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Demonstration of Spontaneous Chiral Symmetry Breaking in Asymmetric Mannich and Aldol Reactions

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ABSTRACT Spontaneous symmetry breaking in reactive systems, known as a rare physical phenomenon and for the Soai autocatalytic irreversible reaction, might in principle also occur in other, more common asymmetric reactions when the chiral product is capable to promote its formation and an element of "nonlinearity" is involved in the reaction scheme. Such phenomena are long sought after in chemistry as a possible explanation for the biological homochirality of biomolecules. We have investigated homogeneous organic stereoselective Mannich and Aldol reactions, in which the product is capable to form H-bridged complexes with the prochiral educt, and found by applying NMR spectroscopy, HPLC analysis, and optical rotation measurements 0.3–50.8% of random product enantiomeric excess under essentially achiral reaction conditions. These findings imply a hitherto overlooked mechanism for spontaneous symmetry breaking and, hence, a novel approach to the problem of absolute asymmetric synthesis and could have also potential significance for the conundrum of homochirality. *Chirality 19:816–825, 2007.*

KEY WORDS: organocatalysis; spontaneous symmetry breaking; asymmetric autocatalysis; Mannich reaction; Aldol reaction; homochirality

INTRODUCTION

Most biomolecules are chiral, i.e., exist in left- and right-handed forms, the enantiomers, with L-amino acids and D-sugars dominating in the earth's biosphere. "Asymmetric Synthesis" aims at introducing elements of asymmetry (e.g. chiral centers) into a molecule with high stereospecifity, using stereoselective organic reactions to produce chiral compounds. Under achiral conditions, a racemic outcome (with 0% ee) of such reactions could be expected. To achieve high product "enantiomeric excess," with more of the desired, less of the unwanted enantiomer, one usually employs chiral catalysts or auxilaries. Proposed as a rare physical phenomenon,[1] a "spontaneous mirror symmetry breaking" due to asymmetric homogenous autocatalytic processes,[2–5] is known to occur in chemistry for an organometallic and irreversible alkylation reaction.[6,7] Such phenomena are long sought after as a possible cause for the homochirality of biomolecules,[3,8] frequently regarded as one of the prerequisites for the origin of life.[9] However, not only evolution biologists and biochemists are intrigued by the homochirality of natural amino acids and sugars—the tangible possibility of such "absolute asymmetric synthesis" in the laboratory,[10,11] i.e., the formation of optically active compounds from achiral starting materials without the intervention of chiral chemical agents or catalysts (For a conceptual overview of the topic of "absolute asymmetric synthesis," see Ref. 11),[11,12] has tantalized chemists since Frank in 1953 has shown theoretically that the combination of asymmetric nonlinear autocatalysis with mutual antagonism in the formation of enantiomers can result in amplification of enantiomeric excess and eventually, homochirality.[12] In 1995, Soai reported the amplification of chirality in the asymmetric autocatalytic irreversible reaction of pyrimidine-5-carbaldehyde with diisopropylzinc,[6] which was later adapted by Singleton to yield an enantiomeric excess of 86% in iterative runs, starting from—for all practical purposes—entirely achiral initial conditions.[7]

Whereas reversible reactions appear unsuitable for an experimental confirmation, as racemization might occur via the back reaction; it was recognized by Saito and Hyuga[13] that only when such a "reverse" reaction is taken into account, amplification of chirality to perfect homochirality is possible, because the reactant, supplied by the decomposition of the enantiomers is "recycled" to produce more of the dominant enantiomer.[13] Kondepudi and Asakura outlined the requirements for spontaneous chiral symmetry breaking under nonequilibrium conditions and that hold also for closed and reversible systems.[8] These authors employed a reaction scheme that involved product formation from the combining reaction of the autocatalytic enantiomeric intermediates. As demonstrated theoretically

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by Plasson et al.,[14] amplification of enantiomeric excess in closed, even noncatalytic systems could result in homochirality if a nonlinear reaction scheme with a "recycling pathway" is employed.[14] Herein, we provide the first experimental demonstrations of spontaneous symmetry breaking under reversible conditions in a homogenous and closed reactive system by the combination of chiral autocatalysis with educt recyclement, for a purely organic ensemble and by involvement of the chiral product alone.

MATERIALS AND METHODS

General

Solvents were purified by standard procedures and distilled before use. Reagents obtained from commercial sources were used without further purification. TLC chromatography was performed on precoated aluminium silica gel SIL G/UV_{254} plates (Marcherey, Nagel & Co.). Flash chromatography was performed using silica gel Merck 60 (particle size 0.040–0.063 mm). ^{1}H NMR spectra were recorded in $CDCl_3$ with Varian Unity 300. ESI mass spectra were recorded with a LCQ Finnigan spectrometer.

Procedure for the Synthesis of N*-PMP-Protected α-Imino Ethyl Glyoxylate 2*

A solution of *p*-anisidine (1.45 g, 11.8 mmol) in dry CH_2Cl_2 (10 ml) was slowly added to a solution of ethyl glyoxylate (1.20 g, 11.8 mmol) in dry CH_2Cl_2 (10 ml). The reaction mixture was stirred at room temperature for 1 h, and then 4-Å molecular sieves (4 g) were added to it. After stirring for an additional 2 h at 40°C the reaction mixture was filtered under Argon. The solvent from the filtrate was evaporated under reduced pressure to afford the almost pure product. Further purification by column chromatography on silica gel under Argon using dry methylene chloride as an eluent gave the imine **2** as a viscous red oil in 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.9 (s, 1H, N=*CH*), 7.3 (d, 2H), 6.9 (d, 2H), 4.4 (q, 2H, O*CH*$_2$CH$_3$), 3.7 (s, 3H, *CH*$_3$O), 1.36 (t, 3H, OCH$_2$*CH*$_3$) ppm. ESI-MS (positive ion): *m/z* = 230.0 $[M + Na]^+$, 436.8 $[2M + Na]^+$.

General Procedure for the Reaction Between N*-PMP Protected α-Imino Ethyl Glyoxylate 2 and Acetone 1*

A solution of *N*-PMP-protected α-imino ethyl glyoxylate **2** (40.19 mg, 0.194 mmol, 1 equiv) in acetone (0.78 ml) was stirred at room temperature for 2–8 days. (Acetone of analytical grade from different suppliers (Acros and Merck) was used). The solvent was evaporated and the residue was purified by chromatography on SiO_2-column under Argon (CH_2Cl_2/EtOAc/Hexane, 1:1:1) to afford the desired product **3** (Table 1). Enantioselectivities were determined by chiral HPLC analysis (Daicel Chiralpak OD, *i*-Propanol/Hexane = 5:95, flow rate 1.0 ml/min, λ = 254 nm) in comparison with authentic racemic material. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.71 (d, 2H), 6.61 (d, 2H), 4.29 (m, 1H), 4.13 (q, 2H, O*CH*$_2$CH$_3$), 3.68 (s, 3H, *CH*$_3$O), 2.91 (d, 2H), 2.12 (s, 3H, CO—*CH*$_3$), 1.20 (t, 3H, OCH$_2$*CH*$_3$) ppm. ESI-MS (positive ion): *m/z* = 288.1 $[M + Na]^+$.

TABLE 1. Yields and enantioselectivities for the Mannich reaction from eq. I with and without added product 3

Entry	*T* (°C)	Solvent	Reaction time (days)	Yield (%)	ee (%)	ee (%)[a]
1[b]	25	Acetone	2	20	9.0 (*S*)	–
2[b]	25	Acetone	4	35	0.5 (*S*)	–
3[b]	25	Acetone	6	35	9.5 (*S*)	–
4[b]	25	Acetone	8	36	2.6 (*S*)	–
5[b]	25	Acetone	4	34	6.8 (*S*)	–
6[b]	25	Acetone	4	33	7.4 (*S*)	–
7[b]	25	Acetone	4	32	2.6 (*S*)	–
8[b]	0	Acetone	4	11	4.4 (*S*)	–
9[c]	0	Acetone	4	13	4.5 (*R*)	–
10[c]	40	Acetone	4	17	2.5 (*S*)	–
11[d]	40	Acetone	4	6	5.2 (*R*)	–
12[d]	40	Acetone	4	15	5.6 (*R*)	–
13[c]	40	DMSO	4	17	0.3 (*S*)	–
14[b]	25	DMSO	19	16	2.4 (*S*)	–
15[b]	0	Toluene	10	15	0.3 (*R*)	–
16[b,e]	25	Acetone	2	14	5.0 (*R*)	–
17[b,e]	25	Acetone	4	17	3.9 (*R*)	–
18[b,e]	0	Acetone	2	12	2.0 (*S*)	–
19[b,e]	−10	Acetone	2	8	3.5 (*S*)	–
20[c,e]	25	DMSO	4	16	2.5 (*R*)	–
21[b]	25	Acetone	2	11	1.5 (*R*)	2.4 (*R*)
22[b]	25	Acetone	2	18	9.1 (*S*)	9.1 (*S*)
23[b]	25	Acetone	4	31	9.4 (*S*)	8.2 (*S*)
24[b]	25	Acetone	4	13	2.1 (*R*)	2.2 (*R*)
25[b]	25	Acetone	6	32	3.8 (*S*)	3.9 (*S*)
26[b]	25	Acetone	8	33	2.0 (*S*)	2.4 (*S*)
27[b]	0	Acetone	4	8	4.6 (*S*)	4.8 (*S*)

[a]Values given show enantioselectivities determined by ^{1}H-NMR in presence of Eu(tfc)$_3$.
[b]Educt concentration 0.25 mol l^{-1}.
[c]Educt concentration 0.50 mol l^{-1}.
[d]Educt concentration 50 mol l^{-1}.
[e]Initially added 15 mol % of racemic product 3. Yields after deduction of initially added product.

General Procedure for the Reaction Between p*-Nitrobenzaldehyde 2 and Acetone 1*

To a stirred solution of *p*-nitrobenzaldehyde **2** (30.24 mg, 0.2 mmol) in acetone (147 μl), DMSO (8 μl) was added and the mixture was stirred at room temperature for 10 days. (Acetone of analytical grade from different suppliers (Acros, Merck and Roth) was used). The reaction mixture was diluted with CH_2Cl_2 to facilitate its handling in the purification of the product by preparative TLC (EtOAc/Hexane, 1:1). Enantioselectivities were determined by chiral HPLC analysis (Daicel Chiralpak AS, *i*-Propanol/Hexane = 25:75, flow rate 1.0 ml/min, λ = 254 nm) in comparison with authentic racemic material. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.2 (d, 2H), 7.53 (d, 2H), 5.24 (m, 1H), 3.58 (br s, 1H), 2.84 (m, 2H), 2.20 (s, 3H).

RESULTS AND DISCUSSION

Recently, we have reported[15] the efficient automultiplication of the asymmetric product in the (reversible) and organocatalytic Mannich[16] and Aldol[17] reactions shown in

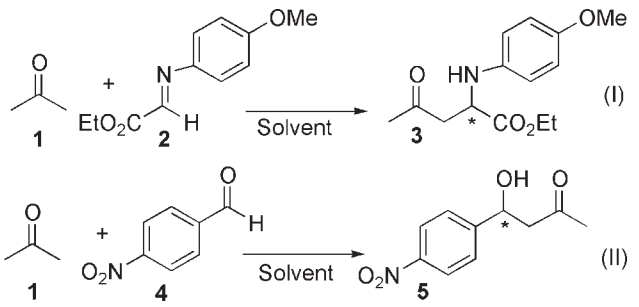

Fig. 1. Investigated Mannich (I) and Aldol (II) reactions.

Figure 1 (eq. I and II, respectively). Both reactions share the capability of the respective product to form complexes via hydrogen bonding with the prochiral substrate. Chemical intuition would have led us to anticipate a racemic outcome in the Mannich reaction of **1** and **2** (Fig. 1) under achiral conditions without addition of external chiral auxiliaries (Table 1, entries 1–15, 21–27).

But surprisingly, we observed significant product enantiomeric excesses ranging from 0.3 (entries 13 and 15) to 9.5% ee (entry 3). Varying solvent, reaction time, reaction temperature, and substrate concentration (Table 1, entry 1–15, 21–27), gave the Mannich product in moderate yields of up to 36% after 8 days reaction time (Table 1, entry 4). Enantiomeric excesses in our first experiments were determined solely by chiral HPLC (Table 1, 1–20). Seeking further confirmation of those findings, we have additionally recorded ^{1}H-NMR spectra for some of our product samples (Table 1, 21–27), employing a chiral Eu(tfc)$_3$ complex as lanthanide shift reagents to allow differentiation between the two product enantiomers. In general, the accordance of the results obtained by the two differing analytical approaches was good and corroborated also our earlier findings (Table 1, entries 1–20) on such spontaneous symmetry breaking in this system. In the minority of cases (9 vs. 18 runs, Table 1), *R* product was formed in excess, albeit sometimes with much lower product yields than in the experiments where *S* product resulted (cf. entries 1 vs. 21, 5 and 6 vs. 24, 10 vs. 11; Table 1).

The reaction in less polar toluene turned out to be slow and gave a "disappointing" enantiomeric excess of only 0.3% (Table 1, entry 15). The dependence of enantiomeric excesses on substrate concentration appeared to be more pronounced than on the change of solvent from acetone to DMSO (cf. entries 10 vs. 11 and 12, 10 vs. 13, respectively). After 4 or more days reaction time in acetone at room temperature, and with the exception of a single result (entry 24), yields remained nearly constant and well above 30% (entries 2–7, 23, 25 and 26), while yields after 2 days had been significantly lower (entries 1, 21 and 22). Higher educt concentrations at 40°C resulted in product ee of 5.2 and 5.6% (Table 1, entries 11 and 12), respectively, but higher ee values of 9.1 and 9.4% ee were obtained in acetone at ambient temperatures and for much lower educt concentrations (Table 1, entries 22 and 23). As a whole, the effect of reaction temperature is not conclusive: both elevated and lowered temperatures resulted in lower yields (Table 1, cf. entries 10–13 vs. 8, 9, 15, 18, 19, 27), however, no resulting ee values higher than 4.6% ee (Table 1, entry 27) was observed for lowered temperatures.

TABLE 2. Yields and enantioselectivities for the Aldol reaction from eq. II carried out at 25°C and stopped after 10 days

Entry	Educt concentration (mol l^{-1})	DMSO Additive (vol %)	Yield (%)	ee (%)	ee (%)[a]
1[b]	0.50	0	1	2.1 (*R*)	–
2[c]	0.50	0	none	–	–
3	3.86	15.4	10	3.6 (*S*)	–
4	4.18	8.4	3	3.9 (*S*)	–
5	5.20	20.8	10	5.1 (*S*)	–
6	5.80	11.6	3	4.1 (*S*)	3.1 (*S*)
7[b]	0.50	0	1.4	2.7 (*S*)	–
8	3.84	15.3	11	3.4 (*S*)	–
9	4.16	8.3	5	15.4 (*S*)	–
10[b]	0.50	0	3	9.4 (*S*)	–
11[b]	0.50	0	1	18.5 (*S*)	–
12	1.28	5.1	10	50.8 (*S*)	65.2 (*S*)
13	1.32	2.1	4	37.8 (*S*)	42.8 (*S*)
14[d]	1.28	5.2	2.5	26.9 (*S*)	–
15	1.36	2.6	0.5	9.4 (*S*)	–
16	0.67	1.3	1.0	13.1 (*S*)	–
17	0.66	2.6	0.6	7.2 (*S*)	–
18	1.28	5.1	5.7	8.7 (*R*)	8.3 (*R*)

[a]Values given show enantioselectivities determined by ^{1}H-NMR in presence of Eu(hfc)$_3$.
[b]A slight turbidity observed could be attributed to educt precipitation.
[c]Toluene as solvent. All other runs employed acetone as solvent.
[d]Optical rotation of product $[\alpha]_D = -31.7°$ ($c = 0.06$, $CHCl_3$).

Some experiments had been carried out with initially present racemic product in 15 mol % concentrations (Table 1, entries 16–20). Product yields were much lower when the racemic product was added at the beginning of the reaction in acetone (entries 16–19). No such different behavior could be found for DMSO as solvent (entry 20). The enantiomeric excesses we observed here ranged from 2.5 to 5.0% ee (entry 20 and 16, respectively), i.e. they were not lower than in most of the experiments without initially added product. It should be noted that the (absolute) maximum error in the HPLC ee% measurements we reported here is found to be of the order 0.1% ee (see supporting information). Calculating the resulting ee values for only the newly formed product, i.e., by deducting the influence of the initially added racemic mixture, revealed to our surprise that some part of the racemic mixture must have deracemized in course of the reaction (Table 1, entry 16–20). This effect probably implies the importance of reversibility for all the results observed, including those without initially added product. In principle, the product added at the beginning might have driven the equilibrium towards the reactant side, or, alternatively, some sort of product inhibition might be in effect.

More stunning results than for the Mannich reaction (eq. I) were obtained for the Aldol reaction of **1** and **4** in Acetone with an additional 0–20.8 vol % of DMSO additive (Fig. 1, II and Table 2). In pure acetone, the prochiral

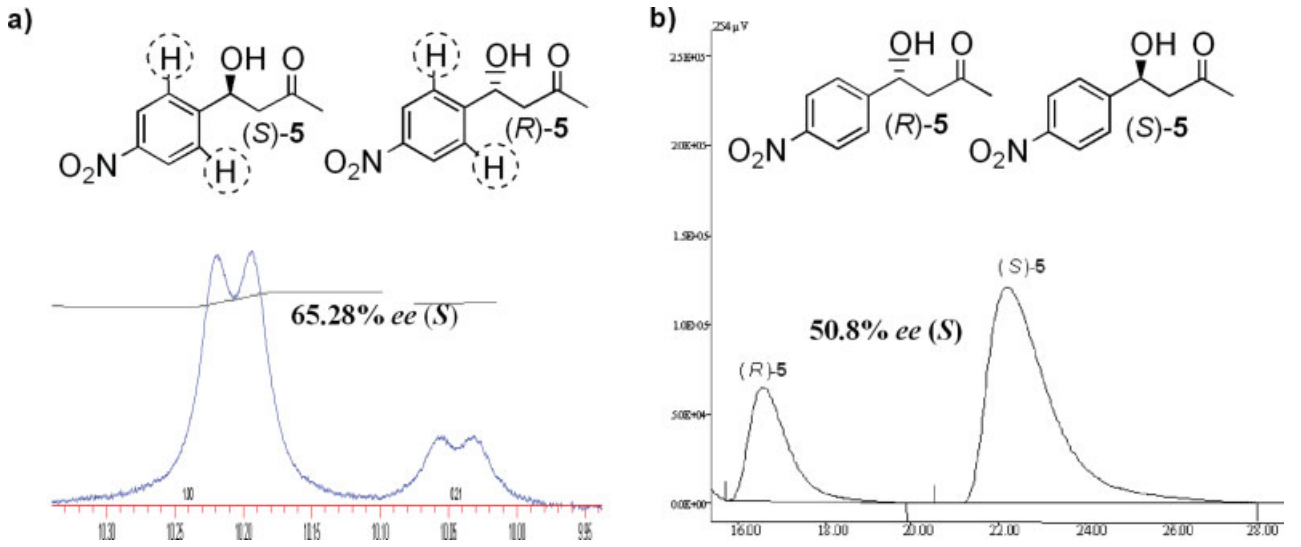

Fig. 2. Measurement of product enantiomeric excess in the Aldol reaction II, run under achiral conditions. (**a**) ^{1}H NMR signals of aromatic protons of (*R*)-**5** (10.05 ppm) and (*S*)-**5** (10.20 ppm) enantiomers at 300 MHz in presence of Eu(hfc)$_3$ complex from a sample of isolated aldol product **5**, depicting the prevalence of the *S*-enantiomer. (**b**) HPLC chromatogram of the same batch of isolated product. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

educt *p*-nitrobenzaldehyde is not fully soluble at the concentration levels employed (entries 1, 7, 10, and 11, Table 2). In the best run, enantiomeric excess values of up to 50.8% (Table 2, entry 12) were achieved when stopping the homogenous reaction after 10 days in acetone and with 5.1 vol % of DMSO additive, starting from entirely achiral conditions. An additional NMR measurement (with 65.2% ee from signal integration, Fig. 2) confirmed the result.

A similar experiment with lower additive amount (about 2.1 vol %, entry 13) gave 37.8% ee, which at least corroborated the remarkable generation and amplification of chirality in a previously symmetric environment. No reaction was observed in toluene (entry 2). Yields on the whole were very low and ranged from 0.5 to 11% ee (Table 2). The accordance of NMR data with the HPLC/UV determination of ee values is usually very good (see e.g. entries 6, 12, 13, and 18, Table 2; entries 21–27, Table 1). In only a small number of experiments (entry 1 and 18, Table 2), an *R* absolute configuration of Aldol product was determined. The reaction was observed to be homogenous (with the possible exception of the runs reported in entries 1, 7, 10, and 11, Table 2, where the observed suspension was most probably due to undissolved educt, though). As the Aldol reactions' educt *p*-nitrobenzaldehyde crystallizes in the chiral space group $P2_1$, we cannot preclude the possibility that enantiomorphous crystallization as a heterogeneous process might have been involved in those experiments where educt precipitation could have occurred. Besides the unpredictable product absolute configuration, we were also unable yet to predict the absolute value of enantiomeric excess in our experiments—the distribution of ee values in the runs (Table 1 and 2) appears to be random (Fig. 3). Attempts to find the factors that govern the size of the generated ee values were, at least until the present, unsuccessful. It should be noted that for both the Mannich and Aldol reactions investigated here, a non-negligible limiting factor for the achievable enantiomeric excess is the extent of the (unavoidable) uncatalyzed "background reaction" that gives racemic product because the chiral product is not involved in it.[15]

Could the work-up process itself have an influence on the measured ee values? In a recent important article, Soloshonok described the "disconcerting" effects of the self-disproportionation of enantiomers in nonracemic samples by achiral-phase chromatography when homo- or heterochiral dimers could be formed by the asymmetric species.[18] To separate the product of interest from by-products which could affect the HPLC measurements through peak overlaps, we purified our samples repeatedly on achiral silica gel (see supporting information). The self-disproportionation effect is due to different physical adsorption properties of monomeric and dimeric or even higher aggregated species. With strongly polar eluents, the effect is mostly eliminated.[18] As we propose the existence and involvement of the (not directly observed) homo- and heterochiral product dimers in this article (*vide infra*), we wanted to make sure that the ee values we measured result from the processes in the reaction bulb, rather than being artefacts of the purification process. Hence, we used "racemic" material, obtained by running reaction II with racemic L,D-proline as external catalyst and investigated the influence of the purification process (as described in the supporting information) on the measured ee-values (see supporting information for a graphical display of results). The results show that small measured product enantiomeric excess values (<2%) are not very reliable for those product species considered here. While there is no clear trend with the number of purification cycles, the concentration of the prepared solutions appears to play a role

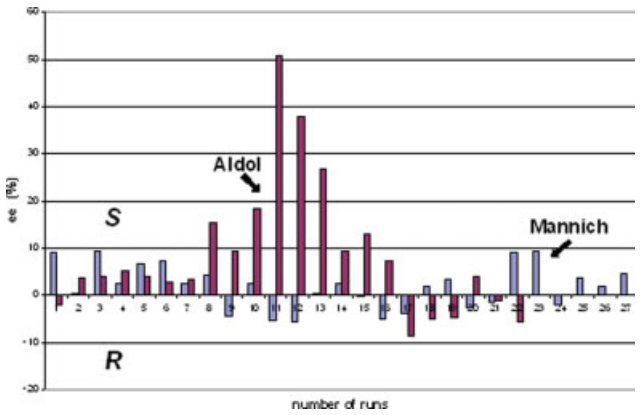

Fig. 3. Graphical display of the results in Tables 1 and 2. The vertical axis shows the measured random product enantiomeric excess values (positive values = excess of *S*-product; negative values = excess of *R*-product), the horizontal axis gives the number of experiments (mostly under varied conditions), for Mannich and Aldol reactions, respectively. This diagram also includes the results of five experiments (run 13–17) which are not listed in the Table 2 and for which only ee values have been determined. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(see supporting information). Furthermore, we asked ourselves how reliable those measured values are, that are somewhat larger (around 5–10%). Hence, we "prepared" a scalemic mixture of 6.2% ee by mixing *S*- and *R*-product enantiomers which were separately prepared from the reaction with L- and D-proline, respectively, and subjected the 0.5 mg/ml solution (see supporting information) of this mixture to repeated cycles of our purification process on silica gel. The result is somewhat encouraging: the "enantiomer self-disproportionation" effect, if present at all, should be rather small and cannot explain why we sometimes observe ee-values that are significantly higher than 2%.

While the existence of a symmetry breaking kinetic mechanism for reactions I and II that is capable to amplify tiny imbalances in the enantiomer concentration, seems now apparent, the exact outcome in terms of product absolute concentration (i.e. *R* or *S*) remains puzzling. If, e.g., thermal fluctuations were the prime cause of those tiny chiral imbalances, a statistical outcome with equal probability for *R*- or *S*-product is anticipated. However, whereas the results were more or less balanced for the Mannich reaction, with a pronounced preference for the *S*-configured products (Table 1), *S*-product resulted in the overwhelming majority of the experiments for the Aldol reaction (Table 2). This might indicate the presence of a systematic cause, e.g., due to a miniscule contamination with chiral substances in our precursors (originating, e.g., from bacteria or dust) and which eluded our analytics, but it could also be the consequence of the random ee generation itself. Our results seem to resemble those of Asakura, who noticed in 1994 the spontaneous symmetry breaking with random generation of enantiomeric excess of up to 30% in the synthesis of an octahedral chiral cobalt complex from supersaturated solutions.[19] Therefore, no bimodal product ee generation, as in the Soai reaction,[20,21] was observed.[19] Hence, a stochastic product ee distribution (with equal probability for *R* and *S* product configuration), frequently regarded as a criterion for "absolute asymmetric synthesis" by chiral autocatalytic processes,[11] might be difficult to demonstrate or not even possible, even in the absence of—hypothetical—trace impurities. Despite such similarities though, is Asakura's system heterogenous, involving, as proposed, crystallization of the product.[19]

A recently published mechanistic proposal (Fig. 4b),[15] supported by quantum-chemical calculations, for the asymmetric weak autocatalysis observed for reaction I, might help to understand on the molecular level the sometimes dramatic amplification of chirality from unobservable per-

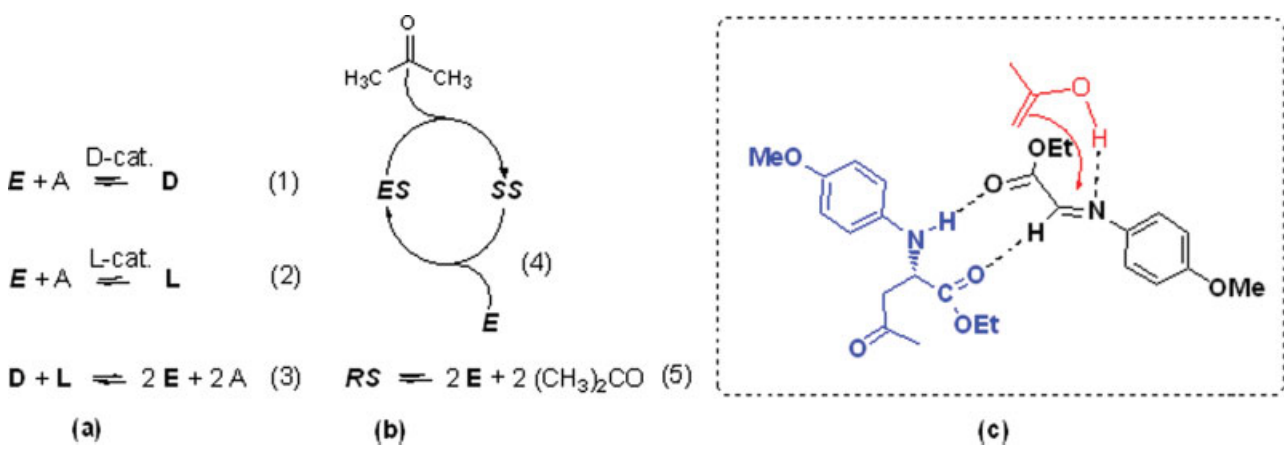

Fig. 4. Juxtaposition of a mechanism, derived from Frank's,[12] with explicit educt recyclement and chiral autocatalytic steps, and the proposed mechanism for reaction I, involving dimer equilibria. (**a**) Schematic representation of the Frank-type mechanism. Equations 1 and 2 depict the formation of the L- and D-enantiomers from a prochiral educt E by attack of a reagent A, catalysed by the respective product. Equation 3 stands for a "lethal interaction" of enantiomers, here to recycle the substrate E. (**b**) Proposed catalytic cycle, (4), for the asymmetric autocatalysis in reaction I (Fig. 1), based on quantum-chemical calculations.[15] Equation 5 shows a hypothetical "back reaction" to explain the experimentally observed educt formation from a solution of racemic mixtures of products 3 and 5 (see text). (**c**) Depiction of the educt-product complex ES from equation (4), based on mass spectrometric data (m/z = 495.0 $[M_{educt} + M_{product} + Na]^+$) and DFT calculations.[15][Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

turbations of yet unknown origin to macroscopically observable optical activity (see Table 2, entry 14) and enantiomeric excess, reported here (Tables 1 and 2): asymmetric product molecules alone are involved in the reaction step that leads to product formation by forming aggregates with the prochiral substrate (Fig. 4c).[15] The product facilitates, thereby, the nucleophilic addition of the ketones' enol form onto the substrate **2** (and probably **4**), by diminishing the activation barrier for this reaction.[15] This contrasts to the situation for the Soai reaction, where the autocatalytic species is most likely a dimeric Zn-alkoxide of the product, not the product alone.[22,23] This is important for two reasons: first, the Soai reaction, being irreversible, requires a nonlinear chiral autocatalytic step to give asymmetric amplification, while the reversible reactions, discussed here, need the asymmetric "catalysis" only to compete favorably with the background reaction—which produces a racemate—and, secondly, to transfer the chirality information selectively from the product to the transition state for the product formation (i.e. "chiral recognition"). The most likely transition state structure for the rate determining step—which also coincides here with the step in which the chiral center is formed—is also reported in our earlier work on the Mannich reaction (eq. I, Fig. 1).[15] Herein does the assistance of an *S*-product molecule slightly and selectively favour the formation of more *S*-product with respect to the formation of an *R*-product molecule. At the same time, the heterochiral adducts of product molecules might react back to the educt under the initially near-racemic conditions in a nonlinear reaction step (as depicted in Fig. 4b, reaction **5**). It should be noted that this is not "reversibility," i.e., the process that is obtained by inverting the movements of the atoms involved in the forward reaction step. The enantiomeric excess obviously increases, when equimolar amounts of the antipodes devolve into the achiral starting compound, because the relative weight of the majority enantiomer grows (see supporting information). Such a process will, therefore, inevitably and with continuously increasing efficiency favour the majority enantiomer, because its molecules, that now outnumber those of its antipode, can assist in the further production of even more of its kind by the aforementioned stereoselective mechanism.[12] That such heterochiral complexes of **3** could form, was shown by density functional calculations.[15] By contrast, educt recyclement originating from homochiral product dimers could not result in asymmetric amplification, because the ratio of *R*- versus *S*-product will not change.

Such a szenario could result in a bistable situation, very similar to that in the Viedma experiment,[24–26] where a racemic mixture of $NaClO_3$ crystals in contact with its saturated solution gradually transforms into an enantiopure assembly. The necessary far-from equilibrium state is preserved as long as the steady growth of the crystals continues (comparable to the formation of product in a reaction mixture with nonequilibrium concentrations), while nonlinearity is introduced here by a continuous abrasion-grinding process, spawning microcrystals of the same chirality as those of the crushed "parent crystal" in conjunction with—as proposed—the process of second nucleation,[24] while the dissolution of the crystallites to achiral ions could be identified with a reverse reaction in a reactive system like I or II (Fig. 1).

Such asymmetric amplification is an inherently nonlinear process, because it requires that the initial chiral symmetry is broken. In principle, nonlinearity of reaction kinetics might be involved in either or both the forward and back reactions, as in reactions (4) and (5), Figure 4b, which could result in exponential growth of initial small differences in enantiomer concentrations.[12,22,24,26] Similar to a possible representation of Frank's mechanism (Fig. 4a), the necessary nonlinearity should stem in our system from the proposed educt recycling reaction step 5, Figure 4b. A merely linear autocatalysis alone, that is implied by the involvement of solely the monomeric product in the formation reaction (as suggested here), is not able to allow the amplification of enantiomeric excess.[8] And we have no reason to assume that a nonlinear forward reaction step takes place, as this would require the involvement of dimeric product or even higher aggregated species in the rate-determining step[8] and we have found no indication for that (e.g. observation of a rate acceleration). Moreover, following the principle of parsimony, the introduction of nonlinearity through educt recyclement from the combining reaction of the enantiomers appeared to us the more straightforward way to do so.

It should be noted, however, that there is a marked difference between our system and the Frank model, though. In Frank's rate equations it is implied that the concentration of the educt is constant (because it is absorbed in the rate constant), as if it is present in excess or is continuously flowed into the system, while in our system it is finite and decreasing, so that a fully chiral "steady state" as in Frank's mathematical model is an idealization that we cannot realistically hope to obtain in our demonstration. In addition, Frank did not explicitly state nor required in his theoretical derivations that the inhibiting nonlinear reaction of the antipodes ("L + D") led back to the starting compound.[12] But the product of this reaction step could in principle be any achiral compound, including the educt itself. Moreover, Frank implicitly assumed that the reaction that produces the enantiomers is 100% stereospecific (i.e. the formation rate of, e.g. "L" only depends on the concentration of "L" and not on the concentration of "D"). Despite these differences remain the basic conclusions the same.

The initial presence of a significant amount of enantiomerically enriched product, as described in our earlier work,[15] somehow appears to "swamp" the effect of the spontaneous symmetry breaking, resulting in enantiomeric product excesses which are in accord with classical catalysis (i.e. with product ee value equal or smaller than the catalyst ee value).[15] We surmise that symmetry breaking effects in a closed system, i.e. under reversible conditions, require a certain realm of initial reactant concentrations (and of relative rate constants). Kondepudi and Asakura provide a theoretical corroboration for that assumption: "spontaneous symmetry breaking" in reactive systems requires that a reaction-specific order parameter is greater than a certain critical value.[8] In their model,

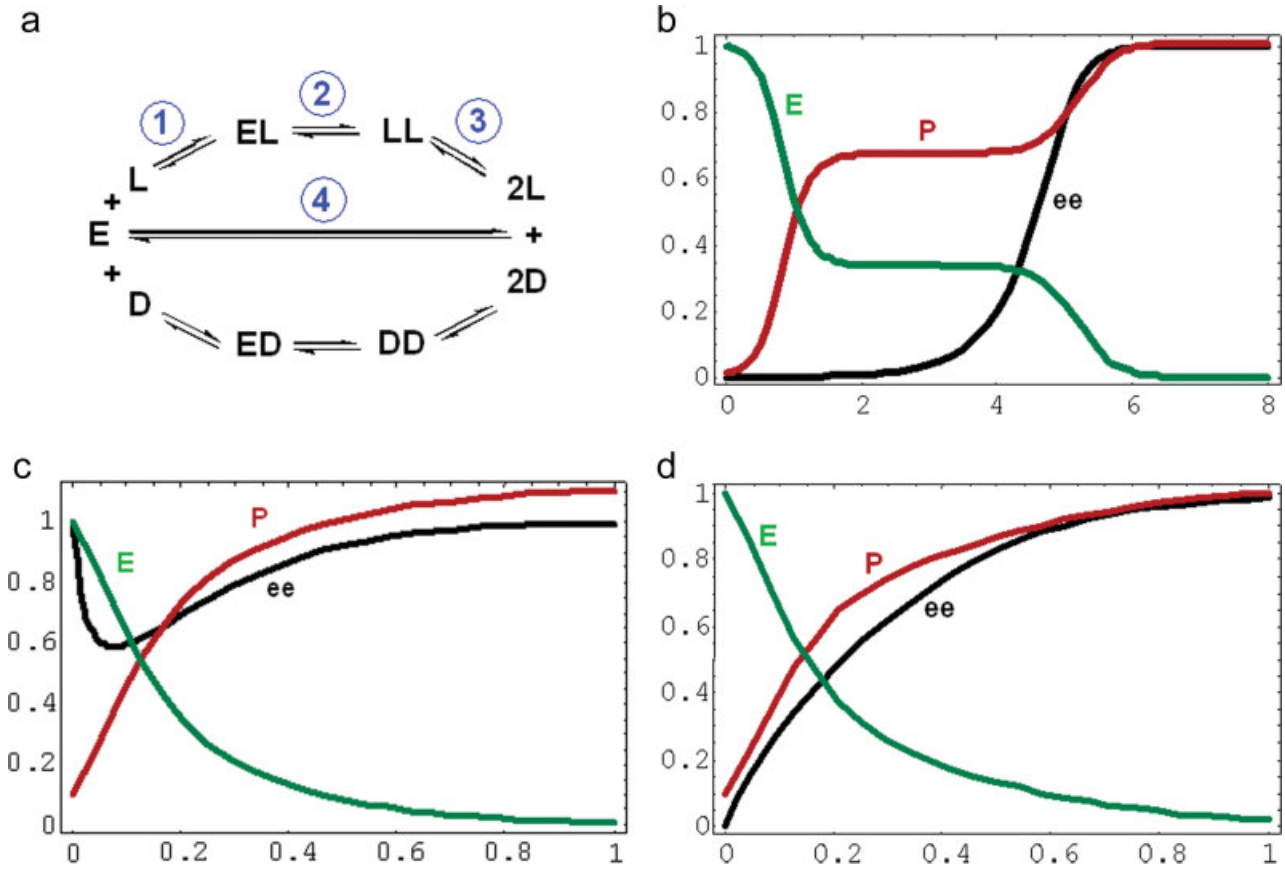

Fig. 5. Numerical solution of a set of coupled differential equations that describe a mechanistic model as depicted in Figure 4b. (**a**) The reaction network that corresponds to the differential equations (given in the supporting information, together with the input parameters). The numbers in circles refer to the four reaction steps. 1: formation of the educt-product pre-complex EL; 2: main branch of the stereoselective reaction step, giving preferentially a homochiral product dimer LL; 3: dissociation of the homochiral product dimer adduct; 4: combining reaction of the antipodes L and D to recycle the educt E. (**b–d**) Educt concentration E, ee value and total yield P = L + D (all normalized to 1) are plotted against an arbitrary time evolution parameter on the horizontal axis. The homochiral state corresponds to ee = 1. (b) achiral initial conditions, no initially added chiral product. (c) chiral initial conditions, almost enantiopure product is present initially. (d) achiral initial conditions, a racemic product mixture is present initially. See text for discussion. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

leading to a bimodal ee value distribution (where the absolute ee value solely depends on the value of the order parameter with the final absolute configuration of the product being principally unpredictable), this parameter was the product of the reactant concentrations.

A cursory mathematical analysis, based on the numerical solution of the set of coupled ordinary differential (rate) equations (see supporting information) and which corresponds to a reaction network (Fig. 5a) that describes a realistic mechanistic model like that depicted in Figure 4b, not only revealed that—at least mathematically (i.e. from a purely kinetic point of view)—100% product ee would, in principle, be achievable on the basis of such a reaction scheme (starting from a tiny chiral imbalance, Fig. 5b), but also confirmed that, as observed, initially present enantiopure product "dampens" the generation of enantiomeric excess (Fig. 5c),[15] while racemic product doesnot (Fig. 5d). The corresponding schematic reaction network with an educt recycling step and on which the differential equations are modeled, is depicted in Figure 5a. We made the simplifying assumption here that free substrate is formed directly from the combining reaction of "L" and "D" product monomers. The plots Figures 5b–5d display a pronouncedly different solution behavior of the differential equations, depending on the initial conditions (see supporting information): when chiral product is absent at the beginning of the reaction, ee increase is slow and follows an S-shaped curve (Fig. 5b). The available experimental data does not allow to verify clearly the occurrence of a "plateau phase" with respect to educt (and product) concentrations, predicted by the mathematical model. While the model itself appears to suggest that we should have just let the reactions run longer, this suggestion is not easy to realize experimentally. Under "real" chemical conditions and particularly in a closed system, many factors might result in the impairment (or even termination) of asymmetric amplification. Among these are thermodynamic restrictions (approach of equilibrium), but also the influence of side reactions, or the instability of educt and reaction products. In our case suffers e.g reaction I from the hydrolysis of the educt **1** to *p*-methoxy-aniline and reaction II is affected by the aldol condensation of the product, resulting in an achiral compound.

It appears also clear that the stereospecifity of the catalyst (i.e. the ratio of *S*-product vs. *R*-product formed under

influence of e.g. enantiopure *S*-product catalyst) should limit the maximum achievable enantiomeric excess. In contrast, an enantioselective crystallization process starting from incipient crystals as in the Viedma experiment, or many enzymatic reactions, is naturally 100% stereospecific. The plots in Figures 5c and 5d show that the initial presence of about 10 mol % of product, depending on its enantiomeric purity, is crucial in the time evolution of both yields and ee values. Not only is a plateau phase (cf. Fig. 5b) in the plotted concentrations absent, the initial presence of nearly enantiopure (99% ee) product results in a "dip" in the ee curve. The reaction, e.g., stopped (or equilibrium reached) before the ee value can "recover" and begin to increase, nonlinear effects are not at once apparent.[15] Conversely, when the initial added product is a racemate (Fig. 5d), ee increases continuously together with the product yield and without a change in the curvature, in contrast to the situation without added product (cf. Fig. 5b).

While the asymmetric induction itself and its efficiency obviously depends on the chiral products' involvement in the reaction step in which the chiral center is formed, this reaction step does in principle not need to coincide with the rate determining step, which would result in linear or nonlinear autocatalysis. Hence, one might wonder, whether the rate-accelerating autocatalysis, as in the Soai reaction,[22] is a prerequisite at all for the occurrence of spontaneous symmetry breaking in reactive homogenous systems, or just a sufficient criterion. The results of Plasson et al. are also stimulating in this context: Their theoretical model, which allowed for the spontaneous evolvement of homochiral states in closed reversible systems, did not require rate accelerating autocatalysis, but rather the combination of a nonlinear reaction network with stereoselective polymerisation and epimerization steps and educt recyclement.[14] The Plasson model differs from ours in an important aspect, though: their closed system model, which, so to speak, "embeds" an open-system representation of the Frank model, allows to reach homochirality, because the necessary nonequilibrium state is preserved by continuous energy intake to recycle the educts.[14]

A cautionary note might be in order in the light of our previous work on the product catalysis in the same system,[15] it cannot be precluded that our laboratory is already "contaminated" with trace impurities, e.g. in dust, containing enantiomerically enriched product. Such "hidden" impurities might also be the probable cause for the deviation from a probabilistic expectation (1:1 for *R*:*S*) for the product configuration in the experiments of Singleton and Vo in 2001.[7] This is because in 2003, Soai et al.[20] and Singleton and Vo[21] independently demonstrated for the Soai reaction that also a stochastic outcome with equal probability for *R* and *S* product is possible. On the basis of these results came Mislow to the conclusion that "it is all but certain that the Soai reaction is capable of producing optically active compounds by an absolute asymmetric synthesis, starting from nominally achiral reagents free of chiral contaminants and run under achiral conditions."[11] However, while there might be some theoretical intrigue involved here, we see only limited practical value in a discussion on how "absolute" an "absolute asymmetric synthesis" has to be, to qualify as such. In our opinion, and for all practical purposes, it does not really matter whether macroscopic chirality in the laboratory evolved from a chiral bias caused by the adventitious presence of trace impurities or from the unavoidable bias because of the likely inequality in the number of enantiomer molecules in a racemic mixture. As a rough estimate, and on statistical grounds alone, a racemic ensemble of $\boldsymbol{n}$ chiral molecules contains a deviation of the order $\boldsymbol{n^{1/2}}$ from exact equality in the number of the antipode molecules. It might not be impossible though that a similarly stochastic outcome as was shown for the Soai reaction, could, in principle, be demonstrated also for reactions I and II (Fig. 1), when carried out many times and under exactly identical conditions.

Theoretical explanations for the biological homochirality focus on two main issues: the origin of the chirality bias in the presumably prochiral prebiotic environment in the first place, and, secondly, its amplification to enantiomeric purity.[2–4] As the amplifying effect reported herein should be a purely kinetic one, the reaction system should drive towards a racemic mixture given enough time and when the initial conditions had been achiral (at least for a reversible reaction in a closed system that is allowed to reach chemical equilibrium). A different result would violate the second law of thermodynamics, or its chemical incarnation, the "principle of microscopic reversibility." This is because there is always a positive "entropy of mixing" of the enantiomers, with a maximum for the racemic mixture (See for a modern treatment of the matter, Ref. 27).[27] Hence, asymmetric amplification under reversible conditions is only possible and will only proceed as long as the system is far from equilibrium.[5,28] The necessary erosion of enantiomeric purity when a reversible, closed reaction system approaches its equilibrium state, though, might be overcome under natural conditions as the earths ecosystem might have greater resemblance to a chemical flow reactor run under nonequilibrium conditions rather than to a closed system.[29]

Clearly, the Soai reaction could serve as a "proof of concept" for the possible role of nonlinear kinetics in engendering biological homochirality. Deriving from Kagan's nonlinear ML_2 model for catalyst aggregation,[30] modified to account for autocatalysis, Blackmond and Brown explained the remarkable findings for the Soai reaction by assuming that both homo- and heterochiral product, i.e. catalyst, dimers form and that only the homochiral dimers are catalytically active.[22] This involves a quadratic dependence of the (forward) reaction rate on the product concentration (the experimental kinetic data for the Soai reaction can be explained assuming either a quadratic autocatalysis or a linear autocatalysis together with dimer equilibria see Ref. 31),[8,22,31] in contradistinction to the linear dependence in Frank's and in our model. Indeed, Frank considered originally a continuous, noniterative process that derives nonlinearity from an additional "lethal interaction" of enantiomers whenever they encounter each other.[12] Hence, Frank's schematic mechanism (Fig. 4a) could principally be satisfied when the enantiomers act either as mutual

anticatalysts (i.e. inhibitors) for the formation of their respective antipodes, or, alternatively, when the *R* and *S* products react to recycle the prochiral substrate while *R* or *S* product molecules alone revert more slowly back to the reactants, resulting thereby in accumulation of the majority enantiomer. In accordance with that mechanism, we have found evidence (through mass spectra, HPLC and ^{1}H-NMR) for educt formation in a solution of racemic product **3** or **5** in acetone, kept at room temperature for 6 and 10 days, respectively. However, also a solution of a nearly enantiopure (98.0% ee) Mannich product slowly reverted to the educt, which could be due to the presence of the minority enantiomer though. No significant enantiomerization was observed in these experiments.

In conclusion, we have demonstrated for the first time that spontaneous random generation of chirality is possible in common asymmetric and fully organic reactions—the Aldol and Mannich reactions, even if they are run under essentially achiral conditions. The product ee values themselves have been verified by applying different analytical techniques. The mechanistic evidence we provided, fits the expectations that could be held in the light of the recent progress in theoretical understanding of such processes. Our results could indicate a potentially novel approach to spontaneous asymmetric synthesis in a purely organic system and under reversible conditions. The underlying, proposed mechanistic idea should be rather general—because only hydrogen bonding is involved—and could, in principle, apply to a wide range of organic reactions and substrates, and might also be of relevance for the origin of the homochirality of biomolecules: the Aldol reaction is involved in important biochemical processes like glycolysis,[32] and was shown to take place nonenzymatically even in pure water (keeping in mind the probably aqueous conditions on early earth),[33] while the Mannich reaction could be employed in the synthesis of amino acids—probably among the first molecules of biological relevance in the pre-biotic stage[34]—and their derivatives.[35]

While theoretically well founded, the spontaneous symmetry breaking we observed when we started from achiral or racemic initial conditions challenges the still well-entrenched belief that macroscopic asymmetry could not result from achiral starting conditions, at least not in a reversible or in a closed homogenous reactive system. Granted, the mechanistic details for the generation and amplification of chirality from achiral starting conditions in the Mannich and Aldol reactions we investigated are not yet sufficiently known to reach a full understanding of all the processes involved. This should justify further studies, both experimental and theoretical. The reproduction of our findings by other researchers should not be too difficult. Investigations, in which the scope and the characteristics of this phenomenon are further explored, are presently carried out in our laboratory.

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