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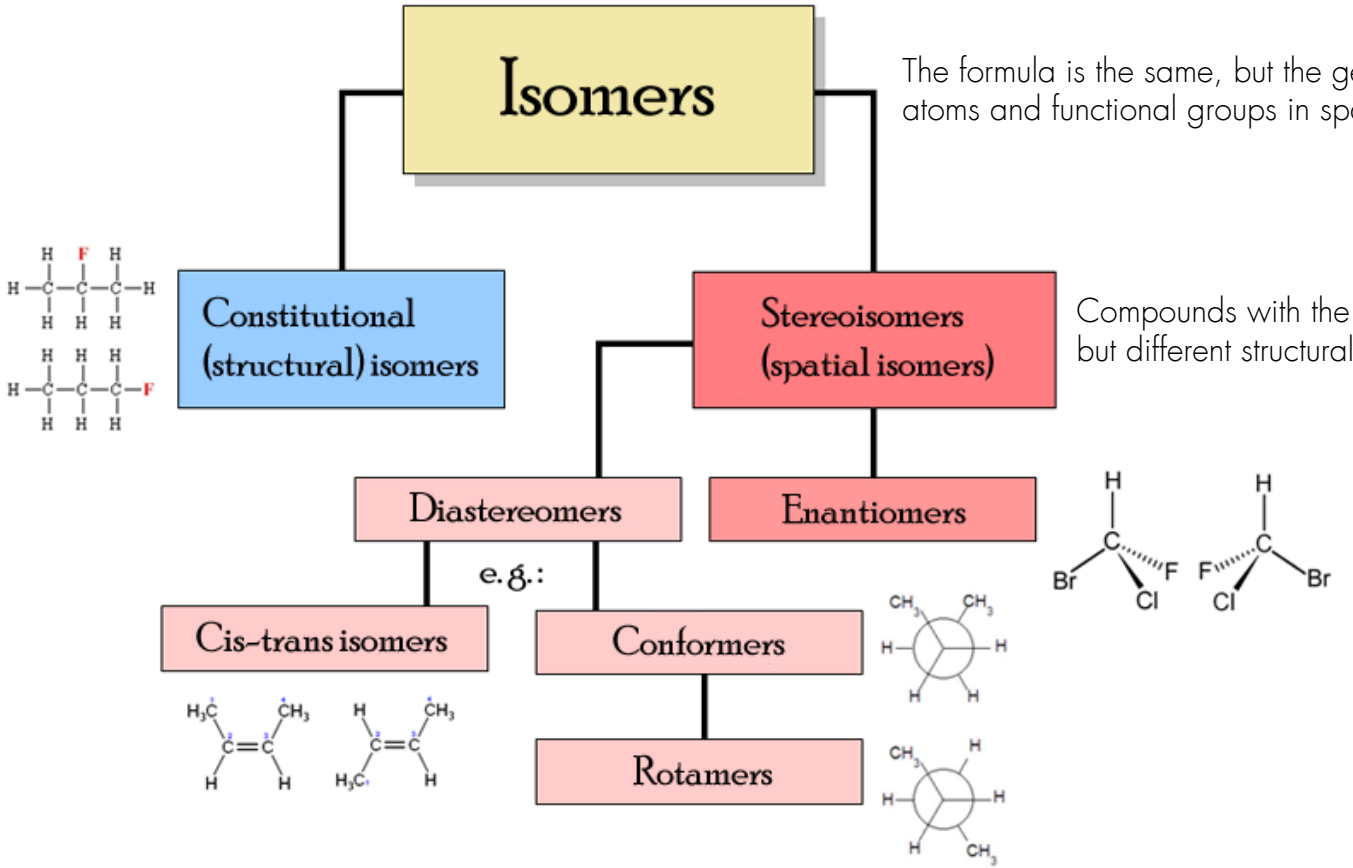
Chiral Resolutions

- Introduction
- Methods for Preparing Chiral Compounds
- Optical Resolutions *via* Diastereomeric Salt Formation
  - Stoichiometry of Resolution
  - Resolving Agents
  - Solvents
  - Selection of the Optimal Parameters
  - Separation and Improve Optical Purity

Chiral Resolutions
Introduction

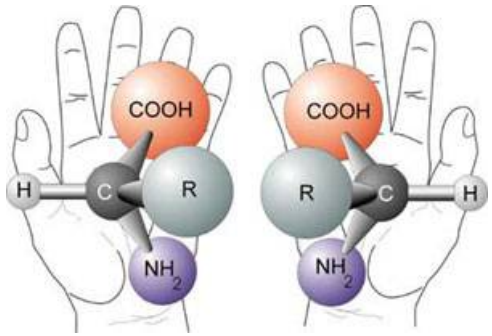


The formula is the same, but the geometrical positioning of atoms and functional groups in space differs

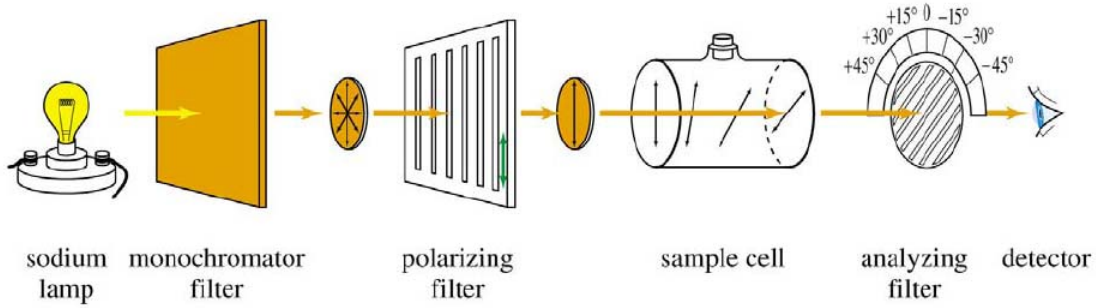
Compounds with the same molecular formula, but different structural formulas

Chiral Resolutions
Introduction

➢ Chirality is a general property of objects and means that an object is not superimposable (not identical) to its mirror image. An example of a pair of chiral objects is the human hands:

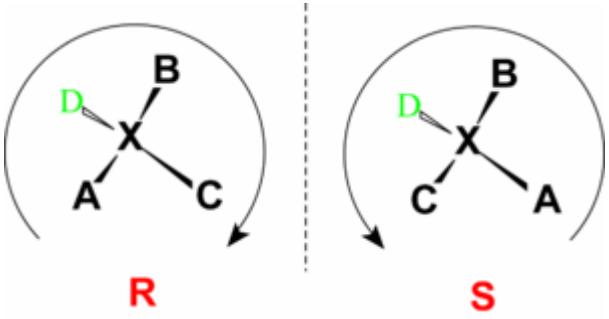


➢ A chiral molecule and its mirror image are called a pair of enantiomers. Enantiomers have identical chemical and physical properties (IR, NMR, mp, mass spectra,…etc), except for their ability to rotate plane-polarized light (+/-) by equal amounts but in opposite directions. A mixture of equal parts of an optically active isomer and its enantiomer is termed racemic and has a net rotation of plane-polarized light of zero

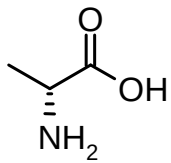


Chiral Resolutions
Introduction

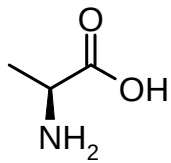
➢ Absolute configuration is the spatial arrangement of the atoms of a chiral group and its stereochemical description e.g. R or S. The assignment of R or S is based on the Cahn-Ingold-Prelog Priority Rules:



➢ Absolute configurations for chiral molecules are traditionally obtained by X-ray crystallography



(*R*)-(-)-aminopropionic acid
D-alanine



(*S*)-(+)-aminopropionic acid
L-alanine

ee=R-S/R+Sx100 ee = enantiomeric excess
ep=R/R+Sx100 ep = enantiomeric purity

Chiral Resolutions
Introduction

- 100 drugs more sold in 1994:

pure enantiomer	20%
racemate	21%
no chiral	33%

- 100 drugs more sold in 1997:

pure enantiomer	30%
racemate	9%
no chiral	57%

- Single–enantiomer drug sales show a continuous growth worldwide and many of the topselling drugs are marketed as single enantiomers

- Annual sales of chiral drugs: $133 billion (2000), $172 billion (2005)

- The drug industry will continue to have a strong growth in chiral compounds, because of the efforts to improve drug efficacy and to cut development costs in the face of regulatory pressures

Chemical & Engineering News 2000, *78*, 55-78
Chemical & Engineering News 2001, *79*, 79-97

Chiral Resolutions
Introduction

Chiral drug sales hurtle past $100 billion and show no sign of slowing			
	Global sales		
$ Millions	1998	1999	2000
Cardiovascular	$21,906	$24,805	$26,012
Antibiotics/antifungals	19,756	20,907	23,265
Hormones/endocrinology	12,297	13,760	17,345
Cancer	8,006	9,420	13,360
Central nervous system	7,027	8,592	13,720
Hematology	6,730	8,580	11,445
Antiviral	6,131	7,540	13,446
Respiratory	4,305	5,087	8,795
Gastrointestinal	1,718	2,998	5,355
Ophthalmic	1,482	1,794	2,070
Dermatological	1,124	1,270	1,540
Analgesics	842	1,045	1,135
Vaccines	568	676	1,100
Other	7,947	8,527	7,425
TOTAL	$99,389	$115,001	$146,013
Source: Technology Catalysts International Corp.			

Chiral Resolutions
Introduction

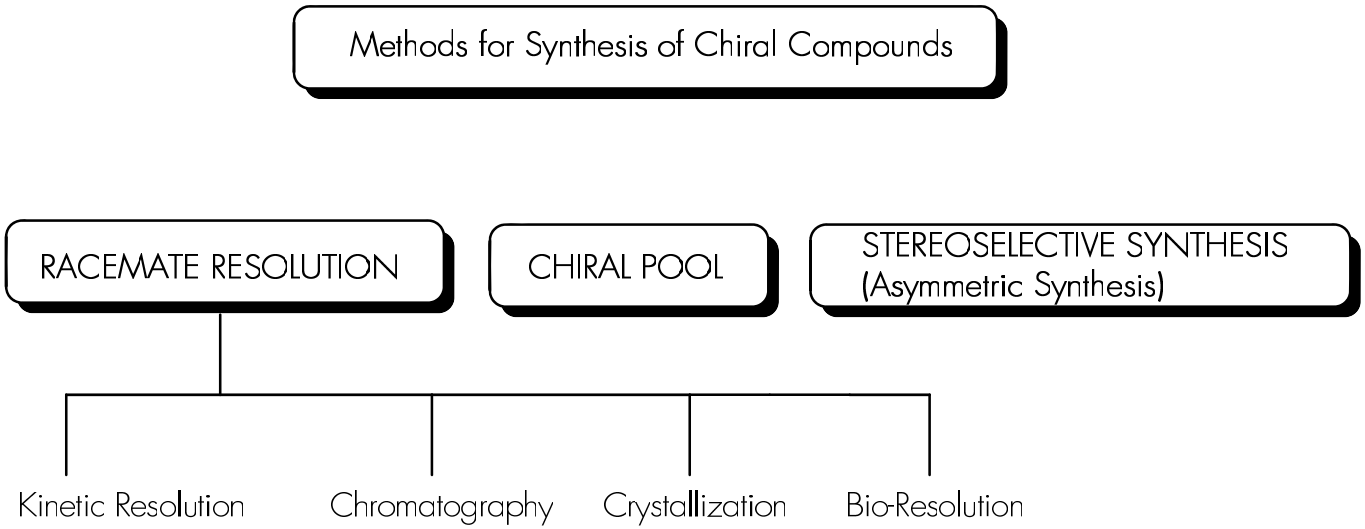
- Some drug companies have patented and developed a racemic drug, with the intention of patenting and developing a single enantiomer later. When the patent on the racemate expires, the company can undercut generic competition by launching the single-enantiomer
- The following table lists pharmaceuticals that have been available in both racemic and single-enantiomer form:

Racemic mixture	Single-enantiomer
Amphetamine (Benzedrine)	Dextroamphetamine (Dexedrine)
Bupivacaine (Marcain)	Levobupivacaine (Chirocaine)
Cetirizine (Zyrtec / Reactine)	Levocetirizine (Xyzal)
Citalopram (Celexa / Cipramil)	Escitalopram (Lexapro / Cipralex)
Methylphinidate (Ritalin)	Dexmethylphenidate (Focalin)
Modafinil (Provigil)	Armodafinil (Nuvigil)
Ofloxacin (Floxin)	Levofloxacin (Levaquin)
Omeprazole (Prilosec)	Esomeprazole (Nexium)
Salbutamol (Ventolin)	Levalbuterol (Xopenex)
Zopiclone (Imovane)	Eszopliclone (Lunesta)

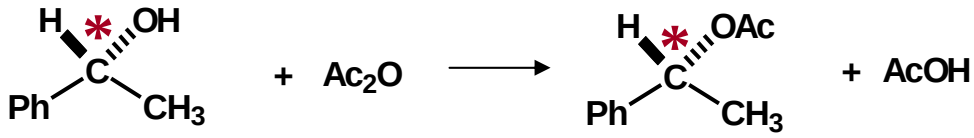
Chemical & Engineering News 2001, *79*, 79-97

Chiral Resolutions

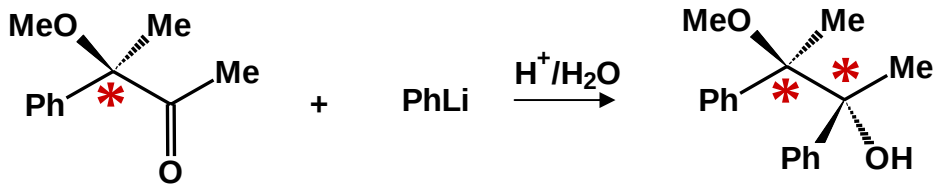
Methods for Preparing Chiral Compounds



- Chiral Pool (functionalizationof a chiral compound):



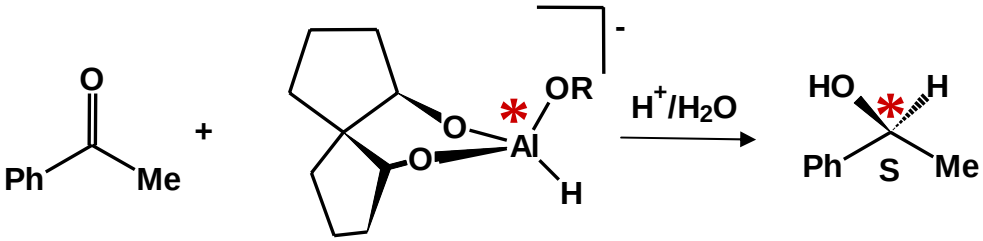
- Stereoselective synthesis: diastereoselective and asymmetric synthesis
  - Diastereoselective Synthesis



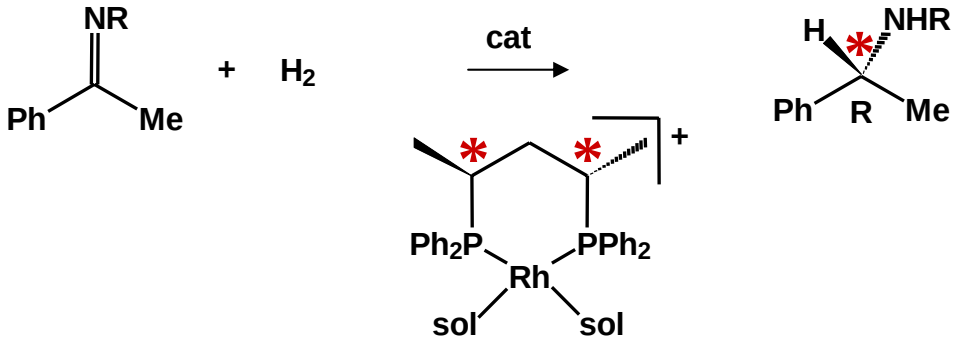
Stereoselective synthesis:

Asymmetric Synthesis

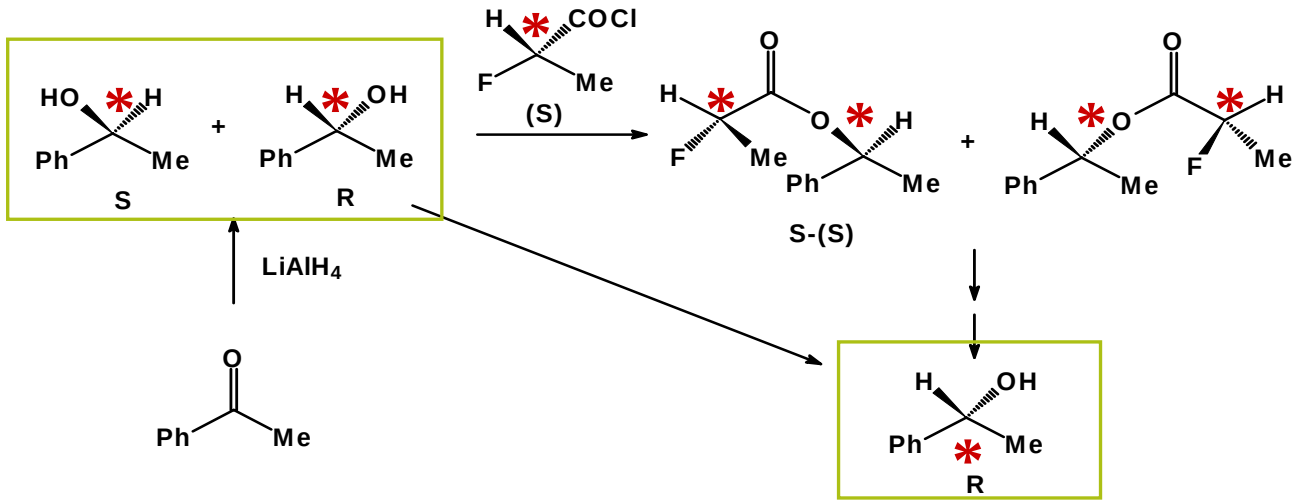
- Use of a chiral reagent:



- Use of a chiral catalyst:



- Resolution:

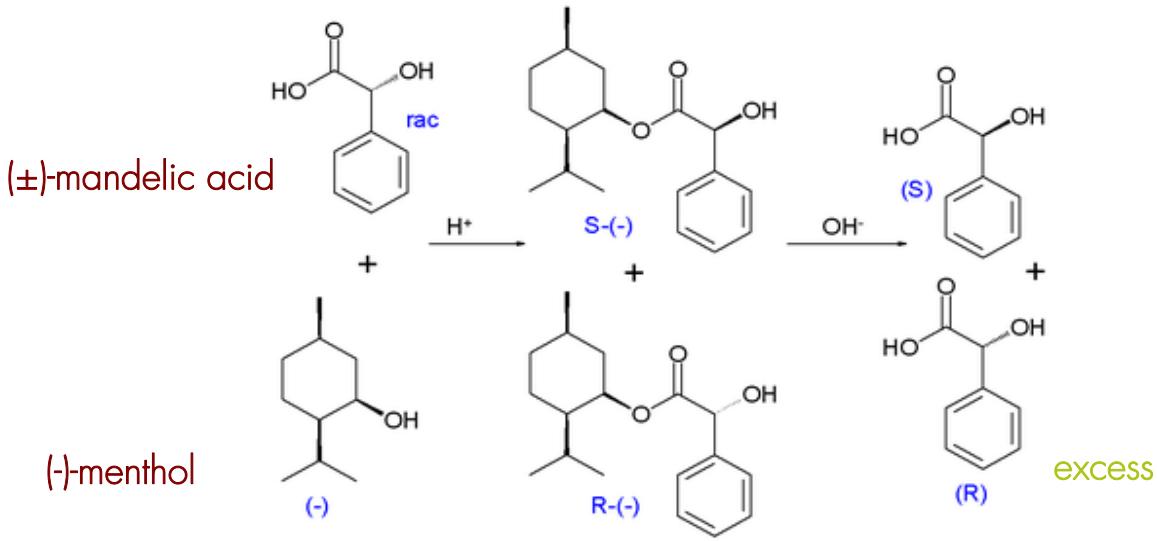

HO
H
Ph
Me
S
+
H
OH
Ph
Me
R
H
COCl
F
Me
(S)
O
H
F
Me
O
H
Ph
Me
S-(S)
+
O
H
Ph
O
Me
H
F
Me
LiAlH4
O
Ph
Me
H
OH
Ph
Me
R

Resolution

Kinetic Resolution

➢ Kinetic resolution is defined as a process in which one of the enantiomers (*R* + *S*) of a racemic mixture is more readily transformed into a product than its mirror image

➢ Kinetic resolution was first observed by Marckwald and McKenzie in 1899 in the esterification reaction of racemic mandelic acid with optically active (-)-menthol

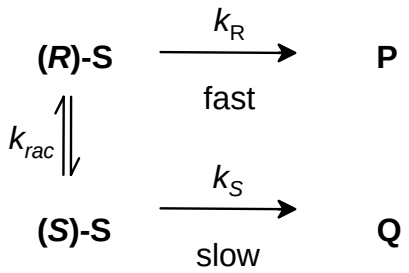


Full hydrolysis of the incomplete esterification mixture gives an excess of (R)-mandelic acid. Taking the reaction to 100% completion will again produce equal amounts of both esters

- Dynamic Kinetic Resolution

➢ A general disadvantage of standard kinetic resolution procedures described so far is that a maximum 50% yield of the desired product enantiomer is obtained based on racemic starting material. To overcome this limitation, recovered starting material may in some cases be racemized and resubmitted to the resolution procedure

Kinetics of "Dynamic Kinetic Resolution"



classic kinetic resolution and the *in situ* racemizaction of the starting material

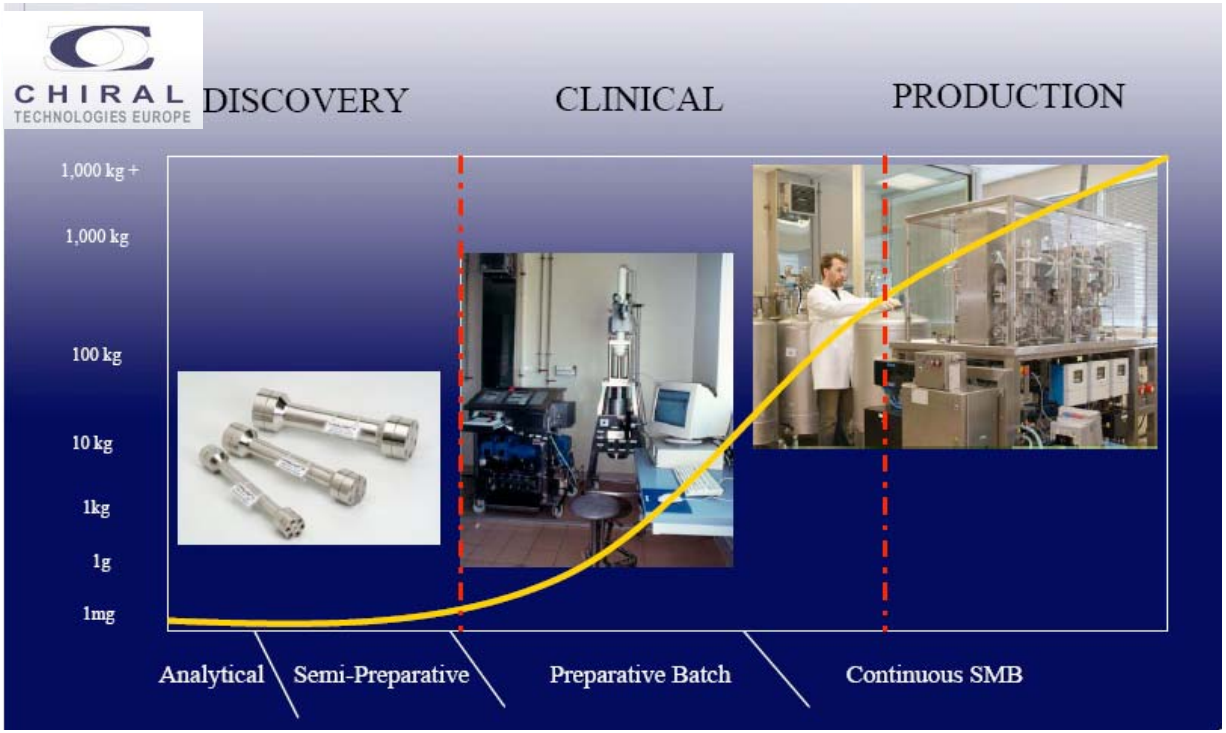
(*R*)-S, **(*S*)-S**: substrate enantiomers
P, **Q**: product enantiomers
k_R, k_S: individual rate constsnts
k_{rac}: racemization constant

- Chromatography

➢ Chiral Chromatography is a branch of chromatography that is oriented towards the exclusive separation of chiral substances

➢ Enantiomeric separations are achieved in chiral chromatography by the use of chiral phases. The mobile phase can be a gas or liquid giving rise to chiral gas chromatography and chiral liquid chromatography

➢ Chiral selectivity is usually achieved by employing chiral stationary phases, although, in chiral liquid chromatography, chiral mobile phases have been successfully employed



Chiral Resolutions
Methods for Preparing Chiral Compounds

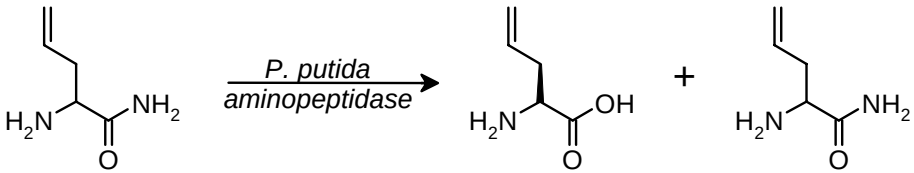
- Chromatography

Copyright: CarboGen

CHIRAL CHROMATOGRAPHY Installation at CarboGen subsidiary of Solutia, Aarau, Switzerland: separates 4 kg of racemate per day

- Bio-Resolution

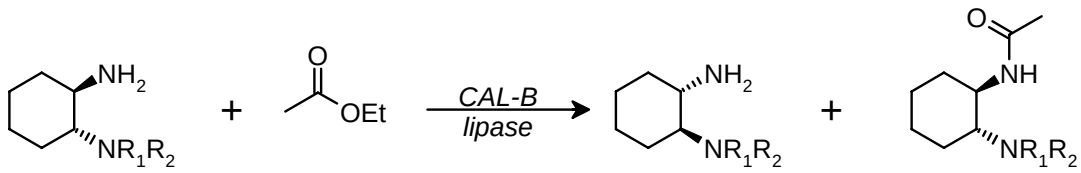
➢ Chiral Enzymatic Resolution employ enzymes as useful catalysts for resolution. Advantages: the commercial availability of these enzymes at mostly reasonable cheap prices, their synthetic flexibility and stability, and the ease of their handling



***rac*-1** **(*S*)-2** ***rac*-1**

Chemical & Engineering News **2000**, *78*, 55-78

enzymatic resolution of racemic diamines *rac*-4



***rac*-4** **(1*S*,2*S*)-4** **(1*R*,2*R*)-6**

Chem. Eur. J. **2004**, *10*, 5788 – 5794

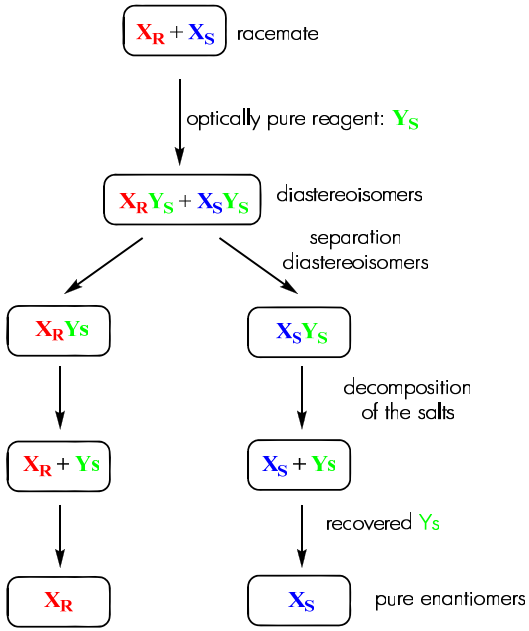
Chiral Resolutions
Methods for Preparing Chiral Compounds

- Crystallization

➢ Racemate resolution via Diastereomeric Salt Crystallization is a process for the separation of racemic compounds into their enantiomers. It is an important tool in the production of optically active drugs

➢ Derivatization of racemic compounds is possible with optically pure reagents forming pairs of diastereoisomers which can be separated by conventional techniques in chemistry (i.e., salt formation between an amine and a carboxilic acid; simple deprotonation affords the pure enantiomer)

➢Two pure enantiomers can be obtained, very important in medicinal chemistry. When the drug is a single-enantiomer, to evaluate the pharmacokinetics of this single enantiomer manufacturers must develop quantitative assays for individual enantiomers in *in vivo* samples early in drug development



Chiral Resolutions
Methods for Preparing Chiral Compounds

- **Kinetic Resolution**

- Evolution of an historical process (> 100 years)
- Based upon different reaction rates of the isomers
- Theoretically 100% yield
- Technology applied on limited scale to date

- **Chromatography**

- Widely used in development laboratories
- Applicable to >99% of small molecules at analytical scale
- Scalable
- Used in manufacturing scale

- **Enzymatic Resolution**

- Selective reduction of a single isomer in a mixture
- Requires a specific enzyme for fast & selective resolution
- Suitable for small molecules (starting materials)
- Suitable for strategic supply as development can be lengthy

- **Diastereomer Crystallisation**

- Most widely used technique in industry
- Widely used in development
- Cheap additives
- Only one isomer recovered per stage

Chiral Resolutions

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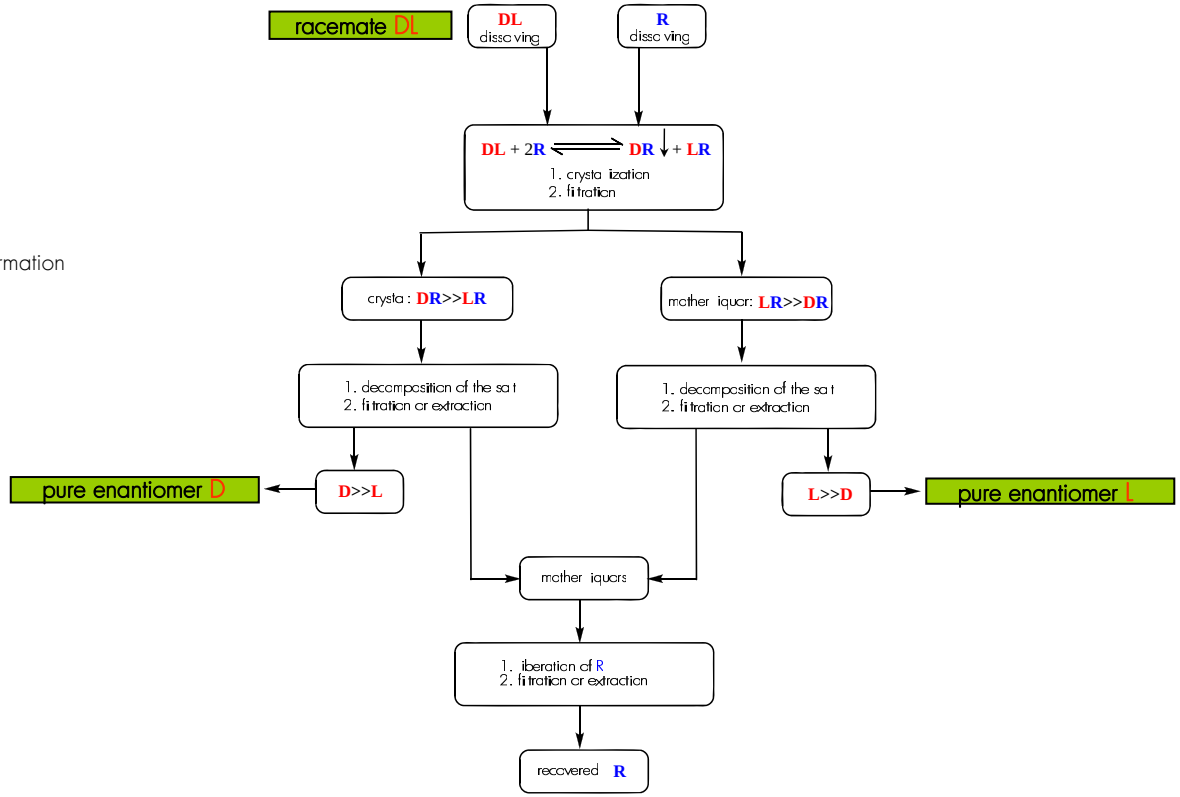
  - Stoichiometry of Resolution
  - Resolving Agents
  - Solvents
  - Selection of the Optimal Parameters
  - Separation and Improve Optical Purity

Chiral Resolutions

Optical Resolutions *via* Diastereomeric Salt Formation

- The method was introduced by Louis Pasteur in 1853 by resolving racemic tartaric acid with optically active (+)-cinchotoxine
- Reaction of a racemic (DL) acid or base with an optically active base or acid (R) gives a pair of diastereomeric salts. Members of this pair exhibit different physicochemical properties (solubility, mp, bp,…) and can be separated owing to these differences. The most important method for the separation of enantiomers is crystallization of diastereomeric salts

flow diagram of resolution by diastereomeric salt formation
DL: racemate
R: resolving agent



❖ Stoichiometry of Resolution

• Resolution with One Equivalent of Resolving Agent

➢ The simplest process consists of reacting the racemate in a suitable solvent with one molar equivalent of the resolving agent, whereupon the less soluble salt preferentially crystallizes, usually contaminated with some of the more soluble diastereomer

➢ Resolution of a basic racemate (DL) with an optically active acid (RH) is described by following equation where the solubility of the diastereomeric salt DHR is less than that of salt LHR

$$DL + 2RH \rightleftharpoons DHR\downarrow + LHR$$

➢ In this equilibrium, two diastereomeric salts, PD and PL, are present in the solid phase and the precipitated salt are in equilibrium with the dissolved salts

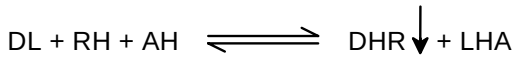
$$PD_{(solid)} \rightleftharpoons DHR_{(solution)}$$

$$PL_{(solid)} \rightleftharpoons LHR_{(solution)}$$

❖ Stoichiometry of Resolution

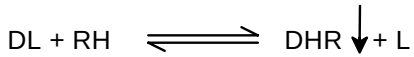
• Resolution with Half Equivalent of Resolving Agent

➢ In resolutions with one equivalent of resolving agent, it is the resolving agent proper that keeps the other enantiomer in solution. The former can be substituted by an achiral reagent of similar character; that is, it is possible to achieve resolution with but one half equivalent of the resolving agent
In this case, the equation of resolution must be modified as follows:



where DHR is the less soluble diastereomeric salt and AH the achiral additive

➢ In practice the achiral additive (AH) is a strong acid or base, most often HCl or NaOH, respectively
➢ The role of the achiral additive can be taken over by the solvent. In this case, the reaction formula of resolution can be written as:



Chiral Resolutions

Optical Resolutions *via* Diastereomeric Salt Formation

❖ Resolving Agents

➢ Any optically pure chiral organic acid or base may be a candidate as resolving agent

➢ Characteristics of an ideal resolving agent:

- It should be a strong acid or base to secure formation of stable salts with weakly basic or acidic racemates, respectively
- The center of chirality should be as close as possible to the functional group involved in salt formation to provide significant differences in the stereostructure of the diastereomeric salts
- Both enantiomers should be available
- It should be chemically stable and should not racemize under the conditions of resolution
- In should be readily recoverable
- It should be non-toxic
- Starting materials for its preparation should be readily available and inexpensive

CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation, 2001, *51-71*

Chiral Resolutions

Optical Resolutions *via* Diastereomeric Salt Formation

❖ Resolving Agents

➢ Despite the availability of several methods to assist in the selection of the resolving agent, this is most often still a matter of trial and error

➢ The usual routine is to try resolving agents available in the laboratory, usually in paralell

Relative Frequencies (%) of the Use of a Selection of Acidic Resolving Agents

Resolving Agent	%
Tartaric acid	34.2
O,O'-Dibenzoyltartaric acid	16.6
Camphor-10-sulfonic acid	9.8
O,O'-Di-4-toluyltartaric acid;	8.4
(*S*)- or (*R*)-Mandelic acid	6.3
(1*R*)- or (1*S*)-3-Bromocamphor-8-sulfonic acid	3.5
N-Acetylleucine	1.7
(*S*)- or (*R*)-Malic acid	1.4
(*R*)-(+)-6,6'-Dinitrobiphenyl-2,2'-dicarboxylic acid	1.0
Camphoric acid	1.0

Relative Frequencies (%) of the Use of a Selection of Basic Resolving Agents

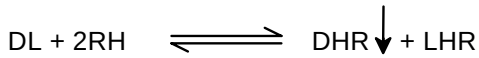
Resolving Agent	%
Brucine	21.3
Quinine	15.6
(*S*)- or (*R*)-1-Phenylethylamine	12.3
Cinchonidine	10.3
Strychnine	6.4
Ephedrine	5.8
Cinchonine	4.2
(1*S*, 2*S*)- or (1*R*, 2*R*)-4-Nitrophenyl-2-aminopropane-1,3-diol	2.6
Morphine	1.7
Amphetamine	1.4
Fenchylamine	0.6
L-Leucinamide	0.6
1-(1-Naphthyl)-ethylamine	0.6
(*S*)- or (*R*)-*O*-Benzyl-2-aminobutan-1-ol	0.6
Tyrosine hydrazide	0.5

Chiral Resolutions

Optical Resolutions *via* Diastereomeric Salt Formation

❖ Solvents

➢ The selection of a suitable solvent is of prime importance. A solvent is often not only a medium of crystallization:



Relative Frequencies of the Use of Various Solvents in Resolutions

Solvent	Racemate: Base (%)	Racemate: Acid (%)
EtOH 96%	19.19	18.50
MeOH	14.51	10.54
H_2O	13.48	15.63
Acetone	8.42	7.84
EtOH-H_2O	5.57	9.61
Abs. EtOH	5.27	3.63
EtOAc	3.15	6.37
iPrOH	2.93	0.97
MeOH-Et_2O	2.34	0.35
MeOH-H_2O	2.05	2.26
EtOH-Et_2O	1.39	0.53
Et_2O	1.10	2.26
EtOH-EtOAc	0.95	0.80
Acetone-EtOH	0.66	0.31
MeOH-EtOAc	0.51	1.73
CH_2Cl_2	0.29	0.29
$CHCl_3$	0.22	0.58
Dioxane	0.07	0.31
Nondefined	5.13	6.55
Other	12.75	11.11

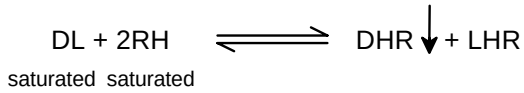
most usual: R-OH, acetone

❖ Selection of the Optimal Parameters

- Determination of the Initial Concentration

  ➢ After having selected the best Solvent and Resolving Agent, the next task is to determine the optimum initial concentration

  ➢ Solutions saturated at their boiling points should be combined



- Initiation of Crystallization

○ Oversaturation:

  ✓ Oversaturation by Cooling

  ✓ Oversaturation by Partial Evaporation of the Solvent (thermally unstable racemates, r.t.)

○ Initiation of Crystallization:

  ✓ Spontaneous Crystallization: in favorable cases, crystallization starts spontaneously from oversaturated solution of the salts. 1 g 100 times more probably than 10 mg. Seed formation can be promoted:
  Sonication
  Cycles of heating/cooling

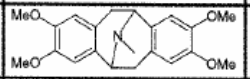
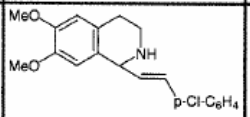
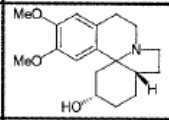
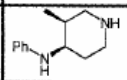
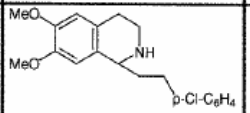
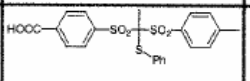
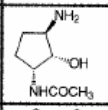
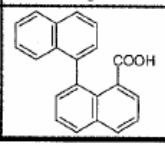
  ✓ Crystallization Using Seed Crystals

  ✓ Precipitation with a Second Solvent

Chiral Resolutions

Optical Resolutions *via* Diastereomeric Salt Formation

✓ Resolutions by Precipitation with a Second Solvent:

No.	Racemate	Resolving Agent	First Solvent	Second Solvent	Ref.
1		Hydroxymethylene camphor	MeOH	H_2O	44
2		DBTA[a]	MeOH	EtOAc	45
3		DBTA[a]	MeOH	Et_2O	46
4		(2*S*,3*S*)-TA[a]	MeOH	Me_2CO	47
5		TA[a]	Me_2CO	MeOH	48
6		DBTA[a]	Me_2CO	Et_2O	49
7		Quinine	EtOH	Petroleum ether	50
8		TA[a]	90% EtOH	Me_2CO	51
9		Brucine	EtOAc	MeOH:H_2O (1:1)	52

[a] DBTA = *O*,*O*′-dibenzoyltartaric acid; TA = tartaric acid.

Chiral Resolutions

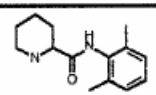
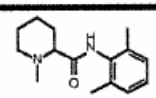
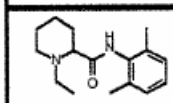
Optical Resolutions *via* Diastereomeric Salt Formation

❖ Selection of the Optimal Parameters

- Purity of Starting Materials
  - ➢ Chemical purity of the racemate may be of prime importance for the initiation of crystallization. Impurities can both promote and inhibit crystallization

- Temperature
  - ➢ Temperature at which the components can be dissolved in the chosen solvent
  - ➢ Temperature of crystallization: reproducibility
  - ➢ Temperature dependence of resolution

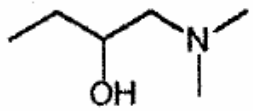
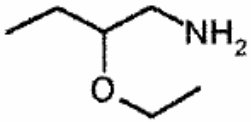
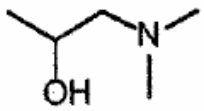
Resolution of Pipecolic Acid Xylylides with *O,O'*-Dibenzoyltartaric acid (DBTA) at various tempeartures (optimun tempetarures in boldface)
o.p.: optical purity

Racemate Solvent	Resolving Agent	Temperature (°C)	Yield (%)	*o.p.* (%)
 iPrOH	DBTA	23	98	72
		25	96.5	77
		35	86	85
		52	75.5	96
		45	**83**	**93**
 abs. EtOH	DBTA	40	97	83
		45	93.5	87
		55	82	95
		60	75	97
		49	**90**	**90**
 iPrOH	DBTA	35	125	84
		50	64	95
		62	34	98
		48	**84**	**93**

Separation and Improve Optical Purity

- For separation of crystals from mother liquor, the routine operation is filtration (laboratory) and centrifugation (industry)
- With crystals that are difficult to filter off, centrifugation usually provides fast separation
- The optical purity (*o.p.*) of the diastereomeric salt is very rarely the adequate. There are two main methods to improve *o.p.* of the salt:
  - ✓ Without a chiral additive: enantiomers liberated and purified
  - ✓ With a chiral additive: the routine procedure is repeat recrystallizations from the original solvent until obtaining an adequate *o.p.* of the salt

Resolutions Involving a High Number of Recrystallizations

Racemate	Solvent	Number of Recrystallizations
	96% EtOH	16
	Abs. EtOH	>20 insufficient to produce an optically pure product
	96% EtOH	24

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