Resolutions of Racemates by Crystallization

Additives and Attrition

Michel S. Leeman

The cover picture shows backlit quartz form Tequisquiapan, Queretaro, Mexico, downloaded from: http://www.sxc.hu/photo/702163.

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Resolutions of Racemates by Crystallization

Additives and Attrition

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The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny ...'

Isaac Asimov (1920 - 1992)

Opgedragen aan Anita

Contents

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'.

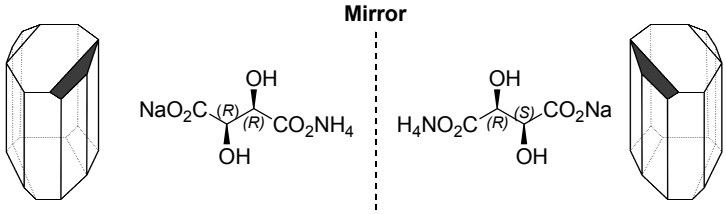
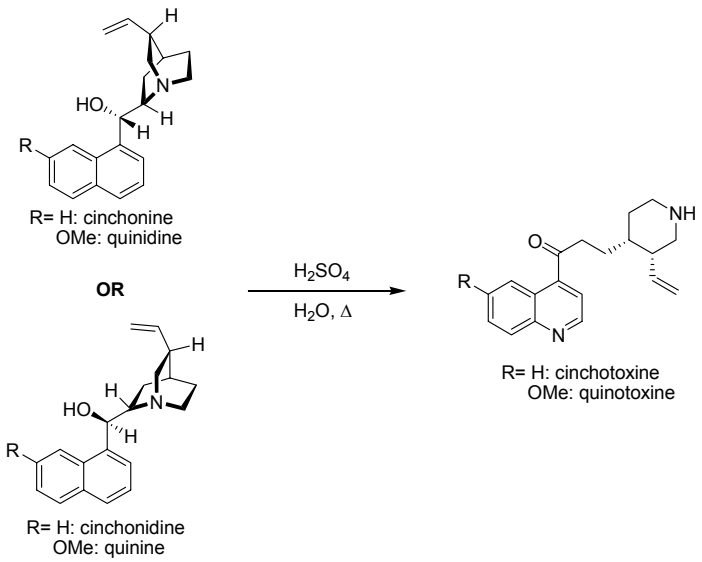


Figure 1.2 *The mirror imaged crystals and enantiomers of sodium ammonium tartrate.*

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.

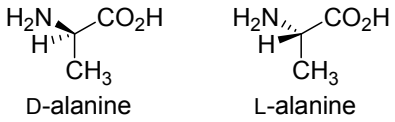


Scheme 1.1 *Synthesis of quinotoxine and cinchotoxine.*

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,

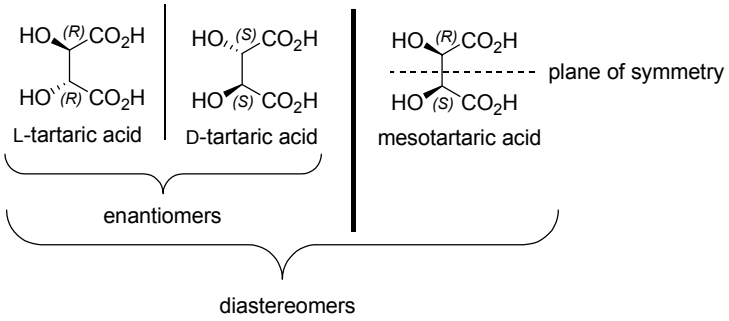
in 1874, Jacobus Henricus van 't Hoff[18] (the first winner of the Nobel prize for chemistry, however, not for the tetraedric carbon[19]) and Joseph Achille Le Bel,[20] nearly simultaneous proposed the theory of the position of atoms in space and the tetrahedral carbon. A carbon atom, surrounded by four 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.

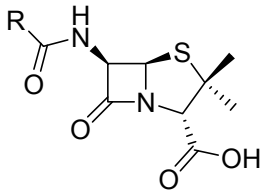


Scheme 1.3 *D- and L-tartaric acid, enantiomers. Mesotartaric acid is a diastereomer of these compounds.*

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]



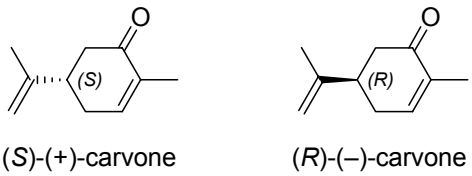
Scheme 1.4 *Penicillin core structure.*

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

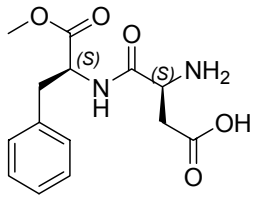
Scheme 1.5 *The enantiomers of limonene.*

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]



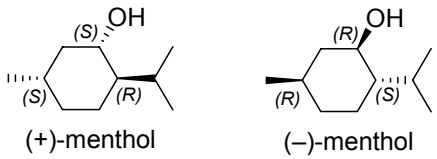
Scheme 1.6 *The enantiomers of carvone.*

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]

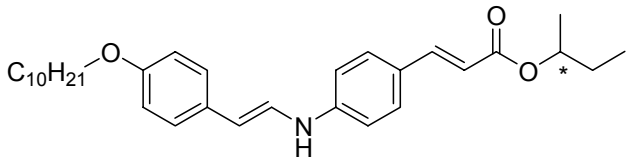


Scheme 1.7 *Aspartame, an artificial sweetener.*

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]

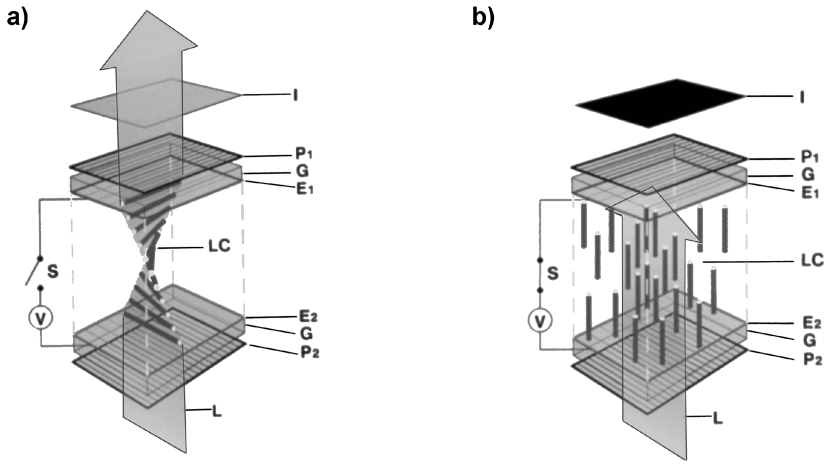


Figure 1.3 *Schematic projection of an LCD pixel.*[33]

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.*

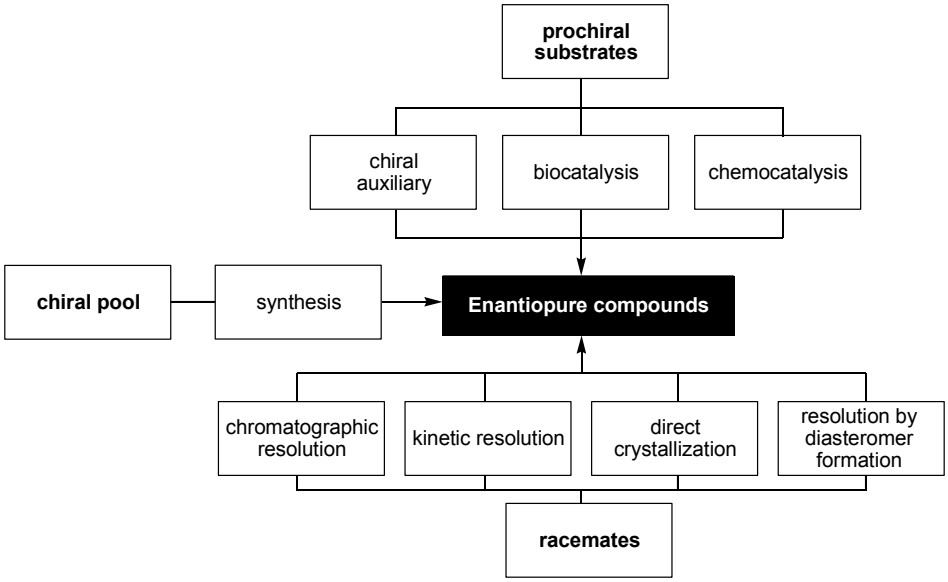
Wherein *de* is the diastereomeric excess of the first salt and *Y* is the yield, both ranging from 0 to 1. In a resolution without racemization, the maximum yield for a single diastereomer is thus 0.5. The factor 2 adjusts the S-factor to a value between 0 and 1.

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.



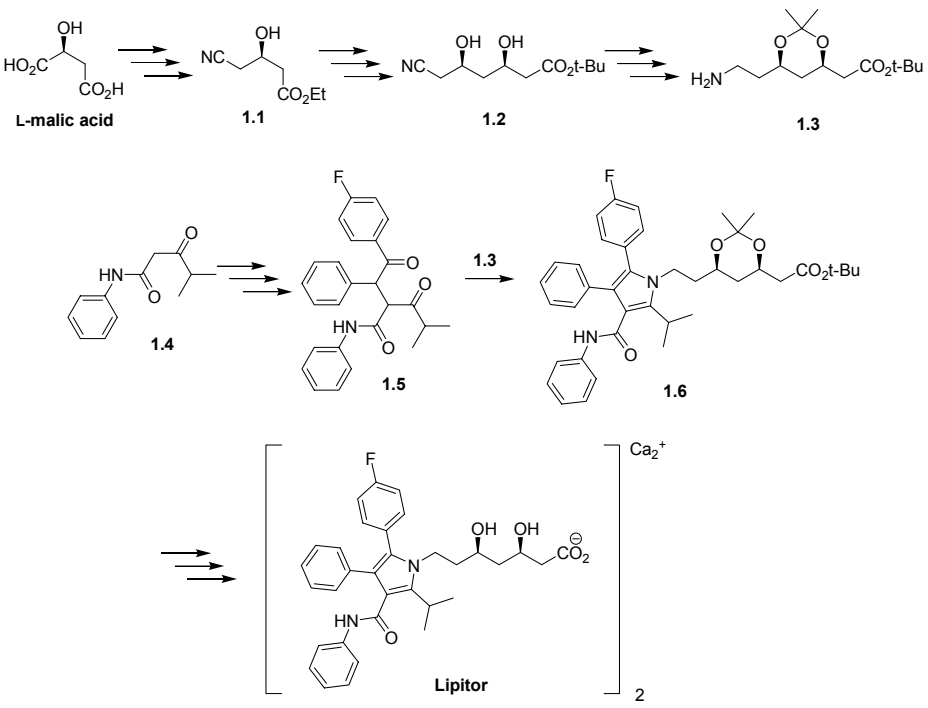
Scheme 1.10 *Routes to enantiopure compounds.*

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]

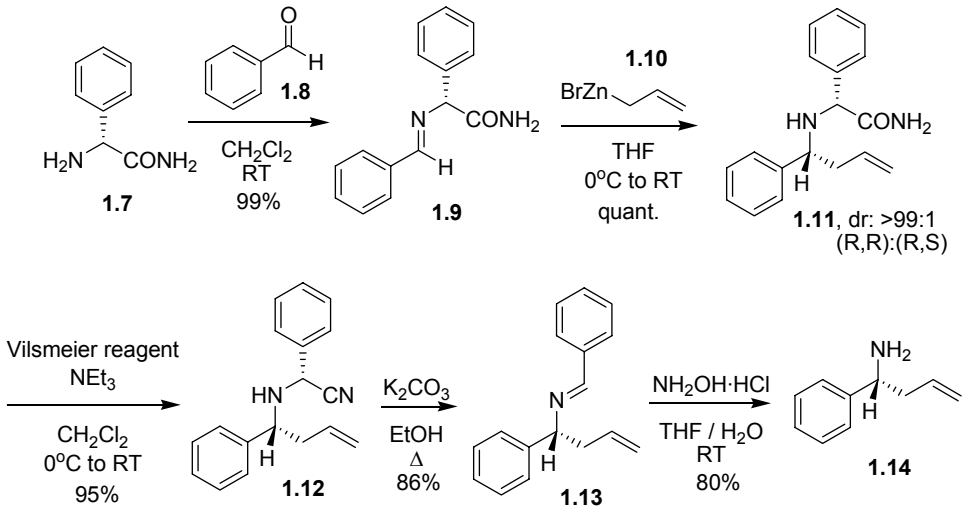
1.4.2.1 Chiral Auxiliary[44]

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).

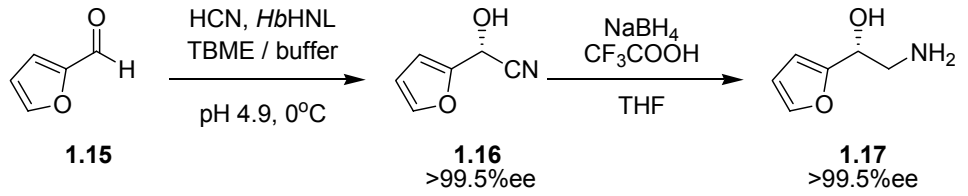
1.4.2.2 Biocatalysis

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.*

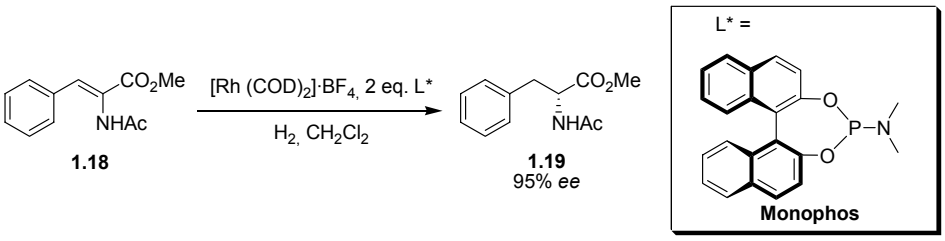
Enzymes may be altered by genetic modifications and thereby improving the activities, stabilities and/or enantioselectivities of the enzyme.[46] Although enzymes can deliver high *ee*'s with high yields, they are notoriously difficult to remove from the reaction mixture.[47] However, by immobilization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

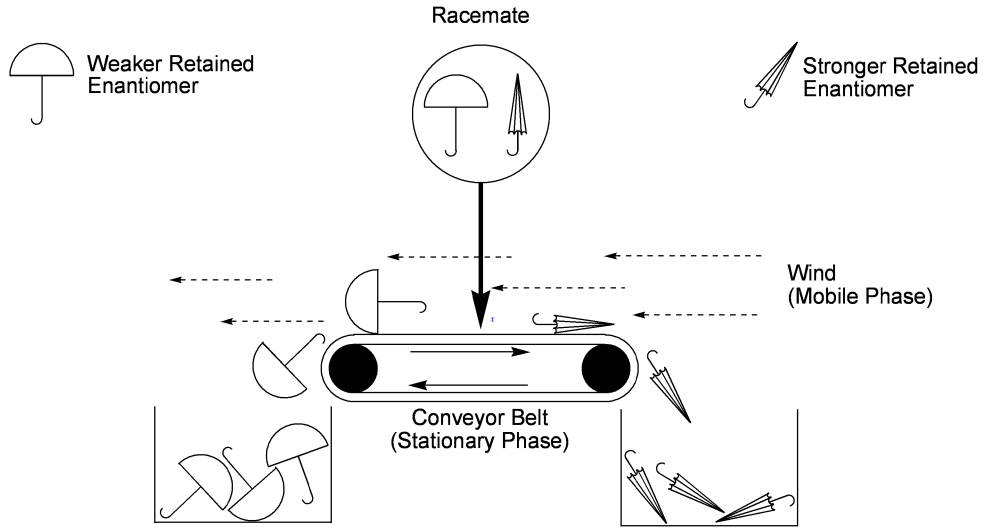


Figure 1.4 *Visualization of the SMBC principle.* [62]

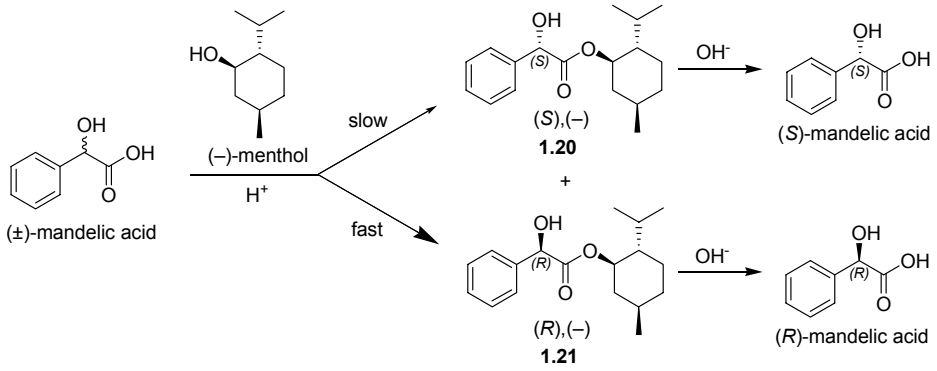
Separated enantiomers are removed *via* the valves and racemate is also introduced *via* these valves.[46] This continuous process is successfully used to separate a racemate on ton-scale and otherwise difficult to separate racemates.[63] The investment costs for SMBC approach are high and it's used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

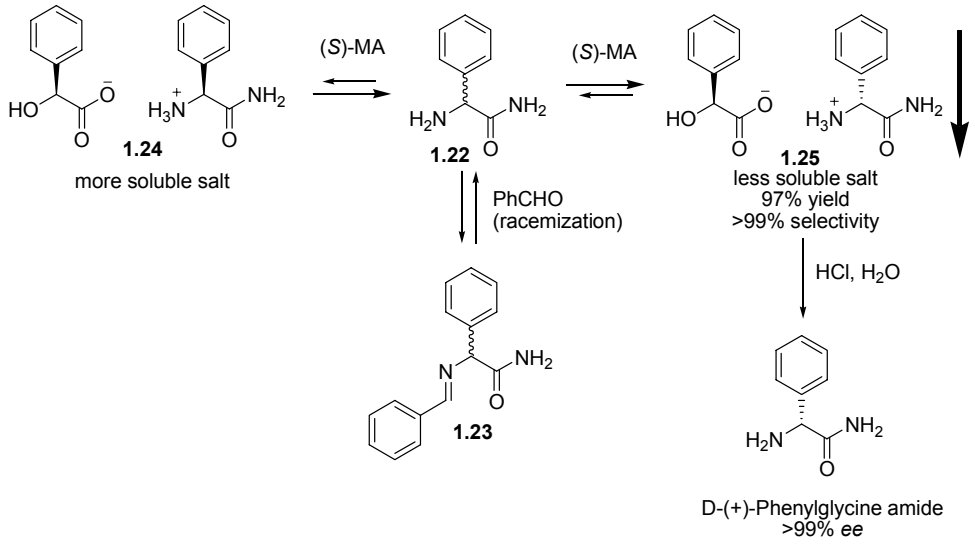
When racemic phenylglycine amide (**1.22**) is mixed with one equivalent of (*S*)-mandelic acid (MA), a diastereomeric salt is formed and the less soluble diastereomer **1.25** crystallizes.[69] The remaining solution is racemized with a small amount of benzaldehyde to produce an easily racemized Schiff 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.

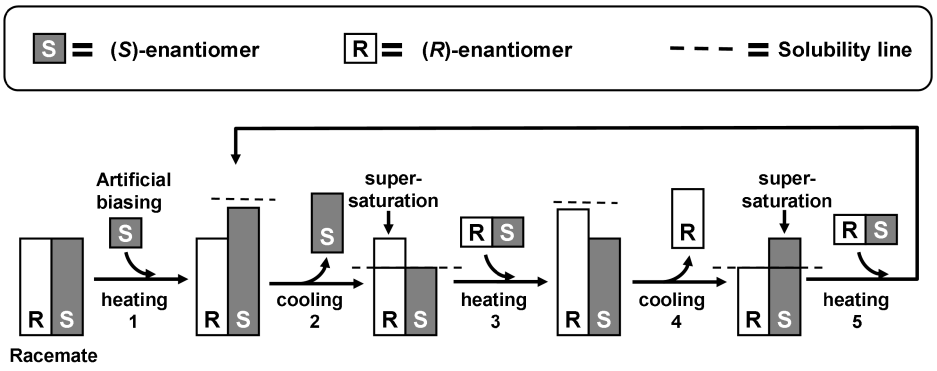


Figure 1.5 *Representation of resolution by entrainment.*

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'.

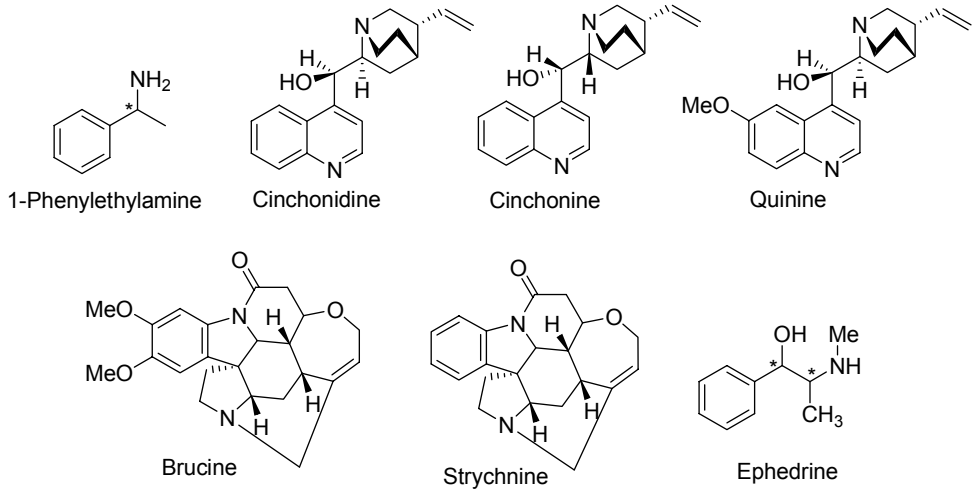
Since the mother liquor is reused, impurities can accumulate in the mother liquor and disturb the crystallization process. This limits the number of cycles that can be performed before the mother liquor needs to be replaced.

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]

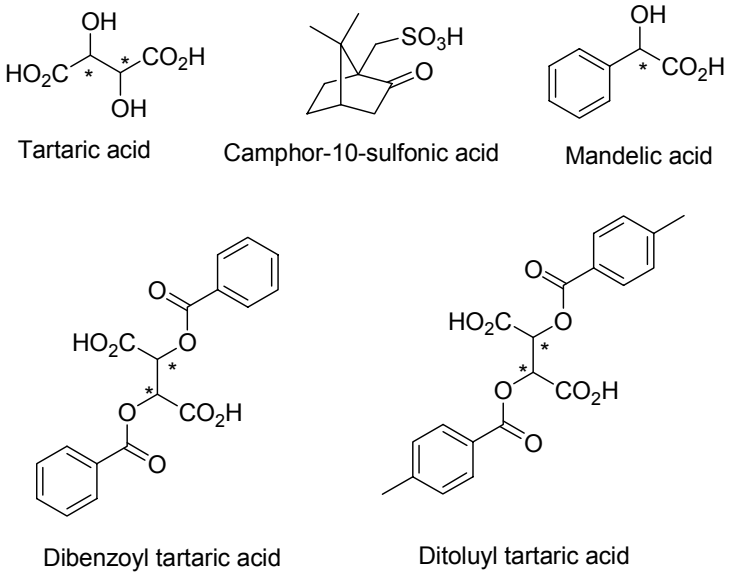
Although brucine and strychnine have been much used resolving agents on lab scale in the past, today these are not used often due to their toxicity. Common acidic resolving agents are given in Scheme 1.18. Although amino acids are cheap chiral compounds with a large diversity, they are not commonly used 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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Chapter 2

Resolution by Crystallization

In this chapter the theoretical aspects of resolutions by crystallization are discussed. Nucleation, crystal growth, nucleation inhibition and polymorphism are considered. Furthermore, several resolution-by-crystallization techniques are highlighted and explained by means of ternary phase diagrams. A proper understanding of the crystallization process under investigation enhances the chance of success and can save time and money.

2.1 Introduction[1]

Lack of understanding in a resolution by crystallization can result in low yields, lengthy recrystallizations, frustration and even isolation of the undesired enantiomer. Although prediction of the outcome of a crystallization process by computer has been performed with increasing success,[2] computing times are still longer (and more expensive) than performing the actual experiment in a laboratory.

2.2 Crystal Growth from Solution

Crystallization from solution starts with nucleation. Nucleation can be divided in two categories: primary and secondary. Primary nucleation is the process in which crystals form spontaneously from a supersaturated solution. On the other hand, one speaks of secondary nucleation when a supersaturated medium is put in contact with an appropriate surface on which the supersaturated compound can crystallize. Examples are seeding, twinning (crystallization of the supersaturated compound on the crystal surface of another compound) and grinding of existing crystals of the supersaturated compound. John Mullin,[4] a highly recognized expert, remarks that the difference between primary and secondary nucleation is sometimes not made clear in the literature. We feel, however, that the definition given here is clear.

2.2.1 Primary Nucleation

To focus on primary nucleation, it is difficult to observe the actual nucleation process since the nuclei are too small to observe with any instrument. When a solution of a compound at a certain temperature (Figure 2.1a) is cooled, a supersaturated solution forms. It has been suggested that very small aggregates, each consisting of a few molecules, form in the supersaturated solution as is depicted in Figure 2.1b.[3]

The small aggregates then grow in the solution or dissolve again as is shown in Figure 2.1c. When the aggregates/nuclei attain a critical size, they can grow into actual crystals. This critical size is determined by the relationship between the volume energy term: ΔG_V of a crystal *versus* the surface area energy term: ΔG_S of this crystal. For a spherical nucleus the total free energy difference ΔG can be calculated with Equation 2.1.[4a]

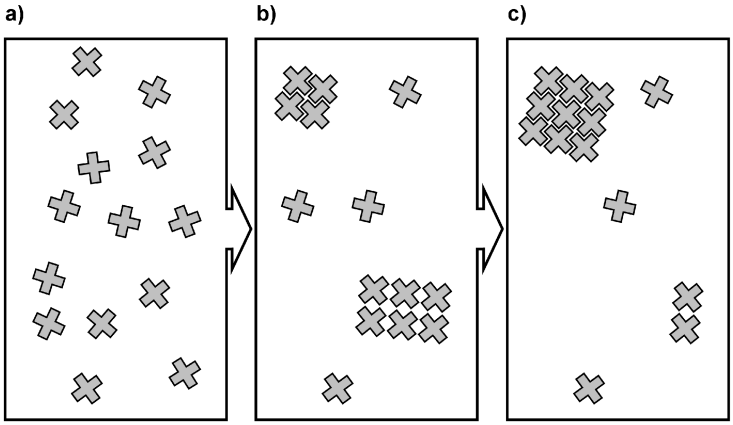


Figure 2.1 *Crystallization from a supersaturated solution. The X-shaped figures represent molecules that can form aggregates.*

Where ΔG is the overall free energy difference, r is the radius of the nucleus, γ is the interfacial tension (surface energy) and ΔG_v is the free energy difference of the transformation per unit volume.

$$\Delta G = \Delta G_S + \Delta G_V = 4\pi r^2 \gamma + \frac{4}{3}\pi r^3 \Delta G_v$$

Equation 2.1 *Calculation of free energy from the radius of a spherical nucleus.*

The graph in Figure 2.2 depicts the development of the free energy difference of the surface term, the volume term and the total free energy difference as a function of the radius of the nucleus.

When the crystal has a radius smaller than r_c, the critical radius, it is energetically more favorable for the nucleus to re-dissolve, as can be told from the graph (line: - - -). For larger sizes than r_c, growth of the nucleus into a crystal is more favorable. Of course, if the nucleus shape resembles the final crystal shape, Equation 2.1 cannot be entirely valid because crystals are never perfect spheres. However, for explanation purposes this picture is useful.

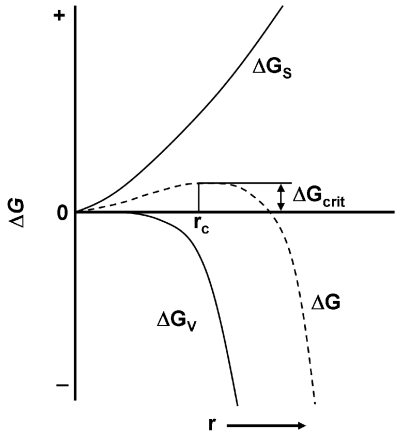


Figure 2.2 *Free energy difference diagram for nucleation of a spherical nucleus with radius r.*

2.2.2 Polymorphism[5]

Often (if not always), aggregates with different molecule packings are formed simultaneously. These aggregates will grow into different crystals, known as polymorphs. Polymorphism is defined as the ability of a compound to exist in more than one crystal form. This means that the internal arrangement of the atoms is different although the chemical composition is the same (*e.g.* solvates, as explained in §2.3.4, are not polymorphs).

Polymorphism is caused, among other things, by the molecule's ability to change conformation, display hydrogen bonding and π-π interactions. The polymorph with the lowest solubility is by definition the most stable. This, however, can be temperature dependent as is shown in Figure 2.3 in which the solubility curves of three polymorphs of a compound are given. Form **III** is the most soluble and thus the least stable polymorph at every temperature and it thus has a monotropic relation with forms **I** and **II**. Form **I** is the most stable polymorph above T_a but below T_a form **II** is the most stable form. This means that form **I** has a so called enantiotropic relation with form **II**.

Fast cooling, as indicated with the horizontal arrow, will produce all forms **I-III**.[6] The formation of a less stable crystal is known as Ostwald's rule of stages: *"An unstable system does not necessarily transform into the most stable state, but into one which closely*

resembles its own, resulting in the smallest loss of energy".[7] This is exemplified in Figure 2.4 where form **A** is the most stable. However, when fast cooling is applied, form **B** will represent the majority of crystals in this kinetically driven crystallization. There are many exceptions to the rule of stages and thus it should not be considered a law.[8]

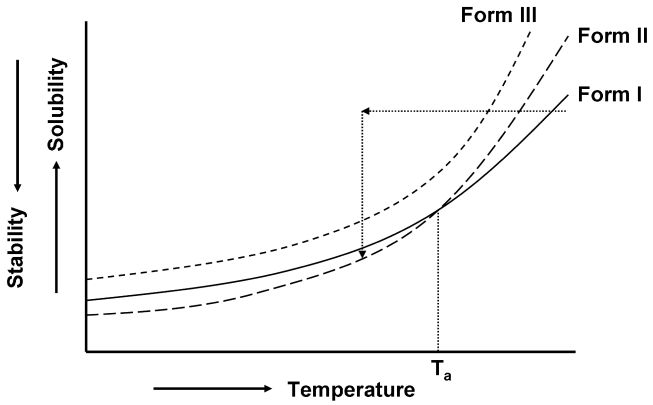


Figure 2.3 *The theoretical dependence of solubility and stability as a function of temperature for a compound that can exist in three polymorphic forms.*

Polymorphism is very common in organic compounds. To quote Walter McCrone: *"Those who study polymorphism are rapidly reaching the conclusion that ALL compounds, organic or inorganic, can crystallize in different forms or polymorphs. In fact, the more diligently any system is studied, the larger the number of polymorphs discovered."* [9]

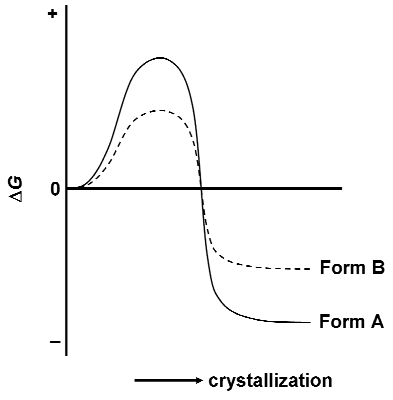


Figure 2.4 *Thermodynamic (—) and kinetically (---) driven crystallization.*

Very fast cooling might lead to amorphous material or even oil formation. In amorphous material the solids show a random orientation of the compound in contrast with crystals which show a very well defined repetitive packing of molecules.

By slurrying a mixture of polymorphs in a saturated solution, the most stable polymorph at a temperature below T_a (form **II** in this case) will eventually be the only polymorph left (vertical arrow in Figure 2.3). The most stable polymorph will consume the other polymorphs *via* a dissolution and crystal growth process known as 'ageing'.[10] However, if the formation of this polymorph is inhibited due to small impurities (nucleation inhibition, see also §2.2.3) the second most stable polymorph will form. However, by seeding with the most stable form or removal of the impurity, the whole system will produce the most stable polymorph. Also a change of solvents can produce different polymorphs (kinetically).[11] A good example is the crystallization of the simplest amino acid, glycine. This can crystallize in three different forms by the interaction of the solvents on the growing aggregates (nucleation inhibition): α (from water), β (from EtOH or MeOH) and γ (from acids or bases).[6,12] Prolonged stirring of the unstable polymorph will eventually result in the most stable polymorph.

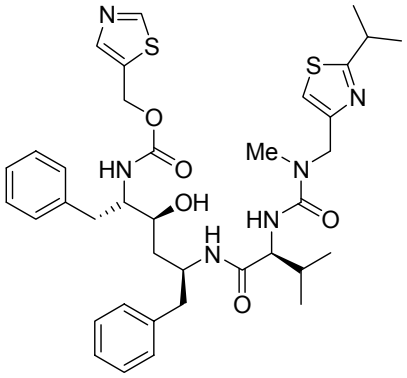
2.2.2.1 (Dis)appearing Polymorphs

A famous example of so called 'disappearing polymorphs' is the production by Abbott of Ritonavir, a HIV protease inhibitor,[13] as shown in Scheme 2.1. This drug was discovered in 1992, commercialized in 1996, and produced as polymorph **I**. However, in 1998 a second, more stable, form **II** appeared unexpectedly. The plant was unable to produce the less stable form **I** and the problem spread to other sites across the world. The biggest problem with this new polymorph was the 5-6 fold lower solubility. The new polymorph could now not provide high enough concentrations of the drug in the patients' body. A decomposition compound was suspected to be the cause of the appearance of form **II**. The workup procedure was altered to produce polymorph **I** again by dehydration of a hydrate.[14] The estimated costs: US$ 1 billion.

2.2.2.2 Polymorphism in Chocolate[15]

Polymorphic transitions in chocolate are one of the most famous examples. Chocolate (actually one of the ingredients: cocoa butter) is known to be able to crystallize in six different polymorphs: form **I** (least stable) to form **VI** (most stable). Form **V**, which does not melt at room temperature but does so in the mouth, is the desired form (melting range of 34.0°C–36.3°C)[16] and can be obtained by a process called 'tempering'. In this

methodology, the chocolate, which contains a mixture of polymorphs **I** to **V**,[17] is heated to ~29°C to melt the lower melting forms **I-IV** and the higher melting form **V** is retained. This mixture is then slowly cooled while it is well mixed to allow secondary nucleation to take place. The whole batch of chocolate will become small crystals of the desired form **V**. If the cooling is too fast also forms **I-IV** will form *via* primary nucleation, resulting in a poor (low melting) quality of chocolate.



Ritonavir

Scheme 2.1 *Ritonavir, a HIV protease inhibitor.*

Furthermore, when chocolate is not handled correctly or stored too long, the most stable form **VI** is formed. This polymorph is formed by partly melting and recrystallizing. The formation of this form **VI** often leads to a white dusty surface which is called 'bloom'. Bloom is the migration of cacao butter to the surface of the chocolate where it crystallizes. The formation of bloom can be prevented by addition of other fats to the cocoa butter, which inhibit the nucleation of form **IV**.[18]

2.2.3 Nucleation Inhibition

By the addition of small amounts of a compound that can partly incorporate in the crystal lattice, the nucleation of the crystallization can be prevented kinetically. In the cartoon in Figure 2.5a a solution of a compound (X-shaped) crystallizes in the presence of an additive (fish shaped). In Figure 2.5b two clusters of different polymorphs have formed. The additive blocks further growth of the top cluster by steric hindrance or crystal forming incompatibility (*e.g.* lack of hydrogen bonding capabilities). However, the bottom polymorph does not suffer from this. Thus, in Figure 2.5c the top polymorph has redissolved and the bottom polymorph can grow without disturbance.[19] This crystal does not distinguish much between the compound that it's made of and the additive and thus

forms a solid solution as discussed further in §2.3.2. Would the bottom polymorph have been inhibited also, this crystallization would have resulted in a clear solution instead of a suspension of crystals.

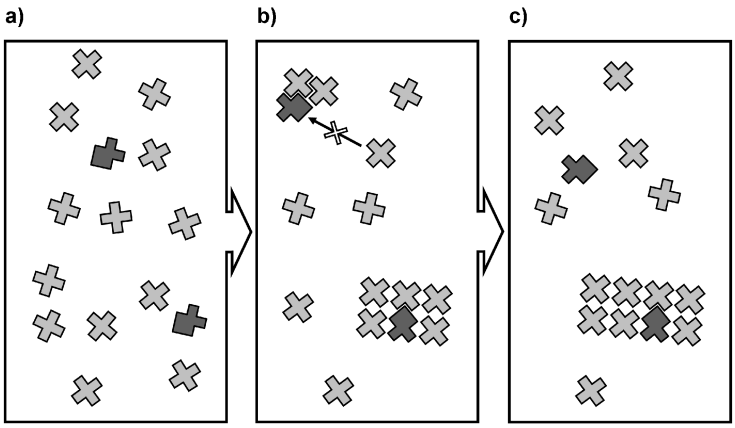
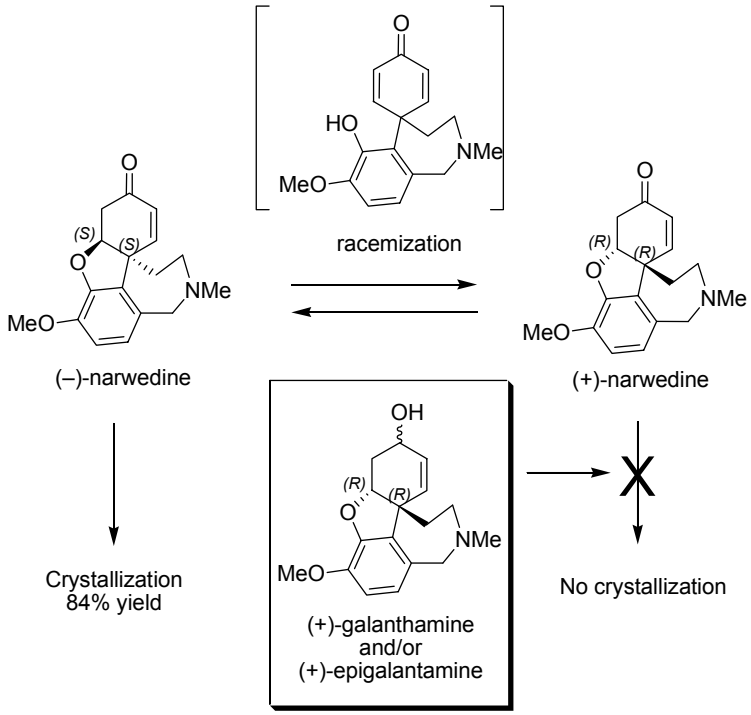


Figure 2.5 *The effect of a nucleation inhibitor. The X-shaped figures represent molecules and the fish-shaped figures represent a nucleation inhibitor.*

Nucleation inhibition was first shown in the Dynamic Kinetic Resolution (DKR) of (±)-narwedine. A relatively large amount of (+)-galanthamine and/or (+)-epigalanthamine (epimers at the alcohol carbon) had been used to prevent the crystallization of (+)-narwedine as shown in Scheme 2.2.[20] The product of this resolution, (–)-narwedine, was used in the total synthesis of (–)-galanthamine.

By racemizing the narwedine in the solution, Barton and Kirby were able to isolate more than 50% yield. It is clear from Scheme 2.2 that galanthamine with the same absolute configuration as narwedine inhibits the nucleation of the latter with the same absolute configuration and thus produces narwedine of the opposite configuration. This is in essence the "rule of reversal" as later postulated by Lahav based on the study of the crystallization behavior of amino acids.[21]

As discussed in §1.5, small amounts of structurally related additives are also used in a resolution by diastereomeric salt formation in the Second Generation Dutch Resolution protocol.[22]



Scheme 2.2 *Resolution of narwedine aided by the addition of (epi)galanthamine.*

2.2.4 Crystal Growth and Habit Modifiers

The effect of an additive, next to nucleation inhibition, can also be on the crystal growth process. Changing the growth of certain faces of a crystal without a change in crystal lattice (polymorph) results in a change of appearance (morphology) of a crystal: habit modification. Since the smallest faces of a crystal are the fastest growing ones, blockage of these faces will make them bigger. An example is the addition of a habit modifier to crystallizing sodium chloride in water as shown in Figure 2.6. The cubic crystal on the right is sodium chloride grown in pure water and the left crystal is sodium chloride grown from water with some formamide as impurity.[24]

Not only achiral crystals may be modified. Addition of small amounts of optically pure impurities can cause the blockage of certain faces of optically pure crystals.[23]

Figure 2.6 *NaCl crystals grown from pure water (right) and water with some formamide.*[24]

2.3 Phase Diagrams: a Necessary Evil on the Path to Salvation/Solvation

The behavior of a mixture of two crystalline compounds and a solvent can be visualized and explained by means of a ternary phase diagram. A ternary phase diagram shows how this mixture of components behaves thermodynamically. Any attempt at a kinetic analysis must be done with great care.

In Figure 2.7a a phase diagram is depicted. The scale of phase diagrams is usually in mole fractions or mole percentages. Each apex represents a pure compound (or solvent). The sides of the triangle represent mixtures of the two compounds that are at the ends of the side. For instance, point **X** represents a 1:1 mixture of (*R*) and (*S*) without any solvent. Point **Y** in the phase diagram represents a 1:1:1 mixture of all three components. Line **Z'** is a dilution line and represents a constant (*R*):(*S*) ratio of 1:3 with a varying amounts of solvent. Point **Z** has the same ratio of (*R*):(*S*) but without solvent.

The phase diagram in Figure 2.7b represents a diastereomeric salt in its solvent. In this diagram, **p** represents the less soluble salt with solubility **p'** and **n** represents the more soluble salt with solubility **n'**. Presuming ideal solubility without dissociation, the solubility line (**p'-E**) of salt **p** runs parallel to line (**S-n**).[25] For the **n** salt the solubility line runs from **n'** to **E** parallel to line (**S-p**). Point **E** represents a mother liquor that is saturated in both **n**

and **p** and is called the eutectic. The parallelogram shaped area above the eutectic (**E,p',S,n'**) represents under-saturated mixtures of **p** and **n** in the solvent.

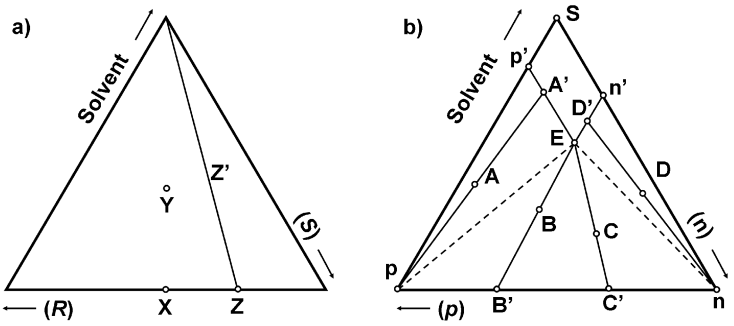


Figure 2.7 *Theoretical ternary phase diagrams.*

The triangle (**n,n',E**) represents the cases in which **n** has crystallized but **p** is under-saturated. The triangle (**p,p',E**), of course, represents the cases in which **p** has crystallized but **n** is under-saturated. For example, if one would have a mixture of composition **A** in equilibrium, this will consist of a mother liquor with composition **A'** and the solids would be pure **p**. In the same manner, a mixture with composition **D**, would have a mother liquor with composition **D'** and solids of pure **n**. A change in concentration at constant temperature will not alter the amount of phases but will only change the composition of the mother liquor (along line (**n',E**)) and the amount of material in the solid phase.

In triangle (**n,p,E**) both diastereomers have crystallized. The composition of the mother liquor in the presence of the solid phases of **p** and **n** will be fixed at a constant temperature. Changing the concentration within triangle (**n,p,E**) will not alter the composition the mother liquor, the composition of the **n** salt nor the composition of the **p** salt. Mixtures with composition **B** or **C** would both have a mother liquor with composition **E** and both mixtures will consist of a mixture of crystals of **n** and crystals of **p**. Although the ratio of **n** and **p** in **B'** and **C'** will be different.

2.3.1 Construction of a Ternary Phase Diagram

Although the construction of a ternary phase diagram is quite laborious and requires at least a racemate and one pure (or properly enriched) enantiomer, it can give more insight if the crystallization needs to be optimized or compared to other resolutions. In principle, the determination of the composition of the mother liquors is sufficient to make a phase diagram (these will furnish the solubility lines). However, important details will be missed

if the composition of the solids and/or system compositions are not taken into account, for instance end-solid solution formation and hydrate/solvate formation (as described in §2.3.4). These can be found when the method of "wet solids" is used.[1c]

Let us start with the theoretical ternary phase diagram in Figure 2.8. One begins with a known mixture of compounds in a known amount of solvent (starting composition: **s**) which is allowed to stir in a sealed flask for a sufficient amount of time (couple of days) at a fixed temperature so that the thermodynamic equilibrium has been reached. The mixture is then filtered, the amount of solvent is determined in the mother liquor by distillation and the composition of the solids in the residue is determined (by weighing and *e.g.* chiral HPLC to determine the *ee*), furnishing point **m**.

The solids on the filter are then sucked 'dry' without evaporating the remaining solvent. The amount of solvent is determined in the wet filter cake by drying and the composition of the resulting solids is determined, delivering point **w**. By extrapolation of the tie line through the points **m**, **s** and **w** one arrives at point **p**: the composition of the crystals.

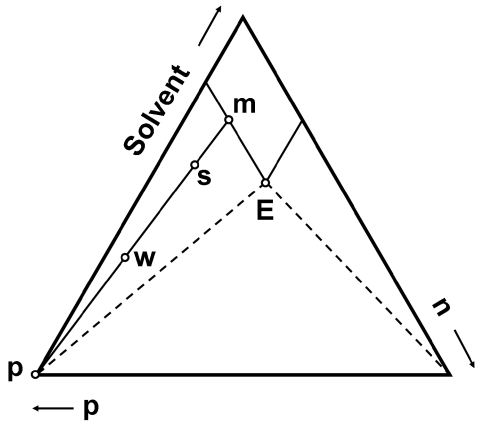


Figure 2.8 *Theoretical ternary phase diagram construction by the method of wet solids.*

Point **p** can also be found by a quick washing of the solids after filtration and then a determination of the *ee*. Although this method may be more convenient, one might miss solvate/hydrate formation and will introduce some error by the unwanted dissolution of some of the material by the washing step.

2.3.2 Conglomerates, Racemic Compounds and Solid Solutions[1c]

Enantiomers can crystallize separately: each in a single crystal or together in one crystal as depicted in Figure 2.9.

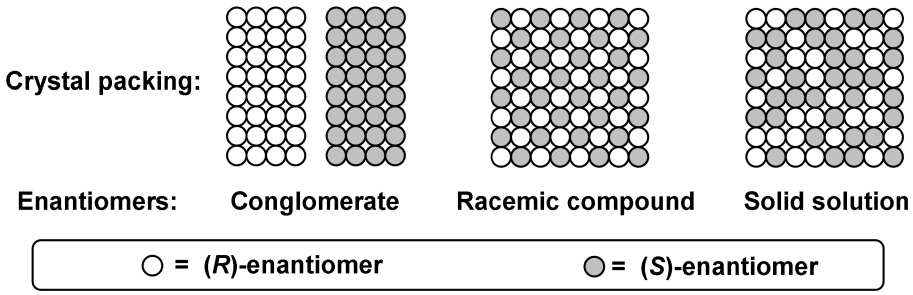


Figure 2.9 *Crystal packing of enantiomers and diastereomers.*

When a racemate crystallizes into two separate crystals of opposite handedness this is called a conglomerate. It is estimated that only 5–10% of all racemates crystallize as a conglomerate. More common (90–95%) is the formation of a racemic compound in which a racemate crystallizes in an ordered fashion. Rare (1%) is a solid solution where a crystal does not distinguish between (*R*)- and (*S*)-enantiomers. This crystal is then randomly packed and thus its overall *ee* depends on the *ee* of the mother liquor it was crystallized from. Typical ternary phase diagrams for these different crystallization behaviors are given in Figure 2.10.

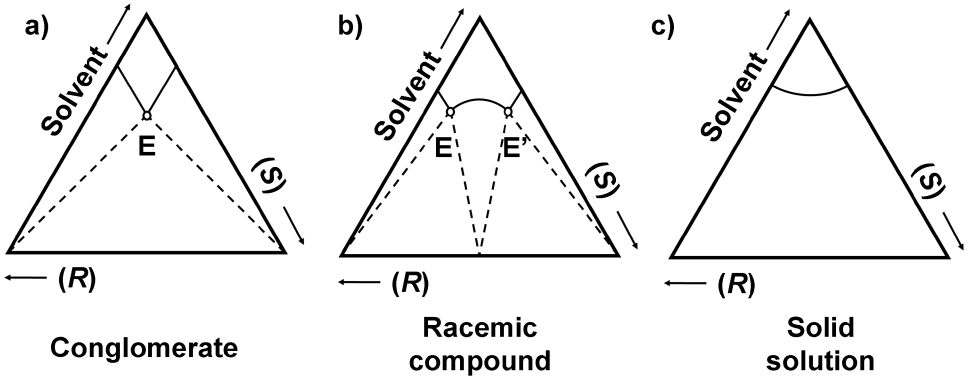


Figure 2.10 *Theoretical ternary phase diagrams for different crystal packings of enantiomers.*

2.3.2.1 Finding Conglomerates

The resolution by direct crystallization can only be performed on conglomerates (see Chapter 1.4.3.3). There are several analytical methods for finding conglomerates, some of which are given below:

- **DSC** If the melting point difference between the pure enantiomer and the racemate is at least 20°C a conglomerate might be expected.[1c]
- **IR/NIR/Raman/UV/Solid state NMR/XRPD** The spectra of the racemate and pure enantiomer should be the same for conglomerates.
- **Solubility** Slurring crystals with ~5% *ee* in a minimal volume of an appropriate solvent will give 0% *ee* (eutectic composition) for conglomerates. Eutectics of racemic compounds will always be >0% *ee* (see also Figure 2.10).[26]
- **SHG (Second Harmonics Generation)** This method only requires the racemate and is explained below.

SHG depends on non-linear optics in which the two photons are combined in a crystal to produce diffuse light with twice the energy ($\lambda/2$) of the irradiated light: the SHG signal.[27] If the sample has crystallized in a non-centrosymmetric space group, as conglomerates do in 95% of investigated cases, a SHG signal is produced. The intensity of the SHG signal depends on the symmetry class to which a crystal belongs. If the compound has crystallized in a centrosymmetric space group, no SHG signal is produced. Racemic compounds are known to crystallize predominantly in centrosymmetric space groups. Since some conglomerates can crystallize in a centrosymmetric space group, and some racemic compounds in a non-centrosymmetric space group, some crystals absorb the irradiated or the SHG photons, leading to false conclusions in an estimated 10% of cases. However, SHG only requires a racemate, is fast, (usually) non-destructive and thus makes it an ideal screening method.

A typical SHG setup is depicted in Figure 2.11 where photons are delivered by a pulsed Nd:Yag laser (1064nm). The photons pass through an energy adjustment device to prevent decomposition of the sample. Subsequently, the photons pass through a filter that removes light from laser flash lamps. The sample container contains powdered crystals and is irradiated. The diffused light from the sample is then collected in a spectrophotometer *via* an optical cable and analyzed for a (usually weak) SHG signal with double the energy compared to the photons of the laser: 532nm.

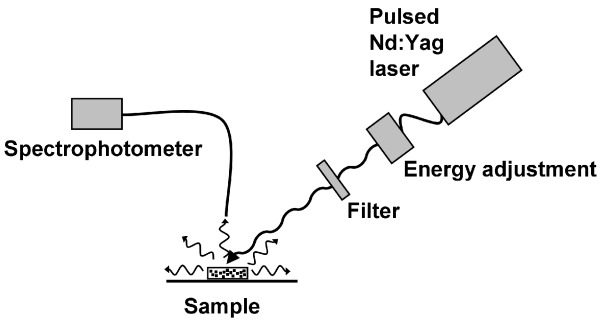


Figure 2.11 *SHG setup.*

2.3.3 Phase Diagrams and Diastereomers

The situation with diastereomers is similar but not identical to that with enantiomers. When a diastereomeric salt is formed from a racemate and an optically pure resolving agent, similar crystal packings are formed as well. The most commonly observed type is the eutectic, which is comparable with conglomerates. Also addition compounds can be formed with different ratios of diastereomers, comparable with racemic compounds. Usually these are 1:1, however, other ratios have been found. For example, from (*R*)-1-phenylethyl amine and (*S*)-mandelic acid it is known that a 1:3 salt can form.[28]

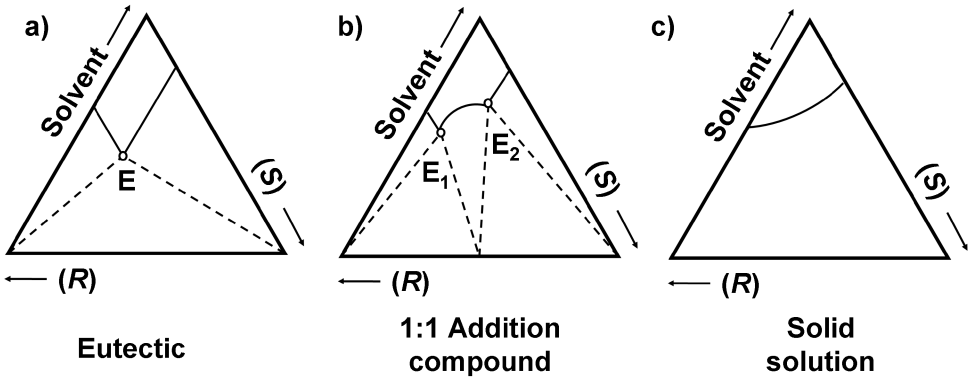


Figure 2.12 *Theoretical ternary phase diagrams for different crystal packings of diastereomers.*

Also, randomly divided solid solutions can be formed in diastereomeric salt formation. However, addition compounds and solid solutions are not very common in diastereomers. Note that a solid solution is not the same as an end-solid solution (as discussed in §2.3.4) which often occur in eutectic mixtures. The phase diagrams for these types of crystallization behaviors are given in Figure 2.12.

2.3.4 End Solid Solutions, Solvates and Hydrates

Often, the resolution efficiency of a diastereomeric salt formation is lower than one would expect from the solubility difference of the two diastereomers which form an eutectic. A close examination of separate crystals will reveal the incorporation of some of one diastereomer in the other diastereomer crystal. This phenomenon is known as a end solid solution (or terminal solid solution) behavior and is very common in diastereomer mixtures.[30] As with (full) solid solutions, the percentage of incorporated 'wrong' material corresponds with the concentration of the latter in the surrounding mother liquor.

Figure 2.13a depicts a diastereomer with an end solid solution for the less soluble salt. Starting with composition **a**, the mother liquor will have composition $\mathbf{a_1}$ and the solids would have composition $\mathbf{a_2}$. On crystallizing the solids in an certain amount of solvent, this will give a suspension of composition **b**. Note that the *ee* remains the same (dotted line). After equilibration, the mother liquor ($\mathbf{b_1}$) is removed and this will result in solids of composition $\mathbf{b_2}$. Another recrystallization delivers $\mathbf{c_2}$, which is still not >99% *de*.

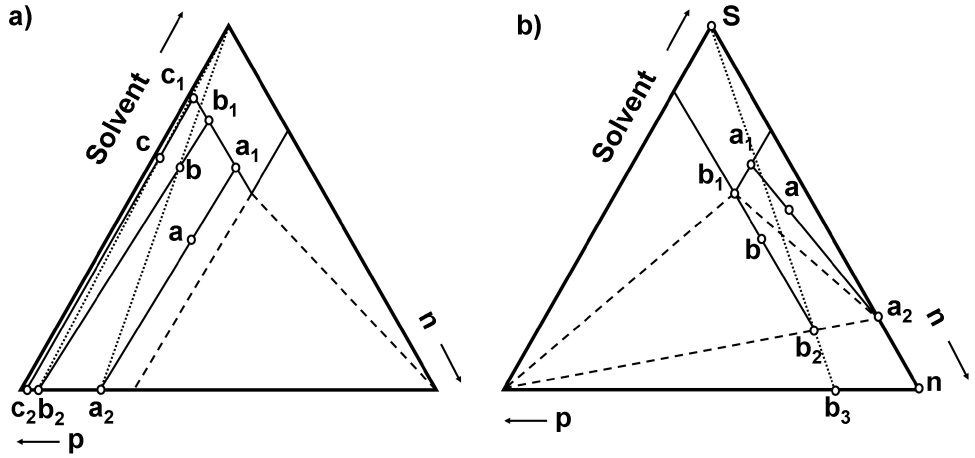


Figure 2.13 *Theoretical ternary phase diagrams of an end solid solution and of a solvate.*

It is clear that isolation of material with >99% *de* requires multiple recrystallizations with serious loss of material.[29] Hence, the efficiency of a resolution does not only depend on the position of the eutectic point, but maybe even more, on the extent of the end solid solution. The resolution of a pair of diastereomers with a poor eutectic point but without end solid solutions can be performed in more dilute solutions and can provide in principle >99% *de* in a single crystallization, perhaps with equally (low) yields as with an end solid solution forming pair of diastereomers and a eutectic point with high *ee*, but with less work.

When performing a resolution by diastereomeric salt formation, often a solvate or hydrate is formed. This is often missed since the salts are usually not specifically analyzed for this. However, the presence of a solvate or hydrate will have profound effect on the outcome of a resolution. Figure 2.13b depicts the ternary phase diagram for the case that the more soluble diastereomer crystallizes as the solvate.

Starting from **a**, the solids will have composition $\mathbf{a_2}$. Point $\mathbf{a_2}$ is on the line (**S**,**n**) because the solids contain some solvent. Furthermore, starting from **b**, the solids will have composition $\mathbf{b_2}$. However, when the solids are dried, the solvent is removed but the composition of the solids remain unchanged ($\mathbf{b_3}$). The triangle (**p**,**n**,**n·solvent**) contains saturated solution, **p**-crystals, **n**-crystals, and **n**-solvate.

2.3.5 Binary *versus* Ternary Phase Diagrams[5,30]

Melting point phase diagrams (binary phase diagrams) are easier to construct than ternary ones. These can be constructed by Differential Scanning Calorimetry (DSC) which requires several mixtures of enantiomers or diastereomers and is a relative fast method. Binary phase diagrams are often used to predict the outcome of a resolution by diastereomeric salt formation.

However, the results can be distorted or even completely invalid for a resolution at room temperature for a number of reasons:

- Polymorphic transition by heating/melting.
- Decomposition during heating.
- Temperature dependent stability of polymorphs (enantiotropic relation).
- Temperature dependent stability of racemic compounds.[31]
- The extent of end solid solution behaviour can change with temperature.
- Grinding of the samples (necessary for DSC measurements) can cause significant changes in the sample.
- Hydrate and solvate formation are not taken into account.

The formation of solvates and hydrates should not be underestimated. Out of 46,460 structures in the Cambridge Structural Database (CSD) there are 9,464 (20%) solvates from which 61.4% are with water.[5]

Therefore, it is better to spend a bit more time to construct a ternary phase diagram which can be trusted rather than use less time to construct a binary phase diagram that might not represent the system under investigation.

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Chapter 3

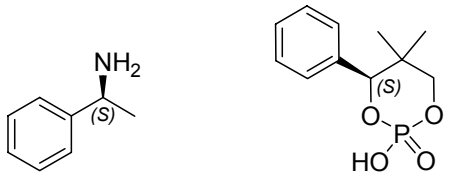
Resolution of Racemates by Diastereomeric Salt Formation with the Aid of Nucleation Inhibitors.

In this chapter[*] the resolution of (±)-1-(3-methoxy-phenyl)ethylamine with (S)-mandelic acid is described. This resolution was aided by the addition of nucleation inhibitors and was performed both on test tube and large scale.

[*] *Parts of this chapter were also published in Angew. Chem. Int. Ed.* ***2008****, 47, 1287–1290 and Tetrahedron Asymm.* ***2009****, 20, 1363–1364.*

3.1 Introduction

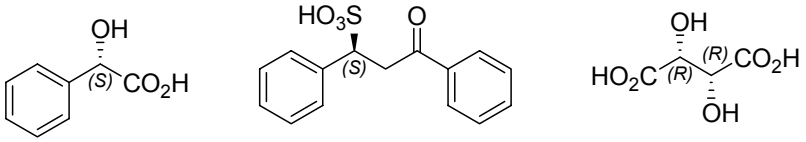
Optically pure 1-phenylethylamine and phencyphos are frequently used resolving agents and for these compounds nucleation inhibitors have been described and proven to be effective.[1,2]



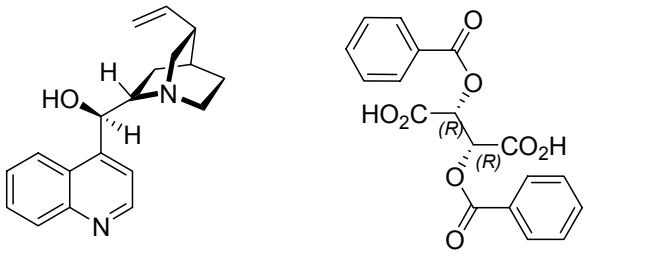
(*S*)-1-Phenylethylamine **(*S*)-Phencyphos**

Scheme 3.1 *Resolving agents for which nucleation inhibitors have been designed.*

However, for other resolving agents like mandelic acid only one example of a successful nucleation inhibitor has been described.[1] For other frequently used resolving agents like dihydrochalcone-3-sulfonic acid, tartaric acid, cinchonidine and *O,O'*-dibenzoyl tartaric no effective nucleation inhibitors have been developed.



(*S*)-mandelic acid **(*S*)-dihydrochalcone-3-sulfonic acid** **L-tartaric acid**



Cinchonidine ***O,O'*-dibenzoyl-L-tartaric acid**

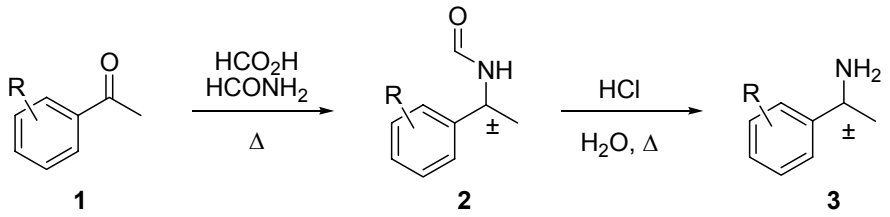
Scheme 3.2 *Commonly used acidic and basic resolving agents.*

3.2 Resolution with Optically Pure Mandelic Acid

The resolution of a racemate with mandelic acid, improved by addition of a nucleation inhibitor should, ideally, meet to the following requirements:

- Chemically and configurationally stable racemate.
- Fast diastereomeric excess (*de*) determination by chiral HPLC or chiral GC, preferably without derivatization or liberation of the salts.
- Salts that precipitate with eutectic behavior without end-solid solution formation.
- Sufficient solubility difference between both diastereomeric salts.

1-Phenylethylamine derivatives were chosen as racemates because of our experience with these compounds, wide variety of low cost starting materials and for their chemical stability. The 1-phenylethylamine derivatives can be synthesized by the Leuckart-Wallach reaction as depicted in Scheme 3.3.[3]



Scheme 3.3 *Lauckart-Wallach synthesis of phenylethylamines.*

Via reductive amination of acetophenones **1** with formic acid and formamide, formyl protected amines **2** were isolated and subsequently deprotected by boiling the latter in aqueous hydrochloric acid to furnish compounds **3** after workup.

3.2.1 Screening for Possible Candidates

The efficiency of resolutions of several 1-phenylethylamine (PEA) derivatives with mandelic acid (MA) have been published,[4] and the results of these resolutions are depicted in Table 3.1. Unfortunately, no experimental details were given (*i.e.* solvent, concentration) so repetition of these experiments can lead to different results. However, these published results might give a fast insight in which diastereomeric salt pair could give the best results.

The racemates that have >95% *de* in the first salts must have eutectic behavior without a large end-solid solution. The racemates which have a low *de* might also be improved by addition of a nucleation inhibitor. Moreover, a solvate or hydrate might be present which

will give different results after a change of solvent. After consideration, the racemates with R = H, 2-Me and 3-MeO (entries 1, 2 and 5) were chosen for further research because these were most promising.

Table 3.1 *Resolution of PEA derivatives with MA.*[4]

Entry	R	Yield (%)	*de* (%)	S-factor[a]
1	H	38	99	0.75
2	2-Me	36	100	0.72
3	2-MeO	35	81	0.57
4	3-Me	47	12	0.11
5	3-MeO	35	99	0.69
6	4-Me	44	4	0.04
7	4-MeO	85	0	0.00
8	4-Cl	17	3	0.01

[a] Resolution efficiency: S-factor = Yield × *de* × 2.[5]

Because any improvement of a resolution with a reasonable high *de* in the precipitated salts by addition of a nucleation inhibitor is difficult to observe, the resolution should be performed at a high concentration. However, a clear solution should be obtained at reflux temperature since nucleation inhibitors cannot inhibit crystals that have already formed. At a high concentration, the more soluble diastereomer can crystallize to a large extent at room temperature and thus give a high yield and poor *de*.

Small scale (1 mmol) experiments were carried out on the 1-phenylethylamines with one equivalent of (*S*)-MA by heating the mixture in an appropriate amount of solvent until complete dissolution was obtained at reflux temperature. The mixtures were allowed to cool to room temperature without stirring and crystals were collected the next day. The results are depicted in Table 3.2. Obviously, the results in the table are not the same as the literature values in Table 3.1. Different solvents, concentrations and other factors will lead to different results.

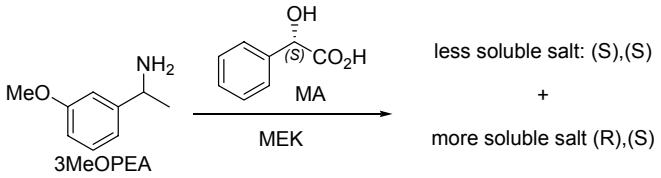
The resolution of racemic 1-phenylethylamine (entry 1, Table 3.2) is too good for experiments to find an effective nucleation inhibitor since the *de* is already quite high and thus improvements will only be marginal.

Table 3.2 *Resolution of PEA derivatives with MA (1 mmol scale) without stirring and controlled cooling.*

Entry	R	Solvent	Yield (%)	*de* (%)	S-factor[c]
1[a]	H	MEK[d] (10 mL)	48	83	0.80
2[b]	2-Me	EtOAc (2 mL)	54	23	0.25
3[b]	3-MeO	MEK[d] (15 mL)	65	9	0.11

[a] Commercially available. [b] See experimental section for preparation. [c] Resolution efficiency: S-factor = Yield × *de* × 2.[5] [d] 2-butanone.

In principle, the resolution of racemic 1-(2-methyl-phenyl)ethylamine (entry 2) is suitable for an improvement by addition of a suitable nucleation inhibitor but an experimental restriction was the requirement of liberation and derivatization before chiral HPLC analysis. Thus, 1-(3-methoxy-phenyl)ethylamine (entry 3) was chosen as an ideal racemate for the resolution with MA as depicted in Scheme 3.4, since it gave high yield, low *de* and required no derivatization or even liberation before the determination of the *de* by chiral HPLC thus allowing a non-laborious workup.



Scheme 3.4 *The resolution of (±)-3MeOPEA with (S)-MA.*

When the resolution was performed by magnetic stirring and controlled cooling, the salts of (±)-1-(3-methoxy-phenyl)ethylamine (3MeOPEA) with (*S*)-MA were isolated in 72% yield and 10% *de*, nearly identical with scouting experiments reported in Table 3.2, entry 3 (see also entry 1 in Table 3.3).

3.2.2 Nucleation Inhibitors Based on the Resolving Agent

Various additives, structurally related to MA, were tested in 2-butanone (MEK) as solvent and the results are given in Table 3.3. In all cases 6 mol% of acid was used with 94 mol% of (*S*)- or (*R*)-MA in order to keep the ratio of acid to base stoichiometric and the system thus neutral. For example, in the case of the double acid in entry 5 (Table 3.3) 3 mol% of this compound was used.

In entry 1, Table 3.3 the *de* of the isolated solids in the blank resolution is 10%, which means that 55% of the solids consists of the less soluble salt and 45% of the more soluble salt. If a nucleation inhibitor were able to block only the more soluble salt, the resolution would furnish instead of the 72% yield in entry 1, the pure less soluble salt in 72% × 0.55 = 40% yield.

Powerful nucleation inhibition effects were seen. These are well illustrated with *O*-acetyl mandelic acid (entry 2a). Instead of synthesizing both enantiomers of each additive, the other enantiomer of the resolving agent was chosen to resolve the racemate according to the Marckwald principle.[6] As can be seen from entries 2a/b, 3b/c and 4a/b, the combination of the same absolute configuration of the nucleation inhibitor with that of the resolving agent leads to high *de*'s in the precipitated salts and thus a strong nucleation inhibition effect. These stereochemical correlations parallel the "rule of reversal" postulated by Lahav *et al* as described in section 2.2.3.[7]

The additive used in entry 3a is racemic and works nearly as well as the optically pure additive in entry 3b. Of course, of the 6% racemic additive used in entry 3a, half consists of (*S*)-enantiomer, which is an excellent nucleation inhibitor. However, the optically pure additive in entry 6a shows strong nucleation inhibition whereas the racemate in 6b shows only minor nucleation inhibition effects. Most likely, the amount of the additive with the same absolute configuration as the resolving agent is too low to still be an effective nucleation inhibitor. Moreover, HPLC analysis of the precipitated salts show that some of this additive is incorporated in the less soluble salts, which makes its concentration in the solution lower and thus less effective as a nucleation inhibitor.

As described before,[2] bifunctional additives based on 1-phenylethylamine show great potential as nucleation inhibitors. In entry 5, it is seen that also bifunctional mandelic acid derivatives can act as nucleation inhibitors. This inhibitor even inhibits the crystallization and/or growth of the less soluble diastereomeric salt as revealed by the low yield.

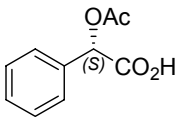
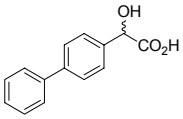
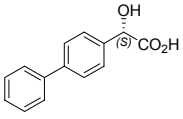
Other chiral and achiral acids that do not resemble mandelic acid closely do not work as nucleation inhibitors as can be seen in entries 9, 15, 28, 29 and 30. Also the multi-functional acids in entries 27 and 28 do not work efficiently as nucleation inhibitors.

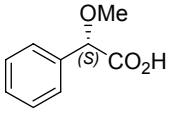
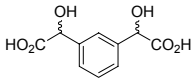
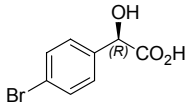
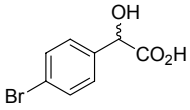
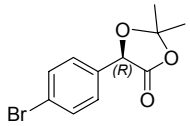
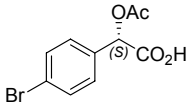
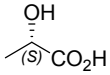
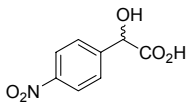
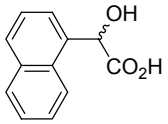
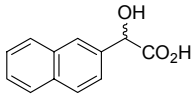
Bulky groups in place of the alpha-proton of mandelic acid also did not give any improvement of the resolution as denoted in entries 14, 16, 20 and 23.

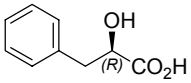
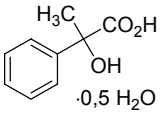
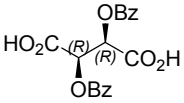
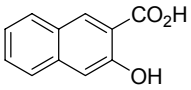
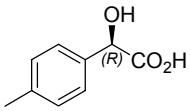
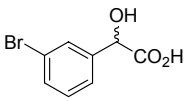
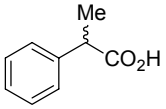
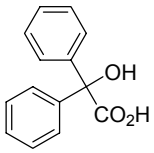
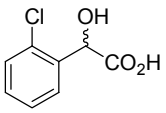
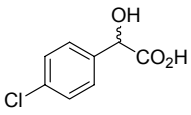
The use of esters (entries 7 and 31), which cannot form salts, do not lead to any improvement in the resolution. Apparently, ability to form salts is crucial for effective nucleation inhibition in this system.

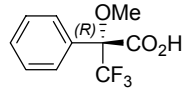
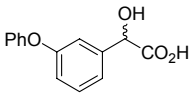
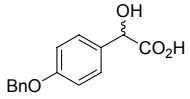
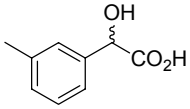
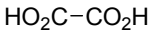
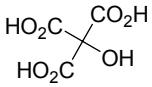
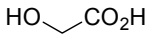
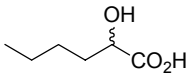
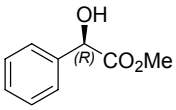
The additives have a great variance in structure and also in effectiveness. Based on the results in Table 3.3, we might conclude that *O*-substituted mandelic acids with the same absolute configuration show potential as nucleation inhibitors. This is most likely because of end-capping of the hydrogen bonding network in the embryo after which this cannot grow further. Also, *para*-substituted mandelic acids with the same absolute stereochemistry work fine as nucleation inhibitors. Most substituents on the *para*-position of the phenyl ring effectively block the nucleation of the more soluble crystal because of sterics, charge and hydrogen bonding abilities. Why the (*R*)-*p*-methyl mandelic acid in entry 17 and the *p*-nitro mandelic acid in entry 10 do not work is unknown.

Table 3.3 *Results of resolutions of racemic 1-(3-methoxy-phenyl)ethylamine with mandelic acid with 6 mol% of additives resembling the resolving agent.*

Entry	Additive	(*R*)- or (*S*)-MA	mol% MA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
1	None	*R/S*	100	-	72	10	0.14	-
2a[a]		*S*	94	6	42	97	0.81	<0.1%
2b[a]	”	*R*	94	6	70	12	0.17	n.d.[f]
3a[b]		*S*	94	6	36	95	0.68	1%
3b[b]		*S*	94	6	42	95	0.80	<0.1%
3c[b]	”	*R*	94	6	68	15	0.20	n.d.[f]

Entry	Additive	(*R*)- or (*S*)-MA	mol% MA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
4a[a]		*S*	94	6	40	96	0.77	<0.1%
4b[a]	”	*R*	94	6	63	11	0.15	n.d.[f]
5[c]		*R*	94	3	16	93	0.30	<0.1%
6a[a]		*R*	94	6	40	93	0.73	4%
6b[a]		*R*	94	6	46	32	0.30	3%
7[c]		*R*	100	6	67	12	0.15	n.d.[f]
8[c]		*S*	94	6	31	99	0.61	<0.1%
9[a]		*S*	94	6	69	13	0.18	n.d.[f]
10[c]		*S*	94	6	60	26	0.31	2%
11[c]		*S*	94	6	66	13	0.17	n.d.[f]
12[c]		*S*	94	6	26	94	0.48	<0.1%

Entry	Additive	(*R*)- or (*S*)-MA	mol% MA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
13[a]		*R*	94	6	67	15	0.20	n.d.[f]
14[a]		*S*	94	6	48	18	0.16	<0.1%
15[a]		*R*	94	3	63	22	0.27	n.d.[f]
16[a]		*R*	94	6	63	16	0.20	n.d.[f]
17[d]		*R*	94	6	64	15	0.19	<0.1%
18[a]		*R*	94	6	63	19	0.23	<0.1%
19[a]		*R*	94	6	62	14	0.17	n.d.[f]
20[a]		*S*	94	6	56	20	0.22	n.d.[f]
21[a]		*S*	94	6	52	15	0.15	n.d.[f]
22[a]		*R*	94	6	33	95	0.62	6%

Entry	Additive	(*R*)- or (*S*)-MA	mol% MA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
23[a]		*R*	94	6	46	32	0.29	n.d.[f]
24[c]		*R*	94	6	61	26	0.32	n.d.[f]
25[a]		*R*	94	6	35	95	0.67	3%
26[a]		*R*	94	6	66	12	0.16	n.d.[f]
27[a]		*R*	94	3	68	10	0.14	n.d.[f]
28[a]		*R*	94	2	67	25	0.30	n.d.[f]
29[a]		*R*	94	6	65	14	0.19	n.d.[f]
30[a]		*R*	94	6	66	19	0.25	n.d.[f]
31[a]		*R*	100	6	74	10	0.15	n.d.[f]

[a] Commercially available. [b] Prepared according to literature.[8] [c] See experimental section for preparation. [d] Prepared according to literature.[9] [e] Resolution efficiency: S-factor = Yield × *de* × 2.[5] [f] not determined.

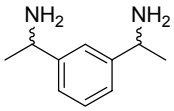
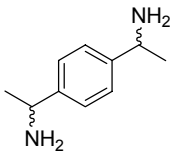
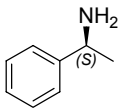
3.2.3 Nucleation Inhibitors Based on the Racemate

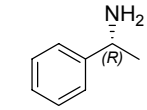
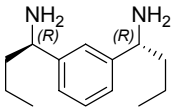
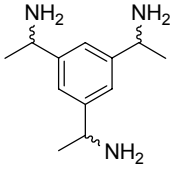
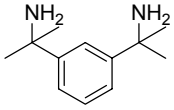
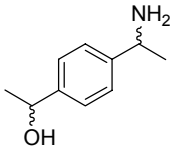
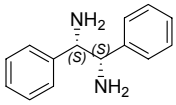
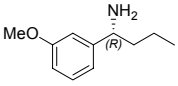
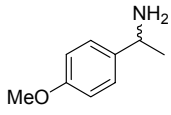
Inhibition should also apply to the racemate to be resolved. However, until now, no example with an increased S-factor has been found to validate this expectation.[1] We had previously observed high activity of certain bifunctional amines as inhibitors of

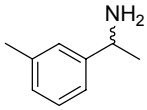
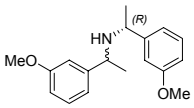
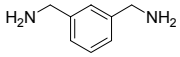
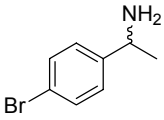
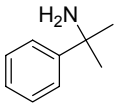
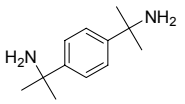
1-phenylethylamine as resolving agent.[2] Some of these compounds have been investigated as inhibitors in the resolution of (±)-3MeOPEA by (*S*)-MA. The results are shown in Table 3.4. As can be concluded from the table, both more than one amine moiety per molecule and steric bulk (see entry 15) seem to be necessary to give a significant change in yield, *de* and S-factor. The tris amine in entry 7 even shows some inhibition of the less soluble salt similar to the bis acid in entry 5 in Table 3.3.

Since none of the mono functionalized amines work, it is impossible to make claims about the use of optically pure additives based on the to be resolved material (entries 4 and 5 or 11a and 11b) and its relation to the rule of reversal. The only enantio pure amine that was tested and has proven to be an effective nucleation inhibitor is the chiral bis-amine in entries 6a and 6b. The additive works equally well in both resolutions based on the Marckwald principle. It seems that the absolute configuration of bi-functional additives is of less importance in this system.

Table 3.4 *Results of resolutions of racemic 1-(3-methoxy-phenyl)ethylamine with mandelic acid with 6 mol% of additives resembling the racemate.*

Entry	Additive	(*R*) or (*S*)-MA	mol% 3MeO PEA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
1	None	*R/S*	100	-	72	10	0.14	-
2[a]		*S*	94	3	32	96	0.61	<0.1%
3[a]		*S*	94	3	35	89	0.63	<0.1%
4[b]		*S*	94	6	43	23	0.20	<0.1%

Entry	Additive	(*R*) or (*S*)-MA	mol% 3MeO PEA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
5[b]		*S*	94	6	46	14	0.13	<0.1%
6a[a]		*S*	94	3	32	97	0.62	<0.1%
6b[a]	”	*R*	94	3	31	96	0.60	<0.1%
7[c]		*S*	94	2	27	95	0.51	4%
8[c]		*S*	94	3	42	97	0.82	5%
	1,3-BAPB							
9[c]		*S*	94	6	75	12	0.18	n.d.
10[b]		*R*	94	3	73	11	0.17	n.d.
11a[a]		*R*	94	6	69	12	0.17	n.d.
11b[a]	”	*S*	94	6	69	13	0.17	n.d
12[d]		*R*	94	6	67	11	0.16	n.d.

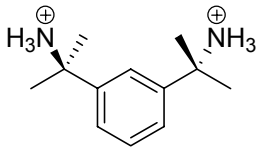
Entry	Additive	(*R*) or (*S*)-MA	mol% 3MeO PEA	mol% NI	Yield (%)	*de* (%)	S-factor[e]	additive in salt
13[d]		*R*	94	6	69	19	0.27	n.d.
14[c]		*S*	94	6	64	10	0.12	<0.1%
15[b]		*S*	94	3	69	13	0.15	1%
16[b]		*S*	94	6	61	15	0.17	2%
17[b]		*R*	94	6	69	14	0.20	n.d.
18[c]		*S*	94	3	Additive insoluble			
	1,4-BAPB							

[a] Prepared according to literature.[10] [b] Commercially available. [c] See experimental section for preparation. [d] Prepared according to literature.[8] [e] Resolution efficiency: S-factor = Yield × *de* × 2.[5] [f] not determined

A remarkable observation is that an achiral bis-amine can be an extremely effective inhibitor. In entry 8 the results with 1,3-bis-[2-amino-2-propyl]benzene (1,3-BAPB) are given. Under the conditions of the resolution 1,3-BAPB is probably doubly protonated. The C2 conformation with the ammonium groups located up and down is chiral as depicted in Scheme 3.5.

Both steric factors and charge repulsion could favor this conformation. Unfortunately an attempt to test this idea of a chiral conformation by use of 1,4-BAPB (entry 18), which

cannot have such a chiral conformation, was foiled by the total insolubility of the bis (*S*)-MA salt in MEK.



Scheme 3.5 *Possible chiral conformation of 1,3-BAPB.*

3.2.4 Nucleation Inhibition in Ternary Phase Diagrams

A graphic representation in the form of a ternary phase diagram of the resolution of (±)-3MeOPEA with (*S*)-MA makes it possible to understand and predict the outcome of a normal resolution and of nucleation inhibitor enhanced resolutions also.

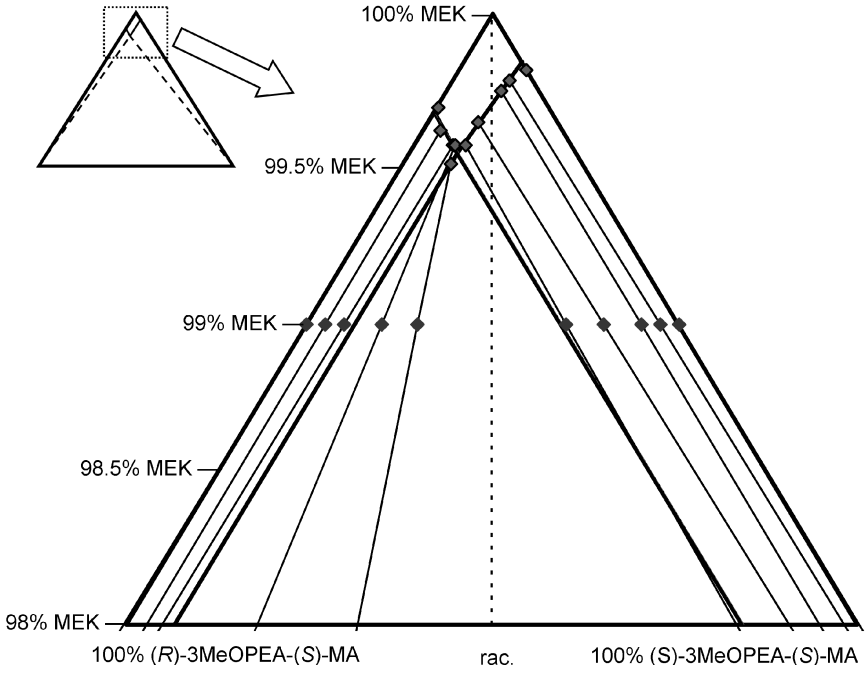


Figure 3.1 *Thermodynamic phase diagram of 3-MeOPEA-(S)-MA. The points in the diagrams represent the start compositions and the mother liquor compositions.*

To determine the thermodynamic ternary phase diagram, suspensions were made with varying compositions of the (*S*)-MA salt of (*R*)- and (*S*)-3MeOPEA and treated like a typical resolution experiment as described in the experimental section. However, the mixtures were stirred at 20°C for 2 additional days to ensure that thermodynamic equilibrium had been reached. The composition of the mother liquors was determined and with the method of algebraic extrapolation the compositions of the solids were determined.[6] The phase diagram showed no end-solid solution behavior for either diastereomer (not shown). The compositions were plotted in a ternary phase diagram, depicted in Figure 3.1. Because of the poor solubility of the diastereomeric salts in MEK, only the top 2% of the phase diagram is shown.

The maximum theoretical yield can be determined from the eutectic composition with Equation 3.1.[11]

$$y_{\max} = \frac{0.5 - x_{eu}}{1 - x_{eu}} \times 100\%$$

Equation 3.1 *Resolvability from the eutectic value.*

Where:

y_{max} = maximum yield of the isolated solids.

x_{eu} = molar fraction of the more soluble diastereomer at the eutectic. A value of 0.5 is racemic, 1.0 and 0.0 are the optically pure opposite enantiomers.

The eutectic in Figure 3.1 lies at $x_{eu} = 0.29$ (= 42% *ee*). This means the maximum yield in a thermodynamically driven resolution at an appropriate concentration is 30%,[12] provided that the material to be resolved is racemic. This means that by increasing the concentration of the salts and by addition of a small amount of nucleation inhibitor as denoted in Table 3.3 and Table 3.4 where up to 97% de with 42% yield were found, the space-time yield should increase by 3–4 fold. In other words: a reactor of a certain volume can give 3 to 4 times more product in the same period of time.

Since the phase diagram in Figure 3.1 is determined after a couple of days stirring at 20°C and the small scale resolutions in Table 3.3 and Table 3.4 were performed in an overnight experiment, a phase diagram was constructed (not shown) under the same conditions as the small scale experiments. When the phase diagrams from this and the previous experiment were superimposed, no significant differences were found. It may be concluded that after a

normal resolution experiment as described above, thermodynamical equilibrium has been reached.

A phase diagram was constructed for the case that 1% (*S*)-MA was replaced by 1% (*S*)-*O*-acetylmandelic acid (a strong nucleation inhibitor, see Table 3.3, entry 2). The results are depicted in Figure 3.2 which is a representation of the top 2% of the full phase diagram.

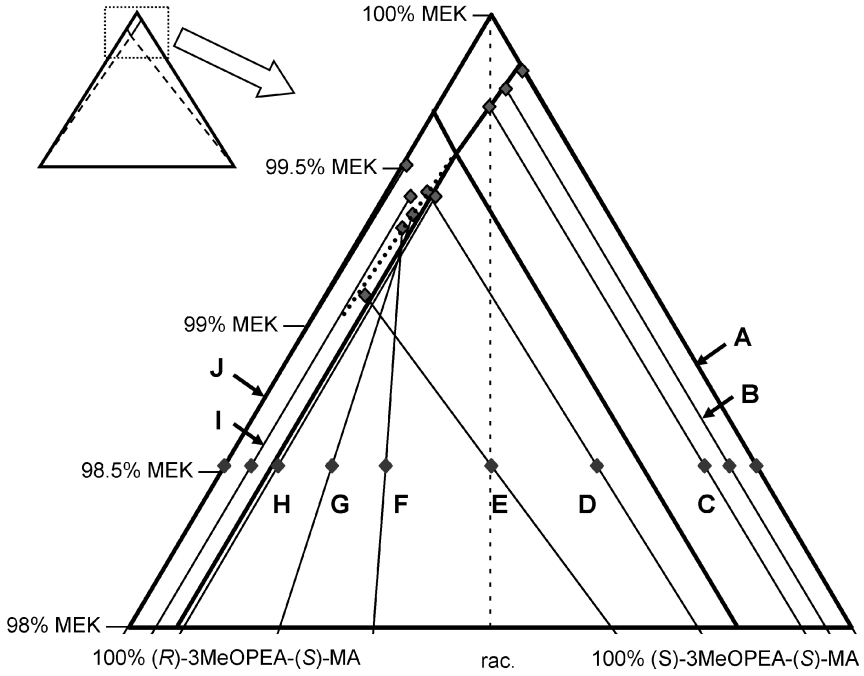


Figure 3.2 *Kinetic phase diagram of 3MeOPEA-(S)-MA with 1% (S)-O-acetylmandelic acid. The points in the diagrams represent the start compositions and the mother liquor compositions.*

In the phase diagram it is clear that the solubility of the less soluble diastereomer is not affected (lines A–C) because the compositions of the mother liquors is on its solubility line. However, at higher fractions of the more soluble diastereomer (lines D and E), the mother liquor is supersaturated in the more soluble diastereomer but does not crystallize. Thus, the composition of the mother liquor follows the elongated solubility line of the less soluble diastereomer (dotted line). At even higher fractions of the more soluble diastereomer (lines F–J), the effect becomes less pronounced and the system returns towards thermodynamic equilibrium (solubility line of the more soluble diastereomer) but does not reach it in this time frame.

3.2.5 Upscaling of the Resolution

A larger scale resolution with 1,3-BAPB as additive (entry 8, Table 3.4) was examined. Starting from 40 gram of (±)-3MeOPEA-(*S*)-MA salt, a resolution in MEK with 0.5% and 1.0% of the 1,3-BAPB-2(*S*)-MA salt as additive was performed and compared to a blank resolution (without additives) with the same concentration as the small scale resolutions from entry 1 in Table 3.3. In Figure 3.3 the blank resolution is shown. Sampling started one hour after the temperature reached 20°C (T_0). The blank resolution illustrates that the *de*'s of the mother liquor and of the unwashed solids do not change much in time.

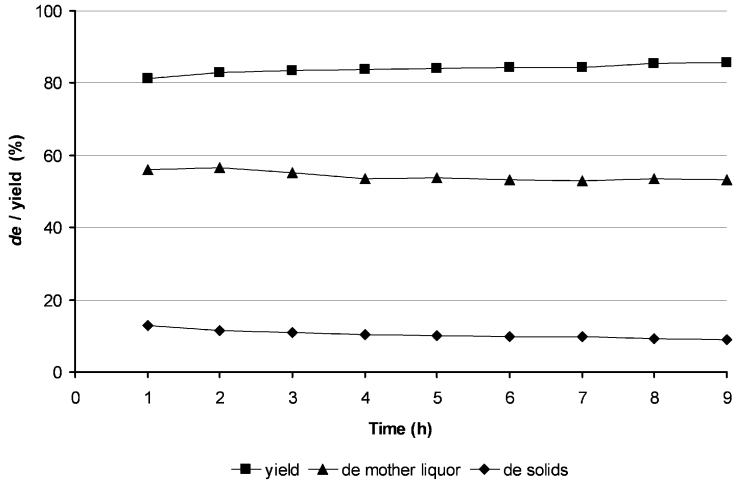


Figure 3.3 *Large scale resolution without nucleation inhibitor. The solids were sucked dry but not washed. Lines are provided as a guide for the eye.*

However, when 0.5 mol% of the 1,3-BAPB-2(*S*)-MA salt was added before the mixture was cooled to 20°C, the first sample of isolated solids after one hour at 20°C had >90% *de* as depicted in Figure 3.4. Note that the solids, collected by filtration, were not washed and hence, contain some mother liquor, lowering the measured *de*. However, a second sample after another hour had only 10% *de* in the collected solids and this percentage remained constant in time. Analysis by HPLC (not shown) established that after one hour, the 1,3-BAPB was for the greater part incorporated in the precipitated less soluble salt. The resulting low concentration of additive in the mother liquor made the system unstable and in the next hour the more soluble salt also precipitated with incorporation of the rest of the additive leaving the system in thermodynamic equilibrium.

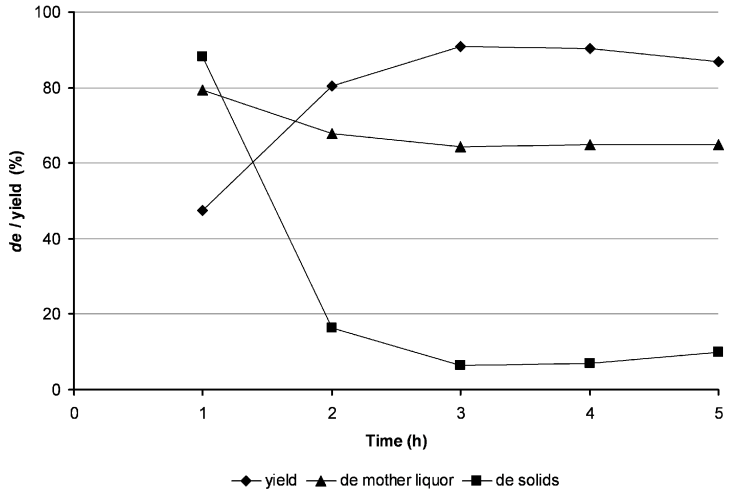


Figure 3.4 *Large scale resolution with 0.5mol% 1,3-BAPB. The solids were sucked dry but not washed. Lines are provided as a guide for the eye.*

However, when 1.0 mol% 1,3-BAPB was used, the more soluble diastereomeric salt remained dissolved for at least 5 days as shown in Figure 3.5.[13] Analysis by HPLC established that only 0.38 % of the additive was still present in the mother liquor, the rest was incorporated in the precipitated less soluble salts. After these 5 days, the solids were collected, washed and dried to give the (*S*)-3MeOPEA-(*S*)-MA in 43% yield and 96% *de* (S-factor=0.83) which is in agreement with the small scale experiment in entry 8, Table 3.4. Apparently at least 0.5% additive is consumed during crystallization.[14] Obviously, also the mother liquor shows more enrichment: 79% *de* with additive compared to 53% *de* in the blank resolution (see Figure 3.3), which is very useful if the other enantiomer is required also.

The latter experiment was repeated but when the mixture reached 20°C, the mixture was seeded with 670 mg of fine crystals of the less soluble (*R*)-3MeOPEA-(*S*)-MA salt. This seeding should stimulate the growth and secondary nucleation of the unwanted diastereomer. The mixture was mechanically stirred continuously and samples were taken regularly. The washed solids had ~95% *de* throughout the experiment (not shown) and after 22 days the solids were isolated by filtration and washing. This yielded the less soluble (*S*)-3MeOPEA-(*S*)-MA salt in yields and high *de*'s similar to the experiment that was not seeded with the more soluble diastereomer. Apparently, 1,3-BAPB is not only an excellent nucleation inhibitor, but also a highly effective growth inhibitor.

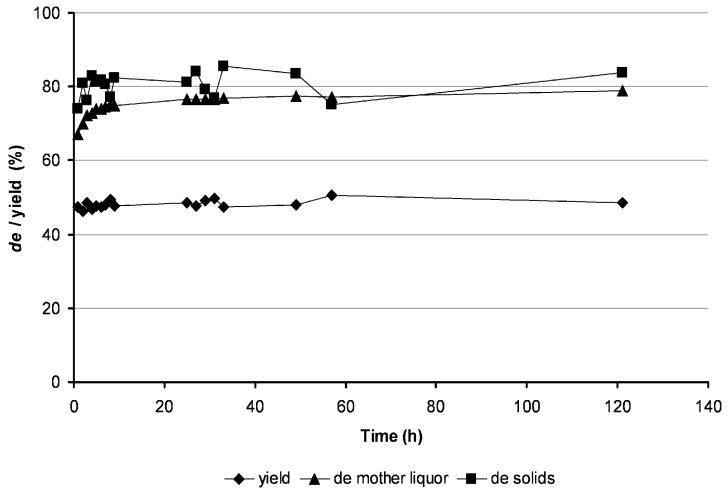


Figure 3.5 *Large scale resolution with 1.0 mol% 1,3-BAPB. The solids were sucked dry but not washed. Lines are provided as a guide for the eye.*

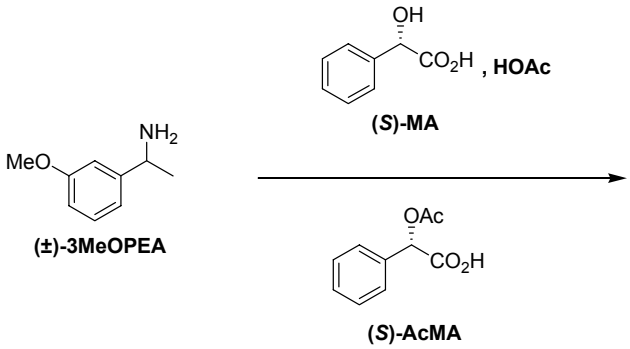
3.3 Pope-Peachey and Nucleation Inhibitors

In principle, a classical resolution requires only one-half equivalent of resolving agent for the less soluble diastereomer to crystallize, which is called the Marckwald method or the method of half-quantities.[6] However, since the solubility of the free racemate is not always high enough in the chosen solvent and double salts are more likely to form in such a resolution, Pope and Peachey[15] proposed a method where the remaining racemate is neutralized with one-half equivalent of an achiral acid or base. Replacing half of the relatively expensive resolving agent by a low-cost acid or base is economically very attractive.

In a classical resolution, the concentration of the resolving agent thus is twice as high as in Pope-Peachey method and thus is the solubility of the less soluble diastereomer salt by the Pope-Peachey method twice as high as in a classical resolution. Note that the crystallization of the less soluble diastereomer still can occur although its solubility will be also doubled. To obtain the same solubility as in a classical resolution, half of the solvent should be taken or a solvent should be chosen in which the diastereomeric salt dissolves less well.

The Pope-Peachey method was applied to the resolution of (±)-3MeOPEA with (*S*)-MA which, as shown in §3.2.2, is aided by addition of (*S*)-AcMA as depicted in Scheme 3.6.

Several achiral acids were screened with both racemic 3MeOPEA as with optically pure 3MeOPEA. Acetic acid (AcOH) was an ideal candidate since this did not gave crystalline salts even after prolonged standing and thus will not crystallize during the resolution. The oily salts with AcOH dissolved smoothly in toluene and ether (Et_2O). Because of the high boiling point of toluene, the salts of (±)-3MeOPEA with (*S*)-MA dissolve at reflux temperature but dissolve poorly at room temperature and thus can produce high yields. At room temperature the salts of (±)-3MeOPEA with (*S*)-MA dissolve less well in Et_2O. However, because of the low boiling point of Et_2O and subsequent low solubility at this temperature, Et_2O could not be used in the resolution. However, the low solubility of the diastereomeric salts in Et_2O at room temperature made it an ideal solvent to wash the isolated crystals.



Scheme 3.6 *Pope-Peachey resolution by addition of an additive.*

In the first experiments, one-half equivalent AcOH, one-half equivalent of (*S*)-MA, varying amounts of (*S*)-AcMA (replacing the AcOH) as a nucleation inhibitor and one equivalent of racemic 3MeOPEA were combined with 10 mL toluene in reactor tubes, heated to reflux (dissolution), and the reactor tubes were stirred for 30 minutes at 70°C and subsequently cooled to 20°C at 0.1°C·min^{-1}. The results are shown in Table 3.5.

Clearly, 1% (*S*)-AcMA (entry 4) prevents the nucleation of the more soluble diastereomer (*R*)-3MeOPEA,(*S*)-MA. This will keep the concentration of (*S*)-MA in solution higher, which will allow more of the less soluble diastereomer to crystallize. This results in nearly the same yield but much higher *de*'s. When less (*S*)-AcMA was added, the nucleation of the more soluble diastereomer could not be inhibited effectively and hence, a lower *de* was obtained. Note that the *de*'s found with these low concentrations of inhibitor are still somewhat higher than in the blank resolution in entry 1.

Table 3.5 *Pope-Peachey resolution with varying amounts of (S)-AcMA with slow cooling starting at 70°C.*

Entry	$\%_{(S)\text{-AcMA}}$	$\%_{AcOH}$	yield (%)[a]	*de* (%)[a]	S-factor[b]
1	none	50.0	47	53	0.51
2	0.01	49.99	46	56	0.50
3	0.1	49.9	47	61	0.56
4	1.0	49.0	43	97	0.83

[a] Results are averages of duplicate experiments. [b] S-factor = yield × *de* × 2.[5]

During these first experiments it was noticed that crystals started to form in the equilibration period of 30 minutes at 70°C. In later experiments, the mixtures were equilibrated at 100°C for 30 minutes and subsequently cooled to 20°C at 0.1°C·min^{-1}. In these experiments it was observed that primary nucleation started at 89°C–84°C. This slower primary nucleation gave strikingly different results as given in Table 3.6.

Table 3.6 *Pope-Peachey resolutions with varying amounts of (S)-AcMA with slow cooling starting at 100°C.*

Entry	$\%_{(S)\text{-AcMA}}$	$\%_{AcOH}$	yield (%)[a]	*de* (%)[a]	S-factor[b]
1	none	50.0	42–43	92–99	0.79–0.83
2	0.05	49.95	41–42	98–99	0.81–0.83
3	0.1	49.9	41–42	99	0.81–0.83
4	0.5	49.5	41–42	99	0.81–0.83
5	1.0	49.0	41–42	98–99	0.81–0.82

[a] Results are the extremes of triplicate experiments. [b] S-factor = yield × *de* × 2.[5]

The high *de*'s in the triplicate experiments without nucleation inhibitor (entry 1) may be explained by the slow primary nucleation together with fast secondary nucleation by the grinding action of the magnetic stirrer. The larger crystal surface generated by the grinding action of the magnetic stirrer will consume the supersaturation faster than the same

experiment without grinding. This will give more crystal mass of the less soluble diastereomer, which consumes (*S*)-MA from the solution and hereby producing a lower supersaturation of the more soluble diastereomer. Apparently, the metastable zone width in the blank resolution of entry 1 is large enough to prevent the primary nucleation of the more soluble diastereomer most of the time. However, addition of very small amounts of nucleation inhibitor does make the process more reproducible (entries 2–5).

The experiment with the lowest *de* (92%), started to crystallize at 84°C whereas the experiments with >98% *de* started to crystallize above 85°C. Screening Pope-Peachey resolutions in a test tube without a proper cooling program will, most likely, result in far from optimal (but thermodynamically driven) results.

All samples were washed with 2 × 2 mL Et_2O instead of 1 × 1 mL MEK in the classical resolution experiments described in §3.2. Better removal of the mother liquor explains the slightly higher *de*'s found in these experiments (99% *de versus* 97% *de*).

The combination of small amounts of nucleation inhibitors, slow primary nucleation, secondary nucleation by the grinding action of the magnetic stirrer and a Pope-Peachey resolution clearly is an economical method to yield more product with less resolving agent. If the resolving agent needs to be recycled, only the precipitated salts need to be liberated since the mother liquor contains only small amounts of resolving agent. Note that the experiments with increased *de* are strictly kinetic in nature. When these experiments are left to stand for a prolonged time (hours, years or even centuries), the experiments will eventually give the same results as the experiment in Table 3.5, entry 1 as this is the thermodynamical outcome of this resolution.

3.4 Experimental Section

General Information: Reagents were obtained from commercial sources and used without further purification.

Chiral HPLC analysis of 1-(3-methoxy-phenyl)ethylamine (3MeOPEA) salts was carried out on a Crownpak CR(-) column with an aqueous solution of $HClO_4$ (pH 2) as eluent at 20°C and 0.6 $mL \cdot min^{-1}$. UV-VIS detection was performed at 192 nm. The salts were dissolved in eluent and injected as such. (*R*)-3MeOPEA R_f: 39.59 min, (*S*)-3MeOPEA R_f: 42.98 min. When 1,3-bis(2-amino-2-propyl)benzene (1,3-BAPB) was used as additive, its percentage (against the sum of areas under both 3MeOPEA peaks) in the precipitated salts and mother liquors was measured with the same HPLC conditions (1,3-BAPB R_f: 9.17 min).

RP-HPLC analysis of other additives was performed by comparing a solution of the precipitated salts with a solution of the additive. The separation was performed on a Zorbax Extend C18 (4.6 × 50 mm, 3.5 μm) column, mobile phase: Solution A: Solution B = 95:5 (3 min) → (5 min) → 0:100 (4 min). Solution A: 9,65 g NH_4Ac; 2250 mL H_2O; 150 mL MeOH; 100 mL Acetonitrile, Solution B: 9.65 g NH_4Ac; 250 mL H_2O; 1350 mL MeOH; 900 mL acetonitrile, at 1.0 mL·min^{-1} and 22°C. Mass detection: API-ES. Amounts were determined relative to the area under the 3MeOPEA or MA peak without internal standard.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury 300MHz machine. Chemical shifts are denoted in δ (ppm) and are referenced to the residual protic solvent. The coupling constant *J* is denoted in Hz. Splitting patterns are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and bs (broad singlet).

Mass spectra were recorded by API-ES (electron spray ionization) by dissolving the samples in MeOH and injecting the solution as such. Mobile phase: acetonitrile : 0.1% formic acid in water 50 : 50 (1 min), flow: 0.2 mL·min^{-1}, injection volume: 5 μL.

Resolutions with additives as described in §3.2: A typical resolution experiment was performed by charging a Kimble reactor tube (Ø 25 × 150 mm) with a PTFE coated egg-shaped magnetic stirring bar (19 mm × 10 mm), 2.5 mL 0.13 M (±)-3MeOPEA in 2-butanone (MEK) and 2.5 mL 0.13M (*S*)- or (*R*)-mandelic acid (MA) in MEK. This mixture was stirred and after some minutes, crystals started to form. When additives were used, an equimolar amount of (±)-3MeOPEA or MA was replaced by a solution of the additive so the whole system remained neutral and of equal volume.

Figure 3.6 *Reactiv8, computer controlled reactor station*

The suspension was heated to dissolution and placed in a Reactiv8 computer controlled reactor station as depicted in Figure 3.6 and stirred magnetically at 600 rpm and 70°C for 30 min. Then the tubes were cooled to 20°C at 0.1°C·min^{-1} and kept at 20°C for an additional 8 hours. The formed crystals were collected on pre-weighed disposable filters and washed with 1.0 mL MEK. The solids were subsequently dried *in vacuo* and weighed and the *de* of the salts was determined.

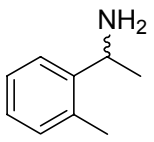
The eutectic composition lies at 71 mol% of the more soluble (*R*),(*S*)-diastereomer in methylethyl ketone (MEK). The solubility of the less soluble (*S*),(*S*)-diastereomer is 4.2 mmol·L^{-1} and that of the (*R*),(*S*)-diastereomer 8.4 mmol·L^{-1}.

Pope-Peachey resolutions with (*S*)-AcMA as described in §3.3: A typical Pope-Peachey resolution experiment was performed by charging a Kimble reactor tube (Ø 25 × 150 mm) with a PTFE coated egg-shaped magnetic stirring bar (19 mm × 10 mm), (±)-3MeOPEA (322 mg, 2.13 mmol, 1.0 eq), (*S*)-MA (162 mg, 1.06 mmol, 0.5 eq), AcOH (63 mg, 1.06 mmol, 0.5 eq) in toluene (10 mL). This mixture was stirred and after some minutes, crystals started to form. When additives were used, an equimolar amount of AcOH and toluene were replaced by a solution of (*S*)-AcMA (41.35 mg·10 mL^{-1} toluene) so the whole system remained neutral and of equal volume.

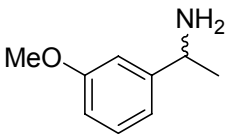
The suspension was heated to reflux (dissolution) and placed in a Reactiv8 computer controlled reactor station as depicted in Figure 3.6 and stirred magnetically at 600 rpm and 70°C (Table 3.5) or 100°C (Table 3.6) for 30 min. Then the tubes were cooled to 20°C with 0.1°C·min^{-1} and kept at 20°C for an additional 8 hours. The formed crystals were collected on pre-weighed disposable filters and washed with 2 × 2.0 mL Et_2O. The solids were dried *in vacuo*, weighted and subsequently the *de* of the salts was determined.

Large scale resolutions with and without 1,3-BAPB: A mixture of (±)-3MeOPEA (19.66 g, 130 mmol, 1.0 eq), (*S*)-MA (19.78 g, 130 mmol, 1.0 eq) and MEK (2.00 L) was mechanically stirred in a thermostated double jacketed 2 L flask at 20°C and the mixture was allowed to crystallize. At this point, 0.5 mol% or 1.0 mol% of 1,3-BAPB-2(*S*)-MA could be added: 323 mg or 646 mg respectively. The mixture was heated to 70°C and then stirred at this temperature for 30 minutes resulting in complete dissolution of the salts, the mixture was then cooled at 0.1°C·min^{-1} to 20°C. When the mixture reached 20°C, samples of ~0.6 mL were taken hourly and the solids were collected and sucked dry. The evaporated mother liquor and filter cake were analyzed by chiral HPLC for *de* and amount of additive. From both *de*'s, the yield can be calculated.[16] Note that small amounts of mother liquor are still present in the solids and hereby lowering and fluctuating the *de* (and thus the yield) of the later. Proper washing of the filter cake of the salts obtained after 5 days from the resolution with 1.0% additive furnished 17.1g (43%) of salts with 96% *de* and contained 1.4% 1,3-BAPB compared to 3MeOPEA.

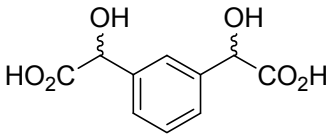
(±)-1-(2-Methylphenyl)ethylamine. A mixture of 2-methyl acetophenone (24.9g, 186 mmol, 1.0 eq), formamide (110 mL) and formic acid (45 mL) was heated to reflux for 2 h. Once the formation of CO_2 ceased, the mixture was cooled to room temperature before it was poured out in water (350 mL) and extracted with TBME (3 × 50 mL). The combined organic layers were washed with brine (25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was heated to reflux in 10% HCl (250 mL) overnight and subsequently allowed to cool to room temperature. The reaction mixture was washed with TBME (3 × 50 mL) and then basified to pH 10 with conc. NaOH. The mixture was extracted with TBME (3 × 50 mL) and the



combined organic layers were washed with brine (25 mL), dried (Na_2SO_4) and concentrated. This yielded a yellow oil with was further purified by distillation (0.03 mbar at 75°C) to furnish the title compound (15.5g, 62% yield) as a colorless oil. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.37 (d, *J*=6.6 Hz, 3H), 1.67 (bs, 2H), 2.37 (s, 3H), 4.37 (q, *J*=6.6 Hz, 1H), 7.13 (d, *J*=0.9 Hz 1H), 7.15 (d, *J*=1.2 Hz, 1H), 7.20-7.27 (m, 1H), 7.48 (d, *J*=7.5 Hz, 1H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 19.0, 24.5, 46.8, 124.0, 124.2, 126.4, 130.3, 134.3, 145.6 ppm. MS (EI): *m/z*= 119 [M-NH_3+H^+].



(±)-1-(3-Methoxyphenyl)ethylamine. A mixture of 3-methyl acetophenone (100 mL, 726 mmol, 1.0 eq), formamide (440 mL) and formic acid (190 mL) was heated to reflux for 2h. After CO_2 evolution stopped, the mixture was cooled to room temperature before it was poured out in water (1 L) and extracted with TBME (3 × 150 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was heated to reflux in 10% HCl (1.0L) overnight and subsequently allowed to cool to room temperature. The reaction mixture was washed with TBME (3 × 100 mL) and then basified to pH 10 with conc. NaOH. The mixture was extracted with TBME (3 × 150 mL) and the combined organic layers were washed with brine (100 mL), dried (Na_2SO_4) and concentrated. This yielded a brown oil which was further purified by distillation (0.006 mbar at 90°C) to furnish the title compound (66.5g, 61% yield) as a colorless oil. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.38 (d, *J*=3.6 Hz, 3H), 1.55 (bs, 2H), 3.81 (s, 3H), 4.09 (q, *J*=6.3, 1H), 6.75-6.79 (m, 1H), 6.90-6.91 (m, 2H), 6.93-7. ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 25.9, 51.5, 55.4, 111.6, 112.3, 129.7, 149.9, 160.0 ppm. MS (EI): *m/z*= 135[M-NH_3+H^+].

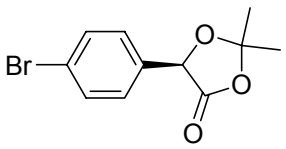


(±)-2-{3-[carboxy(hydroxy)methyl]phenyl}-2-hydroxyacetic acid. A mixture of isophthalaldehyde (1.14 g, 8.5 mmol, 1.0 eq), ZnI_2 (54 mg, 0.17 mmol, 0.02 eq) and I_2 (43 mg, 0.17 mmol, 0.02 eq) were stirred in CH_2Cl_2 (20 mL). To this mixture was added trimethylsilyl cyanide (2.72 mL, 20.4 mmol, 2.4 eq) dropwise upon which the reaction mixture started boiling. The reaction mixture was kept at 40°C overnight. The mixture was subsequently allowed to cool to room temperature and was carefully treated with sat. $NaHCO_3$ (20 mL). CO_2 gas was formed. WARNING: the aqueous layer contains the toxic sodium cyanide. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness. This delivered 2.76 g (65%) (±)-2-(3-{cyano[(trimethylsilyl)oxy]methyl}phenyl)-2-[(trimethylsilyl)oxy]acetonitrile as a brown oil which was used without further purification. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 0.24 (s, 18H), 5.52 (s, 2H), 7.48-7.49 (m, 3H), 7.49 (s, 1H)

ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 0.0, 63.5, 119.1, 124.3, 127.4, 129.9, 137.5 ppm. MS (EI): *m/z*=350 [M+NH_4^+].

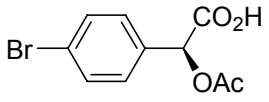
A solution of the cyanohydrin (1.92 g, 5.77 mmol, 1.0 eq) in dioxane (10 mL) was treated with 10% HCl (10 mL) and subsequently heated to 60°C for 6 hours after which the dioxane was removed by distillation. The residue was brought to pH 12 with conc. NaOH and washed with TBME (2 × 20 mL). The aqueous layer was filtered, brought to pH 1 with conc. HCl and washed with TBME (2 × 20 mL). The aqueous layer was concentrated to dryness and the residue stirred in acetone and filtered. The filtrate was concentrated to dryness to furnish 1.01 g (78%) of the title compound as a brown solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 5.03 (s, 2H), 6.90 (bs, 4H), 7.30-7.34 (m, 3H), 7.48 (s, 1H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 72.99, 125.54, 126.83, 126.86, 128.68, 140.77, 140.78, 174.71 ppm. MS (EI): *m/z*=225 [M-H^+], 451 [2M-H^+].

(*R*)-5-(4-bromophenyl)-2,2-dimethyl-1,3-dioxolan-4-one.

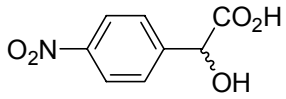


To an ice-cooled solution of (*R*)-4-bromomandelic acid[9] (25 g, 108 mmol, 1.0 eq) in acetone (50 mL) was added concentrated H_2SO_4 (6.34 mL, 119 mmol, 1.1 eq) dropwise in 5 minutes thereby keeping the temperature below 22°C. After addition, Na_2SO_4 (3.5 g) was added to the mixture and stirring was continued for another 4.5 hours after which the reaction mixture was carefully poured out in a mixture of sat. $NaHCO_3$ (350 mL) and ice (250 g). The resulting mixture was extracted with CH_2Cl_2 (1 × 100 mL + 2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4) and concentrated to yield 15.4 g (53%) of the title compound as a yellow oil. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.67 (s, 3H), 1.71 (s, 3H), 5.34 (s, 1H), 7.35 (d, *J*=8.7 Hz, 2H), 7.54 (d, *J*=8.4 Hz, 2H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 26.1, 27.2, 75.1, 111.2, 123.0, 128.0, 131.8, 133.5, 170.9 ppm. MS (EI): *m/z*= 229 [M+H_2O-$CH_3(CO)CH_3$-H^+].

(*S*)-2-(4-bromophenyl)-2-(acetyloxy)acetic acid. (*S*)-4-bromomandelic acid[9] (500 mg, 2.16 mmol, 1.0 eq) was suspended in CH_2Cl_2 (10 mL). Acetyl chloride (0.19 mL, 2.60 mmol, 1.2 eq) and Et_3N (0.33 mL, 2.38 mmol, 1.1 eq) were added dropwise and the resulting mixture was stirred for another hour after which the reaction mixture was poured out in ice water (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with 1M HCl (10 mL) and brine (10 mL), subsequently dried (Na_2SO_4) and concentrated to dryness. This yielded 530 mg (90%) of the title compound as a white solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 2.11 (s, 3H), 5.80 (s, 1H), 7.41 (d, *J*=8.4Hz,

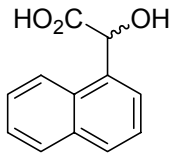


2H), 7.61 (d, *J*=8.4 Hz, 2H), 12.90 (bs, 1H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 21.1, 74.1, 123.0, 130.4, 132.3, 134.5, 170.2, 170.4 ppm. MS (EI): *m/z*= 271 [M-H$^+$].



(±)-4-nitromandelic acid. A mixture of 4-nitrobenzaldehyde (2.57 g, 17 mmol, 1.0 eq), ZnI_2 (54 mg, 0.17 mmol, 0.01 eq) and I_2 (43 mg, 0.17 mmol, 0.01 eq) were stirred in CH_2Cl_2 (20 mL). To this mixture was added trimethylsilyl cyanide (2.72 mL, 20.4 mmol, 1.2 eq) dropwise upon which the reaction mixture started boiling. The reaction mixture was kept at 40°C overnight. The mixture was subsequently allowed to cool to room temperature and was carefully treated with sat. $NaHCO_3$ (20 mL) under expulsion of CO_2 gas. WARNING: the aqueous layer contains the toxic sodium cyanide. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness. This delivered 2.76 g (65%) (±)-2-(4-nitrophenyl)-2-[(trimethylsilyl)oxy]acetonitrile as a brown oil which was used without further purification. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 0.27 (s, 9H), 5.58 (s, 1H), 7.66 (d, *J*=9.0 Hz, 2H), 8.27 (d, *J*=9.0 Hz, 2H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= -0.1, 62.9, 118.4, 124.4, 127.4, 143.1, 148.7 ppm. MS (EI): *m/z*=177 [M+H$^+$].

A solution of the cyanohydrin (2.76 g, 11.0 mmol, 1.0 eq) in dioxane (10 mL) was treated with 10% HCl (10 mL) and subsequently heated to 60°C for 6 hours after which the dioxane was removed by distillation. The residue was brought to pH 12 with conc. NaOH and washed with TBME (2 × 20 mL). The aqueous layer was filtered, brought to pH 1 with conc. HCl and extracted with TBME (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness to furnish 1.29 g (57%) of the title compound as an orange solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 3.90 (bs, 1H), 5.23 (s, 1H), 6.10 (bs, 1H), 7.69 (d, *J*=9.0 Hz, 2H), 8.20 (d, *J*=8.7 Hz, 2H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 72.4, 124.0, 128.4, 147.6, 148.3, 173.8 ppm. MS (EI): *m/z*=196 [M-H$^+$], 393 [2M-H$^+$].

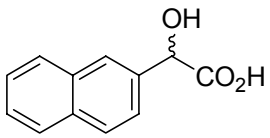


(±)-2-hydroxy-2-(naphthalen-1-yl)acetic acid. A mixture of 1-naphthaldehyde (2.31 mL, 17 mmol, 1.0 eq), ZnI_2 (54 mg, 0.17 mmol, 0.01 eq) and I_2 (43 mg, 0.17 mmol, 0.01 eq) were stirred in CH_2Cl_2 (20mL). To this mixture was added trimethylsilyl cyanide (2.72 mL, 20.4 mmol, 1.2 eq) dropwise upon which the reaction mixture started boiling. The reaction mixture was kept at 40°C overnight. The mixture was subsequently allowed to cool to room temperature and was carefully treated with sat. $NaHCO_3$ (20 mL). CO_2 gas evolved. WARNING: the aqueous layer contains the toxic sodium cyanide. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated

to dryness. This delivered 3.63 g (83%) (±)-2-(naphthalen-1-yl)-2-[(trimethylsilyl)oxy]-acetonitrile as a brown oil which was used without further purification. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 0.19 (s, 9H), 6.04 (s, 1H), 7.47 (t, *J*=7.8 Hz, 1H), 7.51-7.63 (m, 2H), 7.69 (d, *J*=7.2 Hz, 1H), 7.89 (d, *J*=8.1 Hz, 1H), 8.16 (d, *J*=8.7 Hz, 1H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 0.1, 63.0, 119.4, 123.4, 125.3, 125.7, 126.6, 127.3, 129.2, 130.2, 130.7, 131.6, 134.2 ppm. MS (EI): *m/z*= 229 [M-HCN+H$^+$].

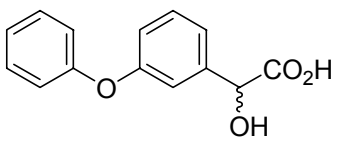
A solution of the cyanohydrin (3.63 g, 14.2 mmol, 1.0 eq) in dioxane (10 mL) was treated with 10% HCl (10 mL) and subsequently heated to 60°C for 6 hours after which the dioxane was removed by distillation. The residue was brought to pH 12 with conc. NaOH and washed with TBME (2 × 20 mL). The aqueous layer was filtered, brought to pH 1 with conc. HCl and extracted with TBME (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness to furnish 1.88 g (65%) of the title compound as a yellow solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 5.65 (s, 1H), 6.10 (bs, 1H), 7.45-7.59 (m, 4H), 7.84-7.94 (m, 2H), 8.25-8.28 (m, 1H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 71.7, 125.3, 126.0, 126.3, 126.4, 126.7, 128.9, 129.1, 131.3, 134.1, 137.0, 175.0 ppm. MS (EI): *m/z*=201 [M-H$^+$].

(±)-2-hydroxy-2-(naphthalen-2-yl)acetic acid. A mixture of 2-naphthaldehyde (2.66 g, 17 mmol, 1.0 eq), ZnI_2 (54 mg, 0.17 mmol, 0.01 eq) and I_2 (43 mg, 0.17 mmol, 0.01 eq) were stirred in CH_2Cl_2 (20mL). To this mixture was added trimethylsilyl cyanide (2.72 mL, 20.4 mmol, 1.2 eq) dropwise upon which the reaction mixture started boiling. The reaction mixture was kept at 40°C overnight. The mixture was subsequently allowed to cool to room temperature and was carefully treated with sat. $NaHCO_3$ (20 mL). CO_2 gas was formed. WARNING: the aqueous layer contains the toxic sodium cyanide. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness. This delivered 3.41 g (78%) (±)-2-(naphthalen-2-yl)-2-[(trimethylsilyl)oxy]acetonitrile as a brown oil, which was used without further purification. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 0.25 (s, 9H), 5.64 (s, 1H), 7.50-7.56 (m, 3H), 7.83-7.92 (m, 4H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 0.07, 64.15, 119.43, 123.91, 125.98, 127.00, 127.19, 128.05, 128.50, 129.36, 133.24, 133.82, 133.84 ppm. MS (EI): *m/z*= 229 [M-HCN+H$^+$].



A solution of the cyanohydrin (3.41 g, 13.4 mmol, 1.0 eq) in dioxane (10 mL) was treated with 10% HCl (10 mL) and subsequently heated to 60°C for 6 hours after which the dioxane was removed by distillation. The residue was brought to pH 12 with conc. NaOH and washed with TBME (2 × 20 mL). The aqueous layer was filtered, brought to pH 1 with

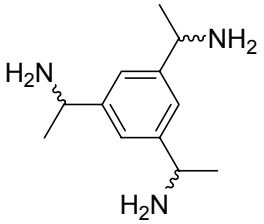
conc. HCl and extracted with TBME (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness to furnish 1.07g (40%) of the title compound as a yellow solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 5.28 (s, 1H), 6.10 (bs, 1H), 7.52-7.65 (m, 3H), 7.91-8.01 (m, 4H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 71.7, 125.3, 126.0, 126.3, 126.4, 126.7, 128.9, 129.1, 131.3, 134.1, 137.0, 175.0 ppm. MS (EI): *m/z*=201 [M-H$^+$].



(±)-3-phenoxymandelic acid. A mixture of 3-phenoxybenzaldehyde (3.37 g, 17 mmol, 1.0 eq), ZnI_2 (54 mg, 0.17 mmol, 0.01 eq) and I_2 (43 mg, 0.17 mmol, 0.01 eq) were stirred in CH_2Cl_2 (20mL). To this mixture was added trimethylsilyl cyanide (2.72 mL, 20.4 mmol, 1.2 eq) dropwise upon which the reaction mixture started boiling. The reaction mixture was kept at 40°C overnight. The mixture was subsequently allowed to cool to room temperature and was carefully treated with sat. $NaHCO_3$ (20 mL). CO_2 gas was formed. WARNING: the aqueous layer contains the toxic sodium cyanide. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness. This delivered 4.65 g (92%) (±)-2-(3-phenoxyphenyl)-2-[(trimethylsilyl)oxy]acetonitrile as a brown oil, which was used without further purification. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 0.20 (s, 9H), 5.44 (s, 1H), 6.98-7.03 (m, 3H), 7.08-7.19 (m, 3H), 7.31-7.37 (m, 3H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= -0.1, 63.4, 116.6, 119.1, 119.5, 120.9, 124.0, 130.0, 130.5, 138.3, 156.7, 158.2 ppm. MS (EI): *m/z*= 315 [M+NH_4^+].

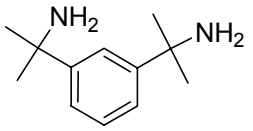
A solution of the cyanohydrin (4.48 g, 15.1 mmol, 1.0 eq) in dioxane (10 mL) was treated with 10% HCl (10 mL) and subsequently heated to 60°C for 6 hours after which the dioxane was removed by distillation. The residue was brought to pH 12 with conc. NaOH and washed with TBME (2 × 20 mL). The aqueous layer was filtered, brought to pH 1 with conc. HCl and extracted with TBME (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness to furnish 3.10g (84%) of the title compound as a brown solid. ^{1}H-NMR (300MHz, DMSO-d6): δ= 5.02 (s, 1H), 5.90 (bs, 1H), 6.88-6.93 (m, 1H), 6.97-7.02 (m, 2H), 7.04-7.06 (m, 1H), 7.09-7.19 (m, 2H), 7.30-7.40 (m, 3H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 72.0, 116.5, 117.7, 118.8, 121.7, 123.6, 129.8, 130.1, 142.4, 156.5, 156.7, 173.8 ppm. MS (EI): *m/z*=243 [M-H$^+$].

(±)-1-[3,5-bis(1-aminoethyl)phenyl]ethan-1-amine. To a solution of 1,3,5-triacetylbenzene (5.00 g, 24.5 mmol, 1.0 eq) in pyridine (90 mL) was added hydroxylamine hydrochloride (17.0 g, 245 mmol, 10 eq) portionwise. The temperature rose to 34°C upon addition. The reaction mixture was heated to 75°C. After 30 minutes, the reaction mixture

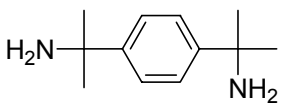


was allowed to cool to room temperature. The mixture was parted between water (3 × 50 mL) and EtOAc (400 mL). The organic layer was washed with brine (50 mL), dried (Na_2SO_4), concentrated and the last traces of pyridine were removed by co-distillation with toluene (3x). This furnished 6.0 g (98%) *N*-(1-{3,5-bis[1-(hydroxyimino)ethyl]phenyl}ethylidene)-hydroxylamine. ^{1}H-NMR (300MHz, DMSO-d6): δ= 2.17 (s, 9H), 7.88 (s, 3H), 11.27 (s, 3H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 11.6, 122.8, 137.2, 152.6 ppm. MS (EI): *m/z*= 191 [M-NH_3+H^+].

The trisoxime (1.0 g, 4.01 mmol, 1.0 eq) was suspended in MeOH (50 mL) and Pd/C (10% Pd, 60 mg) was added carefully. The mixture was hydrogenated at ambient pressure. After 2 days, ^{1}H-NMR showed complete conversion and the suspension was filtered over a path of Celite. The filtrate was concentrated to yield 0.70 g (85%) of the title compound as a colorless oil. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.39 (d, *J*=6.6Hz, 9H), 1.52 (bs, 6H), 4.12 (q, J=6.6Hz, 3H), 7.20 (s, 3H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 25.8, 51.4, 121.6, 148.3 ppm. MS (EI): *m/z*= 208 [M+H^+], 191 [M-NH_3+H^+].



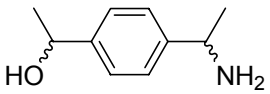
1,3-bis(2-amino-2-propyl)benzene (1,3-BAPB). At 200°C and 0.02mbar $CeCl_3 \cdot 7H_2O$ was dried for 2 hours to a white powder. Dry $CeCl_3$ (11.5 g, 46.8 mmol, 6 eq) was suspended in fresh THF (250 mL) and heated to reflux for 1 hour. The suspension was cooled to -60°C and a solution of MeLi (~1.6M in Et_2O, 29 mL, 46.8 mmol, 6 eq) was added over 5 minutes which resulted in a yellow suspension. After 20 minutes, a solution of 1,3-dicyanobenzene (1.0 g, 7.80 mmol, 1.0 eq) in fresh THF (20 mL) was added over 1 minute. The now orange suspension was allowed to warm to room temperature and after 3 hours the reaction mixture was poured out in water. The resulting mixture was brought to pH 10 with conc. NaOH and was subsequently parted with EtOAc (3 × 200 mL) and solids were removed during the first extraction and discarded. The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4) and concentrated to yield a brown oil which was purified by column chromatography over silica with a gradient of 1M NH_3 in MeOH and CH_2Cl_2. This yielded 0.78 g (52%) of the pure title compound. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.51 (s, 12H), 1.66 (bs, 4H), 7.25-7.37 (m, 3H), 7.67-7.69 (m, 1H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 33.1, 52.7, 121.0, 122.7, 128.1, 150.3 ppm. MS (EI): *m/z*= 159 [M-2NH_3+H^+], 176 [M-NH_3+H^+].



1,4-bis(2-amino-2-propyl)benzene (1,4-BAPB). The compound was prepared in a similar way as the procedure for 1,3-BAPB. This yielded the pure title compound in 0.60 g

(40%) as white solid. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.42 (s, 12H), 1.52 (bs, 4H), 7.39 (s, 4H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 32.9, 52.2, 124.6, 148.3 ppm. MS (EI): *m/z*= 159 [M-2NH_3+H^+], 176 [M-NH_3+H^+].

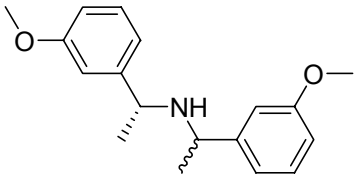
(±)-1-[4-(1-aminoethyl)phenyl]ethan-1-ol. A solution of (±)-1-(4-bromophenyl)ethan-1-amine (2.0 mL, 14.0 mmol, 1.0 eq) in CH_2Cl_2 (20 mL) was treated with Boc_2O (3.66 g, 16.8 mmol, 1.2 eq) and the temperature rose from 18°C to 27°C with gas evolution. After 10 minutes a suspension forms. After 90 minutes the reaction mixture was concentrated and the residue was stirred in Et_2O (10 mL). The solids were collected by filtration and dried on air. This yielded 3.52 g (84%) of (±)-*tert*-butyl-*N*-[1-(4-bromophenyl)ethyl]carbamate as a white solid. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.36-1.43 (m, 12H), 4.71 (bs, 1H), 4.75-4.83 (m, 1H), 7.16 (d, *J*=8.7Hz, 2H), 7.43 (d, *J*=8.4Hz, 2H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 22.7, 28.5, 49.8, 49.7, 120.9, 127.7, 131.7, 143.4, 155.1 ppm. MS (EI): *m/z*= 183 [M-NH_2Boc+H^+], 322 [M+Na^+].



The bromide (3.5 g, 11.7 mmol, 1.0 eq) was dissolved in THF (25 mL) and cooled to -78°C. A solution of n-BuLi (2.5M in hexane, 10.3 mL, 25.6 mmol, 2.2 eq) was added dropwise so the temperature was kept below -65°C. On addition, a very thick suspension was formed and extra THF (5 mL) was added to keep the reaction mixture stirable. Next, acetaldehyde (1.65 mL, 29.3 mmol, 2.5 eq) was added to the suspension in one portion upon which the internal temperature rose to -44°C and a clear solution was formed. The reaction mixture was allowed to warm to room temperature and was subsequently parted between water (20 mL) and EtOAc (2 × 50 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to a colorless oil which was stirred in CH_2Cl_2 (20 mL). The resulting solids were removed by filtration and the filtrate was concentrated. The resulting oil was purified by column chromatography over silica with 25:75 EtOAc:heptanes as eluent. The compound with R_f 0.22 proved to be the desired (±)-*tert*-butyl *N*-{1-[4-(1-hydroxyethyl)phenyl]ethyl} carbamate. This compound was isolated as a colorless oil (2.85 g, 92%). ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.40-1.50 (m, 12H), 4.78 (bs, 1H), 4.86-4.92 (m, 1H), 7.27 (d, *J*=9.0Hz, 2H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 22.7, 25.2, 28.5, 49.9, 70.0, 79.5, 125.7, 126.0, 143.3, 144.9, 155.2 ppm. MS (EI): *m/z*= 288 [M+Na^+].

The Boc protected amine (500 mg, 1.89 mmol, 1.0 eq) was suspended in CH_2Cl_2 (20 mL) and trifluoroacetic acid (0.84 mL, 11.3 mmol, 6 eq) was added after which the reaction mixture became a clear solution and was heated to reflux for 2 days. ^{1}H-NMR showed complete conversion. The reaction mixture was concentrated and stirred at room temperature in a mixture of THF (3 mL) and 1M NaOH (6 mL) for 2. Then the THF was

removed by distillation and the aqueous mixture was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (10 mL) and concentrated to yield 250 mg (80%) of the title compound as a white solid. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.37 (d, *J*=6.6Hz, 3H), 1.49 (d, *J*=6.3Hz, 3H), 1.80 (bs, 3H), 4.10 (q, *J*=6.6Hz, 1H), 4.88 (q, *J*=6.3Hz, 1H), 7.29-7.36 (m, 4H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 25.3, 25.4, 51.0, 69.7, 125.7, 125.8, 126.5, 145.0 ppm. MS (EI): *m/z*= 131 [M-NH_3-H_2O+H^+], 149 [M-NH_3+H^+].



[(*1R*)-1-(3-methoxyphenyl)ethyl][(±)-1-(3-methoxyphenyl)ethyl]amine. A mixture of (*R*)-1-(3-methoxyphenyl)ethan-1-amine (0.895 g, 5.92 mmol, 1.0 eq), 3-methoxyacetophenone (0.816 mL, 5.92 mmol, 1.0 eq), $Ti(O^iPr)_4$ (5.3 mL, 17.8 mmol, 3.0 eq) was stirred for 20 minutes and became warm to the touch. Pd/C (10% Pd, 120 mg) was added and a hydrogen atmosphere was applied by a balloon overnight. The reaction mixture was basified with 1M NaOH (25 mL), EtOAc (20 mL) was added and the resulting mixture was filtered over a pad of Celite. The aqueous layer was extracted with more EtOAc (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and concentrated to dryness. The title compound was isolated as a colorless oil (1.43 g, 85%). RP-HPLC analysis of the material revealed an 1:10 mixture of diastereomers. ^{1}H-NMR (300MHz, $CDCl_3$): δ= 1.28 (d, *J*=6.6Hz, 6H), 3.51 (q, *J*=6.6Hz, 1H), 3.82 (s, 6H), 6.75-6.83 (m, 6H), 7.22-7.28 (m, 2H) ppm. ^{13}C-NMR (75MHz, $CDCl_3$): δ= 25.01, 55.27, 55.30, 112.21, 112.30, 112.36, 112.39, 119.21, 129.48, 147.66, 159.90 ppm. MS (EI): *m/z*= 286 [M+H^+].

3.5 References

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12 The yield of 35% with 99% *de* reported in the literature[4] must then be either kinetic (meaning that the solids were collected before the more soluble diastereomer crystallized) or the result of hydrate or solvate formation.

13 Similar observations in other systems have been made: J.S.C. Loh, W.J.P. van Enckevort, E. Vlieg, C. Gervais, R.F.P. Grimbergen, B. Kaptein, *Cryst. Growth Des.* **2006**, *6*, 861.

14 With 1% additive some additive is initially incorporated but over a period of hours slowly returns into solution.

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16 Determination of the yield from the compositions of both solids and mother liquor determined by chiral HPLC:

Where:

a	=	mass solids precipitated
b	=	mass material in the mother liquor
R_{start} / S_{start}	=	mass of (R) / (S) enantiomer at the start of the experiment
R_{sf} / S_{sf}	=	fraction of (R) / (S) enantiomer in the precipitated salts (range: 0-1)
R_{MLf} / S_{MLf}	=	fraction of (R) / (S) enantiomer in the mother liquor (range: 0-1)
Yield	=	percentage of the precipitated solids.

The overall composition is divided between the crystallized material and the material in the mother liquor:

$$R_{start} = a \times R_{sf} + b \times R_{MLf} \rightarrow a = \frac{R_{start} - b \times R_{MLf}}{R_{sf}} \tag{3.2}$$

$$S_{start} = a \times S_{sf} + b \times S_{MLf} \tag{3.3}$$

When equations 3.2 and 3.3 are combined, equation 3.4 is derived:

$$S_{start} = \frac{R_{start} \times S_{sf}}{R_{sf}} - \frac{b \times R_{MLf} \times S_{sf}}{R_{sf}} + b \times S_{MLf} \tag{3.4}$$

The fraction of total material in solids and mother liquor is, of course, 1:

$$R_{sf} = 1 - S_{sf} \tag{3.5}$$

$$R_{MLf} = 1 - S_{MLf} \tag{3.6}$$

When equations 3.4, 3.5 and 3.6 were combined, equation 3.7 was derived.

$$S_{start} = \frac{R_{start} \times S_{sf}}{1 - S_{sf}} - \frac{b \times S_{sf}}{1 - S_{sf}} + \frac{b \times S_{sf} S_{MLf}}{1 - S_{sf}} + b \times S_{MLf}$$

$$\rightarrow \quad b = \frac{S_{start} - \dfrac{R_{start} \times S_{sf}}{1 - S_{sf}}}{S_{MLf} - \dfrac{S_{sf}}{1 - S_{sf}} + \dfrac{S_{sf} \times S_{MLf}}{1 - S_{sf}}} \tag{3.7}$$

When 'a' and 'b' are calculated from the chiral HPLC data, the yield can be determined:

$$Yield = \frac{a}{a+b} \times 100\% \tag{3.8}$$

When the *ee* of the mother liquor and the solids both approach 0%, then the equations above are not useful to determine the yield since the error will be large.

Chapter 4

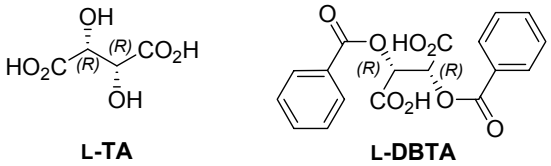
The Effect of Additives

In this chapter the resolutions of racemates by diastereomeric salt formation which are modified by additives are described. Polymorphic transitions and nucleation inhibition have been observed. Furthermore, generation of supersaturation by abrasive grinding followed by dissolution of small particles and resolution by solid-solution formation of cyclic phosphoric acid families is described also.

4.1 Introduction

Crystallization of mixtures of compounds, closely related in structure lead to behavior that differs from that of mixtures without these additives as shown in first[1] and second[2] generation Dutch Resolution. These compounds can be effective nucleation inhibitors[3], habit/growth modifiers[4] and polymorph inhibitors,[5] all of which are of great interest to the chemical industry.

As described in Chapter 3, a resolution by diastereomeric salt formation (hereafter called resolution) can benefit from the addition of certain molecules which resemble either the resolving agent or the racemate. In Chapter 3 the main focus was on the resolution with mandelic acid. For other frequently used resolving agents like tartaric acid (TA) and *O,O'*-dibenzoyl tartaric acid (DBTA) (see Scheme 4.1), additives have not been described. However, for DBTA[1] a family of resolving agents is available, which can be tested as additives.

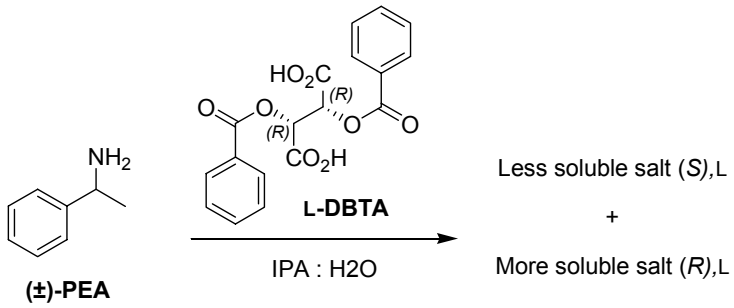


Scheme 4.1 *Frequently used acidic resolving agents.*

In this section the design, synthesis and testing of these compounds as additives in resolutions with these resolving agents is described.

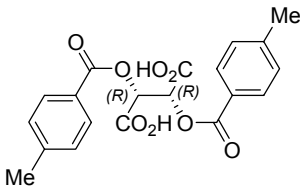
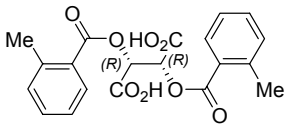
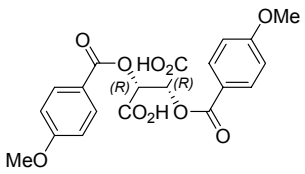
4.2 Resolution of (±)-PEA with DBTA

The resolution of racemic 1-phenylethylamine (PEA) with L-dibenzoyltartaric acid (DBTA), as shown in Scheme 4.2, was investigated. The resolution was performed in a mixture of isopropanol and water (4:1). The blank resolution (entry 1 in Table 4.1) proceeded reasonably but might be improved by the addition of nucleation inhibitors. Several additives that resembled the resolving agent, with the same absolute configuration, were tested.



Scheme 4.2 *Resolution of (±)-1-phenylethylamine with L-dibenzoyl tartaric acid.*

Table 4.1 *Results of resolutions of (±)-PEA with L-DBTA with additives.*

Entry	Additive	% additive[b]	Yield (%)[a]	*de* (%)[a]	S-factor[c]
1	none	-	53	49	0.52
2[d]		6	49	57	0.56
3[d]		6	55	41	0.45
4[d]		6	49	59	0.57

[a] All results are averages of duplicates. [b] Less L-DBTA was added to compensate for the acid groups of the additives so the system was kept neutral and of equal volume. [c] S-factor = yield × *de* × 2.[7] [d] Commercially available.

No large improvements were seen with these additives. The additive in entry 3 even shows a decrease in *de*. The samples were investigated with X-Ray Powder Diffraction (XRPD) and the results are given in Figure 4.1. The top two spectra represent the pure diastereomers, which were crystallized from the same solvent mixture. Careful examination of the XRPD spectra of these separate (*S*)-PEA,L-DBTA (less soluble) and (*R*),L (more soluble) diastereomers and the resolution without additives (**blank**) shows that the blank experiment does not contain these separate pure diastereomer crystals. This can be explained by either (end-)solid solution formation between the diastereomers or polymorphism.

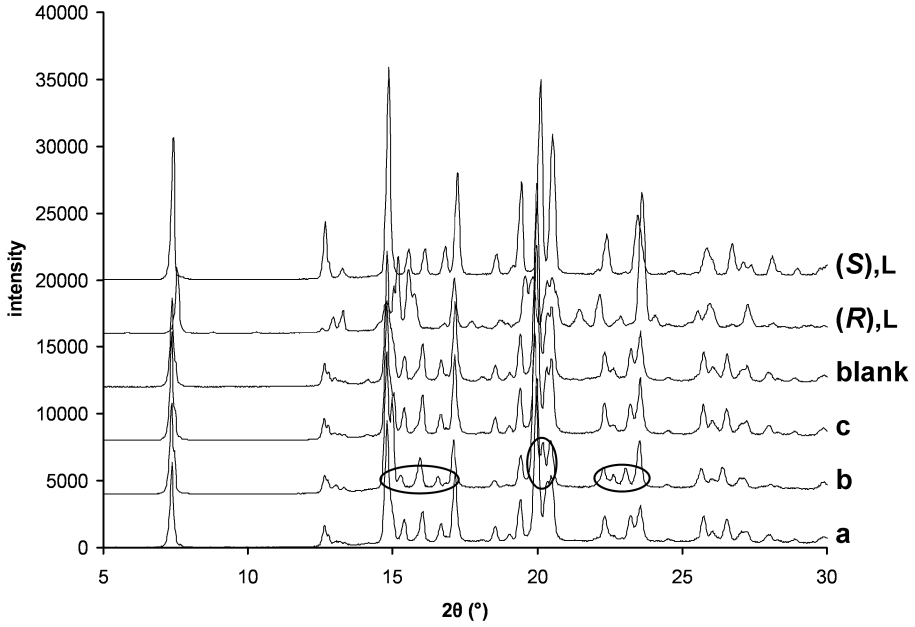


Figure 4.1 *XRPD of solids obtained from single diastereomers and resolutions.*

The solids of the experiments with additives were investigated also. The XRPD spectra **a**, **b** and **c** correspond to entries 2, 3 and 4 in Table 4.1 respectively. On first glance there is no difference. However, small peak changes were found in spectrum **b**, highlighted with circles. This indicates incorporation of the additive in the crystals and/or polymorphism of one or both of the diastereomers. Indeed, HPLC analysis of the solids indicated the presence of the additive. Since the PEA salt of this additive does not crystallize at 20°C at the concentration used in this experiment, it must form a solid solution with one or both

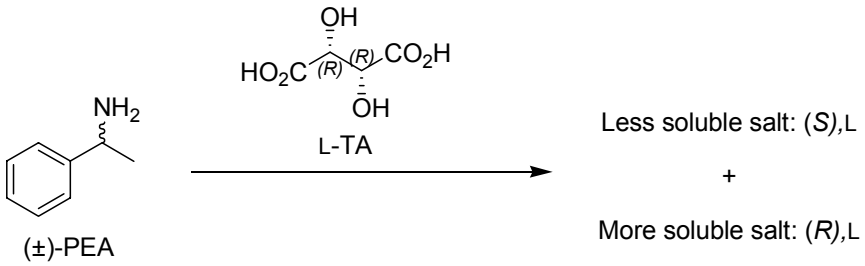
crystallized diastereomers and hence, slightly alter the XRPD. However, with this information, the formation of a different polymorph cannot be ruled out.

The other additives do not reveal a different XRPD than obtained in the blank experiment. The small increase in *de* in entries 2 and 4 can be explained by poor nucleation/growth inhibition and higher dilution since in the experiments with additives, 94% resolving agent was used compared to 100% in the blank resolution in entry 1.

4.3 Resolution of (±)-PEA with TA

The resolution of racemic 1-phenylethylamine (PEA) with tartaric acid (TA) is depicted in Scheme 4.3. This had been described as a low cost experiment which chemistry students could perform.[6] According to the article, the resolution was performed with D-TA and delivered (–)-PEA in a good yield and high *ee* after liberation. However, repeating this experiment delivered the opposite enantiomer, (+)-PEA in a similar yield and *ee*. Most likely, the author made a typing error. Moreover, since the experiment is described to be a low cost experiment, it seems illogical to use the more expensive D-TA than the low cost L-TA. The article also mentions that the formed salt is the *"(–)-amine-(+)-hydrogen tartrate"* which is consistent with the positive optical rotation of L-TA.

The author mentioned that most students obtained (*S*)-PEA in more than 95% *ee* after liberation of the crystals. This indicates that the least soluble diastereomeric salt does not form an end-solid solution (or at least not a large one). The yield of such a resolution could be improved by the addition of nucleation inhibitors if the concentration were to be increased.



Scheme 4.3 *Resolution of (±)-1-phenylethylamine with L-tartaric acid.*

The resolution in the article was performed in boiling methanol. This furnishes the diastereomeric salt in 32% yield and 95% *de* (S-factor: 0.61)[7] which is not a bad resolution as such. However, higher yields could be obtained either by further cooling or utilization of

a higher boiling solvent since the solubility increases at higher temperatures. A phase diagram was constructed for (±)-PEA and L-TA in the higher boiling ethanol in stead of methanol, at 20°C (black) and 70°C (grey) as shown in Figure 4.2.

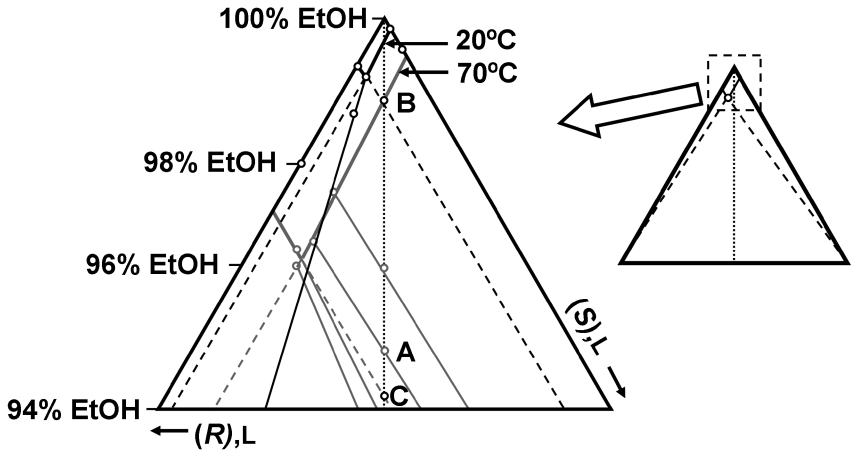


Figure 4.2 *Top 6% of the ternary phase diagram for the L-tartaric acid salt of (±)-1-phenylethylamine at 20°C (black) and 70°C (grey).*

As can be seen from the phase diagram, the solubility of the racemic material is more than four fold higher at 70°C than at 20°C. Furthermore, starting from point **A** at 70°C delivered nearly pure (*S*),L when filtered at 70°C which tells us that (nearly) no end-solid solution is formed. No large differences in end-solid solution formation are expected at 20°C. An additive that inhibits the more soluble diastereomer successfully then should furnish solids with high *de*'s.

Point **B** represents a racemic mixture that is saturated in (*S*),L at 70°C. Thus starting from a racemic mixture, the highest concentration at which a clear solution is obtained at 70°C is point **B**. When this mixture is then cooled to 20°C, point **B** is located in the region where only (*S*),L is supersaturated and will start to crystallize. Since the more soluble diastereomer (*R*),L is not saturated, it will not crystallize and hence, cannot be inhibited by additives.

Since only the nucleation of the more soluble diastereomer has to be inhibited, the starting point does not necessarily need to be located above point **B** where both the more and the less soluble diastereomers are under-saturated. Each mixture between point **B** and **C** represent mixtures in which the more soluble diastereomer is under saturated but the less soluble diastereomer has crystallized at thermodynamic equilibrium. Since only the more soluble diastereomer needs to be inhibited it needs to be dissolved at the start of the cooling

program. The less soluble diastereomer however, may have crystallized at the start of the cooling program since it does not need to be inhibited. Thus a resolution with an effective nucleation inhibitor and starting from a mixture located between points **B** and **C** could deliver pure (*S*),L and will produce higher yields than a mixture with lower concentrations.

A downside to this procedure is that the operator of the resolution now cannot visually inspect whether the more soluble diastereomer has dissolved. However, the phase diagram has shown that this is the case at point **A** and thus an ideal starting point for the resolution aided with a nucleation inhibitor and furnishes 80% yield and 19% *de* (S-factor: 0.30) as given in Table 4.2, entry 1.

The experiments in Table 4.2 are performed with D-TA and not with L-TA which only means that the least soluble diastereomer is (*R*),D and the more soluble diastereomer is (*S*),D. If an additive would work as a nucleation inhibitor on the more soluble (*S*),D diastereomer, this would give 100% *de* and 48% yield (S-factor: 0.95) of the less soluble (*R*),D diastereomer.

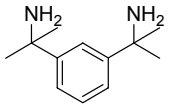
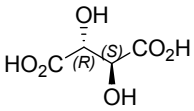
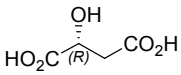
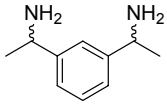
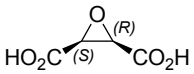
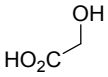
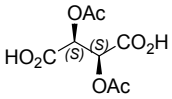
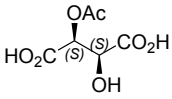
It is clear that the addition of additives did not result in the expected >95% *de*. Only the bi-functional additive in entry 5 and the mixtures in entries 10 and 11 gave a reasonable increase in resolution efficiency. Combinations of additives in entries 10 and 11 do not seem to give higher efficiencies than the bi-functional additive in entry 5 alone.

Since the phase diagram in Figure 4.2 indicated that there was no end-solid solution present it is strange to see that some of the additives give an effect on the resolution but >95% *de* was never found. This might be explained by poor nucleation inhibition performance or by polymorphism. To investigate this, the crystals of some of the resolutions were investigated by XRPD

First, the blank experiment was compared to the separate diastereomers, (*S*),L (less soluble) and (*R*),L (more soluble) which shows that the blank experiment is indeed the "sum" of its separate diastereomers and thus does not form a measurable end-solid solution.

The XRPD spectra **a–d** corresponds to entries 8–11 in Table 4.2 respectively. The duplicate experiment in entry 11 gave two different spectra which are shown in Figure 4.3 as $\mathbf{d_1}$ and $\mathbf{d_2}$. Strikingly, all of the experiments with additives have completely different XRPD spectra than the resolution without additives (**blank**) even though the increase in *de* was minimal. This means that all the additives effectively block the nucleation of both diastereomers present in the blank experiment. For experiments **a**, **b**, **c** and $\mathbf{d_2}$ this is visually most evident in the peak at 2θ: 6.0º but also other peaks are different than the blank experiment. The spectrum $\mathbf{d_1}$ shows even other polymorphs than all the other experiments.

Table 4.2 *Results of resolutions of (±)-PEA with L-TA aided by additives.*

Entry	Additive[a]	% additive[b]	Yield (%)	*de* (%)	S-factor[c]
1	none	-	80	19	0.30
2[d]		3	64	21	0.27
3[e]		6	74	26	0.38
4[e]		6	74	32	0.47
5[e]		3	52	64	0.67
6[e]		6	73	26	0.37
7[e]		12	72	29	0.42
8[f]		6	72	22	0.32
9[f]		6	72	21	0.30
10	entry 5 + entry 8	3 + 6	55	59	0.65
11	entry 5 + entry 9	3 + 6	47	60	0.56

[a] All results are averages of duplicates. [b] Less (±)-PEA and/or L-TA were added to compensate for the additives acidic or basic groups so the system was kept neutral and of equal volume. [c] S-factor = yield × *de* × 2.[7] [d] Synthesis described in chapter 3.
[e] Commercially available. [f] Synthesis described in the experimental section.

It may be concluded that although the effect of an additive does not result in an impressive increase in resolution efficiency, it can have an effect on the polymorph in which the diastereomers crystallize. Perhaps a mixture of carefully chosen additives can prevent the nucleation of less stable polymorphs also and thus give an increase in resolution efficiency.

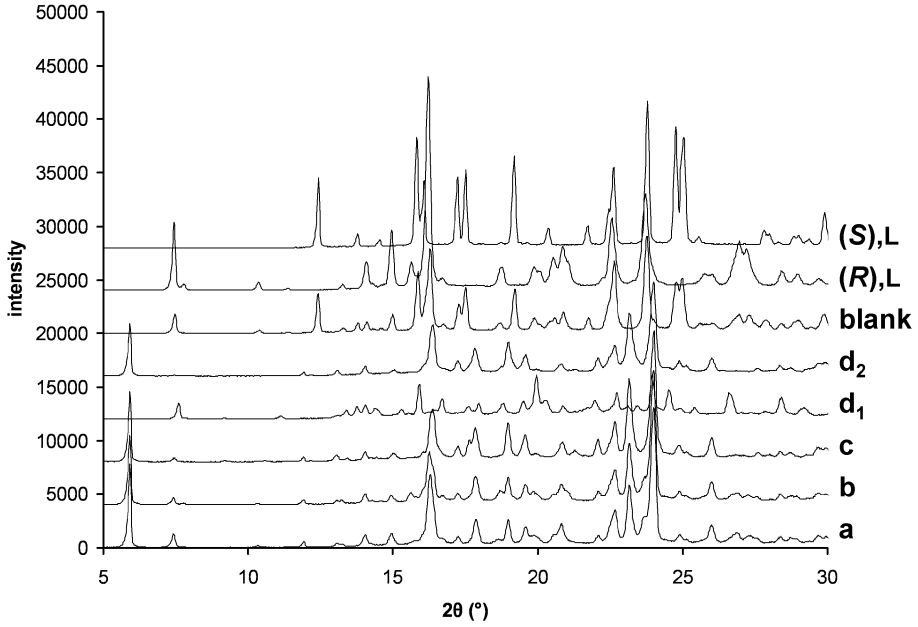


Figure 4.3 *XRPD of the solids collected after resolution experiments.*

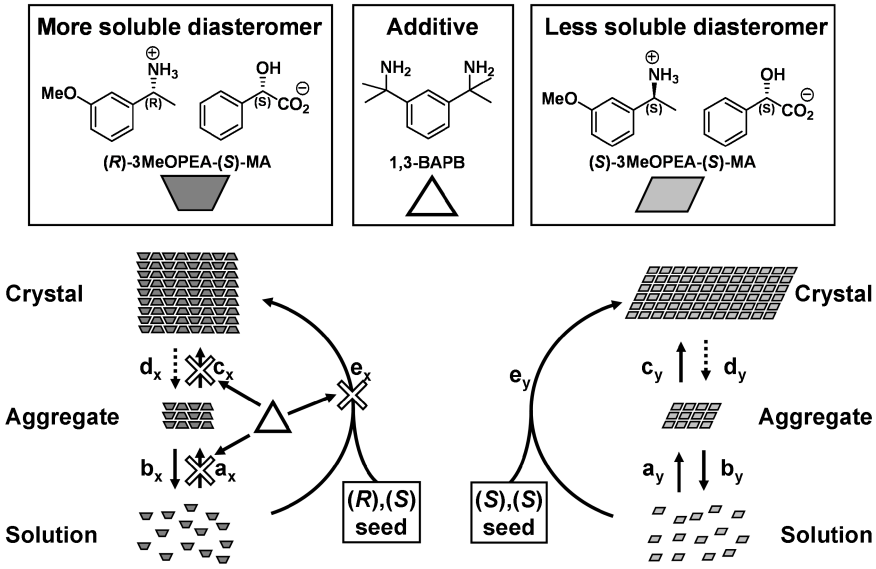
4.4 Dissolution by Abrasive Grinding

Addition of glass beads and an additive to a stirred suspension of crystals can have profound effects as will be made clear in this section and Chapter 5.

4.4.1 Dissolution of Diastereomers in Saturated Mixtures

Nucleation inhibitors can make excellent growth inhibitors as shown in §3.2.5. In that section, seeding of the less soluble (*R*)-3MeOPEA-(*S*)-MA did not result in crystal growth because of the presence of only 1.0 mol% of 1,3-BAPB. This achiral additive inhibits not only the nucleation of the more soluble salt but also blocks the growth of seeds of the more soluble salt.

Depicted in Scheme 4.4 is the crystallization of a diastereomeric salt. Crystallizations take place by forming aggregates in solution (pathways $\mathbf{a}_{x/y}$) which can re-dissolve ($\mathbf{b}_{x/y}$). When these aggregates grow to a certain critical size they form crystals ($\mathbf{c}_{x/y}$) which is know as primary nucleation. Once crystals have formed, these can grow further *via* pathway $\mathbf{e}_{x/y}$ (secondary nucleation).



Scheme 4.4 *Resolution of (±)-3MeOPEA with (S)-MA aided by 1,3-BAPB.*

If an additive (1,3-BAPB in Scheme 4.4) functions as a nucleation and a growth inhibitor only for the more soluble diastereomer, it blocks pathways $\mathbf{a}_x$ and/or $\mathbf{c}_x$ and $\mathbf{e}_x$ but leaves pathways $\mathbf{a}_y$, $\mathbf{c}_y$ and $\mathbf{e}_y$, which describe the crystallization of the less soluble salt, untouched. Of course, had the additive been added after the more soluble salt crystallized, it cannot spontaneously re-dissolve the more soluble diastereomeric salt (pathways $\mathbf{b}_x$ and $\mathbf{d}_x$). When a supersaturated mixture containing a nucleation inhibitor that does not function as a growth inhibitor, thus only blocks pathways $\mathbf{a}_x$ and $\mathbf{c}_x$, is seeded with crystals of the less soluble diastereomer, crystal growth of the seeds will take place *via* pathway $\mathbf{e}_x$.

The Gibbs-Thomson relationship states that extremely small particles have a higher solubility than larger particles.[8] However, small particles suspended in their own saturated solution undergo Ostwald ripening. In this process, larger particles grow by consuming smaller particles *via* the liquid phase.[9] Thus, if crystals are grown in suspension, they have to be milled continuously to keep the average crystal size small. Higher milling efficiencies

will then give smaller particles and thus higher concentrations of the dissolved compound. It must be noted that a noticeable increase in solubility is difficult to achieve. However, if the Ostwald ripening process could be blocked by an effective additive, even relatively slow milling will, in time, produce extremely small particles with a certain higher solubility and could dissolve all material in the solid phase if the mass of particles is not too high.

Furthermore, smaller particles dissolve faster than their larger counterparts since the surface to volume ratio is larger. This phenomenon is important in the washing step of the filter cake during the isolation.[10]

This grinding procedure was applied to the resolution in Scheme 4.4 where the more soluble diastereomer has already crystallized and the additive (that only affects the more soluble diastereomer) is added once the thermodynamic equilibrium has been achieved. Abrasive grinding of the solids should not affect the concentration of the less soluble diastereomer in solution (not *via* pathway $\mathbf{b_y}$ and $\mathbf{d_y}$) much but should dissolve some or all of the more soluble diastereomer *via* pathways $\mathbf{b_x}$ and $\mathbf{d_x}$ and simultaneous blocking pathways $\mathbf{a_x}$, $\mathbf{c_x}$ and $\mathbf{e_x}$.

Four experiments were performed in which the effects of abrasive grinding and the additive were tested. Every experiment contained an egg-shaped magnetic stirrer and a suspension of both more and less soluble diastereomer. This mixture was crystallized overnight and the *de* of the solid phase was 58-68% after washing (t_0). Experiment 1 was left unaltered. Glass beads were added to experiment 2. To experiment 3, 3 mol% of 1,3-BAPB·2(*S*)-MA was added and experiment 4 was charged with both glass beads and 3 mol% 1,3-BAPB·2(*S*)-MA. All four flasks were then stirred at 900 rpm. Samples were taken regularly and the *de*'s were determined of the washed solids and the mother liquors (without washings). The results for the solids are given in Figure 4.4, and the results for the mother liquors are given in Figure 4.5.

The experiments which only grind the available crystals (exp 2 and to a lesser degree, exp 1) do not show a significant increase in *de* in the mother liquor as expected. However, a decrease in *de* in the solids phase was observed. This can be explained by some loss of mother liquor *via* the capillary space between the stopper and the ground glass joint by the strong stirring action which was accompanied with some spattering of the suspension and air pressure differences over the time of the experiment. This was confirmed by some solid salts on the top of the joint that remained after evaporation of the solvent.

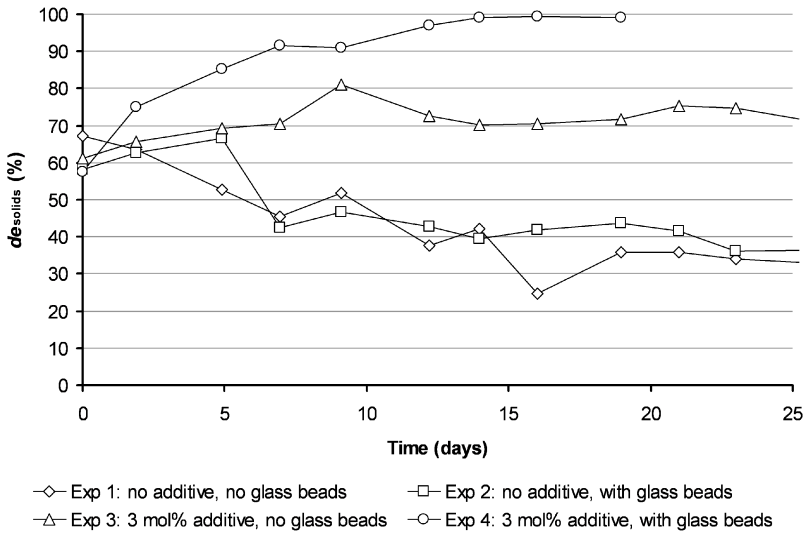


Figure 4.4 *Progression of the de of the solid phases. Lines are provided as a guide for the eye.*

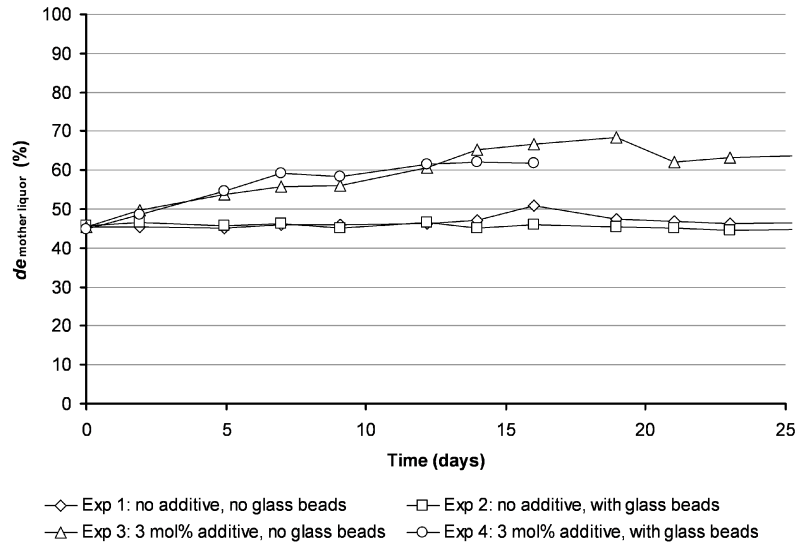


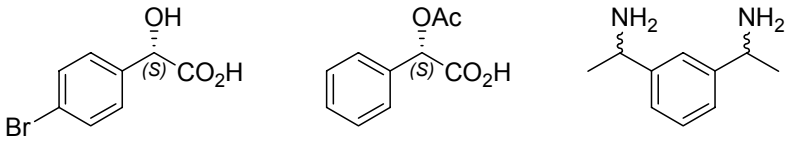
Figure 4.5 *Progression of the de of the mother liquors. Lines are provided as a guide for the eye.*

The mother liquors of experiments 3 and 4, which both contain the growth inhibitor, show an increase in *de* from 45% to approximately 65%. The solid phase from experiment 3 shows a slight increase in *de* but the grinding action of the magnetic stirred alone is not enough to produce the extremely small crystals needed in this experiment. However, should the concentration of this experiment be lowered, the grinding action of the magnetic stirrer alone could be enough to produce complete dissolution of the more soluble diastereomer. This was not further investigated

In experiment 4, where the grinding action does produce extremely small particles, washing of the filter cake dissolved the remainder of small particles and gave the pure less soluble diastereomer within 14 days. Considering that both mother liquors of experiments 3 and 4 have the same *de*'s but the solids of experiment 4 have higher *de*'s than experiment 3 must be the result of crystal size. The difference in these experiments is thus solely the result of faster dissolution of smaller crystals during the washing step.

Abrasive grinding experiments with this additive were performed at higher concentrations also. At the start of the experiment (t_0) the precipitated solids had 23% *de* and the mother liquor had 45% *de*. Then, the additive and the glass beads were added and after 5 days the *de* of the solids was constant at ~40% and the mother liquor at 60% *de*. Doubling the amount of glass beads and increasing the stirring rate from 900 rpm to 1500 rpm increased the *de* of the solids from 40% to 60%. Furthermore, the *de* in the mother liquor increased from 60% to 66%. An attempt to reduce the crystal size even further by sonication (see also §5.4) was foiled by the heat generation of the ultrasonic device. Even when the ultrasonic bath was kept at 20°C by means of a thermostat, the internal temperature could not be kept constant. Since the solubility of the diastereomer is increased by heating, the idea of grinding the salts by sonication was abandoned.

Other successful nucleation inhibitors from §3.2, as depicted in Scheme 4.5, were examined using this procedure. However, addition of these compounds gave *de*'s similar to as the experiment without additives (experiment 2).



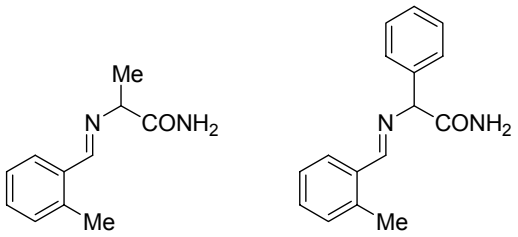
(*S*)-4BrMA **(*S*)-OAcMA** **1,3-BAEB**

Scheme 4.5 *Successful nucleation inhibitors.*

4.4.2 Dissolution of Enantiomers in Saturated Mixtures

Dissolution of compounds in a saturated mixture by grinding is not restricted to diastereomers. However, as can be concluded from the previous paragraph, not every good nucleation inhibitor makes a good growth inhibitor.

Two racemic conglomerates, Ala-Schiff and Phg-Schiff (depicted in Scheme 4.6 and described in §5.6), were suspended in MeCN with some DBU (to racemize the conglomerates in solution) in such a concentration that both materials had crystallized. Deracemization as described in §5.6 was achieved by addition of glass beads and grinding by sonication a sufficient amount of time to give a mixture from which the washed solids were analyzed by chiral HPLC. The ratio between the total area under the Ala-Schiff peaks and the Phg-Schiff peaks was 36:64 at the start of the experiment and when both enantiomers were completely deracemized (>99% *ee*) the ratio was 2:98 at the end of the experiment. Both enantiomers were enriched in the (*R*)-enantiomer.[11]



Ala-Schiff **Phg-Schiff**

Scheme 4.6 *Amino acid based conglomerates.*

The Phg-Schiff in solution proved to be an excellent growth inhibitor for the Ala-Schiff crystals, producing small crystals of the latter which were dissolved in the washing step similar as described in §4.4.1.[12] The small amount Ala-Schiff (2% in area) which was still present might either be crystallized in its own crystal lattice or co-crystallized in the Phg-Schiff crystals as observed in similar experiments in §5.6.2. The experiments described below help in understanding the observations.

It was believed that the addition of D- or L-alanine, a close family member of Ala-Schiff and perhaps of Phg-Schiff also, could provoke faster deracemization of both imines towards the opposite enantiomer of the additive. The additive will then block the growth of crystals with the same handedness as the additive and thus will allow the growth of the opposite enantiomer to proceed unhindered. This phenomenon is known as the rule of reversal.[4a] Addition of (*R*)-alanine to a suspension of (±)-Ala-Schiff under deracemizing

conditions indeed delivered (*S*)-Ala-Schiff in >99% *ee*. In a similar fashion, (*S*)-alanine delivered (*R*)-Ala-Schiff in >99% *ee*. However, addition of (*S*)-alanine to (±)-Phg-Schiff under deracemizing conditions delivered (*S*)-Phg-Schiff in >99% *ee* instead of the expected (*R*)-Phg-Schiff. Also, addition of (*R*)-alanine delivered (*R*)-Phg-Schiff in >99% *ee* under these conditions. These observations seem to contradict the rule of reversal. However, since three of the groups attached to the chiral centers of alanine and Phg-Schiff are different, this observation is not so surprising. If a suspension of (±)-Ala-Schiff and (±)-Phg-Schiff was treated with (*S*)-alanine under deracemizing conditions this should deliver (*R*)-Ala-Schiff and (*S*)-Phg-Schiff if the two compounds crystallized in separate crystal lattices and deliver (*S*)-Ala-Schiff and (*S*)-Phg-Schiff if a solid solution was formed. The experiment with (*S*)-alanine indeed gave Ala-Schiff and Phg-Schiff as the (*S*)-enantiomers. Likewise, addition of (*R*)-alanine gave both Schiff bases in the (*R*)-enantiomers. In both cases the ratio of the area under the peaks of both Ala-Schiff enantiomers and the area under the peaks of both Phg-Schiff enantiomers was 2:98 (the ratio at the start of the experiment was 36:64). These findings demonstrate that indeed Phg-Schiff is an effective growth inhibitor for Ala-Schiff and that some Ala-Schiff is incorporated enantioselective in the crystals of Phg-Schiff.

4.5 Simultaneous Resolution with Cyclic Phosphoric Acids

In Dutch Resolution, a mixture of structurally closely related compounds (family members) is used to resolve a racemate.[1] Two or three resolving agents are used which are often incorporated in the precipitated salts as a solid solution.

Also a mixture of racemic family members can be resolved with one resolving agent. The racemates often form a solid solution or end solid solution between two or three of the components in the precipitated salts. These components usually show high ee's with the same absolute configuration. This process is named Reversed Dutch Resolution.[13]

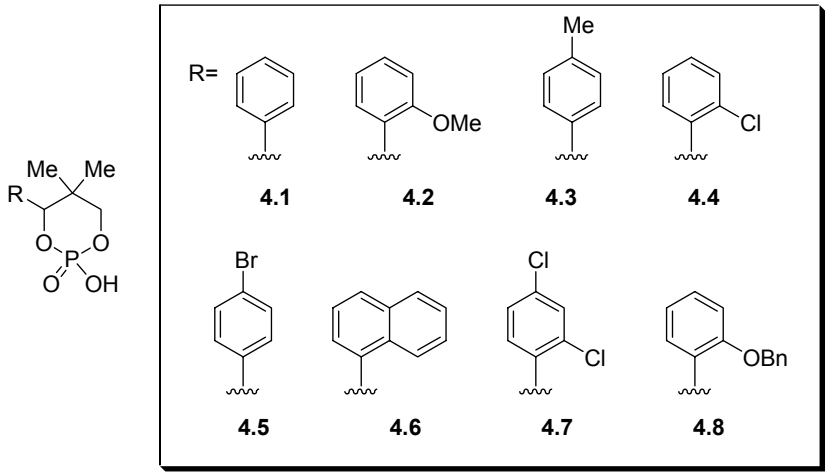
The solid solution formation of the components in the precipitated salts means that usually reasonable amounts (>10%) of these components are co-crystallized, depending on the composition of the mother liquor, of course. In §5.6.2 it is described that when a mixture of amino acid derivative were co-crystallized in the presence of an excess of a conglomerate amino acid derivate, up to 1.22% of the amino acids were incorporated in the crystallized conglomerate. The reason for this low incorporation compared to the solid solution formation in (Reversed) Dutch Resolution is that the crystal lattice of the conglomerate can distinguish between the conglomerate and the other amino acid derivatives because of

relatively large differences. This has been observed before and was confirmed by computer aided calculations.[14]

If a solution of several structurally closely related racemates (family members) were to be co-crystallized in the presence of an excess of an optically pure family member, a mixture should crystallize with the same absolute configuration and with high *ee*'s.[15]

4.5.1 Phencyphos

Seven structurally closely related racemic cyclic phosphoric acids **4.2–4.8** (see Scheme 4.7) were saturated in a water/methanol mixture and then (*R*)-phencyphos (**4.1**) hydrate was added to the solution in such a concentration that a solution was obtained at reflux temperature but the hydrate of (*R*)-**4.1** crystallized when cooled to room temperature. Since the other phosphoric acids were not supersaturated at room temperature, these will not crystallize. The crystals that were collected after cooling, however, contained large quantities of the other phosphoric acids **4.2–4.8** but in variable amounts and all with high enantiomeric excesses with the same absolute configuration as the parent (*R*)-**4.1** as denoted in Table 4.3. Note that in the assignment of the absolute configuration, the priority of the phenyl group changes when a heavy atom is present on the ortho position as is the case with compounds **4.2**, **4.4**, **4.7** and **4.8**. This means that, for example, (*R*)-**4.1** and (*S*)-**4.4** actually have the same spatial configuration. These results are analogous to the results described in §5.6.2.



Scheme 4.7 *Cyclic phosphoric acids.*

The isolated solids however, were very finely divided and only filterable over fine filters (P4). The (*R*)-**4.1**·H_2O crystals grown from aqueous solutions that only contain (*R*)-**4.1**, however, consist of long needles and are collected with ease over a P2 filter. Apparently, the additives **4.2–4.8** block the growth of the (*R*)-**4.1**·H_2O crystals and then more primary nucleation will take place, resulting in more fine crystals. This experiment was repeated with (*S*)-**4.1** resulting in approximately the same incorporation but of opposite handedness. Surprisingly, approximately 30% wt. of the crystal content is not the parent phosphoric acid **4.1**. Thus 70% wt. determines the absolute configuration of the other co-crystallized 30% wt.

Table 4.3 *Compositions of co-crystallized phosphoric acids with optically pure phencyphos (**4.1**) hydrate from water-methanol as solvent.*

Phosphoric acid	*ee* (%)[a,c]	Composition (% wt.)[a]	*ee* (%)[b,c]	Composition (% wt.)[b]
4.1	99.8 (*S*)	70.1	99.9 (*R*)	71.0
4.2	94.3 (*R*)[d]	6.6	84.2 (*S*)[d]	10.5
4.3	98.0 (*S*)	1.7	71.3 (*R*)	1.8
4.4	96.8 (*R*)[d]	11.8	93.9 (*S*)[d]	3.3
4.5	95.0 (*S*)	4.0	92.9 (*R*)	1.9
4.6	90.2 (*S*)	2.0	83.3 (*R*)	0.6
4.7	46.3 (*R*)[d]	2.2	82.7 (*S*)[d]	3.9
4.8	79.8 (*R*)[d]	1.5	74.9 (*S*)[d]	6.8

[a] Single experiment with an excess of (*S*)-PP·H_2O. [b] Single experiment with an excess of (*R*)-PP·H_2O. [c] The letter in parenthesis gives the absolute configuration. These were determined by the elution order of chiral HPLC which showed excellent correlation for phosphoric acids with known absolute configurations: **4.1**,**4.2** and **4.4**. This however, does not necessary need to be true for the other phosphoric acids. [d] The priority of the phenyl group has changed due to a heavy atom on the ortho position. This means that, for example (*R*)-**4.4** has the same spatial configuration as (*S*)-**4.1**.

An attempt to repeat this experiment in pure MeOH did not produce the large quantities of **4.2–4.8** incorporated in the now anhydrous **4.1**. The hydrate formation seems to give an

advantageous crystal lattice in which the other phosphoric acids fit better. Since the phosphoric acids **4.2–4.8** are under-saturated they should remain dissolved, apparently form a solid solution or an end solid solution with the crystallized (*R*)- or (*S*)-**4.1**·H_2O.

4.5.2 Anicyphos and Chlocyphos

Anicyphos (**4.2**) and chlocyphos (**4.4**) were both incorporated in large quantities in the phencyphos (**4.1**) hydrate resolution described above. Since both enantiomers of anicyphos and chlocyphos were available optically pure these were tested also with a mixture of the other racemic phosphoric acids.

Under the same conditions as described above, (*S*)- and (*R*)-anicyphos were crystallized in the presence of a saturated solution of racemic **4.1** and **4.3–4.8**. The results are shown in Table 4.4.

Table 4.4 *Compositions of co-crystallized phosphoric acids with optically pure anicyphos (**4.2**).*

Phosphoric acid	*ee* (%)[a,c]	Composition (% wt.)[a]	*ee* (%)[b,c]	Composition (% wt.)[b]
4.1	-	n.d.[d]	-	n.d.[d]
4.2	99.8 (*R*)	96.7	99.8 (*S*)	97.2
4.3	-	n.d.[d]	-	n.d.[d]
4.4	>99.9 (*R*)	2.0	95.4 (*S*)	1.6
4.5	-	n.d.[d]	-	n.d.[d]
4.6	86.0 (*S*)[e]	0.7	91.0 (*R*)[e]	0.6
4.7	69.8 (*R*)	0.4	86.2 (*S*)	0.4
4.8	90.4 (*R*)	0.2	78.6 (*S*)	0.2

[a] Single experiment with an excess of (*R*)-anicyphos (**4.2**). [b] Single experiment with an excess of (*S*)-anicyphos (**4.2**). [c] The letter in parenthesis gives the absolute configuration. These were determined by the elution order of chiral HPLC which showed excellent correlation for phosphoric acids with known absolute configurations: **4.1, 4.2** and **4.4**. [d] n.d.: not detected [e] The priority of the phenyl group has changed due to a lighter atom on the ortho position. This means that (*R*)-**4.2** has the same spatial configuration as (*S*)-**4.6**.

Although the incorporation of the other cyclic phosphoric acids in anicyphos (**4.2**) is low, all incorporated phosphoric acids in the crystallized **4.2** have the same spatial configuration with high *ee*'s

Even though anicyphos is one of the most incorporated compounds in the phencyphos (**4.1**) hydrate crystals, the anicyphos (**4.2**) does not incorporate significant amounts of phencyphos as show in Table 4.4. This might indicate that anicyphos forms an end solid solution in phencyphos hydrate crystals and not a full solid solution. This observation is remarkable because one would expect that smaller molecules (**4.1** in this case) should incorporate more readily in the crystal lattice of a larger molecule (**4.2** in this case) than *visa versa*. Since **4.1** forms a hydrate from the aqueous solvent mixture, this crystal lattice might provide more room for larger molecules to incorporate by removal of one molecule of water.[15]

Table 4.5 *Compositions of co-crystallized phosphoric acids with optically pure chlocyphos (**4.4**, in the grey bar).*

Phosphoric acid	*ee* (%)[a,c]	Composition (% wt.)[a]	*ee* (%)[b,c]	Composition (% wt.)[b]
4.1	-	n.d.[d]	11.5 (*R*)[e]	1.0
4.2	-	n.d.[d]	11.3 (*S*)	1.2
4.3	-	n.d.[d]	-	n.d.[d]
4.4	>99.9 (*R*)	98.8	>99.9 (*S*)	93.7
4.5	-	n.d.[d]	7.2 (*S*)[e]	0.9
4.6	18.0 (*S*)[e]	0.2	0.4 (*S*)[e]	1.1
4.7	87.8 (*R*)	1.0	39.0 (*S*)	1.5
4.8	-	n.d.[d]	32.5 (*S*)	0.7

[a] Single experiment with an excess of (*R*)-chlocyphos (**4.4**). [b] Single experiment with an excess of (*S*)-chlocyphos (**4.4**). [c] The letter in parenthesis gives the absolute configuration which were determined by the elution order of chiral HPLC which showed good correlation for phosphoric acids with known absolute configurations: **4.1, 4.2** and **4.4**. [d] n.d.: not detected. [e] The priority of the phenyl group has changed due to the lighter atom on the ortho position. This means that (*R*)-**4.4** has the same spatial configuration as (*S*)-**4.6**.

Under the same conditions as described above, (*R*)- and (*S*)-chlocyphos (**4.4**) were crystallized in the presence of a saturated solution of racemic **4.1–4.3** and **4.5–4.8**. The results from these crystallizations are given in Table 4.5.

In the experiment with (*R*)-chlocyphos (**4.4**), some unexpected results were obtained. The enantioselectivity of this experiment is not as good as other experiments in this chapter. This might be because of a poor washing of the filter cake and poor separation of the mixture of phosphoric acids with preparative HPLC (see experimental section), which causes overlap of peaks in the chiral HPLC of the individual phosphoric acids and thus makes the *ee* determination of such low *ee*'s combined with low amounts (~1%) unreliable.

As with anicyphos (**4.2**), chlocyphos (**4.4**) was incorporated in large amounts in the crystallizing phencyphos (**4.1**) hydrate, shown in Table 4.3. However, as can be seen from Table 4.5 phencyphos (**4.1**) is not significantly incorporated in the crystallizing chlocyphos (**4.4**), nor are other cyclic phosphoric acids. This behavior is typical for an end solid solution. Higher incorporation percentages may be found when a mixture is crystallized which have a full solid solution relationship such as described in §6.3.

4.6 Experimental Section

General Information: Reagents were obtained from commercial sources and used without further purification.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury 300MHz machine. Chemical shifts are denoted in δ (ppm) and are referenced to the residual protic solvent. The coupling constant *J* is denoted in Hz. Splitting patterns are denoted as follows: s (singlet), d (doublet), m (multiplet) and bs (broad singlet).

Mass spectra were recorded by API-ES (electron spray ionization) by dissolving the samples in MeOH and injecting the solution as such. Mobile phase: Acetonitrile : 0.1% formic acid in water 50 : 50 (1 min), flow: 0.2 mL·min^{-1}, injection volume: 5 µL.

Procedure for the dissolution of diastereomers by abrasive grinding as described in §4.2 . A typical resolution experiment was performed by charging a Kimble reactor tube (Ø 25 × 150 mm) with a PTFE coated egg-shaped magnetic stirring bar (19 mm × 10 mm), 2.5 mL 0.1106 M (±)-PEA in IPA:H_2O (4:1) and 2.5 mL 0.1106M L-dibenzoyl tartaric acid (DBTA) in IPA:H_2O (4:1). This mixture was stirred and after some minutes, crystals started to form. When additives were used, an equimolar amount of L-DBTA was replaced by a solution of the additive so the whole system remained neutral and of equal volume.

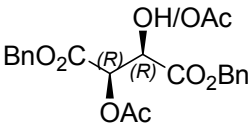
The suspension was heated to dissolution and placed in a Reactiv8 computer controlled reactor station as depicted in Figure 3.6 in Chapter 3 and stirred magnetically at 600 rpm and 70°C for 30 min. Then the tubes were cooled to 20°C at 0.1°C·min^{-1} and kept at 20°C for an additional 8 hours. The formed crystals were collected on pre-weighed disposable filters and washed with 1.0 mL IPA:H_2O (4:1). The solids were subsequently dried *in vacuo* and weighed and the *de* of the salts was determined.

Chiral HPLC analysis of 1-phenylehylamine (PEA) salts was carried out on a Crownpak CR(-) column with an aqueous solution of $HClO_4$ (pH 2) as eluent at 20°C and 0.6 mL·min^{-1}. UV-VIS detection at 200 nm. The salts were dissolved in eluent and 5 µL were injected as such. (*R*)-PEA R_f: 24.87 min, (*S*)-PEA R_f: 37.77 min.

Procedure for the dissolution of diastereomers by abrasive grinding as described in §4.3 . A typical resolution experiment was performed by charging a Kimble reactor tube (Ø 25 × 150 mm) with a PTFE coated egg-shaped magnetic stirring bar (19 mm × 10 mm). The tube was placed in a Reactiv8 computer controlled reactor station as depicted in Figure 3.6 in Chapter 3 and stirred magnetically at 600 rpm at 70°C and further charged with 2.5 mL 0.30 M (±)-PEA in EtOH and 2.5 mL 0.30M L-tartaric acid (TA) in EtOH. After some minutes, crystals started to form at 70°C. When additives were used, an equimolar amount of (±)-PEA and/or L-TA was replaced by a solution of the additive(s) so the whole system remained neutral and of equal volume.

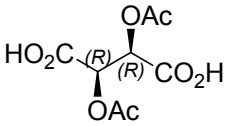
The suspension was kept at 70°C for another 30 min. Then the tubes were cooled to 20°C at 0.1°C·min^{-1} and kept at 20°C for an additional 8 hours. The formed crystals were collected on pre-weighed disposable filters and washed with 1.0 mL EtOH. The solids were subsequently dried *in vacuo* and weighed and the *de* of the salts was determined.

Chiral HPLC was performed with the procedure described above.

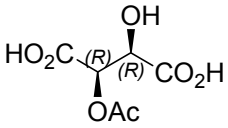


(*2R*,*3R*)-dibenzyl 2,3-diacetoxysuccinate and (*2R*,*3R*)-dibenzyl 2-acetoxy-3-hydroxysuccinate. A solution of (*2R*,*3R*)-dibenzyl 2,3-dihydroxysuccinate (5.0 g, 15.1 mmol, 1.0 eq) in pyridine (50 mL) was treated with acetic anhydride (2.13 mL, 22.7 mL, 1.5 eq) and stirred for two days before it was concentrated to dryness. The mixture was purified by automated column chromatography with EtOAc:heptanes. The diacetylated compound eluded first shortly followed by the monoacetyl compound. The pure fractions were combined and concentrated to deliver 2.38 g (38%) of the diacetylated compound as a colorless oil: (^{1}H-NMR (300MHz, DMSO-d6): δ= 1.91 (s, 6H), 5.12 (d, *J*=12.6Hz, 2H), 5.24 (d, *J*=12.3Hz, 2H), 5.71 (s, 2H), 7.20-7.37 (m, 10H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 19.9, 67.1, 70.3, 128.2, 128.4, 128.5, 135.2, 165.3, 169.1 ppm. MS (EI): *m/z*= 437 [M+Na$^+$]) and 2.31 g (41%) of the mono-acetylated compound as a colorless oil which

crystallized on standing to produce white crystals (^{1}H-NMR (300MHz, DMSO-d6): δ= 1.92 (s, 3H), 4.76 (dd, J_1=6.9Hz, J_2=3.0Hz , 1H), 5.04-5.23 (m, 4H), 6.26 (d, J=6.3Hz, 1H), 7.30-7.37 (m, 10H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 20.1, 66.3, 66.6, 69.9, 73.4, 127.7, 128.1-128.5 (m), 135.4, 135.7, 166.6, 169.6, 169.9 ppm. MS (EI): *m/z*= 395 [M+Na$^+$]).



(2R,3R)-2,3-diacetoxysuccinic acid. A mixture of (*2R*,*3R*)-dibenzyl 2,3-diacetoxysuccinate (1.0 g, 2.41 mmol, 1.0 eq), EtOH (25 mL) and Pd/C (10% Pd, 100 mg) was hydrogenated at atmospheric pressure. A ^{1}H-NMR sample taken after 1.5 hours revealed complete conversion. The reaction mixture was filtered over Celite and concentrated to dryness. This yielded the product as a colorless oil (598 mg, 96%). ^{1}H-NMR (300MHz, DMSO-d6): δ= 2.08 (s, 6H), 4.20 (bs, 2H), 5.49 (s, 2H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 20.3, 70.7, 167.2, 169.4 ppm. MS (EI): *m/z*= 233 [M-H$^+$].



(*2R*,*3R*)-2-acetoxy-3-hydroxysuccinic acid. The above procedure was repeated for (*2R*,*3R*)-dibenzyl 2-acetoxy-3-hydroxysuccinate. Yield: 511 mg (99%) as a colorless oil. ^{1}H-NMR (300MHz, DMSO-d6): δ= 2.06 (s, 3H), 4.54 (s, 1H), 5.22 (s, 1H) ppm. ^{13}C-NMR (75MHz, DMSO-d6): δ= 20.4, 69.8, 73.4, 168.4, 169.7, 172.0 ppm. MS (EI): *m/z*= 191 [M-H$^+$]).

Procedure for the dissolution of diastereomers by abrasive grinding as described in §4.4.1. Four new 250 mL round bottom flasks were each charged with a new PTFE coated egg-shaped stirring bar (2.5 cm × 1.0 cm), (±)-3MeOPEA (907 mg, 6 mmol, 1.0 eq), (*S*)-MA (913 mg, 6 mmol, 1.0 eq) and 2-butanone (12.06 g, 15 mL). The flasks were stoppered and stirred for a couple of hours to ensure complete crystallization of both diastereomeric salts. Then the suspension was further diluted with 2-butanone (108.81 g, total: 150 mL). The resulting suspension was stirred at 900 rpm overnight. Samples were taken from the mother liquor and the solids (P4). The latter was washed with some 2-butanone and the washings were discarded. Glass beads (20 g) were added to flasks 2 and 4. Flasks 3 and 4 were each charged with 1,3-BAPB·2(*S*)-MA (89 mg, 0.18 mmol, 0.03 eq) and the stirring was continued at 900 rpm. Samples of mother liquor and washed solids were taken three times a week in a manner as described above. The methods for chiral HPLC analysis and the procedures to synthesize the compounds are described in the experimental section of Chapter 3.

In the experiments which use either (*S*)-4BrMA-(±)-3MeOPEA, (*S*)-*O*AcMA-(±)-3MeOPEA or (±,±)-1,3-BAEB[16]·2(*S*)-MA, 0.18 mmol of these compounds replaced the 0.18 mmol of 1,3-BAPB·2(*S*)-MA

Procedure for the dissolution of enantiomers by abrasive grinding as described in §4.4.2. A new scintillation vial (20 mL) was charged with 10 g solids glass beads (Ø 2 mm), 0.36 g (±)-Phg-Schiff and/or 0.39 g (±)-Ala-Schiff and 3.6 g MeCN. Depending on the experiment, no alanine, 10 mg D-alanine or L-alanine was added. The bottom part (~1 cm) of the stoppered vial was placed in a ultrasonic cleaning bath (Bandelin Sonorex, RK 106, 35 kHz) which was kept at 20°C by a thermostat (Huber, ministat cc) and was sonicated in the middle of the bath for 5 minutes. Subsequently, fresh 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.20 g) was added and the sonication was continued. After 3 days, samples were taken by filtration (P4), sucked dry and washed with some TBME. The filter cake was analyzed according to Method 1 which is described in the experimental section in Chapter 5 as is the synthesis of (±)-Phg-Schiff and (±)-Ala-Schiff.

Procedure for the resolution of multiple phosphoric acids by co-crystallization as described in §4.5.1. To a 50:50 mixture of water and MeOH (~6 mL) was added sufficient racemic phosphoric acids **4.2**–**4.8** to result in a suspension in which all phosphoric acids were saturated. The mixture was heated to reflux and allowed to cool to room temperature overnight. The solids were removed by filtration (P4). Two tubes were charged with 2.5 mL filtrate each. One tube was charged with enough (*S*)-**4.1**·H_2O and the other with enough (*R*)-**4.1**·H_2O to result in a thin suspension when heated to reflux and was filtered hot. The filtrates were reheated to dissolution and allowed to cool to room temperature overnight with stirring. The solids were collected by filtration (P4). The composition of the mother liquor was 367 mg phosphoric acid mix per mL solvent mixture for the tube containing (*S*)-**4.1** and 396 mg phosphoric acid mix per mL solvent mixture for the tube containing (*R*)-**4.1**. The composition of the filter cake was determined and compensated for the amount of residual mother liquor. The filter cake from the tube containing (*R*)-**4.1** gave 151 mg phosphoric acid mix per mL solvent mixture. The filter cake from the tube containing (*S*)-**4.1** gave 155 mg phosphoric acid mix per mL solvent mixture. The mixtures of phosphoric acids in the filter cakes were separated by HPLC: column: Luna PFP (4.6 × 100 mm, 3 μm). Mobile phase: solution A: solution B = 95:5 (0 min) in 30 min to 60:40 (0 min) in 10 min to 0:100 (0 min). Solution A: 9.65 g ammonium acetate; 2250 mL H_2O; 150 mL MeOH; 100 mL acetonitrile, solution B: 9.65 g ammonium acetate; 250 mL H_2O; 1350 mL MeOH; 900 mL acetonitrile. Flow: 1.0 mL·min^{-1}. Detection: 215 nm. Mass detection: API-ES (electron spray ionization) positive and negative. The samples were measured against a mixture of the phosphoric acids with known concentrations and weight percentages given in Table 4.3.

Table 4.6 *Retention times of phosphoric acids analyzed by chiral HPLC. The absolute configurations of compounds **4.1**, **4.2** and **4.4** are known and given in the table. The absolute configurations of the other compounds are established by the elution order.*

Phosphoric acid	Absolute configuration and Rf first peak (min)	Absolute configuration and Rf second peak (min)
4.1	know: (*S*)-(+): 7.18	know: (*R*)-(–): 8.15
4.2	know: (*R*)-(+): 7.33	know: (*S*)-(–): 9.57
4.3	expected (*S*): 7.61	expected (*R*): 8.49
4.4	know: (*R*)-(+): 7.12	know: (*S*)-(–): 7.59
4.5	expected (*S*): 7.26	expected (*R*): 8.58
4.6	expected (*S*): 7.62	expected (*R*): 17.58
4.7	expected (*R*): 7.01	expected (*S*): 7.49
4.8	expected (*R*): 6.88	expected (*S*): 14.61

The experiments described in §4.5.2 were performed in a similar fashion. For the anicyphos (**4.2**) experiments in Table 4.4, a saturated solution of **4.1, 4.3–4.8** was made and for the chlocyphos (**4.4**) experiments in Table 4.5 a saturated solution of **4.1–4.3, 4.5–4.8** was made.

The separated peaks were collected, concentrated and each analyzed by chiral HPLC: Chiralpak QN-AX column with MeOH:AcOH 97:3 + 0.25g NH_4OAc/100 mL as mobile phase at room temperature and 1.5 $mL \cdot min^{-1}$. UV-VIS detection at 254 nm. The retention times for each of the components are given in Table 4.6. For compound **4.4**, the priority of the phenyl group has changed due to the chlorine on the ortho position. This means that (*R*)-**4.2** and (*R*)-**4.4** have the same spatial configuration as (*S*)-**4.1**. Since the compounds with known configurations (**4.1**, **4.2** and **4.4**) elude in the same order with respect to the spatial configuration, it is expected that the compounds **4.3** and **4.5–4.8** will elude in the same order also. Note that the priority of the phenyl group of compounds **4.7** and **4.8** has changed in the same manner as compounds **4.2** and **4.4**.

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12 The effect of washing of the filter cake on this system was further investigated.[10]

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16 Synthesis described in chapter 3.

Chapter 5

Deracemization by Abrasive Grinding

In this chapter[*] the resolution of racemates that crystallize as conglomerates by means of racemization in the liquid phase and simultaneous grinding of the solid phase is described. Also mixtures of amino acid derivatives have been resolved in similar experiments using a crystalline conglomerate. The other amino acid derivatives co-crystallized with the same absolute configuration. This approach could be a feasible pathway for the origin of homochirality in nature.

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5.1 Introduction

Direct resolution by crystallization of racemic mixtures, induced by seeding or by adding tailor-made additives, forms an attractive alternative to the separation by forming diastereomeric salts.[1] Unfortunately, the overall yield in a single resolution step is low and limited to 50% of each enantiomer and in practice usually lower. Combining a direct resolution with racemization in the solution would lead to a total enantiomeric transformation.[2] Complete resolution in a single operation would be of great practical use. Examples have been very limited, however.

Viedma[3] recently demonstrated the inexorable and random emergence of solid-phase single chirality for the intrinsically achiral inorganic compound $NaClO_3$ or $NaBrO_3$, initially present as a racemic mixture of two enantiomorphic solid phases in equilibrium with the achiral aqueous phase (Figure 5.1a). Grinding the slurry of crystals with glass beads promotes dynamic dissolution / crystallization processes that result in the conversion of one solid enantiomorph into the other *via* the solution. Larger crystals grow by consuming smaller crystals *via* the saturated solution due to the fact that a small surface to volume ratio is energetically favorable. This process is known as Ostwald ripening[4] and is also seen in ice-cream, which forms large ice crystals over time at relative high temperatures and making the ice-cream less smooth. In ice-cream, this process is halted to a large extent by freezing the ice at -18°C.[5]

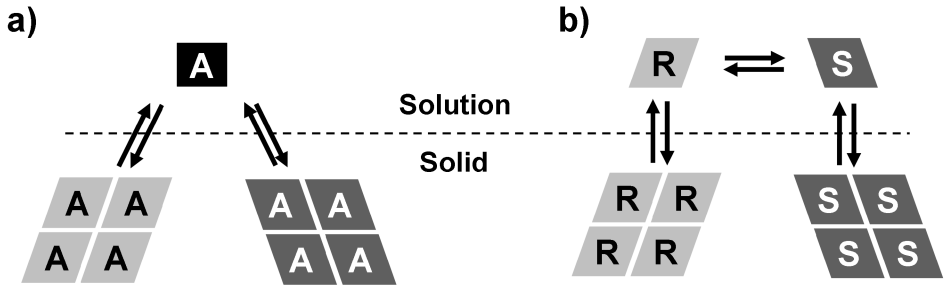


Figure 5.1 *a) Suspension of an achiral compound crystallizing in mirror imaged space groups. b) Suspension of a conglomerate, racemizing in the liquid phase.*

The grinding action of the glass beads gives smaller crystals which dissolve faster than large crystals since the surface to volume ratio is larger for smaller crystals. Had Viedma

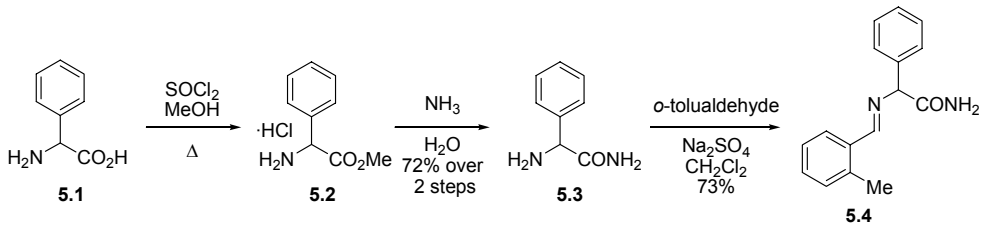
not ground his crystals this deracemization process would also have taken place, however, much slower.[6]

The conversion towards homochiral crystals relies on the fact that the solid-phase chiral identity of the intrinsically achiral $NaClO_3$ or $NaBrO_3$ is lost upon dissolution. Recently similar results have been reported with an achiral organic compound that also crystallizes as a conglomerate[7] as well as an achiral compound that forms left and right handed helices.

During discussions with Prof. Donna Blackmond of Imperial College London, on the occasion of a lecture in Nijmegen, the idea arose to attempt to extend the Viedma observation to an intrinsically chiral organic compound that could be easily racemized,[8] a requisite for complete conversion, and which was also a conglomerate. This process is depicted in Figure 5.1b. It soon became apparent that DSM scientists, with their long experience with amino acids, could likely provide suitable candidates. It was decided by the Radboud University Nijmegen, DSM, Syncom and the Imperial College in London to collaborate in this challenging quest.

5.2 Racemizable Conglomerate

Amino acid amides can be readily racemized under basic conditions when they are converted into their corresponding imines with a functionalized benzaldehyde. Several benzaldehydes were tested with phenylglycinamide (Phg-NH_2, **5.3**) at DSM Geleen and imine **5.4** from *o*-tolualdehyde showed the same XRDP patterns for racemic and optically pure **5.4** which is consistent with conglomerate type behavior, necessary for testing this idea. The synthesis of **5.4** is known from literature[9] and is depicted in Scheme 5.1.



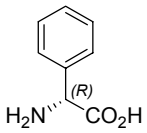
Scheme 5.1 *Synthesis of the imine of phenylglycinamide.*

Racemic phenylglycine (**5.1**) was allowed to react with MeOH and *in situ* generated HCl to deliver the HCl salt of methyl ester **5.2**. This ester was subsequently allowed to react with aqueous ammonia to deliver primary amide **5.3**. This material was treated with

o-tolualdehyde in the presence of a drying agent to remove the water. Compound **5.4** was recrystallized from MeCN to yield pure crystalline material.

5.3 Deracemization by Abrasive Grinding[10]

The method of deracemization by abrasive grinding requires racemization in solution of compound **5.4**. This can be accomplished with a base like 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Several conditions have been tested at DSM and the University of Nijmegen to find a good solvent, amount of solid glass beads, DBU-concentrations, stirring rates and amount of **5.4** in the solid phase.[11,18] Best results were obtained in MeCN as solvent and in general it may be concluded that more glass beads, higher DBU concentrations, faster stirring rates and lower amounts of **5.4** give faster deracemization. More than one hundred experiments were run in four individual laboratories[12] in new glassware and all experiments gave (*R*)-**5.4** as final product as depicted in Figure 5.2 (blank). This can be explained because of small natural (*S*)-amino acid contaminations,[13] which direct the deracemization towards the enantiomer with opposite absolute configuration as dictated by the Rule of Reversal, postulated by Lahav.[14]



(*R*)-Phg

Scheme 5.2 *(R)-Phenylglycine.*

However, by addition of small amounts of an additive with a strong structural resemblance, (*R*)-phenylglycine (Phg), as depicted in Scheme 5.2, the deracemization could be steered towards (*S*)-**5.4**.[15] Addition of larger amounts of (*S*)-Phg makes the deracemization process faster as depicted in Figure 5.2. Of course, addition of (*R*)-Phg steered the deracemization towards (*S*)-**5.4**. The role of the additive is one of growth inhibition in which the **5.4** crystals of opposite handedness are retarded in their growth.

Addition of a small bias of one enantiomer of **5.4** provided this enantiomer optically pure faster than in unbiased systems as depicted in Figure 5.3.

Furthermore, Monte Carlo computer simulations have been performed at the University of Nijmegen to elucidate the kinetics of the abrasive grinding and Ostwald ripening process.[16]

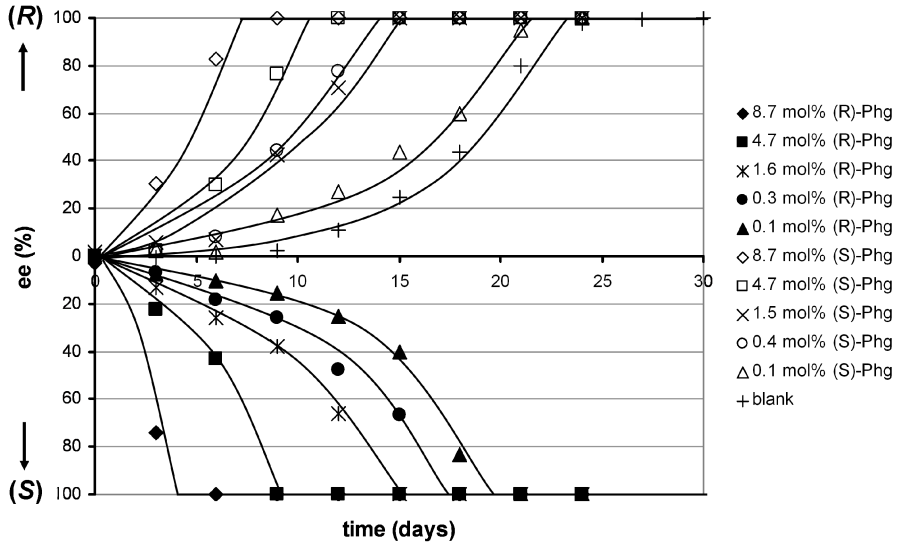


Figure 5.2 *Deracemization aided by (S) or (R)-phenylglycine. Lines are provided as a guide for the eye.*

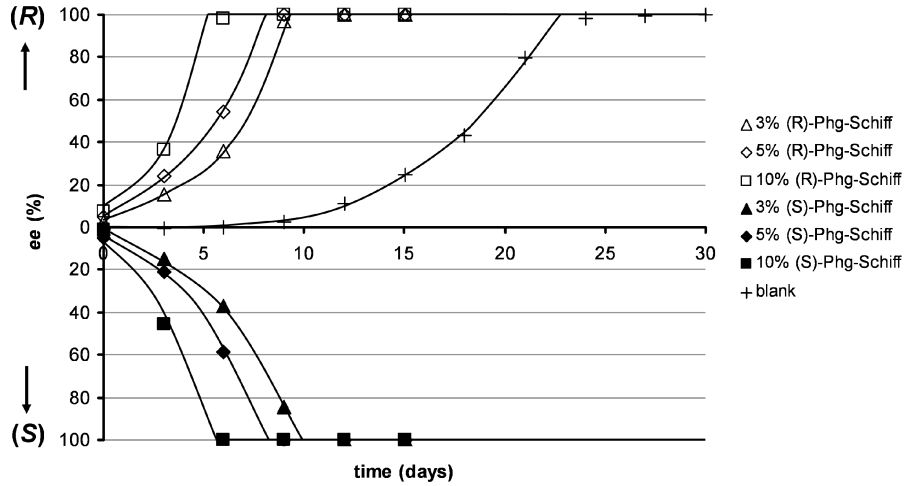


Figure 5.3 *Deracemization with small a small bias in the initial ee. Lines are provided as a guide for the eye.*

5.4 Deracemization by Sonication

Sonication is a way to break up particles in suspension by a process called cavitation. In this process, small bubbles are generated, grown in the vacuum created on passage of the sonic wave, and after a few cycles collapse. The collapsing locally generates extreme pressures (1000 bar), heat (5000 K) and heating and cooling rates (10^{10} K·s^{-1}) in the further cool liquid. With the collapsing of a bubble, a liquid jet is generated which can erode a relative large particle. Moreover, the produced shock waves generate high velocity interparticle collisions.[17] In theory, sonication provides an attractive alternative for abrasive grinding by glass beads and magnetic stirring as described in §5.3 .

A handheld sonicator (Hielscher, UP100H, 100W) with titanium sonotrode (Ø 3mm, 7 mm or 10 mm) was used to mill a suspension of (±)-**5.4** in MeCN with DBU which was placed inside a thermostated double jacketed vessel which kept the suspension at 20°C. Unfortunately, in overnight experiments brown solutions were found. Apparently, the local heat generated by the cavitation process from this relatively powerful device caused the decomposition of **5.4** (confirmed by HPLC analysis). Furthermore, prolonged use of this sonicator caused the titanium sonotrode to wear considerably, resulting in titanium contaminated reaction mixtures.

Sonication in a thermostated bath-sonicator did not lead to this decomposition of the material. However, no significant size reduction of the crystals was observed by optical microscope. When glass beads were introduced to the suspension and then sonicated, fast deracemization was observed leading to >99% *ee* **5.4** within one day.[18] Apparently, the glass beads collided with each other crushing crystals between the beads effectively. Still, the direction of non-biased or non-deliberately contaminated mixtures produced solely (*R*)-**5.4**. Even faster deracemization was achieved by addition of more solid glass beads, more DBU and less **5.4** in the solid phase.

5.5 Deracemization by Crystallization[19]

Nearly 70 years ago, during the second World war, Havinga demonstrated that conglomerate **1**, which racemizes in solution and crystallizes as enantiopure crystals of opposite handedness deposited large single crystals of high enantiomeric purity on slow undisturbed crystallization from a supersaturated solution.[2a,b] The process is illustrated in Figure 5.4. An undersaturated solution (situation I) is cooled to a point where both enantiomers are slightly supersaturated (situation II). In a stochastic process, primary nucleation takes place, arbitrarily illustrated for the (*S*)-enantiomer (situation III). This (*S*)-crystal consumes the supersaturation of the (*S*)-enantiomer in the surrounding liquid.

Because **1** racemizes in solution, the supersaturated (*R*)-enantiomer is consumed also by the growing (*S*)-crystal. This resolution can only be performed slowly, utilizing compounds with a large metastable zone width and on a small scale since one crystal has to consume the supersaturation of the surrounding liquid by diffusion.

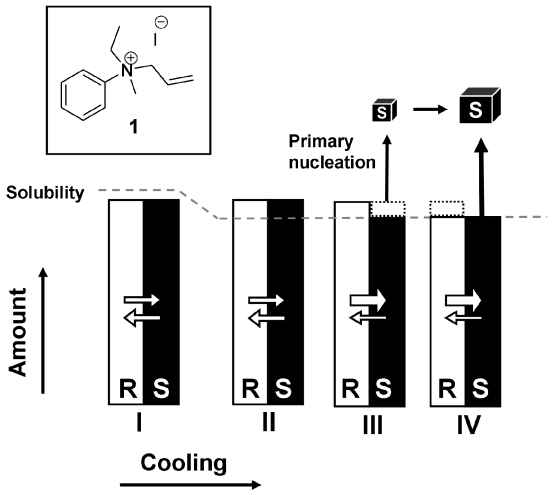


Figure 5.4 *Deracemization of allylethylmethylanilinium iodide (**1**) by slow crystallization from chloroform as performed by Havinga.*

Kondepudi, in 1990, carefully analyzed an analogous process based on $NaClO_3$, a conglomerate when crystalline.[2g] Although intrinsically achiral, $NaClO_3$ is packed in the chiral space group $P2_13$ upon crystallization. The proposed mechanism of this resolution is depicted in Figure 5.5. Starting with situation I where the $NaClO_3$ is completely dissolved, the solvent is slowly allowed to evaporate to produce a supersaturated solution of $NaClO_3$ (situation II). In situation III, one enantiopure crystal of random handedness has formed (the D-enantiomer in this example). Other than the Havinga resolution, this first crystal is ground down by a magnetic stirrer, producing multiple crystals of the same handedness (secondary nucleation) with a larger combined surface than the single crystal.[20] A larger crystal surface leads to faster consumption of the supersaturation (situation III). Higher yields can be obtained by further evaporation of the solvent (situation IV). Kondepudi found that if the solution is not stirred, the supersaturation is not consumed efficiently and primary nucleation of the other enantiomer will be observed also, resulting in a both enantiomeric crystals rather than a stochastic choice of a single chirality. Although this resolution should also be applicable to racemizing enantiomers that crystallize as conglomerates, the process is still relatively slow.

The scale of the experiment of Kondepudi was larger than that of Havinga, 120 mL and 1 mL respectively. Most likely, if Havinga had performed his experiments on a 'Kondepudi scale', the diffusion might have been too slow and then multiple crystals of random handedness would have been formed as was also observed by Kondepudi when no stirring was applied.

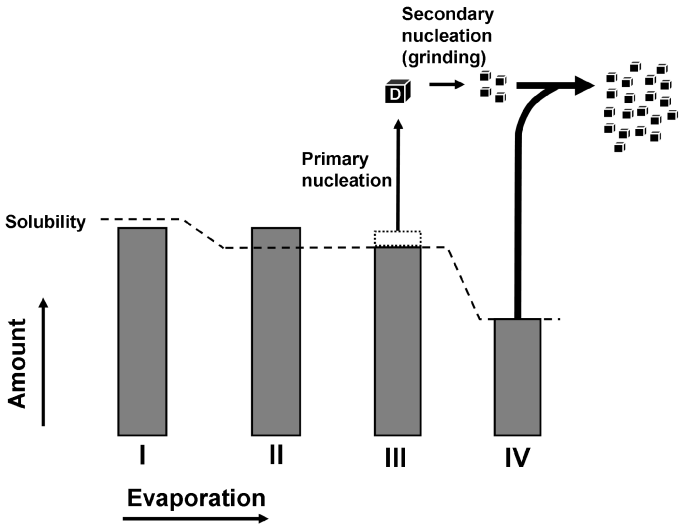


Figure 5.5 *Kondepudi deracemization of achiral $NaClO_3$ by evaporation and grinding.*

This process should also be applicable to the deracemization of (±)-**5.4**. However, evaporation of solvent under ambient atmosphere is a time consuming procedure. Furthermore, this has to be performed under an inert atmosphere since CO_2 in the air will render DBU ineffective due to carbamate formation and hence disable the racemization in solution. An alternative approach is shown in Figure 5.6. An undersaturated solution (I) is cooled slowly to supersaturation of both enantiomers (II). One enantiomer then crystallizes first (III) and is immediately ground down. The large crystal surface readily consumes the supersaturation of both enantiomers via racemization in solution (IV). Primary nucleation cannot take place without supersaturation and upon further cooling, a large volume of crystals of single handedness is generated (V).

It is not impossible to imagine that (*R*)- and (*S*)-crystals are formed nearly simultaneous. In that case, these will deracemize quickly *via* Ostwald ripening because of the small amounts of material present and the high racemization rate at elevated temperatures.

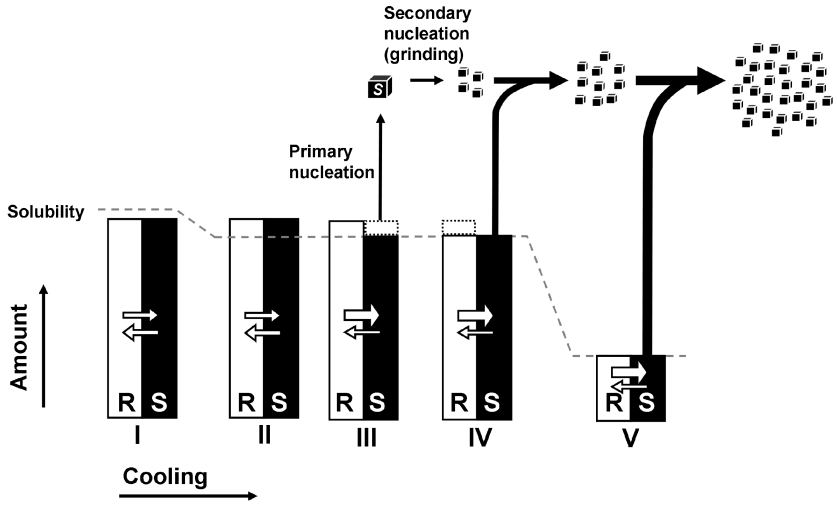


Figure 5.6 *Havinga-Kondepudi resolution of the racemizable conglomerate **5.4** by cooling and grinding*

To a mixture of MeCN and DBU was added (±)-**5.4** so that a suspension was still present at 65°C but at 70°C a clear solution was obtained. The clear solution was subsequently slowly cooled to 20°C at a preset cooling rate combined with magnetic stirring at 1250 rpm in the presence or absence of solid glass beads. A sample was taken from the resulting suspension after reaching 20°C. Solids were collected and analyzed and the results denoted in Table 5.1.

The results show that, analogous to the results of Havinga and Kondepudi, deracemization takes place if the system is allowed to consume the supersaturation by secondary nucleation. The system that was ground with solid glass beads generates more crystal surface than the system with magnetic stirring only, which prohibits the primary nucleation even at faster cooling rates. Only at a cooling rate of 2.0°C·min^{-1} the grinding/secondary nucleation/crystal growth/racemization in solution cannot keep up with the primary nucleation and solids were collected consisting of only 85% *ee*. However, repeated heating and cooling gave decomposition of the material as indicated by HPLC. These findings are similar to those found in §5.4 .

Table 5.1 *Deracemization of **5.4** by crystallization and secondary nucleation.*

Entry	cooling rate (°C·min^{-1})	stirrer[a] *ee* (%); (*R*)/(*S*)[c]	glass beads[b] *ee* (%); (*R*)/(*S*)[c]
1	2.0	25; (*R*)	85; (*R*)
2	1.0	76; (*R*)	>99; (*R*)
3	0.5	83; (*R*)	99; (*R*)
4	0.05	>99; (*R*)	>99; (*R*)

[a] Magnetic stirring at 1250 rpm. [b] Magnetic stirring at 1250 rpm with solid glass beads
[c] enantiomeric excess and absolute configuration.

Mixtures that did not produce enantiopure materials but which did create some *ee* in the solid phase will, of course, on prolonged stirring give enantiopure **5.4** as shown in §5.3.

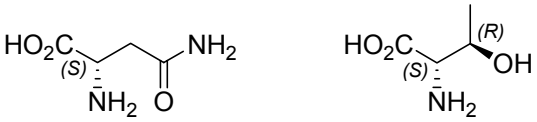
Also, deracemization of another conglomerate amino acid derivative, (±)-**5.5**, depicted in Scheme 5.4 and discussed further in §5.6.1, was complete within one day with this improved protocol. Addition of 4.6 mol% natural (*R*)-alanine (Ala) (depicted in Scheme 5.2) to racemic **5.5** gave optical pure (*S*)-**5.5** in the solid phase. Likewise, (*S*)-Ala delivered optical pure (*R*)-**5.5**, as would be expected from the rule of reversal.

5.6 Deracemization and Co-crystallization

It has been demonstrated previously[21] that under kinetic conditions, asparagine (see Scheme 5.3), a conglomerate amino acid, can incorporate upon crystallization selectively one enantiomer of a certain other (racemic) amino acid. Also, when a mixture of twelve different amino acids were added to the crystallizing racemic asparagine, "*about several percentages at most*"[22] of these amino acids were incorporated into the crystal lattice. By collecting the first crystals that form, often an enantiomeric excess was found.

The formation of enantiomerically enriched material from a racemic mixture can be explained by the fact that the crystallization of a compound from solution will take a certain amount of time with a statistic deviation. If two similar compounds with the same concentration (conglomerate forming racemate) are allowed to crystallize, of course, one enantiomer will crystallizes first. The second enantiomer will start to crystallize within seconds or minutes after the first crystallization event. If the mixture is stirred, the formed

crystals will be ground and secondary nucleation will take place, producing more crystals, which grow faster in mass than a single crystal does.[23] If the solids are collected immediately after a suspension is observed, often high enantiomeric excesses will be found in the solid phase.



L-Asparagine L-Threonine

Scheme 5.3 *Natural occurring forms of amino acids asparagine and threonine. The racemates are conglomerates.*

These results were duplicated with another conglomerate amino acid, threonine (see Scheme 5.3) in water. Nine stirred and slightly supersaturated solutions of racemic threonine were allowed to crystallize. When crystals were observed, these were isolated by filtration and the *ee* was determined. All results are depicted in Figure 5.7.

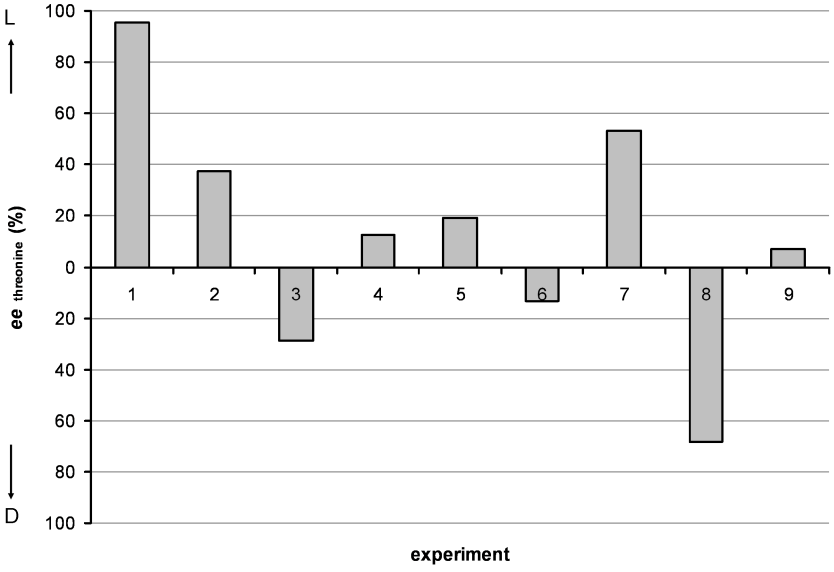


Figure 5.7 *Emergence of kinetic symmetry breaking in threonine.*

Analogously to the results with asparagine, high *ee*'s (experiments 1 and 8) are found. However, when D- and L-threonine crystallize nearly simultaneously, low *ee*'s are found

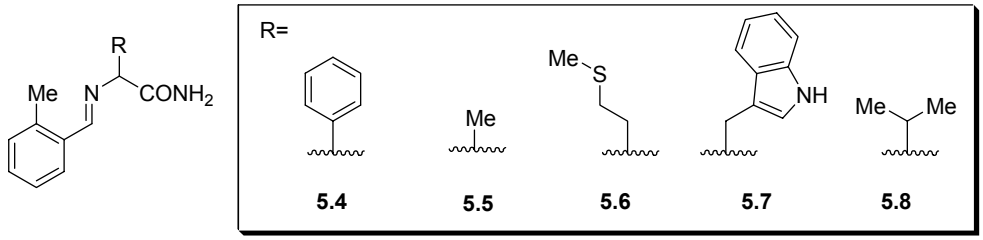
(experiments 3–6 and 9), which is characteristic for these type of experiments. From nine experiments six deliver L-threonine and three D-threonine. This imbalance might be explained by the low amount of experiments, slight initial imbalance or chiral impurities. However, these speculations were not further investigated.

The authors of the asparagine experiments fail to mention that if the mixture is allowed to proceed to equilibrium (within a couple of minutes) the *ee* of the crystallized amino acids will drop to zero. In contrast what the authors claim, without racemization in the solution, this procedure will not give high *ee*'s for a sufficient amount of time to *"account for the predominance of L-amino acids on earth"*.

Because fast racemization of amino acids is difficult to accomplish without harsh conditions and decomposition of the material,[24] amino acids, structurally akin to compound **5.4** were prepared. Imines from alanine, methionine, tryptophan and valine were prepared analogous to the synthetic route as depicted in Scheme 5.1. The synthesized amino acids are depicted in Scheme 5.4.

5.6.1 Second Harmonic Generation[25]

The amino acid derivatives shown in Scheme 5.4 were subjected to Second Harmonic Generation (SHG) experiments.[26] Both compounds **5.4** and **5.5** showed the frequency doubling signal which indicate that these compounds are most likely to be conglomerates. For the other imines, no such signal was found and these compounds are thus most likely racemic compounds.



Scheme 5.4 *Racemizable amino acid derivatives.*

Confirmation of the assignment of conglomerate behavior formation found by SHG was found when deracemization experiments were carried out by crystallization from solution. Of course, racemic compounds cannot deracemize under these conditions. It must be noted that the absence of deracemization in an experiment is not absolute proof for the absence of

a conglomerate; the deracemization might be very slow or hampered by twinning (epitaxy). The results of the experiments are shown in Table 5.2.

Table 5.2 *SHG and deracemization experiments of amino acid derivatives.*

Entry	Racemate	SHG signal[a]	ee (%); (*R*)/(*S*)[b]
1	**5.4**	Yes	>99 (*R*)
2	**5.5**	Yes	>99 (*R*)
3	**5.6**	No	0
4	**5.7**	No	0
5	**5.8**	No	0

[a] of the racemate. [b] enantiomeric excess and absolute configuration

The results show excellent correlation between the SHG experiments and the deracemization experiments. It may be concluded that only **5.4** and **5.5** crystallize as conglomerates and can be racemized under the applied conditions. The other amino acid derivatives **5.6**, **5.7** and **5.8** most likely do not crystallize as conglomerates. This, however, should be confirmed by more reliable techniques since SHG has an estimated accuracy of 90%.

Experiments have shown that the pure enantiomers of all these compounds, were completely racemized with DBU in MeCN overnight. Later, the conglomerate behavior of compounds **5.4** and **5.5** was confirmed by eutectic determination by crystallizing from enriched material without racemization and XRPD determinations of the racemate and the pure enantiomers.[27]

5.6.2 Deracemization with Multiple Compounds

When solutions of each of the other racemic imines are mixed with an excess of **5.4** and the latter is crystallized by the dissolution and crystallization/grinding procedure, as described in §5.5, each experiment shows that the imine is incorporated with high enantiomeric excess into solid **5.4** and with the same absolute configuration (Figure 5.8, experiments 1–4).

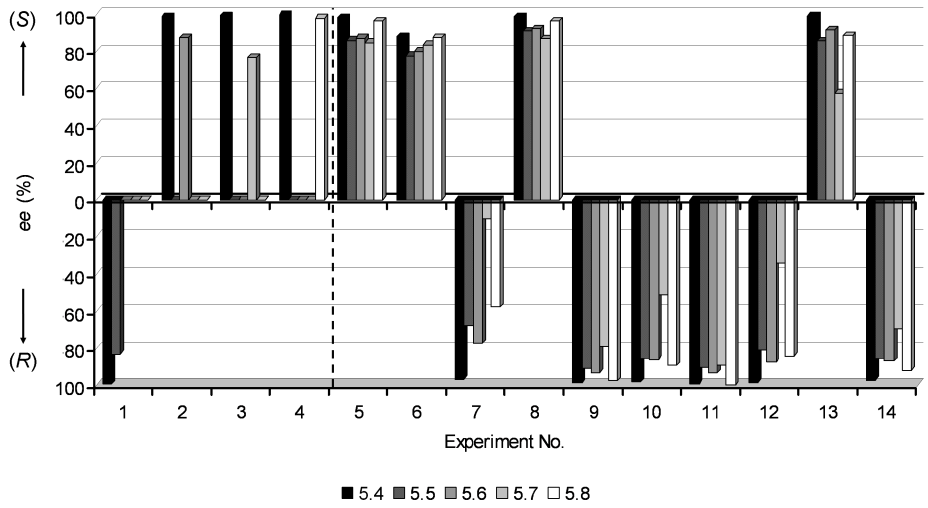


Figure 5.8 *Enantiomeric excess of amino acid derivatives. Experiments 1–4 represent crystallizations of under-saturated separate racemates* ***5.5–5.8*** *in the presence of supersaturated racemic* ***5.4****. Experiments 5–14 represent the experiments with under-saturated mixtures of all racemates* ***5.5–5.8*** *in the presence of supersaturated racemic* ***5.4****.*

When these experiments were repeated with a solution of all four imines and with an excess of **5.4**, the latter is isolated with very high enantiopurity. In contrast to the abrasive grinding experiments of **5.4** alone, the latter process appears to be nearly stochastic; 4 experiments go to (*S*) and 6 experiments go to (*R*). Note that in the mixed experiments all five amino acid imines incorporated in the solid have identical absolute configurations although the particular configuration for the entire experiment appears to be random as depicted in Figure 5.8, experiments 5–14.

The solids of experiments 5–14 were analyzed for content of imines incorporated into the crystallized **5.4** and the averages of these results are given in Table 5.3.

Although 0.29 equivalents of each of the imines has been used in comparison to **5.4**, only small amounts of these imines have co-crystallized. This observation is consistent with the Kojo experiments.[21,22] The low incorporation percentage is most likely a response to small errors in the crystal lattice of **5.4**. In comparison, in (First Generation) Dutch Resolution experiments a solid solution is often formed between the different family members of the resolving agents. The crystal lattice cannot distinguish between the resolving agents but

incorporates whatever available material fits in the crystal lattice and thus largely depends on the composition of the liquid phase.[28] Usually, high incorporation percentages are found because the starting composition of resolving agents is stoichiometric (*e.g.* 1:1 or 1:1:1). The difference between Dutch Resolution and these experiments can be explained by the fact that the family of resolving agents is more similar in structure and size than these imines.[29]

Table 5.3 *Incorporation in the crystalline phase of* ***5.4****.*

Entry	racemate	composition (%)[a]	average *ee* (%)[b]
1	**5.4**	96.76	97
2	**5.5**	0.81	86
3	**5.6**	1.22	86
4	**5.7**	0.22	66
5	**5.8**	0.98	89

[a] mass percentage. [b] always in the same absolute chiral configuration as 5.4.

Since **5.5** is a conglomerate also, the incorporation of the other amino acid derivatives could be enantioselective as well. An experiment similar to that described above was performed with an excess of racemic **5.5** and 0.047 equivalents of each of the other imines described above. The composition of the isolated crystals of a single experiment is shown in Table 5.4. Analogously to the co-crystallization with **5.4**, all components that co-crystallize in the crystal lattice of **5.5** do so with the same absolute configuration.

In these experiments, the average *ee* of each of the components is high. However, the compounds that are poorly incorporated (**5.7** in the **5.4** experiments and **5.4** in the **5.5** experiments) have low *ee*'s also. An explanation can be that small amounts of remaining mother liquor lower the *ee* to a larger extent of the component that is incorporated the least.

As given in the experimental section, these experiments were carried out overnight and worked up the next morning without checking the deracemization in the solid phase. In contrast to the Kojo experiments, the *ee* of the excess conglomerate will become 100% upon longer stirring (Ostwald ripening). Furthermore, abrasive grinding only accelerates the Ostwald ripening (crystals with a smaller volume dissolve faster). If the mixture had not been ground, the same results would have been obtained most likely, but more slowly, of course.

Table 5.4 *Incorporation in the crystalline phase of* ***5.5****.*

Racemate	composition (%)[a]	*ee* (%)[b]
5.4	0.67	36
5.5	93.53	96
5.6	2.24	73
5.7	1.94	74
5.8	1.62	75

[a] mass percentage. [b] all compounds co-crystallized in the (*S*)-enantiomer.

In nature, reasonable fast racemization of amino acids can be performed at high temperatures.[30] For instance, the water in the vicinity of thermal vents on the ocean floor may provide ideal circumstances. The combination of racemization and Ostwald ripening may pose a pathway to homochirality in nature.

5.7 Experimental Section

General Information: Reagents were obtained from commercial sources and used without further purification.

For the chiral HPLC analysis of mixtures of: 2-(((2-methylphenyl)methylidene)amino)-2-phenylacetamide (**5.4**), 2-(((2-methylphenyl)methylidene)amino)propanamide (**5.5**), 2-(((2-methylphenyl)methylidene)amino)-4-(methylsulfanyl)butanamide (**5.6**), 3-(1*H*-indol-3-yl)-2-(((2-methylphenyl)methylidene)amino)propanamide (**5.7**) and 3-methyl-2-(((2-methylphenyl)methylidene)amino)butanamide (**5.8**) two methods have been used because of overlap of some peaks.

Method 1: the separation was carried out on a Chiralcel OJ-H (150 × 4.6mm) column with a gradient of heptane:EtOH as eluent at 20°C and 0.7 mL·min^{-1}: 95:5 (20 min) in 20 min to 90:10 (10 min) in 20 min to 75:25 (40 min). UV-VIS detection was performed at 254 nm. The solids were dissolved in MeOH and injected as such. The areas under the peaks in the chromatogram were measured against a known sample so the percentage of each compound, relative to the total area under all peaks, could be determined. (*S*)-**5.8** R_f: 14.52 min, (*R*)-**5.8** R_f: 20.28 min, (*S*)-**5.5** R_f: 25.23 min, (*S*)-**5.6** R_f: 30.39 min, (*R*)-**5.5** & (*R*)-**5.6**

R_f: 38.81 min, (*S*)-**5.4** R_f: 48.79 min, (*R*)-**5.5** R_f: 64.39 min, (*S*)-**5.7** R_f: 75.87 min, (*R*)-**5.7** R_f: 97.11 min.

Method 2: the separation was carried out on a Chiralcel AD-H (150 × 4.6 mm) column with a gradient of heptane : EtOH as eluent at 20°C and 0.7 mL·min^{-1}: 95:5 (20 min) in 20 min to 90:10 (20 min) in 20 min to 75:25 (20 min). UV-VIS detection was performed at 254 nm. The solids were dissolved in MeOH and injected as such. The areas under the peaks in the chromatogram were measured against a known sample so the percentage of each compound, relative to the total area under all peaks, could be determined. (±)-**5.8** R_f: 38.72 min, (*S*)-**5.5** R_f: 45.55 min, (*S*)-**5.6** & (*S*)-**5.7** R_f: 48.49 min, (*R*)-**5.6** R_f: 51.20 min, (*R*)-**5.5** R_f: 55.35 min, (*R*)-**5.7** R_f: 60.80 min, (*S*)-**5.4** R_f: 62.82 min, (*R*)-**5.4** R_f: 77.65 min.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury 300MHz machine. Chemical shifts are denoted in δ (ppm) and are referenced to the residual protic solvent. The coupling constant *J* is denoted in Hz. Splitting patterns are denoted as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), dt (double triplet), m (multiplet) and bs (broad singlet).

Mass spectra were recorded by API-ES (electron spray ionization) by dissolving the samples in MeOH and injecting the solution as such. Mobile phase: Acetonitrile : 0.1% formic acid in water 50 : 50 (1 min.), flow: 0.2 mL·min^{-1}, injection volume: 5 µl.

Procedure for the deracemization of 5.4 at isothermal conditions as described in §5.3: A new 100 mL round-bottom flask equipped with a new PTFE coated egg-shaped magnetic stirrer (2.5 × 1.3 cm) was charged with 3.0 g (±)-**5.4**, 27 g MeCN and 7.5 g solid glass beads (Ø 2 mm) was stoppered and stirred at 1250 rpm for one hour. Fresh 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 150 µL) was added to the suspension and the stirring was continued. Samples were taken by filtration (P4) and washed with some TBME. The optical rotation of the material on the filter cake was analyzed according to Method 1 (see above). This method was also applied to deracemize 2.0 g (±)-**5.5** (15 g MeCN, 0.67 g DBU and 7 g solid glass beads, stirring at 500 rpm, after 4 days: >99% *ee*).

Procedure for the deracemization by sonication as described in §5.4: A new scintillation vial (20 mL) was charged with 10 g solid glass beads (Ø 2 mm), 0.36 g (±)-**5.4**, 3.5 g MeCN. The bottom part (~1 cm) of the stoppered vial was placed in a ultrasonic cleaning bath (Bandelin Sonorex, RK 106, 35kHz) which was kept at 20°C by a thermostat (Huber, ministat cc) and was sonicated in the middle of the bath for 5 minutes. Subsequently, fresh 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.10 g) was added and the sonication was continued. Samples were taken by filtration (P4) and washed with some TBME. The optical rotation of the material on the filter cake was analyzed according to

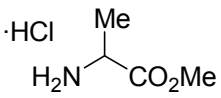
Method 1 (see above). This method was also applied to deracemize 0.46 g (±)-**5.5** under the same conditions as describes above.

Procedure for the deracemization by crystallization as described in §5.5: Two new 100 mL round-bottom flask were each charged with (±)-**5.4** (6.02 g, 23.9 mmol, 1.0 eq), DBU (1.07 mL, 7.16 mmol, 0.3 eq), MeCN (35.0 g) and an PTFE coated egg-shaped stirring bar (1.0 × 2.0 cm). One flask was additionally charged with 15.0 g solid glass beads (Ø 2 mm) also. The loosely stoppered flasks were placed in a stirred water bath (1250 rpm) which was kept at 70°C by a thermostat (Huber, ministat cc) until complete dissolution was observed. The flasks were cooled to 20°C at 0.05, 0.5, 1.0 or 2.0°C·min^{-1}. When the mixture reached 20°C, stirring was continued for another 10 minutes. A sample was taken by filtration (P4), washed with TMBE and dried on air. The *ee* composition of the solids was determined by Method 1 (see above). The suspension was then reheated to dissolution and cooled to 20°C at a different rate. Samples of the mixture at 70°C confirmed the complete racemization of the material upon reheating. After a 5 heating/cooling runs 3.18 g (53%) **5.4** was isolated.

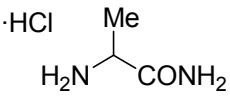
Procedure for the crystallization of (±)-threonine as described in §5.6: A suspension of DL-threonine (22.0 g) in water (100 mL) was heated to dissolution and crash cooled to 20°C. Since the solubility of DL-threonine is 212 mg/g H_2O this solution has a (super)saturation of 103.8%. From this solution, 9 × 10 mL were transferred into separate Kimble reactor tube (Ø 25 × 150 mm) with a PTFE coated egg-shaped magnetic stirring bar (19 × 10 mm). The solutions were heated to 50°C and subsequently cooled to 20°C at 0.3°C·min^{-1} and at 600 rpm in a Reactiv8 computer controlled thermostated stirrer. Suspensions were observed in the tubes between 4h 10min and 5h 50 min. When a tube contained a suspension, the solids were collected immediately and sucked to dryness. A spatula tip of filter cake was mixed with dioxane (1.5 mL), 10% Na_2CO_3 (1.5 mL) and a spatula tip of fresh FMOC-Cl. The mixture was left to stir over night and was subsequently washed with Et_2O (1.5 mL) and acidified with 1M citric acid (5 mL) (under formation of CO_2). The resulting mixture was extracted with EtOAc and evaporated by a flow of N_2. The residue was taken up in MeOH and analyzed by chiral HPLC: Column: Chirobiotic T (250 × 4.6 mm), mobile phase: MeOH/20 mM NH_4OAc in water (30 :70), flow: 0.5 mL·min^{-1}, UV detection at: 220 and 264 nm.

Procedure for the deracemization by co-crystallization as described in §5.6: A mixture of (±)-**5.5** (565 mg, 2.97 mmol 0.29 eq), (±)-**5.6** (744 mg, 2.97 mmol 0.29 eq), (±)-**5.7** (908 mg, 2.97 mmol 0.29 eq), (±)-**5.8** (649 mg, mg, 2.97 mmol 0.29 eq), DBU (356 µL, 2.38 mmol, 0.23 eq) and MeCN (8.75 g) was sonicated to dissolution and filtered (P4) to remove dust particles. The solution was placed in a new 25 mL round-bottom flask, which was charged with a new PTFE coated egg-shaped magnetic stirrer (2.0 × 1.0 cm), 5.0 g solid

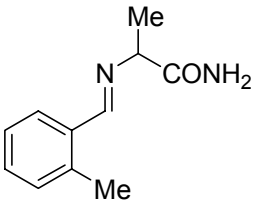
glass beads (Ø 2 mm) and (±)-**5.4** (2.614 g, 10.4 mmol, 1.0 eq). The loosely stoppered flask was placed in a stirred water bath (1250 rpm), which was kept at 70°C by a thermostat (Huber, ministat cc) and complete dissolution was observed. The flask was cooled from 70°C to 20°C at 0.05°C·min^{-1} (16h, 40 min). When the mixture reached 20°C, stirring was continued for another 2.5 hours. The solids were collected by filtration (P4), washed with TMBE and dried on air to yield white solids. The solids were analyzed by both methods 1 and 2 (see above) to determine the relative amount as denoted in Table 5.3 and the *ee* of each component as depicted in experiments 5–14 in Figure 5.8. Experiments 1–4 in Figure 5.8 were performed in the same manner as described above but with exclusion of three imines.



(±)-methyl 2-aminopropanoate hydrochloride (1a). Thionyl chloride (17.4 mL, 0.24 mol, 1.2 eq) was added dropwise to a suspension of (±)-alanine (17.8 g, 0.2 mol, 1.0 eq) in MeOH (100 mL) upon which the reaction mixture started to boil. The reaction mixture was concentrated at 50°C when ^{1}H-NMR showed full conversion after 1.5 hours. The residue was stirred in TBME (200 mL) and 27.4 g (98%) of the title compound was isolated as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.40 (d, *J*=7.2 Hz, 3H), 3.69 (s, 3H), 3.99 (q, *J*=6.9 Hz, 1H), 8.76 (bs, 3H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 15.7, 47.8, 52.8, 170.4 ppm. MS (EI): *m/z*= 104 [M+H$^+$].



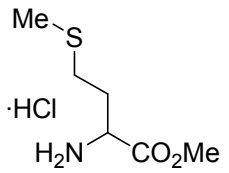
(±)-2-aminopropanamide hydrochloride (2a). To a concentrated solution of ammonia in water (150 mL) was added **1a** (27.4 g, 0.2 mol, 1.0 eq) and the resulting solution was stirred over the weekend. ^{1}H-NMR showed full conversion and the solution was concentrated to dryness and stripped with toluene to remove remaining water. This yielded the title compound (23.0 g, 92%) as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.34 (d, *J*=6.9 Hz, 3H), 3.71 (q, *J*=7.2 Hz, 1H), 7.41 (bs, 1H), 7.98 (bs, 1H), 8.18 (bs, 3H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 17.3, 48.3, 171.5 ppm. MS (EI): *m/z*= 89 [M+H$^+$].



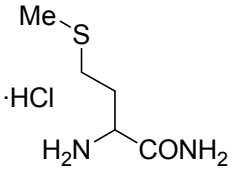
(±)-2-(((2-methylphenyl)methylidene)amino)propanamide (5.5). To a suspension of **2a** (17.7 g, 142 mmol, 1.0 eq) in CH_2Cl_2 (250 mL) was added Na_2SO_4 (25 g), *o*-tolualdehyde (16.4 mL, 142 mmol, 1.0 eq) and DIPEA (23.5 mL, 142 mmol, 1.0 eq). The mixture was stirred overnight at room temperature. Subsequently, the mixture was filtered and the residue washed with CH_2Cl_2. The filtrate was concentrated to a white solid which was washed with TBME (250 mL), H_2O (50 mL) and again TBME (100 mL). The filter cake was dried *in vacuo*. The solid was recrystallized from MeCN to yield 16 g (59%) **5.5** as a white solid.

^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.30 (d, *J*=6.6 Hz, 3H), 3.90 (q, *J*=6.9 Hz 1H), 7.07 (bs, 1H), 7.13 (bs, 1H), 7.23 (t, *J*=7.7 Hz, 2H), 7.33 (t, *J*=8.1 Hz, 1H), 7.91(d, *J*=8.1 Hz, 1H), 8.61 (s, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 19.0, 20.6, 68.3, 125.9, 127.5, 130.5, 130.8, 133.7, 137.8, 160.3, 174.7 ppm. MS (EI): *m/z*= 191 [M+H$^+$]. Optically pure **5.5** was prepared *via* the same method starting from optically pure **2a**.

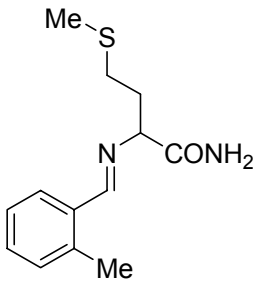
(±)-methyl 2-amino-4-(methylthio)butanoate hydrochloride (1b). Thionyl chloride (17.4 mL, 0.24 mol, 1.2 eq) was added dropwise to a suspension of (±)-methionine (29.8 g, 0.2 mol, 1.0 eq) in MeOH (100 mL) upon which the reaction mixture started to boil. The reaction mixture was concentrated at 50°C when ^{1}H-NMR showed full conversion after 1.5 hours. The residue was stirred in TBME (3 × 200 mL) and 39.0 g (98%) of the title compound was isolated as a yellowish solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 2.03 (s, 3H), 2.07 (q, *J*=6.9 Hz, 2H), 2.50-2.70 (m, 2H), 3.73 (s, 3H), 4.06 (t, *J*=6.3 Hz, 1H), 8.72 (bs, 3H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 14.3, 28.4, 29.3, 50.8, 52.8, 169.6 ppm. MS (EI): *m/z*= 164 [M+H$^+$].



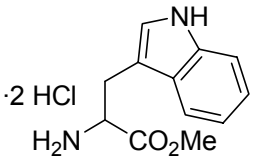
(±)-2-amino-4-(methylthio)butanamide hydrochloride (2b). To a concentrated solution of ammonia in water (150 mL) was added **1b** (39.0 g, 0.2 mol, 1.0 eq) and the resulting brown/orange solution was stirred over the weekend. ^{1}H-NMR showed full conversion and the solution was concentrated to dryness and stripped with toluene to remove remaining water. This yielded the title compound (35.6 g, 96%) as an off-white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.94–2.00 (m, 2H), 2.04 (s, 3H), 2.50–2.60 (m, 2H), 3.76 (t, *J*=6.3 Hz, 1H), 7.51 (bs, 1H), 7.98 (bs, 1H), 8.04 (bs, 3H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 14.6, 28.5, 31.0, 51.7, 170.2 ppm. MS (EI): *m/z*= 149 [M+H$^+$].



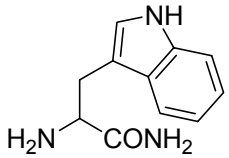
(±)-2-(((2-methylphenyl)methylidene)amino)-4-(methylsulfanyl)butanamide (5.6). To a suspension of **2b** (25.2 g, 136 mmol, 1.0 eq) in CH_2Cl_2 (250 mL) was added Na_2SO_4 (25 g), *o*-tolualdehyde (15.7 mL, 136 mmol, 1.0 eq) and DIPEA (22.5 mL, 136 mmol, 1.0 eq). The mixture was stirred overnight at room temperature. Subsequently, the mixture was filtered and the residue washed with CH_2Cl_2. The filtrate was concentrated to a yellow oil which was stirred with TBME (250 mL). The solids were collected and washed with TBME (50 mL), H_2O (50 mL) and again TBME (100 mL). The filter cake was dried *in vacuo*. The solid was recrystallized from MeCN to yield 26.7 g (79%) **5.5** as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.80–2.10 (m, 2H), 2.01 (s, 3H), 2.30–2.50 (m, 2H), 2.50 (s, 3H), 3.89 (dd, J_1=4.5 Hz, J_2=8.4 Hz, 1H),



7.08 (bs, 1H), 7.21 (bs, 1H), 7.23 (t, *J*=7.5 Hz, 2H), 7.34 (t, *J*=7.8 Hz, 1H), 7.90 (d, *J*=7.8 Hz, 1H), 8.59 (s, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 14.5, 19.0, 29.6, 33.1, 72.4, 126.0, 127.7, 130.5, 130.8, 133.6, 137.9, 161.5, 173.6 ppm. MS (EI): *m/z*= 251 [M+H$^+$]. Optically pure **5.6** was prepared *via* the same method starting from enantiopure **2b**.



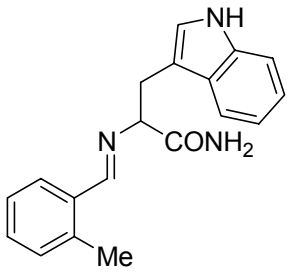
(±)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate dihydrochloride (1c). Thionyl chloride (17.4 mL, 0.24 mol, 1.2 eq) was added dropwise to a suspension of (±)-tryptophan (40.8 g, 0.2 mol, 1.0 eq) in MeOH (100 mL) upon which the reaction mixture started to boil. After 40 minutes, the reaction mixture crystallized as a very thick suspension and the mixture was stirred at 50ºC for 5.5 hours to complete the reaction as indicated by ^{1}H-NMR. The reaction mixture was diluted by addition of TBME (200 mL) and the solids were collected and washed with MeOH and TBME. After drying *in vacuo* this furnished the title compound (50.7 g, 88%) as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 3.20–3.40 (m, 2H), 3.62 (s, 1H), 4.17 (t, *J*=6.0 Hz, 1H), 6.98 (t, *J*=7.5 Hz, 1H), 7.07 (t, *J*=6.3 Hz, 1H), 7.23 (d, *J*=2.1 Hz, 1H), 7.35 (t, *J*=8.1 Hz, 1H), 7.49 (t, *J*=7.8 Hz, 1H), 8.64 (bs, 3H), 11.11 (bs, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 26.1, 52.6, 52.8, 106.4, 111.6, 118.1, 118.6, 121.1, 125.0, 127.0, 136.2, 169.7 ppm. MS (EI): *m/z*= 219 [M+H$^+$], 203 [M-NH$_3$+H$^+$].



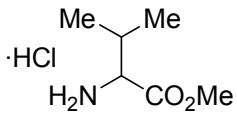
(±)-2-amino-3-(1*H*-indol-3-yl)propanamide (2c). To a concentrated solution of ammonia in water (150 mL) was added **1c** (39.0 g, 0.2 mol, 1.0 eq) and the resulting beige suspension was stirred over the weekend. ^{1}H-NMR showed full conversion and the suspension was cooled to 4°C and left to crystallize overnight. The solids were collected and washed with cold water (50 mL) and TBME (100 mL). The filter cake was dried *in vacuo*. This furnished the title compound (25.0 g, 61%) as white solids. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 1.63 (bs, 2H), 2.70 (dd, J_1=8.1 Hz, J_2=14.1 Hz, 1H), 3.04 (dd, J_1=4.8 Hz, J_2=14.4 Hz, 1H), 3.39 (dd, J_1=4.5 Hz, J_2=8.1 Hz, 1H), 6.93 (bs, 1H), 6.95 (dt, J_d=1.2 Hz, J_t=6.9 Hz, 1H), 7.04 (dt, J_d=0.9 Hz, J_t=7.8 Hz, 1H), 7.14 (d, *J*=2.4 Hz, 1H), 7.31 (d, *J*=7.2 Hz, 1H), 7.31 (bs, 1H), 7.56 (d, *J*=7.8 Hz, 1H), 10.81 (bs, 1H). ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 31.3, 55.5, 111.0, 111.5, 118.4, 118.7, 121.0, 123.9, 127.6, 136.4, 177.5 ppm. MS (EI): *m/z*= 204 [M+H$^+$], 226 [M+Na$^+$], 202 [M-H$^+$].

(±)-3-(1*H*-indol-3-yl)-2-(((2-methylphenyl)methylidene) amino)propanamide (5.7). To a suspension of **2c** (24.4 g, 120 mmol, 1.0 eq) in CH_2Cl_2 (250 mL) was added Na_2SO_4 (25 g) and *o*-tolualdehyde (13.9 mL, 136 mmol, 1.0 eq). The mixture was stirred overnight at room temperature. Subsequently, the mixture was filtered and the residue washed with CH_2Cl_2. The filtrate was concentrated to white solid material. The solids were collected and

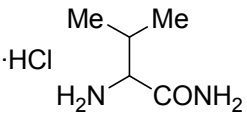
stirred in TBME. The solids were collected and washed with TBME (50 mL) and dried *in vacuo*. This yielded 32.7 g (89%) **5.7** as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 2.50 (s, 3H), 2.99 (dd, J_1=9.3 Hz, J_2=14.1 Hz, 1H), 3.39 (dd, J_1=2.1 Hz, J_2=14.1 Hz, 1H), 4.01 (dd, J_1=4.2 Hz, J_2=9.3 Hz, 1H), 6.92 (t, *J*=6.3 Hz, 1H), 7.00–7.32 (m, 7H), 7.52 (d, *J*=8.1 Hz, 1H), 7.84 (d, *J*=7.8 Hz, 1H), 7.99 (s, 1H), 10.77 (s, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 18.4, 30.2, 74.2, 110.4, 111.4, 118.4, 118.6, 121.0, 124.1, 125.9, 127.3, 127.4, 130.3, 130.6, 133.6, 136.3, 137.6, 160.3, 174.3 ppm. MS (EI): *m/z*= 306 [M+H$^+$], 328 [M+Na$^+$], 304 [M-H$^+$]. Optically pure **5.7** was prepared *via* the same method starting from enantiopure **2c**.



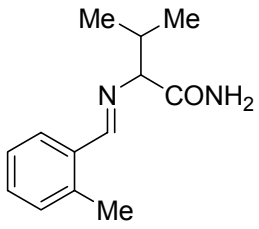
(±)-methyl 2-amino-3-methylbutanoate hydrochloride (1d). Thionyl chloride (27.4 mL, 0.38 mol, 1.9 eq) was added dropwise to a suspension of (±)-valine (23.4 g, 0.2 mol, 1.0 eq) in MeOH (100 mL) upon which the reaction mixture started to boil. The mixture was kept at reflux temperature by heating. The reaction mixture was concentrated at 50°C when ^{1}H-NMR showed full conversion after one day. The oily residue was tritruated in TBME (3 × 200 mL) and the solid was collected by filtration and washed with TMBE (50 mL). The title compound was dried *in vacuo* to yield 26.7 g (80%) as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 0.905 (d, *J*=6.9 Hz, 3H), 0.956 (d, *J*=6.9 Hz, 3H), 2.15–2.20 (m, 1H), 3.71 (s, 3H), 3.77 (bs, 1H), 8.72 (bs, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 17.6, 18.8, 29.3, 52.6, 57.5, 169.1 ppm. MS (EI): *m/z*= 132 [M+H$^+$].



(±)-2-amino-3-methylbutanamide hydrochloride (2d). To a concentrated solution of ammonia in water (150 mL) was added **1d** (26.7 g, 0.16 mol, 1.0 eq) and the resulting solution was stirred over the weekend. ^{1}H-NMR subsequently showed full conversion and the solution was concentrated to dryness and stripped with toluene to remove remaining water. This yielded the title compound (23.9 g, 78%) as an off-white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 0.85–0.95 (m, 6H), 2.09 (q, *J*=5.7 Hz, 1H), 3.53 (d, *J*=5.4 Hz, 1H), 7.48 (bs, 1H), 7.93 (bs, 3H), 7.97 (bs, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 18.1, 18.6, 29.6, 57.4, 169.9 ppm. MS (EI): *m/z*= 117 [M+H$^+$], 139 [M+Na$^+$].



(±)-3-methyl-2-(((2-methylphenyl)methylidene)amino) butanamide (5.8). To a suspension of **2d** (21.2 g, 139 mmol, 1.0 eq) in CH_2Cl_2 (250 mL) was added Na_2SO_4 (25 g), *o*-tolualdehyde (16.1 mL, 139 mmol, 1.0 eq) and DIPEA (23.0 mL, 139 mmol, 1.0 eq). The mixture was stirred overnight at room temperature. Subsequently, the mixture was filtered and the residue washed with CH_2Cl_2. The filtrate was concentrated to a red paste which was



stirred in TBME (250 mL) to yield a pink suspension which was filtered. The filter cake was washed with TBME (50 mL) and the filtrate was concentrated and stripped with toluene. The resulting solid was recrystallized from MeCN to yield 21.0 g (69%) **5.8** as a white solid. ^{1}H-NMR (300MHz, *d6*-DMSO): δ= 0.83 (s, 3H), 0.85 (s, 3H), 2.18 (m, 1H), 2.50 (s, 3H), 3.48 (d, *J*=5.7 Hz, 1H), 6.96 (bs, 1H), 7.15–7.35 (m, 4H), 7.87 (d, *J*=6.9 Hz, 1H), 8.51 (s, 1H) ppm. ^{13}C-NMR (75MHz, *d6*-DMSO): δ= 18.0, 19.2, 19.4, 31.5, 80.0, 126.0, 128.0, 130.4, 130.8, 133.7, 137.7, 161.0, 173.6 ppm. MS (EI): *m/z*= 219 [M+H^{+}], 239 [M+Na^{+}]. Optically pure **5.8** was prepared *via* the same method starting from enantiopure **2d**.

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11 Performed at DSM Geleen and the University of Nijmegen. The results will not be discussed here.

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26 Second Harmonic Generation is further explained in Chapter 2.3.2.1.

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Chapter 6

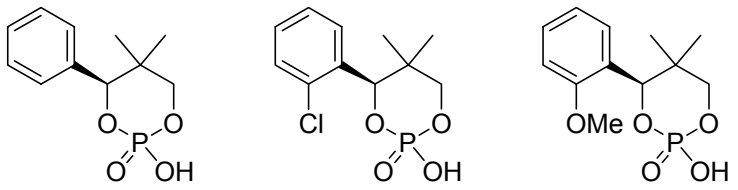
The Resolution of Phencyphos.

In this chapter[*] the resolution of phencyphos by entrainment is described. An overview of the history of phencyphos, the screening for efficient resolving agents and methods to resolve phencyphos on a preparative scale are given. Also, a new method to resolve (±)-phencyphos is devised that relies on a pseudo polymorphic transition by hydrate formation.

[*] *Parts of this chapter have been published in Org. Process Res. Dev. (DOI: 10.1021/op900171k).*

6.1 Introduction

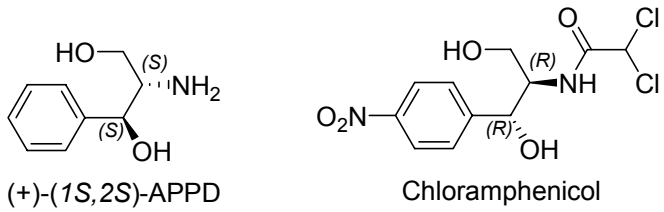
The synthesis and resolution of cyclic phosphoric acids, shown in Scheme 6.1 (together with their trivial names), was described more than 20 years ago.[1] These phosphoric acids show excellent behavior as resolving agents because of their ability to form crystalline salts with high *de*'s with many (weak) bases.



Scheme 6.1 *Cyclic phosphoric acids and their trivial names.*

Racemic chlocyphos and anicyphos are resolved without much effort with standard resolving agents.[1] However, phencyphos, an excellent resolving agent itself, resists resolution with most standard basic resolving agents, a good example that reciprocal resolutions are not by definition successful.[2]

Chloramphenicol (Scheme 6.2) is produced as an antibiotic.[3] Since each enantiomer/diastereomer of most chiral drugs has a different activity and toxicity, the undesired enantiomer of an intermediate used in the synthesis: (+)-(*1S,2S*)-2-amino-1-phenyl-1,3-propanediol (APPD), had to be discarded as waste.



Scheme 6.2 *(+)-2-amino-1-phenyl-1,3-propanediol and Chloramphenicol.*

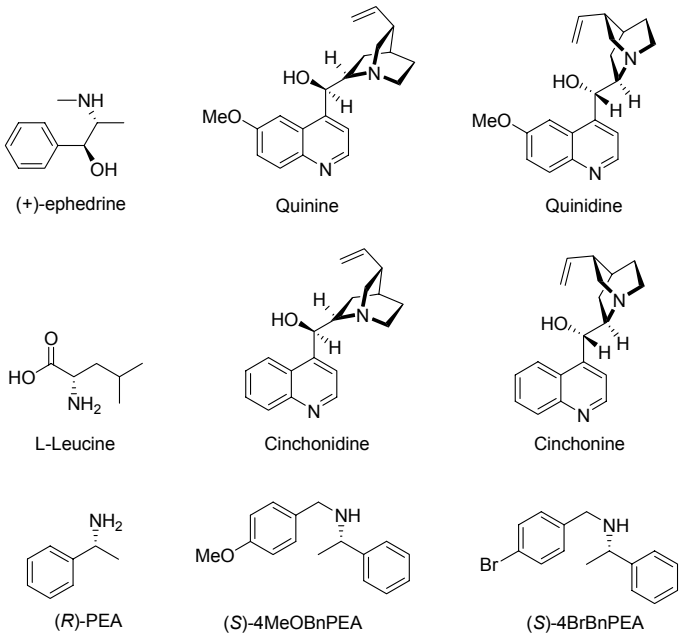
Since the costs of disposal of chemical waste are high, (+)-APPD was put on the market as an inexpensive optically pure basic resolving agent. This (+)-APPD proved to be an excellent resolving agent for phencyphos furnishing both (+) and (–)-phencyphos after

liberation of the isolated salts and mother liquors and subsequent crystallization of the free acids.

However, today the production of Chloramphenicol has been largely ceased for use in western countries because of a rare but serious side effect: *aplastic anemia*.[4] Aplastic anemia is a condition in which bone marrow does not produce sufficient new cells to replenish blood cells.[5] Although the Chloramphenicol is still in use in third-world countries and in eye-drops against eye infections, the synthesis has been altered to an economically more attractive asymmetric route and hereby eliminating the formation of (+)-APPD.[6] Hence, (+)-APPD is no longer available at a low cost, making the resolution of phencyphos both a time-consuming and an expensive business because of the non-quantitative recovery of the resolving agent. A more efficient method had to be found to resolve phencyphos.

6.2 Screening of Resolving Agents

Since large scale resolutions of phencyphos had to be performed, an inexpensive, readily available and easily recoverable resolving agent was required. Several standard basic resolving agents (depicted in Scheme 6.3) were screened to find an alternative for the resolution with (+)-APPD.



Scheme 6.3 *Basic resolving agents.*

Resolutions were performed on 1 mmol scale, in several solvents. Some resolving agents that were tested either failed to produce crystalline salts, failed to form salts or gave hard to filter gel-like precipitates. The results of resolutions that provided crystalline salts are given in Table 6.1.

Table 6.1 *Results of small scale resolutions.*

Entry	Resolving agent	Solvent	Yield (%)	*de*(%); (+/-)[a]
1	(+)-ephedrine	EtOAc	41	0
2	Quinine	IPA	50	14; (+)
3	Quinidine	EtOAc	57	12; (+)
4	Cinchonidine	IPA	18	85; (+)
5	Cinchonine	EtOAc	24	61; (–)
6	(*R*)-PEA	IPA	50	36; (–)
7	(*S*)-4BrBnPEA	Acetone	49	8; (+)

[a] enriched in either (+)-phencyphos or (–)-phencyphos

Quinine and quinidine are considered to be pseudo enantiomers even though their relationship is diastereomeric. In this case one expects that one enantiomer of a racemic acid can be obtained when crystallized with quinine and the other enantiomer by resolution with quinidine. However, as can be seen from Table 6.1, the resolutions of (±)-phencyphos with these pseudo enantiomers do not deliver the opposite enantiomers of phencyphos. On the other hand, the resolutions with cinchonidine or with its pseudo enantiomer cinchonine did deliver the opposite enantiomers.

The resolutions with cinchonidine, cinchonine and PEA (entries 4–6) showed potential and these were investigated further.

6.2.1 Diastereomeric Salt Formation with One Half Equivalent of Resolving Agent

Small scale resolution experiments were performed on 2 mmol scale with the aid of controlled cooling and stirring. Also, resolutions were performed by the Peachey and Pope method in which a half equivalent basic resolving agent is replaced by an inexpensive

achiral base.[2,7] In resolutions by the Pope and Peachey method, the solubility of the less soluble salt is higher than in a normal diastereomeric salt resolution, which could allow for higher reactor loadings if the resolution were to be scaled up. Furthermore, only half of the relatively expensive resolving agent is used and most of it will precipitate as its diastereomeric salt during the resolution. In that event, only the precipitate has to be liberated to recover most resolving agent. The mother liquor will contain mostly the other enantiomer of phencyphos with the achiral base. Since the (±)-phencyphos salt of triethyl amine (TEA) is an oil at room temperature, or at least crystallizes very slowly, it was an ideal achiral base since the salt with phencyphos will remain in the mother liquor. In an attempt to perform a resolution by the method of half-quantities,[2,8,9] in which a half equivalent of resolving agent was left out without neutralization, the poorly soluble phencyphos crystallized as its free (racemic) acid. The results of the resolutions are given in Table 6.2.

Table 6.2 *Resolution of phencyphos with cinchonidine, cinchonine or (R)-PEA.*

Entry	Resolving agent	Achiral base	Solvent	Yield (%)	*de* (%); (+/-)[a]
1	cinchonidine (1.0 eq)	none	IPA	66	25; (+)
2	cinchonidine (0.5 eq)	TEA (0.5 eq)	toluene /EtOH	22	11; (–)
3	cinchonine (1.0 eq)	none	IPA	- [b]	n.d.[c]
4	cinchonine (0.5 eq)	TEA (0.5 eq)	MEK /IPA	- [d]	n.d.[c]
5	(*R*)-PEA (1.0 eq)	none	IPA /H_2O	55	30 ; (+)
6	(*R*)-PEA (0.5 eq)	TEA (0.5 eq)	IPA	- [b]	n.d.[c]

[a] enriched in either (+)-phencyphos or (–)-phencyphos, [b] Gel-like material which was difficult to filter, [c] not determined, [d] slow crystallization

The difference between the resolution with cinchonidine in entry 4 in Table 6.1 and entry 1 in Table 6.2 may be explained by the higher concentration of the latter resolution experiment thus providing a higher yield and lower *de*.

The Pope and Peachey resolution in entry 2 shows enrichment in the opposite enantiomer compared to the resolution in entry 1. Most likely this is because of solvation of the phencyphos salt by 2-propanol, toluene and/or ethanol. Since both yield and *de* are low in entry 2, no further investigation was carried out on the possible occurrence of solvation.

The resolutions with cinchonine (entries 3 and 4) delivered gel-like materials which were very difficult to filter and thus not suitable for large scale resolutions. The previous resolution with cinchonine in ethyl acetate (entry 5 in Table 6.1) did give salts with good filtration behaviour and these results seem due to a solvent (habit modification of solvate formation) or concentration effect. However, since the cinchonine salts of phencyphos dissolves poorly in ethyl acetate, this was no option for up scaling since the reactor loading would be too low.

The resolution with (*R*)-PEA in entry 5 gave a enrichment in (+)-phencyphos which might be improved by a nucleation inhibitor on condition of the absence of an end-solid solution. An attempt to perform a Pope and Peachey resolution (entry 6) was foiled by the formation of difficult to filter gel-like salts.

The resolutions with cinchonidine and (*R*)-PEA were further investigated as function of time in order to allow conclusions about the presence of an end-solid solution or metastable equilibria.

6.2.2 Resolutions Followed in Time

The resolution of (±)-phencyphos with cinchonidine was followed in time during the cooling of the experiment. Samples were taken immediately after material started to precipitate. The *de*'s of both the mother liquor and the (washed) solids were determined. The yield of crystallized solids can be determined from both *de*'s.[10] The results for the resolution with cinchonidine are depicted in Figure 6.1.

As can be concluded from the figure, the slow decrease of *de* of the solids means that the crystallization of the more soluble salt is slow. Furthermore, the first salts which appear during cooling, have 78% *de*. This means the least soluble salt has an end solid solution and the isolated material will need several recrystallizations to arrive at >99% *de*. A better alternative would be to liberate the salt with 78% *de* and recrystallize the free phencyphos to high *ee* as was known to be possible from the original procedure with APPD.[1] After ~15 hours, both mother liquor and precipitated solids have 74% *de* which means the yield is 50%. The maximum yield which can be obtained for each enantiomer, provided phencyphos crystallizes as an conglomerate, is 50% × 74% *de* = 37% yield from a system that contains 50% of each enantiomer.

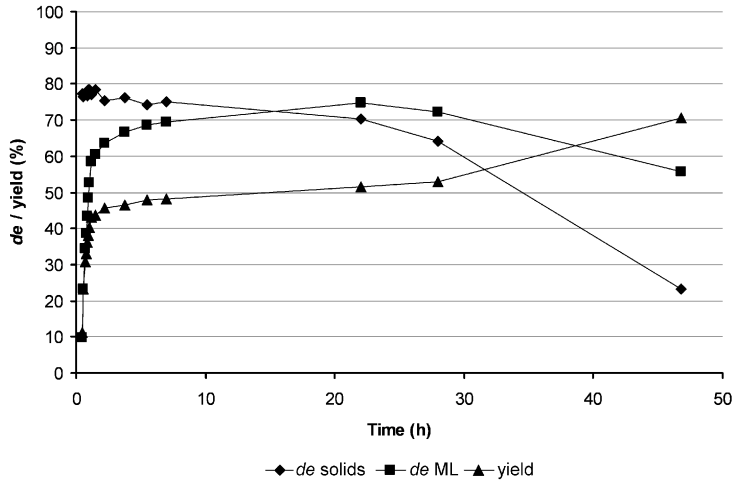


Figure 6.1 *Resolution of (±)-phencyphos with cinchonidine in time. Lines are provided as a guide for the eye.*

The resolution of (±)-phencyphos with (*R*)-PEA was followed in time also. Again, samples were taken and analyzed when crystals started to form. These results are depicted in Figure 6.2.

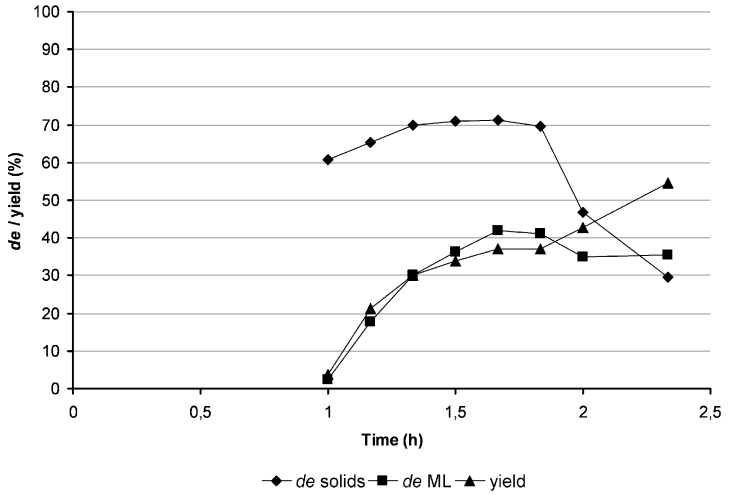


Figure 6.2 *Resolution of (±)-phencyphos with (R)-PEA in time. Lines are provided as a guide for the eye.*

In this resolution, the more soluble diastereomer starts to crystallize within 2 hours of the start of the cooling program. Furthermore, a maximum of 71% *de* can be obtained in this resolution with only 36% yield. This means that if the salt is liberated and crystallized, the maximum yield which can be obtained for (+)-phencyphos, provided phencyphos crystallizes as a conglomerate, is 36% × 71% *de* = 26% yield.

Clearly, the resolution of (±)-phencyphos with cinchonidine is superior to that with (*R*)-PEA but still requires laborious recycling of the resolving agent and recrystallization to achieve high *ee*'s. Another method to obtain complete separation of the enantiomers was investigated: resolution by entrainment.

6.3 Resolution by Entrainment[11]

The resolution of a racemate by entrainment is possible if the racemate crystallizes as a conglomerate (or a racemic compound with very low eutectic values). The ternary phase diagram was constructed in ethanol.

The phase diagram showed that phencyphos crystallizes as a racemic compound with a eutectic at 70 % *ee* (**A** and **A'**) as shown in Figure 6.3 and is thus not suitable for resolution by entrainment.

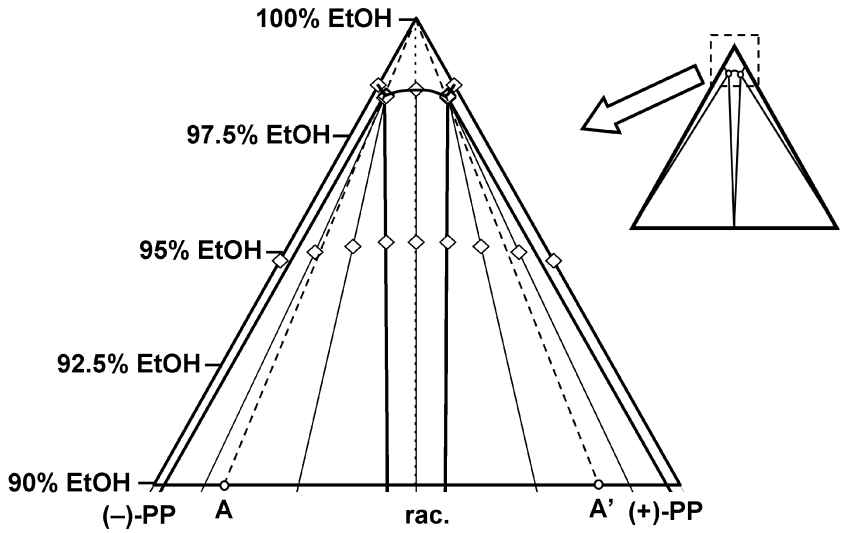


Figure 6.3 *Top 10% of the solubility diagram of phencyphos in ethanol.*

Solvates of phencyphos with either MeOH, EtOH or IPA should give different IR signatures. However, the signatures were similar which indicates that MeOH, EtOH and IPA do not give solvates with (±)-phencyphos as depicted in Figure 6.4.

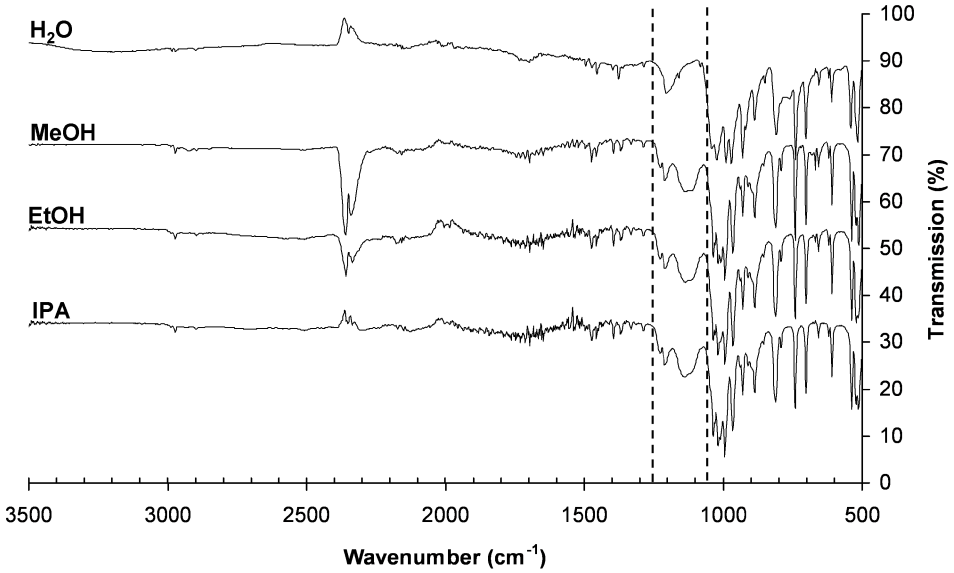


Figure 6.4 *IR spectra from (±)-phencyphos crystallized from several solvents. The peaks at 2350 cm^{-1} are from CO_2.*

However, a recent report in the literature indicated that phencyphos, crystallized from water, can form a hydrate.[12] IR analysis of (±)-phencyphos which was crystallized from water is depicted in Figure 6.4. It is clearly seen that the fingerprint between 1250 cm^{-1} and 1050 cm^{-1} is different in (±)-phencyphos crystallized from H_2O than (±)-phencyphos crystallized from either MeOH, EtOH or IPA. Most likely, this hydrate was also isolated when the APPD-phencyphos salt was liberated with aqueous hydrochloric acid. In the original description of the synthesis of phencyphos the hydrate formation was apparently overlooked. However, it was noted that the isolated phencyphos crystallized as a conglomerate, information that can be found in the experimental section of that article.[1]

Because phencyphos hydrate has a low solubility in pure water (Table 6.3, entry 3), a co-solvent should be added in order to increase the amount of phencyphos in the solution. The higher solubility will make the process economically more attractive. The solubility of

anhydrous phencyphos in dry co-solvents was investigated. A requirement for these solvents is that the co-solvent should be water miscible and should have a high solubility for phencyphos. The solubility data were determined by suspending phencyphos in the appropriate dry solvent and determining the phencyphos content in the filtrate by evaporation. The results are given in Table 6.3.

Table 6.3 *Solubilities of (±)-phencyphos in several water miscible solvents.*

Entry	Solvent	Solubility (mg (±)-phencyphos/mL solvent)
1	MeCN	< 1
2	1,4-dioxane	1.1
3	water	3.2
4	ethylene glycol	4.0
5	acetic acid	4.8
6	1-butanol	5.7
7	sec-butanol	5.8
8	IPA	8.4
9	MeOH	17
10	DMF	81
11	DMSO	142

Most solvents dissolve (±)-phencyphos poorly. However, MeOH, DMF and DMSO show potential to increase the solubility of phencyphos hydrate but still allow the hydrate to crystallize. Solubility diagrams were constructed to determine the minimal water content of the solvent mixture to be able to isolate the hydrated conglomerate and not the anhydrous racemic compound.

6.3.1 Hydration of Phencyphos in DMSO/Water Mixtures

Several mixtures of water and DMSO were prepared and the compositions of the mother liquors were determined and plotted in a phase diagram. Since the removal of DMSO from the filter cake without dehydration of the phencyphos is difficult, the composition of the solids was not determined.

Small scale resolution by entrainment was attempted with enantiomerically enriched (~15% *ee*) in (–)-phencyphos. However, below 25% wt. water, no enrichment was found in the first solids even when the supersaturated solution was seeded with (–)-phencyphos hydrate. The mixtures with less than 25% wt. water were also difficult to filter, indicating a different crystal habit, polymorph, solvate and/or hydrate. The presence of a hydrate solvate which crystallizes as a racemic compound with unknown composition ((±)-phencyphos·x H_2O·y DMSO) might explain this unexpected result. This hypothesis was further investigated by XRPD.

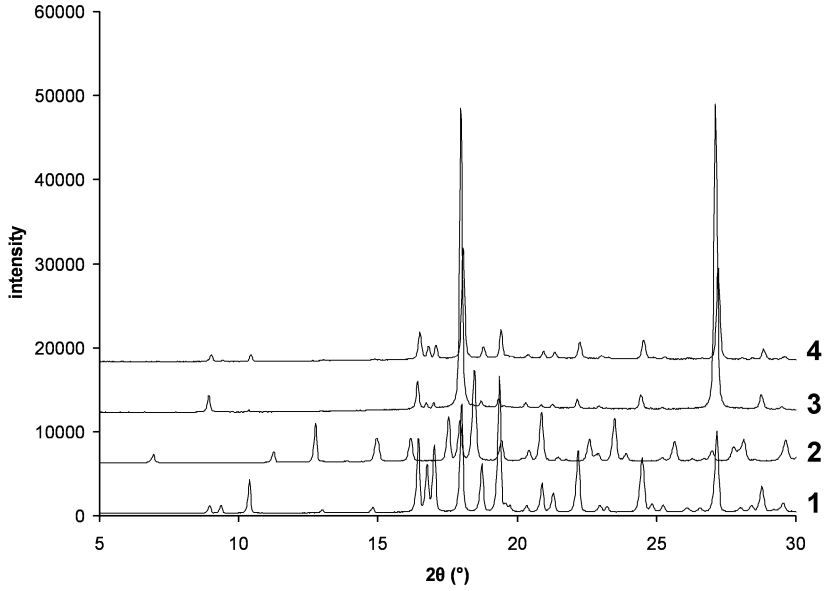


Figure 6.5 *XRPD of racemic phencyphos crystallized from 1) MeOH, 2) 50% H_2O in MeOH, 3) DMSO and 4) 15% H_2O in DMSO.*

Figure 6.5 depicts the XRPD spectra of crystals obtained from 15% H_2O in DMSO (4). The solids were analyzed by XRPD and compared with that of anhydrous (±)-phencyphos from

MeOH (1), phencyphos hydrate from a H_2O:MeOH mixture (2) and anhydrous (±)-phencyphos from DMSO (3). Although peak intensities of spectra 3 and 4 are different from anhydrous (±)-phencyphos (1), the location of peaks are the same. This means that with 15% H_2O:DMSO anhydrous (±)-phencyphos (1) is isolated and these crystals have a different morphology and/or a different orientation of the crystals in the XRPD than the anhydrous (±)-phencyphos crystallized from pure MeOH. This was also confirmed by the more difficult filtration behavior of (±)-phencyphos crystallized from solvent mixtures with less than 25% H_2O in DMSO mixtures.

The phase diagram with (±)-phencyphos, water and DMSO is shown in Figure 6.6.

During an entrainment experiment, the crystallization of the hydrate was slow compared with that of the MeOH/water mixtures (see Chapter 6.3.3). No further investigations were performed on this solvent mixture.

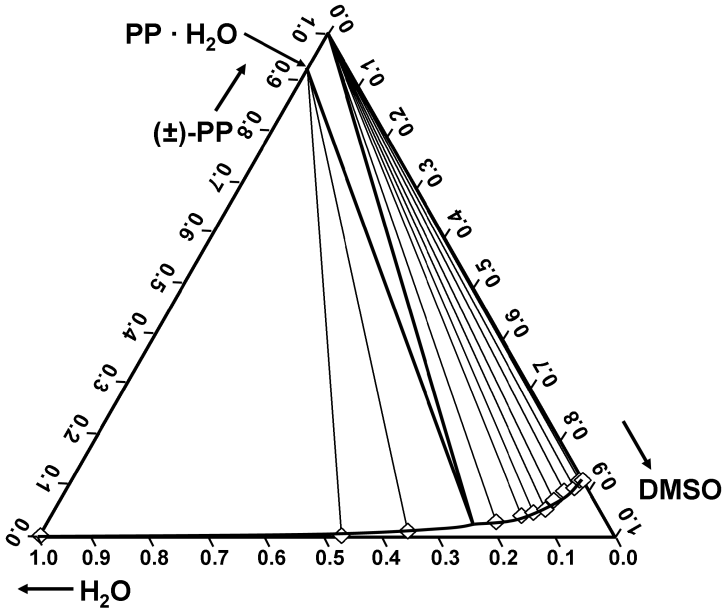


Figure 6.6 *Solubility diagram of (±)-phencyphos, water and DMSO in weight fractions. Open dots are measured points.*

6.3.2 Hydration of Phencyphos in DMF/Water Mixtures

In a similar fashion as the mixtures with DMSO/water, the phase diagram for DMF/water mixtures was determined. Again, we were unable to determine whether the solid phase consisted of phencyphos hydrate without dehydrating the solid phase during the removal of residual DMF. An assumption was made from the shape of the solubility line. The phase diagram is depicted in Figure 6.7 which shows that at least 13% wt. water in DMF is needed to form the hydrate.

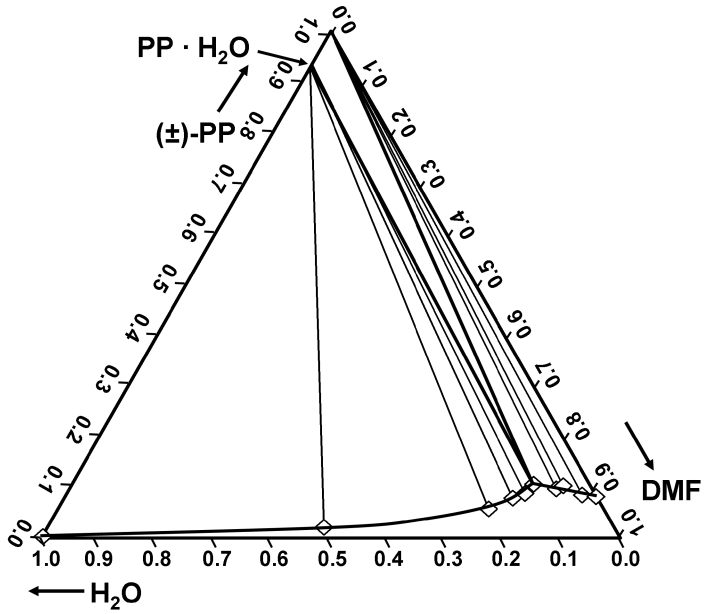


Figure 6.7 *Solubility diagram of (±)-phencyphos, water and DMF in weight fractions. Open dots are measured points.*

Starting from 17% wt. H_2O in DMF and phencyphos hydrate which was enriched in the (–)-enantiomer (15% *ee*) gave high *ee*'s in the first crystals. However, before enrichment in the mother liquor could take place in the (+)-enantiomer, the latter crystallized leaving a racemic mother liquor and crystals with 55% *ee*. Apparently, phencyphos hydrate in this water/DMF mixture lacks fast crystal growth and/or a wide metastable zone width, which makes this system unsuitable for a resolution by entrainment.

6.3.3 Hydration of Phencyphos in MeOH/Water Mixtures

The phase diagram of (±)-phencyphos, water and MeOH was constructed in the same manner as the previous phase diagrams. However, since MeOH is volatile, most MeOH evaporated on exposure to air overnight and after a further drying at 100°C *in vacuo*, the water (hydrate) content could be determined.[13] The phase diagram in Figure 6.8 shows that at least 22% wt. water in MeOH is needed for the formation of phencyphos hydrate. To make sure the process is reproducible even when the water content fluctuates, 30% wt. water was used in the entrainment process.

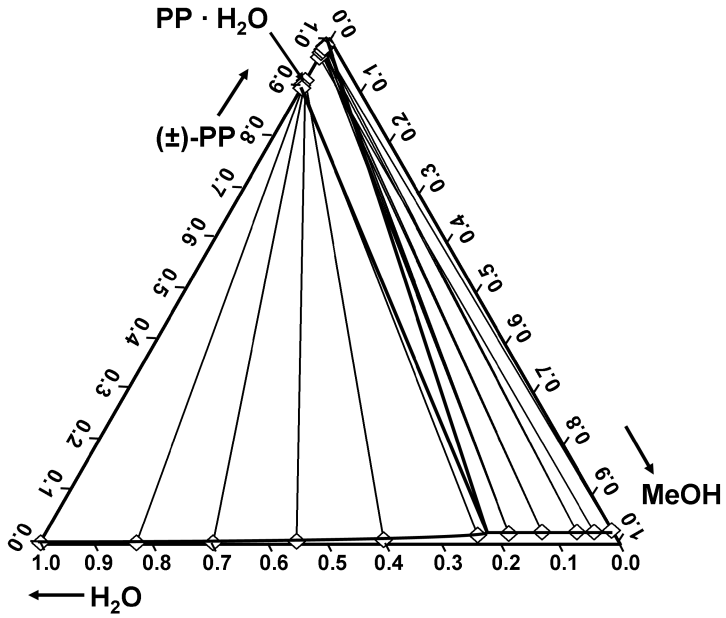


Figure 6.8 *Solubility diagram of (±)-phencyphos, water and MeOH in weight fractions. Open dots are measured points.*

6.3.4 Resolution by Entrainment in MeOH/Water Mixtures

Entrainment experiments starting with 30% wt. water in methanol were tested with different concentrations of phencyphos. The racemic phencyphos from the commercial source contained 5.88% water, which is 0.84 equivalents water compared to phencyphos.[14]

When a concentration of 23.3 mg (±)-phencyphos·0.84H_2O per mL solvent mixture was used, the primary nucleation started 1.5 hours after the start of the cooling of the solution from 54.0°C to 28.9°C. When this mixture was enriched by addition of 3.7 mg (–)-phencyphos hydrate for each mL solvent mixture prior to the cooling, primary nucleation started at 31°C so there was no need to seed the mixture with optically pure phencyphos hydrate. The suspension was left to age for another 30 minutes and then the suspension was filtered. Then, 7.3 mg (±)-phencyphos·0.84H_2O per mL solvent was added to the mother liquor and the mixture was heated to 54.0°C (dissolution) and the cooling cycle was repeated to obtain (+)-phencyphos hydrate. The isolated filter cakes were only sucked dry but not washed to prevent the dilution of the mother liquor. These hydrates had an average optical purity of 93% *ee* due to a remainder mother liquor and premature primary nucleation of the unwanted enantiomer. Combined batches were heated and subsequently cooled in a mixture of 30% wt. H_2O in MeOH to give crystals with >99% *ee* after filtration. In practice a yield of 41% was obtained.

In this manner, phencyphos was resolved on 100 mL, 2L, and 35L scale. On 35L scale, in each run 270 gram of (–)-phencyphos hydrate or (+)-phencyphos hydrate were isolated in alternating turns. With a temperature programmed reactor, 5 batches were performed each day. Because of the metastable zone, the temperature program of the reactor should be controlled internally, precisely and not overshoot during cooling. The latter would result in premature crystallization of the unwanted enantiomer. Furthermore, care should be taken that the crystals are collected before (large amounts of) the unwanted enantiomer crystallizes. A correctly performed resolution by entrainment is schematically depicted in Figure 6.9. In step 1, say 0.5 mol optically pure (+)-phencyphos is added to a mixture of racemic phencyphos in the solvent and subsequently heated to dissolution. In step 2 the solution is cooled to supersaturation of both enantiomers and 1.0 mol (+)-phencyphos hydrate crystallizes and is collected by filtration. In step 3, 1.0 mol racemate is added to the, now enriched in (–)-phencyphos and supersaturated mother liquor and this mixture is heated to dissolution. The mixture is cooled in step 4 and now 1.0 mol (–)-phencyphos hydrate is collected. In step 5, 1.0 mol racemate is added and the mixture is heated to dissolution and hereby producing the same situation as existed after step 1.

The case in which the desired enantiomer, say (+), is isolated before the crystallization is complete is depicted in Figure 6.10. The mixture is first biased in (+)-phencyphos and heated in step 1. After cooling less (+)-phencyphos hydrate is isolated than normal in step 2. The separated mother liquor has a lower *ee* than in a correctly performed entrainment. In step 3 racemate is added and the mixture is dissolved by heating. During the cooling in step 4, the more concentrated (–)-phencyphos starts to crystallize, shortly followed by the now more concentrated than normal (+)-phencyphos. This produces solids with a relatively low

ee and a racemic mother liquor. In step 5, addition of racemate to the mother liquor will not produce the situation after step 1 but the same situation as before step 1.Re-biasing the mother liquor with enough (–) or (+)-phencyphos will allow the entrainment procedure to resume.

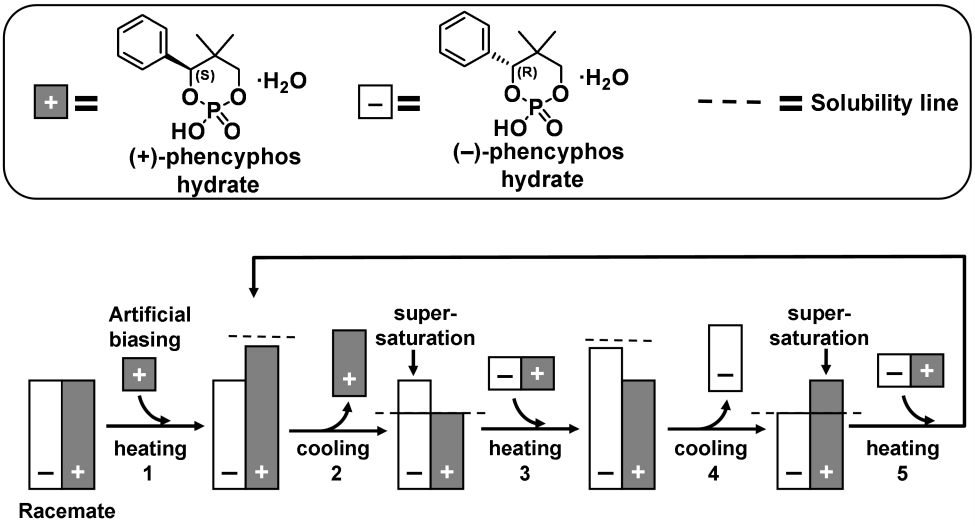


Figure 6.9 *Representation of resolution by entrainment.*

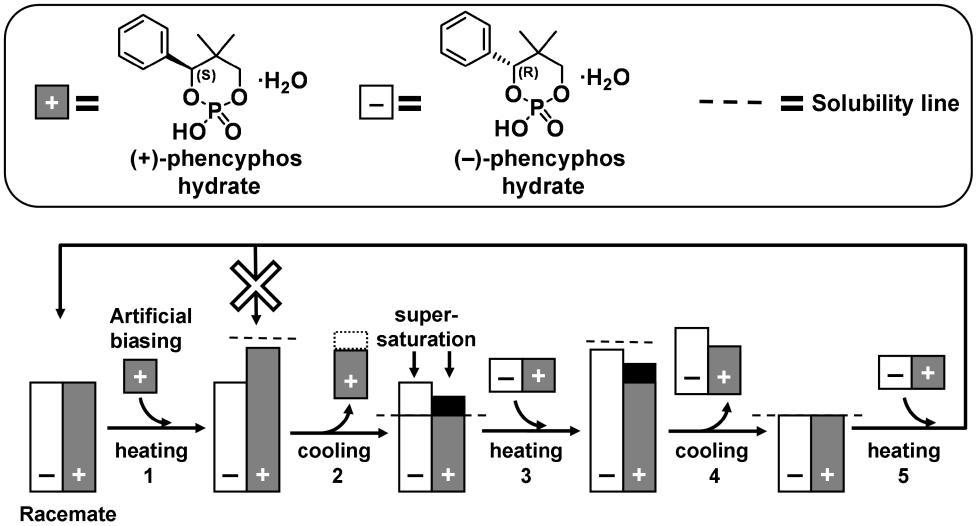


Figure 6.10 *Schematic representation of an entrainment failure.*

Cycles could be repeated up to 50 times without refreshing the mother liquor. Impurities from the starting material were accumulated in the mother liquor during entrainment and started to affect the entrainment after 50 runs by slow crystallization in the now yellow mother liquor. The reactor did not need cleaning between runs because complete dissolution was obtained during the next heating cycle.

6.3.5 Nucleation Inhibition of Phencyphos Hydrate

If nucleation inhibition of phencyphos hydrate by addition of a proper racemic additive could be performed, the process could be performed at higher concentration and thus, a higher yield could be obtained. Nucleation and crystallization of the enantiomer of phencyphos in excess might then start by itself because of the high concentration or be forced to start by addition of enantiopure seeds. Anicyphos and chlocyphos (See Scheme 6.1) were tested as potential nucleation inhibitors against a blank crystallization.

Six suspensions of (±)-phencyphos hydrate in 30% wt. H_2O in MeOH were heated to dissolution and then cooled to 20°C under continuous stirring. On average, primary nucleation was observed after 11 minutes (standard deviation (sd): 1.3 min). When anicyphos (~3% wt.) was added to three of these tubes and the dissolution and cooling procedure was repeated, on average, primary nucleation was observed after 11 minutes (sd: 1.2 min). When chlocyphos (~3% wt.) was added to the other three tubes and the dissolution and cooling procedure was repeated, on average, primary nucleation was observed after 13 minutes (sd: 1.5 min).

Neither anicyphos nor chlocyphos changes the metastable zone width significantly. One may conclude that these compounds are not useful in the resolution of (±)-phencyphos by entrainment. Also, if incorporation of these additives would be found, the recycling of the mother liquor will become more difficult as will be the purification of the resolved phencyphos hydrate which will be contaminated with small amounts of anicyphos or chlocyphos.

6.4 Resolution of Phencyphos by Hydrate Formation

When anhydrous (±)-phencyphos, which was crystallized from dry MeOH, was placed in a moist chamber, no hydration took place.[13] Apparently, the anhydrous (±)-phencyphos cannot interconvert into the hydrate through the solid phase.

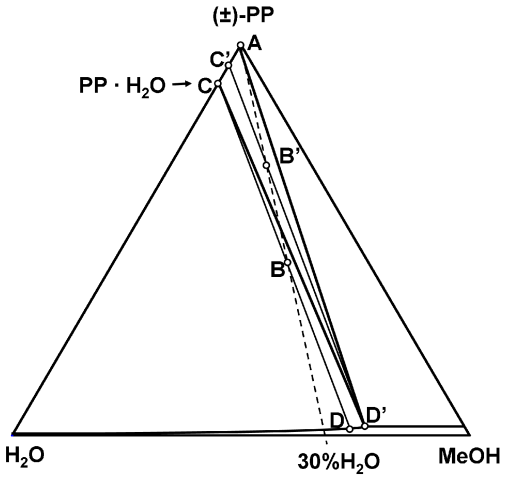


Figure 6.11 *Solubility diagram of (±)-phencyphos in methanol and water.*

However, when anhydrous (±)-phencyphos (**A**) was placed into a mixture of enough 30% wt. water in methanol (dotted line) as depicted in the phase diagram in Figure 6.11, the anhydrous (±)-phencyphos becomes metastable as the system arrives at point **B**. Conversion to (–)- and (+)-phencyphos hydrate can take place through the liquid phase *via* primary nucleation. Since the supersaturation will be low, the primary nucleation will take some time. However, if seeds are added, these will grow and will consume the anhydrous (±)-phencyphos. When all anhydrous (±)-phencyphos is consumed, the solids will have composition **C** and the mother liquor will have composition **D** If the seeds are placed separated in isolated areas (*e.g.* a filter) the seeds can grow and be isolated in a simple manner.

Point **B'** represents the case in which a lack of water is present. Then, when the equilibrium has been reached, the mother liquor will have composition **D'** and the solids composition **C'**. This means that a mixture of (±)-phencyphos, (–)-phencyphos hydrate and (+)-phencyphos hydrate will be isolated.

An experimental setup as depicted in Figure 6.12 was constructed in which the "U" shaped lines represent soxhlet filters which are placed inside a plastic casing. The filter on the left is charged with a suspension of 10 gram of anhydrous (±)-phencyphos in 30% wt. H_2O in MeOH (226 mL). The middle filter is charged with ~200 mg enantiopure (–)-phencyphos hydrate seeds and the filter on the right with ~200 mg enantiopure (+)-phencyphos hydrate. A pump transports the saturated filtrate to the filter on the left where it dissolves some of the metastable anhydrous (±)-phencyphos which gives a solution that is supersaturated in

phencyphos hydrate. This solution passes on to the middle filter in which the supersaturated (–)-enantiomer crystallizes on the seeds of the (–)-phencyphos hydrate. The supersaturated (+)-enantiomer passes though the filter and crystallizes on the seeds of the (+)-phencyphos hydrate in the filter on the right. The filtrate from this filter is then pumped to filter on the left for another run.

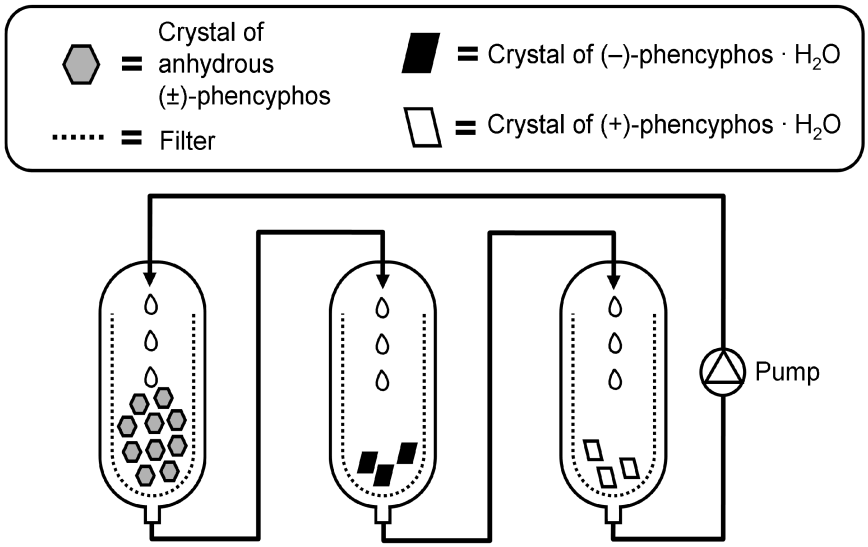


Figure 6.12 *Schematic representation of the resolution of (±)-phencyphos by pseudo polymorphic transition.*

After 3 days the filters were inspected and the filter on the left contained 270 mg of racemic material, the middle filter contained a dense cake of 3.30 gram of (–)-phencyphos hydrate with 98% *ee* (without further washing) and the filter on the right contained a dense cake of 3.70 gram of (+)-phencyphos hydrate with 99% *ee* (without further washing). Because the solution also contained some phencyphos, the sum of all filter contents is not equal to the amount of material put into the top filter.

6.5 Discussion

It has been shown that the resolution of phencyphos can be performed by diastereomeric salt formation with cinchonidine. However, this relatively expensive resolving agent has to be recycled and the isolated diastereomeric salts have a maximum of 74% *de*. These solids have to be recrystallized after liberation to enrich the phencyphos to >99% *ee* with a maximum yield of 37% for each enantiomer. In practice this yield will be lower because of

loss of material on filters and washings, incomplete or unwanted crystallization during filtration of the diastereomeric salt and not optimal conditions during recrystallization of the hydrate.

Since phencyphos hydrate crystallizes as a conglomerate, resolution by entrainment can be performed. This process, although quite laborious, has a yield of 41% for each enantiomer. Almost no waste is produced since the mother liquor is recycled after each run up to 50 runs depending on the purity of the racemate. Furthermore, no expensive resolving agent has to be used (and recycled). Sometimes, premature nucleation of the unwanted enantiomer was observed. This may be prevented to a large extend by filtration at a higher temperature or by a more dilute entrainment procedure. In total, 30 kg of each enantiomer have been prepared.

The resolution by hydrate formation as described in §6.4 seems to be an ideal method to resolve anhydrous (±)-phencyphos by a pseudo-polymorphic transition into the hydrate. However, a large scale application still is troublesome since filters become clogged easily and the process is relatively slow and a lagging supersaturated liquid phase could start primary nucleation and ruin the process. Perhaps with some optimization, this type of resolution might be useful for the resolution of conglomerates which form hydrates/solvate or have a metastable polymorph.

6.6 Experimental Section

General Information: Reagents were obtained form commercial sources and used without further purification.

Chiral HPLC analysis of phencyphos (phencyphos) salts was carried out on a Chiralpak QN-AX column with MeOH:AcOH 97:3 + 0.25g NH_4OAc/100 mL as mobile phase at room temperature and 1.5 $mL \cdot min^{-1}$. UV-VIS detection was performed at 254 nm. The free acid or the corresponding salts were dissolved in MeOH and injected as such. (+)-(*S*)-phencyphos R_f: 7.34 min, (–)-(*R*)-phencyphos R_f: 8.44 min.

IR spectra were recorded on a Thermo Nicolet 380 FT-IR with Smart Orbit.

Procedure for the construction of phase diagrams as depicted in Figure 6.6, Figure 6.7 and Figure 6.8: Just enough (±)-phencyphos·$0.84H_2O$ to achieve a suspension, was slurried in known mixtures of water and DMSO, DMF or MeOH for a couple of days at 20°C. After removal of the solid phase the composition of the mother liquor was determined by weighing the weight loss after evaporation of the solvents *in vacuo*. The solubility of (±)-phencyphos in several pure solvents (Table 6.3) was performed in a similar fashion.

Procedure for the entrainment of phencyphos as described in §6.3.4: A temperature controlled (Huber Unistat 510) 40L double jacketed glass reactor was charged with (±)-phencyphos·0.84H_2O (861 g, 3.35 mol, 1.0 eq), water (9.25 L) and MeOH (27.75 L) and stirred at 300 rpm throughout the entrainment.

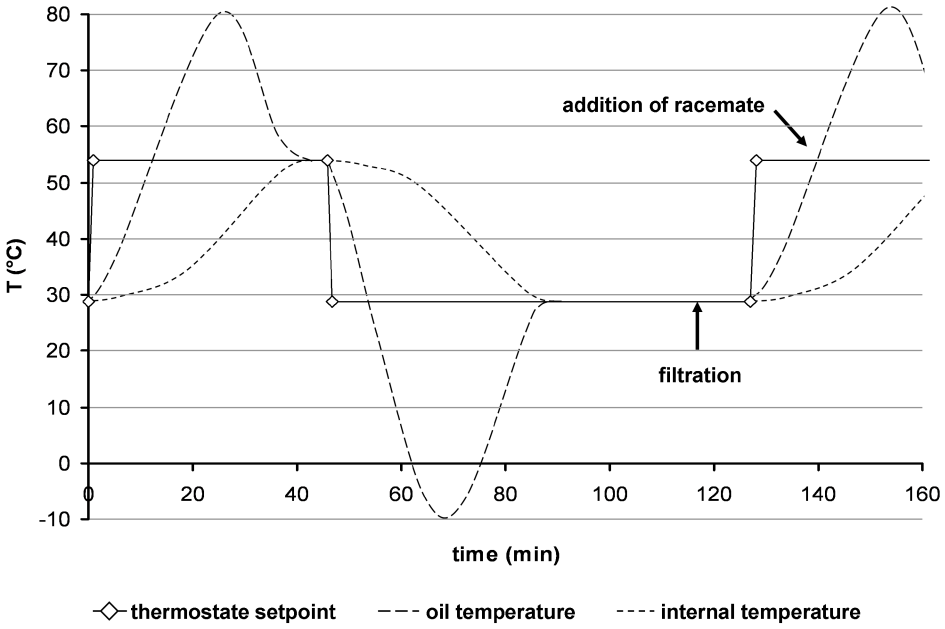


Figure 6.13 *Temperature program for the resolution by entrainment of phencyphos hydrate.*

The mixture was biased with (–)-phencyphos hydrate (137 g, 0.52 mol, 0.16 eq) and a temperature program was run where the internal temperature was heated from 28.9°C to 54.0°C as fast as possible for 46 minutes and then cooled to 28.9°C as fast as possible for 81 minutes and then the temperature program was repeated. The temperature profile is depicted in Figure 6.13.

Solids were collected (P2) 10 minutes prior to the reheating to 54.0°C. Filter cakes were sucked dry but not washed. Chiral HPLC analyses (as described above) on the solids showed were measured. If the solids had an *ee* greater than 85%, (±)-phencyphos·H_2O (270 g, 1.04 mol, 0.31 eq) was added to the filtrate. If the collected solids had 0% *ee*, more water was added and the entrainment repeated with the filter cake. If the collected solids had *ee* between 30% and 85% the entrainment was repeated with the filter cake replacing some

filtrate by MeOH. In a typical run, solids were collected with an average *ee* of 93%. Combined enriched batches were heated and subsequently cooled in a mixture of 30% wt. H_2O in MeOH to give crystals with >99% ee after filtration. In practice the whole resolution process yielded 41% of each enantiomer of optically pure phencyphos hydrate.

Procedure for testing of nucleation inhibition of phencyphos hydrate as described in §6.3.5: Six tubes were charged each with 50 mg (±)-phencyphos·0.84H_2O and 1.70 mL 30% wt. H_2O in MeOH. These mixtures were heated to reflux (dissolution) and subsequently cooled to 20°C with stirring (600 rpm). The time before the first crystals appeared was denoted. Three of these tubes were charged with respectively 1.4, 1.6 and 1.5 mg (±)-anicyphos. The other three tubes were charged with respectively 1.1, 1.4 and 1.2 mg (±)-chlocyphos. The contents of the tubes were recrystallized as described above and the crystallization time was denoted.

Procedure for the resolution by hydrate formation as described in section 6.4 A batch commercial (±)-phencyphos was recrystallized from MeOH to obtain the anhydrous (±)-phencyphos. The top soxhlet filter (35 × 150 mm) was charged with 10.0 gram of this material and the filter was put in a HDPE single-use casing (without filter) for automated column chromatography (44 × 224 mm) from Semco. (–)-(*R*)-phencyphos hydrate was prepared by recrystallization of (–)-phencyphos from 30% wt. H_2O in MeOH. A few drops of this suspension (~200 mg phencyphos) were added to the middle soxhlet filter and plastic casing as described above. The charging of the soxhlet with (+)-(*S*)-phencyphos hydrate was performed in a similar fashion as for (–)-(*R*)-phencyphos hydrate. A mixture of water and MeOH (30% wt. H_2O, 150 mL) was added to the top filter and when the solution arrived at the bottom of the setup, it was pumped to the top filter by a Liquiport NF300 pump with the slowest pump speed. After 3 days the top filter contained 270 mg of racemic material, the middle filter contained a dense cake of 3.30 gram (+)-phencyphos hydrate with 98% *ee* (without further washing) and the bottom filter contained a dense cake of 3.70 gram (with 99% *ee*) (–)-phencyphos hydrate (without further washing).

6.7 References

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3 http://en.wikipedia.org/wiki/Chloramphenicol (retrieved on 29 July, 2009).

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8 a) W. Marckwald, *Ber.* **1896**, *29*, 42. b) W. Marckwald, *Ber.*, **1896**, *29*, 43.

9 J. Read, W.G. Reid, *J. Chem. Soc. Ind.*, **1928**, *47*, 8T.

10 For equation, see Chapter 3, reference 16.

11 For an explanation of entrainment, see Chapter 1.4.3.3.

12 B. Samas, T. Groendyke, A.C. Blackburn, D.B. Godrej, *Acta Cryst. E*, **2007**, *63*, o1276–o1278.

13 F. Querniard, a PhD student from the University of Rouen, France, investigated the hydration and dehydration of phencyphos (unpublished results). Dehydration of the hydrate on at room temperature is only feasible at 0% relative humidity. Since the humidity on the lab is never that low, it may be assumed that no dehydration took place.

14 Later it turned out that dehydrated phencyphos hydrate rehydrates on (humid) air to the original phencyphos hydrate. As was observed also by F. Querniard.[13]

Chapter 7

Future Prospects

In this chapter some future prospects are given for a continuation of the research on direct crystallization of racemic compounds and additives which aid the diastereomeric salt resolution. Furthermore, some processes are described which allow racemic compounds to be resolved by crystallization or attrition enhanced Ostwald ripening. Finally, a method is introduced to determine whether a racemate is a conglomerate or not.

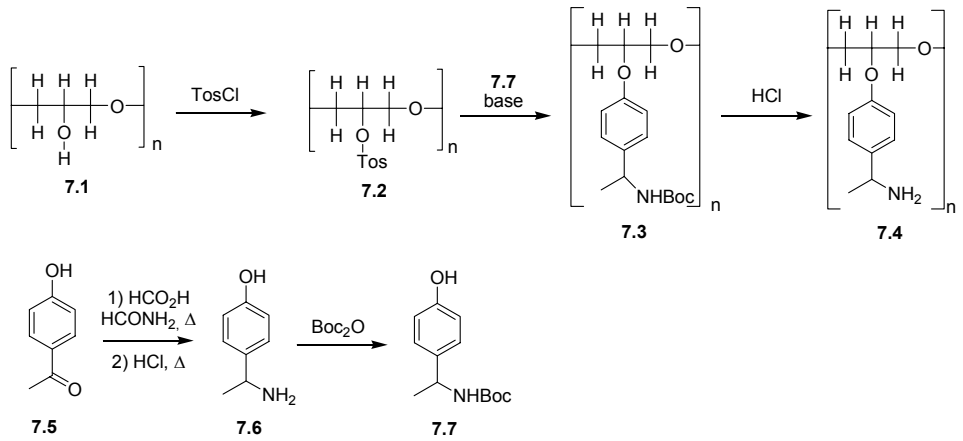
7.1 Nucleation Inhibitors for Diastereomeric Salt Formation

As described in Chapter 3, in a resolution by diastereomeric salt formation, additives with two or three repeating structures that resemble either the resolving agent or the racemate are very effective nucleation inhibitors. A new class of additives can be envisioned that combine even more functional groups. Functionalized soluble polymers have been shown to be effective as nucleation inhibitors in the resolution of conglomerates.[1]

7.1.1 Polyfunctional Nucleation Inhibitors

Once the polymer is in close proximity of a crystal nucleus several 'dockings' can take place since the functional groups in such a polymer are packed closely together. This process should very effective and block the growth of the nucleus.

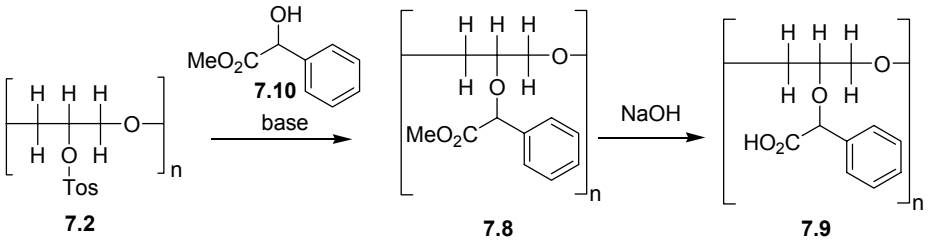
The synthesis of a soluble polymer that might be an effective nucleation inhibitor for 1-phenylethylamine derivatives is shown in Scheme 7.1.



Scheme 7.1 *Suggested synthesis of a polymer nucleation inhibitor based on 1-phenylethylamine*

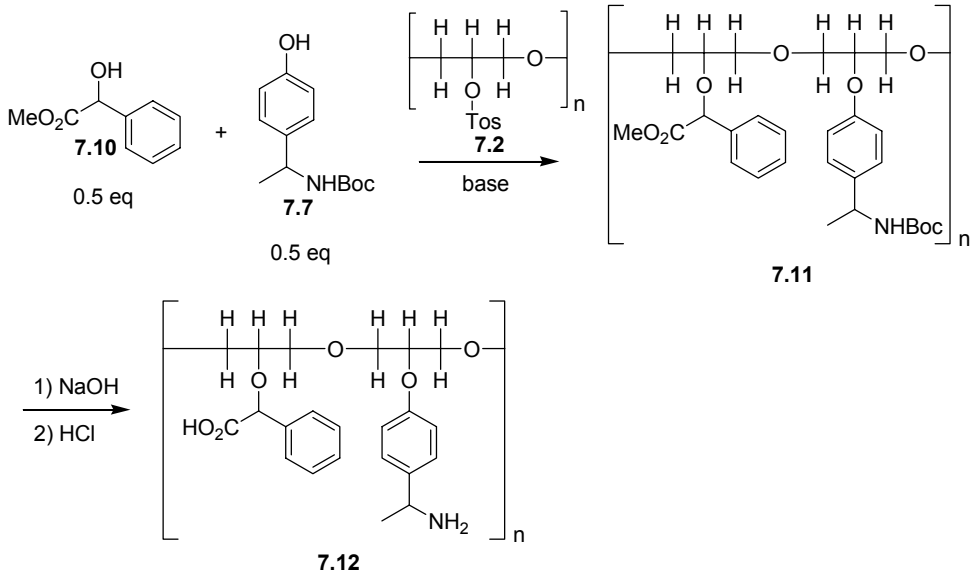
A commercially available polyglycerol **7.1** of an appropriate length and branching reacts with tosyl chloride to furnish compound **7.2**. This compound is allowed to react with phenol **7.7**, which is synthesized from acetophenone **7.5** via a Leuckart-Wallach reaction,[2] hydrolysis and Boc-protection. The reaction of **7.2** with **7.7** gives compound **7.3**.

Deprotection of the Boc-group will furnish **7.4**. The intermediate **7.6** can be resolved with an appropriate resolving agent.



Scheme 7.2 *Suggested synthesis of a polymer additive based on (S)-mandelic acid.*

Furthermore, additives based on mandelic acid may be designed in a similar fashion as depicted in Scheme 7.2. Starting from (optically pure) methyl mandelate **7.10** and tosylate **7.2** compound **7.8** can be isolated. Deprotection of the ester will give the functionalized polymer **7.9**. Although this synthesis is short and simple, the solubility of the polymer might be low.

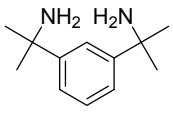


Scheme 7.3 *Suggested synthesis of a polymer based on both mandelic acid and 1-phenylethylamine.*

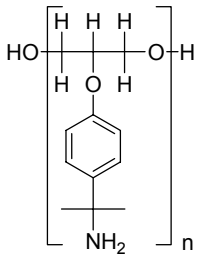
A polymer which is functionalized with both the resolving agent and the racemate might be a very effective nucleation inhibitor. If one-half equivalent of methyl mandelate **7.10** is allowed to react with the tosyl protected polyglycerol **7.2** and after complete conversion, one-half equivalent of Boc-protected amine **7.7** is then allowed to react with the remaining tosylates of the polymer backbone, compound **7.11** is expected as product. Deprotection of the Boc- and methyl ester-groups will furnish compound **7.12**.

Another variation of this 'mixed' polymer is the synthesis of polymers with a family of, say, substituted mandelic acids. More variety in the polymer might promote 'docking' at several places on the nucleus. Such a polymer might be a more generic nucleation inhibitor for several different resolutions with the same resolving agent (or family).

In Chapter 3.2.3, the bifunctional achiral additive **7.13**, depicted in Scheme 7.4, was found to, our surprise, to be a potent inhibitor. This compound was found to be a nucleation inhibitor and a growth inhibitor in the resolution of racemic 1-(3-methoxyphenyl)ethylamine with optically pure mandelic acid. Furthermore, with the same diastereomeric salt pair, this additive proved to be able to redissolve the crystallized more soluble diastereomer as described in Chapter 4.4.1. Perhaps reaction of polyglycerol with 2-(4-fluorophenyl)propan-2-amine could give polymer **7.14**, parallel to the reactions described above.



7.13



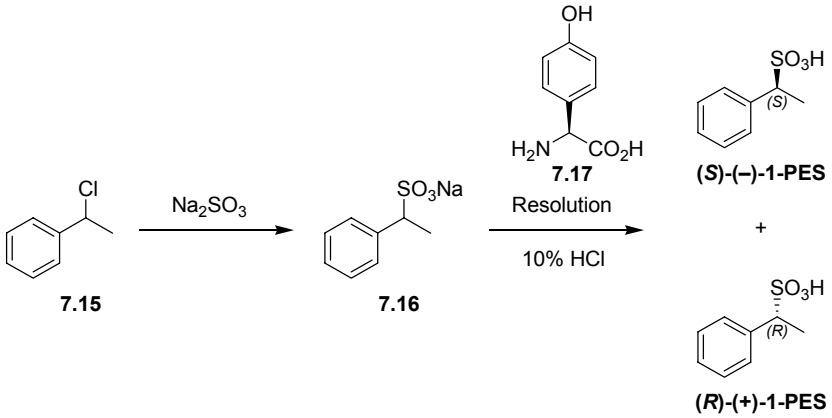
7.14

Scheme 7.4 *Achiral inhibitors and based on 1-phenylethylamine.*

7.1.2 1-PES

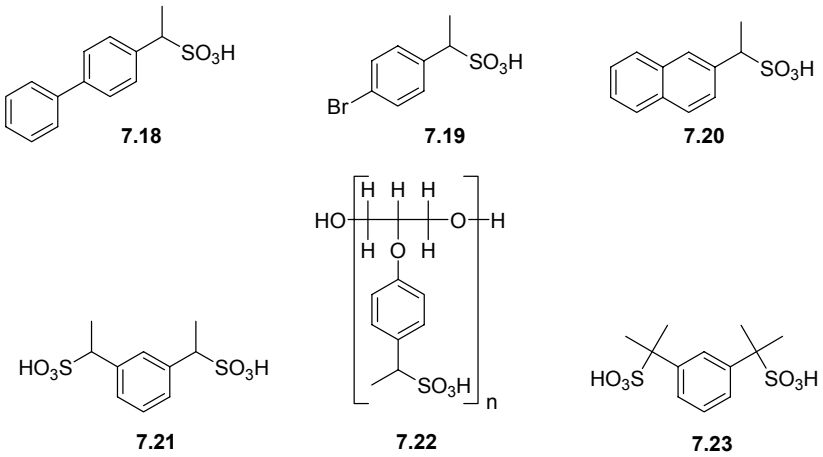
In good resolving agents, the acidic or basic moiety is closely located to the chiral center. In this manner, there is a large structural difference between both diastereomeric salts which can result in different solubilities. Furthermore, it should form crystalline salts, which in practice means the ability to form a hydrogen bonding network.[3]

Although 1-phenylethyl sulfonic acid (**1-PES**) shows great potential as a resolving agent and has been known for some time, it has not been used much in resolutions by diastereomeric salt formation.[4] Actually, the synthesis of **1-PES** is quite easy,[5] as shown in Scheme 7.4.



Scheme 7.5 *The synthesis of optically pure 1-PES.*

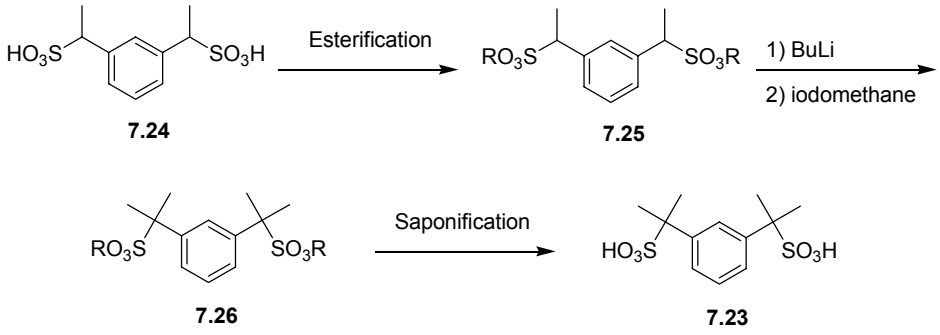
The first step in the synthetic route is the reaction of commercially available racemic 1-chloro-1-phenylethane (**7.15**) with sodium sulfite to give sodium salt **7.16**. Furthermore, synthetic routes that use 1-bromo-1-phenylethane with or without a phase transfer catalyst are also known.[6,7] The racemate can then be resolved with L-4-hydroxyphenylglycine (**7.17**) to produce both enantiomers of **1-PES** optically pure.[7]



Scheme 7.6 *Additives based on 1-PES.*

A suitable resolution (high yield, low *de* and preferably no end-solid solution) with optically pure **1-PES** and a racemate may be improved by use of proper additives. Some possible additives are shown in Scheme 7.6.

The synthesis of compounds **7.18** to **7.22** should not be difficult and may be accessible *via* similar routes as given in Scheme 7.5 and §7.1.1. As described in Chapter 3, racemic additives may be used to screen for a nucleation inhibition effect.



Scheme 7.7 *Proposed synthetic scheme for a achiral bissulfonic acid.*

The achiral bissulfonic acid **7.23** could be synthesized by starting with alkylation of ester **7.24** as is known from the literature.[8] Subsequently, saponification of ester **7.25** would furnish the target compound **7.23** as depicted in Scheme 7.7.

7.2 Racemizable Conglomerates

In a classical resolution, the unwanted enantiomer is regarded as waste. The process will be more economical if the unwanted enantiomer can be racemized and recycled. Such a process, Dynamic Kinetic Resolution (DKR), can produce in principle 100% yield and 100% *ee*.

In Chapter 5.5, it was shown that racemizable conglomerates can be made into optically pure compounds by heating, cooling, racemization in solution and grinding in a short time frame.

It should be possible to adapt this methodology to existing or new drugs by making them racemizable somewhere in the synthetic route. By functionalizing a non crucial part of the molecule (*e.g.* protective group or by salt formation) conglomerates could be found by, for example, Second Harmonic Generation (SHG) as discussed in Chapter 5.6.1 or a new technique as described in section 7.4.

7.3 Like Resolves Like

On crystallizing optically pure phencyphos and when several racemic family members were added, these were selectively incorporated with the same absolute configuration as shown in Chapter 4.4. One could imagine a process in which one poorly soluble pure enantiomer is crystallized in a solution of a very soluble racemic family member. These compounds should form a solid solution for this principle to work as is explained below.

Figure 7.1a represents a theoretical phase diagram for four compounds: pure enantiomer: $\mathbf{A_R}$, both enantiomers of compound **B**: $\mathbf{B_S}$, $\mathbf{B_R}$ and solvent (**S**). The solvent is directed upwards to infinity, hence, the top of the prism is open. The front side of the prism represents solid solution formation of mixtures of $\mathbf{A_R}$ and $\mathbf{B_R}$. The left-back side of the prism represents mixtures of $\mathbf{A_R}$ and $\mathbf{B_S}$. These do not show interaction and thus have conglomerate like solubility lines. The right-back side of the prism represents the conglomerate forming enantiomers of compound **B** ($\mathbf{B_R}$ and $\mathbf{B_S}$). The inside of the prism thus represents mixtures of all these compounds. It is clear from the phase diagram that $\mathbf{A_R}$ (point **a**) has a lower solubility than compound **B** (points **b** and **c**).

In Figure 7.1b the same phase diagrams is represented in a Jänecke projection.[9] The lines in the Jänecke projection represent the monovariant lines (eutectics) of the bivariant surfaces (solubility planes) from Figure 7.1a, viewed from above. The arrows in the Jänecke projection, point to the highest solubility (lowest point in Figure 7.1a). Although some information is lost, such a projection might be easier to read since it is not three dimensional.

Starting from an undersaturated racemic mixture of compound **B**, represented by point **d**, a small amount of $\mathbf{A_R}$ is added so point **e** represents the total composition of the system which is located along line (**d**,$\mathbf{A_R}$). After a recrystallization, the resulting suspension may be filtered. The mother liquor will then have composition **f** and the solids composition **g**. As can be seen from both figures, addition of a small amount of a 'chiral chaperone' $\mathbf{A_R}$ results in solids which consist of only ~15% $\mathbf{A_R}$ and the rest of ~85% consists of $\mathbf{B_R}$ (point **g**). Furthermore, the composition of point **g** is 100% *ee* for both compound **A** and **B**.

Of course, resolution of a racemic conglomerate (compound **B**) as in Figure 7.1 can be performed by direct crystallization as described in Chapter 1.4.3.3 also. However, resolution by direct crystallization of a racemic compound is not possible.[10]

Figure 7.2 represents the case where the to be resolved compound is a racemic compound (compound **B**). In the same manner as the previous example, a mixture of $\mathbf{A_R}$ and a solution of slightly undersaturated racemic **B** (point **d)** will give point **e**. Recrystallization will give a mother liquor (point **f**) and the solids will have composition **g** which has a higher $\mathbf{A_R}$

content compared to the previous example in Figure 7.1. However, the solids will still only contain optically pure $\mathbf{B_R}$. Even higher yields may be obtained when compound **B** is racemized during the crystallization. Since only optically pure $\mathbf{A_R}$ is used, it does not matter whether compound **A** crystallizes as a conglomerate or a racemic compound.

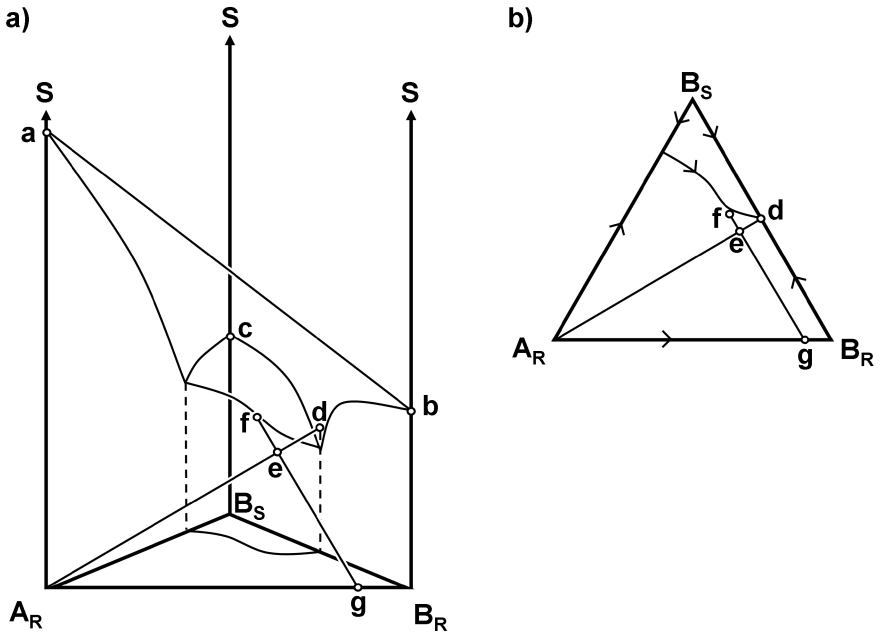


Figure 7.1 *Theoretical phase diagram a) exploded view with solvent to infinity b) Jänecke projection.*

Note that this concept resembles the 'replacing and preferential crystallization' proposed by Shraiwa *et al*[11] where an optically pure additive is used to induce crystallization of a single enantiomer of an supersaturated conglomerate with the same absolute configuration. However, Shraiwa states that a conglomerate is needed for this concept to work and requires an oversaturated solution of the conglomerate. The process described above in this paragraph shows that both are actually not required. Such a resolution has already been described by Collet *et al* although the incorporation was only 8% in mass.[12]

To conclude, a resolution by addition of a chiral chaperone as described above can only work with two structurally related compounds that can form solid solutions forming compounds and thus structurally closely related: family members as is known from the rule of Kitaigorodskii.[13] The rule of Kitaigorodskii states that the ratio between the overlap and

the non-overlap between molecules represents the likelihood of solid solution formation and thus the percentage of co-crystallization. Furthermore, the amount of the chiral chaperone in the isolated solids depends on the solubility difference between $\mathbf{A_R}$ (point **a**) and the solubility of racemic **B** (and thus the position of point **d**).

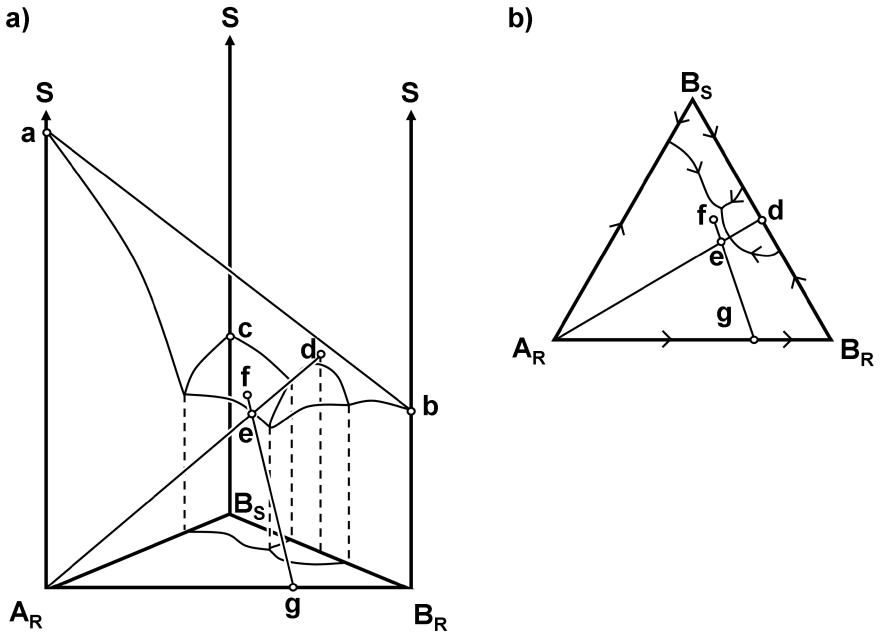
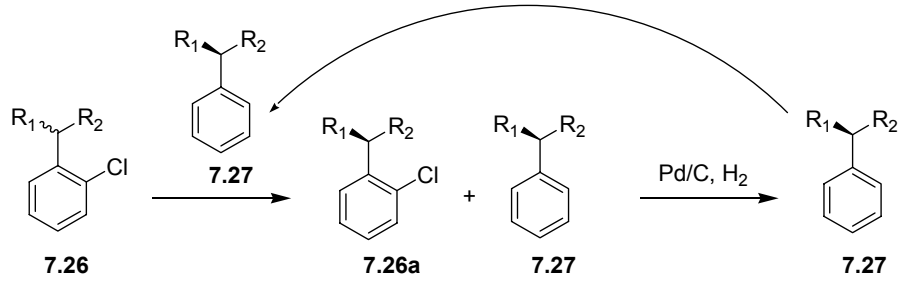


Figure 7.2 *Theoretical phase diagram a) exploded view with solvent to infinity b) Jänecke projection.*

Finally, when a slight excess of $\mathbf{B_S}$ is present, this system can still deliver $\mathbf{B_R}$ when (more) $\mathbf{A_R}$ is added.

A practical application of the above procedure could be two consecutive compounds in a synthesis as depicted in Scheme 7.8. Using a racemic chloride **7.26** and as chiral chaperone the same compound, for example reduced and optically pure (**7.27**), this could deliver an optically pure mixture of the chlorinated and reduced material. By reduction of the chloride **7.26a**, optically pure **7.27** could be isolated and a part could then be used as chiral chaperone for the next resolution batch. Note that, as described above, it does not matter whether either **7.26** or **7.27** is a conglomerate or a racemic compound. The only requirement is that the compounds form a solid solution together. From the rule of Kitaigorodskii[13] it follows that the formation of solid solutions between two compounds

that differ in only one atom will be more likely if the molecules are large and thus the differences between the compounds are minimal.



Scheme 7.8 *Route for the synthesis of an optically pure amine.*

7.4 Finding Conglomerates

Resolution by direct crystallization (described in §1.4.3.3) only is feasible if a racemate crystallizes as a conglomerate. Such a process is attractive since no resolving agent is required. However, since only 5-10% of all racemates are conglomerates,[10a] one finds him/herself screening derivatives of the to be resolved compound in order to find a conglomerate which can be resolved by direct crystallization and subsequently converted into the desired product.

The screening process often requires the racemate and one of the pure enantiomers.[14] Second Harmonic Generation (SHG) requires only the racemate, however, the accuracy is 90% and additional screening has to be performed. If one has the access to an expensive single crystal X-ray diffractor and large crystals of the racemate, the analysis of a single crystal of a conglomerate will show that this crystal contains only one enantiomer.

In §5.6 a slightly supersaturated solution of DL-threonine (a conglomerate) was crystallized under abrasive grinding conditions. The first formed crystal should be either a pure D- or pure L-crystal. This crystal is readily ground up by the grinding action of the stirrer (or even better by addition of glass beads) and produces more crystals and thus more crystal surface which consumes the supersaturation of this enantiomer. Shortly after the primary nucleation event of, say the D-enantiomer, the L-enantiomer will start to crystallize. By filtering the suspension shortly after crystals are observed, often, a significant enantiomeric enrichment is found in one of the enantiomers. By waiting too long before the isolation of the solids, the other enantiomer will have crystallized also and racemic solids will be found.

The process described above might be a general method to find conglomerates. The racemate should then be suspended in a solvent at, say 25.0°C. After a while, the racemate is filtered to produce a solution that is saturated in both enantiomers. This solution is then stirred vigorously and cooled to, say 24.0°C, to produce a slight supersaturation. This solution then is monitored manually for produced solids which are subsequently isolated.

Racemic compounds will, of course always produce completely racemic solids as will solids solutions. Some racemic conglomerates form crystals that display alternating regions (plates or rods) of both pure enantiomers. During the crystallization, an enantiopure crystal forms a template for the other enantiomer to grow on. This phenomenon is known as epitaxy.[15] It may be possible that racemates that display this epitaxial growth may not give a significant *ee* in the crystallized solids. On the other hand, these compounds will not be suitable for a resolution by direct crystallization anyway.

7.5 References

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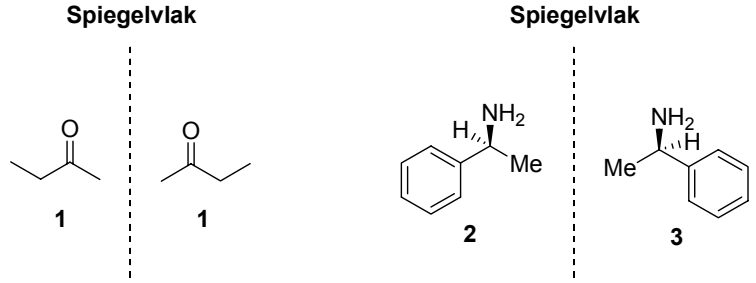
Nederlandse Samenvatting

De Nederlandse samenvatting is een hoofdstuk voor diegene die wel bekend zijn met chemie maar niet zozeer met chiraliteit en het scheiden van zogenoemde enantiomeren door kristallisatietechnieken die beschreven staan in dit proefschrift. Ook worden hier enkele resultaten besproken die tijdens het promotieonderzoek gevonden zijn.

Chiraliteit

Als een molecuul gespiegeld wordt en dit spiegelbeeld niet ruimtelijk identiek is aan het origineel, spreekt men over *chiraliteit*. Zoals te zien is in Figuur 1 levert verbinding **1** geen andere verbinding op als hij gespiegeld is. Bij het roteren van het spiegelbeeld wordt immers het origineel terug gevonden. Maar als verbinding **2**, waarbij het stikstof atoom naar voren staat en het waterstof atoom naar achteren, gespiegeld wordt, levert dit verbinding **3** op. Door het draaien van verbinding **3** wordt nooit verbinding **2** terug gevonden. Deze spiegelbeeldmoleculen worden *enantiomeren* genoemd en een 1:1 mengsel van enantiomeren wordt *racemaat* genoemd.

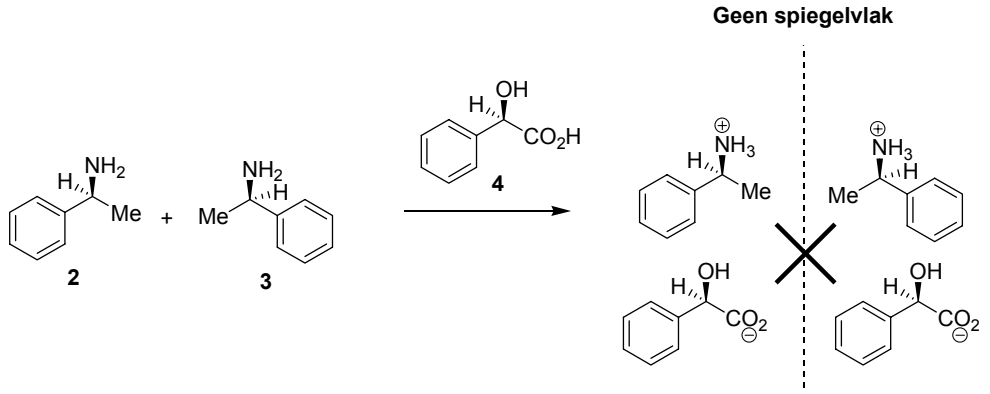


Figuur 1 *Achirale moleculen en chirale moleculen.*

Enantiomeren hebben exact dezelfde fysische eigenschappen (smeltpunt, kookpunt, kleur etc.) en zijn alleen te onderscheiden doordat ze gepolariseerd licht respectievelijk naar links of naar rechts draaien of door verschillende interacties met een andere zuiver enantiomeer.

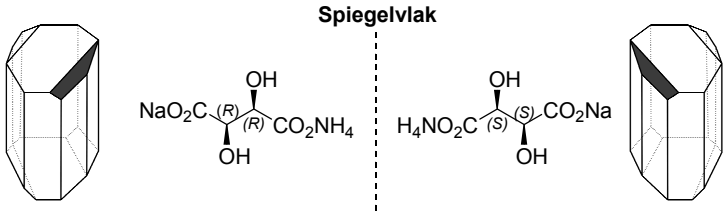
Het proces van het scheiden van enantiomeren wordt een *splitsing* genoemd (ook wel *resolutie*). De meest gebruikte methode voor de splitsing van enantiomeren is door diastereomere zoutformatie zoals hier onder beschreven.

Door een racemaat te laten reageren met een zuiver enantiomeer van een andere verbinding (een zogenaamd *splitsingsmiddel*) ontstaan *diastereomeren*. In het voorbeeld in Figuur 2 wordt een diastereomeer zout gemaakt met amandelzuur (**4**). Diastereomeren zijn geen spiegelbeeld moleculen en hebben dus andere fysische eigenschappen die gebruikt kunnen worden om ze te scheiden. De eenvoudigste manier om deze diastereomeren te scheiden is door ze te laten kristalliseren vanuit een verzadigde oplossing waarbij het ene diastereomeer beter oplost dan het andere. Dit wordt ook wel *klassieke resolutie* genoemd. Er zijn echter ook andere methoden om enantiomeren te scheiden.



Figuur 2 *Diastereomeren, geen spiegelbeelden.*

In ongeveer 10% van alle racematen, kristalliseren beide enantiomeren elk uit in hun eigen kristal. Elk van deze kristallen zijn dus enaniozuiver hoewel alle kristallen bij elkaar racemisch zijn. Deze kristalvorm wordt *conglomeraat* genoemd en is voor het eerst gebruikt in 1848 door Louis Pasteur. Hij nam de kristallen van een racemisch mengsel van het natrium ammonium zout van wijnsteenzuur en kon beide kristalvormen met de hand scheiden omdat de kristallen elkaars spiegelbeeld zijn zoals te zien is in.



Figuur 3 *Beide kristal vormen en enantiomeren van het natrium ammonium zout van wijnsteenzuur.*

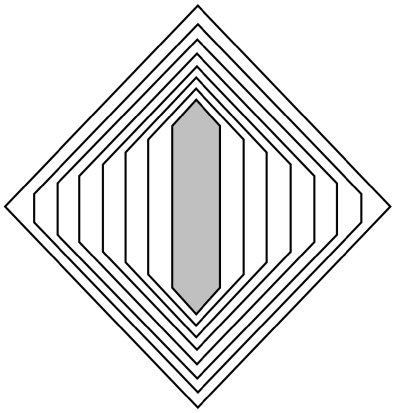
Kristallisatie

Wanneer een oplossing van een verbinding (chiraal of niet) oververzadigd is betekend dit dat het de opgeloste verbinding liever bijvoorbeeld een kristal vormt (uitkristalliseert) dan opgelost blijft. Kristallisatie begint met *nucleatie*, het proces waarbij moleculen van de verbinding in de oplossing beginnen met samenklonteren in een geordende manier. Deze (nog onzichtbare) kernen lossen weer op of groeien dan verder tot zichtbare kristallen. Als

het evenwicht zich ingesteld heeft zal er een verzadigde oplossing met kristallen overblijven.

Het Effect van Onzuiverheden

Onzuiverheden, al dan niet opzettelijk aangebracht kunnen het kristallisatieproces van elk van de verbindingen in oplossing ernstig beïnvloeden. Als een verbinding interactie vertoont met bepaalde vlakken van een kristal dan zal de snelheid waarmee dit vlak groeit langzamer worden. Omdat de rest van de vlakken door blijven groeien, zal het uiterlijk van het kristal anders worden. In het voorbeeld in Figuur 4 wordt de groei de kleine facetten van het grijze kristal afgeremd door een *groei-inhibitor*. Het lange facet van het kristal kan dus doorgroeien en zal kleiner worden totdat het uiteindelijk verdwijnt en er een vierkant kristal overblijft. Dus, hoe groter het facet, hoe langzamer het groeit.



Figuur 4 *Groei-inhibitie van de kleinste vlakken van het grijze kristal.*

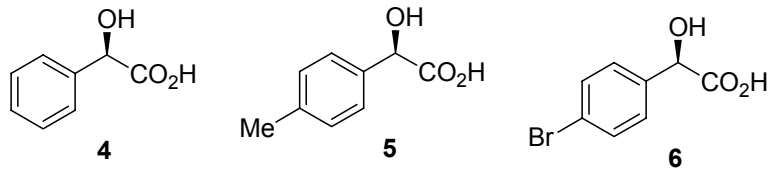
Het is bekend dat het toevoegen van kleine hoeveelheden van optisch zuivere verbindingen die lijken op bijvoorbeeld een conglomeraat dat gaat kristalliseren, het tegenovergestelde enantiomeer oplevert. Met andere woorden, het enantiomeer dat lijkt op de onzuiverheid wordt in de oplossing gehouden doordat deze de nucleatie bemoeilijkt terwijl het andere enantiomeer niet wordt beïnvloed en dus gewoon kan kristalliseren. Een dergelijke onzuiverheid wordt dan een *nucleatie inhibitor* genoemd. Ook de kristallisatie van diastereomere zouten kunnen beïnvloed worden door het toevoegen van nucleatie inhibitoren. Deze moeten dan lijken op het racemaat of het splitsingsmiddel. Het splitsingsmiddel is in beide diastereomere zouten met dezelfde absolute configuratie aanwezig. Maar omdat diastereomeren geen spiegelbeeld moleculen zijn, zullen nucleatie

inhibitoren, die op het splitsingsmiddel lijken, op elk van de diastereomeren anders werken en bijvoorbeeld de kristallisatie van het beter oplosbare diastereomeer wel verstoren maar die van het minder oplosbare diastereomeer niet.

Dutch Resolution

In 1998 hebben Nederlandse wetenschappers een nieuwe methode ontwikkeld om een splitsing uit te voeren door diastereomere zoutformatie, door niet gebruik te maken van één splitsingsmiddel maar van drie tegelijkertijd, een mix. Zo waren ze in staat om splitsingen uit te voeren die met elk van de splitsingsmiddelen apart niet waren gelukt. Deze techniek werd *Dutch resolution* gedoopt en bleek meer kans op succes te geven dan een klassieke resolutie. De kans om in een klassieke resolutie bij de eerste zouten een redelijke verrijking te krijgen is geschat op 20–30% en voor Dutch resolution ligt dit op 90–95%.

De drie splitsingsmiddelen moeten *familieleden* van elkaar zijn, dus structureel veel op elkaar lijken zoals te zien in Figuur 5. De afgebeelde mix van splitsingsmiddelen is gebaseerd op amandelzuur (**4**) en wordt de M-mix genoemd.



Figuur 5 *Familie van amandelzuren: de M-mix*

Het bleek dat van de drie splitsingsmiddelen in een dergelijke mix soms maar twee werden ingebouwd. Als het niet ingebouwde splitsingsmiddel er uit werd gelaten dan bleek soms de splitsing veel minder effectief te zijn dan daarvoor. Dit niet ingebouwde splitsingsmiddel fungeerde dan als nucleatie inhibitor.

Dit Proefschrift

De inhoud van dit proefschrift gaat vooral over het maken van enantiozuivere verbindingen door het toepassen van diverse technieken die het kristallisatieproces beïnvloeden. Eerst wordt in hoofdstuk 1 de geschiedenis weergegeven van chiraliteit en de toepassing van chiraliteit in het dagelijks leven. In hoofdstuk 2 wordt kristalgroei en het effect van additieven hierop beschreven. Tevens worden fase diagrammen beschreven en de noodzaak hiervan voor het voorspellen van splitsingen.

In hoofdstuk 3 wordt klassieke resolutie verbeterd door nucleatie inhibitoren toe te voegen aan splitsingen. De nucleatie inhibitoren zijn zo ontworpen zodat ze familieleden zijn van het splitsingsmiddel of het racemaat. Het bleek dat verbindingen die lijken op twee of meer aan elkaar gekoppelde racemaat moleculen of splitsingsmiddel moleculen zeer potent waren. Ook kon op redelijke schaal een splitsing worden uitgevoerd die verbeterd werd door het toevoegen van een nucleatie inhibitor. Zelfs na het toevoegen van enten van de beter oplosbare diastereomeer, kristalliseerde deze niet verder uit. Naast nucleatie inhibitie werd ook groei inhibitie gevonden zoals in Figuur 4 al is aangegeven.

Additieven die worden toegevoegd aan een splitsing kunnen zorgen voor nucleatie inhibitie en groei inhibitie maar ook andere effecten kunnen gevonden worden. Deze effecten staan beschreven in hoofdstuk 4 waarin ook een methode wordt beschreven om met een één enantiomeer, andere racemische familieleden met dezelfde absolute configuratie te laten kristalliseren in hetzelfde kristal. Ook wordt er een methode beschreven om het diastereomere zout uit hoofdstuk 3 te splitsen door het toevoegen van een additief aan de suspensie van beide diastereomeren en deze te malen.

Het malen van kristallen kan ook andere effecten geven, zoals beschreven staat in hoofdstuk 5. Door een suspensie te nemen van een racemisch conglomeraat dat racemiseert in de oplossing en deze een maand lang fijn te malen bestonden de kristallen slechts uit één enantiomeer. Deze methode is aangepast om met malen binnen één uur één enantiomeer te geven vanuit volledig racemisch materiaal. Als een mengsel van vijf verschillende racemiseerbare aminozuurderivaten werd genomen en deze werden gekristalliseerd onder malen, gaf dit kristallen met een willekeurige absolute configuratie. Wel waren alle aminozuurderivaten ingebouwd in de gevormde kristallen met dezelfde absolute configuratie.

De splitsing van phencyphos staat beschreven in hoofdstuk 6. Phencyphos is een zeer goed splitsingsmiddel maar is als racemaat lastig te splitsen tot enantiozuiver phencyphos. In hoofdstuk 6 staat de route beschreven die is bewandeld om tot een succesvol proces te komen. Dit proces is opgeschaald wordt op mol-schaal uitgevoerd bij Syncom BV. Ook wordt een nieuw proces beschreven waarin een spontane splitsing plaats vindt door hydraat formatie van racemisch phencyphos.

Met de bevindingen van hoofdstuk 3 in het achterhoofd, worden in het laatste hoofdstuk een aantal ideeën gegeven voor nieuwe nucleatie inhibitoren. Tevens staan er een aantal nieuwe splitsingsmethoden in die zijn afgeleid van experimenten uit hoofdstukken 4 en 5.

Dankwoord

Na 4 jaar promotieonderzoek ben ik aangekomen bij het laatste gedeelte: het schrijven van het dankwoord. Zoals zo velen al voor mij schreven: een promotieonderzoek doe je niet alleen. Daarom wil ik een aantal mensen bedanken voor hun bijdrage tijdens mijn promotieonderzoek. Ten eerste wil ik mijn tweede promotor, Prof. Kellogg, erg bedanken voor de goede begeleiding en de leuke en inspirerende discussies die we hebben gehad tijdens het promotieonderzoek. Prof. Minnaard wil ik bedanken om tijdens de laatste fase van mijn promotieonderzoek mijn eerste promotor te worden en voor de snelle correcties van het proefschrift. Verder wil ik via deze weg de algemeen directeur van Syncom B.V., Ton Vries bedanken voor het geven van deze kans en het vertrouwen in een goede afloop van mijn promotieonderzoek.

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A thesis manuscript should be corrected by a reading committee, of course. I would like to thank Prof. Hans de Vries, Prof. Elias Vlieg and Prof. Gérard Coquerel for their fast and thorough corrections.

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