large discrepancies in selectivity between LC and SFC. This result is peculiar to cellulose and amylose-derived CSPs, for which the interactions involved in chiral recognition are not always well balanced. Therefore, in the case of chiral resolution of polar solutes, the analyst should try both LC and SFC so that the more stereoselective one can be chosen. **Figure 12**A–C show some examples of the different selectivities that may exist between LC and SFC for polymer-type CSPs.

Other polymer-type CSPs have been used in SFC, such as those based on polymethacrylates of helical conformation and a polysiloxane CSP (polyWhelk-O), the 'polymeric version' of the commercially available brush-type CSP, Whelk-O 1. For the latter, the comparison was performed between the polymeric CSPs and its brush-type analogue, and it appeared that the polyWhelk-O CSP affords greater enantioselectivity and shorter retention under the same conditions.

Conclusion

Chiral separation is one of the fields where SFC is recognized to have better characteristics than HPLC, both from a kinetic and sometimes thermodynamic point of view.

In general, SFC offers faster separations than LC and often better selectivity values (particularly with cellulosic and amylosic polymer-type chiral stationary phases, and also with brush-type CSPs in particular cases). Consequently, SFC should be considered as a powerful analytical tool for the separation of basic and acidic drugs.

Capillary columns should be chosen for the analysis of chiral compounds having a low or medium polarity. On the other hand, packed columns are preferred for analytes of high polarity for which a polar modifier must be added to the supercritical carbon dioxide mobile phase.

Currently, to meet the requirements of quality control laboratories, most analyses are performed with packed columns. This is mainly due to the progress in SFC instrumentation (full control over many chromatographic parameters and particularly full control of the pressure). Analysts are looking for the chiral column that is best able to achieve racemate separation easily and in a single run. This objective will probably never be achieved, but we can expect that the serial coupling of chiral columns (two or three) will allow some progress in this direction owing to the kinetic advantage exhibited by SFC over LC.

Further Reading

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Synthetic Multiple Interaction ('Pirkle') Stationary Phases

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Introduction

Among the many types of chiral stationary phases (CSPs) that have been developed, synthetic multiple interaction (Pirkle-type, or brush-type) CSPs have proven to be among the most useful for many liquid chromatographic enantiomer separations. These CSPs consist of an enantioenriched small molecule selector immobilized on an inert chromatographic support, typically silica gel. Separation is achieved when the two enantiomers of the analyte are differentially adsorbed by the CSP (**Figure 1**). A combination

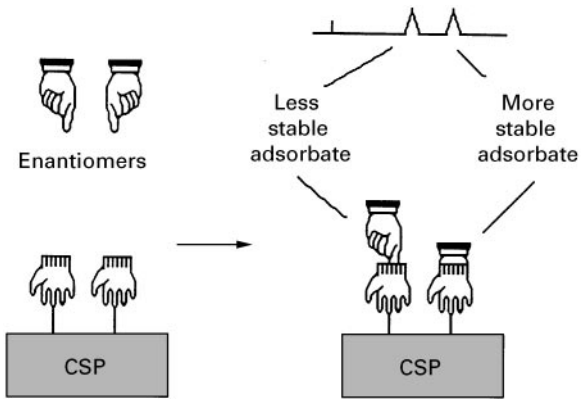


Figure 1 Enantiomer separation on CSPs is made possible by formation of transient diastereomeric adsorbates with differing free energies. In this illustration, the analyte enantiomers are depicted as right and left hands, and the CSP is depicted as immobilized right-handed gloves.

of simultaneous, geometrically constrained, intermolecular interactions utilizing forces such as hydrogen bonding, π-π attraction, ionic interactions, and steric repulsion can result in diastereomeric adsorbates with differing free energies, the prerequisite for enantioseparation.

Brush-type CSPs are just one of the several types of CSPs that have been developed to date. Other types include CSPs based on natural polymers such as polysaccharides or proteins, artificial polymers, and 'imprinted' polymers. Exactly what constitutes a brush-type CSP is a matter of some debate. In the broadest definition, CSPs based on immobilized cyclodextrins, antibiotics, etc. should perhaps be included in this category. However, this article will deal primarily with the synthetic CSPs developed by Professor William H Pirkle.

Background

Although Pirkle's name is strongly associated with the development of synthetic multiple interaction CSPs, several important materials of this type were developed by earlier researchers. Landmark events from the 1960s include the development of TAPA CSPs and chiral GC stationary phases. The 1970s brought a number of further advances, including Davankov's development of ligand exchange CSPs, Baczuk's pioneering development of a synthetic CSP designed specifically for the chromatographic separation of the enantiomers of a particular analyte molecule (DOPA), and Cram's development of chiral crown ether CSPs. During this same decade, Pirkle's investigations into the development of ^{1}H NMR chiral solvating agents led to the development of several useful brush-type CSPs.

Advantages of Brush-Type CSPs

In general, brush-type CSPs possess a number of advantages; since the selector is a small molecule, which is often completely synthetic, a structure which contains no labile or reactive components can usually be developed and the mode of attachment of the selector to the chromatographic support can be chosen for durability. Brush-type CSPs are typically covalently attached to the chromatographic support. Thus, most brush-type phases are chemically robust and are generally quite long-lived. Longevity is desirable for an analytical CSP, but truly essential for a preparative CSP, where continuous operation for several years may be required. The chemical robustness of brush-type CSPs results in the ability of these materials to be used with a wide variety of mobile phases, which provides greater flexibility in method development, especially when poorly soluble analytes are being investigated. The ability to utilize a variety of mobile phases is of even greater importance in preparative chromatography, where compound solubility can drastically limit enantioseparation productivity.

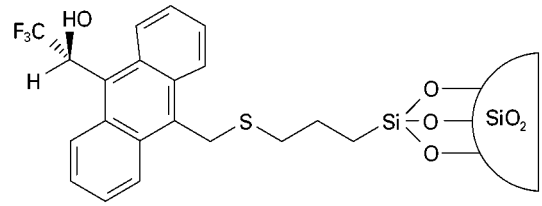
An additional advantage of brush-type phases stems from the fact that the selectors which are used are typically small molecules with molecular mass of less than 11000 Da. Consequently, the selectors can be very densely arrayed on the chromatographic surface, resulting in a phase which is highly resistant to sample overload and which has a very high preparative capacity.

Finally, most synthetic CSPs are available in either enantiomeric form. Consequently, either elution order (+ before − , or − before +) can be chosen. Elution of the minor enantiomer before the major is generally preferred in analysis, while elution of the desired component before the undesired can greatly increase productivity in preparative HPLC.

Survey of Some Important Pirkle-Type CSPs

The Beginnings: A Carbinol CSP

The Work on CSPs in the Pirkle laboratories grew out of studies on ^{1}H NMR chiral solvating agents. Immobilization of an analogue of the enantiopure chiral resolving reagent, trifluoromethyl-9-anthryl carbinol, afforded a CSP capable of separating the enantiomers of electron-deficient aromatic sulfoxides and many other racemates. Further studies showed that 3,5-dinitrobenzamide (DNB) derivatives of the enantiomers of a variety of amines, amino alcohols, amino acids and related compounds were separated with the following CSP structure [I].

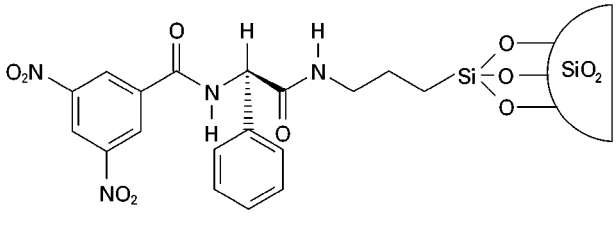


Structure [I]

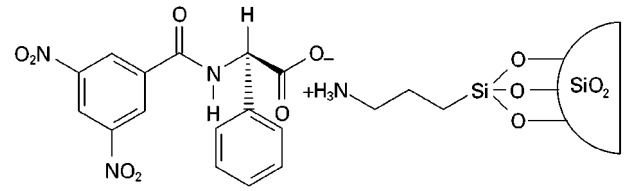
Principle of Reciprocity, and its Importance in CSP Development: Development of DNB Amino Acid CSPs

The observation that the enantiomers of some DNB derivatives are well resolved on CSP structure [I] prompted an investigation of the reciprocal situation. As a general rule, if a CSP is useful for separating the two enantiomers of an analyte, then a second CSP derived from one of the enantiomers of that analyte should be useful for separating the enantiomers of compounds which are structurally related to the chiral selector of the original CSP. This concept is known as the 'principle of reciprocity', and has been of great use in the development of new CSPs by Pirkle and his co-workers. The principle holds true generally, although the manner in which the chiral selector is tethered to the silica surface can influence chiral recognition in either a beneficial or detrimental fashion. After finding that several DNB phenylglycine-derived analytes were particularly well resolved on CSP structure [I], Pirkle and co-workers prepared and evaluated several phases derived from DNB phenylglycine and related compounds. The ready availability of enantiopure (*R*)-phenylglycine, which is utilized in the commercial production of an antibiotic, suggested that phenylglycine-derived CSPs might provide an economical and convenient method for resolving the enantiomers of the highly useful chiral solvating agent, trifluoromethyl-9-anthryl carbinol, and perhaps other alcohols as well.

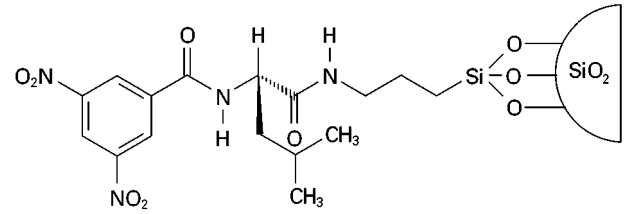
As expected, CSP structure [II] afforded resolution of the enantiomers of the trifluoromethyl-9-anthryl carbinol chiral solvating reagent as well as some related compounds. The related ionically bonded amino acid-derived CSPs structures [III] and [V] were



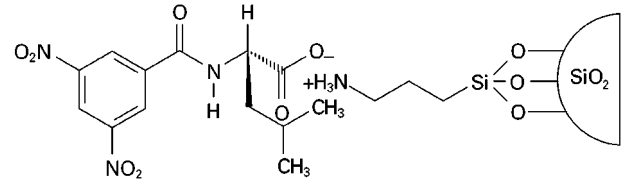
Structure [II] (DNB PG)



Structure [III] (DNB PG-Ionic)



Structure [IV] (DNB Leu)



Structure [V] (DNB Leu-Ionic)

subsequently prepared and also found to be useful for the resolution of the enantiomers of a variety of arylalkylcarbinols, with the phenylglycine-derived CSP structure [II] generally performing better than the leucine-derived CSP structure [IV]. CSP structure [III] was found to be widely useful for the separation of the enantiomers of racemates from a variety of functional group classes, and was shown to be useful for the gram-scale preparative resolution of racemates using automated preparative chromatography. The general ability of CSP structure [III] to resolve the enantiomers of a variety of analytes led to its introduction as the Pirkle 1A CSP by Regis Chemical Company in 1980 – the first commercially available HPLC chiral stationary phase. Somewhat later, CSP structures [II], [IV] and [V] were also made commercially available, and today a wide variety of CSPs incorporating the highly useful DNB moiety have been developed. Commercialization of these early Pirkle phases provided researchers in a number of fields with tools for separating the enantiomers of structurally diverse racemates. Surprisingly, the ionically tethered DNB amino acid CSP structures [III] and [V], are quite long lived when used with relatively non-polar mobile phases, although they are readily degraded when highly polar eluents such as methanol or water are used.

CSPs based on DNB derivatives of the amino acid, tyrosine, were subsequently developed by a team of French researchers including Caude, Rosset, and Tambuté, and a CSP based on DNB naphthylglycine was developed by Ôi and co-workers in Japan. The 3,5-dinitrobenzamide group has proven useful in the development of a number of additional phases, as will be described later.

Second Generation Pirkle CSPs with Electron Rich Aromatic Groups

Studies in the Pirkle laboratories and elsewhere showed that the DNB amino acid-derived CSPs were capable of separating the enantiomers of a wide variety of analytes possessing electron-rich aromatic groups. At this point, it was only natural to again turn to the principle of reciprocity and prepare and evaluate 'reciprocal' materials based on some of these structures. A number of reciprocal phases were prepared and evaluated, although they lacked the requisite generality required of a commercial CSP. Nevertheless, these studies revealed many important design features that would prove useful later.

The enantiomers of aryl hydantoins are well resolved on DNB amino acid-derived CSPs, and several hydantoin-based CSPs were prepared to study the mechanisms for chiral recognition of these types of analytes and to probe the ability of this type of phase to afford separation of various racemates.

Separation of the enantiomers of a number of aryl-substituted phthalides was studied using DNB amino acid-derived CSPs such as CSP structures [**III**] and [V], and a detailed study of the effect of analyte structure on chromatographic performance led to some optimized structures for chiral recognition. One such compound was used to prepare a reciprocal aryl phthalide CSP which, as expected, showed a general ability to resolve a number of racemates containing electron-deficient aromatic systems.

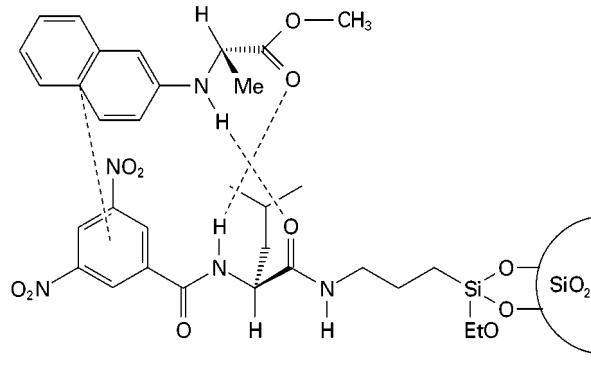
Another class of compounds which are well resolved on the DNB amino acid-derived CSPs are general amide structures bearing at least one aromatic ring. In an extensive series of studies, Pirkle, Hyun and co-workers prepared and evaluated more than 10 CSPs derived from various analogues of (l-naphthyl)ethylamine (α-NEA) in which a variety of different tether geometries and substituent patterns were explored. Study of this group revealed a number of interesting and useful principles of CSP design. However, the CSPs themselves were somewhat limited in that they were useful primarily for separation of the enantiomers of analytes bearing electron-deficient aromatic groups. Similar CSPs were prepared and studied by Ôi and co-workers.

N-Aryl Amino Acid-Derived CSPs

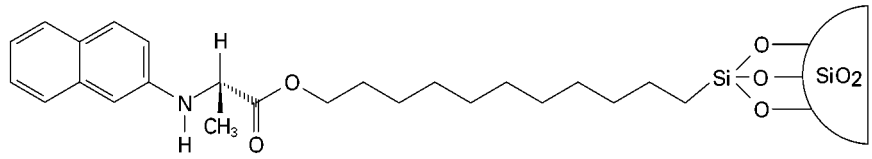
The development of second generation Pirkle phases with electron-rich aromatic groups reached it's zenith with the investigations by Pirkle and Pochapsky of compounds derived from the enantiomers of the readily prepared electron-rich *N*-aryl amino acid derivatives. This is perhaps the best understood chiral recognition system yet studied. In some preliminary studies, it was shown that *N*-aryl amino acid derivatives were well resolved using the DNB Leu-Ionic CSP structure [V]. Subsequently, the structural requirements for chiral recognition were defined, and a chiral recognition rationale was proposed. The high degree of enantioselectivity observed in this system ($\alpha > 10$ in some cases) can be attributed to the intimate association of the two components of the more stable diastereomeric adsorbate as illustrated later. This chiral recognition system has been studied at great length using spectroscopic as well as chromatographic techniques. An X-ray structure of a 1 : 1 complex of soluble analogues of the compounds shows essentially the same adsorbate structure as that initially proposed based upon chromatographic and spectroscopic studies (structure [**VI**]):

A reciprocal *N*-(2-naphthyl)valine-derived stationary phase was prepared and shown to provide unprecedented levels of resolution for a variety of racemates containing electron-deficient aromatic groups. The related alanine-derived CSP structure [**VII**] was subsequently reported, and commercialized, and has proven to be a valuable and widely used research tool:

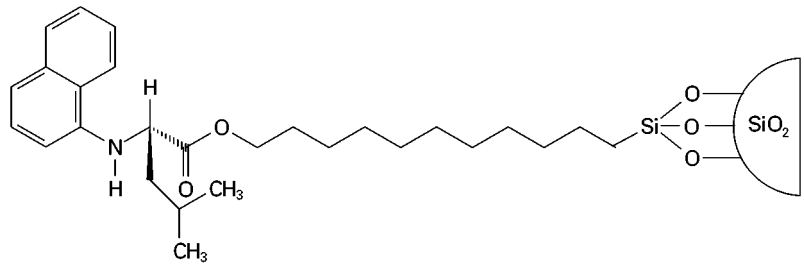
Subsequent structural refinements led to the preparation and commercialization of the leucine-derived CSP structure [**VIII**], which generally affords significantly greater enantioselectivity in the separation of DNB-containing enantiomers than does CSP structure [**VI**]. The predominant effect seems to be a greatly diminished retention of the initially eluted enantiomer, presumably owing to the ability of the more



Structure [**VI**]



Structure [**VII**] (N_2N-Ala CSP)



Structure [**VIII**] (N_1N-Leu CSP)

bulky leucine sidechain to inhibit approach of the least retained enantiomer from the undesired face of the aromatic ring of the CSP.

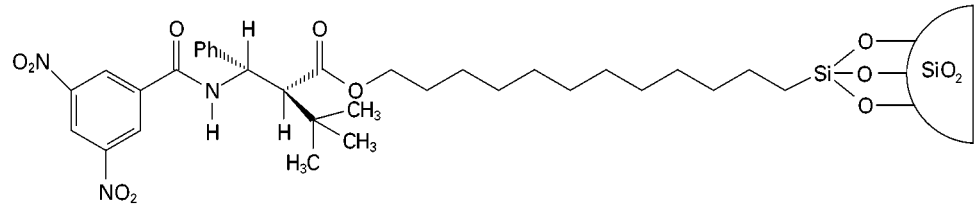
These phases have proven to be widely useful for separating the enantiomers of chiral amines, amino alcohols, amino acids, etc., and are sometimes also useful for separating the enantiomers of chiral alcohols, thiols, etc. However, analysis with these phases generally requires that the analyte be derivatized so as to incorporate an electron-deficient aromatic group. Typically, derivatization reagents such as 3,5-dinitrobenzoyl chloride or 3,5-dinitrophenylisocyanate are used. Despite this somewhat bothersome need to make derivatives, these CSPs remain quite useful and show a tremendous generality.

Recently, a group of CSPs based on proline anilides have been developed in the Pirkle laboratories. These exhibit remarkable enantioselectivities for some DNB derivatives.

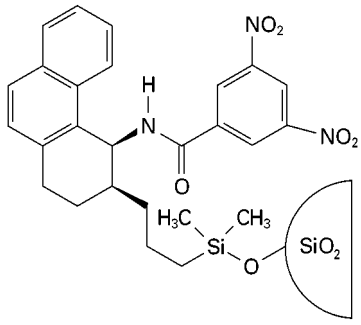
Improved DNB CSPs

The design of CSPs is an evolutionary process so that continuing refinements and improvements in both the understanding of chiral recognition and the design of such phases are constantly being made. Several CSPs, representing improvements upon the widely used DNB phenylglycine-derived CSP structure [**II**] have been developed in the Pirkle laboratories and subsequantly commercialized. The first of these, the β GEM 1 CSP (CSP structure [**IX**]), grew out of studies of the enantiopurity analysis of some chiral β-lactams. The finding that some DNB β-amino acid derivatives were well resolved led to the preparation and evaluation of the DNB β-amino acid-derived structure [**IX**]. An X-ray crystal structure of the selector used in CSP structure [**IX**] shows the t-butyl and phenyl substituents projecting from opposite faces of the stationary phase. The bulky t-butyl substituent at the 2-position is thought to play a major role in controlling the conformation of this phase, preventing intramolecular hydrogen bonding, and restricting access to one face. The β GEM CSP is useful for the separation of the enantiomers of anilide derivatives of aromatic carboxylic acids and *N*-protected amino acids.

As will be related later, the most general CSP yet developed in the Pirkle laboratories, the Whelk-O 1 (CSP structure [**X**]), was actually designed to separate the enantiomers of one particular compound, naproxen. In addition to resolving the enantiomers of



Structure [**IX**] (β-GEM I CSP)



Structure [**X**] (Whelk-O 1 CSP)

naproxen and nearly all related nonsteroidal anti-inflammatory drugs (NSAIDs) this CSP is useful for resolving the enantiomers of analytes from a host of functional group classes.

A very useful DNB-containing CSP based upon diphenylethylenediamine has been prepared and investigated by Uray, Lindner and Maier. This CSP, commercialized as the ULMO CSP (structure [**XI**]), shows a general ability to separate the enantiomers of a number of analytes containing electron-rich aromatic groups. In addition, it shows an ability to separate the enantiomers of aryl carbinols which is unsurpassed among Pirkle-type CSPs.

Another improved DNB-containing CSP based on the relatively inexpensive trans 1,2-diaminocyclohexane (DACH) was developed by the research team of Gasparrini, Misiti, Villani, and co-workers; this phase has never been commercialized, but has been studied extensively.

β-Blocker-Specific CSPs

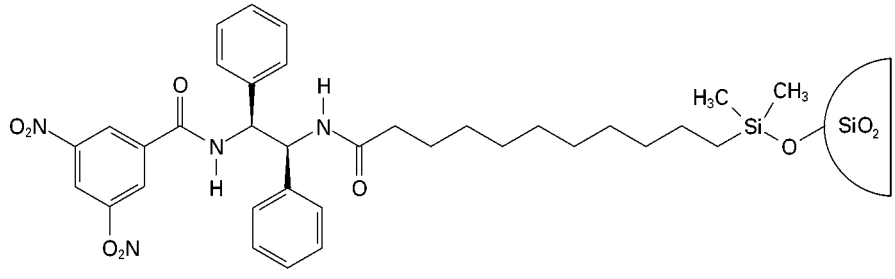
In the mid-1980s, some of the principles of CSP design were becoming fairly well understood in the Pirkle laboratories, and attempts were made to design selectors for particular target racemates of significant scientific and economic importance.

For example, the enantiomers of the β-blocker drug propranolol had been shown to be marginally resolved on the DNB phenylglycine-derived CSP structure [**II**] as any of a variety of *N*-acylated derivatives. A research programme directed at developing a phase capable of separating the enantiomers of underivatized propranolol enantiomers was undertaken. A group of three CSPs were designed, prepared, evaluated, and shown to separate the enantiomers of propranolol with the elution order predicted by the chiral recognition model. Several other β-blockers were similarly resolved, however, the poor chromatographic efficiency of these materials limited their practical utility.

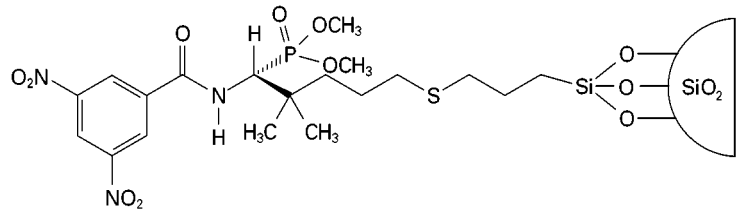
In an effort to overcome this obstacle, a group of amino phosphonic acid-derived CSPs were prepared and shown to provide improved resolution of propranolol. Consequently, CSP structure [**XII**] was prepared, evaluated, and found to provide very good resolution for the enantiomers of propranolol and a number of related β-blockers. This stationary phase was commercialized as the α-Burke I CSP. Subsequent minor improvements in the tethering chemistry led to an improved version commercialized as the α-Burke II CSP (structure [**XII**]).

Naproxen-Specific CSPs Using the Immobilized Guest Method

As mentioned earlier, one of the most general Pirkle-type CSPs resulted from a study aimed at developing something which would be useful for separating the enantiomers of the drug, naproxen. At the time of the inception of this project, separation of the enantiomers of naproxen and related NSAIDs with Pirkle-type CSPs generally required formation of derivatives. The finding that underivatized naproxen enantiomers could be marginally resolved on the β-GEM 1 CSP prompted an investigation into the development of improved separations. Drawing on the familiar principle of reciprocity, a new approach termed the 'immobilized guest method' was utilized in this study. Two CSPs were prepared from enantiopure naproxen, and these were used to investigate the enantioseparation of a variety of different chiral analytes. This study led to an understanding of the structural requirements for enantioselective naproxen recognition, and a new chiral selector incorporating



Structure [**XI**] (ULMO CSP)



Structure [**XII**] (α-Burke I CSP)

many of these key structural features was proposed. This new selector was synthesized and found to be well separated on the immobilized guest naproxen CSPs. Larger scale synthesis, resolution, and immobilization of the selector afforded a CSP which resolved naproxen enantiomers with a very high degree of enantioselectivity ($\alpha = 2.25$). In addition, structurally related NSAIDs such as ibuprofen, ketoprofen, flurbiprofen, etc. are also resolved. Subsequent mechanism-based structural modifications led to the development and commercialization of the Whelk-O 1 CSP (CSP structure [**X**]) which affords improved resolution of NSAID enantiomers. In addition to resolving the enantiomers of NSAIDs, the Whelk-O 1 CSP has proven to be the most general CSP developed to date in the Pirkle laboratories, as it is capable of resolving the enantiomers of racemates from a host of functional group classes.

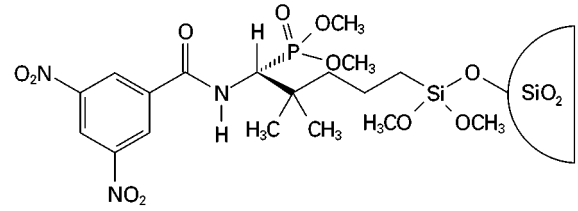
A number of variations on the Whelk-O structure have been developed in the Pirkle laboratories and at Regis Technologies. For example, the same selector immobilized via a trifunctional silane linkage has been commercialized as the Whelk-O II CSP (structure [**XIV**]). This CSP is more resistant to selector cleavage under harsh mobile phase conditions.

Another analogue of the Whelk-O CSP has proven useful for separations utilizing supercritical or subcritical carbon dioxide as an eluent. This phase, commercialized as the PolyWhelk-O CSP, is prepared by first immobilizing the Whelk-O chiral selector onto a polysiloxane polymer, then covalently bonding the resulting polymer to silica gel. The resulting material often shows improved efficiency and enantioselectivity when operated in the SFC mode.

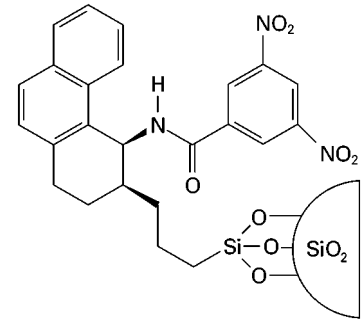
New Trends in Design and Development of Pirkle-Type CSPs

The design and development of new CSPs continues at an active pace in the Pirkle laboratories, with an emphasis on using an array of analytical tools to develop a mechanistic understanding of CSP behaviour. Developing CSPs which will be useful in the preparative chromatographic separation of pharmaceutical enantiomers is another major research area.

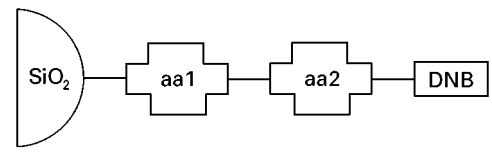
A new method for microscale synthesis and evaluation of libraries of Pirkle-type phases has recently been developed at Regis Technologies. In this technique, libraries of CSPs are prepared on a 50 milligram scale by solid phase synthesis on silica particles. The resulting libraries are then rapidly evaluated using a process which does not require packing into a column. This technique provides a useful tool for rapidly determining which CSP from a library of many hundreds shows the greatest enantioselectivity for a particular analyte. In addition, some of the necessary structural requirements for chiral recognition can be determined by comparing the relative performance of various CSPs in the library. This information can, in turn, suggest further CSP libraries, which can be prepared and evaluated. Ultimately, the process can lead to discovering CSPs which display preparatively useful enantioselectivity for given target racemates.



Structure [**XIII**] (α-Burke II CSP)



Structure [**XIV**] (Whelk-O II CSP)



Structure [**XV**]

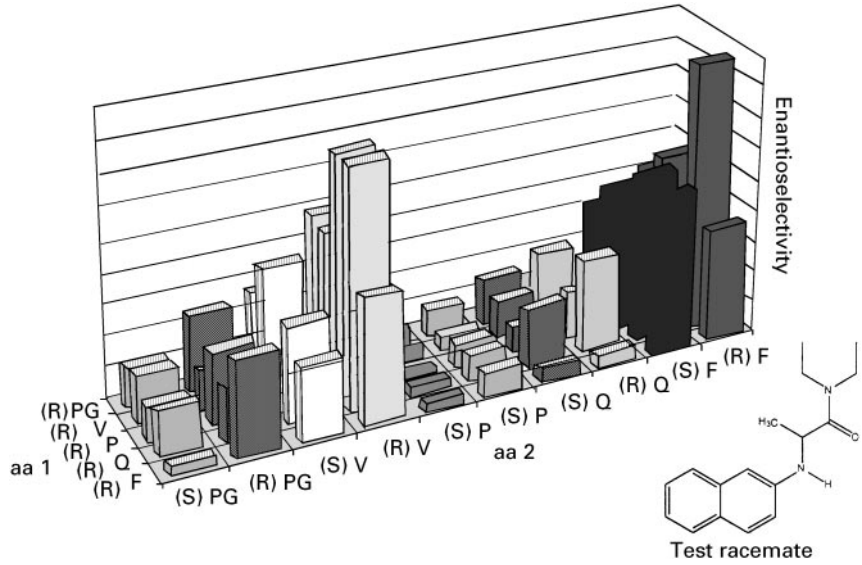


Figure 2 Relative enantioselectivity seen by a library of 50 dipeptide DNB CSPs for the enantiomers of a test racemate.

For example, preparation of a library of 50 dipeptide DNB CSPs and screening for the ability to separate the enantiomers of the test analyte shown (structure [**XV**]) afforded the results presented in **Figure 2**, which suggest that bulky groups in the aa 2 position and hydrogen bonding groups in the aa 1 position are advantageous.

Subsequent preparation and evaluation of such a 'focused' library revealed a number of new CSPs with very high enantioselectivity for the target analyte. One of the better performing members of this library was prepared on a larger scale, packed into a column, and shown to be able to afford exceptionally high productivity for the preparative separation of the enantiomers of the racemic test analyte. This new approach to CSP development promises to be very useful for large-scale preparative chromatographic enantioseparation, and a variety of structurally diverse CSP libraries are now being investigated by a number of different research groups.

Conclusion

A number of Pirkle-type CSPs are of great use in the chromatographic separation of enantiomers. Several of these materials are commercially available and widely used in diverse research disciplines. An ever-increasing understanding of the requirements for chiral recognition and the design of useful CSPs is evident from a survey of Pirkle's work over the past two decades. The entry of other research groups into the area of CSP design suggests that there will be continued developments in this area in coming years.

See also: **III/Chiral Separations:** Amino Acids and Derivatives; Capillary Electrophoresis; Cellulose and Cellulose Derived Phases; Chiral Derivatization; Countercurrent Chromatography; Crystallization; Gas Chromatography; Ion-Pair Chromatography; Ligand Exchange Chromatography; Liquid Chromatography; Molecular Imprints as Stationary Phases; Protein Stationary Phases; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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