Journal of Chromatography, 641 (1993) 11–19
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 091

Chiral stationary phase design

Use of intercalative effects to enhance enantioselectivity

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(Received January 19th, 1993)

ABSTRACT

Two new chiral stationary phases derived from L-proline were designed specifically to separate the enantiomers of N-(3,5-dinitrobenzoyl) amino acid esters and amides and related analytes. The incorporation of structural features which diminish the retention of the first eluting enantiomers has led to the observation of separation factors as high as eighty for selected N-(3,5-dinitrobenzoyl)leucine amides using a normal mobile phase and as high as seven using a reversed mobile phase. The mechanistic rationale by which these chiral stationary phases were designed and the underlying reasons for the high levels of enantioselectivity are discussed.

INTRODUCTION

Owing to the increasingly common need to determine enantiomeric purity, the development of chiral stationary phases for the gas and liquid chromatographic separation of a wide range of enantiomers has been rapid (see, for example, ref. 1). Accompanying this development has been a growing understanding of the mechanisms by which enantioselectivity occurs. For the chemist, the challenge now lies in collecting observations and making deductions relevant to the improvement of existing chiral selectors and the development of new chiral selectors for targeted classes of molecules. Synthetic brush-type chiral phases are particularly suited to these activities.

For example, in endeavoring to design effective chiral stationary phases, one wishes to tailor the chiral stationary phase (CSP) so as to afford high affinity for one enantiomer while reducing affinity for the other. The latter, often more difficult than the former, basically requires that one restrict access of the least retained enantiomer to polar binding sites in the CSP. Polar functionality in the CSP which is not essential to the chiral recognition process is best eliminated, for it is deleterious to enantioselectivity [2]. Additionally, the quasi-membrane-like structure of brush-type phases can introduce subtle, second-order perturbations of the primary chiral recognition mechanism(s). These can often be used to advantage in increasing enantioselectivity. For instance, should one analyte enantiomer intercalate a portion of its structure (*e.g.* a long alkyl group) between the strands of bonded phase, enantioselectivity can be influenced dramatically [3]. Control of the conformational mobility of the CSP can also enhance chiral recognition if properly applied. Clearly, one wishes to populate a conformation which permits only the more retained enantiomer access to sites where bonding interactions occur. Mechanistic

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0021-9673/93/$06.00

insights such as these, combined with a growing understanding of chiral recognition processes, are beginning to enable us to design chiral phases specifically intended to separate the enantiomers of particular classes of substrates [4,5].

Various derivatives of proline have been used as the chiral sorbent in the gas chromatographic separation of enantiomers [6]. Likewise, proline and hydroxyproline have served as selectors in the separation of a number of bidentate-like enantiomers by ligand-exchange chromatography [7]. However, other attempts to utilize a proline-based chiral selector for the liquid chromatographic separation of enantiomers have met with but modest success [8–11].

We had reason to believe that the conformational rigidity of proline could be used to advantage in designing a chiral stationary phase capable of rendering high levels of enantioselectivity for specifiable analytes. We report herein two such chiral phases, discuss the implications of the design, and demonstrate the levels of enantioselectivity which can be realized when one applies those principles of CSP design which are thus far understood.

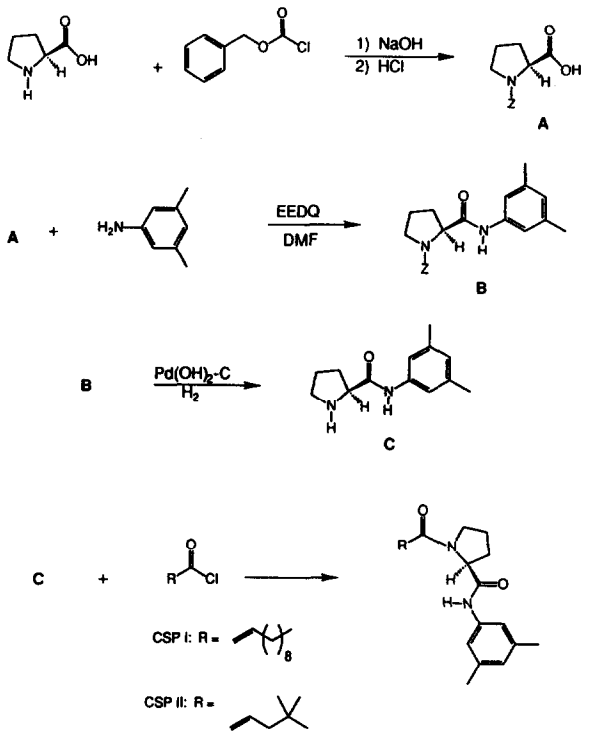


Fig. 1. Synthesis of olefinic precursors to CSPs I and II. EEDQ = 2-Ethoxy-1-ethoxy-carbonyl-1,2-dihydroquinoline; DMF = dimethylformamide.

EXPERIMENTAL

Chromatography

Chromatography was performed with an Aspec-Bischoff Model 2200 isocratic HPLC pump, a Rheodyne Model 7125 injector equipped with a 20-μl sample loop, a Milton-Roy LDC UV Monitor D fixed-wavelength detector operating at 254 nm, and a Hewlett-Packard 3394A recording integrator. A Euromark HPLC column oven was used to maintain a constant temperature of 20°C. Normal-phase void volumes were determined using 1,3,5-tri-*tert.*-butylbenzene, and reversed-phase void volumes were determined using NaI in methanol.

The olefinic precursors to CSPs I and II were prepared according to the scheme shown in Fig. 1.

N-Benzyloxycarbonyl-L-proline (A)

In a 250-ml flask equipped with a magnetic stir bar 11.5 g (0.099 mol) of L-proline was dissolved in 50 ml of 2 *M* NaOH. The mixture was cooled to 0°C and 16 ml (0.105 mol) of benzyl chloroformate and 50 ml of 2N NaOH were added in portions with stirring over the course of 1 h. When the additions were complete, the reaction mixture was stirred for an additional 30 min at 0°C, and then allowed to warm to room temperature and stirred for another 30 min. The alkaline reaction mixture was extracted twice with 75-ml portions of diethyl ether, and the ether extracts discarded. The aqueous layer was acidified to Congo blue with 6 *M* HCl, and extracted three times with diethyl ether. The ether extracts were combined, dried with $MgSO_4$, and concentrated under reduced pressure to give 23.4 g of a colorless solid, yield 95%, m.p. 75–76°C. ^{1}H NMR (C^2HCl_3) 200 MHz δ 7.30 (s, 5H); 5.10 (s, 2H); 4.40 (m, 1H); 3.50 (m, 2H); 2.30–1.80 (m, 4H). IR (mull) cm^{-1} 2980, 2886, 1741, 1358, 1176, 1122, 1089.

N-(Benzyloxycarbonyl)-L-proline-3,5-dimethylanilide (B)

N-Benzyloxycarbonyl-L-proline, 6.91 g (0.027 mol), 7.34 g (0.030 mol) of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), and 50 ml of dry dimethylformamide were placed in a 100-ml flask equipped with a magnetic stir bar. The mixture was stirred until homogeneous, and then 3.70 g (0.030 mol) of freshly distilled 3,5-dimethylaniline was added. The reaction mixture was stirred overnight, and the contents of the flask were poured into a 250-ml separatory funnel containing 75 ml of ethyl acetate. A 75-ml volume of water was added to the separatory funnel and the layers were separated. The organic layer was washed sequentially with two more 75-ml portions of water, two 50-ml portions of 2 *M* HCl, and two 50-ml portions of 5% $NaHCO_3$. The ethyl acetate layer was dried with $MgSO_4$, filtered, concentrated under reduced pressure, and dried *in vacuo* to give 8.08 g of a white solid, 85% yield, m.p. 138–140°C. ^1H NMR (C^2HCl_3) 200 MHz δ 9.00 (br s, 1H), 7.40 (br s, 5H), 7.10 (s, 2H); 6.80 (s, 1 H), 5.20 (s, 2H), 4.50 (m, 1H), 3.50 (m, 2H), 2.60–2.40 (m, 2H); 2.30 (s, 6H), 1.90 (m, 2H). Analysis for $C_{21}H_{24}O_3N_2$: calculated C 71.57; H 6.86; N 7.95; found C 71.13; H 6.90; N 7.84.

L-Proline-3,5-dimethylanilide hydrochloride (C)

N-(Benzyloxycarbonyl)-L-proline-3,5-dimethylanilide, 6.0 g (0.017 mol), was dissolved with gentle heating in 50 ml dry ethanol, placed in a heavy walled pressure bottle, and 600 mg of 20% $Pd(OH)_2$ on carbon (Pearlman's catalyst) was added along with 2 drops of glacial acetic acid. The pressure bottle was installed in a Parr hydrogenator, flushed with nitrogen, and rocked under 20 p.s.i. (1 p.s.i. = 6894.76 Pa) of hydrogen for 12 h. After this time, the pressure bottle was removed from the apparatus, and the contents of the bottle filtered through Celite. The filtrate was concentrated under reduced pressure to give 3.71 g of a pasty white solid (100% yield). The residue was dissolved in dry dichloromethane and converted to the hydrochloride salt with gaseous HCl. m.p. 245°C (decomp). ^1H NMR ([$^2H_6$]dimethyl sulfoxide) 200 MHz δ 10.90 (s, 1H); 7.40 (s, 2H); 6.80 (s, 1H); 4.50 (t, 1H); 3.50 (s, 2H); 3.30 (m, 2H; 2.50 (m, 2H); 2.30 (s, 6H); 200 (br s, 2H). Analysis for $C_{13}H_{19}O_2N_2Cl$: calculated: C 61.29; H 7.52; N 11.00; found: C 61.04; H 7.48; N 11.07.

2,2-Dimethyl-4-pentenoic acid

2,2-Dimethyl-4-pentenoic acid was prepared by oxidation of the corresponding aldehyde [12] using the following variation of a reported procedure [13]. Silver(II) oxide, 10.32 g (0.045 mol), was placed in a 250-ml flask equipped with a magnetic stir bar and covered with 75 ml of water. Sodium hydroxide, 8.91 g (0.223 mol), was added and the reaction mixture heated to 60°C. 2,2-Dimethyl-4-pentenal, 5.0 g (0.045 mol), was added and the reaction mixture was stirred for 15 min at this temperature. Metallic silver forms and coats the walls of the reaction vessel. The mixture was then immediately filtered and the filter cake washed with two 25-ml portions of hot water. The filtrate was acidified with concentrated HCl and extracted 3 times with 75-ml portions of diethyl ether. The ether extracts were combined, dried with $MgSO_4$, concentrated, and dried *in vacuo* to give 4.30 g of a pale yellow oil, yield 75%. ^1H NMR (C^2HCl_3) 200 MHz δ 12.00 (br s, 1H); 5.80 (m, 1H); 5.10 (dd, 2H); 2.30 (d, 2H); 1.10 (s, 6H). IR (neat) cm^{-1} 2926, 1716, 1684, 1651, 1448, 1379, 1329, 1269, 908.

2,2-Dimethyl-4-pentenoyl chloride

2,2-Dimethyl-4-pentenoic acid, 4.0 g (0.031 mol), was dissolved in 20 ml dry dichloromethane and placed in a 50-ml round-bottom flask equipped with a magnetic stir bar. Oxalyl chloride, 5.9 g (0.047 mol) was added carefully, and the mixture was stirred under nitrogen for 40 min, after which time all gas evolution had ceased. The reaction mixture was then concentrated under reduced pressure to afford a yellow oil, 4.60 g, (quantitative yield). This material was used directly, without further purification.

N-(10-Undecenoyl)-L-proline-3,5-dimethylanilide

L-Proline-3,5-dimethylanilide, 1.98 g (0.009 mol), and 1.25 ml (0.009 mol) of triethylamine were dissolved in dry tetrahydrofuran in a 100-ml

flask equipped with a magnetic stir bar and cooled to 0°C. 2.02 g (0.010 ml) of 10-undecenoyl chloride was added in portions over 15 min. When the additions were complete, the reaction mixture was allowed to warm to room temperature and was stirred under nitrogen for 2 h. After this time, the triethylamine hydrochloride precipitate was removed by filtration, and the reaction mixture was concentrated to dryness on a rotary evaporator. The residue was taken up in 75 ml of diethyl ether, and washed with successive 50-ml portions of 5% $NaHCO_3$ and 2 *M* HCl. The organic layer was dried with $MgSO_4$, concentrated, and dried *in vacuo* to yield 3.40 g of a pale yellow syrup (98% yield). ^1H NMR (C^2HCl_3) 200 MHz δ 9.60 (br s, 1H); 7.15 (s, 2H); 6.70 (s, 1H); 5.80 (m, 1H); 4.95 (m, 2H); 4.80 (d, 1H); 3.50 (m, 2H); 2.30 (m, 2H); 2.20–1.60 (messy, 6H); 2.25 (s, 6H); 1.30 (br s, 14H). Analysis for $C_{24}H_{36}N_2O_2$: calculated: C 74.96; H 9.44; N 7.28; found: C 74.97; H 9.46; N 7.29.

N-(2,2-Dimethyl-4-pentenoyl)-L-proline-3,5-dimethylanilide

L-Proline-3,5-dimethylanilide hydrochloride, 1.85 g (0.0073 mol), was placed in a 100-ml flask, and 30 ml of 5% NaOH and 30 ml of dichloromethane was added. The biphasic reaction mixture was stirred vigorously to dissolve the amine hydrochloride. 2,2-Dimethyl-4-pentenoyl chloride, 3.20 g (0.022 mol), was added in portions to the dichloromethane layer. The reaction mixture was stirred vigorously for 1 h. After this time, the layers were separated, the organic layer was washed with two 25-ml portions of 2 *M* HCl, dried with $MgSO_4$, and concentrated under reduced pressure to give 2.00 g of a white solid, yield 84%. The product was purified by flash chromatography using a mobile phase consisting of an ethyl acetate–hexane (1:4) mixture to elute early running impurities, and then dichloromethane to elute the product. Yield after chromatography 1.5 g, 63%. m.p. 128–130°C. ^1H NMR (C^2HCl_3) 200 MHz δ 9.20 (br s, 1H); 7.10 (s, 2H); 6.70 (s, 1H); 5.60–5.90 (m, 1H); 5.00 (m, 2H); 4.80 (dd, 1H); 3.70 (m, 2H); 2.40 (d, 2H); 2.30 (s, 6H); 2.20–1.70 (m, 4H); 1.30 (s, 6H). Analysis for $C_{20}H_{28}O_2N_2$: calculated: C 73.14; H 8.59; N 8.53; found: C 73.25; H 8.52; N 8.26.

N-[11-(Dimethylethoxysilyl)undecanoyl]-L-proline-3,5-dimethylanilide

An oven-dried 100-ml flask equipped with a stir bar, condenser and a nitrogen inlet was charged with 5 ml of $(CH_3)_2SiHCl$, 2.9 g of N-(10-undecenoyl)-L-proline-3,5-dimethylanilide dissolved in 10 ml of dry dichloromethane, and about 30 mg of H_2PtCl_6. The mixture was refluxed for 6 h and then concentrated to dryness under reduced pressure. Residual $(CH_3)_2SiHCl$ was chased with two 25-ml portions of dry dichloromethane. The resulting dark oil was taken up in 30 ml of anhydrous diethyl ether and treated with 10 ml of triethylamine–absolute ethanol (1:1). This mixture was heated to reflux for 10 min, filtered to removed the precipitated triethylamine hydrochloride, the filter cake was washed with two 15-ml portions of diethyl ether, and the filtrate and washings were combined and concentrated under reduced pressure. The residue was chromatographed on silica using 1.5% methanol in dichloromethane. The product was isolated as a pale yellow oil, 1.67 g (47% yield). ^1H NMR (C^2HCl_3) 200 MHz δ 9.2 (br s, 1H); 7.15 (s, 2H); 6.70 (s, 1H); 4.80 (d, 1H); 3.65 (q, 2H); 3.50 (m, 2H); 2.30 (m, 2H); 2.20–1.60 (m, 4H); 2.25 (s, 6H); 1.30 (br s, 16H); 1.00 (t, 3H); 0.50 (t, 2H); 0.00 (s, 6H).

Preparation of CSP I

The purified silane was dissolved in dichloromethane and added to a flask containing 5.0 g of 5 μm, 100 Å Rexchrome silica (Regis, Morton Grove, IL, USA) which had been azeotropically dried with benzene. The slurry was sonicated for several minutes to insure complete coverage of the silica gel with the silane and the solvent was removed under reduced pressure. This procedure was repeated twice, with 1 ml of dimethylformamide being added the last time. The silica and the silane were then heated at 100°C under reduced pressure (1.0 mm Hg = 133.322 Pa) in a Kugelrohr apparatus for 24 h. After cooling, the modified silica gel was washed with several 50-ml portions of methanol and slurry packed into a 250 × 4.6 mm I.D. stainless-steel HPLC column

using methanol. After washing with 100 ml of dichloromethane, CSP I was endcapped using 2 ml of hexamethyldisilazane in 50 ml of dichloromethane. Elemental analysis of residual CSP from the packing procedure showed a loading of 0.22 mmol/g based on C and 0.21 mmol/g based on N.

N-[5-(Dimethylethoxysilyl)-2,2-dimethylpentanoyl]-L-proline-3,5-dimethylanilide

N-[5-(Dimethylethoxysilyl)-2,2-dimethylpentanoyl]-L-proline-3,5-dimethylanilide was prepared using a procedure similar to that described for N - [11 - (dimethylethoxysilyl)undecanoyl] - L - proline-3,5-dimethylanilide. After chromatography, 1.5 g (52% yield) of a pale yellow oil was obtained. ^{1}H NMR (C^2HCl_3) 200 MHz δ 9.20 (br s, 1H); 7.10 (s, 2H); 6.70 (s, 1H); 4.80 (dd, 1H); 3.70 (m, 2H); 3.60 (q, 2H); 2.30 (s, 6H); 2.00–1.40 (m, 6H); 1.30 (s, 6H); 1.10 (t, 3H); 0.50 (m, 2H); 0.00 (s, 6H).

Preparation of CSP II

CSP II was prepared using a procedure similar to that described for the preparation of CSP I. Elemental analysis of residual CSP from the packing procedure showed a loading of 0.23 mmol/g based on C and 0.20 mmol/g based on N.

The analytes

All analytes reported herein were available from previous experiments conducted in these laboratories. The synthesis and characterization of these analytes is reported elsewhere [14,15].

RESULTS AND DISCUSSION

From prior studies in these laboratories, we were aware that the enantiomers of N-protected amino acid anilides can be separated on several π-acidic CSPs [16]. Keeping the aforementioned considerations in mind, Corey Pauling Koltung (CPK) space filling molecular models (Harvard Apparatus, South Natick, MA, USA) were used to aid in the design of a (*S*)-proline-derived chiral stationary phase intended to retain the (*S*)-enantiomers of N-(3,5-dinitrobenzoyl)amino

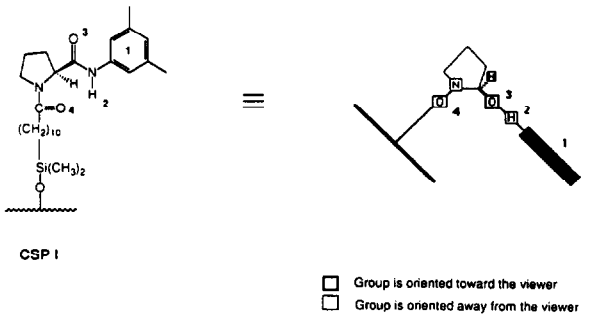


Fig. 2. CSP I and a cartoon-like representation of CSP I.

acids (and their ester and amide derivatives). The structure of this CSP is shown in Fig. 2, along with a cartoon-like representation intended to simplify aspects of the chiral recognition mechanism to be subsequently discussed. The analytes to be discussed are represented using the convention shown in Fig. 3.

The conventions introduced in Figs. 2 and 3 are used in Fig. 4 in an attempt to convey, in two dimensions, our view of the three-dimensional structure of the more stable 1:1 diastereomeric adsorbate expected to result from interaction of the N-(10-undecenoyl)-L-proline-3,5-dimethylanilide-derived CSP with esters or amides of (*S*)-N-(3,5-dinitrobenzoyl)leucine. The important bonding interactions are shown using a double-headed arrow.

The essential bonding interactions, as shown in Fig. 4, were expected to be a face-to-face $\pi-\pi$ interaction between the 3,5-dinitrobenzoyl group and the 3,5-dimethylanilide moiety, a hydrogen bond between the relatively acidic 3,5-dinitrobenzamide proton and the carbonyl oxygen in the CSP's tether to silica, and a hydrogen bond between the 3,5-dimethylanilide amide proton

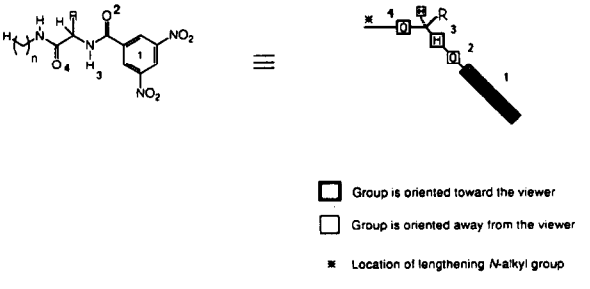


Fig. 3. Analytes and convention used to represent analytes in Figs. 4 and 5.

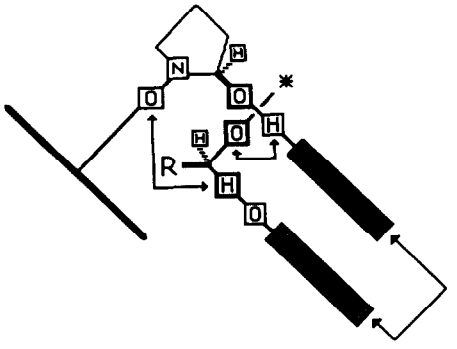


Fig. 4. Representation of the more stable diastereomeric adsorbate expected to form between CSP I and amides of N-(3,5-dinitrobenzoyl) amino acids.

and the carbonyl oxygen in the analyte C-terminal ester or amide. Although conceptually similar CSPs could be derived from other amino acids, proline was selected since, as a secondary amine, nitrogen acylation leaves no extraneous amide N–H. This amide hydrogen is not considered essential to the retention of the more retained enantiomer and its omission was expected to reduce the extent of achiral retention and thus improve enantioselectivity. The conformation imposed by the 5-member ring and the planarity of the 3,5-dimethylanilide system was expected to impart strong affinity for the (*S*)-enantiomers of the target analytes, while the proline ring itself was expected to discourage approach of the analytes' (*R*)-enantiomer from the "backside" of the CSP. By further reducing retention of the (*R*)-enantiomer, enantioselectivity might be enhanced.

A final inference drawn from the models is noteworthy. In the complex containing the more retained enantiomer, the alkoxyl group of the C-terminal ester (or the alkyl group(s) of a C-terminal amide) is directed away from the tether and the silica support. Should the least retained enantiomer bind to the CSP in a similar fashion, so as to enjoy the face-to-face $\pi-\pi$ interaction and the hydrogen bond from the 3,5-dinitrobenzamide N–H to the carbonyl oxygen in the tethering arm of the CSP, its alkoxyl group (if an ester) or its N-alkyl group(s) (if an amide) would then be directed between the adjacent strands of bonded phase and toward the silica support. In a non-polar mobile phase, any alkyl group so directed should exert a destabilizing effect on the complex. This intercalative interaction with the neighboring strands of bonded phase and with the underlying silica support was expected to selectively reduce the retention of the less retained enantiomer, the effect increasing with an increase in the length of the alkoxyl or alkyl group. A representation of this arrangement is shown in Fig. 5.

Chromatographic data for the normal-phase separation of the enantiomers of amides of N-(3,5-dinitrobenzoyl)leucine on CSP I are shown in Table I.

As can be seen, the enantioselectivities encountered are substantial and increase as the length of the N-alkyl amide is increased incrementally, particularly in a dichloromethane mobile phase. This suggests that an intercalative hypothesis is valid. Little retention is observed for the initially eluted enantiomer, this being a major factor in the high levels of enantioselectivity noted for these analytes.

From the foregoing mechanistic rationale, it was expected that shortening the tether which connects the selector to silica would afford still higher levels of enantioselectivity since this would exacerbate the intercalation difficulty encountered by the least retained enantiomers. Adding geminal dimethyl groups on the α carbon of the tether was expected to further compound the difficulty of intercalation. The structure of this modified CSP, II, is shown in Fig. 6.

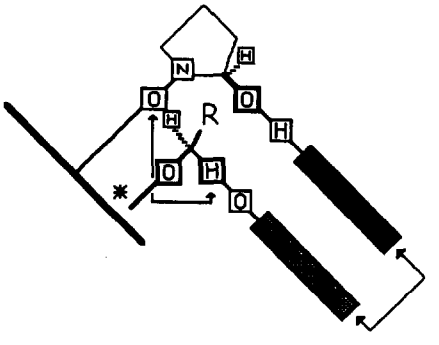
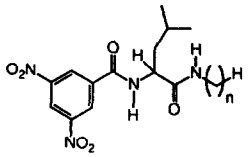


Fig. 5. Representation of the less stable diastereomeric adsorbate expected to form between CSP I and amides of N-(3,5-dinitrobenzoyl) amino acids.

TABLE I

NORMAL-PHASE SEPARATION OF THE ENANTIOMERS OF N-(3,5-DINITROBENZOYL)LEUCINE AMIDES ON CSP I



k_1' = Capacity factor for the first eluting enantiomer; α = chromatographic separation factor; flow-rate 2 ml/min.

n	2-Propanol–hexane (15:85, v/v)		100% Dichloromethane	
	k_1'	α	k_1'	α
1	1.73	18.4	1.26	16.8
2	1.39	23.7	0.88	21.1
3	1.28	26.6	0.70	26.6
6	1.04	26.5	0.53	28.3
10	0.85	26.8	0.49	26.5
14	0.78	25.2	0.36	30.41
18	0.62	28.2	0.31	31.0

When CSP II was prepared and evaluated, it was found to afford greater enantioselectivities than CSP I for all analytes examined. Normal-phase separation factors range from 27 to 50 for the enantiomers of the homologous series of N-(3,5-dinitrobenzoyl)leucine amides shown in Table I. As the analytes' C-terminal amide N–H is not invoked as essential to the retention of the more retained enantiomer, amides derived from secondary amines or bulky primary amines (which are thought to sterically restrict access to the amide N–H) were expected to show even higher levels of enantioselectivity. This is the case, as the data in Table II attest.

Data from the normal- and reversed-phase chromatographic separation of the enantiomers of a homologous series of N-(3,5-dinitrobenzoyl)-α-amino-alkyl phosphonates are presented in Table III.

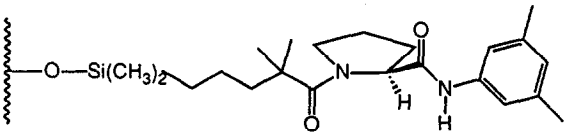
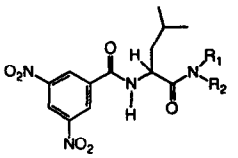


Fig. 6. Structure of CSP II.

TABLE II

NORMAL-PHASE SEPARATION OF THE ENANTIOMERS OF N-(3,5-DINITROBENZOYL)LEUCINE AMIDES ON CSP II



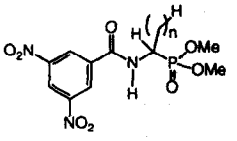
Mobile phase consisting of 20% (v/v) 2-propanol–hexane (20:80, v/v); k_1' = capacity factor for the first eluting enantiomer; α = chromatographic separation factor; flow-rate 2 ml/min.

R_1	R_2	k_1'	α
CH_3	CH_3	0.61	30.4
C_8H_{17}	C_8H_{17}	0.16	48.7
H	Adamantyl	0.32	64.4
H	*tert.*-Butyl	0.22	86.3

From the chiral recognition process shown in Fig. 3, the alkyl group on the stereogenic center of the more retained enantiomer of an N-(3,5-dinitrobenzoyl)-α-amino-alkylphosphonate was

TABLE III

NORMAL- AND REVERSED-PHASE SEPARATION OF THE ENANTIOMERS OF N-(3,5-DINITROBENZOYL)-α-AMINO-ALKYLPHOSPHONATE ESTERS ON CSP I



k_1' = Capacity factor for the first eluting enantiomer; α = chromatographic separation factor; flow-rate 2 ml/min.

n	Normal phase, 2-propanol–hexane (20:80)		Reversed phase, water–methanol (20:80)	
	k_1'	α	k_1'	α
1	3.07	4.57	1.09	1.52
2	2.84	4.31	1.26	1.60
3	2.61	4.31	1.60	1.63
4	2.44	4.31	1.83	1.77
5	2.22	4.35	2.11	1.86
6	2.04	4.28	2.59	1.92
7	1.92	4.15	3.19	1.98
9	1.60	3.95	4.98	2.06

expected to be intercalated between the strands of bonded phase. Should this group be long enough to experience intercalation difficulties under normal phase conditions, enantioselectivity seemed likely to be reduced as a consequence of reduced retention of the more retained enantiomer relative to its non-intercalating antipode. Were a reversed mobile phase used, this trend might be reversed, owing to a hydrophobic incentive for intercalation of alkyl groups between strands of bonded phase. As documented in Table III, these trends are observed. Such trends provide compelling support for the intercalation hypothesis.

CONCLUSIONS

The design, synthesis and chromatographic evaluation of two chiral stationary phases derived from (*S*)-proline has been described. These chiral stationary phases have been used in an ongoing investigation into the effect of intercalative processes on chiral recognition. A mechanistic rationale was used for the *a priori* design of these chiral stationary phases, both of which show unusually high levels of enantioselectivity for the enantiomers of the targeted class of analytes. The elution order of the analyte enantiomers is as expected from the rationale.

While CSPs which afford high levels of enantioselectivity are unnecessary and even undesirable for most analytical determinations of enantiomeric purity, one can utilize a CSP of intentionally lowered enantiomeric purity to reduce run times, perform the chromatography at elevated temperatures, or use a suboptimal mobile phase to reduce enantioselectivity and run time. There are, however, several advantages in having access to high levels of enantioselectivity. A CSP capable of high enantioselectivity will often resolve the enantiomers of compounds which, when chromatographed on CSPs of lesser ability, are either inseparable or of marginal separability. Moreover, the proline-derived chiral phases described herein are not restricted to the separation of the enantiomers of α-amino acid derivatives but suffice to separate the enantiomers of the 3,5-dinitrobenzamides of various chiral amines and the 3,5-dinitrophenyl carbamates of many chiral alcohols. High levels of enantioselectivity are desirable for preparative applications and are essential to new process scale technology using hollow-fiber membranes [17,18]. Finally, high levels of enantioselectivity permit the design of chromatographic and spectroscopic experiments, the results of which can yield unambiguous information concerning the nature of the chiral recognition processes involved. Such information can be instrumental in the design of even better chiral selectors.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the NSF, Eli Lilly and Company, and Glaxo Research Laboratories. Solvents employed for the chromatography were generously supplied by EM Science, a Division of EM Merck. The Euromark HPLC column oven was generously supplied by Euromark Industries in Melrose Park, IL, USA.

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