Chapter-1

General Introduction

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1.1 Chirality and its Origin:

1.1.1 A Chirality Timeline:

Investigating the origin of chirality, revealed that the concept was introduced long ago by *Archimedes of Syracuse* who designed Archimedean water screw and studied its chiral spiral structure as early as 250 *B.C.* which is being utilized presently for various applications.[1] The presence of this phenomena in chemical compounds was later observed by *Dominique Arge* in 1811, who discovered the rotation of plane polarized light in quartz crystal and termed them as chiral. However, French chemist *Jean Baptiste Biot* in 1835 was the first to introduce the modern concept of chirality when he discovered rotation of light in sugar solution.[2]

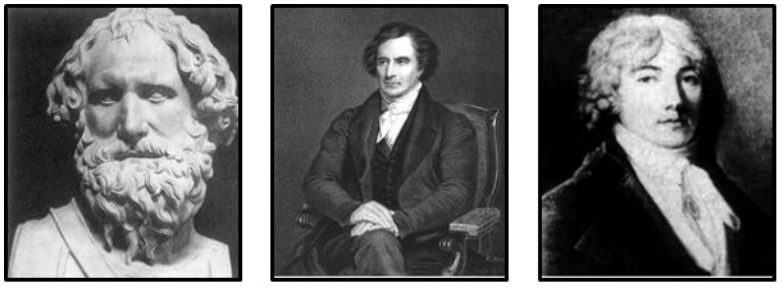


Figure 1 Prominent scientists who made early discoveries contributing to the development of modern concept of chirality

A major breakthrough in understanding the concept of chirality and its significance in chemistry was achieved by *Louis Pasteur* in 1848 who carried out recrystallization of sodium ammonium tartrate. He noticed crystals of two shapes which he then physically separated. Both the types of crystals were optically active, but rotated the plane of polarized light in the opposite direction. He proposed the presence of the molecule in two forms, namely "left handed" and "right handed", together being optically inactive. This finding formed a basis for his famous statement that the universe is chiral (*l'univers est dissymme´trique*).[3,4] Later *Vant Hoff*, a Dutch scientist proposed that a carbon atom attached to four different substituents has a tetrahedral arrangement in space. His proposition faced strong opposition from scientists all over the world, only to be proved correct later, for which he was awarded the first Noble prize in chemistry in 1901.[5] *William Thomson* (Lord Kelvin) in 1893 defined the notion of a chiral object and chirality stating *"I call any geometrical figure or group of points, chiral, and say that it possesses chirality, if*

its image in a plane mirror, ideally realized, cannot be brought to coincide with itself" which is the universally accepted definition of chirality.

Figure 2 Pioneers in the development of the concept of chirality

1.1.2 Classification of Chirality:

The word 'Chirality' originally comes from the Greek word *cheir* which means hand. One of the simplest definition of chirality is given by Mislow: "*An object is chiral if and only if it is not superimposable on its mirror image; otherwise it is achiral*."[6] Human hands are perhaps the most commonly recognized example of chirality, the left hand being non-superimposable mirror image of the right hand; irrespective of how the two hands are oriented, it is impossible for both hands to coincide.

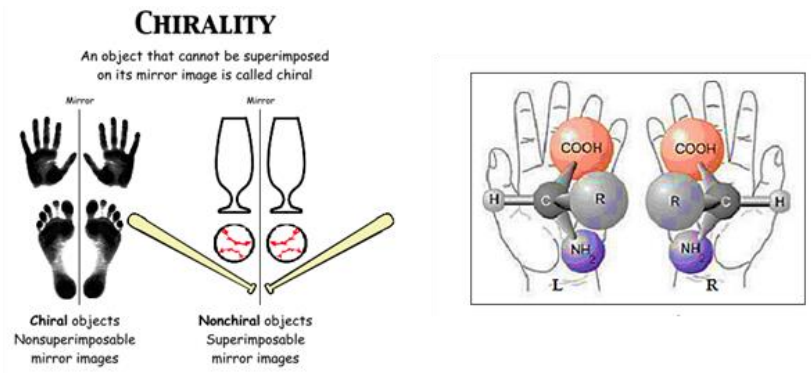


Figure 3 General Concept of Chirality

From definition it is clear that the term *'chiral'* refers to the spatial arrangement of the atoms within a molecule leading to formation of non-superimposable mirror image, termed as *enantiomers*. The study of stereoisomers is one of the most important and significant areas of modern organic chemistry.

Chirality can be induced in a molecule by the presence of: (i) stereogenic center (ii) chiral plane (iii) chiral axis and (iv) helical chirality. Majority of the optically active compounds

reported in literature, owe their chirality to the presence of a sp^3 hybridized asymmetric carbon where a carbon atom is bonded to four different groups, leading to loss in its symmetry. However, other atoms or elements can also act as a stereogenic center *eg.* phosphorus in phosphate triesters, quaternary ammonium group *etc.* The second most commonly observed chirality present in organic compounds is the axial chirality resulting from the non-planar arrangement of groups causing restricted rotation along a C-C σ bond. Biphenyls with bulky *ortho* substituents or binaphthyl derivatives are chiral by the virtue of presence of chiral axis. A relatively less explored type of chirality is the planar chirality where two non-coplanar dissymmetric rings, cannot undergo facile rotation around a chemical bond, constitute this category. Some examples in this class of compounds are substituted paracyclophanes and some poly-substituted metallocenes. A special case of axial chitality is the helical chirality where the molecules tend to adopt a skew shaped structure winding clockwise or anticlockwise along a stereogenic axis. The study of such molecules has gained tremendous impetus recently, due to their close structural relationship with biological entities (DNA) as well as their attractive opto-electronic properties.

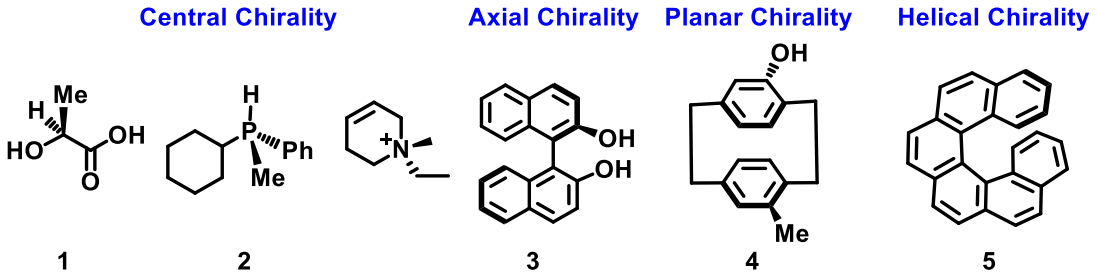


Figure 4 Types of Chirality

The presence of these stereogenic elements causes a loss in symmetry of the molecule leading to configurational isomerism generating a pair of stereoisomers which are non-identical mirror-images, termed as *enantiomers*. However, when an organic entity has the presence of two or more chiral elements, the related stereoisomers are not mirror images of each other as well as non-superimposible and are termed as *diastereomers*. Enantiomers have identical chemical and physical properties but show opposite response towards plane polarized light as well as the interaction with other chiral molecules varies which is of great importance in biology and pharmacology. Diastereomers on the other hand can have very different physical properties and chemical reactivities which is often exploited in synthetic organic chemistry.

1.1.3 Chirality in Nature:

Application of chirality is not only restricted to synthetic organic chemistry, but the natural world around us is filled with structural motifs having an intrinsic handedness. The human body itself is made up of chiral building blocks like amino acids, sugars, proteins *etc*. All the amino acids that constitute the genetic structures in biological system are essentially levorotatory whereas the sugars are all dextrorotatory. Hence, the ribosomes in our body never translate *d*-amino acids or binds specifically only to *d*-sugars. This property also extends to deoxyribose sugar (a precursor of DNA) which occurs in the *d*-form only. The nucleosides, which are nitrogenous bases present in the DNA have all *R*-configuration about the C1 carbon atom. The collective outcome of all these stereochemical selectivities in the living system causes the double helical structure of DNA to be essentially right handed. Hence, biological systems at molecular levels act as chiral entities, have been widely utilized to study their responses towards other chiral guests which has led to the rapid development of medicinal chemistry and drug discovery.

In nature, there are numerous examples where enantiomers have drastically different effects or properties upon interaction with the receptors in our body. One such example is the difference in the fragrance of both the enantiomers of limonene due to the fact that our nasal receptors are made of chiral molecules that interact with these enantiomers differently. Another example is naturally occurring (*R*)-carvone, found in mint leaves, is the principal contributor to the distinctive odour as well as flavour of mint while its enantiomer (*S*)-carvone is found in caraway seeds having a different flavour. Similarly, one enantiomer of asparagine and aspartame tastes sweet while the other tastes bitter.

Clearly living systems are very sensitive to chirality which is extremely important in pharmaceutical industry as many drugs are chiral moieties containing one or more chiral centres. Stereoselectivity in drugs is an essential dimension in the field of pharmacology due to the fact that different stereoisomers interact differently with the receptors present in biological system. It is well established that the opposite enantiomer of a chiral drug often differ significantly in its pharmacological,[7] toxicological,[8] pharmacodynamic and pharmacokinetic properties.[9,10]

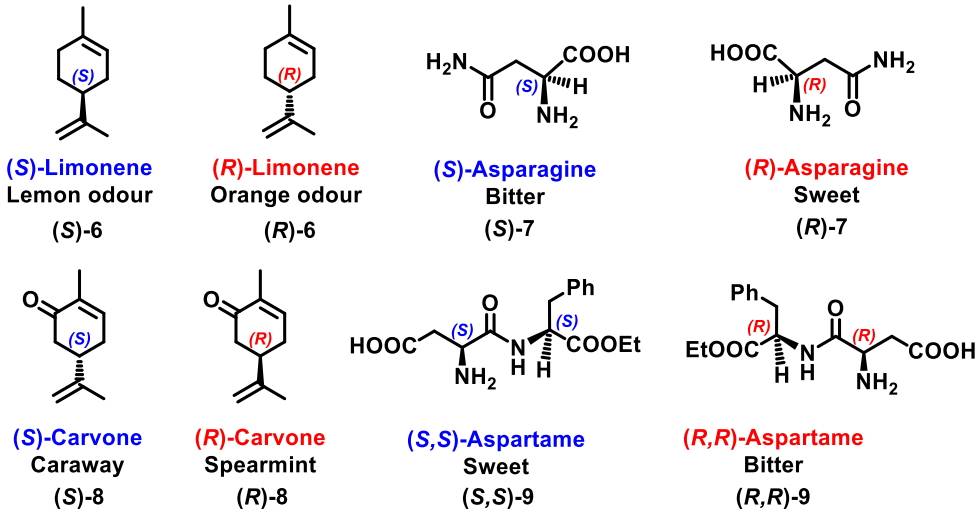


Figure 5 Difference in flavour and odour due to enantiomerism

A renowned example for the drastic difference in the pharmacological action of both the enantiomers of a chiral drug was the thalidomide tragedy. Thalidomide was prescribed to pregnant women in the 1960s to alleviate morning sickness. One of the enantiomeric forms of thalidomide has sedative and anti-nauseatic effect, but the other enantiomer is a potent teratogen, causing severe birth defects in the foetus. Since this tragedy, the significance of enantiopurity of biologically active compounds has received increasing attention and investigation of the stereodynamic properties of chiral molecules has become an integral part of modern drug development.[11]

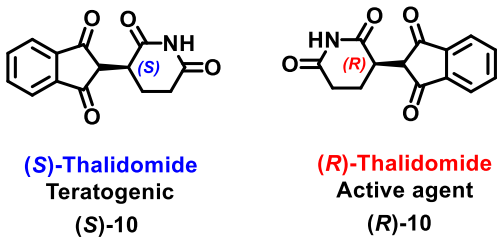


Figure 6 Drastically different pharmacological effect of Enantiomers

Enantiomers can also differ in pharmacokinetic or pharmacodynamic properties, *eg.* the difference in the ease with which they are absorbed or distributed in the body. A well-known example is Warfarin, where the *(S)*-isomer is more potent and metabolized by ring oxidation having a half-life of 32 hours while *(R)*-Warfarin is less potent and metabolized by side chain reduction with half-life of 54 hours. Such selective metabolization can influence drug distribution by selective tissue uptake and its renal excretion. Another example of stereoselective action is that of Ofloxacin which is administered as an antimalarial drug, the

(S)-enantiomer being 128 fold more active against both gram-positive and gram-negative bacteria than its *(R)*-antipode. In some cases, only one of the enantiomer of a drug may add to its therapeutic value while its isomer maybe inactive *eg.* Sotalol, a beta-blocker which is the most widely used pharmaceutical agent for angina, hypertension and arrhythmias. It is known that the (*S*)-enantiomer is active while the (*R*)-enantiomer does not add to the pharmacological effect and does not give any serious side-effects, so it can be considered as *'isomeric ballast'*. Examples are also known in which both the enantiomers of a drug have different biological properties resulting in the marketing of both isomers as different therapeutic indicators. For example, both enantiomers of the drug propoxyphene are available, dextropropoxyphene is administered as an analgesic and the other levopropoxyphene as an antitussive, (-)-methorphan is a potent opioid analgesic while (+)-methorphan is a cough suppressant.

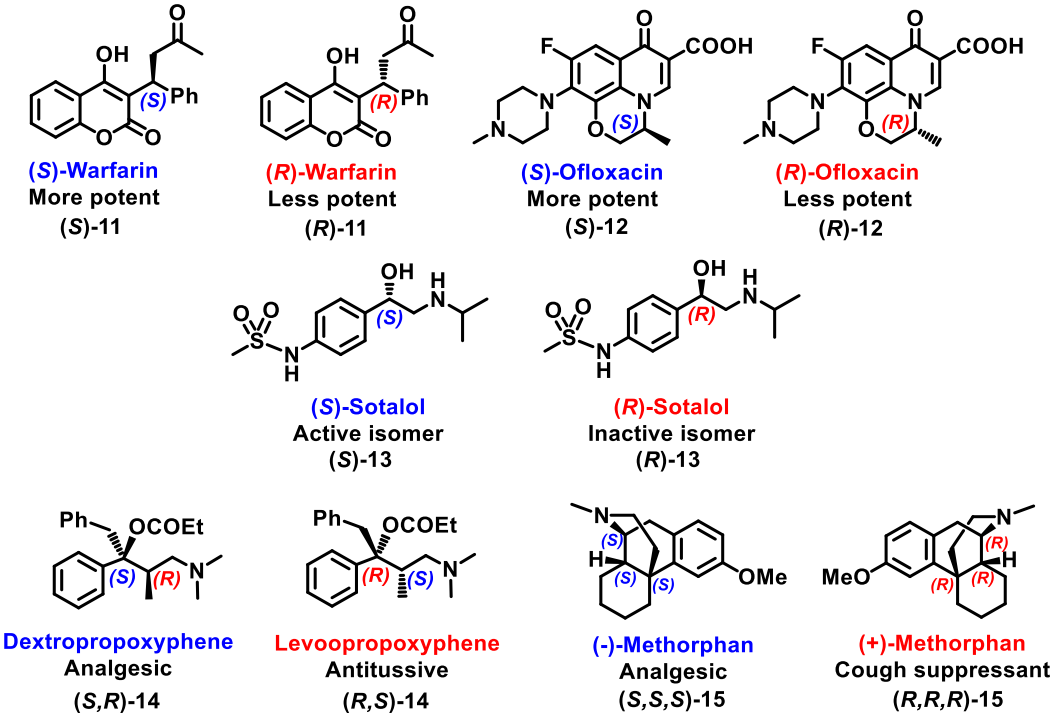


Figure 7 Some well-known drugs having different enantioresponses

Hence, one enantiomer of the drug may be responsible for the activity and its enantiomer could be inactive, may possess some activity of interest, be an antagonist of the active enantiomer or have a separate activity that could be desirable or undesirable. The use of drugs in their enantiomerically pure form has major advantages such as decrease in the overall administered dose, improving drug therapeutic window and accurately estimating the dose response relationship.[12] Therefore, there is a constant need for the development

of various tools and techniques for the synthesis of molecules in their enantiomeric forms and accurate analysis of the enantiopurity of such synthesized molecules.

1.2 Synthesis of Enantiomerically Pure Compounds:

The importance of chiral compounds and the strong need for enantiomerically pure substances has led to the development of versatile methodologies to meet this objective. There are three main approaches for the preparation of chiral compounds (Figure 8)

1. Resolution of Racemates;
2. Chiral pool approach;
3. Stereoselective conversion of prochiral substrates to enantiopure compounds.

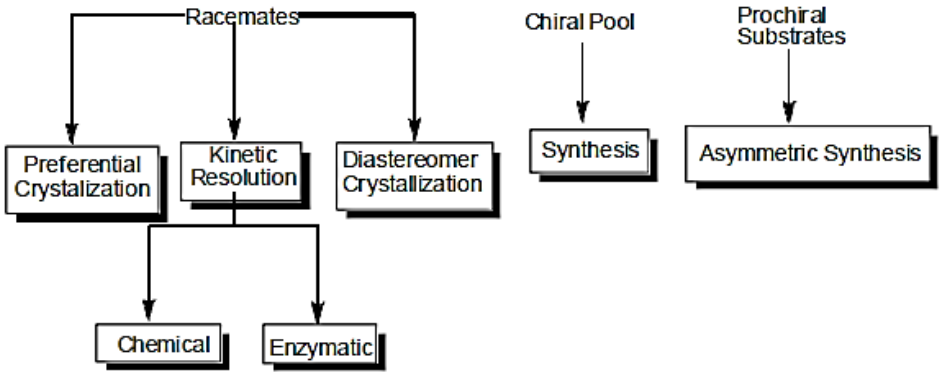


Figure 8 Source of enantiopure chiral molecules

1.2.1 Resolution of Racemates:

Chiral compounds synthesized from achiral starting materials and reagents are generally obtained as racemates. Since enantiomers have same physical and chemical properties, they can seldom be separated using physical methods. However, under the influence of other chiral entities, these enantiomers behave differently and can be subjected to separation. Resolution is the most classical route to obtain enantiopure chiral molecules and can be subdivided into three main techniques.

1.2.1.1 Crystallization:

1.2.1.1.1 Preferential Crystallization:

A breakthrough in the separation of enantiomers by simple crystallization was made by *Louise Pasture* who obtained the enantiomers of sodium ammonium tartrate from a

racemate by carefully observing difference in the morphology of the two enantiomeric crystals. Hence for the first time, the enantiomers of a chiral compound were separated by hand picking of the crystals that differed in morphology. However, both the crystals were formed in equal amounts rendering the overall system as racemic. In some cases, preferential crystallization is possible for racemates that form conglomerates resulting in spontaneous resolution of enantiomers as separate crystals. For such molecules, the crystal structure has a greater affinity for the same enantiomer than for the opposite enantiomer causing both the enantiomers to crystalize out separately and homochirally without the addition of any external chiral entity. Success in this method depends on the fact that for a conglomerate, the racemic mixture is more soluble than either of the pure enantiomers.

This technique still remains the preferred route at the industrial scale for their high selectivity and low cost. The scope of this phenomena is however rare and generally only 5-10% of racemates that form conglomerates fall in this category restricting its scope as a process for resolution.[13–15] Sometimes the resolution of racemates is initiated by inoculation of a saturated solution of the racemate with crystals of one enantiomer, leading to preferential crystallization of a single enantiomer.

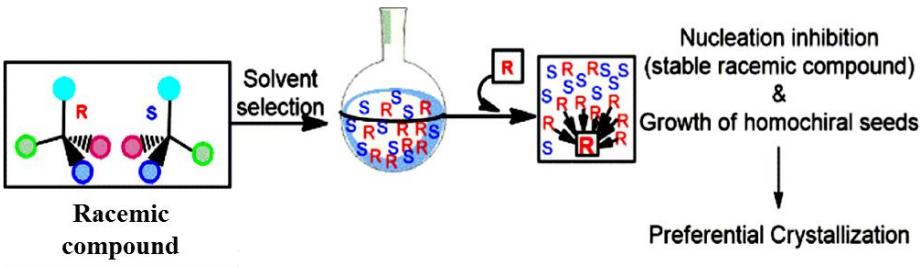


Figure 9 General concept of preferential crystallization

One of the recent report by Petite *et al.* mentions the enantioselective crystallization of proxyphylline, a xanthine-type bronchodilator drug, from its racemate on preparative scale by careful selection of the solvent system used for crystallization by this approach.[16]

1.2.1.1.2 Diastereomeric Crystallization:

The most well-known approach reported in literature involves the conversion of enantiomers into diastereomers and exploit their difference in chemical as well as physical properties. In this approach, racemate interacts with an added enantiopure entity to form diastereomers which can be separated by crystallization due to their difference in the

solubility for a given solvent system. These enantiopure entities are called resolving agents and are usually obtained from the chiral pool, *e.g.* *L*-tartaric acid, *D*-camphor sulfonic acid, quinine, chinchonidine and other alkaloid bases.

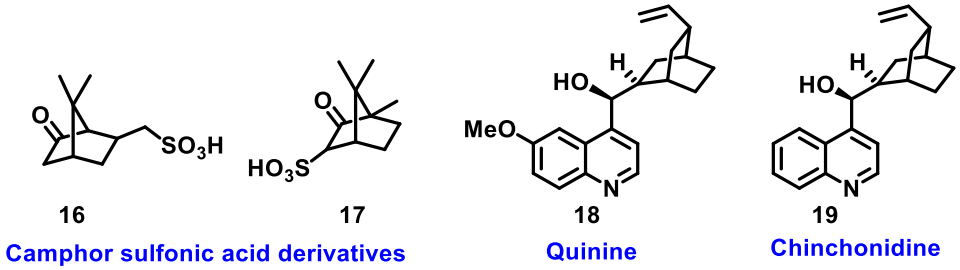


Figure 10 Some Chiral Resolving agents obtained from chiral pool

The resolving agents form diastereomers with the substrates *via* various interactions *eg.* ionic bond, covalent bond, charge transfer or by formation of inclusion complexes. The resolution obtained using this strategy usually exploits the difference in the solubility of the diastereomeric solids or sometimes using chromatographic separation techniques. The desirable characteristics of a good resolving agents are: a) ease of availability in enantiomrically pure form, b) low cost or ease of preparation, c) ease of recovery and reuse without its racemization, d) availability of both enantiomers and e) reasonable solubility.

Various naturally occurring alkaloids like cinchotoxine, quinotoxine, ephedrine**,** menthol and some synthetic bases like 1-phenylethylamine and amphetamine *etc.* have been successfully employed as resolving agents for the resolution of racemic acids. Such basic resolving agents are generally used to transform racemic substrates like carboxylic, sulfonic and variety of phosphorus acids into diastereomeric salts that are separated by crystallization.

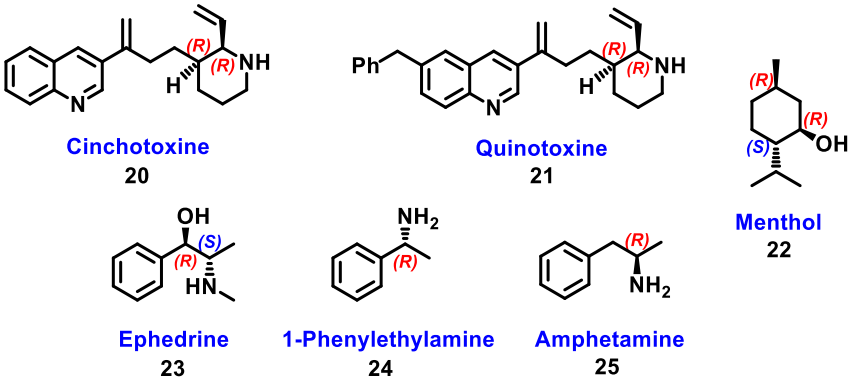


Figure 11 Natural or synthetic bases used as Resolving agents

The resolution of various amines has been achieved by the use of acidic resolving agents like *N*-acetylleucine, phenoxypropionic acid, mandelic acid, tartaric acid and its dibenzoyl derivative, pyroglutamic acid and Moshers acid *etc*.

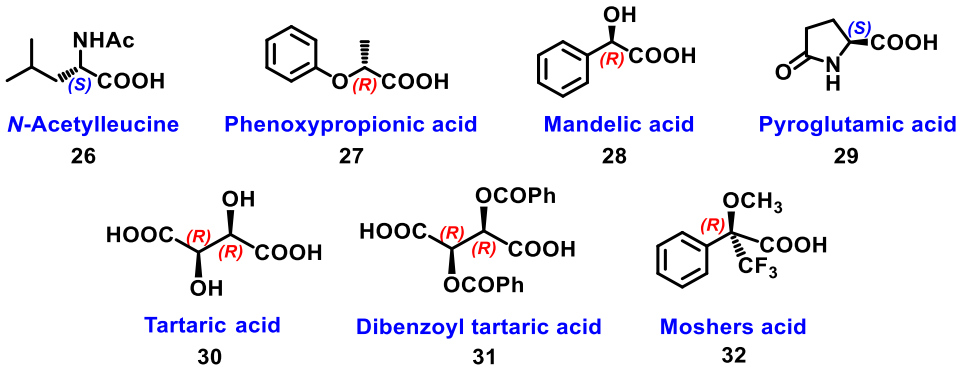
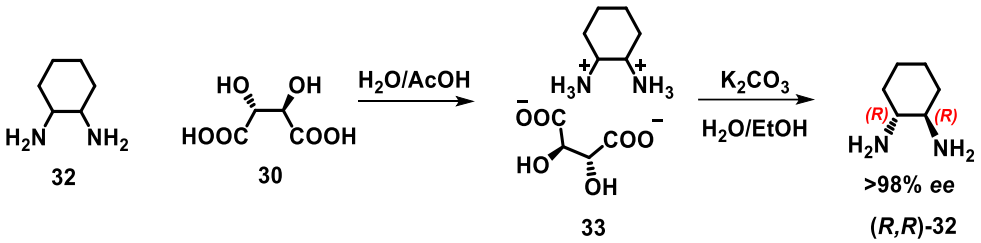


Figure 12 Acidic Resolving agents for various amines

Tartaric acid, an inexpensive chiral dicarboxylic acid found in nature in its optically pure form, has been utilized extensively in the resolution of amines such as 1,2-diamino cyclohexane where protonation of the amines by carboxylic group present in tartaric acid leads to diastereomeric salt formation.



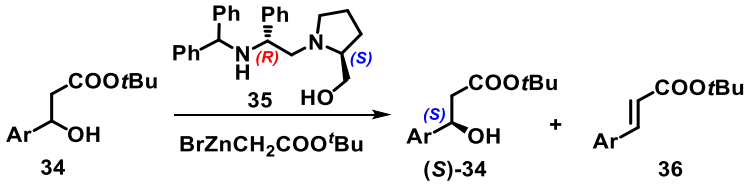
Scheme 1 Synthesis of 1,2-diamino cyclohexane using chiral tartaric acid

These diastereomeric salts can easily be separated and the enantiopure amine can be regenerated by a simple basic workup. The tartaric acid can be recovered from the aqueous layer by addition of dil. HCl without the loss of its optical activity and can be utilized again in the next resolution cycle.[17]

1.2.1.2 Kinetic Resolution:

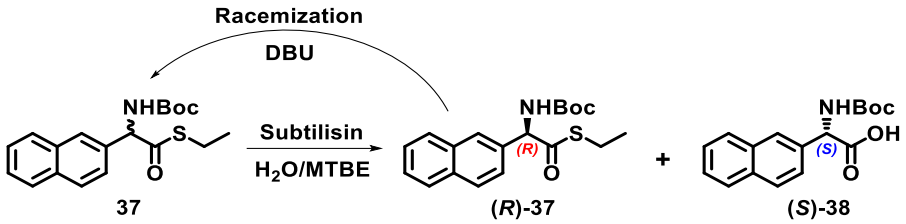
Kinetic resolution can be applied to systems where the rate of reactivity for the two enantiomers with a chiral entity is different. In other words, one enantiomer forms product much more rapidly than the other enantiomer. This difference in the rate of reaction is due to the difference in the activation energy for each enantiomer of the substrate to reach the transition state created due to the formation of a diastereomeric species with the chiral entity. This causes only one enantiomer having lower activation energy to be converted into the

product, whereas the other enantiomer remains unreacted during the course of reaction, leading to resolution. The chiral entity can be a biological catalyst (*e.g.* enzyme) or a chemical catalyst (*e.g.* chiral metal complex or organocatalyst). Scheme 2 shows an example in which racemic β-aryl-β-hydroxy esters with different substituents on the aryl moiety provides preferably the (*R*)-enantiomer with 93-98% *ee* and 32-41% isolated yield.[18]



Scheme 2 Kinetic Resolution using prolinol derivative as organocatalyst

In kinetic resolution, the product is formed from one enantiomer while the other enantiomer remains unreacted leading to a maximum theoretical yield of 50%. If the unwanted enantiomer is racemized *in situ* during resolution, a 100% theoretical yield of the enantiopure product can be reached, this is known as a ***dynamic kinetic resolution***. Tessaro *et al.* have carried out such resolution of naphthyl glycines and alanines simultaneously using protease as the chiral enzyme for resolution and base for *in situ* racemization. The amino acids hence observed were obtained with an *L*-configuration almost quantitatively and with complete enantioselectivity.[19]



Scheme 3 DKR in thioesters of naphthyl glycines using subtilisin

A recent report by Bergens *et al.* used a chiral Rh-complex for hydroacylation of pentenal derivatives with high *ee* and yields ranging from 62-94%.[20]

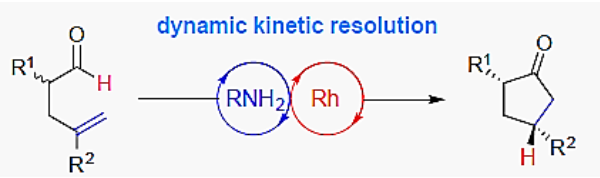


Figure 13 Schematic representation of DKR using a primary amine and Rh-catalyst by Bergens *et al.*

1.2.2 Chiral Pool Approach:

In nature, substances like amino acids, carbohydrates, hydroxy acids, terpenes and alkaloids occur abundantly as pure enantiomers and are often referred as *'chiral pool'*.

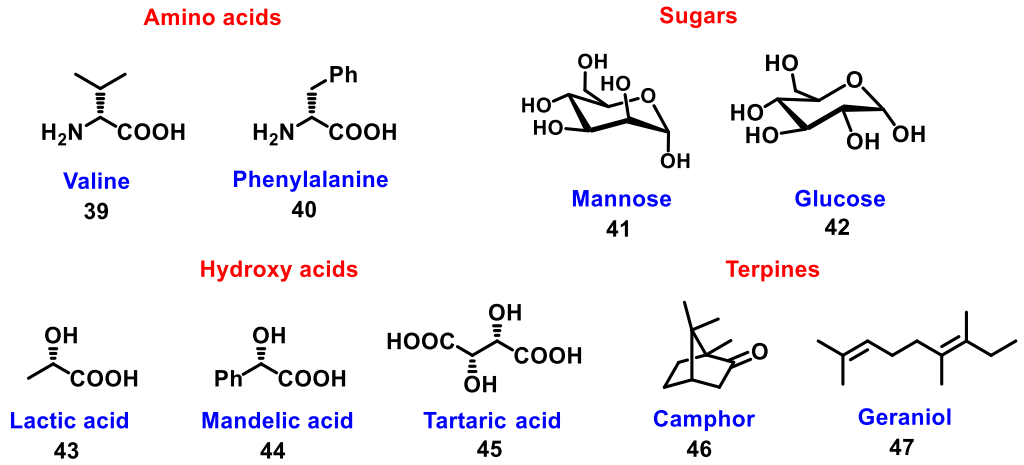


Figure 14 Examples of compounds derived from chiral pool

These substances act as synthons which incorporate chirality into the products using various chemical processes which involve retention of configuration, inversion or chirality transfer. They can be used as chiral substrates for various synthetic targets, or chiral catalyst which is regenerated at the end of a chemical transformation or can act as a chiral reagent, which is lost as a by-product during the reaction.

1.2.2.1 Chiral Substrates:

Naturally occurring (*S*)-amino acids are relatively inexpensive and have been explored greatly as a versatile starting material in organic synthesis. The other enantiomer (*R*) is usually obtained from the resolution of the racemic amino acids.

An example of the application of chiral pool for synthesis of pharmaceutical drugs is the use of *L*-Aspartic acid, a member of chiral pool has been used for the synthesis of Imipenem which is a broad spectrum antibiotic; Tadalafil, used to treat pulmonary arterial hypertension is synthesized from *D*-tryptophan methyl ester are some examples that highlight the importance of amino acids as chiral pool.[21,22] Another example is the synthesis of (*S*)-Vigabatrin, a potent GABA-T inhibitor from (*R*)-methionine which is obtained from a chiral pool was reported by Knaus and Wei with 96% yield and >98% *ee*.[23,24]

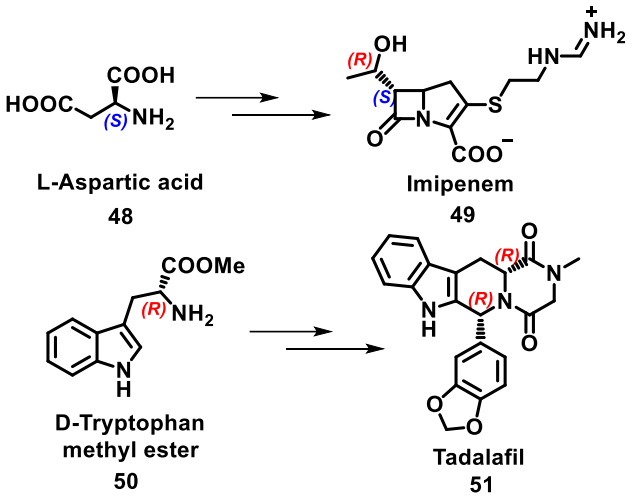
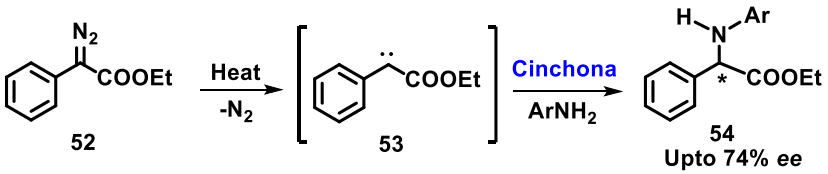


Figure 15 **Amino acids from chiral pool in the synthesis of biologically active drugs**

1.2.2.2 Chiral Catalyst:

Naturally occurring alkaloids like cinchona, ephedrine, strychnine, brucine, sparteine etc. have been used as chiral catalysts in various asymmetric transformations. A recent report by Miyairi *et al.* uses cinchona alkaloid as catalyst for the asymmetric N-H insertion where the chiral induction was dependent on the chirality of cinchona.[25,26]



Scheme 4 **Asymmetric N-H insertion by organocatalyst**

As cinchona based alkaloids are greatly explored as organocatalyst, Kupai *et al.* have synthesized cinchona based compounds with different saturation on the quinuclidine unit (ethyl, vinyl, ethynyl) and compared their activity as organocatalyst in asymmetric Michael addition reactions.[27]

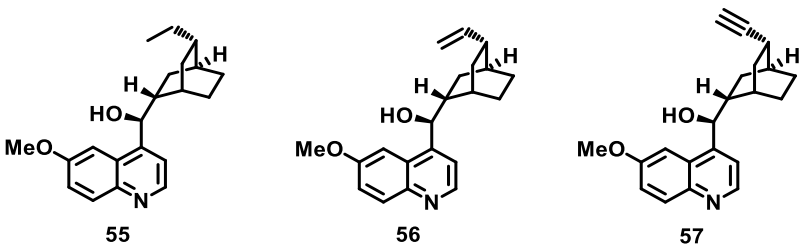
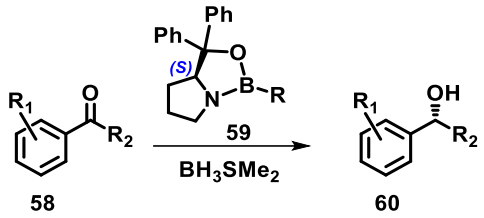


Figure 16 **Cinchona derived organocatalysts by Kupai *et al.***

1.2.2.3 Chiral Reagents:

Various chiral reagents have also been developed from the chiral pool motifs like derivatives of proline or hydroxyl proline for the asymmetric reduction of ketones which is one of the most important chemical transformation to generate optically active stereocenters. The secondary alcohols hence generated are significantly important in a variety of intermediates, chiral building blocks and biologically active components. Prolinol, derived from the reduction of naturally available proline, is used as a chiral reagent in oxazaborolidine-catalyzed asymmetric reduction of ketones has successfully been utilized for synthesis of secondary alcohols in excellent yields ranging from 80-99% and *ee* of 91-99%.[28]



Scheme 5 Oxazaborolidine-catalysed asymmetric reduction of ketones

Despite a large number of functionality available from nature, limited examples are available for large scale synthesis, many of them being expensive. However, with the recent advances in synthetic methods, many new compounds have been added to the chiral pool but they are still limited.

1.2.3 Asymmetric Synthesis:

Asymmetric synthesis involves the formation of a stereogenic centre under the influence of some external or internal chiral inducing agents *ie.* due to the presence of another stereocenter in the reactant molecule itself, the incoming reagent is directed in a stereoselective manner, use of chiral auxiliaries or chiral catalyst which convert the achiral reactant into a chiral entity.

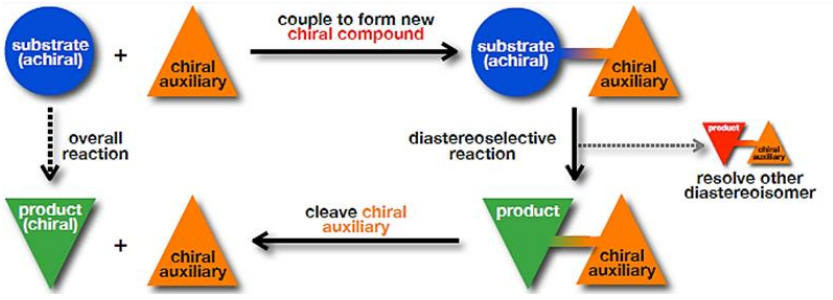


Figure 17 Schematic representation for the use of chiral auxiliary

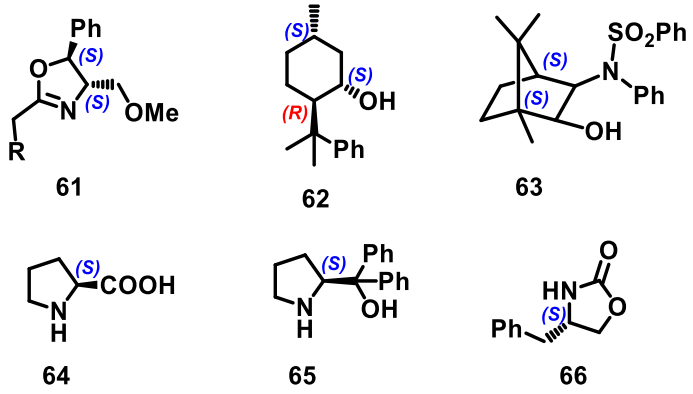
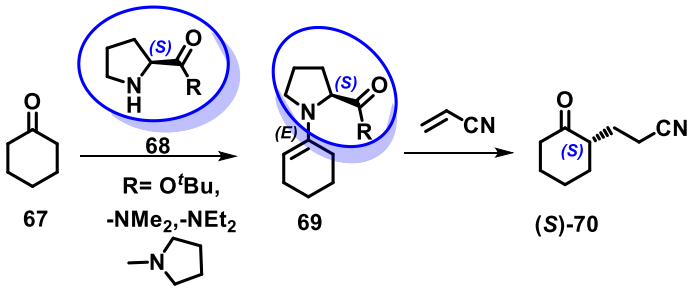


Figure 18 Popularly used Chiral auxiliaries

In other words, chiral auxiliaries lead to a stereochemical control over the substrate, where they are temporarily attached to the substrate, allowing diastereomeric reactions to occur. On their removal, they form products equivalent to those obtained by enantioselective reactions.

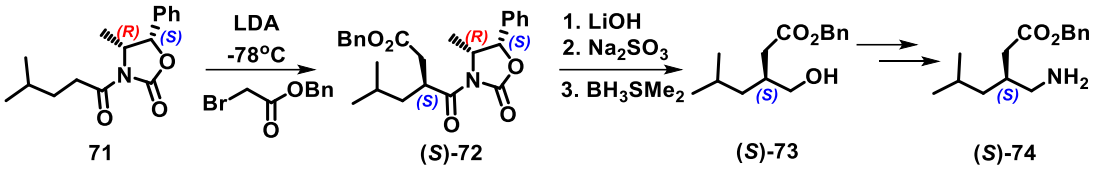
A recent review highlights (−)-8-phenylmenthol, well known as Coreys' auxiliary is the most versatile auxiliary used in asymmetric organic synthesis, along with contributions from the pioneers of this field like Evans, Yamada, Enders, Oppolzer and Kunz. These experts have led to remarkable progress, in the last decade, in the field of asymmetric synthesis and continue to bring development presently.[29] Yamada for the first time reported the use of optically pure proline derivatives for the synthesis of chiral β-substituted cyclohexanones, obtained by asymmetric alkylation of enamines.



Scheme 6 Synthesis of chiral ketones using Yamadas' chiral auxiliary

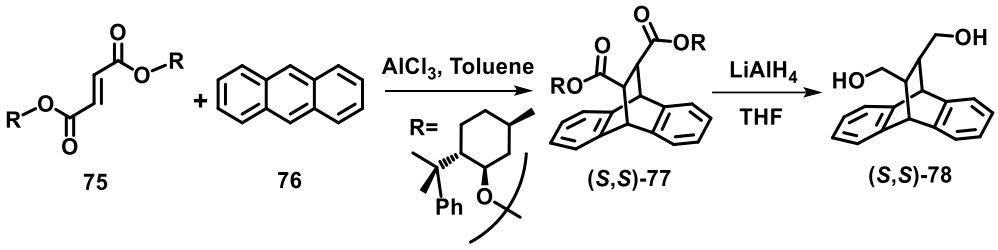
However, the most widely used chiral auxiliary in asymmetric total synthesis is Evans oxazolidinones which have been utilized in a large variety of chemical transformations like α-alkylation, aldol, 1,4-addition reactions, intramolecular Diels-Alder cycloaddition reactions, Michael addition *etc.* Evans oxazolidinones have been used extensively in the enolate chemistry which is very important as it is one of the most common means for C-C bond formation. It has also been successfully utilized in the preparation of pregabalin, an

anticonvulsant for the treatment of neuropathic pain was synthesized enantioselectively for the first time using Evans auxiliary.



Scheme 7 Synthesis of pregabalin using Evans' auxiliary

The field of asymmetric synthesis showed a great development after Corey introducted 8-phenyl menthol as a chiral auxiliary for various transformations. Since then, menthol has been successfully derivatized into a number of compounds due to its easy availability in optically pure form. Chapuis *et al.* have studied and compared a number of chiral auxiliaries derived from menthol in the asymmetric [4+2] cycloaddition reactions of fumarates with dienes. They however concluded that 8-phenyl menthol was the most efficient chiral auxiliary for this reaction giving 76% *de* and 99% yield.[30]



Scheme 8 Coreys' 8-Phenyl menthol as chiral auxiliary in asymmetric Diels-Alder reaction

This method has great applicability in organic synthesis, however it suffers from several drawbacks, some of which are stated below:

1. Limited access to optically pure starting materials having reactive site in close proximity of the chiral centre.
2. A stoichiometric quantity of chiral auxiliary is required.
3. Additional steps involving a chemical bond formation between the chiral auxiliary and prochiral substrate is needed along with its facile cleavage.
4. The stereochemical transformation should not cause any racemization of the newly generated chiral centre or auxiliary.

Although asymmetric synthesis suffers from many such disadvantages, it is cost-effective due to the avoidance of laborious, tedious and time-consuming separation of enantiomers

as well as it does not require the wasteful discarding of the undesired enantiomer, making this field prosperous greatly in organic synthesis of chiral molecules. The importance and significance of asymmetric synthesis was highlighted by the Nobel Prize awarded to W. S. Knowles, R. Noyori and K. B. Sharpless in 2001 for their work on asymmetric catalysis.

1.3 Determination of Optical Purity of Chiral Compounds:

Due to the increasing demand in synthesis of chiral molecules for various applications in the fields of medicine,[31] asymmetric synthesis,[32,33] catalysis,[34] flavour,[35] fragrances,[36] biochemistry and material science chemistry,[37] the determination of optical purity of the newly synthesized compounds has gained tremendous importance. Enantiomeric purity (or optical purity) of a chiral compound is defined as the fractional excess of one enantiomer over the other and is often used to quantify the efficiency of an asymmetric transformation. It is given by the following equation:

$$\%\, ee = \frac{(R - S)}{(R + S)} X100$$

Where, %*ee* is the enantiomeric excess, *R* and *S* are the amount of *R* enantiomer and *S* enantiomer in terms of percentage present in the mixture. The value of enantiomeric excess can range from 0 to 100 *eg.* for a racemic mixture, the %*ee* is zero and for a mixture having 70% *R* and 30% *S* has an enantiomeric excess of 50%. However, to quantify *ee*, the racemate needs to be resolved and the enantiomers should be separated or distinguished which can be efficiently done by providing a chiral environment. The methods for the determination of *ee* relies on the difference in chemical, physical or spectroscopic properties of the diastereomers derived from the enantiomeric mixture. Various analytical techniques used for accurate determination of optical purity of chiral compounds include HPLC or GC involving the use of chiral stationary phases,[38–40] mass spectrometry,[41–44] IR spectroscopy,[45] UV-Vis spectroscopy,[46] CD[47,48] & electrophoresis[49] *etc.*

The simplest method to determine the optical purity of the molecules is by making use of the properties in which the enantiomers differ. Their ability to rotate the plane polarized light to equal but opposite directions has led to the development of SOR and CD as tools for determination of enantiomeric purity. But these methods require the compound to be pure and the results are often unreliable if there is presence of other chiral impurities. These methods are also less sensitive as compared to other available methods, making them a

strategy of less choice for the determination of optical purity. Hence, HPLC and GC are often employed as an efficient tool for such analysis, as they overcome these disadvantages. It involves the use of chiral stationary phases which causes *on-column* derivatization of the enantiomers into diastereomers *via* various non-covalent interactions. They are highly sensitive analytic tools with good accuracy and precision, however they have their own flaws *eg.* the chiral columns involved in these strategies are often expensive and selective to substrate scope and large amount of solvents are required as mobile phase. These methods require a baseline separation of peaks for both the enantiomers for accurate determination of *ee*, which makes them often tedious as well as time consuming. Even though GC and mass spectrometry require a very small amount of sample for analysis, their use is often restricted to samples that are volatile and thermally stable. Capillary electrophoresis is a recently developed method and found to be better than HPLC as it involves the use of small amounts of chiral selector and solvent. As this method is based on the differential rate of migration of a charged species in a capillary tube, it essentially requiring the analyte to be ionisable in the analysis buffer, limiting its scope.[50]

Optical spectroscopy *ie.* UV-Visible and fluorescence spectroscopy have gained attention in molecular sensing due to its numerous advantages. The use of absorbance or emission as a mode of signalling is a quick and efficient tool that requires simple instrumentation. Although fluorescence spectroscopy essentially requires the presence of a fluorophore in the molecule to be analysed, UV-Visible spectroscopy can be carried out for all the compounds which are UV-Visible active and have the presence of a chromophoric and/or auxochromic groups. Hence we have focused our discussion on the use of UV-Visible and NMR spectroscopy as tools for determination of optical purity.

1.3.1 UV-Vis Absorption Spectroscopy:

UV-Visible spectroscopy is one of the most widely used tools for determination of enantiomeric purity due to its high sensitivity and simplicity to study various interactions. A pre-requisite for this technique is the presence of a chromophoric group in the host molecule with the absorption band in the UV-Visible range. This technique usually involves the use of an optically pure UV-Visible sensor (or host) which effectively differentiates between the two enantiomers of the chiral molecule. It is a useful tool for enantiodescrimination when the sensor and its complex with the enantiomers absorb at a

different wavelength. The difference in the absorbance of host and corresponding host-guest complex is used for quantitative determination of *ee* as well as comparing the strength of binding. The association constants related to binding process of the guest enantiomers with the chiral host can be calculated by using the modified Benesie–Hildebrand equation as follows:

$$[H][G] = \frac{1}{Ka\Delta\varepsilon} + \frac{[G]}{\Delta\varepsilon}$$

Further modified equation, where a double reciprocal plot can be made with 1/ΔA as a function of 1/[G]. {where [G] >>> [H]} is generally followed

$$\frac{1}{\Delta A} = \frac{1}{Ka\Delta\varepsilon[H][G]} + \frac{1}{\Delta\varepsilon[G]}$$

where, [H] and [G] are the total concentrations of host and guest, respectively, Δε is the change of molar extinction coefficient between the free and complexed host, ΔA represents the absorption change of host upon the addition of opposite guest enantiomers.[46,51,52] The plots of 1/ΔA against 1/[G] values, usually gives an excellent linear relationship, indicating the corresponding binding process between the host and guest enantiomers. The Δε value can be derived from the intercept, while K_a (association constant) can be calculated from the slope. The binding constants, $K_{(R)}$ or $K_{(S)}$ and associated free energy change (ΔG) for the host molecule upon the complexation are obtained by the curve fitting analysis of observed absorbance changes.

This approach has been utilized for the enantiomeric recognition of a variety of amino acids as they are important building blocks biological sciences as well as in synthetic organic chemistry. In the past decade, naturally occurring cyclodextrins have been extensively used for such molecular recognition of amino acids due to their inner hydrophobic core and outer hydrophilic rim. Literature shows that the surface of cyclodextrins have been modified extensively to enhance their binding affinity, molecular selectivity or enatioselectivities.[53] β-CD has been utilized for the discrimination of enantiomeric composition of a variety of analytes like ephedrine, tryptophan, propranolol and proline with varying enantiomeric compositions. The difference in the absorbtion spectras for the transient diastereomeric species formed were investigated by Warner *et al* using UV-Visible spectroscopy.[54]

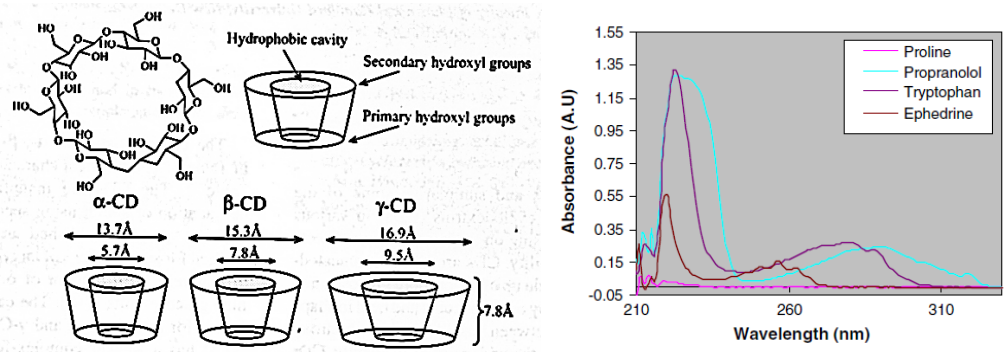


Figure 19 Structure of cyclodextrins and its UV response towards chiral analytes

Another class of chiral compounds consisting of carboxylic acids not only play an important role in various biological processes, but are also important intermediates to a number of pharmaceutical drugs. Various artificial receptors and macrocyclic ligands have been synthesized to study the molecular recognition of carboxylic acids. Chiral calix[4]arenes bearing chiral aminonaphthol moiety has been utilized by Sitit *et al* for the molecular recognition of chiral carboxylic acids by UV-Visible spectroscopy.[55]

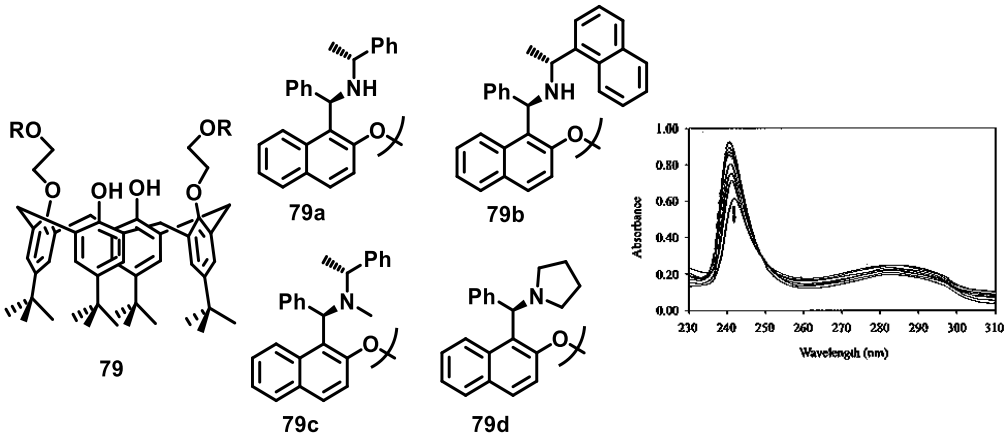


Figure 20 Calix[4]arene derived aminonaphthols for discrimination of (*L*)-benzoyl tartaric acid

Many receptors have been reported in literature for the recognition of carboxylic acids using UV spectroscopy. Huang *et al* have synthesized caloriemetric chemosensor for mandelate ions based on thiourea derivative of BINOL system. These chiral receptors showed a colour change which could be observed with a naked eye with the two enantiomers of mandelate. This was also monitored by UV-Visible spectroscopy, which showed the appearance of a new absorption band at~425nm which increases gradually with the increase in concentration of mandalate ions corresponding to the absorption by complex.[56]

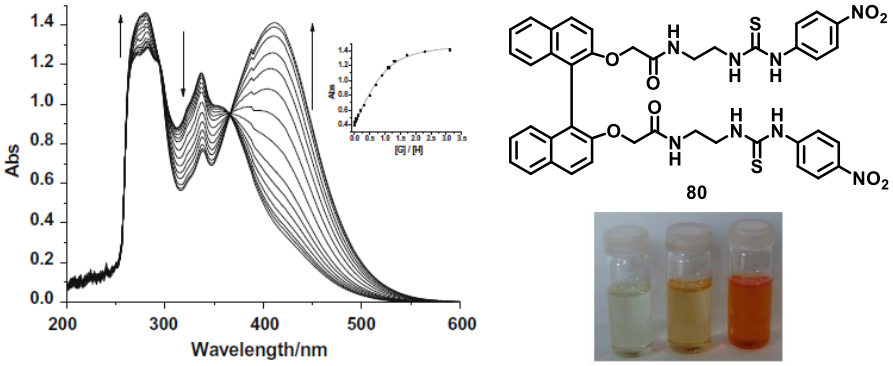


Figure 21 BINOL based thiourea as caloriemetric receptor for mandate ions

UV-Visible spectroscopy not only enables one to study the phenomena of molecular recognition, but also gives useful insights into the mode of interactions, the strength of complexation or binding, as well as gives us the thermodynamic profile of complexation. All this information along with its simplicity and robustness, has made this technique a promising tool with possible utility in the rapid screening of potential drug candidates in pharmaceutical industries. However this method cannot be applied to substrates that are UV inactive. For such molecules, NMR spectroscopy is an efficient tool for determination of optical purity.

1.3.2 Nuclear Magnetic Resonance (NMR) Spectroscopy:

Over the last fifty years, Nuclear magnetic resonance (NMR) spectroscopy has been used as a preeminent tool for characterization and structural elucidation of molecules. However, recently it has been utilized to determine the enantiopurity and assign absolute configuration of chiral compounds.[57] For enantiomers, the NMR active nuclei are isochronous in nature giving no discrimination in their individual spectra. However, in the presence of a chiral environment, the nuclei of the two enantiomers become anisochronous leading to their possible discrimination. This strategy has been most exploited by Raban and Mislow in 1965,[58] which involves the use of an enantiopure chiral reagent to distinguish a pair of enantiomers through the formation of diastereomeric complexes. With the diastereomeric complexes, the resonances of enantiotopic nuclei may split into two resonances, one for the (*R*)-isomer and one for the (*S*)-isomer of the analyte. The area under the peak for the signals obtained due to the two diastereomeric resonances can be used to accurately determine the enantiopurity of the test analyte. Hence, for the enantiomeric discrimination and determination of enantiomeric purity, a chiral entity is required to convert the pair of

enantiomers into diastereomers. The chiral entities used for chiral discrimination in NMR spectroscopy are grouped into three categories: (i) Chiral Solvating Agents (CSA) (ii) Chiral Lanthanide Shift Reagents (CLSR) (iii) Chiral Derivatizing Agents (CDA)

1.3.2.1 Chiral Derivatizing Agents (CDAs):

Derivatization of enantiomers by covalent bond formation with an enantiomerically pure compound remains the most widely used NMR technique for the assay of enantiomeric purity. This method involves the formation of diastereomers which can be differentiated on the NMR time scale.

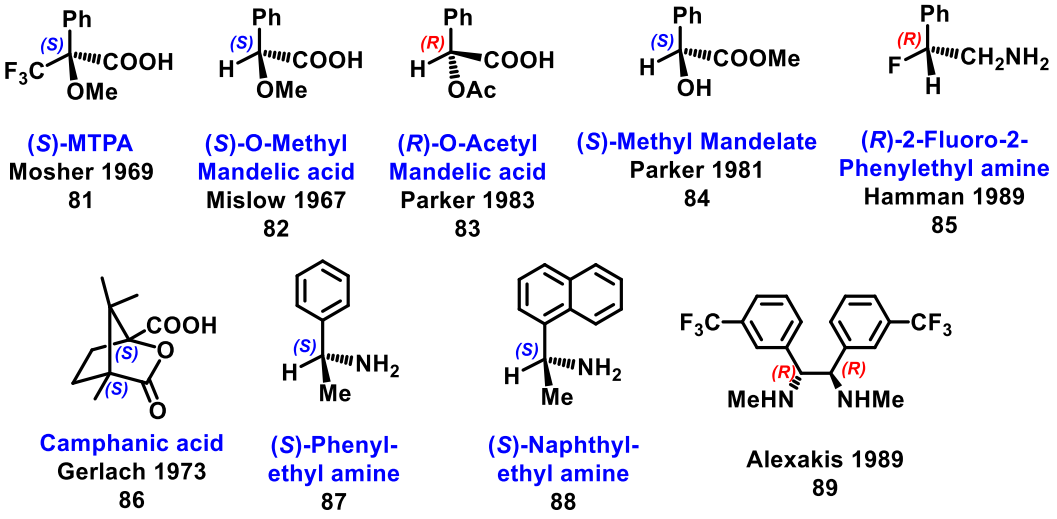


Figure 22 Common Chiral Derivatizing Agents known in literature

One of the most reliable and extensively used CDA was reported by Dale and Mosher in 1973.[59] They synthesized α-methoxy-α-trifluoromethylphenylacetic acid (also known as Moshers acid) for the enantiomeric discrimination of alcohols and amines by the formation of diastereomeric esters or amides using ^{1}H NMR. Since then, a number of modification have been made to Moshers acid to increase its substrate scope as well as to explore other NMR active nuclei.[59–62]

There are two potential concerns with the application of CDAs when determining enantiopurity, (i) the possibility of kinetic resolution, which involves a situation where one enantiomer reacts faster with the CDA than the other and (ii) no racemization should occur during the derivatization reaction. The CDA should also be necessarily enantiomerically pure for the accurate measurement of optical purity of the analyte.

1.3.2.2 Chiral Lanthanide Shift Reagents (CLSRs):

These are usually six-coordinated lanthanide complexes which form a weak addition complex with a large variety of organic compounds. They are usually camphor based complexes of Eu, Pr[63,64] or Yb.[65] Although many lanthanide ions have been used for different specific purposes, Eu(III) has been by far the most commonly used for enantiomeric separations.[66,67] These CLSRs suffer from various drawbacks like line broadening is observed if used in greater stoichiometric amounts as well as they are expensive which greatly limits their usefulness.

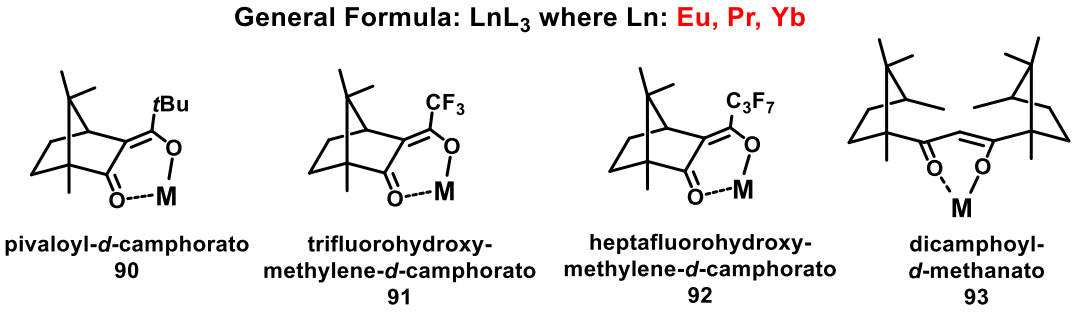


Figure 4 Chiral Lanthanide Shift Reagents

1.3.2.3 Chiral Solvating Agents (CSAs):

CSAs interact with the analyte through non-covalent interactions such as dipole–dipole, ion-pairing, π-π interactions *etc*. Two points need to be considered during the design of CSAs: (i) the host and the guest molecules should have complimentary functionalities which permits their interaction (ii) CSA should have presence of a group with high anisotropy near its asymmetric centre.

The CSAs that have been most widely explored are 2,2,2-trifluoro-lphenylethanol (TFPE), 2,2,2-trifluoro-l-(1-naphthy1)ethanol (TFNE) and 2,2,2-trifluoro-l-(9-anthryl)ethanol (TFAE) used for recognition basic compounds whereas 1-phenylethylamine (PEA) and 1-(1-naphthyl)ethylamine (NEA) are used as CSA for recognition of chiral acidic substrates.

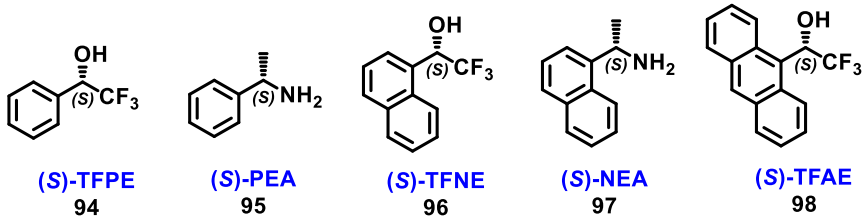


Figure 5 Some Chiral Solvating Agents reported in literature

The resonance of the racemic analyte has an initial chemical shift *δ*. Addition of a CSA perturbs the chemical shift and the difference between the new and original chemical shift value is termed as *induced chemical shift* denoted by Δ*δ*. If the resonance shows anisotropy with a separate peak for each of the enantiomers, the difference in chemical shifts between these two peaks is called *chemical shift non-equivalence* denoted as ΔΔ*δ*. The magnitude of enantio-differentiation of an analyte is directly proportional to the value to chemical shift non-equivalence ΔΔ*δ*.

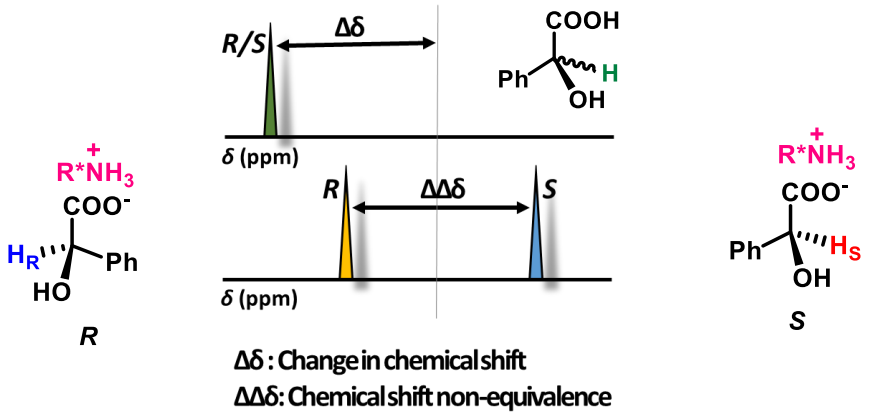


Figure 6 General terms used for description of recognition using CSA

Recently Liu *et al* have used enantiomerically pure BINOL based phosphoric acid derivatives as CSA for the recognition of arylquinazolinones.

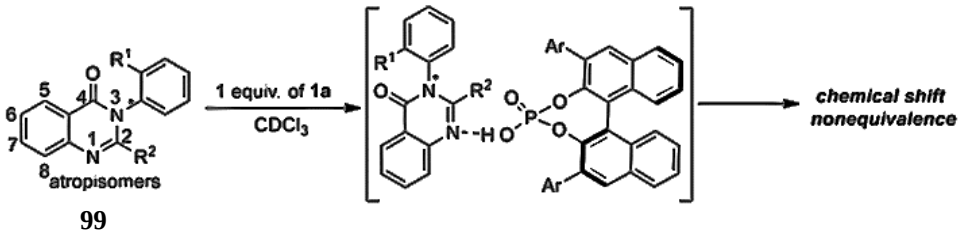


Figure 7 Discrimination of arylquinazolinones using chiral phosphoric acid as CSA

Aryquinazolinones exist as atropisomers due to restricted C-N bond rotations and form an important motif in various biologically active natural products and various drug intermediates causing their determination of optical purity, essential.[68] The determination of optical purity of amino acids is necessary due to their wide applications both in pharmaceuticals as well as synthetic chemistry. However, the analysis of amino acids as such may be difficult due to their low solubility in organic solvents. They are hence, often derivatized and analysed for their optical purity. Pallavicini *et al* have utilized the widely

used (*R*)-Mosher acid for the enantiodiscrimination of a variety of benzyl esters of amino acids. The benzylic protons also act as a handle to accurately determine the enantiomeric excess of the substrates.[69]

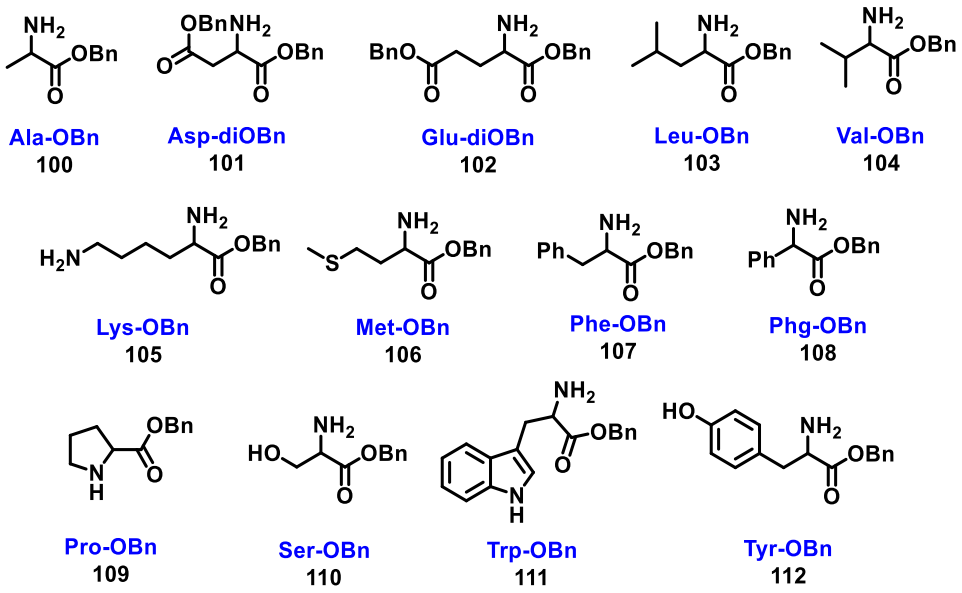


Figure 8 Benzyl esters of amino acid screened by Moshers acid as CSA

The ^{1}H nucleus is most commonly used for spectral analysis in chiral differentiation studies by NMR spectroscopy. However, the ^{1}H NMR spectra of the CSA itself can be crowded (in some cases) or overlapping of the signals of the substrate to be analysed may occur. To overcome such limitations, other NMR active nuclei like ^{19}F,[70–72] ^{31}P,[73–75] ^{13}C[76,77] and ^{77}Se[78–80] can also be used to study optical purity where the interpretation of signals is easy due to less number of signals.[81] There are various advantages of this method *e.g.* it is quick and simple to perform, with no problems of kinetic resolution or sample racemization provided that the complexes remain in solution, requires very low concentrations of host and guest and is very accurate for determination of optical purity as low as 1% *ee*.

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