CHAPTER EIGHTEEN

Chirality and Biological Activity

ALISTAIR G. DRAFFAN
GRAHAM R. EVANS
JAMES A. HENSHILWOOD
Celltech R&D Ltd.
Granta Park, Great Abington, Cambridge, United Kingdom

Contents

1 General Introduction, 782
  1.1 Introduction, 782
  1.2 Definition of Chirality, 783
  1.3 Pharmacology, 785
  1.4 Protein Binding and Metabolism, 786
2 Chromatographic Separations, 787
  2.1 Small-Scale HPLC Examples, 788
  2.2 Chromatographic Diastereoisomer Separation, 788
  2.3 Preparative HPLC/SMB, 789
  2.4 Conclusions, 792
3 Classical Resolution, 793
  3.1 Separation of the Active Pharmaceutical Ingredient, 793
  3.2 Separation of Intermediates to Single Enantiomer Active Pharmaceutical Ingredient, 797
  3.3 Crystallization-Induced Asymmetric Transformation, 798
4 Nonclassical Resolution, 799
  4.1 Preferential Crystallization, 799
  4.2 Enrichment of Enantiomeric Excess by Crystallization, 800
  4.3 Resolution by Direct Crystallization, 802
5 Enzyme-Mediated Asymmetric Synthesis, 804
  5.1 Amide Bond Formation, 804
  5.2 Transesterification and Hydrolysis, 805
  5.3 Oxidation and Reduction, 806
6 Asymmetric Synthesis, 807
  6.1 Chiral Pool, 807
  6.2 Chiral Auxiliary, 810
  6.3 Chiral Reagent, 813
  6.4 Chiral Catalyst, 814
7 Conclusions, 820

Burger's Medicinal Chemistry and Drug Discovery
Sixth Edition, Volume 1: Drug Discovery
Edited by Donald J. Abraham
ISBN 0-471-27090-3 © 2003 John Wiley & Sons, Inc.

1 GENERAL INTRODUCTION

1.1 Introduction

The subtle relationship between the efficacy and chirality of a drug is an area of research that has grown enormously over the past 20 years because of the recognition that single isomer drugs can be more potent and safer than their racemic mixtures. It is intended that this chapter will provide the reader with an appreciation of the area of chirality and biological activity. Indeed, consideration of chirality in drug design has become ubiquitous because of the greater understanding of stereoselective pharmacokinetics, pharmacodynamics, and receptor binding. The enabling technologies of chiral synthesis and analysis have provided the tools to drive these advances in the detailed understanding of the differential biological activity of stereoisomers. Isomers of identical constitution but differing in the arrangement of their atoms in space are defined as stereoisomers (enantiomers and diastereomers are subclasses). Enantiomers consist of a pair of molecular species that are mirror images of each other and are not superimposable.

The term chiralty is broadly used within chemistry and drug development; however, the terms chiralty or chiral are not always well understood by the reader. To clarify the matter, we can consider that two main situations exist. In the first case a sample consists of equal numbers of molecules having an opposite sense of chiralty (heterochiral molecules). This sample is said to be chiral but racemic. The second case occurs when a sample is made up of molecules that all have the same sense of chiralty (homochiral molecules). In this case the sample is said to be chiral and nonracemic.

Recognition of the importance of chirality and biological activity has led to the position where the regulatory authorities will no longer consider the registration of a new racemic compound. Exceptions include cases where the stereoisomers interconvert *in vivo* or where there is a specific advantage or synergy associated with dosing both stereoisomers as a racemate. In addition to the benefit to the patient of a safer and more potent drug, there are numerous advantages to a company in developing a single isomer drug. For example, the cost and complexity of testing is simpler for single isomers, because the Food and Drug Administration (FDA), which regulates the approval and sale of drugs and other products in the United States, requires that both of the isomers within a racemate be tested. Thus, the overall development costs and time are greatly increased because of the requirement for information on all three, the racemate and both isomers separately. The use of a single isomer should also result in a lower dosage, at least one-half that of the racemate. Because of both the therapeutic advantages and the greater regulatory burden of proof associated with a racemate compared with a single isomer, sales of single isomer drugs have increased to over $120 billion dollars, representing more than 30% of the total market in 2000 compared with 3% in 1980 and 9% in 1990. When combined with the declining figures for new drug application approvals by the FDA, the efficient and rapid development of a single isomer drug is imperative (1).

The fundamental reason for the differential activity of stereoisomers is that the majority of molecules that make up living organisms are chiral, and moreover, exist in only one enantiomeric form. Thus, stereoisomers will be seen by the system as different molecules and will have different effects on the biological system. Typically therefore, one of the single enantiomers of a drug will demonstrate greater potency and/or less side effects than the corresponding racemate, and such examples are given within this chapter. For example, Vigabatrin, which is a selective GABA transamidase inhibitor, gained approval in 1997 as a racemate. While the two stereoisomers exhibit the same pharmacokinetics, only the *S*-enantiomer is active as an anti-epileptic. However, there are some limited cases, such as Tramadol, where a synergistic benefit associated with the dosing of the racemate is claimed in comparison with either single enantiomer (2).

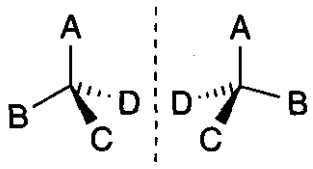


Figure 18.1.

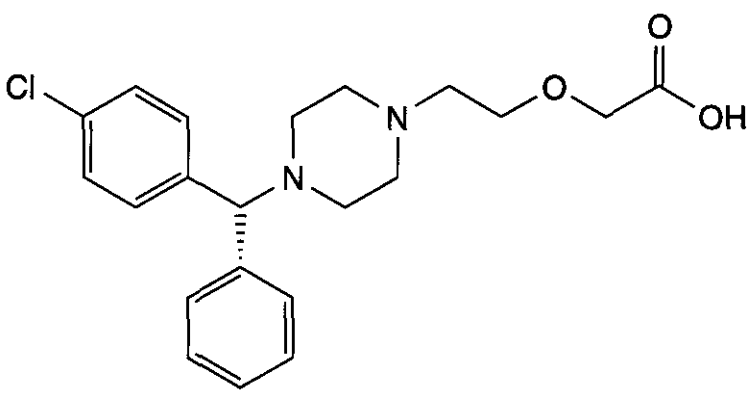
1.2 Definition of Chirality

The definition of chirality and its measurement are described in great detail in a number of texts **(3);** however, a brief introduction to the key issues is given in this section. Specifically, chirality is a term referring to a property of a molecule that is nonsuperimposable on its mirror image as shown in Fig. 18.1, where such a molecule is chiral.

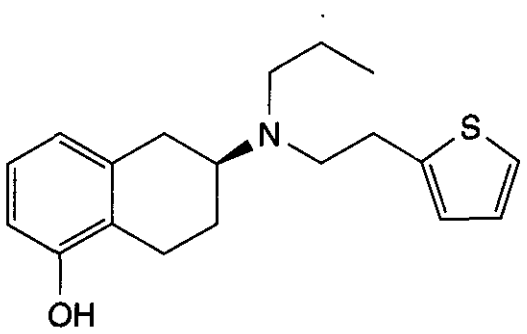
In the majority of cases, chirality results from the three dimensional orientation of four different substituents around a carbon atom forming the chiral center. In addition the orientation of atoms or groups around sulfur, phosphorus, and nitrogen atoms can sometimes form a chiral center. Examples of chiral drugs are numerous but include Certirizine (1), Rotigotine (2), and Ifosfamide **(3).**

When a molecule contains only one chiral center, the two stereoisomers are known as enantiomers. These may be referred to or labeled using the configurational descriptors as either R (*rectus* meaning righthanded) or *S* (sinister meaning left handed), or alternatively, D (dextrorotatory) or L (levorotatory). The D and L configurational descriptors are also commonly used in classifying the configuration of sugars and amino acids (see below). In an achiral environment, enantiomers will behave identically, exhibiting for example, the same melting/boiling point, lipid solubility, nuclear magnetic resonance (NMR), infrared (IR), etc. However, in a chiral environment such as within the macromolecular components of a living system or a chiral high performance liquid chromatograpy (HPLC) system, the enantiomers display different properties, such as a different route and rate of metabolism, different biological activity, and different retention times in chiral HPLC. A frequently quoted analogy for the differing properties of enantiomers is the hand and glove example. The left and right hand are enantiomers of one another, that is they are nonsuperimposable mirror images. If the right hand or "enantiomer" is placed in the right hand glove, or "receptor", there is a good fit. Thus, in the case of a true drug and receptor there will be the desired effect. If the left hand, or "enantiomer" is placed in the right hand glove, there is either a poor fit or no fit.

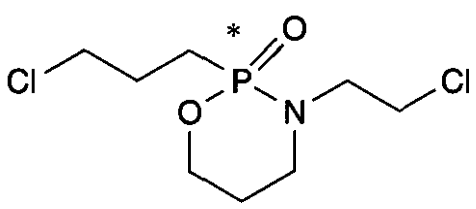
As introduced previously, when a chiral compound is present as exactly a 1:1 mixture of its enantiomers, it is referred to as a racemate or racemic mixture. Thus, if a single enantiomer undergoes racemization, the pure enantiomer is converted to a 1:1 mixture of enantiomers. Perhaps the most famous example of a chiral drug is Thalidomide. In the early



Levocertirizine (1)



Retigotine (2)



Ifosfamide (3)

Figure 18.2.

1960s, Thalidomide was widely prescribed as a sleeping pill and as a treatment for morning sickness, with claims that it was completely safe. We all know of the terrible birth defects suffered by children born to mothers who took the drug during pregnancy. The drug was taken as the racemate, and it has been shown that the R-enantiomer is responsible for the drug's anti-inflammatory activity, whereas the S-enantiomer causes the teratogenicity. Separation of the racemic mixture to give the patient only the R-enantiomer is not a simple answer to the problem. The liver contains an enzyme that converts the R- into the *S*-enatiomer, thus negating the benefit of giving the single enantiomer (4).

As described in this chapter, there are many reactions that can be performed by chemists to create new chiral centers. When these reactions are performed in such a way as to create one enantiomer in greater amounts than the other the process is called asymmetric or stereoselective synthesis. The term enantioselectivity refers to the efficiency with which the reaction produces one enantiomer. This efficiency is quantitatively described as the enantiomeric excess (ee) of the product, which is the percentage by which one enantiomer is produced in excess of the other. Thus a 45:8 mixture of two enantiomers will have an enantiomeric excess of $[(45 - 8)/(45 + 8)] \times 100$, which equals 70%. It should be noted that if neither the startingmaterial or reaction system is chiral and non-racemic, then the product will be formed as an equal mixture of the enantiomers (i.e., a racemate).

Glucose is perhaps the most widely available chiral compound. It is a monosaccharide and part of the sugar group (carbohydrates) that occur naturally. Sugars, along with amino acids, constitute a special example and are commonly classified with a D- or L-configuration. In the case of sugars, the D-configuration is given when the hydroxyl group on the highest numbered chiral carbon atom is on the righthand side (with the structure drawn in the Fischer convention as shown in Fig. 18.3). Likewise for L-configured sugars, the hydroxyl group is on the lefthand side. In the case of the tetrose sugars there are two enantiomer pairs as illustrated in Fig. 18.3. Here, the enantiomer pairs of erythrose (4, 5), namely D-(−)-erythrose (4), and L-(+)-erythrose (5), and threose (6, 7), and L-(−)-threose (6) and D-(+)-threose (7), are shown, with each pair of enantiomers being diasteromeric with the other pair. Diastereomers can be simply defined as stereoisomers that are not enantiomers. The prefixes *erythro*- and *threo*- are applied to such systems that contain two asymmetric carbons where two of the groups are identical and the third is different. The *erythro* pair has the identical groups on the same side, whereas the *threo* pair has them on opposite sides.

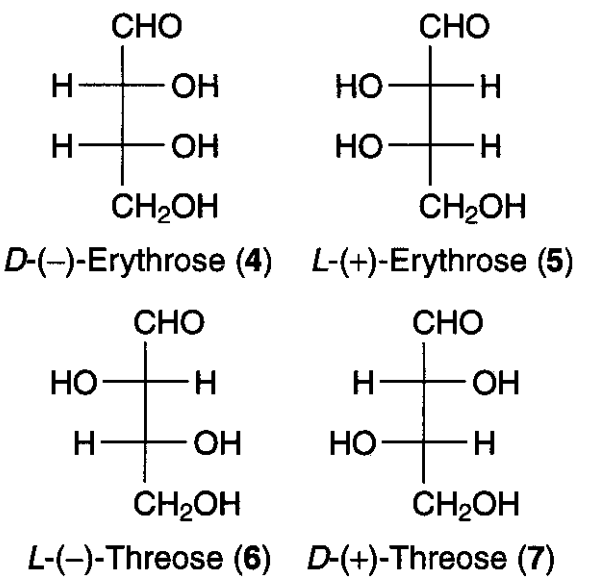


Figure 18.3.

Finally, as further elucidation of this relationship where the molecule contains more than one chiral center the number of stereoisomers increases. In the case of the drug glycopyrrolate which contains two chiral centers, there are four possible stereoisomers as shown in Fig. 18.4. In general, the number of possible isomers can be calculated from the formula 2^n where n is the number of chiral centers.

The four stereoisomers can be divided as shown into two pairs of enantiomers, where the (*R*,*R*)-(8) and (*S*,*S*)-(9) stereoisomers are enantiomers of one another, and the (*S*,*R*)-(10) and (*R*,*S*)-(11) stereoisomers are also an enantiomeric pair. The stereoisomers that do not have an enantiomeric relationship to one another, such as (*R*,*R*)-(8) and (*R*,*S*)-(11) are known as diastereomers. Like enantiomers, these molecules are not superimposable on one another, but unlike enantiomers, they do not exhibit the same physical, chemical, and spectral characteristics. Thus, they have different melting/boiling points, lipid solubility,

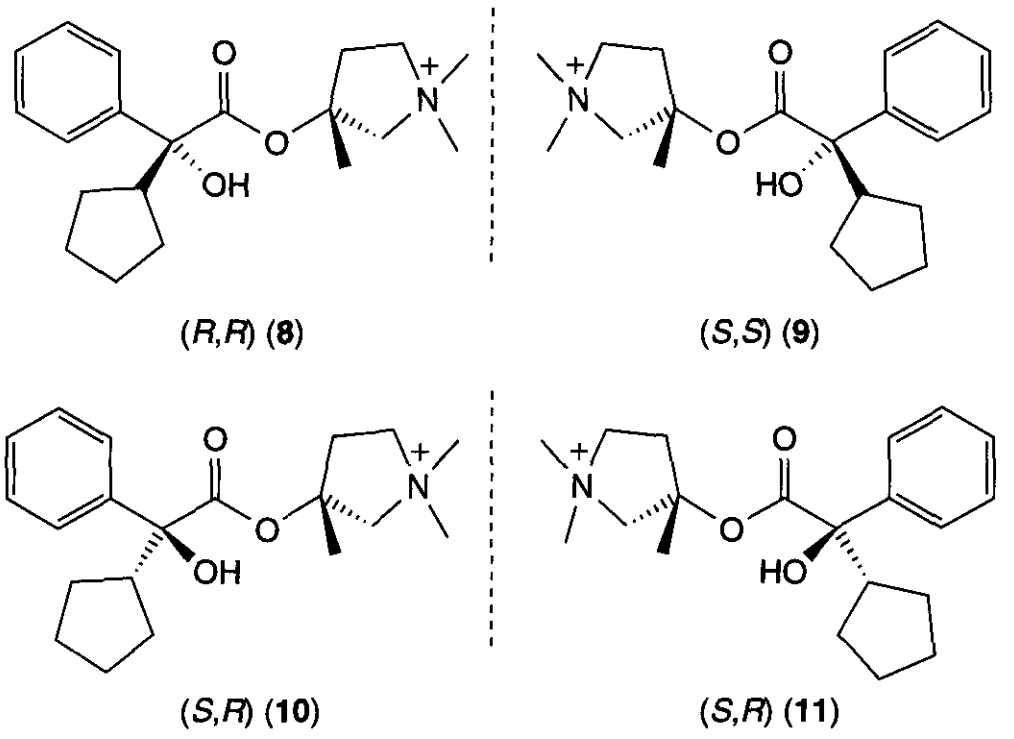


Figure 18.4.

NMR spectra, retention time in HPLC or thin layer chromatography (TLC), and can behave differently in chemical reactions with achiral reagents. The commercial glycopyrrolate product contains only the threo isomers (*S,R*)-(10)and (*R,S*)-(11).

1.3 Pharmacology

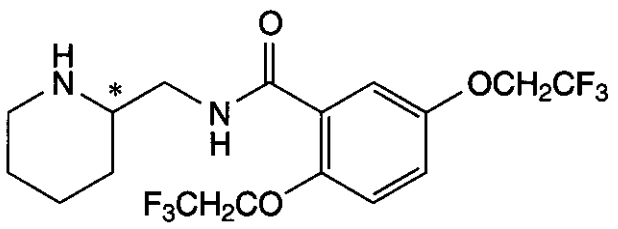
Biological systems are in the main constructed from homochiral molecules such as L-amino acids or D-sugars. Such systems give rise to a highly "chiral environment," and hence, it is not surprising that many drugs possessing asymmetric centers exhibit a high degree of steroselectivity in their interactions with biological macromolecules. In the past 20 years or so, pharmacological and toxicological investigations have clearly demonstrated significant differences in the biological activity of some isomeric pairs. Pharmacokinetic investigations have also led to a better understanding of racemic drug action.

It is important to introduce two other terms that compare the pharmacological activity of a pair of enantiomers. The isomer imparting the desired activity is called the eutomer (in the case of Thalidomide this is the R-enantiomer), whereas the isomer which is inactive or causes unwanted side effects is called the distomer (this is the S-enantiomer for Thalidomide). Comparison of the potencies of the two isomers comes from the eudismic ratio and this can be used in vitro or in *vivo*. With the advancement in analytical and preparative technologies, the researcher is now more able to separate and study individual enantiomers. Pharmacological assessment of the behavior of chiral compounds in early phase research is imperative for selection of the correct isomer for development.

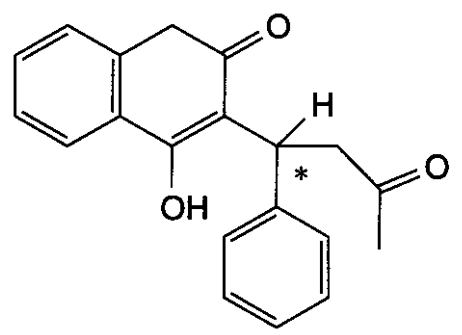
When a racemate is administered, the overall pharmacological effect may have one of three general outcomes described below.

1. All activity resides in one of the isomers, the other antipode being inactive.
2. Both isomers have equal activity.
3. Both isomers have the same activity but differ in potencies.

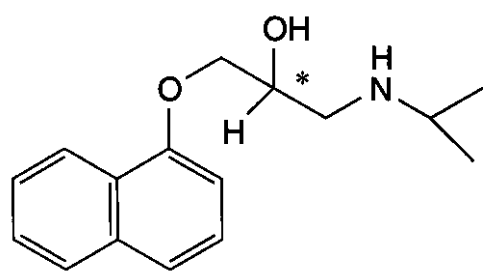
We will briefly highlight some examples that help to elucidate the above general classes with some pertinent examples. The antihypertensive agent a-methyldopa is an example where all the desired antihypertensive activity is confined to a single isomer (the L-enantiomer). It is noteworthy that L-(a)-methyldopa is a prodrug, being metabolized to the L-isomer of the active metabolite, and it is this metabolite that has the required activity (5). L-Dopa is marketed as the single enantiomer; during early development it was noted that the D-isomer exhibited serious side effects such as granulocytopenia (which is defined as a reduced number of blood granulocytes) (6).



Flecainide (12)



Warfarin (13)



Propranolol (14)

Figure 18.5.

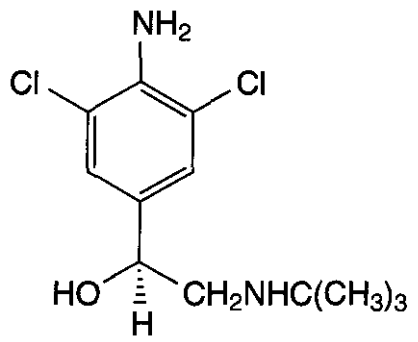
It has been reported that the single **enantiomers** of **Flecainide** (12) (Fig. 18.5) have similar *in vitro* pharmacological activity. Assessment of the effect of each **enantiomer** on the action **potential** characteristics in canine cardisc **Purkinje** fibers gave similar **electrophysiological** effects. The plasma concentration data of the **enantiomers** was only very moderately enantioselective. This gave rise to the authors concluding that there was no advantage in administering a single **enantiomer** of Flecainide over the **racemate** (7). It is, however, relatively more common to find examples where the **enantiomers** have similar qualitative pharmacological activity but differ in their potencies. Two classic examples are **Warfarin** (13)(see Fig. 18.5 for the structure and later on in the crystallization section for it's preparation) and **Propranolol** (14). The potency of ***S*-(−)-Warfarin** *in vivo* is two to five times greater than that of the ***R*-enantiomer** (8). However this difference in potency is offset by the two- to fivefold greater plasma clearance of S-(−)-Warfarin (9). These apparently offsetting properties are only part of the complex pharmacological story surrounding Warfarin, and to this day, it is still administered as the racemate.

β-blocking drugs such as **Propranolol** (14) (Fig, 18.5) have been shown to **stereoselectively** bind to **β-receptors**, and it is the ***S*-(−)-enantiomer** that exhibits the **β-blocking** activity. However, *in vitro*, the binding of the R-and ***S*-enantiomers** varies widely within this class of compound depending on the structure. Again a complex number of **pharmacological** actions come into play with the plasma binding of the ***R*-enantiomer** being much greater than it's antipode. The two **enantiomers** are also stereoselectively metabolized at different rates ($R > S$). Therefore, the pharmacological dynamic outcome can vary greatly between patients who have different P_{450} compositions (10). To add to this already complicated story, the bioavailability of ***S*-(−)-Propranolol** is reduced when given as the single **enantiomer** compared with the racemate. This suggests that the presence of ***R*-(+)-propranolol** has a beneficial effect on the availability of the ***S*-(−)-enantiomer** (11).

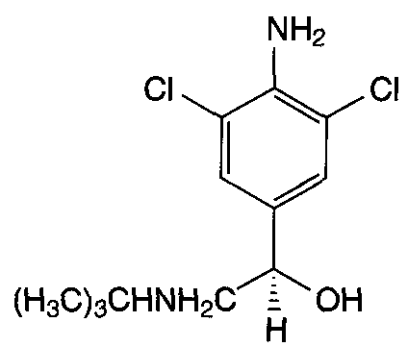
1.4 Protein Binding and Metabolism

The **enantiomers** of a specific drug can bind stereoselectively to plasma proteins. For example, acidic drugs bind in an enantioselective manner to human serum albumin (12). There are two binding sites on albumin, site I (Warfarin) and site II (indole) (13). The binding of L-tryptophan to site II is up to 100 times greater than that of D-tryptophan (14). It has been suggested that binding to albumin can be used as an indication of the extent of the binding to the drug receptor. For basic drugs, α_1-acid **glycoprotein** (AAG) is used and is relatively **non-stereoselective** (12, 15).

Enantioselective metabolism and clearance plays a prominent role in determining the pharmacological effect of a drug. For example, a highly potent rapidly cleared **enantiomer** may be of less benefit clinically than it's lower potency antipode, which is more slowly cleared. Returning to Warfarin, the ***S*-enantiomer** is eliminated mainly by **7-hydroxylation**, whereas the ***R*-enantiomer** is eliminated by ketone reduction and oxidation to 6 and 8-hydroxywarfarin (16). **Tramadol** is a centrally acting analgesic with efficacy and potency



(*R*)-(–)-Clenbuterol (**15**)



(*S*)-(+)-Clenbuterol (**16**)

Figure 18.6.

ranging between weak opioids and morphine, and it is currently used as a racemic mixture (Fig. 18.6). It is metabolized in the liver mainly to *O*-desmethyltramadol (ODT), mono-N-desmethyltramadol, and di-*N*,*O*-desmethyltramadol. Of the metabolites only the *O*-desmthyltramadol is pharmacologically active and the (+)-ODT has ~200 times greater activity for the μ opioid receptor (17). It is thought that this metabolite contributes largely to the analgesic properties of Tramadol, and for this reason numerous studies have been undertaken on the activity of the single enantiomers of Tramadol (17). The complex nature of the interaction of the single enantiomers of a drug with biological systems described in the introduction will have an effect on whether a single enantiomer or racemate is taken through to development.

The following sections describe the available methods for the separation of enantiomers or their preparation using asymmetric synthesis. The first sections involve separations of enantiomers using chromatography or crystallization technology. These are often considered to be the most expedient methods and should deliver the single enantiomers in the shortest timescale. Asymmetric synthesis has developed considerably over the last 20 years and now provides an alternative, and at times more cost-effective, method of preparing the single enantiomers. It is noteworthy that the 2001 Nobel prize for chemistry was awarded to Sharpless, Knowles, and Noyori for their pioneering work in the area of asymmetric synthesis (18).

2 CHROMATOGRAPHIC SEPARATIONS

The separation of enantiomeric drugs and intermediates by chromatographic methods is a well-developed area that is broadly utilized from milligrams to tons (19). Initially, these chromatographic methods were used to determine the enantiopurity of the compound obtained from, for example, a separation process. With the continuing advancement of chiral stationary phases (CSP) and the development of chromatography, separation of enantiomers using chromatographic techniques is increasingly seen as the method of choice because of the speed at which the separation can be achieved (20).

A multitude of chromatographic separation methodologies exist, all of which can be applied to the separation of enantiomers; for example, liquid chromatography (LC) (21), gas chromatography (GC) (22), high performance liquid chromatography (HPLC) (23), capillary electrophoresis (CE) (24), super critical fluid chromatography (SFC) (25), simulated moving bed (SMB) (26), and membrane technologies (27). Once the appropriate technique has been chosen, at the analytical level, run time, sensitivity, and selectivity then need to be enhanced to improve the limits of detection and analysis time. At the preparative scale (milligram to gram), in addition to enantioselectivity, other factors such as high loading capacity, robustness, and chemical compatibility are essential requirements when selecting the CSP (28). In addition, the CSP needs to be readily available and provide a cheaper process when compared with the chemical/biological alternatives that are discussed in this chapter. However a significant advantage of the method is that almost any enantioseparation can be achieved with one of about 200 commercially available chiral selectors by the techniques described above (29). In this section, we will highlight a number of examples,

from the small semi-preparative scale to large-potential manufacturing processes.

2.1 Small-Scale HPLC Examples

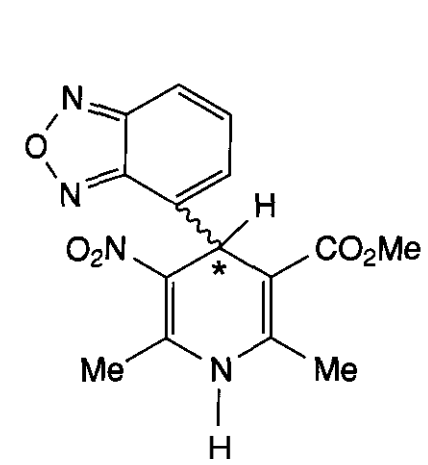
HPLC is now a widely available and user-friendly method employed for qualitative and quantitative analysis and is also one of the most expedient methods for providing the milligram quantities of stereochemically pure material required for initial testing. Often the identification of a suitable CSP to effect separation of a specific pair of enantiomers is seen as being labor intensive and requiring considerable exyerimentation. However, the availability of commercial databases that compile literature on LC enantioseparations makes this process significantly easier (30). The companies that supply CSPs also provide detailed information about a specific columns' suitability towards the separation of certain types of compounds (31). This helps to avoid a "trial and error" approach towards enantioseparations using chromatography. The use of column switchers to test a number of CSPs can also be of enormous assistance in a rational screening program.

One example of separation by HPLC is Clenbuterol, which is an orally active, sympathomimetic agent that has specificity for β2-adrenoceptors. Owing to its bronchodilator properties, it has found use in the treatment of respiratory disorders in humans and animals (32). The two enantiomers of Clenbuterol have been separated using a chirobiotic column, which consists of a macrolide-type antibiotic stationary phase, using a mobile phase with composition of 70% MeOH, 30% acetonitrile, 0.3% acetic acid, and 0.2% triethylamine (33). The enantiomers eluted as follows: *R*-(−)-Clenbuterol (15) with a retention time of 8.35 minutes and *S*-(+)-Clenbuterol (16) with a retention time of 9.12 minutes. The single enantiomers obtained through chromatography were of >95% optical purity. It has been shown that (−)-Clenbuterol was 100–1000 times more potent than (+)-Clenbuterol in β-adrenergic agonist bioassays (34).

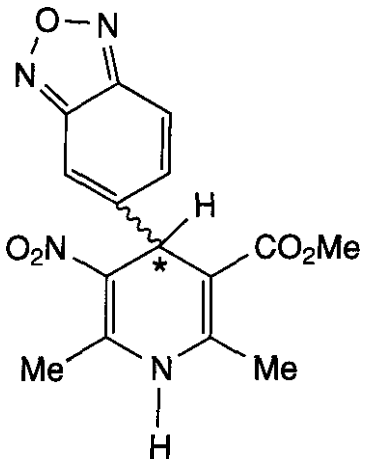
A number of 1,4-dihydropyridines (**17–20**), exhibiting axial chirality (chiralty stemming from the nonplanar arrangement of four groups about an axis), have been separated by small-scale HPLC methods. This is an important class of drugs that are potent blockers of calcium currents and have found use in the treatment of cardiac arrhythmias, peripheral vascular disorders, and hypertension (35). It has been shown that enantiomers of chiral DHP have opposite pharmacological profiles (35). One of the antipodes is a calcium entry activator, while the other is a calcium entry blocker. The analytical and semi-preparative separation using chiral HPLC for a number of DHPs of the structures (Fig. 18.7) has been described **(36).** Here a number of different CSP were utilized and their ability to separate the above DHPs determined.

2.2 Chromatographic Diastereoisomer Separation

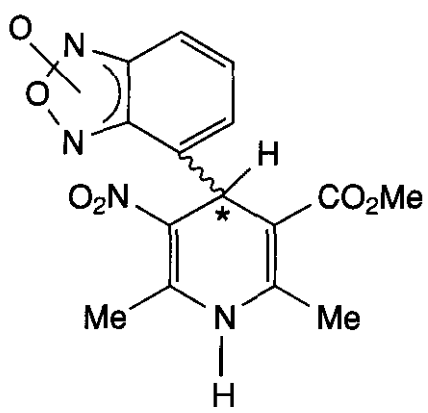
Another approach to the separation of enantiomers by chromatography is to prepare a diastereoisomer of the enantiomer to be separated. As discussed in the introduction to this chapter, diastereomers exist if there is more than one chiral center, but are not enantiomers of one another. As such they do not have identical physical properties. In chromatography, formation of derivatives such as esters, amides, etc., often leads to better separation of the components. In the case of a racemate, if a chiral reagent (i.e., acid or amine) is employed, then a diastereomeric mixture results on treatment with such a derivatizing agent. One such example is the derivatization of Pirlindole, which is a racemic anti-depressant drug. Here the use of amino acid derivatives as chiral derivatizing agents (CDA) was shown to enable an effective and efficient separation (37). Preparation of the L-phenylalanine methyl ester (21) enabled separation of the Pirlindole enantiomers using a medium liquid pressure (MPLC) method. This is highlighted in Fig 18.8, after removal of the CDA the enantiomers of pirlindole were obtained in high optical purity. This gave several grams of each enantiomer, which permitted a study of the stereochemical influence at the pharmacological level. The interaction with monoamine oxidase A (MAO-A) and B (MAO-B) with Pirlindole racemate and single enantiomers using biochemical techniques (in vitro and *ex* vivo determination of rat brain MAO-A and MAO-B activity) was studied. In vitro, the MAO-A IC_{50} of (±)-Pirlindole, *R*-(−)-Pirlin-



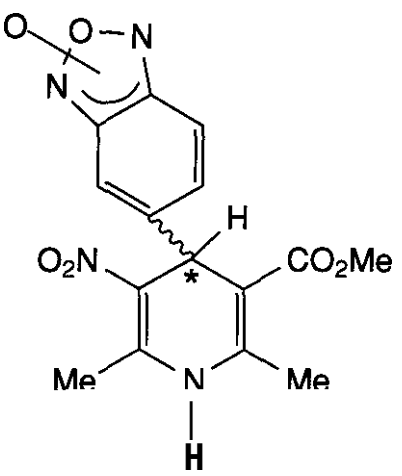
DHP 1 **(17)**



DHP 2 *(18)*



DHP 3 **(19)**



DHP 4 **(20)**

Figure 18.7.

dole (**22**), and S-(+)-Pirlindole (23) were 0.24, 0.43, and 0.18 μM, respectively. The differences between the three compounds were not significant, with a ratio between the two enantiomers R-(−)/S-(+) of 2.2 in vitro (38).

2.3 Preparative HPLC/SMB

In the initial discovery phase of drug research, time is the most important factor where a successful process must be rapidly identified, have a short run time, and have general applicability. As the phase of the project changes to full development, the process needs to be established and cost becomes a crucial factor. Thus, on scale up of an LC method to the preparative level (100 mg and above), a number of additional important aspects become relevant. The selection of a suitable CSP from the plethora available depends on the following factors: CSP availability, loading capacity and selectivity, throughput, and mobile phase.

The most successful and broadly applied chiral stationary phases comprise the cellulose-and amylose-based phases developed by Okamoto (Chiracel and Chiralpak) (39), brush-type phases developed by Pirkle (40), some polyacrylamides (Chiraspher) (41), cross-linked diallyltartramide (42), and to a lesser extent, cyclo-dextrin based phases. Clearly for the larger scale separations, the availability of the CSP in larger quantities is a prerequisite. It should also be noted that at the preparative scale, it seems that up to 90% of racemic compounds tested have been resolved with just four different polysaccharide-based phases (43).

The degree of separation of the two enantiomers obviously plays an important part in the CSP selection. Another equally important parameter is the loading capacity of the stationary phase. The higher the loading capacity, the greater the amount of material that can be separated (44). For example the polysaccharide-based CSPs have a saturation capacity of 5–100 mg/g of CSP; this is clearly dependent on the type of racemate that is beingresolved. On the other hand, protein-based CSPs have lower saturation capacities, of the order 0.1–0.2 mg/g of CSP.

For preparative chromatography, throughput can be defined as the amount of purified material obtained per unit of time and per unit

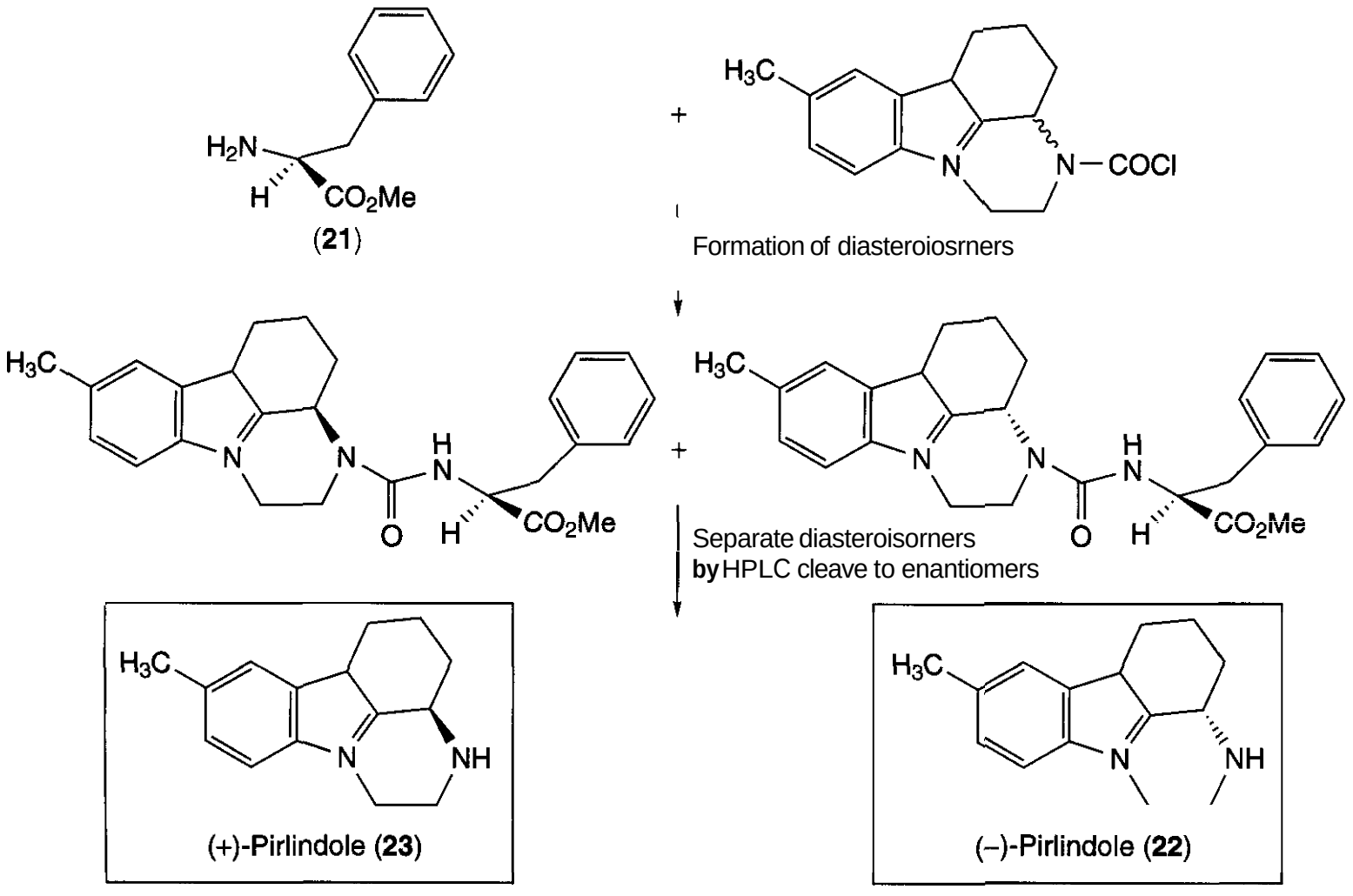


Figure 18.8.

mass of stationary phase. Several factors affect this including loading capacity, column efficiency, selectivity, column size, temperature, cycle time, flow rate, and the solubility of the racemate.

The mobile phase plays a crucial role in the separation process for at least three main reasons. The selectivity of the separation, retention time. and solubility of the racemate are directly affected by the mobile phase composition. Other parameters such as viscosity, solvent recovery, cost, and solvent handling properties also play a prominent role. This brief introduction is also applicable to the criteria for CSP selection for SMB.

An example of a drug separated by preparative HPLC is cetirizine dihydrochloride, a racemic drug that is a second generation antihistamine H_1 receptor antagonist. Studies on the effect of racemic and R **(25)** and S-Cetirizine **(26)** on nasal resistance indicated that both racemic and the R-enantiomer had similar activity. The racemate and R-enantiomer inhibit histamine and induced an increase in nasal resistance, thus indicating the antihistaminic properties of R-Cetirizine **(45).** The *S*-enantiomer was shown not to exhibit these antihistaminic effects. An asymmetric synthesis (46), and resolution of an intermediate have delivered the single enantiomer previously. However, for various reasons, the development of a preparative HPLC method seems to be the method of choice **(47).** The main reasons are the rapid scale up and the improved economics of this approach. Utilization of the amide **(24)** (Fig. **18.9)** gave rise to a highly efficient separation using a Chiralpak AD column in a mixture of acetonitrile/*iso*-propanol 60:40. The efficiency of the separation can be measured by the a value **(2.76)** or the USP resolution **(8.54).** The a value and USP resolution numbers are measurements of how efficient the separation is; typically the higher the number, the better the separation. This enabled the production of **1.6** kg of both the (+) and (−) isomers of high purity.

Like all methods for separating chiral molecules, chromatographic separations do suffer from drawbacks: large quantities of expensive stationary phases are needed and large volumes of mobile phases are used, coupled with the resultant high dilution of separated prod-

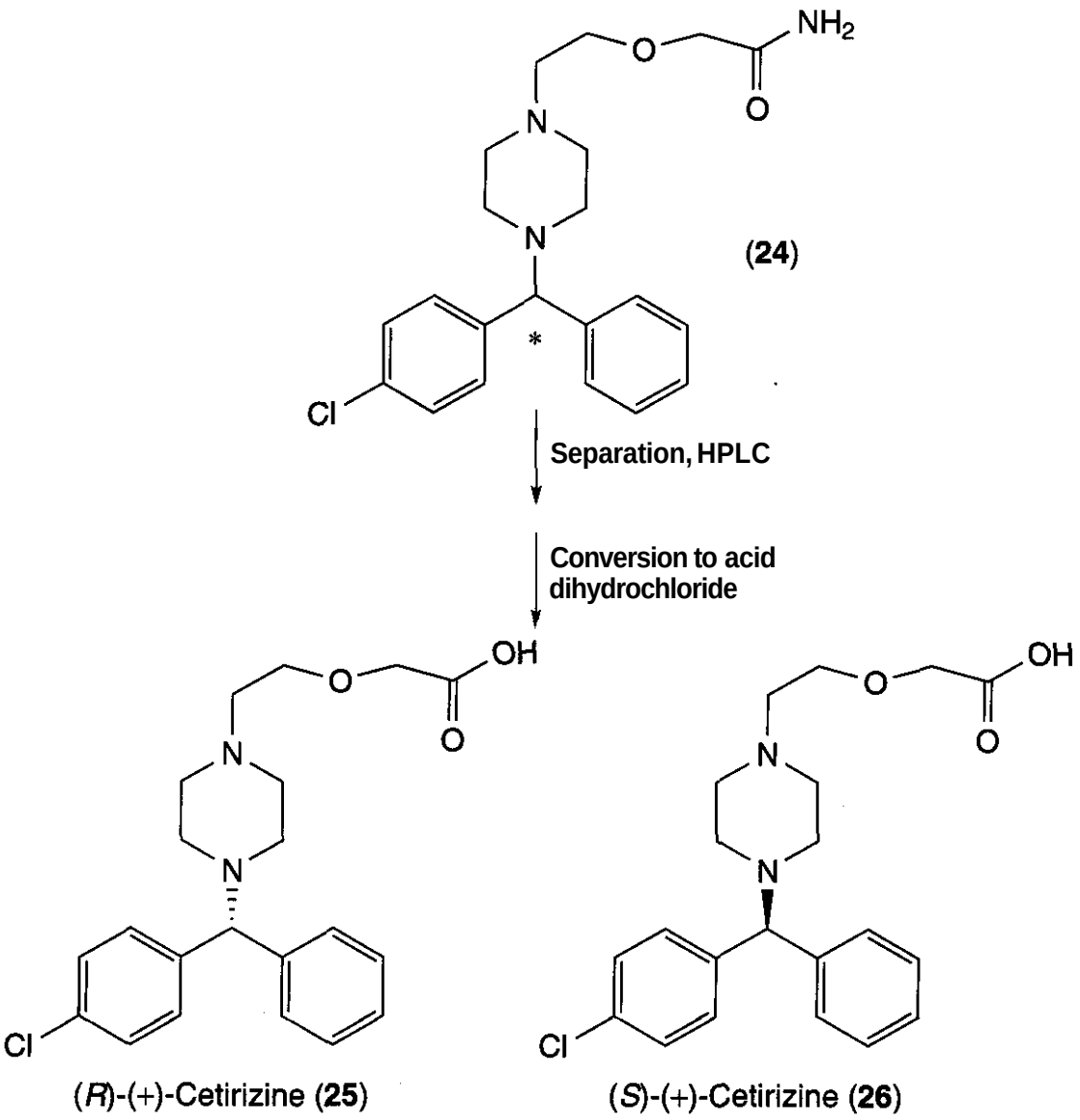
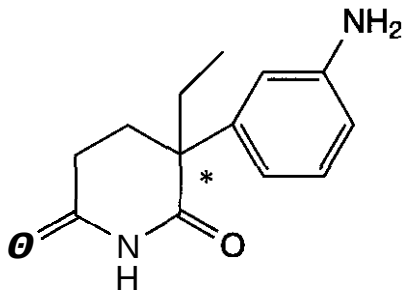


Figure 18.9.

ucts. A number of methods have been introduced in an attempt to improve on this technology, such as recycling (44). Perhaps the biggest advancement in recent times has been the introduction and application of SMB technology in the field of chiral separations (48). This technique was pioneered in the late 1950s by Universal Oil Products in the United States as a useful method for separation of oil derivatives and sugars (49). Initially SMB technology was applied to very large volumes of material. For example, xylene isomers are separated in thousands of ton quantities annually. The application of SMB to the separation of racemic mixtures has led to downsizing and modifications of this technology, but the main principles remain the same. The use of counter-current contact in SMB maximizes the driving force for mass transfer and the contact between the substrate and stationary phase. This provides a more efficient use of the adsorbent capacity than that of a simple batch system (50).

The separation of racemic mixtures is well suited to SMB technology, because these counter current systems can generally only perform two-component separations at a time (51). A detailed description of this technique is given in an excellent article by Guest (52). The SMB system generally consists of several columns, typically 6–12, which are connected in series. An arrangement of pumps and valves are set up to maximize the stationary phase utilization, allowing for better solvent efficiency and adsorbate concentration. This leads to two streams coming off the system in solution, one is termed the raffinate, which is enriched in the less adsorbed component, and the other termed extract, which is enriched in the more adsorbed component. The complex set of conditions and parameters that are required to optimize SMB chromatography has led to the design and process optimization being done by computer simulations (53). A number of examples will be discussed that highlights this growing area of chiral separa-

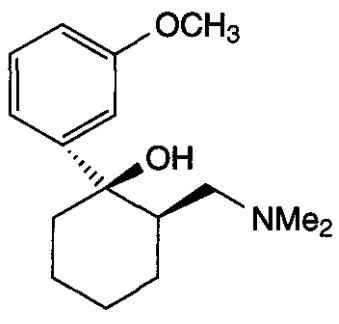


Aminoglutethimide (27)

Figure 18.10. .

tions. It should be noted that the scale of operation is dependent on the column size and can lead to a range from tens of grams to tons of separated isomers. Clearly, the larger quantities separated imply that this technology has industrial applications.

The enantiomers of aminoglutethimide (27) (Fig. 18.10) have been separated using an SMB approach (48) (see also Section 3, Fig. 18.11 for more information on aminoglutethimide). A set of 16 columns (6 × 1.6 cm) containing Chiracel OJ were used. The feed concentration was 1.63% in a mixture of hexane:ethanol (15:85), which was used as the mobile phase. A feed rate of 0.45 ml/min and a mobile phase rate of 6 ml/min gave rise to a production of 5.27 g of each enantiomer per day. The *S*-(−)-enantiomer was obtained as the extract in solution, in a 99.8% purity, while the *R*-(+)-enantiomer also in solution as the raffinate, achieved a 99.9% purity. This would lead to a productivity of 59.9 g of each enantiomer per kilogram of CSP per day. It should be noted that one big advantage of SMB over preparative chromatography are the vast savings on mobile phase consumption; this is generally coupled to thin film evaporators that allow for very high levels of recovery of the solvent. This becomes even more evident when a poorly soluble compound is used.



Tramadol (28)

Figure 18.11.

The two isomers of the racemic analgesic drug Tramadol (28) (Fig. 18.11) display differing affinities for various receptors. (−)-Tramadol mainly inhibits the reuptake of noradrenaline, whereas the (+)-isomer inhibits the reuptake of serotonin. In addition, the (+)-isomer and its primary metabolite, the *O*-desmethyl derivative, are selective agonists of μ opiate receptors (54). Tramadol has been efficiently separated using SMB; in addition, the resolution by crystallization is given in Section 3 of this chapter (55). Comparison between batch chromatography and SMB for the separation of tramadol was made. Use of 12 columns (100 × 21.2 mm ID), each packed with 20 g of Chiralpak AD 20-pm phase, and using a mobile phase composition of 2-propanol/light petroleum/diethylamine (5:95:0.1 v/v/v) with feed concentration of 20 g/L, obtained a very high productivity. Thus, 680 g of racemic tramadol could be separated per liter of stationary phase (which equates to 1.2 kg of racemate per kilogram of stationary phase per day). The solvent consumption of 144 L/kg of racemate should also be noted. This gives both (+)- and (−)-enantiomers of high optical purity, with the extract of 6.33 g/L and the raffinate of 7.69 g/L. Typically, the solvent (mobile phase) is readily recycled by the use of thin film evaporators, which further extends the economic practicality of the process.

2.4 Conclusions

It should be noted that all the techniques described in this chapter can be inter-linked. In other words, if one technique, i.e., asymmetric synthesis, failed to deliver enantiopure material, then another technique such as crystallization can be used to push through the product to the desired purity. As an example of this "double" approach, the application of SMB and crystallization to the separation of mandelic acid is noteworthy (56). When very high levels of enantiopurity are required, the efficiency and cost effectiveness of SMB may not be economical. However, if for example, a lower enantiomeric excess can be coupled with an enhancement by crystallization, then the

SMB approach becomes even more favorable. This can lead to substantial increases in the productivity of the SMB process. Further examples of coupling of two techniques will be given throughout this chapter.

In summary chromatographic separations offer an expedient method for the separation of enantiomers on a small scale. With the development of more efficient stationary phases and the application of SMB, this may become the method of choice for the separation of racemates. Each individual case deserves investigation by all of the techniques/approaches described in this section.

3 CLASSICAL RESOLUTION

Perhaps the most widely used method for the preparation of single enantiomers involves the classical resolution of a racemic mixture, which uses the formation of crystalline diastereomeric salts. As discussed in the introduction to this chapter, by converting a racemic mixture of enantiomers to two diastereomeric salts with differing physical properties, one being crystalline and the other remaining in solution, the molecules can be separated and simply converted back to the two separated enantiomers. With the advent of automation, the classical resolution approach offers a speedy and through racemate separation methodology. This enables the separation of small amounts of material (milligram to gram) and can be directly scaled up to provide an industrial process (kilogram to ton). A number of different approaches to this type of separation are highlighted in the following sections, where it should be noted that diastereomeric salt resolutions have mistakenly been considered to be a mysterious art. In fact, there is considerable information in the literature as to how to perform a resolution and the physical chemistry aspects associated with how to define and conduct the resolution to its optimum capability (57–59). We will not go into this in great detail but will highlight some pertinent points; for greater detail, the reader is directed to the monograph by Jacques et al. (57).

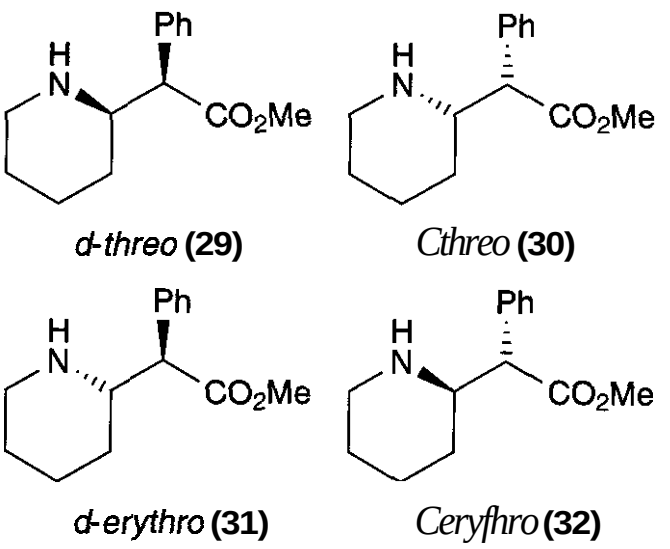


Figure 18.12.

3.1 Separation of the Active Pharmaceutical Ingredient

A number of single isomer switches, that is, where a drug that was previously sold as a racemate is developed and sold as a single isomer, have been isolated through classical resolution (60). This approach to a single isomer offers several advantages; first, the racemate is freely available and can be purchased to high levels of purity and quality. Second, the analytical methods will also be in place. Also, no new synthetic development chemistry is required, and hence this is the fastest route to the single enantiomers at the multigram scale. Generally, this is the first method to be tried. Some of the many available examples demonstrate the different nuances that can be applied in classical resolution to provide the single enantiomers in optimal yields and purities are given in this section.

An efficient and large scale resolution of methylphenidate (ritalin hydrochloride) using dibenzoyl-tartaric acid has been described (61). Ritalin is marketed for the treatment of children with attention deficient disorder (ADHD). Methylphenidate has two chiral centers and originally was marketed as a mixture of two racemates, 20% DL-threo (29, 30) and 80% DL-erythro (**31, 32**) (see Fig. 18.12 for the structures of all four isomers). As introduced previously, the erythro-isomer is defined as the case when the main chain of a molecule (drawn vertically in a Fischer projection) has identical or similar substituents at two adjacent non-identical chiral centers on the same side of the chain, whereas the threo isomer has

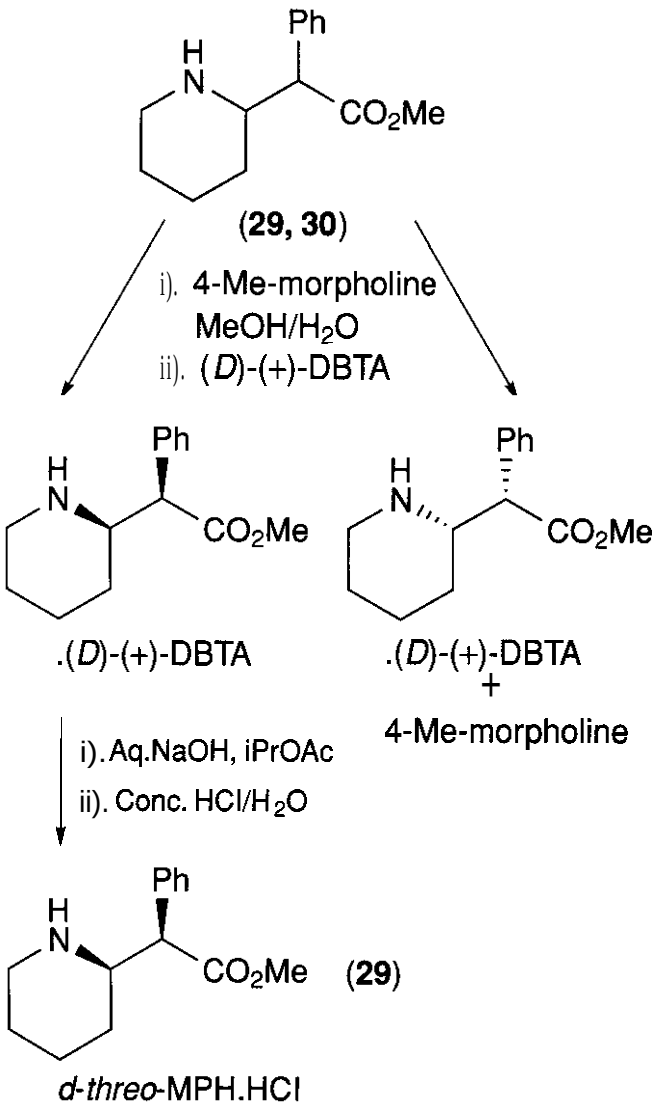


Figure 18.13.

the corresponding substituents on opposite sides. The racemic drug currently used in therapy comprises only the pair of *threo*-enantiomers (29, 30). The mode of action in humans is not completely understood, but methylphenidate presumably activates the brain stem arousal system and cortex to produce its stimulant effect. In addition, there is no specific evidence that clearly establishes the mechanism whereby methylphenidate produces its mental and behavioral effects in children or conclusive evidence regarding how these effects relate to the condition of the CNS. The D-threo (29) enantiomer has, however, been reported to be 5 to 38 times more active than the corresponding L-threo enantiomer (30) (62). The resolution shown in Fig. 18.13 uses the racemic hydrochloride salt as input material. The HCl salt is cracked to the free base *in situ* with 4-Me-morpholine, which then forms a salt with the resolving agent dibenzoyl-tartaric acid (DBTA). The required D-threo-methylphenidate (29) is removed as the crystalline salt of D-(+)-DBTA, leaving the L-threo enantiomer (30) in solution with 4-methylmorpholine hydrochloride. The use of 4-methylmorpholine to effect base release *in* situ helps to streamline the process and to remove 'a costly free base isolation process. The D-threo-methylphenidate, (D)-(+)-DBTA, salt is readily converted into the hydrochloride salt. It is interesting to note that recently, Celgene and Norvatis received a FDA approvable letter for the use of dexmethylphenidate for use in ADHD. This consists of only the D-threo enantiomer (29), in comparison with the original product, which contained all four isomers (29–32).

Chemists at Chiroscience took an alternative approach to the D-*threo*-methylphenidate (29) single enantiomer (63). An efficient resolution using L-(−)-di-toluoyl-tartaric acid (DTTA) was developed. This left the required D-threo diastereoisomer in solution with a diastereomeric excess of 88% yield in 55% chemical yield. Conversion of this salt to the free base and subsequent crystallization of the hydrochloride salt gave >98% ee D-threo methylphenidate in high purity in an overall yield of 42%. The enhancement of the ee is caused by the eutectic point of methylphenidate hydrochloride, which is at 30% ee. A more detailed description of this phenomenon will be discussed later in this section.

(*S*)-Naproxen (36) is a non-steroidal anti-inflammatory drug that was introduced to market in 1976 by Syntex. The *S*-(+)-isomer is about 28 times more effective than the *R*-(−)-isomer (64). The annual sales in 1995 were about $1 billion; thus, a large amount of effort has been spent developing the synthesis of (*S*)-Naproxen (65). The resolution of racemic Naproxen (33), developed by Syntex, approaches the ideal case for a Pope Peachy resolution, that is, resolution using non-stoichiometric quantities of resolving agent (66). Here, a mixture of 1 equivalent (eq) of the racemic acid, 0.5 eq of an achiral amine base, and 0.5 eq of the chiral amine (*N*-alkylglucamine) are used (Fig. 18.13). This results in the formation of two salts: one is the insoluble (*S*)-Naproxen chiral amine (34), obtained in 45–47% yield and optical purity of 99%. The second salt that remains in solution contains (*R*)-Naproxen and the achiral amine (35). The insoluble salt of (*S*)-Naproxen (34) is removed

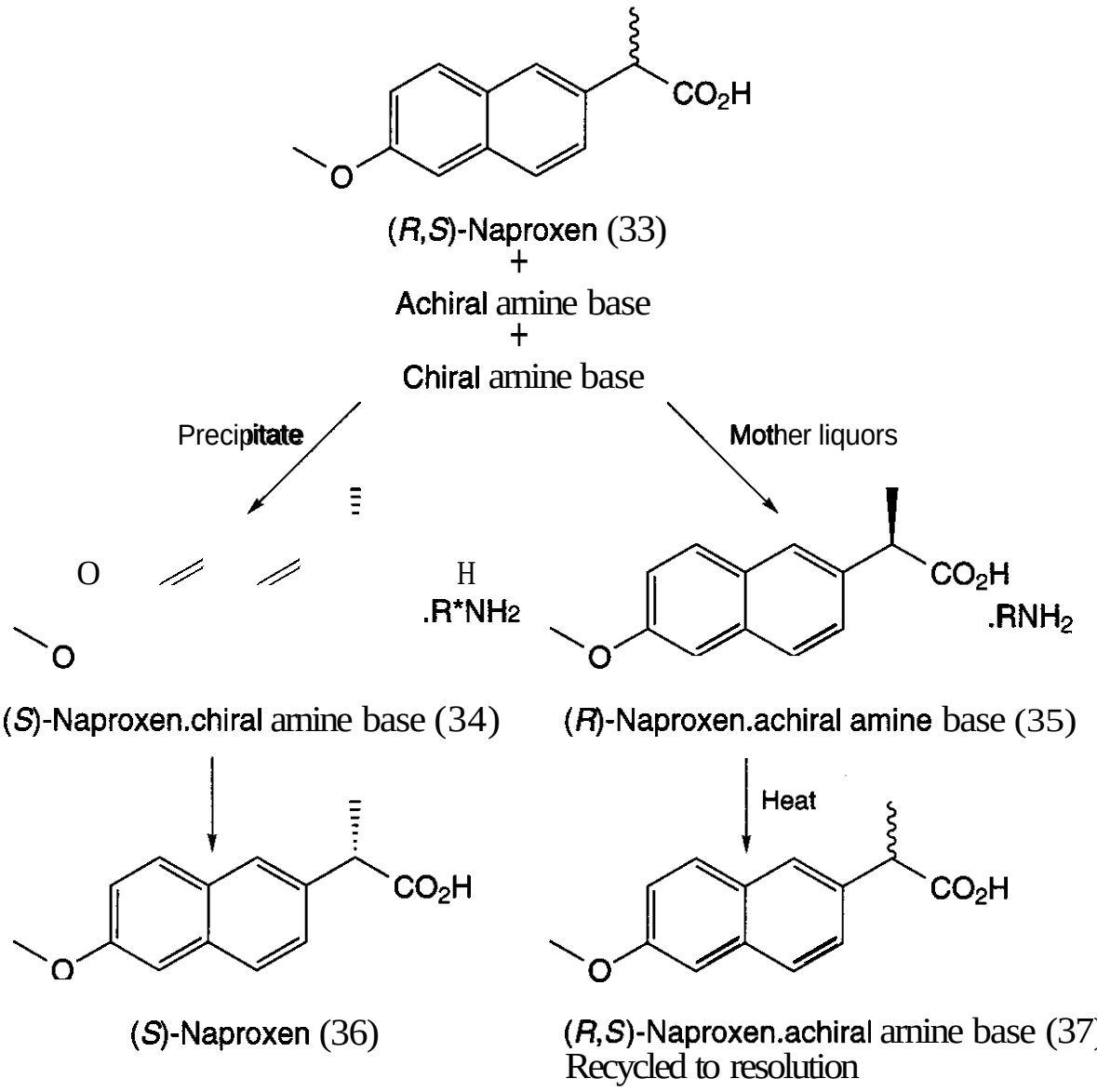


Figure 18.14.

by filtration. The mother liquors are then heated and the achiral amine base catalyzes racemization of the unwanted R-enantiomer. The resulting racemic mixture of the acid (*R*,*S*)-(**37**) can then be put back into the resolution loop. Using this process, the overall yield of (*S*)-Naproxen is >95%, based on the input of racemic acid. To further highlight the efficiency of this process, the *N*-alkylglucamine resolving agent is recovered in >98% per cycle.

Racemic bupivacaine hydrochloride (38, Marcaine) is currently used as an epidural anesthetic during labor and as a local anesthetic in minor operations. Clinical studies have shown that *levo*-bupivacaine (**41**) is less cardiotoxic in man, making it significantly safer than the racemate (67). Separation of the enantiomers was readily achieved using 0.25 eq of D-tartaric acid. This resulted in the isolation of a 2:1 (*S*)-bupivacaine D-tartaric acid salt (39) in 98% de, leaving the (*R*)-bupivacaine free base (40) in solution. Conversion of the tartrate salt to (*S*)-bupivacaine hydrochloride (39) was obtained in 35–40% overall yield based on racemate input. To increase the economics of the process, a racemization of the unwanted R-enantiomer was required. Treatment of the liquors containing the enriched (*R*)-bupivacaine, tartaric acid, propanol, and propionic acid at reflux resulted in complete racemization in 2 h. By pertinent processing, the racemic free base thus obtained is isolated by crystallization and can be put back into the resolution cycle (68). Another fine example by chemists from Eli Lilly involves a clever resolution-racemization-recycle (R-R-R) process in the synthesis of Duloxetine (69).

As discussed in Section 2 of this chapter, Tramadol is a chiral drug substance that is currently used as a high potency analgesic agent. The preparation of Tramadol is shown in Fig 18.16, which results in the formation of all four possible stereoisomers from the Grig-

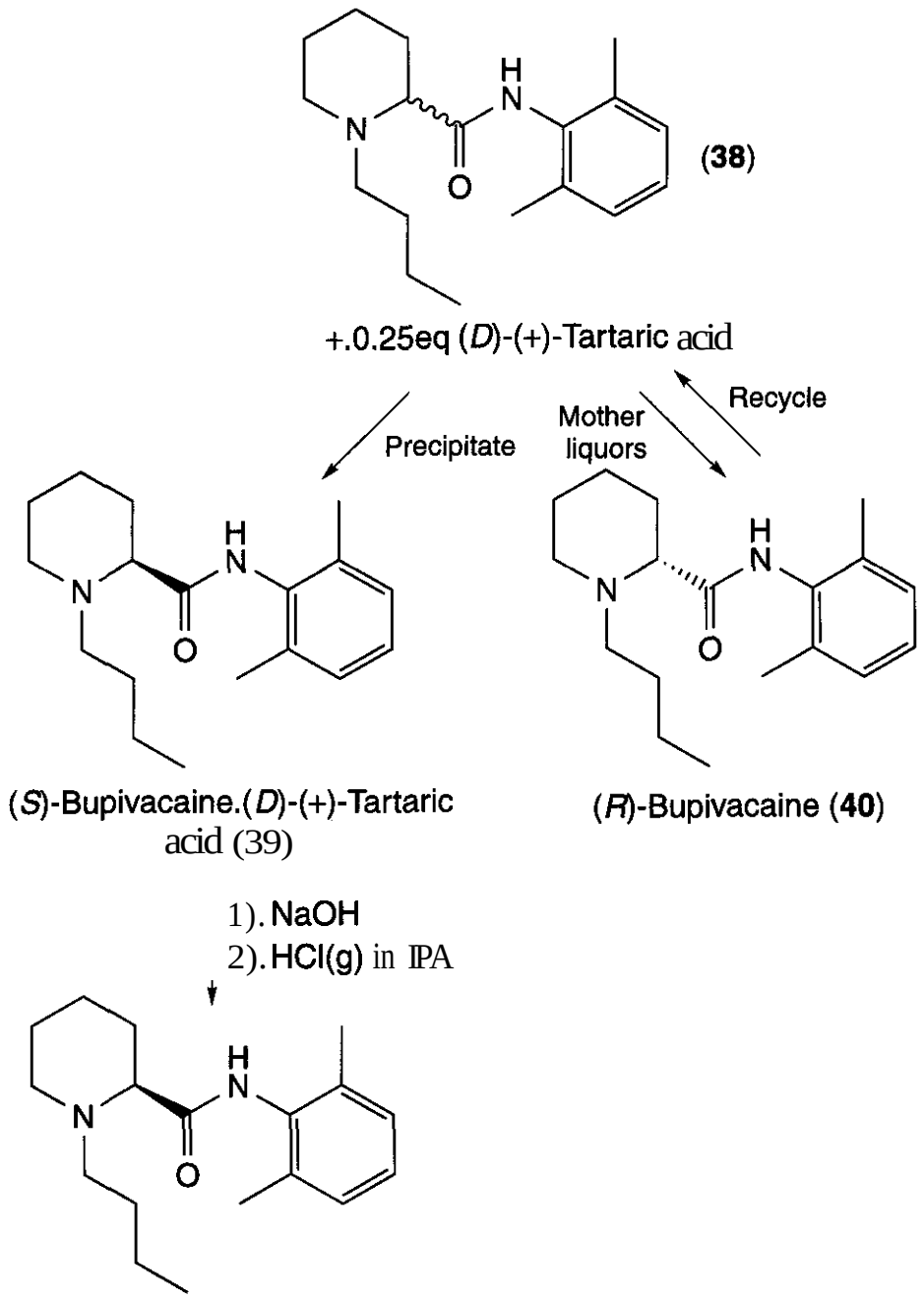


Figure 18.15.

nard reaction (70). The trans isomers (**42, 43**) form over the cis isomers (**44, 45**) in a ratio of ~8:2; the currently marketed racemate consists of only the trans isomers. It is possible to take this crude reaction mixture and selectively isolate either the (+)-trans isomer (**42**), by using di-p-toluoyl->tartaric acid [D-(+)-DTTA] resolving agent or the (−)-trans isomer (43) using L-(−)-DTTA. This highlights the high selectivity that can be achieved when using certain resolving agents. In the case of Tramadol, the cis isomers (**45, 46**) do not form crystalline salts with DTTA and therefore remain in solution. This results in a highly efficient process, where the chiral acid not only separates the single enantiomers (42 or 43) but also removes other impurities (i.e., cis isomers 44 and **45**) at the same time (71).

Another drug that is sold as a racemate is Etodolac (**46**), which is used as a non-steroidal anti-inflammatory agent (NSAID) that also has analgesic properties; it has the ability to retard the progression of skeletal changes in rheumatoid arthritis (72). It has been shown that the majority of therapeutic activity lies in the S-(+)-isomer (73). D-(−)-N-Methylglucamine (meglumine) is obtained by ring opening of D-glucose with methylamine, and hence it is readily available and inexpensive. Scientists at Chiroscience have described the use of meglumine to separate the enantiomers of Etodolac (74). It was shown that the meglumine salt possessed suitable properties to enable its use as a salt for pharmaceutical administration. Therefore, in the case of Etodolac, meglumine can not only be used to separate

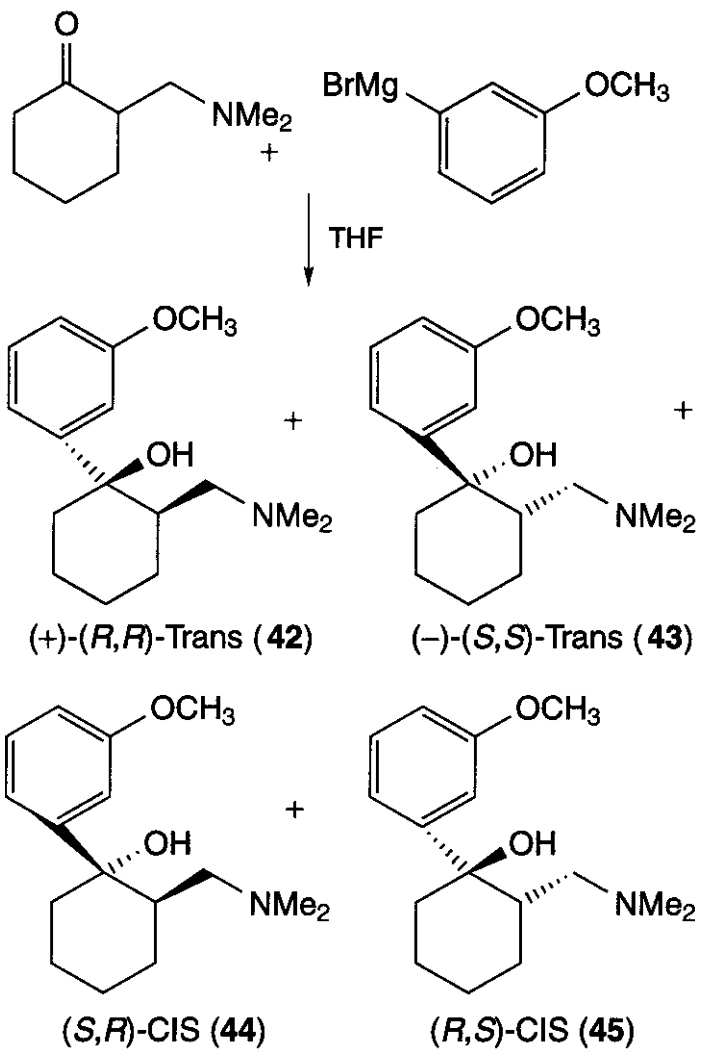


Figure 18.16.

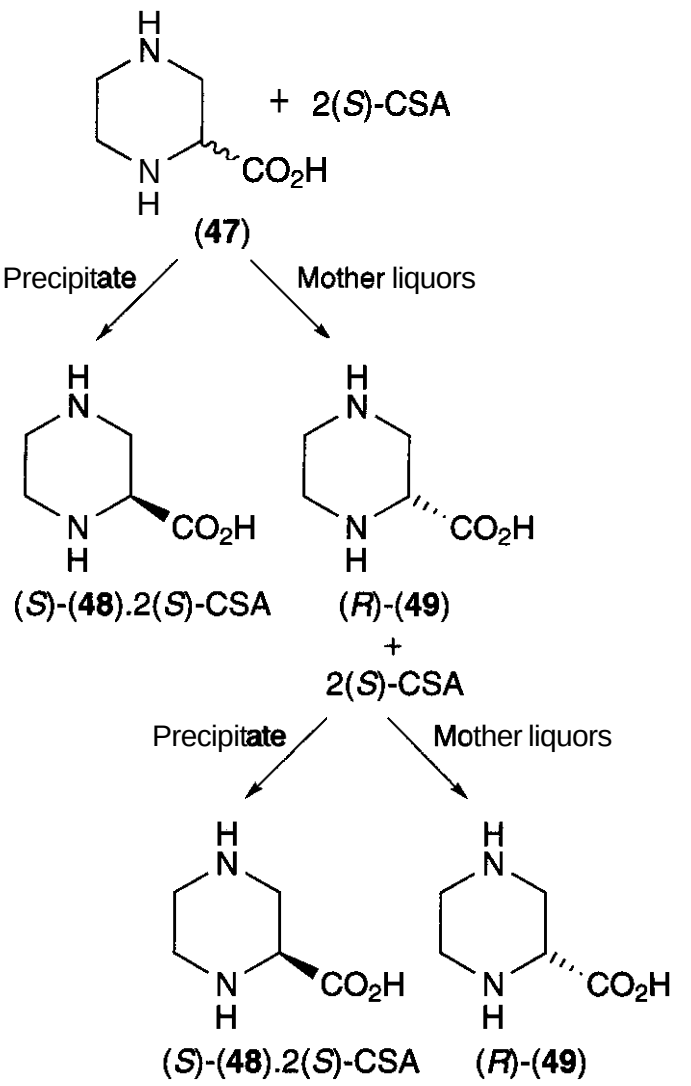
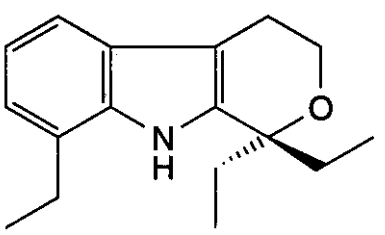


Figure 18.18.

the enantiomers, but it can also be used as the pharmaceutical salt form of choice.

In addition to the racemic drugs discussed in this section, resolutions are also used in the isolation of key building blocks for the pharmaceutical industry. An important class of these intermediates are amino acids, many of which are available as the single isomer from natural sources (see INTRODUCTION). The use of unnatural amino acids and D configured ones are expected to have a greater influence at the biological level. In the drive for molecular diversity and metabolic stability, a number of unnatural amino acids such as the non-proteinogenic piperazine carboxylic acid (47) (Fig. 18.18) have been developed. Specifically,



Etodolac (46)

Figure 18.17.

this amino acid has found use as an intermediate compound of the HIV proteinase inhibitor L-735,525 (75). The racemic cyclic amino acid (47) has been resolved with *S*-camphorsulfonic acid (CSA), which yields the *S*-isomer as the double CSA salt (48) as the precipitate (76). Retained in the mother liquors is the R-isomer (49). This can neatly be racemized to the S-isomer by mixing with S-CSA in a suitable solvent. On seeding with pure (*S*,*S*)-diastereomeric salt, a further quantity of the desired (*S*,*S*) product (48) is obtained, leaving the R-isomer (49) once more in the liquors. The whole cycle can be repeated and has been demonstrated with four complete cycles. To complete the whole process, the resolving agent is also readily recovered and recycled.

3.2 Separation of Intermediates to Single Enantiomer Active Pharmaceutical Ingredient

The previous examples given for diastereomeric salt resolution have all involved separation of the active pharmaceutical ingredient (API) or late stage intermediate. Whereas this

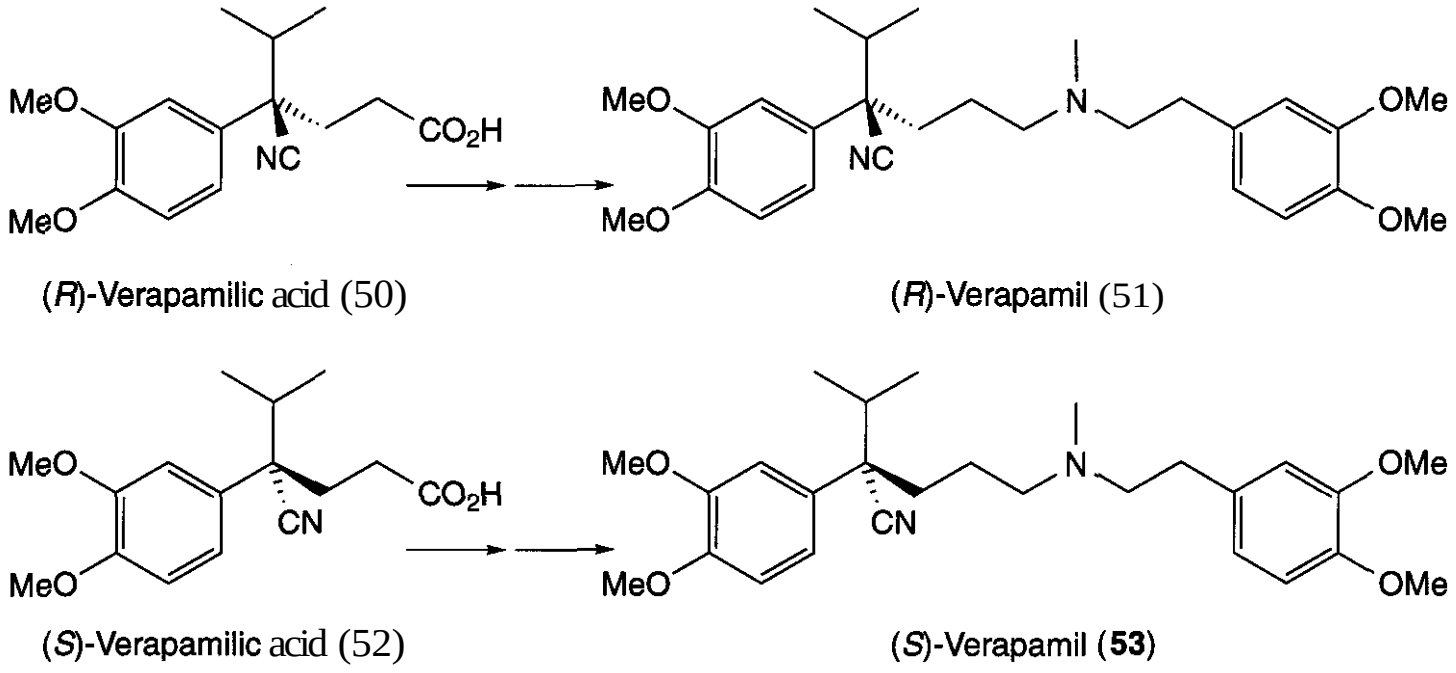


Figure 18.19.

does offer several advantages from the point of view of time and quality aspects, there are also a number of drawbacks. If, for example, a racemization of the unwanted isomer cannot be found, there would be a waste of 50% of material. Therefore, it can often be advantageous to conduct the separation at an earlier stage in the synthesis of the drug. This leads to better atom efficiency compared with resolution of the final product, resulting in a reduction of the overall amount of waste and cost.

One such example is Verapamil, which is a well-established treatment of cardiovascular ailments (77). *S*-(−)-Verapamil (51) has specific transmembrane calcium channel antagonist activity, whereas its antipode (53) influences a wider range of cell pump actions, including those for sodium ions (78). Verapamil has been separated into its single enantiomers by resolution with expensive resolving agents, which required multiple recrystallizations to effect complete separation (79). Looking into the synthetic sequence of Verapamil, several intermediates seemed to be attractive alternatives to Verapamil (80). The intermediate verapamilic acid (Fig. 18.19) was efficiently separated using a-methylbenzylamine (a-MBA), which is an extremely cheap resolving agent (81). Subsequent transformation of the easily obtained R- or S-verapamilic acid (50 or **52**), required a further three to four synthetic steps to yield the active pharmaceutical ingredient.

The racemate aminoglutethimide (**27**) has been shown to be effective in the treatment of hormone-dependent breast cancer (Fig. 18.20). Further studies have shown that the *R*-enantiomer is more potent than its antipode as an aromatase inhibitor (82). The resolution of aminoglutethimide itself has been reported in the literature, using tartaric acid. This resolution suffers from the formation of solid solutions (**83**), which require endless crystallizations to deliver the single enantiomer (**84**). Use of a suitable precursor (54) enabled separation of the intermediate (**55**), by treatment with the alkaloid resolving agent (−)-cinchonidine. This chiral acid was then cyclized to nitroglutethimide, which on reduction, gave the desired R-aminoglutethimide (**56**) (85). It is noteworthy that in the case of aminoglutethimide, the amine functionality is an aniline moiety. Because of the low pK_a associated with this amine (2.5–4.6), the number of acidic resolving agents that can be employed are reduced, because they need to be of relatively high acidity to form a salt.

3.3 Crystallization-Induced Asymmetric Transformation

A number of amino acids have been separated by resolution, in certain cases the yield of the required diastereoisomer has been greater than 50% (86). *p*-Chlorophenylalanine is of considerable pharmacological interest, because of its ability to inhibit serotonin forma-

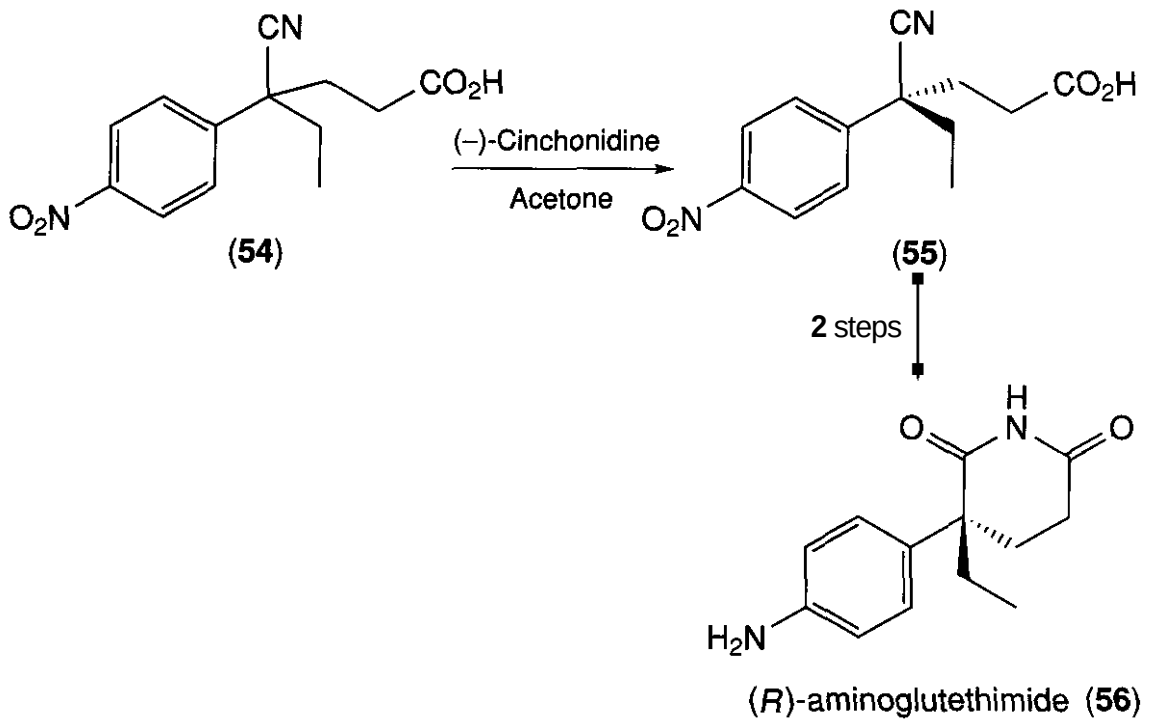


Figure 18.20.

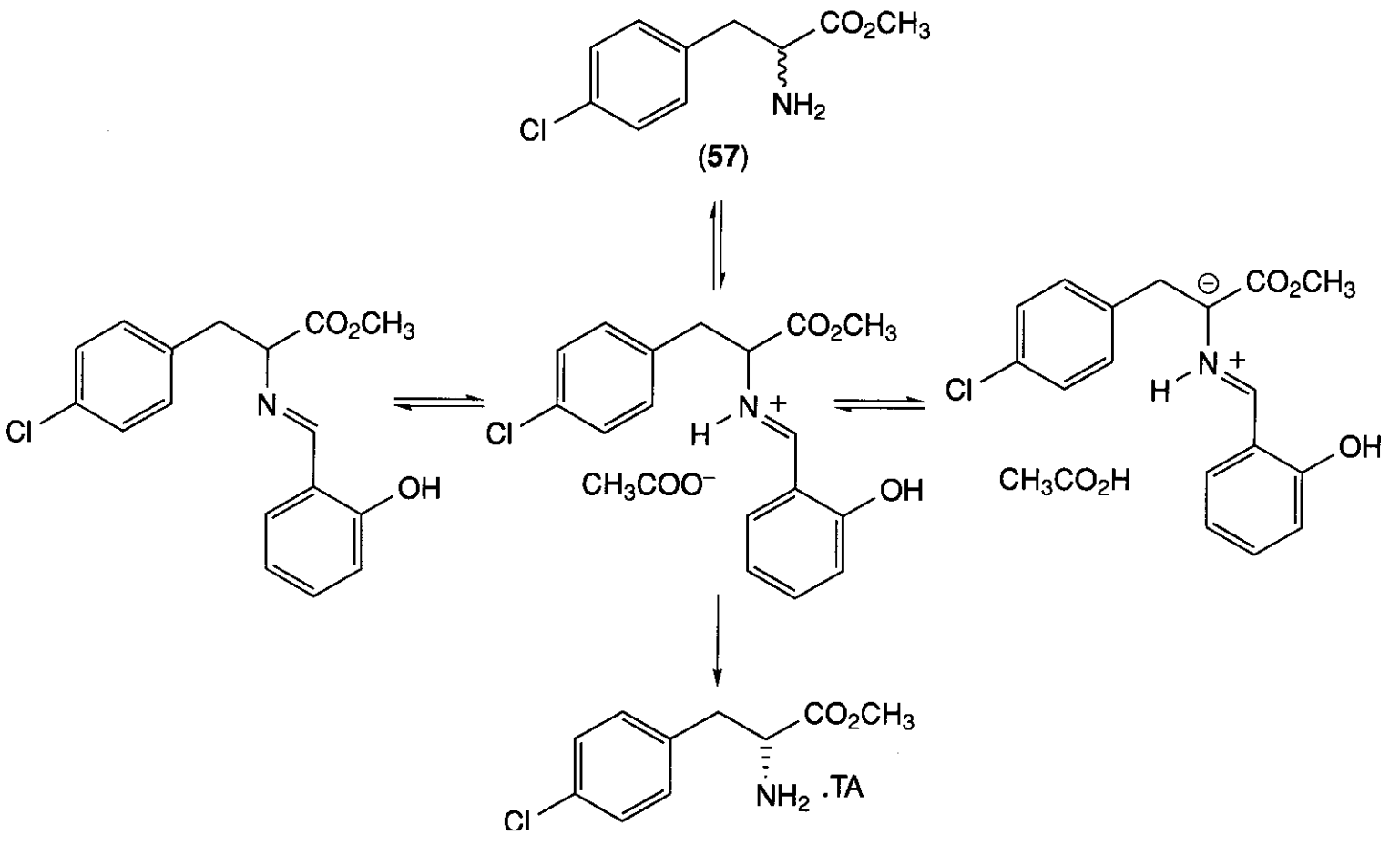
tion in laboratory animals (87). Both the R- and S-enantiomers have also been used as building blocks in the synthesis of other drugs. An ingenious approach to *R-p*-chlorophenylalanine methyl ester, which is based on a one-pot resolution-racemization sequence, is highlighted in Fig. 18.21. Here, treatment of racemic p-chlorophenylalanine methyl ester (57) with 0.5 eq of D-tartaric acid and 0.1 eq of salicylaldehyde in methanol gave a 68% yield of 98% enantiomeric purity of the 2:1 *R-p*-chlorophenylalanine D-tartaric acid salt (58). The reason that the absolute yield is greater than 50% is caused by the S-enantiomer being racemized *in situ*. The 2:1 tartrate salt is crystalline and is therefore removed from the system by virtue of its insolubility. This drives the equilibrium further in favor of the 2:1 *R-p*-chlorophenylalanine D-tartrate salt (88).

While the common goal remains to be the rational design of resolving agents (89), it is clear that we are still away from this actually happening. An alternative "family" approach to classical resolution has been demonstrated by Vries et al. (90). A group of similar resolving agents are mixed simultaneously with the racemate. This was done to shorten the time required to complete the resolving agent screen. Note should be made that the families of resolving agents are very similar and that the crystalline species obtained by this method contained more than one of the resolving agents. As with all screens, analysis of the data is often time consuming and laborious. Bruggink et al. have shown that differential scanning calorimetry (DSC) of the isolated salts can help to quickly determine whether the isolated salt will provide a through resolution (91). However, with a methodical and precise screening protocol, it is nearly always possible to find a suitable resolving agent that effects separation of the enantiomers (92).

4 NONCLASSICAL RESOLUTION

4.1 Preferential Crystallization

A brief description of the type of "racemic" compounds is necessary for the reader to better understand the principles behind the application of crystallization methods to the separation of enantiomers. Three fundamental types of crystalline racemates exist. In the first, the crystalline racemate is a conglomerate, which exists as a mechanical mixture of crystals of two pure enantiomers. The second, which is the most common, consists of the two enantiomers in equal proportions in a well-defined arrangement within the crystal lattice; this is termed racemic compound. The third possibility occurs with the formation of a solid solution between the two enantiomers that coexist in an unordered manner in the crystal. This kind of racemate is called a pseu-



(*R*)-*p*-chlorophenylalanine.0.5eq(*D*)-tartaric acid (58)

Figure 18.21.

doracemate and is rather rare. Conglomerates have been estimated to be approximately 10% of all racemates (93). Diagrammatic representation of the first two types of racemate are shown in Fig. 18.22.

By understanding the appropriate phase diagrams, which describe the melting behavior of the two enantiomers (binary melting point phase diagram) or their solubility behavior in the presence of a solvent (ternary solubility phase diagram), separation of enantiomers can be reproduced. Phase diagrams for the three types of racemate are shown in Fig. 18.23. For a full and detailed explanation of this topic refer to the monograph of Jacques et al. (57).

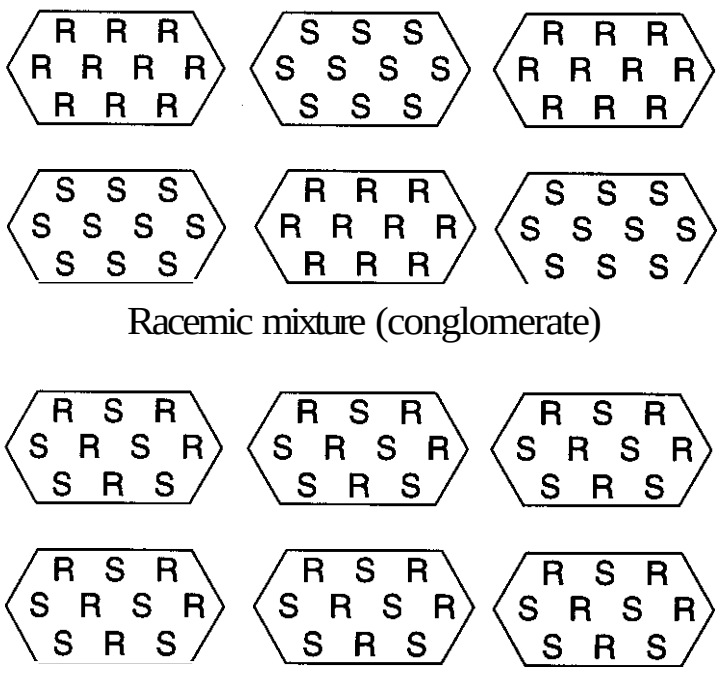
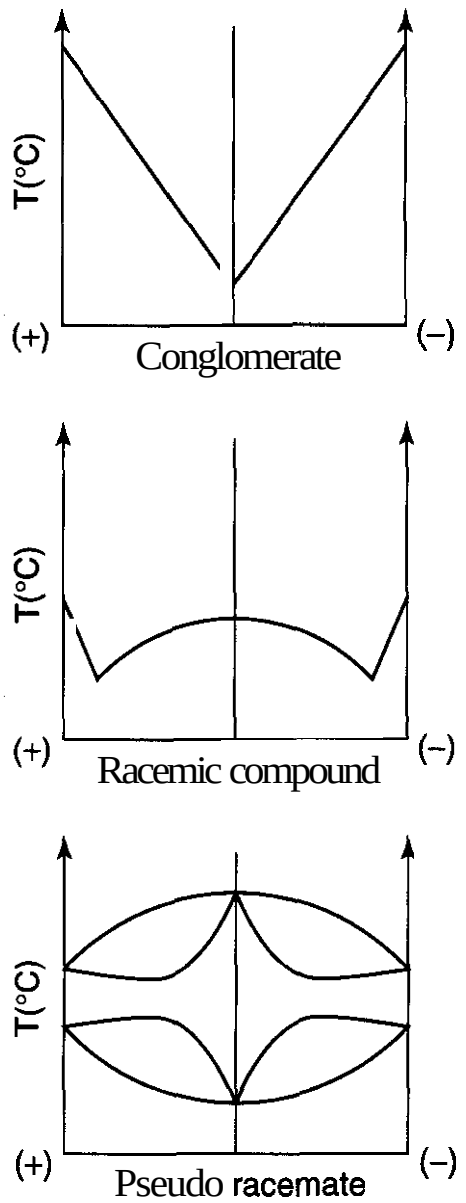


Figure 18.22.

4.2 Enrichment of Enantiomeric Excess by Crystallization

The attainment of high levels of enantiopurity is not always possible by enzymatic or diastereomeric resolutions or by asymmetric syntheses alone. It is however frequently possible to prepare a pure enantiomer from a partially resolved sample by simple recrystallization. For this process to proceed successfully it is necessary that the initial enantiopurity of the mixture is greater than that of the eutectic point in the phase diagram. By utilization of the phase diagram, the optimal quantity of solvent required can be calculated. It is also possible to calculate the maximum expected yield.



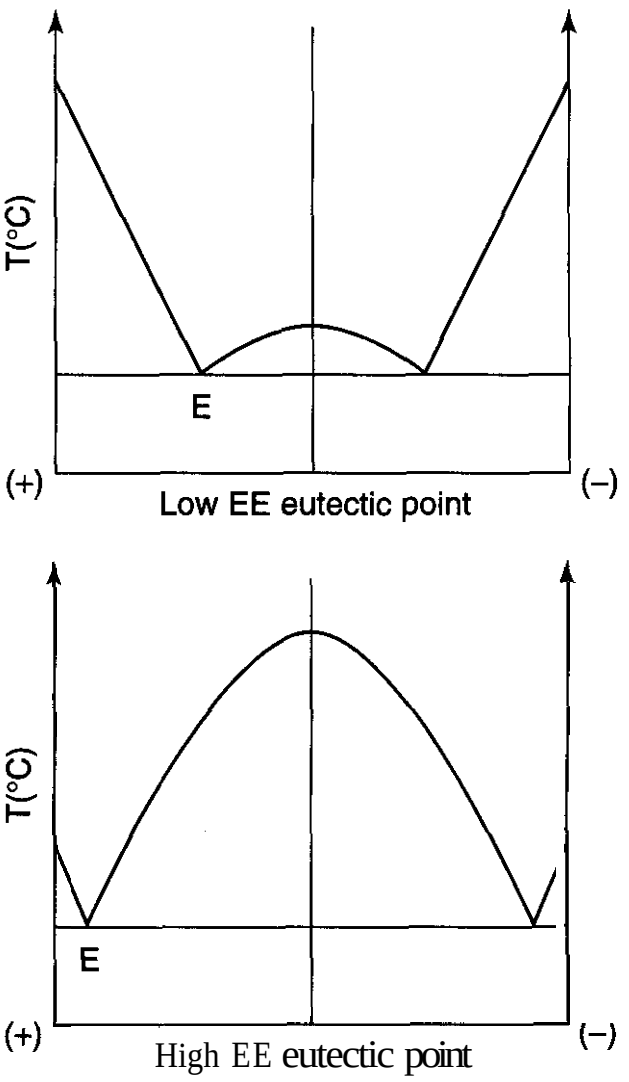


Figure 18.24.

Figure 18.23.

Note should also be made that in some cases recrystallization reduces the enantiomeric excess, which can lead to crystallization of the racemate (94). In these cases the mother liquors contain moderately to highly enriched material. It is therefore important to plan the strategy at which point the enantiomer is recrystallized to optical purity. This may be from an enzymic resolution, or in the event that an asymmetric synthesis has failed, to deliver enantiopure product. As discussed in Section 3, the liquors from the diastereomeric resolution with DTTA of 88%de can be cleaved to the free base, and crystallization of the hydrochloride salt gives >98% ee. This is because of the fact that methylphenidate hydrochloride has a eutectic point of 30% ee. Davies et al. (95) and Winkler et al. (96) have prepared single enantiomer methylphenidate (29). Their approaches use an enantioselective synthesis; the enantiomeric excesses are 86% and 69%, respectively, thus requiring recrystallization to deliver enantiopure product. Another example of this type of compound is Warfarin (13). Chemists at Dupont (97) developed an asymmetric hydrogenation approach, which gave Warfarin in ~80% ee. Simple crystallization in an appropriate solvent yielded optically pure Warfarin, thus indicating that the eutectic point is below 80% ee. (See earlier section on the metabolism and binding properties of the Warfarin enantiomers).

The phase diagrams below highlight two typical cases, the first where the eutectic point E is close to the racemate, and the second where the eutectic approaches the single enantiomer as shown in Fig. 18.24. In the first case, it would be preferable to crystallize the enriched enantiomer to optical purity, e.g., methylphenidate. However, in the second case, a very stable racemic compound exists, giving rise to a high eutectic point. Here crystallization of enriched enantiomer mixture will only be successful at high ee. For example, verapamil hydrochloride requires that the ee be greater than 98% for crystallization to yield

enantiopure product. Below this, the enantiopurity is reduced. In this case, it is advantageous to recrystallize the diastereomeric salt precursor to optical purity before proceeding to final product.

4.3 Resolution by Direct Crystallization

It is important to show how conglomerates are identified. We have already seen that they have specific phase diagrams as shown in Fig. 18.23. Other such data that support identification of a conglomerate are IR, X-ray data, and observation of a spontaneous resolution or resolution by entrainment. Note should be made that in 1848, Louis Pasteur separated the dextrorotatory and levorotatory crystals of sodium ammonium tartrate. This manual sorting of crystals is also known as triage, and by its very nature is time consuming and laborious. The readers are again directed towards the Jaques et al. monograph, which lists over 250 known examples of conglomerates (57). There are two possibilities for separation of enantiomers by direct crystallization. The first uses spontaneous resolution, which occurs when a conglomerate crystallizes. This crystallization may be followed by the mechanical separation of the crystals of the two enantiomers. Various techniques have been developed that aid this separation.

The second type of resolution by direct crystallization is known as entrainment. Here, the differences in the rate of crystallization of the enantiomers in a supersaturated solution give rise to a separation. Strict control of the conditions for the crystallization are required, with the system of crystals and solution not being allowed to come to equilibrium and time playing an important role. The occurrence of conglomerates has been estimated to be approximately 10% of all racemic compounds. We will now illustrate this phenomenon with some pertinent examples.

An example of use of the conglomerate Narwedine (59) in the synthesis of a natural product Galanthamine (61) which is an *Amarylli-daceae* alkaloid and has been used clinically for 30 years for neurological illnesses (98). More recently it has been approved for the use in the treatment of Alzheimer's disease (AD) (99). Galanthamine acts to inhibit acetylcholinesterase (AChE), thus increasing the levels of acetylcholine. An increase in the level of acetylcholine in patients with AD has been shown to improve their cognitive performance. Galanthamine has been extracted from botanical sources; however, several tons of daffodil bulbs are needed to produce 1 kg of product. A synthetic route has been developed that uses a crystallization-induced chiral transformation (Fig. 18.25). This crystallization was first reported by Barton and Kirby (100) and further developed by Shieh and Carlson (101). The success of this transformation is based on two phenomena: narwedine (59), which crystallizes as a conglomerate, and (−)-narwedine (60), which equilibrates with (+)-narwedine through a retro-Michael intermediate. This process has now been developed so that (−)-narwedine (60) is routinely obtained in 80% yield from the racemate input, as shown in Fig. 18.25 (102).

Recently a number of potent 5-HT, receptor antagonists such as Ondansetron have been reported to be clinically effective for the blockade of chemotherapy-induced nausea and emesis (103). The structurally novel compound (62) has also been shown to be a highly potent 5-HT, antagonist (104); specifically, the R-(−)-(62) enantiomer was shown to be the most active. Comparison of the physical data of the racemate and single enantiomer indicated that this structure (62) exists as a conglomerate (104). By careful experimentation, the best concentration, temperature, and time for crystallization were discovered. Table 18.1 highlights the results obtained for the entrainment.

The initial concentration of the solution was 10.0 g of (±)-(62) in 50 g of acetone. In all runs, 10 mg of seed crystals were used. From the 10 runs highlighted in the 18.1, 21.0 g of *R*-(−)-(62) of >92.0% ee and 21.4 g of (*S*)-(+)-(62) of >90% ee are obtained from an input of 50.4 g of racemate. The table also nicely illustrates the continuous nature of the process, which coupled with the fact that no resolving agent, chiral auxiliary, enzyme, or catalyst is needed, underlines the economic advantages of this type of process.

The importance of amino acids as building blocks for asymmetric synthesis is well documented (105). A number of amino acids have been shown to exist as conglomerates. Shi-

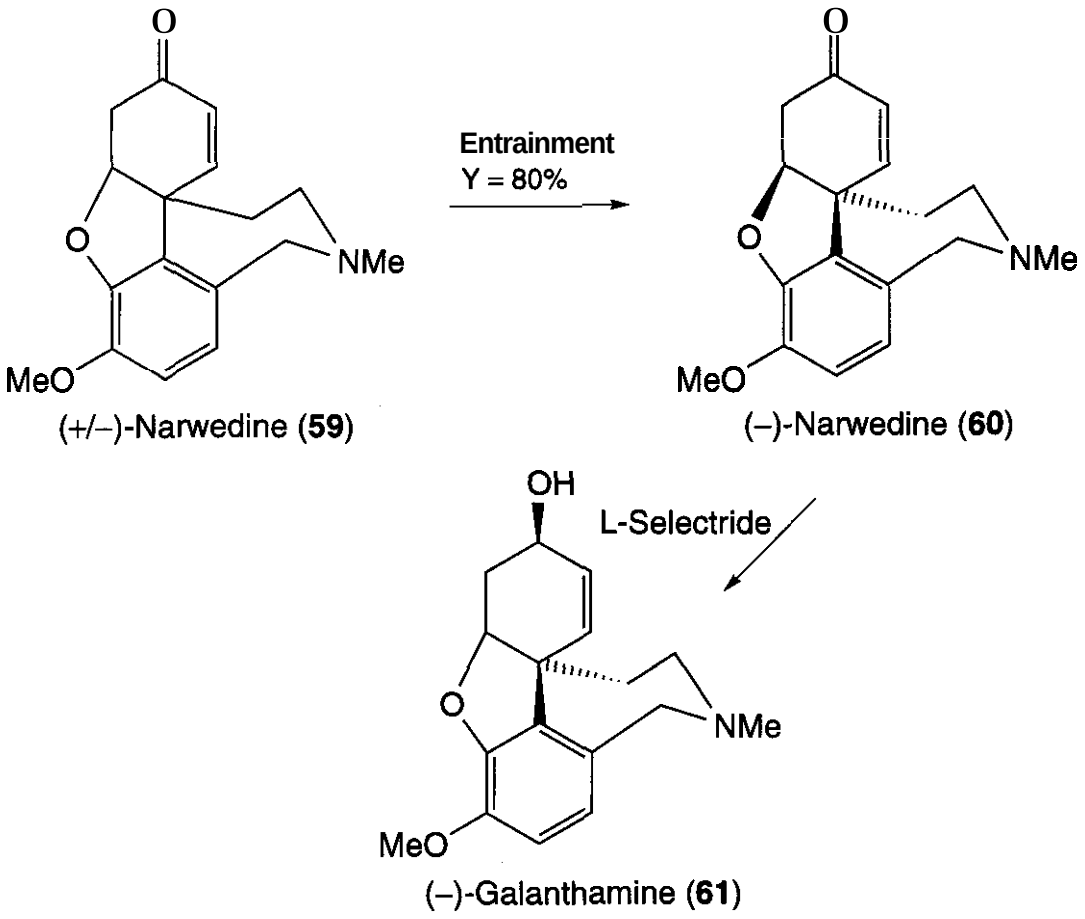
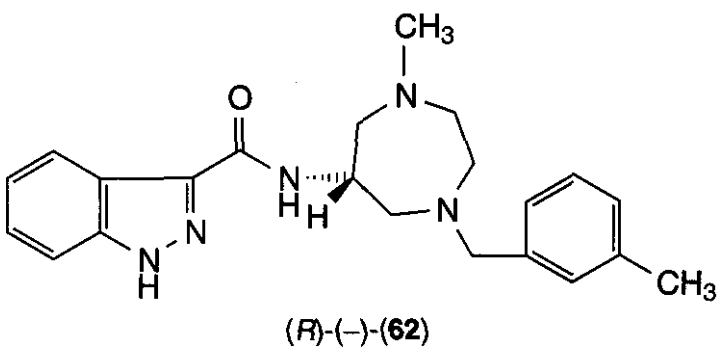


Figure 18.25.

raiwa et al. have described the preferential crystallization of racemic methionine hydrochloride (106). The obtained D- or L-methionine hydrochloride was, however, only —75% optically pure, requiring a further recrystallization to furnish enantiopure product. Shiraiwa et al. have also recently disclosed the resolution of (2*RS*, 3*SR*)-2-amino-3-chlorobutanoic acid HCl again using entrainment (107). Here it was shown to be necessary to conduct the crystallization in an ethanol15 M hydrochloric acid solvent mixture for optimal results. By careful control of the conditions, high levels of enantiomeric excess were obtained in the crystalline salt.

Chemists in Japan have developed an excellent approach to (+)-Diltiazem, which is a coronary vasodilator (108). An intermediate



(*R*)-(–)-(**62**)

Figure 18.26.

is successfully resolved using preferential crystallization. The glycidic acid–substituted phenylesters were prepared; of the 30 synthesized, only one exhibited conglomerate properties (109). This was the 3-(4-methoxyphenyl)glycidic acid 4-chloro-3-methylphenyl ester (63). Table 18.2 summarizes the physical data collected, which is illustrative of the conglomerate nature of this compound.

The obtained single enantiomer (–)-epoxide (64) is then converted into the required (+)-isomer of Diltiazem (**65**) in several steps, as highlighted in Fig. 18.27.

Taxol is a natural product isolated in very low yield from *Taxus* brevifolia and is used in the treatment of cancer (110). The extreme chemical complexity of Taxol makes production by total synthesis uneconomical. However, a semisynthetic approach using the naturally derived 10-deacetylbaccatin III (**66**) condensation with *N*-benzoyl-(2*R*, 3*S*)-3-phenylisoserine (67) does provide an alternative and economic approach (111). *N*-benzoyl-(2*R*, 3*S*)-3-phenylisoserine (67) is also commonly known as the Taxol side-chain and has been prepared in optically active form using chiral auxiliaries or resolving agents (112). It has been shown that the Taxol side-chain is a conglomerate and can therefore be cheaply and

Table 18.1 Resolution of (62) by Preferential Crystallization

Run	(±) Added (g)	Seed	Time (minutes)	EE of Solution (%EE)	Amount of Crystals (g)	Rotation	%EE of Solid
1	—	(*R*)-(−)	220	22.7/+	2.0	−	95
2	4.0	(*S*)-(+)	210	19.4/−	4.6	+	92
3	4.6	(*R*)-(−)	190	21.2/+	3.8	−	95
4	3.8	(*S*)-(+)	205	21.0/−	3.9	+	95
5	3.9	(*R*)-(−)	210	21.5/+	3.8	−	95
6	3.8	(*S*)-(+)	220	16.4/−	5.0	+	90
7	5.0	(*R*)-(−)	200	21.3/+	3.4	−	92
8	3.4	(*S*)-(+)	215	21.2/−	3.9	+	94
9	3.9	(*R*)-(−)	190	22.2/+	4.0	−	93
10	4.0	(*S*)-(+)	220	21.4/−	4.0	+	95
11	4.0	(*R*)-(−)	200	20.8/+	4.0	−	95

Reprinted from H. Harada, *Tetrahedron Asymmetry,* vol. *8, T.* Morie, Y. Hirokawa, and S. Kato, 1997, pp. 2367–2374. Reproduced with permission from Elsevier Science.

efficiently entrained to the single required enantiomer (113).

5 ENZYME-MEDIATED ASYMMETRIC SYNTHESIS

Enzymes have found frequent use in the synthesis of single isomer drugs from racemic or prochiral compounds at the larger manufacturing scales. The use of enzymes to effect chiral transformations in the medicinal chemistry laboratory has been far less frequent; however, the increasing availability of immobilized and stabilized forms of enzymes has made their use easier and the resultant transformations more predictable.

By virtue of their complex macromolecular structure, including a highly defined active site, enzymatic transformations generally proceed with a high degree of chemical selectivity and stereospecificity. Reactions are typically conducted under mild conditions of temperature, pressure, and pH, thus minimizing losses caused by unwanted side reactions or partial racemization. The use of extremophiles or cross-linked enzymes such as CLECs do enable the use of higher temperatures, pressures, and organic solvents.

Enzymes can be utilized to affect a number of transformations; the broad spectrum of reactions, including amide bond formation, hydrolysis, esterification, reduction, oxidation, and carbon–carbon bond formation, has been reviewed elsewhere (114).

5.1 Amide Bond Formation

The use of enzymes to stereospecifically form amide bonds has been described in many texts (115); however, the commercial availability of cross-linked enzyme crystals (CLECs), for example, PeptiCLEC-TR, which is an immobilized form of Thermolysin protease, has been used in the synthesis of D2163 (68), a novel matrix metalloproteinase inhibitor (116). In vitro enzyme screening identified the all-natural SSS-isomer as the active product. The elegant CLEC (117) technology used in this example makes the enzyme stable to typical organic reaction conditions and enables facile removal of the enzyme at the end of the reaction by simple filtration. On this basis, it is

Table 18.2 Properties of (63) Indicating Conglomerate Nature

Compound	MP (°C)	Solubility (g/100 mL) THF	Solubility (g/100 mL) DMF	IR Spectrum
(±)-4.2	123–124	14.0	13.0	Identical
(−)-4.2	139–141	6.7	6.9	Identical

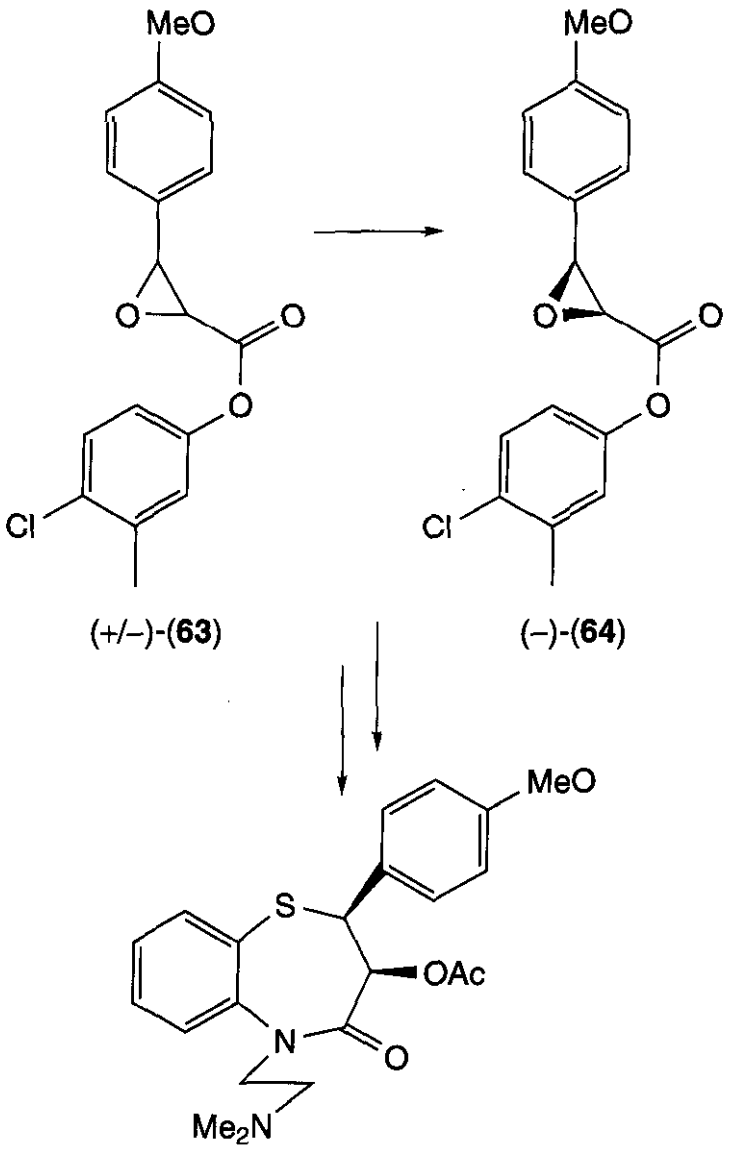


Figure 18.27.

anticipated that medicinal chemists will more commonly use these enzymes in the future.

The coupling of dipeptide (69) to the protected a-thio carboxylic acid (70) was conducted in organic solvent at high concentration with the desired product produced in a few hours with high enantiospecificity.

5.2 Transesterification and Hydrolysis

A widely used technique for separating racemic mixture is the use of enzyme mediated transesterification or hydrolysis. One important example is the separation of Naproxen (**33**), which is a member of the 2-arylpropionic acid class of profens that are broadly used as NSAIDs (see Section 2 for the separation of enantiomers using a crystallization approach). The important association between chirality and biological activity of this class of drugs has been extensively researched, where the role of cyclooxygenase-independent properties of the R-enantiomers in the gastrointestinal toxicity of the racemates and the likelihood that the use of racemates increases the propensity of profens to alter the pharmacokinetics of other drugs has been described (118).

Whereas not all profens are sold as single isomers, Naproxen is sold as the single S-enantiomer (**36**) where various strategies including crystallization, chromatographic separation, asymmetric hydrogenation and enzymatic hydrolysis, and esterification have been used to prepare the single isomer (65). Specific examples include the use of Candida *cylindracea* lipase to enantioselectively prepare single isomer naproxen ester with trimethyl silyl methanol (119) and the use of Candida rugosa lipase in an enantioselective continuous hydrolysis of Naproxen methyl ester (120).

Pipecolic acid is a component of a number of active drugs, including bupivacaine (38) and thioridazine (72) (Fig. 18.30), which has been efficiently resolved as the racemic n-octyl pipecolate with Aspergillus niger. The *S*-isomer is obtained as the free acid in a 40% yield based on the available enantiomer with a 97% ee (121).

Propanolol (**14**) is a broadly used β-adrenergic receptor blocking agent that is sold as the racemate. However, the majority of the activity is associated with the S-enantiomer (**74**) (see Section 2) (122). The asymmetric

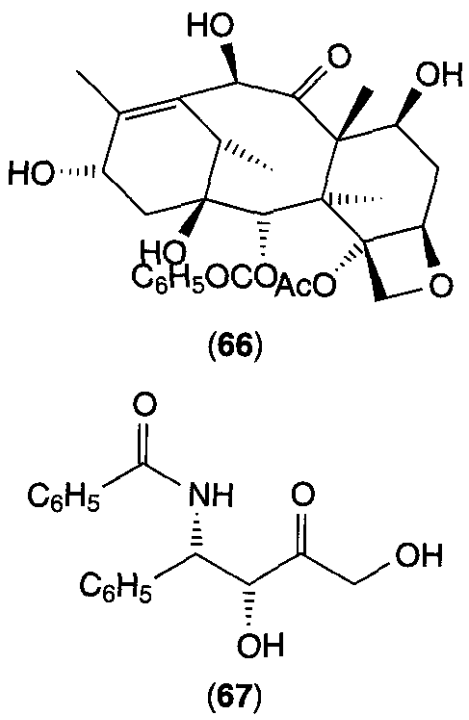


Figure 18.28.

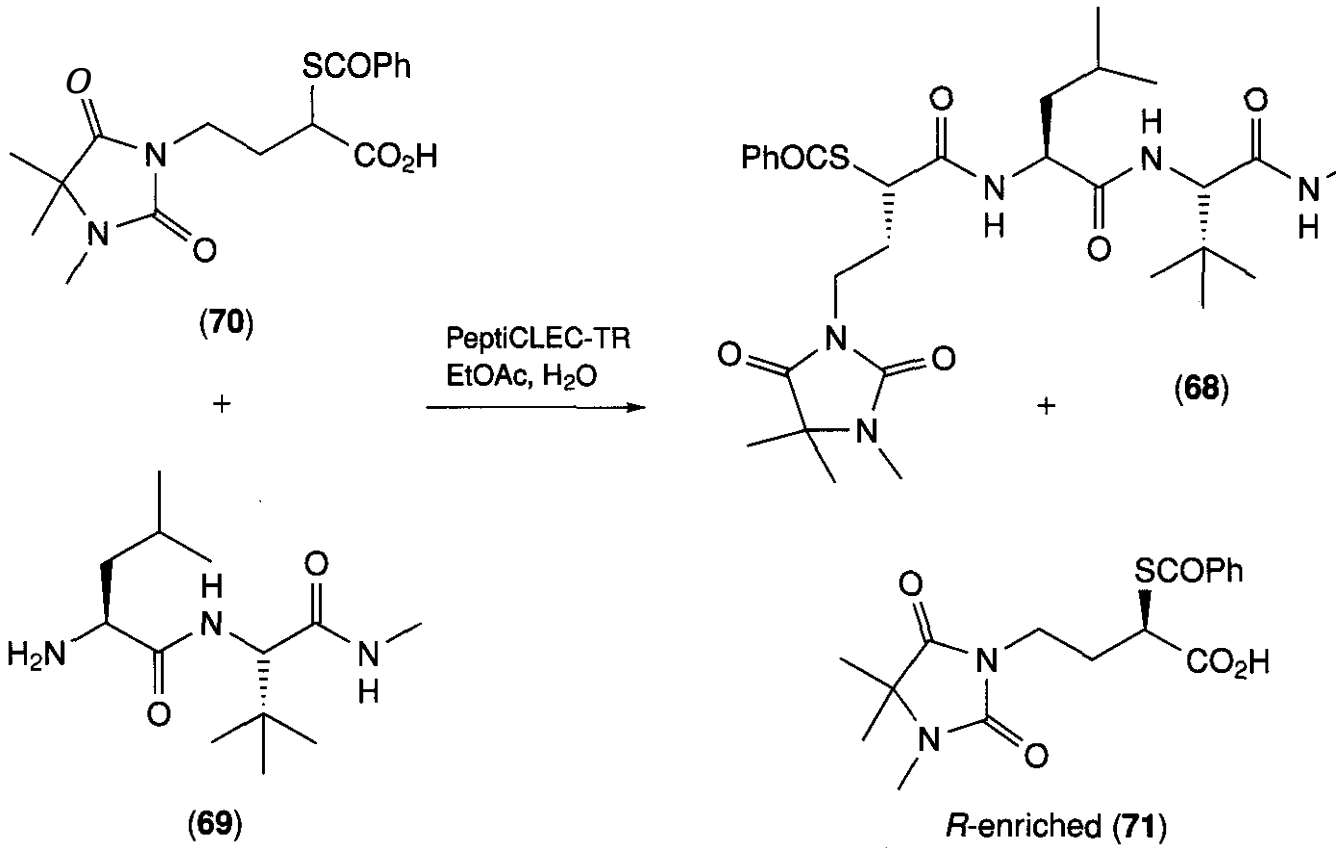
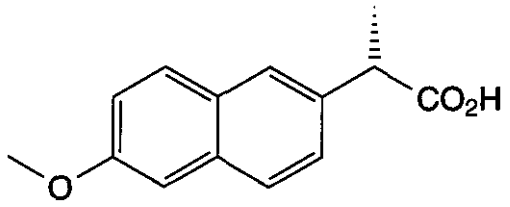
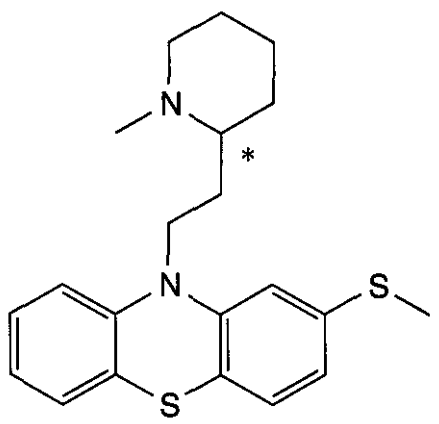


Figure 18.29.

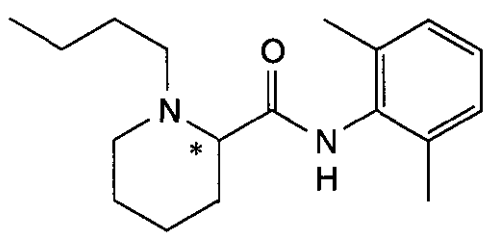
synthesis of the desired S-enantiomer has been achieved by the selective acylation of the R-enantiomer of the key intermediate (73) as shown in Fig. 18.30.



(*S*)-Naproxen (**36**)



Thioridazine (**72**)



Bupivacaine (**38**)

Figure 18.30.

5.3 Oxidation and Reduction

In addition to the widely reported techniques of amide bond formation, transesterification, and hydrolysis, enzymic enantioselective oxidation is also used in the synthesis of single isomer drugs. Patel described the efficient oxidation of benzopyran (**75**), an intermediate in the synthesis of potassium channel openers (123). The transformation was effected with a cell suspension of *Mortierella ramanniana* with glucose over a 48-h period, the isolated product (**77**) was obtained in a 76% yield with an optical purity of 97% and a chemical purity of 98%, as shown in Fig. 18.32.

Reduction with a variety of enzymes has been reported (**114**), including bakers yeast for the reduction of a-methyleneketones to the corresponding a-methylalcohol (**124**), a functionality that is present in a number of drugs. The reduction of an azidoketone (78) using *Pichia angusta* enzyme has been used in the synthesis of S-salmeterol (**79**) (125). Salmeterol (Serevent) is a potent, long-acting β2-adrenoreceptor used as a bronchodilitor in the treatment of asthma. Recently, Sepracor claimed that the S-enantiomer had a higher selectivity for β2 receptors and that it did not cause certain adverse effects associated with the administration of (±)- or (*R*)-salmeterol (126). The synthesis of (*S*)-salmeterol (**79**) is shown in Fig. 18.33.

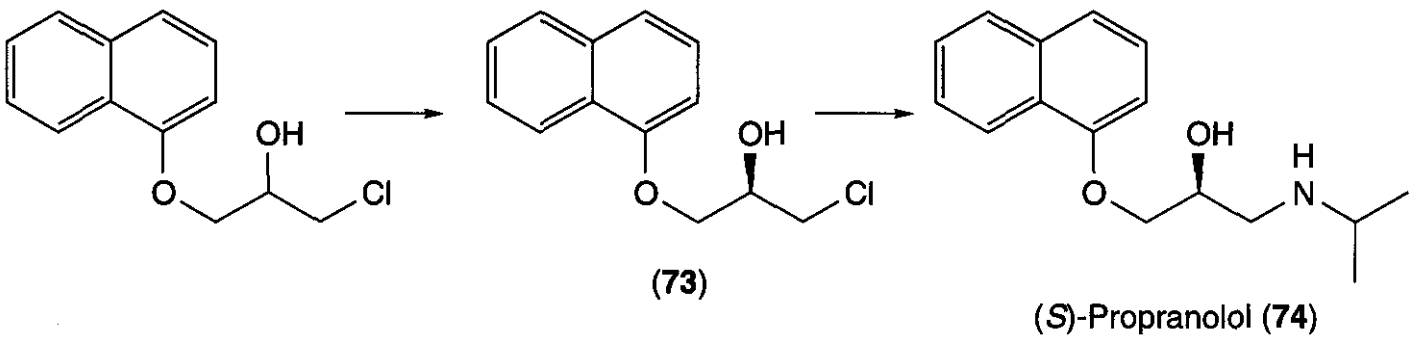


Figure 18.31.

6 ASYMMETRIC SYNTHESIS

Synthetic organic chemists have a vast array of tools at their disposal when faced with the challenge of preparing a chiral compound as a single enantiomer. The purpose of this section is to introduce the reader to some asymmetric approaches toward chiral drugs and medicinal compounds, highlighting examples where the stereoisomers behave differently in biological systems. There are many excellent books and reviews covering methods for asymmetric synthesis and their application to the preparation of pharmaceutical agents and complex natural products (127).

6.1 Chiral Pool

The use of enantiopure starting materials from nature in the synthesis of chiral drugs is not only of great historical significance but remains of critical importance to the pharmaceutical industry. Consideration of the current biggest-selling single enantiomer drugs shows how important this approach is (8of the top 10 in 1996 were obtained from chiral pool starting materials or by synthetic manipulation of fermentation products) (127). The optically pure starting materials that have been used in drug synthesis include amino acids, hydroxy acids, terpenes, alkaloids, carbohydrates, and many more structurally diverse compounds. There are many syntheses involving clever manipulation of chiral pool starting materials and use of these chiral centers to induce further asymmetry (i.e., by diastereoselective reactions). We will briefly consider some examples in which all or most of the chiral centers in the target molecule originate directly from nature.

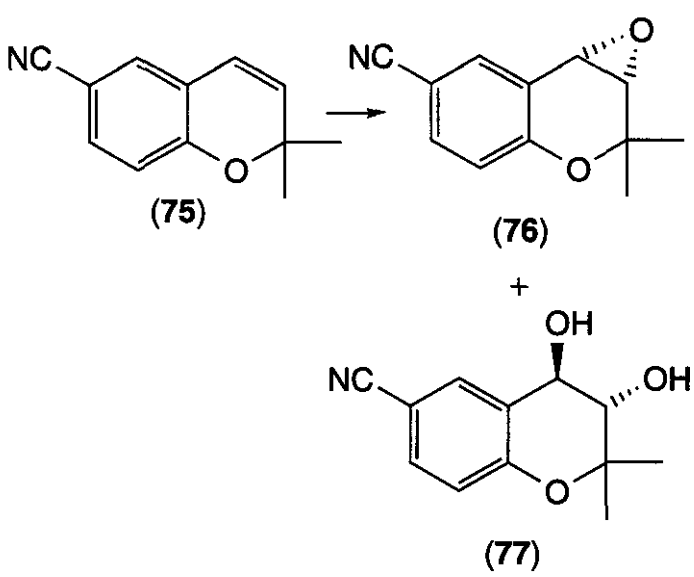


Figure 18.32.

Angiotensin-convertingenzymes (ACE) inhibitors are used mainly for the treatment of cardiovascular disorders and are among the biggest selling drugs worldwide (128). Enalapril (80) is synthesized from the natural amino acids L-alanine and L-proline (129). Lisinopril (81)incorporates a lysine derivative (130). One of the chiral centers in Captopril (82) is derived from proline, but the other is generated by chemical or enzymatic resolution (131). Cilazapril (83) is a conformationally restricted second generation ACE inhibitor developed by Roche, and the core is synthesized from a glutamic acid derivative and an amino acid–derived pyridazine (128, 132).

There are many other examples of drugs based on an amino acid backbone. Stoner et al. recently reported a synthesis of the HIV protease inhibitor ABT-378 (Lopinavir) (84) (133). In a similar synthesis to that of the related compound, Ritonavir, key intermediate (85)is prepared by stepwise diastereoselective reduction of enaminone (86).This means that the existing chiral center, derived from natural L-phenylalanine (protected to 87), controls the formation of the two new stereocenters as

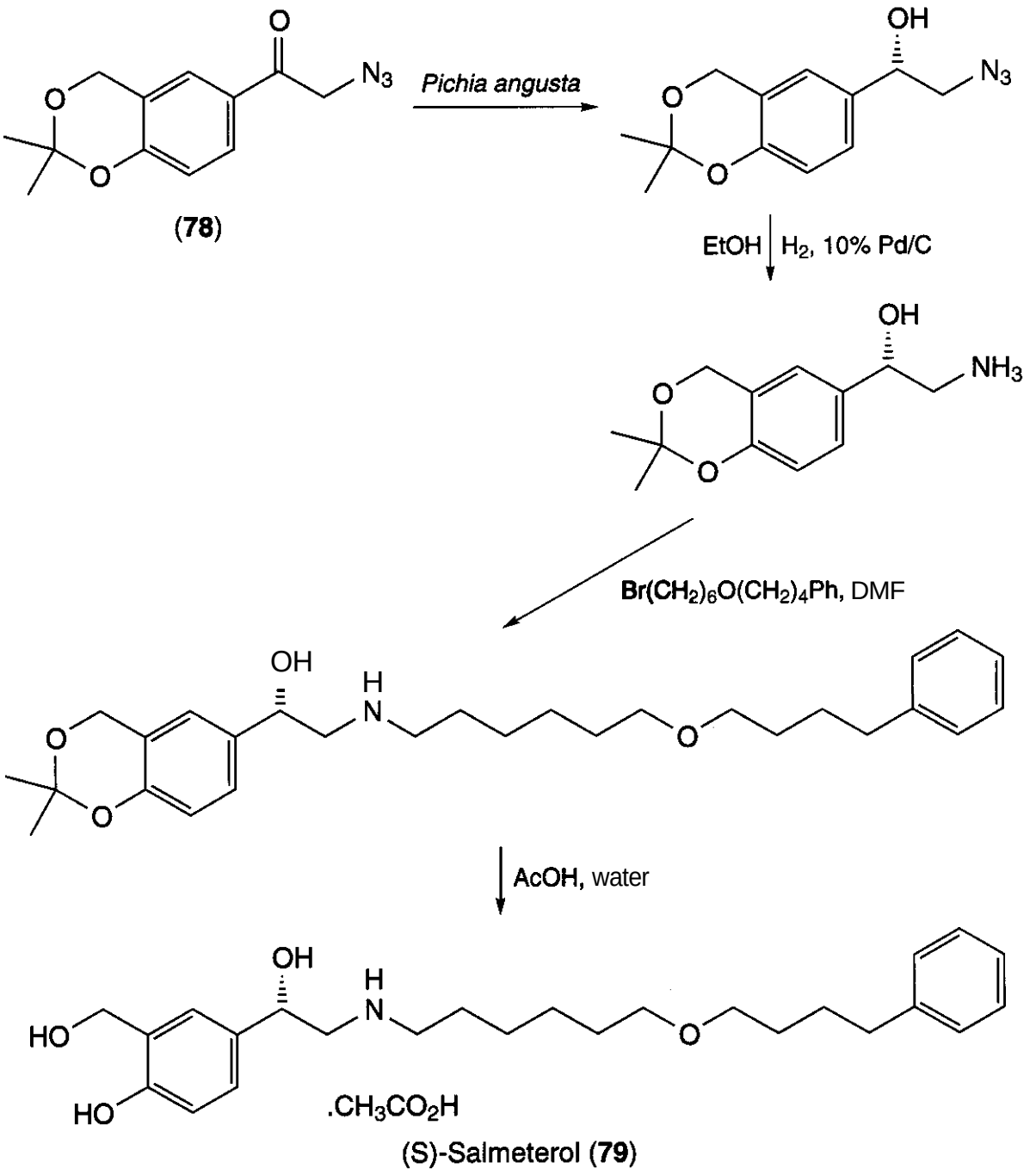


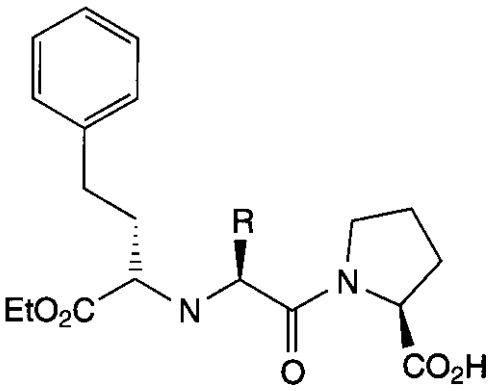
Figure 18.33.

discussed for chiral auxiliaries below. Two acylations then complete the synthesis, with the final chiral center clearly derived from L-valine.

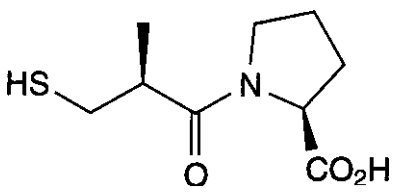
The stereospecificity of binding at the histamine H_3-receptor was investigated by preparing a series of ligands from D- or L-histidine (88)(134). It was found that compounds such as (*S*)-(**89**) had greater affinity for the receptor than their R-enantiomers. In addition, replacing the aromatic moiety with a cyclohexyl group (e.g., 90) switched the activity to agonism for compounds with an amino group in the chain.

Hydroxy acids are important chiral starting materials in the synthesis of many biologically active compounds (135). (*S*)-3-Hydroxy-y-butyrolactone **(91)** is a very useful synthetic unit available from D-pyranoses (136). Workers at Schering-Plough used this as the key starting material in a concise synthesis of Sch 57939 (**92**), a β-lactam–based cholesterol absorption inhibitor (137). The condensation between the dianion of (*S*)-3-hydroxy-γ-butyrolactone and an appropriate diaryl imine proceeded with very high diastereo- and enantioselectivity, generating azetidinone (93) with a *trans*:*cis* ratio of >95:5.

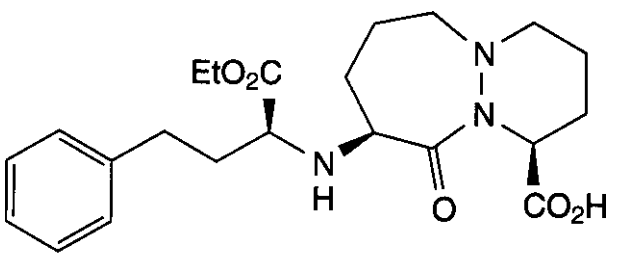
Researchers at Abbott have been investigating the use of pyrrolidinylisoxazoles as nicotinic cholinergic channel activators (138). Until recently, ABT-418 (97) was undergoing clinical trials as a potential treatment for cognitive impairment and decline and for Alzheimer's disease. A short synthesis of ABT-418 was devised starting from commercially avail-



Enalapril R=CH_3 (80)
Lisinopril R=$(CH_2)_4NH_2$ (81)

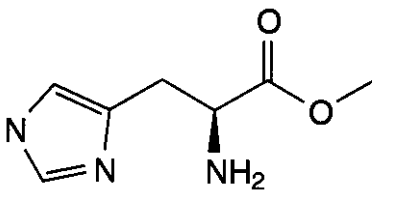


Captopril (82)

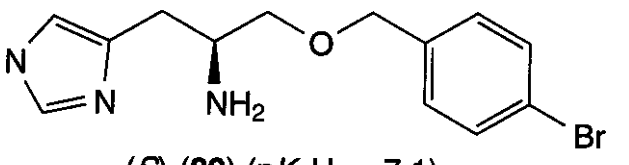


Cilazapril (**83**)

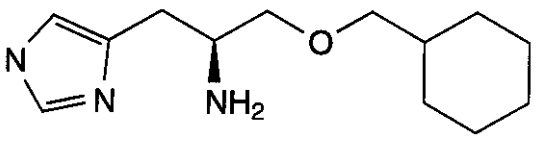
Figure 18.34.



L-histidine methyl ester (88)



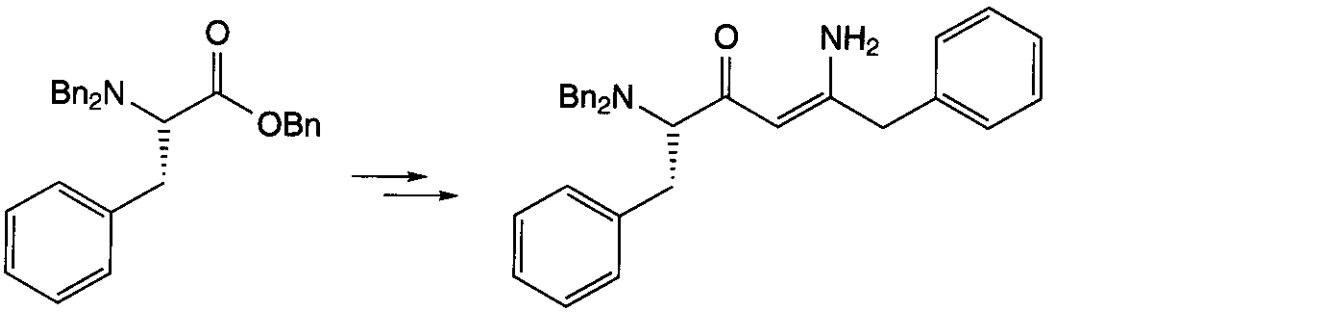
(*S*)-(**89**) (pK_i H_3 = 7.1)
c.f. (*R*)-(**89**) (pK_i H_3 = 6.7)

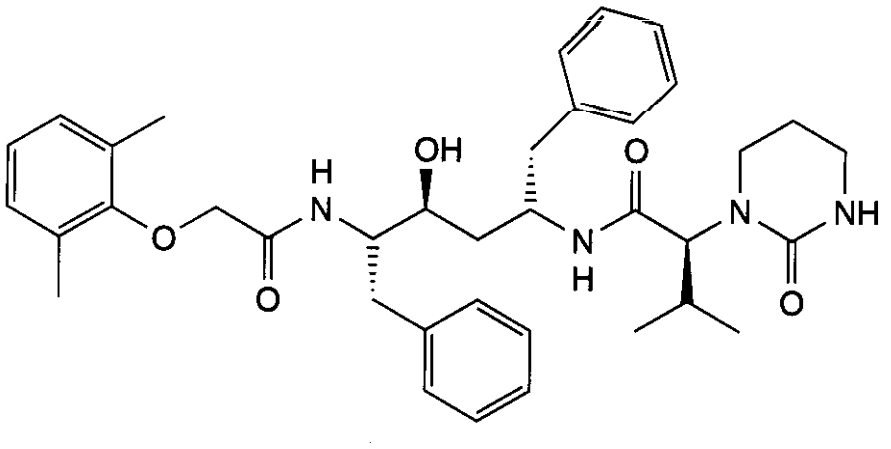


(*S*)-(**90**) (pK_i H_3 = 7.9)
c.f. (*R*)-(**90**) (pK_i H_3 = 6.8)

Figure 18.36.

able (*S*)-**pyroglutamic** acid methyl ester (94) (139). Acetone **oxime dianion** was added to the methyl ester (94) to generate an intermediate (95). Racemization of the **chiral** center was found to occur under basic conditions; however, this was avoided by immediate **treat-**





Lopinavir (87)

(86) (89–93%)

Figure 18.35.

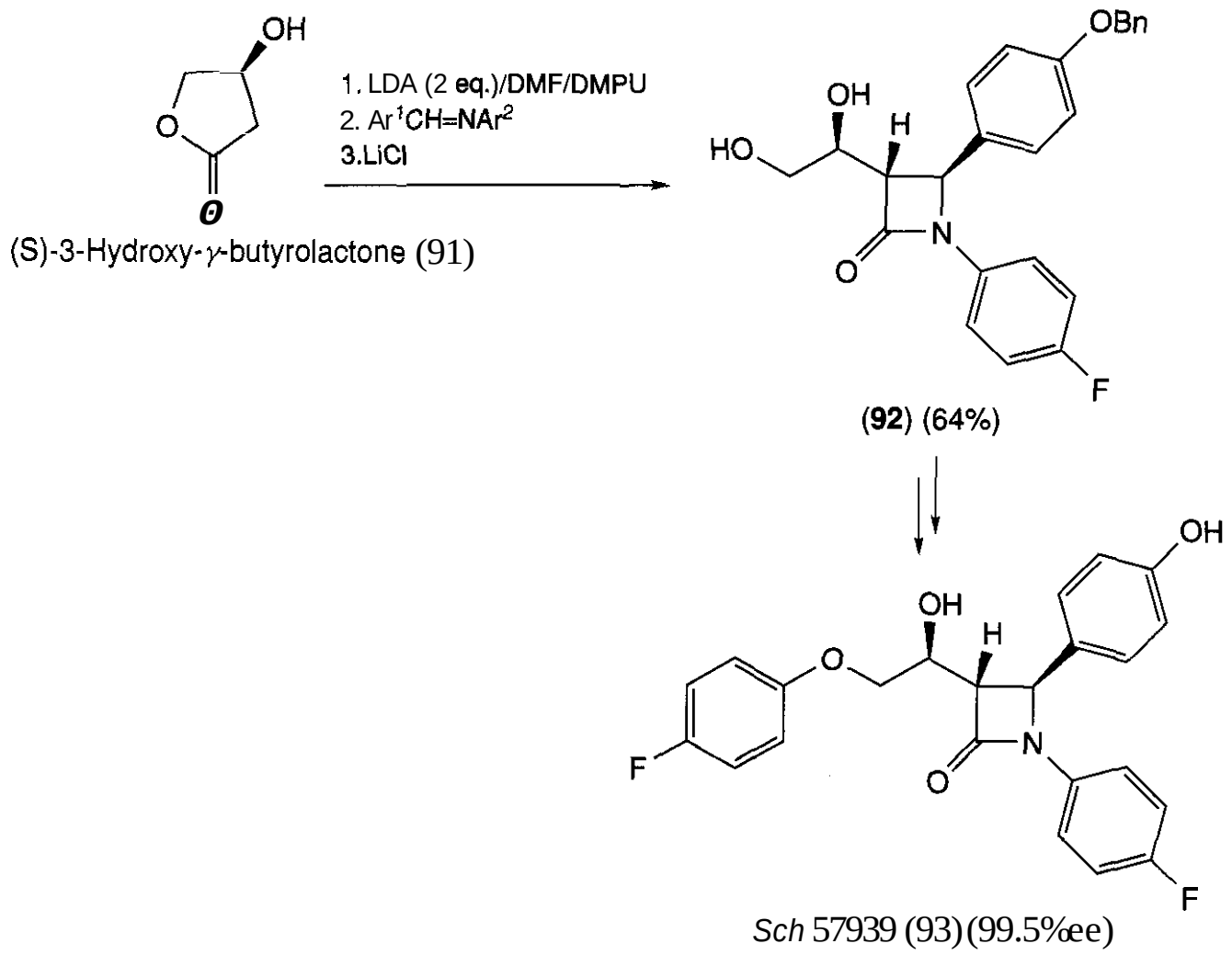


Figure 18.37.

ment with concentrated sulfuric acid resulting in cyclization and dehydration to amide isoxazole (**96**). Reduction and *N*-methylation yielded ABT-418 (**97**). The binding affinity of ABT-418 at neuronal cholinergic channel receptors was measured to be one order of magnitude greater than the corresponding R-enantiomer (K_i = 4.2 versus 44 nM) **(138).**

6.2 Chiral Auxiliary

In this approach the substrate is attached to a chiral, non-racemic unit that controls the formation of one or more new chiral groups. Reaction of the coupled unit with a reagent or prochiral substrate is designed to produce one diastereomeric product in excess. The auxil-

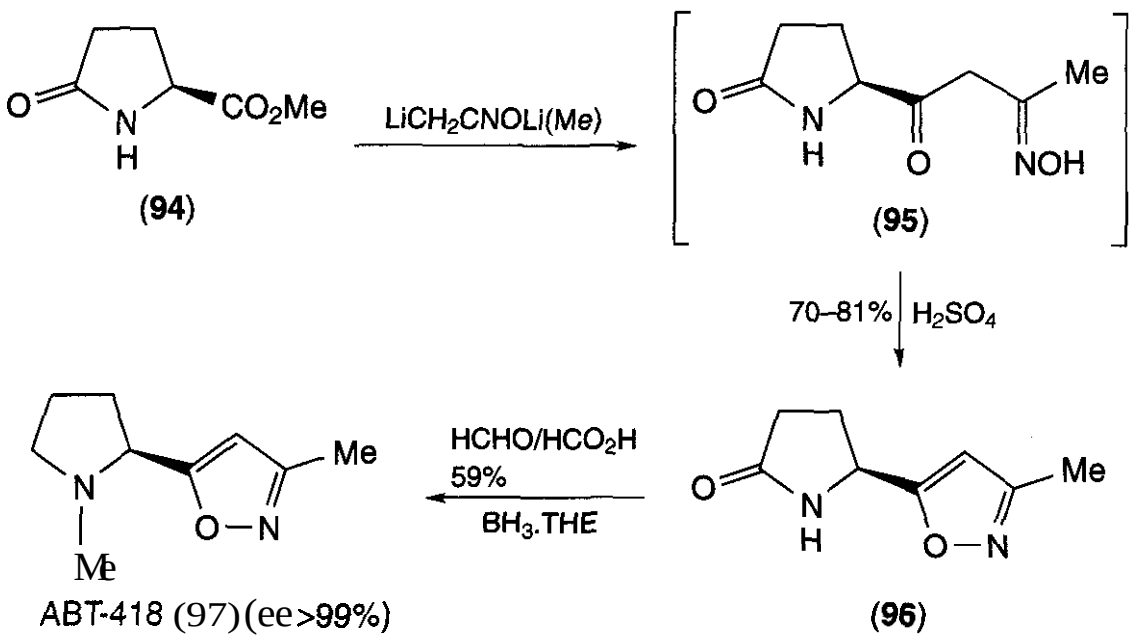


Figure 18.38.

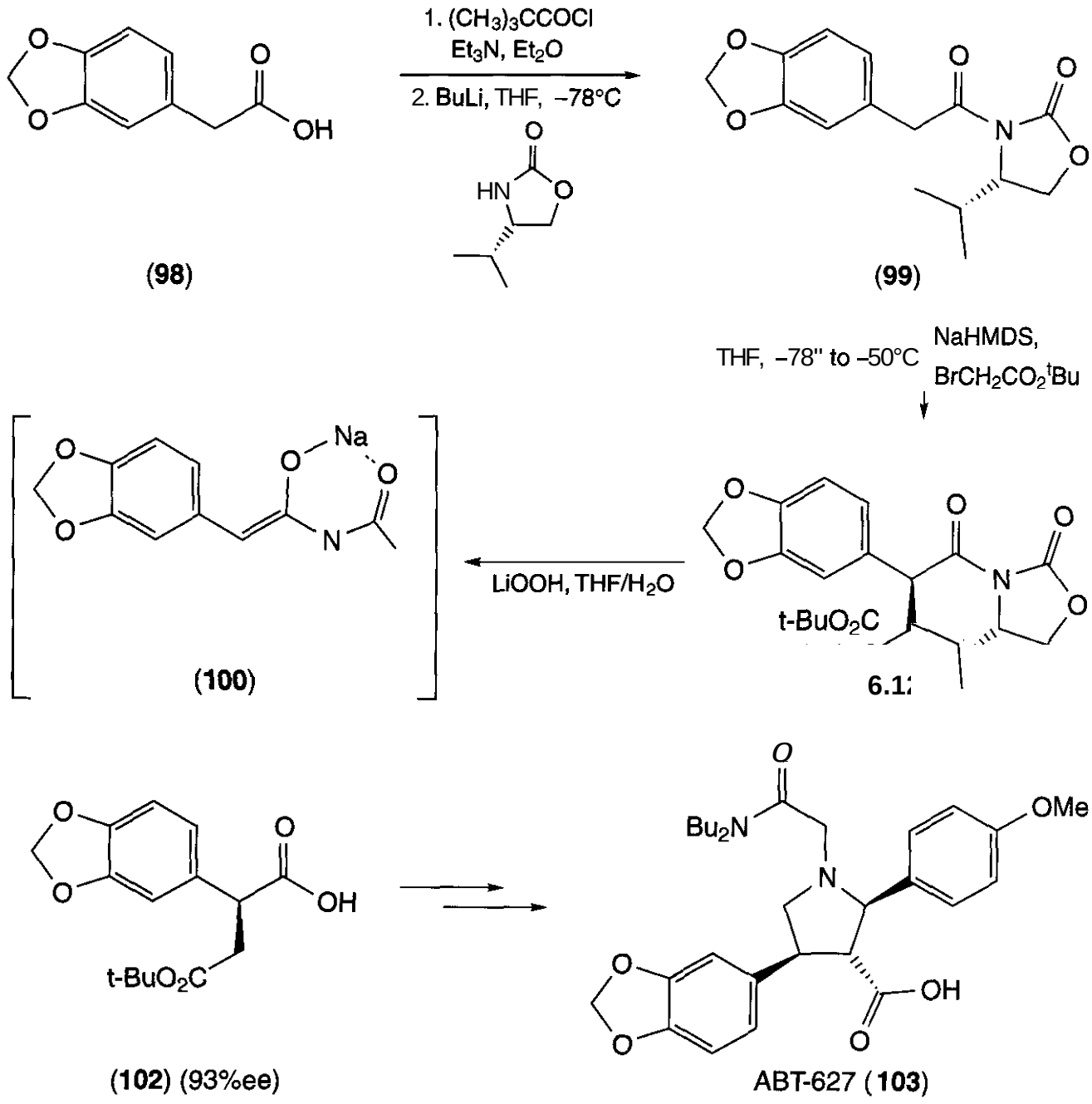


Figure 18.39.

iary is then removed (and preferably recovered), providing the product in high enantiomeric excess. This process is most attractive when both isomers of the auxiliary are readily available in enantiomerically pure form, and where the reaction leads to high levels of stereoselectivity in a predictable manner. Attaching and removing the auxiliary should be straightforward and proceed without loss of stereochemical integrity.

Many auxiliaries currently in use are derived from 1,2-amino alcohols (140). These are readily available from natural sources with little or no synthetic manipulation and can react in a variety of ways to form conformationally well-defined (usually cyclic) auxiliary systems. The use of oxazolidinones in asymmetric synthesis was developed by Evans et al., and these oxazolidinones have been used extensivelvin a variety of different reactions (140, 141). The use of this chiral auxiliary in the preparation of pharmaceuticals is widespread, and there are several large-scale processes using such chemistry (142).

Abbott reported an improved synthesis of ABT-627 (98)involving an asymmetric alkylation of the valine-derived acyl oxazolidinone (99) (143). ABT-627 (Atrasentan) is a selective endothelin ET_A receptor antagonist under development for the treatment of cancer, particularly prostrate cancer. Acid (100) was activated as a mixed anhydride and treated with the lithium anion of the oxazolidinone to give (101). Both of the following deprotonation and alkylation steps must be controlled to give high levels of stereoselectivity. The (Z)-enolate (102) is favored, both kinetically and thermodynamically, by the bulky iso-propyl group

Table 18.3 Stereochemical Variation

(3α, 6)	K_i (nM versus HIV-1)	IC_{95} (μM, cell HIV-1)
R, R Tipranavir	0.008	0.03
R, *S*	0.018	0.14
S, *R*	0.032	0.41
S, *S*	0.22	1.7

and is held rigid by chelation to the carbonyl oxygen of the oxazolidinone. The major stereoisomer then results from alkylation of this chelated enolate anion from the least hindered "upper" face to yield (103) as the major product. There are many strategies for removal and recovery of an oxazolidinone auxiliary (141). In this case, hydrolysis with lithium peroxide provides the acid that is transformed into Atrasentan through a cyclization-ring contraction strategy controlled by the chirality present in (**103**).

Tipranavir (PNU-140690)is a potent third-generation HIV protease inhibitor in clinical development by Boehringer Ingelheim (under license from Pharmacia). The biological activity of such 5,6-dihydro-4-hydroxy-2-pyrone sulfonamides shows considerable stereochemical variation (Table18.3) (**144**). The *R*-configuration is preferred at both chiral centers (3α and 6), and Tripanavir is more than 50 times as potent as its enantiomer in a cell culture assay using $HIV\text{-}1_{IIIB}$–infected H9 cells. An asymmetric synthesis (**145**) begins with the Michael addition of an aryl cuprate (derived from commercially available Grignard reagent 105) to the unsaturated oxazolidinone imide (**104**), yielding the adduct as a single diastereomer (106). The nitrogen protecting group was changed and an acetyl group introduced to give ketone (**107**), which undergoes a stereoselective aldol reaction with an acetylenic ketone (108). The highest diastereoselectivity was obtained for this reaction using $Ti(O^nBu)Cl_3$ as the Lewis acid. Both of the critical asymmetric steps to form new chiral centers are controlled by the (*R*)-phenyl oxazolidinone. The chiral auxiliary is removed when (**109**) is treated with base to form the lactone ring. This is followed by two further steps that generate PNU-140690 (110) as a single enantiomer.

The enantioselective synthesis of dopaminergic benzyltetrahydroisoquinolines and their binding to D_1 and D_2 dopamine receptors was investigated by Cabedo et al. (146). The synthetic route, illustrated by the preparation of the (1s)-isomer involves stereoselective reduction of the isoquinolinium salt (114) with (*R*)-phenylglycinol (introduced in protected form as 112) as the chiral auxiliary. The (1*R*)-enantiomer of (**115**), prepared in an analogous fashion using (*S*)-phenylglycinol, binds to dopamine receptors with considerably less affinity (>100 μM versus D_1 and 61.2 μM versus D_2). In contrast, stereochemical differentiation was not observed at the dopamine uptake site for these compounds.

Two different chiral auxiliary approaches have been applied to the synthesis of NPS 1407 and it's enantiomer (119) (**147**). NPS 1407 is an antagonist of the glutamate NMDA receptor that has in *vivo* activity in neuroprotection and anti-convulsant assays. The *R*-enantiomer was synthesized in four steps from (116)with the chiral center introduced by. a completely stereoselective alkylation of hydrazone (**117**). The chiral auxiliary, *S*-(−)-1-amino-2-(methoxylmethyl)pyrrolidine (SAMP), was introduced by condensation with aldehyde (116) and removed by catalytic hydrogenolysis. In the second method, the *S*-enantiomer was formed in a four-step sequence with the chiral center installed by the Michael addition of chiral amine (121) (formed in one step from the readily available α-methylbenzylamine) to benzyl crotonate (120). NPS 1407 (**123**) was found to be 12 times more potent than it's enantiomer (119)at the NMDA receptor in an in *vitro* assay.

An example of the use of a terpene as a chiral auxiliary is provided by the synthesis of the anti-viral reverse transcriptase inhibitor Lamivudine (148). The nucleoside analog is marketed by Biochem Pharma (now Shire Pharmaceuticals) and Glaxo Wellcome (now GlaxoSmithKline) for the treatment of HIV and hepatitis B virus infection. In the

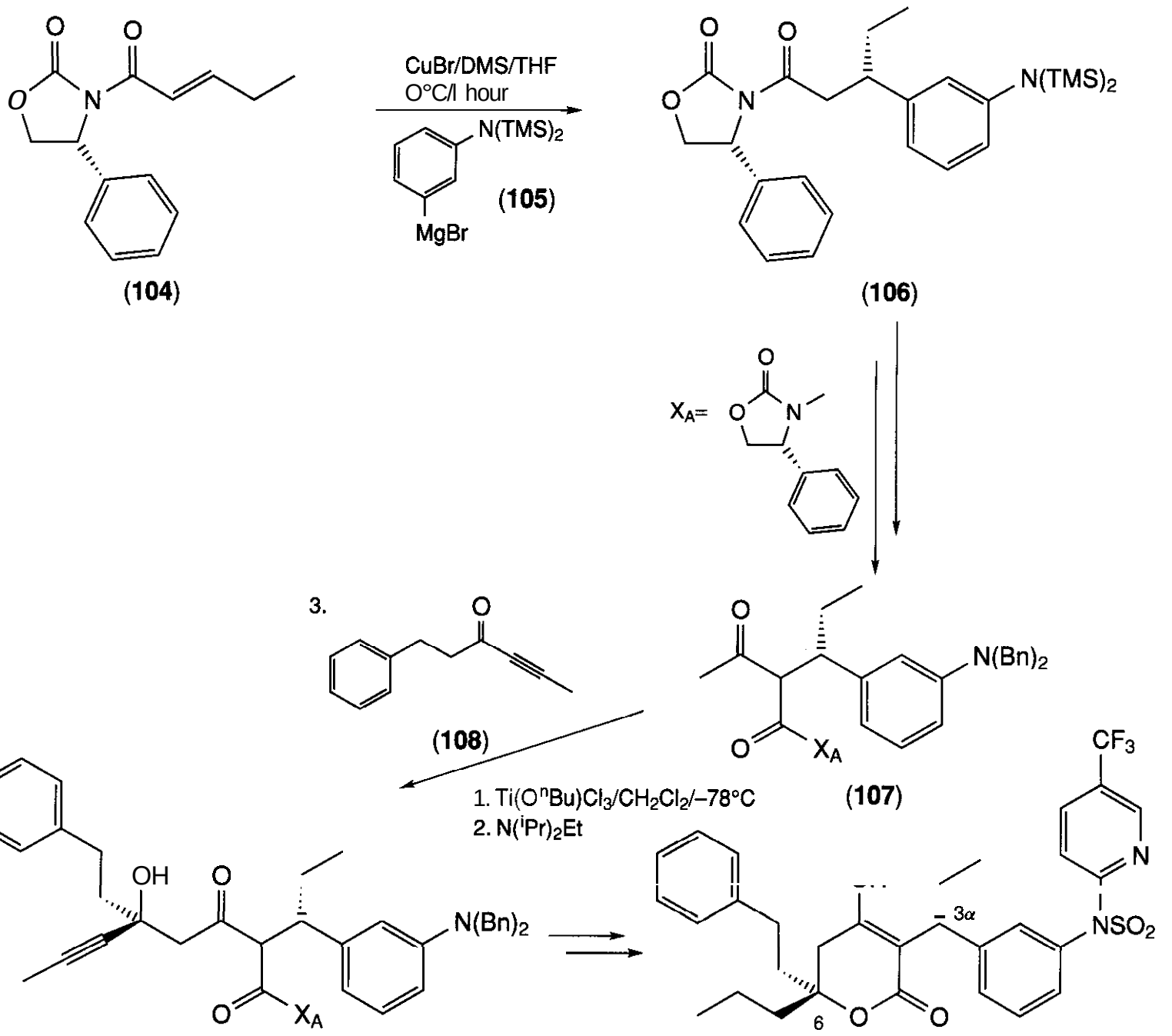


Figure 18.40.

production route, the glycolate derived from (−)-menthol(124) is coupled with thioacetyl dimer (125). The chiral auxiliary directs reaction to install the desired (2*R*)-stereochemistry in (126). In *situ* formation of chloro compound (127) is followed by a stereoselective coupling reaction with trimethylsilyl cytosine again directed by the (−)-menthyl carboxylate. Reductive removal of the auxiliary yields Lamivudine (129) as a single isomer that was found to have favorable toxicological and pharmacokinetic properties to the racemate.

6.3 Chiral Reagent

In this approach, asymmetry is induced in a prochiral molecule or functional group by reaction with a stoichiometric amount of an enantio-enriched reagent system. The reaction proceeds through diastereomeric transition states, resulting in the preferential formation of one enantiomer or diastereomer. Current reagents can lack generality and may be difficult to prepare in both chiral forms. At least one equivalent of the chiral component is required, which can present economical and practical difficulties. Many examples are provided by the reduction of double bonds, especially ketones. Ketone (**130**) was reduced enantioselectively using either (+) or (−)-*b*-chlorodiisopinocampheylborane (149). Reduction with **(–)-b-chlorodiisopinocampheyl**borane generated the alcohol (*S*)-(**131**), which was transformed into the (1*R*,3*S*)-isochroman compound, (1*R*,3*S*)-(**132**), through a ste-

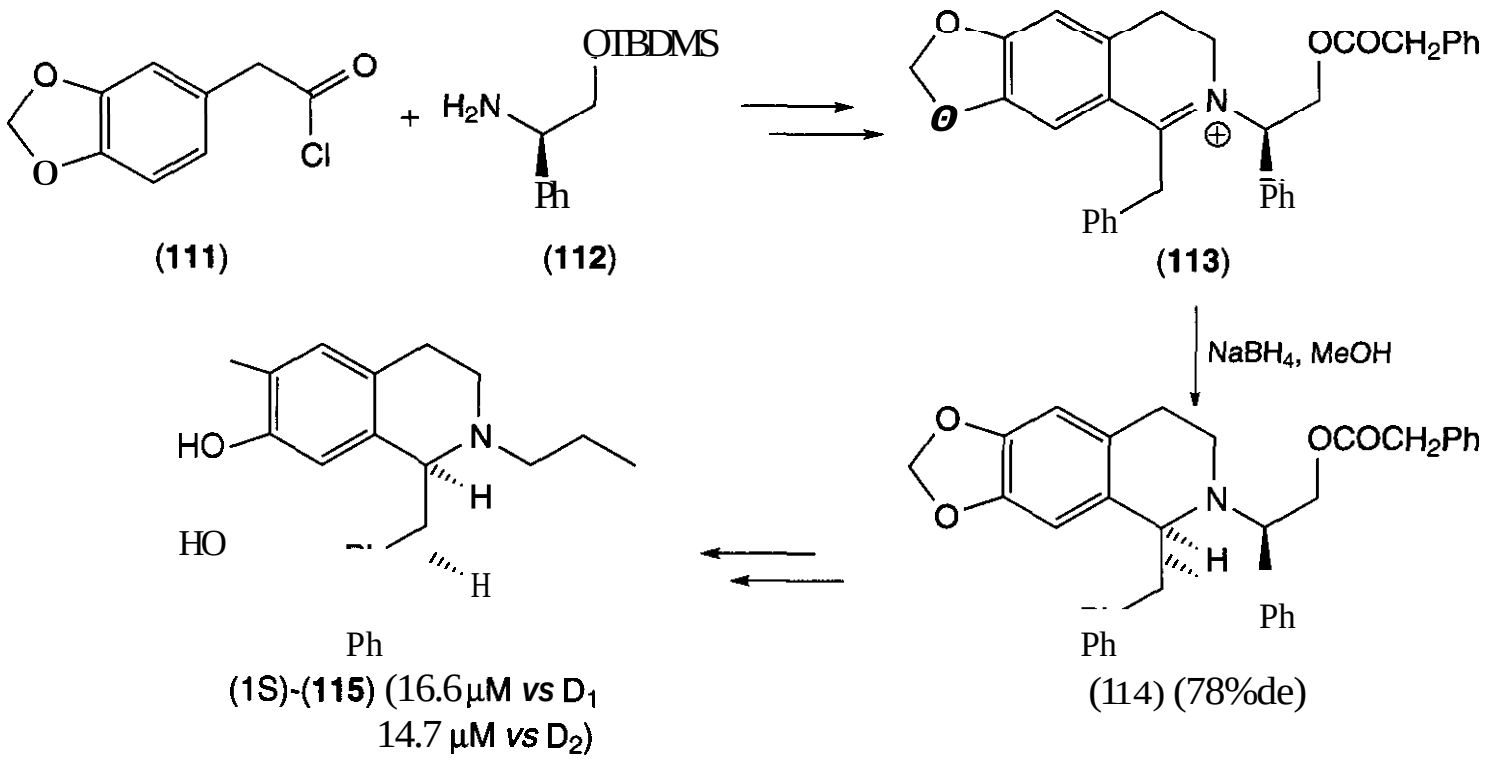


Figure 18.41.

reospecific cyclization to form the cis stereochemistry across the ring. The enantiomer, (1*S*,3*R*)-(**132**), was prepared in a directly analogous manner by reduction with (+)-b-chlorodiisopinocampheylborane. This study represents another example of stereodifferentiation at the dopamine receptors, with nearly a 5000-fold difference in D_1 potency observed between the two isomers in an in vitro assay.

A recent paper by scientists at Bristol-Myers Squibb reports the synthesis of a new class of calcium-activated potassium channel modulators (**150**). The compounds were investigated for their ability to increase channel opening at large conductance (BK or maxi-K) channels and showed a limited degree of stereospecificity. The key step in the synthesis is the direct oxidation of the enolate derived from (133) with either isomer of camphorsulfonyl oxaziridine, a reagent developed by Davis (151). Both enantiomers of the 3-aryl-3-hydroxyindol-2-ones were prepared with very high enantiopurity (>95% ee) using opposite enantiomers of the chiral oxaziridine. (−)-(**134**) was found to be a better activator of a cloned BK channel than the (+)-isomer at 20 μM, generating a current increase of 141% compared with 124% for (+)-(**134**).

6.4 Chiral Catalyst

The use of a chiral catalyst represents the ideal method for asymmetric synthesis because only small amounts of the chiral mediator are required and no modifications of the prochiral substrate are necessary. In many systems both enantiomers of the product cgn be prepared in a predictable and reproducible manner. The pharmaceutical industry is particularly interested in the capability of new catalyst systems to operate as reliable manufacturing processes on a large scale (127,152). Substantial effort continues to be expended by the synthetic organic community with the goal of extending the number of efficient and broadly applicable catalyst systems capable of generating high levels of enantiomeric excess in a wide range of substrates (127).

The reduction of ketones by borane catalyzed by chiral oxazaborolidines such as (**136**), derived from the enantiomeric amino alcohols, has been applied to the synthesis of several drug candidates (127). This system is known as the CBS (Corey, Bakshi, Shibata) reduction (153), and Corey himself has applied it to the synthesis of pharmaceutical compounds (154). A further example is provided by the synthesis of MK-499 (**137**), a

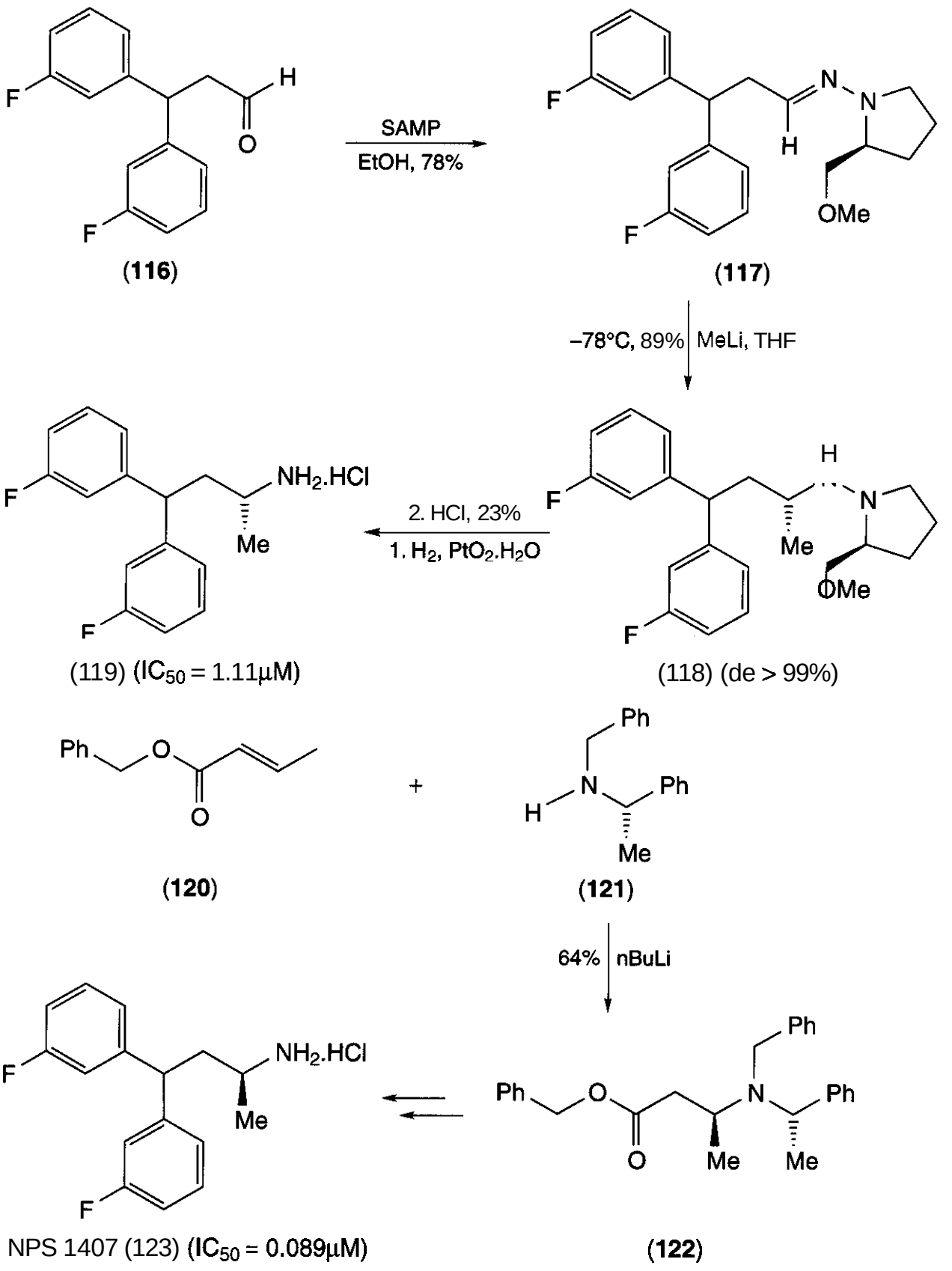


Figure 18.42.

potassium channel blocker that was developed for the treatment of cardiac arrhythmia by Merck (155).

Asymmetric hydrogenations with transition metal catalysts have been applied to single enantiomer synthesis in the pharmaceutical industry with considerable success. ChiroTech and Pfizer developed an improved synthesis of glutarate derivative (**139**), an intermediate required for the synthesis of Candoxatril (140) (156). The drug, a neutral endopeptidase inhibitor, was in clinical development for the treatment of hypertension and congestive heart failure, and its enantiomer does not possess the same biological activity. Several catalysts and conditions were screened before arriving at optimized conditions using cationic rhodium-(*R*,*R*)-MeDuPHOS (**141**), which provided the product with complete enantioselectivity and avoided previously observed problems associated with isomerization of the enone starting material. The reaction could be conducted at a

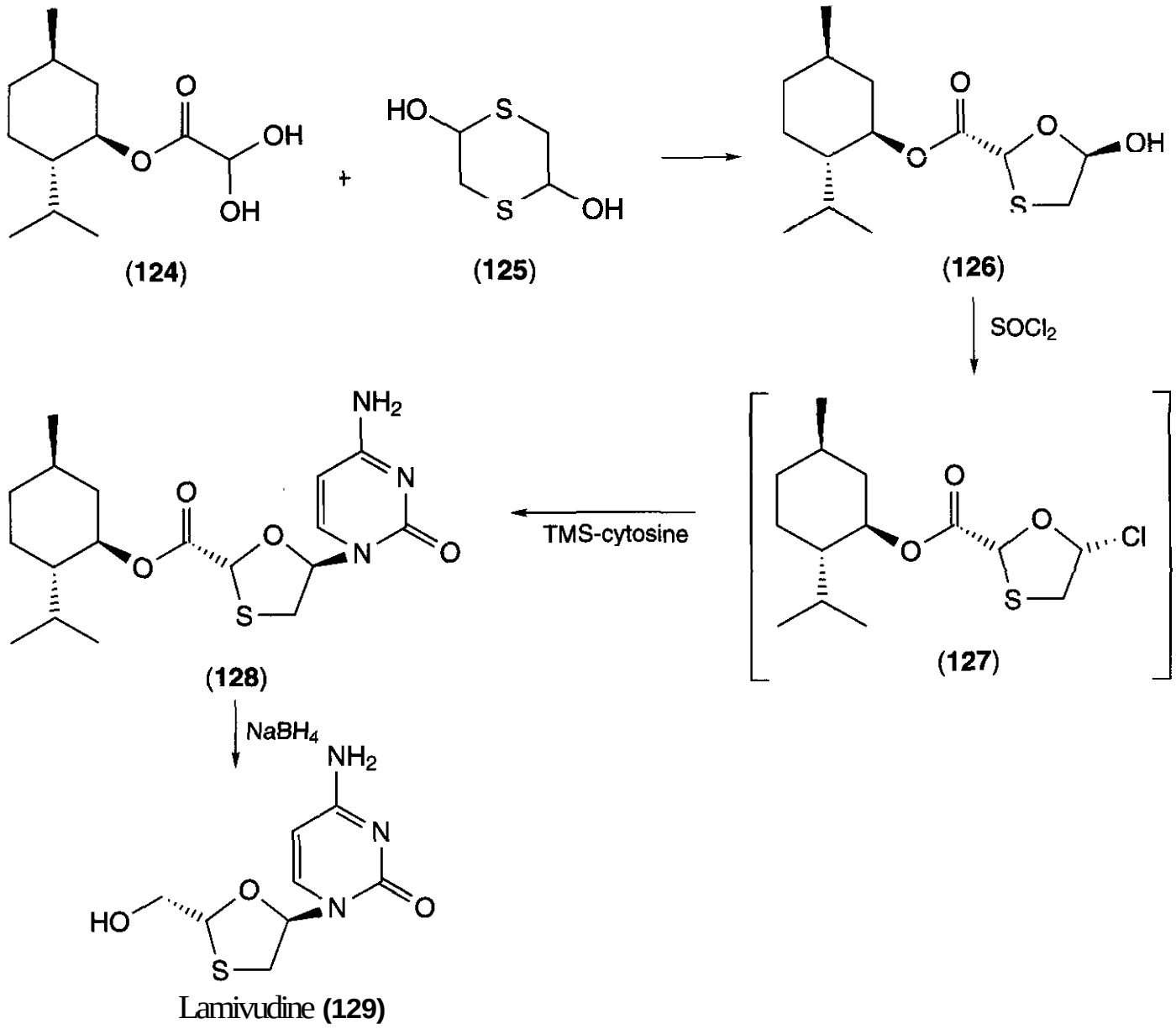


Figure 18.43.

high substrate-to-catalyst ratio of 3500:1 without a detrimental effect on enantiomeric excess or reaction rate. In catalytic asymmetric reactions, it is clearly economically advantageous to minimize the amount of catalyst that may comprise expensive chiral material and transition metals.

A method for the asymmetric dihydroxylation of alkenes to yield cis-diols was developed by the research group of Sharpless using chiral ligands derived from the cinchona alkaloids dihydroquinidine (DHQD) and dihydroquinine (DHQ) with a catalytic amount of osmium tetroxide (157). Although they are diastereomers, the phthalazine ligands act as "pseudo-enantiomeric" ligands, i.e., they give opposite asymmetric induction in a predictable manner. This procedure was recently used to prepare both isomers of combretadioxolane (**144**), a chiral analog of the natural product Combretastatin A-4 (146) (158). Combretastatin A-4 displays antitubulin activity and cytotoxicity to tumor cells and is therefore an interesting lead structure for new anticancer drugs. The asymmetric synthesis of (*S,S*)-combretadioxolane (144) involved treatment of the trans-stilbene (**142**) with "AD-mix-a" [containing $(DHQ)_2$-PHAL] (**145**), whereas the enantiomer (*R,R*)-combretadioxolane resulted from use of AD-mix-& which contains $(DHQD)_2$-PHAL as the chiral ligand. The tubulin polymerization-inhibitory activity of (*S,S*)-combretadioxolane was comparable with combretastatin A-4 ($IC_{50} = 4-6\ \mu M$) in an in *vitro* assay, whereas (*R,R*)-combretadioxolane was essentially inactive ($IC_{50} > 50\ \mu M$). In addition, (*S,S*)-combretadioxolane was 20 times more potent than vincristine as an in *vitro* growth inhibitor of the multidrug-resistant cell line PC-12.

Workers at SmithKline Beecham reported the stereoselective synthesis of inhibitors of

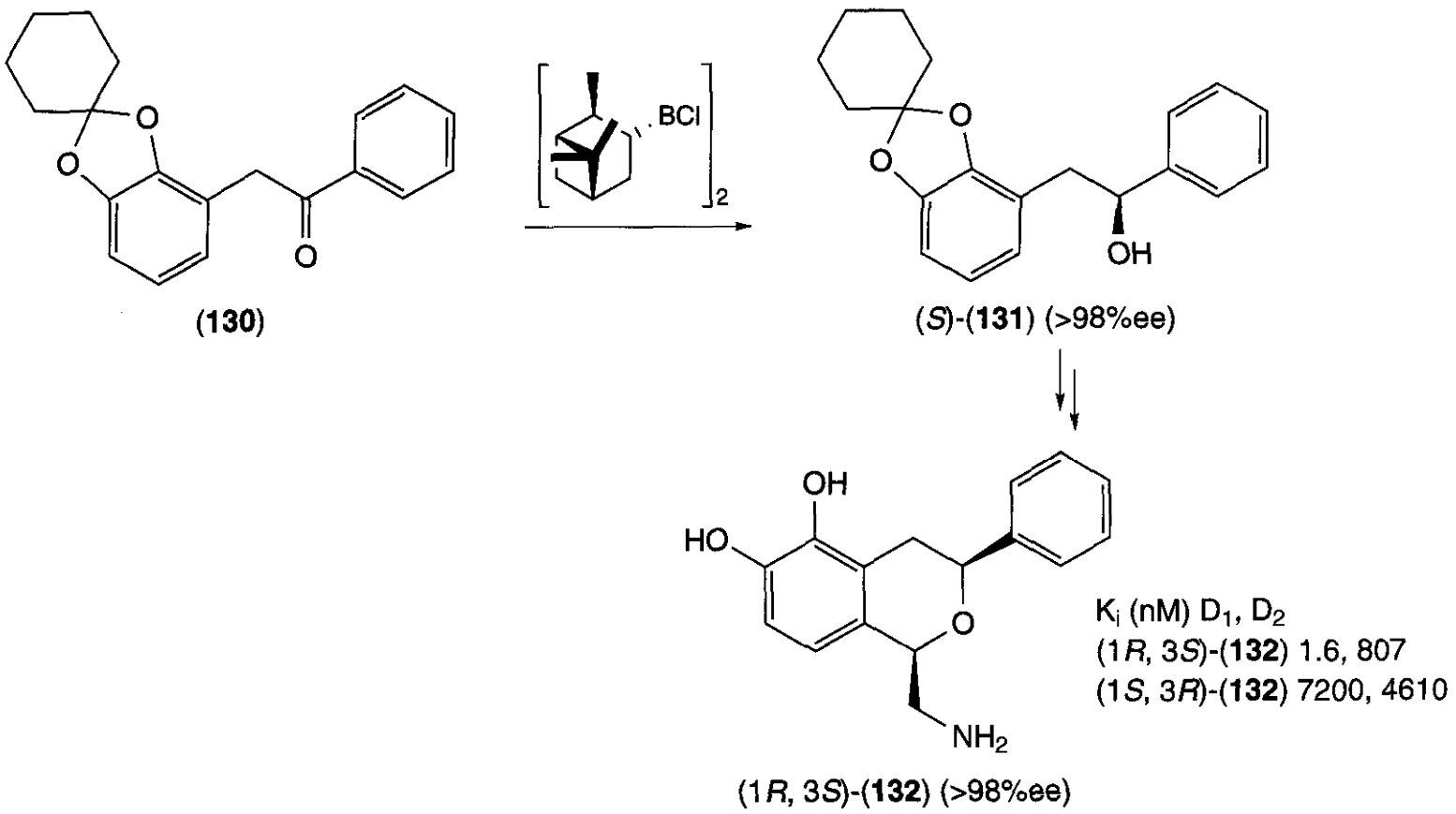


Figure 18.44.

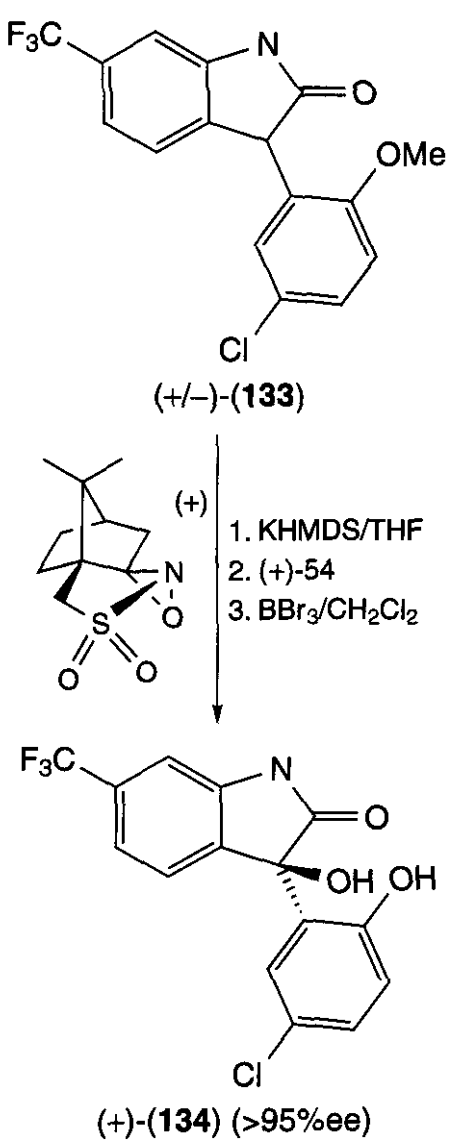


Figure 18.45.

the cysteine protease cathepsin K (159). A procedure was sought to allow preparation of either enantiomer of azido alcohol (148). This was readily achieved by Jacobsen asymmetric desymmetrization of the *meso*-epoxide (**147**) using azidotrimethylsilane catalyzed by chromium salen complex (149) (160). Use of the (*R*,*R*)-salen catalyst shown generated (3*S*, 4*R*)-(**148**), whereas the (*S*,*S*)-catalyst provided the (3*R*,4*S*)-azido silyl alcohol, both with very high enantioselectivity. After removal of the silyl group and reduction of the azido moiety, the resultant enantiomeric amino alcohols were transformed into diastereomers (4*S*)- and (4*R*)-(**150**) by reaction with leucine, amide formation, and oxidation. The cathepsin K activity for the diastereomers showed the (4s)-isomers to be up to 40-fold more potent than the corresponding (4*R*)-(**150**) in an enzyme assay.

A large scale synthesis of the drug Nelfinavir, an HIV protease inhibitor developed by Agouron (now Pfizer) was reported with the amino alcohol derived from (**148**), prepared using the Jacobsen procedure described above (161).

A similar approach uses the chromium-Salen complex (149) to open racemic terminal epoxides in a highly efficient resolution pro-

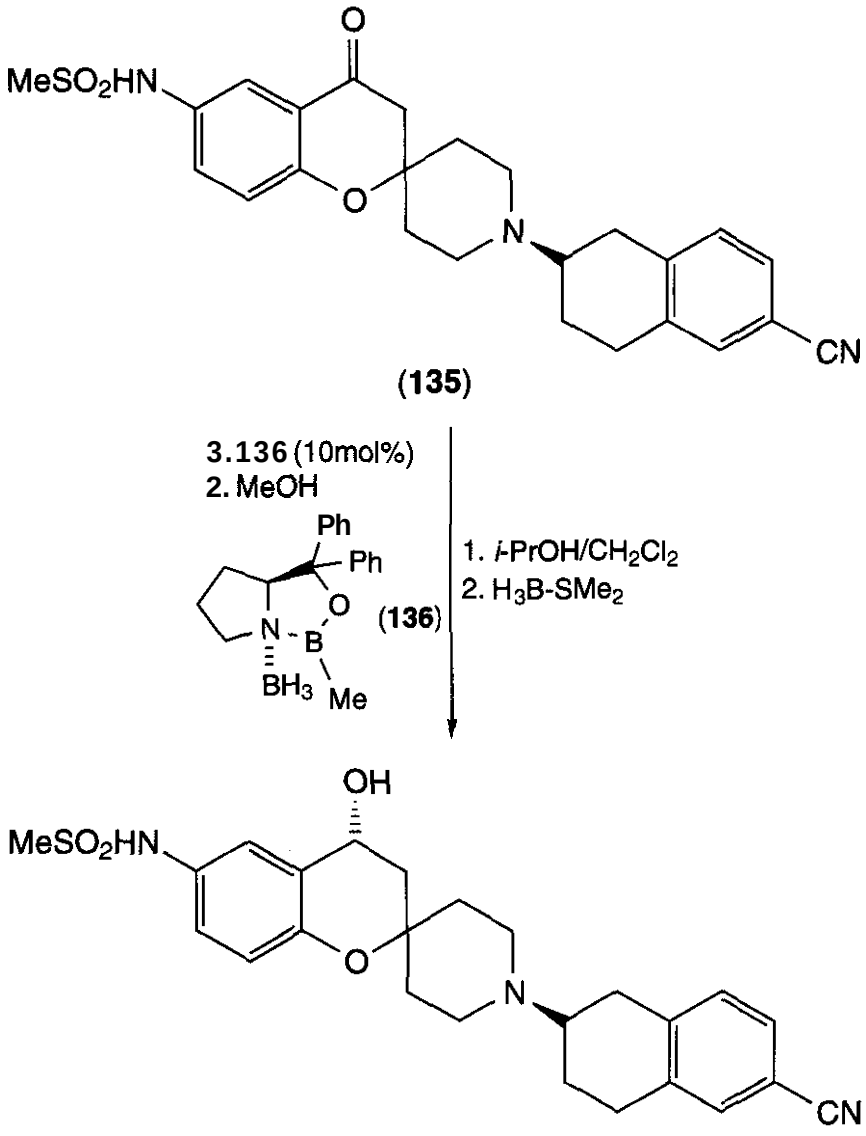


Figure 18.46.

cess that has been applied to the synthesis of biologically active compounds (162). As with any such resolution process, the maximum yield of enantiopure material is 50% based on starting material. Terminal epoxides are easy to prepare in racemic form, and conversely, difficult to prepare as single enantiomers by epoxidation of the corresponding alkene. (*R*)-9-[2-(phosphonomethoxy)propyl]adenine (R-PMPA) is a nucleotide reverse transcriptase

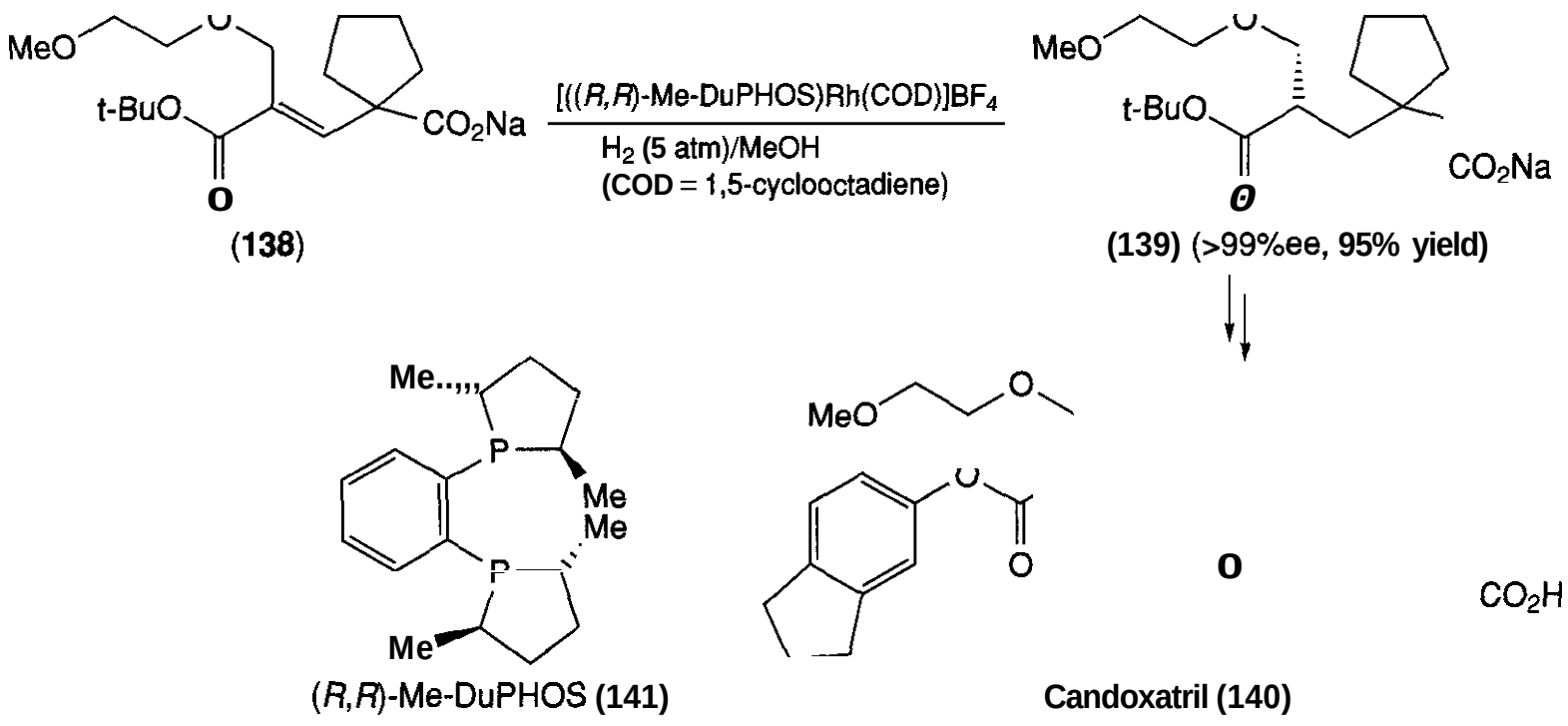


Figure 18.47.

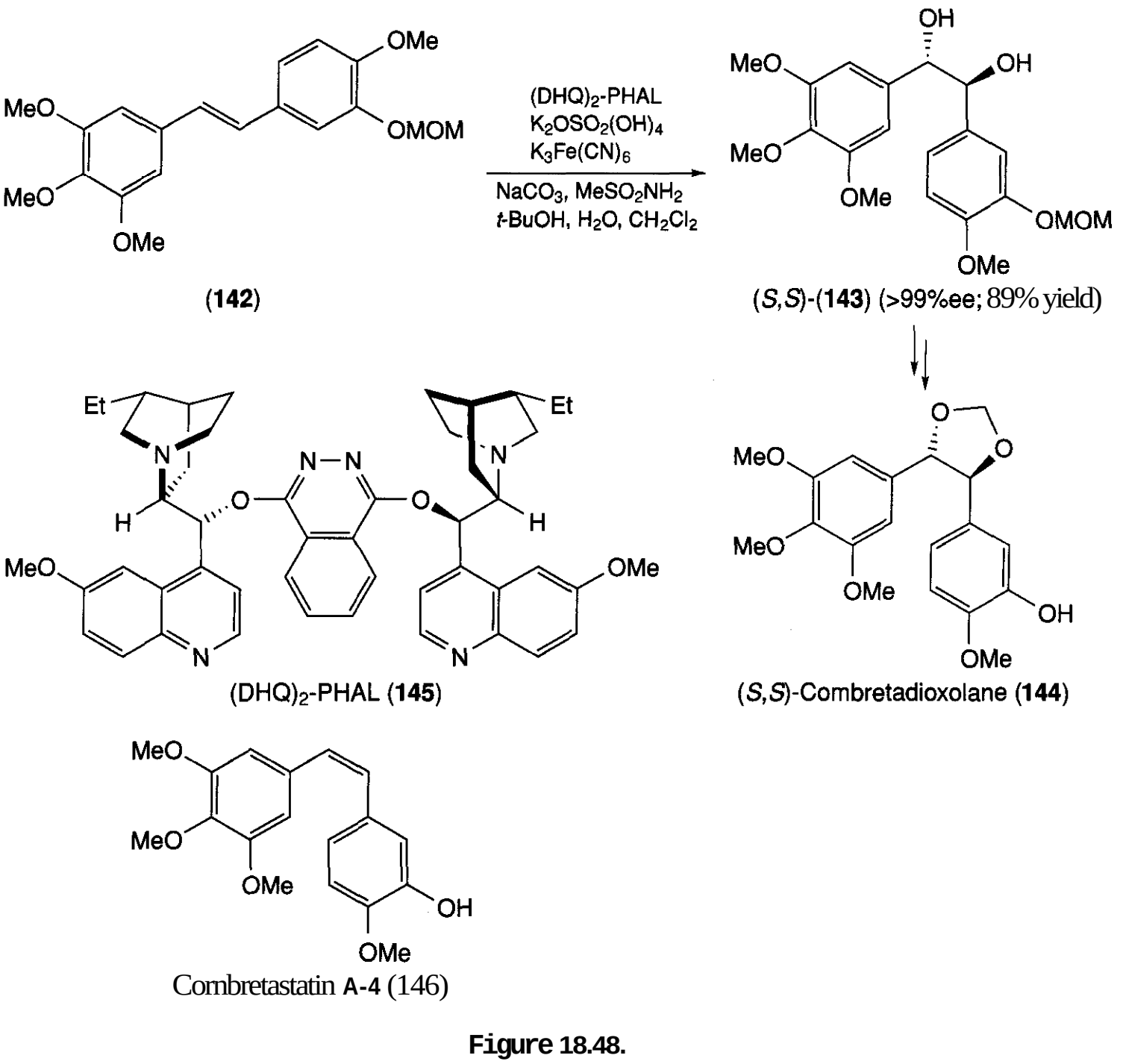


Figure 18.48.

inhibitor being developed by Gilead Sciences and a collaborative group from the University of Washington for the treatment and prevention of HIV infection (163). The compound can be prepared through kinetic resolution of propylene oxide using (*S,S*)-(**149**) and the resultant (*R*)-1-amino-2-propanol (153)was transformed into (*R*)-PMPA (154) in five steps (162).

In 1997, Tokunaga et al. reported the hydrolytic kinetic resolution of racemic terminal epoxides using a Co(III)-Salen catalyst (164). This remarkably general process uses only water as the nucleophile and provides the synthetically useful chiral epoxides and diols in highly enantioenriched form. The catalyst can be recycled and the reactions conducted under solvent-free conditions. The process has been used by academic and industrial groups and is operated by Rhodia ChiRex on a large scale (165).

A wide variety of synthetic processes have been rendered asymmetric through the use of a chiral catalyst. In addition to the types of reaction described above, chiral transition metal catalysts have been used to influence the stereochemical course of isomerization, cyclization, and coupling reactions. As an example, an approach towards the natural product (−)-epibatidine (158) was recently reported by Namyslo and Kaufmann (166). Epibatidine is a potent analgesic and a nicotinic receptor agonist. The synthesis involves an asymmetric Heck-type hydroarylation between the bicyclic alkene (155) and pyridyl iodide (156). A number of bidentate chiral li-

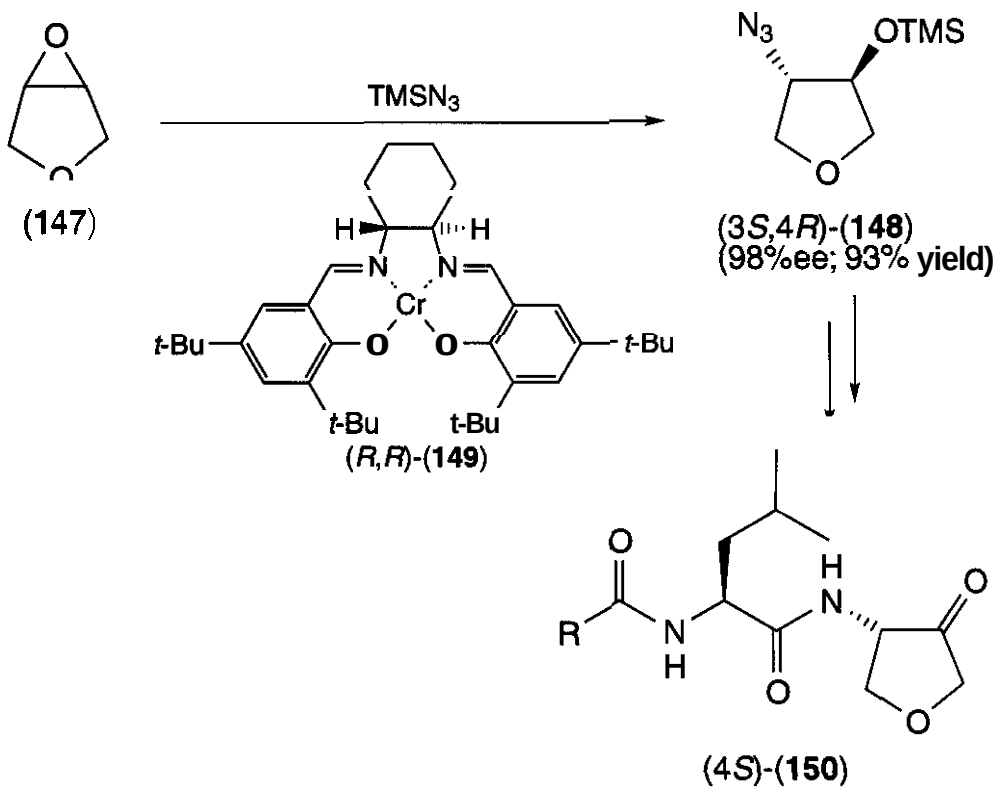


Figure 18.49.

gands were investigated with BINAP (**159**), which were observed to give the highest enantioselectivity. By using the (*R*)- or (*S*)-BINAP ligand, both enantiomers of (**157**) were accessible with about the same level of enantioselection.

The continuing development of efficient and practical asymmetric processes will be one of the major driving forces in the future of drug discovery and development. In particular, the design of new general and practical catalytic processes will help explore the link between chirality and biological activity.

7 CONCLUSIONS

The ultimate focus of the endeavors of medicinal chemists is to develop a successful drug that will cure patients. However, with the increased regulatory requirements within the competitive biotechnology and pharmaceutical industry, the initial research to achieve this objective must be conducted in a rapid and thorough manner. During the drug research and development process, the important and subtle relationship between chirality and biological activity should be carefully considered.

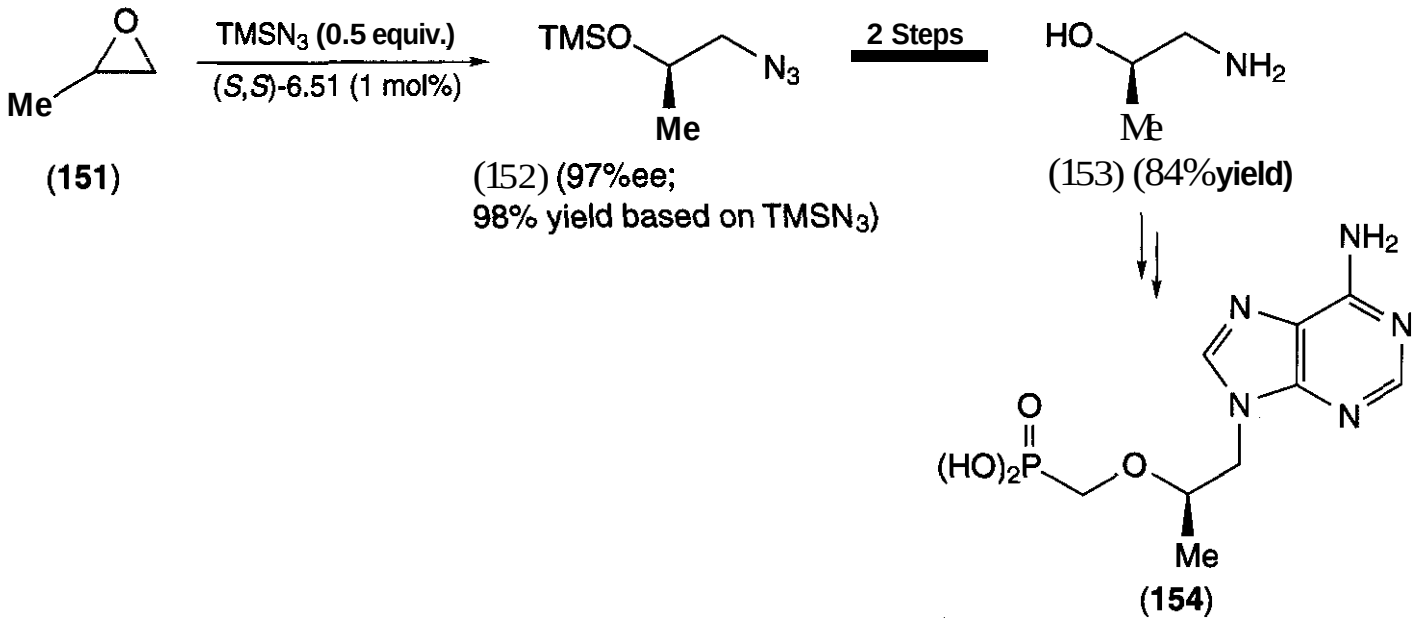
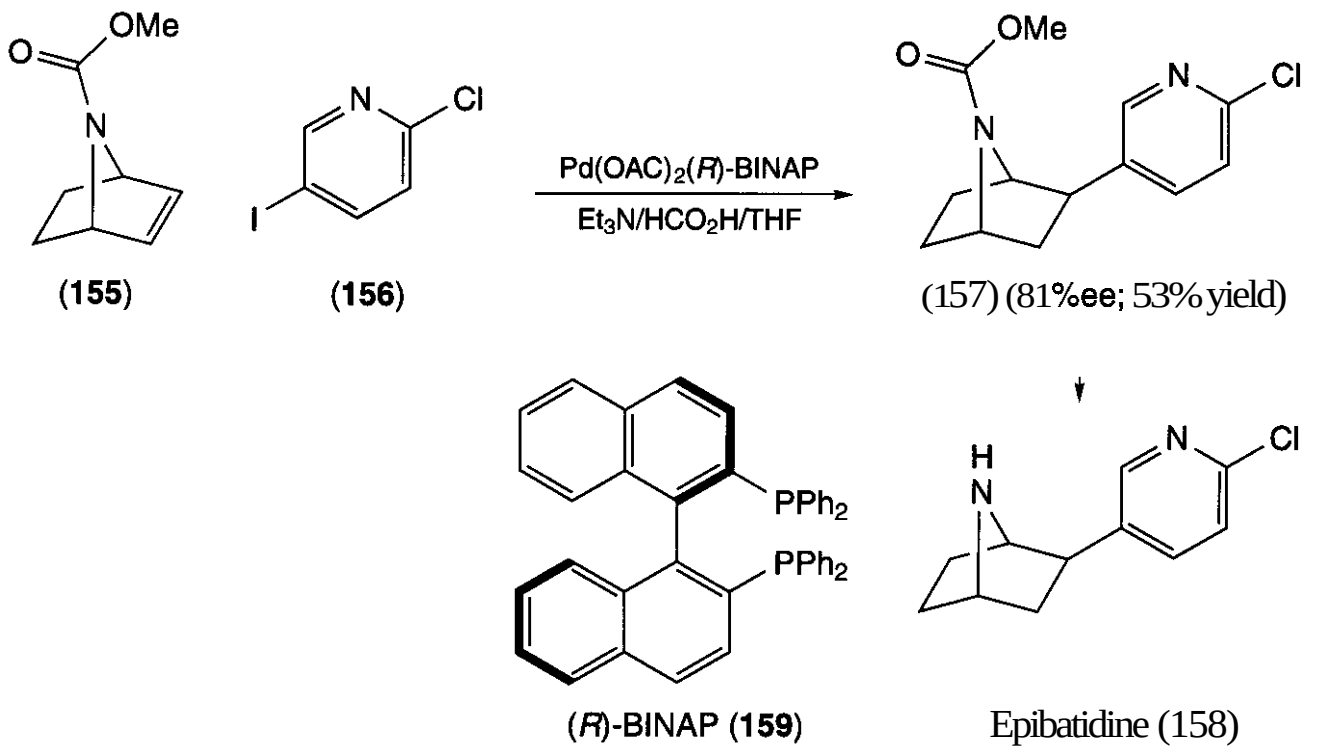


Figure 18.50.



(155) (156) (157) (81%ee; 53% yield)

(*R*)-BINAP (159) Epibatidine (158)

Figure 18.51.

Enantiomers frequently display markedly different biological activity; however, the fact that a large and adaptable toolbox of chemical and biological techniques to obtain single isomers are available allows the medicinal chemist to avoid working with mixtures of stereoisomers.

As reviewed in this chapter, there are numerous synthetic strategies available to the medicinal chemist that offer their own particular drawbacks and advantages. In the early stages of research it may be preferable to separate isomers by chromatography, thus providing both single enantiomers for biological testing. It should be noted that all the techniques described in this chapter can be used in conjunction with one another. That is to say, if one technique such as asymmetric synthesis failed to deliver enantiopure material, then another technique such as crystallization can be used to push through the product to the desired purity. As an example of this "double" approach, the use of SMB and crystallization in the separation of mandelic acid is worthy of note (56). The use of asymmetric hydrogenation followed by asymmetric enzymic transformation to obtain single isomer products has also been described by Taylor et al. at Chiro-Tech (167).

In conclusion, if a chiral center is present in a molecule designed and synthesized by a medicinal chemist, there are a broad number of methods available to prepare or isolate either isomer. From the examples given in this chapter, stereoisomers frequently display markedly different biological properties where the desirable properties associated with one isomer may not be apparent when the corresponding racemic mixture is tested either ***in vivo*** or ***in vitro.***

REFERENCES

1. A. Michaels and J. Fuller, ***Financial Times (Lond.)*, 23, 21 (2001).**
2. **R.** B. Raffa, E. Friderichs, W. Reimann, R. P. Shank, E. E. Codd, J. L. Vaught, H. I. Jacoby, and N. Selve, **J. *Pharmacol. Exp. Ther.*, 267,** 331–340 **(1993).**
3. M. B. Smith and J. March, ***March's Advanced Organic Chemistry, 5th*** ed., Wiley, New York, **2001,** pp. **125-217; E.** L. Eliel, S. H. Wilen, and L. N. Mander, ***Stereochemistry of Organic Compounds,*** Wiley, New York, **1994;** E. L. Eliel, S. H. Wilen, and M. P. Doyle, ***Basic Organic Stereochemistry,*** Wiley, New York, **2001.**
4. T. D. Stephens, ***Chem. Br.*, 37**, 38 **(2001).**
5. J. J. Baldwin and W. B. Abrams in I. W. Wainer and D. E. Drayer, Eds., ***Stereochemically Pure Drugs: An Industrial Perspective,*** Marcel Dekker, New York, **1988,** p. **311.**
6. G. C. Cotzias, P. S. Papavasiliou, R. Gellene, N. *Engl.* **J. *Med.*, 280, 337(1969).**
7. **H. K.** Kroemer, J. Turgeon, R. A. Parker, and D. M. Roden, ***Clin. Pharmacol. Ther.*, 46, 584 (1989).**

8. R. A. O'Reily, Clin. Pharmacol. Ther., 16, 348 (1974).
9. A. Breckenbridge, M. Orme, H. Wesseling, R. J. Lewis, and R. Gibbons, Clin. Pharmacol. Ther., 15,424 (1974).
10. T. Walle, J. G. Webb, E. E. Bagwell, U. K. Walle, H. B. Daniell, and T. E. Gaffney, *Biochem.* Pharamacol., 37, 115 (1988).
11. W. Lindner, M. Rath, K. Stoschitzky, and H. J. Semmelrock, Chirality, 1, 10 (1989).
12. D. E. Drayer in I. W. Wainer and D. E. Drayer, Eds., Drug **Sterochemistry-Analytical** Methods and Pharmacology, Marcel Dekker, New York, 1988, p. 209.
13. K. J. Fehske, W. E. Muller, and U. Wollert, Biochem. Pharmacol., 30,687 (1981).
14. R. H. McMenamy and J. L. Oncley, J. Biol. Chem., 233,1436 (1958).
15. W. E. Muller in I. W. Wainer and D. E. Drayer, Eds., Drug **Sterochemistry-Analytical** Methods and Pharmacology, Marcel Dekker, New York, 1988, p. 227.
16. S. Toon, L. K. Low, M. Gibaldi, W. F. Trager, R. A. O'Reily, C. H. Motley, and D. A. Goulart, Clin. Pharmacol. Ther., 39, 15 (1986).
17. M. A. Campanero, B. Calahorra, M. Valle, I. F. Troconiz, and J. Honorato, Chirality, 11, 272 (1999).
18. R. Stevenson, Chem. Br., **37, 24** (2001).
19. N. M. Maier, P. Franco, and W. Lindner, J. Chromatogr. A, **906**, 3 (2001).
20. L. Miller, C. Orihuela, R. Fronek, D. Honda, and O. Dapremont, J. Chromatogr. A,849,309 (1999).
21. V. M. Meyer, Chirality, 7, 567 (1995); O. P. Kleidernigg, M. Lammerhofer, and W. Lindner, Enantiomer, 1,387 (1996).
22. V. Schurig, J. *Chromatogr.* **441**, 135 (1988);K. Watabe, S. C. Chang, E. Gil-Av, and B. Koppenhofer, Synthesis, 3,225 (1987).
23. E. R. Francotte in S. Ahuja, Ed., Chiral Separations, Applications and Technology, chap. 10, American Chemical Society, Washington DC, 1997, p. 271.
24. K. D. Altria, N. W. Smith, and C. H. Turnbull, Chromatographia, 46,664 (1997); K. D. Altria, M. A. Kelly, and B. J. Clark, Trends Anal. Chem., 17,214 (1998).
25. K. L. Williams, L. C. Sander, and S. A. Wise, J. Pharm. Biomed. Anal., 15, 1789 (1997);N. Bargrnann-Leyder, A. Tambute, and M. Claude, Chirality, 7,311 (1995).
26. M. Juza, M. Mazzotti, and M. Morbidelli, Trends Biotechnol., 18, 108 (2000).
27. J. T. F. Keurentjes and F. J. M. Voermans in A. N. Collins, G. N. Sheldrake, and J. Crosby, Eds., Chirality and industry. II. Developments in the Manufacture of Optically Active Compounds, chap. 8,Wiley, New York, 1997, p. 157.
28. S. C. Stinson, Chem. Eng. News, **73, 44** (1995).
29. E. Francotte, J. Chromatogr. A, 666, 565 (1994).
30. CHIRBASE, available online at http:// chirbase.u-3mrs.fr, accessed on July 29,2002.
31. Daicel Chemical Industries, Ltd., available online at http://www.daicel.co.jp/chiral,accessed on July 29, 2002. NOVASEP, available online at http://www.novasep.com, accessed on July 29, 2002. Astec, available online at http:// www.astecusa.com, accessed on July 29, 2002.
32. D. Boyd, M. O'Keeffe, and M. R. Smyth, Analyst, 119, 1467 (1994).
33. D. A von Deutsch, I. K. Abukhalaf, L. E. Wineski, H. Y. Aboul-Enein, S. A. Pitts, B. A. Parks, R. A. Oster, D. F. Paulsen, and D. E. Potter, Chirality, 12,637 (2000).
34. B. Waldeck, E. Widmark, *Acta* Pharmacol. Toxicol., **56**, 221–227 (1985).
35. D. J. Triggle, D. A. Langs, and R. A. Janis, Med. Res. Rev., 9, 123 (1989);V. C. O. Njar and A. M. H. Brodie, Drugs, 58,233 (1999).
36. S. Visentin, P. Amiel, A. Gasco, B. Bonnet, C. Suteu, and C. Roussel, Chirality, 11, 602 (1999).
37. P. Tullio, A. Ceccato, J-F. Liegeois, B. Pirotte, A. Felikidis, M. Stachow, P. Hubert, J. Crommen, J. Geczy, and J. Delarge, Chirality, 11, 261 (1999).
38. J. Bruhwyler, J. F. Liegeois, J. Gerardy, J. Damas, E. Chleide, C. Lejeuns, E. Decamp, P. De Tullio, J. Delarge, A. Dresse, and J. Geczy, Behav. Pharmacol., 9, 731 (1998).
39. T. Shibata, I. Okamoto, and K. Ishii, J. Liq. Chromatogr., 9,313 (1986);E. Yashima and Y. Okamoto, Bull. Chem. Soc. Jpn., **68,** 3289 (1995).
40. W. H. Pirkle, D. W. House, and J. H. Finn, J. Chromatogr., 192, 143 (1980).
41. J. N. Kinkel in A. Subramanian, Ed., A Practical Approach to Chiral Separations by Liquid Chromatography, VHC, New York, 1994.
42. S. G. Allenmark, S. Andersson, P. Moller, and D. Sanchez, Chirality, 7,248 (1995).
43. M. Meurer, U. Altenhoner, J. Straube, and H. Schmidt-Traub, J. Chromatogr. A,769, 71–79 (1997).
44. M. Schulte, R. Ditz, R. M. Devant, J. N. Kinkel, and F. Charton, J. Chromatogr. A, 769, 93 (1997).

45. D. Y. Wang, F. Hanotte, C De Vos, and P. Clement, Eur. *J. Allerg.* and Clin. Immunol., *56, 339 (2001).*

46. E. J. Corey and C. J. Helal, Tetrahedron Lett., *37,4837* (1996).

47. D. A. Pflum, H. Scot Wilkinson, G. J. Tanoury, D. W. Kessler, H. B. Kraus, C. H. Senanayake, and S. A. Wald, Org. Process Res. Dev., *5, 110 (2001).*

48. E. R. Francotte and P. Richert, *J.* Chromatogr. A, *769, 101* (1997); M. Negawa and F. Shoji, *J.* Chromatogr., *590, 113 (1992).*

49. G. Ganetsos, P. E. Barker, J. A. Johnson, R. G. Kabza, K. Hashimoto, S. Adachi, Y. Shirai, M. Morishita, B. Balannec, G. Hotier, and H. Makai in G. Ganetsos and P. E. Barker, Eds., Preparative and Production Scale Chromatography, chaps. *11–15,* Marcel Dekker, New York, *1993,* p. *233;* D. B. Broughton and C. G. Gerhold, inventors, Universal Oil Prod. Co., assignee, US patent *2,985,589,* May *23, 1961.*

50. D. M. Ruthven, Principles of Adsorption and Adsorption Processes, chap. *12,* Wiley, New York, *1984,* p. *380.*

51. E. R. Francotte, Chim. Nouvelle, *53, 1541 (1996).*

52. D. W. Guest, *J.* Chromatogr. A, *760, 159* (1997).

53. M. Schulte and J. Strube, *J.* Chromatgr. A, *906, 399 (2001).*

54. K. E. Goeringer, B. K. Logan, and G. D. Christian, *J.* Anal. Toxicol. *21, 529 (1997).*

55. E. Cavoy, M.-F. Deltent, *S.* Lehoucq, and D. Miggiano, *J.* Chromatogr. A, **769**, 49 *(1997).*

56. H. Lorenz, P. Sheehan, and A. Seidel-Morgenstern, *J.* Chromatogr. A, *908,201 (2001).*

57. J. Jacques, A. Collet, and S. H. Wilen, *Enantiomers,* Racemates and Resolutions, Krieger: Malabar, Florida, *1994.*

58. A. Collet, *M.* J. Brienne, and J. Jacques, Chem. Rev., *80,215 (1980).*

59. S. H. Wilen in E. L. Eliel, Ed., Tables *of Resolv*ing Agents and Optical Resolutions, University of Notre Dame Press, Notre Dame, IN, *1972;* P. Newman, Optical Resolution Procedures for Chemical Compounds, vol. 1-3, Optical Resolution Center, Manhattan College, New York, *1978–1984.*

60. M. J. Cannarsa, *Chimica Oggi*, **17**, 28 *(1999).*

61. M. Prashad, D. Har, O. Repic, T. J. Blacklock, and P. Giannousis, Tetrahedron Asymmetry, *10,3111 (1999).*

62. R. A. Maxwell, E. Chaplin, S. B. Eckhardt, J. R. Soares, and G. Hite, *J.* Pharmacol. Exp. Ther, *173,158 (1970).*

63. S. Faulconbridge, *H.* S. Zavareh, G. R. Evans, and M. Langston, inventors, Medeva Europe Ltd. (GB), assignee, World patent WO98/*25902,* June *18, 1998.*

64. A. *S.* C. Chan, Chemtech., **3**, 46 *(1993).*

65. *P.* J. Harrington and E. Loderwijk, Org. Process Res. Dev., *1, 72* (1997).

66. W. J. Pope and S. J. Peachey, *J.* Chem. *Soc., 75, 1066* (1899).

67. R. Gristwood, H. Bardsley, H. Baker, and J. Dickens, *J.* Exp. Opin. Invest. Drugs, *3, 1209* (1994).

68. M. Langston, U. C. Dyer, G. A. C. Frampton, G. Hutton, C. J. Lock, B. M. Skead, M. Woods, and H. Zavareh, Org. Process Res. Dev., *4, 530 (2000).*

69. B. A. Astleford and L. O. Weigel in A. N. Collins, G. N. Sheldrake, and J. Crosby, Eds., Chiralty in Industry I, chap. *6,* Wiley, New York, *1997,* p. *99.*

70. K. Flick and E. Frankus, inventors, Gruenenthal Chemie, assignee, US patent *3,652,589,* March *28, 1972.*

71. G. R. Evans, inventor, Darwin Discovery Ltd., *(US)* assignee, World Patent WO00/32554, June *8,2000;* G. R. Evans, J. A. Henshilwood, and J. O'Rourke, Tetrahedron Asymmetry, 12, *1663* (2001).

72. L. G. Humber, *Med.* Res. Rev., **7**, 1 *(1987).*

73. *L.* G. Humber, *J.* Med. Chem., *29,871* (1986).

74. M. Woods, *U.* C. Dyer, J. F. Andrews, C. N. Morfitt, R. Valentine, and J. Sanderson, Org. Process Res. Dev., *4,418 (2000).*

75. D. Askin, *K.* K. Eng, R. M. Purick, K. M. Wells, R. P. Volante, and P. J. Reider, Tetrahedron Lett., *35,673 (1994).*

76. M. Kottenhahn, *K.* Stingl, and K. Drauz, inventors, Degussa (DE), assignee, US patent *6,093,823,* July *25,2000.*

77. M. Eichelbaum, Federation. Proc., *43, 2298 (1984);* M. Eichelbaum, Biochem. Pharmacol., **37**, 93 (1988).

78. H. Echizen, T. Brecht, S. Neidergesass, B. Volgelgesang, and M. Eichelbaum, Am. Heart *J.* **109**, 210 (1985).

79. O. Ehrmann, H. Nagel, and W. Karau, inventors, Knoll Aq (DE), assignee, US patent *5,457,224,* October *10, 1995* and World patent WO94/08950, April *14,1994.*

80. E. J. Trieber, M. Raschack, and F. Dengel, inventors, Knoll Aq (DE), assignee, German patent *2059923,1972.*

81. R. M. Bannister, M. H. Brookes, G. R. Evans, R. B. Katz, and N. D. Tyrrell, *Org.* Process Res. Dev., *4,467 (2000).*

82. P. E. Graves, H. A. Salhanick, *Endocrinology*, **105, 52 (1979).**

83. See ref. **57,** pp. **299–301,382–383.**

84. N. Finch, R. Dziemian, J. Cohen, and B. G. Steinetz, Experientia, **31, 1002 (1975).**

85. M. J. Bunegar, U. C. Dyer, G. R. Evans, R. P. Hewitt, S. W. Jones, N. Henderson, C. J. Richard, S. Sivaprasad, B. M. Skead, M. A. Stark, and E. Teale, *Org*. Process Res. Dev., **3, 442** (1999).

86. See ref. **57,** pp. **374–375.**

87. K. P. Datla and G. Curzon, *Neuropharmacol*ogy, **35, 315 (1996);** S. Salvadori, C. Bianchi, L. H. Lazurus, V. Scaranari, M. Attila, and R. Tomatis, J. Med. Chem., **35,4651** (1992).

88. C. A. Maryanoff, L. Scott, R. D. Shah, and F. J. Villani Jr., Tetrahedron Asymmetry, **9, 3247** (1998).

89. A. Bruggink in A. N. Collins, G. N. Sheldrake, and J. Crosby, Eds., Chirality in Industry 11, chap. **5,** Wiley, New York, **1997,** p. **81.**

90. T. Vries, H. Wynberg, E. van Echten, J. Koek, W. ten Hoeve, R. M. Kellogg, Q. B. Broxterman, A. Minnaard, B. Kaptien, S. van der Sluis, L. Hulshof, and J. Kooistra, *Angew*. Chem. Int. Ed., **37,2349 (1998).**

91. E. Ebbers, G. J. A. Arianns, B. Zwanenburg, and A. Bruggink, Tetrahedron Asymmetry, 9, **2745 (1998);** U. C. Dyer, D. A. Henderson, and M. B. Mitchell, Org. Process Res. Dev., **3, 161** (1999).

92. S. H. Wilen, A. Collet, and J. Jacques, Tetrahedron, **33,2725 (1977).**

93. Z. J. Li, M. T. Zell, E. J. Munson, and D. J. W. Grant, J. Pharm. Sci., **88,337 (1999).**

94. R. Tamura, T. Ushio, H. Takahashi, K. Nakamura, N. Azuma, F. Toda, and K. Endo, Chirality, **9, 220 (1997).**

95. H. M. L. Davies, T. Hansen, D. W. Hopper, and S. A. Panaro, *J*. Am. Chem. *Soc.*, **121, 6509 (1999).**

96. J. M. Axten, R. Ivy, L. Krim, and J. D. Winkler, J. Am. Chem. *Soc.*, **121,6511 (1999).**

97. A. Robinson, H. Y. Li, and J. Feaster, Tetrahedron Lett., **37,8321 (1996).**

98. W. Goppel, W. Betram, Psychiatr. *Neurol*. Med. Psychol., **23, 712 (1971).**

99. H. A. M. Mucke, Drugs Future, **33,259 (1997).**

100. D. H. R. Barton and G. W. Kirby, J. Chem. *Soc.*, **806 (1962).**

101. W. Shieh and J. A. Carlson, J. *Org*. Chem., **59, 5463 (1994).**

102. B. Kuenburg, L. Czollner, J. Frohlich, and U. Jordis, Org. Process Res. Dev., **3,425 (1999).**

103. G. L. Plosker and K. L. Goa, Drugs, **42, 805 (1991).**

104. H. Harada, T. Morie, Y. Hirokawa, and S. Kato, Tetrahedron Asymmetry, **8,2367 (1997).**

105. R. M. Williams in J. E. Baldwin and P. D. Magnus, Eds., Synthesis of Optically Active *'61*-Amino Acids, vol. **7,** Pergamon Press, Oxford, UK **1989;** R. O. Duthaler, Tetrahedron, **50, 1539 (1994).**

106. T. Shiraiwa, H. Miyazaki, T. Watanabe, and H. Kurokawa, Chirality, **9,** 48 **(1997).**

107. T. Shiraiwa, H. Miyazaki, A. Ohta, K. Motonaka, E. Kobayashi, M. Kubo, and H. Kurokawa, Chirality, **9, 656 (1997).**

108. K. Abe, H. Inoue, and T. Nagao, Yakugaku *Zasshi*, **108, 716** (1988).

109. S. Yamada, K. Morimatsu, R. Yoshioka, Y. Ozaki, and H. Seko, Tetrahedron Asymmetry, **9, 1713 (1998).**

110. E. K. Rowinsky, L. A. Cazenav, and R. C. Donehower, J. Natl. Cancer *Inst.*, **82,1247 (1990).**

111. J. N. Denis, A. E. Green, D. Guenard, F. Gueritte-Voegelein, L. Mangatal, and P. Potier, J. Am. Chem. *Soc.*, **110, 5917 (1988).**

112. J. Kearns and M. M. Kayser, Tetrahedron Lett., **35, 2845 (1994).**

113. R. P. Srivastava, J. K. Zjawiony, J. R. Peterson, and J. D. McChesney, Tetrahedron Asymmetry, **5, 1683 (1994).**

114. R. N. Patel, Adv. *Appl*. Microbiol., **47, 33** (2000); V. Gotor, Biocat and Biotrans **18, 87 (2000);** W. A. Loughlin, Bioresource Technology, **74, 49 (2000);** S. M. Roberts, *J*. Chem. *Soc., Perkin* Trans., **1, 1 (1999).**

115. C.-H. Wong and G. M. Whitesides, Enzymes in Synthetic Organic Chemistry, Pergamon, New York, **1994.**

116. A. D. Baxter, J. B. Bird, R. Bannister, R. Bhogal, D. T. Manallack, R. W. Watson, D. A. Owen, J. Montana, J. Henshilwood, and R. C. Jackson in N. Clendeninn and K. Appelt, Eds., Matrix Metalloproteinase Inhibitors in Cancer Therapy, Humana Press, Totawa, NJ, **2000,** pp. **193–221.**

117. N. L. St. Clair and N. L. Nathrough, J. Am. Chem. *Soc.*, **114,7314 (1992);** N. Khalaf, C. P. Govardan, J. J. Lalonde, R. A. Persichetti, Y. F. Wang, and A. L. Margolin, J. Am. Chem. *Soc.*, **118, 5495 (1996).**

118. A. M. Evans, J. *Clin*. Pharmacol.36 (Suppl 12), 7S **(1996).**

119. *S.-W. Tsai and* H.-J. *Wei, Enzyme Microb. Technol.*, 16, 328(1994).

120. *J.-Y. Xin, S.-B. Li, Y. Xy, J.-R. Chui; and C.-G. Xi, J. Chem. Tech. Biotechnol.*, 76, 579(2001).

121. *M. E. Swarbrick, F. Gosselin, and W. D. Lubell, J. Org. Chem.*, 64, 1993-2002(1999).

122. *W. L. Nelson and T. R. Burke, J. Org. Chem.*, 43, 3641(1978).

123. *R. M.* Patel, *Stereosel. Biocatal., Marcel* Dekker, *Inc., New York*, 2000, *pp.* 87-130.

124. *E. P. Siqueira Fihlo, J. A. R.* Rodrigues, *and P. J. S. Moran, Tetrahedron Asymmetry*, 12, 847(2001).

125. *P. A. Procopiou, G. E. Morton, M. Todd, and G. Webb, Tetrahedron Asymmetry*, 12, 2005 (2001).

126. *T. P. Jerusi, inventor,* Sepracor *Inc. (US), assignee, World patent* WO99/13867, *March* 25, 1999.

127. *J. D. Morrison, Ed., Asymmetric Synthesis, Academic Press, San* Diego, *CA*, 1983; *G. Procter, Asymmetric Synthesis, Oxford University Press, Oxford, UK*, 1996; *M.* Nógrádi, *Stereoselective Synthesis, 2nd ed., VCH, Weinheim, Germany*, 1995; *D. J. Ager and M. L. East, Eds., Asymmetric Synthetic Methodology, CRC Press, Boca* Raton, *FL*, 1995; *D. J. Ager, Ed., Handbook of Chiral Chemicals, Marcel* Dekker, *New York*, 1999; *P.* O'Brien, *J. Chem. Soc.*, 1, 95–113 (2001); *H.* Tye *and P. J. Comino, J. Chem. Soc.*, 1, 1729-1747 (2001); *P. I. Dalko and L. Moisan, Chem. Int. Ed.*, 40, 3726–3748 (2001); K. *C. Nicolaou and E. J. Sorensen, Classics in Total Synthesis, VCH,* Basel, *Switzerland*, 1996.

128. *S. Redshaw in C. R. Ganellin and S. M. Roberts, Eds., Medicinal Chemistry,* 2nd *ed., Academic Press, San* Diego, 1993, *pp.* 163-186.

129. *A. A. Patchett, E. Harris, E. W. Tristram, M. J.* Wyvratt, *M. T. Wu, D. Taub, E. R. Peterson, T. J. Ikeler, J. ten Broeke, L. G. Payne, D. L. Ondeyka, E. D. Thorsett, W. J. Greenlee, N. S. Lohr, R. D. Hoffsommer, H. Joshua, W. V. Ruyle, J. W. Rothrock, S. D. Aster, A. L.* Maycock, *F. M. Robinson, R. Hirschmann, C. S. Sweet, E. H. Ulm, D. M. Gross, T. C. Vassil, and C. A. Stone, Nature*, 288, 280 (1980).

130. *T. J. Blacklock, R. F. Shuman, J. W. Butcher, W. E.* Shearin, *J. Budavari, and V. J. J. Grenda, J. Org. Chem.*, 53, 836 (1988).

131. *T.* Ohashi *and J. Hasegawa in A. N. Collins, G. N. Sheldrake, and J. Crosby, Eds., Chirality in Industry II: Developments in the Commercial Manufacture and Applications of Optically Active Compounds, Wiley, Chichester, UK*, 1997, *p.* 269.

132. *M. R. Attwood, C. H. Hassall, A.* Krohn, *G.* Lawton, *and S. Redshaw, J. Chem. Soc.*, 1, 1011-1019 (1986).

133. *E. J. Stoner, A. J. Cooper, D. A.* Dickman, *L.* Kolaczkowski, *J. E. Lallaman, J.-H. Liu, P. A. Oliver-Shaffer, K. M.* Patel, *J. B. Paterson Jr., D. J. Plata, D. A. Riley, H. L. Sham, P. J.* Stengel, *and J.-H. J.* Tien, *Org. Process Res. Dev.*, 4, 264-269 (2000).

134. *J. T. Kovalainen, J. A. M. Christiaans, S.* Kotisaari, *J. T. Laitinen, P. T. Mannisto, L. Tuomisto, and J. Gynther, J. Med. Chem.*, 42, 1193-1202 (1999).

135. *G. M. Coppola and H. F. Schuster, Chiral a -Hydroxy Acids in Enantioselective Syntheses, VCH, Weinheim, Germany*, 1997.

136. G. *Wang and R. Hollinsworth, J. Org. Chem.*, 64, 1036-1038(1999).

137. *G. G. Wu, Org. Process Res. Dev.*, 4, 298–300 (2000); *G. Wu, Y. S. Wong, X. Chen, and Z. Ding, J. Org. Chem.*, 64, 3714–3718 (1999).

138. D. *S.* Garvey, *J. T. Wasicak, M. W. Decker, J. D. Brioni, M. J. Buckley, J. P. Sullivan, G. M.* Carrera, *M. W. Holladay, S. P.* Arneric, *and M. Williams, J. Med. Chem.*, 37, 1055–1059 (1994).

139. *N.-H. Ling, Y. He, and H.* Kopecka, *Tetrahedron Lett.* 36, 2563-2566(1995).

140. *D. J. Ager, I.* Prakash, *and D. R. Schaad, Chem. Rev.*, 96, 835-875(1996).

141. *D. A. Evans, J. M. Takacs, L. R.* McGee, *D. J.* Mathre, *and J.* Bartroli, *Pure Appl. Chem.*, 53, 1109 (1981); *D. A. Evans, M. D. Ennis, and D. J.* Mathre, *J.* Am. *Chem. Soc.*, 104, 1737 (1982); D. *A. Evans, Aldrichimica Acta*, 15, 23 (1982).

142. *D.* R. *Schaad in ref.* 127, *pp.* 287–300.

143. *S. J. Wittenberger and M. A.* McLaughlin, *Tetrahedron Lett.*, 40, 7175-7178 (1999).

144. *S.* R. *Turner, J. W. Strohbach, R. A. Tommasi, P. A. Aristoff, P. D. Johnson, H. I. Skulnick, L. A. Dolak, E. P.* Seest, *P. K. Tomich, M. J. Bohanon, M.-M.* Horng, *J. C. Lynn, K. T. Chong, R. R. Hinshaw, K. D. Watenpaugh, M. N.* Janakiraman, *and S. Thaisrivongs, J. Med. Chem.*, 41, 3467-3476(1998).

145. *T. M. Judge, G. Phillips, J. K. Morris, K. D. Lovasz, K. R. Romines, G. P. Luke, J.* Tulinsky, *J. M. Tustin, R. A. Chrusciel, L. A. Dolak, S. A.* Mizsak, *W. Watt, J. Morris, S. L. Vander Velde, J. W. Strohbach, and R. B. Gammill, J. Am. Chem. Soc.*, 119, 3627-3628(1997).

146. N. Cabedo, I. Andreu, M. C. Ramírez de Arellano, A. Chagraoui, A. Serrano, A. Bermejo, P. Protais, and D. Cortes, *J. Med. Chem.*, **44, 1794-1801** (2001).

147. S. T. Moe, D. L. Smith, E. G. DelMar, S. M. Shimizu, B. C. Van Wagenen, M. F. Balandrin, Y. Chien, J. L. Raszkiewicz, L. D. Artman, H. S. White, and A. L. Mueller, ***Bioorg. Med. Chem. Lett.*, 10, 2411-2415(2000).**

148. M. D. Goodyear, P. O. Dwyer, M. L. Hill, A. J. Whitehead, R. Hornby, and P. Hallett, inventors, Glaxo Group Ltd. (GB), assignee, World patent **WO-09529174,** April **18, 1995.**

149. M. P. DeNinno, R. Schoenleber, R. J. Perner, L. Lijewski, K. E. Asin, D. R. Britton, R. MacKenzie, and J. W. Kebabian, *J. Med. Chem.*, **34, 2561-2569** (1991).

150. P. Hewawasam, N. A. Meanwell, V. K. Gribkoff, S. I. Dworetzky, and C. G. Boissard, ***Bioorg. Med. Chem. Lett.*, 7, 1255-1260(1997).**

151. F. A. Davis and B.-C. Chen, *Chem. Rev.*, **92, 919 (1992).**

152. M. K. O'Brien and B. Vanase, *Curr. Opin. Drug Discovery Dev.*, **3, 793-806 (2000);** R. A. Sheldon, Ed., *Chirotechnology: Industrial Synthesis of Optically Active Compounds*, Marcel Dekker, New York, **1993.**

153. E. J. Corey, R. K. Bakshi, and S. Shibita, *J. Am. Chem. Soc.* **109, 5551(1987);** E. J. Corey, R. K. Bakshi, S. Shibita, C.-P. Chen, and V. K. Singh, *J. Am. Chem. Soc.*, **109, 7925 (1987).**

154. E. J. Corey and J. O. Link, ***Tetrahedron Lett.*, 31, 601 (1990);** E. J. Corey and J. O. Link, *J. Org. Chem.*, **56, 442(1991).**

155. Y.-J. Shi, D. Cai, U.-H. Dolling, A. W. Douglas, D. M. Tschaen, and T. R. Verhoeven, ***Tetrahedron Lett.*, 35, 6409-6412(1994).**

156. M. J. Burk, F. Bienewald, S. Challenger, A. Derrick, and J. A. Ramsden, *J. Org. Chem.*, **64,** 3290–3298 **(1999).**

157. H. C. Kolb, M. S. VanNieuwenhze, and K. B. Sharpless, *Chem. Rev.*, **94, 2483(1994).**

158. R. Shirai, H. Takayama, A. Nishikawa, Y. Koiso, and Y. Hashimoto, ***Bioorg. Med. Chem. Lett.*, 8, 1997-2000** (1998).

159. A. E. Fenwick, A. D. Gribble, R. J. Ife, N. Stevens, and J. Witherington, ***Biorg. Med. Chem. Lett.*, 11, 199-202** (2001).

160. L. E. Martinez, J. L. Leighton, D. H. Carsten, and E. N. Jacobsen, *J. Am. Chem. Soc.*, **117, 5897 (1995);** S. E. Schaus, J. F. Larrow, and E. N. Jacobsen, *J. Org. Chem.*, **62, 4197-4199 (1997).**

161. S. E. Zook, J. K. Busse, and B. C. Borer, ***Tetrahedron Lett.*, 41, 7017-7021** (2000).

162. J. F. Larrow, S. E. Schaus, and E. N. Jacobsen, *J. Am. Chem. Soc.*, **118, 7420-7421 (1996);** S. E. Schaus and E. N. Jacobsen, ***Tetrahedron Lett.*, 37, 7937-7940** (1996).

163. C.-C. Tsai, K. E. Follis, A. Sabo, T. W. Beck, R. F. Grant, N. Bischofberger, R. E. Benveniste, and R. Black, ***Science*, 270, 1197-1199 (1995).**

164. M. Tokunaga, J. F. Larrow, F. Kakiuchi, and E. N. Jacobsen, ***Science*, 277, 936** (1997).

165. J. M. Keith, J. F. Larrow, and E. N. Jacobsen, *Adv. Synth. Catal.*, **343**, 5–26 (2001).

166. J. H. Namyslo and D. E. Kaufmann, *Synlett.*, **804-806 (1999).**

167. S. J. C. Taylor, K. E. Holt, R. C. Brown, P. A. Keene, and I. N. Taylor in R. N. Patel, Ed., ***Stereoselective Biocatalysis,*** Marcel Dekker, New York, **2000,** p. **397.**