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Study of the mechanism of enantioseparation of macrocyclic glycopeptide-based chiral stationary phases

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Major Professor

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For the Major Program

This dissertation is dedicated to

My parents, who raised me and encouraged me,

My teachers, who educated me and challenged me,

My love, Michelle, who completed me.

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ABSTRACT

The purpose of this research has been to investigate the mechanisms of chiral separations by high performance liquid chromatography (HPLC). While both cyclodextrin-based chiral stationary phases (CSPs) and macrocyclic glycopeptide-based CSPs were utilized, the goal was to develop a method of interrogating chiral separations that is applicable to all CSPs. This dissertation focuses on the development and application of this approach. The macrocyclic glycopeptide CSPs were used to experimentally test this model/approach and to provide proof of principle.

The linear solvation energy relationship (LSER), developed by Kamlet, Taft, and Abraham, was selected for its ability to de-convolute the interactions a solute experiences in a biphasic system. The theory states that the logarithm of a partition coefficient of a solute is equal to the sum of the interactions the solute experiences in the two phases. The LSER model has a term for each type of solvation interaction. These include interactions through polarizable n- and π-electrons (*e*E), dipolar interactions (*s*S), hydrogen bond acceptance (*a*A) and donation (*b*B), and dispersion forces (*v*V). There are two components for each of the terms of the LSER equation, one term describing the systems ability to interact in the specified manner, and one term describing the solute's ability to interact in the specified manner. The lower case variables describe the characteristics of the system, and are called system constants. The uppercase variables describe the characteristics of the solute, and are called solute descriptors. The system constant *c* contains the phase ratio.

My initial investigations of the macrocyclic glycopeptide CSPs produced unusual results involving the interactions attributed to polarizable n and π electrons. These results prompted me to first investigate the variety of interactions encoded in the polarizable

electron term (*e*-term) of the LSER equation. My initial interpretation of the interactions encoded by this term was that it described π electron association. I determined the system constants for several chromatographic stationary phases that contained varying aromatic and ionic character, and concluded that both π electron complexation and ion-dipole interactions are encoded by the *e*-term of the LSER model.

Next, I examined the system constants obtained from liquid chromatography utilizing macrocyclic glycopeptide CSPs in the reversed-phase mode and the normal-phase mode. The system constants obtained from macrocyclic glycopeptide CSPs are compared with each other and conventional stationary phases. In the reversed-phase mode, the magnitude of the system constants are very different compared with conventional octadecyl stationary phases and the sign of the dipolar *s*-terms for all four macrocyclic glycopeptide CSPs is opposite that of conventional octadecyl silane-bonded stationary phases. In the normal-phase mode, the signs of the system constants for the four macrocyclic glycopeptide CSPs are the same as for conventional normal-phase stationary phases, although the value of the *e*-term (interaction through polarizable n and π electrons) is indistinguishable from zero.

With knowledge of the system constants for the CSPs, it is possible to determine the solute descriptors of each enantiomer. Using multiple linear regression analysis, with the system constants of a chiral stationary phase (at multiple mobile phase compositions/temperatures) as the independent variables and the logarithm of the retention factor of one enantiomer as the dependent variable, the solute descriptors for each enantiomer are determined. The differences in the solute descriptors reveal the relative importance of each intermolecular interaction in generating enantioselectivity.

I first attempted this method of analysis in the reversed-phase mode with the teicoplanin CSP. Several neutral compounds and amino acids were eluted with ethanol/buffer (0.020M triethylamine acetate, pH=4.1) mobile phases, and the solute descriptors for each enantiomer were determined by using the above described method. The statistical fits of the regression were excellent (the coefficient of correlation, r^2, is typically greater than 0.98). It was found that steric repulsions and ion-dipole interactions had the strongest influence on enantioselectivity, with both types of hydrogen bonding having a weak influence on enantioselectivity. Dipolar interactions were found to be unimportant in generating enantioselectivity.

I next attempted to apply the same method of analysis to data obtained from the normal-phase mode with a teicoplanin CSP. Ethanol/heptane solutions were utilized as mobile phases, and the ratio of ethanol to heptane and the column temperature were varied to generate an array of system constants. The regression fit for each set of system constants was quite good (the coefficient of correlation, r^2, was often greater than 0.98), but there was a high degree of intercorrelation between the sets of system constants (the r^2 was as high as 0.97). Fluorinated solvents were utilized to decrease the degree of intercorrelation. Several neutral compounds were eluted with mobile phases composed of ethanol/heptane, ethanol/ethoxynonafluorobutane, and 2,2,2-trifluoroethanol/ethoxynonafluorobutane. The solute descriptors for the enantiomers of 5-methyl-5-phenyl-hydantoin were determined and then used to determine which intermolecular forces influence enantioselectivity in the normal-phase mode. It was found that dipole-dipole interaction and steric repulsion interactions were most important in generating enantioselectivity, and π electron complexation interactions were important, but less important than dipole-dipole interaction

and steric repulsion interactions. Hydrogen bonding was found to be rather unimportant in generating enantioselectivity.

Chapter 1

General Introduction and Literature Review

1.1 General Introduction

Enantiomeric separations and analyses continue to be an important field in science and technology. It has been well established that the enantiomers of a chiral compound can have different biological activities [1, 2]. In pharmaceutical development, nutrition, and biotechnology, the determination of the activity of each enantiomer is a critical step and one required by regulatory agencies [3]. As new chemical entities are developed, the methods to obtain individual enantiomers for this testing are also needed.

The pathways to obtaining individual enantiomers fall into four categories: asymmetric synthesis and catalysis, biological catalysis, synthesis from the chiral pool, and resolution/separation methods. My focus has been to develop and improve separation methods and better understand the process of chiral recognition. By improving our understanding of the intricacies of the chiral recognition process, one can improve the methods used to obtain individual enantiomers. Additionally, the possibility exists to transpose these findings to other methods used to obtain individual enantiomers.

In my study of enantiomeric separations and chiral recognition, I have chosen to study the macrocyclic glycopeptide chiral stationary phases (CSPs). They are one of the newest and the fastest growing class of chiral selector since their introduction in 1994 by Armstrong [4]. They have proven to be very versatile, having excellent enantioselectivity for amino acids (derivatized and underivatized), di- and tri-peptides, sulfoxides,

dihydrofurocoumarins, hydroxy-acids, amino esters, imides, hydantoins, oxazolidinones, nonsteroidal anti-inflammatories, β-adrenergic blocking agents, as well as other pharmaceuticals and agrochemicals [5]. While there has been a great deal of interest in this class of chiral selector, the vast majority of work has focused on the application of the technology to achieve enantiomeric separations and there have been only a few studies pertaining to the mechanism of enantioseparation.

The goal of this research is to improve our understanding of enantiomeric separations. The investigations performed utilized the macrocyclic glycopeptide CSPs in HPLC and this concept can be extended to other chiral selectors and other separation techniques.

This dissertation begins with a general introduction and a literature review of the fields relevant to the presented research. It is followed by four complete scientific papers including all relevant supporting information. These papers focus on the development and application of the selected method to interrogate and better understand chiral recognition. The final chapter contains general conclusions.

1.2 Literature Review – Macrocyclic Glycopeptides as Chiral Selectors for HPLC

Macrocyclic glycopeptide based chiral stationary phases (CSPs) are among the most broadly applicable CSPs available today. Introduced as chiral selectors in 1994, four CSPs

have been developed into commercially viable products. They are multimodal stationary phases, effective in the normal-phase mode, the reversed-phase mode, and the polar organic mode. Additionally, they have been utilized in sub/supercritical fluid chromatography. They are useful on the analytical scale for the determination of enantiopurity, as well as on the preparative scale to obtain gram-scale or larger quantities of single enantiomers. The four commercially available macrocyclic glycopeptide CSPs are complementary to each other; often if one of the macrocyclic glycopeptide CSPs is able to partially separate a pair of enantiomers, a different macrocyclic glycopeptide CSP is able to produce a baseline separation.

1.2.1 Structural features and properties of the most commonly utilized macrocyclic glycopeptides

The four macrocyclic glycopeptides that are most commonly used as chiral selectors were originally discovered by scientists at Eli Lilly & Company in the 1950s [6]. The three compounds, vancomycin, ristocetin A, and teicoplanin, are produced during fermentation of *Streptomyces orientalis*, *Nocardia lurida*, and *Actinoplanes teichomyceticus*, respectively [6-10]. Teicoplanin aglycon is produced by the removal of the sugar moieties via acid hydrolysis [11].

These four compounds have many structural features in common. Figure 1 gives the structures of vancomycin, ristocetin A, teicoplanin, and teicoplanin aglycon. All four compounds have a peptide chain, beginning with the amine group on the right hand edge of each structure, and terminating at the carboxylic acid residue on the left hand edge of each structure. Both the amine and the carboxylic acid groups are ionizable and are usually

ionized in the presence of aqueous and hydro-organic solvent mixtures [7]. In the case of ristocetin A, the carboxylic acid group is esterified (as a methyl ester). Most of the side chains of the peptide are phenolic residues, and many of these phenolic groups are linked to one another. These cross-linked phenolic residues on the main chain create macrocyclic rings that give these molecules a basket-like shape. Ristocetin A, teicoplanin, and teicoplanin aglycon have four fused macrocyclic rings, while vancomycin has three fused macrocyclic rings.

Molecular modeling was utilized to study the shape of first three macrocyclic glycopeptides (vancomycin, ristocetin A, and teicoplanin) [12]. Energy minimized structures were generated and compared with each other. The three compounds all appeared to have similar "C-shaped" aglycon baskets, but the degree of openness of the basket and the helical twist varied between the three compounds (Figure 2A). Vancomycin was found to have the most open aglycon basket, with an edge-to-edge distance of 9.3 Å, and teicoplanin was found to have the most closed aglycon, having an edge-to-edge distance of 4.5-5.5 Å. Ristocetin A had intermediate edge-to-edge distances of 5.2-8.8 Å. The variations in the edge-to-edge distances of the aglycon baskets were attributed to the helical twist of the aglycon, which makes some edges of the aglycon closer to each other compared with other edge pairs.

Helical twist is another feature found to influence the shape and openness of the aglycon basket (Figure 2B). Helical twist is assessed by defining an "axial ratio" of AR = length of the major axis/length of the minor axis. The axial ratio is a measure of the deformation of a ring system. An axial ratio close to one indicates that the ring system is nearly circular. The axial ratios for vancomycin are ~1.3-1.7, for teicoplanin are ~1.38-1.42, and for ristocetin A are ~1.36-2.13. While none of the axial ratios approach one, teicoplanin

has the least deformed or strained aglycon basket and vancomycin and ristocetin A have the most deformed or strained aglycons [12].

Three of the macrocyclic glycopeptide molecules have several sugar moieties bonded to them. Vancomycin has a single disaccharide consisting of D-glucose and vancosamine. Ristocetin A has two monosaccharides and a tetrasaccharide that is composed of D-arabinose, D-mannose, D-glucose, and D-rhamnose. Teicoplanin has three monosaccharides, two of which are D-glucosamine units and one is a D-mannose unit. One of the amine groups of teicoplanin's D-glucosamine is acetylated, and the amine of the other D-glucosamine possesses a fatty acid chain. There are five related teicoplanin glycopeptides that differ only in the fatty acid chain bonded to the sugar group. The most common glycopeptide has an 8-methyl-nonoic acid chain. Because of the hydrophobic fatty acid chain and the ionizable groups on the aglycon portion of the molecule, teicoplanin is surface active, with a critical micelle concentration of 0.18 mM in unbuffered aqueous solutions [12]. The sugar groups of vancomycin and ristocetin A also possess ionizable amine groups that are usually protonated in the presence of aqueous and hydro-organic solvent mixtures; however, these two macrocyclic glycopeptides are not surface active.

Being composed of both carbohydrates and amino acids, the macrocyclic glycopeptide antibiotics have abundant stereogenic centers. Ristocetin A has the largest number of stereogenic centers at 38, with teicoplanin and vancomycin having 23 and 18, respectively. Teicoplanin aglycon has eight stereogenic centers. The molecular masses of these compounds range from 1197 to 2066 g/mole. They are soluble in water and acidic aqueous solutions but are less soluble at neutral pHs [13]. Additionally, they are moderately

soluble in polar aprotic solvents such as DMSO and DMF but insoluble in most other organic solvents.

The three macrocyclic glycopeptides function *in vivo* as antibiotics. Vancomycin binds to the cell wall of gram-positive bacteria and prevents cell wall growth, which eventually causes rupture and cell death. It was determined that vancomycin binds to D-alanine-D-alanine groups present in the cell wall of gram-positive bacteria [7, 14, 15]. Given this strong binding to a chiral moiety, it was proposed that vancomycin and related compounds would make good chiral selectors.

1.2.2 Macrocyclic Glycopeptides as Chiral Selectors for HPLC

In 1994, macrocyclic glycopeptides were first introduced as chiral selectors for HPLC [4]. Three different CSPs were produced with the macrocyclic glycopeptides vancomycin, rifamycin B, and thiostrepton bonded to silica supports. The three CSPs were evaluated in the reversed-phase mode and normal-phase mode. The most broadly applicable chiral selector was vancomycin, separating the enantiomers of 70 racemates. Only a few enantioseparations were reported for rifamycin B and thiostrepton. There were 45 enantioseparations in the reversed-phase mode and 31 in the normal-phase mode. A large number of classes of compounds were analyzed, including derivatized amino acids, methyl esters, nitrogenous bases, hydantoins, and carboxylic acids.

After the success of the vancomycin CSP, other similar macrocyclic glycopeptide antibiotics were evaluated. The antibiotic teicoplanin was immobilized on a silica support and evaluated as a chiral selector for HPLC [16]. In the reversed-phase mode, 99 enantioseparations were reported, along with 29 enantioseparations in the polar organic mode

and 21 enantioseparations in the normal-phase mode. While there was a great variety in the functionalities and classes of the analytes, ~75% of the analytes that were separated in the reversed-phase mode contained carboxylic acid groups. Additionally, the authors noted the exceptional enantioselectivity of the teicoplanin CSP for native amino acids and peptides.

This exceptional selectivity for amino acid-based analytes was further explored in the reversed-phase mode [17]. The study included all underivatized protenic amino acids, a large number of non-protenic amino acids, as well as several di-peptides. The effects of pH, buffer type and concentration, and organic modifier type and concentration were discussed. It was found that, for most of the compounds, no buffers or salts are required for the elution or enantioseparation of these compounds, allowing for easy isolation of the eluted enantiomers in preparative chromatography. The enantioselectivity of amino acids was found to be rather insensitive to small changes in pH, ionic strength, and organic modifier content. The naturally occurring L-enantiomer of all of the amino acids was eluted first, and the D-enantiomer was retained longer. The enantiomeric elution order of di-peptides was found to be more complicated. While often the D,D-dipeptides were more retained, there were a few cases where the L,D-dipeptides interacted more strongly with the teicoplanin chiral selector.

The performance of a derivatized vancomycin CSP was evaluated in the three modes of HPLC [18]. Vancomycin was derivatized with either *R*- or *S*-(1-napthylethyl)-isocyanate, yielding the corresponding carbamate. This derivative has proven to be effective at improving the performance of other CSPs [19-22]. Additionally, the derivatizing agent is chiral and creates additional interaction sites for chiral recognition. The solid vancomycin CSP was exposed to a 10:1 molar ratio of the isocyanate (10 moles of isocyanate to 1 mole of

vancomycin on the surface of the silica), generating a randomly substituted chiral selector. Of the 60 compounds tested, 45% were separated on both derivatized and underivatized CSPs, 43% were separated on the derivatized CSP only, and 12% were separated on the underivatized CSP only. The compounds tested included: derivatized amino acids, hydantoins, alcohols, amino alcohols, methyl esters, and amines. Many of the separations that were obtained on both stationary phases were very similar. The most noticeable difference was the enantioseparation of dinitrobenzoyl-amino acids, which the derivatized phase was much better at separating. This improved chiral recognition was attributed to the π electron donor - π electron acceptor interactions between the dinitrobenzoyl groups on the amino acids and the napthyl groups of the derivatized CSP. Despite these improved enantioseparations, no further work on the derivatized vancomycin CSP has been found in the literature.

The third macrocyclic glycopeptide that was found to be a broadly applicable CSP for HPLC is ristocetin A [23]. The chiral selector was bonded to silica gel and evaluated in the three modes of liquid chromatography with over 230 chiral analytes, including natural and unusual amino acids, derivatized amino acids, di- and tri-peptides, alcohols, amines, binapthyl compounds, nonsteroidal anti-inflammatories, hydantoins, and amino alcohols. This CSP was shown to be complementary to the other macrocyclic glycopeptide CSPs; compounds that are partially separated into enantiomers by this CSP are often resolved by other macrocyclic glycopeptide CSPs. Similar to the teicoplanin CSP, the ristocetin A-based CSP has excellent enantioselectivity for native and derivatized amino acids and peptides. Unlike the teicoplanin-based CSP, there were a few cases where the naturally occurring L enantiomer of an amino acid was more retained than was the unnatural D enantiomer. This

change in the elution order of enantiomers was thought to be very desirable for analysis of "real-world" samples that are likely to contain a large excess of the L enantiomer.

The final macrocyclic glycopeptide that has proven to be a useful chiral selector is teicoplanin aglycon [11]. This compound was produced by removing the sugar units from teicoplanin by acid hydrolysis. After purification, teicoplanin aglycon was covalently bound to a silica support by the same method as the other macrocyclic glycopeptides and evaluated chromatographically. The teicoplanin aglycon CSP was compared with a commercially available teicoplanin CSP, and the enantioselectivity of both columns for 26 compounds was assessed. The analytes included carboxylic acids, amines, hydantoins, amino alcohols, chiral ethers, and 13 amino acids. Both the teicoplanin and teicoplanin aglycon CSPs were effective at the enantioseparation of the amino acid analytes, with the teicoplanin aglycon CSP often producing greater enantioselectivity and chromatographic resolution. The authors attribute this to two factors: firstly the electrostatic interaction between the ionized amine group on both chiral selectors and the ionized carboxylic acid group of the analytes, and, secondly, access to the aglycon basket portion of the chiral selectors. The authors suggest that the sugar units of teicoplanin block access to the "basket" portion of the aglycon, which seems to be important for the chiral recognition of amino acids. Non amino acids compounds that possess carboxylic acid groups also interact with the ionized amine of the chiral selectors; however, there is no discernible trend regarding whether the sugar units of the chiral selector are beneficial or not (for the enantioseparation of these molecules). The neutral compounds and amine-containing compounds that do not possess an anionic group were generally better separated by the teicoplanin CSP, suggesting a mechanism of separation involving both the aglycon basket and the sugar units. While this was proposed as

a mechanistic study to evaluate the role of the sugar units of teicoplanin in enantioselectivity, the teicoplanin aglycon CSP was so successful that the stationary phase was developed into a commercial product.

Since the introduction and commercialization of the macrocyclic glycopeptides vancomycin, teicoplanin, ristocetin A, and teicoplanin aglycon as chiral selectors for HPLC, there have been many applications of these CSPs to the enantiomeric separation of large groups of molecules. The enantioselectivity of these CSPs for a variety of chiral sulfoxides was examined by Berthod et al. [24]. Twenty-eight substituted dihydrofurocoumarins were analyzed by Xiao et al. [25]. The separation of unusual amino acids has been exhaustively covered by Peter et al. [26-41]. Additionally, Peter has also reported the enantioseparation of β-lactam antibiotics [42] and 1,3-bicyclic-amino-alcohols [43]. There have been several studies that utilize the macrocyclic glycopeptide CSPs to analyze pharmaceuticals and their metabolites in biological fluids [44-50].

1.2.3 Mechanistic Studies on Macrocyclic Glycopeptide CSPs in HPLC

The mechanism of enantioseparation by vancomycin was explored by capillary electrophoresis (CE) [51]. While this research is not conducted using liquid chromatography, the implications to the mechanism of enantioseparation warrant its inclusion in this review. It was found that vancomycin can form an easily isolatable complex of divalent copper, and x-ray crystallography indicated that the copper atom was coordinated with the amine group located on the aglycon portion of vancomycin (as opposed to the amine on the sugar group) [52]. The copper complex was generated in solution, isolated, and used in the run buffer of a CE experiment as the chiral selector. A large number of analytes were analyzed under

slightly acidic conditions, pH 6-7, (a condition where the vancomycin chiral selector is very effective for the analytes tested). No enantioseparations were observed at this condition. Similarly, no enantioseparations were observed at pH 9 with the vancomycin-copper complex. A lower pH of 5 was attempted, and 15 chiral carboxylic acids were partially separated. These compounds were much more effectively separated into enantiomers by the uncomplexed vancomycin. The authors concluded that the copper ion blocks a site on the vancomycin molecule that is critical for chiral recognition. Since the x-ray crystallography data indicated that the copper atom was complexed with the aglycon amine, it was clear that the electrostatic interactions between the amine and anions are critical in generating enantioselectivity.

The complexation of teicoplanin with copper was also explored by HPLC [53]. A teicoplanin CSP was equilibrated with buffered mobile phases containing Cu^{2+}, and the equilibration time was taken as a measure of the amount of copper adsorbed to the CSP. When 0.5 mM Cu^{2+} was in the mobile phase, 9 μmol of copper was estimated to be adsorbed to the stationary phase, corresponding roughly to one copper atom for every 45 teicoplanin molecules. When the concentration of copper was increased to 5.0 mM Cu^{2+} in the mobile phase, the amount of adsorbed copper increased to ~50 μmol, corresponding to approximately one copper atom for every eight teicoplanin molecules. Clearly, this level of copper adsorbed to the stationary phase indicates that divalent copper did not form a strong 1:1 complex with teicoplanin like it did with vancomycin. Chromatographic performance for amino acids was generally worse than with the uncomplexed teicoplanin CSP. While the ability of the copper complexed teicoplanin CSP to resolve the enantiomers of amino acids is reduced, and this is analogous to the results obtained from the vancomycin-copper complex,

the decrease in performance is attributed to the ability of amino acids to form a complex with copper in solution. The performance of the copper complexed CSP for neutral molecules was unpredictable, with some molecules being better separated by the copper complex CSP and some being better separated by the native teicoplanin CSP.

This study also examined isotopic effects on enantioselectivity [53]. Teicoplanin has 101 hydrogen atoms, 25 of which are exchangeable with deuterium, including the amide hydrogen atoms on the peptide chain, phenolic hydrogen atoms, hydroxyl hydrogen atoms on the sugar units, and the hydrogen atoms of the amine groups. Deuterated mobile phases were utilized to replace these hydrogen atoms. No salts, acids, or bases were used in the mobile phase for these experiments. The enantioseparations obtained with the partially deuterated teicoplanin CSP were largely unchanged from those obtained with the native teicoplanin CSP. Amino acids and other amine-containing compounds were retained less, and neutral compounds were retained more. Enantioselectivity was essentially unchanged. The authors attribute this to the isotopic effects changing the interactions between the chiral selector and enantiomers equally and therefore having no net effect on enantioselectivity.

The mechanism of enantioseparation of two series of compounds was investigated in a series of papers by Lehotay and Armstrong et al. [54-58]. Both alkylamino derivatives of aryloxypropanols and alkoxy-substituted esters of phenylcarbamic acids were analyzed with teicoplanin and vancomycin CSPs in the polar organic mode [54]. Electrostatic interactions and hydrogen bonding contributed to enantioselectivity for these compounds in the polar organic mode, when it was previously believed that only hydrogen bonding was active in generating enantioselectivity in this mode. Subsequent papers focused on alkoxy-substituted esters of phenylcarbamic acids. The next publication in this series focused on method

development for the teicoplanin and teicoplanin aglycon CSPs in the polar organic mode and the role of the sugar units of teicoplanin in generating enantioselectivity [55]. The teicoplanin aglycon CSP was found to be more effective at resolving the enantiomers of these compounds. The authors concluded that structural groups that possessed great steric bulk that were in close proximity to the stereogenic center had a large impact on the separation of enantiomers, decreasing the degree of separation.

The remaining papers in this series were thermodynamic van't Hoff studies on the teicoplanin aglycon CSP [56], an experimentally prepared methylated-teicoplanin CSP [57] and the vancomycin CSP and teicoplanin CSPs [58]. The compounds were eluted with polar organic mobile phases at temperatures varying between zero and 50°C. The plots of the natural log of the retention factor vs. the inverse of absolute temperature (van't Hoff plots) were linear across the selected range. The changes in enthalpy of transfer were always greater than the changes in entropy of transfer for these compounds in the polar organic mode. Additionally, the changes in enthalpy and entropy decreased with increasing aliphatic chain length.

The four macrocyclic glycopeptides have proven to be versatile CSPs. They are multimodal stationary phases possessing good chromatographic efficiency and long-term stability. While there has been many applications developed utilizing these CSPs, there have been only a few studies on the mechanism of enantioseparation. This is primarily due to their relatively recent introduction and the overall difficulty in devising and carrying out definitive experiments that provide unambiguous mechanistic information.

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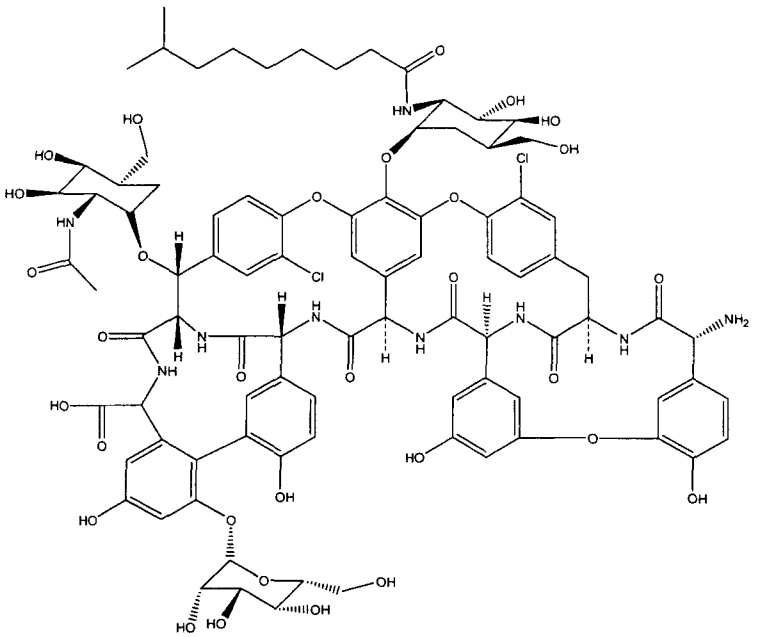
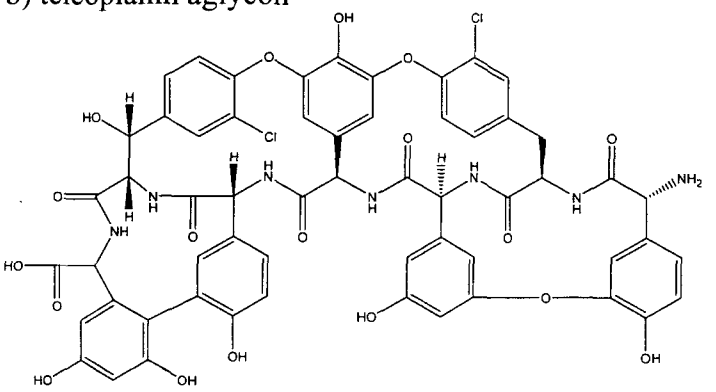
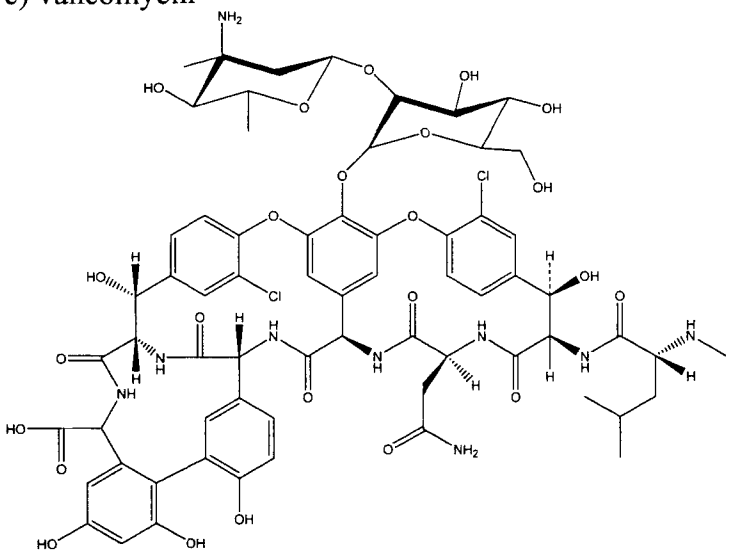
a) teicoplanin



b) teicoplanin aglycon



c) vancomycin



d) ristocetin A

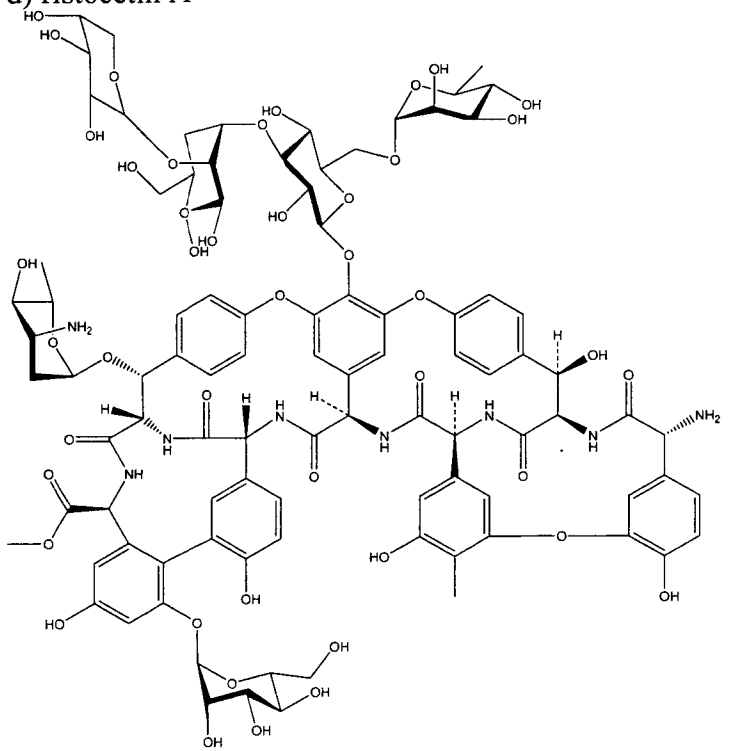


Figure 1. Structure of a) teicoplanin b) teicoplanin aglycon c) vancomycin and d) ristocetin

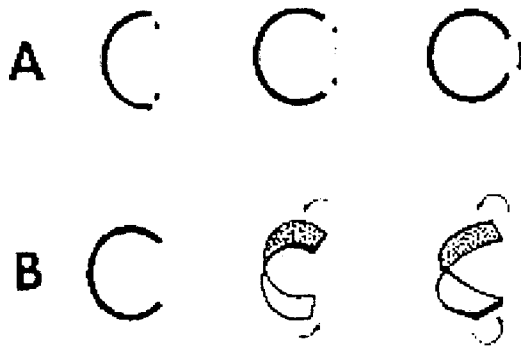


Figure 2. Simplified schematic showing two important morphological characteristics of the aglycon part of glycopeptide antibiotics. (A) End-to-end distance (represented by the length of the arrow) decreases from left to right. (B) The "C-shaped" aglycon also can be twisted to different degrees. The helical twist increases from left to right in this series of three figures. Reproduced with permission from reference 12.

CHAPTER 2

The Manifestation of Charge Transfer Interactions and Ion-Dipole Interactions in the Linear Solvation Energy Relationship

A paper submitted to Journal of Chromatography A

Clifford R. Mitchell, Daniel W. Armstrong

Abstract

The linear solvation energy relationship (LSER) developed by Kamlet, Taft, and Abraham is utilized to characterize chromatographic stationary phases with varying aromatic and ionic character. One of the terms of this LSER method (the *e*-term) quantifies interactions through polarizable n and π electrons between a solute and a stationary phase. Such interactions are stronger in nonpolar solvents and less significant in polar solvents. However, when using this LSER approach, several examples were found where certain stationary phases had larger *e*-terms in the reversed-phase mode (even on stationary phases with no n and π electrons) than in the normal-phase mode. We believe that this can be explained if the *e*-term encodes not only n/π and π/π interactions, but also ion-dipole interactions. This hypothesis was supported with experimental data from a divinylbenzene stationary phase that indicate that some π electron interaction does occur in the reversed-phase mode and by data from an ion exchange stationary phase and a Primesep D cationic stationary phase indicates that ion-dipole interactions also occur in the reversed-phase mode.

It is demonstrated that both interactions are manifested in the *e*-term of the LSER and can be used to properly interpret retention results.

2.1 Introduction

One of the methods we are currently employing to study chiral stationary phases is the linear solvation energy relationship (LSER), developed by Abraham, Kamlet, and Taft. This method is able to deconvolute the intermolecular forces that contribute to retention. The relationship

$$SP = c + eE + sS + aA + bB + vV$$

equates a solute property (such as the logarithm of the partition coefficient) to the interactions the solute experiences between the two phases [1]. The variables E, S, A, B, and V are solute descriptors whose definitions are as follows. E is an excess molar refraction that is obtained from a compound's measured refractive index. S is the solute dipolarity/polarizability. A and B are the hydrogen bond acidity and basicity of a solute, respectively, and V is the McGowan volume (in $cm^3/100$). The corresponding variables, *c*, *e*, *s*, *a*, *b*, and *v*, are system constants obtained by multiple linear regression analysis and are defined as follows. The *e*-term is the system's ability to interact through n and π electrons. The *s*-term is the system's ability to interact via dipolar interactions. The *a*- and *b*-terms are the system's hydrogen bond basicity and acidity, respectively (an acidic solute will interact with a basic phase), and the *v*-term is the system's ability to participate in dispersion interactions. The *c*-term contains the chromatographic phase ratio. This relationship has been used to describe liquid-liquid extraction [2], the uptake of drugs in intestinal absorption

[3] and oral absorption [4], the determination of water solubility [5], the determination of octanol-water partition coefficients [6], gas-liquid chromatography [7, 8], and HPLC [9-14].

During the course of these investigations, we have noted significant variability in the *e*-term, in the normal-phase mode and reversed-phase mode. Our initial interpretation of the interactions encoded by the *e*-term was n/π and π/π stacking interactions. It is generally accepted that n/π and π/π interactions are more significant in nonpolar solvents (e.g., the normal-phase mode) and are diminished in more polar solvents (e.g., the reversed-phase mode) [15, 16]. However, our findings were inconsistent with our chemical intuition; that is, the measured *e*-term was indistinguishable from zero in the normal-phase mode (when π complexation should be relatively strong) and rather strong in the reversed-phase mode (when π complexation should be relatively weak).

Upon re-examination of the seminal papers regarding LSER theory, Abraham indicates that the *e*-term only indicates interactions through polarizable n and π electrons. While this should obviously include n/π and π/π stacking interactions, it may also include ion-dipole interactions (since an ion can polarize n and π electrons). Consequently, the *e*-term could be an indicator of the ionic character of a stationary phase. The goal of this research communication is to examine whether or not both n/π and π/π (aromatic character) and charge manifest themselves in the *e*-term when examining liquid chromatographic systems.

2.2 Experimental

2.2.1 Materials

HPLC grade acetonitrile, trifluoroacetic acid, and triethylamine were purchased from Fisher (Fairlawn, NJ). ACS grade potassium phosphate dibasic was purchased from Fisher. ACS grade potassium phosphate monobasic was purchased from EM Science (Darmstadt, Germany). HPLC grade water was obtained in house using reverse osmosis water filtered through an ion exchange resin. LSER probe molecules were purchased from Aldrich (St. Louis, MO) in high purity grade. The probe solutes are listed in Table I with their solute descriptors.

The Primesep C and D HPLC columns were purchased from SiELC (Prospect Heights, IL) and were 0.46 × 5.0 cm. The Jordi Gel DVB RP 500A HPLC column was purchased from Alltech and was 0.46 × 15.0 cm. The Allsphere SAX column was purchased from Alltech and was 0.46 × 25.0 cm. The C18 HPLC column was purchased from ASTEC (Whippany, NJ) and was 0.46 × 5.0 cm. The Supelcosil LC-DP HPLC column was purchased from Supelco (Bellefonte, PA) and was 0.46 × 5.0 cm. All stationary phases were comprised of 5 μm spherical particles.

2.2.2 HPLC

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett Packard, Palo Alto, CA, USA), and an integrator (3395, Hewlett Packard). Mobile phases were prepared as volumetric ratios and degassed by a helium sparge. UV detection was carried out at 210 nm. All separations were carried out at room temperature (25°C).

2.2.3 Calculations

Retention factors (k) were calculated using the equation $k = (t_r - t_M)/t_M$. Multiple linear regression analysis and statistical calculations were performed using the program Analyse-it, an add in program for Microsoft Excel.

2.3 Results and Discussion

Several HPLC stationary phases of varying aromatic and ionic character were investigated using the LSER approach. The system constants for each system examined are listed in Table II. Mobile phases were composed of acetonitrile and water, and buffers or acids were used to promote ionization of the stationary phase where applicable. The mobile phase organic content to aqueous content ratio was selected to give a broad range of retention for the LSER probe solutes. Due to the wide variety in the retentiveness of these stationary phases, the stationary phases cannot be compared with each other at the same condition using the selected probe solutes. To make comparisons between the stationary phases at the same condition, the log k_w of each solute was determined. The log k_w is a simulated value of the log of the retention factor in a 100% aqueous mobile phase. It is determined for an individual solute by plotting several of the measured log k's versus the mobile phase composition and extrapolating to 0% organic solvent. The log k_w is determined for each solute, and the correlation coefficients for these extrapolations were all 0.98 or better and frequently 0.99 or better.

Figure 1 shows the system constants obtained for the different stationary phases at condition log k_w. Clearly, there is a great deal of variation in all of the system constants for each system, which can be explained by considering the innate ability of each stationary

phase to participate in these interactions (or adsorb mobile phase solvents that can participate in these interactions). Figure 2 shows the *e*-terms from each system. There is also a great deal of variation in each system's ability to participate in the interactions encoded by the *e*-term.

The stationary phase with the weakest interactions based on the *e*-term is the C18 stationary phase from Astec, which has an *e*-term of 0.12 at the condition log k_w. The C18 ligand is bonded to the silica support via a tri functional reagent, and the media is not end-capped. This stationary phase does exhibit tailing when analyzing bases and must therefore have significant silanol activity. Furthermore, since the aqueous portion of the mobile phases used was phosphate buffer at pH 7.0, the silanol groups on the silica surface are predominantly ionized at the conditions used to character the stationary phase. As there are no π electrons available to interact with solutes and the n electrons that are available are located on ionized silanols, it is unlikely that the *e*-value of the C18 stationary phase represents n/π or π/π interaction but rather an ion-dipole interaction. Additionally, it is not unusual for alkyl ligand-based stationary phases to have an *e*-term that is zero or indistinguishable from zero [17].

The diphenyl stationary phase from Supelco has an *e*-term of 0.24 at condition log k_w. This stationary phase is also a silica-based material and therefore a component of the *e*-term likely comes from ionized silanols on the silica surface. Additionally, the presence of the aromatic groups on the surface of the stationary phase creates the possibility for π-electron interactions. The elevated *e*-value for this stationary phase, compared with the C18, is likely due to this.

The Primesep D from SiELC has the next highest *e*-term of 0.36. This stationary phase consists of a C12 alkyl ligand bonded to a silica support, with an amine embedded in the C12 chain. The pKa of the amine is greater than 9 and is therefore ionized across the pH range at which the stationary phase is stable (0.5 – 4.0). The mobile phases used to characterize this stationary phase contained 0.1% trifluoroacetic acid. Given the absence of n and π electrons in the stationary phase and the presence of charged groups, both on the silica surface and in the alkyl chain, the interactions encoded by the e-term for this stationary phase must be the result of polarization of the n and π electrons on the analytes by the charged groups in the stationary phase.

The Jordi Gel stationary phase has an *e*-term of 0.39 at condition log k_w. The support of this stationary phase is composed of divinylbenzene, contains no ligands, and is free of silica or other ionizable compounds. In this case, the *e*-term for this stationary phase can only be due to π-electron interactions.

The stationary phase with the largest e-term, 0.52, is the Allsphere SAX. The system constants for this stationary phase were obtained at 5/95 acetonitrile/30 mM phosphate buffer pH 5.0. The retention of the probe solutes was extremely weak with mobile phases with larger amounts of acetonitrile, and most probe solutes did not elute with a 100% aqueous mobile phase. The stationary phase consists of a trimethyl-propyl-ammonium ligand bonded to a silica support. Clearly, there is a large amount of charge on the surface of this media from the trimethyl-propyl-ammonium group, as well as ionized silanols. The lack of π electrons on the stationary phase and the large amount of charge on the surface suggest that the interaction encoded by the *e*-term is an ion-dipole interaction.

While π-electron complexation and ion-dipole interactions seem to be intermixed in the *e*-term, consideration of the composition of the stationary phase can indicate what type of interactions are occurring in the system. If a stationary phase is devoid of π electrons and has ionizable residues, the e-term most likely indicates ion-dipole interactions. Conversely, if a stationary phase contains no ionizable groups and there are π electrons present, the e-term is likely indicative of π complexation. In the normal-phase mode, the e-term is most likely an indication of π complexation because ions are poorly solvated by normal-phase solvents and any fixed charges in the stationary phase (such as quaternary amines) would exist as an ion pair with a counter-ion.

2.4 Conclusions

While the *e*-term is a rather weak component of retention in the reversed-phase mode, it encodes two different types of interactions: π-electron complexation and ion-dipole. Despite the rather weak interaction strength of π-electron complexation in the reversed-phase mode, the data obtained from the divinylbenzene stationary phase indicates that π-electron complexation does occur in this mode. However, the data obtained from other stationary phases that contain ionized groups and no π-electrons indicate that there must also be some ion dipole interaction encoded in the *e*-term of the LSER.

Acknowledgement

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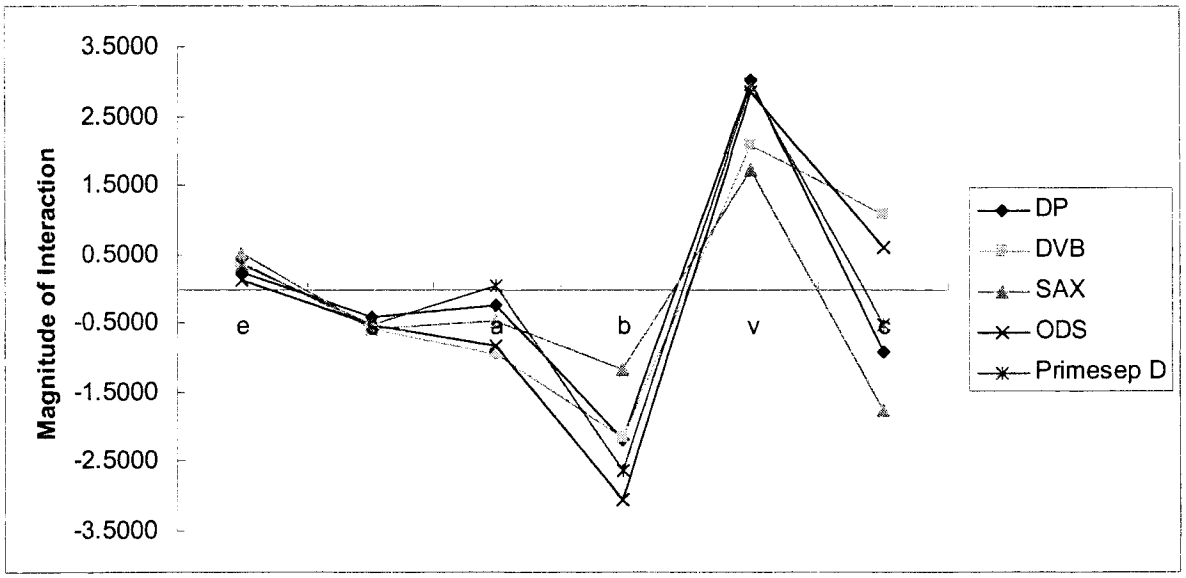

Figure 1. System constants for the stationary phases in this study at log K_w. DP – Supelcosil LC-DP diphenyl column, DVB – Jordi Gel DVB RP divinylbenzene, SAX – Alltech Allsphere anion exchange column, ODS – C18 column from Astec, Primesep D – mixed mode C12/anion exchange from SiELC.

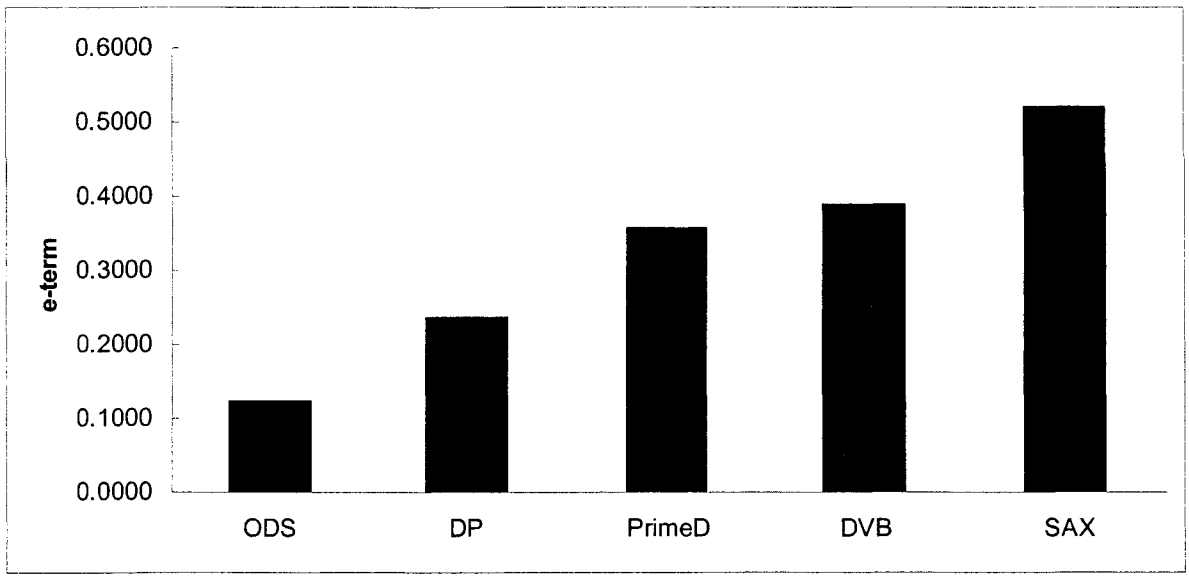

Figure 2. *e*-term system constants for the stationary phases in this study. Column designations are the same as in Figure 1.

Table I. Probe solutes and their solute descriptors.

	E[a]	S	A	B	V
1,3,5-trihydroxybenzene	1.355	1.12	1.40	0.82	0.8925
1,3-benzenediol	0.980	1.00	1.10	0.58	0.8338
1-nitrobutane	0.227	0.95	0.00	0.29	0.8464
4-ethylphenol	0.800	0.90	0.55	0.36	1.0569
3-cyanophenol	0.930	1.55	0.77	0.28	0.9300
benzonitrile	0.742	1.11	0.00	0.33	0.8711
acetophenone	0.818	1.01	0.00	0.48	1.0139
phenol	0.805	0.89	0.60	0.31	0.7751
2-nitrophenol	1.015	1.05	0.05	0.37	0.9490
1-nitropentane	0.212	0.95	0.00	0.29	0.9873
o-cresol	0.840	0.86	0.52	0.30	0.9160
m-cresol	0.822	0.88	0.57	0.34	0.9160
p-cresol	0.820	0.87	0.57	0.31	0.9160
methyl-4-hydroxybenzoate	0.900	1.37	0.69	0.45	1.1313
nitrobenzene	0.871	1.11	0.00	0.28	0.8906
n-ethyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.2722
1-nitrohexane	0.203	0.95	0.00	0.29	1.1282
benzene	0.610	0.52	0.00	0.14	0.7164
4-nitrotoluene	0.870	1.11	0.00	0.28	1.0315
m-trifluoromethylphenol	0.425	0.87	0.72	0.09	0.9691
n-propyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.4131
4-chlorophenol	0.915	1.08	0.67	0.20	0.8970
toluene	0.601	0.52	0.00	0.14	0.8573
o-xylene	0.663	0.56	0.00	0.16	0.9982
m-xylene	0.623	0.52	0.00	0.16	0.9982
p-xylene	0.613	0.52	0.00	0.16	0.9982
3-chlorophenol	0.909	1.06	0.69	0.15	0.8970
valerophenone	0.800	0.95	0.00	0.50	1.4370
n-butyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.5540
1-naphthol	1.520	1.05	0.61	0.37	1.1441
benzophenone	1.447	1.50	0.00	0.50	1.4808
ethylbenzene	0.613	0.51	0.00	0.15	0.9980
3,4-dichlorophenol	1.020	1.14	0.85	0.03	1.0199
naphthalene	1.340	0.92	0.00	0.20	1.0854
hexanophenone	0.720	0.95	0.00	0.50	1.5780
heptanophenone	0.720	0.95	0.00	0.50	1.7180
n-propylbenzene	0.604	0.50	0.00	0.15	1.1391
dibenzo thiophene	1.959	1.31	0.00	0.20	1.3791
biphenyl	1.360	0.99	0.00	0.22	1.3242
n-buytlbenzene	0.600	0.51	0.00	0.15	1.2800
anthracene	2.290	1.34	0.00	0.26	1.4544
phenanthrene	2.055	1.29	0.00	0.26	1.4544
pyrene	2.808	1.71	0.00	0.28	1.5846

a) E, S, A, B, and V are the solute descriptors, defined in the experimental section of the text

Table II. System constants in the reversed-phase mode for the various stationary phases studied. Stationary phase designations are the same as those from Figure 1. r^2 is the coefficient of correlation, SE is the standard error of regression, F is the Fisher statistic, n is the number of solutes in the regression.

SP[a]	MP[b]	e	s	a	b	v	c	r^2	SE	F	n
ODS	60% ACN	0.1170	-0.3789	-0.6076	-1.4019	1.2868	-0.2247	0.99	0.0471	796.6	41
ODS	50% ACN	0.1071	-0.3839	-0.6456	-1.6516	1.4941	-0.0774	0.99	0.0590	838.2	42
ODS	40% ACN	0.1771	-0.5334	-0.6266	-1.6732	1.7437	0.0881	0.99	0.0737	636.5	42
ODS	30% ACN	0.1958	-0.6369	-0.6311	-1.8345	2.0506	0.3068	0.98	0.0817	399.1	33
ODS	L Kw ACN	0.1239	-0.5322	-0.8421	-3.0424	2.8593	0.5963	0.98	0.1012	478.8	40
	Buffer: phosphate pH 7.0 20mM										
DP	40% ACN	0.0431	-0.1374	-0.4694	-1.4438	1.2084	-0.5293	0.99	0.0544	588.2	42
DP	35% ACN	0.0706	-0.1853	-0.4591	-1.5152	1.3599	-0.4951	0.99	0.0535	699.8	42
DP	30% ACN	0.1114	-0.2417	-0.4347	-1.5795	1.5469	-0.4851	0.99	0.0538	808.7	43
DP	10% ACN	0.2459	-0.2374	-0.3028	-1.9566	2.4546	-0.9049	0.99	0.0643	412.4	31
DP	L Kw ACN	0.2366	-0.4213	-0.2470	-2.1803	3.0270	-0.9268	0.99	0.0998	592.9	43
PrimeD	40% ACN	0.0505	-0.0731	-0.1766	-1.6248	1.3039	-0.4778	0.99	0.0477	615.0	42
PrimeD	35% ACN	0.0824	-0.1245	-0.1724	-1.7327	1.4737	-0.4493	0.99	0.0480	745.8	42
PrimeD	30% ACN	0.1241	-0.1848	-0.1398	-1.8268	1.6643	-0.4092	0.99	0.0512	785.5	42
PrimeD	10% ACN	0.1729	-0.2217	-0.0831	-2.0006	1.8741	-0.4857	0.99	0.0595	548.8	39
PrimeD	L Kw ACN	0.3572	-0.5271	0.0513	-2.6150	2.9643	-0.5239	0.98	0.1090	463.4	43
	Buffer: phosphoric acid pH 2.2										
DVB	70% ACN	0.4823	-0.3838	-0.7487	-1.1905	1.0275	-0.0728	0.99	0.0510	963.5	41
DVB	60% ACN	0.4506	-0.4368	-0.7676	-1.2905	1.1855	0.0884	0.99	0.0570	634.3	39
DVB	50% ACN	0.4322	-0.4456	-0.7885	-1.4783	1.3008	0.3114	0.98	0.0640	372.8	33
DVB	L Kw ACN	0.3904	-0.5888	-0.9450	-2.1469	2.1004	1.0791	0.97	0.1287	274.5	39
SAX	5% ACN	0.5199	-0.5655	-0.4694	-1.1745	1.7260	-1.7545	0.94	0.1232	112.0	34
	Buffer: phosphate pH 5.0										

a) SP – stationary phase, b) MP – mobile phase

CHAPTER 3

Characterization of Macrocyclic Glycopeptide-Based Stationary Phases with a Linear Solvation Energy Relationship

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Abstract

The mechanism of retention for four commercially available macrocyclic glycopeptide chiral stationary phases was investigated using a linear solvation energy relationship (LSER) in the reversed-phase mode and the normal-phase mode. The LSER approach is very successful at describing the factors that lead to retention on these stationary phases. The retention data yield excellent statistical fits (correlation coefficients, r^2, greater than 0.98). The differences in the retention mechanism between the two modes of operation on the same stationary phase are easily differentiated by using the LSER. In the reversed-phase mode, the macrocyclic glycopeptide CSPs have significantly different hydrogen bond acidity, basicity, and dipolar system constants compared with octadecyl silane stationary phases. The change in value of these system constants upon changing mobile phase conditions is also significantly different from the system constants of octadecyl silane stationary phases. Retention in the reversed-phase mode was found to be governed by dispersive/hydrophobic interactions, dipolar interactions, and n and π electron interactions. In the normal-phase mode, the system

constants were similar to other conventional normal-phase stationary phases. Retention was found to be governed by hydrogen bonding and dipolar interactions.

3.1 Introduction

Macrocyclic glycopeptide based chiral stationary phases (CSPs) have proven to be extremely useful in enantiomeric separations and are one of the fastest growing classes of CSPs today [1-4]. They have exceptional selectivity for amino acids [5, 6], and have proven to be useful for the enantiomeric separation of a wide variety of molecules [7]. Chemically, they are composed of a diversity of functional groups (Figure 1), including hydroxyls, phenols, amides, aromatic rings, various carbohydrate moieties, ionizable carboxylic acid, and amine groups, as well as a basket-shaped structure [8]. This plethora of functional groups is a stark contrast to the hydrocarbon chains of which the majority of conventional reversed-phase stationary phases are composed. Due to these structural differences, macrocyclic glycopeptide-based stationary phases are capable of participating in a greater variety of intermolecular interactions compared with a conventional brush type stationary phase and can be considered an "alternate selectivity" type of reversed-phase stationary phase. In addition to the differences observed in reversed-phase HPLC, macrocyclic glycopeptide CSPs are effective in the normal-phase mode and polar organic mode and the mechanism of retention and separation is different in each mode of operation [7]. However, no quantitative physicochemical data have been published that supports the assumed differences in retention mechanisms when the same stationary phase is used in the reversed-phase mode versus the normal-phase mode.

It is desirable to be able to describe any stationary phase with objective physicochemical parameters. An objective description of stationary phases would allow the analyst to determine if stationary phases from different manufacturers are nominally equivalent, somewhat similar, or orthogonal. This is especially useful in today's laboratory where there are over 600 different reversed-phase stationary phases available to the analyst, with new and improved products being added all the time [9, 10].

There have been many systems and tests devised to evaluate the physical and chemical properties, as well as the performance, selectivity, and efficiency of chromatographic stationary phases [11-19]. They range in complexity from the rather elegant yet simple tests proposed by Walters [11] and Tanaka [12] to more complicated methods involving spectroscopic analysis [20-22], physical measurements [23], and statistical analyses [24-28]. Many of the simple tests, purported to indicate silanol activity, hydrophobicity, and hydrogen bond capacity, can give results that vary significantly [17]. Often, columns that would be ranked as having low silanol activity by one test are ranked higher when another test procedure is used [15].

The application of these tests to CSPs can give misleading results. It seems that there is a general presumption that, when these types of tests are applied to a typical C18 stationary phase, it is desirable to have low silanol activities and low hydrogen bond capacities. Of course, for a conventional reversed-phase column, this is correct. But in the application of these tests to a CSP (which will most assuredly contain hydrogen bonding groups as part of the chiral selector and could contain ionizable groups as well), the hydrogen bond capacity and silanol activity will be elevated. In this case, the tests are not necessarily flawed, but it must be understood why the results are elevated.

The physical properties that are most frequently cited as being indicative of the quality of a stationary phase are: percent carbon loading, surface area, pore volume and size, and ligand density. These parameters are usually determined by the manufacturer, as they are valuable information for quality control in the manufacturing process [16]. Unfortunately, there is often poor correlation between these parameters and chromatographic performance. The same can be said of spectral measurements made on bulk stationary phases. The data obtained are complex, and there is no clear relation between the data and chromatographic performance. Furthermore, techniques such as solid state NMR are time consuming and are often cost prohibitive.

One of the more comprehensive and broadly applicable systems for the characterization of biphasic systems is the linear solvation energy relationship (LSER) developed by Abraham [26]. It is used to characterize systems by interrogating them with well characterized probe solutes. Information about the system (in terms of defined system constants) is gleaned through linear regression. LSERs have been applied to liquid-liquid extraction [29], the uptake of drugs in intestinal absorption [30] and oral absorption [31], the determination of water solubility [32], the determination of octanol-water partition coefficients [33], gas-liquid chromatography [34, 35]. Abraham and Carr have conducted extensive studies in high performance liquid chromatography [36-41]. The relationship, expressed mathematically, is

$$SP = c + eE + sS + aA + bB + vV$$

where SP is a solute property, such as the logarithm of partition coefficient or retention factor. The variables E, S, A, B, and V are solute descriptors whose definitions are as follows. E is an excess molar refraction that is obtained from a compound's measured

refractive index. S is the solute dipolarity/polarizability. A and B are the hydrogen bond acidity and basicity of a solute, respectively, and V is the McGowan volume (in $cm^3/100$) that can be easily calculated with knowledge of a solutes molecular structure and the appropriate constants for the constituent atoms [42].

The coefficients, c, e, s, a, b, and v, are obtained by linear regression. They are characteristic of the particular system that is described by the SP parameter, and they indicate the relative difference in the strength of a particular interaction a solute experiences between two phases. *e* reflects the system's ability to interact with a solute through n- and π- electron pairs. *s* is a measure of the system's ability to participate in dipolar interactions. *a* is the hydrogen bond basicity of the system, and *b* is the hydrogen bond acidity of the system (an acidic solute will interact with a basic phase/system). *v* is a measure of the system's ability to participate in dispersion interactions and *c* is the phase ratio of the system. The sign of the fitting coefficients indicates which phase dominates in a particular interaction--positive indicating the stationary phase and negative for the mobile phase.

In this article, we will focus on the characterization of macrocyclic glycopeptide-based CSPs by Abraham's LSER in the reversed-phase mode and normal-phase mode. As previously mentioned, the mechanism of retention and selectivity is said to change with the mode of operation. These changes in mechanism should be reflected by the system constants obtained from LSER analysis. It should be noted that this approach is applicable to any CSP or chiral selector.

Some care must be taken when selecting a group of probe solutes to interrogate a CSP. The primary concern should be which mode of operation will be characterized by the LSER theory. Macrocyclic glycopeptide CSPs are multi-modal stationary phases, which can

separate enantiomers in the reversed-phase mode, the normal-phase mode, and the polar organic mode. While there have been many studies utilizing Abrahams LSER theory in the reversed-phase mode, there have been few studies conducted in the normal-phase mode [43-46] and none in the polar organic mode. Additionally, the limited normal-phase studies have used the same probe solutes popularized in the reversed-phase studies. While not strictly inappropriate, many of the probe solutes from these reversed-phase studies are not appreciably retained even in the weakest normal-phase conditions. Because it is generally desirable to have both appreciable retention and a range of retention for the probe solutes, judicious selection of the probe solutes is critical with respect to the mode of operation (see experimental).

3.2 Experimental

3.2.1 Materials

The CSPs were obtained from Advanced Separation Technologies (Whippany, NJ). All stationary phases used consisted of the chiral selector bonded to 5-μm spherical silica gel. The chiral selectors used are the macrocyclic glycopeptide, which are shown in Figure 1. The dimensions of the columns are 50 × 4.6 mm.

Methanol, acetonitrile, heptane, ethanol, and isopropanol were HPLC grade from Fisher (Fairlawn, NJ). All LSER probe solutes were obtained from Aldrich (St. Louis, MO) in high purity grade (99% or greater). It is desirable to utilize a large number of probe solutes for redundancy in each determination. In this study, we have selected 38 compounds for the reversed-phase mode (Table 1) and 51 compounds for the normal-phase mode (Table 2). The probe solute sets contain a diversity of functionalities. Our sets of probe solutes

contain aldehydes, ketones, amides, halogenated phenols, nitro substituted benzenes, nitro substituted alkanes, alkyl benzenes, and polyaromatic hydrocarbons. Care was taken to exclude solutes that interact with the chiral selectors in a manner inconsistent with the majority of the probe solutes (i.e., strongly silanophilic compounds). Additionally, the molecular descriptors span a wide range and do not show large intercorrelation (Tables 3 and 4).

3.2.2 Instrumental

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett Packard, Palo Alto, CA, USA), and an integrator (3395, Hewlett Packard). Mobile phases were degassed by ultra-sonication under vacuum for 5 minutes. UV detection was carried out at 210 nm. All separations were carried out at room temperature (25°C).

3.2.3 Column Evaluation

The retention factor of each probe solute was obtained on each stationary phase in the reversed-phase mode using acetonitrile/water and methanol/water mobile phases. In the normal-phase mode, each stationary phase was evaluated with ethanol/heptane mobile phases. The flow rate was set at 1.0 $mL \cdot min^{-1}$. The column dead time (t_M) was determined in the reversed-phase mode by the negative peak observed upon injection of 25 μL of deuterium oxide and, in the normal-phase mode, by the retention time of tri t-butyl benzene.

3.2.4 Calculations

Retention factors (k) were calculated using the equation $k = (t_r - t_M)/t_M$. Multiple linear regression analysis and statistical calculations were performed using the program Analyse-it, an add in program for Microsoft Excel.

3.3 Results and Discussion

The four commercially available macrocyclic glycopeptide CSPs were evaluated in the reversed-phase mode using acetonitrile/water and methanol/water based mobile phases at a variety of conditions. The normal-phase mode also was evaluated using ethanol/heptane mobile phases at a variety of conditions. The retention factor of the probe solutes was obtained at each condition. LSER fitting coefficients were obtained from these retention data by multiple linear regression analysis. The correlation coefficients were 0.99 and 0.98, and the standard errors were about 0.04 and 0.10 in the reversed-phase mode and normal-phase mode, respectively. Additionally, the LSER equations were determined for the CSPs using log k_w (the logarithm of the retention factor in 100% water). This allows objective comparisons to be made independently of the mobile phase composition [47]. Log k_w was determined by extrapolation of log k data to 0% organic modifier in the mobile phase. Similarly, LSER equations were determined for the CSPs using log k_{hep}, simulating retention in a 100% heptane mobile phase. The correlation coefficient for these extrapolations was 0.98 or better. Log k_w was determined for both methanol- and acetonitrile-based mobile phases and designated log k_w MeOH and log k_w ACN. In principle, log k_w should be independent of the organic modifier and the values extrapolated from methanol/water

retention data should be equal to the values extrapolated from acetonitrile/water retention data. It has been observed from C18 retention data that the extrapolated log k_w retention factor of a compound can differ depending on the identity of the organic modifier [27, 48]. The properties of the organic modifier must have some residual influence on the determination of log k_w, and this influence is manifest in the value of log k_w for a given probe solute and the magnitude of the LSER system constants derived from the retention of these probe solutes. The magnitude of these differences on the macrocyclic glycopeptide CSPs is not as great as those observed on C18 stationary phases and will be noted in the discussion below.

3.3.1 LSER Results in the Reversed-Phase Mode

The coefficients from the LSER equations obtained in the reversed-phase mode are presented in Tables 5 and 6. The general features found in the data reflect the same general trends shown by achiral reversed-phase systems, i.e., a large positive *v*-term (hydrophobicity), a large negative *b*-term (hydrogen bonding in the mobile phase), and a nonzero, negative *a*-term (hydrogen bonding in the mobile phase). Additionally, the *e*-term (n and π electron interaction) is nonzero and positive.

When making specific comparisons of the coefficients obtained at log k_w (Table 5), there are significant differences between C18 stationary phases and macrocyclic glycopeptide CSPs. The sign and magnitude of the *s*-term (dipolarity) for the macrocyclic glycopeptide CSPs is different from a C18 column (0.30 for Chirobiotic T and -0.79 for C18). Obviously, this occurs because of the presence of polar functional groups in the macrocyclic glycopeptide (e.g., hydroxyl, amido, amino, etc). Furthermore, the stationary phase contains

sorbed mobile phase solvent molecules. It is well established that C18 stationary phases contain an enriched ratio of organic modifier to water compared with the mobile phase composition, and that the ratio of solvents in the stationary phase is relatively constant over most of the range of mobile phase compositions [49-52]. Because macrocyclic glycopeptides are much more polar than octadecyl alkyl chains, the macrocyclic glycopeptide CSPs will likely contain a higher proportion of the polar component of the mobile phase mixture. Water, a very polar compound having a dipolarity of 1.09, will influence the dipolarity of the stationary phase when sorbed. The *s*-term in this case is likely elevated, compared with the C18 column, because of the intrinsically more polar macrocyclic glycopeptides and of the sorbed mobile phase solvents.

The magnitude of the *v*-, *b*-, and *a*-terms are quite different between C18 and macrocyclic glycopeptide CSPs. The *v*-term is much larger for the C18 stationary phase (4.65 vs. 1.49 for the Chirobiotic T CSP). This larger *v*-term is easily rationalized considering that a C18 stationary phase is much more hydrophobic. The *b*-term (hydrogen bond acidity of the stationary phase) is elevated for the macrocyclic glycopeptide CSPs (-0.46 and -3.19 for Chirobiotic T and C18, respectively). This difference in the *b*-terms of these two stationary phases can again be rationalized by the presence of polar groups that can participate in hydrogen bonding and the presence of sorbed mobile phase solvents. The macrocyclic glycopeptide molecules contain native polar hydrogen bonding groups, whereas an octadecyl chain contains no hydrogen bond acceptor moieties. Additionally, organic modifiers such as acetonitrile and methanol are weak hydrogen bond acids (0.07 and 0.43, respectively) and will not significantly raise hydrogen bond acidity of the octadecyl stationary phase in which they are sorbed. Water is an extremely strong hydrogen bonding

acid (1.17), and the increased amount of water in the macrocyclic glycopeptide stationary phase has a pronounced effect on the *b*-term.

Compared with the other LSER parameters, the *a*-term (hydrogen bond basicity of the stationary phase) exhibits a greater amount of variation, at the condition log k_w, based on the identity of the organic modifier. When methanol was used as the organic modifier, the C18 stationary phase had a higher *a*-term compared with the macrocyclic glycopeptide CSPs. However, when acetonitrile was used as the organic modifier, the *a*-term was lower for the C18 stationary phase, and was comparable with the macrocyclic glycopeptide CSPs. These differences are due to hydrogen bonding bases in the stationary phase (or lack thereof), as well as mobile phase molecules sorbed to the stationary phase. Acetonitrile and methanol have moderate hydrogen bond basicities (0.32 and 0.47, respectively). The higher hydrogen bond basicity of the stationary phase observed in the methanol data must be influenced by this difference.

Recently, we conducted a study investigating the type of interactions that manifest themselves in the *e*-term [53]. We concluded that both π electron complexation and ion-dipole interactions, both of which are interactions involving n and π electrons, are encoded in the *e*-term. The *e*-term is positive for the C18 and the macrocyclic glycopeptide CSPs and generally the *e*-term for the macrocyclic glycopeptide CSPs can be similar to the C18 column (0.24 for Chirobiotic T and 0.29 for C18) or significantly larger (0.78 for Chirobiotic R). It seems likely that both of the *e*-terms for these two classes of stationary phases are due to the presence of ionized groups, which are both on the silica surface and on the chiral selectors. Foremost, π complexation is a relatively weak interaction in the reversed-phase mode of operation. Additionally, the C18 alkyl chain has no π electrons for π complexation. While

there are many π electrons on the macrocyclic glycopeptides, the normal-phase results, discussed below, seem to indicate that these stationary phases are not strong π complexation stationary phases. For these reasons, we attribute the interactions accounted for in the *e*-term in the reversed-phase mode as ion-dipole interactions.

When the composition of the mobile phase is changed, the system constants of the macrocyclic glycopeptide CSPs change as well. Some of the terms exhibit changes consistent with classical reversed-phase behavior, that is, the absolute value of the terms increases as the mobile phase becomes more polar; other terms exhibit more unusual trends as the composition of the mobile phase changes (Fig. 2 and 3, Table 6). For example, as the amount of water in the mobile phase increases, the *v*-term (hydrophobicity) for Chirobiotic T is positive and increases in magnitude, which is in agreement with classic reversed-phase behavior. Interestingly, several of the system constants for the macrocyclic glycopeptide CSPs exhibit trends that conventional stationary phases do not. The *e*-, *s*-, and *a*-terms are essentially constant for the C18 column (Fig. 4). The small changes in the *s*-term for the C18 column, with changing mobile phase composition, are in the negative direction. Conversely, the *e*- and *s*-term for the macrocyclic glycopeptide CSPs become more positive and the sign of the *s*-term is the opposite of the *s*-term for the C18 column (as mentioned above). Additionally, the *b*-term, which is negative in sign, becomes slightly more positive in several cases (the opposite of what is seen on C18) and the *a*-term becomes more negative.

These changes are due to both the changing ratio of water to organic modifier in the mobile phase and the changing amount of water and organic modifier that is sorbed to the stationary phase. The changes in the mobile phase likely have a greater impact on the magnitude of the system constants. As the composition of the mobile phase shifts to more

water, the ability of the mobile phase to participate in hydrogen bonding and dipolar interactions changes, and the values of the system constants shift to reflect these changes. Additionally, there are changes in the amount and ratio of sorbed mobile phase solvents. In the case of the *a*-term, as the amount of organic modifier in the mobile phase decreases, it is likely that the amount of organic modifier sorbed to the stationary phase changes as well. Since these solvent molecules are moderate hydrogen bond bases, decreasing their concentration in the stationary phase decreases the basicity of the stationary phase (*a*-term). Likewise for the *b*-term and *s*-term, as the amount of water in the mobile phase increases, the relative amount of water sorbed to the stationary phase increases. Because water is a strong hydrogen bonding acid, and very polar, increasing the amount of water absorbed to the stationary phase will increase the acidity of the stationary phase (*b*-term) and dipolarity of the stationary phase (*s*-term). The *e*-term generally decreases in magnitude with the addition of organic modifier. These changes are likely due to the changing amount of organic solvents in the system as well as changes in the ionized state of the charged groups on the chiral selector. It is well established that the ionization constants for acids and bases will shift in the presence of organic solvents (which possess different dielectric constants) [54]. The pKs of the carboxylic acid and amine on the teicoplanin molecule in solution are 2.5 and 9.2, respectively [6]. Slight shifts in the ionization pKs of these groups can decrease the number of charged sites on the chiral selector, thereby reducing the number of ion-dipole interactions and decreasing the value of the *e*-term.

These macrocyclic glycopeptide CSPs can also be compared with each other, at the condition log k_w, to determine which is the most hydrophobic or dipolar or hydrogen bond acidic. Figure 5 shows the system constants for these CSPs at log k_w MeOH and log k_w

ACN. While there are some differences in the system constants between these conditions, the ranking of these CSPs is largely unaffected, unless noted. From Figure 5, it is clear that the Chirobiotic TAG is the most hydrophobic of the examined CSPs, followed by the Chirobiotic T, Chirobiotic R, and Chirobiotic V. Additionally, the Chirobiotic TAG and R are the least H-bond acidic CSPs, while the Chirobiotic T is the most H-bond acidic CSP. There is some variation between the methanol based *b*-term and acetonitrile based *b*-term for the Chirobiotic V phase. The CSP capable of the strongest n and π electron interactions is the Chirobiotic R, which is followed by the Chirobiotic TAG. Chirobiotic T and V are nominally equivalent in this regard. The Chirobiotic R is also the most dipolar phase, with the other 3 having similar *s*-terms (although there is some variation between the methanol and acetonitrile system constants). Finally, the Chirobiotic R is the least H-bonding basic phase, followed by the Chirobiotic T, with Chirobiotic V and TAG being nominally equivalent. These differences between the macrocyclic glycopeptide CSPs at condition log k_w reflect the chemical composition differences between the four chiral selector molecules.

3.3.2 LSER Results in the Normal-phase Mode

The system constants obtained for these macrocyclic glycopeptide CSPs in the normal-phase mode are presented in Tables 7 and 8. The general features are similar to other normal-phase systems described in the literature, i.e., large and positive *s*-, *a*-, and *b*-terms, negative *v*-terms, and statistically insignificant *e*-terms [43-45]. Furthermore, the magnitude of the system constants for the macrocyclic glycopeptide CSPs are similar to conventional stationary phases (bare silica, amino, diol, and cyano phases), although there is a great deal

of variation in the system constants between the different types of conventional stationary phases.

At condition log k_{hep}, the differences in the polar system constants of the four macrocyclic glycopeptide CSPs are readily determined (Figure 6, Table 7). The Chirobiotic TAG is the most H-bond acidic phase (*b*-term), whereas the Chirobiotic V is the least. The Chirobiotic T and Chirobiotic R phases are indistinguishable in this regard. Additionally, the macrocyclic glycopeptide CSPs have nearly identically H-bond basicity (*a*-term). The most dipolar phase is the Chirobiotic V; whereas the Chirobiotic T, TAG, and R are have very similar *s*-terms.

When the composition of the mobile phase changes, the system constants of the macrocyclic glycopeptide CSPs change in a manner consistent with normal-phase systems; that is, when the mobile phase becomes less polar, the magnitude of the system constants increase (Figure 7, Table 8). The trend observed in the *v*-term is due to the change in the solvent properties of the mobile phase. As the amount of heptane in the mobile phase increases, the *v*-term decreases. Because heptane is only capable of dispersive intermolecular interactions, an increase of heptane content in the mobile phase will increase the ability of the mobile phase to participate in dispersive interactions, relative to the stationary phase.

The trends in the *s*-, *a*-, and *b*-terms are also due to the changing mobile phase conditions. The stationary phase is relatively polar, and the polar modifier molecules will interact strongly with the adsorption sites of the stationary phase. As the amount of polar modifier in the mobile phase decreases, fewer adsorption sites of the station phase are occupied by the polar solvent molecules and are available to interact with solutes.

Consequently, the hydrogen bond acidity, hydrogen bond basicity, and dipolarity of the stationary phase increase.

The *e*-terms I measured for all macrocyclic glycopeptide CSPs in the normal-phase mode were indistinguishable from zero. Many conventional normal-phase stationary phases (silica, amino, cyano) often have *e*-terms that are zero [43-46]. However, these stationary phases do not have aromatic functionalities and are not thought to operate as π electron association stationary phases. Conversely, the macrocyclic glycopeptide CSPs do contain many aromatic residues, as well as carbonyl groups and nonbonding electrons, and it has been proposed that π electron complexation is an interaction that contributes to retention and selectivity [7]. These data suggest that n/π & π/π electron interactions do not contribute to retention. However, we are currently investigating the ability of LSER theory to describe π electron complexation in the normal-phase mode.

3.3.3 Changes in separation mechanism described by LSER

The mechanism for retention in the reversed-phase mode and the normal-phase mode are based on different interactions between solutes and the stationary phase. In the reversed-phase mode, retention is predominantly influenced by hydrophobic interactions, and, in the normal-phase mode, hydrogen bonding and dipolar interactions have the greatest influence on retention. These differences in mechanism are readily apparent when considering the LSER system constants for a given macrocyclic glycopeptide CSP in both the reversed-phase mode and the normal-phase mode (Figure 8). Clearly, the LSER theory is very successful at describing these differences in retention mechanism between these two modes of operation. Now that these CSPs have been effectively characterized, the next step is to categorize,

quantify, and evaluate which combination of interactions is responsible for chiral recognition of specific chiral molecules. Furthermore, it may be possible to definitively identify and measure the interaction differences between a CSP and two enantiomers. These will be the foci of following papers.

3.4 Conclusions

The LSER theory is very successful in its ability to describe retention on the macrocyclic glycopeptide CSPs, and the differences between conventional stationary phases and these macrocyclic glycopeptide CSPs are readily apparent. The sign and magnitude of the system constants are in tune with our chemical intuition. The data indicate that, in the reversed-phase mode, the solvents sorbed to the macrocyclic glycopeptide stationary phase are not as enriched in the organic modifier component as they are in a C18 stationary phase. This has a profound effect on the dipolarity, hydrogen bond basicity, and hydrogen bond acidity of these stationary phases. The most noticeable differences are in the dipolarity and hydrogen bond acidity of the macrocyclic glycopeptide CSPs, which, when mobile phase conditions change, have responses opposite to that of C18 stationary phases. In the reversed-phase mode, retention is governed by dispersive, hydrophobic partitioning, whereas in the normal-phase mode, retention is based on competition for hydrogen bonding and dipolar sites on the surface of the stationary phase. These differences in mechanism are easily differentiated by the LSER system constants. These parameters will be useful in the study of chiral interaction mechanisms and the enantioselectivity of CSPs.

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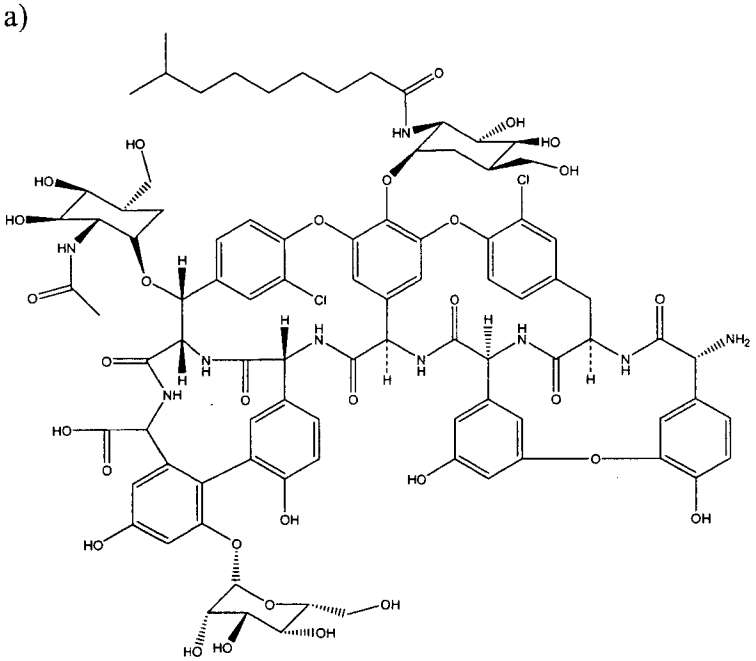
a)



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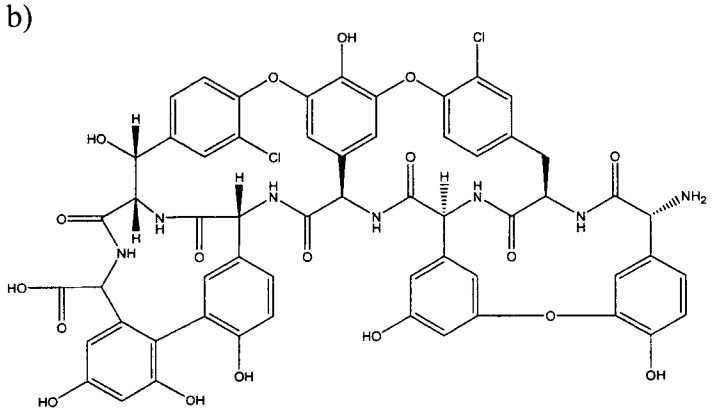

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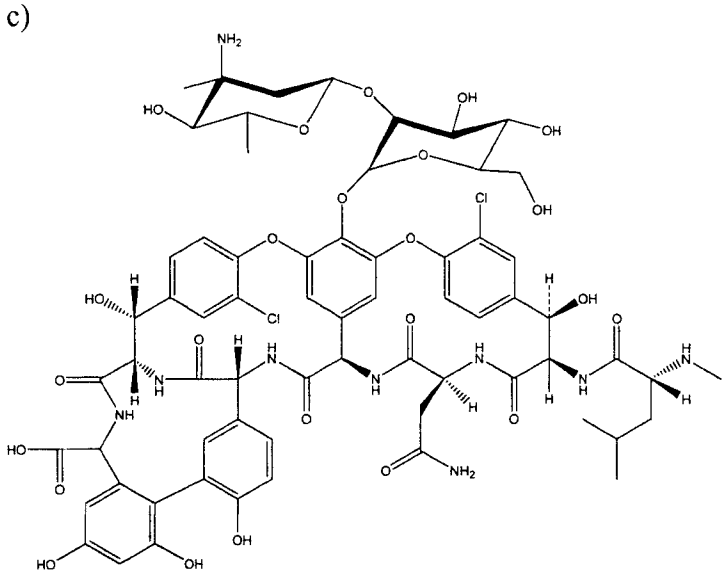

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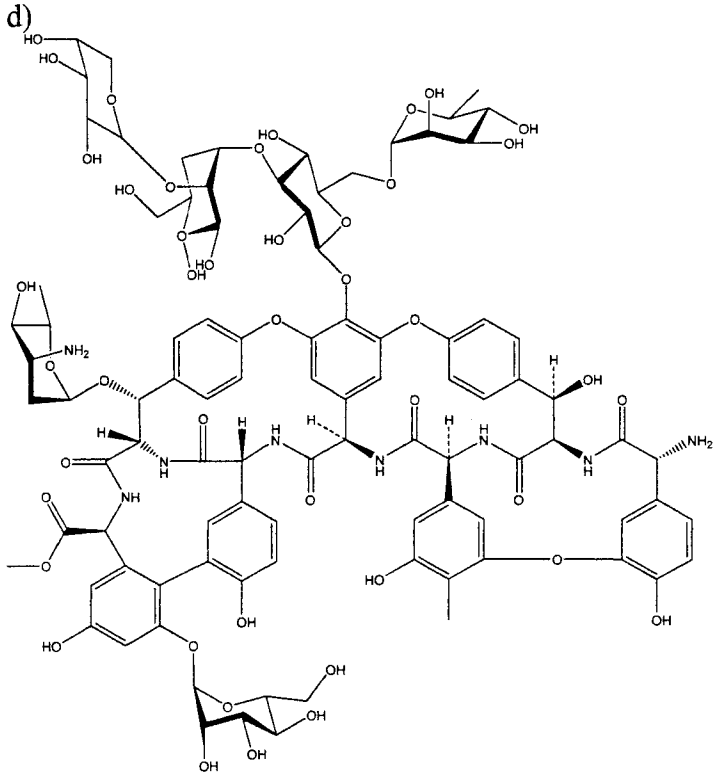


Figure 1. Structures of the four commercially available macrocyclic glycopeptide chiral selectors a) Teicoplanin b) Teicoplanin Aglycon c) Vancomycin and d) Ristocetin A.

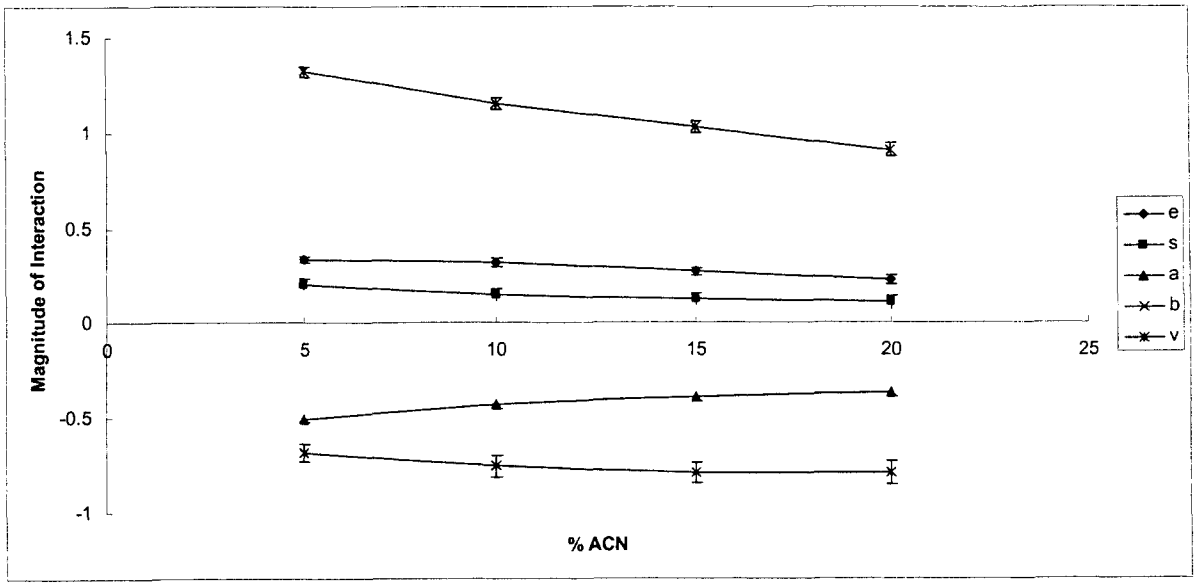


Figure 2. System constants of Chirobiotic T in the reversed-phase mode at varying acetonitrile amounts.

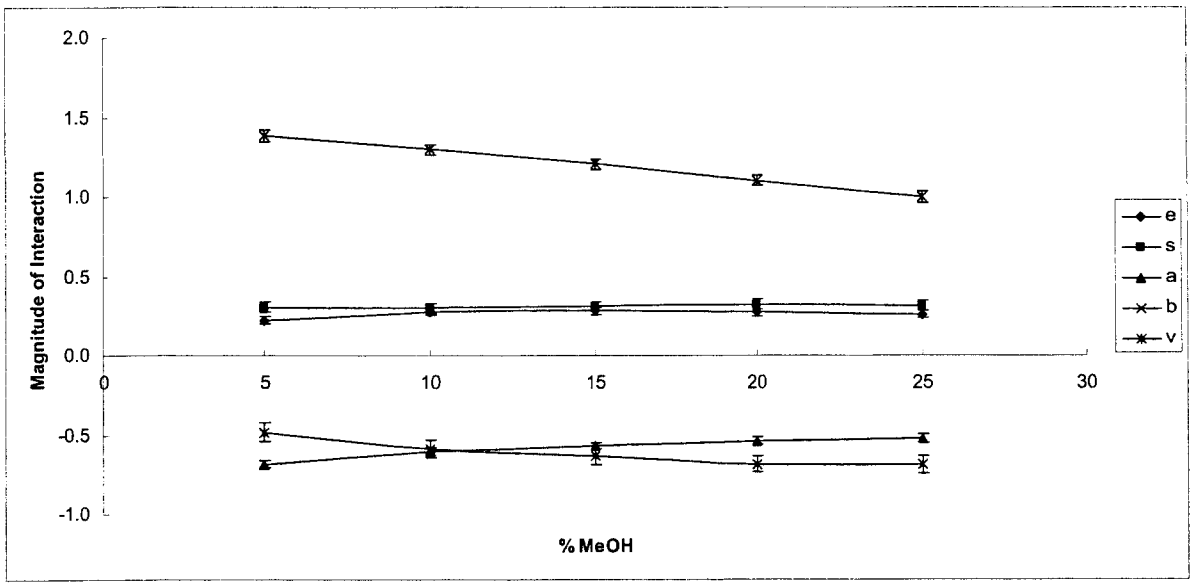


Figure 3. System constants of Chirobiotic T in the reversed-phase mode at varying methanol amounts.

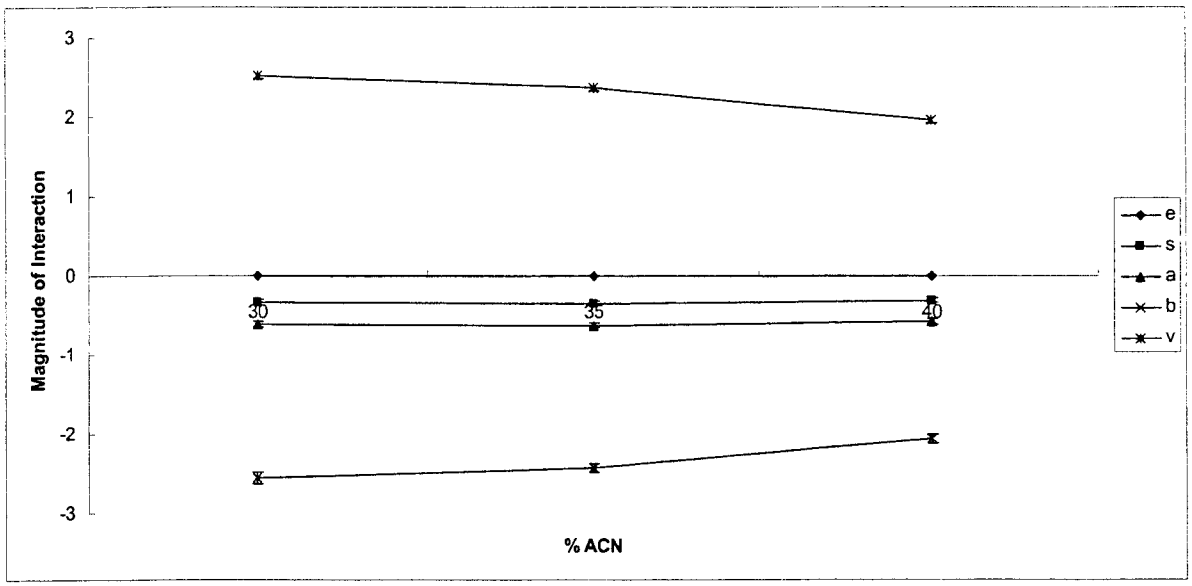
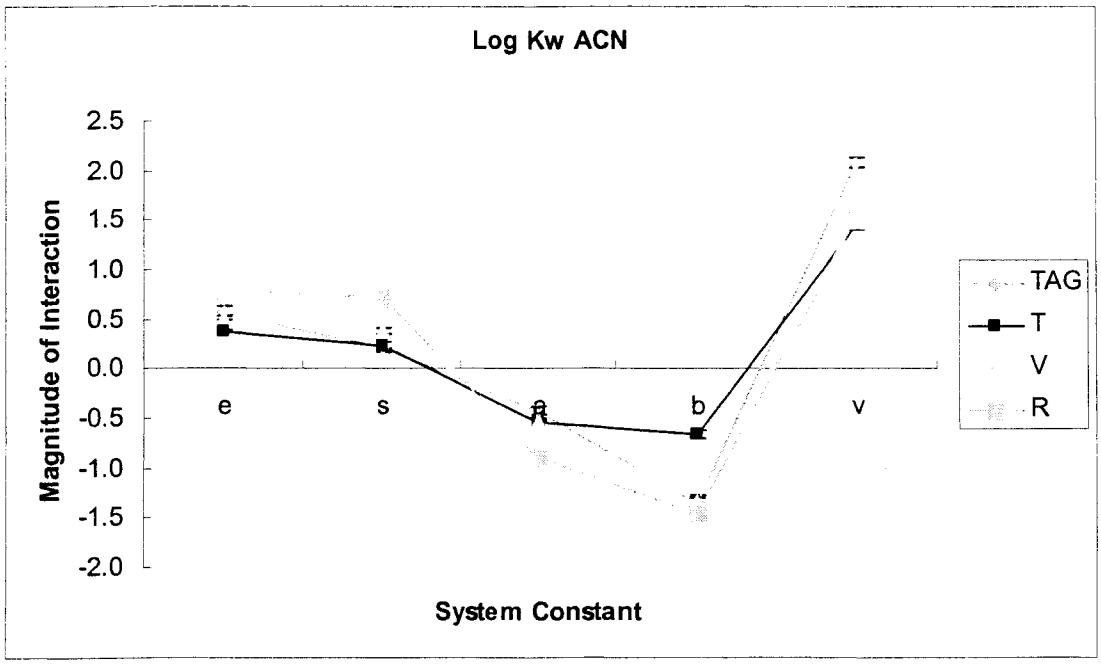


Figure 4. System constants of C18 stationary phase in the reversed-phase mode at varying acetonitrile amounts.

a)



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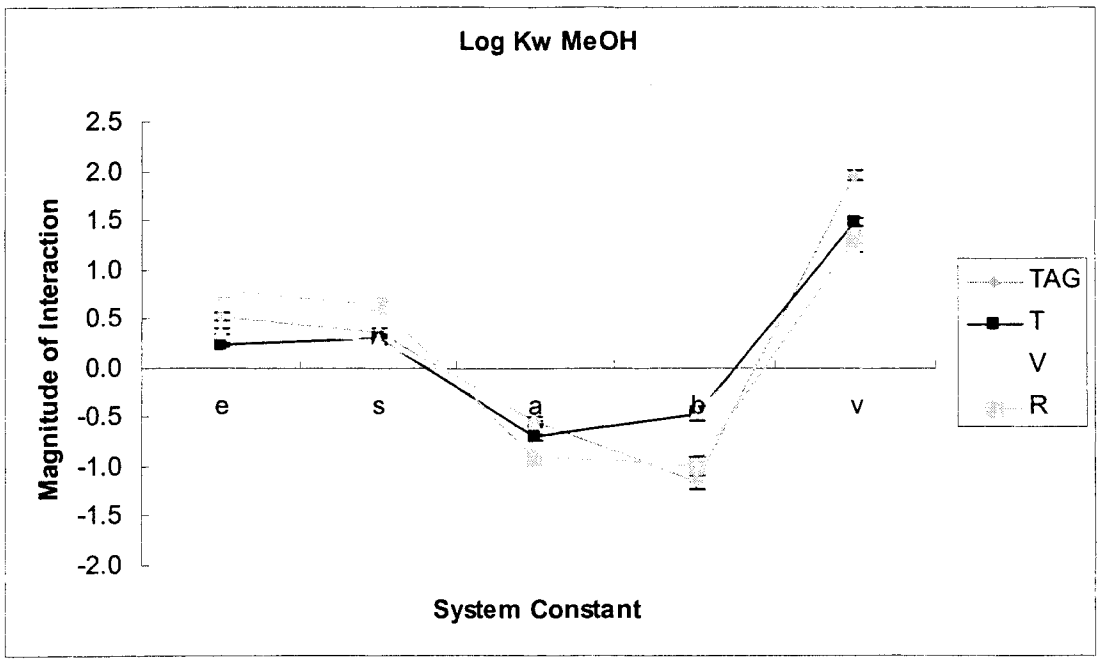

Figure 5. Comparison of four commercially available macrocyclic glycopeptide CSPs at condition log K_w. a) Data extrapolated from acetonitrile retention data. b) Data extrapolated from methanol retention data.

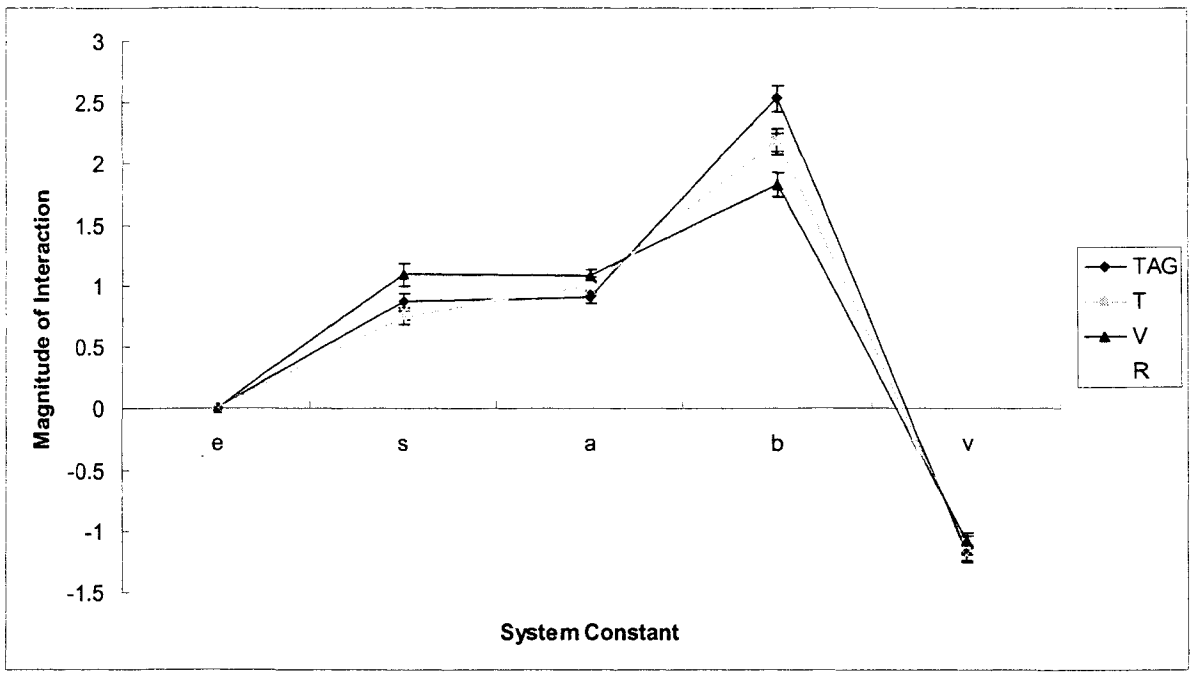

Figure 6. Comparison of four commercially available macrocyclic glycopeptide CSPs at condition log K_{hep}. Data extrapolated from normal-phase retention data.

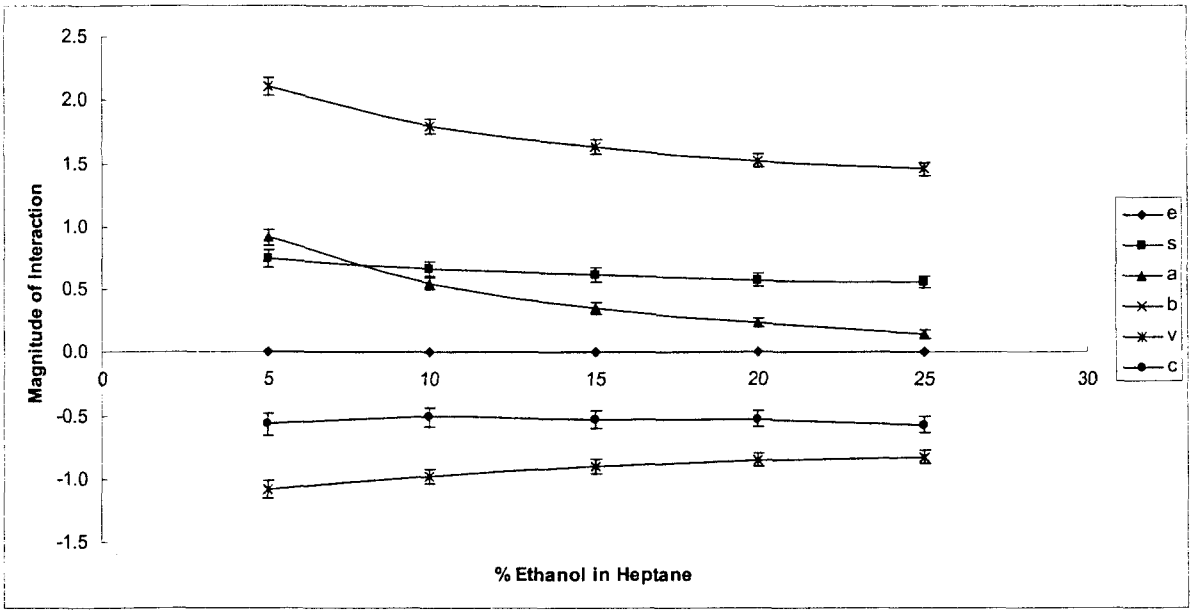

Figure 7. System constants of Chirobiotic T in the normal-phase mode at varying ethanol amounts.

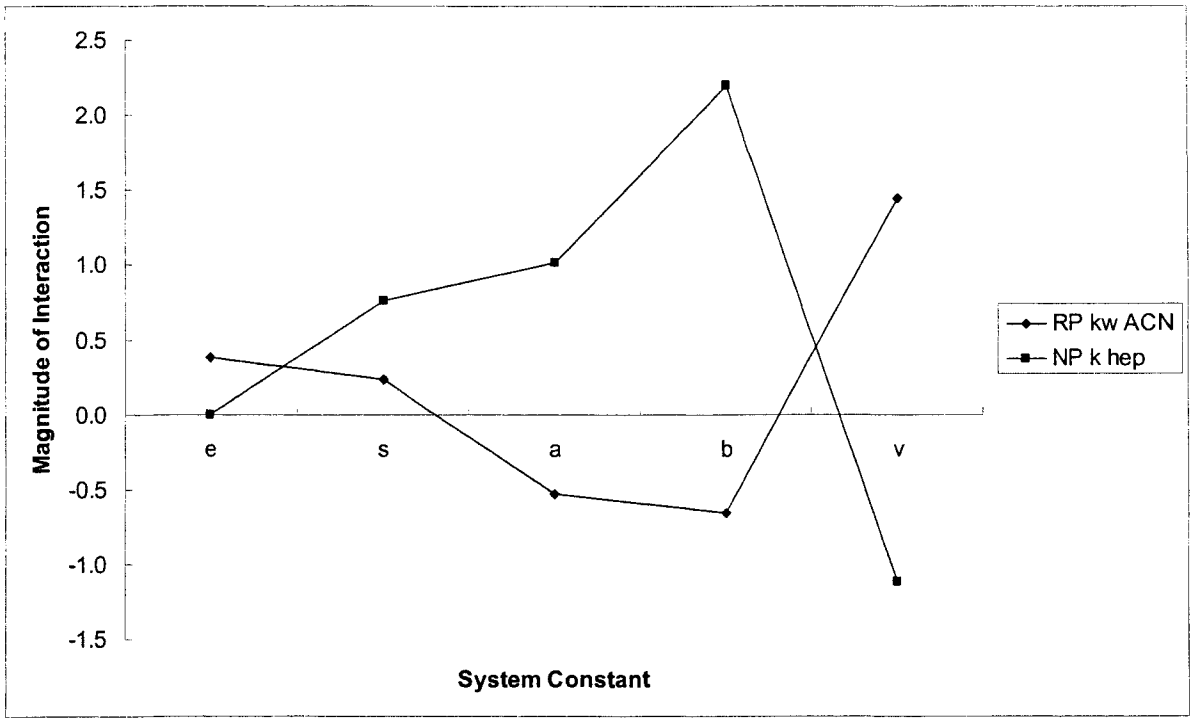

Figure 8. Comparison of system constants in the reversed-phase mode and normal-phase mode. Chirobiotic T at log K_w and log K_{hep}.

Table I. Probe solutes and their solute descriptors used in the reversed-phase mode. Solute descriptors E, S, A, B, and V are defined in the Introduction.

Solute	E	S	A	B	V
1,3,5-trihydroxybenzene	1.355	1.12	1.40	0.82	0.8925
resorscinol	0.980	1.00	1.10	0.58	0.8338
1-nitrobutane	0.227	0.95	0.00	0.29	0.8464
benzaldehyde	0.820	1.00	0.00	0.39	0.8730
3-cyanophenol	0.930	1.55	0.77	0.28	0.9300
benzonitrile	0.742	1.11	0.00	0.33	0.8711
acetophenone	0.818	1.01	0.00	0.48	1.0139
phenol	0.805	0.89	0.60	0.31	0.7751
2-nitrophenol	1.015	1.05	0.05	0.37	0.9490
1-nitropentane	0.212	0.95	0.00	0.29	0.9873
o-cresol	0.840	0.86	0.52	0.30	0.9160
m-cresol	0.822	0.88	0.57	0.34	0.9160
p-cresol	0.820	0.87	0.57	0.31	0.9160
methyl-4-hydroxybenzoate	0.900	1.37	0.69	0.45	1.1313
nitrobenzene	0.871	1.11	0.00	0.28	0.8906
ethyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.2722
1-nitrohexane	0.203	0.95	0.00	0.29	1.1282
benzene	0.610	0.52	0.00	0.14	0.7164
4-nitrotoluene	0.870	1.11	0.00	0.28	1.0315
m-trifluoromethylphenol	0.425	0.87	0.72	0.09	0.9691
n-propyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.4131
4-chlorophenol	0.915	1.08	0.67	0.20	0.8970
toluene	0.601	0.52	0.00	0.14	0.8573
o-xylene	0.663	0.56	0.00	0.16	0.9982
m-xylene	0.623	0.52	0.00	0.16	0.9982
p-xylene	0.613	0.52	0.00	0.16	0.9982
3-chlorophenol	0.909	1.06	0.69	0.15	0.8970
valerophenone	0.800	0.95	0.00	0.50	1.4370
n-butyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.5540
1-naphthol	1.520	1.05	0.61	0.37	1.1441
ethyl benzene	0.613	0.51	0.00	0.15	0.9980
3,4-dichlorophenol	1.020	1.14	0.85	0.03	1.0199
naphthalene	1.340	0.92	0.00	0.20	1.0854
hexanophenone	0.720	0.95	0.00	0.50	1.5780
heptanophenone	0.720	0.95	0.00	0.50	1.7180
n-propylbenzene	0.604	0.50	0.00	0.15	1.1391
dibenzothiophene	1.959	1.31	0.00	0.20	1.3791
n-buytlbenzene	0.600	0.51	0.00	0.15	1.2800

Table II. Probe solutes and their solute descriptors used in the normal-phase mode. Solute descriptors E, S, A, B, and V are defined in the Introduction.

Solute	E	S	A	B	V
pyrene	2.808	1.71	0.00	0.28	1.5846
anthracene	2.290	1.34	0.00	0.26	1.4544
phenanthrene	2.055	1.29	0.00	0.26	1.4544
dibenzothiophene	1.959	1.31	0.00	0.20	1.3791
n-buytlbenzene	0.600	0.51	0.00	0.15	1.2800
ethylbenzene	0.613	0.51	0.00	0.15	0.9980
o-xylene	0.663	0.56	0.00	0.16	0.9982
m-xylene	0.623	0.52	0.00	0.16	0.9982
p-xylene	0.613	0.52	0.00	0.16	0.9982
toluene	0.601	0.52	0.00	0.14	0.8573
biphenyl	1.360	0.99	0.00	0.22	1.3242
naphthalene	1.340	0.92	0.00	0.20	1.0854
heptanophenone	0.720	0.95	0.00	0.50	1.7180
hexanophenone	0.720	0.95	0.00	0.50	1.5780
4-nitrotoluene	0.870	1.11	0.00	0.28	1.0315
nitrobenzene	0.871	1.11	0.00	0.28	0.8906
4-iodophenol	1.380	1.22	0.68	0.20	1.0333
4-bromophenol	1.080	1.17	0.67	0.20	0.9501
4-chlorophenol	0.915	1.08	0.67	0.20	0.8970
benzonitrile	0.742	1.11	0.00	0.33	0.8711
1-naphthol	1.520	1.05	0.61	0.37	1.1441
o-cresol	0.840	0.86	0.52	0.30	0.9160
m-cresol	0.822	0.88	0.57	0.34	0.9160
p-cresol	0.820	0.87	0.57	0.31	0.9160
n-butyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.5540
n-propyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.4131
ethyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.2722
methyl-4-hydroxybenzoate	0.900	1.37	0.69	0.45	1.1313
phenol	0.805	0.89	0.60	0.31	0.7751
nitroethane	0.270	0.95	0.02	0.33	0.5640
nitromethane	0.313	0.95	0.06	0.31	0.4240
3-cyanophenol	0.930	1.55	0.77	0.28	0.9300
4-nitrophenol	1.070	1.72	0.82	0.26	0.9490
aniline	0.955	0.96	0.26	0.53	0.8162
acetanilide	0.870	1.40	0.50	0.67	1.1133
pyridine	0.794	0.87	0.00	0.62	0.6753
resorscinol	0.980	1.00	1.10	0.58	0.8338
hydroquinone	1.063	1.27	1.06	0.57	0.8338
benazamide	0.990	1.50	0.49	0.67	0.9728
dimethylformamide	0.363	1.33	0.00	0.78	0.7877
dimethylacetamide	0.367	1.31	0.00	0.74	0.6468
phenylurea	1.100	1.33	0.79	0.79	1.0726
benzenesulfonamide	1.130	1.55	0.55	0.80	1.0971
paracetamol	1.060	1.78	1.09	0.81	1.1720
1,3,5-trihydroxybenzene	1.355	1.12	1.40	0.82	0.8925
lidocaine	1.010	1.49	0.11	1.27	2.0589

succinimide	0.528	1.84	0.52	0.65	0.6948
theophylline	1.500	1.60	0.54	1.34	1.2223
caffeine	1.500	1.60	0.00	1.33	1.3632
thymine	0.800	1.00	0.44	1.83	0.8925
uracil	0.810	1.00	0.44	1.00	0.7516

Table III. Correlation coefficients (r) between the molecular descriptors for the reversed phase probe solutes. Solute descriptors E, S, A, B, and V are defined in the Introduction.

	E	S	A	B	V
E	1.00				
S	0.46	1.00			
A	0.07	0.31	1.00		
B	0.08	0.47	0.25	1.00	
V	0.46	0.28	-0.21	0.11	1.00

Table IV. Correlation coefficients (r) between the molecular descriptors for the normal-phase probe solutes. Solute descriptors E, S, A, B, and V are defined in the Introduction.

	E	S	A	B	V
E	1.00				
S	0.46	1.00			
A	-0.12	0.29	1.00		
B	-0.11	0.36	0.24	1.00	
V	0.59	0.31	-0.22	0.03	1.00

Table V. System constants in the reversed-phase mode at log k_w for macrocyclic glycopeptide CSPs and C18 stationary phase. r^2 is the coefficient of correlation, SE is the standard error of regression, F is the Fisher statistic, n is the number of solutes in the regression.

SP[a]	MP[b]	e	s	a	b	v	Intercept	r^2	SE	F	n
C18	kw MeOH	0.285	-0.787	-0.294	-3.186	4.65	-0.528	0.99	0.104	849	54
		(0.073)	(0.072)	(0.062)	(0.145)	(0.107)	(0.089)				
TAG	kw MeOH	0.533	0.356	-0.535	-1.148	1.958	-1.190	0.99	0.058	535	36
		(0.042)	(0.047)	(0.034)	(0.076)	(0.048)	(0.057)				
T	kw MeOH	0.243	0.299	-0.701	-0.463	1.490	-1.123	0.99	0.047	755	35
		(0.026)	(0.038)	(0.028)	(0.066)	(0.039)	(0.041)				
V	kw MeOH	0.369	0.279	-0.564	-0.458	1.223	-1.193	0.98	0.052	437	35
		(0.029)	(0.042)	(0.031)	(0.073)	(0.043)	(0.046)				
R	kw MeOH	0.783	0.646	-0.898	-0.984	1.317	-1.531	0.98	0.065	257	31
		(0.064)	(0.058)	(0.053)	(0.094)	(0.056)	(0.069)				
C18	kw ACN	0.120	-0.496	-0.744	-3.813	3.933	-0.001	0.99	0.114	1220	65
		(0.054)	(0.076)	(0.063)	(0.145)	(0.071)	(0.076)				
TAG	kw ACN	0.584	0.214	-0.419	-1.350	2.077	-1.234	0.99	0.062	475	36
		(0.045)	(0.050)	(0.036)	(0.081)	(0.051)	(0.061)				
T	kw ACN	0.381	0.234	-0.534	-0.661	1.443	-1.253	0.99	0.044	745	36
		(0.019)	(0.027)	(0.020)	(0.048)	(0.028)	(0.030)				
V	kw ACN	0.518	0.383	-0.520	-1.339	1.528	-1.607	0.99	0.029	875	32
		(0.020)	(0.026)	(0.018)	(0.053)	(0.023)	(0.026)				
R	kw ACN	0.799	0.716	-0.887	-1.454	1.500	-1.798	0.99	0.045	443	30
		(0.023)	(0.029)	(0.022)	(0.055)	(0.023)	(0.028)				

a) SP – stationary phase, b) MP – mobile phase

Table VI. LSER system constants for macrocyclic glycopeptide CSPs and C18 stationary phase in the reverse phase mode. Entries in bold are indistinguishable from zero.

SP[a]	MP[b]	e	s	a	b	v	Intercept	r^2	SE	F	n
TAG	20% ACN	0.374	**-0.001**	-0.315	-1.202	1.349	-0.779	0.99	0.048	481	35
		(0.029)	(0.039)	(0.027)	(0.061)	(0.040)	(0.043)				
TAG	15% ACN	0.443	**0.037**	-0.334	-1.277	1.550	-0.915	0.98	0.051	419	36
		(0.037)	(0.041)	(0.030)	(0.066)	(0.042)	(0.050)				
TAG	10% ACN	0.488	0.090	-0.349	-1.315	1.730	-1.026	0.98	0.054	451	36
		(0.039)	(0.043)	(0.031)	(0.070)	(0.044)	(0.052)				
TAG	5% ACN	0.530	0.161	-0.394	-1.312	1.913	-1.133	0.99	0.056	499	36
		(0.040)	(0.045)	(0.033)	(0.073)	(0.046)	(0.055)				
TAG	25% MeOH	0.478	0.267	-0.493	-1.211	1.496	-1.109	0.99	0.048	506	36
		(0.035)	(0.039)	(0.028)	(0.063)	(0.040)	(0.047)				
TAG	20% MeOH	0.497	0.265	-0.484	-1.237	1.615	-1.136	0.99	0.050	515	36
		(0.036)	(0.040)	(0.029)	(0.065)	(0.041)	(0.049)				
TAG	15% MeOH	0.527	0.276	-0.492	-1.189	1.695	-1.160	0.99	0.051	547	36
		(0.036)	(0.041)	(0.029)	(0.066)	(0.042)	(0.049)				
TAG	10% MeOH	0.518	0.324	-0.506	-1.164	1.775	-1.192	0.99	0.056	487	36
		(0.040)	(0.045)	(0.032)	(0.073)	(0.046)	(0.054)				
TAG	5% MeOH	0.514	0.346	-0.533	-1.186	1.837	-1.125	0.98	0.056	391	34
		(0.040)	(0.045)	(0.032)	(0.074)	(0.055)	(0.06)				
T	20% ACN	0.228	0.108	-0.368	-0.794	0.909	-0.953	0.99	0.034	558	36
		(0.025)	(0.035)	(0.025)	(0.062)	(0.036)	(0.039)				
T	15% ACN	0.269	0.129	-0.388	-0.792	1.031	-1.017	0.99	0.033	704	36
		(0.021)	(0.031)	(0.022)	(0.054)	(0.032)	(0.034)				
T	10% ACN	0.320	0.148	-0.430	-0.757	1.153	-1.075	0.99	0.039	616	36
		(0.022)	(0.031)	(0.023)	(0.055)	(0.032)	(0.034)				
T	5% ACN	0.335	0.207	-0.503	-0.683	1.322	-1.184	0.99	0.038	829	35
		(0.018)	(0.026)	(0.019)	(0.047)	(0.027)	(0.029)				
T	25% MeOH	0.260	0.315	-0.523	-0.692	1.000	-1.132	0.99	0.039	577	35
		(0.022)	(0.032)	(0.023)	(0.055)	(0.032)	(0.034)				

T	20% MeOH	0.272	0.323	-0.541	-0.684	1.102	-1.154	0.99	0.039	674	35
		(0.022)	(0.031)	(0.023)	(0.054)	(0.032)	(0.034)				
T	15% MeOH	0.282	0.313	-0.568	-0.638	1.207	-1.177	0.99	0.037	847	35
		(0.021)	(0.030)	(0.022)	(0.052)	(0.031)	(0.032)				
T	10% MeOH	0.280	0.299	-0.605	-0.588	1.301	-1.177	0.99	0.039	871	35
		(0.022)	(0.031)	(0.023)	(0.054)	(0.032)	(0.034)				
T	5% MeOH	0.223	0.307	-0.685	-0.484	1.385	-1.084	0.99	0.042	834	35
		(0.024)	(0.034)	(0.025)	(0.060)	(0.035)	(0.037)				
V	20% ACN	0.406	0.176	-0.371	-1.140	0.961	-1.370	0.99	0.024	497	32
		(0.025)	(0.032)	(0.023)	(0.065)	(0.028)	(0.032)				
V	15% ACN	0.454	0.228	-0.388	-1.246	1.138	-1.466	0.99	0.026	571	32
		(0.023)	(0.030)	(0.021)	(0.062)	(0.026)	(0.031)				
V	10% ACN	0.472	0.269	-0.420	-1.278	1.256	-1.499	0.99	0.025	761	31
		(0.021)	(0.028)	(0.019)	(0.056)	(0.024)	(0.028)				
V	5% ACN	0.486	0.335	-0.495	-1.271	1.381	-1.541	0.99	0.028	799	32
		(0.022)	(0.028)	(0.020)	(0.058)	(0.025)	(0.029)				
V	25% MeOH	0.364	0.267	-0.431	-0.803	0.960	-1.338	0.98	0.044	416	35
		(0.024)	(0.035)	(0.026)	(0.062)	(0.036)	(0.039)				
V	20% MeOH	0.369	0.268	-0.443	-0.743	1.026	-1.330	0.99	0.038	598	35
		(0.021)	(0.031)	(0.023)	(0.053)	(0.032)	(0.033)				
V	15% MeOH	0.377	0.287	-0.456	-0.680	1.102	-1.316	0.99	0.044	481	35
		(0.025)	(0.036)	(0.026)	(0.063)	(0.037)	(0.039)				
V	10% MeOH	0.360	0.271	-0.507	-0.617	1.155	-1.277	0.99	0.040	662	35
		(0.022)	(0.032)	(0.024)	(0.056)	(0.033)	(0.035)				
V	5% MeOH	0.294	0.293	-0.581	-0.579	1.177	-1.179	0.98	0.051	437	35
		(0.028)	(0.041)	(0.030)	(0.072)	(0.042)	(0.045)				
R	20% ACN	0.539	0.379	-0.587	-1.164	0.945	-1.594	0.99	0.024	550	30
		(0.042)	(0.053)	(0.041)	(0.102)	(0.042)	(0.052)				
R	15% ACN	0.599	0.450	-0.641	-1.296	1.124	-1.665	0.99	0.027	608	30
		(0.037)	(0.047)	(0.036)	(0.090)	(0.037)	(0.046)				

R	10% ACN	0.664	0.523	-0.708	-1.349	1.252	-1.716	0.99	0.032	564	29
		(0.030)	(0.038)	(0.029)	(0.073)	(0.030)	(0.037)				
R	5% ACN	0.737	0.643	-0.827	-1.363	1.347	-1.736	0.99	0.039	465	30
		(0.025)	(0.032)	(0.025)	(0.062)	(0.025)	(0.031)				
R	25% MeOH	0.689	0.660	-0.816	-1.101	0.930	-1.631	0.99	0.035	531	31
		(0.035)	(0.032)	(0.029)	(0.052)	(0.031)	(0.038)				
R	20% MeOH	0.713	0.661	-0.824	-1.099	1.021	-1.634	0.99	0.038	518	31
		(0.037)	(0.034)	(0.031)	(0.055)	(0.033)	(0.040)				
R	15% MeOH	0.743	0.632	-0.815	-1.050	1.137	-1.651	0.98	0.048	362	31
		(0.047)	(0.043)	(0.039)	(0.070)	(0.042)	(0.051)				
R	10% MeOH	0.764	0.645	-0.850	-1.023	1.182	-1.610	0.98	0.049	365	31
		(0.049)	(0.044)	(0.041)	(0.072)	(0.043)	(0.053)				
R	5% MeOH	0.749	0.658	-0.898	-1.017	1.216	-1.514	0.98	0.057	300	31
		(0.056)	(0.051)	(0.047)	(0.083)	(0.049)	(0.061)				
C18	40% ACN	0	-0.307	-0.575	-2.058	1.960	-0.035	0.99	0.052	1988	65
			(0.028)	(0.029)	(0.057)	(0.029)	(0.035)				
C18	35% ACN	0	-0.349	-0.620	-2.425	2.363	-0.316	0.99	0.052	2468	64
			(0.029)	(0.030)	(0.058)	(0.030)	(0.035)				
C18	30% ACN	0	-0.331	-0.613	-2.558	2.521	-0.064	0.99	0.065	1641	63
			(0.036)	(0.037)	(0.072)	(0.040)	(0.045)				

a) SP – stationary phase, b) MP – mobile phase

Table VII. System constants in the normal phase mode at Log k_{hep} for macrocyclic glycopeptide CSPs.

SP[a]	MP[b]	e	s	a	b	v	Intercept	r^2	SE	F	n
TAG	k hep	0	0.871	0.909	2.536	-1.172	-0.495	0.99	0.109	704	42
			(0.066)	(0.053)	(0.103)	(0.063)	(0.080)				
T	k hep	0	0.756	1.010	2.198	-1.115	-0.537	0.98	0.130	530	44
			(0.074)	(0.061)	(0.097)	(0.071)	(0.091)				
V	k hep	0	1.095	1.080	1.829	-1.077	-0.671	0.99	0.100	775	37
			(0.090)	(0.054)	(0.098)	(0.061)	(0.082)				
R	k hep	0	0.803	0.980	2.168	-1.192	-0.428	0.98	0.122	559	45
			(0.072)	(0.059)	(0.087)	(0.062)	(0.080)				

a) SP – stationary phase, b) MP – mobile phase

Table VIII. LSER system constants for macrocyclic glycopeptide CSPs in the normal phase mode.

SP[a]	MP[b]	e	s	a	b	v	Intercept	r^2	SE	F	n
TAG	25% EtOH Hept	0	0.662	0.056	1.681	-0.917	-0.473	0.98	0.098	449	46
			(0.053)	(0.041)	(0.070)	(0.053)	(0.067)				
TAG	20% EtOH Hept	0	0.691	0.149	1.759	-0.955	-0.460	0.98	0.102	477	46
			(0.055)	(0.042)	(0.073)	(0.055)	(0.070)				
TAG	15% EtOH Hept	0	0.717	0.279	1.875	-1.001	-0.420	0.98	0.104	543	46
			(0.057)	(0.043)	(0.074)	(0.057)	(0.072)				
TAG	10% EtOH Hept	0	0.774	0.473	2.004	-1.014	-0.487	0.98	0.108	568	45
			(0.059)	(0.047)	(0.096)	(0.060)	(0.077)				
TAG	5% EtOH Hept	0	0.839	0.798	2.500	-1.149	-0.504	0.98	0.110	622	42
			(0.067)	(0.053)	(0.105)	(0.064)	(0.082)				
T	25% EtOH Hept	0	0.560	0.144	1.455	-0.826	-0.570	0.97	0.090	432	46
			(0.049)	(0.037)	(0.064)	(0.049)	(0.061)				
T	20% EtOH Hept	0	0.573	0.233	1.520	-0.848	-0.529	0.98	0.093	459	46
			(0.050)	(0.038)	(0.066)	(0.050)	(0.063)				
T	15% EtOH Hept	0	0.615	0.352	1.629	-0.896	-0.529	0.98	0.104	442	46
			(0.057)	(0.043)	(0.074)	(0.057)	(0.072)				
T	10% EtOH Hept	0	0.664	0.542	1.796	-0.979	-0.510	0.98	0.112	506	46
			(0.061)	(0.047)	(0.080)	(0.061)	(0.077)				
T	5% EtOH Hept	0	0.743	0.911	2.107	-1.082	-0.566	0.98	0.125	515	44
			(0.071)	(0.059)	(0.094)	(0.068)	(0.088)				
V	25% EtOH Hept	0	0.699	0.201	1.307	-0.793	-0.587	0.99	0.060	888	39
			(0.044)	(0.027)	(0.052)	(0.035)	(0.048)				
V	20% EtOH Hept	0	0.753	0.296	1.369	-0.824	-0.601	0.99	0.069	773	39
			(0.051)	(0.032)	(0.061)	(0.041)	(0.055)				
V	15% EtOH Hept	0	0.805	0.420	1.446	-0.855	-0.600	0.99	0.077	745	39
			(0.057)	(0.035)	(0.068)	(0.045)	(0.061)				
V	10% EtOH Hept	0	0.871	0.607	1.578	-0.914	-0.611	0.99	0.085	789	39
			(0.063)	(0.039)	(0.074)	(0.050)	(0.068)				
V	5% EtOH Hept	0	1.006	0.966	1.930	-1.006	-0.721	0.99	0.087	859	36
			(0.079)	(0.048)	(0.103)	(0.054)	(0.074)				

R	25% EtOH Hept	0	0.664	0.086	1.372	-0.880	-0.513	0.97	0.103	337	45
			(0.064)	(0.047)	(0.072)	(0.054)	(0.073)				
R	20% EtOH Hept	0	0.656	0.204	1.460	-0.938	-0.453	0.97	0.112	361	49
			(0.059)	(0.048)	(0.069)	(0.055)	(0.071)				
R	15% EtOH Hept	0	0.680	0.331	1.530	-0.971	-0.415	0.97	0.120	367	49
			(0.064)	(0.051)	(0.074)	(0.059)	(0.077)				
R	10% EtOH Hept	0	0.711	0.496	1.824	-1.043	-0.439	0.98	0.112	519	48
			(0.059)	(0.048)	(0.077)	(0.055)	(0.072)				
R	5% EtOH Hept	0	0.760	0.869	2.233	-1.079	-0.557	0.98	0.109	577	43
			(0.066)	(0.053)	(0.099)	(0.063)	(0.081)				

a) SP – stationary phase, b) MP – mobile phase

CHAPTER 4

Factors that Contribute to Enantioselectivity in Macrocyclic Glycopeptide Chiral Stationary Phases in the Reversed Phase Mode

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Abstract

The linear solvation energy relationship (LSER) model was utilized to examine the mechanism of enantioseparation on the Chirobiotic T chiral stationary phase. The solute descriptors of 12 racemates, including 5 neutral compounds, 5 amino acids, and 2 N-blocked amino acids, were determined in the reversed-phase mode with ethanol/buffer mobile phases. LSER system constants and solute descriptors were determined for the systems and solutes studied, and the intermolecular interactions that contribute to retention and enantioselectivity were obtained. It was found that ion-dipole interactions and steric repulsion interactions had the greatest impact on enantioselectivity. Hydrogen bonding, donation and acceptance, had a moderate effect on enantioselectivity, and dipole-dipole interactions have the least effect on enantioselectivity. One chiral sulfoxide was studied with both ethanol/buffer and methanol/water mobile phases, and the LSER model was able to successfully describe the differences in the mechanism of enantioseparation when using these two mobile phases. The retention data yielded excellent statistical fits for the solute descriptors of each enantiomer.

Generally, the neutral compounds experienced a minimum of three different types of intermolecular interactions that contribute to enantioselectivity and the amino acids experienced a minimum of four different types of intermolecular interactions that contribute to enantioselectivity. Theoretically, this approach to the evaluation of enantioselectivity and retention can be used for any chiral stationary phase in any chromatographic mode.

4.1 Introduction

Despite the advances made in enantiomeric separations over the last 25 years, there are only a few widely accepted and utilized approaches for determining the mechanism of chiral recognition. The most prevalent method for studying chiral recognition involves the use of structure-activity relationships [1-6] in which a large group of chiral molecules are analyzed on a given chiral stationary phase (CSP), and changes in enantioselectivity are correlated with structural changes. Although the conclusions drawn from these studies are well founded, they are usually only qualitative, pertain only to the class of molecules analyzed in the study and generally do not definitively reveal all of the interactions occurring between the solute and the chiral selector nor their relative importance (magnitude).

Other methods for studying enantiomeric separations are thermodynamic/van't Hoff studies, X-ray crystallography, NMR spectroscopy utilizing Nuclear Overhauser Effects (NOEs), computational analysis, and statistical methods. Thermodynamic or van't Hoff studies are simple to conduct, give quantitative results, and, when analyzing a large number of similar compounds, can give useful information about the role of structural features in generating enantioselectivity [7-13]. However the results obtained are in terms of changes of free energy, enthalpy, and entropy, and do not explicitly identify the interactions that are

occurring between the chiral selector and the solute. X-ray crystallography does give a precise picture of the interaction of an enantiomer with a chiral selector [14-18]; however, the type of intermolecular interactions must be inferred by the proximity of groups on the chiral selector and analyte. Also, it is necessary to grow a crystal of the diastereomeric complex, which is not possible in many cases. Furthermore, the x-ray structure of the complex only describes interaction in the crystal state and does not necessarily describe what is occurring in a solvated system or capture the differences between the reversed-phase mode and normal-phase mode of HPLC operation. Likewise, computational studies can also produce a detailed picture of the interaction of an enantiomer and chiral selector. However, many studies omit the presence of buffers, counter ions, solvent environments, are carried out with simplified structures for the chiral selector and guest enantiomer, and encounter difficulty in accounting for tethering effects when anchoring the chiral selector to a support [19-21]. Consequently, competition for interactions with the chiral selector by mobile phase solvent molecules and changes in the conformation of the chiral selector due to the polarity/composition of the mobile phase will be neglected in these computational models [19-21]. NMR spectroscopy has been used to study chiral recognition in the solution state via intermolecular NOEs as a single method of analysis [22-24] or in conjunction with some of the other techniques mentioned above [25-28]. Intermolecular NOEs are very useful in describing the orientation of a chiral selector to a single enantiomer. Most of the examples in the literature focus on separations that have large enantioselectivities (α-values greater than 10).

While all of these methods do provide useful information about chiral recognition, the results are often not specific in regard to the exact intermolecular interactions involved and

their relative importance, and do not account for solvation or changes in the mode of operation. From a chiral selector design viewpoint, it is desirable to know which intermolecular interactions contribute to retention and enantioselectivity.

The techniques that have been most successful in describing intermolecular interactions between a chiral selector and chiral solute utilize molecular descriptors of the solutes studied and derived descriptors for the chiral selector. Quantitative structure-activity relationships (QSARs) have been used with varying success, often identifying one or two factors that influence retention or enantioselectivity [29-39]. Linear solvation energy relationships (LSERs) have been used to characterize retention on CSPs [40] and describe enantioselectivity [41-43]. Other studies have utilized regression or correlation to explore intermolecular forces between a chiral selector and enantiomers. For example, Roussel et al. studied enantiomeric separations of N-arylthiazoline-2-(thi)one atropisomers on derivatized cellulosic and amylosic CSPs by factorial design and lipophillic correlation [44, 45]. We desire to study chiral separations via a LSER that is comprehensive in its ability to describe both retention and selectivity with little to no redundancy in the molecular descriptors or ambiguity in the intermolecular interactions they describe.

4.2 Theoretical

The LSER theory developed by Abraham describes partitioning in biphasic systems [46]. The LSER equation

$$\text{Log K} = c + e\text{E} + s\text{S} + a\text{A} + b\text{B} + v\text{V} \qquad (1)$$

contains five terms. The term for interactions through polarizable n and π electrons is (eE), the term for dipole-dipole interactions is (sS), the term for a solute donating a hydrogen atom

and the system donating a lone pair of electrons in a hydrogen bond is (aA), the term for a solute donating a lone pair of electrons and the system donating a hydrogen atom in a hydrogen bond is (bB), and the term for dispersion interactions is (vV). The c term contains the chromatographic phase ratio. Each term contains two components; one component describes a solutes ability to participate in the specified interaction (the upper case letters which are called solute descriptors), and the other component describes the systems ability to participate in the specified interaction (the lower case letters which are called system constants).

To apply the LSER model to enantioselectivity, an expression must be derived relating chromatographic selectivity to the LSER terms. Selectivity is defined by the following relationship

$$\alpha = \frac{k_2}{k_1} = \frac{K_2}{K_1} \qquad (2)$$

Taking the logarithm of the selectivity expression and rearranging gives

$$Log\alpha = Log\frac{K_2}{K_1} = LogK_2 - LogK_1 \qquad (3)$$

Substituting the LSER equation (1) for each log K and rearranging gives

$$Log\alpha = \left[(eE_2 - eE_1) + (sS_2 - sS_1) + (aA_2 - aA_1) + (bB_2 - bB_1) + (vV_2 - vV_1) + (c_2 - c_1)\right] \qquad (4)$$

and can be reduced to the following

$$Log\alpha = \left[(eE_2 - eE_1) + (sS_2 - sS_1) + (aA_2 - aA_1) + (bB_2 - bB_1) + (vV_2 - vV_1)\right] \qquad (5)$$

Note that the c terms are constant and identical for a system and therefore are equal. If we define, for a solute descriptor, ΔX as $X_2 - X_1$, we can simplify the selectivity expression to

$$Log\alpha = e\Delta E + s\Delta S + a\Delta A + b\Delta B + v\Delta V \qquad (6)$$

The terms, such as $s\Delta S$, are components of chromatographic selectivity and are a measure of the importance of the specified interaction, in this case dipole-dipole interactions, to enantioselectivity. With knowledge of the systems constants and the solute descriptors of chiral compounds, it is possible to calculate the importance of each interaction accounted for by the LSER theory to enantioselectivity.

To perform this kind of analysis, it is necessary to be able to determine the system constants as well as the solute descriptors for each enantiomer. Determination of system constants is a fairly routine matter and has been the subject of many publications [47-60]. A given system (e.g., a stationary phase) is interrogated with probe solutes that have well defined solute descriptors. With the log K and solute descriptors of a large number of solutes, the system constants can be determined by multiple linear regression. Solute descriptors can be determined in a similar fashion (although this is performed less frequently) [46, 61-64]; instead of interrogating a system with many well characterized probe solutes, a solute (or racemate) can be interrogated with many well characterized systems. The log K of a given enantiomer will be determined at a given system composition (mobile phase composition, temperature, and chiral selector) that has already been characterized by the LSER theory. Multiple linear regression will then be used to determine the solute descriptors, and the factors that contribute to retention and enantioselectivity can be determined according to equations 1 and 6, respectively.

Normally, the solute descriptors of each enantiomer should be identical because they have identical physical and chemical properties in an isotropic environment. However, they do have different properties in the chiral environment of the HPLC CSP; otherwise, they could not be separated. The solute descriptors determined by our method are likely similar in

magnitude to the solute descriptors determined in an isotropic environment but should not be taken as the actual "universal" solute descriptors.

While a range of system conditions are used (temperature and mobile phase composition), it is necessary to maintain consistency in the mechanism of enantioseparation. Therefore, only one chiral selector and one mode of operation are used in a given determination and the conditions utilized are restricted to a somewhat narrow range.

We envision that this LSER approach can be used in at least three different ways for the study of enantiomeric separations. The first approach would be to identify the exact factors that influence enantioselectivity (as well as their relative importance) for compounds on a given CSP in one mode of operation. This would allow more exact correlations to be drawn between molecular structure and the factors contributing to enantioselectivity. Alternatively, the LSER approach could be used to determine the factors that influence enantioselectivity for a single compound on multiple stationary phases in the same mode of operation. This would reveal the difference in separation mechanism between different CSPs. Finally, the LSER approach can be used to pinpoint the factors that influence enantioselectivity for a single compound on a single CSP in different modes of operation or with different mobile phase compositions. This would reveal the difference in separation mechanisms that are active in each particular mode. In this study, we will perform the first type of analysis listed above. Also, we will show how changes in the nature of the organic modifiers (in a reversed-phase separation) change specific enantioselective interactions.

4.3 Experimental

4.3.1 Materials

The Chirobiotic T stationary phase used was obtained from Advanced Separation Technologies (Whippany, NJ) and consisted of the teicoplanin chiral selector bonded to 5 μm spherical silica gel. The dimensions of the columns are 50 × 4.6 mm.

Methanol, triethylamine, sodium acetate, and phosphoric acid were HPLC grade from Fisher (Fairlawn, NJ). Ethanol was punctilious grade from Aaper alcohol (Shelbyville, KY). HPLC water was obtained by treating in-house reverse osmosis water with an ion-exchange resin. All LSER probe solutes and most chiral solutes were obtained from Aldrich (St. Louis, MO) in high purity grade (99% or greater). The substituted dihydrofurocoumarin was from a previous synthetic preparation [65, 66], as were the chiral sulfoxides [67, 68].

4.3.2 Instrumental

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett Packard, Palo Alto, CA, USA), an integrator (3395, Hewlett Packard), and a Jetstream + column thermostat (Advanced Separation Technologies) capable of regulating column temperature from 0 to 80 °C. Mobile phases were degassed by a sparging with helium for ~5 minutes. UV detection was carried out at 210 nm.

The buffered aqueous component of the mobile phase was composed of 0.1% (v/v) triethylamine and 10 mM sodium acetate (TEAA buffer) in water and brought to pH 4.1 with concentrated phosphoric acid. Mobile phase solutions were made by combining the appropriate amounts of organic solvent and aqueous buffer to achieve the desired ratio.

4.3.3 Calculation

Retention factors (k) were calculated using the equation $k = (t_r - t_M)/t_M$. Enantioselectivity factors (α) were calculated using the formula $\alpha = k_2/k_1$. Multiple linear regression analysis and statistical calculations were performed by using the program Analyse-it, a statistics package for Microsoft Excel.

4.4 Results and Discussion

Twenty-two reversed-phase conditions, each having a unique combination of mobile phase composition and temperature, were characterized by the LSER theory, and system constants were obtained by multiple linear regression (see Supplemental Table I and II for the LSER probe solutes and system constants). Additionally, the retention factors for both enantiomers of the 12 racemates selected for this study (see Figure 1 for structures) were determined at each condition. The reversed-phase mobile phases were ethanol/buffer solutions (10 mM TEAA, pH 4.1). Solute descriptors were determined for each enantiomer by linear regression, and the components of retention and enantioselectivity were determined according to equations 1 and 6, respectively.

The results for the analysis of 5-methyl-5-phenyl-hydantoin are shown in Figure 2. The solute descriptors (Figure 2a) for both enantiomers were determined by linear regression analysis. The coefficient of correlation (r^2) is quite good for both regressions (one for each enantiomer), 0.99 or better, and the standard deviations of the fitting coefficients (solute descriptors) are small, compared with the magnitude of the coefficients (Table I). The differences in these solute descriptors are due to the enantioselective interactions experienced with the chiral selector, teicoplanin. The factors that contribute to retention (log K) are

shown in Figure 2b (Table II). These values are the product of the solute descriptors and the system constants, from equation 1 (the system constants used were for a 25/75 ethanol/buffer mobile phase at 25°C). The factors that contribute to enantioselectivity are shown in Figure 2c (Table III). These values are the terms of equation 6, which are the differences in the factors that contribute to retention. In this type of analysis, it is enlightening to consider both the factors that contribute to selectivity and retention. This is a notable difference in our approach versus the method of Blackwell et al. [42, 43].

Some of the factors that control enantioselectivity (i.e., the different interactions for each enantiomer) are very straightforward to see and understand. Other factors require interpretation. The dipolar ($s\Delta S$) and hydrogen bonding terms ($a\Delta A$ and $b\Delta B$) are straight-forward as to what is occurring between the chiral solute and the stationary phase. The dipolar term ($s\Delta S$) describes dipole-dipole interactions occurring between the enantiomers and the chiral selector. In the case of 5-methyl-5-phenyl-hydantoin, the $s\Delta S$ term is -0.048, which suggests that dipole-dipole interaction do not significantly contribute to enantioselectivity. Note that while the $s\Delta S$ is essentially zero, dipole-dipole interactions do contribute to retention (Fig. 2b). The sS_1 and sS_2 terms are positive, indicating that dipole-dipole interactions contribute to the enantiomers association with the stationary phase (Fig. 2b). The $a\Delta A$ hydrogen bonding term indicates a hydrogen bond being donated by the enantiomers and accepted by the chiral selector, and the $b\Delta B$ hydrogen bonding term indicates a hydrogen bond being donated by the chiral selector and accepted by the enantiomers. In this case, the values of the $a\Delta A$ and $b\Delta B$ terms are 0.33 and 0.23, respectively. Because these values are positive, the second eluting enantiomer experiences more enantioselective hydrogen bonding interactions with the chiral selector, compared with

the first eluting enantiomer. Additionally, only the second eluting enantiomer experiences a significant amount of hydrogen bonding with the stationary phase that contributes with retention (Fig. 2b). The aA_1 and bB_1 factors are zero and -0.10, while the aA_2 and bB_2 factors are 0.33 and 0.14, respectively

The dispersion term ($v\Delta V$) requires more interpretation. A positive value for $v\Delta V$ would suggest dispersion forces and hydrophobic interactions contributing to enantioselectivity. However, for 5-methyl-5-phenyl-hydantoin (and all other compounds in this study) the $v\Delta V$ term has a value of -0.62; the first eluting enantiomer experiences more dispersion forces than the second enantiomer. This is likely an indication of steric repulsions contributing to enantioselectivity. The first eluting enantiomer experiences a steric repulsion with the chiral selector, which is manifested in the larger vV_1 term, causing it to elute before the second enantiomer, which experiences less steric repulsion and has a smaller vV_2 term. It has been well established for the Chirobiotic T CSP, and other CSPs of this family, that steric repulsion interactions are a significant constituent of enantioselectivity [69].

The final term that contributes to enantioselectivity is the excess molar refraction term, $e\Delta E$. This term encodes interaction through polarizable n and π electrons. Recently, we determined that, in the reversed-phase mode, the excess molar refraction term can encode both π electron complexation and ion-dipole interactions [70]. Additionally, we have shown that the macrocyclic glycopeptide CSPs are rather weak π electron complexation stationary phases [71], and it is well established that electrostatics play an important role in retention and enantioselectivity in the reversed-phase mode [69]. In light of this, it is likely that the interactions that are measured by the $e\Delta E$ term are ion-dipole interactions. For 5-methyl-5-phenyl-hydantoin the $e\Delta E$ term is 0.50, and is the generated by ion-dipole interactions

involving the ionized amine residue on the teicoplanin chiral selector (which has proven to be important to enantioselectivity on this CSP [72, 73]).

In addition to determining which factors contribute to retention and enantioselectivity, results are in good agreement with previous studies on the selected CSP and the LSER description of retention and enantioselectivity is very consistent with experimentally obtained results. The enantioselectivity value for 5-methyl-5-phenyl-hydantoin is 2.41 when experimentally determined according to equation 2 (when using a mobile phase of 25/75 ethanol/buffer mobile phase at 25°C). When calculating the enantioselectivity of 5-methyl-5-phenyl-hydantoin with the LSER method (using system constants of the identical condition), equation 6 gives an enantioselectivity value of 2.50, a difference of a few percent (3.66%). Figure 3 is a plot of observed versus calculated selectivity factors for every compound in this study. The coefficient of correlation (r^2) is 0.99, indicating that the LSER's description of enantioselectivity correlates nearly perfectly with experimental values. Additionally, the correlation between calculated and observed log K is also excellent. A significant difference in these values would indicate that there could be inconsistencies in our proposed model or the calculations performed.

The LSER method was used to analyze the retention and enantioselectivity of several other neutral molecules. The compounds ranged from polar (bromacil and 5-methyl-5-phenyl-hydantoin), intermediate polarity (α-naphthalenyl methyl sulfoxide), to nonpolar (the substituted dihydrofurocoumarin and 1,1-dimethyl-3-phenyl-propyl toluyl sulfoxide). Solute descriptors were generated for both enantiomers using linear regression (Table I), and, like the results from 5-methyl-5-phenyl-hydantoin discussed above, the fits of the regressions were quite good.

These neutral compounds have several common features in the factors that contribute to their enantioselectivity. First, all these neutral molecules have positive eΔE terms and negative vΔV terms, indicating that ion-dipole interactions and steric repulsions are important in chiral recognition on the teicoplanin CSP. A second common feature is the near-zero value of the sΔS terms for all compounds, which suggests that, on the teicoplanin CSP in the reversed-phase mode (with the ethanol/buffer mobile phase), dipole-dipole interactions are not a significant component of enantioselectivity. Dipole-dipole interactions do contribute to retention (Table II) but in equal amounts for each enantiomer. Most of the neutral molecules have no interaction through the a-type of hydrogen bonding. This is a consequence of these particular solutes not possessing acidic hydrogen atoms for the a-type of hydrogen bonding. Consequently, the "A" solute descriptor for most of the neutral analytes is zero, and the aΔA term is zero. Finally, additional enantioselective interactions are generated by varying amounts of the b-type of hydrogen bonding.

4.4.1 Comparison of enantioselectivity components from two different mobile phases

In a separate experiment, α-naphthalenyl methyl sulfoxide was eluted with methanol/water mobile phases (as opposed to ethanol/buffer mobile phases). The solute descriptors for α-naphthalenyl methyl sulfoxide were determined from these data by linear regression analysis (Table I). Note that the solute descriptors generated from a different mobile phase system will differ because of changes in the mechanism of enantioseparation. The factors that comprise retention and enantioselectivity were determined according to equations 1 and 6, respectively, and are listed in Tables II and III. Figure 4 compares the factors that contribute to enantioselectivity for α-naphthalenyl methyl sulfoxide in both

mobile phases. While none of the factors derived from the ethanol/buffer mobile phase have the same magnitude as the factors from the methanol/water mobile phase (except for the aΔA term, which is zero because sulfoxides are not capable of this type of hydrogen bonding), the sign and magnitude of some of the terms are similar. The eΔE terms are positive for both mobile phases, and vΔV terms are negative for both mobile phases. In the methanol/water mobile phase, the magnitude of the eΔE term is similar to the magnitude of the methanol/water vΔV term. The same is true of the eΔE and vΔV terms in the ethanol/buffer mobile phases. Additionally, these two terms have the greatest magnitude, and therefore impact on enantioselectivity, of any of the terms (in their respective mobile phases).

There are significant differences in the sΔS and bΔB terms for the two mobile phases. The sΔS term is zero for the ethanol/buffer mobile phase and 0.13 for the methanol/water mobile phase, and the bΔB term is 0.46 for the ethanol/buffer mobile phase and -0.03 for the methanol/water mobile phase. It is well established that the nature of the organic modifier has a strong impact on enantioselectivity in chiral separations, and these changes can only be due to the differences between methanol and ethanol, and the presence of buffer components (triethylamine and acetic acid). Despite the similarities in the terms of enantioselectivity, both enantioselectivities are predicted fairly accurately by the LSER model, the chromatographically observed and LSER predicted values are 1.63 and 1.59 for the ethanol buffer system, and 1.39 and 1.30 for the methanol/water system.

4.4.2 Results from the analysis of amino acids

The macrocyclic glycopeptide CSPs have excellent selectivity for amino acids and are able to baseline separate all 19 chiral protenic amino acids, as well as a wide variety of

non-protenic chiral amino acids [69, 74-84]. Indeed, nature has selected these compounds to discriminate between enantiomers of amino acids. *In vivo* the macrocyclic glycopeptides vancomycin and teicoplanin act as antibiotics by binding to D-alanine-D-alanine residues on the cell walls of enemy bacteria [85-87], preventing cell wall growth and eventually causing cell death. Given this broad selectivity for amino acids, one would expect the enantioseparation mechanism to be fairly consistent, depending only partially on the amino acid side chain.

Several amino acids were examined on the teicoplanin CSP in the reversed-phase mode. Solute descriptors were determined by linear regression. The quality of the regressions were not as good as those for the neutral compounds studied but still sufficient to generate solute descriptors for the enantiomers studied that are statistically different from each other. The solute descriptors for the selected amino acids (arginine, methionine, phenyl glycine, m-tyrosine, and tryptophan) and two N-blocked amino acids (N-acethyl-m-fluoro-phenylalanine and N-benzoyl-phenylalanine) are listed in Table IV, with the factors that comprise retention and enantioselectivity in Tables V and VI, respectively. In Figure 5, the factors that contribute to enantioselectivity for these amino acids are displayed.

When examining the magnitude and sign of these values, there are many common features in the factors that comprise enantioselectivity of each amino acid. Once again, the $e\Delta E$ terms are positive and the $v\Delta V$ terms are negative, and the magnitude of these terms are the largest for each amino acid. Additionally, the $s\Delta S$ terms are all negative in sign and among the smallest in magnitude for each amino acid, with values ranging from -0.06 to -0.32. Finally, the hydrogen bonding terms $a\Delta A$ and $b\Delta B$ are also significant, being positive in value and generally similar in magnitude. These results indicate that ion-dipole

interactions (eΔE), and steric repulsions (vΔV) are the interactions that have the strongest impact on enantioselectivity, both types of hydrogen bonding (aΔA and bΔB) have a weaker impact on enantioselectivity, and dipole-dipole (sΔS) interactions have almost no effect on enantioselectivity.

4.4.3 Normalized factors of selectivity

Given the striking similarity in the factors that control enantioselectivity of amino acids, and the similarity of these factors to the factors that compose enantioselectivity for unrelated compounds, a plot of normalized factors of selectivity was constructed (Table VII). The factors of enantioselectivity for every compound in this study are displayed, normalized to $-v\Delta V$ (Fig. 6). The ranking of the factors of enantioselectivity can vary significantly between the different types of intermolecular interactions. Arginine has values of sΔS and aΔA that are close to the middle of the distribution of values for these interactions, but the bΔB term for arginine is the lowest of all compounds. Bromacil has a value of bΔB that is close the middle of the distribution for this interaction, but the aΔA and sΔS terms are the smallest of all compounds (excluding the compounds that do not possess acidic hydrogen atoms for the aΔA type hydrogen bonding). Additionally, the values of eΔE range from 0.73 to 1.03 and are evenly distributed between these values, and not clustered near one value. From this, we conclude that the similarities in the factors of enantioselectivity for the compounds in this study are not due to interdependent interaction descriptors but are a consequence the mode of operation utilized, and the interactions that the chiral selector teicoplanin is capable of undergoing in that mode.

4.4.4 Contrast in the factors of enantioselectivity for neutral compounds vs. amino acids

While there are several common factors that contribute to the enantioselectivity of both the neutral molecules and amino acids, there are at least three interactions that contribute to enantioselectivity for every case. The three-point model of chiral recognition states that a minimum of three simultaneous yet differing interactions about the stereogenic center are required to differentiate between enantiomers [21, 88-90]. It is possible to have four or more interactions contributing to enantioselectivity, but a minimum of three is required. While some of the neutral molecules have three separate intermolecular interactions that contribute to enantioselectivity, and some have four, all of the amino acids examined have at least four separate interactions that contribute to enantioselectivity (and some have five). This higher number of interactions is most likely due to the constituent groups of amino acids (the carboxylate group, ammonium group, and side chain) participating in multiple interactions. For example, both the ammonium group and the carboxylate groups can participate in ion-dipole interactions and hydrogen bonding. While a molecule's ability to participate in intermolecular interactions is a contributing factor (to the number of interactions that can potentially generate enantioselectivity), it is also possible (and even likely) that the differences observed between the neutral molecules and amino acids are due to the chiral recognition process occurring at different sites on the teicoplanin chiral selector.

4.5 Conclusions

The LSER model can be used to successfully describe both retention and enantioselectivity on the teicoplanin CSP in the reversed-phase mode. The major factors that contribute to the separation of enantiomers are ion-dipole interactions and steric repulsion, with the hydrogen bonding ability of the chiral selector having a moderate impact on enantioselectivity. Dipole-dipole interactions seem to have almost no contribution to enantioselectivity but do contribute to the overall retention of these compounds. The relative importance of three polar interaction terms ($s\Delta S$, $a\Delta A$, and $b\Delta B$) varies for each compound analyzed, as demonstrated by the normalized factors of selectivity. Differences in the mechanism of enantioseparation due to changes in the mobile phase (identity of organic modifier and presence of buffer salts) also can be identified by this method of analysis. All of the compounds examined experience enantioselective interactions from at least three different types of intermolecular interactions, and all of the amino acids examined experience enantioselective interactions from at least four different types of intermolecular interactions.

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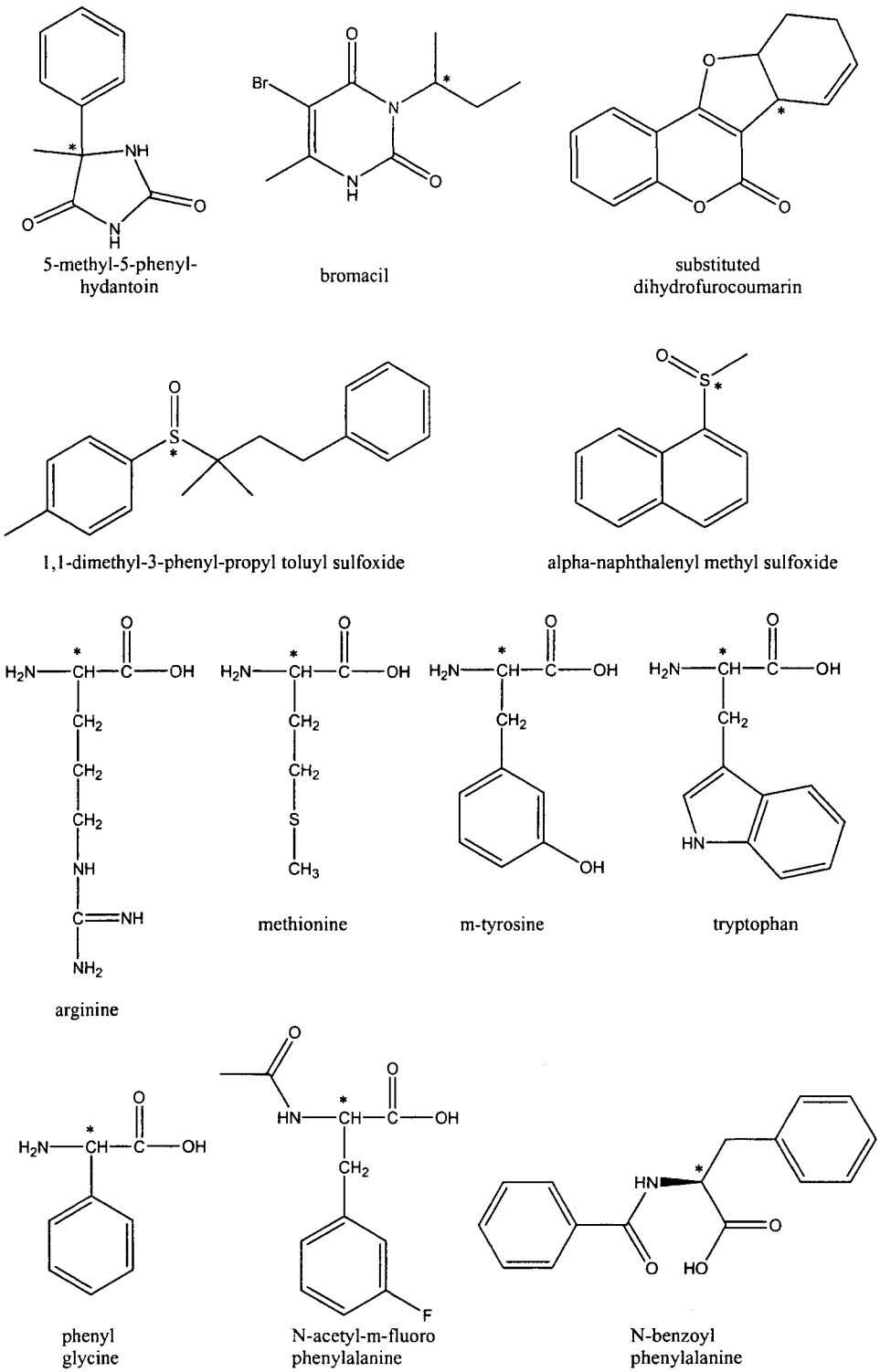
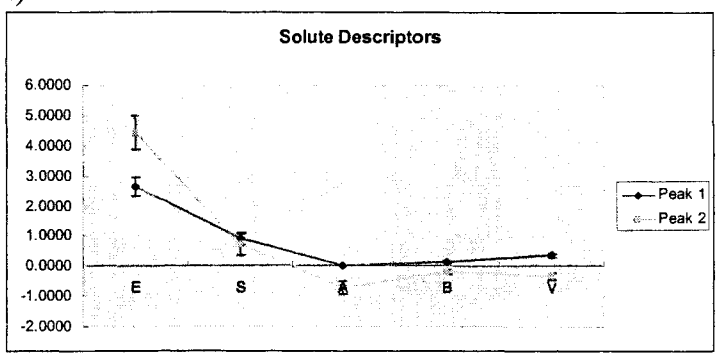
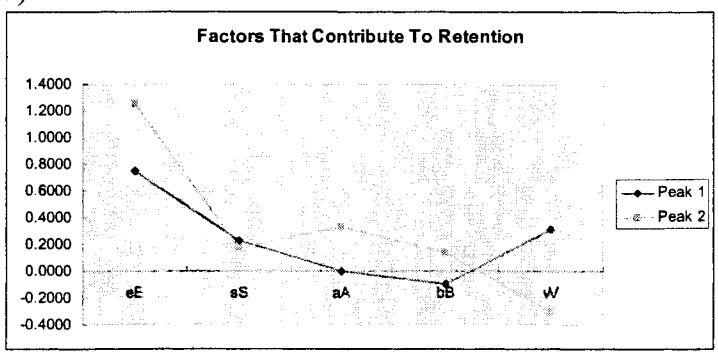


Figure 1. Structure of chiral solutes. Chiral centers are denoted with an asterisk.

a)



b)



c)

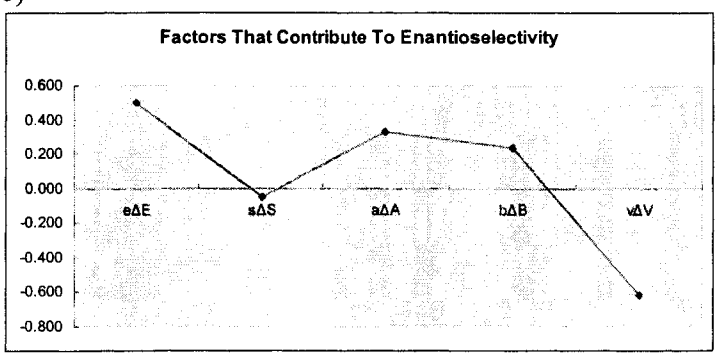


Figure 2. a) Solute descriptors for the enantiomers of 5-methyl-5-phenyl-hydantoin, determined by linear regression (see theoretical & experimental). b) Factors that contribute to the retention of the enantiomers of 5-methyl-5-phenyl-hydantoin. c) Factors that contribute to the enantioselectivity of 5-methyl-5-phenyl-hydantoin. The factors of retention and enantioselectivity are calculated with the solute descriptors obtained from regression and system constants for the teicoplanin CSP at 25/75 ethanol/buffer mobile phase at 25°C, according to equations 1 and 6, respectively.

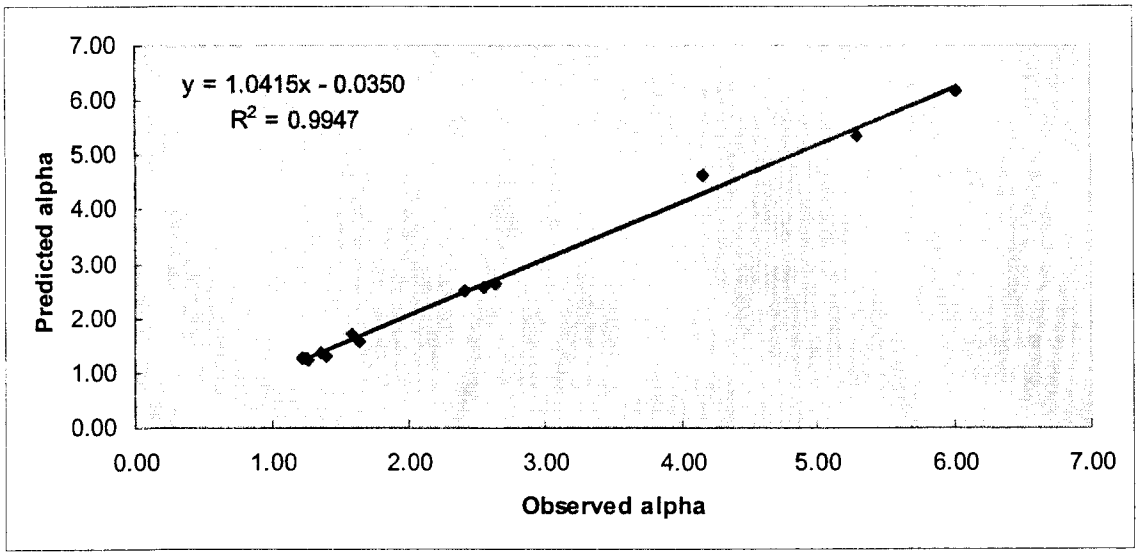


Figure 3. Plot of predicted vs. observed enantioselectivity for all solutes in this study. Predicted enantioselectivities are calculated for the teicoplanin CSP at 25/75 ethanol/buffer mobile phase at 25°C, and the observed enantioselectivity came from measurements at this condition.

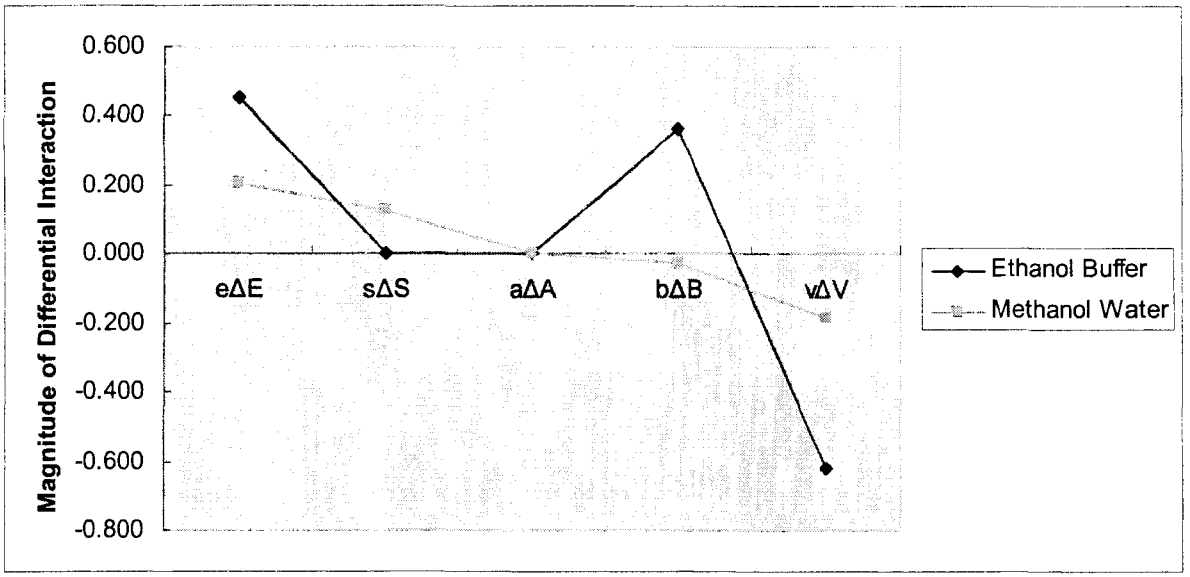


Figure 4. Comparison of factors that contribute to enantioselectivity for α-naphthalenyl methyl sulfoxide in two different mobile phases.

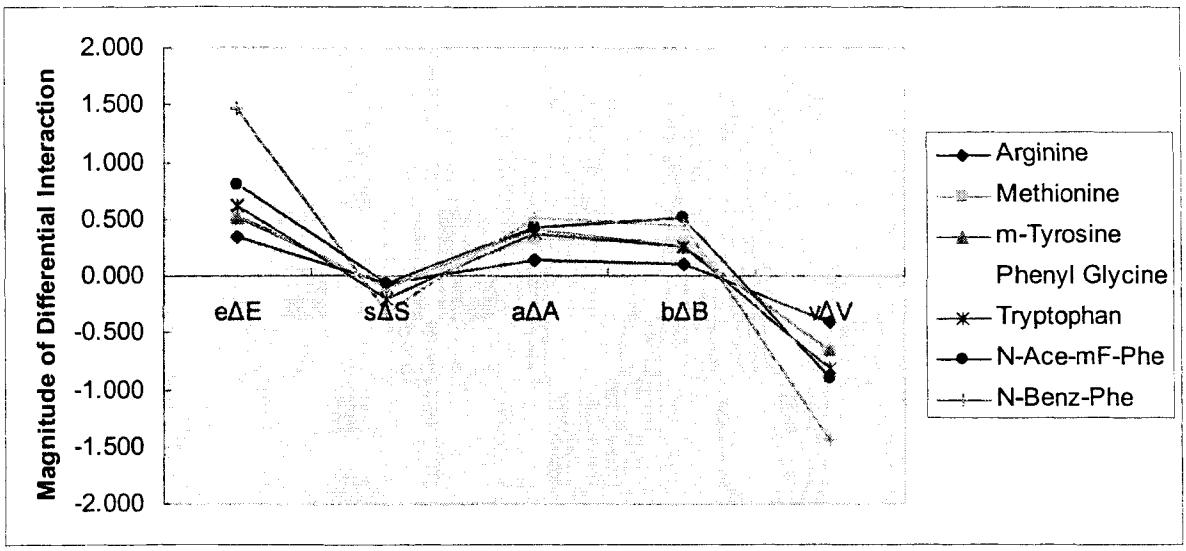


Figure 5. Factors that contribute to the enantioselectivity of amino acids.

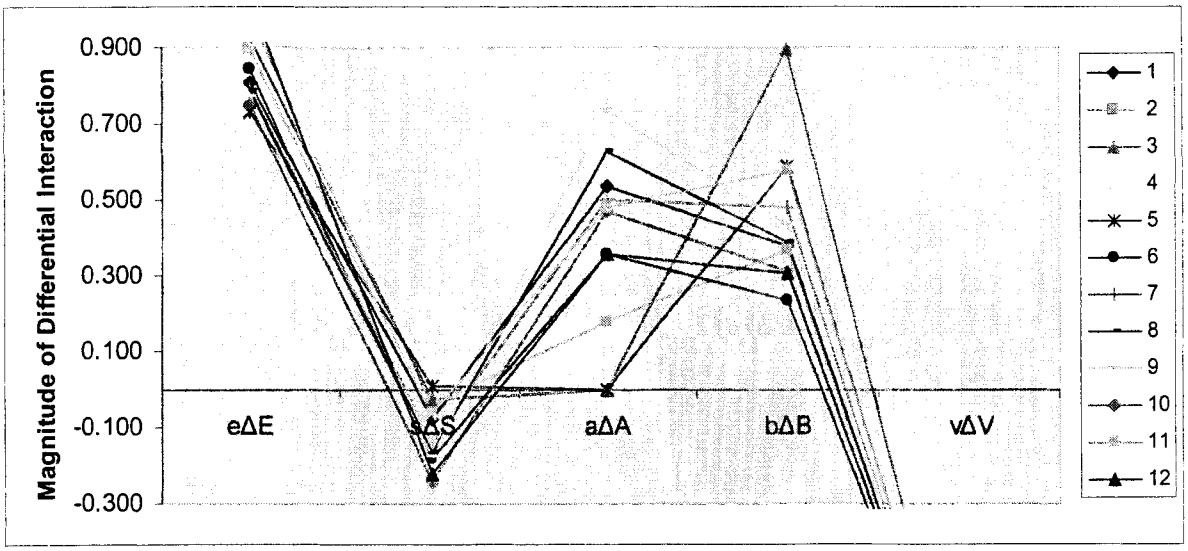


Figure 6. Factors of enantioselectivity, normalized to $-v\Delta V$. The y-axis scale is reduced to enhance the view of the polar interaction terms, $s\Delta S$, $a\Delta A$, and $b\Delta B$. Compound one is 5-methyl-5-phenyl-hydantoin, 2 is bromacil, 3 is dihydrofurocoumarin, 4 is 1,1-dimethyl-3-phenyl-propyl toluyl sulfoxide, 5 is α-naphthalenyl methyl sulfoxide, 6 is arginine, 7 is methionine, 8 is m-tyrosine, 9 is phenyl glycine, 10 is tyrptophan, 11 is N-acetyl-m-fluoro-phenylalanine, and 12 is N-benzoyl-phenylalanine.

Supplemental Table I. LSER probe solutes and their descriptors. Solute descriptors E, S, A, B, and V are defined in the Introduction.

Solute	E	S	A	B	V
1,3-benzenediol	0.980	1.00	1.10	0.58	0.834
1-nitrobutane	0.227	0.95	0.00	0.29	0.846
4-ethylphenol	0.800	0.90	0.55	0.36	1.057
3-cyanophenol	0.930	1.55	0.77	0.28	0.930
benzonitrile	0.742	1.11	0.00	0.33	0.871
acetophenone	0.818	1.01	0.00	0.48	1.014
phenol	0.805	0.89	0.60	0.31	0.775
2-nitrophenol	1.015	1.05	0.05	0.37	0.949
1-nitropentane	0.212	0.95	0.00	0.29	0.987
m-cresol	0.822	0.88	0.57	0.34	0.916
p-cresol	0.820	0.87	0.57	0.31	0.916
methyl-4-hydroxybenzoate	0.900	1.37	0.69	0.45	1.131
nitrobenzene	0.871	1.11	0.00	0.28	0.891
ethyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.272
1-nitrohexane	0.203	0.95	0.00	0.29	1.128
benzene	0.610	0.52	0.00	0.14	0.716
n-propyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.413
4-chlorophenol	0.915	1.08	0.67	0.20	0.897
toluene	0.601	0.52	0.00	0.14	0.857
o-xylene	0.663	0.56	0.00	0.16	0.998
m-xylene	0.623	0.52	0.00	0.16	0.998
p-xylene	0.613	0.52	0.00	0.16	0.998
3-chlorophenol	0.909	1.06	0.69	0.15	0.897
valerophenone	0.800	0.95	0.00	0.50	1.437
n-butyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.554
1-naphthol	1.520	1.05	0.61	0.37	1.144
ethylbenzene	0.613	0.51	0.00	0.15	0.998
naphthalene	1.340	0.92	0.00	0.20	1.085
hexanophenone	0.720	0.95	0.00	0.50	1.578
heptanophenone	0.720	0.95	0.00	0.50	1.718
n-propylbenzene	0.604	0.50	0.00	0.15	1.139
dibenzothiophene	1.959	1.31	0.00	0.20	1.379
n-buytlbenzene	0.600	0.51	0.00	0.15	1.280

Supplemental Table II. System constants for Chirobiotic T CSP at different temperature and ratios of ethanol/buffer. r^2 is the coefficient of correlation, SE is the standard error of regression, F is the Fisher statistic, n is the number of solutes in the regression.

Mobile Phase	Temperature (°C)	e	s	a	b	v	Intercept	r^2	SE	F	n
5% EtOH	40	0.33	0.21	-0.50	-0.80	1.33	-1.38	0.99	0.042	685	34
5% EtOH	25	0.39	0.28	-0.51	-0.69	1.35	-1.42	0.99	0.045	632	34
5% EtOH	10	0.43	0.40	-0.54	-0.40	1.25	-1.40	0.99	0.039	787	34
5% EtOH	5	0.45	0.41	-0.53	-0.45	1.28	-1.44	0.99	0.045	628	34
10% EtOH	55	0.26	0.20	-0.45	-0.83	1.11	-1.24	0.99	0.040	551	34
10% EtOH	40	0.32	0.20	-0.45	-0.77	1.17	-1.26	0.99	0.041	587	34
10% EtOH	25	0.37	0.28	-0.47	-0.66	1.20	-1.33	0.99	0.039	691	34
10% EtOH	10	0.41	0.39	-0.48	-0.62	1.14	-1.41	0.99	0.043	549	34
10% EtOH	5	0.46	0.39	-0.48	-0.57	1.27	-1.45	0.99	0.046	568	34
15% EtOH	40	0.28	0.23	-0.45	-0.85	1.08	-1.26	0.99	0.044	452	34
15% EtOH	25	0.34	0.30	-0.47	-0.80	1.16	-1.36	0.99	0.045	493	34
15% EtOH	10	0.41	0.38	-0.48	-0.66	1.19	-1.43	0.99	0.045	537	34
15% EtOH	5	0.44	0.42	-0.48	-0.64	1.21	-1.47	0.99	0.047	513	34
20% EtOH	25	0.32	0.26	-0.46	-0.73	1.03	-1.30	0.98	0.045	345	33
20% EtOH	10	0.38	0.39	-0.49	-0.69	1.08	-1.36	0.99	0.045	489	34
20% EtOH	5	0.40	0.41	-0.48	-0.67	1.11	-1.38	0.99	0.046	469	34
25% EtOH	25	0.28	0.25	-0.47	-0.72	0.91	-1.23	0.98	0.045	284	33
25% EtOH	10	0.35	0.31	-0.47	-0.68	1.00	-1.25	0.98	0.049	298	33
25% EtOH	5	0.38	0.33	-0.48	-0.68	1.05	-1.28	0.98	0.054	271	33
30% EtOH	25	0.24	0.23	-0.48	-0.67	0.75	-1.13	0.97	0.045	228	33
30% EtOH	10	0.28	0.27	-0.48	-0.65	0.84	-1.09	0.98	0.046	253	33
30% EtOH	5	0.30	0.27	-0.48	-0.69	0.91	-1.10	0.97	0.051	235	33

Table I. Solute descriptors of the enantiomers of the neutral compounds in this study, obtained from ethanol/buffer mobile phase retention data, unless specified. Standard errors of the fitting coefficients are shown in parenthesis.

Compound		E	S	A	B	V	r^2	F	SE	n
5-methyl-5-phenyl-hydantoin	Peak 1	2.65	0.89	0	0.13	0.35	1.00	32767	0.020	22
		(0.30)	(0.18)		(0.03)	(0.06)				
	Peak 2	4.42	0.70	-0.71	-0.19	-0.33	0.99	11971	0.037	22
		(0.56)	(0.35)	(0.20)	(0.08)	(0.12)				
bromacil	Peak 1	1.27	0.98	0.05	0.23	0.99	1.00	288421	0.006	20
		(0.10)	(0.06)	(0.03)	(0.01)	(0.02)				
	Peak 2	2.01	0.94	-0.03	0.12	0.76	1.00	62504	0.015	20
		(0.23)	(0.14)	(0.08)	(0.03)	(0.05)				
substituted dihydrofurocoumarin	Peak 1	1.87	1.35	0	0.39	1.52	1.00	272052	0.012	23
		(0.18)	(0.10)		(0.02)	(0.03)				
	Peak 2	2.27	1.34	0	0.24	1.39	1.00	133689	0.017	23
		(0.26)	(0.15)		(0.02)	(0.05)				
1,1-dimethyl-3-phenyl-propyl toluyl sulfoxide	Peak 1	1.18	1.10	0	0.48	1.83	1.00	259420	0.012	22
		(0.17)	(0.10)		(0.01)	(0.03)				
	Peak 2	1.89	1.14	0	0.29	1.60	1.00	137099	0.017	22
		(0.25)	(0.15)		(0.02)	(0.05)				
α-naphthalenyl methyl sulfoxide	Peak 1	2.02	1.29	0	0.29	1.10	1.00	112530	0.016	23
		(0.23)	(0.14)		(0.02)	(0.05)				
	Peak 2	3.61	1.30	0	-0.21	0.43	0.98	28905	0.032	21
		(0.50)	(0.30)		(0.06)	(0.11)				
α-naphthalenyl methyl sulfoxide [a]	Peak 1	1.93	1.61	0	0.40	1.14	1.00	1034245	0.003	12
		(0.03)	(0.03)		(0.02)	(0.01)				
	Peak 2	2.59	2.19	0	0.50	0.91	1.00	48179	0.013	12
		(0.22)	(0.19)		(0.15)	(0.09)				

a) based on retention data from methanol/water mobile phases

Table II. Factors that contribute to the retention of enantiomers. These values are the product of solute descriptors and system constants for the teicoplanin CSP at 25/75 ethanol/buffer & 25° C, unless specified.

Compound		eE	sS	aA	bB	vV
5-methyl-5-phenyl-hydantoin	Peak 1	0.75	0.23	0.00	-0.09	0.32
	Peak 2	1.25	0.18	0.33	0.14	-0.30
bromacil	Peak 1	0.36	0.25	-0.02	-0.17	0.91
	Peak 2	0.57	0.24	0.02	-0.09	0.69
substituted dihydrofurocoumarin	Peak 1	0.53	0.34	0.00	-0.28	1.39
	Peak 2	0.64	0.34	0.00	-0.17	1.27
1,1-dimethyl-3-phenyl-propyl	Peak 1	0.33	0.28	0.00	-0.35	1.67
toluyl sulfoxide	Peak 2	0.54	0.29	0.00	-0.21	1.46
α-naphthalenyl methyl sulfoxide	Peak 1	0.57	0.33	0.00	-0.21	1.00
	Peak 2	1.02	0.33	0.00	0.15	0.39
α-naphthalenyl methyl sulfoxide [a]	Peak 1	0.59	0.35	0.00	-0.11	0.93
methanol water	Peak 2	0.79	0.48	0.00	-0.14	0.74

a) based on retention data from methanol/water mobile phases

Table III. Factors that contribute to the selectivity of enantiomers. These values are the differences in the factors of retention, calculated for a 25/75 ethanol/buffer mobile phase at 25°C.

Compound	eΔE	sΔS	aΔA	bΔB	vΔV
5-methyl-5-phenyl-hydantoin	0.50	-0.05	0.33	0.23	-0.62
bromacil	0.21	-0.01	0.04	0.08	-0.21
substituted dihydrofurocoumarin	0.11	0.00	0.00	0.11	-0.12
1,1-dimethyl-3-phenyl-propyl toluyl sulfoxide	0.20	0.01	0.00	0.14	-0.21
α-naphthalenyl methyl sulfoxide	0.45	0.00	0.00	0.36	-0.62
α-naphthalenyl methyl sulfoxide [a]	0.20	0.13	0.00	-0.03	-0.19

a) based on retention data from methanol/water mobile phases

Table IV. Solute descriptors of amino acid enantiomers, obtained from ethanol/buffer mobile phase retention data. Standard errors of the fitting coefficients are shown in parenthesis.

Compound	Peak	E	S	A	B	V	r^2	F	SE	n
arginine	1	3.99	-0.37	-0.93	-0.80	-0.73	0.96	12440	0.027	21
		(0.46)	(0.28)	(0.15)	(0.07)	(0.11)				
	2	5.19	-0.66	-1.24	-0.94	-1.17	0.97	16408	0.025	21
		(0.43)	(0.26)	(0.14)	(0.06)	(0.10)				
methionine	1	3.88	0.41	-0.32	-0.18	-0.97	0.97	1405	0.041	22
		(0.62)	(0.39)	(0.23)	(0.09)	(0.14)				
	2	5.75	0.04	-1.02	-0.62	-1.69	0.95	1717	0.056	22
		(0.86)	(0.54)	(0.31)	(0.12)	(0.19)				
m-tyrosine	1	4.19	0.61	-0.08	-0.23	-0.83	0.98	2529	0.041	22
		(0.62)	(0.39)	(0.22)	(0.09)	(0.14)				
	2	5.97	0.18	-0.94	-0.58	-1.54	0.97	2863	0.053	22
		(0.80)	(0.50)	(0.29)	(0.12)	(0.18)				
phenylglycine	1	3.81	0.29	-0.47	-0.29	-0.94	0.97	2190	0.038	22
		(0.58)	(0.36)	(0.21)	(0.08)	(0.13)				
	2	6.40	-0.37	-1.96	-0.86	-1.98	0.96	3376	0.055	22
		(0.83)	(0.52)	(0.30)	(0.12)	(0.18)				
tryptophan	1	3.63	0.60	-0.48	-0.19	-0.46	0.98	6731	0.035	22
		(0.52)	(0.33)	(0.19)	(0.07)	(0.11)				
	2	5.79	-0.19	-1.30	-0.54	-1.35	0.95	2714	0.061	22
		(0.93)	(0.58)	(0.34)	(0.13)	(0.21)				
N-acetyl-m-fluoro-phenylalanine	1	6.28	0.28	-1.01	-0.16	-1.41	0.92	716	0.109	22
		(1.66)	(1.05)	(0.61)	(0.24)	(0.37)				
	2	9.11	0.03	-1.92	-0.88	-2.39	0.94	1824	0.106	22
		(1.61)	(1.02)	(0.59)	(0.24)	(0.36)				

N-benzoyl-phenylalanine	1	2.08	1.28	-0.18	0.45	0.57	0.96	2442	0.070	23
		(1.06)	(0.66)	(0.38)	(0.15)	(0.24)				
	2	7.27	0.03	-1.26	-0.16	-1.00	0.96	2612	0.095	23
		(1.44)	(0.9)	(0.52)	(0.21)	(0.32)				

Table V. Factors that contribute to the retention of amino acid enantiomers. These values are the product of solute descriptors and system constants for the teicoplanin CSP with a 25/75 ethanol/buffer mobile phase at 25° C.

Compound		eE	sS	aA	bB	vV
arginine	Peak 1	1.13	-0.09	0.44	0.58	-0.66
	Peak 2	1.47	-0.17	0.58	0.67	-1.06
methionine	Peak 1	1.10	0.10	0.15	0.13	-0.88
	Peak 2	1.63	0.01	0.48	0.44	-1.54
m-tyrosine	Peak 1	1.19	0.16	0.04	0.17	-0.76
	Peak 2	1.69	0.05	0.44	0.42	-1.40
phenylglycine	Peak 1	1.08	0.07	0.22	0.21	-0.85
	Peak 2	1.81	-0.09	0.92	0.62	-1.80
tryptophan	Peak 1	1.03	0.15	0.23	0.14	-0.42
	Peak 2	1.64	-0.05	0.61	0.39	-1.23
N-acetyl-m-fluoro-phenylalanine	Peak 1	1.78	0.07	0.47	0.12	-1.28
	Peak 2	2.58	0.01	0.90	0.64	-2.18
N-benzoyl-phenylalanine	Peak 1	0.59	0.33	0.09	-0.32	0.52
	Peak 2	2.06	0.01	0.59	0.11	-0.91

Table VI. Factors that contribute to the selectivity of amino acid enantiomers. These values are the differences in the factors of retention, calculated for a 25/75 ethanol/buffer mobile phase at 25°C.

Compound	eΔE	sΔS	aΔA	bΔB	vΔV
arginine	0.34	-0.07	0.14	0.09	-0.40
methionine	0.53	-0.09	0.33	0.31	-0.66
m-tyrosine	0.50	-0.11	0.40	0.25	-0.64
phenylglycine	0.73	-0.17	0.70	0.41	-0.95
tryptophan	0.61	-0.20	0.38	0.25	-0.82
N-acetyl-m-fluoro-phenylalanine	0.80	-0.06	0.43	0.52	-0.90
N-benzoyl-phenylalanine	1.47	-0.32	0.51	0.44	-1.43

Table VII. Factors of enantioselectivity normalized to $-v\Delta V$.

Compound	eΔE	sΔS	aΔA	bΔB	vΔV
5-methyl-5-phenyl hydantoin	0.808	-0.078	0.535	0.377	-1.000
bromacil	0.987	-0.043	0.175	0.366	-1.000
dihydrofurocoumarin	0.927	-0.028	0.000	0.893	-1.000
1,1-dimethyl-3-phenyl-propyl toluyl sulfoxide	0.949	0.042	0.000	0.653	-1.000
α-naphthalenyl methyl sulfoxide	0.733	0.006	0.000	0.588	-1.000
arginine	0.845	-0.180	0.357	0.236	-1.000
methionine	0.793	-0.143	0.501	0.478	-1.000
m-tyrosine	0.785	-0.168	0.630	0.390	-1.000
phenylglycine	0.774	-0.176	0.738	0.432	-1.000
tryptophan	0.751	-0.246	0.470	0.308	-1.000
N-acetyl-m-fluoro-phenylalanine	0.894	-0.071	0.479	0.578	-1.000
N-benzoyl-phenylalanine	1.030	-0.223	0.354	0.306	-1.000

CHAPTER 5

Factors that Contribute to Enantioselectivity in Macrocyclic Glycopeptide Chiral Stationary Phases in the Normal-phase Mode

A paper submitted to Journal of Chromatography A

Clifford R. Mitchell, Daniel W. Armstrong

Abstract

The linear solvation energy relationship, developed by Abraham, is utilized to study enantioselectivity on the Chirobiotic T chiral stationary phase in the normal-phase mode. It was found that the system constants, obtained from ethanol/heptane based mobile phases, were highly intercorrelated (which indicates that solute descriptors derived from these system constants would not be reliably determined). The solvents ethoxynonafluorobutane (ENFB) and 2,2,2-trifluoroethanol (TFE) were also used as mobile phase solvents, and the intercorrelation between the system constants, including those derived from ethanol/ENFB and TFE/ENFB based mobile phases, was sufficiently reduced. The factors that control enantioselectivity for 5-methyl-5-phenyl-hydantoin were determined, and it was found that steric repulsion interactions, dipole-dipole interactions, and π-electron complexation had the largest effect on enantioselectivity, while hydrogen bonding had very little effect on enantioselectivity. The quality of the regressions was sufficient to generate solute descriptors that where statistically different for the enantiomers of 5-methyl-5-phenyl-hydantoin.

5.1 Introduction

The ability to determine which intermolecular interactions contribute to the separation of enantiomers is extremely desirable for the design of a chiral selector. Recently, we demonstrated that the linear solvation energy relationship (LSER), developed by Abraham, can be used to determine which intermolecular interactions occurring between a chiral selector and a pair of enantiomers contribute to retention and enantioselectivity [1]. The study focused on the enantioselectivity of the teicoplanin chiral selector in the reversed-phase mode. It was found that ion-dipole interactions and steric repulsion interactions had the greatest impact on the separation of enantiomers, with varying amounts of hydrogen bonding contributing to enantioselectivity and dipole-dipole interactions having almost no impact the separation of enantiomers (on the Chirobiotic T (teicoplanin) chiral stationary phase (CSP) in the reversed-phase mode).

The normal-phase mode and the reversed-phase mode of liquid chromatography are considered to be different modes of operation because it has been presumed that retention and selectivity are due to different factors, i.e., different intermolecular interactions occurring between the analyte and stationary phase in each mode. While there have been extensive studies of retention theory in both modes of operation, it is rare that a comprehensive theory of retention has been applied to the same stationary phase in both modes of operation. To do so would unambiguously show that retention and selectivity are governed by different intermolecular forces in each mode of operation.

The LSER theory developed by Abraham [2] states that the distribution of a solute in a biphasic system is due to the intermolecular interactions the solutes experiences in both phases, or mathematically:

$$Log\,K = c + eE + sS + aA + bB + vV \quad (1)$$

where the five terms of equation one account for the ability of both the system and the solute to participate in a given intermolecular interaction. The term for interactions through polarizable n and π electrons is (eE), the term for dipole-dipole interactions is (sS), the term for a solute donating a hydrogen atom and the system donating a lone pair of electrons in a hydrogen bond is (aA), the term for a solute donating a lone pair of electrons and the system donating a hydrogen atom in a hydrogen bond is (bB), and the term for dispersion interactions is (vV). The c term contains the chromatographic phase ratio. Each term contains two components; one component describes a solute's ability to participate in the specified interaction (the upper case letters which are called solute descriptors) and the other component describes the system's ability to participate in the specified interaction (the lower case letters that are called system constants).

To apply the LSER to the study of enantioselectivity, the relationship between chromatographic selectivity and the terms of the LSER equation must be established. Chromatographic selectivity is defined by

$$\alpha = \frac{k_2}{k_1} = \frac{K_2}{K_1} \quad (2)$$

Taking the logarithm of this and rearranging gives

$$Log\alpha = Log\frac{K_2}{K_1} = LogK_2 - LogK_1 \quad (3)$$

Substituting a LSER equation for each log K and rearranging gives

$$Log\alpha = \left[(eE_2 - eE_1) + (sS_2 - sS_1) + (aA_2 - aA_1) + (bB_2 - bB_1) + (vV_2 - vV_1) + (c_2 - c_1)\right] \quad (4)$$

which can be reduced to the following

$$Log\alpha = \left[\left(eE_2 - eE_1\right) + \left(sS_2 - sS_1\right) + \left(aA_2 - aA_1\right) + \left(bB_2 - bB_1\right) + \left(vV_2 - vV_1\right)\right] \quad (5)$$

because the c-terms are constant and cancel each other. If we define, for the solute, descriptors ΔX as $X_2 - X_1$, we can simplify the selectivity expression to

$$Log\alpha = e\Delta E + s\Delta S + a\Delta A + b\Delta B + v\Delta V \quad (6)$$

The terms of this equation, such as $s\Delta S$, are components of chromatographic selectivity and indicate the relative importance of the specified interaction, in this case dipole-dipole interactions, to selectivity (or enantioselectivity).

To perform this kind of analysis of enantioselectivity, it is necessary to determine the system constants for a given system (a chiral stationary phase and mobile phase condition) and the solute descriptors for the chiral solute. While both of these tasks have been performed for achiral stationary phases and solutes [2-20], we recently demonstrated that the LSER theory can be applied to chiral separations by determining the system constants [21] and solute descriptors [1] for the analysis of enantioselectivity. Briefly, a large set of system constants of a chiral stationary phase are determined, with achiral solutes, at a variety of conditions (mobile phase composition, temperature). The retention factors for the enantiomers of the selected racemate are also determined at these conditions. Then, with the system constants as the independent variables and the retention factors of a single enantiomer as the dependent variable, multiple linear regression is performed to obtain the solute descriptors of the enantiomer. The process is repeated for the other antipode. With the solute descriptors and system constants of a chiral solute and chiral stationary phase, it is possible to determine which intermolecular interactions generate enantioselectivity, according to equation 6.

An important consideration is the origin of the differences in the solute descriptors of chiral solutes. In an isotropic environment, enantiomers of a chiral molecule have identical physical and chemical properties, and therefore identical solute descriptors. In a chiral environment, those same enantiomers can have different physical and chemical properties. Because of this, any differences in the solute descriptors obtained from this method of analysis are indicative of the differences in solvation interactions that the chiral solute experiences with a chiral stationary phase.

There are several ways that the LSER approach can be used to study enantioseparations. The first approach would be to identify the exact factors that influence enantioselectivity (as well as their relative importance) for compounds on a given CSP, in one mode of operation. This would allow more exact correlations to be drawn between molecular structure and the factors contributing to enantioselectivity. Alternatively, the LSER approach could be used to determine the factors that influence enantioselectivity for a single compound on different stationary phases in the same mode of operation. This would reveal the difference in separation mechanism between different CSPs. Finally, the LSER approach can be used to pinpoint the factors that influence enantioselectivity for a single compound on a single CSP in different modes of operation or with different mobile phase compositions. This would reveal the difference in separation mechanisms that are active in each particular mode. In this study, both the first and third types of comparisons will be made for enantiomeric separations in the normal-phase mode on the Chirobiotic T (teicoplanin) CSP.

5.2 Experimental

5.2.1 Materials

The Chirobiotic T stationary phase used was obtained from Advanced Separation Technologies (Whippany, NJ) and consisted of the teicoplanin chiral selector bonded to 5 μm spherical silica gel. The dimensions of the columns are 50 × 4.6 mm.

Heptane was HPLC grade from Fisher (Fairlawn, NJ), ethanol was punctilious grade from Aaper alcohol (Shelbyville, KY), 2,2,2-trifluoroethanol was reagent plus grade from Aldrich (St. Louis, MO), and the ethoxynonafluorobutane was purchased as Novec Engineered Fluid HFE-7200 from 3M Co. (St. Paul, MN). All LSER probe solutes (Table I) and most chiral solutes were obtained from Aldrich (St. Louis, MO) in high purity grade (99% or greater). The chiral sulfoxides were from a previous synthetic preparation [22, 23].

5.2.2 Instrumental

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett Packard, Palo Alto, CA, USA), an integrator (3395, Hewlett Packard), and a Jetstream + column thermostat (Advanced Separation Technologies) capable of regulating column temperature from 0 to 80 °C. Mobile phases were degassed by sparging with helium for ~2 minutes. UV detection was carried out at 220 nm.

5.2.3 Calculations

Retention factors (k) were calculated using the equation $k = (tr - t_M)/t_M$. Enantioselectivity factors (α) were calculated using the formula $\alpha = k_2/k_1$. Multiple linear

regression analysis and statistical calculations were performed using the program Analyse-it, a statistics package for Microsoft Excel.

5.3 Results & Discussion

The LSER probe solutes and 10 racemates were eluted from the teicoplanin CSP with normal-phase mobile phases at two different temperatures (5°C and 35°C). System constants for each mobile phase/temperature combination were obtained from the retention data of the LSER probe solutes via multiple linear regression, which generated good fits to the LSER equation. Correlation coefficients (r^2) of these linear regressions were typically larger than 0.98, and standard deviations were small in comparison to the fitting coefficients.

Initially, conventional ethanol/heptane mobile phases were utilized at different temperatures. It was found that the system constants obtained from these data are highly intercorrelated (Table II). For example, the coefficient of correlation (r) between all of the "s" system constants and all of the "b" system constants is 0.97. This high degree of intercorrelation indicates that the system constants (for this set of ethanol/heptane data) are not independent variables. When analyzing data by regression, the values of the regressor variables (the system constants in this case) should be independent of each other to properly deconvolute their impact on the value of the dependent variable (log k). Consequently, the solute descriptors obtained from this data set will not be independently determined and should be considered unreliable. It was therefore necessary to use other normal-phase mobile phases that had different properties or were more orthogonal to the ethanol/heptane mobile phases.

Recently, we explored the use of ethoxynonafluorobutane (ENFB) as a replacement for hexane and heptane in normal-phase mobile phases for NP-HPLC/MS [24]. Kagan et al. first demonstrated the feasibility of ENFB as a normal-phase chromatography solvent for NP-LC/MS [25-27]. Novec Engineered Fluid HFE-7200 was initially marketed by 3M Co. as a cleaning fluid, deposition solvent, and heat transfer fluid. HFE-7200 is produced as an azeotropic mixture of ethyl nonafluorobutylether and ethyl nonafluoroisobutyl ether, two compounds with very similar properties [24]. Compared with hexane and heptane, it has lower flammability and volatility, with similar viscosities and UV-cutoffs, making it a good solvent for liquid chromatography.

The LSER probe solutes and 10 racemates were eluted from the teicoplanin CSP with ethanol/ENFB mobile phases, and 2,2,2-trifluoroethanol/ENFB mobile phases. System constants for each mobile phase composition/temperature combination were obtained from the retention factors of the LSER probe solutes via multiple linear regression (Table III). The quality of the linear regressions for the TFE/ENFB system constants was similar to that of the ethanol/heptane (r^2 ~0.98), but the ethanol/ENFB regressions were slightly lower (r^2 ~0.94).

The intercorrelation between system constants of these three mobile phase systems (ethanol/heptane, ethanol/ENFB, TFE/ENFB) was greatly reduced (Table IV), compared with the original ethanol/heptane set of system constants. The correlation between the e- and v-terms is the largest ($r = 0.76$), and some care should be taken when these system constants are used to determine solute descriptors. While most of the racemates were well separated in the ethanol/heptane mobile phase, the enantioselectivity of the examined solutes was often much less in the mobile phases containing fluorinated solvents. Solute descriptors for the 10

racemates were determined from the retention data of each enantiomer and system constants from the three types of mobile phases, ethanol/heptane, ethanol/ENFB, and TFE/ENFB.

Only the solute descriptors for 5-methyl-5-phenyl-hydantoin were statistically different from each other (Fig. 2A, Table V). The quality of the linear regressions for each enantiomer were fair (r^2 ~0.97 and 0.92 for the first and second eluting enantiomer, respectively). The factors that contribute to retention and enantioselectivity were calculated according to equations 1 and 6, respectively (Fig 2B and 2C).

While some of the interactions accounted for in equation 6 require interpretation, others are very clear in that they indicate what is occurring between a solute and a stationary phase (or the enantiomer and the chiral selector). The dipolar ($s\Delta S$) and hydrogen bonding terms ($a\Delta A$ and $b\Delta B$) are straightforward. The $s\Delta S$ term describes dipole-dipole interactions, and for 5-methyl-5-phenyl-hydantoin the $s\Delta S$ is 0.21, and the sS_1 and sS_2 terms are 1.14 and 1.36. These values indicate the dipole-dipole interactions have a positive impact on both retention and enantioselectivity. The $a\Delta A$ hydrogen bonding term indicates a hydrogen bond being donated by the enantiomers, and accepted by the chiral selector, and the $b\Delta B$ hydrogen bonding term indicates a hydrogen bond being donated by the chiral selector, and accepted by the enantiomers. The $a\Delta A$ and $b\Delta B$ terms for 5-methyl-5-phenyl-hydantoin are -0.02 and -0.09, respectively. These values suggest that hydrogen bonding plays a very small role in enantioselectivity, and the first eluting enantiomer experiences more enantioselective hydrogen bonding than the second eluting enantiomer (because the delta terms are negative). The aA_1, aA_2, bB_1, and bB_2 terms are all positive, indicating that hydrogen bonding does have a positive effect on retention. These three polar interactions

parameters indicate the same types of interactions in either the reversed-phase mode or the normal-phase mode.

The $v\Delta V$ term requires more interpretation. In our previous reversed-phase mode study, we found that dispersion interaction had a negative effect on enantioselectivity [1]. We interpreted this as being indicative of a steric repulsion interaction, because the first eluting enantiomer experiences more dispersion forces with the stationary phase than the second eluting enantiomer (indicated by the negative ΔV term), as is the case for separations influenced by steric repulsions. For 5-methyl-5-phenyl-hydantoin, the $v\Delta V$ is 0.25, and we interpret this as a steric repulsion for the same reason as in the reversed-phase mode (the ΔV term is negative). In the normal-phase mode, the "v" system constant is also negative, causing the product of $v\Delta V$ to be positive.

The final interaction covered by the LSER theory is interactions through polarizable n- and π-electrons ($e\Delta E$). In the reversed-phase mode, we attribute interactions in this e-term to ion-dipole interactions [28]. However, in the normal-phase mode, any ionic groups present in the stationary phase will exist as ion-pairs that are not available for solvation interactions, and ionic solutes are poorly soluble in normal-phase solvents. Because ion-dipole interactions are not a possibility in the normal-phase mode, π-electron complexation is the only type of interaction that can be manifested in the e-term. With heptane-based mobile phases, the macrocyclic glycopeptide CSPs seem to have no ability to interact through π-electron complexation (e-term is indistinguishable from zero, Table III) [21]. However, when using ENFB and other fluorinated solvents in the mobile phase, the e-term is positive (Table III). The positive *e*-term is a consequence of highly fluorinated molecules having negative E solute descriptors [2]. Therefore, it seems that fluorinated solvents are good for

promoting π-electron complexation interactions. The eΔE term for 5-methyl-5-phenyl-hydantoin is 0.12, indicating that π-electron complexation has a positive effect on enantioselectivity. Additionally, the eE_1 and eE_2 terms are 0.37 and 0.50, indicating that π-electron complexation also has a positive effect on retention.

5.3.1 Comparison of enantioselectivity in the normal-phase mode and the reversed-phase mode

The factors that make up enantioselectivity for 5-methyl-5-phenyl-hydantoin were determined in the normal-phase mode and the reversed-phase mode (in a previous study) [1]. By comparing the factors contributing to enantioselectivity in each mode, one can determine exactly how the different modes of chromatography are active in separating enantiomers. The factors contributing to enantioselectivity for 5-methyl-5-phenyl-hydantoin in the normal-phase mode and the reversed-phase mode are shown in Figure 3. The only common factor to both modes is the role of steric repulsion interactions (vΔV). The differences in the sign of the vΔV term between the reversed-phase mode and the normal-phase mode are due to the sign of the system constant "v"; the sign of the ΔV term is negative in both modes. There is a great deal of contrast in the relative importance of the three polar interaction terms, sΔS, aΔA, and bΔB. In the reversed-phase mode, we found that both types of hydrogen bonding had a positive effect on enantioselectivity, and dipole-dipole interactions had a negligible effect. In the normal-phase mode, the opposite is true. Dipole-dipole interactions have a positive effect, and the two types of hydrogen bonding have a smaller, almost negligible, negative effect on enantioselectivity. Finally, interactions through polarizable n- and π-electrons (eΔE) have a positive effect in both modes of operation; however, we attribute the

interactions to ion-dipole interactions in the reversed-phase mode and π-electron complexation in the normal-phase mode.

5.4 Conclusions

The LSER approach can be used to examine enantioselectivity in the normal-phase mode of operation; however, a greater variety of mobile phase conditions must be used to offset the intercorrelation in the system constants from ethanol/heptane based mobile phases. Ethoxynonafluorobutane is a suitable replacement for heptane and reduces the intercorrelation in the system constants. It was found that, for 5-methyl-5-phenyl-hydantoin, enantioselectivity is composed of steric repulsion interactions, dipole-dipole interactions, and π-electron complexation. In the reversed-phase mode, enantioselectivity is composed of steric repulsion interactions, ion-dipole interactions, and hydrogen bonding.

Acknowledgement

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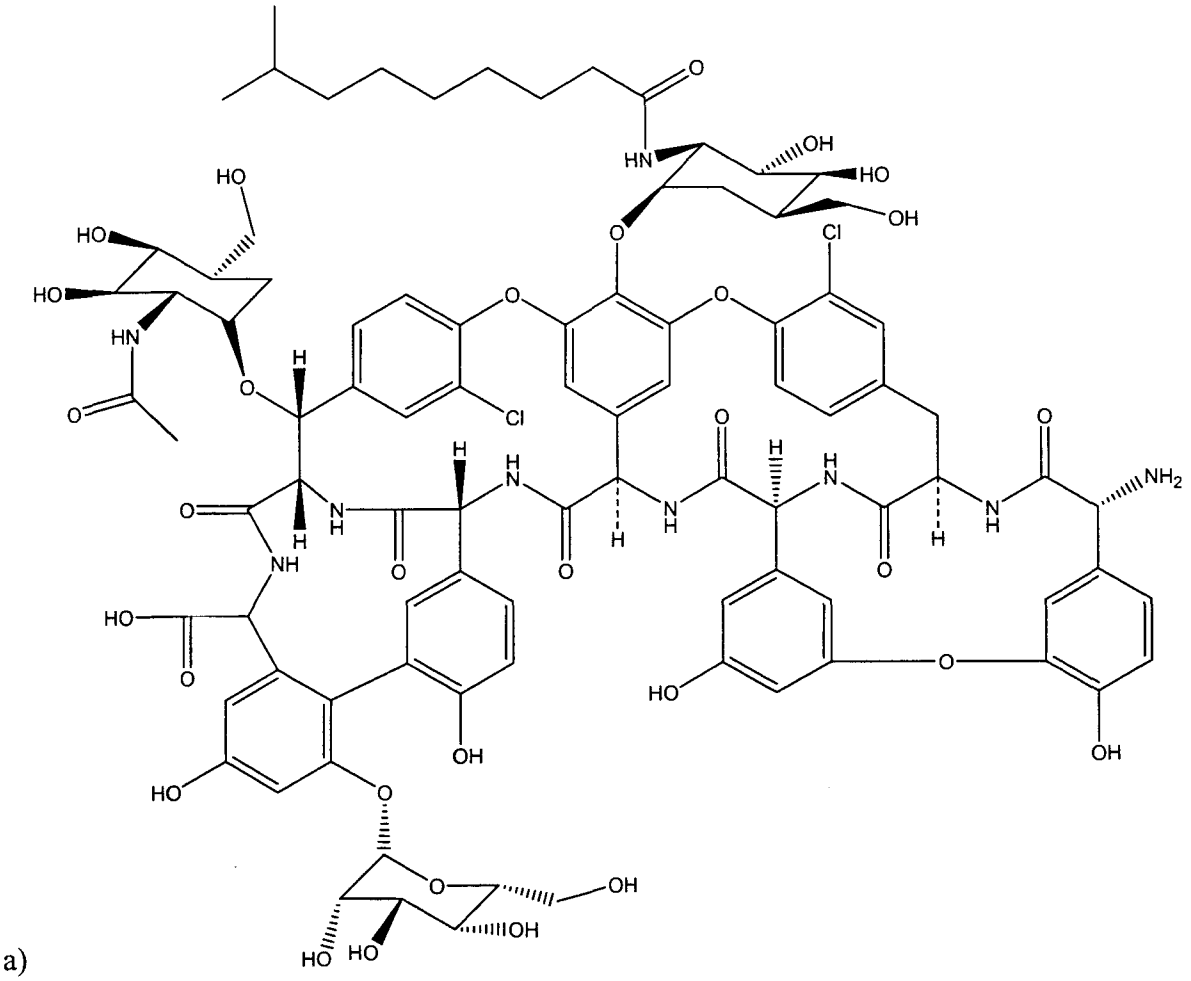
a)



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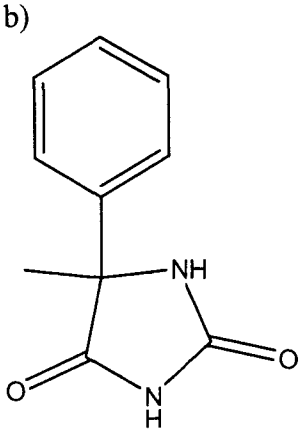
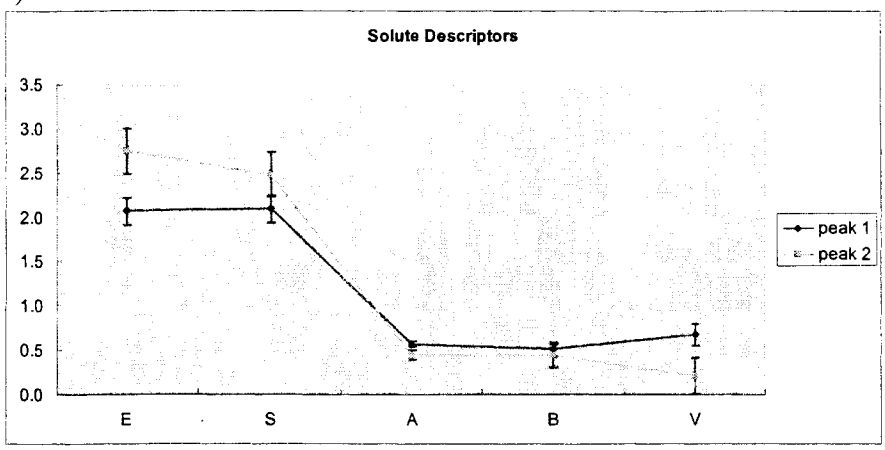


Figure 1. a) Structure of the chiral selector teicoplanin. b) Structure of the solute 5-methyl-5-phenyl-hydantoin.

a)



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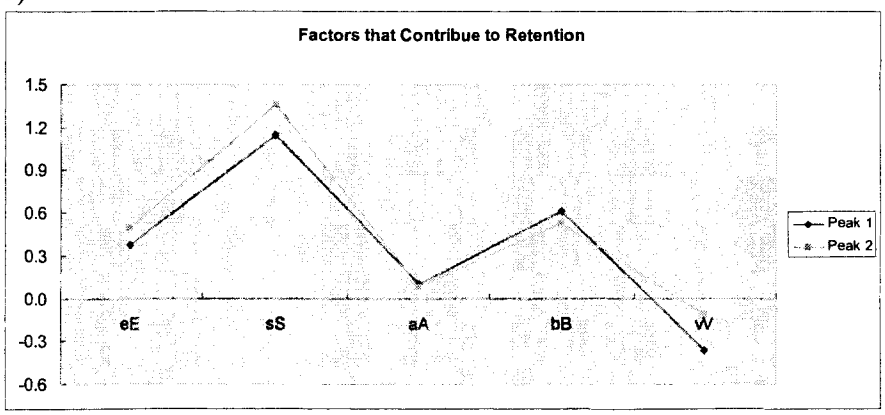

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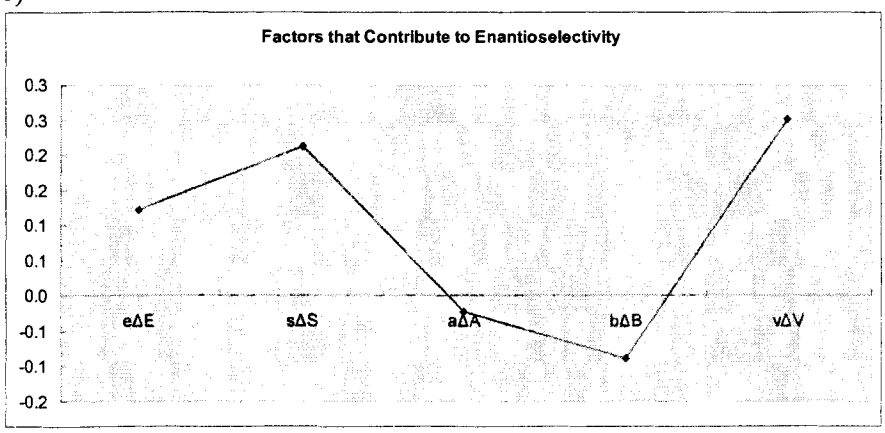

Figure 2. a) Solute descriptors for each enantiomer of 5-methyl-5-phenyl-hydantoin determined in the normal-phase mode on the Chirobiotic T CSP, determined by linear regression. b) The factors that contribute to the retention of each enantiomer of 5-methyl-5-phenyl-hydantoin, determined according to equation 1. c) The factors that contribute to the enantioseparation of 5-methyl-5-phenyl-hydantoin, determined according to equation 6.

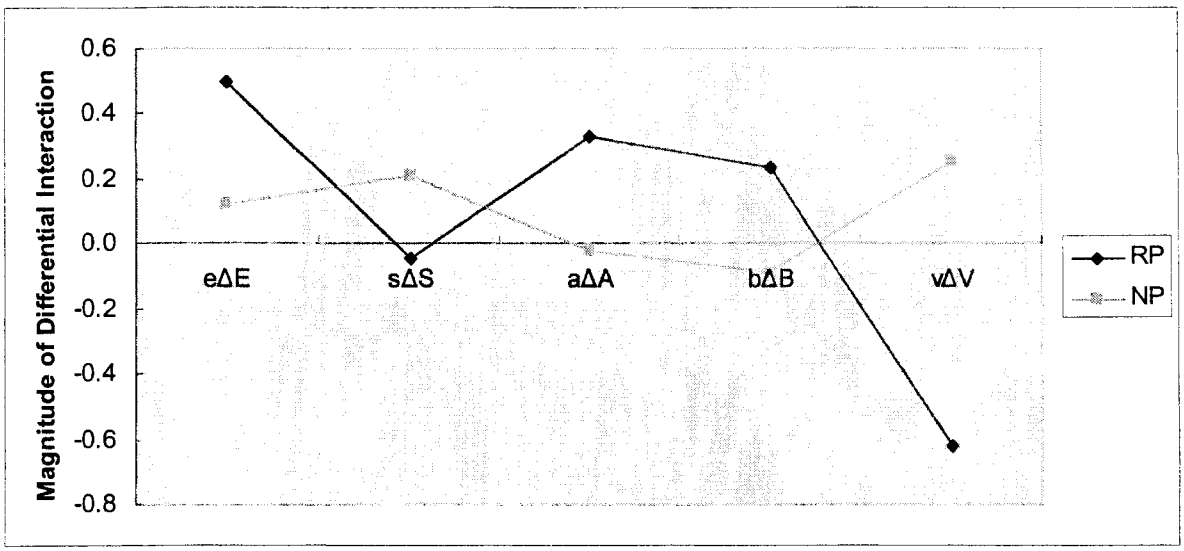

Figure 3. The factors that contribute to the enantioselectivity of 5-methyl-5-phenyl-hydantoin in the reversed-phase mode and the normal-phase mode.

Table I. Solute descriptors of LSER probe solutes. Solute descriptors E, S, A, B, and V are defined in the Introduction.

	E	S	A	B	V
pyrene	2.808	1.71	0.00	0.28	1.5846
dibenzothiophene	1.959	1.31	0.00	0.20	1.3791
phenanthrene	2.055	1.29	0.00	0.26	1.4544
anthracene	2.290	1.34	0.00	0.26	1.4544
n-buytlbenzene	0.600	0.51	0.00	0.15	1.2800
ethylbenzene	0.613	0.51	0.00	0.15	0.9980
p-xylene	0.613	0.52	0.00	0.16	0.9982
toluene	0.601	0.52	0.00	0.14	0.8573
biphenyl	1.360	0.99	0.00	0.22	1.3242
naphthalene	1.340	0.92	0.00	0.20	1.0854
heptanophenone	0.720	0.95	0.00	0.50	1.7180
hexanophenone	0.720	0.95	0.00	0.50	1.5780
4-nitrotoluene	0.870	1.11	0.00	0.28	1.0315
nitrobenzene	0.871	1.11	0.00	0.28	0.8906
4-iodophenol	1.380	1.22	0.68	0.20	1.0333
4-bromophenol	1.080	1.17	0.67	0.20	0.9501
4-chlorophenol	0.915	1.08	0.67	0.20	0.8970
benzonitrile	0.742	1.11	0.00	0.33	0.8711
1-naphthol	1.520	1.05	0.61	0.37	1.1441
o-cresol	0.840	0.86	0.52	0.30	0.9160
n-butyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.5540
n-propyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.4131
ethyl-4-hydroxybenzoate	0.860	1.35	0.69	0.45	1.2722
methyl-4-hydroxybenzoate	0.900	1.37	0.69	0.45	1.1313
phenol	0.805	0.89	0.60	0.31	0.7751
3-cyanophenol	0.930	1.55	0.77	0.28	0.9300
4-nitrophenol	1.070	1.72	0.82	0.26	0.9490
aniline	0.955	0.96	0.26	0.53	0.8162
acetanilide	0.870	1.40	0.50	0.67	1.1133
pyridine	0.794	0.87	0.00	0.62	0.6753
1,3-benzenediol	0.980	1.00	1.10	0.58	0.8338
1,4 benzenediol	1.063	1.27	1.06	0.57	0.8338
benazamide	0.990	1.50	0.49	0.67	0.9728
phenylurea	1.100	1.33	0.79	0.79	1.0726
acetaminophen	1.060	1.78	1.09	0.81	1.1720
1,3,5-trihydroxybenzene	1.355	1.12	1.40	0.82	0.8925
caffeine	1.500	1.60	0.00	1.33	1.3632

Table II. Correlation matrix of system constants obtained from 5-30% ethanol in heptane mobile phases on the Chirobiotic T CSP.

	s	a	b	v
s	1.00			
a	0.87	1.00		
b	0.97	0.79	1.00	
v	-0.89	-0.62	-0.95	1.00

Table III. System constants for Chirobiotic T CSP at various conditions. r^2 is the coefficient of correlation, SE is the standard error of regression, F is the Fisher statistic, n is the number of solutes in the regression.

MP[a]	T	e	s	a	b	v	Intercept	r^2	SE	F	n
30/70 EtOH/heptane	35°C	0	0.497	0.046	1.390	-0.761	-0.621	0.98	0.079	416.6	42
25/75 EtOH/heptane	35°C	0	0.528	0.135	1.448	-0.790	-0.613	0.98	0.083	443.3	42
20/80 EtOH/heptane	35°C	0	0.544	0.223	1.536	-0.826	-0.583	0.98	0.087	461.3	42
15/85 EtOH/heptane	35°C	0	0.576	0.330	1.638	-0.864	-0.576	0.98	0.095	471.5	42
10/90 EtOH/heptane	35°C	0	0.622	0.512	1.802	-0.923	-0.580	0.98	0.104	511.1	42
5/95 EtOH/heptane	35°C	0	0.702	0.831	2.101	-0.996	-0.647	0.98	0.122	560.8	42
25/75 EtOH/heptane	5°C	0	0.563	-0.021	1.664	-0.892	-0.458	0.97	0.104	325.9	42
20/80 EtOH/heptane	5°C	0	0.576	0.065	1.723	-0.925	-0.415	0.97	0.107	339.6	42
15/85 EtOH/heptane	5°C	0	0.595	0.180	1.819	-0.969	-0.358	0.97	0.113	357.5	42
25/75 EtOH/ENFB	35°C	0.181	0.548	0.186	1.201	-0.542	-1.068	0.93	0.129	98.2	37
20/80 EtOH/ENFB	35°C	0.170	0.553	0.286	1.245	-0.527	-1.038	0.94	0.127	116.4	37
15/85 EtOH/ENFB	35°C	0.152	0.524	0.393	1.294	-0.504	-0.942	0.95	0.124	137.7	37
10/90 EtOH/ENFB	35°C	0.142	0.559	0.559	1.423	-0.508	-0.946	0.96	0.131	164.0	37
5/95 EtOH/ENFB	35°C	0.130	0.671	0.914	1.712	-0.544	-1.091	0.96	0.160	177.4	36
25/75 EtOH/ENFB	5°C	0.183	0.567	0.008	1.333	-0.614	-0.896	0.92	0.142	78.7	37
20/80 EtOH/ENFB	5°C	0.187	0.609	0.116	1.380	-0.612	-0.939	0.92	0.154	79.9	37
30/70 TFE/ENFB	35°C	0.178	0.289	0.933	0.911	-0.596	-0.658	0.97	0.111	213.2	34
25/75 TFE/ENFB	35°C	0.161	0.338	1.023	1.053	-0.615	-0.674	0.98	0.109	271.8	34
20/80 TFE/ENFB	35°C	0.116	0.389	1.059	1.121	-0.597	-0.664	0.98	0.116	264.8	34
15/85 TFE/ENFB	35°C	-0.005	0.634	1.040	1.129	-0.573	-0.743	0.98	0.102	332.5	33
30/70 TFE/ENFB	5°C	0.098	0.499	0.798	0.801	-0.701	-0.471	0.97	0.106	189.2	33
25/75 TFE/ENFB	5°C	0.096	0.553	0.909	0.998	-0.733	-0.535	0.97	0.109	236.9	33

a) MP – mobile phase

Table IV. Correlation matrix of system constants obtained from ethanol/heptane, ethanol/ENFB, and TFE/ENFB (5-30% alcohol in nonpolar modifier) mixtures on Chirobiotic T CSP.

	e	s	a	b	v
e	1.00				
s	-0.42	1.00			
a	0.19	-0.27	1.00		
b	-0.56	0.65	-0.38	1.00	
v	0.76	-0.27	0.29	-0.66	1.00

Table V. Solute descriptors, factors that contribute to retention and enantioselectivity in the normal phase mode on the Chirobiotic T CSP for 5-methyl-5-phenyl-hydantoin.

		E	S	A	B	V	r^2	F	SE	n
Solute descriptors	Peak 1	2.07	2.09	0.57	0.51	0.67	0.9727	4556	0.0528	20
	Peak 2	2.74	2.48	0.44	0.44	0.21	0.9237	2549	0.0876	20

		eE	sS	aA	bB	vV
Factors that contribute to retention	Peak 1	0.37	1.14	0.11	0.61	-0.37
	Peak 2	0.50	1.36	0.08	0.52	-0.11

	$e\Delta_E$	$s\Delta S$	$a\Delta A$	$b\Delta B$	$v\Delta V$
Factors that contribute to enantioselectivity	0.12	0.21	-0.02	-0.09	0.25

CHAPTER 6

General Conclusions

The research outlined in this dissertation has demonstrated a unique application of the linear solvation energy relationship (LSER) to the study of enantiomeric separations. For the first time, a comprehensive theory of retention has been applied to enantiomeric separations, and the intermolecular interactions that generate enantiomeric separations have been identified. By using existing methodology, it has proven to be very difficult to comprehensively determine which intermolecular interactions contribute to the separation of enantiomers.

The identification of the intermolecular interactions that are manifested in the e-term of the LSER model was an important development for applying the LSER model to enantiomeric separations. It was found that both ion-dipole and π electron complexation interactions are accounted for in this e-term. While these two intermolecular interactions are not deconvoluted from each other, it is possible to assign the interaction accounted for by the e-term to one of these two interactions based on knowledge of the solute and system. In the normal-phase mode, ionic solutes are poorly solvated by the mobile phase solvents and likely exist as tightly held ion pairs which are unable to participate in solvation interactions. Therefore, any interaction through the e-term in the normal-phase mode is most likely due to π electron complexation interactions. In the reversed-phase mode, π electron complexation is

a very weak interaction, and ionic groups are well solvated by the mobile phase solvents utilized. Therefore, any interaction through the e-term in the reversed-phase mode is most likely due to ion-dipole interactions. Additionally, most reversed-phase stationary phases do not possess nonbonding n or π electrons for π electron complexation. While macrocyclic glycopeptide CSPs (which are the focus of this dissertation) do have numerous nonbonding n or π electrons, they have a zero e-term in the normal-phase mode, indicating they do not readily participate in π electron complexation. In the reversed-phase mode, the amine and carboxylic acid groups will be ionized and able to participate in ion-dipole interactions. Therefore, in the reversed-phase mode, any interaction accounted for by the e-term is attributed to ion-dipole interactions.

The system constants obtained from macrocyclic glycopeptide CSPs revealed the important differences between conventional stationary phases and the macrocyclic glycopeptide CSPs, as well as the differences between the four different macrocyclic glycopeptide CSPs. In the reversed-phase mode, the macrocyclic glycopeptide CSPs are much less retentive because of hydrophobic interactions (compared with conventional octadecyl stationary phases) and capable of participating in greater amounts of polar interactions, such as dipole-dipole and hydrogen bonding interactions. Additionally, the ionizable groups of the stationary phases (amine and carboxylic acid groups) contribute to retention through ion-dipole interactions. In the normal-phase mode, retention was found to be increased by hydrogen bonding and dipole-dipole interactions, while dispersion interactions decreased retention. Interactions through polarizable n and π-electrons had no

impact on retention, which suggests that these stationary phases are not capable of participating in π-electron complexation at the tested conditions.

The characterization of enantioselectivity with the LSER approach is a very interesting and unique way of understanding chiral separations. The factors that contribute to both the retention and separation of the enantiomers of several chiral compounds were determined in the reversed-phase mode and the normal-phase mode. It was found that steric repulsion interactions and ion-dipole interactions have the greatest impact on enantioselectivity, and hydrogen bonding interactions had a lesser effect on enantioselectivity. Dipole-dipole interactions have almost no effect on enantioselectivity, for the compounds examined. Conversely, in the normal-phase mode, dipole-dipole interactions have the greatest impact on enantioselectivity, and hydrogen bonding interactions have the least impact on enantioselectivity. Steric repulsion and π-electron complexation interactions were found to have a moderate impact on enantioselectivity. The normal-phase mode proved to be a more difficult mode of operation for the determination of enantiomeric solute descriptors. This is primarily because of the intercorrelation that exists between the system constants obtained from ethanol/heptane mobile phases. The use of more unconventional solvents resulted in a set of system constants that were independent of each other and permitted the independent determination of solute descriptors for enantiomers in the normal-phase mode.

Future efforts will focus on applying the LSER theory to enantiomeric separations achieved on other broadly applicable chiral stationary phases. By using the LSER approach

outlined in this dissertation, comparisons can be made between the separations achieved on structurally related chiral selectors (such as teicoplanin and teicoplanin aglycon) and unrelated chiral selectors (teicoplanin- and cyclodextrin-based chiral stationary phases). Additionally, the potential to study the differences in the enantiomers of chiral selectors is intriguing. It would be very difficult to obtain the enantiomer of many of the broadly applicable chiral selectors, such as cyclodextrins, linear derivatized carbohydrates, and macrocyclic glycopeptides (which includes the chiral selector utilized in this research, teicoplanin). However, there are a great number of small, low molecular weight chiral selectors that possess one or two stereogenic centers and are readily available in both stereochemical configurations. Comparisons can be made between the solute descriptors of a single enantiomer. obtained from each configuration of chiral selector.

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