# Final report about the work and results carried out between 2017-2022 of the project entitled " Investigation of asymmetrical compounds and their non-linear interactions"

For decades, our research group has been dealing with the separation of racemic compounds (1:1 enantiomer mixtures) with producing pure enantiomers that are essential for the pharmaceutical industry. To this end, we have developed various methods and procedures over the years. The procedures are based on forcing the distribution of diastereomeric salts formed during the resolution between two phases (which can be e.g. liquid-liquid, solid-liquid).

During our work, we not only investigated the preparation of the enantiomers of racemic compounds through the processes that take place during the separation of diastereomeric salts and enantiomeric mixtures but also by observing and (re)interpreting the ongoing reactions and laws, we tried to explain the processes taking place and establish a possible mechanism.

We know that the behaviour of diastereomeric salts and enantiomeric mixtures cannot be described by linear correlations (Figure 1.). During the separation of the enantiomeric mixtures, it was found that in this case, either the enantiomeric excess or the racemic fraction crystallizes. Still, it is a non-linear function of the enantiomeric ratio of the starting mixture. A specific characteristic of the enantiomeric mixtures is that the composition of the phases changes below and above the eutectic composition during the distribution between the two phases.

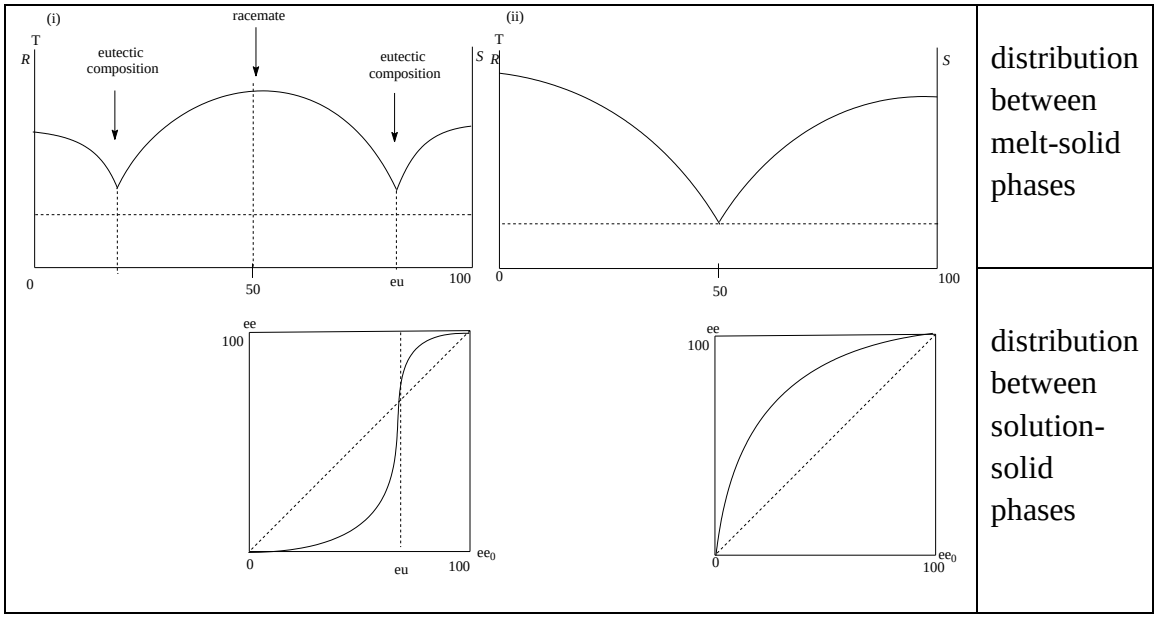



Figure 1. Nonlinear behaviour of enantiomeric mixtures in case of distribution between two phases

If the starting enantiomeric purity ($ee_0$) is above the eutectic composition ($ee_{Eu}$), then the purity of the crystalline precipitation will be higher than the starting composition, but if the enantiomeric purity is under the eutectic composition ($ee_0 < ee_{Eu}$), a less pure crystalline phase

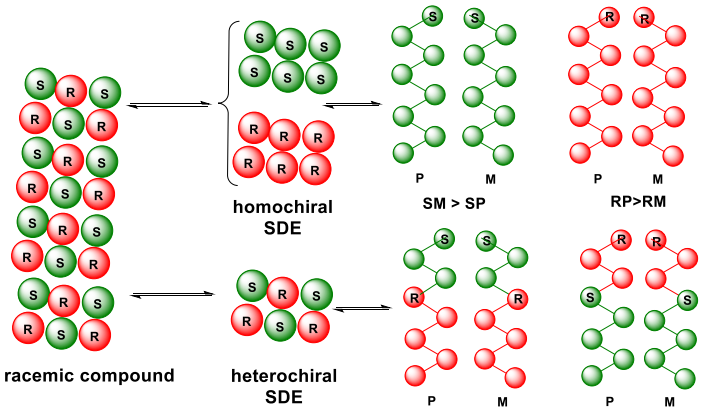
will be obtained. From this, we came to the conclusion that the eutectic composition of the enantiomer mixtures has a decisive role during the separations. So we also wanted to investigate the role of this.

We have observed that in the case of diastereomeric salt-forming resolutions, the composition of the enantiomeric mixture ($ee_{Dia}$) separated from the crystalline diastereomer often approaches (well matches) either the eutectic composition of the racemic compound ($ee_{EuRac}$) or the resolving agent ($ee_{EuResAg}$), i.e. $ee_{Dia}$~$ee_{EuRac}$ or $ee_{Dia}$~$ee_{EuResAg}$.

Examining the results of our previous works from this point of view as well (45 successful resolutions), we found that the average value of the known eutectic composition of the investigated racemic compounds is $ee_{EuRacAv}$ ~ 73%, while the average of the obtained crystalline diastereomeric salts is $ee_{DiaAv}$ ~ 78%.

In the case of 29 resolutions, the average of the eutectic composition of the used resolving agents ($ee_{EuResAgAv}$) is 78%, while the average of the enantiomeric composition of obtained crystalline diastereomeric salts ($ee_{DiaAv}$) is 80%. So at these resolutions, the $ee_{EuResAg}$ was the determinant of $ee_{Dia}$. [1]

We believe that homo- and heterochiral supramolecular associates with a diastereomeric relationship are formed in solutions of enantiomeric mixtures. Thus, the less soluble or faster crystallizing diastereomeric associations with a higher concentration will be crystallized. These are supramolecular helical, double helix-forming associates with M and P helicity. This M and P helicity enables the formation of mirror-image crystals from the enantiomeric mixtures (Figure 2).



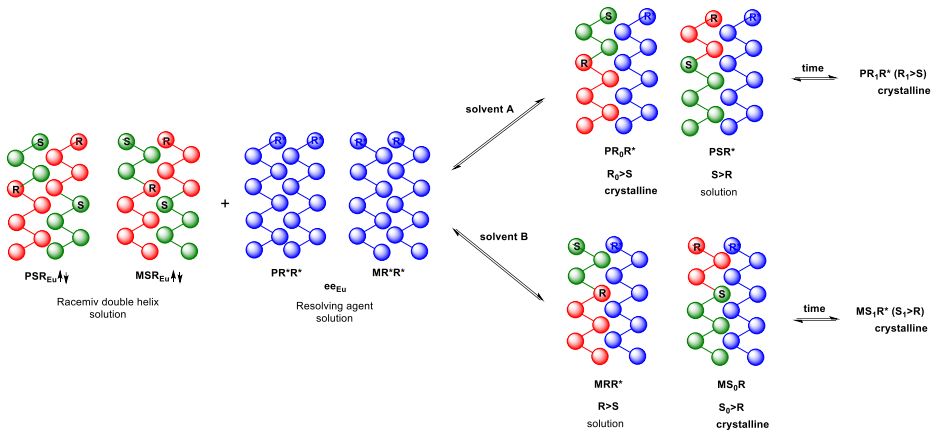



Figure 2. Schematic presentation of the connection of supramolecular associates having M and P helicity formed in solution

Based on the observations of others and our own, we can conclude that supramolecular homo- and heterochiral associations with P and M helicity are formed in solutions of enantiomeric mixtures. At the same time, the solution of the "single" enantiomer also contains the homo- and heterochiral P and M associations. So it is understandable that the crystallization from the solution of the racemic compound and the resolving agent can be determined by the eutectic composition of the racemic compound or the self-reproduction ability of the resolving agent [2]

Based on these, the mechanism of diastereomeric salt precipitation can be explained by the appearance of homo- and heterochiral P and M double helix associations. So, depending on the conditions (solvent, crystallization time, etc.), the formation of $ee_{Dia}$ can be determined by homo- and heterochiral P and M associations of both enantiomers present in the racemic compound, if $ee_{Dia} \sim ee_{EuRac}$. In the case of $ee_{Dia} \sim ee_{EuResAg}$, the P and M associates of the resolving agent (the single enantiomer) are decisive, so the resolving agent carries the code of its enantiomeric mixture even if its enantiomer pair is not present [1].

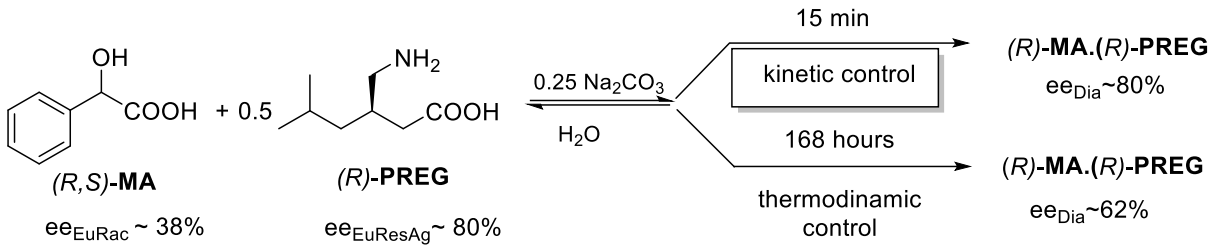
This is possible because the single enantiomer can create a mixture of P and M helicities with its structure, which will be in a similar ratio as when its mirror image pair was next to it. The ratio of conformers in the enantiomeric solution will be the same as the ratio of the conformers formed in the solution of the racemic compound.

The resolving agent can remember and form its racemic compound's M to P ratio. In the next step, it reacts in this ratio with the enantiomeric associations of the racemic compound, and thus $ee_{Dia} \sim ee_{EuRes}$ can be formed [5, 10, 12].

As the eutectic composition of enantiomeric mixtures of chiral compounds determines the distribution/stoichiometry between the phases, different conditions (pH, solvent, crystallization time) can influence the formation of this stoichiometry (sometimes they can even reverse it) [3, 4, 9].

We realized that under kinetic control, the eutectic composition of the resolving agent could be decisive (Figure 3) [5, 7, 12, 13]. We proved with experiments that the favourable effect of kinetic control could be maintained with ultrasound irradiation [3]

a)




b)


Figure 3. Kinetic control during the resolution of a) racemic mandelic acid (**MA**) with pregabalin enantiomer and b) of racemic Anara with tartaric acid (**TA**)

At the same time, we also reported on the influence of the thermodynamic [14] control (Figure 4) on the final result in several publications.

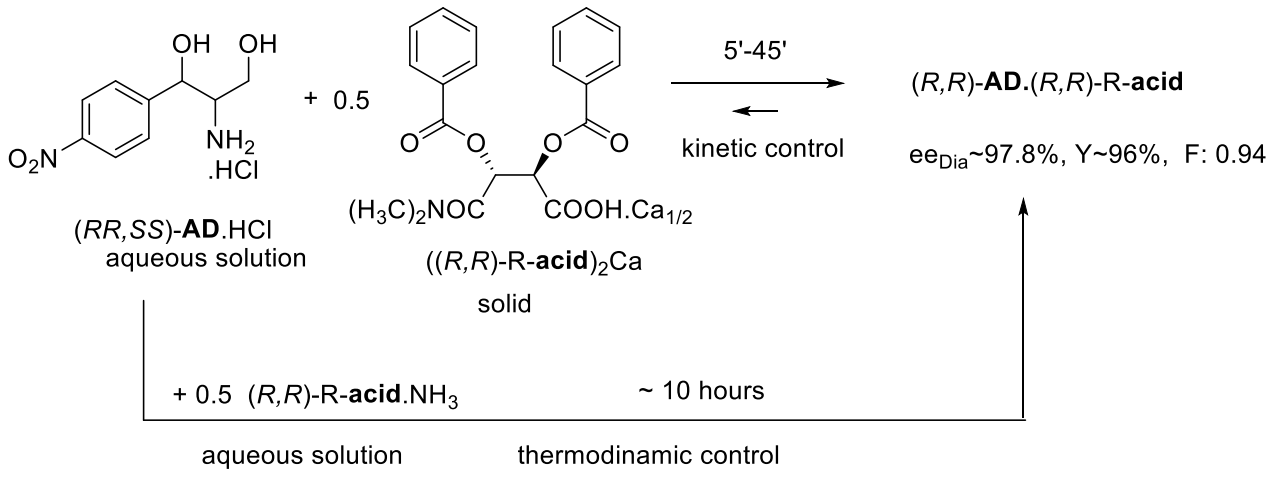



Figure 4. The effect of thermodynamic control in case of resolution of the racemic aminodiol (**AD**) with tartaric acid

The effect of solvents was observed in several ways. Changing the used solvent, made it possible to change the stoichiometry of the crystalline diastereomer in several cases (Figure 5.) [15]. We noticed the importance of solvate formation when this was the condition for the crystallization of the diastereomer (Figure 5, Figure 6) [10, 15].

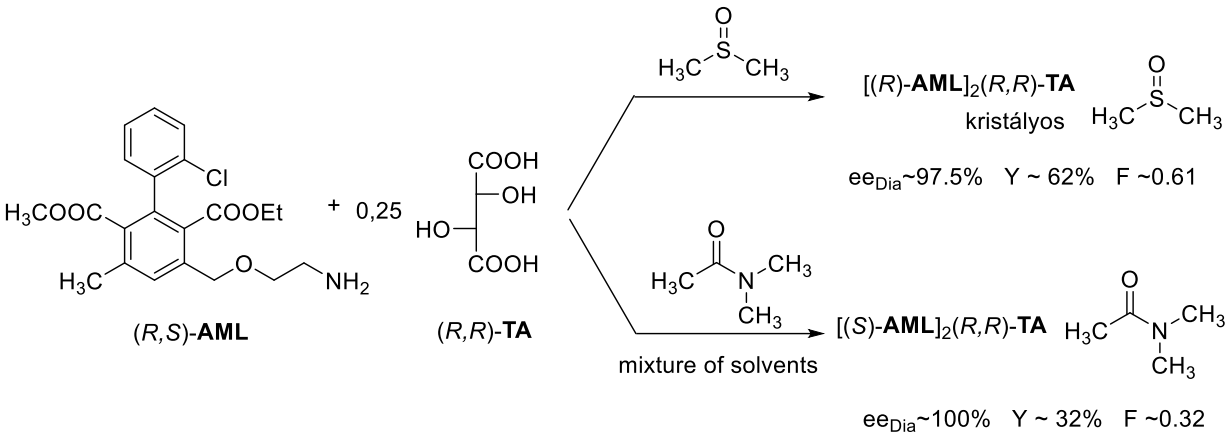



Figure 5. Resolution of amlodipine (**AML**) with tartaric acid

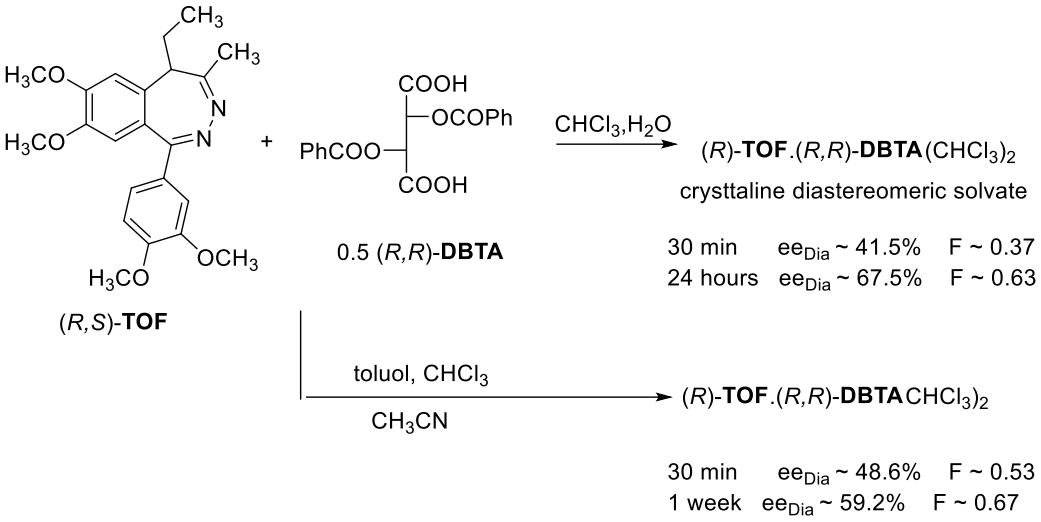



Figure 6. The resolution of Tofizopam (**TOF**) when it became clear that the presence of solvate-forming chloroform in the system is essential for the resolution to work

We also realized that traditional solvents could be used instead of solvate-forming solvents. Still, the solution must contain nonsolvent-forming compounds with a molecular structure similar to solvate-forming solvents, such as urea or thiourea [15], which plays the role of co-crystal (Figure 7.).

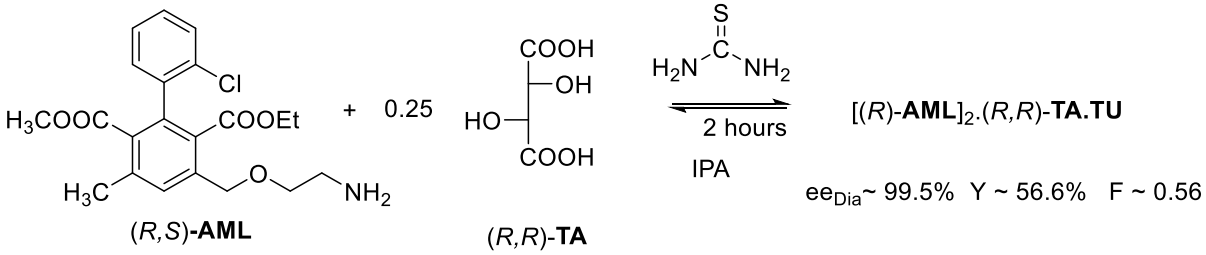



Figure 7. Resolution of Amlodipine in a conventional solvent using a co-crystal.

The positive effect of the presence of achiral compounds with a related molecular structure on the final result can also be observed at the resolution of free amino acids [4].

In the course of our work, we have developed several new methods to increase the efficiency of separations. Such was the case, for example the so-called tandem resolution, when by supplementing the mother liquor of the resolution with the same resolving agent, we succeeded in obtaining the other enantiomer (Figure 8.) [15]

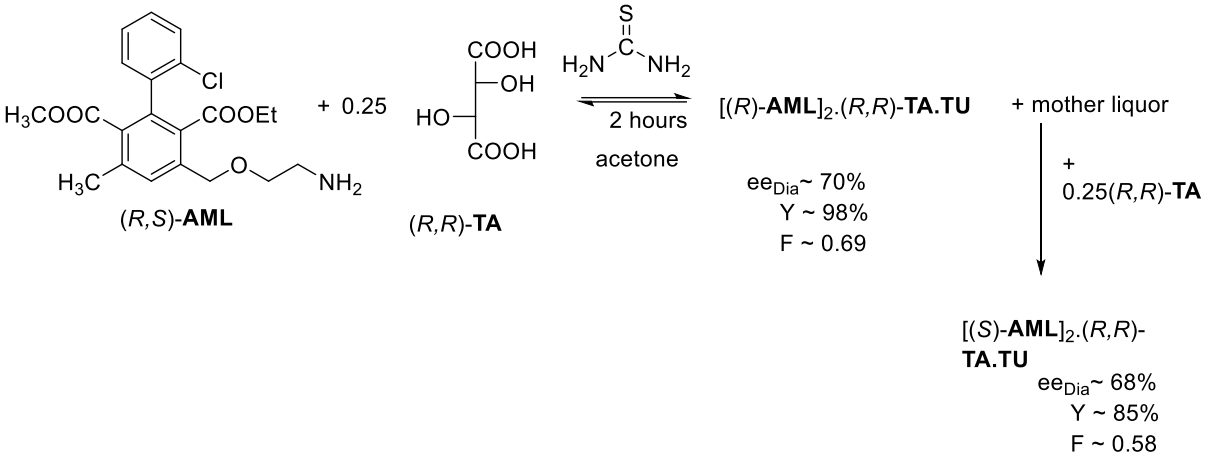



Figure 8. Tandem resolution in the case of Amlodipine (**AML**)

By using quasi-racemic and quasi-enantiomeric resolving agents, we manage to achieve more favorable results [10, 16]. We also observed that the efficiency could be improved using the Na salt of tartaric acid and its derivatives [16].

During this period 9 thesis (5 BSc and 4 MSc) related to the research topic were preparedas a Phd thesis is compiling as well.

We reported on our results in oral presentations held at 5 international (2017 Paris, 2018 Timisoara, 2019 London, Cluj (Pálovics E.) and at 2 Hungarian conferences (Student, Pécs) (poster Cluj (SD))

**Publications**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