Resolution of Racemates: Did You Say "Classical"?

André Collet*

Discovered by Pasteur in 1853, the "classical resolution" method rests upon the combination of a racemate with one enantiomer of a chiral substance (resolving agent) to give a 1:1 mixture of (p,n)-diastereoisomeric salts, which are then separated by crystallization techniques. In spite of its somewhat ambiguous—and no longer justified—reputation of being more an art than a science, classical resolution is still essential in the preparation of a vast majority of the chiral active principles required in enantiopure form for the manufacture of pharmaceuticals and agrochemicals. To cite but a few examples, thousands of tonnes per year of (*S*)-naproxene,[1] D-phenyglycine, and D-4-hydroxyphenyglycine[2] are produced by separation of diastereoisomeric salts rather than by alternative processes based on asymmetric synthesis or bioresolutions. There are good reasons for this: in the context of large-scale enantiomer separations, crystallization techniques are often more straightforward and more economical than any other method. This is the fruit of the labor of academic and industrial researchers in the last decades to gain a better understanding of the physical chemistry concepts upon which separation of diastereoisomers by crystallization is based.[3, 4]

Utilizing phase diagrams generally speeds up the selection of a good resolving agent and the determination of the best crystallization conditions; industrial applications developed in less than six months are not the exception. Three basic types of phase diagrams exist for describing the solid–liquid equilibria for diastereoisomer mixtures. The simple eutectic, without terminal solid solutions, is the most interesting for separation purposes (Figure 1 a). Here the least soluble diastereoisomer (p) will be obtained in pure form after a single crystallization from the 1:1 (p,n) mixture of composition O. The expected yield is $Y = 100 \times (\mathrm{OE/Ep})/C_\mathrm{O}$, where C_O is the concentration of mixture O. This yield approaches its maximum value of 0.5 (or 50 %) when the eutectic E is close to the edges of the phase diagram. Such "good systems" probably represent less than 20 % of the cases. They lend themselves to very economical commercial applications (e.g. naproxen). Often eutectics are complicated by the presence of terminal solid solutions (Figure 1 b). Then several recrystallizations may be required to raise the enantiomeric purity to an acceptable level, and the final yield is less than that given by the above relationship. Terminal solid solutions in diastereoisomer phase diagrams are often major issues in industrial applications.

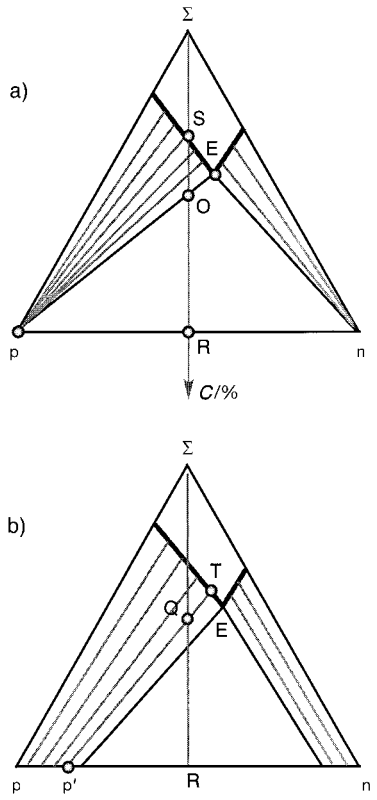


Figure 1. a) Ideal solubility isotherm for (p,n)-diastereoisomers forming an eutectic in solvent Σ. The less soluble diastereomer (p) can be obtained in pure state from solutions of composition between O and S. The maximum yield (Y) in p is obtained from solution O (see text). In the case shown, Y would be around 25 %, a figure which seems relatively common. b) Eutectic phase diagram with terminal solid solutions on both the p and n sides. On crystallization, a system of composition Q will give an impure solid (p′) in a liquid of composition T.

Cocrystallization of the two diastereoisomers to form a continuous series of solid solutions is also relatively common. Such systems are generally unsuitable for resolution, at least in the context of commercial processes.[5] The formation of 1:1 addition compounds (double salts) is also relatively frequent.

[*] Prof. Dr. A. Collet
École normale supérieure de Lyon
Stéréochimie et Interactions Moléculaires (UMR CNRS 117)
F-69364 Lyon Cedex 07 (France)
Fax: (+33) 472-72-84-83
E-mail: andre.collet@ens-lyon.fr

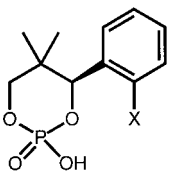
  1433-7851/98/3723-3239 $ 17.50+.50/0

Here no separation of the diastereoisomers is possible by crystallization of their 1:1 mixture.

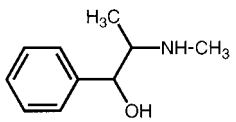
This is why the selection of a good resolving agent, in other words, one which will lead to a very dissymmetric eutectic phase diagram, if possible without terminal solid solutions, is of great importance. In general, this crucial selection is best made by trial and error. Even though such protocols can be made very systematic and efficient,[6] their success is not predictable. There are no established guidelines for predicting what will be the best resolving agent for a given substrate, except perhaps analogies, which may be valid within series of closely related substrates (for instance simple substituted mandelic acids are generally resolved with ephedrine). Although insolvable cases remain the exception (at least in my own experience), in many instances for which classical resolutions have been successfully devised the actual yield is not always close to the 50% limit, a circumstance that may be problematic in commercial applications. This is mostly due to the lack of time for screening enough resolving agents, and also to the limited number of commercially available resolving agents.

A recent paper by Vries et al.[7] conveys a new approach to this long-standing problem. The starting idea has something naively obvious: "It was reasonable to assume that the simultaneous addition of several resolving agents might materially shorten the time required for the hit-and-miss method of finding a resolving agent." Is this idea really as reasonable as it would seem? At first glance a combinatorial version of the usual protocol has little chance of success. The chance to get crystalline materials from such multicomponent mixtures must be very low, because the effective concentration of a given diastereoisomeric salt is reduced by a factor of 3 when a mixture of three resolving agents is used instead of a single one. Accordingly, only exceptionally insoluble salts can be selected by this method. In fact, the authors readily discovered that the use of random combinations of resolving agents did not work so well.

Things improved dramatically, however, when they started to utilize "mixes" consisting of several resolving agents of the same family (usually three). For example, the 1:1:1 mixture of three differently substituted phosphoric acids of the same configuration (**P** mix) constitutes such a family. Ten such



"P mix"
X = H, Cl, OCH_3



(±)-1
ephedrine (1*R*,2*S*)

ternary mixes are presented in the paper by Vries et al.[7] Some are acidic resolving agents, others are basic. In this context, the term "family" is very restrictive: the components of the mix are not only structurally and chemically related, but they are also almost isosteres.[8] For example, tartaric acid is not a member of the dibenzoyltartaric acid family.

According to the authors, when such a mix is added to a solution of a racemic substrate, a crystalline salt usually precipitates immediately. In most cases the substrate contained in the precipitated salt is resolved to about 90–98% *ee*. The important finding is that the salt always contains several resolving agents, which are present in a nonstoichiometric ratio. For instance, (±)-ephedrine ((±)-**1**) combined with the previously mentioned **P** mix in 2-propanol affords a salt having 98% *ee* in (+)-**1** and containing the three phosphoric acids of the **P** mix in a 1:1.2:1.1 ratio. In most cases the mixed salts retain a nonstoichiometric composition on repeated crystallizations. In this example the initial composition drifted to 1:14:55 after 20 crystallizations. This means that the mixed salts are not just mixtures of pure diastereoisomeric salts, but more likely consist of a single crystalline entity where the three resolving agents form a solid solution. The similarity of the structures of the components of the mix is in line with this hypothesis, which is also supported by the resolution of the crystal structure of a binary salt of (+)-ephedrine containing almost equimolecular amounts of two of the phosphoric acid components of **P** mix.

Despite the absence of any relevant phase diagrams or solubility data, I have the feeling that the solid-solution hypothesis provides a good key to a consistent interpretation of the phenomenon. Solid solutions often form between closely related chiral structures of like configuration.[8] This is one of the statements upon which Fredga's method is based.[9] This well-known method establishes the relative configurations of structurally related compounds from their melting point phase diagrams. For instance, *o*-chloro- and *o*-bromomandelic acids of the same configuration form such a solid solution. A solid solution may have a lower solubility and a greater density than the pure components. This is easy to understand: in a single-component crystal, the packing efficiency is restricted to a certain limit by the lattice symmetry requirements, while in a solid solution, even though the average crystal symmetry is maintained, the local symmetry is lowered due to the present of different species. This situation may allow a closer packing, hence a greater density. In turn, a closer packing should result in a better selectivity towards the configuration of the substrate. In other terms, terminal solid solutions between p and n salts (Figure 1b) would form less easily when a mix rather than a single resolving agent is utilized. This is perhaps the origin of the high *ee* values observed for the first precipitated mixed salts.

At this stage, it would be necessary to have more information on the physical properties of the various components of these complex systems and of their mixtures. Thanks to these discoveries, a new field of exploration is now wide open, which should consider various aspects of the question including phase equilibria, crystallization kinetics, the structures and dynamics of the solid and liquid phases, etc.

Whatever the physical chemical explanation, I tend to believe that the main practical interest in this "family approach" is perhaps not in the speeding up of the process of finding the best resolving agent, but rather in its ability to improve the quality of the diastereoisomeric salts, by allowing a sort of self-optimization of the crystal packing which takes advantage of the presence of several slightly different bricks

  1433-7851/98/3723-3240 $ 17.50+.50/0

instead of a single one. DSM Andeno has used this method to resolve racemates on a scale of hundreds of kilograms.[10] Without a doubt "classical resolution" still has a great future, both on the fundamental and applied sides.

German version: *Angew. Chem.* **1998**, *110*, 3429 – 3431

Keywords: chirality · crystallization · diastereoisomers · enantiomeric resolution · resolving agents

[1] P. J. Harrington, E. Lodewijk, *Org. Process Res. Dev.* **1997**, *1*, 72.
[2] J. Kamphuis, W. H. J. Boesten, B. Kaptein, H. F. M. Hermes, T. Sonke, Q. B. Broxterman, W. J. J. van den Tweel, H. E. Schoemaker in *Chirality in Industry* (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, New York, **1994**, p. 189.
[3] J. Jacques, A. Collet, S. H. Wilen, *Enantiomers, Racemates, and Resolutions*, Wiley, New York, **1981**; J. Jacques, A. Collet, *Reissue with corrections*, Krieger, Malabar, FL, USA, **1994**.
[4] E. L. Eliel, S. H. Wilen, N. Mander, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**.
[5] J. Costante, N. Ehlinger, M. Perrin, A. Collet, *Enantiomer* **1996**, 1, 377 – 386.
[6] A. Collet in *Comprehensive Supramolecular Chemistry, Vol. 10* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, D. N. Reinhoudt), Pergamon, Oxford, **1996**, pp. 113 – 149.
[7] T. Vries, H. Wynberg, E. van Echten, J. Koek, W. ten Hoeve, R. M. Kellogg, Q. B. Broxterman, A. Minnard, B. Kaptein, S. van der Sluis, L. Hulshof, J. Kooistra, *Angew. Chem.* **1998**, *110*, 2491; *Angew. Chem. Int. Ed.* **1998**, *37*, 2349.
[8] For a relevant discussion, see: A. Collet, L. Ziminski, C. Garcia, F. Vigné-Maeder in *Supramolecular Stereochemistry* (Ed.: J. S. Siegel), Kluwer, **1995**, pp. 91 – 110 (NATO ASI Series).
[9] A. Fredga, *Tetrahedron* **1960**, 8, 126.
[10] L. A. Hulshof, Q. B. Broxterman, T. R. Vries, H. Wijnberg, E. van Echten (DSM N.V.), EP-B 0 838 448 A1, **1997** [*Chem. Abstr.* **1998**, *129*, 4278].

In Vitro Evolution and Selection of Proteins: Ribosome Display for Larger Libraries**

Ulrich Hoffmüller and Jens Schneider-Mergener*

One of the most rapidly developing research areas since the beginning of the 90s is the generation and exploitation of molecular libraries (for a review see ref. [1]). The catchword is: combinatorial, paired with chemistry, biochemistry, or biology. Although the applied methods have hardly reached the text books, almost every researcher involved in the fields of chemistry and the biosciences is familiar with the term phage display, peptide, and nucleic acid libraries, or combinatorial chemistry. The common strategy as well as the goal of all chemical and biological approaches is always 1) to generate the largest and most comprehensive pool of molecules possible (the "library") and 2) to search through the library for individual substances with the desired properties such as, for example, for a starting substance (lead structure) for a drug or antibody for diagnostic purposes. Libraries are being used increasingly to identify interaction partners in signal transduction, cell – cell recognition, and immunological control processes. These kinds of approaches accelerate not only the handling of many problems, but also often provide the first possible means to solving them. Therefore, numerous chemists and biologists in industry and university labs have begun to develop novel methods that make it possible to create, analyze, and propagate libraries. The best-know procedure amongst the many described[1] is the so-called phage display method,[2, 3] where the peptide or protein library is expressed on the phage surface. The selection of the desired phage is achieved in vitro, whereas the amplification of the phages takes place in bacteria, that is, in vivo. Here lies a decisive problem with the use of this biologically generated repertoire. The size of the library, is limited by the first essential step of introducing the phage DNA, which contains the genes that code for the individual components of the library, into cells. This step is known as transfection or transformation and permits a repertoire size of between 10^7 and 10^9 different components.[4] In addition, the environment of the host cell imposes an additional selection pressure that may work against the desired variant.[5] A further "disadvantage" of phage display is the time consuming shuttling between in vitro and in vivo steps.

These problems could be avoided if the isolated protein synthesis machinery of the cell (ribosome) was used instead of intact cells for the production of peptide and protein libraries. This was successful first of all in the search for peptides with the desired properties by using the so-called polysome display.[6, 7] A polysome is a complex comprised of one mRNA and a number of ribosomes. A breakthrough was achieved by Hanes and Plückthun[8] who worked out a system for the general application of in vitro selection and evolution of proteins, which they called ribosome display. Selection and evolution systems are based on the following criteria: 1) generation of molecular diversity, 2) selection of molecules with

[*] Priv.-Doz. Dr. J. Schneider-Mergener, Dr. U. Hoffmüller
Institut für Medizinische Immunologie
Universitätsklinikum Charité, Humboldt-Universität
Schumannstrasse 20/21, D-10098 Berlin (Germany)
Fax: (+49) 30-2802-6460
E-mail: jsm@charite.de

[**] We would like to thank A. Warsinke and M. Paschke for critical reading of the manuscript. U.H. was supported by the DFG (INK 16A1-1).

  1433-7851/98/3723-3241 $ 17.50+.50/0