white crystalline (1R,1'R)-carbamate **23a** [mp 177-178 °C, $[\alpha]^{23}_D$ -63.7 (c 0.49, $CHCl_3$)] which was used directly in the next step. ^{1}H-NMR: δ 0.19 (9 H, Me_3Si, s), 1.66 (3 H, 2'-CH_3, d, J = 6.6 Hz), 1.90 (3 H, C_8-CH_3, s), 2.80-3.25 (4 H, $2H_2$, $2H_4$, m), 5.20 (1 H, NH, br s), 5.31 (1 H, H_1, apparent br s), 5.67 (1 H, $H_{1'}$, m), 7.80-8.20 (7 H, naphthyl-H, m).

The mother liquors (~244 mg) contained a mixture of (1R,1'R) **23a** and (1S,1'R)-carbamates **23b** enriched in the latter. HPLC analysis (Whatman Partisil 10 Silica Magnum 9, 10% ethyl acetate/hexanes) led to elution of the minor (1R,1'R)-carbamate **23a** followed by the major (1S,1'R)-diastereomer **23b**. The HPLC elution profile is described in the text along with a discussion of studies attesting to configurational assignments and diastereomeric purities. Additional information is described elsewhere in the Experimental Section along with a description of the results of a single-crystal X-ray crystallographic study of the (1S,1'S)-enantiomer.

Biological Evaluation: Intestinal Calcium Absorption and Bone Calcium Mobilization. Intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) were determined in vivo in vitamin D deficient chicks as described previously.[25] Twelve hours before assay, the chicks which had been placed on a zero-calcium diet 48 h before assay, were injected intramuscularly with vitamin D metabolite or analogue in 0.1 mL of ethanol/1,2-propanediol (1:1, v/v) or with vehicle. At the time of assay, 4.0 mg of $^{40}Ca^{2+}$ + 5 μCi of $^{45}Ca^{2+}$ (New England Nuclear) were placed in the duodenum of the animals which had been anesthetized with ether. After 30 min, the birds were decapitated and the blood collected. The radioactivity content of 0.2 mL of serum was measured in a liquid scintillation counter (Beckman LS8000) to determine the amount of $^{45}Ca^{2+}$ absorbed (which is a measure of ICA). BCM activity was estimated from the increase of total serum calcium as measured by atomic absorption spectrophotometry.

1α,25-$(OH)_2$-D_3 Receptor Steroid Competition Assay. A measure of competitive binding to the chick intestinal 1α,25-$(OH)_2$-D_3 receptor was performed by using the hydroxylapatite batch assay.[26] Increasing amounts of 1α,25-$(OH)_2$-D_3 or analogue were added to a standard amount of [^{3}H]-1α,25-$(OH)_2$-D_3 and incubated with chick intestinal cytosol. The relative competitive index (RCI) for the analogues was determined by plotting the percent maximum of 1α,25-$(OH)_2$-D_3 bound × 100 on the ordinate versus [competitor]/[1α,25-$(OH)_2$-[^{3}H]D_3] on the abscissa. The slope of the line obtained for a particular analogue is divided by the slope of the line obtained for 1α,25-$(OH)_2$-D_3; multiplication of this value by 100 gives the RCI value. By definition, the RCI for 1α,25-$(OH)_2$-D_3 is 100.

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Supplementary Material Available: Spectral and analytical data (34 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Design, Synthesis, and Evaluation of an Improved Enantioselective Naproxen Selector

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The design, synthesis, and evaluation of an improved selector for the enantiomers of the nonsteroidal anti-inflammatory drug, naproxen, are described. So as to utilize the principle of reciprocity, two chiral stationary phases (CSPs) derived from (S)-naproxen were produced and HPLC techniques were used to screen candidate naproxen selectors. By determining how the structural features of the candidates influence enantioselective recognition by naproxen, a hypothetical chiral recognition mechanism for enantioselective recognition of naproxen was developed and used to design a selector which was incorporated into a new CSP. This comparatively simple CSP shows significant improvement in the separation of underivatized naproxen enantiomers relative to previous methods. Related compounds such as ibuprofen, ketoprofen, cicloprofen, fenoprofen, etc. are also resolved into their component enantiomers by this CSP.

Introduction

Among the economically significant nonsteroidal anti-inflammatory drugs (NSAIDs), many of which are α-arylpropionic acids, only naproxen, **1**, is sold as a single enantiomer.[1] Consequently, there has been considerable interest in the asymmetric synthesis[2] and chromatographic resolution[3] of naproxen. Several rather complex synthetic receptors intended for enantioselective recognition of naproxen have been developed by Diederich and co-workers[4] In the best case seen to date, an enantioselectivity of 1.21 has been observed.[4d] In this paper, we describe a chromatographic approach which was used to develop a mechanistic rationale which, in turn, led to the design of an enantioselective selector for naproxen. This selector is straightforward in its preparation and shows enhanced

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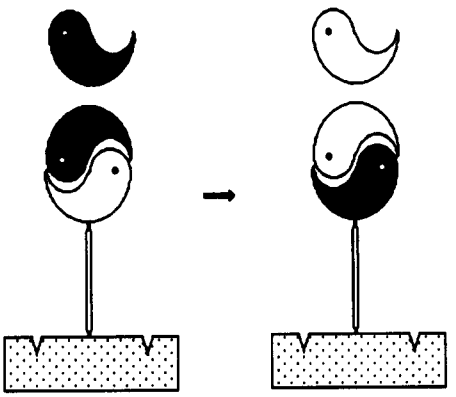
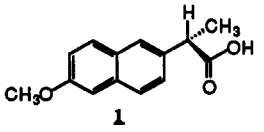


Figure 1. Illustration of the concept of reciprocity: A single enantiomer of a racemate which separates well on the CSP shown on the left, when used to produce a second CSP, shown at the right, will usually afford separation of the enantiomers of analytes which are structurally similar to the chiral selector of the first CSP.

enantioselectivity when compared with previously reported synthetic naproxen selectors.

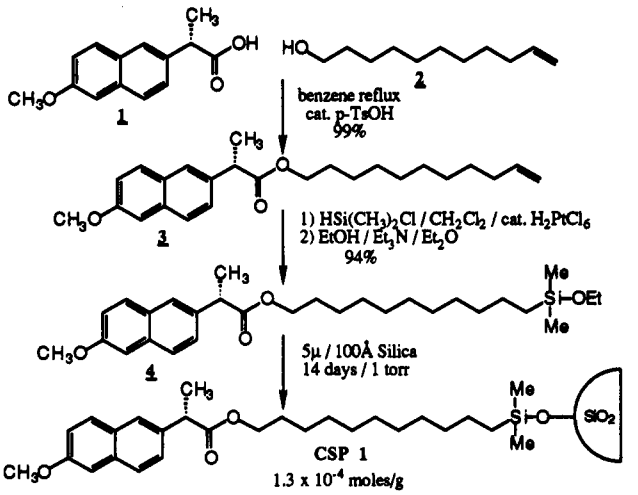


1

(*S*)-naproxen

The notion of reciprocity in chiral recognition has played a considerable role in the design of the chiral selectors and chiral stationary phases (CSPs) emanating from these laboratories.[5] If a single molecule of a chiral selector has different affinities for the enantiomers of another substance, then a single enantiomer of the latter will have different affinities for the enantiomers of the initial selector (Figure 1). Rigorously, the use of immobilized A to chromatographically separate the enantiomers of B is not necessarily the energetic equivalent of the experiment in which immobilized B is used to separate the enantiomers of A. Hence, some degree of care should be taken in extrapolating chromatographically-derived models to solution studies and vice versa. For example, the manner in which the selector is immobilized can influence the energetics of the separation process, both steric repulsion[6] and attractive interactions[7] with the underlying chromatographic support having been observed. In addition, simultaneous interaction of the analyte with more than one strand of bonded phase may result in nonreciprocal behavior. Despite these caveats, there can be considerable similarity between the interactions of a molecule with free and with immobilized selector. Highly enantioselective CSPs have been developed using this approach and separation factors exceeding 100 have been observed in the most extreme examples.[8] From this perspective, we have long maintained that, in order to design a selector to separate the enantiomers of a given target compound, one should first make a CSP from a single enantiomer of the target and use the HPLC data generated with this CSP to better understand how to design a chiral selector for the target. This method has now been reduced to practice.

Scheme I. Preparation of the Ester-Linked (*S*)-Naproxen-Derived CSP 1



Experimental Section

General Methods. All reagents were of pharmaceutical or reagent grade and were used without further purification. Solvents used were HPLC grade or distilled prior to use. (*S*)-Naproxen, (+)-6-methoxy-α-methyl-2-naphthyleneacetic acid, was obtained from Aldrich Chemical Co., Milwaukee, WI. Dimethylchlorosilane was obtained from Petrarch Systems, Bristol, PA. Test analytes were available from previous studies unless otherwise indicated. All ^{1}H NMR spectra were recorded on a 200-MHz NMR spectrometer. Elemental analyses were performed by T. McCarthy and associates of the University of Illinois microanalytical service. All chromatographic experiments were carried out at a nominal flow rate of 2.00 mL/min. Column void time was measured by injection of tri-*tert*-butylbenzene, a presumed unretained solute.[9]

Preparation of CSP 1. A 4.0-mm-i.d. × 14.5-cm-length stainless steel column packed with CSP 1 was prepared from (*S*)-naproxen as outlined in Scheme I and described below.

(*S*)-Naproxen Undec-10-en-1-yl Ester (3). To a solution of 1.50 g of (*S*)-naproxen, 1, and 2.22 g of undec-10-ene-1-ol, 2, in 75 mL of dry benzene was added 20 mg of *p*-toluenesulfonic acid. The stirred mixture was heated to gentle reflux under a nitrogen atmosphere until the starting material had disappeared (thin-layer chromatography assay). The mixture was evaporated to dryness using a rotary evaporator, and the residue was purified by flash chromatography on silica gel using methylene chloride to give 2.46 g of (*S*)-naproxen undecenyl ester, 3, as a clear oil (99% yield). HPLC analysis of 3 using a previously described CSP[10] showed that no racemization had occurred during the course of the reaction. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.10 (bs, 12 H), 1.25 (m, 2 H), 1.45 (d, 3 H, J = 8 Hz), 1.98 (m, 2 H), 3.85 (s, 3 H), 4.0 (m, 3 H), 4.95 (m, 2 H), 5.78 (m, 1 H), 7.14 (dd, 1 H, J = 9.5 and 2.4 Hz), 7.28 (d, 1 H, J = 2.4 Hz), 7.38 (dd, 1 H, J = 8.5 and 1.8 Hz), 7.70 (s, 1 H), 7.76 (d, 1 H, J = 8.5 Hz), 7.79 (d, 1 H, J = 9.5 Hz). Anal. Calcd for $C_{25}H_{34}O_3$: C, 78.49; H, 8.96. Found: C, 78.57, H, 8.92.

(*S*)-10-(Dimethylethoxysilyl)undecyl naproxenate (4). To a solution of 1.00 g of (*S*)-3 in 15 mL of dichloromethane was added 15 mL of dimethylchlorosilane and 10 mg of chloroplatinic acid dissolved in 50 μL of 2-propanol. The stirred mixture was heated at reflux under a nitrogen atmosphere until the starting material had disappeared as indicated by TLC and HPLC analysis of quenched aliquots. The reaction mixture was evaporated to dryness on a rotary evaporator, the crude chlorosilane remaining as a dark oil. Residual dimethylchlorosilane was removed by three successive additions and evaporations of small portions of dichloromethane. A solution of 5 mL of triethylamine, 5 mL of

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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 then filtered to remove triethylamine hydrochloride, concentrated in vacuo, and purified by flash chromatography on silica using 2:1 dichloromethane/hexane as eluent to give 1.19 g of (*S*)-4 as a clear oil (94% yield). HPLC analysis of 4 using a previously described CSP[10] and comparison with a sample of racemic 4 showed no evidence of racemization. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.06 (s, 6 H), 0.51 (m, 2 H), 1.11 (m, 19 H), 1.26 (bs, 2 H), 1.49 (d, 3 H, J = 7.0 Hz), 3.61 (q, 2 H, J = 5.8 Hz), 3.89 (s, 3 H), 3.92 (q, 1 H, J = 7.4 Hz), 4.03 (q, 2 H, J = 6.8 Hz), 7.17 (dd, 1 H, J = 9.0 and 2.2 Hz), 7.31 (d, 1 H, J = 2.4 Hz), 7.40 (dd, 1 H, J = 8.0 and 2.0 Hz), 7.80 (d, 1 H, J = 8.4 Hz), 7.74 (s, 1 H), 7.82 (d, 1 H, J = 9.0 Hz). Anal. Calcd for $C_{29}H_{46}SiO_4$: C, 71.86; H, 9.53. Found: C, 71.33, H, 9.26.

(*S*)-Naproxen-Derived CSP 1. To a slurry of 3.0 g silica gel (5-μm diameter; 100-Å pore size) in 10 mL of dichloromethane was added 690 mg of (*S*)-4 dissolved in 1 mL of dimethylformamide (DMF). The slurry was evaporated under reduced pressure and then placed on a vacuum line (0.5 Torr; room temperature). Small aliquots of silica were periodically removed and washed with ethanol, the washings being checked for racemization of the unbonded silane (HPLC). The extent of silane immobilization was determined by elemental analysis of the washed and dried silica. The slurry was wetted every few days with about 1 mL of DMF and returned to the vacuum line. After 14 days at room temperature (preliminary experiments showed that the high temperature usually used for bonding causes some racemization of the selector), the reaction mixture showed a reasonably high level degree of loading by elemental analysis. The reaction mixture was slurried in absolute ethanol, and the stationary phase was collected using a fine sintered glass filter. The packing was repeatedly washed with ethanol and the methanol and then slurried in methanol and packed into a 4.0-mm × 14.5-cm stainless steel column. Excess silica gel removed from the column packer reservoir was submitted for elemental analysis (C, 4.25; H, 0.67; N, 0.08), indicating a loading of 1.3×10^{-4} mol chiral selector per gram of stationary phase. Residual silanol groups on the silica were endcapped by passing a solution of 2 mL of hexamethyldisilazane in 50 mL of dichloromethane through the methylene chloride equilibrated column at a flow rate of 1 mL/min.

Preparation of CSP 2. A γ-aminopropylsilica column was prepared by slurry packing γ-aminopropyl 7-μm spherical silica gel into a 4.6-mm-i.d. × 25-cm-length stainless steel HPLC column using methanol. The column was then eluted sequentially with 100 mL of 0.2% sodium bicarbonate solution in 90% methanol/water, 100 mL of 90% methanol/water, 100 mL of methanol, and 200 mL of 5% 2-propanol in hexane. A solution of 535 mg of (*S*)-naproxen in 100 mL of 5% 2-propanol in hexane was then recirculated through the column. Evaporation of this solution after 12 h of recirculation gave 300 mg of recovered (*S*)-naproxen. Elution of the column with 20% 2-propanol in hexane initially gave a strongly UV absorbing effluent, the absorbance of which diminished and stabilized after some time.

Preparation of CSP 3. 11-Iodoundec-1-ene. A solution of 93.5 g of undec-10-en-1-ol, **2**, and 100 mL of triethylamine in 500 mL of dry dichloromethane was treated with 68.7 g of methanesulfonyl chloride at 0 °C according to a previously reported method.[11] The crude reaction mixture was then evaporated and partitioned between water and ether. The ether layer was collected, washed with water, then dried over anhydrous magnesium sulfate. Filtration and evaporation gave 142 g of the crude mesylate as a colorless oil, 64 g of which was immediately converted to the iodo compound by treatment with a solution of 137 g of sodium iodide and 1.2 g of dicyclohexano-18-crown-6 in 150 mL of water. After the stirred mixture had been heated on a steam bath for 5 h, the reaction mixture was extracted several times with ether, and the combined ether fractions were washed with water and then dried over anhydrous magnesium sulfate. Filtration and evaporation of the ethereal solution gave the crude iodo compound as an oil which was vacuum distilled to give 62.8 g (90% yield) of 11-iodoundec-1-ene as an almost colorless oil, bp 118–122 °C (4.5 Torr) (lit.[12] bp 104 °C/(2 Torr)).

Racemic 4-Oxo-3-undec-10-en-1-yl-1,2,3,4-tetrahydrophenanthrene (6). In a 1-L three-necked round-bottom flask equipped with a Teflon paddle stirrer, nitrogen inlet, and a Dean-Stark trap was dried 500 mL of benzene by azeotropic water removal. Solid potassium *tert*-butoxide, 12.3 g, was added at 50 °C to give a clear solution. A solution of 8.9 g of 4-oxo-1,2,3,4-tetrahydrophenanthrene[13] (**5**) and 14 g of 11-iodoundec-1-ene in 100 mL of dry benzene was added at 40 °C with stirring, causing the reaction mixture to darken somewhat. Stirring at 40 °C was continued for 45 min, and then the reaction mixture was heated at reflux for 2 h. During this period, approximately 400 mL of benzene was allowed to distill. The cooled reaction mixture was extracted with water, and the organic phase was dried over anhydrous magnesium sulfate. Removal of the drying agent and evaporation of the filtrate gave 19 g of a dark oil which was purified by flash chromatography on silica (1:1 dichloromethane/hexane) to give 9.9 g of the monoalkylated ketone, **6** (63% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.28 (bs, 14 H), 2.0 (m, 5 H), 2.32 (m, 1 H), 2.61 (m, 1 H), 3.16 (t, 2 H, J = 5.4 Hz), 4.98 (m, 2 H), 5.82 (m, 1 H), 7.30 (d, 1 H, J = 9.8 Hz), 7.49 (m, 1 H), 7.62 (m, 1 H), 7.81 (d, 1 H, J = 7.8 Hz), 7.91 (d, 1 H, J = 8.6 Hz), 9.30 (d, 1 H, J = 8.5 Hz). Anal. Calcd for $C_{25}H_{32}O$: C, 86.15; H, 9.25. Found: C, 86.08, H, 9.29.

Racemic 4-Amino-3-undec-10-en-1-yl-1,2,3,4-tetrahydrophenanthrene (7). In a 250-mL thick-walled Parr bottle was mixed 5 g of alkylated ketone **6**, 6 g of sodium cyanoborohydride, 30 g of ammonium acetate, and 100 mL of 2-propanol. After the bottle was securely closed with a rubber stopper and copper wire, the contents were heated to 90–95 °C for 24 h by immersion of the bottle in a steam bath. A safety shield was placed in front of the cloth-wrapped bottle. After the cooled reaction mixture had been concentrated by rotary evaporation, the crude product was partitioned between ether and water and the ether layer was dried with anhydrous magnesium sulfate, filtered, and evaporated to give 5.9 g of an oil which showed no residual ketone by TLC. The crude amine, **7**, was carried on to the next step without further purification.

4-(3,5-Dinitrobenzamido)-3-undec-10-en-1-yl-1,2,3,4-tetrahyrophenanthrene (8). The crude amine, **7**, from the previous reaction was dissolved in 150 mL of dichloromethane and stirred with excess saturated sodium hydrogen carbonate solution. 3,5-Dinitrobenzoyl chloride, 5 g, dissolved in the minimum amount of dichloromethane was then added, and the resulting two-phase mixture was stirred vigorously for 1 h. The organic layer was then dried, concentrated to a volume of approximately 20 mL, and purified by flash chromatography on silica with dichloromethane. Both cis and trans isomers of **8** were obtained in about a 5:1 ratio. Although separation of the diastereomers was incomplete by flash chromatography, pooling of those fractions containing only the cis diastereomer gave 2.2 g of **8** (28% yield from **6**). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.29 (bs, 15 H), 2.03 (m, 5 H), 3.13 (m, 2 H), 4.98 (m, 2 H), 5.82 (m, 1 H), 6.12 (dd, 1 H, J = 10 and 3 Hz), 6.25 (d, 1 H, J = 10 Hz), 7.29 (d, 1 H), 7.51 (m, 2 H), 7.81 (m, 2 H), 8.10 (d, 1 H, J = 8.4 Hz), 8.85 (d, 2 H, J = 2.3 Hz), 9.10 (t, 1 H, J = 2.3 Hz). Anal. Calcd for $C_{32}H_{36}N_3O_5$: C, 70.83; H, 6.69, N, 7.74. Found: C, 70.64, H, 6.81, N, 7.54.

Resolution of Racemic Dinitrobenzamide (8). The enantiomers of **8** were chromatographically separated on a 25-mm × 900-mm column containing a previously described (*S*)-*N*-(1-naphthyl)leucine CSP[8] using 10% 2-propanol in hexane as the eluent.

Organosilane (9). The initially eluting enantiomer of **8** (1.0 g), assigned the (*R*,*R*) absolute configuration by a combination of HPLC, NMR, and X-ray crystallographic evidence (to be described in a separate paper), was dissolved in a mixture of 10 mL of dimethylchlorosilane and 10 mL of dichloromethane. Chloroplatinic acid (about 10 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 2 h, a quenched aliquot of the reaction mixture showed (TLC analysis) no remaining starting material. The reaction mixture was evaporated

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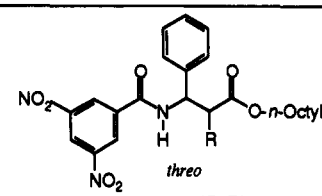
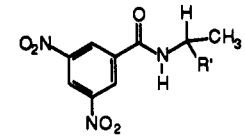
Table I. Separation of the Enantiomers of Several π-Acidic Amine Derivatives on (S)-Naproxen-Derived CSPs 1 and 2[a]

analyte	R	CSP 1 k'_1	CSP 1 k'_2	CSP 1 α	CSP 1 retained*	CSP 2 k'_1	CSP 2 k'_2	CSP 2 α	CSP 2 retained*
									
		threo							
10	*t*-Bu	1.71	2.28	1.33	(*R*,*R*)	1.42	1.70	1.20	(*R*,*R*)
11	*i*-Pr	1.85	2.06	1.11	(*R*,*R*)	2.11	2.38	1.13	(*R*,*R*)
12	Et	1.71	1.71	1.00		2.10	2.32	1.10	(*R*,*R*)
									
13	*t*-Bu	1.75	1.96	1.12	(*S*)	2.03	2.03	1.00	
14	Ph	2.94	2.94	1.00		5.40	5.97	1.11	(*S*)
15	α-Naph	4.28	5.04	1.18	(*S*)	6.58	10.11	1.54	(*S*)

[a] Flow rate = 2 mL/min; mobile phase = 10% 2-propanol in hexane; k'_1 = capacity factor for initially eluted enantiomer, k'_2 = capacity factor for second eluted enantiomer, α = separation factor. * indicates absolute configuration of second eluted enantiomer.

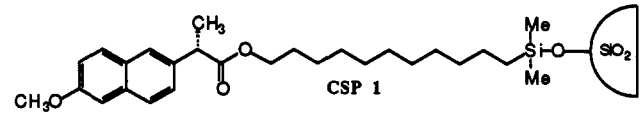
to dryness on a rotary evaporator to give the crude chlorosilane as a dark oil. Residual dimethylchlorosilane was removed by three successive additions and evaporations of small portions of dichloromethane. A mixture of 5 mL of triethylamine, 5 mL of absolute ethanol, and 5 mL of diethyl ether was then 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, **9**, which was used without further purification in the preparation of CSP 3.

(*R*,*R*)-CSP 3. One gram of the ethoxyorganosilane **9**, dissolved in 1 mL dimethylformamide, was added to a dichloromethane slurry of 5 g of Regis Rexchrom silica (5 μm 100 Å) which had been previously dried by azeotropic water removal with benzene. The slurry was carefully evaporated to dryness under reduced pressure and then rocked in a Kügelrohr oven at 90–95 °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 × 250-mm stainless steel HPLC column. Elemental analysis of residual packing (C, 7.27; H, 0.95; N, 0.71) showed a loading of 1.8×10^{-4} mol of chiral selector per gram of stationary phase. The residual silanol groups were endcapped by passing a solution of 2 mL of hexamethyldisilazane in 50 mL dichloromethane through the dichloromethane-equilibrated column at a flow rate of 1 mL/min.

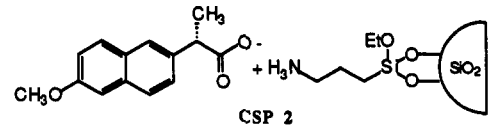
Results and Discussion

At the outset, the design of an enantioselective selector for naproxen was recognized as a challenging task. Although the β-naphthyl system is a potential site for π–π interaction, it is relatively free to rotate, with the two conformers having the methine hydrogen approximately eclipsed with either the 1 or 3 hydrogens of the naphthyl system being of relatively low energy. The carboxyl group is also relatively free to rotate, either oxygen being a potential site for hydrogen bonding. Finally, the methyl substituent on the stereogenic center is not a large group and will exert but a modest effect should chiral recognition entail aspects of size discrimination. As in achiral molecular recognition,[14] chiral recognition is believed to be facilitated by conformational bias and/or rigidity and by spatial localization of interaction sites. Thus, the conformational heterogeneity (i.e., lack of preorganization) of naproxen is expected to reduce the difference in the stability of diastereomeric complexes. However, the recent observation that a β-amino acid-derived CSP[10,15] may be used to separate the enantiomers of underivatized naproxen[16] demonstrates that the problem is not insoluble. Although use of this particular CSP has the advantage of not requiring the prior derivatization of analytes as required by some other approaches,[3,17] the separation factors achieved are modest (α = 1.22) and the enantiomers of α-arylpropionic acids such as ibuprofen or ketoprofen are not resolved. Taking this result as encouragement that a relatively simple compound might function as an enantioselective naproxen selector, we undertook a study of the chiral recognition of naproxen utilizing the principle of reciprocity.

In an effort to develop a naproxen selector of improved enantioselectivity, CSP 1, consisting of (*S*)-naproxen immobilized on silica gel via an ester linkage (Scheme I) and CSP 2, consisting of (*S*)-naproxen immobilized on silica gel via an ionic linkage, were used to screen potential naproxen selectors and to study the structural requirements for chiral recognition by naproxen. Both of these CSPs are easily accessible and simple to use.



CSP 1



CSP 2

(14) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039.

(15) Pirkle, W. H.; McCune, J. *J. Chromatogr.* **1989**, *471*, 271. Pirkle, W. H.; Murray, P. G. *J. Liq. Chromatogr.* **1990**, *13*, 2123.

(16) Pirkle, W. H.; Welch, C. J. *J. Liq. Chromatogr.* **1991**, *14*, 3387.

(17) Excellent separation of the enantiomers of underivatized naproxen have been reported using the protein-based α_1-acid glycoprotein CSP (Hermansson, J.; Eriksson, M. *J. Liq. Chromatogr.* **1986**, *9*, 621. Kern, J. R. *J. Chromatogr.* **1991**, *543*, 355. However, protein-derived CSPs suffer from several drawbacks in the chromatographic separation of enantiomers, including the fact that (i) proteins are available in only one enantiomeric form. Consequently, the reversal of elution order, often desirable in analytical determinations of enantiomeric purity, is usually impossible, (ii) protein-derived CSPs are often less robust than "brush type" CSPs, especially in organic solvents or at extremes of temperature. (iii) Owing to the paucity of binding sites on protein-derived CSPs, the columns are easily overloaded and consequently are unsuited for preparative separations.

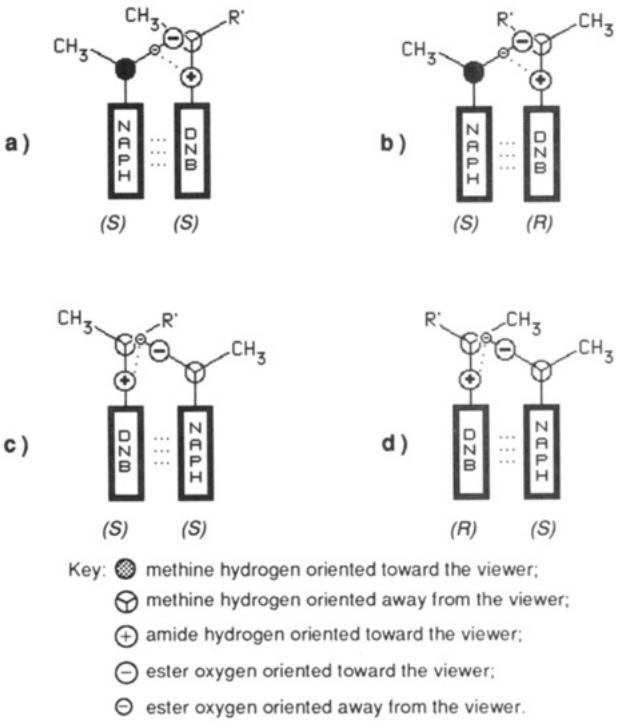


Figure 2. Rationale for enantioselective recognition of π-acidic amine derivatives by (*S*)-naproxen-derived CSPs 1 and 2.

Knowing that underivatized naproxen enantiomers are separable on the *N*-(3,5-dinitrobenzoyl)-β-amino acid-derived CSP,[16] chromatography of racemates of this general type on the (*S*)-naproxen-derived CSPs 1 and 2 shows (Table I) that the size of the alkyl substituent on the α-carbon dramatically influences enantioselectivity. Substitution of isopropyl or ethyl groups for the *tert*-butyl substituent of analyte **10** dramatically decreases the observed separation factor, α. For these analytes, the (*R*,*R*)-enantiomers are more strongly retained.

The enantiomers of a number of other π-acidic compounds were also separated on CSPs 1 and 2; chromatographic data relevant to the separation of *N*-(3,5-dinitrobenzoyl) derivatives of some chiral amines also appear in Table I. A difference in steric bulk between the two alkyl substituents on the stereogenic center is seen to be important for enantioselectivity. Analyte **13**, bearing *tert*-butyl and methyl substituents on the stereogenic center, resolves quite nicely on CSP 1. However, the enantiomers of **13** are not resolved on CSP 2. Interestingly, the enantiomers of **14**, the analyte bearing a phenyl on the stereogenic center, do not separate on CSP 1 but do show a small separation on CSP 2. Introduction of an α-naphthyl substituent on the stereogenic center (analyte **15**) leads to enantiomer separation on both CSPs. This last observation suggests that CSPs similar in structure to the 3,5-dinitrobenzamide of α-(1-naphthyl)ethylamine might separate the underivatized enantiomers of naproxen. Indeed, a previously described CSP[18] incorporating this general structural motif does marginally separate the enantiomers of naproxen ($\alpha = 1.18$).

Four possible diastereomeric adsorbates derived from an (*S*)-naproxen CSP and the two enantiomers of a generalized π-acidic derivative of a chiral amine are represented in Figure 2. Each of the component molecules is represented in conformations which are presumed to be of relatively low energy and hence preferentially populated.

(18) Pirkle, W. H.; Hyun, M. H. *J. Chromatogr.* **1987**, *393*, 357.

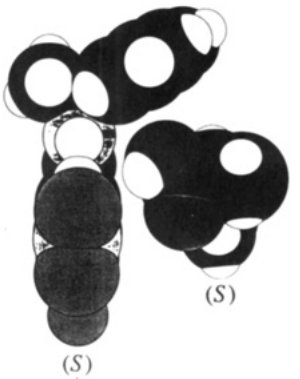


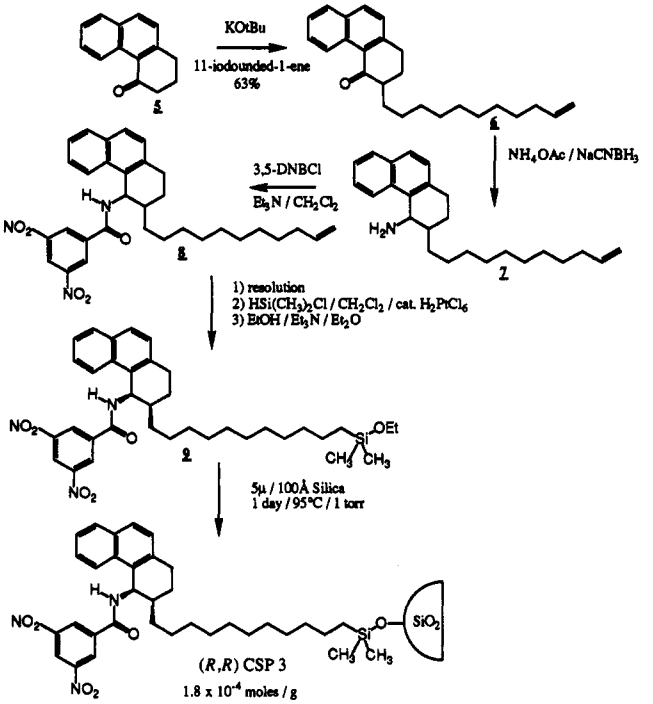
Figure 3. CPK molecular model representation of adsorbate c formed between (**S**)-**15** and (*S*)-naproxen in which the favorable geometry for simultaneous hydrogen bonding, face to face π–π interaction, and face to edge π–π interaction can be seen.

A solid circle at the stereogenic carbon represents a methine hydrogen directed toward the viewer, whereas an open circle represents a methine hydrogen receding from the viewer. π-basic and π-acidic aromatic rings are seen edge on and are represented as rectangles. The (+) and (–) signs represent hydrogen bond donating and accepting functionality, respectively. The two oxygens of the naproxen carboxylate system are represented as small (distal to the viewer) and large (proximal to the viewer) circles. A face to face π–π interaction between the naphthyl ring of naproxen and the dinitrobenzamide system of the analyte and a hydrogen bond between the amide hydrogen of the π-acidic derivative and the carbonyl oxygen of the CSP were expected to provide driving force for complex formation. It is difficult to clearly portray three-dimensional complexes in two-dimensional representations. Hence, the concerned reader is urged to use CPK molecular models to aid in following the subsequent rationale. Computer-generated space-filling molecular model representations of these adsorbates are included as supplementary material.

The predominant mode of adsorption for those analytes where the substituents on the stereogenic center function in a purely steric sense (e.g., analyte **13**) is believed to be as shown in adsorbate a, where the larger of these two steric groups is oriented away from the chiral selector. In the case of CSP 2, where no enantioselectivity was observed for analytes of this type, one must remember that the two oxygens of the carboxylate system are more or less equivalent. Adsorbates c and d depict the utilization of the second carboxyl oxygen as the site of hydrogen bonding. In the ester-linked CSP 1, these adsorbates were thought to contribute to a lesser extent than do adsorbates a and b, owing to the greater basicity of the carbonyl oxygen. If, in the case of ionic-linked CSP 2, the two oxygens behave as if they are nearly of the same basicity, then adsorbate c and d more nearly equal the contribution to analyte retention made by adsorbates a and b. The net result is little or no separation of the enantiomers of **13** on CSP 2.

The chromatographic data suggests that, for analytes bearing aromatic substituents on the stereogenic center, factors other than steric bulk differences are important. Examination of CPK space-filling models indicates that, in adsorbates such as b and c, the aromatic substituent projects toward the CSP in such a way as to make possible a face to edge π–π interaction between the naphthyl ring of the CSP and the aromatic substituent of the analyte. This interaction could provide additional stabilization of adsorbates b and c for analytes bearing aromatic substit-

Scheme II. Synthetic Route for Preparation of CSP 3



uents. Face to edge $\pi-\pi$ complexes are known from crystal structures of proteins,[19] peptides,[20] and small molecules[21] and have been suggested to be important in the binding of aromatic guests by paracyclophane hosts.[22] In addition, a growing body of chromatographic data from these laboratories can be rationalized in terms of face–edge $\pi-\pi$ interactions. Adsorbate c appears to have a geometry favorable for simultaneous face–face and face–edge $\pi-\pi$ interaction and also allows for the possibility that the methine hydrogen of the CSP may form a weak hydrogen bond to the aromatic ring of the analyte. This geometry can be visualized in the CPK molecular model depiction of adsorbate c formed between (*S*)-naproxen and (*S*)-**15** shown in Figure 3. In the case of ester-linked CSP 1, adsorbate c invokes hydrogen bonding at the alkoxyl oxygen, the *less* basic of the two ester oxygens. This is contrary to our a priori supposition and may not, in fact, occur. Rotation of the carboalkoxyl group by 180° may not be energetically very costly and would allow the hydrogen bond in adsorbate c to actually occur at the carbonyl oxygen. Thus, for analytes such as **15**, adsorbate similar to c (either oxygen being the hydrogen bond site) were thought to be the more stable and to lead to the chiral recognition noted on both CSPs 1 and 2.

On the basis of this chiral recognition rationale (the validity of which is presently being studied using spectroscopic techniques), it seemed likely that a selector which allows the usual hydrogen bonding and face to face $\pi-\pi$ stacking but which also allows a more optimal face to edge $\pi-\pi$ interaction would provide improved enantioselectivity. Thus, it was reasoned that compound **8** (Scheme II), in which a cyclohexyl ring (a) contains the stereogenic center bearing the dinitrobenzamide group, (b) controls the orientation of the naphthyl system, and (c) leads to a high degree of conformational rigidity, might offer improved enantioselectivity. It was also thought that the cis-linked alkyl chain would impede approach to the "wrong" face of the dinitrobenzoyl ring system, thus further improving enantioselectivity by reducing the contribution of adsorbate structures such as a and d.

Table II. Separation of the Enantiomers of Underivatized NSAIDs Using CSP 3[a]

compd	k'_1	k'_2	α
naproxen	3.96	8.95	2.25
ibuprofen	0.94	1.05	1.12
ketoprofen	4.53	5.03	1.11
flurbiprofen	1.63	1.94	1.19
pirprofen	2.53	3.49	1.38
fenoprofen	1.48	1.81	1.22
cicloprofen	3.03	5.18	1.71
carprofen	5.95	8.51	1.43
tiaprofenic acid	6.15	6.70	1.09

[a] Conditions: flow rate = 2.0 mL/min, mobile phase = 20% 2-propanol in hexane containing 1 g/L ammonium acetate; k'_1 = capacity factor for initially eluted enantiomer, k'_2 = capacity factor for second eluted enantiomer, α = separation factor.

Ketone **5**, previously described,[11] was alkylated with 11-iodoundecene and reductively aminated to afford a 5:1 mixture of the cis–trans isomers of amine, **7**. This mixture was converted to the 3,5-dinitrobenzamide derivatives, **8**. When chromatographed on the naproxen-derived CSPs 1 or 2, the cis diastereomer was found to show greater enantioselectivity than any analyte examined up to that time (CSP 1, $k'_1 = 6.21$, $\alpha = 1.61$; CSP 2, $k'_1 = 2.18$; $\alpha = 2.11$).

Accordingly, *cis*-**8** was chromatographically purified and resolved on a preparative column containing a CSP derived from (*S*)-*N*-(1-naphthyl)leucine.[8] The initially eluting (*R*,*R*)-enantiomer was hydrosilylated with dimethylchlorosilane using chloroplatinic acid as a catalyst. The resultant chloroorganosilane was converted to the ethoxyorganosilane and bonded to 5-μm spherical silica. The bonded phase, CSP 3, thus obtained was slurry packed into an HPLC column, endcapped, and chromatographically evaluated.

Data relevant to the separation on CSP 3 of not only the underivatized enantiomers of naproxen but also the underivatized enantiomers of several other α-aryl propionic acids are reported in Table II. Use of 2-propanol/hexane mobile phases affords separation of the enantiomers of

(19) Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23.
(20) Siemion, I. Z. *Z. Naturforsch. B* **1990**, *45*(9), 1324.
(21) Muehldorf, A. V.; Van Engen, D.; Warner, J. C., Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561.
(22) Ferguson, S. B.; Sanford, E. M.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410.

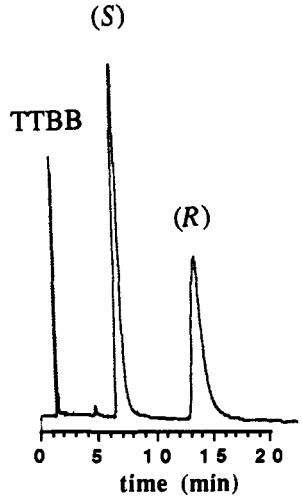


Figure 4. Chromatogram showing separation of the enantiomers of underivatized naproxen using CSP 3. Conditions: flow rate = 2.0 mL/min, mobile phase = 20% 2-propanol in hexane containing 1 g/L ammonium acetate, UV detection at 254 nm, spiked with 1,3,5-tri-*tert*-butylbenzene (TTBB) as void volume indicator.[9]

underivatized NSAIDs, although the peaks are somewhat broadened. Addition of a small amount of acetic acid narrows the peaks, affording complete separation of enantiomers. Modification of the mobile phase by addition of a small amount of ammonium acetate affords a still greater separation factor for naproxen although the separation factors for some of the other NSAIDs are reduced. Figure 4 shows a chromatogram of racemic naproxen obtained using CSP 3. The separation factor of 2.26 for the enantiomers of underivatized naproxen is greater than any yet reported for a synthetic naproxen selector.

CSP 3 should be of immediate utility to many workers. In addition to resolving naproxen, this new CSP separates the enantiomers of many other profens (Table II) and should be amenable to preparative as well as analytical applications. In as much as the aromatic portions of the profens are utilized as π-basic sites, one is not surprised that profens of lesser π-basicity show smaller separation factors. Even so, all of the profens examined to date have been baseline resolved using this CSP. We are still investigating the effect of mobile phase composition on separation of the various profen enantiomers on CSP 3, but can report that excellent separations are obtained in a variety of mobile phases including reversed phase systems such as 60% methanol in water containing a small amount of added acetic acid or ammonium acetate.

Conclusion

In summary, two (*S*)-naproxen-derived CSPs were utilized in the search for naproxen selectors of improved enantioselectivity. A number of candidates were found to display a usable level of enantioselectivity and several factors important for chiral recognition were identified. A mechanistic hypothesis concerning the enantioselective recognition of naproxen was formulated and used to design an improved naproxen selector. Synthesis and resolution of the selector and its incorporation into a silica bonded phase resulted in a CSP which displays the highest level of enantioselectivity toward naproxen yet reported for a synthetic receptor. Significantly, this CSP is capable of separating the enantiomers of a number of other α-arylpropionic acid NSAIDs.

The use of an immobilized target molecule to facilitate the chromatographic evaluation of selectors for that particular target results in a considerable savings in time (and cost) and is much more convenient than the conventional techniques heretofore used in the study of molecular recognition. Although in this example we have undertaken to optimize selectivity (specifically enantioselectivity), the method could also be used to screen for selectors of high affinity. Advantages of the method include the following: (i) only a very small amount of each candidate selector is required for analysis, this being recoverable, (ii) small amounts of impurity in the sample do not affect the outcome, (iii) the stationary phase used for the analysis can also be used for purification and/or resolution of the selector, (iv) analysis time is relatively short, and (v) variable-temperature chromatographic analysis allows the study of the thermodynamic parameters underlying the molecular recognition process.[23] Utilizing this method, selectors of very high affinity and enantioselectivity have been prepared. While high affinities and enantioselectivities are not required for chromatographic separations (and can actually be a hindrance), these properties are desirable in membrane transport[24] or batch adsorption processes. Pharmaceuticals, fermentation products, natural products, and environmental toxins are but a few of the possible targets which could be approached using this technique.

Acknowledgment. This work was supported by a grant from the National Science Foundation, a grant from Eli Lilly and Company, and a Department of Education Advanced Opportunities in Chemistry Graduate Fellowship to C.J.W.

Supplementary Material Available: Space-filling molecular model representations of diastereomeric adsorbates derived from analytes **13** and **15** and (*S*)-naproxen (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(23) Pirkle, W. H.; Welch, C. J. *J. Liq. Chromatogr.* **1991**, *14*, 2027. Pirkle, W. H. *J. Chromatogr.* **1991**, *558*, 1.
(24) Pirkle, W. H.; Doherty, E. M. *J. Am. Chem. Soc.* **1989**, *111*, 4113.