Chiral Resolution

Enantioselective Symmetry Breaking Directed by the Order of Process Steps**

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Since Viedma discovered that a mixture of enantiomorphic NaClO$_3$ crystals can be completely transformed into a solid phase of single handedness by simple grinding of the crystals, this nascent field has rapidly developed and now provides practical access to enantiomerically pure compounds.[1–13] In short, by grinding a racemic mixture of conglomerate crystals in contact with a saturated solution in which racemization of the solute takes place, the solid phase becomes enantiomerically pure. Such systems are sensitive to chiral perturbations, which dictate the final outcome. So far, we have reported that the enantioselective symmetry breaking can be directed by using small initial enantiomeric imbalances, tailor-made additives, and differences in initial crystal-size distributions, and by circularly polarized light.[2,5,11]

Herein, we introduce the novel concept that the configuration of the deracemization product can be controlled simply by the order in which the different reaction-mixture components are combined in the process. The underlying mechanism is based on a subtle balance between enantioselective crystal growth and dissolution. The effect of the order of steps in the experimental procedure was found with the imine **1**, which was previously used for the proof of principle of the deracemization method (Figure 1).[2]

Until now, the experiments were typically performed by first partially dissolving the racemic conglomerate crystals under grinding conditions with glass beads, and then initiating the solution-phase racemization by adding the organic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a racemization catalyst (Figure 1). When we started with a racemic solid
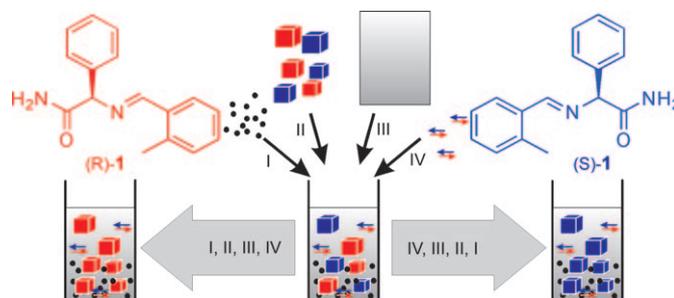

Figure 1. Schematic representation of the traditional experimental procedure (left) and the reverse experimental procedure (right) for the grinding-induced transformation. Until now, the order in which the components were added to the reaction vessel was: I) glass beads, II) racemic **1**, III) the solvent, and then IV) the racemization catalyst. During grinding, these reactions always evolved to give an enantiomerically pure (*R*)-**1** solid phase. A simple reversal of the order of addition of these components to the reaction vessel can lead to the inverse outcome.

phase, we always obtained enantiomerically pure (*R*)-**1** in the solid phase at the end of the experiment (Table 1, entry 1). An explanation for this outcome is that minute amounts of enantiomerically enriched impurities enantioselectively hamper the growth of crystals of one handedness and thereby drive the process toward the formation of the enantiomer of the opposite handedness.[2] We demonstrated this effect by using (*S*)- and (*R*)-phenylglycine ((*S*)- and (*R*)-**2**), which were shown to have the strongest interaction with the (*S*)-**1** and

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Table 1: Deracemization experiments.

Entry	Procedure	Additive **2**	Number of experiments	Final configuration of **1** (*ee* [%])
1	traditional	—[a]	>100	*R* (>99.9)
2	traditional	(*R*)-**2**	12	*S* (>99.9)
3	traditional	(*S*)-**2**	12	*R* (>99.9)
4	reverse[b]	—[a]	12	8x*S* (>99.9), 4x*R* (>99.9)
5	reverse[c]	(*S*)-**2**	4	*S* (>99.9)
6	reverse[c]	(*R*)-**2**	4	*R* (>99.9)

[a] Compound **2** was not added. The starting material contained trace amounts of unintentional chiral impurities. [b] Reverse experiment following the procedure described in Figure 1. [c] Reverse experiment following the procedure described in Figure 2.

(R)-**1** crystals, respectively (Table 1, entries 2 and 3).[2] As a result, the presence of (S)-**2** led to the complete transformation of the racemic solid phase into (R)-**1** and vice versa, according to the rule of reversal proposed by Lahav and co-workers.[14]

In the present experiments, we simply reversed the order in which the components of the reaction mixture were placed in the reaction flask. That is, first the solvent and the racemization catalyst were added, and then the racemic crystals were partially dissolved in this solution (Figure 1); no additive was used. This mixture was gently swirled for 2 h, and then glass beads were introduced, and the slurry was ground in a thermostated ultrasonic cleaning bath. To our surprise, the starting material no longer evolved to the R enantiomer in all experiments; instead, a preference for the formation of the S enantiomer was observed. Of the 12 experiments that were performed, eight led to (S)-**1**, and only four gave the expected product (R)-**1** (Table 1, entry 4).

To understand how it is possible that the order in which the components of the reaction mixture are put in the flask determines the final outcome of this symmetry-breaking process, it is necessary to look in detail at the various process steps. Seminal studies showed that additives can hamper not only the growth, but also the dissolution of crystals.[15] This finding was confirmed by Monte Carlo studies.[16] The reason that the R enantiomer emerged in the earlier experiments is that there is sufficient time during the partial dissolution of the crystals in the solvent for equal concentrations of the R and S enantiomer to be established in the liquid phase, even in the presence of minute concentrations of enantiomerically enriched impurities that hamper the dissolution of S crystals. After the addition of the racemization catalyst, the deracemization process starts. The impurities present in the starting material now enantioselectively hamper the growth of the S crystals and thus steer the transformation of all solid material into an enantiomerically pure R solid phase in all cases.

During the reversed experimental procedure, some subtle differences occur. The impurity that first hampered the growth of the S crystals will now slow down the dissolution of the S enantiomer in the initial step of the reversed procedure and thus cause the R enantiomer to dissolve faster. Owing to the racemization in the solution phase, however, the concentrations of the R and S enantiomers in the solution phase will remain equal through conversion of the excess R enantiomer. As a result, the solid phase will become slightly enantiomerically enriched in the S enantiomer. Full enantiomeric amplification is possible in the subsequent grinding process.

Unfortunately, the enantiomeric enrichment of the solid phase after dissolution is too small to be detected by HPLC on a chiral phase.[17] However, in the four cases (out of 12) that the reversed experiment still gave the R enantiomer, the system took much longer to reach 100% ee. It appears that in these experiments the dissolution process led to a relatively small enantiomeric enrichment in (S)-**1** that was still overruled by the impurity during the grinding; therefore, more time was required to reach an enantiomerically pure end state. Thus, in the reversed procedure we can see the initial

enrichment indirectly, because it competes with the effect of the impurities that hamper the growth of the (S)-**1** crystals to give (R)-**1** in the standard experiments.

To distinguish between the effects of enantioselective dissolution, dominant in the reversed process, and enantioselective growth, we needed to separate these processes. For this purpose we designed a variation of the reversed deracemization route in which the additive was only effective in the dissolution stage (Figure 2; for a detailed description, see the
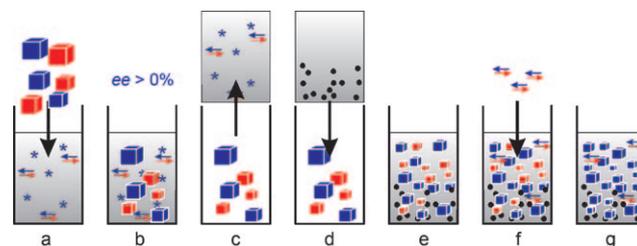

Figure 2. Schematic representation of the experimental procedure used to study the subtle interplay between enantioselective growth and dissolution. a) First, racemic (R,S)-**1** crystals are partially dissolved in a solution containing the racemization catalyst and the enantiomerically pure additive (S)-**2** to give (b). This additive enantioselectively hampers the dissolution of (S)-**1** crystals to create a small enantiomeric enrichment in this handedness. c) The solution is then removed so that the additive does not also hamper crystal growth in the subsequent deracemization step, and d) a freshly prepared saturated solution without the additive or racemization catalyst is added. e) The crystals are then ground. The large crystal surface that results makes possible trace amounts of (S)-**2** ineffective. f) Finally the racemization catalyst is added, and under grinding conditions the small enantiomeric excess in (S)-**1** is fully amplified. g) An enantiomerically pure end state with the same handedness as that of the additive results.

Supporting Information). By using intentional additives at a higher concentration, the effect of enantioselective dissolution can be amplified. We used (S)-phenylglycine (**2**) as an additive, because it had proven to be effective on (S)-**1** crystal surfaces.[2,18] We partially dissolved the two enantiomers in the presence of this additive, subsequently removed the additive, and amplified the enantiomerically enriched S solid phase by the grinding-induced deracemization process. In the dissolution step, the additive needs to be as effective as possible. Therefore, we kept the total crystal surface area relatively small by only gently stirring the reaction mixture (Figure 2a). During the amplification step involving grinding, on the other hand, the effect of trace amounts of the additive remaining after removal of the solution needed to be minimized. The effective concentration of the additive present in trace amounts is lowered by vigorous stirring, which leads to a large total crystal surface area (Figure 2e).[19] The small ee value in the solid phase remains unaffected by the grinding, and the addition of the catalyst drives the process finally to the pure S product (Table 1, entry 5). Conversely, the addition of (R)-**2** resulted in a solid containing only (R)-**1** (Table 1, entry 6).

This study demonstrates the complex interplay of multiple directing processes that are simultaneously involved during symmetry breaking. We have not only unraveled the at first

glance surprising effect of a simple reversal of the order in which the reaction-mixture components in a deracemization process are added, but have also provided a detailed protocol that enables control over the two counteracting processes, growth and dissolution, both of which are hampered by a chiral impurity or additive. Two factors are key for this control: first, the partial dissolution of the racemic conglomerate crystals can be performed under racemizing or non-racemizing conditions; second, the effectiveness of the additive is determined, and can be controlled, by the amount of crystal surface that is affected by this auxiliary.[19]

Over the last two years, a wide variety of procedural tricks, including the use of enantiomeric imbalances, circularly polarized light, shifted crystal-size distributions, or enantioselective additives, have been shown to tip a racemic mixture of conglomerate crystals toward a desired handedness.[2,5,11,12] We have presented herein the surprising observation that even the order in which reaction-mixture components are introduced can determine the final outcome of a symmetry-breaking process. We elucidated the underlying mechanism, which is based on additive-induced enantioselective crystal growth as well as dissolution. Not only do these results provide new insight into the fundamental aspects of this intriguing route to molecules of single handedness, but this modified procedure could be employed to produce enantiomerically pure pharmaceutical intermediates in larger quantities.[4,10,20,21] As the order of addition of the reaction components is such an important factor, it should be taken into account when different experiments are compared. Furthermore, it is relevant to the discussion on the homochirality found in living systems. Thus, by simply mixing solutions and solids in the correct order, it is possible to fully control the sense of chirality of the solid phase that emerges from a grinding-induced deracemization process.

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[17] We observed that even an *ee* value of 0.2% can fully overrule the additive effect and thus dictate the final outcome. However, this enantiomeric excess is too small to measure accurately by HPLC (the limit is approximately 0.5% *ee*).

[18] We confirmed that enantiomerically pure phenylglycine (**2**) can also direct the enantiomeric outcome of the deracemization starting from a clear solution in nucleation experiments with this compound (see the Supporting Information).

[19] The total crystal surface area is especially relevant in terms of the effectiveness of the additive at low concentrations, as in the current experiments.

[20] From another perspective, this technique of reverse grinding may also have a practical application during the screening of new compounds that are amenable to grinding-induced deracemization. Typically, such screening processes begin with the synthesis of a library of racemic derivatives of the target molecule that are prone to racemization.[10] To ascertain whether the derivatives that form a solid crystallize as a racemic conglomerate, second-harmonic generation can be used as a fast indicative tool.[21] Candidates from this screen are then ground under racemizing conditions. In these early studies, often the enantiomerically pure compound is not yet available to direct the symmetry breaking. However, it is often observed that the solid phase diverges spontaneously toward one handedness as a result of contamination by a chiral impurity. A simple reversal of the experimental procedure can now be used to yield the opposite handedness. In this way, elaborate and time-consuming asymmetric synthetic procedures may be avoided.

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