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High-performance liquid chromatographic separation of β-amino alcohols I. Separation of (*R*,*S*)-1-(dialkylamino)-2-alkanols on an amylose-based chiral stationary phase

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Abstract

Direct enantiomeric separations of racemic mixtures of (*R*,*S*)-1-(dialkylamino)-2-alkanols were achieved with a variety of alcohol-modified pentane mobile phases and a Chiralpak AD chiral stationary phase. The effects of the aliphatic component and the alcohol modifier in the mobile phase were studied independently. The best separations were obtained with pentane based mobile phases. A variety of alcohol modifiers were investigated that introduced steric factors or affected hydrogen bonding. Ring size and hetero atom effects of the substituents were noted. Some differences in enantioselectivity between columns were observed.

1. Introduction

Enantiomerically pure β-amino alcohols are important pharmacological agents in medicinal chemistry [1,2]. Therapeutic activity of these molecules can be divided into three categories based on their pharmacological action: vaso constrictors, antihypertensive agents and β-blockers [3]. Many of the β-blockers are marketed as racemic mixtures, but their mode of action is enantioselective [4,5]. The (*S*)-enantiomers are often 50–500-fold more active than their antipode [6]. The binding affinity to the β-receptor has been reported to range from 10 to 1000, for atenolol and pindolol, respectively [1].

The preparation [7–9] and use of β-amino alcohols in organic syntheses has been increasing [9]. Many important transformations of prochiral substrates into chiral compounds of high enantiomeric purity have been achieved using a catalytic amount of an enantiomerically pure β-amino alcohol as a chiral auxiliary [9,10].

There are several methods available for the synthesis of racemic β-amino alcohols [11]. Enantiomerically pure β-amino alcohols are usually obtained either from amino acids or by resolution procedures [12]. The only general asymmetric syntheses of β-amino alcohols currently available are the homogeneous asymmetric hydrogenation of α-amino ketones, using

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(*R*)-(+)- and (*S*)-(−)-2,2'-bis(diphenylphosphine)-1,1'-binaphthyl–ruthenium (BINAP–Ru) complexes with hydrogen pressures of 50–100 atm (1 atm = 101 325 Pa) [13], and the asymmetric reduction of α-amino ketones with the chiral borohydride, K Glucoride [7].

A general synthesis for the preparation of racemic β-amino alcohols via the hydroboration/oxidation of enamines has been developed [11]. This procedure was extended to the preparation of enantiomerically pure β-amino alcohols via the asymmetric hydroboration of enamines using diisopinocampheylborane at 0°C in tetrahydrofuran (THF) generating enantiomeric excesses ranging from 50 to 86% [14].

Accurate determination of the enantiomeric purity of β-amino alcohols is essential to assess their effectiveness as both therapeutic agents and chiral auxiliaries. Many chromatographic techniques [1,15–33] have been employed for the analysis of derivatized and underivatized β-amino alcohols, but the use of HPLC procedures predominate [18]. Pirkle and Burke [34] recently described a N-3,5-dinitrobenzoyl-α-amino phosphonate chiral stationary phase (CSP) that was developed specifically for the separation of β-blockers. Many direct separations of amino alcohols have also been achieved on modified cellulose and amylose CSPs [35–40].

Papers describing enantiomeric separations using cellulose or amylose based CSPs frequently include discussions of the solute-CSP interactions and recognition mechanisms. Hydrogen bonding, dipole and π–π interactions have been identified [35–43] as important interactive forces that may be used to form the diastereomeric solute–CSP complexes which yield the separations. Enantiomeric discrimination may also be influenced by steric fit in the "chiral cavity" of the CSP [43,44]. The composition of the mobile phase is an important factor in these separations. Most of the separations have been obtained using normal-phase conditions with an aliphatic carrier and alcohol modifier in the mobile phase. The type and concentration of the alcohol in the mobile phase has a significant effect on some separations [35,39–44]. Water has been added to a few of the mobile phases to improve resolution [35,45,46].

This article describes the synthesis and direct enantiomeric separation of β-dialkylamino alcohols using a 3,5-dimethylphenyl carbamate modified amylose CSP (Chiralpak AD, Chiral Technologies).

2. Experimental

2.1. Synthesis

The following reagents were purchased from Aldrich and used without further purification: 1,2-epoxyhexane, 1,2-epoxyoctane, 1,2-epoxydecane, styrene oxide, (*R*)-(+)-styrene oxide, pyrrolidine and morpholine. (*R*)-1,2-Epoxyoctane was obtained as a gift from Nippon Mining. All new compounds gave satisfactory C,H,N analyses, and their structures were further confirmed by ^{1}H and ^{13}C NMR spectrometry and Fourier transform (FT) IR spectroscopy.

2.2. Preparation of the amino alcohols

The compounds were prepared by the neat reaction of the appropriate secondary amine and 1,2-epoxyalkane or styrene oxide at reflux. Synthetic procedures will be reported elsewhere [47].

2.3. Materials

HPLC-grade pentane, hexane, *n*-heptane, methanol (MeOH) and 2-propanol (IPA) were purchased from Fisher Scientific. Absolute ethanol (EtOH) was obtained from Quantum Chemical Corp. USI Division and HPLC-grade water from a Barnstead brand NANOpure II water-purification system. Cyclohexanol, 1-octanol, *tert.*-amyl alcohol, 2,2,2-trifluoroethanol and 1,1,1,3,3,3-hexafluoro-2-propanol were purchased from Aldrich and were ≥99% pure. Mobile phases were prepared by blending appropriate volumes of liquids in a 1-l graduated cylinder and mixing with a stirring bar. The

mobile phases were neither vacuum degassed nor sparged with helium before or during their use.

2.4. Instrumentation

Chromatographic separations were achieved using a liquid chromatograph constructed from the following components: a Milton Roy reciprocating piston pump operating at 1–2 ml/min, a Rheodyne Model 7125 injector, a 250 × 4.6 mm I.D. Chiralpak AD column from Chiral Technologies and a Kratos 773 UV absorbance detector. The detector output was stored and reprocessed using a Perkin-Elmer Nelson ACCESS*CHROM data system.

2.5. Chromatography

A series of structurally similar amino alcohols and various mobile phases were used to investigate solute–CSP interactions affecting the separation of amino alcohols on an amylose-based CSP. Racemic mixtures of each compound were analyzed individually and then combined with the other compounds of the series and reanalyzed each time the composition of the mobile phase was changed.

The elution order of selected enantiomers was established by analyzing material of known absolute configuration. Capacity factors and resolutions were computed from the retention times and peak widths. The capacity factors for the first peak of each pair of enantiomers (k_1') and R_s values are provided in the appropriate tables. Mobile phase, substituent and CSP effects are described below.

3. Results and discussion

3.1. Mobile phase effects

Hydrocarbon component

The effect of the aliphatic component in the mobile phase has received little attention. Three hydrocarbons, pentane, hexane and heptane, were selected for evaluation. Pentane was selected to permit the use of a variety of alcohols in the mobile phase. MeOH has limited solubility in hexane and higher alkanes but is miscible in all proportions with pentane. Pentane is also a convenient co-solvent for EtOH and IPA.

Mobile phases containing 5% EtOH and 95% of either *n*-pentane, hexane or heptane were prepared and used to determine the effect of the aliphatic component of the mobile phase. Although slight differences in retention time and resolution were noted for some compounds, elution order, apparent peak shapes and the stereoselectivities were unaffected when only the aliphatic component was varied. However, the results shown in Table 1 for 1-(4-morpholino)-2-octanol and 2-(4-morpholino)-1-phenylethanol, compounds B and D of Table 2, revealed that improvements in resolution were achieved when pentane was used as the aliphatic component of the mobile phase. The poorest resolution was obtained with heptane.

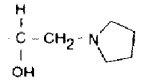
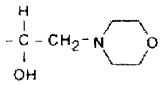
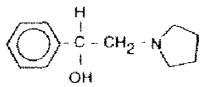
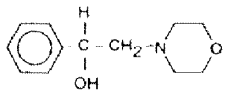
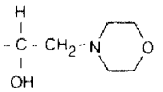
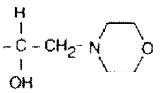
Alcohol component

The effect of the alcohol modifier in the mobile phase on retention and resolution was complex (Fig. 1 and Table 2), yet the (*R*)-enantiomers always eluted first. The results pre-

Table 1
Effect of the aliphatic component in the mobile phase on resolution

Solvent	Compound B			Compound D		
	k_1'	α	R_s	k_1'	α	R_s
Pentane	1.58	1.31	3.48	5.38	1.48	5.02
Hexane	1.52	1.30	2.97	4.78	1.49	4.56
Heptane	1.76	1.31	2.54	5.38	1.49	4.41

Table 2
Chromatographic parameters for β-dialkylamino alcohols

	R1	R2	MeOH			EtOH			IPA		
			k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
A	$CH_3(CH_2)_5$		1.07	1.32	1.57	0.56	1.36	1.79	0.86	1.00	0.00
B	$CH_3(CH_2)_5$		1.92	1.29	1.96	1.34 (1.51)	1.36 (1.63)	4.26 (1.47)	1.33	1.00	0.00
C			2.02	1.61	6.07	1.68	1.75	4.20	2.18	1.27	0.69
D			5.02	1.23	3.50	4.54	1.44	6.26	3.37	1.45	5.64
E	$CH_3(CH_2)_3$		(1.32)	(1.13)	(1.07)	(1.28)	(1.20)	(1.55)	(1.58)	(1.00)	(0.00)
F	$CH_3(CH_2)_7$		(1.11)	(1.15)	(1.23)	(1.06)	(1.18)	(1.34)	(1.17)	(1.00)	(0.00)

Results in parenthesis were obtained with a Chiralpak AD column manufactured in January 1992, all other results were obtained with a column manufactured in December 1990.

sented in Table 2 contain no obvious trends in retention, stereoselectivity or resolution when R_1 was held constant and R_2 was varied. However both retention and resolution increased when R_2 (A vs. C and B vs. D) was constant and R_1 was a phenyl group rather than an aliphatic group. Stereoselectivity was generally better when MeOH or EtOH was used as the alcohol component of the mobile phase.

The enantiomers of compounds A and B contained an aliphatic substituent at R_1 while compounds C and D contained an phenyl substituent in this position. The enantiomers of compounds A and B generally eluted before the enantiomers of compounds C and D, regardless of the other substituents at R_1 and R_2 or the composition of the mobile phase (Fig. 1). The resolution increased significantly in all mobile phases when the R_1 substituent was a phenyl group rather than the aliphatic substituent (A vs. C and B vs. D).

Peak tailing for the pyrrolidino compounds, A and C, increased as the polarity of the alcohol modifier in the mobile phase decreased (Fig. 1). The pyrrolidino group is the smallest of the amine substituents and is also more basic than the morpholino group. Consequently, ring size and the polarity of the mobile phase may play a role in peak shape. All interactions that affect peak shape are moderated by the strength of the alcohol modifier. Mobile phases containing MeOH gave the best peak shape and those containing IPA gave the worst peak shape.

Some differences in column performance and peak shape were noted when the same mixture was injected onto two columns prepared from different lots of packing material. The resolution and peak shapes obtained from the separation of

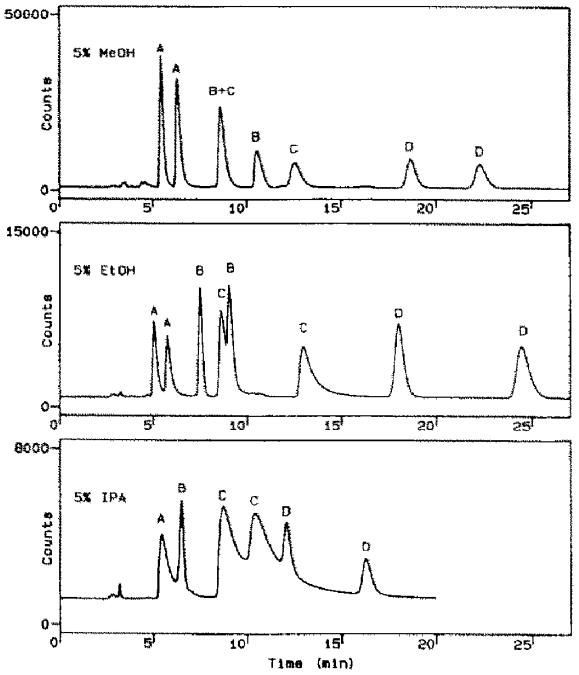


Fig. 1. Effect of MeOH, EtOH and IPA in a pentane-based mobile phase on the direct enantiomeric separation of some β-dialkylamino alcohols. Chromatographic conditions: mobile phase, 95% pentane and 5% MeOH (A), EtOH (B) or IPA (C); flow-rate 1 ml/min; injection volume, 10 μl; analytical column Chiralpak AD; UV detection at 210 nm.

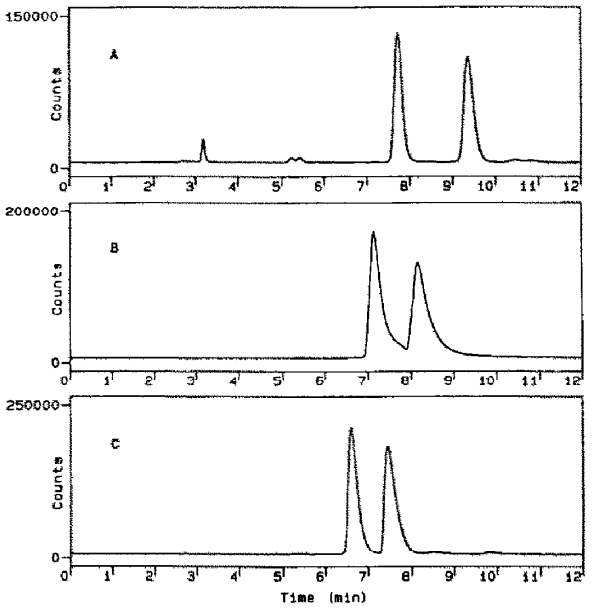


Fig. 2. Representative example of the difference in the separation of 1-(4-morpholino)-2-octanol (compound B) obtained with two different Chiralpak AD columns. Chromatographic conditions: mobile phase, ethanol–pentane (5:95); flow-rate 2 ml/min; injection volume, 10 μl; analytical column Chiralpak AD; UV detection at 210 nm. (A) Column A, manufactured in December 1990 and never exposed to diethylamine; (B) column B, manufactured in January 1992 and never exposed to diethylamine; column B after exposure to 0.1% diethylamine in the mobile phase and reequilibration with a mobile phase of ethanol–pentane (5:95).

1-(4-morpholino)-2-octanol (compound B, Table 2) were significantly different (Fig. 2).

Aboul-Enein and Serignese [39] used diethylamine in hexane-based mobile phases to improve the peak shape of a series of β-blockers that they resolved on a cellulose 3,5-dimethylphenyl carbamate CSP. Adding 0.1–0.4% diethylamine to the mobile phase slightly decreased retention times, led to sharper and more symmetrical peaks, and improved resolution.

We attempted to improve peak shape of 1-(4-morpholino)-2-octanol (Fig. 2B) by conditioning column B for several hours after adding 0.1% diethylamine to the mobile phase. The column was reequilibrated with a mobile phase containing EtOH–pentane (5:95) and the separation repeated (Fig. 2C). Retention was reduced slightly, resolution increased, and the peak tailing was decreased. The cellulose- and amylose-based stationary phases may have an affinity for some additives or compounds. The chromatograms may, therefore, reflect the history of the column or the lot of silica gel used in its manufacturing.

Specific type of alcohol

The alcohol component of the mobile phase affects enantiomeric separations obtained with cellulosic and amylosic CSPs [35,39–44]. We investigated the effect of the alcohol on the separation of 1-(4-morpholino)-2-octanol and 2-(4-morpholino)-1-phenylethanol (compounds B and D of Table 2) by using six different alcohol-modified pentane mobile phases. The modifiers included two aliphatic alcohols, EtOH and 1-octanol, two unusual modifiers, cyclohexanol and *tert.*-amyl alcohol, chosen for their steric

bulk, and two fluorinated alcohols chosen for their potential ability to influence hydrogen bonding interactions. The composition of the mobile phases was based on volume and not molarity as reported by Wainer et al. [44]. Therefore, the number of alcohol molecules available for solvation or competing for active sites was not constant in all mobile phases and may have influenced some of the results.

The effect of the alcohol modifiers is shown in Fig. 3. The following conclusions regarding changes in retention or resolution were drawn from comparisons to the separation obtained with the mobile phase EtOH–pentane (5:95). Retention was shorter when fluorinated alcohols or cyclohexanol were used and longer when *tert.*-amyl alcohol or 1-octanol were used. The best resolution was obtained with EtOH–pentane; the worst when *tert.*-amyl alcohol or cyclohexanol was used. *tert.*-Amyl alcohol and cyclohexanol are the most bulky alcohol modifiers and may interfere with the binding sites near the chiral cavity. Peak shape was affected the most when aliphatic alcohol modifiers were used. Peak tailing was pronounced with *tert.*-amyl alcohol and 1-octanol.

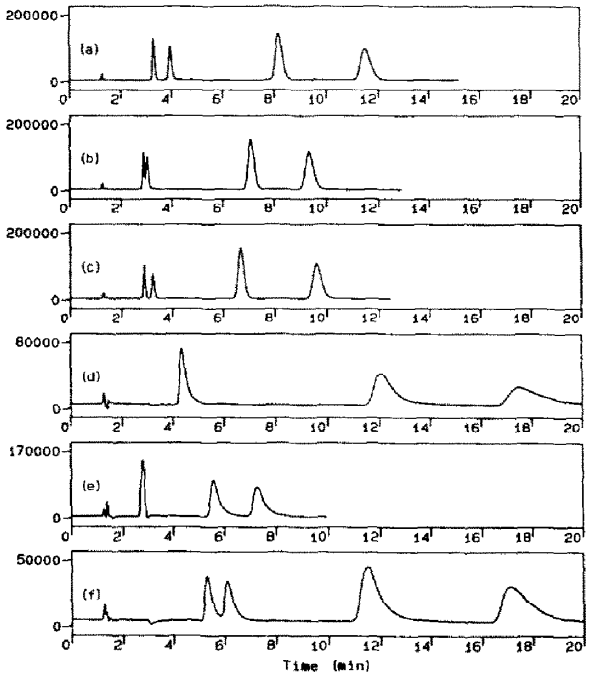


Fig. 3. Effect of alcohol modifiers in a pentane-based mobile phase on the direct enantiomeric separation of 1-(4-morpholino)-2-octanol (compound B) and 2-(4-morpholino)-1-phenylethanol (compound D). Chromatographic conditions: mobile phase 95% pentane and (a) 5% EtOH, (b) 1.5% 2,2,2-trifluoroethanol and 3.5% EtOH, (c) 1.5% 1,1,1,3,3,3-hexafluoropropanol and 3.5% EtOH, (d) 5% *tert.*-amyl alcohol, (e) 5% cyclohexanol, (f) 5% 1-octanol; flow-rate 2 ml/min; injection volume, 10 μl; analytical column Chiralpak AD; UV detection at 210 nm.

The effect of the alcohol modifiers was significantly different when R_1 was a phenyl group, for example, 2-(4-morpholino)-1-phenylethanol (compound D of Table 2). Retention was longer when *tert.*-amyl alcohol or 1-octanol were used and shorter when cyclohexanol was used. The best resolution was obtained in EtOH–pentane. The effect on peak shape was more dramatic for 2-(4-morpholino)-1-phenylethanol than 1-(4-morpholino)-2-octanol. Peak tailing was the most pronounced in *tert.*-amyl alcohol.

The use of 1.5% (v/v) of the fluorinated alcohols reduced retention and resolution regardless of the type of substituent at R_1. Peak shapes were unaffected. The fluorinated alcohols increased the eluotropic strength of the mobile phase without eliminating the hydrogen bonding essential for chiral recognition.

Effect of water

Water has been used as a mobile phase modifier in supercritical fluid chromatography [16,17] and in conventional HPLC [35,45,46] separations of enantiomers. Balmér et al. [45] initially reported that the water content of their mobile phase, 4% IPA and 0.1% diethylamine in hexane, was very important in the enantiomeric separation of metoprolol and its α-hydroxy metabolite. The addition of water did not significantly effect the retention of the (*R*)-enantiomer, but the retention of the (*S*)-isomer was nearly halved. However, the water effect may not be universal. When Balmér et al. [46] used water in the mobile phase to separate the enantiomers of almokalant on Chiracel OD and Chiralpak AD CSPs, they found that the stereoselectivity was affected. The retention of the (*R*)-isomer was decreased on both CSPs. When Balmér et al. [35] studied the effect of water on the separation

of amino alcohols they again found that it had a significant affect and caused a reversal in elution order of one of the solutes. The retention of the (*R*)-enantiomer was almost unaffected while the retention of the (*S*)-enantiomer decreased when the alcohol concentration was held constant and the water increased from 0.1 to about 1.6 g/l.

The effect of water was evaluated in our studies by comparing separations of the enantiomers of 2-(4-morpholino)-1-phenylethanol (compound D of Table 2) with and without water in the mobile phase. The separations were obtained by using mobile phases that contained known amounts of water added to IPA–pentane (5:95). Addition of up to 2000 mg/l of water generally increased retention and resolution. The addition of water significantly decreased peak tailing while increasing both retention and resolution (Fig. 4). These results indicate that it may be beneficial to add water to an IPA–pentane (5:95) mobile phase to improve separations for compounds structurally similar to 2-(4-morpholino)-1-phenylethanol.

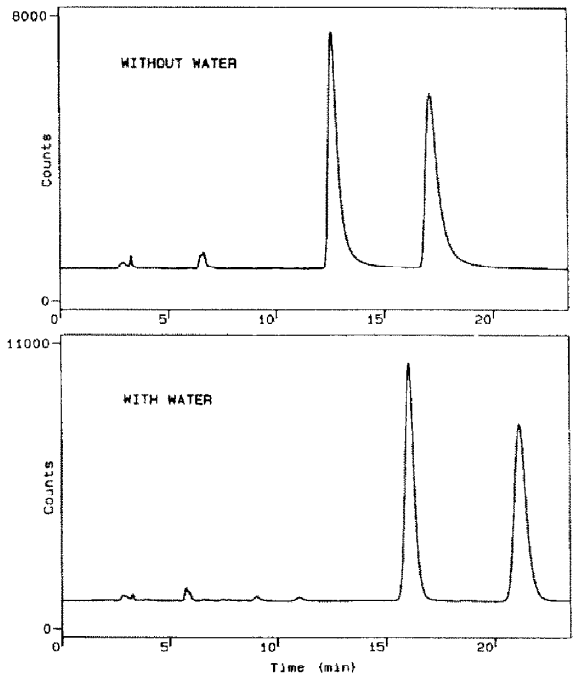


Fig. 4. Effect of adding water to an IPA–pentane mobile phase on the separation of 2-(4-morpholino)-1-phenylethanol (compound D). Chromatographic conditions: mobile phase, 0.15% water in IPA–pentane (5:95); flow-rate 1 ml/min; injection volume, 10 μl; analytical column Chiralpak AD; UV detection at 210 nm.

3.2. Substituent effects

Ring size and heteroatom effects associated with the substituent at R_2 were noted in the various mobile phases (Table 2). Retention increased when the pyrrolidino group was replaced with a morpholino group (compare A vs. B and C vs. D). Resolution also increased in all of the comparisons except for A vs. B in an IPA-modified mobile phase and C vs. D in the MeOH-modified mobile phase. The size of the R_2 group may be important from steric considerations, and the hydrogen bonding capacity of the cyclic amine appears to be enhanced by the presence of an additional heteroatom in the ring system.

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References

[1] C.L. Davies, *J. Chromatogr.*, 531 (1990) 131.
[2] D.T. Witak and M.N. Inbasekaran, in M. Grayson (Editor), *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 17, Wiley, New York, 1982, pp. 311–345.
[3] Q. Yang, Z.-P. Sun and D.-K. Ling, *J. Chromatogr.*, 447 (1988) 208.
[4] E.J. Ariëns, in A.M. Krstulovic (Editor), *Chiral Separations by HPLC: Applications to Pharmaceutical Compounds*, Wiley/Ellis Horwood, Chichester, 1989, pp. 31–68.
[5] B. Waldeck, *Chirality*, 5 (1993) 350.
[6] W. Nelson and T. Burke, *J. Org. Chem.*, 43 (1978) 3641; and references cited therein.
[7] B.T. Cho and Y.S. Chun, *Tetrahedron Asymmetry*, 3 (1992) 341.
[8] K. Soai, S. Yokoyama and T. Hayasaka, *J. Org. Chem.*, 56 (1991) 4264.

[9] K. Soai, M. Okudo and M. Okamoto, *Tetrahedron Lett.*, 32 (1991) 95.
[10] K. Soai, S. Yokoyama, T. Hayasaka and K. Ebihara, *J. Org. Chem.*, 53 (1988) 4148.
[11] C.T. Goralski, B. Singaram and H.C. Brown, *J. Org. Chem.*, 52 (1987) 4014; and references cited therein.
[12] G.M. Coppola and H.F. Schuster, *Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids*, Wiley-Interscience, New York, 1987, pp. 53, 85, 115, 133, 159, 188.
[13] M. Kitamura, T. Ohkuma, S. Inoue, N. Sayo, H. Kumobayashi, S. Akutagawa, T. Ohta, H. Takaya and R. Noyori, *J. Am. Chem. Soc.*, 110 (1988) 629.
[14] G.B. Fisher, C.T. Goralski, L.W. Nicholson and B. Singaram, *Tetrahedron Lett.*, 34 (1993) 7693.
[15] W.-Y. Li, H.L. Jin and D.W. Armstrong, *J. Chromatogr.*, 509 (1990) 303.
[16] L. Siert, N. Bargmann, A. Tambuté and M. Caude, *Chirality*, 4 (1992) 252.
[17] P. Biermanns, C. Miller, V. Lyon and W. Wilson, *LC·GC*, 11 (1993) 744.
[18] G. Egginger, W. Lindner, C. Vandenbosch and D.L. Massarat, *Biomed. Chromatogr.*, 7 (1993) 277.
[19] B. Gallinella, F. La Torre, R. Cirilli and C. Villani, *J. Chromatogr.*, 639 (1993) 193.
[20] L. Olsen, K. Brønnum-Hansen, P. Helboe, G.H. Jørgensen and S. Kryger, *J. Chromatogr.* 636 (1993) 231.
[21] Y. Dobashi and S. Hara, *J. Org Chem.*, 52 (1987) 2490.
[22] I.W. Wainer, T.D. Doyle, F.S. Fry, Jr. and Z. Hamidzadeh, *J. Chromatogr.*, 355 (1986) 149.
[23] E. Delée, L. Le Garrec, I. Jullien, S. Béranger, J.C. Pascal and H. Pinhas, *Chromatographia*, 24 (1987) 357.
[24] K. Iwaki, S. Yoshida, N. Nimura, T. Kinoshita, K. Takeda and H. Ogura, *Chromatographia*, 23 (1987) 899.
[25] S. Yamazaki, T. Takeuchi and T. Tanimura, *J. Chromatogr.*, 540 (1991) 169.
[26] J. Gal, *J. Liq. Chromatogr.*, 9 (1986) 673.
[27] C. Pettersson, E. Heldin and H.W. Stuurman, *J. Chromatogr. Sci.*, 28 (1990) 413.
[28] C. Pettersson and M. Josefsson, *Chromatographia*, 21 (1986) 321.
[29] E. Heldin, K.-J. Lindner, C. Pettersson, W. Lindner and R. Rao, *Chromatographia*, 32 (1991) 407.
[30] F. Gasparrini, D. Misiti, C. Villani and F. La Torre, *J. Chromatogr.*, 539 (1991) 25.
[31] M. Ohwa, M. Akiyoshi and S. Mitamura, *J. Chromatogr.*, 521 (1990) 122.
[32] K.M. Kirkland, K.L. Neilson and D.A. McCombs, *J. Chromatogr.*, 545 (1991) 43.
[33] G. Gübitz, B. Pierer and W. Wendelin, *Chirality*, 4 (1992) 333.
[34] W.H. Pirkle and J.A. Burke, III, *J. Chromatogr.*, 557 (1991) 173.
[35] K. Balmér, P.-O. Lagerström, B.-A. Persson and G. Schill, *J. Chromatogr.*, 592 (1992) 331.
[36] C.B. Ching, B.G. Lim, E.J.D. Lee and S.C. Ng, *Chirality*, 4 (1992) 174.
[37] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
[38] H.Y. Aboul-Enein and M.R. Isalm, *J. Chromatogr.*, 511 (1990) 109.
[39] H.Y. Aboul-Enein and V. Serignese, *J. Liq. Chromatogr.*, 16 (1993) 197.
[40] Th. Hollenhorst and G. Blaschke, *J. Chromatogr.*, 585 (1991) 329.
[41] Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
[42] H.Y. Aboul-Enein and R. Islam, *J. Liq. Chromatogr.*, 13 (1990) 485.
[43] I.W. Wainer, M.C. Alembik and E. Smith, *J. Chromatogr.*, 388 (1987) 65.
[44] I.W. Wainer, R.M. Stiffen and T. Shibata, *J. Chromatogr.*, 411 (1987) 139.
[45] K. Balmér, A. Persson, P.-O. Lagerström, B.-A. Persson and G. Schill, *J. Chromatogr.*, 553 (1991) 391.
[46] K. Balmér, P.-O. Lagerström, S. Larsson and B.-A. Persson, *J. Chromatogr.*, 631 (1993) 191.
[47] G.B. Fisher, C.T. Goralski, L.W. Nicholson and B. Singaram, *J. Org Chem.*, submitted for publication.