Chiral Recognition Mechanisms

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"Most natural organic products, the essential products of life, are asymmetric and possess such asymmetry that they are not superimposable on their image. This establishes perhaps the only well marked line of demarcation that can at present be drawn between the chemistry of dead matter and the chemistry of living matter."

—LOUIS PASTEUR (*1*)

In the middle of the 19th century, Louis Pasteur manually separated the two mirror-image forms of crystallized sodium ammonium tartrate, and interest in stereochemistry ensued (see the art on this page). The word chiral comes from the Greek *cheir*, meaning "the hand". Lord Kelvin first defined chirality in 1904. He said that "any geometrical figure, or group of points," is chiral and has chirality "if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself" (*2*). In 1858, Pasteur stated that interest in molecular chirality came from biological studies. Indeed, living organisms are composed of many chiral biomolecules, such as L-amino acids, D-sugars, proteins, and nucleic acids.

Because of this natural asymmetry, chiral compounds exhibit different properties in biochemical systems, even though they are indistinguishable in most inanimate environments. Two molecules that are mirror images of each other are called an enantiomeric pair, and they have exactly the same physicochemical properties in all isotropic conditions. Because biochemical systems are not isotropic, two enantiomers of a chirally active drug may have dramatically different pharmacologic effects. This is the basis for enantioseparations and for all chiral recognitions: enantiomeric separation always implies interaction with a pure chiral compound, the selector.

A complete understanding of the chiral recognition mechanism, which has not yet been realized, would allow researchers to predict which selector would best separate the enantiomers of chiral compounds. Chiral recognition mechanisms can be studied most effectively when the exact structure of the chiral selector is known, especially for smaller selectors. Unfortunately, most derivatized macromolecules and polymers have little-known structures. However, even with small selectors, the beautiful LC molecular modeling studies of chiral molecule–selector association explain a particular enantioseparation after the fact; they have no predictive value, because they do not account for critical solvent effects.

One common form of molecular chirality is due to the stereogenic centers of sp^3 hybridized carbon atoms that bear four different substituents, as in the case of D-(–)-lactic acid (Figure 1a). Other causes of molecular asymmetry (Figures 1c and 1d) are steric hindrances (e.g., ortho-substituted biaryls) and chiral molecular strain, which is found in substituted binaphthols, helicenes, and natural polymers (e.g., cellulose). A chiral axis is present in substituted allenes with three adjacent sp^2 hybridized carbon atoms (Figure 1b). This article will present an overview of the state of chiral separations.

Three-point (minimum) interaction model

The key step in chiral recognition is the formation of diastereoisomeric complexes between the enantiomers and a chiral selector. Molecular recognition results because of the differences in Gibbs free energy between the two diastereoisomeric enantiomer–selector complexes. Biologists were naturally the first to be interested in chiral recognition mechanisms. In 1933, Easson and Stedman were working on quantitative structure–activity relationships when they proposed that a minimum of three points of attachment were needed between a dissymmetric drug and its target to explain the different physiological activities (*3*). Fifteen years later, Ogston (another biologist) used the three-point model in his work on chiral enzymatic reactions (*4*). Dagliesh later adapted it to TLC (*5*). The model explains the differential binding of the two enantiomers to a chiral three-point site on the selector. Figure 2a shows that one enantiomer can present three substituents to match the selector's three-point site. No matter how its mirror image rotates, the enantiomer can match a maximum of only two sites (Figure 2b).

In the original three-point model, the interactions at all of the sites were attractions. From a modern separations point of view, repulsion and attraction are opposites. However, from a stereochemical point of view, repulsion is considered as productive an interaction as attraction. For example, two of the interactions can be repulsive if the third interaction is strong enough to promote the formation of at least one of the two possible diastereoisomeric selector–ligand complexes (*6*). If the three interactions are all attractive, then the enantiomer in Figure 2a will necessarily be more tightly bound to the receptor than the enantiomer in Figure 2b. The key points in the three-point interaction model are that at least three simultaneous interactions are required and that they should occur with three different substituents attached to the stereogenic center. Two different interactions with the same substituent increase only the selector–ligand binding energy, not the chiral differentiation efficiency.

Although widely accepted, the model was recently challenged (*7*, *8*). In the case of D- and L-isocitrate binding at the active site of isocitrate dehydrogenase, studies established that all four substituents of the asymmetric carbon atom were used (*7*). The researchers presented numerous cases in which three points of interaction were not required (*6*, *8*). For example, the π-complex selectors use large and rigid aromatic associations that may discriminate between two similarly rigid chiral molecules through a pseudo-two-point interaction model (*6*). The researchers pointed out that the three-point interaction model is only a geometrical model. When the π-complex selector involves a docking contact and an interaction with a line or a plane, this agrees with the idea of the three points of interaction because a line is geometrically defined by at least two points and a plane is defined by at least three points.

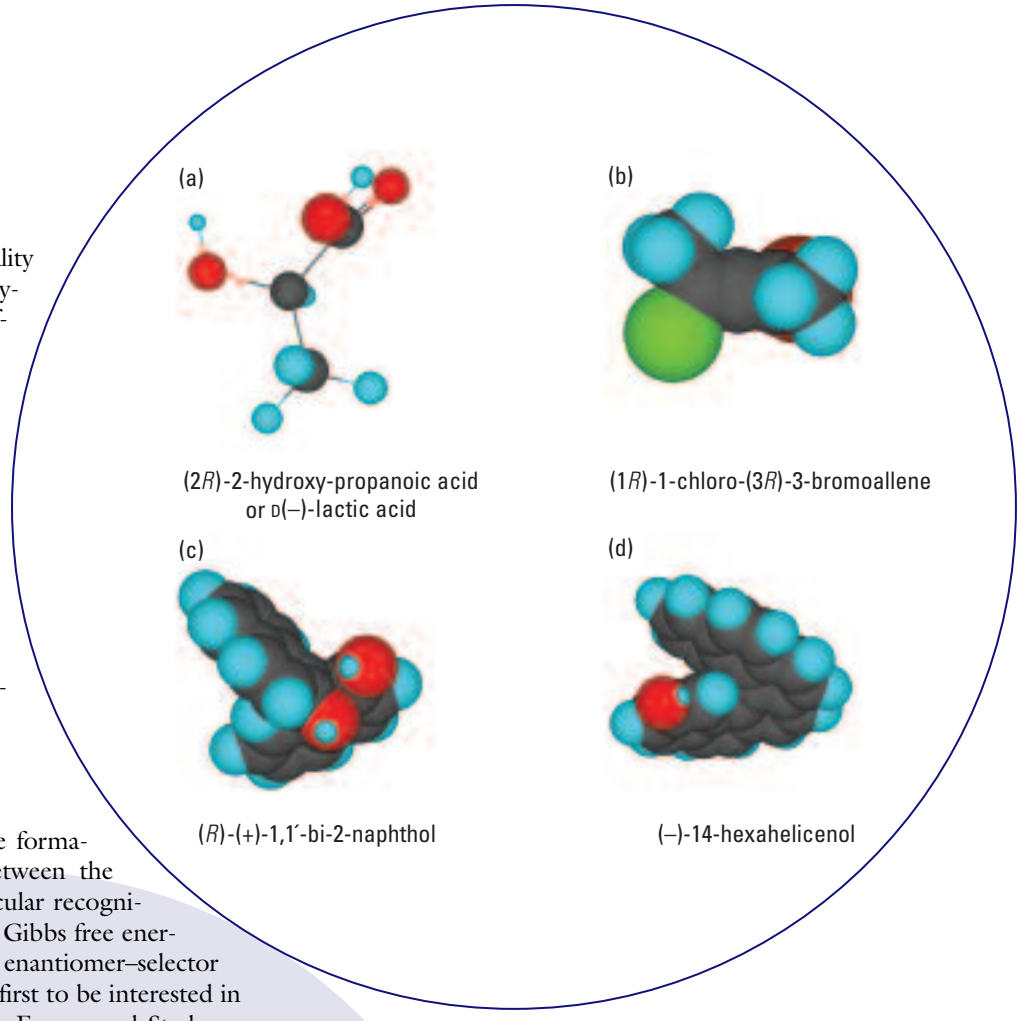


FIGURE 1. Chiral molecules.

(a) The sp^3 hybridized carbon atom that bears four different substituents is by far the most common asymmetric center. (b) The C=C=C allene arrangement forms a chiral axis. The 1-chloro-3-chloroallene would also be chiral. (c) Atropoisomerism occurs when the free rotation around a σ bond is hindered. (d) Steric hindrances create a chiral plane in helicenes.

Molecular interactions

Table 1 lists the intermolecular forces between two enantiomers and the chiral selector. The strongest interaction is obtained with coulomb force—for example, the high cohesion of salts. The hydrogen-bond interaction occurs between the positively polarized hydrogen atom of a hydroxyl or amine group and the negatively polarized oxygen or nitrogen atom of another hydroxyl or amine group. Hydrogen bonds are very strong because the negative site can come very close to the hydrogen atom that is depleted of any remaining repulsive electrons. Steric hindrances are due to the

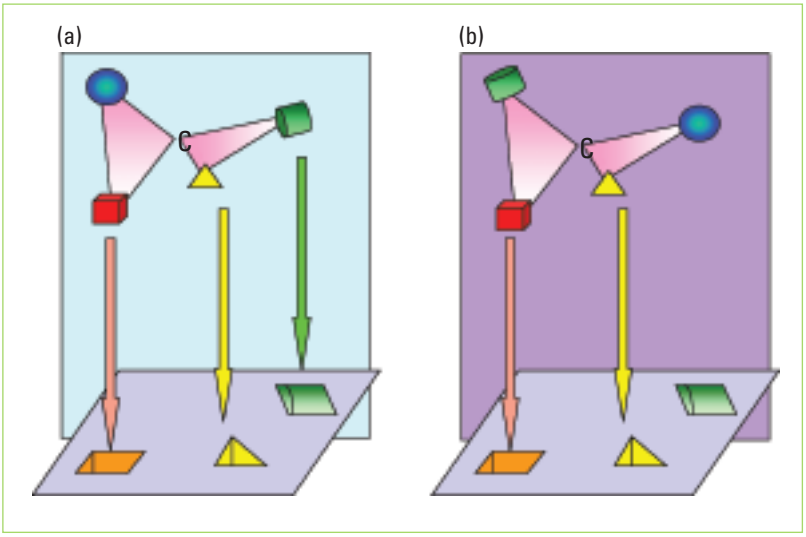


FIGURE 2. The three-point attachment model.

(a) A chiral molecule with an asymmetric carbon atom can present three groups that can match exactly three sites of the selector. (b) Its mirror image, after all possible rotations, can present a maximum of two groups able to interact with only two sites of the selector. The binding constant of the chiral molecule in (a) will be higher than that of its mirror image.

intrinsic room needed per atom or group of atoms; they are repulsive and very strong at very short range.

When π-electron molecular assemblies (mainly aromatic rings) interact with each other, π–π interactions are observed. Aromatic structures are said to be π-accepting, or π-acidic, when the ring has electron-rich substituents, mainly NO_2 groups. They are said to be π-donating, or π-basic, when the π-electron can delocalize, such as in a naphthyl group, or when electron-withdrawing substituents, such as methyl groups, are attached to the aromatic ring. The π–π interactions involved in chiral recognition mechanisms are most often attractive; a π-accepting group of the enantiomer interacts with a π-donating group of the selector, or vice versa.

Ion–dipole, dipole–dipole, and dipole–induced-dipole interactions occur with molecules that have a dipole moment. The strongest ion–dipole interaction involves the coulomb force between the ion and the partial charge of the dipolar molecule. It is always attractive because a permanent dipole structure combines a partial positive charge with an equal partial negative charge. For the same reason, the dipole–dipole interaction is also attractive, although it is weaker than the ion–dipole interaction. The weakest interaction is between a permanent dipolar molecule and a dipole induced by the electric field. The London dispersion forces are the weakest intermolecular forces. They are responsible for the hydrophobic effect and for entropy-driven forces that cause oil to separate from water.

Getting information on chiral recognition mechanisms

The quest for chiral selectors can be separated into the synthetic route and the natural route. The synthetic route evaluates the possible interactions (Table 1) and designs a selector that will interact differently with an enantiomeric form than with its mirror image. The natural route follows Pasteur and is based on the fact that the living world contains countless chiral selectors and produces pure enantiomers. Once chosen, a natural chiral selector is tested with its natural chiral target(s) and many other enantiomers, and the results are used to postulate chiral mechanisms. Actually, neither of these two classes of selectors is 100% pure. A semisynthetic class comes closest to the ideal, because many synthetic selectors are based on a natural molecule and many natural selectors are chemically modified to enhance their initial properties (Table 2).

Most information on chiral recognition mechanisms is obtained by measuring the binding energy of the two chiral-selector–enantiomer complexes. Spectroscopic methods can work with the chiral selector (in solid or in liquid state) associated with the ligand. Circular dichroism and optical rotatory dispersion are important methods for evaluating the structural properties of selector–ligand adducts (*9*). NMR can specifically investigate ^{1}H and ^{13}C atom position and differentiate one enantiomer from the other. X-ray crystallography is a powerful technique for investigating the absolute configuration of diastereoisomeric complexes, but only in the solid state. Fluorescence anisotropy is a polarization technique that measures the rotational motion of a fluorescent molecule or a molecule–selector complex in solution (*10*).

Separation methods use chiral selectors to partition the enantiomers. Multiple selector–ligand association–dissociation reactions occur between a mobile and a stationary phase. In chromatography, the selector is most often attached to the stationary

Table 1. Characteristics of molecular interactions.

Type of interaction	Strength	Direction	Range (d)
Coulomb or electric	Very strong	Attractive or repulsive	Medium ($1/d^2$)
Hydrogen bond	Very strong	Attractive	Long
Steric hindrance	Very strong	Repulsive	Very short
π–π	Strong	Attractive (donor or acceptor) or repulsive	Medium
Ion–dipole	Strong	Attractive	Short
Dipole–dipole	Intermediate	Attractive	Short ($1/d^3$)
Dipole–induced-dipole	Weak	Attractive ($1/d^6$)	Very short
London dispersion or van der Waals	Very weak	Attractive	Very short ($1/d^6$)

Table 2. Chiral selectors.

Selector	Mechanism	Primary interaction
Synthetic selectors		
Ligand exchange	Diastereoisomeric selector–metal-ion–analyte complex	Coulomb or ion–dipole
π-Complex	Transient three-point selector–analyte association	π–π
MIPs	Key-and-lock association	Selective shape interaction with the imprint
Chiral crown ethers	Inclusion complexation	Ion–dipole
Polymers	Diastereoisomeric selector–analyte complex	Hydrogen bond
Natural selectors		
Proteins	Multiple binding sites	Variable
Polysaccharides	Insertion into helical structures	Hydrogen bond, dipolar, or steric
CDs	Inclusion complexation	Hydrogen bond
Macrocyclic glycopeptides	Multiple binding sites	Variable
Cinchona alkaloids	Ion pairing	Coulomb

phase, to produce a chiral stationary phase (CSP). The enantiomers are introduced in the liquid, gas, or supercritical-fluid mobile phase. They move at slightly different speeds according to their binding constants with the chiral selector. In CE, no stationary phase actually exists; the charged chiral selector is added to the electrolyte and moves in the electric field according to its electrophoretic mobility to differentially bind the two enantiomers. The dissolved chiral selector can be treated as a pseudophase. The migration times of the enantiomers provide their binding constants.

Researchers can observe the thermodynamics of chiral mechanisms by varying study temperatures. The slope and intercept of the van't Hoff plots ($\log k$ vs $1/T$, where k is the enantiomer retention factor and T is absolute temperature) contain the enthalpy and entropy variations, respectively, of each enantiomer–selector global (chiral + achiral) interaction. A comparison of the values for the two enantiomers gives information on the chiral part of the interaction (*11*). The thermodynamic parameters, binding constant, and enthalpy or entropy changes correspond to the global ligand–chiral-selector association. Information concerning the enantioselective separation mechanism can sometimes be inferred by changing the experimental conditions in a controlled or sequential manner. The composition, pH, polarity, or ionic strength of the mobile phase can be modified, or a chemical group of the analyte or the selector (or both) can be substituted or derivatized (or both).

A statistical thermodynamic study of the CSP–enantiomer interaction demonstrated that the possible enantioselectivity factor α (the ratio of k_1 to k_2) was not significantly different when the three interactions involved were of comparable strength, or when one interaction dominated the two others. However, in the former case, $\ln \alpha$ should be a linear function of $1/T$; a departure from this van't Hoff behavior would suggest that multiple retention modes are competing (*12*).

Computer methods use chemical theory to establish chiral recognition mechanisms. Software computes the atom's coordinates and calculates the best molecular conformation that minimizes the energy between the chiral selector and the ligand. The resulting beautiful models of chiral-molecule–selector association are particularly useful in crystallography and GC. In LC, they may well explain a particular enantioseparation, but they often have no predictive ability because models ignore critical solvent effects in a particular interaction. Another approach is to compile many results and identify quantitative structure–retention relationships. This approach classifies experimental results, associating conditions, selectors, and enantiomeric pairs successfully separated; however, it does not yield much information on the chiral recognition mechanism (*13*). Nevertheless, such a database, used with probability rules and a statistical approach, has a very good predictive ability (*14*).

Mechanisms and CSPs

The interactions between molecules and the possible chiral selectors are known. Methods that give information on the selector–ligand associations exist. So, it should be possible to understand how the two stereoisomeric complexes form transiently between the enantiomeric ligands and the selector. The problem is that, because several simultaneous interactions are required to discriminate one enantiomer from the other, the selector–chiral-molecule association is never single and simple. All enantioselective chiral mechanisms involve a combination of interactions. The strongest one may be as important as the weakest one for enantiomer discrimination.

To complicate the situation, the critical selector–ligand interaction often is not pure, either. For example, a bulky naphthyl group may have a π-basic character in a given chiral recognition mechanism with a π-acid-containing ligand; on other occasions, the same naphthyl group may interact through steric hindrances with nonaromatic enantiomers. In solution, solvent molecules may completely change the nature of the solute–selector interaction. Water molecules may screen a static charge. Acetonitrile molecules may fill an apolar selector cavity. Solvent molecules are always present at a concentration far in excess of the analyte. However, they are forgotten too often when chiral mechanisms are described. GC, MS, and crystallography do not involve solvents; they allow direct investigation of the chiral recognition mechanism. Nevertheless, an easy chiral recognition mechanism is rare. However, the choice of selectors (Table 2) is mainly controlled by the strongest interaction between the chiral selector and the analyte.

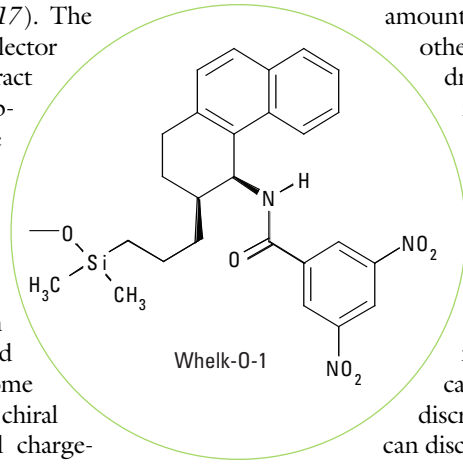
Information concerning the enantioselective separation mechanism can sometimes be inferred by changing the experimental conditions

The chiral ligand-exchange principle was established in the late 1960s (*15*). The basic mechanism involves a metal ion (most often, Cu^{2+}) at the core of a complex with the enantiomers and the chiral selector. For an acceptable chromatographic efficiency to be achieved, the complex must be kinetically labile, forming and dissociating at a high rate. The central metal ion has definite positions in its coordination sphere (six positions for Cu^{2+}), and each can be occupied by a lone electron pair of an organic group or a water molecule. The only chemical functional groups that meet these two requirements—lability and a lone electron pair—are amino, carboxy, hydroxy, amido, and thio derivatives, all of which bear at least one lone electron pair on the hetero atom. The chiral selector is an amino-acid derivative or other analogous chiral bidentate ligand.

Through its amino and carboxy groups, the chiral selector occupies two positions in the copper ion coordination sphere. Small water molecules occupy two positions, leaving two positions for the ligand. The enantiomer analytes must be able to form bidentate chelates, which are α- or β-amino acids, amino alcohols, hydroxyl acids, diamines, amino amides, and dicarboxylic acids. The two interactions described are necessary but not sufficient; the third interaction, required for chiral recognition, is provided by steric or dipole-type interaction with the selector. Bulky and/or rigid groups in the analyte situated close to the stereogenic center will greatly enhance the chiral recognition (*15*).

Molecular adjustment for three-point interaction

The π-donating and π-accepting chiral selectors were introduced in the late 1970s (*16*). Later, the (*R*)-*N*-(3,5-dinitrobenzoyl) phenyl glycine selector was specifically designed to have π-bonding capabilities (*17*). The dinitrobenzoyl group of the selector (which is a π-donator) can interact with an added π-accepting substituent of the enantiomer. The two other necessary interactions can be dipole stacking, hydrogen bonding, or steric repulsion. The concept was demonstrated when, in making the (S) version of the phenyl glycine selector, the elution order of the π-donator-substituted enantiomers was reversed (*18*). Some rigidity in the molecule enhances chiral recognition. The most successful charge-transfer selector at the moment, the Whelk-*O*-1, has two asymmetric centers that are part of a ring and two bonds with two bulky π-electron-rich (acidic and basic) substituents.



Whelk-O-1

Various CSPs

Key-and-lock recognition with MIPs. Molecularly imprinted polymers (MIPs) are prepared in a solvent solution with the pure enantiomer to be imprinted, a functional monomer (e.g., methacrylic acid), a cross-linker (e.g., ethylene glycol dimethacrylate), and an initiator [e.g., 2,2-azobis-(2-methylpropionitrile)]. The mixture is reacted for several hours at elevated temperature. The resultant bulk rigid polymer is ground into a sieved powder and the template enantiomer washed off. Knowing how the MIP was prepared makes it easy to ascertain the strength of the affinity for the enantiomer that served as the template. The interactions are mainly steric, and shape recognition associated with other interactions is solute-dependent (*19*). The drawback is that MIPs are too specific. They play no essential role in enantiomeric separations. They are limited by their poor capacity and the lability of the imprint to varying solvent conditions.

Crown ether host and chiral guest. Chiral crown ether selectors are derivatized forms of polyoxyethylene crown-6 (*20*). This crown ether has a cavity that exactly matches the size of the ionized primary amine group NH_3^+. The host–guest ammonium–crown-ether interaction, which is one point of attachment, is the driving force of the enantiomer with this class of chiral selector. The two other necessary interactions are steric and hydrophobic; they occur between the crown ether substituents and the host substituent. Chiral crown ether can only discriminate chiral molecules with a primary amine group at low pH.

Synthetic polymers. Helical polytriphenylmethyl methacrylate was the first synthetic chiral polymer able to separate a limited number of enantiomers (*21*). Recently a polymerized diacryloyl derivative of *trans*-1,2-diaminocyclohexane [(R, R) or (S, S)] bonded to silica gel in a thin layer was proposed as a new, fully synthetic LC CSP (*22*). This CSP could not resolve many enantiomeric pairs. However, when it could resolve a racemate, the amount that could be loaded was much larger than on most other CSPs; this means that it has many active sites. Hydrogen bonds were pivotal in the chiral recognition mechanism of this CSP. The enantioselectivity was adjusted by varying the methanol content in the organic mobile phase. Polysodium *N*-undecanoyl-L-leucinate and polysodium-L-leucyl-valinate are dipeptide polymers that form micelles and are useful in a broad range of micellar electrokinetic chromatography applications (*23*).

Proteins. Proteins were introduced early as natural chiral selectors (*24*). They are a logical choice because biomacromolecules are responsible for the chiral discrimination of drugs and nutrients in the body. Proteins can discriminate a wide spectrum of charged and neutral molecules. However, they may be difficult to use because small changes in the experimental conditions, pH, ionic strength, and added organic solvent may cancel the enantiorecognition. It is not possible to describe a simple mechanism, because a single protein may contain several sites that can act as chiral selectors. All listed interactions may be involved.

Polysaccharide selectors. Cellulose, amylase, and chitin are the most abundant optically active natural polymers. They can be

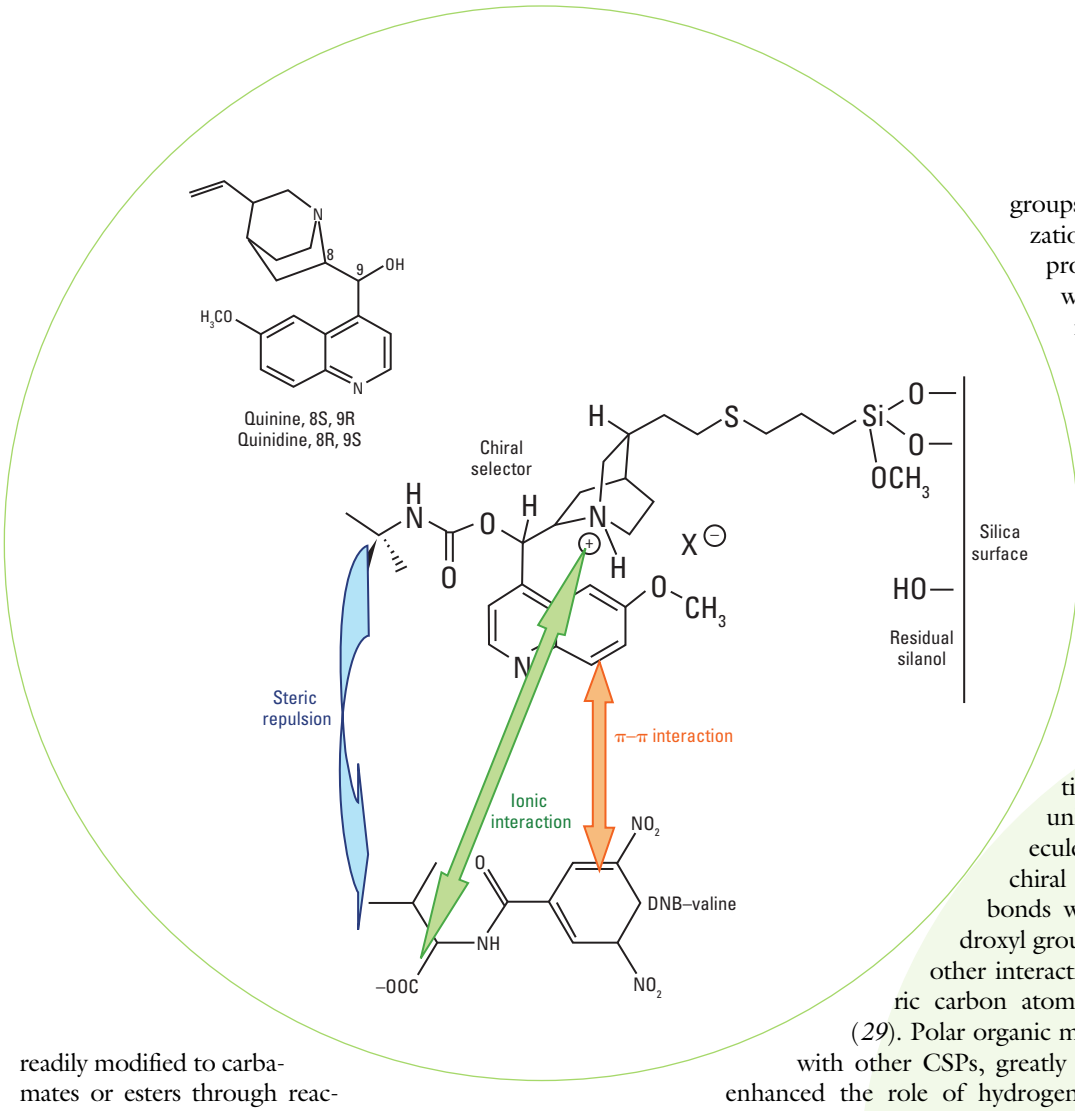


FIGURE 3. Chiral recognition mechanism by a quinine CSP.

The strongest interaction is the ionic docking attraction between opposite charges. DNB-D-valine is more retained by the quinine CSP than its L enantiomer is. DNB-L-valine is more retained by the quinidine CSP than its D enantiomer is.

readily modified to carbamates or esters through reactions with isocyanates or acid chlorides, respectively (*25*). These selectors are broadly discriminating because they have the advantage of chiral individual carbohydrate monomers and a long-range helical secondary structure that effects separations. The most popular selectors (Chiralcel OD, which is a cellulose, and Chiralpack AD, which is an amylose) are derivatized 3,5-dimethylphenyl carbamate (a π-donating or π-basic group). Therefore, π–π interactions will probably be part of the mechanism. However, these chiral polymers have many possible interaction sites. Therefore, many enantiomers can be discriminated (if three different points of interaction are found), but the mechanism can only be partially established.

Inclusion complexation. Cyclodextrins (CDs) are small cyclic polysaccharides that form a cone-shaped cavity with six, seven, or eight glucopyranose units for the α-, β-, or γ-CD, respectively. The interior of the cavity is rather nonpolar with ether groups, and the larger and smaller rims of the cavity are lined with polar primary and secondary hydroxyl groups, respectively. Inclusion complexation is the driving interaction in chiral recognition by CDs. Native CDs were proposed in the early 1980s as chiral selectors (*26*). Polar secondary interactions with the hydroxyl groups were predominant. Derivatization of these hydroxyl groups produced a wide variety of CDs with adjusted polarities and functionalities that can separate a broad spectrum of enantiomers (*27*). For example, naphthyl-ethyl carbamate-substituted CDs, associated π–π interactions, hydrogen bonds, and inclusion complexation widen the applicability of the selector (*28*).

Polar enantiomers can be separated with CDs in a nonaqueous polar medium (e.g., 99% acetonitrile with 1% methanol). In this situation, inclusion complexation is unlikely because the solvent molecules occupy the CD cavity. The chiral mechanism involves hydrogen bonds with the spatially oriented hydroxyl groups at the rims of the cavity and other interactions with numerous asymmetric carbon atoms of the glucopyranose units (*29*). Polar organic mobile phases, which were tried with other CSPs, greatly extended their usefulness and enhanced the role of hydrogen-bond interactions that were screened by water molecules.

Imitating bacteria. Armstrong et al. thought that macrocyclic antibiotics would be wonderful chiral selectors for amino acids because they inhibit the development of Gram-positive bacteria by blocking cell-wall development by binding to the D-Ala-D-Ala terminal of an essential protein (*30*). As expected, these selectors were the best ones for separating native amino acids, because the binding constant of the D form is significantly stronger than that of the L form (*31*). The critical role of the ionized carboxylic acid group was demonstrated. However, methylation of this group cancels all chiral recognition (*32*).

These selectors could do much more than amino acids because of their numerous active groups and many possible mechanisms. The most useful selectors—vancomycin, ristocetin, and especially teicoplanin—have similarities in their complex structures. They contain one or two charged sites, hydroxyl groups, aromatic rings, and polar (e.g., amido) and apolar (e.g., alkyl chain) groups. Thus, the types of interactions listed in Table 1 occur, although the mechanism can be difficult to ascertain (*33*). These three selectors show a complementary separation effect when used in LC. If a partial separation of a given pair of enantiomers is obtained with one glycopeptide, then chances are good that a full baseline separation can be obtained with one of the other two selectors. From a mechanistic point of view, this means that the stereo binding sites of these related selectors have subtle differences.

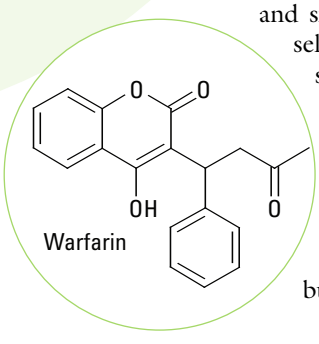
Cinchona alkaloids. Two cinchona alkaloid selectors can be used to delve into a particular chiral recognition mechanism. Quinine is a natural alkaloid extracted from the bark of the South American cinchona tree that is commonly used as an antimalarial drug (Figure 3). It has 8S and 9R configurations. Quinidine, the stereoisomer of quinine, is also found in cinchona bark; it has 8R and 9S configurations. These two alkaloids can easily be derivatized to prepare two useful CSPs with opposite configurations (*34*).

The active site that is responsive to the enantiomer separation can undergo an ionic reaction with quaternary ammonium, π–π interaction with the quinoline group, and dipole and hydrogen bonding or steric hindrance with the carbamoyl substituent. The quinine selector can separate the enantiomers of *N*-3,5-dinitrobenzoyl (DNB)-derivatized amino acids well. Docking is the ionic attraction between the negative carboxylate charge of the DNB amino acid and the positive ammonium group of the CSP. The DNB π-acidic group can then interact with the quinoline π-basic group of the CSP in the second attractive interaction. The third interaction is a repulsive steric hindrance between the bulky tertiobutyl substituent of the carbamate group on the CSP and the substituent of the amino acid (e.g., phenyl group for phenylalanine, isobutyl group for leucine, and methyl group for alanine; Figure 3).

In the case of DNB amino acids, the relevance of the mechanism was established by the following. Methyl esterification of the amino acid carboxylic group cancels all chiral recognition and makes docking impossible. The relationship between log α (α is the enantioselectivity factor) and log k_2 (k_2 is the retention factor of the most retained enantiomer) was found to depend on the size of the amino-acid side chain. The values of k_1 (the retention factor of the DNB amino acids that elute first) were similar to each other (*34*). For the same enantiomeric pairs, the values of α on the quinine and quinidine CSPs are similar; however, the elution order is opposite (*34*). Because these chiral selectors and their own naturally occurring stereoisomers are relatively simple molecules, the chiral recognition mechanism could be fully established in the case of amino-acid enantiomer separation. Most chiral selectors are complicated molecules that make prediction of the chiral recognition mechanism extremely difficult.

Making chiral recognition easier

Enantiomers that have a marked difference in the stereogenic center are always easier to separate. For example, the two enantiomers of warfarin, an anticoagulant whose asymmetric carbon atom has four different substituents that vary greatly in polarity and size, can be separated by many chiral selectors. However, it is a challenge to separate enantiomers, such as the those of 2-butanol or 2-chlorobutane. A common way to differentiate enantiomers is to derivatize them, then analyze them by GC on CD CSPs; this procedure can easily separate the enantiomers of the trifluoroacetyl 2-butanol derivative (*27*).



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