Resolutions of racemates by crystallization

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Chapter 1

Introduction

In this chapter[*] the discovery of chirality, more than 200 years ago, is described and examples are given of optically pure compounds that are a part of our daily life. Furthermore, this chapter describes the most common methods for the production of optically pure compounds synthesized from other optically pure compounds, from pro-chiral compounds or from racemates. At the end of this chapter the aim and outline of this thesis are presented.

[*] *Parts of this chapter have been used for the preparation of Comprehensive Biotechnology 2nd Ed., Chiral Separations, Chapter 128, to be published in* ***2011****.*

1.1 History

More than two hundred years ago, in 1801, the French mineralogist René-Just Haüy observed that quartz crystals showed hemihedral behavior, in other words, certain facets of the crystals are mirror images of each other.[1] In 1815, Jean-Baptiste Biot showed that polarized light, when passed through an (enantiomerically enriched, to use modern terminology) organic liquid or solution, could be rotated clockwise or counterclockwise.[2]

In 1844, Eilhard Mitscherlich, a German scientist, examined the sodium ammonium salts of both enantiomerically pure and racemic tartaric acid. At that time, the main source of optically pure tartaric acid was potassium bitartrate, which is abundant in the sediments from fermenting wine. Often, another form of tartaric acid, "racemic acid" (Latin *racemus* meaning "bunch of grapes"), was found in wine barrels. Mitscherlich found that the crystals from enantiomerically pure and racemic sodium ammonium tartrate were identical in crystalline form, except that upon dissolution the former rotated polarized light whereas the latter did not.[3]

Figure 1.1 *Louis Pasteur working in his laboratory as painted by Robert Thom.*

In 1848, Louis Pasteur (depicted in Figure 1.1) at age 26, was working on his doctorate on crystallization of salts of tartaric acid as a student of Biot. Pasteur could not believe that "tartaric acid" and "racemic acid" were the same and suspected that Mitscherlich had overlooked something.

Indeed, Pasteur observed that the crystals of natural sodium ammonium tartrate were all identical but that the crystals from sodium ammonium tartrate from racemic acid were a mixture of two mirror image crystals, which can be distinguished by the hemihedral facets of the crystals as depicted in Figure 1.2. He separated the right and left handed crystals into two piles. Despite the near universality of the story, since the crystals crumble very easily it is unlikely that Pasteur used a pair of tweezers to pick out the crystals.[4] Upon dissolution of equal amounts of each of these piles, he noticed that the solution from one pile rotated polarized light clockwise (levorotary) and the solution of the other pile provided the same magnitude of rotation but counterclockwise (dextrorotary).[5] The former pile consisted thus of *levo* or L-sodium ammonium tartrate and the latter of *dextro* or D-sodium ammonium tartrate. With this experiment Pasteur performed the first resolution (separation of mirror image compounds: enantiomers) and proved that racemic acid is a 1:1 mixture of left and right handed sodium ammonium tartrate.[6] Today, 1:1 mixtures of opposite enantiomers are called 'racemates'.

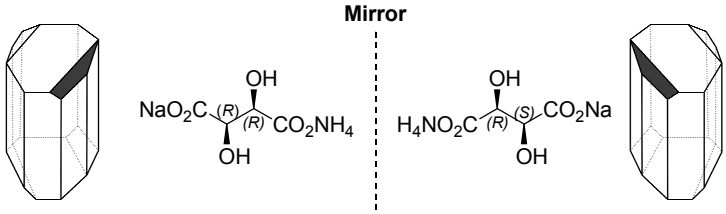
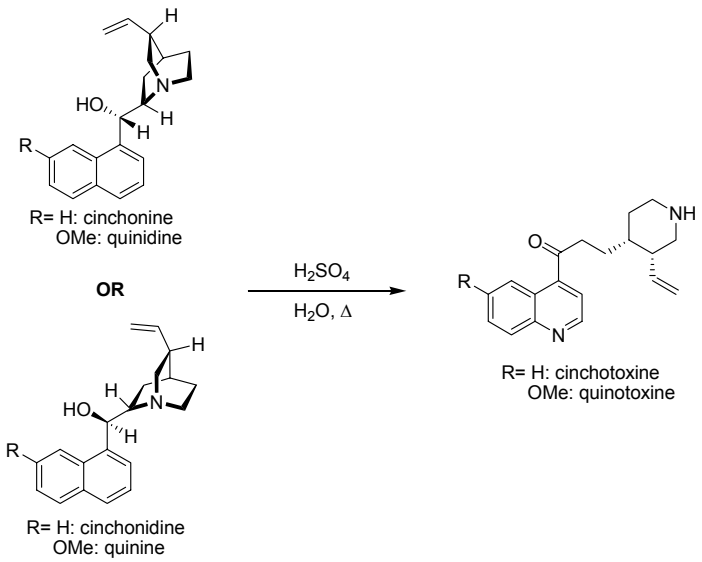


Figure 1.2 *The mirror imaged crystals and enantiomers of sodium ammonium tartrate.*

The story has a fascinating footnote that we can understand today. Pasteur obtained the crystals of racemic sodium ammonium tartrate from a man called Kestner, a French manufacturer. We now know that racemic sodium ammonium tartrate can occur in two crystalline forms: 'conglomerate' and 'racemic compound'. The material Pasteur used was the conglomerate in which the enantiomers crystallize as separate crystals as Pasteur observed. This is relatively rare: only roughly 10% of chiral organic compounds crystallize as conglomerates. The other 90% crystallize as racemic compounds in which the enantiomers pair with each other and cannot be physically separated. With sodium ammonium tartrate the balance between the conglomerate and the racemic compound is extremely delicate: the conglomerate crystallizes as a tetrahydrate below 27°C. However, the racemic compound was only slightly less stable than the conglomerate and thus can form spontaneously by crystallization.[7] Pasteur had the good fortune to get the conglomerate form from Kestner. Other suppliers apparently used a somewhat other

different isolation process and provided the racemic compound used by others who tried, with striking lack of success, to repeat the Pasteur experiments.[8]

A few years later, in 1853, Pasteur synthesized quinotoxine,[9] which is a rearrangement product of quinine or quinidine on heating in dilute sulfuric acid. Two of the four chiral centers are removed in this reaction which is depicted in Scheme 1.1. In a similar fashion, cinchotoxine was prepared from cinchonine or cinchonidine.[10] The salt of quinotoxine with racemic tartaric acid was crystallized and showed enrichment in L-tartaric acid. Furthermore, crystallization of the salts of cinchotoxine with racemic tartaric acid gave salts that were enriched in D-tartaric acid.[11] Nearly one hundred years later, Woodward and Doering repeated this experiment as part of their total synthesis of quinine and showed that Pasteur most likely isolated the hexahydrate of the quinotoxine-L-tartrate.[12] With these experiments, Pasteur gave life to resolution by diastereomeric salt formation, a process left largely unaltered to this day.

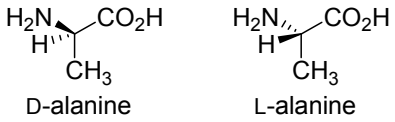


Scheme 1.1 *Synthesis of quinotoxine and cinchotoxine.*

Later, in 1858, Pasteur reported that natural L-ammonium tartrate was digested by the mould *Penicillium Glaucum* and the D-enantiomer was left untouched. Pasteur hereby performed the first kinetic resolution.[13,14] *Penicillium Glaucum* is used in blue cheeses like gorgonzola[15] and, as the name suggests, possesses anti-bacterial activity.[16]

Also in 1858, Friedrich August Kekulé von Stradonitz suggested the tetravalency of carbon, which implies that a carbon atom can form bonds to four other atoms.[17] Several years later,

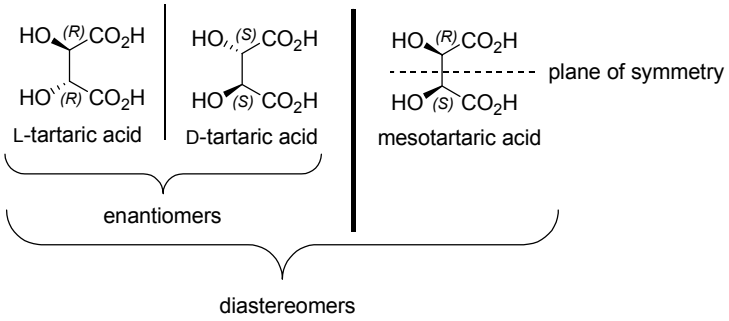
in 1874, Jacobus Henricus van 't Hoff[18] (the first winner of the Nobel prize for chemistry, however, not for the tetraedric carbon[19]) and Joseph Achille Le Bel,[20] nearly simultaneous proposed the theory of the position of atoms in space and the tetrahedral carbon. A carbon atom, surrounded by four different moieties, can exist as two mirror image molecules: enantiomers (Greek *enantios* = opposite, *meros* = part[21]) as depicted in Scheme 1.2. These enantiomers have the same physical properties except for the rotation of polarized light, as Pasteur observed also. In 1883, Lord Kelvin[22] proposed the term 'chirality', derived from the Greek word *kheir* for handedness.



Scheme 1.2 *The enantiomers of alanine.*

In most organisms, amino acids like alanine (Scheme 1.2), only exist in the L-form. Furthermore, sugars exist only in the D-form. This phenomenon whereby compounds closely related in structure (families) have identical absolute configurations is known as homochirality.[23]

Today, the D and L designations are mainly used in biology for amino acids and carbohydrates. However, chemists prefer the designations *R* (for *rectus*) and *S* (for *sinister*) for chiral molecules. The designation depends on the difference in atom weight of the four substituents of the chiral atom (chiral centre). With these designations, each chiral centre can have either an *R* or *S* label and thus multiple chiral centers in a single molecule can be assigned, impossible with the D/L system.

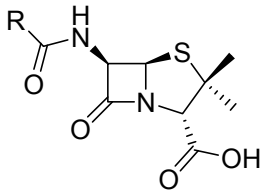


Scheme 1.3 *D- and L-tartaric acid, enantiomers. Mesotartaric acid is a diastereomer of these compounds.*

By definition, stereo isomers that are not each others mirror image are diastereomers. An example is tartaric acid that is shown in Scheme 1.3. D- and L-tartaric acid are mirror images of each other and thus enantiomers. Mesotartaric acid however, has one different chiral center compared to either D- or L-tartaric acid and the latter are thus a diastereomers of mesotartaric acid. Due to the internal plane of symmetry in mesotartaric acid, the two chiral centers cancel each other out and the compound does not rotate light.

1.2 Optically Pure Compounds in Our Daily Life

More than half of the drugs marketed today are chiral and nonracemic.[24] Of course, the human body is also made of chiral molecules like enzymes, DNA and proteins. With this in mind, it is not hard to imagine that the enantiomers of chiral drugs can have different biological activities and toxicities.[25] Therefore, for drugs that enter the market today, the enantiomers have to be tested independently even when the drug is administered as a racemate. For example, penicillins (as depicted in Scheme 1.4) are only active on peptide links of D-alanine that occur in the cell walls of bacteria. The antibiotic can only kill bacteria and not human cells because the latter do not contain D-amino acids.[26]



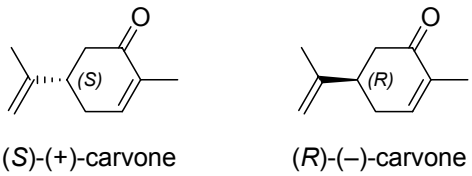
Scheme 1.4 *Penicillin core structure.*

The smell receptors in the nose are a striking example of the working of chirality. A famous example is limonene (depicted in Scheme 1.5). The (*R*)-enantiomer smells like orange whereas the (*S*)-enantiomer smells like lemon.[27] Limonene is also widely used as a biodegradable degreasing agent.[28]

(*R*)-(–)-limonene (*S*)-(+)-limonene

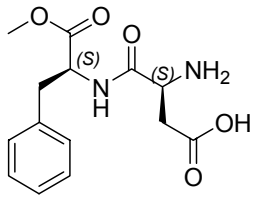
Scheme 1.5 *The enantiomers of limonene.*

The taste receptors on the tongue also can distinguish between enantiomers as demonstrated by carvone as shown in Scheme 1.6. The (*S*)-enantiomer tastes and smells like caraway (anise-like) whereas the (*R*)-enantiomer tastes and smells like spearmint.[27]



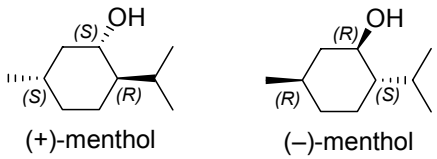
Scheme 1.6 *The enantiomers of carvone.*

Most of the naturally occurring (*S*)-amino acids taste sweet, whereas the unnatural (*R*)-enantiomers taste bitter.[29] The artificial sweetener, aspartame (as shown in Scheme 1.7), is chiral and only the depicted enantiomer tastes sweet whereas the other taste bitter. Aspartame, which is not a sugar but a peptide, is 180–200 times sweeter than ordinary sugar.[30]

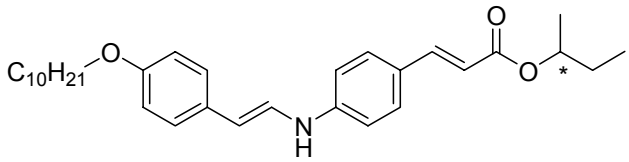


Scheme 1.7 *Aspartame, an artificial sweetener.*

Menthol is also a chiral compound, the (+)- and (–)-enantiomers are depicted in Scheme 1.8. The naturally occurring (–)-menthol can be described as fresh, sweet, minty, cooling and refreshing. The (+)-isomer is similar, but less minty, herbier, with musty, bitter, phenolic and herbaceous notes, and this enantiomer is less refreshing. (–)-Menthol has also about four times more cooling power than the (+)-isomer because the heat receptors in the skin are also chiral.[31]



Scheme 1.8 *Both enantiomers of menthol.*



Scheme 1.9 *Liquid crystal forming chiral compound.*

The role of chirality goes further than living organisms. The working of liquid crystal displays (LCD) is based on optically pure molecules, like the compound in Scheme 1.9. Such compounds are liquid crystals which mean that the liquid compound self orients into *e.g.* helical structures: so called twisted nematic (TN) liquid crystals. Displays containing these TN liquid crystals represent the majority of the consumer LCD's.[32]

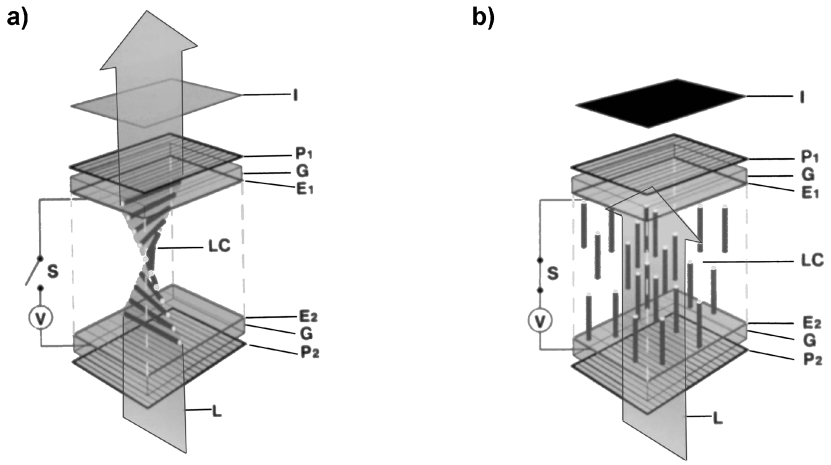


Figure 1.3 *Schematic projection of an LCD pixel.*[33]

The working of an LCD pixel is illustrated in Figure 1.3a. When ordinary light (L) is polarized (P_2) and passed through a cell containing a twisted nematic liquid crystal (LC), which is placed in between two glass plates (G), this polarized light is rotated exactly 90°C. This light then passes through another polarizer (P_1) which is placed perpendicular to P_2. In this manner, the cell (or pixel) looks white. However, if a current is placed (S) over the electrodes (E_1 and E_2), the molecules in the liquid crystal orient themselves towards the

current of the electrons. The polarized light is not rotated in this medium and is then blocked by the perpendicular polarizer P_1. The pixel in this case appears black as shown in Figure 1.3b.[34]

1.3 Calculations

The enantio purity of enantiomers is usually expressed as enantiomeric excess (*ee*) and the diastereomeric purity of diastereomers as diastereomeric excess (*de*). These parameters can be calculated from the relative amounts of each enantiomer (*p* and *q*) or diastereomer (*ap* and *aq*) with Equation 1.1.

For example, the *de* of diastereomeric salts can be measured by chiral HPLC. Samples are liberated before injection or on column by the eluent thus the *ee* is measured by chiral HPLC. The areas under the peaks of both enantiomers are taken for *ap* and *aq*. Presuming the resolving agent is optically pure, the *de* equals the *ee*. In racemates, the area under both peaks is equal thus the *ee* is 0%.

$$ee(\%) = \frac{p-q}{p+q} \times 100 \qquad de(\%) = \frac{ap-aq}{ap+aq} \times 100$$

Equation 1.1 *Calculation of the* ee *and* de *from the amounts of enantiomers or diastereomers.*

To compare resolutions, Fogassy[35] introduced the resolution efficiency or resolvability (S). This S-factor ranges from 0 (no resolution) to 1 (perfect resolution) and is calculated with Equation 1.2.

$$S = de \times Y \times 2$$

Equation 1.2 *The S-factor.*

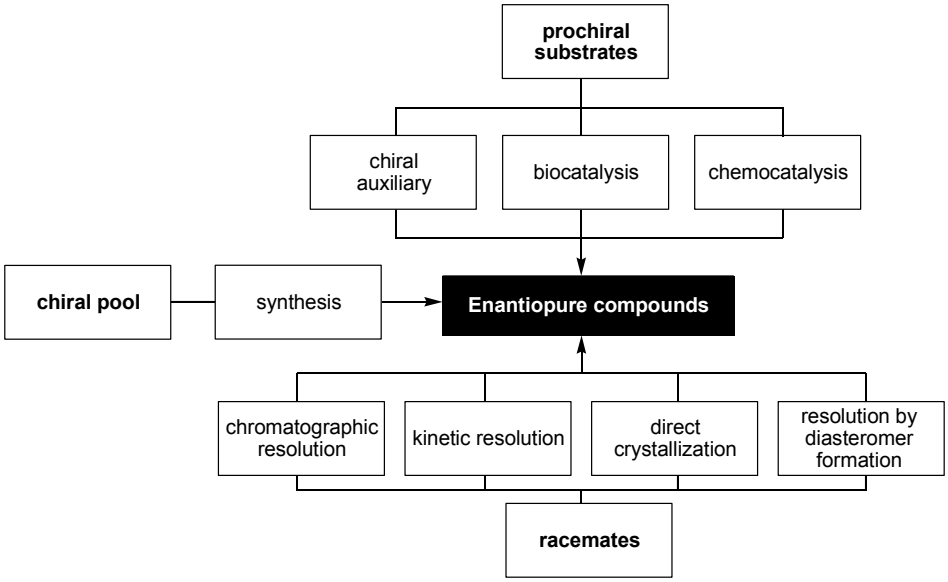
Wherein *de* is the diastereomeric excess of the first salt and *Y* is the yield, both ranging from 0 to 1. In a resolution without racemization, the maximum yield for a single diastereomer is thus 0.5. The factor 2 adjusts the S-factor to a value between 0 and 1.

Often, the purity of the crystals from diastereomeric salts suffer from some incorporation of the one other diastereomer in the other diastereomer producing impure crystals.[71] This phenomenon is known as an end-solid solution.[36] The S-factor is only useful to compare resolutions that have the same amount of end-solid solution. Since the amount of end-solid solution is in general not known and end-solid solutions are very common in diastereomeric

salt formations, it is only suitable for comparing the effectiveness of additives for nucleation inhibition effects as in Chapter 3.

1.4 Methods for the Preparation of Optically Pure Compounds

The sourcing of enantiopure materials has become a very important issue over the years.[37] There are several methods to arrive at a desired optically pure compound. A summary of the most frequently used methodologies is given in Scheme 1.10. A brief explanation and example of each of the techniques is given in the following paragraphs.



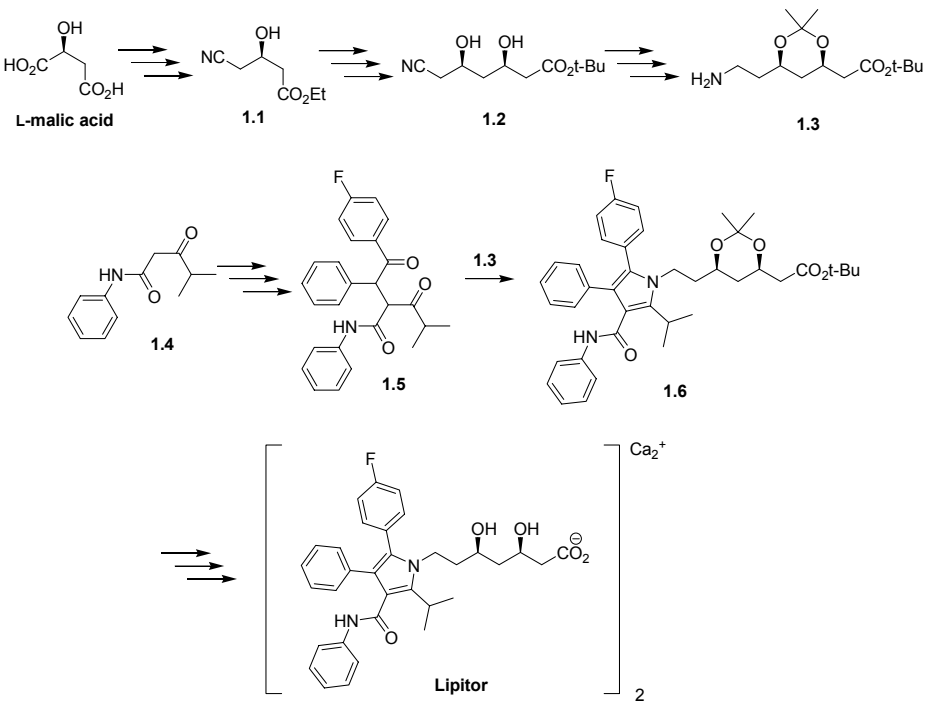
Scheme 1.10 *Routes to enantiopure compounds.*

1.4.1 Chiral Pool

Usually nature prepares chiral compounds an enantioselective fashion. These optical pure or enriched compounds (*e.g.* amino acids, alkaloids and carbohydrates) can then be harvested by extraction from *e.g.* leaves, bark or fermentation processes, can be further functionalized in subsequent synthesis. Although these chiral compounds are often relatively cheap, the subsequent synthesis can become more challenging if transformation to the desired product requires multiple steps.[38] Furthermore, usually only one

enantiomer/diastereomer is available, which might not be the enantiomer/diastereomer of interest. Natural amino acids are the most frequently used compounds from the chiral pool.[39] Other optically pure compounds are also available which are produced on a large scale and thus available at a relatively low price. These compounds form, what is known as, the extended chiral pool.

An example of a chiral drug which is synthesized from a compound from the chiral pool is Lipitor made by Pfizer.[40] Lipitor is a cholesterol lowering agent which is sold as the calcium salt and is the largest selling brand-name drug in the world for the last couple of years with sales of US$6.17 billion in 2007.[41] An overview of the synthesis of Lipitor is given in Scheme 1.11.



Scheme 1.11 *Synthesis of Lipitor starting from* L*-malic acid.*

Starting from L-malic acid (available from the chiral pool) a nitrile group is introduced to give **1.1**. This nitrile group is later used in the coupling with building block **1.5**. Subsequently, a second chiral centre is introduced whose chirality is controlled by the hydroxyl group in compound **1.1**. Compound **1.2** is then reduced and protected to give building block **1.3**. Compound **1.6** is synthesized by a Paal-Knorr condensation of building

block **1.3** and compound **1.5**. The latter was synthesized by a Stetter reaction from compound **1.4**. Deprotection and subsequently isolation as the calcium salt furnishes Lipitor.[42]

1.4.2 Prochiral Substrates

Achiral compounds that can be made into a chiral compound by a chemical reaction are called prochiral substrates. For example, if an addition to a double bond eliminates the planes of symmetry, chiral centers are created. If the stereoselectivity of a reaction is not high enough, one might consider to use a technique from §1.4.3 to raise the optical purity of this partially enriched material to a satisfactory high level.[43]

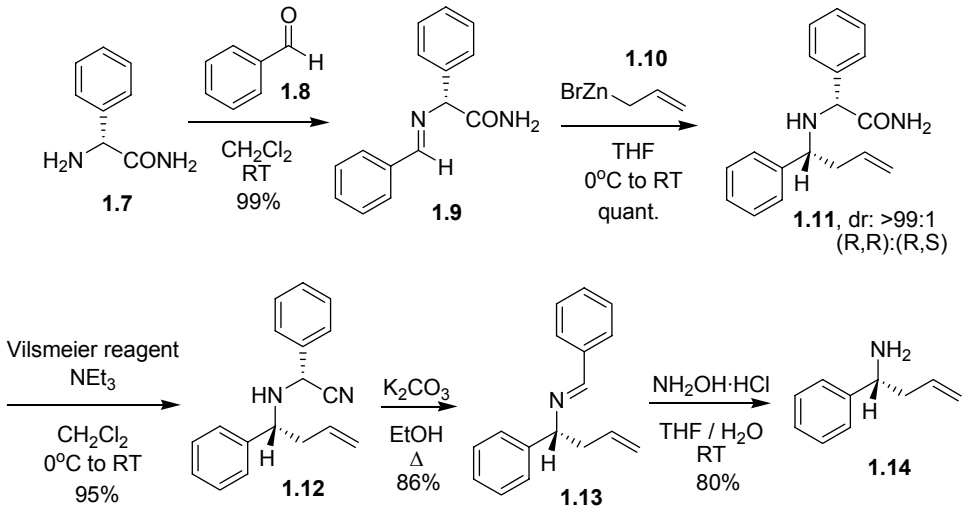
1.4.2.1 Chiral Auxiliary[44]

Another approach is to use the chirality of an inexpensive compound, a chiral auxiliary, to induce new chiral molecules preferably with high stereoselectivity. After the chirality transfer, the chiral auxiliary can be removed completely or a part of the chiral auxiliary might be retained. The latter is exemplified by the synthesis of (*R*)-1-phenylbut-3-en-1-amine (**1.14**) as given in Scheme 1.12.

Imine **1.9** is synthesized starting from (*R*)-phenylglycinamide (**1.7**) and benzaldehyde (**1.8**). Subsequent reaction with allylzinc (**1.10**) gives compound **1.11** in a highly stereoselective manner with a diastereomeric ratio (dr) of 99:1. The authors propose a mechanism which is controlled by chelation of zinc to both the amide oxygen and the imine nitrogen. The non-reductive removal of the chiral auxiliary from **1.11** is performed in three steps to give alkene **1.14** which can then be further functionalized. Although the synthesis is quite lengthy, the overall yield is 65%, which is better than a resolution without racemization (§1.4.3).

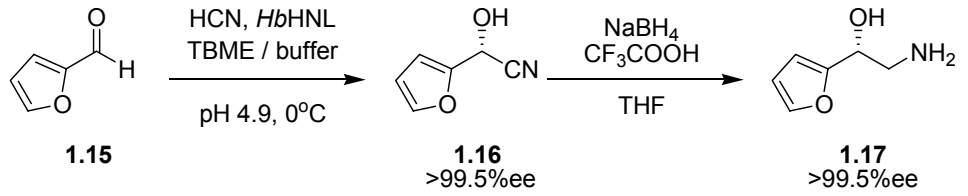
1.4.2.2 Biocatalysis

The synthesis of optically pure compounds can also be achieved by biocatalysis, using enzymes.[38] In the example below, optically pure amino alcohols are prepared by an enzyme catalyzed reaction in which an optically pure cyanohydrin (**1.16**) is synthesized from a prochiral aldehyde (**1.15**) which is then reduced to amino alcohol **1.17** as shown in Scheme 1.13.[45] Although the process delivers amino alcohol **1.17** in high enantiomeric excesses (*ee*), the process requires liquid hydrogen cyanide, which is extremely toxic.



Scheme 1.12 *Synthesis of a optically pure amine which can be further functionalized.*

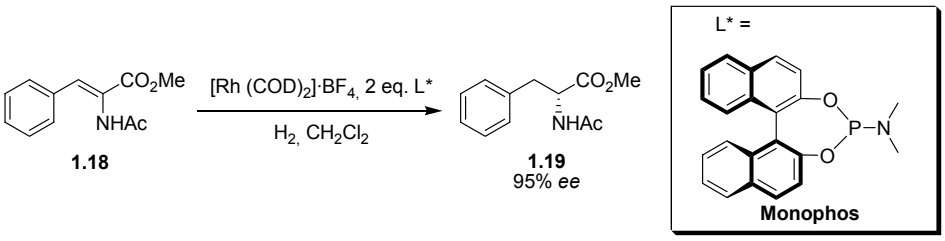
Enzymes may be altered by genetic modifications and thereby improving the activities, stabilities and/or enantioselectivities of the enzyme.[46] Although enzymes can deliver high *ee*'s with high yields, they are notoriously difficult to remove from the reaction mixture.[47] However, by immobilization on a carrier[48] or genetic modification[49] the removal of enzymes can be made easier.



Scheme 1.13 *Synthesis of optically pure (R)-2-amino-1-(2-furyl)ethanol.*

1.4.2.3 Chemocatalysis

Using optically pure catalysts, prochiral substrates can often be converted to chiral compounds with high selectivity. These catalysts can be completely organic in nature (for example proline[50]) or contain a transition metal which coordinates to an organic chiral ligand. An example of the latter is given in Scheme 1.14.



Scheme 1.14 *Asymmetric catalytic homogeneous hydrogenation.*

The *Z*-double bond in compound **1.18** is reduced with hydrogen and a rhodium/Monophos based catalyst to give compound **1.19** in high *ee*.[51] Monophos based catalysts have been used by DSM for large scale synthesis of pharmaceutical intermediates.[52] The downside of metal-based catalysts is that the transition metals used are often very expensive, toxic and often difficult to remove.[53]

1.4.3 Resolution of Racemates[54]

Often, only racemic or partly enriched materials[55] are available and both enantiomers need to be separated. Several techniques are available to chemists and these are described below.

1.4.3.1 Chromatographic Resolution

Chromatographic separation of compounds is a method based on the different affinities of a compound to the stationary phase (column material) and the mobile phase (eluent).[56] This means that a racemate can only be separated on a chiral column or on an achiral column with a chiral eluent.[57] The latter however, utilizes relatively expensive optically pure solvents or additives and hence, not used much.

The bulk of chiral separations with column chromatography is performed on chiral columns with achiral eluents. These columns are available with a wide diversity of functionalized chiral groups *e.g.* cyclodextrins,[58] alkaloids[59] and antibiotics.[60]

For preparative separation of racemates, simulated moving bed chromatography (SMBC) is an attractive method. SMBC utilizes a number of identical columns and valves which simulates an indefinitely long column. By switching the columns one achieves an effect that the enantiomer that has the least affinity with the stationary phase moves in the opposite direction of the stationary phase, hence the name: simulated moving bed.[61] This is exemplified by the cartoon in Figure 1.4.

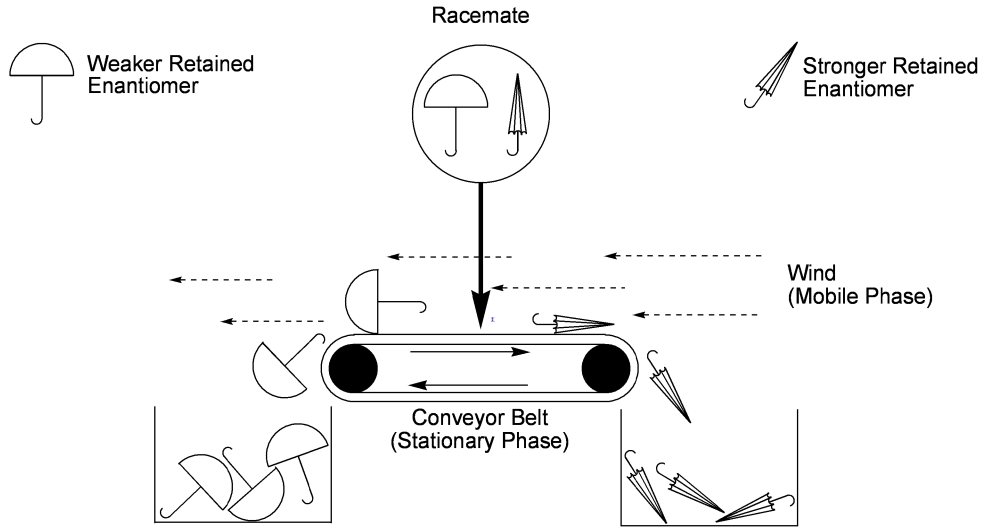


Figure 1.4 *Visualization of the SMBC principle.* [62]

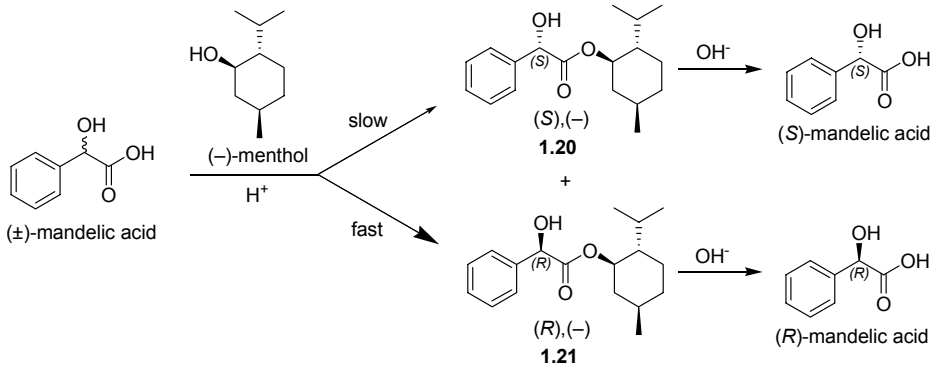
Separated enantiomers are removed *via* the valves and racemate is also introduced *via* these valves.[46] This continuous process is successfully used to separate a racemate on ton-scale and otherwise difficult to separate racemates.[63] The investment costs for SMBC approach are high and it's used almost exclusively in industrial settings if other low-cost solutions fail.

1.4.3.2 Kinetic Resolution

If an enzyme[64] or other chiral catalyst[65] reacts selectively with only one enantiomer, for instance, acylating only the (*R*)-enantiomer, the product and the unreacted (*S*)-enantiomer can be conveniently separated by standard laboratory procedures like chromatography, crystallization, distillation or extraction. In 1858, Pasteur observed that a mold consumed only one enantiomer of ammonium tartrate, as described in §1.1.

Kinetic resolution can also be performed with optically pure chemicals in which one enantiomer of the racemate reacts faster with the optically pure chemical as depicted in Scheme 1.15 for the reaction of racemic mandelic acid with optically pure menthol which was discovered in 1899.[66] Since (*R*)-mandelic acid reacts faster with (–)-menthol than (*S*)-mandelic acid, ester **1.21** is formed faster than ester **1.20** and the former can be isolated by extraction. When this particular reaction is allowed to react to completion, racemic mandelic acid will again be isolated after saponification. It is therefore necessary to isolate

the compounds when the optimal ratio of product and unreacted material is reached. This can also be true for enzyme catalyzed kinetic resolutions, which are not always specific for one enantiomer. Kinetic resolutions will give a maximum yield of 50% and 100% *ee*.



Scheme 1.15 *Kinetic resolution of racemic mandelic acid with menthol.*

When one enantiomer of the racemate is consumed by an enzyme or other chiral catalyst while the remaining unreacted material is racemized, one speaks of Dynamic Kinetic Resolution (DKR).[42,67] This process can in principle give 100% yield and 100% *ee*. An example of DKR is given in Scheme 1.16.[68]

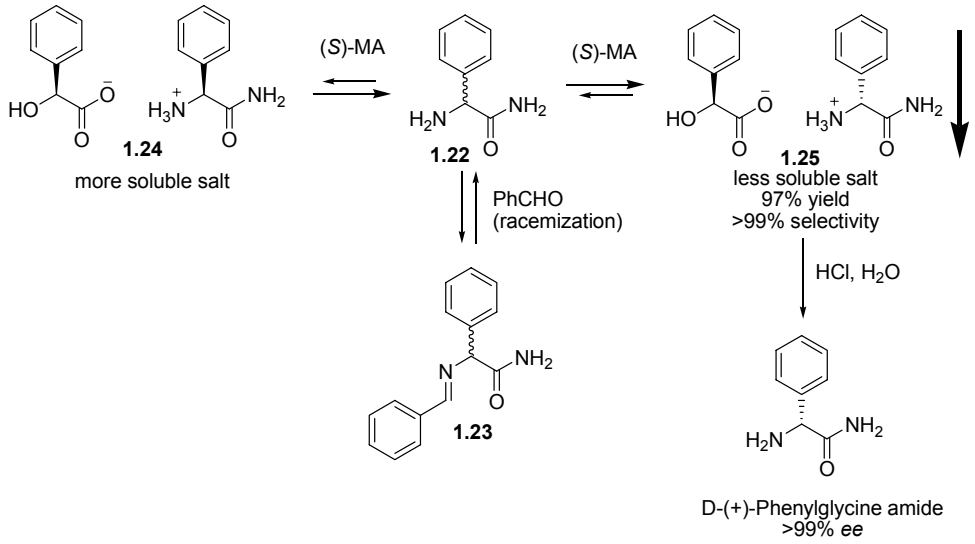
When racemic phenylglycine amide (**1.22**) is mixed with one equivalent of (*S*)-mandelic acid (MA), a diastereomeric salt is formed and the less soluble diastereomer **1.25** crystallizes.[69] The remaining solution is racemized with a small amount of benzaldehyde to produce an easily racemized Schiff base **1.23** which is in equilibrium with **1.22** which makes the solution again racemic in **1.22**. By allowing the material to crystallize under racemizing conditions, a 97% yield is obtained with a *de* of >99%. After liberation of the salt, D-(+)-phenylglycine amide can be isolated with >99% *ee*. D-(+)-phenylglycine amide is used in the synthesis of a semi-synthetic antibiotic, Cephalexin.[70]

1.4.3.3 Direct Crystallization[38,71]

A compound is a conglomerate if both enantiomers of a racemate crystallize in two separate mirror imaged crystals.[72] Because of this behavior, conglomerates can in principle be separated manually.[73] This behavior is essential for resolution by direct crystallization. However, only roughly 10% of all racemates crystallize as conglomerates.[71]

Two different techniques can be applied in preferential crystallization: simultaneous crystallization and resolution by entrainment. In the former technique, a supersaturated

solution of a racemic conglomerate is allowed to crystallize in two separate crystallizers simultaneously. Each crystallizer contains seeds of the respective enantiomer. Before the other enantiomer starts to crystallize, the mixture is filtered and returned to a make-up vessel to restore the concentration to the former concentration. This procedure is then repeated.



Scheme 1.16 *DKR of phenylglycine amide.*

A graphic representation of resolution by entrainment (or preferential crystallization) is shown in Figure 1.5 and explained below.

1. The resolution starts with a racemic mixture in a solvent which is artificially biased in the (*S*)-enantiomer and heated to dissolution.

2. The mixture is cooled to supersaturation of both enantiomers. The enantiomer with the highest concentration, (*S*), will start to crystallize first and its concentration will return to the saturation point. The (*S*)-enantiomer is collected by filtration before the supersaturated (*R*)-enantiomer starts to crystallize.

3. Racemate, with the same weight as the (*S*)-enantiomer which was collected in step 2, is added and the mixture is heated to dissolution. Note that the resulting situation is the same but mirror imaged to the one resulting from step 1.

4. Again, the mixture is cooled to supersaturation of both enantiomers and now the (*R*)-enantiomer crystallizes. The amount of crystals collected after filtration is the same as the amount of racemate added in the previous step.
5. Subsequently, racemate is added and the mixture is heated to dissolution, resulting in the same situation as was obtained after step 1.

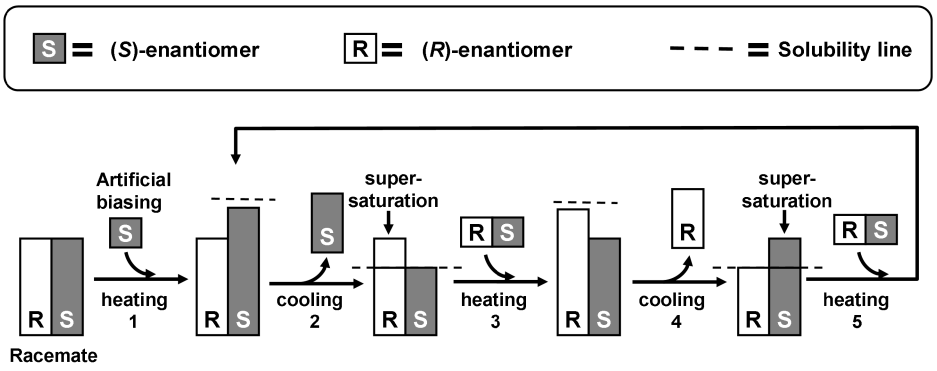


Figure 1.5 *Representation of resolution by entrainment.*

The pure enantiomers do not always form spontaneously or fast enough. In such a case, enantiopure crystals may be added as a template for the enantiomers of the same handedness to crystallize on. This methodology is known as 'seeding'.

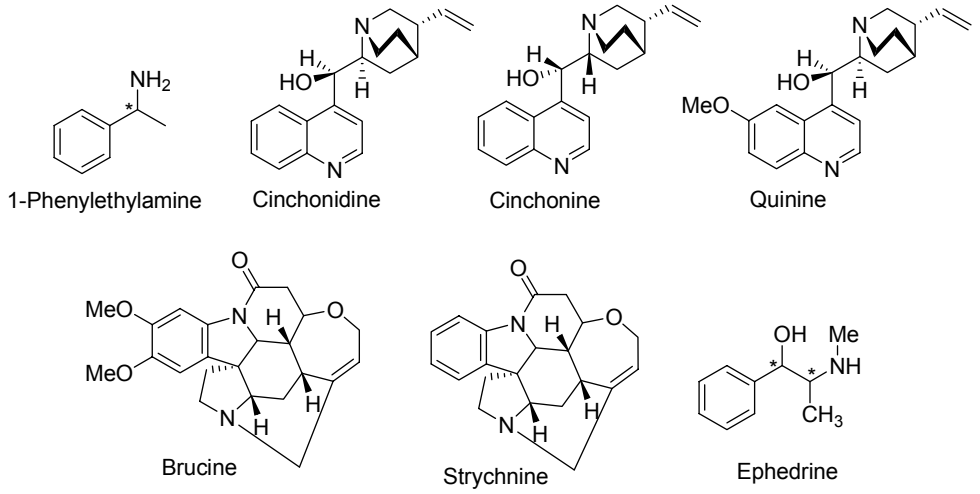
Since the mother liquor is reused, impurities can accumulate in the mother liquor and disturb the crystallization process. This limits the number of cycles that can be performed before the mother liquor needs to be replaced.

1.4.3.4 Resolution by Diastereomeric Salt Formation[71,74]

If a racemate has an acid or base moiety, a salt can be formed with a basic or acidic enantio pure compound (resolving agent), respectively. The diastereomeric salts formed have different physical properties and can thus be separated. The easiest way to do this is by crystallization providing that the least soluble diastereomer crystallizes out of the solution selectively. By crystallizing the salts from a proper solvent, high *de*'s can often be found in the precipitated salts. If necessary, the isolated salt can then be recrystallized (if needed, several times) to high *de* if the *de* of the first salt is too low. The pure enantiomer can be liberated by addition of acid or base. This procedure is known as resolution by diastereomeric salt formation (or classical resolution) and is the most used procedure for isolation of enantiopure compounds.[38] Compounds that do not have an acid or base moiety

like alcohols can be separated by inclusion resolution with an appropriate complexing agent, which will form a diastereomeric complex or be reacted with an enantio pure compound producing covalently bound diastereomers which can be separated by the difference of their physical properties.[75]

Some resolving agents exhibit a larger solubility difference than others or might resist salt or crystal formation with a certain racemate. Thus it is necessary to screen several resolving agents to find a good combination of racemate and resolving agent. Several commonly used basic resolving agents are shown in Scheme 1.17.



Scheme 1.17 *Most frequently used basic resolving agents.*[74]

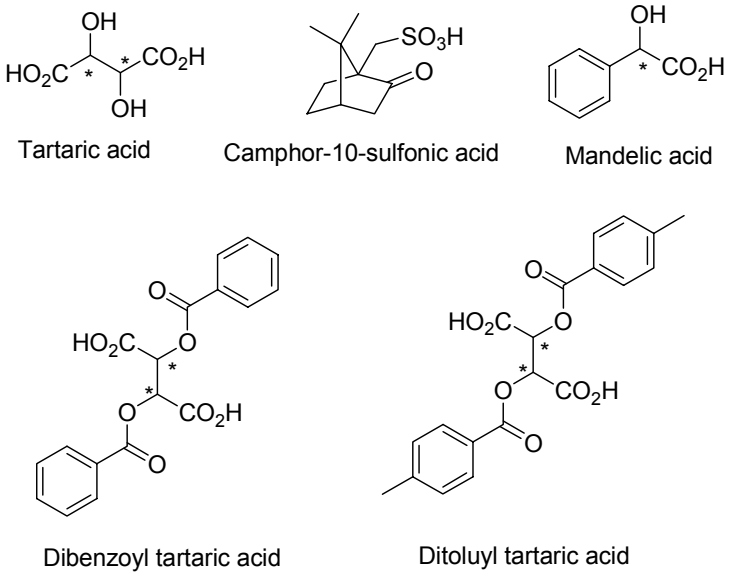
Although brucine and strychnine have been much used resolving agents on lab scale in the past, today these are not used often due to their toxicity. Common acidic resolving agents are given in Scheme 1.18. Although amino acids are cheap chiral compounds with a large diversity, they are not commonly used as resolving agents. The zwitterionic behavior inhibits salt formation with relatively weak acids or bases.

These basic and acidic resolving agents shown in Scheme 1.17 and Scheme 1.18 are in general not expensive and can be isolated from natural sources or produced synthetically.

Solvents also have an impact on the outcome of the resolution and efficiency. Solvate formation, hydrate formation, growth inhibition and polymorphism are effects influenced by solvents.[76,77] As a rule of thumb, the solubility of small organic compounds in organic solvents will be roughly doubled for every 20°C increase in temperature.[78] Not every

compound/solvent combination will show this 'ideal' behavior, of course. To obtain a high yield at room temperature, high boiling solvents can be used *e.g.* water, 2-propanol, 2-butanone and toluene.

The temperature of the resolution process can have an influence on the outcome of the resolution also. (De)hydration, (de)solvation, polymorphism and temperature dependent solubility will be different for each diastereomeric salt and can even invert the outcome of the resolution.[77] As will be discussed in §1.5, small amounts of structural related compounds can drastically influence the outcome of the resolution.



Scheme 1.18 *Most frequently used acidic resolving agents.*[74]

It is estimated that the chance of success of a typical resolution experiment by diastereomeric salt formation is only a disappointing 20–30%.[42,71]

1.5 Dutch Resolution[79]

In 1998, a group of Dutch researchers tried to make the screening process for resolution by diastereomeric salt formation faster by adding stoichiometric amounts of several resolving agents as a mixture to the racemate. They soon discovered that random combinations of resolving agents did not give good results. Only very insoluble salts can be selected using this method. However, when the researchers used structurally related and homochiral[80]

resolving agents (family members) the outcome was different. Often, the combination of these resolving agents gave higher *ee*'s than with each of these resolving agents independently. Moreover, the chance of obtaining solid salts with significant diastereomeric excesses was increased from 20–30% to 90–95%.[71,81]

The reasons for the high success rate of Dutch Resolution are believed to be:

- **Choice of the best resolving agent/racemate combination.** With three resolving agents and one racemate, the least soluble combination of diastereomers will start to crystallize, hereby reducing the chance of encountering a salt that will not form crystalline salts.
- **Solid solution behavior of the family members.** Solid solution behavior means that the crystal lattice does not distinguish much between the several family members that can fit inside the crystal lattice of the salt that is precipitating. Hence, the composition of the crystal depends largely on the composition of the surrounding solution. A solid solution is less soluble than each separate salt and thus will produce crystals in stead of a clear solution. The isolated crystals usually show a non-stoichiometric ratio in the resolving agents although these were added stoichiometric.
- **Peachey-Pope type resolution**. In a Peachey-Pope resolution, instead of one equivalent resolving agent, one-half equivalent of resolving agent is used and supplemented with one-half equivalent of an achiral (low-cost) acid or base like hydrochloric acid or sodium hydroxide to make the system neutral. The achiral supplement should give very soluble salts with the racemate so these will not crystallize and ruin the resolution. The less soluble salt will start to crystallize and will consume most of the resolving agent thus leaving only small amounts of resolving agent for the more soluble diastereomer which, in an ideal case, will not crystallize. The same principle applies to Dutch resolution. When three resolving agents are used, usually, one of these is incorporated the most in the least soluble diastereomer. The concentration of this resolving agent in the solution is subsequently lowered and thus the more soluble diastereomer of this resolving agent cannot crystallize.
- **Nucleation inhibition.** When a family of three resolving agents is used, sometimes only two are incorporated in the crystal lattice. It was found that when a resolving agent is not incorporated in the crystal lattice, this does not mean this compound can be left out without altering the outcome of the resolution. Small amounts of compounds that resemble the resolving agent (or racemate) can inhibit the nucleation of the more soluble (unwanted) diastereomer.

Nucleation inhibition has been further investigated and was found to be very effective for the improvement of a classic resolution by diastereomeric salt formation. By addition of

only a few percent of a structurally related additive, significant improvements were found. This type of resolution was named Second Generation Dutch Resolution.[82,44a]

Furthermore, Reverse Dutch Resolution has been reported[83] where family members of a racemate have been resolved simultaneously with one resolving agent.

1.6 Aim and Outline of This Thesis

The focus of this thesis is the improvements of resolutions of racemates by crystallization and a better understanding of the role of additives/impurities. Furthermore, application of grinding of crystals in resolutions will be addressed. A better understanding will be useful in laboratory scale and industrial scale.

Chapter 2 covers the theoretical aspects of resolutions by crystallization including phase diagrams, crystal growth, nucleation and the inhibition of nucleation.

Chapter 3 deals with the resolution by diastereomeric salt formation and the improvements thereon with additives that can act as nucleation inhibitors and growth inhibitors. For the first time, >95% *de* was achieved by addition of only 1% additive in a diastereomeric salt formation. On a relative large scale, the resolution was performed successfully even after seeding with the more soluble diastereomeric salt. The resolution was even further improved by the use of half-equivalent of resolving agent and grinding of the formed crystals.

Chapter 4 is concerned with the effect of additives on resolutions by diastereomeric salt formation, which do not show great improvement of the resolution. Furthermore, a classical resolution was improved by abrasive grinding of the crystals in the presence of an additive. Also, the crystallization of optically pure phencyphos in the presence of other racemic cyclic phosphoric acid shows a stereoselective incorporation of the latter in high yields.

In Chapter 5 a new concept of deracemization to optical purity by abrasive grinding is given. The stereoselective incorporation of other amino acid derivatives in the precipitating amino acid derivative poses a new pathway to homochirality in nature.

In Chapter 6 the resolution of phencyphos is described for which the original resolving agent was no longer available for a low price. A new method was found and has been adopted on large scale to yield 2.5 moles of each enantiomer each day.

Chapter 7 of this thesis is dedicated to some ideas which might be researched further in the future.

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