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Observations relevant to the differential intercalation of enantiomers between the strands of brush-type chiral stationary phases

William H. Pirkle*, Patrick G. Murray

School of Chemical Sciences, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA

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Abstract

Differential penetration of the enantiomers of certain analytes between the strands of "brush-type" liquid chromatographic chiral stationary phases (CSPs) has been invoked in several instances to explain the progressive change in separation factors as one proceeds, chromatographically, through a homologous series of these analytes. These "intercalative effects" are presumed to arise as a consequence of the semi-ordered, side-by-side bonding of selector strands in conventional brush-type CSPs. Thus, they are not expected to occur in the absence of the stationary phase.

A recently developed proline-derived CSP affords large separation factors for the enantiomers of some esters, thioesters, and amides of N-acylated α-amino acids, the separation factor being dependent upon the length of the C-terminal substituent of the analyte. For example, the separation factors increase with the length of the linear thioalkyl substituent when N-(3,5-dinitrobenzoyl)leucine thioesters are chromatographed on the proline-derived CSP. However, the length of this substituent does not affect the enantiomeric purity attained when the enantiomers of these thioesters are stereochemically equilibrated in solution in the presence of the proline-derived selector employed in the CSP. These observations further support the concept of differential intercalation of enantiomers between the strands of brush-type CSPs and may have implications for asymmetric syntheses conducted with immobilized catalysts or reagents.

Keywords: Chiral stationary phases, LC; Intercalation; Enantiomer separation

1. Introduction

The rapid development of highly enantioselective methods for asymmetric synthesis can, in part, be attributed to the development of fast and accurate methods for determining the enantiomeric purity and absolute configurations of the compounds so synthesized. The development of chiral stationary phases (CSPs) for the chromatographic separation of enantiomers has been particularly important in this regard [1]. Indeed, mechanistic investigations into the origins of enantiodiscrimination have progressed to the point where the a priori design of CSPs for

* Corresponding author.

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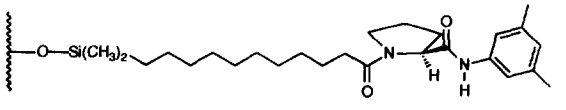
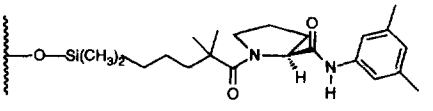
specific target analytes is becoming possible [2,3]. In other instances, enantioselectivity has been increased or the utility broadened by rational improvements to existing CSPs [4].

Because enantiomeric analytes do not utilize the same combinations of interactions with the CSP, they are, on a time-averaged basis, differently oriented with respect to the chiral selector. For some classes of analytes, one enantiomer has been supposed to intercalate a portion of its structure between adjacent selector strands to a greater extent than does its antipode [2]. The energetic consequences of this differential intercalation are superimposed upon the energetics of the fundamental process(es) by which chiral recognition otherwise occurs. In non-polar mobile phases such as mixtures of 2-propanol-hexane, intercalation of simple alkyl groups hastens elution, very possibly for steric reasons. Should it be the least retained enantiomer which undergoes the greater intercalation effect, any structural change in the CSP which exacerbates this effect will enhance enantioselectivity by causing this enantiomer to elute relatively more rapidly than the more retained, non-intercalating enantiomer.

Proline-derived chiral stationary phases **1a** and **1b** (Fig. 1) are specific examples of CSPs which were designed to separate the enantiomers of suitably derivatized amino acid esters and amides, chiral amines, and chiral alcohols [2,5]. High levels of enantioselectivity ($\alpha > 80$) are achieved in some instances, for CSP **1b** enhances enantiodiscrimination by virtue of the intercalative behavior exhibited by some of these analytes. Although CSPs **1a** and **1b** use the same chiral selector and have essentially the same surface coverage [2], CSP **1b** has a shorter and bulkier tether to silica, a change intended to exacerbate intercalation effects. These effects have been postulated to stem from the side-by-side arrangement of the selector strands in conventional brush-type stationary phases. As such, they are not expected to occur in solution without the solid support and the attending preorganized arrangement of selector strands.



CSP **1a**



CSP **1b**

Fig. 1. Proline-derived CSPs **1a** and **1b**.

2. Experimental

All reagents employed were of pharmaceutical or reagent grade. NMR spectra were obtained on a Varian XL FT-NMR operating at 200 MHz in the ^2H lock mode. Elemental analyses were performed by T. McCarthy and associates of the University of Illinois Microanalytical Laboratory. High- and low-resolution mass spectral analyses were performed by R. Milberg and associates at the University of Illinois Mass Spectrometry Laboratory. Low-resolution mass spectra (MS) were obtained on a Varian MAT CH-5 mass spectrometer with 70 eV electron-impact ionization. High-resolution mass spectra (HRMS) were obtained on a Varian 731 mass spectrometer with 70 eV electron-impact ionization. Melting points were taken with a Buchi melting point apparatus and are uncorrected. The preparation of the CSPs used in this study, CSPs **1a** and **1b**, has been reported elsewhere [2].

*2.1. Preparation of thioesters **2a** and **2b***

*(R,S)-S-1-butyl-N-(3,5-dinitrobenzoyl)-leucine thioester (**2a**)*

This procedure is adapted from Ref. [6]. Racemic N-(3,5-dinitrobenzoyl)leucine (4.00 g, 0.0123 mol) was suspended in 150 ml of dry dichloromethane which contained 3 ml (0.0212 mol) of trifluoroacetic anhydride. This solution was stirred until homogeneous and the excess

trifluoroacetic anhydride was removed on a rotary evaporator. The residue was dissolved in 150 ml of dry dichloromethane and 3 ml (0.028 mol) of 1-butanethiol was added to the solution. A 0.60 *M* solution of $SnCl_4$ in dry dichloromethane (30 ml, 0.0183 mol) was added to the reaction mixture dropwise over a period of 5 min. The reaction mixture was allowed to stir for 15 min and was then added to 200 ml of 0.5 *M* HCl. The layers were separated, the organic phase was washed with 200 ml of 5% Na_2CO_3, and then was dried over anhydrous $MgSO_4$. The solution was filtered and the solvent was removed under reduced pressure. The product was crystallized from the residue with hexane to give **2a** as a white powder: 3.22 g (66% yield); m.p. 137–139°C. ^1H NMR (200 MHz) ($CDCl_3$) δ 0.92 (t, 3H); 1.01 (d, 6H); 1.37–1.80 (m, 7H), 2.95 (t, 2H); 5.03 (m, 1H); 6.84 (d, 1H); 8.98 (d, 2H); 9.20 (t, 1H). Analysis for $C_{17}H_{23}N_3O_6S$: Calculated: C, 51.38; H, 5.83; N, 10.52; S, 8.07. Found: C, 51.37; H, 5.87; N, 10.38; S, 7.96.

*(R,S)-S-1-octadecyl-N-(3,5-dinitrobenzoyl)leucine thioester (**2b**)*

Racemic N-(3,5-dinitrobenzoyl)leucine (6.00 g, 0.0185 mol) was suspended in 200 ml of dry dichloromethane which contained 5 ml (0.035 mol) of trifluoroacetic anhydride and 2 ml of dimethylformamide. This solution was stirred until homogeneous and the excess trifluoroacetic anhydride was removed using a rotary evaporator. The residue was dissolved in 150 ml of dry dichloromethane and 8 g (0.028 mol) of octadecylmercaptan was added to the solution in 25 ml of dry dichloromethane. A 0.60 *M* solution of $SnCl_4$ in dry dichloromethane (45 ml, 0.027 mol) was added to the reaction mixture dropwise over a period of 5 min. The reaction mixture was allowed to stir for 15 min and was then added to 200 ml of 0.5 *M* HCl. The layers were separated, the organic phase was washed with 200 ml of 5% Na_2CO_3, and then was dried over anhydrous $MgSO_4$. The solution was filtered and the solvent was removed under reduced pressure. The product was crystallized from the residue with hexane to give **2b** as a white solid: 4.37 g (39% yield); m.p. 98–99°C. ^1H NMR (200 MHz) ($CDCl_3$) δ 0.88 (t, 3H); 1.01 (d, 6H); 1.10–1.90 (m, 35H), 2.95 (t, 2H); 5.00 (m, 1H); 6.70 (d, 1H); 8.98 (d, 2H); 9.18 (t, 1H). Analysis for $C_{31}H_{51}N_3O_6S$: Calculated: C, 62.70; H, 8.66; N, 7.08; S, 5.40. Found: C, 62.38; H, 8.48; N, 7.01; S, 5.26.

*2.2. Preparation of chiral selectors **3a** and **3b***

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

N-Benzyloxycarbonyl-(L)-proline (Aldrich), 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. Then 75 ml 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 and concentrated under reduced pressure to give 8.08 g of a white solid (85% yield); m.p. 138–140°C. ^1H NMR (200 MHz) ($CDCl_3$) 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.

*(S)-N-(Myristoyl)proline-3,5-dimethylanilide (**3a**) and (S)-N-(pivaloyl)proline-3,5-dimethylanilide (**3b**)*

N-(Benzyloxycarbonyl)-(L)-proline-3,5-dimethylanilide, 6.0 g (0.017 mol), was dissolved with gentle heating in 50 ml of dry ethanol and placed in a heavy walled pressure bottle. An amount of 600 mg (10% w/w) of 20% $Pd(OH)_2$ on carbon (Pearlman's catalyst) was added along with two drops of glacial acetic acid. The pressure bottle was installed in a Parr hydrogenator, flushed with

nitrogen, and rocked under 20 psi (137 895.2 Pa) of hydrogen for 12 h. After this time, the pressure bottle was removed from the apparatus and the contents were filtered through Celite. The filtrate was concentrated to give 3.71 g of a pasty white solid (100% yield). Any remaining ethanol was chased with two portions of CCl_4 and the residue was dissolved in dry tetrahydrofuran and split into two equal portions. To each portion was added 0.86 g (0.008 mol) of triethylamine. Trimethylacetyl chloride (Aldrich), 1.12 g (0.0093 mol), was added to the first portion and 2.30 g (0.0093 mol) of myristoyl chloride (Aldrich) was added to the second portion. Each reaction mixture was stirred for 30 min, filtered to remove the triethylamine hydrochloride precipitate, and concentrated in vacuo to remove the solvent. The product was crystallized from the residue using mixtures of ethanol–water (**3a**) or ethyl acetate–hexane (**3b**). Compound **3a**: yield: 3.24 g (89%); ^{1}H NMR (200 MHz) ($CDCl_3$) δ 1.30 (s, 9H); 1.87 (m, 2H); 2.26 (s, 6H); 2.40 (m, 2H); 3.70 (m, 2H); 4.80 (dd, 1H); 6.70 (s, 1H); 7.20 (s, 1H); 9.10 (bs, 1H). Analysis for $C_{18}H_{26}O_2N_2$: Calculated: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.32; H, 8.60; N, 9.24. Compound **3b**: yield 2.57 g (96%); m.p. 73–75°C. ^{1}H NMR (200 MHz) ($CDCl_3$) δ 0.87 (t, 3H); 1.25 (m, 22H); 1.60–2.00 (m, 4H); 2.27 (s, 6H); 2.30 (t, 2H); 3.50 (m, 2H); 4.80 (dd, 1H); 6.71 (s, 1H); 7.17 (s, 2H); 9.63 (bs, 1H). Mass spectrum (70 eV), *m/z* (relative intensity) 428 (M, 39.4), 308 (82.7), 281 (32.3), 280 (100), 211 (99.3), 126 (30.8), 121 (49.4), 113 (69.1), 112 (33.5), 98 (57.7), 85 (41.0), 84 (37.9), 83 (35.1), 71 (100), 70 (110), 69 (91.1), 68 (37.6), 57 (110), 55 (100), 43 (100), 41 (100). High resolution mass spectrum, calculated for: 428.34027, found: 428.34030.

*2.3. Procedure for the deracemization of **2a** and **2b***

Deracemization experiments were conducted in screw-top test tubes, the final reaction mixtures being prepared from standard solutions of each component. Each test tube experiment was 0.25 *M* in chiral selector (**3a** or **3b**), 0.05 *M* in racemic thioester (**2a** or **2b**), and 0.20 *M* in triethylamine, the total volume being 3 ml. The solvent employed for these experiments was 25% (v/v) dichloromethane in cyclohexane. It is important that the caps of the test tubes fit tightly and seal well, lest evaporation of solvent causes concentration of the components. The enantiomeric composition of each of the four test tubes was monitored weekly by high-performance liquid chromatography (HPLC) using chiral stationary phase **1b**.

3. Results and discussion

In 2-propanol-hexane mobile phases, the extent to which CSPs **1a** and **1b** differentiate between the enantiomers of esters or amides of N-(3,5-dinitrobenzoyl)amino acids is influenced by the length of the alkyl substituent on the analyte's C-terminal ester or amide group. To illustrate this, chromatographic data for the separation of the enantiomers of some N-(3,5-dinitrobenzoyl)leucine esters on CSP **1b** are shown in Table 1. Under the conditions used, CSP **1b** affords ca. 13 000 theoretical plates per meter for each enantiomer of each of these analytes. The length of N-terminal alkyl substituents has an even greater effect on enantioselectivity in the case of the corresponding amide derivatives.

We have rationalized these and related data by invoking differential extents of intercalation of the enantiomers between the strands of the

Table 1
Separation of the enantiomers of N-(3,5-dinitrobenzoyl)leucine esters on CSP **1b**

Ester	k_1'[a]	α[b]
Methyl	3.93	7.15
Ethyl	3.27	7.98
Propyl	2.97	8.42
Pentyl	2.59	8.76
Heptyl	2.23	9.34

Mobile phase consists of 10% 2-propanol in hexane.
[a] Capacity factor for the first eluting enantiomer.
[b] Chromatographic separation factor.

immobilized chiral selector. In the present examples, the least retained enantiomer is presumably oriented so as to direct the alkyl substituent of the ester or amide group more or less alongside the selector's tether and between adjacent strands of bonded phase. If sufficiently long, the alkyl substituent may encounter the underlying silica gel support. Thus, the length of the group which undergoes intercalation influences the stability of that diastereomeric adsorbate. The extent to which the stability of the adsorbate is reduced increases with the length of the intercalating group. The chromatographic consequence of this behavior is that the enantiomer suffering this effect has its retention reduced relative to its less intercalating antipode. Thus, enantioselectivity increases as one proceeds through the homologous series. The orientation of the chiral selector (with respect to the solid support) is of importance, for this orientation can determine whether it is the most or the least retained enantiomer which suffers the greater intercalation effect. Depending on whether one wishes to amplify or relieve intercalative effects, the orientation of the selector with respect to the solid support can be changed [7,8].

Soluble analogues of CSPs can sometimes be used to effect first-order asymmetric transformations [9]. For instance, the methine hydrogen on the stereogenic center of N-(3,5-dinitrobenzoyl)leucine thioesters is acidic enough to permit slow interconversion of the enantiomers in the presence of triethylamine at room temperature. When this occurs in the presence of a chiral selector capable of preferentially complexing one enantiomer of the thioester, a thermodynamically driven deracemization occurs and the solution becomes enriched in the more strongly complexed enantiomer. Except in cases where chromatographic intercalative effects are so severe as to override the fundamental chiral recognition process(es) and invert the order of elution of the enantiomers, the solution becomes enriched in the enantiomer which is preferentially retained on a CSP derived from the same chiral selector.

CSPs **1a** and **1b** resolve the enantiomers of thioesters **2a** and **2b**; chromatographic data for these resolutions are shown in Table 2. The

Table 2
Separation of the enantiomers of N-(3,5-dinitrobenzoyl)leucine thioesters on CSPs **1a** and **1b**

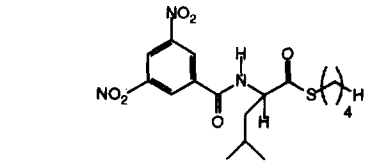
CSP	Thioester	k_1' [a]	α [b]
1a	**2a**	9.29	3.73
1a	**2b**	4.52	4.43
1b	**2a**	5.98	5.70
1b	**2b**	2.88	6.70

Mobile phase consists of 25% dichloromethane in cyclohexane.
[a] Capacity factor for the first eluting enantiomer.
[b] Chromatographic separation factor.

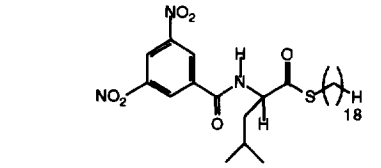
separation factor (α), the measure of the enantiodiscrimination exhibited by CSP **1b** toward the enantiomers of **2a** and **2b**, clearly depends upon the length of the thioester alkyl substituent. However, the length of the thioalkyl substituent would be expected to be essentially immaterial to the degree of chiral recognition observed in solution. The N′-(alkyl)amides of N-(3,5-dinitrobenzoyl)leucine show greater intercalation effects than do the thioesters but do not undergo enantiomer interconversion in the presence of triethylamine. Stronger bases than triethylamine cause other undesirable reactions to occur.

To test the preceding hypothesis, solution-state deracemization experiments were conducted with thioesters **2a** and **2b** using soluble analogues of CSPs **1a** and **1b** (shown in Fig. 2 as **3a** and **3b**) as chiral complexing agents. Fig. 3 shows the enantiomeric enrichment in the deracemization of **2b** with chiral selector **3b** as a function of time, and the results of all four experiments are summarized in Table 3.

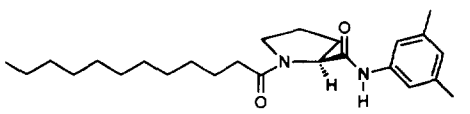
Chiral selector **3b** generates a greater enantiomeric excess in the solution-state deracemization experiments than does **3a**. The bulky pivaloyl group is thought to more extensively populate a conformation favorable for stereochemical discrimination of the enantiomers of **2**. Importantly, the length of the thioalkyl group has no appreciable effect upon the enantiomeric purity at equilibrium in the case of either selector. These results are consistent with differential extents of intercalation occurring for the enantiomers of **2** during chromatography but not during the de-



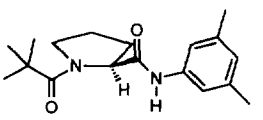
2a



2b



3a



3b

Fig. 2. Thioesters **2a** and **2b** and proline-derived chiral selectors **3a** and **3b**.

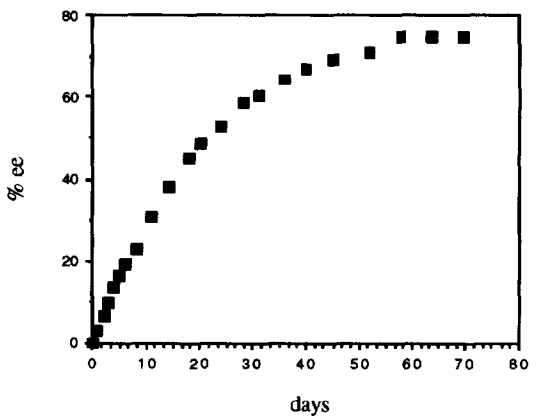


Fig. 3. Enantiomeric excess as a function of time in the deracemization of **2b** with **3b**. Conditions are described in the text.

Table 3
Asymmetric transformation of N-(3,5-dinitrobenzoyl)leucine thioesters **2a** and **2b** with chiral selectors **3a** and **3b**

Chiral selector	Equilibrium % *ee*	
	2a	**2b**
3a	63	63
3b	78	78

Conditions as described in the Experimental section.

racemization in solution. One notes that an *ee* (enantiomeric excess) of 83% corresponds roughly to an 8:1 ratio of the enantiomers, a value which slightly exceeds the relative affinities of the enantiomers for CSP **1b**. The position of the equilibria in solution (this determines the enantiomeric excess) depends upon the solvent, the relative concentrations of the chiral selector and the thioester, the temperature, and, of course, the association constants of the diastereomeric complexes. The enantioselectivity observed in the solution-state asymmetric transformation is a more accurate indicator of the actual ability of the chiral selector to discriminate between the enantiomers of **2a** and **2b** than is the chromatographic separation factor, α. Not only are intercalation effects absent from the solution study, but so are the contributions of unproductive retention processes unrelated to chiral recognition (e.g., interaction of the analyte enantiomers with silanol groups on the surface of the silica gel).

4. Conclusion

Apart from the obvious implications for CSP design, the occurrence of intercalation effects has implications for those who immobilize chiral catalysts or chiral reagents, an area toward which significant efforts have been directed in the recent past [10–15]. In those cases where the catalyst or reagent reacts preferentially with one enantiomer of the substrate, differential intercalation effects might influence reaction rates. Even when the substrate is achiral, the energetics

of the diastereomeric transition states might be influenced by differential intercalation effects. While these effects are more readily visualized on brush-type silica-supported materials than when other types of supports, polystyrene, for instance, are used, it seems likely that intercalation effects will influence both the reaction rates and stereochemical efficiency of catalysts and reagents supported on these less well-defined surfaces. While the chemistry used to anchor catalysts or reagents to silica is less robust than that used to immobilize such substances on polystyrene, the ability to control the role of the solid support in a semi-predictable fashion and to use intercalative effects to enhance selectivity should, in selected instances, be of value to workers in this field.

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