The synthesis of single enantiomer thiolactomycin analogues utilising deracemisation by crystallisation

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3rd July 2024

Declaration

This dissertation is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Strathclyde.

I declare that this dissertation embodies the results of my work and that it has been composed by myself. Nevertheless, following standard academic conventions, I have acknowledged the work of others.

Asma Nashawi Jeddah, 3rd July 20234

Publication Resulting from This Research

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Abstract

Single enantiomers play a crucial role in the pharmaceutical industry. A preferred enantiomer can be obtained through enzymatic kinetic resolution, preferential crystallisation, diastereomeric crystallisation (also called classical resolution), or chromatographic resolution. The use of safe single enantiomers instead of potentially problematic racemic enantiomers has dramatically increased in drug development and design. The objective of this study was to develop a method for converting racemic mixtures into active enantiomers following syntheses of racemates with a thiolactone scaffold. Our deracemisation by crystallisation approach would then provide a basis for the future development of chiral thiolactomycin analogues (TLM) as potential antibiotics.

In the first part of the project, we explored the use of existing crystal structures in an existing database towards the understanding and facilitation in obtaining the conglomerate crystal form necessary for our intended approach. Structural data from compounds with a thiolactone, or related structure, were sourced and processed with Mercury software. The corresponding Hirshfeld surface analysis of these structures disappointingly did not lead to the key structural insights required to guide the subsequent synthesis of racemic TLM analogues.

A synthetic campaign to generate a large library of racemic analogues led to only to the successful generation of three molecules. 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one, 3hydroxy-4-methyl-2*H*-thiophen-5-one, and 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2one. The identification of the crystal form was performed using single-crystal X-ray diffraction, X-ray powder diffraction, and differential scanning calorimetry. These data confirmed the presence of racemic crystal-forming systems and not the conglomerate form required for the subsequent crystallisation and deracemisation experiments.

The final section of the project was concerned with developing a proof-of-concept ibuprofen model for implementation in subsequent crystallisation by racemisation experiments in cases where racemates possessed a conglomerate crystal lattice. Conversation of racemic and enantiopure forms of ibuprofen were converted to their diastereoisomers and studied by NMR spectroscopy and polarimetry analytical methods. The data obtained indicated that this method was valid for the important determination of the % enantiomeric excess, required as part of the overall process to resolve racemates by decracemisation by crystallisation.

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I dedicate this work to my wonderful father, dear mother, twin sister, brothers, and friends, who supported me throughout this project.

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Chapter 1.

Introduction

1.1 The importance of chemistry in biology and medicines

Chemistry is encountered in everyday life. It has become an essential part of modern existence, as in the chemistry of food, biological molecules, and new drugs and technologies. Interest in drug chirality has become an essential theme in drug development, as nearly half of all commonly used drugs are chiral in nature. (1,2) Owing to the increased interest in the chirality of biological properties in general and the chirality of drugs in particular, the preparation of pure stereoisomers has become a subject of great importance. Chirality is the central theme driving the development of the novel methods presented in this thesis. In this chapter, we will introduce small molecules as useful lead-in to medicines that have been subject to clinical trials. We ask how the chirality of a certain molecule should be measured. In addition, the chirality and various available methods of chiral separation will be discussed. We will also consider several ways of creating single isomers and highlight crystallisation techniques.

1.2 Illustrative Examples of Chiral Drugs and the Significance of Chirality in Biological Systems

Chirality is a property found throughout biological systems in the layout of the human body. Small chiral molecules such as amino acids and sugars are building blocks of larger molecules such as proteins and nucleic acids, which are also chiral. In medicines, chiral drugs such as ibuprofen, omeprazole, and others that each enantiomer has its own pharmacological profile. The unequal biological activity of enantiomers highlights the need for the production and isolation of single-enantiomer drugs. (S)-Ibuprofen, for instance, is significantly more effective as a pain reliever than its (R)-enantiomer, which is largely inactive. Other examples include (S)-albuterol (for asthma) and (S)-omeprazole (for gastroesophageal reflux), which demonstrate superior therapeutic activity and reduced side effects compared to racemic mixtures. This widespread importance of chirality extends far beyond pharmaceuticals, as it's observed in numerous biological systems. (3)

Chirality is most likely to be observed in the DNA structure. Generally, the DNA molecule is made up of four chemical bases: adenine, guanine, cytosine, and thymine. Each base attaches to sugar and phosphate molecules to form a nucleotide. The nucleotide exists in nature as an *R* isomer or a right-handed isomer nucleotide. The *S* configuration or lefthanded isomer is prepared only in the laboratory. In the picture, nucleotides are arranged like two strands where the right-hand nucleotide attaches to another nucleotide to form the DNA structure. Each component of a nucleotide contains chirality, causing a twist in the chain, thus giving the spiral double helix of the DNA structure. On the other hand, if the wrong chirality of a nucleotide attaches to the right chirality of a nucleotide, the helical structure does not form. (4)

1.3 Chirality

There is no doubt that the study of stereochemistry, which deals with the three-dimensional (3D) structures of compounds, is obviously difficult. The first stereochemist, Louis Pasteur (1848), discovered chirality when he attempted to separate the two isomers of sodium ammonium tartrate. His work can be summarised as the separation of twin molecules into two optically active components, each having identical properties to the original compound, except that one component rotates clockwise around the plane of polarised light, which is known as dextrorotation. In contrast, the other rotates counterclockwise around the plane of polarisation, which is referred to as levorotation. Later, in 1874, van't Hoff expanded and clarified the concept of chiral molecules. His work reached the same conclusion as that of Joseph Le Bel, with both chemists determining that four different groups on a tetrahedral carbon atom have two configurations (or two isomers) (5). Isomers are of two main classes: constitutional isomers and stereoisomers. Both exhibit connectivity between bonds; the only difference is in the arrangement of atoms in the molecules. The second class, stereoisomers, is made up of diastereoisomers and enantiomers. Diastereoisomers are isomers that do not form mirror images of each other, whereas enantiomers form mirror images. (6)

Chirality is a prominent feature of most biological systems. Many biological molecules such as DNA, amino acids, and sugar are chiral molecules. The word *chiral* is derived from the

Greek word *hand*; the term for the opposite is *achiral*. The models in Figure 1 illustrate the two terms.

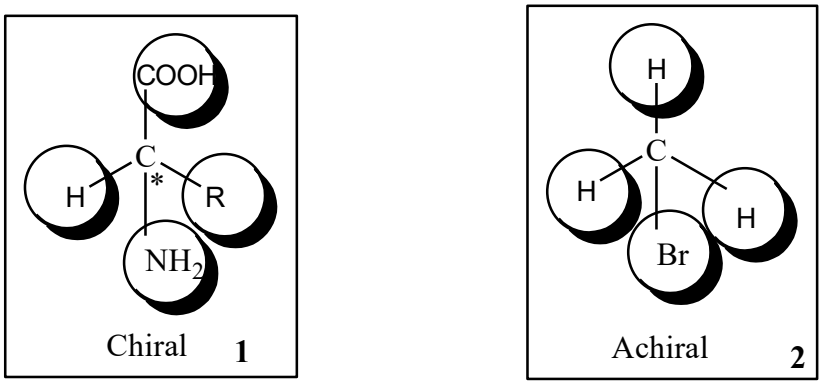


Figure 1: The structure on the left, in which the carbon atom carries four different substituents, is considered chiral opposite to the right achiral one.

This carbon atom can also be described as an asymmetric carbon with a stereogenic centre. Sometimes, this centre is referred to as a centre of chirality and is marked by an asterisk. Chirality is also known as stereoisomerism, enantiomerism, or dissymmetry. The most interesting property of chiral objects is that they are chemically, physically, and energetically the same, except in their interactions with polarised light. This unique property of chiral molecules is called optical activity, which refers to the ability of chiral molecules to rotate either clockwise or counterclockwise around light. The number of stereoisomers that can exist in a compound is determined by 2n, where n is the number of chiral centres. In terms of the nomenclature of chiral molecules, the Cahn-Ingold-Prelog (CIP) rules can be applied to the configuration of the chiral centre, and they can be described according to the priority of each substituent. Therefore, when moving from a higher-priority atom to a lower-priority atom in a clockwise direction, the stereogenic centre is labelled with an R and, vice versa, with an S. The R and S symbols only indicate the arrangement of the atoms around a stereogenic centre and not the direction of the compound in relation to the polarised light. (6,7)

Chiral molecules are frequently encountered in nature; their chirality gives rise to stereoisomers, which may be either enantiomers or diastereomers. Clayden *et al*. (7) provided

the following example of an amino acid, wherein a single asymmetric centre exists as two stereoisomers (Figure 2).

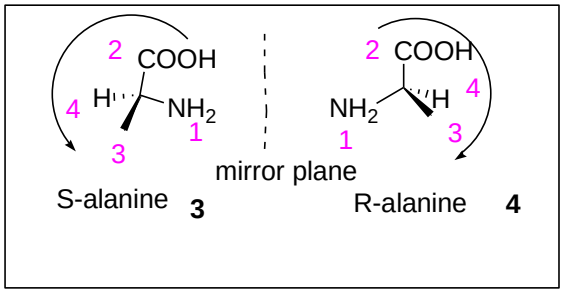


Figure 2: S-alanine **3** and R-alanine **4** are examples of amino acids.

Although R-alanine **4** is found in bacteria, while S-alanine **3** is extracted from plants, the two structures have identical infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectra, and physical properties, with one exception: the S-alanine rotation plane polarises light to the right, while the R-alanine rotates light to the left. Therefore, their structures can be viewed as reflecting the right and left hands, respectively. The two enantiomers, or optical isomers, have different spatial arrangements; each arrangement is completely asymmetrical and cannot be superimposed on the other, so they are considered chiral. (7)

Racemic mixtures composed equally of two enantiomers are called true racemates; a racemic compound is one with a crystallographic unit cell containing two enantiomers.

Drugs can be classified as racemate, enantiomer, or sometimes as achiral. Chemists typically identify diastereoisomers first and then split them into enantiomers. As the chemical and physical properties of diastereoisomers vary, this process allows chemists to separate them through crystallisation or chromatographic methods. (7)

According to Nguyen et al. (1), racemic drugs can be divided into three categories based on their type of activity. The first category is comprised of cases in which one enantiomeric drug exhibits greater therapeutic activity than another. Thus, the second drug is regarded as less potent. For example, the *S* enantiomer of β-blocker drugs is more potent than the *R* enantiomer. (8) In the second category, the two enantiomers of a drug exhibit equal activity, thereby producing a synergistic pharmacological effect when taken by a patient. The third category is comprised of cases in which one enantiomer of a drug is responsible for the drug's

activity, while another could be inactive or might even produce an undesirable pharmacological activity (perhaps due to toxicity) or a completely separate activity. (1) Therefore, the use of the safer single enantiomer, rather than the potentially problematic racemic one, has dramatically increased in the fields of drug development and design.

Although the use of the racemic forms of drugs was more common in pharmacopeias in the past, single enantiomer forms of drugs already accounted for 50% of the drugs approved for the first time in 1998. (1) That year is often considered the ‘golden era’ for the pharmaceutical industry, particularly in terms of the designs of single isomer drugs. It is not surprising that many synthetic chemists find the challenge of preparing single enantiomers of chiral molecules both interesting and rewarding. The development of single-enantiomer forms appears to reduce the total drug doses required, while intersubject variability is reduced both by remaining within the safety range of drug dosages and by being more accurate in relation to evaluating dose-response relationships. (9) Moreover, prescribing a homochiral drug means that only one enantiomer of a drug is present, thereby reducing the possibility of unwanted drug interactions and eliminating the need for the patient to metabolise the less potent isomer. (6)

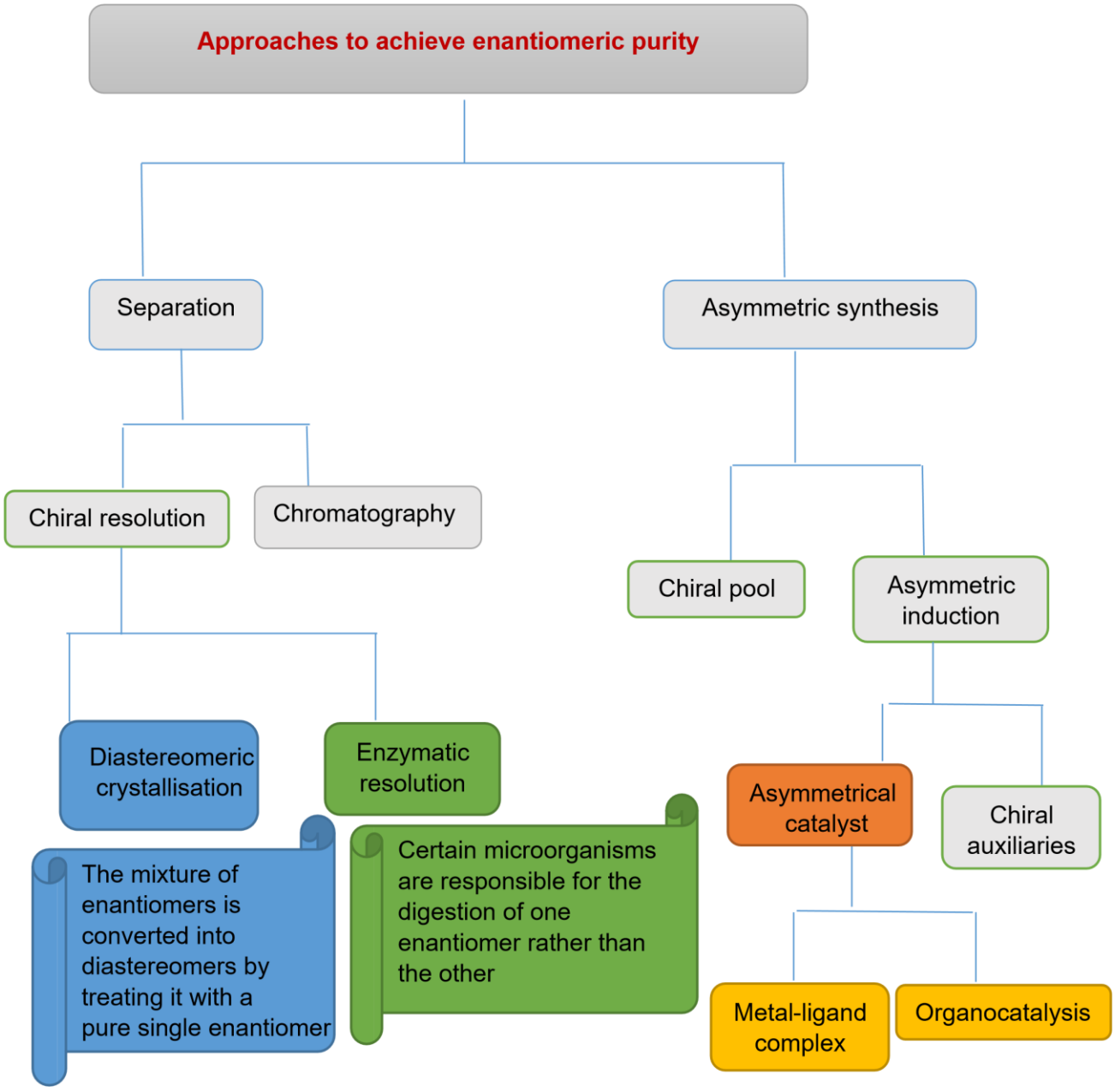
Although some racemic drugs have been redeveloped by a chiral switch into safer single enantiomer forms, many chiral drugs are still approved as racemates. Owing to the difficulty of separating enantiomers and the high cost of techniques for chiral separation, racemic drugs still exist. In addition, the enantiomers of some drugs have shown the same toxicological and pharmacological effects. For these reasons, these drugs have been marketed in racemic form. Moreover, in some cases, a racemate exhibits far greater therapeutic activity than a single enantiomer. Consequently, after a long clinical assessment, a serious decision must be made between a racemate and a single enantiomer and as to whether a single enantiomer is ideal. This evaluation must also consider the activity of the enantiomer in the biological system. (1)

The various potential responses of biological systems to drugs with two enantiomers lead to differences in the pharmacokinetics, pharmacology, and toxicological effects of chiral drugs. Enantiomers exhibit different pharmacodynamics, and the pharmacokinetic properties of the enantiomers present in racemates have been recognised, although the differences between

pairs of enantiomers observed in their pharmacokinetic parameters tend to be relatively modest compared with their pharmacodynamic properties, even though relatively limited pharmacokinetic data are available for the enantiomers of commonly used racemic drugs. Therefore, understanding the biodiscrimination of enantiomers for all chiral drugs during each pharmacokinetic activity could help predict the variation in pharmacological responses between patients. (1) Generally, all biological polymers are considered homochiral or enantiopure. For example, amino acids, comprised of proteins, exist as *S* enantiomers, except for cysteine, while the sugar in DNA and RNA is a D enantiomer. We could say that the *R* enantiomer is right-handed. Thus, it is true that only one enantiomer, which may have either an *R* or *S* structure, is used in chiral biological molecules. (10) Prevalence of chirality in nature and its profound impact on biological function underscore the importance of developing effective methods for obtaining and utilizing enantiopure compounds.

1.4 Methods of Obtaining Enantiopure Molecules: Synthetic and Post-Synthetic Approaches

Understanding chiral discrimination has important implications for the separation of enantiomers. The chemical similarity between them causes difficulties in separation. Thus, a highly efficient means of separation is important. Increasing awareness of the importance of chirality in biological activity has stimulated an increasing demand for efficient methods of industrially synthesising homochiral compounds. Scheme 1 shows strategies for achieving a pure chiral molecule by presenting different methods of separating stereoisomers. (9-11)



Scheme 1: Strategies for obtaining enantiopure molecules. (9,10,11)

In general, a pure enantiomer can be obtained in two ways. One approach is synthetic and involves using asymmetric synthesis, beginning with chiral-pure materials, to avoid a racemic mixture at the end of the synthesis. The other method is post-synthetic and is performed by synthesising a racemic mixture and then separating it into individual isomers using chromatography. (12)

- Synthetic Approaches (Asymmetric Synthesis): These methods aim to produce the desired enantiomer directly through the use of chiral auxiliaries, chiral pools, or asymmetric catalysts, thereby avoiding the need for separation of a racemic mixture. The advantages include high enantiomeric excess (ee) and potential for 100% yield, but they often require extensive optimization and might be limited by substrate scope and catalyst availability.
- Post-Synthetic Approaches (Resolution Techniques): These approaches involve the separation of enantiomers from a racemic mixture. Common techniques include chiral chromatography (such as HPLC), preferential crystallization, diastereomeric salt formation, and enzymatic resolution. While these methods are widely used, they often have a maximum theoretical yield of only 50%, resulting in significant loss of starting material. Furthermore, the cost of chiral resolving agents and specialized equipment can be substantial, particularly for large-scale production.

The method commonly used to separate the two isomers of a racemic compound is known as chiral separation or resolution. The first chiral resolution was achieved in the laboratory by Louis Pasteur in 1848. Pasteur was the first person to separate a racemic mixture of sodium ammonium tartrate salt into two enantiomers through spontaneous resolution at temperatures below 28°C. In rare occurrences, one enantiomer of a racemic mixture forms crystals before the other, forcing other enantiomers to form in a solution. (2,11)

The advancement in chiral technology and the ability to produce enantiomerically pure compounds at the industrial level have created new demands for pharmaceutical firms and regulatory agencies. In the pharmaceutical industry, two techniques are applied to racemic drugs to separate stereoisomers: classical resolution and chromatography. Initially, the classical resolution method, which involves the formation and separation of the diastereomer, was the most widely used method. The mixture of enantiomers is converted into diastereomers by treating them with a pure single enantiomer as a resolving agent. This reaction leads to the formation of two diastereomeric salts, followed by the separation of the diastereomers, either by diastereomer crystallisation or chromatography, to obtain a pure enantiomer. (12) Enzymatic resolution or bio-catalytic resolution, also known as kinetic

resolution, is another classic method of separation that is achievable in the presence of certain microorganisms such as bacteria or moulds that digest one enantiomer rather than the other; they react with one enantiomer and convert it into a different product while not reacting with the remaining enantiomer.

Enzymes specific to particular enantiomers may be used for the separation of enantiomers and obtain only one stereoisomer. (13) Thus, resolution procedures for racemic mixtures provide only a 50% maximum theoretical yield for both enantiomers. (2)

Regardless, this resolution method has low yields due to the loss of at least 50% of the undesired isomer when starting from racemic mixtures. However, the yield of resolution methods can be improved by repeated racemisation and resolution or, more elegantly, by dynamic resolution or *in situ* inversion of the configuration of the unwanted enantiomer. (14)

One technique for chiral resolution is crystallisation. Racemates can be resolved into their enantiomers via three methods: resolution through entrainment (preferential crystallisation, also known as dynamic resolution), kinetic resolution, and classical resolution through crystallisation of diastereomeric salts.

Preferential crystallization: This technique relies on the formation of conglomerates, where the enantiomers crystallize as separate solid forms. This method is relatively straightforward and can be performed using readily available equipment but may be less efficient than using chiral resolving agents.

The second key method of separation involves the use of a chromatographic technique (chiral chromatography). This technique utilizes a chiral stationary phase to separate enantiomers based on their different interactions with the chiral environment. While efficient, this approach requires specialized equipment and expertise.

As enantiomers of the same compound differ in their interactions with other chiral molecules such as the chiral stationary phase in chromatographic separation, this method can be described as one in which a mixture of enantiomers is dissolved in a solvent. This solution is passed through a column. It relies on one enantiomer or homochiral, which interacts less strongly with the chiral stationary phase than the other enantiomer. Thus, the less adsorbed

enantiomer on the chiral chromatography column will be eluted prior to the more strongly adsorbed one. (12) The size of the column, temperature, and composition of the mobile phase all play important roles in the separation of enantiomers.

A wide variety of chromatographic methods use this principle for enantiomeric separation, for example chiral high-performance liquid chromatography (HPLC). HPLC is a very useful and more appropriate available method and is suitable for the large-scale separation of optical isomers. It is divided into two subcategories: direct and indirect. The indirect method is similar to the classic reversed-phase column used to separate the two diastereomers. This highly sensitive technology is more frequently used in biological analysis than in the pharmaceutical industry. (1) Direct HPLC has two types of selectors: the enantiomers are passed through a column containing either a chiral stationary phase or, more commonly, a chiral mobile phase additive. (15)

Similar compounds often require different columns and HPLC separation methods for optimal chromatographic conditions, including optimisation of the mobile phase composition, concentration of enantiomers, and a controlled column temperature. This method is successful owing to its flexibility, simplicity, and economic advantages compared with other methods. (12,15)

A chiral mixture (RS) of the selected molecule is separated by three points of interaction in the chiral stationary phase, to illustrate this point using figure 3 as the key point for structure scaffold choosing TLM example to be the main molecule in this project.

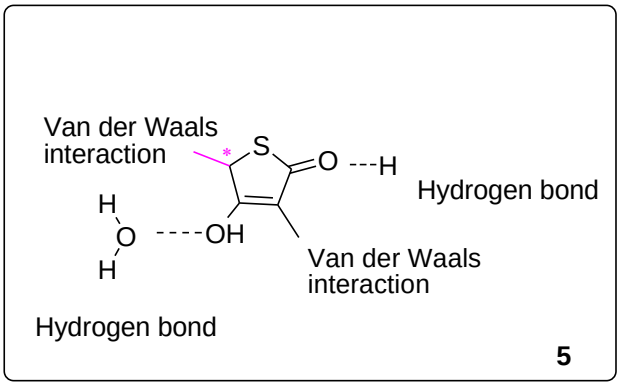
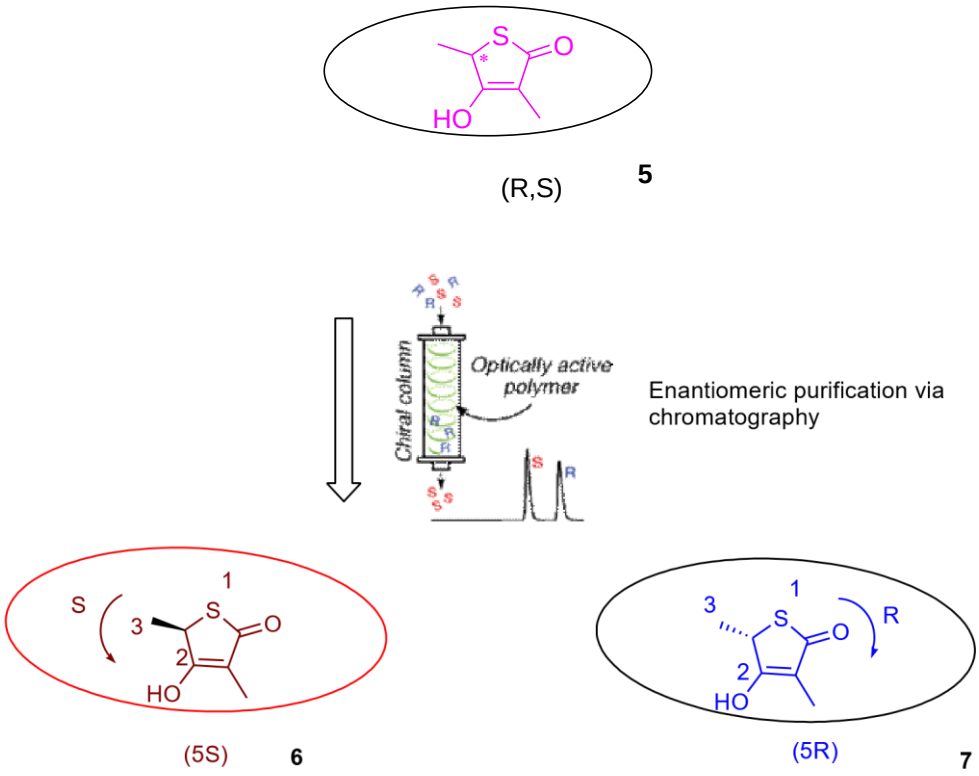


Figure 3. Potential functional group interactions of the TLM scaffold to separate enantiomers by HPLC a molecule of chose on 1.9 subtitle.

Scheme 2 shows an example of enantiomeric purification via chromatography of the TLM analogue using HPLC equipped with a chiral column and an ultraviolet detector. The wavelengths of the detector and the environment of the column oven were set at 254 nm and 40°C, respectively, during the quantification. The concentration of the R- and S isomers must be monitored periodically, and the enantiomeric excess (ee) of the substrate must be calculated.



Scheme 2. Enantiomeric purification of the TLM analogue by chromatography using HPLC.

The ee is the difference between the relative abundance of two enantiomers, that is, the state of enantiomer mixtures where one enantiomer is more abundant than another. The compositions of these mixtures are described by the ee. To obtain pure enantiomers, one must calculate the percentage of ee and prove that racemisation has occurred. The purity percentage of an enantiomer is calculated on the basis of the chromatographic data using Equations 1 and 2, where R and S are the concentrations of the right- and left-hand molecules, respectively. (16)

Enantiomeric excess % = Optical purity X 100

$$= \frac{\text{Observed rotation of mixture}}{\text{Specific rotation of pure enantiomer}} \times 100 \quad (1)$$

$$\textbf{\% ee} = \frac{R-S}{R+S} \times 100 \quad (2)$$

Moving from chiral separation, which produces a single isomer, towards the synthesis of an enantiopure compound (which means that only one enantiomer is present) is important. The demand for a more economical way of producing a single enantiomer has increased in recent years. One such method of synthesis involves a chiral pool, which refers to enantiomerically pure compounds available from natural sources. This strategy offers a more economical means of producing an enantiomerically pure product in the pharmaceutical industry by using a cheap and readily available resolving agent. (7)

The most cost-effective and powerful method of producing a single enantiomer is asymmetrical synthesis. This process is also referred to as enantioselective synthesis. This is based on the idea that if the energy level of the transition state can be lowered, the chance for one enantiomer will be greater than for the other. This is because the transition state of the two enantiomers produces an equal energy level, which is different from diastereomers, where the transition state produces unequal energy. This can be achieved using a molecule as a reagent or catalyst if it is covalently attached to the starting material. (7) Asymmetrical synthesis is divided into two distinct approaches using chiral auxiliaries and asymmetric catalysts. (15,17) Chiral auxiliaries are optically pure compounds that are attached to a substrate and control the stereochemical outcome of the synthesis or reaction. A chiral auxiliary molecule, which contains a stereogenic centre and is enantiomerically pure, comes from a natural product such as an amino acid and is obtained as only one enantiomer of the product. It attaches itself to the starting material during the reaction and can then be easily removed by hydrolysis at the end of the reaction. Therefore, it promotes diastereoselectivity and enhances the enantiomeric excess of the product. (7)

Asymmetry can also be induced by integrating an external chiral ligand into a chiral catalyst. This technology often provides excellent ee. Employing an asymmetrical catalyst has several advantages over using stoichiometric auxiliaries. The term *asymmetrical catalysis* refers to using a small amount of enantiomerically pure catalysts to promote a reaction and form many enantiomerically pure products. This approach has been used by Agustian et al. (8) to synthesise one enantiomer to a much greater extent than that achieved using other methods. (6)

The two types of catalysts are metal-ligand complex catalysts and organocatalysts. The former is based on combining an enantiomeric ligand with a transition metal, which allows for complex formation. The complex then binds to the substrate and transfers its chirality to the product. The second type of catalyst, organocatalysts, uses a sub-stoichiometric amount of an organic molecule to accelerate a chemical reaction. Organocatalysts do not utilise intermediate transition metals. Both methods avoid racemisation during chiral drug preparation. (8)

Recently, the use of asymmetrical synthesis, whether through chiral auxiliaries or chiral catalysts, has dramatically increased. This new method has many applications in modern drug design, as it ensures that only one enantiomer is produced. In theory, the yield of asymmetrical synthesis is 100% because an unwanted isomer is not produced. (2)

Enantiopure products are complicated to develop without adding enantio-enriched materials, despite the availability of several approaches to produce optically pure or enantiomerically pure organic compounds. (2,18) For many decades, chemists have relied on separate asymmetrical synthesis and resolution methods to furnish highly enantiopure molecules; these methods are often complex and expensive and can have significant cost implications in manufacturing and production.

1.5 Deracemization: A New Frontier (A More Efficient Approach to Enantiomer Production)

Owing to the limitations of resolution methods for producing enantiomerically pure compounds, any procedure that can directly convert a racemate to a single enantiomer is highly advantageous. A recently developed resolution procedure called deracemisation has resulted in yields greater than 50%. Deracemization techniques have garnered increasing attention due to their potential for overcoming the challenges associated with traditional resolution methods. These techniques directly convert a racemic mixture into a single enantiomer, offering advantages in terms of efficiency and environmental friendliness. Deracemisation is the opposite of racemisation. It involves the transformation of two enantiomers into a single enantiomer (2). Thus, deracemisation can be defined as converting a racemic mixture into a 100% theoretical yield of a non-racemic product or one enantiomer without intermediate separation of materials. This process reduces costs and waste in relation to chiral compounds and is an innovative method used in the chemical industry (2,18,19). Deracemization strategies include:

1) Repeated Racemization and Resolution: This iterative process utilizes cycles of racemization and resolution, gradually increasing enantiomeric excess.

2) Dynamic Kinetic Resolution (DKR): This method combines kinetic resolution with in situ racemization to drive the reaction toward a single enantiomer.

3) Enantioselective Stereoinversion: This technique involves converting the undesired enantiomer into an achiral intermediate, then irreversibly transforming this intermediate into the desired enantiomer.

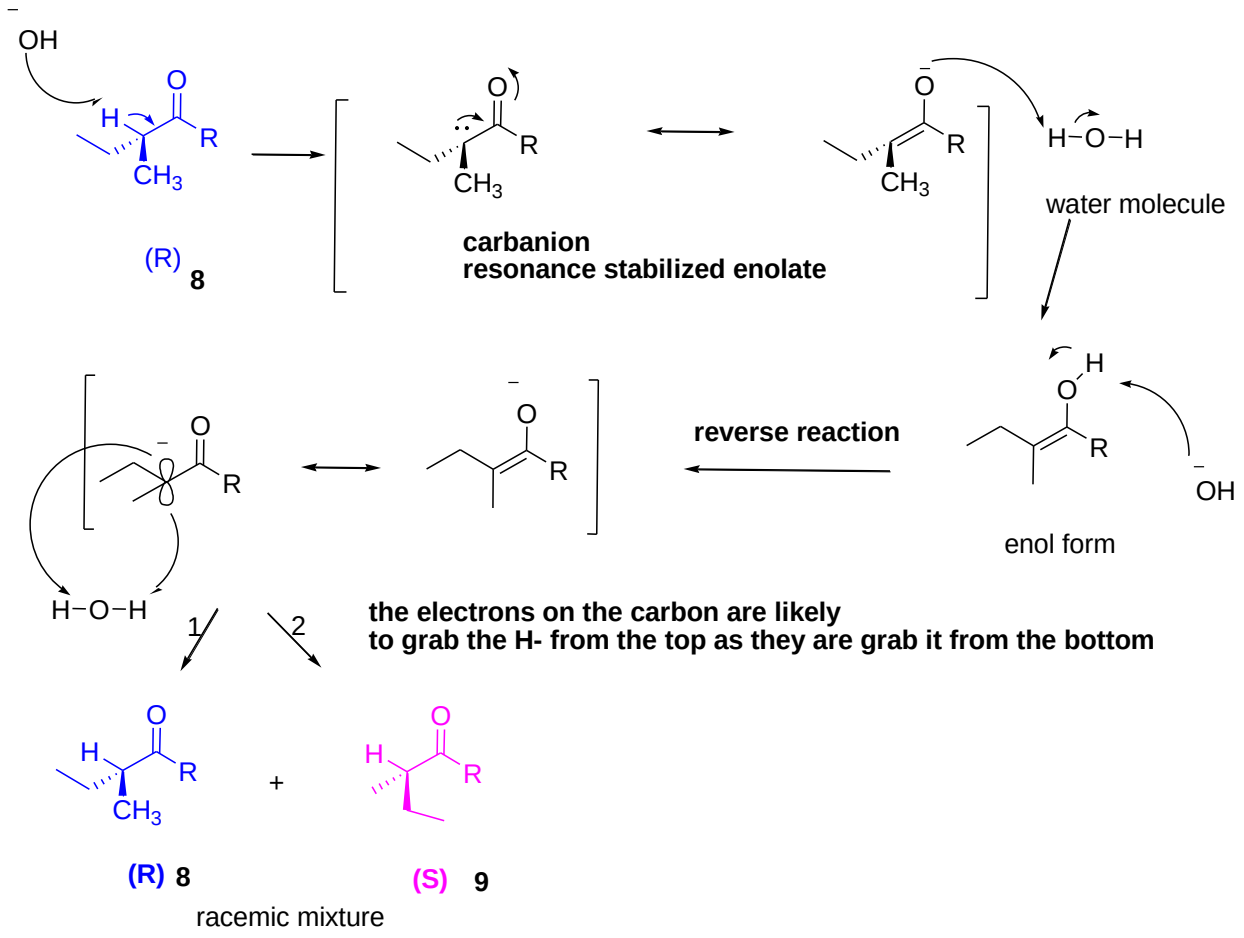
The following sections will delve deeper into these approaches and their implementation within this research.

1) Repeated Racemisation and Resolutions

This section discusses the current state of racemisation as it becomes an economic bottleneck. The loss of half of the substrate in resolution processes can be avoided by conducting the racemisation of the unwanted isomer after it is separated from the desired

enantiomer and by subjecting it again to resolution in the next cycle. This process results in the loss of enantiomeric purity. Racemisation can be achieved through acid- or base-catalysed biological conversion or increased temperature, which is called thermal racemisation. Racemisation is a reaction in which a single enantiomer is restored to its racemic form in a mixture in which more than one enantiomer exists. It starts with a single enantiomer and ends with a mixture of enantiomers. A recycling process for unwanted enantiomers has proven to be the most appropriate production process. The recovery of unwanted isomers often occurs through the racemisation of α-carbon carbonyl atoms under basic conditions. It is used for compounds with stereogenic centres bearing acidic hydrogens at the chiral centres or for α-carbons with low pKa values. The proton adjacent to an electron-withdrawing group, such as the heteroatom with an electron-withdrawing inductive effect, makes racemisation possible. A low-cost base was used. The factors dependent on racemisation are temperature, substrate concentration and solvent type. (20,18)

Organic compounds can undergo racemisation if they are exposed to basic conditions. The reversible transformation of R into its isomer could be done through various base catalysed processes, as shown in Scheme 3.

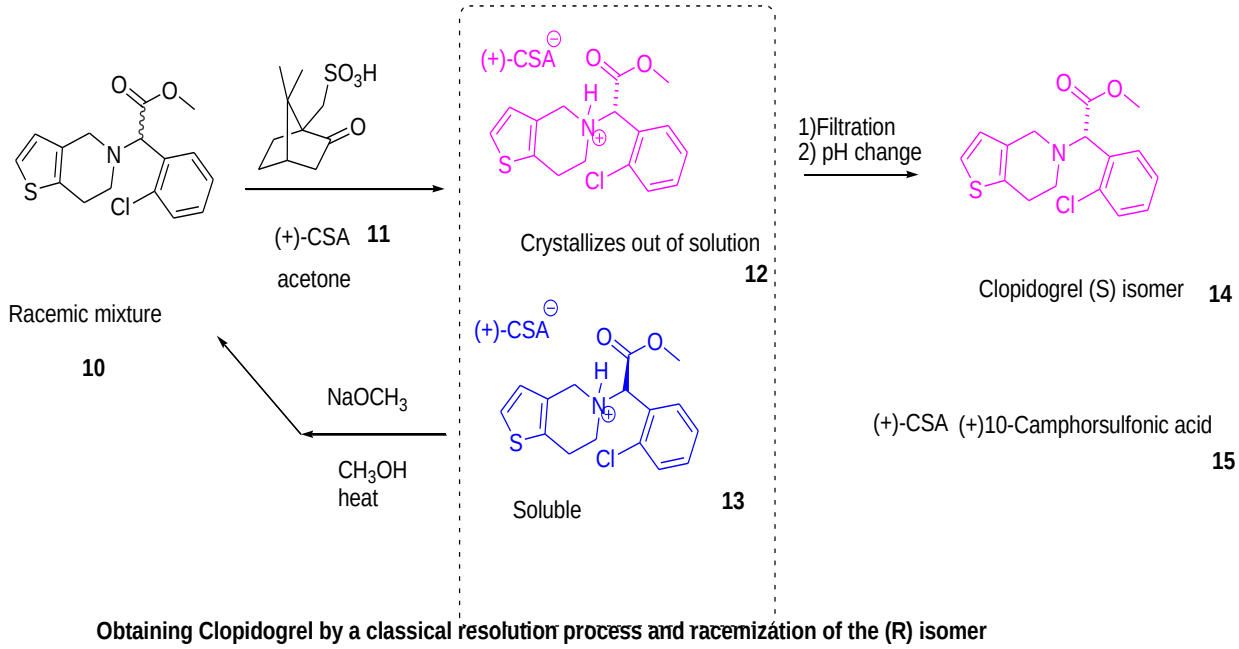


Scheme 3. Proposed mechanism of racemisation via base-catalysed racemisation and related processes.

This reaction is constantly shuttling back and forth, so at any given time, there is a 50:50 mix of both enantiomers. Base-catalysed processes apply to all compounds bearing acidic hydrogen at the stereogenic centre, whereas the acid-catalysed process is limited to compounds capable of keto-enol tautomerism. If an (R)-carbonyl compound is allowed to convert into enol under base or acid catalytic conditions, once the enol product is formed, it will begin to convert back into the carbonyl compound in a reverse reaction commonly referred to as keto-enol tautomerism. Carbonyl groups are sp^2 hybridised; carbon and oxygen both have unhybridised p orbitals which can overlap to form a C = O π bond. These overlapping p orbitals give α hydrogens adjacent to carbonyl weakly acidic properties with a pk_a value of 19–20 because the conjugate base called enolate stabilises the π orbitals of the carbonyl.

Both types of enantiomers will be formed, and the optical activity will be lost. The same result will be obtained in the acid-catalysed version. Base-catalysed racemisation is frequently used in the pharmaceutical industry for the synthesis of optically pure compounds. (16,18, 20, 21,22)

An example of racemisation from the literature is the resolution of a chiral amine and the recovery of an unwanted enantiomer by racemisation (as shown in Scheme 4). (23)



Scheme 4. Example of racemisation from the literature. (23)

Dutch resolution, a new development in crystallisation-induced resolution, combines the classical resolution with in situ racemisation. In the classical resolution, the racemates are converted with a suitable enantiomerically pure resolving agent to provide two diastereomeric salts with different solubilities that make separation by crystallisation easier. The combination of conglomerate formation with enantiomer racemisation in the solution leads to close to 100% conversion to a single enantiomer in crystalline form. These are new routes towards enantiopure products through the combination of reversible synthetic chemistry and

crystallisation. In an industrial resolution, the racemisation of the undesired enantiomer is often one of the major obstacles encountered. In many cases, harsh conditions are required and lead to the decomposition of the substrate, which must be modified to make racemisation possible. (20,22)

2) Dynamic kinetic resolution (DKR): This approach combines a kinetic resolution with an in situ racemization process to drive the reaction towards a single enantiomer. This method offers the potential for achieving high enantiomeric purity and is a promising strategy for deracemizing certain classes of compounds. A widely used technique for deracemisation is DKR. In this process, two enantiomeric species react at different rates such that a fast-reacting enantiomer (S_R) is depleted during the reaction and is readjusted through the racemisation of the slow-reacting enantiomer (S_S), as shown in Figure 4.

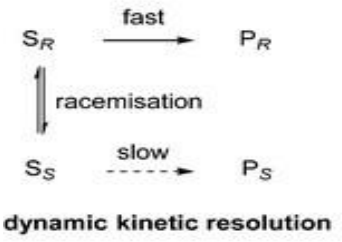


Figure 4. Transformation of a racemate into a single enantiomer. Subtypes of deracemisation: S_R, S_S = substrate enantiomers, P_R, P_S = product enantiomers. (20)

There are numerous examples of racemates resolved through the chemical transformation of a single enantiomer. Organocatalysis uses small chiral organic molecules. Transition metal catalysis induces asymmetric transformations by transitioning metal complexes that withdraw one stereoisomer from the equilibrium to produce a single enantiomerically pure product.

Another type of DKR is attrition-enhanced deracemisation or Viedma ripening. This technique utilizes a combination of grinding and crystallization to achieve enantiomeric enrichment. This method is particularly attractive for its simplicity and its potential for achieving high enantiomeric purity. It employs a compound that crystallises as a conglomerate through

thermodynamic resolution. (2) Viedma ripening is a reliable solid-state method for acquiring the desired enantiomer using preferential crystallisation, as shown in Figure 5.

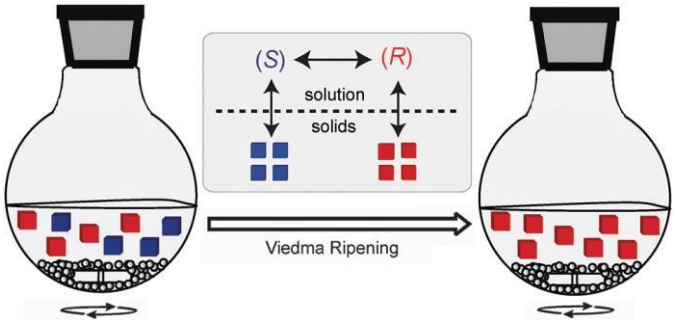


Figure 5. Schematic representation of Viedma ripening. (18)

Viedma ripening and temperature cycling are two methods used for deracemisation in industrial settings. A particularly reliable crystallisation method leads to a high yield of enantiopure crystalline product, in which the initially racemic mixture of crystals is completely transformed into one chiral form. The end state is one in which all crystals are of a single chiral form. (18) Up to now, deracemisation techniques, such as Viedma ripening and total spontaneous crystallisation, have focused on relatively simple chiral molecules. The broad application of Viedma ripening in synthetic organic chemistry and on a large industrial scale is common. It is a reliable method for transforming the crystals of unwanted enantiomers into the desired enantiomers in racemic suspensions of conglomerate crystals in a near-equilibrium state. Temperature cycling is a powerful tool for deracemisation because it expands Viedma ripening 10-fold. When a conglomerate suspension of chiral molecules is subjected to temperature gradients through heating and cooling cycles rather than grinding, as in Videma ripening, full deracemisation occurs. Therefore, applying a temperature-cycling method to speed up the deracemisation process is more convenient and easier to scale up with regard to the Viedma ripening method, but both methods need further optimisation to facilitate deracemisation efficiency.

Five parameters are dependent on deracemisation: total dry mass concentration, catalyst concentration, excess enantiomer concentration, changes in the cooling rate and temperature swings. The deracemisation of racemic mixtures of crystalline compounds leads to

enantiopure high-yield solid-phase products through the continuous grinding of crystals in a solution or suspension. (18)

For example, attrition-enhanced deracemisation is used to produce nonsteroidal anti-inflammatory drugs leading to (S) forms, such as naproxen. Complete deracemisation by crystallisation uses abrasive grinding under near-equilibrium conditions for the racemic mixture (RS), which is solid naproxen suspended in MeOH. The RS solid mixture of naproxen is mixed with S form, which is dissolved in MeOH and mixed with glass beads using a magnetic stirrer. Racemisation is initiated after the addition of NaOMe; seeds of enantiopure solids form because of the lower solubility of (S). This shifts the equilibrium towards the S form and converts all solid racemic mixtures into naproxen's enantiomerically pure (S) form. (20) More than one enantiomer can result from this process because of the larger surface area and faster crystallisation, which shifts the equilibrium of the mixture until one enantiomer is depleted in a solid state. This creates enantiomeric excess, which gradually increases during the reaction. (2,24,25) Another example, a racemic mixture of sodium chlorate crystals of both chiral forms subjected to grinding with glass beads in an isothermally closed system over a period of several days transforms into one chiral form through Viedma ripening.

3. Enantioselective stereoinversion

Deracemisation may be afforded by so-called stereoinversion. In this scenario, the unwanted enantiomer is inverted in situ to achieve the desired isomer in a 100% theoretical yield. Stereoinversion occurs when one enantiomer is transformed into an achiral intermediate I, followed by the irreversible conversion of I to the opposite enantiomer S_R. In this case, the inversion of enantiomer S_S by the chemically stable achiral intermediate I results in no reaction in the counterpart enantiomer S_R. (2,19) Enantioconvergence is a subtype of deracemisation. It occurs when each enantiomer has a different reaction pathway towards a single enantiomer product. In one reaction, S_S must invert the configuration to produce the same enantiomeric product, as shown in Figure 6. (2,19)

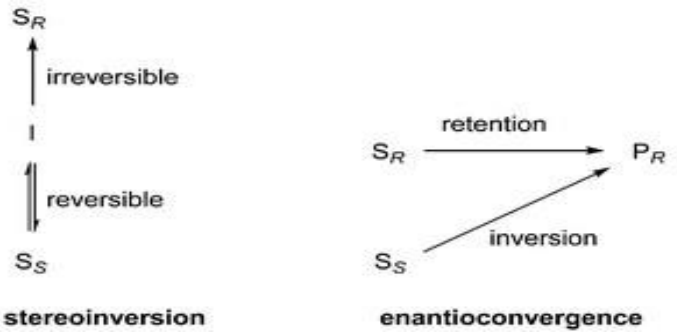


Figure 6. Transformation of a racemate into a single enantiomer; subtypes of deracemisation: S_R, S_S = substrate enantiomers, P_R, P_S = product enantiomers and I = achiral intermediate. (19)

To date, deracemisation by crystallisation has not yet been developed as a systematic workflow to unlock the potential of this interesting technique. However, the development of proof-of-concept chemistry has led to more commercially available drugs through discovery and production using deracemisation by crystallisation. Therefore, we will develop a specific integrated chemistry workflow as a new approach to the manufacture of complex chiral products. This will involve developing new routes to create target chiral compounds that are unachievable with conventional methods by combining reversible synthetic chemistry and selective crystallisation techniques. This will be achieved through the combination of conglomerate formation with enantiomer racemisation in the solution, which has led to an almost 100% conversion to a single

1.6 Understanding solid-state materials and how their spatial arrangement.

Crystallisation, along with asymmetrical synthesis, has been widely used to separate enantiomers. Generally, crystallisation is the most commonly used method for separating mixtures of chiral compounds. (7)

Molecules are packed closely together by intermolecular forces, and free energy is minimised. The unit cell is the basic building block, and its size and shape allow us to distinguish between different crystal structures. The unit cell is the most straightforward repeating unit in a crystal. These unit cells can be characterised by their three edge lengths (*a*, *b*, and *c*) and angles (*α*, *β*, and *γ*). The unit cell also explains the whole structure and symmetry of the crystal through

the positions and axes of its atoms. All crystals can be classified according to the symmetry of their unit cells. Only seven types of unit cells produce crystal structure systems: triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal, and cubic (Figure 7). (26)

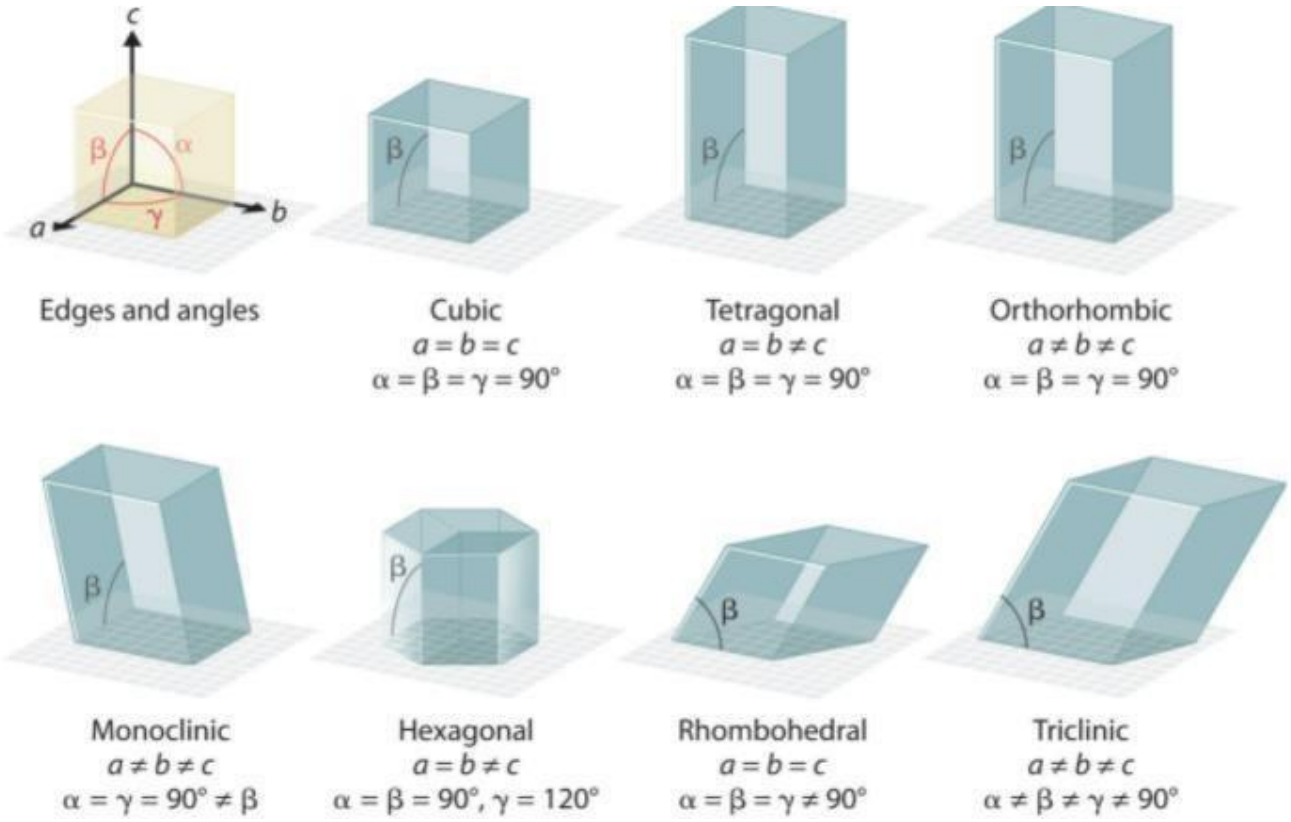


Figure 7. Seven basic unit cells. (26)

Crystallisation is the natural or artificial process by which a solid form as molecules highly organised into a structure, known as a crystal, at a given temperature and pressure. Crystals can be obtained from a solution by dissolving the solid in a solvent and then removing excess solid from the saturated solution by either allowing the solution to cool or by evaporating the solvent until a supersaturated solution results. The solution ultimately ends in nucleation and crystal growth, which is often the final stage of the synthetic process. (27) The main use of crystallisation in organic chemistry is for purifying solid products from chemical reactions. Nearly all active pharmaceutical ingredients are purified and isolated from a suitable solvent through crystallisation. This is a complex process that starts with the appearance of the most soluble form and ends with a stable one. (28, 29) As a realistic picture of how crystallisation is achieved in the laboratory is still lacking, the success of experiments is based on

observations and skills rather than on mathematical or physical predictions. (29) Crystallisation leading to the desired product constitutes an essential function of the pharmaceutical industry, as it remains a molecular level process.

The sugar industry offers an excellent example of industrial crystallisation, as 168 million tonnes are produced annually. The three mechanisms of crystallisation are as follows:

(1) solubility and supersaturation,

(2) nucleus formation or crystal nucleation, and

(3) crystal growth.

The main driving force behind crystallisation can be represented by the supersaturation ratio $S = C/C^*$, where C is the concentration and C^* is the solubility at a specific temperature and pressure. The four means of supersaturation generation within industrial crystallisation are evaporative crystallisation, cooling crystallisation, antisolvent or reactive crystallisation, and precipitation. These are the most frequent types of crystallisation used. (26, 27) The choice between them depends on the properties of the compound to be crystallised, the feed, and the thermodynamics of the system.

Removing a solvent from a boiling solution using evaporation is known as evaporative crystallisation. This process is mainly used to increase yield while reducing energy consumption. However, when the solubility of the suspension at the lowest temperature is low, reducing the temperature of the suspension will also reduce the solubility; in this case, a crystal is formed in a process known as cooling crystallisation. Another way to achieve supersaturation involves either the addition of an antisolvent, which dilutes the suspension and decreases solubility, or precipitation, which is sometimes referred to as reactive crystallisation. (27)

The second mechanism of crystallisation is crystal nucleation, which is a key process in achieving proper control of the crystallisation process. It is divided into primary and secondary nucleation. The primary nucleation present in a clear solution can be either a homogenous or heterogeneous crystal, while the secondary nucleation typically takes place in the presence

of larger parent crystals within the suspension. It is created by the collision of the parent crystals with each other and with the walls and the stirrer. The primary mechanism involved in industrial crystallisation is heterogeneous nucleation, as different heterogeneous particles can barely be avoided. The nucleation processes can be controlled by a solvent or an external energy source such as a laser light, electric or magnetic field, or ultrasound.

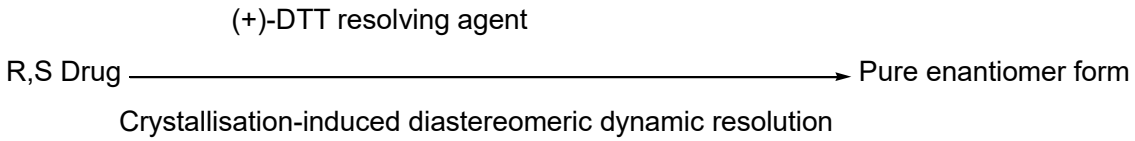
Crystal growth rates represent the third mechanism of crystallisation in which the growth of the crystal increases per unit of time. This process can be controlled by fluid dynamics or solute integration. Sometimes, air bubbles are used instead of stirrers for fluid dynamics. The crystal growth rate is also influenced by the supersaturation in the solution, temperature, pressure, composition of the mother liquor fluid flow, and crystal growth history. It can also be influenced by the presence of solvents, impurities, or additives, which can be considered foreign compounds. (27)

Several factors can affect crystallisation, such as the type of solvent, rate of cooling, agitation, and degree of supersaturation. It is essential to choose an appropriate solvent to create a specific crystal form with different molecule conformations in the crystal lattice and to control crystallisation in the experiment. The cooling rate influences the crystallisation pattern or crystal's habit by altering the degree of supersaturation. For example, when crystallisation occurs at a slower cooling rate, more symmetrical crystals are produced than with faster cooling. In addition, stirring facilitates the rate of nucleation by distributing the molecules in the solvent and breaking larger crystals into smaller ones. (28)

Chiral molecules that crystallise can be easily transformed into an enantiomerically pure compound with a nearly 100% theoretical yield through resolution by crystallisation. (30) One of the most important aims of industrial practice and research is to prepare enantiopure compounds (ee, approximately 100%); however, the resolution of racemic compounds (a 1:1 ratio of two enantiomers with a mirror-image relationship) remains the standard method for producing pure free enantiomers on a large scale. Obtaining pure
enantiomers requires one or more stages of recrystallisation. (31)

Separation of enantiomers by crystallisation can be classified into two main categories: chiral separation through diastereomeric salt formation followed by crystallisation or direct crystallisation of one enantiomer from a racemic mixture.

Diastereomeric salt formation is based on thermodynamic equilibria using a chemically stable, inexpensive, and easily recoverable resolving agent such as dithiothreitol (DTT).
(23)



Direct crystallisation is considered a classic method of enantiomeric separation and is a well-known ‘preferential crystallisation’ of pure enantiomers from conglomerate mixtures, an unusual enantiomeric resolution referred to as ‘preferential enrichment’. Both enantiomers can be obtained without introducing a chiral reagent. It is simpler and more cost-effective than any other method. (30) A less expensive method applicable to industrial production is preferential crystallisation (PC). This is a method in which a supersaturated racemic solution is seeded by one isomer, which then crystallises out preferentially. It is technically feasible only with racemates, which are conglomerates. Therefore, once conglomerate crystals are found, the resolution of the racemic mixture into its enantiomers through PC could be favourable, as there is no need for any chiral auxiliary. This technique is based on the fact that this is a supersaturated racemic solution that does not reach equilibrium and is kinetically relayed through the different crystallisation rates of the enantiomers in the presence of homochiral seeds. (24, 32)

A racemic compound is a mixture of enantiomers that can be crystalised as a conglomerate, a racemic compound, or rarely, as a pseudoracemate, as shown in Diagram 1. (24, 32)

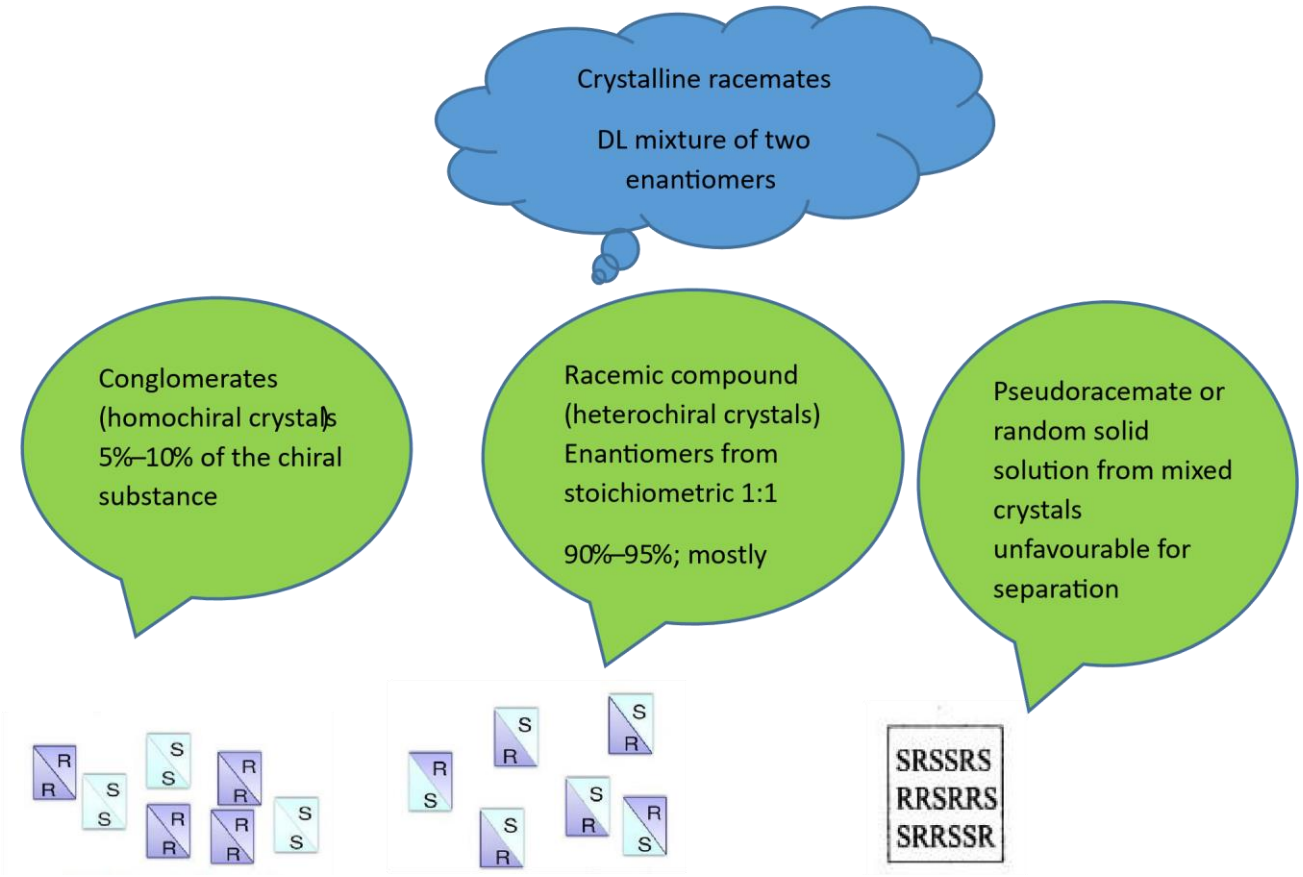


Diagram 1: A racemic mixture of chiral molecules can form racemic, pseudoracemic, or conglomerate crystals. (24,32)

Racemic and pseudoracemic crystals are made up of equal amounts of both enantiomers in an ordered and disordered arrangement, respectively. However, the chirality of conglomerate crystals resides solely in the solid state, which means that only one enantiomer is present in each crystal. (24,32)

It is believed that only 5%–10% of all organic racemates belong to conglomerates and that racemic compounds, rather than conglomerates, tend to crystallise from a racemic solution. The chance for a particular racemate to be a conglomerate is increased if there is salt formation with achiral acid or bases. The occurrence of conglomerates among salts is approximately 2 or 3 times higher than that among covalent compounds. It is possible for one compound to exist as a racemic compound in a stable form at one temperature but as a conglomerate in another. (33)

Chiral compounds most commonly crystallise in one of two forms: a racemic compound, in which crystals contain a 1:1 ratio of D/L molecules, or a conglomerate, in which each crystal is comprised of molecules of a single enantiomer. The crystals themselves are mirror images, with no direct molecular interaction between D and L molecules. Racemic compounds are thermodynamically more stable and prevalent than conglomerates because the D and L crystals of a conglomerate have identical properties, including solubility. (34)

Several methods for detecting conglomerate crystal forms are presented in the sections that follows. However, if the compound crystallises as a racemic form of crystal, then salt or a cocrystal is required for a conglomerate-forming system to achieve resolution through PC or separation via chromatography. (13,24)

1.6.1 Detecting the conglomerate crystal form,

Different strategies can be used to obtain the desired formations, including single-crystal studies, thermal analysis, Raman spectroscopy, and X-ray powder diffraction.

(35,36)

1- Second harmonic generation (SHG). SHG is a rapid, non-destructive, crystal specific, pre-high-throughput technique for identifying chiral discrimination in a solid state.

It is well suited to industrial use, as it requires a small amount of material. It is also a fast and cheap method that does not require any testing of the pure enantiomer. SHG allows selectivity in chiral crystal detection. In the screening of conglomerates, the selectivity of SHG applies to non-centrosymmetric systems. It uses high-power laser light that interacts only with a conglomerate, non-centrosymmetric crystalline structure. The intensity of the SHG signal relies on the symmetry class to which the crystal belongs: centrosymmetric space groups produce inactive SGH, whereas positive SHG is produced by noncentrosymmetric space groups. Conglomerates crystallise in a special category of noncentrosymmetric space groups and are thus expected to present SHG activity. (37)

2- Differential scanning calorimetry (DSC). DSC analyses are performed under a constant helium flow, where a 1-mg sample is heated from 20ºC to 350ºC. These temperatures

designate the melting points of a racemic mixture and its pure enantiomer. The difference between the melting points and the enthalpy of fusion by DSC of the racemates and the corresponding pure enantiomer suggests that the racemate is either conglomerate or racemic. A melting point higher than 130°C for an enantiomer of the TLM compound analogue may indicate a conglomerate. (35,36) It is often used to determine binary phase diagrams. However, many organic compounds decompose before the melting.

3- X-ray powder diffraction (XRPD). XRPD analysis provides a unique 'fingerprint' of the crystals present in a sample. This fingerprint allows the identification of the crystalline form of an unknown solid and its physical properties. The sample rotates in the path of the X-ray beam, while the X-ray detector is mounted on an arm to collect the diffracted X rays and rotates at an angle of 2θ. Peak positions occur where the X-ray beam is diffracted by the crystal lattice. The unique set of d-spacings derived from this pattern can be used for fingerprinting. The d-spacings, which indicate the distance between the atomic layers, were calculated using Bragg's law: $n\lambda = 2d\sin\theta$.

XRPD, solid-state NMR, and solid-state Fourier-transform infrared (FTIR) spectroscopy were used to compare the structural differences between enantiomers and racemates.

XRPD patterns differ between racemic and enantiopure crystals, and a simple powder diffraction fingerprint will not reveal whether a crystal is racemic or enantiopure. (35,36)

4- Single-crystal X-ray diffraction (SC-XRD). SC-XRD confirms that a single crystal of a compound exists in either a conglomerate or racemic form. It is a non-destructive analytical technique that provides information on the internal lattice of a crystalline substance, including the unit cell dimensions, bond lengths, bond angles, and details of site order. The data generated from the X-ray analysis were interpreted and refined to determine the crystal structure. A single crystal is the gold standard source of data that can be used (through mercury) to calculate and export a powder pattern, which can be compared with powder X-ray diffraction (PXRD) data, as shown in Figure 8.

A simple powder diffraction fingerprint cannot be used to determine whether a sample is racemic or enantiopure, but a single crystal from the sample can be used to that end. For

absolute certainty, one should use a single crystal and follow up with powder diffraction PXRD to ensure that the bulk of the crystal is the same. Most people use a single crystal and assume that the bulk is the same. However, although the crystal structure information is more straightforwardly interpreted in this way, the spatial properties of a single crystal may not necessarily be reflective of the bulk solid. Powder samples tend to result in ambiguities in the interpretation of the final structures, while single-crystal diffraction is much less ambiguous. (35,36)

SC-XRD provides much more information than PXRD does. It details, for instance, positions, intensities, and symmetry, allowing for full 3D determination of a molecular structure. The positions of the peak can be transformed into a series of coordinates to recover the underlying crystal lattice dimensions or crystal orientations of the sample of interest. PXRD data are merely averages of single crystal collections in different orientations, which means that several pieces of information are lost. A more common use of PXRD data is for identifying unit cells as fingerprints.

The key difference between X-ray crystallography and X-ray diffraction is that in the former, a single crystal of a material is exposed to X-rays to determine its atomic or the arrangement of atoms within a crystal and molecular structures, whereas XRPD is a chemical technique in which different forms of the material can be used. In the latter, the atoms of a crystal cause an interference pattern in the waves of the incident X-ray beam. XRPD has relatively short sample preparation times and require smaller crystal sizes (less than 10 μm in diameter) than SC-XRD owing to the reduced difficulty in the sample preparation step. XRPD is often easier, faster and more convenient than single crystal diffraction. XRPD is for bulk material that does not form crystal which useful for confirming the identity of a solid material. It is very challenging to grow high-quality single crystals of sufficient size for performing SC-XRD measurements, which is, therefore, often a highly laborious and time-consuming process.

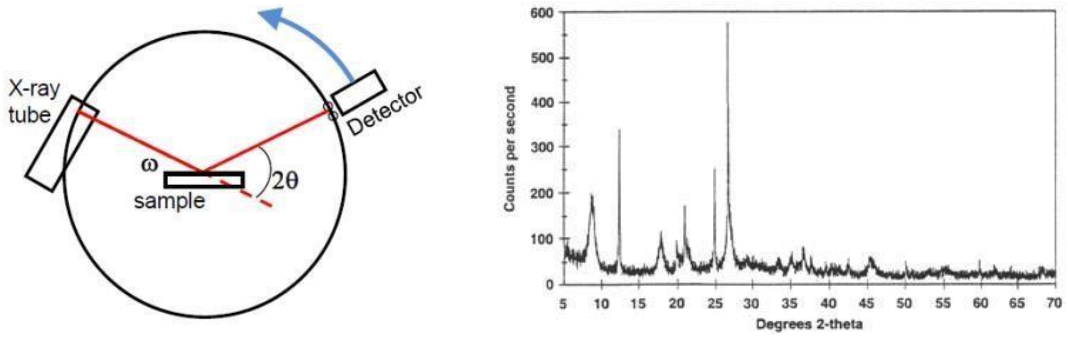


Figure 8. (Left) Single-crystal X-ray diffraction (SC-XRD). When an X-ray is shined on a crystal, it scatters into many different directions. From the angles and intensities of these scattered beams a three- dimensional picture of the density of electrons within the crystal can be produced. The positions of the atoms in the crystal as well as their chemical bonds, their disorder can be determined. (Right) a diffraction pattern plots intensity against the angle of the detector 2θ, typical powder X-ray diffraction pattern data are collected at 2θ from 5° to 70° angles on the X-ray scan, as shown in the right image.

SC-XRD is an increasingly important tool, as it is an easy-to-use and powerful technique for determining the 3D structures of a wide variety of crystals. X-ray crystallography has been fundamental in the development of many scientific fields. With the knowledge of XRD and crystallography, it is possible to determine the crystal structure and molecular formula of a crystalline compound. The PXRD and SC-XRD methods have become workhorse techniques for both new and complex materials and pharmaceutical data collection. (35,36)

5- Solubility measurements. Using a Crystal16™ instrument to take solubility measurements provides comprehensive knowledge of the solid–liquid phase equilibria (SLE) of a chiral compound, as shown in Figure 9. (31) This tool, which is useful for determining solubility curves, makes heating and cooling profiles that perform in an automated and controlled solid state useful for screening for crystallisation conditions. The polythermal method uses the Crystal16™ apparatus (Avantium Technologies) to determine the saturation temperatures of the enantiomeric mixtures in different solvents. This can be accomplished by increasing the temperature of a suspension with a sufficiently slow heating rate until the slurry

becomes a clear liquid and all crystals are dissolved, a state known as the clear point. At a clear point, a solution's composition can be determined. If the crystals dissolve gently rapidly, the thermodynamic saturation temperature can be taken as a clear point. (38,39) A phase diagram screening of enantiomer mixtures in a solution can provide important indications of the possibility of chiral resolution through crystallisation. Phase diagrams are time-consuming and require a large quantity of the compound sampled. Double- or triple-wall jacketed vessels can be connected to a thermostat to ensure isothermal conditions during solubility experiments using the classical isothermal method.

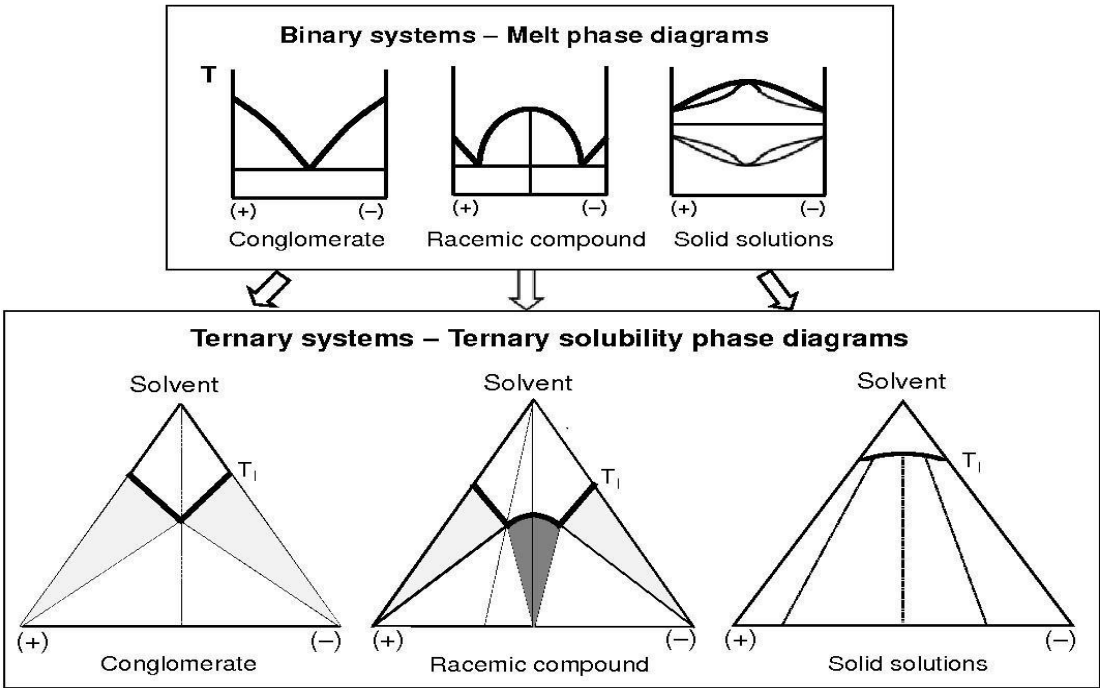
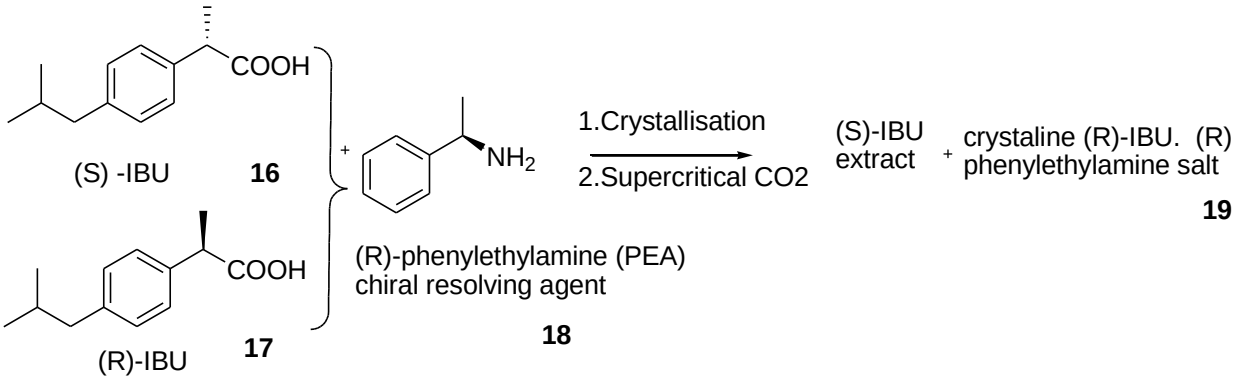


Figure 9. Crystal formed using a binary phase diagram (BPD). (24,31)

6- Polarimetric measurements. Optical activity can be used to predict crystals that are dextrorotatory and laevorotatory. To obtain a polarimetry reading, 10 mg of powder sample was dissolved in 2 ml of solvent. (38,39) The behaviour of a racemic compound may be influenced by the choice of the pH value of the aqueous solution, the polarity of the solvent, or the use of solvent mixtures. It is also determined by the presence of a chiral compound that has a molecular structure analogous to a part of the resolving agent and by the time of crystallisation. The stability of thermodynamic equilibrium requires a more extended time, while faster crystallisation determines the separation of the kinetic conglomerate. (31)

Crystalline material can be separated through filtration, distillation, sublimation, or extraction from residue, and repeated crystallisation steps can be used to increase the product's purity and yield. An example of the separation of enantiomers by extraction is the resolution of racemic ibuprofen (IBU) with chiral resolving agents, as shown in Scheme 5. Chapter 3 will provide more information about classical resolution through diastereomeric salt formation. A free enantiomer (S) can be extracted using supercritical fluid carbon dioxide. (31)



Scheme 5: Enantioseparation of Ibuprofen (IBU) via diastereomeric salt formation (classical resolution) is explained systematically. (18)

Developing a crystallisation method for ee enhancement involves three steps. The first step is to determine the thermodynamically stable phase of the racemate (conglomerate, racemic compound, or psudoracemate) at the temperature of interest. The second is to obtain the key solubility data, and the third is to design the crystallisation process. (33)

1.7 Crystallisation in the pharmaceutical and chemical industries

Continuous crystallisation has greatly improved manufacturing in the pharmaceutical and chemical industries through the efficient use of reagents, solvents, energy, and space. In addition, the small size of the equipment minimises waste production and maximises yield. This crystallisation process results in a well-controlled product quality due to its constant steady state, easily controlled size and morphology, and high product efficiency. Despite the low yield relative to chirality crystal production, this technique improves the safety, economy, and reproducibility of the process. (27,40)

Continuous crystallisation is an attractive process for producing pure enantiomers from racemic mixtures. Conventional crystallisation cannot deal with the chirality issue, so further steps must be taken to produce a pure enantiomer. (15) Researchers have also used a coupled crystalliser, wherein two mixed coupled crystallisers are connected with exchange pipes and equipped with fine dissolution units. Simultaneously, each crystalliser is seeded separately with one of the two enantiomers. Figure 10 presents an example similar to a coupling crystalliser in which the crystals are continuously milled to allow a sufficient surface for crystal growth. If a counter-enantiomer crystal appears during this process, it can easily be recovered.

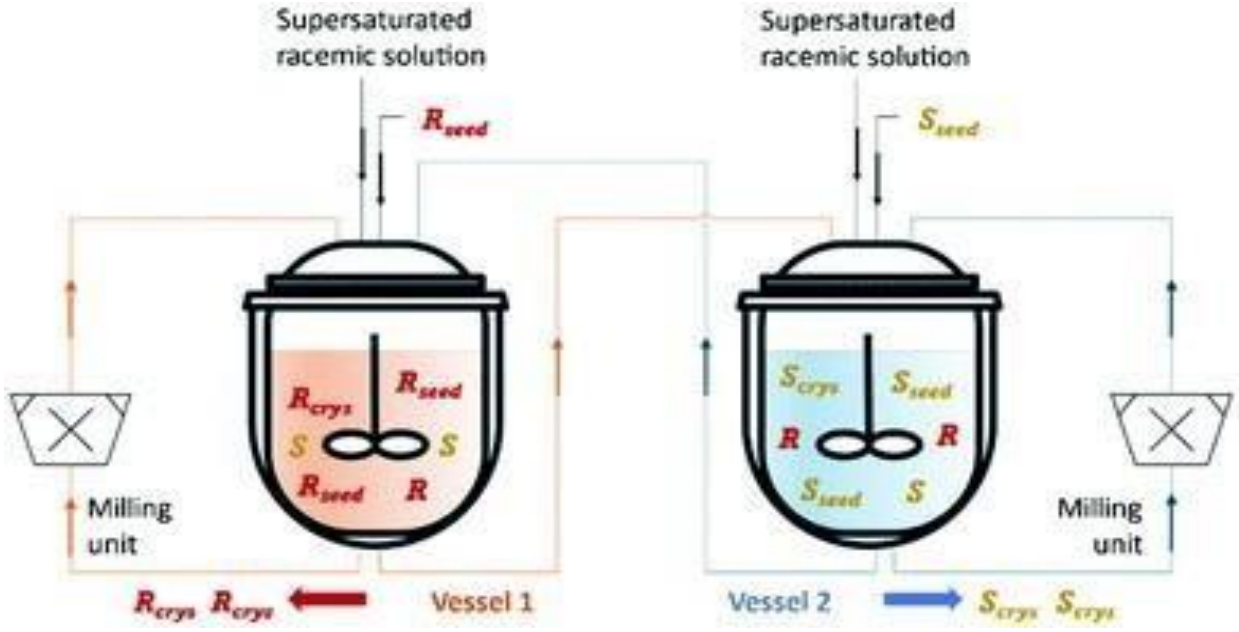


Figure 10. A novel process flow sheet used for continuous preferential crystallisation. (40)
The field of continuous pharmaceutical crystallisation could be further developed in the future through the combination of continuous crystals and a nanofiltration operation unit. This would involve controlling all the operational parameters of continuous crystallisation, such as temperature and residence time, to ensure constant conditions in the steady state and high production efficiency. (27,40)

To summarise, homochirality is predominant in all biological systems. The interest in chirality and its consequences has prompted increasingly high expectations in the last two decades regarding the ethical and environmental reasons for developing homochiral compounds, and

the economic implications are another convincing reason for the development of single-enantiomer-type products. The four main separation methods for obtaining pure enantiomers are asymmetrical synthesis, direct crystallisation, enzymatic resolution, and chiral chromatography separation. Although resolution methods can never exceed a 50% yield, this disadvantage can be overcome by dynamic resolution; stereoinversion, where the unwanted enantiomer is inverted *in situ* to achieve the desired isomer; or repeated racemisation and resolution, known as deracemisation. A complex research procedure is involved in simple racemisation in solution and selective crystallisation into crystallisation enhanced deracemisation. (18)

One technique for chiral resolution is crystallisation. The resolution of a chiral compound strongly depends on the solid state formed upon crystallisation. The crystal of a racemic compound consists of an even ratio of both enantiomers in a regularly structured array. A conglomerate is a physical mixture of pure enantiomer crystals, with the overall mixture being essentially racemic. A solid solution or pseudoracemate forms when both enantiomers compete for the same lattice position in the crystal structure. While the formation of a solid solution is rare, the formation of a racemic compound is common. Most chiral compounds form racemic solutions. A stable conglomerate is formed in approximately 10% of all cases, whereas the formation of a solid solution is relatively rare. A phase diagram related to the solid state of a chiral compound provides important information on the possibility of chiral resolution through crystallisation. Direct crystallisation is applied by increasing the probability of conglomerate formation through careful control of the crystallisation process. (39)

1.8 Suggested workflow to obtain chiral molecules through **deracemisation via crystallisation**

Strategies underpin my PhD dissertation. Diagram 2 shows the workflow and bases of the project that led to the achievement of the aims and objectives of this research without considering last stage as it is not performed.

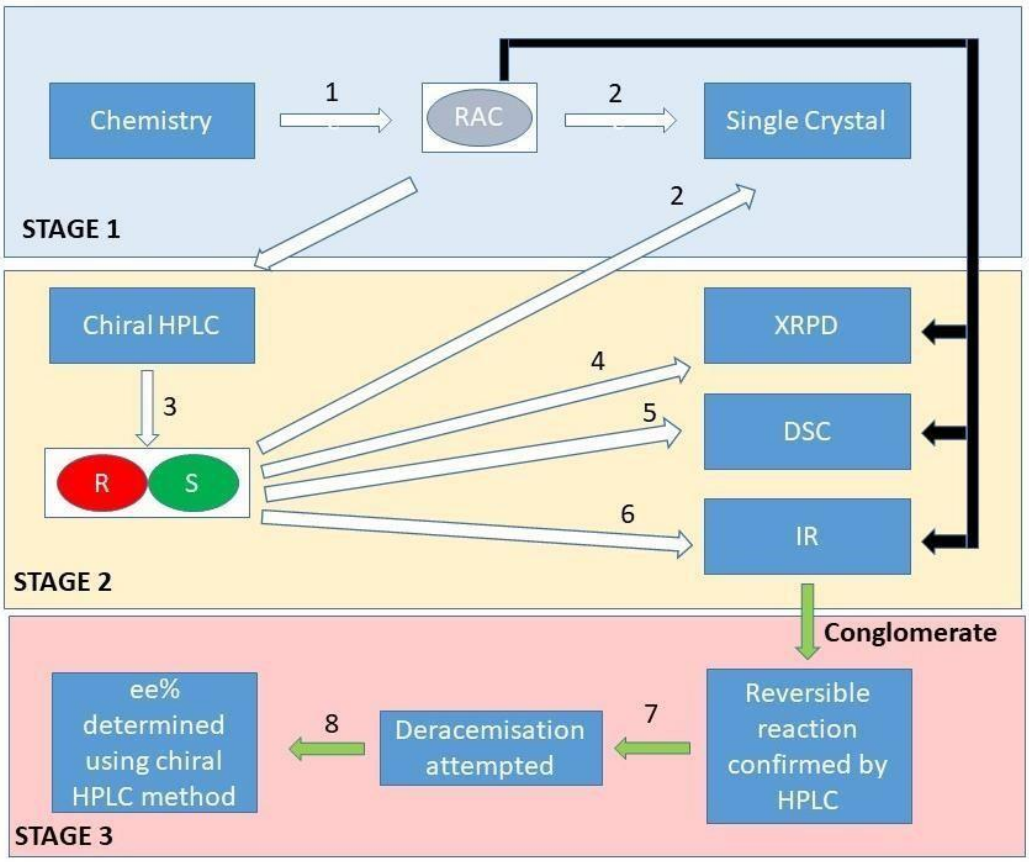
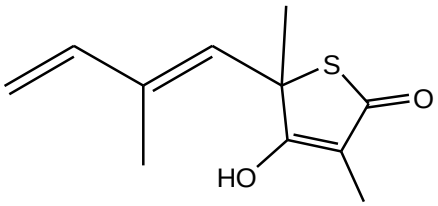


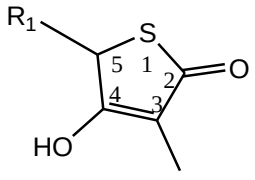
Diagram 2. The suggested overall process that can be exploited to fit with project. An overview of the overall work for TLM scaffold as 'target molecule of interest' is also shown. In stage 1, molecules will be synthesised in the laboratory as TLM analogues are difficult to acquire from other sources. In stage 2, how the structure diversity of the TLM analogues may relate to the formation of a conglomerate crystal form rather than a racemic crystal form was determined. Once the compound crystalises as a conglomerate and moves to stage 3, the resolution of the racemic mixture into its enantiomers through PC could be favourable. The ee% is then determined using the chiral HPLC method to purify chiral molecules and monitor deracemisation.

1.9 Thiolactomycin as a molecule of investigation for deracemisation by crystallisation

An example of a single-enantiomer molecule with important biological properties is 4hydroxy-3,5-dimethyl-5H-thiophen-2-one, also known as (5R)-TLM **20.** (21,41) First isolated from *Nocardia* species, TLM exhibits broad-spectrum antibacterial activity and has been reported to exhibit an *in vivo* activity against *Mycobacterium tuberculosis* by inhibiting fatty acid biosynthesis. (37, 41-47) Specifically, it has been shown to inhibit the beta-ketoacyl carrier protein synthase (beta-ketoacyl-ACP synthase III-condensing enzyme *mt*FabH), which is a vital link between the fatty acid synthase type I and II FAS-I and FAS-II systems in the biosynthesis of both fatty and mycolic acids. (43,48) The minimum inhibitory concentration of TLM for use against *Mtb* is 62.5 μM. (37,49) Various examples in the literature include a synthesised series of substituents at C5 or C3 of the ring analogues of the TLM used to test their biological activity against *mt*FabH compared with the activity of the parent drug, TLM (75 μM). (48) The aim of this study was to develop an environmentally friendly method of converting racemic mixtures into active enantiomers cost-effectively and efficiently. It also aimed to design and synthesise chiral anti-tuberculosis compounds with thiolactone cores based on a structure similar to TLM **20** but with different 5- or 3-substituted derivatives on thiolactone ring **5**, as shown in Figure 11. This is commonly used to target *Mycobacterium tuberculosis.* (21)



Thiolactomycin (TLM) **20**



5

5- substitueted derivatives on Thiolactone ring

Figure 11. Structures of the standard drug thiolactomycin (TLM) **20** and 5-substituted derivatives on thiolactone ring **5**, respectively. (21)

For example, Senior *et al.* illustrated that analogues of TLM have enhanced *in vitro* activity against mtFabH. (43) It is therefore desirable to find a route to the development of analogues with known absolute configurations. To this end, we have chosen this scaffold as the focus of the research project. Thiolactomycin (TLM), a naturally occurring molecule with potent anti-tuberculosis properties, serves as the focus of this study. The compound's unique thiolactone core, highly amenable to derivatization, provides an excellent scaffold for exploring structure–activity relationships and developing novel anti-tuberculosis drugs. While past research has focused primarily on racemic TLM, the unequal biological activity of its enantiomers underscores the critical need to develop efficient methods for generating enantiomerically pure analogues. This thesis will investigate the potential of deracemization via crystallization to address this critical need (44,45).

This research is significant because it contributes to the understanding of the crystallization behaviour of complex molecules, specifically the TLM analogues, and emphasizes the crucial role of experimental validation in complementing computational analysis. Furthermore, the study explores the potential for developing more efficient and environmentally friendly methods for obtaining enantiopure compounds, which is crucial for advancing the field of pharmaceuticals and developing new and effective medications.

1.10 Research Objectives and Thesis Outline

This thesis aims to develop efficient and environmentally friendly methods for obtaining single enantiomers of TLM analogues, focusing on deracemization via crystallization. Referred to diagram 2, this study aimed to find a new route to achieve enantiopure TLM via crystallisation as an affordable alternative to other commonly used methods. This was achieved by preparing a diverse range of racemic compound analogues. The enantiomeric crystal structures of these analogues will be analysed to establish which have conglomerate forms, making racemisation possible. This research project has three specific objectives:

I. The first objective of this study was to search databases and other sources for a set of chiral compounds that include racemic/conglomerate forms, are structurally similar to TLM, and have several pharmacophore features; identify their solid-state behaviours; and find out how

they bond with each other; and determine the exact space group of crystal filling to determine whether a compound tends to crystallise as a racemic compound or as a conglomerate.

II. Understanding how the structural diversity of the TLM analogues may lead to the formation of a conglomerate crystal form is the second objective. To synthesize and characterize the crystal structures of a series of TLM analogues modified at the 3 and 5 positions on the thiolactone core. The analysis will correlate structural features with crystallization behavior.

III. To develop a robust method for determining the enantiomeric excess (%ee) and for implementing a deracemization strategy, using ibuprofen as a model system and applying a suitable method such as polarimetry measurement or chiral column chromatography, specifically HPLC.

The thesis is structured as follows: Chapter 2 explores known TLM analogs and related structures to inform the design of new compounds. Chapter 3 details the synthesis and characterization of the new TLM analogues. Chapter 4 describes the development of a %ee determination method using the ibuprofen model. Chapter 5 concludes the study, summarizes the findings, and outlines potential future research directions.

Chapter 2

Learning from available structures

2.1 Introduction: Understanding the Crystallisation of Racemates and Conglomerates

Demands for single-enantiomer drugs have significantly increased due to their enhanced therapeutic effects and reduced side effects than racemic form of drugs. This chapter focuses on analysing the crystallisation behaviour of thiolactomycin (TLM) analogues and ibuprofen (IBU) using computational approaches. The goals here are to identify structural factors that promote racemic or conglomerate crystal formation and to assess their implications for deracemisation strategies. Understanding these properties can provide insight for the development of more efficient crystallisation techniques for enantiomeric separation.

The crystallisation behaviour of chiral compounds is a key focus of this research because it underpins the development of effective methods for resolving racemic mixtures into pure enantiomers. Crystallisation offers a practical and efficient technique for producing enantiopure compounds, but its success depends on the solid-state behaviour of the compounds in question. In this chapter, I aim to understand why some molecules crystallise as racemates while others do so as conglomerates.

2.2 Background

Chiral drugs have become a particular focus for development, representing the most rapidly growing field within the pharmaceutical market and accounting for over one-third of the drugs produced worldwide. Asymmetrical synthesis is the first mode of production of a pure enantiomer. It involves the chemical reaction of an enantiomeric agent or catalyst with a substrate to produce the desired molecule in the form of a single enantiomer. Although a racemic mixture can be generated as a product with little regard for the synthesis conditions or racemisation, chiral resolution or chiral separation must be performed to recover a pure enantiomer. This can be carried out by chiral chromatography, which takes advantage of the fact that the two enantiomers have different retention times, allowing for their resolution. However, this process is unsuitable for industrial applications because of the slow rate of injection of the product into the column and the long separation time. To overcome these obstacles, crystallisation resolution offers a useful alternative as one of the most efficient and

practical techniques for producing enantiopure compounds. This approach can be classified into two separate subsets: direct crystallisation (preferential crystallisation or spontaneous resolution) and diastereomeric crystallisation. The former can only be applied to conglomerates, which account for 5%–10% of all organic racemates. The latter is far more widely used with diastereomeric crystallisation known as the classic method of resolution. The relationship between the molecular structure and the phase behaviour is of particular importance in diastereomeric crystallisation (32,36,50,51).

In 1853, Louis Pasteur used a chiral resolving agent to achieve the crystallisation of diastereoisomers. Once crystallised, diastereoisomers differ from each other in their symmetry and chemical /physical properties, particularly with regard to solubility. Then, the pure enantiomer can be recovered by salting out the diastereoisomer to remove the resolving agent. To build on this foundation and improve the yield further, Dutch resolution, using equimolar amounts of resolving agents, was recently established. applied to improve the yield. Otherwise, as an alternative to the Pasteurian method, resolution can be performed through preferential crystallisation, where only one enantiomer crystallises in a given period, although both are supersaturated in the mother liquor, and where no resolving agent is required. In the 1990s, preferential crystallisation was explained in the literature using phase diagrams, which communicated detailed information about the behaviours of the two enantiomers in terms of melting and solubility. Accordingly, simple measurements of the melting temperatures of the enantiomers and their corresponding racemic mixture could be used to identify the nature of a sample, either as a conglomerate or as a racemic compound (32,52,53,54). The former is a key step in the application of preferential crystallisation; however, a serious limitation of this resolution approach is the low occurrence of conglomerates among the molecular crystallised compounds (only 5%–10% of the racemic species).(52)

Coquerel (2000) noted that three common types of packing can be observed when crystallising a racemic mixture. An organic compound can exist as a racemic compound (true racemate), which is the most common and represents 90%–95% of racemic species. More specifically, this is a stoichiometric compound in most cases. The second type is a conglomerate compound, which is a mixture of single crystals of homochiral molecules, representing only 5%–10% of racemic species. The third type is a racemic solid solution

(pseudoracemate), in which equal proportions of isomers are packed at random (53). Pseudoracemate crystals are rarely formed in organic compounds, according to Jacques et al. (1994) (52). A conglomerate is formed from the pure crystals of two isomers, each of which has a stronger molecular affinity for the same isomer than for its counterpart. Therefore, the formation of a conglomerate provides a means of obtaining pure enantiomers of organic compounds through direct crystallisation separation, which is simpler and more environmentally friendly and cost-effective than any other method. In this approach, the probability of conglomerate formation can be increased by carefully controlling the crystallisation process to achieve direct crystallisation. Nonetheless, the use of crystallisation is hindered by the tendency of 90% of the compounds to form racemic crystals, as opposed to conglomerates of single enantiomers. A single crystal of a racemic compound is formed against which the two isomers pack regularly at a 1:1 ratio. In this way, the vast majority of organic compounds form racemic compounds, as their crystals have a stronger molecular affinity for the counterpart than for the same isomer (32,36,50, 55).

To date, only racemic compounds have been found to exist for centrosymmetric achiral structures, whereas conglomerates are predominant for non-centrosymmetric chiral structures, although racemic compounds can still crystallise as non-centrosymmetric space groups. A racemic mixture can crystallise in a non-centrosymmetric space group, although the identification of these types of crystals can often be a false positives result from slightly disordered racemic structures. Several spectroscopic techniques can be used to differentiate a racemic mixture from a conglomerate by comparing the racemic pattern with that of the enantiomer. For a conglomerate, the patterns of the pure enantiomer obtained from solid-state NMR or XRPD should be superimposable on those obtained for the racemic mixture (32).

To perform preferential crystallisation, the racemic mixture should crystallise as a stable conglomerate. However, in most cases, the chiral resolution does not fulfil this condition. Owing to the low occurrence of conglomerates, it is often necessary to synthesise a series of derivatives with different co-crystal forms. An interesting alternative is to proceed to the resolution on derivatives such as salts, co-crystals, or solvates that crystallise as conglomerates at the end of the resolution process, at which point the pure enantiomer can easily be recovered by salting out or desolvation. There is interest in combining pure

enantiomer crystallisation with chemically induced racemisation to increase yields for conglomerate slurries, such as in Viedma ripening. Elsewhere, when exploring alternative routes of conglomerate production, Spix et al. (56) used salt derivative formation to achieve conglomerate crystallisation for Viedma ripening.

A crystal structure comprises a basic motif repeated in a 3D space by the symmetry operators of the crystallographic space group. The coordinates of the atoms in this basic motif form an asymmetrical unit. This is the smallest part of a crystal structure from which the complete structure can be built using space group symmetry.

Wilhelm Roentgen discovered X-rays after determining that when high-energy electrons in a tube hit a metal component, they are either slowed down and release energy or remove electrons from the atoms they hit, which triggers a reshuffling that has the same effect of releasing energy. In both cases, energy is emitted in the form of X-rays, a type of electromagnetic radiation with higher energy than visible light. X-rays are sufficiently powerful to pass through many kinds of matter, as if the matter is semi-transparent. They are particularly useful for medical applications, as they can produce images of organs, such as bones, without harmful effects. When X-rays interact with matter, they collide with electrons. X-rays sometimes transfer all of their energy to the matter and are absorbed. At other times, they only transfer some of their energy, and the rest is scattered. The frequencies of these outcomes depend on how many electrons the X-rays are likely to hit. Collisions are likely if a material is dense or made of elements with high atomic numbers, which indicates the presence of many electrons. For example, bones are dense and full of calcium, which has a relatively high atomic number, so they absorb X-rays well. Meanwhile, soft tissue is not as dense and contains mostly elements with lower atomic numbers, such as carbon, hydrogen, and oxygen. For these reasons, more X-rays penetrate tissues such as the lungs and muscles than they do bones, darkening the film. This can make it challenging to visualise internal structures. Therefore, a physician must take X-rays from many angles around the body and piece them together to construct an internal image revealing the change in density that can identify a solid tumour in a patient (or, for instance, a blood clot or infection).

X-rays are generated by the interaction of incident rays directed towards a sample/subject, which produces a diffracted ray that satisfies Bragg's Law ($n\lambda=2d\sin\theta$), where θ is the angle of incidence of the X-ray beam, λ is the wavelength, and *d* is the spacing between atom layers (57). Owing to the wavelength of X-rays being similar to the interatomic spacing in the crystals, the X-rays are diffracted. The diffracted X-rays are then detected through a process that relates the wavelength of electromagnetic radiation to the diffraction angle and lattice spacing in a crystalline sample and then transforms the X-ray photons into an electrical signal, which is sent to a computer for processing. X-ray crystallography and solid-state polarised light microscopy can be used to probe the chirality of conglomerate crystals originating from achiral or chiral molecules.

A 3D object in a single-crystal X-ray image contains internal lattice information on crystalline substances, unit cells (including cell dimensions), and the positions of atoms within the lattice. The unit cell parameters are described by the lengths of the three axes, *a*, *b*, and *c* [in the unit of Ångströms (Å)], and the three angles, α, β, and γ in the unit of degrees, then, the crystal class is determined. The following are the angles between the vectors: *alpha*, the angle between *b* and *c*; *beta*, the angle between *a* and *c*; and *gamma*, the angle between *a* and *b*. Crystallographers usually express torsion angles in the range from −180° to 180°. A clockwise rotation is positive, while an anticlockwise rotation is negative. Moreover, any structure determined in the range of 283–303 K is considered to be a room-temperature structure. The crystallographic R-factor is the conventional figure of merit for crystal structures and provides a measure of how well a refined structure agrees with an experimental model. In terms of *Z*, which is the number of molecules per unit cell, a crystal must belong to one of seven crystal systems, which describes the symmetry of the crystal in terms of space groups. The space groups are made from combinations of the 32 crystallographic point groups belonging to one of the seven crystal systems (57). A space group is a combination of the symmetry of a unit cell, the point group symmetry operation of reflection, and the screw axis and glide plane symmetry operations. The different combinations of these symmetry operations result in 230 different space groups, which can be divided into two sets: (i) 165 space groups that can accommodate achiral crystal structures and (ii) 65 space groups that can accommodate chiral crystal structures. Conglomerate crystals must crystallise in one of the 65 non-

centrosymmetric space groups, which means that these space groups lack a centre of inversion. Once we know the crystal class (e.g. cubic, tetragonal, or orthorhombic), we must identify the space groups to which the crystal belongs. In accordance with the work of Hahn (1996), Table 1 lists the unique space groups categorised by crystal system and Laue class (58,59).

Table 1. Space groups based on crystal system and Laue class

Crystal system	Laue class	Space group
Triclinic	1	*P*1,**Pí**
Monoclinic	2/*m*	P2,*P*21, *C*2, *Pm*, *Pc*, *Cm*, *Cc*, ***P*2/*m***, ***P*2$_1$/*m***, ***C*2/*m***, ***P*2/*c***, ***P*2$_1$/*c***, ***C*2/*c***
Orthorhombic	*mmm*	*P*222, *P*222$_1$, *P*2$_1$2$_1$2, *P*2$_1$2$_1$2$_1$, *C*222, *C*222$_1$, *F*222, *I*222,
		*I*2$_1$2$_1$2$_1$, *Pmm*2, *Pmc*2$_1$, *Pcc*2, *Pma*2, *Pca*2$_1$, *Pnc*2, *Pmn*2$_1$, *Pba* 2, *Pna*2$_1$, *Pnn*2, *Cmm*2, *Cmc*2$_1$, *Ccc*2, *Amm*2, *Aem*2, *Ama*2, *Ae a*2, *Fmm*2, *Fdd*2, *Imm*2, *Iba*2, *Ima*2, ***Pmmm***, ***Pnnn***, ***Pccm***, ***Pb an***, ***Pmma***, ***Pnna***, ***Pmna***, ***Pcca***, ***Pbam***, ***Pccn***, ***Pbcm***, ***Pnnm***, ***P mmn***, ***Pbcn***, ***Pbca***, ***Pnma***, ***Cmcm***, ***Cmce***, ***Cmmm***, ***Cccm***, ***Cm me***, ***Ccce***, ***Fm mm***, ***Fddd***, ***Immm***, ***Ibam***, ***Ibca***, ***Imma***
Tetragonal	4/*m*	*P*4, *P*4$_1$, *P*4$_2$, *P*4$_3$,*I*4, *I*4$_1$, *P*-4, *I*-4, ***P*4/*m*, *P*4$_2$/*m*, *P*4/*n*, *P*4$_2$/*n*** **P4/m, *I*4/*m*, *I*4$_1$/*a***
Tetragonal	4/*mm m*	*P*422, *P*42$_1$2, *P*4$_1$22, *P*4$_1$2$_1$2, *P*4$_2$22, *P*4$_2$2$_1$2, *P*4$_3$22, *P*4$_3$2$_1$2, *I*422, *I*4$_1$22, *P*4*mm*, *P*4*bm*, P4$_2$*cm*, P4$_2$*nm*, *P*4*cc*, *P*4*nc*, P4$_2$*mc*, P4$_2$*bc*, *I*4*mm*, *I*4*cm*, *I*4$_1$*md*, *I*4$_1$*cd*,*P*-42*m*, *P*-42*c*, *P*-42$_1$*m*, *P*-42$_1$*c*, *P*4*m*2, *P*4*c*2, *P*4*b*2, *P*4*n*2, *I*4*m*2, *I*4*c*2, *I*42*m*, *I*42*d*, ***P*4/*mmm***, ***P*4/*mcc***, ***P*4/*nbm***, ***P*4/*nnc***, ***P*4/*mbm***, ***P*4/*mnc***, ***P*4/*n mm***, ***P*4/*mcc***, ***P*4$_2$/*mmc***, ***P*4$_2$/*mcm***, ***P*4$_2$/*nbc***, ***P*4$_2$/*nnm***, ***P*4$_2$/*mb c***, ***P*4$_2$/*mnm***, ***P*4$_2$/*nmc***, ***P*4$_2$/*ncm***, ***I*4/*mmm***, ***I*4/*mcm***, ***I*4/*amd***, ***I*4/ $_1$*acd***
Trigonal	3	*P*3, *P*3$_1$, *P*3$_2$, *R*3, ***P*-3**, ***R*-3**

Trigonal	3*m*	*P*312, *P*321, $P3_112$, $P3_121$, $P3_212$, $P3_221$, *R*32, *P*3*m*1, *P*31*m*, *P*3*c*1, *P*31*c*, *R*3*m*, *R*3*c*, ***P*-31*m*, *P*-31*c*, *P*-3*m*1, *P*-3*c*1, *R*-3*m*, *R*-3*c***
Hexagonal	*6/m*	*P6*, $P6_1$, $P6_2$, $P6_3$, $P6_4$, $P6_5$, *P*-6, ***P6/m***, ***P6*$_3$*/m***
Hexagonal	6/*mm m*	*P*622, $P6_122$, $P6_222$, $P6_322$, $P6_422$, $P6_522$, *P*6*mm*, *P*6*cc*, $P6_3c$ *m*, $P6_3mc$, *P*-6*m*2, *P*-6*c*2 *P*-62*m*, *P*62*c*, ***P*6/*mmm*, *P*6/*mcc*, *P*6$_3$/*mcm*, *P*6$_3$/*mmc***
Cubic	*m*3	*P*23, *F*23, *I*23, $P2_13$, $I2_13$, ***Pm*-3, *Pn*-3, *Pa*-3, *Fm*-3, *Fd*-3, *Im*-3**, *Ia*-3
Cubic	*m*3*m*	*P*432, $P4_232$, $P4_332$, $P4_132$, *F*432, $F4_132$, *I*432, $I4_132$, *P*-43*m*, *P*-43*n*, *F*-43*m*, *F*-43*c*, *I*-43*m*, *I*-43*d*, ***Pm*-3*m*, *Pn*-3*n*, *Pm*-3*n*, *Pn*-3*m*, *Fm*-3*m*, *Fm*-3*m*, *Fd*-3*m*, *Fd*-3*c*, *Im*-3*m*, *Ia*-3*d***

The space groups in **bold** are centrosymmetric, and the *enantiomorphic* space groups are shown in magenta (58,59). In crystallography, a *centrosymmetric point group* has an inversion centre as one of its symmetry elements, such that every point group (*x*, *y*, *z*) in the unit cell has inversion symmetry or point reflection (−*x*, −*y*, −*z*). By contrast, a non-centrosymmetric point group lacks an inversion centre and can be either polar or chiral (58-65).

2.3 Objective and Rationale

The primary objective of the work described in this chapter is to analyse the structural and crystallographic characteristics of TLM analogues and IBU to determine their potential for forming racemic or conglomerate crystals. This should enable us to investigate how molecular structures influence whether a compound crystallises as a racemate or a conglomerate, using TLM analogs described in the literature and computational analysis". The rationale for choosing TLM is its relevance as a promising scaffold in antibacterial drug development and its structural amenability to modification. IBU serves as a control and comparative model due to its well-documented crystallisation properties, which include both racemic and conglomerate forms.

2.4 Methodology

2.4.1 Computational analysis of TLM analogues to predict their crystallisation behaviour

A systematic computational approach to predicting the crystallisation behaviour of TLM analogues was adopted here as follows:

-Software Utilised: Mercury and associated crystallographic tools were used for visualising and analysing crystal structures.

-Data Collection: Structural data for known TLM analogues and structurally related compounds were sourced from crystallographic databases, including the Cambridge Structural Database (CSD).

-Parameters Assessed

-Space Groups: Space groups were identified to determine whether they are centrosymmetric (indicating racemic formation) or non-centrosymmetric (suggesting potential for conglomerate formation).

-Packing Patterns: Molecular packing was analysed to understand interactions that favour enantiomeric or racemic crystals.

-Hirshfeld Surface Analysis: This analysis was used to assess intermolecular interactions and crystal packing efficiency.

2.4.2 Computational study of IBU

A computational study of IBU was included to benchmark the computational approach, as follows:

-Objective: Validate the effectiveness of the computational tools by analysing a compound with known crystallisation behaviour.

-Space Group Analysis: The space group of IBU was determined using crystal data and compared to literature values to confirm the potential for both racemic and conglomerate formation.

-Energy Calculations: The stability of racemic versus enantiomeric forms was evaluated to understand thermodynamic preference under standard crystallisation conditions.

The thiolactone ring acts as a valuable synthetic scaffold for the preparation of synthetic compounds with important pharmacological activity. Thiophenone derivatives are found in many bioactive compounds such as TLM, one of the most important biologically active, thiophenone-based natural products. To explore the opportunities for employing such derivatives in this field, the Crystal Structure Database was searched for a set of compounds similar to TLM analogues. A limited number of compounds were found, and the crystal structures of these were compared. Subsequently, the crystal structure of each molecule was studied, how the molecules bonded was determined, and the space group of the crystal filling was identified to determine whether a compound tended to crystallise as a racemic compound or as a conglomerate. In this context, the following questions arose: When searching databases for compounds similar to TLM analogues and that have structural similarities, as it possible to determine the effects of single-molecular functionality differences on crystallisation? Moreover, what molecular changes lead to a conglomerate rather than a racemic crystal? To answer these questions, the Mercury and Crystal Explorer software programs were used for visualising crystal structures and illuminating intermolecular interactions in the crystalline state. These crystallisation studies were then used to construct a model for predicting new molecules that crystallise as conglomerates instead of racemic forms.

To obtain an understanding of the solid-state behaviour of the thiolactone ring structure in terms of what molecular characteristics would favour the formation of the conglomerate form, the Cambridge Structural Database (CSD) was used. This database contains data from single-crystal X-RPD studies reporting important information on cell parameters, atomic coordinates, and refinements. These data were then used to determine the bond lengths, bond angles, and torsion angles of the molecules via ConQuest software.

The ConQuest interface comprises four principal sections: Build Queries, Combine Queries, Manage Hitlists, and View Results. The Build Queries section enables the user to construct a

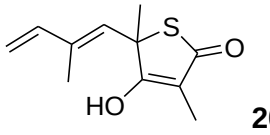
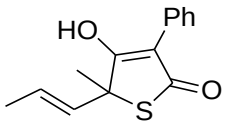
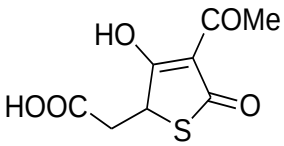
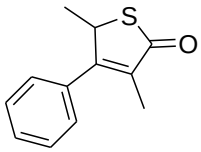
query using various parameters, including formula, space group, and refcode. The latter works based on the unique reference code (refcode) assigned to each entry by the CSD.

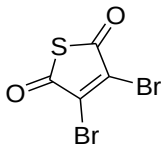
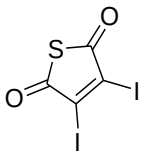
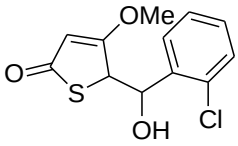
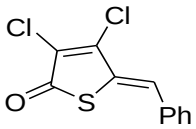
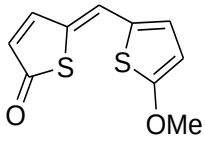
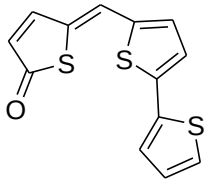
Next, Mercury software, a crystal structure visualisation tool that enables the analysis, design, and prediction of structures, was applied to the selected structures to identify and examine intermolecular hydrogen bond interactions. Crystal Explorer, a free software that aids the analysis of crystal structures by allowing their intermolecular contacts to be observed, was also used. The main objective of this programme is to display and interpret Hirshfeld surfaces, which are effective tools for exploring the experimental information generated through crystallography and visualising intermolecular interactions in the crystalline state.

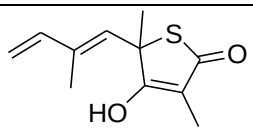
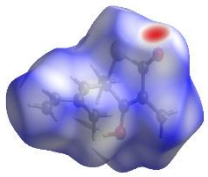
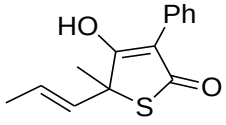
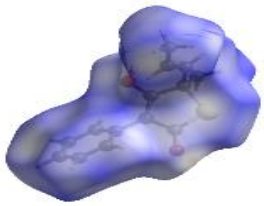
By searching the database through the thiolactone core structure on the ConQuest programme using the Build Queries, various thiophenone derivatives with the potential for structural diversity, that is, compounds bearing structural similarities to thiolactomycin antibiotics, were identified (Table 2). This supports the idea that analysing and resolving data on molecules already present in database may reveal that some chiral compounds can have racemic or conglomerate forms that are structurally similar to TLM analogues.

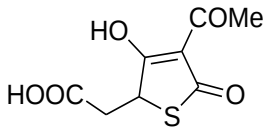
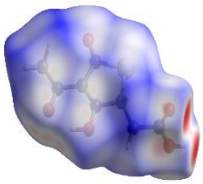
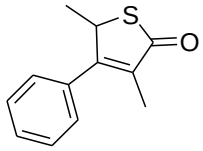
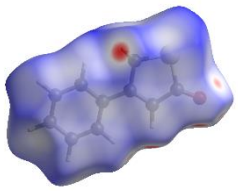
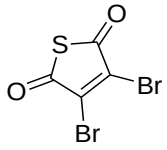
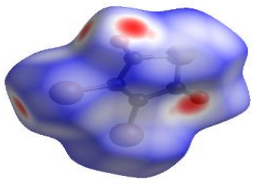
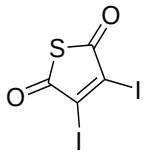
Next, the Mercury programme was used to visualise the crystal structure data, and subsequently, I mapped the Hirshfeld surfaces with dnorm and shaped index functions to obtain further information on the interaction types within the crystals using the Crystal Explorer programme. These compounds were carefully selected to support our explanation and understanding of various concepts in chemistry, such as stereochemistry, functional groups, and symmetry (66).

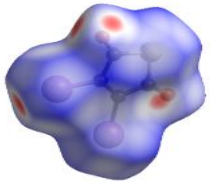
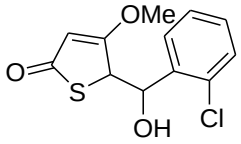
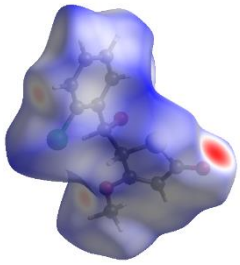
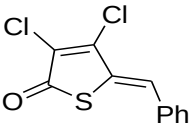
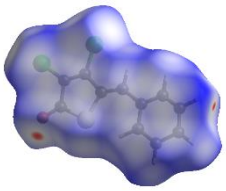
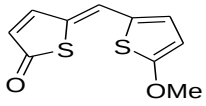
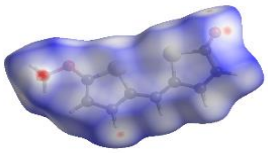
Table 2. Compounds bearing structural similarities to thiolactomycin in the ConQuest database

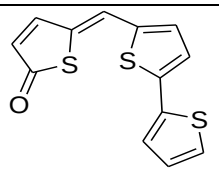
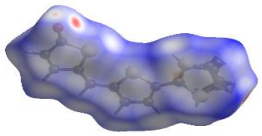
 **20**	 **21**	 **22**	 **23**

 **24**	 **25**	 **26**	 **27**
 **28**	 **29**		

Analogue	**Space Group**	**Unit Cell**	**Crystal System**	**Type**
 **20** 	$P6_5$	a = 9.8514(6) Å α = 90° b = 9.8514(6) Å β = 90° c = 19.954(1) Å γ = 120°	Hexagonal crystal	Conglomerate
 **21** 	$P2_1$	a = 9.696(1) Å α = 90° b = 12.094(2) Å β = 98.61(1)° c = 11.140(2) Å γ = 90°	Monoclinic	Racemic or conglomerate

 **22** 	P1	a = 5.057(2) Å α = 81.017(6)° b = 8.028(4) Å β = 87.832(6)° c = 11.167(5) Å γ = 80.515(6)°	Triclinic	Conglomerate
 **23** 	$P2_1/n$	a = 7.961(1) Å α = 90° b = 6.408(1) Å β = 96.61(1)° c = 16.413(3) Å γ = 90°	Monoclinic	Racemic
 **24** 	$P4_12_12$	a = 7.543(1) Å α = 90° b = 7.543(1) Å β = 90° c = 12.155(4) Å γ = 90°	Tetragonal	Conglomerate
 **25**	$P4_12_12$	a = 7.816 (1) Å α = 90° b = 7.816 (1) Å β = 90 (1)° c = 12.348 (1) Å γ = 90°	Tetragonal	Conglomerate

				
 **26** 	$P2_1/c$	a = 10.8350(12) Å α = 90° b = 9.0220(12) Å β = 108.445(12)° c = 13.321 Å γ = 90°	Monoclinic	Racemic
 **27** 	$P2_1/n$	a = 7.036(2) Å α = 90° b = 11.560(4) Å β = 103.55(2)° c = 13.592 (2) Å γ = 90°	Monoclinic	Racemic
 **28** 	$P2_12_12_1$	a = 3.8585 (6) Å α = 90° b = 10.9330 (17) Å β = 90° c = 22.965 Å γ = 90°	Orthorthombic	Racemic or conglomerate

  **29**	P1	a = 3.828(2) Å α = 100.24(3)° b = 5.892(4) Å β = 94.66(2)° c = 13.221 (9) Å γ = 91.96(3)°	Triclinic	Conglomerate

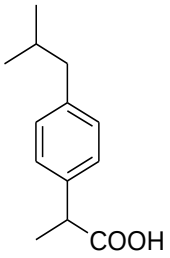
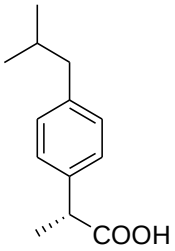
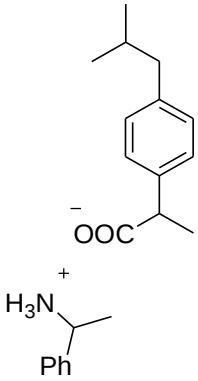
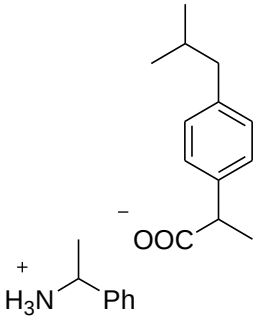
There are various non-random ways of selecting compounds that structurally resemble thiolatomycin or have some form of thiolactone ring similarities to the thiolactone core structure. These similarities were expected to generate ideas of how compounds crystallise as conglomerates. However, the structural similarities failed to provide any relevant insights on this topic. Against this background, it would be beneficial to use surface characteristics or the space group of the crystal filling to determine whether a compound tends to crystallise as a racemic compound or a conglomerate. Obtaining a better understanding of chiral discrimination in the solid state and during the recrystallisation of enantiomers would also be beneficial.

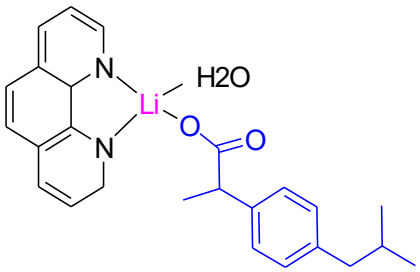
Compound **20** crystallises in a hexagonal crystal shape in the $P6_5$ non-centrosymmetric space group. According to the table, this space group is among the enantiomorphic space groups (referring to its absolute configuration). Compound **21** crystallises in a monoclinic crystal shape in the $P2_1$ non-centrosymmetric space group. Compounds **23** and **27** crystallise in monoclinic crystal shapes in the $P2_1/n$ centrosymmetric space group. Compound **26** crystallises in a monoclinic crystal shape in the $P2_1/c$ centrosymmetric space group. Therefore, compounds **21**, **23**, **26**, and **27** are racemic in nature. Compounds **22** and **29** crystallise in the triclinic P1 non-centrosymmetric space group, indicating that these are conglomerate compounds (P1 represents 95% of the known conglomerates) (25). Compounds **24** and **25** crystallise in tetragonal packing in the $P4_12_12$ non-centrosymmetric space group. Compound **28** crystallises in an orthorhombic crystal shape in the $P2_12_12_1$ non-centrosymmetric space group. Compounds **20**, **22**, **24**, **25**, and **29** crystallise as non-centrosymmetric, most likely to produce conglomerate crystals. In brief, compounds that crystallise in a centrosymmetric space group have mirror images called racemic compounds,

whereas compounds that crystallise in a non-centrosymmetric space group could be racemates or conglomerates.

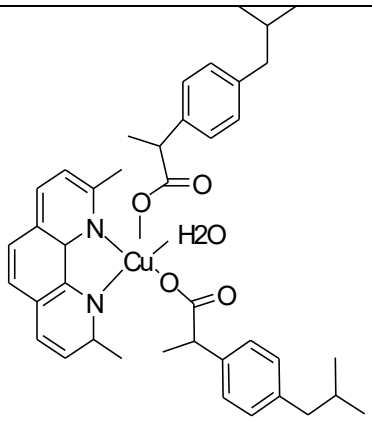
The data from the the ConQuest database were insufficient to identify compounds with structural similarities to TLM analogues. Therefore, IBU was used as a model to determine whether this approch was useful, as there were already IBU compounds in the database. An alternative approach to conglomerate production is to use salt derivatives such as IBU compounds. The potential of salt derivatives to produce conglomerate crystals was studied, as shown in Table 3.

Table 3. Ibuprofen compound and salt derivatives

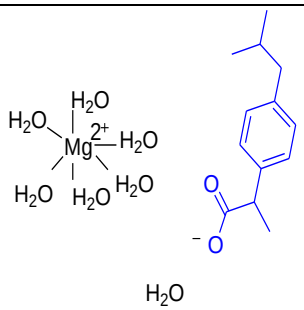
 **30**	 **16**
 **31**	 **19**



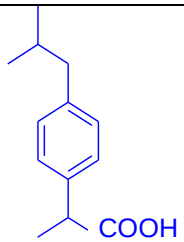
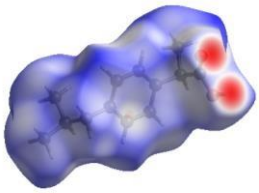
32

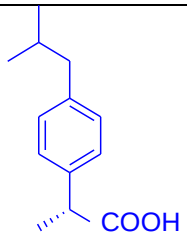
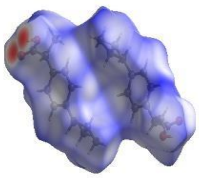
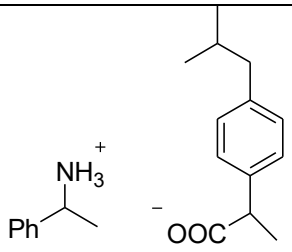
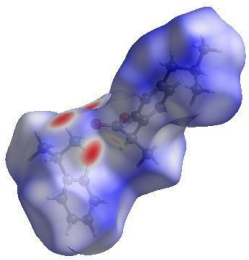
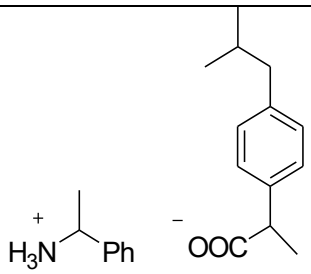


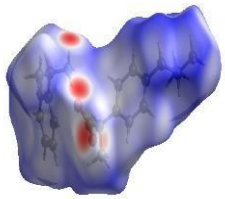
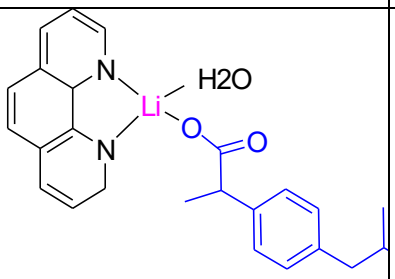
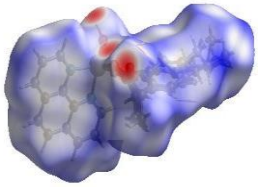
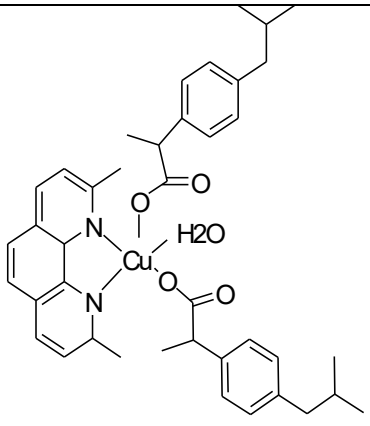
33

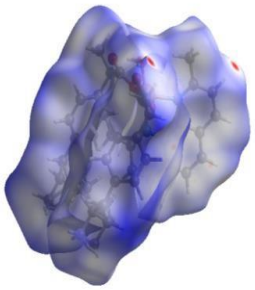
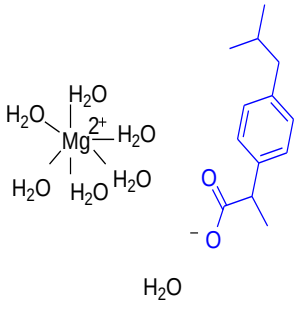
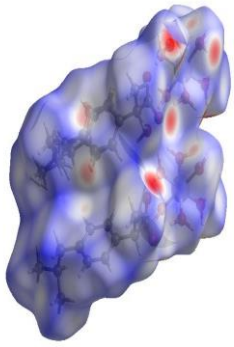


34

Analogue	Space Group	Unit Cell	Crystal System	Type
 **30** 	$P2_1/c$	a = 14.522(7) Å $\alpha = 90°$ b = 7.817(2) Å $\beta = 99.54\ (8)°$ c = 10.619 (13) Å $\gamma = 90°$	Monoclinic crystal	Racemic

Analogue	Space Group	Unit Cell	Crystal System	Type
  **16**	$P2_1$	a = 12.462 (3) Å α = 90° b = 8.035 (3) Å β = 112.89 (3)° c = 13.539 (4) Å γ = 90°	Orthorhombic	Conglomerate
 **31** 	$P2_12_12_1$	a = 5.881 (5) Å α = 90° b = 15.259 (6) Å β = 90° c = 22.318 (2) Å γ = 90°	Orthorhombic	Racemic or conglomerate
 **19**	$P2_12_12_1$	a = 5.8873 (13) Å α = 90° b = 15.257 (4) Å β = 90° c = 22.235 (6) Å γ = 90°	Orthorhombic	Racemic or conglomerate

Analogue	Space Group	Unit Cell	Crystal System	Type
				
 **32** 	P1	a = 7.1787(5) Å α = 96.057 (3)° b = 10.2822 (6) Å β = 101.245° C = 16.1772(10) Å γ = 106.314°	Triclinic	Conglomerate
 **33**	P1	a = 10.3365 (6) Å α = 113.075(3)° b = 12.8412 (6) Å β = 90.900(3)° c = 15.3978 (8) Å γ = 93.3745(19)°	Triclinic	Conglomerate

Analogue	Space Group	Unit Cell	Crystal System	Type
				
 **34** 	P1	a = 6.5483 (2) Å α = 80.677 (2)° b = 10.7963 (3) Å β = 85.513 (2)° c = 23.2598 (7) Å γ = 88.711 (2)	Triclinic	Conglomerate

Clearly, compounds such as compound **30,** which crystallise in a monoclinic crystal shape in the $P2_1/c$ centrosymmetric space group, will produce racemates. Meanwhile, single isomers, either S- (**31**) or R-ibuprofen (**19**), which crystallise in an orthorhombic crystal shape in the $P2_12_12_1$ non-centrosymmetric space group, could produce racemates or conglomerates. They will most likely but not certainly produce conglomerates. It is also uncertain whether a conglomerate or a racemate will be produced for compound **16** (S-ibuprofen), which crystallises in the non-centrosymmetric $P2_1$ space group. To overcome these uncertainties, ibuprofen salt derivatives can be entered into compounds such as lithium, copper, and

magnesium ibuprofen salts (compounds **32**, **33**, and **34**, respectively), all of which crystallise in the triclinic non-centrosymmetric P1 space group. This space group is only known to produce conglomerate compounds and represents 95% of known conglomerates (32).

2.5 Results and Crystallographic Space Group Determination

The results presented here focus on the crystallisation behaviour of the TLM analogues reported on in the literature. The data show that, while some analogues crystallised as racemates, others exhibited conglomerate behaviour.

2.5.1 Crystallographic Data for TLM Analogues

Table 2 presents the crystallographic data for the TLM analogues found in the literature, summarising the space groups, unit cell parameters, and observed packing types.

The crystallographic data were obtained from SC-XRD and confirmed that the analogues crystallised in different space groups. Catagorisation into the space groups provides insight into whether a compound will form a racemic compound or a conglomerate. For example, TLM analogues that crystallised in the **P21 space group** showed a tendency to form conglomerates.

2.5.2 Computational Analysis of IBU

-Confirmed Findings: The computational analysis reported on her validated that IBU can crystallise in both racemic (P21/c) and conglomerate (P21) forms depending on crystallisation conditions. These findings were consistent with those of the established literature, confirming the reliability of the computational tools used.
-Energy Comparison: The energy calculations indicated that the racemic form of IBU was slightly more stable under typical crystallisation conditions, but that the conglomerate form may be preferable when parameters such as seeding and temperature, are modified.

2.6 Discussion: Crystallisation Behaviour and Molecular Structure

2.6.1 Comparison of TLM analogues and IBU

-**Space Group Observations**: As stated earlier, compounds crystallising in **non-centrosymmetric space groups,** such as **P21 and P212121,** are more likely to form conglomerates. This is consistent with the findings from the TLM analogues studied. These space groups are non-centrosymmetric, indicating that the molecules pack as individual enantiomers, leading to conglomerate crystallisation. In contrast, **centrosymmetric space groups** favour racemic compound formation. TLM Analogues that crystallised in space group (P21/c) known for racemic formation. This aligns with the observation that most organic molecules display racemic crystal formation due to its more stable packing arrangements.

-**Potential for Conglomerate Formation**: One TLM analogue exhibited a non-centrosymmetric space group ($P2_12_12_1$), a promising sign for potential conglomerate behaviour. Further experimental investigation could help determine the conditions needed for this analogue to crystallise as a conglomerate.

-**IBU Insights**: The results of the TLM analogues were compared with the model compound. Interestingly, the IBU analogues, which also crystallised in non-centrosymmetric space groups, provided a useful contrast, demonstrating that these space groups do not always guarantee conglomerate formation. This further illustrates the complexity of predicting crystallisation behaviour based solely on space group data.

The comparison with IBU confirmed the reliability of the computational approach and underscored the need for tailored crystallisation conditions to favour conglomerate formation in compounds that do not naturally exhibit this tendency.

2.6.2 Implications for deracemisation strategies

The above results underscore the importance of space group analysis in predicting a compound's crystallisation behaviour. This study reveals that, while some TLM analogues naturally crystallise as racemic compounds, there is potential for achieving conglomerate forms through controlled crystallisation techniques such as seeding with enantiopure crystals or adjusting solvent and temperature conditions. The

success with IBU highlights the potential to apply similar strategies to more complex molecules such as TLM.

2.7 Conclusion

This examination of crystallisation behaviour in TLM analogues has provided valuable insights into the relationship between molecular structure and solid-state behaviour. Compounds crystallising in non-centrosymmetric space groups are more likely to form conglomerates, but additional factors, such as intermolecular interactions, must also be considered. The findings from both TLM and IBU analogues suggest that while space group analysis is a valuable tool, it cannot be used alone to determine whether a compound will crystallise as a racemate or a conglomerate.

The computational study of TLM analogues and IBU provided valuable insights into their crystallisation behaviours, TLM Analogues predominantly crystallise as racemic compounds but show potential for non-centrosymmetric packing under specific conditions, and IBU validation confirmed the effectiveness of the computational approach and highlighted the conditions needed for conglomerate formation.

The findings lay the groundwork for future efforts aimed at deracemisation, particularly through preferential crystallisation methods that exploit non-centrosymmetric properties.

In summary, conglomerate crystals can be resolved using surface characteristics or the space group of crystal filling to determine whether a compound tends to crystallise as a racemic compound or a conglomerate. This is shown in Table 1, which lists the unique space groups categorised by crystal system and Laue class.

Chapter 3:

Chemistry and Determination of Conglomerate Crystal Form

3.1 Introduction

Chapter 3 focuses on the synthesis, crystallographic analysis and spectroscopic study of thiolactomycin (TLM) analogues. The main objective here is to identify the potential of these analogues to form conglomerate crystals, a key step towards effective deracemisation. This chapter details the methodologies used for synthesising TLM analogues, examines their crystallisation properties and provides a comprehensive analysis of their structural and thermal behaviour.

3.2 Synthesis of TLM analogues

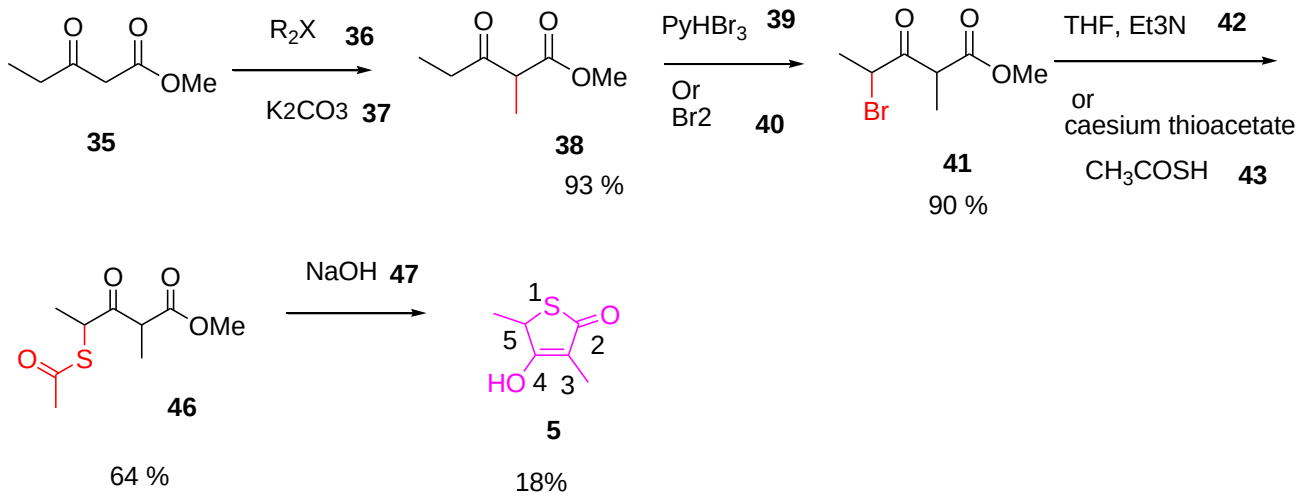
3.2.1 Objective and approach

The synthesis of TLM analogues was undertaken to explore how structural variations influence crystallisation behaviour. The analogues were modified at the 3- and 5-positions to assess the impact of these modifications on the formation of racemic or conglomerate crystals.

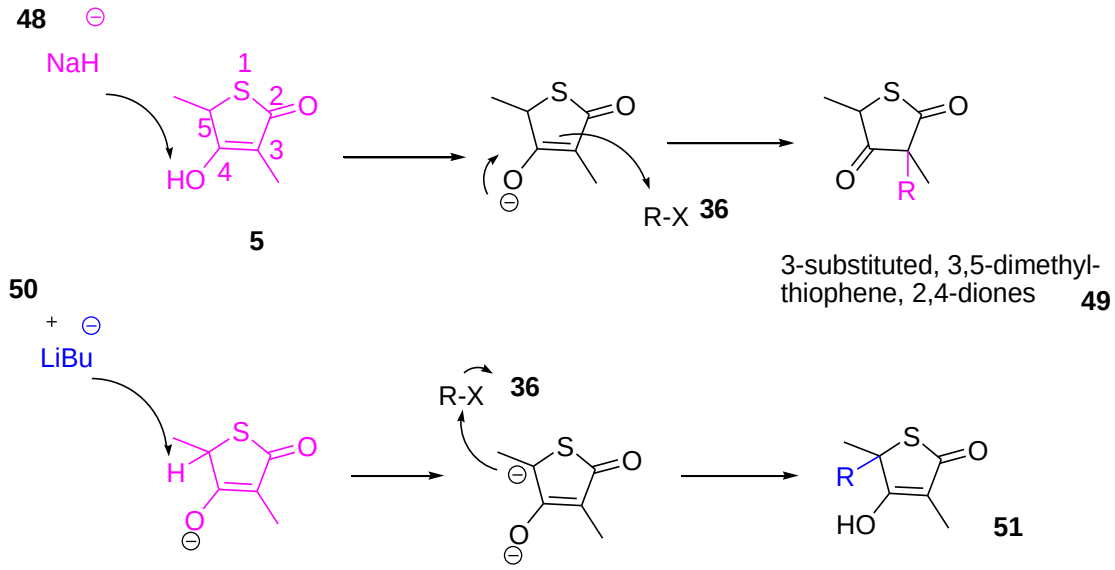
Over the last three decades, efforts aimed at synthesising a single enantiomer of TLM and analogous compounds have involved relatively complex procedures. Specifically, these have employed asymmetric synthesis, diastereomeric recrystallisation or enzymatic resolution, which require many steps and have thus significantly restricted the development of TLM **20**, (5R)-4-hydroxy-3,5-dimethyl-5-[(1E)-2-methylbuta-1,3-dienyl]thiophen-2-one and analogues with a similar scaffold towards clinical application. For example, Chambers et al. (67,68) and McFadden et al. (69) addressed the absolute configuration of (5R)-thiolactomycin and other thiotetronic acids through efficient asymmetric synthesis of naturally occurring (5R)-thiolactomycin using D-alanine as the source of chirality.

However, few reports on enantiomerically pure TLM analogues have been published, and most studies have been performed with racemic mixtures (70). The first total synthesis of a racemic thiolactomycin or thiolactone core used as a key intermediate for further synthesis of a TLM analogue was reported by Wang and Salvino (71). Although Wang and Salvino used

Claisen self-ester condensation of methyl propionate with KH to yield β-keto ester, here I instead used methyl propionylacetate **35** as a starting material. It was treated with iodomethane (alkyl halide) **36** in the presence of anhydrous potassium carbonate (K_2CO_3) **37** as the base, which afforded methylated β-keto ester or alkylated products **38**. Selective bromination of a compound with pyridinium tribromide **39** or even bromine reagent **40** in the presence of acetic acid or chloroform solvent yields a brominated compound as a mixture of diastereoisomers **41**. Displacement of the bromide group was successfully achieved by tri-ethylamine base **42** or another modification of the method reported by Wang and Salvino, which used caesium thioacetate **43** from thioacetic acid **44** and caesium carbonate **45** (due to the caesium effect) in the second step instead of a tri-ethylamine base to produce 2-acetylsulfanyl-2-methyl 3-oxopentanoic acid methyl ester **46**. Finally, cyclisation to the desired thiolactones was accomplished upon treatment of the stirred solution of the thioacetate in ethanol with an aqueous potassium hydroxide solution or sodium hydroxide **47** at an ambient temperature to obtain 3-hydroxy-2,4-dimethylthiophen-2(5H)-one, **5**, as given in Scheme 6 (45,71). If one equivalent of sodium hydride moderate base **48** or a weaker base is used, it will remove the protons of enols, and then treatment with an alkyl halide (RX) **36** would result in alkylation at position 3 of thiolactone ring **49**. Using a very strong base such as *t*-butyl lithium **50** will remove additional protons at C5 of the thiolactone ring. This creates a more reactive anionic centre of the dianion that can be alkylated with RX to yield 5-substituted derivative **51**, which is preferable at position 5, as shown in Scheme 7 (41).

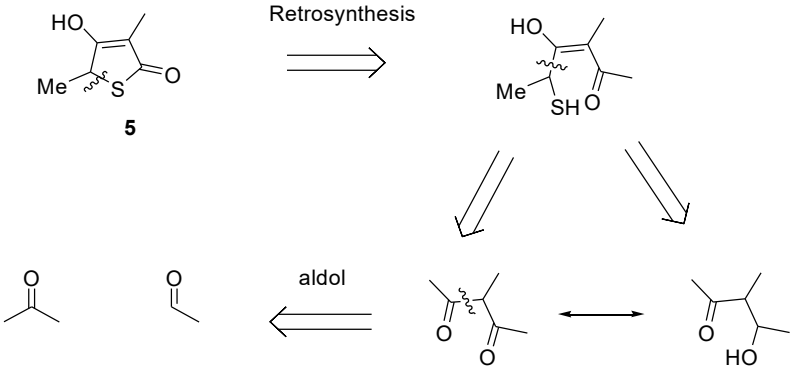


Scheme 6. Strategy and general procedure for the synthesis of TLM analogues (71).

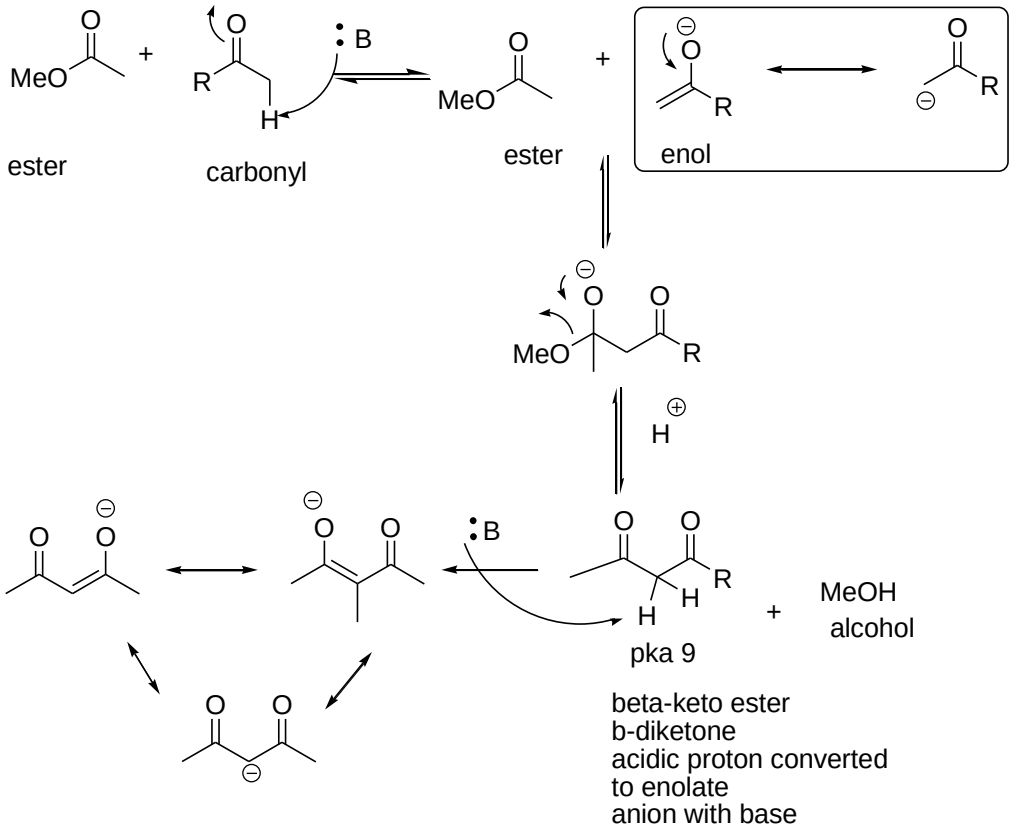


Scheme 7. Different derivatives obtained using weak or strong bases (41).

The retrosynthesis of compound **5** is shown in Scheme 8, and the forward reaction is shown in Scheme 9.

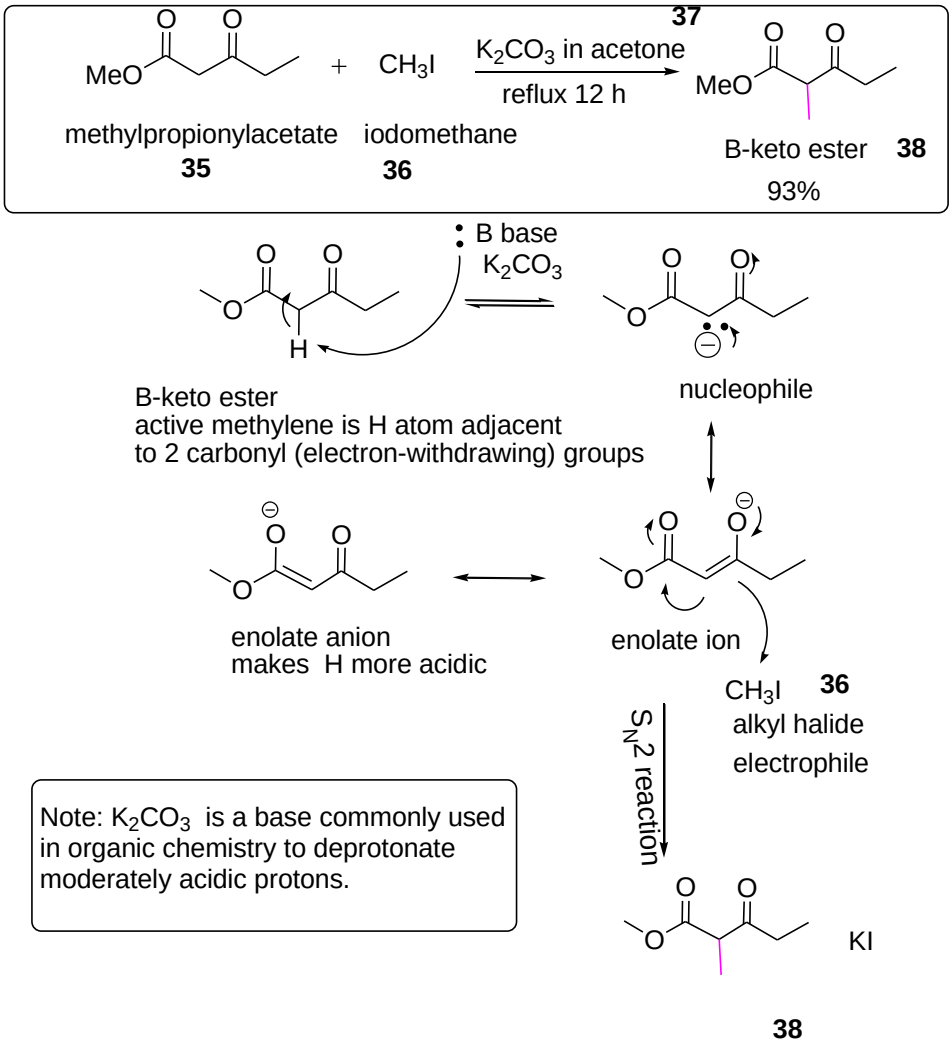


Scheme 8. Retrosynthesis of compound **5**.



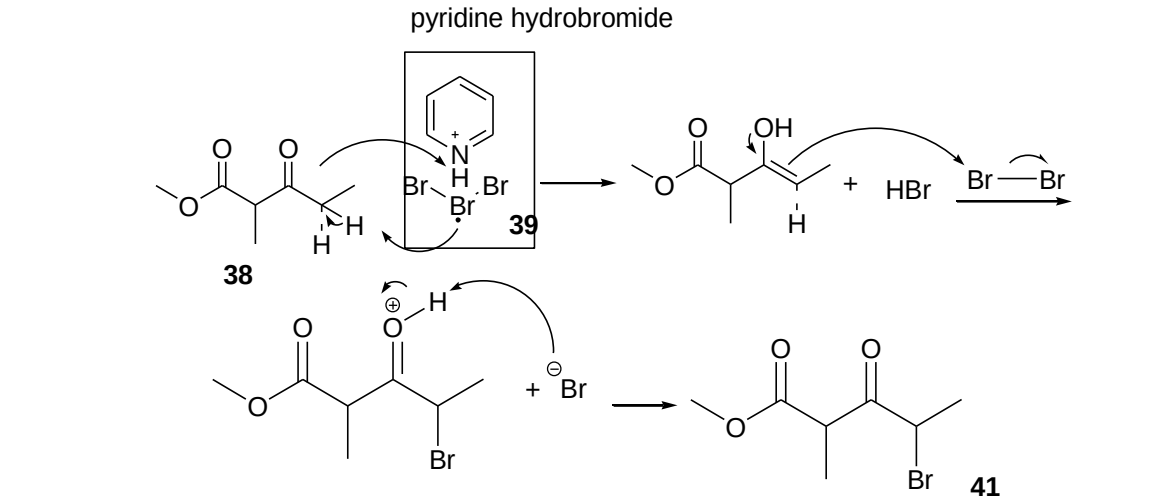
Scheme 9. Forward reaction.

The first compound (methyl-2-methyl 3-oxopentanoate) **38** from methyl 3-oxopentanoate **35** and methyl iodide **36** was successfully synthesised with 93% yield (72). Scheme 10 shows the proposed mechanism by which the first compound is produced.

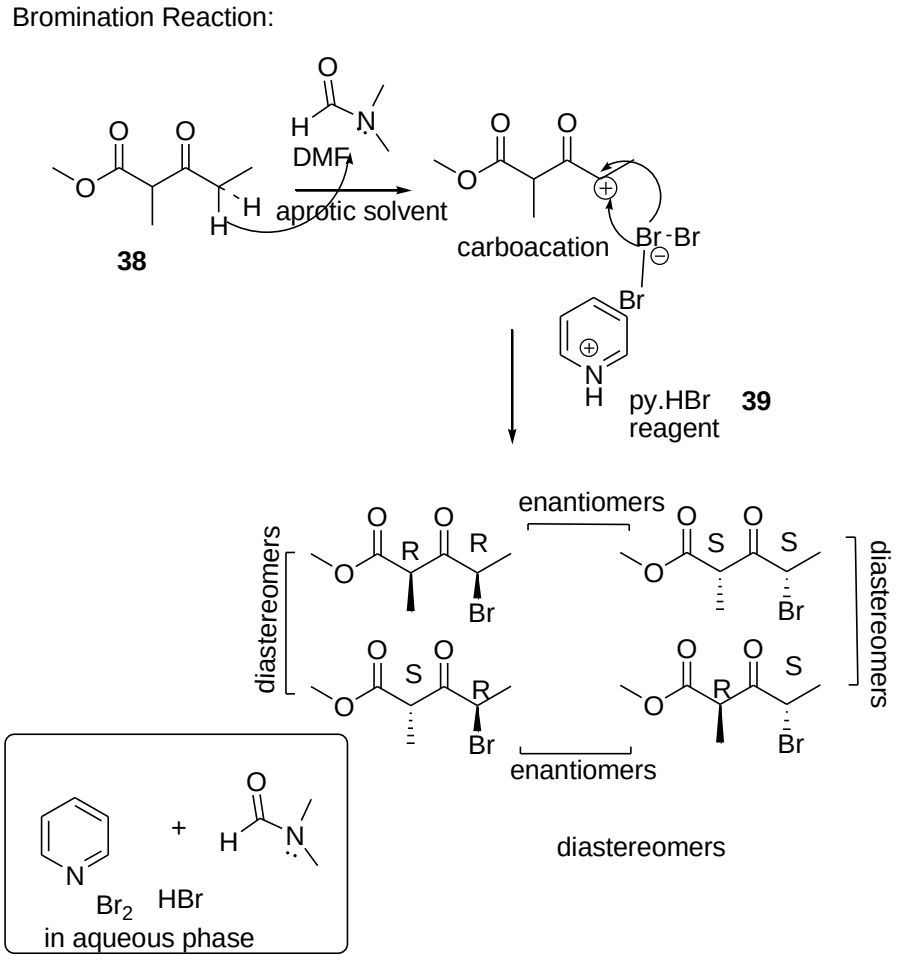


Scheme 10. Proposed mechanism of action for the first step.

The second step is a brominating reaction. Bromination with pyridine hydrobromide (py.HBr_3) **39** is safe and convenient, as it is easy to maintain the stoichiometric ratio during the reaction. Unfortunately, it was not possible to ship this material to Saudi Arabia, as it is considered hazardous and requires special airline approval for transport to Jeddah, KSA. Unlike their non-solid equivalents, solid brominating agents are easier to handle, safer, more convenient and more quickly prepared than liquid bromine **40** brominating agents, which have a tendency to cause eye irritation. The proposed mechanism for bromination using ammonium hydrotribromide salt is shown in Schemes 11 and 12 (73,74).



Scheme 11. Mixture of diastereoisomer brominated compounds after S_N1 reaction (17).

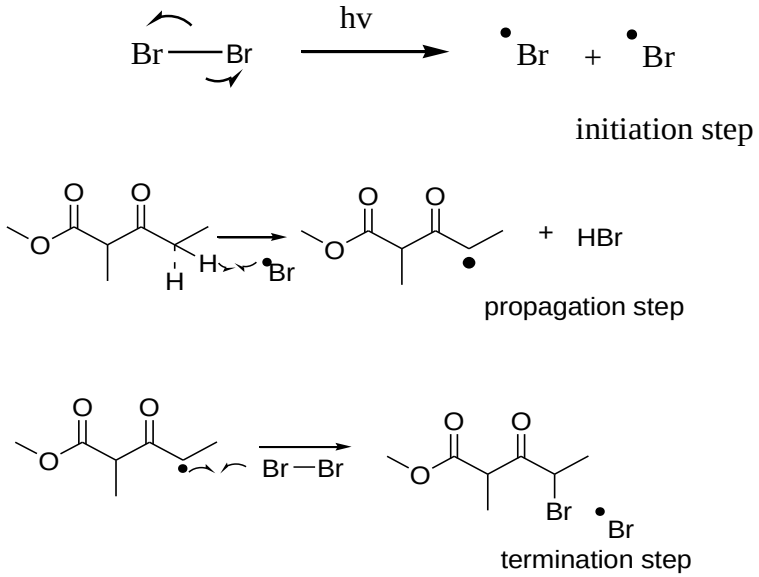


Scheme 12. Mechanism for bromination by ammonium hydrotribromide salt (73,74).

Even when the reaction was run with py.HBr_3 **39** in glacial acetic acid, it could not be completed because laboratory bulb-to-bulb distillation (Kugelrohr distillation) is not available in Saudi Arabia. However, I continued modifying and optimising the conditions of the reaction to obtain the necessary product. Later, the reaction was attempted to dispose acetic acid by adding sodium bicarbonate and silica and performing purification using column chromatography. Compound (2RS,4RS)-4-bromo-2-methyl-3-oxopentanoic acid methyl ester **41** was successfully produced.

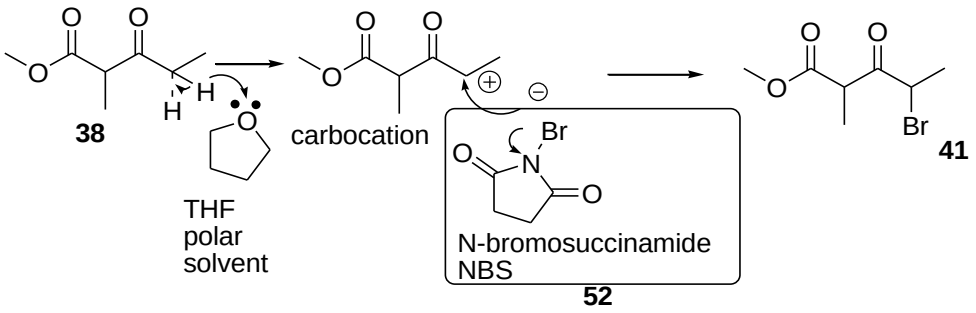
At the same time, we produced using bromine reagent (Br_2) **40**, but it was impure and therefore required further purification before proceeding to the next step.

Generally, all chemical reactions consist of the concurrent breakage and formation of bonds. There are two types of bond cleavage in the bromination reaction, as shown in Schemes 13 and 14. The first is homolytic bond cleavage and free-radical halogenation of alkanes, accompanied by either heat or light (6). In homolytic bond cleavage, two electrons in the bond are divided equally between the products, as shown in Scheme 13.



Scheme 13. Homolytic bond cleavage (17).

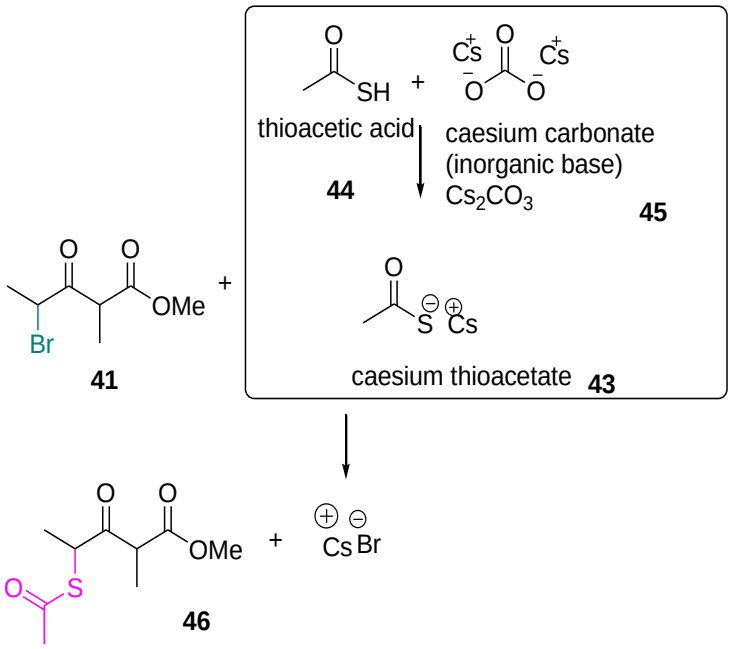
The second is the heterolytic bond cleavage S_N1 reaction, where the heterolytic splitting occurs at the polar bond and the electrons move towards the more electronegative atom using either *N*-bromosuccinamide (NBS) **52** or a liquid bromine reagent **40**, as given in Scheme 14.



Scheme 14. Mechanism of heterolytic bond cleavage.

In summary, homolytic cleavage produces neutral radicals and is unaffected by the nature of the solvent, while heterolytic cleavage depends on the polarity of the solvent. Another difference is that it is more difficult to describe enthalpy changes for heterolytic cleavages than for homolytic ones (17).

For the third step in the synthesis of $CsSCOCH_3$, the synthesis of the thiolactone core was accomplished using caesium carbonate **45**, in accordance with the procedure described by Bhowruth et al.(48), and the mechanism behind the generation of thioacetate is presented in Scheme 15.

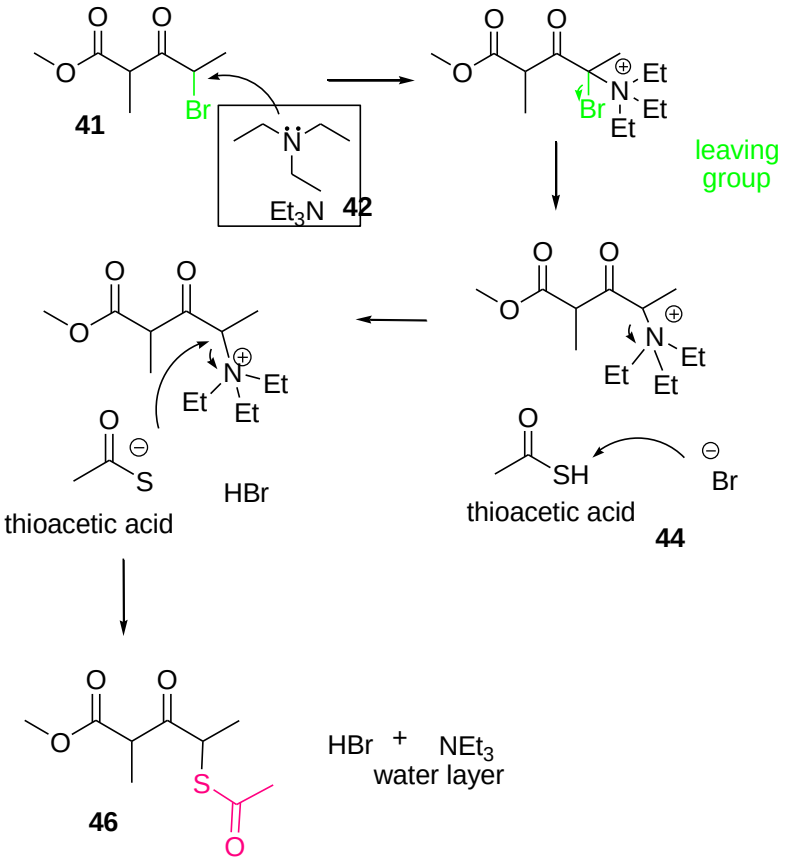


Scheme 15. The proposed mechanism behind the generation of thioacetate (14).

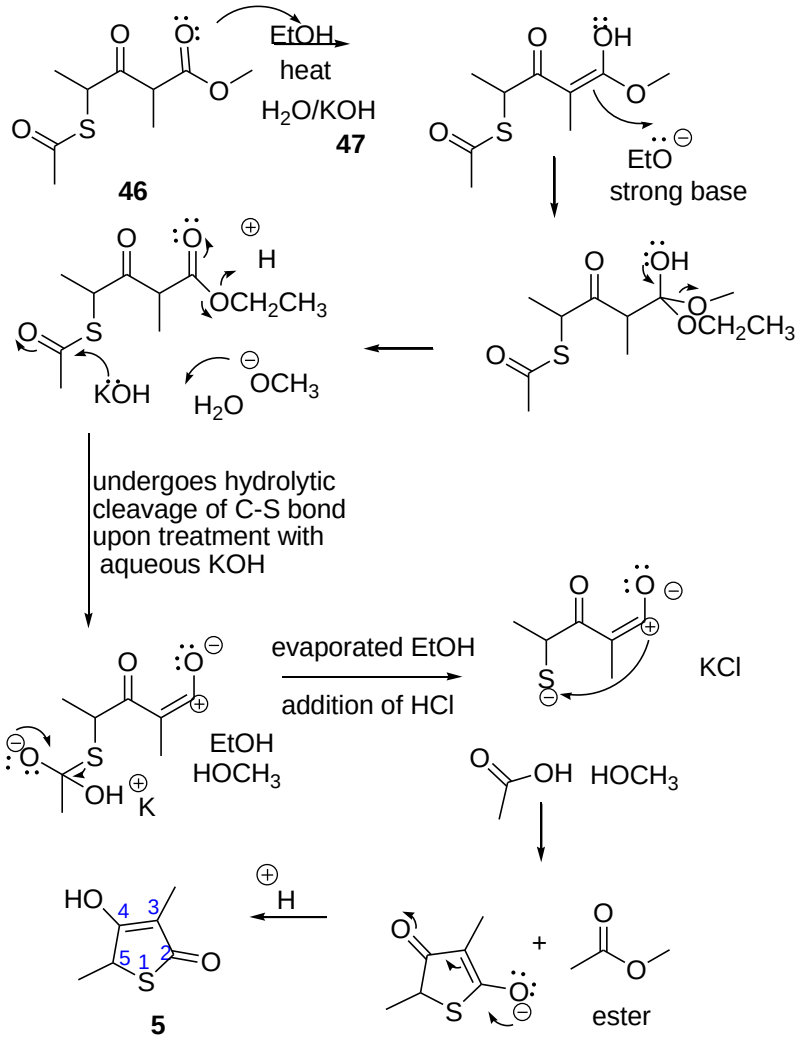
Caesium is the least electronegative element (Pauling scale: 0.79) in the periodic table, while fluorine is the most electronegative one (Pauling scale: 3.98). Caesium is a larger atom with one electron in its outermost shell and is likely to give up electrons, so it is less electronegative (sometimes called electropositive) and less attractive to its positive nucleus, making it easier for it to give away its own electrons. Within the periodic table, electronegativity increases upon passing from left to right along a period and decreases upon descending within a group.

Thiol (R-SH) is a stronger acid than alcohol (R-OH) and has proven excellent nucleophilicity in the S_N2 reaction of alkyl halide, given that the nucleophilicity of sulphur is much greater than that of oxygen. Owing to the electronegativity of sulphur being 2.58 compared with 3.44 for oxygen, sulphur is less electronegative than oxygen.

The proposed mechanism behind the generation of thioacetate using tri-ethylamine base **42** instead of caesium carbonate is shown in Scheme 16 (17). The proposed mechanism of hydrolysis and cyclisation is shown in Scheme 17 (see Supplementary Document).



Scheme 16. Mechanism behind the generation of the third thioacetate compound using a triethylamine base (17).

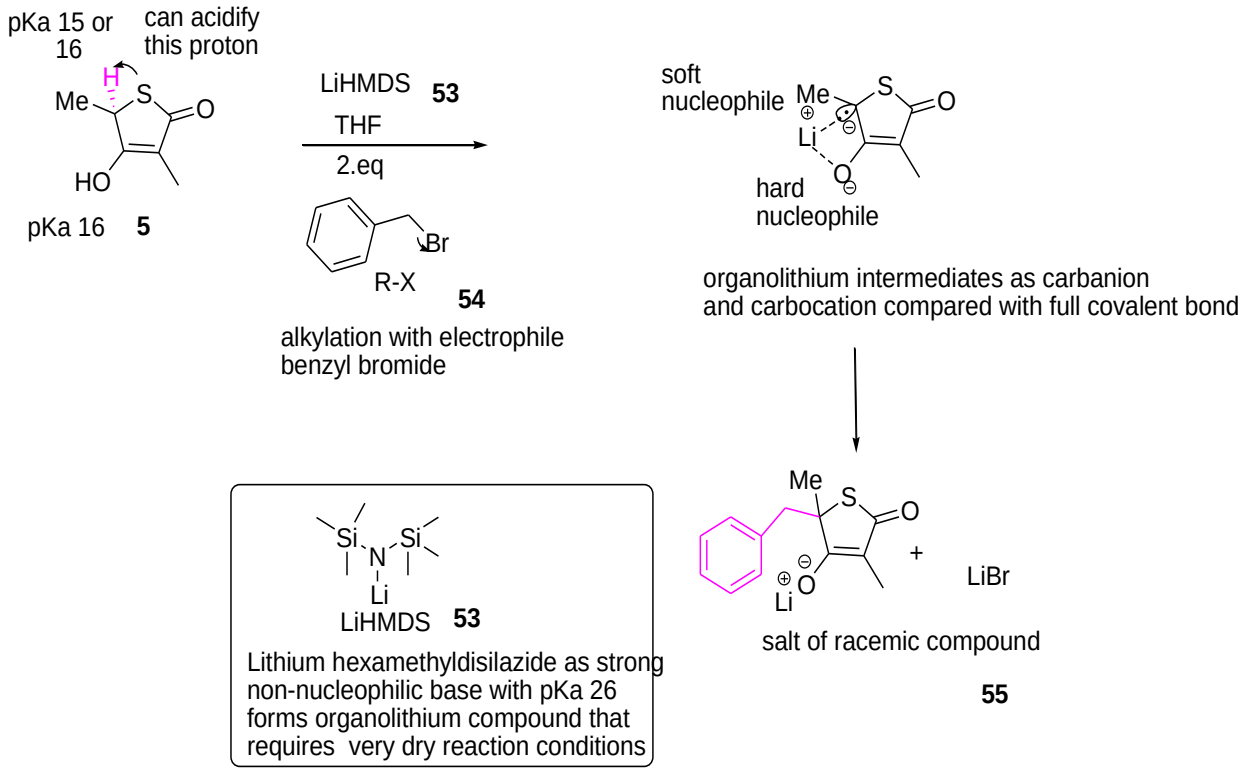


Scheme 17. Proposed mechanism of hydrolysis and cyclisation**.**

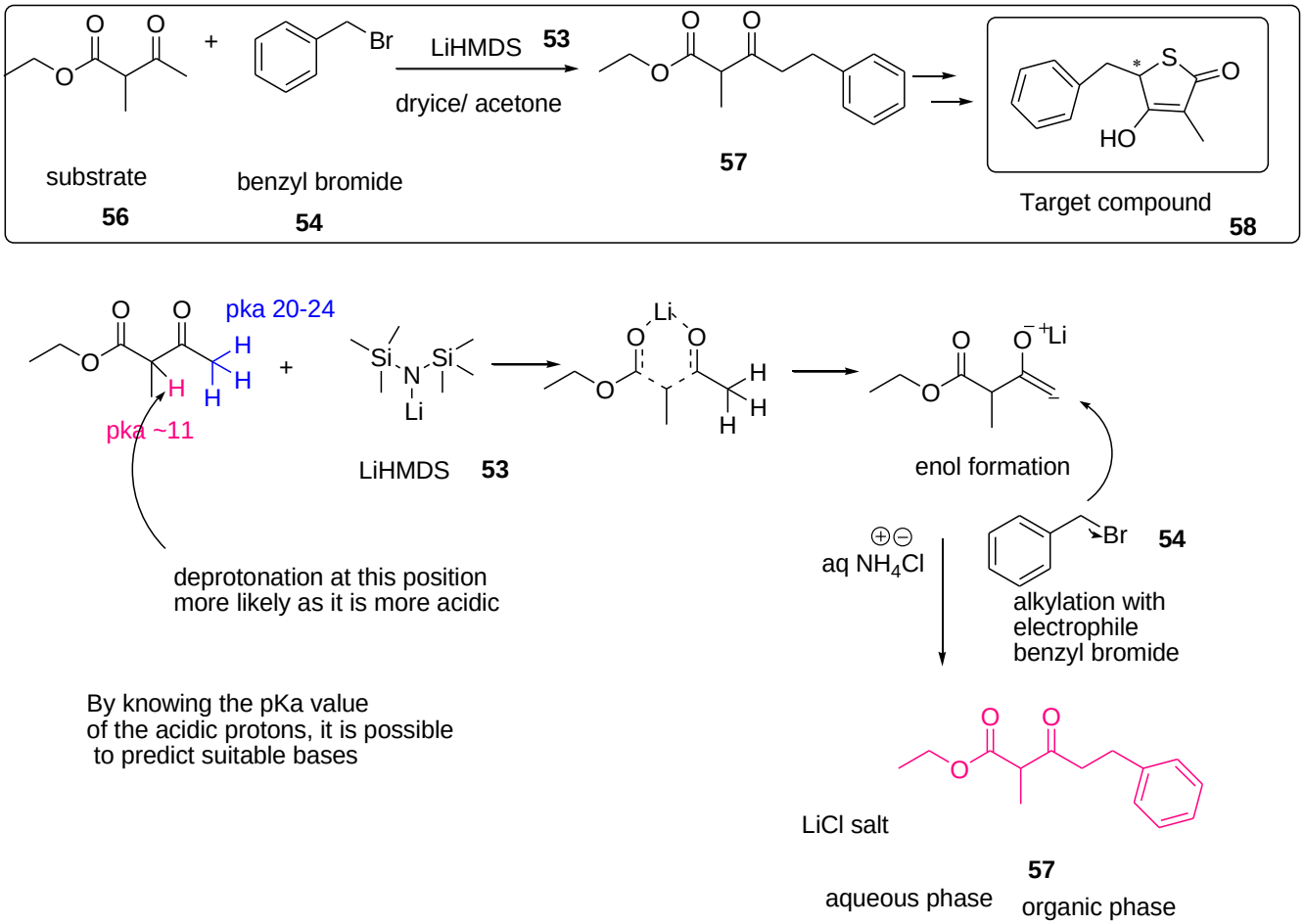
When strong non-nucleophilic lithium hexamethyldisilazide **53** is used, it will remove protons at C5 of the thiolactone ring in the preparation of the derivatives of TLM analogues **55,** as shown in Scheme 18 and 19.

The strength of an acid (its pKa value) depends directly on which proton is transferred from the acid to a base, which in turn is determined by the stability of the atom to which the acidic proton is attached. The factors that affect acidity are electronegativity, bond energies, inductive and steric effects, hybridisation, resonance stabilisation and aromaticity. Generally, thiophene can be metallated at the C5 position with alkyl lithium reagents; thus, C5 is more

prone to deprotonation than C3 because of the effect of the heteroatom of inducing electron withdrawal. Although C and S have similar electronegativity, sulphur is a larger atom than carbon, so the electron density in C–S preferentially shifts and resides on it. The C–S bond tends to polarise because of the polarisability of sulphur. Therefore, the size of the sulphur atom and its polarisability influence the stabilisation of these electrons. Specifically, the sulphur behaves as if the bond were polar, causing the electron density to shift towards the S atom (17).



Scheme 18. Preparation of the derivatives of TLM analogues.



Scheme 19. Proposed mechanism of the synthesis of the intermediate compound to synthesise the target compound **58**.

Synthesis of the intermediate compound **57** to synthesise target compound **58** was laborious and time-consuming in this work. To optimise these reaction conditions, two methods can be used. The first involves screening different bases such as the moderately strong base lithium hexamethyldisilazane (HMDS), the strong base *n*-butyllithium (n-BuLi) and lithium diisopropylamide. The second option, which I chose to apply, involves the use of a safer, less hazardous base that can be easily handled, such as an HMDS base. Various alternative approaches were applied in which the equivalent of the HMDS base was varied for each reaction, as shown in Table 4.

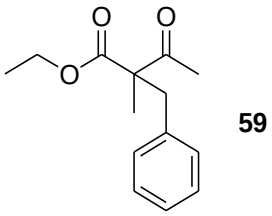
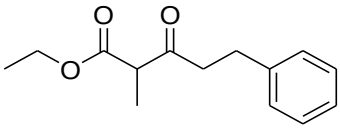
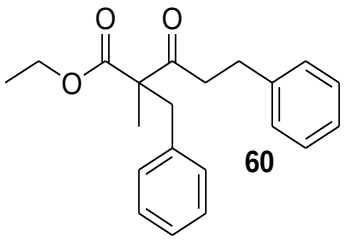
Base Used	**Result (Mass Spectrometry, NMR)**		
Equivalent of the HMDS base	 **59** A	 **57** B	 **60** C
1.1 equiv.	✓		
2.5 equiv.	✓	✓	✓
3 equiv.	✓	✓	✓

Table 4. The only variable that was changed in each reaction was the equivalent of the HMDS base.

It is clear that, when treating the starting material **56** with a 1.1 equivalent of an HMDS base, the proton at the active methylene group is more acidic, so it is most likely to deprotonate first and result in compound 3-position analogue A. However, when using a 2.5 equivalent of an HMDS base, the result is the target compound 5-position analogue B and 3-position analogue A, and a dimer or dibenzyl compound C. This can be observed when considering the data from the low-resolution mass spectrometry (liquid chromatography coupled to mass spectrometry: LCMS), HPLC and nuclear magnetic resonance (NMR) together for each fraction separated by preparative HPLC. Taken together, the data strongly suggest that the 5-methylbenzylate precursor **57** was made and ready for the next step. However, to be certain that the peak in the LCMS at approximately 8 min with an M^+ of 234 is the compound that will produce the 5-position analogue and not the 3-position analogue, NMR had to be performed. The peak that appeared at approximately 9 min with an M^+ of 324 resembled dibenzyl **60**. The NMR of this peak proved that it was also a dimer. In addition, the NMR data, the purified HPLC and the LCMS provided the proof. Taking all of the findings together, we obtained the 3-position analogue A, 5-position analogue B and dimer (dibenzyl) C. In conclusion, this reaction

resulted in the intermediate compound that yielded target compound 5-position analogue B and the by-product compounds 3-position analogue A and a dimer or dibenzyl compound C. However, the purification by preparative HPLC took a long time, with low yield, as the purification was achieved from other by-products, as shown in Figures 12–14. When this experiment was performed using another base (n-BuLi), the same results were obtained as when the HMDS base was used (see Supporting Documents).

HPLC data

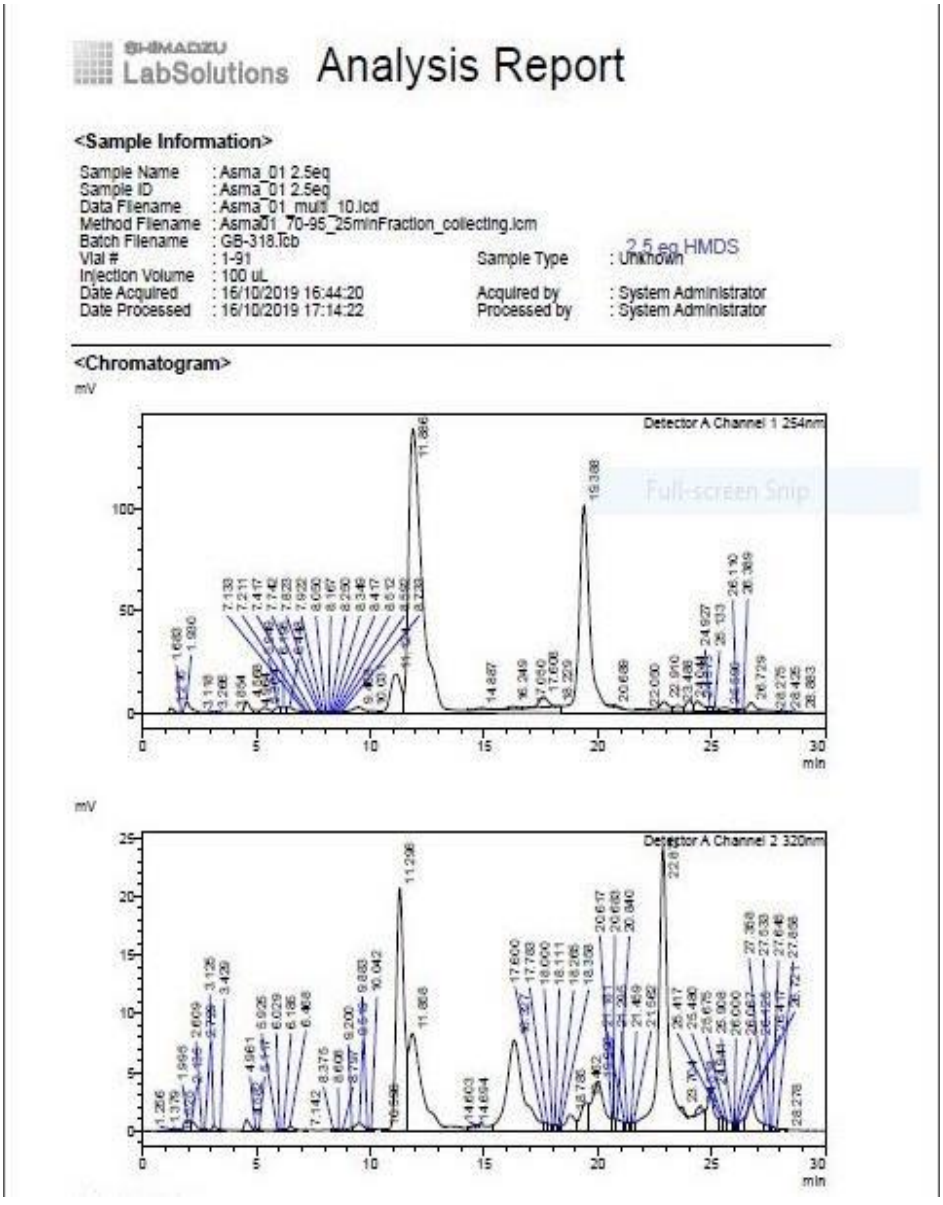


Figure 12. Chromatogram HPLC analysis report.

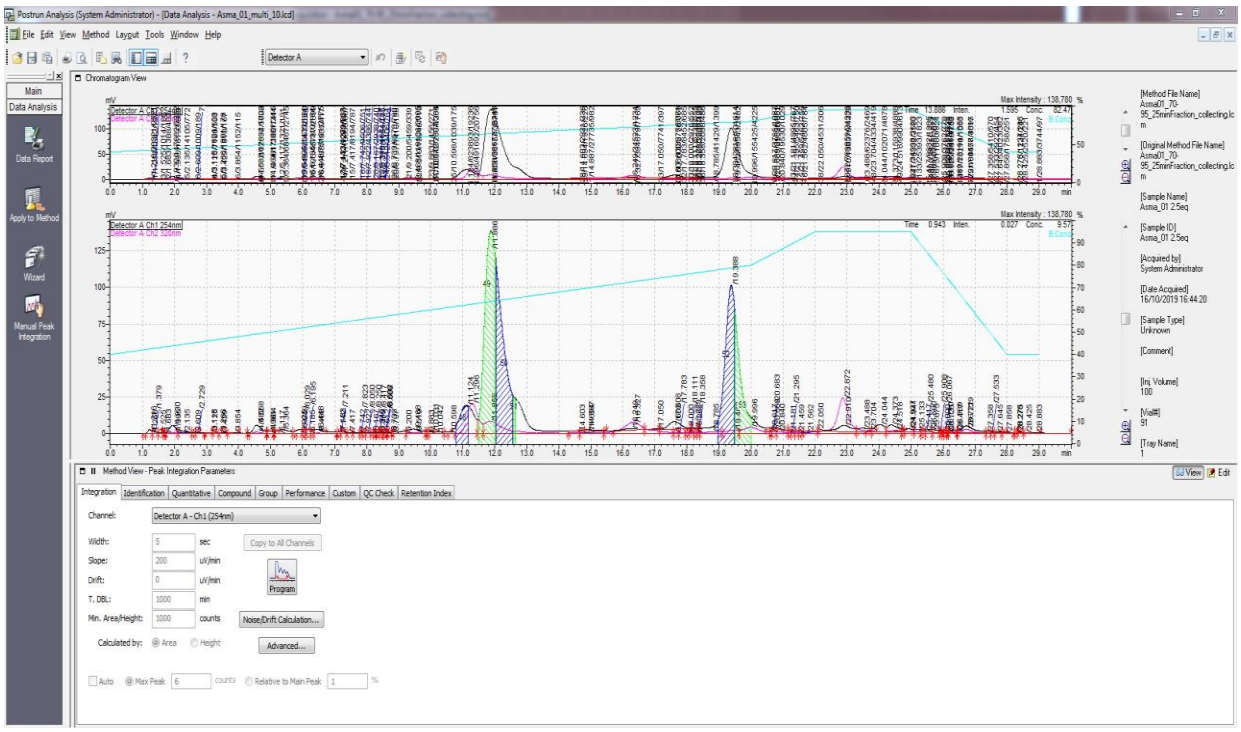


Figure 13. Preparative HPLC separates the intermediate component results into three compounds, as shown by the three peaks in the preparative HPLC results.

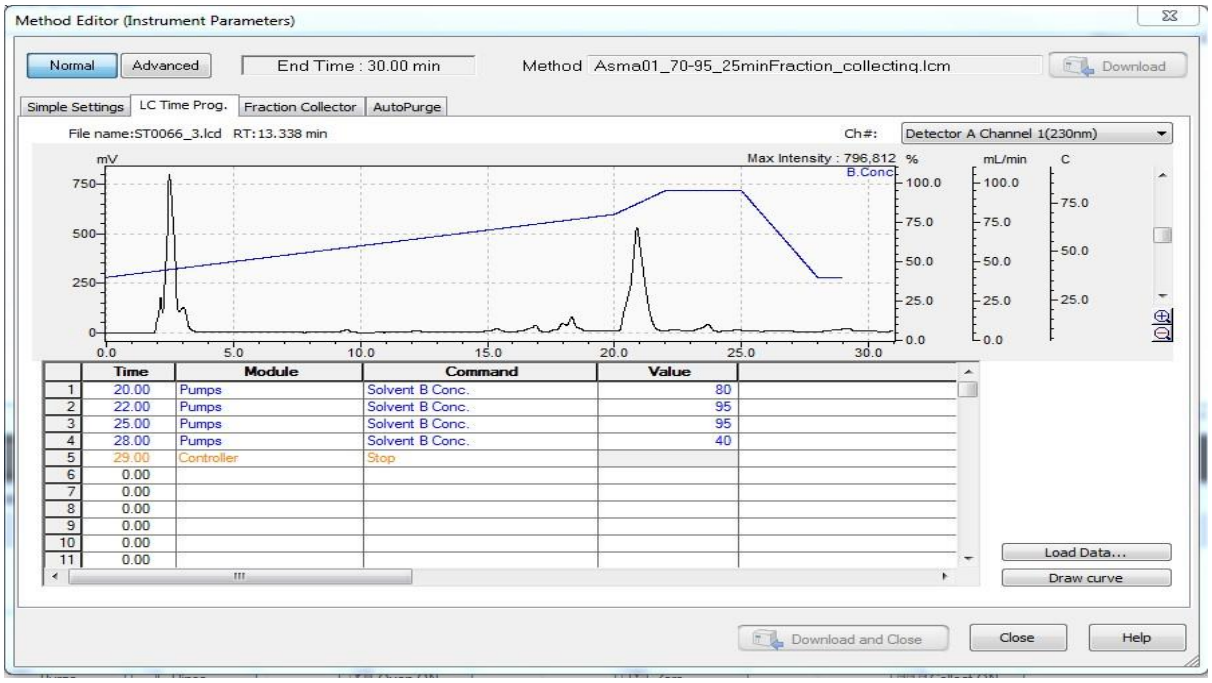
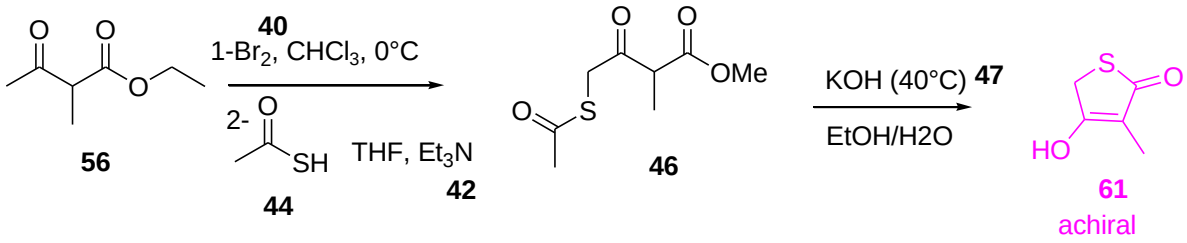


Figure 14. The preparative HPLC method applied to purify the product using acetonitrile in water.

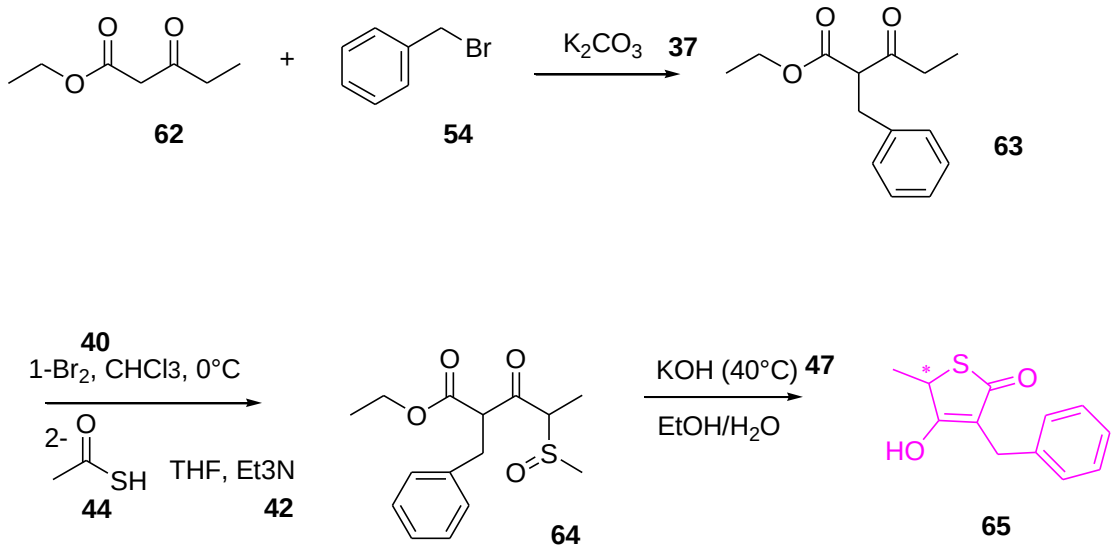
The second total synthesis of achiral compound (4-hydroxy-3-methyl-5H-thiophen-2-one, also known as 3-hydroxy-4-methyl-2H-thiophen-5-one) **61** was similar to that of racemic thiolactomycin or thiolactone core used as a key intermediate for further synthesis of the TLM analogue, which was reported by Wang and Salvino (71). Bromine **40** was added to a solution of 2-methyl-3-oxo-butyric acid ethyl ester **56**, yielding a brominated compound composed of a mixture of diastereoisomers. Displacement of the bromide group was successfully achieved by tri-ethylamine base **42** to produce 4-acetylsulfanyl-2-methyl-3-oxo-butyric acid ethyl ester **46**. Finally, cyclisation to the desired thiolactones was accomplished upon treatment of the stirred solution of the thioacetate in ethanol with aqueous potassium hydroxide solution **47** at an ambient temperature to obtain 4-hydroxy-3-methyl-5H-thiophen-2-one, **61**, as presented in Scheme 20 (75) (see Supporting Documents).



Scheme 20. Strategy and general procedure for the synthesis of the second TLM analogues.

The third total synthesis of the third TLM analogue derivative (3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one) involved a similar synthetic procedure related to racemic thiolactomycin or thiolactone core used as a key intermediate for further synthesis of the TLM analogue, which was reported by Wang and Salvino (71). Similar to the work of Wang and Salvino, we used ethyl propionyl acetate **62** as a starting material. It was treated with benzyl bromide (alkyl halide) **54** in the presence of anhydrous potassium carbonate (K_2CO_3) **37** as the base, which afforded alkylated product 3-benzyl ethyl propionyl acetate **63**. Bromine **40** was added to a solution of 3-benzyl ethyl propionyl acetate **63**, yielding a brominated compound as a mixture of diastereoisomers. Displacement of the bromide group was successfully achieved by a tri-ethylamine base **42** to produce intermediate compound **64**. Finally, cyclisation to the desired thiolactones was accomplished upon treatment of the stirred solution of the thioacetate in ethanol with an aqueous sodium hydroxide solution **47** at an ambient temperature to obtain

3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one **65**, as given in Scheme 21 (see Supporting Documents).



Scheme 21. Strategy and general procedure towards the synthesis of third TLM analogues (71).

3.3 Crystallographic analysis

3.3.1 Methods of crystallographic analysis

In this section, I outline the specific crystallographic methods employed to investigate the synthesised TLM analogues. These include single-crystal X-ray diffraction (SC-XRD), powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC).

3.3.2 X-ray crystallography

X-ray crystallography was employed to determine the space groups and confirm the molecular structures of the synthesised TLM analogues. Here, an experiment was performed generating numerous feature results of TLM analogue compounds and involving the crystallisation of these compounds to determine the crystal type of packing (i.e. either conglomerate or racemic) for the compound that I created. To extend my knowledge of solid-state behaviour, I

used CSD to identify closely related structures of the racemic 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one, 3-hydroxy-4methyl-2*H*-thiophen-5-one and 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one, as shown in Table 5.

Table 5 cont. Crystallographic data for synthesised TLM analogues.

	5	**61**
Crystal data		
Chemical formula	$C_6H_8O_2S$	$C_5H_6O_2S$
M_r	144.18	130.16
Crystal system, space group	Orthorhombic, *P b c a*	Monoclinic, $P2_1/c$
Temperature (K)	123	100
a, *b*, *c* (Å)	9.286 (1), 11.4809 (8), 12.6469 (10)	4.1054 (3), 22.9727 (13), 6.1928 (5)
α, β, γ (°)	90, 90, 90	90, 103.728 (7), 90
V (Å^3)	1348.3 (2)	567.37 (7)
Z	8	4
Radiation type	Cu *K*α	Mo *K*α
μ (mm^{-1})	3.63	0.46
Crystal size (mm)	0.55 × 0.08 × 0.04	0.12 × 0.02 × 0.01
Data collection		
Diffractometer	Oxford Diffraction Gemini S	Rigaku XtaLAB AFC12
Absorption correction	Analytical (*CrysAlis PRO*; Rigaku OD, 2019 ▸)	Multi-scan (*CrysAlis PRO*; Rigaku OD, 2019 ▸)
T_{min}, T_{max}	0.323, 0.847	0.766, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	4293, 1323, 1115	2121, 2121, 1955
R_{int}	0.049	0.026
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.620	0.650

	5	61
Refinement		
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.053, 0.148, 1.07	0.050, 0.113, 1.19
No. of reflections	1323	2121
No. of parameters	88	79
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.67, −0.29	0.43, −0.33

Table 5. Experimental details.

	65
Crystal data	
Chemical formula	$C_{12}H_{12}O_2S$
M_r	220.28
Crystal system, space group	Monoclinic, $P\,2_1/n$
Temperature (K)	100
a, b, c (Å)	6.5924 (5), 10.8402 (8), 14.8871 (15)
α, β, γ (°)	90, 93.796(8), 90
V (Å^3)	1061.54 (16)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.280
Crystal size (mm)	0.08 × 0.01 × 0.01
Data collection	
Diffractometer	Rigaku XtaLAB AFC12
Absorption correction	Multi-scan (*CrysAlis PRO*; Rigaku OD, 2019 ▸)

	65
T_{min}, T_{max}	0.59975, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	15,882, 1865, 141
R_{int}	0.1789
$(\sin \theta/\lambda)_{max}$ ($Å^{-1}$)	0.620
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.0499, 0.1197, 1.017
No. of reflections	1865
No. of parameters	141
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e $Å^{-3}$)	0.292, −0.41

The single-crystal data of the compound were collected using the Oxford Diffraction Gemini and Xcalibur instruments fitted with variable temperature cryosystems. Both Mo and Cu sources are available, which means that a wide range of chemical types can be examined, and that absolute structure determination is possible even for structures containing light atoms. All empirical absorption corrections were applied using the Mercury Soft database programme. The structure was solved using direct methods, which yielded the positions of all non-H atoms. These were initially refined isotropically and then anisotropically. Normally, when we refine a crystal structure, we first arrange the atoms isotropically and then use the ANIS command in SHELX to refine them anisotropically. The thermal motion of the atoms in this case is made isotropic, where the motion of the atom is constrained to be the same in all three directions. We do this for H atoms or atoms that misbehave or are not well defined. If the atoms are well defined, we let them remain anisotropic, where their thermal motion is not equivalent in all three directions. All errors made in crystallographic measurements can be seen in the displacement parameters, and if incorrectly assigned, an atom will exhibit highly anomalous thermal parameters. If the crystal structure is measured at a low temperature, then

the thermal motion is reduced such that the displacement parameters are smaller and more predictable (13,33,76, 79-83).

Three-dimensional (3D) space groups are used by crystallographers to describe the symmetry of crystal structures or the space group for atoms at general positions *x, y* and *z*. In general, the rotational axes are listed before screw axes, and the mirror planes are indicated before glide planes. The molecule 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one crystallises in the chiral orthorhombic space group Pbca. The primitive centrosymmetric orthorhombic space group with only screw axes and glide planes is called *Pbca.* The full space-group symbol, P2, C2, emphasises the presence of rotation and screw axes not shown in the short space-group symbol. Some space groups have no symmetry element that can change the handedness of an object; these are termed *enantiomorphic* space groups (also called non-centrosymmetric), which can be polar and could not be seen in our compound (13,76,82).

The racemic compound 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one **5** has the chemical formula $C_6H_8O_2S$. It crystallises with six solvents [tetrahydrofuran (THF), ether, chloroform, ethyl acetate, toluene and water] through a slow evaporation method involving vials sealed with a pierced paraffin film at room temperature and atmospheric pressure for determining the crystal structure. Producing suitable crystals from the initial synthesis of a compound can be difficult, unpredictable and time-consuming and is not guaranteed to succeed. Any compound can adopt various crystal structures depending on the crystallisation conditions (different solvents and temperatures) to which it is exposed. The phenomenon through which a compound can have two different crystalline forms is called polymorphism. Polymorphs created by this process can have different properties. For example, one polymorph of a substance can be active, while the other is inactive, even though they are derived from the same chemical substance. Polymorphic forms have different packing arrangements and crystal symmetries, but the molecules themselves are the same in both structures.

Table 5 presents the results of an experiment in which single crystals of 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one are placed in THF solvent in a copper X-ray tube with a characteristic X-ray wavelength of 1.54184 Å (see Table 5 for the rest of the measurements for this crystal). In the cell unit structure, all angles are equal to 90° while the side lengths are

unequal. The number of reflections collected is 4293, and the number of unique reflection measures is 1323. Each spot observed in the X-ray pattern can be labelled by the three indices *hkl,* which contribute to each atom's *xyz* position in the unit cell. The unit cell's geometry has a - $x + {}^{3}/_{2}$, $y + ½$, z coordinates. The chains lying parallel to the crystallographic *b*-axis are composed of alternate R and S enantiomers.

The space group can be used to ascertain whether the compound is centrosymmetric or non-centrosymmetric. The dataset shown in Table 1 can indicate the presence or absence of certain elements of symmetry, particularly those relating to centrosymmetry. As with centrosymmetric structures, the effects of scattering on the opposite reflections *h, k* and *l* and $-h, -k$ and $-l$ are equal. In other words, the structure has no inversion symmetry, which means that the crystal phase will be either 0° or 180°. For non-centrosymmetric structures, the effects of scattering do not cancel out. In crystallography, a centrosymmetric point group contains an inversion centre such that every point (*x, y, z*) in the unit cell has an indistinguishable point ($-x, -y, -z$). The lack of an inversion centre reveals that the structure is non-centrosymmetric and it may be a chiral compound.

The combined left- and right-hand racemic structures are separated by crystallisation to find a pure enantiomer. A pure enantiomer must have a non-centrosymmetric space group, must not have a mirror of the three axes (*a, b* and *c*) and can only have rotation along the screw axis. The most common non-centrosymmetric space groups are **P2, $P2_1$, C2** and **$P2_12_12$,** which do not have mirror images or inversions of symmetry.

In the racemic example, the Pbca space group is the target of enquiry, where "P" indicates a primitive unit cell with lattice points only at its corners and "bca" describes translation along a mirrored glide plane. Translation occurs as follows: the symmetries of b, c and a glide along the *a*, *b* and *c* axes, respectively. The structure of the racemic 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one compound is *centrosymmetric*, which means that it should have a mirror or a centre of inversion. The structure of 3-hydroxy-2,4-methyl-2*H*-thiophen5-one is likely to be twinned. Twinning can seriously hinder the determination of a crystal structure. As that is the case, the twin molecule of the 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one compound cannot

be separated by the crystallisation technique. The other option for separation is chiral chromatography.

The similarity between the calculated and observed diffraction patterns indicates that the atoms in the model structure were almost in the right positions. For a correct and complete crystal structure derived from well-measured data, the *R*-factor is typically 0.02–0.07. The least-squares method can be used to find the best fit of two sets of data and to refine the crystal structure. How well it fits describes how well the calculated diffraction pattern corresponding to the model structure agrees with the observed diffraction pattern. Table 5 shows the *R*-factor, which is widely used to assess the quality of an X-ray experiment. As shown, the observed and calculated reflection intensities yielded a model structure of 5% and 6%, respectively. The R-value is 0.062, which indicates that the observed and calculated models are in agreement on the basis of the good fit of the model's structure with the actual data.

For the second compound, 3-hydroxy-4-methyl-2*H*-thiophen-5-one, **61**, with the chemical formula $C_5H_6O_2S$, is crystallised in a chloroform solution using molybdenum as the target material for the X-ray tube (see Table 5 for full details). All lengths of the crystals were unequal, with only the *b*-axis demonstrating symmetry due to the crystal's unique monoclinic shape. As before, each spot observed in the X-ray diffraction pattern is labelled with the three indices *hkl*, which reveal each atom's *xyz* position in the unit cell. These chains propagate only through translation, corresponding to $x + 1$, y $z + 1$, which are the coordinates relative to the unit cell's geometry. The structure of the achiral 3-hydroxy-4-methyl-2*H*-thiophen-5-one shows its mirror image or inversion centre. There are three possible points of monoclinic symmetry, each with a corresponding space group (Table 6).

Crystal system	Point symmetry	Space group
Monoclinic	Enantiomorphic polar	P2, $P2_1$, C2
	Polar	Pm, Pc, Cm, Cc
	Centrosymmetric	P2/m, $P2_1$/m, C2/m, P2/c, $P2_1$/c, C2/C

Table 6. Monoclinic points of symmetry and their space groups (58,59).

The structure of 3-hydroxy-4-methyl-2*H*-thiophen-5-one is likely to be twinned, having a combination of two inversion-related components of approximately equal amounts. Twinning can seriously hinder the determination of a crystal structure. This can result when unit cells join together to form a complete crystal during growth. A twinned crystal is one in which two orientations or mirror images of the same structure occur and have a well-defined relationship. Twinning commonly arises when a chiral structure grows together with its opposite enantiomer in a single crystal. This can occur if both forms of the material are present in a solution, as the unit cell shape is identical for both enantiomers, which means that the crystal lattice is always centrosymmetric even if the overall structure is not. In this case, the crystals of achiral 3-hydroxy-4-methyl-2*H* thiophen-5-one is twinned by a 180° rotation in the reciprocal 001 direction; twinning is revealed when determining the absolute configuration by refinement (60).

As stated previously, the similarity between the calculated and observed diffraction patterns indicates that the atoms of the model structure are approximately in the right positions, with R typically ranging from 0.02 to 0.07. The least-squares refinement of the crystal structure is also calculated. As shown in Table 5, the *R*-factor of the reflection intensities measured in the experiment is R1 = 0.0500 (model structure 5%), and that calculated for the model is R1 = 0.0560 (actual data, 5%). These two results are almost the same. In the final refinement, 5% of the data agreed with the observed and calculated models in terms of the model structure's fit to the actual data.

In the third experiment, the 3-benzyl,5-methyl-4-hydroxy-5*H*-thiophen-2-one compound, **65**, with chemical formula $C_{12}H_{12}O_2S$, was grown in three solvents (THF, ether and chloroform)

using a slow evaporation method (vials sealed with pierced paraffin film) at room temperature and atmospheric pressure for determining the crystal structure. Although the overall quality of the structure was good, all solvents transformed into racemic crystals. This led me to surmise that the solvent and temperature do not affect whether the structure is racemic or conglomerate. When molybdenum was used as the target material for the X-ray tube (see Table 5 for full details), all lengths of the crystal were unequal, with only the *b*-axis demonstrating symmetry due to the unique monoclinic shape of the crystal. As before, each spot observed in the X-ray diffraction pattern was labelled with the three indices, *hkl*, which contribute to determining each atom's *xyz* position in the unit cell. The compound 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one has $P2_1/n$, where n indicates that the glide plane translates molecules in a direction on the plane perpendicular to the screw axis. In addition, it is in a centrosymmetric space group, as it has a mirror image or inversion centre ($-x$, $-y$, $-z$ in its symmetry).

The molecule 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one crystallises in the chiral monoclinic space group $P2_1/n$. The crystals in this system refer to three axes of unequal lengths (*a, b* and *c*), where *a* is perpendicular to *b* and *c*, but *b* and *c* are not perpendicular to each other. The monoclinic space group with $P2_1/n$ is distinguished by a single axis called an axis of twofold symmetry, in which the cell can be rotated 180° without changing its appearance. In the monoclinic unit cell, all three axes are unequal in length, and the two axes are perpendicular to each other (13,76,82).

There are three possible points of monoclinic symmetry, each with a corresponding space group, as shown in Table 6. The unit cell shape is identical for the two enantiomers, where a crystal lattice is always centrosymmetric, even if the overall structure is not.

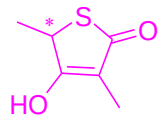
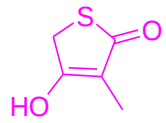
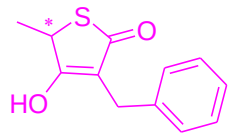
As stated previously, the similarity between the calculated and observed diffraction patterns indicates that the atoms of the model structure are approximately in the right positions, with R typically around 0.02–0.07. The least-squares refinement of the crystal structure was also calculated. As shown in Table 5, the *R*-factor of the reflection intensity measured experimentally is R1 = 0.0801, and that calculated for the model is R1 = 0.0499. In the final refinement, 5% of the data agreed with the observed and calculated models in terms of the fit

of the model structure to the actual data. The crystal system can easily be predicted from the unit cell; however, it cannot predict the exact space group of its crystal unless a free software programme is used.

Single-crystal X-ray diffraction data for the crystals revealed identical unit cells but different chiralities. The two enantiomers crystallise together, do not form separate colonies, and possess identical crystal morphology, colour and solubility. It is not possible to identify whether racemic or conglomerate crystals form. Alternatively, if appropriate salts have a higher tendency to crystallise as conglomerates than as neutral organic molecules (13,61). Although the two enantiomers can be distinguished by single-crystal X-ray diffraction data, their separation cannot be achieved chemically. The differential behaviour of the racemic compound illustrates the complexity associated with spontaneous resolution or conglomerate formation of chiral products. This type of crystal could not be distinguished from the colour or tendency to crystallise; therefore, we were unable to separate the two enantiomers (13,21,33,76,83). The chiral resolution is confirmed by a comparison of crystallographic and thermal analyses (76).

The table below (Table 7) outlines the crystallographic details of the synthesised TLM analogues, including space groups, unit cell parameters and crystal systems.

Table 7. Crystallographic data for synthesised TLM analogues.

Analogue	Space Group	Unit Cell Parameters	Crystal System	Type (Racemic/Conglomerate)
1 	Pbca	a = 9.2860(10) Å α = 90° b = 11.4809(8) Å β= 90° c = 12.6469(10) Å γ = 90°	Orthorhombic	Racemic
2 	$P2_1/c$	a = 4.1054(3) Å α = 90° b = 22.9727(13) Å β = 103.728(7)° c = 6.1928(5) Å γ = 90°	Monoclinic	Potential conglomerate
3 	$P2_1/n$	a = 6.5924(5) Å α = 90° b = 10.8402(8) Å β= 93.796(8)° c =14.8871(15) Å γ = 90°	Monoclinic	Racemic

Single-crystal X-ray diffraction (SC-XRD) was performed to determine the detailed structural properties of each TLM analogue. The analysis confirmed the symmetry and packing types as follows:

- **Analogue 1**: Displayed typical racemic packing with centrosymmetric space group Pbca, which is consistent with the formation of racemic compounds.
- **Analogue 2**: Showed a non-centrosymmetric space group ($P2_1/c$), suggesting potential for conglomerate formation under optimised conditions.
- **Analogue 3**: Crystallised in ($P2_1/n$), confirming racemic properties.

The space group identification involved an iterative process, starting with initial data collection and then refinement of intensity data to confirm the final space group.

Once a structure model was established, it was refined using the collected intensity data to confirm the space group. To address difficulties in space group determination, the challenges in differentiating between centrosymmetric and non-centrosymmetric groups in borderline cases were explained.

Searching for a non-centrosymmetric space group is not as simple as expected. When separating a mixture of left- and right-hand compounds using crystallisation, and when in pursuit of a pure enantiomer, there must be a non-centrosymmetric space group that exhibits only rotation, with no inversion symmetry or mirror image. Two compounds synthesised in the laboratory gave centrosymmetric space groups that crystallise in the racemic form instead of the conglomerate form. To prevent this, one option could be to synthesise these compounds as salt derivatives, which may help to achieve conglomerate crystallisation, as can be seen in the examples of ibuprofen salt compounds **32**–**34**.

3.3.3 Hydrogen bonding feature

The main structural feature of the three compounds is the hydrogen-bonded chains formed between the OH and C=O groups, producing racemic instead of conglomerate crystals. These strong hydrogen bond chains lie parallel to the crystallographic axis and are composed of alternate R and S enantiomers. The only modification in the synthesis of the two compounds was the presence of the benzyl ring at the 3-position of the thiophene ring. With a large molecular weight, this molecule is likely to form a conglomerate enantiopure crystal. However, the molecules become near-racemic as they form RR and SS isomers, and only the pi-stacking interaction occurs between the two enantiomers. Therefore, to crystallise a molecule in a conglomerate or enantiopure form, the best approach is to synthesise it with a large functional group, such as a molecule with a large molecular weight and weak hydrogen bonds. The existence of weak hydrogen bonds (which may be maintained regardless of which enantiomer docks at the surface) may lead to the formation of a homochiral crystal layer. Accordingly, when seeking to design a molecule that does not form a racemic compound

through structural prediction, OH and C=O groups should be avoided. In other words, the lack of –OH…O=C intermolecular interactions observed in the experimental structure appears to be dominated by ring stacking, which predicts a conglomerate. A pure enantiomer with a melting point higher than that of a racemic compound indicates a conglomerate nature (36,50,51).

The Mercury programme was used to visualise the crystal structure data for these three structures and identify and examine the intermolecular hydrogen bond interactions. Single crystals of 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one were grown in THF solvent using a slow evaporation method at room temperature and atmospheric pressure for crystal structure determination. Data were calculated and refined to provide the attached structures shown in Figures 15–19.

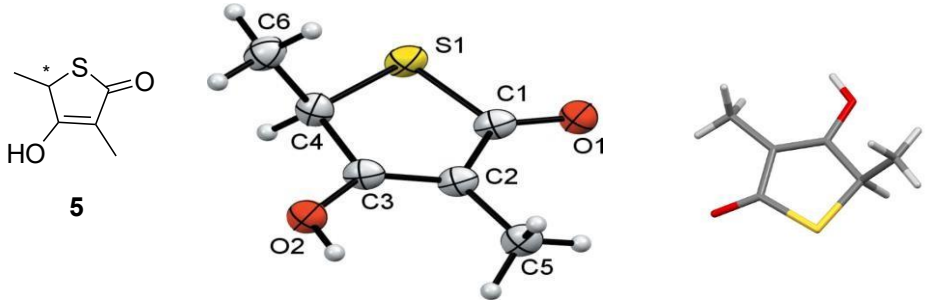


Figure 15. Molecular structure of 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one, with non-H atoms shown as 50% probability ellipsoids. H atoms are drawn as small spheres of arbitrary size.

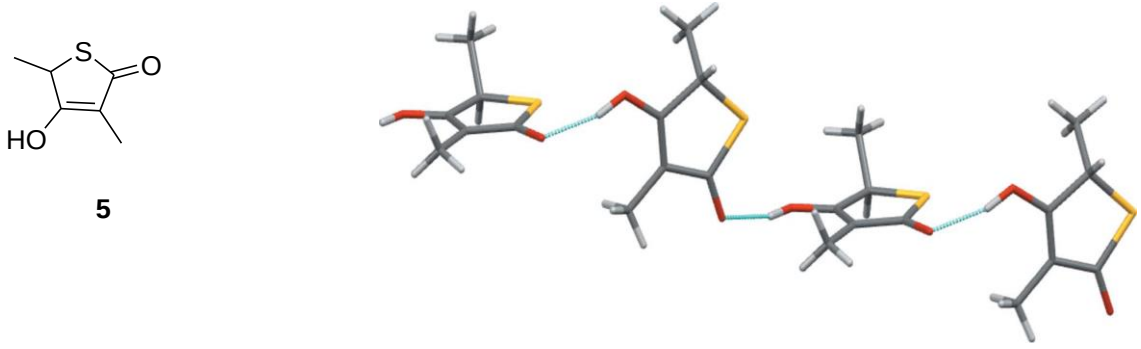


Figure 16. Hydrogen-bonded chain motif in the 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one structures. The chain extends parallel to the crystallographic *b*-axis. Strong H-bonding interactions occur between the carbonyl of one molecule and the hydroxyl of the other.

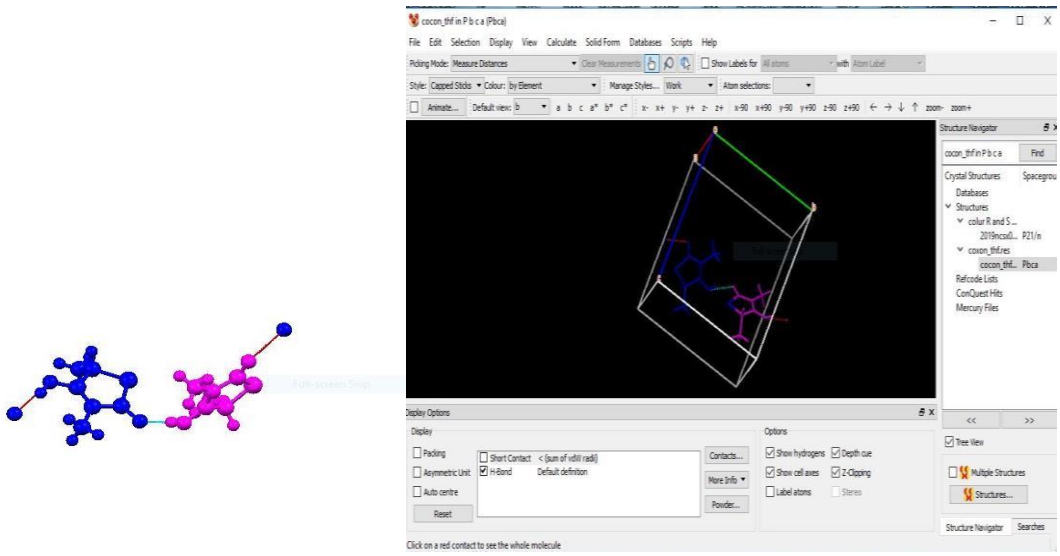


Figure 17. Both left- and right-handed structures are produced with equal probability in the crystal structure.

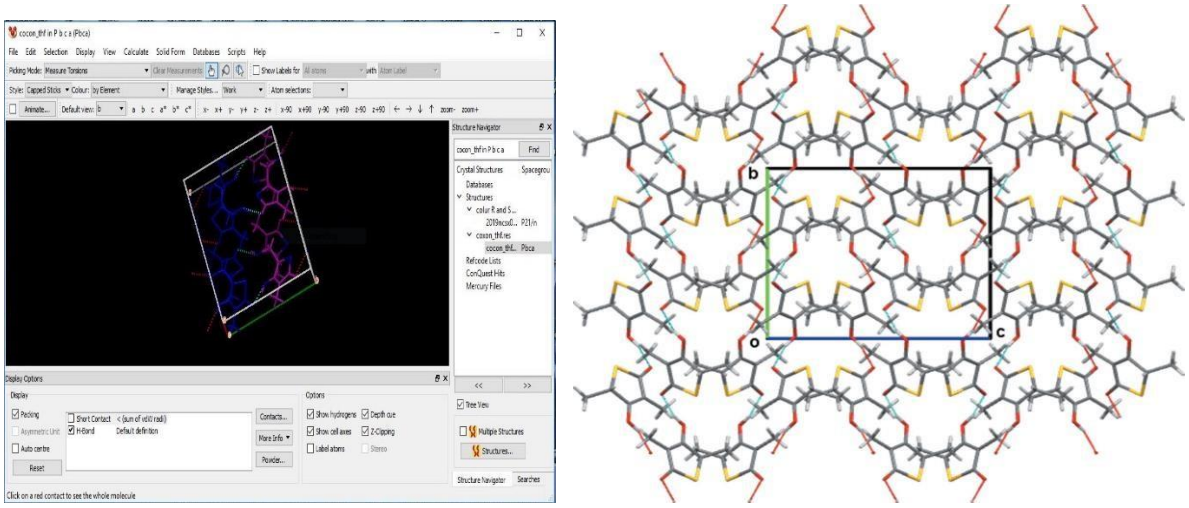


Figure 18. Packing diagram for a racemic compound with a view down the *a*-axis. Given the packing of chiral 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one as eight molecules in the unit cell, it is called a centrosymmetric molecule because it is symmetrical at its centre.

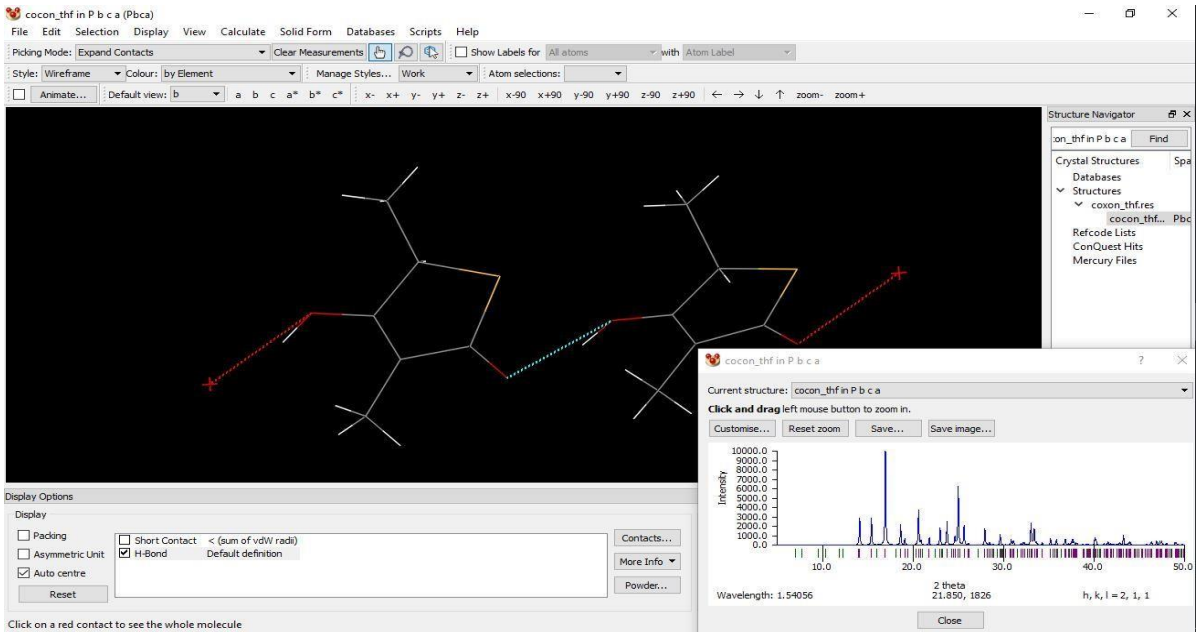
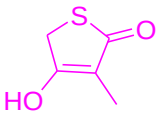


Figure 19. Crystal structure of the R and S isomers in the same unit cell and predicted chart using XRPD from the Mercury Soft database.

Moving to the second derivative, single crystals of 3-hydroxy-4-methyl-2H-thiophen-5-one were grown in chloroform solvent using a slow evaporation method at room temperature and atmospheric pressure for crystal structure determination. Data were calculated and refined to provide the attached structure (Figures 20–22). It is a novel structure not found in the crystallographic database.



61

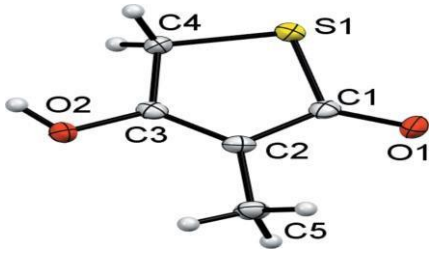


Figure 20. Molecular structure of 3-hydroxy-4-methyl-2H-thiophen-5-one with non-H atoms shown as 50% probability ellipsoids. H atoms are drawn as small spheres of arbitrary size.

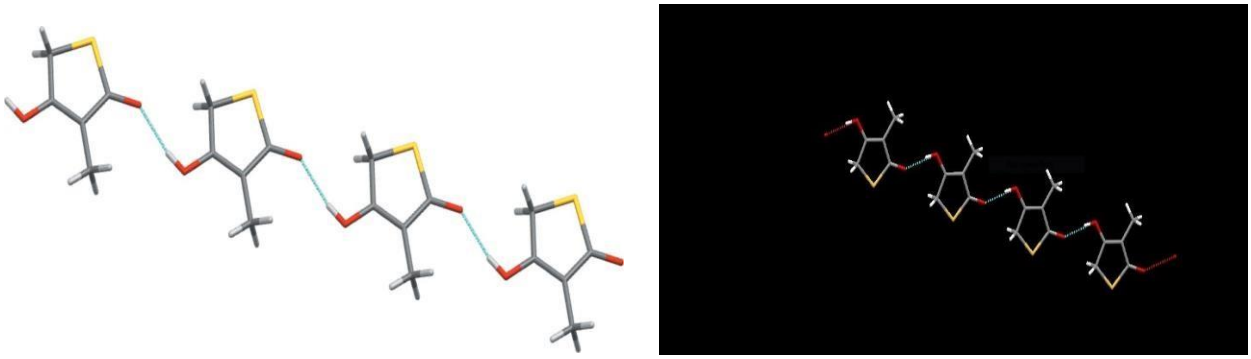


Figure 21. Hydrogen-bonded chain motif in the structure of 3-hydroxy-4-methyl-2H-thiophen-5-one. The chain extends parallel to the [101] direction.

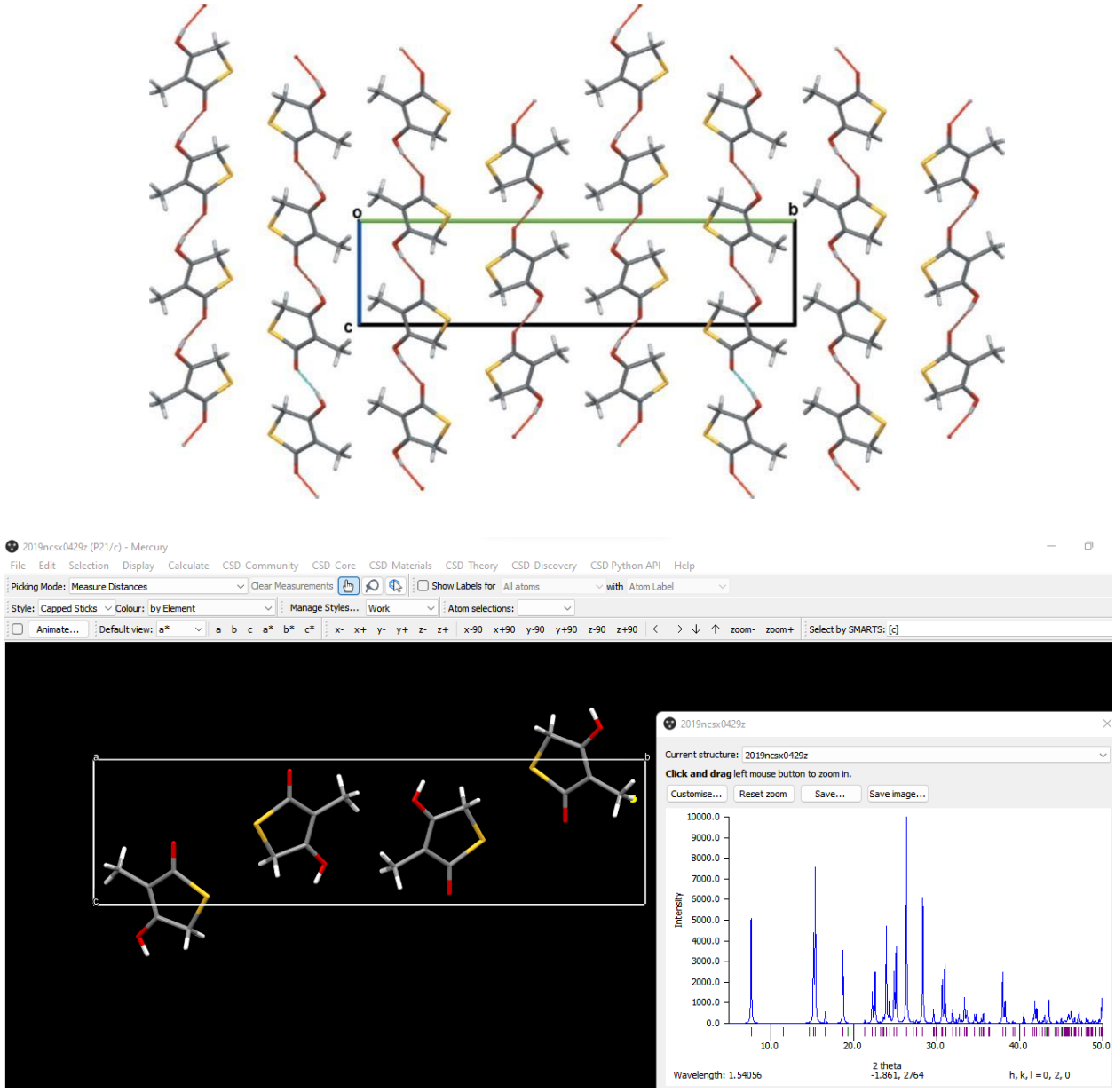
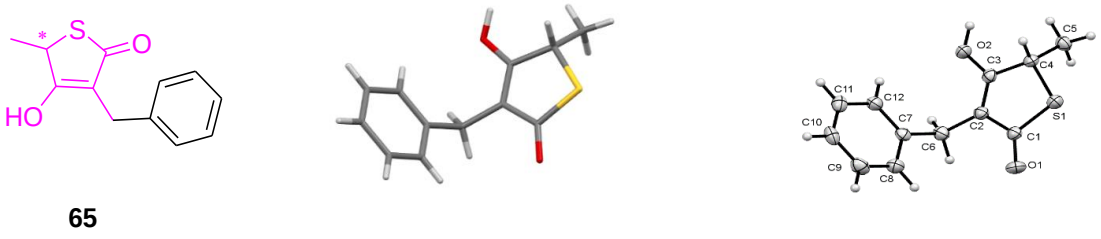


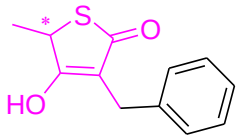
Figure 22. Packing diagram for compound 3-hydroxy-4-methyl-2H-thiophen-5-one in a view down the *a*-axis. Note the alternating Me_ _ _Me and S_ _ _S layers and predicted chart using XRPD from the Mercury Soft database.

Moving to the third derivative compound **65**, the crystal structure of its compound, the intermolecular hydrogen bond interaction and predicted XRPD data from the Mercury soft database are shown in Figures 23–26.



65

Figure 23. Crystal structure systems for the compound 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one.



65

Figure 24. Hydrogen bonds between R and R and between S and S in the same chain.

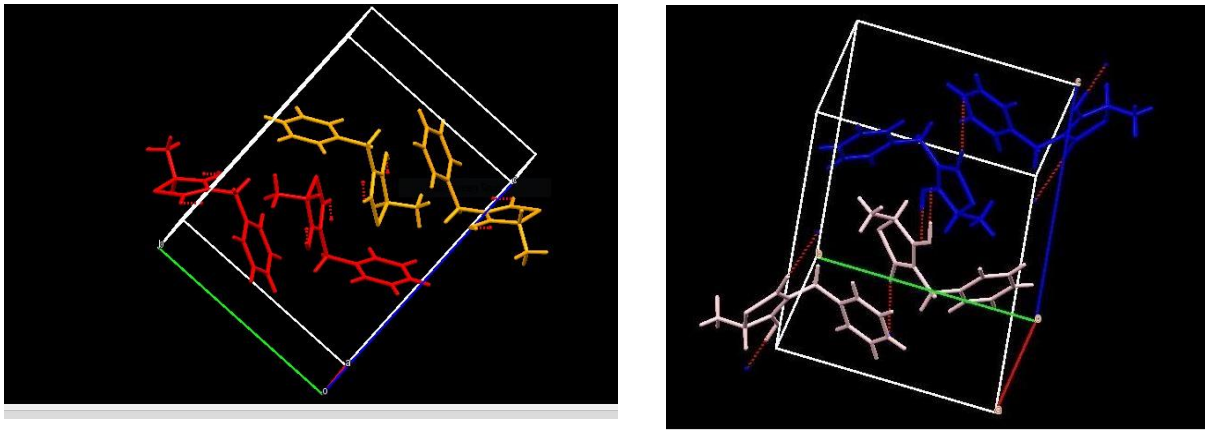


Figure 25. The red colour indicates the R isomer, and the sandy colour indicates the S isomer.

Attractive non-covalent interactions between aromatic rings called pi-stacking or π–π-

stacking bonds between R and S were observed. The RR and SS isomers belong to the same unit cell, referred to as racemic or near-racemic crystal packing.

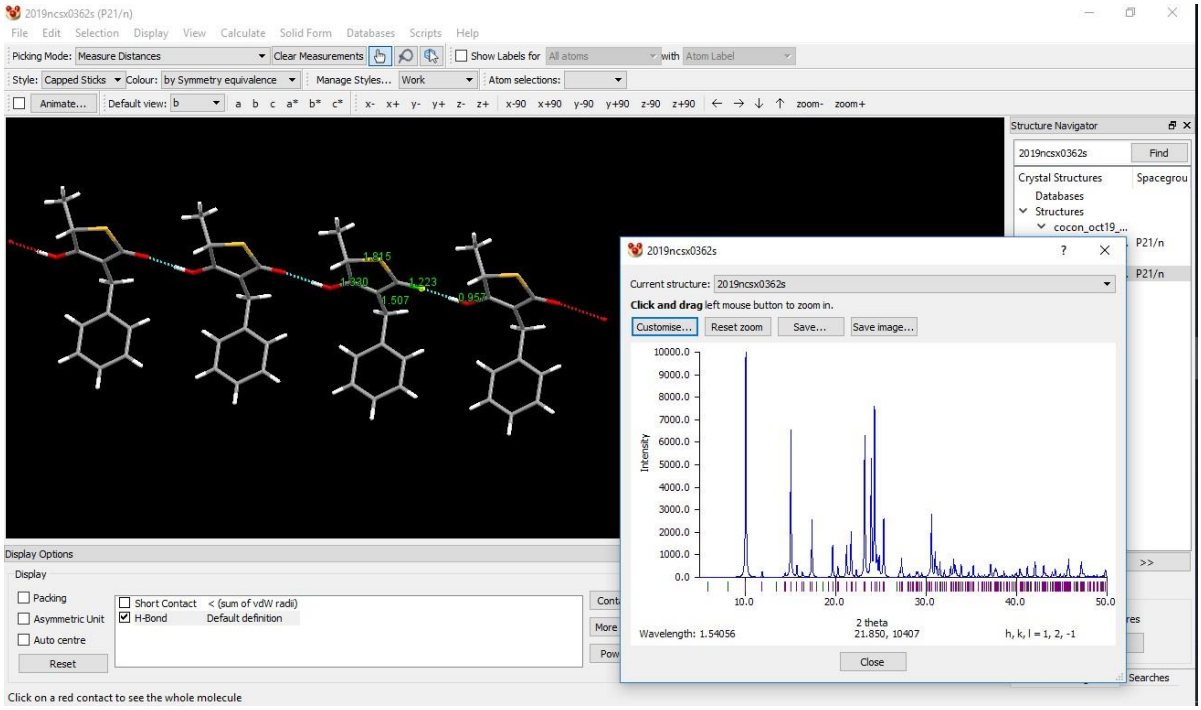


Figure 26. Crystal structure of R and S isomers in the same unit cell and predicted XRPD from the Mercury Soft database.

3.3.4 Hirshfeld surface analysis

Hirshfeld surface analysis was used to examine intermolecular interactions within the crystal structures, providing insight into how these interactions influence crystallisation behaviour.

The Crystal Explorer programme is often used to visualise intermolecular interactions between atoms by generating the Hirshfeld surfaces of structures with standardised X–H distances. The application of a colour scale onto the Hirshfield surface, known as decoration, has proven to be a powerful approach for gaining insights into molecular environments in the crystalline state. The total area of the Hirshfeld surface is used to report the percentage contribution of the interaction. In Crystal Explorer, the electron densities were used to compute the Hirshfeld surfaces by integrating the electron density belonging to the molecule.

In this study, Hirshfeld surface analysis was performed for racemic 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one.

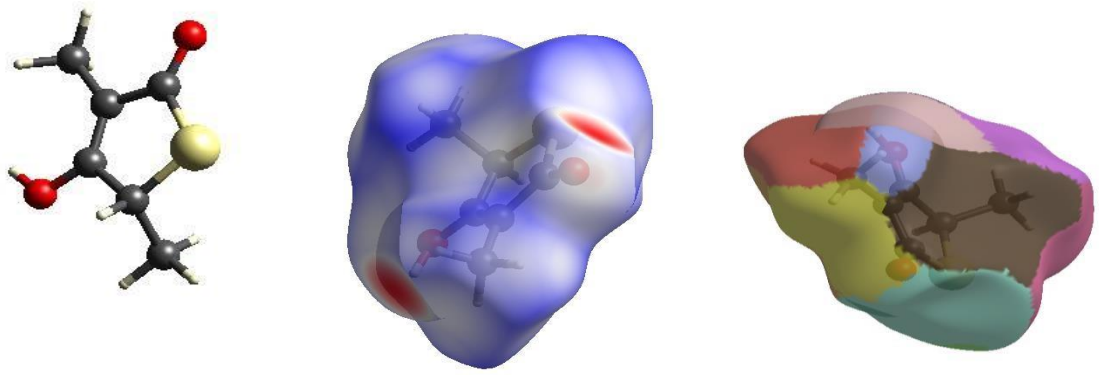


Figure 27. Fragment patch Hirshfeld surface analysis results for racemic 3-hydroxy-2,4-dimethyl-2*H*-thiophen-5-one.

In this figure 27, the red portions of the surface represent the closest contacts, which are shorter than the sum of the van der Waals radii. The surface analysis revealed two red spots, which indicate close contact between the neighbouring molecules throughout those atoms. Intermolecular interaction can be observed primarily in the O–H bonding and C=O groups. Meanwhile, the blue and white portions of the surface indicate that the contacts are longer than and equal to the sum of the van der Waals radii, respectively.

A Hirshfeld analysis was also performed for achiral 3-hydroxy-4-methyl-2*H*-thiophen-5-one (see Figure 28 for the fragment patch Hirshfeld surface diagram). The red portions of the surface represent the closest contacts, which are shorter than the sum of the van der Waals radii. The surface analysis revealed two red spots, which indicate close contact between the neighbouring molecules throughout those atoms, from which we can surmise that intermolecular interaction occurs primarily through O–H bonding. The blue and white portions of the surface highlight where the contacts are longer than and equal to the sum of the van der Waals radii, respectively.

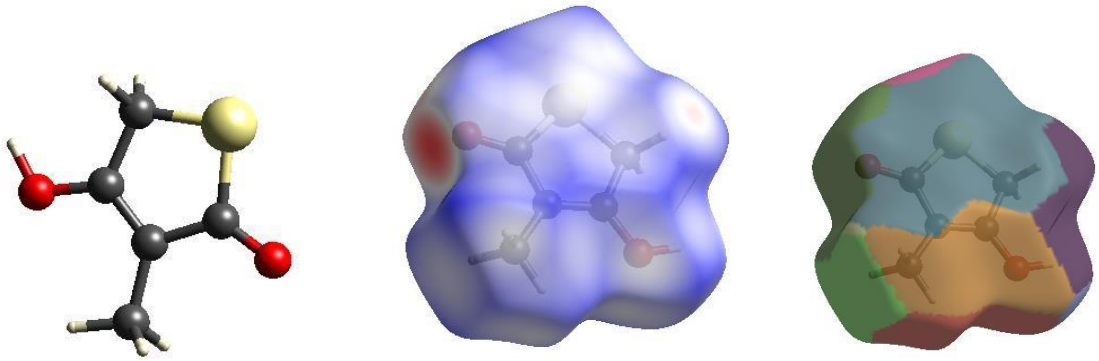


Figure 28. Fragment patch Hirshfeld surface analysis results for achiral 3-hydroxy-4-methyl-2*H*-thiophen-5-one.

A Hirshfeld analysis was also performed for the 3-benzyl,5-methyl-4-hydroxy-5*H*-thiophen-2-one compound (see Figure 29 for the fragment patch Hirshfeld surface diagram). The red portions of the surface represent the closest contacts, which are shorter than the sum of the van der Waals radii. The surface analysis revealed two red spots, which indicate close contact between the neighbouring molecules throughout those atoms, from which we can surmise that intermolecular interaction occurs primarily through O–H bonding. The blue and white portions of the surface highlight where the contacts are longer than or equal to the sum of the van der Waals radii, respectively.

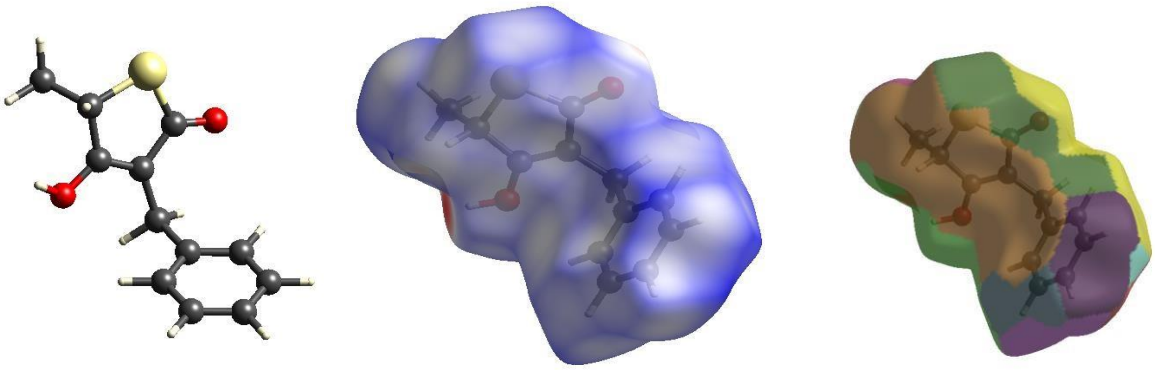


Figure 29. Fragment patch Hirshfeld surface analysis results for the 3-benzyl,5-methyl-4-hydroxy-5*H*-thiophen-2-one compound.

Another way of comparing the two structures is by Hirshfeld surface analysis. In such analysis, fingerprint plots similar to Hirshfeld surface diagrams which can be used to analyse the intermolecular interactions of structures. Figure 30 presents a complete fingerprint plot of the racemic compound, showing different atomic contacts across the surface. The 2D fingerprint plot has two axes: one corresponds to the distance from the molecule core to the surface (di), or in another sense, the distance from the Hirshfeld surface to the nearest nucleus beneath the surface. The other corresponds to the distance from the neighbouring atom to the surface (de), or from the Hirshfeld surface to the nearest nucleus outside the surface. The 2D fingerprint breakdown reveals close contact O–H information. The dark blue areas contribute the least to the surface, the light blue areas contribute more, and the green areas contribute the most.

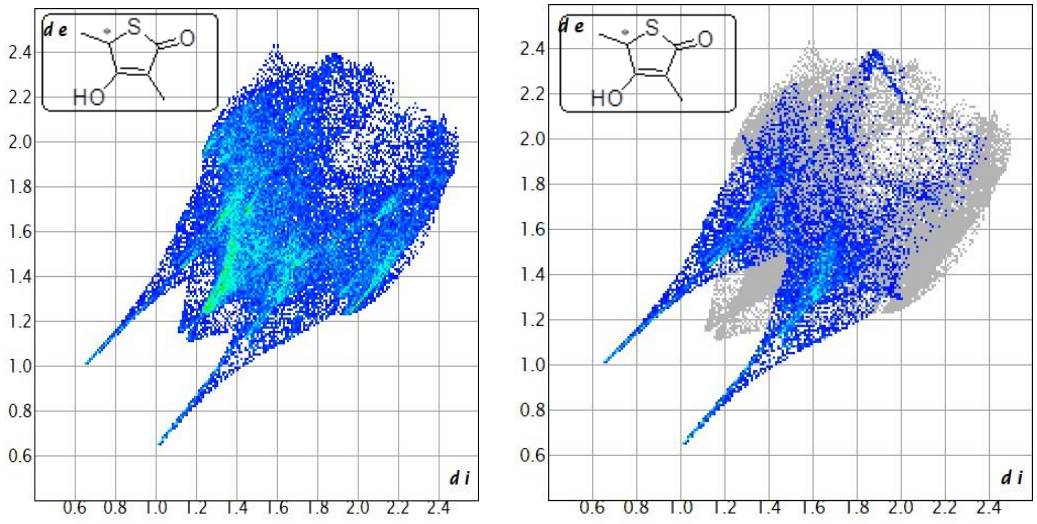


Figure 30. Full fingerprint plot of racemic 3-hydroxy-2,4-dimethyl-2*H*-thiophene-5-one (right) and a breakdown to show O–H contact (left).

The dominant interaction in this compound is clearly hydrogen–oxygen contact, as indicated by the small amounts of light blue and green on the plot shown in Figure 31. Breakdown of the fingerprint plot for the above racemic compound **shown in Figures 32-35**.

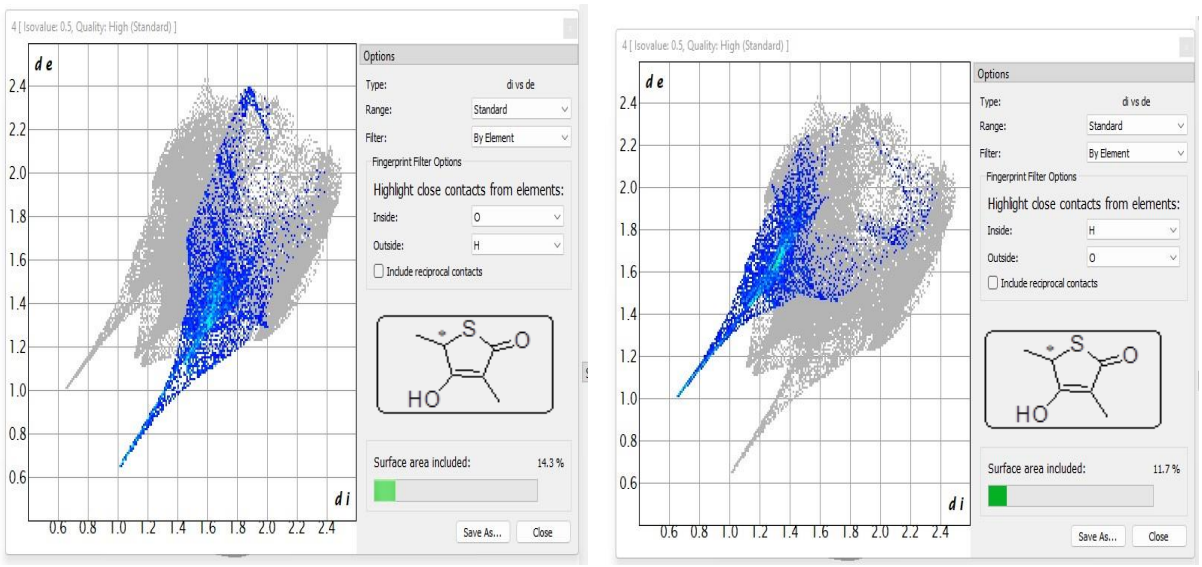


Figure 31. Breakdowns of the fingerprint plot to show O–H (left) and H–O (right) contacts.

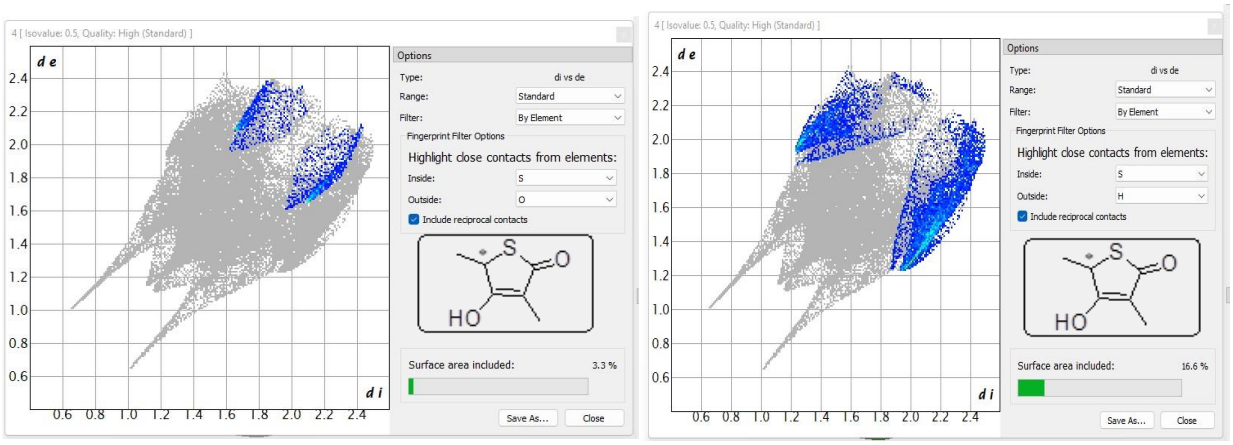


Figure 32. Breakdown of the fingerprint plot for the above racemic compound. This investigation was based on the assumption of a 3.3% close contact between the S atom of the molecule and the neighbouring O atom outside the surface (left). A 16.6% close contact between the S atom and H atom neighbouring to the surface was observed (right).

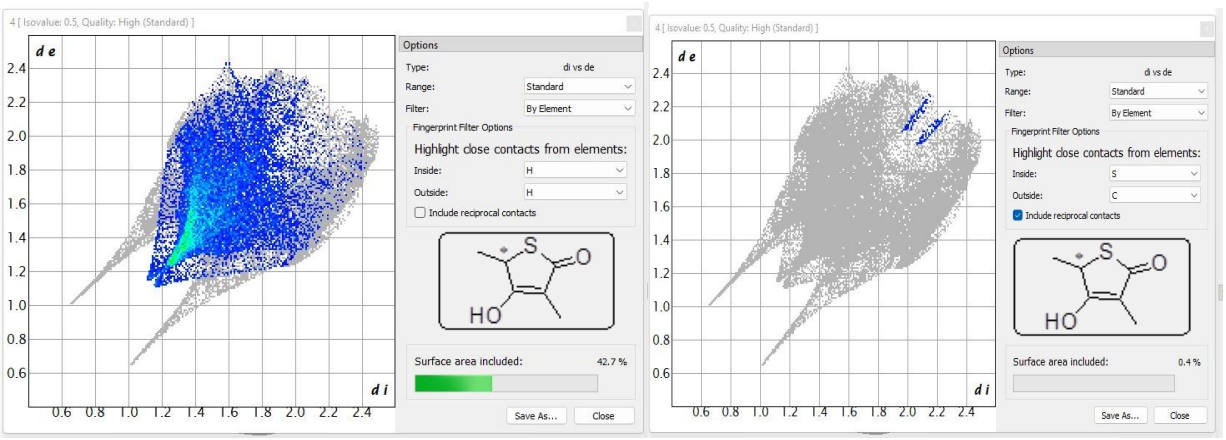


Figure 33. This investigation was based on the assumption of a 42.7% close contact between the H atom of the molecule and the neighbouring H atom to the surface (left). The close contact between the S atom and the neighbouring C atom to the surface was 0.4% (right).

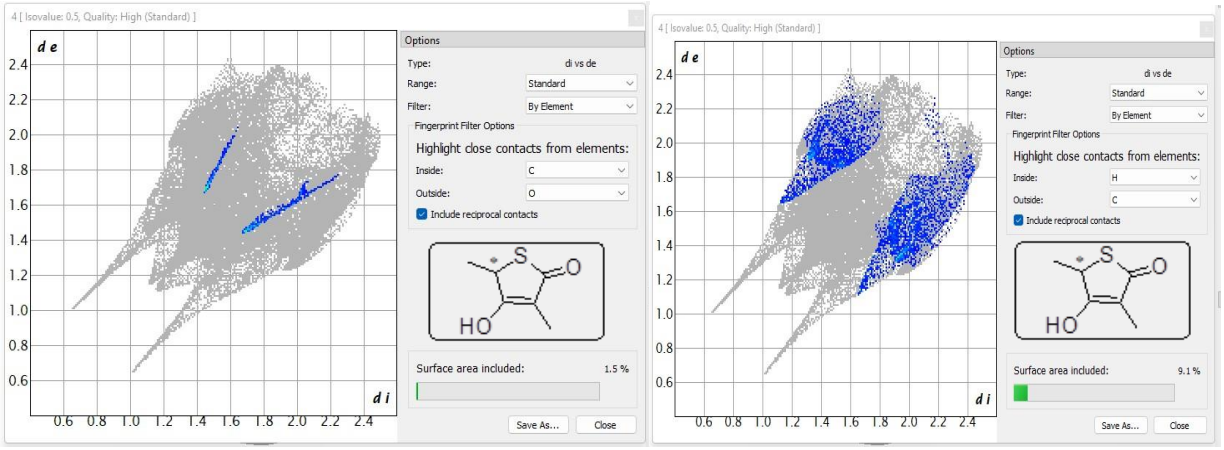


Figure 34. This investigation was based on the assumption of a 1.5% close contact between the C atom of the molecule and the neighbouring O atom to the surface (left). A 9.1% close contact between the H atom and the neighbouring C atom to the surface was found (right).

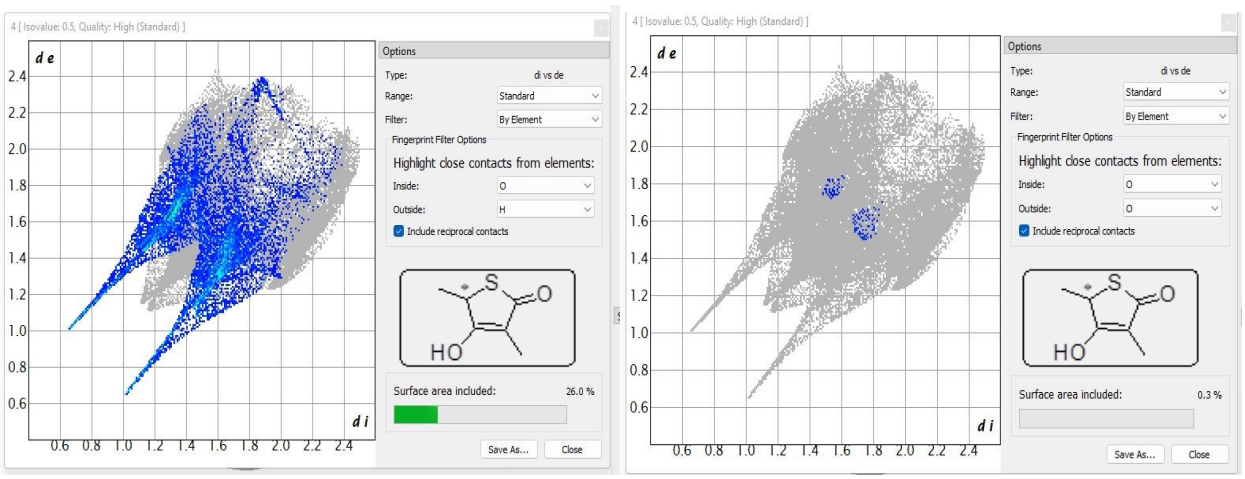


Figure 35. This investigation was based on the assumption of a 26% close contact between the O atom of the molecule and the neighbouring H atom to the surface (left). The close contact between the O atom and the neighbouring O atom to the surface was 0.3% (right).

Chart 1 confirms that oxygen–hydrogen interactions dominate this structure. Hydrogen–hydrogen contacts also contribute a relatively large portion of the surface compared with other atomic contacts. This is typical, as H atoms tend to dominate at the outside of organic molecules; however, while those interactions are numerous, they are generally considered weak. Oxygen–oxygen interactions contribute the least (a negligible amount) to the overall surface shown in **Figures 32-35**.

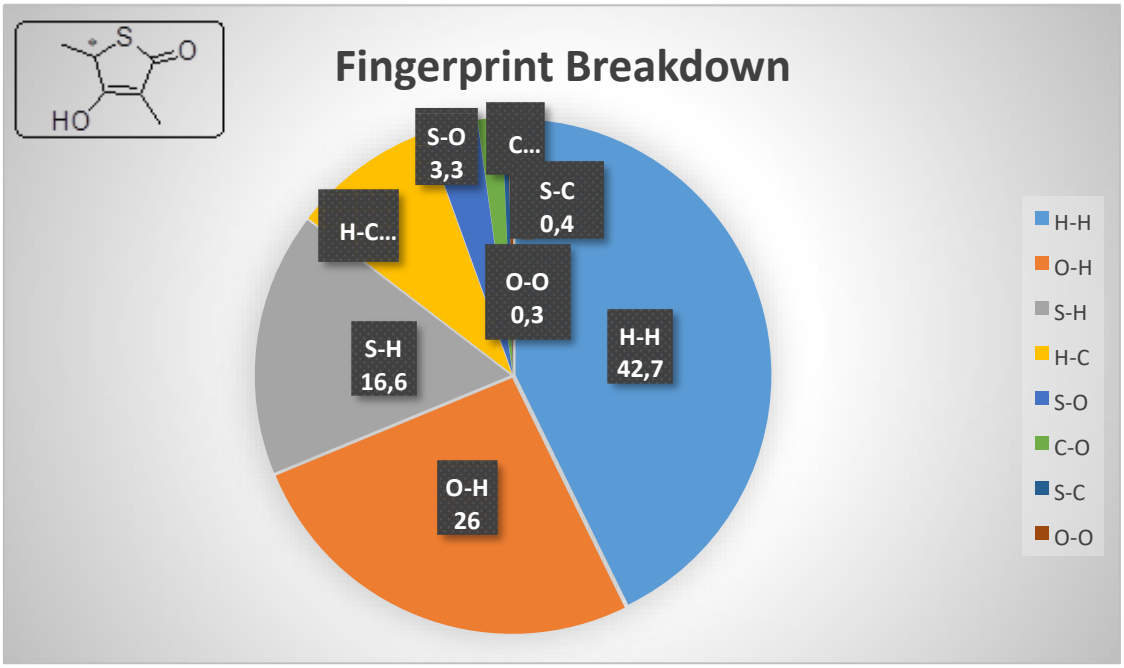


Chart 1. Pie chart of the contributions of different atomic contacts across the surface.

Additionally, the complete fingerprint plot of the achiral structure, it shows different atomic contacts across the surface, as shown in Figure 36. The 2D fingerprint plot has two axes: one corresponds to the distance from the molecule core to the surface (di), and the other corresponds to the distance from the neighbouring atom to the surface (de). A 2D fingerprint breakdown, as shown in Figure 36, reveals close contact O–H information. The dark blue areas contribute the least to the surface, the light blue areas contribute more, and the green areas contribute the most.

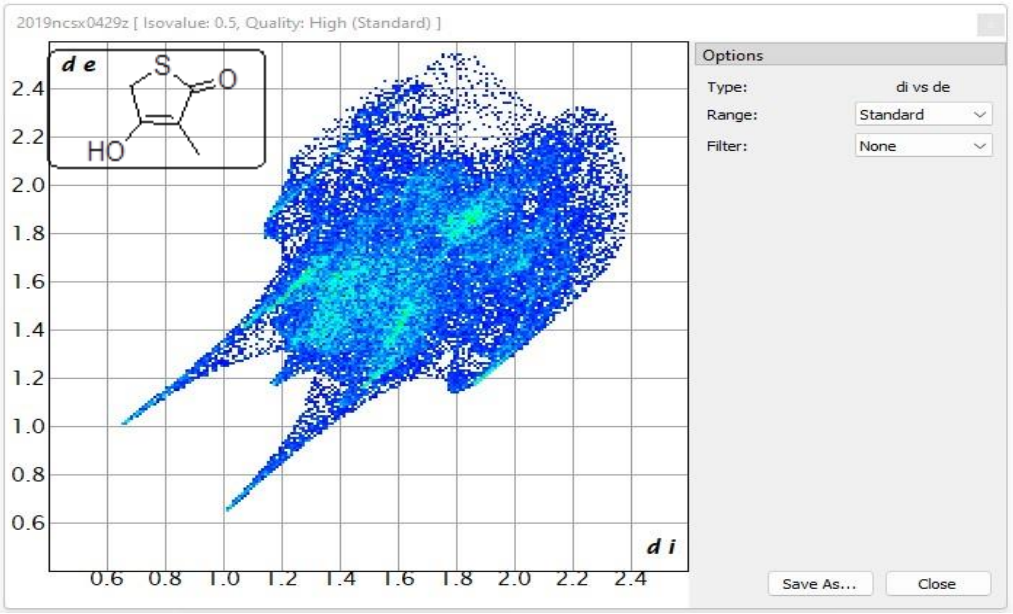


Figure 36. Full fingerprint plot of achiral 3-hydroxy-4-methyl-2*H*-thiophen-5-one.

The dominant interaction in this compound is clearly hydrogen–oxygen contact, as indicated by the multiple portions of light blue and green in Figure 36. Breakdown of the fingerprint plot for achiral 3-hydroxy-4-methyl-2-H-thiophen-5-one **shown in Figures 37-41**

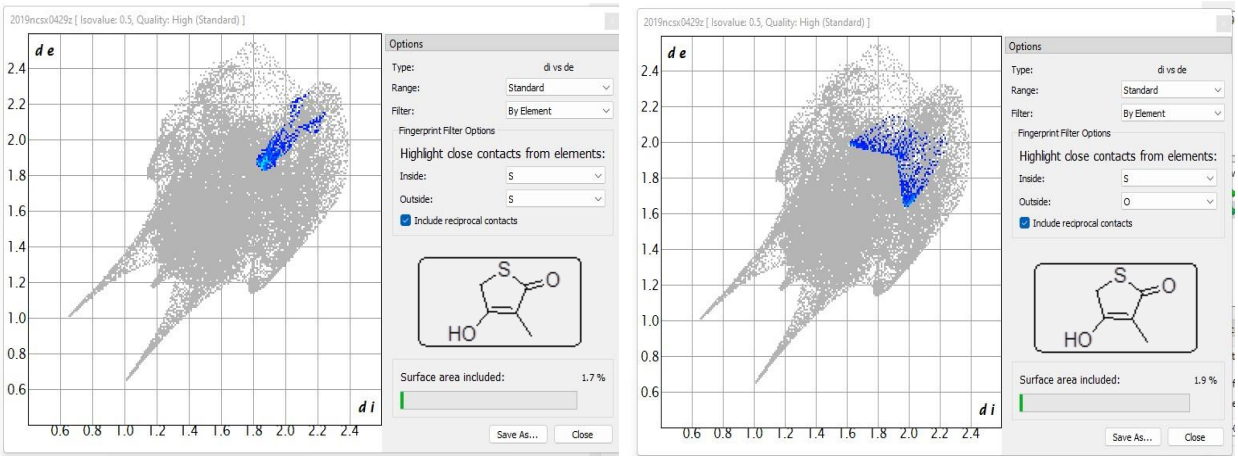


Figure 37. Breakdown of the fingerprint plot for achiral 3-hydroxy-4-methyl-2-H-thiophen-5-one. This investigation was based on the assumption of a 1.7% close contact between the S atom of the molecule and the neighbouring S atom to the surface (left). The close contact between the S atom and the neighbouring O atom to the surface was found 1.9 % (right).

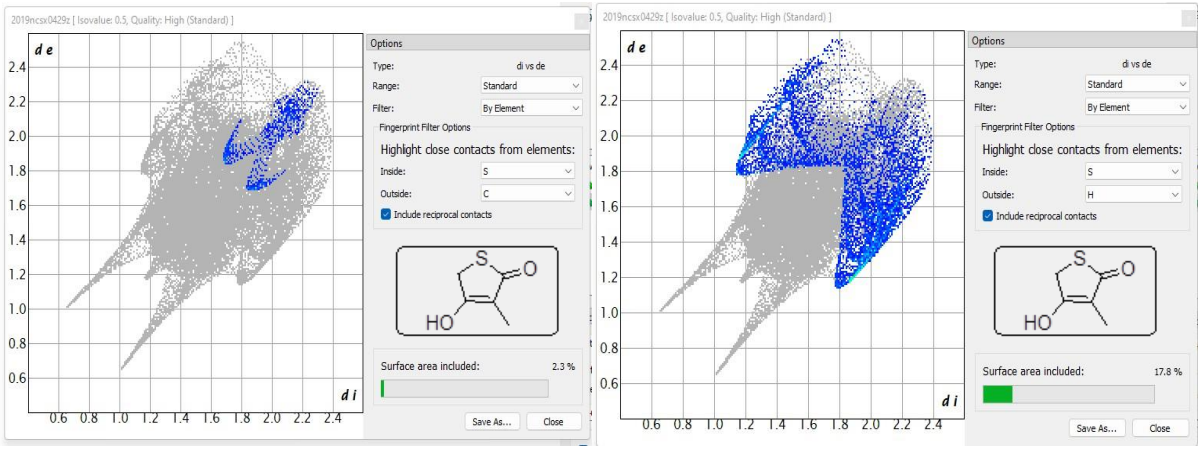


Figure 38. This investigation was based on the assumption of a 2.3% close contact between the S atom of the molecule and the neighbouring C atom to the surface (left). The close contact between the S atom and the neighbouring H atom to the surface was 17.8% (right).

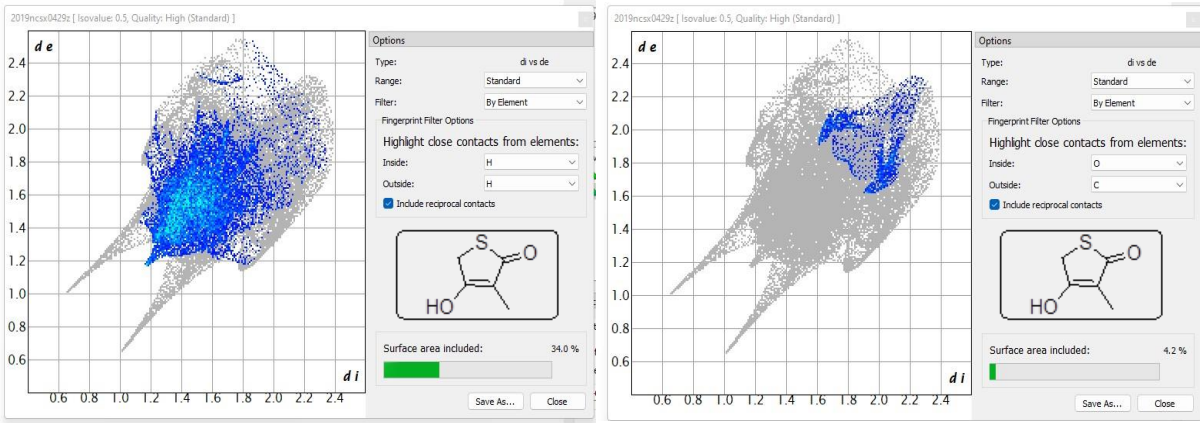


Figure 39. This investigation was based on the assumption of a close contact of 34% between the H atom of the molecule and the neighbouring H atom to the surface (left)**.** The close contact between the O atom and the neighbouring C atom to the surface was 4.2% (right).

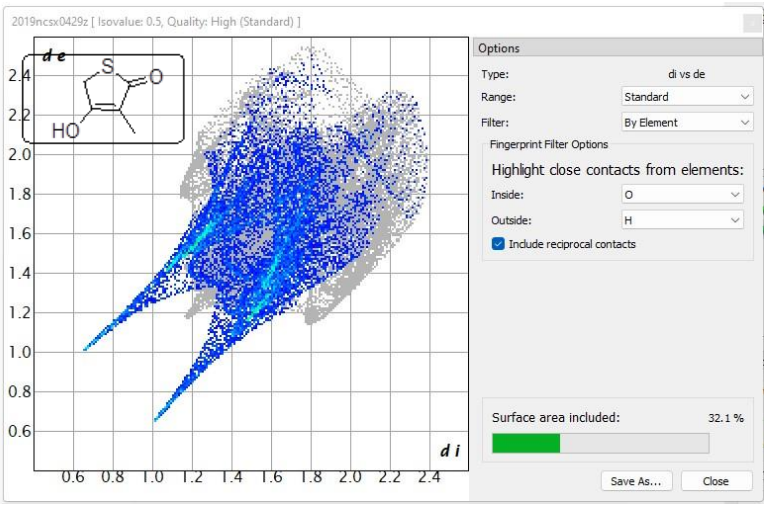


Figure 40. This investigation was based on the assumption of a 32.1% close contact between the O atom of the molecule and the neighbouring H atom to the surface.

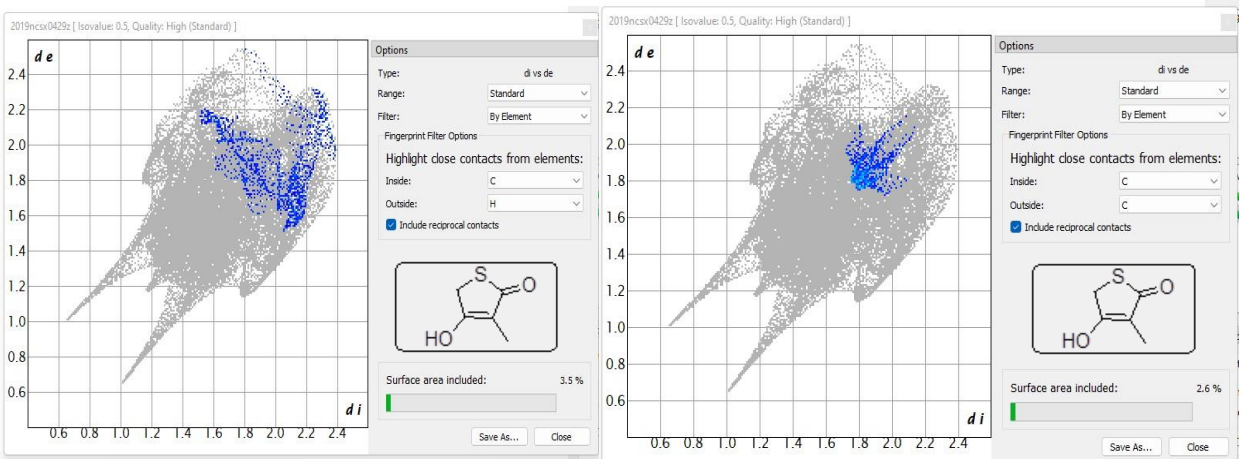


Figure 41. This investigation was based on the assumption of a 3.5% close contact between the C atom of the molecule and the neighbouring H atom to the surface (left). The close contact between the C atom and the neighbouring C atom to the surface was 2.6% (right).

Chart 2 confirms the findings illustrated by the figures: oxygen–hydrogen interactions dominate in this structure. Hydrogen–hydrogen contacts also contribute a relatively large

portion of the surface compared with other atomic contacts. Sulphur–sulphur interactions contribute the least (a negligible amount) to the overall surface shown in Figure **37-41**.

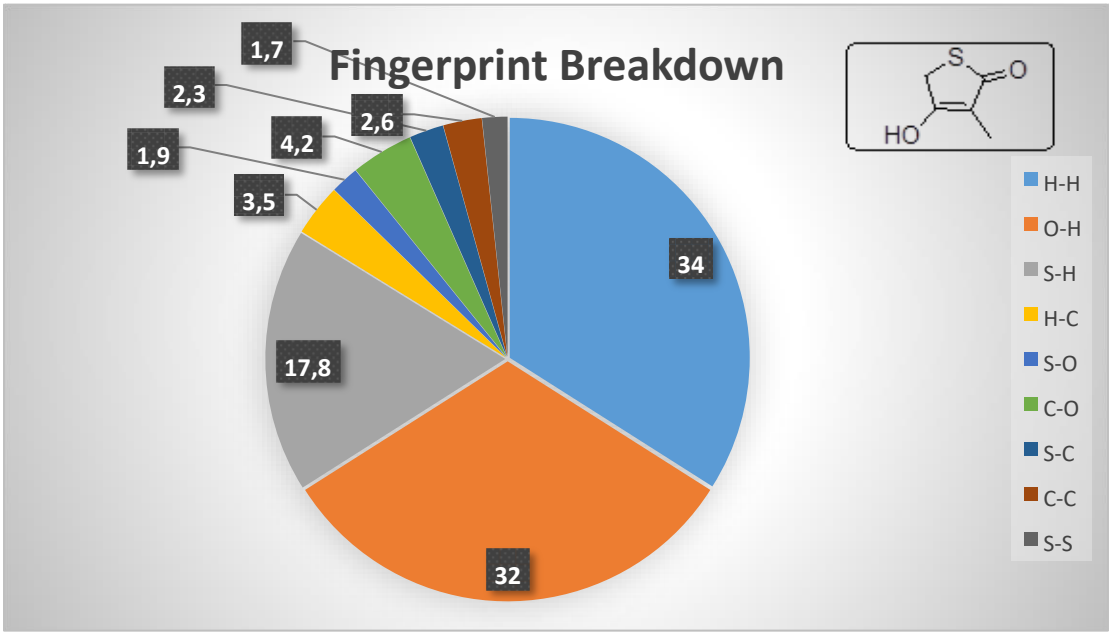


Chart 2. Pie chart of the achiral compound contributions of different atomic contacts to the surface.

To summarise the data, results and discussion of the data by comparing hydrogen bonding between the two compounds, we observed that oxygen–hydrogen interactions dominate for achiral 3-hydroxy-4-methyl-2H-thiophen-5-one, constituting approximately 32.1% of the compound's surface area. Meanwhile, oxygen–hydrogen interactions dominate at 26% for racemic 3-hydroxy-4-methyl-2H-thiophen-5-one, as shown in Figure 42 (to facilitate Comparison of Figure 35 and 40).

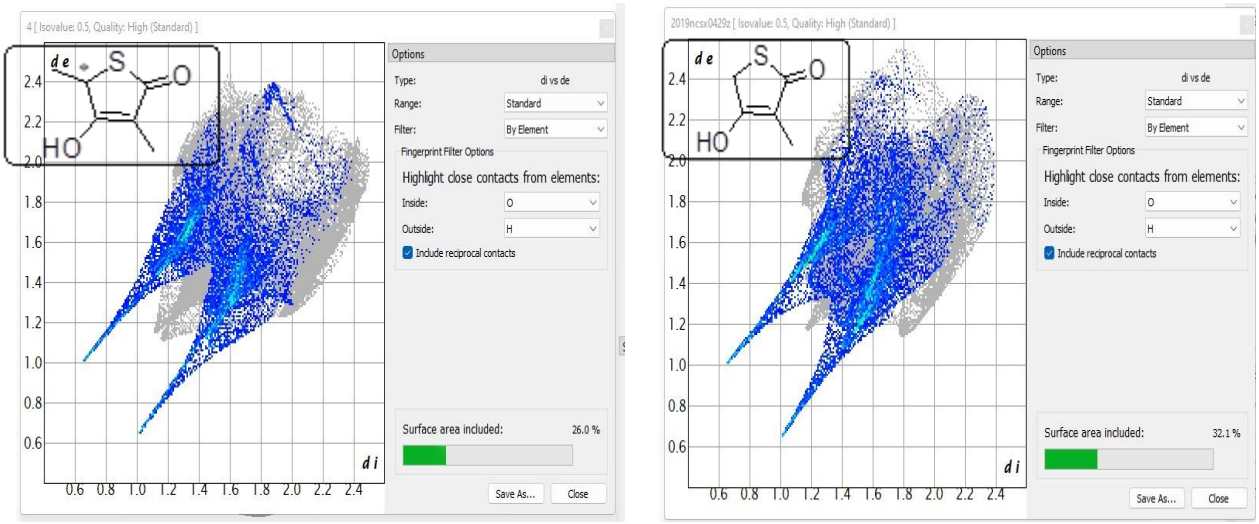


Figure 42 (to facilitate Comparison of **Figures 35 and 40**) Breakdown fingerprint plots showing O–H contacts for racemic 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one (left) and achiral 3-hydroxy-4-methyl-2H-thiophene-2-one.

In completing this experimental work, the crystal or Hirshfeld surfaces of the structures and 2D fingerprint plots were generated, and energy frameworks were visualised using Crystal Explorer software. Percentages of the surface area corresponding to different types of contacts can reveal important packing features within crystal packing. Hirshfeld surface analysis was proven to be valuable for quickly gaining insight into the molecular environment in the crystalline state. It is a valuable tool for understanding the structures of molecular crystals.

3.3.5 Differential Scanning Calorimetry (DSC)

Thermal analysis differential scanning calorimetry (DSC) can be performed on powders obtained by the grinding of a single-crystal sample. DSC is a technique used to determine the thermodynamic properties of solids, such as their melting points, polymorphism or solvate formation. DSC was conducted to analyse the thermal behaviour of the racemic and conglomerate crystal forms. For example, the racemic 3,5-dimethyl-4-hydroxy-5H-thiophene-2-one compound exhibited a melting point of 131.6°C, as discussed later.

The analyses were performed with a DSC 214 Polyma from Netzsch calibrated using highly pure indium as a standard. The melting point measurements were performed with a constant heating rate of 0.5°C·min^{-1} in suitable temperature ranges under a high-purity helium (99.999%) atmosphere. For analysis, 2.38 mg of racemic 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one was weighed in pierced aluminium crucibles. DSC measurements allow us to determine the melting point and enthalpy of fusion. Owing to the temperature programme, two cycles of heating/cooling were performed with the aim of identifying potential different equilibria for the racemic mixture (see Figure 43 and Table 8).

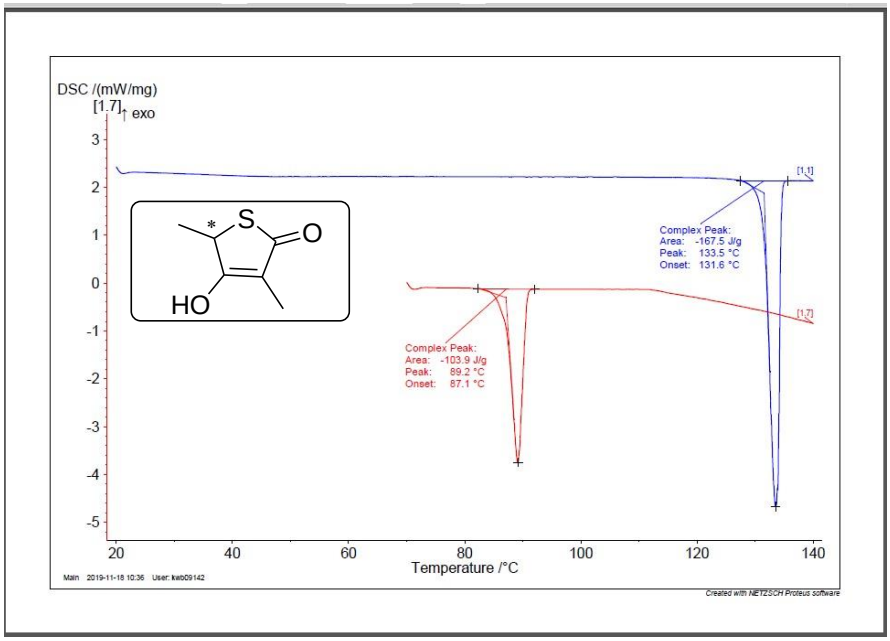


Figure 43. DSC thermograms for racemic 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one (blue represents the first heating cycle, generating an onset temperature of 131.6°C, while red represents the second heating cycle with an onset temperature of 87.1°C).

Thermodynamic property	Racemic 3,5-dimethyl-4-hydroxy-5H-thiophene-2-one		
Melting temperature	[ºC]	87.1	131.6
Enthalpy of fusion	[kJ·mol^{-1}]	103.9	167.5

Table 8. Melting temperatures and heat of fusion of the racemic compound via DSC at a heating rate of 0.5°C·min^{-1} and temperature range of 20–140°C.

Indeed, the melting point of the racemic 3,5-dimethyl-4-hydroxy-5H-thiophene-2-one compound was 131.6°C. The second cycle produced something different, so the racemic mixture could have a metastable racemic compound or polymorph; this compound appears to play a role in polymorphism. When differential scanning calorimetry (DSC) was performed for racemic compound **5**, it was shown to act as a polymorph; that is, it exists in two crystalline forms (13,82). In conclusion, the twin molecule 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one could not be separated using a crystallisation technique. Another option to achieve this would be the chiral chromatographic technique.

The analyses were also performed with a DSC 214 Polyma from Netzsch calibrated using high-purity indium as a standard. Melting point measurements were carried out with a constant heating rate of 0.5°C·min^{-1} in suitable temperature ranges under a high-purity helium (99.999%) atmosphere. For the analysis, 2.26 mg of racemic of 3-benzyl,5-methyl-4-hydroxy-5H- thiophen-2-one was weighed in pierced aluminium crucibles. DSC measurements allow determination of the melting point and enthalpy of fusion. In terms of the temperature programme, two cycles of heating/cooling were performed to identify potential different equilibria for the racemic mixture (see Figure 44 and Table 9).

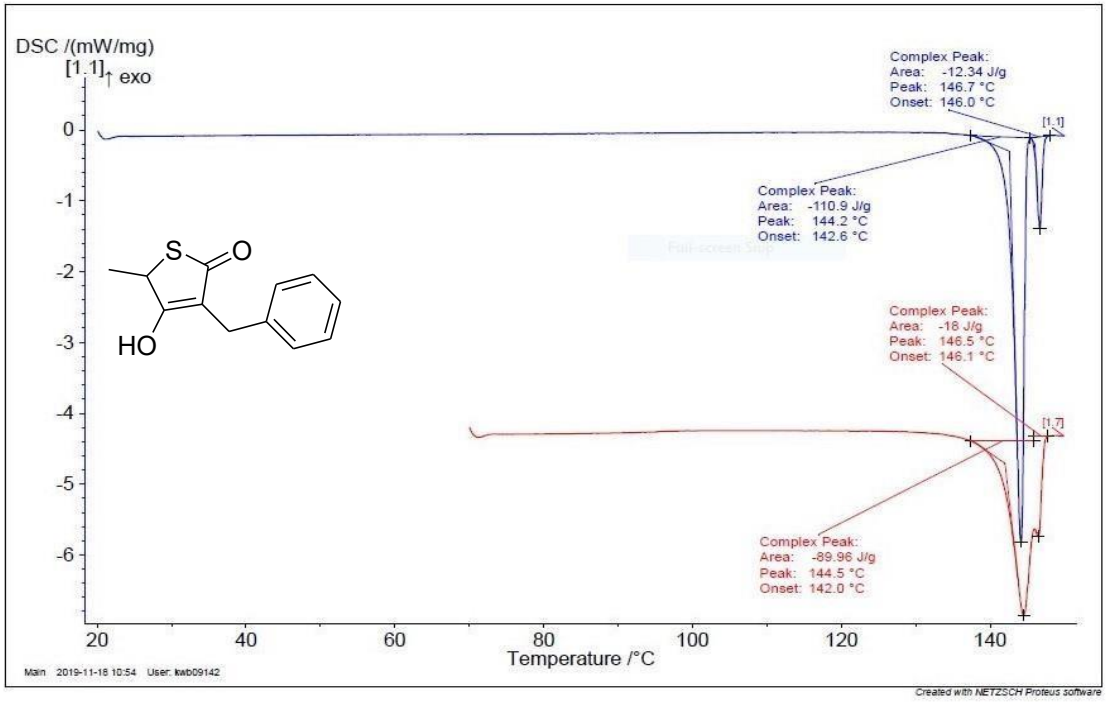


Figure 44. DSC thermograms for racemic 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one. Blue represents the first heating cycle, generating an onset temperature of 142.6°C, while red represents the second heating cycle with an onset temperature of 142.0°C.

Thermodynamic properties	**Racemic 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one**		
Melting temperature	[°C]	142.0	142.6
Enthalpy of fusion	[kJ·mol^{-1}]	89.96	110.9

Table 9. Melting temperatures and heat of fusion of 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one via DSC, obtained at a heating rate of 0.5°C·min^{-1} and a temperature range of 20–150°C.

From the first cycle, the melting point of the racemic 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one compound was 142.6°C. The second cycle gave the same result. However, the racemic mixture presented impurities, as another melting point at 146°C was identified, and the enthalpy of fusion was 12.34 $kJ \cdot mol^{-1}$ (13,82).

- **Results**:
  - **Analogue 1**: Showed a clear melting point at 131.6°C. The second cycle indicated a potential polymorphism, as evidenced by a slight shift in melting behaviour.
  - **Analogue 2**: Displayed unique melting behaviour, suggesting that metastable states could contribute to conglomerate formation.

Detailed explanations are provided for statements regarding differences observed in multiple DSC cycles and potential polymorphic behaviour.

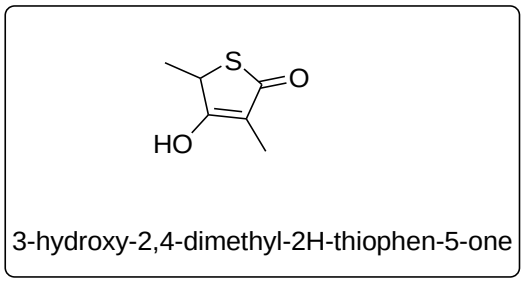
3.3.6 Structural refinement and analysis

For structural refinement, data from SC-XRD and spectroscopic analyses were combined:

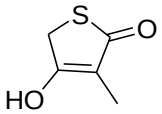
- **Refined models**: The process included iterative adjustments to resolve coordinates and ensure consistency with observed data.
- **Clarification of atomic coordinates**: The statement that “only half the atom coordinates are determined” refers to symmetry operations generating the remaining structure. This step ensures precise modelling of the crystal lattice.

3.3.7 Structural commentary

A comparison of molecular structures between compounds 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one and 3-hydroxy-4-methyl-2H-thiophen-5-one is shown below (figure 45).



3-hydroxy-2,4-dimethyl-2H-thiophen-5-one



3-hydroxy-4-methyl-2H-thiophen-5-one

Figure 45.

A notable structural difference between these structures is the orientation of the hydroxyl groups containing the O_2 atoms. In both structures, this O atom is coplanar with the SC_4 ring, but in 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one, the H atom points towards C5 and is eclipsed by the C2=C3 double bond. Meanwhile, in 3-hydroxy-4-methyl-2H-thiophen-5-one, the H atom points towards the CH_2 group and is eclipsed by the C3–C4 single bond. This change in orientation is associated with a change in the bond angles involving O_2 [compare C4–C3–O2 angles of 113.4° (2) and 119.7°]. The equivalent geometric parameters in the two structures are similar. According to the selected geometric parameters, the largest difference in bond length is found for the S1–C4 values [1.816 (3) and 1.799 (3) A°], as shown in Tables 10 and 11.

Table 10. Selected geometric parameters (A°) for 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one.

S1—C1 1.778 (3)	C3—C2 1.351 (4)
S1—C4 1.816 (3)	C3—C4 1.507 (4)
O1—C1 1.238 (3)	C2—C1 1.434 (4)
O2—C3 1.326 (3)	
C1—S1—C4 92.62 (12)	C3—C2—C1 112.3 (2)
O2—C3—C4 113.4 (2)	C2—C1—S1 112.1 (2)
C2—C3—C4 117.8 (2)	C3—C4—S1 105.16 (18)

Table 11. Selected geometric parameters (A°) for 3-hydroxy-4-methyl-2H-thiophen-5 one

S1—C1 1.775 (3)	C1—C2 1.440 (4)
S1—C4 1.799 (3)	C2—C3 1.347 (4)
O1—C1 1.231 (4)	C2—C5 1.502 (4)

O2—C3 1.332 (4)	C3—C4 1.499 (4)
C1—S1—C4 92.33 (15)	O2—C3—C4 119.7 (3)
C2—C1—S1 112.0 (2)	C2—C3—C4 117.2 (3)
C3—C2—C1 112.2 (3)	C3—C4—S1 106.2 (2)

The main supramolecular feature of both structures is a one-dimensional C (6) hydrogen-bonded chain using OH as the donor group and O1 as the acceptor group.

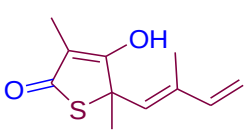
A significant difference was found for 3-hydroxy-4-methyl-2H-thiophen-5-one. The chains propagated by translations corresponded to $x + 1$, y, $z + 1$. This propagation by translation alone provides the repeating pattern shown in Figure 22, where all of the SC_4 rings of the hydrogen-bonded unit are coplanar, and all S atoms lie on the same side of the chain. When travelling along the *b*-axis direction, neighbouring chains bear their S atoms on different sides, producing the layered structure shown in Figure 22.

By contrast, for the structure 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one, the chain propagates through $x + 3/2$, $y + 1/2$, z operation, creating a chain parallel to the crystallographic *b*-axis direction. As shown in Figures 16 and 17, this results in the neighbouring R and S enantiomers of the racemic chain having perpendicular relationships between the planes of their SC_4 rings. This different chain geometry provides a packing arrangement that is very different from that of the other structure (see Figure 18).

Data on 3-hydroxy-4-methyl-2H-thiophen-5-one were collected by the National Crystallography Service. The crystals were found to be twinned by a 180° rotation in the reciprocal 001 direction. In the final refinement, the twin ratio was refined to 0.568 (2):0.432 (2) (84). The main structural feature of both compounds is the hydrogen-bonded chains that form between the OH and C=O groups. In achiral $C_5H_6O_2S$, these chains propagate only by translation, corresponding to $x + 1$, y, $z + 1$. By contrast, for racemic $C_6H_8O_2S$, the hydrogen-bonded chains propagate through a $– x + {}^3/_2$, $y + ½$, z operation, creating chains parallel to the crystallographic *b*-axis direction that are composed of alternating R and S enantiomers.

From a search of the Cambridge Structural Database (version 5.40; Groom et al., 2016), only three other structures with similar 4-hydroxy-thiophen-2-one cores were found (84). These

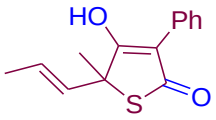
are TLM itself (BIHKIM, Nawata et al., 1989) and two other derivatives (FIVKEA, Chambers et al., 1987; POXZOS, Kikionis et al., 2009) (68,86,87), as shown in Diagram 3.



BIHKIM

P65 space group

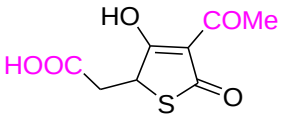
20



FIVKEA

P21 space group

21



POXZOS

P1 space group

22

Diagram 3. Three structures with similar 4-hydroxy-thiophen-2-one cores are available in the database.

These three structures have geometric parameters that are generally similar to those of 3-hydroxy-4-methyl-2H-thiophen-5-one and 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one. Two of the database structures have the same hydroxy group orientation as the 3-hydroxy-4-methyl-2H-thiophen-5-one (**61**) achiral compound. Only POXZOS has the same hydroxy orientation as 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one (**5**), and here this orientation is predetermined by the OH group participating in an intramolecular six-membered hydrogen-bonded ring. As with both structures in the database, the orientation of the OH group is associated with systematic changes to the C–C–O bond angles involving OH. The only interchain contact significantly shorter than the sum of van der Waals radii in either structure occurs in 3-hydroxy-4-methyl-2H-thiophen-5-one. This is a C–H_ _ _O contact between the CH_2 group and the ketone O atom. Of the 3-hydroxythiophen-2-one structures described in the literature, both FIVKEA and BIHKIM (TLM) display the same C(6) hydrogen-bonded chain motif as both structures. In both cases, the geometrical detail of the chain is similar to that found in 3-hydroxy-4-methyl-2H-thiophen-5-one, with the difference being that both examples in the literature are enantiopure. The final structure, POXZOS, contains additional carboxylic acid and carbonyl groups, and these strong hydrogen-bonding groups dominate the intermolecular contacts formed and thus prohibit the formation of the otherwise common C(6) motif.

The comparison of the crystal structure of the molecules of 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one with that of 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one showed that the only modification in the synthesis of the two compounds is the benzyl ring at the 3-position of the thiophen ring. When a molecule with a large molecular weight, such as a benzyl ring at the 3-position of the thiophen ring, and with a large Log P is synthesised, it is more likely to form a conglomerate enantiopure crystal. In this case, however, the molecules become near-racemic, as they form RR and SS isomers, and only pi-stacking interaction occurs between the two enantiomers (see figure 46).

Therefore, in future work, synthesising a molecule with a large functional group, such as a molecule with a large molecular weight and an increasing Log P, would be better for crystallising the molecule in conglomerate or enantiopure form.

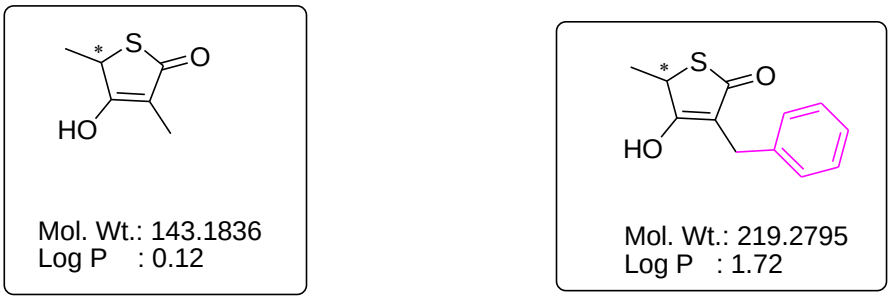


Figure 46.

Although the two enantiomers can be distinguished by single-crystal X-ray diffraction data, their separation cannot be achieved chemically. The differential behaviour of the racemic compound illustrates the complexity associated with the spontaneous resolution or conglomerate formation of chiral products. This type of crystal could not be distinguished by its colour or tendency to crystallise, so we could not separate the two enantiomers (13,76,82). Chiral resolution is confirmed by a comparison of crystallographic and thermal analysis results (83).

3.4 Discussion and Conclusions

3.4.1 Analysis of results

- **Crystallisation tendencies**: Most TLM analogues favoured racemic packing, consistent with their centrosymmetric space groups. However, one analogue demonstrated potential for non-centrosymmetric crystallisation.
- **Implications**: The data suggest that, while TLM analogues predominantly crystallise in racemic forms, careful adjustment of crystallisation conditions could yield conglomerate crystals.

3.4.2 Comparative insights (TLM vs. IBU)

- **Lessons learned**: This study revealed that TLM analogues follow crystallisation patterns similar to those of many chiral compounds where racemic forms dominate. The IBU example highlights the feasibility of tuning crystallisation conditions to favour conglomerates.

In summary, Crystal Explorer is a valuable tool for understanding the structure and properties of molecular crystals made up of three compounds.

The space group of crystal filling is required to determine whether a compound tends to crystallise as a racemic compound or conglomerate. Conglomerate crystals can be resolved using a space group of crystals, as shown in Table 1, which lists the unique space groups categorised by the crystal system. In crystallography, a *centrosymmetric point group* has an inversion centre as one of its symmetry elements, such that every point group (x, y, z) in the unit cell has inversion symmetry or point reflection ($-x$, $-y$, $-z$). By contrast, a non-centrosymmetric point group lacks an inversion centre and can be chiral. When separating a mixture of left- and right-hand compounds using crystallisation and pursuing a pure enantiomer, there must be a non-centrosymmetric space group that only exhibits rotation, with no inversion symmetry or mirror image.

Three compounds could not be separated using a crystallisation technique. The difficulties caused by the COVID-19 restrictions and the inability to return to Strathclyde University

hindered me from separating these three compounds using another chiral chromatographic technique. The percentages of ee and the purity of each enantiomer can be calculated on the basis of chromatographic data. Once we have the pure enantiomer of the three compounds, we can compare the result of a single crystal structure of a pure enantiomer to that of a single crystal structure of a racemic one. This would enable understanding of the difference in behaviour between pure and racemic enantiomer.

Chapter 4: Development of a Method to Determine the %ee of Ibuprofen Model Compound towards Deracemisation

4.1 Introduction to Deracemisation Techniques

The resolution of racemic mixtures into enantiomerically pure compounds remains a significant challenge in pharmaceutical and chemical synthesis. Deracemisation, the process of converting a racemic mixture into a single enantiomer, is a powerful technique for achieving this (2). This chapter details the method developed in this study to determine the enantiomeric excess (%ee) of the model compound used, particularly as it relates to the process of deracemisation.

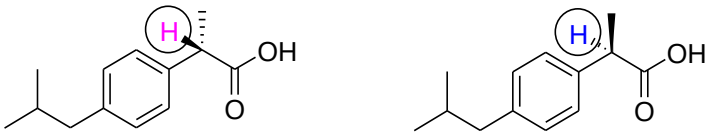
4.2 Objective:

The objective of the work described in this chapter is to develop and validate a method for determining the %ee of the model compound, using polarimetry and high-performance liquid chromatography (HPLC), and to apply this method in the context of deracemisation experiments.

4.3 Model Compound Selection and Rationale

The model compound chosen for this study was **ibuprofen (IBU)**, a chiral drug that exists as a racemic mixture. Ibuprofen was selected due to its well-known crystallisation properties and its use in previous deracemisation studies. Additionally, the presence of both racemates and conglomerates in its crystallisation makes ibuprofen an ideal candidate for exploring the techniques of enantiomeric separation and %ee determination.

Ibuprofen, a widely used non-steroidal anti-inflammatory drug (NSAID) for pain and inflammation, exists as two enantiomers: (S)-(+)-ibuprofen and (R)-(−)-ibuprofen. While both have anti-inflammatory activity, the (S)-enantiomer **16** exhibits significantly greater potency and a better safety profile than the (R)-enantiomer **17**, as shown in Figure 47 (88, 89). This difference in activity has led pharmaceutical companies to focus on producing and distributing enantiopure (S)-ibuprofen.



(S)-(-) Ibuprofen **16**
active

(R)-(-) Ibuprofen **17**
undesirable

Figure 47. Chemical structures of (S)-ibuprofen and (R)-ibuprofen.

If we superimpose the (R)- and (S)-enantiomers, they will not match because of differences in their spatial orientations. When racemisation occurs, a mixture of the two enantiomers at a 1:1 ratio is always obtained. This occurs in conditions resembling physiological ones.

Ibuprofen is commonly used for the treatment of rheumatoid arthritis. When it is used for such treatment and the (R)-enantiomer is present, it can produce the primary side effects of gastrointestinal ulceration and haemorrhage. Meanwhile, (S)-ibuprofen provides three times faster relief with fewer side effects than the racemic mixture. To avoid the disadvantages of the (R)-enantiomer, it can be converted into the active form (S)-enantiomer through metabolic inversion" (88). However, if enantiomeric resolution is used to obtain the (S)-enantiomer, it, has the disadvantage of producing a maximum of 50% yield of the desired enantiomer and the rest is waste. Motivated by the goal, my colleagues and I developed an environmentally friendly and inexpensive process to convert racemic mixtures into active enantiomers in a cost-effective and efficient manner (89).

(R) undesired enantiomer $\xrightarrow{\text{Racemisation}}$ RS racemic mixture

According to our literature review, the enzymatic resolution of ibuprofen and the separation of (S)-ibuprofen are followed by the forced racemisation of (R)-ibuprofen by deprotonating the α-position of a carboxylate salt to form a planar double-enolate intermediate. This procedure is carried out via at least an 8 h reflux in a 1:1 solution of dimethyl sulfoxide and a 2 M NaOH

base. However, this is an inefficient method, as it requires repetition of the resolution and racemisation steps until enough (S)-ibuprofen has been produced (89). In a previous study, the saturation temperature of a racemic mixture of ibuprofen in hexane was found to be 44.5°C, whereas that of the enantiopure sample in hexane was 18.1°C, in which case the racemic compound was highly stable compared with the enantiopure one (39).

Enantiopure compounds can be obtained through various methods, as described below.

4.3.1 Conventional Resolution Methods

- **Chiral resolution:** This involves separating a racemic mixture (a 1:1 mixture of both enantiomers) into its individual enantiomers using various techniques, such as chiral chromatography or preferential crystallisation (12, 30).
- **Chiral chromatography:** This uses a chiral stationary phase to separate enantiomers based on their different interactions with the chiral environment (12).
- **Preferential crystallisation:** This relies on the formation of conglomerates, in which the enantiomers crystallise as separate solid forms (30).
- **Enantioselective stereoinversion:** This involves designing a synthetic route that selectively produces only the desired enantiomer. This technique involves transforming the unwanted enantiomer into an achiral intermediate that can then be converted into the desired enantiomer. It holds potential for achieving high yields and for simplifying the deracemisation process (2, 19).

4.3.2 Recent Advancements: Deracemisation

In recent years, there has been growing interest in deracemisation techniques, including the following

- **Dynamic kinetic resolution (DKR):** This approach couples kinetic resolution with an in situ racemisation process to drive the reaction towards a single enantiomer. It is an attractive approach for achieving high enantiomeric purity (2, 19).

- **Attrition-enhanced deracemisation (Viedma ripening):** This technique involves a combination of grinding and crystallisation to achieve enantiomeric enrichment. It has the potential to improve the efficiency and environmental friendliness of the process (18).

The primary objective of this research was to develop a method for determining the enantiomeric purity (%ee) of ibuprofen towards deracemisation.

Specifically, this research aimed to:

1. **Identify the most suitable method for resolving the racemic mixture of ibuprofen.** This involved investigating different approaches for resolving racemic mixtures and identifying the most suitable method among them for this specific compound.
2. **Develop a reliable method for determining the %ee of ibuprofen:** This involved investigating different analytical techniques and validating their accuracy for measuring enantiomeric purity.
3. **Evaluate the feasibility of using deracemisation techniques for resolving ibuprofen:** This involved determining the potential of directly converting a racemic mixture of ibuprofen into a single enantiomer using deracemisation techniques.

4.4 Methodology

Several approaches for determining the %ee of ibuprofen were investigated here, including the following:

1. Classical resolution using chiral resolving agents: This involves reacting the racemic mixture with a chiral resolving agent to form diastereomeric salts. These diastereomeric salts have different physical properties, such as solubility, and can therefore be separated by fractional crystallisation. This involved reacting the racemic mixture of ibuprofen with a chiral resolving agent, (S)-(-)-alpha-methylbenzylamine, to form diastereomeric salts.

This approach is widely used in the pharmaceutical industry, but it can be challenging for certain compounds, particularly those with low solubility or that form conglomerates.

- **Rationale:** The use of a chiral resolving agent is a well-established method for separating enantiomers.
- **Advantages:** This method is relatively straightforward, often provides good yields, and can be performed using readily available reagents.

2. Chromatographic resolution: This technique, commonly used in the pharmaceutical industry, involves separating a racemic mixture based on the different interactions of the enantiomers with a chiral stationary phase.

- **Advantages:** High efficiency and the ability to achieve high enantiomeric purity.
- **Limitations:** Requires specialised equipment and expertise.

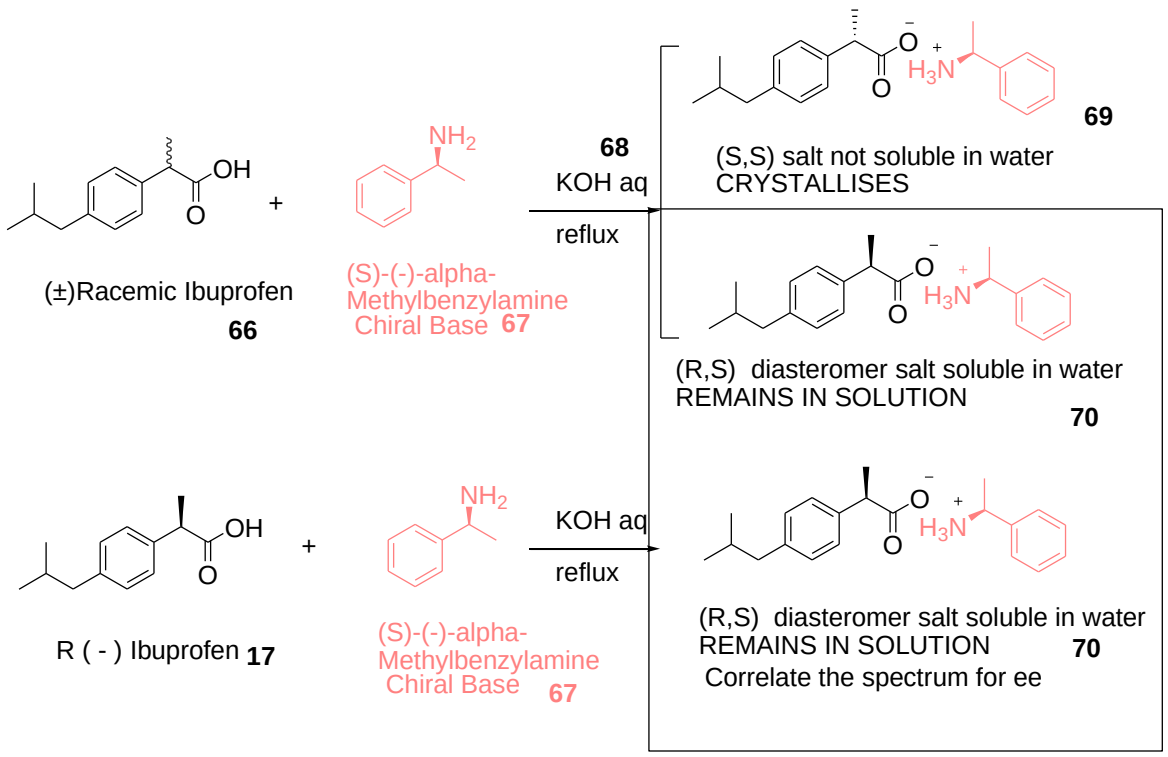
4.4.1 Application of the classical resolution for the separation of RS-ibuprofen (methodology)

Our goal was to develop a separation system that enables resolution of the enantiomers of ibuprofen in the form of diastereomeric salts, as illustrated in Scheme 22. This is a practical way of synthesising optically active S-ibuprofen anti-inflammatory drugs. The resolution of compound **66** carried out using half the equivalent of the resolving agent **67** allowed the formation of diastereomeric salts, namely compounds **69** and **70**. This was followed by the crystallisation of compound **69** or separation by crystallisation-induced diastereomer transformation, in which compound S-ibuprofen **16** was obtained, as shown in Scheme 23. Therefore, using a specific quantity of resolving agent causes the two enantiomers to be prepared simultaneously (9).

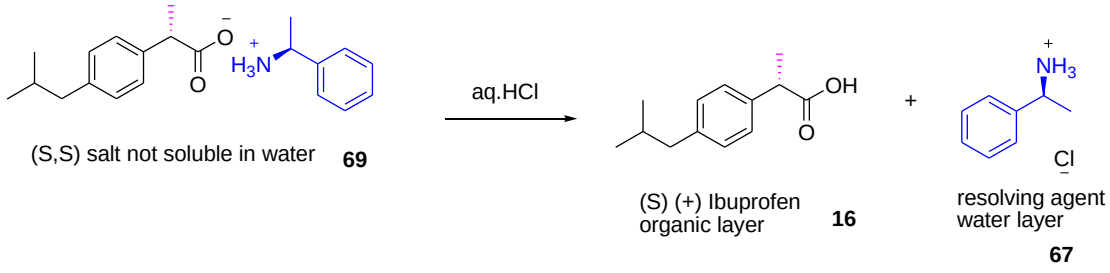
The selected study model was as follows:

Racemic ibuprofen + chiral resolving agent $\longrightarrow$ diastereomeric salt

R- ibuprofen + chiral resolving agent $\longrightarrow$ diastereomeric salt



Scheme 22. Chiral resolving agent: a) Resolution of the enantiomers of ibuprofen in the form of diastereomeric salts. b) The single-enantiomer reaction procedure using the reaction of the specific enantiomer R-ibuprofen with a chiral amine to form diastereomeric salts (90).



Scheme 23. Recovery of the desired enantiomer using acid.

For our experiments, we chose α-methylbenzylamine, as it is the first base of choice to separate enantiomeric acid by chiral resolution; it is a base that is cheap and widely available, for which the (S)-enantiomer is used as an agent to resolve ibuprofen. (S)(−)-α-

Methylbenzylamine, also known as (S)(−)-1-phenylethylamine (abbreviated as (S)(−)-PEA) was selected for this study as one of most common resolving agents for racemic ibuprofen.

Generally, both proton and carbon NMR spectra can be used to measure the percentage of diastereomeric excess of the two diastereomers de%. However, here, only the carbon NMR spectrum was used.

In this study, the chiral resolution of racemic ibuprofen using (S)(−)-α-methylbenzylamine as a resolving agent was used as the model system. Recovery of the desired enantiomer (S)-ibuprofen as well as recycling of the resolving agent could be used to reduce the cost and carbon footprint, to ensure compliance with a green chemistry approach.

4.5 Results and Discussion

The experimental results demonstrated that classical resolution using a chiral resolving agent is a suitable and efficient method for resolving racemic ibuprofen and obtaining enantiopure (S)-ibuprofen. The results of the experiments indicated that ibuprofen is a suitable candidate for deracemisation using classical resolution with a chiral resolving agent. Specifically, the following findings were made:

- **Classical resolution using a chiral resolving agent:** This method was successful in resolving the enantiomers of ibuprofen using (S)-(−)-alpha methylbenzylamine as a chiral resolving agent. This resulted in the formation of two diastereomeric salts: (R,S)-salt and (S,S)-salt. The (S,S)-salt was less soluble in water and crystallised out of solution, due to its lower solubility in water, allowing it to be isolated and subsequently converted back to (S)-ibuprofen. Meanwhile, the (R,S)-salt remained in solution. This allowed isolation of the (S,S)-salt, which was then treated with an acid to recover the desired (S)-ibuprofen. This approach provided high enantiomeric purity (93.5%) and a good yield (64%), making it an efficient method for resolving ibuprofen.

Owing to the simplicity and availability of ibuprofen racemate and single-enantiomer compounds, I chose them to verify reversible equilibrium. Such verification may involve the

comparison of ibuprofen with a single enantiomer. Upon comparing the resulting compounds when racemic ibuprofen was used versus when R-ibuprofen was used with the same chiral amine, I found that almost the same (R, S)-salt was obtained. A smart NMR experiment may be the best method to use with polarimetry or derivatisation to a diastereoisomer. Once the racemic mixture is crystallised in racemic form, it is allowed to react with resolving agents, such as a chiral base, to form a diastereomeric salt. This is one method of separating a racemic compound to obtain a diastereomer salt. The experiment described above was conducted in relation to the overall flow of my PhD project. The experiment complete the dataset and ensure that they provide useful data to support future TLM research on the racemisation of ibuprofen organic compounds and determining enantiomeric excess values, as depicted in Schemes 22 and 23. In this work, we developed an economical and effective method for the preparation of S-ibuprofen on a laboratory scale by using (S)-(−)-α-methylbenzylamine as a chiral resolving agent. The R-ibuprofen enantiomer was relatively well resolved by a chiral resolving agent, with the product having a high yield of 49% without racemisation. The applied method is simple, does not require a complex apparatus and involves conditions that can be produced in small-scale research laboratories.

The rapid measurement of enantiomeric excess using NMR is based on the formation of diastereomeric complexes between the chiral analyte and the chiral resolving agent, which leads to two species with no symmetrical relationship. When a resolving agent is used, the separation of a mixture of enantiomers is achieved with a high % ee value. The CH peaks of the ibuprofen unit related to the two diastereoisomers are completely separated, allowing us to integrate them accurately. Maximum resolution was afforded through the use of water, as this most polar solvent may be preferable in chiral resolution; it is cheap and safe and can form hydrogen bonds with the diastereomeric salt, resulting in preferential crystallisation. The proton or carbon NMR spectra of diastereomeric salt were used to confirm the success of the chiral separation. The degree of chiral resolution is usually measured by determining the percentage of diastereomeric excess of the two diastereomers.

In the ^{13}C-NMR spectrum of ibuprofen, the scale spans from 0 to 250 parts per million (ppm) regardless of the nucleus observed. The different carbons of the given structure show different chemical shifts. In the ^{13}C-NMR spectrum, the signals are all singlets, with the

exception of the solvent, which is deuterated chloroform ($CDCl_3$), giving the triplet centred at 77.2 ppm. For the signals of the isopropyl group $(CH_3)_2C$, these two methyl carbons appear to have the same chemical shift; their carbons will be equivalent, while the other methyl group appears at a very different location and therefore differs in chemical shift.

For the ibuprofen unit, there are two kinds of CH groups: four that belong to the ring and two outside the ring. They can be clearly discerned because of the simplicity of the ^{13}C spectrum compared with the ^{1}H spectrum. It should be noted that four signals at 22.4, 45.0, 127.2, and 129.4 ppm have double the intensity compared with that in the pure enantiomer, usually indicating the equivalency of two carbons or the chemical shift of two carbons. The ^{13}C-NMR spectra of racemic ibuprofen and pure R-ibuprofen in $CDCl_3$ are shown in Figures 48 and 49, respectively. The 1D carbon experiment is sensitive to all ^{13}C nuclei in the sample. It clearly resolves nine resonances. An interesting feature evident in these spectra is that the peak at 45 ppm is made up of CH and CH_2 resonances with identical chemical shifts.

NMR is a rapid analytical technique, but the chemical shifts obtained when using it are identical for D- and L-enantiomers. This is because the number of peaks observed is equal to the number of protons attached. For ^{13}C, the number of protons attached to carbon causes splitting of carbon atom. The chemical shift value of ^{13}C nuclei in a sample is detected to deduce the different environments of those nuclei in the above structure. The signal for carbonyl carbons is the furthest downfield at 180 ppm because of sp^2 hybridisation and the double bond to oxygen. The ^{13}C isotope makes up only 1% of total carbon and has a magnetic dipole moment, just like a proton. It is a weak signal, and it takes a long time to acquire a spectrum. A ^{13}C-NMR spectrum provides useful information, for the number of signals that indicates the number of different carbons atoms present. Each peak indicates a carbon atom in different environments in the same molecule. The external magnetic field surrounding the carbon nuclei is affected by the electronegativity of the atoms attached to them. This means that more significant chemical shifts occur when more electronegative atoms are attached.

The detailed carbon-NMR spectra shown in Figure 50 appear like fingerprints with slight differences between them, depending on the rotation of the molecule with the magnetic field. When comparing the results obtained with racemic ibuprofen and R-ibuprofen as starting

materials, similar results were obtained, with the magnitude of the chemical shift of chiral carbon showing a 2:1 ratio between these samples, as shown in Figures 48–51.

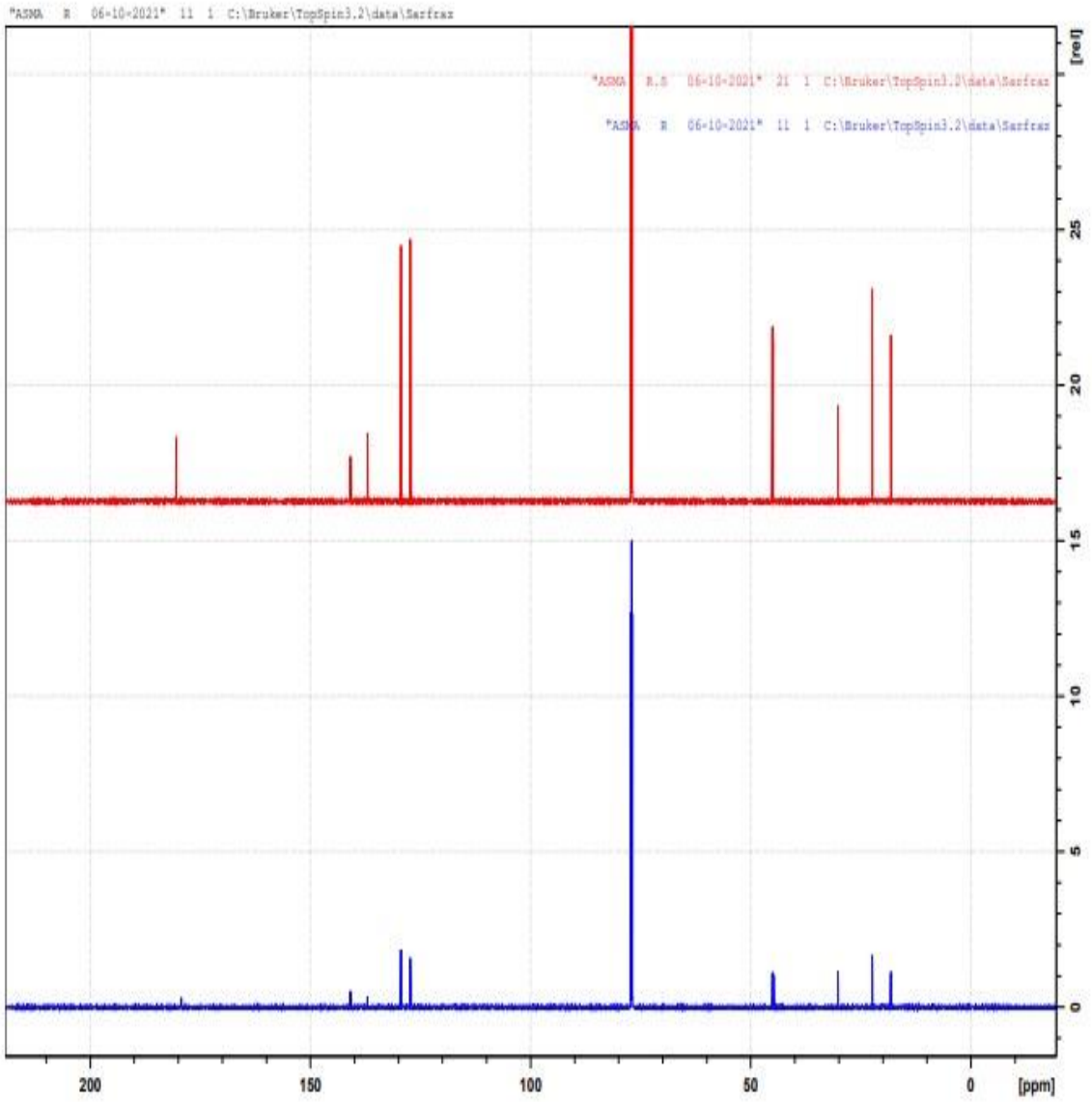


Figure 48. Carbon-NMR data when the starting materials were racemic ibuprofen (top) and R-ibuprofen (bottom), with similar results showing the magnitude of the chemical shift of chiral carbon showing a 2:1 ratio between these samples.

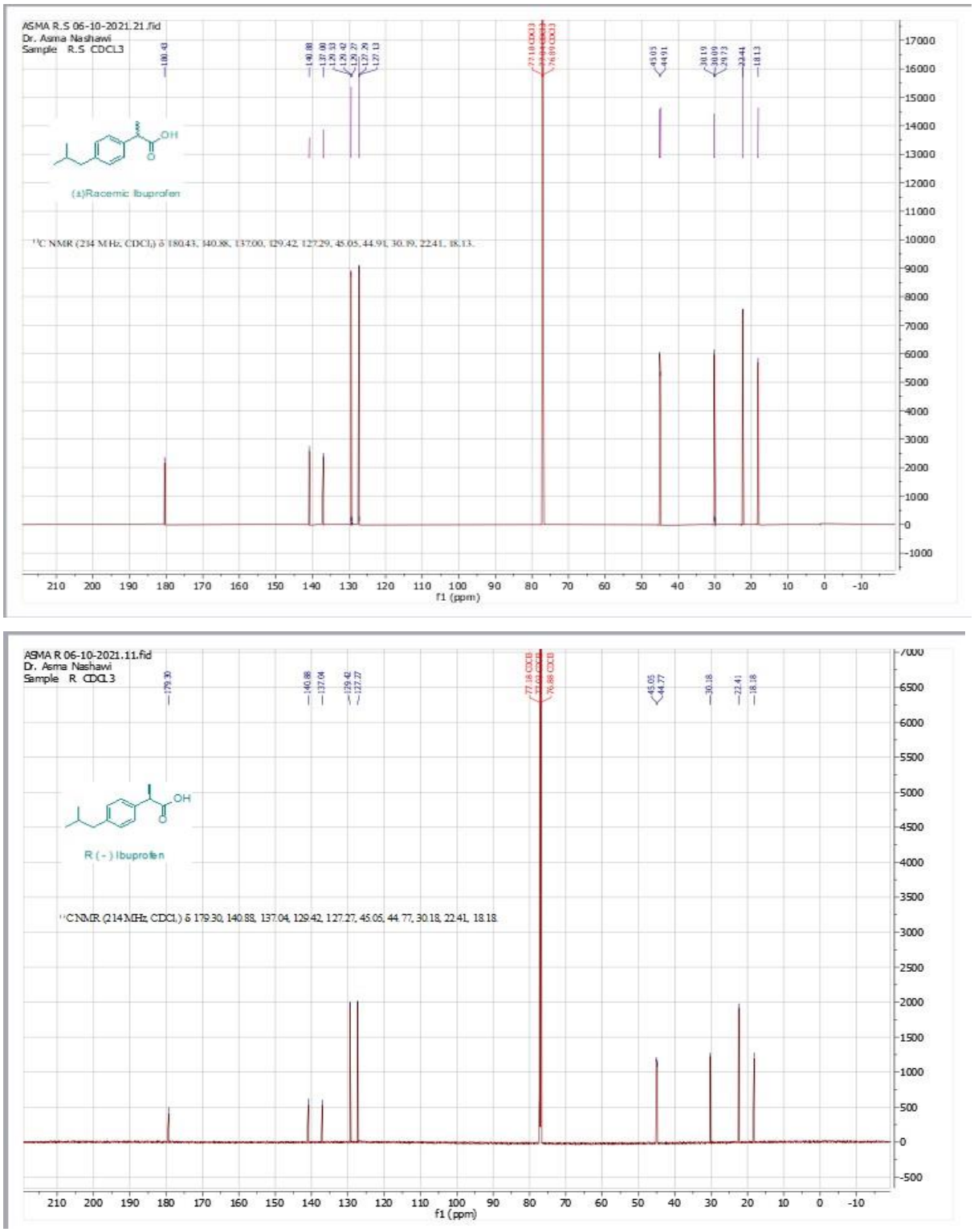


Figure 49. Carbon-NMR data when the starting materials were racemic ibuprofen (top) and R-ibuprofen (bottom).

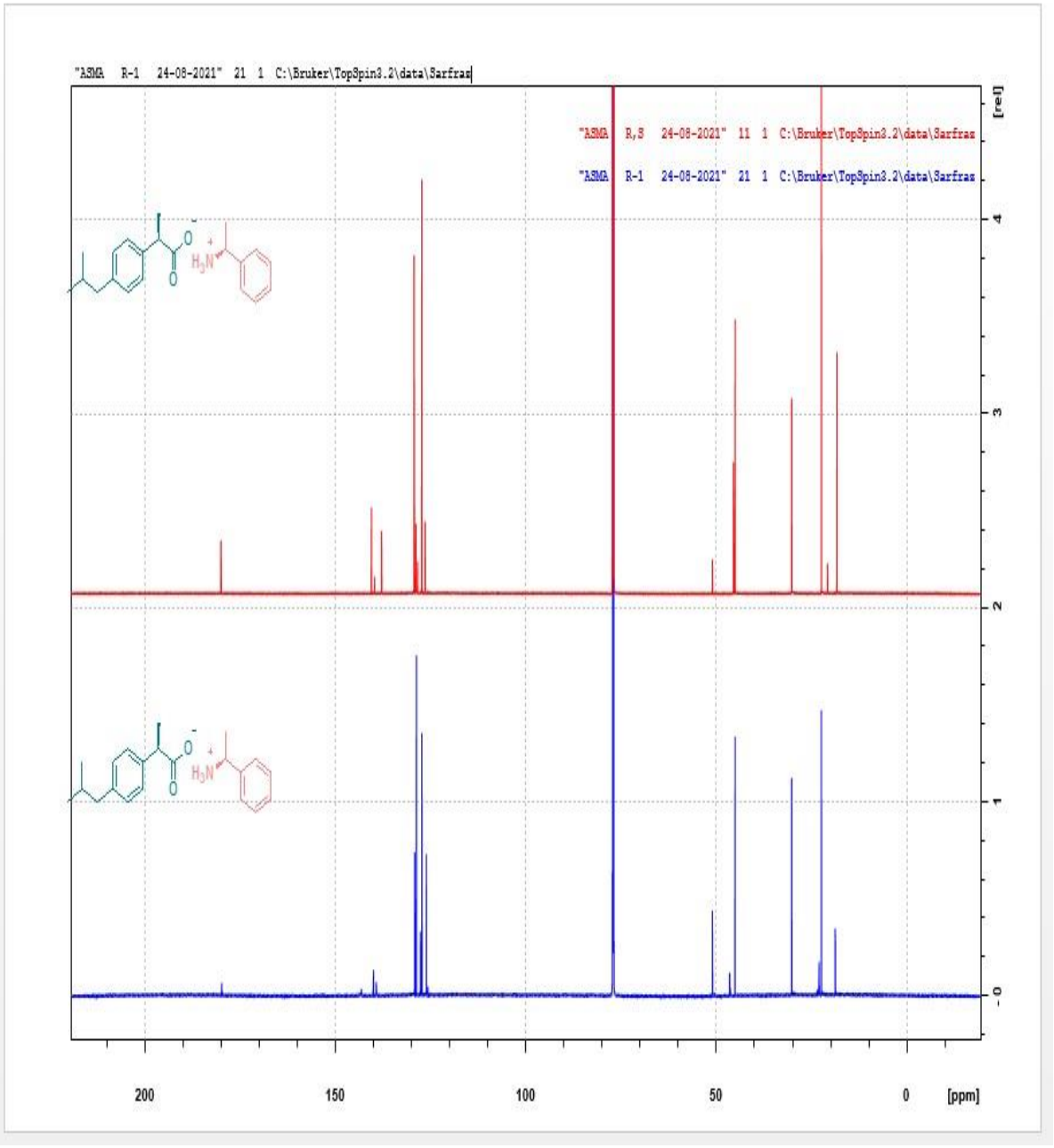


Figure 50. Carbon-NMR data of the target analogue when racemic ibuprofen (top) and (R)-ibuprofen were used with a chiral base (bottom).

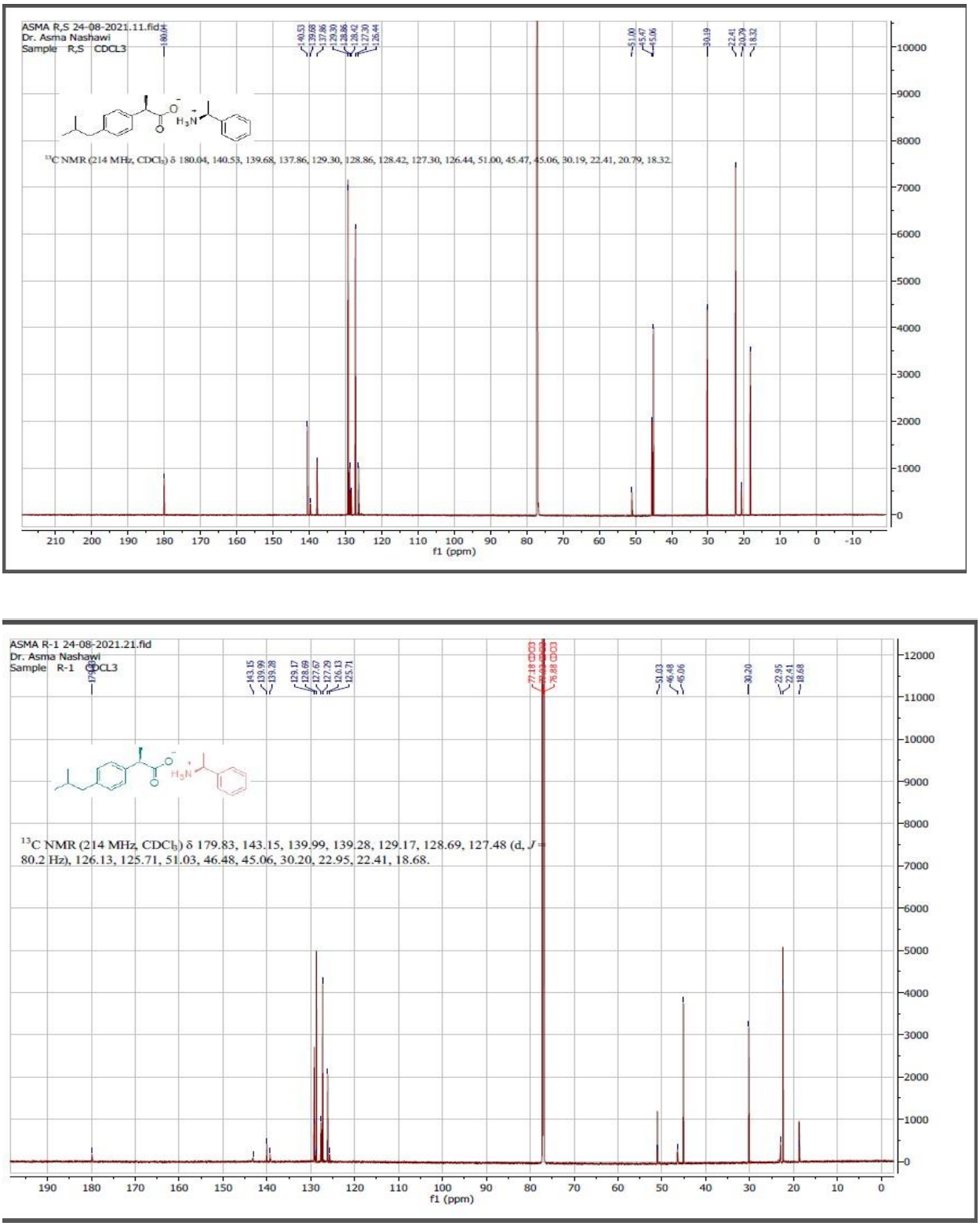
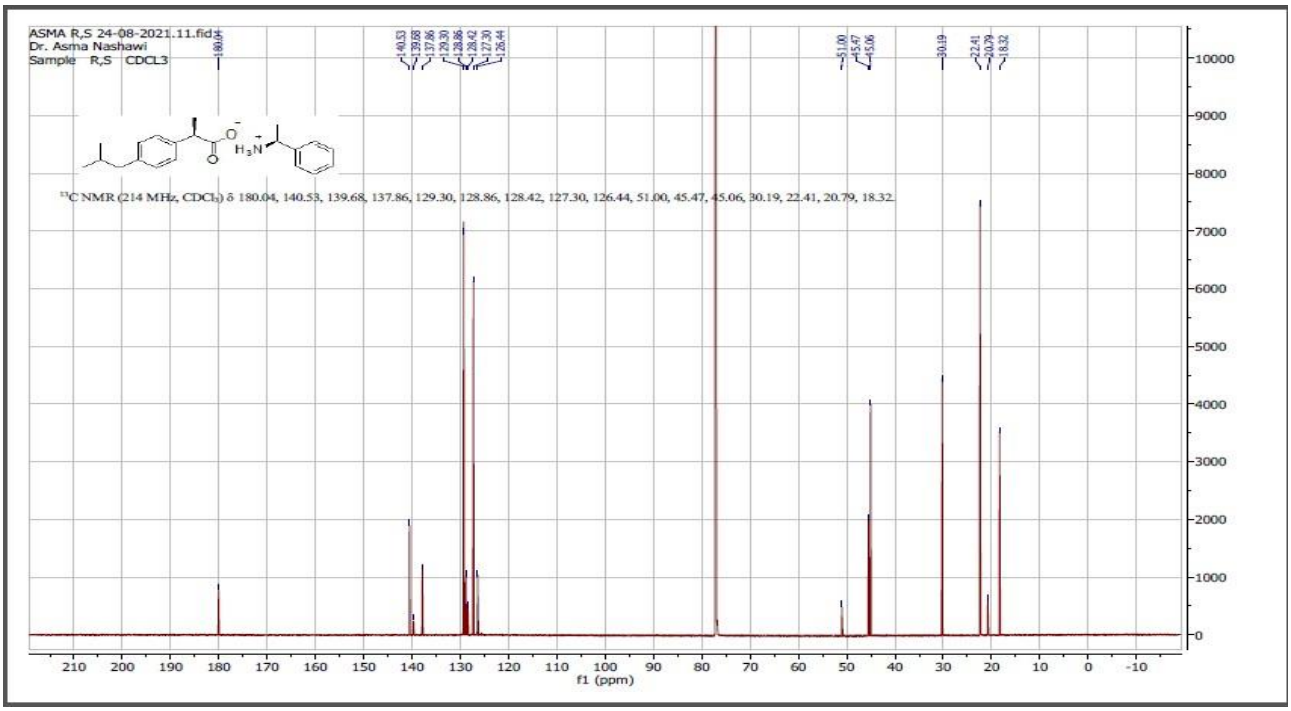


Figure 51. Carbon-NMR spectra of (R, S)-salt compounds when racemic ibuprofen (top) and (R)-ibuprofen were used with a chiral base (bottom).

The interpretation and comparison of both carbon spectra of the two diastereomers show that one results from a racemic mixture of enantiomers and the other from a pure enantiomer. The result is two duplet peaks at 45.47–45.06 ppm for the (R,S)-salt from the racemic one, while one peak signal at 45.06 is split from the other peak at 46.48 ppm for the (R,S)-salt from a single enantiomer.

From Figure 52 below, the two diastereomers showed two different peaks appearing as duplets at 45.47–45.06 ppm for the (R,S)-salt of the diastereomer pair as two signals, in contrast to one peak at 44.93 ppm for the (S,S)-salt. Furthermore, for CH attached to amine NH_3, the signal appearing at 50.76 for the (S,S)-salt is slightly different from that for the (R,S)-salt, which can be seen at 51 ppm.

The use of an enantiopure chiral agent reacting with the chiral analyte enables the formation of covalent bonds with enantiomers and their conversion into stable diastereoisomers. Among the detectable nuclei in organic molecules, carbon-13 NMR can be observed. Recording ^{13}C-NMR spectra is advantageous in terms of the clarity of discriminating enantiomers due to the differences in their magnitudes, leading to the robust determination of ee values calculated by peak integration. NMR-based methods, such as ^{13}C-NMR, have been used to evaluate enantiomeric recognition and determine the enantiomeric excess of chiral compounds. NMR techniques provided useful information about models of the enantio-separation of enantiomeric ibuprofen with a chiral amine base example. Therefore, chiral discrimination through the formation of complexes, which might be extended to separation techniques, was realised.



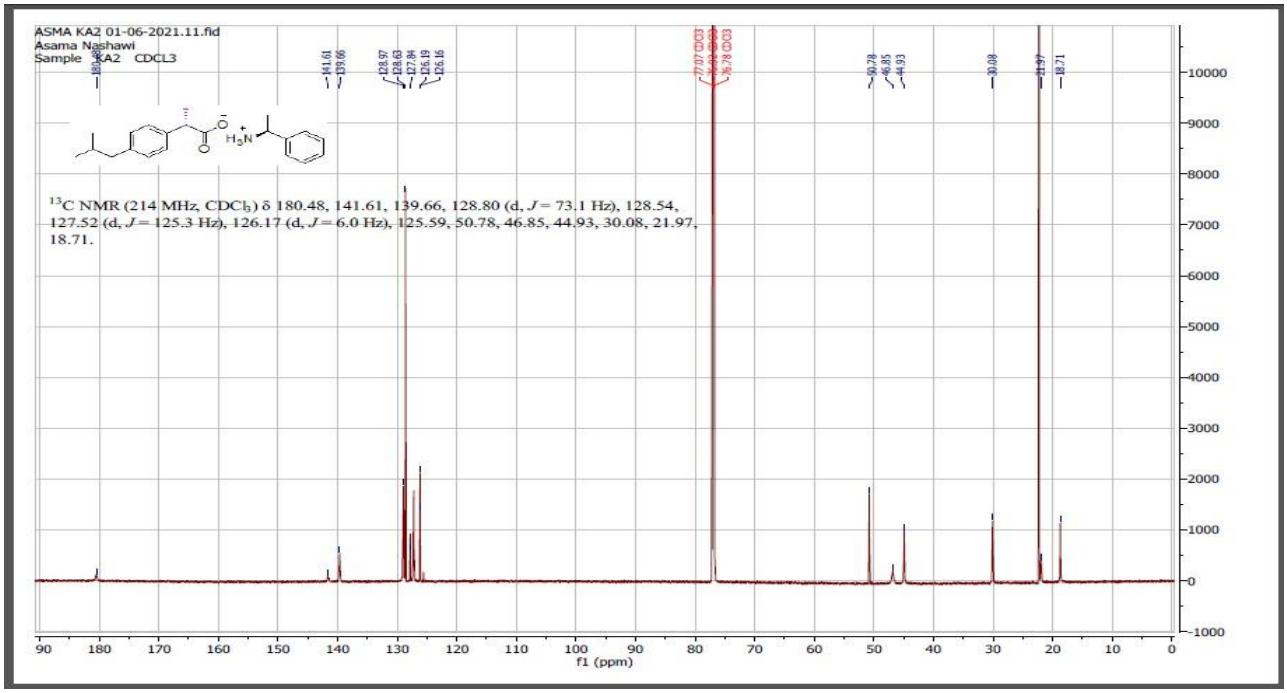


Figure 52. Carbon-NMR spectra of the (R,S)-salt compounds (top) and (S,S)-salt compounds when racemic ibuprofen (bottom) was used.

4.6 Conclusion

Based on the experimental results, it was concluded that classical resolution using a chiral resolving agent is a suitable and efficient method for resolving racemic ibuprofen and obtaining enantiopure (S)-ibuprofen. This method is readily accessible, provides good yields and is a valuable tool for the pharmaceutical industry.

4.7 Future Work

While the classical resolution method proved successful for resolving ibuprofen, future research could focus on exploring alternative deracemisation techniques for ibuprofen, such as the following:

1. Deracemisation techniques: This work involved different deracemisation techniques, such as dynamic kinetic resolution (DKR) and attrition-enhanced deracemisation (Viedma ripening), which directly convert a racemic mixture into a single enantiomer. These approaches converted the racemic mixture of ibuprofen into its enantiopure form through repeated crystallisation cycles. In each cycle, a supersaturated solution of racemic ibuprofen was seeded with the enantiopure form, and the resulting crystals were analysed to determine the %ee. The racemic ibuprofen was dissolved in ethanol, and the solution was heated to form a saturated solution. The solution was then cooled slowly to induce crystallisation. A small amount of enantiopure ibuprofen was added as a seed, promoting the selective crystallisation of the desired enantiomer. This process was repeated until a high %ee was achieved. The effectiveness of this process was confirmed through both polarimetry and chiral HPLC measurements. These methods have attracted increasing attention in recent years due to their potential for achieving higher yields and for being more environmentally friendly than conventional resolution methods. However, these approaches may be more challenging to implement and require further optimisation for specific compounds (18-20).

2. Chromatographic resolution: This technique, commonly used in the pharmaceutical industry, involves separating a racemic mixture based on the different interactions of the enantiomers with a chiral stationary phase. In future work, chiral high-performance liquid

chromatography (HPLC) will be employed as a more precise method for determining %ee. A chiral stationary phase will be used to separate the R- and S-enantiomers of ibuprofen, and their relative concentrations will be measured using an ultraviolet (UV) detector set at a wavelength of 254 nm. The %ee can be calculated from the chromatographic data using the following formula: %ee = (R−S) / (R+S) × 100, where R and S represent the concentrations of the right- and left-handed enantiomers, respectively (12, 16)

This research has highlighted the challenges associated with resolving racemic mixtures and the importance of considering the crystalline properties of the compound. Given this background, future work should focus on developing more efficient and environmentally friendly methods for resolving ibuprofen and understanding the complex interplay between molecular structure and the formation of conglomerates.

4.8 Methods for Determining %ee

The accurate determination of enantiomeric excess is critical for evaluating the success of the deracemisation process. Primary method was employed in this study: polarimetry and other method which was the chiral HPLC will be used in the future. Both techniques allow the quantification of the ratio of enantiomers in a racemic mixture and provide insights into the efficiency of the deracemisation process.

4.8.1 Polarimetric Studies

Polarimetry is a widely used technique for measuring optical rotation, which is directly related to the enantiomeric composition of a chiral sample. Here, polarimetry was employed to determine the %ee of ibuprofen. A solution of the racemic and enantiopure forms of ibuprofen was prepared, and the optical rotation was measured at a wavelength of 589 nm. The %ee was calculated using the following formula:

$$\%\ ee = \frac{|\text{observed } \alpha|}{|\alpha \text{ of pure enantiomer}|} \times 100\%$$

where *{observed* α*}* is the optical rotation of the sample, and *[α of pure enantiomer*} is the optical rotation of the pure enantiomer.

4.8.1.1 Introduction

Polarimetry is one of the most widely used methods for studying the racemisation of chiral organic compounds and determining %ee (91). It is a sensitive, non-destructive technique for measuring the optical activity of organic compounds that can be used to determine the purity and concentration of a substance. A compound is considered optically active if polarised light rotates when it passes through it. Plane-polarised light is created by passing light through a polarising device. Enantiomers rotate plane-polarised light to an equal degree but in the opposite directions: clockwise (+) or counterclockwise (–). The polarisation plane can be determined using an instrument called a polarimeter. Chemists use polarimeters to investigate the effects of compounds composed of either right- or left-handed molecules on plane-polarised light when examining a single enantiomer. (91)

4.8.1.2 Instrument

A P3000 polarimeter (A. Krüss Optronic GmbH, Hamburg, Germany was used to measure the optical rotation produced by the ibuprofen sample. After measurement, Biot-Savart law was applied to calculate the specific rotation as follows:

$$[\alpha]_{\lambda}^{T} = \frac{\alpha_{\lambda}^{T}}{c \cdot l}$$

where [*α*] is the specific rotation, *α* is the observed rotation, *c* is the concentration of the sample in grams per millilitre (g/mL), l is the length of the sample container in decimetres (1 dm = 10 cm), *λ* is the wavelength and *T* is the temperature in degrees Celsius (°C). The light source was the D-line of a sodium lamp at 589 nm and 25°C (92, 93).

4.8.1.3 Methodology, Results and Discussion

In the first step, the device was calibrated with a blank containing no solvent (only air), and the reading was 0.02°. (S)-(+)-Ibuprofen was prepared by dissolving 100 mg of optically active

material in 1 mL of methanol (MeOH). The measurement was performed in a 10 cm cell. The observed optical rotation for three replicates of the (S)-ibuprofen sample at a mean concentration of 0.1 g/mL was +5.19°, as shown in Figure 53. The experiment was repeated three times to ensure reproducibility.

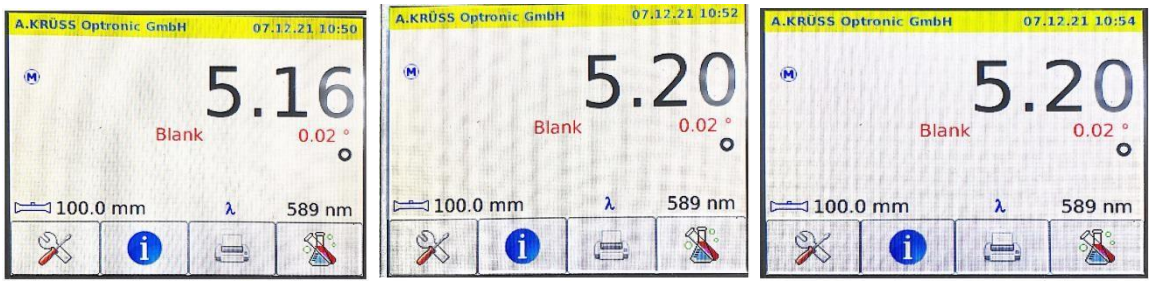


Figure 53. Three readings of the observed optical rotation (°) in the polarimetric measurement of the S-enantiomer of ibuprofen.

Thus, the specific rotation calculated on the basis of Biot-Savart law as follows (92-94):

$[a]_D^{25}$ = 5.19/(0.1 X1) = 51.9°.

The ee was calculated as follows:

ee = (observed specific rotation) / (specific rotation of pure enantiomer) × 100%

(S)-Ibuprofen was found to have a specific rotation in its pure form of 54.5° (dextrorotatory) in MeOH (94-96).

For this ibuprofen example, ee = (51.9 / 54.5) × 100% = 95.2%.

This means that the sample contains 95.2% excess S-ibuprofen. As the percentages of the S- and R-enantiomers must sum to 100%,

$R + S = 100,$ $S = R + 95.2,$ $R + R + 95.2 = 100$

$2R + 95.2 = 100,$ $2R = 4.8,$

$$R = \frac{4.8}{2} = 2.4\ \% \qquad S = 2.4 + 95.2 = 97.6\%.$$

Therefore, the percentages of *(R)*- and *(S)*-ibuprofen in the sample were 2.4% and 97.6%, respectively.

For R-ibuprofen, the device was calibrated with a blank containing no solvent (only air), and the reading was 0.02°. (R)-(−)-Ibuprofen was prepared by dissolving 100 mg of optical material in 1 mL of MeOH. The measurement was performed in a 10 cm cell. The observed optical rotation for three replicates of the (R)-ibuprofen sample at a concentration of 0.1 g/mL had an average of −5.1°, as shown in Figure 54.

Figure 54. Three readings of the observed optical rotation (°) in the polarimetric measurement of the R-enantiomer of ibuprofen.

Thus, the specific rotation based on Biot-Savart law was calculated as follows:

$[a]_D^{25}$ = 5.1 / (0.1 X1) = −51°

(R)-Ibuprofen was found to have a specific rotation in its pure form of −54.5° in MeOH (94-96).

For the R-ibuprofen example, ee = (−51 / −54.5) × 100% = 93.5%. This means that the sample contains a 93.5% excess of R-ibuprofen. As the percentages of the S- and R-enantiomers must add up to 100%,

$$R + S = 100, \qquad R = S + 93.5, \quad S + S + 93.5 = 100$$

$$2S + 93.5 = 100, \qquad 2S = 6.5,$$

$$S = \frac{6.5}{2} = 3.2\ \% \quad and\ R = 3.2 + 93.5 = 96.8\%.$$

Therefore, the percentages of (*R*)- and (*S*)-ibuprofen in the sample were 96.8% and 3.2%, respectively.

For racemic ibuprofen, the device was calibrated with a blank using an empty tube containing no solvent (only air), and the reading was 0.01°. Racemic (R/S) -(±) ibuprofen was prepared by accurately dissolving 100 mg of optical material in 1 mL of MeOH. The measurement was performed in a 10 cm cell. The observed optical rotation for the (R/S)-ibuprofen sample at a concentration of 0.1 g/mL was 0.00°, as shown in Figure 55. Thus, there were equal amounts of R- and S-enantiomers in the sample; that is, no optical activity was observed, as each cancelled out the optical rotation of the other.

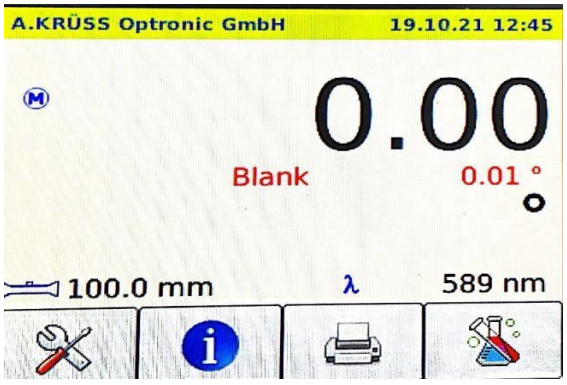


Figure 55. Readings of the observed optical rotation (°) in the polarimetric measurement of racemic ibuprofen.

In summary, the measurement results obtained using the specific light from a sodium atomic spectrum with a wavelength of 589 nm, which is called the sodium D-line from a sodium lamp, are used in most polarimetric measurements. The observed optical rotation depends

on the length of the tube, the concentration of the sample and the temperature. The results obtained here prove that it is feasible to rapidly predict the enantiomeric excess value based on a polarimetry technique. The optical rotation of the single-enantiomer S-ibuprofen was in the clockwise (+) direction, whereas for the R-ibuprofen, it was counterclockwise (−, described as laevorotatory). Meanwhile, the optical activity was lost for racemic ibuprofen.

In conclusion, this study successfully developed and validated methods for determining the %ee of ibuprofen, a model compound for deracemisation studies. The combination of polarimetry and chiral HPLC allowed for the accurate and reliable measurement of enantiomeric excess, providing critical insights into the efficiency of the deracemisation process. Polarimetry, while simple and rapid, was less precise than chiral HPLC with regard to the above results. However, it was sufficient for providing an initial estimate of the enantiomeric purity. Chiral HPLC, meanwhile, allowed for more detailed analysis of the enantiomeric composition, confirming the success of the deracemisation procedure. Future work should focus on extending these methods to other chiral compounds and further optimising the crystallisation conditions to improve the efficiency of the deracemisation process.

Chapter 5:
Conclusion and Future Work

5.1 Conclusion

Chirality is an important aspect of modern drug design. Nowadays, there is a preference for creating single-isomer drugs because of the significant advantages that they offer to patients. Only one enantiomer is acceptable in a chiral environment, because biological systems distinguish between the two enantiomers as two different components, leading to different responses. Driven by the need for enantiomerically pure compounds, technological advancements have led to the development of new methods for separating racemic mixtures.

Two different methods are discussed of this kind are discussed in this thesis: classical resolution and the chromatographic technique. The chromatographic technique, particularly HPLC, is used in the pharmaceutical industry for the large-scale separation of compounds in racemic mixtures. Meanwhile, the synthesis of an enantiopure compound can be achieved using two approaches: the chiral pool method and asymmetric synthesis. Recently, the latter of these, with either chiral auxiliary or chiral catalysts, has seen a dramatic increase in its usage. This new method has many applications in modern drug design by ensuring the selective production of only a single enantiomer. (1,6,10,11).

Following crystallisation resolution, one of four main separation methods for obtaining pure enantiomers (see Fig. 56) can be applied: enzymatic resolution, asymmetric synthesis, direct crystallisation, and chiral chromatography separation of any chiral molecule. Crystallisation resolution, which occurs by either direct crystallisation or diastereomeric crystallisation, is one of the most efficient and practical techniques for producing enantiopure compounds.

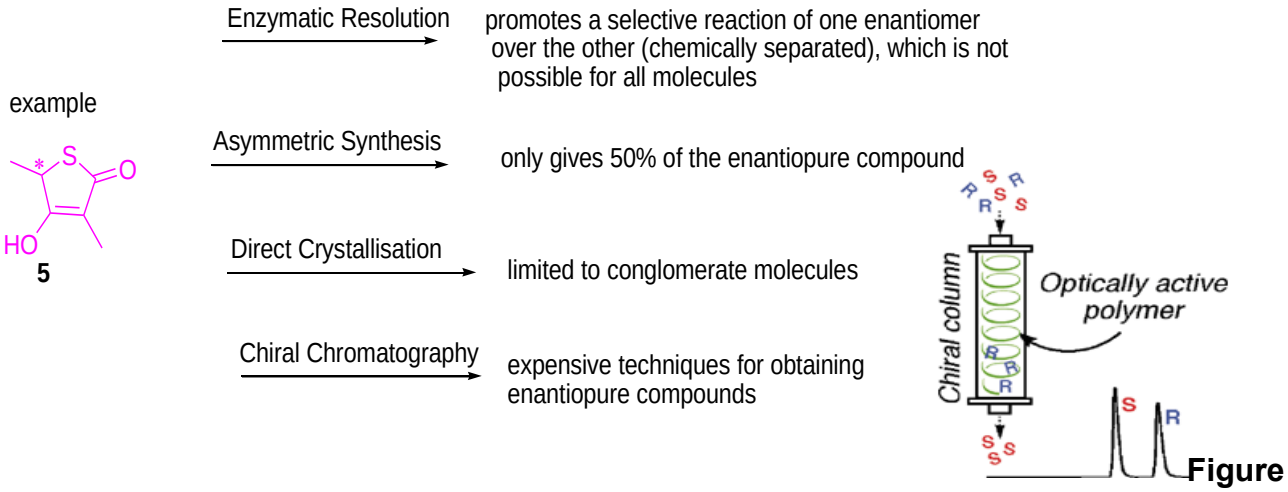


Figure 56. Four main separation methods for obtaining a target chiral molecule.

This research aimed to develop a novel method for obtaining a single enantiomer of thiolactomycin (TLM) using deracemisation via crystallisation, offering a more efficient and cost-effective alternative to conventional methods. TLM is a class of natural products with potent antibacterial activity against Mycobacterium tuberculosis. It has significant potential as an antibiotic, but synthesizing it, is complicated by the need to obtain it in its enantiomerically pure form. This work was undertaken to understand the intricate relationship between molecular structure and crystallisation behaviour, aiming to overcome the challenge of achieving high enantiomeric purity for these complex molecules (20). The following three main objectives guided this research:

1. **Understanding the solid-state behaviour of TLM analogues:** This research analysed the crystal structures of a series of TLM analogues to identify which ones tended to form racemic compounds and which ones formed conglomerates. This analysis revealed that the presence of specific functional groups can influence the formation of conglomerate crystals. This study focused on exploring the relationship between the molecular structures of the compounds and their tendency to form conglomerates, which is essential for successful deracemisation.
2. **Identifying key structural features for conglomerate crystal formation:** To delve deeper into the factors influencing conglomerate formation, a series of TLM analogues with varying substituents at the 3- and 5-positions were synthesised. Detailed analysis of their

crystal structures was undertaken to pinpoint the specific structural characteristics that contribute to the formation of conglomerates.

3. **Developing a method for determining the enantiomeric excess (% ee) of a model compound towards deracemisation.** This involved investigating different methods for determining the enantiomeric excess (%ee) of a model compound, including repeated racemisation and resolution, dynamic kinetic resolution, and enantioselective stereoinversion. This research found that the most effective method was repeated racemisation and resolution, which can achieve almost 100% conversion to a single enantiomer in crystalline form.

This research highlighted several key findings:

This study emphasised the crucial role of experimental validation in complementing computational analysis. While computational methods can provide initial predictions, they cannot fully capture the complexities of crystal formation and enantiomeric purity. Experimental techniques, such as single-crystal X-ray diffraction, are essential for accurate structural analysis and determining the precise enantiomeric purity of the synthesised compounds.

An understanding of why some molecules crystallise as racemates while others crystallise as conglomerates was obtained by searching the database for compounds similar to thiolactomycin antibiotics by using the thiolactone core structure as a query in the ConQuest program. Analyses of the crystal structures present in the database using crystal structure visualisation tools, such as Mercury software, Crystal Explorer software, or Hirshfeld surface analysis, allowed the examination of intermolecular contacts. Unfortunately, the database contained insufficient data on compounds with structural similarities to TLM analogues. To overcome this limitation, conglomerate crystals can be resolved using the surface characteristics or the space group of crystal filling to determine whether a compound tends to crystallise as a racemic compound or a conglomerate. A centrosymmetric point group has an inversion centre that indicates that it is racemic in nature. In contrast, a non-centrosymmetric point group lacks an inversion centre and can be either polar or chiral.

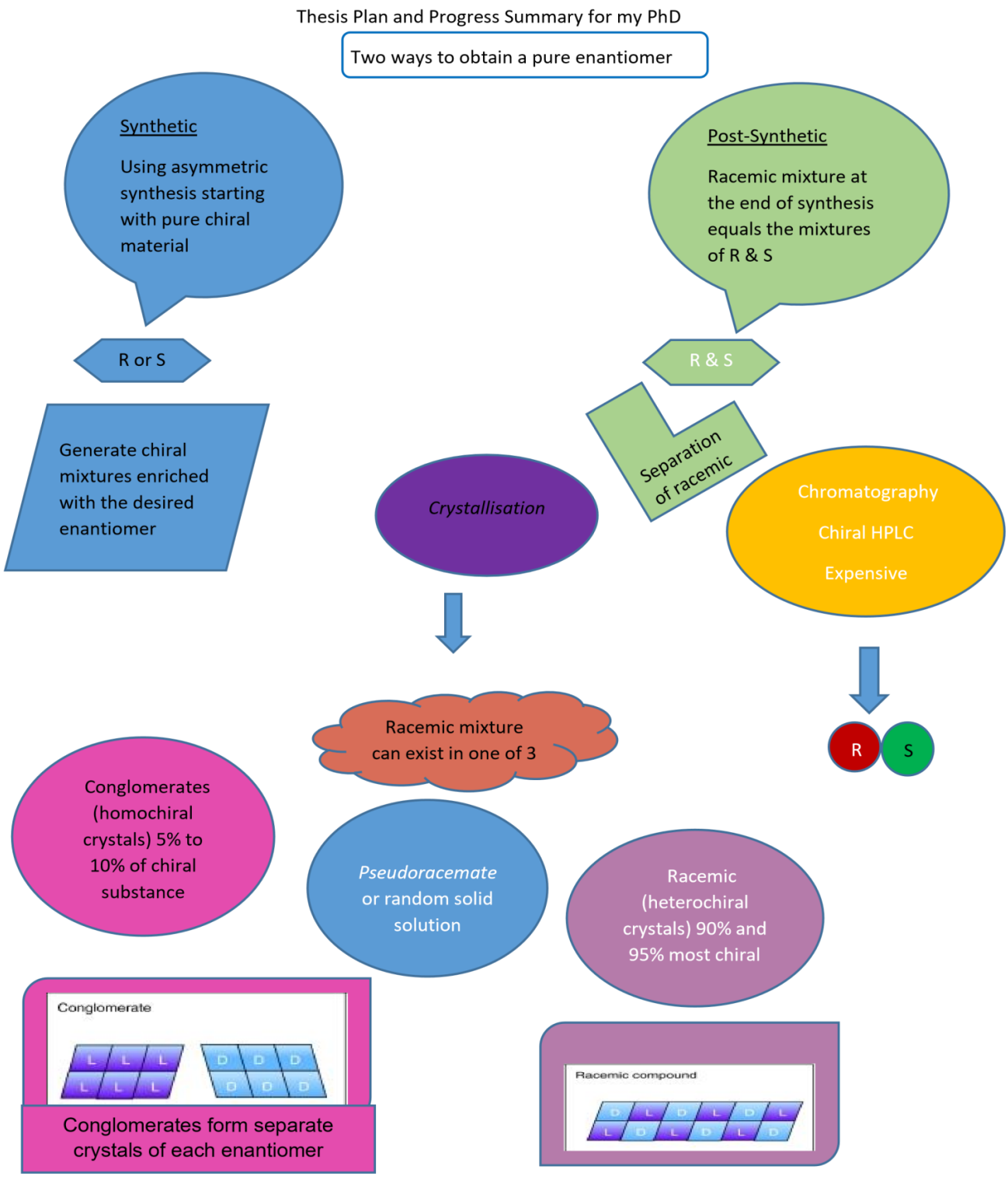
Searching for a non-centrosymmetric space group was not as simple as expected. When separating a mixture of left- and right-handed compounds using crystallisation in pursuit of a pure enantiomer, there must be a non-centrosymmetric space group that only exhibits rotation with no inversion symmetry or mirror image. Three TLM analogue compounds

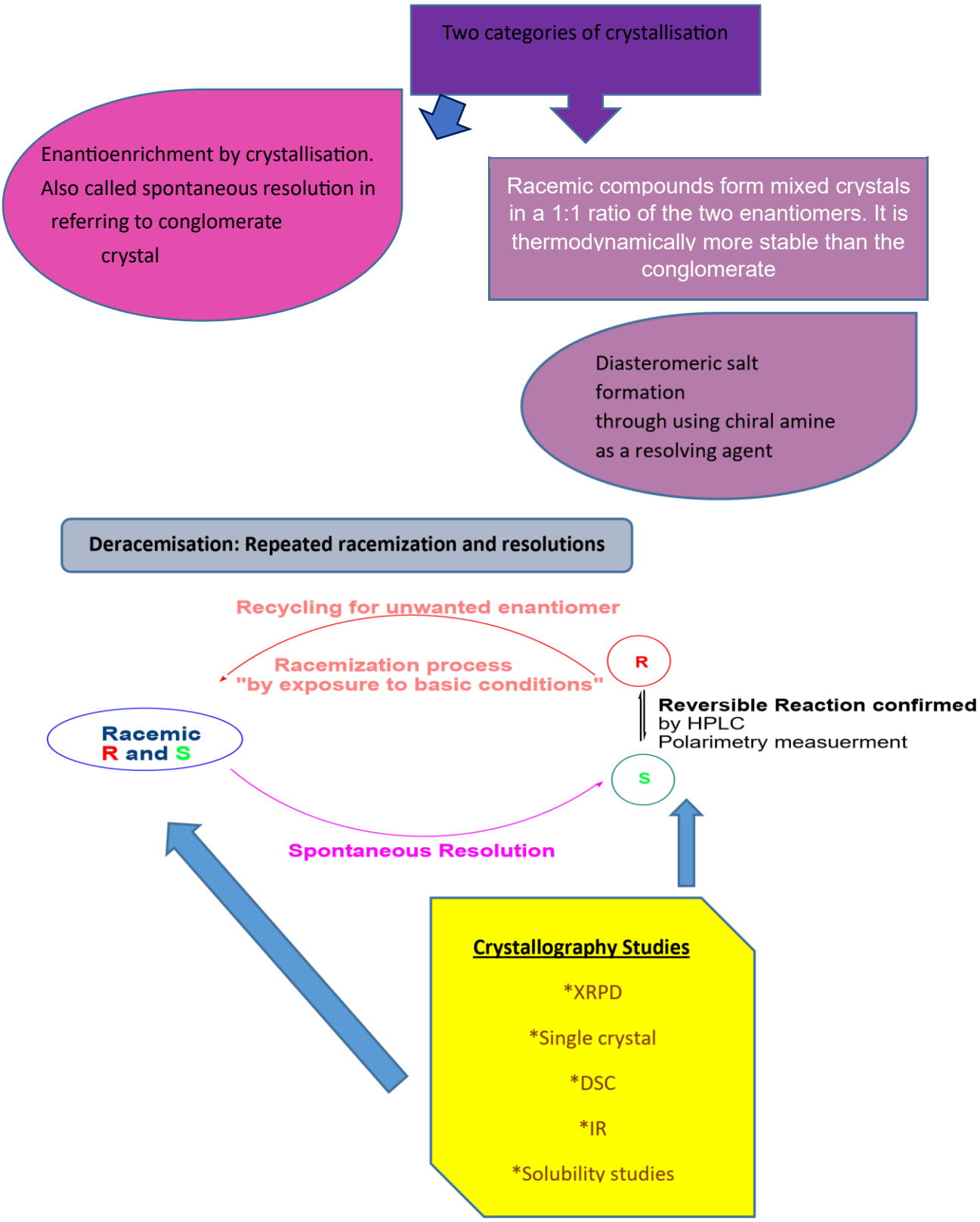
synthesised in the laboratory produced centrosymmetric space groups that crystallised in racemic form instead of conglomerate form. Among the measurements of each of their single crystals, which were racemic crystal-forming systems, the results showed that enantioseparation via direct crystallisation is the most challenging technique for obtaining crystalline racemates. This conclusion is in good agreement with the literature (82).

To overcome this difficulty of enantioseparation via direct crystallisation, one option is to synthesise these compounds as salt derivatives, which may help achieve conglomerate crystallisation. Thus far, the three compounds have not been separated using a crystallisation technique. The additional chiral chromatographic technique could be performed in future studies.

Here, an economically effective method for recovering unwanted R-ibuprofen by forming diastereomers was performed using a chiral resolving agent. Then, the measurement of enantiomeric excesses using NMR was based on the formation of diastereomeric complexes between the chiral analyte and the chiral resolving agent. Racemisation of an unwanted enantiomer was restored to its racemic form and is then subjected to the next cycle of resolution, called deracemisation, in which racemic materials are converted to desired products and waste is reduced in relation to the chiral compound. In addition, the prediction of the enantiomeric excess value based on a polarimetry technique was used for the ibuprofen model compound. For a summary of this procedure and the entirety of the work described in this thesis, see Scheme 24.

Thesis Plan and Progress Summary for my PhD





Scheme 24. Summary of the PhD thesis.

5.2 Future Work

Specifically, future work could focus on the following:

- **Expanding the library of TLM analogues:** Synthesising new derivatives of TLM and screening them for conglomerate crystal formation. This work showed that the presence of specific functional groups can influence the formation of conglomerate crystals. Therefore, synthesising new TLM derivatives with different functional groups could lead to the identification of new compounds that form conglomerate crystals. As shown in Figure 57, several compounds can be produced, and screening for conglomerate crystal formation can be performed. The crystal structure of TLM and its derivatives bound to the active site of FAS II *M. tuberculosis* can be explored in detail in future research.

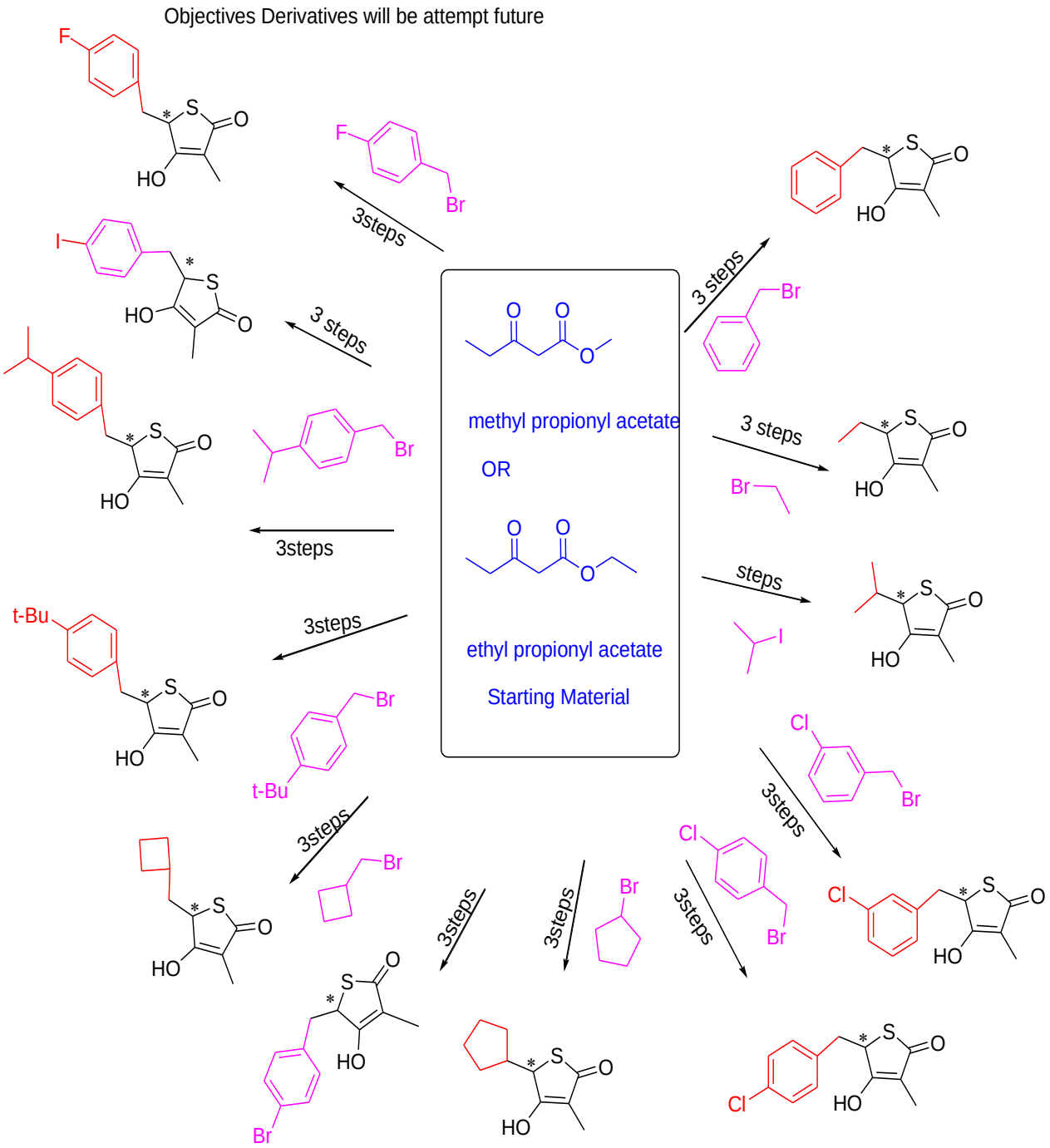
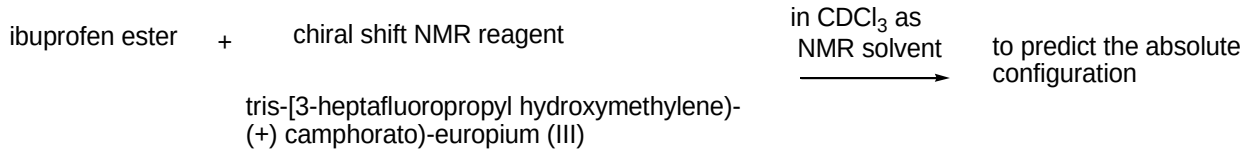


Figure 57. Several compounds for which production and screening for conglomerate crystal forms are planned for future work. If we confirm that any of these derivatives crystallise as conglomerates, racemisation is possible.

- **Exploring chiral shift reagents:** This research suggested that the use of a chiral shift reagent could be a powerful way of determining the absolute configuration of TLM analogues. Further exploration of this technique could be beneficial for drug development and understanding the stereochemical properties of these compounds.
- **Alternative chiral resolving agents:** This research revealed the difficulty of achieving high enantiomeric purity using conventional resolution methods for the TLM analogues. Exploring alternative chiral resolving agents, specifically those that can more effectively form conglomerates or racemates with these compounds, could lead to more efficient separation and higher enantiomeric purity.

An alternative method using a chiral shift reagent could be used in the future for ibuprofen model chemistry.



Chiral NMR shift reagents, which were first developed in the 1970s, are essential tools for determining the enantiomeric purity of a range of substrates. Methods using them are more rapid and efficient than polarimetry, which requires an optically pure reference sample (97,98). Unfortunately, NMR and HPLC are known to distinguish diastereomers rather than a mixture of enantiomers. Enantiomer mixtures can only be separated through two analytical methods: covalent attachment and non-covalent attachment. The first of these involves the covalent attachment of enantiomers to a chiral derivatising agent or a chiral auxiliary that can convert enantiomers into a diastereomer form, so that they can be easily separated according to their solubility. The second method uses the non-covalent interactions of enantiomers with chiral NMR shift reagents, taking advantage of the formation of diastereomeric complexes (98).

Chiral shift reagents include lanthanide shift reagents, which are useful for determining optical purity using a lanthanide chiral ligand. *Tris*-[3(trifluoromethylhydroxymethylene)*d*-camphorato]europium(III) Eu(tfc), *tris*-(3-trifluororacetyl-*d*-camphorato) europium(III) Eu(facam)3, and *tris*-(3-heptafluorobutyryl-d-camphorato)europium(III) are effective reagents

for resolving the signal overlap in NMR spectra based on the differential shifts induced upon the addition of a stable paramagnetic lanthanide complex (97). The (R)- and (S)-enantiomers could be sufficiently separated to determine enantiomeric purity by NMR integration. The enantiomeric composition of ibuprofen in a bulk drug was determined using proton NMR with an achiral lanthanide chelate or by taking an ibuprofen ester with *tris*[(3-heptafluoropropyl hydroxy methylene)-(+)camphorato]-europium(III) as a chiral shift NMR reagent, on which future work is planned. Unfortunately, chiral recognition reagents are expensive and not easily prepared (99).

This research has demonstrated the potential of deracemisation via crystallisation as a valuable tool for obtaining enantiopure compounds. However, this study also highlighted the need for further research in several areas:

- **Developing more robust deracemisation methods:** This study showed the potential of repeated racemisation and resolution, but there is a need to optimise and refine this method to improve its efficiency and reduce the time required for purification.
- **Novel deracemisation techniques:** Exploring novel deracemisation techniques, including:

1. **Attrition-enhanced deracemisation (Viedma ripening):** This technique, using a combination of grinding and crystallisation, shows potential for overcoming the challenges associated with forming conglomerates (18). Optimising crystallisation conditions, such as temperature, solvent, and concentration, on the formation of conglomerates or racemates could provide valuable insights for optimising the process.

2. **Dynamic kinetic resolution (DKR):** This technique, coupling kinetic resolution with an in situ racemisation process, has the potential to achieve high enantiomeric purity (19).
3. **Enantioselective stereoinversion:** This technique, transforming the unwanted enantiomer into an achiral intermediate that can then be converted into the desired enantiomer, might simplify the deracemisation process (2,19).

- **Comprehensive structure–crystallisation relationship analysis:** A more in-depth study, systematically analysing the effect of different structural features of the TLM analogues

on their crystallisation behaviour, could deepen our understanding of the complex interplay between molecular structure and crystal formation.

- **Exploring applications of synthesised TLM analogues:** Once a reliable and efficient method for obtaining enantiopure TLM analogues is established, future research could focus on evaluating their potential applications in diverse fields, such as medicine, agriculture, and materials science.

5.3 Summary

This research highlighted the complexities associated with resolving racemic mixtures and the importance of a multifaceted research approach combining both theoretical and experimental methods to obtain a comprehensive understanding of complex chemical systems. Future research focusing on exploring novel deracemisation techniques and optimising crystallisation conditions holds potential for achieving enantiopure TLM analogues and furthering their application in diverse fields.

This thesis offers valuable insights into the challenging but crucial field of deracemisation, providing a fundamental understanding of the factors influencing crystal formation and the development of a promising new approach for obtaining single enantiomers of thiolactomycin. This work has demonstrated the potential of using repeated racemisation and resolution to achieve high enantiomeric purity and has identified several key structural features that influence the formation of conglomerate crystals. With further research, the insights gained from this work could contribute significantly to the development of new and more effective methods for synthesising and purifying enantiomerically pure TLM analogues, potentially leading to the discovery of new antibiotics against Mycobacterium tuberculosis.

Supplementary Document

Experimental procedures and spectral data

All chemicals used in this study were of analytical grade and were obtained from Sigma Aldrich, Labseeker Inc., and Lancaster Synthesis. These commercial reagents and materials were used as received. All solvents were of reagent grade, and drying solvents were used. To determine the purity of a substance, thin-layer chromatography (TLC) was used to separate the mixtures. TLC was generally used to monitor the progress of reactions and was carried out using 32 silica gel 60 F254 pre-coated aluminium sheets from EM Science. TLC was run for these plates with 2:1 petroleum ether-ethyl acetate as a mixture of polar and nonpolar solvents, unless otherwise noted. Spots were detected with UV light (254 nm). This procedure allows a suitable solvent to be chosen for purification via column chromatography. Column chromatography is one of the most commonly used purification methods. In this technique, a solvent is run through a stationary phase (SiO_2 column), and crude reaction mixtures are loaded onto the column, which is created by tightly packing a slurry of Fisher matrix silica 60 into a glass chromatographic column, ensuring that no air bubbles are present. The compound is then eluted with an appropriate solvent system or mobile phase, as determined via TLC. Fractions were then collected in several test tubes and identified on the basis of the previous TLC conditions. Nuclear magnetic resonance (NMR) spectroscopy was used to elucidate the structures of any organic compounds involved in the experiment. Chemical proton shifts and ^{13}C NMR chemical shifts are reported in ppm relative to residual $CHCl_3$ ($\delta = 7.26$). The spectra were recorded on a Bruker DPX 400 spectrometer (850 and 213 MHz for 1H and 13C NMR, respectively. The samples were dissolved in chloroform D (CDCl3), and chemical shifts were obtained in ppm with respect to (CDCl3). This procedure allowed for the purity to be assessed after column chromatography or recrystallisation. A mass spectrometer was also used. Samples were first subjected to a beam of electrons with sufficient energies to ionise them. Thus, the samples that ionised into cations and anions were separated according to their mass and charges, and subsequently measured. Each bar in the mass spectrum represents the specific mass-to charge ratio of an ion, and the length of the bar represents the abundance of the ion. (100)

LCMS Data Results

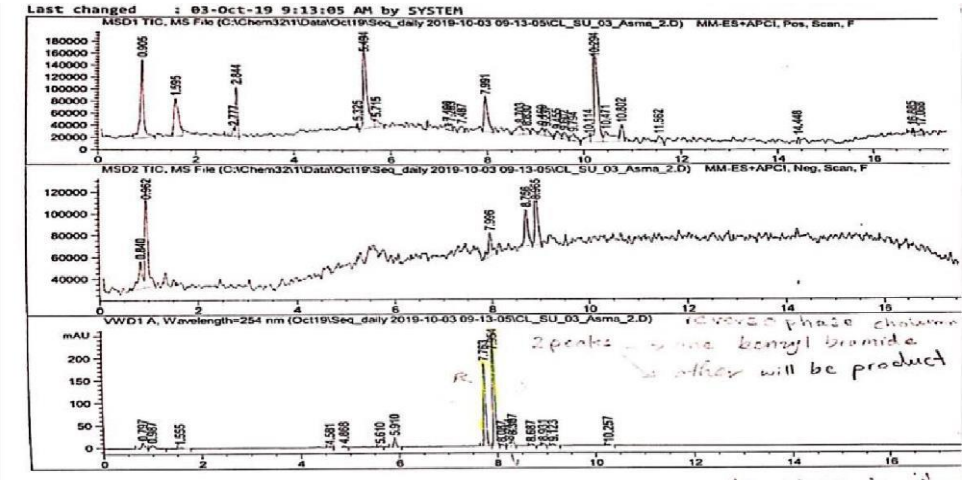


Figure 58. LCMS data obtained using a 1.1 equivalent HMDS base are consistent with compound A in Table 4.

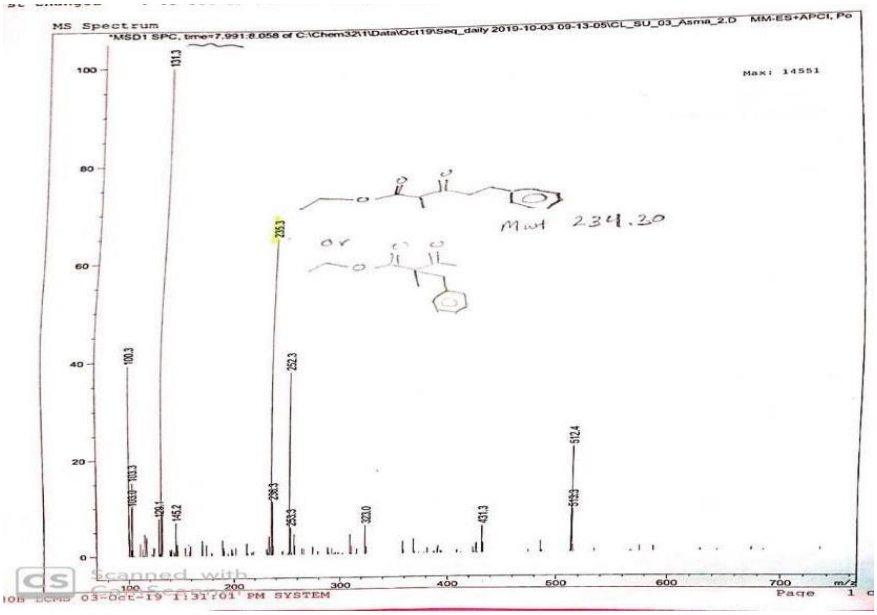


Figure 59. LCMS data obtained using a 1.1 equivalent HMDS base are consistent with compound A in Table 4.

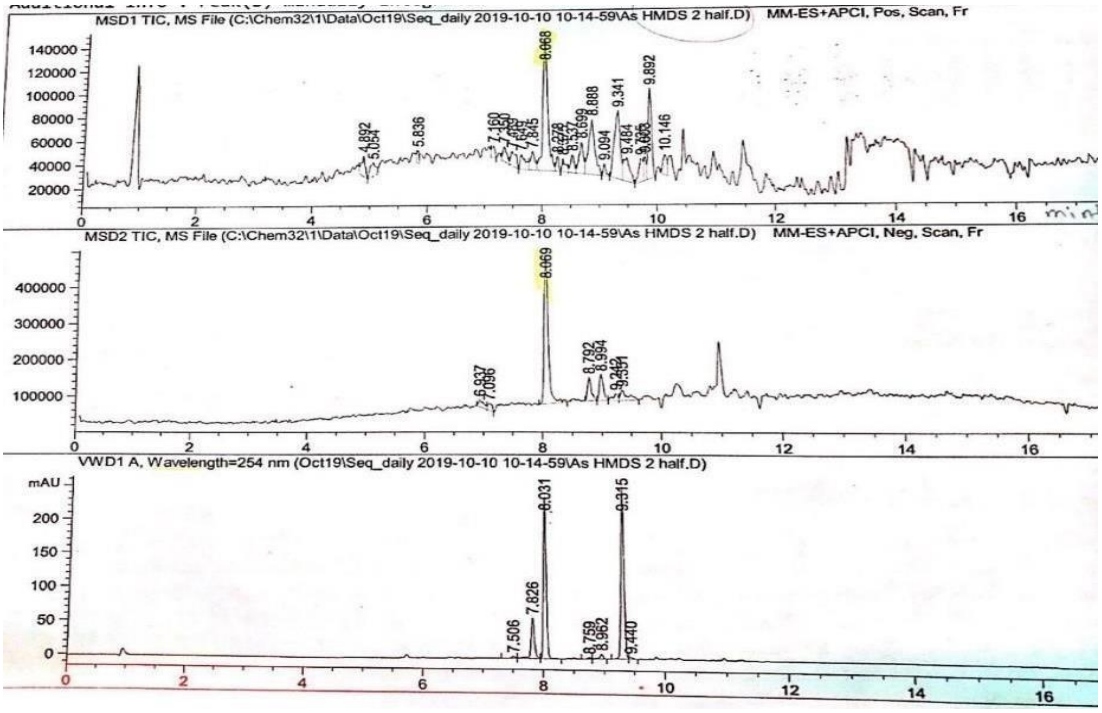


Figure 60. LCMS data obtained using a 2.5 equivalent HMDS base are consistent with compounds A, B, and C in Table 4.

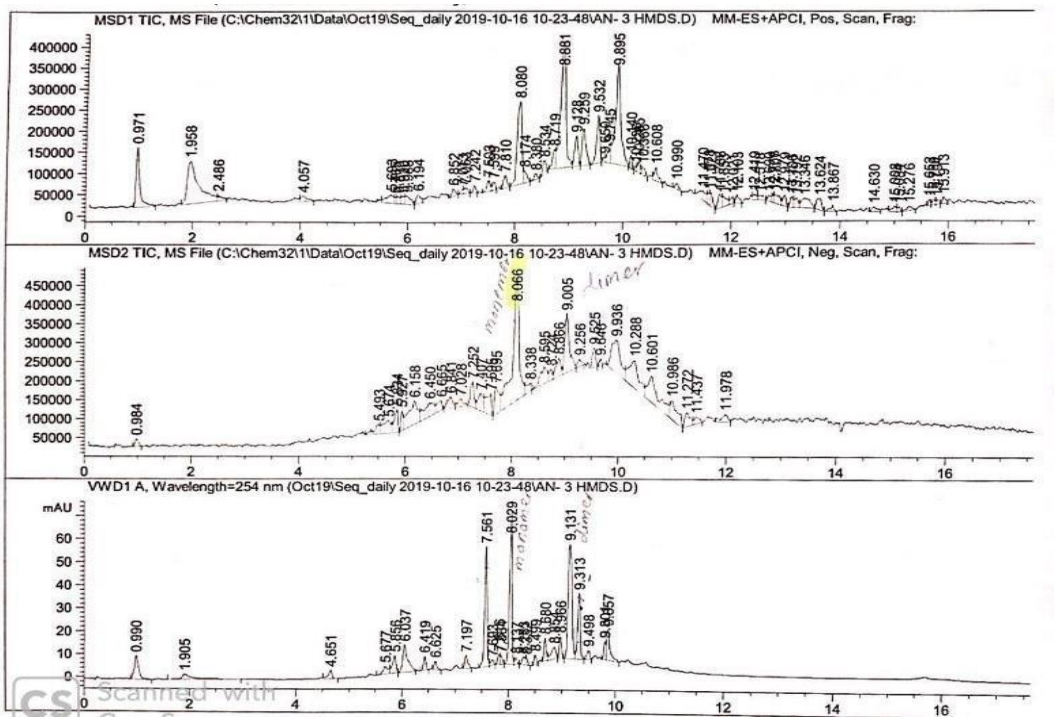


Figure 61. LCMS data obtained using 3 equivalent HMDS bases are consistent with compounds A, B, and C in Table 4.

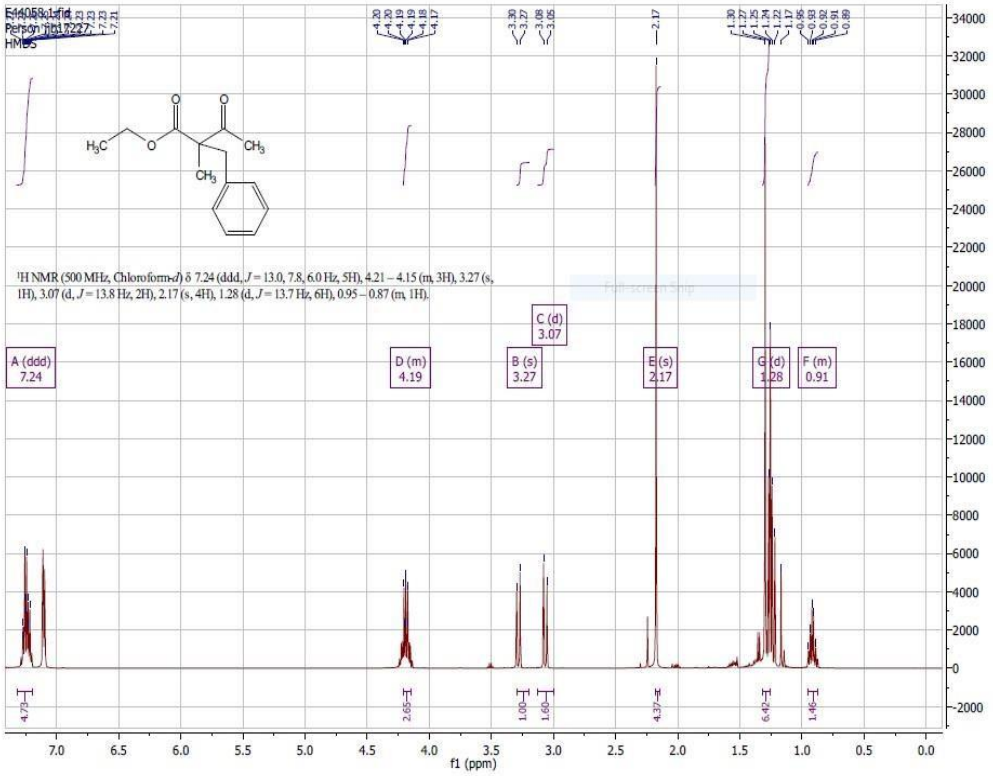


Figure 62. Proton NMR data obtained using a 1.1 equivalent HMDS base, resulting in a 3-position analogue that produced compound A only.

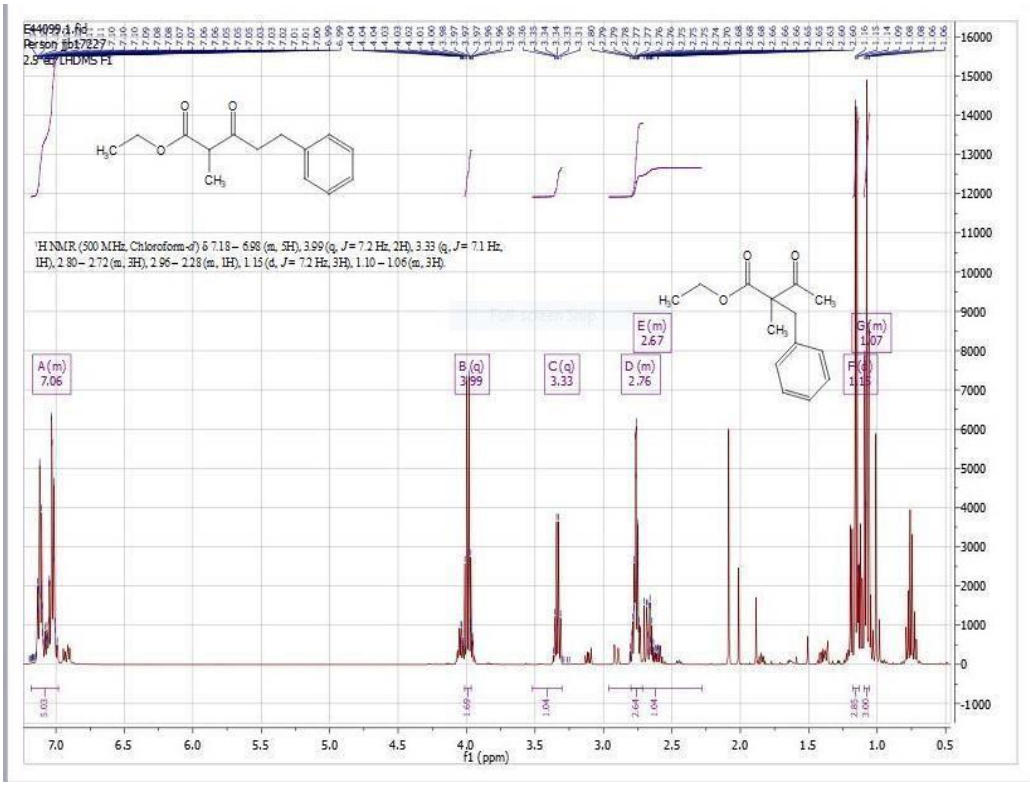


Figure 63. Proton NMR image showing that a mixture of 3- and 5position analogues, which produce compounds A and B, resulted from using a 2.5 equivalent HMDS base.

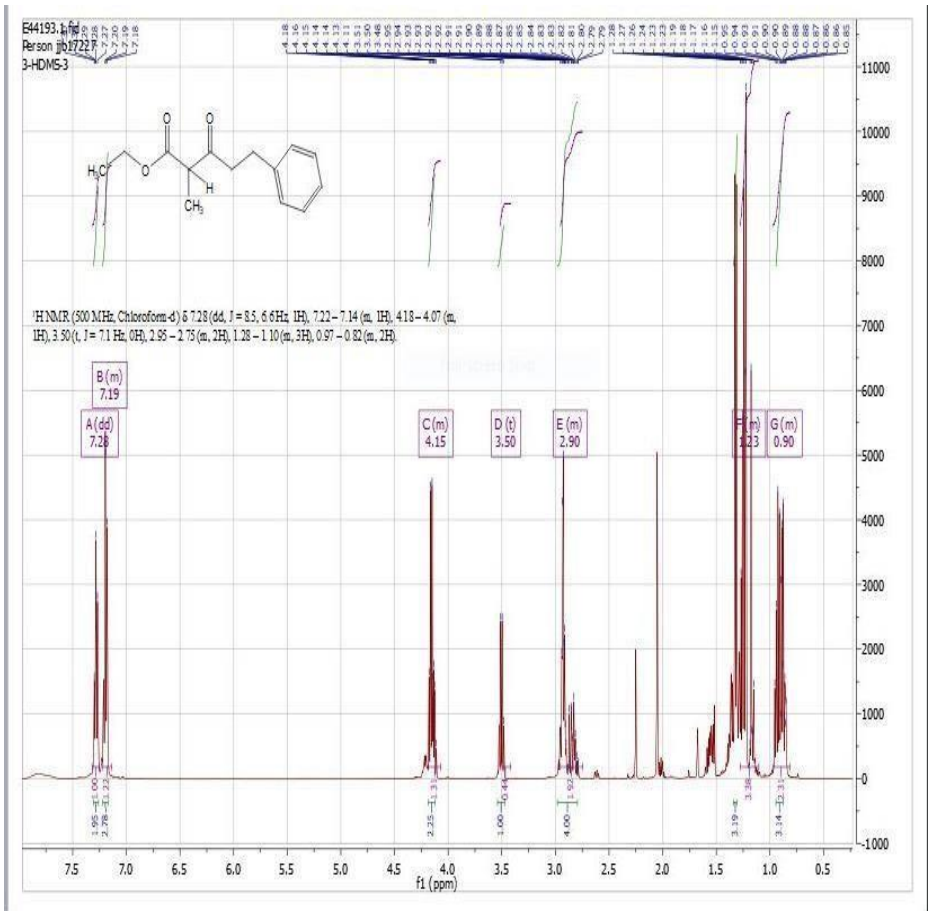


Figure 64. Proton NMR data obtained using a 3 equivalent HMDS base, resulting in a mixture of compounds A, B, and C (3- and 5-position analogues).

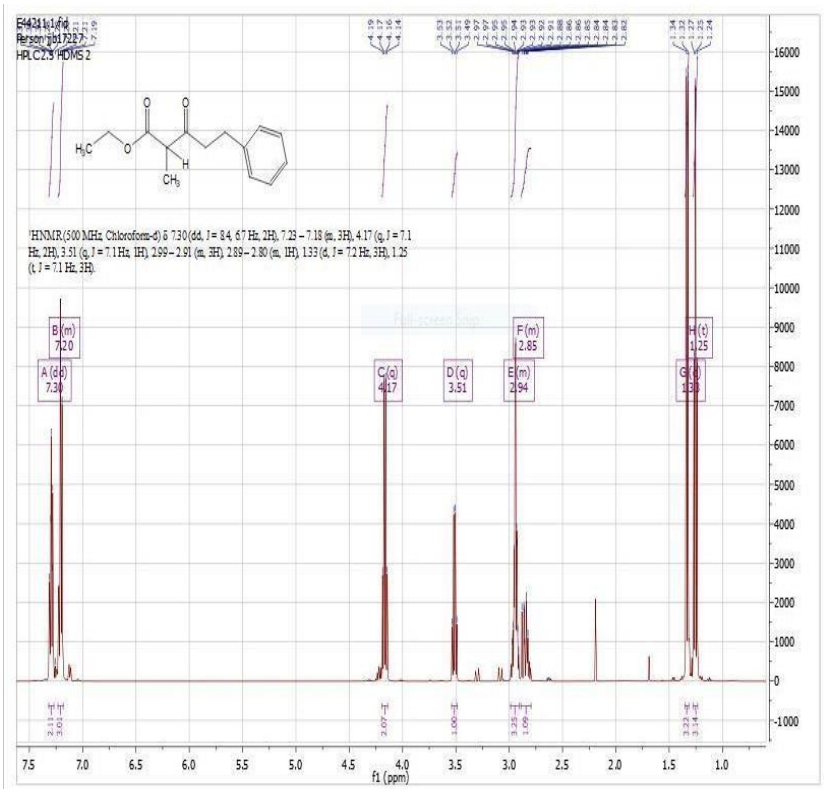


Figure 65. Proton NMR data obtained using a 2.5 equivalent HMDS base, resulting in compound **57,** which produces a 5-position analogue after preparative HPLC purification

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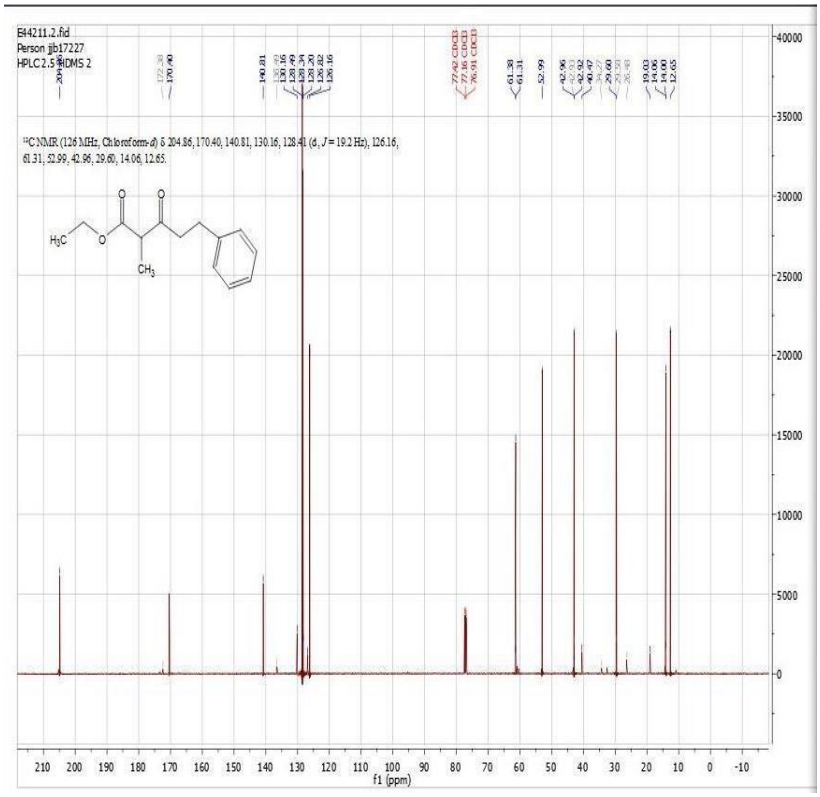
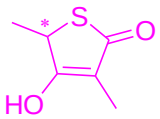


Figure 66. Carbon NMR data obtained using a 2.5 equivalent HMDS base, resulting in the target analogue **57** after preparative HPLC purification.

One-pot synthesis was also used as follows:

Synthesis of (3-hydroxy-2, 4-dimethyl-2H-thiophen-5-one) 5



Bromine **40** (5.55 g, 34.72 mmol, 1 eq) was added to a solution of methyl-2-methyl-3oxopentanoate **38** (5 g, 34.7 mmol, 1eq) in 50 mL of chloroform at 0ºC. The reaction mixture was then stirred for 20 h at room temperature. The solvent was then evaporated in vacuum, and the resulting mixture **41** (approximately 6 g) was used in the next step without further purification. Tetrahydrofuran (THF; 50 mL), triethylamine **42** (3.64, 36 mmol), and thioacetic acid **44** (2.74 g, 2.5 mL, 36 mmol) were added to the mixture under argon atmosphere and stirred overnight at room temperature. Next, the THF was evaporated, and CH_2Cl_2 (50 mL) and water (50 mL) were added to the resulting mixture. The organic layer was dried with sodium sulphate and concentrated in vacuo. Finally, the orange residue was purified by column chromatography using petroleum ether/diethyl ether (3:1) to produce 5.5 g (93%) of 2-acetylsulfanyl-2-methyl-3-oxopentanoic acid methyl ester **46** as a yellow oil.

Ten millilitres of sodium hydroxide solution **47** (1.9 g, 47.9 mmol) in water was added to a solution of 2-acetylsulfanyl-2-methyl-3-oxopentanoic acid methyl ester **46** (5.5 g, 25.22 mmol) in methanol (20 mL). The mixture was stirred overnight at room temperature. Methanol was evaporated in vacuo and acidified to pH 5 with HCl 1N. The mixture was extracted with ethyl acetate (10 mL X 2), and then the organic layer was dried over $MgSO_4$ and concentrated in vacuo. Finally, the creamy yellowish solid residue was purified by recrystallisation using diethyl ether and petroleum ether (1 to 0.5) to produce the key intermediate crystal compound 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one **5** as off-white solid weighing (1.1 g, 22%): m.p 408–409 K; ^{1}H NMR (500 MHz, chloroform-*d*), δ 4.22 (qd, *J* = 7.1, 1.2 Hz, 1H), 1.78 (d, *J* = 1.2 Hz, 3H), and 1.67 (d, *J* = 7.1 Hz, 3H) ; ^{13}C NMR (126 MHz, CDCl3) δ 197.16, 177.63, 111.32, 42.99, 18.80, and 7.62; as shown in Figures 67, 68. m/z (EI) 144.0245. The HRMS calculated for $C_6H_9O_2S$ [MH^+] 145.0329 found 144.9782. (82,101)

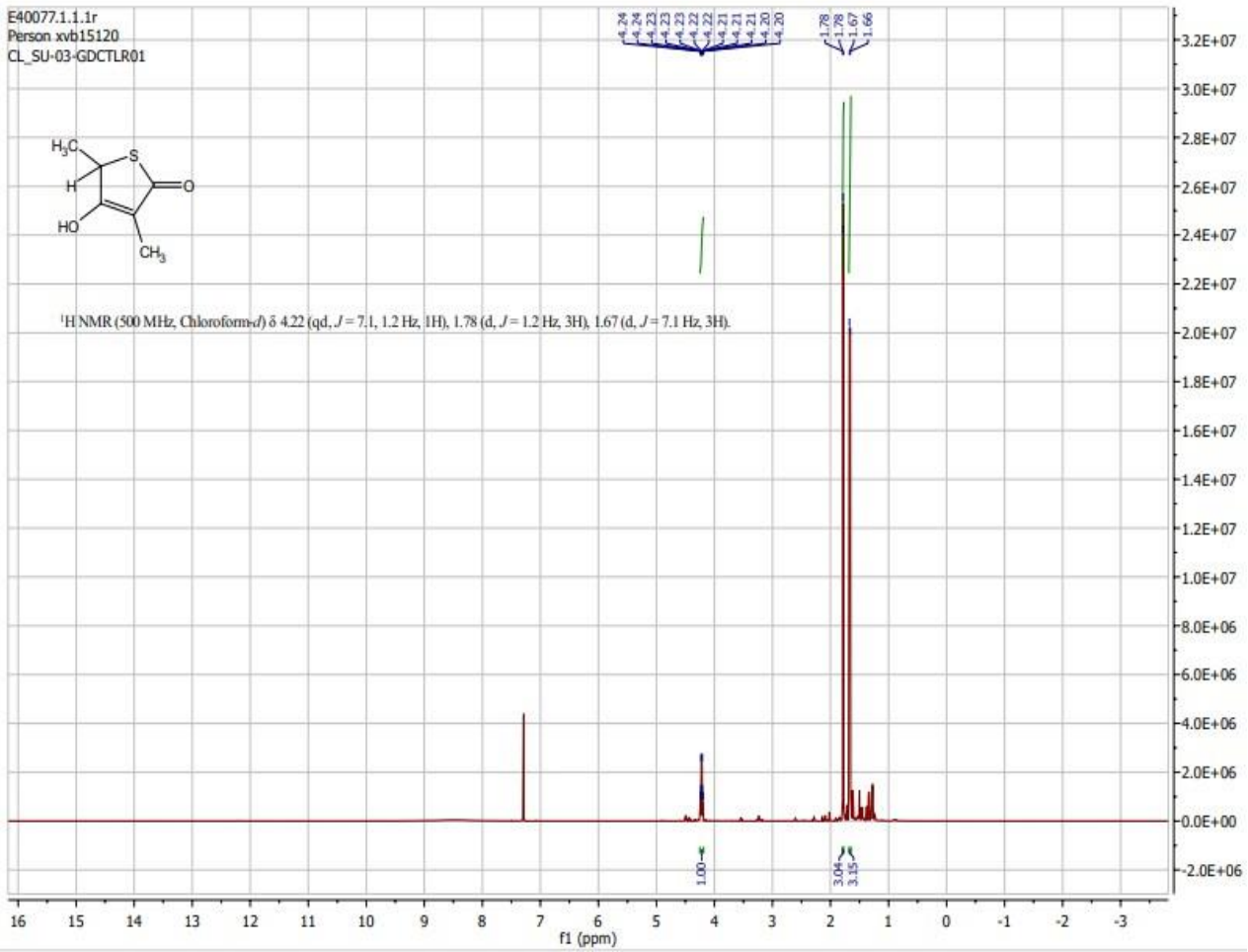


Figure 67. ^{1}H NMR spectra for the first TLM analogues (3,5-dimethyl-4-hydroxy5Hthiophen-2-one)

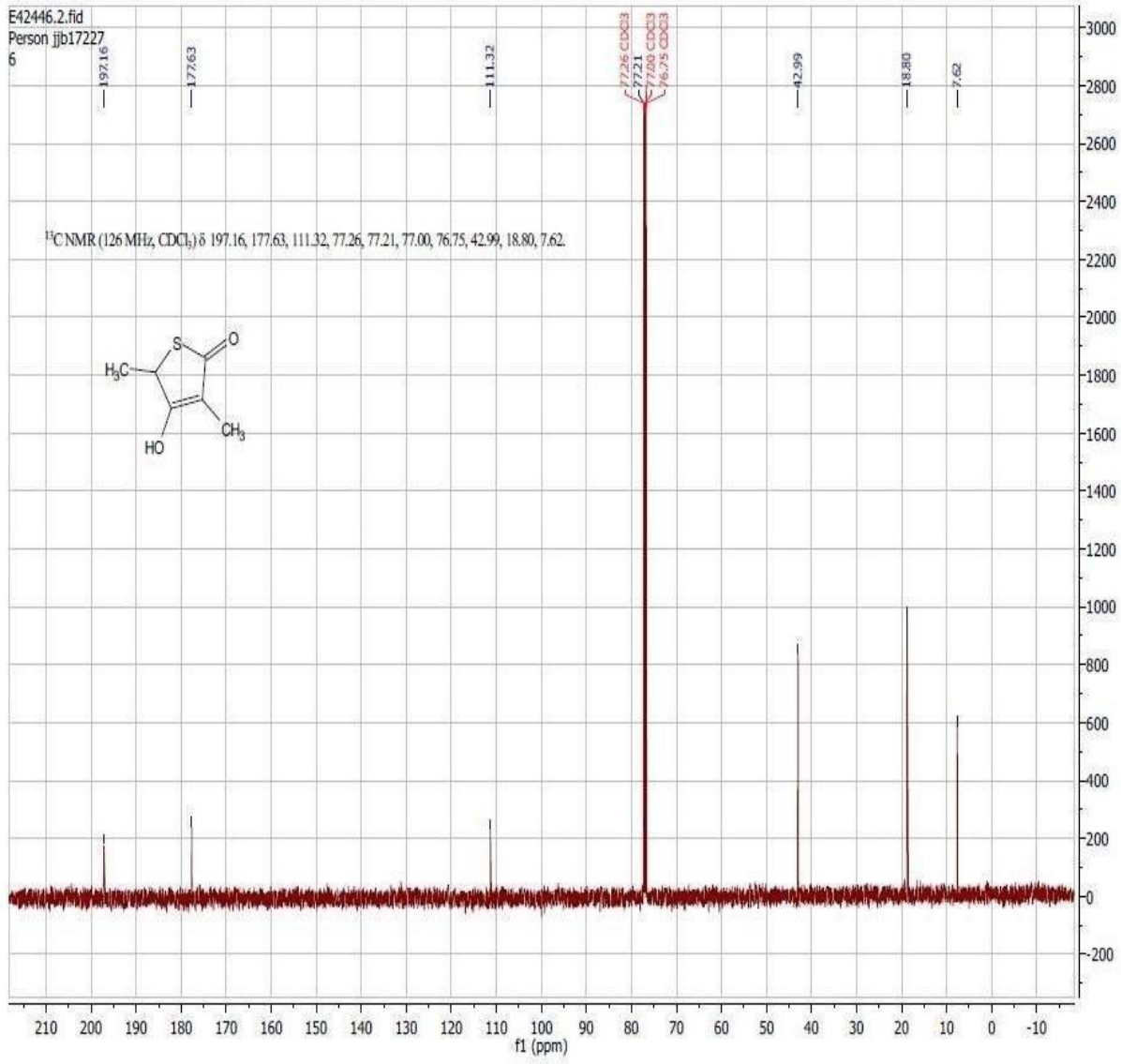


Figure 68.[13]C NMR spectra for the first TLM analogues (3,5-dimethyl-4-hydroxy-5Hthiophen-2-one)

Solubility Experiments

For the solubility experiments, 5 or 10 mg of the compound (3-hydroxy-2,4-dimethyl- 2H-thiophen-5-one) were taken and added to 0.5 mL of various solvents, as shown in Table 12.

Table 12.

Solvent	Methanol	Ethanol	Ethyl acetate	Chloroform	Diethyl ether	Dichloromethane	Petroleum ether
Solubility	Dissolved well				Partially dissolved	Not dissolved	

The single crystals of 3-hydroxy-2,4-dimethyl-2H-thiophen-5-one (also known as 3,5dimethyl-4-hydroxy-5H-thiophen-2-one) were grown in six solvents (tetrahydrofuran (THF), ether, chloroform, ethyl acetate, toluene solution, and water) by a slow evaporation method (vials sealed with pierced paraffin film) at room temperature and atmospheric pressure for crystal structure determination. Although the overall quality of the structure was good, all solvents turned into racemic crystals; therefore, solvent and temperature had no effect on racemic compounds compared with conglomerates, as shown in Table

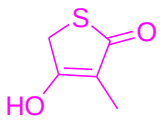
13.

Table 13. Using different solvent for crystallisation study.

Solvent **Usage**	**Toluene**	**Diethyl ether**	**(THF)**	**Ethyl acetate**	**Chloroform**	**H_2O**
	6 mL in 100 mg	10 mL in 100 mg	1 mL in 100 mg	2 mL in 100 mg	1 mL in 100 mg	2 mL in 100 mg
Crystal formation	Heated until dissolved and then left at room temperature . Crystals formed after 2 h	Heated until dissolved and then filtrated and left at room temperature. Crystals formed after 2 days	Crystals formed the next day	Crystals formed after 2 days	Crystals formed by the next day	Heated until dissolved and then left at room temperature. Crystals formed after 2 h

NOTE: After crystals formed, a similar cold solvent was added to each vial and decanted to eliminate impurities.

The second TLM analogue derivative is



61

Synthesis of 4-hydroxy-3-methyl-5H-thiophen-2-one (also known as 3-hydroxy-4methyl-2H-thiophen-5-one) 61.

Bromine **40** (5.55 g, 34.7 mmol, 1 eq) was added to a solution of 2-methyl-3-oxo-butyric acid ethyl ester **56** (5 g, 34.7 mmol) in 50 mL of chloroform at 0ºC. The reaction mixture was then stirred for 20 h at room temperature. Subsequently, the solvent was evaporated in vacuum, and the resulting mixture (~4 g) was used in the next step without further purification. Tetrahydrofuran (THF; 50 mL), triethylamine **42** (3.64 mL, 35.5 mmol), and thioacetic acid **44** (2.7 g, 2.5 mL, 35.5 mmol) were added to the mixture under an argon atmosphere and stirred overnight at room temperature. Next, the THF was evaporated, and DCM (CH_2Cl_2) (50 mL) and water (50 mL) were added to the resulting mixture. The organic layer was dried with sodium sulphate and concentrated in a vacuum. Finally, the orange residue was purified by column chromatography using petroleum ether/diethyl ether (4:1) to give 1.9 g (51%) of 4-acetylsulfanyl-2-methyl-3-oxo-butyric acid ethyl ester **46** as brown oil.

Ten millilitres of sodium hydroxide solution **47** (0.7 g, 17.50 mmol) in water was added to a solution of 4-acetylsulfanyl-2-methyl-3-oxo-butyric acid ethyl ester **46** (1.9 g, 9.2 mmol) in methanol (20 mL). The mixture was stirred overnight at room temperature. Methanol was evaporated in vacuo and acidified to pH 5 with HCl 1N. The mixture was extracted with ethyl acetate (10 mL X 2), and then the organic layer was dried over $MgSO_4$ and concentrated in vacuo. Finally, the red solid residue was purified by reverse phase C18 column

chromatography using water and 1% to 2% acetonitrile to produce achiral compound **61** as a yellow fluffy solid weighing 0.024 g (2%) see Figure 69. m.p. 397–398 K. ^{1}H NMR (500 MHz, acetone-d_6) δ 3.94 (s, 2H), 1.68 (s, 3H). This result agrees with the literature. (75)

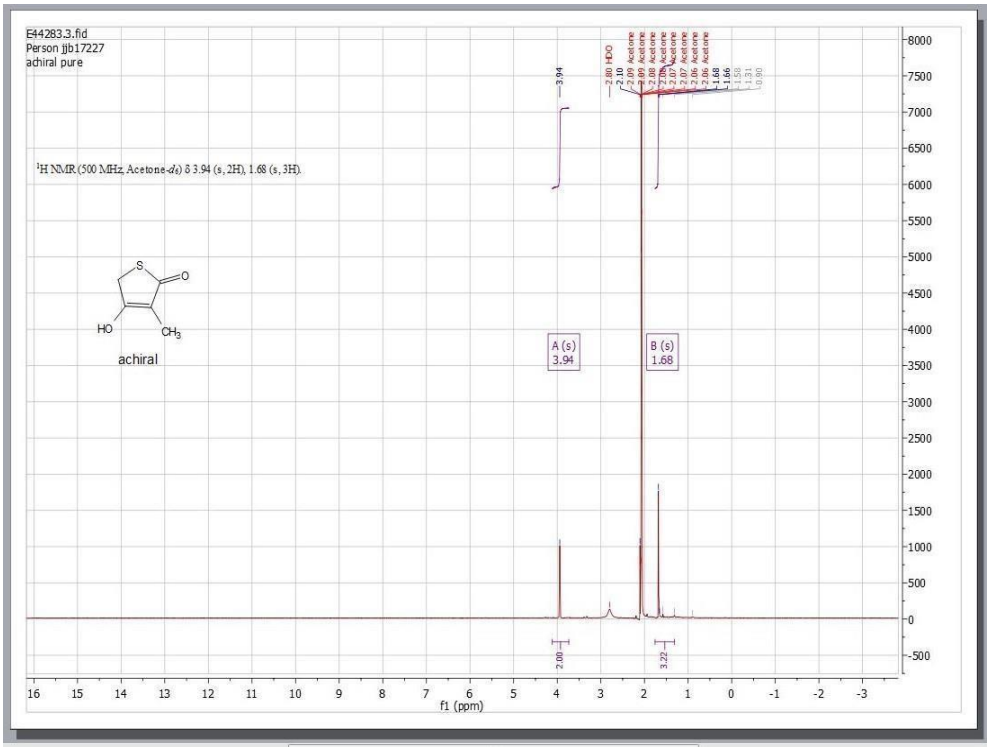


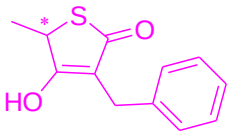
Figure 69. ^{1}H NMR spectra for achiral (4-hydroxy-3-methyl-5H-thiophen-2-one).

Solubility Experiments

The single crystals of 4-hydroxy-5H-thiophen-2-one were grown in chloroform solvent (7 mg in 2 mL chloroform heated and filtrated till clear yellow solution and seeded with tiny amount) using a slow evaporation method (vials sealed with pierced paraffin film) at room temperature and atmospheric pressure for crystal structure determination. Data were calculated and refined to provide the attached structure. It is a new structure that does not seem to be in the crystallographic database, but it is racemic of an achiral molecule.

The third derivative of TLM analogues

Synthesis of 3-benzyl, 5-methyl-4-hydroxy-5H-thiophen-2-one



65

K_2CO_3 **37** (4.9 g, 34.68 mmol,1 equivalent) was added to a solution of ethyl propionyl acetate **62** (5 mL, 34.68 mmol, 1 equivalent) in dry acetone (20 mL), and the solution was stirred for 5 min. Then, benzyl bromide **54** (5 mL, 41.61 mol, 1.2 equivalent) was added slowly and stirring was continued for 10 min. The reaction mixture was refluxed for 12 h and allowed to cool to room temperature. The reaction was worked up through the addition of diethyl ether (40 mL) to the mixture, which was then filtered, dried over

($MgSO_4$) and evaporated under reduced pressure to produce **63** as a yellow liquid (7 g, 86% of the yield).

Bromine **40** (4.7 g, 29.89 mmol, 1 eq) was added to a solution of the alkylation product 3-benzyl ethyl propionyl acetate (7 g, 29.89 mmol, 1eq) in 50 ml of chloroform at 0ºC. The reaction mixture was then stirred for 20 h at room temperature. The solvent was then evaporated in vacuum, and the resulting mixture (approximately 10 g) was used in the next step without further purification. Tetrahydrofuran (THF; 50 mL), triethylamine **42** (4.9, 48.7 mmol), and thioacetic acid **44** (3.7 g, 3.4 mL, 48.7 mmol) were added to the mixture under an argon atmosphere and stirred overnight at room temperature. Next. The THF was evaporated, and CH_2Cl_2 (50 mL) and water (50 mL) were added to the resulting mixture. The organic layer was dried with sodium sulphate and concentrated in vacuo. Finally, the orange residue was

purified by column chromatography using hexane/diethyl ether (3:1) to give 6.7 g (62%) of thioacetate compound **64** as orange to reddish oil. Ten millilitres of sodium hydroxide solution **65** (1.68 g, 41.3 mmol) in water was added to a solution of thioacetate compound **59** (6.7 g, 21.74 mmol) in methanol (20 mL). Next, the mixture was stirred overnight at room temperature. Methanol was evaporated in vacuo and acidified to pH 5 with HCl 1N. The mixture was extracted with ethyl acetate (10 mL X

2), and then the organic layer was dried over Na_2SO_4 and concentrated in vacuo. Finally, the black to reddish solid-residue compound was purified by column chromatography using diethyl ether solvent only, resulting in a white solid compound of 0.29 g **65** (6%). (H 400 MHz, $CDCl_3$) 7.37 (d, *J*= 1.1 Hz, 2 H), 7.35 (s. 3H) 4.13 (m, *J*=7.1, 1.0 Hz, 1H) 3.6 (d, *J*=4.9, 2H). 1.6 (s, 3H). ^{13}C NMR δc (101 MHz, $CDCL_3$) 195.39, 177.64, 136.66, 128.67, 128.12, 126.62, 113.51, 42.23, 28.74, and 14.72, as shown in Figures 70 and 71.

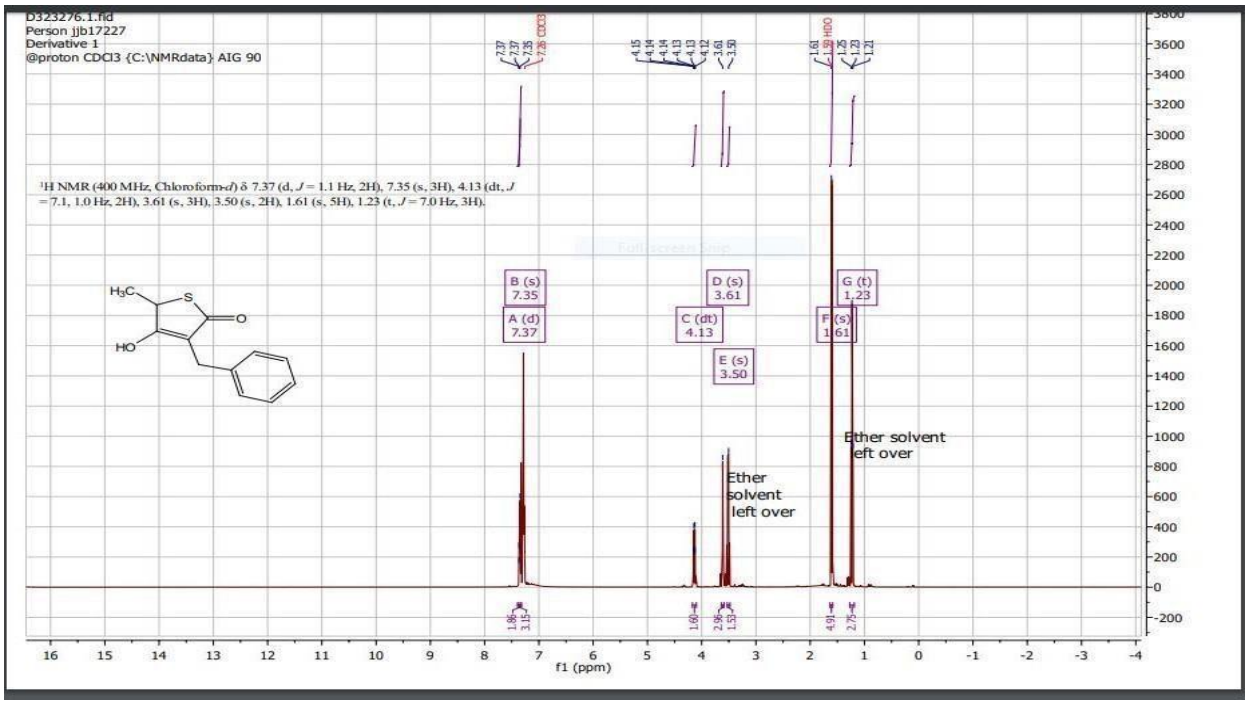


Figure 70. ^1H NMR spectra for third TLM analogue derivative 3-benzyl,5-methyl4hydroxy-5H-thiophen-2-one.

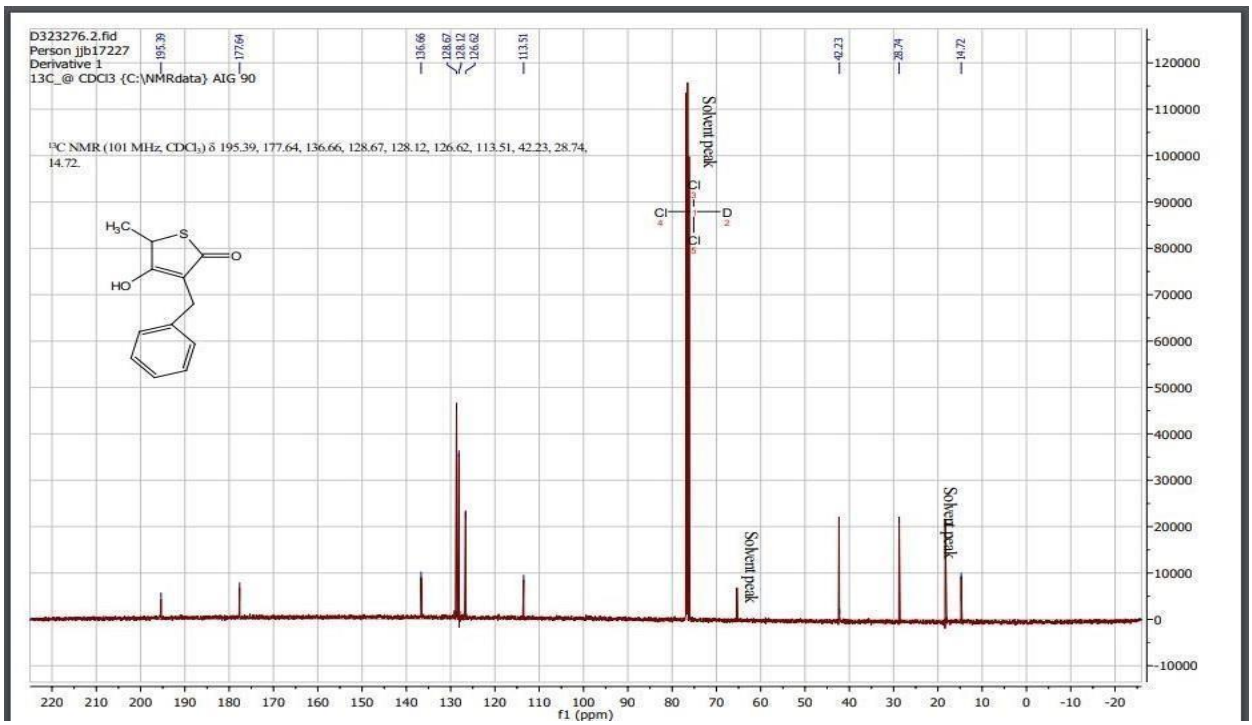


Figure 71. ^{13}C NMR spectra for the third TLM analogue derivative 3-benzyl, 5-methyl4hydroxy-5H-thiophen-2-one.

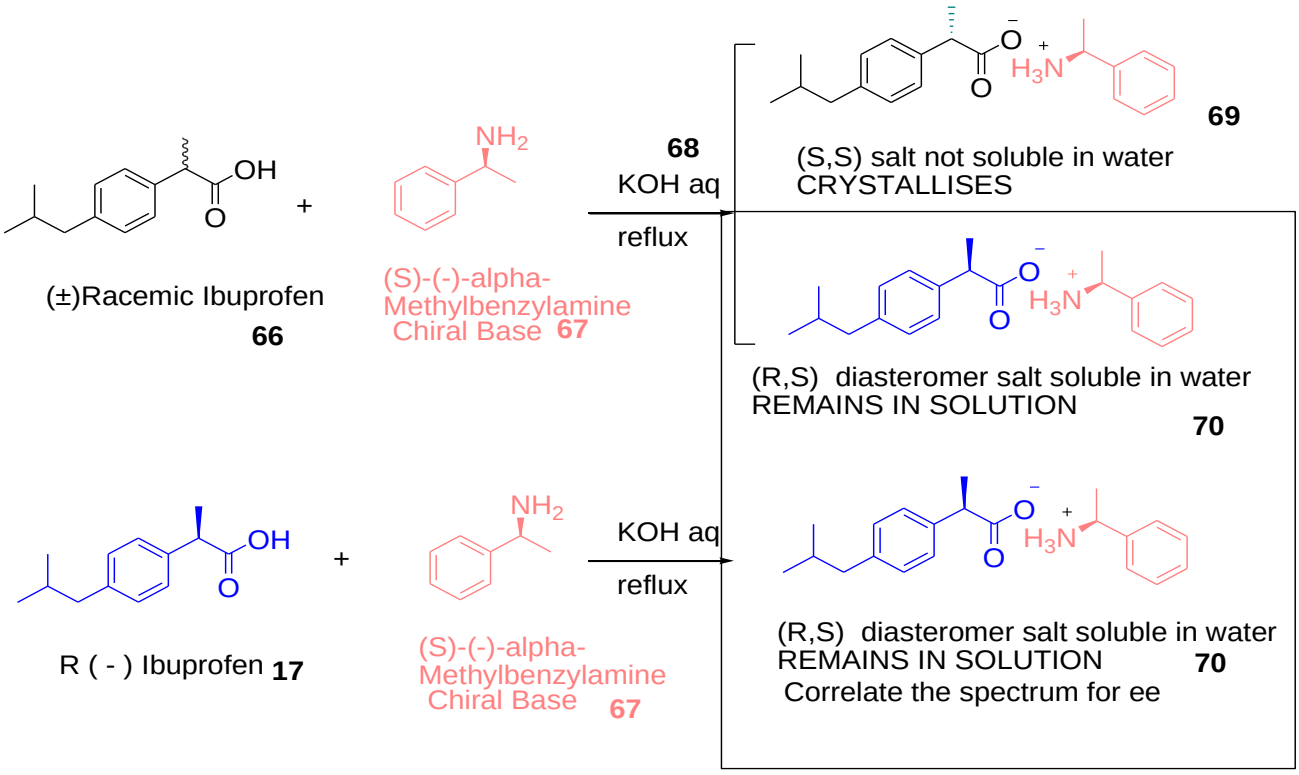
Solubility Experiments

For the solubility experiments, 5 or 10 mg of the compound 3-benzyl,5-methyl-4-hydroxy5H-thiophen-2-one was taken and added to 0.5 mL of various solvents, as shown in Table 16. The single crystals of 3-benzyl,5-methyl-4-hydroxy-5H-thiophen-2-one were grown in three solvents (THF, ether, and chloroform) by a slow evaporation method (vials sealed with pierced paraffin film) at room temperature and atmospheric pressure for crystal structure determination. Although the overall quality of the structure was good, all solvents turned into racemic crystals; therefore, the solvent and temperature had no effect on racemic compounds compared with conglomerates, as shown in Table 14.

Table 14.

Solvent **Use**	**Diethyl ether**	**Tetrahydrofuran** **(THF)**	**Chloroform**
	10 mL in 96 mg ~100 mg	1 mL in 100 mg	1 mL in 12 mg
Crystal formation	Crystals formed after 2 days	Crystals formed after 2day	Crystals formed on the next day

NOTE: After crystals formed, a similar cold solvent was added to each vial and decanted to eliminate impurities.



Scheme 22. Compared with the resulting compounds when racemic ibuprofen was used and R-ibuprofen was used with the same chiral amine, almost the same (R, S) salt was obtained.

For part a), a solution of racemic (±) ibuprofen (1 g, 4.847 mmol, 1 eq) and KOH (0.13 g, 2.423 mmol, 0.5 eq) in water (10 mL) was stirred and heated at 85ºC at reflux. By then, most of the ibuprofen had dissolved gradually. A chiral base called (S)-(−)-αmethylbenzylamine (0.293 g or 0.3 mL, 2.423 mmol, 0.5 eq) was added dropwise for 20 min. After the heating was continued at a reflux temperature for 20 min, the mixture was allowed to cool to an ambient temperature, as shown in Scheme 22. The precipitated solids were collected by filtration at ambient temperature, washed with ice-chilled water, and dried in vacuo to produce the expected crude white solid salt (S, S; 0.64 g, 64% yield; m.p. = 92–97ºC). ^{13}C NMR (214 MHz, $CDCl_3$) δ 180.48, 141.61, 139.66, 128.80 (d, *J* = 73.1 Hz), 128.54, 127.52 (d, *J* = 125.3 Hz), 126.17 (d, *J* = 6.0 Hz), 125.59, 50.78, 46.85, 44.93, 30.08, 21.97, and 18.71, as shown in Figures 72. (90)

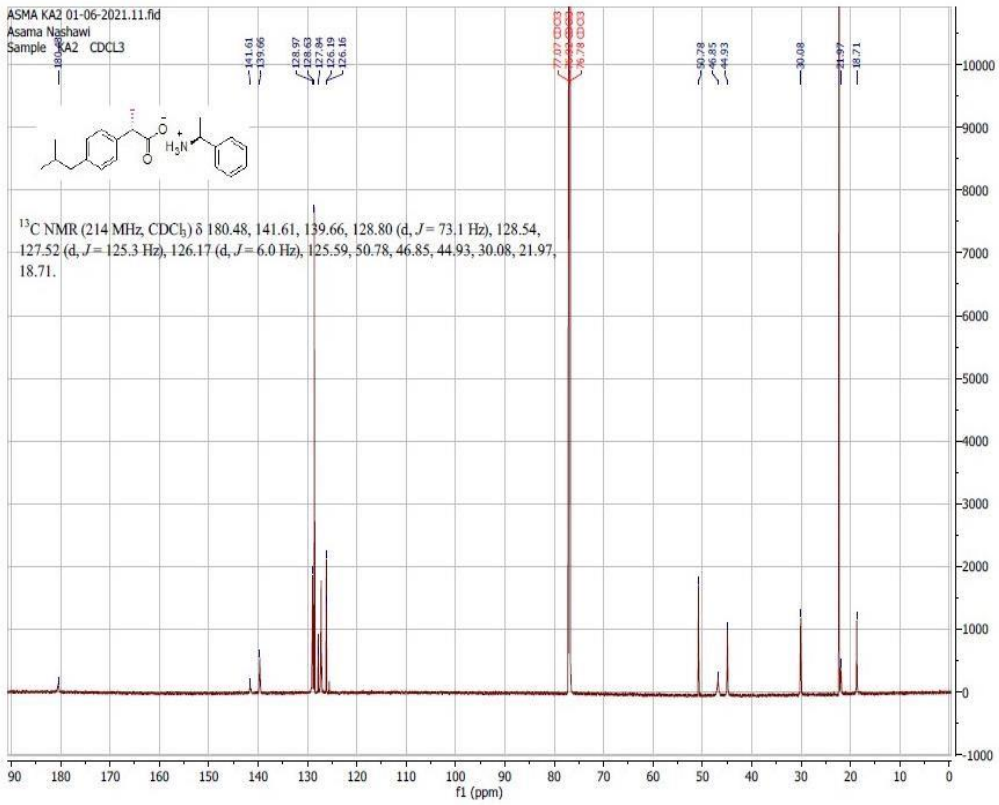


Figure 72. Carbon NMR spectrum of (S, S) salt compounds.

The filtration from the reaction was saved in a clear container labelled (R, S) diastereomer salt end with a fluffy white compound (0.07 g, 7%) ^{13}C NMR (214 MHz, $CDCl_3$) δ 180.04, 140.53, 139.68, 137.86, 129.30, 128.86, 128.42, 127.30, 126.44, 51.00, 45.47, 45.06, 30.19, 22.41, 20.79, and 18.32, as shown in Figures 73. (90)

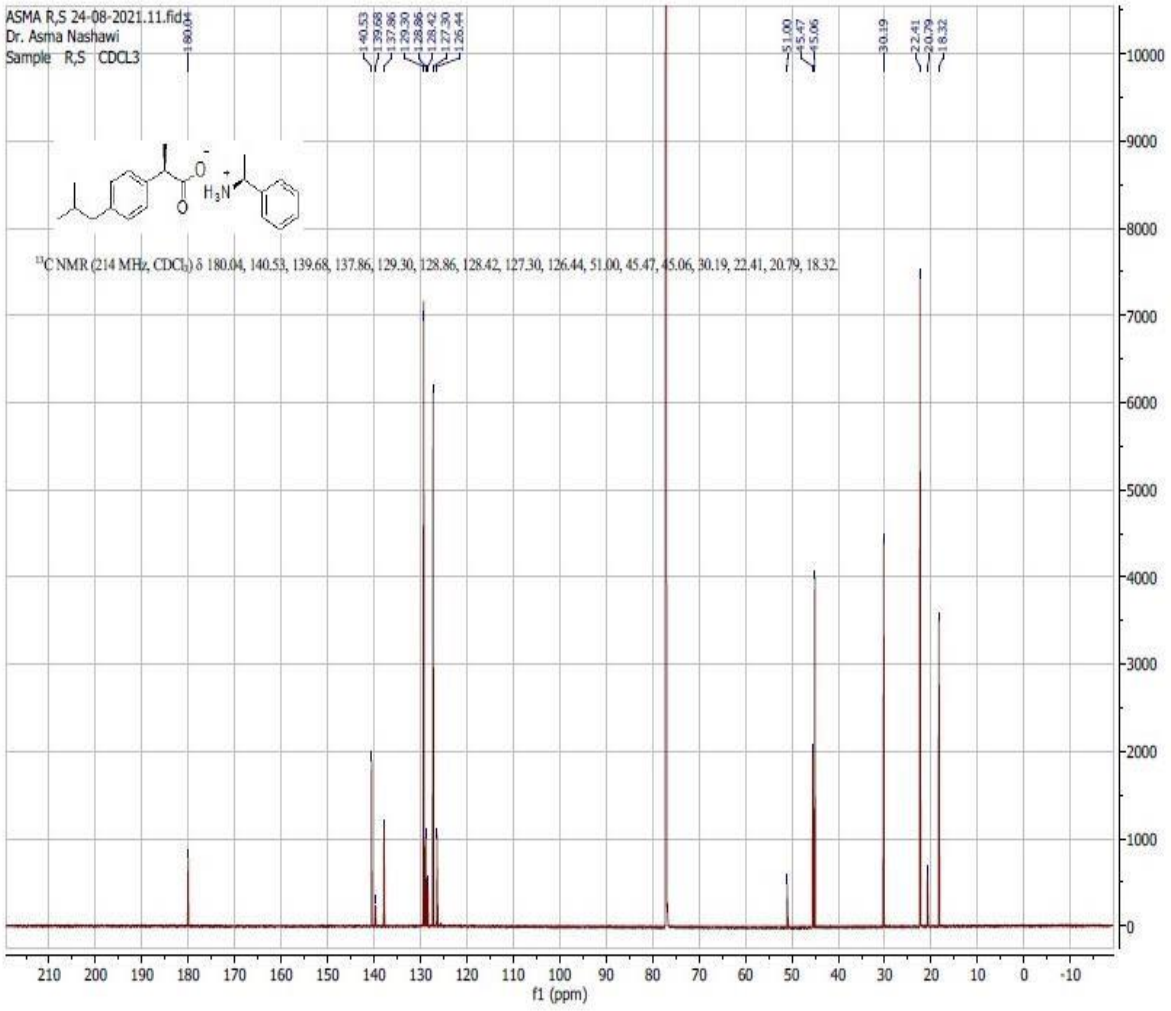


Figure 73. Carbon NMR spectrum of (R, S) salt compounds when racemic ibuprofen was used with a chiral base.

For part b), after a solution of R (−) ibuprofen (0.1 g, 0.484 mmol, 1 eq) and KOH (0.013 g, 0.242 mmol, 0.5 eq), water (10 mL) was stirred in and heated at a reflux temperature of 85ºC. By then, most of the ibuprofen had gradually dissolved. A chiral base called (S)(−)-α-methylbenzylamine (0.029 g or 0.03 mL, 0.242 mmol, 0.5 eq) was added dropwise for 20 min. After the heating was continued at a reflux temperature for 20 min, the mixture was allowed to cool to an ambient temperature, as shown in Scheme 22. Then, the filtered reaction produced a fluffy white compound (0.049 g, 49% yield; m.p. = 130–140ºC). ^{13}C NMR (214 MHz, $CDCl_3$) δ 179.83, 143.15, 139.99, 139.28, 129.17, 128.69, 127.48 (d, *J* = 80.2 Hz),

126.13, 125.71, 51.03, 46.48, 45.06, 30.20, 22.95, 22.41, and 18.68, as shown in Figures 74. (90)

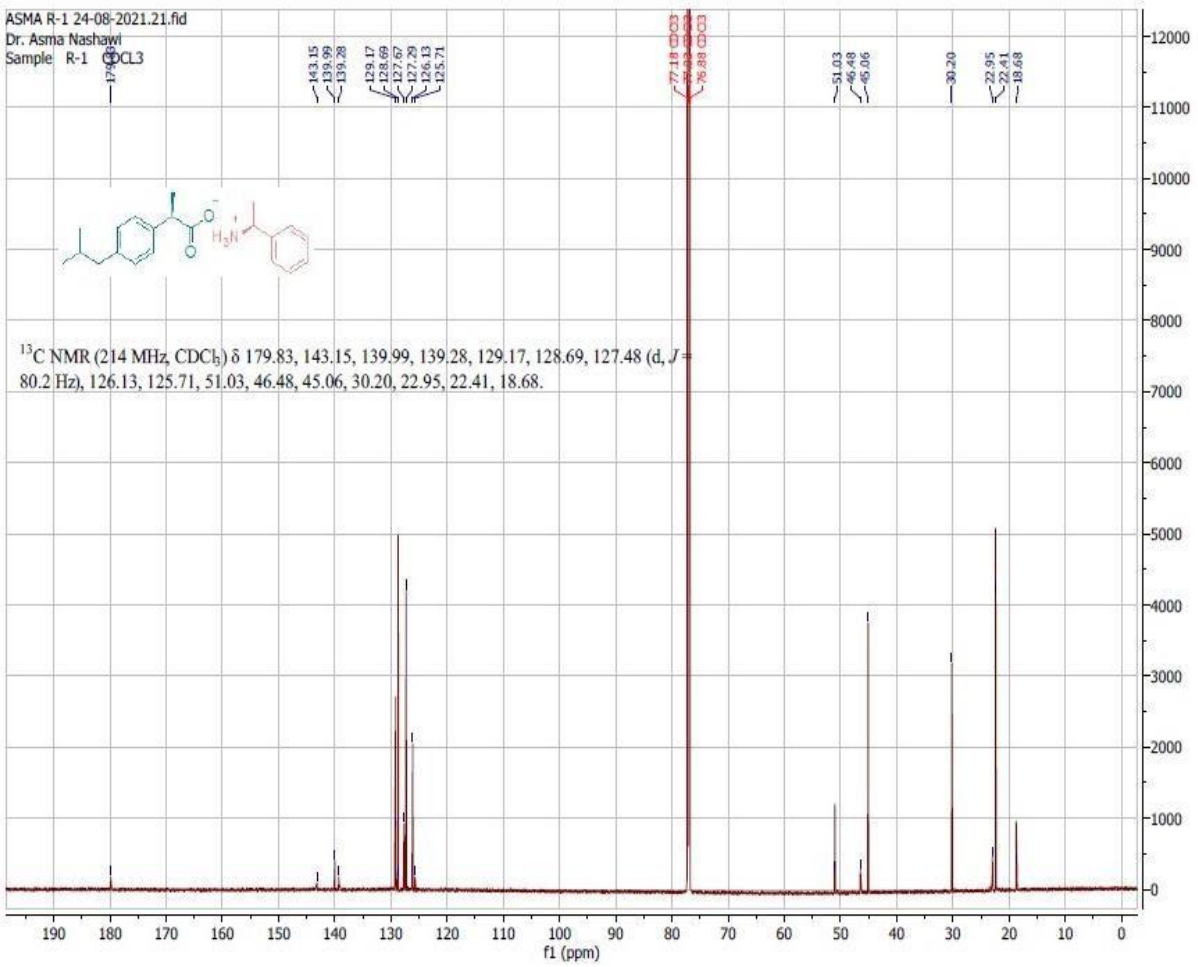


Figure 74. Carbon NMR spectrum of (R, S) salt compounds when (R)-ibuprofen was used with a chiral base.

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