Chapter 7

USE OF LIPASES FOR ORGANIC SYNTHESES

The application fields of lipases are not only those in lipid-modification. Lipases are also very powerful tools fo organic synthesis, where non-lipid substrates are reacted. One of such applications is kinetic resolution and asymmetric synthesis, in which enantio- and stereospecificity of lipases are employed. Here, apart from oil chemistry, the use of lipases for the production chiral chemicals are described.

7-1 Stereochemistry

7-1-1 Chirality

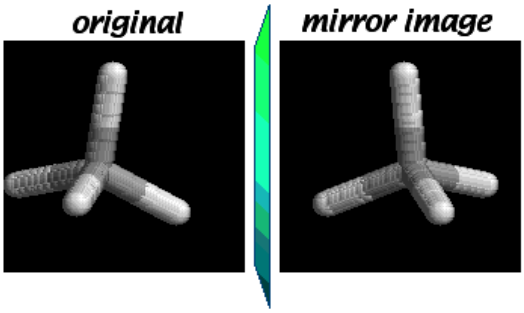

Figure 7-1-1: Achiral structure. The four identical ligands (white) are attached to the carbon (grey).

Consider an organic compound with tetrahedral structure. If the mirror image of the compound is not superimposable on it's mirror image, the compound is **chiral**. If superimposable, the the compound is **achiral**.

Figure 7-1-1 shows one of the cases of achiral compound. The four ligands (white) attached to the carbon atom (grey) are all identical.

Figures 7-1-2 and 7-1-3 show the second and the third cases of achiral structures, respectively. Among the four ligands, three (white) are identical and the rest (red) is different from the three (Figure 7-1-2), and two (white) are identical and the other two (red) are also identical (Figure 7-1-3).

The fourth case of achiral structure is shown in Figure 7-1-4. It has two identical (white) substituents and two (red and blue) different ones. This structure is a type of achiral, but also called **prochiral** structure. **Prochirality** is explained later.

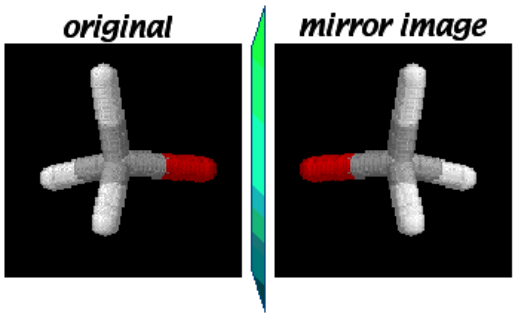

Figure 7-1-2: Achiral structure three (white) of the four functional groups are identical.

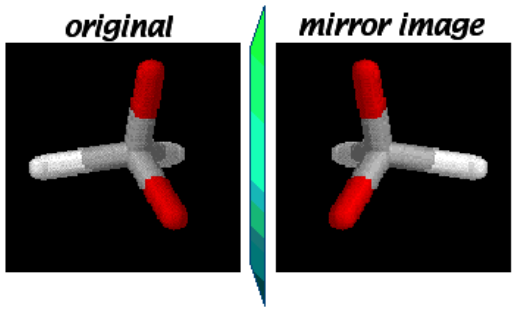

Figure 7-1-3: Achiral structure with two (white) identical ligans and other two (red) identical ones among four.

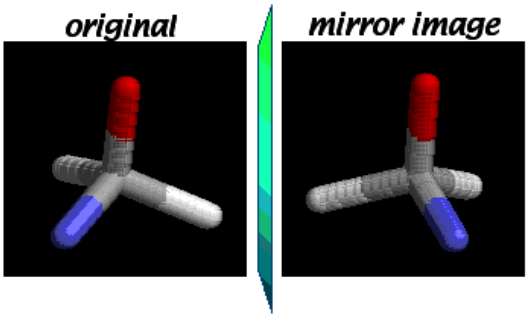

Figure 7-1-4: Achiral structure with two (white) identical ligans and two (red and blue) different ones. This is a prochiral compound.

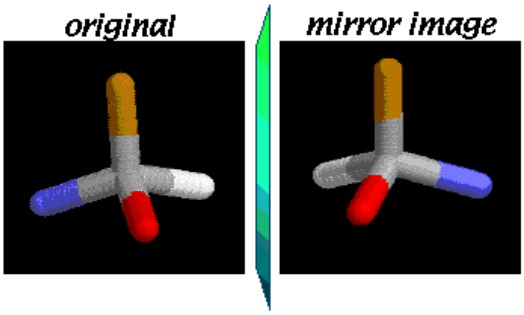

Figure 7-1-5: Chiral structure with four different ligans attached to the stereocenter.

Figure 7-1-5 shows chiral structure. It has four different (white, red, blue and yellow) ligans. The mirror image can not be superimposed on the original whichever it is turned. In such a case, the original and the mirror image compounds are **enantiomer** of each other. The carbon atom with the four different ligans are attached is called **stereocenter** .

7-1-2 Notation of enantiomers

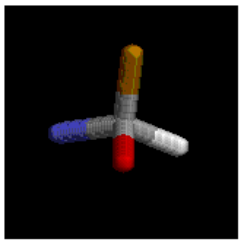

Figure 7-1-6: *R*-configuration.

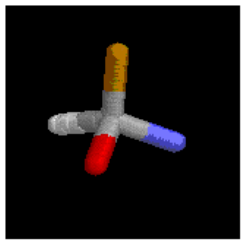

Figure 7-1-7: *S*-configuration.

Configuration of enantiomers are notated by R/S **convention** as follows.

(1) Rank the four ligands according to the priority rules (described later). In Figures 6-1-6, and 6-1-7, suppose that the priority of the ligands is Red → Blue → Yellow → White.

(2) Rotate the molecule so that the ligand with the lowest priority is far from you. Look at the molecule from the opposite side of the lowest priority ligand.

(3) Draw a circular arrow from the highest priority ligand to the next highest to the third highest. If the circular arrow is in clockwise, the configuration is R (R-form). If counter clockwise, the molecule is S (S-form).

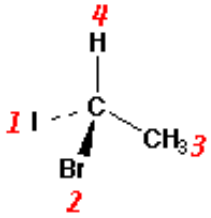

Figure 7-1-8: Rank the ligands by comparing the atoms directly bound to the stereocenter.

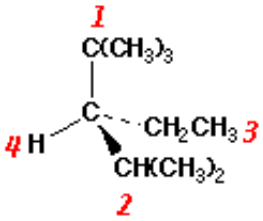

Figure 7-1-9: If some of the directly-bound atoms are the same, compare the next ones.

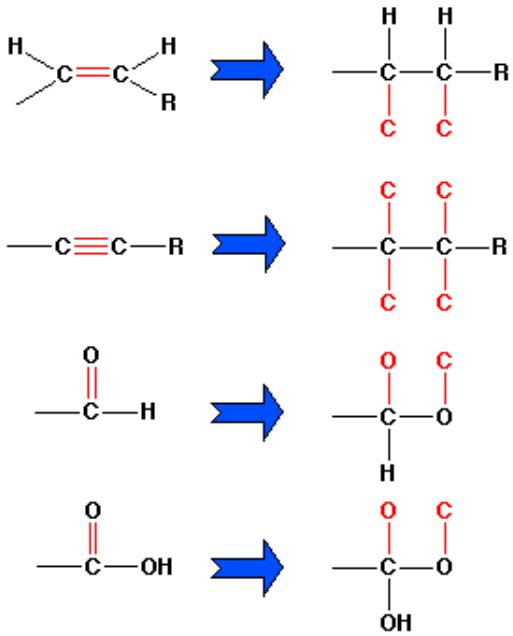

Figure 7-1-10: Multiple bonds are regarded as single bonds assuming that the bond-forming atoms were multiply substituted with the other counterpart atoms.

Priority rules

(1) Compare the atoms which are directly bound to the stereocenter. Ones with larger atomic numbers have higher priority (Figure 7-1-8).

(2) When some of the atoms directly bound to the center are at the same priority, compare the next atoms (second atoms from the center). If they are still the same, continue comparing similarly the third, fourth ... until a difference is found (Figure 7-1-9).

(3) Double and triple bonds are regarded as single bonds. In this case, each of the two atoms connected by double or triple bonds are considered to be substituted with the other counterpart atom twice or three times, respectively (Figure 7-1-10).

7-1-3 Molecules with two or more stereocenters

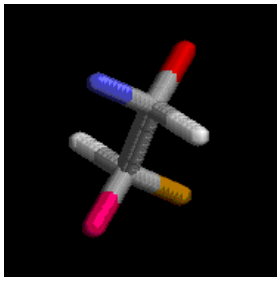

Figure 7-1-11: Notation of the stereo configuration of a compound with two stereo centers.

For a molecule having two stereocenters (grey) shown in Figure 7-1-11, the stereo configuration of each stereocenter can be determined. Here, let us suppose that the ranking of the atoms is **Red** → **Blue** → **Yellow** → **Pink** → **Grey** → **White**. Applying the notation method described above, the stereo configuration of the upper stereocenter is *R*, and the lower one is also *R*. Therefore, the molecule in Figure 7-1-11 is *R*,*R*-form.

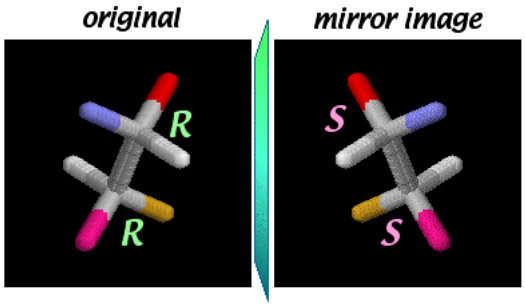

Figure 7-1-12: Enantiomers having two stereo centers.

Figure 7-1-12 compares the structure given in Figure 7-1-11 with its mirror image. Since the mirror image is not superimposable on the original structure, they are enantiomers. Both of the two stereocenters in the mirror image are in *S* configuration (the molecule is *S*,*S*-form), contrary to the original (*R*,*R*-form).

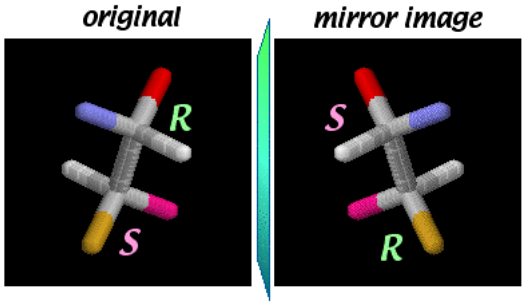

Figure 7-1-13: *R*,*S*- and *S*,*R*-forms are enantiomers of each other.

Consider other isomers, in which the configuration of only one stereocenter is different. Figure 7-1-13 shows such isomers with *R*,*S* and *S*,*R* configuration. The *S*,*R* form is the mirror image of the *R*,*S* form and is not superimposable. Therefore, the *R*,*S* and the *S*,*R* are enantiomers.

However, *R*,*S* and *S*,*R* are not mirror images of *R*,*R* and *S*,*S*. Therefore, *R*,*S* or *S*,*R* are not enantiomers of *R*,*R* or *S*,*S*. These isomers in which the configuration of one of the two stereocenters is different (like *R*,*R* vs *R*,*S*, *R*,*R* vs *S*,*R*, *S*,*S* vs *R*,*S* and *S*,*S* vs *S*,*R*) are called **diastereomers**. Figure 7-1-14 summarizes the the relationships of enantiomers and diastereomers.

Consider another structure with two stereocenters. In this case, two stereocenters have the same ligands (Figure 7-1-15). The *(R,R)*-form and its mirror image, *(S,S)*-form are enantiomers, because they are not superimposable. In contrast, their diastereomers *(S,R)*-form and *(R,S)*-form (mirror image) are superimposable (identical). Therefore, they are achiral, although they have some stereocenters. This type of diastereomer is called **meso form**.

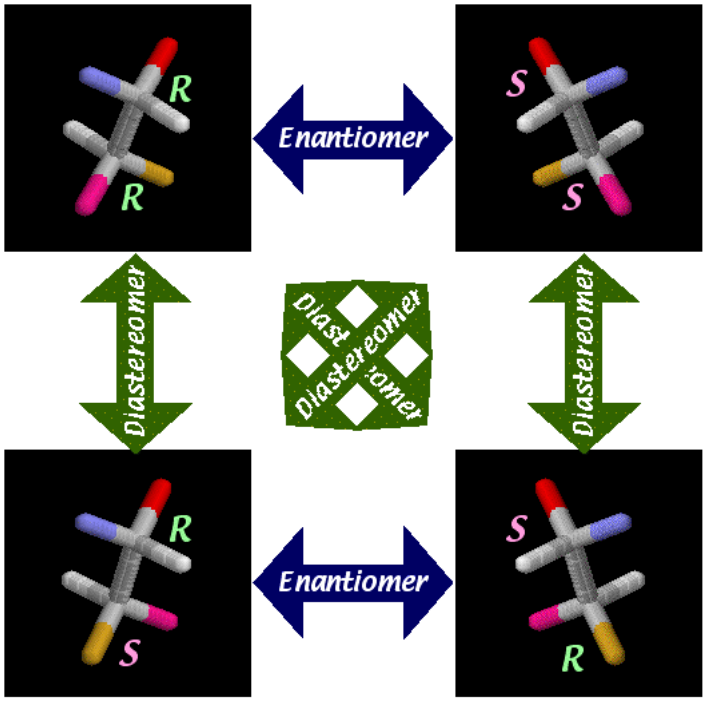

Figure 7-1-14: Enantiomer and diastereomer.

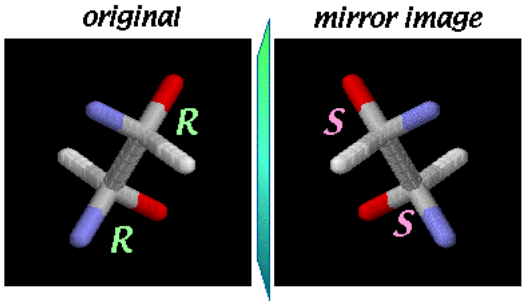

Figure 7-1-15: Structure with two stereo centers both of which have the same ligands.

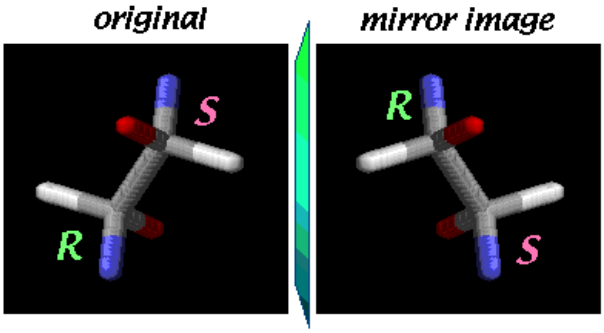

Figure 7-1-16: Meso-form. The original and the mirror image are identical.

7-1-4 Prochirality

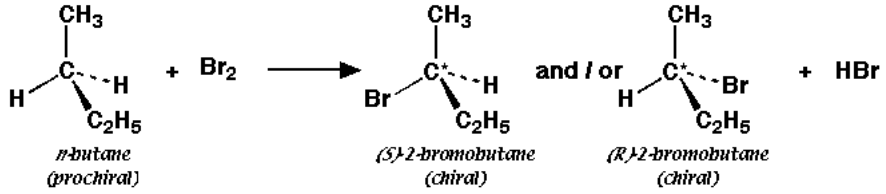

Figure 7-1-17: The sp^3-prochirality.

Prochirality is a structural feature of achiral organic compounds. If a compound can be converted to chiral one by one replacement or addition, the compound is prochiral (it does not matter whether the reaction really runs or not).

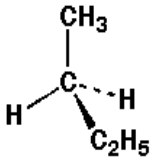

Figure 7-1-18: Notation of *pro-R* and *pro-S* ligands.

As shown in Figure 7-1-17, achiral *n*-butane can be converted to chiral 2-bromobutane by a replacement of one hydrogen at C2 with bromine. Therefore, *n*-butane is prochiral. The stereoconfiguration of the product (2-bromobutane) depends on which one of the two hydrogen atoms in *n*-butane is replaced with bromide atom. This type of prochirality (tetrahedral structure with two identical ligands and two different ligands) is called **sp^3-prochirality**. In sp^3-prochirality, the two identical (enantiotopic) ligands (the two hydrogen in *n*-butane) are not equivalent, and are distinguishable. Notation of the two enantiotopic ligands in an sp^3-prochiral molecule is done as follows.

(1) Rank the four ligands according to the priority rules similarly to the *R*/*S*-convention. For the two identical enantiotopic ligands, choose **either one arbitrarily**, and give it **higher priority than the other**.

(2) Look at the molecule from the opposite side of the lowest priority ligand.

(3) Draw a circular arrow from the highest priority ligand to the next highest to the third highest. If the circular arrow is in clockwise, the ligand chosen and given higher priority is ***pro-R*** ligand, and the other ligand is ***pro-S*** ligand. If counter clockwise, the ligand chosen is called ***pro-S***, and the other is ***pro-R***.

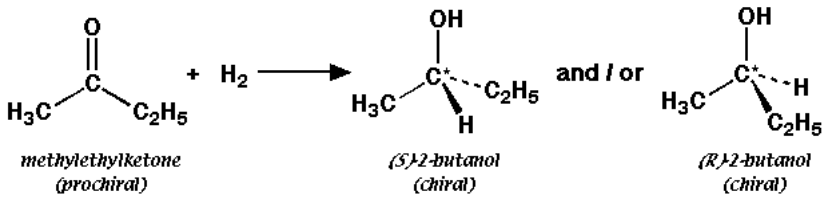

Figure 7-1-19: The sp^2-prochirality.

Figure 7-1-19 shows another case of prochirality. Reduction (addition of hydrogen) of an achiral ketone (methylethylketone) gives chiral secondary alcohol (2-butanol). Therefore, the ketone is prochiral. The stereoconfiguration of the product (2-butanol) depends on from which side the ketone is attacked. This type of prochirality (trigonal system with double bonds) is called **sp^2-prochirality**. In sp^2-prochirality, the molecule has a plane structure. The two faces of the plane are not equivalent, and are distinguishable. Notation of the two faces in an sp^2-prochiral molecule is done as follows.

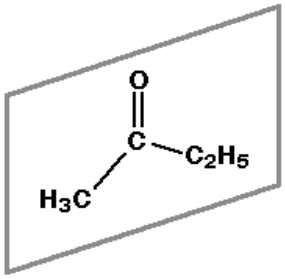

Figure 7-1-20: Notation of *Re* and *Si* faces.

(1) Rank the three ligands according to the priority rules similarly to the *R*/*S*-convention.

(2) Choose arbitrarily either one of the two faces of the molecular plane. Look at the molecule from the face chosen.

(3) Draw a circular arrow from the highest priority ligand to the next highest to the third highest. If the circular arrow is in clockwise, the face the viewer is looking from is ***Re*-face**, and the other (opposite) face is ***Si*-face**. If counter clockwise, the face chosen is ***Si*-face**, and the other is ***Re*-face**.

7-2 Importance of chiral compounds

7-2-1 Why chiral compounds are important?

Chirality is a major concern in the modern pharmaceutical industry. This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects.The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects.

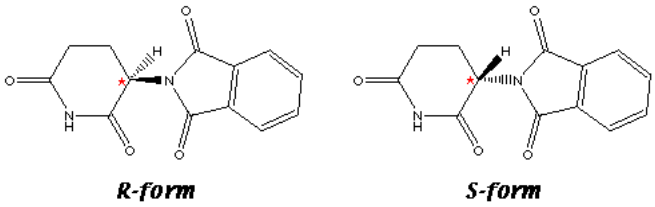

Figure 7-2-1: Structure of Thalidomide. R-form (left) is an effective sedative, while S-form (right) is teratogenic. Red asterisk indicates the stereo center.

Consider the tragic case of the racemic drug of n-phthalyl-glutamic acid imide that was marketed in the 1960's as the sedative Thalidomide. Its therapeutic activity resided exclusively in the R-(+)-enantiomer. It was discovered only after several hundred births of malformed infants that the S-(+)-enantiomer was teratogenic.

The U.S. Food and Drug Administration, in 1992, issued a guideline that for chiral drugs only its therapeutically active isomer be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways. In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs are both synthetic and chiral. However, a large number of chiral drugs are still marketed as racemic mixtures. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.

7-2-2 Asymmetric synthesis

To prepare only one enantiomer, one strategy is **asymmetric synthesis**. In an asymmetric synthesis, an excess of either one of the enantiomers is produced. The starting substrate for these synthesis is prochiral or meso. So the point is to make chiral product from achiral substrate. This is done using a catalyst which can act on the substrate stereoselectively (usually the catalyst itself is chiral molecule).

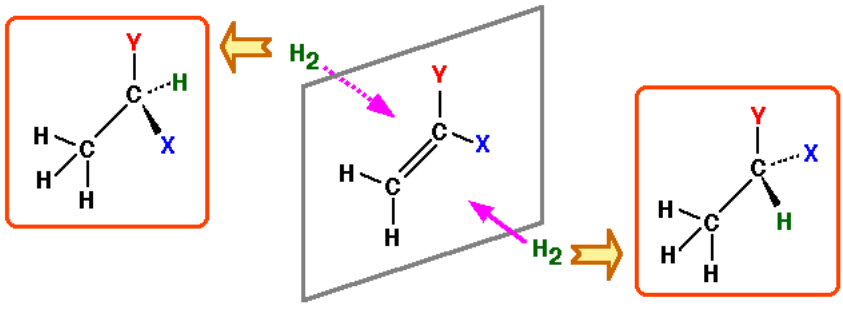

Figure 7-2-2: Asymmetric synthesis. Asymmetric hydrogenation of double bond.

Figure 7-2-2 shows a typical example of asymmetric synthesis, asymmetric hydrogenation of C=C double bond. The chirality of the product depends on which side (*Re* or *Si* face) of the substrate H_2 attacks.

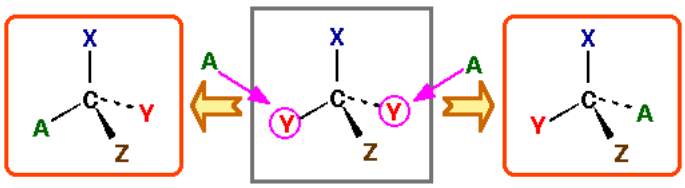

Figure 7-2-3: Asymmetric substitution of a prochiral molecule.

Figure 7-2-3 shows asymmetric substitution of a prochiral molecule. Substitution (or modification) of either one of the two enantiotopic ligands (Y) gives the chiral product. The chirality of the product depends on which ligands (*pro-R* or *pro-S*) is modified.

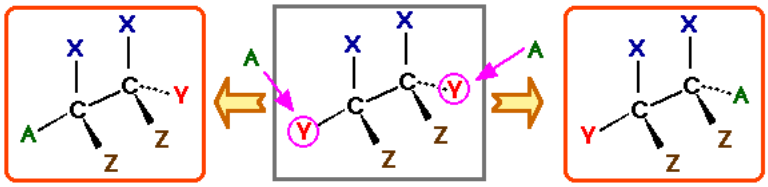

Figure 7-2-4: Asymmetric substitution of a meso molecule.

Figure 7-2-4 shows asymmetric substitution of a meso molecule. Substitution (or modification) of either one of the two ligands (Y) gives the chiral product having two stereocenters. The chirality of the product depends on which ligands is modified.

7-2-3 Optical resolution

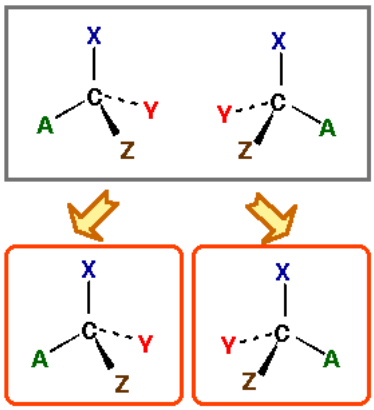

Figure 7-2-5: Direct optical resolution.

Optical resolution is another strategy to prepare only one enantiomer. This is to to separate the desired enantiomer from the other counterpart (Figure 7-2-5).

Figure 7-2-5 shows direct optical resolution without derivatization (chemical conversion) of the target compounds. This is done for example, by using a chiral-phase column liquid chromatography.

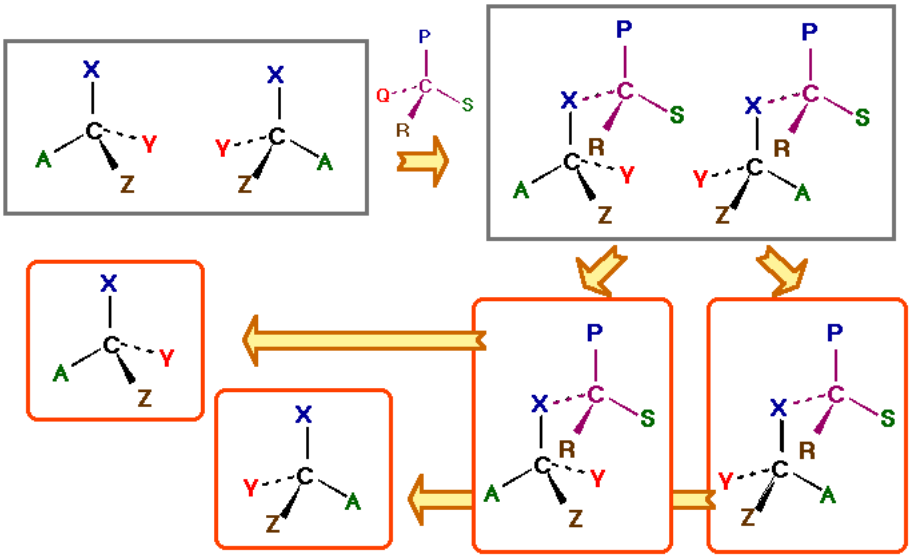

Figure 7-2-6: Diastereomer method.

Figure 7-2-6 shows an alternative, diastereomer method. Mixture of enantiomers are first derivatized to diastereomers by reacting with another chiral reagent (with the ligands P,Q,R,S). The diastereomers are separated from each other by a conventional means (because diastereomers are different from each other in many physical properties). Finally, the isolated diastereomers are restored to the original structure.

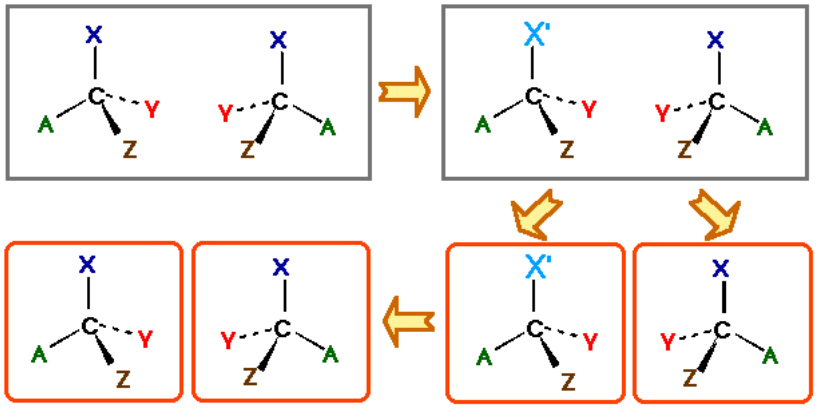

Figure 7-2-7: Kinetic resolution.

Figure 7-2-7 is another optical resolution, kinetic resolution. Mixture of enantiomers are subjected to derivatization. In this case, special catalysts promote the conversion of one enantiomer faster than the other enantiomer. After the reaction, the modified and the unmodified enantiomers are separated.

7-3 Production of chiral compounds by lipase-mediated reactions.

Many many researches are reported for the synthesis of chiral compounds using lipase-catalyzed kinetic resolution of racemates or asymmetrical modification of prochiral or *meso* compounds. Some interesting examples are shown in the followings.

7-3-1 C3-chiral synthons for β-blockers

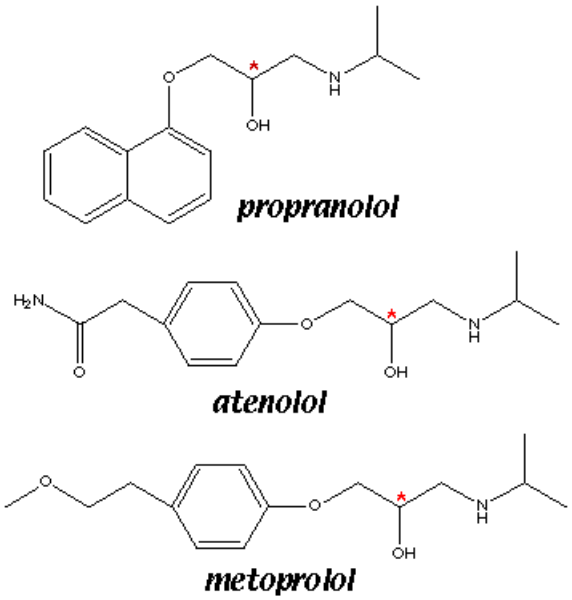

Figure 7-3-1: Structures of some β-blockers.

The β-blockers (or β-adrenergic blocking agents) have been very successful group of antihypertensive agents. They are chiral molecules as shown in Figure 7-3-1. The commercially most important ones such as propranolol, atenolol and metoprolol are all marketed as racemic mixtures, although the active drugs are *(S)*-enantiomers.

An enormous effort has been made for the establishment of the synthetic route for chiral *(S)*-enantiomer of β-blockers. Many of the investigated routes involve synthesis of C3-synthons, often using enzymatic kinetic resolution. For example, Figure 7-3-2 shows an example of the commercial production of optically active glycidyl derivatives via lipase-catalyzed enantioselective hydrolysis of racemic glycidylbutyrate.

Unfortunately, the racemic beta blockers are not yet replaced by the *(S)*-enantiomers; they are still sold as racemates. So the chiral C3-synthons are not utilized for the production of chiral beta-blockers. But, fortunately, these chiral synthons have commercial utility in the synthesis of many optically active drugs.

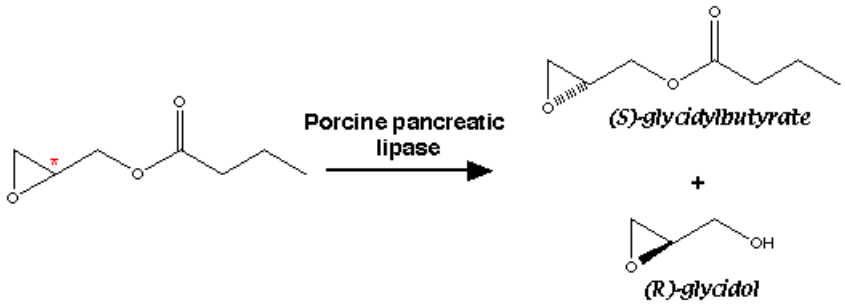

Figure 7-3-2: Lipase-catalyzed kinetic resolution of glycidylbutyrate for the production of chiral C3-synthons.

7-3-2 Diltiazem hydrochloride

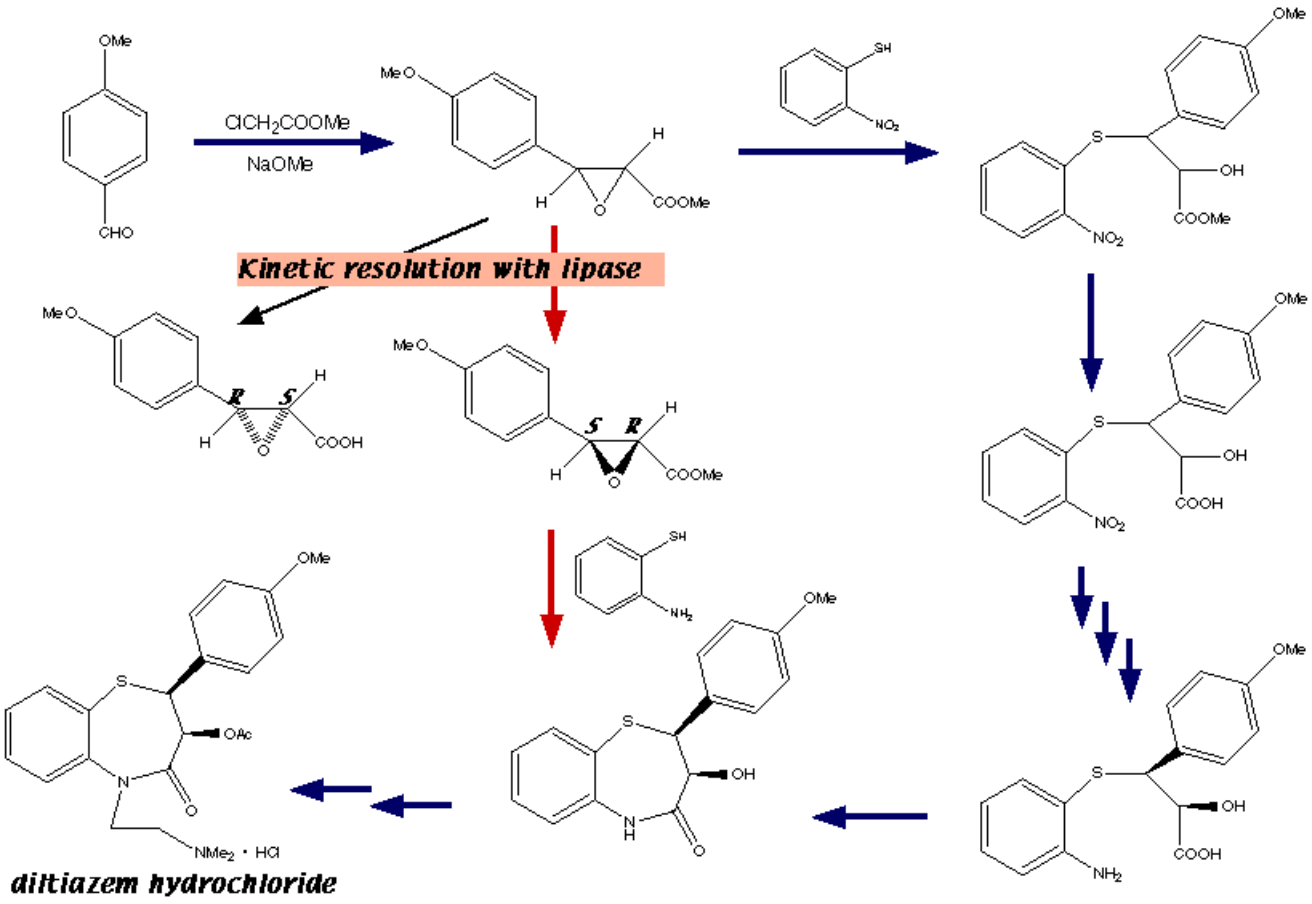

Figure 7-3-3: Production route of diltiazem hydrochloride.

Diltiazem hydrochloride, a calcium channel blocker, is one of the ten best selling drugs in the world. A key intermediate in its synthesis is a chiral glycidic ester. The conventional production route of diltiazem hydrochloride involved 9-step chemical reaction. This route has been now superseded by a more economical process. The racemic intermediate, *trans*-3-(4-methoxyphenyl)glycidic acid methylester is subjected to lipase-mediated kinetic resolution to afford *(2R, 3S)*-ester and *(2S, 3R)*-acid. The *(2R, 3S)*-ester is further converted by chemical means to the chiral target drug. The use of lipase-mediated kinetic resolution enables the production route in shorter route (5-steps).

7-3-3 Chiral pantoic acid

D (or *R*)-Pantoic acid and its derivatives are used as additives for animal feeds and as pharmaceutical products. The commercial production of D-pantoic acid has been dependent exclusively on chemical synthesis including the optical resolution of racemic pantolactone. A drawback of this chemical process is the troublesome resolution of racemic pantolactone, which requires the use of an expensive alkaloid or chiral amine as a resolving reagent.

To solve the problem, an enzyme-mediated kinetic resolution has been developed, The process employs an enzyme, lactone hydrolase (a kind of esterase), which cleaves the lactone

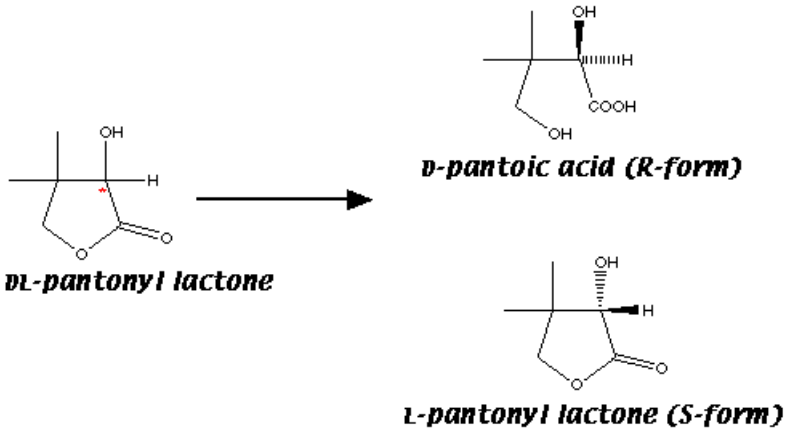

Figure 7-3-4: Kinetic resolution of DL-pantolactone.

ring of only D-pantonyl lactone but not L-pantonyl lactone. This process is commercialized for the production of optically pure D-pantoic acid.

7-3-4 Asymmetrical hydrolysis of a prochiral diester

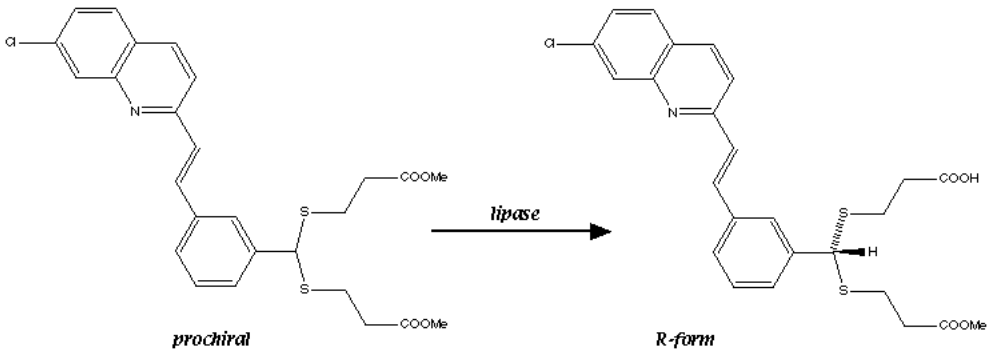

Figure 7-3-5: Asymmetrical cleavage of a prochiral diester.

This example is asymmetrical ester cleavage of a prochiral diester for the synthesis of a leukotriene D4 antagonist developed for treatment of asthma. Treatment of the diester with a lipase cleaves only one of the ester bonds, generating a stereo center.