

Review of Stereochemistry

Stereoisomers are compounds made up of the same atoms connected by the same sequence of bonds, but having different three dimensional structures.

Major kinds of stereoisomers are :

Enantiomers (mirror image stereoisomers)

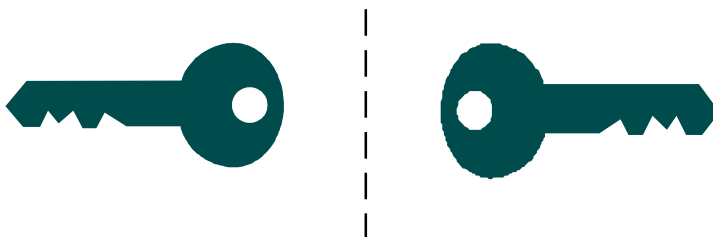
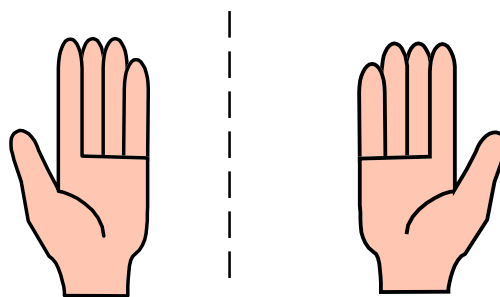
Other Stereoisomers (cis-trans isomers, diastereomers etc.)

Enantiomers and Chirality

A **chiral** object is not superimposable on its mirror image.
Examples include hands, screws, propellers, and keys

Acknowledgements:

Dr. Chris Welch
Ted Szczerba
Scott Perrin

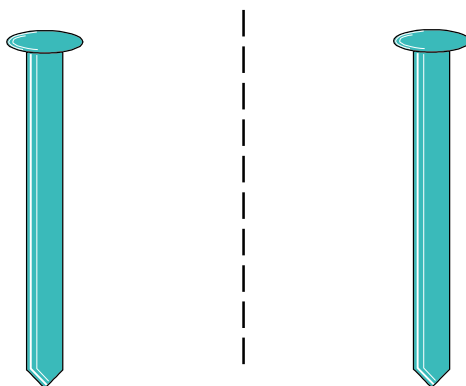


An **Achiral** object is superimposable on its mirror image. Examples include ball, buckets, nails, and T-shirts (no pockets). Achiral objects possess either a plane, center, or alternating axis of symmetry.

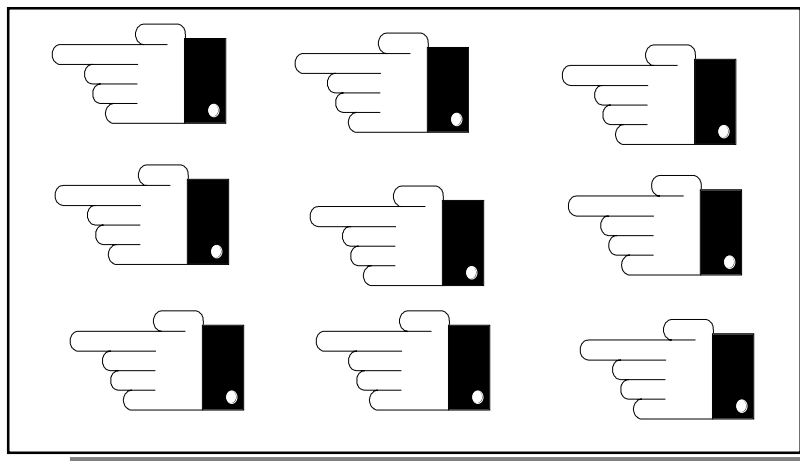
References:

1). J. March, Advanced Organic Chemistry: Reactions, Mechanism, and Structure, 4th Edition, Wiley, New York, 1992

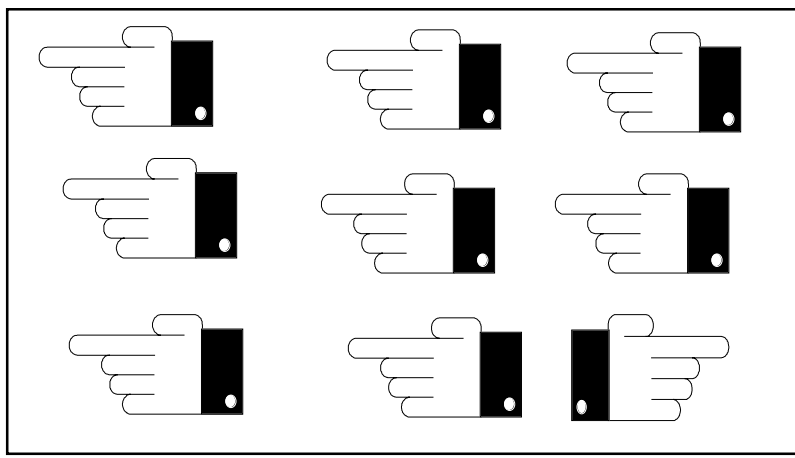
2). Sokolov, Introduction to Theoretical Stereochemistry, Gordon & Breach, New York, 1991



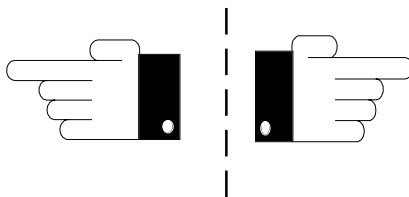
A collection containing only one enantiomeric form of a chiral molecule is called: **Enantiopure, Enantiomerically pure, or Optically Pure.**



A mixture containing predominantly one enantiomer is termed: **Enantiomerically Enriched, or Enantioenriched.**



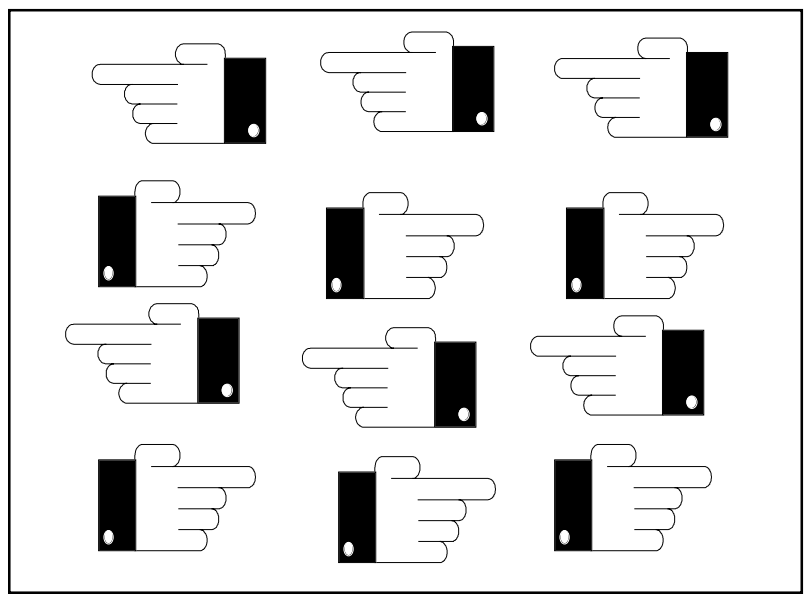
The two forms of a chiral object are known as **Enantiomers**



Unlike other stereoisomers, enantiomers have identical physical properties and consequently are difficult to separate and quantitate.

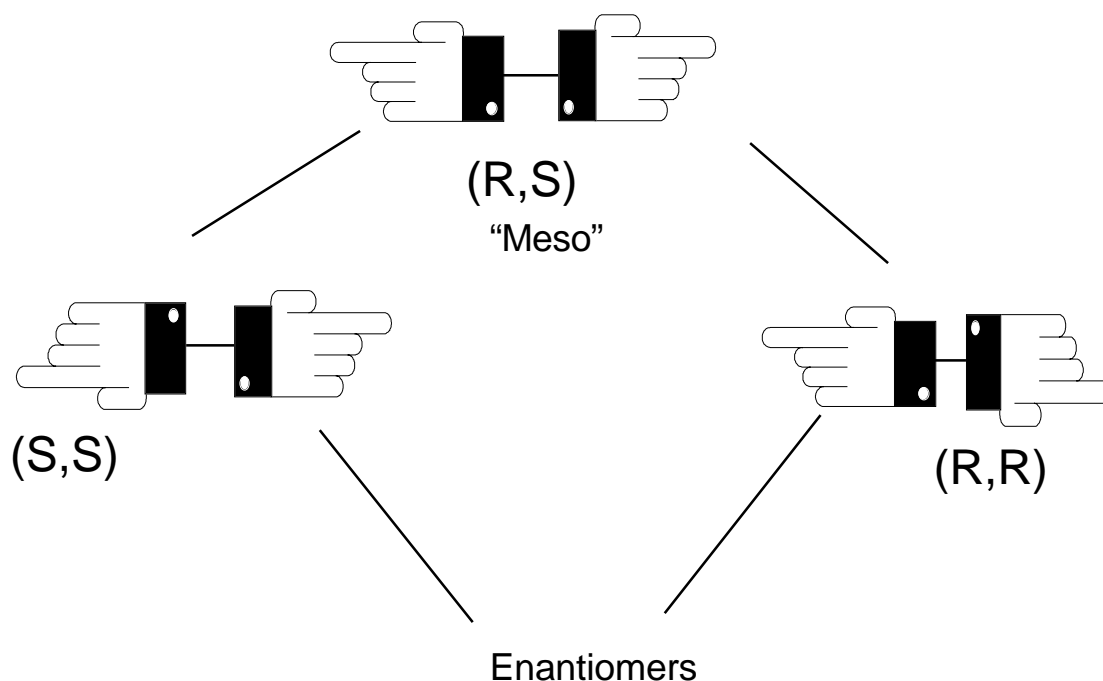
A process wherein enantiomers are separated is called a **Resolution**.

A collection containing equal amounts of the two enantiomeric forms of a chiral molecule is called a: **Racemic Mixture or Racemate**.



Diastereomers

Diastereomers are non-enantiomeric isomers arising when more than one stereocenter is present in a molecule.

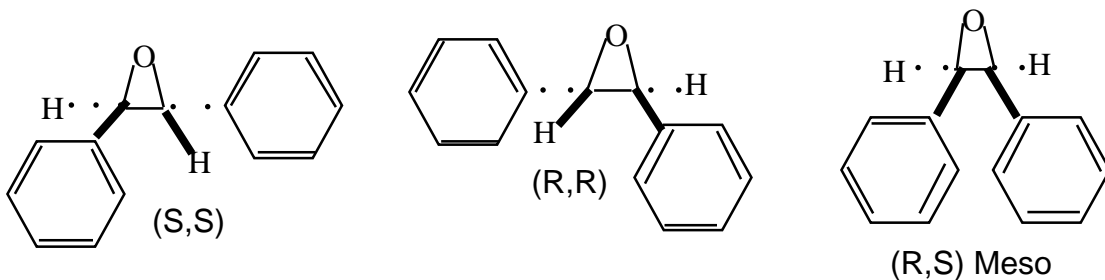


Compounds which differ in the absolute configuration at a single stereogenic center are called **Epimers**.

The process of changing the absolute configuration at one of the stereogenic centers of a diastereomer is called **Epimerization**.

Example of Diastereomers

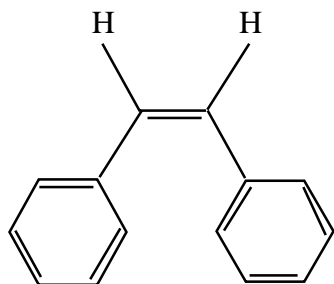
Stilbene Oxide Diastereomers



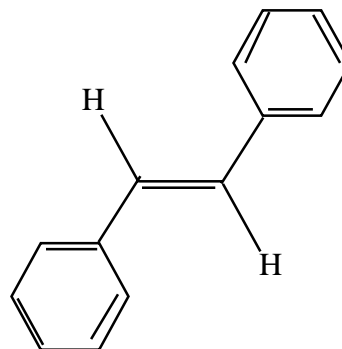
Cis-Trans

Cis-Trans Isomers are the simplest stereoisomers. Cis-Trans Isomerism in Olefinic Compounds.

Example: Stilbene



Cis isomer
Z (zusammen = "together")

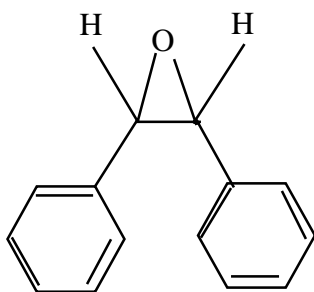


Trans isomer
E (Entgegen = "opposite")

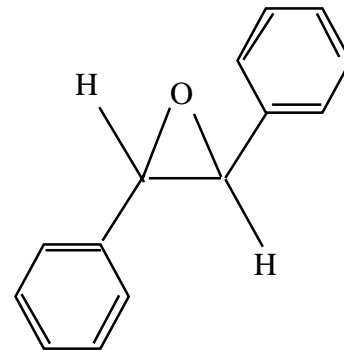
Mnemonic: Z = "zame zide"

Cis-Trans Isomerism in Cyclic Compounds

Example: Stilbene Oxide

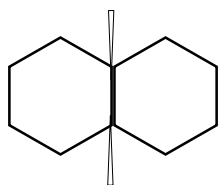


Cis isomer

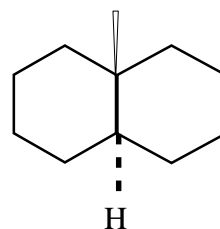


Trans isomer

The E,Z Nomenclature is not used for cis-trans isomerism of cyclic compounds.



Cis-decalin



Trans-decalin

Cahn-Ingold-Prelog System

This is the system most frequently used for designating absolute configurations of chiral compounds

1. Arrange the ligands associated with an element of chirality into order of priority.

Priority Rules

- a). Higher atomic number is given higher priority.
- b). Higher atomic mass is given higher priority.
- c). When the proximate atom of two or more of the ligands are the same, the atomic number of the next atom determines the priority.
- d). Double and triple bonds are counted as if they were split into two or three single bonds, respectively.
- e). Cis is given higher priority than trans.
- f). Like pairs [(R,R) or (S,S)] are given priority over unlike pairs [(R,S) or (S,R)].
- g). Lone-pair electrons are regulated as an atom with atomic number 0.
- h). Proximal groups take priority over distal groups.

2. View the molecule with the lowest priority group pointing away from the viewer.

3. Count the remaining ligands in order of decreasing priority.

- If the path traced is clockwise, the **(R)** (rectus) absolute configuration is assigned.

- If the path traced is counterclockwise, the **(S)** (sinister) absolute configuration is assigned.

Reference:

1). Klyne, Buckingham, *Atlas of Stereochemistry*, 2nd Edition, Oxford University Press, 1978

Optical Rotation Nomenclature

Enantiomers can rotate the plane of polarization of plane-polarized light.

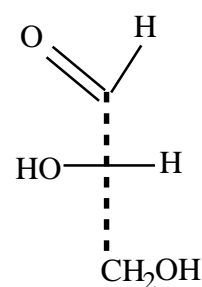
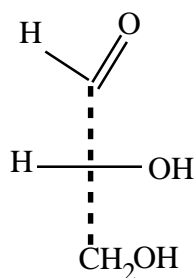
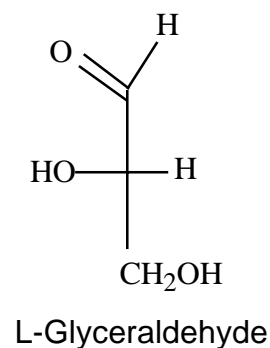
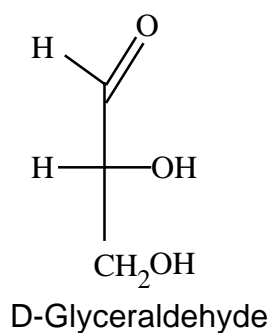
Dextrorotatory (+) enantiomer giving a positive optical rotation

Levorotatory (-) enantiomer giving a negative optical rotation

The “dl” nomenclature system previously used to designate the sign of optical rotation is no longer used, **(+)** and **(-)** symbols are now preferred.

Fischer Projections and the DL Nomenclature System

This was invented by Fischer in 1891. It works by having horizontal bonds in front of the plane and vertical bonds behind the plane.

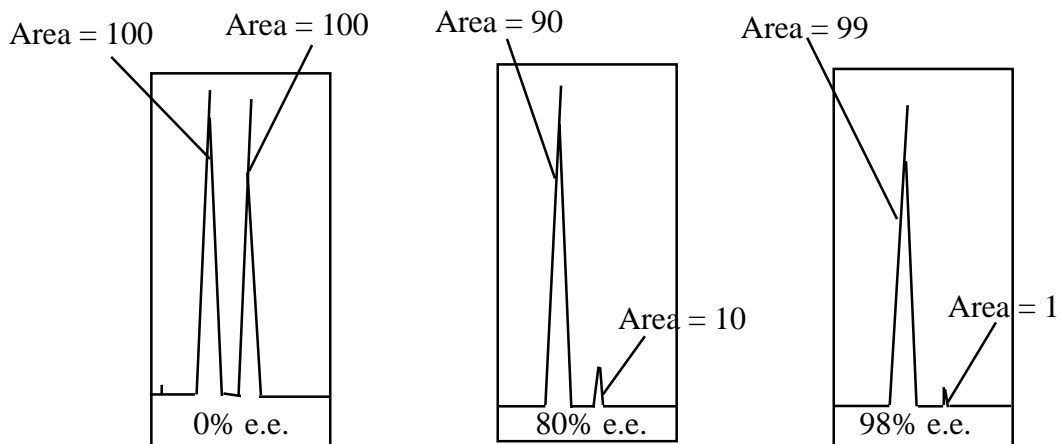


Still used for sugars, but generally discouraged.

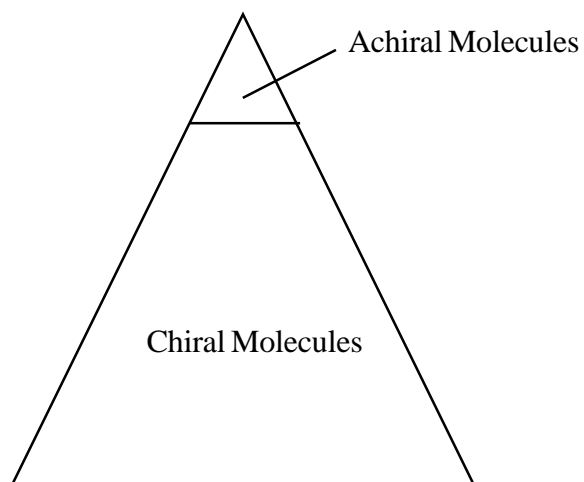
**Quantitating
Enantiopurity****Enantiomeric Excess (%e.e.)**

Enantiopurity is usually reported in terms of “**enantiomeric excess**” (e.e.).

$$\%e.e. = \frac{\text{Major} - \text{minor}}{\text{major} + \text{minor}} \times 100$$



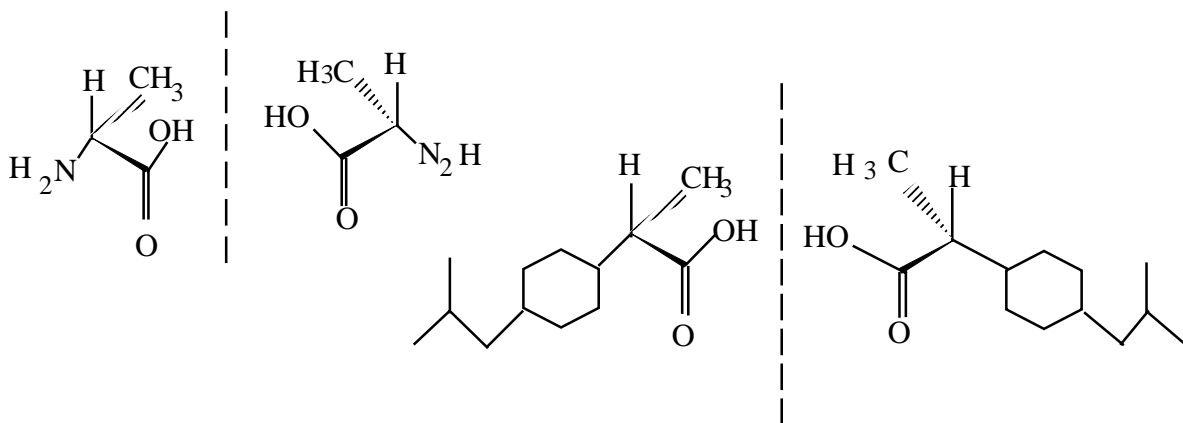
Most organic molecules are chiral



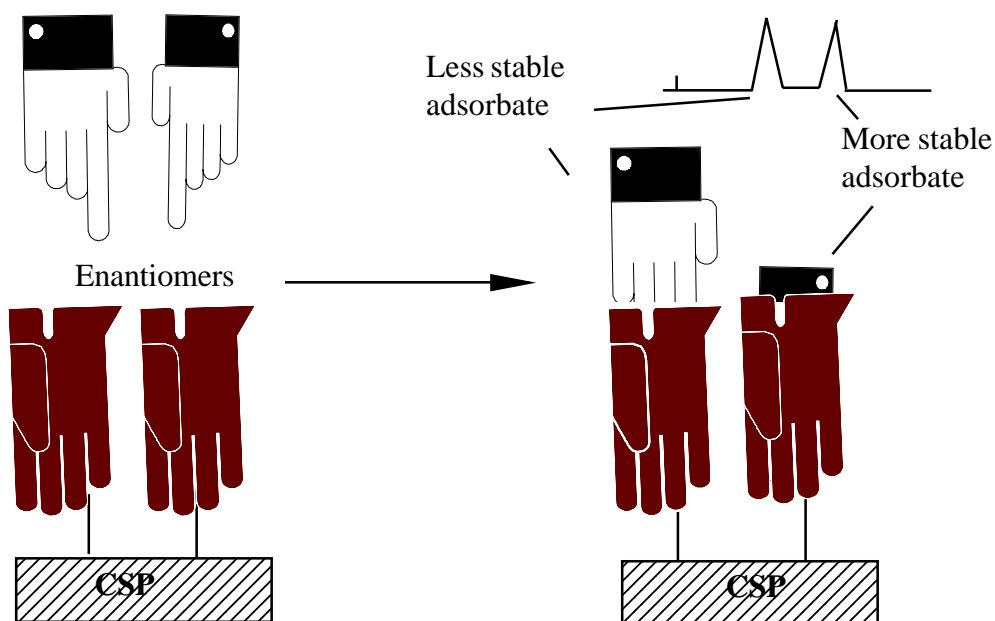
Chiral molecules possess either: an asymmetrically substituted atom or an overall chiral shape.

Chiral molecules containing asymmetrically substituted atoms are the most frequently encountered.

Chiral Molecules with an Asymmetrically Substituted Carbon Atom



Enantiomeric Separation

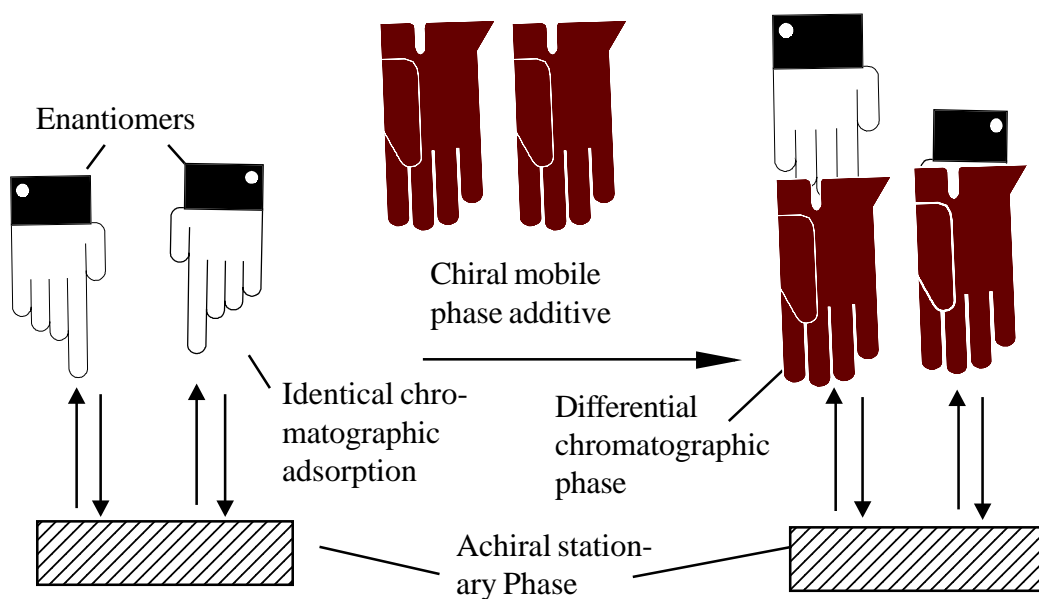


Enantiomer Separation on CSP's depends upon formation of transient diastereomeric adsorbates of differing free energy.

Stability differences as small as 10 calories can result in detectable HPLC separation.

The above illustrates the use of a stationary phase containing an immobilized enantioenriched selector molecule.

An alternative approach utilizes an enantioenriched selector molecule which is added to the mobile phase.

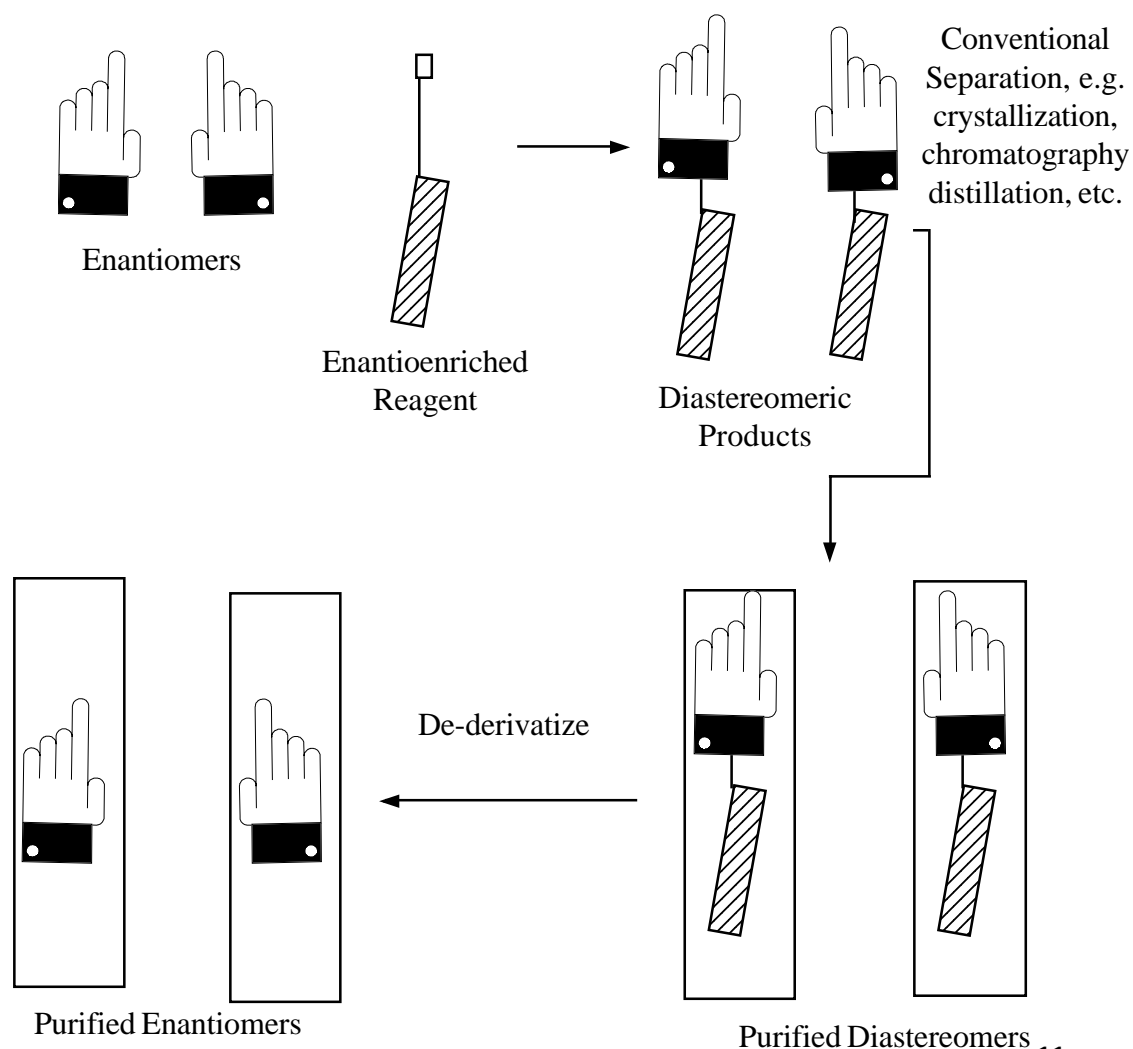


Classical Resolution

Enantiomers have identical physical properties, and consequently cannot be directly separated by conventional methods such as distillation, crystallization, sizing, or chromatography on conventional stationary phases.

Physical separation of the enantiomers comprising a racemic mixture requires the use of some external enantiopure or enantioenriched material or device.

Classically, enantiomers have been separated by forming diastereomeric salts or derivatives with enantioenriched chiral pool reagents. Since these diastereomeric derivatives are no longer enantiomers, they can be separated by conventional separation methods such as crystallization, or chromatography on silica or other conventional stationary phases.



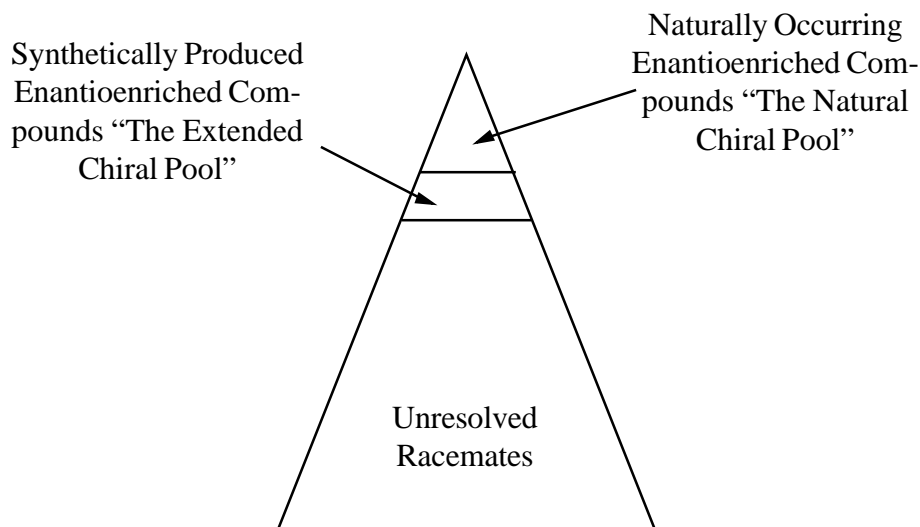
Methods for Obtaining Enantioenriched Compounds

1. Directly from Chiral Pool
2. Synthesis from enantioenriched Chiral Pool starting products.
3. Synthesis using enantioenriched Chiral Pool auxiliaries.
4. Enantioselective catalysts.
5. Classical resolution & Chromatographic enantioseparation.
6. Spontaneous resolution.

Most Chiral Compounds are Unavailable in Enantioenriched Form

Reference:

Sharpless, K.B.;
Amberg, W.;
Bennani, Y.L.;
Crispino, G.A.;
Hartung, J.;
Jeong, K.S.;
Kwong, H.L.;
Morikawa, K.;
Wang, Z.M.; Xu,
D.; Zhang, X.L.,
J. Org. Chem.,
57, 2769, 1992

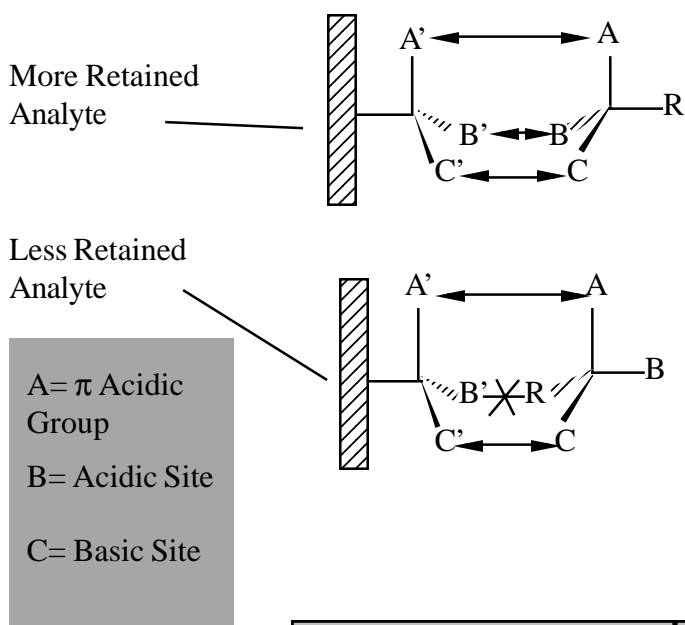


Most of the molecules of importance to living systems are enantioenriched.

Examples include: Amino Acids; Sugars; Proteins; Nucleic Acids; Vitamins; Terpenes; Alkaloids; and Steroids.

Pirkle Chiral Columns

Prof. William Pirkle, of the University of Illinois was an early pioneer in the development of chiral stationary phases. His rationale for the development of the Pirkle Chiral Stationary Phase was based on the following: If a chiral molecule is to have different affinities for enantiomers, it must have a minimum of three points of interaction- at least one of these being stereochemically dependent.



With Pirkle CSP's, chiral recognition occurs at binding sites. Major binding sites are classified as π -basic or π -acidic aromatic rings, acidic sites, basic sites, and steric interaction sites. Aromatic rings are potential for π - π interactions. Acidic sites supply hydrogens for potential intermolecular hydrogen bonds. Basic sites, such as π electrons may also form hydrogen bonds. Steric interactions may also occur between large groups.

Pirkle CSP's generally fall into three

Pirkle π -Electron Acceptors/ π -Electron Donors	π -Electron Acceptors	π -Electron Donors
Whelk-0 ULMO	Phenylglycine Leucine β -Gem 1 α -Burke 2 Pirkle 1-J	Naphthylleucine

classes:

π -electron acceptors

π -electron donors

π -electron acceptors and π -electron donors

Enantiomer Separation

The Pirkle CSP's manufactured by Regis Technologies can separate a wide variety of enantiomers in numerous compound groups. Examples include:

Aryl Propionic Acid Non-Steroidal Anti-Inflammatory Drugs.
Agricultural Compounds.
Natural Products.
 β -Blockers.
Many Pharmaceuticals.

Please see Chart 1, Recommended Column Selection Sequence for Compound Types, page 15.

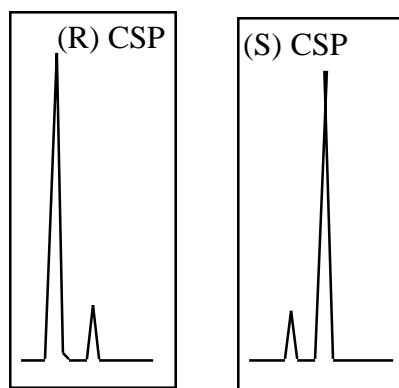
Column Durability

The Pirkle Chiral Stationary Phases are covalently bonded to the silica, providing excellent column durability. The covalent bonding of the CSP to the silica offers the following:

Universal Solvent Compatibility
Long-lasting columns.
The CSP coating will not leach off.
Capacity to tolerate sample overload.

Ability to Invert Elution Order

An important advantage of the Pirkle Chiral Stationary Phase is the ability to invert elution order by using the same type of CSP, but with the opposite absolute configuration. As a result it is possible to have



the trace enantiomer elute before the major- a desirable feature for enantiomeric purity determinations. For preparative separations it is beneficial to elute the desired component first.

**Recommended
Column Selec-
tion Sequence
For Compound
Types**

<u>Class of Compounds</u>	<u>Regis Recommended</u>	<u>Chiral Column</u>
Amino Acids	Davankov (LEC)	Ligand Exchange Column
Acids		
Amines		
Alcohols		
Amides		
Esters		
Sulfoxides		
Carbamates		Whelk-O
Ureas		ULMO
Thiols		Pirkle 1J
Amino Alcohols		Alpha-Burke
Succinamides		Beta-Gem
Hydantoins		Phenylglycine
Binaphtols		Leucine
Beta-Lactams		Naphthylleucine
Cyclic drugs		
Aromatic drugs		
Lactones		
Cyclic ketones		
Alkaloids		
Dihydropyridines		
Oxazolindones		
NSAIDS		

The Pirkle Chiral Columns are given in the order that we recommend screening the compound.

Regis Pirkle Columns

Compatible with any mobile phase?	Can be used in Normal or Reverse Phase modes. Compatible with any conventional mobile phase.
Ability to invert elution order?	Can invert elution order.
Separates broad range of enantiomers?	Yes.
CSP covalently bonded to silica?	Yes. The CSP will not leach off. This covalent bonding makes the CSP very durable.
Durability of CSP?	The most durable column on the market.
CSP covalently bonded to silica?	Yes.
Durability of CSP?	Yes.
Can be used for SFC (Super Critical Fluid" Chromatography ?	Yes. This allows you to clean out the column after use.
Can be used in SMB?	Yes.
Column can be reversed?	Yes. This allows you to clean out the column after use.

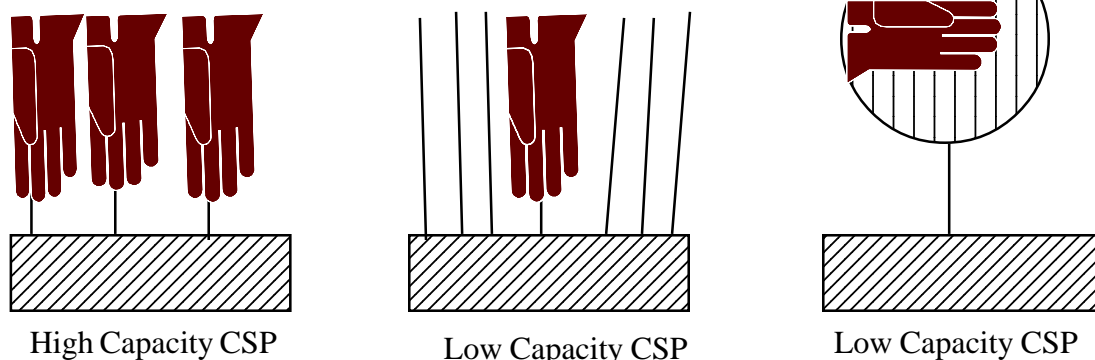
The Pirkle Chiral columns are very versatile, allowing separation of a broad range of enantiomers in either Reverse-Phase or Normal-Phase.

Regis' Pirkle Chiral Stationary Phases are excellent for both analytical and preparative separations.

Chromatographic Efficiency

Unlike many chiral columns, the Pirkle Chiral HPLC Columns show excellent chromatographic efficiency. The Pirkle CSP's also have a high density of binding sites which permits injection of larger amounts of samples without changes in columns performance.

Pirkle Type CSP



Enantiomeric Purity Determination

Pirkle CSP's are particularly useful for the accurate determination of enantiomeric purity, especially in trace analysis because they provide excellent resolution. Such determinations are fast, accurate, precise, and sensitive; and utilize small amounts of sample, which does not need to be chemically pure.

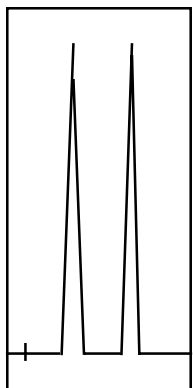
Universal Solvent Compatibility

Choice of mobile phase is not a limitation with the Pirkle HPLC columns. They are compatible with most mobile phases. The pH of the mobile phase, however, must be between 2.5 and 7.5. Both normal-phase and reversed-phase modes can be used, although normal phase is more common.

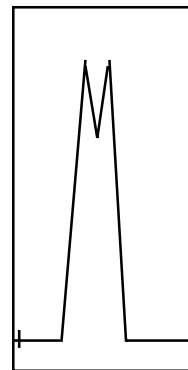
Analytical to Preparative-Sized Columns

All of Regis' Pirkle Columns are available in both analytical and preparative sizes. Since all of the phases are manufactured on-site, Regis can pack special or custom-sized columns quickly and easily at no extra charge.

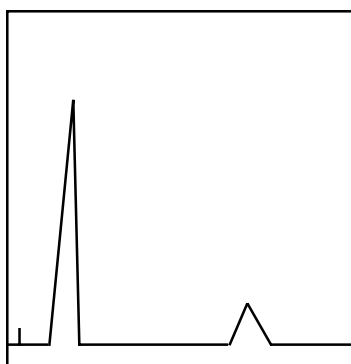
Resolution Of Enantiomers



Baseline Resolution ($R_s > 1.5$) allows for convenient quantitation of e.e.



Resolution < 1.5 (overlapping peaks) complicates e.e. determinations



Resolution $\gg 1.5$ may also complicate e.e. determination, but is useful for preparative separations.

Calculating Resolution

$$R_s = \frac{(t_2 - t_1)}{0.5 (t_{w1} + t_{w2})}$$

where:

t_1 = retention time of 1st eluted peak

t_2 = retention time of 2nd eluted peak

w_1 = peak width at baseline of 1st eluted peak

w_2 = peak width at baseline of 2nd eluted peak

Other Types of CSP's

Modified Polysaccharides:

Chemically modified polysaccharides (starch or cellulose) are absorbed on silica.

Advantages

- Resolve a wide variety of enantiomers
- Reasonably good efficiency
- Reasonably good capacity

Disadvantages

- The CSP has poor stability/ durability
- Poor solvent compatibility
- Cannot invert elution order
- Often unpredictable structure resolution properties

Cyclodextrins:

Cyclodextrins can be used as a mobile phase additive or stationary phase for both GC and LC. They can be considered short circuited starch molecules.

Advantages

- High capacity
- Stable
- Compatible with a wide range of solvents

Disadvantages

- Limited to compounds which can enter cyclodextrin cavity
- Small changes in analyte structure often lead to unpredictable effects upon resolution.
- Cannot invert elution order
- Often poor efficiency

Protein CSP's

In the protein CSP's a protein selector is immobilized on a chromatographic support.

Advantages

- Show broad generality in chiral recognition of great diversity of enantiomers
- Good efficiency

Disadvantages

- Low Capacity- unsuitable for preparative separations
- Poor solvent compatibility
- Cannot invert elution order

Pirkle CSP's

All of Regis' Pirkle Columns are available in both analytical and preparative sizes. Since all of the phases are manufactured on-site, Regis can pack special or custom-sized columns quickly and easily at no extra charge.

Whelk-01
Whelk-02
ULMO
Leucine
Phenylglycine
 β -Gem 1
 α -Burke 2
Pirkle 1-J
Naphthylleucine

Whelk-o1

- Completely Synthetic
- Originally designed for Naproxen
- Broad generality
 - Amides, epoxides, esters, ureas, carbomates, ethers
 - aziridines, phosphonates, aldehydes, ketones
 - carboxylic acids, and alcohols

Selected Applications

- **Naproxen**
- **Bupivacaine**
- **Mephénytoin**
- **Nirvanol**
- **Permethrin**
- **Devrinol**

Whelk-02

- Covalent Trifunctional Version of the Whelk-O 1
- Retains Same Chiral Selector as Whelk-O 1
- Improves Resistance of the Stationary Phase to Hydrolysis While Using Strong Organic Modifiers
- Bonded on 10mm, 100A
- Ideal for Preparative Separations

α -Burke

- Originally Designed for β -Blockers
- Best Column for Amino Alcohols
- Compounds That Can be Resolved
 - aromatic sulfoxides, phthalides, lactams, succinimides,
 - hydantoins, and acetamides

Selected Applications

- Metoprolol
- Atenolol
- Pronethalol

Leucine

- Based on the 3,5-dinitrobenzoyl Derivative of Leucine Bonded to Aminopropyl Silica
- Available in L- and D- Configurations
- Enhanced Enantioselectivities for Benzodiazepines and other racemates

Selected Application

- Hexobarbital

β -Gem 1

- Derived from N-3,5-Dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethyl)-propranoate
- Covalently bonded via an Ester Linkage

Resolves the anilide derivatives of a wide variety of carboxylic acids, including Nonsteroidal Anti-Inflammatory Drugs

Phenylglycine

- Based on 3,5-dinitrobenzoyl phenylglycine
- Covalently Bonded to Amino-propyl Silica
- Available in L- and D- Configurations

Resolves Compounds with p-basic Groups

- aryl-substituted cyclic sulfoxides
- bi- β -naphthol and its analogs
- α -indanol
- α -tetralol analogs
- aryl-substituted hydantoins

Pirkle 1-J

- β -lactase structure
- Useful for the direct separation of underivatized β -blockers, arylpropionic acid NSAIDs, and other drugs
- More Economical than the α -Burke
- Selected Applications

Naphthyleucine

- N-(2-naphthyl) Derivative of Leucine
- Covalently bound via an ester linkage to undecanysilica (C11)

Works with derivatized: alcohols, amines, thiols, amino acids, amino alcohols, and carboxylic acids

ULMO

- Regis' latest Pirkle Chiral column
- Developed by Austrian Researchers Uray, Lindner and Maier
- Particularly good at separating aryl carbinols

Separates enantiomers of many racemate classes

**Marketing and
Technical Support**

Regis has just published a new Chiral Application Guide. The Guide contains applications for our new columns along with new applications for our older columns.

Most of our applications can also be found on our web site (www.registech.com/chiral/applications). In fact the chiral application section of our web site attracts a large number of visitors every week.

Regis also offers complete technical support for all of our chiral columns.

**Free Chiral
Screening
Service.**

If you are not sure which chiral column will separate your compound, we can help. The first thing we ask is to see the structure of the compound. If necessary we will sign a secrecy agreement. From the structure we can determine whether or not one of our Pirkle columns should be able to separate the compound. At this point we are then willing to take a small sample and screen it on all of our Pirkle columns for free. If we are successful in separating the compound we will give the method we used and chromatograph we obtained.

Scale Up

If your customer would like to scale up to a preparative separation we will help them through the process. From information obtained from the separation obtained on an analytical column, we will help them determine exactly what size of prep column then need to obtain their required separation.