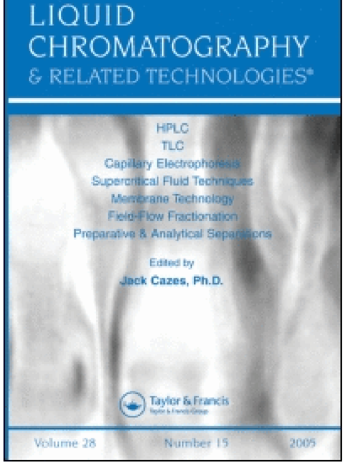
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

MECHANISTIC ASPECTS OF CHIRAL DISCRIMINATION ON A MOLECULAR IMPRINTED POLYMER PHASE

Thomas P. O'Brien[a]; Nicholas H. Snow[a]; Nelu Grinberg; Louis Crocker
[a] Chemistry Department, Seton Hall University, South Orange, NJ, U.S.A.

Online publication date: 28 January 1999

To cite this Article O'Brien, Thomas P. , Snow, Nicholas H. , Grinberg, Nelu and Crocker, Louis(1999) 'MECHANISTIC ASPECTS OF CHIRAL DISCRIMINATION ON A MOLECULAR IMPRINTED POLYMER PHASE', Journal of Liquid Chromatography & Related Technologies, 22: 2, 183 — 204
To link to this Article: DOI: 10.1081/JLC-100101653
URL: http://dx.doi.org/10.1081/JLC-100101653

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 22(2), 183–204 (1999)

MECHANISTIC ASPECTS OF CHIRAL DISCRIMINATION ON A MOLECULAR IMPRINTED POLYMER PHASE

Thomas P. O'Brien,[1] Nicholas H. Snow,[1,*]
Nelu Grinberg,[2,*] Louis Crocker

[1] Seton Hall University
Chemistry Department
South Orange, NJ 07079, USA

[2] Merck Research Laboratories
R80Y-115
P. O. Box 2000
Rahway, NJ 07065, USA

Downloaded At: 10:36 24 January 2011

ABSTRACT

The mechanism of chiral selectivity of a difunctional polymer imprinted with dansyl-L-phenylalanine was investigated using the polymer as an HPLC stationary phase. Temperature studies revealed that the mass transfer of the imprinted enantiomer with the polymer was sluggish at low temperatures, leading to a non-equilibrium migration down the column. Conversely, retention of the non-imprinted enantiomer was controlled by a thermodynamic equilibrium over the entire temperature range of the study. Variation of the structure of the analyte indicated a single leading interaction between both enantiomers of dansyl-phenylalanine and the polymer phase; a hydrogen bonding interaction between the carboxylic acid group of dansyl-phenylalanine and pyridinyl sites on the polymer.

Copyright © 1999 by Marcel Dekker, Inc. www.dekker.com

Secondary processes contributing to enantioselectivity were also deduced; a hydrogen bonding interaction occurring between the imprinted enantiomer and carboxyl sites on the polymer and a precise steric fit of the amino acid side chain into the imprinted sites. Studies varying the mobile phase hydrogen bond competitor agree with the results obtained by the structural studies.

INTRODUCTION

Interest in molecular imprinting, a technique that allows for preparation of adsorbents with sites tailored to recognize a particular molecule, is rapidly growing.[1,2] An imprinted polymer is formed by polymerizing a solution of functional monomers and a crosslinking agent in the presence of a template molecule. The functional monomers are chosen such that their functionality complements those of the template molecule.[3] When the polymerization is complete, the template molecule is removed, leaving sites in the polymer that are complementary in shape and functionality to the template. The polymer can then be used to selectively rebind the original template molecule.

Two approaches to molecular imprinting, covalent and non-covalent, have been developed. In covalent molecular imprinting, the functional monomer is chemically bound to the template molecule during the polymerization.[4] This approach has been used to produce polymers selective for a host of compounds including sugars,[5,6] amino acid derivatives,[7,8] and ketones.[9] The non-covalent approach utilizes functional monomers capable of undergoing interactions with the template such as hydrogen bonding and ion-pairing.[1]

Methacrylic acid has been commonly used as the functional monomer in non-covalent imprinting because it can undergo hydrogen bonding interactions with acids, esters, and amides and ionic interactions with amines.[10,11] To exploit these interactions, most non-covalent molecular imprinted polymers (MIP's) have been prepared in organic solvents.[12]

Molecular imprinted polymers have recently been used as artificial antibodies to determine the drugs theophylline and diazepam in human serum, producing results that were comparable to a traditional immunoassay.[13,14] MIP's have also been used as substrate selective sensors[15-16] and as catalysts for organic reactions.[17,19] Perhaps the most widely studied application of these polymers has been in chiral separations. MIP particles have been used as HPLC chiral stationary phases to separate the enantiomers of amino acids and amino acid derivatives[10,11,20-23] as well as pharmaceuticals.[24,25]

Downloaded At: 10:36 24 January 2011

Several fundamental studies have been conducted in attempt to describe the processes by which an imprinted polymer recognizes the imprint molecule. Prior to polymerization, NMR studies have shown evidence of complex formation between methacrylic acid and phenylalanine anilide in solution.[26] It was proposed that these complexes are preserved during the polymerization, resulting in an arrangement of methacrylic acid residues in the polymer complementary to phenylalanine anilide.[11] Recently, results from a similar NMR study showing an assembly of 9-ethyladenine and butyric acid in solution led the authors to prepare an MIP for 9-ethyladenine.[3]

Other studies using amino acid imprinted polymers as chiral stationary phases have supported the premise that the selectivity stems from the presence of complementary hydrogen bonding sites on the phase. In one report, a polymer imprinted with amino acids using methacrylic acid was prepared in solvents of varying hydrogen bonding capacity.[27] Greater enantioselectivity was observed for the polymers prepared in less polar solvents. In another report, a similar imprinted polymer prepared for a dipeptide in chloroform showed marked enantioselectivity (α=17.8)[28] when the mobile phase was non-aqueous, but was substantially lowered by addition of small percentages of water.[29] It was suggested that this reduction in enantioselectivity was due to water interfering with the hydrogen bonding interactions between the analyte and the polymer.

There is also evidence that an imprinted polymer recognizes the shape of the original template. For amino acid derivative imprinted polymers, substituents on the amine,[11,23,30] carboxyl group,[28] and amino acid side chain[23] have also shown to be important for recognition on certain imprinted phases. Interestingly, for a Z-L-phenylalanine imprinted phase, the selectivity factor for Z-D,L-alanine was significantly less than for Z-D,L-phenylalanine.[23] The authors proposed that a stabilizing interaction between the amino acid side chain and the imprinted polymer might explain this observation.

The polymer used for this study has previously been used as a biomimetic sensor to selectively bind Dns-L-Phe from its optical antipode.[31] For this polymer, both methacrylic acid and 2-vinylpyridine were used as the functional monomers. Better enantioselectivity was obtained for this difunctional polymer as compared to similar polymers prepared using only one of the two functional monomers.[32] It was assumed that the acid group of Dns-Phe hydrogen bonds with vinyl pyridine and the dimethylamino and sulfonamide groups of Dns-Phe hydrogen bond with methacrylic acid during the polymerization.[32] Presumably, the hydrogen bonding monomers become locked into their hydrogen bonding positions after polymerization, leaving sites in the polymer that are complementary to Dns-L-Phe.

Downloaded At: 10:36 24 January 2011

The Dns-L-Phe imprinted polymer incorporating both acid and pyridine groups was chosen as a model for our investigations because little attention has been given to the study of MIP's using multiple functional monomers. We believe that the use of multiple functional monomers for molecular imprinting will be important in further expanding this technique to more sophisticated molecules, such as pharmaceutical drugs. In this paper, we explore the types of interactions occurring between this polymer and its imprint molecule, Dns-Phe, in an attempt to provide further insight into the mechanism of selectivity on this difunctional imprinted polymer phase.

EXPERIMENTAL

Equipment

The HPLC system consisted of a Spectra-Physics (Piscataway, N.J., USA) SP-8700 pump and a Waters (Medford, MA., USA) 715 ULTRA WISP autosampler. The column temperature was controlled by a Neslab RTE-110 (Newington, NH, USA) recirculating water / ethylene glycol bath. An Applied Biosystems (Foster City, CA, USA) 908 Fluorescence Detector (Excitation = 305 nm, Detection < 375 nm.) and a 757 absorbance detector set at 250 nm was used. The chromatographic data analysis was performed using P.E. Nelson (Cupertino, CA, USA) Access*Chrom software. The statistical analysis of the data were done using Origin software (Microcal, Northampton, Ma, USA). The FTIR spectrometer was a Nicolet (Madsion, WI) and the particle size analysis was done by image analysis (Leica RMRD) with Qwin software (Cambridge, UK).

Reagents

The monomers ethylene glycol dimethacrylate (EDMA), methacrylic acid (MAA), and 2-vinyl-pyridine were obtained from Aldrich (Milwaukee, WI). Both enantiomers of dansyl-phenylalanine (Dns-Phe), dansyl-leucine (Dns-Leu), dansyl-alanine (Dns-Ala) and dansyl-valine (Dns-Val) were obtained from Sigma (St. Louis, MO). The AIBN initiator was obtained from Pfaltz and Bauer (Waterbury, CT).

Synthesis of Dansyl-Phenylalanine Methyl Ester

Each enantiomer of dansyl-phenylalanine methyl ester (Dns-Phe-Me-ester) was prepared by dissolving 300 mg (1.4 mmol) of either L-or D- phenylalanine methylester hydochloride (Aldrich) in 100 mL of 70/30 v/v water/acetonitrile in

Downloaded At: 10:36 24 January 2011

a 500 mL roundbottom flask. To this solution was added 100 mL of 3 equivalents (4.2 mmol) of sodium bicarbonate in water. As the solution was mixed with an overhead stirrer, 1.1 equivalents (1.5 mmol) of dansyl-chloride (Aldrich) in 50 mL of acetonitrile was slowly added (~0.5 hr) to the phenylalanine solution. The reaction progress was monitored by HPLC. After complete addition of dansyl-chloride, the solution was stirred overnight. The solution was extracted with 250 mL of toluene. The toluene layer was evaporated to dryness to afford the product as a yellow oil.

Synthesis of Napthyl-Sulfonyl-Phenylalanine

Each enantiomer of napthyl-sulfonyl-phenylalanine (Napth-Phe) was prepared in a similar manner to that of dansyl-phenylalanine methyl ester using L- and D-phenylalanine as the substrate and 1-naphthylsulfonic acid as the derivatizing agent. After the overnight age, the pH of the reaction solution was adjusted to 2.5 with formic acid and the product was extracted into toluene. The toluene layer was evaporated to dryness to afford the product as an oil.

Downloaded At: 10:36 24 January 2011

Polymer and Column Preparation

The imprinted polymer was prepared by combining EDMA (65.5mmol), MAA (13.09mmol), 2-vinyl-pyridine (13.08mmol), dansyl-L-phenylalanine (1.64 mmol), AIBN initiator (100mg), and 20 mL of dry acetonitrile (60 µg/mL water by Karl Fischer titration) in a 200 mL pyrex bottle. The solution was sparged with nitrogen for 5 min. The bottle was capped tightly and placed in an oven at 55°C for 36 hours. The resulting solid polymer was removed from the bottle, ground with a mortar and pestle, and washed with 2 liters of 10% acetic acid in acetonitrile. The polymer particles were sized by passing them through 38 and 25 µm sieves. Approximately 1gram of the 25-38 µm sized particles was slurry packed (in 10% acetic acid in acetonitrile) into an HPLC column (15 cm x 0.46 cm) at ~ 80 mL/min using a Waters Preparative HPLC pump (Medford, MA., USA). The remaining particles were washed with methanol, dried under vacuum, and characterized by Diffuse-Reflectance IR spectroscopy: 3300cm^{-1} (O-H), 1742cm^{-1} (ester), 1636cm^{-1} (vinyl), 1569cm^{-1} (pyridine), 1550cm^{-1} (pyridine). The mean particle size was determined to be 33 µm by image analysis.

The imprinted polymer column was connected to the HPLC system and equilibrated with mobile phase. Each time the mobile phase composition or temperature was adjusted, the column was re-equilibrated at the new conditions for at least 60 min. The samples were prepared by dissolving 5 mg of each Dns-Phe enantiomer in 100 mL acetonitrile. A 5 µL volume of the sample was

Downloaded At: 10:36 24 January 2011

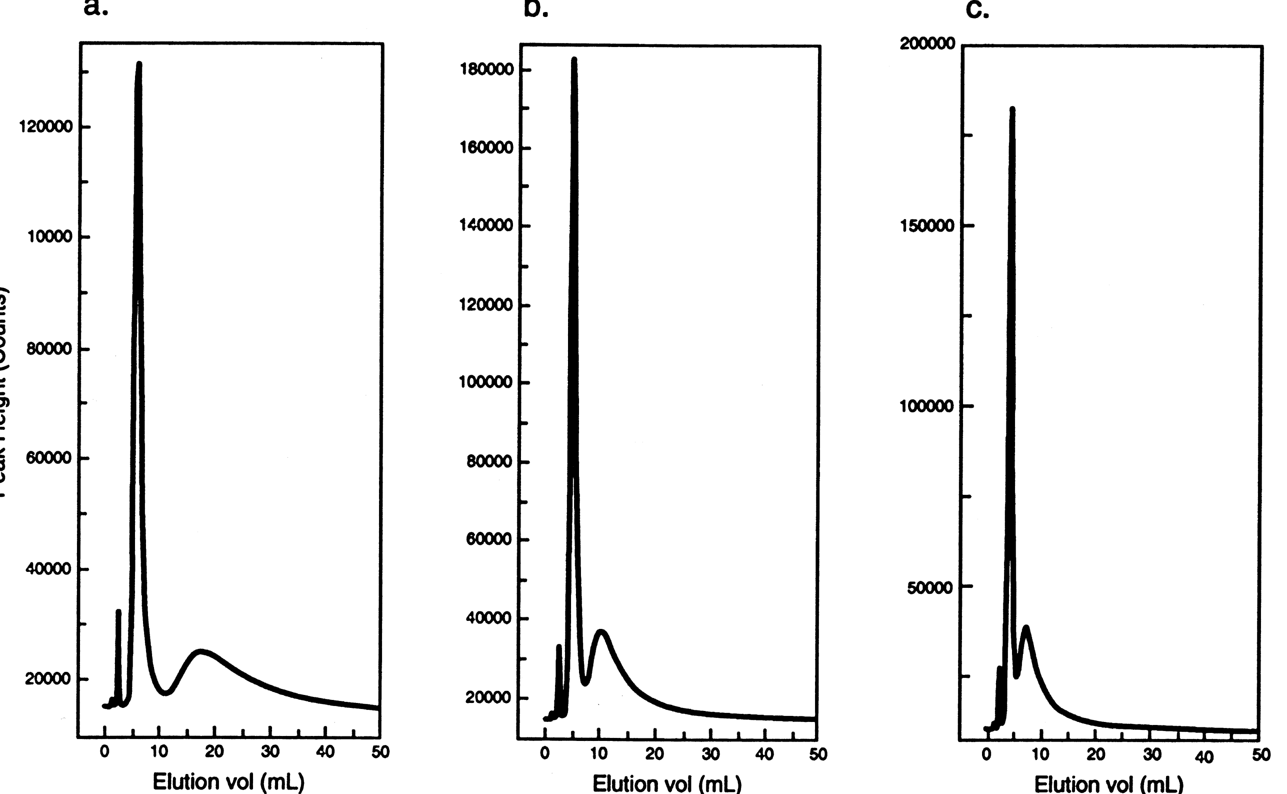


Figure 1. Chromatograms of racemic dansyl-phenylalanine. a.) Mobile phase: 4% acetic acid in acetonitrile; b.) Mobile phase: 6% acetic acid in acetonitrile; c.) Mobile phase: 8% acetic acid in acetonitrile; column temperature: 60°C; flowrate: 0.5mL/min; sample size: 300 ng each enantiomer; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm.

injected onto the column. For the structural variation studies, the samples were prepared in a similar manner with the exception that each enantiomer was dissolved and injected separately.

RESULTS AND DISCUSSION

Typical Chromatograms

The mobile phase used to separate racemic Dns-Phe on the imprinted polymer column was acetonitrile modified with varying amounts of acetic acid. Figure 1. shows the effect of increasing acetic acid concentration at a constant temperature (60°C).

The second eluting broad peak is the imprinted (Dns-L-Phe) enantiomer. The broadness of this peak indicates that it undergoes a slower mass transfer with the polymer relative to the non-imprinted (D) enantiomer.[33] As the concentration of the acetic acid is increased from 4-8%, mass transfer kinetics increase and the peaks sharpen. At the same time, this increase in acetic acid concentration results in a decrease in enantioselectivity (from $\alpha = 4.4$ down to $\alpha = 2.7$). Figure 2. shows the effect of increasing temperature at a constant acetic acid concentration.

There is a significant increase in resolution with temperature as a result of faster mass transfer kinetics.

Downloaded At: 10:36 24 January 2011

Influence of Sample Size

Prior to the selectivity studies, it was necessary to evaluate the capacity of the imprinted polymer column for each Dns-Phe enantiomer. It is known that imprinted polymers have a limited capacity.[12] Figure 3. gives a plot of retention factor (k') for each enantiomer versus the log of the weight of Dns-Phe injected.

For the imprinted (L) enantiomer, the retention factor (k') is approximately constant for sample loads between ~30 to 300 ng, after which there is a smooth decrease in k' with further increases in sample size. This behavior indicates that, for sample sizes above above 300 ng, the binding of this enantiomer becomes non-linear[34] as the capacity of the sites available to this enantiomer is approached. On the other hand, k' remains essentially constant for the non-imprinted (D) enantiomer over the same loading range. These results suggest that there are two distinct types of binding sites on the polymer. There are a limited number of imprinted sites that bind the imprint enantiomer and there are also non-specific sites that occupy the bulk of the polymer. Apparently, it is at

Downloaded At: 10:36 24 January 2011

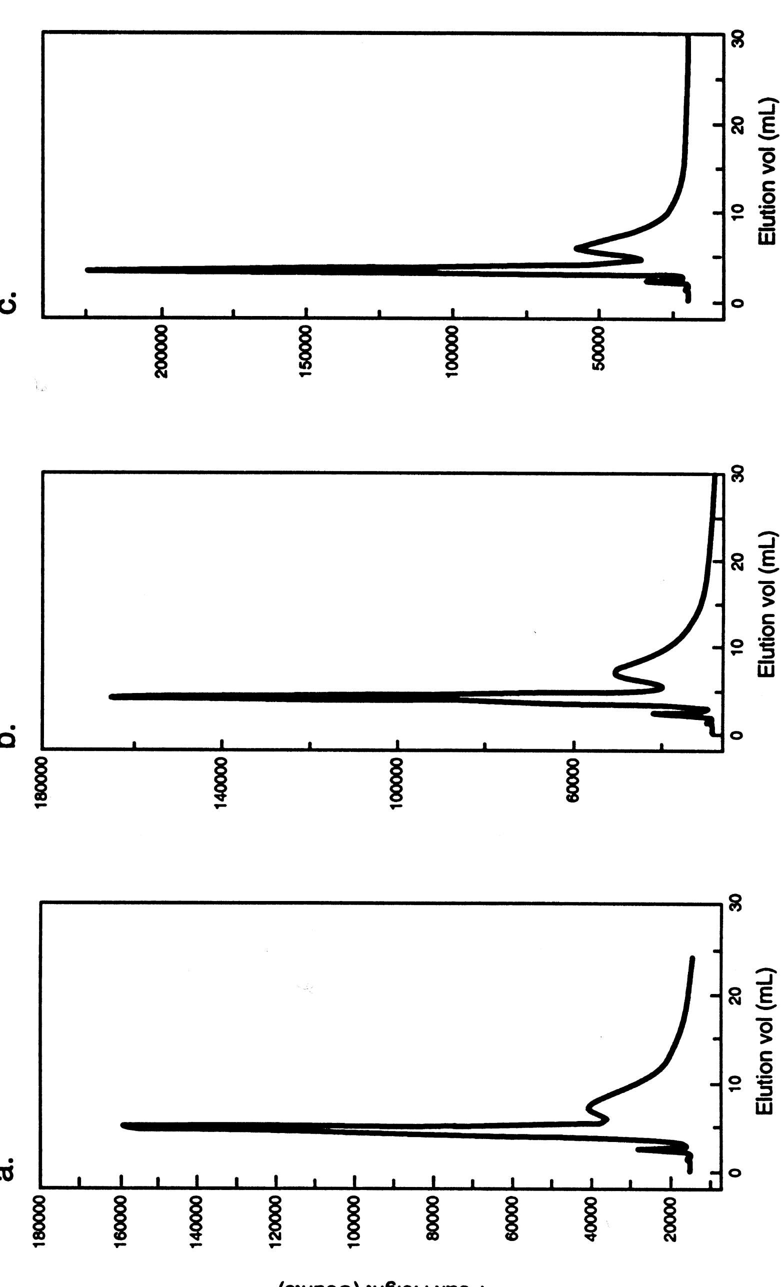


Figure 2. Chromatograms of racemic dansyl-phenylalanine. a.) Column temperature: 40°C; b.) Column temperature: 60°C; c.) Column temperature: 80°C; mobile phase: 8% acetic acid in acetonitrile; flowrate: 0.5mL/min; sample size: 300 ng each enantiomer; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm.

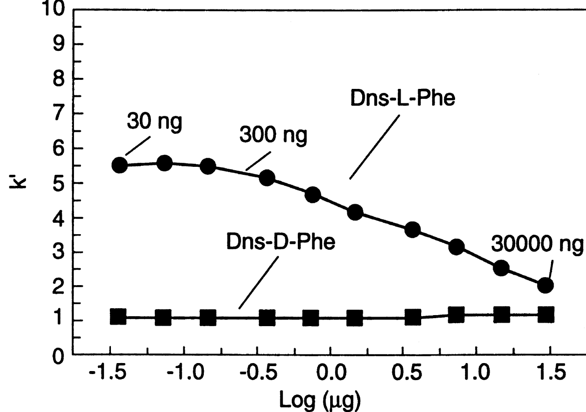


Figure 3. Effect of Sample Load on k' for Each Dansyl-phenylalanine Enantiomer. Mobile phase: 4% acetic acid in acetonitrile; flowrate: 0.5mL/min; sample size: 300 ng each enantiomer; column temperature: 60°C; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm.

Downloaded At: 10:36 24 January 2011

the bulk non-selective sites where the non-imprinted enantiomer interacts because overloading of this enantiomer is not observed. For further experiments, a sample size of 300 ng of each enantiomer was used to ensure that overloading effects did not impact the results.

Influence of Temperature

The retention factor (k') can be related to the change in enthalpy (ΔH) and entropy (ΔS) of an analyte when it moves from the mobile phase to stationary phase in a chromatographic system by the van't Hoff equation:

$$\ln k' = - (\Delta H/RT) + (\Delta S/R) + \ln \phi \quad (1)$$

where: ϕ is the phase ratio T is the absolute temperature

Equation (1) predicts that a plot of ln k' vs. 1/T will be a straight line with a slope of $-(\Delta H/R)$ and an intercept of $[(\Delta S/R) + \ln \phi]$ provided that ϕ is independent of the temperature. If the stationary phase undergoes a change in conformation at a certain temperature, the enthalpy and the entropy of the retention process will change, and the van't Hoff plot will show a change in slope and intercept at the transition temperature.

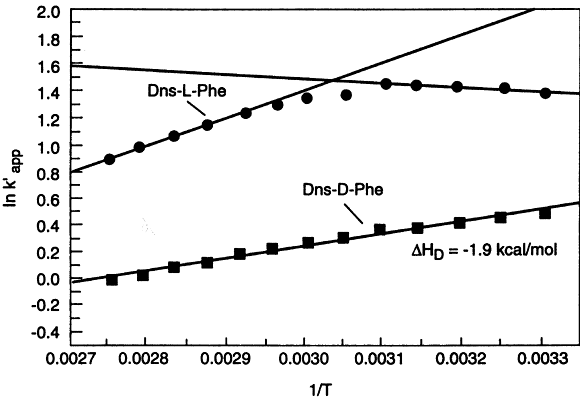


Figure 4. Typical van't Hoff plots for dansyl-phenylalanine enantiomers. Mobile phase: 6% acetic acid in acetonitrile; flow rate: 0.5mL/min; sample size: 300 ng each enantiomer; detection: fluorescence- excitiation λ=305 nm, detection $\lambda < 375$ nm.

Downloaded At: 10:36 24 January 2011

van't Hoff plots in k' were constructed for each Dns-Phe enantiomer at 3 different acetic acid concentrations (4,6,8% in acetonitrile) between 25°C and 90°C. The retention factors (k') were calculated from the peak maxima of each enantiomer. Initially, an assumption was made that the peak maximum was a point of equilibrium between each Dns-Phe enantiomer and the polymer phase.[33] A typical plot at 6% acetic acid is given in Figure 4.

For the non-imprinted (D) enantiomer, the plot is a straight line with a positive slope, indicating a favorable enthalpy change (-1.9Kcal/mol) when this enantiomer interacts with the stationary phase. On the other hand, a distinct curvature was observed in the van't Hoff plot for the imprinted enantiomer. At higher temperatures (60°C to 90°C), the plot was linear with a positive slope, but leveled off below 60°C with a slope approaching zero.

Prior to interpretation of the van't Hoff plots, it was necessary to test the assumption that an equilibrium exists at the peak maximum of each enantiomer. As shown in Figures 1 and 2, the band profile for the imprinted (L) enantiomer is broad and tailing, suggesting that there may be a kinetic contribution to the retention of this enantiomer.[35] It has been shown that slow adsorption / desorption kinetics can lead to a non-equilibrium migration of an analyte down a chromatographic column.[34-35] For a peak to be at equilibrium, the apparent retention factor (k') should be independent of the mobile phase flow rate. Thus, van't Hoff plots were reconstructed at a range of flow rates varying between 0.1 mL/min and 1.5 mL/min. An overlay of these plots is presented in Figure 5.

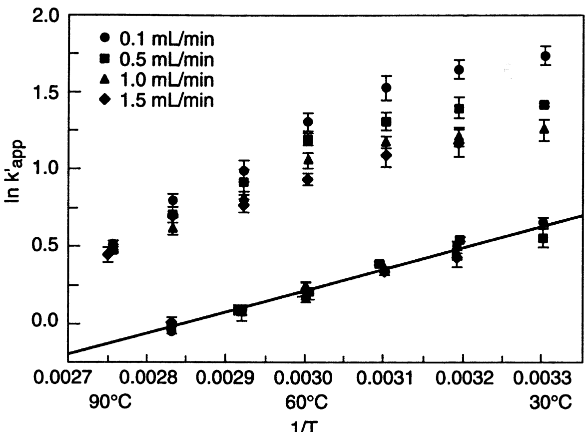


Figure 5. van't Hoff plots for dansyl-phenylalanine enantiomers at different flow rates. Mobile phase: 6% acetic acid in acetonitrile; sample size: 300 ng each enantiomer; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm.

Downloaded At: 10:36 24 January 2011

For Dns-D-Phe, k' is essentially independent of flow rate over the temperature range studied. Indeed, an equilibrium is achieved for this peak and it is, therefore, reasonable to use the slope of this plot to determine ΔH. However, at lower temperatures, a dramatic difference is seen in the apparent k' (k'_{app}) for Dns-L-Phe at each flow rate, with a larger k'_{app} observed at lower flow rates. As the temperature is increased gradually, smaller changes in k'_{app} with flow rate are observed up to 80°C-90°C when the retention factor becomes essentially independent of flow rate. The cause of the non-linear van't Hoff plot for Dns-L-Phe was, therefore, attributed to a strong contribution of slow kinetics to the retention factor at low temperature. It is only at high temperatures (80°C-90°C), that the mass transfer of this enantiomer is rapid enough to achieve equilibrium and thermodynamic treatments become valid. For the structural studies presented below, the experiments were performed at elevated temperatures (90°C) to ensure that comparison of thermodynamic parameters such as selectivity (α) and ΔΔG are reasonable.

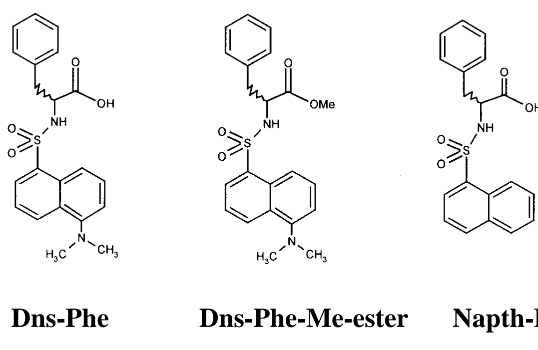
Influence of Structure

The processes underlying recognition on this Dns-L-Phe MIP was investigated by performing chromatographic experiments using the imprint molecule and several structurally related compounds. In an initial study, our goal was to characterize the role interactions at the carboxylic acid and

Downloaded At: 10:36 24 January 2011

Table 1

Effect of Varying the Functionality of the Amino Acid Derivative*



	Dns-Phe	**Dns-Phe-Me-ester**	**Napth-Phe**
k'D-enantiomer	1.0	0.0	1.1
ΔH (kcal/mol)	-1.8	0.0	1.9
k'L enantiomer	2.2	0.0	1.7
α	2.2	---	1.5

* Mobile phase: 6% acetic acid in acetonitrile; flowrate: 0.5 mL/min; sample size: 300 ng each enantiomer; column temperature: 90°C; detection: fluorescence-excitiation λ = 305 nm, detection λ <375 nm.

dimethylamino groups of Dns-Phe play in the separation process. This experiment was performed by synthesizing two amino acid derivatives that would block the potential interaction at each of these groups. Enantiomers of Dansyl-phenylalanine methyl ester (Dns-Phe-Me-ester) were synthesized to assess the importance of interaction between the carboxylic acid group and the polymer. Alternatively, each enantiomer of Napthyl-sulfonyl-phenylalanine (Napth-Phe) was synthesized to probe the importance of interaction at the dimethylamino group. The capacity factors and selectivity factors for Dns-Phe and these derivatives are listed in Table 1. below the structures of each compound.

The results in Table 1. indicate that blocking the carboxyl group on the amino acid causes each enantiomer of Dns-Phe-Me-ester to travel through the column unretained. Apparently, interaction between this group and the polymer is the leading interaction providing retention of the enantiomers. It was hypothesized that this interaction occurs primarily between the carboxyl group of Dns-Phe and pyridinyl groups on the polymer. Two experiments were performed to confirm this hypothesis. First, a similar MIP was prepared for

Dns-L-Phe omitting the pyridinyl monomer in the synthesis. For a column prepared with this polymer, no retention was observed for either enantiomer of Dns-Phe. This eliminated the possibility of a leading interaction existing between Dns-Phe and acid sites on the polymer. Further, Diffuse Reflectance IR experiments were performed on the original polymer. A comparison was made between the IR spectrum of dry polymer and a spectrum of polymer doped with acetic acid. The spectrum for the polymer doped with acetic acid showed a distinct shift for one of the pyridinyl ring stretch bands (from $1590cm^{-1}$ to $1600cm^{-1}$) as compared to the dry polymer. This observation is consistent with the hypothesis that pyridinyl groups incorporated into the polymer are accessible for hydrogen bonding with a carboxylic acid group.

Further analysis of Table 1. reveals that the D-enantiomers of Dns-Phe and Napth-Phe have approximately the same retention factor (k') and enthalpy (ΔH) of interaction with the polymer (as determined by van't Hoff plots). However, the retention factor for the L-enantiomer of Napth-Phe is reduced by 20% relative to that of Dns-L-Phe. As a result, a 30% decrease is observed in the enantioselectivity (α) for Napth-Phe.

The above results suggest that the carboxyl group of the amino acid participates in a leading interaction with pyridinyl groups on the polymer to retain the enantiomers, while the role of the dimethylamino group is to provide for a secondary cooperative interaction with acid sites on the polymer. This dimethylamino interaction occurs solely at the imprinted sites to stabilize inclusion of the imprinted enantiomer and is unimportant to non-selective binding to the polymer. This hypothesis is further supported by the mobile phase modifier studies presented below.

In a second study, our goal was to assess the importance of molecular shape on the selectivity of this polymer phase. To perform this experiment, the side chain of the amino acid was varied by using the compounds Dns-Ala, Dns-Leu, and Dns-Val. Changing the side chain of the amino acid does not alter the potential points of interaction with the polymer, but does change the steric bulk of the molecule. The capacity factors and selectivity factors for Dns-Phe and these derivatives are listed in Table 2. below the structures of each compound.

As shown in Table 2., the L-enantiomers of each derivative are selectively retained over their D-antipodes, suggesting that the L-enantiomers have access to the imprinted sites. It is also important to note here that the apparent van't Hoff plots for the L-enantiomers (not shown) of these amino acids showed a curvature similar to Dns-L-Phe while plots of the D-enantiomers were straight lines. This observation suggests that slow kinetics also contributes to the retention of the L-enantiomers of these compounds. While there is selectivity for these enantiomers, the selectivity factors are lower than that of Dns-Phe.

Downloaded At: 10:36 24 January 2011

Table 2

Effect of Varying the Steric Bulk of the Side Chain of the Amino Acid*

	Dns-Ala	**Dns-Leu**	**Dns-Val**
k-D-enantiomer	0.8	0.5	0.5
k'L-enantiomer	1.0	0.6	0.7
α	1.3	1.2	1.4

* Mobile phase: 6% acetic acid in acetonitrile; flowrate: 0.5 mL/min; sample size: 300 ng each enantiomer; column temperature: 90°C; detection: fluorescence-excitiation $\lambda = 305$ nm, detection $\lambda = <375$ nm.

Downloaded At: 10:36 24 January 2011

This is consistent with the results of Kempe and Mosbach,[20] who noted a decrease in enantioselectivity when injecting the enantiomers of Z-alanine on a Z-phenylalanine polymer imprinted using methacrylic acid as the functional monomer. Such a loss in selectivity can be due to an improper fit of the side chain into the imprinted cavities. While the side chain may not interact with the polymer, a tight fit at the imprinted sites can be a secondary process that enhances the hydrogen bonding interactions at the imprinted sites.

The difference in the free energy of binding of the two enantiomers ($\Delta\Delta G$) to the polymer phase can be deduced from the selectivity factors by the following equation:

$$\Delta\Delta G = -RT\ln\alpha \qquad (2)$$

The $\Delta\Delta G$ for each amino acid derivative was calculated using eqn. (2) and the results are presented in Table 3. By comparing the $\Delta\Delta G$ of Dns-Phe and the Napth-Phe, it is seen that approximately half of the enantioselective energy is lost when the dimethylamino group is removed from the molecule. This is consistent with the loss of one of two hydrogen bonds (that are similar in

Table 3

ΔΔG for the Enantiomers of Different Amino Acid Derivatives*

	α	ΔΔG
Dns-Phe	2.2	-570 cal/mol
Napth-Phe	1.5	-292 cal/mol
Dns-Ala	1.3	-190 cal/mol
Dns-Le	1.2	-130 cal/mol
Dns-Val	1.4	-240 cal/mol

* Mobile phase: 6% acetic acid in acetonitrile; flowrate: 0.5 mL/min; sample: 300 ng each enantiomer; column temperature: 90°C; detection: fluorescence-excitiation λ = 30 nm, detection λ <75.

Downloaded At: 10:36 24 January 2011

energy) at the imprinted sites. For the derivatives with different side chains, a reduction in ΔΔG of similar magnitude is also observed. Regardless of whether the side chain is less sterically hindered (Dns-Ala) or more sterically hindered (Dns-Val) than Dns-Phe (as determined by the bulk around the β carbon), enantioselectivity is reduced to a similar extent.

This further supports the premise that a tight steric fit of the side chain enhances the stability of the hydrogen bonding interactions occurring at the imprinted sites.

Influence of Mobile Phase Modifier

To further investigate the interactions responsible for enantioselectivity, the concentration of the mobile phase competitor was varied. For these studies, a temperature of 60°C and flow rate of 0.5 mL/min were used. Under these conditions, it was assumed that retention of the imprinted enantiomer is dominated by thermodynamics. This assumption is reasonable based on the relatively small difference observed in k' at 0.5 mL/min (k'=3.3) and 0.1 mL/min (k'=3.7) at 60°C.

Initially, the molarity of acetic acid in acetonitrile was varied from 0.1M - 1M. Figures 6a. and 6b. give plots of k' and α respectively for Dns-Phe and Napth-Phe.

Downloaded At: 10:36 24 January 2011

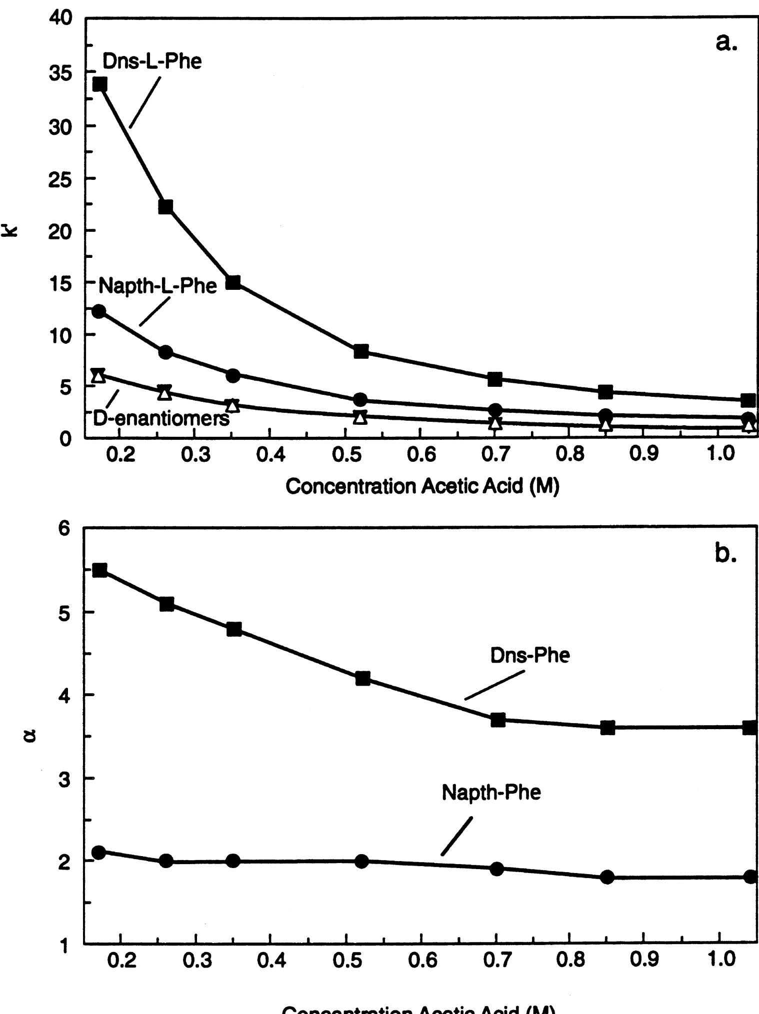


Figure 6. a) Effect of mobile phase acetic acid concentration on the k' of the Dns-Phe and Napth-Phe enantiomers. Flowrate: 0.5mL/min; sample size: 300 ng each enantiomer; column temperature: 60°C; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm. b) Effect of mobile phase acetic acid concentration on α of Dns-Phe and Napth-Phe. flowrate: 0.5mL/min; sample size: 300 ng each enantiomer; column temperature: 60°C; detection: fluorescence- excitiation λ=305 nm, detection λ < 375 nm.

Downloaded At: 10:36 24 January 2011

For both enantiomers of each derivative, k' decreases with increasing acetic acid concentration. This decrease is expected because retention of the analyte is determined by hydrogen bonding between the carboxyl group of the amino acid and pyridinyl groups on the polymer. By increasing the acetic acid concentration in the mobile phase, the strong hydrogen bonding interactions between the analyte and polymer are diminished as acetic acid competes more effectively for these sites on the polymer. In addition, these data further support the conclusion that non-specific interactions occurring between each derivative and the polymer are similar as the retention factors of the D-enantiomers are equal over the entire acetic acid concentration range.

The nature of the enantioselective interactions occurring between the analyte and polymer are further clarified by looking at the dependence of the selectivity factor (α) for each derivative with increasing acetic acid concentration. For Dns-Phe, Figure 6b. shows an initial decrease in α with increasing acetic acid concentration between 0.1M-0.7M, after which α levels off with further increases in acetic acid. For Napth-Phe, only a relatively weak dependence of α is seen with changing acetic acid concentration. It is possible to explain this behavior by considering the enhanced solvation of the analyte and polymer by increasing the acetic acid concentration in the mobile phase.

From the structural studies presented above, it was proposed that an enantioselective interaction occurs between the dimethylamino group of Dns-L-Phe and carboxyl groups on the polymer. By increasing the acetic acid concentration, Dns-Phe and the polymer become more solvated with acetic acid. As a result, the enantioselective interaction between the dimethylamino group and the polymer is reduced as it becomes more difficult for this group to lose acetic acid to interact with the polymer. As a result, a significant decrease in the selectivity is observed. For the Napth-Phe, this secondary enantioselective interaction cannot occur. As a result, the enantioselectivity remains essentially unchanged over the entire acetic acid concentration range.

It is important to note the selectivity factor for the enantiomers of Dns-Phe appear to level off at higher acetic acid concentration rather than approach that of the Napth-Phe. An explanation of this behavior is not straightforward, but may indicate that interaction between the dimethylamino group and the polymer cannot be completely suppressed or there is also a significant contribution of the shape of the dimethylamino group to the enantioselectivity.

In a second study, the retention and selectivity of Dns-Phe was studied using a mixture of pyridine and acetic acid as the mobile phase modifier. The total modifier concentration was held constant at 0.52M while the individual amounts of pyridine and acetic acid were varied from 0-0.52M. Figures 7a. and 7b. give plots of k' and α respectively for the enantiomers of Dns-Phe.

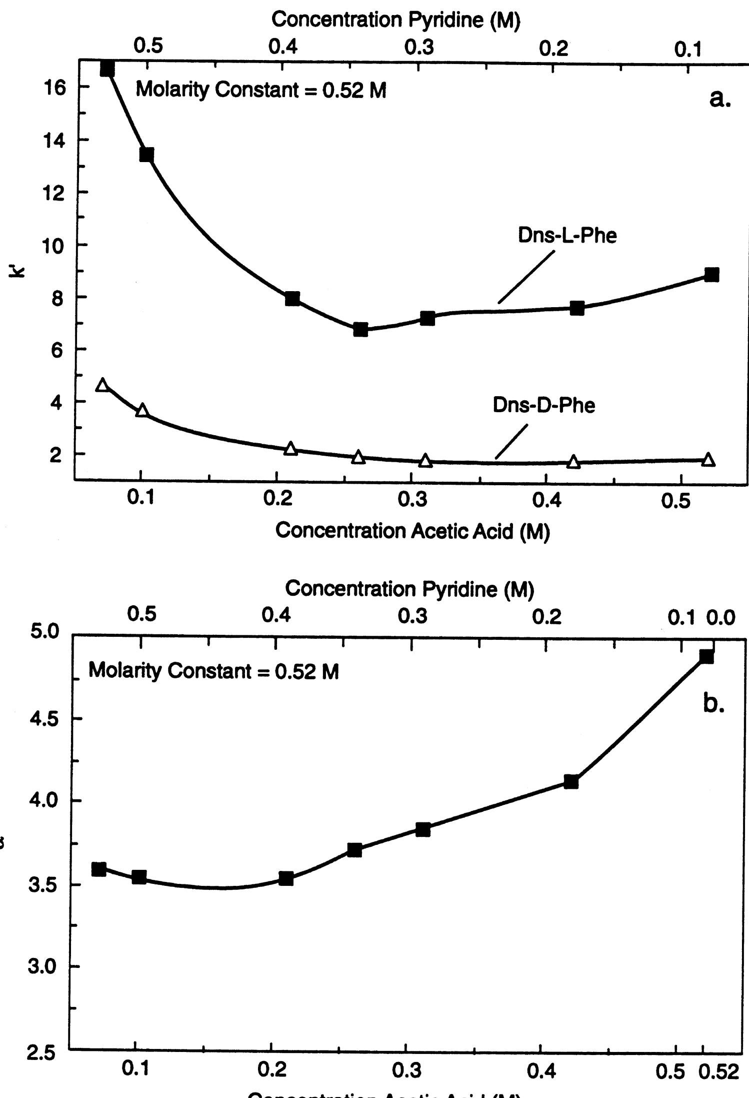


Figure 7. a) Effect of mobile phase pyridine/acetic acid concentration on the k' of the Dns-Phe enantiomers. Flowrate: 0.5mL/min; Sample Size: 300 ng each enantiomer; Column Temperature: 60°C; Detection: Fluorescence- Excitiation λ=305 nm, Detection λ < 375 nm. b) Effect of Mobile Phase Pyridine/Acetic Acid Concentration on α of the Dns-Phe Enantiomers, flow rate: 0.5 mL/min; sample size: 300 ng each enantiomer; column temperature: 60°C; detection: fluorescence-excitiation λ=305 nm, detection λ <375 nm.

Downloaded At: 10:36 24 January 2011

Introduction of pyridine to the system results in strong hydrogen bonding between pyridine and the carboxyl groups on the amino acid and carboxyl sites on the polymer. These interactions reduce the retention factor of each enantiomer relative to the system with the same concentration of acetic acid in the absence of pyridine (Figure 6a.). As pyridine is replaced by acetic acid, the retention factor of each enantiomer is initially reduced. Apparently, the competition between acetic acid and Dns-Phe for pyridinyl sites on the polymer is more effective in eluting the analyte than solvation of Dns-Phe by pyridine in the mobile phase. This is supported by the fact that when the acetic acid concentration was reduced to 0M (0.52M pyridine), neither enantiomer eluted.

Figure 7a. also shows that when the concentrations of acetic acid and pyridine in the mobile phase become approximately equal (~0.26M) and pyridine is no longer in excess, the retention factor of Dns-L-Phe reaches a minimum while that of Dns-D-Phe levels off. This behavior can be explained by the differences in solvation pyridine and acetic acid exhibit on the system. At high pyridine/low acetic acid concentration, the retention factor of each enantiomer is relatively large because there is little acetic acid to compete with the analytes for the pyridinyl sites on the polymer. As pyridine is replaced by acetic acid, the retention factors drop as acetic acid begins to compete for these sites on the polymer. At the same time, the strong hydrogen bonding between pyridine and carboxyl sites on the polymer is reduced, fostering the secondary enantioselective interaction between the dimethylamino group of the imprinted enantiomer (Dns-L-Phe) and the carboxyl sites on the polymer. As a result, a minimum is observed in the retention factor for Dns-L-Phe as this secondary interaction becomes more pronounced.

Based on this argument, it is expected that the enantioselectivity for Dns-Phe will increase as pyridine is replaced by acetic acid. This is the case, as shown in Figure 7b., which gives a plot of the selectivity factor of Dns-Phe for this study.

CONCLUSIONS

Chromatographic experiments performed on a polymer imprinted with Dns-L-Phe using carboxyl and pyridine functional monomers indicated that the adsorption/desorption kinetics at the imprinted sites are slow. Structural studies indicated that the leading interaction is between the carboxyl group of Dns-Phe and the pyridinyl sites on the polymer. Further, interaction between the dimethylamino group of Dns-Phe and the polymer occurs only at the imprinted sites and is relatively unimportant to non-selective binding on the polymer. From these data, we propose that chiral recognition on this polymer is a cooperative process. There must first be a leading interaction between the carboxyl group of Dns-Phe and the pyridinyl groups on the polymers to retain

Downloaded At: 10:36 24 January 2011

the enantiomers. This is followed by secondary stabilizing processes that contribute to enantioselectivity; interaction at the dimethylamino group and correct steric fit of the amino acid side chain into the imprinted cavities. We believe that this fundamental study further contributes to the understanding of the processes behind chiral separations on imprinted polymer phases, in particular, one in which multiple functional groups were used to enhance the enantioselectivity.

ACKNOWLEDGMENTS

The authors would like to thank Charles Moeder and Dr. Richard Thompson for their thoughtful review of this paper. We would also like to thank Merck and Company for their support of this project.

Downloaded At: 10:36 24 January 2011

REFERENCES

1. C. Yu, K. Mosbach, J. Org. Chem., **62,** 4057 (1997).

2. H. Sreenivasan, Die Angew. Makrom. Chem., **65**, 246 (1997).

3. D. Spivak, M. Gilmore, K. J. Shea, J. Am Chem. Soc., **119**, 4388 (1997).

4. M. Kempe, M. Mosbach, J. Chromatogr., **694**, 3 (1995).

5. G. Wulff, W. Vesper, J. Chromatogr, **167**, 171 (1978).

6. G. Wulff, S. Schauhoff, J. Org. Chem., **56**, 395 (1991).

7. L. I. Andersson, K. Mosbach, J. Chromatogr., **516**, 313 (1990).

8. L. I. Andersson, D. J. O'Shannessy, K. Mosbach, J. Chromatogr. **513**, 167 (1990).

9. G. Wulff, W. Best, A. Akelah, Reactive Polymers, **2**, 167 (1984).

10. G. Wulff, J. Vietmeier, J. Makromol. Chem., **190**, 1717 (1989).

11. K. Shea, D. Sasaki, J. Am. Chem. Soc., **113**, 4109 (1991).

12. K. Mosbach, Trends Biochem. Sci., **19**, 9 (1994).

13. G. Vlatakis, L. I. Andersson, R. Muller, K. Mosbach, Nature, **361**, 645 (1993).

14. L. I. Andersson, ACS Symposium Series, **586**, 89 (1995).

15. E. Heborg, Sensors and Actuators, **37-8**, 796 (1993).

16. D. Kriz, K. Mosbach, Anal. Chim. Acta., **300**, 71 (1995).

17. B. Sellergren, K. J. Shea, Tetrahedron, **5**, 403 (1994).

18. R. Miller, L. I. Andersson, K. Mosbach, Makromol. Chem., **14**, 637 (1993).

19. J. V. Beach, K. J. Shea, J. Am. Chem. Soc., **116**, 379, (1994).

20. M. Kempe, K. Mosbach, J. Chromatogr., **691**, 317 (1995).

21. D. O'Shannessy, B. Ekberg, K. Mosbach, Anal. Biochem., **177**, 144 (1989).

22. M. Kempe, K. Mosbach, Tet. Lett., **36**, 3563 (1995).

23. M. Kempe, K. Mosbach, Int. J. Peptide Res., **44**, 603 (1994).

24. L. Fischer, R. Muller, B. Ekberg, K. Mosbach, J. Am. Chem. Soc., **113**, 9358 (1991).

25. M. Kempe, K. Mosbach, J. Chromatogr., **664**, 276 (1994).

26. B. Sellergren, M. Lepisto, K. Mosbach, J. Am. Chem. Soc., **110**, 5853 (1988).

27. B. Sellergren, K. J. Shea, J. Chromatogr., **635**, 31 (1993).

28. O. Ramstrom, I. A. Nicholls, K. Mosbach, Tetrahedron, **5**, 649 (1994).

29. I. A. Nicholls, O. Ramstrom, K. Mosbach, J. Chromatogr., **691**, 349 (1995).

30. D. J. O'Shannessy, L. I. Andersson, K. Mosbach, J. Mol. Recog., **2**, 1 (1989).

31. D. Kritz, O. Ramstrom, A. Svensson, K. Mosbach, Anal. Chem., **67**, 2142 (1995).

32. O. Ramstrom, L. I. Andersson, K. Mosbach, J. Org. Chem., **58**, 7562 (1993).

33. J. Calvin Giddings, **Unified Separation Science**, Wiley, Chichester, 1991.

Downloaded At: 10:36 24 January 2011

34. G. Guichon, S. G. Shirazi, A. M. Katti, **Fundamentals of Preparative and Nonlinear Chromatography**, Academic Press, Boston, 1994.

35. T. Fornstedt, Z. Guoming, G. Guiochon, J. Chromatogr., **742**, 55 (1996).

Received June 4, 1998
Accepted June 10, 1998
Manuscript 4799

Downloaded At: 10:36 24 January 2011

Downloaded At: 10:36 24 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JLC100101653