Chapter 6*

Understanding Dutch Resolution: the role of nucleation inhibition

This Chapter is a uniquely important part of this thesis, since it demonstrates that the success of Dutch Resolution can most likely be explained by the phenomenon of nucleation inhibition, together with other factors. Turbidity measurements were used to quantify our observations of kinetic effects and a second generation of Dutch Resolution experiments was developed on the basis of these findings. The concept of nucleation inhibition seems to be reasonably general among several resolving agents. The potential for industrial applications of these new resolution experiments is discussed, as well as the synthesis of some potent nucleation inhibitors.

6.1 Introduction

Since the original description by Pasteur, the separation of enantiomers via diastereomeric salt formation has remained largely unchanged in technique. It is, however, still the most widely used method for obtainment of enantiomerically pure compounds in industry (see also Chapter 1). The discovery of Dutch Resolution marked an important change in this classic technique, by using mixtures of resolving agents instead of a single one.

One of the remarkable findings was the fact that the success rate was 90-95% compared to the usual 20-30% estimated in one of the few studies published. Another striking feature was the non-stoichiometric ratio of resolving agents found in the salt, starting from a 1:1:1 ratio in most of the cases. Some regularly used families, the term used for the mixtures, of resolving agents are shown in Figure 6.1.

![Figure 6.1](image)

Figure 6.1 Families of resolving agents

The structural similarity among family members is clear, although in initial experiments the choice of the family members was arbitrary in the sense that it depended on the derivatives available in the lab. Variations consist of substituents on aryl rings. It was assumed that the substituents should be more or less of the same ‘size’. Obviously, more research was necessary to investigate the importance of the different substituents and, more importantly, to find a rationale for to the high success rates.

In this Chapter, the role of nucleation inhibition will be discussed and it will become clear that this is most likely one of the critical factors that is important in Dutch Resolution. Also, turbidity measurements being vital for the construction of the hypothesis of nucleation inhibition will be described. In addition, a second generation of Dutch Resolution
experiments will be described which overcomes some of the disadvantages of the ‘classic’ Dutch Resolution.

6.2 The dog that didn’t bark

In most of the cases reported in the original publication, three structurally related resolving agents were used in a 1:1:1 ratio. A mixture of these resolving agents was usually found in the first salt, but in non-stoichiometric ratios. In general, the families of resolving agents show solid solution behavior (see Section 2.1.1), which means that the crystal lattice can randomly accommodate the three different resolving agents. Or, in other words, the lattice does not ‘sense’ the difference between the resolving agents. As a result, it is possible to incorporate relatively large amounts of the different resolving agents in the lattice. This differs with the work on the ‘tailor-made additives’ as described by Lahav, since in these experiments only a few percent of the additive could be incorporated in the crystal.

In 46 examples of resolutions of single racemates reported, in ten cases no detectable amount of one (or more) of the three resolving agents was present in the salt. In three other cases one of the resolving agents was present in <10 mol%. In the majority of examples the ratios differed appreciably from the originally used stoichiometries.

It is difficult to compare directly resolutions using a mixture of three resolving agents with a resolution of the same racemate using, for instance, two or one of the three resolving agents, because the solubilities of the various diastereomeric salts will differ. Also, from our experience in the lab with these resolutions, it was anticipated that kinetic effects would play an essential role. Moreover, the strong feeling arose that resolutions proceeded less well in the absence of the non (or poorly) incorporated resolving agents. The suspicion arose that they might be "the dog that didn’t bark" (Box 6.1).

To test this idea, a model resolution was designed with two resolving agents: one being the "parent" resolving agent and the other being the "additive" that had been observed not - or only poorly - to be incorporated. Solubility differences between the pure "parent" and "parent/additive" system are kept to a minimum. The model system chosen was the resolution of (rac)-mandelic acid \(6.1\) with (S)-phenylethylamine \(6.2\) (Scheme 6.1).

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**Box 6.1 The dog that didn’t bark**

...but I saw by the inspector's face that his attention had been keenly aroused.

"You consider that to be important?" he asked. "Exceedingly so."

"Is there any point to which you would wish to draw my attention?"

"To the curious incident of the dog in the night-time." "The dog did nothing in the night-time."

"That was the curious incident," remarked Sherlock Holmes.
A 1:1 ortho:para mixture of nitro-substituted (S)-phenylethylamines 6.3 was chosen as additive. This mixture together with 6.2 forms the family PE-II and had been observed not, or barely, to be incorporated in "family" resolutions. One resolution was done with one equivalent of 6.2 and the other with 0.9 eqv of 6.2 and 0.1 eqv 6.3.

The concentration of the experiment was chosen in such a way that the diastereomeric excess of the less-soluble salt 6.4 was not high, so improvements could be easily detected.

At the non-optimum concentrations chosen the resolution of 6.1 with (S)-6.2 delivers the first salt with a $de$ of 14% and S-factor of 0.19 (Table 6.1). In the presence of 0.1 eqv of (S)-6.3 and 0.9 eqv (S)-6.2 the $de$ of the first salt increased from 14% to 55% and the S-factor to 0.41.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additive</th>
<th>Yield (%)</th>
<th>$De$ (%)</th>
<th>S-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>68</td>
<td>14</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>10% (S)-6.3</td>
<td>37</td>
<td>55</td>
<td>0.41</td>
</tr>
</tbody>
</table>

No detectable amount of either ortho or para (S)-6.3 was incorporated in the salt. This followed the experimental observation that in the presence of the (yellow) additive, upon filtration of the less soluble salt, the mother liquor was yellow and the salt white, as is shown in the pictures in Figure 6.2.
Figure 6.2 Resolution of 6.1 in the absence (left tube) and presence (right tube) of additive (left): precipitation in the presence of additive (middle) and salt in the presence of additive (right)

Crystallization in the presence of (S)-6.3 as additive to (S)-6.2 was observed qualitatively to begin at a lower temperature than with (S)-6.2 alone. To quantify this observation, turbidity measurements were carried out.

6.3 Turbidity measurements

At the Center for Particle Technology at DSM in Geleen a special Medium Throughput Experimentation (MTE) setup has been developed for automated solubility and nucleation measurements (Figure 6.3).

Figure 6.3 Experimental set-up for turbidity measurements

This MTE experimental setup consists of four identical double-walled glass vessels, which are connected (in series) to a thermostated bath to control the temperature. Each vessel is equipped with a magnetic stirring bar, a Pt100 thermocouple and a He/Ne-laser with a detector.
A value of 5 (arbitrary units) registered by the apparatus corresponds to complete opaqueness due to crystallization. A value close to zero is indicative of a clear solution. Several heating and cooling runs were carried out subsequently, in order to establish an average value. For the less soluble salt the error in the temperatures is estimated at 0.3°C; for the more-soluble salt this is somewhat larger.

### 6.3.1 o-Nitrophenylethylamine 6.3 as additive

In these experiments the diastereomers (S)/(S)-6.4 and (S)/(R)-6.5 were studied separately and therefore at different concentrations. In the first model experiment a mixture of 1:1 o,p-nitrophenylethylamine was used, to stay close to the original experiments with the PE-II-mix. The o- and p-substituted nitrophenylethylamines were synthesized separately (see Section 6.8) and resolved. In the following experiment only o-nitrophenylethylamine was used as an additive, since this compound was available in larger amounts than the p-nitrophenylethylamine as well as for reasons of simplicity.

For the less-soluble diastereomer (S)/(S)-6.4 it is observed that the dissolution temperature is 70.2°C and the nucleation temperature 64.2°C (Figure 6.4 solid line).

![Figure 6.4 Turbidity measurements on the less soluble salt 6.4](image)

Replacement of 10 mol % of (S)-6.2 by (S)-o-nitrophenylethylamine 6.6 led to a shift in nucleation temperature from 64.2°C to 61.6°C. The meta-stable zone (see also Section 2.1.3) is enlarged from 6.0 to 7.1°C, which change is significantly beyond the experimental error.
For comparison the effect of 10% (R)-o-nitrophényl ethylamine 6.7 is also shown in Figure 6.4. The (R)-additive has no statistically significant effect on either the nucleation or the dissolution temperature.

In the case of the more-soluble diastereomer (S)/(R)-6.5 (at a much higher concentration) also a clear effect on the nucleation temperature is observed (Figure 6.5).

![Figure 6.5 Turbidity measurements on the more-soluble salt 6.5](image)

The meta-stable zone width increases from 11.4 to 28.0°C on substitution of 0.1 eqv of (S)-6.2 by (S)-6.6. The dissolution temperatures differ somewhat, but since the concentration is so high in this experiment, the error margins are probably somewhat larger than in the case of the less-soluble diastereomer.

The nucleation of both diastereomers starts at a lower temperature in the presence of the additive (S)-6.6. This effect is called nucleation inhibition. Recognition of the additive appears to be enantioselective; the additive should have the same absolute configuration as the resolving agents to be effective. This bears similarity to the work of Lahav, since it was found that either enantiomer of the additive displayed a different effect on the nucleation and the crystal habit.

The use of achiral additives such as substituted benzylamines was also tested, but this did not have a significant effect on the resolution efficiency.
6.3.2 Substituted mandelic acids as additives

Another frequently used family of resolving agents contains mandelic acid \( 6.8 \), as well as \( p \)-methyl mandelic acid \( 6.9 (p\text{-MeMA}) \) and \( p \)-bromo mandelic acid \( 6.10 (p\text{-BrMA}) \) (M-mix, Scheme 6.2, see also Figure 6.1). The diastereomers with racemic phenylethylamine \( 6.11 \) were used for turbidity measurements, in which the less-soluble salt \( (S)/(S)-6.4 \) and the more-soluble salt \( (S)/(R)-6.12 \) were measured separately.

Scheme 6.2 Diastereomers of \( (S)-6.8 \) with \( (\text{rac})-6.11 \) in the presence of \( (S)-6.9 \) and \( (S)-6.10 \)

At a certain concentration of the less-soluble diastereomer \( (S)/(S)-6.4 \) the dissolution temperature is \( 68.9^\circ \text{C} \) and the nucleation temperature \( 65.6^\circ \text{C} \) (Figure 6.6). Replacement of 10% of \( 6.8 \) by \( (S)-6.9 \) induced a shift in nucleation temperature to \( 63.6^\circ \text{C} \) and the meta-stable zone was again enlarged.

Figure 6.6 Turbidity measurements on the less soluble salt \( (S)/(S)-6.4 \) with \( (S)-6.9 \) as additive
For the more soluble diastereomer 6.12 (at a much higher concentration) a similar effect is observed (Figure 6.7).

![Figure 6.7](image)

**Figure 6.7** Turbidity measurements on the more soluble salt (S)/(R)-6.12 with (S)-6.9 as additive

The effect of a bromo substituent on the less-soluble salt was also investigated. The use of 10% (S)-6.10 also resulted in nucleation inhibition (Figure 6.8). The nucleation temperature shifted from 64.8°C to 62.8°C and the dissolution temperatures were again similar.

![Figure 6.8](image)

**Figure 6.8** Turbidity measurements on the less-soluble salt (S)/(S)-6.4 with (S)-6.10 as additive
Compared to the addition of (S)-6.9, the bromo additive seems to be slightly more effective in nucleation inhibition, which can be seen in Figure 6.9 in which the meta-stable zone widths are compared.

![Figure 6.9](image)

**Figure 6.9 Meta-stable zone widths of the less soluble salt under influence of (S)-6.9 and (S)-6.10**

(S)-p-Phenyl mandelic acid 6.13 was also investigated as potential nucleation inhibitor. The addition of 10% (S)-6.13 resulted in a dramatic effect on the nucleation temperature, but now also the dissolution temperature was affected by the additive. Apparently, the solubility of 6.13 substantially differs from the solubility of (S)-6.8.

### 6.4 Ternary phase diagram

A qualitative ternary phase diagram (at constant temperature) can be constructed to correlate the results of the model resolution with the outcome of the turbidity measurements. In Figure 6.10 the area that would lead to 100% de in the less-soluble salt ((S)-phenylethylamine/(S)mandelic acid, (S)PEA(S)MA), without additives, is represented by the triangle n-E-6.4. Starting from the 1:1 mixture (the line Se), the line ac shows the concentration range in which diastereomerically pure salt will be obtained, without the use of additives. The dashed lines p’E’ and n’E’ represent the meta-stable nucleation lines that come into play on use of the additive. The intersection points of triangle n’E’-6.4 with line Se define the line bd, which is longer than the line ac. This implies that the operating window for a successful resolution is larger. Also, it can be seen that high de should be obtainable at higher concentrations than in the situation without additive.
This ternary phase diagram might account for the high success rate observed in Dutch Resolution experiments. When screening for an unknown resolution, typically several test tubes are filled with racemate and resolving agents and as little solvent as possible to obtain a clear solution on heating. In this way, one has the highest chance to obtain a salt, even when its solubility is low in that particular solvent (see also Section 2.2.1). One may conclude from this ternary phase diagram that the chance to obtain a high de using Dutch Resolution is higher at higher concentrations compared to a classical resolution using a single resolving agent.

To verify this latter conclusion, the resolution of racemic 6.1 with (S)-6.2 was performed at different concentrations, with and without 5% (S)-6.3. The yields, enantiomeric excesses and S-factors are shown in Figure 6.11.

In general, on going to a higher concentration, the yield increases and the ee decreases. This is what could be expected, since at a higher concentration the more-soluble diastereomer precipitates to a larger extent, resulting in a higher yield and lower ee.

The resolution efficiency in the presence of the additive is high at 0.50 M in isopropanol, whereas the resolution efficiency without additive drops dramatically somewhere between 0.25 and 0.33 M. This experiment supports the hypothesis drawn from the phase diagram, namely that the resolution in the presence of an additive gives a higher ee, even at higher concentrations.
To establish generality, additional resolutions were carried out all based on the same principle: the use of 10% of a non-incorporated family member of the resolving agent as suspected nucleation inhibitor together with the resolving agent. The additives were initially chosen from the pool of known families of resolving agents [Figure 6.1]. The 2-nitro-substituted phosphoric acid (entry 8b) was not reported in ref. 2 but was investigated on the basis of the present developments. In nine out of the ten systems given in Table 6.2 significant improvements in de of the first salt and S values are achieved. Improvements are sometimes dramatic, for example, entries 8a/b. Only for the resolution of p-chloromandelic acid, as shown in entries 4a/b, no significant improvement was observed. The resolutions were deliberately performed in such a way that the de without additive was not high due to simultaneous crystallization of both diastereomeric salts, so that improvements could be easily detected. Concentrations and conditions for the experiments with additives were analogous to those without additives. The resolutions given in entries 9 and 10 were performed in a reverse manner, i.e. an enantiomerically pure family member of the racemate was added instead of a compound structurally related to the resolving agent.
It can be seen that this second generation Dutch Resolution experiments seem to work with the same principle: on using an additive, the yield can be lower, but the enantiomeric excess will be higher.

Table 6.2 Second generation Dutch Resolution experiments

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Resolving agent</th>
<th>Additive</th>
<th>Yield (%)</th>
<th>De (%)</th>
<th>S-factor</th>
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<td>23</td>
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<td>0.31</td>
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<tr>
<td>1b^a</td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>86</td>
<td>0.50</td>
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<tr>
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<td></td>
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<td>30</td>
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<td></td>
<td>36</td>
<td>89</td>
<td>0.64</td>
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</table>
### Reverse resolutions with a family member of the racemate as additive

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<td><img src="image" alt="Chemical Structure" /></td>
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</tr>
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<tr>
<td></td>
<td>36</td>
<td>86</td>
<td>0.62</td>
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Reverse resolutions with a family member of the racemate as additive

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<thead>
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<tbody>
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<tr>
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<td>38</td>
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<td>0.49</td>
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<tr>
<td>10a</td>
<td><img src="image" alt="Chemical Structure" /></td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>44</td>
<td>0.34</td>
</tr>
</tbody>
</table>
In this case some of the additive is incorporated in the crystal, but always less than 10%.

b The resolution of mandelic acid 6.1 with (S)-6.2 in this experiment was performed at a different concentration than the one previously described, hence the difference in results.

In some of the cases (entries 1b, 2b, 3b and 5b) the additive is found in the salt, but less than 10% of the original amount. It is not clear at this point whether the additive is located at the outside of the crystal or is incorporated in the crystal lattice and forms a solid solution with the ‘parent’ resolving agent. Additional research is needed to answer this question.

6.6 Crystal habit

The salts obtained in the presence of additives were sometimes difficult to filter or sticky. On closer examination under a microscope, it was found that in general the crystals were smaller and/or the crystal habit less well defined.

As an example, the salts obtained in the resolution of racemic 6.1 with (S)-6.2 in the presence of 6.3 were examined under a microscope. On the left in Figure 6.12 the salt is shown that was obtained in the experiment without additives and on the left the salt that was obtained on addition of 6.3.

**Figure 6.12 On the left: no additive and on the right: in the presence of 10% (S)-6.3**
The salt on the right is still crystalline, but the crystals are smaller than the crystals in the picture on the left. These observations point to the involvement of a kinetic effect of the additive as habit modifier. Since the crystallization starts at a lower temperature, the supersaturation is higher. This means that a larger number of nuclei per time unit is produced, resulting in smaller crystals.\(^{10,11}\) Apart from the fact that the morphology will be different by the influence of the additive, the smaller crystal size is most likely caused by the higher supersaturation.

Habit modification of a diastereomeric salt has been reported by Saigo,\(^{12}\) but resolutions in the presence of a habit modifier have not been reported. Habit modification of fine chemicals by the addition of additives has been extensively studied to improve e.g. filtration behavior.\(^{13}\) Also, kinetic effects in resolutions have been studied,\(^{14}\) but the connection between nucleation inhibition and resolution efficiency has not been reported, to the best of our knowledge.

As an example of nucleation inhibition of an achiral compound, the crystallization of paracetamol was investigated in the presence and absence of \(p\)-acetoxyacetanilide as additive (Figure 6.13).\(^{15}\)

![Figure 6.13 Paracetamol (host compound) and \(p\)-acetoxyacetanilide (additive)](image)

Some of the additive was incorporated in the crystal, which was attributed to the molecular similarity of the additive and the host molecule. It was found that the nucleation induction time was increased, most likely through blocking the development of a critical nucleus. The nucleation induction time increased with increasing levels of the additive, which is shown in Figure 6.13 for two levels of supersaturation.
Moreover, the morphology of the crystals clearly changed from plate-like to columnar on increasing additive levels (Figure 6.15).

These observations on achiral molecules and our results on the turbidity measurements have a clear similarity with the work on tailor-made additives by Lahav et al. in the resolution of conglomerates by preferential crystallization. By addition of small amounts of certain structurally related compounds, the crystallization rate of one of the enantiomers was retarded.
dramatically, thereby leading to efficient resolution. A selective adsorption of an additive to a certain crystal face resulted in a change of crystal habit (see also Figure 2.3).

A closer examination of the morphology of the salts obtained in our experiments is definitely necessary to gain more insight in the mechanism of nucleation inhibition. In some of the cases reported in Section 6.5 no detectable amounts of nucleation inhibitor was found in the salt, which is not the case in the work of Lahav or in the work on paracetamol described above. Dynamic light scattering will be one of the tools to be used in further research, in order to investigate the particle size and distribution thereof during crystallization.

As was discussed in Chapter 2, aggregates are formed in solution prior to nucleation (Section 2.4.1). These embryos form nuclei once they reach a critical size. When the nucleation is inhibited by an additive, the additive must have an effect on these aggregates. The exact mechanism of action of the nucleation inhibitors on these embryos is worth investigating.

Also, it is desirable to gain more insight in the substituent effects that play a role, or in other words, which substituents make a successful nucleation inhibitor. Highly pertinent in this respect is the work of Kitaigorodskii, who developed a coefficient to determine the degree of isosterism (a measure of the analogy of the shapes and volumes of the compounds), in order to establish if two compounds are able to form solid solutions or not. The coefficient of isomorphism $\varepsilon$ is defined as follows:

$$\varepsilon = 1 - \frac{V_{no}}{V_o}$$

In this equation $V_o$ is the volume of overlap, or the volume that two molecules have in common. $V_{no}$ is the volume of nonoverlap. According to Kitaigorodskii no solid solution behavior is observed when $\varepsilon < 0.8$, whereas full solid solution behavior can be expected if $\varepsilon \geq 0.9$. The coefficient of isomorphism $\varepsilon$ can be determined either from the experimental X-ray data or via calculation a priori. Kitaigorodskii described an equation to calculate the overlapping volume $V_o$ between an atom with radius $R$ and $i$ other atoms with radius $R_i$ at a distance $d_i$ away:

$$V_o = \frac{4}{3} \pi R^3 - \sum_{i} \frac{1}{3} h_i^3 (3R - h_i)$$
In which \( h_i \) is given by:

\[
h_i = R - \frac{R^2 + d_i^2 - R_i^2}{2d_i}
\]

If Van der Waals radii are used to calculate the volume increment, the following data are obtained (Table 6.3).

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Volume increment (Å(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>-NO(_2)</td>
<td>23.0</td>
</tr>
<tr>
<td>-CH(_3)</td>
<td>22.1</td>
</tr>
<tr>
<td>-Br</td>
<td>35.4</td>
</tr>
<tr>
<td>-Cl</td>
<td>29.0</td>
</tr>
</tbody>
</table>

Nitro and methyl substituents have nearly identical volume increments. From the experiments described it follows that usually the nitro group is a very effective nucleation inhibitor and the methyl group far less so. Therefore, the effect cannot be attributed to a ‘size’ effect alone and probably the dipole moment plays an important role as well. Also, the halogens give rise to a substantial volume increment, but this does not seem to make them better nucleation inhibitors than the nitro group.

It is definitely worthwhile to investigate if a correlation exists between the coefficient of isomorphism and possible nucleation inhibitors. It could be that nucleation inhibition is most effective if a solid solution cannot be formed, which means that the \( \varepsilon \) should be smaller than 0.8. On the other hand, it has been shown that small amounts of resolving agent can be incorporated, but still nucleation inhibition is observed. Obviously, there is no clear borderline between solid solution behaviour and effective nucleation inhibitors.

It is known that the nucleation induction time decreases on increasing supersaturation, or in other words: the lower the supersaturation, the longer it takes before the first nuclei appear. This is shown in Figure 6.16 for paracetamol.
The turbidity measurements on the separate diastereomers were conducted at different concentrations, but in the process of a resolution, the initial concentration of both diastereomers is the same. The turbidity measurements show that the nucleation induction time increases under the influence of the additive. For the more-soluble salt, this effect will be even larger in a resolution process: the more-soluble salt is less supersaturated and therefore the effect on the nucleation induction time will be much larger. This can be seen in Figure 6.17.

For the less-soluble salt the effect in nucleation induction time \( \tau \) is \( \Delta \tau_1 \) and this effect is smaller than for a situation of lower supersaturation, shown as \( \Delta \tau_2 \).
6.7 ‘Classic’ Dutch Resolution versus second generation Dutch Resolution

The Dutch Resolution approach with families of resolving agents provides a high chance of finding a successful resolution in which nucleation inhibition can be involved. At this stage we obviously cannot predict with certainty all structural aspects that are essential for a compound to be an effective nucleation inhibitor.

It is clear that substitution in an aromatic ring can be especially effective, particularly with nitro as well as methoxy and sometimes halo groups. Also, sterically demanding groups, such as the phenyl group (entry 9b) seem to work. The position of the substituent must also be a contributing factor. In this respect, the work of Saigo is important.20 The effect of a substituent on the aromatic ring of phenylethylamine was investigated in the resolution with mandelic acid derivatives. It was found that the electron withdrawing or donating character of the substituent was important for the resolution efficiency, as well as the position of the substituent, involving steric effects in the crystal lattice. Obviously, the amount of additive to be used needs further optimization and probably depends on the resolution system.

Dutch Resolution provides a simple search procedure for discovery of potential nucleation inhibitors. If a component of a Dutch Resolution is found not, or only poorly, to be incorporated one may then turn to a single resolving agent approach aided by the "missing component" as potential nucleation inhibitor. This procedure seems to be reasonably general among several available families of resolving agents.

In this Chapter it was shown that nucleation inhibition and associated kinetic effects can play a role in Dutch Resolution. This leads to a second generation of Dutch Resolution, namely the use of small amounts of a certain family member of the resolving agent as additive.

This new method may well have industrial applications. A two-component resolution system, especially one in which the minor component is not incorporated, has clear advantages particularly from an industrial viewpoint. In this way the resolving agent can easily be recycled from the less-soluble salt. This is not possible in the ‘classic’ Dutch Resolution approach, when always a non-stoichiometric ratio was found in the salt. In general, the mixtures of resolving agents could not be separated and the mixture of resolving agents in non-stoichiometric ratio has only limited use, especially in view of the nucleation inhibition results.

In addition, it was shown that the resolution in the presence of an additive gives still high ee at a higher concentration. The use of higher concentrations is obviously beneficial, since more material can be resolved in one batch.
6.8 Synthesis of the nucleation inhibitors

6.8.1 Nitro-substituted phenylethylamines

The nitro-substituted phenylethylamines are not commercially available and can be synthesized from the corresponding acetophenones 6.14-6.16 via a Leuckardt reaction (Scheme 6.3). The first step consists of an in situ imine formation with formamide and subsequent reduction by formic acid, forming the $N$-formyl derivative 6.17-6.19. Hydrolysis with 10% HCl affords the free amine.

\[
\begin{align*}
\text{o-NO}_2: & \quad 6.14 \\
\text{m-NO}_2: & \quad 6.15 \\
\text{p-NO}_2: & \quad 6.16 \\
\text{o-NO}_2: & \quad 6.17 \\
\text{m-NO}_2: & \quad 6.18 \\
\text{p-NO}_2: & \quad 6.19 \\
\end{align*}
\]

Scheme 6.3 Synthesis of nitro-substituted phenylethylamines via the Leuckardt reaction

This procedure can also be used to synthesize other substituted phenylethylamines. The presence of the nitro-substituents caused some problems during work-up. The reaction mixtures were very dark and extraction was troublesome. Therefore, the yields are somewhat lower than can be expected on the basis of known experience with other substituted phenylethylamines (Table 6.4). Moreover, the amines needed to be distilled after the acid-base extraction after the second reaction step, in contrast to other substituted phenylethylamines, which are normally pure enough.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Yield (%) 1st step</th>
<th>Yield (%) 2nd step</th>
<th>Overall yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{o-NO}_2$</td>
<td>83</td>
<td>48</td>
<td>40</td>
</tr>
<tr>
<td>$\text{m-NO}_2$</td>
<td>94</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>$\text{p-NO}_2$</td>
<td>86</td>
<td>67</td>
<td>57</td>
</tr>
</tbody>
</table>

Table 6.4 Synthesis of nitro-substituted phenylethylamines

Subsequently, the amines needed to be resolved. Several 1 mmol test experiments were performed and it turned out that $\text{o}$-nitrophenylethylamine could be successfully resolved with mandelic acid. Chlocyphos was effective for $\text{m}$-nitrophenylethylamine and phenecyphos for $\text{p}$-
nitrophenylethylamine. The resolution efficiency for \( p \)-nitrophenylethylamine is somewhat low. Several resolving agents have been tried, but none of them resulted in an improvement of the current results. Since this compound is an effective nucleation inhibitor, the resolution of this compound remains a point for further research.

Table 6.5 Resolution of nitro-substituted phenylethylamines

<table>
<thead>
<tr>
<th></th>
<th>Yield (after rec.)(%)</th>
<th>Ee (after rec.)(%)</th>
<th>S-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-( o )-NO(_2)-PEA 6.6</td>
<td>35(^b)</td>
<td>99+(^b)</td>
<td></td>
</tr>
<tr>
<td>(R)-( o )-NO(_2)-PEA 6.7</td>
<td>45 (35)</td>
<td>84 (99+)</td>
<td>0.76</td>
</tr>
<tr>
<td>(S)-( m )-NO(_2)-PEA 6.18</td>
<td>44 (25)</td>
<td>84 (99+)</td>
<td>0.74</td>
</tr>
<tr>
<td>(R)-( m )-NO(_2)-PEA 6.19</td>
<td>28(^b)</td>
<td>99(^b)</td>
<td></td>
</tr>
<tr>
<td>(S)-( p )-NO(_2)-PEA 6.20</td>
<td>3(^b,c)</td>
<td>99+(^b,c)</td>
<td></td>
</tr>
<tr>
<td>(R)-( p )-NO(_2)-PEA 6.21</td>
<td>47 (11)(^a)</td>
<td>46 (98)(^a)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^a\): After 4 recrystallizations  
\(^b\): From the mother liquor  
\(^c\): After 3 recrystallizations

6.8.2 Phenyl-substituted mandelic acid

\((S)-p\)-Phenylmandelic acid 6.13 was synthesized according via a Suzuki reaction with \((S)-p\)-bromo mandelic acid 6.22 and phenyl boronic acid 6.23, with \( \text{Na}_2\text{CO}_3 \) as a base and \( \text{Pd/C} \) as catalyst. No additional ligands were necessary to perform the reaction. Enantiomerically pure \((S)-p\)-bromo mandelic acid was used and the product was enantiomerically pure.

\[ \text{Scheme 6.4 Synthesis of 6.13 via a Suzuki reaction} \]

6.9 Experimental section

For general remarks: see Section 3.10; HPLC-methods are given at the end of this section.

General procedure for the synthesis of nitro-substituted phenylethylamines: A mixture of 0.2 mol nitro-substituted acetophenone, 120 mL formamide and 50 mL formic acid was heated to reflux. The mixture was refluxed for several hours until the reaction was complete.
(followed by $^1$H-NMR). After cooling to room temperature, 200 mL water was added and the mixture was extracted with TBME (3 x 150 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated to a red oil or semi-solid which was refluxed in 250 mL 10% HCl overnight. After cooling to room temperature, the mixture was extracted with TBME (2 x 100 mL). The combined organic layers were washed with water. To the combined waterlayers was added concentrated NaOH-solution to pH 10. The mixture was extracted with TBME (3 x 250 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated to a brown oil. Kugelrohr distillation at 130 ºC and 0.069 mbar furnished the amines as yellow oils.

**o-Nitrophenylethylamine (6.6 + 6.7):** Yield after Kugelrohr over two steps 40%. $^1$H-NMR (CDCl$_3$): $\delta$ 1.42 (d, 3H), 1.56 (bs, 2H), 4.56 (q, 1H), 7.34 (t, 1H), 7.57 (t, 1H), 7.74 (m, 2H); $^{13}$C-NMR (CDCl$_3$): $\delta$ 24.74 (q), 46.00 (d), 123.85 (d), 127.42 (d), 132.99 (d), 142.07 (s).

**m-Nitrophenylethylamine (6.18 + 6.19):** Yield after Kugelrohr over two steps 56%. $^1$H-NMR (CDCl$_3$): $\delta$ 1.36 (d, 3H), 1.51 (bs, 2H), 4.21 (q, 1H), 7.46 (m, 1H), 7.66 (d, 1H), 8.03 (m, 1H), 8.30 (s, 1H); $^{13}$C-NMR (CDCl$_3$): $\delta$ 25.66 (q), 50.63 (d), 120.70 (d), 121.61 (d), 129.27 (d), 132.29 (d), 149.93 (s).

**p-Nitrophenylethylamine (6.20 + 6.21):** Yield after Kugelrohr over two steps 57%. $^1$H-NMR (CDCl$_3$): $\delta$ 1.39 (d, 3H), 1.50 (bs, 2H), 4.24 (q, 1H), 7.52 (d, 2H), 8.17 (d, 2H); $^{13}$C-NMR (CDCl$_3$): $\delta$ 25.65 (q), 50.85 (d), 123.62 (d), 126.68 (d), 155.38 (s).

**Resolution of o-nitrophenylethylamine with (S)-mandelic acid:** Racemic o-nitrophenylethylamine (10.0 g; 60 mmol) was dissolved in iPrOH (120 mL). (S)-Mandelic acid (9.16 g; 60 mmol) was added and a clear mixture was obtained at reflux. The solution was allowed to crystallize while stirring. The resulting solid was removed by filtration to obtain 8.54 g salt (45% yield, 84% $ee$). This salt was recrystallized from iPrOH (210 mL), furnishing 7.03 g salt (37%) with 99+% $ee$. Mp 154.2-155.3 ºC. The salt was stirred with 12.5 % NH$_3$-solution (200 mL ) and extracted with TBME (3 x 150 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated to a yellow oil. Yield 3.48 g (35%; 99+% $ee$) 6.7. The first mother liquor was concentrated in vacuo. To the resulting solid was added 12.5% NH$_3$-solution (200 mL ) and liberation of the free amine was carried out as described above, yielding 4.7 g of a yellow oil, which was subsequently dissolved in iPrOH (50 mL). (R)-Mandelic acid was added (4.0 g; 0.93 eq) and iPrOH (210 mL ) to obtain a clear solution at reflux. The solution was allowed to crystallize while stirring. The resulting solid was removed by filtration, yielding 6.6 g salt with 99+% $ee$. Liberation of the free amine was carried out with 12.5 % NH$_3$-solution as described above, yielding 3.4 g of 6.6 (35%; 99+% $ee$).
Resolution of \(m\)-nitrophenylethylamine with (-) chlocyphos: \(m\)-Nitrophenylethylamine (12.4 g; 75 mmol) was dissolved in \(iPrOH\) (200 mL). (-) Chlocyphos (20.7 g; 75 mmol) was added together with \(iPrOH\) (200 mL) and water (250 mL) until a clear solution was obtained at reflux. The solution was allowed to crystallize while stirring. The resulting solid was removed by filtration under suction, yielding 19.7 g salt (44%; 84% ee of the \((S)\)-enantiomer 6.18). Recrystallization from \(iPrOH\) (300 mL) and water (200 mL) gave 13.1 g salt (30%; 99+% ee). Mp 291.7°C (decomp.). The salt was suspended in 10% HCl (200 mL) and the resulting solid ((-)-chlocyphos) was removed by filtration and thoroughly washed with water. To the filtrate was added concentrated NaOH-solution to pH 10. The mixture was extracted with TBME (3 x 150 mL). The combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to a yellow oil. Yield 4.2 g (25%; 99+% ee) 6.18. The first mother liquor was concentrated \textit{in vacuo}. To the resulting solid was added 10% HCl (150 mL) and the free amine was liberated as described above, yielding 5.76 g amine (88% ee of the \((R)\)-enantiomer 6.19). The amine was dissolved in \(iPrOH\) (50 mL) and 9.0 g (+) \((0.96 \text{ eq})\) chlocyphos was added, together with \(iPrOH\) (250 mL) and water (300 mL). A clear solution was obtained at reflux. The resulting salt was removed by filtration, furnishing 9.8 g salt with 99+% ee. The free amine was liberated as described above, yielding 3.5 g of a yellow oil (28%, 99+% ee) 6.19.

Resolution of \(p\)-nitrophenylethylamine with (-) phencyphos: \(p\)-Nitrophenylethylamine (13.0 g; 78 mmol) was dissolved in MeOH (300 mL) and water (10 mL). (-) Phencyphos was added and the mixture was heated to reflux until a clear solution was obtained. The solution was allowed to crystallize while stirring. The resulting solid was collected via filtration under suction, furnishing 14.9 g (47%) salt with 46% ee. ((R)-6.21 in excess). This salt was recrystallized from MeOH (250 mL) and water (3 mL) yielding 8.4 g (27%) salt with 83% ee. A second recrystallization from MeOH (160 mL) and water (2 mL) gave 5.2 g (16%) salt with 93% ee. A final recrystallization yielded 3.4 g (11%) salt with 98% ee. Mp 268.5°C (decomp.). The free amine was liberated by stirring the salt in 10% HCl (150 mL). The resulting solid ((-)-phencyphos) was removed by filtration and thoroughly washed with water. To the filtrate was added 25% NH\(_3\)-solution to pH 10. The mixture was extracted with TBME (3 x 100 mL). The combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to a yellow oil. Yield 1.12 g (9%) of the free amine 6.21 (ee still 98%). The first mother liquor (46% ee of the \((S)\)-6.20) was concentrated \textit{in vacuo} and the resulting solid was stirred in 10% HCl (250 mL). The resulting solid was removed by filtration and the filtrate was added 25% NH\(_3\)-solution to pH 10. Similar extraction gave 5.6 g of the yellow free amine, which was dissolved in MeOH (170 mL) and water (2 mL). (+) Phencyphos (6.3 g; 0.77 eq) was added and a clear mixture was obtained at reflux. The solution was allowed to crystallize while stirring. The resulting solid was removed by filtration yielding 5.5 g salt (17% yield; 76% ee). This salt was recrystallized from MeOH (125 mL) and water (2 mL), furnishing 3.1 g salt (10%; 88% ee). After a second and a third recrystallization the ee was 99+% and the yield 1.06 g (3%). Liberation of the free amine as described above gave 0.39 g 6.20 (3%; 99+% ee).
(S)-p-Phenyl mandelic acid (6.13): (S)-p-Br-mandelic acid 6.22 (10.8 g; 46.5 mmol) and phenyl boronic acid 6.23 (5.4 g; 51.2 mmol) were suspended in iPrOH (12 mL) and water (36 mL). Pd/C (ca. 500 mg) was added and carefully a solution of Na₂CO₃ (6.16 g; 58.2 mmol) in water (24 mL) was added. The mixture was heated to 64°C and the reaction was followed by LC-MS. After 4 hrs the reaction was not complete yet, so the mixture was stirred overnight at 64°C. After cooling to room temperature, a mixture of iPrOH/water/2N NaOH (70:15:1; 100 mL) was added. The catalyst was removed by filtration under suction over Celite and washed with the same solvent mixture. 10% HCl (ca. 20 mL) was added to pH 2. A white solid precipitated. Most of the iPrOH was evaporated in vacuo and the white solid was removed by filtration and washed with water. The crude product was recrystallized from acetic acid, giving 5.3 g (50%) of a white solid. ¹H-NMR (DMSO): δ 5.15 (s, 1H), 6.42-7.73 (m, 9H); ¹³C-NMR (DMSO): δ; mp 256.8°C (decomp.); HPLC: 99+% ee.

HPLC-methods

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>HPLC-column</th>
<th>Conditions</th>
<th>Ret. Times (min.)</th>
</tr>
</thead>
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<td>1</td>
<td><img src="crownpak.png" alt="Image" /></td>
<td>Crownpak</td>
<td>HClO₄, pH=2, flow 0.8 mL/min, T=5°C, UV 268 nm</td>
<td>7.1; 25.6</td>
</tr>
<tr>
<td>2</td>
<td><img src="chiralpak.png" alt="Image" /></td>
<td>Chiralpak AS</td>
<td>Heptane/EtOH/Et₃NH 95:5:0.2, flow 1.0 mL/min, UV 260 nm</td>
<td>15.8; 17.5</td>
</tr>
<tr>
<td>3</td>
<td><img src="crownpak.png" alt="Image" /></td>
<td>Crownpak</td>
<td>HClO₄, pH=2, flow 0.8 mL/min, T=5.0°C, UV 268 nm</td>
<td>9.8; 12.2</td>
</tr>
<tr>
<td>4</td>
<td><img src="crownpak.png" alt="Image" /></td>
<td>Crownpak</td>
<td>UV 200 nm, HClO₄, pH=2, flow 1.0 mL/min</td>
<td>11.8; 25.6</td>
</tr>
<tr>
<td>5</td>
<td><img src="crownpak.png" alt="Image" /></td>
<td>Crownpak</td>
<td>HClO₄, pH=2, flow 0.8 mL/min, T=5°C, UV 200 nm</td>
<td>6.0; 7.6</td>
</tr>
</tbody>
</table>
Chiralpak AD  Heptane:iPrOH:Et2NH=90:10:0.2, flow 1.0 mL/min, UV 240 nm  12.7; 24.6

Chiralpak AD  Heptane/EtOH/TFA = 85:15:0.2, 1.0 mL/min, UV 220 nm  24.6; 36.8

Chiralpak OJ  Heptane/EtOH/TFA = 90:10:0.2, flow 1.0 mL/min, UV 220 nm  23.6; 27.2

Chiralpak AD  Heptane:iPrOH:TFA = 95:5:0.1, flow 1.0 mL/min, T=10ºC, UV 218 nm  7.5; 9.3

\[ \text{a } 16.3 \text{ g 70\% } \text{HClO}_4 \text{ in } 1.0 \text{ L bidest. } 100 \text{ mL of this mixture was diluted with bidest to } 1 \text{ L and used as eluent.} \]
\[ \text{b Analysis on the phenylureum derivative: the amine was liberated from the salt, dissolved in dichloromethane and 1 drop of NEt}_3 \text{ was added and subsequently 1 drop of phenylisocyanate. From the resulting mixture a few drops were diluted with the eluent and analyzed.} \]

6.10 References

1 Pasteur, L. C. R. Acad. Sci. 1848, 26, 535.
5 Doyle, A.C. Silver Blaze, 1894.
7 1H-NMR and mass spectrometry were used to investigate the presence of 6.3.
8 The error in resolution efficiency is estimated at 5%.


(a) Kitaigorodskii, A.I. *Organic Chemical Crystallography*, Consultant Bureau, New York, **1961**, p. 230; (b) ref. 3 p. 128-129.


Bondi, A.A. *Physical Properties of Molecular Crystals, Liquids and Glasses*, Wiley, New York, **1968**.

Ref. 16, p.13


Chlocyphos is 4-(2-chlorophenyl)-2-hydroxy-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane and phenecyphos is 2-hydroxy-5,5-dimethyl-2-oxo-4-phenyl-1,3,2-dioxaphosphorinane. These cyclic phosphoric acids were developed in our lab: ten Hoeve, W.; Wynberg, H. *J. Org. Chem.* **1985**, **50**, 4508; Eur. Pat. 180,276; US Pat. 4,814,477

Undergraduate student Ronald Boelhouwer also worked on these phenyl-substituted mandelic acids.
