Journal of Chromatography, 589 (1992) 45–51
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 720

Effect of superfluous remote polar functionality on chiral recognition

William H. Pirkle* and Christopher J. Welch

School of Chemical Sciences, University of Illinois, Urbana, IL 61801 (USA)

(First received July 30th, 1991; revised manuscript received September 2nd, 1991)

ABSTRACT

During the liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs), non-specific adsorption processes diminish the observed enantioselectivity. The role of those polar groups in the analyte which are not specifically required for chiral recognition was investigated. A series of racemic analytes bearing various non-essential polar groups spatially removed from the sites of chiral recognition were synthesized and chromatographically evaluated. Four scenarios for the interaction of these remote polar groups with the CSP were considered and used to rationalize experimental findings. Cases were observed where enantioselectivity decreased, increased or remained the same on incorporation of a remote polar group.

INTRODUCTION

The separation of enantiomers on chiral stationary phases (CSPs) is dependent on the formation of transient diastereomeric adsorbates with different free energies (Fig. 1). Although this process can be conveniently represented as the formation of a more and a less stable adsorbate pair as in Fig. 1, the actual situation represents the contribution of a number of different adsorbate structures. Nevertheless, we have found that by considering the most probable adsorbate structures, reasonably accurate predictions concerning CSP performance and enantiomer elution order can be made. Using this approach, we have successfully designed and produced a number of CSPs with both broad scope and high enantioselectivity, achieving separation factors (α) greater than 50 in some instances [1].

On these CSPs, enantiomer separation requires that the analyte contain an appropriate combination of suitably located functionality for interaction with complementary sites in the CSP. However,

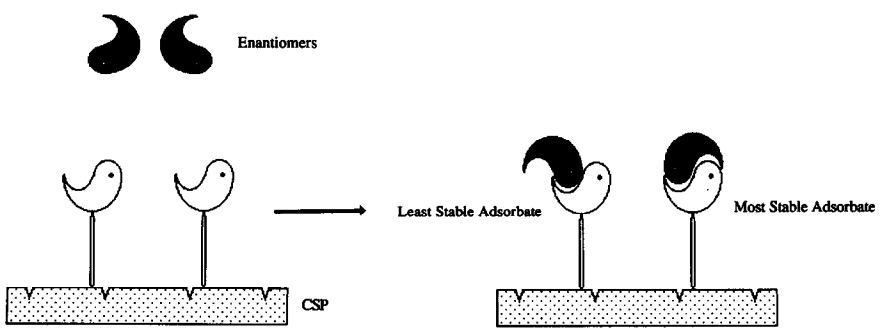


Fig. 1. Formation of transient diastereomeric adsorbates with different free energies permits enantiomer separation on CSPs.

0021-9673/92/$05.00

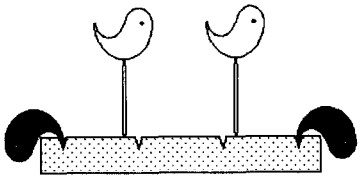


Fig. 2. Achiral retention, illustrated here as an interaction with the underlying chromatographic support, typically increases retention and decreases enantioselectivity.

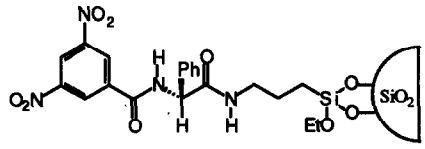
many analytes which do possess such an arrangement also contain interaction sites not required in the chiral recognition process. In this investigation, we focused on the influence of these superfluous polar groups on chiral recognition. To investigate this phenomenon, a series of derivatives of the well studied N-(3,5-dinitrobenzoyl)leucine were studied chromatographically using several different CSPs.

BACKGROUND

Non-specific adsorption stemming from sites on the CSP

Non-specific adsorption stemming from superfluous sites on a CSP is known to lead to diminished enantioselectivity (Fig. 2). For example, it is well known that end-capping residual silanol groups on the underlying silica support often decreases retention and increases enantioselectivity [2].

Tandem column experiments such as the example shown in Fig. 3 clearly illustrate this principle. When a racemate such as β-binaphthol is chroma-



CSP 1

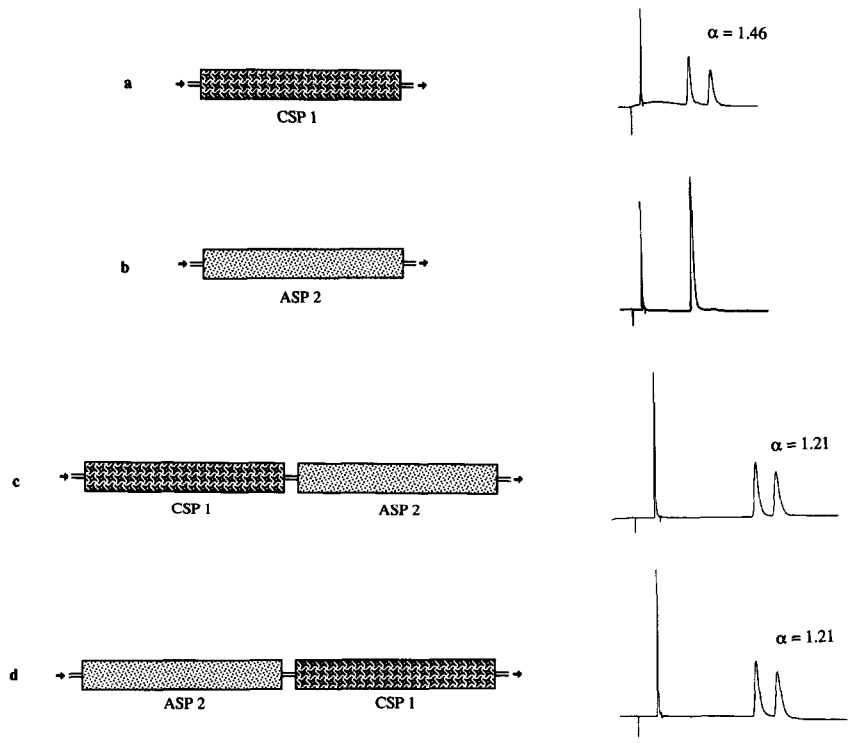
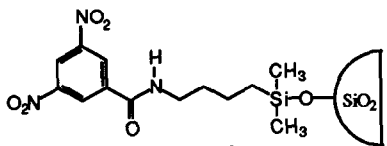


Fig. 3. Tandem column experiment illustrating decrease in enantioselectivity due to achiral retention. CSP 1 = Regis (*R*)-DNB-phenylglycine; ASP 2 = DNB-aminobutyl; mobile phase = 2-propanol–hexane (5:95); flow-rate = 2.00 ml/min; void volume indicator = tri-*tert.*-butylbenzene [5].

tographed on CSP 1 [3], the enantiomers are resolved (Fig. 3a), the separation factor being derived from their relative retention. An achiral stationary phase (ASP) such as ASP 2 gives retention, but no



ASP 2

separation of enantiomers (Fig. 3b). When racemic β-binaphthol is chromatographed on a tandem column arrangement such as that shown in Fig. 3c or d, increased retention is observed. However, the interval between the elution of the enantiomer peaks remains the same. Hence the observed separation factor (α) decreases relative to the single column experiment.

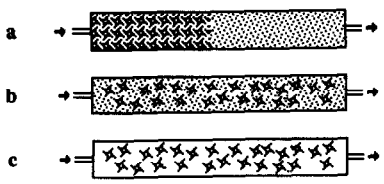


Fig. 4. Tandem column arrangement is equivalent to a single column containing regions of chiral and achiral packing (a), or to a column where these regions are intermingled (b). Elimination of sites for non-specific retention leads to improved CSPs (c).

The tandem column arrangement shown in Fig. 3c or d is conceptually identical with a single column having either separate or intermingled regions of chiral and achiral packing (Fig. 4a and b). Elimination of sites of non-specific analyte adsorption (Fig. 4c) leads to CSPs with improved enantioselectivites, as seen in the end-capping studied cited pre-

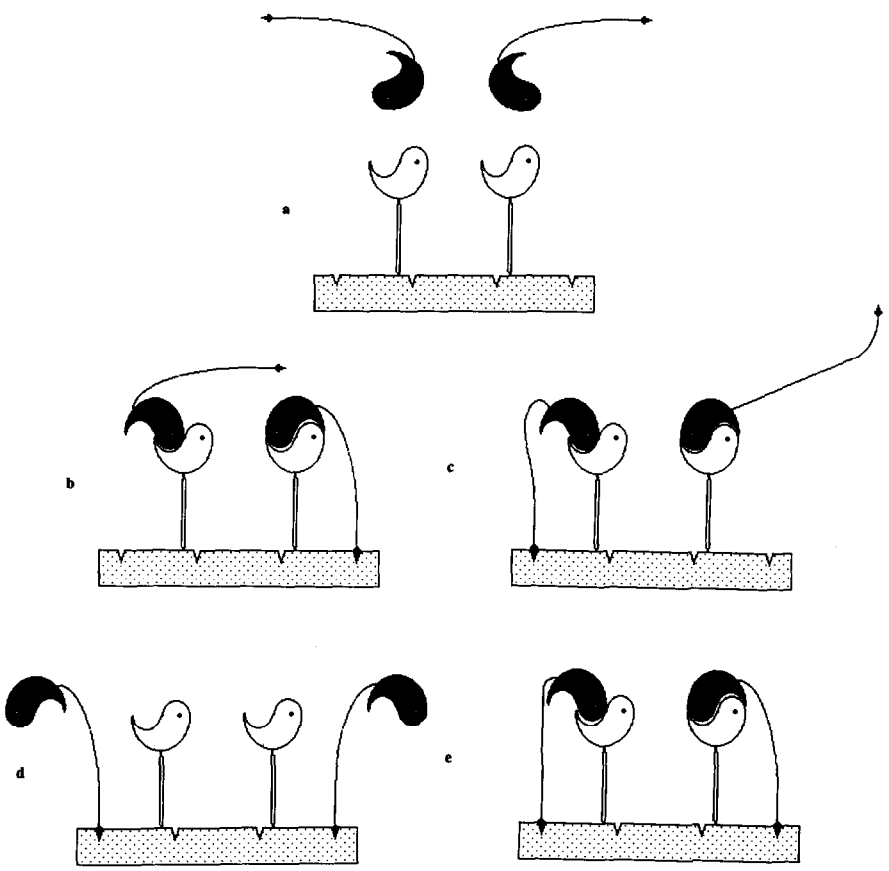


Fig. 5. Possible adsorption modes for analytes bearing remote polar groups.

viously. Although this message may seem obvious, its importance is not generally appreciated even though it has profound implications regarding CSP design. Many CSPs reported in the literature (or offered for sale) contain adsorption sites in excess of those required for chiral recognition. A forthcoming paper will deal specifically with the design of improved CSPs by deleting sites of non-productive adsorption. This study focuses on the effect of non-essential interaction sites present in the analyte.

Non-specific adsorption stemming from sites in the analyte

A racemic analyte bearing a remote polar group can be imagined to interact with a CSP in several different ways, all of which contribute to the observed retention (Fig. 5). Fig. 5 specifically illustrates the remote polar group interacting with the underlying chromatographic support. However, the remote polar group might also interact at sites in the selector or in the connecting tether. The consequences of these interactions will be similar regardless of their sites provided the interaction site is not one required for chiral recognition. Fig. 5a depicts the analyte enantiomers prior to adsorption by the CSP. Fig. 5b depicts the more strongly adsorbed enantiomer being selectively stabilized owing to an additional interaction between its remote polar group and the CSP. In the absence of a similar interaction for the less retained enantiomer, enantioselectivity is increased. Fig. 5c shows the converse of this situation where it is now the less stable diastereomeric adsorbate which is selectively stabilized by interaction of the remote polar group. This situation leads to a decrease in the observed enantioselectivity. In an extreme case, the elution order might even be reversed as the formerly less stable diastereomeric adsorbate becomes the most stable. Fig. 5d illustrates the situation where both enantiomers interact solely through the remote polar group. These achiral processes compete with those which afford chiral recognition, attenuating the observed enantioselectivity. The situation pictured in Fig. 5d is analogous to the tandem column and pre-end-capping situations described earlier, situations which were shown to afford a decrease in enantioselectivity. The final example (Fig. 5e) depicts the remote polar group of each enantiomer interacting equally well with the CSP during the normal chiral recognition process. This situation is expected to give rise to increased retention but no change in enantioselectivity as it increases binding energies but does not alter the difference in the binding energies.

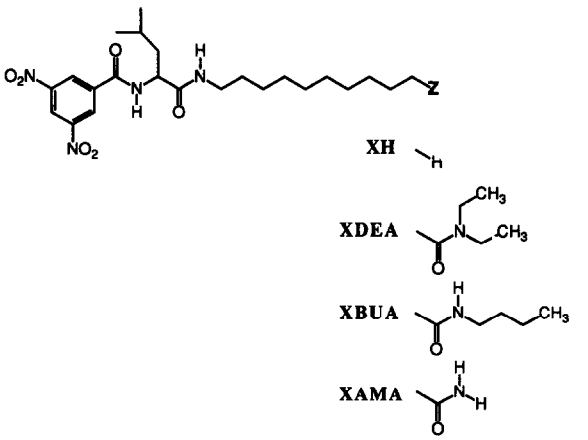


Fig. 6. The group of four N-(3,5-dinitrobenzoyl)leucine derivatives used in the study.

In an effort to assess the contribution of the various adsorption modes to actual chiral separations, four derivatives of the well-studied N-(3,5-dinitrobenzoyl)leucine system were prepared and analyzed on several CSPs. The four analytes, shown in Fig. 6, contain groups of varying polarity at Z. Using the symbol X to designate the DNB-leucine moiety containing ten methylene units, the four derivatives are termed XH, XDEA, XBUA and XAMA. The last three terminal groups are amides, good hydrogen bond acceptors having sizable dipole moments. These have, respectively, no, one and two acidic hydrogen bond donor sites as well.

EXPERIMENTAL

Apparatus

Chromatographic analysis was performed using a Beckman-Altex Model 100-A pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Beckman 153A UV absorbance monitor (254 nm) and a Hewlett-Packard HP 3394A integrating recorder.

Materials

Solvents used were of HPLC grade or distilled

prior to use. The four N-(3,5-dinitrobenzoyl)leucine derivatives were prepared by addition of the appropriate amine to the corresponding N-hydroxysuccinimide active ester in acetonitrile solvent, followed by purification of the derivative by flash chromatography on silica. All were satisfactorily characterized by ^{1}H NMR spectrometry.

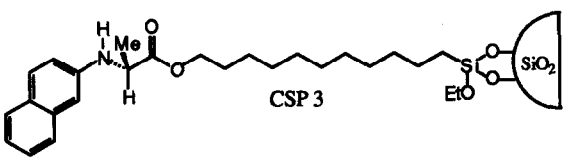
CSPs 1, 3, 4 and 6 are the commercial versions (Regis Chemical, Morton Grove, IL, USA) of CSPs developed in our laboratories. CSPs 2, 5 and 7 were produced in our laboratories and will be described in subsequent publications. CSPs 8 and 9 were available from a previous study [4].

Methods

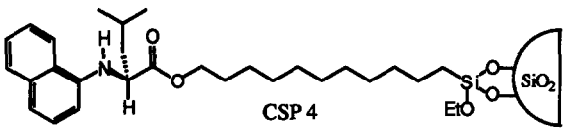
All chromatographic experiments were carried out at a nominal flow-rate of 2.00 ml/min. The void time was determined by injection of 1,3,5-tri-*tert.*-butylbenzene [5].

RESULTS AND DISCUSSION

We have previously reported [6] that the N-(2-naphthyl)alanine-based CSP 3, affords high enantioselectivities for the separation of the enantiomers of N-3,5-dinitrobenzoyl (DNB) derivatives of amino acids. The data concerning the separation of the enantiomers of the four remote polar group analytes on this CSP are given in Table I. The compounds undergo a dramatic increase in retention as the polarity of the Z group is increased. However, the separation factor remains relatively unchanged throughout the series, indicating a predominant mode of interaction such as that shown in Fig. 5e.



The recently developed N-(1-naphthyl)leucine CSP 4 is mechanistically similar to CSP 3 but has been shown to afford greater enantioselectivity in some instances [1]. Data pertinent to the separation of the enantiomers of three of the four remote polar group analytes on this CSP are given in Table I. A dramatic increase in retention occurs as the polarity of the Z group is increased with α remaining more or less constant throughout the series, again indicating an interaction mode such as that shown in Fig. 5e.



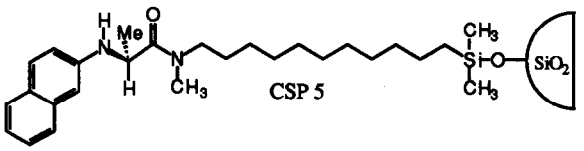
The recently developed CSP 5 represents an alternative anchoring scheme for the selector shown in CSP 3. Owing to the greater electron density on the carbonyl oxygen in this CSP, increased enantio-

TABLE I

CHROMATOGRAPHIC DATA FOR THE SEPARATION OF THE FOUR REMOTE POLAR GROUP ANALYTES ON DIFFERENT CSPs

CSP	Mobile phase[a]	XH			XDEA			XBUA			XAMA		
		k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
CSP 3	A	1.35	17.45	12.93	5.09	68.40	13.44	4.04	51.19	12.67	6.60	83.19	12.66
CSP 4	A	1.11	26.56	23.93	2.92	68.38	21.71	2.39	51.19	21.67	–	–	–
CSP 5	A	1.07	33.72	31.51	2.43	67.34	27.71	3.02	78.11	25.86	4.81	115.6	24.04
CSP 3	B	2.15	10.95	5.09	2.08	10.08	4.85	1.29	6.05	4.69	0.95	4.40	4.63
CSP 6	A	0.87	1.30	1.49	4.59	6.75	1.47	3.97	5.87	1.48	8.99	13.13	1.46
CSP 7	A	0.58	1.20	2.07	2.05	3.83	1.87	2.11	3.81	1.81	5.08	9.01	1.77
CSP 8	A	1.77	1.77	1.00	7.65	10.48	1.37	9.53	11.67	1.22	23.44	37.26	1.59
CSP 9	A	0.73	1.34	1.84	3.79	4.94	1.30	4.35	5.75	1.32	13.37	13.37	1.00

[a] Mobile phase: (A) 2-propanol–hexane (20:80); (B) methanol–water (80:20).

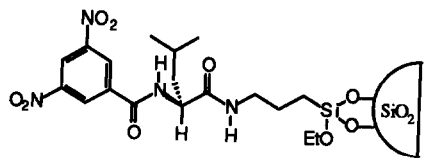


CSP 5

selectivity relative to CSP 3 is often noted for analytes using this carbonyl oxygen as an essential interaction site. Data for the separations of the enantiomers of the four remote polar groups analytes on CSP 5, given in Table I, show greatly increased retention and relatively constant α throughout the series. The slight decrease in enantioselectivity observed as the polarity of the Z group is increased may reflect some contribution to retention by adsorbates such as that shown in Fig. 5d. As Z groups of increased polarity decrease enantioselectivity more dramatically on CSP 5 than on either CSP 3 or 4, one presumes that the amide carbonyl oxygen of the CSP may be involved in additional achiral interaction.

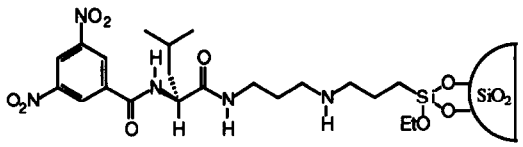
Reversed-phase separation of the enantiomers of the four remote polar group analytes on CSP 3 is also shown in Table I. As is frequently the case, enantioselectivities are reduced relative to those obtained under normal-phase conditions [6,7]. Not unexpectedly, the retentions decrease with increasing Z group polarity, reflecting the increased solvation of the more polar Z groups. Again, a slight decrease in α with increasing Z group polarity is noted, perhaps indicating the contribution of adsorbates such as that shown in Fig. 5d.

An interesting property of DNB-amino acid derivatives is their modest capacity for self-recognition. Thus, a DNB-leucine CSP such as CSP 6 is



CSP 6

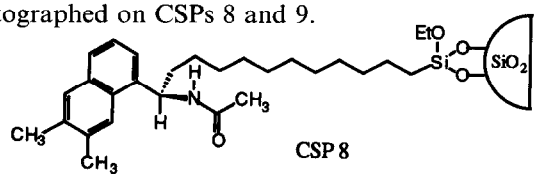
capable of serparating the enantiomers of racemic DNB-leucine derivatives. Data for the separation of the four analytes on CSP 6, given in Table I, again exhibit a dramatic increase in retention with increasing Z group polarity. However, α remains constant throughout the series, indicating that the predominant adsorbate may be represented as shown in Fig. 5e.



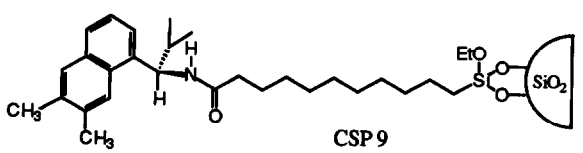
CSP 7

In CSP 7, an analogue of CSP 6, a superfluous amino group has been incorporated into the linking tether. Although this CSP shows the typical increase in retention with increasing Z group polarity, its enantioselectivity decreases for those analytes having more polar Z groups. This result may be indicative of the contribution of adsorbate structures (such as that shown in Fig. 5d) where the polar Z groups are capable of achiral interaction with the superfluous amine moiety.

Perhaps the most interesting observations come from the analysis of the remote polar group analytes on CSPs 8 and 9. These CSPs are very similar except for their mode of attachment to silica. When used to separate the enantiomers of DNB derivatives of a homologous series of α-arylaminoalkanes, the alkyl-linked CSP 8 shows increased enantioselectivity as the length of the alkyl substituent of the analyte is increased, whereas the acyl-linked CSP 9 shows decreased enantioselectivity [4]. This behavior was explained in terms of differential intercalation of the alkyl substituents of analyte enantiomers between strands of bonded phase. Differential intercalation influences enantioselectivity and the mode of attachment of the chiral selector to the silica determines whether it is the initially eluted or the more retained enantiomer which has the more severe intercalation problem. Differential intercalation also seems to explain the behavior of the remote polar group analyte enantiomers when chromatographed on CSPs 8 and 9.



CSP 8



CSP 9

Data pertinent to the separation of the enantiomers of the remote polar group analytes on CSPs 8 and 9 are presented in Table I. On the alkyl-linked CSP 8, an increase in enantioselectivity occurs with an increase in Z group polarity, whereas on the acyl-linked CSP 9, enantioselectivity decreases as Z becomes more polar. Although the chain of ten methylene units causes intercalation difficulties, a polar group at its end makes possible a bonding interaction with (presumably) the underlying silica support. The mode of attachment of the selector to the silica support determines which enantiomer most effectively intercalates its Z group. This situation is depicted in Fig. 5b and c for CSPs 8 and 9, respectively. Consistent with this interpretation, when a homologous series of DNB-leucine *n*-alkylamides is chromatographed on CSPs 8 and 9, an increase in the length of the *n*-alkyl group causes a decrease in enantioselectivity on CSP 8 but an increase on CSP 9. Moreover, the elution orders noted (an *R* CSP selectively retains the *R* analyte enantiomer) are consistent with this mechanistic picture.

CONCLUSIONS

Four racemic derivatives of N-(3,5-dinitrobenzoyl)leucine bearing non-essential polar functionalities remote from those sites essential for chiral recognition have been examined on several CSPs. Four possible scenarios for the interaction of these remote polar groups with the CSP and the consequences thereof (in terms of the effect on enantioselectivity) have been considered and used to rationalize the experimental observations. The case in which the retention increases dramatically and the enantioselectivity remains more or less constant on incorporation of a remote polar group seems to be common. Such cases, owing to the greatly increased retentions, would commonly necessitate the use of more polar mobile phases to achieve reasonable analysis times. As enantioselectivity generally diminishes with increasing mobile phase polarity [7], the remote polar groups may have the effect of indirectly reducing enantioselectivity.

ACKNOWLEDGEMENTS

This material was presented in part at the *17th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, Cleveland, OH, October 1990*. This research was funded in part by a Department of Education Advanced Opportunities in Chemistry Graduate Fellowship. Special thanks are due to Qing Yang for the preparation of CSP 7.

REFERENCES

1 W. H. Pirkle, K. C. Deming and J. A. Burke, *Chirality*, 3 (1991) 183.
2 W. H. Pirkle and R. S. Readnour, *Chromatographia*, 31 (1991) 129.
3 W. H. Pirkle and J. L. Schreiner, *J. Org. Chem.*, 46 (1981) 4988.
4 W. H. Pirkle, M. H. Hyun and B. Bank, *J. Chromatogr.*, 316 (1984) 585.
5 W. H. Pirkle and C. J. Welch, *J. Liq. Chromatogr.*, 14 (1991) 1.
6 W. H. Pirkle and T. C. Pochapsky, *J. Am. Chem. Soc.*, 108 (1986) 352.
7 W. H. Pirkle and C. J. Welch, *J. Liq. Chromatogr.*, 14 (1991) 2027.