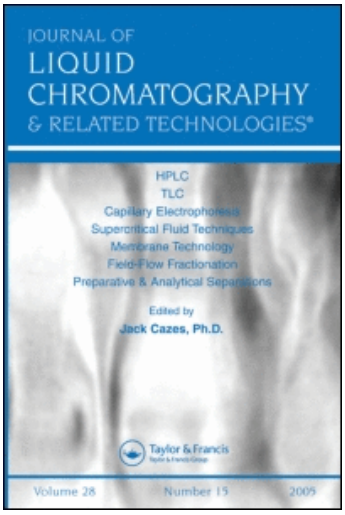

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An Improved Chiral Stationary Phase for the Chromatographic Separation of Underivatized Naproxen Enantiomers

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AN IMPROVED CHIRAL STATIONARY PHASE FOR THE CHROMATOGRAPHIC SEPARATION OF UNDERIVATIZED NAPROXEN ENANTIOMERS

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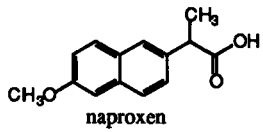
ABSTRACT

A mechanistic rationalization of the ability of a recently developed chiral stationary phase (CSP) to separate the enantiomers of underivatized naproxen (as well as a number of other non-steroidal anti-inflammatory drugs) suggested that an analog of this CSP containing a shortened tether might afford greater enantioselectivity. This is indeed the case. A separation factor of 2.93 obtained at room temperature with this CSP is the greatest enantioselectivity reported to date for the differential complexation of naproxen enantiomers by a synthetic selector.

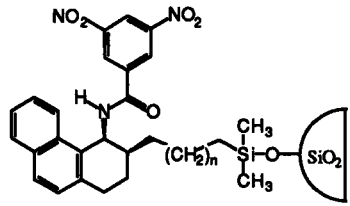
INTRODUCTION

We recently described[1] the design, synthesis, and evaluation of a chiral stationary phase (CSP) engineered specifically for the chromatographic separation of the underivatized enantiomers of the anti-inflammatory drug, naproxen, 1. This chiral stationary phase (CSP 1) affords much improved separations for the underivatized enantiomers of naproxen and a number of other non-steroidal anti-inflammatory drugs (NSAIDs) relative to other brush-type CSPs.[2] In fact, this CSP provides the highest level of enantioselectivity yet reported for the underivatized enantiomers of naproxen using any of the several naproxen-specific selectors designed for this purpose.[2,3] We now report that CSP 2, an analog of CSP 1 linked to silica via a shortened tether, shows even greater enantioselectivities in the separation of the underivatized enantiomers of NSAIDs. Considering the robustness and sample capacity of brush-type CSPs and the

enantioselectivities afforded, it seems likely that CSP 2 will be rather useful to many researchers.



naproxen



CSP 1 n = 9
CSP 2 n = 1

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using a Altex model 100A pump, a Rheodyne model 7125 injector with a 20 µl sample loop, a Linear UVIS 200 variable wavelength absorbance monitor, set at 254 nm, and a Hewlett-Packard HP 3394A integrating recorder. All ^{1}H NMR spectra were recorded on a Varian XL 200 FT NMR spectrometer. Elemental analyses were performed by T. McCarthy and associates of the University of Illinois microanalytical service.

Methods

All chromatographic experiments were carried out at a flow rate of 2.00 ml/min. Column void time was measured by injection of tri-*t*-butylbenzene, a presumed unretained solute.[4] ^{1}H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane.

Materials

All reagents were of pharmaceutical or reagent grade and were used without further purification. Solvents used were HPLC grade or distilled prior to use. Test analytes were available from previous studies. Dimethylchlorosilane was obtained from Petrarch Systems, Bristol, PA. Rexchrom 5µ / 100Å silica gel was obtained from Regis Chemical Co., Morton Grove, IL.

Preparation of Racemic 4-oxo-3-allyl-1,2,3,4-tetrahyrophenanthrene (3).

Transformation of 4-oxo-1,2,3,4-tetrahydro-phenanthrene[5], 1, to the racemic precursor, 3, was accomplished using the previously reported method described for the

synthesis of CSP 1.[1] Alkylation of 1 with allyl bromide gives ketone 2. Reductive amination of 2, followed by acylation with 3,5-dinitrobenzoyl chloride, gives the racemic precursor of CSP 2, 3. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.60 (m, 1 H), 2.10 (m, 3H), 2.65 (m, 1H), 3.10 (m, 2H), 5.12 (m, 2H), 6.00 (m, 1H), 6.18 (dd, 1H, *J* = 10 Hz and 3 Hz), 6.34 (d, 1H, *J* = 10 Hz), 7.29 (d, 1H), 7.45 (m, 2H), 7.79 (m, 2H), 8.05 (d, 1H, J = 8.4 Hz), 8.88 (d, 2H, *J* = 2.3 Hz), 9.11 (t, 1H, *J* = 2.3 Hz),

Resolution of the Racemic Precursor (3).

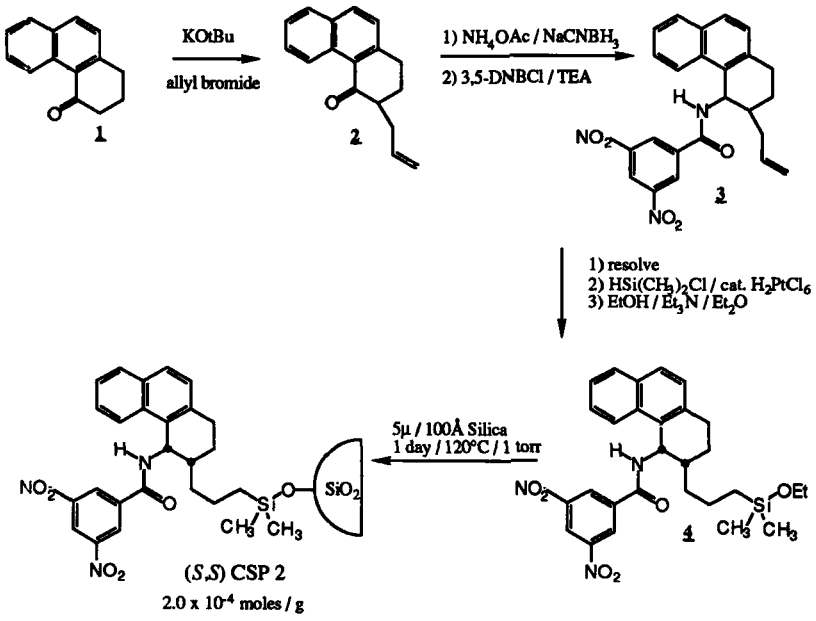
The enantiomers of 3 were chromatographically separated on a 25 mm x 900 mm column containing a previously described[6] (*S*)-*N*-(1-naphthyl) leucine CSP using 10% 2-propanol in hexane as the eluent.

Preparation of Ethoxyorganosilane (4).

The second eluted enantiomer of 3 (0.85 g), assigned the (*S*,*S*) absolute configuration by a combination of HPLC, NMR, and X-ray crystallographic evidence (to be described in a separate publication), was dissolved in a mixture of 10 ml of dimethylchlorosilane and 10 ml of dichloromethane. Chloroplatinic acid (about 5 mg) dissolved in a minimum amount of 2-propanol was then added and the reaction mixture was heated at reflux under a nitrogen atmosphere. After 1 h, a quenched aliquot of the reaction mixture showed (TLC analysis) no remaining starting material. The reaction mixture was evaporated to dryness on a rotary evaporator to give the crude chlorosilane as a dark oil. Residual dimethylchlorosilane was removed by three successive additions and evaporations of small portions of dichloromethane. A mixture of 5 ml of triethylamine, 5 ml of absolute ethanol, and 5 ml of diethyl ether was then added to the crude chlorosilane and the mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. The mixture was filtered to remove triethylamine hydrochloride and evaporated to afford the crude ethoxyorganosilane which was purified by flash chromatography on silica using 5% ethanol/dichloromethane as eluent to give 0.93 g of ethoxyorganosilane 4 (88% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.09 (s, 6 H), 0.62 (m, 2H), 1.18 (t, 3 H), 1.60 (m, 5H), 2.05 (m, 2H), 3.10 (m, 2H), 3.68 (q, 2H), 6.11 (dd, 1H), 6.33 (d, 1H), 7.29 (d, 1H), 7.45 (m, 2H), 7.79 (m, 2H), 8.10 (d, 1H), 8.85 (d, 2H), 9.10 (t, 1H).

Preparation of (*S*,*S*)-CSP 2.

A solution of 0.93 g of ethoxyorganosilane 4, dissolved in 1 ml of dimethylformamide, was added to a dichloromethane slurry of 5 g of Regis Rexchrom silica (5μ,100Å) which had been previously dried by azeotropic water removal with



Scheme 1: Synthetic route for preparation of CSP 2.

benzene. The slurry was carefully evaporated to dryness under reduced pressure, then heated at 120°C / 1 torr for 24 h. The silica gel was washed extensively with ethanol and then methanol, slurried in methanol and packed into a 4.6 mm x 250 mm stainless steel HPLC column. Elemental analysis of residual packing (C 4.95%; H 0.61%; N 0.56%) showed a loading of 2.0 x 10^{-4} moles of chiral selector per gram of stationary phase. The residual silanol groups were endcapped by passing a solution of 2 ml of hexamethyldisilazane in 50mL dichloromethane through the dichloromethane equilibrated column at a flow rate of 1 ml/min.

RESULTS AND DISCUSSION

From the rationale advanced to explain the separation of naproxen enantiomers upon CSP 1,[1] it was suspected that an analog containing a shortened tether might afford improved enantioselectivity. An overview of that rationale follows.

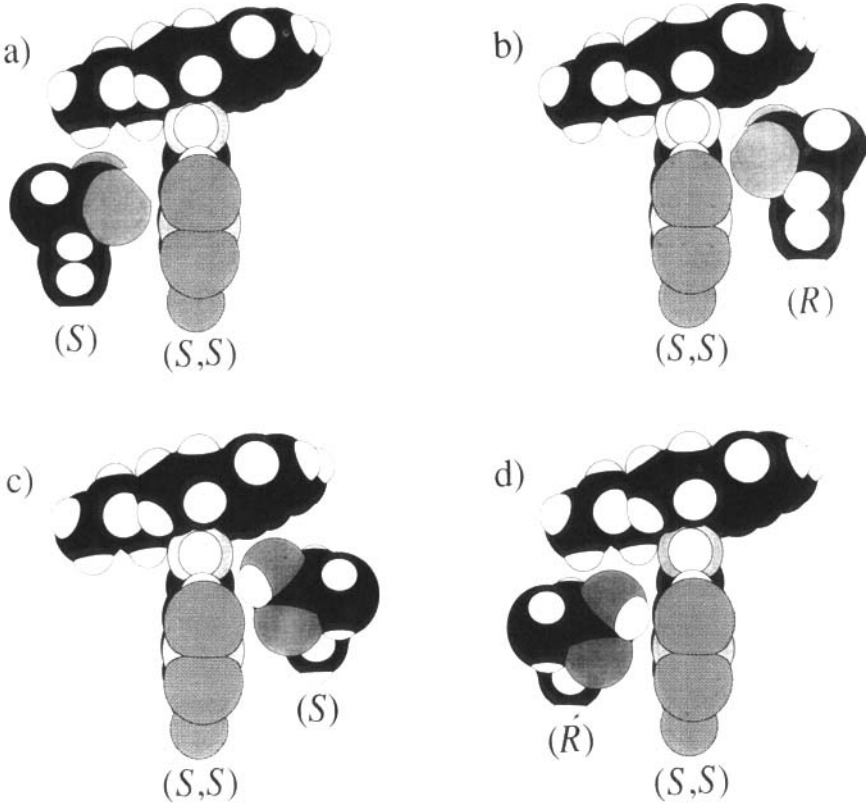


Figure 1: Exploded view of diastereomeric adsorbates derived from interaction of naproxen enantiomers with (*S,S*)-CSP 1.

Four possible diastereomeric adsorbates derived from an (*S,S*)-CSP and the two enantiomers of naproxen are represented in Figure 1 using computer-generated space-filling molecular model representations. It is difficult to clearly portray three dimensional complexes in a two dimensional representation, thus the concerned reader is urged to use CPK molecular models to aid in following the subsequent rationale. The alkyl tether of the CSP is represented as an *n*-propyl substituent. The distances between the two components of the diastereomeric complexes have been exaggerated for the sake of clarity. Each of the component molecules is represented in a conformation which is presumed to be of relatively low energy and hence preferentially populated. The dinitrobenzoyl ring system

Table 1: Separation of the enantiomers of underivatized NSAIDs using CSP 2. Conditions: Flow rate = 2.0 mL/min, mobile phase = 20% 2-propanol in hexane containing 1g / l ammonium acetate; k'_1 = capacity factor for initially eluted enantiomer, k'_2 = capacity factor for second eluted enantiomer, α = separation factor.

Separation of Underivatized NSAID Enantiomers on CSPs 1 and 2

	CSP 1			CSP 2		
compound	k'_1	k'_2	α	k'_1	k'_2	α
naproxen	3.96	8.95	2.26	1.71	5.01	2.93
ibuprofen	0.94	1.05	1.12	0.19	0.28	1.47
ketoprofen	4.53	5.03	1.11	1.39	1.79	1.29
flurbiprofen	1.63	1.94	1.19	0.37	0.59	1.59
pirprofen	2.53	3.49	1.38	0.85	1.54	1.81
fenoprofen	1.48	1.81	1.22	0.38	0.61	1.61
cicloprofen	3.03	5.18	1.71	1.16	2.50	2.15
tiaprofenic acid	6.15	6.70	1.09	2.02	2.48	1.23

of the (*S*,*S*)-CSP is viewed edge on, with the amide hydrogen projecting toward the viewer. A face to face π-π interaction between the naphthyl ring of naproxen and the dinitrobenzamide system of the CSP and a hydrogen bond between the amide hydrogen of the CSP and one of the two carboxylate oxygens of naproxen are believed to provide driving force for complex formation. Since naproxen contains two carboxylate oxygens, either of which might be involved in this hydrogen bonding interaction, the four adsorbates pictured in Figure 1 might each undergo these bonding interactions. In addition, adsorbates b) and c) are each thought to undergo a face to edge π-π interaction involving the naphthyl group of the selector and the aryl substituent of the NSAID. Adsorbate c) might also allow weak hydrogen bonding of the methine hydrogen of the NSAID to the π cloud of the naphthyl ring of the CSP. Face to edge π-π interactions are known from crystal structures of proteins,[7] peptides,[8] and small molecules,[9] and have recently been invoked to rationalize a growing body of chromatographic data in these laboratories.[10] Adsorbate c) is believed to be the predominant mode of interaction between the CSP and the more retained enantiomer, while adsorbate b) is believed to be the predominant mode of interaction undergone by the less retained enantiomer.

In order to increase enantioselectivity, one would typically like to either increase the stability of adsorbate c) or decrease the stability of adsorbate b). While attempts to

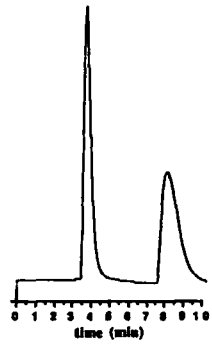


Figure 2: Actual chromatogram showing the separation of underivatized naproxen enantiomers using CSP 2. $k'_{(R)} = 1.71$; $k'_{(S)} = 5.01$; $\alpha = 2.93$. Conditions: Flow rate = 2.0 ml/min, mobile phase = 20% 2-propanol in hexane containing 1g / l ammonium acetate, UV detection at 254 nm, ambient temperature.

accomplish this by modification of the structure of the chiral selector are currently being pursued, it was deemed worthwhile to shorten the tether and move the selector closer to the silica surface.

The *cis* stereochemistry of the alkyl tether of CSP 1 was specifically incorporated into this selector to reduce the contribution of adsorbates a) and d) by sterically impeding approach to the face of the dinitrobenzamide system remote from the naphthyl ring (*i.e.* the *exo* face). We presently have no way of knowing the extent to which the contributions of *exo* face adsorbates a) and d) influence chiral recognition, but we were concerned that their contributions might reduce the overall enantioselectivity of CSP 1. A shorter tether might provide some bias against *exo* face approach of the analytes by using the underlying chromatographic support as a steric barrier to approach from the *exo* direction.

CSP 2, a short tether analog of CSP 1, was prepared in a manner analogous to the preparation of the previously described[1] CSP 1 (Scheme 1). Alkylation of ketone 1 with allyl bromide (instead of undecenyl iodide, as in the preparation of CSP 1) gives allyl ketone 2, which was reductively aminated, then acylated with 3,5-dinitrobenzoyl chloride to afford racemic precursor 3. The enantiomers of 3 were then separated preparatively using a CSP derived from (*S*)-*N*-(1-naphthyl)leucine.[6] The second eluted enantiomer of 3 was hydrosilylated to afford (*S*,*S*)-ethoxysilane 4, which was, in turn, bonded to silica to afford (*S*,*S*)-CSP 2.

CSP 2 does indeed show improved enantioselectivity in the separation of the enantiomers of naproxen and other underivatized NSAIDs as shown in Table 1. Increased

enantioselectivity relative to CSP 1 is noted for all of the NSAIDs examined, with a separation facto (α) of nearly three being obtained for naproxen. Analyte capacity factors (k's) are consistently less on the short tethered CSP 2, especially for the less retained enantiomer. These observations are consistent with the hypothesis that a shortened tether diminishes the contribution of *exo* face adsorbates (adsorbates a and d, Figure 1) to the overall retention of the analytes. The validity of this hypothesis is currently being studied using chromatographic as well as spectroscopic methods.

A chromatogram showing the separation of the enantiomers of underivatized naproxen on CSP 2 is shown in Figure 2. This separation represents the highest level of enantioselectivity observed for any of the several synthetic naproxen selectors which have been prepared to date.

CONCLUSION

A mechanistic rationalization of the separations of the enantiomers of underivatized naproxen (and a number of other NSAIDs) on CSP 1 suggested that a short tether analog of this CSP might afford improved enantioselectivity. Synthesis and evaluation of CSP 2, which contains a three carbon tether to silica, shows that it does indeed afford more facile separations of the underivatized enantiomers of naproxen and other NSAIDs. A separation factor (α) of 2.93 at room temperature was obtained for the separation of the enantiomers of naproxen, this being the highest level of enantioselectivity reported to date for naproxen using a synthetic selector. Although this result, as well as the design of CSP 1 according to the originally proposed mechanistic rationale[1], clearly shows the value of mechanistic insight in CSP design, further chromatographic and spectroscopic studies designed to test the validity of these rationales are underway.

ACKNOWLEDGEMENTS

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