Resolution of Enantiomers

Doç.Dr. Zühal KILIÇ KURT

Resolution of Enantiomers

Many of the building blocks of biological systems such as sugars and amino acids are produced exclusively as one enantiomer. As a result, living systems possess a high degree of chemical chirality and will often react differently with the various enantiomers of a given compound.
Examples of this selectivity include:

- **Flavour:** the artificial sweetener aspartame has two enantiomers. L-aspartame tastes sweet whereas D-aspartame is tasteless.
- **Odor:** *R*-(–)-carvone smells like spearmint whereas *S*-(+)-carvone smells like caraway.
- **Drug effectiveness:** the antidepressant drug Citalopram is sold as a racemic mixture. However, studies have shown that only the (*S*)-(+) enantiomer is responsible for the drug's beneficial effects.
- **Drug safety:** D-penicillamine is used in chelation therapy and for the treatment of rheumatoid arthritis whereas L-penicillamine is toxic as it inhibits the action of pyridoxine, an essential B vitamin.

https://en.wikipedia.org/wiki/Enantioselective_synthesis

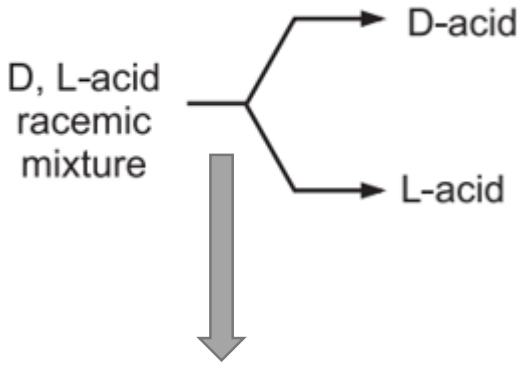
Separation of Stereoisomers

Resolution of Racemic Mixtures: The separation of a racemic mixture into the individual pure enantiomers is called resolution.

Enantiomers have identical physical properties:

- solubility
- boiling point
- chromatographic retention time

they can not be resolved by common physical techniques such as crystallization, distillation or basic chromatography.

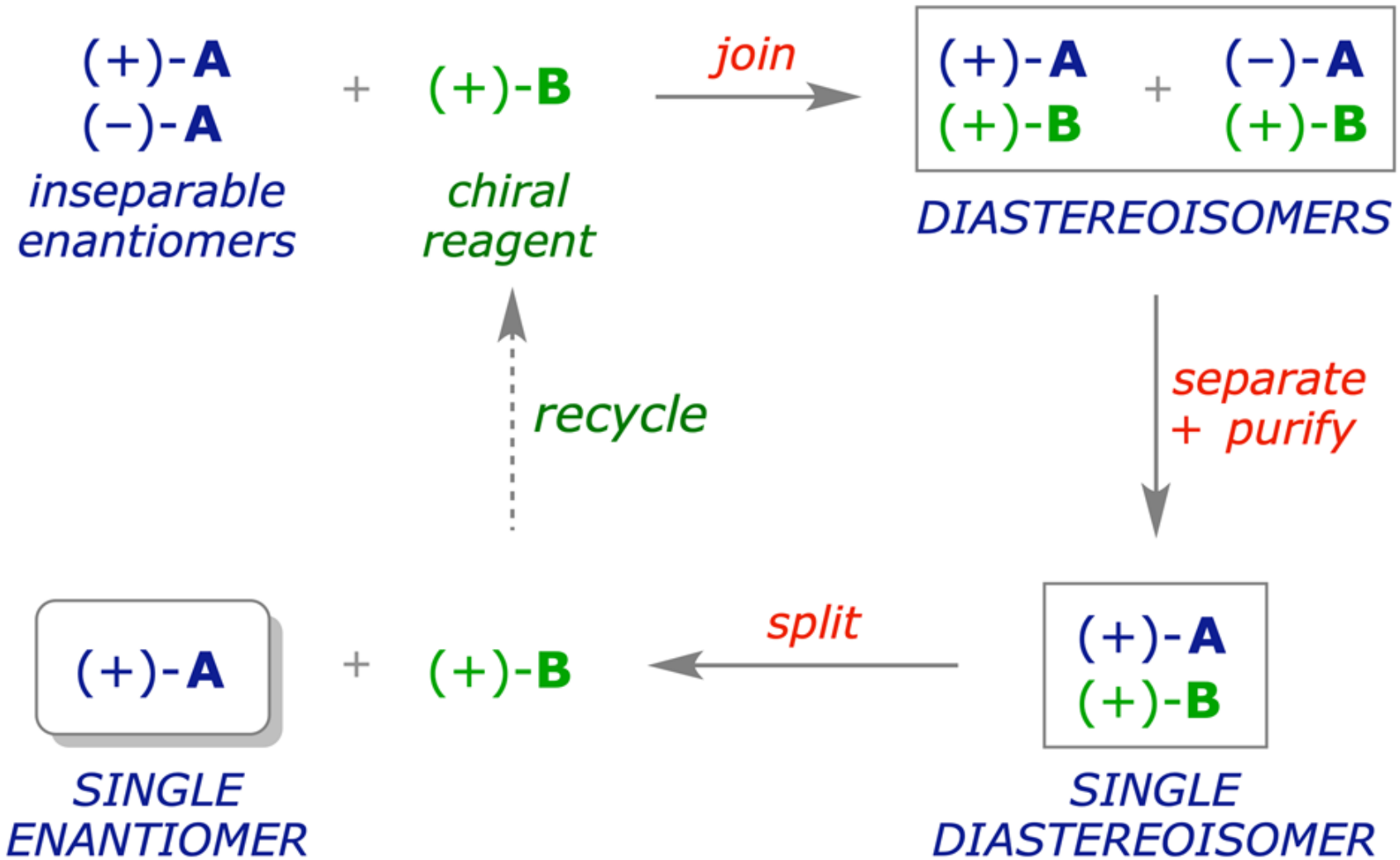


Resolution

Separation of Stereoisomers

- In oder to separate enantiomers, the enantiomeric relationship must be converted into a ***diastereoisomeric*** one.
- Diastereomers have different physical properties and can be separated by conventional physical techniques.
- Diastereomeric interactions are initiated by placing the racemic mixtures in a chiral environment.
- This requires another source of chirality, *e.g.* an enantiomerically pure ***chiral reagent (resolving reagent)***.

RESOLUTION: A GENERALISED SCHEME

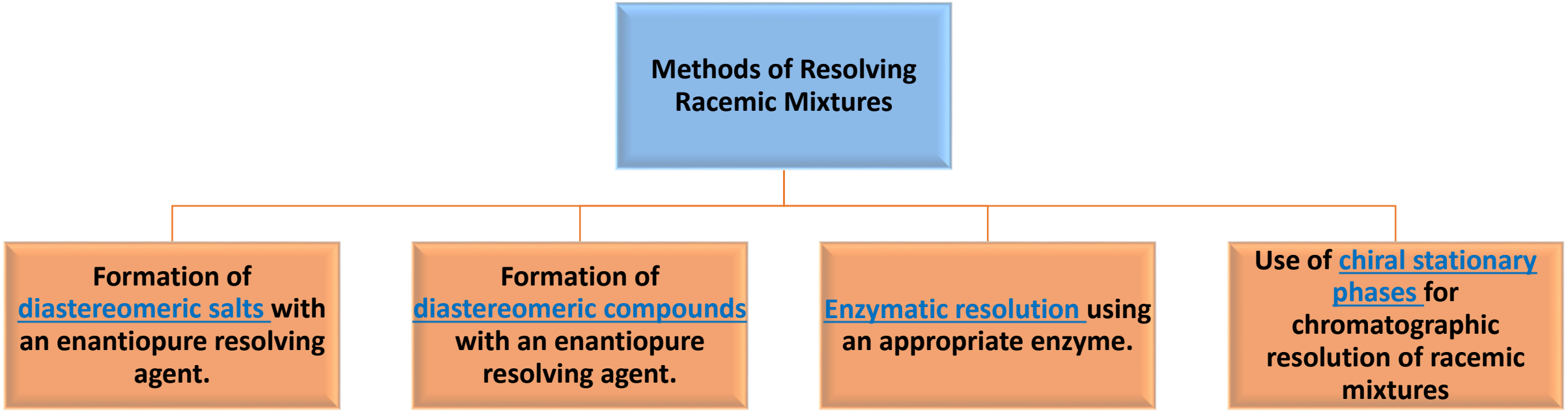


Separation of Stereoisomers

All methods for separating or characterizing enantiomers exploit the formation of diastereomeric interactions.

In principle, diastereoisomeric intermediates resulting from any type of interaction between **A** and **B** might be useful, including:

- **Ionic bonding**
- **Covalent bonding**
- **Weak coordinate bonding** (hydrogen bonds, dipole-dipole interactions, pi/pi interactions, *etc.*)


Methods of Resolving Racemic Mixtures
Formation of diastereomeric salts with an enantiopure resolving agent.
Formation of diastereomeric compounds with an enantiopure resolving agent.
Enzymatic resolution using an appropriate enzyme.
Use of chiral stationary phases for chromatographic resolution of racemic mixtures

Resolution *via* diastereomeric salt formation (ionic bonding)

- The simplest examples of resolution involve the neutralisation of **acids** or **bases** in order to convert them into a separable pair of diastereoisomeric **salts**.

Resolution of racemic acids → using an amine as the resolving agent

Resolution of racemic bases → using an acid as the resolving agent

Resolution *via* diastereomeric salt formation (ionic bonding)

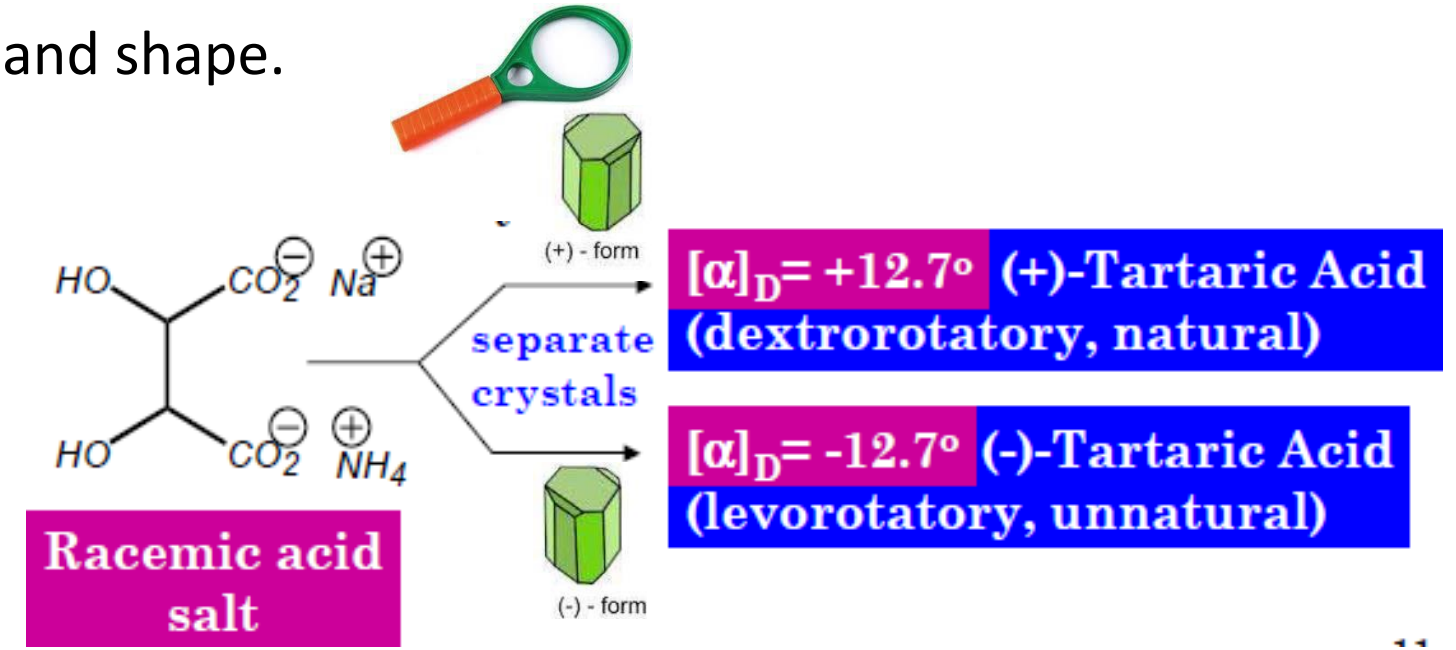
This type of resolution is generally efficient for the following reasons:

- Diastereoisomeric salts can often be separated by crystallisation. Recrystallisation gives high diastereoisomeric purity and the process is practical on a large scale.

- The pure enantiomers can easily be separated from the resolving agent at the end of the process by simple solvent (acid/base) extraction, which is again practical on a large scale.

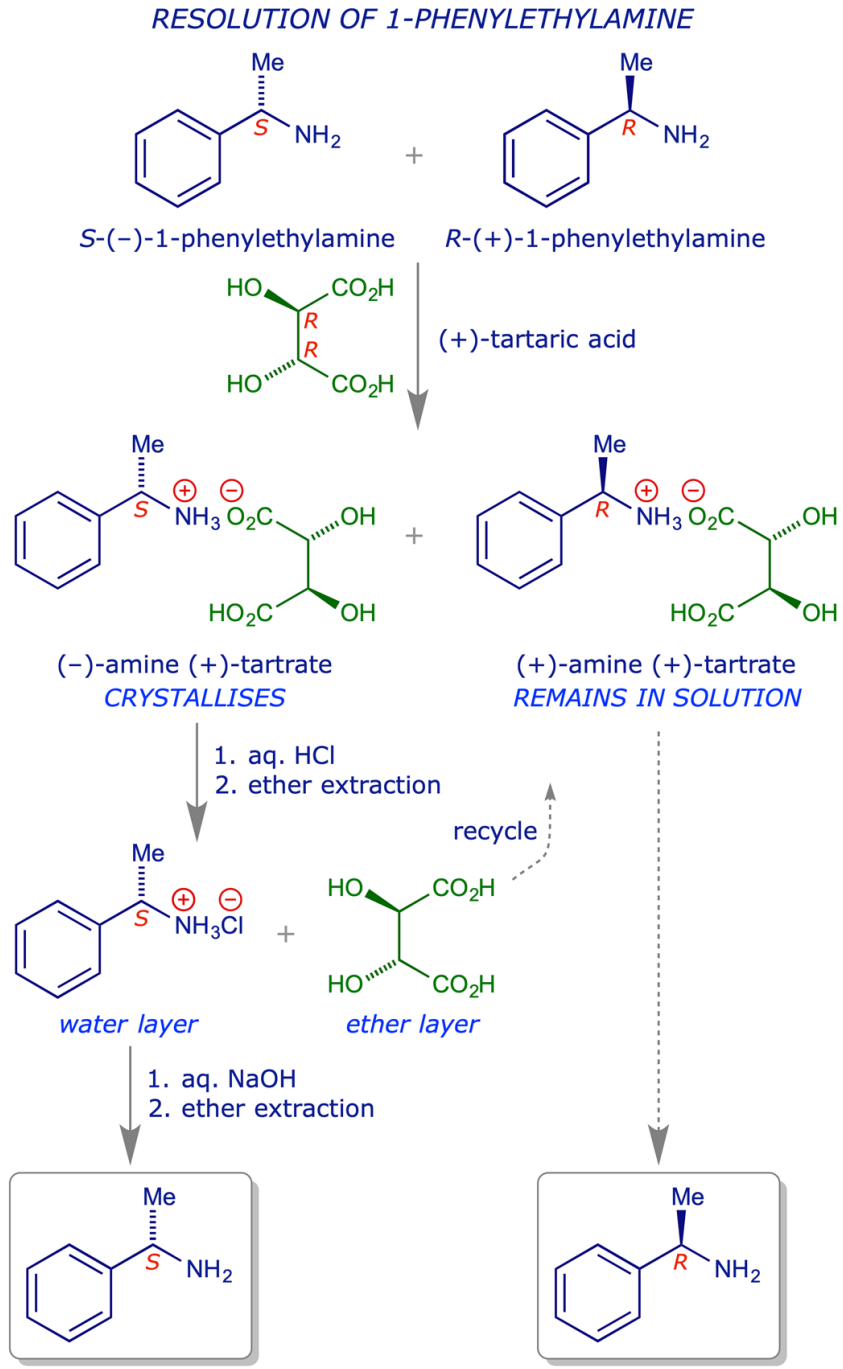
Resolution *via* diastereomeric salt formation (ionic bonding)

- In 1849, Louis Pasteur, in his pioneering work, was able to isolate the enantiomers of tartaric acid because they crystallize from solution as crystals with differing symmetry and shape.

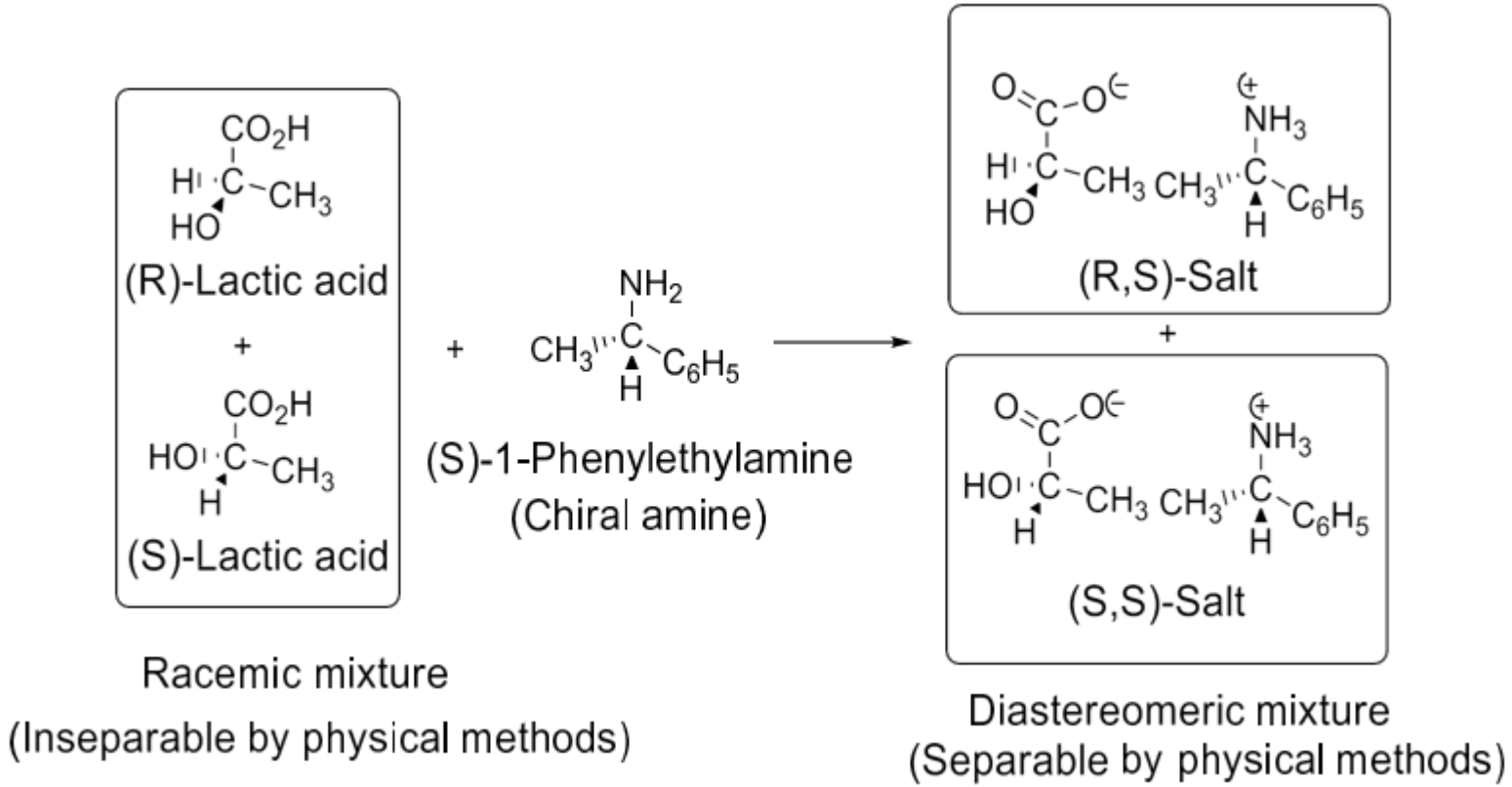


- Due to this observation, concept of enantiomerism was born.

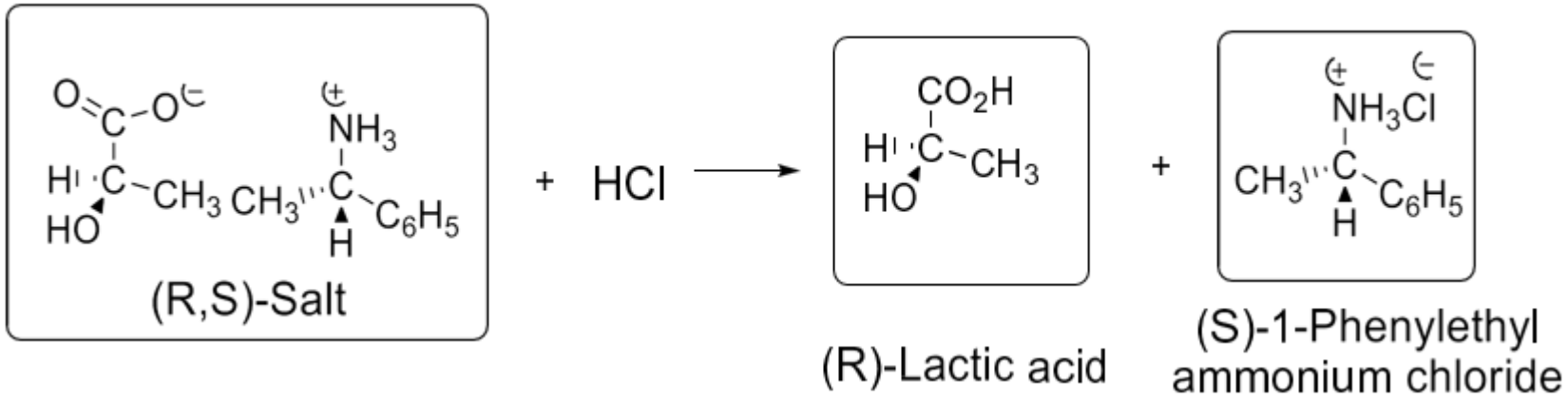
- The title amine has long been recognised as a versatile chiral reagent, both of the pure enantiomers being inexpensive and available in quantity.
- It can be resolved using the natural form of tartaric acid.



- (S)-1-Phenylethylamine combines with a racemic mixture of lactic acid to form diastereomeric salts.
- The diastereomers are separated by fractional crystallization.

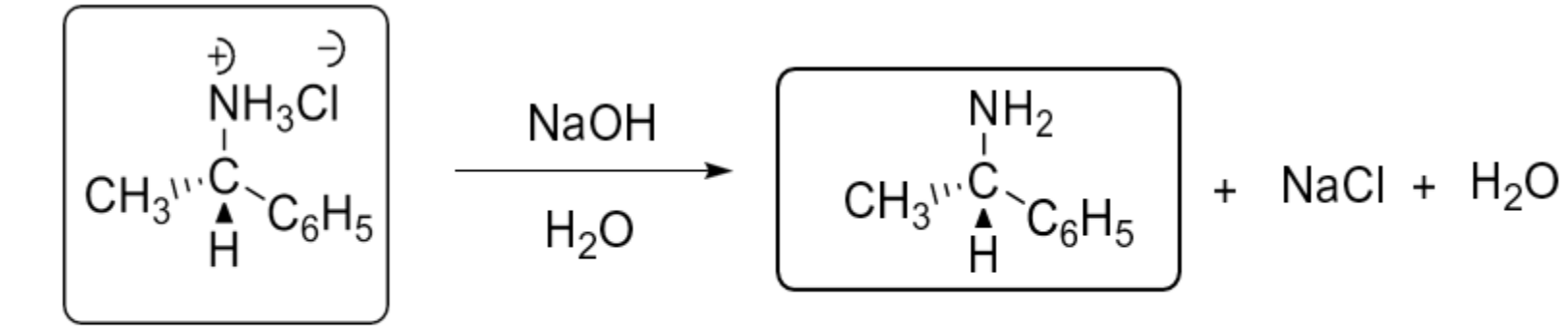


After the separation process, each of the diastereomers is subsequently treated with a strong acid such as HCl to regenerate the corresponding enantiomer of lactic acid.



Note that since lactic acid would be soluble in the organic layer, while the ammonium salt of the resolving agent would be in the water layer, routine solvent extraction with an organic solvent would allow recovery of R-lactic acid.

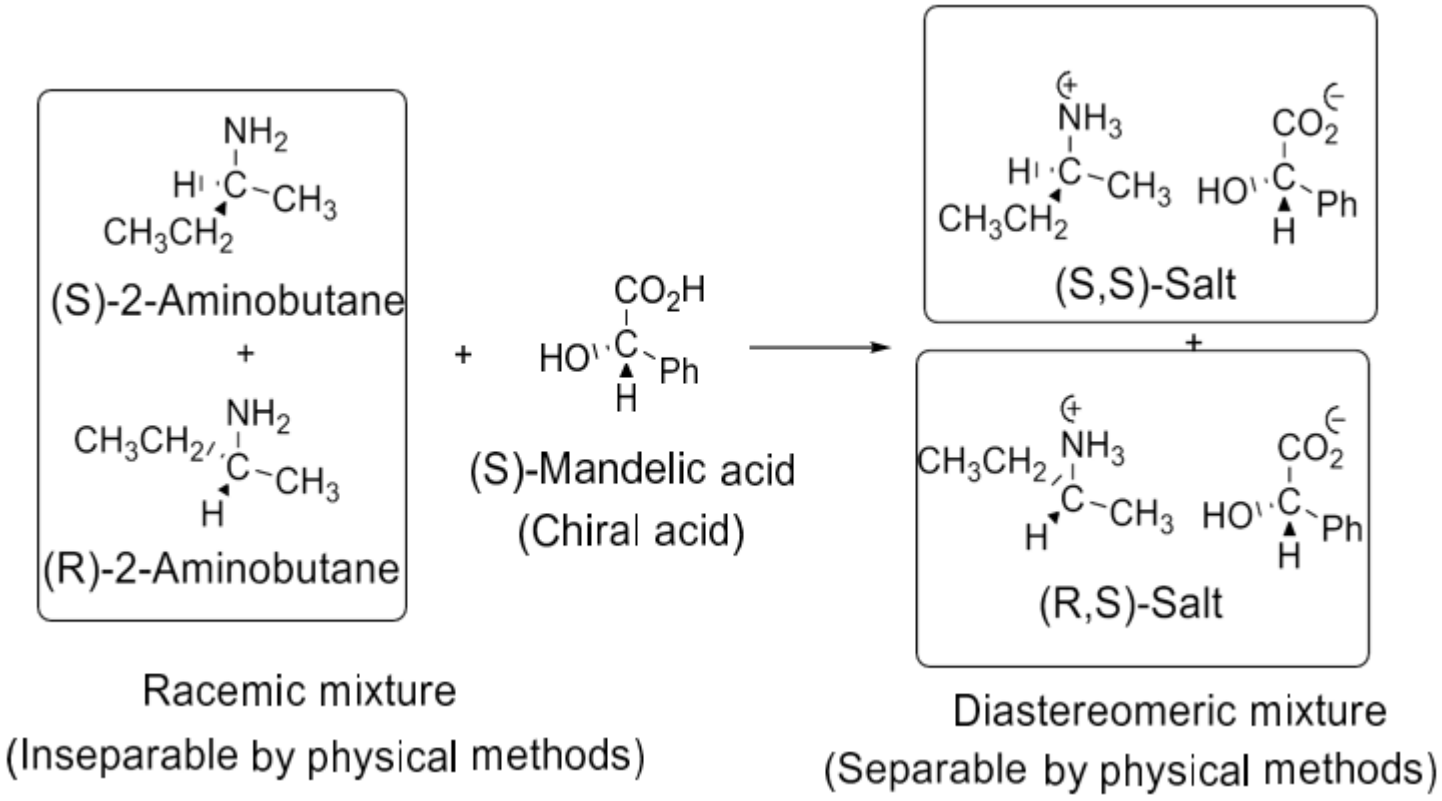
- Since enantiomerically pure compounds are very expensive, it is usually necessary to recover and reuse the chiral amine.
- This is achieved by treating the (S)-1-phenylethyl ammonium chloride salt with a strong base such as sodium hydroxide to regenerate and recover the chiral amine



(S)-1-Phenylethyl ammonium chloride

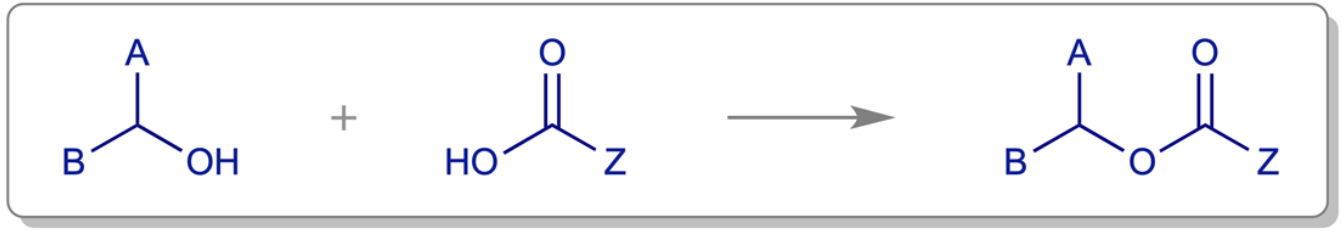
(S)-1-Phenylethylamine (Chiral amine)

The natural enantiomer, (S)-(+)-mandelic acid (sweet almonds), combines with a racemic mixture of 2- aminobutane to form diastereomeric salts.



Resolution *via* diastereomeric compounds formation

- In principle any functional group of a **racemic compound** can be reacted with a **chiral reagent** so as to generate **diastereoisomeric products**, which might then be separable.



(i) (*R*,*S*)-alcohol — *ENANTIOMERS*

(*R*)-carboxylic acid — *chiral derivatising agent*

(*R*)-alcohol (*R*)-carboxylate
(*S*)-alcohol (*R*)-carboxylate
DIASTEREOISOMERS

(ii) (*R*)-alcohol — *chiral derivatising agent*

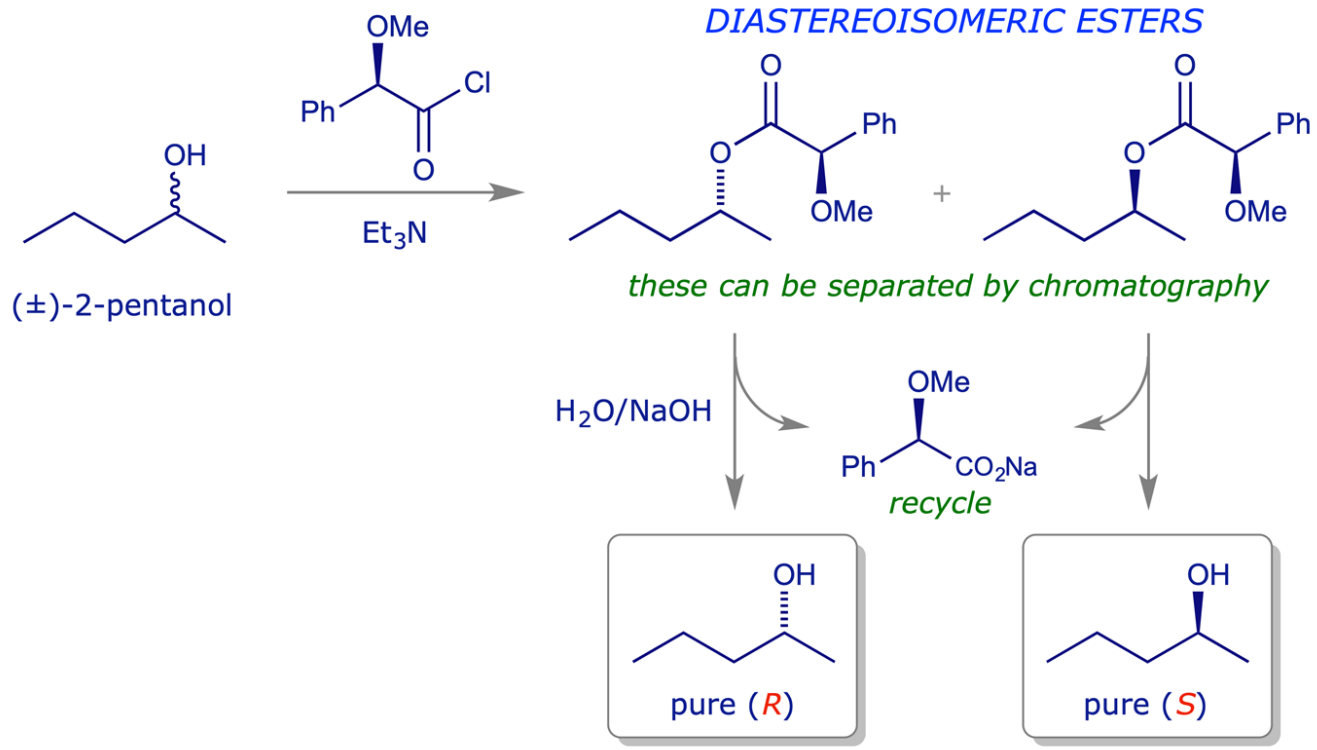
(*R*,*S*)-carboxylic acid — *ENANTIOMERS*

(*R*)-alcohol (*R*)-carboxylate
(*R*)-alcohol (*S*)-carboxylate
DIASTEREOISOMERS

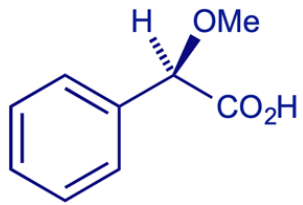
- However, for an effective resolution it must be possible to reverse the reaction used for diastereoisomer formation.

- This imposes a limit on the number of reactions which are useful for resolution purposes, and the most common covalent reaction used is ***esterification***.

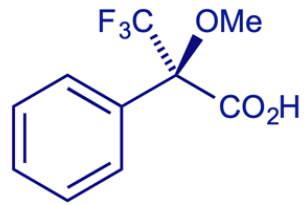
- The chemistry is of course complementary — it can be used to resolve (i) chiral alcohols **or** (ii) chiral carboxylic acids.



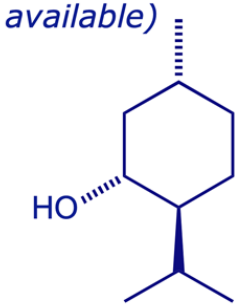
Typical resolving agents (inexpensive, both enantiomers available)



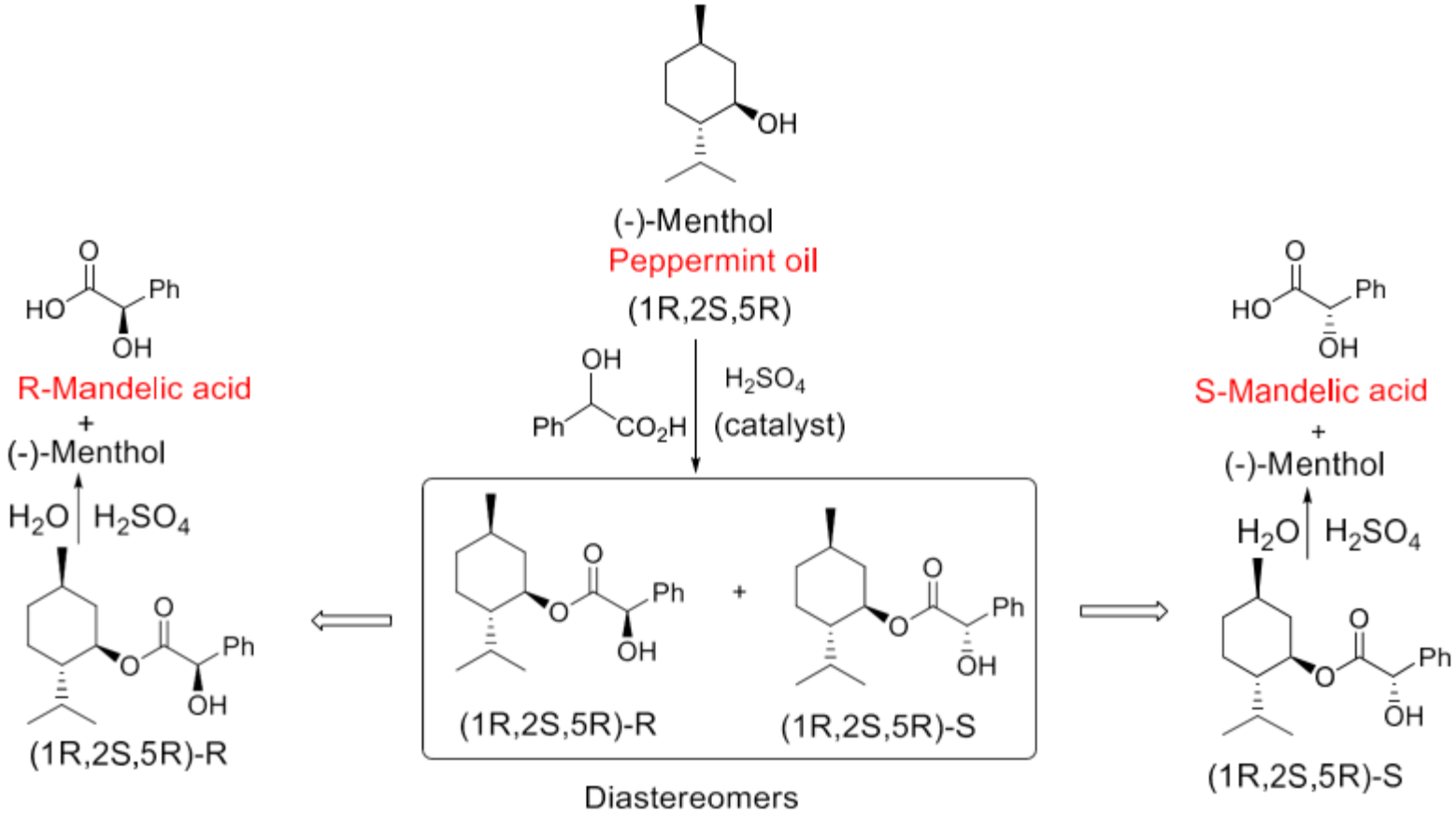
O-methylmandelic acid



Mosher's acid

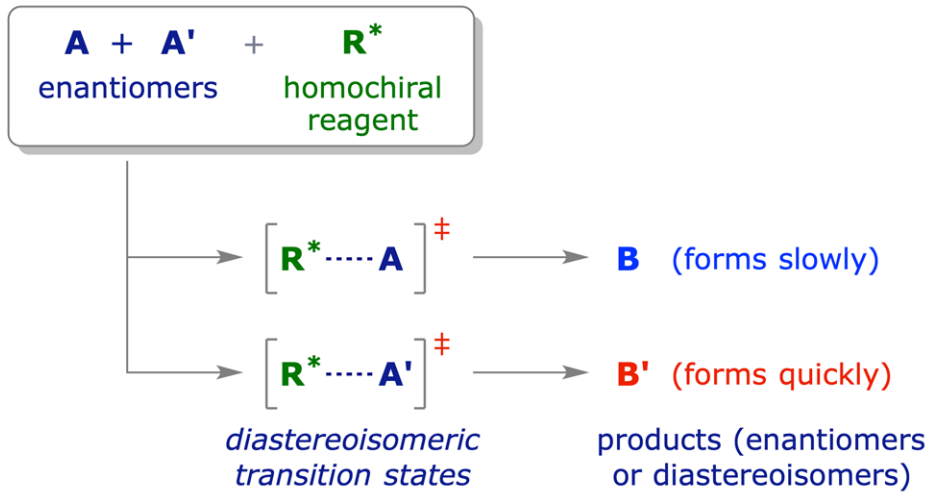


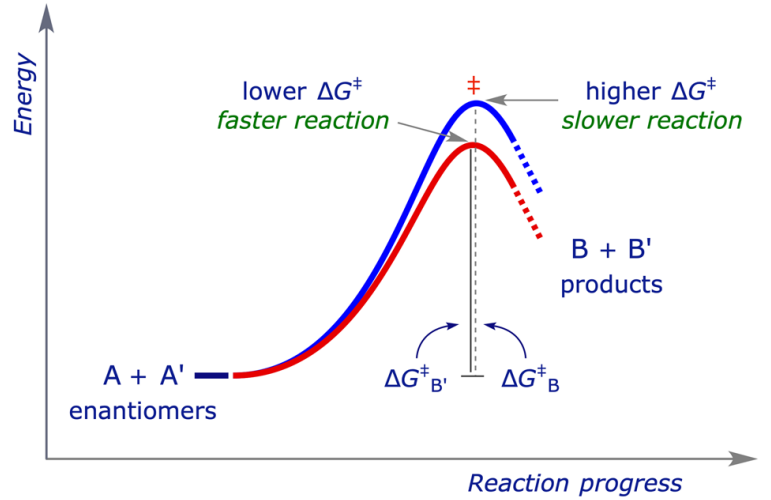
menthol



Enzymatic or Kinetic Resolution

- When a racemic substrate reacts with a homochiral reagent two reactions occur, one with each enantiomer of the substrate.
- Each process has a transition state which incorporates the same enantiomer of the reagent, but opposite enantiomers of the substrate.
- The transition states for the two processes have a ***diastereoisomeric*** relationship, and the respective activation energies are different.
- Therefore the reactions proceed at *different rates*. If the rates of the two reactions are sufficiently different, the reaction can be used for separating the enantiomeric reactants.



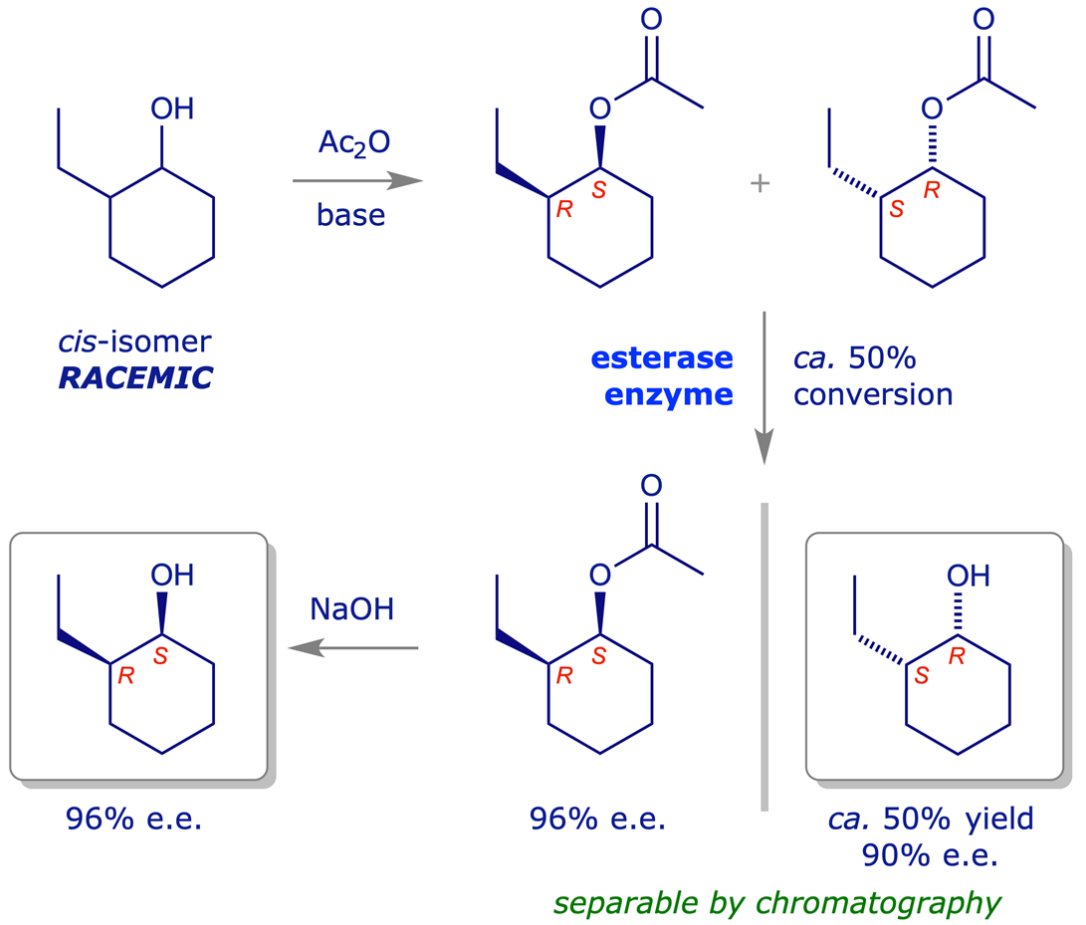


N.B. For a 10-fold rate difference at 25°C, $\Delta\Delta G^{\ddagger} = 5.7$ kJ mol^{-1}

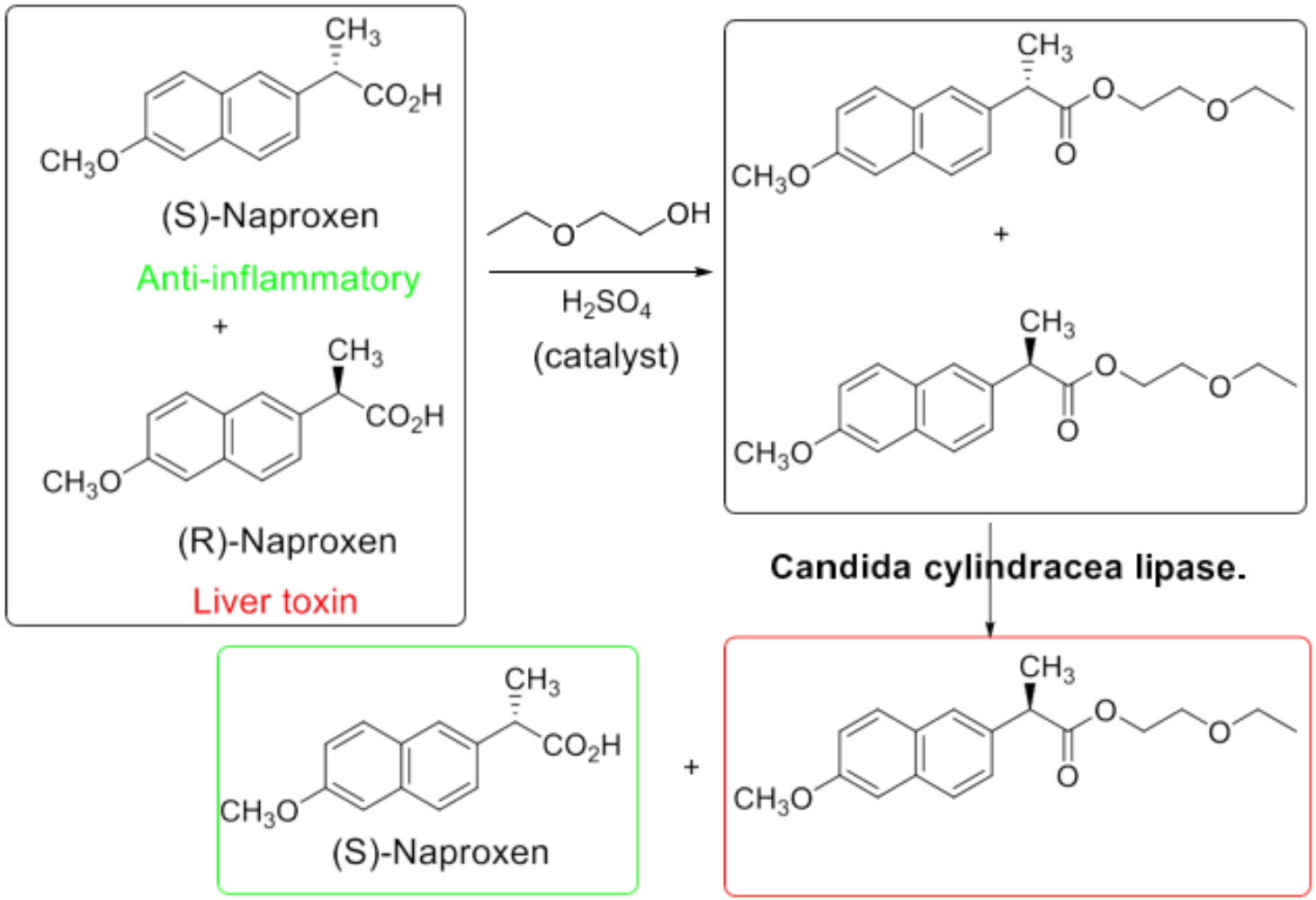
- There are some chemical processes which provide effective kinetic resolution, but the commonest examples involve the use of ***enzymes***.
- Being themselves chiral, enzymes can discriminate between enantiomers,
- If the diastereoisomeric transition states formed when each enantiomer binds to the enzyme are significantly different in energy, the enantiomers will be 'processed' by the enzyme at different rates.

- **Ester hydrolysis** (or **esterification**) using an esterase enzyme can be particularly effective, proceeding with high selectivity under mild conditions and at various scales.

- It is one of the most commonly used kinetic resolution processes.



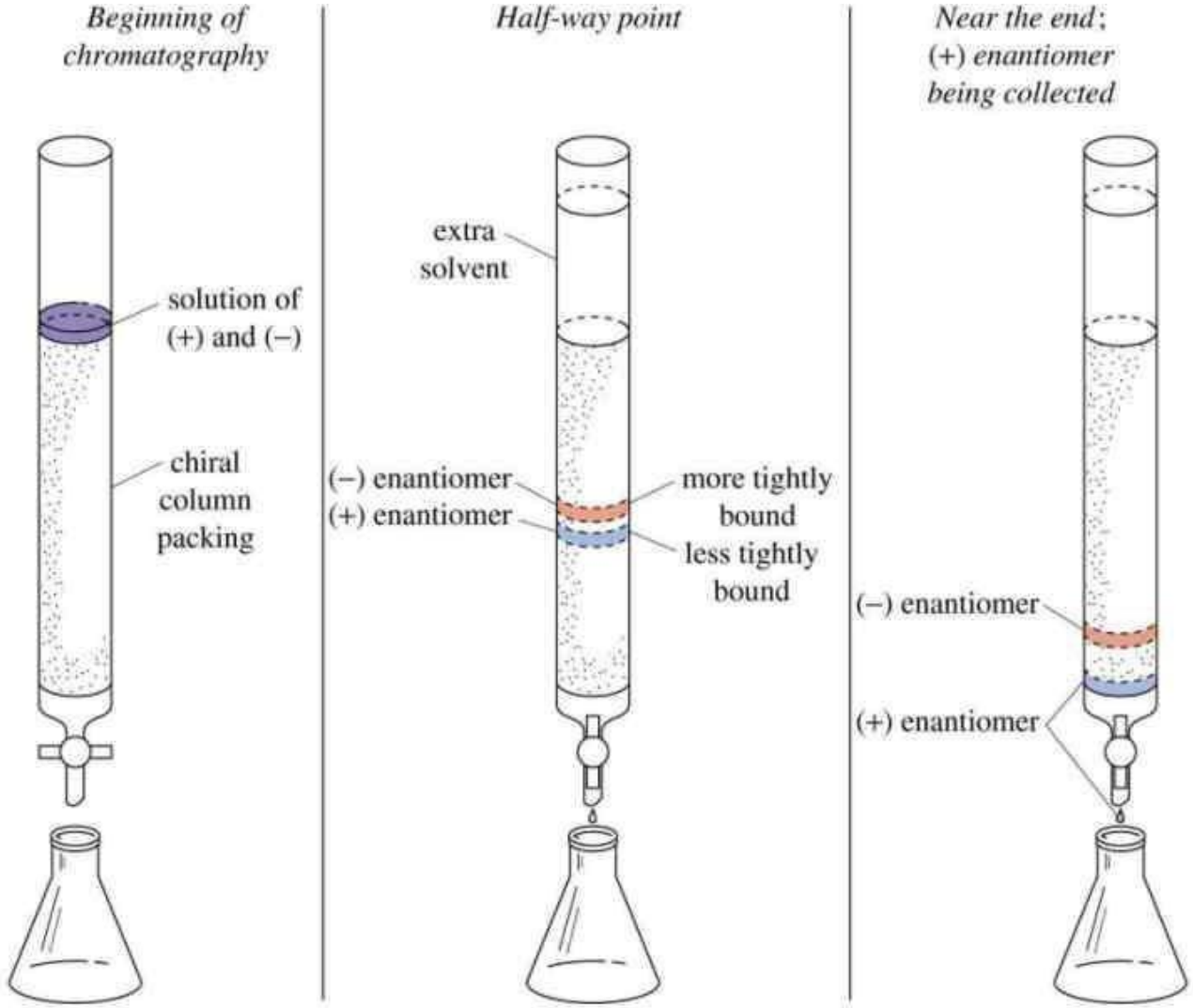
Enzymatic or Kinetic Resolution



Chiral Stationary Phases for Chromatographic Resolution

- A more versatile method for resolving a racemic mixture is through the use of **chromatography** on **chiral stationary phases**.
- These are applied in **gas** chromatographic and **liquid** chromatographic techniques.
- In the resolution of **racemic 2-aminobutane** on a chromatographic system, enantiomer of **mandelic acid** is attached to a stationary phase.
- Diastereomeric interactions between 2- aminobutane and the stationary phase lead to separable diastereomers with different retention times.

- As the solution passes through the column, the enantiomers form weak complexes, usually through hydrogen bonding, with the chiral column packing.
- These diastereomeric complexes have different physical properties.
- One of the two enantiomers will spend more time complexed with the chiral column packing.
- The solvent flows continually through the column, and the dissolved enantiomers gradually move along.
- Compounds that are adsorbed strongly spend more time on the stationary particles
- They come off the column later than less strongly adsorbed compounds, which spend more time in the mobile solvent phase.



Chromatographic resolution of enantiomers. The enantiomers of the racemic compound form diastereomeric complexes with the chiral material on the column packing. One of the enantiomers binds more tightly than the other, so it moves more slowly through the column.

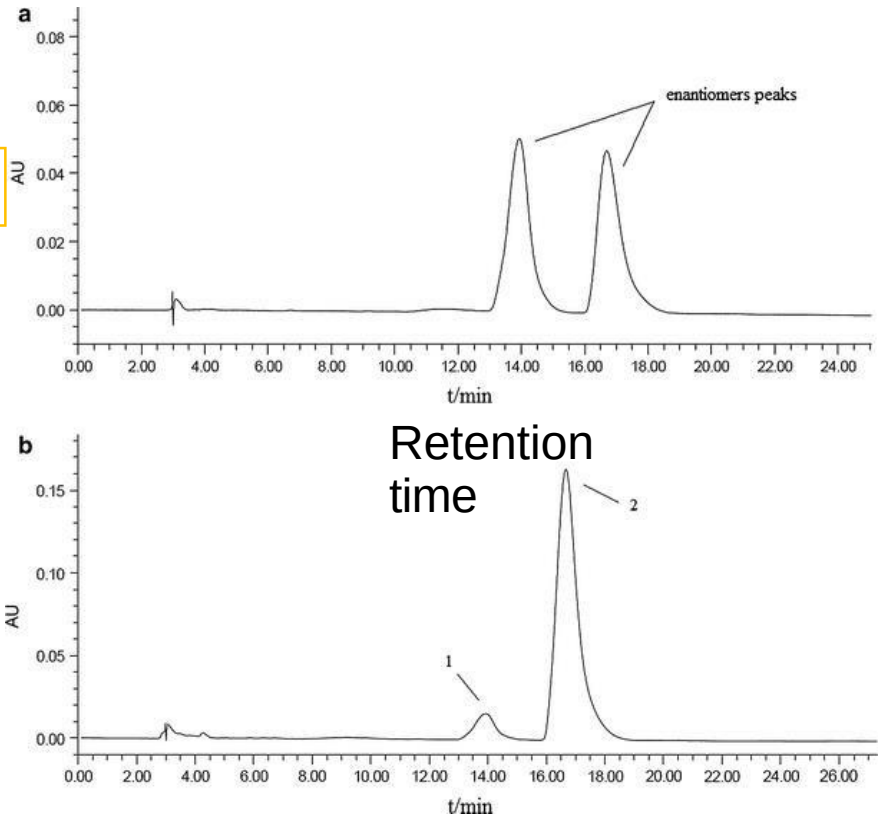
Assessing the Efficacy of a Resolution

- Using a chromatogram of the resolved compound on a chiral stationary phase, the percentage composition can be determined.

- The efficacy of the different strategies for separating a racemic mixture can be determined by assessing the purity of each of the enantiomers obtained.
- The purity of each of the enantiomers is usually expressed as enantiomeric excess (ee).

Racemic mixture

Enantiomerically enriched



Calculating Enantiomeric excess (ee) by Percentage

- Enantiomeric excess (optical purity) is a measure of how pure an enantiomer is (i.e. the extent to which one enantiomer is present in excess of the racemic mixture).
- It is denoted by the symbol *ee and calculated as a %.*

ee= % of major enantiomer -% of the minor enantiomer.

Enantiomeric excess

- If the percentages of each of the two enantiomers in the mixture is calculated from the area under the peak in a chromatogram, the enantiomeric excess can be calculated directly using the formula below.
- Consider the case of a mixture containing 95% of one enantiomer and 5% of the other, the enantiomeric excess of the mixture is

95% - 5% = 90%.

Calculating Enantiomeric excess (ee) by Mass

- The enantiomeric excess (purity) of a mixture can be calculated using the masses of each of the enantiomers in the mixture.
- If the masses of each of the pure enantiomers isolated after separation is known, the enantiomeric excess can be calculated using the equation below

$$ee = \left(\frac{(R - S)}{(R + S)} \right) \times 100$$

Calculating Enantiomeric Excess (Assignment)

- The enantiomeric excess can also be calculated if the specific rotation [α] of a mixture and the specific rotation [α] of a pure enantiomer are known.

$$ee = ([\alpha] \text{ mixture} / [\alpha] \text{ pure enantiomer}) \times 100$$

- A sample of mandelic acid analysed in a polarimeter gave an observed specific rotation of -75 degrees. If the specific rotation of (S)-mandelic acid is +154 degrees;

(i) Which enantiomer is in excess? (R or S)

(ii) Calculate the enantiomeric excess of the mixture.

(iii) Calculate the percentage of each enantiomer in the mixture.

Calculating Enantiomeric Excess (Assignment)

- If (S)-mandelic acid has a specific rotation of +154 degrees then its enantiomer has a specific rotation of -154 degrees. As the specific rotation of the mixture is negative, (R)-mandelic acid is the dominant one.

$$ee = [\alpha]obs / [\alpha]max \times 100$$

where ee is the enantiomeric excess and [ ] is the modulus sign that makes negative values positive.

$$ee = (75 /154) \times 100 = 48.7\%$$

- Let the % of R-enantiomer be R, that of the S-enantiomer be S, then R + S = 100,

while R – S = enantiomeric excess.

R – (100 – R) = enantiomeric excess = 48.7 2R – 100 = 48.7,

which implies that 2R = 148.7 The major R-enantiomer = 74.4%

The minor S-enantiomer = (100 – R) = 25.6%