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Structural optimization of a chiral selector for use in preparative enantioselective chromatography

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Abstract

Utilizing the immobilized-target strategy, the structure of a proline-derived chiral stationary phase was optimized for use in the preparative chromatographic separation of the enantiomers of two chiral selectors used in commercial chiral stationary phases. In this study, various *N*-acylated proline anilides were prepared and chromatographed on the commercial Pirkle-1J and α-Burke 2 chiral stationary phases. The analyte which displayed the greatest retention without sacrifice of enantioselectivity (the 3,5-dimethoxyanilide of *N*-undecenoyl proline) was chosen for incorporation into the preparative chiral stationary phase. Once prepared, this phase shows increased analyte retention and enantioselectivity comparable to that of earlier phases derived from 3,5-dimethyl anilides of proline. The increased retention allows one to use mobile phases in which the target analytes are more soluble, hence greatly facilitating an increase in the through-put of a column of a given size.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Preparative chromatography; *N*-Acetylproline anilides

1. Introduction

For a number of years, we have been interested in the design and development of low-molecular mass-chiral selectors for use in enantioselective chromatography [1,2]. Through systematic studies of selector–analyte interactions, we have been able to develop a number of successful chiral stationary phases (CSPs), many of which are now commercially available. Our general approach toward selector design has been to optimize the enantiodifferentiation of the selector toward a certain target analyte or class of analytes. For analytical purposes, the retention of the analyte can be adjusted through mobile phase composition so as to minimize the time required for analysis. It is even acceptable to use a mobile phase in which the analyte is but sparingly soluble, since so little material is needed for analysis. However, when performing a preparative resolution, substantial substrate solubility in the mobile phase is highly desirable. For given chromatographic parameters (retention factors, enantioselectivity, band shape considerations) the through-put on a given chiral column is also affected by the solubility of the substrate in the mobile phase. In this paper, we report our efforts toward optimizing one of our recently developed chiral selectors for preparative separations.

The analytes of interest, **1** and **2**, are the olefinic precursors of CSPs developed in this laboratory [3,4]. Because these racemates are resolved chro-

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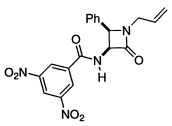
matographically in the production of the commercial versions of these chiral columns, a CSP tailored to the needs for preparative resolution of **1** and **2** was desired. Both analytes are well resolved on a recently reported proline-derived CSP using hexane–2-propanol mobile phases [5–8]. However, both racemates are poorly soluble in these mobile phases. Our goal was to optimize the structure of the chiral selector to produce a CSP which would produce comparable retention and separation of the test analytes, but in a mobile phase in which these racemates are much more soluble so as to allow a larger amount of material to be loaded on the column per run, hence increasing through-put.



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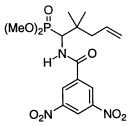

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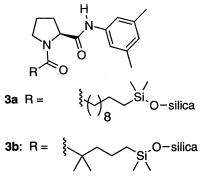
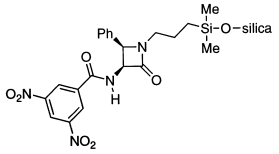


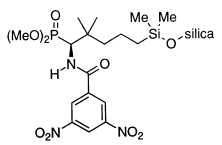
2. Experimental

2.1. General experimental

Analytes **1** and **2**, were obtained from Regis Technologies (Morton Grove, IL, USA). (*S*)-CSP **3a** and (*S*)-CSP **3b** packed in 250×4.6 mm stainless steel high-performance liquid chromatography (HPLC) columns were available from previous studies. Analytes **4**, **5**, **6** and **7** were prepared as outlined in Scheme 1. (3*R*,4*S*)-CSP **8** and (*S*)-CSP **9** packed in 250×4.6 mm stainless steel HPLC columns were obtained from Regis Technologies under the trade names, Pirkle-1J and α-Burke 2, respectively. (*S*)-CSP **13** was prepared as outlined in Scheme 2. Reaction solvents were purified as follows: methylene chloride was distilled from calcium hydride; tetrahydrofuran (THF) was distilled from sodium/benzophenone. Chromatography was carried out at ambient temperature, unless otherwise noted, with the mobile phase composition and flow-rate indicated in the appropriate table. Tri-*tert*.-butylbenzene was used as the void volume marker.



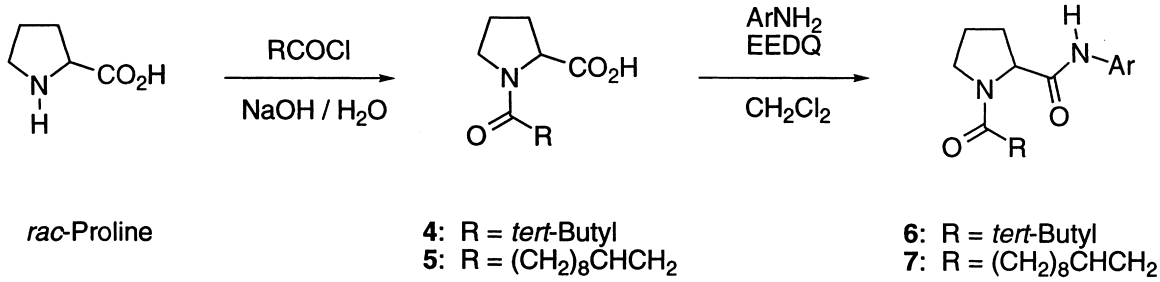
CSP 8



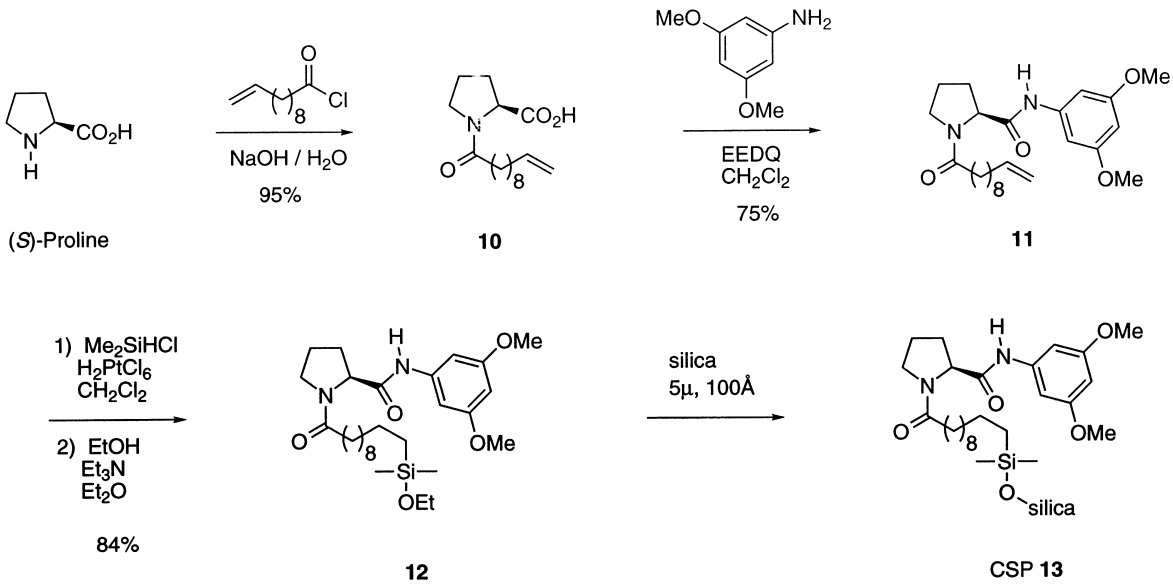
CSP 9

2.2. Preparation of racemic N-pivaloyl proline (*4*)

To a solution of racemic proline (1.15 g, 10.0 mmol) in 20 ml of a 2 *M* NaOH solution cooled in an ice-water bath was added pivaloyl chloride (1.23 ml, 10.0 mmol). After 30 min, the reaction mixture was allowed to warm to room temperature and stir for an additional 2 h. Ether (50 ml) was then added, the layers were separated and the ether layer was discarded. The aqueous layer was acidified to pH 1 with concentrated hydrochloric acid and extracted with methylene chloride (2×50 ml). The combined extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo. Recrystallization of the remaining solid from hexane–methylene chloride yielded 985 mg of pure **4**. Removal of the solvent from the mother liquor followed by recrystallization yielded an additional 382 mg of 4 (68.6% total yield). melting point (m.p.): 143–144°C. ^{1}H



Scheme 1.



Scheme 2.

nuclear magnetic resonance (NMR) ($CDCl_3$, 500 MHz) 9.6–10.4 (br s, 1H), 4.56 (dd, $J=7.8$, 4.1, 1H), 3.72 (m, 2H), 2.17 (m, 1H), 1.94–2.12 (m, 3H), 1.27 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz) 178.8, 175.3, 61.5, 48.4, 39.0, 27.2, 26.9, 25.9. Anal. calculated for: C, 60.28; H, 8.60; N, 7.03; found: C, 60.29; N, 6.99; H, 8.61.

*2.3. Preparation of anilides of pivaloyl proline (**6**)*

To a solution of **4** (20 mg, 0.10 mmol) dissolved in methylene chloride (0.5 ml) was added EEDQ (25 mg, 0.10 mmol) followed by 0.10 mmol of the appropriate aromatic amine. Each solution was allowed to stand overnight, then was diluted with ethyl acetate (10 ml), washed with 2 *M* HCl (2×10 ml), and finally washed with a saturated $NaHCO_3$ solution (2×10 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo. Each sample was then used for chromatography without any further purification.

*2.4. Preparation of racemic N-undecenoyl proline (**5**)*

To a solution of racemic proline (576 mg, 5.0 mmol) in 10 ml of a 2 *M* NaOH solution while cooled in an ice-water bath was added undecenoyl chloride (1.07 ml, 5.0 mmol). After 30 min, the reaction mixture was allowed to warm to room temperature and was then stirred for an additional 2 h. Ether (25 ml) was then added, the layers were separated and the ether layer was discarded. The aqueous layer was acidified to pH 1 with concentrated hydrochloric acid and extracted with methylene chloride (2×25 ml). The combined extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo to yield 1.31 g of **6** as a white solid. ^1H NMR ($CDCl_3$, 500 MHz) 5.79 (m, 1H), 4.95 (m, 2H), 4.35 (m, 1H), 3.62 (m, 1H), 3.37 (m, 1H), 1.7–2.4 (m, 9H), 1.5–1.6 (m, 2H), 1.2–1.4 (m, 10H). ^{13}C NMR ($CDCl_3$, 125 MHz) 180.2, 173.2, 139.1, 114.2, 61.3, 47.7, 34.7, 33.8, 29.7, 29.5, 29.5, 29.4, 29.1, 29.0, 28.9, 24.6. High-resolution mass spectrometry (HRMS) calculated for $C_{16}H_{27}NO_3$, 281.1991; found, 281.1991.

*2.5. Preparation of anilides of undecenoyl proline (**7**)*

To a solution of **6** (28 mg, 0.10 mmol) dissolved in methylene chloride (0.5 ml) was added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (25 mg, 0.10 mmol) followed by 0.10 mmol of the

appropriate aromatic amine. Each solution was allowed to stand overnight, then was diluted with ethyl acetate (10 ml), washed with 2 *M* HCl (2×10 ml), and finally washed with a saturated $NaHCO_3$ solution (2×10 ml). The organic layer was then dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo. Each sample was then used for chromatography without any further purification.

*2.6. Preparation of (S)-N-undecenoyl proline (**10**)*

(*S*)-Proline (13.82 g, 120 mmol) was dissolved in 200 ml of 2 *M* NaOH and the solution was cooled in an ice-water bath. Undecenoyl chloride (21.48 ml, 100 mmol) was then added dropwise, keeping the temperature of the reaction mixture to below 8°C (internal thermometer). After addition was complete, the reaction was kept for 1 h, then allowed to warm to room temperature and stirred for an additional hour. The reaction mixture was poured into a separatory funnel containing 200 ml ether, the layers were separated and the ether layer discarded. The aqueous layer was acidified to pH 1 with concentrated hydrochloric acid and extracted with methylene chloride (2×200 ml). The extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo to yield 26.8g of **10** as a clear oil (95.2% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) 9.2–9.8 (br s, 1H), 5.80 (ddt, J=16.8, 10.3, 6.7, 1H), 4.95 (m, 2H), 4.60 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 2.41 (m, 1H), 2.37 (m, 2H), 2.01 (m, 5H), 1.63 (m, 2H), 1.2–1.4 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz) 175.4, 172.5, 139.1, 114.1, 59.8, 47.8, 34.4, 33.7, 29.3, 29.2, 29.2, 29.0, 28.8, 27.4, 24.7, 24.5. HRMS calculated for $C_{16}H_{27}NO_3$, 281.1991; found, 281.1986.

*2.7. Preparation of (S)-N-undecenoyl proline 3,5-dimethoxyanilide (**11**)*

To a solution of **10** (2.81 g, 10 mmol) in methylene chloride (20 ml) was added EEDQ (2.72 g, 11 mmol) followed by 3,5-dimethoxyaniline (1.53 g, 11 mmol). After 12 h, the reaction was concentrated to ca. 5 ml on a rotary evaporator, then diluted with ethyl acetate (50 ml), washed with 2 *M* HCl (2×50 ml), and finally washed with saturated $NaHCO_3$ (2×50 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo. Flash chromatography, eluting with ether–hexane (3:1, v/v), yielded 3.12 g of **11**, as a light yellow oil (74.8% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) 9.78 (s, 1H), 6.75 (d, J=2.2, 2H), 6.16 (t, J=2.2, 1H), 5.79 (ddt, J=16.5, 10.2, 6.7, 1H), 4.9–5.0 (m, 2H), 3.74 (s, 6H), 3.4–3.6 (m, 2H), 2.54 (m, 1H), 2.34 (m, 2H), 2.16 (m, 1H), 2.01 (m, 3H), 1.81 (m, 1H), 1.66 (m, 2H), 1.2–1.4 (m, 10H). ^{13}C NMR ($CDCl_3$, 125 MHz) 174.3, 169.2, 160.8, 140.1, 139.1, 114.1, 97.6, 96.8, 60.5, 55.3, 47.6, 34.6, 33.7, 29.4, 29.3, 29.3, 29.0, 28.8, 26.5, 25.0, 24.7. HRMS calculated for $C_{24}H_{37}NO_5$, 416.2675; found, 416.2676.

*2.8. Preparation of ethoxy silane (**12**)*

To a solution of **11** (1.70 g, 4.08 mmol) dissolved in methylene chloride (20 ml) was added dimethylchlorosilane (9.06 ml, 81.6 mmol). Chloroplatinic acid (8.2 mg) dissolved in THF (0.5 ml) was then added. A reflux condenser was attached to the reaction flask and the system was purged with nitrogen, and the reaction mixture was heated to reflux for 12 h. The solvents were then removed in vacuo. The remaining residue was dissolved in methylene chloride (20 ml) and 10 ml of a solution of triethylamine–ethanol (1:1, v/v) was added, and the resulting solution was heated to reflux for 45 min. The volatiles were then removed in vacuo and the residue purified by flash chromatography (2% methanol in methylene chloride) yielding 1.78 g of **12** as a light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) 9.77 (br s, 1H), 6.78 (d, J=2.3, 2H), 6.19 (t, J=2.3, 1H), 4.79 (d, J=7.8, 1H), 3.76 (s, 6H), 3.65 (q, J=7.0), 3.4–3.6 (m, 2H), 2.59 (m, 1H), 2.34 (t, J=7.4, 2H), 2.16 (m, 1H), 2.04 (m, 1H), 1.79 (m, 1H), 1.66 (m, 1H), 1.2 –1.4 (m, 14H), 1.18 (t, J=7.0, 2H), 0.58 (m, 2H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) 174.6, 169.0, 160.9, 140.1, 97.7, 96.8, 60.5, 58.2, 55.4, 47.7, 34.7, 33.4, 29.5, 29.5, 29.4, 29.4, 29.3, 26.2, 25.1, 24.8, 23.2, 18.6, 16.3, −2.1.

*2.9. Preparation of CSP **13***

Silica gel (4.60 g, 5 μ, 100 Å) was azeotropically

dried by refluxing in toluene (20 ml) with a Dean–Stark trap. After cooling, a solution of ethoxy silane **12** dissolved in toluene (5 ml) was added and the slurry was refluxed for 24 h. The toluene was then removed under reduced pressure, dimethylformamide (DMF) (2 ml) was added, and the slurry was sonicated for several min to break up aggregates of silica particles. The mixture was then heated on a Kugelrohr apparatus for 38 h (bath temperature: 110°C, pressure = 1.0 mmHg; 1 mmHg = 133.322 Pa). The silica was then slurried in DMF (5 ml), sonicated for several min, and returned to the Kugelrohr for 22 h. The derivatized silica was slurried in methanol, collected by filtration, and washed sequentially with methanol (60 ml), ethyl acetate (30 ml), methylene chloride (30 ml), hexane (30 ml), methanol (30 ml) and ether (30 ml). The derivatized silica was then slurried in methanol and packed into a 250×4.6 mm stainless steel HPLC column. A portion of the left-over silica was dried and submitted for elemental analysis: C, 10.16; H, 1.46; N, 0.83. This corresponds to a loading of 0.33 mmol/g based on C, 0.34 mmol/g based on H, and 0.30 mmol/g based on N.

2.10. Van't Hoff plots

Analytes **1** and **2** were chromatographed on CSP **3a**, CSP **3b** and CSP **13** at temperatures of 4.0, 13.5, 23.0, 33.0, 44.0 and 55.0°C, eluting with 2-propanol–hexane (30:70) at a flow-rate of 2 ml/min. Plots of the natural logarithm of the retention factor versus reciprocal absolute temperature yielded a straight line in all cases ($r > 0.999$).

3. Results and discussion

From previous studies, we had available to us two versions of the proline-derived CSPs, **3a** and **3b**, which differ only in the alkyl tether which covalently links them to the silica surface [5–8]. The short-tethered version affords, relative to the long-tethered CSP, less retention for both analytes but greater enantioselectivity for analyte **2** (Table 1). The shorter tether positions the selector closer to the surface of the silica which enhances the intercalation effects previously observed with these CSPs. Since the intercalation effect benefits the enantioselectivity of **2** but reduces that of **1**, the choice of tether length was tentatively made on the basis of the commercial availability of the longer tether.

Table 1
Separation of the enantiomers of analytes **1** and **2** on CSP **3a** and **3b**[a]

Analyte	CSP	k_1	α
1	**3a**	3.45	3.44
1	**3b**	1.83	2.59
2	**3a**	2.34	3.48
2	**3b**	1.46	4.95

[a] Conditions: 2-propanol–hexane (20:80, v/v), 2 ml/min.

To determine the optimal aromatic group for the proline-derived selector a reciprocal study was employed. A variety of substituted anilides of pivaloyl proline, **6**, and undecenoyl proline, **7** was prepared (as outlined in Scheme 1) by condensation of the appropriate acid chloride with racemic proline followed by coupling to a substituted aniline. These anilide analytes were then screened on the commercial versions of the CSPs derived from analytes **1** and **2**, CSPs **8** and **9**, respectively, as shown in Tables 2–5. Our goal in carrying out this reciprocal study was to determine how the substituents on the

Table 2
Separation of the enantiomers of type **6** analytes on CSP **8**[a]

Entry	Ar	k_1	α	R_s
1	Ph	2.15	2.75	4.81
2	α-Naph	12.05	1.10	0.71
3	β-Naph	6.89	2.22	4.01
4	4-F-Ph	1.57	2.17	3.55
5	4-Cl-Ph	1.52	2.16	3.57
6	4-Br-Ph	1.60	2.16	3.60
7	4-I-Ph	1.67	2.23	3.72
8	4-Me-Ph	2.55	3.13	5.58
9	4-SMe-Ph	3.85	2.39	4.42
10	4-OMe-Ph	4.98	2.36	4.24
11	3,5-Cl-Ph	0.83	2.37	3.40
12	3,5-CF_3-Ph	0.32	1.72	1.57
13	3,5-Me-Ph	2.73	3.14	5.38
14	3,5-OMe-Ph	6.90	4.02	10.32
15	3,4,5-Cl-Ph	0.86	1.93	3.02
16	3,4,5-OMe-Ph	8.81	2.67	7.72
17	2-Me-Ph	3.51	1.50	2.77
18	2,4-Me-Ph	4.03	1.59	2.94
19	2,4,6-Me-Ph	2.34	1.05	0.40

[a] Conditions: 2-propanol–hexane (20:80, v/v), 2 ml/min.

Table 3
Separation of the enantiomers of type **6** analytes on CSP **9**[a]

Entry	Ar	k_1	α	R_s
1	Ph	1.20	3.76	6.31
2	α-Naph	4.30	1.24	1.57
3	β-Naph	2.87	4.09	5.87
4	4-F-Ph	0.96	3.24	5.17
5	4-Cl-Ph	0.95	3.68	5.44
6	4-Br-Ph	0.99	3.80	5.25
7	4-I-Ph	1.02	3.86	5.22
8	4-Me-Ph	1.36	4.43	6.98
9	4-SMe-Ph	1.90	3.79	5.73
10	4-OMe-Ph	2.32	3.53	5.92
11	3,5-Cl-Ph	0.68	6.10	6.12
12	3,5-CF_3-Ph	0.34	5.09	4.70
13	3,5-Me-Ph	1.39	6.03	8.26
14	3,5-OMe-Ph	3.38	6.78	8.66
15	3,4,5-Cl-Ph	0.78	5.77	5.16
16	3,4,5-OMe-Ph	4.16	5.02	6.91
17	2-Me-Ph	1.67	1.26	1.43
18	2,4-Me-Ph	1.83	1.31	1.79
19	2,4,6-Me-Ph	0.96	1.00	0.00

[a] Conditions: 2-propanol–hexane (20:80, v/v), 2 ml/min.

anilide rings influence both enantioselectivity and retention. Substituents which afford increased retention without sacrifice of enantioselectivity would be candidates for use in the final selector structure, the presumption being that an analyte which is strongly retained on CSPs **8** and **9** would afford, when bonded to silica, a CSP which would afford greater retention than CSP **3**. This would allow the use of mobile phases in which analytes **1** and **2** are more soluble, thus increasing the amount one could load on the column and thus leading to greater through-put.

Table 4
Separation of the enantiomers of type **7** analytes on CSP **8**[a]

Entry	Ar	k_1	α	R_s
1	Ph	2.61	2.34	4.19
2	4-Cl-Ph	1.88	1.94	2.30
3	4-Me-Ph	3.07	2.41	4.39
4	4-OMe-Ph	6.92	2.01	3.50
5	3,5-Cl-Ph	0.97	2.03	2.29
6	3,5-CF_3-Ph	0.32	1.49	0.72
7	3,5-Me-Ph	3.45	2.24	3.91
8	3,5-OMe-Ph	8.31	3.06	6.41
9	3,4,5-Cl-Ph	1.06	1.74	1.63
10	3,4,5-OMe-Ph	11.61	2.46	4.69

[a] Conditions: 2-propanol–hexane (20:80, v/v), 2 ml/min.

Table 5
Separation of the enantiomers of type **7** analytes on CSP **9**[a]

Entry	Ar	k_1	α	R_s
1	Ph	1.20	2.13	3.44
2	4-Cl-Ph	0.95	2.08	2.76
3	4-Me-Ph	1.38	2.29	4.06
4	4-OMe-Ph	2.45	2.04	3.58
5	3,5-Cl-Ph	0.69	2.88	3.49
6	3,5-CF_3-Ph	0.32	2.47	2.26
7	3,5-Me-Ph	1.47	2.54	4.59
8	3,5-OMe-Ph	3.27	3.18	6.16
9	3,4,5-Cl-Ph	0.76	2.88	3.56
10	3,4,5-OMe-Ph	4.06	2.81	5.11

[a] Conditions: 2-propanol–hexane (20:80, v/v), 2 ml/min.

From inspection of Table 2, it can be seen that addition of electron withdrawing groups to the aromatic group of type **6** analytes (entries 4, 5, 6, 7, 11, 12 and 15) diminishes both retention and enantioselectivity on CSP **8** compared to the unsubstituted analyte (entry 1). Electron donating substituents (entries 8, 9, 10, 13, 14 and 16) increase retention on this CSP. Relative to the unsubstituted anilide, the separation factors for the enantiomers are increased by 4-methyl-, 3,5-dimethyl- and 3,5-dimethoxy-substituents but are diminished for all other substituents in Table 2. The two naphthyl-subsituted analytes (entries 2 and 3) show a marked increase in retention times but suffer from diminished separation factors. Comparison of the various methyl-substituted analytes (entries 8, 13, 17, 18 and 19) shows the 3,5-substitution pattern to be desirable when considering both retention and separation.

From inspection of Table 3, it can be seen that type **6** analytes display retention trends on CSP **9** similar to those observed on CSP **8**. Electron withdrawing substituents reduce retention (entries 4, 5, 6, 7), although on CSP **9**, separation factors are comparable to that of the unsubstituted analyte. Addition of multiple electron withdrawing groups enhances the separation factor while further reducing the retention (entries 11, 12 and 15). Analytes with electron donating substituents increase both retention (entries 8, 9, 10, 13, 14 and 16) and enantioselectivity with the exception of the 4-methoxy substituted phenyl (compared to the unsubstituted analyte). As before, the α-naphthyl substituted analyte and the analytes having a substituent in the 2-position show

diminished enantioselectivities (entries 2, 17, 18 and 19). On both CSPs, the analyte which has the largest separation factor and the longest retention of the second eluted enantiomer is the analyte where the aryl group is 3,5-dimethoxyphenyl.

We next turned our attention to the undecenoyl proline derived type **7** analytes in order to determine whether the trends in the data show any dependency on the length of this chain (R in Chart 1). The chromatographic data for the type **7** analytes on CSPs **8** and **9** are shown in Tables 4 and 5, respectively. As before, electron withdrawing substituents (entries 2, 5, 6 and 9) reduce retention on both CSPs. Additionally, the separation factors of these analytes are reduced on CSP **8**, but increased on CSP **9** for the di- and trisubstituted analytes compared to the unsubstituted analyte (entry 1). Analytes with electron donating substituents (entries 3, 4, 7, 8 and 10) display increased retention and the separation factors are increased in all but one case, the 4-methoxyphenyl analyte (entry 4). In this series of analytes, the one affording the greatest retention of the second eluted enantiomer is the 3,4,5-trimethoxyphenyl analyte (entry 10).

Taking all the reciprocal data together, it was decided that 3,5-dimethoxy derivative would be the best overall choice as a selector for the preparative CSP. Even though the greatest retention for the second eluted enantiomer occurs with the type **7** 3,4,5-trimethoxy anilide, the separation factors for the enantiomers of this compound are diminished relative to those of the 3,5-dimethoxy anilide derivatives. The synthesis of the 3,5-dimethoxy-substituted selector is outlined in Scheme 2. Condensation of (*S*)-proline with undecenoyl chloride, followed by EEDQ promoted coupling with 3,5-dimethoxy aniline, yields the olefinic selector, **11**. Hydrosilylation with dimethylchlorosilane, followed by treatment with ethanol and triethylamine, yields the ethoxysilane, **12**, which, after bonding to silica, yields the derivatized silica, CSP **13**.

Chromatographic data, in various mobile phases, for analytes **1** and **2** on both CSPs **3b** and **13**, are shown in Tables 6 and 7. Retention is greater on CSP **13** than on CSP **3b** for both analytes, while the separation factors are comparable and similarly dependent on polar modifier for both CSPs. The greater retention afforded by CSP **13** allows one to use a stronger mobile phase and still produce a separation comparable to that afforded by CSP **3b**.

Table 6
Chromatographic data for analyte **1** on CSP **3b** and CSP **13**

Mobile phase	CSP **3b**		CSP **13**	
(2 ml/min)	k_1	α	k_1	α
2-Propanol–hexane (25:75)	2.85	3.44	6.71	3.77
THF–hexane (25:75)	3.40	2.64	6.14	2.61
EtOAc–hexane (25:75)	4.30	2.87	7.54	2.90
EtOAc–hexane (50:50)	1.03	2.79	1.48	2.80

Table 7
Chromatographic data for analyte **2** on CSP **3b** and CSP **13**

Mobile phase	CSP **3b**		CSP **13**	
(2 ml/min)	k_1	α	k_1	α
2-Propanol–hexane (25:75)	1.91	3.50	4.07	3.30
THF–hexane (25:75)	4.19	5.76	5.76	6.04
EtOAc–hexane (25:75)	5.60	4.99	6.12	5.58
EtOAc–hexane (50:50)	1.25	5.40	1.32	6.10

To demonstrate the greater suitability of CSP **13** (relative to CSP **3a**), preparative runs were carried out using CSP **13** with both analytes **1** and **2**. Injection of 11.9 mg of analyte 1 dissolved in THF (0.1 ml), eluting with THF–2-propanol–hexane (1:9:10, v/v/v) at a flow-rate of 2 ml/min afforded complete enantioseparation (Fig. 1a). Assay of both fractions collected from this run were ≥99% ee

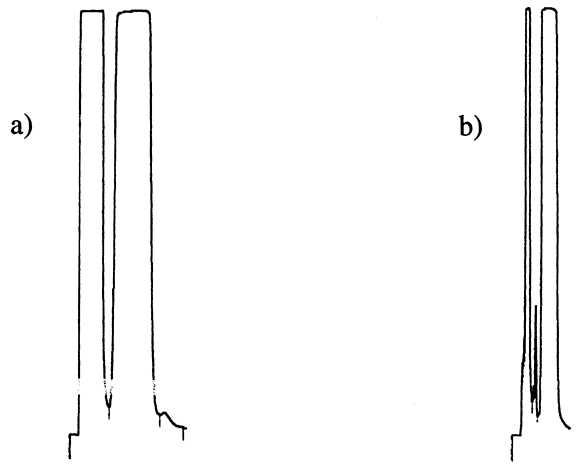


Fig. 1. Preparative separation of the enatiomers of (a) analyte **1**, (b) analyte **2** on CSP **13**.

(enantiomer excess). Total time for this run was 15 min. Injection of 25.4 mg of analyte **2** dissolved in THF–hexane (1:1, v/v) (0.4 ml), and eluting with THF–hexane (1:1, v/v) at a flow-rate of 2 ml/min afforded complete enantioseparation (Fig. 1b). Assay of both fractions collected from this run were ≥99% ee. Total time for this run was 9 min.

For comparison, similar preparative runs were conducted using CSP **3a** with both analytes **1** and **2**. A solution of **1** in THF was prepared with a concentration of 120 mg/ml. Complete enantioseparation was observed for injections up to 25 μl, eluting with THF–2-propanol–hexane (1:9:10, v/v/v) at a flow-rate of 2 ml/min, which corresponds to a maximum loading of 3 mg of **1**. Total time for each run was 10 min. Similarly, a solution of **2** was prepared with a concentration of 100 mg/ml, dissolved in THF–hexane (1:1, v/v). Complete enantioseparation was observed for injections up to 80 μl, eluting with THF–hexane (1:1, v/v) at a flow-rate of 2 ml/min, which corresponds to a maximum loading of 8 mg of **2**. Total time for each run was 9 min. Sample loadings greater than those stated above (for CSP **3a**) caused the two chromatographic bands to overlap.

4. Conclusions

A proline-derived chiral selector intended for preparative chromatographic separation of the enantiomers of the selectors used in two commercial CSPs is described. A reciprocal study, utilizing proline-derived analytes, was conducted to select structural features which maximize retention without sacrificing enantioselectivity. The selector of the determined structure was then synthesized in quantity, bonded to silica, and evaluated. The developed CSP shows increased retention when using the same mobile phase as an earlier CSP, with comparable separation factors. This allows one to use a mobile phase in which the analytes are more soluble in order to achieve a comparable separation but with a greater amount of sample on the column per run.

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