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Isotopically-Directed Symmetry Breaking and Enantioenrichment in Attrition-Enhanced Deracemization

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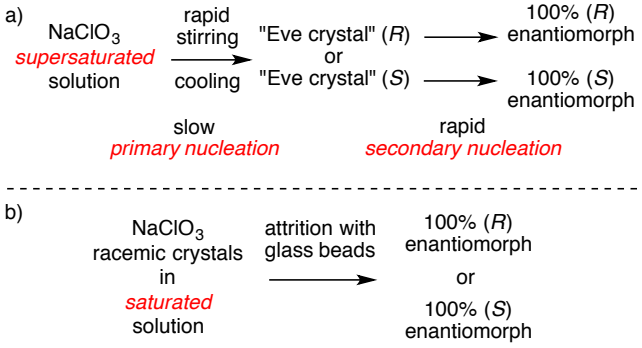
ABSTRACT: The evolution of homochirality via attrition-enhanced deracemization (AED) of enantiomorphic solids is carried out using molecules that differ only in the isotopic composition of a phenyl group positioned remote from the chiral center. Enantioenrichment consistently favors the enantiomorph containing a deuterated phenyl group over the protio or ^{13}C version, and the protio version is consistently favored over the ^{13}C version. While these isotopic compounds exhibit identical crystal structures and solubilities, the trend in deracemization correlates with melting points. Understanding the origin of this isotope bias provides fundamental clues about overcoming stochastic behavior to direct the stereochemical outcome in attrition-enhanced deracemization processes. The energy required for breaking symmetry with chiral bias is compared for this near-equilibrium AED process and t the far-from-equilibrium Soai autocatalytic reaction. Implications for the origin of biological homochirality are discussed.

INTRODUCTION

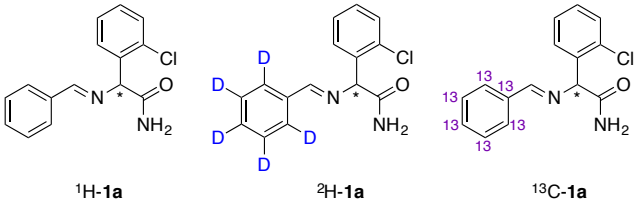
Symmetry breaking in crystallization processes of molecules that form enantiomorphic mirror-image crystals (known as racemic conglomerates) has fascinated scientists since the end of the 19th century, when the phenomenon was first observed in the formation of mirror image crystals of the achiral molecule $NaClO_3$.[1] In the last decade of the 20th century, Kondepudi demonstrated and rationalized this phenomenon in terms of the far-from equilibrium dynamics of slow primary nucleation ("Eve crystal") followed by rapid secondary nucleation processes (Scheme 1a).[2] Viedma reported an intriguing further demonstration of symmetry breaking in this system in 2005 with his observation of slurries of racemic $NaClO_3$ crystals stirred under attrition in which the system evolved inexorably and stochastically over time to one of the two enantiomorphic solids (attrition-enhanced deracemization, AED, or "Viedma ripening", Scheme 1b).[3] A key contrast between the processes shown in Scheme 1a and 1b is that the AED process involves only secondary nucleation and remains close to equilibrium at all times.[4+]

The observation of attrition-enhanced deracemization in Scheme 1b has since been extended to intrinsically chiral molecules including amino acids[5] and derivatives,[6] such as members of the family of phenylglycine amide Schiff bases[7] that includes the precursor to the blockbuster drug Plavix™ (Scheme 2a). Rapid solution-phase racemization of enantiomers serves as a conduit between the two enantiomorphic solids, analogous to the role of the achiral solution phase $NaClO_3$ in Scheme 1b. This process has been shown to be a practical industrial deracemization method[8] and has been studied in the context of probing the origin of biological homochirality.[4]

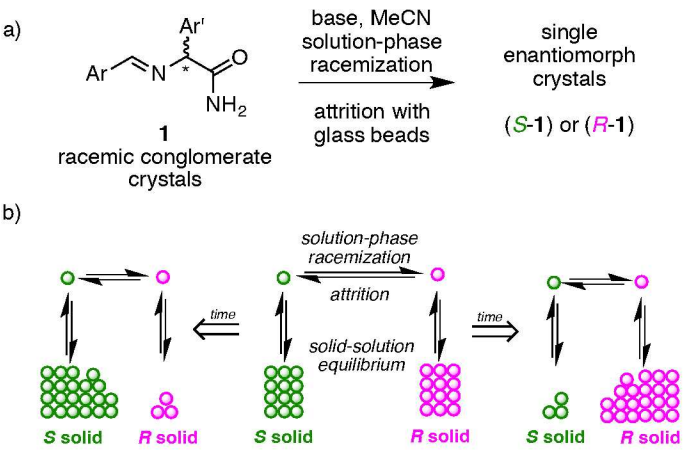
Scheme 1. Stochastic Emergence of a Single Enantiomorph of $NaClO_3$ in a) Far-From Equilibrium[2] or b) Near-Equilibrium Process.[3]



In the present work, we exploit the near-equilibrium nature of the attrition-enhanced deracemization process to probe the influence of isotopic substitution on chiral symmetry breaking by employing mixtures of three isotopomers of **1a**: ^{1}H-**1a**, ^{2}H-**1a**, and ^{13}C-**1a**.



Scheme 2. Emergence of a Single Enantiomorph in AED of Intrinsically Chiral Molecules. a) Molecule Structure; b) Stochastic Outcome.



RESULT AND DISCUSSION

Our interest in the role of isotopes were partly inspired by previous work showing that in some cases the aggregation of molecules in the solid state may be affected by deuterium substitution, which can act as a weak directing group to influence hydrogen bonds and hence the molecular arrangement of molecules in a crystal.[9] For example, pyridine, pyridine-N-oxide, and acridine all exhibit altered crystal structures due to changes in H (D) bonding that causes perturbations in molecular arrangement. In addition, the H/D isotope effect on the molar volume and expansivity of benzene vs. d^6-benzene has been probed, with most recent studies[10] revealing that the molar volume of benzene is ca. 0.5% greater than that of d^6-benzene and is not influenced by temperature, as previously thought.[11] However, in the case of racemic conglomerate **1a**, we confirmed that, within the limits of the measurement, the single crystal structure remained unaltered by either ^{2}H or ^{13}C substitution in a phenyl ring of **1**, which is remote from the racemizable chiral center (Figure 1, Table 1). Indeed, the high-quality crystal structure of enantiopure ^{1}H-**1a**,^{2}H-**1a**, and ^{13}C-**1a** exhibit near-perfect overlay. The unit cell shows the phenyl rings of neighboring molecules nearly orthogonal to one another, removed from H-bonding interactions with heteroatoms in the structure. The same structure is obtained for mixtures of ^{1}H-**1a** and ^{2}H-**1a** of the same chirality, implying that the isotopic molecules are interchangeable in the unit cell. Thus the crystal remains agnostic to the isotopic content of the molecules while it recognizes like – and rejects opposite – chirality.

Our aim in the present work is to examine whether the outcome of the AED process is influenced by isotopic substitution as in the molecules of Scheme 2b. Racemic mixtures were prepared with the *R*- and *S*-enantiomorphs comprised of different isotopic forms of **1a**. Consideration of all pairwise combinations of isotopes (^{1}H-**1a**, ^{2}H-**1a**, and ^{13}C-**1a**) and chirality (*R*-**1a** and *S*-**1a**) leads to a total of six separate experimental protocols, as shown in Table 2. Pairwise mixtures of the isotopic versions of **1a** were prepared under carefully controlled conditions[12] to minimize potential influences from effects such as differences in particle size and to ensure a chemically racemic starting composition for attrition enhanced deracemization.

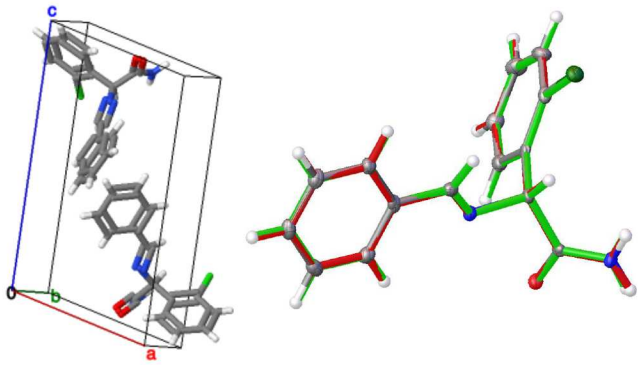


Figure 1. X-ray crystal structure for ^{1}H-**1a**, ^{2}H-**1a** and ^{13}C-**1a** showing unit cell (left) and overlay for the three isotopomers (right).

Table 1. Crystal Unit Cell Parameters.

compound (R^2)	a	b	c
^{1}H-1a (0.032)	8.5127 (±0.0016)	5.0847 (±0.0012)	14.994 (±0.004)
^{2}H-1a (0.047)	8.5059 (±0.0015)	5.0765 (±0.0009)	14.976 (±0.003)
^{13}C-1a (0.033)	8.5213 (±0.0006)	5.0768 (±0.0003)	14.9966 (±0.001)

Table 2. Racemic Mixtures of 1a Employed and Stereochemical Outcome in Attrition Enhanced Deracemization.

Exp.	*R*-solid	*S*-solid	outcome
A	^{1}H-1a	^{2}H-1a	*S*-solid
A'	^{2}H-1a	^{1}H-1a	*R*-solid
B	^{13}C-1a	^{2}H-1a	*S*-solid
B'	^{2}H-1a	^{13}C-1a	*R*-solid
C	^{13}C-1a	^{1}H-1a	*S*-solid
C'	^{1}H-1a	^{13}C-1a	*R*-solid

We have previously demonstrated the stochastic nature of the AED process for ^{1}H-**1a** when a racemic mixture of crystals is employed, and we confirmed that this is the case for ^{2}H-**1a** as shown in Figure 2. Multiple trials exhibited enantioenrichment towards either the *R* or the *S* enantiomorph without discrimination.

Figure 3 shows that when racemic mixtures were prepared from chemically racemic mixtures of 50% ^{1}H-**1a** and 50% ^{2}H-**1a** of opposite chirality, as in entries A and A' in Table 2, the deracemization proceeded inexorably towards the enantiomer initially present as ^{2}H-**1a**, regardless of whether the *R*- or the *S*-enantiomer of **1a** contained the deutero component. The final result is a homochiral solid phase containing 50% of each isotopomer. This same result was found in every one of 48 separate trials.

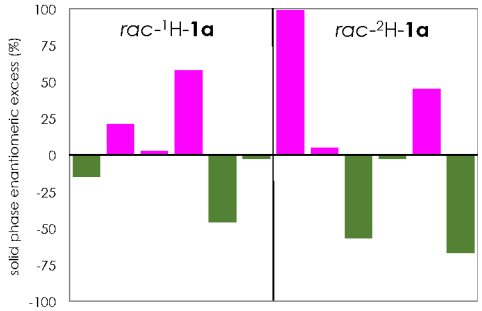


Figure 2. Attrition-enhanced deracemization in separate flasks of *rac*-**1a** (left) and *rac*-*d*5-**1a** (right) showing stochastic behavior over multiple trials: 72 hours using 2.75 mmol racemic crystals in 0.8 ml MeCN, 0.1 M DBU.

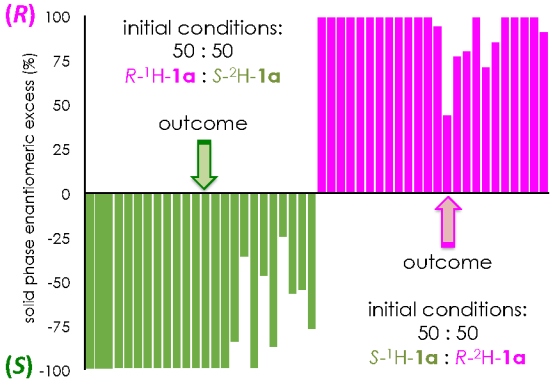


Figure 3. Attrition-enhanced deracemization of racemic mixtures **a** prepared using 50% protio *R*-^{1}H-**1a**/50% deutero *S*-^{2}H-**1a** (left) or 50% deutero *R*-^{2}H-**1a**/50% protio *S*-^{1}H-**1a** (left). Multiple trials with conditions as in Fig 2.

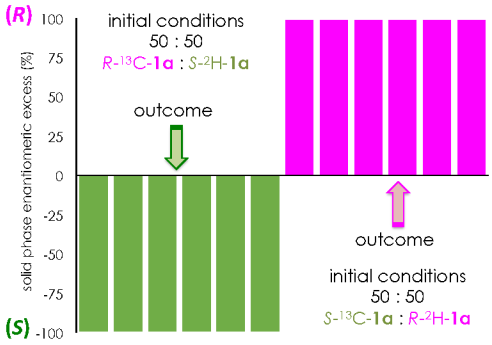


Figure 4. Outcome of attrition-enhanced deracemization of racemic mixtures prepared using 50% *R*-^{13}C-**1a** / 50% *S*-^{2}H-**1a** (left) or 50% *R*-^{2}H-**1a** / 50% *S*-^{13}C-**1a** (left). Multiple trials with conditions as in Fig 2.

The consistent isotopic bias observed in Figs. 3-5 reveals that the emergence of homochirality trends as ^{2}H-**1a** > ^{1}H-**1a** > ^{13}C-**1a**. The origin of the bias clearly does not correlate with the most obvious difference, the molecular mass of the compound, which trends as ^{13}C-**1a** > ^{2}H-**1a** > ^{1}H-**1a**. Indeed, if "racemic" mixtures prepared on the basis of equal mass of the isotopic combinations rather than equal moles, the enantiomeric excess for a mass-based racemic mixture decreases in the order of ^{1}H-**1a** and ^{13}C-**1a** (1.09% ee_{mass} towards ^{13}C-**1a**), ^{1}H-**1a** and ^{2}H-**1a** (0.91% ee_{mass} towards ^{2}H-**1a**, and for ^{2}H-**1a** and ^{13}C-**1a** (0.18% ee_{mass} towards ^{13}C-**1a**).

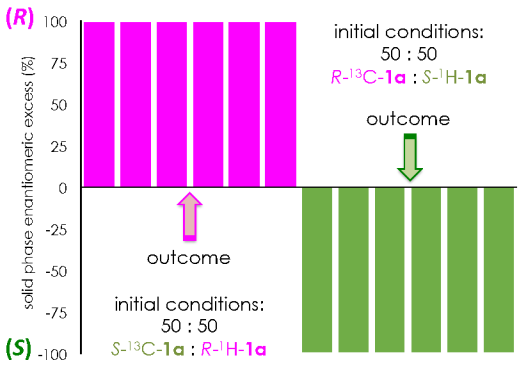
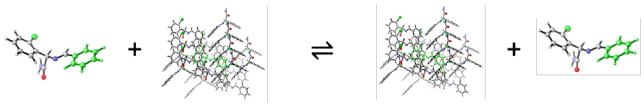


Figure 5. Outcome of attrition-enhanced deracemization of racemic mixtures **a** prepared using 50% *R*-^{13}C-**1a** / 50% *S*-^{2}H-**1a** (left) or 50% *R*-^{2}H-**1a** /5 0% *S*-^{13}C-**1a** (left). Multiple trials with conditions as in Fig 2.

Calculating the effect of isotopic substitution reveals a different trend. In particular, we used density functional theory to estimate the thermodynamic preference for exchanging two different isotopomers between the crystal lattice and the gas phase. (To model a molecule in the crystal lattice, we included all neighboring molecules within 5 Å in a geometry constrained to match the experimental crystal structure. We also considered only vibrational contributions to the enthalpy and free energy for the molecule in the crystal lattice, not translational or rotational contributions.) We found that isotope effects were greater in the crystal lattice than in the gas phase, as a molecule in a more confined space has higher vibrational frequencies than an isolated molecule in the gas phase. Thus, it is thermodynamically favorable to exchange a molecule with heavy atoms into a crystal lattice. Moreover, as with other isotope effects, the magnitude of the effect is greater for ^{2}H-**1a** than for ^{13}C-**1a** owing to the greater relative change in mass. Putting these effects together, the free energy preference for a molecule of ^{2}H-**1a** to exchange into the lattice of ^{1}H-**1a** is $\Delta G = -0.154$ kcal/mol, while the free energy preference for a molecule of ^{13}C-**1a** to exchange into the lattice of ^{1}H-**1a** is $\Delta G = -0.005$ kcal/mol (Table 3). Thus, this calculated free energy preference for being in the crystal lattice over the gas phase, which trends as ^{2}H-**1a** > ^{13}C-**1a** > ^{1}H-**1a**, does not correlate with the emergence of homochirality in AED experiments, which trends as ^{2}H-**1a** > ^{1}H-**1a** > ^{13}C-**1a**. While the computed preference for ^{2}H-**1a** to exchange into the lattice of ^{1}H-**1a** is consistent with the AED experiments, the computational model falls short in predicting the experimental behavior of ^{13}C-**1a**, suggesting that more exact modeling of other features such as collective vibrations or intermolecular interactions during the melting process are necessary to fully explain the experimental results.

Table 3. Thermodynamic Parameters for Isotopomer Substitution Between Crystal Lattice and Gas Phase.



Isotopomer Exchange Reaction	ΔZPE	ΔH	ΔG
^{2}H (gas phase) + ^{1}H (crystal) ⇌ ^{2}H (crystal) + ^{1}H (gas phase)	−0.197	−0.183	−0.154
^{13}C (gas phase) + ^{1}H (crystal) ⇌ ^{13}C (crystal) + ^{1}H (gas phase)	−0.023	−0.009	−0.005

We then sought to observe any measurable differences in chemical and physical properties of these molecules as well as differences in the rates of the chemical and physical processes that occur during AED. A prominent theory of AED postulates that a transient, stochastically generated difference in crystal size may induce a small difference in solubility between *R* and *S* enantiomorphic solids, as dictated by the Gibbs-Thomson rule. In analogy to crystallization-induced diastereomeric transformations,[13] such a solubility difference could induce the net flow of molecules from one enantiomorphic solid to the other via the conduit of solution-phase racemization.

Figure 6 shows that enantiopure ^{1}H-**1a** and ^{2}H-**1a** in separate flasks and a 50/50 mixture of the same enantiomer ^{1}H-**1a** and ^{2}H-**1a** in a single flask exhibit identical solubilities. Racemic mixtures show twice the solubility of the enantiopure, whether comprised of all protio, all deutero, or a mixture of the two. These observations confirm that these conglomerates ^{1}H-**1a** and ^{2}H-**1a** do not act as distinctly different species in solution/solid equilibrium; since the solubility exhibited is that for the enantiomer regardless of ^{2}H content, the protio and deutero molecules must inhabit the same crystal indiscriminately.

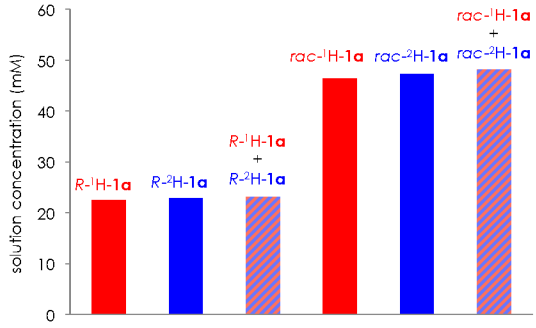


Figure 6. Solubility measurements of enantiopure and racemic ^{1}H-**1a** and ^{2}H-**1a**, and 50/50 mixtures.

This conclusion is further supported by the fact that the double solubility rule is exhibited for racemic mixtures without regard to isotopic composition. These solubility characteristics are in accord with the finding of identical crystal structures for ^{1}H-**1a**, ^{2}H-**1a**, and mixtures of the two. Similar results were observed for ^{1}H-**1b** and ^{2}H-**1b**, even while this molecule exhibits an order of magnitude higher absolute solubility. These findings indicate that only the chirality, and not the deuterium content, is relevant to the crystal form and solubility behavior of **1**.

The AED process involves both chemical and physical rate processes. Solution phase racemization is the chemical reaction that converts molecules from one stereochemical form to the other and ultimately allows molecules to move from one solid enantiomorph to the other. Dissolution from, and accretion onto, the solid enantiomorphs is a physical process. We probed both processes for ^{1}H-**1a** and ^{2}H-**1a** via ReactIR spectroscopic monitoring. Adding base to an enantiopure solid-solution mixture allows us to measure the racemization rate by monitoring the increase in solution concentration as the system obeys the double solubility rule. Similarly, monitoring the increase in solution concentration after adding one solid enantiomorph to a saturated solution of the other in the absence of base measures the rate of dissolution. As shown in Figures 7 and 8, both dissolution and racemization are rapid. No difference could be observed comparing ^{1}H-**1a** and ^{2}H-**1a** in either process.

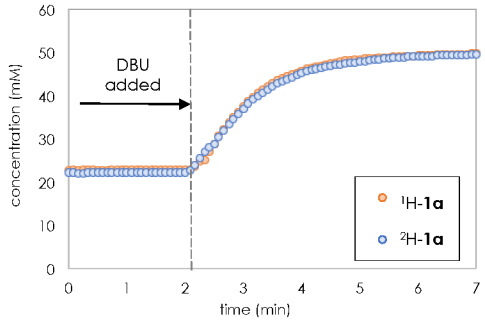


Figure 7. Solution-phase racemization of enantiopure ^{1}H-**1a** and ^{2}H-**1a** carried out by adding 1.5 mM DBU to solutions in equilibrium with the enantiopure solid.

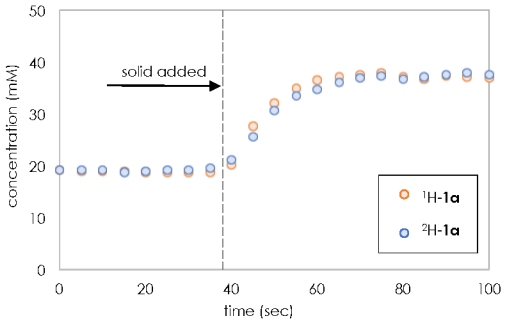


Figure 8. Rate of dissolution of solid crystals of ^{1}H-**1a** and ^{2}H-**1a**.

We have previously demonstrated a correlation between eutectic composition and strength of homochiral vs. heterochiral composition of enantiomorphs by comparing eutectic values, fusion temperatures, and selectivity in sublimation of nonracemic mixtures of enantiomorphs. Attempts to monitor selectivity in sublimation of mixtures of ^{1}H-**1a** and ^{2}H-**1a** were unsuccessful since racemization occurs when samples are heated to sublimation temperatures. However, employing differential scanning calorimetry to measure melting points provided a key measurable difference in properties between these isotopic compounds. Figure 9 shows melting point phase diagrams for all three compounds. For the enantiopure compounds, melting points decrease in the order ^{2}H-**1a** > ^{1}H-**1a** > ^{13}C-**1a**, the same trend shown in the isotopic bias of the AED experiments, with a ca. 2 °C difference in melting points between the protio and deutero molecules. The trend holds for racemates of ^{2}H-**1a** and ^{1}H-**1a**, which each exhibit a ca. 19 °C difference in melting point for racemic compared to enantiopure samples, characteristic of compounds that form conglomerates. For ^{13}C-**1a**, however, the difference is less than 11 °C, and the melting point for

its racemate equals that of ^{1}H-**1a**. ^{13}C-**1a** also shows much more curvature in the phase diagram than do the other two isotopomers.

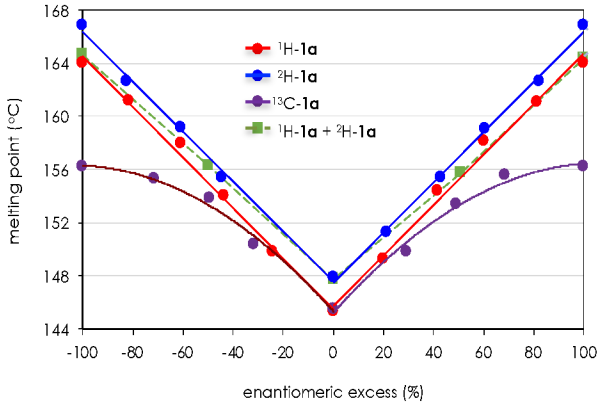


Figure 9. Melting point phase diagrams for ^{1}H-**1a**, ^{2}H-**1a** and ^{13}C-**1a** by differential scanning calorimetry using a scan rate of 5 °C/min.

We also explored the melting point for mixtures at different enantiomeric excess values comprised of 50% ^{1}H-**1a** and 50% ^{2}H-**1a** (green symbols and green dashed line in Fig. 9), which mimicks the path followed during the attrition-enhanced deracemization process. Interestingly, the racemic mixture (the starting point for the experiments shown in Figure 3), exhibits a melting point similar to ^{2}H-**1a**, but as the system trends towards the melting point of ^{1}H-**1a** as the enantiomeric excess increases.

Table 4 show that enthalpies of fusion follow the same trend for racemates of the three isotopomers. As with the melting points, the trend in enthalpies for the enantiopure substances correlates with the trend in AED. A racemic mixture comprised of 50% ^{1}H-**1a** of one hand and 50% ^{2}H-**1a** of the other (mimicking the starting point in AED) gives a heat of fusion lower than that of an enantiopure mixture of equal amounts of ^{2}H-**1a** and ^{1}H-**1a** (mimicking the end point of AED). Thus the extremely subtle difference between the isotopes, which has been found to give a ca. 0.5% volume difference between H and D benzene but is too small to be revealed in the crystal structures of Figure 1 and Table 1, is manifested in small differences in enthalpy that are consistent with the trends for deracemization of the conglomerate solids.

Table 4. Enthalpy of Fusion for Enantiopure and Racemate Samples of Isotopomers.

sample	enthalpy (kJ/mol)	standard deviation
^{2}H-1a	47.9	0.59
^{1}H-1a	45.6	0.63
^{13}C-1a	41.2	0.7
(^{1}H-*S*+^{2}H-*S*)-1a	45.1	0.34
(^{1}H-*R*+^{2}H-*S*)-1a	43.0	0.23

The melting point behavior provides the first property measurement that correlates to the deracemization trends for the isotopic crystals, following the clear bias towards the deuterated, then protonated, then ^{13}C.This demonstrates that the melting point data offer a more sensitive measure of the very small differences in stability of the compounds than could be discerned from the long-range crystal structure data.

Since a racemic system of ^{1}H-**1a** (either *R* or *S*) and ^{2}H-**1a** of the other hand consistently evolves to enantiopurity towards the hand of ^{2}H-**1a**, we may seek to probe conditions under which this bias toward ^{2}H-**1a** could be overcome. Two questions arise to address this issue: 1) how large must an initial enantiomer imbalance toward ^{1}H-**1a** be to overcome this natural bias? and 2) at what deuterium level in one enantiomer does a racemic mixture revert to stochastic deracemization behavior? Quantitative analysis of these two points will may provide fundamental information about the energy required to effect the near-equilibrium process of attrition-enhanced deracemization.

AED experiments were carried out to address these two questions as shown in Figures 10 and 11. using mixtures with an initial nonracemic enantiomeric excess with ^{1}H-**1a** (either *R* or *S*) in excess and the minor enantiomer comprised of ^{2}H-**1a** showed that the bias toward ^{2}H-**1a** previously observed in Fig. 3 could be overcome and reversed if the initial enantiomeric excess was ca. 3% *ee* or greater towards ^{1}H-**1a** (Fig. 10). Racemic mixtures in which the deuterium content is varied (Fig. 11) show that trend toward the enantiomer enriched in ^{2}H-**1a** holds when its deuterium fraction is above 96%, below which stochastic behavior is restored.

These results further quantify the chiral bias required to overcome what appears to be an intrinsic bias toward the deutero-substituted compound. A value of 3% *ee* corresponds to a transition energy difference of 0.15 kJ/mol. Thus the ability of the isotope to control the deracemization process is found to be quite subtle.

This energy requirement for the emergence of homochirality from this near-equilibrium process may be compared to recent experimental and computational studies of the Soai reaction, which serves as an autocatalytic model for a far-from-equilibrium process for symmetry breaking and asymmetric amplification towards homochirality. Combining experiments using isotopically chiral initiators[14] and stochastic modeling of Soai reaction kinetic profiles allowed us to determine that the initial enantiomer imbalance required to break symmetry faithfully towards one enantiomer in the Soai reaction lies between 10^{-7} – 10^{-8} % *ee*, or 2 x 10^{-8} kJ/mol.[15] This value is more than one million-fold lower than the energy requirement determined here for attrition-enhanced racemization. AED processes exhibit the characteristic sigmoidal profile of an autocatalytic process. The reversibility of the AED process distinguishes it from the irreversible autocatalytic Soai reaction system. Together these results demonstrate that the energy "tipping" required to break symmetry in a near-equilibrium process is significantly greater than that required in a far-from-equilibrium process.

Both of these symmetry breaking scenarios may be compared to the fundamental energy difference between enantiomers due to parity violation of the weak force (parity violation energy difference, PVED). While a been direct measurement of PVED has never been made, calculations estimate it to be ca. 10^{-12} – 10^{-15} kJ/mol.[16] This suggests that PVED is unlikely to be the driving force for the emergence of homochirality in either the near- or far-from-equilibrium processes discussed here.

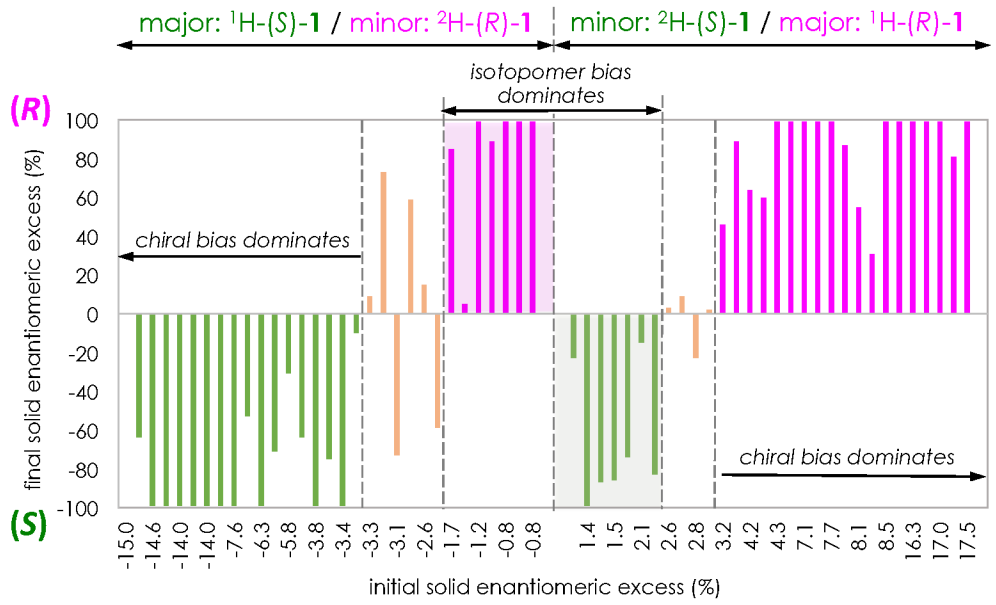


Figure 10. Attrition-enhanced deracemization carried out with mixtures of ^{1}H-**1a**/^{2}H-**1a** varying initial enantiomeric excess with ^{1}H-**1a** as the major enantiomer. Conditions as in Fig. 2.

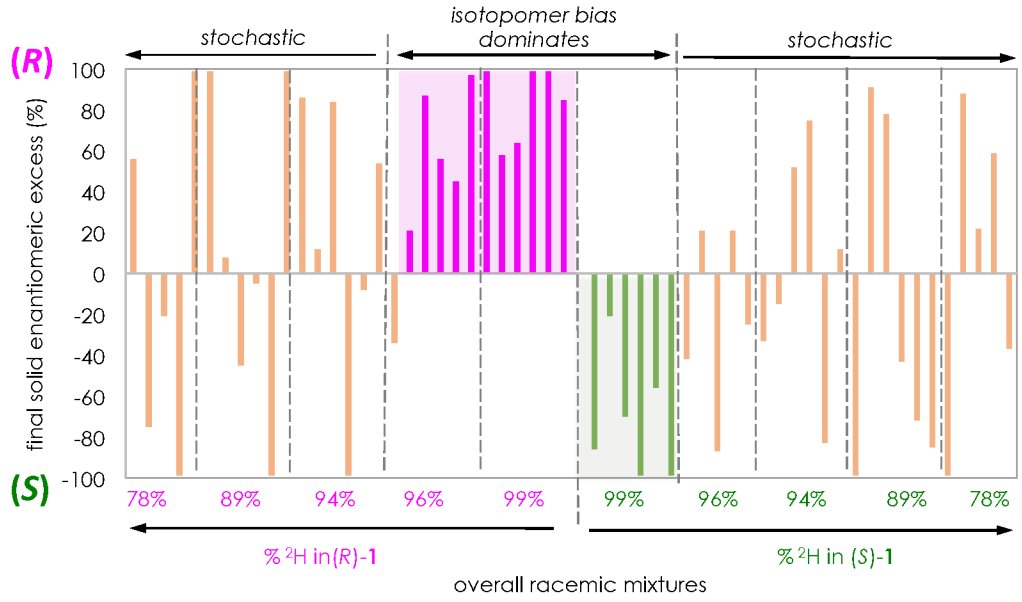


Figure 11. Attrition-enhanced deracemization carried out with racemic mixtures of ^{1}H-**1a**/^{2}H-**1a** varying deuterium content of one enantiomer in racemic mixtures of ^{1}H-**1a**/^{2}H-**1a**. Conditions as in Fig. 2.

CONCLUSIONS

In conclusion, the emergence of homochirality in attrition-enhanced deracemization has been investigated in the context of understanding the directing influence of the isotopic composition of the molecules. Investigations were carried out with isotopic compounds that differ only in the isotopic composition of a phenyl group positioned remote from the chiral center. Racemic mixtures of these compounds were prepared with one enantiomer as ^{1}H, ^{2}H, or ^{13}C and the other enantiomer as one of the isotopes. We demonstrated that these molecules in pure or racemic form exhibit identical physical properties including crystal structures and solubilities. However, attrition-enhanced deracemization experiments revealed that enantioenrichment consistently favors the enantiomorph containing a deuterated phenyl group over the protio or ^{13}C version, and the protio version is consistently favored over the ^{13}C version. These trends in deracemization were shown to correlate with melting points of the different isotopic compounds. Removing the bias in the ^{1}H-^{2}H mixture to return the system to stochastic behavior

required either a small enantiomeric excess toward ^{1}H-**1a** or a small excess of ^{2}H-**1a** in the ^{1}H-**1a** enantiomer in a racemic mixture. These experiments demonstrate that the energy required to overcome this natural bias toward the ^{2}H isotope is small, ca. 0.15 kJ/mol. Understanding the origin of this isotope bias may provide fundamental clues about symmetry breaking and directing the stereochemical outcome in attrition-enhanced deracemization processes.

Supporting Information. Synthetic details and general procedures for attrition enhanced deracemization, solubility and melting point measurements, FTIR and NMR spectroscopy and chiral HPLC measurements, crystallography, computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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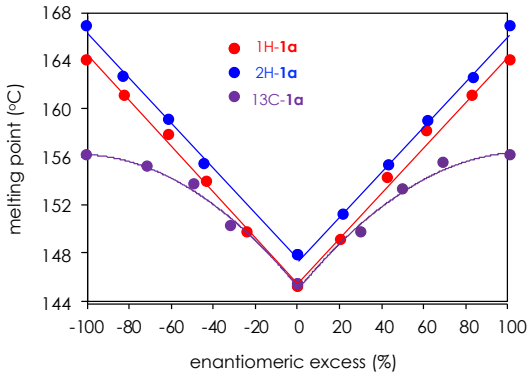
168
164
160
156
152
148
144
melting point (°C)
1H-1a
2H-1a
13C-1a
-100 -80 -60 -40 -20 0 20 40 60 80 100
enantiomeric excess (%)