Journal of Chromatography, 585 (1991) 195–206
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 537

New chiral covalently bonded, π-donor stationary phases for high-performance liquid chromatography, based on derivatives of optically active 1-(1-naphthyl)ethylamine

Part II☆

Rolf Straub☆☆, Markus Pfister and Hans Arm*

Institute of Organic Chemistry, University of Berne, Freiestrasse 3, CH-3012 Berne (Switzerland)

(Received March 26th, 1991)

ABSTRACT

On the basis of optically active 1-(1-naphthyl)ethylamine as the chiral selector part, several derivatives with different N-acylamide substituents were synthesized and attached to 3-glycidoxypropyl-derived silica to give "brush-type" stationary phases for high-performance liquid chromatography. Their suitability for enantiomer separation was tested with aromatic amide derivatives including two homologous series. The best separations on every phase were found for samples with strong π-acceptor groups, such as the 3,5-dinitrophenyl group.

INTRODUCTION

There has been significant progress in the direct separation of enantiomers by high-performance liquid chromatography (HPLC) in recent years [1], especially with the aid of chiral stationary phases (CSPs) [2,3] with proteins, ligand-exchange sites, helical polymers or chiral cavities [4,5]. Amongst the most successful CSPs are the "brush-type" phases which consist of a silica matrix with covalently bonded chiral groups. The π-acceptor 3,5-dinitrobenzoylphenylglycine CSP developed by Pirkle and co-workers [6,7] is a well known phase of this type, and a great variety of similar CSPs have been reported and in some instances commercialized [8,9].

The mechanistic hypothesis formulated from the study of N-acyl-1-arylalkylamines with these π-acceptor CSPs were instrumental in the design of "reciprocal" CSPs intended to resolve analytes derived with the π-acidic 3,5-dinitrobenzoyl (DNB) group [10,11]. The π-donor 1-arylalkylamide-derived chiral stationary phases are especially interesting from the mechanistic point of view, as they utilize multiple and competing chiral recognition mechanisms depending on the types of analytes and the chiral selector of the CSPs. The proposed model introduces the intercalative and the conflicting non-intercalative process. Chromatographic experiments with suitable homologous series, in which stereochemistry and elution order are correlated, help to distinguish different arrangements of the diatereomeric complex formed out of the chiral selector and the analyte [12].

These or similar "brush-type" CSPs are capable of separating many different enantiomers of phar-

☆ For part I, see ref. 14.
☆☆ This paper is part of the dissertation of R. Straub [19].

0021-9673/91/$03.50

maceutical interest. Preparative-scale separations are also possible if a sufficient amount of the chiral stationary phase is available [13].

In an earlier paper [14] we described chiral stationary phases for HPLC based on optically active 1-(1-naphthyl)ethylamine. (*R*)-4-(N-1-Pivaloylamino-1-ethyl)-1-naphthylamine {(*R*)-2,2-dimethyl-N-[1-(4-amino)naphthylethyl]propanamide} fixed either on a silica support or in a glass capillary showed remarkable separation capabilities for a variety of chiral amides and a broad range of applications in HPLC, supercritical fluid chromatography and gas chromatography [15]. The "brush-type" CSP on a silica matrix has been subject of intense chromatographic and mechanistic investigations during the last few years [16,17]. In subsequent studies computational chemistry was applied to understand the separation mechanism and to design similar CSPs with improved separation capabilities for chiral DNB-amide derivatives [18]. From theoretical calculations (computer-aided molecular modelling, CAMM) [18] and chromatographic data [14,17] it can be deduced that a π-donor naphthyl group and a bulky pivaloylamide substituent attached to the chiral centre of the selector are suitable for enantioseparation. In this paper we examine the chromatographic behaviour of four new π-donor "brush-type" CSPs with different carboxamide substituents on the chiral centre and compare them with the mentioned CSP.

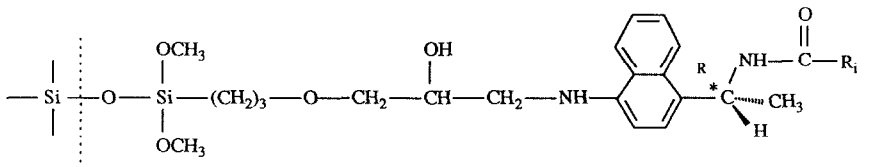
Fig. 1 shows the common basic structure of the phases with amide groups R_i of different rigidity and polarity. To test the separation capabilities of the CSPs we used different chiral amides, shown in Table I.

Homologous series of 1-phenylalkylamides **1a–l** and esters of DNB-amino acids **30a–j**, given in Fig. 2, were used to examine the retention mechanisms which make significant contributions to enantioselectivity.

EXPERIMENTAL

General

Five chiral stationary phases with different N-acyl groups R_i on the chiral selector part were synthesized starting from commercially available (*R*)-1-(1-naphthyl)ethylamine. The preparation of the CSPs was done in five steps according to the procedures described for CSP I (PIV) by Däppen *et al.* [14] and Brügger *et al.* [15] but with different alkyloyl chlorides for the amination step [19]. Cyclohexanecarbonyl chloride was used for CSP II (CYH), adamantanecarbonyl chloride for CSP III



$R_i = R_1 =$ —$C(CH_3)_3$ CSP I (PIV)

$R_i = R_2 =$ CSP II (CYH)

$R_i = R_3 =$ CSP III (ADA)

$R_i = R_4 =$ CSP IV (CPE)

$R_i = R_5 =$ —CF_2—CF_2—CF_3 CSP V (HFP)

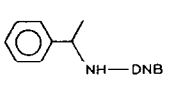
Fig. 1. Chiral stationary phases (CSPs) I–V, N-acyl derivatives of (*R*)-4-(N-1-alkyloylamino-1-ethyl)-1-naphthylamine.

TABLE I

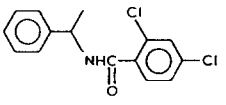
SAMPLES **1–29** USED IN CHROMATOGRAPHIC EXPERIMENTS

DNB = 3,5-Dinitrobenzoyl; DNP = 3,5-dinotrophenyl. **8** = DNB amide of cathinone; **9** = DNB amide of merucathinone; **10** = di-DNB derivative of merucathine; **24** = DNB amide of propranolol; **25** = DNB amide of pindolol; **26** = DNB amide of *erythro*-dihydrosphingosine; **27** = DNB amide of *threo*-dihydrosphingosine.

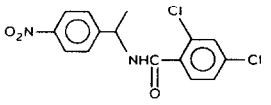
Phenyl-containing samples



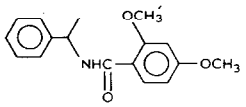
1



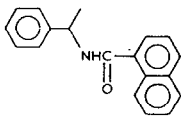
2



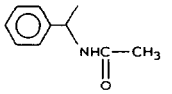
3



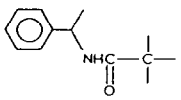
4



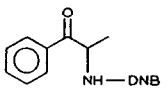
5



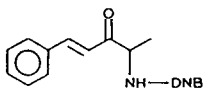
6



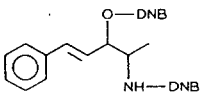
7



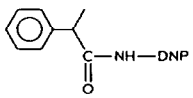
8



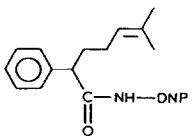
9



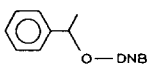
10



11

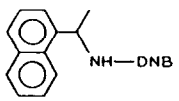


12

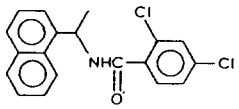


13

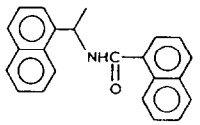
Naphthyl- and indolyl-containing samples



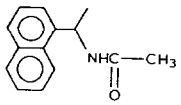
14



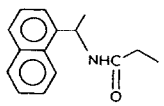
15



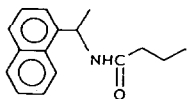
16



17



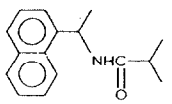
18



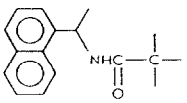
19

(Continued on p. 198)

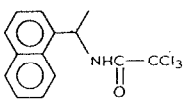
TABLE I (*continued*)



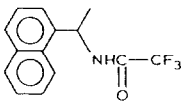
20



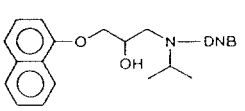
21



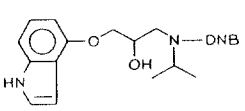
22



23

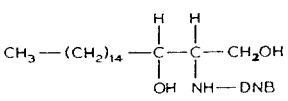


24

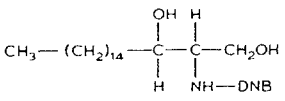


25

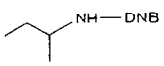
Miscellaneous DNB-derived samples



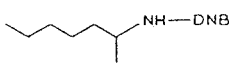
26



27

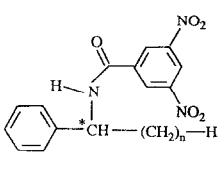


28

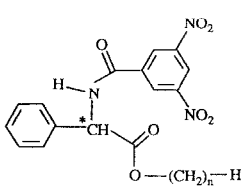


29

(ADA), 3-cyclopentylpropionyl chloride for CSP IV (CPE) and heptafluorobutyryl chloride for CSP V (HFP). The general synthetic procedure for the aromatic nitration of the naphthalene ring in the *para* position, the catalytic reduction of the introduced aromatic nitro group, the bonding procedure to the silica matrix by 3-glycidoxypropyltrimethoxysilane as a spacer and the filling mode into stainless-steel columns were identical for all five CSPs, except for small variations in the purification steps and in the yields. Only the yield, the measured physical parameters of the different intermediates and elemental analysis data of the bonded phases are reported.



1a-l



30a-j

Fig. 2. N-3,5-Dinitrobenzoyl (DNB) derived homologous series **1a–l** and **30a–j**.

Materials

For the preparation of CSPs LiChrospher Si 100 (Merck, Darmstadt, Germany) with a particle size of 5 μm and a specific surface area S_{BET} = 264.4 ± 5.4 m^2/g was used. It was dried at 150°C and 0.01 mbar for 6 h prior to the bonding procedure. All chemicals were purchased from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland).

Analysis

The NMR data were recorded on a 60-MHz Varian EM 360L NMR spectrometer (Varian, Zug, Switzerland) in [^{2}H]chloroform with tetramethylsilane as internal standard. The polarimetric measurements were done on a Perkin-Elmer Model 241 polarimeter. The IR spectra were recorded on a Perkin-Elmer Model 782 infrared spectrophotometer in chloroform and the UV spectra on a Perkin-Elmer Model 554 UV–VIS spectrophotometer in ethanol (Perkin-Elmer, Überlingen, Germany). The mass spectra were recorded on a Varian MAT CH7 A mass spectrometer (electron impact ionization, 70 eV). Only the mass spectrometric (MS) *m/z* value, the relative intensity of the molecular ion and the base peak are given. The elemental analyses were done with a routine analyser in the Microanalytical Department of Ciba-Geigy, Basle, Switzerland.

Preparation of CSP I (PIV)

(R)-1-(N-1-Pivaloylamino-1-ethyl)naphthalene. This amide was prepared by amination of (*R*)-1-(1-naphthyl)ethylamine in dry dioxane. Yield, 70%; m.p. 133.0–135.0°C; $[\alpha]_D = 39.1°$ ($c = 0.11$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.20–7.68 (m, 3H), 7.68–7.40 (m, 4H), 6.15–5.65 (m, 2H), 1.90 (m, 3H), 1.15 (s, 9H). IR (cm^{-1}): 3460, 3060–2880, 1650, 1600, 1500, 1450. MS: *m/z* 255 (65, M$^{+\cdot}$), 155 (100).

(R)-1-Nitro-4-(N-1-pivaloylamino-1-ethyl)-naphthalene. The nitration of the aromatic ring was done in a mixture of 65% nitric acid and of 98% acetic acid. Yield after several purification steps, 48%; m.p. 152.0–153.0°C; $[\alpha]_D = +50°$ ($c = 0.11$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.65–8.05 (m, 3H), 7.85–7.40 (m, 3H), 6.25–5.65 (m, 2H), 1.65 (d, $J = 6$ Hz, 3H), 1.20 (s, 9H). IR (cm^{-1}): 3460, 3010–2880, 1660, 1600, 1520, 1350. MS: *m/z* 300 (41, M$^{+\cdot}$), 57 (100).

(R)-4-(N-1-Pivaloylamino-1-ethyl)-1-naphthylamine. The aromatic amine was obtained by reduction with hydrazine monohydrate in methanol and a catalytic amount of palladium on activated charcoal. Yield, 63%; m.p., 131.0–131.5°C; $[\alpha]_D = +111.2°$ ($c = 0.107$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.20–7.70 (m, 2H), 6.75 (d, $J = 8$ Hz, 1H), 6.10–5.50 (s, 2H), 4.50–3.65 (s, 2H), 1.65 (d, $J = 6$ Hz, 3H), 1.20 (s, 9H). IR (cm^{-1}): 3460, 3010–2870, 1650, 1625, 1500, 1470-1450. MS: *m/z* 270 (100, M$^{+\cdot}$).

CSP I (PIV). The aromatic amine, 3-glycidoxypropyltrimethoxysilane and dried silica were stirred in methanol. Analysis: found, C 6.99, H 1.51, N 0.69%; calculated, 0.25 mmol of (*R*)-ligand/g stationary phase (based on N) and 0.23 mmol (*R*)-ligand/g stationary phase (based on C).

Preparation of CSP II (CYH)

(R)-1-(N-1-Cyclohexylcarboxamido-1-ethyl)-naphthalene. Yield, 82.3%; m.p., 170.0–170.5°C; $[\alpha]_D = +29.95°$ ($c = 0.177$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.20–7.33 (m, 7H), 6.20–5.50 (m, 2H), 2.20–0.80 (m, 11H), 1.66 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3620–2860, 1660, 1600, 1500, 1450. UV–VIS (nm): 288 (sh), 278, 268, 260 (sh), 222. MS: *m/z* 281 (4, M$^{+\cdot}$), 155 (100).

(R)-1-Nitro-4-(N-1-cyclohexylcarboxamido-1-ethyl)naphthalene. Yield, 44.5%; m.p., 193.5–194.5°C; $[\alpha]_D = +44.83°$ ($c = 0.029$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.60–7.90 (m, 3H), 6.20–5.66 (m, 2H), 2.20–0.80 (m, 11H), 1.66 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3440, 3000–2860, 1670, 1530, 1500, 1450, 1340. UV–VIS (nm): 328, 246, 220. MS: *m/z* 326 (67, M$^{+\cdot}$), 83 (100).

(R)-4-(N-1-Cyclohexylcarboxamido-1-ethyl)-1-naphthylamine. Yield, 71.66%; m.p., 176.0–176.5°C; $[\alpha]_D = +89.7$ ($c = 0.017$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.16–7.20 (m, 5H), 6.75 (d, 1H), 6.10–5.40 (m, 2H), 4.12 (s, 2H), 2.20–0.70 (m, 11H), 1.66 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3010–2860, 1650, 1628, 1500, 1450. UV–VIS (nm): 324, 242, 218. MS: *m/z* 296 (57, M$^{+\cdot}$), 171 (100).

CSP II (CYH). Analysis: found, C 6.43, H 1.34, N 0.63%; calculated, 0.22 mmol (*R*)-ligand/g stationary phase (based on N) and 0.20 mmol (*R*)-ligand/g stationary phases (based on C).

Preparation of CSP III (ADA)

(R)-1-(N-1-Adamantylcarboxamido-1-ethyl)-naphthalene. Yield, 81%; m.p., 189.0–190.0°C; $[\alpha]_D = +5°$ ($c = 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.16–7.33 (m, 7H), 6.10–5.80 (m, 2H), 2.10–150 (m, 15H), 1.76 (d, $J = 10$ Hz, 3H). IR (cm^{-1}): 3450, 3000, 2910, 2850, 1650, 1600, 1500, 1450. UV–VIS (nm): 290, 278, 268, 260 (sh), 226, 220 (sh). MS: *m/z* 333 (36, M$^{+\cdot}$), 135 (100).

(R)-1-Nitro-4-(N-1-adamantylcarboxamido-1-

ethyl)naphthalene. Yield, 38%; m.p., 201.0–203.0°C (decomposition); $[\alpha]_D = +10°$ ($c = 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.70–7.40 (m, 6H), 6.10–5.80 (m, 2H), 2.13–1.60 (m, 15H), 1.80 (d, $J = 10$ Hz, 3H). IR (cm^{-1}): 3450, 3000, 2910, 2850, 1660, 1600, 1500, 1450, 1340. UV–VIS (nm): 328, 246, 218. MS: *m/z* 378 (12, $M^{+\cdot}$), 135 (100).

(R)-4-(N-1-Adamantylcarboxamido-1-ethyl)-1-naphthylamine. Yield, 67%; m.p., 191.0°C (decomposition); $[\alpha]_D = +52°$ ($c = 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.20–7.40 (m, 6H), 6.78 (d, $J = 8$ Hz, 2H), 5.90–5.50 (m, 2H), 2.00–1.10 (m, 15H), 1.75 (d, $J = 10$ Hz, 3H). IR (cm^{-1}): 3630, 3450, 3000, 2910, 2850, 1640, 1500, 1450. UV–VIS (nm): 324, 242, 214. MS: *m/z* 348 (100, $M^{+\cdot}$).

CSP III (ADA). Analysis: found, C 5.75, H 1.04, N 0.3%; calculated, 0.15 mmol (*R*)-ligand/g stationary phase (based on N) and 0.11 mmol (*R*)-ligand/g stationary phase (based on C).

Preparation of CSP IV (CPE)

(R)-1-(N-1-Cyclopentylpropionylamino-1-ethyl)naphthalene. Yield, 94%; m.p., 131.5–132.0°C; $[\alpha]_D = +39°$ ($c = 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 7.47–8.23 (m, 7H), 5.97–5.77 (m, 2H), 2.35–0.70 (m, 13H), 1.67 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3620, 3440, 3000, 2950, 2870, 1660, 1600, 1500, 1450. UV–VIS (nm): 290 (sh), 278, 268, 226. MS: *m/z* 295 (32 $M^{+\cdot}$), 155 (100).

(R)-1-Nitro-4-(N-1-cyclopentylpropionylamino-1-ethyl)naphthalene. Yield, 47%; m.p., 159.0–160.0°C; $[\alpha]_D = +49°$ ($c = 0.1$ in ethanol, 25°C), ^{1}H NMR (ppm): 8.67–7.53 (m, 6H), 5.93 (s, 2H), 2.30–0.80 (m, 13H), 1.65 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3620, 3440, 3000, 2970, 2860, 1670, 1600, 1520, 1500, 1450, 1340. UV–VIS (nm): 334, 246, 222. MS: *m/z* 340 (22, $M^{+\cdot}$), 258 (100).

(R)-4-(N-1-Cyclopentylpropionylamino-1-ethyl)-1-naphthylamine. Yield, 30%; m.p., 156.0–158.0°C; $[\alpha]_D = 106°$ (c) $= 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.17–7.40 (m, 6H), 6.73 (d, $J = 8$ Hz), 5.67 (s, 2H), (s, 2H), 2.36–0.82 (m, 13H), 1.62 (d, $J = 6$ Hz, 3 H). IR (cm^{-1}): 3610, 3430, 3000, 2950, 2860, 1650, 1640, 1490, 1450. UV–VIS (nm): 324, 238, 222. MS: *m/z* 310 (31, $M^{+\cdot}$), 171 (100).

CSP IV (CPE). Analysis: found, C 5.98, H 1.10, N 0.5%; calculated, 0.18 mmol (*R*)-ligand/g stationary phase (based on N and C).

Preparation of CSP V (HFP)

(R)-1-(N-1-Heptafluorobutyrylamino-1-ethyl)-naphthalene. Yield, 100%; m.p., 104–105°C; $[\alpha]_D = +40°$ ($c = 0.1$ in ethanol, 20°C). ^{1}H NMR (ppm): 8.17–7.35 (m, 7H), 6.64 (s, 1H), 5.97 (q, 1H), 1.73 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3620, 3420, 2970, 1720, 1600, 1520, 1450. UV–VIS (nm): 288 (sh), 278, 269 (sh), 260 (sh), 206. MS: *m/z* 367 (100, $M^{+\cdot}$).

(R)-1-Nitro-4-(N-1-heptafluorobutyrylamino-1-ethyl)-1-naphthalene. Yield, 46%; m.p., 133.0–135.0°C; $[\alpha]_D = +54°$ ($c = 0.1$ in ethanol, 25°C). ^{1}H NMR (ppm): 8.63–7.50 (m, 6H), 6.87 (s, 1H), 6.00 (q, 1H), 1.75 (d, $J = 6$ Hz, 3H). IR (cm^{-1}): 3610, 3420, 2970, 1720, 1520, 1450, 1350. UV–VIS (nm): 324, 242, 218. MS: *m/z* 378 (12, $M^{+\cdot}$), 135 (100).

(R)-4-(N-1-Heptafluorobutyrylamino-1-ethyl)-1-naphthylamine. Yield, 86%; m.p., 137.5–138.5°C; $[\alpha]_D = +95°$ ($c = 0.1$ in ethanol, 25°C), ^{1}H NMR (ppm): 8.00–7.25 (m, 6H), 6.58 (s, 1H), 5.87 (q, 1H), 4.13 (s, 2H), 1.75 (d, $J = 6$ Hz, 3 H). IR (cm^{-1}): 3610, 3420, 2970, 1710, 1620, 1510, 1450. UV–VIS (nm): 306, 222, 198. MS: *m/z* 382 (100, $M^{+\cdot}$).

CSP V (HFP). Analysis: found, C 4.58, H 0.84, N 0.3, F 1.11%; calculated, 0.11 mmol (*R*)-ligand/g stationary phase (based on N) and 0.16 mmol (*R*)-ligand/g stationary phase (based on C).

Liquid chromatography

To eliminate fines the CSPs were sedimented five times in methanol. Stainless-steel tubes (25 cm × 3.2 mm I.D.) were used as columns. A slurry prepared from 1.9 g of the phase and 30 ml of methanol–triethylene glycol (1:9) was filled into the column with a Model 27486-4 air-driven fluid pump (Haskel Engineering and Supply, Burbank, CA, USA) at a pressure of 680 bar. The columns were conditioned with methanol and *n*-hexane.

Chromatography was performed using a Model 110 solvent metering pump (Altex, Berkeley, CA, USA); detector Hitachi Model 100-10 variable-wavelength UV detector (Kontron, Zürich, Switzerland), detection at 254 nm; sampling device, Rheodyne (Berkeley, CA, USA) Model 7125 syringe-loading sample injector with a 20-μl loop; and recording devices, Tarkan W & W recorder 600 (Kontron) and HP 3396 A integrator (Hewlett-Packard, Widen, Switzerland).

The mobile phases used were (a) *n*-hexane–2-pro-

panol (78:22) (b) *n*-hexane–tetrahydrofuran (THF) (75:25) and (c) *n*-hexane–THF (85:15) at a flow-rate of 1 ml/min. The columns and the mobile phase container were maintained at 20°C (Assistant WTE var 3185 thermostat; R.C. Kuhn, Berne, Switzerland).

Toluene as a non-retained standard, dissolved in the appropriate mobile phase, was used to determine the dead time, t_0, and number of theoretical plates, N_0. The measured values were in the range $1.46 \leqslant t_0 \leqslant 1.72$ min and $3300 \leqslant N_0 \leqslant 7600$ for the five phases tested.

RESULTS AND DISCUSSION

Structure of bonded phases

All synthesized phases I–V shown in Fig. 1 are identical except for the bulky aliphatic N-acyl group R_i. The loading density of chiral ligands and the packing quality of the five columns tested are comparable. From the results of elemental analyses we calculated surface densities of *ca.* 0.5–0.7 groups/nm^2, which correspond to only one eighth of the available silanol groups [20]. It was not possible to increase the amount of chiral ligands by using higher reactant concentrations in the bonding procedure. Reasons are the bulkiness of the ligands and the limited reactivity of the Si–OCH_3 group of the spacer molecule used.

Samples

Table II gives the chromatographic results obtained with all samples, different amides, most of them derived with a π-acidic DNB or 3,5-dinitrophenyl (DNP) group for increased charge-transfer (CT) interaction with the naphthalene group of the phases [17]. Every sample contains one or more aromatic groups. Compounds with strong π-acceptors such as DNB or DNP are strongly retained and, in general, sufficient resolution of enantiomers is achieved, as reported previously [14]. On CSP I (PIV), non-aromatic racemates were hardly or not resolved owing to a lack of strong CT interaction between aromatic systems [16]. It was found that a π–π interaction is important for enantiomer separation on all five stationary phases. All have a high selectivity for amides with a π-acidic N-acyl substituent, especially for the DNB-containing compounds **1**, **8**, **9**, **10**, **14**, and **26–29**. The highest separation factors were obtained with compound **14** on all five CSPs; **14** contains a π-basic naphthalene and a DNB-group on the chiral centre. The naphthalene group increases the π-acidity of the DNB group additionally.

Weaker π-acidic aromatic N-acyl substituents lower the ability for chiral recognition, as can be seen with compounds **2** and **15**. Compounds with π-basic or aliphatic N-acyl groups, such as **5–7** and **16–23** have lower separation factors on all five CSPs. Aliphatic N-acyl groups with larger and bulkier alkyl tails are slightly better resolved as shorter tailed homologues. Electronegative substituents increase the acidity of the amide group. The N-trichloro- and N-trifluoroacetyl derivatives are better resolved as the analogous N-acetyl derived amines. The aliphatic N-acyl "tails" increase the hydrophobic character of the samples. This leads to shorter elution times with the same mobile phase in comparison with analogous amines derived with the polar DNB group.

If the π-acidic site of the enantiomers is not fixed to the N-acyl group, as in **3**, chiral recognition is more difficult or even completely lost. The reason may be that several similarly stable diastereomeric complexes between the enantiomers and the chiral selectors are possible.

On CSP V (HFP) all samples have the poorest resolution and also the shortest retention times. Owing to the substitution with fluorine atoms the N-acyl group R_5 is very apolar and relatively small in comparison with the other R_i groups. A change in the polarity of the mobile phase (Table III) increases retention and the separation capability of the stationary phase considerably. Nevertheless, the separation factors for all the tested samples are still smaller than with the other phases.

Correlation experiments

Two homologous series, the racemic 1-phenylalkyl amines **1a–l** and the 1-phenylglycine derivatives **30a–j**, both as DNB derivatives (Fig. 2), were separated with *n*-hexane–2-propanol (78:22) as mobile phase (Tables IV and V and Figs. 3–6).

Chromatographic investigations on other aryl-based π-donor CSPs led to a hypothesis dealing with two competing recognition mechanisms, the non-intercalative and the intercalative process. Hydrogen bonding is an important contribution in the

TABLE II

RESOLUTION OF ENANTIOMERS **1–29** ON STATIONARY PHASES I–V

HPLC conditions: (a) *n*-hexane–2-propanol (78:22); (b) *n*-hexane–tetrahydrofuran (75:25); (c) *n*-hexane–tetrahydrofuran (85:15); flow-rate, 1 ml/min; column, 25 cm × 3.2 mm I.D., 5 μm; detection, UV (254 nm). No. = number of sample given in Table I; k'_1 = capacity factor of the first-eluted enantiomer; α = separation factor; configuration = configuration of last-eluted enantiomer; n.r. = no resolution.

No.	CSP I (PIV)		CSP II (CYH)		CSP III (ADA)		CSP IV (CPE)		CSP V (HFP)		Mobile phase	Configuration
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α		
1	4.92	2.17	5.52	2.34	4.95	2.45	6.95	1.87	0.96	1.33	b	*R*
2	2.51	1.08	3.02	1.06	0.78	1.17	1.43	1.10	0.88	n.r.	b	*R*
3	2.82	1.01	3.56	n.r.	2.50	1.09	3.50	1.08	1.37	n.r.	b	*R*
4	2.51	1.08	3.02	1.06	1.90	1.06	3.19	1.05	3.28	1.05	b	*R*
5	1.44	1.08	1.64	1.07	1.51	1.11	2.80	1.07	2.04	n.r.	b	*R*
6	2.75	1.08	2.91	1.06	5.01	1.05	6.21	1.05	5.48	1.03	b	*R*
7	0.52	1.12	0.59	1.12	0.21	1.47	0.80	1.18	0.66	n.r.	b	*R*
8	3.39	3.46	3.10	3.63	3.92	3.73	3.97	2.72	1.10	1.32	b	*R*
9	4.26	2.57	4.19	2.60	4.39	2.68	5.00	2.09	1.26	1.27	b	*R*
10	8.92	1.32	9.55	1.27	8.45	1.38	12.33	1.31	1.78	1.11	b	3*S*,4*R*
11	10.62	2.28	8.80	2.63	9.57	2.52	12.89	2.53	1.01	1.36	b	
12	9.44	2.16	4.76	2.01	7.43	3.25	9.26	3.14	1.15	n.r.	b	
13	0.61	1.13	0.68	1.08	0.48	1.20	0.84	1.16	0.95	1.14	a	*R*
14	8.19	4.67	7.68	4.84	19.88	4.23	10.33	3.00	3.69	2.83	a	*R*
15	0.94	1.14	1.11	1.08	0.92	1.18	1.63	1.12	0.94	n.r.	b	*R*
16	2.03	1.09	1.73	1.08	1.70	1.14	2.99	1.09	2.11	n.r.	b	*R*
17	5.30	1.05	3.66	1.07	6.02	1.05	7.19	1.04	7.07	n.r.	b	*R*
18	2.95	1.08	3.01	1.05	1.98	1.12	3.16	1.08	2.69	n.r.	b	
19	1.92	1.09	1.97	1.05	1.37	1.58	2.43	1.10	1.98	n.r.	b	
20	3.42	1.12	2.89	1.10	2.91	1.23	3.74	1.17	2.93	n.r.	c	*R*
21	0.50	1.16	0.51	1.16	0.31	1.55	0.82	1.27	0.68	n.r.	b	*R*
22	1.06	1.14	1.05	1.12	0.65	1.41	1.16	1.24	0.61	n.r.	c	*R*
23	1.06	1.14	1.05	1.12	0.64	1.40	1.13	1.22	0.52	n.r.	c	*R*
24	2.97	1.10	3.24	1.09	2.12	1.11	3.52	1.08	1.29	1.06	b	
25	6.66	1.05	7.12	1.05	5.65	1.05	9.63	1.03	2.85	n.r.	b	
26	13.01	1.33	11.27	1.51	14.45	1.63	19.23	1.49	2.69	n.r.	b	
27	11.20	1.41	9.11	1.42	13.07	1.44	15.58	1.44	3.01	n.r.	b	
28	4.03	1.24	5.29	1.26	3.28	1.24	4.71	1.18	0.77	n.r.	b	
29	2.90	1.22	2.82	1.29	2.84	1.29	4.17	1.21	0.49	n.r.	b	

TABLE III

RESOLUTION OF ENANTIOMERS **1–29** ON STATIONARY PHASE V (HFP) WITH MOBILE PHASE *n*-HEXANE–2-PROPANOL

HPLC conditions: *n*-hexane–2-propanol (78:22); flow-rate, 1 ml/min; column, 25 cm × 3.2 mm I.D., 5 μm; detection, UV (254 nm). No. = number of sample given in Table I; k'_1 = capacity factor of the first-eluted enantiomer; α = separation factor; configuration = configuration of the last-eluted enantiomer; n.r. = no resolution.

No.	k'_1	α	Configuration	No.	k'_1	α	Configuration	No.	k'_1	α
1	0.96	1.33	*R*	**14**	3.69	2.83	*R*	**26**	1.24	n.r.
2	1.19	1.07	*R*	**15**	1.49	1.08	*R*	**27**	0.81	n.r.
3	2.26	1.03	*R*	**16**	3.05	1.06	*R*	**28**	0.77	n.r.
4	2.17	1.07	*R*	**17**	2.47	1.04	*R*	**29**	1.83	1.04
5	2.44	1.05	*R*	**18**	1.69	1.04	*R*			
6	1.66	1.04	*R*	**19**	1.41	1.06				
7	0.97	n.r.		**20**	1.29	1.05	*R*			
8	2.96	1.63	*R*	**21**	0.86	1.12	*R*			
9	3.03	1.47	*R*	**22**	0.74	1.13	*R*			
10	2.06	1.13	*R*	**23**	1.35	n.r.				
11	1.78	1.53		**24**	3.02	n.r.				
12	7.83	2.39		**25**	7.84	n.r.				
13	0.95	1.14	*R*							

diastereomeric complex formation for the resolution of alkyl ester derivatives. On the other hand, the homologous N-alkylamide derivatives form a complex with a dominant dipole stacking of the amide dipoles [2,11,12].

If the alkyl chain of the enantiomer intercalates between the strands of the bonded phase and is directed toward the underlying silica support, the separation factor α would diminish with longer alkyl chains more and more owing to unfavourable steric repulsion. If the alkyl chain of the solute is in a more rectangular position relative to the strands of the bonded phase, as in the proposed non-intercalative process, the separation factor α would increase

TABLE IV

RESOLUTION OF N-3,5-DINITROBENZOYL-1-PHENYLALKYLAMINES **1a–l** ON STATIONARY PHASES I–V

HPLC conditions: *n*-hexane–2-propanol (78:22); flow-rate, 1 ml/min; column, 25 cm × 3.2 mm I.D., 5 μm; detection, UV (254 nm). *n* = Number of carbon atoms in the alkyl chain; k'_1 = capacity factor of the first-eluted enantiomer; α = separation factor; configuration = configuration of last-eluted enantiomer.

n	CSP I (PIV)		CSP II (CYH)		CSP III (ADA)		CSP IV (CPE)		CSP V (HFP)		Configuration
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	
1	9.48	2.08	8.54	2.19	9.82	1.92	6.80	1.80	1.64	1.49	*R*
2	9.94	2.50	8.76	2.61	10.62	1.97	7.44	1.83	1.66	1.56	*R*
3	9.69	2.52	9.08	2.67	10.69	2.36	7.41	2.23	1.42	1.81	*R*
4	10.27	2.38	10.22	2.55	11.70	2.31	7.72	2.15	1.36	1.81	*R*
5	10.29	2.50	10.69	2.64	11.61	2.44	7.51	2.22	1.27	1.87	
7	9.29	2.47	9.62	2.77	10.97	2.51	7.13	2.33	1.12	1.92	
8	8.77	2.52	8.77	2.85	10.33	2.56	6.34	2.35	1.05	1.93	
9	8.43	2.57	8.61	2.94	10.24	2.62	6.49	2.39	1.00	1.95	
10	8.14	2.59	8.56	2.99	9.62	2.61	6.32	2.43	0.94	1.99	
13	7.57	2.67	7.75	3.08	8.56	2.68	6.12	2.45	0.80	2.02	
15	7.00	2.68	7.16	3.14	8.48	2.74	5.46	2.48	0.73	2.11	
17	5.53	2.70	6.96	3.19	6.26	2.76	5.05	2.52	0.68	2.31	

TABLE V

RESOLUTION OF *n*-ALKYL ESTERS OF N-3,5-DINITROBENZOYL-1-PHENYLGLYCINE **30a–j** ON STATIONARY PHASES I–V

HPLC conditions: *n*-hexane–2-propanol (78:22); flow-rate, 1 ml/min; column, 25 cm × 3.2 mm I.D., 5 μm; detection, UV (254 nm). *n* = Number of carbon atoms in the alkoxy chain; k'_1 = capacity factor of the first-eluted enantiomer; α = separation factor; configuration = configuration of last-eluted enantiomer.

n	CSP I (PIV)		CSP II (CYH)		CSP III (ADA)		CSP IV (CPE)		CSP V (HFP)		Configuration
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	
0	12.05	1.21	10.06	1.16	10.85	1.26	8.47	1.29	7.07	1.11	
1	10.16	1.20	8.02	1.17	8.83	1.29	6.34	1.27	5.69	1.03	*R*
2	7.29	1.20	6.13	1.15	7.14	1.28	5.38	1.27	4.50	1.04	*R*
3	7.13	1.22	5.53	1.16	6.11	1.29	4.63	1.29	3.73	1.06	*R*
4	6.67	1.22	5.23	1.15	5.74	1.30	4.22	1.30	3.35	1.08	
5	6.25	1.23	4.99	1.16	5.62	1.30	4.08	1.31	3.15	1.08	*R*
6	5.63	1.24	4.75	1.16	5.24	1.30	3.74	1.32	2.87	1.09	
8	5.21	1.23	4.68	1.16	5.04	1.31	3.67	1.34	2.56	1.09	
10	4.63	1.23	4.31	1.15	4.64	1.30	3.31	1.35	2.28	1.09	
12	4.34	1.23	4.01	1.15	4.48	1.31	3.09	1.34	2.13	1.09	

with a longer alkyl chain owing to a larger hydrophobic interaction.

For the homologous 1-phenylalkyl amide derivatives **1a–l** (Table IV and Fig. 3), on all five phases there is an increase in the separation factor α with increasing carbon number *n* of the *n*-alkyl "tail". The relative maximization of α at *n* = 2 and the different slopes of the connecting lines between the measured values with *n* < 4 and *n* > 4 point to different arrangements of the solutes in the diastereomeric complex.

Däppen *et al.* [18] calculated four stable complexes with (*R*)-2,2-dimethyl-N-{1-[1-(4-amino)naphthyl]ethyl}propanamide as a model phase and (*R*/*S*)-dinitro-N-(1-phenylethyl)benzamide (**1a**) as test solute. Based on CAMM calculations they pro-

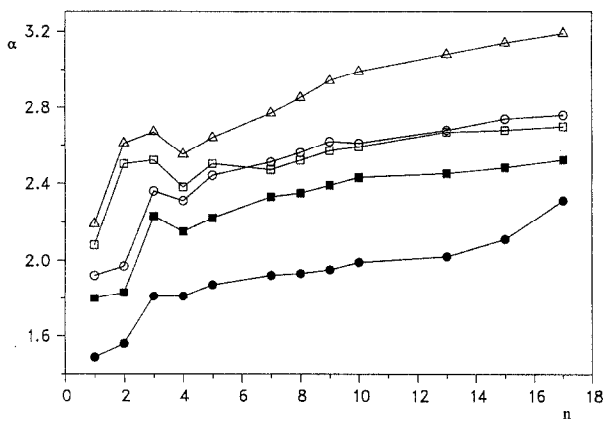


Fig. 3. Separation of phenylalkyl DNB derivatives **1a–l** on CSP I–V using *n*-hexane–2-propanol (78:22) as mobile phase. Separation factor α *versus* carbon number *n*. □ = CSP I (PIV); △ = CSP II (CYH); ○ = CSP III (ADA); ■ = CSP IV (CPE); ● = CSP V (HFP).

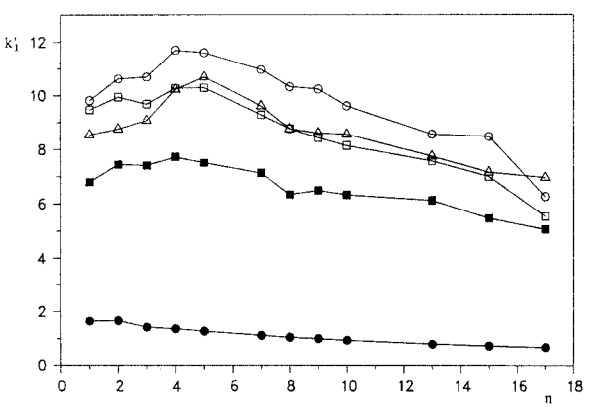


Fig. 4. Separation of phenylalkyl DNB derivatives **1a–l** on CSP I–V using *n*-hexane–2-propanol (78:22) as mobile phase. Capacity factor k'_1 *versus* carbon number *n*. □ = CSP I (PIV); △ = CSP II (CYH); ○ = CSP III (ADA); ■ = CSP IV (CPE); ● = CSP V (HFP).

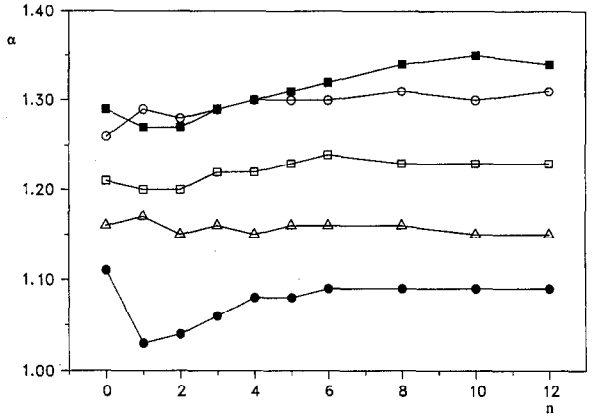


Fig. 5. Separation of phenylglycine DNB derivatives **30a–j** on CSP I–V using *n*-hexane–2-propanol (78:22) as mobile phase. Separation factor α *versus* carbon number *n*. □ = CSP I (PIV); △ = CSP II (CYH); ○ = CSP III (ADA); ■ = CSP IV (CPE); ● = CSP V (HFP).

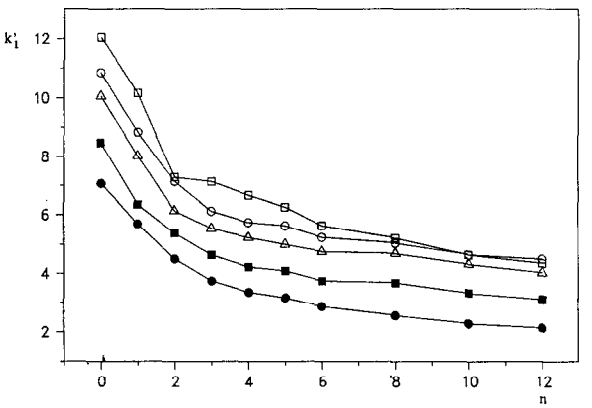


Fig. 6. Separation of phenylglycine DNB derivates **30a–j** on CSP I–V using *n*-hexane–2-propanol (78:22) as mobile phase. Capacity factor k'_1 *versus* carbon number *n*. □ = CSP I (PIV); △ = CSP II (CYH); ○ = CSP III (ADA); ■ = CSP IV (CPE); ● = CSP V (HFP).

posed an arrangement for the most stable complexes without parallel stacking of the aromatic π-donor and the π-acceptor system.

From this result and from our measurements, it is probable that shorter "tailed" enantiomers are capable of attaching with different orientations to the strands of the bonded phase and with partial inclusion between them. However, the short *n*-alkyl chains may not necessarily be directed straight toward the underlying silica support. Against that, solutes with longer alkyl chains have an orientation of the tails almost rectangular to the strands of the bonded phase. The measured α values increase with increasing chain length after $n > 4$ (butyl). This points to a non-intercalative mechanism for these longer "tailed" aryl containing enantiomers.

The *R*-enantiomer usually elutes last, and also there are no signs of inversion of the elution order, although not all pure enantiomers were available to check this completely. For butyl ($n = 4$) or pentyl ($n = 5$) the capacity factors k'_1 go through a maximum on phases I–III. On CSP IV (CPE) this tendency is less pronounced and on CSP V (HFP) the k'_1 values are minimal and nearly constant (Fig. 4).

A homologous series of 1-phenylalkylamine derivatives, **30a–j**, was eluted using hexane–2-propanol (78:22). Table V and Fig. 5 show the relationship between the separation factor α and the carbon number *n* of the analyte's alkyl chain. The corresponding dependence of the capacity factors k'_1 is seen in Fig. 6. For these analytes, the non-intercalative process is initially dominant, presumably because of the added hydrogen-bonding site, the carboalkoxy carbonyl oxygen [2,11,12].

On all five phases the separation factors remain nearly constant and there is a large decrease in the k'_1 values with increasing carbon number *n*. Hence the bulky alkyl "tails" of these samples do not influence the recognition mechanism of all five phases. This behaviour can only be explained with an orientation of the "tails" towards the mobile phase. The smallness of the separation factors is a result of the weaker rotational stability of the molecules, favouring more conformations in the diastereomeric complexes. An explanation for this type of solute was given by Pirkle and co-workers [21,22].

Comparison of bonded phases

All five CSPs show a similar separation behaviour but a different separation performance for chiral amides (Tables II–V, Figs. 3–6). Large differences are found in the magnitude of the separation factors α and in the elution times of the tested analytes. The enantioselectivity of the bonded phases decreases, in general, with the following order of the sterically determinative group: R_2 = cyclohexyl > R_1 = *tert.*-butyl > R_3 = adamantyl > R_4 = cyclopentylethyl ⩾ R_5 = heptafluoropropyl. For some types of solutes there are small differences between CSP I, II and III in this general order.

The N-acyl group R_2 of CSP II (CYH) has the same molecular cross-sectional area as R_3 of CSP III (ADA) but no rigid and bulky spherical shape and, therefore, it is able to reach an energetically favourable position by turning of the cyclohexyl ring.

R_3 is the most bulky and rigid N-acyl substituent. R_1 of CSP I (PIV) has a similar volume but more freedom of rotation. This is better for the resolution of short "tailed" enantiomers.

The cyclopentyl ring of CSP IV (CPE) has a smaller diameter than the cyclohexyl ring but its ethyl bridge is freely moveable towards the amide group of the selector. This also allows a more favourable displacement of R_4 in the diastereomeric complex with the analyte. Owing to the smaller dimension of the cyclopentyl ring, the steric interaction site of the chiral selector is smaller than that with the larger R_2.

The CSP V (HFP) with the small and apolar group R_5 shows the poorest resolution and the shortest elution times of all phases. Very polar solutes are badly resolved. With a more hydrophobic analyte a better interaction with the stationary phase is possible owing to a larger extent of dispersional forces.

CONCLUSIONS

Using N-1-(1-naphthyl)ethylamine as starting material, chiral stationary π-donor phases with different separation performances for chiral amine derivatives are accessible. Derivatization of these amines with a strong π-acceptor group such as N-3,5-dinitrobenzoyl or 3,5-dinitrophenyl gives the best results. A variation of the N-acyl substituent of the chiral selector changes the separation performance and the polarity of the bonded phase, indicating a significant contribution of steric interactions in the chiral recognition process.

From correlation experiments with homologous series of analytes, two non-intercalative chiral recognition processes can be assumed. In addition to π–π interactions between aromatic moieties of the solute and the CSP, the steric fit between the molecules within the complex determines the magnitude of the resolution. Bulky but flexible N-acyl groups such as cyclohexylcarbonyl or pivaloyl attached to the chiral centre of the selector increase the discrimination between the two chiral antipodes.

ACKNOWLEDGEMENTS

We thank Mrs. Vera Rätz for valuable assistance with the synthetic and analytical work, the Intercantonal Office for the Control of Medicaments, Berne, for kindly providing samples, Mr. H. Gfeller for mass spectrometric measurements, Dr. W. Padowetz of Ciba Geigy, Basle, for elemental analyses and Mrs. H. Murray for linguistic advice.

REFERENCES

1 S. G. Allenmark, *Chromatographic Enantioseparation, Methods and Applications*, Wiley, New York, 1989.
2 W. H. Pirkle, M. H. Hyun, A. Tsipouras, B. C. Hamper and B. Bank, *J. Pharm. Biomed. Anal.*, 2 (1984) 173.
3 W. Lindner and C. Petterson, in I. W. Wainer (Editor), *Liquid Chromatography in Pharmaceutical Development, an Introduction*, Aster, Springfield, 1985, p. 63.
4 I. W. Wainer, *Trends Anal. Chem.*, 6 (1987) 125.
5 H. Lienne, M. Caude, A. Tambuté and R. Rosset, *Analusis*, 15 (1987) 431.
6 W. H. Pirkle, J. M. Finn, J. L. Schreiner and B. C. Hamper, *J. Am. Chem. Soc.*, 103 (1981) 3964.
7 W. H. Pirkle, J. M. Finn, B. C. Hamper, J. L. Schreiner and J. R. Pribish, *ACS Symp. Ser.*, 185 (1982) 245.
8 R. Däppen, H. Arm and V. Meyer, *J. Chromatogr.*, 373 (1986) 1.
9 A. M. Krstulovic, *J. Pharm. Biomed. Anal.*, 6 (1988) 641.
10 W. H. Pirkle and M. H. Hyun, *J. Org. Chem.*, 49 (1984) 3043.
11 W. H. Pirkle, M. H. Hyun and B. Bank, *J. Chromatogr.*, 316 (1984) 585.
12 W. H. Pirkle and M. H. Hyun, *J. Chromatogr.*, 328 (1985) 1.
13 V. R. Meyer, *Chromatographia*, 24 (1987) 639; and references cited therein.
14 R. Däppen, V. R. Meyer and H. Arm, *J. Chromatogr.*, 361 (1986) 93.
15 R. Brügger, P. Krähenbühl, A. R. Marti, R. Straub and H. Arm, *J. Chromatogr.*, 557 (1991) 163.
16 R. Brügger, A. R. Marti, V. R. Meyer and H. Arm, *J. Chromatogr.*, 440 (1988) 197.
17 R. Däppen, H. R. Karfunkel and F. J. J. Leusen, *J. Chromatogr.*, 469 (1989) 101.
18 R. Däppen, H. R. Karfunkel and F. J. J. Leusen, *J. Comput. Chem.*, 11 (1990) 181.
19 R. Straub, *Dissertation*, University of Berne, Berne, 1990.
20 K. K. Unger, *Porous Silica (Journal of Chromatography Library, Vol. 16)*, Elsevier, Amsterdam, 1979, p. 57.
21 W. H. Pirkle, D. W. House and J. H. Finn, *J. Chromatogr.*, 192 (1980) 143.
22 W. H. Pirkle, C. J. Welch and M. H. Hyun, *J. Org. Chem.*, 48 (1983) 5022.