ELSEVIER

Journal of Chromatography A, 694 (1995) 135–150

JOURNAL OF CHROMATOGRAPHY A

Comparison of (*S*)-N-(3,5-dinitrobenzoyl)tyrosine derivatives as chiral selectors for high-performance liquid chromatographic enantioseparations

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Abstract

The synthesis of a new chiral stationary phase (CSP), based on immobilized (*S*)-N-(3,5-dinitrobenzoyl)tyrosine methyl-ester, is described and its chromatographic behaviour and enantioselectivity are compared with those of a well known dinitrobenzoyltyrosine-derived stationary phase. In addition, a new "end-capping" procedure for the CSP to reduce polar but non-stereoselective selectand–selector interactions was developed and evaluated by analyzing a broad range of chiral compounds [e.g., ibuprofen-1-naphthylamide, benzoic acid 1-phenylethylamide, 1-(1-naphthyl)ethylphenylurea, sulfoxides, propranolol oxazolidin-2-one].

1. Introduction

Chiral stationary phases (CSPs) based on N-(3,5-dinitrobenzoyl)amino acids as chiral selectors (SOs) are widely used for the enantioseparation of compounds of pharmaceutical and biological interest (selectands, SAs). The (*S*)-N-(3,5-dinitrobenzoyl)phenylglycine phase, introduced 1980 by Pirkle and co-workers [1–3], is a resolving phase with a relatively broad range of applicability and the underlying chiral recognition mechanisms are based on hydrogen bonding and/or dipole–dipole stacking paired with charge-transfer ($\pi-\pi$), steric and hydrophobic interactions. Convincing SO-SA models have been drawn for certain groups of analytes [4]. Because of the broad applicability of this type of CSP, several groups have been searching for new π-acid-type CSPs owning to their different chromatographic behaviour (e.g., [5–11]). Thus many 3,5-dinitrobenzoylated (DNB) amino acid derivatives (SOs) were synthesized in the course of developing new CSPs; however, those based on DNB-tyrosine seem to have some special features [12]. There are two possibilities of immobilizing DNB-Tyr on silica gel. The first is a similar method to the usual way of binding DNB-phenylglycine (and DNB-Leu or DNB-Val, etc.) via its carboxyl function (either ionically or covalently to an amino group), but an alternative approach is via an alkyl linkage to the phenolic hydroxyl group (see Fig. 1). Tambuté et al. [13] synthesized several tyrosine-derived CSPs containing different residues including π-acids and π-bases on either the carboxyl or the amino function. Generally, all of these CSPs were immobilized via an allyl ether group obtainable by alkylation of the phenolic hydroxyl group of tyrosine followed by a radical addition reaction

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0021-9673/95/$09.50
SSDI 0021-9673(94)00792-6

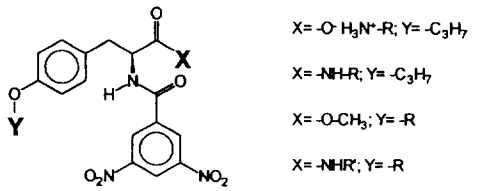


Fig. 1. DNB-Tyr derivatives as chiral selectors. The different possibilities of immobilizing DNB-Tyr on silica gel are indicated via the corresponding X and Y residues, whereas R represents various kinds of linkers between the chiral SO and the silica gel surface.

on to 3-mercaptopropylsilica gel (see CSP-Ia and CSP-Ib in Fig. 2).

In this paper we describe an alternative approach to binding DNB-Tyr covalently to silica gel, namely the formation of an allylurethane of DNB-Tyr-OMe, which was subsequently radically immobilized on 3-mercaptopropylsilica gel, resulting CSP-II (see Fig. 2). As observed by Siret et al. [14], the replacement of the methyl ester group of DNB-Tyr-OMe by a primary or secondary amide group (compare CSP-Ia and CSP-Ib in Fig. 2) causes a substantial change in the steroselectivity of the new CSPs. However, by changing the immobilization strategy of DNB-Tyr-OMe by introducing a carbamoyl group instead of an ether group (compare CSP-Ia and CSP-II), one also ends up with two amido functions but maybe less favourably positioned with respect to chiral recognition via hydrogen bonding bridges.

In order to reduce the (re)activity of the remaining mercapto groups of the starting mercaptopropylsilica material, it was "end-capped" with 1-hexene, thus reducing polar but non-stereoselective interactions of the mercapto groups and also of the silanols of the silica gel. The resulting CSP is referred to as "end-capped" CSP-IaE (see Fig. 3). The aim of this study was to compare the effects of these small structural changes to the chiral selectors on the overall

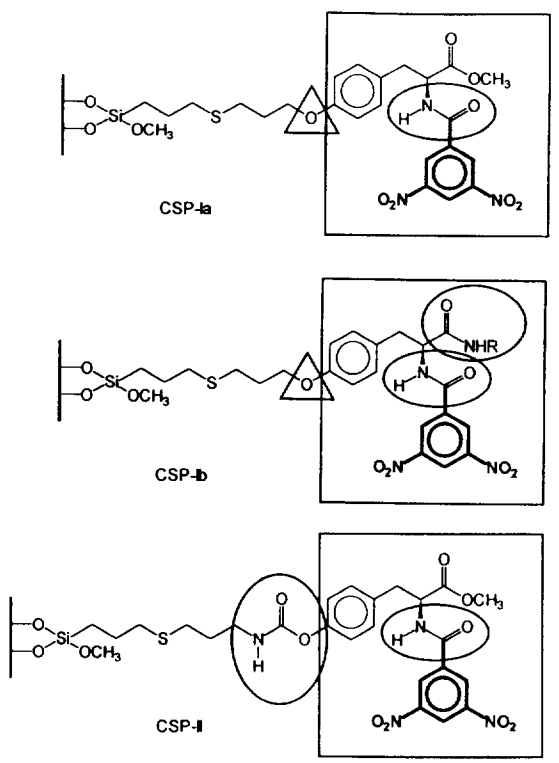


Fig. 2. Structures of CSP-Ia, CSP-Ib and CSP-II. The representation should assist the visualization of structural elements (e.g., π-acid, hydrogen donor acceptor, stereogenic centre) responsible for intermolecular interactions with suitable guest molecules. Square = identical chiral selectors; ellipse = secondary amide; triangle = ether bridge; R may contain an additional stereogenic centre and a π-basic group (see also [12]).

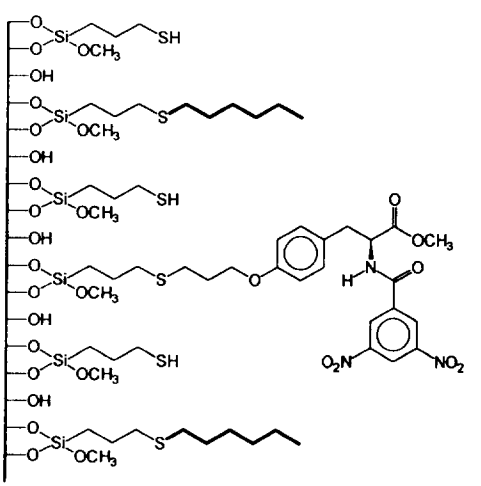


Fig. 3. Structure of CSP-IaE in the non-solvated status; the randomly selected surface structure is presented in relation to the calculated coverage of the "chiral stationary phase" to imagine the complex structure and interaction possibilities with diverse guest solutes. About 25% of the mercapto groups were reacted with the chiral SO and about 30% were alkylated with 1-hexene.

chromatographic behaviour and subsequently to draw some conclusions on chiral recognition mechanisms.

2. Experimental

2.1. Apparatus

Liquid chromatography was performed with a modular liquid chromatograph (Merck–Hitachi, Darmstadt, Germany), equipped with a Model L-6200 intelligent pump, a Model AS-2000A autosampler with a 100-μl loop, a Model L-4250 UV–Vis detector, controlled via a Model D-6000 chromatography data station software, HPLC Manager Vers. 2.09. Temperature was controlled with a column thermostat (B.O. Electronics, Austria). Standard operating conditions were detection at 254 nm and a column temperature of 20°C. A guard column (LiChroCART 4-4, LiChrosorb Si 60, 5 μM; Merck, Darmstadt, Germany) was used throughout.

2.2. Chemicals

(*S*)-Tyrosine methyl ester hydrochloride was obtained from Bachem (Switzerland) and 3,5-dinitrobenzoyl chloride, propene oxide, dibutyltin (IV) acid dilaurate, azobisisobutyronitrile (AIBN) and allyl isocyanate from Aldrich (Germany). Other solvents and reagents were of analytical-reagent grade from Merck.

3-Mercaptopropylsilicagel for CSP-Ia and CSP-IaE was prepared from Kromasil-100-A spherical silica (5 μm) (EKA Nobel, Nobel Industries, Sweden) and 3-mercaptopropyltrimethoxysilane (Aldrich) in the same manner. Elemental analysis indicated 788 μmol of bonded 3-mercaptopropyl residues per gram of modified silica gel (based on C analysis; found, 4.73% C).

3-Mercaptopropylsilicagel for CSP-II was prepared from LiChrosorb Si-100 (5 μm) using 3-mercaptopropyltrimethoxysilane (Aldrich) as described previously [15]. Elemental analysis indicated 784 μmol of bonded 3-mercaptopropyl residues per gram of modified silica gel (based on C analysis: found, 4.83% C; starting material 0.12% C, a value which should be zero according to the materials specification).

The specific surface areas of the two starting silica gels were 360 and 380 m^2 according to the provided information by the suppliers.

Mobile phases were prepared from *n*-heptane of HPLC grade and 2-propanol and diethylamine of analytical-reagent grade from Merck.

The racemic and optically pure test compounds were available from previous studies.

2.3. Chiral stationary phases

CSP-Ia

The starting material [(S)-N-(3,5-dinitrobenzoyl)tyrosine-O-(2-propen-1-yl)methylester] was kindly provided by A. Tambuté (Centre d'Études du Bouchet, Vert-le-Petit, France). CSP-Ia was prepared by refluxing 6 g of 3-mercaptopropylsilica gel with 3.15 mmol (1.35 g) of CS-Ia and 100 mg of AIBN in 200 ml of azeotropically dried chloroform for 40 h. The modified silica gel was successively washed with chloroform, methanol, acetonitrile, methanol and diethyl ether and air dried at 40°C. After sieving (25 μm), 6.2 g of modified silica gel were obtained. Elemental analysis afforded C 9.40, H 1.54, N 0.81%. The calculated coverage was 192 μmol of chiral selector bonded per gram (based on N analysis) and 194 μmol of chiral selector per gram (based on C analysis), respectively.

The final CSP-Ia material was packed into a 150 × 4.0 mm I.D. stainless-steel column using $CHCl_3$–MeOH (9:1) as slurry and heptane as packing solvent.

CSP-IaE

CSP-Ia (4 g), 20 mmol (1.96 g) of 1-hexene and 0.66 g of AIBN were refluxed in azeotropically dried chloroform for 35 h. The modified silica was washed with chloroform, methanol, diethyl ether and light petroleum and dried in air at 40°C. After sieving (25 μm), 3.2 g of slightly yellow silica gel were obtained. Elemental analysis afforded C 11.17, H 1.80, N 0.93%. An additional coverage of 246 μmol of hexyl groups per gram (based on C analysis) was calculated.

The microanalytical data for CSP-Ia and CSP-IaE show differences in C, H and surprisingly

also in N values. The unexpected higher content of nitrogen may be due to the usual deviations of C, N analysis of modified silica gels or may be caused by remaining residues from AIBN and/or its degradation products; therefore, the calculated additional coverage with hexyl residues is an approximate value and the effectiveness of the "end-capping" procedure should preferably be evaluated from chromatographic results.

The final CSP-IaE material was slurry packed in the same manner as CSP-Ia into a 150 × 4.0 mm I.D. stainless-steel column.

CSP-II

The preparation of CSP-II is outlined and briefly described in Fig. 4.

Elemental analysis of CSP-II afforded C 10.28, H 1.58, N 1.42%. A coverage of 253 μmol of chiral selector bonded per gram (based on N analysis) and 216 μmol of chiral selector per gram (based on C analysis), respectively, was calculated (note again the difference depending whether the C or the N analysis data are taken as a basis).

The final CSP-II material was slurry packed in the same manner as CSP-Ia into a 250 × 4.6 mm I.D. stainless-steel column.

3. Results and discussion

As mentioned above, small structural changes in a chiral selector may have a pronounced influence on the overall enantioselectivity of so-called "Pirkle-type" CSPs, in particular when the position of the amido function is shifted within an SO molecule. To focus on such phenomena, two slightly different CSPs based on differently immobilized L-Tyr-OMe (see CSP-Ia and CSP-II) were compared.

Futhermore, we investigated the effect of "end-capping" (comparison of CSP-Ia and CSP-IaE; see also Figs. 2 and 3) on the overall chromatographic behaviour of two CSPs based on identical chiral SO molecules. When evaluating chromatographic results one must bear in mind that the observed retention and resolution data are due to the sum of stereoselective and non-stereoselective interactions of the solutes with the chiral selector (SO) moiety and the additional anchor and spacer groups and/or surface-modifying molecule increments, as was discussed by Däppen *et al.* [16] and Pirkle and Readnour [17]. This touches to some extent on the question of the input of "end-capping" on non-stereoselective retention characteristics.

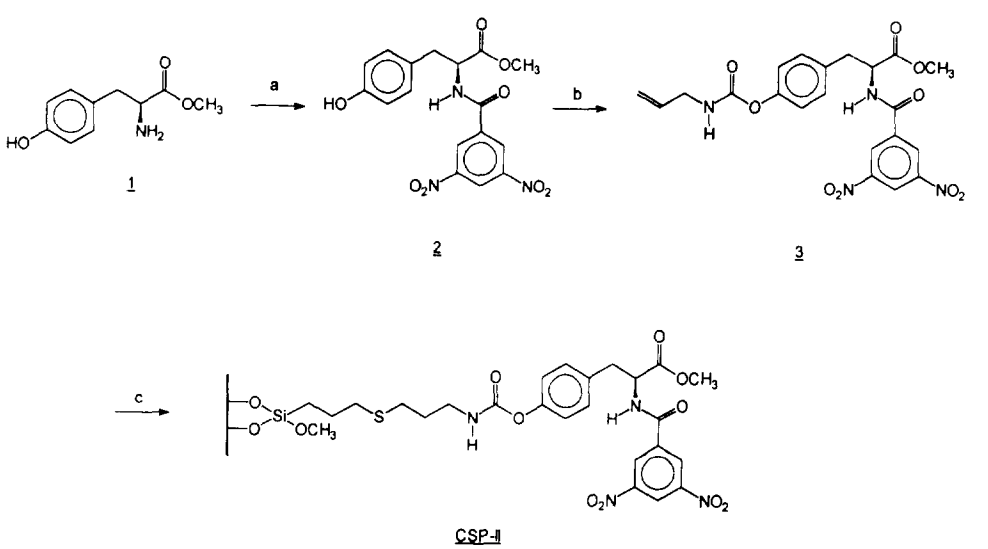


Fig. 4. Reaction scheme for preparing CSP-II. (a) 3,5-Dinitrobenzoyl chloride (1.1 equiv.), propene oxide (6 equiv.), dioxane, 2.5 h at 40°C. (b) Allyl isocyanate (1.1 equiv.), dibutyltin (IV) acid dilaurate (catalytic amount), dioxane, 2 h reflux. (c) 1.5 g of **3**, 2 g of 3-mercaptopropylsilica gel, 100 mg of AIBN, $CHCl_3$, 40 h reflux.

As can be seen in Fig. 2 (compare CSP-Ia and CSP-II), the structure of the chiral SO molecules is similar in many respects (as indicated by the square), but with one exception. For CSP-Ia the derivatized (*S*)-Tyr-OMe is immobilized on silica gel via an ether bond (indicated by a triangle in Fig. 2), whereas in CSP-II this ether bond is replaced by a carbamate bridge (ellipse in Fig. 2). In terms of possible intermolecular SO–SA interactions, the inert ether group (triangle in Fig. 2) has been replaced by a polar and hydrogen bonding amide group (or carbamoyl function) (ellipse in Fig. 2). As a consequence, the two CSPs may have different enantioselectivities. Despite the variation of the selector molecule and the locating of a binding group far away from the stereogenic centre, the observed overall enantioselectivity of a CSP is a result of all interactions of the chiral solutes with the chiral selector, both stereoselective and non-stereoselective; by varying only one intermolecular binding element we thought we could recognize some specific stereoselective SO–SA interaction domains by synthesizing CSP-Ia, CSP-IaE and CSP-II.

CSP-Ia is known to resolve a broad range of analytes [12], e.g., DNB-amino acids (derivatized to esters or amides), alkyl N-arylsulfinamoyl esters, phosphine oxides, ibuprofen derivatives and lactams. For this study, we selected a representative collection of chiral acids, amines, alcohols (derivatized to amides, ureas and carbamates, respectively) as analytes; they had already been used in previous studies with (*S*,*S*)diphenylethylethanediamine derivatives as chiral model compounds [5,6,18]. The chromatographic results obtained with CSP-Ia, CSP-IaE and CSP-II are summarized in Tables 1–3 and are discussed below.

3.1. Using equal mobile phase conditions

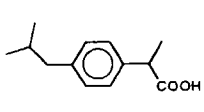
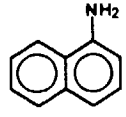
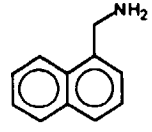
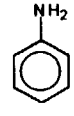
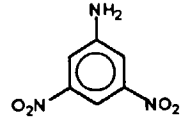
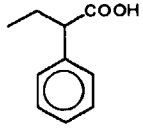
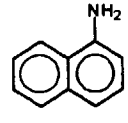
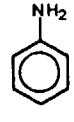
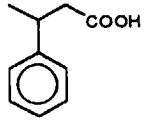
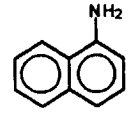
First we compared the behaviours of CSP-Ia, CSP-IaE and CSP-II using identical mobile phase conditions in order to elucidate increments responsible for non-stereoselective and stereoselective retention characteristics. As can be clearly seen, the enantioselectivities were, with exceptions, relatively similar, although the absolute retention varied and in particular was lower for the "end-capped" CSP-IaE. For simply structured analytes such as amides or ureas, the 3,5-dinitrobenzamide function seems to be the most important binding domain and the additional amido (carbamoyl) group of CSP-II has little influence on the total enantioselectivity, whereas for more complex analytes such as oxazepam and indapamide this carbamate group seems to take part. The additional amido group of CSP-II, being fairly distant from the stereogenic centre, seems to compete for binding sites of the analytes. Oxazepam and indapamide were resolvable to a small extent on CSP-Ia, whereas CSP-II showed no enantioselectivity, despite the fact that both drugs show higher retention times on CSP-II than on CSP-Ia.

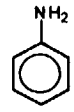
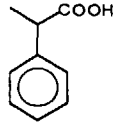
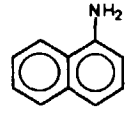
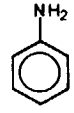
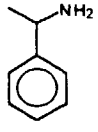
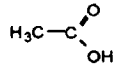
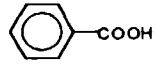
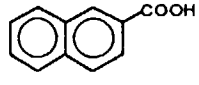
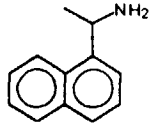
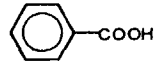
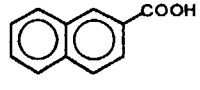
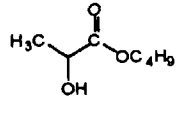
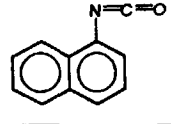
Interestingly, the ibuprofen naphthylamide and anilide enantiomers were resolvable on both CSPs Ia and II, but ibuprofen naphthylmethylamide was only resolved on CSP-Ia. Changing the naphthyl or phenyl group to the more flexible naphthlymethyl, group which should be accompanied by a lower steric hindrance of the chiral solutes, resulted in a general diminishment of chiral recognition on CSP-II. In contrast, there were also solutes resolvable on CSP-II and not on CSP-Ia, namely 2-phenylpropionic acid anilide and 2-naphthoic acid 1-phenylethylamide.

Another interesting phenomenon is the opposite elution order of chiral acids as amides (*S* before *R*) and chiral amines as amides (*R* before *S*). Changing the chiral centre from the carboxyl to the amine function caused a reversal of the elution order, indicating a chiral recognition mechanism based on a combination of charge-transfer interaction, hydrogen bonding and dipole–dipole stacking.

For 1-phenylethylamine and 1-(1-naphthyl) ethylamine (derivatized to the corresponding amides with 1-naphthoic acid), an unusual retention behaviour was observed. The former optical antipodes showed higher retention on CSP-Ia, whereas the latter enantiomers were more strongly retained on CSP-II. Similar differences were observed for the β-blocking agents

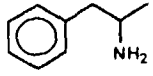
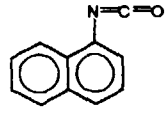
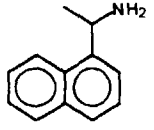
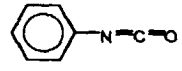
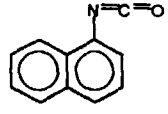
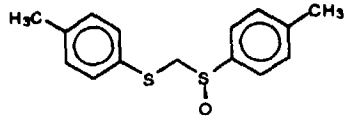
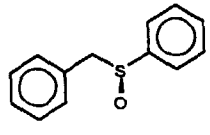
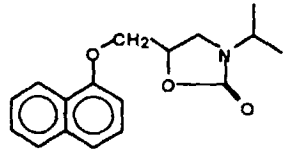
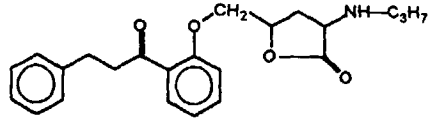
Table 1
Enantioseparation of representative analytes on CSP-Ia, CSP-IaE and CSP-II

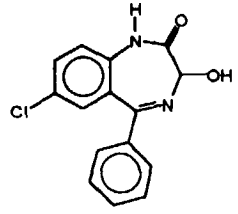
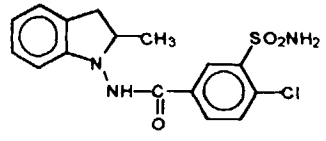
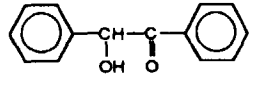
Compound	Derivatizing reagent	CSP-Ia			CSP-IaE			CSP-II		
		k_1' [a]	α [b]	R_s [c]	k_1'	α	R_s	k_1'	α	R_s
Acids as amides										
		3.16	1.15	1.66	1.96	1.17	1.53	2.87 (*S*)	1.15	2.24
		2.63	1.04	<0.7	1.70	1.00	–	2.68	1.00	–
		0.62	1.15	<0.7	0.42	1.14	<0.7	0.85 (*S*)	1.11	0.97
		0.99	1.00	–	0.79	1.00	–	1.16	1.00	–
		3.70	1.11	0.73	2.49	1.09	0.75	3.75	1.07	<0.7
		0.87	1.00	–	0.61	1.00	–	1.09	1.00	–
		3.82	1.00	–	2.47	1.00	–	3.94	1.00	–

		0.78	1.10	<0.7	0.62	1.02	<0.7	1.02	1.07	<0.7
		4.51	1.09	1.08	2.95	1.12	1.11	4.52 (*S*)	1.08	1.15
		0.98	1.00	–	0.76	1.01	<0.7	1.19 (*S*)	1.05	0.49
Amines as amides										
		0.83 (*R*)	1.21	1.09	0.50	1.28	0.83	1.05 (*R*)	1.12	1.05
		1.62 (*R*)	1.14	1.23	1.13	1.16	1.15	1.89 (*R*)	1.12	1.38
		6.75	1.00	–	4.34	1.00	–	4.14 (*R*)	1.04	<0.7
		4.00	1.41	4.1	2.66	1.46	4.09	4.11 (*R*)	1.41	5.23
		9.64	1.15	2.03	6.03	1.22	2.36	14.88 (*R*)	1.10	1.18
Alcohols as carbamates										
		1.00	1.15	0.84	0.75	1.18	<0.7	1.23	1.08	<0.7

(Continued on p. 142)

Table 1 (*continued*)
Enantioseparation of representative analytes on CSP-Ia, CSP-IaE and CSP-II

Compound	Derivatizing reagent	CSP-Ia			CSP-IaE			CSP-II		
		k_1' [a]	α [b]	R_s [c]	k_1'	α	R_s	k_1'	α	R_s
Amines as ureas										
								3.54	1.07	0.91
		3.26	1.39	3.11	2.32	1.50	2.82	2.94 (*R*)	1.41	3.54
		9.15	1.34	3.24	6.54	1.42	3.58	9.47	1.36	3.48
Sulfoxides										
		1.46	1.07	<0.7	1.01	1.09	<0.7	1.56	1.06	<0.7
		1.37	1.00	–	0.96	1.00	–	1.44	1.00	–
β-Blockers as oxazolidin-2-ones										
		5.79	1.12	1.52	4.29	1.12	1.07	7.61 (*S*)	1.15	1.53
		6.01	1.00	–	4.35	1.00	–	7.22	1.00	–

Miscellaneous Oxazepam		2.34	1.06	<0.7	1.82	1.00	–	3.04	1.00	–
Indapamid		5.89	1.05	<0.7	4.56	1.00	–	8.84	1.00	–
Benzoin					0.40	1.48	1.13	1.01	1.00	–

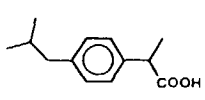
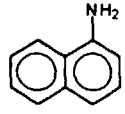
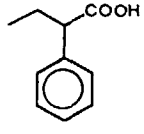
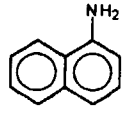
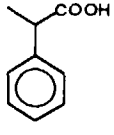
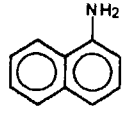
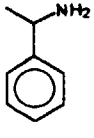
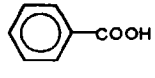
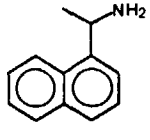
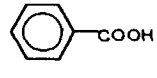
Mobile phase: *n*-heptane–2-propanol–diethylamine (DEA) (70:30:0.1, v/v/v).

[a] Capacity factor: $k' = (t_r - t_0)/t_0$.

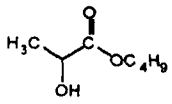
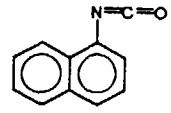
[b] Selectivity: $\alpha = k'_2/k'_1$.

[c] Resolution: $R_s = 1.18\ (t_{r_2} - t_{r_1})/(w_{1/2_1} + w_{1/2_2})$.

Table 2
Enantioseparation of representative analytes on CSP-Ia and CSP-II under isoeluotropic conditions

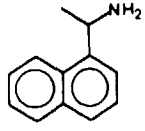
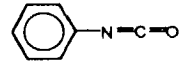
Compound	Derivatizing reagent	CSP-Ia				CSP-II			
		k_1' [a]	k_2'	α [b]	R_s [c]	k_1'	k_2'	α	R_s
Acids as amides									
		3.16	3.36	1.15	1.66	2.87 (*S*)	3.30	1.15	2.24
		3.70	4.11	1.11	0.73	3.75	4.01	1.07	<0.7
		4.51	4.92	1.09	1.08	4.52 (*S*)	4.88	1.08	1.15
Amines as amides									
		1.62 (*R*)	1.85	1.14	1.23	1.70 (*R*)	1.86	1.09	0.95
		4.00	5.64	1.41	4.41	4.11 (*R*)	5.80	1.41	5.23

Alcohols as carbamates

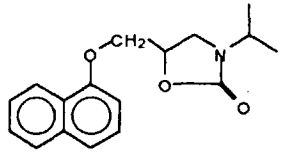
1.00	1.15	1.15	0.84	1.05	1.13	1.08	<0.7

Amines as ureas

3.26	4.53	1.39	3.11	3.32 (*R*)	4.56	1.37	2.91

β-Blockers as oxazolidin-2-ones

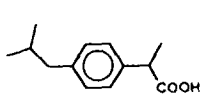
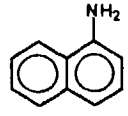
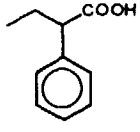
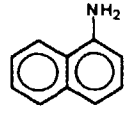
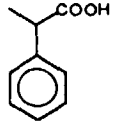
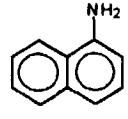
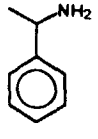
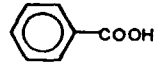
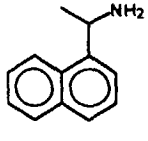
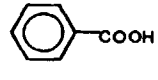


5.79	6.49	1.12	1.52	5.88 (*S*)	6.59	1.12	1.18

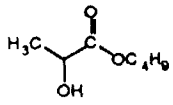
Mobile phases: CSP-Ia, *n*-heptane–2-propanol–diethylamine (DEA) (70:30:0.1), v/v/v); CSP-II, *n*-heptane–2-propanol–diethylamine (DEA) (between 65:35:0.1 and 72:28:0.1, v/v/v).

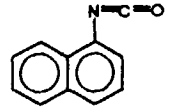
[a–c] See Table 1.

Table 3
Enantioseparation of representative analytes on CSP-Ia and CSP-IaE under isoeluotropic conditions

Compound	Derivatizing reagent	CSP-Ia				CSP-IaE			
		k_1' [a]	k_2'	α [b]	R_s [c]	k_1'	k_2'	α	R_s
Acids as amides									
		3.16	3.36	1.15	1.66	2.83	3.32	1.17	2.14
		3.70	4.11	1.11	0.73	3.72	4.04	1.09	1.04
		4.51	4.92	1.09	1.08	4.54	5.00	1.10	1.02
Amines as amides									
		1.62 (*R*)	1.85	1.14	1.23	1.60	1.84	1.15	1.48
		4.00	5.64	1.41	4.41	3.97	5.77	1.45	5.06

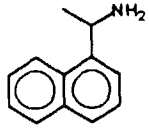
Alcohols as carbamates

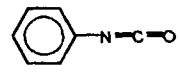




1.00	1.15	1.15	0.84	1.04	1.18	1.14	0.90

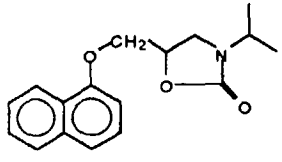
Amines as ureas





3.26	4.53	1.39	3.11	3.01	4.44	1.47	2.15

β-Blockers as oxazolidin-2-ones



5.79	6.49	1.12	1.52	5.94	6.65	1.12	1.50

Mobile phases: CSP-Ia, *n*-heptane–2-propanol–diethylamine (DEA) (70:30:0.1, v/v/v); CSP-IaE, *n*-heptane–2-propanol–diethylamine (DEA) (between 78:22:0.1 and 73:27:0.1, v/v/v).

[a–c] See Table 1.

propranolol and propafenone (as their oxazolidinones) and the antihypertonic acting drug indapamide, which were more strongly retained on CSP-II, probably owing to additional interactions with the second amido group of CSP-II.

To obtain further information about the chromatographic properties of CSP-Ia and CSP-II, we focused on the retention behaviour caused by $\pi-\pi$ interactions. In general, and as also observed by others, the naphthyl derivatives were more retained than their phenyl analogues. Within the ibuprofen derivatives both CSPs showed decreasing capacity factors on going from the naphthyl- to 1-naphthylmethylamide and to the anilide, caused by weaker $\pi-\pi$ interactions with the DNB residue of the stationary phase within this series. Interestingly, the 3,5-dinitroanilide, a π-acid compound, showed higher k' values than the anilide. This behaviour may be explained by interaction of the π-acid solute with the weak π-basic phenyl ring of the stationary phases. As was mentioned above, this phenyl ring has different electron densities in CSP-Ia and CSP-II owing to the stronger electron-withdrawing effect of the carbamate function compared with the ether function enhancing the electron density within the aromatic ring.

3.2. Using isoeluotropic mobile phase conditions and judging the "end-capping"

As already pointed out, the capacity factors for many compounds are similar on CSP-Ia and CSP-II, although these two CSPs have a slightly different loadings of the SO on a molecular basis (N values 192 μmol of SO per gram of CSP-Ia and 253 μmol of SO per gam of CSP-II, corresponding to C values of 194 and 216 μmol, respectively). Therefore, the chiral SO of CSP-Ia seems to cause stronger retention of the analytes than the chiral SO of CSP-II. However, retention of analytes may also be caused by interactions with remaining silanol and other polar groups stemming from the silica gel and its degree of premodification. However, stereoselective retention can only be generated by SO–SA interactions, and the question remains of how strong the influence of the SO density on the surface is on the observed enantioselectivity. "End-capping" reduces the polar interaction sites on the CSP and should result in reduced retention under normal-phase conditions. When comparing two CSPs (e.g., CSP-Ia and CSP-IaE), it should be possible to extract the increment responsible only for chiral recognition within the chromatographic process by normalizing the retention of the second eluting peak of resolved pairs of enantiomers by adjusting the mobile phase and measuring the resulting α values.

For such isoeluotropic conditions, the data are summarized in Tables 2 and 3 and we found that the stereoselectivity did not change significantly whether we compare CSP-Ia with CSP-II or CSP-Ia with CSP-IaE. The observed differences in the enantioselectivity of CSP-Ia and CSP-II seems to be mainly due to their different selector molecules and not to their different grafting rates. Hence, the usual arguments that one can only compare CSPs and their stereoselectivities for a set of chiral analytes when the CSPs are based on the same silica or silica gel modification and with the same selector density per square metre of surface area seem doubtful. However, by examining also the resolution values of the resolved pairs, one might obtain additional information on unwanted polar interactions and/or packing characteristics of the columns.

As was indicated previously, an additional "end-capping" procedure was developed and the CSPs were evaluated by comparison of CSP-Ia and CSP-IaE. The remaining mercapto and silanol groups represent polar residues able to influence the overall selectivity of the chromatographic system. About 40% (judged by C analysis data) of the remaining free mercapto groups of CSP-Ia reacted with 1-hexene to give the corresponding 1-hexane thioether (see Fig. 3), resulting in the reduction of polar but non-stereoselective interactions of the solutes with the remaining polar groups, as was described in a previous paper [15]. In general, the capacity factors of almost all analytes were smaller on CSP-IaE than on the comparable CSP-Ia, but at

the same time the α values did not change significantly and the resolution was inconsistently slightly increased or decreased. In the present case, the interaction of the analytes with the chemically modified silica surface to which the chiral selector groups are bound does not play a significant role in the course of chiral recognition.

Concerning the comparison of CSP-Ia and CSP-IaE under isoeluotropic conditions, these stationary phases showed almost identical α-values, indicating that the "end-capping" procedure does not influence the stereoselective intermolecular interactions of the chiral SO and SA moiety, in contrast to the non-stereoselective interactions.

Increased retention will cause higher enantioselectivity but only if the main retention mechanism is enantioselective, as was stressed by Boehm *et al.* [19]. The enhanced retention of the analytes on CSP-Ia in contrast to retention on CSP-IaE seems to be due to polar, non-stereoselective interactions of the solutes with the stationary phase.

The column efficiency of CSP-Ia and CSP-IaE was calculated either for pure aromatic compounds without functional groups (e.g., benzene) and pure heptane as eluent or for the above-mentioned mobile phase and test solutes containing a π-basic site and additional polar functional groups. For benzene, the reduced plate heights were 4.8 for CSP-Ia and 4.5 for CSP-IaE, as is more or less usual for this type of CSP. Calculating the reduced plate heights for ibuprofen-1-naphthylamide the values were 20.2 and 24.3, respectively. As can be extracted from these data, solutes containing polar functional groups interacting with polar groups of the total CSP (see Fig. 3) showed slow mass transfer and/or pronounced adsorption phenomena. However, the calculated reduced plate heights are similar for both CSPs and from this point of view the "end-capping" procedure did not lead to better results. The symmetry factor for the more strongly retained ibuprofen-1-naphthylamide enantiomer were calculated as 0.96 for CSP-Ia and 1.2 for the "end-capped" CSP; however, for a clear interpretation of this behaviour the number of data is not sufficient. These discouraging data show the discrepancies between theoretical calculations and virtual effects of retention mechanisms on chiral supports that have to be taken into account when working with CSPs. However, and as additional assumption, it seems possible to obtain the same enantioselectivity and almost the same resolution but within a shorter analysis time by using "end-capped" CSPs and non-aqueous mobile phases.

4. Conclusions

The two chiral selectors derived from L-tyrosine presented in this paper proved to be enantioselective for a broad range of chiral analytes. The synthesis of CSP-Ia and CSP-II is comparable with regard to costs and time consumption. They do show comparable enantioselectivity and resolution and the substitution of the ether group with a carbamate group does not influence the chromatographic behaviour to a great extent. Working with similar chiral selectors, a carbamate linkage sufficiently distant from the dominating interaction groups or/and the stereogenic centers may be chosen if there are any problems by conveniently synthesizing an ether bond. The "end-capping" method presented offers a simple route to enhance the applicability of the CSPs due to a shortening of the analysis time but maintaining the enantioselectivity and resolution.

Acknowledgements

The authors express their grateful thanks to Dr. A. Tambuté (Centre d'Études du Bouchet, Vert-le-Petit, France) for the gift of the chiral selector [(*S*)-N-(3,5-dinitrobenzoyl)tyrosine-O-(2-propen-1-yl)methyl ester]. Acknowledgement is made to the Austrian Fonds zur Förderung der wissenschaftlichen Forschung, project number P-8898-CHE, for the support of this project.

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