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Review

Evolution of chiral stationary phase design in the Pirkle laboratories

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ABSTRACT

An historical review of the design of chiral stationary phases (CSPs) in the Pirkle laboratories is presented. Beginning with the discovery of the non-equivalence of nuclear magnetic resonance signals arising from enantiomers in the presence of a chiral solvating agent more than 25 years ago the Pirkle group has been at the forefront of the study of the enantioselective interactions of chiral molecules. Dozens of CSPs have been synthesized and evaluated, and several of these CSPs have subsequently been commercialized and are now widely used by researchers around the world. Several recently developed CSPs are also presented, and general principles which have guided the design of these CSPs are discussed.

CONTENTS

1. INTRODUCTION

The liquid chromatographic separation of enantiomers using chiral stationary phases (CSPs) has become an indispensable tool in many areas of modern research [1]. Indeed, the current explosion of interest in enantiopure pharmaceuticals and enantioselective synthetic methods may be attributed, at least in part, to the recent appearance of convenient methods for the pre-

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cise measurement of enantiopurity. As the inventor of the first commercially available CSP and as a leading researcher in the field of chromatographic enantioseparation, Professor William H. Pirkle has played a major role in making possible the current "chirotechnology revolution".

Professor Pirkle's research is best characterized by an ever-present rational strategy discernible on even casual overview. Whether it be the design of chiral NMR shift reagents, the chromatographic separation of enantiomers as diastereomeric derivatives, or the development of new CSPs, Professor Pirkle's research is always guided by a rational model constructed from a constantly evolving understanding of molecular interactions. Inferences made from such a model are methodically tested, and the model is honed and refined. Repeatedly, a model emerges that is both well understood and of considerable predictive power.

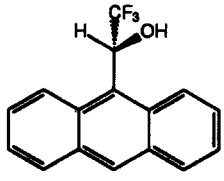
The evolution of CSP design in the Pirkle laboratories aptly illustrates this approach. Beginning with models derived from the study of chiral NMR shift reagents and using an ingenious reciprocal approach to CSP design, succeeding generations of CSPs have been designed and evaluated over the years. Chiral recognition has been elucidated by a battery of analytical techniques, and a truly comprehensive understanding of at least some of these systems has been obtained. Within the Pirkle group, the record enantioselectivity for the separation of the enantiomers of an analyte containing a single stereogenic center using a CSP with a single stereogenic center has risen steadily over the years, and now exceeds the almost incredible value of 125 —surely a result that testifies to the merits of the "Pirkle approach".

In this review an historical overview of the development of CSP design in the Pirkle laboratories is presented, with emphasis on the evolutionary nature of this research. Every attempt has been made to excavate and reconstruct this history in its entirety. However, a number of "evolutionary dead ends" in this research program (*i.e.* CSPs with poor performance or stability) were frequently unreported, and consequently remain buried in the strata of old notebooks, reports and chromatograms in Professor Pirkle's office. Nevertheless, an updated and somewhat comprehensive review of this topic, which was the subject of a 1988 review by Finn [2], is presented. The interested reader is encouraged to consult a number of additional review articles containing valuable information on this topic [3–9].

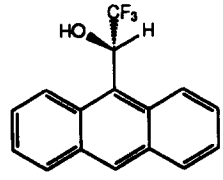
2. DEVELOPMENT OF NMR CHIRAL SOLVATING AGENTS

Pirkle's 1966 discovery of the non-equivalence of NMR signals arising from enantiomers in the presence of a chiral solvating agent (CSA) [10] had a dramatic effect upon the future course of the study of chiral molecular recognition. While this topic was reviewed in 1982 [11], a brief mention of the highlights of this research effort are of interest in the present context.

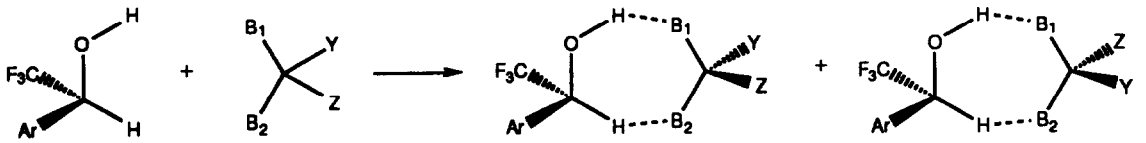
The first reported CSA consisted of enantioenriched α-phenylethylamine (α-PEA) which was used to show spectral non-equivalence for the enantiomers of 2,2,2-trifluoro-1-phenylethanol by ^{19}F NMR [10]. Pirkle soon thereafter reported the ^{1}H NMR non-equivalence of aryl carbinol enantiomers in the presence of similar amino CSAs [12] and the utility of this technique in the assignment of absolute configuration to a number of arylalkylcarbinols [13]. Subsequently, the reciprocal situation was investigated, and enantioenriched trifluoromethyl aryl carbinols were shown to be broadly useful in the determination of enantiomeric purity and assignment of absolute configuration of amines [14] and analytes from a broad assortment of functional group classes including sulfoxides [15,16], sulfinamides [17], sulfinates [17,18], sulfites [17],



(*R*)-CSA 1



(*S*)-CSA 1



thiosulfinates [17], phosphine oxides [17], amine oxides [17,19], amino acids [20], lactones [21,22], oxaziridines [23,24] and allenes [25]. During this time, a number of CSAs were evaluated, and 1-(9-anthryl)-2,2,2-trifluoroethanol (CSA 1) was found to be the most generally useful. This reagent was commercialized (Aldrich) and has subsequently been used extensively by numerous researchers.

A general model, illustrated above, was advanced to account for the ability of CSA 1 to afford non-equivalence in the NMR spectra of a variety of compound classes [11]. In this model, the CSA was proposed to form a two-point, chelate-like complex with analytes containing two different electron-rich interaction sites. The carbinyl hydrogen, made slightly acidic by the adjacent trifluoromethyl and aryl groups, was recognized as a key component of the CSA. This proton serves as a weak hydrogen-bond donor, interacting with the weaker of two electron-rich H-bond acceptor sites in the analyte.

This same general model was shown to be highly accurate in allowing enantiopurity determinations and predictions of absolute configuration for a number of analytes containing two hydrogen-bond acceptor sites of differing basicity. Interestingly, this method, relying as it does on weak molecular interactions, permits the study of some very subtle aspects of molecular recognition. For example, the ability of the π-cloud of an aromatic system to function as a hydrogen bond acceptor, a topic which has received considerable recent attention [26,27], was observed repeatedly in a number of systems [11].

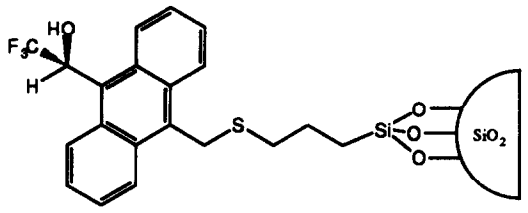
Analytes containing electron-deficient aromatic rings were found to undergo additional interaction with the electron-rich aromatic ring of CSA 1. Using a very clever experimental approach, one such analyte, methyl 2,4-dinitrophenylsulfoxide, was shown to form diastereomeric adsorbates of differing free energies with CSA 1 [28]. This breakthrough was of great importance because while NMR methods for enantiopurity determination require only the formation of diastereomeric derivatives with differing geometries, the formation of diastereomeric adsorbates with differing free energies can permit physical separations and manipulations based upon this thermodynamic difference. For example, recrystallization of racemic methyl 2,4-dinitrophenylsulfoxide in the presence of (*R*)-CSA 1 was found to afford partial resolution [28] and, in what may be the first example of chromatographic enantioseparation using a chiral mobile phase additive, (*R*)-CSA 1 was used to chromatographically separate the enantiomers of the sulfoxide using a silica column [29].

3. 9-AC CSP

In a natural extension of this work, a single enantiomer of an analogue of CSA 1, referred to as 9-Ac in the Pirkle group, was immobilized on a silica support to afford a stable CSP capable of separating the enantiomers of π-acidic sulfoxides and a variety of π-acidic racemates [30,31]. This method provides an exquisitely sensitive tool for the study of small differences in the free energy of formation of diastereomeric adsorbates. For example, separation factors (α values) as small as 1.02 were observed with the original column, corresponding to a free energy difference of only 12 cal/mol (1 cal = 4.1868 J) at room temperature!

4. DINITROBENZOYL AMINO ACID CSPs

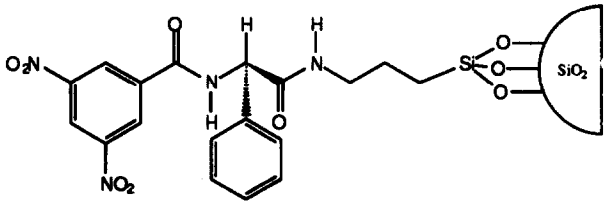
In the course of the evaluation of CSP 1, it was found that the enantiomers of not only π-acidic sulfoxides, but also those of a number of



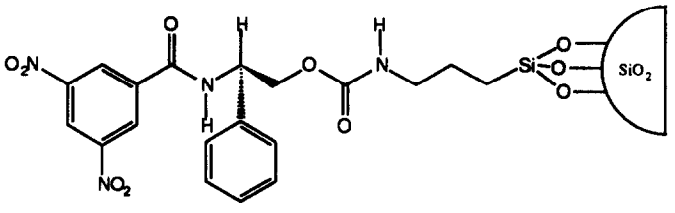
CSP 1

other compounds possessing π-acidic groups could be separated as well [30,31]. For example, 3,5-dinitrobenzoyl (DNB) derivatives of aryl alcohols, amines and thiols, amino acids, amino alcohols, amino ethers, amino esters, amino amides and amino phosphonates are all resolved upon CSP 1 [30]. DNB derivatives of some amino acids, most notably phenylglycine, show quite large separation factors ($\alpha > 1.75$) on CSP 1. This observation prompted an investigation of the reciprocal situation and several DNB-phenylglycine-derived CSPs were prepared and evaluated [31]. The ready availability of enantiopure (*R*)-phenylglycine (utilized in the commercial production of an antibiotic) and the high degree of enantiodifferentiation afforded by CSP 1 for various derivatives of DNB-phenylglycine suggested that phenylglycine-derived CSPs might provide an economical and convenient method for resolving the enantiomers of the highly useful CSA 1.

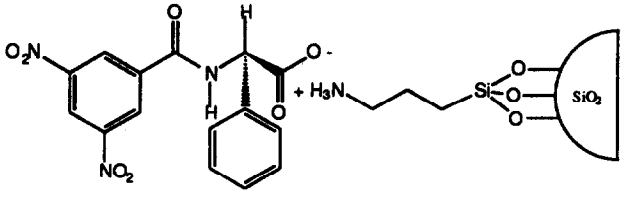
Both CSPs 2 and 3 afford resolution of the enantiomers of CSA 1 and related compounds, with the amino acid-derived CSP 2 generally affording superior separations [31]. The related ionically bonded amino acid-derived CSPs 4, 5 and 6 were subsequently prepared and found also to be useful for the resolution of the enantiomers of a variety of arylalkylcarbinols [32], with the phenylglycine-derived CSP 4 generally performing better than the valine or leucine-derived CSPs 5 and 6. CSP 4 was found to be widely useful for the separation of the enantiomers of racemates from a variety of functional group classes [33–35], and was shown



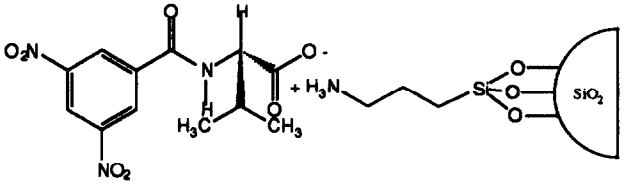
CSP 2



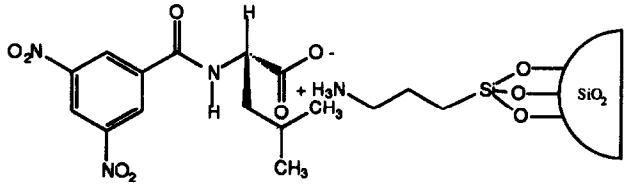
CSP 3



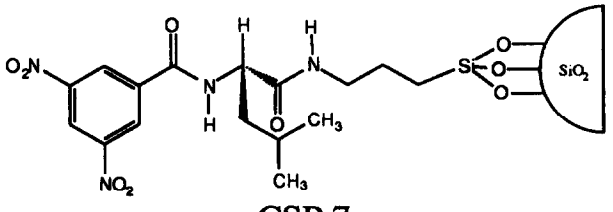
CSP 4



CSP 5



CSP 6



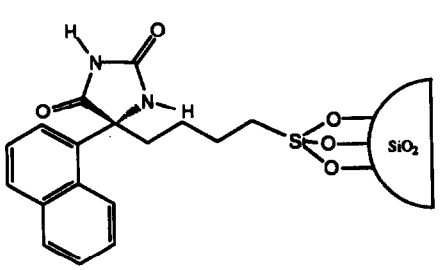
CSP 7

to be useful for the gram-scale preparative resolution of racemates using a previously developed automated preparative chromatography apparatus which employs continuous solvent redistillation and recycling [36]. The general ability of CSP 4 to resolve the enantiomers of a variety of analytes led to its introduction as the first commercially available HPLC CSP by Regis Chemical Company in 1980. Somewhat later, CSPs 2, 6 and 7 were also made commercially available, and today a wide variety of CSPs incorporating the highly useful DNB moiety have been reported.

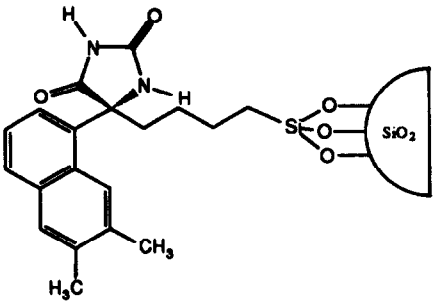
5. HYDANTOIN-DERIVED CSPs

The enantiomers of aryl hydantoins were shown to be well resolved on DNB-amino acid-derived CSPs. Consequently it was deemed important to design a hydantoin-based CSP both to study the mechanisms for chiral recognition of these types of analytes and to probe the ability of this type of CSP to afford separation of various racemates [37]. Toward this end, hydantoin-derived CSPs 8–10 were prepared and evaluated. It is of interest to note that CSP 8, prepared several years before it was described in print, was the first CSP developed in the Pirkle group to be prepared via a route involving hydrosilylation of a terminal olefin precursor, an approach which subsequently became widely used.

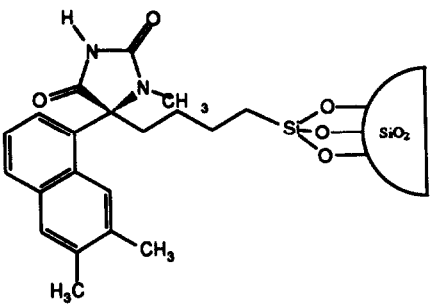
In general, CSP 9, which bears additional methyl groups on its naphthyl ring, was shown to afford superior enantioseparation relative to CSP 8. The electron-donating capability of these methyl substituents is believed to increase the π-basicity of the aromatic ring. The ability of



CSP 8



CSP 9



CSP 10

these methyl groups to serve as a steric barrier to control conformation of the face-to-face π–π complex may also be important, as may be the ability of these methyl groups to participate in weak hydrogen-bonding interactions, particularly with the π-cloud of a complexed analyte.

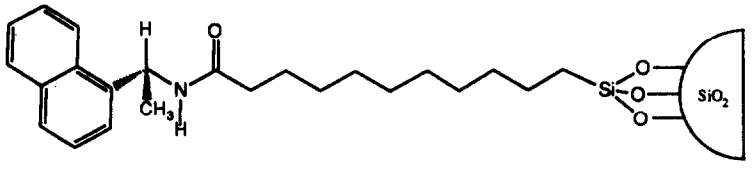
Substitution of the amide hydrogen of CSP 9 with a methyl group afforded the analogous CSP 10, which shows some very interesting behavior. The chiral recognition scheme which was originally advanced for the resolution of the enantiomers of this type of analyte did not invoke this amide hydrogen as an interaction site. Consequently, by effectively removing this non-essential interaction site, the enantioselectivity afforded by CSP 10 was expected to be superior to that observed with CSP 9. The actual results with CSP 10 turned out to be rather surprising: not only was the performance of this CSP oftentimes inferior to that of CSP 9, but the elution order of DNB-amino acid enantiomers was often reversed. This result suggests that the amide hydrogen does indeed participate in chiral recognition for this CSP.

6. 2-ARYLAMIDOALKANE-BASED CSPs

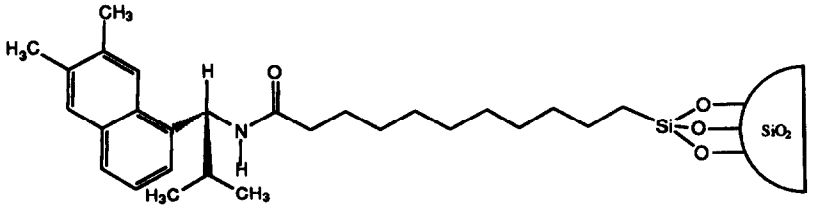
The DNB-amino acid-derived CSPs such as CSP 2 were shown to have broad utility for the separation of the enantiomers of a variety of amide derivatives, with quite large separation factors being observed for some 2-arylamidoalkanes [38–40]. A rationale to account for the face to face approach of selector and analyte needed for enantioselectivity was proposed to involve the formation of hydrogen-bonded and dipole-stacked arrangements of CSP and analyte [38,41]. While this rationale has been useful in rationalizing the experimental data, permitting the determination of absolute configuration, and offering insight into the design of new CSPs, certain aspects of this model have recently been revised [42]. In particular, an alternative hydrogen-bonded adsorbate has been suggested to be more likely than the previously advanced "dipole-stacked" adsorbate. Any chiral recognition model should be considered as an oversimplified first approximation of reality and subject to refinement as additional information becomes available.

As with the hydantoin CSPs, the incorporation of a chiral selector containing the more π-basic dimethylnaphthyl aromatic system is generally found to be advantageous, as is an increase in the steric bulk of the alkyl substituent at the stereogenic center. Consequently, CSPs 12 and 13, which contain both of these modifications, generally outperform CSP 11 [43,44].

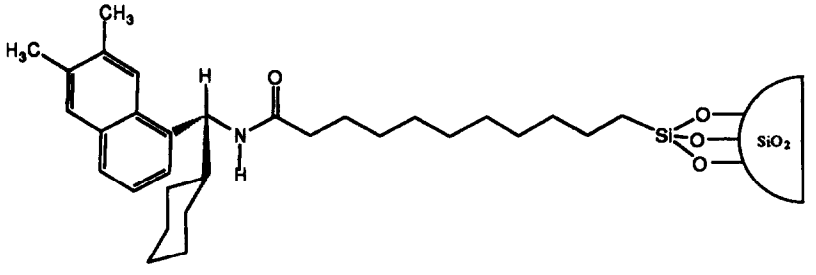
Both the length and the geometry of attachment of the tether connecting the chiral selector to the chromatographic support was found to have a dramatic influence on the separation of the enantiomers of certain analytes containing



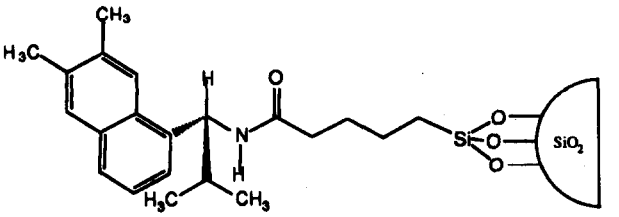
CSP 11



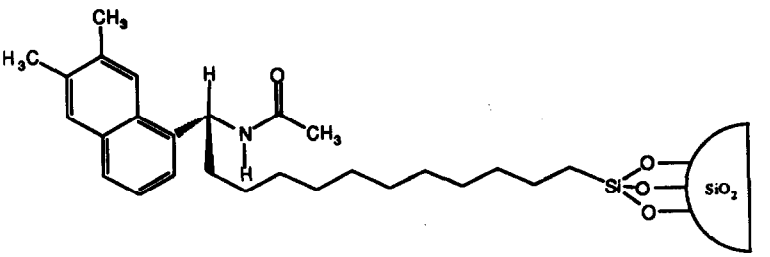
CSP 12



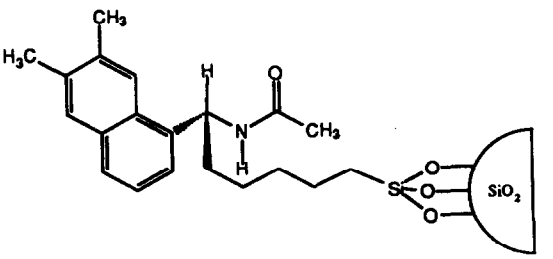
CSP 13



CSP 14



CSP 15

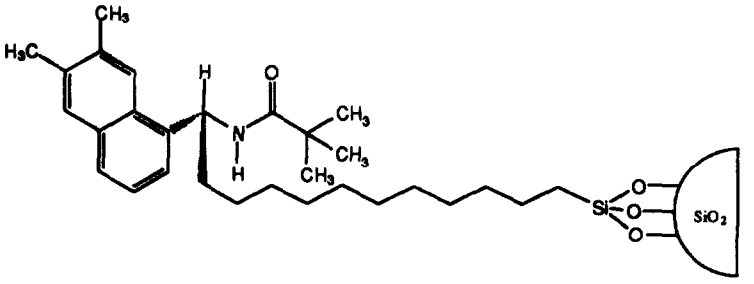


CSP 16

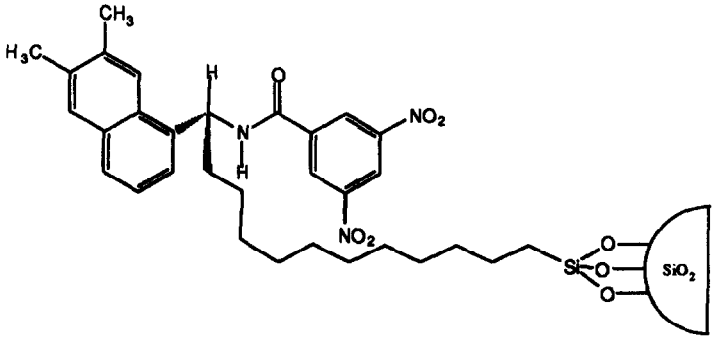
long alkyl chains. These long alkyl chains are thought to sterically impede normal adsorbate formation by interacting with the underlying chromatographic support. CSPs 12, 14, 15 and 16 were prepared and evaluated in a study of this "intercalation" phenomenon, and a general model was developed to rationalize the results [42,45,46].

The synthetic route for the preparation of the "turned phase" CSP 15 permits introduction of a variety of acyl groups, making possible the formation of a series of new CSPs. Acylation with the bulky pivaloyl group affords CSP 17 [45], which generally shows improved performance relative to CSP 15, and which is useful for resolving the enantiomers of a number of DNB-containing analytes [47]. Acylation with the π-acidic DNB group produced "hybrid" CSP 18 which incorporates both π-acidic and π-basic functionality. Consequently, this CSP is capable of separating the enantiomers of either π-acidic or π-basic analytes [48].

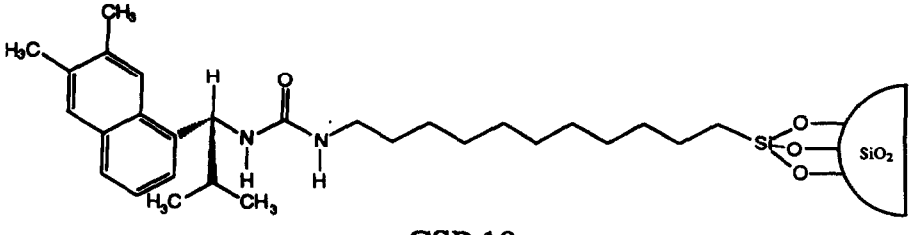
In a study comparing the influence of amide and urea linkages on chiral recognition, CSPs 19 and 20 were prepared and compared with their amide-linked counterparts, CSPs 12 and 14 [49]. CSP 20, which had previously been described by Ôi *et al.* [50], was also prepared for evaluation.



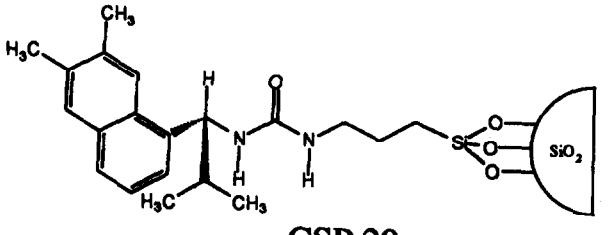
CSP 17



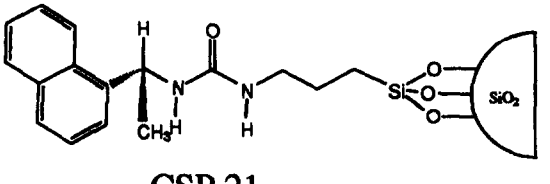
CSP 18



CSP 19



CSP 20

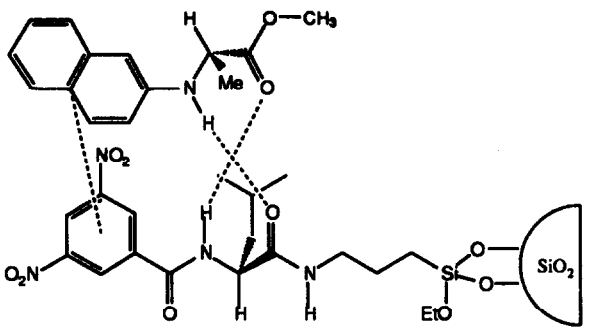


CSP 21

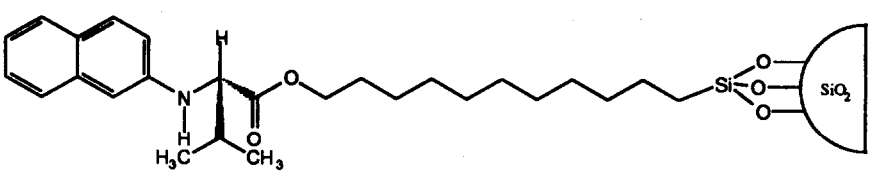
The preparation and evaluation of this series of reciprocal 2-arylamidoalkane-based CSPs by the Pirkle group is illustrative of how CSP design can be used in the study of subtle aspects of chiral recognition. Despite the proven utility of this class of CSPs in providing useful resolutions for a number of racemates, the mechanistic complexity of their mode of action has also been well documented. Such ambiguities in chiral recognition are undesirable in general-purpose CSPs and limit both the generality of the CSP and the degree of certainty with which absolute configurations can be assigned. Nevertheless, it is by studying and understanding such ambiguous chiral recognition systems that advances in CSP design are made.

7. N-ARYL AMINO ACID-DERIVED CSPs

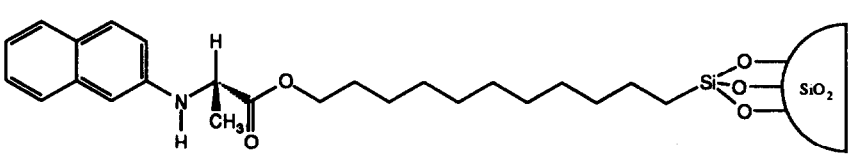
The separation of the enantiomers of the readily prepared π-basic N-aryl amino acid derivatives [51] on DNB-amino acid CSPs (*e.g.* CSP 6) is perhaps the best understood and least ambiguous chiral recognition system yet studied in the Pirkle laboratories. Originally, an assortment of racemates were examined chromatographically using CSP 6. The structural requirements for chiral recognition were defined, and a chiral recognition rationale was proposed [52]. 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 below. This chiral recognition system has been studied in great detail using a variety of spectroscopic as well as chromatographic techniques [53,54]. An X-ray structure of a 1:1 complex of soluble analogues of the compounds shown below shows essentially the same adsorbate structure as that initially proposed based upon chromatographic and spectroscopic studies [55].



A reciprocal N-(2-naphthyl)valine-derived stationary phase (CSP 22) was prepared and shown to provide unprecedented levels of resolution for a variety of π-acidic racemates [56]. The related alanine-derived CSP 23 was subsequently reported [57] and commercialized (Regis), and has proven to be a valuable and widely used research tool.



CSP 22



CSP 23

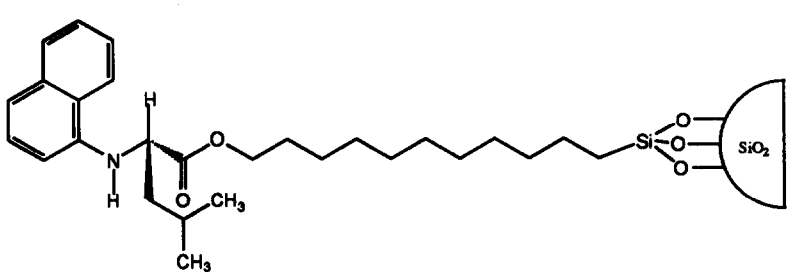
Subsequent structural refinements led to the preparation and commercialization (Regis) of the leucine-derived CSP 24, which generally affords significantly greater enantioselectivity in the separation of DNB-containing enantiomers than does CSP 23 [58]. The predominant effect seems to be a greatly diminished retention of the initially eluted enantiomer, presumably owing to the ability of the more bulky leucine sidechain to inhibit approach of the least retained enantiomer from the undesired face of the aromatic ring of the CSP.

8. PHTHALIDE-DERIVED CSPs

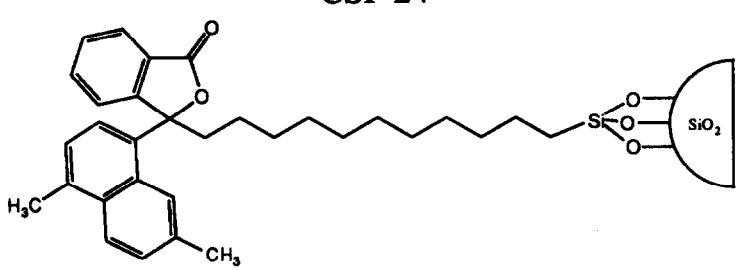
Resolution of the enantiomers of a variety of aryl-substituted phthalides was shown to be possible using DNB-amino acid-derived CSPs such as CSPs 2 and 7 [59]. A detailed study of the effect of analyte structure on chromatographic performance showed that analytes bearing a large alkyl group and a methyl-substituted naphthyl ring system afforded the greatest enantioselectivity. A reciprocal CSP embodying these features (CSP 25) was prepared and evaluated for its ability to resolve the enantiomers of a number of π-acidic analytes [60]. As expected, this CSP shows a general ability to resolve a number of π-acidic racemates, albeit with a degree of enantioselectivity which is less than that obtained with some other reciprocal π-basic CSPs. Nevertheless, a rationale accounting for the observed chiral recognition was proposed, and a useful correlation of the circular dichroism spectra and absolute configuration of aryl phthalides was advanced [61].

9. β-GEM 1: AN IMPROVED DNB-AMINO ACID CSP

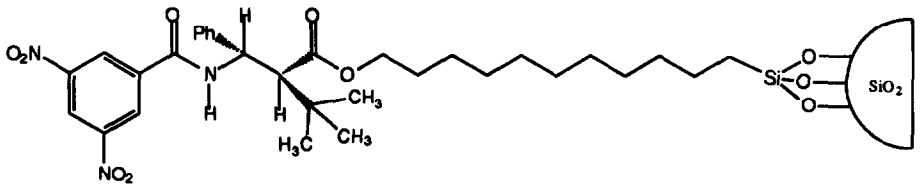
CSP design being an evolutionary process, continuing refinements and improvements in both the understanding of chiral recognition and the design of CSPs are constantly being made. One such improvement upon the widely used DNB-phenylglycine-derived CSP 2 was the development of the β-GEM 1 CSP (CSP 26). The separation of the enantiomers of a number of DNB derivatives of β-amino acids was shown to be readily accomplished with the arylamide-derived CSP 12, providing a convenient method for determining the enantiomeric purity of a number of β-lactams [62,63]. The finding that some DNB-β-amino acid derivatives were well resolved on this and other π-basic CSPs led to the preparation and evaluation of the DNB-β-amino acid-derived CSP 26 [64]. An X-ray crystal structure of the selector used in CSP 26 shows



CSP 24



CSP 25



CSP 26

the *tert.*-butyl and phenyl substituents projecting from opposite faces of the stationary phase [65]. The bulky *tert.*-butyl substituent at the 2-position of this CSP is thought to play a major role in controlling the conformation of this CSP, preventing intramolecular hydrogen bonding, and restricting access to one face of the CSP.

The β-GEM CSP was shown to be quite useful for the separation of the enantiomers of anilide derivatives of aromatic carboxylic acids [66] and N-protected α-amino acids [67] and to oftentimes afford improved resolution of the enantiomers of π-basic analytes relative to the DNB-phenylglycine-derived CSP 2 [64].

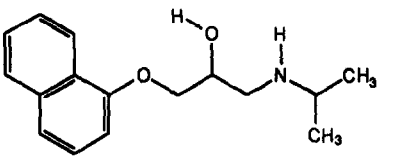
10. DESIGNED CSPs I: β-BLOCKER-SPECIFIC CSPs

The strategy utilized by the Pirkle group to develop rationally designed selectors for chromatographic enantioseparation was now well demonstrated. However the focus had heretofore been principally on the design of selectors and stationary phases for mechanistic study. While these might display high enantioselectivity for some racemates, these racemates were often of limited interest. Even so, the CSPs resulting from these fundamental studies are of the great utility for a number of practical applications. In the mid-1980s, some of the principles of CSP design were sufficiently understood so that attempts were made to design selectors for particular target racemates of significant economic and scientific importance [68].

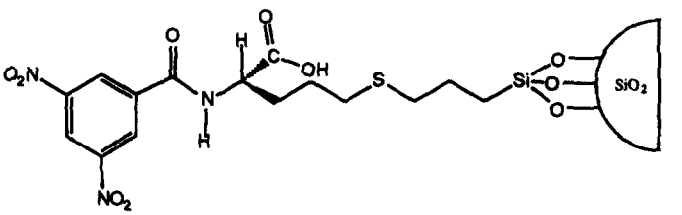
For example, the enantiomers of the β-blocker drug propranolol had been shown to be marginally resolved on the DNB-phenylglycine-derived CSP 2 as any of a variety of N-acylated derivatives [34]. A research program directed at developing a stationary phase capable of separating the enantiomers of underivatized propranolol enantiomers was undertaken. It was thought that a DNB-amino acid-derived CSP in which the carboxyl group was not involved in tethering, but instead was free and ionized, would provide not only the familiar π–π and hydrogen-bonding interactions, but should also be capable of an electrostatic interaction with the protonated amine functionality of otherwise underivatized propranolol enantiomers. To this end, the "turned phase" DNB-amino acid CSPs 27–29 were prepared and evaluated [69].

These CSPs do indeed resolve the enantiomers of propranolol with the elution order predicted by the interaction model. Several other β-blockers were similarly resolved. However, the strength of the electrostatic interaction leads to long retention and poor band shape, limiting the utility of these CSPs. As a general rule, the degree of chiral recognition stems from the weaker, as opposed to the stronger, of the essential interactions. Consequently, vastly stengthening a given interaction often serves only to increase the retention to such an extent as to require a very strong eluent (which often *decreases* the strength of the remaining interactions). To remedy this situation, the amino phosphonic acid-derived CSP 30 was prepared, the idea being that the more diffused charge on the phosphonate anion would result in a weaker ion pair with the complexed analyte enantiomers.

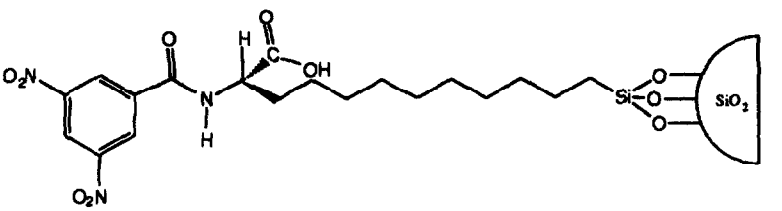
The amino phosphonic acid-derived CSP 30 does provide improved resolution of propranolol enantiomers relative to the analogous amino acid-derived CSP 29. However, it still shows undesirably strong retention for propranolol enantiomers [70]. The amino phosphonate-derived CSP 31 [71] shows dramatically decreased retention of propranolol enantiomers relative to the phosphonic acid-derived CSP 30. In addition,



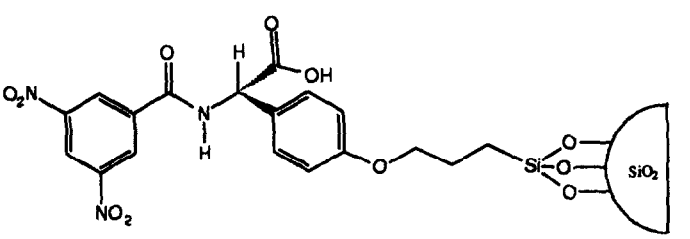
Propranolol



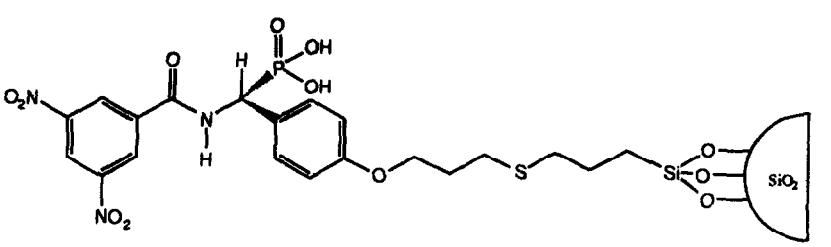
CSP 27



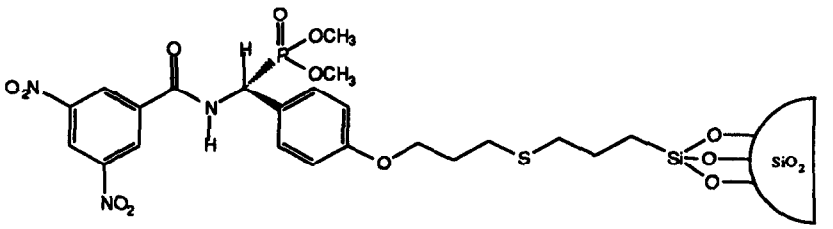
CSP 28



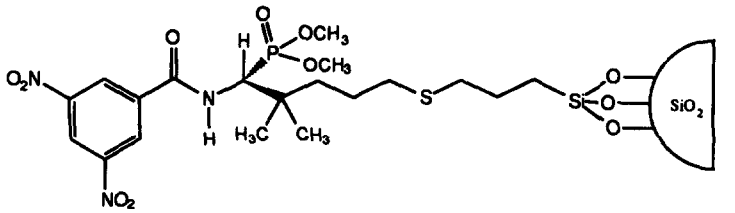
CSP 29



CSP 30



CSP 31



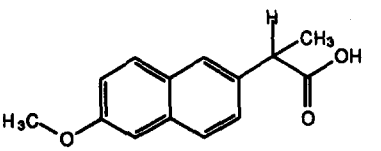
CSP 32

it also shows improved resolution of the enantiomers of propranolol and related compounds.

Owing to the well-recognized fact that aromatic groups can often serve as sites for hydrogen bonding [57] or face-to-edge $\pi-\pi$ interaction [42], it was deemed desirable to prepare a stationary phase in which the aromatic ring in the tether of CSP 31 was replaced with group which would function in a purely steric sense. Consequently, CSP 32 was prepared, evaluated, and found to provide very good resolution for the enantiomers of propranolol and a number of related β-blockers [70]. This stationary phase has been commercialized as the α-Burke 1 CSP (Regis) and has proven to be quite useful to researchers in the pharmaceutical field.

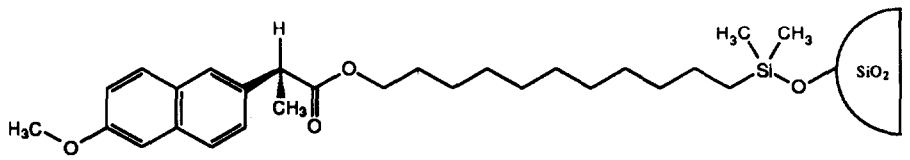
11. DESIGNED CSPS II: NAPROXEN-SPECIFIC CSPs

Another example of the *de novo* design of a CSP for a particular target analyte involves a series of CSPs designed for resolution of the enantiomers of the non-steroidal anti-inflammatory drug (NSAID), naproxen. The resolution of the enantiomers of a number of NSAIDs, including naproxen, has been demonstrated using the β-GEM 1 CSP (CSP 27), provided that the carboxylic acid functionality of the analyte is first converted to an anilide derivative [72]. A subsequent study demonstrated that, under certain mobile phase conditions, the enantiomers of naproxen can be marginally resolved on this CSP without derivatization [73]. As chemical derivatization in pharmaceutical analysis is generally to be avoided whenever possible, this result prompted an investigation into the design of improved selectors for the enantiomers of naproxen.

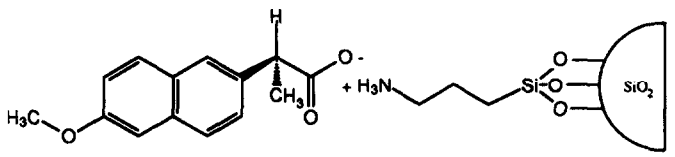


naproxen

The strategy which was used in this research program is termed the immobilized guest method, and is a natural extension of the many studies conducted with reciprocal CSPs in the Pirkle laboratories. A single enantiomer of the target naproxen molecule was immobilized to form two naproxen-derived CSPs (CSPs 33 and 34) which were used to screen candidate enantioselective naproxen selectors. In essence, this was a reciprocal study of the structural requirements for enantioselective naproxen recognition [74].



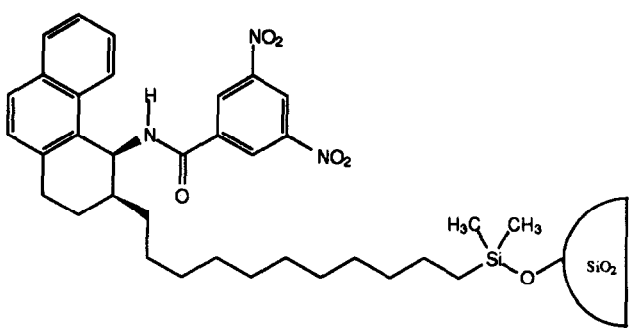
CSP 33



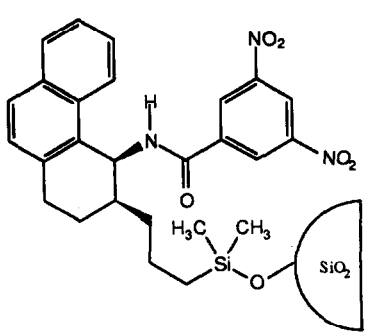
CSP 34

Both CSPs 33 and 34 afford resolution of the enantiomers of several π-acidic analytes. Of the many analytes screened from the extensive library of chiral compounds available in the Pirkle laboratories, an analogue of the selector used in the β-GEM 1 CSP, 27, showed the highest enantioselectivity. This CSP had previously been shown to afford only modest resolution of naproxen enantiomers [73], so this was a somewhat disappointing result. Nevertheless, data obtained from the few analytes which do resolve with these CSPs revealed some features important for the enantioselective recognition of naproxen, namely, the importance of face-to-edge $\pi-\pi$ interaction in addition to previously considered hydrogen bonding and face-to-face $\pi-\pi$ interaction. A rationale for the observed resolutions was advanced and a chiral selector incorporating all of the features felt to be essential was proposed [74].

Initial synthesis of this candidate selector on a small scale afforded a material which showed much greater enantioselectivity on naproxen-derived CSPs 33 and 34 than any other selector which had been examined. Larger-scale synthesis, resolution, and immobilization of the selector afforded CSP 35. This CSP resolves naproxen enantiomers with a degree of enantioselectivity ($\alpha = 2.25$) greater than any previously developed enantioselective naproxen selector [74]. In addition, structurally related NSAIDs such as ibuprofen, ketoprofen, flurbiprofen, etc. are also resolved. Subsequent mechanism-based structural modifications led to the development of CSP 36 which affords improved resolution of NSAID enantiomers [75]. This stationary phase was subsequently commercialized as the Whelk-O 1 CSP (Regis). In addition to resolving the enantiomers of NSAIDs, this CSPs has proven to be the most general CSP developed to date in the Pirkle laboratories, being capable of resolving the enantiomers of racemates from a host of functional group classes [76].

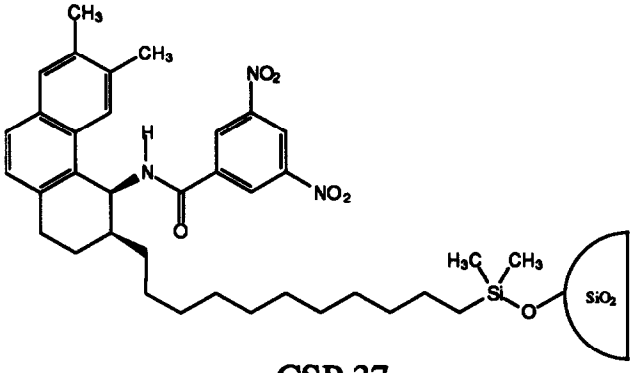


CSP 35

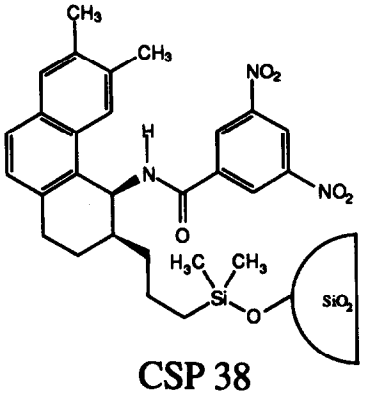


CSP 36

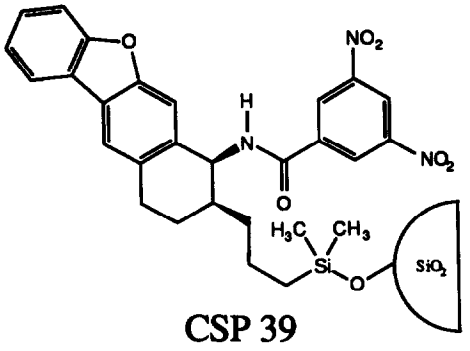
Several variations on this general structural motif have been investigated in the Pirkle laboratories. For example, the incorporation of methyl substituents on the aromatic ring affords CSPs 37 and 38, which generally show enhanced enantioselectivity relative to CSPs 35 and 36 [77]. The analogous CSPs 39 and 40, which contain the dibenzofuran aromatic system, generally show slightly poorer performance relative to the commercially available CSP 36 [78].



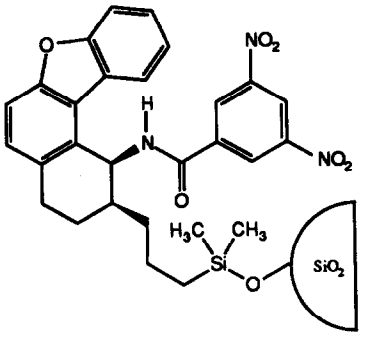
CSP 37



CSP 38



CSP 39



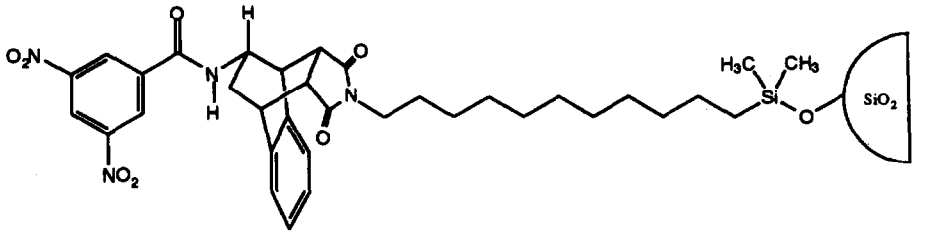
CSP 40

12. CSPs IN DEVELOPMENT I: CSPs UTILIZING FACE-TO-EDGE $\pi-\pi$ INTERACTION

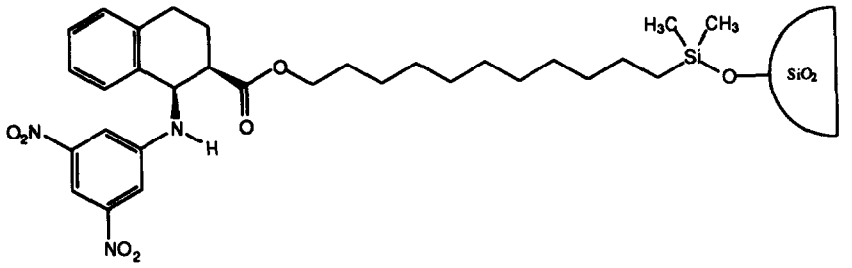
Recent work in the Pirkle laboratories has resulted in the preparation of several new families of chiral selectors which take advantage of the simultaneous face-to-face and face-to-edge $\pi-\pi$ interactions which are believed to be crucial to performance of the above described CSPs. One promising candidate, CSP 41, has recently been prepared and shown to have considerable potential in the resolution of the enantiomers of NSAIDs and other racemates [79].

Another series of CSPs has recently been prepared in which varying ring size in the selector results in differing dispositions of the π-acidic and π-basic rings. Not surprisingly, these CSPs (CSPs 42 and 43) show markedly different behavior in the separation of some racemates [80].

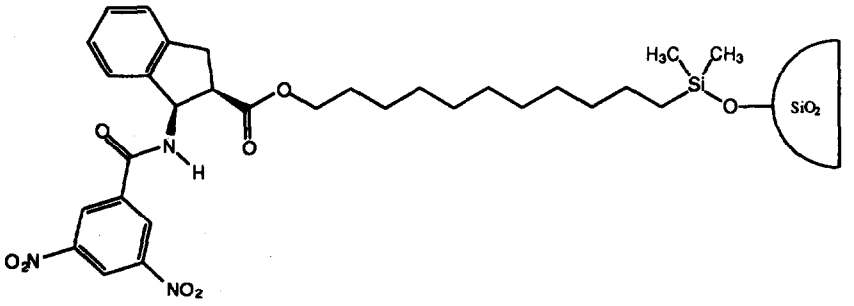
The recent preparation of a series of CSPs containing β-lactam structural units (*e.g.* CSPs 44–47) has resulted in a number of very promising CSPs [81]. In particular, CSP 45 affords improved resolution of β-blocker enantiomers relative to previously developed CSPs such as CSP 32. In addition, these CSPs also show a general ability to resolve the enantiomers of other classes of analytes. While this class of CSPs is still in the development stage, this line of research is certain to result in CSPs of great utility to a number of researchers.



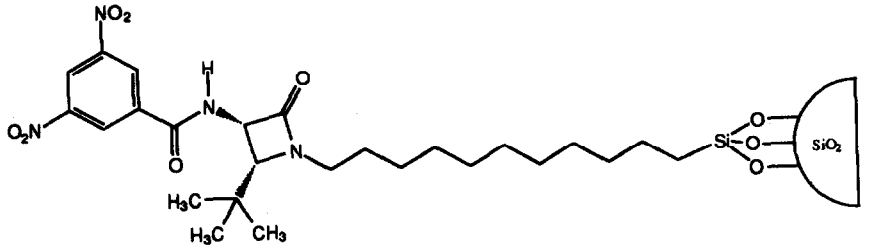
CSP 41



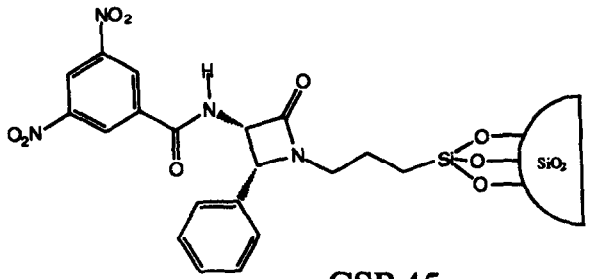
CSP 42



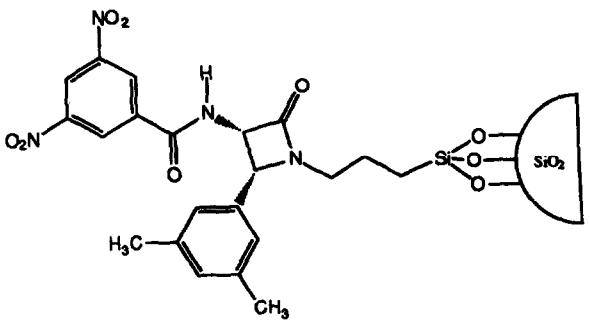
CSP 43



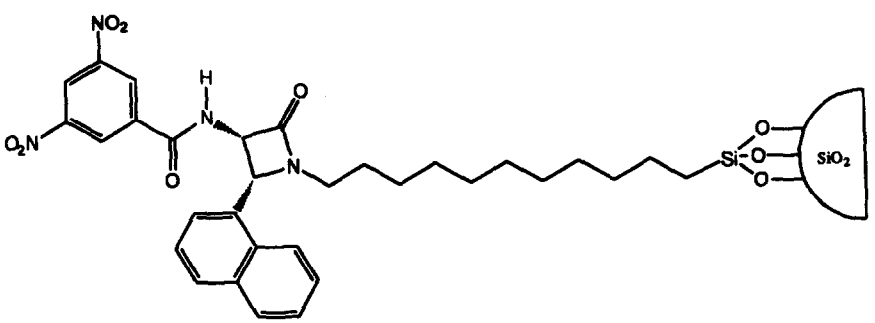
CSP 44



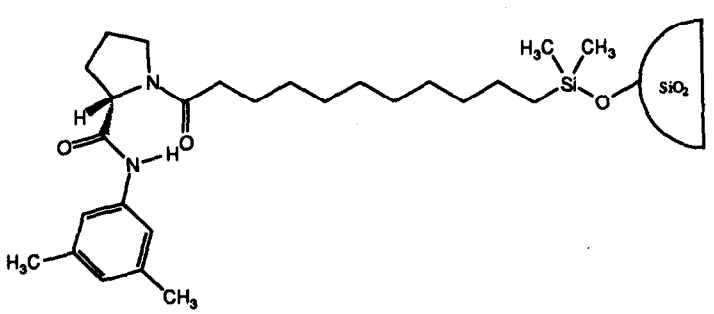
CSP 45



CSP 46



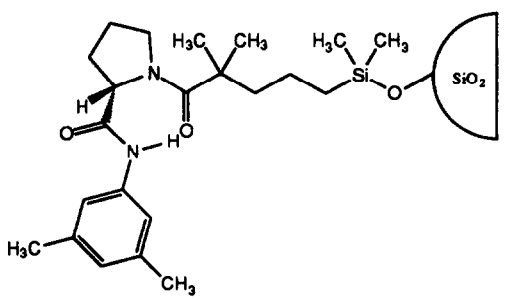
CSP 47



CSP 48

13. CSPs IN DEVELOPMENT II: PROLINE ANILIDE CSPs

Two π-basic proline-derived CSPs, designed to take advantage of the beneficial aspects of "intercalative effects", have recently been prepared and shown to afford very high enantioselectivities for DNB-amino acid derivatives ($\alpha > 85$ in one case) [82]. The facile preparation of these CSPs from a readily available enantiopure starting material (proline) suggests that they may provide an economical method for preparative enantiomer separations.

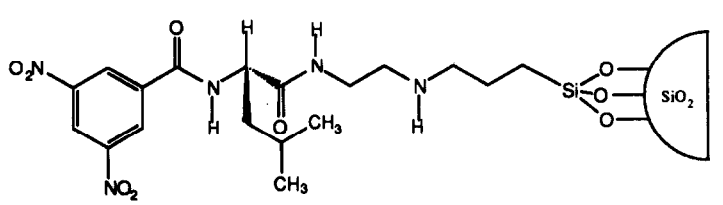


CSP 49

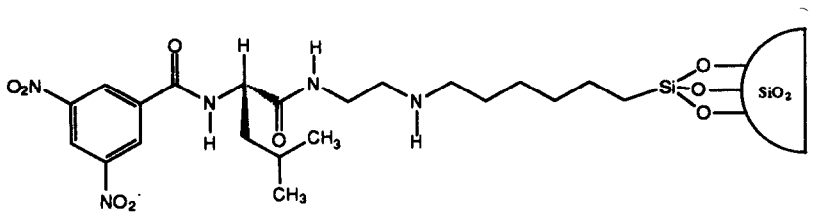
14. CSPs IN DEVELOPMENT III: "FINE TUNING" OF EXISTING CSPs

A number of improvements and modification of existing CSPs have recently been investigated in the Pirkle group. One such investigation focused on a series of analogues of the DNB-leucine-derived CSP 7 which incorporate an amino group in the tether portion of the CSP [83]. The influence of this amino group on chiral recognition has been investigated and found to have a marginal effect for most analytes. However, the presence of this basic group in the tether of CSPs 50 and 51 permits its protonation and allows the formation of ion pairs utilizing anions of varying size, steric bulk and enantiomeric composition. All of these factors influence, to a greater or lesser extent, the degree of chiral recognition afforded by the phase. Using this approach, a single CSP can be reversibly modified so as to form a host of CSPs, each displaying unique properties.

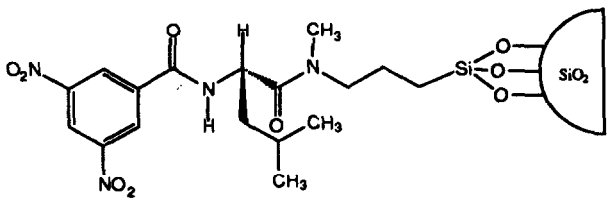
In another study involving tether modifications, a series of CSPs were produced in which non-productive adsorption sites were deleted from the tethers of existing CSPs [84]. The presence of nonproductive adsorption sites in CSPs is well known to increase analyte retention and decrease enantioselectivity [85]. Consequently, by replacing the amide hydrogen in the tether of the leucine-derived CSP 7 (an adsorption site which is believed to normally play no role in chiral recognition) with a methyl group (CSP 52) a CSP with improved performance was expected. This is indeed the case, and CSP 52 shows improved resolution of the enantiomers of many, but not all racemates.



CSP 50



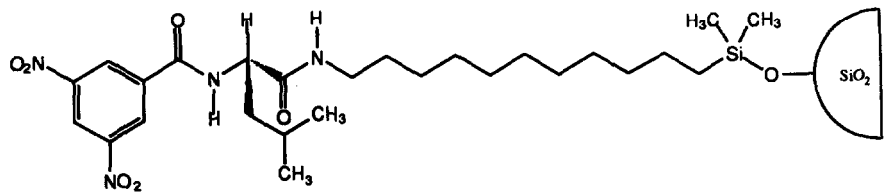
CSP 51



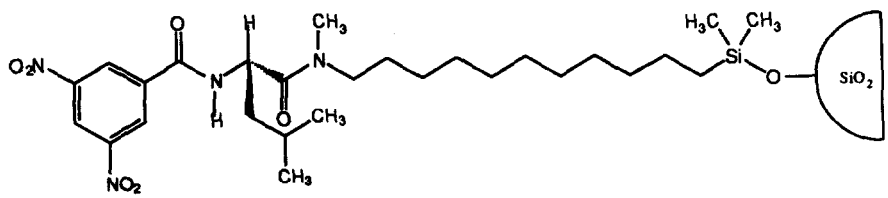
CSP 52

Likewise, CSPs 53–56 behave in a similar fashion, and the doubly linked CSP 57 oftentimes affords the greatest enantioselectivities encountered in the entire series of DNB-leucine-derived CSPs. CSPs 53 and 54, which contain long alkyl tethers, generally perform similarly to the short-tether counterparts, CSPs 55 and 56, under normal-phase conditions. Interestingly, under reversed-phase conditions, these long tether CSPs oftentimes afford increased retention and decreased enantioselectivity relative to their short-tether counterparts, presumably owing to non-specific hydrophobic adsorption arising from the alkyl tether.

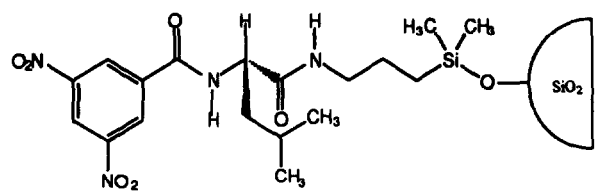
A similar series of naproxen-derived CSPs also illustrates the importance of the deletion of non-



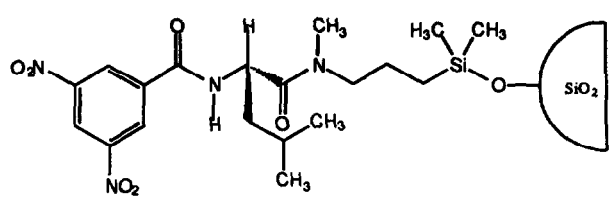
CSP 53



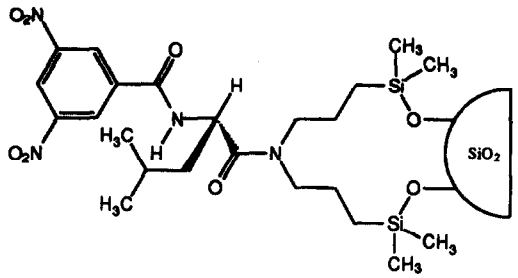
CSP 54



CSP 55



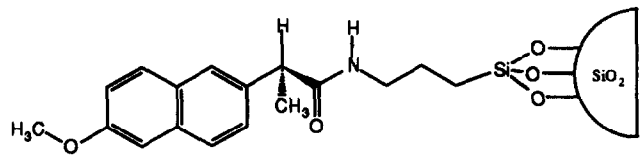
CSP 56



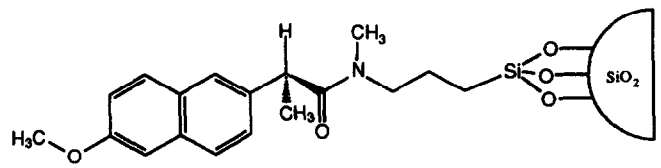
CSP 57

productive adsorption sites. CSP 58, originally described by Doyle *et al.* [86], is a useful CSP for the separation of π-acidic analytes. The amide hydrogen of this CSP was suspected to play no role in the separation of the enantiomers of a number of DNB-containing analytes, and CSPs 59 and 60 were prepared for comparison [84]. Again, the CSPs in which this non-productive adsorption site has been blocked show significantly greater enantioselectivity in the separation of a number of π-acidic racemates, with CSP 61 generally affording the greatest enantioselectivity of the series [87]. All of these amide-linked CSPs perform better than the ester or ionic-linked naproxen-derived CSPs 33 and CSP 34 used to develop the selector used in CSPs 35 and 36.

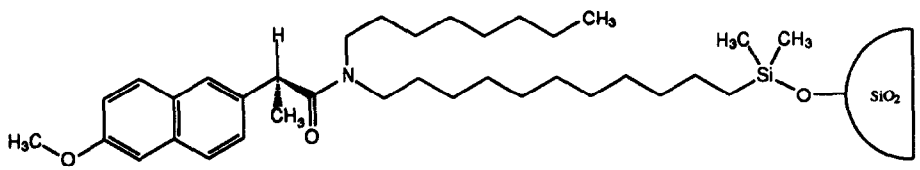
Recently, the application of alternate tethering



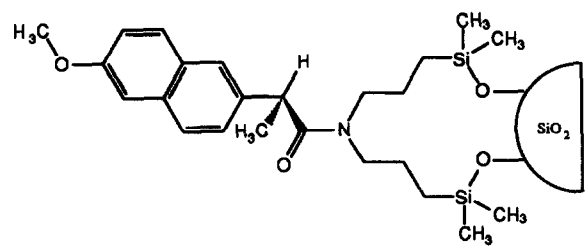
CSP 58



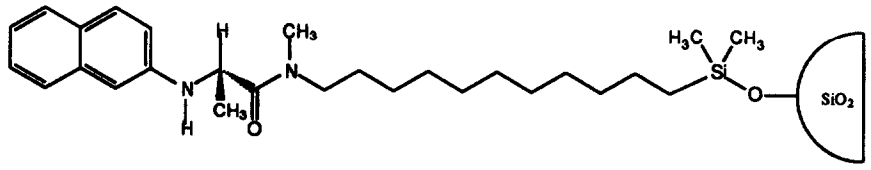
CSP 59



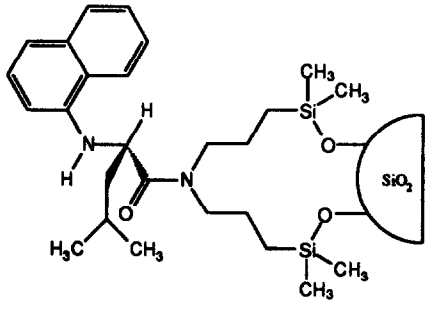
CSP 60



CSP 61



CSP 62



CSP 63

schemes to N-aryl amino acid-derived CSPs such as CSPs 23 and 24 has proven quite successful. The amide-linked N-(2-naphthyl)alanine-derived CSP 62 generally affords enantioselectivities for DNB-amino acid derivatives which are more than double those obtained with the analogous ester-linked CSP 23 [88] The amide-linked N-(1-naphthyl)leucine-derived CSP 63 affords even greater enantioselectivity for these compounds, and separation factors in excess of 125 have recently been obtained for analytes bearing a single stereogenic center using this CSP [89] (arbitrarily large separation factors can be obtained for analytes bearing multiple stereogenic centers [90]). The enhanced enantioselectivity of the amide-linked CSPs can be attributed to the greater electron density of the amide carbonyl oxygen, a hydrogen-bonding site, relative to the ester carbonyl oxygen. In addition to providing enhanced enantioselectivity, these amide-linked CSPs can be expected to be more resistant to selector hydrolysis under reversed-phase conditions.

15. OVERVIEW OF IMPORTANT PRINCIPLES IN CSP DESIGN

Some important principles which have proven useful in the Pirkle group for the design and development of CSPs are herein presented, with the hope and expectation that they will prove useful to other researchers in this field.

(1) Take advantage of the reciprocal nature of chiral recognition. Reciprocal CSPs have proven invaluable in the Pirkle group, both for the study of chiral recognition and the development of improved CSPs.

(2) Eliminate heterogeneity in CSP design. The nature of chiral recognition is difficult enough to elucidate with a selector of a specified structure. The design of CSPs which contain an assortment of different regioisomeric or diasteromeric forms may be synthetically convenient, but the resulting structural complexity makes these CSPs extremely difficult to understand mechanistically.

(3) Keep interaction sites to a minimum. Eliminating superfluous interaction sites affords CSPs which give greater enantioselectivity, less retention, better band shapes, more easily understood mechanisms of action, and greater predictability.

(4) Choose systems for study which go beyond what is already known. Any enantioenriched compound, when immobilized so as to form a CSP, will resolve the enantiomers of *some* racemates. Therefore, the design of a "novel" CSPs can be seen to be a trivial task. Unless such CSPs serve a useful purpose or add to our knowledge of molecular interactions, they are little more than a waste of time and resources.

(5) Do not be afraid to go beyond the chiral pool. While the convenience and economics of chromatographic enantiomer separations using CSPs derived from the chiral pool of enantiopure starting materials cannot be denied, it is highly unlikely that the ideal selector for a given task will be readily available from an inexpensive enantiopure starting material.

(6) Study chiral recognition using all available chromatographic and spectroscopic techniques and methods.

(7) Utilize variable temperature chromatographic experiments to study the thermodymanic basis of chiral recognition [91–94].

(8) Formulate and *put to the test* hypotheses concerning chiral recognition.

(9) Avoid weak linkages in CSP design. CSPs which change properties or have a limited lifetime owing to selector cleavage or leaching are of restricted utility.

16. CONCLUSIONS

The history of CSP design and development in the Pirkle laboratories is replete with examples of how the rational study of chiral recognition can result in products of great utility to the chemical community. While this review has focused primarily upon CSP design, a number of related research areas deserve mention, including the use of chiral selectors in non-chromatographic applications such as deracemization [94] and membrane transport [95], and the application of the principles of CSP design to the development of improved stationary phases for the separation of non-chiral analytes [96]. Given this rich history of innovation and productivity, we can look forward with great anticipation to future technologies emerging from the research laboratories of Professor William H. Pirkle.

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