The Dutch Resolution Variant of the Classical Resolution of Racemates by Formation of Diastereomeric Salts: Family Behaviour in Nucleation Inhibition


Abstract: The resolution of racemates through their diastereomeric salts can be positively affected by the addition of small amounts of suitable nucleation inhibitors. This discovery is a logical extension of “Dutch Resolution”, in which equimolar amounts of resolving agents that are members of the same family (i.e., structurally related) are used. We conducted a systematic search for nucleation inhibitors of the resolving agent 1-phenylethylamine. A wide range of amines that bear possible family resemblances to 1-phenylethylamine was investigated. It was found that (R)-1-phenylbutylamine is a good inhibitor of (R)-1-phenylethylamine. Results of turbidity measurements showed that, for the model case of mandelic acid resolution, the chief effect of this inhibitor was to widen the metastable zone for the more soluble diastereomer. This observation is in accordance with previous experience. Further scouting for possible family members revealed a wide variation in the effectiveness of inhibitors, dependent on their structure. By far the most effective inhibitors are bifunctional 1-phenylethylamine and/or 1-phenylbutylamine analogues. The effect of racemic inhibitors was found to approach that of enantiomerically pure inhibitors of the same absolute configuration of the 1-phenylethylamine used for resolution. The most effective inhibitors were tested for the resolution of a structural variety of racemates, and were shown to be broadly applicable.

Keywords: chiral resolution · chirality · crystal growth · diastereoselectivity · enantioselectivity

Introduction

“Dutch Resolution” is the use of families of resolving agents in the otherwise classical Pasteur separation of racemates through their diastereomeric salts.[1,2] As first described an equimolar mixture of, in general, three resolving agents of the same family (i.e., with close structural analogy, common absolute stereochemistry) was used. Non-stoichiometric incorporation of resolving agents and often improved diastereomeric excesses of the first salts were observed.

Further investigations revealed that certain family members of resolving agents failed to be, or were only slightly, incorporated into the salts, although they had a positive effect on the resolution.[3] An example is the ortho/para-nitro mixture (R)-2b and the classical resolving agent 1-phenylethylamine (R)-2a, as illustrated for the case of the resolution of mandelic acid (1) in Scheme 1. On the basis of turbidity measurements, it was shown that 2b is an effective nucleation inhibitor. It is not detectably incorporated into the less soluble salt, which is obtained in significantly higher diastereomeric excess in the presence of 2b (55% de compared to 14% de without inhibitor under the experimental conditions used). Inhibitor 2b acts by widening the width of the metastable zone of supersaturation (the temperature zone between dissolution and the lower temperature at
Concentration = ly available (in both enantiomeric forms) 1-phenylbutyl-ally. In orientational experiments, it was observed that readily the side-chain rather than the aryl ring is modified structurally.

Table 1. Diastereomerically pure salts cannot be obtained 2a 2c of 6mol% 5620 as possible for nucleation inhibitors among other potential opportunities for selective precipitation of the less soluble diastereomeric salt. [6]

The use of a single resolving agent with a small amount of additive (nucleation inhibitor) instead of a mixture is technologically simpler, but how can nucleation inhibitors in the context of Dutch Resolution be identified? Here, we describe some guidelines derived from the study of amines, and indicate how these can be applied.

Results and Discussion

The model system that was used for scanning is that shown in Scheme 1, and the primary goal was to search as quickly as possible for nucleation inhibitors among other potential “family members” related to the parent resolving agent 2a.

We first looked at analogues of resolving agent 2a, in which the side-chain rather than the aryl ring is modified structurally. In orientational experiments, it was observed that readily available [5] (in both enantiomeric forms) 1-phenethylamine (R)-2c induced significant improvements if used as an additive with (R)-2a. Notably, no (R)-2c can be detected in the precipitated salt. Yields and de values are shown in Table 1. [8] Diastereomerically pure salts cannot be obtained in more than 50% yield. We prefer the use of Fogassy S-factors (2 × yield × de) to express the overall efficiencies. [7] Scanning of 2a/2c ratios revealed that the optimal concentration for nucleation inhibition was 6 mol% of 2c (see Supporting Information).

Rather unexpectedly, it was observed that racemic 2c is also nearly as effective as (R)-2c (Table 1, entry 3) and that the “wrong” enantiomer, (S)-2c, also has some positive effect (entry 4). Notably, (R)-2c alone is not a resolving agent for 1 (entry 5). The yield, de and S-factor of the reaction corresponding to entry 2, performed on a 10 g (65.8 mmol) scale, were within 1% of the results obtained on the 2 mmol scale reported in Table 1.

Turbidity measurements (see Supporting Information) revealed that, for (R)-2c, analogously to 2b, the metastable zone width for the more soluble (S)-1/(R)-2a diastereomeric salt is increased from 7.3°C without inhibitor to 28.5°C (zone width 25.3°C for racemic 2c and 13.0°C for (S)-2c), whereas for the less soluble (R)-1/(R)-2a diastereomeric salt, the zone width increases from 3.9°C without inhibitor to only 7.0°C (5.9°C for racemic 2c and 7.0°C for (S)-2c). The conclusion is clear; 2c, of the same relative configuration as resolving agent 2a, is an effective nucleation inhibitor that affects chiefly the more soluble diastereomeric salt by increasing the metastable temperature zone width; the more soluble diastereomer remains in solution for a longer period of time. [8] An analogous effect is observed in slightly diminished form for racemic 2c, and relatively weakly for the “wrong” (S) enantiomer of 2c.

Although 2c is clearly an inhibitor, it seems unlikely that every small change in the structure of a resolving agent will result in a nucleation inhibitor. Are there predictable structural considerations and, if so, what are they? To examine further the effects of substitution in the aryl ring, the compounds 3–25 (all enantiomerically pure (R)) shown in Figure 1 were investigated as inhibitors for the resolution of mandelic acid (1) by 1-phenylethylamine (2a), as depicted in Scheme 1. [9] All experiments were performed in triplicate and the values of the S-factors are reproducible to within (±) 5%. If a “hit” is arbitrarily defined as S ≥ 0.35, relative to S = 0.24 without additive (see farthest right bar in Figure 1, in which the concentration of resolving agent 2a is identical to that in the inhibition experiment), then 2c itself, the 3-nitro (26), 2-hydroxy (23), 1-naphthyl (24), 2-fluoro (22), 2-methoxy (21) and 2-naphthyl (20) derivatives are active inhibitors (7 out of a library of 24). Note that the halo-substituted derivatives 3–7 as additives lead to significantly lower S-values. The other compounds have only modest effects.

By contrast, the effects of structural modification of the side-chain are more profound (Figure 2). In this case, not only compounds with the (R) absolute configuration analogous to (R)-2a were examined, but also racemates and “wrong” (S) enantiomers. By using the same arbitrary definition of a “hit”, 17 of the 21 compounds examined show significant effects. The achiral additives 26, 28, 32 and 36 have virtually no effect. Cyclization of the side-chain (33–35) produces no dramatic improvements. Branching of the side-chain can, however, be quite effective, as exemplified by 41. [9]

However, by far the most potent inhibitors are the di-amine derivatives (42–45). [10] We included these compounds in the screen because molecules with repeating functionality in their structure often have a greater tendency to aggregate,

![Scheme 1. Resolution of mandelic acid (1) with (R)-1-phenylethylamine 2a. Additives are (R)-2b and (R)-2c.](image-url)
and might influence nucleation in a different manner (gemini effect). \[11\]

The proof of the pudding is in the tasting. Do the better nucleation inhibitors identified by screening also work in the resolution of other compounds? Those shown in Figure 3 are clearly the most potent. Selected results for the resolution of $\alpha$-methylphenylacetic acid (46), $\alpha$-methoxyphenylacetic acid (47) and N-acetylleucine (48) with (R)-2a are summarised in Table 2. \[15\]
In all cases, the de values of the first-isolated salts increase to very acceptable values, as the yields decrease. In our experience, this often, but not always, happens. We also emphasise that the experiments described here may not be optimal with regard to the concentration of inhibitor and temperature. Nevertheless, we consider these non-optimised results to be extremely promising.

**Summary**

We conclude that:

1) Nucleation inhibition is often involved in Dutch Resolution.

2) The effect of nucleation inhibition is greatest on the more soluble diastereomeric salt.[4c,16]

3) A search for inhibitors is best carried out among “family members” of the resolving agent.

4) There are, at least at this stage, no hard and fast rules for the identification with regard to structure, and all of the suspects must be subjected to screening.

5) Absolute stereochemistry is not an absolute prerequisite, and racemates may be used for screening purposes.

6) Although we are now able to identify inhibitors by using screening methods, our understanding of the inhibition phenomenon itself and of the stereochemical aspects in particular remains insufficient.

In future work, we intend to develop automated protocols for the screening of inhibitors, to develop specific inhibitors for the most commonly used resolving agents for which families exist, to find methodologies based on the discoveries described in this paper that permit application to large scale resolutions and to understand better the mechanism of action of nucleation inhibitors.

### Experimental Section

**General procedure for the small-scale nucleation inhibition experiments described in Table 1:** In a Kimble reactor tube (dimensions 25 × 150 mm), provided with a cylindrical, PTFE-coated magnetic stirring bar (10 × 6 mm), 0.12 mmol (0.06 mol equiv) of additive 2c and 1.88 mmol (0.94 mol equiv) of (R)-1-phenylethylamine (2a) were mixed in 3.33 mL of CH₃CH(OH)CH₃. Subsequently, 2.0 mmol racemic mandelic acid (1) (1.0 mol equiv) in 1.67 mL CH₃CH(OH)CH₃ was added. The mixture was heated until a clear solution was obtained. After the reactor tube was sealed with a rubber stopper, it was placed in the Varian thermostatted bath and mechanically stirred at 78°C for 30 min. The tubes were gradually cooled to 20°C at a ramp rate of 10°C per hour and stirred at that temperature for 12 h. The precipitated salts were collected by filtration using a VacMaster® 20, then each was washed with 1.5 mL of CH₃CH(OH)CH₃ and dried. HPLC analysis was used to determine the diastereomeric excesses of the salts. To ensure accurate de determination, the racemic substrate was measured first in each case. The composition of the salt was determined by conducting mass spectrometry, and ¹H and ¹³C NMR spectroscopy. The resolution experiments described in Tables S4, S5 and S6 of the Supporting Information were performed analogously to this general procedure, with either 0.03 or 0.06 mol equiv of additive (and, respectively, 0.97 or 0.94 mol equiv of parent resolving agent 2a) with respect to the 1.0 mol equivalent of racemic substrate. The solvent(s) and concentrations used are listed at the bottom of each table. The conditions used in the HPLC analysis for each substrate are given in Figure 3.

### Table 2. Selected results for the resolution of racemates 46–48 with (R)-2a, obtained by using the most effective nucleation inhibitors shown in Figure 3.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Racemate</th>
<th>Additive (mol %)</th>
<th>Yield [%]</th>
<th>de [%]</th>
<th>S-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>–</td>
<td>60</td>
<td>7</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>(±)-42 (3 %)</td>
<td>36</td>
<td>59</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>(±)-43 (3 %)</td>
<td>54</td>
<td>52</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>(R,R)-44 (6 %)</td>
<td>18</td>
<td>97</td>
<td>0.35</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>–</td>
<td>65</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>(±)-42 (6 %)</td>
<td>16</td>
<td>96</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>58</td>
<td>13</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>(±)-43 (3 %)</td>
<td>35</td>
<td>75</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>(R,R)-44 (3 %)</td>
<td>44</td>
<td>48</td>
<td>0.43</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>(R,R)-45 (6 %)</td>
<td>56</td>
<td>32</td>
<td>0.36</td>
</tr>
</tbody>
</table>

[a] Salt isolated after 6 days.
General procedure for the Leuckart synthesis of additives 2c, 26, 27, 29, and 31–33, 37, 38 and 40–43: A mixture of the corresponding aldehyde or ketone (10 mmol), formamide (20 mL) and formic acid (10 mL) was heated to reflux and then refluxed for 1 h. After cooling to ambient temperature, 30 mL of water was added and the mixture was extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated to furnish the intermediate formamide. Aqueous HCl (20 mL, 30%) was added and the reaction mixture was refluxed for 1 h. After cooling to ambient temperature, 20 mL of water was added. The reaction mixture was carefully adjusted to pH 10 with aqueous NaOH (33%) and extracted with diethyl ether (3×20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated to furnish the corresponding primary amine.

1-Propyl-Butylamine (26): Colourless liquid, 55% yield after Kugelrohr distillation; ¹H NMR (400 MHz, CDCl₃): δ = 0.87–1.26 (m, 4H), 1.49–1.59 (m, 2H), 2.77–2.92 ppm (m, 3H); ¹³C NMR (50 MHz, CDCl₃): δ = 14.90 (q), 34.22 (s), 126.28 (d), 128.14 (d), 137.61 ppm (s); MS (CI): m/z = 116 [M⁺]^+.

(±)-2,2-Dimethyl-1-phenyl-1-propanamine (27): Pale yellow oil, 95% yield; ¹H NMR (300 MHz, CDCl₃): δ = 1.27–1.83 (m, 4H), 2.62–2.70 ppm (m, 2H); ¹³C NMR (50 MHz, CDCl₃): δ = 16.34 (q), 36.78 (t), 125.99 (d), 128.17 (d), 130.17 ppm (s); MS (EI): m/z = 162 [M⁺]^+.

(±)-1-Phenyl-1-pentanamine (31): Yellow oil, 87% yield; ¹H NMR (300 MHz, CDCl₃): δ = 0.82 (t, J = 6.7 Hz, 3H), 1.06–1.27 (m, 4H), 1.41 (brs, 2H), 1.57–1.63 (m, 2H), 1.85 (m, 2H); ¹³C NMR (50 MHz, CDCl₃): δ = 17.41 (t), 25.33 (t), 26.04 (t), 43.21 (t), 61.64 (d), 126.51 (d), 127.67 (d), 128.17 (d), 144.70 ppm (s); MS (EI): m/z = 162 [M⁺]^+.

(±)-6,8,9-Tetrahydro-5-H-benz[cyclohepten-5-amine (33): Colourless liquid, 53% yield after Kugelrohr distillation; ¹H NMR (300 MHz, CDCl₃): δ = 1.52–2.02 (m, brs 8H), 2.82–2.87 (m, 2H), 4.21–4.25 (m, 1H), 7.10–7.26 (m, 4H), 7.42–7.45 ppm (m, 1H); ¹³C NMR (50 MHz, CDCl₃): δ = 13.79 (q), 22.46 (t), 28.55 (t), 39.21 (t), 56.08 (d), 126.07 (d), 126.54 (d), 128.14 (d), 146.69 ppm (s); MS (EI): m/z = 163 [M⁺]^+.

(±)-1-Methylbutylamine (37): Colourless oil, 65% yield after Kugelrohr distillation; ¹H NMR (200 MHz, CDCl₃): δ = 0.81–0.88 (m, 3H), 0.98 (d, J = 6.34 Hz, 3H), 1.10–1.39 (m, 4H), 1.63 (brs 2H), 2.77–2.86 ppm (m, 1H); ¹³C NMR (50 MHz, CDCl₃): δ = 13.86 (q), 19.29 (t), 23.63 (q), 42.18 (t), 46.37 ppm (s); MS (EI): m/z = 88 [M⁺]^+.

(±)-2-Methyl-1-phenyl-1-propanamine (41): Yellow oil, 83% yield; ¹H NMR (200 MHz, CDCl₃): δ = 0.72 (d, J = 6.59 Hz, 3H), 0.92 (d, J = 6.59 Hz, 3H), 1.41 (brs 2H); 1.74–1.85 (m, 1H), 3.54 (d, J = 7.32 Hz, 1H), 7.17–7.28 ppm (m, 5H); ¹³C NMR (50 MHz, CDCl₃): δ = 18.81 (q), 19.70 (q), 35.36 (d), 62.37, 128.03 (d), 129.61 (d), 128.03 (d), 150.91 ppm (s); MS (CI): m/z = 150 [M⁺]^+.

Note that in the cases of 42 and 43, two stereocenters are present and a meso compound is possible. After the Leuckart reductive amination of the corresponding bis-aldehydes, the reaction mixture contained a racemic ratio of 85:15 and 80:20, respectively, according to ¹H NMR analyses. After bulb-to-bulb distillation, the only products isolated were racemic 42 and 43 (rac > 99:1) as a colourless oil, 53% yield after bulb-to-bulb distillation; ¹H NMR (300 MHz, CDCl₃): δ = 1.28 (d, J = 6.59 Hz, 6H), 2.63 (brs 4H), 3.98 (q, J = 6.59 Hz, 2H), 7.07–7.17 ppm (m, 4H); ¹³C NMR (50 MHz, CDCl₃): δ = 25.07 (q), 49.64 (d), 122.85 (d), 124.10 (d), 126.80 (d), 147.29 ppm (s); MS (CI): m/z = 165 [M⁺]^+.

Procedure for the synthesis of (R)-phenylglycine amide (PGA) diimines: The disubstituted benzaldehyde (100 mmol) was added to a suspension of (R)-phenylglycine amide (200 mmol, 30.0 g) in CH₂Cl₂ (200 mL) at ambient temperature. The reaction mixture was stirred overnight at room temperature. After removal of the CH₂Cl₂, the residual solid was recrystallised once from acetone/hexane (1:20).

(2R)-2-[[((R)-[1-[(1R)-2-Amino-2-oxo-1-phenylethyl]amino]methyl)methyl]phenyl][methy]lene[imin]o]amine (49): White solid, 96% yield. M.p. 143.8–144.5°C; ¹H NMR (300 MHz, CDCl₃/D₂O): δ = 4.55 (s, 2H), 6.60 (brs, 2H), 6.63 (brs, 2H), 7.62–7.59 ppm (m, 4H), 8.47 (d, J = 7.99 Hz, 2H), 8.77 (s, 1H), 7.94 ppm (s, 2H); ¹³C NMR (50 MHz, CDCl₃/D₂O): δ = 31.52 (d), 60.74 (d), 123.37 (d), 128.67 ppm (s); elemental analysis calc (%) for C₂₀H₁₇N₃O₂: C 61.79, H 5.91, N 13.15; MS (EI): m/z = 337 [M⁺]+.

Procedure for the alkylation of (R)-PGA diimines 49 and 50: A solution of allylzinc bromide (67 mmol) was added to a suspension of (R)-PGA diimine (50 mmol) in THF (200 mL). The solution of allylzinc bromide was cooled to room temperature and 97.3 mmol of the imine in THF (50 mL) was added at 0°C. The reaction mixture was warmed to room temperature and then poured into water (100 mL). Ethyl acetate (70 mL) was added and the mixture was stirred vigorously. After filtration through Celite, the organic phase was separated and the water layer was extracted with ethyl acetate (2×100 mL). The combined organic phase was dried over sodium sulfate and the ethyl acetate was evaporated to furnish the PGA alkyllamines 51 or 52, which in both cases crystallised on standing.
7.10, N 11.61; found: C 74.66, H 7.10, N 11.61; MS (CI): m/z: 483 [M+H]+.

(2R)-2-t-[(1R)-1-[(1R)-2-Amino-2-oxo-1-phenylethyl]ami-no]-3-butenyl]phenyl]-3-butenyl]amino)-2-phenylethanamide (52) Pale yellow brittle solid, 99% yield, 98% dr. M.p. 96.5–98.9°C. 1H NMR (300 MHz, CDCl3): δ = 2.16–2.44 (m, brs, 5H), 3.70 (dt, J = 6.2 Hz, 2H), 4.05 (s, 2H), 4.95–5.10 (m, 4H), 5.61–5.69 (m, 2H), 6.26 (brs 2H), 7.31–7.09 ppm (m, 16H); 13C NMR (50 MHz, CDCl3): δ = 24.33 (d), 127.41 (d), 127.49 (d), 128.38 (d), 128.96 (d), 134.46 (d), 138.43 (s), 141.25 (s), 175.90 ppm (s); elemental analysis cald (%) for C30H34N4O2: C 74.66, H 7.10, N 11.61; found: C 74.70, H 7.06, N 11.62; MS (Cl): m/z: 483 [M+H]+.

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[6] Details of an efficient HPLC method to allow rapid and accurate analysis are given in the Supporting Information.


[8] All these compounds were available by means of procedures described in ref. [5].

[9] A 1:1 mixture of enantiopure 2a, 37 and 42, the latter two of which appear to be nucleation inhibitors (Table 2) has been used in the past (ref. [1]) as a family of resolving agents (“PE-III mix”).

[10] The preparation of these materials by means of a Leukart reaction and subsequent easy isolation is described in the Supporting Information.


[12] Conc. = 0.30 mmol mL−1 in CH3(CH2)OH:CH3:HO (20:1). On addition of additive, the crystallized material contained the (R)-2a (S)-46 in excess.

[13] Conc. = 0.25 mmol mL−1 in CH3(CH2)OH:CH3:HO (20:1). On addition of additive, the crystallized material contained the (R)-2a(R)-46 in excess.

[14] Conc. = 0.75 mmol mL−1 in CH3(CH2)OH:CH3:HO (5:2). On addition of additive, the crystallized material contained the (R)-2a(R)-46 in excess.

[15] The “habit modifier” (R)-bis(2-methylbenzyl)amine [K. Sakai, Y. Maezawa, K. Saigo, M. Sukegawa, H. Murakami, H. Nohira, Bull. Chem. Soc. Jpn. 1992, 65, 1747–1750], the secondary amine analog of 1-phenylethylamine 2a, was only modestly effective and raised the S-factor for the resolution of 1 to 0.26 and that of 46 to 0.41; these results were not sufficient to encourage further investigation.

[16] From turbidity measurements performed to date, we have observed that “Dutch Resolution inhibitors” have the greatest effect on the more soluble diastereomeric salt. However, not every inhibitor has been subjected to turbidity measurements.

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