressure, then water (10 cm3) was added to the residue and the mixture was extracted with dichloromethane (3 × 10 cm3). The organic phases were combined and dried over magnesium sulphate. The solvent was removed under reduced pressure and the residue was purified by column chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10) to give the product as a white solid once the solvent was removed. Yield 65%, 1.14 g, 3.05 mmol.

MS m/z (ES) 373 (100%, MH+); 1H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.80 (1 H, d, J=8.0 Hz), 6.59 (1 H, d, J=8.0 Hz), 6.01 (2 H, d, J=3.1 Hz), 6.01 (2 H, s), 4.58 - 4.64 (1 H, m), 4.10 (2 H, br d, J=15.5 Hz), 4.05 - 4.14 (2 H, m), 3.72 (1 H, d, J=15.5 Hz), 3.23 - 3.49 (5 H, m), 3.00 - 3.20 (2 H, m), 2.61 (1 H, dd, J=15.5, 3.0 Hz), 2.43 (3 H, s), 2.12 (1 H, ddd, J=13.0, 3.0 Hz), 1.93 (1 H, ddd, J=15.5, 5.0, 2.0 Hz), 1.59 (1 H, ddd, J=14.0, 3.5, 2.0 Hz), 1.20 (6 H, dt, J=27.1, 6.8 Hz), 13C NMR (101 MHz, CHLOROFORM-d) δ ppm 153.0 (C=O), 148.9 (C10), 134.7 (C9), 134.2 (C6), 133.3 (C10a), 127.7 (C4), 126.4 (C4a), 121.3 (C7), 121.0 (C8), 88.0 (C1), 61.7 (C3), 60.8 (C6), 53.7 (C12), 48.6 (C10b), 42.5 (NCH2CH3), 42.1 (NCH2CH3), 42.0 (OCH3), 33.1 (C11), 30.1 (C2), 14.1 (NCH2CH3), 13.2 (NCH2CH3).
(4aS,6R,8aS)-6-((tert-Butyldimethylsilyl)oxy)-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 168

The O-carbamate protected sanguinine, 167, (0.9309 g, 2.499 mmol, 1 eq.) was dissolved in dichloromethane (5.0 cm$^3$) and tert-butyldimethylsilyl trifluoromethanesulfonate (1.72 cm$^3$, 7.49 mmol, 3 eq.) was added slowly, then 2,6-lutidine (0.875 cm$^3$, 7.5 mmol, 3 eq.). The reaction was stirred overnight. The reaction was then diluted with dichloromethane (30 cm$^3$) and washed with saturated aqueous sodium hydrogen carbonate (20 cm$^3$) and then brine (20 cm$^3$). The organic phase was then dried over magnesium sulfate and the solvent removed under reduced pressure to leave a thin golden oil. The oil was purified by column chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10) to give the product as a golden oil. Yield 53%, 0.6409 g, 1.317 mmol.

$[\alpha]_D^{22}$: -45.1$^\circ$ (c. 1, Chloroform); MS m/z (ES+) 509 (100%, MNa$^+$); HRMS: found 487.2976, MNa$^+$ requires 487.2987; $^1$H-NMR (400 MHz, CHLOROFORM-d) $\delta$ ppm 6.9 (1 H, d, $J$=8.5 Hz, H7), 6.5 (1 H, d, $J$=8.5 Hz, H8), 6.1 (1 H, m, $J$=10.3 Hz, H4a), 5.8 (1 H, dd, $J$=10.5, 4.5 Hz, H4), 4.5 - 4.6 (1 H, m, H1), 4.2 - 4.3 (1 H, m, H3), 4.2 (1 H, d, $J$=15.0 Hz, H6), 3.7 (1 H, d, $J$=15.0 Hz, H6), 3.3 - 3.5 (5 H, m, H12, NCH$_2$CH$_3$), 3.0 (1 H, m, $J$=14.5 Hz, H12), 2.4 (3 H, s, NCH$_3$), 2.3 - 2.4 (1 H, m, H11), 2.1 (1 H, ddd, $J$=13.0, 3.0 Hz, H2), 2.0 (1 H, ddd, $J$=15.5, 5.0, 4.0 Hz, H11), 1.5 (1 H, br dtd, $J$=13.5, 2.0, 2.0, 1.5 Hz, H2), 1.1 - 1.3 (6 H, m, NCH$_3$CH$_3$), 0.9 (9 H, s, SiC(CH$_3$)$_3$), 0.1 (3 H, s, SiCH$_3$), 0.0 (3 H, s, SiCH$_3$); $^{13}$C NMR (101 MHz, CHLOROFORM-d) $\delta$ ppm 153.6 (C=O), 149.8 (C10), 134.5 (C9), 133.7 (C6), 133.3 (C10a), 128.5 (C4), 127.1 (C4a), 122.2 (C7), 120.6 (C8), 87.0 (C1), 61.8 (C3), 60.1 (C6), 53.6 (C12), 48.1 (C10b), 42.1 (NCH$_2$CH$_3$), 41.8 (NCH$_2$CH$_3$), 41.1 (NCH$_3$), 34.2 (C11), 32.2 (C2), 25.8 (SiC(CH$_3$)$_3$), 18.0 (SiC(CH$_3$)$_3$), 14.1 (NCH$_2$CH$_3$), 13.3 (NCH$_2$CH$_3$), -4.4 (SiCH$_3$), -4.7 (SiCH$_3$).
13.1.a General method for the ortholithiation of the \(O\)-diethylcarbamate-\(O^{\prime}\)-TBDMSsanguinine

TMEDA (0.310 cm\(^3\), 2.07 mmol, 10 eq.) was added to THF (3.0 cm\(^3\)) and the solution was cooled to -78 °C, sec-butyllithium 1.03 mol dm\(^{-3}\) (0.500 cm\(^3\), 0.515 mmol, 2.51 eq.) was added slowly and the mixture was stirred for ten minutes. \(O\)-diethylcarbamate-\(O^{\prime}\)-TBDMSsanguinine, 168, (0.0997 g, 0.205 mmol, 1 eq.) in THF (0.535 cm\(^3\)) was added slowly. The reaction was stirred for 1 hour at -78 °C, then the electrophile source was added dropwise and the reaction was stirred for a further hour. The reaction was then quenched by the addition of methanol (1 cm\(^3\)) and allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue was worked up from saturated aqueous ammonium chloride (10 cm\(^3\)) and extracted with dichloromethane (4 × 10 cm\(^3\)). The organic phases were combined, dried over magnesium sulphate and then the solvent was removed under reduced pressure.

\((4aS,6R,8aS)-6-((\text{tert-Butyldimethylsilyl})oxy)-11\text{-methyl-2-(methylthio)}-4a,5,9,10,11,12\text{-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl} \text{diethylcarbamate, 169} \)

The electrophile source used was dimethyl disulfide. Crude yield 92.4 mg approximately 85%.

\(R_f\): 0.388 (9:1, dichloromethane:methanol-35% aqueous ammonia (9:1)); \text{MS} m/z (ES\(^{+}\)) 533 (30%, MH\(^{+}\)); \text{\(^1\)H-NMR} (500 MHz, CHLOROFORM-\(d\)) \(\delta\) ppm 6.46 (1 H, s H7), 6.03 (1 H, d, \(J=10.5\) Hz, H4), 5.79 (1 H, dd, \(J=10.5, 4.5\) Hz, H4a), 4.59 (1 H, t, \(J=3.5\) Hz, H1), 4.21 - 4.25 (1 H, m, H3), 4.18 (1 H, d, \(J=15.5\) Hz, H6), 3.65 (1 H, d, \(J=15.5\) Hz, H6), 3.44 - 3.54 (1 H, m, H12), 3.29 - 3.43 (4 H, m, NCH\(_2\)CH\(_3\)), 3.02 (1 H, m, \(J=15.0\) Hz, H12), 2.37 (3 H, s, NCH\(_3\)), 2.37 (3 H, s, SCH\(_3\)), 2.28 - 2.35 (1 H, m, H11), 2.06 - 2.15 (1 H, m, H2), 1.99 (1 H, ddd, \(J=15.0, 5.0, 4.0\) Hz, H11), 1.38 - 1.51 (1 H, m, \(J=13.5\) Hz, H2), 1.27 (3 H, br t, \(J=6.5\) Hz, NCH\(_2\)CH\(_3\)), 1.18 (3 H, br t, \(J=6.5\) Hz, NCH\(_2\)CH\(_3\)), 0.80 - 0.99 (9 H, s, SiC(CH\(_3\))\(_3\)), 0.62 (1 H, s, SiCH\(_3\)), 0.02 (3 H, s, SiCH\(_3\)).
The electrophile source used was 1,2-dibromoethane. Crude yield 115.0 mg.

**MS** m/z (ES+) 565 (90%, MH+ (79Br)), 567 (100%, MH+ (81Br)); **1H NMR** (500 MHz, CHLOROFORM-d) δ ppm 6.80 (1 H, s, H7), 6.03 (1 H, d, J=10.0 Hz, H4a), 5.83 (1 H, dd, J=10.0, 4.5 Hz, H4), 4.63 (1 H, br t, J=3.0 Hz, H1), 4.22 - 4.25 (1 H, m, H3), 4.20 (1 H, br d, J=15.5 Hz, H6), 3.64 (1 H, d, J=15.5 Hz, H6), 3.45 - 3.54 (1 H, m, H12), 3.32 - 3.43 (4 H, m, NCH2CH3), 2.38 (3 H, s, NCH3), 2.35 - 2.38 (1 H, m, H11), 2.12 (1 H, ddd, J=13.5, 3.0 Hz, H2), 1.99 (1 H, br ddd, J=15.5, 5.0, 4.0 Hz, H11), 1.46 (1 H, d, J=13.5 Hz, H2), 1.28 (3 H, t, J=7.0 Hz), 1.20 (3 H, br t, J=7.0 Hz), 0.86 (9 H, s, SiC(CH3)3), 0.07 (3 H, s, SiCH3), 0.03 (3 H, s, SiCH3).

(4aS,6R,8aS)-6-((tert-butyldimethylsilyl)oxy)-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 171

**MS** m/z (ES+) 559 (100%, MH+).
**13.1.b General method for the TBDMS deprotection of the allylic alcohol of the O-diethylcarbamate-sanguinine**

TBDMS-O-diethylcarbamate-sanguinine residue from the ortholithiation (compounds 169 - 172) was dissolved in ethanol-1% concentrated hydrochloric acid (4 cm$^3$) and stirred for 1 hour. The reaction mixture was quenched with sodium hydrogen carbonate. The organic solvent was then removed and the reaction worked up from saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane (3 × 10 cm$^3$). The organic fractions were combined, dried over magnesium sulphate and then the solvent was removed under reduced pressure. The residues were purified by preparative TLC, eluting with 95:5 dichloromethane:methanol-35% aqueous ammonia (9:1).

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The deprotected analogue was almost clean after work up; crude yield 78%, 67.6 mg, 0.162 mmol. Attempted purification using column chromatography eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10) had negligible impact on the purity but reduced the yield to 74% 54.1 mg.
0.129 mmol. The compound was finally purified using preparative TLC eluting with 95:5 dichloromethane:methanol-35% aqueous ammonia (9:1). Yield 47%, 40.6 mg, 0.097 mmol (over two steps).

Rf: 0.306 (9:1, dichloromethane:methanol-35% aqueous ammonia (9:1)); MS m/z (ES+) 419 (100%, MH+); HRMS: found 419.1989, MH+ requires 419.1999; IR νmax (film)/cm⁻¹

¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.49 (1 H, s, H7), 6.01 (2 H, s, H4 and H4a), 4.62 - 4.67 (1 H, m, H1), 4.14 (1 H, d, J=15.5 Hz, H6), 4.06 - 4.12 (1 H, m, H3), 3.72 (1 H, d, J=15.5 Hz, H6), 3.31 - 3.58 (4 H, m, N(CH₂CH₃)₂), 3.26 - 3.34 (1 H, m, H12), 3.03 - 3.17 (1 H, m, H12 and OH), 2.62 (1 H, br dd, J=15.5, 2.5 Hz, H2), 2.44 (3 H, s, NCH₃), 2.39 (3 H, s, SCH₃), 2.13 (1 H, ddd, J=13.5, 3.0 Hz, H11), 1.92 (1 H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.58 (1 H, m, J=13.5 Hz, H11), 1.30 (3 H, br t, J=7.0 Hz, NCH₂CH₃), 1.18 (3 H, br t, J=7.0 Hz, NCH₂CH₃); ¹³C NMR (101 MHz, CHLOROFORM-d) δ ppm 152.3 (C=O), 149.6 (C₁₀), 134.1 (C₆), 132.0 (C₉), 131.1 (C₈), 130.7 (C₁₀a), 127.7 (C₄), 126.4 (C₄a), 119.1 (C₇), 88.6 (C₁), 61.6 (C₃), 60.8 (C₆), 53.6 (C₁₂), 48.5 (C₁₀b), 42.8 (NCH₂CH₃), 42.3 (NCH₂CH₃), 41.5 (NCH₃), 32.8 (NCH₂CH₃), 30.2 (C₁₂), 15.6 (SCH₃), 14.2 (NCH₂CH₃), 13.3 (NCH₂CH₃).

(4aS,6R,8aS)-2-Bromo-6-hydroxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 174

Purified by purified using preparative TLC eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) (95:5). Yield 47%, 43.6 mg, 0.0966 mmol.

MS m/z (ES+) 451 (100%, MH⁺ ⁷⁹Br), 453 (90%, MH⁺ ⁸¹Br); HRMS: found 451.1229, MH⁺ ⁷⁹Br requires 451.1232; ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 6.85 (1 H, s, H7), 6.03 (1 H, dd, J=10.5, 4.0 Hz, H4), 6.00 (1 H, dd, J=10.0, 1.0 Hz, H4a), 4.65 - 4.68 (1 H, m, H1), 4.12 (1 H, d, J=15.5 Hz, H6), 4.07 - 4.13 (1 H, m, H3), 3.68 (1 H, d, J=15.5 Hz, H6), 3.38 - 3.56 (2 H, m, NCH₂CH₃), 3.26 - 3.42 (3 H, m, NCH₂CH₃ and H12), 3.00 - 3.12 (2 H, m, H12 and OH), 2.58 - 2.65 (1 H, m, H2), 2.42 (3 H, s, NCH₃), 2.13 (1 H, td, J=13.0, 3.0 Hz, H11), 1.93 (1 H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.57 (1 H, ddd, J=13.5, 3.5,
1.5 Hz, H11), 1.30 (3 H, br t, J=7.0 Hz, NCH$_2$CH$_3$), 1.19 (3 H, br t, J=7.0 Hz, NCH$_2$CH$_3$).‡

(4aS,6R,8aS)-2-(Dimethoxyboranyl)-6-hydroxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 175

Purified by purified using preparative TLC eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) (95:5). Yield 11%, 9.6 mg, 0.022 mmol.

MS m/z (ES+) 445 (100%, MH$^+$); $^1$H NMR was still too noisy to fully characterize.

(4aS,6R,8aS)-6-Hydroxy-2-iodo-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-3-yl diethylcarbamate, 176

Purified by purified using preparative TLC eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) (95:5). Yield 14%, 14.5 mg, 0.0291 mmol.

$[\alpha]^{22}_D$: -214.8° (c. 1, CHCl$_3$); MS m/z (ES+) 499 (100%, MH$^+$); HRMS: found 499.1079, MH$^+$ requires 499.1088; IR $\nu_{\text{max}}$ (film)/cm$^{-1}$; $^1$H NMR (500 MHz, CHLOROFORM-d) $\delta$ ppm 7.07 (1 H, s, H7), 6.04 (1 H, dd, J=10.5, 5.0 Hz, H4), 6.00 (1 H, d, J=10.5 Hz, H4a), 4.64 - 4.68 (1 H, m, H1), 4.12 (1 H, d, J=15.5 Hz, H6), 4.07 - 4.14 (1 H, m, H3), 3.69 (1 H, d, J=15.5 Hz, H6), 3.43 - 3.58 (2 H, m, NCH$_2$CH$_3$), 3.25 - 3.43 (1 H, m, NCH$_2$CH$_3$), 3.25 -

‡ Unfortunatley two products were confused and ths compound was disposed of before it was completely characterized. However, the data is consistent with what would be expected when compared to other similar compounds.
3.36 (1 H, m H12), 3.05 - 3.13 (1 H, m, H12 and OH), 2.63 (1 H, dd, J=15.5, 1.5 Hz, H2), 2.43 (3 H, s, NCH3), 2.09 - 2.18 (1 H, m, H11), 1.93 (1 H, m, J=15.5 Hz, H2), 1.58 (1 H, m, J=13.5 Hz, H11), 1.33 (3 H, br t, J=7.0 Hz, NCH2CH3), 1.20 (3 H, br t, J=7.0 Hz, NCH2CH3); 13C NMR (126 MHz, CHLOROFORM-d) δ ppm 151.98 (C=O), 149.32 (C10), 135.76 (C6), 135.34 (C9), 134.02 (10), 130.59 (H7), 128.13 (C4), 126.00 (C4a), 89.16 (C8), 88.59 (C1), 61.55 (C3), 59.99 (C6), 53.47 (C12), 48.78 (C10b), 42.76 (NCH2CH3), 42.35 (NCH2CH3), 41.39 (NCH3), 32.55 (C11), 30.08 (C2), 14.38 (NCH2CH3), 13.23 (NCH2CH3).

13.2 The compounds of Chapter 8: Urea-mediated lithiation chemistry for galanthamine C6 functionalization

13.2.a General method for the synthesis of carbamoyl chlorides

The method was adapted from the work of Lasne et al.151 Briefly triphosgene (1 eq.) in dichloromethane (3.0 volumes) was cooled to -78 °C. Pyridine (2.2 eq.) was added dropwise and the reaction was stirred for 5 minutes, after which time the N-methylaniline analogue (2.2 eq.) in dichloromethane (0.07 volumes) was added dropwise, very slowly. After 5 minutes the reaction was allowed to warm to room temperature and was stirred for 2 hours. The reaction was quenched with 1 mol dm⁻³ hydrochloric acid (2 volumes) and stirred for 30 minutes. The mixture was extracted with dichloromethane (3 × 2 volumes), the organic phase was washed with saturated aqueous sodium hydrogen carbonate (6 volumes) and then the organic solvent was removed carefully under reduced pressure. The residue was an oil that solidified upon cooling. Carbamoyl chlorides were characterized by IR and 1H NMR.

Methyl(o-tolyl)carbamic chloride, 183

\[
\begin{aligned}
\text{Cl} & \quad \text{N} \\
\text{183} \\
\end{aligned}
\]

IR νmax (film)/cm⁻¹ 1727 (C=O); 1H-NMR (CHLOROFORM-d, 300 MHz) δ ppm 7.23 - 7.33 (3H, m, Aryl-H), 7.12 - 7.20 (1H, m, Aryl-H), 3.30 (3H, s, NCH3), 2.29 (3H, s, CH3).
Methyl(m-tolyl)carbamic chloride, 184

\[
\begin{align*}
\text{Cl} & \quad \text{N} \\
\text{184}
\end{align*}
\]

\text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1742 \text{ (C=O); } ^1\text{H-NMR (CHLOROFORM-d, 300 MHz) } \delta \text{ ppm 7.28} - 7.35 \text{ (1H, m, ArH), 7.14} - 7.24 \text{ (1H, m, ArH), 6.99} - 7.12 \text{ (2H, m, ArH), 3.37 (3H, s, NCH}_3\text{), 2.40 (3H, s, CH}_3\text{).}

Methyl(p-tolyl)carbamic chloride, 185

\[
\begin{align*}
\text{Cl} & \quad \text{N} \\
\text{185}
\end{align*}
\]

\text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1731 \text{ (C=O); } ^1\text{H-NMR (CHLOROFORM-d, 300 MHz) } \delta \text{ ppm 7.22} \text{ (2H, d, } J=8.0 \text{ Hz, ArH), 7.11} \text{ (2H, d, } J=8.0 \text{ Hz, ArH), 3.35 (3H, s, NCH}_3\text{), 2.39 (3H, s, CH}_3\text{).}

(2-Methoxyphenyl)(methyl)carbamic chloride, 186

\[
\begin{align*}
\text{Cl} & \quad \text{N} \\
\text{186}
\end{align*}
\]

\text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1735 \text{ (C=O); } ^1\text{H-NMR (CHLOROFORM-d, 300 MHz) } \delta \text{ ppm 7.41} - 7.32 \text{ (1H, m, ArH), 7.16} - 7.23 \text{ (1H, m, ArH), 6.93} - 7.03 \text{ (2H, m, ArH), 3.88 (3H, s, OCH}_3\text{), 3.28 (3H, s, NCH}_3\text{).}
(3-Methoxyphenyl)(methyl)carbamic chloride, 187

\[ \text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1732 \text{ (C=O)}; \text{ } ^1\text{H-NMR (CHLOROFORM-d, 500 MHz) } \delta \text{ ppm 7.34 (1H, dd, } J=8.0 \text{ Hz, ArH), 6.74 - 6.98 (3H, m, ArH), 3.84 (3H, s, OCH}_3) , 3.38 (3H, s, NCH}_3). \]

(4-Methoxyphenyl)(methyl)carbamic chloride, 188

\[ \text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1739 \text{ (C=O)}; \text{ } ^1\text{H-NMR (CHLOROFORM-d, 300 MHz) } \delta \text{ ppm 7.16 (2H, d, } J=8.5 \text{ Hz, ArH), 6.93 (2H, d, } J=9.0 \text{ Hz, ArH), 3.84 (3H, s, OCH}_3) , 3.35 (3H, s, NCH}_3). \]

(4-Fluorophenyl)(methyl)carbamic chloride, 190

\[ \text{IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} 1733 \text{ (C=O)}; \text{ } ^1\text{H-NMR (CHLOROFORM-d, 500 MHz) } \delta \text{ ppm 7.24 (2H, dd, } J=8.0, 8.0 \text{ Hz, ArH), 7.12 (2H, dd, } J=8.5, 8.5 \text{ Hz, ArH), 3.36 (3H, s, NCH}_3). \]
(2-Chlorophenyl)(methyl)carbamic chloride, 191

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{Cl}
\end{array}
\]

\text{191}

**\text{IR} \nu_{\text{max}} (\text{film})/\text{cm}^{-1} 1737 (\text{C}=\text{O})**; ¹\text{H}-\text{NMR} (\text{CHLOROFORM-}d, 500 \text{ MHz}) \delta \text{ ppm} 7.55 - 7.48 (1\text{H, m, ArH}), 7.30 - 7.40 (3\text{H, m, ArH}), 3.33 (3\text{H, s, NCH}_3).

Methyl(pyridin-2-yl)carbamic chloride, 194

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{N}
\end{array}
\]

\text{194}

The method was adapted from the work of Lasne et al.¹⁵¹ Briefly triphosgene (1 eq.) in dichloromethane (3.0 volumes) was cooled to -78 °C. Pyridine (2.2 eq.) was added dropwise and the reaction was stirred for 5 minutes, after which time the \text{N-methylaniline} analogue (2.2 eq.) in dichloromethane (0.07 volumes) was added dropwise, very slowly. After 5 minutes the reaction was allowed to warm to room temperature and was stirred for 2 hours. The reaction was quenched with distilled water (2 volumes) and stirred for 15 minutes. The mixture was extracted with dichloromethane (3 \times 2 volumes), then the organic solvent was removed carefully under reduced pressure. The residue was a colourless oil.

**\text{IR} \nu_{\text{max}} (\text{film})/\text{cm}^{-1} 1739 (\text{C}=\text{O})**.

13.2.b General method for the synthesis of galanthamine based ureas

Dichloromethane (3.4 volumes) was added to norgalanthamine, 19, (1 eq.) and carbamoyl chloride (1 eq.) and then triethylamine (1.1 eq.) was added. The reaction was stirred overnight and then quenched with saturated aqueous sodium hydrogen carbonate (17 volumes). The reaction mixture was extracted with dichloromethane (3 \times 17 volumes), the organic phases were combined and dried over magnesium sulphate and then the solvent was removed under reduced pressure. The residue was purified by columned chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10).
(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-methyl-N-phenyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 195

Yield 99%, 0.3684g 0.9060 mmol.

**RF**: 0.695 (9:1:0.1, dichloromethane:methanol:35% aqueous ammonia); **MS** m/z (ES+) 407 (50%, MH⁺); **HRMS**: found 407.1966, MH⁺ requires 407.1965; **IR** νmax (film)/cm⁻¹ 3546 (OH), 1633 (C=O), 1263 (O-H); **¹H-NMR** (CHLOROFORM-d, 300 MHz) δ ppm 7.26 - 7.36 (2H, m, ArH), 7.05 - 7.16 (3H, m, ArH), 6.60 (1H, d, J=8.0, H7), 6.45 (1H, d, J=8.0, H8), 5.98 (1H, dd, J=10.0, 4.5, H4), 5.91 (1H, dd, J=10.0, 1.0, H4a), 4.43 - 4.57 (2H, m, H1, H6), 4.05 - 4.17 (2H, m, H3, H12), 4.01 (1H, d, J=16.0, H6), 3.83 (3H, s, OCH₃), 3.26 (1H, ddd, J=15.0, 12.5, 2.0, H12), 3.14 (3H, s, NCH₃), 2.67 (1H, ddd, J=15.5, 3.0, 1.5, H2), 2.42 (1H, b, OH), 1.98 (1H, ddd, J=15.5, 5.0, 2.5, H2), 1.86 (1H, ddd, J=13.0, 13.0, 3.0, H11), 1.64 (1H, ddd, J=14.0, 3.5, 1.5, H11); **¹³C-NMR** (CHLOROFORM-d, 75.5 MHz) δ ppm 161.2 (C=O), 147.0 (Aryl), 146.1 (Aryl), 144.0 (Aryl), 132.2 (Aryl), 129.5 (Aryl), 128.9 (Aryl), 127.8 (C4), 126.5 (C4a), 124.8 (Aryl), 123.7 (Aryl), 120.7 (Aryl), 111.13 (Aryl), 88.3 (C1), 61.8 (C3), 55.8 (OCH₃), 52.5 (C6), 48.0 (C10b), 47.0 (C12), 40.1 (NCH₃), 36.2 (C11), 29.7 (C2).

(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-methyl-N-(o-tolyl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 196

Yield 84% 0.3256 g 0.7743 mmol.

**IR** νmax (film)/cm⁻¹ 3549 (w, OH), 2930 (w, CH), 1629 (s, C=O); **¹H-NMR** (CHLOROFORM-d, 500 MHz) δ ppm 7.21 (1H, ddd, J=7.0, 5.5, 3.5 Hz, ArH), 7.10 - 7.15 (2H, m, ArH), 7.02 (1H, ddd, J=9.5, 3.8, 3.8 Hz, ArH), 6.60 (1H, d, J=8.0, H8), 6.50 (1H,
E. W. D. Burke

d, J=8.5 Hz, H7), 5.94 (1H, ddd, J=10.5, 5.0, 1.5 Hz, H4), 5.87 (1H, d, J=10.0 Hz, H4a),
4.63 (1H, d, J=15.5 Hz, H6), 4.44 (1H, br ddd, H2), 4.08 (1H, ddd, J=11.5, 4.5, 4.5 Hz,
H3), 3.95 (1H, d, J=15.5 Hz, H6), 3.90 (1H, br ddd, J=15.5 Hz, H12), 3.82 (3H, s, OCH3),
3.21 (1H, ddd, J=15.5, 10.5, 4.0 Hz, H12), 3.02 (3H, s, NCH3), 2.63 (1H, ddd, J=16.0, 4.0,
2.0 Hz, H2), 2.38 (1H, d, J=11.0 Hz, OH), 1.91 (1H, d, J=15.7, 5.1, 2.5 Hz, H2), 1.54-1.44 (2H,
m, H11); 13C-NMR (CHLOROFORM-d, 125 MHz) δ ppm 161.8 (C=O), 146.0 (Aryl), 145.4 (Aryl), 144.0 (Aryl), 134.3 (Aryl), 132.3 (Aryl),
131.6 (Aryl), 129.3 (Aryl), 127.7 (C4), 127.3 (Aryl), 126.7 (Aryl), 126.6 (Aryl), 126.6
(C4a), 121.0 (C8), 111.1 (C7), 88.3 (C1), 61.9 (C3), 55.8 (OCH3), 52.6 (C6), 48.1 (C10b),
47.3 (C12), 39.5 (NCH3), 36.2 (C11), 29.7 (C2), 17.6 (ArCH3).

(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-methyl-N-(m-tolyl)-4a,5,9,10-tetrahydro-6H-

Yield 86%, 0.3300 g, 0.7847 mmol.

1H-NMR (CHLOROFORM-d, 500 MHz) δ ppm δ 7.18 (1H, t, J=7.5 Hz, H22), 6.88 - 6.94
(2H, m, H21, H23), 6.86 (1H, s, H19), 6.58 (1H, d, J=8.0 Hz, H7), 6.40 (1H, d, J=8.0, H8),
5.97 (1H, ddd, J=10.5, 5.0, 1.0 Hz, H4), 5.91 (1H, d, J=10.5 Hz, H4a), 4.57-4.88 (2H, m,
H6, H1), 4.18-4.06 (2H, m, H12, H3), 4.01 (1H, d, J=16.0 Hz, H6), 3.82 (3H, s, OCH3),
3.25 (1H, ddd, J=13.5, 12.5, 1.0 Hz, H12), 3.12 (3H, s, NCH3), 2.67 (1H, ddd, J=16.0, 4.0,
2.0 Hz, H2), 2.39 (1H, d, J=11.0 Hz, OH), 2.26 (3H, s, CH3), 1.98 (1H, ddd, J=16.0, 5.0,
2.5 Hz, H2), 1.84 (1H, ddd, J=13.5, 3.5, 1.0 Hz, H11), 1.65 (1H, ddd, J=13.5, 4.0, 2.0 Hz,
H11); 13C-NMR (125 MHz) δ ppm 161.33 (C=O), 147.0 (Aryl), 146.1 (Aryl), 144.0
(Aryl), 139.5 (Aryl), 132.3 (Aryl), 129.3 (Aryl CH), 129.0 (Aryl), 127.8 (C4a), 126.6
(C4a), 125.6 (Aryl CH), 124.6 (C19), 120.9 (Aryl CH), 120.8 (C7), 111.1 (C8), 88.4 (C1),
61.9 (C3), 55.9 (OCH3), 52.7 (C6), 48.1 (C10b), 47.0 (H12), 40.3 (NCH3), 36.3 (C11),
29.8 (C2), 21.3 (CH3).

197
(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-methyl-N-(p-tolyl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 198

Yield 84% 0.3244, 0.7714 mmol.

**MS** m/z (ES+) 443 (100%, MNa+), 421 (13%, MH+); **HRMS**: found 443.1947, MNa+ requires 443.1942; IR ν max (film)/cm⁻¹ 3549 (w, OH), 2920 (CH), 1634 (C=O); ¹H-NMR (CHLOROFORM-d, 500 MHz) δ ppm 7.09 (2H, d, J=8.0 Hz, ArH), 6.97 (2H, d, J=8.0 Hz, ArH), 6.57 (1H, d, J=8.0 Hz, H7), 6.41 (1H, d, J=8.0 Hz, H8), 5.97 (1H, ddd, J=10.5, 5.0, 1.0, H4), 5.90 (1H, dd, J=10.5 Hz, H4a), 4.54 (1H, br s, H1), 4.49 (1H, d, J=16.0 Hz, H6), 4.16-4.06 (2H, m, H3, H12), 3.99 (1H, d, J=16.0 Hz, H6), 3.82 (3H, s, OCH₃), 3.23 (1H, br t, J=12.5 Hz, H12), 3.11 (3H, s, NCH₃), 2.67 (1H, ddd, J=16.0, 3.5, 2.0 Hz, H2), 2.39 (1H, d, J=11.0 Hz, OH), 2.32 (3H, s, ArCH3), 1.98 (1H, ddd, J=16.0, 5.0, 2.5 Hz, H2), 1.87 (1H, dt, J=13.0, 3.0 Hz, H11), 1.64 (1H, ddd, J=14.0, 4.0, 1.5 Hz, H11); ¹³C-NMR (CHLOROFORM-d, 125 MHz) δ ppm 161.4 (C=O), 146.0 (Aryl), 144.5 (Aryl), 144.0 (Aryl), 134.7 (Aryl), 132.2 (Aryl), 130.1 (Aryl), 129.1 (Aryl CH), 127.8 (C4), 126.6 (C4a), 124.0 (Aryl CH), 120.8 (C8), 110.9 (C7), 88.4 (C1), 61.9 (C3), 55.8 (OCH₃), 52.7 (C6), 48.1 (C10b), 47.0 (H12), 40.4 (NCH₃), 36.3 (C11), 29.8 (C2), 20.8 (ArCH3).

(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-(2-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 199

Yield 72%, 0.2859 g, 0.6549 mmol.

**MS** m/z (ES+) 459 (100%, MNa+), 437 (17%, MH+); **HRMS**: found 459.1885, MNa+ requires 459.1891; IR ν max (film)/cm⁻¹ 3549 (OH), 2929 (CH), 1627 (C=O); ¹H-NMR
(CHLOROFORM-\textit{d}, 500 MHz) δ ppm 7.15 (1H, tdd, \textit{J} = 8.0, 1.5, 0.5 Hz, H21), 7.04 (1H, dd, \textit{J} = 7.5, 1.5 Hz, H23), 6.86 (1H, td, \textit{J} = 7.5, 1.5 Hz, H22), 6.82 (1H, dd, \textit{J} = 8.0, 1.5 Hz, H20), 6.56 (1H, d, \textit{J} = 8.5, H7), 6.43 (1H, d, \textit{J} = 8.0 Hz, H8), 5.94 (1H, ddd, \textit{J} = 10.5, 4.5, 1.0 Hz, H4), 5.90 (1H, dd, \textit{J} = 10.5, 1.0 Hz, H4a), 4.76 (1H, d, \textit{J} = 16.0 Hz, H6), 4.38 (1H, br s, H1), 4.12-4.06 (1H, br m, H3), 4.05-3.99 (1H, br m, H12), 3.97 (1H, d, \textit{J} = 16.0 Hz, H6), 3.82 (3H, s, O\textit{CH}_3), 3.66 (3H, s, \textit{H}24), 3.24 (1H, td, \textit{J} = 12.0, 1.5 Hz, H22), 3.05 (3H, s, N\textit{CH}_3), 2.63 (1H, ddt, \textit{J} = 16.0, 3.5, 1.5 Hz, H19), 2.36 (1H, d, \textit{J} = 11.5 Hz, OH), 1.92 (1H, ddd, \textit{J} = 16.0, 5.0, 2.5 Hz, H2), 1.46 (1H, ddd, \textit{J} = 13.5, 4.0, 2.0 Hz, H11), 1.26 (1H, ddd, \textit{J} = 13.0, 3.0, 0.5 Hz, H11); \textsuperscript{13C-NMR} (, 125 MHz) δ ppm 162.4 (C=O), 154.2 (Aryl), 145.8 (Aryl), 143.8 (Aryl), 135.2 (Aryl), 132.2 (Aryl), 129.4 (Aryl), 127.8 (C23), 127.6 (C4), 127.4 (C21), 126.8 (C4a), 121.2 (C22), 121.0 (C8), 111.9 (C20), 110.9 (C7), 88.4 (C1), 61.9 (C3), 55.9 (O\textit{CH}_3), 55.2 (C24), 52.4 (C6), 48.0 (C10b), 47.4 (C12), 39.0 (N\textit{CH}_3), 36.1 (C11), 29.7 (C2).

Yield 80%, 0.3214 g, 0.7363 mmol.

**MS** m/z (ES+) 437.5 (100%, MH\textsuperscript{+}); \textsuperscript{1H-NMR} (CHLOROFORM-\textit{d}, 500 MHz) δ ppm 7.21 (1H, t, \textit{J} = 8.0 Hz, H22), 6.70 (1H, ddd, \textit{J} = 8.0, 2.0, 0.5 Hz, ArH), 6.65 (1H, ddd, \textit{J} = 8.0, 2.5, 0.5 Hz, ArH), 6.59 (1H, t, \textit{J} = 2.0 Hz, H19), 6.59 (1H, d, \textit{J} = 8.0, H7), 6.44 (1H, d, \textit{J} = 8.0 Hz, H8), 5.97 (1H, ddd, \textit{J} = 10.5, 5.0, 1.5 Hz, H4), 5.91 (1H, ddd, \textit{J} = 10.0, 0.5 Hz, H4a), 4.57-4.50 (2H, m, H1, H6), 4.17-4.08 (2H, m, H3, H12), 4.02 (1H, d, H6), 3.82 (3H, s, O\textit{CH}_3), 3.71 (3H, s, H24), 3.27 (1H, td, \textit{J} = 12.5, 2.0 Hz, H12), 3.12 (3H, s, N\textit{CH}_3), 2.66 (1H, ddt, \textit{J} = 16.0, 3.47, 2.0 Hz, H2), 2.38 (1H, d, \textit{J} = 11.5 Hz, OH), 1.98 (1H, ddd, \textit{J} = 15.8, 5.0, 2.5 Hz, H2), 1.85 (1H, td, \textit{J} = 13.5, 3.5 Hz, H11), 1.66 (1H, ddd, \textit{J} = 14.0, 4.0, 2.0 Hz, H11); \textsuperscript{13C-NMR} (CHLOROFORM-\textit{d}, 125 MHz) δ ppm 161.2 (C=O), 160.5 (Aryl), 148.2 (Aryl), 146.1 (Aryl), 144.1 (Aryl), 132.3 (Aryl), 130.2 (C22), 129.0 (Aryl), 127.9 (C4), 126.5 (C4a), 120.8 (C8), 116.1 (Aryl CH), 111.1 (C7), 110.1 (Aryl CH), 109.8 (C19), 88.4 (C1),
61.9 (C3), 55.9 (OCH₃), 55.2 (C24), 52.7 (C6), 48.1 (C10b), 47.1 (C12), 40.1 (NCH₃), 36.3 (C11), 29.8 (C2).

(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-(4-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 201

\[\text{HO} \quad \text{H} \quad \text{N} \quad \text{N} \quad \text{OH} \]

Yield 86%, 0.3463 g, 0.7933 mmol.

**MS** m/z (ES+) 437 (100%, MH⁺); **HRMS**: found 459.1895, MNa⁺ requires 459.1891; **IR** ν_{max} (film)/cm⁻¹ 3549 (O-H), 2930 (C-H), 1634 (C=O); **¹H-NMR** (CHLOROFORM-d, 500 MHz) δ ppm 7.01 (2H, td, J=10.0, 3.5, 2.5 Hz, ArH), 6.80 (2H, td, J=10.0, 3.5, 2.0 Hz, ArH), 6.57 (1H, d, J=8.0 Hz, H7), 6.39 (1H, d, J=8.0 Hz, H8), 5.96 (1H, ddd, J=10.5, 4.5, 1.0, H4), 5.90 (1H, dd, J=10.0, 0.5 Hz, H4a), 4.53 (1H, dt, J=4.0, 2.0 Hz, H1), 4.50 (1H, d, J=16.0 Hz, H6), 4.17-4.07 (2H, m, H3, H12), 3.99 (1H, d, J=16.0 Hz, H6), 3.82 (3H, s, OCH₃), 3.78 (3H, s, H24), 3.23 (1H, td, J=13.0, 2.0 Hz, H12), 3.09 (3H, s, NCH₃), 2.66 (1H, ddt, J=16.0, 3.47, 2.0 Hz, H2), 2.38 (1H, d, J=11.0 Hz, OH), 1.98 (1H, ddd, J=16.0, 5.0, 2.5 Hz, H2), 1.84 (1H, td, J=13.5, 3.5 Hz, H11), 1.64 (1H, ddd, J=14.0, 4.0, 2.0 Hz, H11); **¹³C-NMR** (CHLOROFORM-d, 125 MHz) δ ppm 161.5 (C=O), 157.1 (Aryl), 146.0 (Aryl), 144.0 (Aryl), 140.0 (Aryl), 132.3 (Aryl), 129.2 (Aryl), 127.8 (C4), 126.6 (C4a), 125.7 (Aryl CH), 120.8 (C8), 114.7 (Aryl CH), 111.0 (C7), 88.4 (C1), 61.9 (C3), 55.8 (OCH₃), 55.4 (C24), 52.7 (C6), 48.1 (C10b), 47.1 (C12), 40.9 (NCH₃), 36.4 (C11), 29.8 (C2).

Yield 84%, 0.3414 g, 0.7743 mmol.

^1H-NMR (CHLOROFORM-d, 500 MHz) δ ppm 7.40 - 7.45 (1H, m, ArH), 7.10 - 7.16 (2H, m, ArH), 7.03 - 7.08 (1H, m, ArH), 6.56 (1H, d, J=8.5 Hz, H7), 6.48 (1H, d, J=8.0, H8), 5.95 (1H, ddd, J=10.5, 5.0, 1.0 Hz, H4), 5.87 (1H, d, J=10.5 Hz, H4a), 4.59 (1H, d, J=16.0 Hz, H6), 4.46 (1H, dt, J=4.0, 2.0 Hz, H1), 4.09 (1H, dtd, J=11.0, 4.0, 2.0 Hz, H3), 4.02 (1H, d, J=16.0 Hz, H6), 3.96 - 4.05 (1H, m, ArH), 3.81 (3H, s, OCH₃), 3.24 (1H, ddd, J=15.0, 10.5, 3.8 Hz, H12), 3.05 (3H, s, NCH₃), 2.64 (1H, dtd, J=16.0, 3.5, 1.5 Hz, H2), 2.38 (1H, d, J=16.0 Hz, H11).

^3C-NMR (CHLOROFORM-d, 125 MHz) δ ppm 161.6 (C=O), 146.0 (Aryl), 144.0 (Aryl), 143.2 (Aryl), 131.2 (Aryl), 130.9 (Aryl CH), 128.8 (Aryl), 128.1 (Aryl CH), 127.8 (Aryl CH), 127.8 (C4), 127.6 (Aryl CH), 126.6 (C4a), 121.1 (C8), 111.1 (C7), 88.3 (C1), 61.9 (C3), 55.8 (OCH₃), 52.8 (C6), 48.0 (C10b), 46.8 (C12), 38.8 (NCH₃), 36.3 (C11), 29.7 (C2).

(4aS,6R,8aS)-N-(3-Chlorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 203

Yield 70%, 0.2259 g, 0.5123 mmol.

MS m/z (ES+) 463 (100%, MNa⁺), 441 (90%, MH⁺); HRMS: found 463.1393, MNa⁺ requires 463.1396; IR νmax (film)/cm⁻¹ 3549 (OH), 2929 (C-H), 1645 (C=O); ^1H-NMR (CHLOROFORM-d, 500 MHz) δ ppm 7.17 - 7.22 (1H, t, J=8.5 Hz, H22), 7.02 - 7.07 (2H,
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m, ArH), 6.95 (1H, ddd, J=8.5, 2.0, 1.0 Hz, ArH), 6.57 (1H, d, J=8.0 Hz, H7), 6.37 (1H, d, J=8.0, H8), 5.98 (1H, ddd, J=10.5, 5.0, 1.0 Hz, H4), 5.91 (1H, d, J=10.0 Hz, H4a), 4.55 - 4.59 (1H, m, H1), 4.48 (1H, d, J=16.0 Hz, H6), 4.15 - 4.22 (1H, m, H12), 4.12 (1H, dt, J=10.5, 5.0 Hz, H3), 4.08 (1H, d, J=16.0 Hz, H6), 3.82 (3H, s, OCH3), 3.29 (1H, td, J=12.5, 1.5 Hz, H12), 3.12 (3H, s, NCH3), 2.68 (1H, ddt, J=16.0, 3.5, 1.5 Hz, H2), 2.39 (1H, d, J=11.5 Hz, OH), 1.99 (1H, ddd, J=16.0, 5.0, 2.5 Hz, H2), 1.92 (1H, td, J=13.5, 3.0 Hz, H11), 1.72 (1H, ddd, J=13.5, 4.0, 2.0 Hz, H11); ¹³C-NMR (CHLOROFORM-d, 125 MHz) δ ppm 160.8 (C=O), 148.0 (Aryl), 146.2 (Aryl), 144.2 (Aryl), 135.0 (Aryl), 132.1 (Aryl), 130.4 (C22), 128.5 (Aryl), 128.0 (C4), 126.4 (C4a), 124.5 (Aryl CH), 123.4 (Aryl CH), 121.2 (Aryl CH), 120.6 (C8), 111.1 (C7), 88.4 (C1), 61.8 (C3), 55.9 (OCH3), 53.0 (H6), 48.1 (C10b), 46.9 (C12), 39.8 (NCH3), 36.4 (C11), 29.7 (C2).

(4aS, 6R, 8aS)-N-(4-Chlorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 204

![Chemical Structure](image)

Yield 50%, 0.1886 g, 0.4277 mmol.

**MS** m/z (%) 463 (88%, MH+), 465 (45%, MH+); **HRMS:** found 463.1388, MH+ requires 463.1396; **IR** νmax (film)/cm⁻¹ 3549 (O-H), 2929 (C-H), 1640 (C=O); ¹H-NMR (CHLOROFORM-d, 500 MHz) δ ppm 7.16 - 7.21 (2H, m, ArH), 6.95 - 7.00 (2H, m, ArH), 6.54 (1H, d, J=8.0 Hz, H7), 6.26 (1H, d, J=8.0 Hz, H8), 5.97 (1H, ddd, J=10.0, 5.0, 1.5, H4), 5.89 (1H, d, J=10.5 Hz, H4a), 4.56 (1H, br-s, H1), 4.45 (1H, d, J=15.5 Hz, H6), 4.26-4.19 (1H, m, H12), 4.12 (1H, ddd, J=11.0, 5.5, 4.7 Hz, H3), 4.05 (1H, d, J=16.0 Hz, H6), 3.82 (1H, s, OCH3), 3.25 (1H, t, J=13.5 Hz, H12), 3.11 (1H, s, NCH3), 2.67 (1H, ddt, J=16.0, 3.5, 1.5 Hz, H2), 2.39 (1H, d, J=11.5 Hz, OH), 1.99 (1H, ddd, J=16.0, 5.0, 2.5 Hz, H2), 1.89 (1H, td, J=13.0, 3.0 Hz, H11), 1.71 (1H, ddd, J=13.5, 4.0, 2.0 Hz, H11); ¹³C-NMR (, 125 MHz) δ ppm 161.0 (C=O), 146.0 (Aryl), 145.5 (Aryl), 144.0 (Aryl), 132.0 (Aryl), 130.0 (Aryl), 129.4 (Aryl CH), 128.5 (Aryl), 127.9 (C4), 126.4 (C4a), 124.9 (Aryl CH), 120.6 (C7), 110.9 (C8), 88.3 (C1), 61.8 (C3), 55.8 (OCH3), 53.1 (C6), 48.1 (C10a), 46.8 (C12), 40.1 (NCH3), 36.5 (C11), 29.7 (C2).
(4aS,6R,8aS)-N-(3-Fluorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 205

Yield 93%, 0.1458 g, 0.3435 mmol.

**MS** m/z (100%, MNa‘); **HRMS:** found 447.1686, MNa+ requires 447.1691; **IR** ν_{max} (film)/cm⁻¹ 3549 (O-H), 2929 (C-H), 1645 (C=O); **¹H-NMR** (CHLOROFORM-d, 500 MHz) δ ppm 7.23 (1H, td, J=8.5, 6.5 Hz, ArH), 6.87 - 6.83 (1H, m, ArH), 6.76 - 6.82 (2H, m, ArH), 6.60 (1H, d, J=8.0 Hz, H7), 6.44 (1H, d, J=8.5, H8), 5.99 (1H, ddd, J=10.0, 5.0, 1.0 Hz, C4), 5.91 (1H, d, J=11.0 Hz, C4a), 4.57 (1H, br s, H1), 4.47 (1H, d, J=15.5 Hz, H6), 4.09 - 4.16 (2H, m, H3, H12), 4.06 (1H, d, J=16.0 Hz, H6), 3.82 (3H, s, OCH₃), 3.29 (1H, ddd, J=15.0, 12.0, 1.5 Hz, H12), 3.12 (3H, s, NCH₃), 2.68 (1H, ddt, J=16.0, 4.0, 1.5 Hz, H2), 2.39 (1H, d, J=11.1 Hz, OH), 1.99 (1H, ddd, J=16.0, 5.0, 2.5 Hz, H2), 1.94 (1H, td, J=13.0, 3.15 Hz, H11), 1.71 (1H, ddd, J=14.0, 4.0, 1.5 Hz, H11); **¹³C-NMR** (, 125 MHz) δ ppm 163.3 (d, J_{CF} 246.0, C20), 160.8 (C=O), 148.4 (d, J=10.0, C18), 146.2 (Aryl), 144.2 (Aryl), 132.2 (Aryl), 130.6 (d, J_{CF} 9.0, C23), 128.6 (Aryl), 128.0 (C4), 126.4 (C4a), 120.7 (C8), 118.4 (d, J_{CF} 2.5, C23), 111.2 (d, J_{CF} 22.0, Aryl CH), 111.1 (C7), 110.1 (d, J_{CF} 23.5, Aryl CH), 88.4 (C1), 61.8 (C3), 55.9 (OCH₃), 52.9 (C6), 48.1 (C10a), 47.0 (C12), 39.7 (NCH₃), 36.4 (C11), 29.7 (C2).

Figure 13.1: X-Ray structure of 205.
(4aS,6R,8aS)-N-(4-Fluorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 206

Yield 95%, 0.3725 g, 0.8776 mmol.

\[ ^1H-NMR \text{(CHLOROFORM-}d, 500 MHz) \delta \text{ ppm } 7.02 - 7.06 \text{ (2H, m, H19), 6.93 - 6.99 (2H, m, H20), 6.57 (1H, d, } J=8.2 \text{ Hz, H7), 6.36 (1H, d, } J=8.2 \text{ Hz, H8), 5.97 (1H, ddd, } J=10.0, 5.0, 1.0 \text{, H4), 5.89 (1H, d, } J=10.0 \text{ Hz, C4a), 4.54 (1H, dt, } J=3.5, 2.0 \text{ Hz, H1), 4.47 (1H, d, } J=15.5 \text{ Hz, H6), 4.08 - 4.18 (2H, m, H3, H12), 4.02 (1H, d, } J=16.0 \text{ Hz, H6), 3.82 (3H, s, OCH}_3, 3.25 (1H, ddd, } J=15.0, 12.5, 1.5 \text{ Hz, H12), 3.10 (3H, s, NCH}_3, 2.67 (1H, ddt, } J=16.0, 4.0, 2.0 \text{ Hz, H2), 2.39 (1H, d, } J=11.5 \text{ Hz, OH), 1.98 (1H, ddd, } J=16.0, 5.0, 2.5 \text{ Hz, H2), 1.84 (1H, td, } J=13.0, 3.0 \text{ Hz, H11), 1.67 (1H, ddd, } J=14.0, 4.0, 2.0 \text{ Hz, H11); } ^{13}C-NMR \text{(CHLOROFORM-}d, 125 MHz) \delta \text{ ppm } 161.3 \text{ (C}=O), 159.9 \text{ (d, } 1JC_F 245.5, \text{ C18), 146.1 \text{ (Aryl), 144.1 \text{ (Aryl), 143.1 (d, } 4JC_F 3.5, \text{ C18), 132.2 \text{ (Aryl), 128.8 \text{ (Aryl), 128.0 (C4), 125.5 (C4a), 125.7 (d, } 1JC_F 8.5, \text{ C19), 120.7 (C8), 116.3 (d, } 2JC_F 23.0, \text{ C20), 111.1 (C7), 88.4 (C1), 61.9 (C3), 55.9 \text{ (OCH}_3, 52.9 (C6), 48.1 \text{ (C10a), 47.0 \text{ (C12), 40.7 \text{ (NCH}_3, 36.4 \text{ (C11), 29.8 (C2).}}}

(4aS,6R,8aS)-N-Ethyl-6-hydroxy-3-methoxy-N-(naphthalen-1-yl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 207

Yield 100%, 0.4173 g, 0.9140 mmol.

\[ ^1H-NMR \text{(CHLOROFORM-}d, 400 MHz) \delta \text{ ppm } 7.97 - 8.03 \text{ (1H, m, H23), 7.85 - 7.91 (1H, m, H20), 7.70 (1H, d, } J=8.5 \text{ Hz, H25), 7.50 - 7.58 (2H, m, H21, H22), 7.31 (1H, t, } J=8.0 \text{, H26), 7.14 (1H, dd, } J=7.5, 1.5 \text{ Hz, H27), 6.47 (1H, d, } J=8.5 \text{ Hz, H7), 6.03 (1H, br d,}}

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$J=9.0$ Hz, H8), 5.89 (1H, ddd, $J=10.5$, 5.0, 1.5 Hz, C4), 5.78 (1H, d, $J=10.0$ Hz, C4a), 4.49 (1H, d, $J=16.0$ Hz, H6), 4.43-4.39 (1H, m, H1), 3.94 - 4.09 (2H, m, H3, H12), 3.89 (1H, d, $J=16.0$ Hz, H6), 3.80 (3H, s, OCH$_3$), 3.56 - 3.68 (2H, m, H28), 3.14 (1H, br t, $J=13.4$ Hz, H12), 2.61 (1H, ddt, $J=15.5$, 3.5, 1.5 Hz, H2), 2.35 (1H, br s, OH), 1.90 (1H, ddd, $J=16.0$, 5.0, 2.5 Hz, H2), 1.59 (1H, td, $J=13.0$, 3.0 Hz, H11), 1.45 (1H, br dd, $J=14.0$, 2.5 Hz, H11), 1.22 (3H, t, $J=7.0$ Hz, H29); $^{13}$C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 161.8 (C=O), 145.9 (Aryl), 143.9 (Aryl), 141.0 (Aryl), 135.0 (Aryl), 132.0 (Aryl), 129.7 (Aryl), 129.0 (Aryl), 128.6 (C20), 127.6 (C4), 126.9 (C25), 126.7 (Aryl CH), 126.6 (C4a), 126.3 (Aryl CH), 125.6 (C26), 124.6 (C27), 122.9 (C23), 120.9 (C8), 110.9 (C7), 88.2 (C1), 61.8 (C3), 55.8 (OCH$_3$), 52.6 (C6), 48.0 (C10a), 47.1 (C28), 46.8 (C12), 36.1 (C11), 29.7 (C2), 13.3 (C29).

(4aS,6R,8aS)-6-Hydroxy-3-methoxy-N-methyl-N-(pyridin-2-yl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 208

Yield 89%, 0.1323 g, 0.3247 mmol.

**MS** m/z (ES+) 430 (100%, MNa$^+$); **HRMS**: found 430.1732, MNa$^+$ requires 430.1738; **IR** $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3549 (O-H), 2912 (C-H), 1652 (C=O); $^1$H-NMR (CHLOROFORM-d, 500 MHz) δ ppm 8.3 (1H, ddd, $J=5.0$, 2.0, 0.5 Hz, H20), 7.46 (1H, ddd, $J=8.5$, 7.0, 2.0 Hz, H22), 6.85 (1H, ddd, $J=7.5$, 5.0, 1.0 Hz, H21), 6.82 (1H, dt, $J=8.0$, 1.0 Hz, H23), 6.58 (1H, d, $J=8.0$, H7), 6.46 (1H, d, $J=8.0$ Hz, H8), 6.01 (1H, ddd, $J=10.5$, 5.0, 1.5 Hz, H4), 5.96 (1H, dd, $J=10.5$, 1.0 Hz, H4a), 4.55 - 4.62 (2H, m, H1, H6), 4.18 - 4.30 (2H, m, H12, H6), 4.10 - 4.17 (1H, m, H3), 3.82 (3H, s, OCH$_3$), 3.41 (1H, td, $J=12.5$, 2.0 Hz, H12), 3.20 (3H, s, NCH$_3$), 2.69 (1H, ddt, $J=16.0$, 3.5, 1.5 Hz, H2), 2.39 (1H, d, $J=11.5$ Hz, OH), 1.95 - 2.05 (2H, m, H2, H11), 1.79 (1H, ddd, $J=14.0$, 4.0, 2.0 Hz, H11); $^{13}$C-NMR (CHLOROFORM-d, 125 MHz) δ ppm 160.5 (C=O), 157.3 (Aryl), 148.3 (C20), 146.2 (Aryl), 144.2 (Aryl), 137.5 (C22), 132.1 (Aryl), 128.4 (Aryl), 128.2 (C4), 126.3 (C4a), 121.0 (C8), 117.4 (C21), 113.6 (C23), 111.2 (C7), 88.4 (C1), 61.8 (C3), 55.8 (OCH$_3$), 53.1 (C6), 48.1 (C10a), 46.6 (C12), 36.7 (C11), 36.1 (NCH$_3$), 29.7 (C2).
13.2.c Intramolecular aryl migration; migration and purification

The galanthamine derived urea (1 eq., approx. 100 mg) was dissolved in THF (4.0 cm$^3$) cooled to -42 °C (acetonitrile dry ice bath). LDA was added dropwise and the reaction was left to stir overnight. The reaction was then quenched with methanol (1 cm$^3$) and then saturated aqueous ammonium chloride (1 cm$^3$). The organic solvent was then removed under reduced pressure. Saturated aqueous ammonium chloride (10 cm$^3$) was added to the residue which was then extracted with dichloromethane (4 × 10 cm$^3$), the organic phases were combined, dried over magnesium sulphate and then the solvent was removed under reduced pressure. The crude reaction mixture was purified by column chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10).

For the purpose of analysis the diastereoisomers from the rearrangement of the ureas were separated via reverse phase HPLC. In each case the method was developed using an analytical scale ACE C18-AR column, particle size 5 μm, column dimensions 250 mm × 4.6 mm (internal diameter), with a sample concentration of approximately 1 mg ml$^{-1}$. There was no equipment available to regulate the temperature of the preparative HPLC, but the laboratory temperature was reasonably stable between about 27 °C and 28 °C, hence the analytical methods were developed at 27.5 °C using thermostatic control. The flow rate was 1 ml min$^{-1}$. The Thermo Scientific Isocratic Method Transfer tool§ was used to determine the conditions needed for a preparative scale HPLC column; a C18-AR, particle size 10 μm, column dimensions 250 mm × 21.2 mm (internal diameter). In each case this

§ Available online at http://hplctransfer.com/IsocraticMethod.aspx
resulted in an increase of flowrate to 15 ml min\(^{-1}\), using the same isocratic solvent mixture and run at approximately the same temperature. The sample concentration was increased to 0.1000 g ml\(^{-1}\).

**Migration of the phenyl group**

Rearrangement gave a 1:1.7 mixture of diastereoisomers, as determined by NMR. Yield 94%, 0.0964 g, 0.2372 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 1 ml min\(^{-1}\) and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 6.187 min and 7.718 min (dr 1:1.6). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min\(^{-1}\) and room temperature at 27–28 °C. This method gave two sharp symmetrical peaks eluting at 8.458 min and 10.288 min (dr 1:1.6):

\((4aS,6R,8aS,12R)-6\text{-Hydroxy-3-methoxy-N-methyl-12-phenyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 209}\)

\[\text{Rf: 0.419 (9:0.9:0.1, dichloromethane:methanol:35\% aqueous ammonia); } [\alpha]_D^{22}: -40.8^\circ (c. 1, CHCl}_3)]\]

**MS** m/z (ES\(^+\)) 407 (40%, MH\(^+\)), 429 (100%, MNa\(^+\)); **HRMS**: found 429.1777, MNa\(^+\) requires 429.1785; **IR** \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3549 (O-H), 3382 (N-H), 2929 (C-H), 1625 (C=O); \(\lambda_{\text{max}}\) 290 nm, \(^1\text{H-NMR}\) (CHLOROFORM-\(d\), 500 MHz) \(\delta\) ppm 7.27 - 7.35 (3H, m, H20, H21, H22), 7.08 (2H, J=7.5 Hz, H19, H23), 6.83 (1H, d, J=8.0 Hz, H7), 6.78 (1H, d, J=8.0 Hz, H8), 6.59 (1H, br s, H6), 5.78 (1H, dd, J=10.5, 5.0 Hz, H4), 5.40 (1H, d, J=10.0 Hz, H4a), 4.67 (1H, q, J=4.5 Hz, NH), 4.52 - 4.57 (1H, m, H1), 4.01 - 4.08 (1H, m, H3), 3.88 (3H, s, OCH\(_3\)), 3.68 - 3.83 (1H, m, H12a), 3.36 (1H, br td, J=14.0 Hz, H12b), 2.84 (3H, d, J=4.5 Hz, NCH\(_3\)), 2.65 (1H, dd, J=15.5, 2.5 Hz, H2), 2.49 (1H, br s, OH), 1.96-1.87 (2H, m, H2, H11), 1.59 (1H, dd, J=13.0, 3.0 Hz, H11); 

\(^{13}\text{C-NMR}\) (CHLOROFORM-\(d\), 100 MHz) \(\delta\) ppm 158.6 (C=O), 147.3 (C10), 144.5 (C9), 140.2 (C18), 132.1 (C10a), 130.3 (C6a), 128.4 (C20, C22), 128.4 (C4a), 128.0 (C19, C23), 127.4 (C21), 127.4 (C4), 123.4 (C7), 111.3 (C8), 88.2 (C1), 62.4 (C6), 61.6 (C3), 55.9 (OCH\(_3\)), 48.7 (C10b), 39.0 (C12), 36.2 (C11), 29.7 (C2), 27.9 (NCH\(_3\)).
(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-N-methyl-12-phenyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 210

$R_f$: 0.400 (9:0.9:0.1, dichloromethane:methanol:aqueous ammonia 35%); $[\alpha]_D^{22}$: -109.6º (c. 1, CHCl$_3$); MS m/z (ES$^+$): 407 (100%, MH$^+$), 429 (80%, MNa$^+$); HRMS: found 429.1773, MNa$^+$ requires 429.1785; IR $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3549 (O-H), 3361 (N-H), 2932 (C-H), 1623 (C=O); $\lambda_{\text{max}}$ 288 nm, $^{1}H$-NMR (CHLOROFORM-d, 500 MHz) $\delta$ ppm 7.23 - 7.33 (3H, m, H$_{20}$, H$_{21}$, H$_{22}$), 7.06 (2H, d, $J$=7.5 Hz, H$_{19}$, H$_{23}$), 6.76 (1H, d, $J$=8.5 Hz, H$_8$), 6.61 (1H, d, $J$=8.5 Hz, H$_7$), 6.53 (1H, br s, H$_6$), 5.96 (1H, dd, $J$=10.5, 5.0 Hz, H$_4$), 5.61 (1H, d, $J$=10.0 Hz, H$_{4a}$), 4.57 - 4.66 (2H, m, H$_1$, NH$_1$), 4.77 (1H, dt, $J$=11.0, 5.5 Hz, H$_3$), 3.81 - 3.95 (4H, m, H$_{12}$, OCH$_3$), 3.14 - 3.25 (1H, m, H$_{12}$), 2.86 (3H, d, $J$=4.5 Hz, NCH$_3$), 2.64 (1H, ddt, $J$=15.5, 4.0, 2.0 Hz, H$_2$), 2.44 (1H, d, $J$=11.5 Hz, OH$_1$), 2.00 (1H, ddd, $J$=15.5, 5.0, 2.0 Hz, H$_2$), 1.83 (1H, dt, $J$=13.0, 6.0 Hz, H$_{11}$), 1.67 (1H, dt, $J$=13.5, 7.0 Hz, H$_{11}$); $^{13}C$-NMR (CHLOROFORM-d, 100 MHz) $\delta$ ppm 158.9 (C=O), 147.0 (C10), 144.2 (C9), 140.8 (C18), 133.1 (C10a), 129.0 (C4a), 128.4 (C6a), 128.4 (C20, C22), 128.0 (C4), 128.0 (C19, C23), 127.5 (C21), 122.0 (C7), 111.8 (C8), 89.0 (C1), 61.6 (C3), 60.8 (C6), 56.0 (OCH$_3$), 47.7 (C10b), 39.8 (C12), 34.6 (C11), 29.8 (C2), 28.0 (NCH$_3$).

Migration of the $m$-methylphenyl group

Rearrangement gave a 1:2.28 mixture of diastereoisomers, as determined by NMR. Yield 93%, 0.0969 g, 0.2304 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 1 ml min$^{-1}$ and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 5.960 min and 7.353 min (dr 1:2.25). The prep HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 15 ml min$^{-1}$ and room temperature at 27 – 28 °C. This method gave two fairly sharp peaks, with only very minor tailing, eluting at 8.152 min and 9.733 min (dr 1:2.28):
(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-N-methyl-12-(m-tolyl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 215

[α]D22: -37.6° (c. 1, CHCl₃); MS m/z (ES+) 443 (60%, MNa⁺); HRMS: found 421.2111, MH⁺ requires 421.2122; IR νmax (film)/cm⁻¹ 3554 (O-H), 3355 (N-H), 2925 (C-H), 1622 (C=O); λmax 290 nm; ¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 7.20 (1H, t, J=7.5 Hz, H22), 7.09 (1H, d, J=8.0 Hz, H23), 6.93 (1H, br s, H19), 6.86-6.79 (2H, m, H7, H21), 6.78 (1H, d, J=8.5 Hz, H8), 6.52 (1H, br s, H6), 5.80 (1H, ddd, J=10.5, 5.0, 1.5 Hz, H4), 5.46 (1H, d, J=10.0 Hz, H4a), 4.52 - 4.60 (2H, m, H1, NH), 4.06 (1H, ddd, J=11.5, 6.0, 5.0 Hz, H3), 3.89 (3H, s, OCH₃), 3.71 - 3.85 (1H, m, H12), 3.36 (1H, t, J=14.0 Hz, H12), 2.85 (3H, d, J=4.5 Hz, NCH₃), 2.67 (1H, ddt, J=15.5, 3.5, 1.7 Hz, H2), 2.49 (1H, d, J=11.5 Hz, OH), 2.32 (3H, s, H24), 1.86 - 1.98 (2H, m, H2, H11), 1.60 (1H, dd, J=12.0, 3.5 Hz, H11); ¹³C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 158.6 (C=O), 147.3 (C10), 144.5 (C9), 140.2 (C18), 138.2 (C20), 132.1 (C6a), 130.4 (C10a), 128.5 (C4a), 128.5 (C19), 128.3 (C22), 128.2 (C21), 127.3 (C4), 125.2 (C23), 123.3 (C7), 111.3 (C8), 88.2 (C1), 62.4 (C6), 61.7 (C3), 55.9 (OCH₃), 48.7 (C10b), 39.0 (C12), 36.2 (C11), 29.7 (C2), 27.9 (NCH₃), 21.5 (C24).

(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-N-methyl-12-(m-tolyl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 216

[α]D22: -114.8° (c. 1, CHCl₃); MS m/z (ES+) 421 (30%, MH⁺); HRMS: found 421.2113, MH⁺ requires 451.2122; IR vmax (film)/cm⁻¹ 3553 (O-H), 3366 (N-H), 2926 (C-H), 1622 (C=O); λmax 288 nm; ¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 7.18 (1H, t, J=7.5 Hz, H22), 7.08 (1H, d, J=7.5 Hz, H21), 6.93 (1H, s, H19), 6.80 (1H, d, J=8.0 Hz, H23), 6.77 (1H, d, J=8.5, H8), 6.60 (1H, d, J=8.5 Hz, H7), 6.44 (1H, br s, H6), 5.98 (1H, dd,
J=10.5, 5.0 Hz, H4), 5.62 (1H, d, J=10.5 Hz, H4a), 4.60 - 4.65 (1H, m, H1), 4.53 (1H, q, J=5.0 Hz, NH), 4.12 (1H, dt, J=11.5, 5.0 Hz, H3), 3.86 - 3.99 (1H, m, H12), 3.88 (3H, s, OCH3), 3.15 - 3.29 (1H, m, H12), 2.87 (3H, d, J=4.5 Hz, NCH3), 2.66 (1H, ddt, J=15.5, 4.0, 1.5 Hz, H2), 2.42 (1H, d, J=11.5 Hz, OH), 2.31 (3H, s, H24), 2.02 (1H, ddd, J=15.5, 5.0, 2.5 Hz, H2), 1.85 (1H, dt, J=13.5, 6.0 Hz, H11), 1.65 - 1.75 (1H, m, H11); ¹³C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 159.0 (C=O), 147.0 (C10), 144.2 (C9), 140.7 (C18), 138.2 (C20), 133.1 (C6a), 129.0 (C4a), 128.7 (C19), 128.6 (C10a), 128.4 (C21), 128.3 (C22), 128.1 (C4), 125.1 (C23), 121.9 (C7), 111.8 (C8), 89.0 (C1), 61.6 (C3), 60.9 (C6), 56.0 (OCH3), 47.7 (C10b), 39.9 (C12), 34.7 (C11), 29.9 (C2), 28.0 (NCH3), 21.4 (C24).

Migration of the p-methylphenyl group

Rearrangement gave a 1:1.6 mixture of diastereoisomers, as determined by NMR, and no noticeable starting material. Yield 100%, 0.1030 g, 0.2449 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 1 ml min⁻¹ and the temperature set to 27.5 ºC. This method gave sharp symmetrical peaks eluting at 6.271 min and 7.813 min (dr 1:1.46). The prep HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 15 ml min⁻¹ and room temperature at 27 – 28 ºC. This method gave sharp peaks eluting at 8.467 min and 10.162 min (dr 1:1.55):

(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-N-methyl-12-(p-tolyl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 217

Mpt. 163-165 ºC (chloroform); [α]D²²: -36.8º (c. 1, CHCl3); MS m/z (ES+) 421 (100%, MH⁺); HRMS: found 421.2120, MH⁺ requires 421.2122; IR νmax (film)/cm⁻¹ 3555 (O-H), 3353 (N-H), 2927 (C-H), 1622 (C=O); ¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 7.12 (2H, d, J=8.0 Hz, H20, H22), 6.95 (2H, d, J=8.0 Hz, H19, H23), 6.81 (1H, d, J=8.5 Hz, H7), 6.77 (1H, d, J=8.5 Hz, H8), 6.51 (1H, br s, H6), 5.80 (1H, dd, J=10.5, 5.5 Hz, H4), 5.45 (1H, d, J=10.5 Hz, H4a), 4.58 - 4.68 (1H, m, NH), 4.57-4.51 (1H, m, H1), 4.05 (1H, dt, J=11.0, 5.0 Hz, H3), 3.87 (3H, s, OCH3), 3.66 - 3.83 (1H, m,
H12), 3.29 - 3.43 (1H, m, H12), 2.84 (3H, d, J=4.5 Hz, NCH3), 2.65 (1H, ddd, J=16.0 Hz, H2), 2.49 (1H, d, J=11.5 Hz, OH), 2.35 (3H, s, H24), 1.85 - 1.97 (2H, m, H2, H11), 1.59 (1H, ddd, J=13.5, 3.0 Hz, H11); 13C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 158.6 (C=O), 147.3 (C10), 144.5 (C9), 137.1 (C18, C21), 132.0 (C10a), 130.5 (C6a), 129.1 (C20, C22), 128.5 (C4a), 127.9 (C19, C23), 127.2 (C4), 123.3 (C7), 111.2 (C8), 88.2 (C1), 62.2 (C6), 61.7 (C3), 55.9 (OCH3), 48.7 (C10b), 38.9 (C12), 36.2 (C11), 29.7 (C2), 27.9 (NCH3), 21.0 (C24).

(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-N-methyl-12-(p-tolyl)-4a,5,9,10-tetrahydro-6H-benzofuro[2,3-cd]azepine-11(12H)-carboxamide, 218

Mpt: 140-142 °C (chloroform); [α]22: -119.6° (c. 1, CHCl3); MS m/z (ES+) 421 (100%, MH+); HRMS: found 421.2118, MNa+ requires 421.2122; IR νmax (film)/cm⁻¹ 3551 (O-H), 3360 (N-H), 2923 (C-H), 1621 (C=O); λmax 288 nm; 1H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 7.10 (2H, d, J=8.0 Hz, H20, H22), 6.95 (2H, d, J=8.0 Hz, H19, H23), 6.75 (1H, d, J=8.5 Hz, C8), 6.60 (1H, d, J=8.5 Hz, C7), 6.46 (1H, br s, H6), 5.96 (1H, ddd, J=10.5, 5.0 Hz, H4), 5.62 (1H, d, J=10.5 Hz, H4a), 4.64-4.52 (2H, m, H1, NH), 4.11 (1H, dt, J=11.0, 5.5 Hz, H3), 3.98-3.82 (1H, m, H12), 3.87 (3H, s, OCH3), 3.12 - 3.26 (1H, m, H12), 2.85 (3H, d, J=4.5 Hz, NCH3), 2.65 (1H, dt, J=15.5, 2.0 Hz, H2), 2.43 (1H, d, J=11.5 Hz, OH), 2.33 (3H, s, H24), 2.01 (1H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.83 (1H, dt, J=13.5, 6.0 Hz, H11), 1.68 (1H, dt, J=14.0, 7.5 Hz, H11); 13C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 158.9 (C=O), 146.9 (C10), 144.1 (C9), 137.8 (C18), 137.3 (C21), 133.1 (C10a), 129.1 (C20, C22), 129.0 (C4a), 128.7 (C6a), 128.0 (C4), 127.9 (C19, C23), 121.9 (C7), 111.8 (C8), 89.0 (C1), 61.6 (C3), 60.7 (C6), 56.0 (OCH3), 47.7 (C10b), 39.8 (C12), 34.7 (C11), 29.8 (C2), 28.0 (NCH3), 21.0 (C24).
And

(4aS,6R,8aS,12R)-12-(3-Chlorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 222

Rearrangement gave a 1:1 mixture of diastereoisomers, as determined by NMR. Yield 26%, 0.0288 g, 0.0065 mmol, some starting material was also recovered, 47%, 0.0509 g, 0.1154 mmol.

An apparently successful analytical HPLC method was shown to be inadequate for preparative HPLC resulting in the loss of significant material and no separation of the diastereoisomers. In a further attempt to separate the diastereoisomers thin layer chromatography was used, however, this only resulted in a minuscule amount of one diastereoisomer being collected, and this was dirty. Therefore only a proton NMR of the mixed diastereoisomers after column chromatography was collected, this consists mainly of a mixture of diastereoisomers; a few clear peaks highly indicative of the successful rearrangement were visible.

$^1$H NMR (400 MHz, CHLOROFORM-$d$) δ ppm 7.25 - 7.37 (6H, m, ArH), 7.04 - 7.13 (4H, m ArH), 6.75 - 6.86 (3H, m, ArH), 6.62 (1H, d, J=8.5 Hz, ArH), 5.98 (1H, dd, J=10.5, 5.0 Hz, H4), 5.79 (1H, dd, J=10.5, 5.0 Hz, H4), 5.63 (1H, br d, J=10.5 Hz, H4a), 5.41 (1H, d, J=10.5 Hz, H4a), 4.49 - 4.64 (4H, m, H1, H1, H3 and H3), 4.01 - 4.17 (2H, m, H12 and H12), 3.88 (6H, s, OCH$_3$ and OCH$_3$), 3.28 - 3.44 (1H, m, H12), 3.16 - 3.26 (1H, m, H12), 2.87 (3H, br d, J=4.5 Hz, NCH$_3$), 2.85 (3H, br d, J=4.5 Hz, NCH$_3$), 2.62 - 2.70
(2H, m, H2 and H2), 2.48 (1H, d, J=11.5 Hz, OH), 2.42 (1H, d, J=11.5 Hz, OH), 1.79 - 2.05 (4H, m, H2, H2, H11 and H11), 1.57 - 1.73 (2H, m, H11 and H11).

**Migration of the p-chlorophenyl group**

Rearrangement gave a mixture of products, starting material and other unidentified degradation products. The NMR of the crude reaction mixture indicated a 1:7.5 mixture of diastereoisomers, after column this had changed to a dr of 1:2.6. Yield 37%, 0.0400 g, 0.0907 mmol. Starting material was also reclaimed 31%, 0.0339 g, 0.0769 mmol.

The analytical HPLC method was run with an isocratic flow of acetonitrile and water (50:50), with a flow rate of 1 ml min\(^{-1}\) and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 6.267 min and 7.174 min (dr 1:2.31). The prep HPLC method was run with an isocratic flow of acetonitrile and water (50:50), with a flow rate of 15 ml min\(^{-1}\) and room temperature at 27 – 28 °C. This method gave peaks eluting at 12.845 min and 14.219 min (dr 1:1.91):

\[(4aS,6R,8aS,12R)-12-(4-Chlorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 223\]

\[\alpha^2\]D: -21.6º (c. 1, CHCl\(_3\)); IR \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3545 (O-H), 3356 (N-H), 2955 (C-H), 1623 (C=O); \(\lambda_{\text{max}}\) 290 nm; \(^1\)H-NMR (CHLOROFORM-d, 400 MHz) \(\delta\) ppm 7.30 (2H, d, \(J=9.0\) Hz, H21, H22), 7.02 (2H, d, \(J=8.0\) Hz, H19, H23), 6.83 (1H, d, \(J=8.5\) Hz, H7), 6.79 (1H, d, \(J=8.5\) Hz, H8), 6.59 (1H, br s, H6), 5.83 (1H, ddd, \(J=10.5, 5.3, 1.5\) Hz, H4), 5.37 (1H, d, \(J=10.5\) Hz, H4a), 4.54 - 4.60 (2H, m, H1, NH), 4.07 (1H, dt, \(J=10.5, 5.5\) Hz, H3), 3.88 (3H, s, OCH\(_3\)), 3.65 - 3.79 (1H, m, H12), 3.33 (1H, br dd, \(J=16.0, 13.0\) Hz, H12), 2.85 (3H, d, \(J=4.5\) Hz, NCH\(_3\)), 2.67 (1H, ddt, \(J=15.5, 3.5, 1.5\) Hz, H2), 2.48 (1H, d, \(J=11.5\) Hz, OH), 1.86 - 1.98 (2H, m, H2, H11), 1.58 - 1.66 (1H, m, H11); \(^{13}\)C-NMR (CHLOROFORM-d, 100 MHz) \(\delta\) ppm 158.4 (C=O), 147.4 (C10), 144.7 (C9), 138.9 (C18), 133.3 (C21), 132.0 (C10a), 129.8 (C6a), 129.4 (C19, C23), 128.7 (C21, C22), 128.1 (C4a), 127.7 (C4), 123.4 (C7), 111.4 (C8), 88.2 (C1), 61.8 (C6), 61.6 (C3), 55.9 (OCH\(_3\)), 48.6 (C10b), 39.0 (C12), 36.1 (C11), 29.7 (C2), 27.9 (NCH\(_3\)).
(4aS,6R,8aS,12R)-12-(4-Chlorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-

\[
\begin{align*}
&\text{HO} \\
H &\text{N} \\
\text{Cl} &\text{O}
\end{align*}
\]

\[\text{224}\]

\([\alpha]_D^{22} = -110^\circ \text{ (c. 1, CHCl}_3\text{)}; \ MS \ m/z \ (ES-) \ 441 \ (45\%, \ M^{(37}\text{Cl})H^+)\), \ 439 \ (20\%, \ M^{(35}\text{Cl})H^+); \ HRMS: \ \text{found 441.1572, } \ M^{(37}\text{Cl})H^+ \ \text{requires 441.1576; } \ IR \ \nu_{\text{max}} \ (\text{film})/\text{cm}^{-1} \ 3548 \ (\text{O-H}), \ 3358 \ (\text{N-H}), \ 2923 \ (\text{C-H}), \ 1622 \ (\text{C=O}); \ \lambda_{\text{max}} \ 288 \text{ nm}; \ \text{¹H-}NMR \ (\text{CHLOROFORM-d}, 400 \text{ MHz}) \ \delta \text{ ppm} \ 7.27 \ (2\text{H, d, } J=8.5 \text{ Hz, H20, H22}), \ 7.03 \ (2\text{H, d, } J=8.5 \text{ Hz, H19, H23}), \ 6.77 \ (1\text{H, d, } J=8.5 \text{ Hz, C8}), \ 6.60 \ (1\text{H, d, } J=8.5 \text{ Hz, C7}), \ 6.59 \ (1\text{H, br s, H6}), \ 5.97 \ (1\text{H, ddd, } J=10.5, 5.0, 1.0 \text{ Hz, H4}), \ 5.6 \ (1\text{H, d, } J=10.5 \text{ Hz, H4a}), \ 4.61 \ (1\text{H, dt, } J=4.0, 2.0 \text{ Hz, H1}), \ 4.54 \ (1\text{H, q, } J=4.5 \text{ Hz, NH}), \ 4.16-4.08 \ (1\text{H, m, H3}), \ 3.88 \ (3\text{H, s, OCH}_3), \ 3.74 - 3.86 \ (1\text{H, m, H12}), \ 3.14 - 3.27 \ (1\text{H, m, H12}), \ 2.87 \ (3\text{H, d, } J=4.5 \text{ Hz, NCH}_3), \ 2.67 \ (1\text{H, ddt, } J=15.5, \ 3.5, 1.5 \text{ Hz, H2}), \ 2.41 \ (1\text{H, d, } J=11.5 \text{ Hz, OH}), \ 2.02 \ (1\text{H, ddd, } J=15.5, 5.0, 2.5 \text{ Hz, H2}), \ 1.87 \ (1\text{H, dt, } J=13.5, 6.0 \text{ Hz, H11}), \ 1.67 \ (1\text{H, br dt, } J=14.0, 7.0 \text{ Hz, H11}); \ ¹³C-NMR \ (\text{CHLOROFORM-d}, 100 \text{ MHz}) \ \delta \text{ ppm} \ 158.8 \ (C=O), \ 147.0 \ (C10), \ 144.2 \ (C9), \ 139.6 \ (C18), \ 133.4 \ (C21), \ 132.9 \ (C10a), \ 129.5 \ (C19, C23), \ 128.9 \ (C4a), \ 128.6 \ (C20, C22), \ 128.1 \ (C4), \ 128.0 \ (C6a), \ 122.0 \ (C7), \ 111.9 \ (C8), \ 89.0 \ (C1), \ 61.5 \ (C3), \ 60.1 \ (C6), \ 56.0 \ (OCH}_3), \ 47.6 \ (C10b), \ 39.8 \ (C12), \ 34.7 \ (C11), \ 29.8 \ (C2), \ 28.0 \ (NCH}_3).\]

**Migration of the o-methoxylphenyl group**

Rearrangement gave a 1:1.1 mixture of diastereoisomers, as determined by NMR. Yield 99%, 0.1068 g, 0.2447 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (65:35), with a flow rate of 1 ml min\(^{-1}\) and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 8.030 min and 11.052 min (dr 1:1.0). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min\(^{-1}\) and room temperature at 27 – 28 °C. This method gave two fairly sharp peaks with a small amount of tailing eluting at 8.480 min and 10.613 min (dr 1:1.2):
(4aS,6R,8aS,12S)-6-Hydroxy-3-methoxy-12-(2-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 212

Mpt: 152-153 °C (Chloroform); [α]D22: 87.2° (c. 1, CHCl3); MS m/z (ES+): 437 (40%, MH+); HRMS: found 459.1885, MNa+ requires 459.1890; IR νmax (film)/cm⁻¹: 3554 (O-H), 3357 (N-H), 2927 (C-H), 1621 (C=O); λmax 280 nm,¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm: 7.31 (1H, td, J=8.0, 1.5 Hz, H21), 6.92 (1H, d, J=8.0 Hz, H20), 6.86 (1H, td, J=7.5, 1.0 Hz, H22), 6.77-6.68 (3H, m, H7, H8, H23), 6.42 (1H, br s, H6), 5.96-5.87 (2H, m, H4, H4a), 4.70 (1H, br s, NH), 4.54 - 4.59 (1H, m, H1), 4.09 (1H, dt, J=11.0, 4.5 Hz, H3), 3.86 (3H, s, OCH₃), 3.85 (3H, s, H24), 3.74 - 3.88 (1H, m, H12a), 3.06 (1H, br t, H12b), 2.85 (3H, d, J=4.5 Hz, NCH₃), 2.67 (1H, dd, J=16.0, 4.0 Hz, H2), 2.60 (1H, d, J=11.5 Hz, OH), 2.02-1.91 (2H, m, H2, H11), 1.62 (1H, dd, J=13.0, 4.0 Hz, H11); ¹³C-NMR (CHLOROFORM-d, 100 MHz) δ ppm: 158.2 (C=O), 157.9 (C19), 147.3 (C10), 144.4 (C9), 132.6 (C10a), 131.1 (C23), 131.0 (C6a), 129.4 (C21), 129.4 (C4a), 128.1 (C18), 127.2 (C4), 122.5 (C7), 119.8 (C22), 111.4 (C8), 110.7 (C20), 88.4 (C1), 61.7 (C3), 60.4 (C6), 55.9 (OCH₃), 55.8 (C24), 49.0 (C10b), 38.6 (C12), 36.6 (C11), 29.7 (C2), 28.0 (NCH₃).

(4aS,6R,8aS,12S)-6-Hydroxy-3-methoxy-12-(2-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 225

Mpt: 215-216 °C (methanol); [α]D²²: -140.4° (c. 1, CHCl₃); MS m/z (ES-): 435 (45%, M-H+); IR νmax (film)/cm⁻¹: 3545 (O-H), 3366 (N-H), 2936 (C-H), 1623 (C=O); λmax 280 nm,¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm: 7.30 (1H, td, J=8.0, 2.0 Hz, H21), 6.94
(1H, d, J=8.0 Hz, H20), 6.87 (1H, td, J=7.5, 1.0 Hz, H22), 6.80 (1H, d, J=7.5 Hz, H23), 6.70 (1H, d, J=8.5, H8), 6.39 (1H, d, J=8.5 Hz, H7), 6.33 - 6.45 (1H, br s, H6), 5.96 (1H, ddd, J=10.0, 5.0, 1.0 Hz, H4), 5.71 (1H, d, J=10.5 Hz, H4a), 4.92 (1H, br s, NH), 4.64 - 4.70 (1H, m, H1), 4.12 (1H, dt, J=11.0, 4.5 Hz, H3), 4.10 - 4.16 (1H, m, H12), 3.88 (3H, s, H24), 3.85 (3H, s, OCH3), 3.09 (1H, dt, J=14.0, 7.0 Hz, H12), 2.85 (3H, d, J=4.5 Hz, NCH3), 2.65 (1H, ddt, J=15.5, 4.0, 2.0 Hz, H2), 2.40 (1H, d, J=11.5 Hz, OH), 2.04 - 2.14 (1H, m, H11), 1.75 (1H, dt, J=13.5, 6.5 Hz, H11);

¹³C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 159.0 (C=O), 157.0 (C19), 146.8 (C10), 144.1 (C9), 133.2 (C10a), 129.8 (C4a), 129.3 (C21), 129.1 (C23), 129.0 (C6a), 127.4 (C4), 121.4 (C7), 120.7 (C22), 111.8 (C8), 110.3 (C20), 89.4 (C1), 61.7 (C3), 56.9 (C6), 55.9 (OCH3), 55.8 (C24), 47.8 (C10b), 40.0 (C12), 35.5 (C11), 30.0 (C2), 28.0 (NCH3).

Migration of the m-methoxyphenyl group

Rearrangement gave a 1:3.8 mixture of diastereoisomers, as determined by NMR, and no noticeable starting material. Yield 73%, 0.0785 g 0.1800 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 1 ml min⁻¹ and the temperature set to 27.5 °C. This method gave fairly sharp symmetrical peaks eluting at 5.15 min and 6.17 min (dr 1:3.50). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min⁻¹ and room temperature at 27–28 °C. This method gave peaks eluting at 14.883 min and 20.370 min (dr 1:3.55):

(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-12-(3-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 226

[α]D²²: -39.6° (c. 1, CHCl3); MS m/z (ES+) 459 (80%, MNa⁺); HRMS: found 459.1881, MNa⁺ requires 459.1890; IR v_max (film)/cm⁻¹ 3554 (O-H), 3354 (N-H), 2927 (C-H), 1621 (C=O); λ_max 282; ¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 7.23 (1H, t, J=8.0 Hz, H22), 6.80 - 6.86 (2H, m, H7, H21), 6.78 (1H, d, J=8.0 Hz, H8), 6.67 (1H, s, H19), 6.63 (1H, d, J=8.0, H23), 6.53 (1H, br s, H6), 5.81 (1H, dd, J=10.5, 5.0 Hz, H4), 5.49 (1H, d,
Migration of the p-methoxylphenyl group

Rearrangement gave a 1:1.9 mixture of diastereoisomers, as determined by NMR, and no noticeable starting material. Yield 99%, 0.1060 g, 0.2428 mmol.
The analytical HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 1 ml min\(^{-1}\) and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 6.341 min and 7.856 min (dr 2.11:1). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min\(^{-1}\) and room temperature at 27 – 28 °C. This method gave sharp peaks eluting at 7.106 min and 8.220 min (dr 1.29:1):

\((4aS,6R,8aS,12R)-6\text{-Hydroxy-3-methoxy-12-(4-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11}(12H)-\text{carboxamide, 229}\)

\[\text{229}\]

\([\alpha]_D^{22} = -36.2^\circ \text{ (c. 1, CHCl}_3); \text{ MS } m/z \text{ (ES+)} \quad 437 \text{ (50\%, MH}^+\text{), 459 (35\%, MNa}^+\text{); HRMS: found 459.1890, MNa}^+ \text{ requires 459.1890; IR } \nu_\text{max}(\text{film})/\text{cm}^{-1} \quad 3546 \text{ (O-H), 3354 (N-H), 2930 (C-H), 1620 (C=O); } \lambda_\text{max} \text{ 286 nm; } ^1\text{H-NMR (CHLOROFORM-d, 400 MHz) } \delta \text{ ppm 6.97 (2H, d, } J=9.0 \text{ Hz, H19, H23), 6.83 (2H, d, } J=9.0 \text{ Hz, H20, H22), 6.81 (1H, d, } J=8.5 \text{ Hz, H7), 6.76 (1H, d, } J=8.5 \text{ Hz, H8), 6.50 (1H, br s, H6), 5.79 (1H, dd, } J=10.0, 5.0 \text{ Hz, H4), 5.43 (1H, d, } J=10.5 \text{ Hz, H4a), 4.70 (1H, q, } J=4.5 \text{ Hz, NH}, 4.49 - 4.55 (1H, m, H1), 4.04 (1H, dt, } J=11.0, 5.5 \text{ Hz, H3), 3.86 (3H, s, OCH}_3, 3.80 (3H, s, H24), 3.69 - 3.79 (1H, m, H12), 3.35 (1H, br td, } J=14.5, 3.5 \text{ Hz, H12), 2.82 (3H, d, } J=4.5 \text{ Hz, NCH}_3, 2.64 (1H, ddd, } J=16.0, 3.5, 2.0 \text{ Hz, H2), 2.49 (1H, d, } J=11.5 \text{ Hz, OH), 1.84 - 1.96 (2H, m, H2, H11), 1.58 (1H, dd, } J=13.5, 3.5 \text{ Hz, H11); } ^{13}\text{C-NMR (CHLOROFORM-d, 100 MHz) } \delta \text{ ppm 158.8 (C21), 158.6 (C=O), 147.2 (C10), 144.4 (C9), 132.0 (C10a), 130.6 (C6a), 129.1 (C19, C23), 128.5 (C4a), 127.3 (C4), 123.2 (C7, C18), 113.7 (C20, C22), 111.2 (C8), 88.2 (C1), 61.8 (C6), 61.6 (C3), 55.8 (OCH}_3, 55.2 (C24), 48.6 (C10b), 38.7 (H12), 36.2 (H11), 29.6 (H2), 27.8 (NCH}_3).}
(4aS,6R,8aS,12R)-6-Hydroxy-3-methoxy-12-(4-methoxyphenyl)-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 228

\[ \alpha^D \text{D} \text{22} \text{8}: -116.8^\circ \text{ (c. 1, CHCl}_3); \text{ MS } m/z \text{ (ES+) 437 (100\%, MH\textsuperscript{+}); HRMS: found 437.2075, MH\textsuperscript{+} requires 437.2076; IR } \nu_{max} \text{ (film)/cm}^{-1} \text{ 3545 (O-H), 3365 (N-H), 2930 (C-H), 1622 (C=O); } \lambda_{max} \text{ 284 nm; } ^1\text{H-NMR} \text{ (CHLOROFORM-d, 400 MHz) } \delta \text{ ppm 6.98 (1H, d, } J=9.0 \text{ Hz, H19, H23), 6.82 (1H, d, } J=9.0 \text{ Hz, H20, H22), 6.76 (1H, d, } J=8.5 \text{ Hz, H8), 6.60 (1H, d, } J=8.5 \text{ Hz, H7), 6.45 (1H, br s, H6), 5.97 (1H, dd, } J=10.5, 5.0 \text{ Hz, H4), 5.61 (1H, d, } J=10.0 \text{ Hz, H4a), 4.61 (1H, dt, } J=4.0, 2.0 \text{ Hz, H1), 4.54 (1H, q, } J=4.5 \text{ Hz, N-H), 4.11 (1H, dt, } J=10.5, 5.5 \text{ Hz, H3), 3.84 - 3.96 (1H, m, H12), 3.87 (3H, s, OCH}_3\text{), 3.79 (3H, s, H24), 3.12 - 3.25 (1H, m, H12), 2.86 (3H, d, } J=4.5 \text{ Hz, NCH}_3\text{), 2.65 (1H, ddt, } J=15.5, 4.0, 1.5 \text{ Hz, H2), 2.42 (1H, d, } J=11.5 \text{ Hz, OH), 2.01 (1H, ddd, } J=15.5, 5.0, 2.5 \text{ Hz, H2), 1.84 (1H, dt, } J=14.0, 6.0 \text{ Hz, H11), 1.69 (1H, ddd, } J=13.5, 8.0, 6.5 \text{ Hz, H11); } ^{13}\text{C-NMR} \text{ (CHLOROFORM-d, 100 MHz) } \delta \text{ ppm 158.9 (C=O), 158.9 (C21), 147.0 (C10), 144.1 (C9), 133.1 (C10a), 132.8 (C18), 129.2 (C19, C23), 129.0 (C4a), 128.9 (C6a), 128.0 (C4), 121.9 (C7), 113.7 (C20, C22), 111.9 (C8), 89.0 (C1), 61.6 (C3), 60.4 (C6), 56.0 (OCH}_3\text{), 55.2 (C24), 47.7 (C10b), 39.7 (C12), 34.7 (C11), 29.8 (C2), 28.0 (NCH}_3\text{).}

**Migration of the p-Fluorophenyl group**

Rearrangement gave a 1:1.2 mixture of diastereoisomers, as determined by NMR, and no obvious starting material, however, the reaction gave a dirtier mixture of products after work up than some of the other rearrangements, making the determination of the diastereotopic ratio harder and less accurate. Yield 73\%, 0.0785 g 0.1800 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 1 ml min\textsuperscript{-1} and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 7.011 min and 8.533 min (dr 1:1.49). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min\textsuperscript{-1} and room temperature at 27 – 28 °C. This method gave peaks eluting at 14.042 min and 16.823 min (dr 1:1.57).
(4aS,6R,8aS,12R)-12-(4-Fluorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 230

\[
\begin{align*}
&\text{Mpt: } 164-165 \, ^\circ \text{C (chloroform); } [\alpha]_D^{22} \text{: } -45.2^\circ \text{ (c. 1, CHCl}_3); \text{ MS } m/z \text{ (ES+) 425 (70\%, MH}^+)\text{, 447 (50\%, MNa}^+)\text{; HRMS: found 425.1885, MH}^+\text{ requires 425.1871; IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} \text{ 3554 (O-H), 3354 (N-H), 2927 (C-H), 1622 (C=O); } \lambda_{\text{max}} \text{ 290 nm, } ^1\text{H-NMR} \\
&\text{(CHLOROFORM-d, 400 MHz) } \delta \text{ ppm 6.89 - 7.01 (4H, m, H19, H20, H22, H23), 6.75 (1H, d, } J=8.5 \, \text{Hz, C7), 6.71 (1H, d, } J=8.5 \, \text{Hz, C8), 6.50 (1H, br s, H6), 5.74 (1H, d, } J=10.0, \\
&5.0, 1.5, H4) 5.30 (1H, d, } J=10.5 \, \text{Hz, H4a), 4.51 (1H, q, } J=4.5 \, \text{Hz, NH}, 4.45 - 4.50 \, \text{(1H, m, H1), 3.99 (1H, dt, } J=10.5, 4.5 \, \text{Hz, H3), 3.80 (3H, s, OCH}_3), 3.58 - 3.74 \, \text{(1H, m, H12), 3.26 (1H, dd, } J=15.5, 13.0 \, \text{Hz, H12), 2.77 (3H, d, } J=5.0 \, \text{Hz, NCH}_3), 2.59 \, \text{(1H, ddt, } J=15.5, \\
&3.5, 1.5 \, \text{Hz, H2), 2.40 (1H, d, } J=11.5 \, \text{Hz, OH), 1.79 - 1.90 \, \text{(2H, m, H2, H11), 1.54 (1H, dd, } J=13.0, 3.5 \, \text{Hz, H11); } ^{13}\text{C-NMR} \text{ (CHLOROFORM-d, 100 MHz) } \delta \text{ ppm 162.0 (} ^1\text{JCF 247.0, C21), 158.5 (C=O), 147.4 (C10), 144.6 (C9), 136.0 (} ^1\text{JCF 3.0, C18), 132.0 (C10a), 130.1 \text{ (C6a), 129.6 (} ^1\text{JCF 8.0, C19, C23), 128.1 (C4a), 127.6 (C4), 123.3 (C7), 115.3 (} ^1\text{JCF 21.5, \\
&C20, C22), 111.4 (C8), 88.2 (C1), 61.7 (C6), 61.6 (C3), 55.9 (OCH}_3), 48.6 (C10b), 38.9 \text{ (C12), 36.2 (C11), 29.7 (C2), 27.9 (NCH}_3).}
\end{align*}
\]

(4aS,6R,8aS,12R)-12-(4-Fluorophenyl)-6-hydroxy-3-methoxy-N-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 231

\[
\begin{align*}
&\text{Mpt: } 132-133 \, ^\circ \text{C (chloroform); } [\alpha]_D^{22} \text{: } -103.2^\circ \text{ (c. 1, CHCl}_3); \text{ MS } m/z \text{ (ES+) 425 (90\%, MH}^+)\text{, 447 (40\%, MNa}^+)\text{; HRMS: found 425.1876, MH}^+\text{ requires 425.1871; IR } \nu_{\text{max}} \text{ (film)/cm}^{-1} \text{ 3554 (O-H), 3365 (N-H), 2927 (C-H), 1622 (C=O); } \lambda_{\text{max}} \text{ 288 nm, } ^1\text{H-NMR} \\
&\text{ (CHLOROFORM-d, 400 MHz) } \delta \text{ ppm 7.02 - 7.10 (2H, m, H19, H23), 6.94 - 7.02 \, \text{(2H, m, H20, H22), 6.77 (1H, d, } J=8.5 \, \text{Hz, H8), 6.59 (1H, d, } J=8.5 \, \text{Hz, H7), 6.62 - 6.53 \, \text{(1H, br s,}
\end{align*}
\]
Migration of the 1-naphthyl group

Rearrangement gave an approximately 1:1.9 mixture of diastereoisomers, as determined by NMR, however, there was a degree of peak overlap in the crude NMR and traces of starting material. Yield 80%, 0.0922 g, 0.2019 mmol. The majority of the material was separated by column chromatography. For comparative purposes; the analytical HPLC method was run with an isocratic flow of methanol and water (75:25), with a flow rate of 1 ml min⁻¹ and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 9.637 min and 11.469 min (dr 1:1.79).

(4aS,6R,8aS,12R)-N-Ethyl-6-hydroxy-3-methoxy-12-(naphthalen-1-yl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 232

Mpt: the colour and appearance changed at 137 °C and 170 °C but had still not melted by 200 °C, a fresh sample heated directly to 200 °C melted immediately °C (chloroform);

$[\alpha]_{D}^{22}$: 53.6° (c. 1, CHCl₃); MS m/z (ES+) 471 (75%, MH⁺); HRMS: found 471.2275, MH⁺ requires 471.2278; IR $\nu_{\max }$ (film)/cm⁻¹ 3553 (O-H), 3355 (N-H), 2926 (C-H), 1620 (C=O); $\lambda_{\max }$ 284 nm; $^1$H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 8.16 (1H, d, $J$=8.0 Hz, H23), 7.86 - 7.92 (1H, m, H25), 7.82 (1H, d, $J$=8.5 Hz, H21), 7.49 - 7.58 (2H, m, H24, H26), 7.33 (1H, t, $J$=7.5, H20), 6.93 (1H, d, $J$=7.5 Hz, H19), 6.85 (1H, d, $J$=8.5 Hz, H7),
6.78 (1H, d, J=8.5 Hz, H8), 5.87 - 5.98 (2H, m, H4, H4a), 4.54 - 4.65 (2H, m, H1, NH), 4.04 - 4.13 (1H, m, H3), 3.88 (3H, s, OCH3), 3.32 - 3.48 (2H, m, NCH2CH3), 2.92 - 3.30 (2H, m, H12), 2.69 (1H, dd, J=16.0, 3.5 Hz, H2), 2.63 (1H, d, J=10.5 Hz, OH), 1.89 - 2.01 (2H, m, H2, H11), 1.57 (1H, d, H11), 1.19 (3H, t, J=7.5 Hz, NCH2CH3); ¹³C-NMR (CHLOROFORM-d, 100 MHz) δ ppm 156.7 (C=O), 147.3 (C10), 144.4 (C9), 136.8 (C18), 133.9 (C22), 132.1 (C10a), 131.7 (C27), 131.6 (C6a), 129.2 (C4a), 129.1 (C21), 129.0 (C19), 128.7 (C25), 127.4 (C4), 126.8 (C24), 126.0 (C26), 124.3 (C20, C23), 123.1 (C7), 111.7 (C8), 88.3 (C1), 61.6 (C3), 61.0 (C6), 55.9 (OCH3), 49.0 (C10b), 38.8 (C12), 36.0 (NCH2CH3), 35.9 (C2), 29.7 (C2), 15.7 (NCH2CH3). The ¹H NMR did not show a peak for the H6 proton, there is a slightly larger than expected integration for H24, H26, given that the H6 peak is broad in all the other analogues of this series and the position of the H6 peak in the other diastereoisomer of this analogue (~7 ppm) this is not an unreasonable assignment.

(4aS,6R,8aS,12R)-N-Ethyl-6-hydroxy-3-methoxy-12-(naphthalen-1-yl)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 233

**Mpt:** 148-151 °C (chloroform); [α]D²²: -163.2° (c. 1, CHCl3); MS m/z (ES+) 471 (90%, MH+); HRMS: found 471.2282, MH+ requires 471.2278; IR νmax (film)/cm⁻¹ 3552 (O-H), 3365 (N-H), 2927 (C-H), 1620 (C=O); λmax 284 nm ¹H-NMR (CHLOROFORM-d, 400 MHz) δ ppm 8.05 (1H, d, J=8.5 Hz, H26), 7.89 (1H, dd, J=8.0, 1.0 Hz, H24), 7.79 (1H, d, J=8.5 Hz, H21), 7.57 (1H, td, J=7.0, 1.5 Hz, H25), 7.53 (1H, td, J=7.0, 1.5, H23), 7.28 (1H, t, J=8.0 Hz, H20), 7.02 (1H, br s, H6), 6.79 (1H, d, J=8.5 Hz, H8), 6.73 (1H, d, J=7.5 Hz, H19), 6.67 (1H, d, J=8.5 Hz, H7), 6.00 (1H, dd, J=10.5, 5.0 Hz, H4), 5.65 (1H, d, J=10.5 Hz, H4a), 4.72-4.63 (2H, m, H1, NH), 4.11 (1H, dt, J=11.0, 5.0 Hz, H3), 3.78 - 4.06 (1H, m, H12), 3.89 (3H, s, OCH3), 3.29 - 3.50 (2H, m, NCH2CH3), 2.67 (1H, ddt, J=15.5, 4.0, 2.0 Hz, H2), 2.53 - 2.72 (1H, m, H12), 2.49 (1H, d, J=11.5 Hz, OH), 2.02 (1H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.82 - 1.95 (1H, m, H11), 1.65 - 1.76 (1H, m, H11), 1.19 (3H, t, J=7.0 Hz, NCH2CH3); ¹³C-NMR (CHLOROFORM-d, 100 MHz) δ 158.0 (C=O), 147.0 (C10), 144.3 (C9), 136.5 (C18), 134.1 (C10a), 133.5 (C22), 131.9 (C27), 129.3
(C6a), 128.9 (C21), 128.8 (C24), 128.6 (C4), 128.6 (C4a), 127.2 (C25), 127.1 (C19), 126.0 (C23), 124.6 (C20), 123.1 (C26), 121.7 (C7), 112.3 (C8), 89.0 (C1), 61.6 (C3), 58.6 (C6), 56.0 (OCH₃), 47.9 (C10b), 39.0 (C12), 36.2 (NCH₂CH₃), 34.6 (C11), 29.8 (C2), 15.8 (NCH₂CH₃).

**Migration of the 2-pyridyl group**

Rearrangement gave a 1:1.2 mixture of diastereoisomers, as determined by NMR, and no obvious starting material but the reaction did give a dirtier mixture of products after work up than some of the rearrangements. Yield 73%, 0.0785 g 0.1800 mmol.

The analytical HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 1 ml min⁻¹ and the temperature set to 27.5 °C. This method gave sharp symmetrical peaks eluting at 3.820 min and 4.192 min (dr 1.15:1). The prep HPLC method was run with an isocratic flow of methanol and water (70:30), with a flow rate of 15 ml min⁻¹ and room temperature at 27 – 28 °C. This method gave peaks eluting at 6.716 min and 7.972 min (dr 1:1):

$$(4aS,6R,8aS,12S)-6$-Hydroxy-3-methoxy-N-methyl-12-(pyridin-2-yl)-4a,5,9,10$-
$\text{tetrahydro-6H$-\text{benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)$-carboxamidex, 234}$$

\[\alpha\]D²²: -20.4° (c. 1, CHCl₃); **MS** m/z (ES+) 408 (100%, MH⁺); **HRMS**: found 408.1921, MH⁺ requires 408.1918; **IR** νmax (film)/cm⁻¹ 3554 (O-H), 3346 (N-H), 2925 (C-H), 1622 (C=O); \(\lambda_{max}\) 262 nm and 290 nm; **¹H-NMR** (CHLOROFORM-d, 400 MHz) δ ppm 8.64 (1H, dd, \(J=5.0, 2.0\) Hz, H20), 7.64 (1H, td, \(J=7.5, 2.0\) Hz, H21), 7.20 (1H, dd, \(J=8.0, 5.0\) Hz, H22), 7.12 (1H, d, \(J=8.0\) Hz, H23), 6.86 (1H, d, \(J=8.5\) Hz, H7), 6.78 (1H, d, \(J=8.5\) Hz, H8), 6.74 (1H, br s, H6), 5.82 (1H, ddd, \(J=10.5, 5.0, 1.0\) Hz, H4), 5.50 (1H, d, \(J=10.5\) Hz, H4a), 4.90 (1H, q, \(J=4.5\) Hz, NH), 4.55 (1H, dt, \(J=4.0, 2.0\) Hz, H1), 4.05 (1H, dt, \(J=11.5, 5.5\) Hz, H3), 3.86 (3H, s, OCH₃), 3.82-3.71 (1H, m, H12a), 3.32 (1H, dd, \(J=16.5, 13.0\) Hz, H12b), 2.83 (3H, d, \(J=4.5\) Hz, NCH₃), 2.66 (1H, ddt, \(J=15.5, 3.5, 1.5\) Hz, H2), 2.46 (1H, d, \(J=11.5\) Hz, OH), 2.02-1.89 (2H, m, H2, H11), 1.62 (1H, dd, \(J=13.0, 3.5\) Hz, H11); **¹³C-NMR** (CHLOROFORM-d, 100 MHz) δ ppm 159.9 (C18), 158.5 (C=O), 149.4 (C20), 147.1 (C10), 144.5 (C9), 136.6 (C22), 131.9 (C10a), 130.1 (C6a), 128.2 (C4a), 127.4 (C4), 230
123.7 (C7), 123.4 (C23), 122.2 (C21), 111.3 (C8), 88.1 (C1), 64.1 (C6), 61.7 (C3), 55.8 (OCH₃), 48.6 (C10b), 39.7 (C12), 36.2 (C11), 29.7 (C2), 27.9 (NCH₃).

\( (4aS,6R,8aS,12S) - 6\text{-Hydroxy-3-methoxy-N-methyl-12-(pyridin-2-yl)} - 4a,5,9,10\text{-tetrahydro-6}H\text{-benzo[2,3]benzofuro[4,3-cd]azepine-11(12}H\text{)-carboxamide, 235} \)

\[ [\alpha]^{22}_D: -126^\circ \text{ (c. 1, CHCl}_3\text{)}; \text{ MS m/z (ES+) 408 (100\%, MH}^+\text{)}; \text{ HRMS: found 408.1929, MH}^+\text{ requires 408.1918; IR } \nu_{\text{max}}/\text{cm}^{-1} 3545 (\text{O-H}), 3359 (\text{N-H}), 2924 (\text{C-H}), 1622 (\text{C=O}); \lambda_{\text{max}} 264 \text{ nm, 288 nm; } \text{¹H-NMR (CHLOROFORM-d, 400 MHz) } \delta \text{ ppm 8.57 (1H, ddd, } J=5.0, 2.0, 0.5 \text{ Hz, H20), 7.69 (1H, td, } J=8.0, 2.0 \text{ Hz, H22), 7.29 (1H, d, } J=8.0 \text{ Hz, H23), 7.27-7.23 (1H, m, H21), 6.69 (1H, d, } J=8.5, \text{ H8), 6.48 (1H, br s, H6), 6.33 (1H, d, } J=8.5 \text{ Hz, H7), 5.90 (1H, dd, } J=10.0, 5.5 \text{ Hz, H4), 5.76 (1H, d, } J=10.0 \text{ Hz, H4a), 5.51 - 5.70 (1H, m, NH), 4.71 (1H, dt, } J=4.0, 2.0 \text{ Hz, H1), 4.12 (1H, dt, } J=10.5, 5.5 \text{ Hz, H3), 3.84 (3H, s, OCH}_3\text{), 3.77-3.61 (2H, m, H12), 2.83 (3H, d, } J=5.0 \text{ Hz, NCH}_3\text{), 2.67 (1H, ddt, } J=15.5, 4.0, 2.0 \text{ Hz, H2), 2.30 - 2.44 (2H, m, H11, OH), 2.04 (1H, ddd, } J=15.5, 5.0, 2.5 \text{ Hz, H2), 1.70 - 1.80 (1H, m, H11); } \text{¹³C-NMR (CHLOROFORM-d, 100 MHz) } \delta \text{ ppm 161.2 (C18), 159.0 (C=O), 149.1 (C20), 146.9 (C10), 144.0 (C9), 137.1 (C22), 131.9 (C10a), 131.1 (C4a), 128.3 (C6a), 126.0 (C4), 122.9 (C21), 122.8 (C23), 121.8 (C7), 111.7 (C8), 89.5 (C1), 63.9 (C6), 61.4 (C3), 55.9 (OCH}_3\text{), 46.8 (C10b), 41.0 (C12), 36.7 (C11), 30.0 (C2), 27.7 (NCH}_3\text{).} \)

\( N\text{-Boc-norgalanthamine or tert-butyl (4aS,6R,8aS)-6\text{-Hydroxy-3-methoxy-4a,5,9,10\text{-tetrahydro-6}H\text{-benzo[2,3]benzofuro[4,3-cd]azepine-11(12}H\text{-carboxylate, 162} \)\)

This method was adapted from the method reported by Rouleau and Guillou for the synthesis of \( N\text{-Boc-sanguinine}. \text{ Briefly dichloromethane (20 cm}^3\text{) and triethylamine} \)
(0.560 cm³, 4.018 mmol, 1.1 eq.) were added to norgalanthamine (0.9933 g, 3.637 mmol, 1 eq.) and di-tert-butyl dicarbonate (0.7903 g, 3.621 mmol, 1.0 eq.). The reaction was stirred overnight and then quenched with saturated aqueous ammonium chloride (20 cm³). The mixture was extracted with dichloromethane (3 × 20 cm³), the organic fractions were combined, dried over magnesium sulphate and then the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10). The product was a white solid that existed as rotamers on the NMR time scale. Yield 83%, 1.12 g, 3.02 mmol.

**MS** m/z (ES+) 396 (45%, MNa⁺), 769 (100%, MMNa⁺); **HRMS**: found 396.1787, MNa⁺ requires 396.1787; **IR** νmax (film)/cm⁻¹ (O-H), 1687 (C=O); **¹H NMR** (400 MHz, CHLOROFORM-d) δ ppm 6.79 (0.37 H, br d, J=8.0 Hz, H7), 6.64 - 6.07 (1.63 H, m, H7 and H8), 5.94 - 6.06 (2 H, m, H4 and H4a), 4.88 (0.37 H, br d, J=15.5 Hz, H6), 4.69 (0.63 H, br d, J=16.0 Hz, H6), 4.57 - 4.61 (1 H, m, H1), 4.29 (0.63 H, m, J=15.0 Hz, H12), 4.02 - 4.19 (2.37 H, m, 3H, H6, H6, H12), 3.84 (1.89 H, br s, OCH₃), 3.83 (1.11 H, br s, OCH₃), 3.26 - 3.44 (1 H, m, H12), 2.66 - 2.73 (1 H, m, J=16.0 Hz, H2), 2.37 - 2.44 (1 H, m, OH), 1.69 - 2.07 (3 H, m, H2, H11, H11), 1.42 (3.4 H, s, CH₃), 1.37 (5.6 H, s, CH₃); **¹³C NMR** (101 MHz, CHLOROFORM-d) δ ppm 154.9 (C=O), 154.6 (C=O), 146.4 (C11), 146.3 (C10), 144.2 (C9), 132.4 (C10a), 132.1 (C10a), 129.8 (C6a), 129.7 (C6a), 128.0 (C4), 127.9 (C4), 126.7 (C4a), 126.5 (C4a), 121.5 (C7), 120.9 (C7), 111.2 (C8), 110.8 (C8), 88.4 (C1), 88.2 (C1), 79.8 (C10b), 61.9 (C3), 55.8 (OCH₃), 51.9 (C6), 51.3 (C6), 48.3 (C10b), 45.6 (C12), 45.4 (C12), 37.4 (C11), 36.4 (C11), 29.8 (C2), 28.4 (CH₃), 28.3 (CH₃).

(4aS,6R,8aS)-N,N-Diethyl-6-hydroxy-3-methoxy-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-ed]azepine-11(12H)-carboxamide, 238

![Norgalanthamine](image)

Norgalanthamine (0.2511 g, 0.9193 mmol, 1 eq.) was dissolved in dichloromethane (4.0 cm³) and to this was added diethylcarbamoyl chloride (0.120 cm³, 0.947 mmol, 1 eq.) and then triethylamine (0.140 cm³, 1.00 mmol, 1 eq.). The reaction was stirred overnight and then quenched with saturated sodium hydrogen carbonate solution (10 cm³) and then extracted with dichloromethane (3 × 10 cm³). The organic phases were combined, dried
over magnesium sulphate and then the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with dichloromethane:methanol-35% aqueous ammonia (9:1) in a gradient (98:2, 96:4, 94:6, 92:8, 90:10). The product was a white solid. Yield 85%, 0.2903 g, 0.7794 mmol.

**MS m/z (ES+) 395 (100%, MNa+); HRMS: found 373.2124, MH+ requires 373.2122; IR v_{max} (film)/cm^{-1} 3549 (O-H), 1624 (C=O); ^1H NMR (500 MHz, CHLOROFORM-d) \delta ppm 6.67 (1 H, d, J=8.0 Hz), 6.63 (1 H, d, J=8.0 Hz), 5.96 (1 H, br dd, J=10.0, 4.5 Hz), 5.92 (1 H, d, J=10.0 Hz), 4.53 - 4.58 (1 H, m), 4.44 (1 H, br d, J=16.0 Hz), 4.19 (1 H, br d, J=16.0 Hz), 3.97 - 4.12 (2 H, m), 3.78 (3 H, s), 3.27 - 3.40 (1 H, m), 3.12 (4 H, q, J=7.0 Hz), 2.58 - 2.67 (1 H, m), 2.42 (1 H, br d, J=10.0 Hz), 1.99 (1 H, ddd, J=16.0, 5.0, 2.0 Hz), 1.92 (1 H, td, J=13.0, 3.5 Hz), 1.73 - 1.84 (1 H, m), 1.05 (6 H, t, J=7.0 Hz); ^13C NMR (126 MHz, CHLOROFORM-d) \delta ppm 164.7 (C=O), 146.1 (C10), 144.0 (C9), 132.8 (C10a), 129.1 (C6a), 127.9 (C4), 126.8 (C4a), 120.6 (C7), 111.1 (C8), 88.3 (C1), 61.7 (C3), 55.7 (OCH3), 54.0 (C6), 47.9 (C10b), 46.6 (C12), 42.1 (CH2), 36.8 (C11), 29.7 (C2), 13.1 (CH3).

**13.2.d General method for the C6 lithiation and functionalization of galanthamine derived N-diethyl ureas**

Urea, 238, (0.1000 g, 0.2685 mmol, 1 eq.) was dissolved in dry THF and TMEDA (0.400 cm³, 2.68 mmol, 10 eq.) was added. The mixture was cooled to -78 °C and then sec-butyllithium (4 eq., approx. 1.04 cm³) was added slowly. The reaction was stirred for 1 hour and then quenched with the electrophile source and stirred for 1 hour, the reaction was then quenched with methanol (1 cm³). The solvent was removed under reduced pressure, saturated aqueous ammonium chloride (10 cm³) was added to the residue and then the mixture was extracted with dichloromethane (3 \times 10 cm³). The organic phases were combined, dried over magnesium sulphate and evaporated under reduced pressure.
The residue of the reaction was purified by filtering through silica gel and then prep HPLC to separate the starting material and the product. The preparative HPLC method was run with an isocratic flow of acetonitrile and water (40:60), with a flow rate of 15 ml min\(^{-1}\) and room temperature at 27 – 28 °C. This method gave sharp peaks eluting at 14.294 min (remaining starting material) and at 15.188 min (product). No conditions could be found to separate the diastereoisomers. Yield 35%, 0.0390 g, 0.0932 mmol. A further product was isolated but not identified.

**MS** m/z (ES+) 441 (35%, MNa\(^+\)), 427 (100%, M(-CH\(_3\)))+Na; **\(^1\)H NMR** (500 MHz, CHLOROFORM-\(d\)) \(\delta\) ppm 6.85 (1 H, br d, \(J=8.5\) Hz, ArH), 6.83 (1 H, br d, \(J=8.5\) Hz, ArH), 6.75 (1 H, d, \(J=8.5\) Hz, ArH), 6.71 (1 H, d, \(J=8.5\) Hz, ArH), 6.57 (1 H, d, \(J=10.0\) Hz, Alkene H), 6.09 (1 H, s, H6), 6.00 (1 H, s, H6), 5.96 - 6.05 (2 H, m, Alkene H), 5.75 (1 H, d, \(J=10.0\) Hz, Alkene H), 4.58 - 4.63 (1 H, m, H1), 4.54 - 4.58 (1 H, m, H1), 4.22 (1 H, m, \(J=16.0\) Hz, H12), 4.04 - 4.18 (3 H, m, H3, H3, H12), 3.85 (3 H, s OCH\(_3\)), 3.84 (3 H, s, OCH\(_3\)), 3.64 - 3.77 (2 H, m, H12, H12), 3.20 - 3.35 (4 H, m, CH\(_2\)CH\(_3\)), 2.93 - 3.08 (4 H, m, CH\(_2\)CH\(_3\)), 3.64 - 3.77 (2 H, m, H2, H2), 2.50 (1 H, d, \(J=11.5\) Hz, OH), 2.41 - 2.49 (1 H, m, H2), 2.40 (1 H, d, \(J=11.5\) Hz, OH), 2.17 (3 H, s, SCH\(_3\)), 2.07 (1 H, s, SCH\(_3\)), 1.98 - 2.10 (1 H, m, H11), 1.83 - 1.93 (2 H, m, H11), 1.70 - 1.82 (1 H, m, H11), 1.08 - 1.16 (12 H, m, CH\(_2\)CH\(_3\)).

**The stannylation of a galanthamine based urea via lithiation**

This was done using the standard method described above (13.2.d). The crude reaction mixture was columned three times to remove the excess tin and separate the diastereoisomers. The eluent was pentane:ethyl acetate (100 cm\(^3\) 100:0, 200 cm\(^3\) 75:25, 100 cm\(^3\) 50:50, 100 cm\(^3\) 25:75 and then 100% ethyl acetate until complete).
(4aS,6R,8aS,12R)-N,N-Diethyl-6-hydroxy-3-methoxy-12-(tributylstanny)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 240

Yield 7%, 0.0376 g, 0.0568 mmol.

**MS** m/z (ES+) 663 (100%, MH⁺); **HRMS** found 663.3135, MH⁺ requires 663.3184; **IR** νmax (film)/cm⁻¹ 3556 (O-H), 1632 (C=O); **¹H NMR** (400 MHz, CHLOROFORM-d) δ ppm 6.66 (1 H, d, J=8.5 Hz, H8), 6.48 (1 H, d, J=8.5 Hz, H7), 5.96 (1 H, dd, J=10.0, 5.0 Hz, H4), 5.84 (1 H, d, J=10.0 Hz, H4a), 5.01 - 5.27 (1 H, m, H6), 4.53 - 4.57 (1 H, m, H1), 4.27 - 4.43 (1 H, m, H12), 4.09 - 4.17 (1 H, m, H3), 3.82 (3 H, s, OCH3), 3.01 - 3.18 (5 H, m, H17 and H12), 2.68 (1 H, m, J=15.5 Hz H2), 2.51 (1 H, d, J=11.5 Hz, OH), 2.03 (1 H, ddd, J=15.5, 5.0, 1.5 Hz, H2), 1.76 - 1.92 (2 H, m, H11), 1.34 - 1.53 (6 H, m, H21), 1.22 - 1.34 (6 H, s, H20), 1.09 (6 H, t, J=7.0 Hz, H18), 0.82 - 0.94 (15 H, m, H19 and H22); **¹³C NMR** (100 MHz, CHLOROFORM-d) δ ppm 164.9 (C=O), 146.6 (C10), 141.9 (C9), 135.5 (C6a), 130.0 (C10a), 127.6 (C4a), 127.1 (C4), 118.2 (C7), 112.0 (C8), 88.0 (C1), 61.9 (C3), 58.5 (C6), 56.0 (OCH3), 48.3 (C10b), 47.0 (C12), 42.7 (C17), 38.0 (C11), 29.8 (C2), 29.0 (3JSnC 19.8 Hz, C21), 27.4 (2JSnC 56.5 Hz, C20), 13.6 (C22), 13.3 (C18), 11.4 (1JSnC 281.0, 293.4 Hz, C19).

(4aS,6R,8aS,12R)-N,N-Diethyl-6-hydroxy-3-methoxy-12-(tributylstanny)-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 241

Yield 32%, 0.1821 g, 0.2753 mmol.
[α]D22: -91.1° (c. 1, CHCl3); MS m/z (ES+) 663 (100%, MH+); HRMS: found 663.3135, MH+ requires 663.3184; IR νmax (film)/cm⁻¹ 3555 (O-H), 1624 (C=O); 1H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.66 (1 H, d, J=8.5 Hz, H8), 6.41 (1 H, d, J=8.5 Hz, H7), 5.95 (1 H, dd, J=10.5, 5.0 Hz, H4), 5.76 (1 H, d, J=10.5 Hz, H4a), 4.94 (1 H, d, JH,Sn=76.0 Hz, H6), 4.49 - 4.55 (1 H, m, H1), 4.07 - 4.16 (1 H, m, H2), 3.83 - 3.95 (1 H, m, H12), 3.81 (3 H, s, OCH3), 3.17 - 3.31 (3 H, m, H12 and H17), 2.95 - 3.07 (2 H, m, H17), 2.66 (1 H, m, J=16.0 Hz, H2), 2.50 (1 H, d, J=11.5 Hz, OH), 1.80 - 2.08 (3 H, m, H2, H11 and H11), 1.31 - 1.49 (6 H, m, H21), 1.19 - 1.30 (6 H, m, H20), 1.10 (6 H, t, J=7.0 Hz, H18), 0.77 - 0.91 (15 H, m, H19 and H22); 13C NMR (100 MHz, CHLOROFORM-d) δ ppm 165.1 (C=O), 146.6 (C10), 142.0 (C9), 134.6 (C6), 131.0 (C10a), 129.3 (C4), 128.3 (C4a), 115.8 (C7), 112.4 (C8), 89.0 (C1), 61.8 (C6), 56.1 (OCH3), 54.1 (C6), 46.7 (C10b), 45.7 (C12), 42.4 (C17), 32.6 (C11), 29.8 (C2), 28.8 (JSnC 19.8 Hz, C21), 27.3 (JSnC 57.2 Hz, C20), 13.6 (2C, H18 and H22), 11.3 (JSnC 292.7, 280.2 Hz, C19).

(4aS,6R,8aS,12R)-N,N-Diethyl-6-hydroxy-3-methoxy-12-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine-11(12H)-carboxamide, 243 and 244

The prep HPLC method for the separation of the diastereoisomers was run using an isocratic flow of acetonitrile and water (30:70), with a flow rate of 15 ml min⁻¹ and room temperature at 27 – 28 °C. This method gave peaks eluting at 23.237 min and 24.440 min (dr 1:1.1).

Peak at 23.237 min,
From lithiation and iodomethane quench: Yield 5%, 5.6 mg, 0.014 mmol.
From tin-lithium exchange and quench with iodomethane: Yield 7%, 7.1 mg, 0.018 mmol.

[α]D22: -13.6° (c. 1, CHCl3); MS m/z (ES+) 409 (100%, MNa+); HRMS: found 387.2287, MH+ requires 387.2278; 1H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.75 (1 H, d, J=8.5 Hz, H8), 6.70 (1 H, d, J=8.5 Hz, H7), 6.00 (1 H, dd, J=10.5, 5.0 Hz, H4), 5.81 (1 H, br d, J=10.5 Hz, H4a), 4.78 - 4.97 (1 H, br s, H6), 4.57 - 4.62 (1 H, m, H1), 4.11 - 4.18 (1 H, m, H3), 3.85 (3 H, s, OCH3), 3.76 (1 H, ddd, J=13.5, 13.5, 7.0 Hz, H12), 3.51 - 3.64 (1 H, m, H12), 3.00 - 3.30 (4 H, m, H19), 2.69 (1 H, ddt, J=16.0, 3.5, 1.5, 1.5 Hz, H2), 2.50 (1 H, d, J=11.5 Hz, OH), 2.06 (2 H, ddd, J=15.6, 4.8, 1.8 Hz, H2 and H11), 1.83 - 1.96 (1
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H, m, H11), 1.53 (3 H, br d, J=7.3 Hz, H17), 1.11 (6 H, t, J=7.0 Hz, H20); $^{13}$C NMR (100 MHz, CHLOROFORM-d) δ ppm 165.1 (C=O), 146.4 (C10), 143.4 (C9), 134.1 (C6), 128.8 (C10a), 128.0 (C4a), 127.9 (C4), 119.6 (C7), 112.0 (C8), 88.8 (C1), 61.9 (C3), 57.6 (C6), 56.0 (OCH3), 47.7 (C10b), 42.1 (C19), 41.0 (C12), 34.6 (C11), 29.7 (C2), 22.3 (C17), 13.3 (C20).

Peak at 24.440 min:

From lithiation and iodomethane quench: Yield 5%, 5.3 mg, 0.014 mmol. $[^{1}]{\alpha}D_{22}^{2}: -17.2º (c. 1, CHCl3); MS m/z (ES+) 409 (100%, MNa$^+$); HRMS: found 409.2112, MNa$^+$ requires 409.2098; $^1$H NMR (400 MHz, CHLOROFORM-d) δ ppm 6.71 (1 H, d, J=8.5 Hz, H8), 6.69 (1 H, d, J=8.5 Hz, H7), 6.06 (1 H, dd, J=10.5, 1.0 Hz, H4a), 6.02 (1 H, dd, J=11.5, 5.5, 4.5 Hz, H3), 3.94 (1 H, m, J=15.5 Hz, H12), 3.84 (3 H, s, OCH3), 3.59 (1 H, dd, J=15.5, 12.0 Hz, H12), 3.05 - 3.22 (4 H, m, H19), 2.69 (1 H, m, J=15.7, 3.6 Hz, H2), 2.51 (1 H, d, J=11.5 Hz, OH), 2.03 (1 H, ddd, J=15.5, 5.0, 2.0 Hz, H2), 1.88 - 1.98 (1 H, m, H11), 1.81 (1 H, dd, J=13.5, 3.5 Hz, H11), 1.59 (4 H, d, J=7.5 Hz, H17), 1.10 (6 H, t, J=7.0 Hz, H20); $^{13}$C NMR (101 MHz, CHLOROFORM-d) δ ppm 165.3 (C=O), 146.5 (C10), 143.6 (C9), 134.7 (C6), 131.4 (C10a), 128.9 (C4), 127.9 (C4a), 121.3 (C7), 111.5 (C8), 88.5 (C1), 61.8 (C3), 58.4 (C6), 55.8 (OCH3), 48.7 (C10b), 42.5 (C19), 38.8 (C12), 37.6 (C11), 29.7 (C2), 21.8 (C17), 13.2 (C20).

Galanthaminium bromide or (4aS,6R,8aS)-6-hydroxy-3-methoxy-11-methyl-4a,5,9,10-tetrahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-11-ium bromide, 245

Galanthaminium bromide was synthesised using the method of Hametner et al. Briefly galanthamine (0.5735 g, 2.001 mmol) was dissolved in dichloromethane (29.0 cm$^3$), to this was added N-bromosuccinimide (0.3574 g, 2.008 mmol). The reaction was stirred at room temperature for 12 minutes. The mixture was filtered, washing with ice cold dichloromethane and then air dried to give a pale greenish-yellow powder. Yield 80%, 0.5814 g, 1.587 mmol. $^1$H-NMR (METHANOL-d4, 300 MHz) δ 8.92 (1H, s, H6), 7.55 (1H, d, J=8.7, ArH), 7.16 (1H, d, J=8.5, ArH), 6.07 (1H, dd, J=10.2, 4.7, H4), 4.78 (1H, m, H1), 4.40-4.21 (2H, br m, H12), 4.15 (1H, m, H1), 4.00 (3H, s, NCH3), 3.87 (3H, s,
OCH3), 2.59 (1H, m, H2), 2.35-2.16 (3H, br m, H2, H11); \(^{13}\text{C}-\text{NMR (METHANOL-}\text{D}_4, 75.5 \text{ MHz}) \ \delta 169.5 \text{ (C6), 154.4 (Aryl), 148.5 (Aryl), 138.8 (Aryl), 135.6 (Aryl), 130.6 (C4), 128.2 (C4a), 116.6 (Aryl), 114.4 (Aryl), 89.3 (C1), 61.6 (C3), 57.6 (OCH3), 55.0 (C12), 52.6 (NCH3), 48.8 (C10b), 33.0 (C11), 30.8 (C2). NMR in agreement with the literature.}^{165}

6-Methylgalanthamine or (4aS,6R,8aS,12R)-3,6-dimethoxy-11,12-dimethyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepine, 246

Galanthaminium (0.1000 g, 0.2730 mmol) in ether was cooled to 0 °C, to this 1 mol dm\(^{-1}\) methyl magnesium bromide in ether (1.00 cm\(^3\), 1.00 mmol) was added dropwise. The reaction mixture was then allowed to warm to room temperature and stirred for 2 hours. The reaction was quenched with concentrated aqueous ammonia (1.0 cm\(^3\)) diluted with water (10.0 cm\(^3\)) and then extracted with dichloromethane (3 \times 20.0 cm\(^3\)). The organic phase was dried over magnesium sulphate and evaporated under vacuum to give the title compound as mixture of diastereoisomers, a white solid (75.1 mg, 0.249 mmol, 91%). Diastereoisomers remain unseparated but the reaction mixture was otherwise clean after work up, crude NMR in agreement with the literature.\(^{165}\)
13.3 The compound of Chapter 9: Functionalisation at the C3 hydroxyl site of galanthamine

\[(4aS,6R,8aS)-3\text{-}\text{Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-6-yl methyl(pyridin-2-yl)carbamate, 265}\]

\[^1H\text{ NMR (400 MHz, CHLOROFORM-d) } \delta \text{ ppm 8.32 (1 H, ddd, } J=5.0, 2.0, 1.0 \text{ Hz, H24), 7.81 (1 H, d, } J=8.5 \text{ Hz, H22), 7.54 (1 H, ddd, } J=8.5, 8.0, 2.0 \text{ Hz, H23), 6.95 (1 H, ddd, } J=7.0, 5.0, 1.0 \text{ Hz, H21), 6.67 (1 H, d, } J=8.0 \text{ Hz, H8), 6.58 (1 H, d, } J=8.0 \text{ Hz, H7), 6.30 (1 H, d, } J=10.0 \text{ Hz, H4a), 5.97 (1 H, dd, } J=10.0, 5.0 \text{ Hz, H4), 5.30 - 5.36 (1 H, m, H3), 4.58 - 4.62 (1 H, m, H1), 4.11 (1 H, d, } J=15.5 \text{ Hz, H6), 3.82 (3 H, s, OCH}_3, 3.66 (1 H, m, } J=15.5 \text{ Hz, H6), 3.42 (3 H, s, H19), 3.25 - 3.35 (1 H, m, H12), 3.05 (1 H, m, } J=14.5 \text{ Hz, H12), 2.79 (1 H, ddt, } J=16.0, 3.0, 1.5, 1.5 \text{ Hz, H2), 2.39 (3 H, s, NCH}_3, 2.05 - 2.20 (2 H, m, H2 and H11), 1.59 (1 H, d, } J=14.0 \text{ Hz, H11); } ^{13}C\text{ NMR (101 MHz, CHLOROFORM-d) } \delta \text{ ppm 154.8 (C18), 154.6 (C20), 147.1 (C24), 146.4 (C10), 143.8 (C9), 136.9 (C22), 132.0 (C10a), 130.8 (C4a), 129.4 (C6a), 122.8 (C4), 121.2 (C7), 119.1 (2C, C21 and C23), 111.0 (C8), 86.3 (C1), 64.6 (C3), 60.4 (C6), 55.7 (OCH}_3, 53.7 (C12), 48.0 (C10b), 41.9 (NCH}_3, 34.2 (C11), 34.0 (C19), 27.8 (C2).}\]

13.4 The compounds of Chapter 10: The structural modification of the galanthamine core via Diels Alder reactions

Mesylation of galanthamine

Galanthamine (49.9 mg, 0.174 mmol, 1 eq.) was dissolved in dichloromethane (1.0 cm\(^3\)) and cooled to -78 °C. Methanesulfonyl chloride (0.020 cm\(^3\), 0.258 mmol, 1.49 eq.) was added drop wise and then triethylamine (0.035 cm\(^3\), 0.251 mmol, 1.44 eq.) was added drop wise. The reaction was stirred for three hours at -78 °C and then removed from the cold bath and water (0.4 cm cubed) was added to quench the reaction. A small volume of
additional water was added and then the mixture was extracted with dichloromethane (3 × 10 cm³). The organic fractions were combined, dried over magnesium sulphate and then evaporated under reduced pressure. The residue was purified by flash column chromatography using a gradient solvent system (dichloromethane/methanol/35% ammonia in water; 95:4:1, 94:5:1, 92:7:1, 90:9:1). Three main compounds were eluted from the column, but the first two were not completely clean. 3-Deoxy-3-chlorogalanthamine yield 20%, 10.6 mg, 0.0347 mmol and galanthamine (contaminated with noticeable epigalanthamine in 4.24:1 ratio) recovered yield 10%, 7.0 mg 0.017 mmol and epigalanthamine yield 66%, 33.2 mg, 0.166 mmol.

3-Deoxy-3-chlorogalanthamine, 255

![Chemical structure](image)

**MS** m/z (ES+) 308 (36%, MH⁺ ³⁷Cl), 306 (100%, MH⁺ ³⁵Cl); **¹H-NMR** (CHLOROFORM-d, 400 MHz) δ ppm 6.66 (1H, d, J=8.0 Hz, H8), 6.60 (1H, br d, J=8.5 Hz, H7), 6.08 (1H, dt, J=10.5, 2.0 Hz, H4a), 5.87 (1H, dt, J=10.5, 2.0 Hz, H4), 4.88 (1H, J=11.0, 6.0, 2.0 Hz, H3), 4.57 (1H, d, J=3.5, 2.0 Hz, H1), 4.06 (1H, d, J=15.0 Hz, H6), 3.65 (1H, d, J=15.0 Hz, H6), 3.26 (1H, ddd, J=14.5, 12.5, 2.5 Hz, H12), 3.03 - 3.12 (1H, m, H12), 2.97 (1H, dddd, J=14.5, 5.5, 3.5 Hz, H2), 2.38 (3H, s, NCH₃), 2.18 (1H, ddd, J=14.0, 13.0, 3.0 Hz, H11), 2.09 (1H, ddd, J=14.5, 11.0, 2.5 Hz, H2), 1.71 (1H, ddd, J=14.0, 4.0, 2.0 Hz, H11); **¹³C-NMR** (CHLOROFORM-d, 100 MHz) δ ppm 146.4 (Aryl), 143.9 (Aryl), 132.3 (Aryl), 129.4 (C4a), 129.4 (Aryl), 127.4 (C4), 121.8 (C7), 111.0 (C8), 88.0 (C1), 60.2 (C6), 55.8 (OCH₃), 53.7 (C12), 51.4 (C3), 47.7 (C10b), 41.8 (NCH₃), 33.9 (C11), 33.4 (C2).
E. W. D. Burke

Epigalanthamine, 17

\[
\text{\begin{align*}
\text{HO} & \text{H} \\
\text{N} & \text{O} \\
\text{17}
\end{align*}}
\]

**MS** \(m/z\) (ES+) 288 (100%, \(\text{MH}^+\)), 270 (20%, \(\text{M-OH}^+\)); \(^1\text{H}-\text{NMR}\) (CHLOROFORM-\(d\), 400 MHz) \(\delta\) ppm 6.44 (1H, \(d, J=8.0\) Hz, H8), 6.58 (1H, \(d, J=8.0\) Hz, H7), 6.07 (1H, \(dt, J=10.5, 2.0\) Hz, H4), 5.82 (1H, \(dt, J=10.5, 2.0\) Hz, H4a), 4.65 (1H, \(ddt, J=10.5, 6.0, 2.0\) Hz, H3), 4.61 (1H, \(dt, J=3.5, 2.0\) Hz, H1), 4.08 (1H, \(d, J=15.0\) Hz, H6), 3.85 (3H, s, OCH\(_3\)), 3.64 (1H, \(d, J=15.0\) Hz, H6), 3.27 (1H, \(dd, J=14.5, 12.5, 2.0\) Hz, H12), 3.06 (1H, \(dt, J=14.5, 4.0\) Hz, H12), 2.79 (1H, \(dddd, J=13.5, 13.0, 3.0\) Hz, H11), 1.72 (1H, \(dd, J=14.0, 10.5, 2.5\) Hz, H2), 1.65 (1H, \(ddd, J=14.0, 4.0, 1.5\) Hz, H11); \(^{13}\text{C}-\text{NMR}\) (CHLOROFORM-\(d\), 100 MHz) \(\delta\) ppm 146.6 (Aryl), 143.8 (Aryl), 132.9 (Aryl), 131.6 (C4a), 129.2 (Aryl), 126.5 (C4), 121.5 (C7), 110.8 (C8), 88.4 (C1), 63.1 (C3), 60.4 (C6), 55.8 (OCH\(_3\)), 54.0 (C12), 48.1 (C10b), 42.0 (NCH\(_3\)), 34.3 (C11), 32.4 (C2).

2-Methoxy-6-methyl-5,6,7,8-tetrahydrodibenzo[c,e]azocin-1-ol, 259

\[
\text{\begin{align*}
\text{HO} & \text{H} \\
\text{N} & \text{O} \\
\text{259}
\end{align*}}
\]

Concentrated phosphoric acid 85 wt.% (2 cm\(^3\)) was added to galanthamine (25.1 mg, 0.0873 mmol), heated to 140° C and stirred at this temperature for 25 min. The reaction mixture was allowed to cool before being diluted with water (50 cm\(^3\)) and basified by the addition of sodium carbonate. When the reaction mixture reached pH 10, as determined by litmus paper, it was extracted with dichloromethane (3 \(\times\) 50 cm\(^3\)), the organic fractions were combined, dried over magnesium sulphate and evaporated under reduced pressure to give the product as a white solid; no further purification was required. Yield 95% 0.0223 g, 0.0828 mmol.

**MS** \(m/z\) (ES+) 270 (100%, \(\text{MH}^+\)); **HRMS**: found 270.1486, \(\text{MH}^+\) requires 270.1489; **IR** \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3529 (O-H), 2937 (C-H); \(^1\text{H}-\text{NMR}\) (CHLOROFORM-\(d\), 500 MHz) \(\delta\) ppm
7.42 (1H, dd, J=7.5, 2.0 Hz, ArH), 7.37 (1H, ddd, J=8.0, 6.5, 1.5 Hz, ArH), 7.31-7.27 (2H, m, ArH), 6.95 (1H, d, J=8.0 Hz, H7), 6.89 (1H, d, J=8.0 Hz, H8), 3.94 (3H, s, OCH3), 3.52 (1H, d, J=13.5 Hz, H6), 3.24 (1H, dtd, J=11.5, 7.0, 0.5 Hz, H12), 3.03 (1H, d, J=13.5 Hz, H6), 2.70-2.63 (1H, ddd, J=12.5, 7.0, 5.5 Hz, H11), 2.57-2.51 (2H, m, H11, H12), 2.43 (3H, s, NCH3); \(^{13}C\)-NMR (CHLOROFORM-d, 125 MHz) δ ppm 145.7 (Aryl), 142.2 (Aryl), 142.1 (Aryl), 134.4 (Aryl), 131.8 (Aryl), 130.3 (Aryl CH), 129.8 (Aryl CH), 128.4 (Aryl CH), 126.4 (Aryl), 125.4 (Aryl CH), 122.1 (C7), 110.0 (C8), 58.9 (C12), 57.6 (C6), 56.1 (OCH3), 45.3 (NCH3), 32.3 (C11).

3,4-Didehydro-3-deoxygalanthamine or anhydro galanthamine, 141

3-deoxy-3-chlorogalanthamine (10.6 mg, 0.0347 mmol) was stirred with potassium \(\text{tert-}
\text{butoxide (17.1 mg, 0.152 mmol, 4.4 eq.) in tetrahydrofuran (1.3 cm}^3)\), at room temperature, overnight. Then the reaction mixture was refluxed for 6.5 hours, before being quenched with water (1.0 cm\(^3\)). The solvent was removed under reduced and then saturated sodium hydrogen carbonate aqueous solution (20 cm\(^3\)) was added and the mixture extracted with dichloromethane (3 × 20 cm\(^3\)). The organic fractions were combined, dried over magnesium sulphate and evaporated under reduced pressure to give the crude product.

This was then purified using gradient columned chromatography (dichloromethane/35% ammonia in water in methanol (1:9); 98:2, 96:4, 94:6, 92:8, 90:10) to give the product, yield 43%, 4.0 mg, 0.015 mmol.

\(^1H\)-NMR (CHLOROFORM-d, 500 MHz) δ ppm 6.57 (2H, s, H7 and H8), 6.22 (1H, dd, J=9.5, 5.5 Hz, H3), 6.13 (1H, dt, J=9.5, 1.5 Hz, H4a), 6.07 (1H, ddd, J=9.5, 5.5, 1.5 Hz, H2), 5.98 (1H, ddd, J=9.5, 5.5, 0.5 Hz, H4), 4.79 (1H, dd, J=5.5, 0.5 Hz, H1), 4.16 (1H, d, J=15.0 Hz, H6), 3.80 (3H, s, OCH\(_3\)), 3.66 (1H, dd, J=15.0, 1.0 Hz, H6), 3.34 (1H, ddd, J=14.5, 13.0, 2.0 Hz, H12), 3.00 (1H, dt, J=14.5, 4.0 Hz, H12), 2.37 (3H, s, NCH3), 1.98 (1H, td, J=13.0, 3.0 Hz, H11), 1.65 (1H, ddd, J=13.5, 4.0, 2.0 Hz, H11); \(^{13}C\)-NMR (CHLOROFORM-d, 125 MHz) δ ppm 147.6 (Aryl), 143.7 (Aryl), 131.1 (Aryl), 129.6 (C4a), 129.4 (Aryl), 127.5 (C3), 121.6 (C7), 120.6 (C4), 119.7 (C2), 110.0 (C8), 85.0 (C1), 60.2 (C6), 55.7 (OCH\(_3\)), 53.9 (C12), 48.2 (C10b), 41.7 (NCH3), 35.0 (C11).

3,4-didehydro-3-deoxygalanthamine (62.1 mg, 0.230 mmol, 1 eq.) in toluene (5.0 cm³) was added to 4-phenyl-1,2,4-triazoline-3,5-dione (40.3 mg, 0.230 mmol, 1 eq.) and stirred, at room temperature, for 5 minutes. A small volume of water was added and then the mixture was extracted with dichloromethane (3 × 10 cm³). The organic fractions were combined, dried over magnesium sulphate and then evaporated under reduced pressure. The residue was purified by flash column chromatography using a gradient solvent system dichloromethane/35% ammonia in water in methanol (1:9; 98:2, 96:4, 94:6, 92:8, 90:10) to give the product and remaining starting material in a 1.94:1 mixture, yield 42%, 43.2 mg, 0.0972 mmol, starting material present 28%, 17.1 mg, 0.0501 mmol. As the product and starting material were not separated, the yields and masses were calculated based on the ratio of product to starting material found by 1H NMR.

\(^1\)H-NMR (CHLOROFORM-d, 500 MHz) \( \delta \) ppm 7.43 - 7.48 (4H, m, H13, H14, H16, H17), 7.35 - 7.40 (1H, m, H15), 6.65 (1H, d, ArH), 6.61 (1H, d, ArH), 6.44 (1H, ddt, \( J=8.0, 5.5, 1.5 \) Hz, H3), 6.40 (1H, ddd, \( J=8.0, 6.0, 2.0 \) Hz, H4), 5.62 (1H, d, \( J=6.0 \) Hz, H4a), 5.37 (1H, ddd, \( J=5.5, 4.5, 2.0 \) Hz, H2), 4.86 (1H, d, \( J=4.5 \) Hz, H1), 4.18 (1H, d, \( J=15.0 \) Hz, H6), 3.82 (3H, s, OCH₃), 3.58 (1H, d, \( J=15.0 \) Hz, H6), 3.38 - 3.46 (1H, m, H12), 2.99 - 3.11 (1H, m, H12), 2.27 - 2.36 (1H, m, H11), 2.31 (3H, s, NCH₃), 1.97 - 2.05 (1H, m, H11).
14 Appendix

14.1 Is nevirapine atropisomeric? Experimental and computational evidence for rapid conformational inversion


The work for the following publication was undertaken as an aside to the bulk of this project in response to the (now retracted) publication by Chattopadhyay et al. claiming to have isolated optically active nevirapine from a natural source. Chattopadhyay et al. retracted the publication before this response was published, since that time they have republished some of this work claiming to have isolated nevirapine from a natural source, although all claims to it being chiral are absent. They state that the extraction was repeated on the same batch of seeds to confirm the presence of nevirapine, unfortunately the use of the same batch of seeds does not eliminate the possibility that it was the seeds that were contaminated and not the extraction process.

The author’s contribution to this publication was the preparation and running of NMR samples and the use of gNMR to model bandshapes in comparison with the experimental data and finally to determine values of $\Delta H^\ddagger$, $\Delta G^\ddagger$ and $\Delta S^\ddagger$. 
Is nevirapine atropisomeric? Experimental and computational evidence for rapid conformational inversion†

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The non-nucleoside reverse transcriptase inhibitor nevirapine displays in its room temperature 'H-NMR spectrum signals characteristic of a chiral compound. Following suggestions in the recent literature that nevirapine may display atropisomerism—and therefore be a chiral compound, due to slow interconversion between two enantiomeric conformers—we report the results of an NMR and computational study which reveal that while nevirapine does indeed possess two stable enantiomeric conformations, they interconvert with a barrier of about 76 kJ mol⁻¹ at room temperature. Nevirapine has a half life for enantiomerisation at room temperature of the order of seconds, is not atropisomeric, and cannot exist as separable enantiomers.

Introduction

Nevirapine (Fig. 1) is a non-nucleoside reverse transcriptase inhibitor developed by Boehringer Ingelheim in the 1990s. It received approval for use in the treatment of HIV between 1996 and 1998 and its efficacy has been explored in a number of clinical trials since then, some of them controversial. Nevirapine's X-ray crystal structure reveals a butterfly-like shape in which the planar pyridyl rings are bent upwards or downwards from the puckered central diazepine; their planes intersecting at an angle of 121°. Despite potential delocalisation of its lone pair into the adjacent pyridyl rings, the nitrogen atom of the cyclopropylamine is significantly pyramidalised, and the cyclopropyl ring adopts a position almost perpendicular to the plane of the diazepine, opposite to the two pyridyl rings. Vibrational spectroscopy and computational studies confirm a similar conformation in solution and when bound to the drug's protein target.

Despite nevirapine's lack of stereogenic centres, this favoured conformation has no plane of symmetry, and can interconvert with its mirror image by flexing of the diazepine, with inversion of the nitrogen atom accompanied by a butterfly-wing motion of the pyridyl rings as the cyclopropyl group passes between the pyridyl nitrogen atoms. The relatively slow interconversion of the enantiomeric conformers of benzo[c]quinones has been exploited for the enantiomeric construction of quaternary stereogenic centres by "memory of chirality." Furthermore, related benzalactams (albeit with a fully substituted lactam N) display atropisomery: that is, the interconversion of their conformers is slow enough for those conformers to exist as separable, chiral stereoisomers. Structurally related sterically encumbered aromatic amidines, amides, ureas, and (thio)ethers may also display atropisomerism. As with all chiral compounds, atropisomers may display biological activity that is dependent on their absolute configuration and for this reason unforeseen atropisomerism has recently been highlighted as a potential pitfall in the development of drug candidates. Although conformational motion in the cyclopropyl sidechain of nevirapine has been studied computationally it is remarkable that the rate of conformational inversion of nevirapine kᵢᵣ and whether it may be low enough for enantiomeric atropisomers to exist, has never been reported.

The question of atropisomerism in nevirapine was further highlighted early in 2011 in a paper since withdrawn, claiming an optical rotation for a sample of nevirapine purportedly isolated from a natural source—something possible only if nevirapine can indeed exist as a pair of atropisomeric enantiomers. In response to this paper, and to the discussion it generated, we have quantified, by spectroscopic and computational methods, the barrier to conformational inversion of nevirapine, and in this paper we report our results.

Results and discussion

NMR spectroscopy

Lack of planarity in the diazepine ring is evident in the 'H NMR spectrum of nevirapine. Although the initial characterisation of the molecule reported multiplets (3.62, 1H, H₇; 0.88, 2H, H₅; 0.35, 2H, H₆) for the protons around the cyclopropyl ring, close
Table 1  Chemical shifts and coupling constants at 303 K for the protons around the cyclopropyl ring

<table>
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<th>$\delta$ (ppm)</th>
<th>$J_\text{H-N}$ Hz</th>
<th>$J_\text{N-H}$ Hz</th>
<th>$J_\text{H-H}$ Hz</th>
<th>$J_\text{H}1$ Hz</th>
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<td>--</td>
<td>10.5</td>
<td>--</td>
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<tr>
<td>H$_5$</td>
<td>1.5685</td>
<td>10.5</td>
<td>--</td>
<td>6.5</td>
<td>--</td>
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<tr>
<td>H$_3$</td>
<td>0.6835</td>
<td>--</td>
<td>6.8</td>
<td>--</td>
<td>9.0</td>
</tr>
<tr>
<td>H$_4$</td>
<td>0.6841</td>
<td>6.5</td>
<td>--</td>
<td>9.9</td>
<td>--</td>
</tr>
<tr>
<td>H$_5$</td>
<td>6.0153</td>
<td>3.9</td>
<td>2.9</td>
<td>6.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

inspection of the $^1$H NMR spectrum of a sample of nevirapine (0.02 M in $d_6$-DMSO) at room temperature revealed symmetrical bandshapes at 0.88 and 0.35 ppm corresponding to the ABCD region of an ABCDX system in which coupling constants are symmetry-related but chemical shifts are not. The chemical shifts and coupling constants shown in Table 1 were determined by comparison with simulation. The pairs of protons H$_{5a}$ and H$_{5b}$ are distinguished by lying trans or cis to the cyclopropylamine nitrogen whereas the symmetry of the rest of the molecule, but the chemical inequivalence of H$_5$, H$_4$, and H$_3$, and H$_6$, excludes a plane of symmetry through the rest of the molecule, rendering these pairs diastereotopic.

Inversion of the nitrogen and flipping of the diazepinone (see Fig. 2) leads to exchange of the protons H$_2$, H$_3$, and H$_4$ and H$_6$, resulting in an AA'BB'X spin system under fast exchange, and we set out to determine the temperature dependence of the rate of exchange by dynamic NMR techniques. $^1$H NMR spectra were acquired in $d_6$-DMSO at a range of temperatures from 298 K (25 °C) to 350 K (117 °C). Coalescences were observed between H$_3$ and H$_6$, and H$_4$ and H$_5$, at around 60–70 °C, indicating a shift from the slow to the fast exchange regime.

![Figure 2](image2.png)

**Fig. 2** Chemical exchange and conformational inversion of nevirapine and its enantiomeric conformers.

The bandshapes over the full temperature range were modelled both by direct density matrix calculation using Mathematica and by using the commercial program gNMR. The linewidth was set at 1 Hz, and the average chemical shifts for protons A and B and protons C and D were used to match the positions of the two experimental bandshapes. The rate of exchange was adjusted by eye to give the best fit between the experimental and modelled bandshapes for both multitriplet at each temperature (Fig. 3).

The value of $k$ at 60 °C, where the line shape is most sensitive to variations in $k$, is $19 \pm 4$ s$^{-1}$, which corresponds to a value of $\Delta G^\circ$ for the enantiomerisation process of 73.7 ± 0.45 kJ mol$^{-1}$ by plotting $\ln(k/RT)$ against $1/T$ for data points close to coalescence. We estimated values for $\Delta H^\circ$ of 81 ± 4.3 kJ mol$^{-1}$ and $\Delta S^\circ$ of $+22 \pm 5.4$ J mol$^{-1}$ K$^{-1}$. While we have high confidence in the rate of exchange, and therefore the value of $\Delta G^\circ$, close to the coalescence point, the activation parameters $\Delta H^\circ$ and $\Delta S^\circ$ estimated by this method are considerably less certain, because of the unusual nature of the underlying spectrum. The large number of individual transitions contributing to this very strongly coupled spectrum

![Figure 3](image3.png)

**Fig. 3** (a) Experimental and (b) modelled bandshapes for H$_2$-H$_6$.

means that the bandshape at low and high temperatures is very sensitive to instrumental line broadening and $T^{-1}$ relaxation, while above coalescence there is no direct means of assessing any changes in the chemical shift differences between protons A and B and C and D.

Nonetheless, a value of $\Delta S^\circ$ of this magnitude suggests that $\Delta G^\circ$ for the enantiomerisation of nevirapine varies between 71 and 75 kJ mol$^{-1}$ over the range of temperatures studied. At 25 °C, we estimate a half-life for the enantiomerisation process of 1.5 s, or a half life for racemisation ($k_{\text{racem}} = 2k_{\text{en}}$) of less than a second. Nevirapine is not atropisomeric at room temperature, and would be separable into enantiomeric atropisomers only below about –30 °C.

**Computation**

To illuminate the process by which conformational inversion occurs, and to model the barrier computationally, electronic structure calculations, using the Gaussian09 suite of programs, were carried out using density functional theory methods to map out the potential energy surface (PES) across which inversion at the nitrogen atom occurs. We first explored the main features of the PES using the M06-1 functional in conjunction with a 6-31G** basis. Thermodynamic corrections were evaluated using the rigid rotor, harmonic oscillator approximation to give the free energies that we quote here.

The initial step from the minimum energy structure (Fig. 4a) leads to the transition structure (TS1; Fig. 4b), which is close to planarity at the inverting nitrogen. The corresponding barrier
is calculated to be 58 kJ mol⁻¹. This TS leads to a local energy minimum (Fig. 4c) 18 kJ mol⁻¹ above the global minimum. Rotation of the cyclopropyl group is needed for the global minimum energy structure to be reached again. The barrier for this step was calculated to be 16 kJ mol⁻¹, this TS (Fig. 4d) being 22 kJ mol⁻¹ below the TS for nitrogen inversion. We note that there is an alternative local energy minimum to 4c, in which the cyclopropyl group is rotated, and whose energetics are close to those of 4c.

Since the initial barrier to inversion at the nitrogen will determine the kinetics observed experimentally, we have evaluated this barrier using a more realistic model. We have employed a 6-311G(2d, 2p) basis set together with the M06-2X functional, which is more computationally demanding than M06-L, but has been shown to describe long range interactions more accurately.¹⁹ Solvation effects were included firstly by adding a single DMSO molecule, whose general position, hydrogen-bonded to the NH group, was previously identified from molecular dynamics simulations.¹⁴ The remaining bulk solvation was included by a single point calculation using the CPCM model employing a dielectric of 46.8 (DMSO). This procedure led to a free energy barrier to nitrogen inversion of 76 kJ mol⁻¹.

**Conclusion**

Nevirapine exists as two enantiomeric non-planar conformers which interconvert with a barrier of about 75 kJ mol⁻¹ at room temperature, determined both experimentally and computationally. We therefore conclude that while nevirapine’s room temperature NMR spectrum displays asymmetry, it is not an atropisomeric compound at this temperature and cannot be isolated as a single enantiomer above a temperature of about −30 °C.

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Notes and references


14 The reason for withdrawal was not related to the question of atropisomerism; see S. K. Chattopadhyay, A. Chatturret, S. Tandon, P. R. Mallick and R. Kanti, Tetrahedron, 2011, 67, 452.


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