The Design and Synthesis of Novel Chiral Ionic Liquids for Testing as Chiral Selecting Agents in GC Stationary Phases

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School of Applied Sciences
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Statement of authenticity

I certify that except where due acknowledgement has been made; the thesis comprises only my original work. This thesis has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out during the official commencement date of the approved research program.

Chewe Chifuntwe
Acknowledgments

First and foremost I would like to thank God, without whom we can do nothing but through whom all things are possible. Secondly, I would like to thank my supervisors Assoc Prof. Helmut Hügel and Prof Philip Marriott for taking a leap of faith in taking on a biologist and turning me into a chemist. Your help, patience and guidance have truly been appreciated. I hope you will continue in that spirit of adventure and continue to invest your time and knowledge in students from various scientific backgrounds. I would also like to thank my family for their encouragement and for making it easier to continue studying for this long. My parents (Kalonga and Happy Chifuntwe) for letting me live at home for this long so I could continue studying, and my brothers Chabala, Misongo, and Numa for being so much fun and making life a joy. Thank you to all the past and present students who have been a great source of knowledge and support throughout the years, I’ve had some of the greatest discussions in the office covering the most profound questions of life. I wish you all success in your careers and in finding the answers to life’s big questions. You’ve certainly challenged me to continue searching. Let me not forget to thank all the RMIT staff members that make it possible for us to do our research. Your efforts were greatly appreciated.

What more is there to say, but, that life is meant to be a challenge taken in fullness of joy. But, I must concede that doing this PhD has been one of the toughest challenges I have ever put myself through. I wouldn’t do it twice, but it’s given me the courage to be able to take on anything. It has been very challenging but, extremely rewarding. The knowledge and wisdom I have gained through the years go far far beyond chemistry. One of the most valuable lessons I’ve learned is that a challenge can either make you or break you, but that choice is yours.
Publications

Dynamic interconversion of chiral oxime compounds in gas chromatography.

*Publications in preparation:*

Synthesis of novel 2,4,5-triphenylimidazolinium ionic liquids.
Authors: Chewe Chifuntwe, Philip J. Marriott, Helmut Hügel.

Synthesis of new per-2,3-O-acetyl-6-deoxy-6-(N-imidazolium/ or pyridinium) ionic cyclodextrins.
Authors: Chewe Chifuntwe, Philip J. Marriott, Helmut Hügel.

Synthesis of amino acid derived chiral imidazolinium ionic liquids
Authors: Chewe Chifuntwe, Philip J. Marriott, Helmut Huegel
Abstract

As the production of new chiral products such as drugs continually increases, the currently available CSPs (chiral stationary phases) are not guarantees to provide adequate enantioseparation for new products. With the increased demand from drug regulatory agencies for drug manufacturers to provide safety data by way of enantiomeric purity, there is a need for the development of more chiral selectors for application in GC/ or LC stationary phases for the analysis of chiral drug products.

Given that GC is one of the preferred methods of chiral analysis recommended by drug regulatory agencies, it is important to have a range of GC stationary phases which possess diverse physical and chemical properties to allow researchers to conduct not only routine chiral analysis but provide a selection of stationary phases with the appropriate properties required to conduct specialised enantioselective analysis experiments.

Ionic liquids have been identified as good candidates for application as stationary phases in GC. Their negligible vapour pressure, good thermal stability, multiple solvation interaction and their tuneable physical and chemical properties make them ideal candidates for application in the design of new stationary phases.

In this work the design, synthesis, and testing of novel chiral ionic liquids (ILs), for enantioselective capability in GC stationary phases is presented. First, new chiral cis- and trans-2,4,5-triphenylimidazolinium ILs are synthesised as well as their achiral 2,4,5-triphenylimidazolium IL counter parts. Following which the asymmetrical N-derivatisation of trans-2,4,5-triphenylimidazoline with various amino acids is described, yielding new 2,4,5-triphenylimidazolines, which upon alkylation with an alkylhalide served as precursors for the synthesis of novel chiral ILs. In addition to these 2,4,5-triphenylimidazoline bases ILs, we describe the synthesis of new asymmetrical chiral imidazolinium ILs with various side groups from simple amino acids. This method proved to be quite versatile, permitting the incorporation of various functional groups at positions 2, 3, 4, and 5 on the imidazoline moiety, allowing for the production of a wide range of imidazolinium ILs with tunable physical and chemical properties.

In addition to the imidazoline based ILs, new ionic cyclodextins (CD) were synthesised from per-6-iodo-2,3-hydroxy-β-CD, per-6-iodo-2,3-O-acetyl-β-CD, per-6-iodo-2,3-O-
acetyl-γ-CD to afford various \(\text{per-6-imidazolium-2,3-hydroxy-β-CD iodide, per-6-imidazolium-2,3-O-acetyl-β-CD iodide, and per-6-imidazolium-2,3-O-acetyl-γ-CD iodide}\) as well as their pyridium ionic CD counterparts.

A selection of these chiral ILs were incorporated into capillary columns as chiral selectors diluted in OV-1701, by static coating and evaluated their effect on phase polarity and their enantioselective capabilities. Despite the addition of IL to the relatively non-polar OV-1701 phase the resultant mixed phases remained relatively non-polar while displaying markedly altered retention behaviours for various analytes.

A good example of the need for a wider selection of chiral stationary phases which possess a variety of chemical properties for specialized applications was illustrated in the study we conducted with chiral oximes which undergo dynamic molecular interconversion between their \(E\) & \(Z\) isomeric forms during the chromatographic elution process on wax stationary phases. The study was conducted on wax column coupled to a chiral column to allow the sequential examination of the interconversion process and chiral resolution. It would be ideal to examine the interconversion process and enantiomer resolution simultaneously, however, this would be achievable on a stationary phase capable of both chiral oxime resolution while simultaneously inducing the interconversion. Nonetheless, a column with such as phase is not currently available on the market, illustrating the need for the development of new chiral stationary phases (CSPs) for both routine and specialised enantioselective analysis.
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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</tr>
<tr>
<td>AcOH</td>
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<td>ADME</td>
<td>ADME=absorption, distribution, metabolism, and elimination</td>
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<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
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<tr>
<td>AILs</td>
<td>Aprotic ionic liquids</td>
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<td>Benzoyl-</td>
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<td>CDCl₃</td>
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<td>Capillary Electrophchromatography</td>
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<td>Central nervous system</td>
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<td>Carbon dioxide</td>
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</tr>
<tr>
<td>δ</td>
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</tr>
<tr>
<td>°C</td>
<td>Degrees celcius</td>
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<td>Detector-2</td>
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<td>Dimethylsulfoxide</td>
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<td>Deoxyribonucleic acid</td>
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<td>DNB</td>
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<td>Entgegen</td>
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<td>Ethanolammonium nitrate</td>
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<td>(E_i’)</td>
<td>activation energy</td>
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<td>Enzyme</td>
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<td>ES</td>
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<td>EWG</td>
<td>Electron withdrawing group</td>
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<td>Grams</td>
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<td>Gas chromatography quadrupole mass spectrometry</td>
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<td>Gas-solid chromatography</td>
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<td>H</td>
<td>Plate height</td>
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<td>HETP</td>
<td>Height Equivalent to One Theoretical Plate</td>
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<td>HH-COSYGPS</td>
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<td>Hours</td>
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<td>IBS</td>
<td>Imidazoline binding sites</td>
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<td>i.d</td>
<td>Internal diameter</td>
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<td>IL</td>
<td>Ionic liquid</td>
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<td>Ionic liquids</td>
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<tr>
<td>J_{HH}</td>
<td>Hydrogen Nuclear spin-spin coupling</td>
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<td>Definition/Abbreviation</td>
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<td>$^2J$, $^3J$, $^4J$, $^5J$</td>
<td>Nuclear spin-spin coupling through 2, 3, 4, and 5 bonds</td>
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<td>$k$</td>
<td>Retention factor</td>
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<td>LB</td>
<td>Lewis base</td>
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<td>LC</td>
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<td>LMSC</td>
<td>Longitudinal modulation cryogenic system</td>
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<tr>
<td>m</td>
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<tr>
<td>mbar</td>
<td>Millibar</td>
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<tr>
<td>$M^+$</td>
<td>Positively charged molecular mass</td>
</tr>
<tr>
<td>$M+H^+$</td>
<td>Positively charged molecular mass plus hydrogen</td>
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<td>Me</td>
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<td>Minute</td>
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<td>Minutes</td>
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<tr>
<td>$\mu$m</td>
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<td>MIPs</td>
<td>Molecular imprinted polymers</td>
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<td>mL</td>
<td>Millilitre</td>
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<td>ml/min</td>
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<td>mm</td>
<td>Millimeters</td>
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<td>MOPs</td>
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<td>Non-$N$-methyl-D-aspartate receptors</td>
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<td>Nuclear Magnetic Resonance</td>
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<td>p</td>
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<td>PA-CD</td>
<td>per-2,3,6-acetyl-$\beta$-CD</td>
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<td>PEMFC</td>
<td>Polymer electrolyte membrane fuel cells</td>
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<td>PILs</td>
<td>Protic ionic liquids</td>
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<td>PM-CD</td>
<td>per-2,3,6-methyl-$\beta$-CD</td>
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<tr>
<td>ppm</td>
<td>Part per million</td>
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<tr>
<td>psi</td>
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<tr>
<td>PVP</td>
<td>Polyvinylpyridine</td>
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<td>Poly-4-vinylpyridine</td>
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<td>4-PVP-XL</td>
<td>Poly-4-vinylpyridine, 2% crosslinked, methyl chloride quaternary salt</td>
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<td>Quartet</td>
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<td>Quintet</td>
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<td>$\mu$L</td>
<td>Microlitre</td>
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<tr>
<td>$R$</td>
<td>Rectus (right)</td>
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<tr>
<td>$R_s$</td>
<td>Resolution</td>
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<td>$R_r$</td>
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<td>S</td>
<td>Sinister (left)</td>
</tr>
<tr>
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<td>-------------</td>
</tr>
<tr>
<td>S&lt;sub&gt;N&lt;/sub&gt;2</td>
<td>Bimolecular nucleophilic substitution</td>
</tr>
<tr>
<td>S</td>
<td>Singlet (in NMR) or seconds (elsewhere)</td>
</tr>
<tr>
<td>S</td>
<td>Substrate</td>
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<td>SFC</td>
<td>Supercritical Fluid Chromatography</td>
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<td>Sextet</td>
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<td>T</td>
<td>Temperature</td>
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<td>t&lt;sub&gt;SZ&lt;/sub&gt;</td>
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<tr>
<td>W</td>
<td>Watts</td>
</tr>
<tr>
<td>WCOT</td>
<td>Wall coated open tubular</td>
</tr>
<tr>
<td>w&lt;sub&gt;h&lt;/sub&gt;</td>
<td>Peak width at half height</td>
</tr>
<tr>
<td>Z</td>
<td>Zusammen</td>
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<thead>
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"The universe is asymmetric and I am persuaded that life, as it is known to us, is a direct result of the asymmetry of the universe or of its indirect consequences. The universe is asymmetric."

*Louis Pasteur*
1 Chapter 1

*General introduction*
1.1 The mystery of chirality

1.1.1 Introduction to chirality

One of the most remarkable facts in biology is that the biomolecular chirality, be it in a virus, in a bacterium, or in a human brain cell, is everywhere the same. All cells contain DNA in the form of a right-handed double helix, all proteins consist of L-amino acids, and carbohydrates are derived from D-sugars, where ‘L’ stands for laevum, meaning ‘left in latin and ‘D’ signifies dextrum, ‘right. These very complex molecules that make up a living organism, such as DNA, RNA, proteins, and sugars, are thus all chiral. RNA contains the carbohydrate moiety D-ribose, and DNA D-2-deoxyribose. Life on earth is based on enantiomeric biomolecules. On the other hand, left handed DNA do not occur naturally in nature. While D-amino acids and L-sugars only occur in trace amounts in nature.\(^1\) This remarkable selectivity is called biological homochirality. And it is this phenomenon that drives research into chiral molecules in the world of synthetic chemistry.

From the chemical standpoint, a chiral molecule and its enantiomer should, under identical external conditions have exactly the same energy. In a thermodynamic equilibrium with their surroundings, both enantiomers would consequently have the same probability of existing. However, from a particle physics aspect, it must be taken into

\(^1\) Life on earth is based on enantiomeric biomolecules.
account the elementary particle interactions called parity violating weak forces, whereby it has been suggested that there must exist a very small energy difference favouring one chiral form with respect to the other, resulting in the observed biological homochirality.

Pasteur postulated that the peculiar selectivity of living processes for one or the other of enantiomeric forms of the same molecule might be the manifestation of asymmetric forces of the environment acting upon the living organism during the synthesis of protoplasmic constituents.\textsuperscript{2,3} As we know on the Earth, the biological systems are based on D-sugars and L-amino acids rather than L-sugars and D-amino acid.\textsuperscript{4}

![A-Form and B-Form DNA](image)

**Figure 1.1:** A- and B-DNA in the form of a right-handed double helix.

### 1.1.2 The discovery of natural optical activity

In a chiral medium, the plane of polarization of linear polarized light is rotated. This phenomenon is called natural optical rotation or natural optical activity. The angle of rotation is specific for the molecular property of the medium. In enantiomeric medium
under the same conditions, the angle of rotation is exactly opposite for each enantiomer. This very fundamental effect was discovered by two French scientists in the early 19th century.\textsuperscript{5}

In 1811 Francois Arago noticed optical rotation in slabs of α-quartz. The observation that optical activity not only is a property of a particular crystals, but that it occurs in liquids, for instance, in sugar solutions, was demonstrated four years later by Jean-Baptiste Biot. The angle of rotation is measured with a polarimeter.

**Figure 1.2: Portrait of Louis Pasteur\textsuperscript{6}**

Arago’s and Biot’s discovery in the early 19th century stimulated much research into the optical properties of matter. An important breakthrough for the future development of chemistry was achieved in 1848 by Louis Pasteur. He investigated solutions of sodium ammonium tartarate that were indifferent to polarized light (not optically active). Letting the solutes crystallize, Pasteur discovered that the crystals turned out to be hemihedral, indicating that they were chiral. Some crystals turned out to be hemihedral to the right, others hemihedral to the left, showing the presence of both enantiomorphous forms. Selectively redissolving the right handed crystals, Pasteur found the new solutions rotated the plane of linearly polarized light to one side, while the solution made from left handed crystals rotated the plane of linearly polarized light to the other. The original, optically inactive solutions were racemic, containing equal amounts of both enantiomers of the compound, achieving the first resolution of a racemic mixture into its chiral components. Pasteur was fortunate enough to have found the perfect sample molecules. However, racemic solutions often
form racemic crystals,⁷ and the resolution of the enantiomers has to be carried out by other procedures.

1.1.3 Enantioselective separation of racemic mixture into isolated enantiomers.

If one is as fortunate as Pasteur was with the tartaric salt, and the racemic mixture crystallizes forming distinguishable chiral, enantiomorphic crystals, one may separate the crystals by inspection to obtain the molecular enantiomers. Unfortunately, many racemic solutions under laboratory conditions form racemic crystals.⁷ Furthermore, if chiral crystals indeed are formed, the procedure of manually selecting these crystals enantioselectively is tedious and does not lend itself well to separations on a large scale.

One method of resolution relies on selective absorption to an external chiral medium by chiral chromatography. The chromatographic material (stationary phase) must be chemically inert, yet contain numerous, asymmetrically configured polar groups. Carbohydrates polymers such as cellulose or cyclodextrin, or macromolecules like chiral crown ethers, lend themselves well to this purpose.
Figure 1.3: Sodium ammonium tartrate salt crystals; (a) an illustration of the left and right handed salt crystals, (b) large crystals formed by seeding method (Left, (−)-enantiomer; right, (+)-enantiomer).
1.2 Chirality in drug design and development

1.2.1 Introduction

Chiral drugs are a group of drug substances that contain one or more stereogenic centers. More than one half of marketed drugs are chiral. It has been well established that the opposite enantiomer of a chiral drug often differs significantly in its pharmacological, toxicological, pharmacodynamic, pharmacokinetic properties. Therefore from the points of view of safety and efficacy, the pure enantiomer is preferred over the racemate in many marketed dosage forms. However, chiral drugs are often synthesized in the racemic form, and it is frequently costly to resolve the racemic mixture into the pure enantiomers. The decision whether to market the raceme or the enantiomer of a chiral drug is mainly based on pharmacology, toxicology, and economics. From a pharmaceutical perspective, the physical properties of both the racemate and the enantiomer need to be characterized in detail in order to develop a safe, effective and reliable formulation, no matter whether the racemate or the enantiomer is chosen as the marketed form.

1.2.2 Drug Stereochemistry

1.2.2.1 Stereoselectivity in Drug Action.

Stereoselectivity in drug action has been known since the early years of the last century, but apart from a relatively few instances, it was not understood and hence overlooked between the 1950s and the early 1970s, a time period stamped as the golden age of drug discovery and development. As a result of this neglect, by the late 1980s 25% of the products available in a survey of 1675 drugs were racemic. However, over the last 15 to 20 years there has been a change in safety regulations with respect to chiral pharmaceuticals. This change has catalysed medicinal chemistry research advances in methodology associated with the stereoselective synthesis and stereospecific analysis of chiral drug molecules, coupled together with an increased appreciation of the potential for significant differences in biological properties of the enantiomers of chiral drugs.
administered as racemates. As a consequence of the advances in technology and increasing safety concerns,\textsuperscript{16} drug chirality has become the highest priority for both the regulatory agencies and the pharmaceutical industry,\textsuperscript{17} driving a move toward the development of single stereoisomer products.\textsuperscript{9,17,18}

1.2.2.2 Biological Discrimination of Stereoisomers

The differential biological activity of stereoisomers is a long known phenomenon that was not previously understood. In 1858, Pasteur demonstrated that the mold \textit{Penicillium glaucum} metabolized (+)-tartrate more rapidly than the (-)-enantiomer.\textsuperscript{19} This was followed in 1886 by Piutti’s observation that (+) asparagine had a sweet taste whereas the (-)-enantiomer was insipid (Figure 1.4). Following Puitti’s report, Pasteur made the remark that “the nervous tissue might itself be dissymmetric,” an observation regarded as the first mention of stereoselectivity of a receptor.\textsuperscript{19} These differences in taste have been established for other amino acids where by the D-isomers are sweet while the L-isomers are either bitter or tasteless.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(+/-) of tartrate and asparagine}
\end{figure}

The $\alpha,\beta$-unsaturated terpenoid ketones ($R$)- and ($S$)-carvone were isolated and shown to have odors of spearmint and caraway, respectively (Figure 1.5).\textsuperscript{20-22} Similarly, the related terpine enantiomers ($R$)- and ($S$)-limonene have odors of orange and turpentine respectively.\textsuperscript{20} This physiological sensivity to chirality is nicely demonstrated by the olfactory sense.
The interaction between a drug and a receptor is associated with bonding interactions between the functionalities in the drug and complementary sites on the receptor, the three-dimensional spatial arrangement of such functionalities being of considerable significance. It was postulated by Easson and Stedman\(^\text{23}\) that the differences in activity arise as a result of differential binding of the pair of enantiomers to a common site, resulting in a difference in the fit of the enantiomers to the receptor and, the total energy of the interaction.

Chiral recognition is a fundamental molecular phenomenon. Chiral recognition models continue to be a matter of considerable interest, not only with respect to biological activity but also with molecular recognition phenomena in chemistry and particularly the separation sciences.\(^\text{24-27}\)
1.2.2.3  Eudismic Ratio and Enantiomeric Purity

The differential pharmacodynamic activities of drug stereoisomers are described as being either the eutomer or distomer. The enantiomer with the greater affinity, or activity, is termed the eutomer, whereas that with the lower affinity or activity is the distomer. The eudismic ratio (ratio of affinities or activities, of eutomer to distomer) refers to a single activity of a drug and it is important to recognize that for a dual action drug the eutomer for one activity may be the distomer for the other.

Determination of the eudismic ratio is dependent on the stereochemical purity of the material under examination. This is particularly true in the case for the less active enantiomer, and as the eudismic ratio increases, the significance of trace quantities of the eutomer as an impurity of the distomer also increases. Enantiomeric purity is frequently not reported in the pharmacological literature, and when presented, is presented in terms of optical rotation, particularly in the older literature, which is not a sensitive technique at levels of enantiomeric contamination of a few per cent. Even if values of the specific rotation of a given analyte are known, then experimental factors such as temperature, solvent, and wavelength need to be reproduced to ensure that accurate and comparable data is obtained.\(^{17}\) An example of the significance of stereochemical purity may be illustrated by a consideration of the activity of the selective \(\beta_2\)-adrenoceptor agonist Formoterol (Figure 1.7).\(^{28}\) This compound contains two stereogenic centers in its structure, and therefore four stereoisomers are possible. Initial investigations indicated that the \(\beta_2\)-agonist activity resided in the \(R,R\) stereoisomer with a ranking order of potency of \(R,R > R,S > S,S >> S,R\). Subsequent investigations reported much greater differences, the eudismic ratio \(R,R/S,S\) increasing from 50 to 850 when the impurity of the eutomer in the distomer decreased from approximately 1.5 % to <0.1 %. And, the increased stereoisomeric purity resulted in an altered ranking order of potency ratio to \(R,R>>R,S > S,R >> S,S\).\(^{29}\)
Having seen examples of the effect of enantiomeric impurities in determining eudismic ratios we can see the importance of the continuous development of stereospecific analytical methodologies in the separation sciences, such as gas chromatography. With these developments in analytical methodology, it should be considered unacceptable to present pharmacological data on enantiomers without quoting their stereochemical purity.

![Formoterol](image)

**Figure 1.7**: Formoterol

### 1.2.2.4 Pharmacodynamic complexities

There are relatively few examples of drugs in which the pharmacodynamic activity is restricted to a single stereoisomer, and its enantiomer being totally devoid of activity. Similarly, there are few examples where the required beneficial activity resides in a single stereoisomer and the adverse effects, or toxicity, reside in its enantiomer. Instances are also known where the activity of a pair of enantiomers differs sufficiently that both are marketed with different therapeutic indications. It is obvious that the evaluation of the activity of a racemate does not provide a clear indication of the properties of the drug and it is essential to separate the enantiomers to evaluate the pharmacodynamic activities of each stereoisomer.

### 1.2.3 Drug Metabolism and toxicology

#### 1.2.3.1 Thalidomide and related teratogenic agents

A compound frequently cited, as an example where the use of a single stereoisomer would have prevented a tragedy, is the teratogenic hypnotic sedative agent, thalidomide
An investigation in the late 1970s reported that following administration of the individual enantiomers to mice, both enantiomers possessed hypnotic activity, whereas the teratogenic activity resided solely in the S-enantiomer.\textsuperscript{30} However, studies in a more sensitive test species, New Zealand White rabbits, indicated that both enantiomers, and the racemate, were teratogenic,\textsuperscript{31} while further research found the drug to readily undergo racemization in biological media.\textsuperscript{32-34} These findings indicated that even if the single R-enantiomer had been commercially available in the early 1960s, patients would still have been exposed to both enantiomers of the drug as a result of its racemization. Thalidomide is therefore not a particularly useful example to cite in support of single stereoisomer products. But rather an example of the great need for chromatographic separation technologies that allow the investigation of the effects of not only the individual enantiomers but rather the analysis of metabolite enantiomeric compositions as well. In seeing this demand we strive to advance research into developing new chiral stationary phases to make such analysis possible by providing a new range of separation media, in the way of ionic liquids, with chiral selective properties.

![Thalidomide](image)

**Figure 1.8:** Chemical structure of Thalidomide

 Creating Chiral Molecular Environments in Synthesis and Separation

The enantioselective biochemical synthesis of enantiopure products as produced in nature is an ideality that is rarely achieved in reality in the laboratory. The necessity to obtain and deliver enantiomerically pure pharmaceuticals and chemicals has produced an enormous growth in fields such as asymmetric synthesis, asymmetric catalysis, and in separation science. In asymmetric catalysis or in enantiomeric discrimination there are a
limited number of generic or key activation modes that are important in understanding the interactions of catalysts or chiral selectors with their substrates or analytes. For example in the enantioselective bromocycloetherification of olefins 1, the combination of an achiral Lewis base (LB) and a chiral Bronsted acid (BA*) are the key activators that afford reasonable product enantioselectivities in the cyclization of $Z$ or $E$ configured 5-arylpentenols to tetrahydrofuran derivatives. The chiral complex formed by the hydrogen bonding of the BINOL-related chiral phosphoric acid catalyst to the Br$^+$ electrophile and to the hydroxyl group of the pentenol, reacted with the alkene nucleophile and facilitated the enantioselective bromoetherification to give the anti-Markovnikov 5-exo chiral ether cyclization products as outlined in Figure 1.9.

![Figure 1.9: Enantioselective synthesis of arylbromomethyl tetrahydrofurans](image)

The consequent task of product enantiomeric analysis is quite challenging because of the small differences in reactivity or binding energies between the enantiomers. The analysis and determination of enantiomeric composition and concentration of the products mainly relies on separation-based techniques such as gas chromatography, high-performance liquid chromatography and capillary electrophoresis.
1.3 Chiral analysis techniques

1.3.1 Enantioseparation by Gas Chromatography

Although pharmacologists have long been aware of the pharmacological enantioselectivity of drug action, pharmacokineticists became focused on the possibility of stereoselectivity in drug disposition only in the past 25 years. Part of this delayed response was caused by limitations in analytical chemistry. With the early development of gas chromatography and high performance liquid chromatography, significant inroads into quantitative determination of xenobiotics in biological specimens were made, (Xenobiotics being substances such as antibiotics used in a species in which they are not naturally produced). A variety of methods based on chromatographic techniques such as Supercritical Fluid Chromatography (SFC), Thin Layer Chromatography (TLC) have been developed during the past three decades, and more recently, Capillary Electrophoresis (CE), Capillary Electrochromatography (CEC) and Countercurrent Chromatography (CCC) have also been shown to be useful techniques for the purpose of separating enantiomers.

Analytical separation of enantiomers can be achieved by the use of two quantitative chemistry approaches, to facilitate the physical separation of enantiomers. One approach involves molecular modification of the enantiomers by derivatization with an enantiopure reagent. In so doing, the enantiomers are converted to diastereomers, which possess different physicochemical properties and which can then be separated using conventional chromatography. The second method involves changing the molecular environment to interact with the enantiomers during the separation procedure. This is facilitated by use of special chromatographic columns which consist of chiral materials that confer a three dimensional structure to the stationary phase. These stationary phases consist of enantiopure compounds bonded or coated onto the chromatography column. As the racemic mixture passes through such a column, the enantiomers interact differently with the stationary phase resulting in separation. These columns have become increasingly popular over the past decade as their reliability and durability have improved.
1.3.2  Chiral stationary phases for Gas Chromatography

1.3.2.1  Introduction

The heart of gas chromatography resides in the column which contains the stationary phase. The stationary phase functions as the separation medium. For chiral analysis, chiral stationary phases (CSPs) are used to separate enantiomers. Today, the most popular columns are open tubular capillary columns made of fused silica with the stationary liquid phase coated on the inside surface of the capillary wall as shown in Figure 1.10.

![Figure 1.10: An illustration of an open tubular capillary columns made of fused silica with the stationary liquid phase coated on the inside surface of the capillary wall.]

Chiral stationary phases use chiral selectors to partition the enantiomers (the mechanism associated with enantiomer resolution is discussed in detail in Section 1.3.2.2). The selector is either attached to or mixed with the stationary phase, to produce a chiral stationary phase (CSP). The enantiomers are introduced in the mobile phase and move at slightly different speeds according to their binding constants with the chiral selector as
they partition between the mobile phase and the CSP. It is the culmination of these interaction mechanisms which is known as enantiomeric resolution. And these interactions vary according to the molecular structure of both the chiral selector and the ligand (or analyte).

### 1.3.2.2 Chiral recognition mechanisms

#### 1.3.2.2.1 Three-point interaction model

The key step in chiral recognition is the formation of non-covalent diastereoisomeric complexes between the enantiomers and a chiral selector. Molecular recognition results from the differences in Gibbs free energy between the two diastereoisomeric enantiomer–selector complexes. Naturally biologists were the first to be interested in the chiral recognition mechanisms. While working on quantitative structure–activity relationships, Easson and Stedman, in 1933, proposed that a minimum of three points of attachment were needed between a asymmetric drug and its target to elicit the different physiological activities observed.\(^{36}\) Fifteen years later, another biologist, Ogston used the three-point model in his work on chiral enzymatic reactions.\(^{37}\) And Dalgliesh followed by adapting the same model to TLC.\(^{38}\) The model explains the differential binding of the two enantiomers to a chiral three-point site on the selector (Figure 1.11).
**Figure 1.11**: Easson-Stedman Model of drug-receptor interaction or 3-point interaction model, the above stereoisomer represents 3 points of interaction simultaneously, while the other enantiomer is only capable of 2-points of interaction simultaneously, irrespective of orientation to the binding site.

The original three-point model only involved attraction interactions at all three points. From a separations point of view, repulsion and attraction forces are considered opposites. However, from a stereochemical aspect, repulsion is considered as productive an interaction as attraction. For example, two of the interactions can be repulsive if the third interaction is strong enough to promote the formation of at least one of the two possible diastereoisomeric selector–ligand complexes.\(^{39}\) The key points in the three-point interaction model are that at least three simultaneous interactions are required and that they should occur with three different substituents attached to the stereogenic center. Although widely accepted, the model has been challenged.\(^{40,41}\) Additional investigations,\(^{42,43}\) resulted in the conclusion that the Easson–Stedman hypothesis only holds for sites of direct action.\(^{42,43}\)
Sokolov and Zefirov developed the Ogston enzyme in which they described a “rocking tetrahedron” model. In this model, enantioselectivity is examined in dynamic terms rather than the static terms. In the rocking tetrahedron model the substrate binds to the enzyme via two interactions and has conformational flexibility. The two enantiotopic groups (A and A*, Figure 1.12) occupy overlapping but identical volumes, and the enantioselectivity of the enzymatic transformation is dependent on the orientation and interaction between the active site and the “volume”. If the interaction is at right angles to the plane of the substrate (from point X, Figure 1.12, then enantioselectivity is not observed, whereas if the interaction is in the plane (e.g., from point Y), the process is potentially highly enantioselective, or enantiospecific. Thus in compounds with “small” highly flexible enantiotopic functionalities, minimal stereoselectivity would be observed. In contrast, if the enantiotopic groups are large bulky substituents, which are conformationally less flexible, then greater selectivity, tending towards specificity, is expected.

\[ \text{Figure 1.12: “Rocking tetrahedron” model} \]

Chiral recognition models continue to be a matter of considerable interest, not only with respect to biological activity but also with recognition phenomena in chemistry and particularly the separation sciences. The rocking tetrahedron concept has been expanded to a conformationally driven chiral recognition mechanism by Booth et al., in terms of
chromatographic recognition. They proposed a four stage chiral recognition process. The initial step involving the formation of a selectand–selector complex (tethering), followed by a conformational change of the complex in order to optimize the molecular interactions, complex activation via additional interactions and finally an expression of molecular fit, that is, chiral recognition. They proposed that this general process describes enantiomeric discrimination for all cases of chiral selection. In recent years there has been extensive discussion as to the minimal requirements for chiral recognition. Current consensus appears to be that a diastereomeric intermediate must be involved, and that interactions between eight centers (and hence a four-point attachment) are required.

1.3.2.2 Intermolecular forces involved in enantiomer-selector complexation interactions.

Table 1.1 lists a range of intermolecular forces that can exist between two enantiomers and a chiral selector in the stationary phase. Coulomb force are the strongest interaction, this is well illustrated by the high cohesion of salts. The hydrogen bond interactions are also very strong forces, they occur between a positively polarized hydrogen atom of a hydroxyl or amine group and a negatively polarized oxygen or nitrogen atom. Steric hindrance is another strong force which result from the intrinsic room needed per atom or group of atoms; they are repulsive in action and very strong at very short range. \( n-n \) interactions are observed when \( n \)-electron molecular assemblies, mainly aromatic rings, interact with each other. The \( n-n \) interactions involved in chiral recognition mechanisms are most often attractive; a \( n \)-accepting group of the enantiomer interacts with a \( n \)-donating group of the selector, or vice versa. Ion–dipole, dipole–dipole, and dipole–induced-dipole interactions occur with molecules that have a dipole moment. The strongest force being ion–dipole interactions which involve the attractive coulomb force from the ion and the partial charge from the dipolar molecule. The weakest interactions are dipole-induced dipole forces which occur between a permanent dipolar molecule and a dipole induced by the electric field. The weakest intermolecular forces are London
dispersion forces. They are responsible for hydrophobic effects and for entropy driven forces that cause oil to separate from water.

Table 1.1: Characteristics of molecular interactions.

<table>
<thead>
<tr>
<th>Type of interaction</th>
<th>Strength</th>
<th>Direction</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulomb or electric</td>
<td>Very strong</td>
<td>Attractive or repulsive</td>
<td>Medium (1/d^2)</td>
</tr>
<tr>
<td>Hydrogen bond</td>
<td>Very strong</td>
<td>Attractive</td>
<td>Long</td>
</tr>
<tr>
<td>Steric hinderance</td>
<td>Very strong</td>
<td>Repulsive</td>
<td>Very short</td>
</tr>
<tr>
<td>(n-n)</td>
<td>Strong</td>
<td>Attractive or acceptor or repulsive</td>
<td>Medium</td>
</tr>
<tr>
<td>Ion-dipole</td>
<td>Strong</td>
<td>Attractive</td>
<td>Short</td>
</tr>
<tr>
<td>Dipole-dipole</td>
<td>Intermediate</td>
<td>Attractive</td>
<td>Short (1/d^3)</td>
</tr>
<tr>
<td>Dipole-induced dipole</td>
<td>Weak</td>
<td>Attractive</td>
<td>Very short (1/d^6)</td>
</tr>
<tr>
<td>London dispersion or Van der Waals</td>
<td>Very weak</td>
<td>Attractive</td>
<td>Very short (1/d^6)</td>
</tr>
</tbody>
</table>

1.3.2.2.3 Designing chiral selectors.

Chiral selectors can be separated into two subgroups, synthetic chiral selectors and the natural chiral selectors. The ability to synthesise various chiral selectors permit researches to study interaction mechanisms (Table 1.2) and consequently design a chiral selector that will interact preferentially with one enantiomeric form than with its mirror image. Chiral selectors derived by natural routes are based on the fact that the living world contains numerous chiral molecules which can function as chiral selectors and are
produced as pure enantiomers. Once a natural product is chosen for application as a chiral selector, the natural chiral selector is tested with its natural chiral target(s) and many other enantiomers, to derive information pertaining to the molecular interactions involved, and the data used to postulate the mechanisms involved in chiral recognition. However, neither of these classes of selectors are 100% synthetic or natural. These chiral selectors are actually semisynthetic; because many synthetic selectors are based on a natural product and many natural selectors are chemically modified to enhance their initial properties (Table 1.2).

Most information on chiral recognition mechanisms is obtained by measuring the binding energy of the two chiral selector–enantiomer complexes. Multiple selector–ligand association–dissociation reactions occur in the CSP as the analytes partition between the mobile phase and the stationary phase. The enantiomers introduced into the mobile phase move at slightly different speeds through the mobile phase according to the sum of their binding constants with the chiral selector in the stationary phase. The retention time (or migration times) of the enantiomers provide their binding constants.

Researchers can observe the thermodynamics of chiral reaction mechanisms by varying study temperatures. The thermodynamic parameters, binding constant, and enthalpy or entropy changes correspond to the ligand–chiral selector association.

### 1.3.2.3 Chiral Stationary Phases

#### 1.3.2.3.1 A brief historical perspective of chiral GC and ILs stationary phases

- **1959** Barber *et al.*, describe the first separation by GC using ILs (molten salts).
- **1966** Gil-Av, Feibush and Sigler reported the first direct enantioseparation of a derivatised $\alpha$-amino acids by GC on a CSP containing the involitile $\alpha$-amino acid derivative $N$-trifluoroacetyl-$L$-isoleucine lauryl ester. Organic ILs of the type currently
used today were used as GC stationary phases by Gordon et al. in 1966, however, little was known of their general properties.\textsuperscript{52}

- **1977** Frank, Nicholson and Bayer developed Chirasil-L-Val, by bonding a valine diamine chiral selector to a copolymer consisting of carboxy-alkylmethylsiloxane and dimethylsiloxane, officially combining the desirable properties of silicone (renowned for producing high resolution and efficiencies) and the enantioselectivity of the chiral selector.\textsuperscript{53} The result was a thermally stable, non-volatile, enantioselective chiral polysiloxane-valine diamide CSP.\textsuperscript{53} Schurig provides an excellent account of the historical development, optimization and utilization of GC analysis of derivatized α-amino acids on CSP.\textsuperscript{51}

- **1980s** Cyclodextrins [CDs] and derivatives considered the most versatile and efficient chiral selectors for the enantiomeric separation\textsuperscript{54-56} of volatile and thermally stable compounds.

- **1980-1990s** The unique properties of ionic liquids plus their solubilizing properties were shown to be beneficial for GC\textsuperscript{57} and many other areas of separation technology.

- **2000-12** Various chiral materials such as: ionic liquids, polymeric ionic liquids, ionic-CDs, chiral metal organic frameworks are under investigation as CSPs.

The chemical asymmetric synthesis impetus is driven by the established science that individual enantiomers of chiral compounds exhibit unique biological, pharmacological and physical responses. In achiral media, enantiomers show identical physical and chemical properties, so scientists have developed and utilized natural and synthetic chiral materials and compounds to facilitate enantiomer separation. Table 1.2 lists the classes of chiral selectors used in chiral stationary phases, the mechanism involved in enantiomer resolution and the primary intermolecular interaction involved in the enantiomeric-complexation mechanism.
Table 1.2: Chiral selectors and their mechanisms of interaction.

<table>
<thead>
<tr>
<th>Class of Selector</th>
<th>Chiral Selectors</th>
<th>Mechanism</th>
<th>Primary interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic selectors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ligand exchange</td>
<td>Amino acid chiral selectors and their derivatives.\textsuperscript{58,59} Metal ions Cu(II), Co(II), Cd(II), Ni(II), Zn(II).\textsuperscript{59}</td>
<td>Diasteromeric selector-metal ion-analyte complexation</td>
<td>Coulomb or ion-dipole</td>
</tr>
<tr>
<td>Molecular imprinted polymers (MIPs)</td>
<td>Amino acids\textsuperscript{60} amino acid derivatives,\textsuperscript{61,62} peptides,\textsuperscript{63} carboxylic acids,\textsuperscript{64,65} \beta-blocking,\textsuperscript{66,67} cinchona alkaloids,\textsuperscript{66,67} NSAIDs,\textsuperscript{68,69} and benzodiazepines.\textsuperscript{70}</td>
<td>Key and lock association</td>
<td>Selective shape interaction with imprint</td>
</tr>
<tr>
<td>Chiral crown ethers</td>
<td>Derivatized forms of polyoxyethylene crown-6.\textsuperscript{71}</td>
<td>Inclusion complex, steric and hydrophobic</td>
<td>Ion-dipole</td>
</tr>
<tr>
<td>Polymers</td>
<td>Polyacrylamide and Poly(methacrylamide),\textsuperscript{72} Poly(trphenylmethylmethacrylate)\textsuperscript{73}</td>
<td>Diastereomeric selector-analyte complex</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>Natural selectors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>Bovine serum albumin (BSA),\textsuperscript{74} Human serum albumin (HSA),\textsuperscript{75} Ovomucoid from chicken egg white,\textsuperscript{76} Trypsin\textsuperscript{77} and (\alpha)-Chymotrypsin\textsuperscript{78} Lysozyme\textsuperscript{79} and Pepsin\textsuperscript{80} Cellobiohydrolases\textsuperscript{81,82}</td>
<td>Multiple binding sites</td>
<td>Variable</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Cyclodextrin, Cellulose,\textsuperscript{32,33} chitosan,\textsuperscript{34,83} chitin,\textsuperscript{84} amylase and amylopectin\textsuperscript{85,86}</td>
<td>Insertion into helical structures, plus individual chiral carbohydrate monomers</td>
<td>Hydrogen bond, dipolar, or steric</td>
</tr>
<tr>
<td>Cyclodextrins (CDs)</td>
<td>Most common derivatives are methylated, acetylated, carboxymethylated and hydroxypropylated CDs</td>
<td>Inclusion complexation</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>Macrocyclic glycopeptides</td>
<td>Vancomycin,\textsuperscript{87} teicoplanin,\textsuperscript{88} Ristocetin A,\textsuperscript{89} and Avoparcin\textsuperscript{90} Thiostrepton\textsuperscript{67} and Rifamycin B\textsuperscript{87}</td>
<td>Multiple binding sites</td>
<td>Variable</td>
</tr>
<tr>
<td>Cinchona alkaloids</td>
<td>Ionic Quinidine, and Quinine (N)-derivatives\textsuperscript{91}</td>
<td>Ion pairing</td>
<td>Coulombs</td>
</tr>
</tbody>
</table>
1.3.2.3.2  Innovative chiral stationary phases for GC.

A snapshot of some innovative chiral stationary phases that are currently utilized or under investigation is presented in Figure 1.14.

![Figure 1.13: An outline of the innovative CSPs currently utilised and/or being investigated for GC enantiomeric analysis.](image)

Chiral metal-organic frameworks

A novel application of chiral metal organic frameworks (MOFs) as GC stationary phases has been reported to produce excellent enantiomer recognition ability towards a mixture of aldehydes, ketones, organic acids, amino acids, and alcohols. Crystal structure analysis revealed that the stationary phase possesses a 3-dimensional left-handed helical chiral channel framework with a 2.5 nm screw pitch, with the formula \([\text{Cu}(\text{sala})_n] \text{H}_2\text{sala} = \text{N-}(2\text{-hydroxybenzyl})\text{-L-alanine}\). The chiral recognition mechanism for the MOFs phase is reported to depend on surface interactions, primarily steric fit between the chiral framework and conformation of the analyte. Dispersion forces, dipole-dipole, and hydrogen bonding interactions are also said to play a role in the chiral recognition mechanism. Besides their excellent chiral recognition ability, these new types of GC CSP have many positive attributes which make them ideal candidates for application in
GC phases such as selective adsorption, high porosity, and high thermal and chemical stability. Further studies are being undertaken to exploit inherently chiral natural products to create function helical metal organic frameworks from $\gamma$-CD co-ordinated to alkaline metal cations. $^{93-95}$ $\alpha$-Cyclodextrin MOFs are also being developed with Rb$^+$ ions to afford left handed helical crystals with pores running through the structure. $^{96}$ The $\alpha$-CD rings in the CD metal-coordinated frameworks are co-ordinated to Rb$^+$ cations, with each Rb$^+$ ion binding to the $\alpha$-1-4-D-glucose units on either the primary or secondary face via C2, C3, C6, and ring oxygen atoms. $^{96}$

Amino acid derivatives

There is one popular non-cyclodextrin based CSP that is widely used, Chirasil-Val (Figure 1.15), supplied by Agilent Technologies (Santa Clara, California, USA). This phase was based on the work of E. Bayer's group, $^{53}$ it is one of the earliest commercially available CSPs, and yet it is still the most popular for the separation of D/L amino acids. $^{51,56}$ Chirasil-Val is prepared by covalently bonding L-Valine-tert-butylamine units to a polysiloxane backbone. $^{51}$ Although it is a versatile phase it has limited capabilities in other areas.

![Figure 1.14: Chirasil-Val phase available in the D or L enantiomeric form.](image)

In order to overcome some of the limitations of Chirasil-Val, particularly its inadequate separation of Proline, a new diamide chiral polymethysiloxane phase based on (S)-(\textendash)\textasciitilde t-leucine-(S)-1-(-)-(\textalpha-naphthyl)-ethylamide was synthesised and applied to the separation of N-pivaloyl derivatised amino acids. $^{55}$ This phase was shown to produced baseline separation of most proteinogenic amino acid N-pivaloyl derivatives, including Proline, but
was found to be unsuccessful at separating multi-functional amino acids such as, Asp, Glu, Orn, Lys and Trp.\textsuperscript{55} The same research group also introduced another Chirasil-Type CSP based on (S)-(\text{-})-t-leucine-(S)-(\text{-})-1-phenylethylamide for the separation of warfarin enantiomers following conversion to O-perfluoroacyl derivative.\textsuperscript{55} Another Chirasil-Type GC phase known as Chirasil-Calix was prepared by covalently bonding resorcinarenes, which contain L-valine-\text{tert}-butylamide moieties, to a dimethylpolysiloxane.\textsuperscript{55} However, this phase did not display any improvement in enantioselectivity when compared with chirasil-Val.

ChDA is a co-polymeric \((1R-\text{trans})-N,N'\text{-}1,2\text{-cyclohexylene-}bis\text{-}benzamide oligodimethylsiloxane \text{(ChDA)}\) developed for investigation as a new chiral stationary phase for GC.\textsuperscript{55} This phase exhibited high enantioselectivity for a broad spectrum of chiral molecules, however, it was limited to an operating temperature range of 110-260 °C. Such a high minimum operating temperature is disadvantageous to enantiomer resolution, because enantiomer resolution is known to take place at low temperatures (the lower the elution temperature, the greater selectivity differences observed between enantiomers). In addition this phase only showed high efficiencies at temperature above 150 °C, which is higher than the temperatures at which enantioreolution typically takes place.

Metal chelates

The design and synthesis of a diverse series of novel chiral metal-containing ionic liquids (CMILs) was reported by Song \textit{et al.},\textsuperscript{97} and used as chiral cocatalysts in the enantioselective cycloaddition of CO\textsubscript{2} to epoxides. These novel chiral salts consist of natural amino acid anions and crown ether-chelated alkali metal (potassium/ sodium) cations.\textsuperscript{97} They are reported to have melting points below than 200 °C and thermal stabilities ranging from 138 °C up to 222 °C.\textsuperscript{97} Application of these novel CMIL as GC stationary phases has not been reported and may prove to be potentially applicable to enantiomeric separation of chiral analytes such as amino acids.
Cyclodextrins

Separation techniques like HPLC employ a wide variety of stationary phases (such as crown ethers, macrocyclic antibiotics and proteins, among others), whereas cyclodextrins are ubiquitous to capillary GC. Cyclodextrins (CDs) are small cyclic polysaccharides that form a cone-shaped cavity with six, seven, or eight glucopyranose units known as α-, β-, and γ-CD, respectively. The interior is a hydrophobic cavity consisting of ether groups, while the outer rim of the cavity are lined with the polar hydroxyl groups making it hydrophilic. Inclusion complexation is the driving interaction in chiral recognition by CDs. Derivatization of these hydroxyl groups produced a wide variety of CDs with adjusted polarities and functionalities that can separate a broad spectrum of enantiomers. Several CD derivatives have been developed showing improved enantioselectivity in many cases and are available commercially (see Table 3.1). In addition to inclusion complexation, hydrogen-bondings, $n$-$n$ interactions, hydrophobic interactions and steric repulsion are assumed to be responsible for chiral recognition.

The two most popular CD chiral selectors employed in enantioselective GC are the 2,3-di-O-methyl-6-tert-butyldimethylsilyl-β-CD and the 2,6-di-O-pentyl-3-trifluoroacetyl-γ-CD. The 2,3-di-O-methyl-6-tert-butyldimethylsilyl-β-CD being the leading commercial CD derivative in the field from an application standpoint, and has largely replaced the straight 2-3-6-tri-O-methyl version. The tert-butyl group in the 6-position has little effect on chiral selectivity but provides improved solubility of the CD in the different polysiloxane carriers which are used. This chiral selector has been shown to provide ~60 % of all chiral separations possible on capillary GC phases. The maximum operating temperature of this phase is usually the highest of all the CD phases at 220–230 °C isothermal, and is extended to 250 °C when using temperature programing.

The 2,6-di-O-pentyl-3-trifluoroacetyl-γ-CD phase is produced by Sigma-Aldrich/Supelco (Bellefonte, Pennsylvania, USA). The mechanism for this phase is a surface dipole-
dipole interaction and as a result covers a broader area using the γ-CD as opposed to the β-CD. This phase is highly selective for oxygen-containing analytes, separating not only the widest variety of enantiomers, but also the greatest number within certain classes. Its main limitations are that its sensitivity to moisture and its isothermal upper temperature limit of 180 °C.

The mechanisms of separation of CD based stationary phases fall into two basic categories: surface interactions vs. inclusion complexation. Certain CD derivatives involve largely surface interactions, being more efficient and with higher capacity, while other CDs involve the inclusion complexation phenomena typically associated with cyclodextrins. Because the internal cavity of a cyclodextrin molecule tends to be more hydrophobic than its exterior, most nonpolar molecules (or molecules with nonpolar moieties) prefer to reside in the cyclodextrin cavity. This inclusion phenomenon in capillary GC is associated with the type and number of derivatives linked to the 3-position hydroxyl as opposed to the 2-position of the glucose units that make up the CD structure. When more polar groups are placed in the 3-hydroxyl position of the CD glucose unit, chiral interaction does not appear to involve the inclusion phenomena. These types of CSPs typically have higher efficiency, faster separations and capacities as high as 10–50 times greater than those relying on the inclusion phenomena.

Thermodynamic relationships and sample loading studies are used in the determination of the dominant chiral recognition mechanism. Enantiomers that have large $H^0$ and $S^0$ values also have low loading capacities, which is suggestive that inclusion complexes are involved in the enantioselective mechanism. Enantiomers that have smaller $H^0$ and $S^0$ values also have higher sample loading capacities, which is indicative that inclusion complexes play a less dominant, or negligible, role in the enantioselective mechanism. The involvement of surface interactions in chiral recognition mechanisms can be determined by observing the peak efficiencies of the enantiomers. It has been observed that peak efficiencies are much higher for those CD
derivatives that involve surface interaction mechanisms as opposed to an inclusion mechanism.\textsuperscript{56}

A complete understanding of the chiral recognition mechanism, would allow researchers to predict which selector would best separate the enantiomers of chiral compounds. However, given that a comprehensive understanding of the chiral recognition mechanism has not yet been realized, there is a need for the development of a wide range of chiral selectors to permit the separation of a wide variety of chiral compounds. This is the driving force behind research into the development of new classes of chiral selectors, such as ionic liquid selectors for application in stationary phases. Here in we discuss the properties of ionic liquids, their various applications, but most importantly, their application in Gas Chromatography, as well as the properties which make ILs good candidates for application in stationary phases.

### 1.4 Ionic liquids

Ionic liquids are defined as salts with melting points below 100 °C. Although the first observation of an ionic liquid occurred in 1914, it was the development of modern ionic liquids that really accelerated research in this area during the last decade.

![Figure 1.15: Typical IL synthesis reaction scheme.](image)

The more conventional ionic liquids are generally prepared in a two-step procedure from the corresponding amines in Figure 1.16. Alkylation leads to quaternization of the heteroatom and then anion metathesis can be performed if desired. Because they need to be prepared, RTILs are less environmentally friendly in this respect than many other alternative solvents. However, they are perfect solvents for many applications.
1.4.1 Ionic liquids and their properties.

Ionic liquids (ILs) can be composed from a large number of cations and anions. Knowledge of the physical properties of ILs is important for evaluating and selecting ILs for each application as well as process design. More recently, ILs have become very popular as potential solvents for industrial applications in different disciplines of science and environment. Although ILs have various fields of application our focus is on the synthesis of new chiral ionic liquids (CILs) for application in the field of analytical chemistry, more specifically in GC stationary phases. Indeed, because each IL has its own unique properties, it is possible to design an IL, to suit particular applications. We have taken advantage of this property of tunability to design chiral ILs for application as chiral selectors in GC stationary phases.
1.4.1.1 Effects of the structure on physical properties.

The physical–chemical properties of ILs depend on the nature and size of both their cation and anion constituents. Their application in science and industries is merited because ILs have some unique properties, such as a negligible vapour pressure, good thermal stability, tunable viscosity and miscibility with water, inorganic and organic substances, a wide electrochemical window, high conductivity, high heat capacity and solvents available to control reactions. Despite their wide range of polarity and hydrogen-bonding ability, these new solvents are liquid from -93 °C (glass transition) to ∼327 °C.

Possible choices of cation and anion allow the formation of numerous ILs. The most popular well-known classes of ILs are: imidazolium, pyridinium, pyrrolidinium quaternary ammonium, and tetra alkylphosphonium ILs. Of these, the most used in experimental laboratory work worldwide are undoubtedly 1,3-dialkylimidazolium salts, primarily due to their attractive and easy-tailored physical properties.

By changing the cation or anion, their solvent properties can differ considerably from one another as well as from traditional molecular solvents. Two different ILs that have essentially identical polarity ratings or descriptors can produce very different results when used as solvents for organic reactions, gas–liquid chromatography (GLC), or extraction. ILs with additional functional groups are capable of having additional interactions with other solvents or dissolved molecules. Due to the diversity of their structure and functionalities, they are capable of most types of interactions such as dispersive, n–n, n–π, hydrogen bonding, dipolar, and ionic/charge–charge interactions.

The viscosity of ILs is determined by van der Waals forces and hydrogen bonded structures. Electrostatic forces and the shift of charge at the anion may also play an important role. For the same cation (N-butyl-N-methylimidazolium), the viscosities for typical anions decrease in the order: [I]− > [PF6]− > [BF4]− > [TfO]− > [CF3CO2]− > [Tf2N]−. For a series of 1-alkyl-3-methylimidazolium cations with [PF6]− and [BF4]−
anions, increasing the alkyl chain length from butyl to octyl increases the hydrophobicity and the viscosity of the IL, while densities and surface tension values decrease.\textsuperscript{107} This results from the stronger Van der Waals forces between cations, leading to an increase in the energy required for molecular motion.\textsuperscript{107}

1.4.1.2 \textit{Melting point, glass transition, and thermal stability.}

Ionic liquids have been defined as having melting points below 100 °C and most of them are liquid at room temperature. Both, the cation and anion influence the melting points of ILs. The size and symmetry of the cation have a significant impact on the melting points of ILs. Generally, increasing the size of the anion and having an asymmetric substitution pattern leads to a decrease in the melting point. For the short chain alkyl substituents in 1,3-dialkylimidazolium salts, an increase in the alkyl chain decreases the melting temperature. The melting points of imidazolium salts also increase with the degree of chains branching.\textsuperscript{108}

Popular ILs are thermally stable up to around \textasciitilde427 °C.\textsuperscript{109} Thermal stability is limited by the same factors that contribute to the melting temperature.\textsuperscript{109} Usually, the difference in the decomposition temperature between particular salts with different cations is rather insignificant.\textsuperscript{109} Branching of the alkyl chain, as with the melting temperature, decreases the thermal stability of imidazolium ILs.\textsuperscript{110}

1.4.2 \textit{Various applications for ionic liquids.}

Ionic liquids (ILs) are solvents that have captured the interest of academia and industry. As a result ILs are currently being introduced into a number of industrial processes worldwide. The widespread interest in ILs has drawn scientists from all disciplines to study ILs resulting in the increase in knowledge and understanding of these unique solvents in the last decade. Because the properties (melting point, density, viscosity) and behaviour of the ILs can be adjusted to suit an individual processes, they can truly be
described as designer solvents. Thus, ILs have found applications in various processes including biological application as immunotoxins, protein renaturation and crystallization studies, \textsuperscript{111-116} biocatalysts, \textsuperscript{117} and as potentially useful antimicrobials. \textsuperscript{118,119} They have even found applications in bacterial endospore detection.\textsuperscript{120} Polymer membrane fuel cells are considered to be one of the most promising clean energy sources since they do not generate toxic gases and other hazardous compounds.\textsuperscript{121,122} Ionic liquids are also used in explosive formulations for use as propellants or explosives.\textsuperscript{123-125,126} They also have applications as Lubricants.\textsuperscript{127}

\subsection*{1.4.3 Applications in organic synthesis}

A wide range of ILs usually containing imidazolium cations have been used as the solvents and catalysts for a number of different organic reactions. There are major potential benefits in using ILs as solvents and catalysts for organic reactions. The main driving force for their use is the environmental advantage of using the relatively benign ILs to replace organic solvents and what are usually highly acidic catalysts.

The main application of these ILs is as catalysts in reactions that require a Lewis or Brønsted acidic catalyst or, more rarely, a basic catalyst.\textsuperscript{128-136} These ILs are typically easy and cheap to produce, can be easily recycled and reused, usually involve simple reaction processes, and often only produce one byproduct, water. In comparison, most traditional methods for these organic reactions involve large amounts of acidic waste, waste molecular solvents, and either waste catalyst or expensive and time-consuming catalyst-recycling procedures. The driving force behind the use of ILs in this field has been to find “greener” alternatives; hence, high conversions, high selectivities, and the ability to reuse the IL are important. No additional catalysts were required, and compared to conventional methods, yields were higher, the reaction times were shorter, less hazardous solvents were used, there were milder conditions, and the ILs could be reused.\textsuperscript{137,138}
In addition to the reactions listed in Table 1.3 Beckmann Rearrangement,\textsuperscript{139} Condensation reactions,\textsuperscript{137-145} Isomerization,\textsuperscript{146} Ether Cleavage,\textsuperscript{147} Chlorination,\textsuperscript{148} Nitration\textsuperscript{149} and Friedel-Craft alkylation\textsuperscript{150-152} reactions have also been reported to have also been successfully conducted using ILs as both catalysts and solvents.

More recently copper- and phosphine-free Sonogashira coupling reactions in biodegradable ionic liquids derived from nicotinic acid were reported by Harjani et al.\textsuperscript{153,154} Of particular interest was 3-\((n\text{-butoxycarbonyl})\)methylpyridinium bis(trifluoromethanesulfonyl)imide which proved to be biodegraded within 7 days of treatment with waste water organism.\textsuperscript{153} A number of typical 1,3-dialkylimidazolium based ILs have proven to be resilient to biodegradation, however, it was demonstrated that when an ester functionality was built into the imidazolium salt sidechain the resulting ILs biodegrade within 28 days.\textsuperscript{155,156}

In addition to the presumed environmental advantage of using relatively benign ILs to replace organic solvents and the use of catalysts, this characteristic of biodegradability\textsuperscript{156-158} bodes well for the application of ILs as “greener” reaction solvents/catalysts in organic synthesis.
Table 1.3: A list of organic reactions which use ILs as either solvents or catalyst.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Mechanism</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diels-Alder Reaction&lt;sup&gt;159&lt;/sup&gt;</td>
<td><img src="image" alt="Diels-Alder Reaction" /></td>
<td>solvents and catalysts</td>
</tr>
<tr>
<td>Henry Reaction&lt;sup&gt;160&lt;/sup&gt;</td>
<td><img src="image" alt="Henry Reaction" /></td>
<td>catalysts</td>
</tr>
<tr>
<td>Direct-Aldol condensation Reaction&lt;sup&gt;161&lt;/sup&gt;</td>
<td><img src="image" alt="Direct-Aldol condensation Reaction" /></td>
<td>basic catalyst</td>
</tr>
<tr>
<td>Mannich Reaction&lt;sup&gt;161&lt;/sup&gt;</td>
<td><img src="image" alt="Mannich Reaction" /></td>
<td>solvents and catalysts</td>
</tr>
<tr>
<td>Fischer Esterification&lt;sup&gt;150,162-165&lt;/sup&gt;</td>
<td><img src="image" alt="Fischer Esterification" /></td>
<td>solvents and catalysts</td>
</tr>
<tr>
<td>Dehydration of Fructose&lt;sup&gt;166&lt;/sup&gt;</td>
<td><img src="image" alt="Dehydration of Fructose" /></td>
<td>solvents and catalysts</td>
</tr>
<tr>
<td>Biginelli Reaction&lt;sup&gt;167,168&lt;/sup&gt;</td>
<td><img src="image" alt="Biginelli Reaction" /></td>
<td>solvents and catalysts</td>
</tr>
<tr>
<td>(Z)-to(E)-Alkene Isomerisation&lt;sup&gt;146&lt;/sup&gt;</td>
<td><img src="image" alt="Biginelli Reaction" /></td>
<td>solvents and catalysts</td>
</tr>
</tbody>
</table>

There are strong expectations for the use of ILs to increase in laboratory and industrial applications, as replacements for many organic solvents which are currently in use. In addition to their environmental benefits (non-volatile, re-usable and biodegradable), ILs also have a major advantage over conventional molecular solvents in the number of potential combinations of cations and anions that could be produced and, hence, the
ability of ILs to be designed for specific applications, one of which is as stationary phases in analytical chemistry.  

1.4.4 Application of ionic liquids as stationary phases in Gas Chromatography.

1.4.4.1 Introduction

ILs possess many unique physicochemical and solvation properties that can be varied and tuned for specific applications. It is this tunability that makes these ILs so desirable for a large variety of applications. In addition, most ILs possess negligible vapor pressure and high thermal stability, producing few volatile organic compounds. Because of their unique solvation properties ILs have been employed as replacements of traditional solvents. Furthermore, IL-based solvent systems typically exhibit enhanced reaction kinetics resulting in the efficient use of time and energy. The potential for producing unique task-specific ILs can be achieved by the simple modification of the cation and anion structure. The resulting ILs can be used to carry out designated applications or to serve a functional purpose in a method or device.

1.4.4.2 Chromatographic relationship between the structure and properties of ionic liquid as stationary phases for gas chromatographic separations.

In the field of gas chromatography (GC), new stationary phases that exhibit unique separation selectivity, high efficiency, and high thermal stability are in high demand and are particularly sought after. These properties have allowed ILs to function as stationary phases which are described as possessing unique dual-nature retention selectivity providing highly selective separations. Figure 1.18 is a good illustration of the “dual-nature” retention behaviour of IL stationary phases as described by Armstrong et al. The Figure 1.18 illustrates the comparison of the retention behaviour of 8 analytes on an IL phase (Figure 1.18 B) and a commercial phase (Figure 1.18 A) of identical dimensions and similar polarities, under identical
conditions.\textsuperscript{57} It was shown that non-acidic or basic nonpolar analytes, separated on the ionic liquid stationary phases in much the same manner as on non-polar commercial stationary phase.\textsuperscript{57} However, polar analytes (Figure 1.18 B: compounds 4, 5, and 7) are strongly retained by the ionic liquid stationary phases.\textsuperscript{57}

In addition to providing highly selective separations, there are a multitude of other desired characteristics that a gas chromatographic stationary phase should possess. These properties include high viscosity, low surface tension allowing for wetting of the fused silica capillary wall, and low vapour pressure at elevated temperatures. This section primarily describes the desired characteristics of ILs namely viscosity, thermal stability, and surface tension which largely dictate the quality and integrity of the stationary phase coating.

\textbf{Figure 1.17.}\textsuperscript{57} A comparative analysis of the separation of 8 compounds on an ionic liquid phase [BuMIm][PF\textsubscript{6}] (B) versus a commercial DB-5 phase (A). The columns were of identical dimensions (15 m × 0.25-mm i.d.), under identical conditions, and similar polarity. The test compounds are: 1) butyl acetate; 2) n-heptanol, 3) \(\rho\)-dichlorobenzene,
4) o-cresol, 5) 2,5-dimethylphenol, 6) n-dodecane, 7) 4-chloroaniline, and 8) n-tridecane.

Poole and coworkers evaluated liquid organic salts as stationary phases in GLC in the early 1980s. Ethylpyridinium bromide was used to separate various organic compounds containing large dipoles or functional groups capable of hydrogen bonding. Ethylpyridinium bromide has a melting point of 110 °C, below this melting point, this stationary phase exhibited poor efficiency and peak asymmetry. However, due to the tunability of ILs, the temperature range of the molten salts was expanded by the design of new classes of RTIL stationary phases such as tetra-n-hexylammonium benzoate, 1-methyl-3-ethylimidazolium chloride, tri-n-butylyphosphonium chloride, and tetra-n-butylammonium tetrafluoroborate.

1.4.4.2.1 Thermal stability characteristics of ionic liquids

One of the most chromatographically important physical properties possessed by many ILs is their high thermal stabilities. In GLC, the thermal stability of the stationary phase is one of the most important considerations as it governs the onset of column bleed and ultimately dictates the lifespan of the stationary phase.

For ILs based on the 1,3-dialkyl imidazolium cation, it was found that the thermal stability can be increased by incorporating large, bulky cations paired with triflate anions as shown in Figure 1.19, IL structures A and B. Not only did these bulkier ILs exhibiting higher thermal stabilities, but also demonstrated improved separation selectivity for solutes containing nonbonding and n-electrons as well as hydrogen bond basic character. This was likely due to the ability of the electron-rich phenyl ring in IL B being able to interact strongly via n–n interactions with aromatic solutes, whereas the phenyl ring in IL A is insulated from the imidazolium ring by the methylene group, slightly decreasing its propensity for n–n interactions. This is an excellent demonstration of the tunability of ILs for enhanced thermal stability while also producing two stationary
phases that exhibit quite unique selectivities. It has also been observed that the thermal stability of geminal dicationic ILs is considerably higher than their monocationic analogs.\textsuperscript{180}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{imidazolium_cations.png}
\caption{Bulky imidazolium cations paired with a triflate anion.}
\end{figure}

\subsection{1.4.4.2.2 Development of highly viscous stationary phases}

Ideal stationary phases in GC possess high viscosities as well as exhibit small changes in viscosity with large ranges in temperature. ILs possessing the highest viscosities typically contain halide anions that exhibit superior hydrogen bond coordinating behavior. However, despite having high viscosities, these ILs tend to exhibit lower thermal stabilities and tenaciously retain analytes capable of hydrogen bonding.

\subsection{1.4.4.2.3 IL surface tension and wall-coated open tubular columns}

To perform gas chromatographic separations in wall coated open tubular (WCOT) capillary columns, it is essential that the stationary phase be evenly coated as a thin film on the wall of the capillary column. It is imperative that the stationary phase retains an even film over the surface of the capillary column without forming droplets, neither during the coating process or chromatographic separation when the oven temperature is increased. The surface tension of the IL is crucial in forming a thin and homogeneous coated layer.

\subsection{1.4.4.2.4 Anion and cation effects on chromatographic selectivity}
Selectivity is one of the most important characteristics of any chromatographic stationary phase. Typically, GLC stationary phases are classified in terms of their polarity, in order to achieve separation the polarity of the employed stationary phase should closely match that of the analytes being separated. ILs can give rise a multitude of different solvation interactions that result in unique interactions with the solute molecules. However, an evaluation of imidazolium-based ILs with different combinations of cations and anions revealed that the anion contributes the most to the unique selectivity exhibited by the IL stationary phase.\(^{179}\)

### 1.4.4.3 Application of ionic liquids as chiral stationary phase solvents

Two approaches have been employed to incorporate ILs as new stationary phases for chiral GLC. One method involves coating a pure chiral IL as the stationary phase in WCOT GLC.\(^{181}\) The second approach, involves dissolving chiral selectors such as cyclodextrins in an achiral IL and the mixture coated onto the wall of the capillary column.\(^{182}\) Both approaches can separate a variety of different analytes, but the observed enantioselectivities and efficiencies do not rival those observed with commercially available chiral stationary phases (CSPs). The first application of a chiral IL as a CSP was first demonstrated by Armstrong, Welton, and coworkers.\(^{181}\) The chiral ILs evaluated were based on the \(N,N\)-dimethylephedrinium cation paired with the bis[trifluorormethylsulfonyl]amide anion [Tf\(_2\)N]- (Figure 1.20).

![Figure 1.19](image-url)
These IL CSPs were enantioselective for four classes of chiral analytes: alcohols (including diols), sulfoxides, epoxides, and acetylated amines (Figure 1.21). And when the opposite enantiomer of the chiral IL was employed as the stationary phase the elution order of the chiral analytes could be reversed.\textsuperscript{181}

![Figure 1.20](image)

**Figure 1.20:**\textsuperscript{181} Enantiomeric separation of sec-phenethyl alcohol, 1-phenyl-1-butanol, and trans-1,2-cyclohexanediol on a \((1S,2R)-(+)\)-\(N,N\)-dimethylephedrinium bis(trifluoromethane sulfon)imidate ionic liquid stationary phase.

As mentioned earlier, the chiral selector can also be dissolved in the IL solvent and be subsequently coated on the capillary wall.\textsuperscript{182} Using this approach, the achiral [\(N\)-butyl-\(N\)-methylimidazolium] \(\text{Cl}^-\) IL was used to dissolve permethylated \(\beta\)-cyclodextrin (PM-\(\beta\)CD) and dimethylated \(\beta\)-cyclodextrin (DM-\(\beta\)CD). The chromatographic separations obtained from these two columns were compared to two commercially available CSPs based on PM-\(\beta\)CD and DM-\(\beta\)CD dissolved in polydimethylsiloxane. From a set of 64 chiral molecules separated by the commercial PM-\(\beta\)CD column, only 21 of the molecules were enantioresolved by the IL-based PM-\(\beta\)CD column. Likewise, from a collection of 80 analytes separated by the DM-\(\beta\)CD column, only 16 analytes could be separated on the IL-based DM-\(\beta\)CD column.\textsuperscript{182} A considerable enhancement in the separation efficiency of
the IL-based CSPs was observed. The enhancement in separation efficiency, coupled to
the loss of enantioselectivity for most separations, suggested to be the result of the
imidazolium cation occupying the cavity of the cyclodextrin preventing the analyte–
cyclodextrin inclusion complexation that is crucial for chiral recognition.\textsuperscript{182-183}

1.4.4.4 \textit{Binary mixtures of ionic liquids as high-selectivity stationary phases}

Although IL-based stationary phases exhibit unique selectivity compared to many non-
ionic stationary phases, it is not always possible to completely resolve all analytes in
complex mixtures during the separation. As a result stationary phases consisting of a
mixture of ILs have been used in GLC to achieve selectivities that is not achievable with
neat IL stationary phases.\textsuperscript{184} A complex mixture of alcohols (both cyclic and aliphatic)
and analytes with aromatic functionality were subjected to separation on a stationary
phase consisting of neat $N$-butyl-$N$-methylimidazolium [Tf$_2$N]$^-$ IL.\textsuperscript{184} This stationary
phase, though selective for most of the molecules, exhibited poor resolution.\textsuperscript{184} Columns
were prepared consisting of a mixture of different percentages of the ILs $N$-butyl-$N$-
methylimidazolium [Cl]$^-$ and $N$-butyl-$N$-methylimidazolium [Tf$_2$N]$^-$ (Figure 1.22).
Through tuning the composition of the stationary phase mixture, the separation
selectivity and resolution of most analytes were varied. It was observed that the
hydrogen bond basicity increased linearly as the concentration of chloride anion (in the
form of $N$-butyl-$N$-methylimidazolium Cl$^-$) was increased.\textsuperscript{184} It was found that the
retention factors of short-chained alcohols increased by as much as 1100 \% on the
column containing the highest percentage of chloride anion compared to the neat $N$-
butyl-$N$-methylimidazolium [Tf$_2$N]$^-$.\textsuperscript{184} Most alcohols exhibited a reversal of elution order
as the stationary phase hydrogen bond basicity increased.\textsuperscript{184} By utilizing stationary
phases based on IL mixtures it has been demonstrated that the separation selectivity,
resolution, and elution order of analytes can be tuned systematically,\textsuperscript{184} adding another
parameter in the design aspect to ILs based stationary phases for gas chromatographic
separations.
1.4.4.5 Polymeric ionic liquid stationary phases for high-temperature separations.

Although ILs possess a variety of favourable properties rendering them excellent candidates for application as stationary phases, their most significant drawback lies with their drop in viscosity with increasing temperature. This characteristic results in an increased propensity for flowing of the IL within the capillary, producing pooling of the IL and irregular coating and film thickness throughout the column. These factors often have detrimental effects on retention time reproducibility as well as separation efficiency.

In an attempt to combat the problem of IL on column flow during separation at increased temperature a method of immobilizing the IL onto the capillary column was devised. This method involved on-column polymerisation of mono- and di-cations vinyl-substituted imidazolium cations (Figure 1.23) with a free-radical initiator (azobisisobutyronitrile, AIBN) in dichloromethane. This mixture was coated onto the wall of the capillary column by the static coating method and the capillary was sealed at both ends and heated to initiate polymerization. Three types of polymerised stationary phases were produced with this method, linear polymerised, partially crosslinked and fully crosslinked stationary phases.

Figure 1.22: Mono- and di-cations vinyl-substituted imidazolium ILs; (a) 1-vinyl-3-hexylimidazolium and (b) 1,9-di-(3-vinylimidazolium)nonane bis-(trifluoromethane sulfonyl)imidate.
The formation of a linear IL polymer stationary phase was performed by the free radical polymerization of monocationic monomers. These stationary phases exhibited the lowest thermal stability, producing significant column bleed and dramatic decreases of efficiency above 300 °C.\textsuperscript{185} Partially crosslinked stationary phases were synthesized by polymerizing a blend of monocationic and dicationic crosslinking monomers. Using this approach, the stationary phase consisting of an equal ratio of mono- and dicationic ILs exhibited the best thermal stability up to ~280 °C.\textsuperscript{185} The third stationary phases consisted of a completely crosslinked IL polymer formed by the polymerization of geminal dicationic IL monomers.\textsuperscript{185} This matrix exhibited high stability and enhanced efficiencies at separation temperatures over 350 °C.\textsuperscript{185} It was shown that mono-cationic IL matrixes were best suited for low-temperature analysis, whereas the partially crosslinked and fully crosslinked stationary phases exhibited higher thermal stabilities, thus, were ideal for moderate (100–280 °C) and high (200–380 °C) temperature gas chromatographic separations, respectively (as illustrated in Figure 1.24).\textsuperscript{185} In addition it was found that the solvation interaction parameters of the polymerized IL stationary phases were largely unchanged compared to their monomeric IL analogs.\textsuperscript{185} Further work into the development of IL polymeric stationary phases has continued,\textsuperscript{186} including the development of racemic 2-(1H-imidazol-1-yl)cyclohexanol based polymeric phase which exhibited good thermal stabilities (240–300 °C), very high efficiencies (3120–4200 plates/m) and excellent selectivity.\textsuperscript{187} This is one of the very few chiral IL GC stationary phases reported, however, only a racemic form of the stationary phases was reported to have been synthesised and so no enantiomeric separations were reported.
Figure 1.23: Separation of C$_6$-C$_{24}$ fatty acid methyl esters on a partially cross-linked ionic liquid stationary phase. Conditions: 100 °C, hold for 2 mins, 15 °C/min to 260 °C.

Despite the increased interest in the development on IL stationary phases for application in GC analysis and the increasing availability of IL stationary phases on the market, there are very few examples of chiral ionic liquid GC stationary phases in the literature and none are commercially available. This is in part due to the very large number of achiral ionic liquids being developed and comparatively few chiral ionic liquids. In addition, the most common (and effective) chiral selecting agents used in chiral GC stationary phases are cyclodextrins based chiral selecting agent. A great deal of research has gone into the development of various CD derivatives for application as chiral selecting agents and much less effort into the development of new chiral selectors. In this study we endeavour to design and synthesise novel chiral ionic liquids and test them for enantiomer resolving capabilities.
1.5 The aim and scope of this research

The various properties exhibited by ILs make them ideal stationary phases in GLC. ILs exhibit a unique dual-nature selectivity that allows them to separate polar molecules like a polar stationary phase and nonpolar molecules like a nonpolar stationary phase. In addition, the combination of cations and anions can be tuned to add further selectivity for more complex separations. Viscosity, thermal stability, and surface tension are vital properties that dictate the quality and integrity of the stationary phase coating and are additional characteristics that can be controlled when custom designing and synthesizing ILs. IL-based stationary phases also hold great promise in GC mass spectrometry where the dual-nature selectivity of the stationary phase eliminates the need for frequent changing of columns.

Several simultaneous interactions are required to discriminate between enantiomers; the diastereoisomeric selector–ligand complexations required for chiral discrimination involve a combination of interactions. Chapter 2 describes the synthesis of new imidazole based chiral ionic liquids with various functional groups designed to be able to promote a variety of molecular interactions.

In chapter 3 the new chiral IL are incorporated into OV-1701 polysiloxane stationary phases as chiral resolving agents and tested for enantionselective capability by gas chromatography. In these studies we look at the thermal stability of the CILs which impacts a column’s maximum operation temperature, the ability of the various functional groups on the CILs to form diastereoisomeric selector–ligand complexation interactions between enantiomers and also study the dynamic interconversion process of chiral oximes on polysiloxane chiral stationary phases and achiral polyethylene glycol stationary phases.

The aim of this work is to endeavour to assess whether these new classes of ILs can be used as chiral resolving agents in common achiral stationary phases such as polysiloxane...
gums. In doing so we strive to develop a new class of IL chiral stationary phases that can be used to tackle the chemists’ problem of assessing the enantiomeric purity of new synthetic products, as well as, provide an extra avenue for pharmaceutical industries and the like, to be able separate and assess enantiomers and provide the data required by regulatory agencies with respect to chiral drugs.
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Chapter 2

The synthesis of novel ionic liquids for application as chiral selecting agents
2.1 Synthesis of simple imidazole and pyridine based ionic liquids

2.1.1 Introduction

The first reported application of ionic salts, previously known as molten salts, as GC stationary phases was by Barber et al in 1959.\textsuperscript{1} In 1982 1-alkyl-3-methylimidazolium cation based room temperature ionic liquids were first reported by Wilkes et al.\textsuperscript{2} paving the way for the development of air and moisture stable ionic liquids (ILs) which could be used for a wider range of applications. Common organic ILs consist of an asymmetrically substituted nitrogen containing cation such as imidazoles or pyridines with a counter anion such as a halide ion, PF\textsubscript{6} -, BF\textsubscript{4} - or Tf\textsubscript{2}O - anion. The wide range of anion and cation combinations which can be coupled, allows for the synthesis of various room temperature ionic liquids (RTILs) with customized physical and chemical properties suited to specific applications.

More recently, imidazole based room temperature ionic liquids have been employed as stationary phases in gas chromatography. Ionic liquids have been shown to possess separation capabilities by Anderson et al.\textsuperscript{3} who stated they had “dual nature” retention capabilities. That is, ionic liquid stationary phases can separate both polar molecules as do polar stationary phases and nonpolar molecules as do nonpolar stationary phases, on the one phase. In 2004 Ding et al.\textsuperscript{4} reported the first enantiomeric separations using imidazole based chiral ionic liquid stationary phases in gas chromatography. The utility of imidazole based ionic liquids as GC stationary phases has stimulated the development of new imidazole based ionic liquids. Because the physical and chemical properties of ionic liquids can be tuned by altering the structure and functional groups of the cation and anion, they make ideal candidates for application in the development of new stationary phases with a wide range of tunable physical and chemical properties for application in analytical chemistry separations.
Moreover, a few chiral imidazolinium salts have recently been reported as chiral ionic liquids.\(^5,6\) However, examples of chiral ionic liquids remain few compared to achiral ionic liquids.\(^7,8\) This research was undertaken to contribute to the development of new chiral ionic liquids. Some simple methods for the synthesis of imidazole based ionic liquids, and some new chiral ionic liquids are presented.

### 2.1.2 Results and Discussion

**Imidazolium ionic liquids**

Ionic liquids were synthesised from the 1H-imidazole dissolved in anhydrous DMF. To this solution was added excess alkylhalide and heated for ~6 hrs at 90 \(^\circ\)C under a nitrogen atmosphere. The solution was concentrated under negative pressure and the remaining residues of DMF and alkylhalide were removed by kugelrohr bulb-to-bulb distillation to afford \(N,N\)-dialkylimidazolium ionic liquids (reaction shown in Figure 2.1). Using this method the simple room temperature ionic liquids 1-3 were synthesized as depicted in Figure 2.2.

\[
\begin{align*}
\text{N} & \quad \text{DMF/alkylhalide} \\
\text{H} & \quad 90^\circ\text{C, 6hrs} \\
\rightarrow & \quad \text{R} \quad \text{N} \quad \text{N} \\
\text{R} & \quad \text{X} \\
\end{align*}
\]

**Figure 2.1**: Typical reaction scheme for the synthesis of \(N,N\)-dialkylimidazolium ILs. 1 molar equivalent of imidazole with excess alkylhalide in DMF heated to 90 \(^\circ\)C for 6 hrs affords dialkylimidazolium halide.
Figure 2.2: Imidazolium ILs 1-3 were synthesised by heating 1-{H-imidazole with the alkylhalides ethyliodide, benzylchloride and \( n \)-bromobutane in DMF at 90 °C for 6 hrs under nitrogen. ILs 4-5 were synthesised from either \( N \)-benzoylhistidine or \( N \)-acetylhistidine by heating with benzylchloride or ethyliodide, repectively, in DMF at 90 °C for 6 hrs.

These ILs are achiral, however, using the same method novel chiral imidazolium ILs were synthesised from (L)-\( N \)-benzoylhistidine (44) and (L)-\( N \)-acetylhistidine by alkylating with benzylchloride and ethyliodide, repectively. By heating \( N \)-benzoylhistidine (44) or (L)-\( N \)-acetylhistidine with either benzylchloride or ethyliodide at 90 °C for 6 hrs, 4 and 5 are afforded in quantitative yield (Figure 2.2 and 2.3). DMF is removed by rotary evaporation and the product further purified by recystallisation. These chiral ILs are ideal for application as chiral selecting agents.
**Figure 2.3:** Synthesis of histidine derived ionic liquid 4. Reflux (L)-N-benzoylhistidine with benzyl chloride in anhydrous DMF at 90 °C for 6 hrs.

Chiral epoxides are also very useful compounds for producing chiral imidazoles which can then be used for the synthesis of chiral ionic liquids. Epoxides are simply cyclic ethers, but because of their cyclic structure, the strain from their three-membered ring structure gives them unique chemical reactivity when compared to open chain ethers. Unlike linear ethers, epoxide can be cleaved by both base as well as by acid. Whereas the ether oxygen is normally a poor leaving group in an $S_N2$ reaction, epoxides react with imidazole at elevated temperatures.

Glas and Thiel$^9$ reported a facile, high yield synthesis of a chiral imidazole derived from (R)-styrene epoxide under microwave conditions. Following this procedure, irradiating a 1:1 mixture of imidazole and (+/-)-styrene epoxide for 3 minutes at 360 W afforded (+/-)-2-(1-imidazolyl)-1-phenylethanol (6), the pure product was precipitated upon addition of ethyl acetate in 77 % yield (Figure 2.4).

**Figure 2.4:** Microwave irradiation of a 1:1 mixture of of imidazole and (R)-styrene epoxide at 360 W for 3 mins, affords (1R)-2-(1-imidazolyl)-1-phenylethanol (6).
Base-catalysed epoxide ring opening is a typical $S_N2$ reaction in which attack by the nucleophile takes place at the least hindered epoxide carbon. The basic nitrogen on the imidazole acts as the nucleophile attacking the least hindered carbon on the three-membered ring. Given that styrene epoxide has primary and secondary carbons, nucleophilic attack takes place at the primary carbon via $S_N2$ mechanism producing a single isomeric product.

![Figure 2.5](image1.png)

**Figure 2.5:** Alkylation of (+/-)-2-(1-Imidazolyl)-1-phenylethanol (6) to afford ILs 7-10.

(+/-)-2-(1-Imidazolyl)-1-phenylethanol was then alkylated with the alkylhalides ethyliodide, bromobutane, benzylchloride, and allylbromide to afford the corresponding new imidazolium salts 7-10 (Figure 2.5 & 2.6) in quantitative yields with melting points ranging from RTIL-125 °C.

![Figure 2.6](image2.png)

**Figure 2.6:** Racemic ionic liquids synthesised from (+/-)-2-(1-imidazolyl)-1-phenylethanol.
**Figure 2.7:** $^{13}$C-NMR spectrum of IL 9 in CDCl$_3$. (iii) DEPT135 spectrum of carbon atoms which are bonded to hydrogen atoms ($CH$ and $CH_3$ positive, $CH_2$ negative), (ii) DEPT90 showing only $CH$ carbons and, (i) $^{13}$C-NMR spectra showing $CH$, $CH_2$, $CH_3$, and quaternary carbons.
Table 2.1: Imidazolium IL melting points (mp) and yields.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mp °C</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RTIL</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>RTIL</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>RTIL</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>RTIL</td>
<td>Q</td>
</tr>
<tr>
<td>5</td>
<td>RTIL</td>
<td>Q</td>
</tr>
<tr>
<td>6</td>
<td>141-142</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>124-125</td>
<td>92</td>
</tr>
<tr>
<td>8</td>
<td>72-74</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>RTIL</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
<td>120-122</td>
<td>83</td>
</tr>
</tbody>
</table>

Q= quantitative yield.

**Pyridinium ILs**

Pyridine also undergoes S_N2 alkylation with an alkylhalide, due to its basic sp$^2$ hybridised lone-pair electrons. Refluxing neat pyridine-4-carboxaldehyde with excess alkylhalide, such as ethyliodide or benzylchloride, forms N-alkylpyridinium halide ILs (Figure 2.8). The excess alkylhalide can be removed to give pure 11-12 in quantitative yields.

![Figure 2.8: Formation of pyridinium ILs](image)

**Figure 2.8:** Formation of pyridinium ILs.
Given the ease with which pyridines can be alkylated, the possibility of manipulating the physical and chemical properties of poly-4-vinylpyridine (4-PVP) polymers by alkylating the pyridine pendant groups to form poly-4-vinylpyridinium ILs was explored. Being a polymer the solubility of 4-PVP is limited to acetic acid, t-butanol, DMF, DMSO, lower alcohols and water at low pH. In converting the pyridine pendant groups into ionic liquids we endeavoured to expand the solubility of these polymers in less polar organic solvents, because their insolubility rendered them difficult to use in many applications including their application as GC stationary phases.

Figure 2.9: Polyvinyl pyridines (a) poly(4-vinylpyridine) (4-PVP) and (b) poly(4-vinylpyridine) 2% crosslinked with divinylbenzene (4-PVP-XL).

Alkylation of the PVP’s was conducted under the same conditions as the imidazolium ILs, in DMF with the alklyhalides ethyliodide, 1-bromobutane or benzylchloride with a longer reaction time of 24 hrs owing to the lower solubility of the PVP’s. The resultant PVP ILs (13-15 and 16-18) were synthesised in good yields (Figure 2.11), however, the solubility of these IL polymers in nonpolar solvents was not significantly increased by N-alkylation. With the knowledge that anion metathesis can increase the solubility of ILs, anion metathesis was applied to these ionic polymers unsuccessfully. The ionic polymers remained insoluble in most organic solvents thus anion exchange did not take place. Due to their insolubility in less polar common organic solvents their incorporation into GC stationary phases is not possible. Further investigation into appropriate solvent systems for anion exchange metathesis may yield ionic PVP polymers with improved solubility. Perhaps ionic liquids themselves could be used to dissolve PVP based ionic polymers. Generally the crosslinked PVP’s were less soluble than the non-crosslinked PVP’s. Thus
employing a non-crosslinked 4-PVP of smaller molecular mass than ~60,000 perhaps, for synthesising ionic PVP polymers is recommended and worthwhile investigating. Such ionic polymers may be soluble enough in less polar solvents such as DCM to permit their investigation as novel GC stationary phases.

![Figure 2.10: Alkylation of 4-PVP with the alkylhalides ethyliodide, bromobutane, and benzylchloride.](image)

**Figure 2.10:** Alkylation of 4-PVP with the alkylhalides ethyliodide, bromobutane, and benzylchloride.

**Thermogravometric analysis of 4-PVP ILs**

In order to evaluate the thermal stability of the 4-PVP ILs, thermogravimetric analysis tests were carried out. Thermogravimetric analysis scans were obtained on a Mettler thermogravimetric balance at a heating rate of 20 °C/min from 50 to 800 °C under an inert nitrogen atmosphere flowing at 20 mL/min, switching to air at 600 °C. As can be seen in Figure 2.12 (A) compounds 13-15 begin to show weight loss indicative of decomposition at 233 °C, 200 °C, and 208 °C, respectively, compared to the underivatised 4-PVP which begins to decompose at 320 °C.
Figure 2.11: The poly-(4-vinylpyridinium halide) ionic liquids synthesised.

Underivatised 2 % crosslinked 4-PVP begins to decompose at 341 °C, while its derivative ILs 16-18 began decomposing at 245 °C, 203 °C, and 227 °C, respectively (Figure 2.12 B). It can be seen that the thermal stabilities of the ionic 4-PVP were significantly reduced by the derivatisation process. It was observed that the N-benzyl derivative produced the least thermally stable ILs while N-ethyl derivatives showed the greatest stability out of the 3 derivatives synthesised for both the 2 % cross-linked and non-crosslinked polymers. Despite the reduced thermal stability these ionic PVPs are stable at high enough temperatures to be functional as stationary phases. Although these ionic PVP may not be novel compounds, their application as GC stationary phases would be novel and make an interesting study which could pave the way for the application of chiral ionic polymers derived from chiral polymers such as chitosan, for example.
**Figure 2.12:** A) TGA analysis of PVP ILs, 14, 15, and 13 compared to non cross-linked poly(4-vinylpyridine) (4-PVP). B) TGA analysis of 4-PVP-XL ILs, 17, 18, and 16 compared with 2 % cross-linked poly(4-vinylpyridine) (4-PVP-XL).

### 2.1.3 Conclusion

Simple methods for the synthesis of achiral and chiral imidazolium ionic liquids as well as their dicationic imidazolium analogues were presented. The possibility of manipulating the physical and chemical properties of poly-4-vinylpyridine polymers by converting the pyridine pendant groups into pyridinium salts were also explored for the purpose of improving the solubility of the polymers which would permit their application in GC stationary phases. However, no significant changes in solubility were observed, and the TGA analysis of the ionic PVPs were found to have lower thermal stability than their precursors. Although their lowered thermal stabilities do not diminish their usefulness in stationary phases, their limited solubility impedes their ability to be incorporated into GC capillary columns as stationary phases, which is the focus of our research. The potential use of ILs to solubilize ionic PVP polymers has never been explored and should be investigated as a possible solution to overcoming the problem of insolubility. These investigations into the synthesis of ionic polymers pave the way for future work to prepare highly soluble ionic PVP polymers as well as chiral ionic carbohydrate based polymers which would be highly desirable for application as chiral resolving agents in separation media such as GC stationary phases.
2.1.4 Experimental

2.1.4.1 Reagents

DMF, ethyl acetate, ethyl iodide, 1,2-diiodoethane, benzylchloride, 1-bromobutane, (R)- and (+/-)-styrene epoxide, imidazole, N-methylimidazole, N-benzylimidazole, N-Butylimidazole, N-acetyl-L-histidine monohydrate, (L)-N-benzoylhistidine, poly-4-vinylpyridine (4-PVP), poly-4-vinylpyridine 2 % cross-linked with divinylbenzene (4-PVP-XL), and pyridine-4-carboxaldehyde were all purchased from Sigma Aldrich.

2.1.4.2 Instruments

DMF was removed by bulb-bulb distillation using a Buchi Kugelrohr (manufactured in Switzerland). Melting points were measured with a Gillkhenhamp Melting Point apparatus (manufactured England). NMR spectra analysis was conducted on a Bruker 300 spectrometer. All NMR structure elucidation was done from $^1$H-NMR, $^{13}$C-NMR, DEPT135, DEPT90, HSQCGP, and HH-COSYGPSW experiments. Mass spectroscopy data were obtained by Electro-spray ionization (model) in positive ion mode (in 1:1 acetonitile/H$_2$O). Thermogravimetric analysis (TGA) was conducted on a Perkin-Elmer TGA-7 instrument. The scan was obtained at 20 °C/min under an inert nitrogen atmosphere flowing at 20 mL/min from ambient temperature to 800 °C switching to air at 600 °C. The thermostabilities of the ILs were determined on a Mettler thermogravimetric balance at a heating rate of 10 °C/min.

2.1.4.3 Synthesis

General N-alkylation procedure:

To a stirring solution of imidazole in dry DMF under nitrogen was added an alkyl halide and heated for 6hr at 90 °C under a nitrogen atmosphere. The solution was concentrated by rotary evaporation at 80 °C and the remaining residues of DMF were removed by
bulb-to-bulb distillation using a kugelrohr. Further purification was carried out either by flash chromatography or recrystallization from ethyl acetate and hexane.

**Imidazoles**

*N,N’-Diethylimidazolium iodide 1*

1 was synthesised following the general alkylation procedure from 3.0 g (44.08 mM) imidazole and excess ethyl iodide (7.8 mL) in 7mL of DMF to afford 1 as a dark brown ionic liquid; yield: 10.8 g (97 %): RTIL; ¹H-NMR (300 MHz DMSO-d₆) δ= 9.23-9.08 (m, 1H, -NC₃H₅N-), 7.80-7.67 (m, 2H, -NCH), 4.20 (dq, ³J_H,H = 7.5 Hz, 4H, -CH₂), 1.41 (t, ³J_H,H =7.5 Hz, 6H, -CH₃); ¹³C-NMR (75MHz, DMSO-d₆) δ = 135.2, 134.9, 122.0, 121.6, 120.1, 119.4, 44.2(CH₂), 43.8(CH₂), 15.9(CH₃), 15.7(CH₃); ESI-MS: calcd m/z = 125.19 (M⁺), found m/z = 125.1 (M⁺).

*N,N’-Dibenzylimidazolium chloride 2*

2 was synthesised following the general alkylation procedure from 3.0 g (44.08 mM) imidazole and excess benzyl chloride (11.2 mL) in 7 mL of DMF to afford 2 as a a brown glass; yield: 9.77 g (78 %): RTIL; ¹H-NMR (300 MHz, DMSO-d₆) δ= 9.49 (s, 1H, -NC₃H₅N-), 7.86 (s, 2H, -NCH), 7.50-7.36 (m, 10H, Ar-H), 5.47 (s, 4H, -CH₂); ¹³C-NMR (75MHz, DMSO-d₆) δ= 136.2, 129.0, 128.9, 128.8, 128.6, 128.4, 128.2, 122.8, 41.7(CH₂); ESI-MS: calcd m/z = 249.33 (M⁺), found m/z = 249.1 (M⁺).

*N,N’-Dibutylimidazolium bromide 3*

3 was synthesised from 3 g (44.08 mM) of imidazole following the general alkylation procedure using excess n-bromobutane (10.45 mL) in 7 mL of DMF to afford 3 as a light brown RTIL; yield: 11.4 g (99 %): RTIL; ¹H-NMR (300 MHz, CDCl₃) δ= 8.5 (m, 1H, -NCHN-), 6.30-6.71 (m, 2H, -NCH), 3.57-3.47 (m, 4H, -NCH₂), 1.03 (m, 4H, -NCH₂CH₂-), 0.45 (m, 4H, -CH₂CH₃). 0.02 (m, 6H, -CH₂CH₃); ¹³C-NMR (75MHz, CDCl₃) δ= 135.2, 134.0, 132.9, 122.1, 121.5, 119.33, 118.6, 49.0(CH₂), 48.8(CH₂), 31.5(CH₂), 31.4(CH₂), 18.7(CH₂), 12.9(CH₃); ESI-MS: calcd m/z = 181.30 (M⁺), found m/z = 181.1 (M⁺).
5-(2-Benzoylamino-2-carboxy-ethyl)-1,3-dibenzyl-imidazolium chloride 4

4 was synthesised from 44 (3 g, 11.57 mM) following the general alkylation procedure using excess benzyl chloride (4.2 mL) in 7 mL of DMF with K$_2$CO$_3$. Following the removal of DMF by bulb-bulb distillation the crude product was dissolved in methanol leaving K$_2$CO$_3$ as a white precipitate. The solution was filtered and the methanol removed by evaporation to afford 4 as a brown glass in quantitative yield: 5.7 g: RTIL; $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ = 9.67 (s, 1H, -OH), 9.05 (d, $J_{H,H} = 7.8$ Hz, 1H, -CHIm), 7.86 (d, $J_{H,H} = 7.5$ Hz, 4H, Ar-H), 7.74-7.08 (m, 12H, Ar-H/CH$_3$), 5.60 (s, 2H, -CH$_2$), 5.45 (s, 2H, -CH$_2$), 4.71 (m, 1H, -CH), 3.21 (m, 2H, -CH$_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$ = 171.9, 170.2, 166.6, 166.4, 142.6, 136.6, 135.7, 134.8, 134.0, 133.9, 133.6, 133.2, 132.2, 131.5, 131.4, 131.0, 130.2, 129.2, 129.0, 128.8, 128.6, 128.6, 128.5, 128.3, 128.1, 128.0, 127.8, 127.7, 127.5, 127.4, 127.2, 126.5, 126.4, 120.8, 51.9, 51.10, 49.7, 25.2; ESI-MS: calcd m/z = 443.54 (M$^+$), found m/z = 440.3

5-(2-Acetylamino-2-carboxy-ethyl)-1,3-diethyl-imidazolium iodide 5

5 was synthesised from N-acetyl-L-histidine monohydrate (2 g, 10.14 mM) following the general alkylation procedure using ethyl iodide in 7 mL of DMF with K$_2$CO$_3$. Following the removal of DMF by bulb-bulb distillation the crude product was dissolved in methanol leaving K$_2$CO$_3$ as a white precipitate. The solution was filtered and the methanol removed by evaporation to afford 5 as a dark brown glass in quantitative yield: 3.9 g: (RTIL); $^1$H-NMR (300 MHz, D$_2$O) $\delta$= 7.35 (s, 1H, -NCHN-), 4.48 (m, $J_{H,H} = 4.7$ Hz, 1H, -CH$_2$CH), 4.38 (s, 1H), 4.21 (dq, $J_{H,H} = 7.4$ Hz, 4H, -CH$_2$CH$_3$), 3.34-3.01 (m, $J_{H,H} = 4.7$ Hz, 2H, -CH$_2$CH$_3$), 2.03 (s, 3H, -CH$_3$), 1.5 (dt, $J_{H,H} = 7.4$ Hz, 6H, -CH$_3$CH$_3$); $^{13}$C-NMR (75MHz, D$_2$O) $\delta$= 176.7, 173.7, 131.4, 119.8, 55.4, 49.2, 44.9, 42.2, 26.2, 22.2, 14.8, 14.4; ESI-MS: calcd m/z = 257.33 (M$^+$), found m/z = 257.1.
Microwave reaction:

Styrene epoxides  

(1R)- or (+/-)-2-(1-Imidazolyl)-1-phenylethanol 6

A 1:1 mixture of imidazole (0.51 g) and styrene epoxide (838 µL) was irradiated for 3 minutes in a microwave oven at 360 W. To the oily crude product was added ethyl acetate to afford 2-(1-imidazolyl)-1-phenylethanol 6 as a white crystalline precipitate. The precipitate was filtered and air dried: 1.1 g (77%): mp 141-142 °C; ¹H-NMR (300 MHz, DMSO-d₆) δ = 7.46 (s, 1H, -NC₃H₅N _), 7.37-7.21 (m, 5H, Ar-H), 7.06 (s, -CH₃im), 6.82(s, 1H, -CH₃im), 5.70 (s, 1H, -OH), 4.82 (dd, Jₕₕ =7.7, 4.1 Hz, 1H, -CH₂CH₃), 4.17-3.99 (m, 2H, -CH₂CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ= 142.5q, 137.6, 127.9, 127.6, 127.2, 125.9, 119.9, 79.3, 78.8, 78.4, 72.1, 53.7(CH₂); ESI-MS: calcd m/z = 189.23 (M+H⁺), found m/z = 189.1 (M+H⁺).

(+/-) 2-(N-Ethyl-imidazolium iodide)-1-phenylethanol 7

IL 7 was synthesised from the racemic imidazole 6 (0.5 g) following the general alkylation procedure using 260 µL of ethyl iodide in 3 mL of DMF to afford 7 as a dark brown glass/RTIL at 97 % yield (0.89 g) : mp 124-125 °C; ¹H-NMR (300 MHz, CDCl₃/DMSO -d₆) δ = 9.21 (s, 1H, -NCHN-), 7.53 (s, 2H, -NCH₃im, 7.41-7.22 (m, 5H, Ar-H), 5.01 (dd, Jₕₕ =7.9, 2.8 Hz, 1H, -CH₂CH₃), 4.48 (dd, Jₕₕ =13.7, 2.8 Hz, 1H, -CH₂CH₃), 4.22 (m, Jₕₕ =14.4, 7.5 Hz, 3H, -CH₂CH-(1H) & -CH₂CH₃ (2H)), 1.47 (t, Jₕₕ =7.5 Hz, 3H, -CH₃); ¹³C-NMR (75MHz, CDCl₃/DMSO-d₆) δ= 145.3q, 141.0, 133.4, 133.0, 130.8, 128.5, 126.2, 75.9, 61.2(CH₂), 49.8(CH₂), 20.4(CH₃); ESI-MS: calcd m/z = 217.29 (M⁺), found m/z = 217.1.

(+/-) 2-(N-Benzyl-imidazolium chloride)-1-phenylethanol 8

8 was synthesised synthesised from the racemic imidazole 6 (0.5 g) following the general alkylation procedure using 370 µL of benzyl chloride in 3 mL of DMF to afford 6 as a light brown glass at 97 % yield (0.81 g) : mp 72-74 °C; ¹H-NMR (300 MHz, CDCl₃) δ = 9.80
(s, 1H, -NCHN-), 7.41-7.16 (m, 11H, Ar-H), 7.06 (s, 1H,-NCH$_\text{im}$), 5.79 (s, 1H, -OH), 5.29 (s, 2H, -CH$_2$), 5.20 (dd, $J_{\text{H,H}}$ = 4.9, 2.5 Hz, 1H, -CH$_2$CH$^-$), 4.65 (dd, $J_{\text{H,H}}$ =13.9, 2.5 Hz, 1H, -CH$_2$CH$^-$), 4.36 (m, $^3J_{\text{H,H}}$=13.9, 1H, -CH$_2$CH); $^{13}$C-NMR (75MHz, CDCl$_3$) $\delta$ = 140.4$_\text{q}$, 137.0, 132.9$_\text{q}$, 129.4, 128.8, 128.6, 127.9, 126.1, 123.7, 120.8, 71.0, 56.8(CH$_2$)$_3$, 53.3(CH$_2$); ESI-MS: calcd m/z = 279.36 (M$^+$), found m/z = 279.2.

(+/-) 2-(N-Butyl-imidazolium bromide)-1-phenylethanol 9

9 was synthesised synthesised from the racemic imidazole 6 (0.5 g) following the general alkylation procedure using butyl bromide in 3 mL DMF to afford 9 as a brown glass in good yield: 0.85 g (98 %): (RTIL); $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ = 9.53 (s, 1H), 7.44-7.19 (m, 7H, Ar-H/2xH$_\text{im}$), 5.23 (dd, $J_{\text{H,H}}$ =7.1, 2.8 Hz, 1H, , -CH$_2$CH$^-$), 4.72 (dd, $J_{\text{H,H}}$ =13.8, 2.9 Hz, 1H, -CH$_2$CH$^-$), 4.42 (dd, $^3J_{\text{H,H}}$ =13.7, 7.2, 2H, -CH$_2$CH$^-$), 4.41 (s, -OH), 4.10 (t, $^3J_{\text{H,H}}$ =7.3 Hz, 2H, -NCH$_2$CH$_2$-$^-$), 1.76 (dt, $^3J_{\text{H,H}}$ =15.1, 7.5 Hz, 2H, -CH$_2$CH$_2$CH$_3$), 1.25 (dq, $^3J_{\text{H,H}}$ =14.6, 7.3 Hz, 2H, -CH$_2$CH$_3$), 0.89 (t, $^3J_{\text{H,H}}$ = 7.3 Hz, 3H, -CH$_3$); $^{13}$C-NMR (75MHz, CDCl$_3$) $\delta$ = 139.8$_\text{q}$, 136.6, 128.6, 128.0, 126.1, 123.67, 121.0, 70.9, 56.6(CH$_2$)$_3$, 49.8(CH$_2$)$_3$, 31.8(CH$_2$)$_3$, 19.4(CH$_2$)$_3$, 13.4(CH$_3$); ESI-MS: calcd m/z = 245.34 (M$^+$), found m/z = 245.2.

(+/-) 2-(N-Allyl-imidazolium iodide)-1-phenylethanol 10

10 was synthesised synthesised from the racemic imidazole 6 (0.35 g) following the general alkylation procedure using allylbromide in 3 mL of DMF to afford 10 as a brown/grey solid in good yield: 0.47 g (83 %): mp 120-122 °C; $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ = 9.25 (s, 1H, -CH), 7.83 (s, 1H, -CH), 7.76 (s, 1H, -CH), 7.52 - 7.22 (m, 5H, Ar-H), 6.03 (ddt, $J_{\text{H,H}}$ = 16.1, 10.3, 5.9 Hz, 1H, =CH), 5.29 (ddd, $J_{\text{H,H}}$ = 18.3, 13.7, 1.2 Hz, 2H, =CH$_2$), 5.01 (dd, $J_{\text{H,H}}$ = 8.4, 2.8 Hz, 1H, -CH), 4.90 (d, $J_{\text{H,H}}$ = 5.8 Hz, 2H, -CH$_2$), 4.38 (ddd, $J_{\text{H,H}}$ = 22.2, 13.6, 5.9 Hz, 2H, -CH$_2$); $^{13}$C-NMR (75MHz, DMSO-$d_6$) $\delta$ = $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$ = 141.1$_\text{q}$, 136.5, 131.8, 131.7, 129.0, 128.2, 127.7, 126.9, 126.0, 123.3, 122.6, 121.9, 120.3(CH$_2$)$_3$, 120.0(CH$_2$)$_3$, 70.4, 55.7(CH$_2$)$_3$, 50.9(CH$_2$)$_3$, 50.7(CH$_2$)$_3$; ESI-MS: calcd m/z = 229.30 (M$^+$), found m/z = 229.1.
1-Ethyl-4-formyl-pyridinium iodide 11

RTIL 11 was synthesised from pyridine-4-carboxaldehyde (2.64 mL) by alkylating with excess ethyl iodide (3 mL) in DCM and heating to 50-60 °C for 24 hrs. The DCM and excess alkylhalide was removed by evaporation to afford 11 in qualitative yield as a RTIL; \(^1\)H-NMR (300 MHz, DMSO-d6) \(\delta= 10.22 (s, 1H, O=CH), 9.35 (d, J_{H,H} = 6.6 Hz, 2H, Ar-H), 8.50 (d, J_{H,H} = 6.2 Hz, 2H, Ar-H), 4.84–4.56 (dq, J_{H,H} =7.3 Hz, 2H, -CH\(_2\)), 1.54 (dt, J_{H,H} = 7.3, 5.9 Hz, 3H, -CH\(_3\)); \(^{13}\)C-NMR (75 MHz, DMSO-d6) \(\delta= 190.0, 161.4, 146.4, 144.4, 126.4, 124.9, 87.5, 57.0\(_{CH2}\), 55.9\(_{CH2}\), 16.2\(_{CH3}\); ESI-MS: calcd m/z = 136.17 (M\(^+\)), found m/z = 136.0 (M\(^+\)).

1-Benzyl-4-formyl-pyridinium chloride 12

RTIL 12 was synthesised from pyridine-4-carboxaldehyde (4.4 mL) by alkylating with excess benzyl chloride (10.8 mL) in DCM and heating to 50-60 °C for 24 hrs. The DCM and excess alkylhalide was removed by evaporation to afford 12 in qualitative yield as a RTIL; \(^1\)H-NMR (300 MHz, DMSO-d6) \(\delta= 9.37 (d, J_{H,H} = 6.9 Hz, 2H, Ar-H), 8.48 (d, J_{H,H} = 6.7 Hz, 2H, Ar-H), 7.61-7.36 (m, 5H, Ar-H), 5.96 (s, 2H, -CH\(_2\)); \(^{13}\)C-NMR (75 MHz, DMSO-d6) \(\delta= 168.6, 151.3, 139.2, 134.5, 134.2, 132.9, 53.8\(_{CH2}\); ESI-MS: calcd m/z = 198.24 (M\(^+\)), found m/z = 198.1 (M\(^+\)).

**Poly-(4-vinylpyridine)**

\(N\)-Ethyl-(4-Polyvinylpyridinium iodide) 13

Ionic salt 13 was synthesised from 0.5 g of 4-PVP following the general alkylation procedure using excess ethyl iodide (500 \(\mu\)L) in 2 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 13 as a a fluorescent yellow/green solid in ~72 % yield (w/w) (0.74 g): mp >350 °C; \(^1\)H-NMR (300 MHz, DMSO-d6) \(\delta= 8.88 (b, Ar-H), 7.96 (b, Ar-H), 4.56 (b, -CH\(_2\)),...
3.38 (b, -CH), 1.59(b, -CH3); \(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 162.3_{(q)}, 144.2, 126.8, 55.8_{(CH2)}, 34.4_{(CH)}, 15.8_{(CH3)}\).

\(\textit{N}-\text{Benzyl-(4-polyvinylpyridinium chloride) 14}\)

Ionic salt 14 was synthesised from 0.5 g of 4-PVP following the general alkylation procedure using excess benzyl chloride (658 \(\mu\)L) in 2 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 14 as a brown solid in \(~66\) % yield (w/w) (0.61 g): mp >350 °C; \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 9.40 \text{ (b, Ar-H)}, 8.59 \text{ (b, Ar-H)}, 7.74 \text{ (b, Ar-H)}, 7.42 \text{ (b, Ar-H)}, 6.07 \text{ (CH2)}, 3.66 \text{ (CH)}; \(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 163.6_q, 144.5, 134.3_q, 129.1, 62.1_{(CH2)}, 34.0_{(CH)}\).

\(\textit{N}-\text{Butyl-(4-polyvinylpyridinium bromide) 15}\)

Ionic salt 15 was synthesised from 0.5 g of 4-PVP following the general alkylation procedure using excess \(n\)-bromobutane (615 \(\mu\)L) in 2 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 15 as a purple solid which turned brown upon exposure to air in \(~64\) % yield (w/w) (0.61 g): mp >250 °C; \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 9.10 \text{ (b, Ar-H)}, 8.42 \text{ (b, Ar-H)}, 8.05 \text{ (b, Ar-H)}, 4.62 \text{ (b, -CH2)}, 3.46 \text{ (b, -CH)}, 1.94 \text{ (b, -CH2)}, 1.31 \text{ (b, -CH2)}, 0.90 \text{ (b, CH3)}; \(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta 1= 63.0_{q}, 144.5, 127.0, 59.6_{(CH2)}, 34.2_{(CH)}, 32.3_{(CH2)}, 18.9_{(CH2)}, 13.4_{(CH3)}\).

\(\textit{N}-\text{Ethyl-(4-polyvinylpyridinium iodide) 2 \% cross-linked 16}\)

Ionic salt 16 was synthesised from 1.0 g of 2% cross-linked 4-PVP following the general alkylation procedure using excess ethyl iodide (\(\mu\)L) in 10 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 16 as a brown glass; yield: 1.7 g (\(~81\) %): mp >350 °C; FT-IR (KBr); 3121, 3038, 2975, 2934, 1970, 1841, 1715, 1640, 1601, 1570, and 1516 cm\(^{-1}\).
$N$-Benzyll-(4-polyvinylpyridinium chloride) 2 % cross-linked 17

Ionic salt 17 was synthesised from 0.5 g of 2 % cross-linked 4-PVP following the general alkylation procedure using excess benzyl chloride (657 μL) in 2 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 17 as a brown solid in ~77 % yield (w/w) (0.71 g): mp >350 °C; FT-IR (KBr); 3120, 3040, 2701, 2410, 2106, 1984, 1842, 1638, 1569, 1515 cm⁻¹.

$N$-Butyl-(4-polyvinylpyridinium bromide) 2 % cross-linked 18

Ionic salt 18 was synthesised from 0.5 g of 2% cross-linked 4-PVP following the general alkylation procedure using excess $n$-bromobutane (615 μL) in 2 mL DMF. However, instead of removing the DMF by bulb-bulb distillation, the product was precipitated by addition of acetone to afford 18 as a solid in ~68 % yield (w/w) (0.65 g): mp >350 °C; FT-IR (KBr) 3121, 3012, 2961, 2937, 2874, 2744, 2619, 2059, 1866, 1640, 1570, 1516 cm⁻¹.
2.2 Synthesis of 2,4,5-triphenylimidazolium ionic liquids

2.2.1 Introduction

The synthesis of enantiomerically pure compounds is an important and an intensely pursued undertaking in organic chemistry.\textsuperscript{10} This increasing need for pure enantiomers is coupled with an increasing awareness of the differences in molecular interaction between living organisms and two enantiomeric substances which result from the chirality of receptors, enzymes and other functionalities sited in living organisms. Chemical and pharmaceutical companies are obligated to demonstrate and investigate the differences in activity of the stereoisomers of racemic drugs and other bioactive compounds for environmental and commercial reasons.\textsuperscript{11} This need to analyse and investigate the individual enantiomers of a widening array of chiral compounds has lead to an increase in demand for a wider range of chiral selectors for analysis and/or separation of chiral compounds. The demand can in part be met by enantioselective gas chromatography (GC) and the design of new chiral selecting agents for utilisation in stationary phases in order to separate enantiomers.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.13.png}
\caption{3 step ring opening of trans-2,4,5-triphenylimidazoline to produce (R,R) and (S,S) enantiomers of stilbenediamine. 1) DCC, DCM. \(-20\,^{\circ}\text{C to r.t, 5 hrs.}\) 2) HCl, H\(_2\)O, THF, reflux 2 hrs. 3) HBr, AcOH, reflux 24 hrs.\textsuperscript{12}}
\end{figure}
On the other hand, the design of catalysts which provide high stereoselectivity in reactions is an emerging field of modern organic chemistry. For this purpose, researchers are carrying out extensive studies to discover new complex forming ligands which can be easily modified. From this point of view, 1,2-ethylenediamine derivatives have generated considerable interest. The enantiomers of these diamines have been employed for stereoselective synthesis in various reactions. Enantiopure C2-symmetrical 1,2-diphenylethane-1,2-diamines (stilbenediamine Figure 2.13), (1S,2S) and (1R,2R), and their N-modified derivatives have been widely incorporated into diverse reagents for highly asymmetric dihydroxylation, allylation, propargylation and aldol reactions. Moreover, they have been used as ligands in important catalytic asymmetric processes such as ruthenium-catalysed hydrogenation of ketones, manganese-catalysed epoxidation of alkenes, aluminium-catalysed Diels–Alder reactions and magnesium-catalysed merged enolisation-aminations. However, the range of vicinal diamines is presently limited to 1,2-diphenyl-ethylene-1,2-diamine (stilbenediamine Figure 2.13) and their derivatives. Since a general method for the synthesis of vicinal diamines is lacking.

The utility of the (R,R) and (S,S) enantiomers of stilbendiamine in asymmetric synthesis has stimulated the development of a number of new processes for their preparation. As the resolution of racemic (+/-)-stilbenediamine can be carried out efficiently to produce the two pure enantiomers, the principal need is for a very simple and economical synthesis of the racemic stilbenediamine. The most effective process by which these diamines can be produced is via the synthesis of amarine and iso-amarine (cis- and trans-2,4,5-triphenylimidazoline). Extensive research has gone into devising methods for the synthesis of cis- and trans-2,4,5-triarylimidazolines from which 1,2-diaryl-ethylene-1,2-diamines can be derived via ring opening of the imidazolines to produce derivatives of vicinal diamines (Figure 2.13). However, we have foreseen the potential to exploit these chiral imidazolines for the synthesis of new chiral imidazoline ionic liquids.
Very few chiral imidazolinium ionic liquids have been reported.\textsuperscript{5,6} Examples of this emerging class of chiral imidazoline based ionic liquids remain scarce compared to other types of chiral ionic liquids.\textsuperscript{7,8} Here, the synthesis of new chiral 2,4,5-triphenylimidazoline based ionic salts from \textit{trans} 2,4,5-triphenylimidazoline (\textit{iso}-amine) and their potential application as chiral selecting agents was investigated.

\subsection*{2.2.2 Results and Discussion:}

\textit{trans} (+/-)-2,4,5-Triphenylimidazoline (\textit{iso}-amine) captured our interest because of its inherent stereochemistry, it has two chiral centers and is synthesised as a racemic mixture consisting of (RR) and (SS) enantiomers. From these enantiomers there is the potential for application in the synthesis of new enantiomerically pure chiral ionic salts. The \textit{iso}-amine was synthesised from benzaldehyde, the enantiomers isolated, and ultimately utilised in the synthesis of new chiral \textit{trans} (4R5R)-2,4,5-triphenylimidazolinium ionic liquids. As well as the synthesis of 2,4,5-triphenylimidazole based ionic salt analogues of the 2,4,5-triphenylimidazolines.

\textbf{Hydrobenzamide} (1,3,5-triphenyl-2,4-diazapentadiene) \textsuperscript{19}

Research began with the synthesis of 1,3,5-triphenyl-2,4-diazapentadiene (hydrobenzamide \textsuperscript{19}, see Figure 2.16), from readily available benzaldehyde. Hydrobenzamide is an important intermediate because the two imidazolines (\textsuperscript{20} and \textsuperscript{21}) and imidazole \textsuperscript{22}, the precursors required to synthesise ionic salts, can be directly synthesised from hydrobenzamide in a one-step reaction by varying the reaction conditions and in the case of \textsuperscript{20} and \textsuperscript{21} with high stereo-specificity. The versatility of hydrobenzamide and the high stereospecificity with which it can be converted to \textsuperscript{20} and \textsuperscript{21} makes it a highly desirable starting material from which to synthesis the desired imidazolines and imidazoles.

There are various methods for synthesising amarine from hydrobenzamide, but the simplest and cleanest method was described by Williams and Bailar\textsuperscript{27} whereby
hydrobenzamide 19 is converted to amarine in high yields. Hydrobenzamide was synthesised by the addition of distilled benzaldehyde to excess liquid ammonia at -78 °C, and the solution allowed to warm to room temperature.27 When the mixture was left to stand at room temperature the ammonia evaporated leaving the product as a white crystalline solid which was used without further purification. However, if required, 19 can be further purified by recrystallisation from cyclohexane to afford the purified crystalline product in 89 % yield.

Amarine (cis-2,4,5-triphenylimidazoline) 20

As shown in Figure 2.16, amarine was prepared by cyclising 19 either with a strong base or under thermal conditions. Under thermal conditions amarine was prepared by heating neat hydrobenzamide between 90-120 °C in a kugelrohr under negative pressure (10⁻² Torr) for 4-6 hrs.30 The resultant product was obtained in high yield (98 %) in a very pure state and could be used without further purification for synthesising iso-amarine. Treatment of hydrobenzamide with a strong base such as n-butyllithium or potassium-t-butoxide (t-BuOK) afforded amarine in excellent yields (Table 2.2). Base catalysed cyclisation of hydrobenzamide with 0.4 N potassium-t-butoxide in t-butanol at 60 °C for 1 hour led to immediate conversion to amarine, with isomerization occurring with a very high degree of stereospecificity to give exclusively the less stable isomer amarine via an anionic intermediate.31 The stereospecific formation of 2,4,5-triphenylimidazolines occurs via an electrolytic pericyclic ring closure which involves the cyclisation of the conjugated polyene hydrobenzamide via a 2,4-diazapentadienyl anion. The intermediacy of a carbanion was suggested by the observation of a deep blue colour upon treatment of hydrobenzamide in tetrahydrofuran with 0.4M n-butyllithium at -78 °C.31 Upon warming to room temperature the blue colour faded to a light yellow appearance.

The anionic intermediate shown in Figure 2.14 accounts for the interconversion between amarine and iso-amarine.31 Although multiple geometries are available for the anionic
intermediate, the most stable geometry required for cyclization is a U-shape. Conrotatory cyclization (maintaining an axis of symmetry) of this ionic intermediate leads to iso-amarine while the disrotatory motion (plane of symmetry) produces amarine.

**Figure 2.14:** Anion intermediate U-shape

Pericyclic reactions occur by a concerted process in which all bonding changes occur simultaneously in a single step. For bond formation to take place, the outermost $n$-bond must rotate so that favourable bonding interaction is achieved, a positive lobe with a positive lobe, or a negative lobe with a negative lobe. If two lobes of like signs are on the same side of the molecule, the two orbitals rotate in the opposite directions. This kind of motion is referred to as disrotatory. Conversely, if lobes of like sign are on opposite sides of the molecule, both orbitals rotate in the same direction, either clockwise or counter clockwise by conrotatory motion.

Simple orbital symmetry considerations lead to the prediction that a disrotatory process is preferred for the ground electronic state of the carbanion. Thus orbital symmetry control in the cyclization of the U-shaped anion is consistent with the observed dominant formation of the less stable amarine. Orbital symmetry further predicts that a planar $n$, $n^*$ state would preferentially produce iso-amarine by conrotatory motion.

**Figure 2.15:** Pericyclic ring closure cyclisation mechanism.
**Iso-amine** (*trans*-2,4,5-triphenylimidazoline) **21**

In addition, to being prepared from hydrobenzamide **19**, *iso*-amine **21** can also be prepared from isomerisation of its meso isoform amarine **20** as illustrated in Figure 2.16 (a). By changing the reaction conditions (basic or thermal) one can obtain either the *cis* or *trans* isomer as shown in Figure 2.16. Under basic conditions amarine is isomerised into *iso*-amine (Figure 2.16 (b)) by deprotonation by a strong base (such as *t*-BuOK in *t*-BuOH or NaOH in diethylene glycol) forming a delocalised benzylic anion intermediate, which then cyclises by a conrotatory pericyclic ring closure to give *trans*-2,4,5-triphenylimidazoline as a racemic mixture of *SS* and *RR* enantiomers. Isomerisation can be accomplished by boiling *cis*-2,4,5-triphenylimidazoline in a solution of sodium hydroxide, water, and diethylene glycol at 155 °C for 45 minutes during which a sodium salt of *iso*-amine precipitates forming a thick slurry (Figure 2.16 (d)). The slurry was allowed to cool then treated with glacial acetic acid dissolving most of the product. 95 % Ethanol was then added and the solution was heated under reflux. Then allowed to cool and neutralised with excess concentrated ammonia to afford the product as a tan crystalline precipitate in 62 % yield. Alternatively, iso-amine can be synthesised directly from Hydrobenzamide by heating at 60 °C with *t*-BuOK in THF for 2 hrs in 88 % yield (Figure 2.16 (c)).

*Iso*-amine was also prepared from hydrobenzamide **19** on a smaller scale by microwave irradiation of **19** at 500 W with 1.0 molar equivalent of *t*-BuOK in *t*-BuOH for 5 minutes produced *iso*-marine **21**, and amarine **20** was produced upon irradiation for 2 minutes, thereby drastically reducing the reaction times required by conventional heating from 6 hours to just minutes. **35**
Figure 2.16: Synthesis of hydrobenzamide 19 from benzaldehyde in liquid ammonia, and the cyclisation to 2,4,5-triphenylimidazolines 20 and 21. a) Benzaldehyde in excess liquid ammonia at -78 °C, b) kugelrohr 120 °C for 6 hrs at 10^{-2} Torr, c) t-BuOK in anhydrous THF at 60 °C 2 hrs, d) Diethylene glycol H_2O/NaOH at 155 °C for 45 mins, e) Swern oxidation, and f) Kugelrohr 140-160 °C for 5 hrs at 10^{-2} Torr.

Without a doubt the quickest method of preparing amarine and *iso*-amarine in small scale is by microwave irradiation. This method has proved to be quick, 2 minutes for amarine and 5 minutes for *iso*-amarine, highly stereospecific and affords the products in good yield (67%).

However, the most effective and efficient method of preparing amarine and *iso*-amarine on large scales is via conventional heating under basic conditions. Although the reaction times take 1-6 hours, the product is formed in high yield with high stereospecificity. The thermal cyclisation of 19 was also found to be a very clean and efficient method of synthesising amarine on larger scales. With microwave irradiation the *cis*:trans product
ratio can vary with scale up of the reaction, understandably due to the very short time in which the reactions are run. Isomerisation of cis to trans is dependant on the base used for the cyclisation, the reaction temperature, and, for microwave irradiation, the reaction time.

**Product characterisation**

2,4,5-triphenylimidazolines have been studied extensively with respect to structure elucidation.\(^{27}\) The cis- and trans- isomers can be readily identified from their C4 and C5 proton \(^1\text{H}\) NMR signals. The proton signals from C4 and C5 differ for the cis- and trans-isomers whereby the cis-2,4,5-triphenylimidazoline proton signal is downfield shifted at \(\delta\) 5.31 ppm compared to trans-2,4,5-triphenylimidazoline at \(\delta\) 4.79 ppm, in addition their C4 and C5 \(^1\text{H}\) chemical shift are \(\delta\) 70.60 ppm and \(\delta\) 74.62 ppm, respectively. The determined configuration of the imidazoline isomers from NMR spectrum were validated by the observed differences in their melting point temperatures, which is higher for the trans-isomer (202 °C) compared to cis-isomer (129-130 °C), which is consistent with the literature (198-201 °C and 128-131 °C, respectively).\(^{27}\)

Once the target compound (+/-) iso-amarine was synthesised, the next challenge was to isolate the individual enantiomers (RR) and (SS). The most desirable way for obtaining enantiomerically pure substances is by the enantioselective synthesis. However, this is not always feasible as was the case with iso-amarine. Fractional crystallization is an efficient and practical method for obtaining optically pure compounds from a conglomerate mixture of chiral compounds. Diastereomeric crystallization is a classic method and also the most widely applied for resolving racemic compounds. Acidic or basic racemates such as carboxylic acids and amines, are most applicable for this method as they readily react with an optically active amine or carboxylic acid counterpart to afford a pair of diastereomeric salts. Here, (S)-Mandelic acid was used as the enantiopure resolving agent.
Figure 2.17: Fractional crystallization: a) Reflux (+/-) iso-amarine and (S)-mandelic acid in iso-propanol for 1 hr. b) Cool to 0 °C for crystallization and salt isolation. c) Treat with 1N aqueous NaOH to remove mandelic acid.
When racemic iso-amine 21 was refluxed with (S)-mandelic acid, a pair of diastereomeric salt derivatives formed (Figure 2.17). These diastereoisomers differ in their chemical and physical properties, therefore, the diastereomeric salt crystals could be separated by fractional crystallization and their respective iso-amine enantiomers were recovered by decomposing by treatment with an aqueous solution of NaOH.

**Imidazoline ionic salts**

Once the SS and RR enantiomers of iso-amine were isolated, they were then transformed into ionic salts by alkylation with an alkyl halide. Under a nitrogen atmosphere to a stirring solution of imidazoline in dry DMF was added excess alkyl halide and heated for 6 hrs at 90 °C. The resulting solution was concentrated in vacuo. The remaining residues of DMF were removed by bulb-to-bulb distillation using a kugelrohr apparatus to afford the ionic salt as a glass and further purified by re-crystallisation from ethyl acetate and n-hexane to afford a white crystalline salt.

**Table 2.2**: Melting points and yields of the imidazolines synthesized and their ionic salts.

<table>
<thead>
<tr>
<th>Compounds*</th>
<th>mp °C</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>129-132</td>
<td>98*</td>
</tr>
<tr>
<td>23</td>
<td>202-204</td>
<td>91</td>
</tr>
<tr>
<td>24</td>
<td>228-233</td>
<td>74</td>
</tr>
<tr>
<td>25</td>
<td>144-46</td>
<td>83</td>
</tr>
<tr>
<td>21</td>
<td>202</td>
<td>88</td>
</tr>
<tr>
<td>26</td>
<td>78-80</td>
<td>98</td>
</tr>
<tr>
<td>27</td>
<td>190-3</td>
<td>85</td>
</tr>
<tr>
<td>28</td>
<td>79-81</td>
<td>92</td>
</tr>
</tbody>
</table>
Thermal cyclization reaction at 120 °C under vacuum in a kugelrohr. *Refer to Figure 2.21 for their chemical structures.

\[ \text{(RR)-21} \xrightarrow{\text{RX/ DMF}} \text{(RR)-26-28} \]

**Figure 2.18:** Alkylation reaction, heat imidazoline with excess alkyhalide in DMF at 90 °C for 6 hrs.

From the resonance structures (Figure 2.19) of 2,4,5-triphenylimidazoline the imine nitrogen acts as an electron withdrawing group (EWG) by resonance effect through the \( n \)-bond delocalisation, stabilising the resultant anionic nitrogen thus, increasing the basicity of the amine making it a stronger nucleophile.

**Figure 2.19:** Resonance structures of (R,R)-2,4,5-triphenylimidazoline 21.

Substituents with electron-withdrawing resonance effect on a benzene ring have the general structure illustrated in Figure 2.20 with a more electronegative \( Z \) atom attached to a less electronegative \( Y \) atom.
**Figure 2.20:** General structure of substituent with electron-withdrawing group attached to a benzene ring.

As a consequence the nitrogen acts as an EWG by resonance effects, when one equivalence of alkylhalide is used in the alkylation reaction a mixture of di- and mono-alkylated imidazolines are formed. The reaction proceeds to the di-alkylated product due to the increased resonance/nucleophilicity of both N atoms. The mono-substituted product can be isolated by chromatography separation from the mixture but in relatively low yield. As a result of the low yield the mono-substituted product was not isolated and only the \(N,N'\)-dialkyl-2,4,5-triphenylimidazolinium ionic salts were synthesised (Figure 2.21).
Figure 2.21: Ionic compounds synthesised from alkylation of the 2,4,5-triphenylimidazoline 20 and 21, and lophine 22. (* synthesised with t-BuOK in anhydrous THF at 60 °C 2 hrs)

NMR analysis of cis N,N'-diethyl-2,4,5-triphenylimidazolinium iodide ionic liquid 23 reveals a symmetrical structure (Figure 2.22). The NMR spectrum in the aliphatic region can be explained if the methylene (CH₂) protons are diastereotopic (that is chemically and magnetically non-equivalent) due to the two chiral carbons in the imidazoline ring. Geminal coupling of protons on a saturated carbon having different chemical shifts commonly occurs in a chiral molecule with a tetrahedral stereocenter adjacent to a methylene group. The geminal protons are labeled Hₐ and Hₖ and have chemical shifts of δ 3.15 ppm and δ 3.37 ppm appearing as sextets (Figure 2.22). These protons split each other into doublets which are then split by methyl CH₃ protons into quartets with coupling constants 14.4 Hz and 7.3 Hz. The larger coupling constant is typical of geminal coupling and the small split typical of vicinal coupling. The sextet observed is actually 2 overlapping quartets while the methyl group is a triplet with a coupling constant of 7.3 Hz.

The structure of N,N'-diethyl-2,4,5-triphenylimidazolinium iodide 23 is more accurately depicted as a resonance form, in which each of the nitrogens in the imidazoline moiety share half the positive charge making the molecule symmetrical. This symmetry is observed through the chemical shift equivalences observed for both ethyl groups in both the 13C- and ¹H-NMR spectra, as well as the single peak observed for both the C₄ and C₅ methine carbons at δ 67.6.
Figure 2.22: NMR spectrum of cis-(+/-)-N,N-diethyl-2,4,5-triphenylimidazolinium iodide 23 in CDCl$_3$. iv) $^1$H-NMR spectra with an expansion of the apparent sextets (which are actually overlapping double quartets), i) $^{13}$C-NMR spectra showing DEPT135 (iii) and DEPT90 (ii) experiments.

This is confirmed by $HH$-COSY in which methylene $H_a$ and $H_b$ protons are correlated to one another as well as to the methyl protons of the ethyl substituent (Figure 2.23).
Figure 2.23: 2D NMR spectra of cis N,N-diethyl-2,4,5-triphenylimidazolinium iodide in CDCl₃. Determination of HH connectivity by HH-COSYGPSW analysis.

These chiral ionic salts have a symmetrical structure, thus to reduce this symmetry a more selective method for the differential substitution of the two amines was employed. As it has proved to be a challenge to selectively alkylate the secondary amine on 2,4,5-triphenylimidazolines without alkylating the tertiary (imine) amine, an alternative method of differential substitution of amine using a more selective method typically employed for forming peptide bonds was adopted.

**N-acyl-N’-alkyl-trans-2,4,5-triphenylimidazolines**

A very useful method of forming peptide or other amide bonds was reported by Sheenan and Hess in 1955.³⁷ The two components, one containing a free carboxyl function and the other a free amino group, couple directly and rapidly in high yield on treatment with
$N,N'$-dicyclohexylcarbodiimide at room temperature. DCC is a highly selective dehydrating coupling agent and this remarkable selectivity is attested to by the successful use of carbobenzoxyserine as an acylating moiety without the need to protect its hydroxyl group. In addition, no racemization has been detected when employing DCC as the coupling agent with optically active amino acids. This is also advantageous when using enantiomerically purified iso-amarine as the starting material as this would permit the differential substitution of the amine without racemisation of iso-amarine taking place during the reaction.

This method was used for the enantiomeric separation of trans-(+/-)-2,4,5-triphenylimidazoline by forming diastereoisomers with enantiomerically pure (S)-amino acids as illustrated in Figure 2.24. Not only was this method used for the separation of the (RRS)-32b and (SSS)-32a diastereoisomers of trans-2,4,5-triphenylimidazoline, but, it was also applied as a tool for asymmetrically derivatising the enantiomerically purified trans-(4R,5R)-2,4,5-triphenylimidazoline permitting the synthesis of a wide variety of novel imidazolines with various mono-$N$-substituents to be synthesised from one isomer of iso-amarine (Figure 2.26). These compounds are not only desirable for the synthesis of new asymmetrically substituted trans-(4R,5R)-2,4,5-triphenylimidazolinium ionic salts, but, they may also possess potential applications as imidazoline-based drugs for various therapeutic targets.

To a solution of (+/-)-trans 2,4,5-triarylimidazoline and $N$-acyl amino acid (or any carboxylic acids) in DCM at -78 °C under nitrogen was added $N,N'$-dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to warm to room temperature and left to react for 5 hours. During which the carbodiimide precipitated out of solution as a white solid which was then removed by filtration. The filtrate was collected and DCM was removed under reduced pressure to afford the crude product as a solid mixture of imidazoline diastereoisomers at 96 % yield. The diastereoisomers were isolated by column chromatography (46 % yield).
**Figure 2.24:** Selective acylation of the secondary amine of iso-amarine. DCC mediated acylation reaction with an N-acetylphenylalanine to form the diastereoisomers 32a and 32b plus N,N’-dicyclohexylurea, which were isolated by flash chromatography.

Figure 2.25 illustrates how dicyclohexylcarbodiimide was used to convert the carboxylic acid from an amino acid into a reactive acylating agent which could then undergo nucleophilic acyl substitution with amines. The carboxylic acid from the amino acid first adds to one of the double bonds of the carbodiimide to give O-acylisourea which is a very reactive acylating agent resembling carboxylic acid anhydrides. Then the nucleophilic amine from the imidazoline attacks at the carboxyl carbon on the O-acylisourea yielding a tetrahedral intermediate. The intermediate then dissociates to an amide losing N,N’-dicyclohexylurea as a co-product. The co-product, N,N’-dicyclohexylurea, has a very low solubility in most organic or aqueous solvents, and is easily separated by precipitation.
**Figure 2.25:** Reaction mechanism for the nucleophilic acyl substitution with *trans* (+/-)-2,4,5-triphenylimidazoline.

Using this method it was possible to couple the carboxylic acids of N-acetylphenylalanine, N-benzyolhistidine derivatives, and (S)-mandelic acid to *trans*-(4R,5R)-2,4,5-triphenylimidazoline. Glutamic acid having two carboxylic acid groups allowed for the coupling of 2 imidazolines to the one amino acid to afford the bis-2,4,5-triphenylimidazoline 39 (Figure 2.27).
The tertiary amines of the mono-\(N\)-substituted \(\text{trans-2,4,5}\)-triphenylimidazolines were then alkylated with an alkylhalide by refluxing in DMF at 90 °C to afford differentially substituted \(\text{trans-2,4,5}\)-triphenylimidazoline ionic salts.
Following the successful synthesis of chiral cis- and trans-2,4,5-triphenylimidazoline based ionic salts we endeavoured to synthesise their achiral imidazole based ionic liquid analogues from lophine (2,4,5-triphenylimidazole). These achiral analogues would be particularly interesting for studying the effect of the chiral centres if these novel imidazolines are found to possess potential applications as imidazoline-based drugs for therapeutic targets.

Figure 2.27: Differentially substituted trans-2,4,5-triphenylimidazoline ionic salts.
**Lophine**

Lophine (2,4,5-triphenylimidazole) was synthesised from amarine by Swern oxidation (Figure 2.28) or under thermal conditions.\(^3\) The overall transformation that takes place in the formation of lophine is an oxidation of the imidazoline ring [elimination of 2 H atoms]. However, given that H is not a good leaving group, either the C or N of cis-2,4,5-triphenylimidazoline must have an attached H replaced by a leaving group before an elimination reaction can take place. Given that the N of the imidazoline is nucleophilic under basic conditions, the elimination reaction can take place first by attachment of a leaving group to N of the imidazoline, and then proceeds to a retro-hetero-ene elimination reaction.

![Figure 2.28: A scheme for the synthesis of Lophine by Swern oxidation and the resultant side products.](image)

When 2,4,5-triphenylimidazoline is treated with a reaction mixture derived from oxalyl chloride, DMSO, and triethylamine (Et\(_3\)N), it is oxidized. And it is important to note that the order of addition of these reagents is critical. The role of oxalyl chloride is to activate the DMSO. Activation of DMSO with oxalyl chloride produces an unstable chlorosulfonium salt intermediate (an electrophile) which ultimately becomes a leaving group when attached to the imidazoline N.

First oxalyl chloride was added to DMSO at -60 °C. DMSO is nucleophilic on the oxygen and reacts with the electrophilic oxalyl chloride by addition-elimination displacing a chloride (Figure 2.29).
Figure 2.29: Addition-elimination reaction of DMSO with oxalyl chloride to form the dimethylchlorosulfonium chloride intermediate plus CO₂ and CO gases.

The sulfur now having a good leaving group is attacked by the eliminated Cl⁻, displacing the oxalate, which decomposes to give off CO₂, CO and produces dimethylchlorosulfonium chloride, in which the sulfur is a good electrophile. Then 2,4,5-triphenylimidazoline and Et₃N were added, and the reaction mixture was allowed to warm to room temperature. The imidazoline N-H is deprotonated by Et₃N and the resultant nucleophilic N anion attacks the sulfur on dimethylchlorosulfonium chloride, displacing the chloride forming a sulfonium ion intermediate (Figure 2.30).

Figure 2.30: The nucleophilic attack by the imidazoline N anion on the electrophilic dimethylchlorosulfonium chloride to form the bis-sulfonium salt intermediate.
Now there’s a good leaving group attached to the nitrogen of the imidazoline, so elimination ensues. In the currently accepted mechanism, the elimination ensues by a retro-hetero-ene reaction (Figure 2.31).

Retro-hetero-ene reaction is a subclass of an ene reaction, one of four major classes of pericyclic reactions. Retro-hetero-ene reactions are used in some elimination procedures, which occurs through a concerted retro-ene mechanism. These reactions are driven by the gain in entropy, by charge neutralisation and sometimes by cleavage of a weak σ-bond (eg N-S) in favour of a C=C π-bond. All retro-hetero-ene reactions involve a 1,2-dipole such as a sulphur ylide, whereby the CH₃ attached to sulphur is deprotonated by Et₃N to give a sulphur ylide. Ylides are neutral dipolar molecules that have two oppositely charges atoms directly bonded to each other, each with an octet of electrons. The electron distribution of the ylide is highly polarised towards the carbon making the carbon electron rich. The carbon has much of the character of a carbanion and acts as a nucleophile toward the H in the imidazoline ring. The nucleophilic carbon attacks the imidazoline ring proton creating a five membered ring transition state. The ensuing reaction result in the formation of a new C=N and the decomposition of the sulphur ylide by cleavage of the N-S σ-bond releasing 2 molecules of dimethylsulfide (Me₂S).

The tertiary nitrogen also acts as a nucleophile, which also attacks the dimethylchlorosulfonium chloride displacing the chloride forming the second sulfonium ion intermediate (Figure 2.31). Elimination ensues via the retro-hetero-ene reaction, to afford the imidazole product lophine in 74 % yield (mp 272-273 °C) and Me₂S, CO₂, CO, and Et₃NH⁺Cl⁻ as side products.
Lophine can also be synthesised under thermal conditions from amarine by heated to 160 °C for 6 hrs under negative pressure (0.01-0.05 mbar) to afford lophine as the dehydrogenation product,\textsuperscript{30} as well as via various methods by microwave irradiation.\textsuperscript{39-43} Lophine was then alkylated with excess alkylhalide by refluxing in DMF at 130-140 °C to afford the \(N,N\)-dialkyl-2,4,5-triphenylimidazolium ionic liquids 29-31 (Figure 2.21) in 75-84 % yields.

### 2.2.3 Conclusion

The synthesis of new 2,4,5-triphenylimidazoline based ionic salts from \(cis\)- (20) and \(trans\)-(+/-)-2,4,5-triphenylimidazoline (21) have been prepared and the individual \((RR)\) and \((SS)\) enantiomers of \(trans\)-2,4,5-triphenylimidazoline 21 separated by fractional crystallisation with (S)-mandelic acid. From the enantiomerically purified \((4R,5R)\)-2,4,5-triphenylimidazoline 21, \((4R,5R)\)-\(N,N\)-dialkyl-2,4,5-triphenylimidazolinium halide ionic liquids were synthesised by alkylation with alkyl halides. The reaction proceeded to the \(N,N\)-diakylated rather than the monoalkylated product due to the increased nucleophilicity of the tertiary amine.
To overcome the problem of imidazoline $N,N'$-dialklylation DCC mediated peptide bond formation method was used for differentially derivatising the $\text{trans}-(+/-)-2,4,5$-triphenyimidazoline 21 nitrogens with the amino acid $N$-acetylphenylalanine followed by the separation and isolation of the resultant $(RRS)-32b$ and $(SSS)-32a$ diastereoisomers. This method was applied both as a tool for selective derivation as well as a means for the formation of diastereoisomers from $\text{trans}-(+/-)-2,4,5$-triphenyimidazoline 21 which can be isolated by column chromatography. Using this method a large variety of imidazolines were synthesised, compounds which may also possess potential applications as imidazoline-based drugs for various therapeutic targets. The chiral ionic salts subsequently synthesised from these imidazolines hold potential for application in biological, chemical, or industrial settings. Their potential application as chiral selecting agents for GC station phases will be explored in chapter 3.
2.2.4 Experimental

2.2.4.1 Reagents:

$N$-Benzoyl-(L)-histidine, $N$-acetyl-(L)-phenylalanine, ammonia (gas), concentrated aqueous ammonia, benzaldehyde, benzyl chloride, $n$-bromobutane, cyclohexane, diethylene glycol, dicyclohexylcarbodiimide (DCC), DCM, DMF, 95 % ethanol, ethyl acetate, ethyl iodide, glacial acetic acid, $n$-hexane, iso-propanol, $(S)$-$(+)$-mandelic acid, methanol, MgSO$_4$, Na$_2$CO$_3$, oxalyl chloride, potassium-$t$-butoxide, sodium hydroxide, THF, and triethylamine. All chemicals were purchased from Sigma Aldrich.

2.2.4.2 Instruments

Ionic salts were characterised by NMR, ESI-MS, and melting point. $^1$H and $^{13}$C-NMR data were recorded on a Bruker 300 spectrometer and the chemical shift values were recorded in ppm. All NMR structure elucidation was done from $^1$H-NMR, $^{13}$C-NMR, DEPT135, DEPT90, HSQCGP, and $HH$-COSYGPSW experiments. Mass spectroscopy data were obtained by Electro spray ionization (model) in positive ion mode (in 1:1 acetonitrile/H$_2$O). Melting points were measured with a Gillikhenamp Melting point apparatus (manufactures in England) and are uncorrected.

2.2.4.3 Synthesis:

Synthesis of $N,N'$-di(phenylmethylidene)phenylmethane (Hydrobenzamide) 19

Hydrobenzamide 19 was prepared by the procedure as outline by Williams and Bailar.$^{27}$ Distilled benzaldehyde (10 g, 0.094 M) was added to an excess amount of liquefied ammonia at -78 °C. The solution was stirred and allowed to warm to room temperature. The mixture was allowed to stand until the ammonia evaporated, and the crude product was purified by recrystallization from cyclohexane to afford 19 as a white crystalline solid in good yield: 8.9 g (95 %): mp 97-100 °C; $^1$H-NMR (300 MHz, CDCl$_3$-d) $\delta$= 8.64 (s, 2H,
N=CH), 7.90 (m, 4H, Ar-H), 7.65 (d, 2H, Ar-H), 7.60-7.33 (m, 9H, Ar-H), 6.72 (s, 1H, N=CH=N); $^{13}$C-NMR (75MHz, CDCl$_3$-d) δ = 160.8, 141.9$_q$, 136.1$_q$, 131.1, 129.8, 128.0, 127.9, 127.4, 92.7; ESI-MS: calcd $m/z$ = 299.39 (M+H$^+$), found $m/z$ = 299.2 (M+H$^+$).

Synthesis of cis-2,4,5-triphenylimidazoline (amarine) 20

Following the Chou et al.$^{30}$ procedure, hydrobenzamide (5 g, 16.76 mM) was cyclised to form 20 in a bulb-to-bulb distillation chamber by heating at 120 °C for 5 hrs at a constant pressure of $10^{-2}$ Torr to afford a yellow glass which was used in further reactions without further purification in good yield: 4.9 g (98 %): mp 129-132 °C; $^1$H-NMR (300 MHz, CDCl$_3$-d) δ = 7.87 (d, 2H, Ar-H), 7.48-6.81 (m, 13H, Ar-H), 5.31(s, 2H, NCH); $^{13}$C-NMR (75MHz, CDCl$_3$-d) δ = 164.7$_q$, 138.8$_q$, 131.3, 129.7$_q$, 128.8, 128.7, 128.4, 127.7, 127.5, 127.4, 126.9, 70.6; ESI-MS: calcd $m/z$ = 299.39 (M+H$^+$), found $m/z$ = 299.2 (M+H$^+$).

Synthesis of (+/-) trans-2,4,5-triphenylimidazoline (iso-amarine) 21

Racemic iso-amarine was prepared following the procedure as reported by Williams and Bailar.$^{27}$ A stirred solution of amarine (11.6 g, 0.039 M), diethylene glycol (7.9 mL), water (15 mL) and 2.5 g of sodium hydroxide was boiled in a beaker until temperatures reached 155 °C. The temperature was maintained at 155 °C for 45 minutes, during which time the sodium salt of iso-amarine precipitated and the solution becomes a thick slurry. The slurry was allowed to cool, followed by treatment with glacial acetic acid (5.7 g). When most of the product had dissolved, 95 % ethanol (25 mL) was added and the solution heated to boiling. After cooling, the solution was neutralized with excess concentrated aqueous ammonia. Iso-amarine precipitated slowly as a mass of light tan crystals, which was filtered and washed with cold 95 % ethanol to afford 21 in 62 % yield.

Alternatively, iso-amarine was prepared by a modified procedure of Lozinskaya et al.$^{35}$ in which hydrobenzamide (5 g, 16.76 mM) was dissolved in anhydrous THF (50 mL) and the solution placed under nitrogen. Potassium-t-butoxide (1.5 g, 13 mM) was added to the
vigorously stirring solution, resulting in an intense blue colour change. The mixture was heated to 60 °C for 24 hrs and the reaction stopped by the addition of a triple excess of cold water. The product was extracted with ethyl acetate dried with MgSO₄, and filtered. The solvent was removed under negative pressure to afford 21 as a white solid in good yield: 4.4 g (88 %): mp 202 °C; ¹H-NMR (300 MHz, CDCl₃) δ= 7.89-7.82 (m, J_H,H = 8.29, 6.97, 1.32 Hz, 2H, Ar-H), 7.46-7.14 (m, J_H,H = 8.29, 6.97, 1.32, 13H, Ar-H), 4.79 (s, 2H, NCH); ¹³C-NMR (75MHz, CDCl₃) δ = 163.3q, 143.1q, 131.4, 129.3q, 128.8, 128.7, 127.68, 127.6, 126.6, 74.6; ESI-MS: calcd m/z = 299.39 (M+H⁺), found m/z = 299.2 (M+H⁺).

**General N-alkylation procedure**

To a stirring solution of imidazoline in dry DMF under nitrogen was added an alkyl halide and heated for 6 hrs at 90 °C. The solution was concentrated by heating at 80 °C under negative pressure. The remaining residues of DMF were removed by bulb-to-bulb distillation using a kugelrohr. Further purification was carried out by recrystallisation from ethyl acetate and n-hexane.

**cis N,N'-diethyl-2,4,5-triphenylimidazolinium iodide 23**

23 was synthesised following the general alkylation procedure from amarine 20 (8.47 g) using 2.1 molar equivalent of ethyl iodide (4.8 mL) in 20 mL of DMF to afford 23 as a solid; yield: 12.46 g (91 %): mp 202-204 °C; ¹H-NMR (300 MHz, CDCl₃-d) δ= 8.25 (s, 1H, Ar-H), 7.71 (s, 4H, Ar-H), 7.14-6.97 (m, 10H, Ar-H), 6.45 (s, 2H, N=CH ), 3.37 (dq, ³J_HH = 14.37, 7.27 Hz, 2H, -CH₂), 3.15 (dq, ³J_HH =14.37, 7.27 Hz, 2H, -CH₂), 7.17 (t, ³J_HH =7.27 Hz, 6H, -CH₃); ¹³C-NMR (75MHz, CDCl₃-d) δ= 167.7q, 132.9, 131.5q, 130.4, 130.0, 129.0, 128.8, 128.6, 128.3, 127.5, 122.8q, 67.9, 41.9(CH₂), CH₃ 13.3(CH₃); ESI-MS: calcd m/z = 355.50 (M⁺), found m/z = 355.3 (M⁺).

**cis N,N'-dibenzyll-2,4,5-triphenylimidazolinium chloride [nnDBTPICl] 24**
was synthesised from 20 (5.0 g, 16.76 mM) following the general alkylation procedure using 2.1 molar equivalence of benzyl chloride (4.05 mL) in 20 mL DMF to afford 24 as a solid; yield: 1.28 g (74 %): mp 228-233 °C; 1H-NMR (300 MHz, CDCl$_3$) δ= 8.16 (m, 2H, Ar-H), 7.63 (m, 4H, Ar-H), 7.41-6.73 (m, 19H, Ar-H), 5.20 (s, 2H, -NC$_2$H), 4.58 (d, $^3$J$_{HH}$ =15.2 Hz, 2H, -CH$_2$), 4.34 (d, $^3$J$_{HH}$ =15.2 Hz, 2H, -CH$_2$); 13C-NMR (75MHz, CDCl$_3$) δ= 168.0$_{q}$, 133.7$_{q}$, 133.0, 132.7$_{q}$, 130.1, 130.0, 129.7, 129.5, 129.0$_{q}$, 128.9, 128.8, 128.6, 128.5, 128.4, 128.1, 127.9, 127.6, 122.5$_{q}$, 66.9, 45.3$_{(CH_2)}$, 28.4$_{(CH_2)}$, 18.9$_{(CH_2)}$, 13.2$_{(CH_3)}$; ESI-MS: calcd m/z = 479.63 (M$^+$), found m/z = 479.4 (M$^+$).

Entantiomeric purification of *iso*-amarine

(4R,5R)-2,4,5-triphenylimidazoline [(R,R)-iso-amarine] 21

The procedure used for the preparation of 21 is a modification of the method of Braddock *et al.* Racemic *iso*-amarine (4.1 g, 0.014 M) and (S)-(+) -mandelic acid (2.1 g, 0.014 M) were dissolved in 30 ml of refluxing *iso*-propanol and allowed to reflux for 1 hr. After 1 hr, heating was stopped and the flask was left in oil to slowly cool to room temperature with gentle stirring. After 16 hrs at room temperature the solution was place on ice and cooled to 0 °C and stirred for a further 4 hrs. White crystals formed which were filtered,
collected and dried under vacuum, and the filtrate containing a crude mixture of diastereoisomer salts \((SRR)^{-21a}/(SSS)^{-21b}\) was collected for isolation of the \((S,S)-iso\)-amarine enantiomer. The product was recrystallised from \(iso\)-propanol to afford the 1:1 \((S)-(\pm)-mandelic\) acid: \((R,R)-iso\)-amarine diastereomeric salt \(21a\) as white a crystalline solid. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)/CDCl\(_3\)-\(d\)) \(\delta = 8.11\ (d, J_{HH} = 8.3\) Hz, 2H, \(Ar-H\)), 7.59 \((t, J_{HH} = 7.4\) Hz, 1H, \(Ar-H\)), 7.44 \((t, J_{HH} = 7.6\) Hz, 2H, \(Ar-H\)), 7.38-7.33 \((m, 6H, Ar-H)\), 7.25-7.22 \((m, 6H, Ar-H)\), 7.14-7.05 \((m, 3H, Ar-H)\), 5.01 \((s, 2H, -NCIm)\), 4.64 \((s, 1H, -CH_{(mandelic.a)})\); \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)-\(d\)) \(\delta = 181.1\) q, 168.9 q, 147.3 q, 144.8 q, 138.7, 133.0, 134.88, 133.5, 132.6, 131.5, 131.3, 128.6 q, 78.8, 75.4.

The salt was suspended in DCM and 1N NaOH (aq) was added and the bi-phasic mixture was stirred rapidly until all of the crystals dissolved. The organic layer containing the iso-amarine was collected and the aqueous layer was re-extracted with DCM. The organic layer was combined, washed with water and dried over MgSO\(_4\). The solution was filtered and concentrated to afford \((R,R)-iso\)-amarine \(21\) as a white crystalline solid; yield: 1.84 g (90 %): mp 202 °C; \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 8.01\ (d, J_{HH} = 7.8\) Hz, 2H), 7.53-7.43 \((m, 3H, Ar-H)\), 7.37-7.24 \((m, 10H, Ar-H)\), 4.76 \((s, 2H, -NCH-)\); ESI-MS: calcd \(m/z = 299.38\) (M+H\(^+\)), found \(m/z = 299.2\) (M+H\(^+\)).

\((S,S)-iso\)-amarine \(21\) was isolated from the filtrate collected containing the crude mixture of diastereoisomeric salts \((RRS)^{-21a}/(SSS)^{-21b}\). The filtrated was concentrated under negative pressure to give a light yellow solid. The diastereoisomeric mixture was purified by flash column chromatography (ethyl acetate: methanol, gradient elution 4:0 to 3:1) to yield pure diastereoisomer \((SSS)^{-21b}\). The salt was suspended in DCM and 1M NaOH was added and the bi-phasic mixture was stirred rapidly until the solid dissolved. The organic layer containing the iso-amarine was collected and the aqueous layer was re-extracted with DCM. The organic layer was combined, washed with water and dried over MgSO\(_4\). The solution was filtered and concentrated to afford \((S,S)-iso\)-amarine \(21\) as a white crystalline solid; 62 % yield (1.28 g). Spectral data and melting point comparable with \((R,R)\)-isomer and \((+/-)-iso\)-amarine \(21\) enantiomer.
(4R,5R)-N,N'-diethyl-2,4,5-triphenylimidazolinium iodide \([\text{nnDiEtTrPhlineI}]_26\)

Ionic salt 26 was synthesised from (S,S)-iso-amarine 21 (0.4 g, 1.34 mM) following the general alkylation procedure using 280 \(\mu\)L (2.6 eq.) of ethyl iodide in DMF (3 mL) to afford 26 as a solid in very good yield: 0.64 g (98%): mp 78-80 °C; \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 8.02-7.99\) (m, 2H, Ar-H), 7.81-7.47 (m, 13H, Ar-H), 5.42 (s, 2H, -NCH), 3.25 (dq, \(^3\)J\(_{HH}\) = 14.5, 7.2 Hz, 2H, -CH\(_2\)), 2.99 (dq, \(^3\)J\(_{HH}\) = 14.3, 7.1 Hz, 2H, -CH\(_2\)), 0.91 (t, \(^3\)J\(_{HH}\) = 7.2 Hz 3H, -CH\(_3\)); \(^1^3\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 165.7_{q}, 135.7_{q}, 133_{q}, 130.9, 130.8, 129.7, 129.4, 129.1, 128.9, 128.5, 128.2, 127.1, 122.2_{q}, 70.9, 41.2_{(CH2)}, 12.7_{(CH3)}\); ESI-MS: calcd m/z = 355.50 (M\(^+\)), found m/z = 355.3 (M\(^+\)).

(4R,5R)-N,N'-dibenzyl-2,4,5-triphenylimidazolinium chloride \([\text{nnDiBnTrPhlineCl}]_27\)

27 was synthesised from 0.4 g (1.34 mM) of (S,S)-21 following the general alkylation procedure using 2.5 molar equivalence (390 \(\mu\)L) of benzyl chloride in 3 mL of DMF to afford 27 as a solid; yield 0.59 g (85%): mp 190-193 °C; \(^1\)H-NMR (300 MHz, CDCl\(_3\)-d) \(\delta = 8.67\) (d, \(^3\)J\(_{HH}\) = 7.6 Hz, 2H, Ar-H), 8.12-8.10 (s, 2H, Ar-H), 7.75 (s, 4H, Ar-H), 7.60-6.76 (m, 17H, Ar-H), 5.05, 4.64 (d, \(^3\)J\(_{HH}\) = 15.2 Hz, 2H), 4.25 (d, \(^3\)J\(_{HH}\) = 15.0 Hz, 2H); \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)-d) \(\delta = 164.1_{q}, 139.2_{q}, 134.5_{q}, 134.0_{q}, 133.7, 132.0_{q}, 130.5, 130.3, 129.8, 129.2, 129.1, 128.6, 128.4, 128.2, 127.8, 126.2, 121.9_{q}, 72.2, 50.7_{(CH2)}\); ESI MS: calcd m/z = 479.63 (M\(^+\)), found m/z = 479.4 (M\(^+\));.

(4R,5R)-N,N'-dibutyl-2,4,5-triphenylimidazolinium bromide \([\text{nnDiBuTrPhlineBr}]_28\)

28 was synthesised from 0.4 g (1.34 mM) of (S,S)-21 following the general alkylation procedure using 2.2 molar equivalence of \(n\)-bromo butane (319 \(\mu\)L) in DMF (3 mL) to afford 28 as a solid; yield: 0.61 g (92%): mp 79-81 °C; \(^1\)H-NMR (300 MHz, CDCl\(_3\)-d) \(\delta = 8.57\) (d, \(^3\)J\(_{HH}\) = 8.0 Hz, 2H, Ar-H), 7.89-6.93 (m, 13H, Ar-H), 5.05, 3.29 (m, \(^3\)J\(_{HH}\) = 7.6 Hz, 1H, -NCH\(_2\)), 3.17 (m, \(^3\)J\(_{HH}\) = 7.1 Hz, 1H, -NCH\(_2\)), 1.52-1.17 (m, \(^3\)J\(_{HH}\) = 7.6, 7.0 Hz, 2H-CH\(_2\)CH\(_2\)CH\(_3\)), 1.12-0.85 (m, \(^3\)J\(_{HH}\) = 7.3, 7.0 Hz, 2H, -CH\(_2\)CH\(_3\)), 0.64 (t, \(^3\)J\(_{HH}\) = 7.3 Hz, 1H, -CH\(_3\)); \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)-d) \(\delta = 164.1_{q}, 138.8_{q}, 134.3, 133.4, 130.5, 130.4, \)
Synthesis of 2,4,5-triphenylimidazole (Lophine) 22

Lophine was synthesized from cis-amarine by Swern oxidation following the procedure described by Chou et al.30 To a stirred solution of oxalyl chloride (2.77, 1.6 eq.) in 49.4 mL DCM at -78 °C was added 3.6 mL (3.2 eq.) DMSO in DCM (24.7 mL) dropwise over 5 minutes. After 10 minutes, cis-amarine (4.0 g 0.0134 M) in DCM (25 mL) was added dropwise over 10 minutes, followed by triethylamine (9.5 mL) dropwise over 10 minutes. The mixture was stirred and allowed to gradually warm to room temperature overnight. The organic phase was washed with brine and dried over MgSO₄. The organic phase was then filtered and concentrated under negative pressure to afford a crude product, which was further purified by flash chromatography using 2:1 n-hexane: ethyl acetate to afford 22 as a yellow solid; 2.95 g (74 %): mp 272-273 °C; ¹H-NMR (300 MHz, CDCl₃-d/DMSO-d₆) 7.89 (m, 2H, Ar-H), 7.37-7.25 (m, 4H, Ar-H), 7.22-6.94 (m, 9H, Ar-H); ¹³C-NMR (75MHz, CDCl₃-d/DMSO-d₆) 150.8q, 137.2q, 134.2, 133.5, 133.4, 133.2, 130.7; ESI-MS: calcd m/z = 297.37 (M+H⁺), found m/z = 297.2 (M+H⁺).

N,N’-Diethyl-2,4,5-triphenylimidazolium iodide [DETPzole I] 29

29 was synthesized from 22 (0.4 g, 1.34 mM) following the general alkylation procedure using ethyl iodide (130 μL, 2.2 eq.) in DMF (3 mL) to afford a 29 as a solid; yield: 0.54 g (84 %): mp 174 °C: ¹H-NMR (300 MHz, DMSO-d₆) δ = 8.10 (d, 3JHH = 7.2 Hz, 2H, Ar-H), 7.78 (m, 2H, Ar-H), 7.61-7.31(m, 13H, Ar-H), 3.84 (q, 4H, 3JHH = 7.1 Hz, -CH₂), 0.95 (t, 3JHH = 7.1 Hz, 6H, -CH₃); ¹³C-NMR (75MHz, DMSO-d₆) δ= 145.3q, 142.8q, 132.5, 132.2q, 130.9, 130.9, 130.7, 130.1, 129.7, 129.2, 129.0, 128.9, 128.8, 128.5, 128.2, 127.9, 127.5, 126.4, 125.7q, 125.5, 122.2q, 41.8(CH₂), 14.8(CH₃); ESI-MS: calcd m/z = 353.48 (M⁺), found m/z = 353.3 (M⁺).
**N,N’-Dibenyl-2,4,5-triphenylimidazolium chloride** [DBnTPzole Cl] 30

30 was synthesised from 22 (0.4 g, 1.34 mM) following the general alkylation procedure using 342 µL (2.2 eq.) of benzyl chloride in DMF (3 mL) to afford 30 as a solid in good yield: 0.54 g (78 %): mp 93 °C: ¹H-NMR (300 MHz, CDCl₃-d) δ = 8.19-8.08 (m, 2H, Ar-H), 7.68-7.16 (m, 23H, Ar-H), 5.24 (s, 2H, -CH₂), 5.06 (s, 2H, -CH₂); ¹³C-NMR (75 MHz, CDCl₃-d) δ = 153.2, 144.2, 133.3, 132.6, 131.6, 131.4, 131.0, 129.5, 129.7, 129.0, 128.8, 128.7, 128.2, 127.6, 127.0, 126.3, 126.0, 122.7, 48.0 (CH₂); ESI-MS: calcd m/z = 477.62 (M⁺), found m/z = 477.4 (M⁺).

**N,N’-Dibutyl-2,4,5-triphenylimidazolium bromide** [DBuTPzole Br] 31

31 was synthesised from 22 (0.4 g, 1.34 mM) following the general alkylation procedure using n-bromo butane (321 µL, 2.2 eq.) in DMF (3 mL) to afford 31 as a solid; yield: 0.5 g (75 %): ¹H-NMR (300 MHz, CDCl₃-d) δ = 8.37 (m, 2H, Ar-H), 7.61-7.04 (m, 13H, Ar-H), 3.11 (m, 4H, -NC₃H₂CH₂), 1.27 (qui, 3J_HH = 7.9, 7.4 Hz, 2H, -CH₂CH₂CH₃), 0.91 (sxt, 3J_HH = 7.5, 7.4 Hz, 4H, -CH₂CH₃), 0.47(t, 3J_HH = 7.5 Hz, 6H, -CH₃); ¹³C-NMR (75 MHz, CDCl₃-d) δ = 144.4, 132.6, 131.8, 131.3, 130.9, 129.5, 129.2, 129.1, 128.8, 128.7, 128.5, 128.4, 128.2, 127.6, 127.2, 126.4, 122.5, 48.1 (CH₂), 31.5 (CH₂), 19.3 (CH₂), 13.1 (CH₃); ESI-MS: calcd m/z = 409.59 (M⁺), found m/z = 409.4 (M⁺).

**DCC mediated coupling of amino acids to trans 2,4,5-triphenylimidazoline**

N-[1-Benzyl-2-oxo-2-(2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl)-ethyl]-acetamide 32a and 32b.

To a stirring solution of (+/-)-iso-amarine 21 (1.0 g, 3.35 mM) and an amino acid (in this case (S)-N-acetylphenylalanine (0.83 g, 1.2 eq.) in DCM (11 mL) at -78 °C under nitrogen was added 0.69 g (1.2 eq.) of Dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to react at room temperature for 5 hrs during which N,N'-dicyclohexylurea precipitated out of solution as a white solid. The mixture was filtered and the filtrate concentrated by rotary evaporation to afford 32 at 96 % yield (1.56 g) as
a mixture of 32a (SSS) and 32b (SRR). 1H-NMR (300 MHz, CDCl3-d) δ = 8.38 (s, 2H, Ar-H), 7.76-6.69 (m, 18H, Ar-H), 5.23 (s, 2H, 2x(-CH2)), 4.72 (m, 1H, -CH), 4.38 (m, 1H, -CH), 3.25-3.07 (m, 2H, -CH2), 3.00 (m, 2H, -CH2), 2.02 (s, 3H, -CH3), 1.84 (s, 3H, -CH3); 13C-NMR (75 MHz, CDCl3-d) δ = 173.8q, 170.7q, 135.9q, 135.1q, 133.7q, 129.5, 129.4, 128.6, 128.5, 128.0, 127.3, 127.1, 65.9, 65.3, 53.5, 37.1(CH2), 36.9(CH2), 23.0(CH3), 15.0(CH3). The two diastereoisomers were isolated by flash chromatography with Ethyl acetate and methanol at 3:1. 32a (SSS) was isolated at 46 % yield (0.75 g), Rf =0.8, mp 75-77 °C; ESI-MS: calcd m/z = 488.60 (M+H+), found m/z = 488.4 (M+H+). 32b (SRR) was isolated at 44 % yield (0.72 g), Rf =0.38, mp 132-138 °C; ESI-MS: calcd m/z = 488.60 (M+H+), found m/z = 488.4 (M+H+).

N-(1-Benzyl-2-imidazol-1-yl-2-oxo-ethyl)-acetamide 33

33 was synthesised from imidazole (0.1 g, 1.47 mM) by DCC mediated coupling with of DCC (0.3g, 1.2 eq.) and the N-acetyl-(L)-phenylalanine (0.37g, 1.2 eq.) in DCM (3 mL) to afford 33 as a solid in good yield: 0.32 g (85 %): mp 88-90 °C; 1H-NMR (300 MHz, CDCl3-d) δ = 8.38 (s, 1H, CHim), 7.22 (m, 7H, Ar-H/CHim), 4.72 (dd, 1H, -CH), 4.38 (m, 2H, -CH), 3.25-2.89 (m, 4H, -CH2), 2.02 (s, 3H, -CH3), 1.84 (s, 3H, -CH3); 13C-NMR (75 MHz, CDCl3-d) δ = 178.0q, 169.8q, 168.9q, 163.0q, 136.4, 135.2q, 134.4q, 131.5, 129.5, 129.2, 128.9, 128.5, 127.7, 127.2, 116.1, 66.0, 53.3, 38.8(CH2), 36.9(CH2), 23.0(CH3), 15.1(CH3); ESI-MS: calcd m/z = 258.30 (M+H+), found m/z = 258.1 (M+H+).

2-Hydroxy-2-phenyl-1-(2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl)-ethanone 34

34 was synthesised from a 0.39 g (1.307 mM) mixture of (RR)-21 and (SS)-21 (with an excess of the SS enantiomer) by DCC mediated coupling using 0.35 g of DCC and (S)-mandelic acid (0.26 g, 1.3 eq.) to afford 34 as a white solid in good yield: 0.43 g (76 %): mp 160 °C; 1H-NMR (300 MHz, DMSO-d6) δ = 8.05 (m, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 7.57-7.28 (m, 17H, Ar-H), 4.89 (s, 1H, -CH), 4.69 (s, 1H, -CH), 3.42 (s, 1H, -CH); 13C-NMR (75 MHz, DMSO-d6) δ = 162.3q, 156.7q, 144.4q, 144.1q, 130.6, 130.2q, 128.6,
128.4, 128.3, 127.5, 127.3, 126.9, 126.7, 126.2, 79.5, 69.2, 54.7; ESI-MS: calcd m/z = 433.52 (M⁺), found m/z = 433.2 (M⁺).

1,3-Dibenzyl-5-[2-benzylamino-3-oxo-3-(2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl)-propyl]-3H-imidazol-1-imid; chloride 35.

35 was synthesised from 0.28 g (0.938 mM) of (SS)-21 by DCC mediated coupling with 0.53 g (1.2 eq.) of the N-benzyl-(L)-histidine derived IL 4 to afford 35 as an solid; 72% yield (0.50 g): mp 64-66 °C; ¹H-NMR (300 MHz, DMSO-d₆) δ = 9.55 (s, 1H, -OH), 8.23-6.97 (m, 32H, Ar-H/-CH₃), 5.57 (m, 2H, -CH₂), 5.43 (m, 2H, -CH₂), 4.74 (s, 1H, -CH), 4.51 (s, 1H, -CH), 4.39 (m, 1H, -CH), 3.07 (m, 2H, -CH₂); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 167.9q, 165.8q, 163.1q, 156.9q, 154.4q, 141.2q, 140.6q, 139.7q, 134.7q, 134.2q, 133.9q, 133.2, 133.0, 131.6q, 131.2, 130.2, 129.2, 129.0, 128.9, 128.5, 128.40, 128.3, 128.3, 128.1, 128.1, 128.0q, 127.7, 127.5, 127.3, 126.9, 126.7, 126.4, 125.7q, 66.9, 62.8, 51.9(CH₂), 51.0, 49.6(CH₂), 25.3(CH₂); ESI-MS: calcd m/z = 720.88 (M⁺), and 360.95 (M+H)²⁺, found m/z = 720.6 (M⁺) and 360.8 (M+H)²⁺.

N-[4-Oxo-1-(2,4,5-triphenyl-4,5-dihydro-imidazole-1-carbonyl)-4-(2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl)-butyl]-benzamide 36

36 was synthesised from 0.4 g (0.37 mM) of (SS)-21 by DCC mediated coupling with 0.17 g (0.5 eq.) of N-benzoyl-L-glutamic acid using 0.4 g (1.4 eq.) of DCC to afford 36 as a white crystalline solid; yield: 0.4 g (53 %): ¹H-NMR (300 MHz, CDCl₃-d) δ = 8.41 (d, 3J₆,₇ = 7.7 Hz, 1H, Ar-H), 7.66 (d, 3J₆,₇ = 8.1 Hz, 1H, Ar-H), 7.51-6.94 (m, 33H, Ar-H), 5.28 (s, 1H, -CH), 5.12 (s, 1H, -CH), 5.07 (s, 1H, -CH), 4.91 (s, 1H, -CH), 4.03 (m, 1H, -CH), 1.70 (m, 2H, -CH₂), 1.47 (m, 2H, -CH₂); ¹³C-NMR (75 MHz, CDCl₃-d) δ = 170.2q, 169.3q, 168.5q, 168.0q, 162.4q, 159.6q, 155.6q, 152.4q, 140.0q, 138.6q, 137.0q, 135.1q, 134.0q, 132.9, 132.7q, 130.9q, 129.8, 129.2q, 128.8, 128.2, 127.7, 127.5, 127.4, 127.3, 127.2q, 127.2, 127.0, 126.7, 126.6, 126.3, 126.3, 126.2, 126.1, 125.7, 125.2, 124.8, 124.4, 124.2, 123.7, 119.9, 76.3, 70.5, 67.2, 55.2, 51.5(CH₂), 51.3, 50.4, 35.9(CH₂) ESI-MS: calcd m/z = 407.0 (M+H)²⁺, found m/z = 406.7 (M+H)²⁺.
3-(2-Acetylamino-3-phenyl-propionyl)-1-ethyl-2,4,5-triphenyl-4,5-dihydro-3H-imidazolin-1-ium; iodide 37a (SSS) and 37b (SRR).

37a (SSS) and 37b (SRR) were synthesised from 0.2 g (0.410 mM) of 32a and 32b, respectively, following the general alkylation procedure using excess ethyl iodide (100 μL, 3 eq.) in 3 mL DMF to afford the 37a (SSS) and 37b (SRR), respectively, in good yields: 37a (SSS); yield: 0.26 g (97 %): RTIL: 1H-NMR (300 MHz, DMSO-d₆) δ = 8.09-7.16 (m, 20H, Ar-H), 5.35 (s, 2H, -CH), 4.37 (m, 1H, -CH), 3.34 (q, 3J_H,H = 7.3 Hz, 2H, -CH₂), 3.25 - 2.95 (m, 2H, -CH₂), 1.93 (s, 3H, -CH₃), 1.16 (t, 3J_H,H = 7.3 Hz, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 171.5q, 169.5q, 163.8q, 138.0q, 135.0, 129.5, 129.4, 129.1, 129.0, 128.9, 128.2, 127.3, 121.9q, 68.9, 53.7, 41.8(CH₂), 36.6(CH₂), 22.3(CH₃), 8.1(CH₃); ESI-MS: calcd m/z = 516.65 (M⁺). found m/z = 516.5 (M⁺). 37b (SRR); yield: 0.26 g (97 %): mp 92-97 °C: ¹H-NMR (300 MHz, DMSO-d₆) δ = 7.74 (m, 20H, Ar-H), 5.37 (s, 2H, -CH), 4.42 (dd, 3J_H,H = 14.0, 8.3 Hz, 1H, -CH), 3.34 (q, 3J_H,H = 7.3 Hz, 2H, -CH₂), 3.00-2.81 (m, 2H, -CH₂), 1.77 (s, 3H, -CH₃), 1.19 (t, 3J_H,H = 7.3 Hz, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 171.6q, 169.5q, 163.8q, 138.1q, 137.1q, 135.0, 129.5, 129.4, 129.2, 129.1, 129.0, 128.9, 128.2, 127.3, 126.5, 121.9q, 68.9, 63.8(CH₂), 53.7, 36.6(CH₂), 22.3(CH₃), 8.0(CH₃); ESI-MS: calcd m/z = 516.65 (M⁺), found m/z = 516.5 (M⁺).

1,3-Dibenzyl-5-[2-benzylamino-3-(3-ethyl-2,4,5-triphenyl-4,5-dihydro-imidazolin-1-ium; iodide)-3-oxo-propyl]-3-H-imidazol-1-ium; chloride 38

38 was synthesised following the general alkylation procedure from 35 (0.26 g 0.35 mM) and excess ethyl iodide in 3 mL DMF to afford 38 as a dark oil; yield: 0.28 g (88 %): dark oil: ¹H-NMR (300 MHz, DMSO-d₆) δ = 8.33-7.93 (m, 2H, Ar-H), 7.87-7.19 (m, 30H, Ar-H), 5.37 (s, 1H, -CH), 4.43 (m, 1H, -CH), 4.10 (q, 3J_H,H = 7.3 Hz, 2H, -CH₂), 3.36 (m, 2H, -CH₂), 2.97 (s, 4H, -CH₂), 2.77 (s, 3H, -CH₃), 1.18 (t, 3J_H,H = 7.3 Hz, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 171.6q, 169.4q, 163.8q, 138.2q, 135.0, 129.5, 129.4, 129.2, 129.1, 129.0, 128.9, 128.2, 127.2, 127.0q, 126.5, 121.9q, 68.9, 57.9(CH₂), 53.7, 51.8(CH₂),
N-[1-(3-Ethyl-2,4,5-triphenyl-4,5-dihydro-imidazolium-1-carbonyl)-4-(3-ethyl-2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl)-4-oxo-butyl]-acetamide 39

39 was synthesised following the general alkylation procedure from 0.17 g (0.227 mM) of 36 and excess ethyl iodide (500 μL) in 3 mL DMF to afford 36 as a solid in good yield: 0.23 g (96 %): mp 72-74 °C: ¹H-NMR (300 MHz, DMSO-δ6) δ = 8.20-7.38 (m, 35H, Ar-H), 5.42 (s, 1H, -CH), 5.38 (s, 1H, -CH), 5.35 (s, 1H, -CH), 5.32 (s, 1H, -CH), 3.89 (q, Jₜₜ = 7.2 Hz, 1H, -CH), 3.50 (td, J₁ = 14.5, 7.2 Hz, 1H, -CH₂), 3.26 (td, J₁ = 14.7, 7.3 Hz, 1H, -CH₂), 3.11 (dt, J₁ = 21.1, 6.8 Hz, 1H, -CH₂), 3.00 (dt, J₁ = 20.2, 6.3 Hz, 1H-CH₂) 1.80 (m, 1H, -CH₂), 1.73 (m, 1H, -CH₂), 1.60 (m, 1H, -CH₂), 1.30 (m, 1H, -CH₂), 1.24 (d, J₁ = 7.5 Hz, 1H, -CH₂), 0.94 (t, Jₜₜ = 7.2 Hz, 6H, -CH₃); ¹³C-NMR (75 MHz, DMSO-δ6) δ = 166.4, 165.8, 165.8, 165.7q, 165.3q, 163.8q, 143.0q, 140.6q, 138.4q, 138.0q, 136.5q, 135.8q, 135.0, 134.8q, 133.5, 133.0, 132.6, 130.9q, 130.9, 130.6, 130.1, 129.8, 129.7, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.5, 128.2, 128.2, 128.1, 127.9, 127.2q, 127.2, 127.0, 126.8, 125.7q, 122.9q, 122.2q, 122.1q, 72.7, 70.9, 69.1, 67.4, 48.3, 41.8, 41.2, 40.8, 32.4, 25.2, 24.9, 14.7, 12.7, 12.6; ESI-MS: calcd m/z = 435.05 (M²⁺), found m/z = 434.8 (M²⁺).

2-Benzylamino-1-[3-(2-(3-[2-benzylamino-3-(1,3-dibenzyl-1H-imidazol-4-yl)-propionyl]-2,4,5-triphenyl-4,5-dihydro-3H-imidazol-1-yl)-ethyl]-2,4,5-triphenyl-4,5-dihydro-imidazol-1-yl]-3-(1,3-dibenzyl-1H-imidazol-4-yl)-propan-1-one 40

40 was synthesised following the general alkylation procedure from 35 (0.18 g, 0.24 mM) using 0.5 molar equivalents of 1,2-diiodoethane (0.034 g) in 3 mL of DMF to afford 40 as a solid in good yield: 0.17 g (81 %): dark oil: ¹H-NMR (300 MHz, DMSO-δ6) δ = 8.17-7.12 (m, 64H, Ar-H/ CH₃), 5.51 (m, 4H, -CH₂), 5.43 (m, 4H, -CH₂), 4.64 (s, 1H, -CH), 4.40 (s, 1H, -CH), 3.39 (m, 4H, -CH₂), 3.33 (m, 1H, -CH), 3.12 (m, 4H, -CH₂), ¹³C-NMR (75 MHz, DMSO-δ6) δ = 170.1, 163.8, 138.0, 135.0, 132.9, 131.0, 129.5, 129.4, 116
129.1, 129.1, 128.9, 128.9, 128.4, 128.2, 128.0, 127.8, 127.3, 121.7, 68.9, 55.3, 51.9(CH2), 49.3, 49.2, 30.3(CH2), 28.6(CH2), 24.7(CH2), 24.5(CH2), 23.8(CH2), 23.6(CH2); ESI-MS: calcd m/z = 367.45 (M4+), found m/z = 367.3 (M4+).
2.3 Synthesis of amino acid derived imidazolinium ionic liquids

2.3.1 Introduction

The synthesis and use of imidazolines has expanded dramatically in the past decade, driven both by their increased applications in catalysis and especially by the development of imidazoline-based drugs for various therapeutic targets. Human imidazoline binding sites (IBS) have been identified, and the nature of these receptor subtypes and their preferred ligands have been explored in detail. Imidazoline drugs are now in development for a remarkable array of disease states including oncology, depression and neuroprotection, hypertension, inflammation, and analgesia.

An example of their biological activity is well illustrated by the unique anti-cancer properties of syn-imidazolines (nutlins) and anti-imidazolines (SP-4-84). The syn-imidazolines were found to be inhibitors of MDM2, a protein that negatively regulates the activity of the pro-apoptotic transcription factor p53. The anti-imidazoline SP-4-84, on the other hand, was found to be a drastic enhancer of the chemotherapeutic efficacy of anti-cancer agents and modulator of the anti-apoptotic NF-κB signaling pathway.

High-throughput screening has identified 2,4,4-triphenyimidazoline and several of its derivatives to be potent neuropeptide Y Y5 (NPY Y5) receptor agonists. Neuropeptide Y (NPY) is thought to play a major role in the physiological control of energy homeostasis. Pharmaceutical data suggests that the NPY Y5 receptor is involved in feeding regulation. Administration of Y5 antagonists suppresses Y5 agonist-induced food intake and diet-induced body weight gain. Hence, Y5 antagonists have been targeted by pharmaceutical companies as potential anti-obesity drugs.
In addition to their diverse biological activity, imidazolines have been utilized as building blocks for biologically interesting scaffolds and recently attracted considerable interest as ligands for asymmetric catalysis.

The stereochemical diversity of these heterocyclic molecules presents novel territory not only in terms of their biological applications, but hold potential for application as chiral selecting agents in analytical chemistry. Given that these imidazoline molecules have stereogenic centers, it was anticipated that their ionic salt analogues may hold great potential as chiral selecting agents for gas chromatographic separations. Approaches to the efficient synthesis of imidazolinium ionic liquids from simple amino acids, with diverse functionalities, was the focus of this research investigation.

### 2.3.2 Results and Discussion

**Synthesis of N-benzoyl amino acids:**

The synthesis of imidazolines begins with the \( N \)-Acylation of simple and readily available amino acids following the procedure outlined by Steiger, which was first introduced by Baum. It is important to note that the choice of acetylating agent used at this stage, is a major determining factor on the stereoisomeric composition of imidazoline product later on in the reaction scheme (Figure 2.36).
Acylamino acids are most conveniently prepared by the action of an acid chloride upon amino acids in aqueous solution in the presence of a base, generally sodium hydroxide. Using this method, large quantities of N-acyl amino acids can be prepared efficiently in high yields. The amino acids used can be either as a pure enantiomer or a racemic mix as this enantiomeric purity of the amino acid will not affect the stereoisomeric composition of the imidazolines produced.

Following the procedure as outlined by Steiger, an ice cold aqueous solution containing one mole of amino acid and one mole of sodium hydroxide was treated with one mole of an acid chloride and one mole of sodium hydroxide while vigorously stirring and shaking the reaction mixture:

\[
\text{R}^2\text{COOH} + \text{NaOH (aq)} \rightarrow \text{R}^2\text{CONa} + \text{H}_2\text{O} + \text{R}^2\text{COCl}/\text{NaOH (aq)}
\]

**Figure 2.33:** Amino acid acylation reaction. Amino acids 41-45 have a phenyl group at R\(^1\) while compounds 45-49 have a benzyl at R\(^1\) with R\(^2\) being either methyl, phenyl, methylindole, and 4-methylimidazole from the sidechains of their respective amino acids alanine, phenylalanine, tryptophan, and histidine.

Benzoyl chloride (Figure 2.34) was used as an acylating agent, however, phenylacetyl chloride was also utilized to produce N-benzyllamino acids 41-45 and N-phenylacetylamino acids 46-49, respectively:
It’s important to note that enough sodium hydroxide must be used to neutralize the hydrochloric acid liberated in the reaction, to maintain the sodium salt of the benzoyl compound in solution until the reaction is complete, and to destroy any excess of benzoyl chloride. An additional amount of sodium hydroxide may be required to prevent the premature precipitation of unreacted amino acids. Failure to do so results in reduced yields.

The reaction was complete when the reaction mixture had a clear yellow appearance and no oil droplets remained at the bottom of the flask. Upon completion, the reaction mixture was treated with one mole of hydrochloric acid to precipitate the N-benzoyl amino acid. To convert all the unreacted amino acid into the hydrochloride a further quantity of hydrochloric acid was added, giving yields of purified benzoyl derivatives as high as, 95 % (see Table 2.3). These N-benzoyl amino acids were then used to synthesise enantiomerically pure oxazolones.
Table 2.3: N-Benzoyl and N-phenylacetyl amino acids.

<table>
<thead>
<tr>
<th>N-Acyl amino acids</th>
<th>R¹</th>
<th>R²</th>
<th>Stereoisomer</th>
<th>Mp (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>phenyl</td>
<td>methyl</td>
<td>L</td>
<td>94-7</td>
<td>95</td>
</tr>
<tr>
<td>42</td>
<td>phenyl</td>
<td>phenyl</td>
<td>DL</td>
<td>157-61</td>
<td>74</td>
</tr>
<tr>
<td>43</td>
<td>Phenyl</td>
<td>3-methyl-1H-indole</td>
<td>DL</td>
<td>180-4</td>
<td>84</td>
</tr>
<tr>
<td>44</td>
<td>Phenyl</td>
<td>4-methyl-1H-imidazole</td>
<td>L</td>
<td>179-82</td>
<td>92</td>
</tr>
<tr>
<td>45</td>
<td>phenyl</td>
<td>Propionic acid</td>
<td>L</td>
<td>114-6</td>
<td>86</td>
</tr>
<tr>
<td>46</td>
<td>phenylacetyl</td>
<td>methyl</td>
<td>L</td>
<td>86-89</td>
<td>65</td>
</tr>
<tr>
<td>47</td>
<td>phenylacetyl</td>
<td>phenyl</td>
<td>DL</td>
<td>109-12</td>
<td>62</td>
</tr>
<tr>
<td>48</td>
<td>phenylacetyl</td>
<td>3-methyl-1H-indole</td>
<td>DL</td>
<td>146-8</td>
<td>83</td>
</tr>
<tr>
<td>49</td>
<td>phenylacetyl</td>
<td>4-methyl-1H-imidazole</td>
<td>L</td>
<td>oil</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 2.36: The synthesis of imidazolinium ILs 65-77 from N-benzoyl and N-phenylacetyl amino acids (41-48), where R¹ = phenyl or benzyl, R² = methyl, phenyl, indole or imidazole, R³ = phenyl or pyridine R⁴ = ethyl or benzyl, and X⁻ = iodide or chloride anion, via oxazolones 50-55.

Synthesis of oxazolones

Oxazolones are commonly synthesized under mild conditions by reacting N-acylated amino acid derivatives with dehydrating reagents. These dehydrating reagents usually consist of activated anhydrides or carbodiimides. Enantiomerically pure oxazolones were prepared from different N-benzoyl- and N-phenylacetyl-amino acids via an EDCI-mediated dehydration reaction in high yields using soluble N-ethyl,N’-(γ-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) in DCM at 0 °C followed by removal of urea and any starting material by washing with water and aqueous sodium hydrogen carbonate.
The solution of $N$-acylated amino acid and $N$-ethyl,$N'$-($\gamma$-dimethylaminopropyl)-carbodiimide hydrochloride in DCM was allowed to react for 1 hour for racemic amino acids and 15 minutes for enantiomerically pure amino acids at 0 °C. The carbodiimide converts the carboxylic acid into a reactive acylating agent by deprotonating the hydroxyl group, as shown in Figure 2.37, which in turn attacks the nucleophilic carbon on the carbodiimide to give an O-acylisourea intermediate. The remaining imide abstracts the proton on the amino acid amine resulting in the formation of an enolate ion. The enolate ion then attacks the carbonyl carbon to yielding a cyclised tetrahedral intermediate. The tetrahedral intermediate dissociates losing $N$-ethyl,$N'$-($\gamma$-dimethylaminopropyl)-urea as a co-product which is highly soluble in water, and is easily removed by washing with water as well as any unreacted amino acids. The oxazolones are unstable compounds which decompose at room temperature, thus all work up must be done at 0 °C and the pure oxazolone stored at -20 °C. The very short reaction time and the ease of the work up to produce enantiomerically pure oxazolones, combined with the high yields produced make this procedure highly effective and desirable for synthesising oxazolones.

**Figure 2.37:** Mechanism for the synthesis of oxazolones from derivatised amino acids.
Table 2.4: The yields and properties of the oxazolones synthesised.

<table>
<thead>
<tr>
<th>Oxazolones</th>
<th>R¹</th>
<th>R²</th>
<th>Mp (˚C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>phenyl</td>
<td>Methyl</td>
<td>oil</td>
<td>67</td>
</tr>
<tr>
<td>51</td>
<td>Phenyl</td>
<td>Phenyl</td>
<td>oil</td>
<td>87</td>
</tr>
<tr>
<td>52</td>
<td>phenyl</td>
<td>3-methyl-</td>
<td>solid</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1H-indole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>phenyacetyl</td>
<td>Methyl</td>
<td>oil</td>
<td>79</td>
</tr>
<tr>
<td>54</td>
<td>phenyacetyl</td>
<td>Phenyl</td>
<td>oil</td>
<td>93</td>
</tr>
<tr>
<td>55</td>
<td>phenyacetyl</td>
<td>3-methyl-</td>
<td>solid</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1H-indole</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The enantiomeric purity of the oxazolones results from kinetic resolution which occurred during the process of the reaction. Classic kinetic resolution is defined as a process by which the two enantiomers of a racemate are transformed to their isomeric product at different rates. If the kinetic resolution is efficient, one of the enantiomers of the racemic mixture is transformed to the desired product while the other is recovered unchanged.

Figure 2.38: Classic kinetic resolution and dynamic kinetic resolution $S=$ substrate, $P=$ product.
Classical kinetic resolution has led to the development of the dynamic kinetic resolution principal, which combines the resolution step of classic kinetic resolution, with an in situ equilibration or racemisation of the chiral substrate. In such a process, one can in principle obtain a quantitative yield of one of the enantiomers.

Dynamic kinetic resolution allows for the selective synthesis of an enantiomer when the reaction takes place, along with the creation of a new stereogenic center. The reaction proceeds preferentially with one of the two enantiomers of the racemate forming an irreversible product. At the same time, the unreactive enantiomer undergoes epimerization to the more reactive enantiomer allowing for the complete conversion of the racemate to the desired stereoisomer.

![Reaction diagram](image)

**Figure 2.39: Racemisation of oxazolones**

Under conditions which are not conducive for the racemisation of the product all of the substrate is converted into a single isomeric product. Typically, racemisation of a substrate can take place chemically, enzymatically, or even spontaneously. In the case of this EDCI-mediated dehydration reaction racemisation occurs spontaneously yielding a single enantiomer of oxazoline as the product. And because these oxazolones racemise very slowly in solvents such as chloroform and DCM the pure oxazolones were obtained before racemisation took place.
**Figure 2.40:** The Oxazolone products derived from the amino acids: alanine, phenylalanine, and tryptophan.

The oxazolone ring system contains numerous reactive sites allowing for a wide variety of transformations (Figure 2.39). The use of Lewis acids with the oxazolones results in the formation of either the 1,3-dipole (also known as a münchnone) or the reactive ketene intermediate (Figure 2.41), each yielding the possibility of synthesizing novel heterocyclic compounds, such as imidazolines, via cycloaddition reactions.77
Figure 2.41: Lewis acid mediated formation of a münchnone 1,3-dipole intermediate.

**Synthesis of imidazolines**

Imidazolines can be synthesised from oxazolones via a silicon mediated 1,3-dipolar cycloaddition with imines. Imidazolines can be produced via a one-pot reaction of an imine and an *in situ* Lewis acid-generated münchnone. The münchnone and imine are allowed to react at slightly elevated temperatures to give imidazoline products. 1,3-Dipolar cycloaddition reactions with oxazolones provide a general route for the syntheses of heterocyclic compound such pyrroles, pyrrolines, imidazoles and imidazolines with various functionalities attached to their heterocyclic scaffolds.

It was discovered that alkenes disubstituted with aryl and acyl groups react with münchnones to afford pyrrolines. Similar to pyrrole synthesis, imidazolines can be synthesised via the reaction of oxazolones with a variety of imines in the presence of the lewis acid chlorotrimethylsilane (TMSCl). 1,3-dipolar cycloaddition is promoted when the oxazolone is in its münchnone form, wherein it acts as a dipole and reacts with dipolarophiles such as imines. The TMSCl promotes the cycloaddition reaction of oxazolones and imines to afford the imidazolines.
Synthesis of amino acid derived imidazolines: 78

To synthesize the imidazolines a solution of aldehyde and benzylamine in dry DCM was refluxed under a nitrogen atmosphere for 2 hrs to permit the formation of the imine. To this solution was added the oxazolone followed by TMSCl to promote the formation of the münchnone, then refluxed at 40 °C for 6-10 hrs under a nitrogen atmosphere. Because oxazolones epimerize readily under mild reaction conditions the münchnone and imine were only allowed to react at slightly elevated temperatures. The mixture was left to react at room temperature overnight to afford the imidazoline. The DCM was removed by rotatory evaporation and the product isolated by flash chromatography. This method permits the production of imidazolines with a wide range of substituents at R₁, R², R³ and R⁴ in very good yields (Table 2.5).

Given that these imidazolines were intended for application as chiral selecting agents, there was a need to produce imidazolines with high stereoselectivity. As a result it was determined to prepare (4S,5S) anti-imidazolines, because (4S,5S) anti-imidazolines could be prepared with high stereoselectivity via this trimethylsilyl chloride mediated 1,3-dipolar cycloaddition reaction (Figure 2.42). The high diastereoselectivity of the trimethylsilyl chloride mediated 1,3-dipolar cycloaddition reaction is substrate controlled, thus varying the substrates at R₁, R², and R³ enables influence over the formation of either syn- or anti-imidazolines. 78,83,84
Figure 2.42: Proposed mechanism for the synthesis of anti-imidazolines from 1,3-dipolar cycloaddition of imines to oxazolones.

Reaction mechanism

It has been shown that when $R^1$ is phenyl the anti diastereoisomer is exclusively formed, regardless of the nature of $R^2$, $R^3$, or $R^4$.\textsuperscript{78,84} This observation indicates that $R^1$ controls the branch point of the stereochemical diversity. This is why the acylating agent used in the preparation of the $N$-acylamino acids was of significant importance as mentioned earlier, as this determines the $R^1$ substituent and consequently the stereoisomeric product. In the case of 2-phenylimidazolines where $R^1$ is a phenyl group, the high diastereoselectivity was attributed to a (1,3) strain in the azlactone dipole, which prevents co-planarity of Ph, TMS, and $R^2$ groups (Figure 2.42). To prevent steric interaction between the bulky silyl group of the azlactone and the $R^3$ group of the imine, the endo approach of the imine was favoured resulting in the anti-isomer as the sole product.\textsuperscript{78}

Reducing the resonance stabilization of the carbocation in the dipole by changing the electronic nature of the $R^1$ substituent, has been shown to significantly change the
diastereoselectivity of the reaction. Replacement of the R^1 phenyl group with a benzyl or a methyl moiety has been shown to significantly erode the stereoselectivity. When R^1 was methyl and R^2 methyl, benzyl, or isopropyl a mixture of diastereoisomers were obtained. However, when R^1 was an alkyl substituent the syn-imidazolines were obtained with high diastereoselectivity as the major or sole products only when the R^2 substituent is an aryl group.

\[
\begin{align*}
\text{Figure 2.43:} & \quad \text{Imidazolines 56-58 and their respective ILs 65-70, synthesised from } N\text{-phenyl amino acids.}
\end{align*}
\]

Figure 2.43 shows the imidazolines synthesised with a phenyl group at R^1 and R^3, while various functional groups at R^2 were inherited from the side groups of amino acids utilised in the synthesis of the oxazolones. The amino acids utilised were alanine,
phenylalanine, and tryptophan which gave a methyl, benzyl, or and indole side groups, respectively. Having a phenyl at R\textsuperscript{1} gave the desired (4S,5S) \textit{anti}-imidazoline products 56-58 as the major product with greater than 95 \% ee by NMR analysis. From these imidazolines ionic liquids 65-70 were synthesised by alkylating the \textit{sp}\textsuperscript{2} hybridised tertiary amine imidazolines with the alkyl halides iodoethane or benzyl chloride. These compounds are novel chiral ionic liquids with greater than 95 \% ee of the (4S,5S) \textit{anti} stereoisomer. These chiral ionic liquids hold potential for application as chiral selecting agents not only in GC stationary phases but also for chiral NMR analysis.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{imidazolines.png}
\caption{Imidazolines 59-61 and their respective ionic liquids 71-75 synthesised from N-phenylacetyl amino acids.}
\end{figure}

Imidazolines 59-61 were also synthesised with a benzyl group at R¹ and phenyl at R³ (Figure 2.44). Similarly imidazolines 62-64 had a benzyl group at R¹ but pyridine at R³. These imidazolines were alkylated to form the ionic salts (71-75) from their imidazolines 59-61 and the di-cationic salts 76-77 from their imidazoline 62 (Figure 2.45). However, having a benzyl group at R¹ eroded the stereoselectivity otherwise favoured by a phenyl substituent resulting in imidazoline products with a mixture of the syn and anti diastereoisomers.

Table 2.5: Imidazolines 56-64 yields and properties.

<table>
<thead>
<tr>
<th>Imidazolines</th>
<th>no.</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>phenyl</td>
<td>Methyl</td>
<td>phenyl</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Phenyl</td>
<td>Phenyl</td>
<td>Phenyl</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Phenyl</td>
<td>Indole</td>
<td>Phenyl</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>phenylacetyl</td>
<td>Methyl</td>
<td>phenyl</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>phenylacetyl</td>
<td>Phenyl</td>
<td>phenyl</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>phenylacetyl</td>
<td>Indole</td>
<td>phenyl</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>phenylacetyl</td>
<td>Methyl</td>
<td>pyridine</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>phenylacetyl</td>
<td>Phenyl</td>
<td>pyridine</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>phenylacetyl</td>
<td>Indole</td>
<td>pyridine</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.45: Imidazolines 62-64, and the di-cationic liquids 76-77 synthesised from imidazoline 62.

2.3.3 Conclusion

An efficient method for synthesising imidazolines which permit the addition of a variety of functional groups to the imidazoline scaffold, with a high level of diastereoselectivity, has been employed for the development of novel chiral ionic salts. These chiral ionic salts may hold future potential for a variety of applications including application as chiral selective agents in analytical separation techniques such as gas chromatography. A selection of ionic salts derived from the (4S,5S) anti-imidazolines were incorporated into GC stationary phases and tested for enantioselective capabilities and these results are discussed in Section 3.2.
2.3.4 Experimental

2.3.4.1 Reagents:

Alanine, phenylalanine, tryptophan, N-histidine monohydrate, NaOH, benzoyl chloride, Phenylacetyl chloride, conc. HCl, \( N-(3\text{-dimethylaminopropyl})-N'\text{-ethyl-carbodiimide} \) hydrochloride, DCM, Na\(_2\)CO\(_3\), MgSO\(_4\), chlorotrimethylsilane, Benzylamine, Benzaldehyde, and pyridine-4-carboxaldehyde. All chemicals were purchased from Sigma Aldrich.

2.3.4.2 Instruments

Residual DMF was removed from the ionic salts by bulb-bulb distillation using a Buchi Kugelrohr (manufactured in Germany). Ionic salts were characterised by NMR, ESI-MS, and melting point. \(^1\)H and \(^{13}\)C-NMR data were recorded on a Bruker 300 spectrometer and the chemical shift values were recorded in ppm. All NMR structure elucidation was done from \(^1\)H-NMR, \(^{13}\)C-NMR, DEPT135, DEPT90, HSQC, and \( HH\text{-COSYGPSW} \) experiments. Mass spectroscopy data were obtained by Electro spray ionization (model) in positive ion mode, (in 1:1 acetonitrile/ \( \text{H}_2\text{O} \)). Melting points were measured with a Gillkhenhamp Melting point apparatus (manufactures in England) and are uncorrected.

2.3.4.3 Synthesis

\textit{N-acylation of amino acids: general procedure}

One mole of amino acid in 1000 mL of 1\(N\) sodium hydroxide was stirred rapidly at 0 \(^\circ\)C until a clear solution is obtained. To the solution was added 116 mL (1 \( N \)) of benzoyl chloride dropwise. The solution was stopper and shaken vigorously until the solution was completely mixed. The pH was monitored and an additional solution of 6\(N\) NaOH was added to maintain an alkaline pH. In reactions of more than one mole of amino acid, alkali of a higher concentration, 6\(N\) sodium hydroxide was used to dissolve the amino acid provided that the sodium salt of the benzoyl compound was sufficiently soluble at 0
°C to remain in solution. The reaction is complete when the reaction mixture has a clear yellow appearance and no oil droplets remain in the solution. The clear solution was made strongly acid to Congo red to precipitate the crude product by the slow addition of conc. hydrochloric acid with continuous stirring and cooling in ice/water. The suspension was filtered and the crystals washed with ice-cold water. The product was dried in a vacuum desiccator over phosphorus pentoxide and sodium hydroxide, to remove any hydrochloric acid retained by the wet crystals.

2-Benzoylamino-proprionic acid (N-Benzoyl-alanine) 41

L-Alanine (4 g, 44.9 mM) in 100 mL of water was acylated following the general N-acylation procedure using 10 mL of a 6N sodium hydroxide solution and 6.31 mL (1 eq.) of benzoyl chloride. The pH was monitored and an additional solution of 6N sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to Congo red with conc. hydrochloric acid to afford 41 in good yield: 8.26 g (95%); mp 94-97 °C; 1H-NMR (300 MHz, CDCl₃-d/DMSO-d₆): δ = 9.77 (s, 1H, -OH), 6.88-6.72 (m, 3Jₕ,ₕ=14.1, 7.7, 7.2, 6.8 Hz, 5H, Ar-H), 3.92 (m, 3Jₕ,ₕ=14.1, 7.7, 6.8 Hz, 1H, -NCH), 0.84-0.80 (d, 3Jₕ,ₕ=7.2 Hz, 3H, -CH₃); 13C-NMR (75MHz, CDCl₃-d/DMSO-d₆): δ = 179.6, 171.9, 138.6, 136.3, 134.0, 133.1, 132.2, 53.3, 22.5 (CH₃); ESI-MS: calcd m/z = 194.21 (M+H)⁺, found m/z = 194.0 (M+H)⁺.

2-Benzoylamino-3-phenylprorionic acid (N-Benzoyl-phenyalanine) 42

DL-Phenylalanine (3.74 g, 22.6 mM) in 200 mL of water was acylated following the general N-acylation procedure using 40 mL of a 6N sodium hydroxide solution and 2.63 mL (1 eq.) of benzoyl chloride. The pH was monitored and an additional 6N solution sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to Congo red with conc. hydrochloric acid to afford 42 in good yield: 5.1 g (74%); mp 160-164 °C; 1H-NMR (300 MHz, CDCl₃-d/DMSO-d₆): δ = 7.56 (s, 1H, -OH), 7.45-6.62 (m, 9H, Ar-H), 4.25 (m, 1H, -NCH), 2.76-2.56 (m, 2H, -CHCH₂); 13C-NMR (75MHz, CDCl₃-d/DMSO-d₆): δ = 178.3, 172.9, 171.9, 142.5, 139.0, 137.5, 136.3,
135.8\textsubscript{q}, 134.4, 134.1, 133.1, 133.1, 132.3, 131.4, 59.0, 41.8(CH\textsubscript{2}); ESI MS: calcd \textit{m/z} = 270.30 (M+H)\textsuperscript{+}, found \textit{m/z} = 270.1 (M+H)\textsuperscript{+}.

2-Benzoylamino-3-(1H-indol-3-yl)-propionic acid (N-Benzoyl-tryptophan) \textsuperscript{43}

DL-Tryptophan (4 g, 19.0 mM) in 200 mL of water was acylated following the general \textit{N}-acylation procedure using 40 mL of a 6\textit{N} sodium hydroxide solution 2.3 mL (1 eq.) of benzoyl chloride. The pH was monitored and an additional 6\textit{N} solution of sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to congo red with conc. hydrochloric acid to afford \textsuperscript{43} in good yield: 4.9 g (84\%): mp 174-178 °C; \textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}-d/DMSO-d\textsubscript{6}): \(\delta = 9.82\) (s, 1H, -OH), 7.27-6.41 (m, \textit{J}_{H,H} = 15.1, 14.3, 7.0 Hz, 10H, Ar-H), 4.34 (d, \textit{J}_{H,H} = 7.0 Hz, 1H, -NCH), 2.84 (m, \textit{J}_{H,H} = 15.1, 14.3 Hz, 2H, -CH\textsubscript{2}H\textsubscript{2}); \textsuperscript{13}C-NMR (75MHz, CDCl\textsubscript{3}-d/DMSO-d\textsubscript{6}): \(\delta = 178.7\textsubscript{q}, 173.0\textsubscript{q}, 171.8\textsubscript{q}, 141.2\textsubscript{q}, 139.0\textsubscript{q}, 137.5, 136.2, 135.8, 134.4, 133.1, 132.4\textsubscript{q}, 132.2, 128.4, 126.1, 123.6, 123.3, 116.4, 114.8\textsubscript{q}, 58.5, 32.0(CH\textsubscript{2}); ESI-MS: calcd \textit{m/z} = 309.34 (M+H)\textsuperscript{+}, found \textit{m/z} = 309.2 (M+H)\textsuperscript{+}.

2-Benzoylamino-3-(1H-imidazol-4-yl)-propionic acid (N-Benzoyl-histidine) \textsuperscript{44}

L-Histidine-monohydrate (4 g, 28.7 mM) in 50 mL of water was acylated following the general \textit{N}-acylation procedure using 20 mL of a 6\textit{N} sodium hydroxide solution and 2.4 mL (1 eq.) of benzoyl chloride. The pH was monitored and an additional solution of 6\textit{N} sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by the acidifying to congo red with conc. hydrochloric acid to afford \textsuperscript{44} in good yield: 6.2 g (92\%): mp 179-184 °C; \textsuperscript{1}H-NMR (300 MHz, D\textsubscript{2}O): \(\delta = 8.44\) (s, 1H, -OH), 7.52 (d, \textit{J}_{H,H} = 7.5 Hz, 2H, Ar-H), 7.42 (m, \textit{J}_{H,H} = 14.9, 9.7, 7.2 Hz, 1H, Ar-H), 7.33-7.28 (m, \textit{J}_{H,H} = 14.9, 7.2 Hz, 3H, Ar-H/Im), 7.14 (s, 1H, -CH\textsubscript{im}), 4.77 (m, \textit{J}_{H,H} = 9.2, 5.3 Hz, 1H, -NCH), 3.32-3.24 (m, \textit{J}_{H,H} = 14.9, 9.2, 5.3 Hz, 2H, -CH\textsubscript{2}H\textsubscript{2}); \textsuperscript{13}C-NMR (75MHz, D\textsubscript{2}O): \(\delta = 173.4\textsubscript{q}, 170.4\textsubscript{q}, 134.1\textsubscript{q}, 133.3, 132.5, 132.4\textsubscript{q}, 128.8\textsubscript{q}, 128.7, 127.1, 126.4\textsubscript{q}, 118.2, 117.1, 52.1, 26.0(CH\textsubscript{2}), 25.0(CH\textsubscript{2}); ESI-MS: calcd \textit{m/z} = 260.27 (M+H)\textsuperscript{+}, found \textit{m/z} = 259.9 (M+H)\textsuperscript{+}.
2-Benzoylamino-pentanedioic acid (N-Benzoyl-glutamic acid) 45

L-Glutamic acid (4.0 g, 27.2 mM) in 200 mL of water was acylated following the general N-acylation procedure using 40 mL of a 6N sodium hydroxide solution 3.2 mL (1 eq.) of benzoyl chloride. The pH was monitored and an additional solution of 6N sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to congo red with conc. hydrochloric acid to afford 45 in good yield: 5.9 g (86%): mp 114–116 °C; \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 8.67\) (b, 2H, -OH), 8.06–7.86 (m, 2H, Ar-H), 7.73–7.54 (m, 1H, Ar-H), 7.54–7.40 (m, 2H, Ar-H), 4.54–4.37 (m, 1H, -CH), 2.39 (t, \(^3\)J\(_{H,H} = 7.4\) Hz, 2H, -CH\(_2\)) \(13\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 173.9\)q, 173.4q, 166.6q, 131.3, 130.5q, 128.8, 127.4, 52.0, 30.4(CH\(_2\)), 25.9(CH\(_2\)); ESI-MS: calcd m/z = 252.24 (M+H\(^+\)), found m/z = 524.1 (M+H\(^+\)).

2-Phenylacetylamino-propionic acid (N-Phenylacetyl-alanine) 46

L-Alanine (4.0 g, 44.9 mM) in 50 mL of water was acylated following the general N-acylation procedure using 5.7 mL of a 6N sodium hydroxide solution 5.95 mL (1 eq.) of phenyl-acetylchloride. The pH was monitored and an additional solution of a 6N solution of sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to congo red with conc. hydrochloric acid to afford 46 in good yield: 6.1 g (65%): mp 84-92 °C; \(^1\)H-NMR (300 MHz, CDCl\(_3\)-d/DMSO-\(d_6\)): \(\delta = 7.42\) (s, 1H, -OH), 6.75-6.73 (m, 5H, Ar-H), 3.83 (m, 1H, -NCH), 2.99 (s, 2H, -CH\(_2\)), 0.81 (d, 3H, -CH\(_3\)); \(^13\)C-NMR (75MHz, CDCl\(_3\)-d/DMSO-\(d_6\)): \(\delta = 179.4\)q, 175.5q, 140.8q, 134.0, 133.2, 131.4, 52.8, 47.4(CH\(_2\)), CH\(_3\) 22.6(CH\(_3\)); ESI-MS: calcd m/z = 208.23 (M\(^+\)), found m/z = 208.1 (M\(^+\)).

3-Phenyl-2-phenylacetylamino-propionic acid (N-Phenylacetyl-phenylalanine) 47

DL-Phenylalanine (4 g, 24.2 mM) in 50 mL of water was acylated following the general N-acylation procedure using 5.7 mL of a 6N sodium hydroxide solution 3.0 mL (1 eq.) of phenyl-acetyl chloride. The pH was monitored and an additional solution of 6N sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by
acidifying to congo red with conc. hydrochloric acid to afford 47 in good yield: 4.3 g (62%): mp 109-112 °C; 1H-NMR (300 MHz, CDCl3-d/DMSO-d6): δ= 7.5-7.11 (m, 8H, Ar-H), 6.93 (s, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 4.82 (m, 1H, -NCH), 3.56 (d, 2H, -CH2), 3.49 (m, 2H, -CH2); 13C-NMR (75MHz, CDCl3-d/DMSO-d6): δ= 173.0q, 170.7q, 136.1q, 134.7q, 129.3, 129.3, 128.8, 128.4, 128.3, 127.1, 126.7, 53.0, 43.4(CH2), 37.2(CH2); ESI-MS: calcd m/z = 284.33 (M+), found m/z = 284.1 (M+).

3-(1H-indol-3-yl)-2-phenylacetylamino-propionic acid (N-Phenylacetyl-tryptophan) 48

DL-Tytophan (4 g, 19.6 mM) in 50 mL of water was acylated following the general N-acylation procedure using 5.7 mL of a 6N sodium hydroxide solution 2.6 mL (1 eq.) of phenyl-acetyl chloride. The pH was monitored and an additional solution of 6N sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to congo red with conc. hydrochloric acid to afford 48 in good yield: 5.2 g (83%): mp 146-148 °C; 1H-NMR (300 MHz, CDCl3-d): δ=7.23-7.10 (m, 9H, Ar-H), 6.91(s, 1H, Ar-H), 4.74-4.68 (dd, JH,H= 13.3, 5.7 Hz , 1H, -NCH), 3.45 (s, 2H, -CH2), 3.09-2.91 (m, 2H, -CH2); 13C-NMR (75MHz, CDCl3-d/DMSO-d6): δ=178.4q, 175.4q, 141.1q, 139.9q, 134.1, 133.3, 133.2, 132.5q, 131.6, 128.3, 126.1, 123.7, 123.3, 116.22, 114.2q, 57.9, 47.9(CH2), 32.1(CH2); ESI-MS: calcd m/z = 323.37 (M+), found m/z = 323.2 (M+).

3-(1H-Imidazol-4-yl)-2-phenylacetylamino-propionic acid (N-Phenylacetyl-histidine) 49

L-Histidine-monohydrate (4 g, 20.9 mM) in 50 mL of water was acylated following the general N-acylation procedure using 5.7 mL of a 6N sodium hydroxide solution 2.8 mL (1 eq.) of phenyl-acetyl chloride. The pH was monitored and an additional solution of 6N sodium hydroxide was added to maintain an alkaline pH. The product was precipitated by acidifying to congo red with conc. hydrochloric acid to afford 49 in good yield: 5.1 g (89%): RTIL; 1H-NMR (300 MHz, DMSO-d6) δ = 9.00 (s, 1H, -OH), 8.80 (d, JH,H = 7.5 Hz, 1H, -CH(t)), 7.50-7.14 (m, 6H, Ar-H/-CH(t)), 4.53 (m, 1H, -CH), 3.45 (s, 2H, -CH2), 3.10 (m, 2H, -CH2); 13C-NMR (75 MHz, DMSO-d6) δ = 172.6q, 172.0q, 171.0q, 170.6q, 170.4q,
169.4, 136.0_, 135.9_, 134.9_, 133.9, 133.3, 129.3, 128.9, 128.3, 128.2, 128.1, 127.09, 126.5, 126.3, 117.9, 117.0, 116.8, 52.2, 51.3, 51.1, 41.9(CH2), 40.7(CH2), 25.9(CH2), 25.08(CH2); ESI-MS: calcd m/z = 274.30 (M+H)+, found m/z = 273.9 (M+H)+.

**Synthesis of oxazolones: general procedure**

A solution of \(N\)-acylated amino acid and \(N\)-ethyl,\(N\)’-(\(\gamma\)-dimethylaminopropyl)-carbodiimide hydrochloride in DCM were stirred at 0 °C for 1 hour for racemic amino acids and 15 minutes for enantiomerically pure amino acids. The solution was washed successively with ice water, followed by an ice cold aqueous solution of sodium hydrogen carbonate, and finally ice water. The DCM solution was then dried over magnesium sulphate, filtered, and DCM removed by rotary evaporation to give a pure oxazolone as an oil or crystals.

4-Methyl-2-phenyl-4\(H\)-oxazol-5-one 50

50 was synthesised following the general procedure for the synthesis of oxazolones using 41 (3.0 g, 15.5 mM) in 155 mL of DCM and 2.98 g (1 eq.) of DCC to afford 50 as an oil in good yield: 1.8 g (67 %): \(^1\)H-NMR (300 MHz, CDCl3-\(d\)): \(\delta = 8.07\) (d, \(^3J_{H,H}= 8.3\) Hz, Ar-\(H\)resonance), \(7.90\) (d, \(^3J_{H,H}= 8.1\) Hz, 2H, Ar-\(H\)), \(7.56\)-7.36 (m, \(J_{H,H}= 8.1, 7.3, 6.2, 1.3\) Hz, 3H, Ar-\(H\)), \(4.36\) (q, \(^3J_{H,H}= 7.7\) Hz, 1H, -NCH), \(1.50\) (d, \(^3J_{H,H}= 7.7\) Hz, 3H, -CH3); \(^{13}\)C-NMR (75MHz, CDCl3-\(d\)); \(\delta = 179.0_\text{q}, 162.4_\text{q}, 161.6_\text{q}, 134.6, 132.8, 130.6, 128.9, 128.8, 127.9, 125.8_\text{q}, 61.0, 16.9_{(CH3)}\).

4-Benzyl-2-phenyl-4\(H\)-oxazol-5-one 51

51 was synthesised following the general procedure for the synthesis of oxazolones from 42 (4 g, 14.9 mM) in 150 mL of DCM and 2.85 g (1 eq.) of DCC to afford 51 as an oil in good yield: 3.2 g (87 %): \(^1\)H-NMR (300 MHz CDCl3-\(d\)): \(\delta = 8.07\) (d, \(^3J_{H,H} =7.7\), 1H, Ar-\(H\)), \(7.82\) (d, \(^3J_{H,H} =7.4\), 2H, Ar-\(H\)), \(7.60\)-7.06 (m, 7H, Ar-\(H\)), \(4.59\) (t, \(^3J_{H,H} =5.1\) Hz, 1H, -NCH), \(3.23\)-3.04 (m, \(J_{H,H} = 5.1\) Hz, 2H,CH2); \(^{13}\)C-NMR (75MHz, CDCl3-\(d\)): \(\delta = 177.6_{\text{q}},\)
4-(1H-Indol-2-ylmethyl)-2-phenyl-4H-oxazol-5-one 52

52 was synthesised following the general procedure for the synthesis of oxazolones from 43 (4 g, 12.97 mM) in 130 mL of DCM and 2.49 g (1 eq.) of DCC to afford 52 in good yield: 3.4 g (90 %): ¹H-NMR (300 MHz, CDCl₃-d): δ= 8.21-8.04 (m, 3 Jₕ,ₕ = 8.5, 4.7 Hz, 1H, Ar-H), 7.88-6.93 (m, 3 Jₕ,ₕ =8.5, 5.7 Hz, 9H, Ar-H), 4.65 (m, 3 Jₕ,ₕ =14.7, 5.7 Hz, 1H, -NCH), 3.47-3.24 (m, Jₕ,ₕ =14.7, 8.5, 4.7 Hz, 2H, CH₂); ¹³C-NMR (75MHz, CDCl₃-d): δ= 178.0 q, 161.9 q, 136.0 q, 134.7, 132.7, 130.6, 129.0, 128.7, 127.9, 127.4 q, 125.7 q, 123.6, 122.0, 119.6, 119.2, 111.1, 109.4 q, 66.5, 27.3(CH₂).

2-Benzyl-4-methyl-4H-oxazol-5-one 53

53 was synthesised following the general procedure for the synthesis of oxazolones from 46 (2.77 g, 13.4 mM) in 145 mL of DCM and 2.75 g (1 eq.) of DCC to afford 53 as a oil in good yield: 2.0 g (79 %); ¹H-NMR (300 MHz, CDCl₃-d): δ= 7.29-7.11 (m, 5H, Ar-H), 4.12 (q, 3 Jₕ,ₕ =7.8 Hz, 1H, -NCH), 3.70 (s, 2H, -CH₂), 1.37 (d, 3 Jₕ,ₕ =7.8, 3H, -CH₃); ¹³C-NMR (75MHz CDCl₃-d): δ= 178.87 q, 164.5 q, 132.8 q, 129.2, 128.9, 127.7, 60.5, 35.8(CH₂), 16.6(CH₃).

2,4-Dibenzyl-4H-oxazol-5-one 54

54 was synthesised following the general procedure for the synthesis of oxazolones from 47 (2.2 g, 7.77 mM) in 82 mL of DCM and 1.58 g (1 eq.) of DCC to afford 54 as an oil in good yield: 1.9 g (93 %).

2-Benzyl-4-(1H-indol-2-ylmethyl)-4H-oxazol-5-one 55
was synthesised following the general procedure for the synthesis of oxazolones from (4.9 g, 15.2 mM) in 160 mL of DCM and 3.1 g (1 eq.) of DCC to afford 55 in good yield: 4.2 g (91 %).

**Synthesis of amino acid derived imidazolines, general procedure:**

A solution of 1 molar equivalent of aldehyde and benzylamine in dry DCM was refluxed under nitrogen atmosphere for 2 hours. To the solution was added the oxazolone and 1.3 molar equivalents of chlorotrimethylsilane and refluxed for 6-10 hours then stirred over night at room temperature. The solvent was removed by rotatary evaporation and the product isolated by flash chromatography with 100 % ethyl acetate then 100% methanol.

1-Benzyl-4-methyl-2,5-diphenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid 56

To a solution of benzaldehyde (580 μL) and benzylamine (620 μL) in dry DCM (84 mL) was added oxazolone 50 (1 g, 5.71 mM) and chlorotrimethylsilane (580 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 56 in good yield: 0.97 g (46 %): mp 57-60 °C; ¹H-NMR (300 MHz, DMSO-d₆) δ = 7.96-6.94 (m, 15H, Ar-H), 5.42-4.43 (b, 1H, -OH), 4.87 (d, ²J_HH =16.3 Hz, 1H, -CH₂), 4.63 (s, 1H, -CH), 4.14 (d, ²J_HH =16.3 Hz, 1H, -CH₂), 1.59 (s, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 173.6q, 164.1q, 134.5q, 133.2, 133.2q, 132.6, 129.8, 129.3, 129.2, 128.9, 128.6, 128.5, 128.38q, 127.9, 123.1q, 73.3, 69.6q, 48.4(CH2), 26.5(CH3); ESI-MS: calcd m/z = 371.45 (M+H)+, found m/z = 371.3 (M+H)+.

1,4-Dibenzyl-2,5-diphenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid 57

To a solution of benzaldehyde (410 μL) and benzylamine (440 μL) in dry DCM (84 mL) was added oxazolone 51 (1 g, 0.00398 M) and chlorotrimethylsilane (410 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 57 in good yield: 0.91 g (51 %): mp 65 °C; ¹H-NMR (300 MHz, DMSO-d₆) δ = 7.81-6.97 (m,
19H, Ar-H), 6.22 (d, $^3J_{HH} = 7.4$ Hz, 1H, Ar-H), 4.81 (s, 1H, -CH), 3.94 (m, 2H, -CH$_2$), 3.25 (m, 2H, -CH$_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ = 166.1$q$, 161.9$q$, 138.7$q$, 135.3$q$, 133.7$q$, 132.8, 131.4$q$, 130.2, 129.2, 129.1, 129.0, 128.9, 128.9, 128.6, 128.5, 128.3, 128.2, 127.7, 127.5, 127.1, 127.0, 126.3, 123.3$q$, 74.1, 71.3$q$, 48.0(CH$_2$)$_v$, 43.4(CH$_2$)$_v$; ESI-MS: calcd m/z = 447.55 (M+H$^+$), found m/z = 447.4 (M+H$^+$).

1-Benzyl-4-(1H-indol-3-ylmethyl)-2,5-diphenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid 58

To a solution of benzaldehyde (350 µL) and benzylamine (380 µL) in dry DCM (84 mL) was added oxazolone 52 (1 g, 3.44 mM) and chlorotrimethylsilane (350 µL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 58 in good yield: 1.1 g (63 %): mp 185 $^\circ$C; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 7.95-6.90 (m, 19H, Ar-H), 6.03 (d, $^3J_{HH} = 7.5$ Hz, 1H, Ar-H), 4.76 (s, 1H, -CH), 4.26 (m, 2H, -CH$_2$), 3.26 (m, 2H, -CH$_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ = 168.0$q$, 161.8$q$, 139.6, 136.0$q$, 131.2$q$, 130.8$q$, 129.0, 128.7$q$, 128.5$q$, 128.4, 128.3, 128.2, 127.9, 127.9, 127.5, 127.3, 127.0, 126.8, 118.7$q$, 110.2$q$, 70.5, 63.9$q$, 48.6(CH$_2$)$_v$, 47.9(CH$_2$)$_v$; ESI-MS: calcd m/z = 486.58 (M+H$^+$), found m/z = 486.4 (M+H$^+$).

1,2-Dibenzy1-4-methyl-5-phenyl-4,5-dihydro-1H-imidazole-4-carboxylic acid 59

To a solution of benzaldehyde (810 µL) and benzylamine (850 µL) in dry DCM (100 µL) was added oxazolone 53 (1.2 g, 6.34 mM) and chlorotrimethylsilane (990 µL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 59 in good yield: 2.37 g (97 %): mp 206-208 $^\circ$C; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 8.75 (s,1H, -OH), 7.69-6.97 (m, 14H, Ar-H), 6.70 (d, $^3J_{HH} = 6.9$ Hz, 1H, Ar-H), 5.15 (m, 1H, -CH), 5.04 (s, 1H, -CH), 4.49 (s, 2H, -CH$_2$), 3.91-3.82 (m, 1H, -CH$_2$), 1.63 (s, 3H, -CH$_3$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ = 169.3$q$, 167.3$q$, 134.1$q$, 132.8$q$, 132.1$q$, 129.6, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.4, 128.3, 128.1, 128.0, 127.7, 70.0$q$, 69.4, 47.7(CH$_2$)$_v$, 30.9(CH$_2$)$_v$, 25.4(CH$_3$)$_v$; ESI MS: calcd m/z = 385.48 (M+H$^+$), found m/z = 385.3 (M+H$^+$).

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1,2,4-Tribenzyl-5-phenyl-1H-imidazole-4-carboxylic acid 60

To a solution of benzaldehyde (450 μL) and benzylamine (480 mL) in dry DCM (85 μL) was added oxazolone 54 (1.0 g, 3.77 mM) and chlorotrimethylsilane (410 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 60 in good yield: 1.4 g (83 %): mp 136.1-39 ˚C; 1H-NMR (300 MHz, DMSO-d6) δ = 8.67 (s, 1H, -OH), 8.53 (d, 3JHH = 8.5 Hz, 1H, Ar-H), 7.62-7.05 (m, 18H), 6.57 (d, 3JHH = 7.2 Hz, 1H, Ar-H), 4.57 (m, 1H, -CH), 4.28 (m, 2H, -CH2), 3.44 (m, 2H, -CH2), 2.95 (m, 2H, -CH2); 13C-NMR (75 MHz, DMSO-d6) δ = 171.2q, 169.9q, 133.7q, 132.4q, 132.2q, 131.0q, 130.7q, 130.0, 129.4, 129.2, 129.0, 128.6, 128.5, 128.3, 128.2, 128.0, 127.7, 127.0, 126.6, 126.2, 126.1, 73.8q, 70.4, 47.6(CH2), 37.8(CH2), 30.9(CH2); ESI-MS: calcd m/z = 461.57 (M+H)+, found m/z = 461.4 (M+H)+.

1,2-Dibenzyl-4-(1H-indol-3-ylmethyl)-5-phenyl-1H-imidazole-4-carboxylic acid 61

To a solution of benzaldehyde (410 μL) and benzylamine (430 μL) in dry DCM (75 μL) was added oxazolone 55 (1 g, 3.29 mM) and chlorotrimethylsilane (370 mL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 61 in good yield: 1.6 g (96 %): mp 146-148 ˚C; 1H-NMR (300 MHz, DMSO-d6) δ = 10.84 (s, 1H, -OH), 8.53 (t, JHH = 5.9 Hz, 1H, Ar-H), 8.33 (d, 3JHH = 8.2 Hz, 1H, Ar-H), 7.64 (d, 3JHH = 7.8 Hz, 1H, Ar-H), 7.37 (d, 3JHH = 8.0 Hz, 1H, Ar-H), 7.31 - 6.94 (m, 16H, Ar-H), 4.64 (m, 1H, -CH), 4.29 (d, JHH = 5.9 Hz, 2H, -CH2), 3.41 (s, 2H, -CH2), 3.10 (m, 2H -CH2); 13C-NMR (75 MHz, DMSO-d6) δ = 171.5q, 169.9q, 139.2q, 136.3q, 136.1q, 129.0, 128.2, 128.1, 127.3q, 127.0, 126.6, 126.2, 123.7, 120.8, 118.5, 118.2, 111.2, 110.0q, 74.5q, 70.1, 42.1(CH2), 42.0(CH2), 28.1(CH2); ESI-MS: calcd m/z = 500.61 (M+H)+, found m/z = 500.4 (M+H)+.

1,2-Dibenzyl-4-methyl-5-pyridin-4-yl-1H-imidazole-4-carboxylic acid 62
To a solution of pyridine-4-carboxaldehyde (550 μL) and benzylamine (550 μL) in dry DCM (60 μL) was added oxazolone 53 (0.8 g, 4.23 mM) and chlorotrimethylsilane (460 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 62 in good yield: 1.63 g (100 %): mp 76-80 °C; 1H-NMR (300 MHz, DMSO-d6) δ = 8.80-8.44 (m, 3H, Ar-H/-OH), 7.80-6.98 (m, 11H, Ar-H), 6.53 (d, $^3$JHH = 7.3 Hz, 1H, Ar-H), 5.05 (s, 1H, -CH), 4.41 (s, 2H, -CH2), 3.85 (m, 2H, -CH2), 1.65 (s, 3H, -CH3); 13C-NMR (75 MHz, DMSO-d6) δ= 172.6, 155.2, 155.0, 154.7, 134.5, 134.4, 134.0, 133.8, 133.6, 133.5, 133.2, 133.0, 132.8, 132.1, 131.7, 131.4, 128.0, 73.4, 72.8, 47.6(CH2), 36.7(CH2), 25.4(CH3); ESI-MS: calcd m/z = 386.47 (M+H+). found m/z = 386.3 (M+H+).

1,2,4-Tribenzyl-5-pyridin-4-yl-4,5-dihydro-1H-imidazole-4-carboxylic acid 63

To a solution of pyridine-4-carboxaldehyde (420 μL) and benzylamine (480 μL) in dry DCM (60 μL) was added oxazolone 54 (1.0 g, 3.77 mM) and chlorotrimethylsilane (410 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 63 in good yield: 1.6 g (94 %): mp 92-96 °C; 1H-NMR (300 MHz, DMSO-d6) δ = 8.80-8.41 (m, 3H, Ar-H/-OH), 7.82 (d, $^3$JHH = 5.9 Hz, 1H, Ar-H), 7.61-7.08 (m, 16H, Ar-H), 6.55 (d, $^3$JHH = 7.2 Hz, 1H, Ar-H), 5.14 (s, 1H, -CH), 4.52 (s, 2H, -CH2), 3.42 (m, 2H, -CH2), 2.88 (m, 2H, -CH2); 13C-NMR (75 MHz, DMSO-d6) δ = 171.2, 166.3, 150.5, 150.1, 133.4, 132.8, 132.2, 130.8, 130.0, 129.1, 129.2, 129.1, 129.0, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.4, 127.0, 126.6, 126.3, 126.1, 122.8, 71.6, 69.2, 48.0(CH2), 43.9(CH2), 31.0(CH2); ESI-MS: calcd m/z = 462.56 (M+H+). found m/z = 462.4 (M+H+).

1,2-Dibenzy1-4-(1H-indol-3-ylmethyl)-5-pyridin-4-yl-1H-imidazole-4-carboxylic acid 64

To a solution of pyridine-4-carboxaldehyde (374 μL) and benzylamine (430 μL) in dry DCM (60 μL) was added oxazolone 55 (1.0 g, 3.29 mM) and chlorotrimethylsilane (370 μL) and refluxed for 6-10 hrs. The product was isolated by flash chromatography to afford 64 in good yield: 1.3 g (80 %): mp 156 °C; 1H-NMR (300 MHz, DMSO-d6) δ = 8.88-8.41 (m, 3H, Ar-H/-OH), 7.84-6.94 (m, 14H, Ar-H), 6.59 (d, $^3$JHH = 7.2 Hz, 1H, Ar-
$H), \text{6.40 (d, } J_{HH} = 7.1 \text{ Hz, 1H, Ar-H}, \text{6.17 (d, } J_{HH} = 7.3 \text{ Hz, 1H, Ar-H}, \text{5.02 (s, 1H, -CH), 4.52 (s, 2H, -CH$_2$), 3.78 (m, 2H, -CH$_2$), 2.73 (m, 1H, -CH$_2$); \text{C-NMR (75 MHz, DMSO-d$_6$) } \delta = 171.6, 169.2, 150.0, 149.6, 132.2, 131.5, 131.5, 129.5, 129.1, 129.0, 128.7, 128.5, 128.5, 128.3, 127.8, 127.6, 127.3, 126.3, 125.5, 121.3, 120.8, 119.1, 118.2, 111.7, 111.4, 110.0, 74.5, 68.7, 47.6, 42.1, 27.8; \text{ESI-MS: calcd } m/z = 501.60 (M+H$^+$), found m/z = 501.4 (M+H$^+$).}

**General N-alkylation procedure**

To a stirring solution of imidazoline in dry DMF under nitrogen was added an alkyl halide and heated for 6 hrs at 90 °C. The solvent was evaporated by heating at 70-80 °C under negative pressure. The remaining residues of DMF were removed by bulb-to-bulb distillation using a kugelrohr. Further purification was carried out by recrystallisation from ethyl acetate and hexane.

3-Benzyl-5-carboxy-1-ethyl-5-methyl-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 65

Ionic salt 65 was synthesised from 56 (0.15 g, 0.405 mM) and 240 μL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 65 in good yield: 0.19 g (93 %): mp 54-56 °C; $^1$H-NMR (300 MHz, DMSO-d$_6$) $\delta$= 8.35 (b, 1H, OH), 7.89-6.91 (m, 15H, Ar-H), 5.34 (s, 1H, -CH), 4.77 (dd, $J_{HH} = 21.5, 16.2$ Hz, 1H, -CH$_2$), 4.04 (dd, $J_{HH} = 21.5, 10.7$ Hz, 1H, -CH$_2$), 3.56, 3.48 (dq, $J_{HH} = 16.3, 7.1$ Hz, 2H, -CH$_2$-CH$_3$), 0.70 (t, $J_{HH} = 7.1$ Hz, 3H, -CH$_2$-CH$_3$); $^{13}$C-NMR (75 MHz, DMSO-d$_6$) $\delta$ = 174.4, 166.0, 133.9, 133.5, 132.8, 132.6, 129.8, 129.3, 129.2, 129.0, 128.7, 128.5, 128.3, 127.7, 122.5, 73.0, 69.0, 61.3, 48.7, 25.6, 13.3; ESI-MS: calcd $m/z = 399.50$ (M$^+$), found $m/z = 399.3$ (M$^+$).

1,3-Dibenzyl-5-carboxy-5-methyl-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium;  chloride 66
Ionic salt 66 was synthesised from 56 (0.15 g, 0.405 mM) and 90 µL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 66 in good yield: 0.23 g (97 %): mp 66-70 °C; 1H-NMR (300 MHz, DMSO-d$_6$) δ = 9.43, (s, 1H, OH), 7.94-6.84 (m, 20H, Ar-H), 5.02-4.94 (m, 1H, -CH), 4.81 (d, $^2$J$_{HH}$ = 15.3 Hz, 1H, -CH$_2$), 4.63 (d, $^2$J$_{HH}$ = 12.5 Hz, 1H, -CH$_2$), 4.40 (d, $^2$J$_{HH}$ = 12.6 Hz, 1H, -CH$_2$), 4.12 (d, $^2$J$_{HH}$ = 15.3 Hz, 1H, -CH$_2$), 1.74 (s, 3H, -CH$_3$); 13C-NMR (75 MHz, DMSO-d$_6$) δ = 168.5$_{q}$, 164.7$_{q}$, 135.2$_{q}$, 134.1$_{q}$, 133.6, 133.2, 132.8, 132.3$_{q}$, 129.4, 129.3, 129.2, 129.1, 128.8, 128.5, 128.3, 127.6, 127.5, 126.4, 122.5$_{q}$, 73.3, 68.7$_{q}$, 66.8(CH$_2$)$_2$, 48.8(CH$_2$)$_2$, 24.8(CH$_3$)$_3$; ESI-MS: calcd m/z = 461.57 (M$^+$), found m/z = 461.4 (M$^+$).

3,5-Dibenzyl-5-carboxy-1-ethyl-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 67

Ionic salt 67 was synthesised from 57 (0.11 g, 0.247 mM) and 41 µL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 67 in good yield: 0.14 g (95 %): mp 102 °C; 1H-NMR (300 MHz, DMSO-d$_6$) δ = 8.24 (b, 1H, -OH), 7.74-6.93 (m, 19H, Ar-H), 6.04 (d, $^3$J$_{HH}$ = 7.3 Hz, 1H, Ar-H) 5.09 (s, 1H, -CH), 3.33 (q, $^3$J$_{HH}$ = 7.3 Hz, 2H, -CH$_2$CH$_3$), 2.98 (m, 2H, -CH$_2$), 2.73 (m, 2H, -CH$_2$), 1.14 (t, $^1$J$_{HH}$ = 7.3 Hz, 3H, -CH$_3$); 13C-NMR (75 MHz, DMSO-d$_6$) δ = 170.2$_{q}$, 165.3$_{q}$, 136.2$_{q}$, 133.5$_{q}$, 131.8$_{q}$, 129.9, 129.3, 129.0, 128.8$_{q}$, 128.7, 128.6, 128.4, 128.3, 126.7, 126.4, 118.8, 74.9, 70.4$_{q}$, 58.0(CH$_2$)$_2$, 49.5(CH$_2$)$_2$, 41.9(CH$_2$)$_2$, 11.0(CH$_3$)$_3$; ESI-MS: calcd m/z = 475.60 (M$^+$), found m/z = 475.4 (M$^+$).

1,3,5-Tribenzyl-5-carboxy-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium; chloride 68

Ionic salt 68 was synthesised from 57 (0.12 g, 0.269 mM) and 64 µL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 68 in good yield: 0.14 g (91 %): mp 116-118 °C; 1H-NMR (300 MHz, DMSO-d$_6$) δ = 9.21 (b, 1H, -OH), 8.01-6.61 (m, 24H, Ar-H), 6.07 (d, $^3$J$_{HH}$ = 7.8 Hz, 1H, Ar-H), 5.14 (s, 1H, -CH), 4.27 (s, 1H, -CH$_2$), 4.10 (s, 1H, -CH$_2$), 3.18 (s, 1H, -CH$_2$), 3.08 (s, 1H, -CH$_2$), 2.71 (s, 2H, -CH$_2$); 13C-NMR (75 MHz, DMSO-d$_6$) δ = 169.6$_{q}$, 165.7$_{q}$, 131.9$_{q}$, 131.2, 131.0, 130.5$_{q}$, 130.4, 130.2, 129.5, 129.3, 129.2, 128.7$_{q}$, 128.5, 128.5, 128.3, 128.2, 127.7, 127.5, 127.4,
126.5, 125.6, 120.0, 75.3, 64.9, 59.2(CH2), 58.6(CH2), 49.6(CH2); ESI-MS: calcd m/z = 537.67 (M⁺), found m/z = 537.5 (M⁺).

3-Benzyl-5-carboxy-1-ethyl-5-(1H-indol-3-ylmethyl)-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 69

Ionic salt 69 was synthesised from 58 (0.15 g, 0.309 mM) and 50 μL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 69 in good yield: 0.19 g (98 %): mp 48 °C: 1H-NMR (300 MHz, DMSO-d6) δ = 7.72-6.89 (m, 19H, Ar-H), 6.28 (d, 3JHH = 7.4 Hz, 1H, Ar-H), 4.90 (s, 1H, -CH), 4.44 (m, 1H, -CH2), 3.75 (m, 1H, -CH2), 3.33 (q, 3JHH = 7.1 Hz, 1H, -CH2CH3), 3.04 (q, 3JHH = 7.1 Hz, 1H, -CH2CH3), 2.98 (s, 2H, -CH2), 1.12 (t, 3JHH = 7.1 Hz, 3H, -CH3); 13C-NMR (75 MHz, DMSO-d6) δ = 170.0, 164.9, 138.7, 134.6, 133.4, 132.8, 132.5, 131.4, 130.9, 130.7, 130.1, 129.9, 129.5, 129.4, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.6, 127.4, 127.2, 127.0, 126.9, 122.5, 75.1, 70.3, 51.7(CH2), 48.3(CH2), 41.7(CH2), 9.5(CH3); ESI-MS: calcd m/z = 514.64 (M⁺), found m/z = 514.4 (M⁺).

1,3-Dibenzyl-5-carboxy-5-(1H-indol-3-ylmethyl)-2,4-diphenyl-4,5-dihydro-3H-imidazol-1-ium; chloride 70

Ionic salt 70 was synthesised from 58 (0.15 g, 0.309 mM) and 86 μL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 70 in good yield: 0.18 g (96 %): mp 54-56 °C: 1H-NMR (300 MHz, DMSO-d6) δ = 7.95-6.95 (m, 22H, Ar-H), 6.68 (m, 2H, Ar-H), 6.33 (d, 3JHH = 7.1 Hz, 1H, Ar-H), 4.75 (m, 1H, -CH), 4.52 (s, 2H, -CH2), 3.95 (d, 2JHH = 15.8 Hz, 1H, -CH2), 3.52 (d, 2JHH = 15.8 Hz, 1H, -CH2), 3.23 (s, 2H, -CH2); 13C-NMR (75 MHz, DMSO-d6) δ = 170.3, 165.1, 138.5, 137.9, 134.9, 133.9, 133.6, 132.9, 132.7, 132.5, 132.4, 131.2, 131.0, 130.9, 130.2, 130.1, 129.9, 129.5, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.9, 126.5, 126.2, 124.0, 121.3, 70.1, 66.8, 49.3(CH2), 48.3(CH2), 42.1(CH2); ESI-MS: calcd m/z = 576.71 (M⁺), found m/z = 576.5 (M⁺).
2,3-Dibenzyl-5-carboxy-1-ethyl-5-methyl-4-phenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 71

Ionic salt 71 was synthesised from 59 (0.4 g, 1.04 mM) and 133 μL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 71 in good yield: 0.50 g (89 %): RTIL: $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 8.18 (s, 1H, -OH), 7.90-678 (m, 14H, Ar-H), 6.36 (d, $^3$J$_{HH}$ = 6.0 Hz, 1H, Ar-H), 5.16 (s, 1H, -CH), 4.96 (d, $^2$J$_{HH}$ = 15.0 Hz, 1H, -CH$_2$), 4.12 (d, $^2$J$_{HH}$ = 15.0 Hz, 1H, -CH$_3$), 0.95 (t, $^3$J$_{HH}$ = 7.1 Hz, 3H, -CH$_3$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ = 172.7, 169.3, 133.2, 132.3, 129.5, 129.3, 129.2, 129.0, 128.9, 128.6, 128.5, 128.3, 128.0, 127.8, 127.7, 71.9, 69.2, 49.4, 47.5, 42.3, 25.6, 19.1; ESI-MS: calcd m/z = 413.53 (M$^+$), found m/z = 413.3 (M$^+$).

1,2,3-Tribenzyl-5-carboxy-5-methyl-4-phenyl-4,5-dihydro-3H-imidazol-1-ium; chloride 72

Ionic salt 72 was synthesised from 59 (0.5 g, 1.43 mM) and 187 μL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 72 in moderate yield: 0.4 g (54 %): mp 176-178 °C: $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 8.64 (s, 1H, -OH), 7.60-6.86 (m, 19H, Ar-H), 6.72 (d, $^3$J$_{HH}$ = 7.0 Hz, 1H, Ar-H), 5.16 (m, , 1H, -CH$_2$), 5.05 (s, 1H, -CH), 4.45 (s, 2H, -CH$_2$), 3.92 - 3.83 (m, 1H, -CH$_2$), 1.63 (s, 3H, -CH$_3$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) δ = 172.7, 167.3, 132.8, 132.3, 132.0, 131.0, 129.6, 129.2, 129.2, 128.9, 128.8, 128.6, 128.5, 128.3, 128.1, 127.9, 127.7, 72.4, 69.4, 47.7, 42.1, 31.0, 25.5, 19.7; ESI-MS: calcd m/z = 475.60 (M$^+$), found m/z = 475.4 (M$^+$).

2,3,5-Tribenzyl-5-carboxy-1-ethyl-4-phenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 73

Ionic salt 73 was synthesised from 60 (0.4 g, 0.868 mM) and 120 μL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 73 in good yield: 0.46 g (86 %): dark oil: $^1$H-NMR (300 MHz, DMSO-$d_6$) δ = 8.27 (s, 1H, -OH), 7.90 (d, $^3$J$_{HH}$ = 8.3
2,3-Dibenzyl-5-carboxy-1-ethyl-5-(1H-indol-3-ylmethyl)-4-phenyl-4,5-dihydro-3H-imidazol-1-ium; iodide 74

Ionic salt 74 was synthesised from 61 (0.5 g, 1.0 mM) and mL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 74 in moderate yield: 0.28 g (43 %): mp dark oil: ¹H-NMR (300 MHz, DMSO-d₆) δ = 10.89 (s, 1H, -OH), 8.56 (s, 1H, Ar-H), 8.37 (d, ³JHH = 8.2 Hz, 1H, Ar-H), 7.73 – 6.87 (m, 19H, Ar-H), 4.60 (dd, ³JHH = 14.0, 8.3 Hz, 1H, -CH), 4.26 (m, 2H, -CH₂), 3.45 (s, 2H, -CH₂), 3.26 – 2.89 (m, 4H, -CH & -CH₂), 0.98 (t, ³JHH = 7.2 Hz, 3H); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 171.6, 169.9, 139.2, 136.3, 136.1, 134.1, 128.9, 128.9, 128.5, 128.4, 128.1, 128.0, 127.3, 126.9, 126.6, 126.1, 123.7, 120.8, 118.5, 118.2, 111.2, 110.0, 74.1, 71.6, 54.4, 42.1, 42.0, 28.1, 15.6; ESI-MS: calcd m/z = 528.66 (M⁺), found m/z = 528.5 (M⁺).

1,2,3-Tribenzyl-5-carboxy-5-(1H-indol-3-ylmethyl)-4-phenyl-4,5-dihydro-3H-imidazol-1-ium; chloride 75

Ionic salt 75 was synthesised from 61 (0.5 g, 1.0 mM) and 155 µL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 75 in good yield: 0.47 g (75 %): mp 132-136 °C: ¹H-NMR (300 MHz, DMSO-d₆) δ = 11.03 (s, 1H, -OH), 8.67 (m, 1H, Ar-H), 8.50 (d, ³JHH = 8.2 Hz, 1H, Ar-H), 7.66-6.95 (m, 23H, Ar-H), 4.64 (dd, ³JHH = 13.9, 8.2 Hz, 1H, -CH), 4.29 (m, 2H, -CH₂), 4.02 (s, 2H, -CH₂), 3.49 (s, 2H, -CH₂), 3.29
- 2.99 (m, 2H, -CH₂); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 171.7q, 170.1q, 139.2q, 136.3q, 136.1q, 135.9q, 134.5q, 134.0q, 132.6q, 132.2q, 131.6, 130.2, 129.0, 128.8, 128.7, 128.5, 128.3, 128.2, 128.1, 127.6, 127.3q, 127.0, 126.6, 126.2, 123.8, 120.8, 118.5, 118.2, 111.3, 110.0q, 71.8, 70.0q, 54.4(CH₂), 42.1(CH₂), 42.0(CH₂), 28.1(CH₂), 25.1(CH₂); ESI-MS: calcd m/z = 590.73 (M⁺), found m/z = 590.5 (M⁺).

**Dicationic pyridinium IL**

2-Benzyl-5-carboxy-1-ethyl-4-(1-ethyl-pyridin-4-ium; iodide)-5-methyl-3-(2-methylene-pent-3-enyl)-4,5-dihydro-3H-imidazol-1-ium; iodide 76

Di-cationic salt 76 was synthesised from 62 (0.3 g, 0.779 mM) and 177 µL of ethyl iodide in 3 mL of DMF following the general N-alkylation procedure to afford 76 in good yield: 0.47 g (87 %): mp RTIL °C: ¹H-NMR (300 MHz, DMSO-d₆) δ = 8.27 (m, 3H, Ar-H/ -OH), 7.56-6.80 (m, 12H, Ar-H), 5.03 (s, 1H, -CH), 4.67 (s, 2H, -CH₂), 4.24 (d, JHH = 6.1 Hz, 2H, -CH₂), 3.46 (m, 2H, -CH₂), 1.52 (m, 3JHH = 7.2 Hz, 6H, -CH₃), 1.24 (t, 3JHH = 7.1 Hz, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 172.7q, 164.3q, 145.2, 139.2q, 136.2q, 136.1q, 129.3, 129.0, 128.5, 128.2, 128.1, 127.1, 126.9, 126.7, 126.6, 126.3, 72.3, 69.8q, 42.3(CH₂), 42.1, 41.8(CH₂), 40.3(CH₂), 18.3(CH₃), 17.2(CH₃), 16.3(CH₃); ESI-MS: calcd m/z = 221.79 (M²⁺), found m/z = 221.5 (M²⁺).

1,2,3-Tribenzyl-4-(1-benzyl-pyridin-4-ium; chloride)-5-carboxy-5-methyl-4,5-dihydro-3H-imidazol-1-ium; chloride 77

Di-cationic salt 77 was synthesised from 62 (0.3 g, 0.779 mM) and 150 µL of benzyl chloride in 3 mL of DMF following the general N-alkylation procedure to afford 77 in good yield: 0.4 g (81 %): mp 158-162 °C: ¹H-NMR (300 MHz, DMSO-d₆) δ = 9.16 (b, 1H, -OH), 8.60-8.52 (m, 2H, Ar-H), 7.59-7.04 (m, 21H, Ar-H), 6.67 (s, 1H, Ar-H), 5.20 (m, 1H, -CH), 4.23 (s, 2H, -CH₂), 4.10 (s, 2H, -CH₂), 3.47 (m, 2H, -CH₂), 1.20 (m, 3H, -CH₃); ¹³C-NMR (75 MHz, DMSO-d₆) δ = 172.5q, 170.0q, 139.3q, 136.3q, 129.1, 129.0, 129.0, 128.6, 128.4, 128.1, 128.1, 127.1, 126.9, 126.6, 126.2, 72.2, 69.8q, 48.6(CH₂), 152
47.8\textsubscript{(CH2)}, 41.9\textsubscript{(CH2)}, 41.8\textsubscript{(CH2)}, 18.3\textsubscript{(CH3)}, 17.2\textsubscript{(CH3)}; ESI-MS: calcd $m/z = 283.86$ (M$^{2+}$), found $m/z = 283.7$ (M$^{2+}$).
2.4 Synthesis of cyclodextrin ionic liquids.

2.4.1 Introduction

Cyclodextrins (CDs) have been catapulted into prominence in the last few decades because of their catalytic, enzyme mimic, complexation, drug encapsulation, asymmetric, and molecular recognition behaviour. They are also used in purification, stabilization of products, polymerization, food preservation, chemical treatment, and other industrial processes. Since structural and available functional group limitations have hindered their utility, these molecules have been subjected to exhaustive synthetic manipulations and a large number of practical methods have been developed for their modifications.

As chiral entities, selectively modified CDs have the potential for utilization in asymmetric synthesis, molecular recognition, molecular switches, and chiral separations. Since they are expected to be nontoxic and form complexes with various flavours, perfumes, vitamins, and essential oils, they have a promising future in biodegradable materials and health-related products. Synthesis of artificial receptors that recognize and bind with transition metals is an important field which could find application in waste treatment. Modified cyclodextrins show better complexing behaviour than native cyclodextrins and hold greater potential for the formulation of slow-releasing drugs. CDs are spectroscopically inert and can be converted to photosensitive molecules by attachment of chromophores to function as chemosensors. Theoretical studies of cyclodextrins and their derivatives with computer-generated 3D modelling provided information about their lipophilic, hydrophobic, and complexation behaviour. This insight has led to a better understanding in devising new synthetic approaches and applications for CD molecules.
The native cyclodextrin (α, β and γ cyclodextrins) are cyclic oligosaccharides, consist of six, seven and eight 1-4 linked α-D-glucopyranose units, respectively. Interest in these compounds stems from the fact that they act as host molecules, forming inclusion complexes with a wide variety of guest molecules (Figure 2.47). These CDs naturally exist as a single enantiomer, consequently, when they act as host molecules to a racemic guest, they can form inclusion complexations which lead to the formation of diastereoisomeric complexes of differing thermodynamic stability resulting in chiral discrimination. This has been intensively studied, most notably through the work of Armstrong et al. in the development of CD based chromatographic systems. The extent of chiral discrimination displayed by CDs is typically quite modest, with efficient resolution of racemates predominantly being attributed to the repeated interactions with the cyclodextrin. This low enantioselectivity is associated with the inherent symmetry of these oligosaccharides. In addition, inclusion complex formation often occurs principally as a result of interaction between the hydrophobic portion of the cyclodextrin with the guest molecule within the hydrophobic annulus of the cyclodextrin, rather than interaction with the CDs chiral centers. Thus, chiral discrimination can be increased with modified CDs whereby, the degree of asymmetry of the cyclodextrin is increased and there is increased interaction between the chiral sites of the cyclodextrins with those of the guests.

**Figure 2.46:** Structure of β-cyclodextrin.
Modifying cyclodextrins and their complexing characteristics usually involves derivatisation of one or more of the C-2, C-3 and C-6 hydroxy groups. The modifications can be divided into two categories, symmetric and asymmetric substitution patterns. Symmetric modification can be accomplished by two methods; the symmetric substitution to give a single modified cyclodextrin product (e.g., all the hydroxy groups may be substituted). Secondly, random substitution resulting in a complex mixture of cyclodextrins where by the over all effect is equivalent to that of symmetric substitution. Symmetric substitutions tend not to alter the symmetry of the cyclodextrin. Thus, symmetrically substituted cyclodextrins tend to show no better chiral discrimination than the naturally occurring CDs. With asymmetric modification patterns, a single or a specific combination/ or number of substituents is introduced. This tends to induce significant changes in the asymmetry of the cyclodextrin and results in additional and more specific interactions between the chiral area of the guest and the asymmetry of the host. As a result this may restrict the host binding geometry, leading to greater enantioselectivity.

Although native CDs are able to resolve some enantiomers\textsuperscript{108} this ability can be enhanced in many cases by their derivatisation. Each glucose unit of the native CDs has two secondary hydroxy groups on C-2 and C-3 positions and one primary hydroxy group on the C-6 position which can be derivatized readily by a wide variety of substituents. Therefore, additional interactions, such as $n-n$ interactions, dipole–dipole interactions, ion-paring, electrostatic and steric repulsive effects can be introduced between the

\textbf{Figure 2.47:} Guest-host inclusion complexation between CD and a guest molecule.
guest-analytes and the appropriately derivatized CDs. In this way, the chromatographic properties and the chiral recognition as well as the solubility and complex-forming capacity of the CDs can be improved. Therefore, the range of enantiomeric compounds that can be resolved with CDs has been greatly expanded.\textsuperscript{109-111}

Selective functionalization of the primary and the secondary hydroxy groups is complicated by statistical and steric problems. Several strategies have been developed to selectively functionalise either all the primary hydroxy groups or all the secondary hydroxy groups, however the complexity of the problem is increased if control on the degree of functionalisation such as mono-, di- or tri-substitutions, is desired. The methods available to achieve such control are often tedious and produce low yields.\textsuperscript{112} Among these methods, selective monotosylation at the C6 positions (Figure 2.48) is straight forward,\textsuperscript{113} however, the secondary side (inner rim) has been shown to be the most important for CD binding studies.\textsuperscript{114} Although functionalisation of the secondary hydroxy groups is the most difficult, they are more important in the design and synthesis of chiral discriminating cyclodextrins.

![Figure 2.48: Selective β-CD monotosylation product.](image)

In order to apply various derivatization methods for the syntheses of new CDs, it is important to understand the statistical and steric problems, as well as, various chemical factors which complicate the selective modification of the primary and the secondary hydroxy groups. The rotation of the secondary hydroxy groups at the 2- and 3-positions of adjacent glucose units is restricted forming of a ‘belt’ of intramolecular hydrogen
bonds which gives the oligosaccharide structural rigidity and the wider rim on the secondary side of the CD cavity, while the rotation of the primary hydroxy groups is not restricted, which is attributes to the smaller cavity diameter observed on the primary side of the molecule.\textsuperscript{104} The presence of the polar primary and secondary hydroxy groups makes the upper and lower end of the molecule hydrophilic while the cavity of cyclodextrins is hydrophobic due to the presence of glycosidic oxygens and C-H groups. The internal diameter of the cavity on the secondary side varies in size from 5.7 to 9.5 Å as the number of glucose units increases from α-CD to γ-CD, respectively, with β-CD having an internal diameter of 7.8 Å.\textsuperscript{115} NMR analysis of native β-CD in DMSO-\textit{d}_6 illustrates the symmetric nature of cyclodextrin structure. Figure 2.49 shows the $^{13}$C-NMR spectra (i) and DEPT experiments which allow the determination of CH from CH$_2$ carbons. DEPT135 experiment (iii) shows the C6 CH$_2$ carbons as an inverse peak at 59.9 ppm, while the DEPT90 (ii) shows only the CH peaks corresponding to C1-C5 carbons. Modification of cyclodextrin yields complex chemical shift patterns depending on the type of modification carried out and the substituents used in the modification.
Two main factors need to be considered in the chemical modification of cyclodextrins, the nucleophilicity of the hydroxy groups and the ability of cyclodextrins to form complexes with the reagents used. All modifications of cyclodextrins take place at the hydroxy groups. Of the three different hydroxyl groups present in cyclodextrins, the C6 primary hydroxy units are the most basic, with C2 secondary hydroxy groups being the most acidic, while C3 secondary hydroxyl groups are the most sterically inaccessible.\textsuperscript{116,117} Consequently, an electrophilic reagent is more inclined to react with the C6 primary hydroxy groups. However, a more reactive reagent will attack the hydroxy groups with less selectivity. Thus, the more reactive reagents will not only react with C6 hydroxy groups but also with those on the secondary side C2 and C3 positions, whereas, a less reactive reagent will react selectively with the C6 hydroxy groups. An example of this is the application of the derivatising agents tert-butyldimethylsilyl chloride (TBDMS) and trimethylsilyl chloride (TMSCl). The less reactive more bulky reagent TBDMS reacts selectively with hydroxy groups at the 6-positions,\textsuperscript{118} to give 90\% of the major product under optimal conditions and the derivative can be easily isolated by flash column chromatography or by recrystallization.\textsuperscript{119} The smaller, more reactive TMSCl reacts with all the hydroxy groups non-selectively (Figure 2.50).\textsuperscript{120}
The ability of cyclodextrins to form complexes introduces an additional factor in determining the substitution pattern.\textsuperscript{121} If the complex formed with the reagent is very strong, the predominant product formed is dictated by the orientation of the reagent within the complex. If the complex is weak, then the relative nucleophilicities of the hydroxy groups dictate the product formed. Solvents play an important role in determining the strength and the orientation of the complex formed between the reagent and cyclodextrin. This is evident when tosyl chloride reacts with $\alpha$-cyclodextrin in pyridine to give the 6-tosylated product, whereas, in aqueous base, the 2-tosylated derivative is the product.\textsuperscript{122} The size of the cyclodextrin cavity also has a marked effect on the strength and the orientation of the reagent complex, thus, affects the product of the reaction. For example, reaction of tosyl chloride in aqueous solution with $\alpha$-cyclodextrin gives the 2-substituted product; whereas with $\beta$-cyclodextrin, the 6-substituted product is formed (Figure 2.48).\textsuperscript{112}

for the synthesis of C2 and C3 derivatives, the per-6-iodo-cyclodextrins is a very useful intermediate product from which further functionalisation with imidazolium or pyridinium moieties at the C6 position is permitted to afford novel ionic cyclodextrins with improved solubilities in less polar organic solvents than native CDs. The synthesis of these new ionic cyclodextrins per-2,3-O-acetyl-6-imidazolium-$\beta$-cyclodextrin iodide, per-2,3-O-acetyl-6-pyridinium-$\beta$-cyclodextrin iodide, per-2,3-O-acetyl-6-imidazolium-$\gamma$-cyclodextrin iodide, and per-2,3-O-acetyl-6-pyridinium-$\gamma$-cyclodextrin iodide is discussed and their thermal stabilities assessed for applicability as GC stationary phases.

\textbf{2.4.2 Results and discussion}

Primary hydroxy groups are more nucleophilic than their secondary counterparts, they are easily transformed into other functionalities. Therefore our focus was directed toward the selective derivatisation of these primary hydroxyl groups. Ideally the best cyclodextrin candidates for application as GC chiral selectors are asymmetrically substituted CDs. Modification of one -OH functional group is the simplest way to produce
an asymmetrical mono-substituted CD. A convenient and widely used intermediate for modifying of one of the C6 hydroxy group on the primary side of CD into other functional groups such as amino,\textsuperscript{113,123} thioalkyl,\textsuperscript{124} halo,\textsuperscript{113} and formyl\textsuperscript{125} is mono-6-(p-tolylsulfonyl)-6-OH-β-CD \textbf{78} (Figure 2.51). Thus this mono-6-(p-tolylsulfonyl)-6-OH-β-CD was synthesised with the aim to directly synthesise a mono-6-imidazolium-6-OH-β-CD (Figure 2.52).

\begin{center}
\includegraphics[width=0.8\textwidth]{structure.png}
\end{center}

\textbf{Figure 2.51:} Structure of mono-6-(p-tolylsulfonyl)-6-OH-β-CD (78).

\textit{Mono-6-(p-tolylsulfonyl)-6-OH-β-CDs are typically prepared by the reaction of CD with p-toluuenesulfonyl chloride in dry pyridine\textsuperscript{112,113} or in aqueous acetonitrile at alkaline pH.\textsuperscript{112,113,124,126} This method has produced very low yields of 6 \%,\textsuperscript{126} 11 \%,\textsuperscript{123} or 17 \%,\textsuperscript{124} and forming multi-tosylated by-products from which \textbf{78} must be isolated by chromatography.\textsuperscript{112} However, in 1998 Zhong et al.\textsuperscript{127} reported an easy large-scale synthesis of \textbf{78} as a single product in reasonable yield, avoiding the production of the multitosylated byproducts generated by other methods. Following this method p-toluuenesulfonic anhydride (Ts\textsubscript{2}O) was added to an aqueous solution of β-CD, subsequent addition of 10 \% aqueous NaOH solution induced the tosylation reaction, affording \textbf{78} as the sole product in 49 \% yield without the need for further purification. After filtration to remove the unreacted Ts\textsubscript{2}O, the product was isolated by precipitation at 4 °C overnight in alkaline medium (pH 8 with aq. NH\textsubscript{4}Cl).}
Figure 2.52: i) β-CD and Ts₂O at r.t for 2 hrs, add 10 % (aq) NaOH, react for 10 mins, ii) heat 78 and imidazole or pyridine for 24 hrs at 90 °C in DMF, iii) Reflux 78 with KI in DMF for 1 hrs, iv) heat mono-6-iodo-6-OH-β-CD with an imidazole or pyridine in DMF at 90 °C for 24 hrs.

The tosylate substituent has been described by Muderawan et al. to be displaced by 3-methylimidazole (as well as pyridine and alkylamines) upon heating of 6-monotosyl-β-CD with imidazole at 90 °C in DMF for 48 hrs to afford a mono-6-(3-methylimidazolium)-β-CD iodide in 99 % yield.³²⁸ Replication of these experiments did not yield the imidazole substituted β-CD (only starting material 78). Comparison of the melting point of the isolated CD 78 (195-196 °C) with those reported in literature by Melton and Slessor (159-162 °C), suggesting that mono-6-(p-tolylsulfonyl)-6-OH-β-CD might not have been isolated. No melting point were reported by Zhong et al., so a comparison with their CD isolate was not possible.

Unable to substitute the tosyl group with an imidazole or pyridine, our focus should have been directed towards substituting the CD-tosyl group with iodine. Sulphonate groups are described as being readily displaceable by nucleophilic substitution with a variety of nucleophiles including iodine to afford mono-6-iodo-6-OH-β-CD by Melton and Slessor, in hindsight we recommend the further pursuit to displace the tosylate group with an iodide following the method described by Melton and Slessor in which 78 is boiled in water or DMF with excess NaI to afford mono-6-iodo-6-OH-β-CD.¹¹³
CD nucleophilic substitution with an imidazole would take place more readily to yield the desired ionic mono-6-imidazolium-6-OH-β-CD iodide. Using this ionic mono-6-substituted CD, a series of asymmetrical mono-ionic CDs with various derivatives at the remaining hydroxyl groups with improved solubilities could potentially be synthesised for application as GC chiral selecting agents. Such mono-ionic CDs would be novel and highly desirable for this application.

Selective per-modification of all the primary hydroxyl groups is easier than mono-, di-, or trisubstitution because symmetrical substitution is achieved when the reaction is allowed to run for a longer time with appropriate amounts of reagents. The formation of regioisomeric products (Figure 2.53) further complicates this situation when selective di- or trisubstituted cyclodextrins are required to be prepared, so that the reaction products require chromatographic purification.

![Figure 2.53](image)

**Figure 2.53:** An illustration of the possible regioisomers for di- and tri-substitution patterns of β-CD where by each circle represents one derivatized glucose subunit.

Given the difficulty in synthesizing mono-6-iodo-6-OH-β-CD, our focus was directed towards the preparation of per-substituted 6-iodo-β-cyclodextrin 79 and 6-iodo-γ-cyclodextrin 80 from which per-2,3-O-acetyl-6-iodo-β-cyclodextrin 81 and per-2,3-O-
acetyl-6-iodo-γ-cyclodextrin 82 can be synthesised (see Figure 2.56). From these per-2,3-acetyl-6-iodo-cyclodextrins it is possible to synthesise a large variety of highly charged ionic CDs with seven or eight ionic substituents, respectively, on the primary side in high yields.

**Figure 2.54:** Synthesis of per-6-deoxy-6-iodo-β-CD To a solution of triphenylphosphine and iodine in DMF was added cyclodextrin and reacted at 80 °C for 18 hrs.

Per-6-deoxy-6-iodo-β-cyclodextrin was synthesised from native β-cyclodextrin and native γ-cyclodextrin by nucleophilic substitution of the C6 hydroxy substituents with iodine using triphenylphosphine (Ph3P) dissolved in anhydrous DMF (Figure 2.56).129 The crude product was isolated by precipitation in ice-water and collected by filtration to afford an off-white product at 90 % (79) and 87 % (80) yield. The 13C-NMR spectrum of per-6-iodo-β-cyclodextrin in DMSO-d6 is shown in Figure 2.55 (ii); in the sugar region it is similar to that of native β-cyclodextrin (Figure 2.55 (ii)) with the exception of C6, substitution of the C6 hydroxyl groups with iodine leads to an upfield shift of the α-carbon (C6) from 59.9 to 9.5 ppm, proportional to the electronegativity of the substituent, resulting from iodine being less electronegative than oxygen, but a smaller upfield shift of the β-carbon (C5) typical of the electron-withdrawing inductive effect, while the γ-carbon (C4) experiences a downfield shift. This is illustrated in Figure 2.55 by 13C-NMR and DEPT experiments.
Figure 2.55: $^{13}$C-NMR spectra of (ii) Per-6-deoxy-6-iodo-β-cyclodextrin compared with (i) native β-cyclodextrin (DMSO-$d_6$).

Per-6-deoxy-6-halogenated cyclodextrins are an important class of compounds which can be used for the selective functionalization of the primary face because of their great stability. However, their use is limited because of their insolubility in less polar solvents. They are only soluble in polar high boiling solvents such as pyridine, DMF, DMSO, or HMPA. Their solubility in nonpolar solvents can be increased by methylation or esterification of the secondary hydroxyl groups. The secondary side of the halogenated cyclodextrins can be acetylated with acetic anhydride in pyridine in high yields for further selective functionalization.$^{130,131}$
**Figure 2.56:** Reaction scheme for the synthesis of per-6-deoxy-6-iodo-cyclodextrins 79-80 from native β-cyclodextrin, followed by the synthesis of C2- and C3-O-acetylated per-6-deoxy-6-iodo-cyclodextrins 81-82 and their ionic cyclodextrin derivatives 83-88.

**Acetylation of per-6-deoxy-6-iodo-β-Cyclodextrin**

Per-alkylation or per-acetylation of the cyclodextrin hydroxy groups improves their solubility in organic solvents, rendering them more soluble in less polar solvents than unmodified cyclodextrins132 widening their potential for further exploitation. Not only does derivatisation improve their solubility, but, the *secondary side* is stated to be catalytically very important,114,133 and therefore, modifications of this face are believed to produce valuable derivatives for catalysis, enzyme mimics, etc. Both of these characteristics are desirable qualities to incorporate onto these novel CD selecting agents.

In order to improve solubility of per-2,3-hydroxy-6-deoxy-iodo-cyclodextrins in DCM, a solvent commonly used as the medium for dissolving the stationary phase in the procedure for static coating of capillary columns, the C2 and C3 secondary hydroxy groups were derivatised by non-selective acetylation by dissolving CD 79 or 80 in anhydrous pyridine under a nitrogen atmosphere, followed by the slow addition of acetic anhydride. The mixture was stirred for 4 hrs at room temperature in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) to afford 81 in 85 % yield and 82 in 78 % yield. This procedure gives the per-2,3-O-acetyl-6-deoxy-6-iodo-cyclodextrins in good yield and the products were simple to isolate and purify.

Following acetylation of the C2- and C3-positions there was significant improvement in the solubility of the iodo-CDs in the less polar solvents. In Figure 2.57 show the $^{13}$C-NMR spectra and DEPT experiments for per-2,3-acetyl-6-deoxy-6-iodo-β-CD. Here $^{13}$C-NMR spectra are used as the primary means of characterising the CD derivatives given that the $^{13}$C-NMR shifts are a much better indicator of the structure variations than $^{1}$H-NMR,
due to the fact that substituent-induced proton shifts do not follow a regular pattern as do carbon shifts. Figure 2.57 shows $^{13}$C-NMR spectra of the symmetrical substitution pattern as expected from per-substitution of the secondary hydroxyl groups of CD 81.

Figure 2.57: Per-2,3-acetyl-6-deoxy-6-iodo-β-CD; i) $^{13}$C NMR, ii) DEPT 135, iii) DEPT 90 spectra in CDCl$_3$.

The $^{13}$C-NMR spectrum of Per-2,3-O-acetyl-6-deoxy-6-iodo-β-cyclodextrin in DMSO-$d_6$ is shown in Figure 2.57; $^{13}$C-NMR spectra of CD 81 is similar to that of per-6-deoxy-iodo-β-cyclodextrin (79) in the sugar region with the presence of two acetyl quaternary at 170.6 ppm and 169.4 ppm, as well as CH$_3$ at 22.7 ppm which are confirmed by DEPT experiments in which the signals for the two quaternary carbons do not appear in the DEPT experiments (Figure 2.57) (ii) and (iii) while the methyl carbon is present in the DEPT 135 (which show all proton bound carbons in positive amplitude with the exception of CH$_2$ in negative amplitude) but disappears from the DEPT 90 (iii) spectra (which only show CH carbons). Acetylation of C2 and C3 can be confirmed as having gone to completion owing to the observed symmetry in the sugar region on the carbon spectra. C1 to C6 of each of the 7 glucose subunits of CD 81 appear as single peaks as a result of the symmetrical substitution pattern.
Figure 2.58: Preparation of per-6-substituted ionic CDs 89-94 by refluxing per-6-deoxy-6-iodo-CD 81-82 in DMF at 90 °C with either various i) pyridine or ii) imidazole derivatives.

The ionic cyclodextrin compounds 89-94 per-2,3-O-acetyl-6-deoxy-6-(imidazolium or pyridinium) β or γ-CD were prepared from compound 81 and 82 (per-2,3-O-acetyl-6-deoxy-6-iodo-β- or γ-CD) by nucleophilic displacement of iodine with either excess imidazole or pyridine in DMF (or neat if the pyridine or imidazole used is liquid) at 90-100 °C for 24 hrs, similar to the method described by Thatcher et al.126 DMF was removed under reduced pressure and per-6-deoxy-6-imidazolium or per-6-deoxy-6-pyridinium-β-cyclodextrin ionic liquids were isolated. The ionic CDs synthesised are listed in Table 2.6.
Table 2.6: A table of the CD compounds synthesied.

<table>
<thead>
<tr>
<th>C6-substituents</th>
<th>2,3-OH-β-CD</th>
<th>2,3-Ac-β-CD</th>
<th>2,3-Ac-γ-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per-6-deoxy-6-iodo-β-CD</td>
<td>79 (80 is γ-CD)</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>-6-Methylimidazolium I</td>
<td>83</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td>-6-Benzylimidazolium I</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6-Butylimidazolium I</td>
<td>85</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td>-6-(-2-(1-imidazolium)-1-phenylethanol) I</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6-Pyridinium I</td>
<td>87</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>-6-(4-dimethylamino pyridinium) I</td>
<td>88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.59: Preparation of per-2,3-OH-6-deoxy-6-(N-dimethylaminopyridinium)-β-CD iodide 88 by refluxing per-2,3-OH-6-deoxy-6-iodo-β-CD 79 in DMF at 90 °C with either various pyridines or imidazoles substituents (in this case pyridine). $^{13}$C-NMR spectra; i)
Native-β-CD, ii) per-6-deoxy-6-iodo-β-CD, and iii) per-6-deoxy-6-(N-dimethylamino pyridinium iodide)-β-CD in DMSO-d$_6$.

Figure 2.59 shows the sequential $^{13}$C-NMR spectral shift changes in the 2 step synthesis of β-CD IL 88 starting from native β-CD (i) undergoing nucleophilic substitution with iodine to afford per-6-deoxy-6-iodo-β-CD (ii) followed by the nucleophilic substitution with pyridine to give ionic β-CD 88 (iii) in 66 % yield. The $^{13}$C-NMR spectra of ionic CD 88 (iii) is similar to that of per-6-deoxy-6-iodo-β-cyclodextrin (ii) in the sugar region with the exception of the downfield shifted C6, resulting from the substitution of the less electronegative iodine with the more electronegative nitrogen from pyridine having a greater electron attracting power resulting in the de-shielded effect on the C6 carbon atom.
Table 2.7: Properties of per-2,3-substituted-6-deoxy-6-substituted β-CD ILs.

<table>
<thead>
<tr>
<th>C6-substituents</th>
<th>mp [°C] Per-6-deoxy-6-iodo-β-CD</th>
<th>mp [°C] 2,3-OH-β-CD</th>
<th>mp [°C] 2,3-Ac-β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per-6-deoxy-6-iodo-β-CD</td>
<td>207-210</td>
<td>198-200</td>
<td>190-192</td>
</tr>
<tr>
<td><img src="image1" alt="Iodo-substituent" /></td>
<td>234</td>
<td>RTIL</td>
<td>RTIL</td>
</tr>
<tr>
<td><img src="image2" alt="Acetate-substituent" /></td>
<td>218</td>
<td>RTIL</td>
<td>RTIL</td>
</tr>
<tr>
<td><img src="image3" alt="Hydroxyl-substituent" /></td>
<td>141-145</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image4" alt="Protonated-substituent" /></td>
<td>215-216</td>
<td>220-224</td>
<td>198-202</td>
</tr>
<tr>
<td><img src="image5" alt="Imidazole-substituent" /></td>
<td>242-243</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Thermogravimetric Analysis**

It is of significant importance to determine the thermal stability of the synthesised cyclodextrins as this directly reflects the maximum allowable operating temperature of the GC column in which these compounds will be incorporated. For application as chiral selectors in GC stationary phases it is of significant importance to know the temperatures at which these ionic CD are stable and the temperature at which decomposition begins to take place. The thermostabilities of the derivatised cyclodextrins were determined and compared with the thermal stabilities of native β-cyclodextrin as well as per-2,3,6-O-
methyl-β-CD and *per*-2,3,6-*O*-acetyl-β-CD. Thermogravimetric analysis scans were obtained on a Mettler thermogravimetric balance at a heating rate of 20 °C/min from 50 to 800 °C under an inert nitrogen atmosphere flowing at 20 mL/min, switching to air at 600 °C.

The thermogravimetric analysis of β-CD in Figure 2.60 showed mass loss in two different temperature regions. The first mass loss at around 200-400 °C being due to the melting and decomposition of the glucose unit in β-CD, and the second mass loss around 600 °C due to the introduction of oxygen. The first mass loss is the region of interest as this is indicative of the decomposition of the glucose and imidazolium/ or pyridinium units. Following TGA analysis of *per*-2,3-OH-6-deoxy-6-ido-β-CD decomposition took place at 221 °C, that is 23 °C lower than native-β-cyclodextrin which decomposed at 244 °C. Derivatisation of native-β-CD by *per*-2,3,6-methylation and *per*-2,3,6-acetylation significantly increased their thermal stabilities by 50 °C and 76 °C decomposing at 294 °C and 320 °C, respectively.
Figure 2.60: A) TGA analysis of native β-CD CD, per-6-deoxy-6-iodo-β-CD, per-2,3,6-methyl-β-CD, and per-2,3,6-acetyl-β-CD. B) TGA analysis of the per-2,3-acetylated ionic β-CDs 89, 91, and 93, represented in blue, green and black, respectively, as well as per-2,3-O-acety-6-deoxy-6-iodo-β-CD (81).

Following nucleophilic displacement of iodide with an imidazole or pyridine substituent thermal analysis showed per-6-deoxy-6-benzylimidazolium 84 and per-6-deoxy-6-methylimidazolium 83 β-CDs to have slightly higher thermal stabilities compared to that of the native β-CD while a diminished thermostability was observed for CD 86 decomposing at 160 °C.
Table 2.8: Thermal decomposition temperatures of per-6-deoxy-6-substituted CDs.

<table>
<thead>
<tr>
<th>Per C6-substituents</th>
<th>$T_D$ [°C]</th>
<th>$T_D$ [°C]</th>
<th>$T_D$ [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per-6-deoxy-6-iodo-</td>
<td>222</td>
<td>243</td>
<td>232</td>
</tr>
<tr>
<td>~~~~N+_N–I</td>
<td>256</td>
<td>202</td>
<td>188</td>
</tr>
<tr>
<td>~~~~N+_N–I</td>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~~~~N+_N–I</td>
<td>293</td>
<td>204</td>
<td>204</td>
</tr>
<tr>
<td>~~~~N+_N–OH</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>~~~~N+_I</td>
<td>225</td>
<td>227</td>
<td>223</td>
</tr>
<tr>
<td>~~~~N+_I</td>
<td>250</td>
<td></td>
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</table>

Similarly, the pyridinium IL CD derivative generally showed a diminished thermal stability (ranging from 180-215 °C) compared to native β-CD, with per-2,3-OH-6-deoxy-6-(4-dimethylaminopyridinium)-β-CD having a slightly higher thermal stability compared to native β-CD decomposing at 250 °C.

**TGA analysis of per-2,3-acetyl-CD ILs**

Following the thermogravimetric analysis of per-2,3,6-O-methyl-β-CD and per-2,3,6-O-acetyl-β-CD it was evident that derivatisation at C2 and C3 significantly increased the
thermal stability of the cyclodextrin products. Thus it was expected that acetylation at C2 and C3 would in improved thermal stability our imidazolium and pyridinium ionic CDs. However, the thermales stabilities were significantly diminished by up to 17-42 °C for the 2,3-O-acetyl ionic β-CDs when compared with native β-CD and diminished by 21-89 °C when compared with their 2,3-hydroxy ionic β-CD analogues. An interesting observation was that the pyridinium CDs 87, 93, and 94 maintained their thermal stabilities at 225 °C, 227 °C, and 223 °C, respectively, regardless of whether C2 and C3 substituent was an acetyl group or hydroxy groups. Whereas, the thermal stabilities of the imidazolium CDs were affected by the C2 and C3 substituents, with the C2 and C3 acetyl groups generally lowering the thermal stabilities. The size of the CD ring had a minor affect on the thermal stability of the iodo-CDs with per-2,3-acetyl-6-deoxy-iodo-β-CD decomposing at 243 °C while per-2,3-acetyl-6-deoxy-6-iodo-γ-CD decomposed at 232 °C a difference of 11 °C. However, the size of the CD ring had little to no effect on the thermal stabilities of the ionic CDs when comparing the ionic γ-CDs and β-CDs analogues (see Table 2.8).

**Selective secondary face modification**

The internal secondary side is more crowded than the primary side due to the presence of twice the number of hydroxy groups. The hydrogen bonding between hydroxy groups at the C2- and C3-positions makes them rigid and less flexible as compared to C-6 hydroxy groups. The hydroxy groups at the C3-positions are the least reactive probably due to their involvement in hydrogen bonding with C-2 hydroxy groups. The C3-position is more sterically hindered and not easily available for further modification. All these factors make the *secondary side* less reactive and harder to selectively functionalize than the outer primary face.

Ideally the best cyclodextrin derivative candidates for application as GC chiral selectors are asymmetrically substituted CDs. To reduce the symmetry of the ionic CDs the synthesis of *mono*-6-deoxy-6-iodo-6-OH-β-CD was attempted initially, from which *mono*
ionic CDs could have potentially been synthesised by nucleophilic substitution of the iodide with imidazoles or pyridines. Given that per-2,3-O-acetylated ionic CDs that were synthesised in this work are highly symmetrical products, it was sought to reduce the symmetry of these CDs by selectively per-methylating at the C2 positions and acetylating at C3 positions as described by Bicchi et al.\textsuperscript{134} It has been shown that asymmetrically substituted per-2-O-methyl-3-O-acetyl- or per-2-O-methyl-3-O-ethyl-CDs can extend enantioselectivity in comparison to that of the corresponding per-2,3-O-methyl or per-2,3-O-acetyl symmetrical derivatives, in terms of both enantiomer resolution and number of chiral compounds separated by GC.\textsuperscript{134,135}

And so work was directed towards the selective C2 per-methylation of the per-6-ido-β-cyclodextins \textbf{79} and \textbf{80} with the intentions of per-acetylation of the C3 hydroxy groups. Using these selectively derivatised iodo-CDs one can synthesise imidazolium or pyridinium ionic CDs by nucleophilic substitution of the C6 iodo substituent. Selective per-methylation is carried out with BaO and Ba(OH)\textsubscript{2} monohydrate, a weaker base than NaH and thus more selective in its action as described by Bicchi.\textsuperscript{134} The procedure for selective C2 methylation were applied to CDs \textbf{79} and \textbf{80} by stirred with excess BaO and Ba(OH)\textsubscript{2} monohydrate in anhydrous DMF at room temperature for 3 hrs, then cooled to 0 °C. Following which methyl iodide was slowly added and the mixture allowed to react at 40 °C for 24 hrs. The reaction mixture was worked up by diluting with a 10 % ammonia solution filtered through a pad of silica gel and washed with water. The C2-O-methyl-CD derivative was supposed to be eluted with ethyl acetate, followed by purification by washed with brine, and drying over MgSO\textsubscript{4}. The solvent would then have been evaporated under vacuum to afford the derivative as an oily residue which is then to be further purified by column chromatography. However, our reaction product did not elute with ethyl acetate and as a result the C2-O-methyl derivative products were not isolated. This is possibly due to the difference in polarity of the compound we were trying to synthesise (per-2-O-methyl-3-OH-6-deoxy-6-iodo-CD) in comparison to the compound synthesised by Bicchi (per-2-O-methyl-3-OH-6-O-THDMS-γ-CD). Preliminary attempts
were made to isolate the product, however, due to time constraints the method for isolating these C2 per-methylated iodo-CDs was not further developed. The isolation of per-2-O-methyl-3-OH-6-deoxy-6-iodo-CD was unsuccessful as we were not able to confirm the product by NMR. A significant change in the solubility of the reaction product in methanol compared to the starting material (per-2,3-OH-6-deoxy-6-iodo-CD) was found. Per-2,3-OH-6-deoxy-6-iodo-CD was insoluble in methanol while the reaction product readily dissolved in methanol. Analysis by TLC in 4:1 Ethyl acetate/methanol given an \( R_f \) value of 0 for both per-2,3-OH-6-deoxy-6-iodo-\( \beta \)-CD and per-2,3-OH-6-deoxy-6-iodo-\( \gamma \)-CD compare to an \( R_f \) value of 0.54 for the reaction products per-2-methyl-3-OH-6-deoxy-6-iodo-\( \beta \)-CD and per-2-methyl-3-OH-6-deoxy-6-iodo-\( \gamma \)-CD.

Further effort to isolate these compounds is part of the planned future work. Synthesis of per-2-O-methyl-3-OH-6-deoxy-6-iodo-CD would pave the way for the synthesis of the desirable differentially substituted per-2-O-methyl-3-O-acetyl-6-deoxy-6-iodo-CD and allow for the synthesis of per-2-O-methyl-3-O-acetyl-6-deoxy-6-imidazolium-CD ionic salts (Figure 2.61), novel compounds which have not yet been synthesised and are highly desirable for study as chiral selecting agents.

![Reaction scheme for the synthesis of asymmetrically substituted ionic CDs.](image)

**Figure 2.61:** Reaction scheme for the synthesis of asymmetrically substituted ionic CDs.
2.4.3 Conclusion

We have reported a simple route for the synthesis of a range of pre-2,3-OH-6-deoxy-6-imidazolium and of per-2,3-OH-6-deoxy-6-pyridinium substituted ionic CDs as well as their per-2,3-O-acetylated derivatives in good yields, some of which possess improved thermal stabilities as well as improved solubility in less polar organic solvents making their coating onto capillary columns via the static method possible, given that the insolubility of native β-cyclodextrin in organic solvents make them difficult to coat onto capillary columns. These new CDs possess ionic substitutents making them interesting candidates for future application as stationary phases in chromatographic separations for enantiomeric resolution of racemates. This study paves the way for these new ionic CDs to be employed as potential selecting agents with chiral molecular recognition abilities in GC separation.

The synthesis of the asymmetrically derivatised per-2-O-methyl-3-O-acetyl-6-deoxy-6-iodo-CDs from which ionic CDs with reduced symmetry is part of future studies to be undertaken. To reduce the symmetry of the charged CD salts via the synthesis of mono-6-deoxy-6-iodo-CD will be further investigated. These CDs may lead to the synthesis of novel per-2-O-methyl-3-O-acetyl-6-deoxy-6-(imidazolium or pyridinium)-CD ionic salts with reduced symmetry, as well as various mono-6-(imidazolium or pyridinium) substituted ionic CDs which may possess enhanced enantioselective capabilities.
2.4.4 Experimental

2.4.4.1 Reagents

β-Cyclodextrin, γ-cyclodextrin, DMF, triphenylphosphine, sodium, MeOH, NaH, acetic anhydride, pyridine, DCM, BaO, Ba(OH)$_2$, 1-methylimidazole, 1-butylimidazole, 1-benzylimidazole, pyridine, pyridine-4-carboxaldehyde, 2-imidazoyl-1-phenylethanol, 4-dimethylaminopyridine (DMAP), and 2,6-dihydroxyppyridine. All chemicals were purchased from Sigma Aldrich.

2.4.4.2 Instruments

DMF was removed by bulb-bulb distillation using a Buchi Kugelrohr (manufactured in Germany). Melting points were measured with a Gillkhenhamp Melting Point apparatus (England). NMR spectral analysis was recorded on a Bruker 300 spectrometer and the chemical shift values were recorded in ppm. All NMR structure elucidation was done from $^1$H-NMR, $^{13}$C-NMR, DEPT135, DEPT90, HSQC, and HH-COSY experiments. Mass spectroscopy data were obtained by Electro-spray ionization (model) in positive ion mode, (in 1:1 acetonitile/ H$_2$O). Thermogravimetric analysis (TGA) was conducted on a Perkin-Elmer TGA-7 instrument. The scan was obtained on a Mettler thermogravimetric balance at a heating rate of 20 °C/min under an inert nitrogen atmosphere flowing at 20 mL/min from 50 to 600 °C switching to air from 600 °C to 800 °C.

2.4.4.3 Synthesis

$\textbf{Mono-6-(p-tolylsulfonyl)-6-OH-β-CD 78}$

A suspension of β-CD hydrate (5.09 g, 4.48 mM) and p-Tolylsulfonylic anhydride (Ts$_2$O) (2.17 g, 6.65 mM) in 110 mL of water was stirred at room temperature for 2 hrs. A solution of NaOH (2.2 g in 22.1 mL of dH$_2$O) was added, and after 10 mins unreacted Ts$_2$O was removed by filtration. The filtrate was brought to pH 8 by the addition of NH$_4$Cl.
(5.9 g), and cooled to 4 °C overnight to affording 78 as a precipitate; 2.83 g (49 %) yield, mp 195-196 °C (Lit. 159-162 °C);\(^{113}\) \(^1\)H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta = 7.48\) (d, \(J_{HH} = 8.1\) Hz, 2H, Ar-H(TsO)), 7.12 (d, \(J_{HH} = 7.9\) Hz, 2H, Ar-H(TsO), 5.80-5.63 (m, 14H, OH\(_{2,3}\)), 4.88 (s, 1H, H\(_1\)), 4.84 (s, 6H, H\(_1\)), 4.53 (s, 1H, OH\(_6\)), 4.48 (s, 5H, OH\(_6\)), 3.91-3.85 (m, 4H, H\(_{3,5,6}\)), 3.63-3.54 (m, 24H, H\(_{2,4,6}\)), 3.36 (m, 14H, H\(_{2,4}\)), 2.29 (s, 3H, CH\(_3(TsO)\)); \(^{13}\)C-NMR (75 MHz, DMSO-d\(_6\)) \(\delta = 144.7\) (TsO), 138.2 (TsO), 128.2 (TsO), 125.4 (TsO), 102.1, 101.8, 101.5, 82.6, 81.7, 81.4, 73.0, 72.6, 72.3, 72.1, 71.9, 70.1, 59.8 (CH\(_2\)), 45.3 (CH\(_2\)), 20.8 (CH\(_3\)).

**Synthesis of per-6-deoxy-6-iodo-β-cyclodextrin 79.**\(^{129}\)

Triphenylphosphine Ph\(_3\)P (21.0 g) was dissolved with stirring in dry DMF (80 mL). To this solution was added 20.51 g of iodine over 10 minutes with the evolution of heat (the solution reaches approximately 50 °C). Dry β-cyclodextrin (4.32 g, 3.81 mM) was then added to this dark brown reaction mixture and the temperature was raised to 70 °C and allowed to stir under N\(_2\) atmosphere for 18 hrs. After which heating was discontinued and the solution concentrated under reduced pressure by the removal of DMF. A 3N solution of NaOMe in MeOH was then prepared by adding 4.14 g sodium to 60 mL MeOH under inert atmosphere with cooling. This NaOMe solution was then added to the reaction mixture with cooling, the reaction mixture was stirred for 30 minutes. The reaction was worked up by pouring the reaction mixture into MeOH to form a precipitate. The precipitate was washed with MeOH and air dry to leave an off white solid. The product was further purified by Soxhlet extraction with MeOH until there was no discolouration of the solvent. The pure product was air dried and then further dried under vaccum; yield: 6.53 g (90 %): mp 207-210 °C; \(^1\)H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta = 6.02\) (d, \(J_{HH} = 6.3\) Hz, 7H, -OH), 5.91 (s, 7H, OH\(_3\)), 4.99 (s, 7H, H\(_1\)), 3.82-3.78 (d, 7H, H\(_3\)), 3.73-3.52 (m, 7H, H\(_3\)), 3.51-3.20 (m, 28H, H\(_{2,4,6}\)); \(^{13}\)C-NMR (75 MHz, DMSO-d\(_6\)) \(\delta = 102.1, 85.91, 72.1, 71.9, 70.9, 9.4\) (CH\(_2\)).

**Per-6-deoxy-6-iodo-γ-cyclodextrin 80**
80 was synthesised following the procedure described for the synthesis of 79 using 21.2 g of \( \text{Ph}_3\text{P} \), in 80 mL of DMF, with 20.0 g of iodine and 5.0 g of \( \gamma \)-CD (3.85 mM). The reaction solution was treated with 3\( N \) solution of NaOMe and the mixture precipitated in MeOH to afford 80 as a solid in good yield: 8.21 g (87 %): mp \( 204 \, ^\circ \text{C} \); \( ^1\text{H}-\text{NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta = 6.03 \text{–} 5.98 \) (m, 16H, OH\( _{2,3} \)), 5.03-5.02 (d, 8H, H\( _1 \)), 3.83-3.8 (m, \( J_{\text{HH}}=9.7 \) Hz, 8H, H\( _3 \)), 3.60-3.57 (m, 8H, H\( _2 \)) 3.45-3.25 (m, 32H, H\( _{2,4,6} \)); \( ^{13}\text{C}-\text{NMR} \) (75 MHz, DMSO-\( d_6 \)) \( \delta = 101.9, 85.2, 72.4, 71.8, 71.1, 9.3 \) (CH\( _2 \)).

**Synthesis of per-6-deoxy-6-(\( N \)-alkylimidazolium or pyridinium iodide)-\( \beta \)-cyclodextrin ILs**

Mix per-6-deoxy-6-iodo-\( \beta \)-cyclodextrin with excess imidazole or pyridine (28 \text{ mol eq.}.) in DMF and heat under nitrogen atmosphere at \( 90 \, ^\circ \text{C} \) for 48 hrs. Allow to cool to room temperature then remove half of the DMF by concentrating the reaction mixture under vacuo. Alternatively, when using a liquid imidazole or pyridine, the CD was refluxed neat in excess imidazole or pyridine without DMF. The crude product was precipitated from the reaction solution by addition of either acetone or ethyl acetate. Wash the product repeatedly with the precipitating solvent and further purify by soxhlet extraction.

**Per-6-deoxy-6-(\( N \)-methylimidazolium iodide)-\( \beta \)-cyclodextrin 83**

\( \beta \)-Cyclodextrin 79 (0.3 g, 0.156 mM) was refluxed with \( N \)-methylimidazolide in 3 mL of DMF to afford 83 in good yield: 0.28 g (73 %): mp \( 234 \, ^\circ \text{C} \); \( ^1\text{H}-\text{NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta = 9.13 \) (s, 7H, H\( _{\text{Im}} \)), 7.73 (s, 14H, H\( _{\text{Im}} \)), 7.56 (s, H\( _{\text{Im}} \)), 7.10 (s, H\( _{\text{Im}} \)), 6.86 (s, H\( _{\text{Im}} \)), 6.18 (d, 7H, OH\( _2 \)) 5.94 (s, 7H, OH\( _3 \)), 5.06 (s, 7H, H\( _1 \)), 4.47-3.33 (m, 63H, H\( _{2,3,4,5,6,\text{CH}3\text{Im}} \)); \( ^{13}\text{C}-\text{NMR} \) (75 MHz, DMSO-\( d_6 \)) \( \delta = 137.6, 123.8, 123.4, 101.9, 82.0, 72.2, 71.5, 68.9, 49.5 \) (CH\( _2 \)), 32.8 (CH\( _3 \)); ESI-MS: calcd \( m/z = 354.05 \) (M\( ^{7+} \)), found \( m/z = 353.9 \) (M\( ^{7+} \)).

**Per-6-(\( N \)-benzylimidazolium iodide)-\( \beta \)-cyclodextrin 84**

\( \beta \)-Cyclodextrin 79 (0.3 g, 0.156 mM) was refluxed with \( N \)-benzylimidazolide in 3 mL of DMF to afford 84 in good yield: 0.33 g (71 %): mp \( 234 \, ^\circ \text{C} \); \( ^1\text{H}-\text{NMR} \) (300 MHz, DMSO-
$d_6$) $\delta = 9.35$ (s, 7H, $H_{Im}$), 7.83-7.78 (m, 14H, $H_{Im}$), 7.47-7.30 (m, 35H, $Ar-H$), 6.18 (d, 7H, OH$_2$), 5.96 (s, 7H, OH$_3$), 5.49 (m, 14H, -CH$_2$-), 5.03 (s, 7H, $H_1$), 4.52-3.67 (m, 28H, H$_{2,3,4,5}$), 3.32 (m, 14H, $H_6$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta = 137.2$, 134.5, 128.8, 128.4, 124.0, 122.3, 102.0, 82.0, 72.2, 71.4, 68.8, 51.9$(CH$_2$), 49.7$(CH$_2$) ; ESI-MS: calcd $m/z = 430.24$ (M$^{2+}$), found $m/z = 430.0$ (M$^{2+}$).

Per-6-(N-butylimidazolinium iodide)-$\beta$-cyclodextrin 85

$\beta$-Cyclodextrin 79 (0.3 g, 0.156 mM) was refluxed with N-butylimidazole in 3 mL of DMF to afford 85 in good yield: 0.35 g (80 %): mp 218 °C: $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta = 9.24$ (s, 7H, $H_{Im}$), 7.84 (s, 7H, $H_{Im}$), 7.77 (s, 7H, $H_{Im}$), 7.22' (s, $H_{Im}$), 6.95' (s, $H_{Im}$), 6.18 (d, 7H, OH$_2$), 5.95 (s, 7H, OH$_3$), 5.06 (s, 7H, $H_1$), 4.51-3.42 (m, 42, $H_{2,3,4,5}$), [4.23 (t, 14H, -$CH_2$-$Im$)], 1.77 (qui, 14H, -$CH_2$-$CH_2$-$Im$), 1.26 (sxt, 14H, $CH_2$-$CH_3$), 0.89 (t, 21H, -$CH_3$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta = 137.2_{Im}$, 123.5$_{Im}$, 122.7$_{Im}$, 101.9, 82.1, 72.2, 71.4, 68.8, 49.6$(CH$_2$), 48.8$(CH$_2$), 45.9$(CH$_2$)$_{2r}$, 32.5$(CH$_2$), 31.3$(CH$_2$), 19.1$(CH$_2$), 18.9$(CH$_2$), 13.3$(CH$_3$); ESI-MS: calcd $m/z = 396.22$ (M$^{2+}$), found $m/z = 395.9$ (M$^{2+}$).

Per-6-(N-1-hydroxy-1-phenylethylimidazolinium iodide)-$\beta$-cyclodextrin 86

$\beta$-Cyclodextrin 79 (0.1 g, 0.0525 mM) was refluxed with (+/-)-2-(1-Imidazolyl)-1-phenylethanol 6 in 3 mL of DMF to afford 86 in moderate yield: 0.11 g (63 %): mp 141-145 °C: $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta = 9.11$ (s, 7H, $H_{Im}$), 7.76 (s, 14H, $H_{Im}$), 7.54-7.24 (m, 35H, $Ar-H$), 6.27 (s, OH$_1$), 6.02 (s, 14H, OH$_{2,3}$), 5.03 (s, 7H, $H_1$), 4.68-4.09 (m, 14H, $H_3, H_3, H_3$), 3.78-3.25 (m, 28H, H$_{2,4,5}$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta = 141.0_{q, 137.5_{Im}, 128.4, 128.0, 126.1, 123.3_{Im}, 102.0, 82.0, 72.1, 71.4, 70.4_{Im}, 68.8, 55.5$(CH$_2$), 49.6$(CH$_2$); ESI-MS: calcd $m/z = 460.27$ (M$^{2+}$), found $m/z = 460.1$ (M$^{2+}$).

Per-6-(pyridinium iodide)-$\beta$-cyclodextrin 87

$\beta$-Cyclodextrin 79 (0.3 g, 0.156 mM) was refluxed with excess pyridine (3 mL) to afford 87 in good yield: 0.32 g (82 %): mp 215-216 °C: $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta= 8.95$ (m, 7H, $Ar-H$), 8.64 (m, 7H, $Ar-H$), 8.23-8.13 (m, 7H, $Ar-H$), 6.32 (s, 7H, OH$_2$), 6.08 (s,
7H, OH), 5.97′ (s, OH), 5.20 (s, 7H, H1), 5.11′ (s, H1), 4.87-3.36 (m, 42H, H2,3,4,5,6); 
13C-NMR (75 MHz, DMSO-d6) δ = 146.6, 145.9, 128.4, 101.6, 81.9, 72.1, 71.2, 68.9, 60.5(CH2); ESI-MS: calcd m/z = 351.14 (M+), found m/z = 350.7 (M+).

Per-6-(4-dimethyaminopyridinium iodide)-β-cyclodextrin 88

β-Cyclodextrin 79 (0.3 g, 0.156 mM) was refluxed with 4-dimethyaminopyridine in 3 mL of DMF to afford 88 in good yield: 0.32 g (74%): mp 242-243 °C: 1H-NMR (300 MHz, DMSO-d6) δ = 8.09 (s, 14H, Ar-H), 7.04 (s, 14H, Ar-H), 6.36 (s, 7H, OH2), 6.13 (s, 7H, OH3), 5.07 (s, 7H, H1), 4.37-3.35 (m, 42H, H2,3,4,5,6), 3.10 (s, 42H, -CH3); 13C-NMR (75 MHz, DMSO) δ = 155.4q, 142.9, 107.8, 101.6, 82.5, 72.1, 71.56, 69.2, 56.8(CH2), 40.0(CH3); ESI-MS: calcd m/z = 394.21 (M+), found m/z = 393.8 (M+).

Per-acetylation of secondary hydroxyl groups

Per-2,3-O-acetyl-6-deoxy-6-iodo-β-cyclodextrin 81

CD (79) (2.0 g, 1.05 mM) was dissolved in anhydrous pyridine (9 mL) and stirred under nitrogen. Acetic anhydride (7.5 mL) was slowly added, and the mixture was stirred for 4 hrs at room temperature in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP). The pyridine and acetic anhydride were removed by rotatory evaporation and the reaction mixture dissolved in CH2Cl2 (15 mL) and washed with H2O and the combined organic layers successively washed with 3N HCl, saturated aqueous NaHCO3, and brine. After drying over MgSO4 and removal of the solvent afforded 1.96 g of CD 81 (85 % yield): mp 200-203 °C: 1H-NMR (300 MHz, CDCl3) δ = 5.36-5.25 (m, 3JHN = 9.8 Hz, 7H, H3), 5.13 (d, 3JHN = 3.9 Hz, 1H, H1), 4.76 (dd, 3JHH = 9.9, 3.8 Hz, 7H, H2), 3.80-3.64 (m, 14H, H5,6), 3.63-3.50 (m, 14H, H4,6), 2.00 (d, 42H, -CH3); 13C-NMR (75 MHz, CDCl3) δ = 170.6q, 169.4q, 96.5Cl, 80.5C4, 70.4C2, 70.2CS, 70.1CS, 20.7(CH3), 8.0(CH2).

Per-2,3-O-acetyl-6-deoxy-6-iodo-γ-cyclodextrin 82
\( \gamma \)-Cyclodextrin 80 (2.0 g, 0.817 mM) reacted with pyridine (9 mL), acetic anhydride (7.5 mL) and a catalytic amount of DMAP following the procedure described in the synthesis of CD 81, to afford 82 in good yield: 1.77 g (78%): mp 200-203 °C: \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \( \delta = 5.30 \) (d, \( \text{J}_{HH} = 9.8 \text{ Hz}, 8\text{H}, \text{H}_3 \)), 5.18 (d, \( \text{J}_{HH} = 3.8 \text{ Hz}, 8\text{H}, \text{H}_1 \)), 4.70 (dd, \( \text{J}_{HH} = 10.0, 3.8 \text{ Hz}, 8\text{H}, \text{H}_2 \)), 3.73-3.70 (m, 16H, \( \text{H}_{5,6} \)), 3.59 - 3.41 (m, 16H, \( \text{H}_{4,6} \)); \(^13\)C-NMR (75 MHz, CDCl\(_3\)) \( \delta = 170.6_{q}, 169.3_{q}, 96.1_{c1}, 79.6_{c4}, 70.8_{c2}, 70.3_{c5}, 69.6_{c3}, 20.8_{(\text{CH}_3)}, 20.7_{(\text{CH}_3)}, 7.8_{(\text{CH}_2)} \).

**per-2,3-O-acetyl-\( \beta \)-CD ILs**

\( \beta \)-Cyclodextrin 81 (0.2 g, 0.091 mM) was refluxed with excess \( N \)-methylimidazole to afford 89 in good yield: 0.22 g (86%): RTIL: \(^1\)H-NMR (300 MHz, DMSO-\( d_6 \)) \( \delta = 9.30 \) (s, \( 7\text{H},\text{H}_\text{im} \)), 7.87 (s, \( 14\text{H}, \text{H}_\text{im} \)), 5.33-3.91 (m, 49H, \( \text{H}_{\text{CD}_{1,2,3,4,5,6}} \)), 3.75 (s, 21H, -NC\(_\text{H}_2\)); \(^13\)C-NMR (75 MHz, DMSO-\( d_6 \)) \( \delta = 175.2_{q}, 174.4_{q}, 143.27_{\text{Im}}, 128.6_{\text{Im}}, 102.2_{c1}, 83.0_{c4}, 74.7_{(\text{C}_2,3,5)}, 54.6_{(\text{CH}_2)}, 41.6_{(\text{CH}_3)}, 25.8_{(\text{CH}_3)} \); ESI-MS: calcd \( m/z \) = 396.17 (\( \text{M}^+ \)), found \( m/z \) = 395.8 (\( \text{M}^+ \)).

\( \beta \)-Cyclodextrin 81 (0.2 g, 0.091 mM) was refluxed with excess \( N \)-butylimidazole to afford 91 in good yield: 0.26 g (94%): RTIL: \(^1\)H-NMR (300 MHz, DMSO-\( d_6 \)) \( \delta = 9.38 \) (s, \( 7\text{H}, \text{H}_\text{im} \)), 7.85 (s, \( 14\text{H}, \text{H}_\text{im} \)), 5.23-4.15 (m, 49H, \( \text{H}_{\text{CD}_{1,2,3,4,5,6}} \)), \( 4.17 \) (t, \( \text{J}_{HH} = 7.4 \text{ Hz}, 14\text{H}, -\text{NCH}_2 \)), 2.01 (m, 42H, O=CC\(_3\)), 1.77 (q, \( \text{J}_{HH} = 14.8, 7.4 \text{ Hz}, 14\text{H}, -\text{NCH}_2\text{CH}_2 \)), 1.24 (sxt, \( \text{J}_{HH} = 14.7, 7.3 \text{ Hz}, 14\text{H}, -\text{CH}_2\text{CH}_3 \)), 0.89 (t, \( \text{J}_{HH} = 7.3 \text{ Hz}, 21\text{H}, -\text{CH}_2\text{CH}_3 \)); \(^13\)C-NMR (75 MHz, DMSO-\( d_6 \)) \( \delta = 169.9_{q}, 137.5_{\text{im}}, 123.1_{\text{im}}, 122.2_{\text{im}}, 97.1, 78.1, 69.3_{(\text{C}_2,3,5)}, 48.7_{(\text{CH}_2)}, 47.9_{(\text{CH}_2)}, 31.5_{(\text{CH}_2)}, 20.6_{(\text{CH}_3)}, 18.8_{(\text{CH}_2)}, 13.3_{(\text{CH}_3)} \); ESI-MS: calcd \( m/z \) = 438.25 (\( \text{M}^+ \)), Found \( m/z \) = 438.1 (\( \text{M}^+ \)).
β-Cyclodextrin 81 (0.2 g, 0.091 mM) was refluxed with excess pyridine to afford 93 in good yield: 0.24 g (95%); mp 220-224 °C: 1H-NMR (300 MHz, CDCl3-d/DMSO-d6) δ = 9.22 (m, 7H, Ar-Hpyr), 8.69 (m, 7H, Ar-Hpyr), 8.17 (m, 7H, Ar-Hpyr), 5.30-3.60 (m, 49H, H1,2,3,4,5,6), 2.02 (m, 42H, O=CCH3); 13C-NMR (75 MHz, CDCl3-d/DMSO-d6) δ = 169.6 qr, 145.8, 144.6 qr, 128.7, 96.4 78.9, 68.0(CH2), 20.5(CH3); ESI-MS: calcd m/z = 393.17 (M8+), found m/z = 393.0 (M8+).

**Per-2,3-O-acetyl-γ-CD ILs**

**Per-2,3-O-acetyl-6-deoxy-(N-methylimidazolium iodide)-γ-cyclodextrin 90**

γ-Cyclodextrin 82 (0.15 g, 0.0539 mM) was refluxed in excess N-methyimidazole to afford 90 in good yield: 0.17 g (92%); RTIL: 1H-NMR (300 MHz, DMSO-d6) δ = 8.69 (s, 8H, -CHIm), 7.54 (s, 8H, -CHIm), 7.43 (s, 8H, -CHIm), 5.14-3.47 (m, 56H, -CHCD1,2,3,4,5,6), 3.81 (s, 24H, -CH3), 1.94 (s, 24H, -CH3); 13C-NMR (75 MHz, DMSO-d6) δ = 172.1 qr, 169.9 qr, 169.4 qr, 137.0, 136.2, 124.2, 123.3, 122.4, 121.9, 97.0, 77.2, 69.6, 49.2, 34.8(CH3), 21.1(CH3), 20.6(CH3); ESI-MS: calcd m/z = 430.18 (M8+), found m/z = 429.8 (M8+).

**Per-2,3-O-acetyl-6-deoxy-(N-butylimidazolium iodide)-γ-cyclodextrin 92**

γ-Cyclodextrin 82 (0.15 g, 0.0539 mM) was refluxed with excess N-butylimidazole to afford 92 in good yield: 0.18 g (88%); RTIL: 1H-NMR (300 MHz, DMSO-d6) δ = 9.75 (s, 1H, -CHIm), 9.12 (s, 8H, -CHIm), 8.17 (s, 8H, -CHIm), 7.94 (s, 8H, -CHIm), 7.78 (s, 8H, -CHIm), 7.63 (s, 8H, -CHIm), 5.19-3.63 (m, 56H, CH(CD)1,2,3,4,5,6), 4.23 – 4.16 (m, 16H, -CH2), 2.00 (s, 24H, -CH3), 1.76 (qui, 3JHH = 7.3 Hz, 16H, -CH2), 1.23 (sxt, 3JHH = 7.4 Hz, 16H, -CH2), 0.87 (t, 3JHH = 7.3 Hz, 24H, -CH3); 13C-NMR (75 MHz, DMSO-d6) δ = 172.0 qr, 169.9 qr, 169.3 qr, 137.5, 135.2, 123.1, 122.1, 121.8, 120.34, 96.8, 77.4, 69.3, 58.0(CH2), 49.1(CH2), 48.6(CH2), 48.0(CH2), 31.5(CH2), 31.3(CH2), 31.0(CH2), 21.1(CH3), 20.5(CH3), 18.8(CH2), 18.7(CH2), 13.3(CH3), 13.2(CH3); ESI-MS: calcd m/z = 472.26 (M8+), found m/z = 471.9 (M8+).
Per-2,3-O-acetyl-6-deoxy-6-(N-pyridinium iodide)-γ-cyclodextrin 94

γ-Cyclodextrin 82 (0.15 g, 0.0539 mM) was refluxed with excess pyridine to afford 94 in good yield: 0.17 g (95%): mp 198-202 °C: ¹H-NMR (300 MHz, CDCl₃-d/DMSO-d₆) δ = 9.26 (m, 8H, Ar-H_pyr), 8.53 (m, 8H, Ar-H_pyr), 8.18 (m, 8H, Ar-H_pyr), 5.41-3.47 (m, 56H, H_(CD)₁,₂,₃,₄,₅,₆), 2.02 (m, 48H, O=CCH₃); ¹³C-NMR (75 MHz, CDCl₃-d/DMSO-d₆) δ = 169.5_q, 146.1, 145.5_q, 128.0, 96.2, 78.0, 69.4(CH₂), 20.7(CH₃); ESI-MS: calcd m/z = 427.18 (M⁺), found m/z = 426.9 (M⁺).
2.5 References


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3 Chapter 3

Chiral analysis by Gas Chromatographic
3.1 Introduction to Chapter 3

There is evermore a need for the continual development of new chiral stationary phases (CSPs) as the production of new chiral products such as drugs continually increases and no guarantee that the currently available CSPs will provide adequate enantioseparation. With the increased demand from drug regulatory agencies for drug manufacturers to provide safety data by way of enantiomeric purity, there is a need for the development of more chiral selectors for application in GC/ or LC stationary phases for the analysis of chiral drug products. A single column cannot separate all chiral compounds, consequently analysts need to select (or introduce new) specific phases that permit separation and analysis of novel chiral drug products. Given that GC is one of the preferred methods of chiral analysis recommended by drug regulatory agencies, it is important to have a range of GC stationary phases which possess diverse physical and chemical properties to allow researchers to conduct not only routine chiral analysis but provide a selection of stationary phases with the appropriate properties required to conduct specialised enantioselective analysis experiments.

An example of the need for a selection of chiral columns which possess a variety of chemical properties for specialized applications is the study of species that undergo interconversion or some other type of structural change during the chromatographic elution process, known as dynamic molecular interconversion, where each molecule undergoes a mechanism of structural change into their counterpart. The results of the evaluation for enantioselective capabilities of the novel chiral ionic liquid stationary phases are presented in chapter 3.2. While in chapter 3.3, the results of the study of the dynamic molecular interconversion of chiral oximes is presented.
3.2 An investigation into the potential application of novel chiral ionic salts as chiral selecting agents in gas chromatography stationary phases.

3.2.1 Introduction

The most common liquid stationary phases in GC columns are polysiloxanes and polyethylene glycol phases. Polysiloxane based stationary phases are primarily utilized for the routine analysis of the less polar analytes. The polysiloxane can be modified to contain various substituent groups to change the polarity of the phase. Dimethyl polysiloxane (such as OV1 and OV101 phases, see Figure 3.1) are classically nonpolar phases, and can be modified to be more polar by increasing the percentage of phenyl, cyanopropyl, fluoro groups, or other substituents on the polymer. For routine analysis of very polar analytes, polyethylene glycol (PEG), also known as Carbowax (Figure 3.2), is commonly used as the stationary phase.

![Figure 3.1: Structure of dimethylpolysiloxanes such as OV-1 and DB-1.](image)

For chiral analysis currently available columns consist primarily of the less polar polysiloxane stationary phases, impregnated with chiral selecting agents by either; chemically bonding the chiral selecting agent to siloxane pendent groups or by dissolving the chiral selecting agent in the liquid polymer.

![Figure 3.2: Structure of Carbowax 20M a polyethyleneglycol polymer with an average molecular weight of 20,000.](image)

Cyclodextrin derivatives (CDs) are the most common and widely used chiral selecting agent in chiral stationary phases (CSPs) for GC. This is due to their broad applicability to
enantioselective separation, ease of derivatisation, compatibility with column manufacture and ability to separate underivatized enantiomers of different volatilities. When used in enantioselective GC, CDs are dissolved in non-polar to moderately polar phases such as polysiloxanes in order to obtain highly efficient capillary GC columns.\(^1\)\(^-\)\(^3\)

**IL bonded polysiloxane phase**

Derivatives of polysiloxane\(^4\) cover a majority of the commercially available and lab-made stationary phases due to their good film-forming ability, chemical stability and thermal stability. Since a complex sample is usually composed of various polar and nonpolar components, a given stationary phase cannot offer good separation for both polar and nonpolar components in one run. Fortunately, ionic liquid stationary phases possess the unique advantage of “dual nature”\(^5\)\(^,\)\(^6\) characteristics over traditional stationary phases. It was noted that this unique characteristic may be beneficial to the analysis of a complex samples comprising of components with a wide polarity range.

The earliest report on the GC stationary phase of organic molten salts was contributed by Barber \textit{et al.} in 1959.\(^7\) Later, Poole and co-workers studied the stationary phases of organic molten salts, such as ethylammonium nitrate and ethylpyridinium bromide.\(^8\)\(^,\)\(^9\) These stationary phases exhibited separation ability for many compounds, but their thermal stabilities were not favourable. Hence, little attention was paid to this kind of stationary phase in GC until the publication of IL stationary phase based on an imidazole cation 1-butyl-3-methylimidazolium coupled with hexafluorophosphate and chloride anions by Armstrong’s group.\(^5\) These stationary phases were able to separate a wide range of organic compounds and exhibited the special characteristic “dual nature”, reviving research into IL GC stationary phases. Subsequently, various imidazole bases ILs have been investigated as stationary phases, one example being the di-cationic vinylimidazole 1,9-di(3-vinyl-imidazolium)nonane bis(trifluoromethylsulfonyl) imidate IL, which is produced commercially as SLB IL-100 (Figure 3.3). Since the ionic liquids were primarily compounds of small molecular mass they did not possess high thermal stabilities and good coating stability simultaneously. As a result a method of in-column
polymerization was proposed to prepare polymeric stationary phases from IL monomers composed of vinylimidazole. This kind of IL polymer was shown to withstand higher temperatures of up to 350 °C, however, the reactions have been deemed to be complicated and time consuming.

![Figure 3.3: Structure of the ionic liquids 1,9-di(3-vinyl-imidazolium)nonane bis(trifluoromethylsulfonyl)imidate.](image)

Since various substituted polysiloxanes have been employed extensively as stationary phases in GC owing to their high thermal stability and wide variability in modification, combining the characteristics of polysiloxane phases with the “dual nature” separation capabilities of ILs was seen to have the potential to improve the performance of IL stationary phase. Therefore, ILs were diluted in commercial stationary phases (e.g., OV-1, OV-1701 and FFAP) and evaluated. Qi and Armstrong investigated the “dual nature” of the geminal dicationic IL, 1,9-di(3-vinylimidazolium)nonane bis(trifluoromethylsulfonyl)imidate, for the purpose of separating both nonpolar and polar compounds of complex samples such as essential oils that contain a great variety of compounds. The dicationic IL was utilised as a stationary phase for capillary GC either as a neat IL phase or as a mixed stationary phase with a polysiloxane as the diluent. Interestingly, it was found that the mixed stationary phase exhibited a much better selectivity for polar and nonpolar compounds than either the dicationic IL or the polysiloxane, suggesting that a kind of synergistic effect occurred when these stationary phases were combined.

Compared with neat IL stationary phases, the mixed stationary phases provided excellent wetting ability on the inner wall of the capillaries. However, the durability of the physical
mixture seemed to be doubtful because of the significant difference between the ILs and commercial stationary phases. Subsequently, two different synthesis routes for ionic liquid bonded polysiloxanes stationary phases were reported.\textsuperscript{21,22} The method by Wei \textit{et al.}\textsuperscript{21} was based on hydrosilylation between poly(methylhydrosiloxane) and 1-vinyl-3-hexylimidazolium hexafluorophosphate [VHIm][PF\textsubscript{6}]. The column efficiency of this phase was relatively low (only 2500 plates/m) with conditioning temperature of 200 °C. Sun \textit{et al.}\textsuperscript{22} proposed an alternative way via the quaterisation of γ-chloropropyl-polysiloxane with methylimidazolium. The content of the imidazolium moiety in ionic liquids was about 10 %. This kind of IL stationary phase was more stable than the physical mixture and offered better wetting ability on inner wall of capillaries than neat IL stationary phases. Nevertheless, it could not be used over 220 °C. Thus, improving the thermal stability of IL bonded polysiloxanes and an increase in their separation efficiency is still crucial to the development of IL stationary phases. In that work, a novel methylimidazolium bistrifluoromethanesulfonylimide ionic liquid bonded to polysiloxane ([PSOMIM][NTf\textsubscript{2}]) was prepared.\textsuperscript{23} For the purpose of comparison, another ionic liquid methylimidazolium chloride ([PSOMIM][Cl]) was tested with an imidazolium ionic liquid unit content of 30 %.\textsuperscript{23} The synthesized polymers were then used as GC stationary phases and their selectivity was compared to that of DB-1.\textsuperscript{23} The results suggested that the ionic liquid bonded polysiloxane [PSOMIM][NTf\textsubscript{2}] has great potential for practical application as stationary phases for high temperature and high selectivity GC analysis.\textsuperscript{23}

\textbf{Figure 3.4:} Polysiloxane bonded methylimidazolium ionic liquids [PSOMIM][NTf\textsubscript{2}] and [PSOMIM][Cl].
Methylimidazolium bis(trifluoromethanesulfonyl)imidate-polysiloxane exhibited good coating ability and durability. Moreover, the solvation parameter evaluation revealed that hydrogen bond basicity of [PSOMIM][NTf₂] was expectedly lower than that of [PSOMIM][Cl] under the test conditions.²³ It was shown that the anion had significant influence on the thermal stability, polarity and selectivity of the IL bonded polysiloxanes.²³ The [PSOMIM][NTf₂] column possessed higher separation efficiency than [PSOMIM][Cl] column, and was found to have good selectivity for analytes of a wide range of polarities including aromatic isomers, fatty acid methyl esters (FAMEs), polychlorinated biphenyls (PCBs) and aromatic amines.²³ These new IL bonded polysiloxane [PSOMIM][NTf₂] have great potential in high temperature and highly selective GC analysis.

Work has been undertaken to combine the unique attributes of polysiloxanes such as high thermal stability with the unique “dual nature” characteristic of ILs which allows for the separation of both polar and nonpolar analytes on the same column. However, in our work the need to develop new enantioselective IL chiral selectors was addressed, striving to make a contribution toward the continual need for development of new chiral separation media for GC stationary phases. Highly enantioselective IL chiral selectors can be useful in the development of new stationary phases such as polysiloxane bonded ILs. Advancements in chromatography permit the separation and isolation of a larger variety of chiral synthetic compounds, thus, making available to research chemists and drug companies alternative chiral separation media for obtaining the data required by regulatory agencies.

3.2.2 Results and Discussion

Strategies to engineer CDs which possess and exploit the desired chromatographic properties of the desired stationary phases, such as increased enantioselectivity, is to either combine two chiral selectors into a single phase²⁴-²⁸ or create a hybrid selector.
A good example of a mixed CD phase was demonstrated by Ruderisch et al.,\(^{24}\) in which two stationary phases, Chirasil-Dex and Chiralsil-Calixval, where combined by chemically bonding the associated chiral selectors; \(\text{mono-kis-O-octenyl-permethyl-}\beta\text{-cyclodextrin}\) and \(\text{octakis-O-}[\text{(L-valine-}\text{tert-butylamide)-N-acetyl]}\text{-C-decenyl-resorcinarene}, \) to polysiloxane forming the Chiralsil-Calixval-Dex phase.

An engineered hybrid stationary phase was investigated by Bicchi et al. whereby \(\text{per-6-O-THDMS-3-O-acetyl-2-O-methyl-}\gamma\text{-CD and 6-O-THDMS-2-O-acetyl-3-O-methyl-}\gamma\text{-CD}\) hybrid chiral selectors were syntheses (Figure 3.5) and tested for enantioselectivity and their performance compared to their analogous phases \(2,3\text{-di-O-methyl- and 2,3-di-O-acetyl-}\gamma\text{-CD, not with unequivocal results.}^{29}\) A general conclusion was not made regarding the enantioselective behaviour of the hybrid phases given that some analytes showed improved separation while others did not and had better separation on the non-hybrid columns. However, it was found that all the analytes tested were separated by all the phases that had an acetyl substituent at C3. Other hybrid phases have been reported by Bicchi et al. more recently where the asymmetrically substituted \(2\text{-O-methyl-3-O-ethyl- and 2-O-ethyl-3-O-methyl-6-O-TBDMS-}\beta\text{-CD showed extended enantioselectivity compared to their symmetrical 2,3-O-methyl and 2,3-O-ethyl derivatised counter parts.}^{30}\)

![Figure 3.5: per-6-O-THDMS-3-O-acetyl-2-O-methyl-\(\gamma\)-CD and 6-O-THDMS-2-O-acetyl-3-O-methyl-\(\gamma\)-CD hybrid chiral selectors.](image)

The CD ring size and substituents at C-2, C-3 and C-6 positions of the sugar units strongly influence the CD’s chemical and physical properties and enantioselectivity. We have chosen to use the intermediate size \(\beta\)-CDs. It has been shown that CD derivatives with the "small" substituents such as acetyl, methyl or ethyl groups on the secondary face, with a bulky group on the primary side produce good enantioselectivity by GC.
Keeping this concept in mind, CDs with bulky imidazolium or pyridinium groups on the primary face were prepared that complemented the design by substituting the secondary face with \( \text{per-2,3-}\text{O-acetyl substituents.} \) The influence of the substituents in positions 2, 3, and 6 of the CD ring has been discussed extensively by Bicchi et al., and Shitangkoon et al.\textsuperscript{31-33} Amongst the most effective derivatives used as CSP for enantioselective GC are 6-TBDMS-\( \beta \)-CDs substituted with methyl, ethyl or acetyl groups at C-2 (OR\textsuperscript{1}) and C-3 (OR\textsuperscript{2}) positions (Figure 3.6).\textsuperscript{34,35}

\[ \text{Figure 3.6: 6-TBDMS-\( \beta \)-CDs substituted with methyl, ethyl or acetyl groups at C-2 (OR}\textsuperscript{1}) \] and C-3 (OR\textsuperscript{2}).

There are many CD GC capillary columns currently commercially available for enantioseparation application. ASTEC produces Chiradex capillary GC column phases consisting of derivatives of \( \alpha \)-, \( \beta \)-, or \( \gamma \)-cyclodextrin for the separation of enantiomers as illustrated in Table 3.1. These columns can routinely separate a variety of underivatised non-aromatic enantiomers and several aromatic enantiomers including compounds that are starting materials or intermediates for chiral synthesis, biochemical and pharmaceutical intermediates, metabolites, environmental contaminants, flavours, etc., permitting the resolution of small chiral compounds. The various compounds separated by the different Chiralaldex columns are presented in Table 3.1. These cyclodextrin derivatives are stable, high boiling liquids, which makes them effective stationary phases for gas-liquid chromatography. Their maximum allowable operating temperatures (MAOT) both for isothermal and temperature programmed conditions range between 180-220 °C (Table 3.1).
<table>
<thead>
<tr>
<th>Column</th>
<th>Derivatisation</th>
<th>Compounds separated on column</th>
<th>Interaction Mechanism</th>
<th>MAOT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiradex TA</td>
<td>2,6-di-O-pentyl-3-trifluoroacetyl</td>
<td>$\alpha$-TA Small epoxides, alcohols, amino alcohols, amino alkanes, and diols.</td>
<td>Strong dipole-dipole interactions</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\beta$-TA Alkyl alcohols, halo acid esters, amino alkanes, amino acid derivatives, halocycloalkanes, certain lactones, diols, alkyl halides, and furan and pyran derivatives.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\gamma$-TA Chiral alcohols, diols, polyols, hydrocarbons, lactones, amino alcohols, halocarboxylic acid esters, furan and pyran derivatives, epoxides, glycidyl analogs, and haloepihydrins.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiradex DP</td>
<td>2,3-di-O-propionyl-6-t-butylsilyl</td>
<td>$\alpha$-DP Acid esters, amines, lactones, alcohols, and some diols.</td>
<td>Mostly surface interactions</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\gamma$-DP Acid esters (long chain), fused ring/bulky amines, and lactones.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiradex PN</td>
<td>2,6-di-O-pentyl-3-propionyl</td>
<td>$\gamma$-PN Epoxides, secondary amines, alcohols, esters, lactones, and diols.</td>
<td>There is little evidence of inclusion formation</td>
<td>220</td>
</tr>
<tr>
<td>Chiradex BP</td>
<td>2,6-di-O-pentyl-3-butyryl</td>
<td>$\gamma$-BP Aromatic acids, certain primary amines, and furans.</td>
<td>primary surface interactions</td>
<td>220</td>
</tr>
<tr>
<td>Chiradex DM</td>
<td>2,3-di-O-methyl-6-t-butylsilyl</td>
<td>$\beta$-DM Aromatic alcohols, short chain alcohols, amines, amino alcohols, epoxides, cyclic ketones, diols, aromatic acids, and esters</td>
<td>Size selectivity Fewer structural</td>
<td>220</td>
</tr>
<tr>
<td>Chiradex PM</td>
<td>2,3,6-tri-O-methyl</td>
<td><strong>β-PM</strong> Acids, alcohols, barbitals, diols, epoxides, esters, hydrocarbons, ketones, lactones, and terpenes.</td>
<td>Inclusion complexes</td>
<td>220</td>
</tr>
</tbody>
</table>
| Chiradex DA | 2,6-di-O-pentyl-3-methoxy | **α-DA** Small cyclic and aromatic amines, alcohols, and epoxides.  
**β-DA** Heterocyclics, some multi-ring lactones, aromatic amines, sugars, certain amino acid derivatives, bicyclics, and epoxides.  
**γ-DA** Aromatic amines containing 2 or more rings, large cyclic diols, some heterocyclics, multi-ring compounds, or compounds with bulky substituents. | Inclusion complexes | 220 |
| Chiradex PH | (S)-2-hydroxy propylmethylether | **α-PH** Small linear and saturated amines, alcohols, esters, and epoxides.  
**β-PH** Linear and cyclic amines and alcohols, lactones, amino alcohols, sugars, bicyclics, epoxides, haloalkanes, aromatic and cyclic hydrocarbons. | Inclusion complexes | 220 |

**Table 3.1:** A list of commercial ASTEC columns, the compounds they separate, the primary enantiorecognition mechanisms involved and their maximum operating temperatures.\textsuperscript{36,37} (data obtained from www.sigmaaldrich.com).
There are currently a few ionic liquid stationary phase GC capillary columns available on the market (Table 3.2). These columns range from polar to highly polar with maximum allowable operating temperatures ranging from 270-300 °C comparable to polyethylene glycol (PEG) phases which have a MAOT of ~280 °C, with the exception of IL column SLB-IL 100 with a MAOT of 230 °C. The ionic liquid used in SLB-IL 100 is the di-cationic vinylimidazole 1,9-di(3-vinyl-imidazolium)nonane bis(trifluoromethylsulfonyl)imidate (Figure 3.3). Of the commercial ionic liquid GC capillary columns on the market there are no chiral IL based stationary phases available for application in enantiomer resolution application.

Table 3.2: Commercial non-bonded IL stationary phases.

<table>
<thead>
<tr>
<th>Column</th>
<th>MAOT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLB-IL 59</td>
<td>300</td>
</tr>
<tr>
<td>SLB-IL 76</td>
<td>270</td>
</tr>
<tr>
<td>SLB-IL 82</td>
<td>270</td>
</tr>
<tr>
<td>SLB-IL 100</td>
<td>230</td>
</tr>
<tr>
<td>SLB-IL 111</td>
<td>270</td>
</tr>
</tbody>
</table>

Consequently, we endeavoured to address the need for the development of new enantioselective IL chiral selectors for application in GC stationary phases. New chiral IL were diluted in the polysiloxane OV-1701 and incorporated into GC capillary columns for accessing enantioselectivity.

**Static coating method**

**Film thickness**

All capillary columns were prepared by static coating, as outlined in Section 3.2.4.3, with a chiral IL and polysiloxane OV-1701 at a proportion of 30 % chiral selector to 70 % OV-1701 (w/w) (or Carbowax 20 where indicated).
In general columns with a thinner film stationary phase have slightly higher efficiencies than a corresponding thicker film column. All the columns prepared were coated using a 0.4 % (w/v) solution of DCM giving a stationary phase with a standard film thickness of 0.25 µm calculated with Equation 1;

\[ d_f = \frac{d_c \times c}{400} \]

\[ = \frac{250 \mu m \times 0.4\%}{400} \]

\[ = 0.25 \mu m \quad \text{Eqn. (1)} \]

where \( d_c \) is the diameter of the capillary column (µm) and \( c \) is the percentage weight of stationary phase dissolved in the solvent (DCM).

**Percentage composition of ionic chiral selector in OV-1701**

The dilution of cyclodextrin derivatives in a polysiloxane stationary phase has proven to have several advantages enabling the operating temperature range of the column to be wider, and making it possible to conduct chiral analysis at temperatures below the melting points of the chiral selector (in this case the ionic salts). Finally, improved column efficiencies can be achieved due to the favourable coating properties of polysiloxanes. The amount of chiral selector in the polymeric stationary phase can have a large influence on enantioselectivity. As expected, Schmarr et al. reported that low cyclodextrin concentrations reduce enantioresolution. Hardt and König also reported a significant increase in selectivity as the amount of octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin in OV-1701 increased, but the separation factor levelled off when the concentration of the chiral selector neared 50 % (w/w). Bicchi et al. achieved separation of 1,8-epoxy-9-(3-methyl-6-buten-1-yl)-p-methane using 30 % permethylated β-CD in OV-1701 but not when the permethylated β-CD was at a concentration of 10 % (w/w). Jung et al. demonstrated both theoretically and experimentally that different solutes exhibited different behaviours; that is, the apparent enantioselectivity factor for each racemate reached a plateau at different amounts of chiral selector. In general, however, it is expected that the highest possible concentration of the CD derivative
compatible with its solubility in the polymeric solvent should be used to achieve the highest enantioselectivity factor for most solutes. As a general rule, less polar polymers lead to enhanced enantioselectivity. Therefore OV-1701 (a moderately polar phase) was chosen as the achiral diluent phase for the chiral selectors being tested for enantioselectivity.

The main dilemma with using OV-1701 as our achiral phase is that the solubility of the most polar CD derivatives in nonpolar or moderately polar polysiloxanes is rather limited at relatively low temperatures, while low elution temperatures are required to enhance the molecular interactions that lead to enantioseparation. Ideally, conditions that favour low chromatographic temperatures should be selected to maximise the resolution of enantiomers. The maximum solubility that allows GC separations at low temperatures has to be critically studied for each mixture of the ionic salt and the achiral stationary phase in which it is diluted. However, given the number of chiral selectors being tested in this study, it was not practical to determine the optimum concentration that would produce the best chromatographic separations at low operating temperatures for each chiral selector. For practical consideration a medium concentration of 30 % chiral selector was chosen; and assumed to be high enough to permit enantioselectivity studies and low enough for the chiral selectors to be soluble at the lower operating temperature.

All of the columns prepared by the static method consisted of 30 % (w/w) chiral selector dissolved in OV-1701 (with the exception of per-2,3,6-acetylated-β-CD which was also diluted in Carbowax 20 (designated PA-CD wax) as well as OV-1701). The chiral selectors tested are illustrated in Figure 3.7, the non-cyclodextrin IL CSPs are named according the compound number of the chiral ionic salt they are impregnated with, while the CD CSPs are designated with an acronym (see Figure 3.7).
Figure 3.7: Chemical structures of the ionic chiral selecting agents tested.

**Column conditioning**

Long term conditioning of newly coated columns at high temperatures is not necessary and column bleeding is negligible if pure ILs are used. However, all columns were
conditioned to remove any possible residual solvent from the coating process. The columns were conditioned from 40 °C to 180 °C at 10 °C/min and then held at 180 °C for 50 mins. The capillary columns were then installed into a GC followed by injection of the test mixtures. Stationary phase polarity was determined using the Rohrschneider-McReynolds classification system, and column efficiencies determined with naphthalene at 100 °C isothermal with a constant column pressure of 2.5 psi.

**Evaluation of column polarities**

*Rohrschneider–McReynolds classification system*

To characterize and categorize the solute selectivities of GC stationary phases, the Rohrschneider–McReynolds system\textsuperscript{42,43} is the most widely utilized stationary phase classification system, based on the retention of five probe molecules; benzene, butanol, 2-pentanone, nitropropane, and pyridine. Each probe molecule has the ability to undergo distinct interactions with the stationary phase. Benzene (X) measures dispersive interactions with weak proton acceptor properties; butanol (Y) measures dipolar interactions with both proton donor and proton acceptor capabilities; 2-pentanone (Z) measures dipolar interactions with proton acceptor but not proton donor capabilities; nitropropane (U) measures weak dipolar interactions; and pyridine (S) measures weak dipolar interactions with strong proton acceptor but not proton donor capabilities.
Figure 3.8: Separation of the alkane series C₇-C₁₃ on the MEGA commercial chiral column (A) and on IL 26 column (B).
**Kovats’ Retention Index**

To find the Kovats’ index ($I$) for the probe molecules on a given stationary phase, members of the alkane homologous series were chromatographed. Then the probe molecules were run under the same conditions and the retention values of the probe molecules and the homologous series, were used to determine Kovats’ index using Equation (2). It is desirable that the alkanes chosen, bracket the retention value of the analyte. If the flow rate is kept constant during the analysis, then adjusted retention times can be used.

$$I = 100 \times \left[ \frac{\log t'_{R,X} - \log t'_{R,Cn}}{\log t'_{R,Cn+1} - \log t'_{R,Cn}} \right] + 100n \quad \text{Eqn. (2)}$$

Figure 3.8 shows the analysis of the C$_7$-C$_{13}$ alkane series on (a) the commercial column (MEGA) and (b) the ionic phase 26. Comparison of the phases revealed a significant difference in the retention behaviour of alkanes despite both phases being of intermediate polarity (see Table 3.5 for a comparison of polarities). The C$_{13}$ alkane eluted from the 23 m MEGA column in under 22 mins and eluted from the 10 m ionic 26 phase in just over 22 mins. The shorter ionic phase column with a slightly higher polarity tended to retain the nonpolar alkanes more strongly than the less polar commercial column.

The value of each phase constant (i.e., X, Y, Z, U, and S) was determined by subtracting the retention index of the probe on a squalane stationary phase ($I_{SQ}$) from the retention index of the probe on the stationary phase being characterized ($I_{TP}$). For example, the phase constant of benzene (X) would be calculated as shown in Equation 3.

$$\Delta I_{(X)} = \Delta I_{(benzene)} = I_{TP(benzene)} - I_{SQ(benzene)} \quad \text{Eqn. (3)}$$
Figure 3.9: Squalane a saturated highly branched C30 hydrocarbon

The difference in these indices provides a measure of the increased relative polarity of the test phase relative to squalane (which is known as the universal nonpolar standard). All five probes were run on squalane and on the stationary phase whose polarity is to be determined, and a set of five $\Delta I$ values were determined. The sum of the five retention indices ($\Sigma \Delta I$) then gave the relative phase polarity of the phase being evaluated relative to a squalene stationary phase, while the overall polarity of the stationary phase was determined by taking the average of all five phase constants. Table 3.5 and Table 3.6 show the retention indices and phase polarities of some non-ionic liquid commercial columns and ionic liquid commercial columns, respectively.
Table 3.3: The Rohrschneider–McReynolds system applied to stationary phases of commercials columns.\textsuperscript{37}

<table>
<thead>
<tr>
<th>McReynolds Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stationary phases</strong></td>
</tr>
<tr>
<td><strong>Squalane</strong></td>
</tr>
<tr>
<td><strong>Apolane 87</strong></td>
</tr>
<tr>
<td><strong>OV-1 or DB-1</strong></td>
</tr>
<tr>
<td><strong>OV-101</strong></td>
</tr>
<tr>
<td><strong>Dexsil 300</strong></td>
</tr>
<tr>
<td><strong>Equity-1701\textsuperscript{*}</strong></td>
</tr>
<tr>
<td><strong>OV-17</strong></td>
</tr>
<tr>
<td><strong>OV-225</strong></td>
</tr>
<tr>
<td><strong>Carbowax 20M</strong></td>
</tr>
<tr>
<td><strong>DEG</strong></td>
</tr>
<tr>
<td><strong>OV-275</strong></td>
</tr>
</tbody>
</table>

(*data from www.sigmaaldrich.com), \textsuperscript{®} = Registered trademark

Armstrong and coworkers determined the Rohrschneider–McReynolds constants for two imidazolium-based ILs: 1-butyl-3-methylimidazolium chloride \([\text{C4C1Im}]\text{Cl}\) and 1-butyl-3-methylimidazolium hexafluorophosphate \([\text{C4C1Im}]\text{PF}_6\).\textsuperscript{5} A comparison of the phase constants for \([\text{C4C1Im}]\text{PF}_6\) and \([\text{C4C1Im}]\text{Cl}\) to two common commercial GC stationary phases, DB-5 (phenylmethyl polysiloxane; 5% phenyl) and OV-22 (phenylmethylidiphenyl polysiloxane; 65% phenyl) revealed the average polarity of the two ILs (\([\Sigma \Delta I]_{\text{Av}}=216\) and \([\Sigma \Delta I]_{\text{Av}}=218\), respectively) to be very similar to that of the OV-22 stationary phase (\([\Sigma \Delta I]_{\text{Av}}=215\)).\textsuperscript{5} Both the ILs exhibited significant proton accepting and dipolar interactions with solute molecules.\textsuperscript{5} It was also observed that different anions influenced the magnitude of the phase constants, but did not affect the
overall polarity of the stationary phase. Although the overall polarities of the studied ILs were similar to each other and other polysiloxane stationary phases, their separation selectivities were very different.

The effect of the chiral ionic additive on the polarity of the OV-1701 phase was investigated using the Rohrschneider–McReynolds system. The retention indices of each of the polysiloxane-based chiral ionic phases are presented in Table 3.5 along with a 100 % OV-1701 phase for comparison. The retention indices given for each phase was the average of 3 injections at either 60 °C or 70 °C normalised to squalane. Table 3.5 shows the phase polarities of the polysiloxane-based ionic phases to range from $\sum \Delta I_{\text{Av}}$ 75 to 187 compared to 100 % OV-1701 phase with an overall phase polarity of $\sum \Delta I_{\text{Av}}$ 131. It would be expected that the chiral additive would increase the overall polarity of OV-1701, however, this was not necessarily the case.

**Table 3.4:** The Rohrschneider–McReynolds system applied to commercial IL phases.

<table>
<thead>
<tr>
<th>Stationary phases</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>U</th>
<th>S</th>
<th>$\Delta I_{\text{Av}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLB-IL59</td>
<td>338</td>
<td>505</td>
<td>549</td>
<td>649</td>
<td>583</td>
<td>525</td>
</tr>
<tr>
<td>SLB-IL76</td>
<td>456</td>
<td>690</td>
<td>643</td>
<td>845</td>
<td>745</td>
<td>676</td>
</tr>
<tr>
<td>SLB-IL82</td>
<td>532</td>
<td>676</td>
<td>701</td>
<td>921</td>
<td>808</td>
<td>728</td>
</tr>
<tr>
<td>SLB-IL100</td>
<td>602</td>
<td>853</td>
<td>884</td>
<td>1017</td>
<td>1081</td>
<td>888</td>
</tr>
<tr>
<td>SLB-IL111</td>
<td>766</td>
<td>930</td>
<td>957</td>
<td>1192</td>
<td>1093</td>
<td>988</td>
</tr>
</tbody>
</table>

PM-CD (per-2,3,6-methyl-$\beta$-CD) and PA-CD (per-2,3,6-acetyl-$\beta$-CD) diluted in OV-1701 both have a polarity of 98 which is significantly lower than that of pure OV1701, while PM-CD diluted in carbowax 20 (PM-CD wax) has a significantly higher polarity ($\sum \Delta I_{\text{Av}}$ 2041).
220) as expected, (pure carbowax 20 has a polarity of 462). Derivatised cyclodextrins, either permethylated or peracetylated, lower the overall polarity of the stationary phase in which they are diluted. While carbowax 20 is a highly polar phase, the dilution of 30% permethylated β-CD (PM-CD) lowered the overall polarity of the stationary phase from highly polar to one of intermediate polarity.

**Figure 3.10:** Visual representation of the polarity scale of the tested phases in comparison to various commercial phases; the polarities of the phases tested range from 75-220.

When the polarities of PM-CD and PA-CD diluted in OV-1701 are compared with those of the ionic CD phases there is only a minor increase in the polarities for the ionic β-CD derivatives. The ionic substituent had little effect on the overall polarity of the phase while the size of the cyclodextrin had a more profound effect on the phase polarity. The ionic liquid P23A6BuImCD (92) has a phase polarity of $[\Sigma \Delta I]_{av}$ 89 (making it a nonpolar phase) which is significantly lower than its β-CD analogue (P23A6BuImβCD (91)) with a polarity of $[\Sigma \Delta I]_{av}$ 120 (intermediate polarity). All the ionic salts tested significantly reduced the polarity of the OV-1701 polysiloxane polymer in which they were diluted with the exception of phase 69 which increased the phase polarity and 4 which had no effect on the polarity of the phase resulting in a similar polarity to OV-1701. The ionic liquid stationary phases currently available on the market, shown in Table 3.4, have polarities ranging from highly polar to extremely polar ($[\Sigma \Delta I]_{av}$ 525 to 988). Whereas the polarity range of the polysiloxane-based ionic stationary phases do not differ greatly from pure OV-1701, yet the retention behaviours of the probe molecules analysed varied
significantly as a result of the differences in the chemical structures of the chiral ionic additives.

**Table 3.5:** The Rohrschneider–McReynolds system applied to the chiral ionic phases.

<table>
<thead>
<tr>
<th>McReynolds Constants</th>
<th>Stationary phases</th>
<th>( X )</th>
<th>( Y )</th>
<th>( Z )</th>
<th>( U )</th>
<th>( S )</th>
<th>Av. ( \Delta I )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>OV-1701</strong></td>
<td>46</td>
<td>161</td>
<td>127</td>
<td>201</td>
<td>121</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>MEGA</td>
<td>36</td>
<td>178</td>
<td>93</td>
<td>169</td>
<td>94</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>171</td>
<td>129</td>
<td>214</td>
<td>125</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>26</td>
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<td>129</td>
<td>86</td>
<td>136</td>
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</tr>
<tr>
<td>39</td>
<td>33</td>
<td>130</td>
<td>97</td>
<td>166</td>
<td>98</td>
<td>105</td>
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<td>33</td>
<td>93</td>
<td>68</td>
<td>112</td>
<td>70</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>36</td>
<td>174</td>
<td>118</td>
<td>190</td>
<td>-11</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>46</td>
<td>158</td>
<td>220</td>
<td>172</td>
<td>338</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td><strong>PM-CD wax</strong></td>
<td>93</td>
<td>321</td>
<td>141</td>
<td>314</td>
<td>232</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td><strong>PM-CD</strong></td>
<td>8</td>
<td>144</td>
<td>77</td>
<td>165</td>
<td>94</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td><strong>PA-CD</strong></td>
<td>3</td>
<td>149</td>
<td>42</td>
<td>187</td>
<td>110</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td><strong>P23Ac6Bu(\beta)CD (91)</strong></td>
<td>43</td>
<td>156</td>
<td>99</td>
<td>177</td>
<td>127</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>P23Ac6Me(\beta)CD (89)</strong></td>
<td>33</td>
<td>176</td>
<td>83</td>
<td>192</td>
<td>128</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td><strong>P23Ac6Buy(\beta)CD (92)</strong></td>
<td>31</td>
<td>106</td>
<td>80</td>
<td>141</td>
<td>86</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

The relative contributions of the chiral additives to the McReynolds constants of the OV-1701 phase are shown in Table 3.6. These results were derived by subtracting the McReynolds constants for pure OV-1701 from the constants of the OV-1701 phases impregnated with a chiral compound. When normalised to OV-1701 we can see the contribution the additives make to the distinct interactions each probe molecule
undergoes with the stationary phase. P23A6BuImβCD (91) and P23A6MeβIm-CD (89) stationary with having similar overall polarities of 120 and 122, respectively, showed distinctly different molecular interaction with the probe molecules (see Table 3.6). P23A6BuImβCD (91) phase showed a decrease in dipolar interactions with proton acceptor but not proton donor capabilities (Z) as well as a decrease in weak dipolar interactions (U), while P23A6MeImβCD (89) phase not only showed a decrease in dipolar interactions with proton acceptor but not proton donor capabilities (Z), but a marked increase in dipolar interactions with both proton donor and proton acceptor capabilities (Y). These differences are most likely attributed to the differences in the chemical structure of the imidazolium cationic component of the ionic salt given that all the ionic CDs have the same counter anion (iodine).

Not only does the chemical structure of the imidazolium substituent affect the strength of interaction, but the size of the CD ring itself has a marked effect on how the probe molecules interact with stationary phases. Comparison of P23A6BuImβCD (91) and P23A6BuImγCD (92) indicates their markedly different polarities, of 120 and 89 respectively, one being of moderate polarity and the other non-polar on the polarity scale. Although both additives have the same butylimidazolium cation derivative bonded to the C6, the difference in the size of the cyclodextrin ring cavity exerts a significant effect on the strength of the molecular interactions between the probe molecules and the stationary phase (Table 3.6). The γ-CD ionic phase P23A6BuImγCD (92) showed a distinct reduction in all the molecular interactions represented by the phase constants, X, Y, Z, U, and S, in comparison to the β-CD ionic stationary phase P23A6BuImβCD (91).
Table 3.6: The relative contribution of the additives to McReynolds constants derived from the Rohrschneider–McReynolds system and normalised to OV-1701.

<table>
<thead>
<tr>
<th>Stationary phases</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>U</th>
<th>S</th>
<th>Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1701</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>10</td>
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<td>1</td>
<td>-32</td>
<td>-41</td>
<td>-65</td>
<td>-34</td>
<td>-34</td>
</tr>
<tr>
<td>39</td>
<td>-13</td>
<td>-31</td>
<td>-30</td>
<td>-35</td>
<td>-23</td>
<td>-26</td>
</tr>
<tr>
<td>35</td>
<td>-13</td>
<td>-68</td>
<td>-59</td>
<td>-89</td>
<td>-51</td>
<td>-56</td>
</tr>
<tr>
<td>65</td>
<td>-10</td>
<td>13</td>
<td>-9</td>
<td>-11</td>
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<td>-30</td>
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<tr>
<td>69</td>
<td>0</td>
<td>-3</td>
<td>93</td>
<td>-29</td>
<td>217</td>
<td>56</td>
</tr>
<tr>
<td>PM-CD</td>
<td>-38</td>
<td>-17</td>
<td>-50</td>
<td>-36</td>
<td>-27</td>
<td>-34</td>
</tr>
<tr>
<td>PA-CD</td>
<td>-43</td>
<td>-12</td>
<td>-85</td>
<td>-14</td>
<td>-11</td>
<td>-33</td>
</tr>
<tr>
<td>P23Ac6BuβCD (91)</td>
<td>-3</td>
<td>-5</td>
<td>-28</td>
<td>-24</td>
<td>6</td>
<td>-11</td>
</tr>
<tr>
<td>P23Ac6MeβCD (89)</td>
<td>-13</td>
<td>15</td>
<td>-44</td>
<td>-9</td>
<td>7</td>
<td>-9</td>
</tr>
<tr>
<td>P23Ac6BuyCD (92)</td>
<td>-15</td>
<td>-55</td>
<td>-47</td>
<td>-60</td>
<td>-35</td>
<td>-42</td>
</tr>
</tbody>
</table>

However, there are limitations with the application of the Rohrschneider–McReynolds method for characterising a stationary phase. The Rohrschneider–McReynolds approach is helpful in illustrating differences between ILs in terms of the types of interactions they exhibit with the probe molecules. Any one probe can indicate a particularly strong interaction, indicating that the phase interacts strongly with compounds of comparable characteristic to the probe molecule. However, the model has deficiencies that preclude it from fully characterizing individual solvation interactions. The method utilizes probe molecules that are too volatile (e.g., benzene, 2-pentanone, and nitropropane) having short retention times often eluting with the dead volume of the column. In addition, a
single parameter polarity scale has the disadvantage of describing a weighted average of all solute–solvent interactions.\textsuperscript{44} Ideally what is required is to ascertain the individual solvation interactions the ionic additive contributes to the properties of the OV-1701 phase. Given that the additives vary significantly in their chemical structures, they may undergo variable solvation interactions. To determine the individual solvation interactions imparted by the cation and anion, the method must be capable of describing more than a single polarity, or phase constant. The solvation parameter model (linear solvation free energy relationship [LSFER] model) developed by Abraham,\textsuperscript{45,46} has been used to characterize liquid- or gas-phase interactions between solute molecules and liquid phases. It is highly recommended that such a model be applied to gain a better understanding of the individual solvation interactions contributed by the chiral ionic salt additives to the polysiloxane phase.

**Column efficiency**

*Number of theoretical plates (N)*

The most common measure of the efficiency of a chromatographic system is the Number of theoretical plates or plate number, $N$. For a chromatogram containing many peaks, the values of $N$ for individual peaks may vary (increasing slightly with retention time). It is common practice, however, to assign a value to a particular column based on only one measurement even though an average value would be better. Here, naphthalene has been assigned as the analyte for assessing the column efficiencies.

Column efficiency is the relationship between solute retention time and the amount of band broadening. Symmetrical peaks with small width are desired. Narrow peaks can be close together and still be resolved. Broader peaks of the same distance apart will not be resolved as well and may co-elute. Column efficiency is expressed as the number of theoretical plates ($N$), and is calculated from Equation 4;

\[
N = 5.545 \frac{I_R}{W_h} \quad \text{Eqn. (4)}
\]
where $t_R$ is the peak retention time and $w_H$ is the peak width at half height expressed in units of time. The higher the number of theoretical plates, the higher the column efficiency and its potential to resolve two closely eluting solutes.

**Table 3.7:** Column efficiencies; plate number and plate height of naphthalene on the ionic test phases compared with OV-1701 and the commercial column (MEGA).

<table>
<thead>
<tr>
<th>Columns</th>
<th>$t_R'$</th>
<th>$N$</th>
<th>$H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1701</td>
<td>6.99</td>
<td>20231</td>
<td>494</td>
</tr>
<tr>
<td>MEGA</td>
<td>9.7</td>
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<td>1376</td>
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<td>69</td>
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</tr>
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<td>PM-CD OV1701</td>
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<td>11616</td>
</tr>
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<td>PM-CD wax</td>
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<td>2605</td>
<td>14242</td>
</tr>
<tr>
<td>PA-CD</td>
<td>17.66</td>
<td>633</td>
<td>39962</td>
</tr>
<tr>
<td>P23Ac6BuImβCD (91)</td>
<td>7.73</td>
<td>228</td>
<td>73156</td>
</tr>
<tr>
<td>P23Ac6MeImβCD (89)</td>
<td>7.75</td>
<td>309</td>
<td>33914</td>
</tr>
<tr>
<td>P23Ac6BuImγCD (92)</td>
<td>10.97</td>
<td>430</td>
<td>33781</td>
</tr>
</tbody>
</table>

*Unable to determine $N$ for 850 & 870 due to extreme peak broadening of Naphthalene. (The green number is the compound number of the chiral selector used in the phase).
Plate height ($H$)

Another related parameter which expresses the efficiency of a column is the plate height ($H$), also called the Height Equivalent to One Theoretical Plate (HETP), where $L$ is the length of the column (mm) and $N$ is number of theoretical plates (Equation (5)). Plate height is better than the number of theoretical plates for comparing efficiencies of columns of differing lengths. A good column will have a large $N$ and a small $H$ value.

$$H = \frac{L}{N} \quad \text{Eqn. (5)}$$

Table 3.7 compares the column efficiencies of the ionic stationary phases with 100 % OV-1701 and the commercial column (MEGA). The chiral ionic additives significantly reduced the efficiency of the stationary phase for naphthalene, with the exception of phase 65 with a plate height of 1376 which is better than the plate number produced by the commercial column (plate height 2409).

Chiral analysis

The capability of the chiral ionic stationary phases to chromatographically resolve enantiomeric mixtures was tested against the series of chiral analytes illustrated in Figure 3.11. Of the 54 racemic compounds tested only the most significant results will be reported here. No separation was observed for other terpenes, including limonene and $\alpha$-pinene, with any of the columns tested. The commercial column (MEGA) however, separated 19 out of the remaining 39 chiral analytes tested. Over all, the commercial column resolved 50 % of the chiral compounds analysed.
Figure 3.11: List of racemic analytes tested.

Figure 3.12 shows chiral resolution of γ-valerolactone using the commercial column (MEGA) as well as with the PA-CD (per-2,3,6-acetyl-β-CD) phase. The enantiomeric resolution of γ-valerolactone with a stationary phase prepared and coated in our lab validated the effectiveness of the coating procedure.
**Figure 3.12:** Chromatographic spectra of ketones, lactones and alkanes separated on the MEGA, and PA-CD columns. 1) 3-methylpentanone 2) δ-decalactone 3) γ-valerolactone 4) 3-methylhexane 5) 3-methylpentane.
Figure 3.13 shows a comparison of the separation of a test mixture of the aromatic and cyclic chiral analytes limonene, mentone, styrene oxide, and 1-chloro-2,3-epoxypropane (epichlorohydrin) on stationary phases OV-1701, MEGA, PA-CD, PM-CD, 39, 26, P23Ac6MeImβCD (89), and P23Ac6BuImβCD (91). The differences in retention time, peaking broadening, and peak symmetry show just how differently the analytes behave on the different phases. For example, 1-chloro-2,3-epoxypropane is not strongly retained and co-elutes with the solvent peak on phase 39 and the ionic CD phases, whereas the peak is present in the spectra of the remaining phases. Severe peak broadening was observed for styrene epoxide on both ionic CD phases to the extent where the analyte could not be seen in the spectra where as it was detected in the remaining stationary phases. Enantiomeric resolution of 1-chloro-2,3-epoxypropane was observed on PA-CD stationary phase while the MEGA column resolved limonene and styrene epoxide.
**Figure 3.13:** A comparison of the separation of a test mixture of aromatic and cyclic chiral analytes on the OV-1701, MEGA, PA-CD, PM-CD, 39, 26, P23Ac6MeImβCD (89), and P23Ac6BuImβCD (91) stationary phases. 1) Limonene, 2) Mentone and *iso*-methone, 3) Styrene oxide, 4) 1-chloro-2,3-epoxypropane (epichlorohydrin).

The chromatographic separation of aldehydes and oximes on OV-1701, MEGA, 39 and PA-CD stationary phases is illustrated in Figure 3.14. The commercial MEGA column was able to enantiomerically resolve 2-methylpentane aldehyde, and enantiomers of the *E* and *Z* isomers of the oximes. The remaining ionic phases did not resolve the enantiomers of either the aldehydes or oximes, but the *E* and *Z* isomers of the oximes were resolved on all the ionic phases tested.
Figure 3.14: Chromatographic separation of aldehydes and oximes on OV-1701, MEGA, 39 and PA-CD stationary phases. 1) 2-methylbutyraldehyde, 2) 2-methylpentanealdehyde, 3) 2-methylbutyraldehyde oxime, 4) 2-methylpentanealdehyde oxime.
The retention factor \( k \) is a measure of how much time a solute spends in the stationary phase relative to the time it spends in the mobile phase (carrier gas). All solutes spend the same amount of time in the mobile phase, thus the \( k \) value is directly related to the retention caused by the stationary phase. It has a unitless value calculated using Equation 6;

\[
\hat{k} = \frac{t_R - t_M}{t_M}
\]

Eqn (6)

where \( t_R \) is the solute retention time and \( t_M \) the retention time of non-retained solute such as methane gas. Table 3.8 shows the partition ratios of selected compounds analysed on the ionic phases 26, 4, P23Ac6MeImβCD (89) and P23Ac6BuImγCD (92), compared with the 100 % OV-1701 phase, PA-CD phase and the MEGA (commercial column). The commercial column having a different stationary phase to OV-1701 and consisting of a different cyclodextrin is expected to show considerably different retention behaviours for each test analyte. However, a comparison of the differences in retention behaviour of the chiral analytes of the OV-1701 based ionic stationary phases verses the 100 % OV-1701 stationary phase reveals the retention caused by the chiral ionic additives.

A further comparison of the effect of the differences in the chemical structure of the cyclodextrin additive can be made by studying the difference in the retention behaviours of the chiral analytes on the PA-CD stationary phase consisting of 30 % per-2,3,6-acetyl-β-CD additive in OV-1701, verses P23Ac6MeImβCD (89) ionic stationary phase composed of 30 % per-2,3-acetyl-6-(N-Methylimidazolium iodide)-β-CD in OV-1701. These β cyclodextrin derivatives have a similar chemical structure with an acetyl group at C2 and C3 but, differing at C6 with P23Ac6MeImβCD (89) having a methylimidazolium substituent instead of an acetyl group as per PA-CD. By comparing these two phases the effect of the C6 imidazolium substituent can be seen by comparing the \( k \) value in Table 3.8. The effect of the size of the cyclodextrin ring on the retention of the analytes can be
seen by comparing P23Ac6BuImβCD (91) and P23Ac6BuImγCD (92) stationary phases. Both CD derivatives have identical substituents at C2, C3 and C6, the only difference being that one is a β-CD derivative and the other a γ-CD derivative, respectively.
Table 3.8: A comparison of the retention factors ($k$) of selected chiral analytes on the various stationary phases.

<table>
<thead>
<tr>
<th>Chiral Analytes</th>
<th>MEGA</th>
<th>OV-1701</th>
<th>PA-CD</th>
<th>PA23Ac 6MeImβCD</th>
<th>P23Ac 6BuImγCD</th>
<th>PA23Ac 6BuImβCD</th>
</tr>
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<tr>
<td></td>
<td>$k^1$</td>
<td>$k^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2-Butanol</td>
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<td>4.0</td>
<td>1.3</td>
<td>3.1</td>
<td>0.9</td>
<td>1.7</td>
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<td>7.5</td>
<td>2.7</td>
<td>5.5</td>
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<td>2.1</td>
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<td>2-Methyl-1-butanol</td>
<td>10.1</td>
<td>4.0</td>
<td>8.7</td>
<td></td>
<td>3.87</td>
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<td>1-Phenylethanol</td>
<td>23.1</td>
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<td>20.3</td>
<td>47.4</td>
<td>61.30</td>
<td>38.30</td>
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<td>2-Octanol</td>
<td>16.3</td>
<td>31.1</td>
<td>24.2</td>
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<td>2,3-Butanediol(rr/ss)</td>
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<td>43.4</td>
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<td>γ-Valerolactone</td>
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<td>Value 3</td>
<td>Value 4</td>
<td>Value 5</td>
<td>Value 6</td>
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<td></td>
</tr>
<tr>
<td>Compound</td>
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<td>3</td>
<td>4</td>
<td>5</td>
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<td>--------------------------------</td>
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<td>------</td>
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<td>------</td>
<td>------</td>
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</tr>
<tr>
<td>2-Methylbutyraldehyde oxime</td>
<td>17.2</td>
<td>18.5</td>
<td>11.0</td>
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<td>9.0</td>
<td>6.98</td>
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<td>14.90</td>
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<td>2-Methyl pentanealdoxime</td>
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<td>20.9</td>
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<td>34.2</td>
<td>17.8</td>
<td>15.5</td>
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<td>Malic acid methyl ester</td>
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<td>23.9</td>
<td>46.2</td>
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<td>49.92</td>
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<td>Mandelic acid methyl ester</td>
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<td>2-Hydroxydecanoic acid methyl ester</td>
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<td>30.9</td>
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</tr>
<tr>
<td>D/L Alanine methyl ester</td>
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<td>13.8</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D/L Aspartic acid methyl ester</td>
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<td>23.2</td>
<td>53.8</td>
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<td>D/L Asparagine methyl ester</td>
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<tr>
<td>D/L Leucine methyl ester</td>
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<td>D/L methionine methyl ester</td>
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<td>49.0</td>
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<td>31.3</td>
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<td>D/L Serine methyl ester</td>
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<td>23.3</td>
<td>53.0</td>
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</tr>
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<td>D/L Threonine methyl ester</td>
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<td>22.4</td>
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<td>D/L Valine methyl ester</td>
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<td>14.1</td>
<td>20.8</td>
<td></td>
<td>11.3</td>
<td></td>
</tr>
</tbody>
</table>

* Methone was present as two isomers (as methone and iso-menthone) in the test mixture, each isomer was present as a racemic mixture which were not resolved by the chiral ionic liquids.
Amino acid analysis

![Amino acid derivatisation into methyl esters](image)

**Figure 3.15:** Amino acid derivatisation into methyl esters.

Amino acids and carboxylic acids were derivatised into their methyl esters, following a procedure by Li and Sha, by mixing the amino acid or carboxylic acids with an excess molar equivalent of freshly distilled chlorotrimethylsilane (TMSCl) in methanol and stirring at room temp for 24 hrs to afford the methyl ester hydrochlorides as a product (Figure 3.15). Methanol was removed by evaporation and the methyl esters dissolved in DCM for injection.

**Table 3.9:** Derivatised D/L amino acid and R/S carboxylic acid methyl esters

<table>
<thead>
<tr>
<th>Alanine</th>
<th>Aspartic acid</th>
<th>Asparagine</th>
<th>Leucine</th>
<th>Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>Serine</td>
<td>Tryptophan</td>
<td>Threonine</td>
<td>Valine</td>
</tr>
<tr>
<td>Malic acid</td>
<td>Mandelic acid</td>
<td>Tartaric acid</td>
<td>2-Hydroxydecanoic acid</td>
<td>Citrulline</td>
</tr>
</tbody>
</table>

Of the amino acid and carboxylic acid methyl esters analysed the commercial column separated 8 out of the 15 methyl esters. However, neither the ionic or, PM-CD and PA-CD selecting agents showed chromatographic resolution of the enantiomers of the methyl esters. The ionic phases 26, P23Ac6MeIm-BCD (89), as well as the 100 % OV-1701 phase strongly retained the amino acid and carboxylic acid methyl esters, with the exception of the ionic phase 4 (derived from the alkylation of the amino acid N-acetylhistidine) which eluted the majority of the amino acid methyl esters analysed. Despite having similar a polarity as 100 % OV-1701 (Av. ΔI =131) the IL additive 4 in stationary phase 4 (Av. ΔI=137) had a significant effect on the retention behaviour of this ionic stationary phase. Given that OV-1701 strongly retained the amino acid and
carboxylic acid methyl esters it is recommended that an alternative phase be used to dilute the chiral ionic additives for application in amino acid and carboxylic acid analysis, one that will not strongly retain the analytes.

**Discussion of chiral analysis**

Permethylated-β-CD is one of the most extensively used chiral selectors. From the comprehensive work of Schurig and co-workers,\textsuperscript{48-50} it is evident that a wide range of chiral analytes can be enantioseparated with this selector. Usually, the selector is dissolved in the moderately polar polysiloxane OV-1701 or in SPB-35 (35 % diphenyl, 65 % dimethylpolysiloxane); that are commercially available. However, the solubility of PM-β-CD in the OV-1701 polymer is limited.\textsuperscript{1} According to Bicchi et al.,\textsuperscript{51} the minimum operating temperature for columns containing PM-β-CD were 110 °C for the column coated with undiluted PM-β-CD, 75 °C for the column coated with 30 % PM-β-CD in OV-1701, 55 °C for the column coated with 10 % PM-β-CD in OV-1701, and 0 °C for the column coated with 5 % PM-β-CD in the same polysiloxane. Generally, permethylated cyclodextrin is only sparingly soluble in nonpolar dimethylpolysiloxanes and enantiomer separations below 80 °C have proven to be unsuccessful on this stationary phase.\textsuperscript{50} To overcome this problem Schürig et al.\textsuperscript{50} chemically bonded permethylated 5-Pent-1-enyl-β-cyclodextrin derivative to a polysiloxane backbone. The resulting chiral stationary phase is known commercially as Chirasil-Dex.\textsuperscript{50}

Thus it is important to know/ or determine the solubility of the chiral selective agent in the polysiloxane in which it is to be diluted. Ideally the maximum percentage of chiral selective agent added to the polysiloxane should be determined based on the solubility of chiral selector in the phase, however, given that the chiral ionic salts are novel compounds for which such data is not yet available, a concentration of 30 % (w/w) was selected. It is unknown whether this quantity of ionic selective agent is optimal for chromatographic resolution of enantiomers to be observed. However, it is important to note that spectroscopic discrimination does not necessarily correlate with the
thermodynamic discrimination associated with the diastereomeric complexes involved in enantiomer resolution.

It was reported by Bicchi et al. that increasing the amount of 2,6-dimethyl-3-pentyl-β-cyclodextrin from 10 % to 30 % in OV-1701 increased enantioresolution of some racemates but caused complete loss of resolution for the menthol racemate. On the other hand, the separation of another group of analytes was not affected by increasing the concentration of the chiral selector in OV-1701.

Research has been carried out with several other commonly used polymers with the aim of obtaining a higher PM-β-CD solubility, and presumably better enantioseparations. More recently however, ionic liquids have been investigated as chiral stationary phases for application in chiral analysis by GC. To date there have only been three reported applications for chiral ILs as stationary phase solvents and chiral selectors in GC analysis. One method involved dissolving the chiral selector in an ionic liquid and coating the stationary phase on the capillary wall. Cyclodextrin derivatives (permethylated-β-cyclodextrin and dimethylated-β-cyclodextrin) were dissolved in 1-butyl-3-methylimidazolium chloride and the performance of these IL-based chiral stationary phases were compared with analogous polysiloxane based commercial columns (Chiraldex BPM and Chiraldex BDM) employing the same chiral selectors. Although the chiral IL phases did show enantioselectivity, the observed enantioselectivities of these IL-based columns did not rival those of the commercial columns.

The second application involved using chiral ILs directly as chiral stationary phases, as opposed to the method described above whereby an achiral IL was impregnated with a chiral selector. N,N-dimethylephedrinium bis(trifluoromethanesulfonyl)imidate-based ILs were the first class of 100 % chiral ionic liquid stationary phase used for the separation of enantiomers in GC. Enantiomers including alcohols, diols, sulfoxides, epoxides, and acetylated amines were resolved. The successful use of a pure ionic liquid phase in chiral resolution of a selective group of analytes suggests that perhaps in this study using
higher concentrations of the chiral ionic salts may have possibly improved chiral resolution capability of the tested ionic stationary phases. It is possible that the 30 % ionic salt concentration used in polysiloxane may not have been sufficient to observe the chromatographic resolution.

The third application involved the use of chiral ionic cyclodextrin derivatives as chiral selectors diluted in an ionic liquid phase. When compared with commercial polysiloxane based phases which possess analogous CD selectors to the ionic CD phases, the ionic CD phases were reported as having improved enantioselectivity for more than one third of the chiral analytes tested and also separated some compounds that were not separated on the commercial phases tested.55 Achiral RTIL’s have been reported as being able to dissolve highly polar compounds including carbohydrates56 and appear to work well as a stationary phase matrix for dissolving cyclodextrin selecting agents. It is possible that the ionic CDs (and imidazolinium salts) synthesised are not highly soluble in OV-1701, and possibly raises the minimum operating temperatures of the stationary phase well above the temperature ranges in which enantioselectivity occurs for the individual chiral analytes tested in this study. Perhaps if an achiral RTIL had been employed as the stationary phase matrix, instead of a polysiloxane for which the solubility of CDs (such as PM-CD) is known to be limited, the enantioselectivity of the chiral ionic phases may have been improved. The degree of miscibility of the chiral ionic salts studied in this work with OV-1701 (and polysiloxanes in general) has not been established and limited solubility cannot be eliminated a possible contributing factor toward the poor performance of the ionic salts as chiral resolving agents.

Chiral diastereomeric complexation interaction mechanisms

The cyclodextrins exist as a single enantiomer, with the consequence that as host molecules, their interaction with a racemic guest may lead to the formation of diastereoisomeric complexes of differing thermodynamic stabilities. This chiral discrimination by unmodified cyclodextrins has been extensively studied, most notably
through the work of Armstrong et al.\textsuperscript{57,58} in the development of cyclodextrin based chromatographic systems.

In GC the enantioselective separation of enantiomers by CD derivatives has been recognized to be the result of fast kinetics and is governed entirely by thermodynamics.\textsuperscript{49,59} As a consequence, enantioselective separation is strongly dependent on temperature. The discrimination of two enantiomers results from a small difference in the energy of association during complexation interactions, between each enantiomer and the CD selector.\textsuperscript{41,48} It has been noted previously that no correlation exists between the retention times of molecules on cyclodextrin-based chromatography phases and the thermodynamic stability of the inclusion complexes formed in solution between those molecules and cyclodextrins.\textsuperscript{48} Thus, spectroscopic discrimination does not necessarily correlate with thermodynamic discrimination.

**Chiral resolution on IL columns**

It was also observed by Ding et al. that the configuration of the chiral center of the \textit{N,N}-dimethylephedrinium bis(trifluoromethanesulfonyl)imdate IL CSPs played a significant role in the observed enantioselectivity.\textsuperscript{54} For example, (1\textit{S},2\textit{S}) \textit{N,N}-dimethylephedrinium bis(trifluoromethanesulfonyl)imdate IL CSP exhibited no enantioselective separation of chiral alcohols but exhibited similar enantioselectivity to its (1\textit{S},2\textit{R}) IL analogue in the separation of chiral sulfoxides. It was found that prolonged exposure of these \textit{N,N}-dimethylephedrinium IL CSPs to oven temperatures \(\geq 140\) °C resulted in enantioselectivity losses for some classes of molecules such as alcohols, but not sulfoxides. These enantioselectivity losses were attributed to the formation of a dehydration product (Figure 3.16) via a thermally induced epimerization process that may also result in racemisation.\textsuperscript{54} It was concluded that a fixed configuration of the first stereogenic center was required for enantiorecognition and enantiomeric resolution of the sulfoxide analytes, but not the second stereogenic center.\textsuperscript{54} However, enantiomeric resolution of the alcohols not only requires the configuration of both stereogenic centers, but, that they have opposite absolute configuration, either (1\textit{S},2\textit{R}) or (1\textit{R},2\textit{S}).\textsuperscript{54}
*N*,*N*-dimethylephedrinium chiral hydroxyl group was found to be imperative in separating all the sulfoxide analytes tested, but not sufficient alone for the enantioseparation of chiral alcohols, epoxides, and acetylated amines.\(^{54}\)

![Dehydration and Epimerisation](image)

**Figure 3.16:** The dehydration and epimerisation product of \((1S,2R)-N,N\)-dimethylephedrinium cation.

Given that the configuration of the chiral stereogenic center of the IL CSP has been demonstrated to be of significant importance to chiral recognition, it must be considered that the configuration of the stereogenic centers of the 2,4,5-triphenylimizolinium ILs (26, 35, and 39) tested in this study (all being of \((4S,5S)\) configuration isolated from *trans* (+/-)-2,4,5-triphenylimizoline) may not have been the appropriate stereogenic configuration to produce enantiomeric resolution. It would be of great value in future work to examine the enantioselectivities of the \((4R,5R)\), \((4R,5S)\), and \((4S,5R)\) isomers of the 2,4,5-triphenylimizolinium IL chiral selecting agents tested in this study.

The single report which appeared in 2001 on the use of achiral ILs as stationary phase solvents for derivatized cyclodextrins,\(^{53}\) in which permethylated-\(\beta\)-CDs (BPM) and dimethylated-\(\beta\)-CDs (BDM) were dissolved in 1-butyl-3-methylimidazolium chloride (at concentrations of 26.4 % and 27.7 % (w/w), respectively) and the column performances evaluated and compared with those of analogous polysiloxane-bases commercial columns (ChiralDEX BPM and ChiralDEX BDM), were found to have column efficiencies up to ten times higher than the commercial columns, but they separated only about a fifth to a third of the racemic analytes that could be separated on the commercial columns.\(^{53}\) The narrow range of enantioselectivity was attributed to the small BMIM-Cl ion pair being included in the CD cavity and consequently reducing the inclusion complexation interaction between the chiral selector and the analyte. The only enantiomers resolved
were separated by an external adsorption process rather than inclusion complexation, with excellent resolution as a result of the high efficiencies reported on these IL-based matrix columns.

In order to reduce the accessibility of IL matrices in the CD cavity and combine the higher efficiency of the IL matrix based chiral stationary phases with the excellent enantioselectivity of the polysiloxane based chiral stationary phases, charged cyclodextrins, (permethyllated mono-6-(butylimidazolium)-cyclodextrin (BIM-BPM) and permethylated mono-6-(tripropylphosphonium)-cyclodextrin (TPP-BPM)), were synthesised and dissolved in ionic liquid matrices and examined as GC chiral stationary phases. Although charged cyclodextrin derivatives have been widely employed as chiral selectors in capillary electrophoresis (CE), the use of charged cyclodextrins as chiral selectors in GC has only been reported once.55

The first successful application of the ionic cyclodextrins to the enantiomeric separation of a variety of chiral molecules by GC was demonstrated by Huang et al in 2010.55 The synthesised ionic cyclodextrins (BIM-BPM and TPP-BPM) were dissolved in ionic liquid matrices rather than a polysiloxane matrix. The selectivity of these ionic CD phases were compared with the commercial stationary phase Chiraldex BPM. It was found that the cationic CD chiral selector made a significant contribution to the enhanced column efficiency while the type of ionic functional group on the CD had a major impact on the chiral recognition capability of the stationary phase.55 When compared with the commercial column, the IL-based stationary phases showed improved enantioseparation for more than one third of the analytes tested and separated some compounds that were not separated on the commercial column.55 This demonstrated the feasibility of using charged cyclodextrins as chiral selectors in GC stationary phases, opening a new avenue of research and development of potentially useful GC chiral stationary phases.
**Figure 3.17:** An illustration of column bleed during analysis on phase 35.

**Thermal stability of ionic Phases**

Iionic CDs were shown to be thermally stable at the maximum operating temperature of 150 °C reached during analysis. Thermal gravimetric analysis of the ionic CDs showed decomposition temperature ranging from 160 °C - 250 °C (see Section 2.4.2) by TGA analysis, far beyond the maximum operating temperatures reached during GC analysis. No significant column bleed was observed during analysis with the exception of phase 35, which showed significant column bleed at high operating temperatures (Figure 3.17) while 65 showed a marked reduction in the selectivity of some compounds following exposure to high temperature ranges. Subsequent analysis of the same test mixture showed poor resolution for selected compound on phase 65 as illustrated in Figure 3.18. This could be a result of pooling of the stationary phase following operation at high temperature resulting in an unevenly coated phase in subsequent analyses, or the ionic chiral additive may be unstable at high temperatures. Given that the thermal stabilities of the imidazolinium ionic salts have not been determined by TGA analysis it cannot be discounted that the ionic salts may be unstable at the higher operating temperatures. Neither can the possibility of isomerisation or racemisation occurring during high
temperature analysis be discounted, particularly for trans-2,4,5-triphenylimidazoline 21 derived ionic salts such as 26, 39, and 35. Because 21 can be synthesized from either cis-2,4,5-triphenylimidazoline 20 or hydrobenzamide 19 via an anionic intermediate, the possibility of isomerisation or racemisation occurring on the column cannot be overlooked.

The stereospecific formation of 2,4,5-triphenylimidazolines occurs via an electrolytic pericyclic ring closure which involves the cyclisation of the conjugated polyene hydrobenzamide via a 2,4-diazapentadienyl anion. The anionic intermediate in Figure 2.14 has been accounted for the interconversion between amarine 20 and iso-amarine 21.63 Busacca et al.64 studied the racemisation of chiral imidazolines upon exposure to base and found that strong inorganic bases could induce racemisation, however, racemisation occurred only when nitrogen on the imidazoline was un-substituted, and they proposed a symmetry-allowed thermal disrotatory ring opening and closure via a diazapentadienyl anion as the mechanism by which racemisation occurred. Given that both nitrogens on the imidazolinium salts were substituted it was expected that racemisation or isomerisation were unlikely occurring during GC analysis.
Figure 3.18: An illustration of the temperature effect on phase performance. A) First temperature programed run on phase 65, B) subsequent analysis of the same test mixture on phase 65 showing markedly reduced resolution.
3.2.3 Conclusion

The design and synthesis of chiral selecting agents is difficult and unpredictable. Although enantiomer resolution was not observed for the series of chiral analytes tested in this study, their application as chiral ionic salts for enantioselective separation yielded stationary phases of similar polarities with significantly different retention patterns. However, the ionic stationary phases need be evaluated against a wider variety of chiral analytes to determine their full potential as chiral selecting agents, as well as conducting a comprehensive investigation into the optimum concentration of ionic salt necessary in polysiloxane to achieve optimal resolution. Perhaps different polysiloxanes also need to be investigated to determine how they affect chiral resolution.

Given that per-2,3-\(O\)-acetylation yields a highly symmetrical product it’s recommended to reduce the symmetry of these CDs by selectively permethylating at the C2 positions and acetylation at C3 positions. The enantioselectivity of the symmetrically substituted novel per-2,3-acetylated ionic CDs was comparable to that of the known symmetrically substituted per-2,3,6-methyl-\(\beta\)-CD (PM-CD) where no enantioresolution was observed, while per-2,3,6-acetyl-\(\beta\)-CD only marginally performing better by enantiomerically resolving 2 chiral analytes out of the 69 tested. It has been shown that asymmetrically substituted methyl/acetyl CDs can extend enantioselectivity in comparison to that of the corresponding per-2,3,6-O-methyl or per-2,3,6-\(O\)-acetyl symmetrical CD derivatives, in terms of both enantiomer resolution and number of chiral compounds separated.\(^{29}\) It has also been reported that mono-substituted ionic CDs display superior enantioselectivity when compared with commercial columns with comparable non-ionic CD derivative analogues.\(^{55}\) Synthesis of per-substituted ionic CD analogues with a higher degree of asymmetry is recommended to improve the enantioselectivity of the novel ionic CD chiral selectors beyond that of the symmetrically substituted PM-CD and PA-CD chiral selectors tested in this study. This study opens the door for the development and application of new chiral imidazolinium ionic salts as well as imidazolium cyclodextrin ionic salts as potential chiral selecting agents in GC stationary phases.
The growing need for new chiral selecting agents with better enantioseparation capacity to increase the number of chiral compounds separated with a single chiral selector, and/or improve their resolution, has not been resolved. New chiral selecting agents with better performances can actively contribute to increasing the adoption of the “one column for one problem” approach and, as a consequence, extend the use of enantioselective GC in routine analysis and specialised chiral analysis.
3.2.4  Experimental

3.2.4.1  Sample preparation

All stock test mixtures were diluted in 10 mL dichloromethane (DCM) except in the case of the probe molecules which were used neat. The alkane test mixture was made up with 200 μL of each alkane for C_7-C_{13} in 10 mL DCM. Naphthalene was made up to a concentration of 0.2 g in 10 mL of DCM. While the stock chiral test mixtures were made up in 10 mL DCM with 30 μL 1-Octanol as the internal standard. Samples were prepared for injection by diluting 500 μL of the stock test mixture (as prepared above) with 1500 μL DCM in a sample.

3.2.4.2  Methyl esterification^47

100-200 mg of amino acid or carboxylic acid were placed in sample vials with excess methanol (2-4 mL depending on the solubility of the starting material). Then excess TMSCl was added to the sample vials, the solution mixed manually and capped. The solutions were left to react at room temperature for 24 hrs with occasional mixing. The sample vials were placed under vacuum to remove the methanol and TMSCl, leaving the product as a methyl ester hydrochloride.

3.2.4.3  Column preparation by static coating

Into a 2 mL degassed solution of DCM was added 4 mg chiral IL to make a 0.4 % (w/v) stationary phase solution. Similarly, 4 mg of OV-1701 (or Carbowax 20M where polysiloxane was not used as the stationary phase) was added to a 2 mL degassed solution of DCM. These two 0.4 % (w/v) solutions where mixed together at a proportion consisting of 30 % IL solution to 70 % (v/v) OV-1701 solution. The capillary was filled with the 0.4 % mixed solution and the other end of the capillary column sealed with a viscous solution of sodium silicate. Air bubbles must be rigorously excluded during the
coating process. After consolidation of the sodium silicate plug overnight, the capillary is mounted in a water bath at room temperature and a vacuum pump is connected on the open end. It is essential that the sodium silicate plug is sight and that the solvent is removed slowly and steadily. The presence of any air bubbles will cause the solution to be sucked out partially or totally. In this case the coating procedure has to be repeated. After the complete evaporation of the solvent a few centimetres of the column ends were cut off. The capillary then installed into a GC and conditioned prior to testing.

All capillary columns were coated with ionic salts diluted in the polysiloxane OV-1701 at 30 % IL to polysiloxane (w/w) unless otherwise stated. The ionic salts were coated onto deactivated inner column surface by static procedure on a 10m fused silica capillary column. Following the coating process, the column was conditioned from 40 °C to 150 °C at 10 °C/min. Column efficiency was determined with naphthalene at 100 °C at 2.9 psi. A Shimadzu GC-17A GC with FID, split injection (split ratio 50:1) and hydrogen as a carrier gas, injection temperature of 250 °C and detector temperature of 250 °C. All analytes were dissolved in DCM with the exception of the probe molecules which were used neat.

Isothermal analysis of alkanes and probe molecules for calculating Kovats retention indices

Isothermal analysis was conducted at 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C at a column pressure of 2.5 psi, with a split ratio of 50 (for the alkane series) and 100 (for the probe molecules).

Temperature programming for chiral analysis.

Chiral analytes were analysed by temperature program starting at 40 °C (0mins) with a ramp rate of 3 °C/min to 100 °C followed by a ramp rate of 4 °C/min to 150 °C which was held for 5 mins.
3.3 Dynamic interconversion of chiral oxime compounds in gas chromatography.

Published in:

3.3.1 Introduction

The study of species that undergo interconversion or some other type of structural change during chromatographic elution is a small but interesting topic area, which can be subdivided into two distinct or different processes. One is a structural change that is irreversible, such as in decomposition processes, a good example is the decomposition of dicyclopentadiene to cyclopentadiene. The other process is that of dynamic molecular interconversion, a process where each molecule undergoes a mechanism of a reversible structural change into their counterpart (Figure 3.20). The most important features of dynamic interconversion being that (i) the two molecules should be resolvable on the phase and under the conditions applied, and (ii) the physical conditions applied allow the observation of the interconversion process (i.e. the temperature and/or time of analysis is adequate to permit interconversion). The general scheme of the dynamic interconversion process is shown in Figure 3.19, a chromatogram of an interconverting system $A \rightleftharpoons B$, with sketched hypothetical distributions of each of the isomers underlying the overall response. The interconversion region corresponds to compounds that undergo an isomerisation step, and therefore are largely resolved from each of the original isomers $A$ and $B$. Thus the total band of the compound comprises the original narrow peaks of $A$ and $B$, and the broad zone of interconversion.
Figure 3.19: Chromatogram of interconversion process between two isomers A and B, with the net effect shown in bold, and sketched outlines showing the A isomer distribution dotted, and B isomer dashed.

A number of researchers have been prominent in this area, in particular Schurig’s group which has investigated a wide range of molecular processes, including chiral molecules in a variety of enantioselective separation methods, including HPLC and GC. Schurig has reviewed this general area.\textsuperscript{66-72} Rotational energy barriers for chiral cyclophanes determined by using GC were reported by Hochmuth and König.\textsuperscript{75} The dynamic effect of the secondary equilibria of prolines in reversed-phase HPLC were discussed by Melander\textit{ et al.}\textsuperscript{76} While the theoretical interpretation of the interconversion process and a computer program to derive physical constants were developed by Trapp,\textsuperscript{77-79} Krupcik compared the experimental data and the simulated results of various examples of interconversion.\textsuperscript{80}
The observation of sterically hindered rotation of phenanthrene and anthracene molecules, organometallics and inorganic complexes was reported in a series of papers by Marriott et al., who also studied the oxime system using one-dimensional gas chromatography systematically.\textsuperscript{81-83}

The enantiomerization (inversion) barriers for 1-chloro-2,2-dimethylaziridine in the gas phase, with a range of $\Delta G^\circ$ values from 70 kJ mol\(^{-1}\) to 200 kJ mol\(^{-1}\) was determined using enantioselective stopped-flow multidimensional gas chromatography (MDGC). Gas phase enantiomerization of the heart-cut fraction of one single enantiomer was performed in the second (reactor) column at increased temperature after GC separation of the enantiomers in the first column. This fraction was subsequently separated into the enantiomers in the third column.\textsuperscript{84} This process was recently reviewed.\textsuperscript{85}

More recently, Marriott et al. applied comprehensive two-dimensional gas chromatography (GC×GC) to the study of the interconversion processes of oximes.\textsuperscript{86,87} Oximes can exist in 2 discrete geometrically isomeric forms: the $E$ isomer and the $Z$ isomer, owing to the relative rigidity of the carbon-nitrogen double bond. In solid state, both oximes show high configurational stability and discrete existence. However, in solution, equilibrium between both isomers is rapidly established, favouring the most thermodynamically stable isomer. In these GC×GC experiments a modulator programmed to collect effluent from the first dimension (1D) column every 2-8s and pulsing it rapidly to a 2D short, fast elution column. With the proper choice of 2D column phase, it was possible to resolve the different isomer forms of the compound produced during the interconversion process, providing an instantaneous distribution of species at any given point over the chromatographic profile.\textsuperscript{86,87} Trapp et al. interpreted the GC×GC experiment based on theoretical treatment and derived kinetic data for the isomerisation process.\textsuperscript{88} The separation of two pairs of diastereoisomers for chalcogran under conditions of dynamic interconversion were proposed in a review of molecular structure – retention relationships in GC×GC,\textsuperscript{89} with the assumption of adequate resolution of all species on the two columns, however this system had not been experimentally verified.
Based on this background, the present study extended the oxime study to chiral molecular species, and incorporated enantioselective GC column phases as the separation medium.

\[ \text{Figure 3.20: } E \text{ and } Z \text{ isomers of chiral aldoximes} \]

### 3.3.2 Results & Discussion

#### 3.3.2.1 Relative elution and interconversion on single column systems.

Initial analysis on the chiral column was found to not generate the characteristic isomerisation profile shown in Figure 3.1, but rather only separated the compounds. The chiral column resolved both $E$ and $Z$ isomers, and did so more effectively than the SolGel wax or the BP20 wax phases. For the chiral oximes, it was generally found that the $E$ isomers were resolved into their ($R$) and ($S$) enantiomers better than the $Z$ isomers. For instance, for 2-methylbutraldehyde oxime on the chiral phase at 90 °C and 20 kPa $H_2$ pressure, the $R_s$ of $E$ isomers was found to be about 8.5, whereas for the $Z$ isomers $R_s$ was only approximately 1.2-1.5. The $Z$ isomer normally eluted earlier than the earliest eluting $E$ enantiomer. In contrast, for 2-methylpentanaldehyde oxime the $Z$ isomers often elute after the first $E$ enantiomer. The $Z$ isomers exhibited a much greater change in retention factor as temperatures were varied, such that it could elute earlier than the first $E$ enantiomer, and then co-elute with this isomer, and eventually could co-elute with the later of the $E$ enantiomers as the temperature was increased (see Figure 3.21). This was more pronounced for the 2-methylpentanaldoxime compound than for the 2-methylbutanaldoxime.
Figure 3.21: Isobaric 20 psi, 2-methylbutanaldehyde oxime analysis on a wax-chiral column configuration. (A) 90 °C, (B) 100 °C, (C) 110 °C, (D) 120 °C, (E) 130 °C, (F) 140 °C.

An additional observation was that the low polarity phase produced less isomerisation. Thus the BPX5 phase was able to resolve the $E$ and $Z$ isomers but little interconversion was observed. While the wax-type (polyethylene glycol) phases, SolGel Wax and BP20, led to much greater interconversion. The chiral phases tested produced to negligible isomerisation, presumably due to the low polarity of polymer phase in which the chiral selector is dissolved. Only the Mega phase gave effective resolution of the enantiomers of both the $E$ and $Z$ isomers and so was chosen for further study. Unfortunately, there currently does not seem to be a phase that is able to simultaneously resolve the geometrical isomers and enantiomers, and also strongly generate isomerisation. These characteristic would be expected from a phase with a cyclodextrin chiral selector embedded in a polar wax-type phase, and columns with this characteristic do not seem to be widely available. This is surprising, given that wax-type phases are compatible with essential oil components, which often exhibit chirality, and often require determination of the chiral composition of those compounds. Thus to circumvent the unavailability of columns which permit the simultaneous study of the isomerisation process and enantiomer resolution, the approach reported here was to join a chiral to a wax phase column, so that both chiral resolution and interconversion can be observed, though this is a sequential process rather than simultaneous.

On an achiral column, the chiral molecules lead to only two peaks, $E$ and $Z$, with almost negligible interconversion barrier under conditions which promote this process. For 2-methylbutyraldehyde oxime the $Z$ isomer elutes rather later than the $E$ isomer, so the relative retentions of $Z$ and $E$ were reversed on the chiral and achiral columns.

The effect of increased oven temperature ($T$) on extent of interconversion, for the chiral 2-methylbutanaldehyde compound on the wax column, can be seen in Figure 3.22. Even at 80 °C there was evident interconversion, and at 150 °C, the distribution resembled a smooth but broad band, with no evidence of the $E$ and $Z$ antipodes. This corresponds to
rapid interconversion, but not so rapid that the band then becomes a narrow peak of similar magnitude to a non-interchanging internal standard, which would imply $t_{RE} \sim t_{RZ}$.

**Figure 3.22**: Isobaric (20 kPa) 2-methylbutyraldehyde oxime analysis on a wax column. Isothermal oven temperatures are (A) 80 °C; (B) 110 °C; (C) 130 °C; (D) 150 °C.

### 3.3.2.2 The dual column system

There are three approaches which can be taken to interpretation of the results of the dual column system.

(i) decide what happens to the $Z$ isomer  
(ii) decide what happens to the $E$ isomer  
(iii) decide how to treat the interconversion region on the first column, as it enters then is eluted from the second column.
In this case it was informative to study the effect of each column separately. The various studies below report such observations, denoted as detector-1 (DET-1), and detector-2 (DET-2).

The two columns perform different functions; the chiral column gives enantiomer separation and $E/Z$ separation, but little isomerisation; the wax phase produces a much larger extent of isomerisation, but also gives $E/Z$ separation. The assumption here is that $E/Z$ isomerisation occurs only within the particular enantiomer ($R$) or ($S$), i.e. $E(S) \rightleftharpoons Z(S)$ or $E(R) \rightleftharpoons Z(R)$, and $E/Z$ isomerisation is not accompanied by enantiomerisation.

The dual column arrangement used here comprised a chiral column and a wax column, coupled in either order. The dual column arrangement was first used to analyse achiral oximes in order to facilitate understanding of the processes that occur without the complexity that comes with chiral separation, then the chiral oxime compounds were studied. The need for both wax-chiral and chiral-wax column arrangements to be tested was due to the different types of processes that can be observed on each column. A detector was provided between the columns in some experiments and the dual FID system was employed when a separate evaluation of the effect of each column was required. For the GC × GC experiments (see Section 3.3.4.5) the most appropriate column order was selected based on the analysis results obtained from the Dual FID system.

**Achiral compounds**

**Wax-chiral arrangement.** The wax column separated the $E/Z$ isomers, and depending on conditions (temperature ($T$), and time ($t$) spent in the column), promoted interconversion to varying extents.

The chiral column will then;

(i) separate the individual $E$ and $Z$ enantiomers according to the retention factor of each; and

(ii) cause the interconversion region to move apart as specific bands. Here it cannot be stated that the chiral column will ‘resolve’ the interconversion
region, because this is a broad overlapping band, and it is possible that the broad \(E\) and \(Z\) bands that comprise the interconversion zone will not be fully resolved.

Figure 3.23 is an example of the achiral compound heptanaldoxime progressively undergoing an increasing extent of isomerisation as the carrier flow (as indicated by the pressure setting) is decreased from 50psi to 10psi. The first column (wax) reveals a smooth distribution comprising the interconversion zone, which reflects the type of ‘plateau’ conventionally found for this system and as seen in Figure 1. The trace at the end of the second column (chiral) is no longer a smooth plateau shape, but is distorted by the separation that the chiral column generates for the interconversion zone (see Figure 3.23 (C)(ii)). The distortion results from the specific retentions of the \(E\) and \(Z\) isomers comprising the interconversion zone, on the chiral column. Note that even though there is considerable interconversion on the wax column, there is little additional interconversion on the chiral column, as indicated by comparison of Figure 3.23 C(i) and C(ii); the \(E\) and \(Z\) isomers change very little in respect of their areas, compared to the total area of the band. The relative retentions of the \(E\) and \(Z\) isomers do not appear to have varied greatly on the chiral phase, but \(Z\) elutes slightly closer to \(E\). For the wax column, \(k_E \approx 1.65\), and \(k_Z \approx 1.98\); for the chiral column, \(k_E \approx 1.6\), and \(k_Z \approx 1.7\). Figure 3.25 D(i) generates a single peak, although its width (\(\approx 1\) min) implies that there is still considerable influence of the \(E\) and \(Z\) isomers in defining the total width. Figure 3.25 D(ii) shows the \(E\) and \(Z\) isomers delivered to the chiral column, but some separation occurs (without additional interconversion). Peak width now being about 2.5 min.
Figure 3.23: Isothermal (130 °C) heptanaldoxime analysis. Dual detector wax–chiral column system, respectively. (i) FID1 detector (DET-1); (ii) FID2 detector (DET-2), carrier gas pressures are (A) 50 psi; (B) 40 psi; (C) 30 psi; (D) 10 psi. As an indication of the linear carrier velocities in each case, these were estimated to be 191, 97, 74 and 25 cm/s, respectively.
Chiral-wax arrangement. The chiral column causes $E/Z$ separation, but little interconversion. Then on the wax column the individual $E$ and $Z$ isomers undergo an isomerisation process, but essentially now on physically resolved isomers from the first (chiral) column (see Figure 3.24). Thus, an observation somewhat like a decomposition process $^6$ might be expected rather than the more familiar reversible interconversion process of the oximes due to the prior resolution on the wax column. The extent of resolution of the $E$ and $Z$ isomers on the wax column will most likely be different to that of the input distribution from the chiral column. This will be determined by the individual retention factors on each column. This means it should be possible to consider this to be a sum of the effect of the wax column on the $E$ isomer, and the effect of the wax column on the $Z$ isomer. The actual distribution will therefore not be the same as that for the analysis of $E$ and $Z$ on a single wax column but will more likely be a complex linear combination of the two effects.
**Figure 3.24:** Isothermal (130 °C) heptanal oxidime analysis. Dual detector chiral-wax column system, respectively. (i) FID1 detector (DET-1); (ii) FID2 detector (DET-2), carrier gas pressures are (A) 50 psi; (B) 40 psi; (C) 30 psi; (D) 10 psi.
Chiral compounds:

**Wax-chiral arrangement.** The process on the wax column is the same as for the achiral compound above (E/Z isomer separation, with isomerisation). However, when the chiral compound reaches the chiral column, each of the $E$ enantiomers ($(R)E$ and $(S)E$) and $Z$ enantiomers will commence to resolve. This is shown in Figure 3.25. The swapping of positions is clear in this figure. The chiral 2-methylbutanaldoxime molecule exhibits a small extent of interconversion on the wax column at 100 °C (Figure 3.25 A(i) and B(i)) and only a minor plateau is given even at 20 psi. The chiral column selectivity towards the $E$ and $Z$ isomers is readily seen to vary greatly from that of the SolGel column, as the isomers swap positions with the $Z$ isomer showing a large reduction in relative retention on the chiral phase. The relative resolution of the two enantiomers for $E$ are also very much greater than that for the $Z$ enantiomers. Thus for Figure 3.25 A(i) and B(i) $R_s$ is from 3 to 4, whilst for A(ii) and B(ii) $R_s$ of the $(R,S)E$ enantiomers is from 7 to 8, and for the $Z$ enantiomers, $R_s$ is < 1.0.

There appears to be a small shoulder (marked with the arrow (a) Figure 3.25 A(iii)) preceding the $Z$ isomers, which is taken to arise from the $Z$ isomer zone at the interconversion region marked (a) in Figure 3.25 A(i), eluting earlier than the $Z$ enantiomers of the unconverted compounds. Likewise the small shoulder after the $E$ enantiomers are the $E$ enantiomers of the interconversion region moving to a later retention than the non-converted $E$ enantiomers, which is more clearly visible in Figure 3.25 B(ii).
Figure 3.25: Isothermal (100 °C) 2-methylbutyraldehyde oxime analysis. Dual detector wax–chiral column system. (i) FID1 detector; (ii) FID2 detector. Carrier gas pressures are (A) 50 psi and (B) 20 psi. (a) Indicates the interconversion region which for data in (ii) shows this region moves earlier than the Z isomer peaks.

Chiral-wax arrangement. The chiral compounds are resolved into (R) and (S) species (here, each of E and Z are racemic) so two pairs of peaks (R,S)E and (R,S)Z were obtained. Note that E and Z show markedly different chiral resolutions, and often (R,S)Z may partially overlap one enantiomer of E. Then on the wax column, (R)Z and (R)E will interconvert, and also (S)Z and (S)E, but again from a starting basis of an input distribution that has already resolved the E and Z isomers. Thus, it is necessary to identify and interpret (i) the peaks that interconvert, and (ii) the extent of interconversion and how it is displayed in the final chromatogram.
Following the use of the system shown in Figure 3.34 (System A) for the non-modulated dual column – dual FID system, the Figure 3.34 (System B) arrangement was tested, but with only a single detector at the outlet of the triple column arrangement.

To some extent, the experience with the achiral compound will inform the understanding of interpretation of such a process. It is important to note that the interconversion could lead to a product that has either a greater or lesser retention on the second column, and this will lead to different presentation of the overall distribution. Since often the $Z$ isomers are not well resolved from one of the $E$ isomers, this makes the interconversion process difficult to distinguish with certainty when using 1D GC. But only one $E$ isomer is affected by overlap with the $Z$ isomers. The $Z$ isomers also exhibit small resolution on the chiral column, making it difficult to recognize the individual $(R)Z$ and $(S)Z$ isomers (Figure 3.26 shows this). The $Z$ isomers overlap the earlier $E$ enantiomer after passage through the wax column. Under 20psi isobaric conditions, at $T = 90 \, ^\circ C$, the $Z$ peaks elute just before the $E$ peak, while at $110 \, ^\circ C$, one of the $Z$ peaks elutes just after the $E$ enantiomer (see Figure 3.27).
**Figure 3.26**: Isothermal (100 °C) 2-methylbutyraldehyde oxime analysis. Dual detector chiral–wax column system. (i) FID1 detector; (ii) FID2 detector. Carrier gas pressure at (A) 50 psi and (B) 20 psi.
**Figure 3.27:** Isobaric (100 °C) 2-methylbutyraldehyde oxime analysis. Dual detector chiral–wax column system. (i) FID1 detector; (ii) FID2 detector. Carrier gas pressure at (A) 50 psi and (B) 20 psi.
**3.3.2.2 Comprehensive 2D GC**

Previous reports have revealed interesting chromatographic data from the use of GC×GC for interconverting species. Based on the prior experimental data, it was decided to adopt the wax-chiral column arrangement for GC×GC experimentation, which results in separation of the $E/Z$ isomers with interconversion, followed by chiral resolution, where applicable.

**Achiral compound:**

In the present case, only a wax-chiral column set was employed for $(1^1D_1+1^1D_2)$, with a wax column as the $2^D$ column. Figure 3.28 is the GC×GC result for the achiral heptanaldoxime compound, with the 1D insert shown in Figure 3.28 (B) being similar to the chromatographic result shown in Figure 3.23 D(ii). Given that a slightly higher oven temperature was used here, and that there is no chiral separation for the achiral compound, only a broad peak was obtained. Figure 3.28(A) shows that at 140 °C, the heptanaldoxime isomers show considerable interconversion having a broadened distribution on the $1^D$ column set. There is almost baseline resolution of $E$ and $Z$ on the $2^D$ column, with the first peak being the $E$ isomer. The $Z$ isomer elutes just after the $E$, as reported in Figure 3.28(C); their retention difference on the 2D column is about 0.4 s. This clearly demonstrates the ability of GC×GC to provide detailed chromatographic information on each compound compared with the apparent single broad ‘hump’ in Figure 3.28(B). The broadened bands for both $E$ and $Z$ are indicative of peak broadening (coalescence) of the forth kind (according to Schurig) which is a result of extensive interconversion between the isomers, but not so extensive that the peaks become rapidly interconverting on the GC time scale. Rapid interconversion would generate a peak of conventional peak width (as indicated by comparison with an internal standard). This will only happen if the rate of interconversion is extremely fast and leads to the two compounds effectively having the same chromatographic distribution constant. Whilst Schurig referred to 4 types of coalescence for enantioselective complexation GC, type 4 would appear to be generally applicable to any rapidly interconverting case.
Figure 3.28: Isothermal (140 °C) GC×GC heptanaldoxime analysis. PM = 3 s; 5.0 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in A.

Chiral compounds:

Figure 3.29 is the modulated (GC×GC) case for 2-methylbutyraldoxime at 100 °C. Figure 3.29(A) is the linear presentation of chromatographic data i.e. the non-transformed detector response. The (R) and (S) enantiomers of E isomer are located at about 3.3 s on the second dimension, whilst the Z enantiomer is well resolved from E on the second column, and is located at about 3.6 s (Figure 3.29(C)). Compared with the rather difficult to interpret 1D GC result in Figure 3.29(B), the GC×GC reveals the individual
overlapping compounds and the ‘tails’ that exist for each compound which should permit a rational model to be developed to describe the chromatographic result. Here it is easy to note the different effect on the \( E \) isomers and \( Z \) isomer. There appears to be a tail before the \( Z \) isomer, but the tail is after the \( E \) isomers. The tails appear to be of about the same magnitude in these two cases. The two isomers of \((R,S)Z\) are not well resolved, and this is reflected in the apparent single peak for \( Z \). Inset B shows the non-modulated result for the same compounds and conditions. The small preceding tail for \( Z \) (and the tail after the \( E \) isomer) is also seen in this inset. The 2D GC×GC plot indicates this preceding tail for \( Z \) to in fact due to the presence of the \( Z \) isomer. This result is interpreted as due to the \( Z \) isomer in the interconversion zone on the wax column eluting earlier than the unconverted \( Z \) isomer, on the chiral phase column.
Figure 3.29: Isothermal (100 °C) GC×GC 2-methylbutanaldehyde analysis. PM = 4 s. Chiral-wax column system. 1.5 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

Injection of 2-methylpentanaldehyde at 110 °C under GC×GC operation leads to the result shown in Figure 3.30. In contrast to 2-methylbutanaldehyde, the Z isomer elutes after the earlier E enantiomer. At this temperature, retention is relatively long. The Z isomer also appears to show partial resolution into its enantiomers (see inset, Figure 3.30(B)) although the transformed data in Figure 3.30(C) does not seem to show this so clearly for isomer Z. However, this is confirmed by the pattern of modulation of the Z peak in Figure 3.30(A) at 18.5 min, which does not exhibit the classical in-phase, 180° out-of-phase nor intermediate phase peak shape, but rather has a broader distribution that can be interpreted as emerging peak separation. The Z isomer elutes close to, but just after, the first eluting E isomer. The tailing on the peaks (earlier for Z, later for E) is still seen. There is now considerable resolution between the E enantiomers, and there is a wide baseline region between the Z isomers and later E enantiomer.

The above 2-methylpentanaldehyde compound exhibits considerable shift in relative retentions of the Z and E isomers in 2D space with change in T.
**Figure 3.30**: Isothermal (110 °C) 2-methylpentanaldehyde oxime analysis. PM = 4 s; 1.5 mL/min flow rate. (A) Modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

At 10 °C higher oven temperature, (120 °C; Figure 3.31) the Z enantiomers (now not well resolved on the chiral column) elute closer to the later eluting E enantiomer. The tails reported for Figure 3.29 and Figure 3.30 are now extended somewhat in Figure 3.31. The vertical alignment of the tails (E and Z) in the 2D plot would classically be interpreted as an interconversion region between the first E enantiomer and the Z peak as shown in Figure 3.31(B). The resolution provided by GC×GC operation clarifies the contribution of the E and Z isomers to this overlap region. The Z isomer here almost obeys 180° out-of-phase modulation (Figure 3.31(A)), which suggests practically
unresolved $R$ and $S$ enantiomers. Between the $Z$ and later eluting $E$ isomers, the response almost returns to baseline (Figure 3.31(B)).

![Figure 3.31](image)

**Figure 3.31:** Isothermal (120 °C) 2-methylpentanaldehyde oxime analysis. PM = 2 s; 1.5 mL/min flow rate. (A) modulated GC result; (B) equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

At 130 °C, (Figure 3.32) the $(R,S)Z$ enantiomers, which are still unresolved, elute very close to the later $E$ enantiomer. Now, there is no baseline resolution between the isomers on the 1D column. The tails referred to above are still seen. Overall resolution of the $E$ enantiomers has decreased significantly, as expected for higher temperature operation on a chiral column.
Figure 3.32: Isothermal (130 °C) 2-methylpentanaldehyde oxime analysis. PM = 2 s; 1.5 mL/min flow rate. (A) Modulated GC result; (B) Equivalent 1D GC analysis on the same column set; (C) 2D representation of data in (A).

3.3.2.3 Development of a model for chiral interconversion in GC×GC

Based on the observations above, a schematic model of the process of wax-chiral sequential column separation of the chiral compound is proposed in Figure 3.33. Considering the separation on the wax column on 1D₁ in Figure 3.33(A), the effect of chiral resolution on 1D₂ of each compound in turn (here taking the E and Z isomers separately) is shown in Figure 3.33 (B)(i) and Figure 3.33(B)(ii). The interconversion zone is separately illustrated in Figure 3.33 (B)(iii), and this interconversion zone comprising (R,S)Z and (R,S)E now can be considered to move apart on the chiral phase (with Z isomers not so well resolved as stated earlier). The tailing of the Z isomer to
earlier retention arises due to interconversion zone of the $Z$ isomer (Figure 3.19), and is caused by this zone eluting earlier on the chiral phase than the original $Z$ isomers. The tailing to longer retention of the $E$ isomer arises from the interconversion zone of the $E$ isomers eluting later on the chiral column. The two $E$ enantiomers of this zone will move to locate just after their respective enantiomers. Here, the $(R)$ enantiomer is shown to elute earlier than the $(S)$, but this has not yet been confirmed. It is shown for demonstrating purposes only. The overall distribution is then given by the summation of $(B)(i) - (B)(iii)$, as given in Figure 3.33(C). Figure 3.33(C) bears close resemblance to Figure 3.33(B), and so represents but one chromatographic condition of relative retentions of $E$ and $Z$ isomers with respective chiral resolution and extent of interconversion. Note that at this stage, development of a more rigorous model must await further experimentation and interpretation, and the present proposal is offered as a means to inform consideration of future, more complete description of the chromatographic results for the behaviour reported in this study.
Figure 3.33: Model of analysis of a chiral compound on a wax–chiral column set, in the case where there is interconversion of the compound. The two processes of what is believed to occur for the \( E \) and the \( Z \) enantiomers are isolated. (A) is the initial separation into \( E \) and \( Z \) forms on the wax phase; (B)(i) and (B)(ii) are the individual \((R,S)E\) and \((R,S)Z\) enantiomer separations, whilst (B)(iii) is the chiral resolution of the interconversion zone from the wax column. (C) is the net effect shown as a summation of each of the parts given in B(i)-(iii). Here, the \((R)\) enantiomer is shown to elute before the \((S)\) enantiomer, however this has not been proven at this stage.

3.3.3 Conclusion

A study of chiral oxime compounds which undergo \( E/Z \) isomerisation on polar (wax-type) columns is described. Since the wax column does not lead to enantioseparation, a chiral (cyclodextrin) column was used to provide enantiomer separation. The chiral compounds
undergo classical isomerisation on the wax column as has been seen in previous achiral studies, however the chiral column does not appear to provide isomerisation. The relative retentions and hence isomer separation of $E$ and $Z$ can swap on the two phases depending on the compound and conditions employed. In order to examine the effect of chiral resolution, and provide some measure of isomerisation, a dual column arrangement with either a wax-chiral or chiral-wax arrangement was implemented. In one method, an FID was located between the two columns to assist in understanding of the separate effect of the two columns. The resulting distribution exhibited a complex overlap of enantiomer separation and interconversion. The use of comprehensive 2D GC with a wax-chiral arrangement chosen, assisted in resolving the different effects that these compounds undergo.

A model is proposed to aid interpretation of the results of the process observed. At this stage, kinetic data for interconversion have not been derived for this system, however this should be possible. Since interconversion does not appear to arise on the chiral column, only kinetic data on the wax column will be obtainable. The use of a single column that simultaneously provides both chiral and $E/Z$ resolution, along with $E/Z$ interconversion, would be an interesting system to investigate.
3.3.4 Experimental

3.3.4.1 Reagents and chemicals

The aldehydes 2-methylbutyraldehyde and 2-methylpentanaldehyde used to synthesise the oximes were purchased from Aldrich, and used without further distillation. Absolute ethanol (Ajax), hexane (Ajax), hydroxylammonium chloride (BDH) and sodium hydroxide (BDH), all of analytical reagent grade, were also used in the synthesis of oximes.

3.3.4.2 Synthesis

2-methylbutyraldehyde oxime, and 2-methylpentanaldehyde oxime, were synthesised from their aldehydes, 2-methylbutyraldehyde, and 2-methylpentanaldehyde, respectively, following a modified procedure from Boucher et al. in which an 80 mmol solution of hydroxylammonium chloride in 20 mL of ethanol was stirred at room temperature. To this solution was added 20 mL of an 80 mmol aqueous solution in sodium hydroxide, followed by a 40 mmol solution of aldehyde dissolved in ethanol. The reaction mixture was stirred at room temperature for 4 hrs. After 4 hrs the solution was filtered to remove the sodium chloride precipitate, and ethanol was removed by rotary evaporation at 50 °C under reduced pressure. The oxime was extracted with either hexane or petroleum ether, dried with anhydrous MgSO₄ and the solution filtered. Solvent was removed by rotary evaporation under reduced pressure at 50 °C to afford the crude product. The crude product was purified by bulb-to-bulb distillation in a kugelrohr and characterised by NMR and GC-MS.

\[
\begin{align*}
\text{HO} & \text{N} \\
\text{Z} & \text{E}
\end{align*}
\]

2-methylbutyraldehyde oxime 95
$^1$H-NMR (300MHz CDCl$_3$): $\delta = 8.97_{(Z)}$ (s, 1H, -OH), 8.76$_{(E)}$ (s, 1H, -OH), 7.23$_{(E 60\%)}$ (d, $^3J_{H,H}=$7.0 Hz, 1H, -NCH), 6.44$_{(Z 40\%)}$ (d, $^3J_{H,H}=$7.7 Hz, 1H, -NCH), 3.10-2.98 (Z) (m, 1H, -NCH), 2.34-2.10 (E) (m, 1H, -NCH). $^{13}$C-NMR (75MHz CDCl$_3$): $\delta =$157.3$_{(Z)}$ (CH), 156.4$_{(E)}$ (CH), 36.0$_{(E)}$ (CH), 31.1$_{(Z)}$ (CH), 27.5$_{(E+Z, CH_2)}$, 17.5$_{(E, CH_3)}$, 17.1$_{(Z, CH_3)}$, 11.6$_{(Z, CH_3)}$, 11.4$_{(E, CH_3)}$.

2-methylpentanealdehyde oxime 96

$^1$H-NMR (300MHz CDCl$_3$): $\delta = 9.41$ (s, 1H, -OH), 9.19 (s, 1H, -OH), 7.04 (d, $^3J_{H,H}=$ 7.2 Hz, 1H, -NCH), 6.26 (d, $^3J_{H,H}=$ 7.7 Hz, 1H, -NCH), 2.90 (m, 1H, -NCH), 1.17-1.02 (m, 4H, -CH$_2$CH$_2^-$), 0.83-0.78 (dd, 3H, -CHCH$_3$), 0.67-0.56 (m, -CH$_2$CH$_3$); $^{13}$C-NMR (75MHz CDCl$_3$): $\delta = 157.3_{(Z)}$, 156.5$_{(E)}$, 36.8$_{(E+Z, CH_2)}$, 34.1$_{(E)}$, 29.3$_{(Z)}$, 20.4$_{(Z, CH_2)}$, 20.2$_{(E, CH_2)}$, 18.0$_{(E, CH_3)}$, 17.4$_{(Z, CH_3)}$, 14.1$_{(Z, CH_3)}$, 14.0$_{(E, CH_3)}$.

3.3.4.3 Sample preparation:

Following synthesis samples of 2-methylbutyraldehyde oxime and 2-methylpentanaldehyde oxime were prepared for GC analysis in dichloromethane (B&J ACS; HPLC grade) with N,N-dimethylformamide (Ajax; AR grade) used as an internal standard, heptanaloxime was prepared in absolute ethanol (Scharlau; HPLC grade) and 1-octanol (Ajax; LR grade) as an internal standards.

3.3.4.4 Instrumentation:

NMR
All oximes were prepared in CDCl$_3$-d solvent (Merck) and characterised by NMR on either a Brucker Avance 300 or Bruker 500 instrument. 2-methylbutyraldehyde oxime and 2-methylpentanal oxime were characterised using the Brucker Avance 300 NMR.

**GC-MS**

A quadrupole mass spectrometry system (GC-qMS) was used for characterisation of the synthesised oximes. The system consisted of an Agilent model 6890 GC fitted with an Agilent model 7683 autoinjector, coupled to a model 5973 MS detector with fast electronics upgrade (Agilent Technologies, Burwood, Australia). A BPX5 column (SGE International, Ringwood Australia) of dimensions 30 m × 0.25 mm i.d × 0.25 µm film thickness (d$_f$) was used. Data acquisition and processing was afforded by Agilent ChemStation software.

**GC-FID**

Once the oximes were characterised, preliminary single dimension GC experiments were conducted on an Agilent model 5890 GC system, subsequently, a Shimadzu model GC-17A with autosampler model AOC-17 (Shimadzu, Rydalmere, Australia) was used for single dimension GC experiments. Both systems were fitted with a flame ionisation detector (FID).

Single dimension GC experiments were conducted to find the most suitable wax and chiral columns to employ for our experiments. The column found to be the most suitable for chiral analysis was a MEGA column (diethyl-t-butyl-β-cyclodextrin phase) capillary column, of dimensions 23 m × 0.25 mm ID × 0.25 µm d$_f$.

For interconversion experiments, a polyethylene glycol (wax) phase was found to be most suitable, and so a SolGel Wax phase (SGE International; 30 m × 0.25 mm ID × 0.25 d$_f$) column was used. In order to combine the chiral separation properties and the molecular interconversion properties of the respective columns, the two columns were coupled together to form one long column (dual column).
For coupled column analysis (dual ¹D columns), the above MEGA and SolGel wax columns were joined in either chiral-wax or wax-chiral order. To investigate the contribution of each column on the overall chromatogram a FID was positioned at the interface of the coupled columns. Whilst other chiral columns were tested (Rt-BDex cst-TM from Restek Corp, Bellefonte, PA) they were not successful in resolving both of the enantiomers of the $E$ and $Z$ isomers, of the chiral compounds of interest.

A range of GC conditions were employed, according to the needs of the analysis, to either limit or promote interconversion. This was accomplished by a combination of temperature and pressure (flow rate) settings. Generally isothermal conditions were used, but on occasions temperature programmed analyses were conducted. Hydrogen carrier gas was used throughout. The injector and detector temperatures were both 230 °C. Shimadzu Class GC-10 software was used for data acquisition.

**GC×GC-FID**

The gas chromatograph, equipped with a flame ionisation detector (GC×GC-FID), used in the study was an Agilent 6890 system with a Longitudinal Modulation Cryogenic System (LMCS, Chromatographic Concepts Pty Ltd, Doncaster, Australia). The dual ¹D (wax-chiral) column arrangement was used for GC×GC studies. Since the ²D column should be of wax type to ensure adequate ²D separation, either a Stabilwax (1.1 m × 0.1 mm i.d × 0.1 µm df, Restek) or a BP20 (3.1 m × 0.1 mm i.d × 0.1 µm df; SGE) column was used, the latter providing better resolution.

The injector and detector temperatures were 230 °C and 250 °C, respectively. A sampling frequency of 100 Hz was used for GC×GC analysis. A high sampling frequency of 100 Hz was used for GC×GC analysis. A high sampling frequency is required to monitor very fast GC peaks at the end of the 2D column. Hydrogen was used as a carrier gas at various flow rates as indicated on the figures, and the temperature was usually isothermal, at various temperatures as indicated on figure captions. A range of modulation periods between 2-5 s were used, according to the retention time on the ²D column and the duration of the overall peak distribution on the ¹D column. The
The temperature of the modulator system ($T_m$) was held at 0 °C. CO$_2$ was used as a coolant in the LMCS and nitrogen as a flush gas at a pressure of 15 psi. The modulator in GC×GC operation was commenced at a time usually 2 min prior to the elution of the first peak of interest. Agilent ChemStation software was used for modulation control, data acquisition and processing.

3.3.4.5 Description of instrument arrangements and conditions:

For analyses which employed a dual column set-up, a FID was positioned at the interface and/or at the end of the dual column arrangement. Figure 3.34 is a schematic diagram describing the different geometries of the column arrangements developed in this study. Collectively, the system can be operated as:

(i) a single GC column ($^1D_1$), with a single FID (FID1);
(ii) a dual GC column ($^1D_1 + ^1D_2$), with a single FID (FID2);
(iii) a dual GC column ($^1D_1 + ^1D_2$), with a dual FID (FID1 + FID2);  
(iv) a dual GC column as first dimension ($^1D_1 + ^1D_2$), with a single column second dimension ($^2D$) and a single FID (FID2), for GC×GC operation.
**Figure 3.34:** Dual column arrangement. (System A) Single dimension coupled column with an FID detector (FID1) between column $^{1}D_{1}$ and $^{1}D_{2}$, and FID detection (FID2) after $^{1}D_{2}$. The two columns can be either a wax–chiral set, or a chiral–wax set. The single column arrangement is trivial. (System B) Two-dimensional coupled column with a single FID detector (FID2) after the 2D column, as shown by the dotted line. Only a wax–chiral column arrangement for $^{1}D_{1} + ^{1}D_{2}$ was employed in this case, with a wax 2D column. A modulator M is located between the 1D columns and the 2D column.

Figure 3.34 (System A) describes a one-dimensional system even when comprising two columns, since the columns are essentially directly connected and there is no heart-cutting or other process at their interface. Figure 3.34 (System B) describes a comprehensive 2D system, since the modulator performs the classical function of sub-sampling the first dimension effluent into the $^{2}D$ column for rapid separation.

For 1D analysis, a variety of single chiral columns, and two different wax-type columns were used. For dual column operation, the wax and chiral columns were swapped around as required with either wax-chiral or chiral-wax arrangement. For GC×GC operation, it was decided to choose the chiral-wax column arrangement for ($^{1}D_{1} + ^{1}D_{2}$), with both short (1.1 m) Stabilwax and longer (3.1 m) SolGel wax columns as $^{2}D$, each of 0.1 mm ID and 0.1 $\mu$m df. The longer column provided improved separation in the second dimension. Table 3.10 presents an overview of different experiments that were conducted for the different columns and system arrangements. The most important variables are temperature setting and retention times, and these can generally be found on the Figure legends and from the chromatographic traces. Thus either pressure or linear carrier flow rates are only of value as a means to change overall retention.

**3.3.4.6 Data conversion**

Data were converted to .csv format, and exported in ascii format for processing using Origin (Microcal Software, Northampton, MA, USA). Contour plots were displayed using Transform software (Fortner Research, VA, USA).
Table 3.10: Details of experimental arrangements investigated, and conditions employed.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D GC; 5890 GC; BP5 and wax columns</td>
<td>Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime, and phenylacetaldoxime</td>
</tr>
<tr>
<td>1D GC; GC-17 GC; single column; chiral columns and wax columns</td>
<td>Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime, and phenylacetaldoxime</td>
</tr>
<tr>
<td>1D GC; GC-17 GC; dual columns; wax-chiral &amp; chiral-wax columns</td>
<td>Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime</td>
</tr>
<tr>
<td>1D GC; dual FID GC-17 GC; dual columns; wax-chiral &amp; chiral-wax columns</td>
<td>Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime</td>
</tr>
<tr>
<td>GC × GC; 6890 GC; dual ¹D columns (wax-chiral) and ²D wax column;</td>
<td>Heptanaldoxime, 2-methylbutyraldehyde oxime, 2-methylpentanaldehyde oxime</td>
</tr>
</tbody>
</table>

Conditions: Generally conditions were varied over a range of temperatures from 70 °C to 150 °C, and pressure settings were generally varied over the range from 10 psi to 50 psi. Resulting flow rates depended upon pressure, column lengths and i.d., and the temperature. In general, neither the linear carrier flow rate nor pressure setting are critical in determining the extent of reaction, but rather the temperature setting and the time on the column (as given by the retention time of the species) are the critical parameters. Hence for ease of presentation, the pressure settings are generally provided.
3.4 References


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