Liquid Chromatography

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Remarkable Amplification of the Self-Disproportionation of Enantiomers on Achiral-Phase Chromatography Columns**

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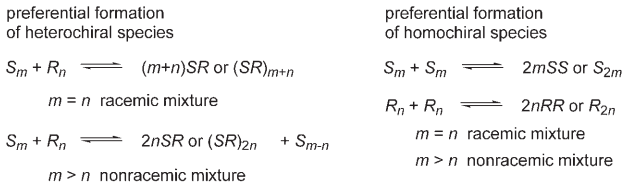
Dedicated to Professor Yuri Nikolaevich Belokon on the occasion of his 67th birthday

Two distinct modes of association can be envisioned when considering the intermolecular interactions between the enantiomers of a chiral compound in solution, namely, modes based on the preferential formation of either heterochiral or homochiral species (Scheme 1). In both cases, the



Scheme 1. Modes of association of the enantiomers of a chiral compound in solution.

formation of dimeric or more complex oligo/polymeric associates can be expected. When the ratio between the amounts of *S* and *R* enantiomers is equal ($m = n$, a racemic mixture), any type of association will give rise to isoenergetic enantiomeric species that display identical chemical and physical properties. However, when a nonracemic mixture is considered ($m > n$), the outcome will be drastically different. Thus, when heterochiral associations are preferred one can expect the formation of racemic species $2n\,S,R$ or $(S,R)_{2n}$ plus an excess of enantiomer *S*, which are different chemical entities and therefore may be separated without the application of any *external element of chirality*. In the case when homochiral associations are preferred, the situation is more subtle as the formation of dimers will result in different numbers of enantiomeric *S*,*S* and *R*,*R* pairs. However, when oligo/polymeric associations are favored, the formation of aggregates of different molecular weight is possible and therefore their separation may be achieved.

The first physical observation of the enantiomer associations discussed above, and therefore a proof of concept,[1] was reported more than 35 years ago by Williams et al.,[1a] who studied the NMR spectra of nonracemic dihydroquinines. They detected two sets of peaks for some protons, with peak areas being proportional to the relative ratio of the enantiomers. These associations are believed to be responsible for several remarkable phenomena observed for nonracemic compounds, such as the nonlinear behavior of optical rotation[2] and UV absorbance,[3] as well as nonlinear effects in asymmetric catalysis.[4]

However, the most fascinating opportunity offered by the enantiomer associations is the possibility of using the *internal chirality* of nonracemic mixtures for their optical purification/separation by achiral-phase chromatography.[5] Thus, in 1983 Cundy and Crooks reported on the separation of an excess of ^{14}C-labeled (*S*)-(−)-nicotine enantiomer (second fraction) from the racemate (first fraction) on an achiral HPLC system.[6] Since that time a handful of sporadic publications on such separations of various organic compounds by HPLC,[7] medium-pressure liquid chromatography (MPLC),[8] and flash[9] and regular[10] chromatography have appeared in the literature. However, this phenomenon still remains virtually unknown[11] to most practitioners in academic and industrial laboratories, and routine column chromatography on silica gel is generally accepted as a safe method for the purification of optically active compounds that does not alter the original enantiomeric composition.

Moreover, authors use different terminology to describe the observed phenomenon, most frequently calling it "enantiomeric enrichment on achiral-phase chromatography".[12] This definition is clearly incorrect and rather misleading, as part of a nonracemic sample does indeed undergo enantiomeric enrichment while the rest of the sample becomes more racemic. Herein, we introduce the term "self-disproportionation of enantiomers" or "enantiomer self-disproportionation" to describe the phenomenon, and report on the truly remarkable amplification of this effect that is induced by a trifluoromethyl group.

In the course of our studies on the asymmetric biomimetic transamination of perfluoroalkyl keto compounds **1** to amines and amino acids **4** by the DBU-catalyzed isomerization of imine **2** to Schiff base **3** (Scheme 2),[13] we encountered an irritating problem of poor reproducibility in the *ee* values. Thus, for determination of the enantiomeric purity of amino compounds **4** by HPLC analysis on chiral stationary phases, we used a method that required preparation of the dinitrobenzoyl derivatives **6**. As the reaction of highly electrophilic acid chloride **5** with amino compounds **4** generated substantial amounts of by-products, the chromatographic purification of derivatives **6** was inevitable.

Detailed analysis of the research data revealed that, as a consequence of the overlap of target compounds **6** with the by-products, preparative TLC or column chromatography allowed the recovery of 65 to 80% of pure derivatives **6** from the crude reaction mixture. Then we found that the enantiomeric purity of the samples strongly depended on the amount

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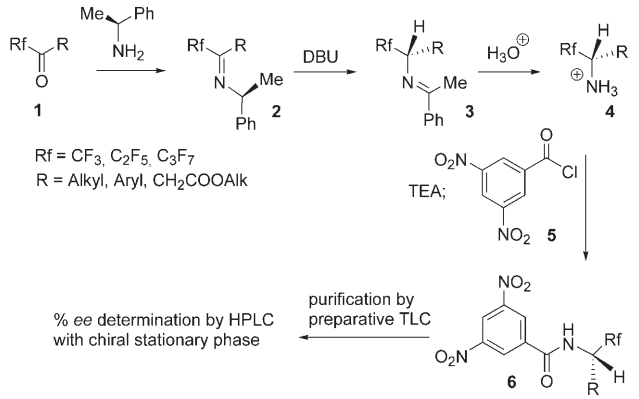


Scheme 2. Asymmetric biomimetic transamination of perfluoroalkyl keto compounds. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; TEA = triethylamine.

of recovered **6**, and increased with increasing percentage recovery. These observations led us to an experiment in which we loaded a sample of β-amino acid **6** (Rf = CF_3, R = CH_2COOEt) of 66.6% *ee* on a regular silica gel column and subjected the sample to chromatography with hexane/EtOAc = 5:1 as mobile phase.[14] We were expecting to find some effect of self-disproportionation of enantiomers leading to different *ee* percentage values in different fractions. However, the results obtained were truly remarkable (Table 1): the

Table 1: Chromatography of compound (*S*)-**6** (Rf = CF_3, R = CH_2COOEt; 66.6% *ee*) with elution by hexane/EtOAc = 5:1.

Fraction	*ee* [mass%]	Fraction	*ee* [mass%]
1	8.1 (3.3)	11	94.3 (91.9)
2	15.7 (7.9)	12	95.9 (93.5)
3	32.2 (12.1)	13	96.9 (95.2)
4	43.9 (25.3)	14	97.3 (96.3)
5	56.5 (39.2)	15	96.9 (97.9)
6	66.3 (52.6)	16	98.6 (97.9)
7	76.1 (64.5)	17	99.5 (98.5)
8	82.2 (74.0)	18	>99.9 (99.1)
9	86.2 (81.6)	19	>99.9 (99.6)
10	91.0 (87.6)	20	>99.9 (>99.9)

first fraction was substantially more racemic than the later fractions, which afforded virtually enantiomerically pure product of >99.9% *ee*.

The magnitude of this effect, which amounted to an unprecedented[15] 91.8% difference between the lowest and highest *ee* values obtained, was totally unexpected. To investigate the possible application of the observed self-disproportionation of enantiomers on achiral silica gel, for the practical optical purification of optically active samples by using their internal chirality, we conducted a series of experiments in which the starting *ee* value of the samples was varied and different eluents were used. Consideration of the results summarized in Table 2 leads to the following conclusions: to obtain enantiomerically pure samples, at least under the conditions studied so far, the *ee* value of the starting nonracemic mixture should be higher than 61% (Table 2, entries 1 versus 2). Next, very high (95% *ee*; Table 2, entry 1) and low (31.1% *ee*; Table 2, entry 2) *ee* values lead to diminished enantiomer self-disproportionation. Application of a more polar solvent also results in this reduced effect (Table 2, entry 6).

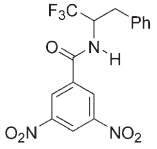
Table 2: Self-disproportionation of enantiomers of compound **6** (Rf = CF_3, R = CH_2COOEt) on achiral silica gel chromatography.

Entry	Starting %*ee* (*S*)	Eluent[a]	%*ee* min	%*ee* max
1	95.0	Hex/EtOAc = 5:1	83.3	99.9
2	31.1	Hex/EtOAc = 5:1	20.9	41.8
3	61.1	Hex/EtOAc = 5:1	12.6	95.5
4	66.6	Hex/EtOAc = 5:1	8.1	99.9
5	66.6	Hex/Et_2O = 1:1	54.8	99.9
6	66.6	$CHCl_3$	65.5	68.2

[a] Hex = hexane.

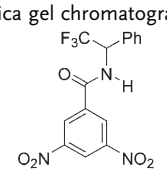
Similar results for the enantiomer self-disproportionation were also obtained for compounds **6** derived from (2-benzyl)trifluoroethylamine (Table 3) and (2-phenyl)trifluoroethylamine (Table 4). The magnitude of the effects observed was less pronounced than that of the β-amino acid derivative

Table 3: Self-disproportionation of enantiomers of compound **6** (Rf = CF_3, R = CH_2Ph) on achiral silica gel chromatography.



Entry	Starting %*ee* (*S*)	Eluent	%*ee* min	%*ee* max
1	95.0	Hex/EtOAc = 5:1	90.8	97.6
2	31.1	Hex/EtOAc = 5:1	27.1	53.4
3	61.1	Hex/EtOAc = 5:1	49.9	92.3
4	66.6	Hex/EtOAc = 5:1	58.4	99.9
5	66.6	Hex/Et_2O = 1:1	55.7	95.2
6	66.6	$CHCl_3$	65.8	68.6

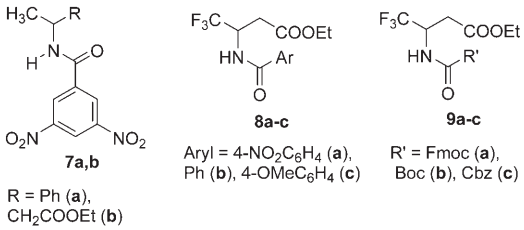
Table 4: Self-disproportionation of enantiomers of compound **6** (Rf = CF_3, R = Ph) on achiral silica gel chromatography.



Entry	Starting %*ee* (*S*)	Eluent	%*ee* min	%*ee* max
1	95.0	Hex/EtOAc = 5:1	91.0	99.6
2	31.1	Hex/EtOAc = 5:1	28.9	39.1
3	61.1	Hex/EtOAc = 5:1	55.1	70.3
4	66.6	Hex/EtOAc = 5:1	59.9	78.8
5	66.6	Hex/Et_2O = 1:1	64.8	72.1
6	66.6	$CHCl_3$	66.6	66.6

6 (Table 1); however, enantiomerically pure samples (99.9% *ee*) were obtained in both cases. It is interesting that in the latter case, the effect was not observed when $CHCl_3$ was used as eluent.

With these results in hand, we next focused our attention on determining what structural feature in **6** might be responsible for the enantiomer self-disproportionation effect. One may agree that the best "suspect" would be a highly polar dinitrobenzoyl moiety. Therefore, we prepared samples of the corresponding derivatives of phenylethylamine (*R*)-**7a** and β-aminobutyric acid (*R*)-**7b** of 66.6% *ee* and subjected them to chromatography under standard conditions (Scheme 3).[14] Surprisingly, the enantiomer self-

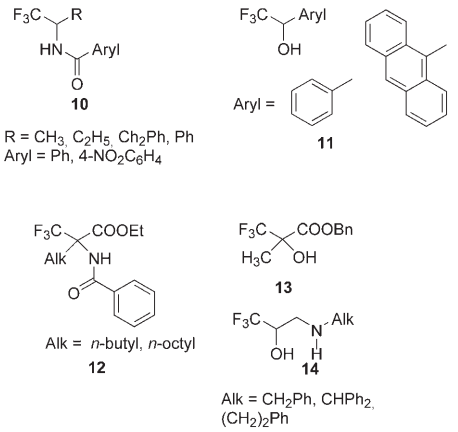


Scheme 3. Compounds used to examine the structural features of **6**. Fmoc = 9-fluorenylmethoxycarbonyl; boc = *tert*-butoxycarbonyl; cbz = benzyloxycarbonyl.

disproportionation effect was not observed for either of these compounds as all the collected fractions showed 66.6 ± 0.05% *ee*. We reasoned that the combination of a polar trifluoromethyl group and a dinitrobenzoyl moiety in the same molecule could explain the chromatographic behavior of **6**. To this end we prepared compounds (*S*)-**8a–c** of 66.6% *ee* bearing *p*-nitrobenzoyl, unsubstituted benzoyl, and *p*-methoxybenzoyl groups, respectively. Again, quite surprisingly, in all three cases we detected a strong enantiomer self-disproportionation effect on achiral silica gel. Moreover, β-amino acid derivatives (*S*)-**9a–c** of 66.6% *ee*, with the typical protection groups Fmoc, Boc, and Cbz, respectively, were also found to undergo enantiomer self-disproportionation.

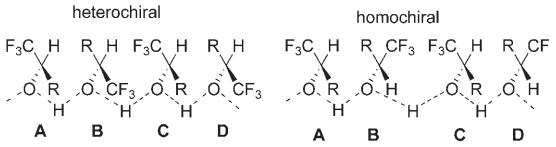
All these data strongly suggest that a trifluoromethyl group directly bonded to a stereogenic carbon center may be solely responsible for the phenomenon observed. To evaluate the generality of this finding we studied the chromatographic behavior[14] of a series of compounds that contained a trifluoromethyl group directly bonded to a stereogenic center, which were either commercially available or readily prepared by synthetic methods. The results were truly impressive, as in all the examples studied so far (Scheme 4) we observed a substantial enantiomer self-disproportionation effect. Considering that these examples include different classes of compounds, such as amines **10**, alcohols **11**, β-amino acids **6**, α-amino acids **12**, α-hydroxy acids **13**, and amino alcohols **14**, the enantiomer self-disproportionation effect should be generally expected for compounds containing a trifluoromethyl group directly bonded to a stereogenic carbon center.

The true nature of the role of a trifluoromethyl group (or, more generally, a perfluoroalkyl group) may be quite



Scheme 4. Compounds with a trifluoromethyl group directly bonded to a stereogenic center. Bn = benzyl.

complicated and awaits a comprehensive study. However, at this point we can suggest a general feature that should be taken into consideration by any plausible model for explaining enantiomer self-disproportionation on trifluoromethyl-containing compounds. It is well documented[16] that in transition states or supramolecular associations a trifluoromethyl group tends to be in a position as far as possible from another trifluoromethyl group or any negatively charged substituents. Therefore, it is reasonable to assume that this principle will also be valid for the formation of the corresponding dimers, trimers, and so on. Thus, by considering the possible arrangements of trifluoromethyl alcohol molecules through hydrogen bonding, one can envision two models that describe heterochiral and homochiral interactions



Scheme 5. Models of heterochiral and homochiral interactions of trifluoromethyl alcohol molecules.

(Scheme 5). As can be clearly seen in Scheme 5, the trifluoromethyl groups point away from each other in the heterochiral *R*,*S*,*R*,*S* arrangement, which minimizes the destabilizing electrostatic repulsive interactions. By contrast, in the homochiral arrangement the trifluoromethyl groups of molecules **B** and **C** point toward each other, thus rendering this association unstable. Therefore, we assume that heterochiral interactions would lead to the formation of macromolecular structures of relatively high molecular weight, whereas only dimers would be expected in the case of homochiral interactions. This assumption is in perfect agreement with the experimental data, which show that the excess enantiomer of alcohol **11** is always eluted first followed by the more racemic fractions.[17]

In summary, we have demonstrated that compounds containing a trifluoromethyl group directly bonded to a stereogenic carbon center are prone to a strong enantiomer

self-disproportionation effect on achiral silica gel. One of the serious consequences of this finding is that all fluorine-containing chiral reagents and drugs currently on the market, as well as all previously reported literature data on the stereochemical outcome of asymmetric transformations involving fluorine-containing compounds, should be carefully reevaluated. On the basis of the results described herein and the literature data,[6–10] one may conclude that any type of column chromatography is not a safe method for the purification of nonracemic mixtures in the determination of their enantiomeric purity. We would like to suggest that chemists dealing with nonracemic compounds should first run an "enantiomer self-disproportionation test" before using chromatography for any purification steps. As shown herein, an increase in the polarity of an eluent usually leads to elimination of the enantiomer self-disproportionation effect. Once a "safe" eluent has been identified, chromatography can be used for sample purification. The full scope of the enantiomer self-disproportionation induced by trifluoromethyl and other polar groups, and application of this effect to the purification of nonracemic compounds using the internal chirality of a sample, are currently under study.

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[15] In three previous examples of self-disproportionation of enantiomers on achiral silica gel, the greatest *ee* percentage differences between the lowest and highest *ee* values obtained were: 83%,[10a] 31%,[10b] and 21%.[10c]

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