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EXAMPLE OF THE CONCENTRATION DEPENDENCE OF ELUTION ORDER IN THE RESOLUTION OF ENANTIOMERS ON MICROCRYSTALLINE TRIACETYLCELLULOSE CHIRAL STATIONARY PHASE

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SUMMARY

The resolution of 3-(2-propylphenyl)-4-methyl-4-thiazolin-2-one atropisomers by liquid chromatography on microcrystalline triacetylcellulose shows an unprecedented inversion of the capacity factors with increasing amount of sample injected. The two enantiomers behave independently on the chiral stationary phase. The occurrence of such different isotherms for the two enantiomers is related to the presence of a propyl group and provides experimental proof of the intervention of different sites for chiral recognition in the supramolecular structure of microcrystalline triacetylcellulose.

INTRODUCTION

In recent years, the separation of enantiomers by liquid chromatography on chiral stationary phases (CSPs) and the design of new CSPs have aroused wide interest[1–15]. Among the various commercially available phases of this type, microcrystalline triacetylcellulose (MTAC)[16–20] is attractive as it has been used with success in separating various racemates on a preparative scale (Pirkle and Hamper[21] "preparative" separations as those involving the collection of the resolved materials for subsequent utilization, whether this isolation affords sub-milligram or multi-gram amounts of material).

During a systematic study of the factors that affect chiral discrimination on MTAC for a series of atropisomers of N-arylthiazolin(thi)ones, we found that 3-(2-propylphenyl)-4-methyl-4-thiazolin-2-one (3a) shows an unprecedented behaviour on MTAC; chromatography of racemic 3a with polarimetric detection indicates that the first eluted enantiomer is dextrorotatory for an analytical-scale sample (3 mg), whereas the first eluted enantiomer is laevorotatory for semi-preparative-scale injections (50 mg). We believe that this observation may open the way to a better understanding of the type of molecular recognition involved in these chiral separations.

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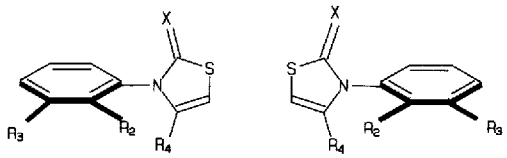


1a $R_2=C_3H_7$, $R_3=H$, $R_4=CH_3$, X=S

3a $R_2=C_3H_7$, $R_3=H$, $R_4=CH_3$, X=O

1b $R_2=CH_3$, $R_3=CH_3$, $R_4=CH_3$, X=S

3b $R_2=CH_3$, $R_3=CH_3$, $R_4=CH_3$, X=O

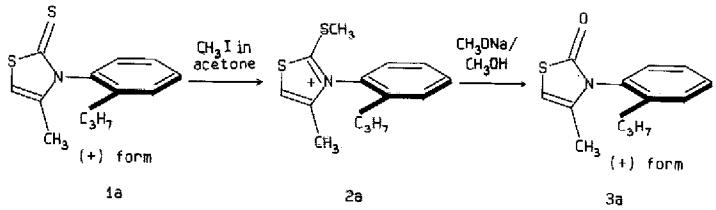
Scheme 1.

EXPERIMENTAL AND RESULTS

N-Arylthiazoline-2-thiones and their oxygen analogues bearing alkyl substituents in positions 4 and 2′ have a steric barrier to rotation around the N–aryl bond larger than 29 kcal mol^{-1}, giving rise to stable atropisomers which can be eventually separated at room temperature on MTAC (Scheme 1) [the lower limit was determined for thiazoline with methyl groups in the 4 and 2′ positions in diglyme; in ethanol the barriers are larger owing to the specific solvation of the (thio)carbonyl group[22,23]. For a general discussion of steric barriers in atropisomers in heterocycles, see ref. 24]. These compounds are interesting models for a study of molecular recognition on MTAC and on other CSPs because (i) a wide variety of steric requirements through alkyl substituents can be regioselectively introduced in various positions on the aryl ring and in the 4 and 5 positions on the heterocycle by unequivocal syntheses, (ii) X-ray data indicate that the two rings are almost perpendicular in the crystalline state[22–24] and the absolute configurations of the 3-(2′-alkylphenyl)-4-alkyl-4-thiazoline-2-thiones are known [the absolute configuration has been determined on optically pure (+)-3-(2′-methylphenyl)-4-*tert.*-butyl-4-thiazoline-2-thione by X-ray analysis of the salt obtained by reaction with an optically active menthyl derivative of known configuration[25]], (iii) optically pure thiazoline-2-thiones can be converted into optically pure thiazolin-2-ones without racemization at room temperature and (iv) the two parts of these compounds have very different dipolar requirements, basicities and hydrogen bonding abilities. In order to investigate the effect of the lipophilicity of the alkyl chain in the 2′ position on the aryl ring on the separation on MTAC, we prepared 3-(2-propylphenyl)-4-methyl-4-thiazoline-2-thione (1a) and its oxygen analogue (3a).

The determinations of the chromatographic parameters (capacity factors and separation coefficients) were performed on a thermostated (20°C) 200 mm × 25 mm I.D. MTAC (15–25 nm) (Merck) column eluted with 95% ethanol at a flow-rate of 138 ml/h with UV and polarimetric detection. The dead volume of the column was determined from the elution volume of 1,3,5-tri-*tert.*-butylbenzene, which was injected together with the sample in ethanol using a 5-ml injection loop[26].

In the general experimental procedure, the compounds studied were injected in an analytical run (*ca.* 2 mg) in racemic form. If baseline separation was observed, the chromatographic parameters were extracted directly from the chromatogram using either UV or polarimetric data. If partial resolution was obtained, UV detection gave



1a 2a 3a

Scheme 2.

a useless single envelope whereas the polarimeter indicated the order of elution and the shape of the resulting signal. In the latter instance, a larger amount of the compound was injected in order to collect the very beginning and the very end of the peak, and this procedure was repeated until enantiomerically pure samples were obtained in milligram amounts, which could be injected separately so as to determine the chromatographic parameters.

The thiazolinethione 1a showed a baseline separation, the dextrorotatory enantiomer appearing first, whereas the thiazolinone 3a was partially resolved with again the dextrorotatory enantiomer appearing first. On applying the general procedure described above to a larger amount of 3a, we found that the first eluted enantiomer is the laevorotatory form. In order to study this unexpected behaviour,we prepared optically pure (+)-3a and (−)-3a from the corresponding thiones (+)-1a and (−)-1a after preparative separation of the latter on MTAC (Scheme 2). Known amounts of optically pure samples of (+)-3a were injected into the column and the peak shapes recorded using polarimetric detection. Digitization of the experimental curves and treatment by computer afforded the series of positive curves depicted in Fig. 1 together with the experimental area and the position of the barycentre [the barycentre (moment of mass) was determined instead of the peak maximum to account for the asymmetry of the curves] (Table I).

It appears that the capacity factor k'^{+} increased from 1.37 to 1.64 when the amount of compound injected was increased from 1.82 to 16.36 mg. The same experiments performed on the laevorotatory enantiomer (−)-3a for the same amounts gave the series of negative curves depicted in Fig. 1, and it appears that the capacity factor k'^{-} decreased when the amount of compound injected was increased.

Simultaneous treatments of the two independent curves obtained for the pure enantiomers in order to simulate the response of the corresponding racemic injections are shown in Fig. 1. They account perfectly for the observed experimental inversion of the elution order on increasing the amount of compounds injected. Fig. 1c is particularly instructive as the calculated resulting curve obtained by combination of the two separate injections of 4.55 mg of each enantiomer has a very unusual and sensitive shape owing to the contrasting shapes of the two constituent curves. Such an unusual shape with more than two maximal was obtained experimentally by injection of 9.10 mg of racemic sample. It is worth noting that the different shapes of the two curves indicate that under conditions of identical capacity factors on would still observe a signal with polarimetric detection. We were able to reproduce experimentally the calculated curves for racemic injections over the whole range of concentration, and it follows that the two enantiomers show completely independent behaviour on the

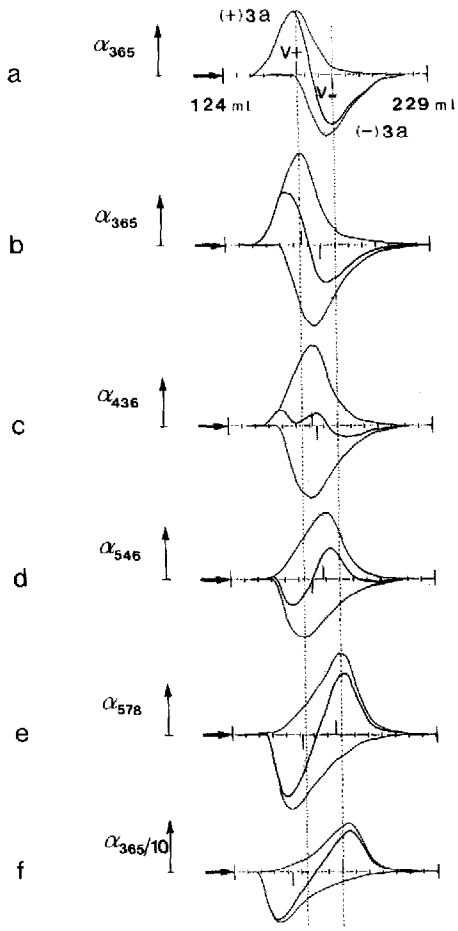


Fig. 1. Concentration dependence of elution order for 3a on MTAC (polarimetric detection). Experimental positive or negative curves were obtained by independent injections of pure (+)-3a and (−)-3a, respectively: (a) 1.82 mg; (b) 2.73 mg; (c) 4.55 mg; (d) 7.30 mg; (e) 10.45 mg; (f) 16.36 mg. The resulting curves are computer simulations of the corresponding racemic form.

column and that a displacement process is not involved in the observed phenomena.

Fig. 2 reports the variation of capacity factors with the amount of sample injected. An explanation of the behaviour of (+)-3a, which shows an increase in capacity factor with increase in the amount of sample injected, based on self-association (which is generally involved in such behaviour mainly on zeolites) seems improbable, as it should be enantioselective in order to account for the fitting of the experimental chromatogram of a racemic injection with the calculated result obtained by combination of the experimental chromatograms for each enantiomer.

It was therefore interesting to study the influence of concentration on the capacity factors of the thiazolinethione 1a. For this purpose, increasing amounts of optically pure sample were injected separately. The resulting curves do not overlap and the separation is excellent (Fig. 3 and Table I). In the same rang of concentration as for

TABLE I

CAPACITY FACTORS AND SEPARATION COEFFICIENTS FOR COMPOUNDS 1a, 3a, 1b AND 3b AS A FUNCTION OF THE AMOUNT OF SAMPLE INJECTED ON MTAC

Compound	*Fig.*	*Amount in 5 ml of ethanol (mg)*	k'^{+}*	k'^{-}	k'^{+}/k'^{-}	α**
(+)-3a	1a	1.82	1.37			
(−)-3a	1a	1.82		1.64	0.84	1.19
(+)-3a	1b	2.73	1.41			
(−)-3a	1b	2.73		1.53	0.92	1.09
(+)-3a	1c	4.55	1.43			
(−)-3a	1c	4.55		1.49	0.96	1.04
(+)-3a	1d	7.30	1.52			
(−)-3a	1d	7.30		1.44	1.06	1.06
(+)-3a	1e	10.45	1.605			
(−)-3a	1e	10.45		1.35	1.19	1.19
(+)-3a	1f	16.36	1.645			
(−)-3a	1f	16.36		1.27	1.30	1.30
(+)-1a	3a	1.82	1.04			
(−)-1a	3a	1.82		3.09	0.366	2.98
(+)-1a	3b	4.54	1.02			
(−)-1a	3b	4.54		2.88	0.354	2.82
(+)-1a	3c	9.10	1.00			
(−)-1a	3c	9.10		2.64	0.379	2.64
(+)-1a	3d	13.64	0.98			
(−)-1a	3d	13.64		2.51	0.391	2.55
(+/−)-1b	4a	3.27	0.97	3.29	0.296	3.38
(+/−)-1b	4b	24.55	0.93	3.35	0.277	3.60
(+/−)-3b	4c	3.57	0.83	2.00	0.415	2.41
(+/−)-3b	4d	25.57	0.835	1.97	0.425	2.35

* Defined by $V^{+} - V^{ref}/V^{ref}$ using barycentric calculation to account for tailing.
** Defined as the ratio of the capacity factor of the more bound to that of the less bound enantiomer.
*** Injected in racemic form.

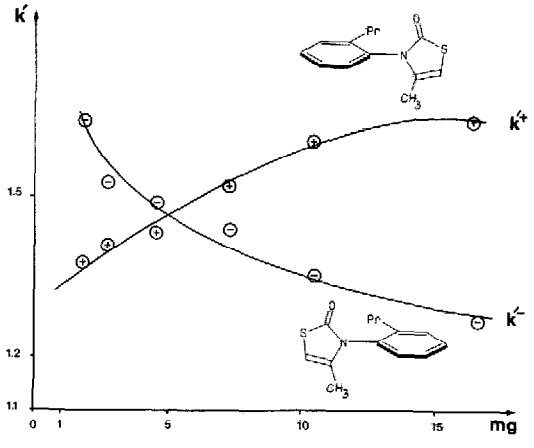


Fig. 2. Variation of the capacity factors k'^{+} and k'^{-} for 3a as a function of amount injected.

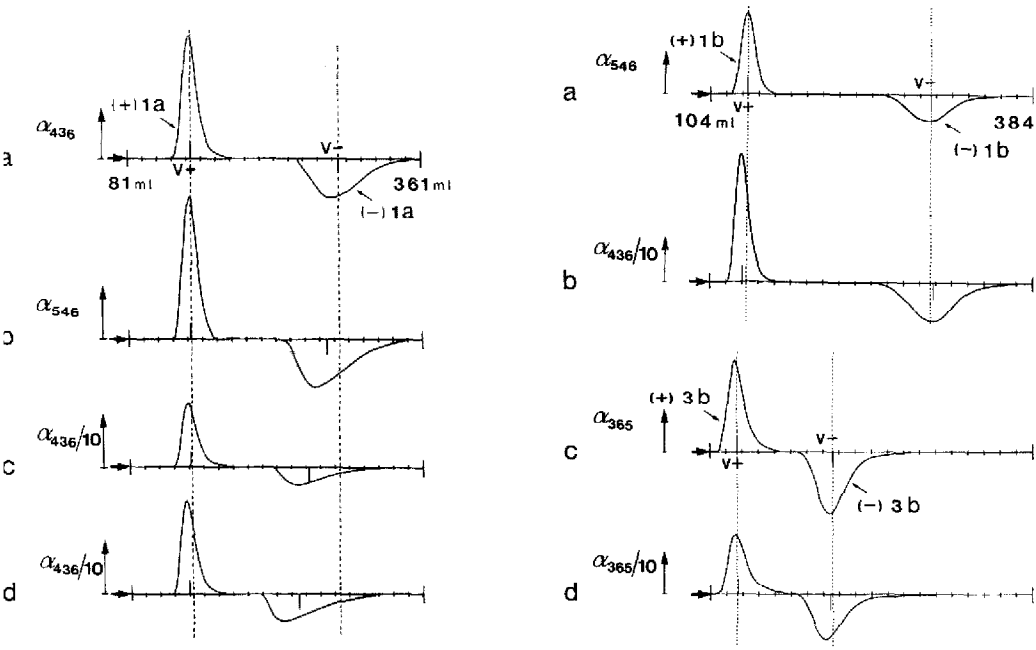


Fig. 3. Concentration dependence of the chromatographic behaviour of 1a on MTAC. Positive and negative curves were obtained by independent injections of pure (+)-1a and (−)-1a, respectively: (a) 1.82 mg; (b) 4.54 mg; (c) 9.10 mg; (d) 13.64 mg.

Fig. 4. Chromatographic behaviour of 1b [(a) and (b)] and 3b [(c) and (d)] as a function of the amount of sample injected (Table I). These curves were obtained by direct analysis of the racemic form; (a) 3.27 mg; (b) 24.55 mg; (c) 3.57 mg; (d) 25.57 mg.

3a, the capacity factor of the positive enantiomer decreases slightly whereas that of the negative enantiomer is much more dependent on concentration. In order to verify the independence of enantiomer behaviour on the CSP as already discussed for 3a, we prepared samples in wich 1.82 mg of the negative enantiomer was mixed with 13.6 mg of the positive enantiomer. The resulting capacity factors for bot enantiomers agree perfectly with those obtained in the respective separate injections.

The chromatographic behaviours of 1a and 3a are in sharp contrast with those reported in Table I for 3-(2,3-dimethylphenyl)-4-methyl-4-thiazoline-2-thione (1b) and its oxygen analogue (3b) (the syntheses of these compounds have been described elsewhere[22]). The capacity factors for these two compounds display very little dependence on the amount of sample injected in the concentration range studied (Fig. 4 and Table I). The results indicate that in the concentration range considered the number of suitable sites is large enough to prevent saturation phenomema for compounds very similar to 1a and 3b. Hence it appears that the presence of a lipophilic propyl group in 1a and 3a might account for the concentration dependence of the capacity factors.

To the best of our knowledge, very little attention has been paid to the effect of the amount of racemates injected on resolution on MTAC[27] and on other CSPs. Some

examples were provided by Soah and Cram[28,29] for the resolution of enantiomers of amino acid and ester salts in host–guest complexation studies[28,29]. No inversion of the elution order was observed but the chiral recognition was dependent on the host:guest ratio and it was suggested that "the sterically more confined sites which exhibit higher chiral recognition were engaged in binding as the amounts of guest were increased". We believe such an explanation might hold for compounds 3a. An example of the inversion of elution order with increasing sample size for achiral compounds as been reported recently and was accounted for by isotherm crossing, but in that instance both compounds exhibited a differential decrease in capacity factors with increasing sample size.[30]

We are currently extending this type of study to tailored racemates with suitable substitution to improve our knowledge of the mechanism of chiral recognition on MTAC and similar CSPs. So far our experimental results confirm the concurrence of various sites for chiral recognition in the supramolecular structure of MTAC[31,32] which are dramatically discriminatory towards the lipophilic effect.

Synthesis of compounds

3-(2-Propylphenyl)-4-methyl-4-thiazoline-2-thione (racemic) (1a). This was prepared by reaction of the corresponding ammonium dithiocarbamate with chloropropanone[33]. Yield 64%; m.p. = 75–76°C; R_F (silica gel, chloroform as eluent) = 0.53. ^{1}H NMR (C^2HCl_3): δ (ppm) 0.95 (3H,t), 1.3–1.8 (2H,m), 1.82 (3H,s), 2.39 (2H,t), 6.35 (1H,s), 7–7.6 (4H,m). ^{13}C NMR (C^2HCl_3): δ (ppm) 14.20, 15.83, 22.32, 32.78, 106.73 (C-5), 127.40, 128.23, 129.87, 130.18, 136.30, 140,03, 140.03, 189.23 (C=S). Mass spectrum, (70 eV): *m/z* (%) 249(27), 217(15), 216(100), 214(15), 206(10), 200(9), 188(10), 187(25), 91(11), 77(12), 45(8), 41(7), 39(8). UV (95% ethanol); $\lambda(\varepsilon)$ = 320 nm (15400). Analysis: calculated for $C_{13}H_{15}NS_2$, C 62.7, H 6.0, N 5.6, S 25.7; found, C 62.65, H 6.09, N 5.62, S 25.55%.

Racemic thiazoline-2-thione (525 mg in ten injections) was separated into 235 mg of the (+)-form and 255 mg of the (−)-form. (+)-form (20.8 mg in 2 ml of 95% ethanol, 25°C): λ (nm), α, [α], Φ 436, +4.31, +414.5, +1032; 546, +1.970, +183.4, +471.6; 578, +1.667, +160.3, +399.1; 589, +1.58, +151.9, +378.2. (−)-form (23.1 mg in 2 ml of 95% ethanol, 25°C): λ (nm), α, [α], Φ 436, −4.573, −395.9, −985.8; 546, −2.088, −180.8, −450.2; 578, −1.768, −153.1, −381.2; 589, −1.677, −145.2, −361.5.

2-Methylthio-4-methyl-3-(2-propylphenyl)thiazolium iodide (racemic)(2a). Compound 1a (500 mg) in 12.5 ml of dry acetone was allowed to react for 2 h at room temperature with 0.75 ml of methyliodide. After partial evaporation, the solid was collected and washed with anhydrous diethyl ether (415 mg; yield 53%; m.p. = 131–132°C). ^{1}H NMR (C^2HCl_3): δ (ppm) 0.8–1 (3H,m), 1.4–1.8 (2H,m), 2–2.3 (2H,m), 2.15 (3H,s), 2.33 (3H,s), 7.5–7.6 (4H,m), 8.35 (1H,s). UV (95% ethanol): λ (ε) = 296 nm. (10900).

3-(2-Propylphenyl)-4-methyl-4-thiazolin-2-one (racemic) (3a). This was synthesized by an adaptation of the procedure for conversion of pyrimidine-2-thione into pyrimidine-2-one[34]. Compound 2a (350 mg) was treated at room temperature for 1 h with a mixture of sodium methoxide (810 mg) in 7.8 ml of methanol. After extraction, washing with water and drying, 211 mg of 3a were obtained as a colourless oil (quantitative yield), which after further purification by liquid chromatography on

MTAC crystallized (m.p. = 58°C). Total yield from 1a = 40%. R_F (silica gel 60, chloroform as eluent) = 0.36. ^{1}H NMR (C^2HCl_3): δ (ppm) 0.9 (3H,t), 1.5 (2H,m), 1.75 (3H,s), 2.40 (2H,m), 5.9 (1H,s), 7–7.5 (4H,m), ^{13}C NMR (C^2HCl_3): δ (ppm) 14.16, 15.63, 22.87, 33.17, 96.31, 127.19, 129.00, 129.59, 130.25, 132.72, 134.37, 141.01, 172.29 (C=O). Mass spectrum (70 eV): *m/z* (%) 233(22), 216(9), 201(16), 160(11), 144(10), 130(9), 91(3), 77(11), 41(8), 39(8). Analysis: calculated for $C_{13}H_{15}NOS$, C 67, H 6.4, N 6, S 13.7; found, C 66.45, H 6.44, N 5.95, S 13.65%.

(+)-3-(2-Propylphenyl)-4-methyl-4-thiazolin-2-one [(+)-3a]. Optically pure (+)-3a was obtained as an oil from (+)-1a by the same procedure as for the preparation of racemic 3a [total isolated yield from (+)-1a 57%]. Optical properties (19.1 mg in 2 ml of 95% ethanol, 25°C): λ (nm), α, [α], Φ 365, +3.0, +315, +734; 436, +1.646, +172.4, +401.7; 546, +0.882, +92.4, +215.2; 578, +0.764, +80., +186.4; 589, +0.727, +76.1, +177.4.

(−)-3-(2-Propylphenyl)-4-methyl-4-thiazolin-2-one [(−)-3a]. Optically pure (−)-3a was obtained as an oil from (−)-1a by the same procedure as for the preparation of racemic 3a [total isolated yield from (−)-1a 64%]. Optical properties (21.1 mg in 2 ml of 95% ethanol, 25°C): λ (nm), α, [α], Φ 365, −3.142, −297.8, −694; 436, −1.718, −162.8, −379.3; 546, −0.920, −87.2, −203.2; 578, −0.796, −75.5, −175.8; 589, −0.759, −71.9, −167.6.

REFERENCES

1 R. W. Souter, *Chromatographic Separations of Stereoisomers*, CRC Press, Boca Raton, FL, 1985, Ch. 3, pp. 87–183.
2 I. W. Wainer, *Trends Anal. Chem.*, 6 (1987) 125.
3 D. Johns, *Int. Lab.*, (1987) 66.
4 R. Däppen, H. Arm and V. R. Meyer, *J. Chromatogr.*, 373 (1986) 1.
5 D. W. Armstrong and W. Demond, *J. Chromatogr. Sci.*, 22 (1984) 411.
6 Y. Okamoto, *Chemtech.*, (1987) 176.
7 S. Allenmark, *J. Liq. Chromatogr.*, 9 (1986) 425.
8 T. J. Ward and D. W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407.
9 G. Wulff, H. G. Poll and M. Minarik, *J. Liq. Chromatogr.*, 9 (1986) 385.
10 V. A. Davankov, A. A. Kurganov and A. S. Bochkov, *Adv. Chromatogr.*, 21 (1984) 1.
11 W. H. Pirkle and J. Finn, in J. D. Morrison (Editor), *Asymmetric Synthesis*, Vol. 1, Academic Press, New York, 1983, pp. 87–124.
12 J. N. Kinkel, W. Fraenkel and G. Blaschke, *Kontakte (Darmstadt)*, 1 (1987) 3.
13 D. W. Armstrong, *Anal. Chem.*, 57 (1987) 84A.
14 M. Lienne, M. Caude, A. Tambute and R. Rosset, *Analusis*, 15 (1987) 431.
15 M. Zief and L. J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988.
16 A. Mannschreck, H. Koller and R. Wernicke, *Kontakte (Darmstadt)*, 1 (1985) 40.
17 E. Francotte and D. Lohmann, *Helv. Chim. Acta*, 70 (1987) 1569.
18 G. Blaschke, *Angew. Chem.*, 92 (1980) 14.
19 T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
20 A. Ichiba and L. Shibata, in M. Zief and L. J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988, p. 219.
21 W. H. Pirkle and B. C. Hamper, in B. A. Bidlingmeyer (Editor), *Preparative Liquid Chromatography (Journal of Chromatography Library*, Vol. 38), Elsevier, Amsterdam, 1987, p. 236.
22 C. Roussel, M. Adjimi, A. Chemlal and A. Djafri, *J. Org. Chem.*, 53 (1988) 5076.
23 C. Roussel and A. Djafri, *Nouv. J. Chim.*, 10 (1986) 399.
24 R. Gallo, C. Roussel and U. Berg, *Adv. Heterocycl. Chem.*, 43 (1988) 173.
25 C. Roussel and A. Chemlal, *New J. Chem.*, 12 (1988) 945.
26 H. Koller, K. H. Rimbock and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89.

27 G. Hesse and R. Hagel, *Justus Liebigs Ann. Chem.*, (1976) 996.
28 G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 101 (1979) 3035.
29 G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 98 (1976) 3038.
30 M. J. Gonzalez, A. Jaulmes, P. Valentin and C. Vidal-Madjar, *J. Chromatogr.*, 386 (1987) 333.
31 E. Francotte, R. M. Wolf, D. Lohman and R. Mueller, *J. Chromatogr.*, 347 (1985) 25.
32 G. Hesse and H. Hagel, *Chromatographia*, 9 (1976) 62.
33 C. Roussel and A. Djafri, *Nouv. J. Chim.*, 10 (1986) 399.
34 C. Kashima and A. Katoh, *J. Chem. Soc., Perkin Trans. 1*, (1980) 1599.