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Short communication

Liquid chromatographic separation of the stereoisomers of thiazide diuretics

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Abstract

A number of racemic thiazide diuretics and analogues were resolved on two diastereomeric chiral stationary phases (CSPs) prepared from (*S*)- or (*R*)-α-[1-(6,7-dimethyl)naphthyl]-10-dodecenylamine and (*S*)-2-phenylpropanoic acid. Of the two diastereomeric CSPs, the (*S*,*S*) and the (*R*,*S*), the former is found to be better than the latter in separating the enantiomers of the racemic thiazide diuretics and their analogues with complete separation being observed on the (*S*,*S*)-CSP. Chiral recognition is controlled principally by the (*R*)- or (*S*)-α-[1-(6,7-dimethyl)naphthyl]-10-dodecenylamine portion of the CSPs. The second stereogenic center of the CSP provides but secondary effects on the chiral recognition presumably involving, in the case of the (*S*,*S*)-CSP, face-to-edge π–π interaction between the aromatic ring of the analytes and the phenyl on the second stereogenic center.

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

1. Introduction

Enantiomers of racemic drugs often show different pharmacological activities [1]. For example, the activities of β-adrenergic blocking drugs, nonsteroidal anti-inflammatory drugs such as α-arylpropionic acids, and central nervous system stimulants are known to be stereochemically dependent [2,3]. Several racemic benzo-1-thia-2,4-diazines, **1** (Fig. 1), also termed thiazides, have been used as diuretics and it is conceivable that the two enantiomers of these compounds might elicit different pharmacological responses. Another chiral thiazide, IDRA 21 (7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine *S*,*S*-dioxide) shows cognition-enhancing activity, only the (+)-enantiomer being identified as pharmacologically active [4]. For the study of the stereochemical dependence of the pharmacokinetics and pharmacological activities of chiral drugs, direct chromatographic chiral resolution techniques are often used [2,3]. In the view of their structures and means of synthesis, separation of thiazides on chiral stationary phases would seem to be essential to the elucidation of the pharmacokinetic and pharmacological properties of these enantiomers. Previously, the enantiomers of several thiazides have been separated by high-performance liquid chromatography by several groups and on several types of CSPs [4–8]. However, a comprehensive report documenting the ability of those CSPs to resolve thiazides in general has not appeared.

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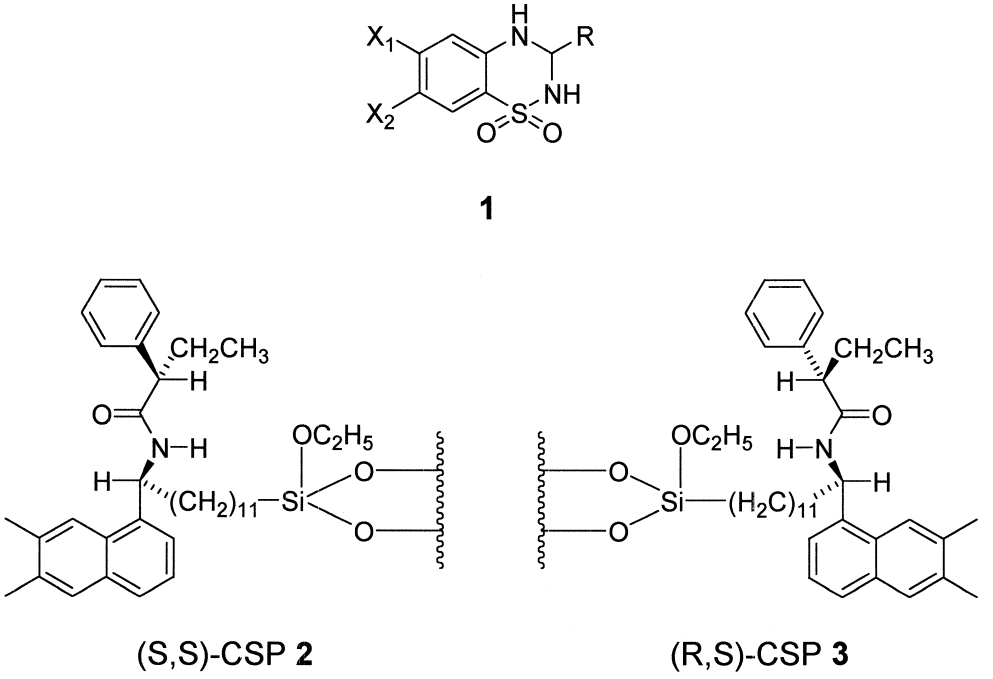

Fig. 1. Structure of thiazides **1** and CSPs **2** and **3**.

In this study, various thiazides, **1**, were chromatographed on diastereomeric CSPs **2** and **3** (Fig. 1), typically with acceptable resolution on the (*S*,*S*)-CSP. While the chromatographic results provide some insights into the possible chiral recognition process, it is not completely clear as to how such recognition occurs. Owing to the fact that absolute configurations of these thiazides are unknown, no correlation between elution order and absolute configuration can presently be made.

2. Experimental

2.1. General

^{1}H NMR spectra were recorded on a Varian XL-200 Spectrometer, IR spectra on a Nicolet 7199 FT-IR Spectrometer. High resolution mass spectra were obtained on a varian 731 Mass Spectrometer. Melting points were taken on a Buchi apparatus and are uncorrected. Enantiomeric purities of starting materials were determined by chromatography using a CSP derived from cis-3-(*tert*.-butyl)-4-phenyl-2-azetidinone [9].

Chromatography was performed by using an Anspec HPLC pump, a Rheodyne Model 7125 Injector with a 20-μl sample loop, LDC/Milton Roy UV Monitor D fixed Wavelength Detector (254 nm and 280 nm) and a Kip-Zonen BD 41 recorder. All chromatographic experiments were carried out at a nominal flow-rate of 2 ml/min. Column void volume was measured by injection of tri-*tert*.-butylbenzene [10]. A Rudolph Autopol III polarimeter containing a 20-cm flow cell was used to monitor the optical activity of the eluting enantiomers at 589 nm.

2.2. Materials

CSPs **2** and **3** are prepared by the procedure shown in Fig. 2 and the details for the preparation are described in the synthesis section. (*S*)-2-Phenylbutanoic acid is available commercially from Aldrich. Thiazide diuretics and their analogues were provided by other workers or prepared by simply treating 2-aminobenzensulfonamides with aldehydes

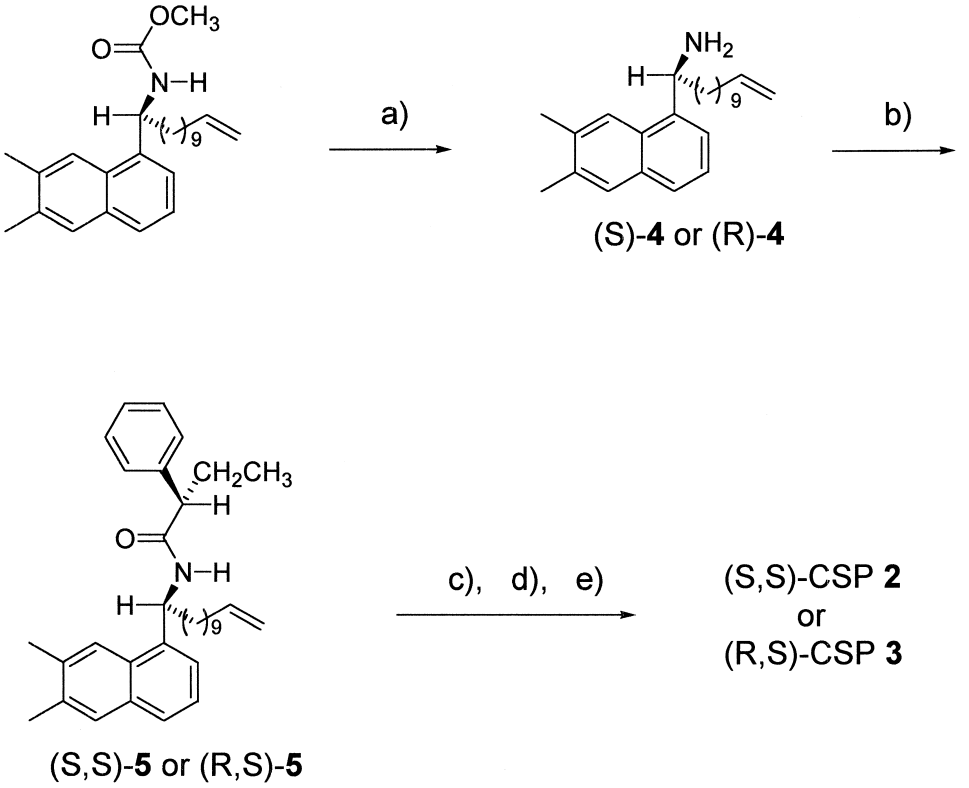

Fig. 2. Preparation of CSPs **2** and **3**. (a) KOH, dimethylsulfoxide, room temperature, (b) (*S*)-2-Phenylbutanoyl chloride, triethylamine, CH_2Cl_2, room temperature, (c) $HSiCl_3$, chloroplatinic acid, reflux, (d) Ethyl alcohol/triethylamine, CH_2Cl_2, (e) 5 μm Silica gel, 100°C (Kugelrohr), reduced pressure.

in ethanol containing a catalytic amount of hydrogen chloride [11].

*2.3. Synthesis of CSPs **2** and **3***

*2.3.1. (R)-α-[1-(6,7-dimethyl)naphthyl]-11-dodecenylamine, (r)-**4***

Preparation of this amine from the corresponding enantiomerically pure *N*-methoxycarbonyl derivative (carbamate derivative) which had been resolved using a preparative chiral column has been described previously [12]. However, in this study (*R*)-**4** was prepared by a more convenient method. The resolved carbamate (1.50 g, 0.0038 mole) was dissolved in 115 ml of dimethylsulfoxide containing 3.5 g of KOH pellets and the mixture was stirred at room temperature for 12 h. After dilution with 1000 ml of water, the strongly basic (pH>11) solution was extracted twice with diethyl ether. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness to afford the amine as a yellow liquid which was used directly for the next reaction.

*2.3.2. (R)-N-[(S)-1-phenylbutanoyl]-α-[1-(6,7-dimethyl)naphthyl]-11-dodecenylamine, (R,S)-**5***

Crude amine (*R*)-**4**, obtained as above, and 1 ml of triethylamine were dissolved in 150 ml of methylene chloride. To the stirred solution was added (*S*)-2-phenylbutanoyl chloride prepared by the reaction of (*S*)-2-phenylbutanoic acid (0.821 g, 0.005 mole) with thionyl chloride in refluxing benzene. This mixture was stirred at room temperature for 20 min, then washed sequentially with 1 N NaOH solution, 1 N HCl solution and then water. The methylene chloride solution was dried over anhydrous $MgSO_4$, concentrated and flash chromatographed to afford 1.70 g (overall 92% yield from carbamate) of amide (*R,S*)-**5** [99+% ee as determined by HPLC using a CSP

derived from cis-3-(*tert*.-butyl)-4-phenyl-2-azetidinone] as a white solid. m.p. 126–128°C. ^{1}H NMR ($CDCl_3$): δ 0.83(t, 3 H), 1.10–1.40(broad m, 14 H), 1.70–2.30(m, 6 H), 2.42(s, 3 H), 2.45(s, 3 H), 3.15(t, 1 H), 4.88–5.05(m, 2 H), 5.55–5.92(m, 3 H), 7.20–7.40(m, 7 H), 7.58–7.70(m, 2 H), 7.90(s, 1 H). IR (KBr): cm^{-1} 3300, 3060, 2920, 2860, 1630, 1600, 1530. High resolution mass spectrum: calcd. for $C_{34}H_{45}NO$ 483.3501; found 483.3514

*2.3.3. (S)-N-[(S)-1-phenylbutanoyl]-α-[1-(6,7-dimethyl)naphthyl]-11-dodecenylamine, (S,S)-**5***

Amine, (*S*)-**4**, obtained from 1.40 g (0.0035 mole) of carbamate as described above was treated with (*S*)-2-phenylbutanoyl chloride [prepared from (*S*)-2-phenylbutanoic acid (0.821 g, 0.005 mole)] as described above to afford 1.60 g of (*S,S*)-**5** (overall 93% yield from carbamate) of greater than 99% ee as a white solid. m.p. 140–142°C. ^{1}H NMR ($CDCl_3$): δ 0.90(t, 3 H), 1.18–1.44(m, 14 H), 1.70–2.30(m, 6 H), 2.33(s, 3 H), 2.40(s, 3 H), 3.23(t, 1 H), 4.88–5.05(m, 2 H), 5.60–5.92(m, 3 H), 7.05–7.38(m, 7 H), 7.52–7.63(m, 2 H), 7.82(s, 1 H). IR (KBr): cm^{-1} 3280, 3060, 2920, 2860, 1630, 1600, 1530. High resolution mass spectrum: cald. for $C_{34}H_{45}NO$ 483.3501; found 483.3514

*2.3.4. Chiral stationary phase (S,S)-**2***

Amide, (*S,S*)-**5** (1.40 g, 0.003 mole) was dissolved in 20 ml of trichlorosilane, 1.5 ml of the chloroplatinic acid solution (71.5 mg of $H_2PtCl_6 \cdot 6\ H_2O$ in 20 ml of isopropyl alcohol) was added and the mixture was heated to reflux for 1.5 h. Excess trichlorosilane was removed by simple distillation and chased with methylene chloride several times. The oily residue was dissolved in 30 ml of methylene chloride and 1.5 ml of ethyl alcohol and 2 ml of triethylamine were added in one portion. After stirring the mixture for 20 min, all the solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel to afford the triethoxysilane derivative of the amide. ^{1}H NMR [($CDCl_3$) δ 0.57–0.68 (m, 2 H), 0.90(t, 3 H), 1.18–1.45(m, 27 H), 1.68–2.30(m, 4 H), 2.33(s, 3 H), 2.40(s, 3 H), 3.23(t, 1 H), 3.82(q, 6 H), 5.60-5.78(m, 2 H), 7.05–7.38(m, 7 H), 7.52–7.63(m, 2 H), 7.82(s, 1 H)]. This chiral silane was bonded to 5 μm Spherisorb silica gel as follows: water was azotropically removed (Dean-Stark trap) from a slurry of 7.5 g of 5 μm Spherisorb silica gel in 50 ml of benzene. The chiral silane dissolved in 50 ml of dry methylene chloride was added to the silica slurry and the mixture was sonicated briefly before the solvent was removed under reduced pressure. The residual slurry was heated to 100°C for 15 h in a Kugelrohr apparatus. The modified silica gel was then washed thoroughly with methylene chloride, methyl alcohol, ethyl acetate, diethyl ether and pentane and was packed into 250×4.6 mm I.D. stainless steel column using methyl alcohol as a packing solvent. The column was end-capped by passing hexamethyldisilazane in methylene chloride through the column. Micro analysis for CSP-(*S,S*)-**3**: found C, 9.30%; H, 1.25%; N, 0.29%, calcd. 0.215 mmoles of chiral selector/g of stationary phase based on C, 0.207 mmoles of chiral selector/g of stationary phase based on N.

*2.3.5. Chiral stationary phase (R,S)-**3***

This CSP was prepared from 1.50 g (0.0031 mole) of (*R,S*)-**5** and 7.5 g of 5 μm Spherisorb silica gel and then packed into 250×4.6 mm I.D. stainless-steel column and end-capped as described above. ^{1}H NMR of the triethoxysilylated amide ($CDCl_3$) δ 0.57–0.68(m, 2 H), 0.83(t, 3 H), 1.10–1.45(m, 27 H), 1.68–2.30(m, 4 H), 2.42(s, 3 H), 2.45(s, 3 H), 3.15(t, 1 H), 3.82(q, 6 H), 5.55–5.80(m, 2 H), 7.20–7.40(m, 7 H), 7.58–7.70(m, 2 H), 7.90(s, 1 H). Micro analysis: found C, 9.38%; H, 1.30%; N, 0.28%, calcd. 0.217 mmoles of chiral selector/g of stationary phase based on C, 0.200 mmoles of chiral selector/g of stationary phase based on N.

3. Results and discussion

A number of CSPs derived from variously *N*-acylated α-(1-naphthyl)ethylamines have been reported and several are available as commercially packed high-performance liquid chromatography (HPLC) columns [13–16]. Typically, the *N*-acyl group also serves as a tether to silica even in those instances where the *N*-acyl group is itself chiral. We can only assume this is done for the sake of convenience since we have previously shown that tethering through the *N*-acyl group often reduces the

enantioselectivity of the resulting CSP [13]. In the mid-1980s, we described several CSPs derived from α-(1-naphthyl)ethylamine and from several structurally related amines, the structures of which were chosen to improve enantioselectivity (when *N*-acylated) and to avoid tethering through the *N*-acyl group. This allows a free choice of the *N*-acylating agent. For example, *N*-acylation of optically active (*R*)- and (*S*)-amine **4** with (*S*)-2-phenylbutanoyl chloride leads to two diastereomeric amides, lower melting (*R*,*S*)-**5** and higher melting (*S*,*S*)-**5**. Hydrosilylation of these amides with trichlorosilane in the presence of chloroplatinic acid and subsequent treatment with ethanol-triethylamine affords triethoxysilylated amides. These triethoxysilylated amides were bonded to 5 μm Spherisorb silica gel to afford CSPs **2** and **3** (Fig. 2).

While there is nothing particularly novel in diastereomeric CSPs similar to α-(1-naphthyl)ethylamines acylated with a chiral moiety, we had a rational basis for preparing CSPs **2**and **3**; the phenyl in the acyl substituent is a potential site for face to edge π–π interactions with selected analytes.

Various racemic thiazides, **1**, some pharmaceuticals and some prepared for this study, were chromatographed on CSPs **2** and **3** using 20% 2-propanol in hexane as the mobile phase. A 254 nm ultraviolet detector and a 589 nm polarimetric detector coupled in series allowed, in most instances, determination of the signs of the optical rotation of the enantiomers as they eluted. These data are presented in Table 1.

As shown in Table 1, all racemic thiazides tested were resolved with modest but reasonable separation factors on CSP-(*S*,*S*)-**2**. The (−)-enantiomers are found to always elute first with the exception of methylchlothiazide (entry 5). However, the resolutions of the thiazides on CSP-(*R*,*S*)-**3** are less satisfactory, several not being resolved. In contrast to the resolutions on CSP-(*S*,*S*)-**2**, the (+)-enantiomers typically are found to elute first on CSP-(*R*,*S*)-**3**. In the case of bemetizide (entry 6) which has two chiral centers, the enantiomers of each diastereomer are resolved on both CSPs **2** and **3**. On CSP-(*S*,*S*)-**2**, the sign of optical rotation of the first eluted two enantiomers of bemetzide are consistent with other samples. However, on CSP-(*R*,*S*)-**3**, the elution order of the enantiomers of one diastereomer are reversed relative to the other entries in Table 1.

From the general trends in the sign of rotation of the two enantiomers on CSP-(*S*,*S*)-**2** and CSP-(*R*,*S*)-**3**, it seems that the enantioselectivity is controlled principally by the α-[1-(6,7-dimethyl)naphthyl]-10-undecenylamine portion of the CSP. From our gener-

Table 1
Resolution of thiazide diuretic drugs and analologues on (*S*,*S*)-CSP **2** and (*R*,*S*)-CSP **3**[a]

Entry[b]	Diuretics **1**			(*S*,*S*)-CSP **2**			(*R*,*S*)-CSP **3**		
	X_1	X_2	*R*	k'_1	k'_2	α	k'_1	k'_2	α
1	CF_3	SO_2NH_2	$CH_2C_6H_5$	6.97 (−)	8.70 (+)	1.25	4.90 (+)	5.33 (−)	1.09
2	CF_3	SO_2NH_2	$(CH_2)_4CH_3$	5.64 (−)	6.54 (+)	1.16	3.99	3.99	1.00
3	Cl	SO_2NH_2	CH_2-cyclo-Pentyl	7.21 (−)	13.03 (+)	1.81	5.14 (+)	7.36 (−)	1.43
4	Cl	SO_2NH_2	$CH_2CH(CH_3)_2$	5.94 (−)	10.99 (+)	1.85	4.33 (+)	6.14 (−)	1.42
5	Cl	SO_2NH_2	CH_2Cl	13.59 (+)	16.31 (−)	1.20	8.14	8.14	1.00
6	Cl	SO_2NH_2	$CH(CH_3)C_6H_5$	5.01 (−)	6.23 (+)	1.24	4.07 (+)	5.21 (−)	1.28
				12.29 (−)	14.83 (+)	1.21	8.69 (−)	10.29 (+)	1.18
7	Cl	SO_2NH_2	$(CH_2)_8CH{=}CH_2$	6.93 (−)	8.43 (+)	1.22	4.86	4.86	1.00
8	Cl	SO_2NH_2	CH_2CH_3	8.26 (−)	9.33 (+)	1.13	5.71	5.71	1.00
9	Cl	SO_2NH_2	$CH_2CH_2CH_3$	8.63 (−)	10.89 (+)	1.26	6.21 (+)	6.71 (−)	1.08
10	Cl	SO_2NH_2	Cyclohexyl	9.29 (−)	11.61 (+)	1.25	7.21 (+)	8.29 (−)	1.15
11	H	H	$(CH_2)_6CH_3$	2.44 (−)	3.74 (+)	1.53	1.93 (+)	2.41 (−)	1.25
12	H	H	$(CH_2)_{10}CH_3$	2.13 (−)	3.29 (+)	1.54	1.60 (+)	1.97 (−)	1.23

[a] Chromatographic conditions: mobile phase, 20% isopropyl alcohol in hexane; flow-rate, 2.00 ml/min, ambient temperature. k'_1, Capacity factor for the first eluted enantiomer. k'_2, Capacity factor for the second eluted enantiomer. α, Separation factor. The sign of the optical rotation of each enantiomer is in the parenthesis.

[b] Commercial names of diuretic drugs are as follows: Entry 1, bendroflumethiazide; 2, penfluthiazide; 3, cyclopenthiazide; 4, buthiazide; 5, methylchlothiazide; 6, bemetizide.

al experience with CSPs similar to **2** and **3**, one might imagine that the aromatic portion of the thiazides act as a π-acidic site owing to the fact that trifluoromethyl, chloro and/or sulfonamide substituents are electron withdrawing groups [13]. While there may be face-to-face π–π interaction between the aromatic ring of the analytes and the 6,7-dimethylnaphthyl portion of the CSPs, no such assertion is presently made even though such an assumption would facilitate the rationalization of why the stereochemistry of the α-[1-(6,7-dimethyl)naphthyl]-10-undecenylamine portion of the CSP seems to typically control the elution order of the enantiomers. Note that entries 11 and 12 lack the trifluoromethyl, chloro, or primary sulfonamide substituents and show increase separation factors (α values) and reduced retention. Presumably, this stems from the fact that the primary sulfonamide substituent is not essential to the chiral recognition process but instead leads to achiral retention processes.

The degree of the chiral recognition of thiazides, **1**, on CSPs **2** and **3** is affected by the stereochemistry of the second stereogenic group of the CSP. The conformations of CSPs **2** and **3** shown are thought to be heavily populated based on the conformational preferences of other amides [13,17,18]. In the case of (*S*,*S*)-CSP **2**, the phenyl and naphthyl substituents are syn, projecting from the same face of the *Z*-amide group, and their planes are roughly orthogonal to each other creating a kind of 'pocket' or cleft. However, the low energy conformation of the (*R*,*S*)-CSP **3** lacks such a pocket as the aryl substituents project from opposite sides of the *Z*-amide group. Face-to-edge π–π interactions are receiving increased attention as an important element in chiral recognition [19–21]. The greater retention and enantioselectivities shown by the enantiomers of thiazides on (*S*,*S*)-CSP **2** than on (*R*,*S*)-CSP **3** are consistent with the occurrence of face to edge π–π interaction on the former CSP but not the latter.

In conclusion, diastereomeric CSPs **2** and **3** prepared from (*S*)- or (*R*)-α-[1-(6,7-dimethylnaphthyl)]-10-undecenylamine and (*S*)-2-phenylbutanoic acid can be used for the resolution of the enantiomers of thiazide diuretics and analogues. In general, the sense of enantioselectivity seems to be controlled by the stereochemistry of the amine portion of the CSP although the degree of the enantioselectivity is affected by the stereochemistry of the acyl portion. The chiral pocket developed by the two aryl groups of (*S*,*S*)-CSP **2** may be responsible for simultaneous face-to-face and face-to-edge π–π interaction with the aromatic portion of the thiazides but, while plausible, this view is speculative. Elucidation of the chiral recognition mechanisms used by (*S*,*S*)-CSP **2** and (*R*,*S*)-CSP **3** for the resolution of thiazides and analogues must await further study and determination of the absolute configurations of several representative thiazides.

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References

[1] R. Crossley, Chirality and the Biological Activity of Drugs: New Directions in Organic and Biological Chemistry, CRC Press, Boca Raton, FL, 1995.
[2] A.M. Krstulovic (Ed.), Chiral Separations by HPLC: Applications to Pharmaceutical Compounds, Ellis Horwood Ltd, Chichester, 1989.
[3] W.J. Lough (Ed.), Chiral Liquid Chromatography, Blackie, Glasgow, 1989.
[4] D.P. Uzunov, I. Zivkovich, W.H. Pirkle, E. Costa, A. Guidotti, J. Pharm. Sci. 84 (1995) 937.
[5] G. Blaschke, J. Maibaum, J. Pharm. Sci. 74 (1985) 438.
[6] D.W. Armstrong, Y. Tang, S. Chen, Y. Chou, C. Bagwill, J.R. Chen, Anal. Chem. 66 (1994) 1473.
[7] D.W. Armstrong, Y. Liu, K.H. Ekborg-Ott, Chirality 7 (1995) 474.
[8] S.G. Allenmark, S. Andersson, P. Moller, D. Sanchez, Chirality 7 (1995) 248–256.
[9] W.H. Pirkle, J.E. McCune, J. Chromatogr. 441 (1988) 311.
[10] W.H. Pirkle, C.J. Welch, J. Liq. Chromatogr. 14 (1991) 1.
[11] C.W. Whitehead, J.J. Traverso, H.R. Sullivan, F.J. Marshall, J. Org. Chem. 26 (1961) 2814.
[12] M.H. Hyun, W.H. Pirkle, J. Chromatogr. 393 (1987) 357.
[13] W.H. Pirkle, M.H. Hyun, B. Bank, J. Chromatogr. 316 (1984) 585.
[14] C.J. Welch, J. Chromatogr. A 666 (1994) 3.
[15] N. Oi, H. Kitahara, R. Kira, J. Chromatogr. 535 (1990) 213.
[16] N. Oi, H. Kitahara, F. Aoki, J. Chromatogr. A 694 (1995) 129.

[17] G. Helmchen, H. Volter, W. Schuhle, Tetrahedron Lett. 18 (1977) 1417.
[18] G. Helmchen, G. Nill, D. Flockerzi, W. Schuhle, M.S.K. Youssef, Angew. Chem. Int. Ed. Engl. 18 (1979) 62.
[19] W.H. Pirkle, C.J. Welch, Tetrahedron Asymm. 5 (1994) 777.
[20] M.H. Hyun, C.S. Min, Y.J. Cho, M.S. Na, J. Liq. Chromatogr. 18 (1995) 2527.
[21] W.H. Pirkle, Y. Liu, J. Chromatogr. A 749 (1996) 19.