The racemate cage. Influence of p_1,n_1 salt occurrence on enantiomer separation processes. The case of *trans*-chrysanthemic acid

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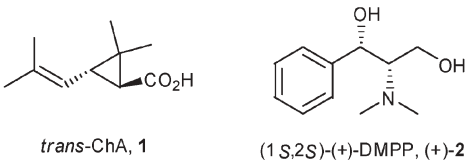
The occurrence of p_1,n_1 salt when accompanied by substrate self-association can have profound effects on enantiomer separation processes of non-racemic mixtures, impeding the complete recovery of the major enantiomer through formation of an inescapable racemate cage.

In a recent Communication to this journal[1] we reported that racemic *trans*-chrysanthemic acid (ChA) forms p_1,n_1 salts when treated with the pure enantiomers of the base *threo*-2-dimethyl-amino-1-phenyl-1,3-propanediol (DMPP) (Fig. 1).

p_1,n_1 Salts may appear during resolution processes through formation and separation of crystalline diastereomeric salts between racemic substrates and optically pure resolving agents[2–4] (Scheme 1).[5] They have been supposed to be the often overlooked "culprits" of many resolution failures.[6]

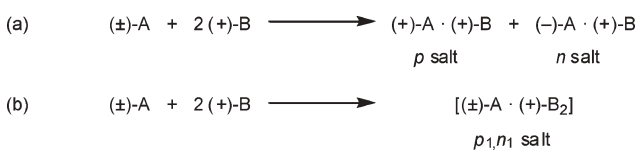
In the same paper, we also presented how p_1,n_1 salt occurrence can be exploited to recover the exceeding fraction of the major enantiomer from non-racemic mixtures, along with a practical application of this new concept to the industrially relevant case of non-racemic mixtures of *trans*-ChA (Fig. 2).[7]

Spurred by these results, we set out to explore the system formed by non-racemic **1** and the enantiopure base **2**,[8] in diisopropyl ether as the solvent.[9] We wish to report here, that using the other enantiomer of the agent DMPP [(−)-**2**] led to totally different and unexpected results, revealing an inescapable racemate "cage".



trans-ChA, **1** (1*S*,2*S*)-(+)-DMPP, (+)-**2**

Fig. 1 *trans*-ChA and (+)-DMPP structures.



Scheme 1 Reaction of a racemic acid (A) with an enantiopure base (B): formation of: (a) a *p* and *n* diastereoisomeric pair of salts; (b) p_1,n_1 salt.

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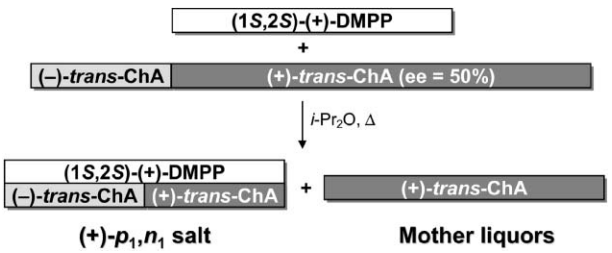


Fig. 2 Recovery of the excess enantiomer from a non-racemic mixture of *trans*-ChA through p_1,n_1 salt formation.

Racemic *trans*-ChA when treated with enantiopure DMPP of any rotation sign, invariably furnishes the p_1,n_1 salt enantiomer of the same rotation of the DMPP enantiomer used.

When a (+)-*trans*-ChA enriched mixture (50% ee) was treated with (+)-DMPP, the first 0.5 equivalents of base added caused the precipitation of the p_1,n_1 salt (Fig. 2).

The same experiment was performed using the same 50% ee (+)-*trans*-ChA enriched mixture, but changing the enantiomer of the DMPP base [Fig. 3, this time (−)-DMPP was used]. A completely different behaviour was found: the addition of 0.5 equivalents of the base – that is, stoichiometric to the excess of (+)-*trans*-ChA in the starting mixture – caused the formation of the *n* salt between (+)-*trans*-ChA and (−)-DMPP, *this time leaving the racemate in solution*.

Using mixtures of *trans*-ChA with different enrichments in (+)-enantiomer did not change the outcome: additions of (−)-DMPP base stoichiometric to the exceeding fraction of (+)-*trans*-ChA enantiomer produced the formation of the *n* salt only.

In brief, when a non-racemic mixture of *trans*-ChA is treated with the pure enantiomer of DMPP with the same sign of rotation (Fig. 2) the p_1,n_1 salt is formed leaving the exceeding enantiomer of *trans*-ChA in solution. On the contrary, when an enriched mixture of *trans*-ChA is treated with the pure enantiomer of DMPP with

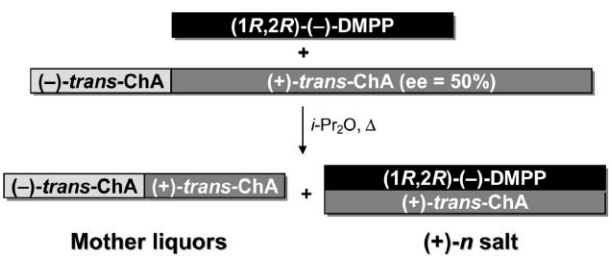


Fig. 3 Use of the other DMPP enantiomer. Formation of the *n* salt.

Table 1 Characterization data for the three salts

	Mp/°C	DSC Peak/°C	DSC ΔH^f/J g^{-1}	$[\alpha]_D$[a] (c/g (100 mL)$^{-1}$)
(+)-*p* salt	90.0–92.0	88.81	−111.2	+39.7 (0.97)
(+)-*n* salt	132.6–134.0	131.27	−126.7	+12.3 (0.93)
(+)-p_1n_1 salt	110.0–111.5	110.97	−146.4	+26.8 (1.04)

[a] Chloroform solution at 23 °C.

opposite sign of rotation (Fig. 3) and stoichiometric to the exceeding fraction of the *trans*-ChA enantiomer, *n* salt always forms first, leaving the racemate of *trans*-ChA in solution.

Characterization[10] and solubility data of the salts (Tables 1 and 2) did not fully explain this absolute specificity for *n* salt formation.

In the case depicted in Fig. 3, the system has the opportunity to choose between the precipitation of the racemate as the p_1,n_1 salt and that of the exceeding enantiomer as the *n* salt. In such cases a specific and quantitative precipitation of the *n* salt has always been observed.

We thought that, because of this great selectivity for the formation of the *n* salt, the possibility of a *quantitative* recovery of the major *trans*-ChA enantiomer was close at hand. Instead of recovering only the *exceeding* fraction of the *trans*-ChA enantiomer (as in Fig. 3), the addition of 0.75 eq. of (−)-**2**, should allow the precipitation of *all* (+)-**1** as the *n* salt.

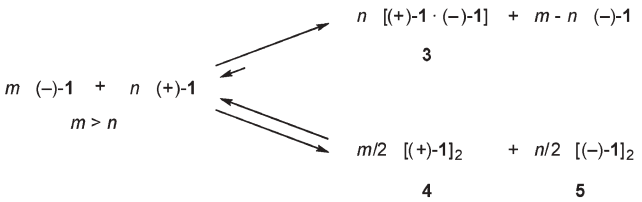
The result was disappointing: instead of a quantitative recovery of the major *trans*-ChA enantiomer, the salt isolated furnished *trans*-ChA with only 66% ee. This % ee value can be ascribed to 2 : 1 mixture of *n* and p_1,n_1 salts respectively.

To better understand this result we performed a set of ten crystallization experiments in a parallel reactor. The ten vessels were charged with increasing amounts of (−)-DMPP (from 0.1 equiv. in vessel #1 up to 1 equiv. in vessel #10, in linear steps of 0.1 equiv.). Then all the ten vessels were added with 1 equiv. of the scalematic mixture of (+)-*trans*-ChA with 50% ee in *i*-Pr_2O. The parallel reactor was heated at reflux for 30 min and then the ten mixtures were let to crystallize. Chrysanthemic acid obtained from solids and mother-liquors of each vessel was collected and separately analyzed. The analyses showed that *n* salt precipitation occurs first (vessels 1–5) up to the point were all *the exceeding* (+)-*trans*-ChA enantiomer is consumed (0.5 equiv. of base for a 50% ee of ChA) leaving in solution the *trans*-ChA racemate. Larger additions of the same DMPP base enantiomer (vessels 6–10) cause the precipitation of the *trans*-ChA racemate as the p_1,n_1 salt. This behaviour may be explained by considering *trans*-ChA enantiomers self-association in solution.

Table 2 Solubilities of the salts in *i*-Pr_2O[a]

	Solubilities in *i*-Pr_2O[a]/mg ml^{-1}		
T/°C	*n* salt	p_1n_1 salt	*p* salt
15	4.21	4.84	N.d.[b]
31	7.78	8.72	N.d.[b]
68	33.0	46.6	Soluble

[a] Performed by equilibrating the solid/liquid mixture of the salt for 24 h at the given temperature. [b] *p* Salt is soluble in refluxing *i*-Pr_2O. Once dissolved, it forms metastable solutions from which precipitation is achieved only by scratching the container's walls.



Scheme 2 Dimeric aggregates in a non-racemic *trans*-ChA solution, with the preferential formation of the heterodimer **3**, over homodimers **4** and **5**.

In solution, the two enantiomers of a chiral substance can give rise to the formation of three distinct dimers (Scheme 2), namely the diastereomeric heterodimer **3** and the two enantiomeric homodimers **4** and **5**.[11] Three different situations can be considered. (a) *Initial racemic composition* (Scheme 2, $m = n$): independently of the system preference for either **3** or **4** and **5**, equal amounts of the two enantiomeric homodimers are always formed ($m = n$) and no free unmatched enantiomers is left after the formation of **3** ($m - n = 0$). (b) *Initial non-racemic composition* (Scheme 2, $m \neq n$) *with a preference for homodimers*: the enantiomeric homodimers are formed in the same initial *m*/*n* ratio. (c) *Initial non-racemic composition* (Scheme 2, $m \neq n$) *with a preference for the heterodimer*: after the formation of **3**, the exceeding fraction of the major enantiomer remains free in solution; this is the only case where the initial *m*/*n* ratio between enantiomeric species is broken. This latter phenomenon has been exploited in a few cases for the achiral separation of the exceeding enantiomer from the racemate of a non-racemic mixture of a chiral substance.[12,13]

The assumption that, in solution, *trans*-ChA strongly prefers to form heterodimers,[14] can explain the different results obtained with two enantiomers of DMPP.

Thus, in a solution of non-racemic *trans*-ChA, the heterodimer actually acts as a sequestrant of the minor enantiomer.[15] Any base added to the system would preferentially react with the fraction of the major enantiomer that remains free after the aggregation, without disrupting the stable heterodimer **3**.[16]

Given this picture, the addition of either of the DMPP enantiomers makes a big difference. If the DMPP base added has the *same rotation sign* as the exceeding *trans*-ChA enantiomer [(−)-**2**, in Scheme 2], reaction with the free (−)-**1** would form the *p* salt, which is soluble (Table 2) and does not precipitate. The only alternative for this base is to disrupt aggregate **3** and form the p_1,n_1 salt (as in Fig. 2). If the added enantiopure DMPP has the *opposite rotation sign* with respect to the exceeding *trans*-ChA enantiomer [(+)-**2**, in Scheme 2], reaction with the free (−)-**1** precipitates the less soluble *n* salt (Table 2). When the free fraction of (−)-**1** is consumed up to the *n* salt solubility level, further additions of the base would precipitate the heterodimer as the p_1,n_1 salt.

We therefore came to the conclusion that, considering its behaviour with respect to the base DMPP, the major enantiomer in a *trans*-ChA non-racemic mixture it is not all the same. The part of the major enantiomer that exceeds the racemate forms the *n* salt, while the fraction of the major enantiomer that is part of the racemate behaves in a totally different way, forming the p_1,n_1 salt.

In the context of the recent ongoing debate whether "% ee makes sense as expression of the enantiomeric composition",[17] we came to the different conclusion that, in this case, % ee makes perfect sense in describing a non-racemic mixture as composed of

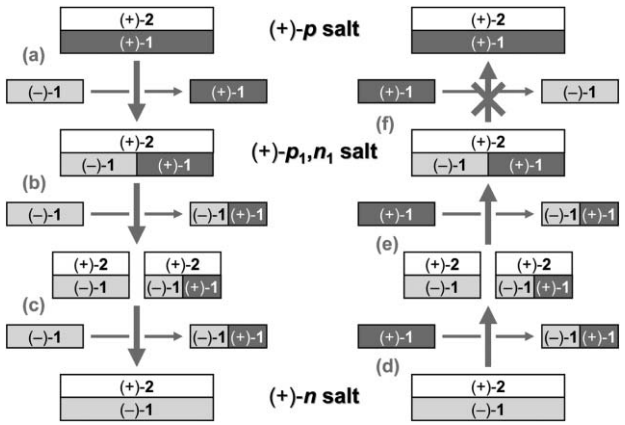


Fig. 4 Relative stability of the *n*, p_1,n_1 and *p* salts.

an exceeding enantiomer in a % ee fraction, and of a racemate in 100 − % ee fraction. In our case, describing this composition in terms of enantiomer ratio[18] *q* would be misleading. For example, the non-racemic mixture of *trans*-ChA in Fig. 3 has ee = 50% and *q* = 3. Considering its ability to form the *n* salt with (−)-**2**, the ee value exactly matches the fact that only (and no more than) 50% of this mixture can form the *n* salt. While by considering the *q* value only, the misleading information that three parts out of four of the mixture are the same is conveyed.

In an attempt to study the relative stability of the *n*, p_1,n_1 and *p* salts we performed the set of experiments depicted in Fig. 4. In any case a salt was suspended in hot *i*-Pr_2O, then 0.5 eq. of a pure enantiomer of **1** were added. The mixture was kept at reflux for 30 min and then filtered. Enantiomer composition of *trans*-ChA obtained from the salts and the mother-liquors was determined. The results were the following (Fig. 4): (a) addition of 0.5 eq. of (−)-**1** to (+)-*p* salt displaces 0.5 eq. of (+)-**1** from the salt to form the more stable and insoluble (+)-p_1,n_1 salt; (b) addition of 0.5 eq. of (−)-**1** to (+)-p_1,n_1 salt, *does not displace* the remaining 0.5 eq. of (+)-**1** from the salt to form the *n* salt: it only displaces half of the remaining (+)-**1** to form a 1 : 1 mixture of *n* and p_1,n_1 salts, so that a racemic composition is attained in solution; (c) *n* salt formation is completed by a addition of further 0.5 equiv. of (−)-**1** and again attaining a racemic composition in solution. Experiments (d)–(f) show that it is possible to convert the more stable and less soluble *n* salt into the p_1,n_1 salt, provided that a racemic composition of **1** is attained in solution: (d) addition of 0.5 eq. of (+)-**1** to *n* salt leads to the formation of the 1 : 1 mixture of *n* and p_1,n_1 salts and a racemic solution of **1**; (e) addition of further 0.5 eq. of (+)-**1** to this mixture of salts leads to the p_1,n_1 salt and a racemic solution of **1**; and (f) addition of 0.5 eq. of (+)-**1** to p_1,n_1 salt causes no change since this would form the less stable and more soluble *p* salt without the possibility to achieve a racemic solution of **1**.

In conclusion, the results reported here show the influence of the p_1,n_1 salt occurrence in the enantiomer separation process through diastereomeric compounds formation. When this phenomenon is accompanied by the substrate self-aggregation, an inescapable racemate "cage" may form both in the solid phase (the p_1,n_1 salt) on one side and in the solution phase self-aggregation of the substrate with preference for the heterodimer aggregate. This cage impedes the complete recovery of the major enantiomer of the substrate in a non-racemic mixture, allowing to recover only the exceeding part of it.

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Notes and references

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5 Letter *p* is used to designate the diastereoisomers resulting from reaction of two constituents having *like* sign of rotation and the letter *n* to designate the diastereoisomer formed from constituents of *unlike* sign. See ref. 2, p. 326 and ref. 3, p. 251.
6 "Careful culling of the literature would produce many more examples of this phenomenon." Taken from ref. 3, p. 295.
7 For a very efficient resolution process of racemic *trans*-ChA from technical mixtures of *trans*/*cis*-ChA, see: G. Rosini, C. Ayoub, V. Borzatta, E. Marotta, A. Mazzanti and P. Righi, *Green Chem.*, 2007, DOI: 10.1039/b615785h.
8 p_1,n_1 Salts are enantiopure compounds. For example, in Scheme 1(b), the p_1,n_1 salt that might be expected to arise from the use of (−)-B, would be the enantiomeric [(±)-A·(−)-B_2].
9 All the experiments performed by dissolving DMPP in hot diisopropyl ether and then adding the *trans*-ChA mixture. To make sure that no kinetic effect was playing any role, the system was allowed to equilibrate at reflux. The outcome in terms of salt composition and yield did not change over time.
10 For X-ray crystal structures of p_1,n_1 and *n* salts, see ref. 1.
11 The picture might well be more complicated by the formation of oligomeric aggregates. See: A. Gavezzotti and G. Filippini, *Chem. Commun.*, 1998, 287.
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